Title
Re-Initiation Promoters: Genetic Elements that Modify Cell Cycle Control of Adjacent DNA Replication Origins

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Publication Date
2014

Peer reviewed|Thesis/dissertation

Re-Initiation Promoters: Genetic Elements that Modify Cell Cycle Control of Adjacent DNA Replication Origins

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biochemistry and Molecular Biology

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

[redacted]

Acknowledgments

This dissertation, which serves as a record of my graduate training, would not have been possible without the help, support, and advice of countless people. It is my intention to recognize and thank my coworkers, friends, and family for the profound effect they have had on my development as a scientist and as a man.

I want to start by thanking my advisor, Joachim Li, whose contributions over the last eight years have improved both my intellect and my character. Joachim taught me to think through a project from the premises to the conclusion without eliding or outsourcing any of the details – no matter how esoteric. He supplemented this focus on clear and comprehensive thought with a similarly rigorous approach to developing clear expression. His insistence that I speak every week at round robin lab meetings helped me overcome my considerable fear of public speaking. Beyond the technical aspects of my training, I would also like to recognize Joachim for the courage and integrity that he shows as a scientist. I have seen him manage crises involving data falsification (note: not mine!) and results that complicate our pretty models. His instincts are always to get to the truth regardless of how inconvenient or uncomfortable that process might be. I hope that I've absorbed some of this boldness. Lastly, I'd like to thank Joachim for the unbelievable level of preparedness that he showed when teaching. Clear thought, clear expression, great teaching, and great ethics. I will never forget the standards that he set.

I would also like to recognize the members of my thesis committee for their invaluable contributions to my work. Pat O'Farrell, in particular, always provided great critical feedback on my ideas and results. His skepticism about yeast as a model system and suggestions that re-replication might be a spandrel both challenged and strengthened my project. Hiten Madhani gave freely from his encyclopedic knowledge of yeast biology and experimental techniques, providing great suggestions for pathways that might play a role in re-replication. He and his lab also helped immensely when I had to learn ChIP-qPCR. Finally, while he is not actually a member of my thesis committee, I would like to thank Joe DeRisi for developing the microarray platform that I used extensively. Joe's relentless optimism and fearless hurdling of technical boundaries made a big impression on me - and provided yet another example that I hope to emulate.

Thanks are also due to the former and current members of the Li Lab. Brian Green and Richard Morreale did not overlap with me in the lab but left a very large legacy. I am grateful to them for developing the experimental system that I worked with AND the software tools that I used to analyze my data. Muluye Liku overlapped with me briefly, provided me with some great advice, and left me with one incredible bon mot that I will never be able to forget, nor commit to writing. Thanks Muluye, and I hope you found your peace with honor. I must also thank Ken Finn and Stacey Hanlon for being great friends and collaborators, especially during stressful microarray print runs. Ken showed me what a young, independent scientist looks like. His tenaciousness, technical skill, and sheer

work ethic set the benchmark that I will always compare myself against. Stacey always impressed me with her willingness to question the accepted wisdom. There were many occasions during my graduate career where something that I thought I “knew” turned out to be poorly supported or incorrect. More often than not it was Stacey who pointed this out. And last but certainly not least I would like to thank Francisco Castro. Frank is Joachim’s consigliore: media technician, glass washer, lab manager, and technician all rolled into one. He rarely gets the respect or thanks that he deserves, but his work enables everyone else in the lab to focus on experiments instead of media preparation. Thanks, Frank.

None of these experiences over the last eight years would have been possible without the support of the UCSF Tetrad program. The open, positive, and fun environment fostered by the faculty, students, and administrators made this a great place to work. So: my thanks go out to UCSF as well as my classmates in the Tetrad program for making me feel welcome and for being so generous with their expertise and resources.

Whatever abilities UCSF developed in me were first recognized and nurtured by Andy Dillin at the Salk Institute in San Diego. I am grateful for the direct role that his mentorship and influence played in my admission to UCSF as well as the indirect role that he continues to play in my career. Someone once told me that Andy was successful because he never thought himself out of an experiment. This was not meant as a slight. Rather, I think it is one secret to

success. Scientists can be very critical when evaluating each other's ideas and experiments. Internalizing the form of this criticism without evaluating its validity is an easy way to lose motivation and interest in one's work. Andy showed me that optimism and drive are an effective way to avoid this trap and, as a result, I have tried to cultivate these qualities in myself.

Outside of work, I am grateful for the many, many excellent human beings in my life. I will start by thanking the cast of characters who kept me sane during my PhD career. To my friends outside the program, specifically Chris Krauskopf, Peter Seliga, Mike Cardellini, Adam Degregorio, Braden Levine, Ben Moore, and Megan Taylor: thanks for distracting me. My life is immeasurably richer for having you as friends. You have given me so many great memories that I don't even regret the two or three additional (but wonderful) years you collectively added to my graduate tenure.

On the topic of distractions, I owe huge debts to Larry Baskin and Bill Moore for taking me sailing and trusting me to do complicated things with expensive sails that I am totally unqualified to do. Sailing, in addition to being tremendous fun, made me a better scientist because by reminding me what it feels like to be engaged and motivated. I always tried to work hard on the boat. When science went poorly and my desire to cut corners grew, an effective antidote was usually to ask, "What would I think if someone on a race crew acted this way?"

I am deeply grateful for the love and support provided by my parents, Matthew Bone and Elaine Richardson. My parents raised my sister and me to be kind, to value education and science, and to set ambitious goals for ourselves. This in itself is a wonderful achievement but they outdo themselves by continuing to support and encourage me. My sister, Jennifer Bone, joins them in their unwavering faith that I will succeed. My family believes that I can perform at the highest level and the knowledge that three people in the world think I can succeed has shepherded me through many crises of confidence. As my sister begins her own graduate education, I look forward to providing her with the same support and encouragement.

Saving the best for last, I would like to recognize the role that my girlfriend and best friend Brooke Gardner played in my doctoral training. Brooke is an excellent scientist and provided me with great advice and encouragement in personal and professional matters. More importantly, she provided love, and compassion, and respect, and generosity when I needed these emotions most. I love you, Brookie.

The work described in Chapter 2 is a reprint of “Regulatory Mechanisms That Prevent Re-Initiation of DNA Replication Can Be Locally Modulated at Origins by Nearby Sequence Elements” Christopher D. Richardson and Joachim J. Li (2014) *PLoS Genet*. http://www.plosgenetics.org/doi/pgen.1004358. The Public Library of Science (PLoS), the non-profit publisher of *PLoS Genetics*, permits unrestricted reuse of published material by the authors without acquiring specific permission. Christopher D. Richardson performed the experiments and analyzed the data. Christopher D. Richardson and Joachim J. Li contributed to the study design, writing of the manuscript, and editing of the manuscript.

Abstract

Re-Initiation Promoters: Genetic Elements that Modify Cell Cycle Control of Adjacent DNA Replication Origins

Christopher Douglas Richardson

Replication control is fundamental to genomic stability as aberrant replication within a single cell cycle can induce high rates of segmental amplification, chromosomal aneuploidy, and possibly other genomic instabilities. Current models for how eukaryotic cells prevent such re-initiation focus on the global cell-wide inhibition of replication proteins involved in loading the Mcm2-7 replicative helicase at origins (e.g. cyclin dependent kinase, CDK, inhibition of ORC, Cdc6, Cdt1, Mcm2-7). By preventing this initial step of initiation from reoccurring once S phase begins, re-initiation can be effectively prevented. Such models, however, treat origins as generic interchangeable elements and cannot account for the diverse efficiencies with which origins re-initiate when global control mechanisms are disrupted. These varied re-initiation efficiencies also cannot be explained by the well-documented diversity in origin timing and efficiency observed during normal S phase initiation. Instead, we now have evidence of a novel mechanism that contributes to the diversity in origin re-initiation efficiency.

Chapter 2 of this dissertation details the identification and characterization of genetic elements near ARS317 and ARS1238 that confer preferential re-replication on these and other origins when cell cycle control of MCM2-7 and Cdc6 is disrupted. These elements do not confer any detectable change on the replication efficiency or timing of adjacent origins, suggesting that their regulatory effect is specific to origin re-initiation. Hence, we refer to these elements as Re-Initiation Promoters (RIPs). The two RIPs mapped are AT rich sequences 40-50bp in size and exert their effects on adjacent origins in an orientation and distance dependent manner. Analysis of Mcm2-7 association with origins suggests that RIP elements allow local escape from the residual CDK inhibition of helicase loading when global CDK inhibition of Mcm2-7 and Cdc6 is disrupted.

Such local modulation of origin control suggests that there is a complex genomic landscape of re-replication potential, particularly when mechanisms preventing re-replication are partially or sporadically disrupted. Hence, if re-replication does contribute to genomic alterations, as has been speculated for cancer cells, some regions of the genome may be more susceptible to these alterations than others.

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Chapter 1

General Introduction

DNA replication requires the coordinated activity of many individual origins.

DNA is the basic unit of biological inheritance in all known cellular organisms. Maintaining the integrity of this molecule is critical to the viability of a given cell, and successful cell division is likewise dependent on near-perfect duplication and segregation of DNA. As such, DNA replication is one of the most regulated processes in all of biology, requiring tight coordination of the thousands of complicated protein machines that carefully copy a cell's genetic information while simultaneously surveying for and repairing damage. This dissertation explores one aspect of the highly regulated and staggeringly complicated control of DNA replication, illustrating that while all origins of DNA replication are subject to global licensing and firing regulation, there are also local determinants that act on individual replication origins.

DNA replication is constrained by a number of physical parameters, including the speed at which it can be copied. Eukaryotic replisomes – the protein complexes that replicate DNA [1] – can copy 1-2 kilobases of DNA per minute under ideal conditions [2] while making on the order of 1 mistake per billion nucleotides [3]. The processivity and fidelity of these complexes are impressive, but a human cell contains around 6 billion basepairs of diploid DNA [4]. Duplicating a genome of this size would take over 30 days if initiated from a single replication origin. In reality, the synthesis phase (S-phase) in the human cell cycle lasts a matter of hours [5]. This massive increase in speed comes from

the simultaneous initiation of replication from many origins scattered throughout the genome. In the case of mammalian cells, the number of origins utilized can exceed 50,000.

Parallelization of DNA replication imposes another constraint on the system. Because replication proceeds from multiple origins, perfect duplication of a genome requires not just the extraordinary fidelity with which a single replisome copies DNA, but also the coordinated assembly and firing of replisomes from hundreds to thousands of replication origins. *Under-* or *over-* replication from these origins alters the copy number of large stretches of the genome and can provide a substrate for undesirable or toxic genomic rearrangement. As a result, replication from each of these origins is tightly regulated to occur once and only once from each origin during a normal cell cycle.

The basis of this control is a licensing system that temporally separates the cell cycle into two stages [6-9]. The first stage (G1) licenses replication origins but does not permit replication initiation. The second stage (S-G2) activates licensed replication origins while preventing additional rounds of licensing. Thus, an origin that is licensed in G1 and activated in S-G2 cannot be licensed again until G1 of the subsequent cell cycle. Cell cycle signals enforce this temporal separation. The absence of such signals during G1 permits the assembly of pre-replicative complexes (pre-RCs; explained below) onto replication origins. At the completion of this phase, cell cycle signals are turned

on, simultaneously initiating assembled pre-RCs and inhibiting their re-assembly. Eukaryotic cells deploy multiple overlapping regulatory mechanisms to enforce this control. The identity of each of these overlapping mechanisms varies in different species, but the principle of overlapping control ensures a single round of replication initiation occurs in all eukaryotes [10].

Studying DNA replication in budding yeast.

The budding yeast *S. cerevesiae* is an ideal model system for studying eukaryotic DNA replication. The organism is genetically tractable and excellent functional annotations exist for many genes. The *S. cerevisiae* genome was first sequenced nearly twenty years ago [11] and comprehensive genomic sequencing of a given strain is well within the capabilities of a small-medium research lab. Easy genome manipulation, excellent functional annotations, and rapid monitoring of strain genotype make yeast reverse genetics an especially powerful approach for understanding cell biology and biochemistry. Another advantage that yeast offer the study of DNA replication is well-defined replication origin sequences of 100-200 basepairs in size [12]. Unlike metazoans, yeast initiate replication from specific origins instead of from large replication zones [13]. This property allows for detailed molecular genetic study of the DNA sequence determinants that play a role in origin assembly and initiation. Moreover, the defined replication origin sequences can be easily moved around the genome to

test the effects that location and context have on their activities. Thus, the efficiency of yeast reverse genetics and molecular genetics allows for rapid testing of both the *trans*- and *cis*- factors that regulate DNA replication.

Replication Control in *Saccharomyces cerevisiae*

Replication control in budding yeast, as in all eukaryotes, specifically regulates the initiation step. During G1, pre-RCs consisting of the DNA binding origin recognition complex (ORC), the helicase complex Mcm2-7, and two loading factors, Cdc6 and Cdt1 assemble onto origin DNA. An increasing body of evidence supports a model in which loading of the Mcm2-7 complex around origin DNA is the key step in the licensing process [14]. This is the point at which cyclin dependent kinase signaling shifts from repressing replication activity to stimulating it. Cdc6, Cdt1, and Mcm2-7 association with origin DNA are all inhibited by cyclin dependent kinase (CDK) activity and will assemble onto origin DNA in G1 only when CDK activity is low. ORC is present at origin DNA throughout the cell cycle but does not allow pre-RC assembly when CDK activity is present. After Mcm2-7 is loaded around DNA, any CDK activity promotes DNA replication. In a normal cell cycle, the rise in CDK activity at the end of G1 and beginning of S phase recruits additional components that incorporate loaded Mcm2-7 into two separate multi-protein replisome complexes that replicate DNA bidirectionally away from the origin [7].

The origin recognition complex (ORC) binds to DNA and acts as the site of recruitment for other pre-RC components [6,7,15]. ORC subunits were originally identified in *S. cerevisiae*, and at least in this organism, appear to be bound to origin DNA throughout the cell cycle [7,16-18]. Five of the six ORC subunits (Orc1-5) are members of the AAA+ ATPase family, though only Orc1 retains a functional ATPase [19]. ATP binding by ORC (but not its hydrolysis) is essential for stable binding of ORC to DNA [20]. Early models speculated that ATP hydrolysis by ORC could reshape origin DNA to promote interaction between the DNA and other replication factors. However, it does not appear that ORC activity drives any structural changes at origin DNA. Rather, ORC hydrolysis of ATP is required for the loading of Mcm2-7 onto origins [21-23]. Thus, the first pre-RC components to assemble onto origin DNA appear to control whether or not the key step in initiation is completed.

Two additional proteins, Cdc6 and Cdt1 also play a role in loading Mcm2-7 around DNA. Cdc6, another AAA+ ATPase, associates with ORC and hydrolyzes ATP to drive the loading of Mcm2-7 at origins [21]. Cdt1 is one of the two non-AAA+ ATPase members of the pre-RC (the other being Orc6) and is thought to play a role in regulating the association between Mcm2-7 and ORC assembled at origin DNA [24,25]. The working model is that Cdt1 enters the nucleus as part of a heptameric complex with Mcm2-7 and promotes the association – but not the loading – of this complex with ORC-Cdc6 assembled at origin DNA [24-26].

Mcm2-7 is a heterohexameric protein complex composed of the AAA+ ATPases Mcm2, 3, 4, 5, 6, and 7. Various subassemblies of this complex have helicase activity in vitro [27], and it has been shown to travel with the replication fork [28,29]. For these reasons, there has long been controversy about whether or not Mcm2-7 is the replicative helicase. The dominant model in the field proposes that a CMG complex composed of Mcm2-7 and other protein factors Cdc45 and GINS is the helicase acting at replication forks [29,30]. There is, however, no controversy that Mcm2-7 loading around DNA is critical for replication. The details of this step remain to be observed in vivo, but in vitro data supports a model in which Mcm2-7 first transiently associates with ORC-Cdc6 and is then topologically loaded onto DNA [24]. This loading reaction is thought to configure Mcm2-7 as a head-to-head double hexamer encircling double-stranded DNA [31]. Regardless, once Mcm2-7 hexamers are loaded at origins, these origins are said to be licensed to initiate.

Immediately after licensing, loaded Mcm2-7 molecules are in an inactive state and do not move along DNA. Activation of licensed origins - or replication initiation - requires the reconfiguration of Mcm2-7 into a helicase capable of unwinding double stranded DNA [32,33]. This transition requires the action of two kinases, Cyclin-dependent kinase (CDK) and Dbf4-dependent kinase (DDK). These kinases promote the assembly of Cdc45 and GINS onto Mcm2-7 to form the CMG complex in a loading reaction that requires Sld2, Sld3, and Dpb11 [32,33]. The essential role of CDK in this process is to phosphorylate Sld2 and

Sld3 and promote their interaction with Dpb11 and proteins assembled at the origin [34,35]. DDK's role is to phosphorylate Mcm2-7 subunits. The precise function of Mcm2-7 phosphorylation is not entirely clear, but it is thought to promote conformational change of Mcm2-7 [36] and might drive association with other replisome components. The transition from CMG complex to active replication fork has not been fully elucidated but requires the incorporation of other factors including RPA, DNA polymerases, and PCNA [36].

The ordered assembly of replisome components onto origin DNA suggests many points where regulatory intervention could prevent aberrant re-replication events. In budding yeast and other eukaryotes, all known mechanisms that block re-replication act prior to the key step where Mcm2-7 is topologically loaded around origin DNA. Thus, these blocks act to prevent steps during pre-RC assembly. *S. cerevisiae* relies on CDK inhibition of the pre-RC components ORC, Mcm2-7, and Cdc6. Metazoans retain CDK-dependent controls while adding two CDK-independent mechanisms that prevent re-replication through inhibition of Cdt1 [37-39]. CDK phosphorylation of ORC disrupts the association between ORC and Cdt1 and prevents loading of Mcm2-7 [24,40]. Cdc6 activity is eliminated by transcriptional downregulation [41], as well as direct [42] and indirect [43] inhibition by Clb-CDK. Cdt1/Mcm2-7 is exported from the nucleus to prevent its association with other pre-RC components. Collectively, these regulatory mechanisms prevent the assembly of ORC, Cdc6, and Cdt1/Mcm2-7 into functional pre-RC complexes. As discussed above, once

MCMs are loaded onto origin DNA, CDK activity promotes their incorporation into functional replisomes.

These CDK-dependent regulatory mechanisms clearly block aberrant replication events because, in budding yeast, their disruption leads to additional rounds of replication within a single cell cycle [44-46]. Formally, these re-replication blocks could work in a redundant manner, where one block is sufficient to prevent re-replication, or an overlapping manner, where disruption of individual mechanisms results in a progressive increase in the amount of re-replication. Published data from our lab and others support the latter model, as we see a progressive increase in re-replication when two [Chapter 2, Figure S1], three [45], or four ([46] and Li lab unpublished) CDK-dependent pathways are disrupted. The sensitivity of microarray assays limits our ability to directly observe re-replication below the level produced by the simultaneous deregulation of MCM and Cdc6. However, by monitoring a downstream consequence of re-replication, Ken Finn's dissertation [47] showed that deregulation of a single CDK-dependent pathway increases the rate of re-replication induced genomic rearrangement. This finding needs to be extended, but is consistent with the block to re-initiation working in an overlapping manner. The redundant-overlapping dispute is critically important in establishing the broader relevance of re-replication. Re-replication is a major insult to cells, causing large regions of the genome to be duplicated and potentially upsetting gene dosage or causing instability. If re-replication blocks work in a redundant manner, then sporadic

induction of re-replication by random mutation is exponentially less likely to occur due to the constraint that multiple mutations must occur in a given cell before re-replication occurs. On the other hand, if re-replication blocks work in an overlapping manner, then single genetic lesions could cause small but significant amounts of re-replication.

Re-replication causes genomic instability

Extensive re-replication in budding yeast causes activation of the DNA damage checkpoint and cell death. Cells subjected to this also show clear evidence of chromosome fragmentation and double strand breaks [48,49]. Re-replication has also been observed in metazoan cells when Cdc6 and Cdt1 are overexpressed or when Geminin, a repressor of Cdt1, is depleted [50-53]. Metazoan re-replication causes double strand breaks and foci of the damage-induced histone variant H2AX, indicating that DNA damage occurs [51,54,55]. Thus, if extensive re-replication induces the DNA damage checkpoint and cell death, then what are the consequences of lower levels of re-replication?

Biologists have long speculated that re-replication could cause heritable genomic changes [56] though no direct support for this hypothesis was produced. The first conclusive evidence came from Brian Green and Ken Finn who developed an assay that drove re-replication through repetitive sequences and

monitored the consequences [44]. The authors demonstrated that transient deregulation of MCM and Cdc6 resulted in preferential re-replication from primarily one origin, a transplanted fragment containing *ARS317*. This re-replication could be monitored by microarray CGH and did not result in severe cell lethality. Use of a copy-number reporter system allowed the authors to explore a linkage between re-replication and gene amplification. Re-replication induced genomic amplification not only occurred, but did so with shocking efficiency, with approximately 1 in 20 re-replication events causing a heritable segmental amplification.

Subsequent studies in the Li lab adapted this system to drive re-replication through centromeres, heterochromatic regions, and repetitive regions that are predisposed to form extrachromosomal amplifications. In these cases re-replication dramatically increased aneuploidy, loss of silencing, and extrachromosomal amplifications in cells. Thus, we speculate that aberrant re-replication is a general cause of genomic – and epigenomic – change.

The perturbations discussed above could be important in human disease. Gene amplifications, for example, are thought to play a major role in the chemotherapeutic drug resistance of many cancers [57]. The role of gene amplification in the etiology of cancer is not as well worked out, but certain oncogene amplifications are structurally consistent with re-replication induced gene amplification [58,59]. In a similar fashion, the adaptability and evolvability

of cancer cells could be conferred by aneuploidy [60-62], and re-replication induced aneuploidy could contribute to this diversity. Finally, the emerging field of cancer epigenomics introduces the possibility that the epigenetic state of a tumor cell could determine its drug resistance or epithelial-mesenchymal plasticity [63]. Thus, loss of silencing caused by re-replication might alter one or both of these parameters.

Beyond human disease, re-replication induced genomic or epigenomic changes could play a role in creating the diversity acted upon by evolution [64,65]. Gene neofunctionalization is thought to proceed by a mechanism in which one copy of the duplicated gene provides the necessary biological function while the second copy acquires new capabilities. In such a model, re-replication induced duplication of large (aneuploidy) or small (segmental amplification) regions of the genome could play a substantial role in providing the diversity acted upon by classical evolution.

My predecessors in Joachim's lab identified some of the blocks to re-replication in budding yeast [66,67] and established that these blocks function in an overlapping manner [45]. My colleagues in the lab demonstrated that re-replication occurs preferentially from some origins and not universally from all replication origins as the licensing model would predict [45]. They went on to show that preferential re-replication is contained within short (~500bp) origin-containing fragments [44], a completely counterintuitive observation that

suggested DNA elements near replication origins might influence how they responded to inhibition by CDK. Finally, my colleagues showed that re-replication potently stimulates genomic rearrangements.

This dissertation establishes one mechanism behind preferential re-replication and explains how some replication origins re-initiate more effectively than others. I never addressed the question of why a cell would want to re-replicate one region of the genome instead of another. Perhaps the efficiency with which re-replication causes genomic rearrangements offers an explanation. My favorite (untested) hypothesis is that preferential re-replication provides a mechanism for adaptive evolution. By recruiting specific factors to replication origins, a cell might locally stimulate re-replication and thus genomic instability. This model is completely speculative, but there is precedent for cells using odd mechanisms to rearrange their genome. 400 million years ago, the common ancestor of jawed vertebrates was invaded by a transposon. The responsible RAG1/2 transposase complex now forms the basis for adaptive immunity across the majority of Chordata [68]. Briefly, RAG1/2 has been tamed into a site-specific recombinase that reorganizes human immunoglobulin and T cell receptor loci by V(D)J recombination. Maybe preferential re-replication works by a similar mechanism in which recruitment of some factor to a replication origin increases the chance that the adjacent origin will re-replicate and thus rearrange the adjacent genomic region.

Dissertation Summary

In this dissertation, I will describe my efforts to understand the *cis* acting elements that confer preferential re-replication on *S. cerevisiae* replication origins when CDK inhibition of MCMs and Cdc6 is disrupted. Chapter 2 describes my identification of genetic elements that are necessary and sufficient for preferential re-replication. This provides the first evidence that the blocks to re-initiation do not act universally on all replication origins. Other experiments described in Chapter 2 establish a likely mechanism of interaction between these genetic elements and adjacent origins as well as the specific point in the pre-RC assembly process that they deregulate.

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Chapter 2

Regulatory Mechanisms That Prevent Re-Initiation of DNA Replication Can Be Locally Modulated at Origins by Nearby Sequence Elements

Abstract

Eukaryotic cells must inhibit re-initiation of DNA replication at each of the thousands of origins in their genome because re-initiation can generate genomic alterations with extraordinary frequency. To minimize the probability of re-initiation from so many origins, cells use a battery of regulatory mechanisms that reduce the activity of replication initiation proteins. Given the global nature of these mechanisms, it has been presumed that all origins are inhibited identically. However, origins re-initiate with diverse efficiencies when these mechanisms are disabled, and this diversity cannot be explained by differences in the efficiency or timing of origin initiation during normal S phase replication. This observation raises the possibility of an additional layer of replication control that can differentially regulate re-initiation at distinct origins. We have identified novel genetic elements that are necessary for preferential re-initiation of two origins and sufficient to confer preferential re-initiation on heterologous origins when the control of re-initiation is partially deregulated. The elements do not enhance the S phase timing or efficiency of adjacent origins and thus are specifically acting as re-initiation promoters (RIPs). We have mapped the two RIPs to ~60 bp AT rich sequences that act in a distance- and sequence-dependent manner. During the induction of re-replication, Mcm2-7 reassociates both with origins that preferentially re-initiate and origins that do not, suggesting that the RIP elements can overcome a block to re-initiation imposed after Mcm2-7 associates with origins. Our findings identify a local level of control in the block to re-initiation.

This local control creates a complex genomic landscape of re-replication potential that is revealed when global mechanisms preventing re-replication are compromised. Hence, if re-replication does contribute to genomic alterations, as has been speculated for cancer cells, some regions of the genome may be more susceptible to these alterations than others.

Author Summary

Eukaryotic organisms have hundreds to thousands of DNA replication origins distributed throughout their genomes. Faithful duplication of these genomes requires a multitude of global controls that ensure that every replication origin initiates at most once per cell cycle. Disruptions in these controls can result in re-initiation of origins and localized re-replication of the surrounding genome. Such re-replicated genomic segments are converted to stable chromosomal alterations with extraordinarily efficiency and could provide a potential source of genomic alterations associated with cancer cells. This publication establishes the existence of a local layer of replication control by identifying new genetic elements, termed re-initiation promoters (RIPs) that can locally override some of the global mechanisms preventing re-initiation. Origins adjacent to RIP elements are not as tightly controlled and thus more susceptible to re-initiation, especially when these global controls are compromised. We speculate that RIP elements

contribute to genomic variability in origin control and make some regions of the genome more susceptible to re-replication induced genomic instability.

Introduction

The initiation of eukaryotic DNA replication is tightly regulated so that it occurs at most once per cell cycle [1]. This regulation is critical because re-replication of a chromosomal segment makes that segment highly susceptible to genomic alterations [2]. Preventing re-replication throughout the genome is particularly challenging for eukaryotic cells because their genomes contain hundreds to thousands of replication origins. Hence, each individual origin must be tightly controlled if a genome is to avoid any re-initiation events [3].

The basic strategy eukaryotic cells use to prevent re-initiation is to prevent the reassembly of replication initiation complexes at origins that have fired. The critical assembly step that is regulated is the loading of the core replicative helicase Mcm2-7, which forms a toroidal complex that encircles the origin DNA [4]. This loading is carried out by four factors: the origin recognition complex (ORC), Cdc6, Cdt1, and Mcm2-7 [5,6]. In the budding yeast, *Saccharomyces cerevisiae*, cyclin dependent kinases (CDKs) use multiple mechanisms targeting each of these proteins to prevent the reloading of Mcm2-7 once cells enter S phase [7]. In other organisms, additional CDK-independent mechanisms have been identified that inhibit Cdt1. The precise mechanisms used differ among

species, but the reliance on multiple mechanisms targeting each of the initiation proteins involved in Mcm2-7 loading is highly conserved [3,5].

The paradigm that has thus developed for the cell cycle control of replication initiation is that a multitude of overlapping mechanisms collaborate to globally inhibit initiation proteins throughout the cell, thereby minimizing the odds of re-initiating at any origin [8]. Consistent with this paradigm, disruption of individual mechanisms often does not lead to measurable re-replication even though the suspected consequences of re-replication, e.g. DNA damage or genomic alterations, have been observed [9-11]. Therefore, any investigation into the role of individual regulatory mechanisms in the block to re-initiation must be conducted in a sensitized system where a number of other overlapping mechanisms have been disrupted and re-replication can be readily detected.

Development of such sensitized systems revealed that origins re-initiate with diverse efficiencies and challenged the implicit assumption that all replication origins are uniformly regulated by global inhibition mechanisms [12,13]. For example, when ORC, Cdc6, and Mcm2-7 are deregulated, many (~100) origins detectably re-initiate, but many more (~200) do not. Moreover, the amount of re-initiation from each origin varies widely. This diversity of re-initiation efficiency does not correlate with the diversity of S-phase origin timing and efficiency and thus cannot be explained by the chromosome context effects that are responsible for the latter [14]. Instead, the diversity in re-initiation efficiencies suggests that origins are not solely and uniformly regulated by global controls. Thus, we believe the paradigm for re-initiation control needs to be

modified by the addition of a local layer of control that can modulate how tightly the global regulatory mechanisms inhibit re-initiation at specific origins.

Here, we explore the workings of this local control by asking why some budding yeast origins re-initiate more readily than others when global restrictions on re-initiation are partially inactivated. We show that local sequence elements adjacent to these origins specifically promote their re-initiation without enhancing their initiation activity. Furthermore, these elements act independently of the chromosomal context and silencing effects that regulate S-phase origin timing and efficiency. These elements, which we term re-initiation promoters (*RIPs*), map to ~60 bp segments that work in a distance- and sequence- dependent manner. Analysis of the re-association of Mcm2-7 with origins suggests that these *RIP* elements antagonize an inhibitory mechanism that operates after Mcm2-7 association with origins. These findings provide our first insight into how diversity can be introduced in the regulation of eukaryotic replication origins.

Results

Several Origins Preferentially Re-Initiate When Origin Controls Are Deregulated

To investigate the mechanisms underlying the diversity of origin regulation in the block to re-initiation, we examined *S. cerevisiae* origins whose ability to escape this regulation stood out the most from other origins. We previously

reported that re-initiation occurs predominantly from *ARS317* in a strain where a subset of global replication controls was disrupted [12]. This "*MC2Ao*" strain was deregulated in three ways: (1) (*M*) - the CDK driven export of Mcm2-7 from the nucleus [15-17] was blocked by fusing a constitutive nuclear localization signal onto the endogenously expressed Mcm7; (2) (*C2A*) – the CDK inhibition of Cdc6, which occurs through transcriptional regulation [18], phosphorylation-directed degradation [8,19,20], and direct CDK binding [21], was completely disrupted by expressing an extra copy of Cdc6 lacking CDK phosphorylation and binding sites under a galactose-inducible promoter; and (3) (*o*) - the CDK inhibition of ORC by phosphorylation of Orc2 and Orc6 was minimally perturbed by eliminating one of four CDK consensus phosphorylation sites on Orc6 [2]. We note that this ORC deregulation was not necessary for the preferential re-initiation of *ARS317*, but enhanced it approximately 3-fold (Figure S1). Importantly, of the known mechanisms preventing re-initiation in budding yeast, two are retained in this strain: (1) CDK phosphorylation of Orc2 and Orc6 (9 out of 10 CDK consensus phosphorylation sites remain unmutated) [7]; and (2) Clb5-Cdc28 binding to an RXL docking site on Orc6 [22].

Re-initiation was not detectable in the *MC2Ao* strain until the deregulated Cdc6 was induced. We could thus arrest cells at metaphase with a normal 2C DNA content across the genome, induce the deregulated Cdc6, and detect re-initiation and re-replication as a >2C DNA copy number using array comparative genomic hybridization (aCGH). Although the primary re-initiation event after a 3 hr induction of re-replication was at *ARS317* [12], the re-replication profiles

showed hints of additional re-replication peaks at other genomic loci. At least two of these peaks were readily confirmed with a longer 6 hr induction of re-replication, one on the right arm of Chr 5 near position 575 kb, and one on the right arm of chromosome 12 near position 890 kb (Figure 1A). The latter was dependent on *ARS1238*, establishing that this origin also preferentially re-initiated in the *MC2Ao* strain (Figure 1B). Because *ARS317* and *ARS1238* were among the two most efficient re-initiating origins, we focused on them to investigate why some origins are more susceptible to re-initiation than others.

Preferential Re-Initiation of *ARS317* and *ARS1238* Is Conferred by Local Sequence Determinants

We first sought to determine whether the preferential re-initiation of *ARS317* and *ARS1238* was conferred by the origin and immediate surrounding sequences or required a broader chromosomal context that spans kilobases of DNA. An example of the latter is the poorly understood chromosome position effect that has been implicated in the diversity of yeast origin timing and efficiency during normal S phase initiation (discussed in [14,23]). We and others had previously shown that there was no correlation between this diversity of origin activity in S phase and the diversity of re-initiation efficiency displayed in strains where many origins re-initiate due to complete deregulation of ORC, Mcm2-7, and Cdc6 [12,13]. Nonetheless, a different chromosomal context could be conferring preferential re-initiation on *ARS317* or *ARS1238* in the *MC2Ao* strain.

To distinguish between local sequence determinants and a broader chromosomal context, we investigated whether small fragments containing the *ARS317* or *ARS1238* origins could preferentially re-initiate when transplanted to ectopic genomic loci. We focused initially on fragments that we hoped would be small enough to dissect at the nucleotide level but large enough to encompass the origin and any possible additional sequences that might be needed for preferential re-initiation. A 537bp fragment previously shown to contain *ARS317* [24] preferentially re-initiated when transplanted from its endogenous location to sites on other chromosomes (ChrIV_567kb, ChrIV_1089kb) [2,12]. In all cases, the amount of re-initiation induced after 3hr (2.7-3.0 C) at the ectopic locus was comparable to the amount of re-initiation at the endogenous locus (2.8 -3.2 C) [2,12]. Hence, neither the chromatin context nor the replication timing (early or late in S-phase) of the transplant location were key determinants of the re-replication activity on these origins. Consistent with this notion, Figure 2A shows that an even smaller 406 bp fragment containing *ARS317* preferentially re-initiates when transplanted to position ChrIV_567kb. At this same location, a 233 bp *ARS1238* fragment that contains the ORC binding site (OBS) and 100 bp of flanking sequence on either side [25] also re-initiates (Figure 2A). Thus, the preferential re-initiation of *ARS317* and *ARS1238* is conferred by local sequence determinants and is independent of a broader chromosomal context.

Preferential Re-Initiation at *ARS317* and *ARS1238* Does Not Require Silencing Proteins or Forkhead Transcription Factors

ARS317 is a core element of a 138 bp transcriptional silencer *HMR-E*, one of several silencers that recruit the silencing proteins Sir1-4 to organize the surrounding DNA into a heterochromatin-like structure (reviewed in [26]). The entire *HMR-E* silencer is included within the transplanted *ARS317*-containing fragments described above, so the preferential re-initiation of this fragment could be associated with its organization into heterochromatin [27,28]. Such a connection is reminiscent of reports that heterochromatin preferentially re-replicates in budding yeast and Drosophila [13,29]. To test this possibility, we individually deleted each of the four *SIR* genes and analyzed the re-replication profiles around *ARS317* for each *sir* mutant. These profiles resembled those from the wild-type *SIR* control strains (Figure 2B and Figure S2A), indicating that none of the Sir proteins are required for the preferential re-initiation of *ARS317*. We also observed re-replication in a truncated *ARS317* clone lacking the RapI and AbfI binding sites that are critical for *HMR-E* silencer function [28] (Figure 2C). We conclude that a silent chromatin state is not necessary for the preferential re-initiation of *ARS317*. *ARS1238* is not assembled into heterochromatin, so one would expect its preferential re-initiation to be independent of Sir proteins. Our data are consistent with this expectation (Figure S2C), although the profiles are not as clear-cut.

Other factors known to influence nearby origin function are the forkhead transcription factors Fkh1 and Fkh2. Association of these proteins with origins and ORC has been implicated in the spatial organization of origins in the nucleus. This organization is thought to alter the S phase replication timing of some

origins, including *ARS1238* [30]. Although Fkh proteins do not influence *ARS317* replication timing, searches for their proposed binding motifs have identified predicted binding sites within a few kilobases of both *ARS317* and *ARS1238* [31,32]. To test whether Fkh1 or Fkh2 are critical for re-initiation of either origin, we examined the re-replication profiles in *fkh1*Δ, *fkh2*Δ, and *fkh1*Δ*fkh2*Δ strains. At both *ARS317* (Figure 2D and Figure S2B) and *ARS1238* (Figure S2D), *fkh*Δ strains re-replicated significantly more than negative control strains that lack re-replicating origins at these loci**.** These results confirm that the forkhead proteins are not essential for the preferential re-initiation of either origin. We did observe a partial reduction of re-replication in the *fkh1*Δ*fkh2*Δ background, so we cannot rule out a role for these proteins in supporting re-initiation. However, we suspect that this reduced re-replication may be an indirect consequence of the severe growth defect and cell clumping exhibited by the double mutant during growth in liquid media [30].

A Distinct Element Confers Preferential Re-Initiation on *ARS317* and *ARS1238*

The preferential re-initiation activity seen in transplanted fragments containing *ARS317* and *ARS1238* could be intrinsic to the origin sequences themselves, or be conferred on these origins by neighboring sequences that are dispensable for initiation activity. The former possibility is particularly relevant for *ARS317*, whose especially tight interaction with ORC appears to govern the activity of this origin in S phase [33]. If this possibility is correct, any minimal

segment containing origin activity should also exhibit preferential re-initiation. In contrast, if the latter possibility is correct, the fragments should be separable into an origin segment that can initiate but not preferentially re-initiate, and an adjacent segment that can neither initiate nor preferentially re-initiate on its own but confers preferential re-initiation on the origin segment. To test this separability, of functions for both *ARS317* and *ARS1238* we generated subclones of the transplanted fragments described in Figure 2A and assayed them for both initiation and re-initiation activity.

Initiation activity requires a 33 bp consensus ORC binding site (*OBS*) and less well-defined flanking sequences [34,35]. The *OBS* is comprised of a 17 bp extended *ARS* consensus sequence (e*ACS*), formerly known as the A domain, and a WTW sequence [36] formerly known as the B1 subdomain. The required flanking sequences usually lie 3' of the T-rich strand of the *OBS*, where they comprise the rest of the B domain (B2 and B3), but occasionally can lie 5' of the *OBS*, where they are referred to as C domain sequences [37]. We numbered nucleotides in our subclones relative to the *OBS* [35], assigning +1 and +33 to the first and last nucleotide, respectively, of the T-rich strand of the *OBS*. In this scheme, B domain sequences outside the *OBS* are numbered +34 and higher, and C domain sequences have negative coordinates (Figure 3A). The 406 bp preferentially re-initiating fragment containing *ARS317* is thus designated *317(+300..-106)*, and the equivalent 233 bp fragment for *ARS1238* is designated *1238(+133..-100)*.

The initiation activity of an origin can be assayed by the ability of a plasmid containing the origin to be maintained in cells. One measure of this ability is the mitotic stability assay, which measures the steady state percentage of cells containing the plasmid in a culture grown under selection for the plasmid [38,39]. The mitotic stability of several subfragments containing *ARS317* showed that full origin activity was retained by *317(+76..-106)* (Figure 3B). This origin segment failed to re-initiate when inserted at ChrIV_567kb (Figure 3C), demonstrating that *ARS317* does not have an intrinsic ability to re-initiate. The adjacent segment *317(+300..+77)* was also not able to re-initiate when examined in the context of a slightly larger fragment *317(+300..+34)* at ChrIV_567kb (Figure 3D). This adjacent segment does contain sequences that are essential for a weak cryptic origin (Figure 3A) [36], but a mutation that disrupts this cryptic origin did not reduce the ability of these adjacent sequences to induce re-initiation (Figure S3; mutant A). In contrast, a mutation of the *ARS* consensus sequence in the *ARS317 OBS* did eliminate re-initiation, confirming that the re-initiation is dependent on *ARS317* ([12], also Figure S3 mutant E). These data show that the preferentially re-initiating fragment *317(+300..-106)* can be separated into an *ARS317* origin segment *317(+76..-106)* and an adjacent segment *317(+300..+77)* that confers preferential re-initiation on *ARS317* in the *MC2Ao* strain. We call the sequence element that confers this activity a re-initiation promoter (*RIP*) and will refer to it as *RIP317*.

We used a similar approach to identify a subsegment of *1238(+69..-100)* that retains full *ARS1238* origin activity (Figure 3B) but is not sufficient to

preferentially re-initiate. This inability to re-initiate was demonstrated in the context of a slightly larger segment *1238(+83..-100)* at ChrIV_567kb (Figure 3E). Further evidence that neither origin segment nor adjacent segment have re-initiation activity on their own comes from insertion mutations (discussed later) that separate the two segments by 153 bp, and abolish re-initiation. In addition, the adjacent segment *1238(+133..+70)* does not contain the origin activity needed to support maintenance of an autonomous plasmid. Thus, like *ARS317*, *ARS1238* acquires its ability to preferentially re-initiate from an adjacent re-initiation promoter, which we will refer to as *RIP1238*.

Mapping the Re-Initiation Promoters

In order to map *RIP317* with finer resolution, we first analyzed the re-initiation efficiency of a nested series of deletions extending into the left border (plus side) of the 406bp *317(+300..-106)* fragment. These deletion constructs were introduced into ChrIV_567kb, and their re-initiation efficiency measured by normalizing the amount of re-initiation for each deletion (i.e. the copy number increase above 2C) against the amount of re-initiation for the full-length fragment. Deletions up to nucleotide +153 had limited effect on re-initiation efficiency, but further deletion into the fragment caused a precipitous drop (Figure 4A). Thus, nucleotide +153 in the 259 bp deletion fragment *317(+153..-106)* defines a left-hand boundary for *RIP317*.

To further map *RIP317* we used *317(+153..-106)* as the parent sequence for a linker scan analysis of *RIP317* structure (Figure 4A; bold line). Most of the

linker mutations that showed a noticeable reduction in *ARS317* re-initiation efficiency were from L4 to L15, which covers the 51 bp from nucleotide +137 to +87 (Figure 4B). On the left end of this 51 bp region were linker mutations (L4-L7), which drastically reduced or eliminated *ARS317* re-initiation and identified sequences that are critical for *RIP* function. Other linker mutations (L8-L15) showed less striking reductions in re-initiation individually (Figure 4B), but eliminated re-initiation when combined together (Figure S4A). Thus, the sequences mutated by linkers L8-L15 are also important for *RIP* function but may contain partially redundant sequence elements.

In contrast to linker mutations L4-L15, the remaining linker mutations from L16-L32 each had limited effects on *ARS317* re-initiation (Figure 4B). We note that *ARS317* differs from most yeast origins in that the WTW sequence of its *OBS* is dispensable for initiation activity [36,40]. Linker L29, which mutates the WTW sequence, and linkers L30 and L31, which intrude further into the OBS, still leave intact the 17 bp extended ARS consensus sequence (*eACS*), which forms the core of the ORC binding site [41]**.** Thus, although these linkers mutate parts of the *OBS*, they presumably do not disrupt *ARS317* re-initiation efficiency because they leave *ARS317* origin activity intact. Linker L33, on other hand, does mutate part of the *eACS*, so its partial disruption of *ARS317* re-initiation is likely due to impairment of origin function. Replacement of the entire sequence covered by L17-L31 (nucleotides +86 to +23) with sequence of similar AT content did not have much effect on *ARS317* re-initiation (Figure S4B). Additional replacement of sequences covered by L1-L3 decreased re-initiation efficiency by

a third, indicating that these sequences contribute to optimal *RIP317* activity (Figure S4B). These results suggest that *RIP317* resides in the 67 bp from nucleotides +153 to +87 and contains a core region of approximately 19 bp (+137 to +119) that is crucial for its function.

As discussed above, we had narrowed down *RIP1238* to a 64 bp segment from nucleotide +133 to +70. Linker scan analysis revealed that linker mutations spanning 40 bp (+117 to +78) of this segment abolished *ARS1238* re-initiation, while the remaining mutations showed a more modest reduction (Figure S4C). Thus, like *RIP317*, *RIP1238* has a core segment that is crucial for RIP function and surrounding sequences that enhance this function.

RIP Function Is Not Simply Dependent on High AT Content

The most obvious common feature of *RIP317* and *RIP1238* is the high AT-content of these sequences (92% and 84% AT respectively). Regions of high AT-content have been postulated to exclude nucleosomes (Reviewed in [42]) or to provide regions of reduced helical stability that facilitate DNA unwinding during replication initiation [43]. Therefore, we wondered if *RIP* elements were stimulating re-initiation through such a positioning or thermodynamic mechanism.

To test this possibility we generated various mutants that preserved the AT content of *RIP317* while altering its sequence identity. Neither predicted nucleosome exclusion [44] nor predicted DNA helical stability [45] of *RIP317-ARS317* is changed by these mutations. These mutations profoundly compromised re-initiation activity, with many of the mutants showing no re-

initiation even after 6 hours of induction (Figure S5). These findings suggest that *RIP* elements do not simply act as a DNA unwinding element or a nucleosome exclusion site. We do note that many of the mutations disrupted a palindrome in *RIP317* (5'-TTTATAAA-3') that is also present in shorter form in *RIP1238* (5'-TTATAA-3'). However, the palindrome in *RIP1238* is not necessary for *RIP* function (Figure S4C, mutant B), and the palindrome in *RIP317* is not sufficient (Figure S5, mutant D2). Thus, although our mutational data does not rule out a role for the palindrome that is specific for *RIP317*, the sequence dependence we observed is consistent with the *RIP* acting as a recruitment site for factors that promote re-initiation.

RIP Function Is Distance Dependent

The origin proximal boundary of *RIP317* is 53 bp away from the B-side boundary of the *ARS317 OBS*. To determine whether the size of this spacing is important for *RIP317* function, *RIP-OBS* spacing was increased by inserting randomly generated DNA of 38% AT-content (the average AT-content of genomic DNA in *S. cerevisiae*) between *RIP317* and *ARS317* and decreased by deleting portions of *ARS317* in this 53 bp spacing (See Materials and Methods and Table S1). The resulting clones were analyzed for re-initiation efficiency (Figure 5A). Re-initiation declined with increased spacing and was abolished by 153 bp, suggesting that *RIP317* must be relatively close to the origins to confer preferential re-initiation. Re-initiation could tolerate a decline in spacing to 37 bp but was significantly reduced by a spacing of 21 bp. The latter reduction,

however, could simply be a secondary consequence of excessive removal of the B domain, which lies in the 53 bp spacing. Nonetheless, the overall finding is that re-initiation requires close but not precise spacing (within ~35 to ~75 bp) between the *RIP* and the *OBS.*

A spacing of only 36 bp separates *RIP1238* from the *OBS* of *ARS1238*. This short spacing suggested that *ARS1238* might re-initiate less efficiently than *ARS317* because the spacing is suboptimal. We thus performed a similar analysis of the spacing requirements between *RIP1238* and the *OBS* of *ARS1238* (Figure 5B). Like *ARS317*, re-initiation of *ARS1238* also required relatively close spacing of the *RIP* and *OBS* (~25 to ~55 bp). Moreover, re-initiation levels were relatively constant across this range of spacings, indicating that the lower levels of re-initiation for *ARS1238* versus *ARS317* cannot be attributed to suboptimal *RIP-OBS* spacing for the former. This requirement for close proximity between *RIP* and origin raise the possibility that proteins bound to both sites must closely interact in some manner to facilitate re-initiation.

RIP Elements Confer Preferential Re-Initiation on Heterologous Origins

If the *RIP* elements promote preferential re-initiation by influencing common regulatory pathways controlling origins, they should be able to promote re-initiation from heterologous origins. To test this possibility, we fused *RIP317* and *RIP1238* to other replication origins, keeping the spacing between *RIP* and origin *OBS* between 46-53 bp, within the optimal range of spacing determined for

both *ARS317* and *ARS1238*. These *RIP*-origin chimeras were then assayed at ChrIV_567 kb for re-initiation in an *MC2Ao* strain.

RIP317 promoted preferential re-initiation from *ARS1021* and *ARS301* (Figure 6A) at levels comparable to the re-initiation it promoted from *ARS317* (Figure 2D) following a 3 hr induction of re-replication (2.8-3C), while fusions to a non-functional rip317 (equivalent to Figure 4B linker 6) failed to re-initiate. *RIP317* also stimulated re-replication from *ARS305, ARS209, and ARS1238*, but a longer 6 hr induction of re-replication was needed to show an unequivocal stimulation (Figure S6A and S6B). *RIP1238* was similarly able to promote preferential re-initiation from *ARS1021* and *ARS301* (Figure 6B). In these cases the re-initiation levels (4-4.5C) were comparable to the re-initiation *RIP317* promoted at *ARS317* following a 6 hr induction of re-replication (compare to Figure 1). Thus, both *RIPs* can promote preferential re-initiation on heterologous origins.

We did observe some origins (*ARS306*, *ARS702*) that exhibited no detectable preferential re-initiation when fused to *RIP317* (Figure S6B). One possible reason is that the optimal spacing between the origin *OBS* and the *RIP* element places constraints on the size of the B domain that can fit between these two elements. Origins requiring larger B domains would be expected to have their initiation, and thus any re-initiation, compromised in their corresponding RIP fusion constructs. Consistent with this explanation, the truncated *ARS306* and *ARS702* fragments fused to *RIP317* displayed defective origin function when assayed by plasmid mitotic stability (Figure S6C).

RIP Elements Do Not Enhance The S Phase Initiation Activity of Adjacent Origins

Just as compromising origin function can reduce re-initiation efficiency, one can imagine that *RIP* elements might promote re-initiation by simply enhancing the intrinsic initiation efficiency of an origin. Such an effect was difficult to detect by plasmid mitotic stability because origins that re-initiate when fused to *RIP317 (ARS317, ARS1021, and ARS301*) appear to have maximal mitotic stability in this assay (Figure 7A). However, when integrated in the chromosome, *ARS317*, *ARS1021*, and *ARS301* exhibited much lower initiation activity, allowing us to look for stimulation of this activity by *RIP317*.

We used array CGH analysis of S phase replication to assay the activity of these origins with, and without, a functional *RIP317* element. In the resulting replication profiles, the heights of the peaks represent a combination of the efficiency and timing of origin initiation in S phase. Low but measurable peak heights for the origins are ideal, because they leave open the maximal dynamic range for detecting a stimulation of origin activity by *RIP317.*

We observed no measurable difference in replication peak heights for *ARS317*, *ARS1021*, and *ARS301* with or without a functional *RIP317* (Figure 7B). At its endogenous location *ARS317* initiates in approximately 10-15% of cells each S phase based on 2-dimensional gel analysis of initiation bubble intermediates [46,47]. Such origin activity at ChrIV_567kb would be at the limit of detection for our aCGH replication assay, and any significant *RIP317* stimulation

of *ARS317* activity should have been detectable as a larger peak. More striking is the detection of clear origin activity from *ARS1021* and the absence of any stimulation of this activity from *RIP317*. These results argue that *RIP317* does not advance the timing or enhance the initiation efficiency of adjacent origins. We thus favor a model in which *RIP* elements specifically promote re-initiation by antagonizing a mechanism(s) that prevents re-initiation.

RIP Elements Facilitate A Step after Mcm2-7 Association with Origins

In vitro studies have shown that the loading of Mcm2-7 at origins can be subdivided into a sequence of discrete steps: (1) binding of ORC to origins; (2) recruitment of Cdc6 to ORC; (3) recruitment of Cdt1-Mcm2-7 to ORC-Cdc6; and (4) loading of a double hexamer of Mcm2-7 as a ring around the duplex origin DNA [48]. The numerous global mechanisms used by CDKs to prevent Mcm2-7 loading are thought to inhibit one or more of these steps, because once Mcm2-7 loading is complete, origins are primed to be activated by CDKs [49-51]. The partial deregulation of these mechanisms in the *MC2Ao* strain presumably allows some but not all of these steps to proceed, accounting for why the majority of origins do not re-initiate. *RIP* elements could therefore function by locally releasing an origin from the remaining block(s), allowing the origin to complete a re-initiation cycle. Thus, to gain insight into the mechanism of *RIP* action, we investigated which step in the loading process was blocked for the majority of origins that do not re-initiate in *MC2Ao* strains.

We examined Mcm2-7 ChIP association at three origins that do not re-initiate in *MC2Ao* strains: *ARS305*, *ARS418*, and *ARS1420*. As expected, Mcm2-7 associated more with these origins relative to nonspecific DNA in G1 phase (Figure 8B) but not in M phase (Figure 8C). After a 90 minute induction of re-replication, Mcm2-7 became enriched 2-4x at these origins but not at a non-origin locus *ACT1* (Figure 8D). ChIP also detected a similar degree of re-replication-induced association of Mcm2-7 with the two re-initiating origins, *ARS317* and *ARS1238* (Figure 8D). As expected, given the association of Mcm2-7 with origins that cannot re-initiate, preventing re-initiation of *ARS317* by disrupting its adjacent *RIP317* did not prevent the association of Mcm2-7 with *ARS317* (Figure 8D). On the other hand, disrupting the ORC binding site in *ARS317*, did lead to loss of Mcm2-7 association, specifically with this origin (Figures 8C, 8D). This result is consistent with the *in vitro* dependence of Mcm2-7 origin association on ORC binding [52].

Taken together, our data indicate that the global deregulation of re-initiation in the *MC2Ao* strain allows Mcm2-7 to associate with most origins. Thus, in this strain the *RIP* elements must promote re-initiation at adjacent origins by facilitating or deregulating a step that is blocked after this association. As discussed below, determining more precisely which step is involved will require better *in vivo* tools to distinguish between the two types of association (Mcm2-7 recruitment versus loading) that have been identified *in vitro*.

Discussion

RIP Elements Contribute to The Diversity of Origin Re-Replication Control

Preventing re-initiation at the hundreds to thousands of replication origins in a eukaryotic genome is critical for preserving genome stability [2]. Models for how such tight regulation can be achieved emphasize the importance of using numerous overlapping inhibitory mechanisms to reduce the probability that any origin will re-initiate [3,8]. These mechanisms all inhibit the loading of the Mcm2-7 core replicative helicase onto origins, and each does so by reducing the total cellular activity of one of the four proteins required for this step: ORC, Cdc6, Cdt1, or Mcm2-7 [5,6]. Given their global nature, these regulatory mechanisms are presumed to act equally at all origins throughout the genome. Thus, current models cannot account for the broad range of efficiencies with which origins re-initiate when global mechanisms are compromised. This diversity suggests that the models may be missing the contribution of local factors that can modulate the regulation of individual origins.

Our work here demonstrates that such a local layer of regulation does indeed exist by identifying a local control that makes *ARS317* and *ARS1238* more susceptible to re-initiation when global regulation of Cdc6 and Mcm2-7 is removed. Our analysis of this control establishes some of its key mechanistic properties and constraints. First, this control specifically enhances the propensity of an origin to re-initiate and not its efficiency or timing during normal S phase initiation. Second, this preferential re-initiation is not imposed by a diffuse chromosomal context but is conferred by discrete sequence elements that are

adjacent to but distinct from the origin. Third, these elements, which we call re-initiation promoters (*RIPs*), have specific sequence requirements and function best within a narrow range of distances close to the origin. Finally, these *RIPs* appear to overcome inhibitory mechanisms that block a step in initiation that follows the association of Mcm2-7 with origins. These results provide a paradigm for the local control of origin re-initiation and lay the groundwork for a more detailed molecular analysis of this control.

Our results do not address the question of whether the presence and activity of these *RIPs* is incidental to some other genomic function of these elements or whether they arose for the purpose of modulating replication control in cells with intact replication controls. Nonetheless, as discussed below, the existence of *RIP* elements has potential biological ramifications in both mutant and wild-type settings.

Is *RIP* Function Mediated by Protein Binding?

One of the questions raised by our results is whether *RIP* function is mediated by proteins that specifically recognize these sequences or is mediated by some other property of these elements. The two *RIP* sequences we identified, *RIP317* and *RIP1238*, are both AT-rich, especially in their core regions. They do not share an obvious consensus sequence, and in fact, their AT-rich character makes it difficult to find meaningful conservation of these elements throughout the genome. Importantly, this AT-rich character raises the possibility that these elements just act thermodynamically to facilitate the DNA unwinding needed to

re-initiate DNA replication. Another possibility is that they simply influence nucleosome positioning around origins, as AT-rich DNA tends to be excluded from nucleosomes [53]. These hypotheses, however, are not sufficient to account for *RIP* function, because we were able to abrogate *RIP317* function using mutations that preserved AT content without significantly perturbing their calculated unwinding potential or predicted propensity to exclude nucleosomes [44,45].

These considerations suggest to us that *RIP* elements may act through proteins that bind to them. Such a possibility is compatible with the poor nucleosome occupancy over *RIP317* that has been observed at its endogenous chromosomal location [35,54,55]. A quick attempt to uncover such proteins by screening through yeast transcription factors with potential binding motifs [56,57] in both *RIP317* and *RIP1238* did not yield any promising candidates (See Materials and Methods); deletions in *NHP6A NHP6B, YAP1, SUM1, YNR063W, GAT4, SMP1, or YOX1* failed to disrupt the function of either *RIP*. Hence, we are pursuing more systematic studies to identify proteins that bind *RIP* elements in vivo and are essential for *RIP* function. If *RIPs* do indeed work by recruiting proteins near an origin, the distance dependence of *RIP* function suggests that these proteins may have to interact in close proximity with specific initiation or regulatory protein that assemble at origins.

RIP Elements Deregulate A Block to Re-Initiation That Occurs after Mcm2-7 Associates with Origins

Our work also demonstrates that origins that do not re-initiate in the MC2Ao strain associate with Mcm2-7 by ChIP analysis and thus can at least recruit Mcm2-7 to origins. Apparently, these origins are blocked at an initiation step subsequent to Mcm2-7 recruitment, and the *RIP* elements confer preferential re-initiation on neighboring origins by deregulating this step.

Exactly which step is deregulated by *RIP* elements is not resolved by our experiments, but there are two major possibilities. The elements could be deregulating the transition between Mcm2-7 recruitment and Mcm2-7 loading, which has been defined *in vitro* [58] but not yet demonstrated *in vivo*. Alternatively, they could be deregulating a step following Mcm2-7 loading. We favor the former possibility because the latter requires us to violate a fundamental principle of the current paradigm for re-initiation control [3,5], namely that this control only targets steps preceding Mcm2-7 loading. Nonetheless, resolution of this question must await the development of more sophisticated *in vivo* protein-DNA binding assays that are capable of distinguishing recruited from loaded Mcm2-7 at individual origins.

Importantly, this role in enabling a step of initiation subsequent to Mcm2-7 origin association distinguishes *RIP* elements from B2 elements, one of the core elements of budding yeast origins. Both elements are AT rich, positioned 3' of the T-rich strand of the *OBS*, and have relaxed positioning requirements relative to the *OBS*. However, the B2 elements are needed for Mcm association with origins [59], and *RIP* elements are not. This distinction provides further support

for a model in which *RIP* elements antagonize an inhibitory mechanism, rather than simply promote a normal initiation function.

Possible Inhibitory Pathways Targeted by *RIP* Elements

How might *RIP317* and *RIP1238* locally override a block to Mcm2-7 loading that prevents origins from re-firing in the *MC2Ao* background? The simplest model is that the block is imposed by one or more of the regulatory mechanisms that remain intact in *MC2Ao* strains, e.g. CDK phosphorylation of Orc2 and Orc6 [7] or CDK binding to Orc6 [22]. According to this model, *RIP* elements locally antagonize some or all of these mechanisms, relieving enough of the block to allow detectable re-initiation at *RIP*-associated origins. This model is consistent with *in vitro* studies that indicate these inhibitory mechanisms still permit ORC binding and some Mcm2-7 recruitment to origins, but completely block Mcm2-7 loading onto origins [58]. The model is also consistent with our observation that globally antagonizing CDK phosphorylation of ORC in the *MC2Ao* background by mutating all CDK consensus phosphorylation sites on Orc2 and Orc6 allows many origins to join *ARS317* and *ARS1238* in re-initiating at detectable levels [12]. However, direct support for this model will require analysis of ORC phosphorylation and *CDK* binding at origins to determine if they are indeed reduced at *RIP*-associated origins as might be predicted by the model.

We note that the induction of re-initiation in the *MC2Ao* strain is limited and slow compared to the usual efficiency of origin initiation in a normal S phase. After 3 hr of induction, over one and a half cell cycles for this strain, only 50%

and 25% of *ARS317* and *ARS1238*, respectively, have re-initiated. This inefficient re-initiation suggests that *RIP317* and *RIP1238* only partially antagonize the inhibitory mechanisms blocking Mcm2-7 loading. Such incomplete relief of inhibition may explain why completely antagonizing inhibitory phosphorylation of Orc6 on one CDK consensus site (S116A) can further enhance *ARS317* and *ARS1238* re-initiation in the *MC2Ao* strain relative to the *MC2A* strain (Figure S1).

Parallels to Localized Re-Initiation during Development

The preferential re-initiation of *ARS317* and *ARS1238* is reminiscent of the localized re-initiation that occurs in several cases of developmentally programmed gene amplification [60]. One of the best characterized is the amplification of the chorion gene locus in Drosophila ovarian follicle cells during oogenesis. Like the *RIP* elements identified in this work, an Amplification Control Element (*ACE3*) of ~320 bp has been identified that has little origin function on its own and confers preferential re-initiation on a nearby origin (*ori-beta*). However, the mechanism by which *ACE3* and other potential ACE elements promote re-initiation at a select group of origins remains a mystery [60].

Our work in budding yeast offers a conceptual framework for exploring the mechanism of developmentally regulated gene amplification, even if the details prove to be different. For example, characterizing how far the initiation reaction can proceed on the majority of origins that don't re-initiate may give insight into the key step that allows amplification origins to re-initiate. Similarly, it may be

informative to investigate the status of inhibitory modifications on initiation proteins associated with re-initiating origins to see if these modifications are reduced relative to the bulk protein population.

Preferential Re-Initiation May Predispose Genomic Regions to Re-Replication Induced Genetic Variation

In addition to its established role in developmentally programmed gene amplification, there are several hints that DNA re-replication may also contribute to the amplifications and abundant duplications observed in cancer cells. First, we have shown in budding yeast that re-replication arising from deregulated replication initiation proteins can be an extremely efficient source of segmental amplification [2]. Second, overexpression of initiation proteins in murine models has been shown to promote oncogenesis [61-63]. Third, overexpression of replication initiation proteins has been observed in some human cancer cells [64-67]. And finally, the tandem direct repeat structure of some oncogene amplifications and many of the duplications detected in cancer cells is consistent with the structures that could arise from re-replication [68].

Should re-replication prove to be a new source of copy number variation (and possibly other genomic alterations) in cancer cells, local modulation of origin control, such as that described in this work, could make some regions of the genome more susceptible to re-replication induced genetic alterations than others. One can therefore imagine that an irregular genomic landscape of re-initiation susceptibility could give rise to an irregular genomic landscape of

genetic instability in cancer cells. Preliminary indication for such position dependent variability in genetic instability has been obtained by experiments showing that the frequency and structure of DHFR amplification in a cancer cell line was different for different genomic positions of DHFR [69].

Copy number variation may also play an important role in normal cells. For example, gene duplications are thought to provide the functional redundancy that enables the functional diversification of genes during molecular evolution [70]. In addition, copy number increases, which occur with high prevalence in normal human genomes [71], may directly provide phenotypic variation that can be selected for during evolution. In both examples, the mechanism of copy number change is not clear. We speculate that extremely rare re-initiation events may occur despite the presence of normal re-initiation controls and contribute to copy number increases. Should re-initiation drive some of these copy number increases, variable susceptibility of origin re-initiation throughout the genome would be expected to make some regions of the genome more subject to evolutionary change than others. Thus, the presence of a local layer of re-initiation control provided by *RIP*-like elements may have far reaching ramifications on oncogenesis and evolution.

Materials and Methods

Plasmids

Integrative plasmids were used to test *RIP*-origin re-replication or replication activity in a chromosomal context. These plasmids were all derived from pBJ2889 [2]. This plasmid contains a portable re-replication integration cassette made up of the following elements: Homology Left (sequences centromere proximal to *ARS419,* which is located at 567kb on Chromosome IV), the *kanMX6* reporter gene [72], the *ade3-2p* color reporter gene [73], a polylinker, which includes the XbaI restriction site, and Homology Right (sequences centromere distal to *ARS419*). SpeI – XbaI fragments containing *RIP*-origin inserts and additional restriction sites were integrated into the XbaI site of the pBJL2889 polylinker, creating a SpeI/XbaI fusion site (TCTAGT) on the *ade3-2p* side of the insert and re-creating an XbaI (TCTAGA) site on side adjacent to Homology Right. We report the sequence of these clones in Table S1 from the SpeI/XbaI fusion site to the intact XbaI site. The re-replication integration cassette was excised from the plasmid using SacI-NotI or SacI-SalI and introduced into yeast using standard techniques. Integration of these cassettes at *ARS419* destroyed its origin activity.

The *ARS* activity of RIP-origin constructs was measured by mitotic stability assays utilizing centromere-containing plasmids. These *CEN-ARS* plasmids were derived from pFJ11 [36], a plasmid containing *ARS317* and *CEN4*. As a preliminary step, the BamHI site adjacent to *CEN4* was destroyed by BamHI digestion, klenow fill-in of the cut overhangs, and blunt-end ligation. The *ARS317* in this modified pFJ11 was then replaced with our origin or *RIP*-origin constructs by cloning these constructs into the HinDIII and EcoR1 sites of the

plasmid (exact sequences listed in Table S1). These plasmids were transformed into YJL310 [74] using standard techniques.

Altering *ARS317* and *ARS1238 RIP-OBS* Spacing

The full sequence of all insertion and deletion mutants used to alter RIP-OBS spacings are listed in Table S1. They were generated as follows:

Inserting sequence. To increase the distance between *RIP1238* and the *OBS* of *ARS1238* we first randomly generate a 117 nucleotide sequence of 38% GC content (matching the average GC content of *S. cerevisiae*) DNA sequence then manually altered it to be free of yeast transcription factor binding motifs:

5′ –

ATAGCCTGCCCATAGGATATAGAGATACCAATAGTTGTTTGTGAACAGCAAAGAAGGAT

CCAGAAGATCAGTCGCACGATATTGATGTGAATACTAGGTTTATAGGATAGTCGTACA

– 3′

Various sized segments of this sequence, all spanning the BamHI site (underlined), were inserted between nucleotides +69 and +70 of *ARS1238*. A 100 nucleotide sequence was similarly generated to insert sequences between *RIP317* and the *OBS* of *ARS317*:

5’ –

CCCATAGGATATAGAGATACCAATAGTTGTTTGTGAGCAACAAAGAAGGATCCAGAAGG

TCGATCGCACGATATTGATGTGAATACTAGTTGTAGTAATG – 3′

Deleting sequence. For *ARS317* BamHI linker mutants L19, L21, L23, and L27 described in Figure 4B were digested with BamHI and ligated together to produce 8 bp (L23-L27), 16 bp (L21-L27), and 32 bp (L19-L27) deletions. For *ARS1238*, sequences +69..+55 and +69..+51 were deleted from the left border of *ARS1238*.

Strains

Genotypes and derivations for all strains used in this manuscript can be found in Table S2. Almost all the *MC2Ao* yeast strains in this paper were generated from the previously published strain YJL3758 [2] by one or more of the following genetic alterations: (1) integration of a re-replication cassette (described in Plasmids above and detailed in Table S1); (2) deletion of *ARS317*, *ARS418*, or *ARS1238* (Table S3); (3) deletion of *SIR* or *FKH* genes (Table S3) [72,75,76]. *MC2A* strains YJL8923 and YJL8924 are congenic to YJL3758 but have wildtype *ORC6* instead of *orc6(S116A)*.

Oligonucleotides

Oligonucleotides used to PCR marked deletion fragments for deleting origins or genes encoding transcription factors are listed in Table S3. Oligonucleotides used in quantitative PCR are listed in Table S4.

Strain Growth and Induction of Re-Replication

Synthetic complete medium containing 2% wt/vol dextrose (SDC) was made up as described [77] except that we used twice the concentration of amino acids and purines for all but leucine, which was added to a final concentration of 120 µg/mL, and serine, which was added to a final concentration of 200 µg/mL. Drop out media like SDC-URA, simply lacked the indicated component. For nonselective rich media cells were grown in YEPD (YEP + 2% wt/vol dextrose) or YEPRaf (YEP + 3% wt/vol raffinose + 0.05% wt/vol dextrose). All cell growth was performed at 30° C.

To induce re-replication, freshly thawed log phase cultures in YEPD were extensively diluted into YEPRaff and grown for 12-15 hr until they reached an OD600 of 0.2-0.8. At this cell density (approximately 1 x 10^7 cells/ml), nocodazole (US Biological N3000) was added to a final concentration of 15 µg/mL for 120-135 min to arrest cells in metaphase. *GAL1* promoter driven *pGAL-Δntcdc6,2A* was then expressed by the addition of 2-3% galactose for 3 hr or 6 hr where indicated.

Strain Growth for Replication Arrays

Strains were grown overnight in YEPD at 30°C to an OD600 of 0.2-0.4. At this cell density, 50 ng/mL alpha factor was added to arrest cells in G1 phase. Arrested cells were released into fresh YEPD media containing 0.1 M hydroxyurea (US Biological H9120), 100 µg/mL pronase (EMD 53702), and 15 µg/mL nocodazole (US Biological N3000) to permit a single, slowed S phase to occur. Cultures were harvested after 135 minutes when 30-60% of the genome

was replicated as verified by FACS analysis [78]. To increase the sensitivity of detecting initiation activity from the integrated re-replication cassettes, we deleted the closest early origin *ARS418* so that its forks would not run through the origins in the cassettes and preclude their initiation.

Genomic DNA Preparation for aCGH Analysis

Method 1. Genomic DNA was extracted from yeast as described [2,79]. Briefly, 10-25 OD units of cells were harvested and lysed by bead beating. DNA was extracted by phenol:chloroform:isoamyl extraction, ethanol precipitated, and resuspended in 50 µL of 2 mM Tris-Cl (pH 7.8). Typical yields were 2-5 µg of DNA.

Method 2: Larger cultures (>100 OD units) were subjected to a more extensive purification consisting of organic extraction, enzymatic removal of protein and RNA, detergent (cetyltrimethylammonium bromide) treatment, and DNA isolation using anion-exchange columns (Qiagen #10243 100/G tips). Typically, this protocol was performed to produce 50-120 µg of M phase arrested DNA for aCGH. Full details of this protocol are described in [2] and [12].

Array CGH: DNA Labeling, Hybridization, and Scanning

aCGH analysis of whole genome (Used in Figure 1). A single large (>250 OD units) culture was the initial source for both reference (non-induced) and re-replicated DNA. Half of this culture was harvested at the arrest (0 hr) to generate the uninduced reference DNA. The remaining culture was induced with

galactose for 6 hr before harvesting to generate the induced re-replicated DNA. 1.5-2 µg of reference DNA (prepared using Method 2 above) was labeled with Cy3, and 1.5-2 µg of 6 hr induced DNA was labeled with Cy5 essentially as described [12]. The labeled DNA was hybridized as previously described [12].

aCGH analysis of re-replication (Used in Figures 2-6 & Figures S1-S6). 2-2.5 µg of each DNA sample (prepared using Method 1 above) was labeled with Cy5 and 1.5-2 µg of purified M phase reference DNA from YJL7695 (prepared using Method 2 above) was labeled with Cy3 using a low-throughput [12] or high-throughput [80] method. All samples were hybridized as described [12].

aCGH analysis of replication (Used in Figure 7). 1.5-2 µg of each experimental DNA sample (prepared using Method 2 above) was labeled with Cy5, and 1.5-2 µg of purified M phase reference DNA from YJL7695 (prepared using Method 2 above) was labeled with Cy3 essentially as described [12]. The labeled DNA was hybridized as previously described [12].

Array CGH: Data Analysis

Full details of array CGH data analysis are described in [12]. Briefly: arrays were scanned on a GenePix 4000B scanner and quantified using GenePix 6.0 (Axon Instruments). The Cy5/Cy3 ratios were normalized such that the average ratio was equivalent to DNA content for that specific point in the cell cycle (e.g. 2C for M arrested or induced samples, and 1.5C for S phase samples). Medians for these raw normalized data were then calculated across a 10kb moving window. Smoothed curves were calculated from this moving median

using Fourier Convolution Smoothing (FCS). The degree of smoothing is determined by a parameter called the convolution kernel [81], and for the chromosomes we display we used the following values optimized for re-replication profiles: Chromosome III, 9; Chromosome IV, 11.25; Chromosome V, 9; Chromosome XII, 10.75. For S phase replication profiles, the convolution kernel for Chromosome IV was set to 6.25. For presentation purposes, smoothed lines for each individual re-replication or S-phase profile were averaged into one composite profile. Most figures in the manuscript show these composite profiles as black lines surrounded by a gray zone representing ±1 standard deviation. The raw data and the smoothed lines for each individual experiment performed for this work can be seen in Document S1.

We note that, because of cross hybridization among the various repetitive sequence elements, these elements (tRNA genes, subtelomeric repeats, Ty elements and long terminal repeats) were removed from the analysis. In the Saccharomyces Genome Database, the two rDNA genes representing the large rDNA repeat arrays are adjacent to a Ty element and additional repeated sequences, so the entire ~44 kb region between YLR153C and YLR163C was omitted from the analysis.

Also, because each chromosome was effectively circularized during the calculation of the moving window median and the FCS, deviations of the smoothed curve from baseline values at one chromosome end can artifactually cause the curve to deviate from baseline at the other end [82]. Thus, when *ARS317* preferentially re-initiated at its endogenous location near the right end of

Chromosome III, it caused the smoothed re-replication curves to rise at the left end. We have masked the left 20 kb of the smoothed re-replication curves for Chromosome III in Figures 1A, 2B, and S1A, but left the curves unmasked in the individual experimental profiles shown in Document S1.

Comparison of Array Profiles in Bar Graph Format

Bar graphs were generated to compare the amount of re-initiation seen in experimental vs control strains. aCGH re-initiation peak heights were measured relative to the expected G2/M copy number (2C) for both experimental and control strains. Replicates of each array were then averaged (x_{exp} and x_{cont}) and a standard deviation calculated (s_{exp} and s_{cont}). The ratio x_{ratio} formed by x_{exp} divided by x_{cont} was converted to a percentage and plotted as shown. The error for this ratio was calculated by solving the equation:

$$s_{ratio} = \sqrt{\left(\frac{s_{exp}}{x_{exp}}\right)^2 + \left(\frac{s_{cont}}{x_{cont}}\right)^2} \times x_{ratio}$$

Statistical Analysis of Array Profiles for sirΔ and fkhΔ Strains

Re-replication of each experimental strain (n=2) was measured at one of the following re-replicating loci: ChrIII_292kb (endogenous *ARS317*), ChrIV_567kb (transplanted locus), or ChrXII_889kb (endogenous *ARS1238*). Relevant control strains lacking (negative control) a re-replicating origin at each location were measured to provide a background (i.e. non re-replicating) baseline.

Sample size for these negative control strains ranged from n=5 to n=10 as indicated in figure legends. Mean profile heights of the experimental and negative control strains were compared using Welch's t-test. Significant ($p<0.05$) results reject the null hypothesis and confirm that re-replication of *sir*Δ and *fkh*Δ strains is significantly different from re-replication of the relevant negative control strain.

Mitotic Stability Assay

CEN-ARS plasmids containing *RIP*-origin, *RIP,* or origin constructs were transformed into YJL310 [77], a strain with intact re-replication controls. Three independent transformants were inoculated into media selective for the plasmids (SDC-URA) and grown overnight to saturation. Cultures were subsequently diluted back into fresh selective media and grown overnight to an optical density of 0.1-0.6. Each log phase culture was plated to five selective (SDC-URA) and five non-selective plates (SDC) at a density of 200-400 cfu/plate. Plates were grown for 3-4 days and the fraction of cells harboring a plasmid was determined by dividing the number of colonies on the selective plates over the number on non-selective plates. Values reported are averaged from the three independent plasmid transformants.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments were performed with approximately 20 OD units of cells in a media volume of 50mL. Cultures were handled as described above for re-

replication cultures except induction was restricted to 90 minutes. We reasoned that anti-Mcm ChIP would work best immediately after Mcms were re-loaded onto origin DNA but before most of these origins had re-fired and distributed Mcms throughout the genome. Thus, we selected the 90-minute induction time point as this was the latest induction time before re-replication became visible by array CGH. This rationale is similar to that used in earlier ChIP-chip analysis of re-replicating strains [13].

Terminal cultures were fixed by addition of formaldehyde (37% w/v) to a final concentration of 1%. Fixation proceeded for 15 minutes at room temperature and was quenched by the addition of glycine to a final concentration of 0.125M. Fixed cells were harvested by centrifugation, washed once in 1x TE pH 7.5, and frozen at -80C.

Cell pellets were resuspended in 500 µL lysis buffer (50 mM HEPES/KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton, 0.1% Na-Deoxycholate) with protease inhibitors (Roche mini complete #04693159001 + 2 mM PMSF) and transferred into 2 mL screw-cap tubes (Sarstedt #72.694.006). 0.5 mm glass beads (Biospec Products 11079-105) were added to the level of the meniscus and cells were disrupted using a FastPrep 24 for two cycles of 45 sec at 6.0 m/s with 2 min incubation on ice in between. All subsequent steps were performed in low adhesion DNAse/RNAse free 1.5 mL microfuge tubes at 4°C unless otherwise indicated. Lysates were cleared by centrifugation at 20,000 rcf for 10 min and pellets (containing chromatin) were resuspended in 500 µL of fresh lysis buffer + protease inhibitors. Each pellet was sonicated using a 1/8” tapered

microtip attached to a Branson 450 sonicator for 4 cycles of 30 sec at setting 1.5 with >2 min on ice in-between. The resulting slurry was cleared again by centrifugation at 20,000 rcf for 10 min and the supernatant was retained as whole cell extract (WCE).

Immunoprecipitation, washes, and elution were performed on 80-90% of the WCE volume using methods described in [83]. These extracts were exposed to UM174 antibodies (rabbit polyclonal anti-Mcm2-7, 1:500 dilution) [58] (generous gifts from Steve Bell) in the presence of 30 uL slurry of Protein G Dynabeads (Life Technologies, 10004D). Immunoprecipitations were performed for 20 hr at 4° C. Beads were washed 3x with 1 mL of Wash Buffer (10 mM Tris-Cl pH 8, 250 mM LiCl, 0.5% NP-40, 0.5% Na-Deoxycholate, 1 mM EDTA) and 1x with 1 mL of TE (10 mM TrisCl pH 8, 1 mM EDTA) with 50mM NaCl. DNA was eluted from the beads by incubating them in 100 µL of 65° C Elution Buffer (50mM Tris-Cl pH 8, 10mM EDTA, 1% SDS) for ten minutes.

Crosslink reversal and DNA purification was performed essentially as described in [84]. Briefly, IP samples were digested in proteinase K (final concentration 1mg/mL) for 2 hr at 37° C and incubated at 65° C for 6 hr to reverse crosslinks. WCE samples omitted the proteinase K but were otherwise subjected to the same incubation conditions. DNA from both IP and WCE were purified using PCR purification columns (Qiagen Inc 28106) and eluted into 300 µL of 1x TE pH 8.

Quantitative Real-Time PCR (qPCR)

For each genotype, three independent cultures were analyzed and the average fold enrichments of origin DNA by ChIP were reported. The IP and WCE DNA samples from each individual culture were analyzed in triplicate on a Stratagene MX3000P qPCR machine using primer pairs listed in Table S4. Each reaction was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) in a total volume of 20 µL with primers at a final concentration of 300 nM. Because of the AT-rich nature of template origin DNA, we used an annealing temperature of 57°C and an extension temperature of 65°C. Fold enrichment of the assayed DNA segments over the average of two non-origin DNA segments (*ADH1* and *SLH1*) was calculated using the $2^{-\Delta\Delta Ct}$ method essentially as described [85].

Identifying and Testing Candidate RIP Binding Factors

The UNIPROBE database of *in vitro* DNA binding specificities [57] was searched using *RIP317* and *RIP1238* sequences. The search was restricted to *S. cerevisiae* datasets and the stringency filter was set to the lowest setting. Nonessential candidate RIP-binding proteins found in both sequences were *NHP6A NHP6B, YAP1, SUM1, YNR063W, GAT4, SMP1,* and *YOX1*. These factors were knocked out genetically and the resulting strains were tested for re-replication activity at *ARS317* and *ARS1238.*

Accession Numbers

All array CGH data from this study have been deposited in the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo) database (Series Accession #GSE55420).

Acknowledgments

We thank Steve Bell and Michael Weinreich for antibodies and plasmids, and May Szeto for conducting some of the microarray experiments. We appreciate helpful discussions with David MacAlpine, Conrad Nieduszynski, and Oscar Aparicio. We thank Hiten Madhani, David Toczyski, David Morgan, and members of the Li lab for comments on the manuscript.

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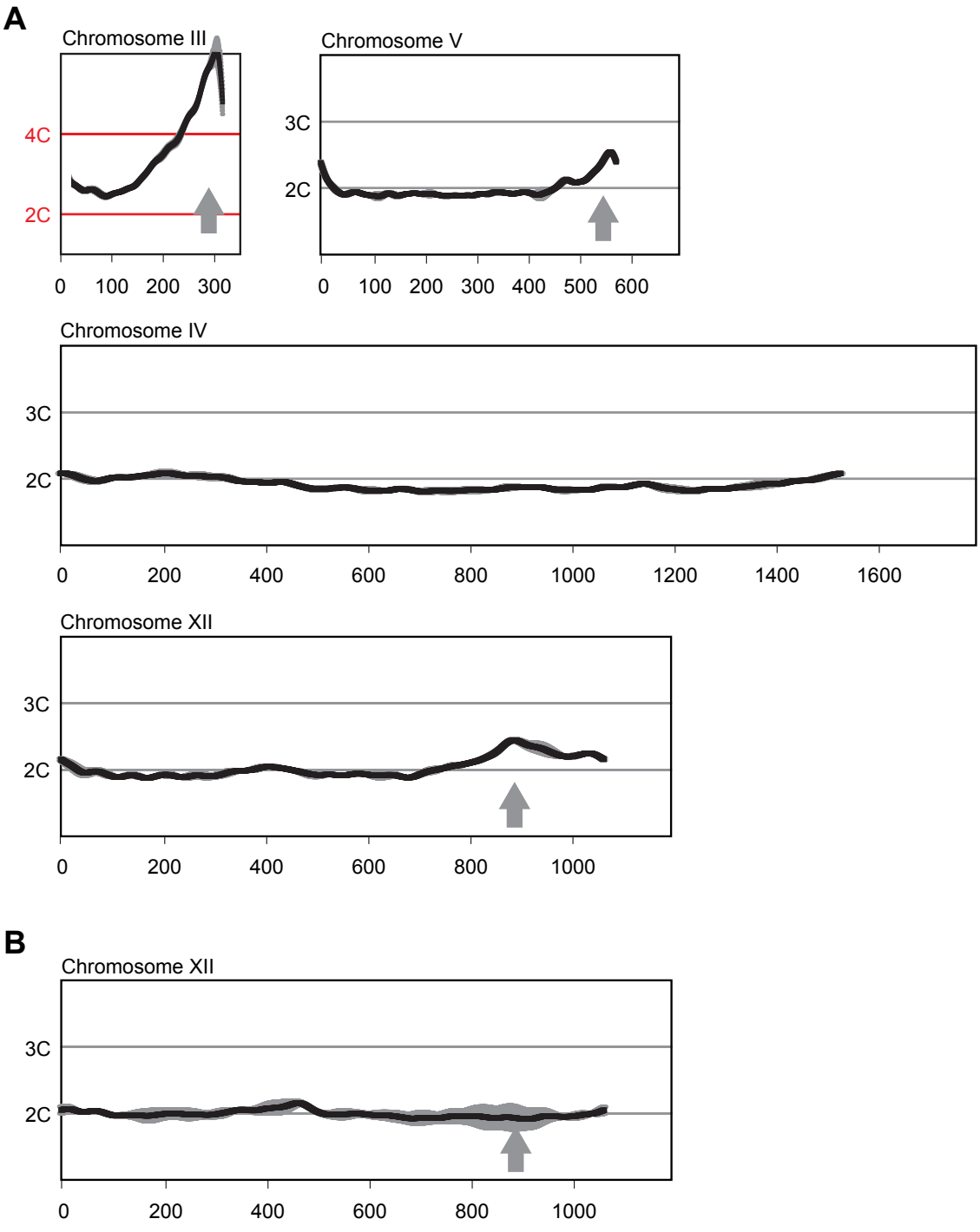


Figure 1. Multiple sites preferentially re-replicate when re-replication controls are deregulated in the *MC2Ao* strain background. (A) Re-

replication profiles of Chromosomes III, V, and XII showing apparent sites of re-replication (gray arrows) in isogenic *MC2Ao* strains (YJL3758 and YJL3759) that were induced to re-replicate for 6 hr at an M phase arrest (nocodazole). For each strain, competitive genomic hybridization was performed against genomic DNA from the same strain arrested in M phase but before induction of re-replication (0 hr) (see Materials and Methods). DNA content from array CGH is plotted against chromosome position in kb. Chromosome IV shows the baseline ~2C DNA content displayed by most of the genome. Data shown as mean of two profiles from the isogenic pair of strains (dark trace) ± SD (light trace). (B) Re-replication peak on Chromosome XII is dependent on *ARS1238*. Re-replication profile of Chromosome XII from YJL9152 was generated as described in A. YJL9152 is congenic to YJL3758/YJL3759 except for deletion of *ARS317* and *ARS1238* (arrow). Data shown as mean of duplicate profiles from YJL9152 (dark trace) ± SD (light trace).

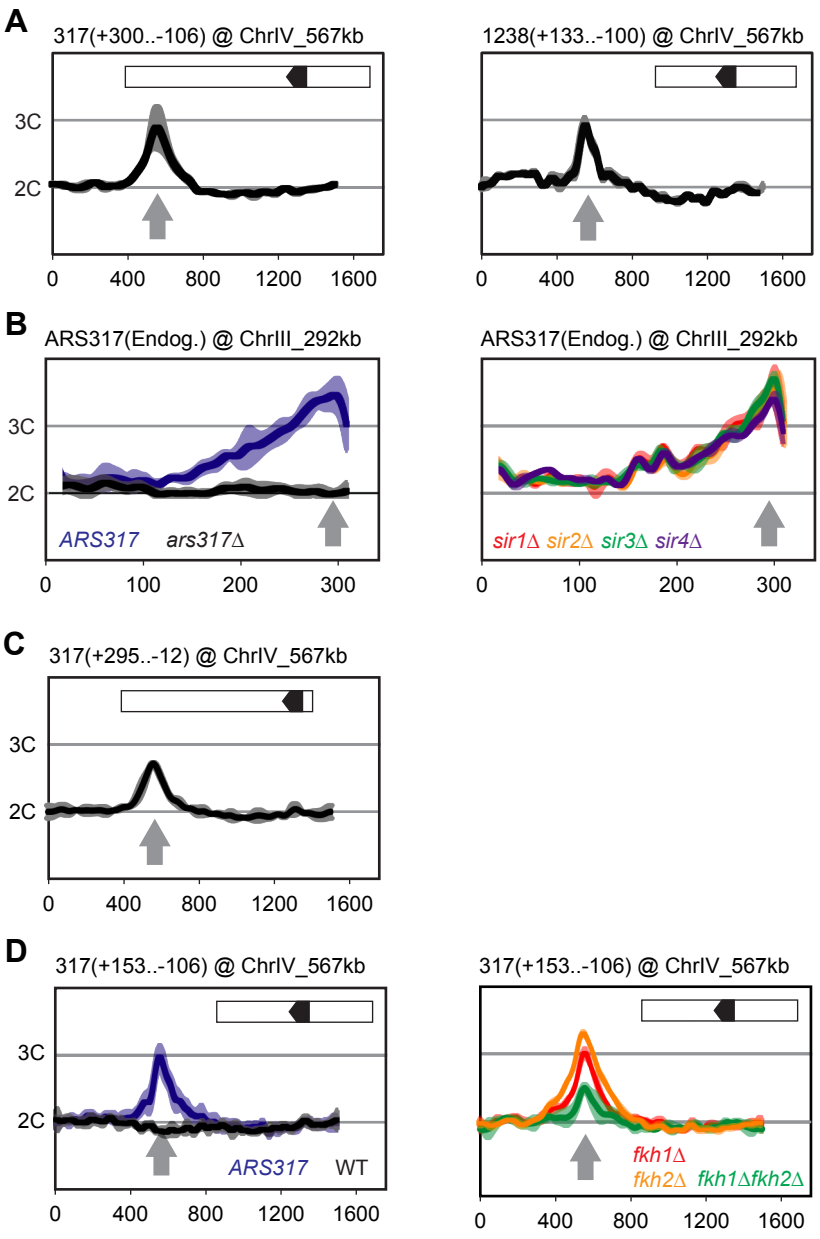


Figure 2. Local determinants and not chromatin context confer preferential re-initiation on *ARS317* and *ARS1238.* Re-replication profiles for *ARS317* at its endogenous location on Chromosome III (ChrIII_292kb) or at a transplanted location on Chromosome IV (ChrIV_567kb). Profiles were obtained by array CGH of genomic DNA from MC2Ao strains induced to re-replicate for 3 hr at an

M phase (nocodazole) arrest against genomic DNA from an M phase arrested strain (YJL7695; see Materials and Methods). The same protocol was used for *ARS1238* transplanted to ChrIV_567kb except re-replication was induced for 6 hr. Boundaries of the transplanted fragments are listed in parentheses above their panels using nucleotide positions relative to the T-rich strand of the ORC binding sites (OBS) (+1 to +33 in the 5' to 3' direction). Inset shows schematic of transplanted fragments: black arrowhead – OBS pointing in 5' to 3' direction of T-rich strand; white – additional sequences adjacent to *ARS317* or *ARS1238* at their endogenous loci that are transplanted along with the origins. (A) DNA fragments containing *ARS317* and *ARS1238* confer preferential re-initiation when transplanted to an ectopic locus. Left panel: *MC2Ao* strains with a 406 bp *ARS317*-containing fragment (YJL7700 and YJL7701) integrated at ChrIV_567kb (gray arrow). Right panel: *MC2Ao* strains with a 233 bp *ARS1238*-containing fragment (YJL9566 and YJL9567). Data shown as mean of two profiles from the isogenic pair of strains (dark trace) ± SD (light trace). (B) Preferential re-initiation of *ARS317* is independent of the transcriptional silencing genes *SIR1-4*. Re-replication profiles of *ARS317* at endogenous locus on Chromosome III (ChrIII_292kb, gray arrow). Left panel: *MC2Ao* strains that are wild-type for the *SIR* genes and contain either *ARS317* (YJL3758; n=5) or *ars317Δ* (YJL8398; n=10) at the endogenous locus. Data shown is mean of the indicated number of profiles for each strain (dark trace) ± SD (light trace). Right panel: strains congenic to YJL3758 but containing *sir1Δ* (YJL6893 and YJL6894, red), *sir2Δ*

(YJL6896 and YJL6897, yellow), *sir3Δ* (YJL6899 and YJL6900, green), or *sir4Δ* (YJL6902 and YJL6903, blue). Data shown as mean of two profiles from each isogenic pair of strains (dark trace) ± SD (light trace). (C) Preferential re-initiation of *ARS317* is independent of the transcriptional silencer element *HMR-E*. A 307 bp fragment that contains the *ARS317 OBS* but lacks the other two essential subelements of *HMR-E* (Rap1 and Abf1 binding sites) was integrated at ChrIV_567 kb (gray arrow) in *MC2Ao* strains YJL8256 and YJL8257 (which are congenic to YJL7700 and YJL7701). Data shown as mean of two profiles from the isogenic pair of strains (dark trace) ± SD (light trace). (D) Forkhead proteins are not essential for preferential re-initiation of *ARS317*. A 259 bp fragment that is sufficient to confer preferential re-initiation of *ARS317* was integrated at ChrIV_567 kb (gray arrow) in *MC2Ao* strains. Left panel: *FKH* strains with (*ARS317*, YJL8398, n=10) or without (*WT*, YJL3758, n=5) *ARS317* transplanted to ChrIV_567kb. Data shown is mean of the indicated number of profiles for each strain (dark trace) ± SD (light trace). Right panel: strains congenic to YJL8398 but containing *fkh1Δ* (YJL8745 and YJL8746, red*), fkh2Δ* (YJL8701 and YJL8702, yellow*),* or *fkh1Δ fkh2Δ (*YJL8749 and YJL8750, green*).* Data shown as mean of two profiles from each isogenic pair of strains (dark trace) ± SD (light trace).

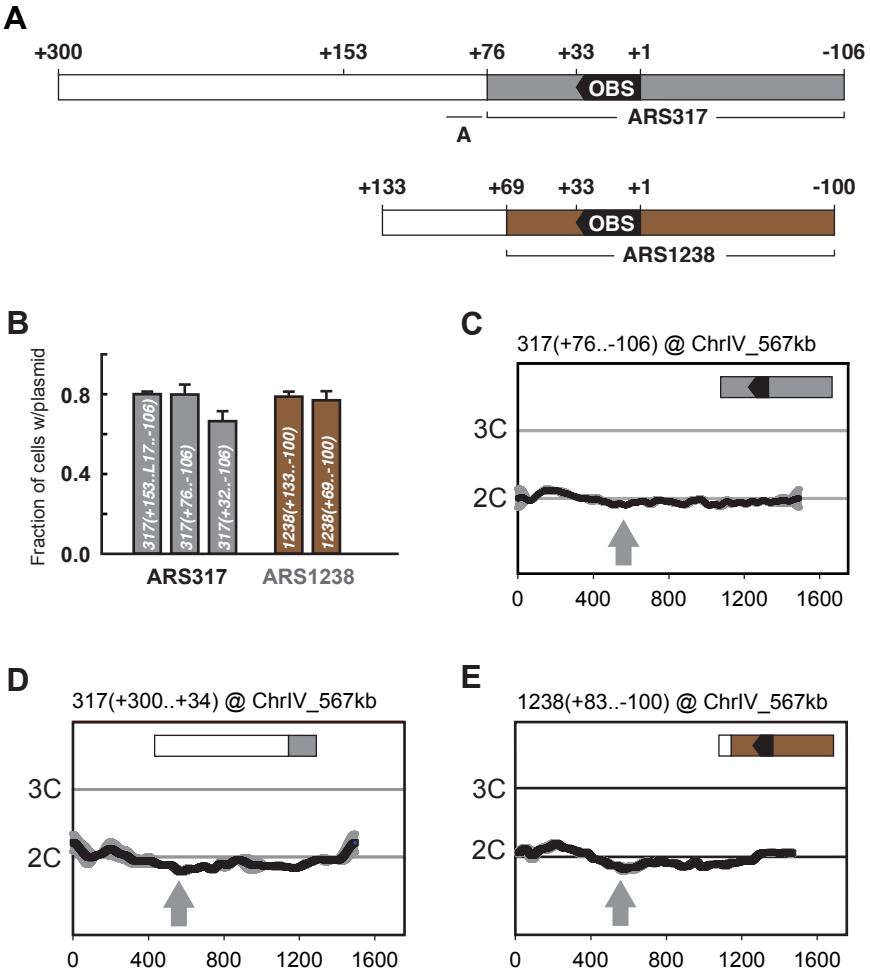


Figure 3. Preferential re-initiation of *ARS317* and *ARS1238* requires additional sequences flanking the origins. (A) Schematic of origin segments contained within preferentially re-initiating fragments. Nucleotide positions are defined relative to the *OBS* for each origin (+1 to +33 on T-rich strand). In the upper schematic, gray indicates sequences (+76…-106) sufficient for optimal *ARS317* ARS activity (see B). The full segment (+300…-106) corresponds to the fragment responsible for preferential re-initiation in Figure 2A, left panel. +153 indicates the left boundary of the minimal sequence required for preferential re-

initiation of *ARS317* (see Figure 4A). A indicates the location (+95 to +77) of previously mapped near matches to the ARS consensus sequence that confers cryptic origin activity independent of *ARS317* [36]**.** In the lower schematic, brown indicates sequences (+69…-100) sufficient for optimal *ARS1238* ARS activity (see B). The full segment (+133…-100) corresponds to the fragment responsible for preferential re-initiation in Figure 2A, right panel. (B) Identification of sequences sufficient for origin activity in preferentially re-initiating fragments. Plasmids containing indicated cloned DNA segments were assayed in wild-type strain YJL310 by measuring their mitotic stability, i.e. fraction of cells growing under selection for the plasmid that contain the plasmid. Plasmids and DNA segments assayed were pCR133*, 317(+153..L17..-106)*-; pCR339, *317(+76..-106)*; pCR287, *317(+32..-106)*, pCR221, *1238(+133..-100)*; and pCR321, *1238(+69..-100);* where the numbers in parentheses indicate nucleotide boundaries of the segment. L17 is an 8 bp linker substitution mutation of nt +86 to +79 (see Figure 4B), which disrupts the cryptic origin activity mentioned in A and allows *ARS317* origin activity to be assayed on its own. pCR287 contains the *HMR-E* silencer fragment that originally identified *ARS317* [28], but this fragment contains suboptimal origin activity. Mitotic stabilities presented as mean ± SD, n = 3. (C) *ARS317* origin cannot preferentially re-initiate by itself. The 182 bp *ARS317* origin segment shown in A and B, *317(+76..-106)*, was integrated at ChrIV_567 kb (gray arrow) in strains YJL10444 and YJL10445. Re-replication profiles were generated and displayed as in Figure 2. (D) The

sequence flanking the *ARS317* origin cannot preferentially re-initiate by itself. A 267 bp segment (nt +300 to +34) containing the 224 bp segment flanking the *ARS317* origin mapped in B (nt +300 to +77) was integrated at ChrIV_567 kb (gray arrow) in YJL7717. Re-replication profiles were generated and displayed as in Figure 2 for *ARS317*, except the mean of two profiles, both from YJL7717, is shown. (E) *ARS1238* origin cannot preferentially re-initiate by itself. A 183 bp segment (nucleotides +83 to -100), containing the 169 bp *ARS1238* origin segment assayed in B, *1238(+69..-106)*, was integrated at ChrIV_567 kb (gray arrow) in strains YJL9707 and YJL9708. Re-replication profiles were generated and displayed as in Figure 2 for *ARS1238* in the congenic strains YJL9566 and YJL9567.

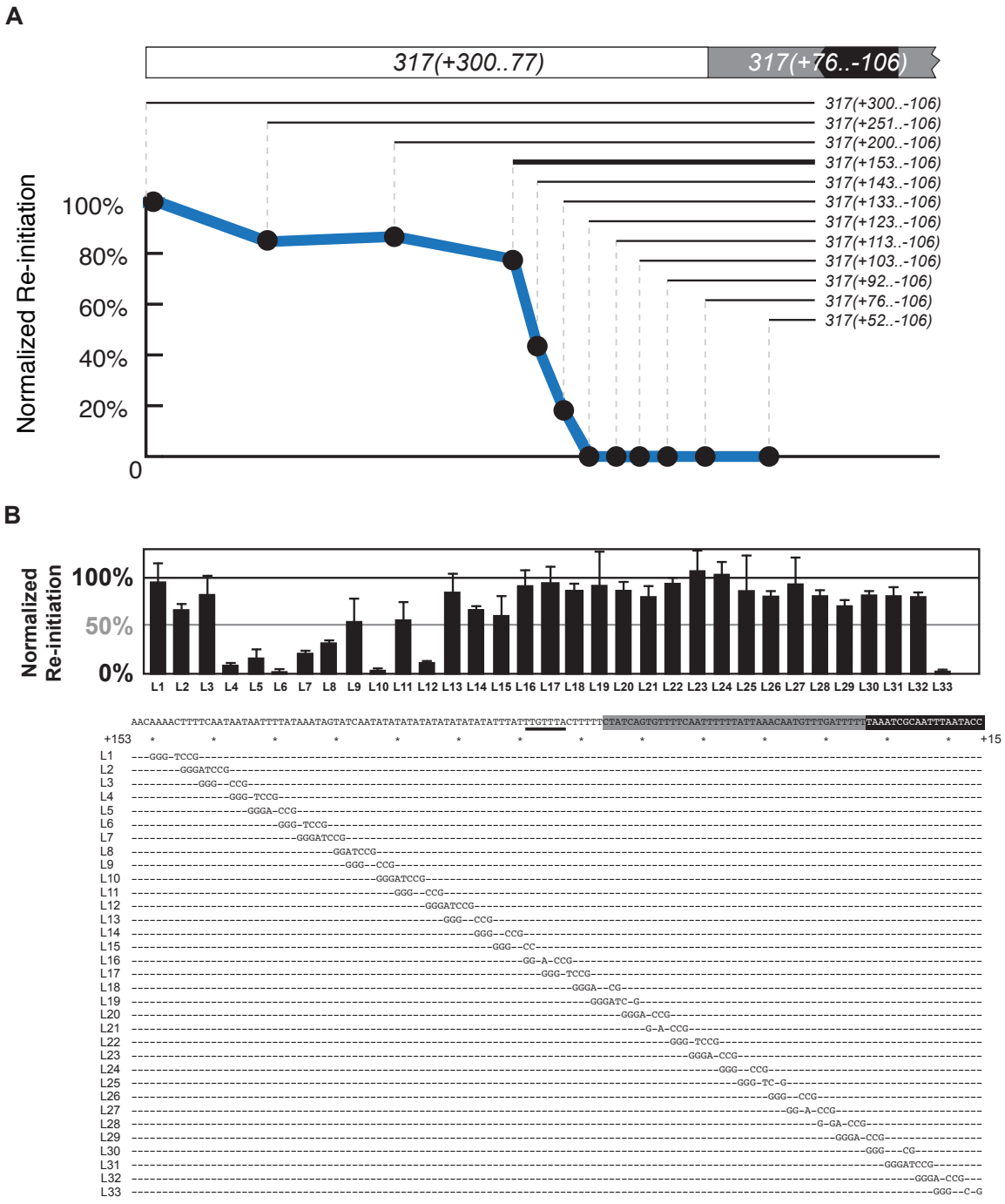


Figure 4. Mapping the re-initiation promoter for *ARS317*. (A) Identifying the left boundary of the Re-Initiation Promoter for *ARS317*. A nested series of left-side deletions (lines with nucleotide coordinates) of the *ARS317* re-initiating fragment *317(+300..-106)* were individually inserted at ChrIV_567 kb. Bold line

represents deletion segment used for linker scan analysis in B. Mean re-replication profiles were obtained as described in Figure 2, except each mean profile was calculated from duplicate experiments of the same strain. Re-initiation efficiency was calculated by normalizing the mean peak height for each deletion fragment against the mean peak height for the full length *317(+300..-106)* fragment in the congenic reference strain YJL7700. The strains used for each deletion are listed in Table S2. (B) Structure of Re-Initiation Promoter for *ARS317*. An overlapping series of linker substitution mutations (L1 – L33) constructed with an 8 bp GGGATCCG linker in the preferentially re-initiating segment *317(+153..-106)* were assayed for re-initiation efficiency as described in A, except efficiencies were normalized against the congenic reference strain YJL8398, which contains the wild-type *317(+153..-106)* sequence (partially shown below graph). Sequences of linker mutations are represented by letters for changed nucleotides and dashes for unchanged nucleotides. Position of the linker mutant HMRE-A (See Figure S3) used to disrupt cryptic origin activity is indicated by a thick black line at position +89..+83. The strains tested in duplicate for each linker substitution are listed in Table S2.

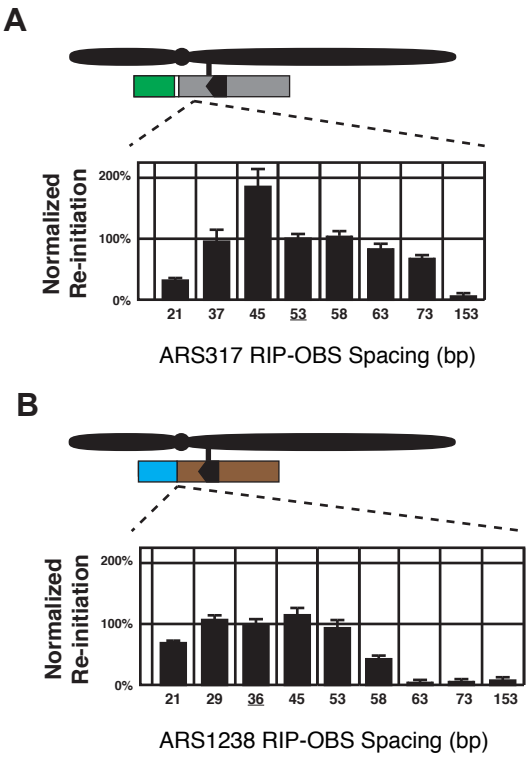


Figure 5. Re-initiation promoters function in close proximity to their origins. (A) Re-initiation efficiency for insertion and deletion mutants that alter the distance between the 67 bp *RIP317* (nt +153 to +87) and the *ARS317 OBS* (nt +33 to +1) in the context of the preferentially re-initiating segment *317(+153..-106)* were determined as described in Figure 4B. These mutant strains change the spacing between the *RIP* and the *OBS* from the wild-type spacing of 53 bp (YJL8398) to 153 bp (YJL8785), 73 bp (YJL8783), 63 bp (YJL8781), 58 bp (YJL8779), 45 bp (YJL8912), 37 bp (YJL8910), or 21 bp (YJL8908). (B) Re-initiation efficiency for insertion and deletion mutants that alter the distance between the 64 bp *RIP1238* (nt +133 to +70) and the *ARS1238 OBS* (nt +33 to +1) in the context of the preferentially re-initiating segment *1238(+133..-100)*

were determined as described in A, except re-replication was induced for 6 hr and isogenic strain pairs were normalized against YJL9566 and YJL9567 to obtain efficiencies. Mutant strains change the spacing between the *RIP* and the *OBS* from the wild-type spacing of 36 bp (YJL9566 and YJL9567) to 153 bp (YJL10287 and YJL10288), 73 bp (YJL10289 and YJL10290), 63 bp (YJL10291 and YJL10292), 58 bp (YJL10293 and YJL10294), 53 bp (YJL10158 and YJL10159), 45 bp (YJL10295 and YJL10296), or 21 bp (YJL10299 and YJL10300).

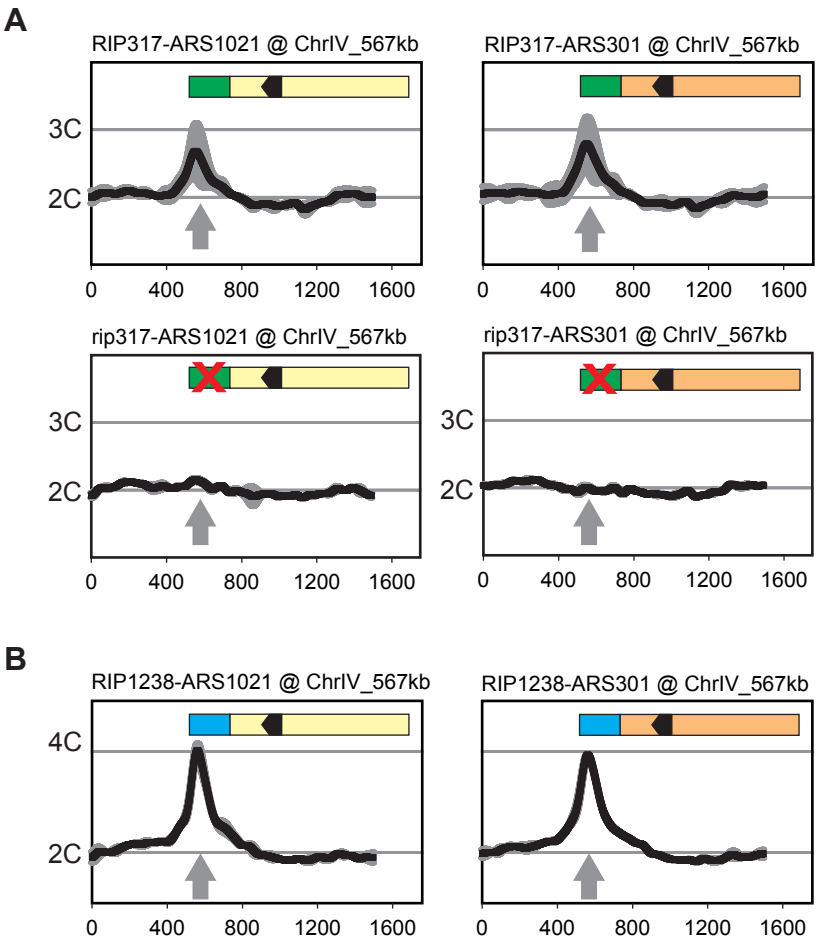


Figure 6. Re-initiation promoters can confer preferential re-initiation on exogenous origins. (A) *RIP317* confers preferential re-initiation on *ARS1021* and *ARS301*. *RIP317-ARS1021* fusion (top left, YJL9078), *rip317-ARS1021* fusion (bottom left, YJL9221), *RIP317-ARS301* fusion (top right, YJL9080), and *rip317-ARS301* fusion (bottom right, YJL9225) were inserted at ChrIV_567kb (gray arrow) in an MC2Ao strain. The mutant *rip317* has linker L6Xho disrupting the same nucleotides as linker L6 (see Figure 4B). Re-replication profiles (shown for Chromosome IV) were obtained as described for *ARS317* in Figure 2 except the mean profile was generated from duplicate experiments of the indicated

strains. (B) *RIP1238* confers re-replication when fused to *ARS1021,* and *ARS301*. A *RIP1238-ARS1021* fusion (left, YJL9999 and YJL10000) or a *RIP1238-ARS301* fusion (right, YJL10001 and YJL10002) was inserted at ChrIV_567kb (gray arrow) in an *MC2A0* strain. Re-replication profiles were obtained as described for *ARS1238* in Figure 2 using a 6 hr induction of re-replication.

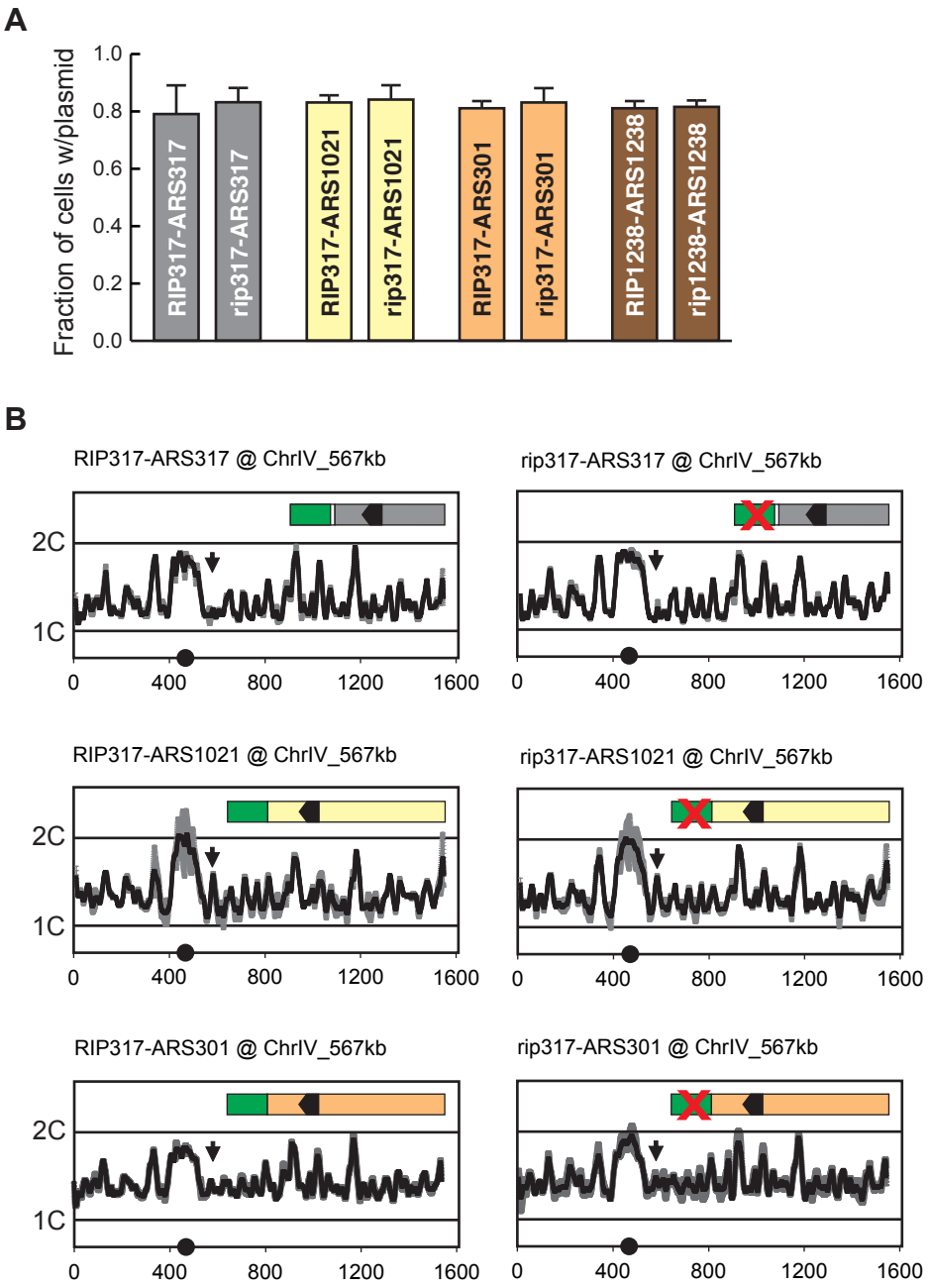


Figure 7. Re-initiation promoters do not alter the initiation activity of origins. (A) *RIP* elements do not enhance mitotic stability of adjacent origins. DNA segments containing combinations of *RIP* elements and origin sequences were cloned into the following plasmids: pCR133 (*RIP317-ARS317*); pCR165

(*rip317-ARS317*); pCR136 (*RIP317-ARS1021*); pCR169 (*rip317-ARS1021*); pCR137 (*RIP317-ARS301*); pCR171 (*rip317-ARS301*); pCR221 (*RIP1238-ARS1238*); pCR313 (*rip1238-ARS1238*). Mitotic stability of these plasmids was measured as described in Figure 3B. (B) *RIP*-origin fragments described in A were inserted at ChrIV_567kb (arrow) in the following MC2Ao strains: YJL9175 (*RIP317-ARS317*); YJL9248 (*rip317-ARS317*); YJL9177 (*RIP317-ARS1021*); YJL9229 (*rip317-ARS1021*); YJL9179 (*RIP317-ARS301*); YJL9233 (*rip317-ARS301*). Inset shows schematic of *RIP-ARS* fusions: green – *RIP317*; green with cross – *rip317*; black arrowhead – OBS. Strains were synchronously released from an alpha factor arrest into media containing 0.1M HU and collected in S phase when 30-60% of the genome was replicated. Replicating DNA from these strains was hybridized against nonreplicating DNA from M phase arrested YJL7695. Profiles show DNA content (1C to 2C) from array CGH of each strain plotted against position (in kb) of Chromosome IV. Data shown as mean of two profiles from duplicate experiments of each strain (dark trace) ± SD (light trace).

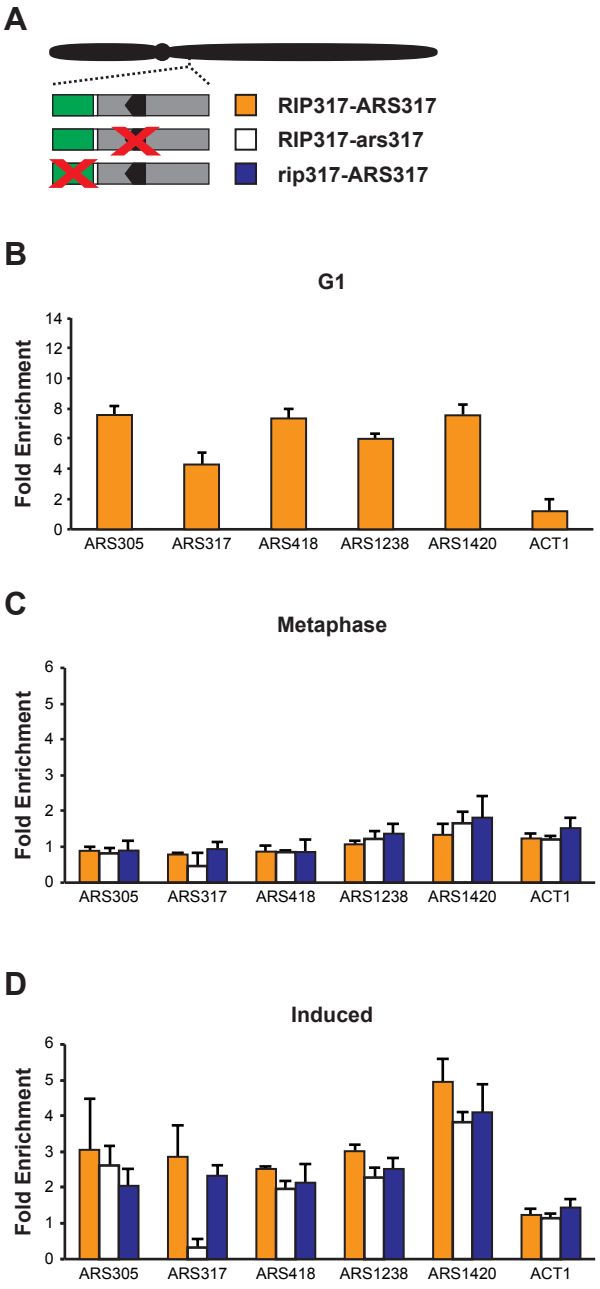


Figure 8. Deregulation of Cdc6 and Mcm2-7 allows Mcm2-7 to re-associate with origins that do not preferentially re-initiate. (A) Schematic of *RIP*-origin fragments inserted at ChrIV_567kb In *MC2Ao* strains. YJL8398 (*RIP317-ARS317,* orange) is described in Figure 4B. YJL8541 (*RIP317-ars317*; white) has

ACSs of both *ARS317* and the nearby cryptic origin disrupted using the mutations *HMRE-A* and *HMRE-E* (described in Figure S3). YJL9244 (*rip317-ARS317*; blue) has the *RIP* disrupted with linker L6 as described in Fig 4B. In all strains, the endogenous *ARS317* is deleted. (B) Mcm2-7 associates with origins at a G1 arrest. ChIP association with the indicated origins and non-origin control ACT1 was measured using anti-Mcm2-7 polyclonal antibodies in alpha factor arrested cells. Data shown as mean +/- SD (n = 3) of DNA enrichment relative to two non-origin segments (ADH1 and SLH1). (C) Mcm2-7 does not associate with origins in an M phase arrest. Mcm2-7 association with DNA segments shown in A was measured as described in B at a metaphase arrest before induction of re-replication. (D) Mcm2-7 associates with origins when re-replication is induced. Mcm2-7 association with DNA segments shown in A was measured as described in B at a metaphase arrest after 1.5 hr of re-replication induction.

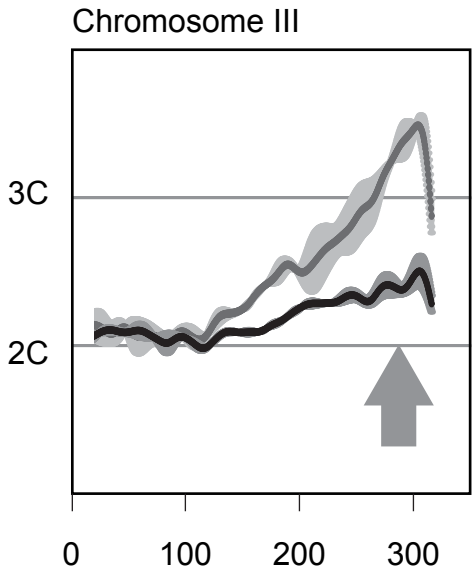


Figure S1. Preferential re-replication is enhanced by, but does not require, the *orc6-S116A* allele. Re-replication profiles for Chromosome III containing *ARS317* at its endogenous locus were generated from isogenic *MC2A* strains (YJL8923 and YJL8924, black trace) and isogenic *MC2Ao* strains (YJL3758 and YJL3759, gray trace) and displayed as in Figure 2. Data shown as mean of two profiles (dark trace) ± SD (light trace).

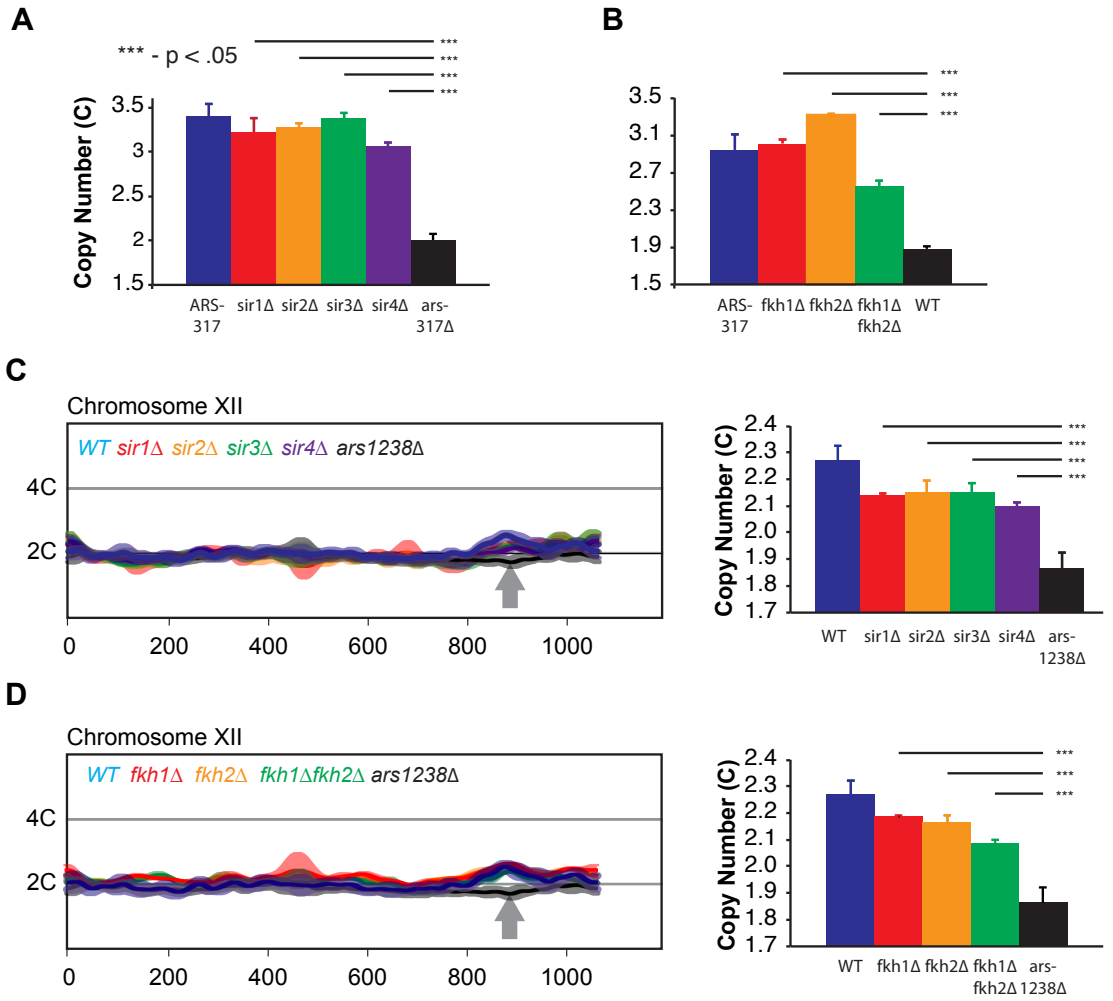


Figure S2. Preferential re-initiation of *ARS1238* does not require *SIR* and *FKH* genes. (A) Quantification of re-replication profile peak heights for *sir*Δ strains described in Figure 2B. DNA copy number at the endogenous *ARS317* locus (ChrIII_292kb) was plotted as a bar graph. Black lines indicate significant difference ($p < 0.05$ for Welch's t-test) between experimental and negative control (*ars317*Δ) strains. (B) Quantification of re-replication peak heights for *fkh*Δ strains described in Figure 2D. DNA copy number at ChrIV_567kb was plotted as a bar graph. Black lines indicate significant difference ($p < 0.05$ for Welch's t-test) between experimental and negative control (*ars317*Δ) strains. (C)

Preferential re-initiation of *ARS1238* is independent of transcriptional silencing genes *SIR1-4*. Left panel: re-replication profiles of Chromosome XII containing *ARS1238* at its endogenous location (gray arrow). Profiles for positive (WT, YJL8398, n=10) and negative (*ars1238*Δ, YJL9152, n=7) control strains are overlaid with *sir*Δ strains described in Figure 2B. Right panel: DNA copy number at the endogenous *ARS1238* locus (ChrXII_889kb) plotted as a bar graph. Black lines indicate significant difference ($p < 0.05$ for Welch's t-test) between experimental and negative control (*ars1238*Δ) strains. Culture conditions as described in Figure 2B. (D) Preferential re-initiation of *ARS1238* does not require forkhead proteins. Left panel: re-replication profiles of Chromosome XII containing *ARS1238* at its endogenous location (gray arrow). Profiles for positive (WT, YJL8398, n=10) and negative (*ars1238*Δ, YJL9152, n=7) control strains are overlaid with *fkh*Δ strains described in Figure 2D. Right panel: DNA copy number at the endogenous *ARS1238* locus (ChrXII_889kb) plotted as a bar graph. Black lines indicate significant difference ($p < 0.05$ for Welch's t-test) between experimental and negative control (*ars1238*Δ) strains. Culture conditions as described in Figure 2D.

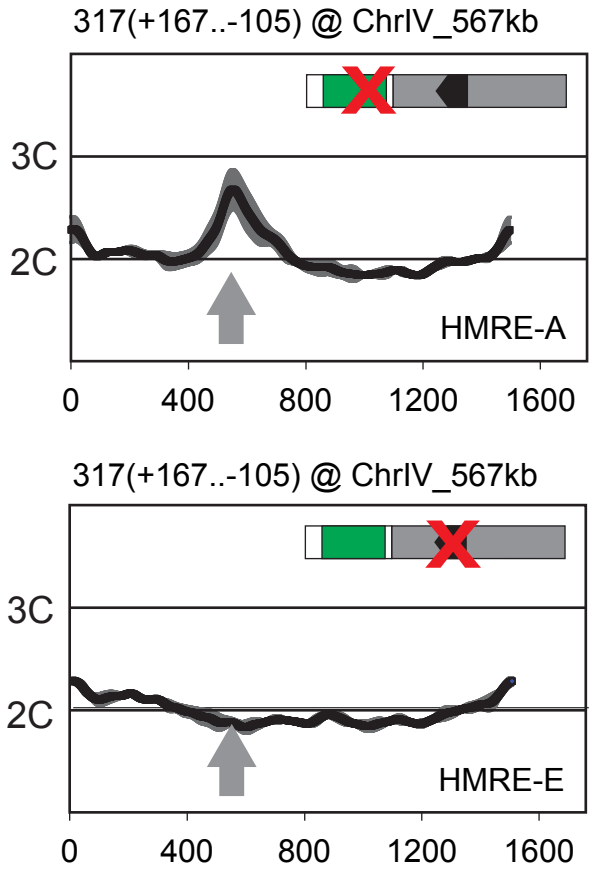


Figure S3. Preferential re-initiation of *ARS317* does not require a nearby cryptic origin. Mutations that disrupt the cryptic origin close to *ARS317* (*HMRE-A*, YJL8526) or the OBS of *ARS317* (*HMRE-E*, YJL8538) (see [36]) were introduced into the preferentially re-initiating fragment *317(+167..-105)* and integrated at ChrIV_567 kb of an *MC2Ao* strain. Re-replication profiles of Chromosome IV were generated and displayed as in Figure 2, except the mean of two profiles was obtained from duplicate experiments on individual mutant strains and not isogenic strain pairs.

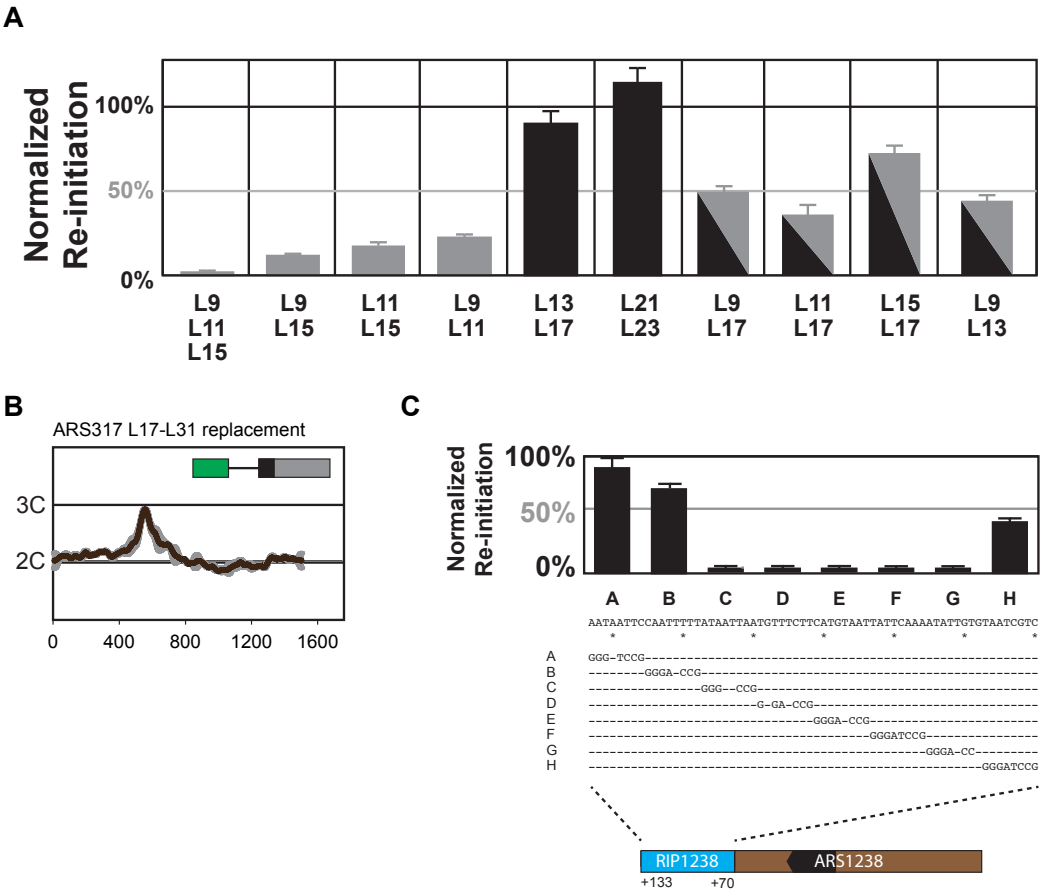


Figure S4. Mapping re-initiation promoters for *ARS317* and *ARS1238*. (A) Partial disruption of preferential re-initiation of *ARS317* by linkers L9, L11, and L15 is additive. Combinations of linker mutation L9, L11, and L15 were introduced into the preferentially re-initiating fragment *317(+153..-106)* and integrated at ChrIV_567 kb of an *MC2Ao* strain (gray bars). As controls L9, L11, and L15 were combined with linkers L13 or L17, which have no effect on preferential re-initiation (black and gray bars), and linkers with no effect were combined with each other (L13 L17, L21 L23; black bars). Re-initiation efficiencies were obtained as described for Figure 4B. Strains used are listed in Table S2. (B) Sequences from nucleotide +153 to +87 are sufficient to confer

preferential re-initiation on *ARS317*. Sequences covered by linkers L17 to L31 (nucleotides +86 to +23), each of which alone had little or no effect on preferential re-initiation of ARS317, were replaced by randomly generated sequence of similar AT content (25%) in the preferentially re-initiating fragment *317(+153..-106)* fragment. The resulting clone was integrated at ChrIV_567 kb in *MC2Ao* strain YJL8838 (top panel). A similar strain YJL9713 (bottom panel) was generated that in addition had nucleotides +153 to +135 (covering linkers L1 to L3) replaced. Re-replication profiles of Chromosome IV were generated and displayed as in Figure 2, except for each mutant strain, the mean of two profiles was obtained from duplicate experiments on the single strain. (C) Structure of Re-Initiation Promoter for *ARS1238*. A series of linker substitution mutations (A-H) constructed with an 8 bp GGGATCCG linker were introduced into the segment adjacent to the *ARS1238* origin in the preferentially re-initiating fragment *1238(+133..-100)* and integrated at ChrIV_567kb of an *MC2Ao* strain. The mutant fragments were assayed for re-initiation efficiency as described in Figure 5B. Wild type sequence is shown beneath graph. Sequence of linker mutations are represented by letters for changed nucleotides and dashes for unchanged nucleotides. Isogenic strain pairs used for each linker substitution are listed in Table S2.

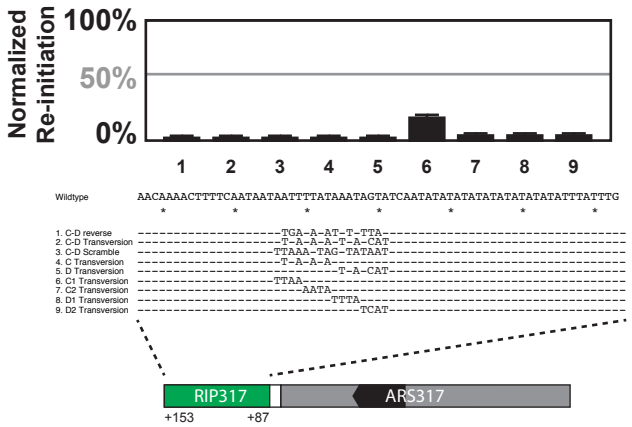


Figure S5. *RIP317* AT-content is not sufficient for re-initiation. Re-initiation efficiency for *317(+153..L17..-106)* fragments with mutations in region *317(+134..+109)* that preserve AT-content while altering sequence. Wild type sequence of *RIP317* (nucleotides +153..+87) is shown beneath graph. Mutated sequence is represented by letters for changed nucleotides and dashes for unchanged nucleotides. Mutant fragments were assayed for re-initiation efficiency as described in Figure 5B, except that normalization was performed against the mean peak height for the full length *317(+153..-106)* fragment in reference strains YJL8398 and YJL8399. Isogenic strain pairs used for each mutation are listed in Table S2.

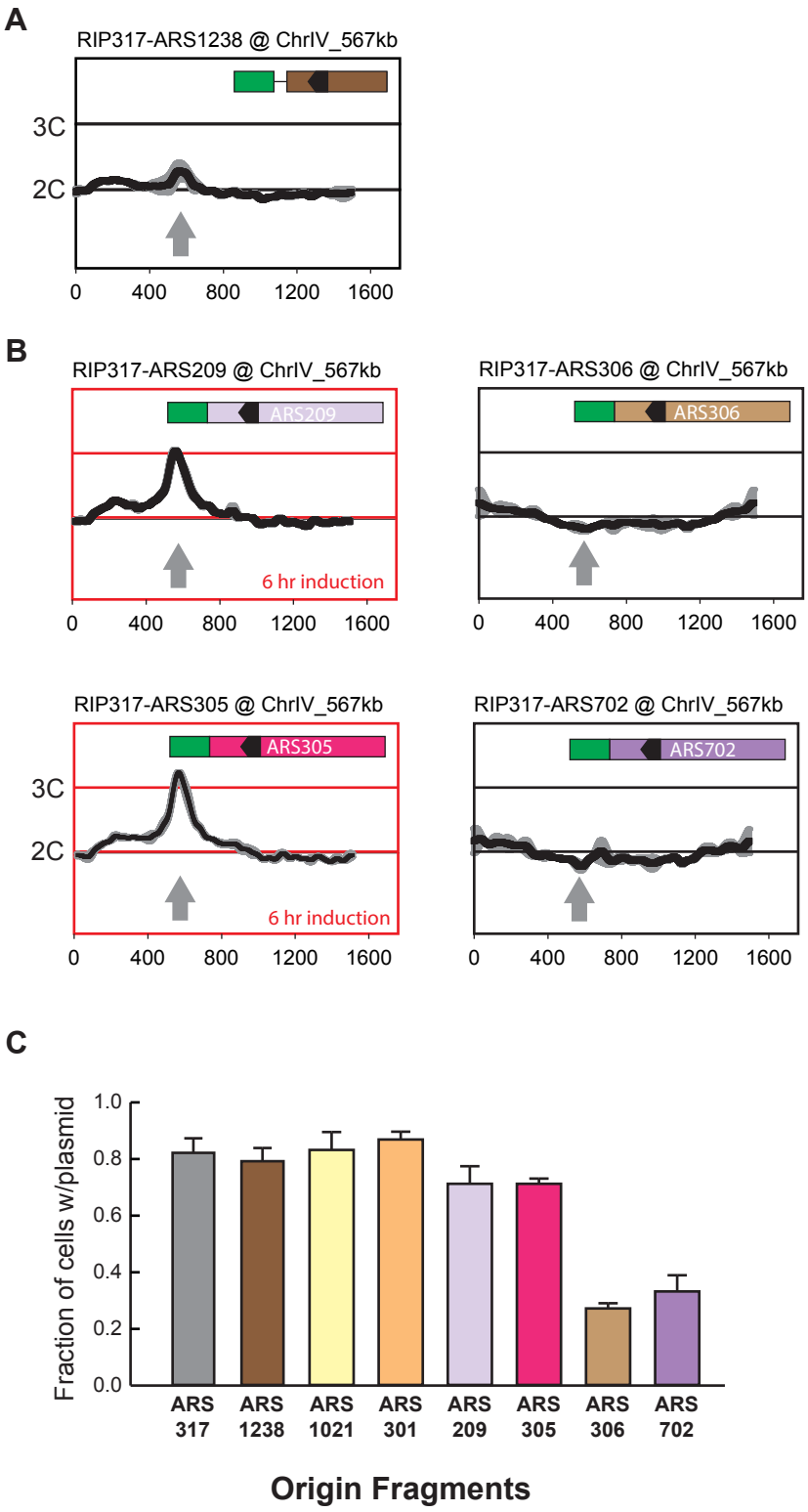


Figure S6. Susceptibility of additional origins to RIP function. (A) Hybrid *RIP317-ARS1238* fusion fragments re-initiate, but not as efficiently as the endogenous

RIP317-ARS317 fragment. *RIP317* was fused to *ARS1238* while maintaining the spacing between *RIP* and *OBS* that normally occurs between *RIP317* and *ARS317* (53 nt). The fragment was integrated at ChrIV_567 kb in isogenic *MC2Ao* strains YJL10160 and YJL10161 and a re-replication profile for Chromosome IV was generated and displayed as in Figure 1. (B) *RIP317* does not confer significant re-replication on all origins. *RIP317* was fused to the following origin fragments and the fusion constructs integrated at ChrIV_567 kb in the *MC2Ao* strain background. For each origin, nucleotide boundaries, *RIP-OBS* spacing, and yeast strain analyzed are indicated in parentheses. Nucleotide numbering is based on +1 to +33 for the T-rich strand of the OBS. *ARS209* (nt +91..-241, 58, YJL9088), *ARS305* (nt +83..-249, 50, YJL9082), *ARS306* (nt +89..-245, 56, YJL9084), *ARS702* (+84..-247, 51, YJL9086). Re-replication profiles of Chromosome IV were generated and displayed as in Figure 2 except the mean of two profiles was obtained from duplicate experiments on each individual mutant strain. Re-replication was induced for 3 hr (black borders) or 6 hr (red border). (C) Mitotic stability of origin fragments fused to *RIP317*. The *ARS* activity of the origin fragments described in B was assayed by mitotic stability in plasmids containing *CEN4* and *URA3*. *ARS317* (pCR339), *ARS1238* (pCR321), *ARS1021* (pCR146), *ARS301* (pCR147), *ARS209* (pCR154), *ARS305* (pCR149), *ARS306* (pCR150), or *ARS702* (pCR152) were assayed for mitotic stability as described in Figure 3B.

Table S1. Description of integrative and mitotic stability plasmids used in this manuscript. Each entry lists plasmid name, key plasmid features, and the sequence of the *RIP*-origin fragment inserted into XbaI (Integrative *RIP*-origin plasmids; pBJL2889 derived [2]) or HindIII-EcoRI (mitotic stability; pFJ11 derived [36]) restriction sites. See Materials and Methods for a complete description of plasmid construction.

Integrative RIP-origin Plasmids	**Sequence: SpeI/XbaI to XbaI (inclusive)***	
pBJL2889	ChromIV_567kb::{ade3-2p,kanMX}	TCTAGA
pBJL2933	ChromIV_567kb::{kanMX6,ade3-2p,317(+300..-106)}	TCTAGTCTAGTACTTAAAAAAACTGTAGT TTCAGTGCAAAAAAGTTTTAACATTACGT ATCTTGTACCCTTTTTATTGCATATAGAA AGGTCAAATAATCCTTCACATCATGAAAT ATAAGCTAAATCGCATTTCTTTTCGTCCAC ATTTGCAAACAAAACTTTTCAATAATAAT TTTATAAATAGTATCAATATATATATATA TATATATATTTATTTGTTTACTTTTTCTA TCAGTGTTTTCAATTTTTTATTAAACAAT GTTTGATTTTTTAAATCGCAATTTAATAC CTAAATATAAAAAATGTTATTATATTGCA AAAACCCATCAACCTTGAAAAAAAGTAGA AACGTTTTATTTAATTCTATCAATACATC ATAAAATACGAACGATCCCCGTCCAAGTT ATGAGTCTAGA
pBJL2935	ChromIV_567kb::{kanMX6,ade3-2p,317(+300..+33)}	TCTAGTCTAGTACTTAAAAAAACTGTAGT TTCAGTGCAAAAAAGTTTTAACATTACGT ATCTTGTACCCTTTTTATTGCATATAGAA AGGTCAAATAATCCTTCACATCATGAAAT ATAAGCTAAATCGCATTTCTTTTCGTCCAC ATTTGCAAACAAAACTTTTCAATAATAAT TTTATAAATAGTATCAATATATATATATA TATATATATTTATTTGTTTACTTTTTCTA TCAGTGTTTTCAATTTTTTATTAAACAAT GTTTGATTTTTTTCTAGA
pCR013	ChromIV_567kb::{kanMX6,ade3-2p,317(+294..-13)}	TCTAGTCGTACGACTTAAAAAAACTGTAG TTTCAGTGCAAAAAAGTTTTAACATTACG TATCTTGTACCCTTTTTATTGCATATAGA

		AAGGTCAAATAATCCTTCACATCATGAAA TATAAGCTAAATCGCATTTCTTTTCGTCC ACATTTGCAAACAAAACTTTTCAATAATA ATTTTATAAATAGTATCAATATATATATA TATATATATATTTATTTGTTTACTTTTTC TATCAGTGTTTTCAATTTTTTATTAAACA ATGTTTGATTTTTTAAATCGCAATTTAAT ACCTAAATATAAAAAATGTTATTATATTG CTTCGAATCTAGA
pCR016	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+251..-106)}	TCTAGTCGTACGCGTATCTTGTACCCTTTT TATTGCATATAGAAAGGTCAAATAATCCT TCACATCATGAAATATAAGCTAAATCGCA TTTCTTTTCGTCCACATTTGCAAACAAAAC TTTTCAATAATAATTTTATAAATAGTATC AATATATATATATATATATATATTTATTT GTTTACTTTTTCTATCAGTGTTTTCAATT TTTTATTAAACAATGTTTGATTTTTTAAA TCGCAATTTAATACCTAAATATAAAAAAT GTTATTATATTGCAAAAACCCATCAACCT TGAAAAAAAGTAGAAACGTTTTATTTAAT TCTATCAATACATCATAAAATACGAACGA TCCCCGTCCAAGTTATGAGTTCGAATCTA GA
pCR019	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+200..-106)}	TCTAGTCGTACGATCATGAAATATAAGCT AAATCGCATTTCTTTTCGTCCACATTTGCA AACAAAACTTTTCAATAATAATTTTATAA ATAGTATCAATATATATATATATATATAT ATTTATTTGTTTACTTTTTCTATCAGTGT TTTCAATTTTTTATTAAACAATGTTTGAT TTTTTAAATCGCAATTTAATACCTAAATA TAAAAAATGTTATTATATTGCAAAAACCC ATCAACCTTGAAAAAAAGTAGAAACGTTT TATTTAATTCTATCAATACATCATAAAAT ACGAACGATCCCCGTCCAAGTTATGAGTT CGAATCTAGA
pCR020	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+167..-106)}	TCTAGTCGTACGCGTCCACATTTGCAAACA AAACTTTTCAATAATAATTTTATAAATAG TATCAATATATATATATATATATATATTT ATTTGTTTACTTTTTCTATCAGTGTTTTC AATTTTTTATTAAACAATGTTTGATTTTT TAAATCGCAATTTAATACCTAAATATAAA AAATGTTATTATATTGCAAAAACCCATCA ACCTTGAAAAAAAGTAGAAACGTTTTATT TAATTCTATCAATACATCATAAAATACGA ACGATCCCCGTCCAAGTTATGAGTTCGAA

		TCTAGA
pCR022	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATATATATATATTTATTTGTTTACTTTTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR024	ChromIV_567kb::{kanMX6,ade3-2p,317(+92..-106)}	TCTAGTCGTACGTATTTGTTTACTTTTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR026	ChromIV_567kb::{kanMX6,ade3-2p,317(+52..-106)}	TCTAGTCGTACGTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR041	ChromIV_567kb::{kanMX6,ade3-2p,317(+167..-106)HMRE-A}	TCTAGTCGTACGCGTCCACATTTGCAAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATATATATATATTTATGCTCGAGCTTTTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR045	ChromIV_567kb::{kanMX6,ade3-2p,317(+167..-106)HMRE-E}	TCTAGTCGTACGCGTCCACATTTGCAAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATATATATATATTTATTTGTTTACTTTTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTTAAATCGCAATTTAATACCGGTCGACAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATT

		TAATTCTATCAATACATCATAAAATACGA ACGATCCCCGTCCAAGTTATGAGTTCGAA TCTAGA
pCR047	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+167..- 106)HMRE-A/E}	TCTAGTCGTACGCGTCCACATTTGCAAACA AAACTTTTCAATAATAATTTTATAAATAG TATCAATATATATATATATATATATATTT ATGCTCGAGCTTTTTCTATCAGTGTTTTCA ATTTTTTATTAAACAATGTTTGATTTTTT AAATCGCAATTTAATACCGGTCGACAAAA AATGTTATTATATTGCAAAAACCCATCAA CCTTGAAAAAAAGTAGAAACGTTTTATTT AATTCTATCAATACATCATAAAATACGAA CGATCCCCGTCCAAGTTATGAGTTCGAATC TAGA
pCR051	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+143..-106)}	TCTAGTCGTACGTTCAATAATAATTTTAT AAATAGTATCAATATATATATATATATAT ATATTTATTTGTTTACTTTTTCTATCAGT GTTTTCAATTTTTTATTAAACAATGTTTG ATTTTTTAAATCGCAATTTAATACCTAAA TATAAAAAATGTTATTATATTGCAAAAAC CCATCAACCTTGAAAAAAAGTAGAAACGT TTTATTTAATTCTATCAATACATCATAAA ATACGAACGATCCCCGTCCAAGTTATGAG TTCGAATCTAGA
pCR052	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+133..-106)}	TCTAGTCGTACGATTTTATAAATAGTATC AATATATATATATATATATATATTTATTT GTTTACTTTTTCTATCAGTGTTTTCAATT TTTTATTAAACAATGTTTGATTTTTTAAA TCGCAATTTAATACCTAAATATAAAAAAT GTTATTATATTGCAAAAACCCATCAACCT TGAAAAAAAGTAGAAACGTTTTATTTAAT TCTATCAATACATCATAAAATACGAACGA TCCCCGTCCAAGTTATGAGTTCGAATCTA GA
pCR053	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+123..-106)}	TCTAGTCGTACGTAGTATCAATATATATA TATATATATATATTTATTTGTTTACTTTT TCTATCAGTGTTTTCAATTTTTTATTAAA CAATGTTTGATTTTTTAAATCGCAATTTA ATACCTAAATATAAAAAATGTTATTATAT TGCAAAAACCCATCAACCTTGAAAAAAAG TAGAAACGTTTTATTTAATTCTATCAATA CATCATAAAATACGAACGATCCCCGTCCA AGTTATGAGTTCGAATCTAGA
pCR054	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+113..-106)}	TCTAGTCGTACGATATATATATATATATA TATTTATTTGTTTACTTTTTCTATCAGTG

		TTTTCAATTTTTTATTAAACAATGTTTGA TTTTTTAAATCGCAATTTAATACCTAAAT ATAAAAAATGTTATTATATTGCAAAAACC CATCAACCTTGAAAAAAAGTAGAAACGTT TTATTTAATTCTATCAATACATCATAAAA TACGAACGATCCCCGTCCAAGTTATGAGT TCGAATCTAGA
pCR055	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+103..-106)}	TCTAGTCGTACGATATATATATTTATTTG TTTACTTTTTCTATCAGTGTTTTCAATTTT TTATTAAACAATGTTTGATTTTTTAAATC GCAATTTAATACCTAAATATAAAAAATGT TATTATATTGCAAAAACCCATCAACCTTG AAAAAAAGTAGAAACGTTTTATTTAATTC TATCAATACATCATAAAATACGAACGATC CCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR061	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker1}	TCTAGTCGTACGAACGGGATCCGTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR062	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker3}	TCTAGTCGTACGAACAAAACTTTGGGATC CGAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR063	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker5}	TCTAGTCGTACGAACAAAACTTTTCAATA ATGGGATCCGAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA

pCR064	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker7}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATGGGATCCGTCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR065	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTAGGGATCCGTAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR066	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker15}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATAGGGATCCGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR067	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker19}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT GGGATCCGGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR068	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker23}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCGGGATCCGATTAA ACAATGTTTGATTTTTTAAATCGCAATTT

		AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR069	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker25}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTGGGAT CCGATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR070	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker27}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAGGGATCCGTTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR072	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker11}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATAGGG ATCCGTATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR073	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker13}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATAGGGATCCGTTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA

pCR074	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker17}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR075	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker21}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGGGATCCGAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR076	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker29}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGAGGGATCCGATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR077	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker31}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAGGGATCCGTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR078	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker33}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATGG

		GATCCGTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR088	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker9,11,15}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTAGGGATCCGGGG ATCCGTATATATAGGGATCCGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR089	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker9,15}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTAGGGATCCGTAT ATATATATATATAGGGATCCGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR090	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker11,15}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATAGGG ATCCGTATATATAGGGATCCGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR091	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker9,11}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTAGGGATCCGGGG ATCCGTATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA

pCR092	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)58bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGGATCCATTTTTTAAATCGC AATTTAATACCTAAATATAAAAAATGTTA TTATATTGCAAAAACCCATCAACCTTGAA AAAAAGTAGAAACGTTTTATTTAATTCTA TCAATACATCATAAAATACGAACGATCCC CGTCCAAGTTATGAGTTCGAATCTAGA
pCR093	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)63bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGAAGGATCCAGATTTTTTAA ATCGCAATTTAATACCTAAATATAAAAAA TGTTATTATATTGCAAAAACCCATCAACC TTGAAAAAAAGTAGAAACGTTTTATTTAA TTCTATCAATACATCATAAAATACGAACG ATCCCCGTCCAAGTTATGAGTTCGAATCT AGA
pCR094	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)73bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGCAAAGAAGGATCCAGAAGG TATTTTTTAAATCGCAATTTAATACCTAA ATATAAAAAATGTTATTATATTGCAAAAA CCCATCAACCTTGAAAAAAAGTAGAAACG TTTTATTTAATTCTATCAATACATCATAA AATACGAACGATCCCCGTCCAAGTTATGA GTTCGAATCTAGA
pCR095	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)153bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGCCCATAGGATATAGAGATA CCAATAGTTGTTTGTGAGCAACAAAGAAG GATCCAGAAGGTCGATCGCACGATATTGA TGTGAATACTAGTTGTAGTAATGATTTTT TAAATCGCAATTTAATACCTAAATATAAA AAATGTTATTATATTGCAAAAACCCATCA ACCTTGAAAAAAAGTAGAAACGTTTTATT TAATTCTATCAATACATCATAAAATACGA ACGATCCCCGTCCAAGTTATGAGTTCGAA

		TCTAGA
pCR096	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker13,17}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATAGGGATCCGTTTATTTGGGGATCCGTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR097	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker21,23}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATATATATATATTTATTTGTTTACTTTTTCTATCAGGGATCCGGGGATCCGATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR098	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,17}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTAGGGATCCGTATATATATATATATATTTATTTGGGGATCCGTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR099	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker11,17}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATAGGGATCCGTATATATATTTATTTGGGGATCCGTTCTATCAGTGTTTTCAATTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCTATCAATACATCATAAAATACGAACGATCCCCGTCCAAGTTATGAGTTCGAATCTAGA
pCR100	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker15,17}	TCTAGTCGTACGAACAAAACTTTTCAATAATAATTTTATAAATAGTATCAATATATATATATATATAGGGATCCGGGGATCCGTTCTATCAGTGTTTTCAATTTTTTATTAA

		ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR101	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker9,13}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTAGGGATCCGTAT ATATAGGGATCCGTTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR102	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)L17-L31replacement}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGAAAGTAAG TATACATAACTACTTTGACTTGGATCCTA GAATAGTATTTGATATGTATCAATAGATT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR107	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)21bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT GGGATCCGTTTTTTAAATCGCAATTTAAT ACCTAAATATAAAAAATGTTATTATATTG CAAAAACCCATCAACCTTGAAAAAAAGTA GAAACGTTTTATTTAATTCTATCAATACA TCATAAAATACGAACGATCCCCGTCCAAG TTATGAGTTCGAATCTAGA
pCR108	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)37bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCGGGATCCGTTTTTT AAATCGCAATTTAATACCTAAATATAAAA AATGTTATTATATTGCAAAAACCCATCAA CCTTGAAAAAAAGTAGAAACGTTTTATTT AATTCTATCAATACATCATAAAATACGAA CGATCCCCGTCCAAGTTATGAGTTCGAATC TAGA

pCR109	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)45bpRIP-OBS}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTGGGAT CCGTTTTTTAAATCGCAATTTAATACCTA AATATAAAAAATGTTATTATATTGCAAAA ACCCATCAACCTTGAAAAAAAGTAGAAAC GTTTTATTTAATTCTATCAATACATCATA AAATACGAACGATCCCCGTCCAAGTTATG AGTTCGAATCTAGA
pCR113	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker2}	TCTAGTCGTACGAACAAAACGGGATCCGA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR114	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker4}	TCTAGTCGTACGAACAAAACTTTTCAATG GGATCCGTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR115	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker8}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGGGATCCGATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR116	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker10}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATGGGATC CGATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT

		AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR117	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker12}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATGGGATCCGATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR118	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker14}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATGGGATCCGTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR119	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker18}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACGGG ATCCGTCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR120	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker20}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTAGGGATCCGTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA

pCR121	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker22}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTGGGATCCGTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR122	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker26}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA GGGATCCGTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR123	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker28}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTGGGATCCGTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR124	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker30}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTGGGATCCGAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR125	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker32}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCGGGAT

		CCGACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR126	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker6}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTGGGATCCGGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR129	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker16}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATGGGATCCGTTT TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR130	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)Linker24}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGTTTACTTT TTCTATCAGTGTTTTCAATTTGGGATCCG ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR156	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS1021(+72..-253)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT CTATTTTCTGCTATTCATCCAAAATATAT TTTCTCACAAAAGCTGTTAATTTGAAACT AATGTTAAACAAAACATCTCACTTCAAAT TGAAGCCAAATTAGAACCTAAGTAACCAA CACACGCACAACTTTCATCCCTTATCCCAC TTCTTTGAAAACTTTTAGAAGCCATTGAT GGTATTGTACATTTACCTATCTTAGCAGA

		TTCCTTATTAGCCGCCGAAATGGGTAATA AGTATTATAATCACTAAATACGAAAAATT TATTCATACCAACCCGCAACATGAACTAC AAACCTTTGGGTATATAATGACAAAGACG TATCTTCGAATCTAGA
pCR157	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS301(+78..-247)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCA TTGTTCTTTCATTAATAATTTGAATTCCA AATAAGTCCGTGCCGAAAACTTTAATGTT TTAAAAAAACATAAAAAAATCAAAAACCC ATTCATAAGATACTGTGTTGATCCATATA TTAATAGGTTTTAAGTACATATAGAATAC TTAAGAAATTACATTCCATTGCGATACAC CTATTTGATTCTGATTGTGTTGAAGTCTG TATAAAAGGCGAAAAAAATAAAATGAAAA TAAGAAAATAACTGCGCTACTTGTCTTCT AAATTATCTTCTGGTGTCTCGTCCTTTTTT GGTATGATTAATTTCTTATTGGATGAAAA ACTATTCGAATCTAGA
pCR159	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS305(+76..-249)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT TTCTTTGATAAATTCTTGTTTTCATATCCT AAAATTAAAGGGAAAATAAACAATACATA ACAAAACATATAAAAACCAACACAATAAA AAAAAGGATCAAATACTCATTAAAGTAAC TTACACGGGGGCTAAAAACGGAGTTTGAT GAATATTCACAAGATAAAAATCATATGTA TGTTTCTGATATATCGATATACAATCAAA CACTTTCAAGAATTTGTTTGTAGACTTTT TGCTAGAGACCTCATCAAAGTGCTACCAA CTAAGATCAACTTATACTTCTTTTAGAGA AAATTTTTTTCAATGTACTCCAAAGAGAT TTAGTTCGAATCTAGA
pCR160	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS306(+82..-245)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT TTTTGGAAGTGTTTTTCGACAAAAGTTGC ATTTTTACGAAGGATATGTAAACTCAAGA AGTACTAAATAAATATAAATACAATTCGA AAATTTGAAAAAAGTAGGAGACAACACTT TCCTTCCTCACGAAGAAGTTAAGCTTGGG TTTGTGACTTACTAACGTCAACGTACAAT CGCGTTACAAACAAGATGCTTGCATTCTT

		ACCTTACATTACAAGGACTTAGACGAAGA AAAAGTTCAGAAACACTGCTTACACTATT CACCAGACCCGCTCCTTCTCCTAACATCAA TAACGAACAGCACTATTGATGTAAGAACG TCTTAGTTCGAATCTAGA
pCR162	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS702(+77..-247)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT GGCCCCCAATAGGCTTCGTAATTTTTACAT GTCACAAAATTAAATAAGGCAAAACGAGA AACAAAATATTAAAAATGTAGGAAAAAAA AAGTCAAAATTGGTTTGACGACTTAACCT AATCAATTTAAGTCGGAATGATGATCAAC AATAACATCTTTAATTTTATCATGAATAT CTAATTCGGTTAATTGACCCAATTCATCT ATGAGCTCGGCAACTTTATCATGCAACTT AACATCCTCCTCGTGAACAATTTCATTCTC ATCCATTGTGGATGGATGAGCTGGTTTAC TCATATCAGCTTTTTCCAAAAAGTCCTGG TTTTCGAATCTAGA
pCR164	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+153..+87)- ARS209(+84..-241)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT ATTTTTTTTTCTTCATTCCGTAACTCTTCT ACCTTCTTTATTTACTTTCTAAAATCCAA ATACAAAACATAAAAATAAATAAACACAG AGTAAATTCCCAAATTATTCCATCATTAA AAAATACGAGGCGCGTGTAAGTTACAGAC AAGCGATCCCTATTCCATGCAAGTTCGGT AAGTAGCAGAAATAATCAAACTGTTTAAA CCCAATTAAAATTAAATTAAATACCCTTT ATATGTTTATAATTGTACATATTTCTCCT AAACCCGCTATAATACACTCATATTTGTA GAAGAAAAACCGGGCAGTTGAATACGAAT CCCATTCGAATCTAGA
pCR187	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153…- 106)Linker6Xho}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTGGCTCGAGGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA

pCR191	ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS1021(+72..-253)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTGGCTCGAGGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCT CTATTTTCTGCTATTCATCCAAAATATAT TTTCTCACAAAAGCTGTTAATTTGAAACT AATGTTAAACAAAACATCTCACTTCAAAT TGAAGCCAAATTAGAACCTAAGTAACCAA CACACGCACAACTTTCATCCCTTATCCCAC TTCTTTGAAAACTTTTAGAAGCCATTGAT GGTATTGTACATTTACCTATCTTAGCAGA TTCCTTATTAGCCGCCGAAATGGGTAATA AGTATTATAATCACTAAATACGAAAAATT TATTCATACCAACCCGCAACATGAACTAC AAACCTTTGGGTATATAATGACAAAGACG TATCTTCGAATCTAGA
pCR193	ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS301(+78..-247)}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTGGCTCGAGGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCA TTGTTCTTTCATTAATAATTTGAATTCCA AATAAGTCCGTGCCGAAAACTTTAATGTT TTAAAAAAACATAAAAAAATCAAAAACCC ATTCATAAGATACTGTGTTGATCCATATA TTAATAGGTTTTAAGTACATATAGAATAC TTAAGAAATTACATTCCATTGCGATACAC CTATTTGATTCTGATTGTGTTGAAGTCTG TATAAAAGGCGAAAAAAATAAAATGAAAA TAAGAAAATAACTGCGCTACTTGTCTTCT AAATTATCTTCTGGTGTCTCGTCCTTTTTT GGTATGATTAATTTCTTATTGGATGAAAA ACTATTCGAATCTAGA
pCR217	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR234	ChromIV_567kb::{kanMX6,ade3-2p,1238(+83..-100)}	TCTAGTCGTACGATTGTGTAATCGTCTAT TTGAATTCGTTTTCACGGCAGCATATTTG AAAAAAAAATTAATTTTTACATCTAAACA TAAAAAACCTAATCGTTTTCTTTCCATGC TGTTATAGAGACATTTATTAGTACGAATA TTTTCCTTTCTTTACGAGCACTATAGACA

		GTAATTTATATAACTAAGAAATTCGAATC TAGA
pCR238	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)L1-L3,L17- L31replacement}	TCTAGTCGTACGTGAAAGTGTGTGATGTA CAAATTTTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG ACATAGTTGTTTGTGAGCAACAAAGAAAG AAGGTCGATCGCACGATATTGGCTAGCTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR274	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP1238(+133..+70)- ARS1021(+72..-253)}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCGGGATCCGTCT ATTTTCTGCTATTCATCCAAAATATATTT TCTCACAAAAGCTGTTAATTTGAAACTAA TGTTAAACAAAACATCTCACTTCAAATTG AAGCCAAATTAGAACCTAAGTAACCAACA CACGCACAACTTTCATCCCTTATCCCACTT CTTTGAAAACTTTTAGAAGCCATTGATGG TATTGTACATTTACCTATCTTAGCAGATT CCTTATTAGCCGCCGAAATGGGTAATAAG TATTATAATCACTAAATACGAAAAATTTA TTCATACCAACCCGCAACATGAACTACAA ACCTTTGGGTATATAATGACAAAGACGTA TCTTCGAATCTAGA
pCR275	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP1238(+133..+70)- ARS301(+78..-247)}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCGGGATCCGATT GTTCTTTCATTAATAATTTGAATTCCAAA TAAGTCCGTGCCGAAAACTTTAATGTTTT AAAAAAACATAAAAAAATCAAAAACCCAT TCATAAGATACTGTGTTGATCCATATATT AATAGGTTTTAAGTACATATAGAATACTT AAGAAATTACATTCCATTGCGATACACCT ATTTGATTCTGATTGTGTTGAAGTCTGTA TAAAAGGCGAAAAAAATAAAATGAAAATA AGAAAATAACTGCGCTACTTGTCTTCTAA ATTATCTTCTGGTGTCTCGTCCTTTTTTGG TATGATTAATTTCTTATTGGATGAAAAAC TATTCGAATCTAGA
pCR289	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)53bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCAAGAAGGATCC

		AGAAGATATTTGAATTCGTTTTCACGGCA GCATATTTGAAAAAAAAATTAATTTTTAC ATCTAAACATAAAAAACCTAATCGTTTTC TTTCCATGCTGTTATAGAGACATTTATTA GTACGAATATTTTCCTTTCTTTACGAGCA CTATAGACAGTAATTTATATAACTAAGAA ATTCGAATCTAGA
pCR290	ChromIV_567kb::{kanMX6 ,ade3- 2p,RIP317(+150..+87)- ARS1238(+69..- 100)53bpRIP-OBS}	TCTAGTCGTACGAAAACTTTTCAATAATA ATTTTATAAATAGTATCAATATATATATA TATATATATATTTATTTGAAGAAGGATCC AGAAGATATTTGAATTCGTTTTCACGGCA GCATATTTGAAAAAAAAATTAATTTTTAC ATCTAAACATAAAAAACCTAATCGTTTTC TTTCCATGCTGTTATAGAGACATTTATTA GTACGAATATTTTCCTTTCTTTACGAGCA CTATAGACAGTAATTTATATAACTAAGAA ATTCGAATCTAGA
pCR295	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)LinkerA}	TCTAGTCGTACGGGGATCCGCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR296	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)LinkerB}	TCTAGTCGTACGAATAATTCGGGATCCGA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR297	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)LinkerC}	TCTAGTCGTACGAATAATTCCAATTTTTG GGATCCGTGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR298	ChromIV_567kb::{kanMX6	TCTAGTCGTACGAATAATTCCAATTTTTA

	,ade3-2p,1238(+133..-100)LinkerD}	TAATTAAGGGATCCGCATGTAATTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR299	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerE}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTGGGATCCGTATTCA AAATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR300	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerF}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATGGGATC CGATATTGTGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR301	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerG}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAGGGATCCGTAATCGTCTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR302	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerH}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGGGGATCCGTATTTGAATTC GTTTTCACGGCAGCATATTTGAAAAAAAA ATTAATTTTTACATCTAAACATAAAAAAC CTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR303	ChromIV_567kb::{kanMX6	TCTAGTCGTACGAATAATTCCAATTTTTA

	,ade3-2p,1238(+133..-100)153bpRIP-OBS}	TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCATAGCCTGCCC ATAGGATATAGAGATACCAATAGTTGTTT GTGAACAGCAAAGAAGGATCCAGAAGATC AGTCGCACGATATTGATGTGAATACTAGG TTTATAGGATAGTCGTACATATTTGAATT CGTTTTCACGGCAGCATATTTGAAAAAAA AATTAATTTTTACATCTAAACATAAAAAA CCTAATCGTTTTCTTTCCATGCTGTTATAG AGACATTTATTAGTACGAATATTTTCCTT TCTTTACGAGCACTATAGACAGTAATTTA TATAACTAAGAAATTCGAATCTAGA
pCR304	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)73bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTGTGAACAGCA AAGAAGGATCCAGAAGATCAGTCGCATAT TTGAATTCGTTTTCACGGCAGCATATTTG AAAAAAAAATTAATTTTTACATCTAAACA TAAAAAACCTAATCGTTTTCTTTCCATGC TGTTATAGAGACATTTATTAGTACGAATA TTTTCCTTTCTTTACGAGCACTATAGACA GTAATTTATATAACTAAGAAATTCGAATC TAGA
pCR305	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)63bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCACAGCAAAGAA GGATCCAGAAGATCAGTATTTGAATTCGT TTTCACGGCAGCATATTTGAAAAAAAAAT TAATTTTTACATCTAAACATAAAAAACCT AATCGTTTTCTTTCCATGCTGTTATAGAG ACATTTATTAGTACGAATATTTTCCTTTC TTTACGAGCACTATAGACAGTAATTTATA TAACTAAGAAATTCGAATCTAGA
pCR306	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)58bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCGCAAAGAAGGA TCCAGAAGATCTATTTGAATTCGTTTTCA CGGCAGCATATTTGAAAAAAAAATTAATT TTTACATCTAAACATAAAAAACCTAATCG TTTTCTTTCCATGCTGTTATAGAGACATT TATTAGTACGAATATTTTCCTTTCTTTAC GAGCACTATAGACAGTAATTTATATAACT AAGAAATTCGAATCTAGA
pCR307	ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA

	100)45bpRIP-OBS}	AAATATTGTGTAATCGTCAAGGATCCATA TTTGAATTCGTTTTCACGGCAGCATATTT GAAAAAAAAATTAATTTTTACATCTAAAC ATAAAAAACCTAATCGTTTTCTTTCCATG CTGTTATAGAGACATTTATTAGTACGAAT ATTTTCCTTTCTTTACGAGCACTATAGAC AGTAATTTATATAACTAAGAAATTCGAAT CTAGA
pCR308	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)29bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCATTCGTTTTCA CGGCAGCATATTTGAAAAAAAAATTAATT TTTACATCTAAACATAAAAAACCTAATCG TTTTCTTTCCATGCTGTTATAGAGACATT TATTAGTACGAATATTTTCCTTTCTTTAC GAGCACTATAGACAGTAATTTATATAACT AAGAAATTCGAATCTAGA
pCR309	ChromIV_567kb::{kanMX6 ,ade3-2p,1238(+133..- 100)21bpRIP-OBS}	TCTAGTCGTACGAATAATTCCAATTTTTA TAATTAATGTTTCTTCATGTAATTATTCA AAATATTGTGTAATCGTCTCACGGCAGCA TATTTGAAAAAAAAATTAATTTTTACATC TAAACATAAAAAACCTAATCGTTTTCTTT CCATGCTGTTATAGAGACATTTATTAGTA CGAATATTTTCCTTTCTTTACGAGCACTAT AGACAGTAATTTATATAACTAAGAAATTC GAATCTAGA
pCR322	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)C-DReverse}	TCTAGTCGTACGAACAAAACTTTTCAATA ATATGATAAATATTTTAATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR323	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)C-DTransversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATATTATAAAATAAACATTCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC

		AAGTTATGAGTTCGAATCTAGA
pCR324	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DScramble}	TCTAGTCGTACGAACAAAACTTTTCAATA ATTTAAATTAGATATAATTCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR325	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)CTransversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATATTATAAAAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR326	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)DTransversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATATAAACATTCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR327	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C1Transversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATTTAATTATAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR328	ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C2Transversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTAATAAAATAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA

		ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR329	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)D1Transversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATTTTAAGTATCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR330	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+153..- 106)D2Transversion}	TCTAGTCGTACGAACAAAACTTTTCAATA ATAATTTTATAAATTCATTCAATATATAT ATATATATATATATTTATTTGGGGATCCG TTCTATCAGTGTTTTCAATTTTTTATTAA ACAATGTTTGATTTTTTAAATCGCAATTT AATACCTAAATATAAAAAATGTTATTATA TTGCAAAAACCCATCAACCTTGAAAAAAA GTAGAAACGTTTTATTTAATTCTATCAAT ACATCATAAAATACGAACGATCCCCGTCC AAGTTATGAGTTCGAATCTAGA
pCR340	ChromIV_567kb::{kanMX6 ,ade3-2p,317(+76..-106)}	TCTAGTCGTACGCTATCAGTGTTTTCAAT TTTTTATTAAACAATGTTTGATTTTTTAA ATCGCAATTTAATACCTAAATATAAAAAA TGTTATTATATTGCAAAAACCCATCAACC TTGAAAAAAAGTAGAAACGTTTTATTTAA TTCTATCAATACATCATAAAATACGAACG ATCCCCGTCCAAGTTATGAGTTCGAATCT AGA

Mitotic Stability Plasmids		
Name	**Description**	**Sequence: HinDIII to EcoRI (inclusive)**
pFJ11	CEN4, URA3	Described in [36]
pCR133	317(+153..- 106)Linker17; (RIP317- ARS317)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCGTTCTATCAGTGTTTTCAA TTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAA TTTAATACCTAAATATAAAAAATGTTATTATATTGCA

		AAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTT ATTTAATTCTATCAATACATCATAAAATACGAACGAT CCCCGTCCAAGTTATGAGTTCGAATCTAGAGAATTC
pCR136	RIP317(+153.. +87)- ARS1021(+72.. -253)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTCTATTTTCTGCTATTCATC CAAAATATATTTTCTCACAAAAGCTGTTAATTTGAAA CTAATGTTAAACAAAACATCTCACTTCAAATTGAAGC CAAATTAGAACCTAAGTAACCAACACACGCACAACTT TCATCCCTTATCCCACTTCTTTGAAAACTTTTAGAAGC CATTGATGGTATTGTACATTTACCTATCTTAGCAGAT TCCTTATTAGCCGCCGAAATGGGTAATAAGTATTATA ATCACTAAATACGAAAAATTTATTCATACCAACCCGC AACATGAACTACAAACCTTTGGGTATATAATGACAAA GACGTATCTTCGAATCTAGAGAATTC
pCR137	RIP317(+153.. +87)- ARS301(+78..- 247)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCATTGTTCTTTCATTAATAA TTTGAATTCCAAATAAGTCCGTGCCGAAAACTTTAAT GTTTTAAAAAAACATAAAAAAATCAAAAACCCATTCA TAAGATACTGTGTTGATCCATATATTAATAGGTTTTA AGTACATATAGAATACTTAAGAAATTACATTCCATTG CGATACACCTATTTGATTCTGATTGTGTTGAAGTCTG TATAAAAGGCGAAAAAAATAAAATGAAAATAAGAAA ATAACTGCGCTACTTGTCTTCTAAATTATCTTCTGGTG TCTCGTCCTTTTTTGGTATGATTAATTTCTTATTGGAT GAAAAACTATTCGAATCTAGAGAATTC
pCR139	RIP317(+153.. +87)- ARS305(+76..- 249)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTTTCTTTGATAAATTCTTG TTTTCATATCCTAAAATTAAAGGGAAAATAAACAATA CATAACAAAACATATAAAAACCAACACAATAAAAAAA AGGATCAAATACTCATTAAAGTAACTTACACGGGGGC TAAAAACGGAGTTTGATGAATATTCACAAGATAAAAA TCATATGTATGTTTCTGATATATCGATATACAATCAA ACACTTTCAAGAATTTGTTTGTAGACTTTTTGCTAGA GACCTCATCAAAGTGCTACCAACTAAGATCAACTTAT ACTTCTTTTAGAGAAAATTTTTTTCAATGTACTCCAA AGAGATTTAGTTCGAATCTAGAGAATTC
pCR140	RIP317(+153.. +87)- ARS306(+82..- 245)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTTTTTGGAAGTGTTTTTCG ACAAAAGTTGCATTTTTACGAAGGATATGTAAACTCA AGAAGTACTAAATAAATATAAATACAATTCGAAAATT TGAAAAAAGTAGGAGACAACACTTTCCTTCCTCACGA

		AGAAGTTAAGCTTGGGTTTGTGACTTACTAACGTCAA CGTACAATCGCGTTACAAACAAGATGCTTGCATTCTTA CCTTACATTACAAGGACTTAGACGAAGAAAAAGTTCA GAAACACTGCTTACACTATTCACCAGACCCGCTCCTTC TCCTAACATCAATAACGAACAGCACTATTGATGTAAG AACGTCTTAGTTCGAATCTAGAGAATTC
pCR142	RIP317(+153..+87)-ARS702(+77..-247)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTGGCCCCCAATAGGCTTCGT AATTTTTACATGTCACAAAATTAAATAAGGCAAAACG AGAAACAAAATATTAAAAATGTAGGAAAAAAAAAGTC AAAATTGGTTTGACGACTTAACCTAATCAATTTAAGT CGGAATGATGATCAACAATAACATCTTTAATTTTATC ATGAATATCTAATTCGGTTAATTGACCCAATTCATCT ATGAGCTCGGCAACTTTATCATGCAACTTAACATCCTC CTCGTGAACAATTTCATTCTCATCCATTGTGGATGGAT GAGCTGGTTTACTCATATCAGCTTTTTCCAAAAAGTCC TGGTTTTCGAATCTAGAGAATTC
pCR144	RIP317(+153..+87)-ARS209(+84..-241)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTTATAAATAGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTATTTTTTTTTCTTCATTCC GTAACTCTTCTACCTTCTTTATTTACTTTCTAAAATCC AAATACAAAACATAAAAATAAATAAACACAGAGTAAA TTCCCAAATTATTCCATCATTAAAAAATACGAGGCGC GTGTAAGTTACAGACAAGCGATCCCTATTCCATGCAA GTTCGGTAAGTAGCAGAAATAATCAAACTGTTTAAAC CCAATTAAAATTAAATTAAATACCCTTTATATGTTTA TAATTGTACATATTTCTCCTAAACCCGCTATAATACAC TCATATTTGTAGAAGAAAAACCGGGCAGTTGAATACG AATCCCATTCGAATCTAGAGAATTC
pCR146	ARS1021(+72..-253); BglII-BamHI digest and recircularization of pCR136	AAGCTTAGATCCTCTATTTTCTGCTATTCATCCAAAAT ATATTTTCTCACAAAAGCTGTTAATTTGAAACTAATG TTAAACAAAACATCTCACTTCAAATTGAAGCCAAATT AGAACCTAAGTAACCAACACACGCACAACTTTCATCCC TTATCCCACTTCTTTGAAAACTTTTAGAAGCCATTGAT GGTATTGTACATTTACCTATCTTAGCAGATTCCTTATT AGCCGCCGAAATGGGTAATAAGTATTATAATCACTAA ATACGAAAAATTTATTCATACCAACCCGCAACATGAA CTACAAACCTTTGGGTATATAATGACAAAGACGTATC TTCGAATCTAGAGAATTC
pCR147	ARS301(+78..-247); BglII-BamHI digest and recircularizatio	AAGCTTAGATCCATTGTTCTTTCATTAATAATTTGAA TTCCAAATAAGTCCGTGCCGAAAACTTTAATGTTTTA AAAAAACATAAAAAAATCAAAAACCCATTCATAAGAT ACTGTGTTGATCCATATATTAATAGGTTTTAAGTACA TATAGAATACTTAAGAAATTACATTCCATTGCGATAC

	n of pCR137	ACCTATTTGATTCTGATTGTGTTGAAGTCTGTATAAA AGGCGAAAAAAATAAAATGAAAATAAGAAAATAACTG CGCTACTTGTCTTCTAAATTATCTTCTGGTGTCTCGTC CTTTTTTGGTATGATTAATTTCTTATTGGATGAAAAA CTATTCGAATCTAGAGAATTC
pCR149	ARS305(+76..-249); BglII-BamHI digest and recircularization of pCR139	AAGCTTAGATCCTTTCTTTGATAAATTCTTGTTTTCAT ATCCTAAAATTAAAGGGAAAATAAACAATACATAACA AAACATATAAAAACCAACACAATAAAAAAAAGGATCA AATACTCATTAAAGTAACTTACACGGGGGCTAAAAAC GGAGTTTGATGAATATTCACAAGATAAAAATCATATG TATGTTTCTGATATATCGATATACAATCAAACACTTT CAAGAATTTGTTTGTAGACTTTTTGCTAGAGACCTCA TCAAAGTGCTACCAACTAAGATCAACTTATACTTCTTT TAGAGAAAATTTTTTTCAATGTACTCCAAAGAGATTT AGTTCGAATCTAGAGAATTC
pCR150	ARS306(+82..-245); BglII-BamHI digest and recircularization of pCR140	AAGCTTAGATCCTTTTTGGAAGTGTTTTTCGACAAAA GTTGCATTTTTACGAAGGATATGTAAACTCAAGAAGT ACTAAATAAATATAAATACAATTCGAAAATTTGAAAA AAGTAGGAGACAACACTTTCCTTCCTCACGAAGAAGT TAAGCTTGGGTTTGTGACTTACTAACGTCAACGTACA ATCGCGTTACAAACAAGATGCTTGCATTCTTACCTTAC ATTACAAGGACTTAGACGAAGAAAAAGTTCAGAAACA CTGCTTACACTATTCACCAGACCCGCTCCTTCTCCTAA CATCAATAACGAACAGCACTATTGATGTAAGAACGTC TTAGTTCGAATCTAGAGAATTC
pCR152	ARS702(+77..-247); BglII-BamHI digest and recircularization of pCR142	AAGCTTAGATCCTGGCCCCCAATAGGCTTCGTAATTTT TACATGTCACAAAATTAAATAAGGCAAAACGAGAAAC AAAATATTAAAAATGTAGGAAAAAAAAAGTCAAAATT GGTTTGACGACTTAACCTAATCAATTTAAGTCGGAAT GATGATCAACAATAACATCTTTAATTTTATCATGAAT ATCTAATTCGGTTAATTGACCCAATTCATCTATGAGCT CGGCAACTTTATCATGCAACTTAACATCCTCCTCGTGA ACAATTTCATTCTCATCCATTGTGGATGGATGAGCTG GTTTACTCATATCAGCTTTTTCCAAAAAGTCCTGGTTT TCGAATCTAGAGAATTC
pCR154	ARS209(+84..-241); BglII-BamHI digest and recircularization of pCR144	AAGCTTAGATCCTATTTTTTTTTCTTCATTCCGTAACT CTTCTACCTTCTTTATTTACTTTCTAAAATCCAAATAC AAAACATAAAAATAAATAAACACAGAGTAAATTCCCA AATTATTCCATCATTAAAAAATACGAGGCGCGTGTAA GTTACAGACAAGCGATCCCTATTCCATGCAAGTTCGGT AAGTAGCAGAAATAATCAAACTGTTTAAACCCAATTA AAATTAAATTAAATACCCTTTATATGTTTATAATTGT ACATATTTCTCCTAAACCCGCTATAATACACTCATATT TGTAGAAGAAAAACCGGGCAGTTGAATACGAATCCCA TTCGAATCTAGAGAATTC

pCR165	317(+153..-106)Linker6Xho; (rip317-ARS317)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTGGCTCGAGGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCGTTCTATCAGTGTTTTCAA TTTTTTATTAAACAATGTTTGATTTTTTAAATCGCAA TTTAATACCTAAATATAAAAAATGTTATTATATTGCA AAAACCCATCAACCTTGAAAAAAAGTAGAAACGTTTT ATTTAATTCTATCAATACATCATAAAATACGAACGAT CCCCGTCCAAGTTATGAGTTCGAATCTAGAGAATTC
pCR169	rip317(+153..+87)Linker6Xho-ARS1021(+72..-253)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTGGCTCGAGGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCTCTATTTTCTGCTATTCATC CAAAATATATTTTCTCACAAAAGCTGTTAATTTGAAA CTAATGTTAAACAAAACATCTCACTTCAAATTGAAGC CAAATTAGAACCTAAGTAACCAACACACGCACAACTT TCATCCCTTATCCCACTTCTTTGAAAACTTTTAGAAGC CATTGATGGTATTGTACATTTACCTATCTTAGCAGAT TCCTTATTAGCCGCCGAAATGGGTAATAAGTATTATA ATCACTAAATACGAAAAATTTATTCATACCAACCCGC AACATGAACTACAAACCTTTGGGTATATAATGACAAA GACGTATCTTCGAATCTAGAGAATTC
pCR171	rip317(+153..+87)Linker6Xho-ARS301(+78..-247)	AAGCTTAGATCTCGTACGAACAAAACTTTTCAATAAT AATTTGGCTCGAGGTATCAATATATATATATATATAT ATATTTATTTGGGGATCCATTGTTCTTTCATTAATAA TTTGAATTCCAAATAAGTCCGTGCCGAAAACTTTAAT GTTTTAAAAAAACATAAAAAAATCAAAAACCCATTCA TAAGATACTGTGTTGATCCATATATTAATAGGTTTTA AGTACATATAGAATACTTAAGAAATTACATTCCATTG CGATACACCTATTTGATTCTGATTGTGTTGAAGTCTG TATAAAAGGCGAAAAAAATAAAATGAAAATAAGAAA ATAACTGCGCTACTTGTCTTCTAAATTATCTTCTGGTG TCTCGTCCTTTTTTGGTATGATTAATTTCTTATTGGAT GAAAAACTATTCGAATCTAGAGAATTC
pCR221	1238(+133..-100) (RIP1238-ARS1238)	AAGCTTAGATCTCGTACGAATAATTCCAATTTTTATA ATTAATGTTTCTTCATGTAATTATTCAAAATATTGTG TAATCGTCTATTTGAATTCGTTTTCACGGCAGCATATT TGAAAAAAAAATTAATTTTTACATCTAAACATAAAAA ACCTAATCGTTTTCTTTCCATGCTGTTATAGAGACATT TATTAGTACGAATATTTTCCTTTCTTTACGAGCACTAT AGACAGTAATTTATATAACTAAGAAATTCGAATCTAG AGAATTC
pCR287	317(+32..-106) (ars317)	AAGCTTAGATCTCGTACGAAATCGCAATTTAATACCT AAATATAAAAAATGTTATTATATTGCAAAAACCCATC AACCTTGAAAAAAAGTAGAAACGTTTTATTTAATTCT ATCAATACATCATAAAATACGAACGATCCCCGTCCAA GTTATGAGTTCGAATCTAGAGAATTC

pCR313	1238(+133..-100)LinkerD (rip1238-ARS1238)	AAGCTTAGATCTCGTACGAATAATTCCAATTTTTATA ATTAAGGGATCCGCATGTAATTATTCAAAATATTGTG TAATCGTCTATTTGAATTCGTTTTCACGGCAGCATATT TGAAAAAAAAATTAATTTTTACATCTAAACATAAAAA ACCTAATCGTTTTCTTTCCATGCTGTTATAGAGACATT TATTAGTACGAATATTTTCCTTTCTTTACGAGCACTAT AGACAGTAATTTATATAACTAAGAAATTCGAATCTAG AGAATTC
pCR321	1238(+69..-100); (ARS1238)	AAGCTTAGATCTCGTACGTATTTGAATTCGTTTTCACG GCAGCATATTTGAAAAAAAAATTAATTTTTACATCTA AACATAAAAAACCTAATCGTTTTCTTTCCATGCTGTT ATAGAGACATTTATTAGTACGAATATTTTCCTTTCTT TACGAGCACTATAGACAGTAATTTATATAACTAAGAA ATTCGAATCTAGAGAATTC
pCR339	317(+76..-106); (ARS317)	AAGCTTAGATCTCGTACGCTATCAGTGTTTTCAATTTT TTATTAAACAATGTTTGATTTTTTAAATCGCAATTTA ATACCTAAATATAAAAAATGTTATTATATTGCAAAAA CCCATCAACCTTGAAAAAAAGTAGAAACGTTTTATTT AATTCTATCAATACATCATAAAATACGAACGATCCCC GTCCAAGTTATGAGTTCGAATCTAGAGAATTC
* First and last 12 nucleotides of each sequence are flanking restriction sites for cloning purposes: XbaI/SpeI-BsiWI and BstBI-XbaI, respectively.		

Table S2. Description of all yeast strains used in this publication. Yeast strain numbers are presented with: 1) the genotype for each strain, and 2) the plasmid used to integrate the re-replication cassette at ChrIV_567kb. Sequence of the *RIP*-origin region of the re-replication cassette plasmid can be found in Table S1.

Strain	Genotype	Derivation
YJL310	leu2, ura3-52, trp1-289, bar1::LEU2	Described in [77]
YJL3155	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	Described in [2]
YJL3158	ORC2-(NotI,SgrAI), ORC6, leu2, ura3-52, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	derived as described for YJL3155 but resulting with ORC6 instead of orc6(S116A)
YJL3758	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	Described in [2]
YJL3759	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	isogenic sister isolate of YJL3758
YJL6893	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir1Δ::kanMX	sir1Δ::kanMX (Table S3) into YJL3758
YJL6894	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir1Δ::kanMX	sir1Δ::kanMX (Table S3) into YJL3758
YJL6896	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir2Δ::kanMX	sir2Δ::kanMX (Table S3) into YJL3758
YJL6897	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir2Δ::kanMX	sir2Δ::kanMX (Table S3) into YJL3758
YJL6899	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	sir3Δ::kanMX (Table S3) into YJL3758

	sir3Δ::kanMX	
YJL6900	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir3Δ::kanMX	sir3Δ::kanMX (Table S3) into YJL3758
YJL6902	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir4Δ::kanMX	sir4Δ::kanMX (Table S3) into YJL3758
YJL6903	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, sir4Δ::kanMX	sir4Δ::kanMX (Table S3) into YJL3758
YJL6905	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX	ars317Δ::natMX into YJL3758
YJL7700	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+300..-106)}	pBJL2933/SacI-SalI into YJL6905
YJL7701	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+300..-106)}	pBJL2933/SacI-SalI into YJL6905
YJL7717	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+300..+33)}	pBJL2935/SacI-SalI into YJL6905
YJL8256	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+294..-13)}	pCR013/SacI-NotI into YJL6905
YJL8257	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+294..-13)}	pCR013/SacI-NotI into YJL6905
YJL8386	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR016/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+251..-106)}	
YJL8387	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+251..-106)}	pCR016/SacI-NotI into YJL6905
YJL8392	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+200..-106)}	pCR019/SacI-NotI into YJL6905
YJL8393	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+200..-106)}	pCR019/SacI-NotI into YJL6905
YJL8398	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	pCR022/SacI-NotI into YJL6905
YJL8399	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	pCR022/SacI-NotI into YJL6905
YJL8401	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+92..-106)}	pCR024/SacI-NotI into YJL6905
YJL8402	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+92..-106)}	pCR024/SacI-NotI into YJL6905
YJL8404	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+52..-106)}	pCR026/SacI-NotI into YJL6905
YJL8405	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR026/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+52..-106)}	
YJL8526	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+167..-106)HMRE-A}	pCR041/SacI-NotI into YJL6905
YJL8538	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+167..-106)HMRE-E}	pCR045/SacI-NotI into YJL6905
YJL8541	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+167..-106)HMRE-A/E}	pCR047/SacI-NotI into YJL6905
YJL8553	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+143..-106)}	pCR051/SacI-NotI into YJL6905
YJL8556	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+133..-106)}	pCR052/SacI-NotI into YJL6905
YJL8559	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+123..-106)}	pCR053/SacI-NotI into YJL6905
YJL8562	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+113..-106)}	pCR054/SacI-NotI into YJL6905
YJL8565	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+103..-106)}	pCR055/SacI-NotI into YJL6905
YJL8644	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR061/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker1}	
YJL8647	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker3}	pCR062/SacI-NotI into YJL6905
YJL8650	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker5}	pCR063/SacI-NotI into YJL6905
YJL8653	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker7}	pCR064/SacI-NotI into YJL6905
YJL8656	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9}	pCR065/SacI-NotI into YJL6905
YJL8659	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker15}	pCR066/SacI-NotI into YJL6905
YJL8662	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker19}	pCR067/SacI-NotI into YJL6905
YJL8665	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker23}	pCR068/SacI-NotI into YJL6905
YJL8668	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker25}	pCR069/SacI-NotI into YJL6905
YJL8671	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR070/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker27}	
YJL8677	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker11}	pCR072/SacI-NotI into YJL6905
YJL8680	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker13}	pCR073/SacI-NotI into YJL6905
YJL8683	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker17}	pCR074/SacI-NotI into YJL6905
YJL8686	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker21}	pCR075/SacI-NotI into YJL6905
YJL8689	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker29}	pCR076/SacI-NotI into YJL6905
YJL8692	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker31}	pCR077/SacI-NotI into YJL6905
YJL8695	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker33}	pCR078/SacI-NotI into YJL6905
YJL8701	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, fkh2Δ::TRP1, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	fkh2Δ::TRP1 (Table S3) into YJL8398
YJL8702	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	fkh2Δ::TRP1 (Table S3) into YJL8398

	ars317Δ::natMX, fkh2Δ::TRP1, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	
YJL8745	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, fkh1Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	fkh1Δ::hphMX (Table S3) into YJL8398
YJL8746	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, fkh1Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	fkh1Δ::hphMX (Table S3) into YJL8398
YJL8749	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, fkh1Δ::hphMX, fkh2Δ::TRP1, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	fkh1Δ::hphMX (Table S3) into YJL8701
YJL8750	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, fkh1Δ::hphMX, fkh2Δ::TRP1, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)}	fkh1Δ::hphMX (Table S3) into YJL8701
YJL8771	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,11,15}	pCR088/SacI-NotI into YJL6905
YJL8773	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,15}	pCR089/SacI-NotI into YJL6905
YJL8775	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker11,15}	pCR090/SacI-NotI into YJL6905
YJL8777	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,11}	pCR091/SacI-NotI into YJL6905
YJL8779	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR092/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)58bpRIP-OBS}	
YJL8781	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)63bpRIP-OBS}	pCR093/SacI-NotI into YJL6905
YJL8783	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)73bpRIP-OBS}	pCR094/SacI-NotI into YJL6905
YJL8785	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)153bpRIP-OBS}	pCR095/SacI-NotI into YJL6905
YJL8826	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker13,17}	pCR096/SacI-NotI into YJL6905
YJL8828	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker21,23}	pCR097/SacI-NotI into YJL6905
YJL8830	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,17}	pCR098/SacI-NotI into YJL6905
YJL8832	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker11,17}	pCR099/SacI-NotI into YJL6905
YJL8834	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker15,17}	pCR100/SacI-NotI into YJL6905
YJL8836	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR101/SacI-NotI into YJL6905

	ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker9,13}	
YJL8838	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)L17-L31replacement}	pCR102/SacI-NotI into YJL6905
YJL8908	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)21bpRIP-OBS}	pCR107/SacI-NotI into YJL6905
YJL8910	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)37bpRIP-OBS}	pCR108/SacI-NotI into YJL6905
YJL8912	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)45bpRIP-OBS}	pCR109/SacI-NotI into YJL6905
YJL8923	ORC2-(NotI,SgrAI), ORC6, leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	Derived as described for YJL3758 but from YJL3158
YJL8924	ORC2-(NotI,SgrAI), ORC6, leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1	Derived as described for YJL3758 but from YJL3158
YJL8973	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker2}	pCR113/SacI-NotI into YJL6905
YJL8975	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker4}	pCR114/SacI-NotI into YJL6905
YJL8977	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker8}	pCR115/SacI-NotI into YJL6905

YJL8979	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker10}	pCR116/SacI-NotI into YJL6905
YJL8981	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker12}	pCR117/SacI-NotI into YJL6905
YJL8983	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker14}	pCR118/SacI-SalI into YJL6905
YJL8985	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker18}	pCR119/SacI-SalI into YJL6905
YJL8987	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker20}	pCR120/SacI-SalI into YJL6905
YJL8989	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker22}	pCR121/SacI-SalI into YJL6905
YJL8991	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker26}	pCR122/SacI-SalI into YJL6905
YJL8993	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker28}	pCR123/SacI-SalI into YJL6905
YJL8995	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker32}	pCR125/SacI-SalI into YJL6905

YJL9016	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker6}	pCR126/SacI-SalI into YJL6905
YJL9018	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker16}	pCR129/SacI-SalI into YJL6905
YJL9020	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker24}	pCR130/SacI-SalI into YJL6905
YJL9022	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker30}	pCR124/SacI-SalI into YJL6905
YJL9078	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS1021(+72..-253)}	pCR156/SacI-SalI into YJL6905
YJL9080	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS301(+78..-247)}	pCR157/SacI-SalI into YJL6905
YJL9082	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS305(+76..-249)}	pCR159/SacI-SalI into YJL6905
YJL9084	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS306(+82..-245)}	pCR160/SacI-SalI into YJL6905
YJL9086	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS702(+77..-247)}	pCR162/SacI-SalI into YJL6905

YJL9088	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS209(+84..-241)}	pCR164/SacI-SalI into YJL6905
YJL9152	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars1238Δ::{tACT1-hphMX}	ars1238Δ::{tACT1-hphMX} (Table S3) into YJL6905
YJL9173	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX,	ars418Δ::hphMX (Table S3) into YJL6905
YJL9175	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)Linker17}	ars418Δ::hphMX (Table S3) into YJL8683
YJL9177	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS1021(+72..-253)}	ars418Δ::hphMX (Table S3) into YJL9078
YJL9179	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+153..+87)-ARS301(+78..-247)}	ars418Δ::hphMX (Table S3) into YJL9080
YJL9221	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS1021(+72..-253)}	pCR191/SacI-NotI into YJL6905
YJL9225	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS301(+78..-247)}	pCR193/SacI-NotI into YJL6905
YJL9229	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289,	pCR191/SacI-NotI into

	ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS1021(+72..-253)}	YJL9173
YJL9233	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,rip317(+153..+87)Linker6Xho-ARS301(+78..-247)}	pCR193/SacI-NotI into YJL9173
YJL9248	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153…-106)Linker6Xho}	pCR187/SacI-NotI into YJL9173
YJL9566	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)}	pCR217/SacI-NotI into YJL6905
YJL9567	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)}	pCR217/SacI-NotI into YJL6905
YJL9707	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+83..-100)}	pCR234/SacI-NotI into YJL9173
YJL9708	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+83..-100)}	pCR234/SacI-NotI into YJL9173
YJL9713	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX,ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)L1-L3,L17-L31replacement}	pCR238/SacI-NotI into YJL9173
YJL9999	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-	pCR274/SacI-

	52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP1238(+133..+70)-ARS1021(+72..-253)}	SalI into YJL9173
YJL10000	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP1238(+133..+70)-ARS1021(+72..-253)}	pCR274/SacI-SalI into YJL9173
YJL10001	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP1238(+133..+70)-ARS301(+78..-247)}	pCR275/SacI-SalI into YJL9173
YJL10002	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP1238(+133..+70)-ARS301(+78..-247)}	pCR275/SacI-SalI into YJL9173
YJL10158	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)53bpRIP-OBS}	pCR289/SacI-SalI into YJL9173
YJL10159	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)53bpRIP-OBS}	pCR289/SacI-SalI into YJL9173
YJL10160	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+150..+87)-ARS1238(+69..-100)53bpRIP-OBS}	pCR290/SacI-SalI into YJL9173
YJL10161	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1,	pCR290/SacI-SalI into YJL9173

	ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,RIP317(+150..+87)-ARS1238(+69..-100)53bpRIP-OBS}	
YJL10271	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerA}	pCR295/SacI-SalI into YJL9173
YJL10272	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerA}	pCR295/SacI-SalI into YJL9173
YJL10273	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerB}	pCR296/SacI-SalI into YJL9173
YJL10274	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerB}	pCR296/SacI-SalI into YJL9173
YJL10275	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerC}	pCR297/SacI-SalI into YJL9173
YJL10276	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerC}	pCR297/SacI-SalI into YJL9173
YJL10277	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-	pCR298/SacI-SalI into YJL9173

	100)LinkerD}	
YJL10278	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerD}	pCR298/SacI-SalI into YJL9173
YJL10279	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerE}	pCR299/SacI-SalI into YJL9173
YJL10280	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerE}	pCR299/SacI-SalI into YJL9173
YJL10281	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerF}	pCR300/SacI-SalI into YJL9173
YJL10282	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerF}	pCR300/SacI-SalI into YJL9173
YJL10283	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerG}	pCR301/SacI-SalI into YJL9173
YJL10284	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerG}	pCR301/SacI-SalI into YJL9173
YJL10285	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289,	pCR302/SacI-SalI into YJL9173

	ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerH}	
YJL10286	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)LinkerH}	pCR302/SacI-SalI into YJL9173
YJL10287	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)153bpRIP-OBS}	pCR303/SacI-SalI into YJL9173
YJL10288	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)153bpRIP-OBS}	pCR303/SacI-SalI into YJL9173
YJL10289	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)73bpRIP-OBS}	pCR304/SacI-SalI into YJL9173
YJL10290	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)73bpRIP-OBS}	pCR304/SacI-SalI into YJL9173
YJL10291	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)63bpRIP-OBS}	pCR305/SacI-SalI into YJL9173
YJL10292	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-	pCR305/SacI-SalI into YJL9173

	100)63bpRIP-OBS}	
YJL10293	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)58bpRIP-OBS}	pCR306/SacI-SalI into YJL9173
YJL10294	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)58bpRIP-OBS}	pCR306/SacI-SalI into YJL9173
YJL10295	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)45bpRIP-OBS}	pCR307/SacI-SalI into YJL9173
YJL10296	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)45bpRIP-OBS}	pCR307/SacI-SalI into YJL9173
YJL10297	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)29bpRIP-OBS}	pCR308/SacI-SalI into YJL9173
YJL10298	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)29bpRIP-OBS}	pCR308/SacI-SalI into YJL9173
YJL10299	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)21bpRIP-OBS}	pCR309/SacI-SalI into YJL9173
YJL10300	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289,	pCR309/SacI-SalI into YJL9173

	ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ars418Δ::hphMX, ChromIV_567kb::{kanMX6,ade3-2p,1238(+133..-100)21bpRIP-OBS}	
YJL10319	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DReverse}	pCR322/SacI-SalI into YJL6905
YJL10320	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DReverse}	pCR322/SacI-SalI into YJL6905
YJL10321	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DTransversion}	pCR323/SacI-SalI into YJL6905
YJL10322	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DTransversion}	pCR323/SacI-SalI into YJL6905
YJL10323	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DScramble}	pCR324/SacI-SalI into YJL6905
YJL10324	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C-DScramble}	pCR324/SacI-SalI into YJL6905
YJL10325	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)CTransversion}	pCR325/SacI-SalI into YJL6905
YJL10326	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)CTransversion}	pCR325/SacI-SalI into YJL6905
YJL10327	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-	pCR326/SacI-

	52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)DTransversion}	SalI into YJL6905
YJL10328	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)DTransversion}	pCR326/SacI-SalI into YJL6905
YJL10329	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C1Transversion}	pCR327/SacI-SalI into YJL6905
YJL10330	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C1Transversion}	pCR327/SacI-SalI into YJL6905
YJL10331	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C2Transversion}	pCR328/SacI-SalI into YJL6905
YJL10332	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)C2Transversion}	pCR328/SacI-SalI into YJL6905
YJL10333	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)D1Transversion}	pCR329/SacI-SalI into YJL6905
YJL10334	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)D1Transversion}	pCR329/SacI-SalI into YJL6905
YJL10335	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)D2Transversion}	pCR330/SacI-SalI into YJL6905
YJL10336	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-	pCR330/SacI-

	52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+153..-106)D2Transversion}	SalI into YJL6905
YJL10444	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+76..-106)}	pCR340/SacI-SalI into YJL6905
YJL10445	ORC2-(NotI,SgrAI), orc6(S116A), leu2, ura3-52::{pGAL-delntcdc6-cdk2A(6,8),URA3}, trp1-289, ade2, ade3, MCM7-2NLS, bar1::LEU2, CAN1, ars317Δ::natMX, ChromIV_567kb::{kanMX6,ade3-2p,317(+76..-106)}	pCR340/SacI-SalI into YJL6905

Table S3. Primers used in strain generation. Transcription factors and origin DNA were disrupted by one-step gene replacement. The primers, targets, sequences, and template DNA used for PCR amplification of these disruption fragments are listed along with the marker used (in parentheses).

Transcription Factors		
Oligo	**Gene**	**Sequence (5' > 3') - upper case hybridizes to template**
OJL1450	Sir1	GCGAGCGAGTCAGCAAGCAG
OJL1451	Sir1	GGATGAGCTAGTTCGCCAAC
OJL1340	Sir2	CTTTTCCAAGCTACATCTAGCACTC
OJL1341	Sir2	ACCTGCCCTTCTTACATTAAGCTAT
OJL1351	Sir3	GCAATGACTGATACACAAAGAAATG
OJL1352	Sir3	TTGTGACATCTAGTCATTTTGGGTA
OJL1741	Sir4	ATATTTTTATCGTTGAGAACGAACG
OJL1742	Sir4	TGTGATGTTGATATCGGTACTATGG
OJL2252	Fkh2 (outer)	tgcagcttagccatttctcattcatttctttagtcttagtgattcaccttg
OJL2263	Fkh2 (inner)	tagtcttagtgattcaccttgtttcttgtcGAGCAGATTGTACTGAGAG TGC
OJL2254	Fkh2 (outer)	aaatattggtgtgctccctccgtttcctttattgaaactttatcaatgcg
OJL2264	Fkh2 (inner)	ctttattgaaactttatcaatgcgcaagaaCTGTGCGGTATTTCACAC CG
OJL2240	Fkh1 (outer)	cttaacgggtctttgttctttattgtttaataatacatatgggttcgacg
OJL2241	Fkh1 (inner)	taatacatatgggttcgacgacgctgaattCATCGATGAATTCGAGCT CG
OJL2242	Fkh1 (inner)	GAAAGGCTTGGAGAGACACAGTAATAATAACGGATCCCCG GGTTAATTAAGG
OJL2243	Fkh1 (outer)	agtgtgtaaattgtgcgttcaattagcaaagaaaggcttggagagacacag
Origins		
Oligo	**Gene**	**Sequence (5'-3') - upper case hybridizes to template**
OJL1639	ARS317	attaaacaatgtttgatttttaaatcgcaatttaataccCGGATCCCCGGG TTAATTAA
OJL1640	ARS317	atttttatggaagattaagctcataacttggacggggatcCATCGATGAAT TCGAGCTCG
OJL2767	ARS418	aatttttaggatttttcttagcatttgcatatattttcatagacacagtacttacatt taCGGATCCCCGGGTTAATTAA
OJL2768	ARS418	aagaaaagcatttaacaattgaacacctctatatcaacgaagaatattactttgt ctctaCATCGATGAATTCGAGCTCG
OJL2497	ARS1238	ccttaattaacccggggatccgTATGATACACGGTCCAATGG
OJL2498	ARS1238	ccattggaccgtgtatcataCGGATCCCCGGGTTAATTAAGG

OJL2499	ARS1238	gttagtatccacagaatgagagaccctgagagacagaataattccaatttttat aattaaTCTCTGCTTTTGTGCGCG
OJL2500	ARS1238	ccagaaatacctgttccagagattcatgatttccctaagagtcatattgttgattt ttgaCATCGATGAATTCGAGCTCG
* ACT1 terminator (tACT1) from OJL2497/OJL2499 PCR was fused to hphMX from OJL2498/OJL2500 PCR by fusion PCR using OJL2499/OJL2500 to create ars1238Δ::{tACT1-hphMX}		

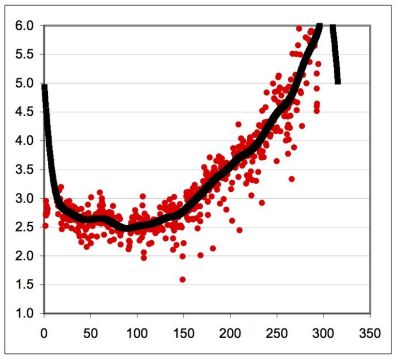
Table S4. Primers used in qPCR analysis. Primer names, targets, and sequences are listed.

Oligo	Gene	Sequence (5' > 3')
OJL3395	ARS305	GGCCACAGCAAGACCG
OJL3396	ARS305	ATCAAACTCCGTTTTTAGCCC
OJL3090	ARS317	CAGTGTTTTCAATTTTTTATTAAACAATGTTTG
OJL3091	ARS317	AATTAAATAAAACGTTTCTACTTTTTTTCAAGG
OJL3399	ARS418	TGCATATATTTTCATAGACACAGTACTTAC
OJL3400	ARS418	GTAAAATGTGTACGATCTCTATATGGG
OJL3401	ARS1238	ATCCACAGAATGAGAGACCC
OJL3402	ARS1238	GTCATATTGTTGATTTTTGAGCATACC
OJL3403	ARS1420	TCTTGAGGTTCTATAGTTTCAAAATAGC
OJL3404	ARS1420	ACACACTTGTCTTCTTAAAAGAGG
OJL3405	ACT1	ATTCCAAACCCAAAACAGAAGG
OJL3406	ACT1	TGGCCGGTAGAGATTTGAC
OJL3407	ADH1	TGTCTGTCACACTGACTTGC
OJL3408	ADH1	AGACAAGTCAGCGTGAGG
OJL3409	SLH1	GTATGAGCAGTGAATTTGACGG
OJL3410	SLH1	TTCTATTGACACCTATTAAAAACAATGC

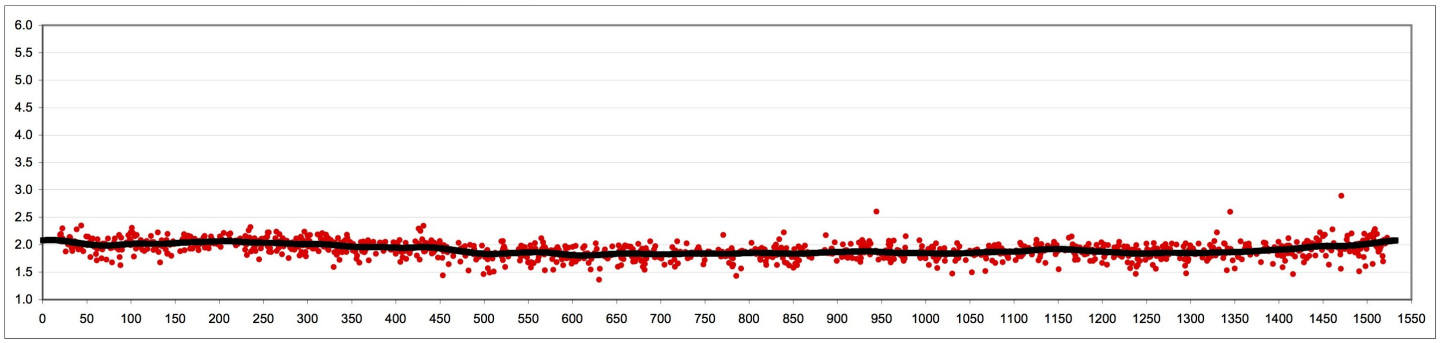
Document S1: Raw normalized data (red dots) and smoothed line (black line) used to generate composite profiles or percent re-replication efficiency for all the other figures of this manuscript.

Figure 1A

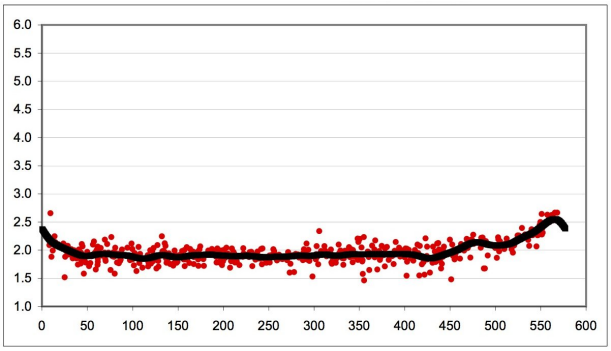
YJL3758 Chr_III



YJL3758 Chr_IV



YJL3758 Chr_V



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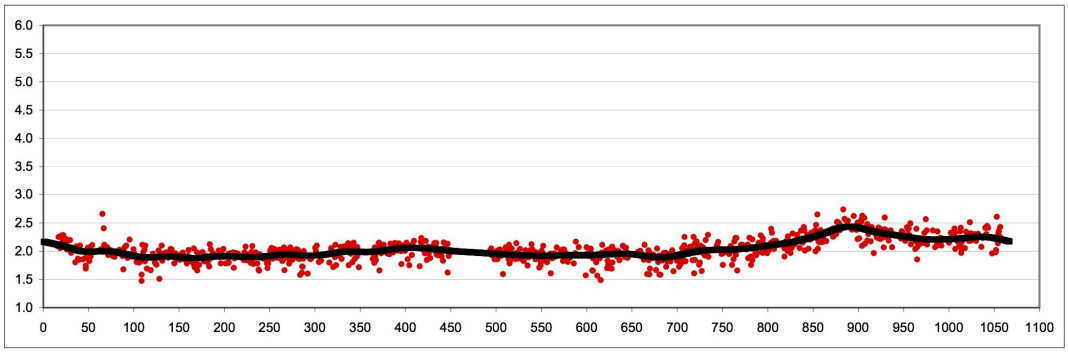

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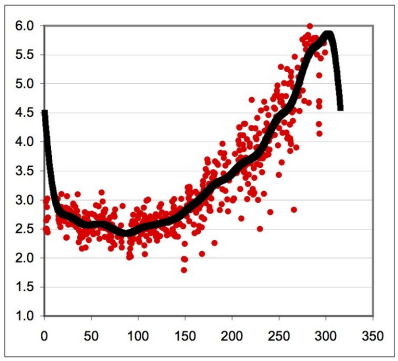

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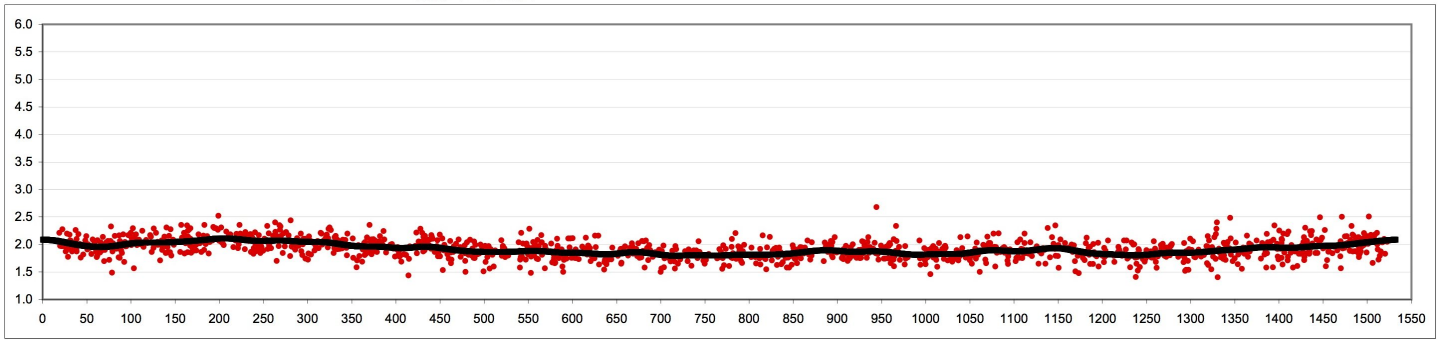

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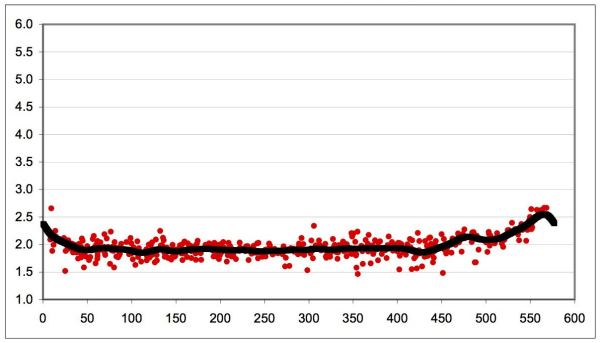

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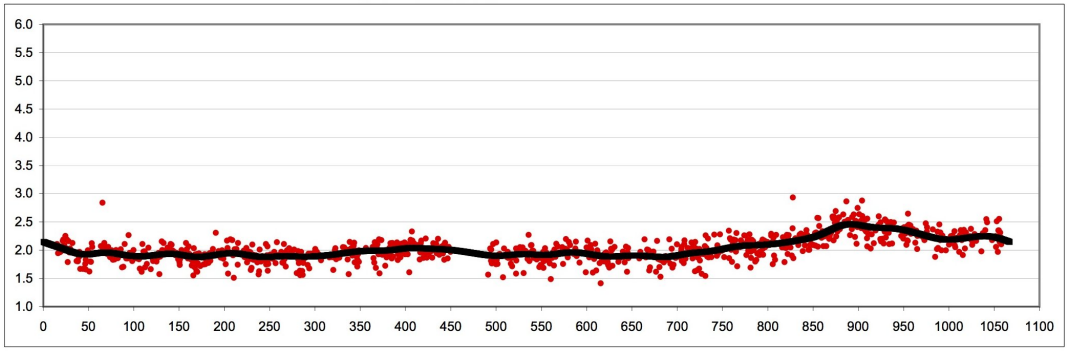
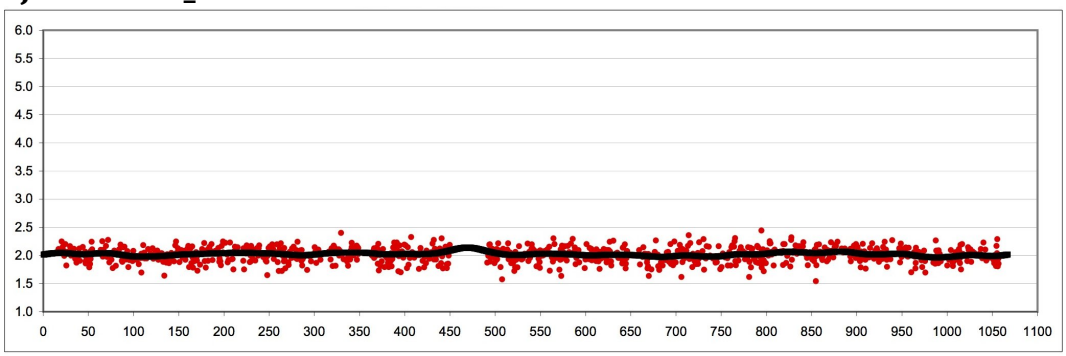


Figure 1B
YJL9152 Chr_XII



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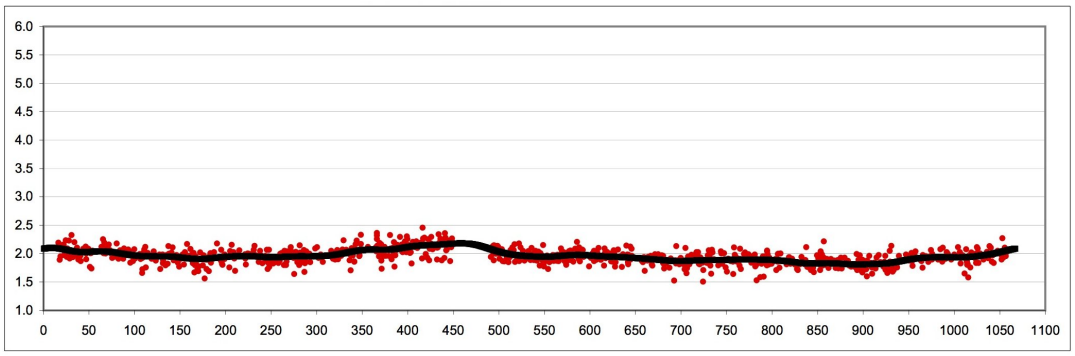
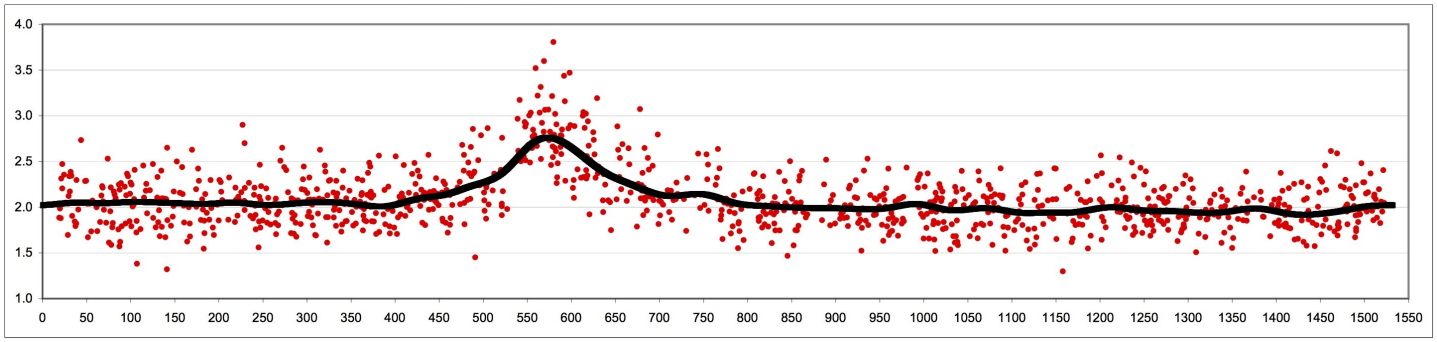


Figure 2A
YJL7700



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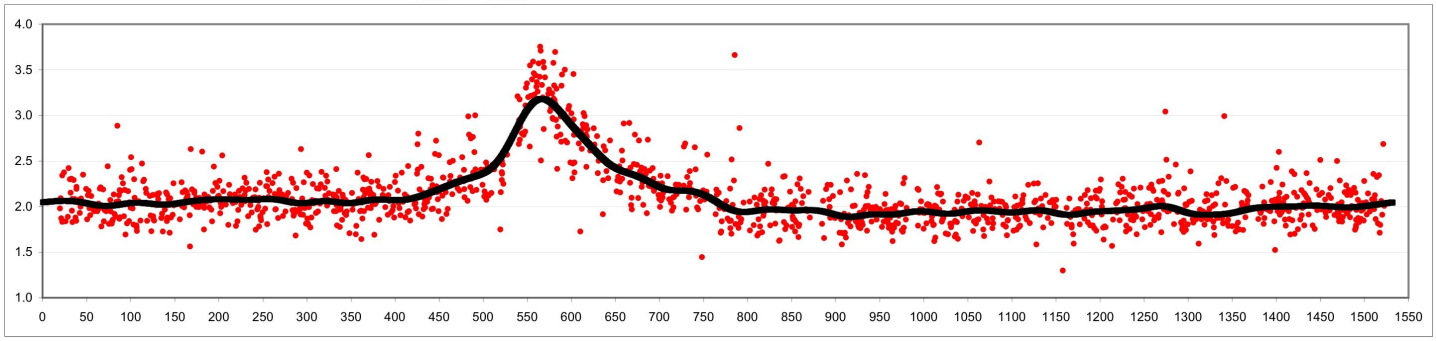

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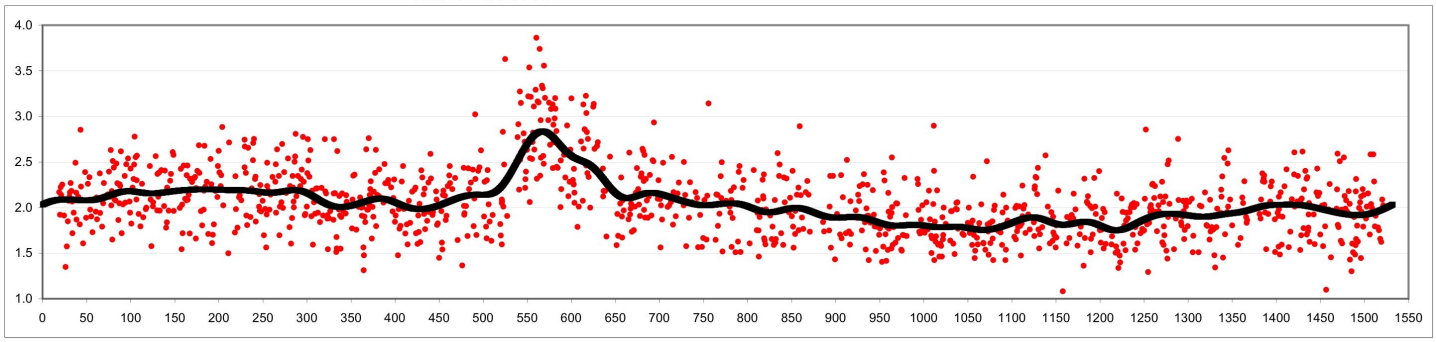

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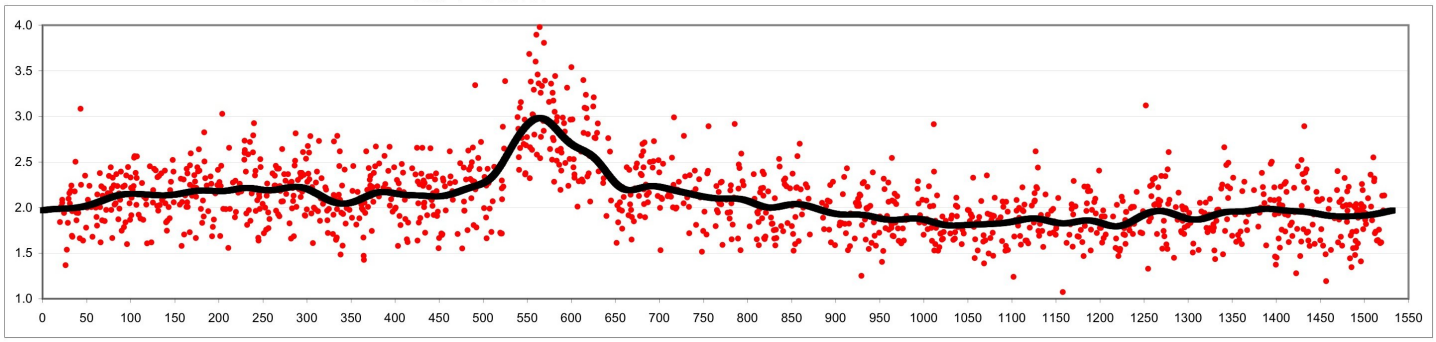

Figure 2B

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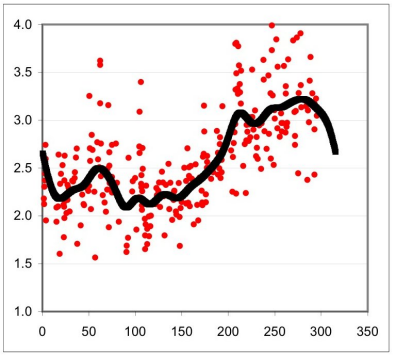

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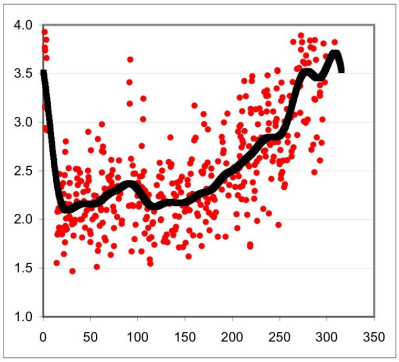

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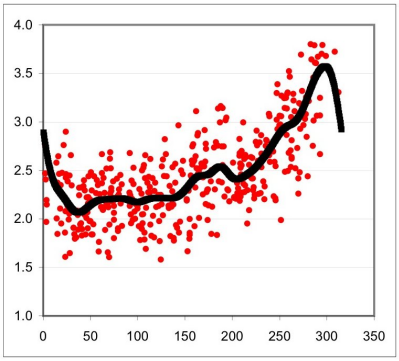

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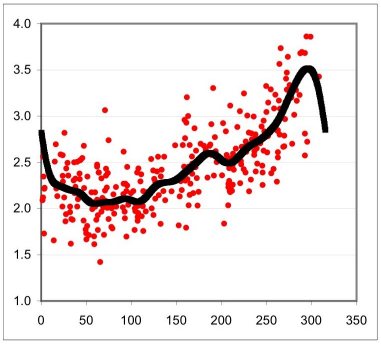

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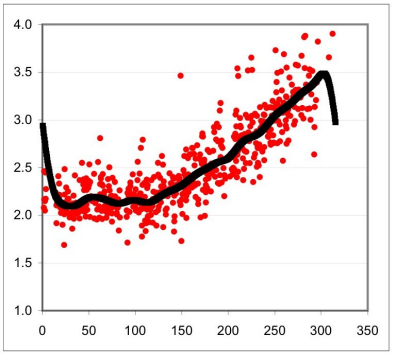

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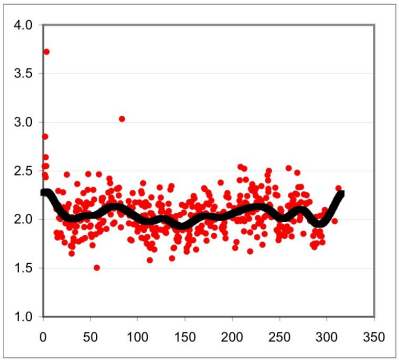

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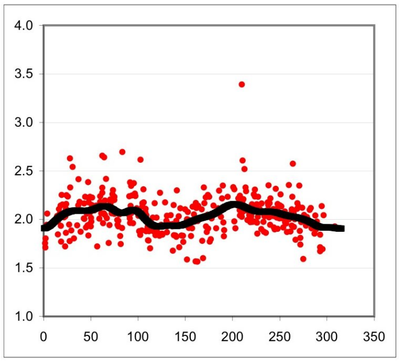

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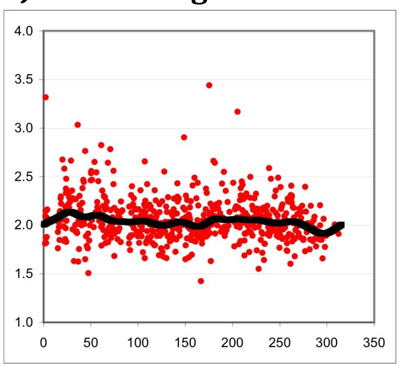

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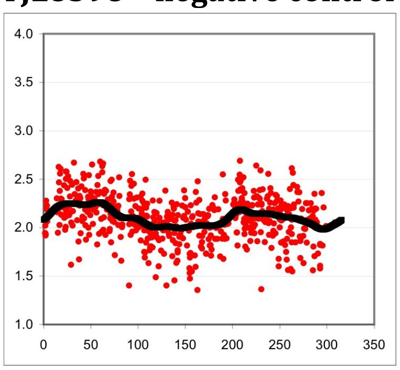

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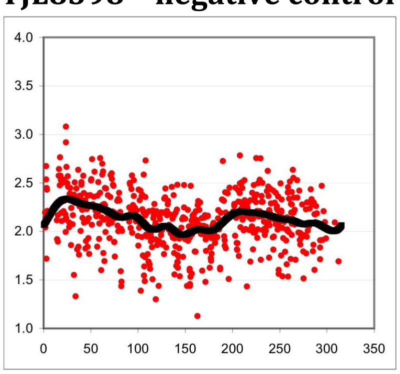

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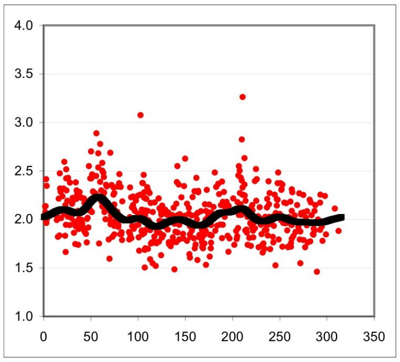

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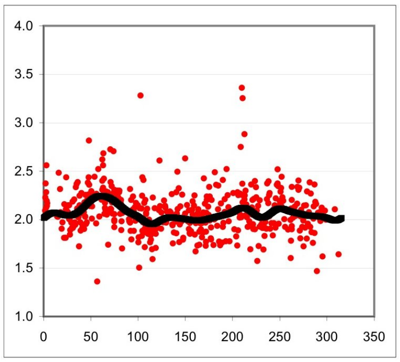

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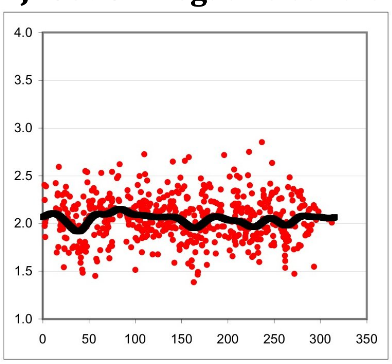

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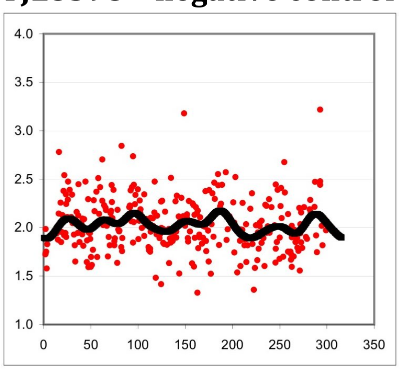

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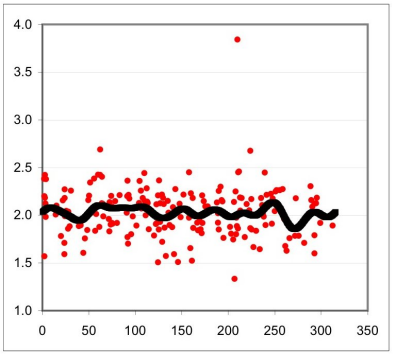

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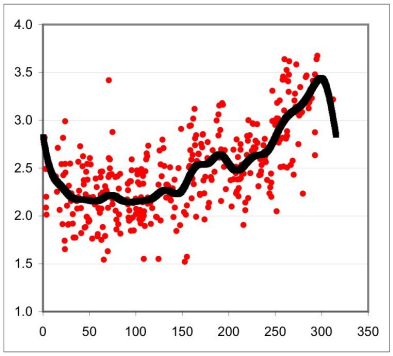

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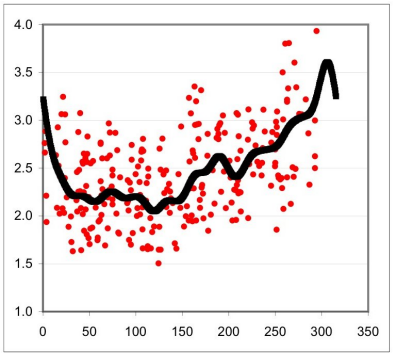

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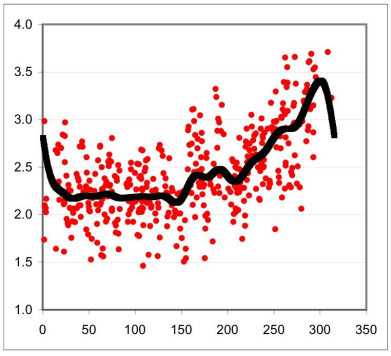

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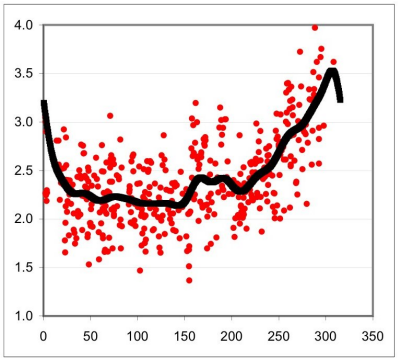

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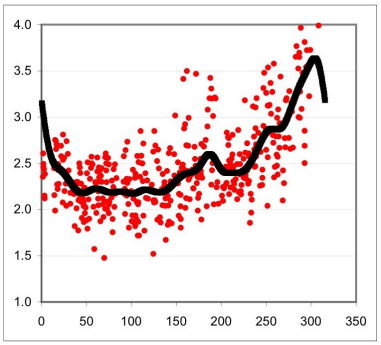

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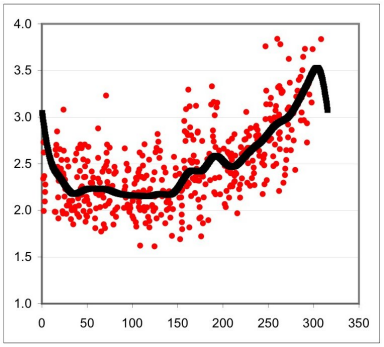

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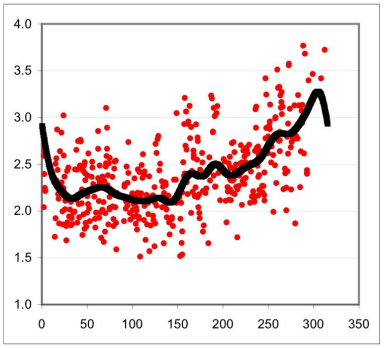

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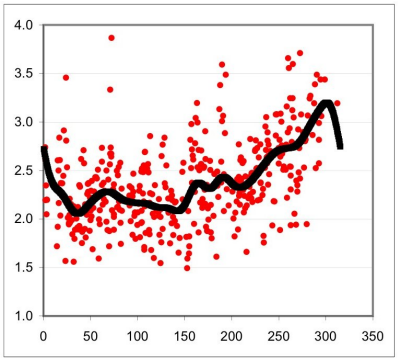
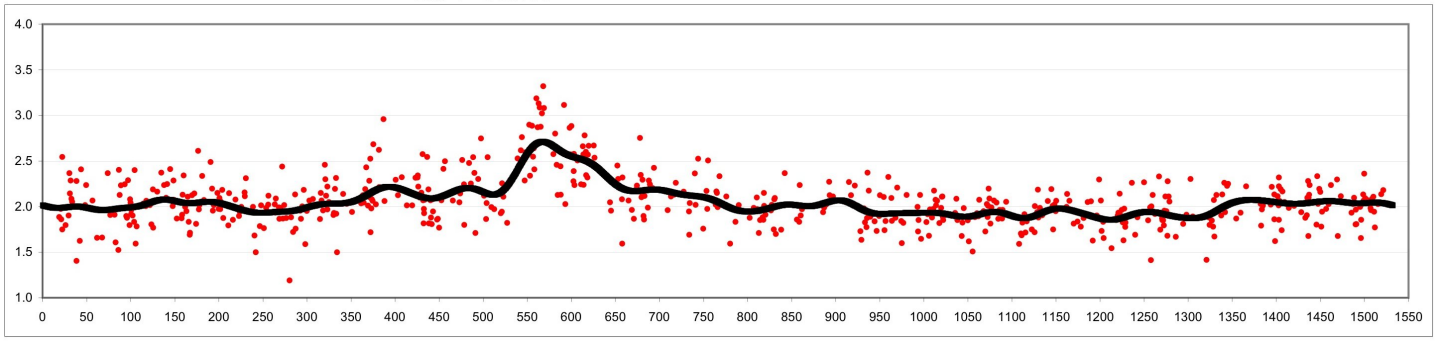


Figure 2C
YJL8256



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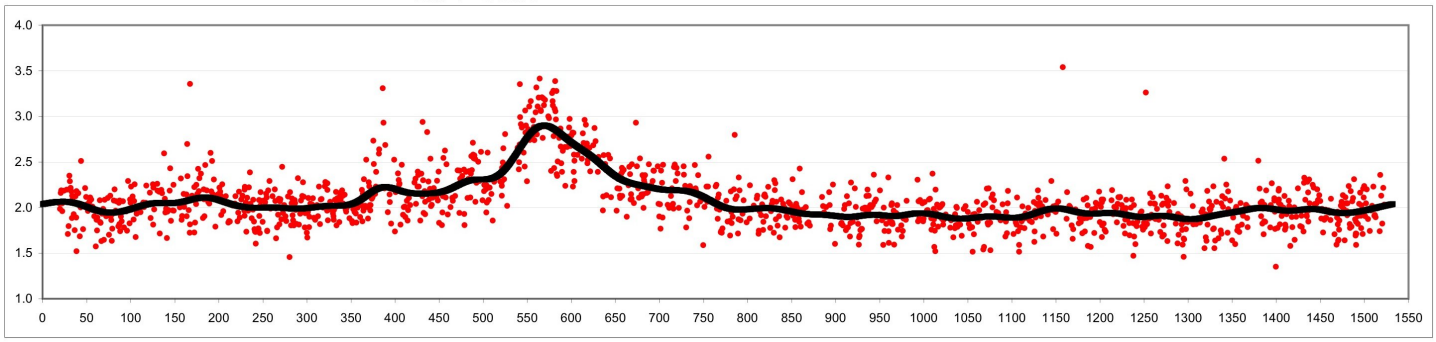
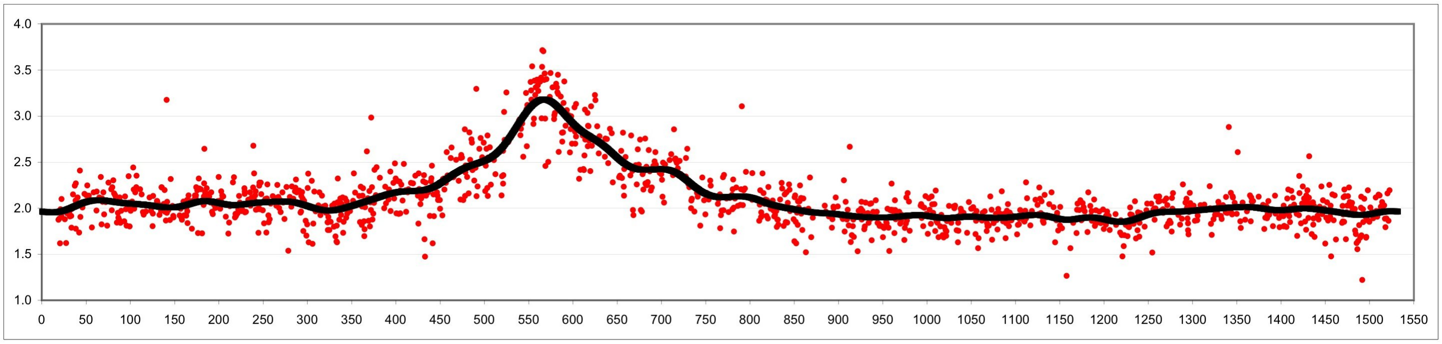


Figure 2D
YJL8398 – Positive Control



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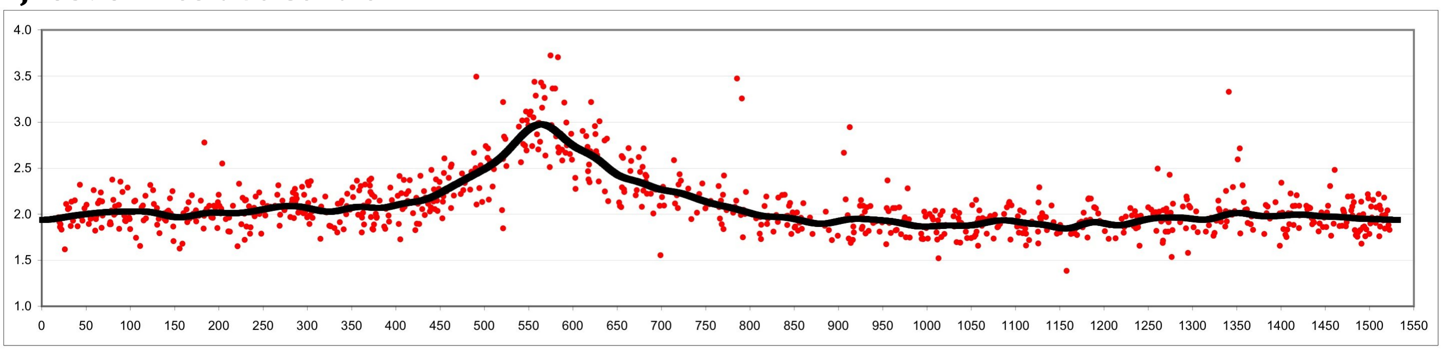

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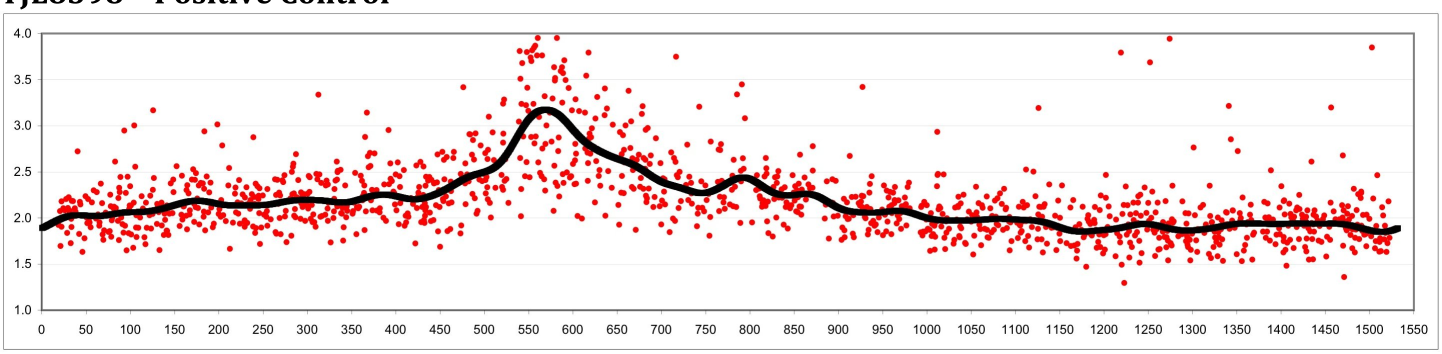

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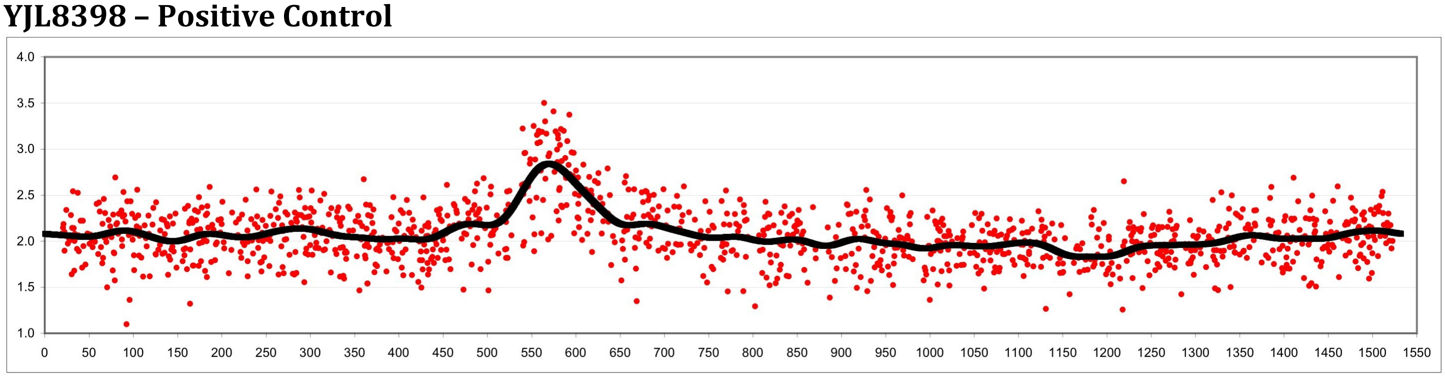

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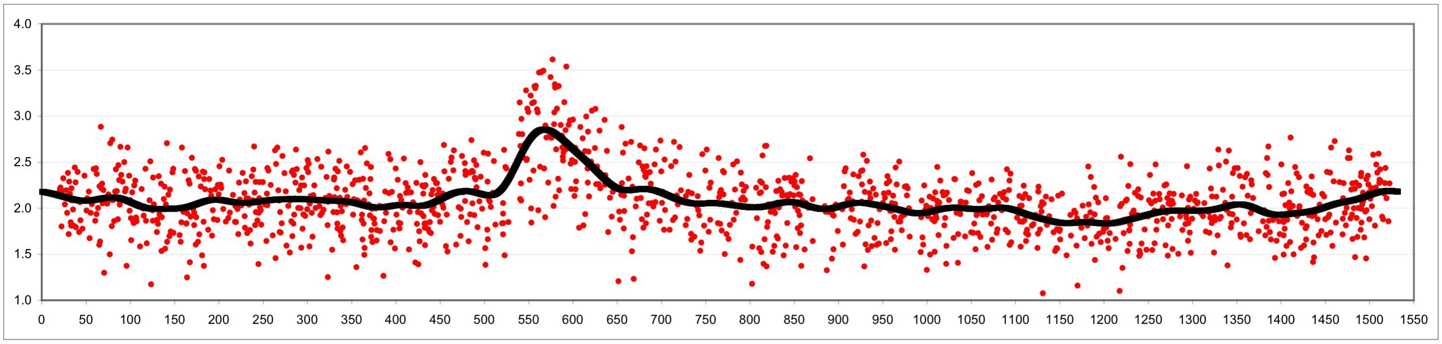

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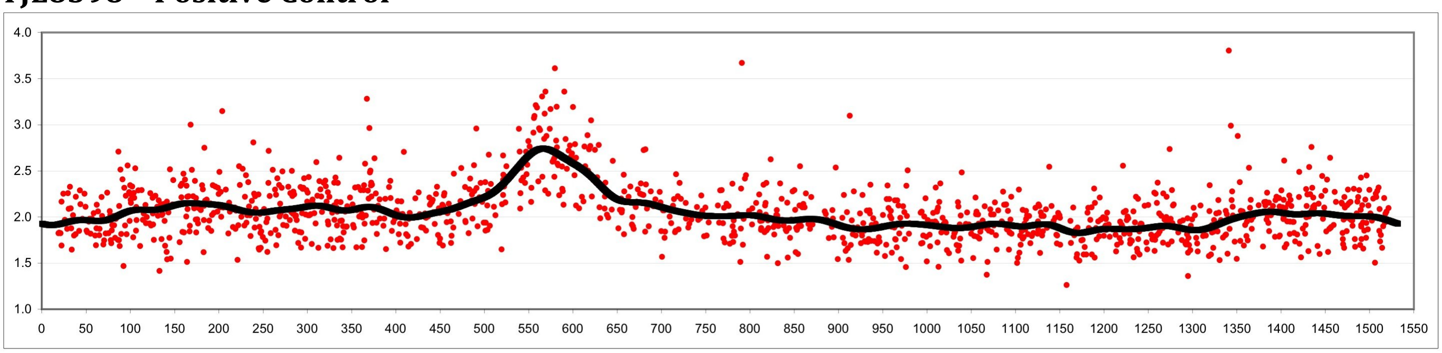

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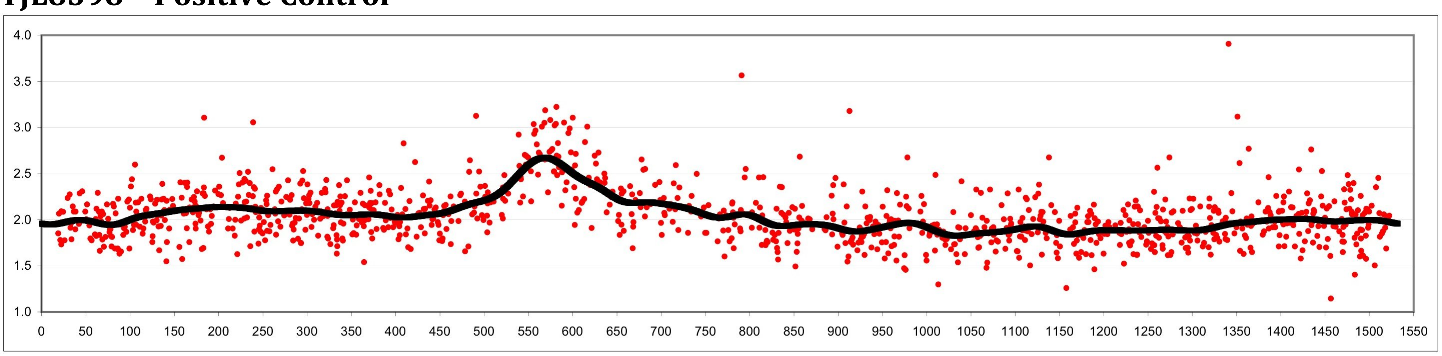

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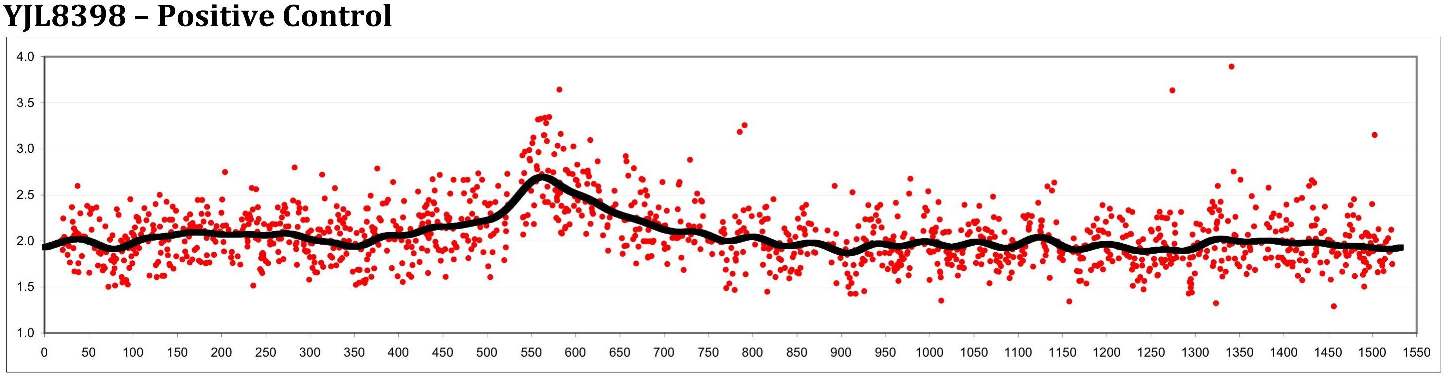
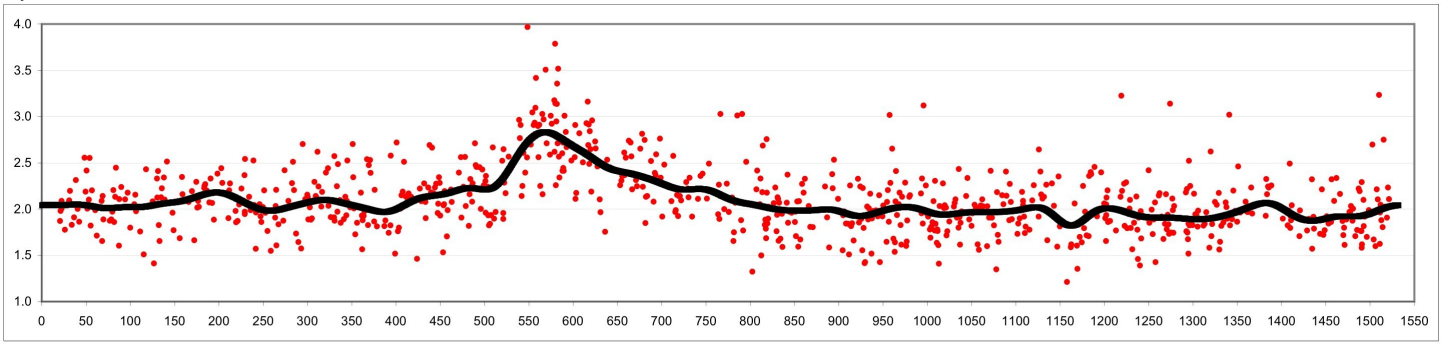
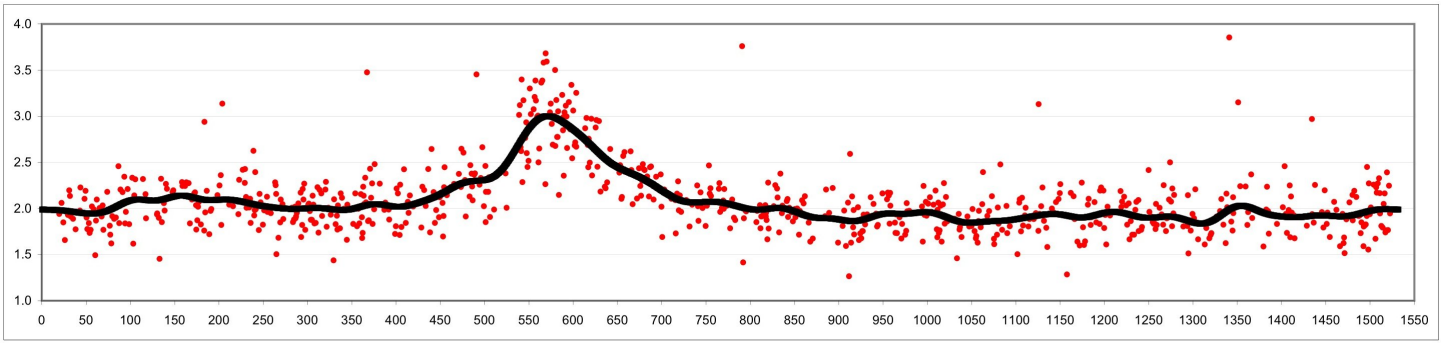


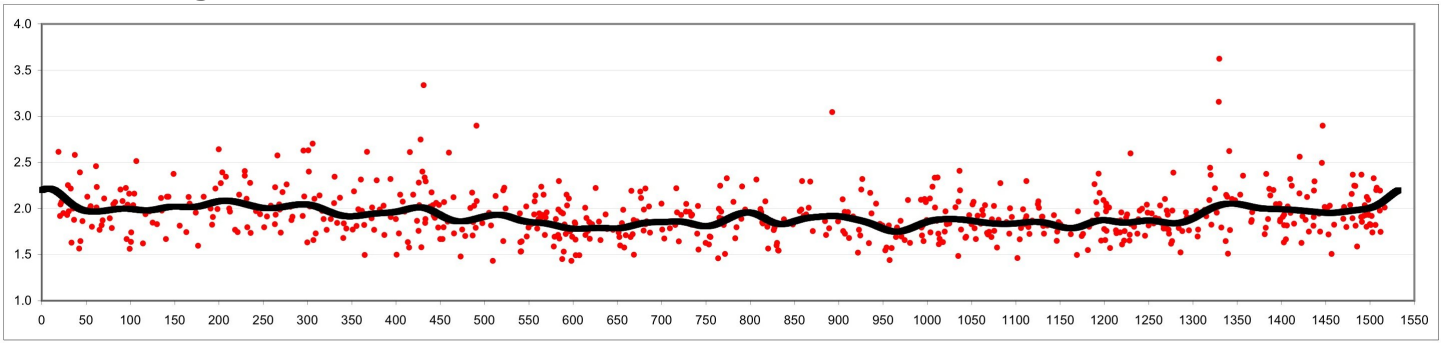
YJL8398 – Positive Control



YJL8398 – Positive Control



YJL3758 – Negative Control



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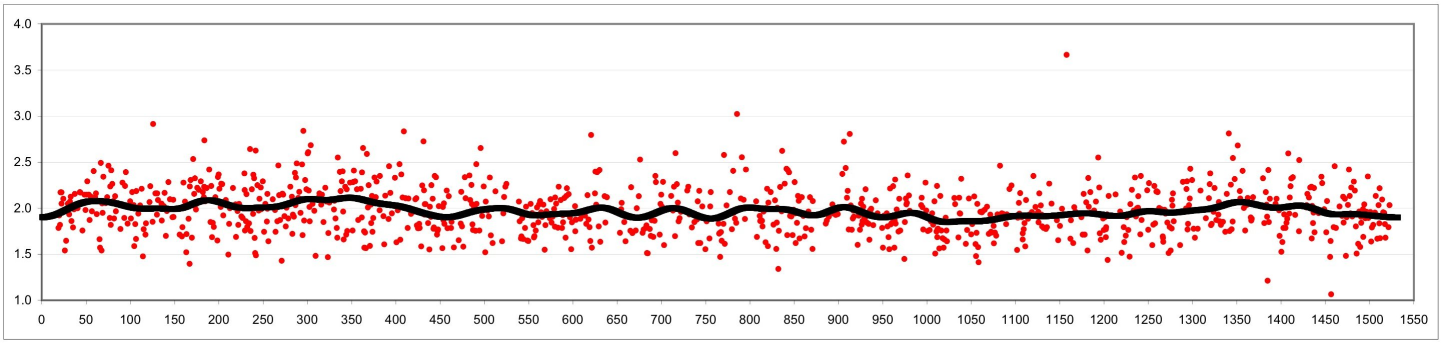

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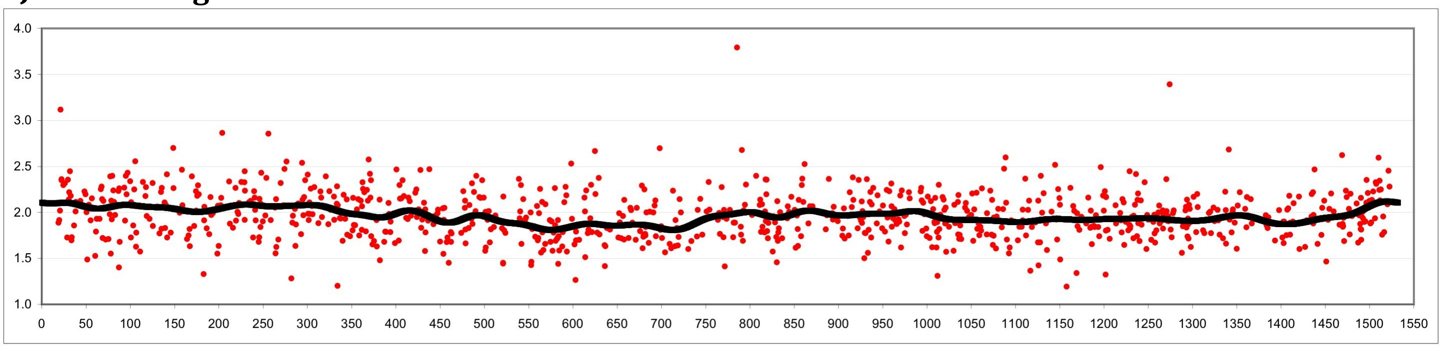

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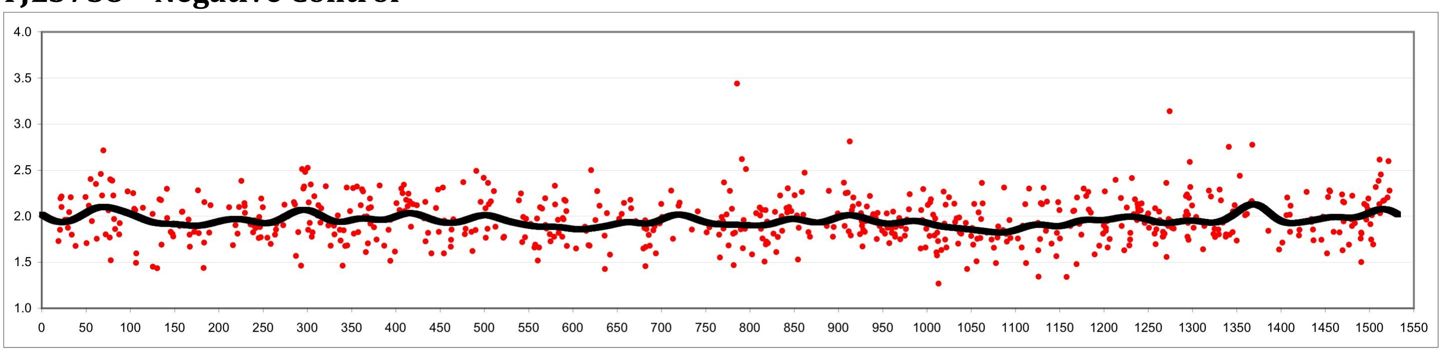

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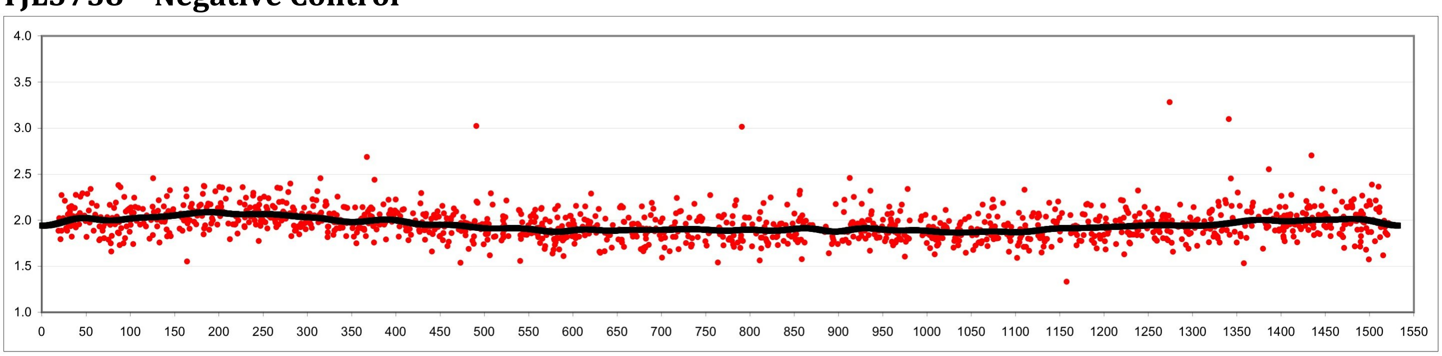

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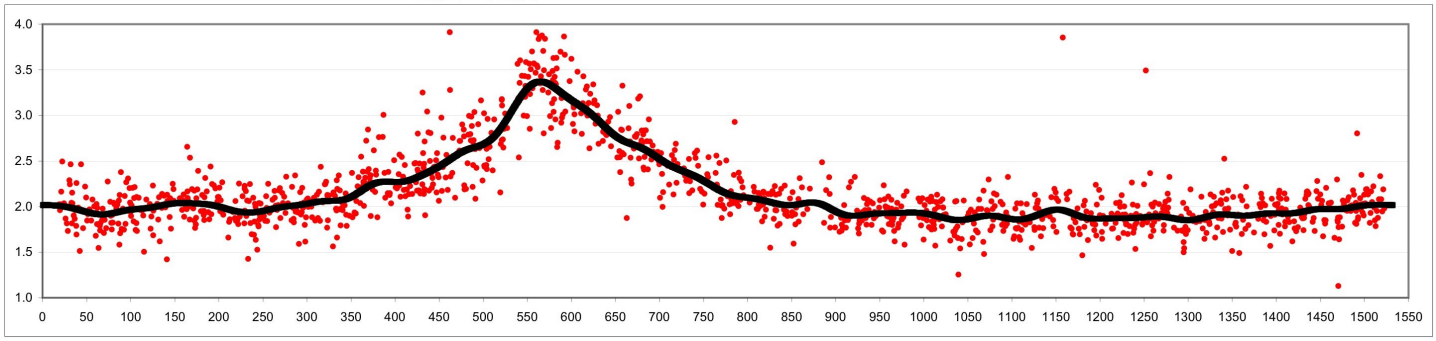
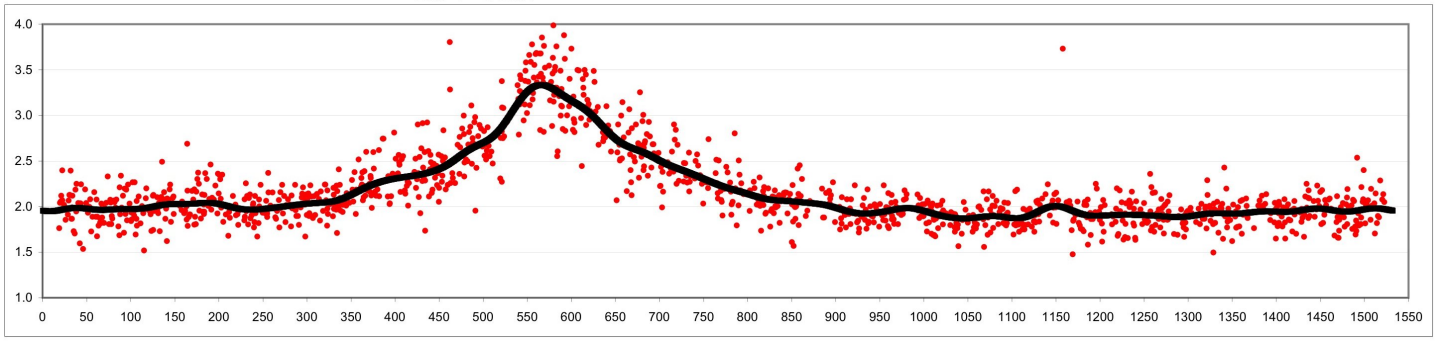


YJL8702



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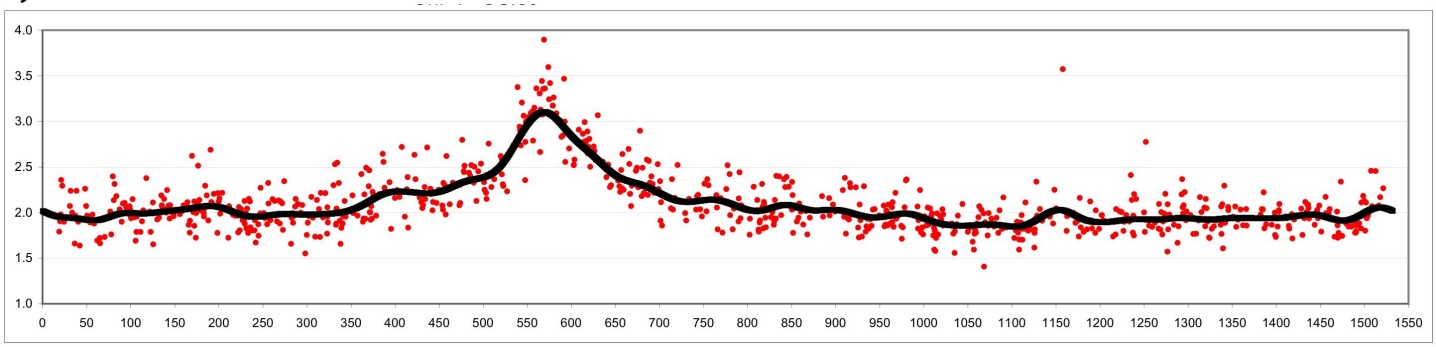

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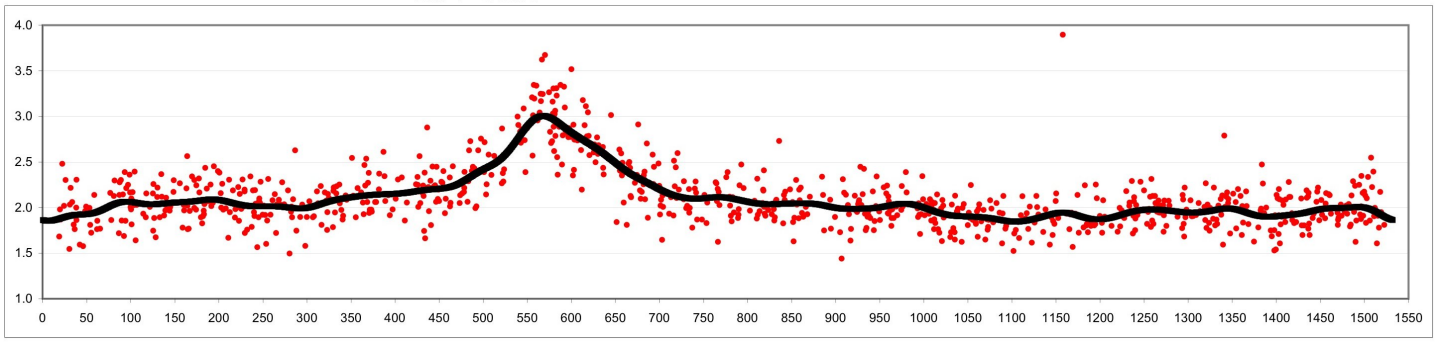

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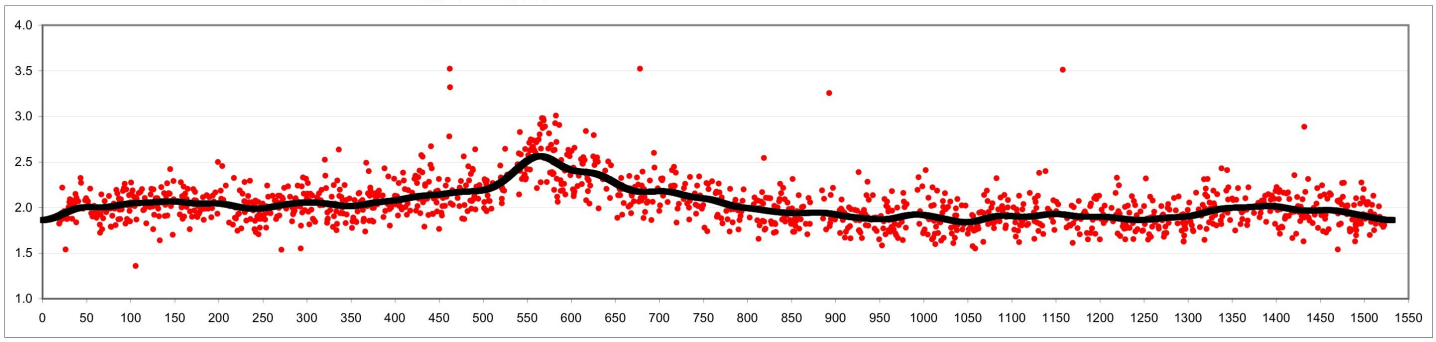

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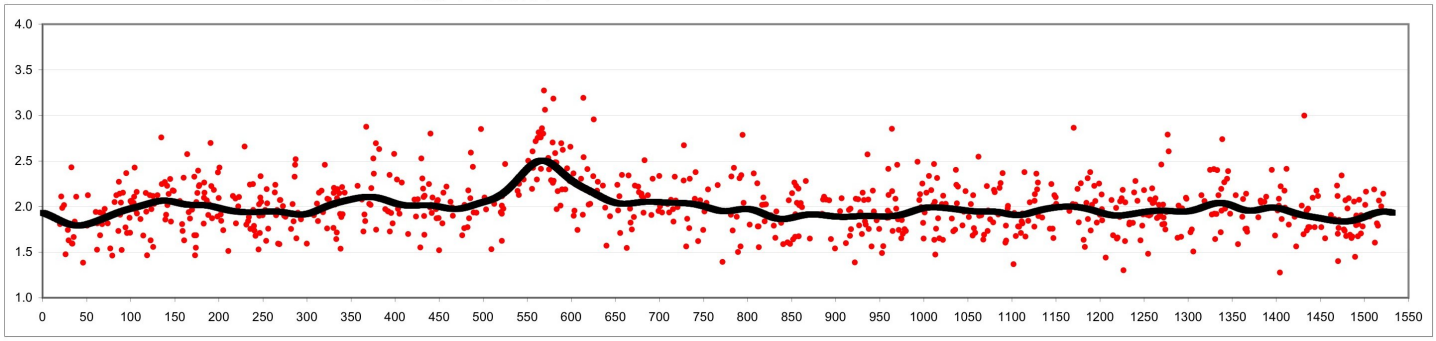
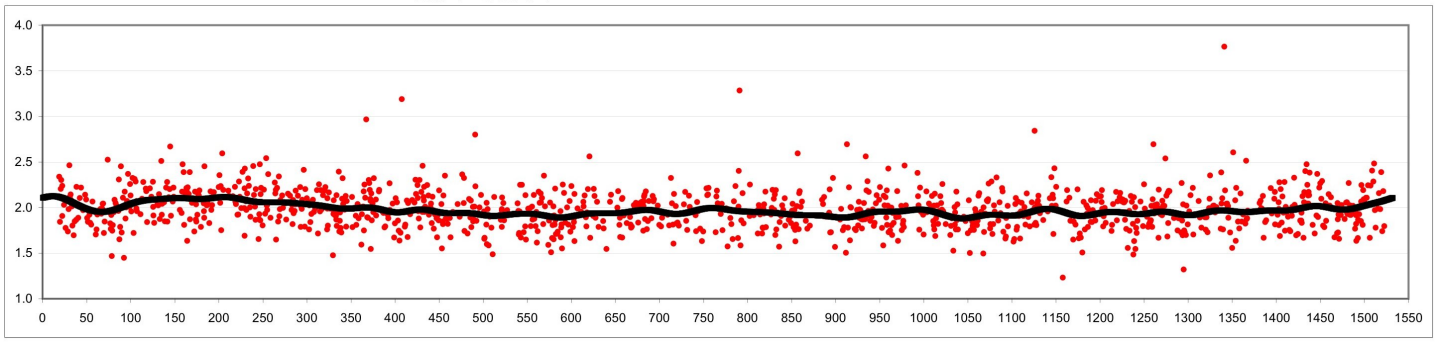


Figure 3C
YJL10444



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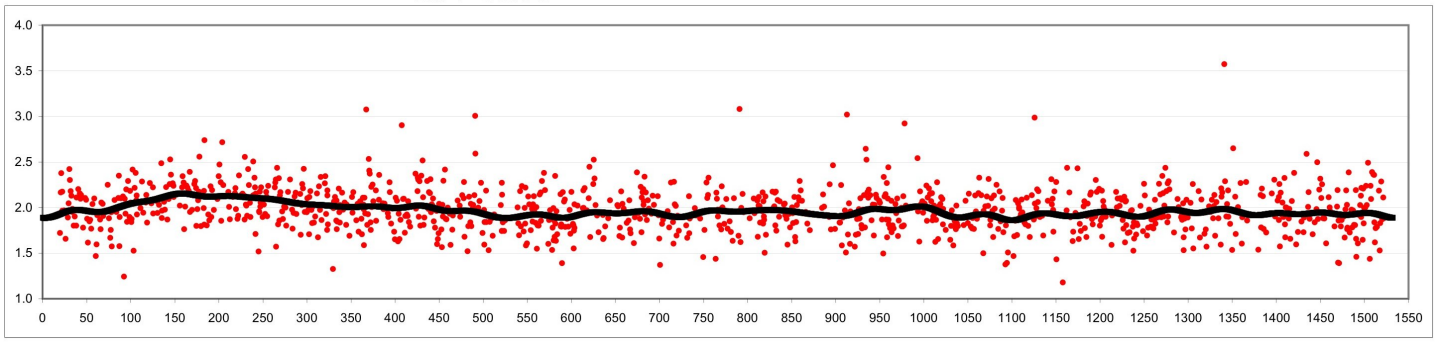
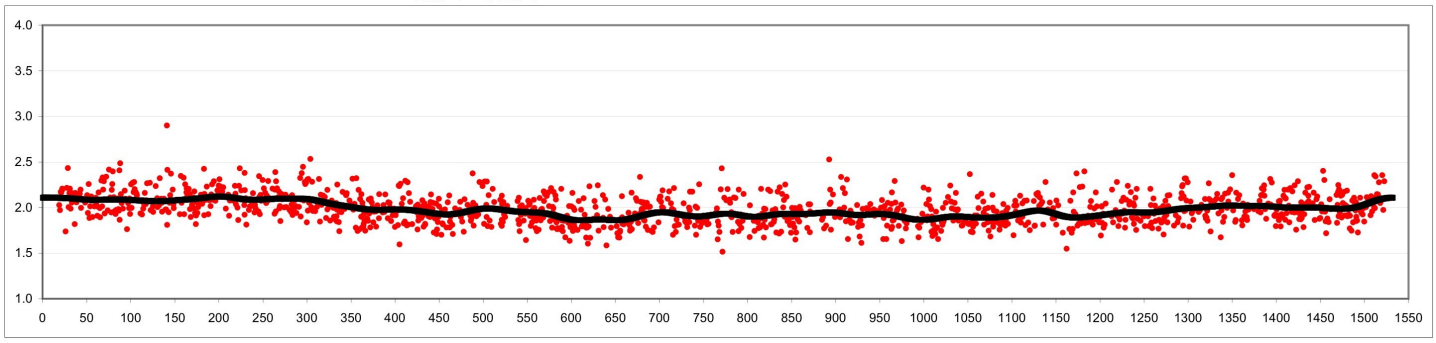


Figure 3D

YJL7717



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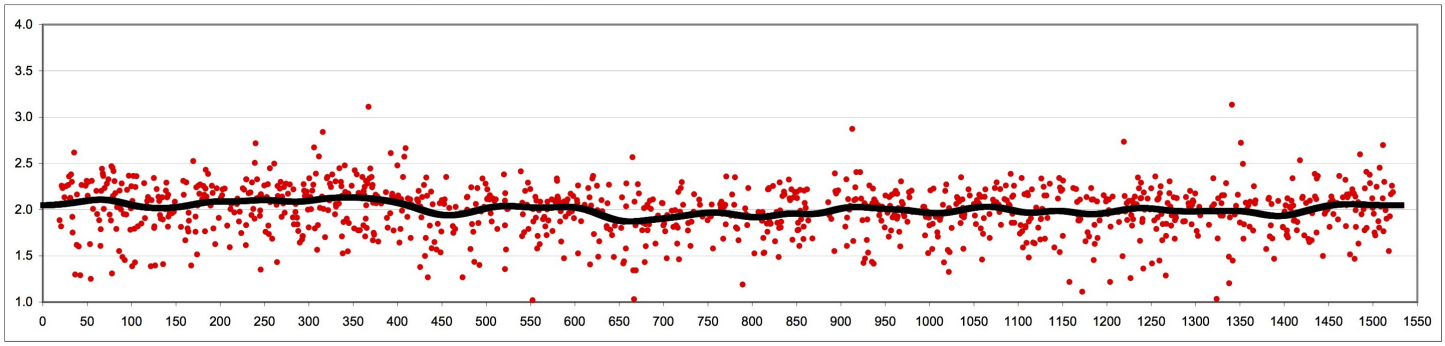
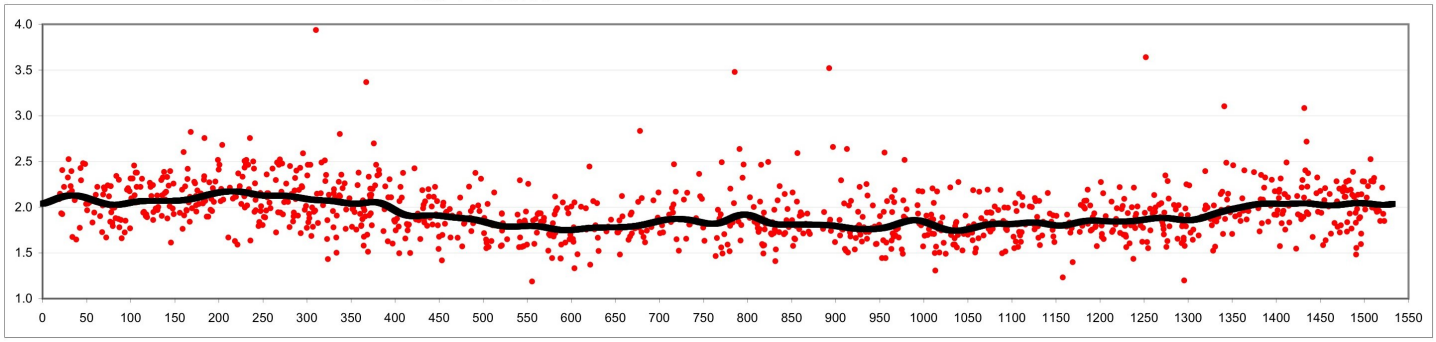


Figure 3E

YJL9707



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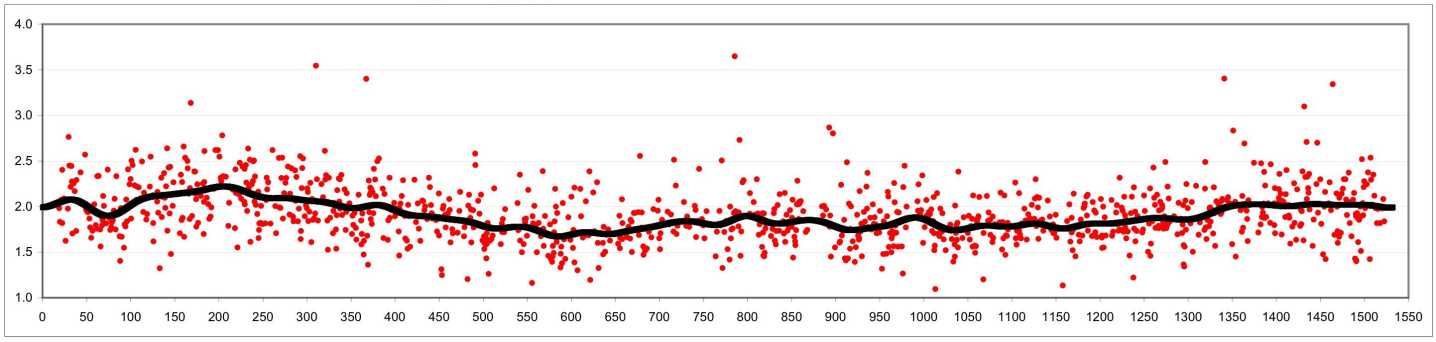
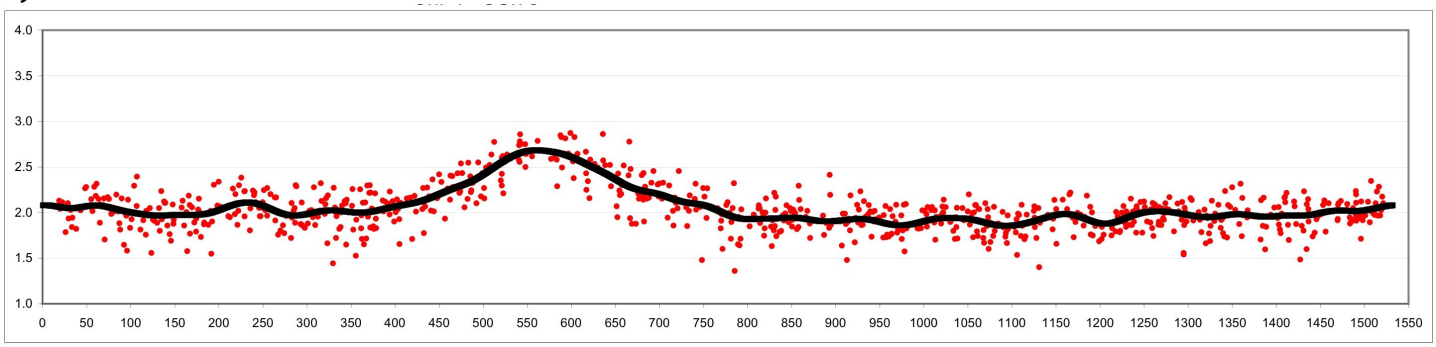
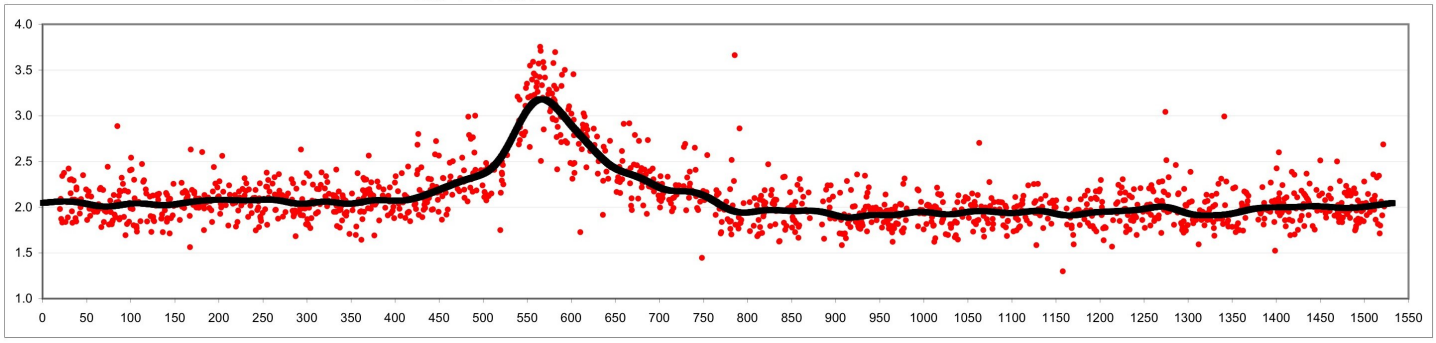


Figure 4A

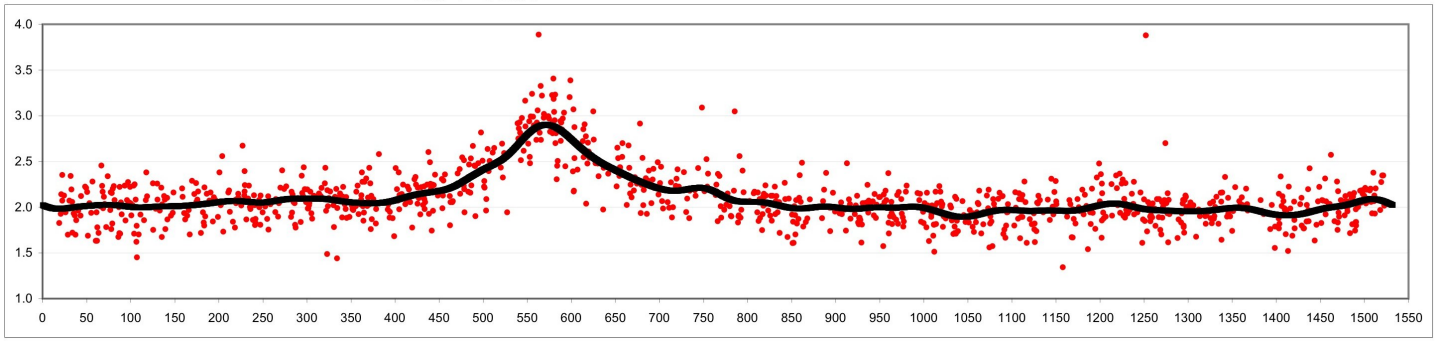
YJL7700 – control



YJL7701 – control



YJL8386



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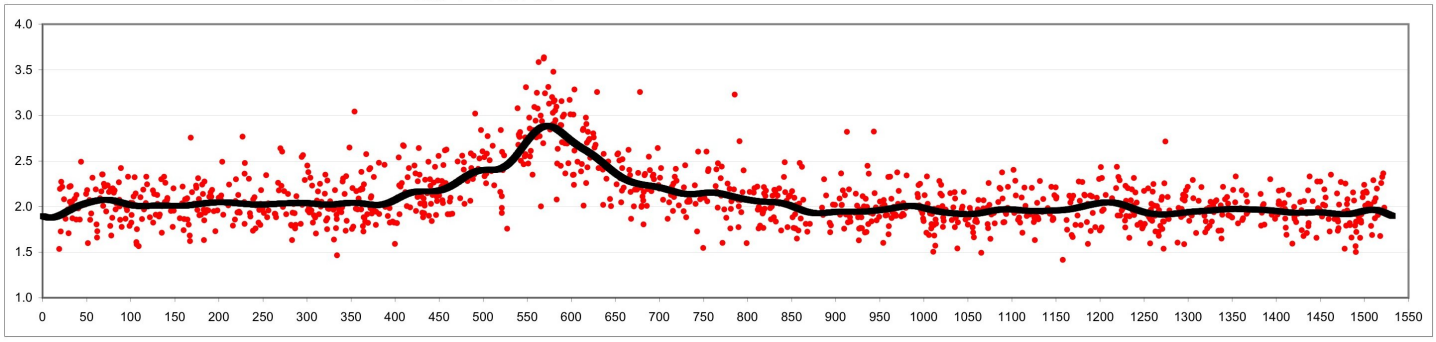
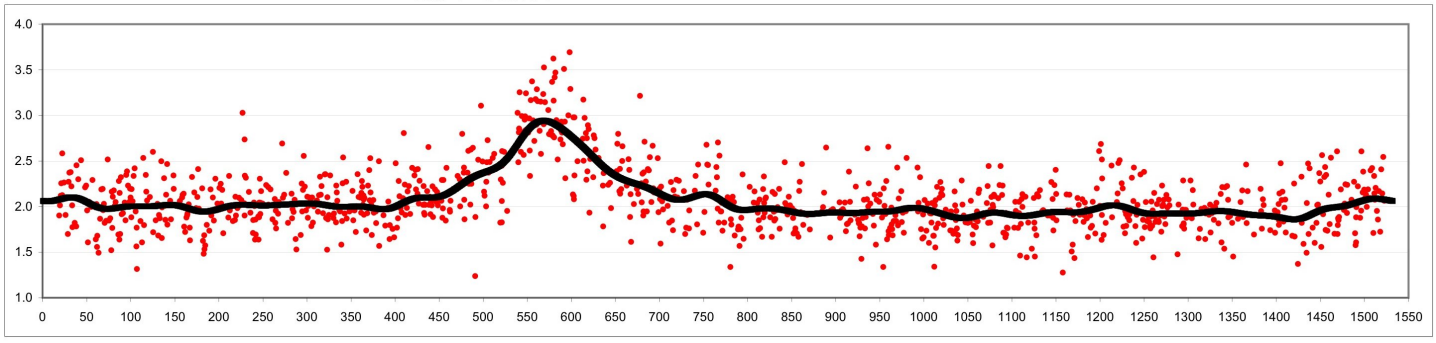


YJL8392



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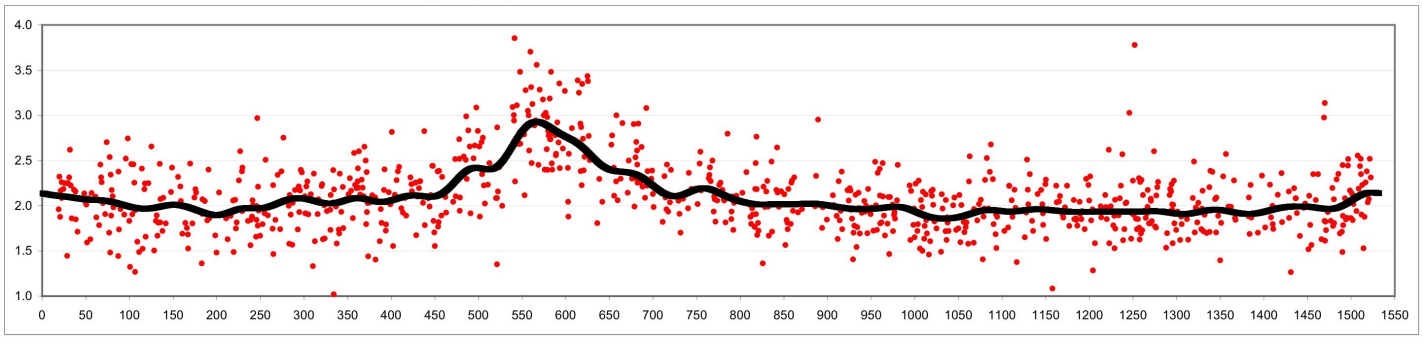

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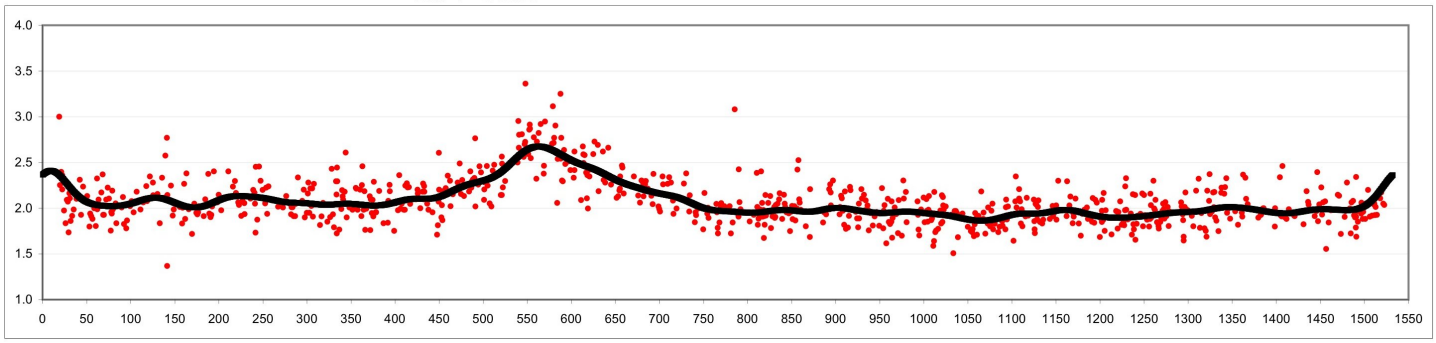

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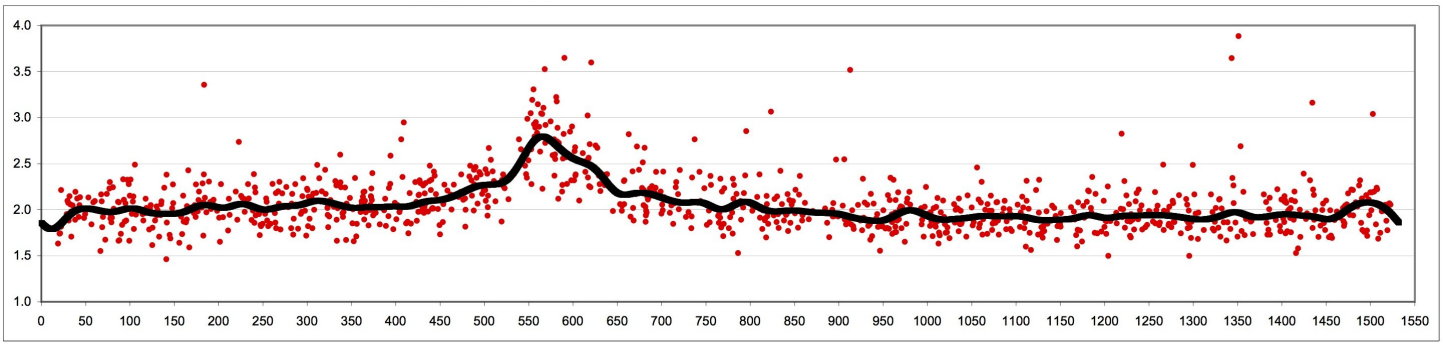

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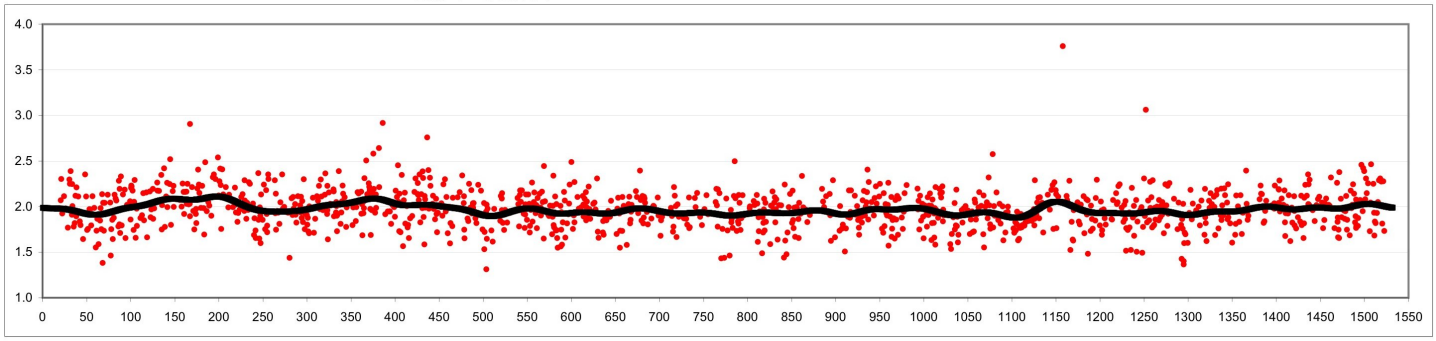

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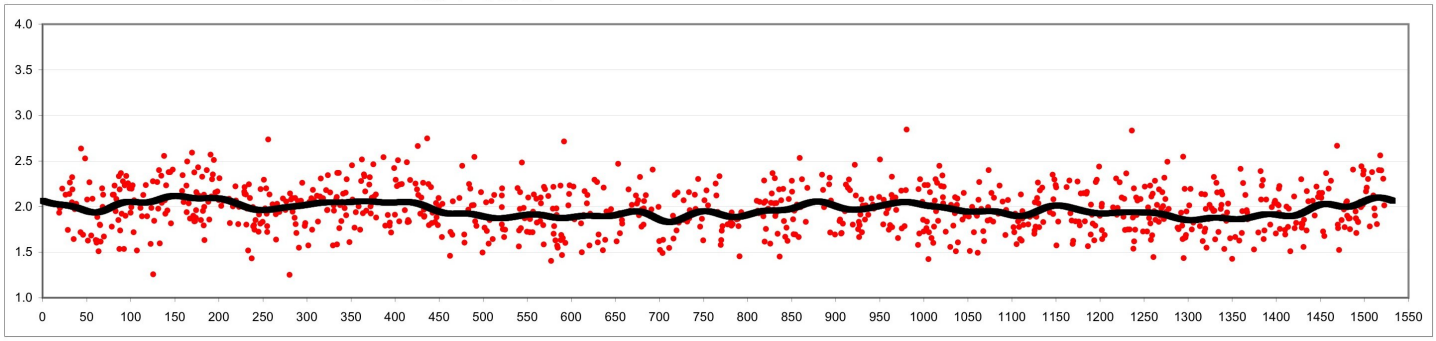

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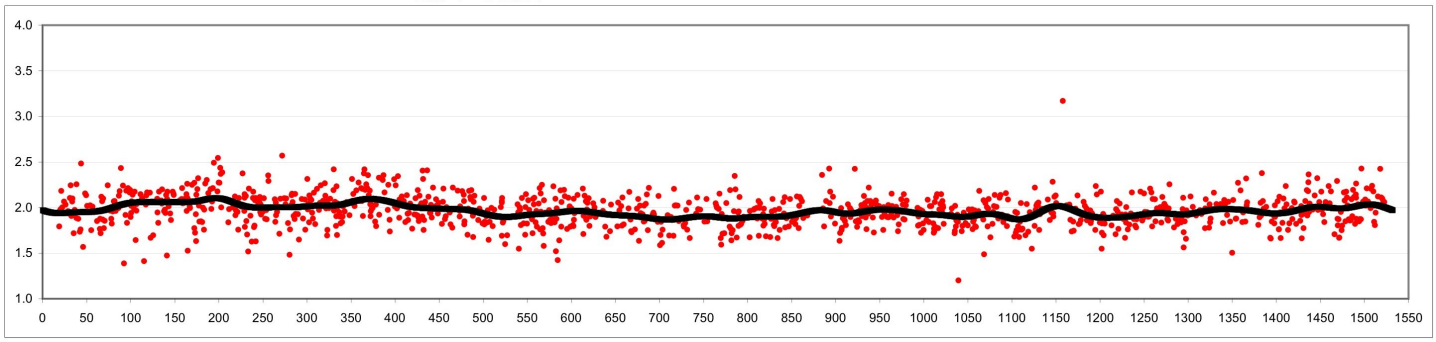

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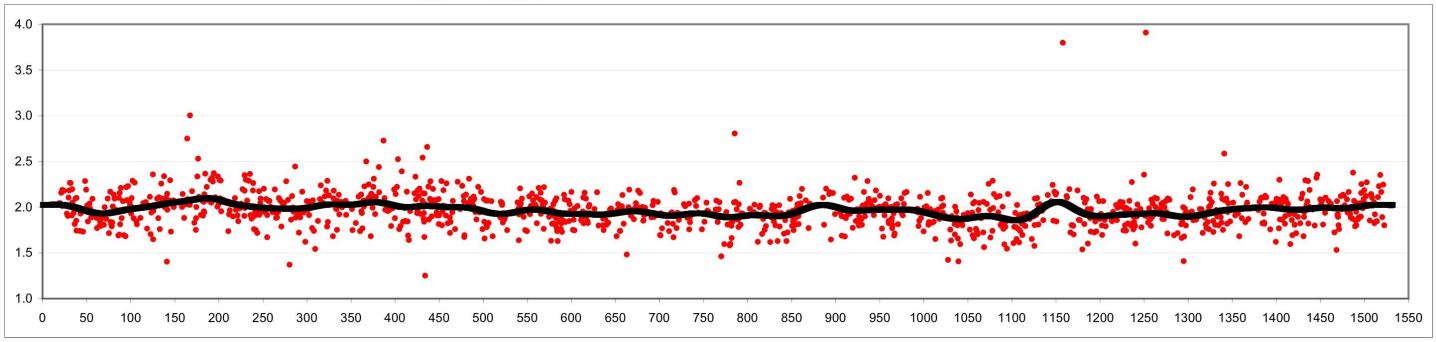

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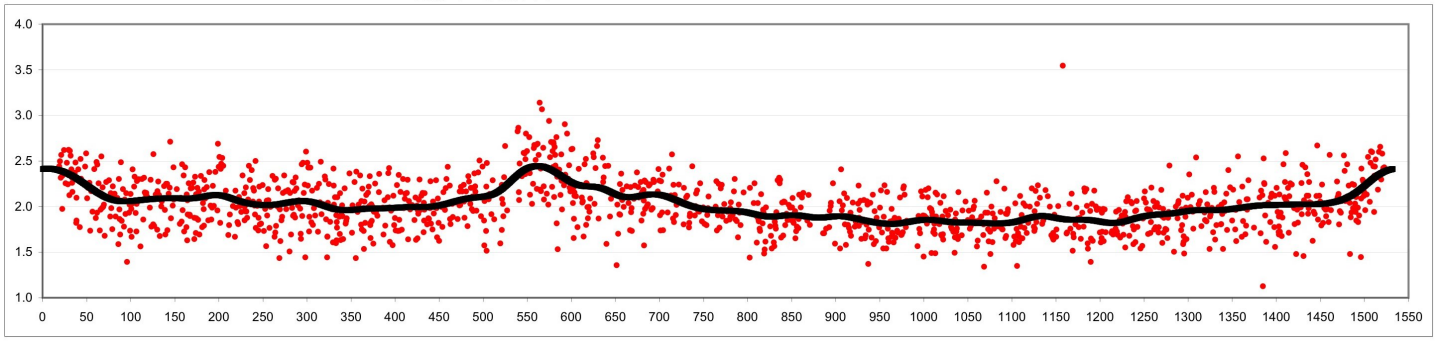

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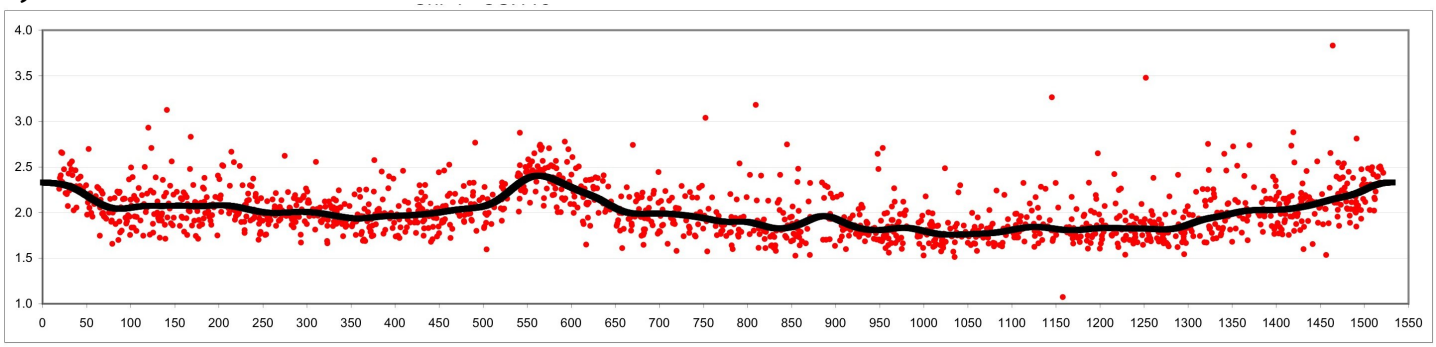

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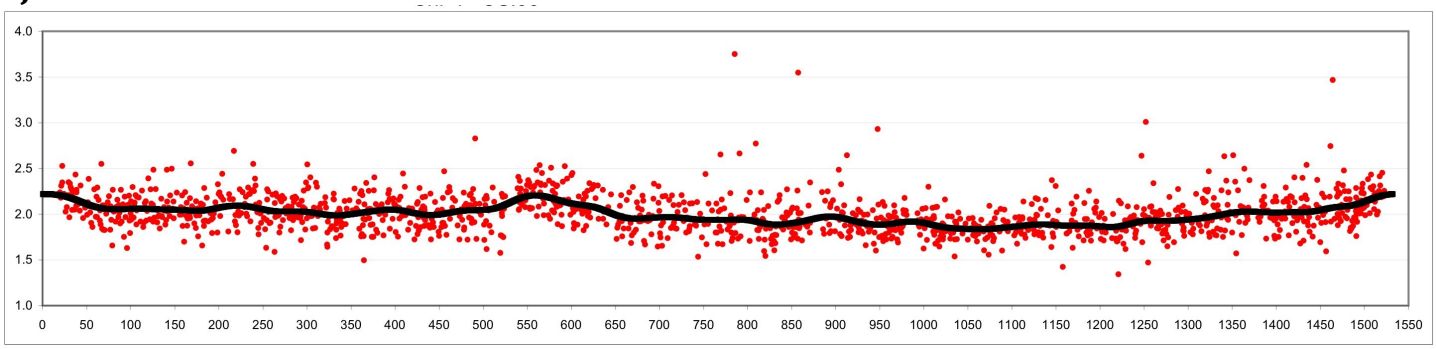

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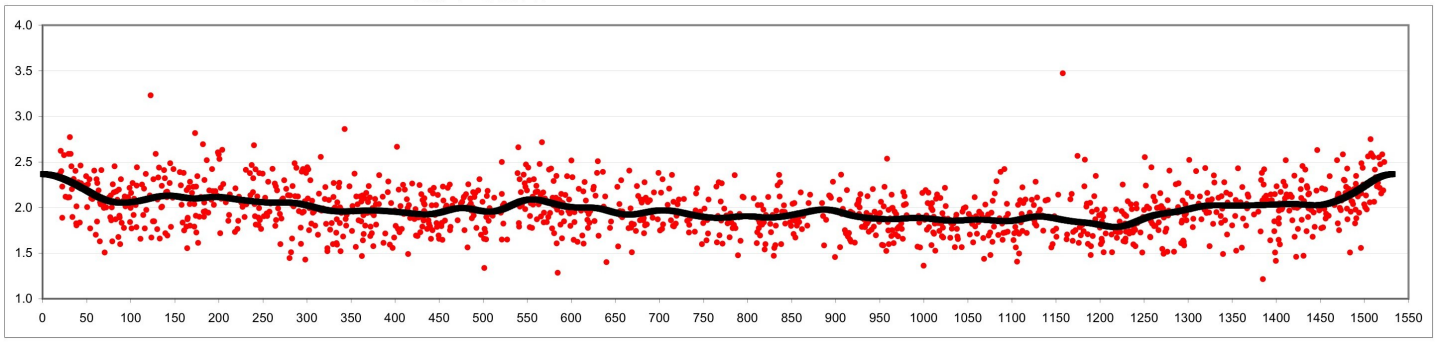

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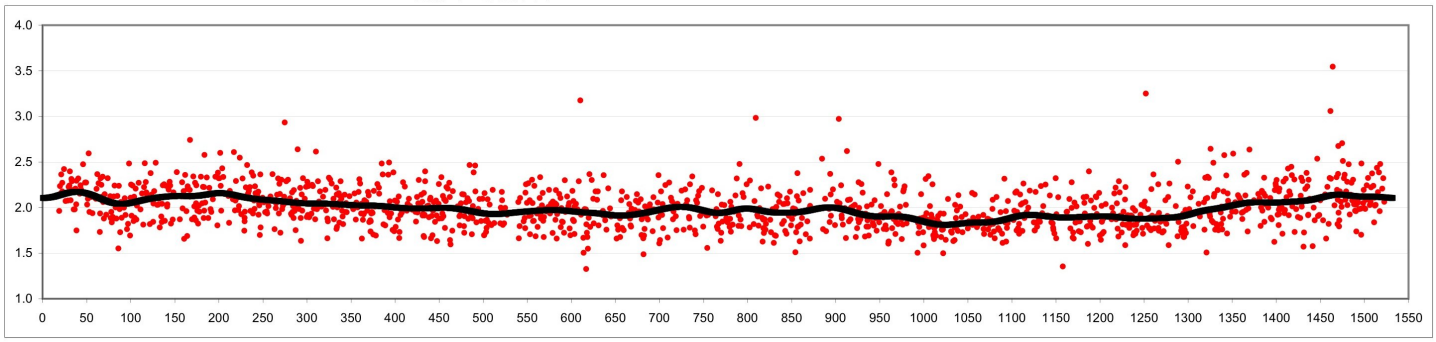

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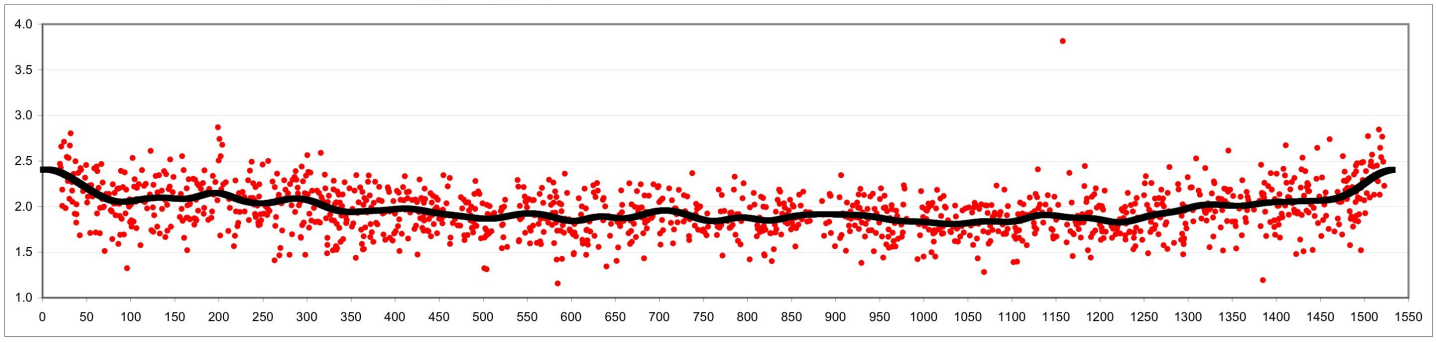

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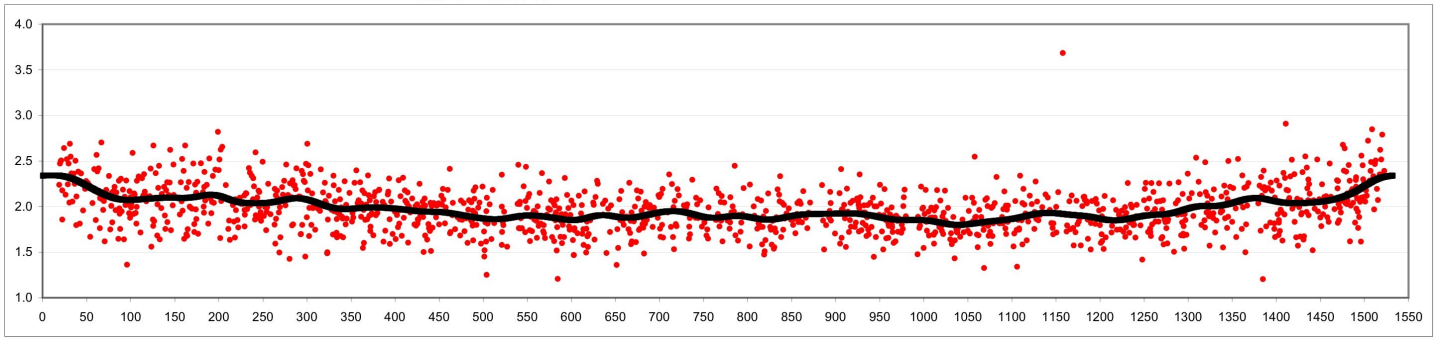

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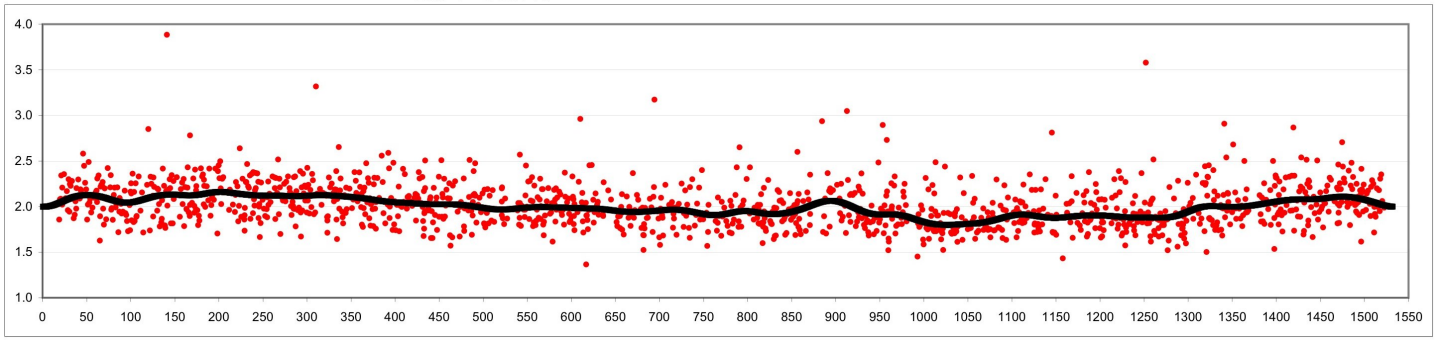

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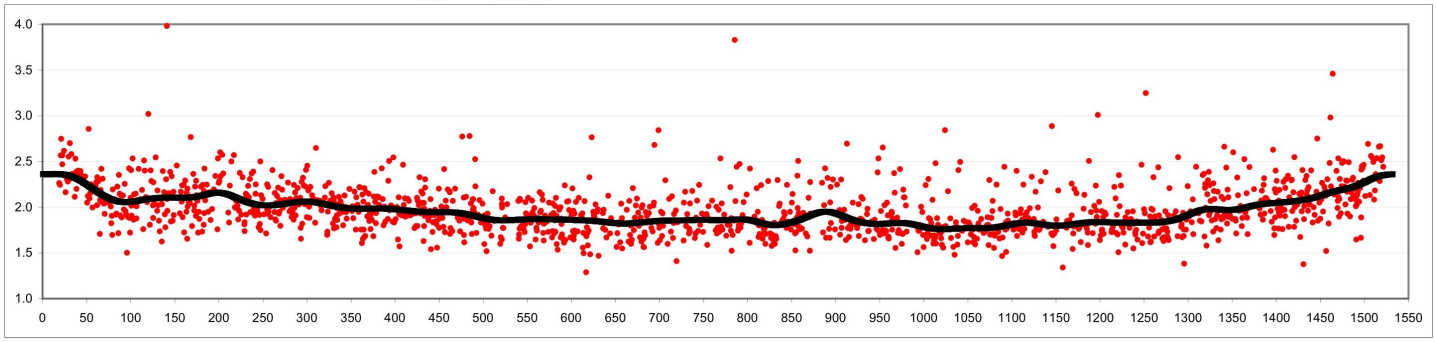

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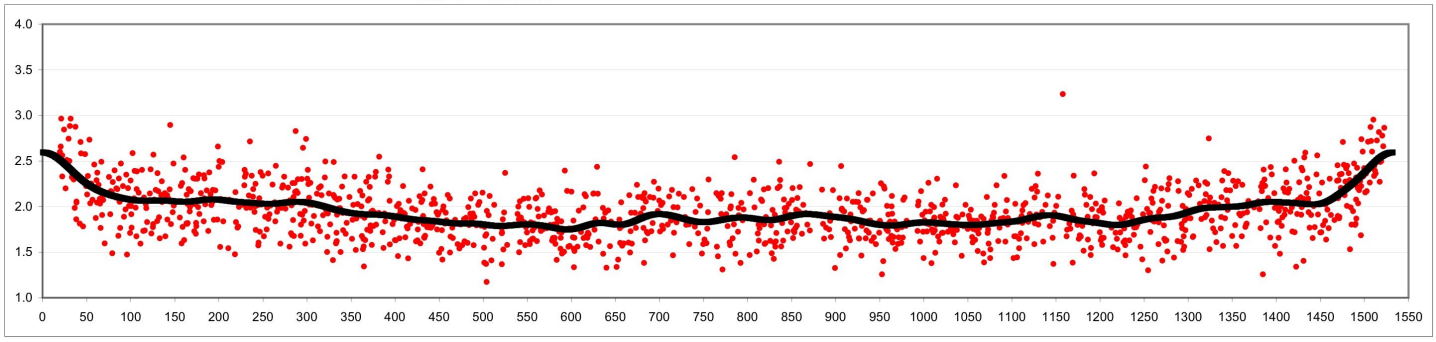

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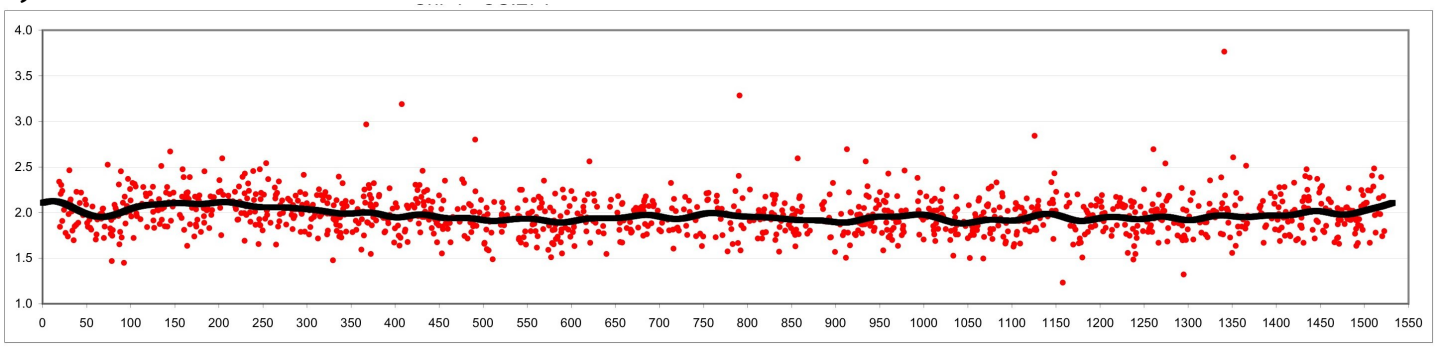

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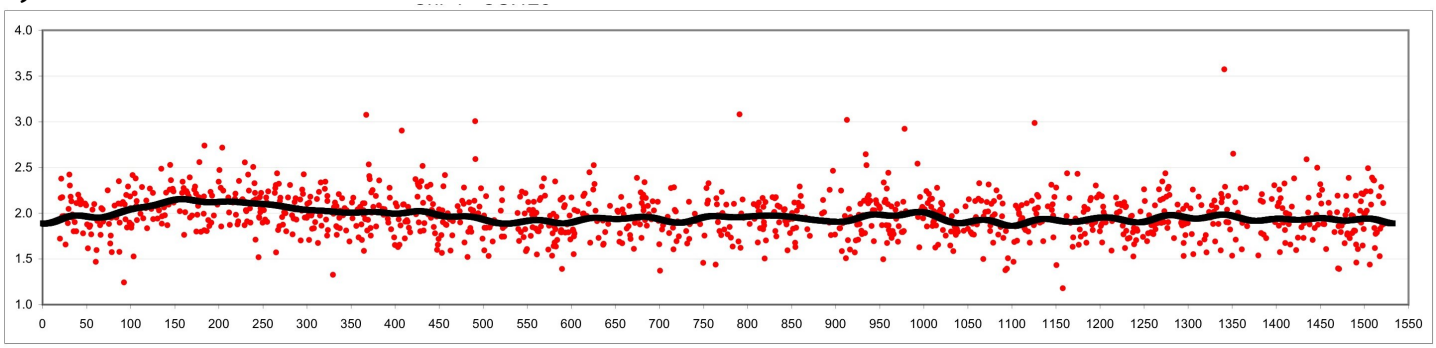
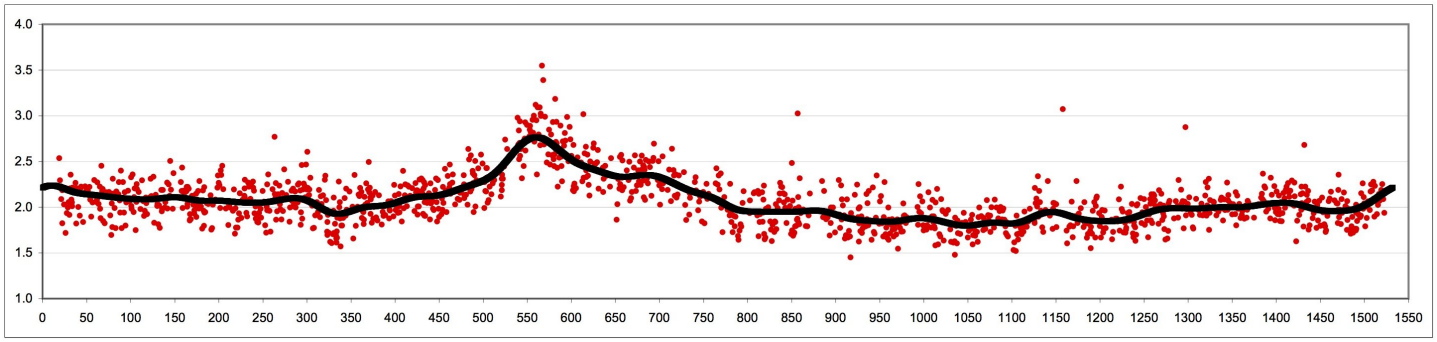
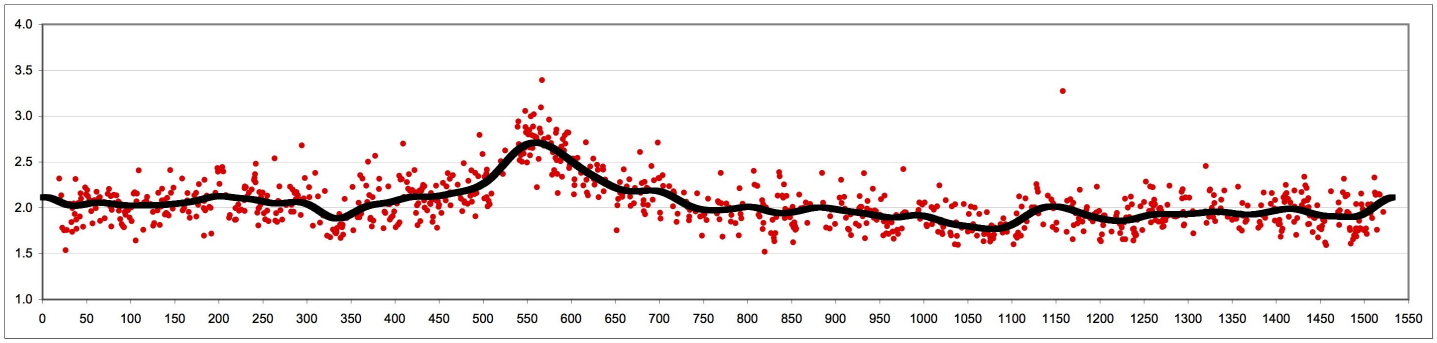


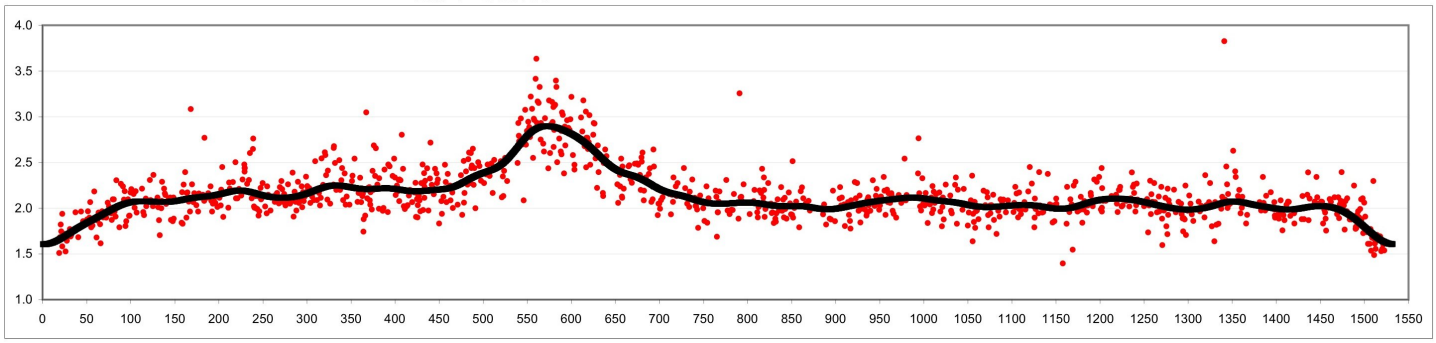
Figure 4B
YJL8398 – Control for odd linkers



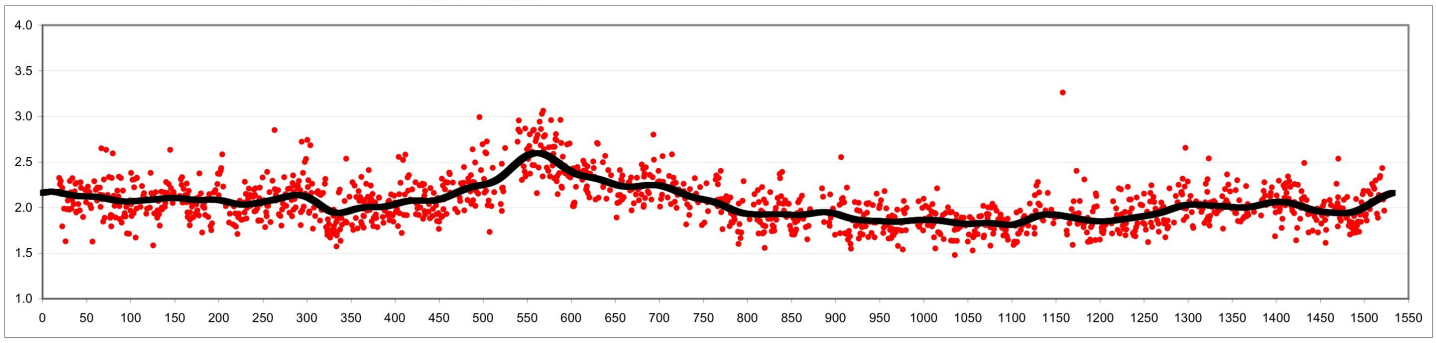
YJL8398 – Control for odd linkers



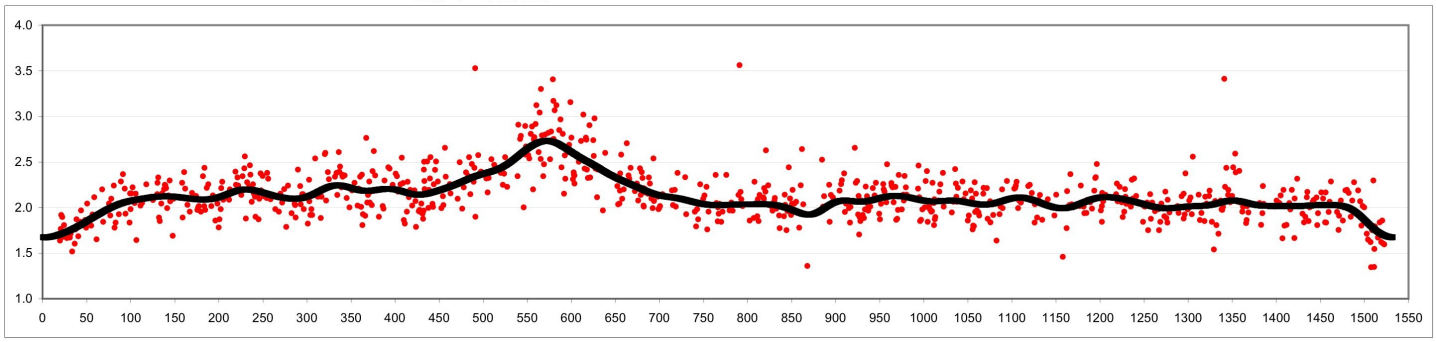
YJL8644 – Linker L1



YJL8644 – Linker L1



YJL8647 – Linker L3



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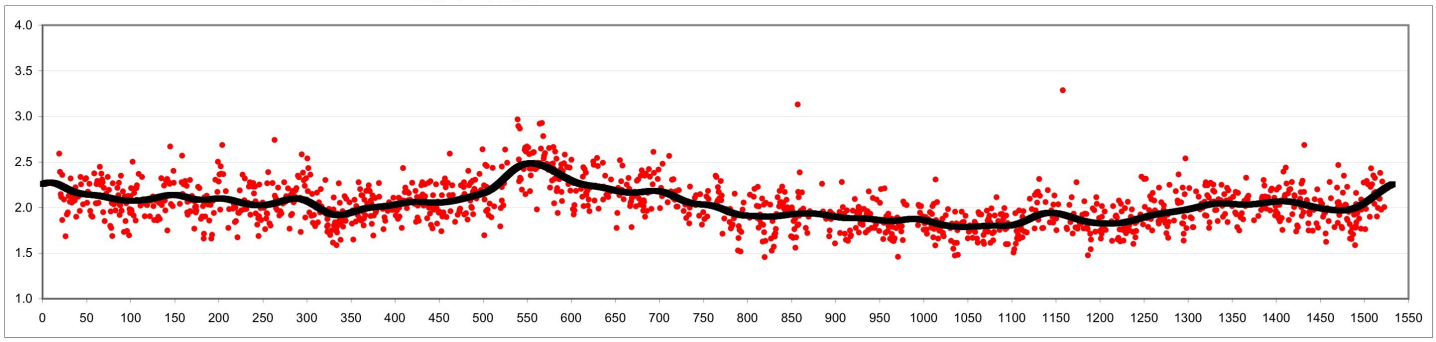
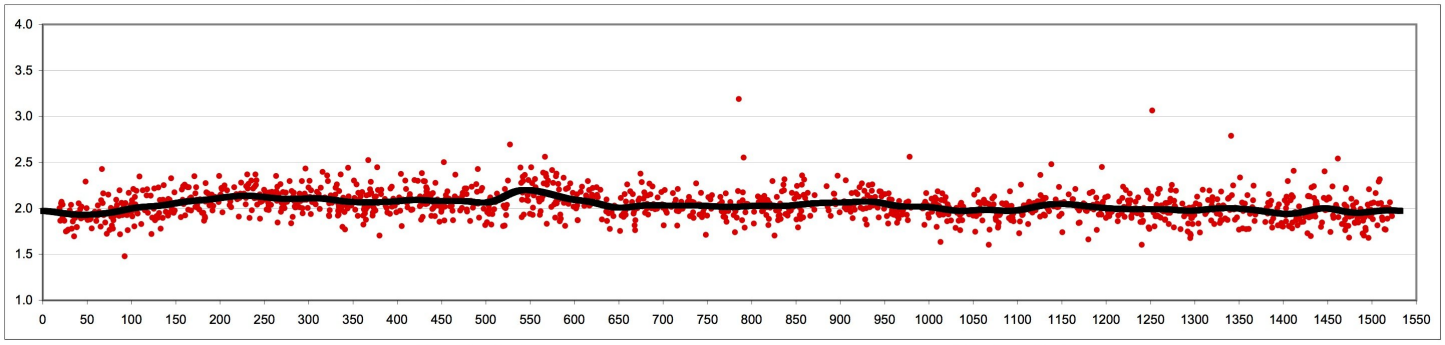


YJL8650– Linker L5



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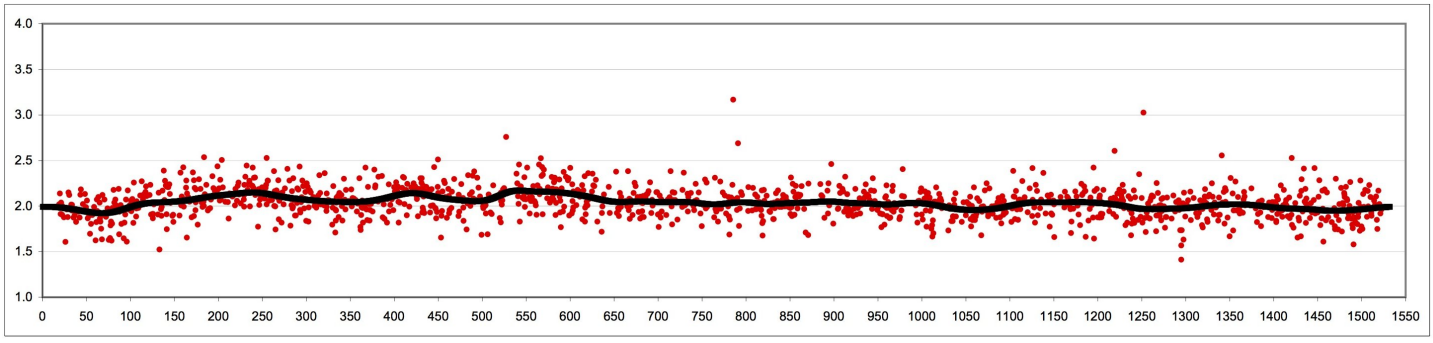
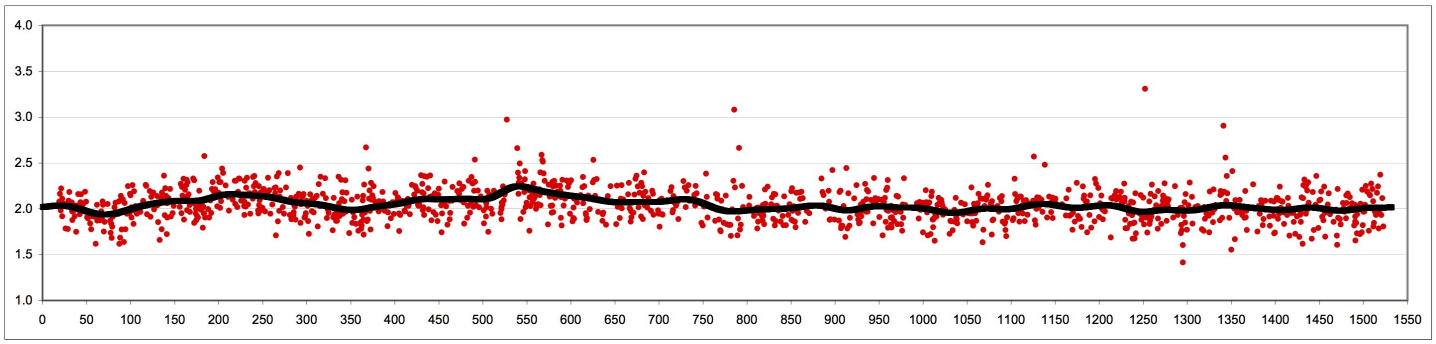


YJL8653 – Linker L7



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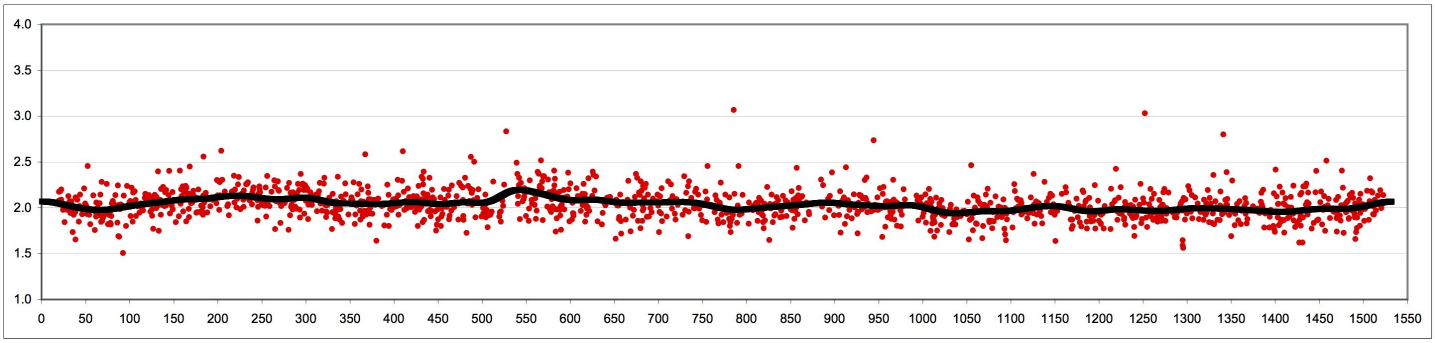
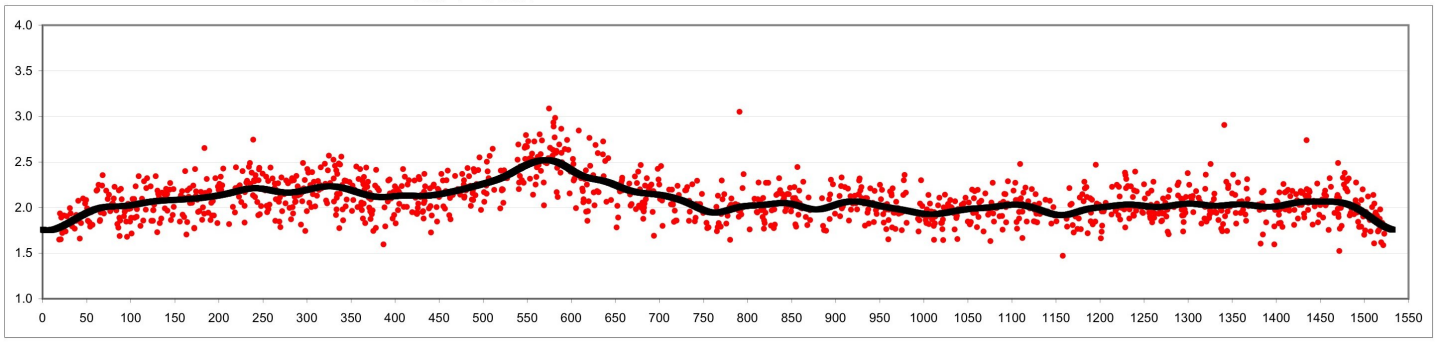
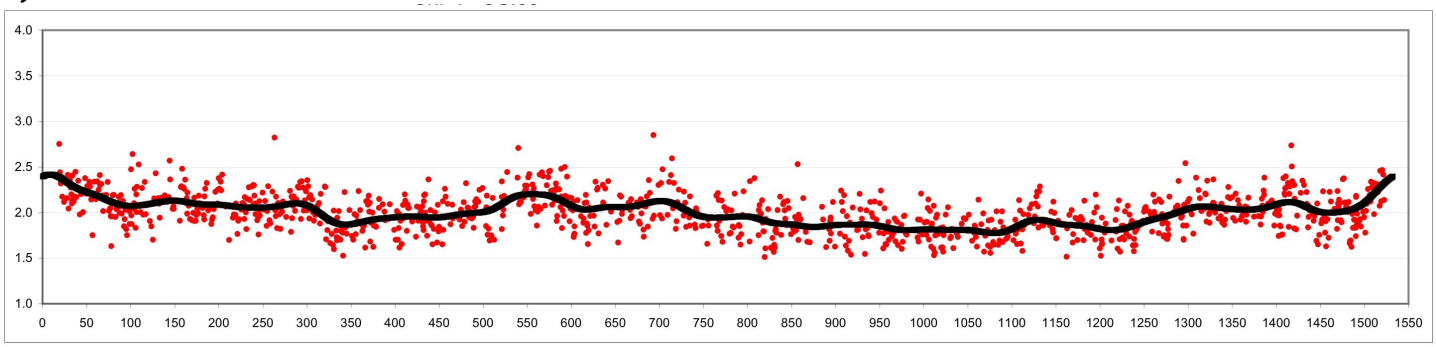


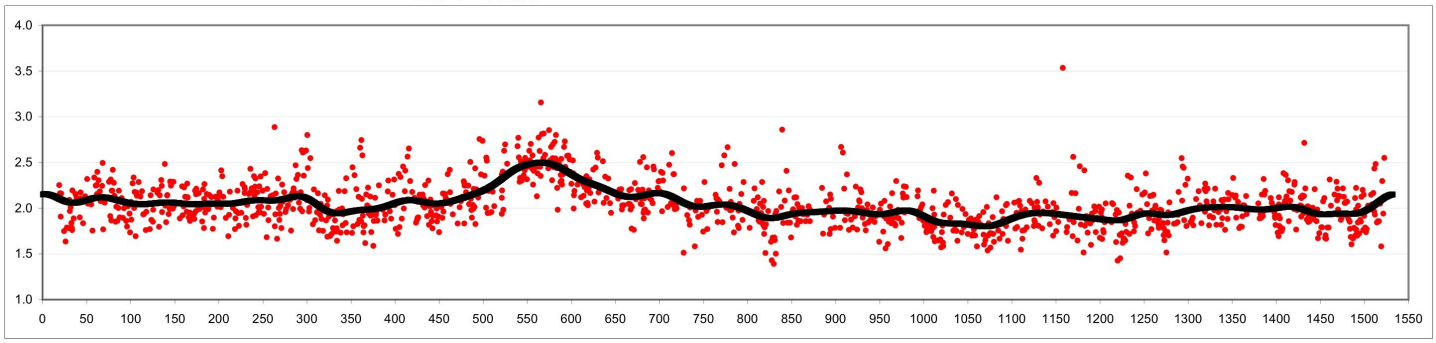
YJL8656- Linker L9



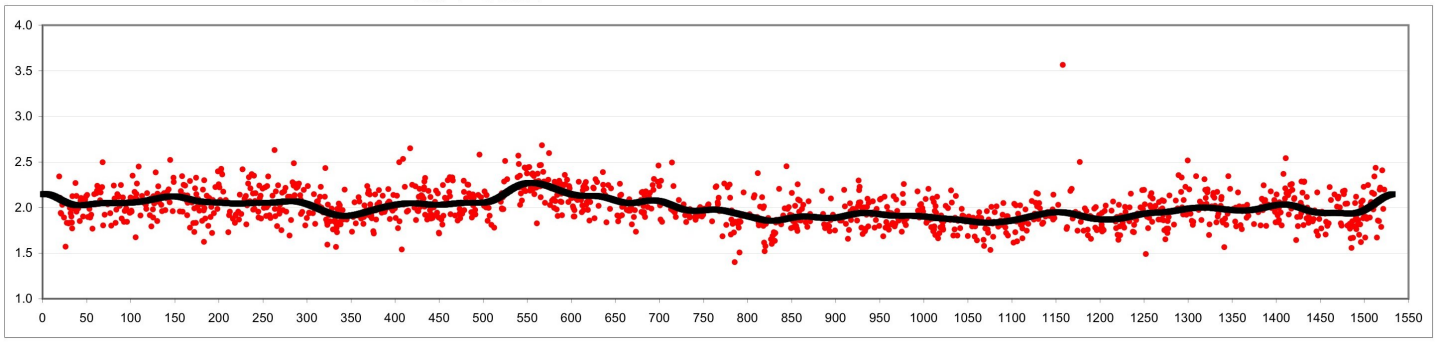
YJL8656- Linker L9



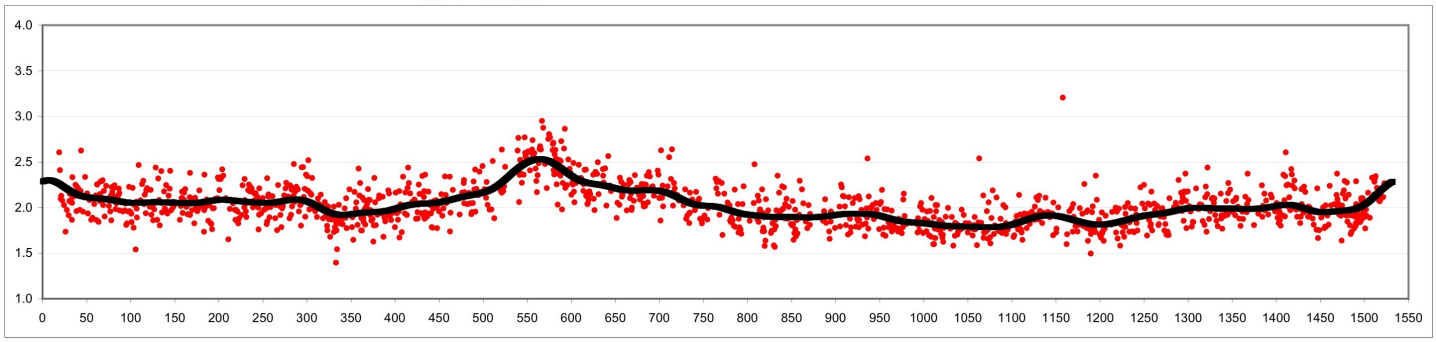
YJL8677 – Linker L11



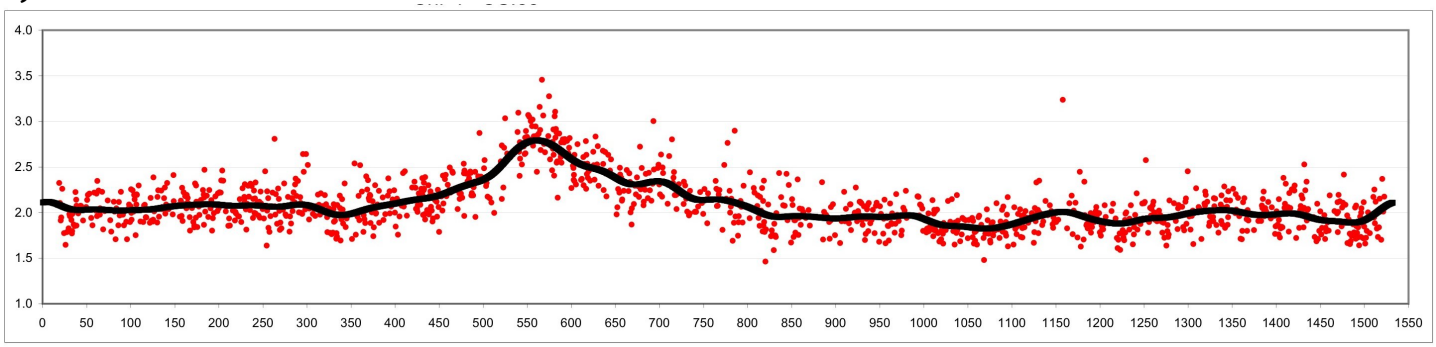
YJL8677 – Linker L11



YJL8680 – Linker L13



YJL8680 – Linker L13



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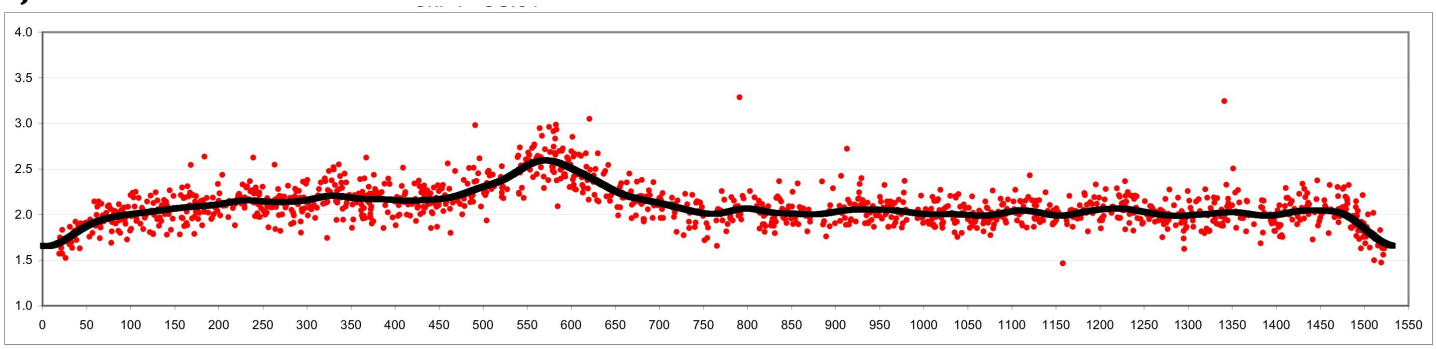

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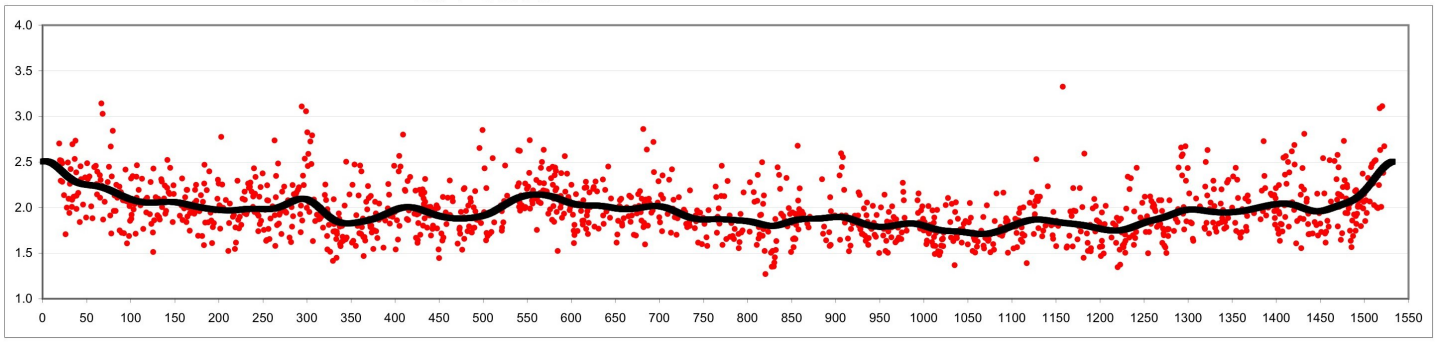

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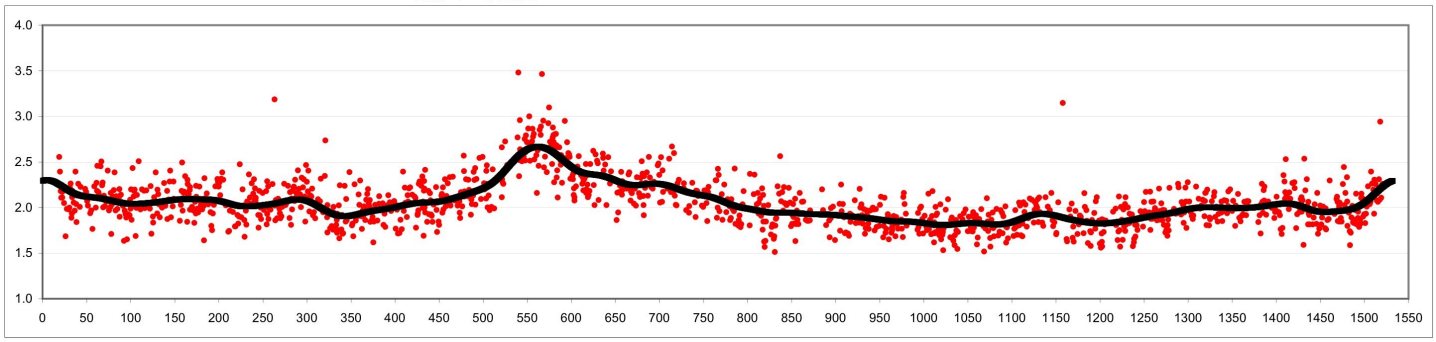

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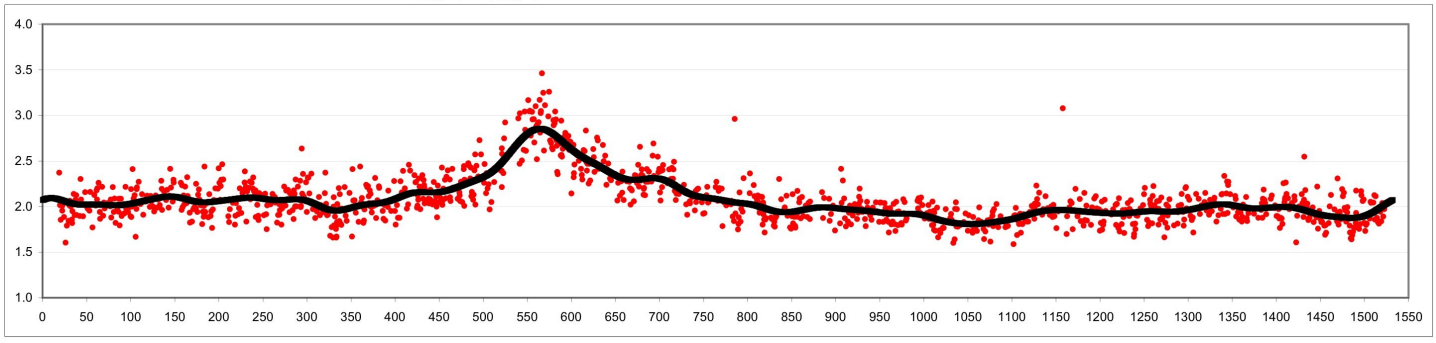

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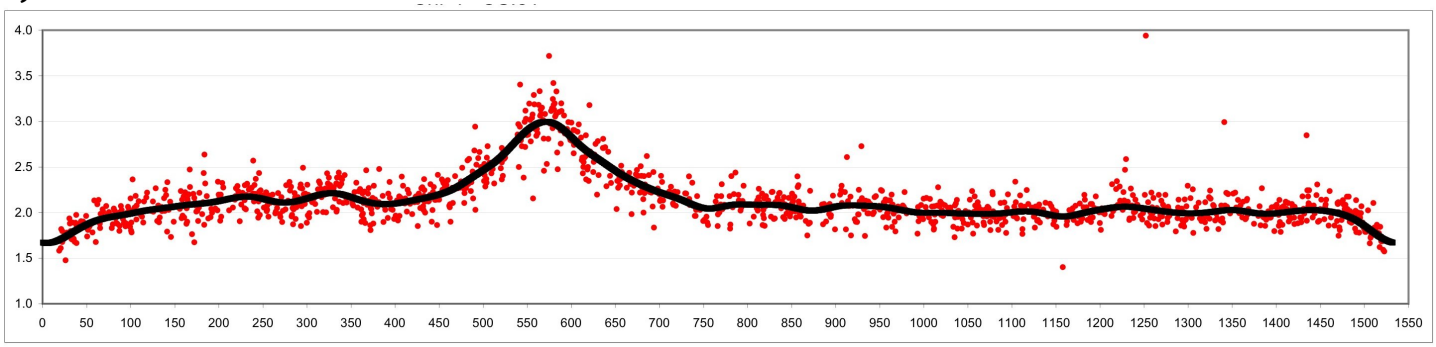

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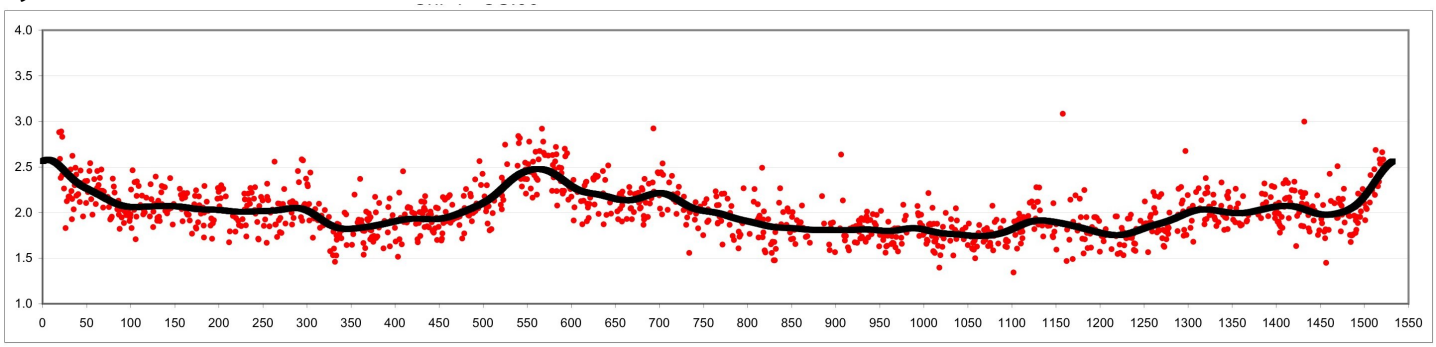

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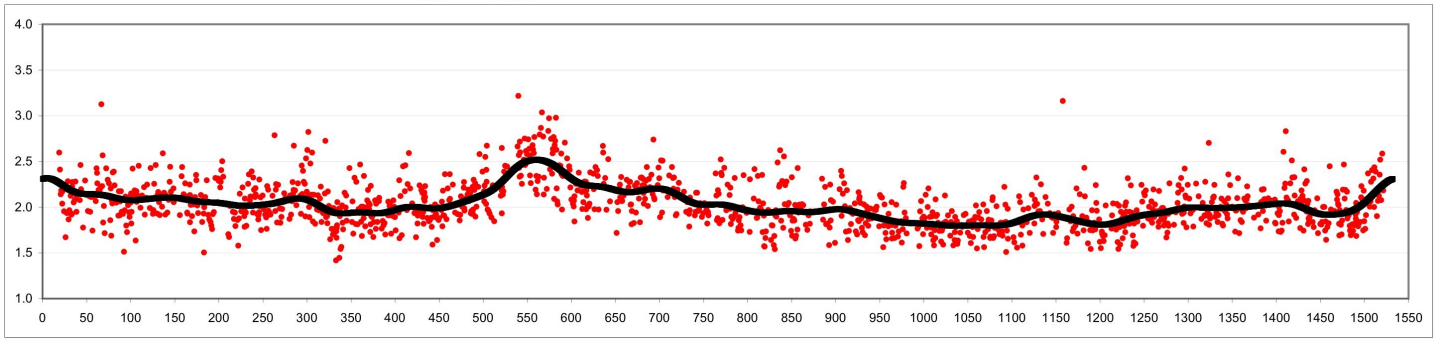

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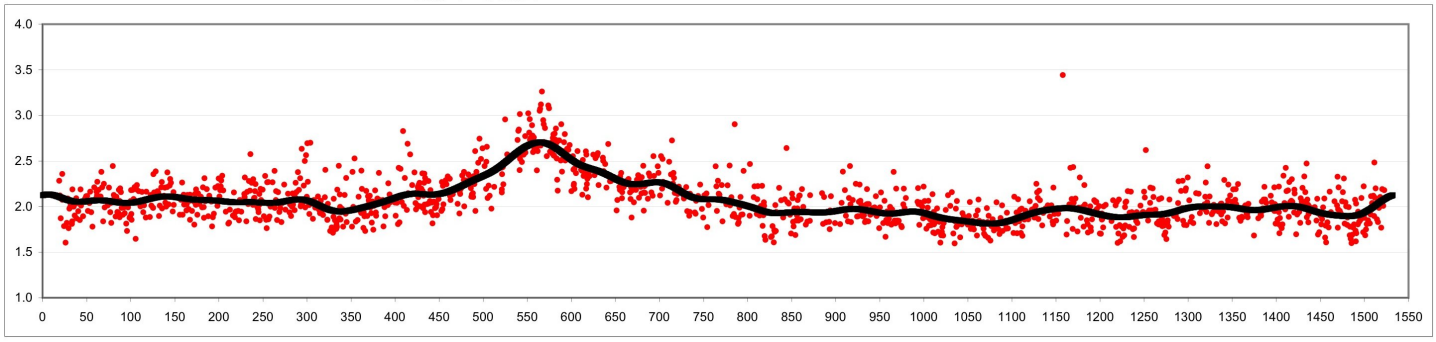

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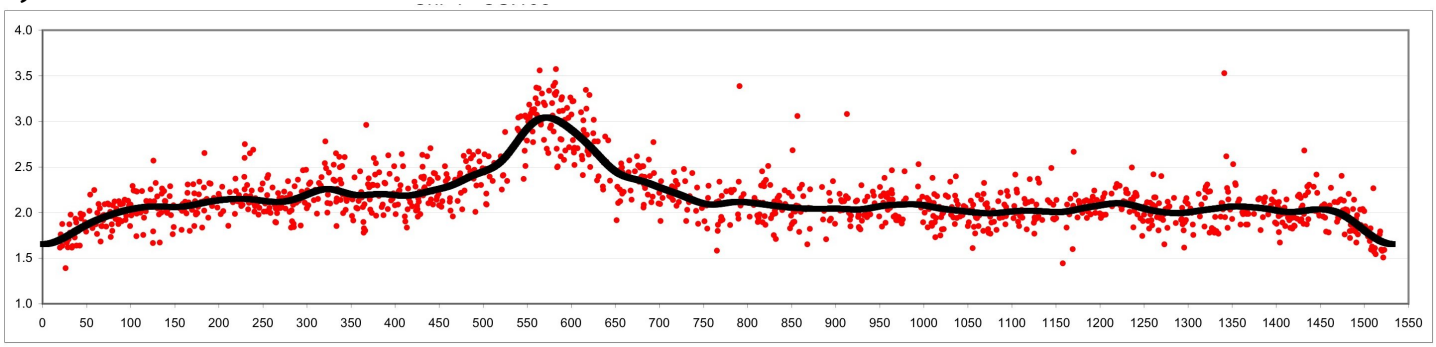

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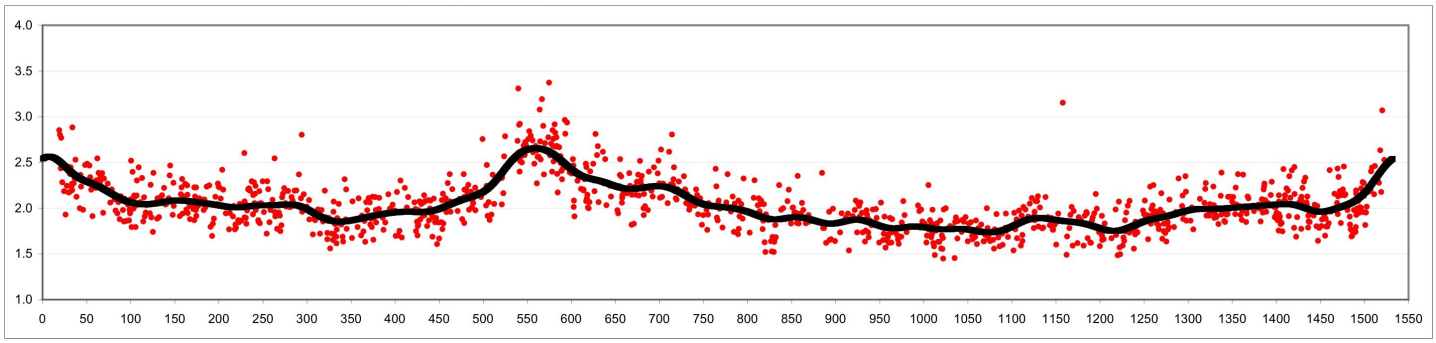

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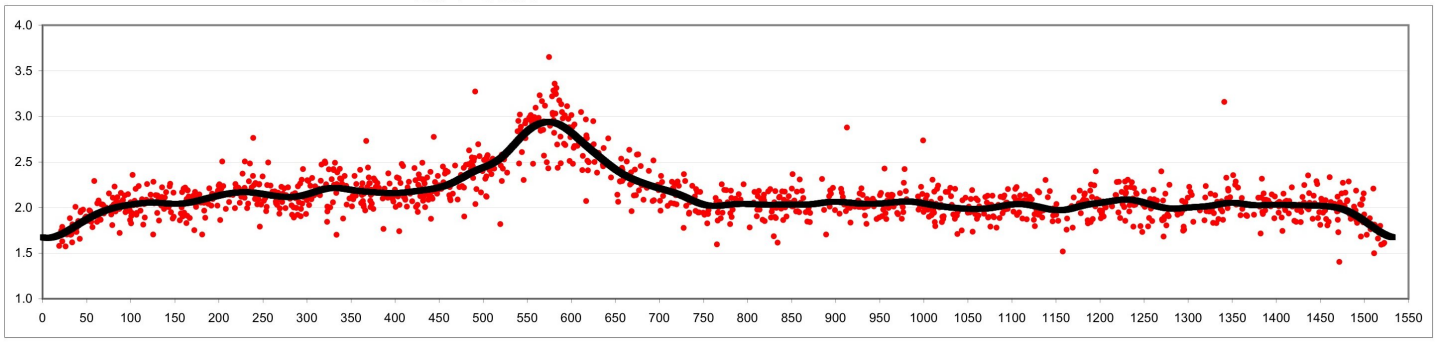

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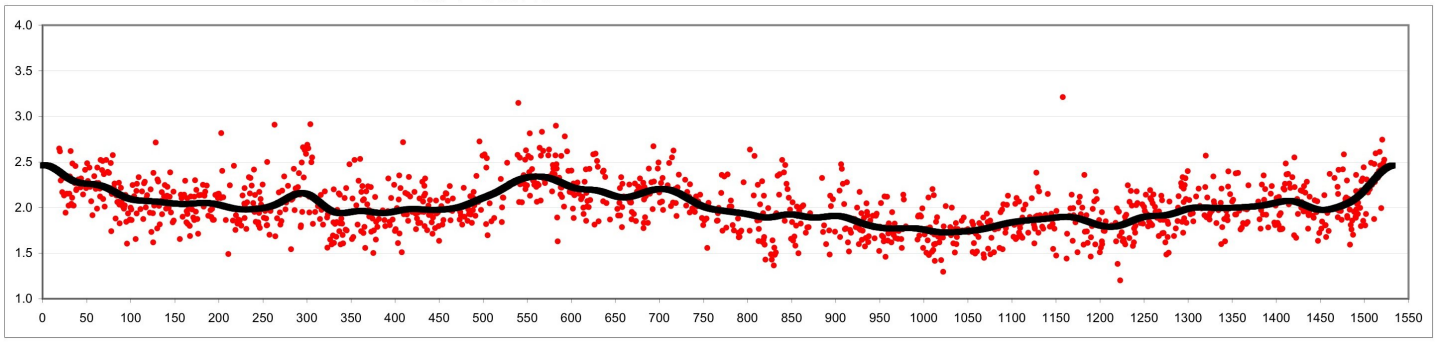

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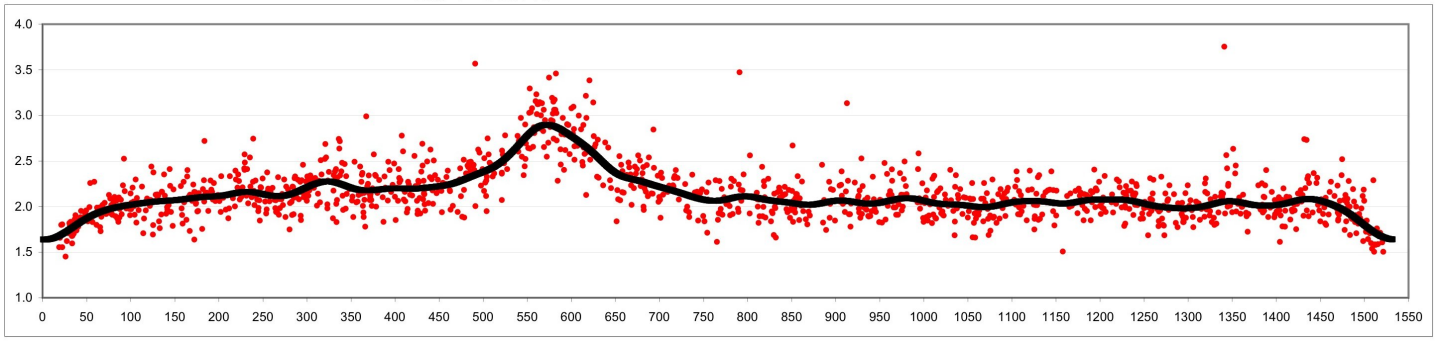

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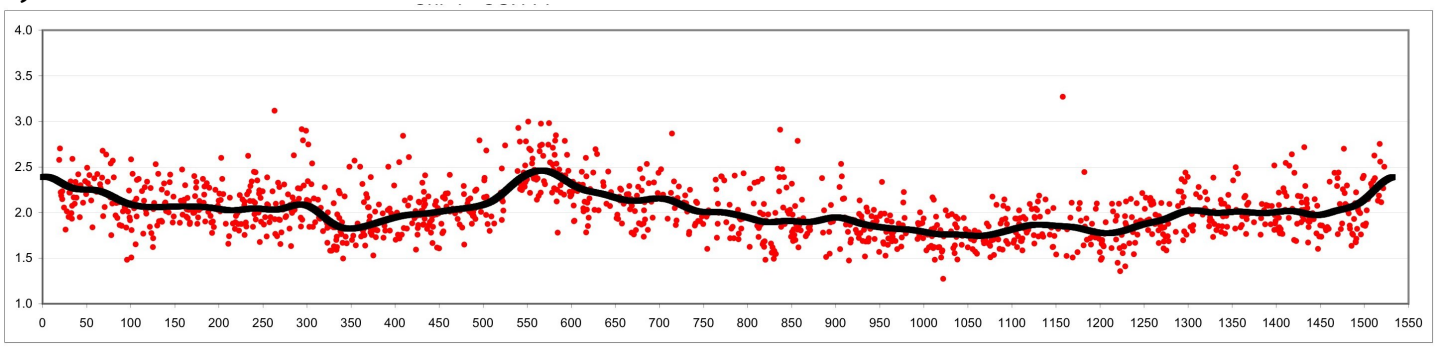

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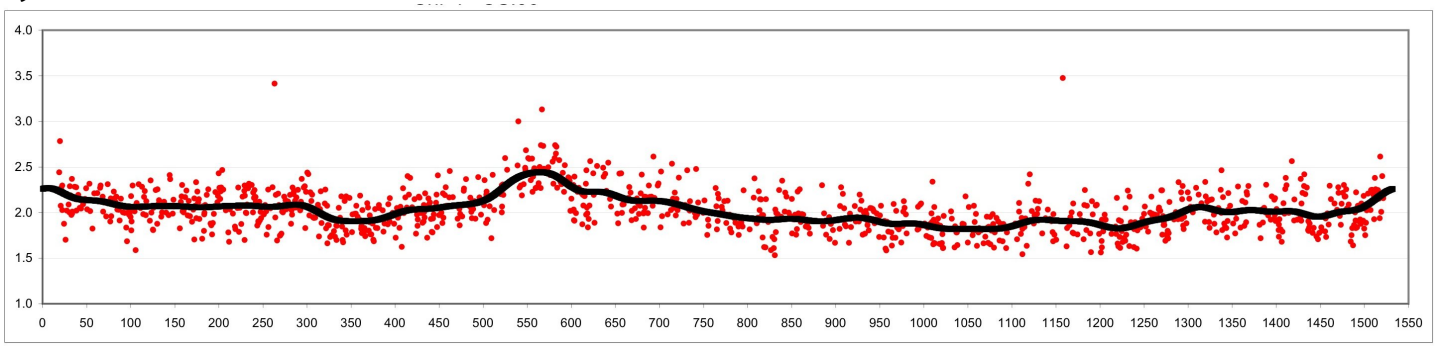

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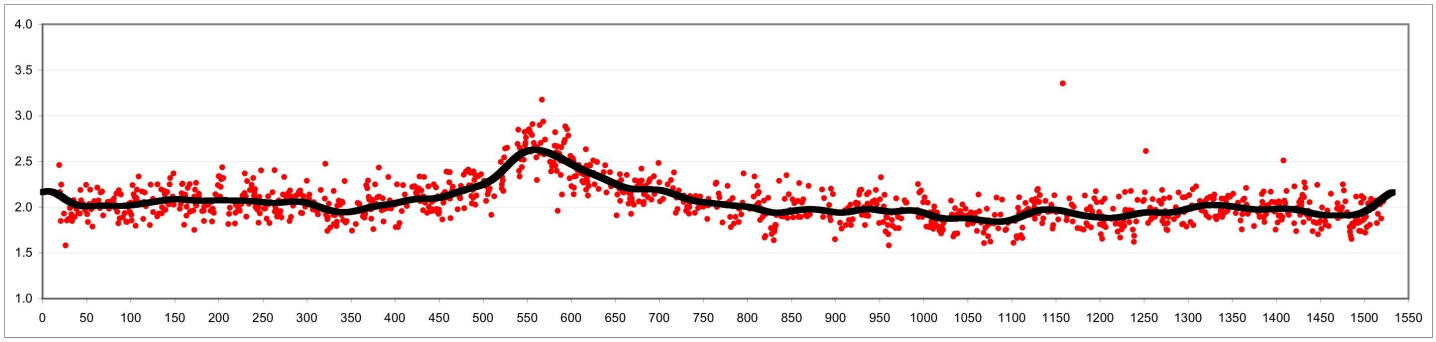

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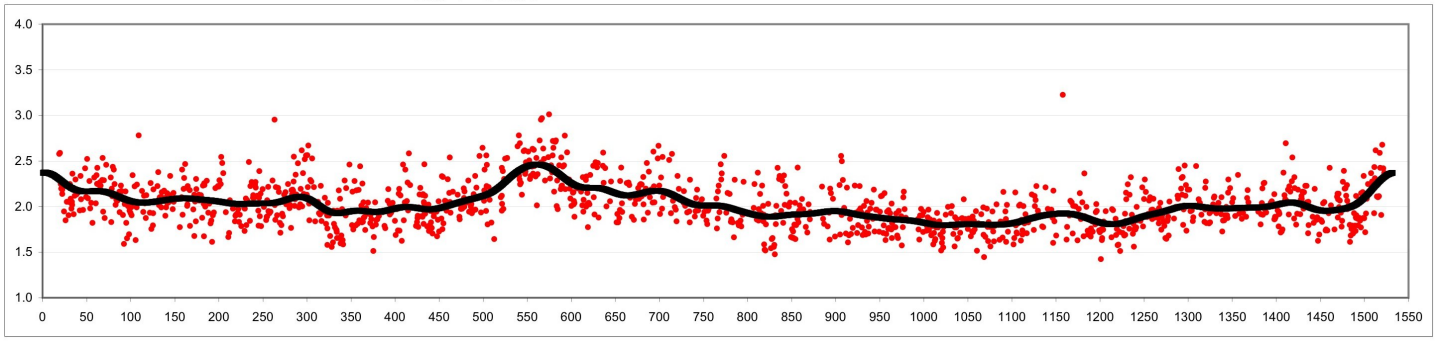

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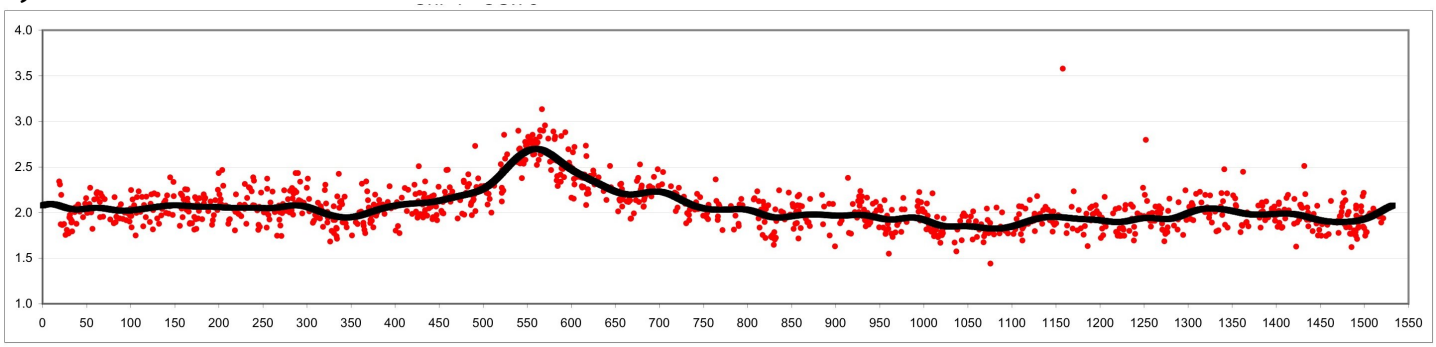

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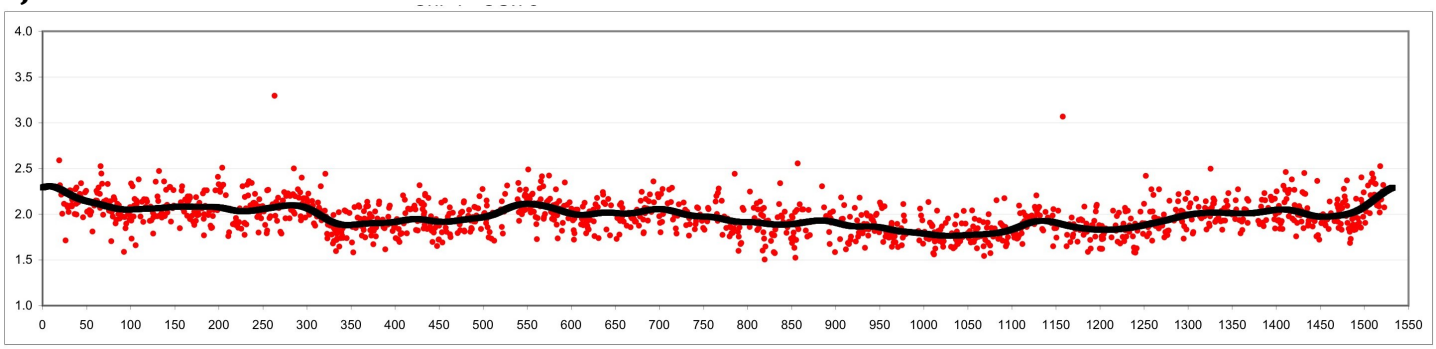

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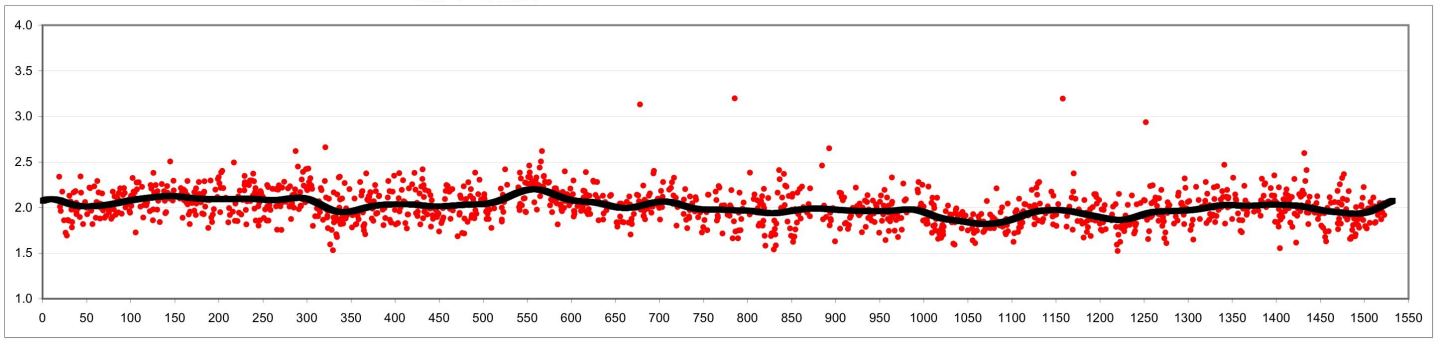
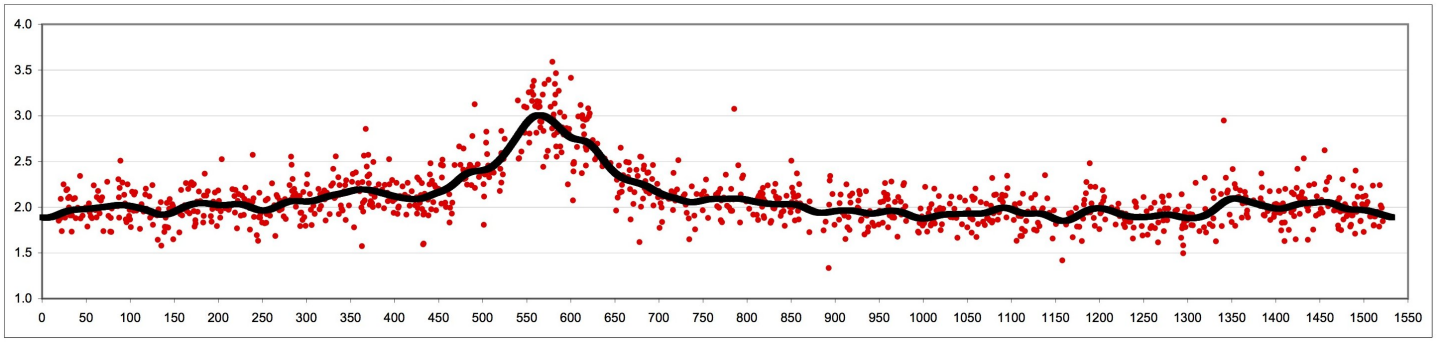
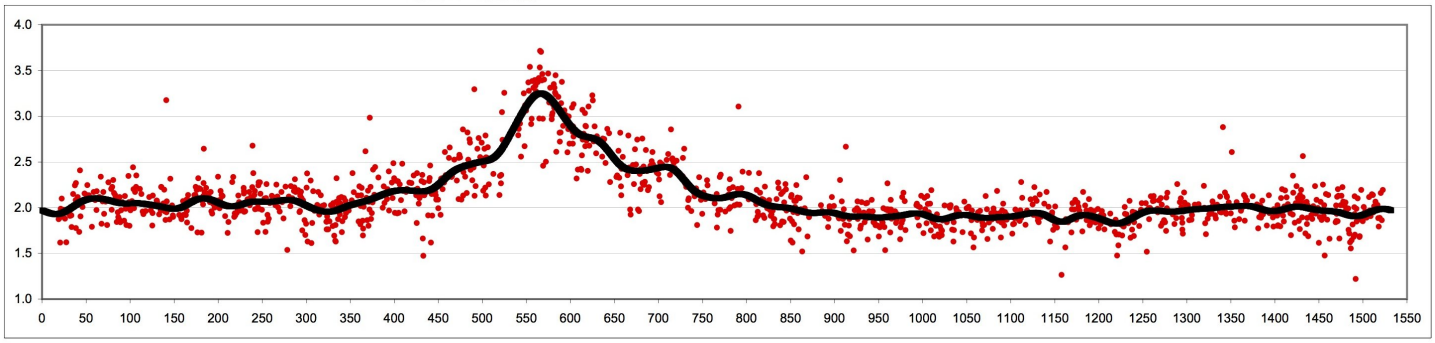


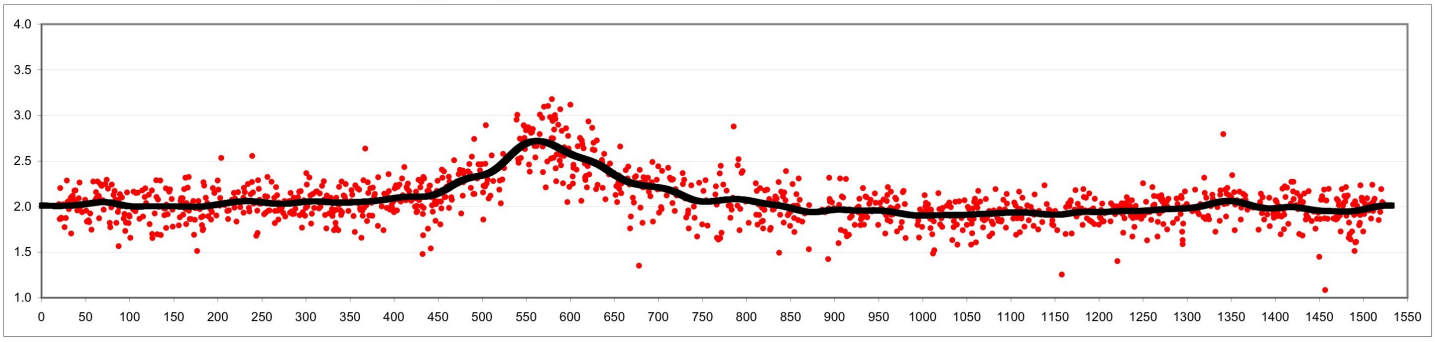
YJL8398 – control for even linkers



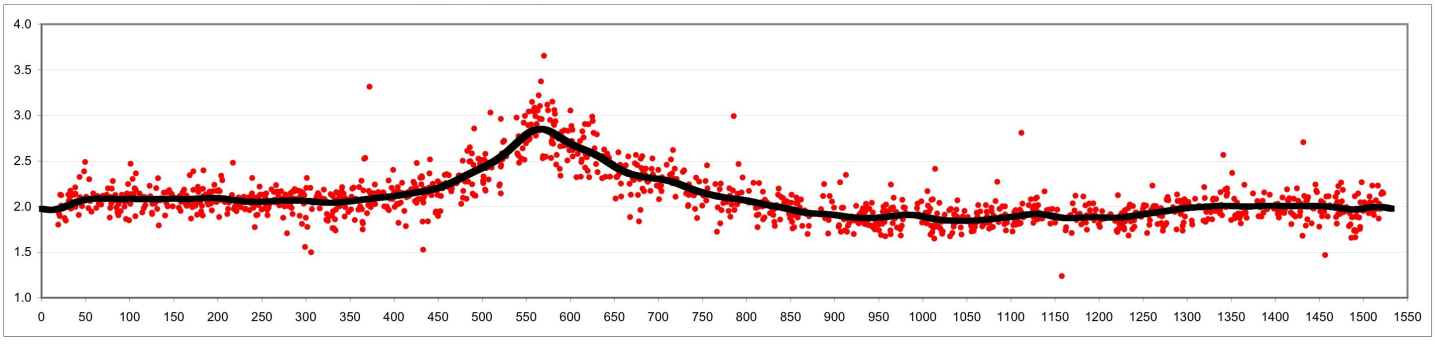
YJL8398 – control for even linkers



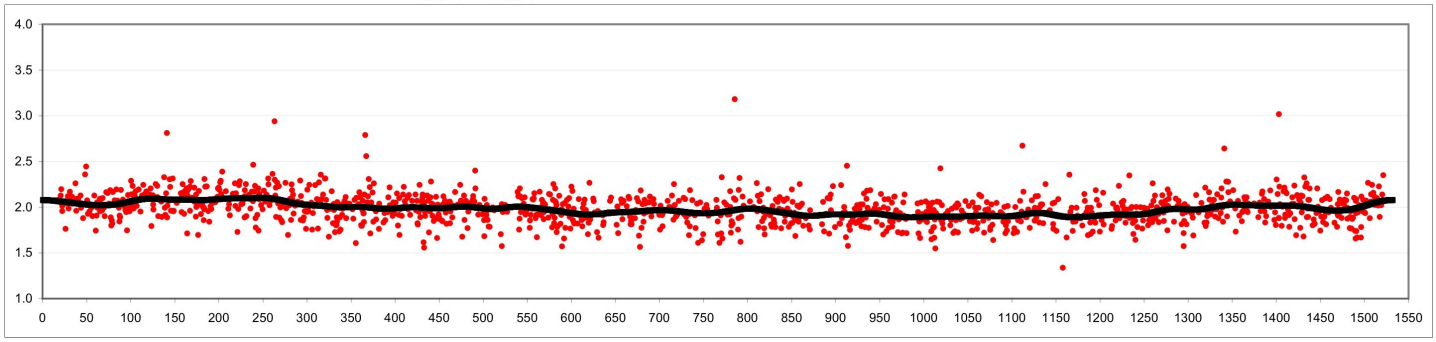
YJL8973 – Linker L2



YJL8973 – Linker L2



YJL8975 – Linker L4



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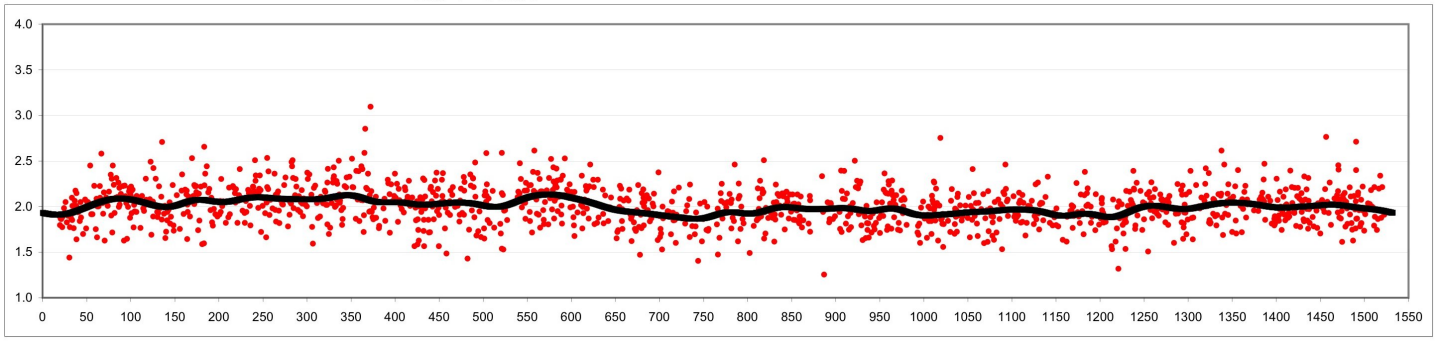
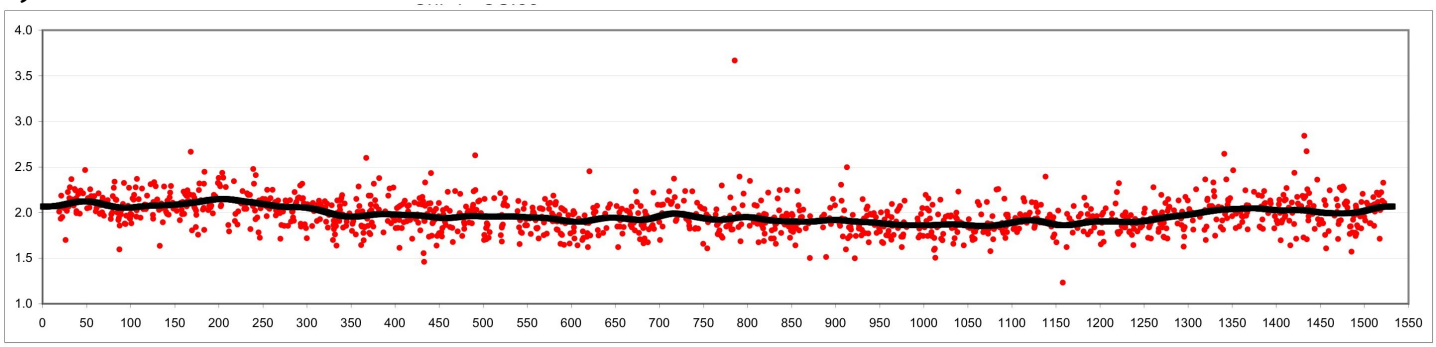


YJL9016 – Linker L6



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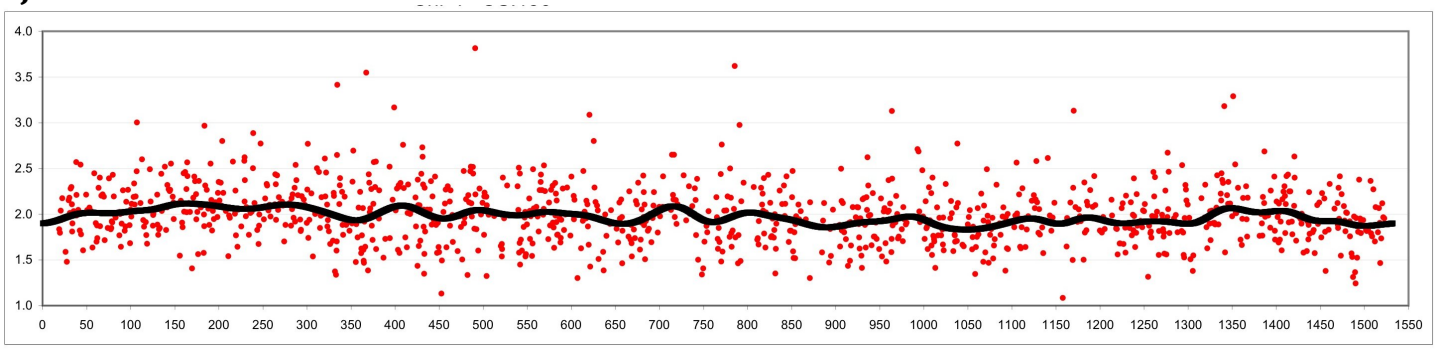
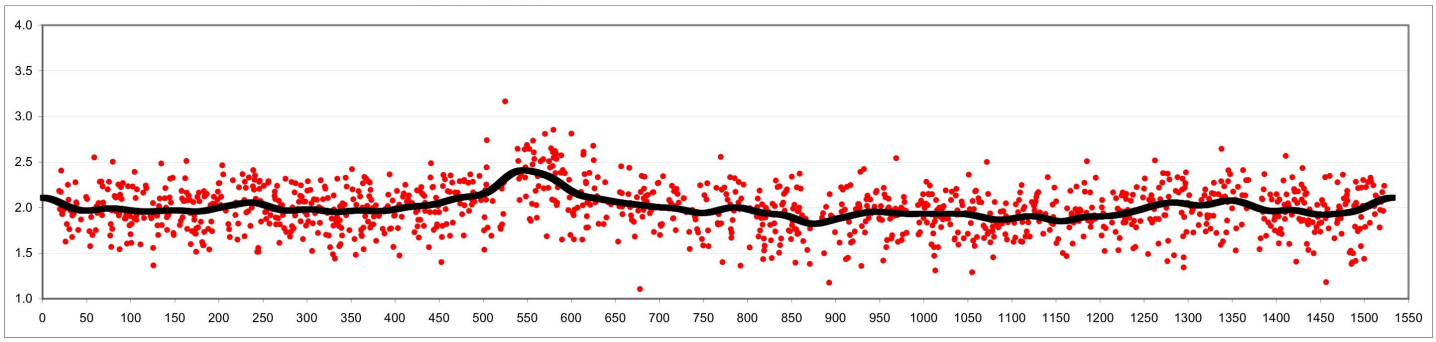


YJL8977 – Linker L8



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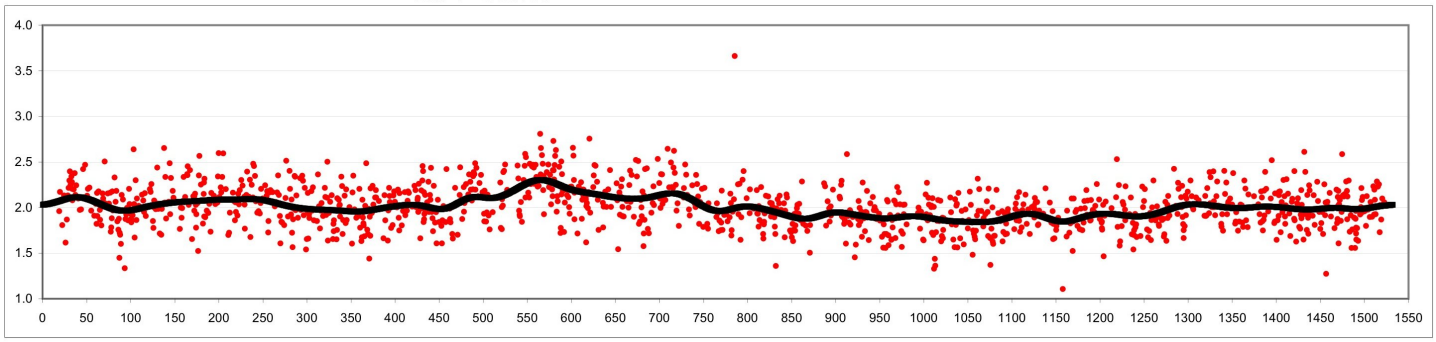

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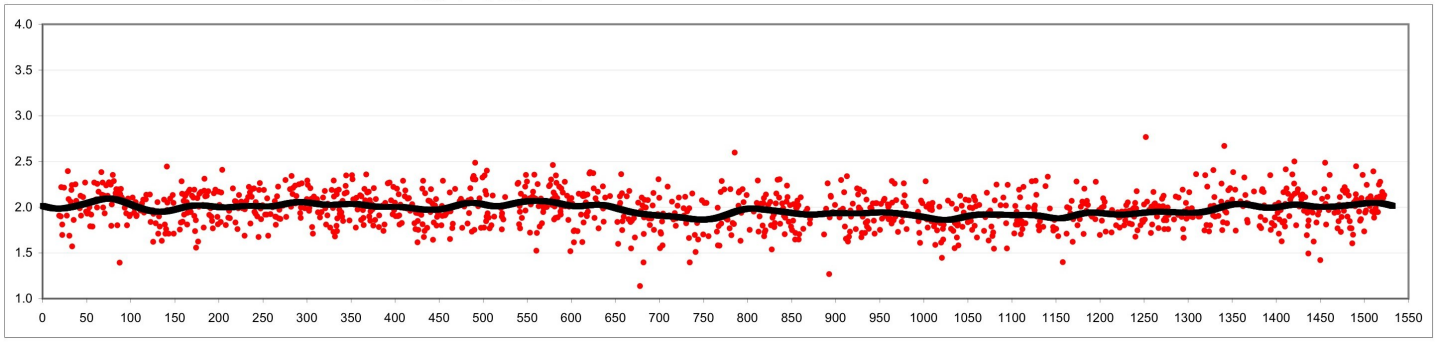

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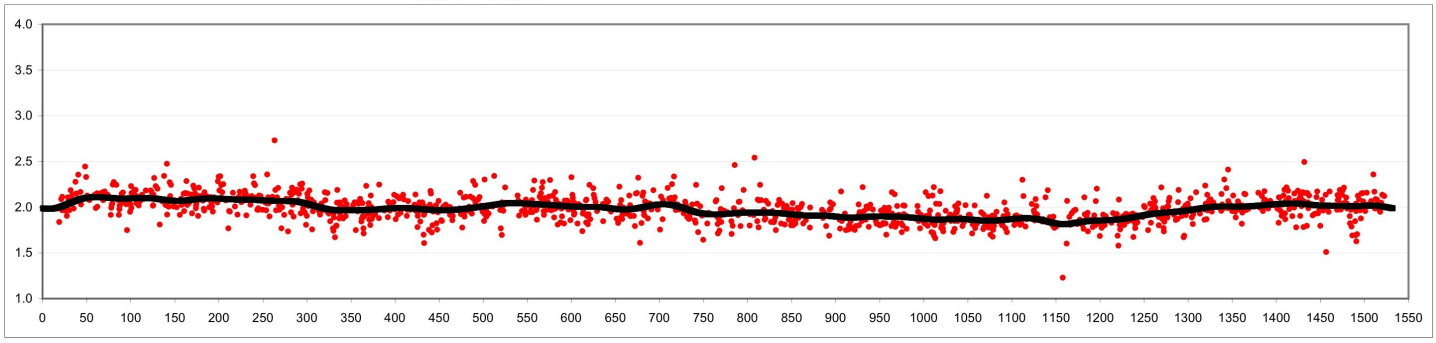
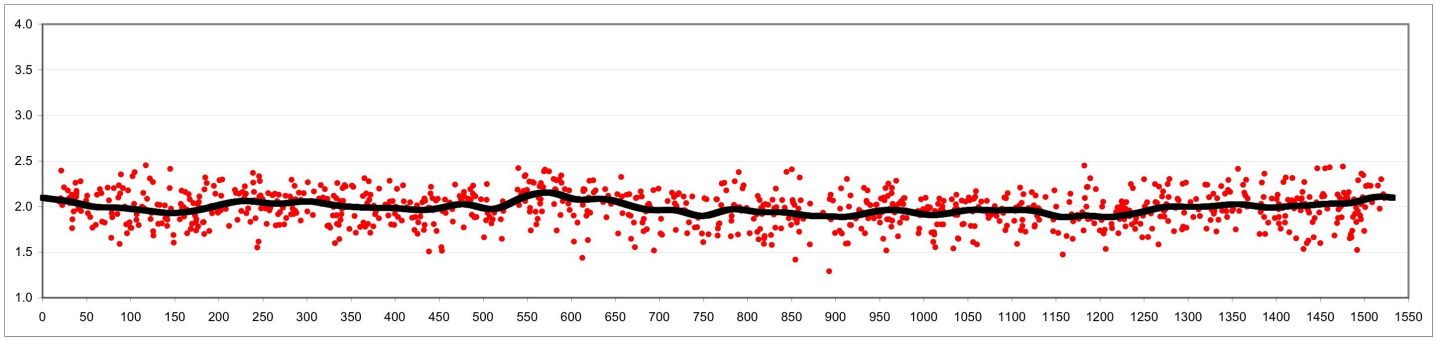
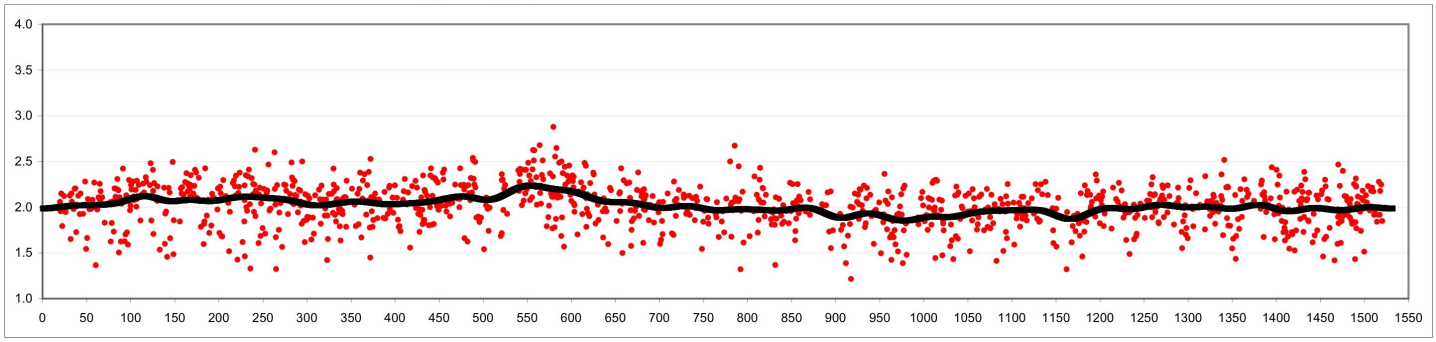


YJL8981 – Linker L12



YJL8981 – Linker L12



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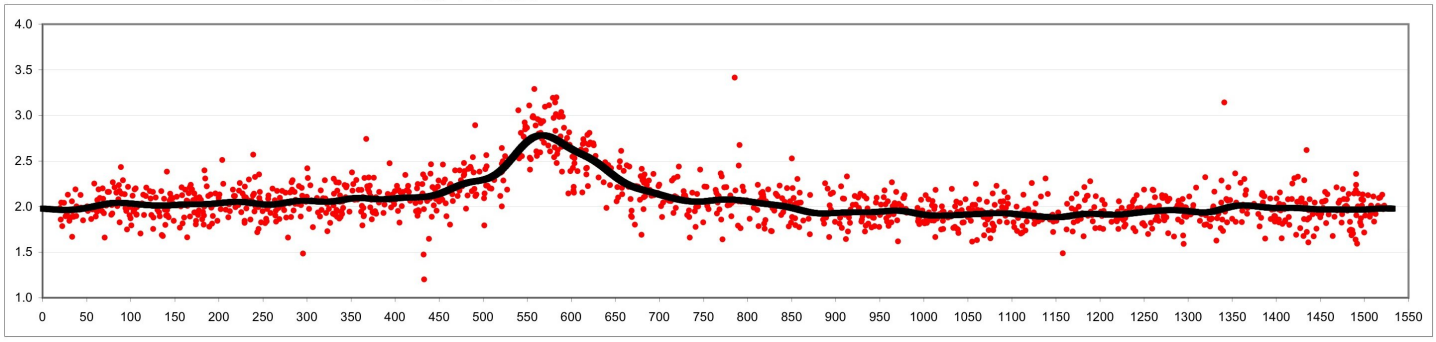

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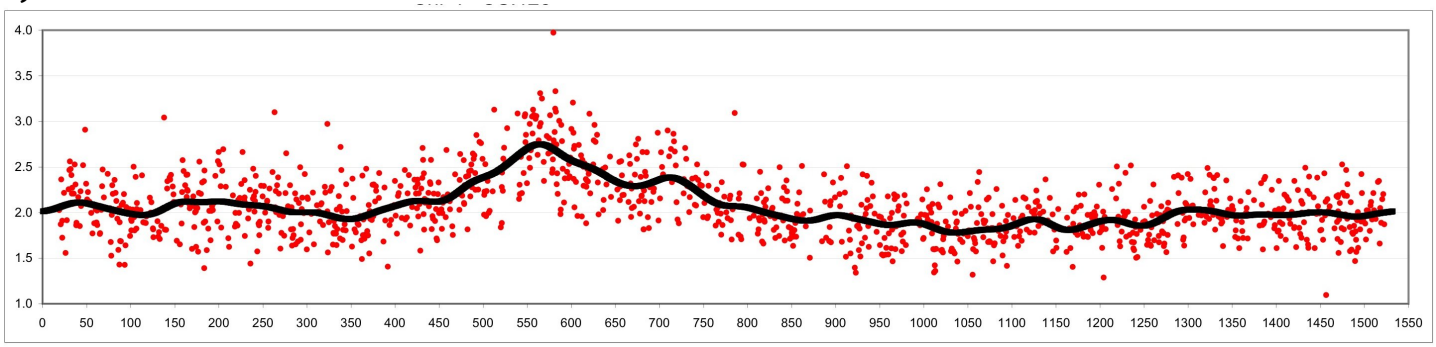
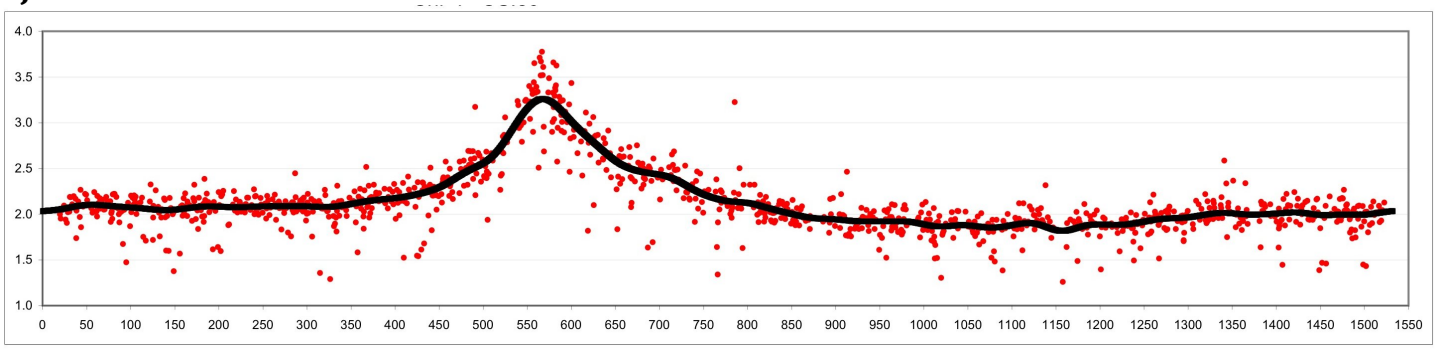
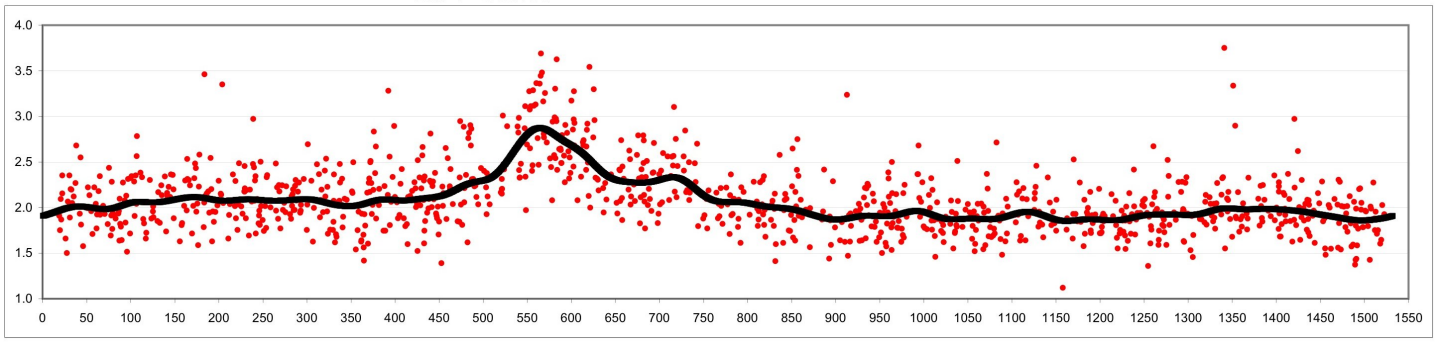


YJL9018 – Linker L16



YJL9018 – Linker L16



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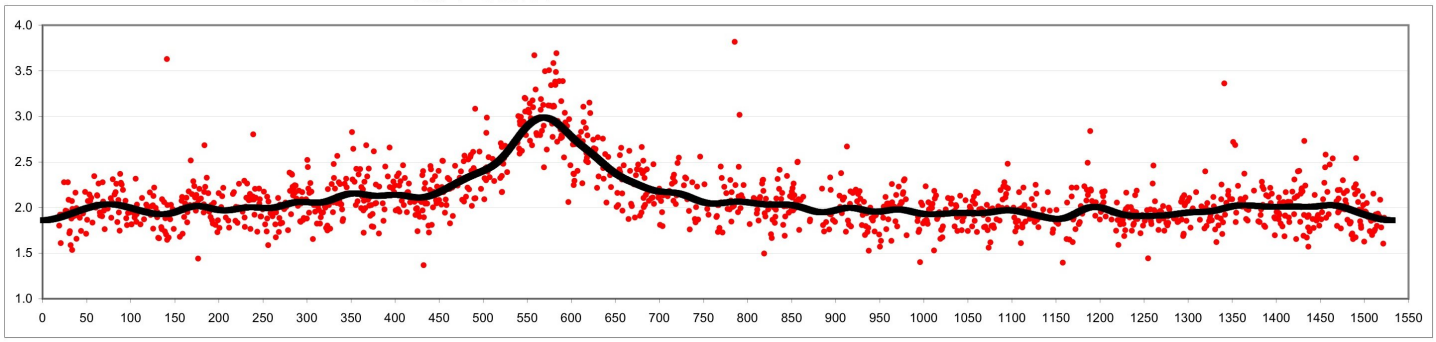
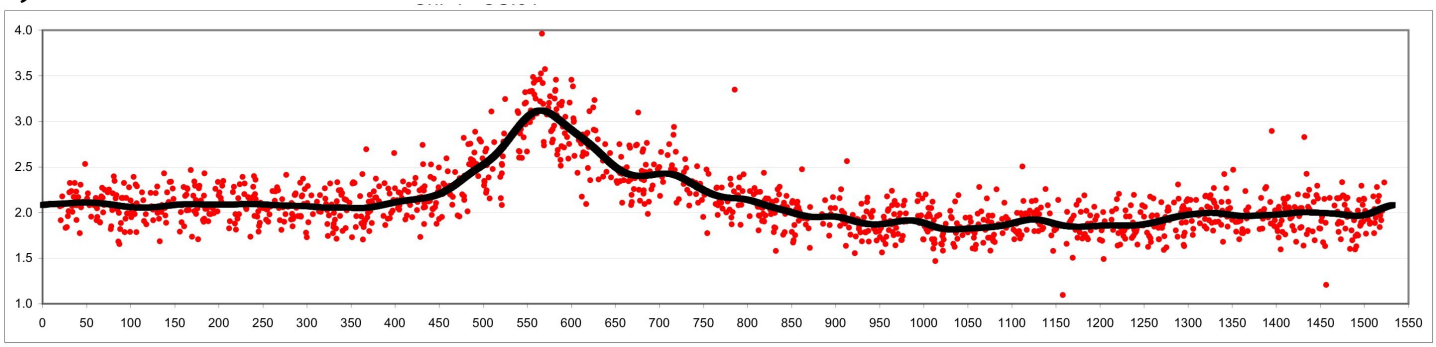
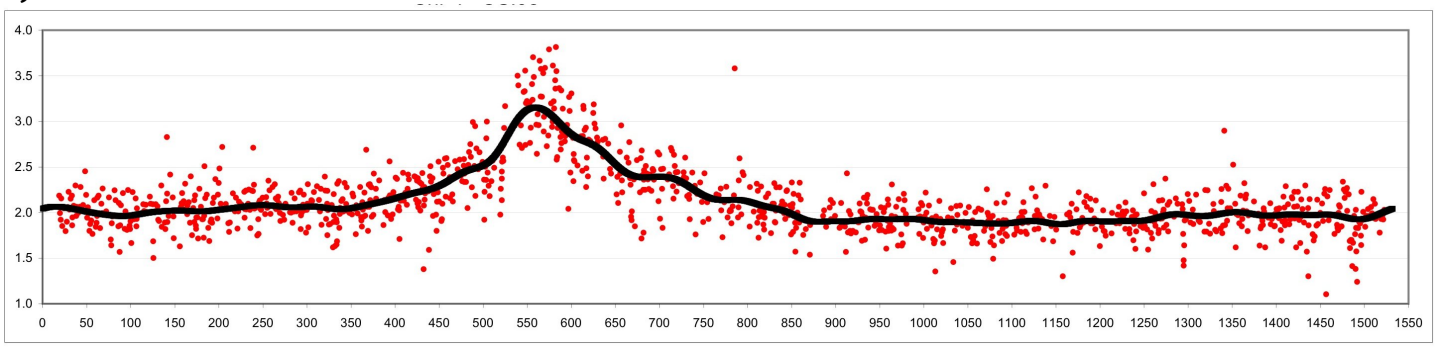


YJL8985 – Linker L18



YJL8987 – Linker L20



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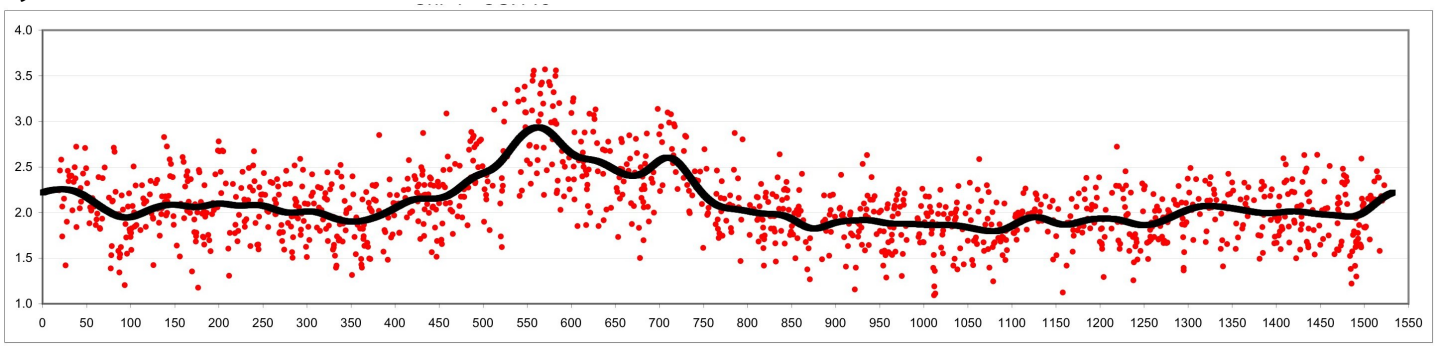

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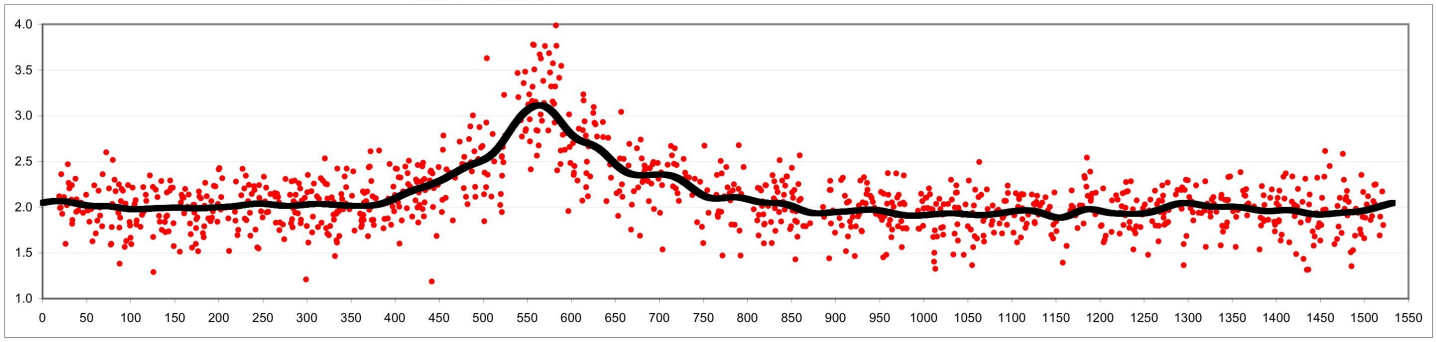

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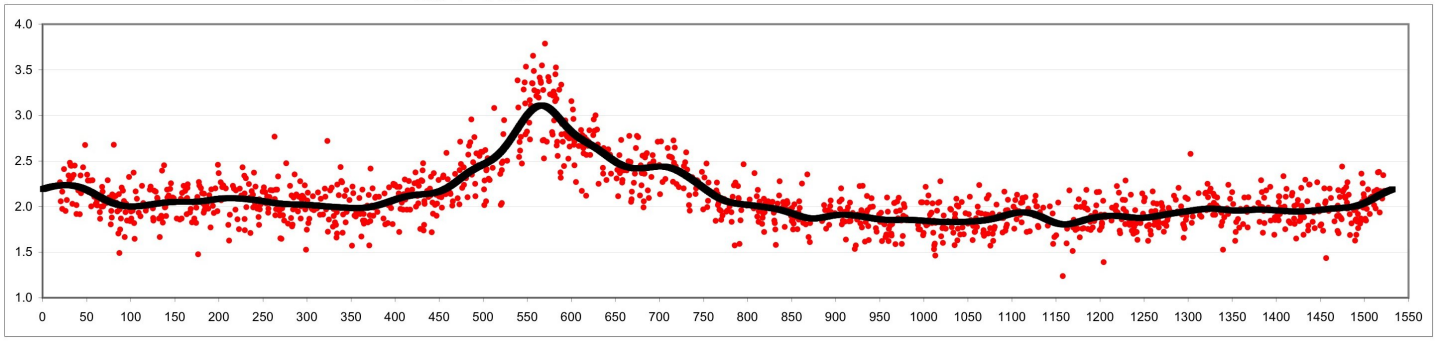
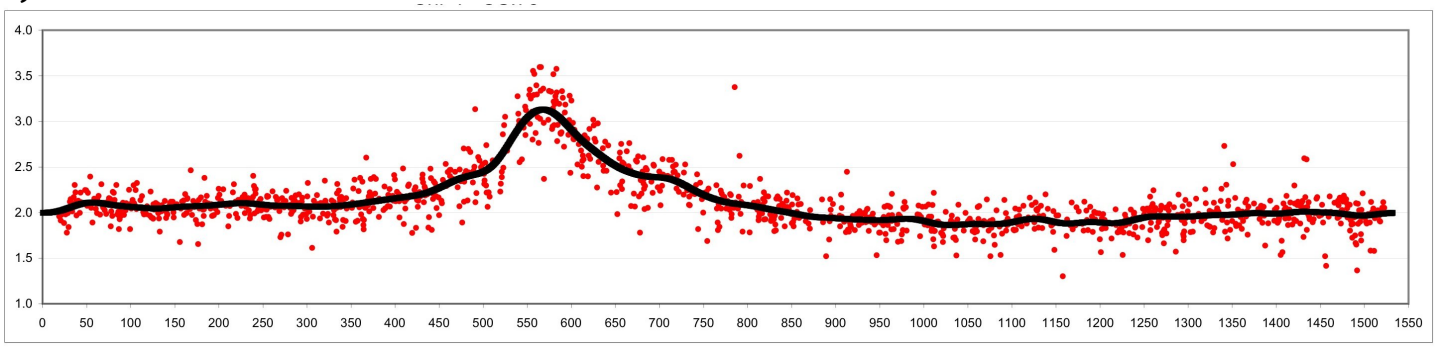


YJL9020 – Linker L24



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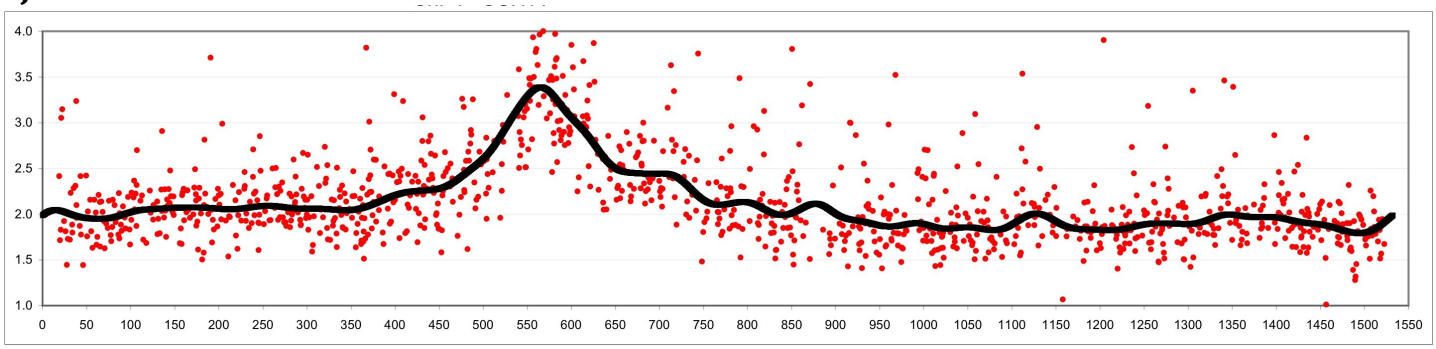
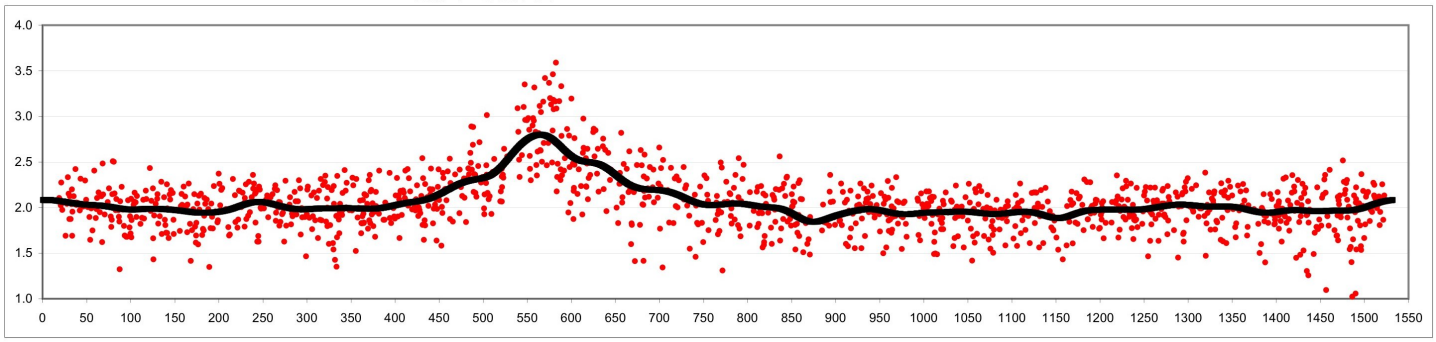


YJL8991 – Linker L26



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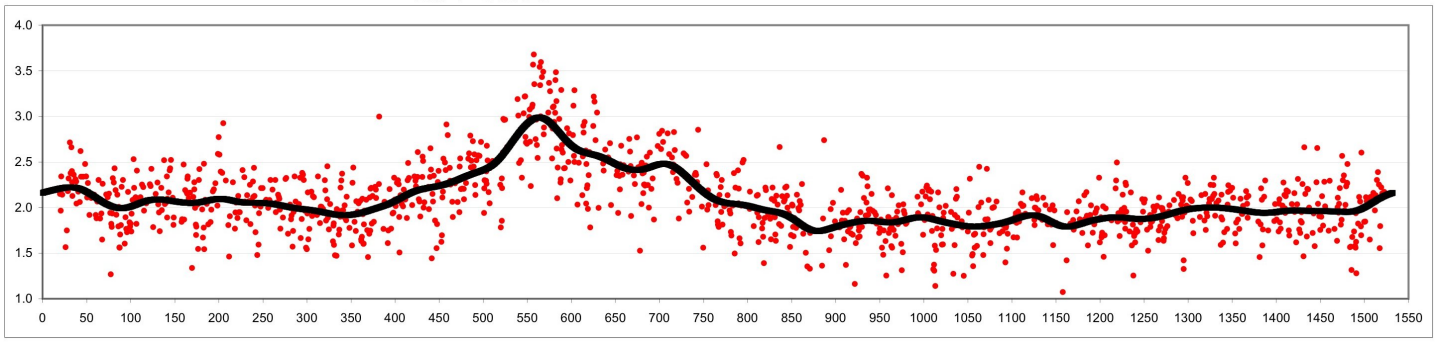

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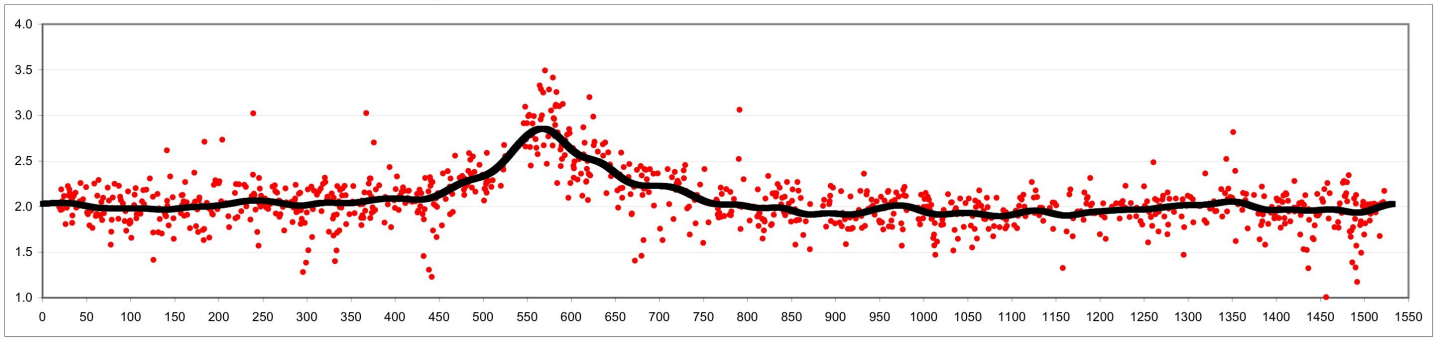

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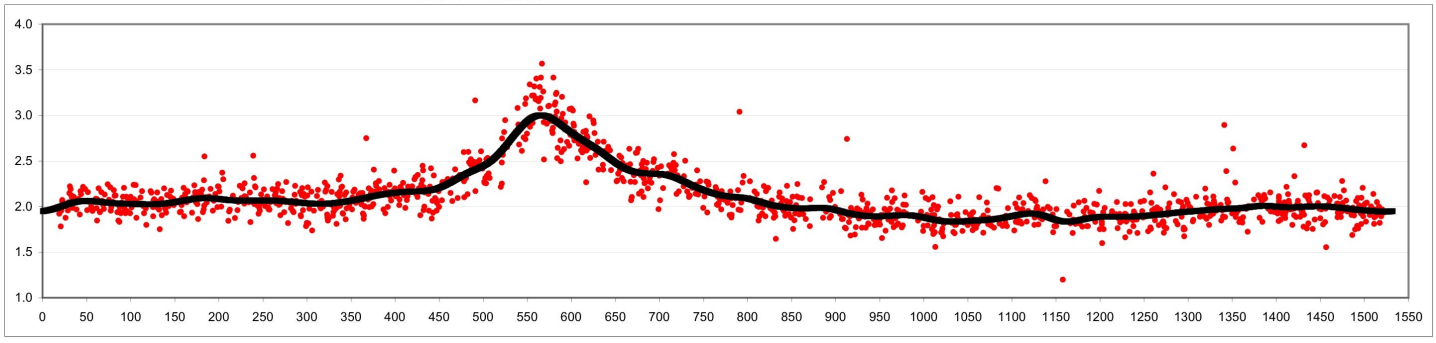
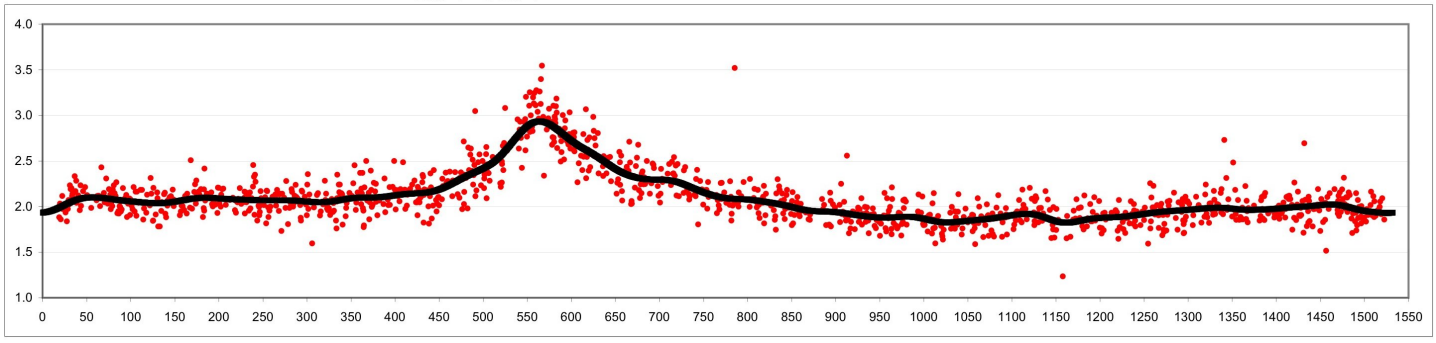


YJL9022 – Linker L30



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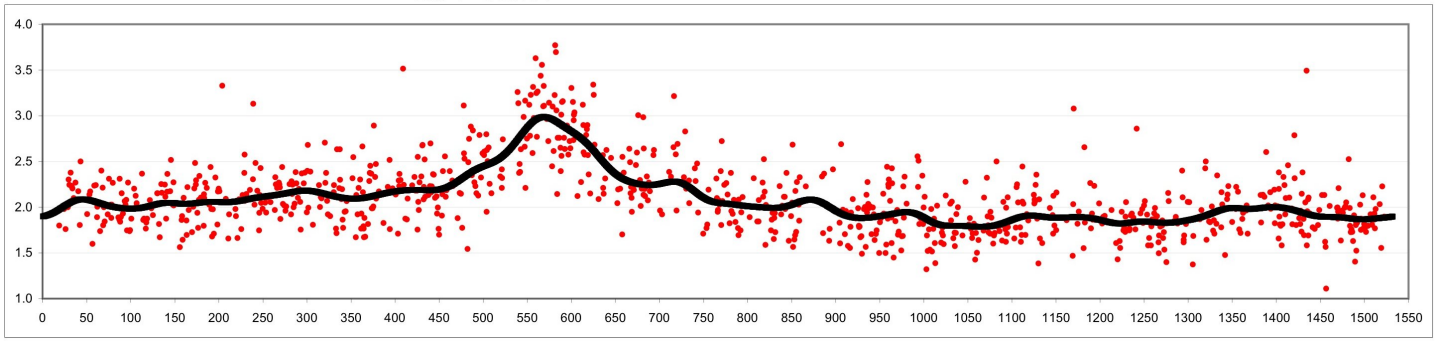

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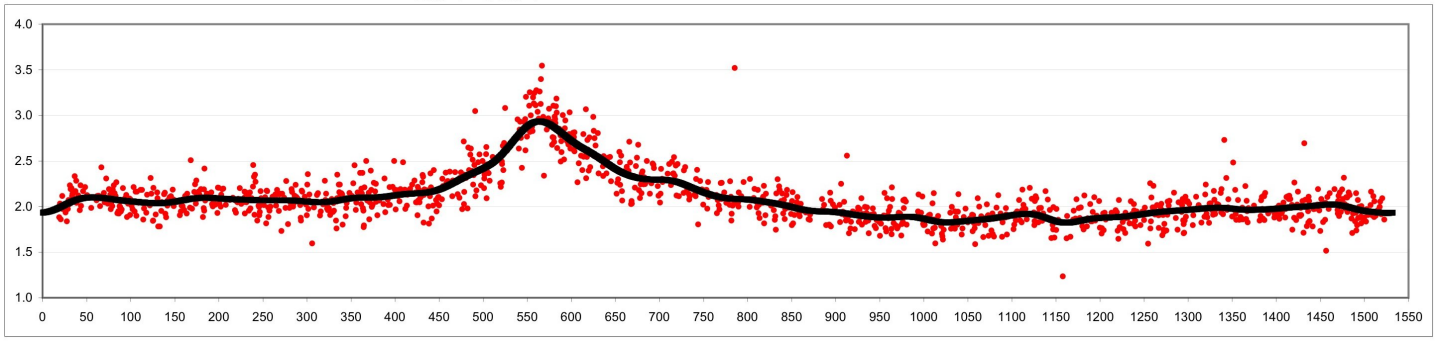

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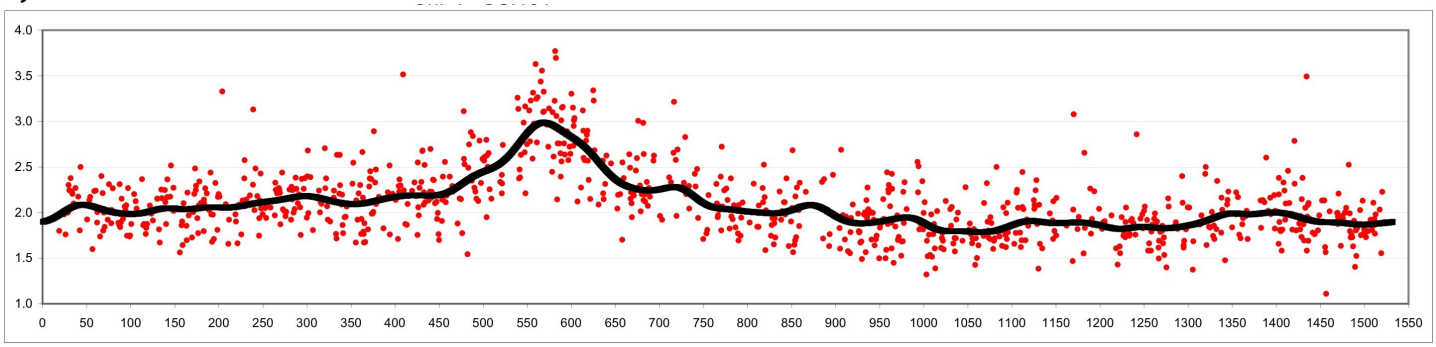
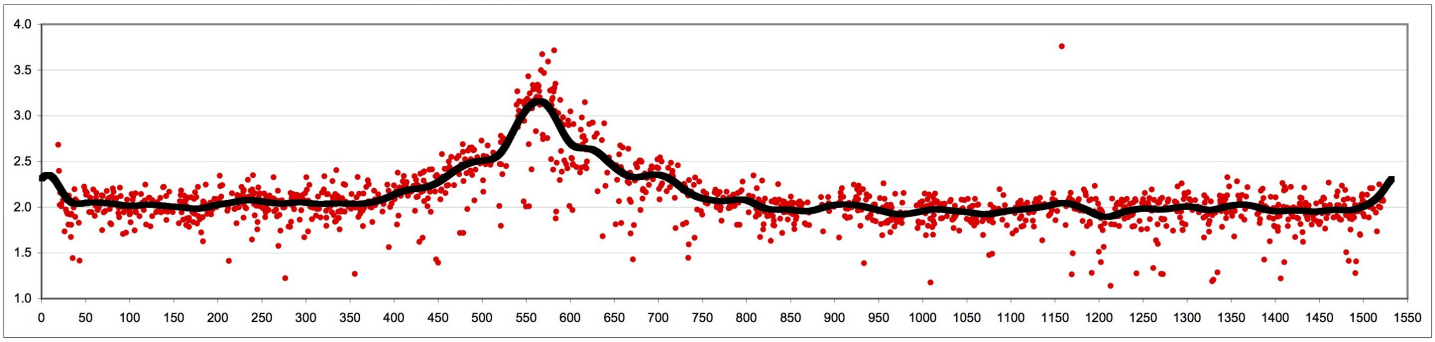
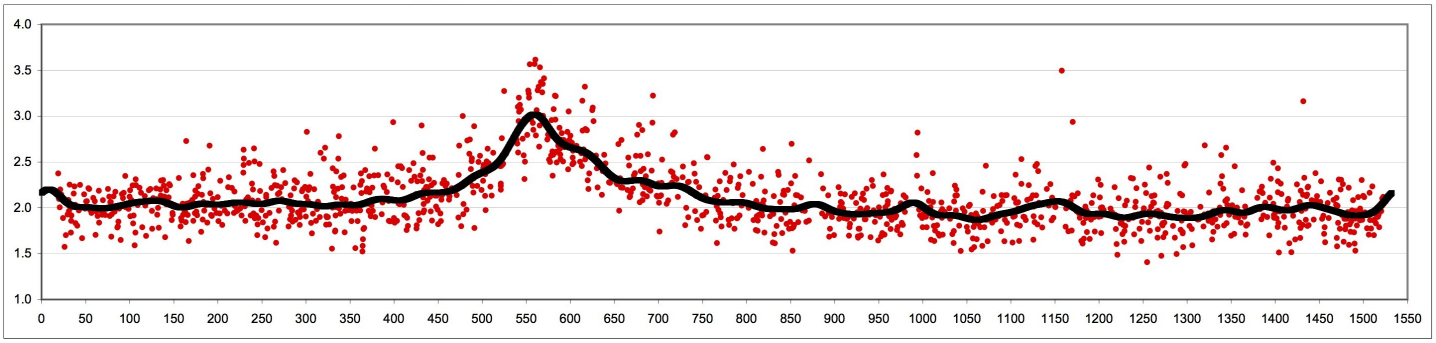


Figure 5A
YJL8398 – Control Strain



YJL8398 – Control Strain



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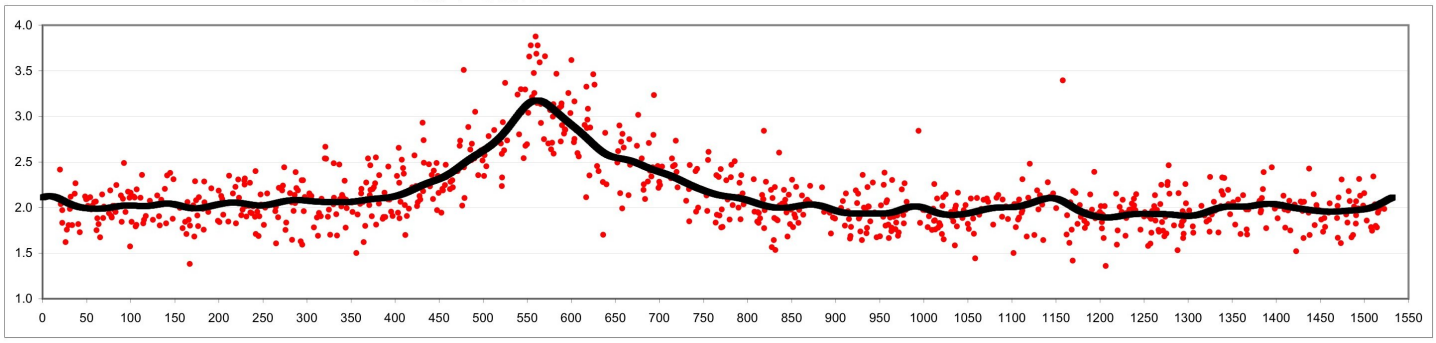

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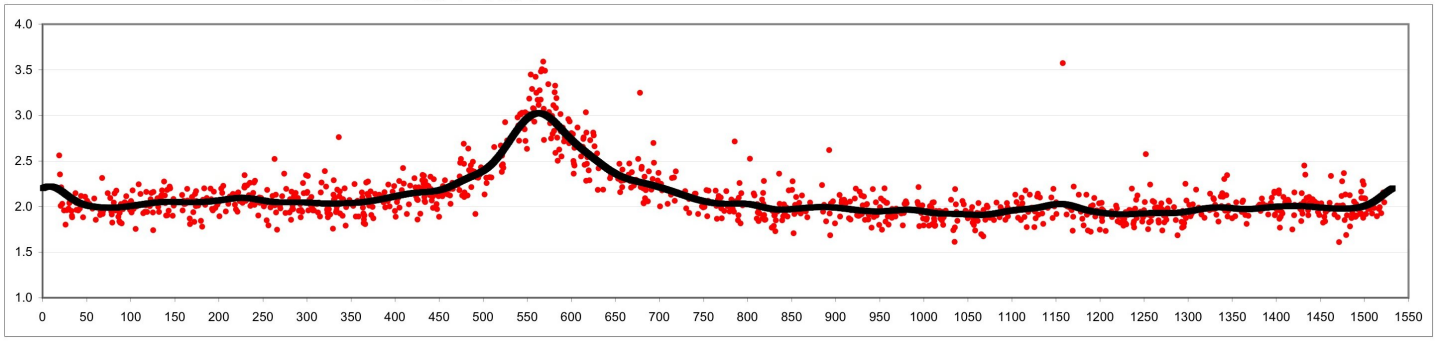

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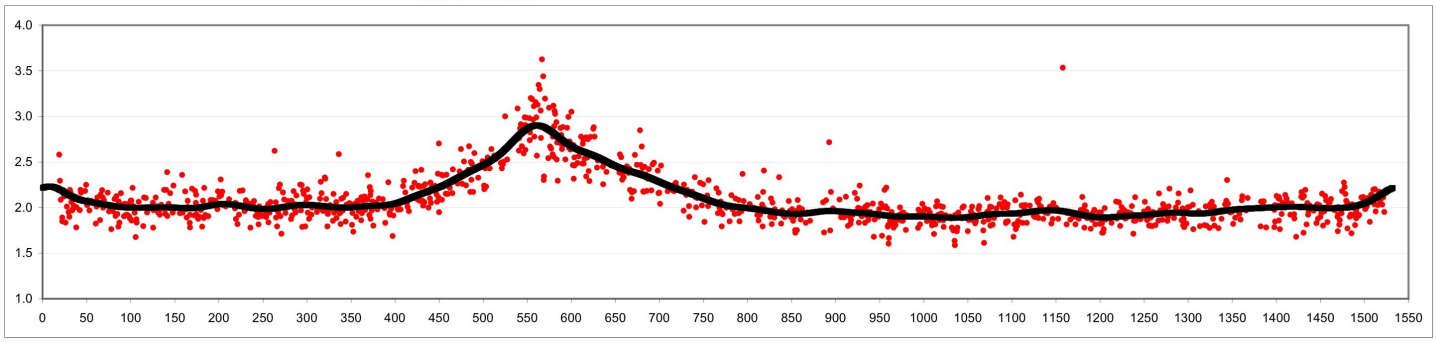

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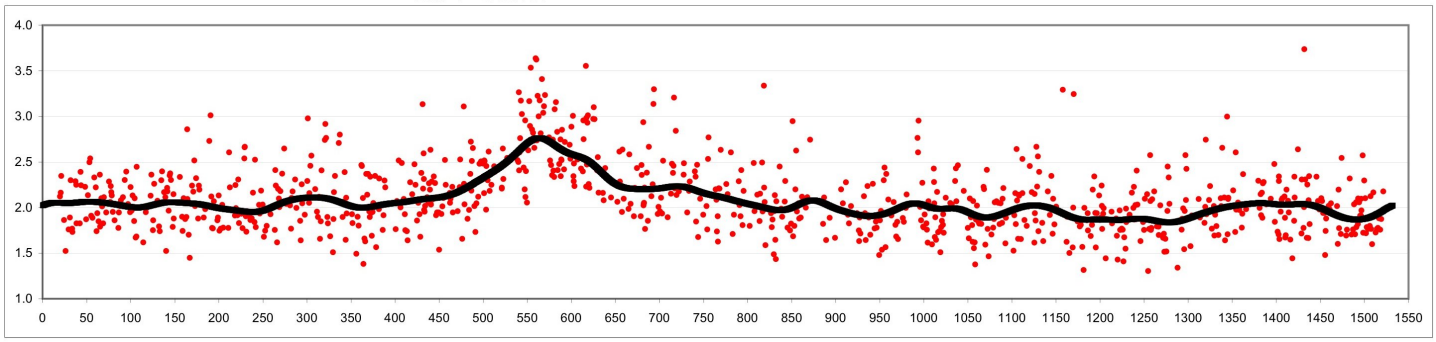

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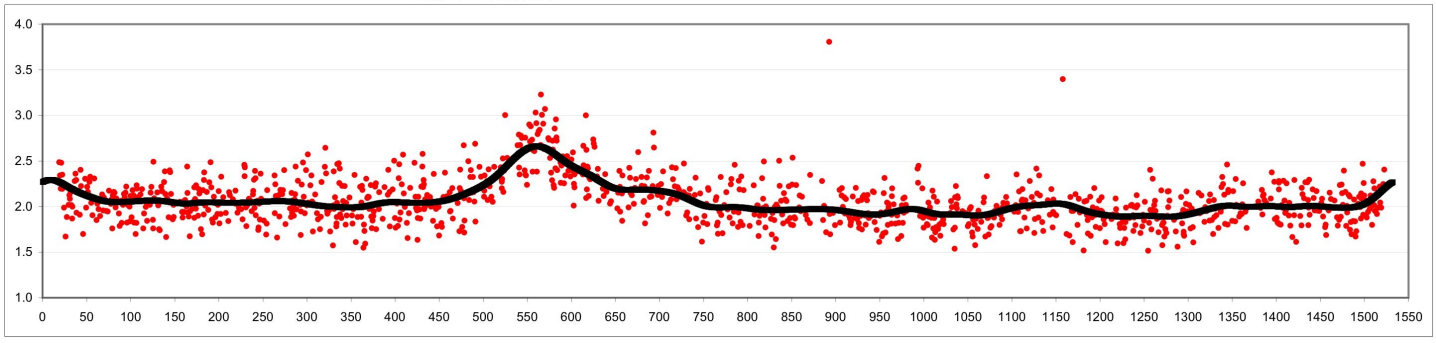

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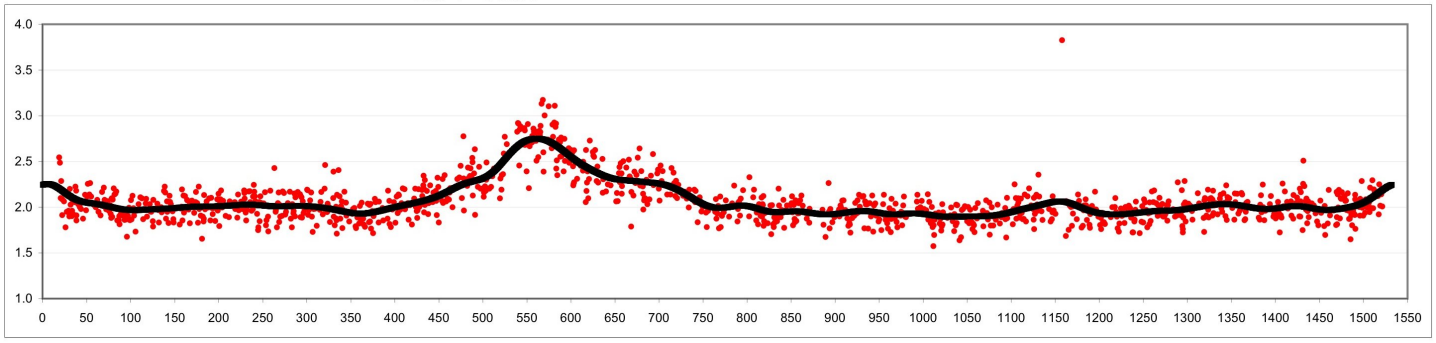

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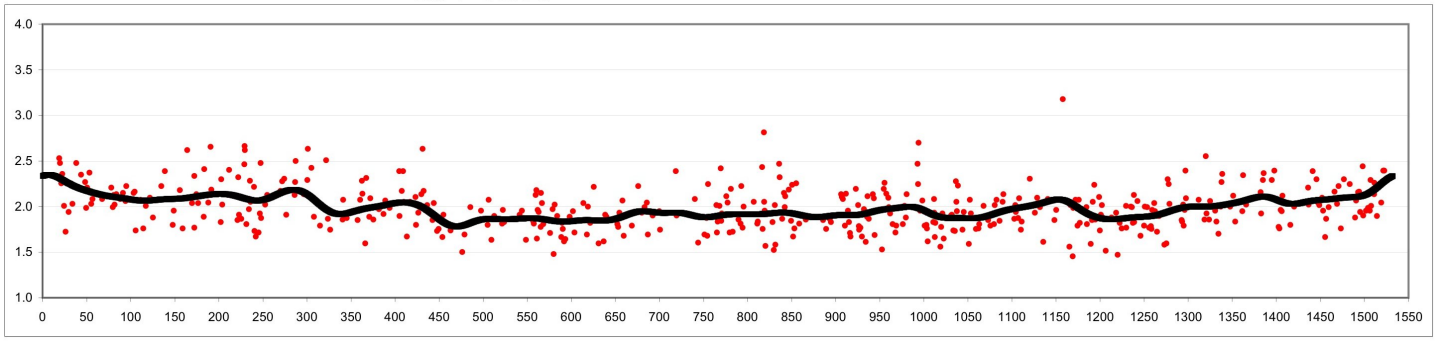

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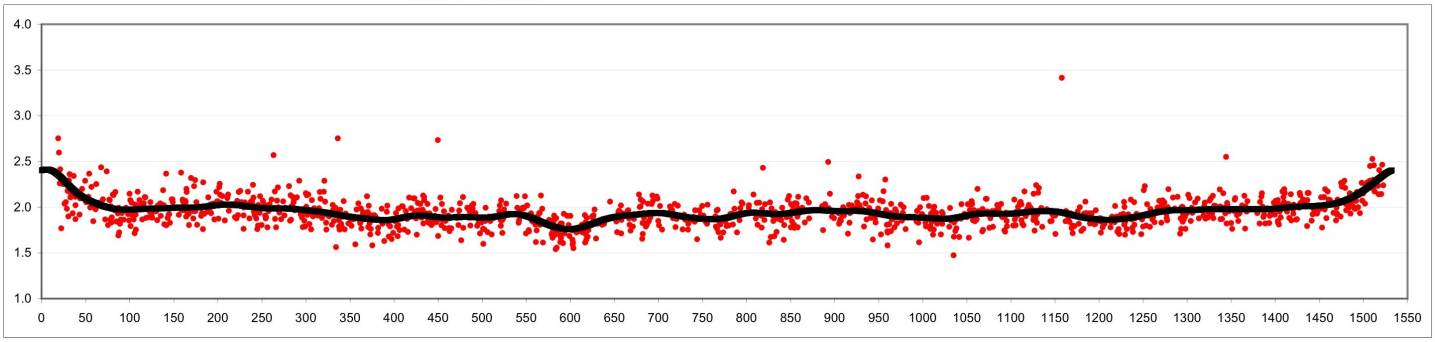

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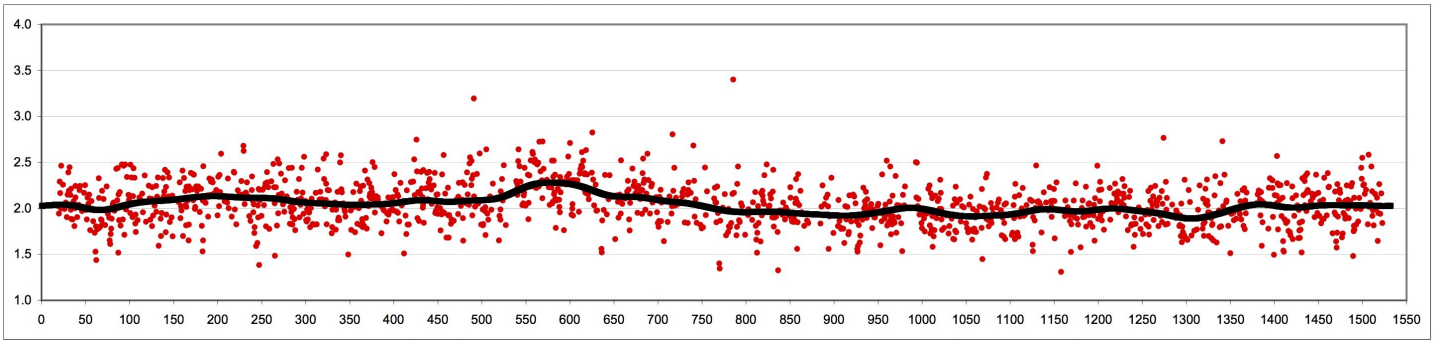

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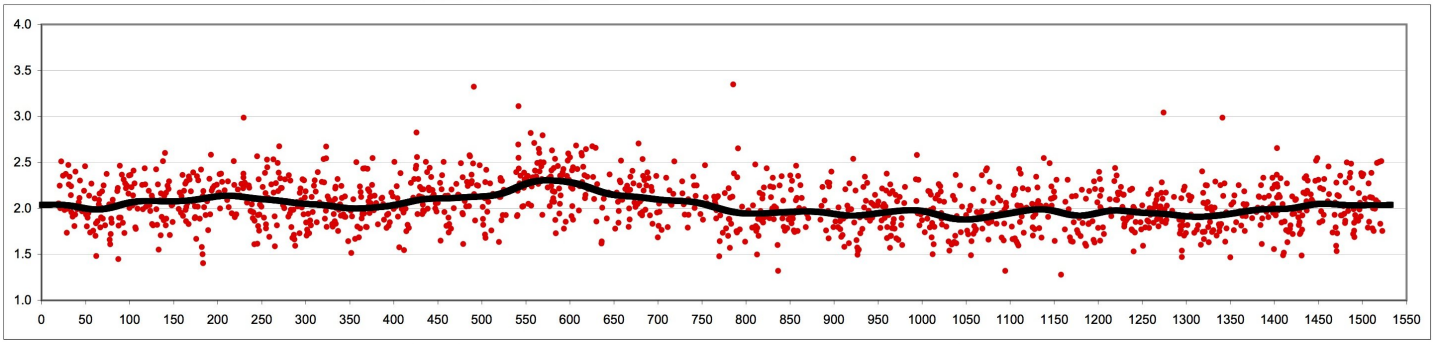

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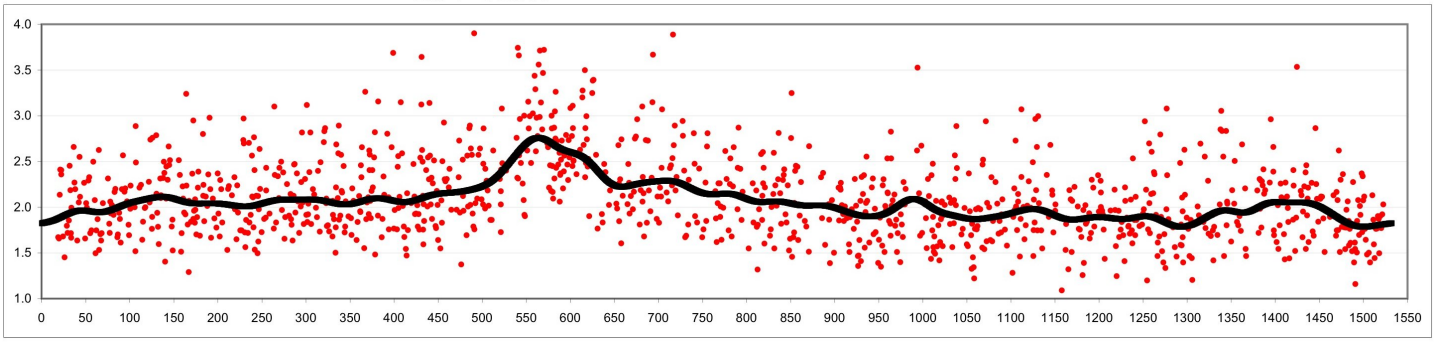

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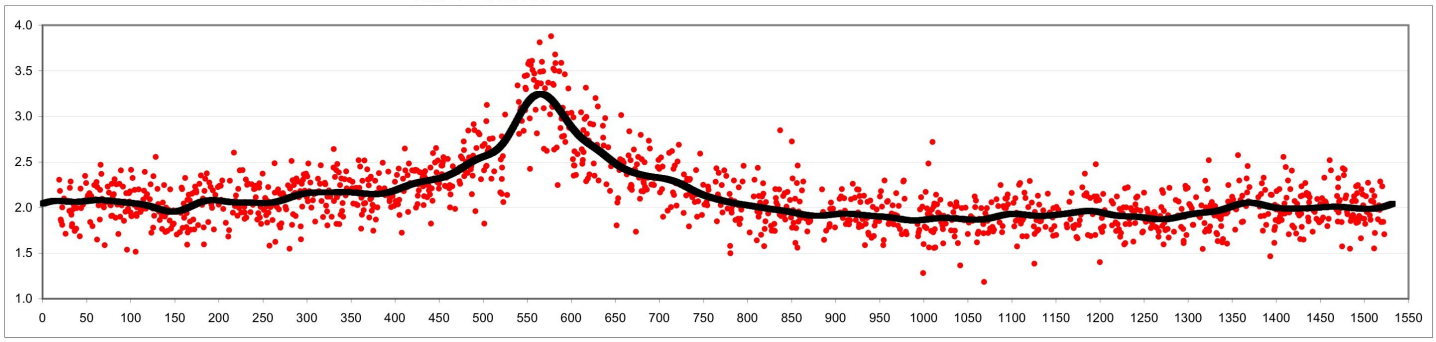

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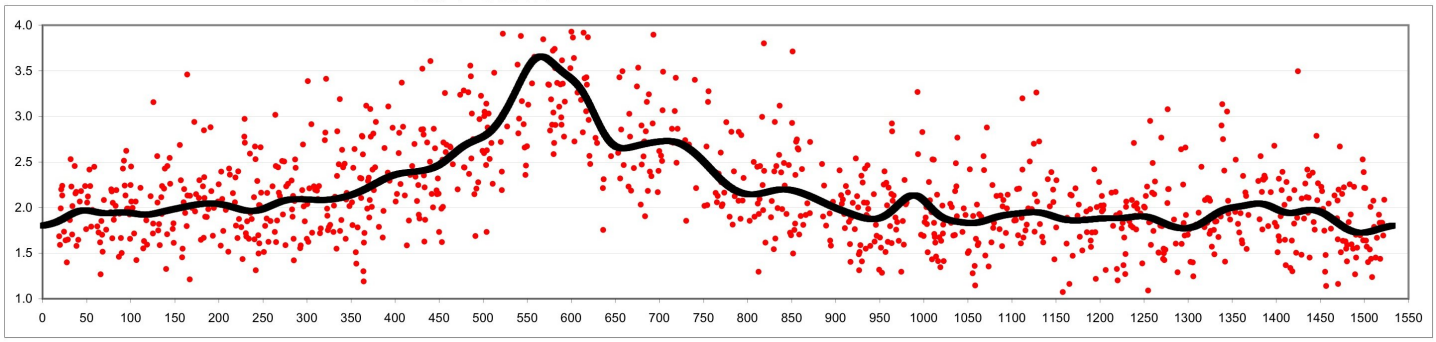

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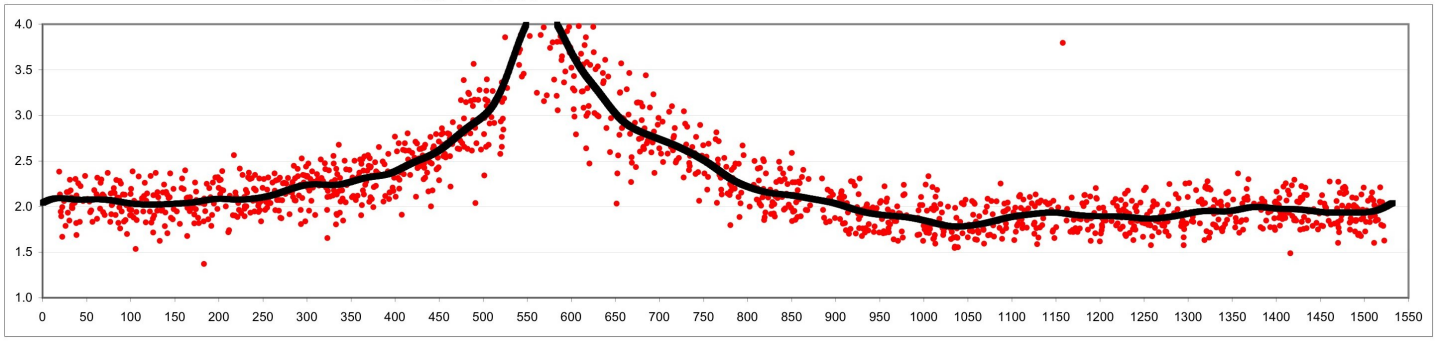
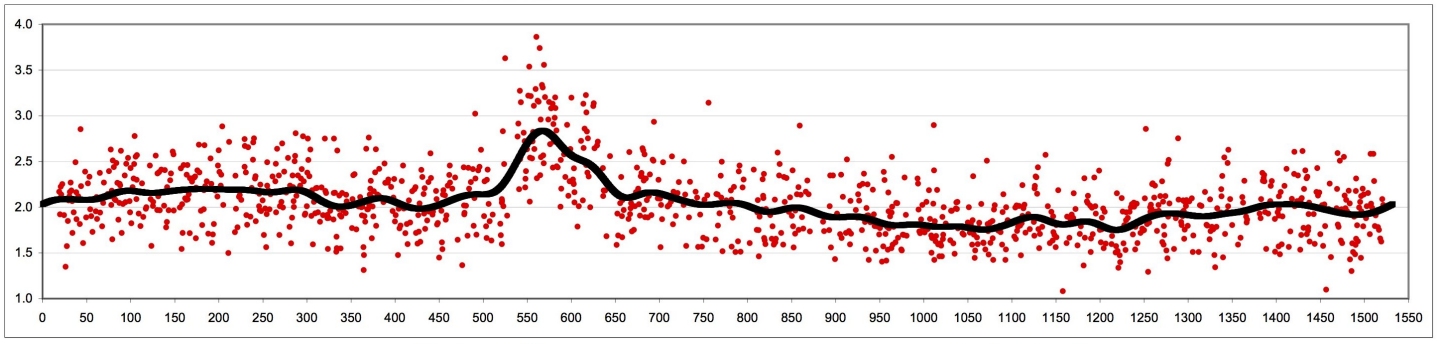


Figure 5B

YJL9566 – Control



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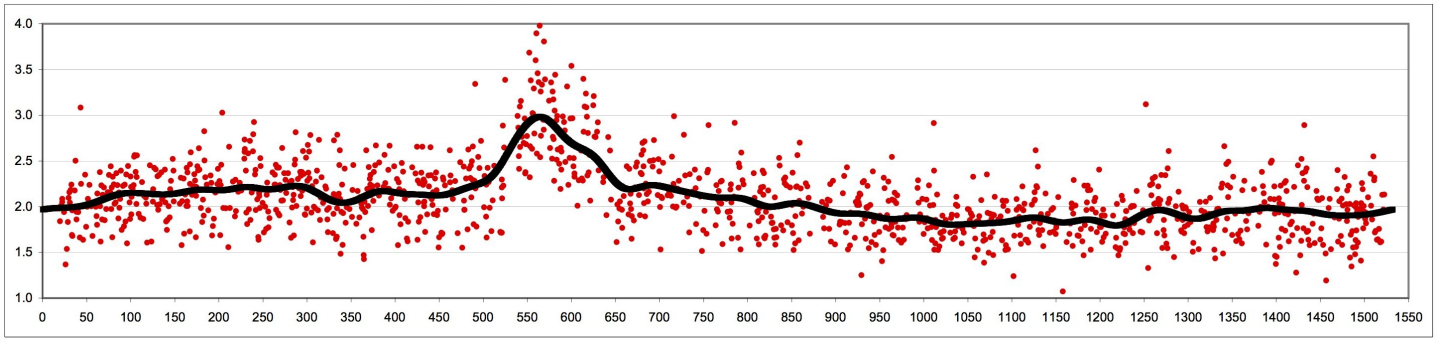

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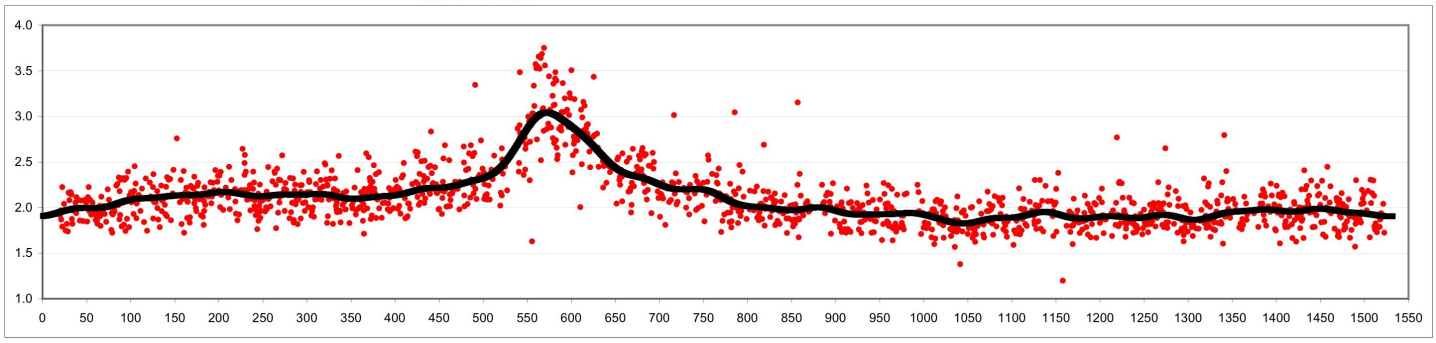

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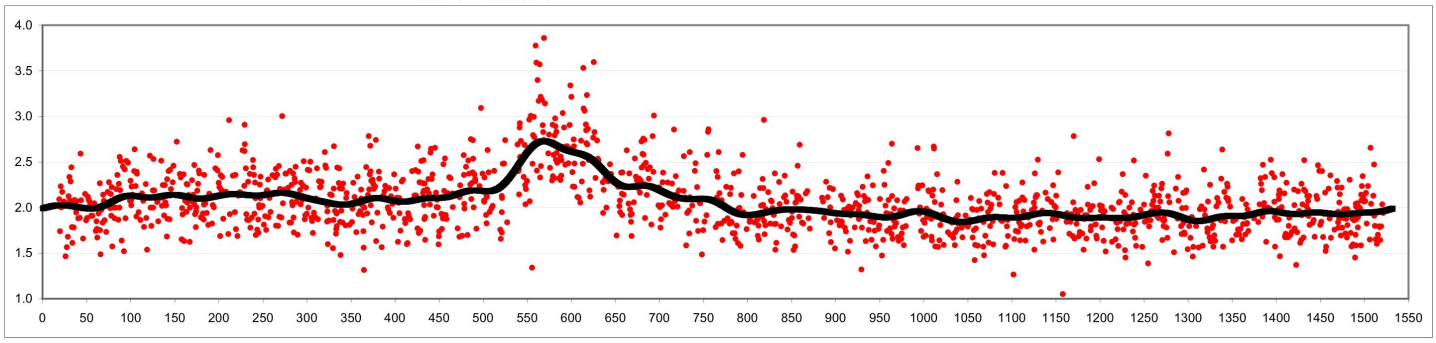

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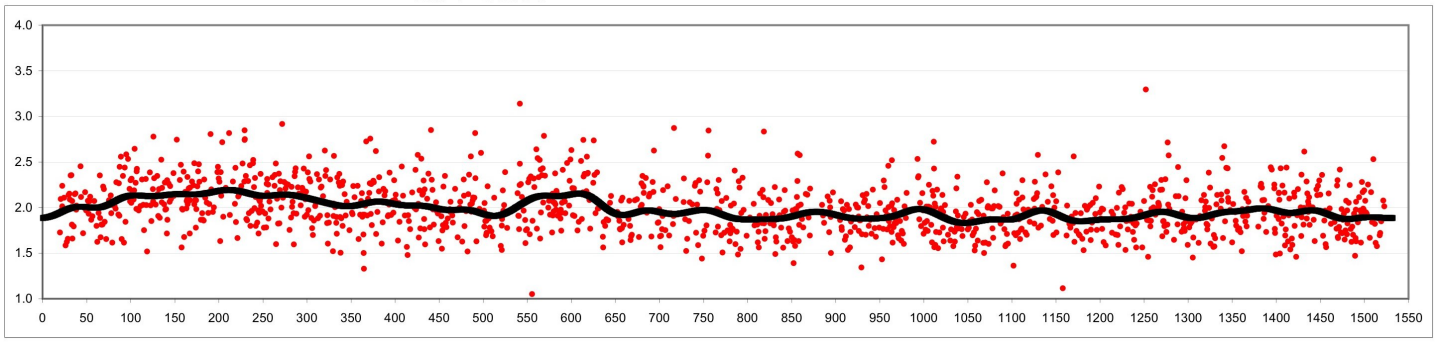

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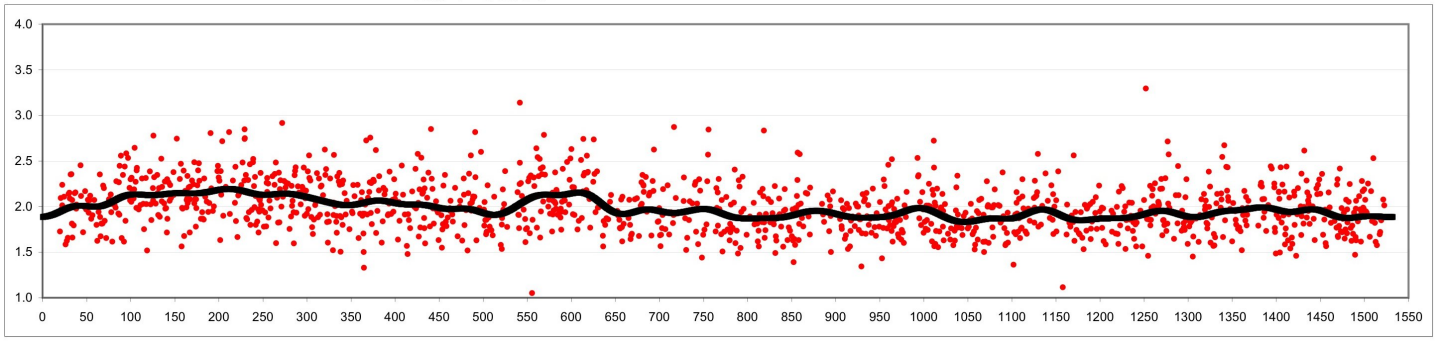

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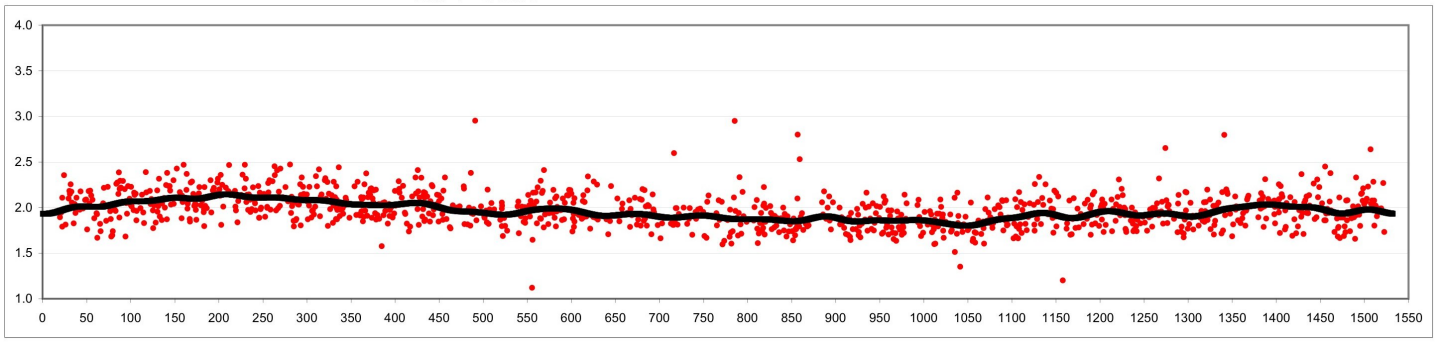

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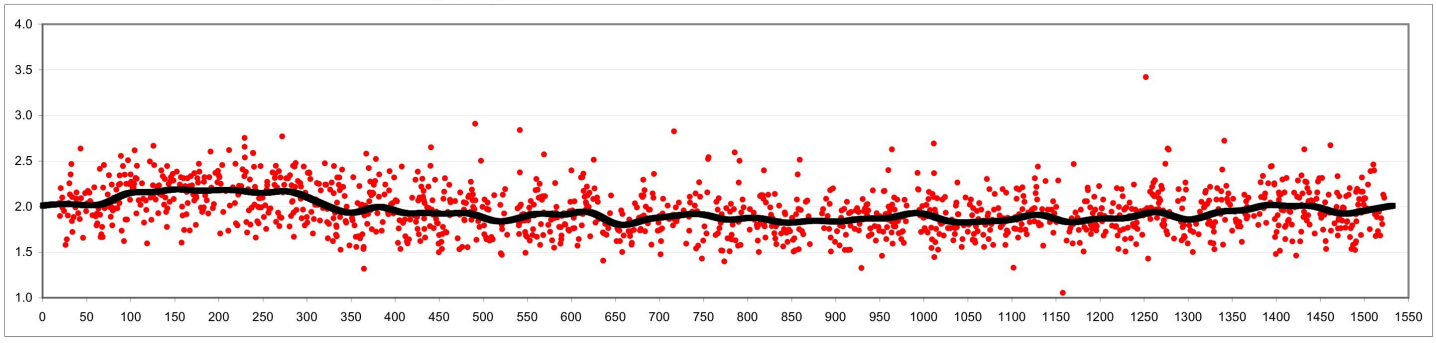

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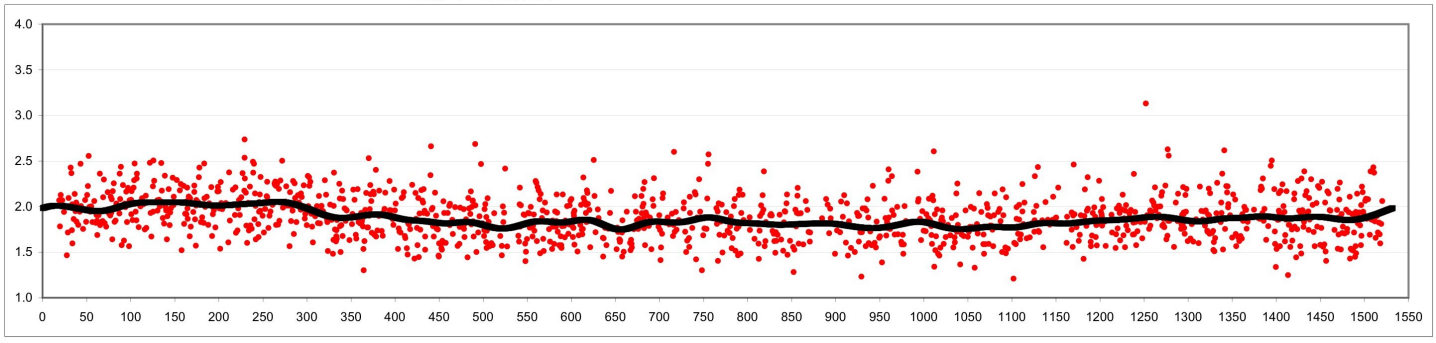

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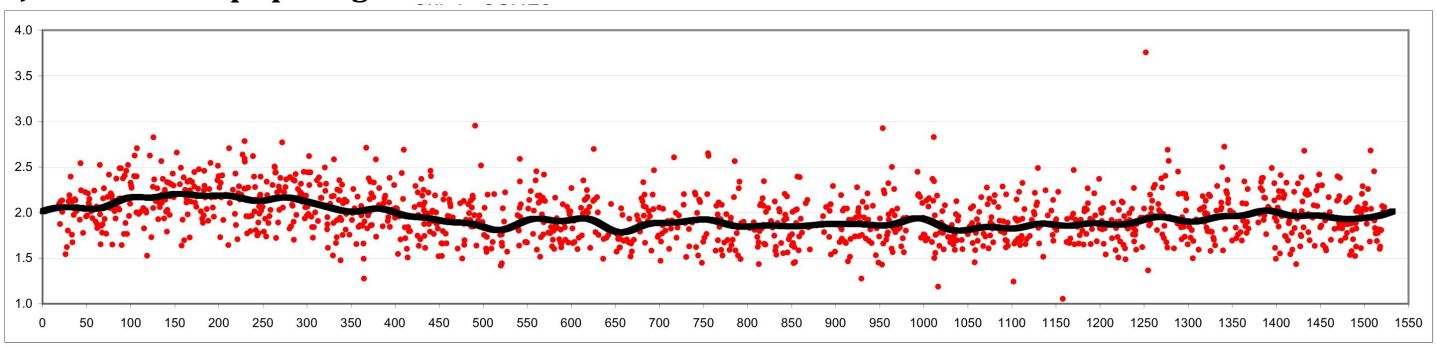

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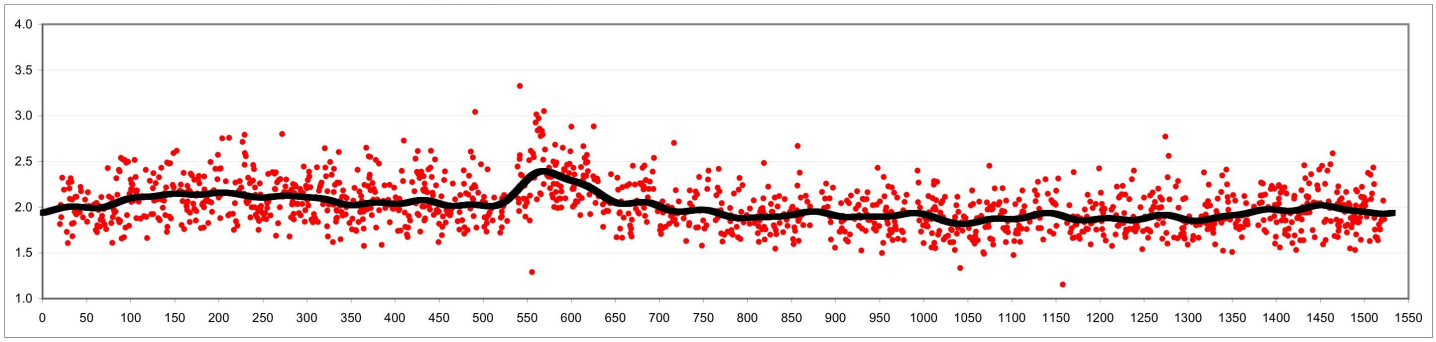

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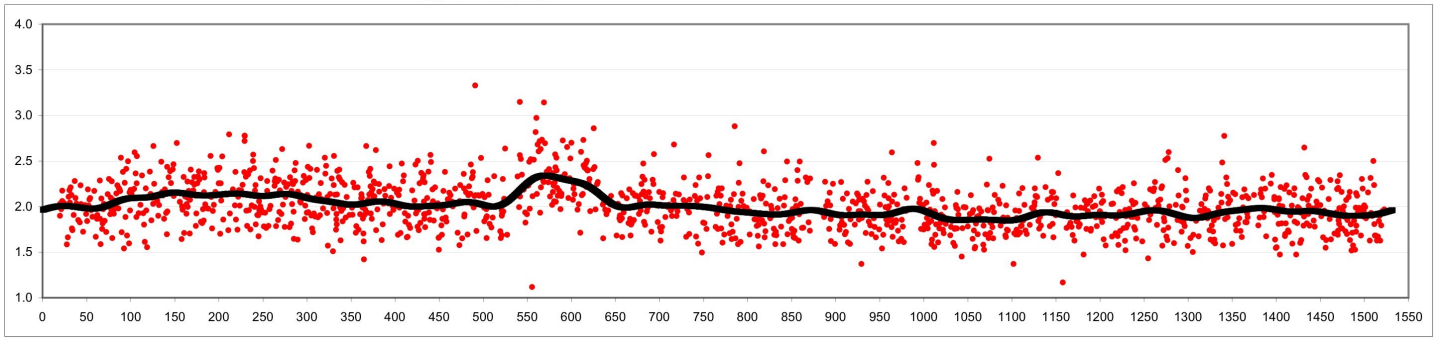

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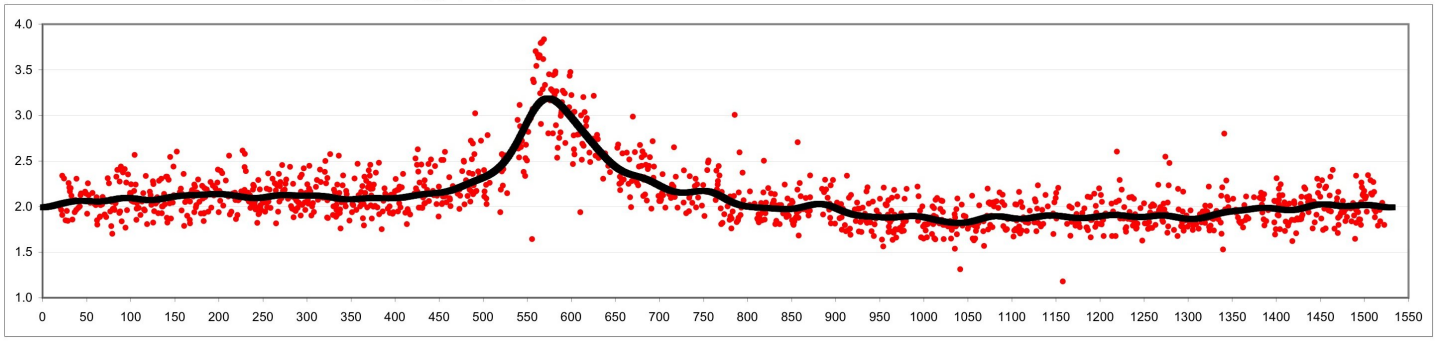

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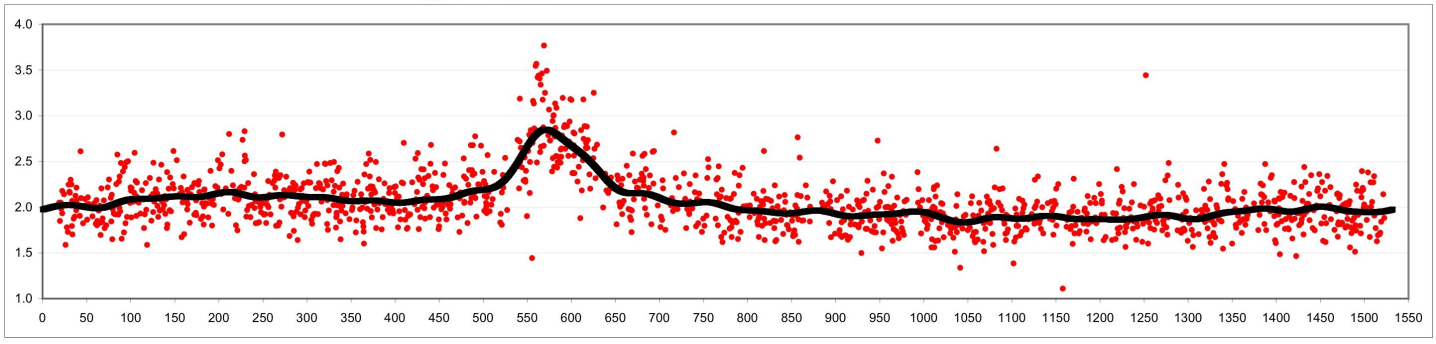

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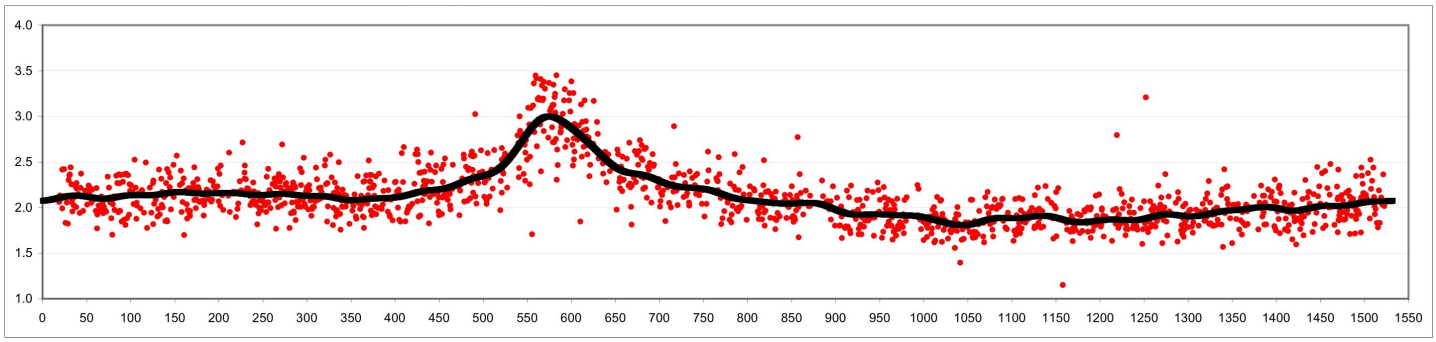

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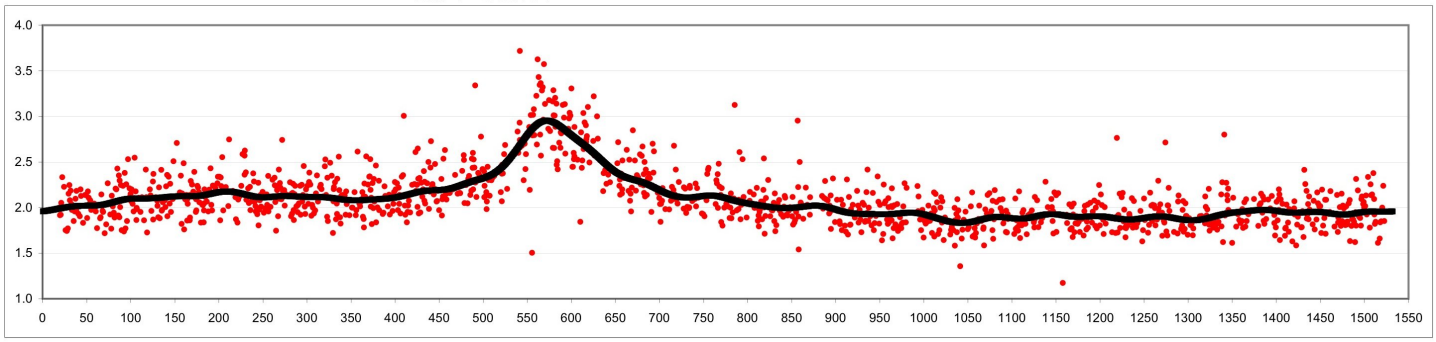

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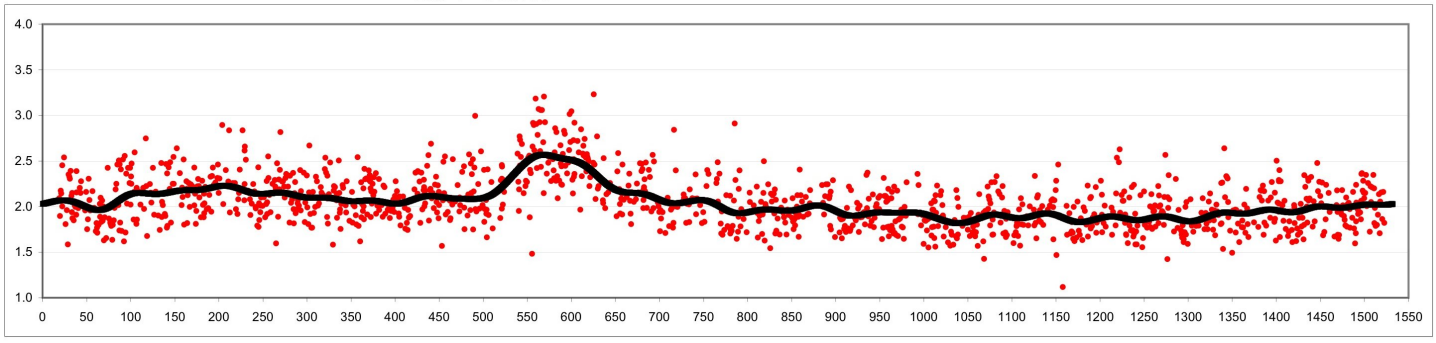

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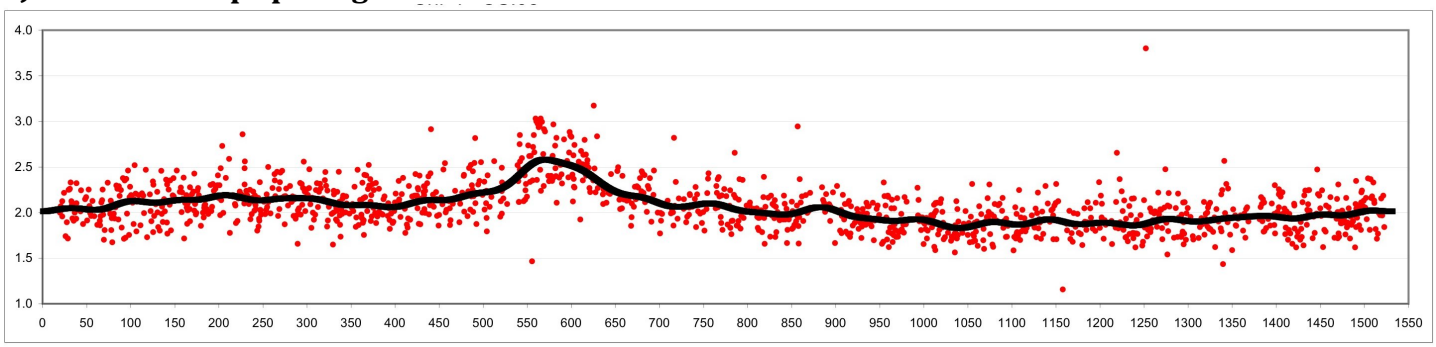
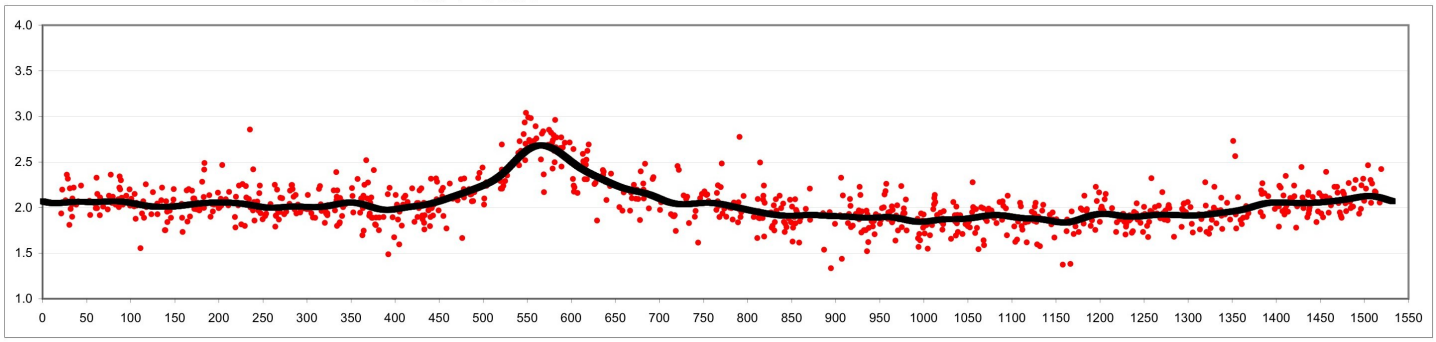


Figure 6A
YJL9078



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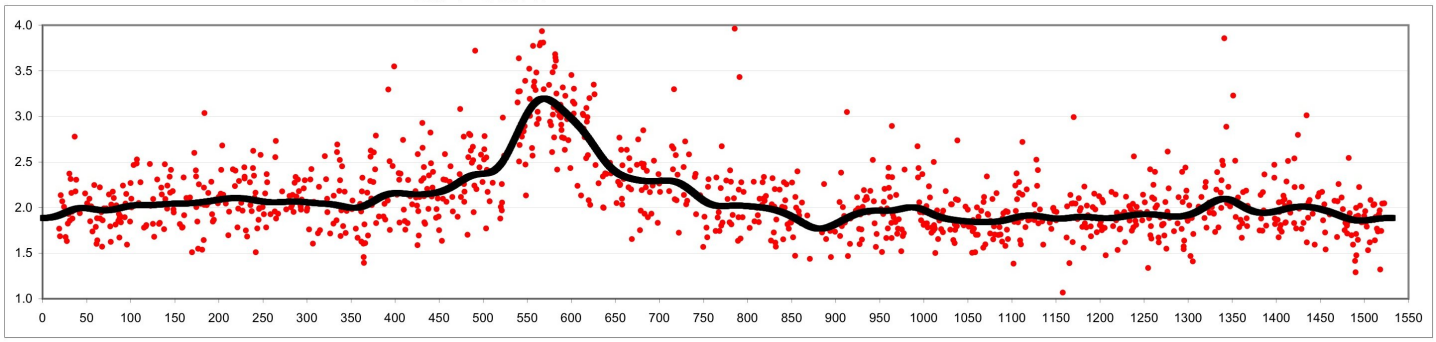

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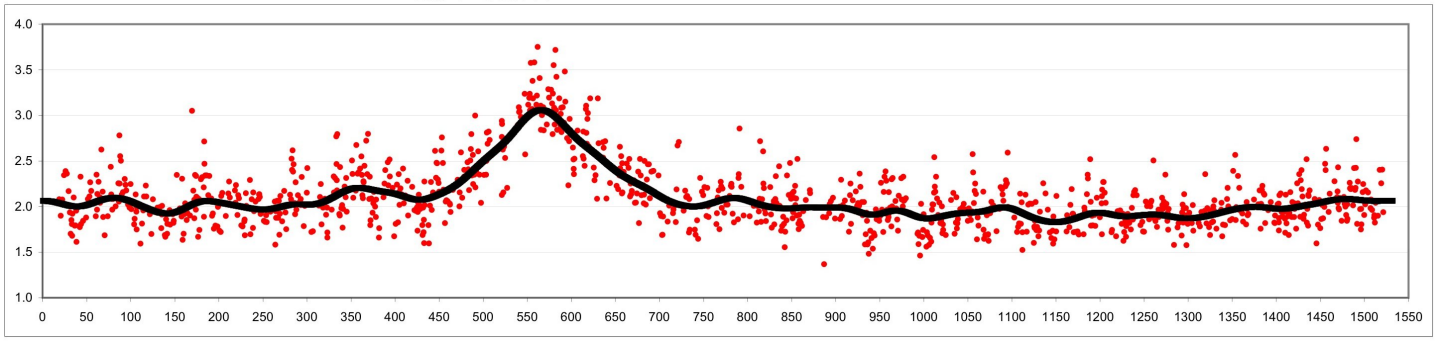

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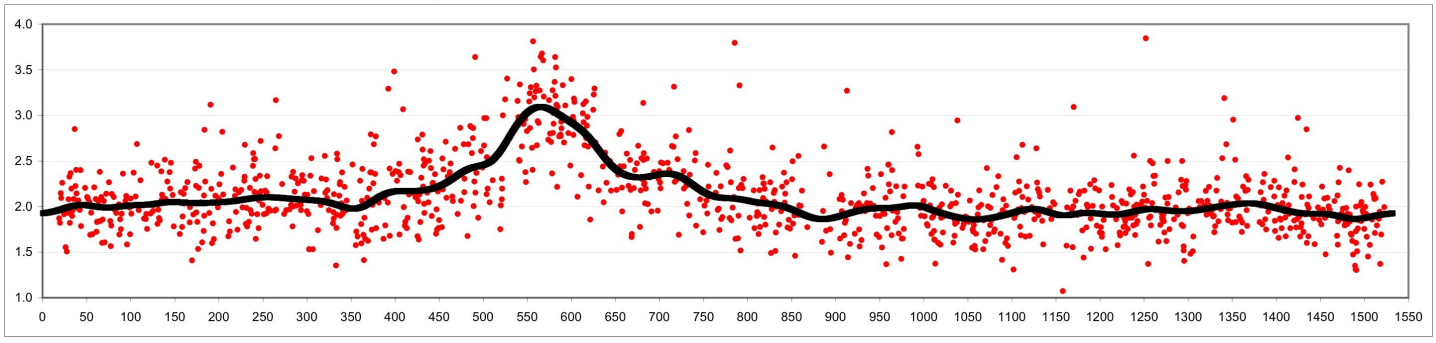

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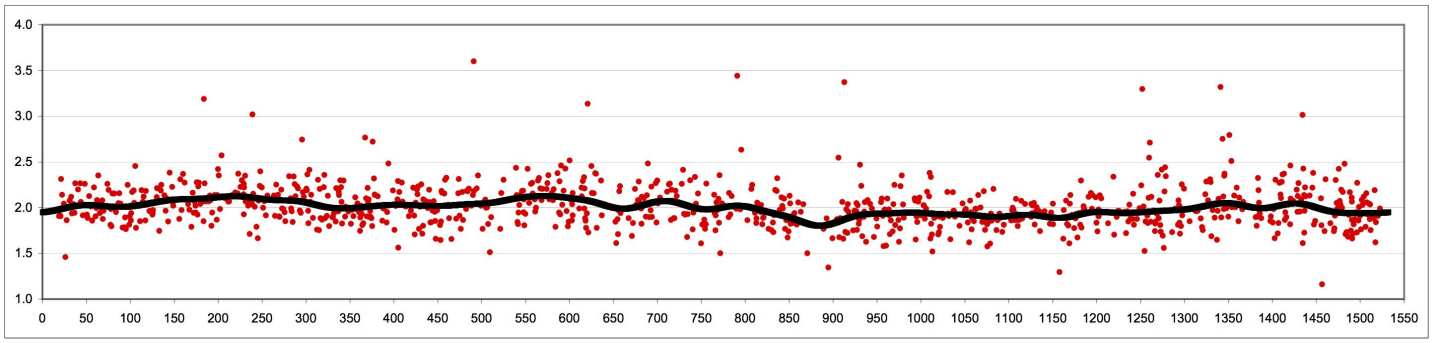

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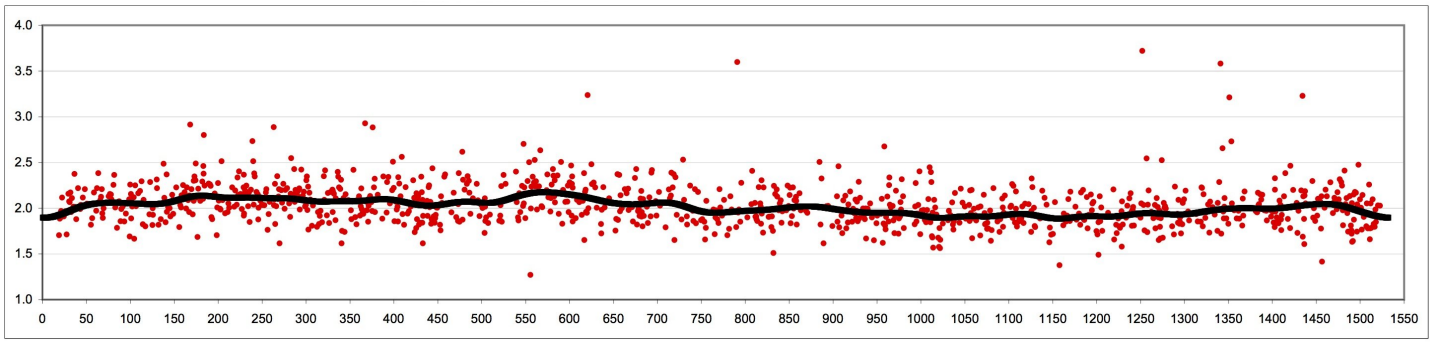

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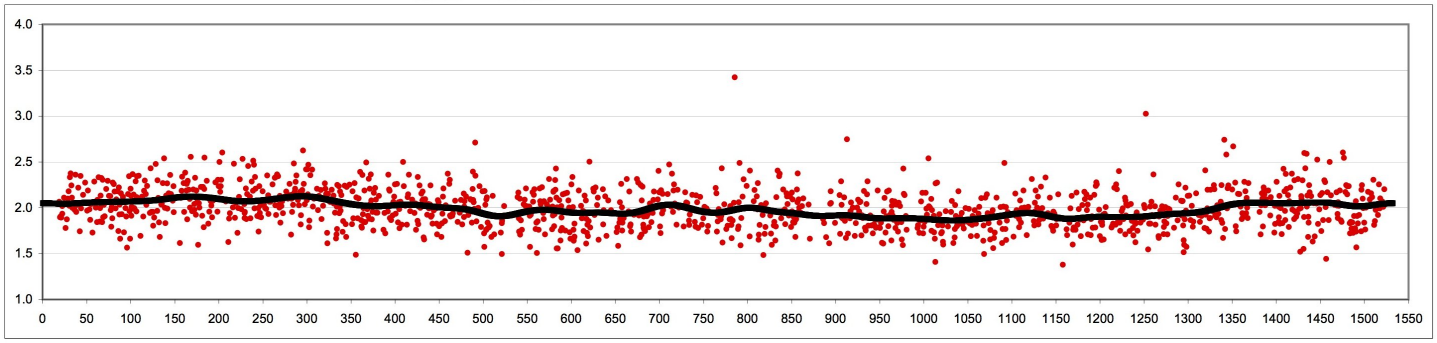

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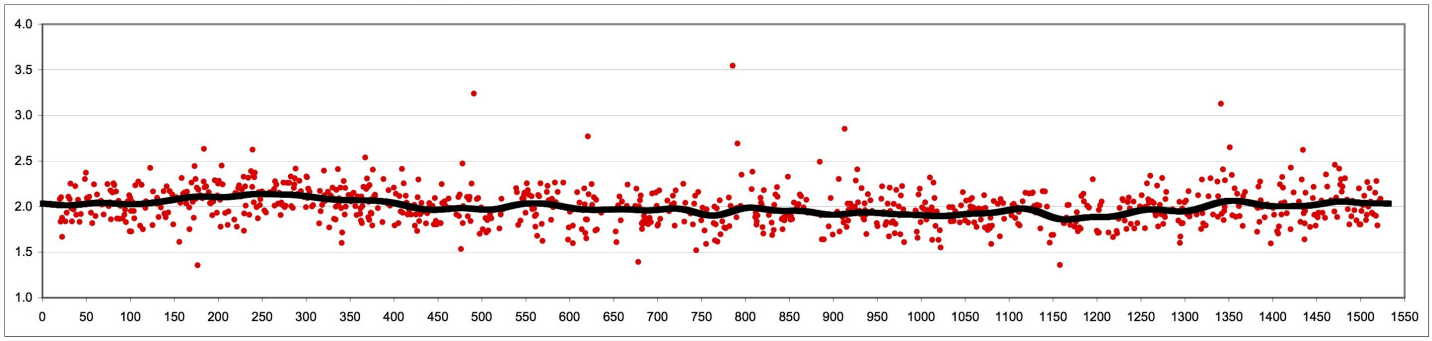
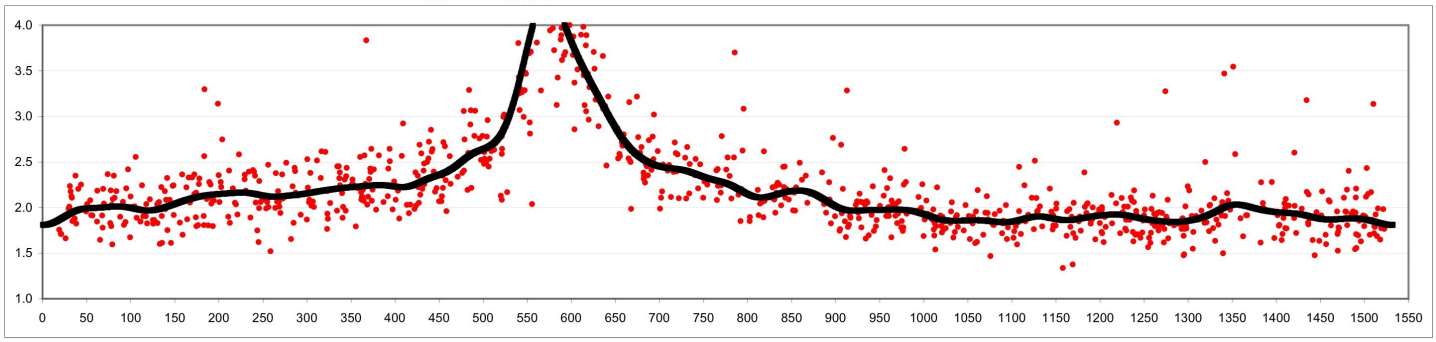


Figure 6B
YJL9999



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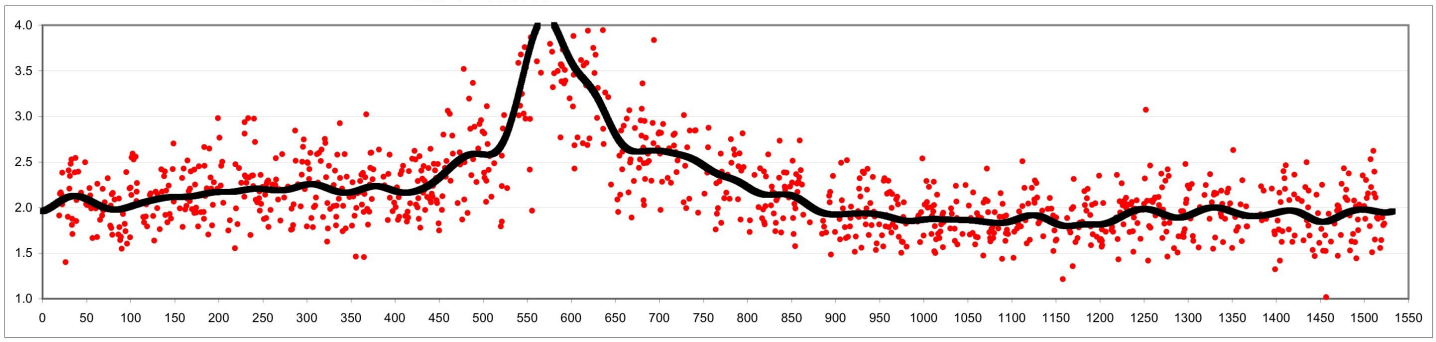

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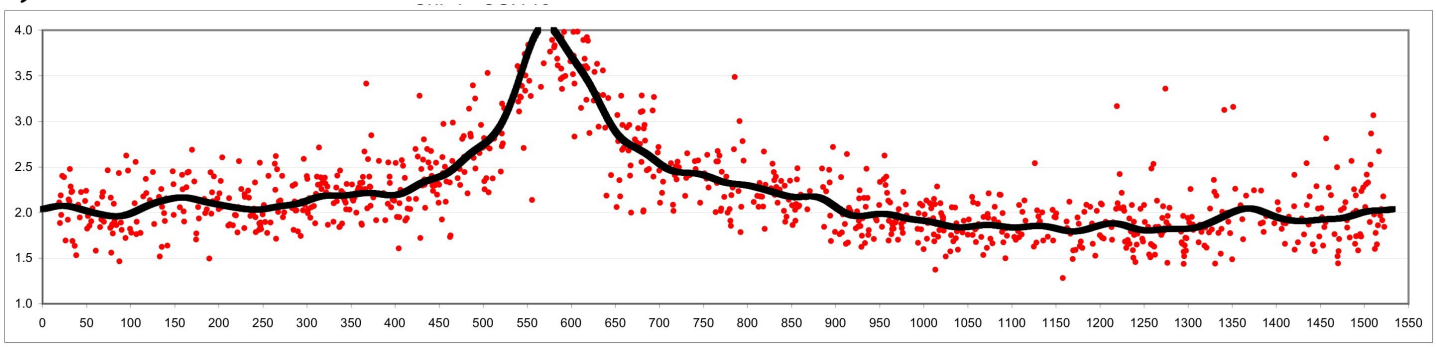

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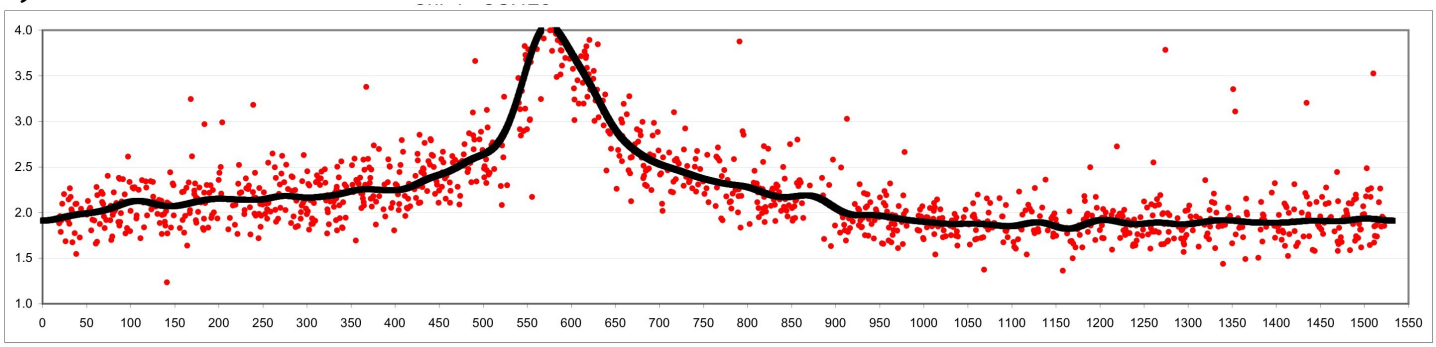
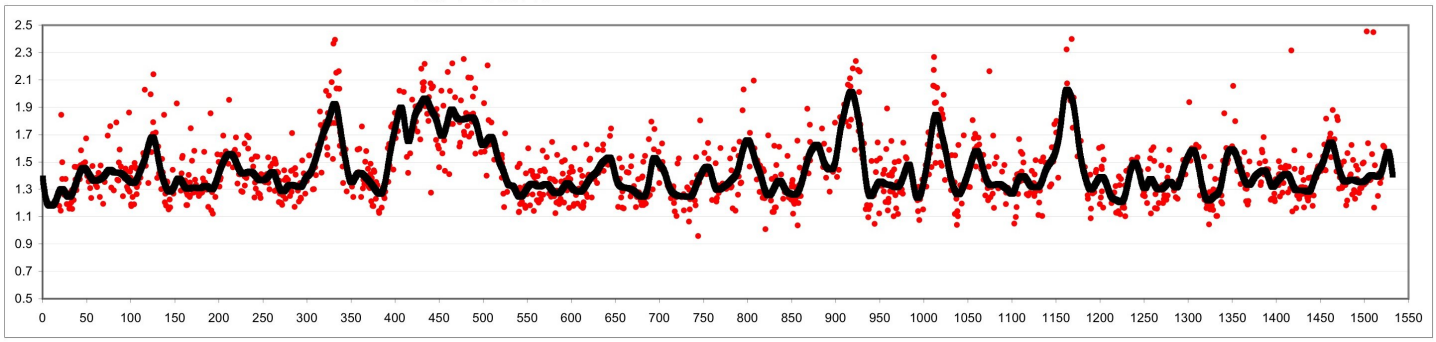


Figure 7
YJL9175



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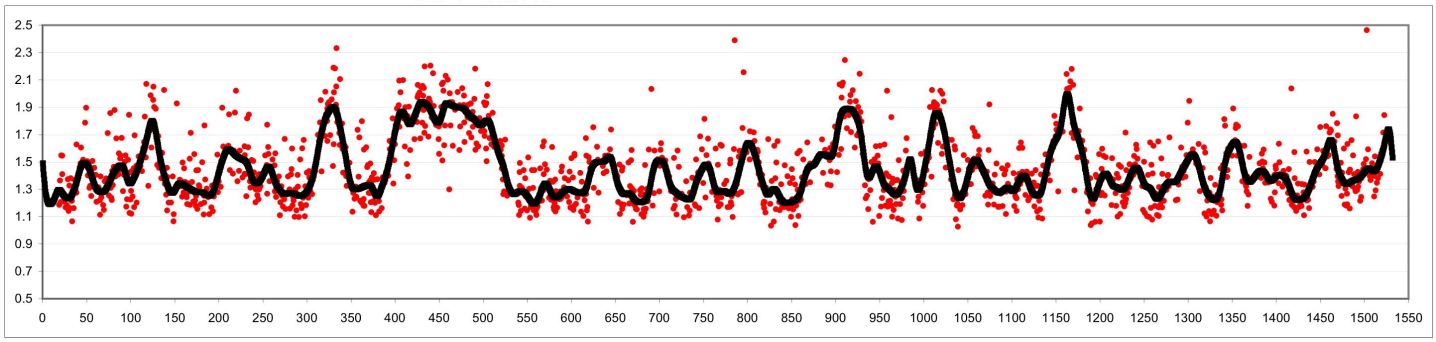

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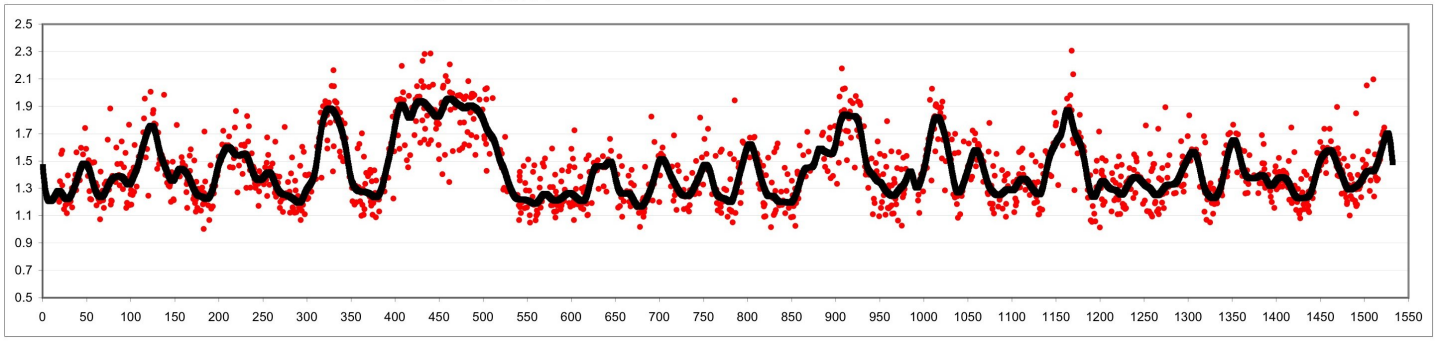

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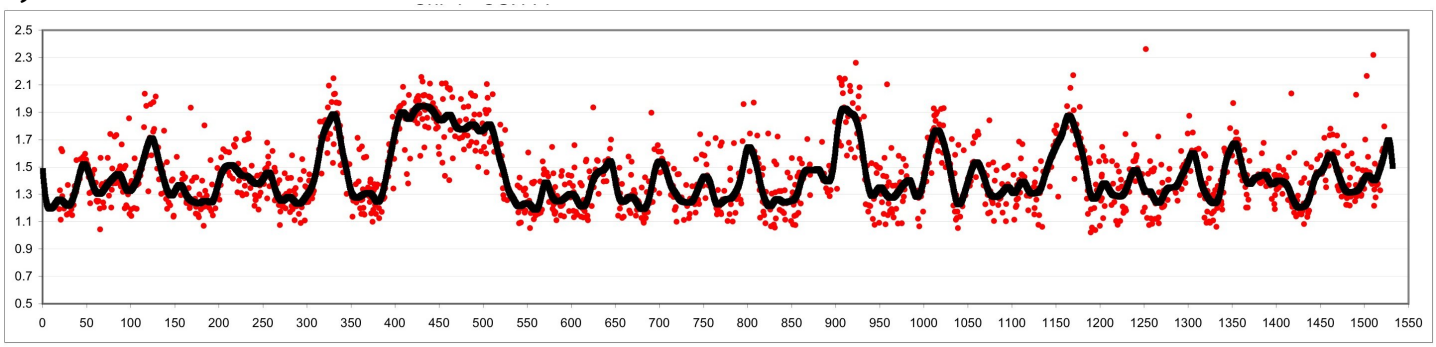

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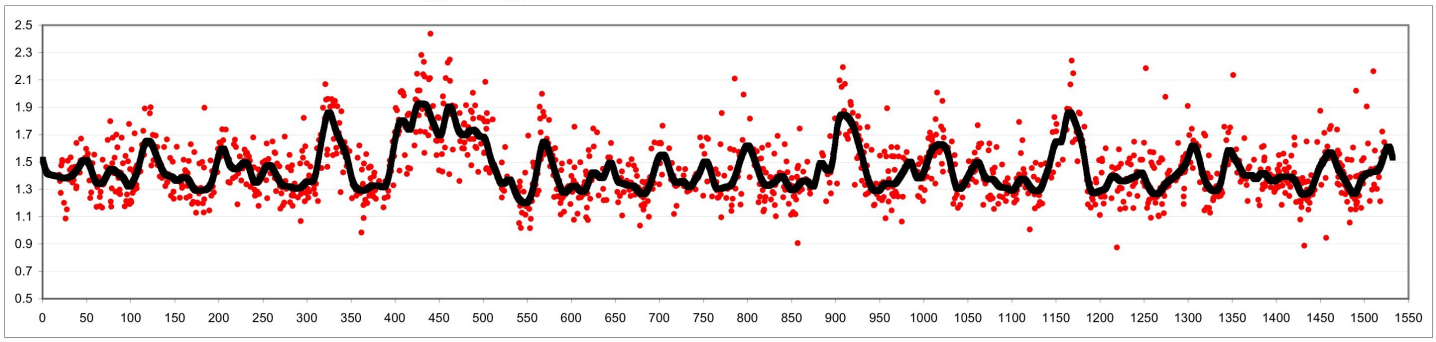

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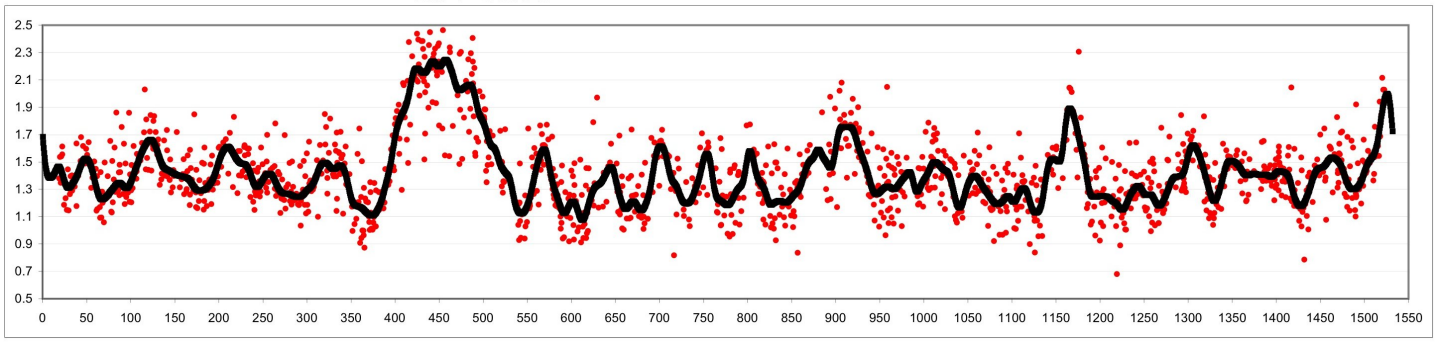

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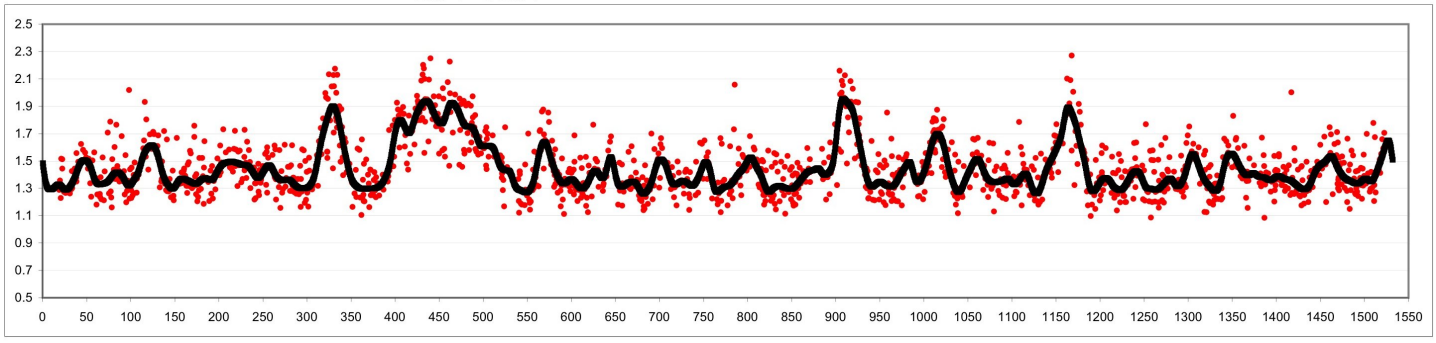

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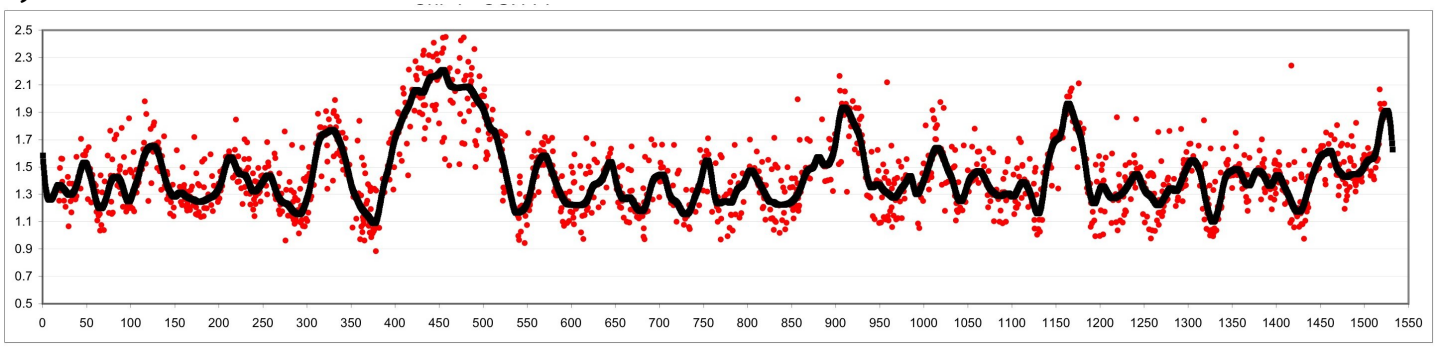

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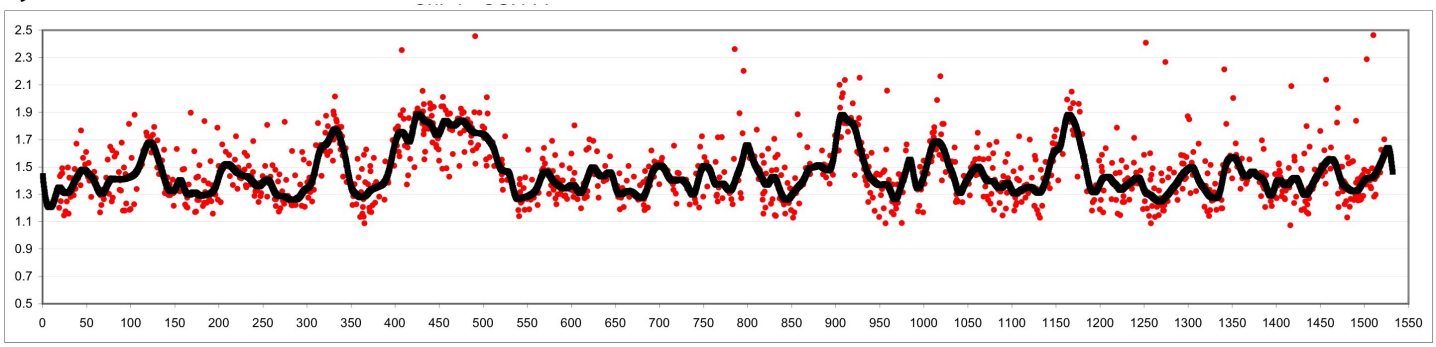

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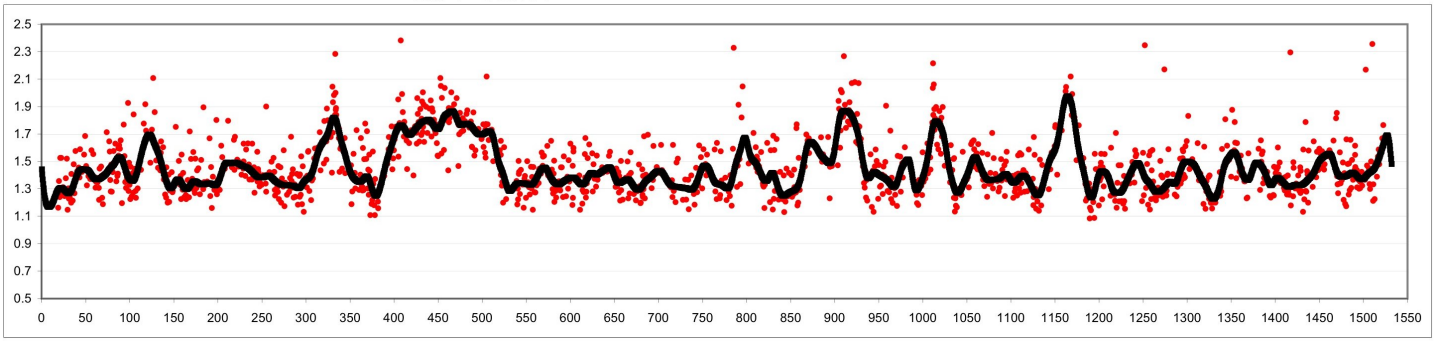

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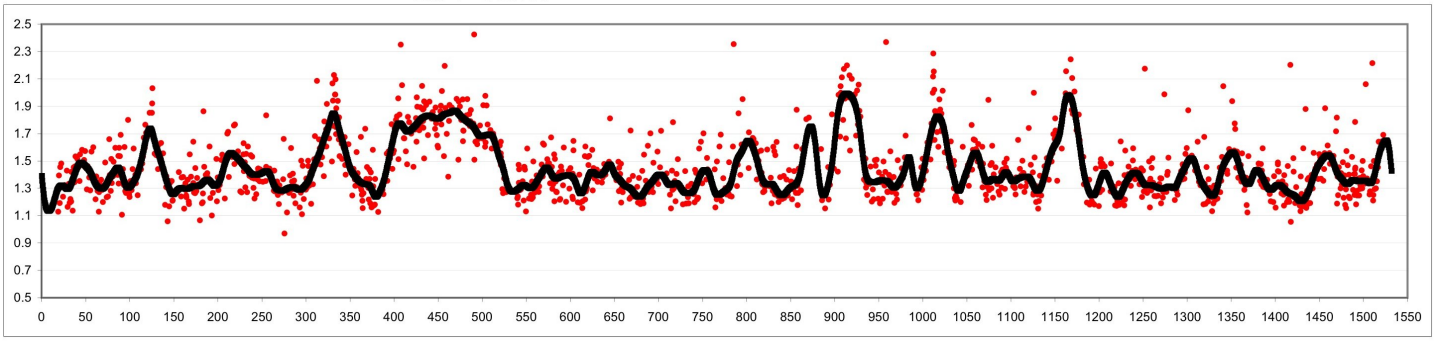

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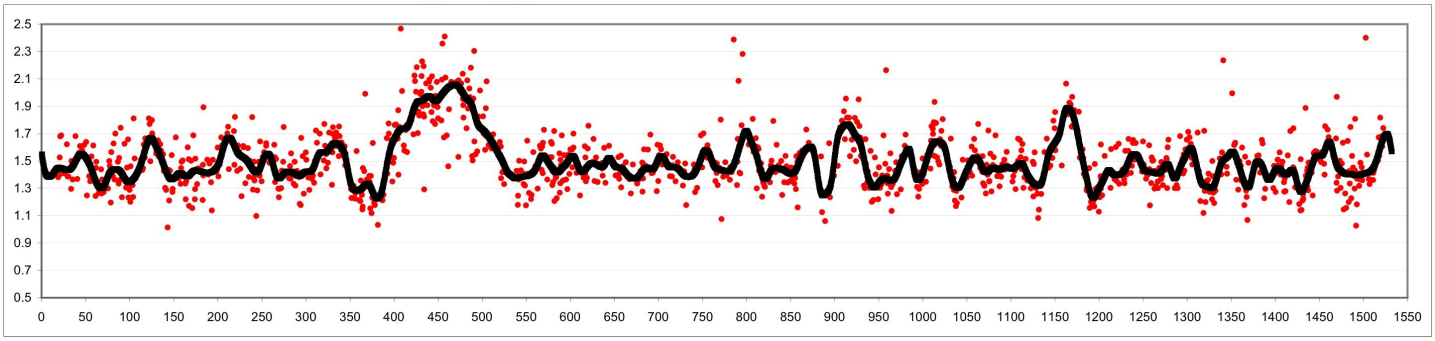
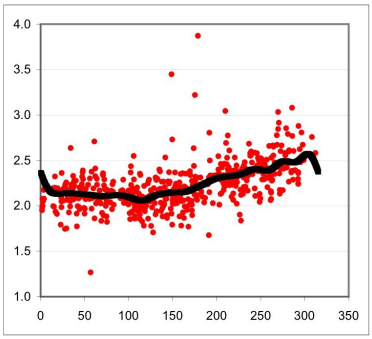


Supplemental Figure 1
YJL8923



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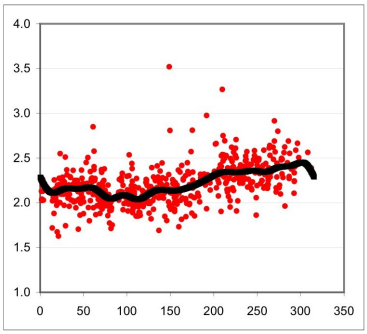

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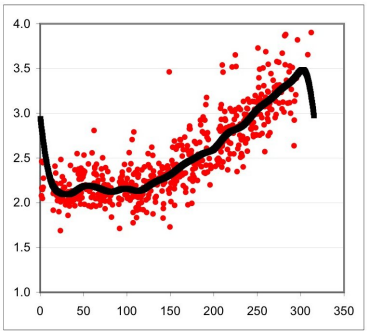

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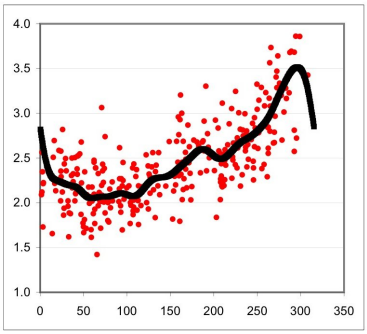
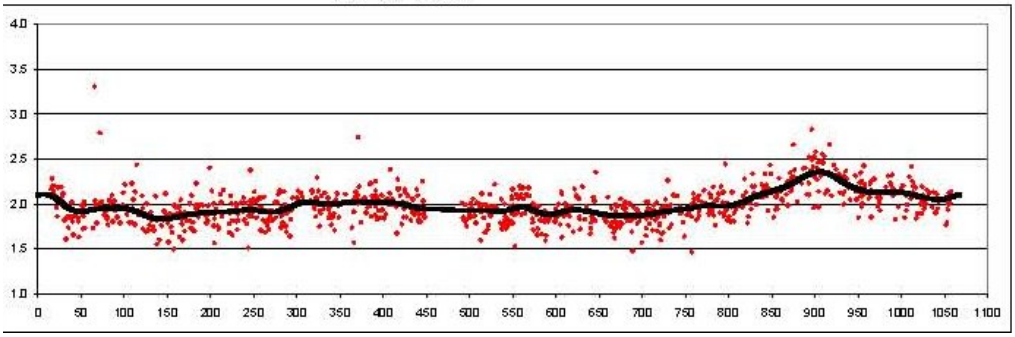


Supplemental Figure 2C
YJL8398 – Positive Control



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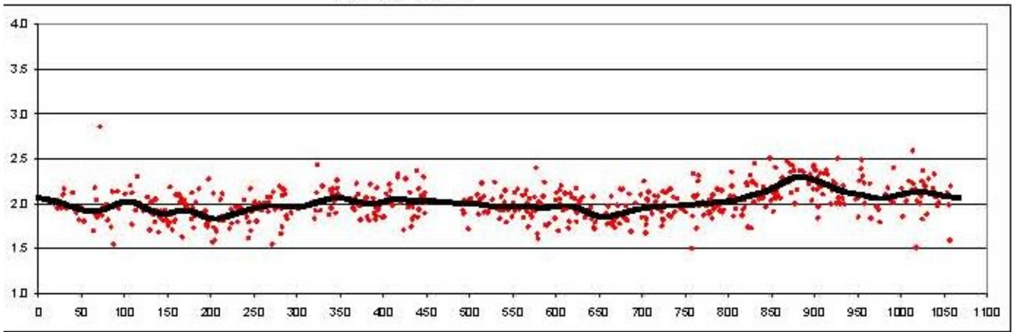

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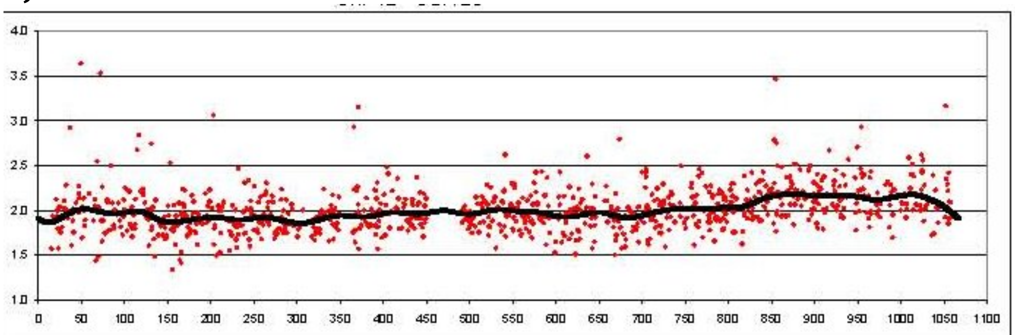

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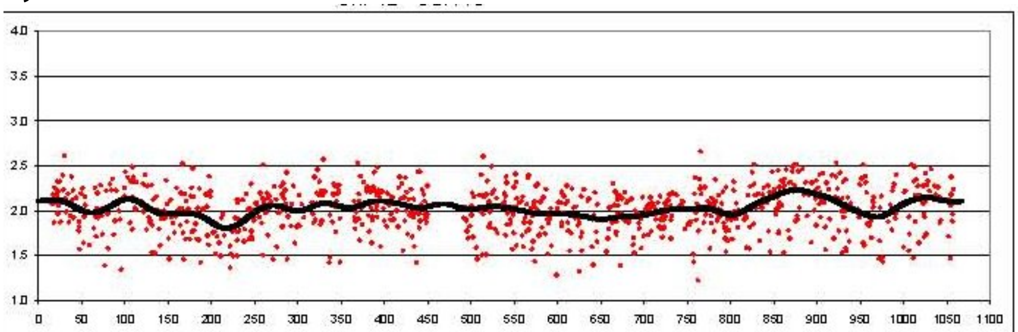

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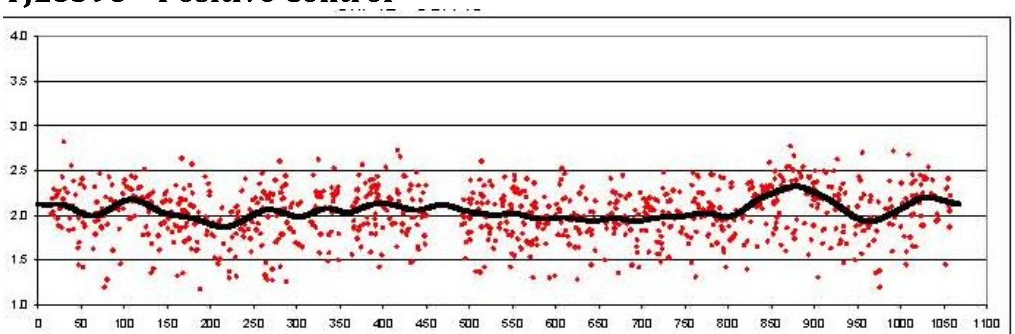

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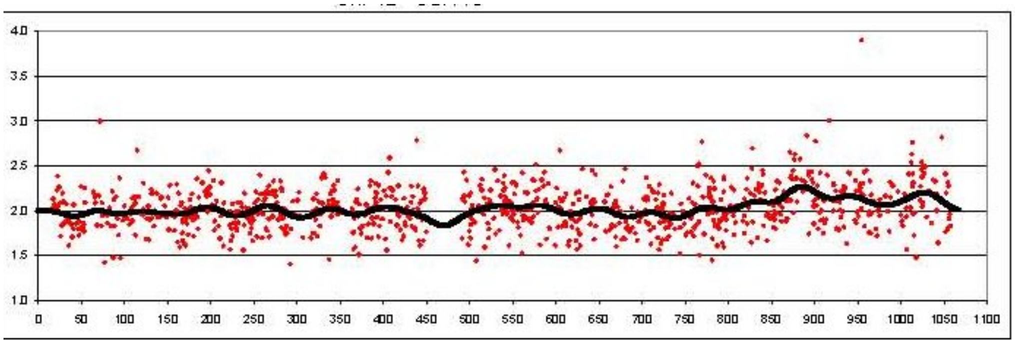

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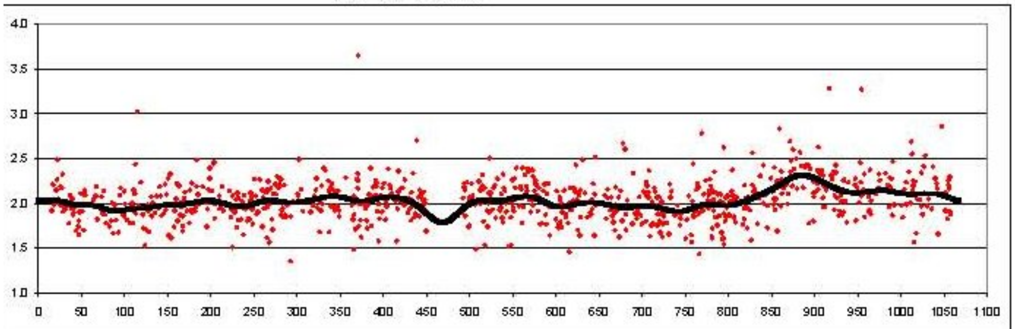

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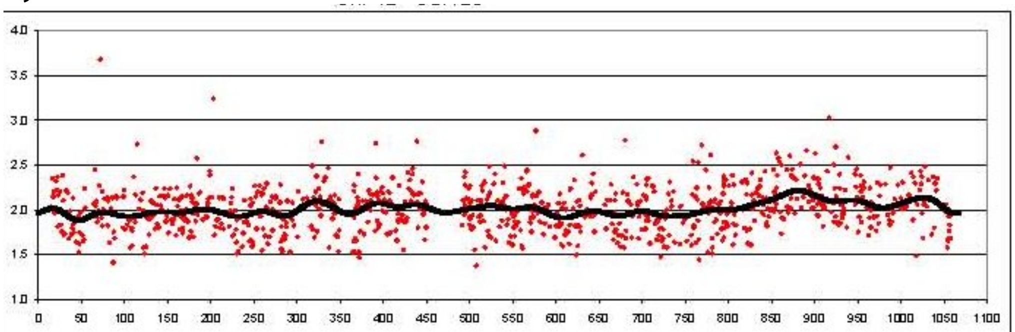

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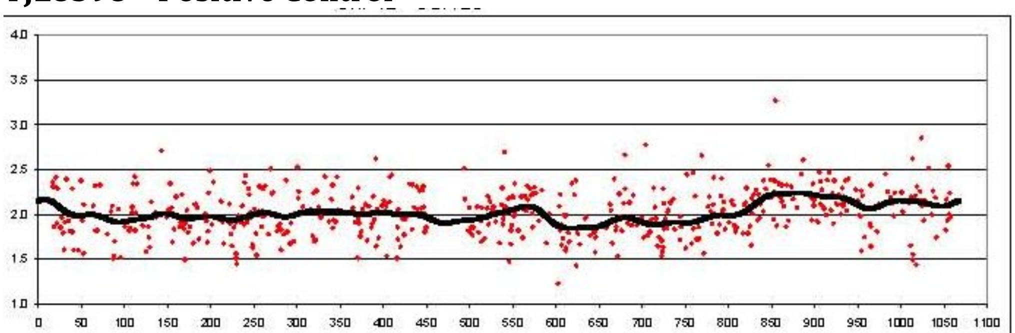

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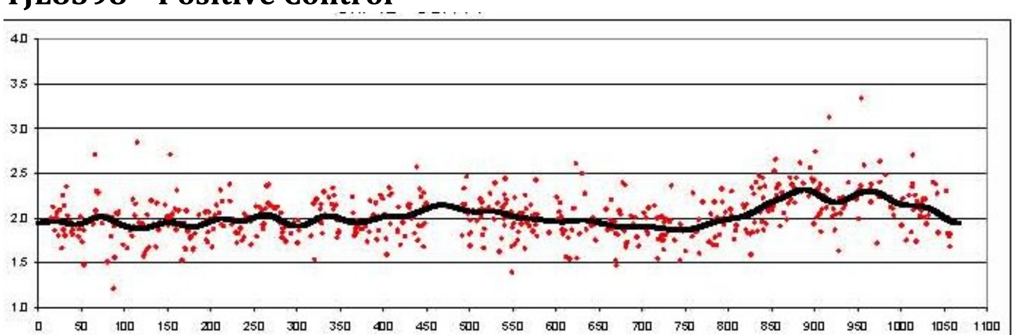

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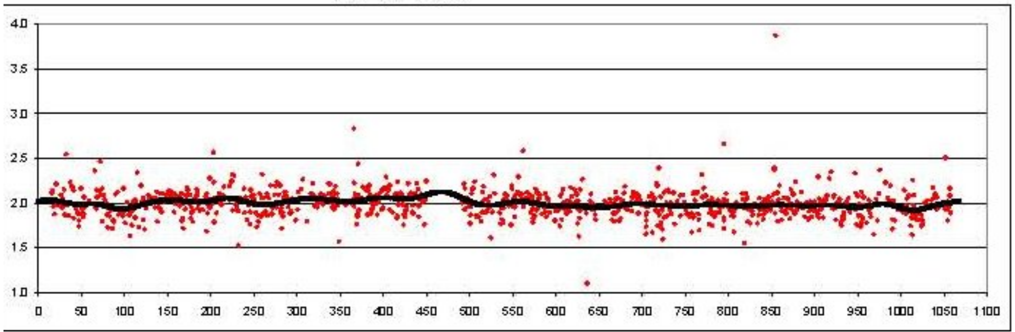

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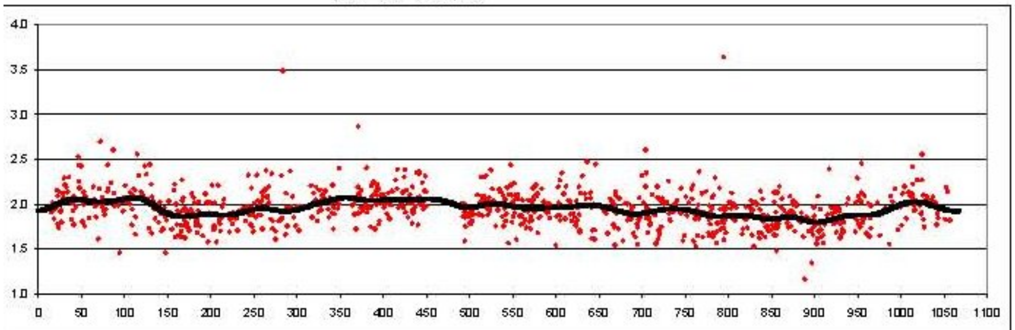

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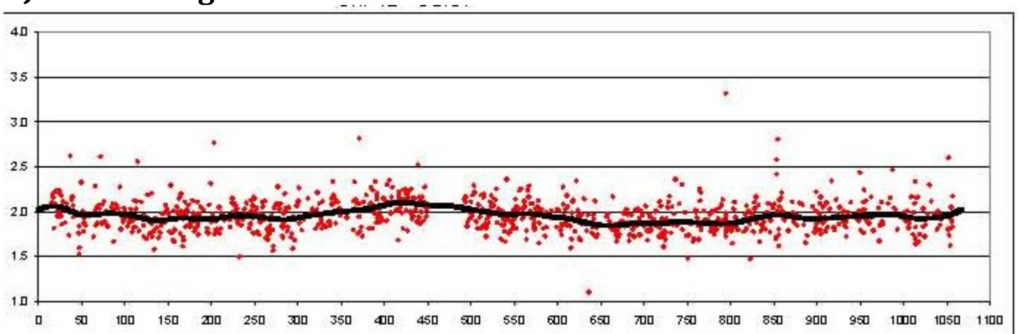

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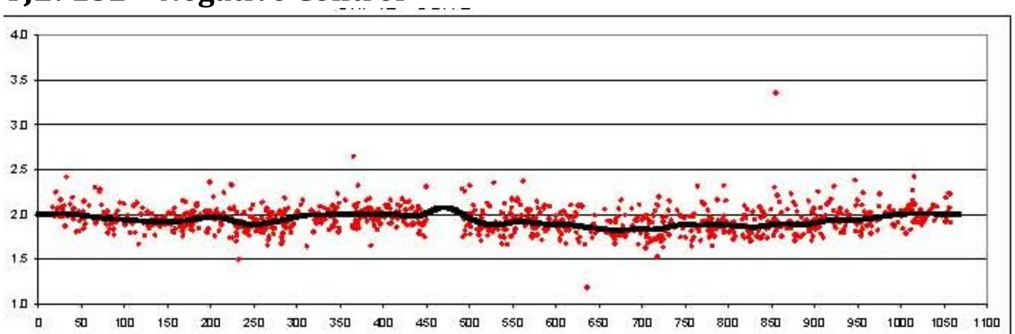

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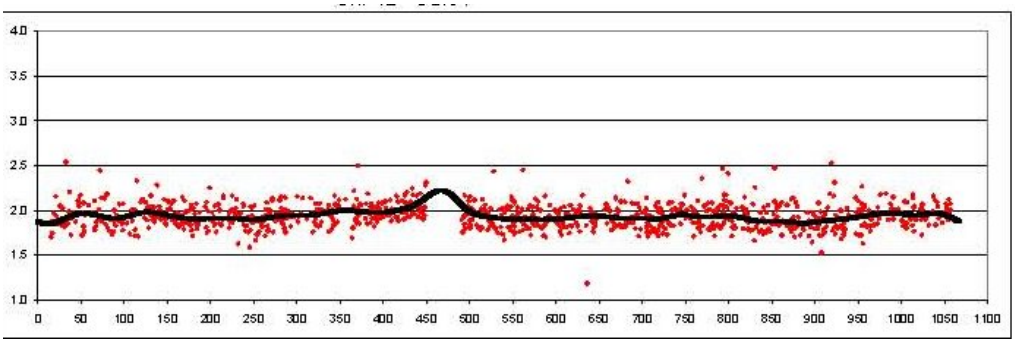

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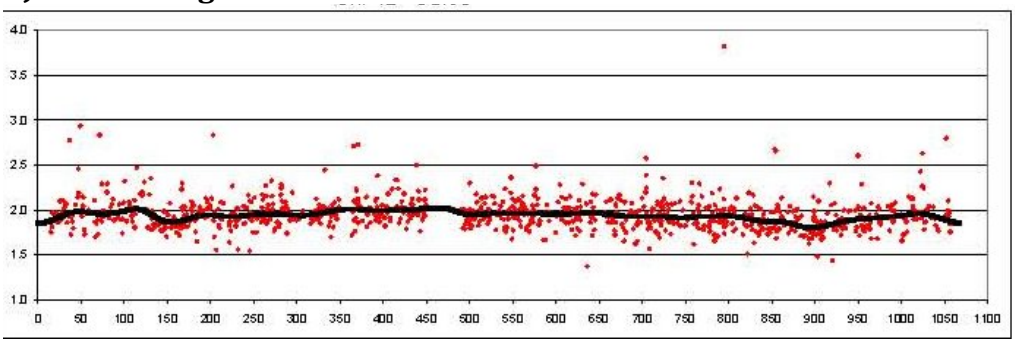

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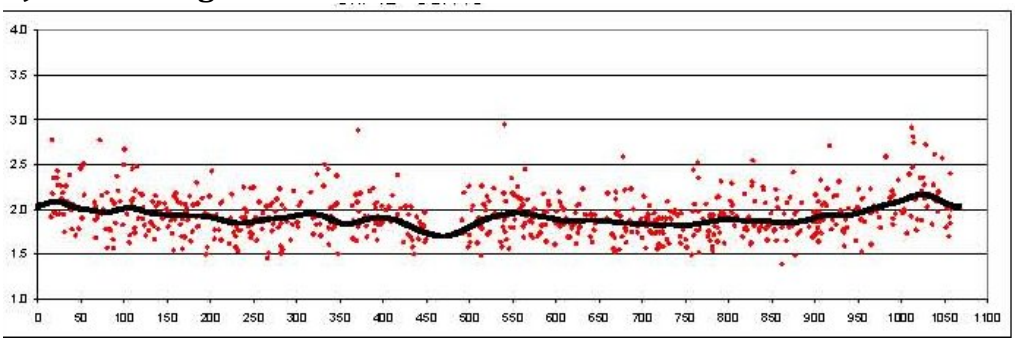

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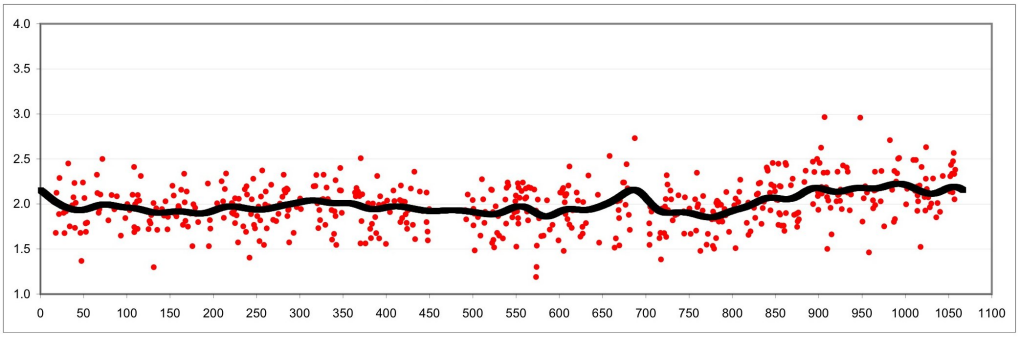

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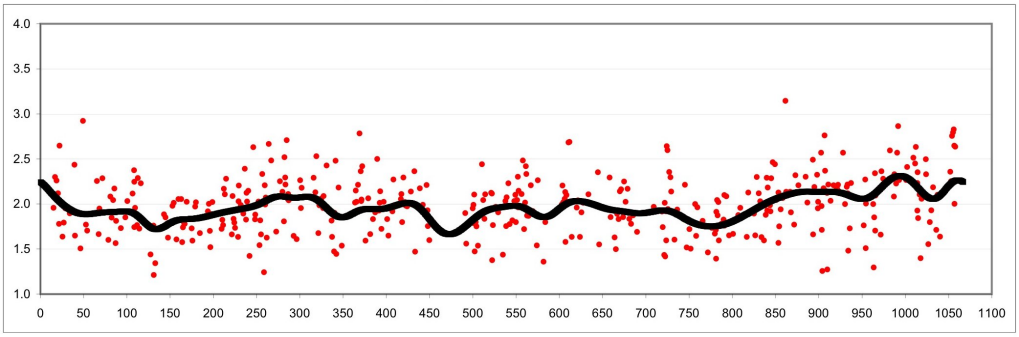

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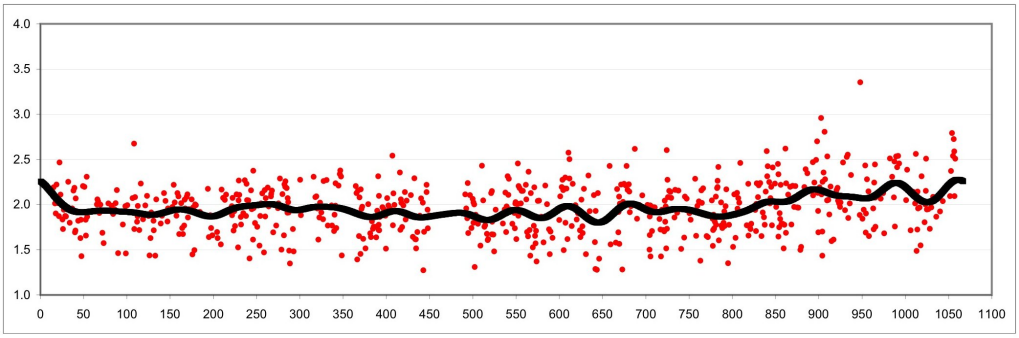

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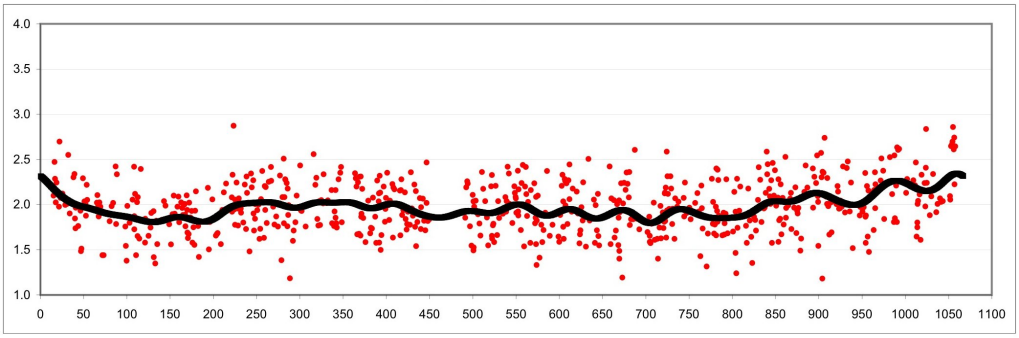

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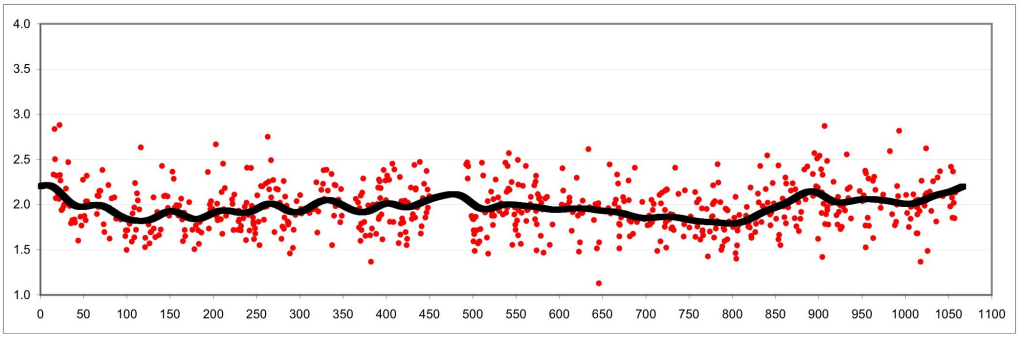

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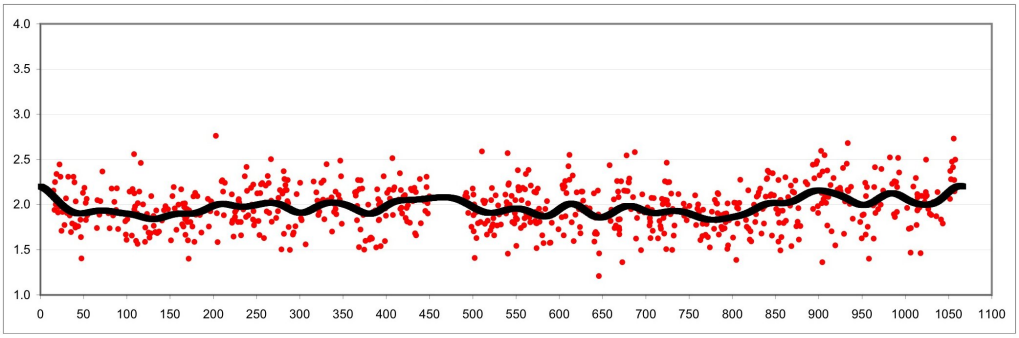

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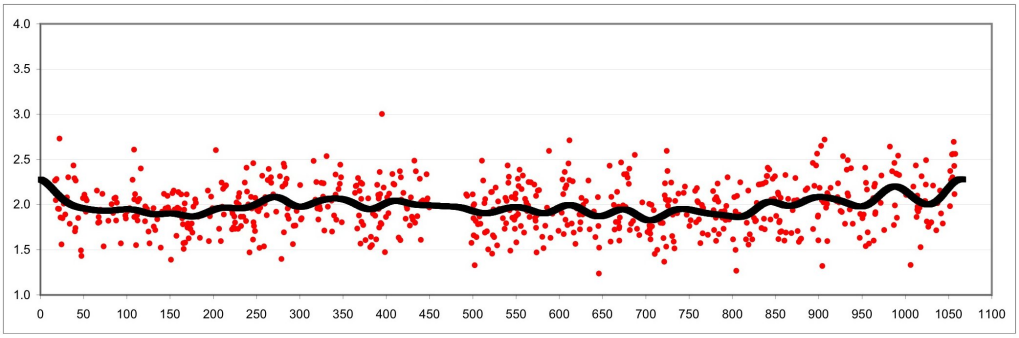

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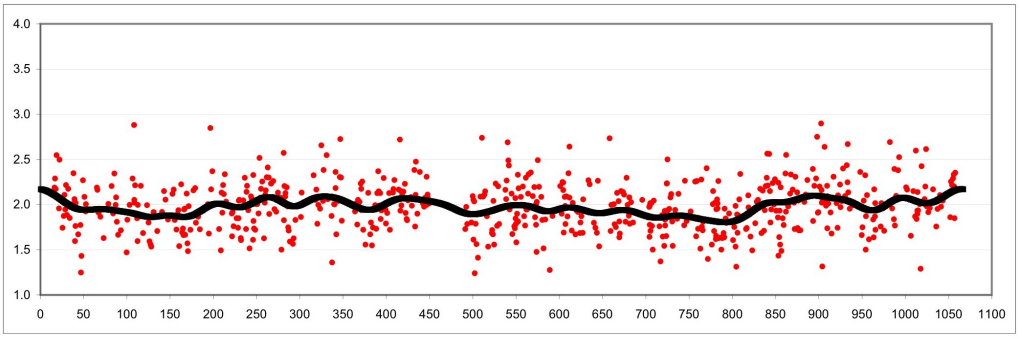
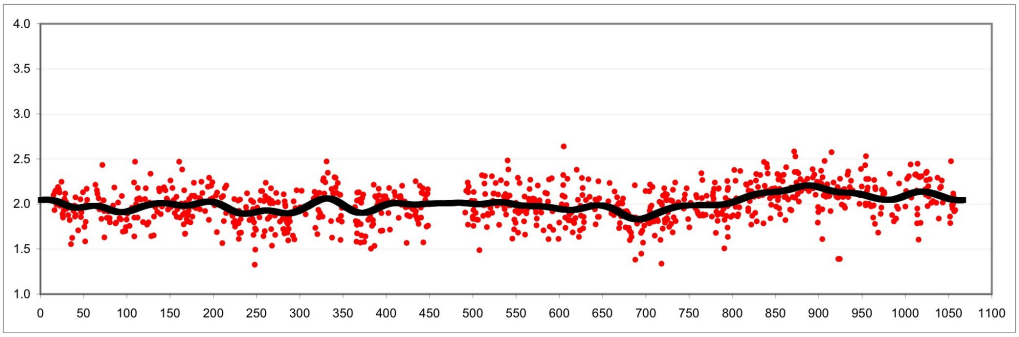


Supplemental Figure 2D

YJL8701



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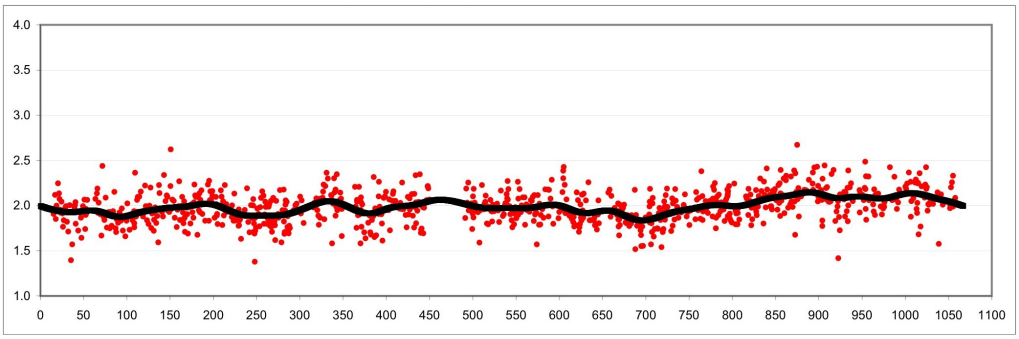

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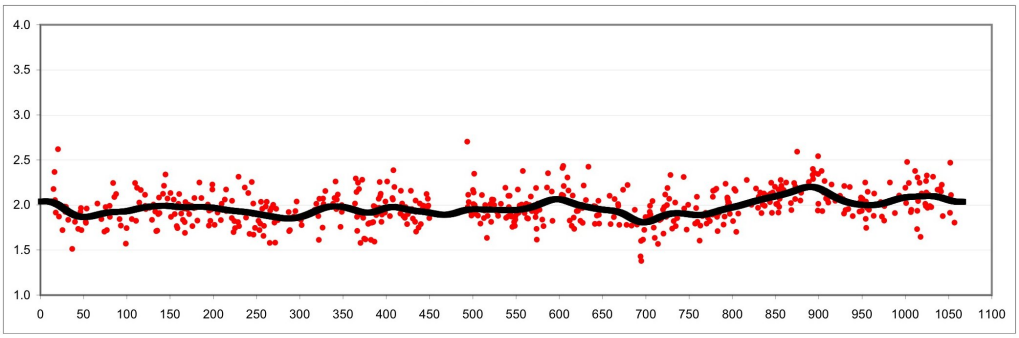

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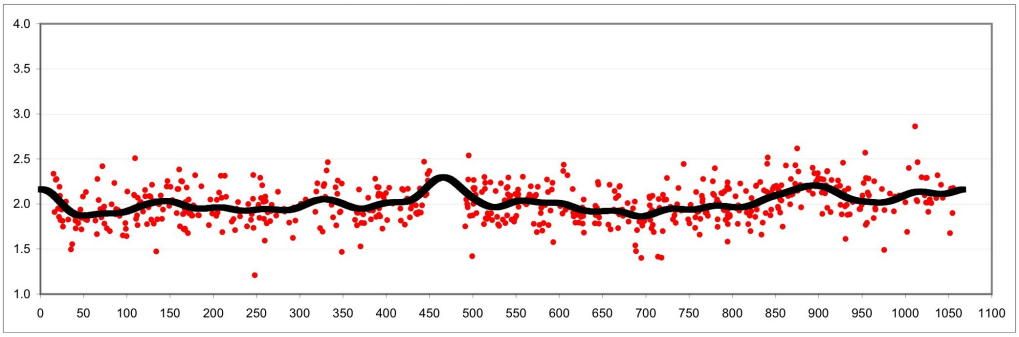

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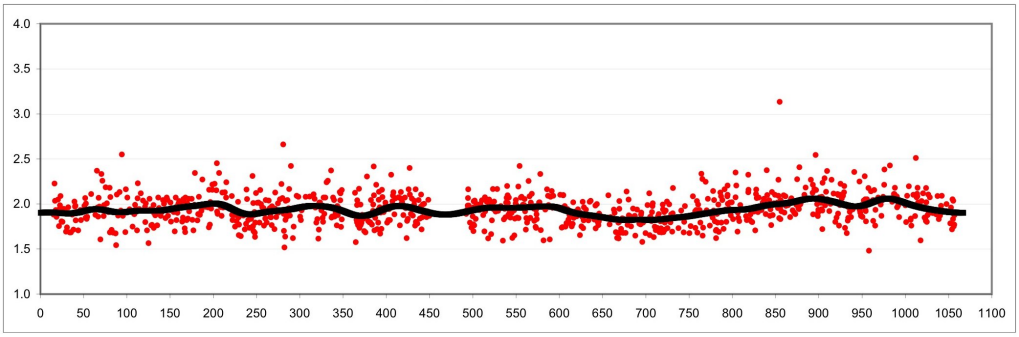

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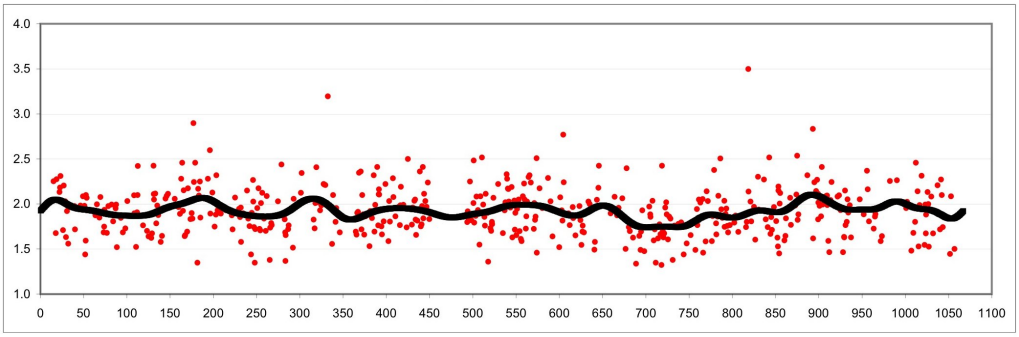
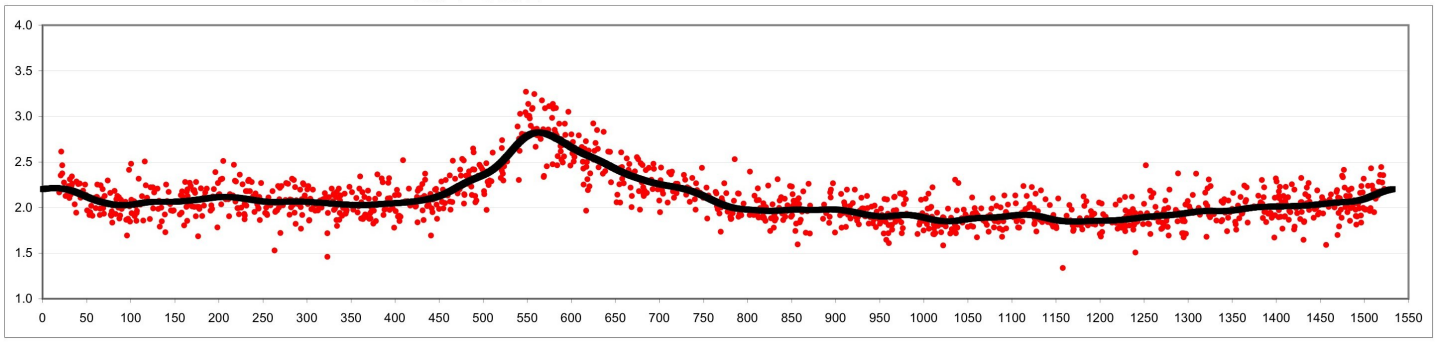


Supplemental Figure 3
YJL8526



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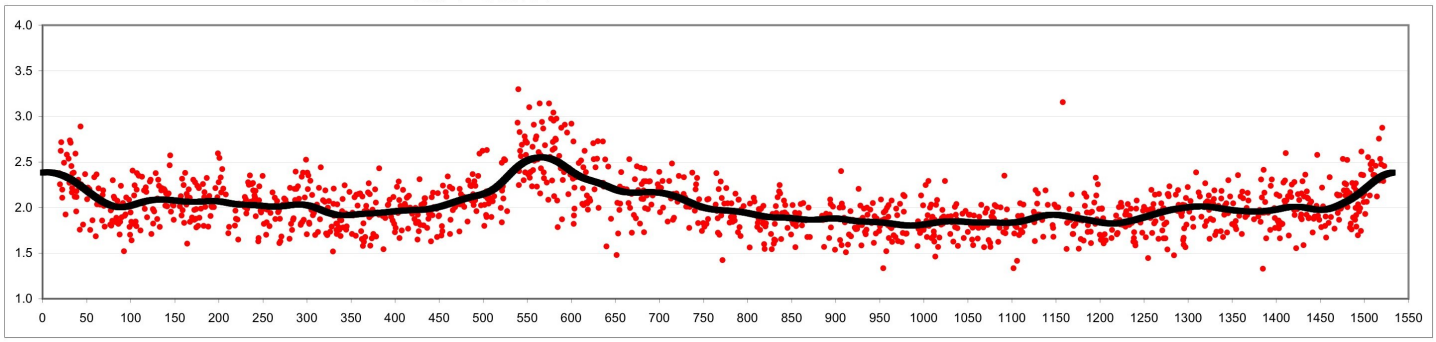

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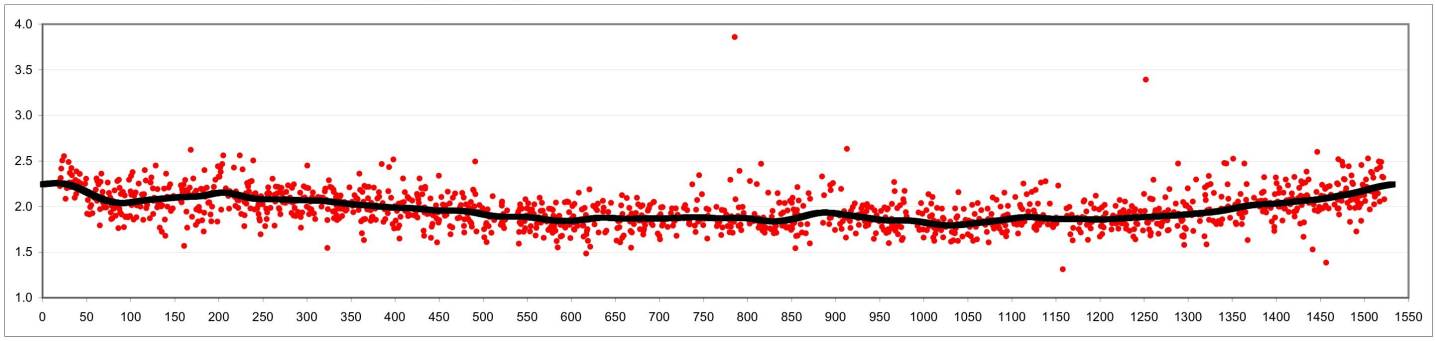

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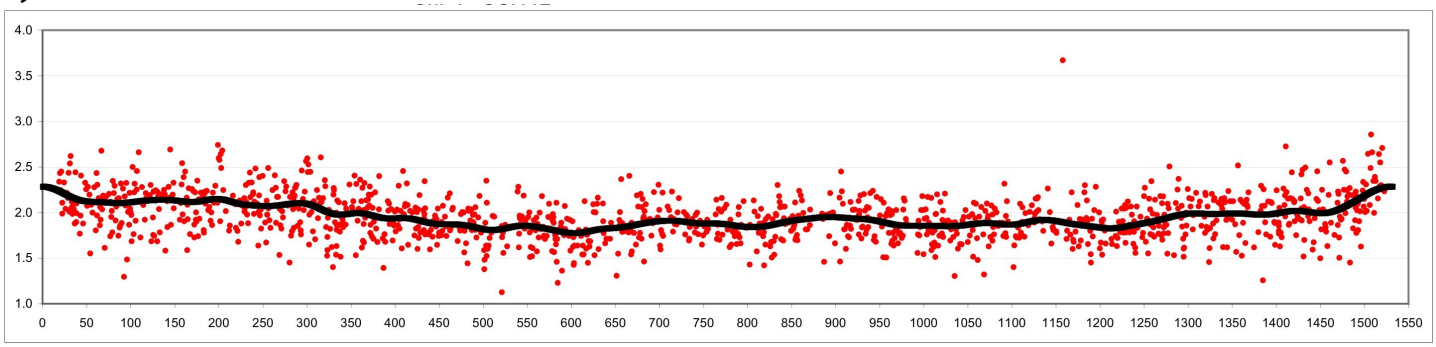
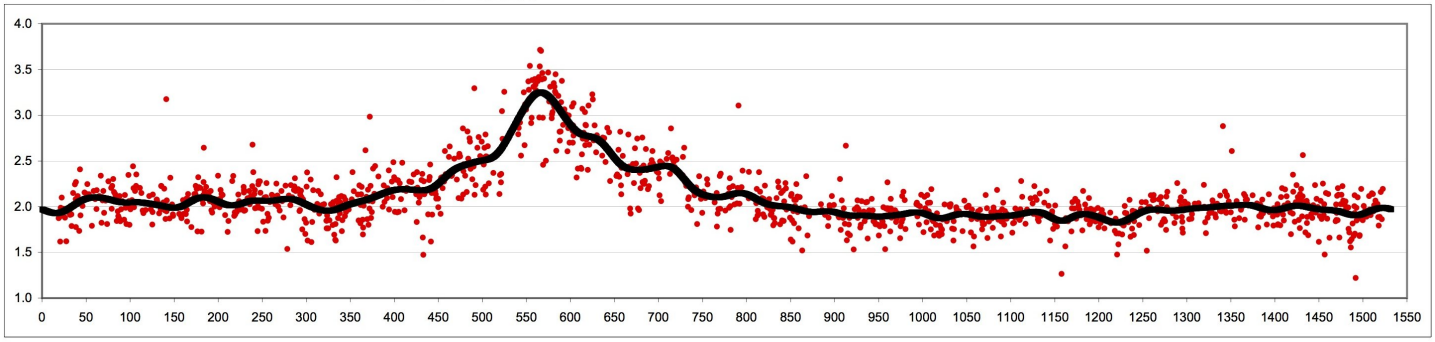
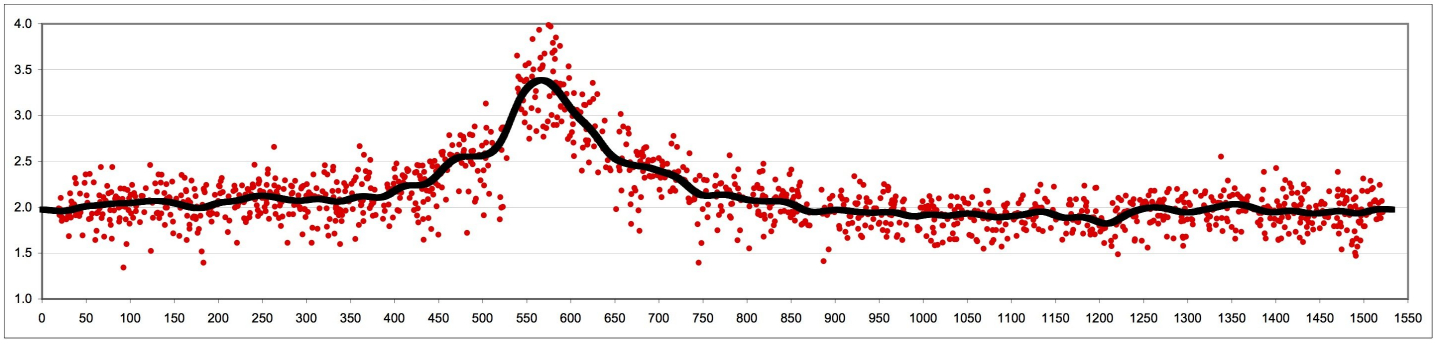


Supplemental Figure 4A

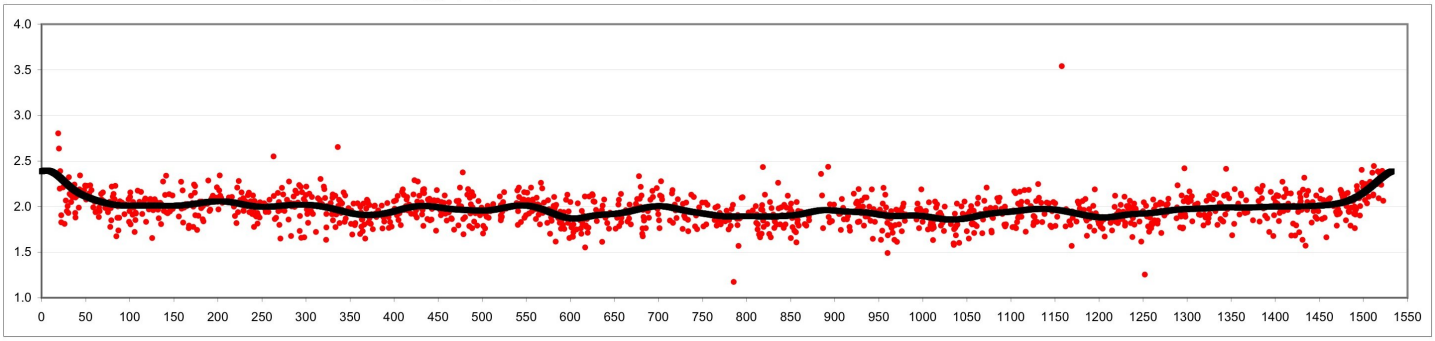
YJL8398 – control



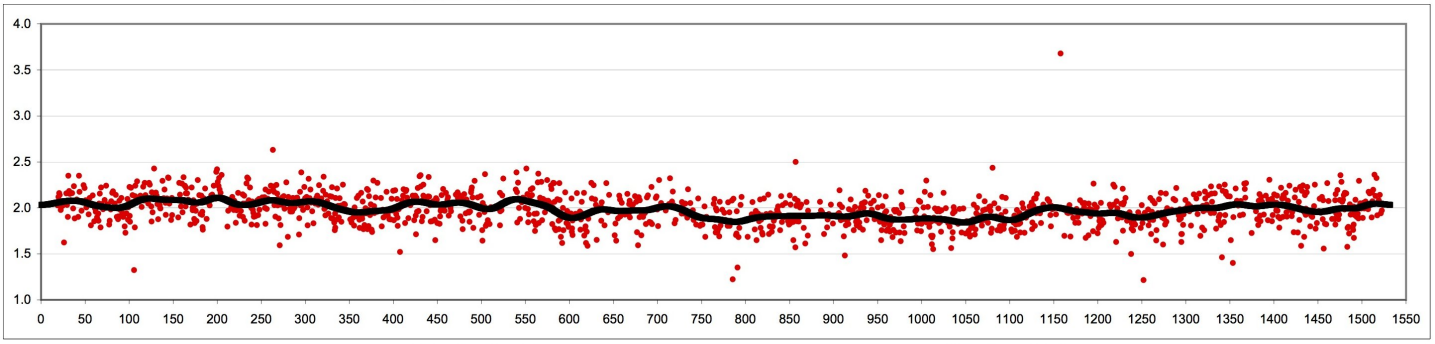
YJL8398 – control



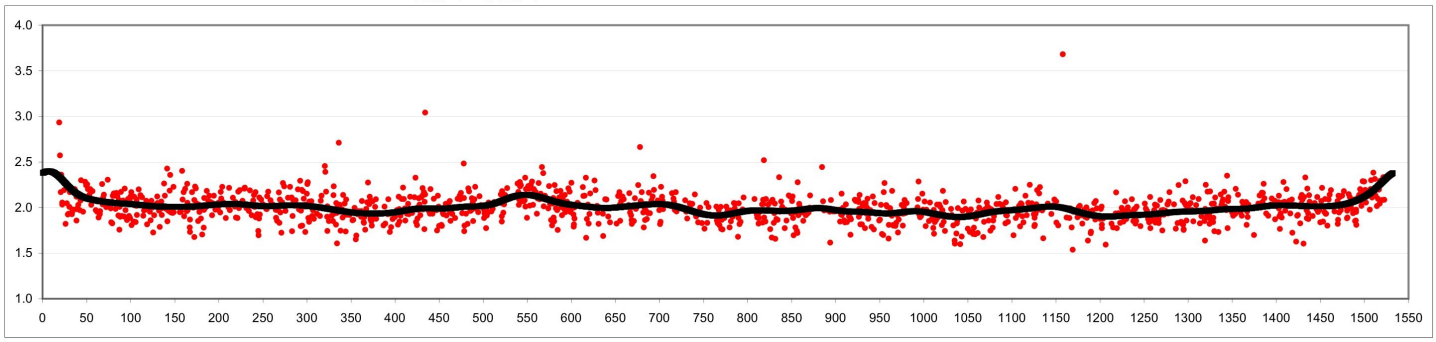
YJL8771 – Linker L9+L11+L15



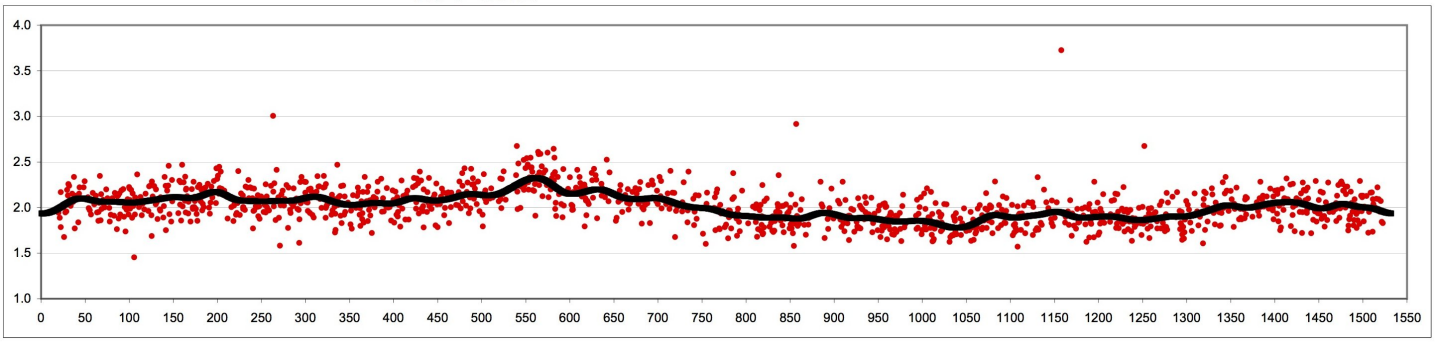
YJL8771 – Linker L9+L11+L15



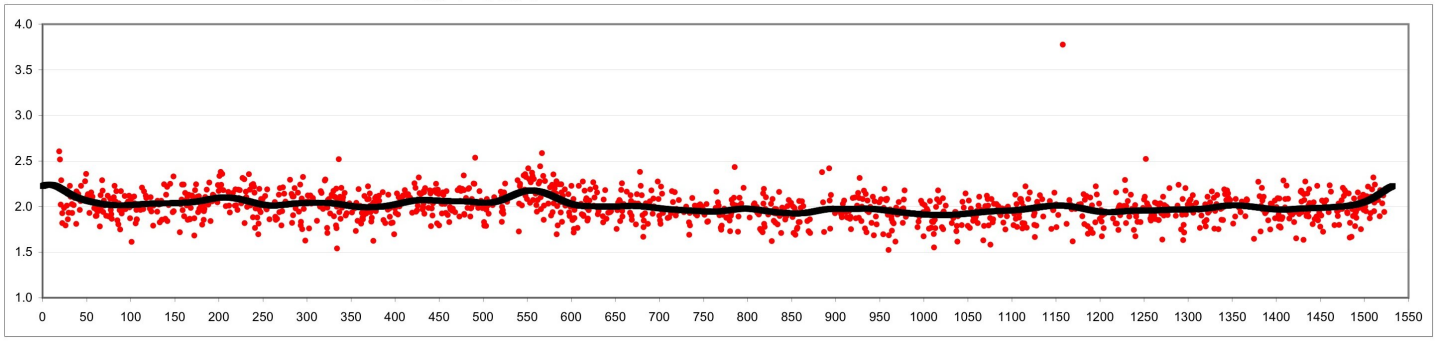
YJL8773 – Linker L9+L15



YJL8773 – Linker L9+L15



YJL8775 – Linker L11+L15



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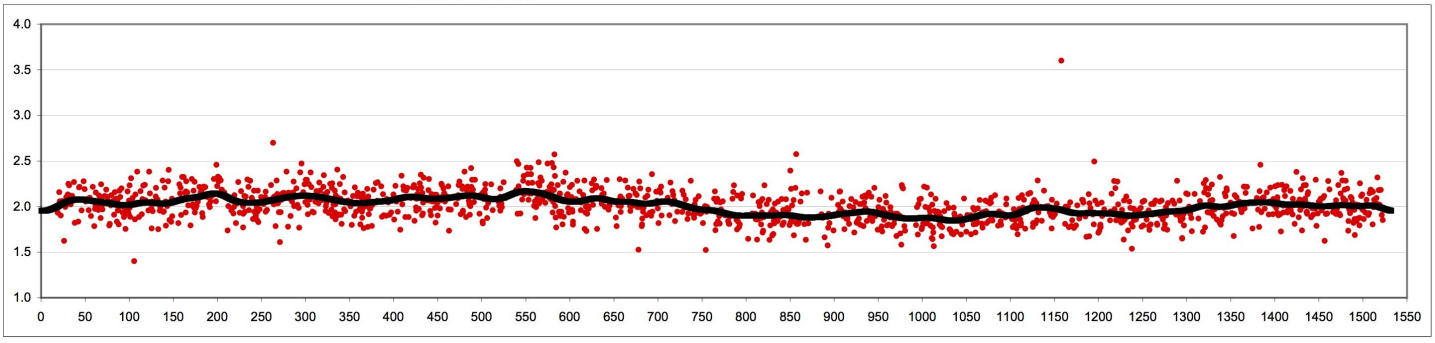
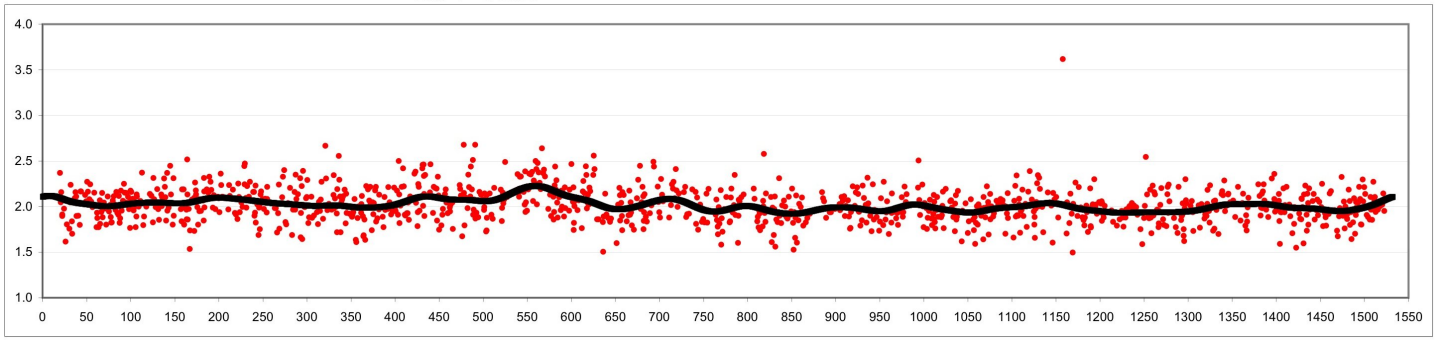
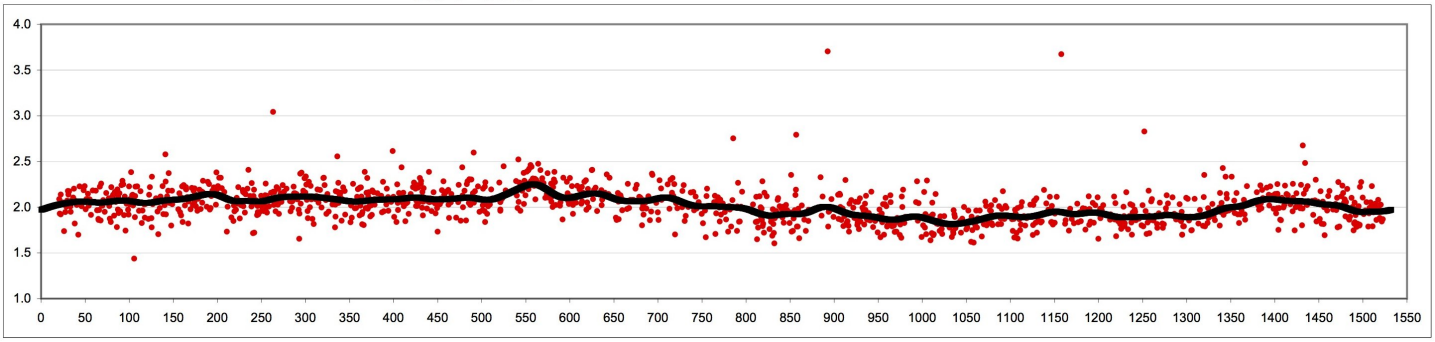


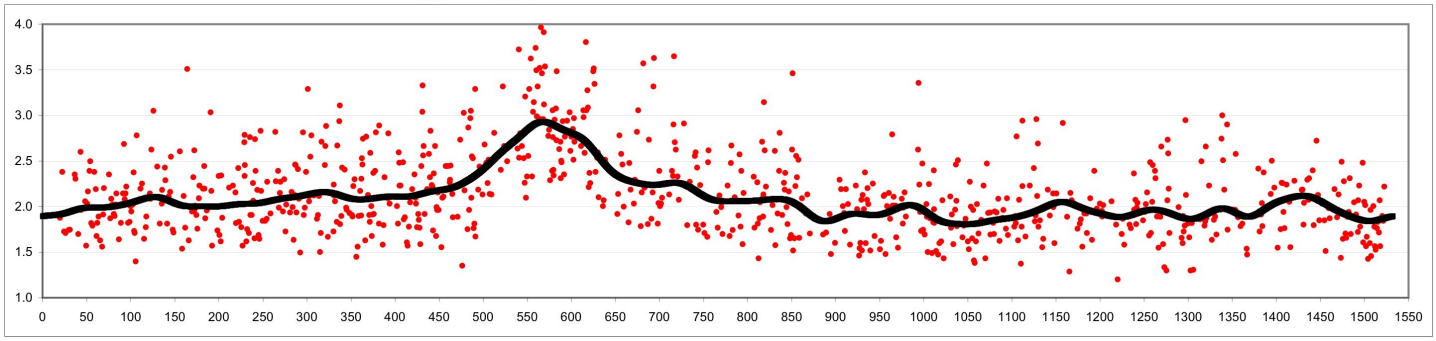
YJL8777 – Linker L9+L11



YJL8777 – Linker L9+L11



YJL8826 – Linker L13+L17



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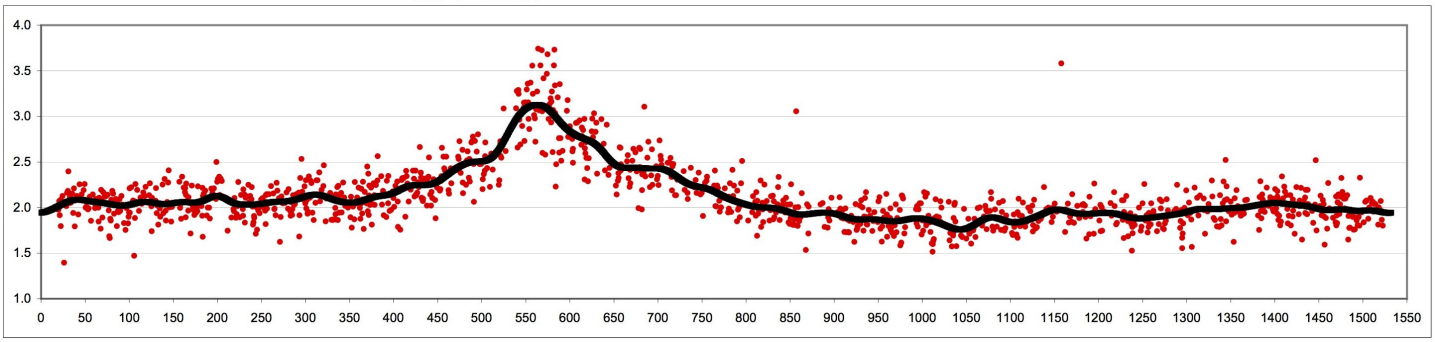
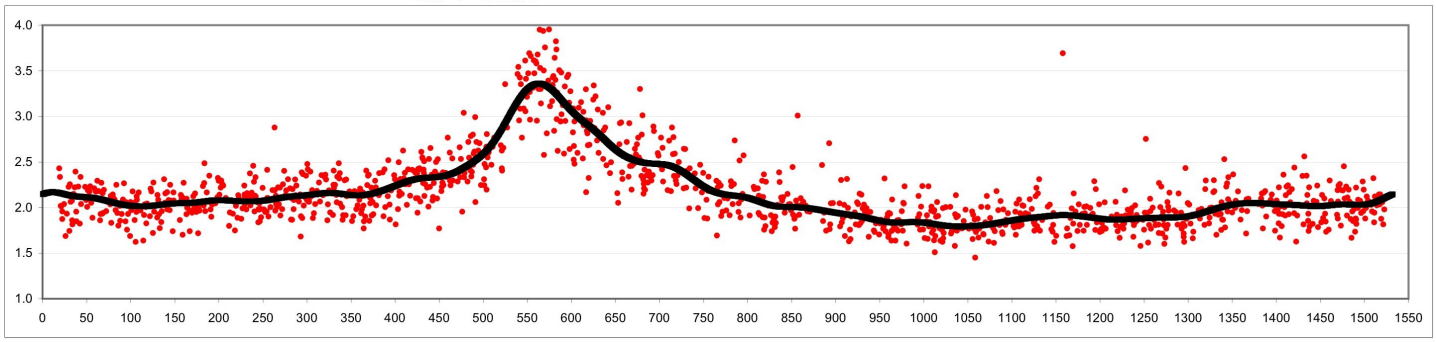


YJL8828 – Linker L21+L23



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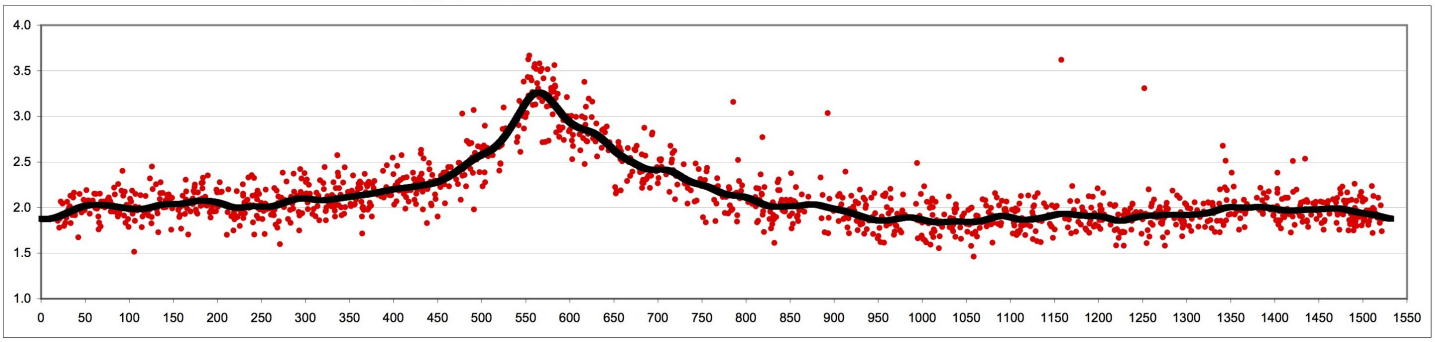
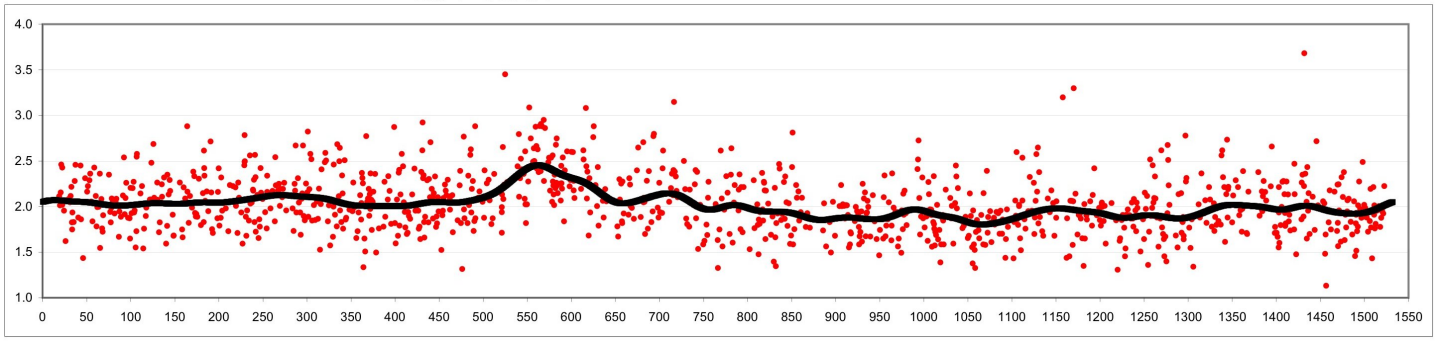


YJL8830 – Linker L9+L17



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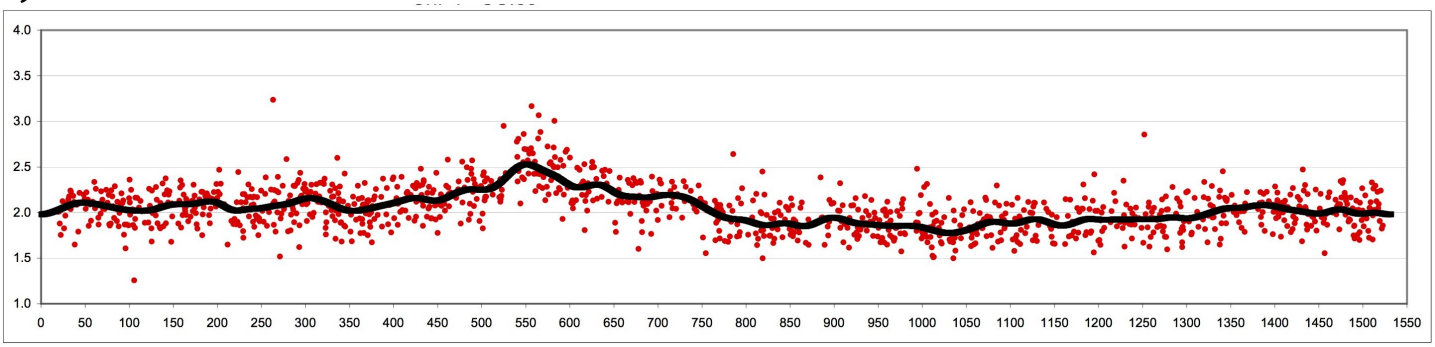

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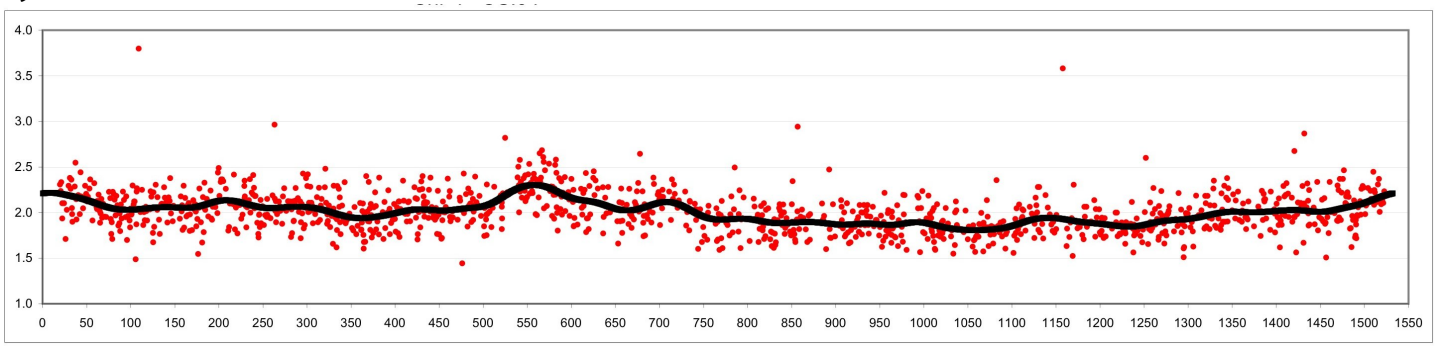

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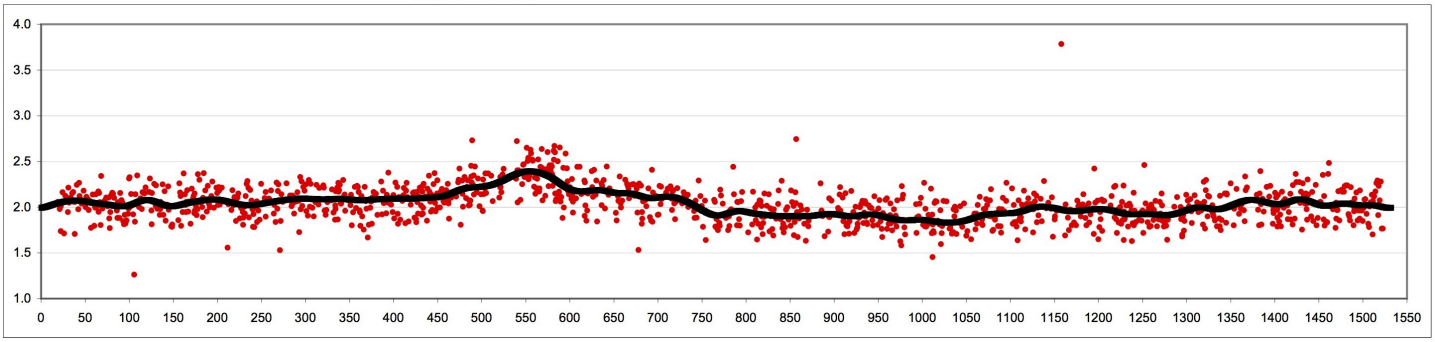

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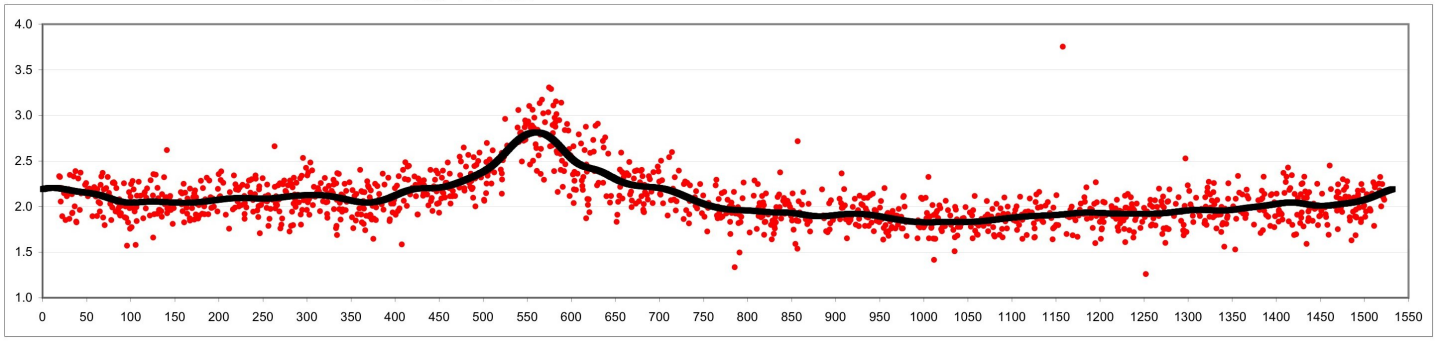
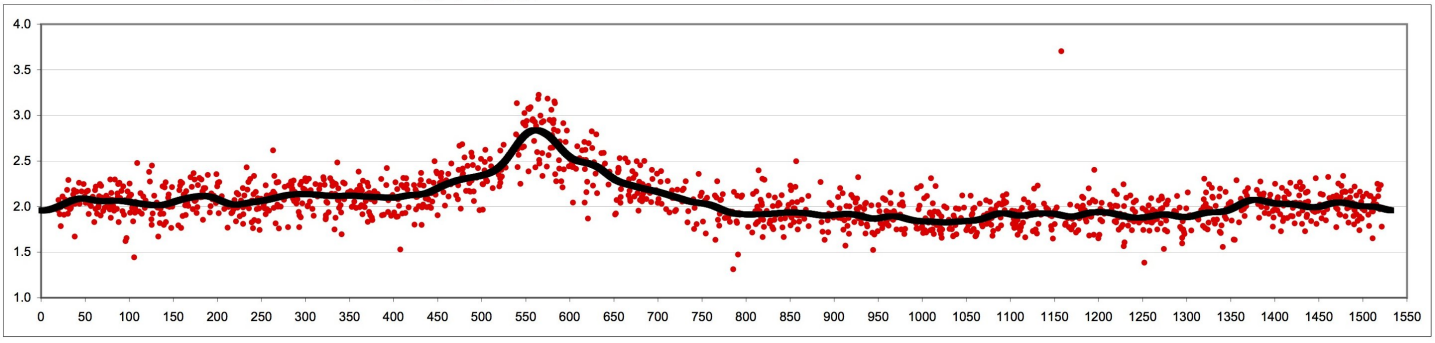
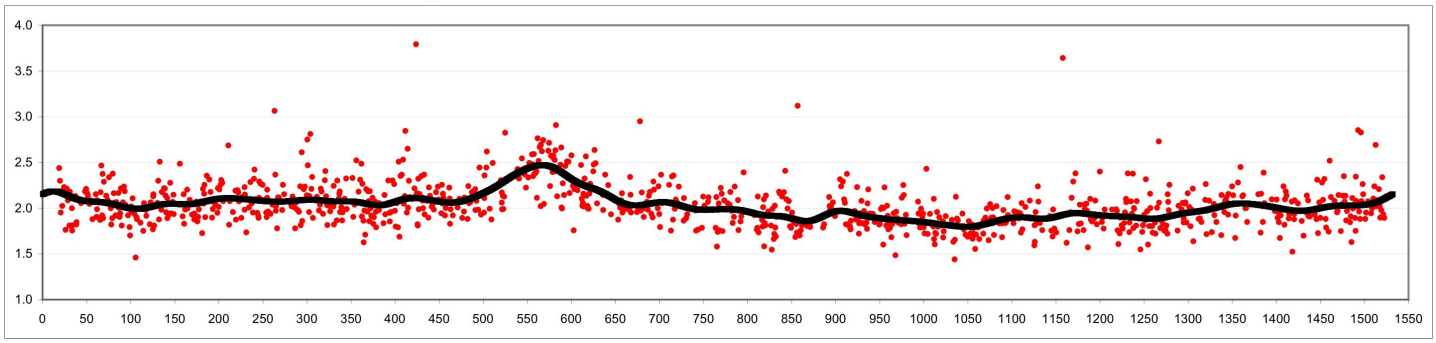


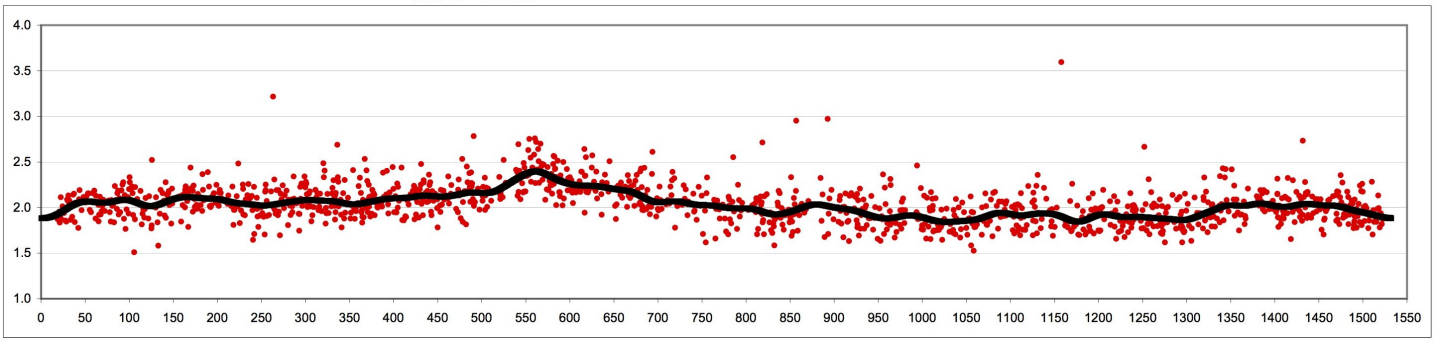
YJL8834 – Linker L15+L17



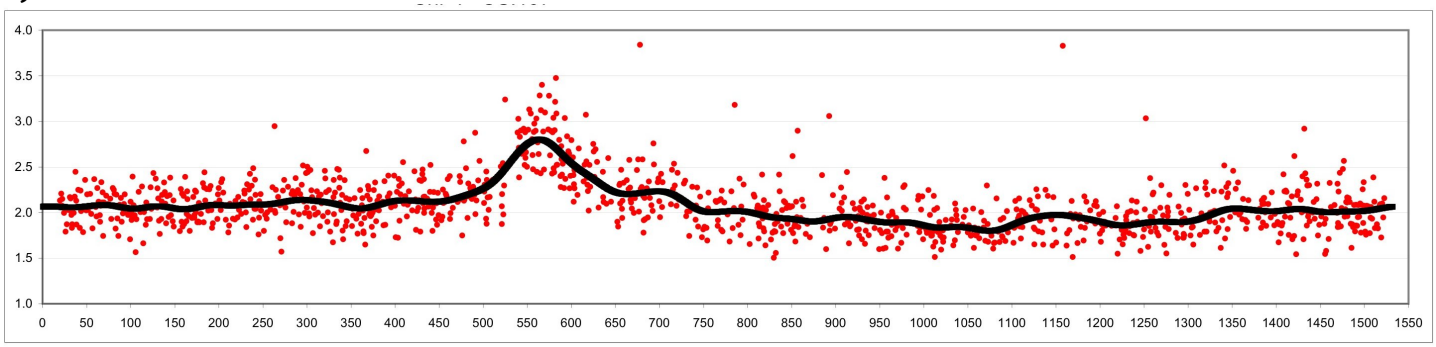
YJL8836 – Linker L9+L13



YJL8836 – Linker L9+L13



Supplemental Figure 4B
YJL8838



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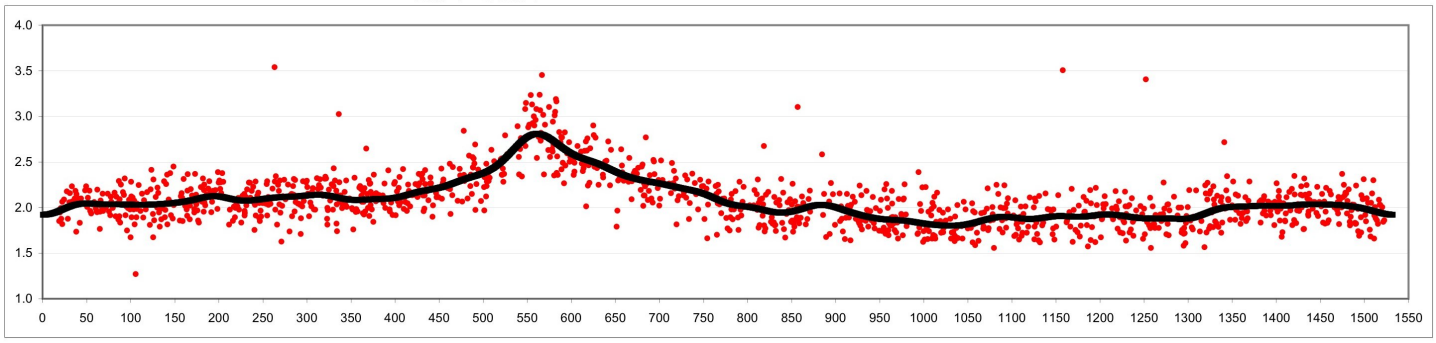
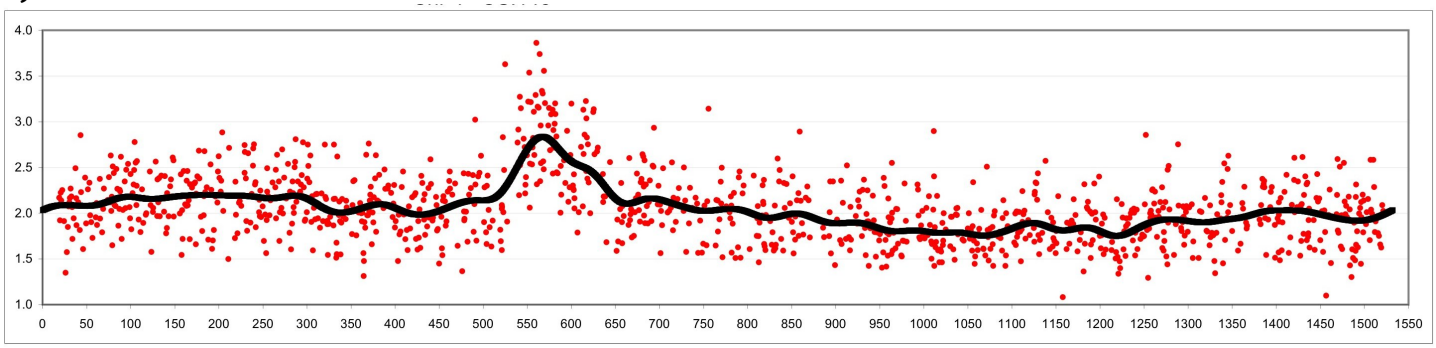
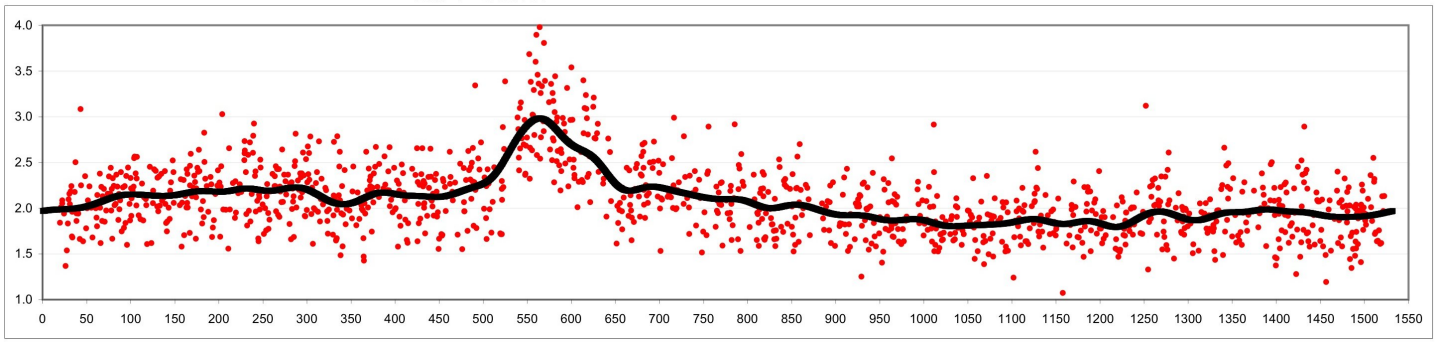


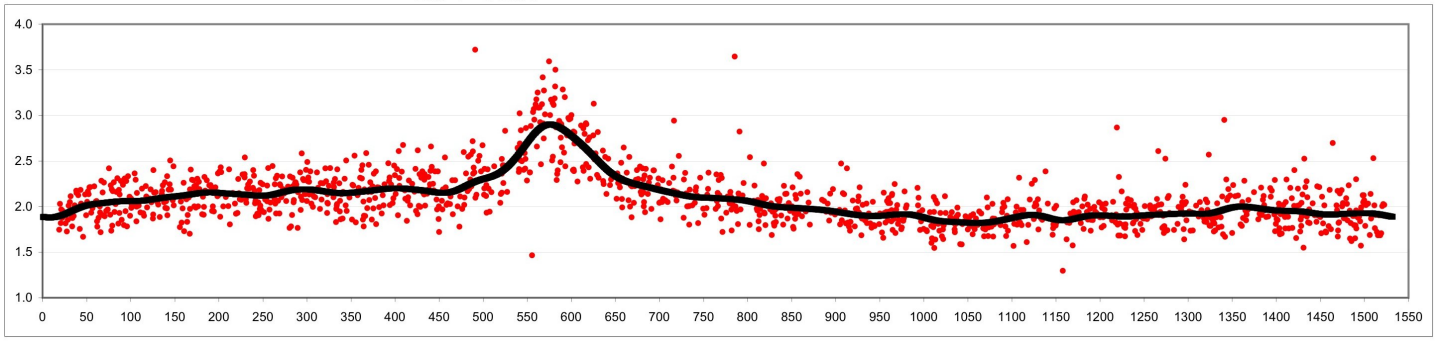
Supplemental Figure 4C
YJL9566 – ARS1238 control



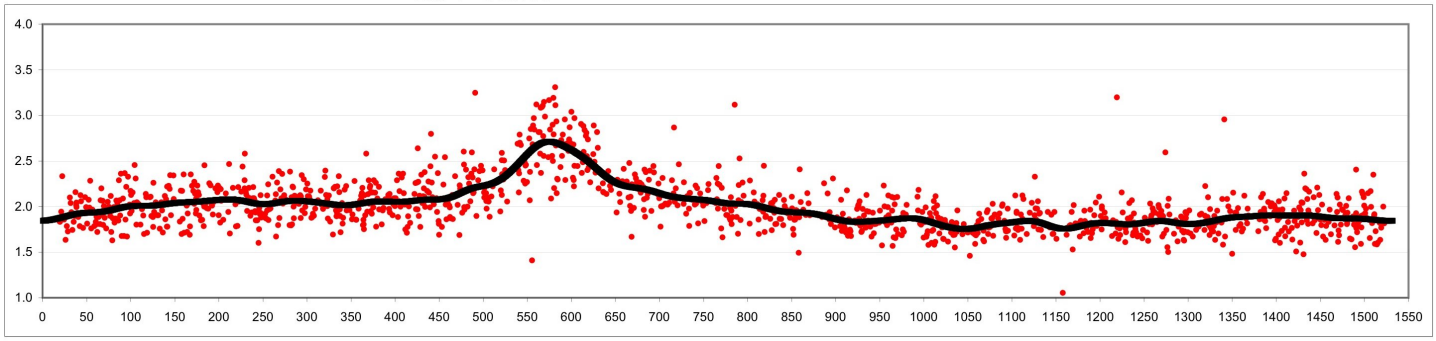
YJL9567 – ARS1238 control



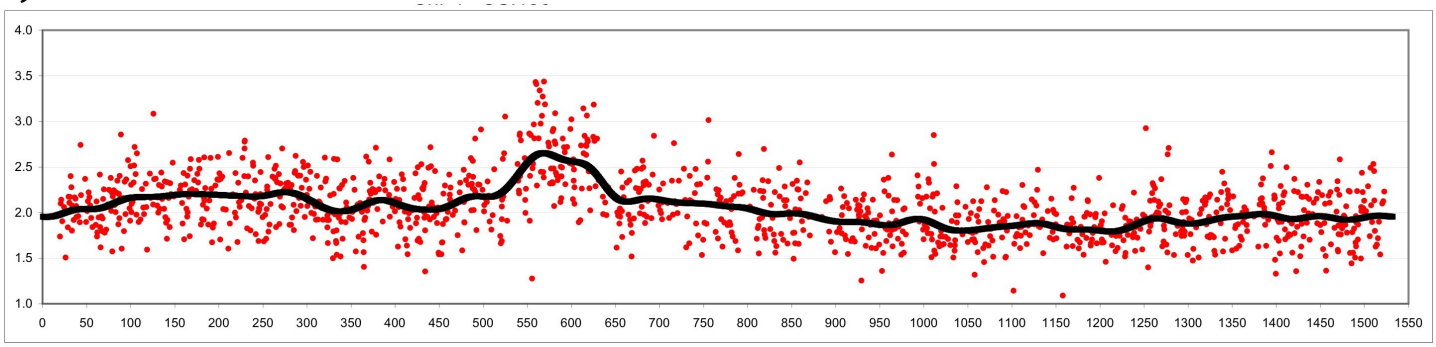
YJL10271 – ARS1238 Linker A



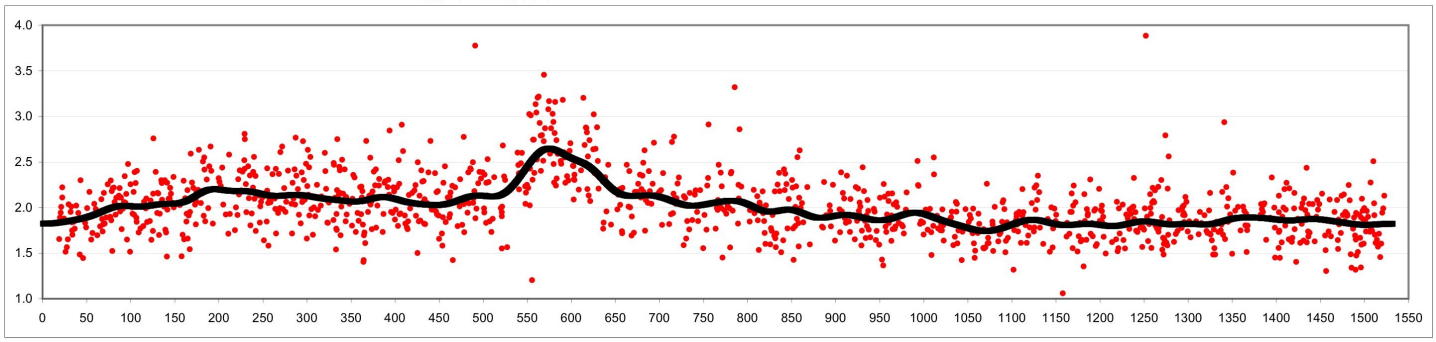
YJL10272 – ARS1238 Linker A



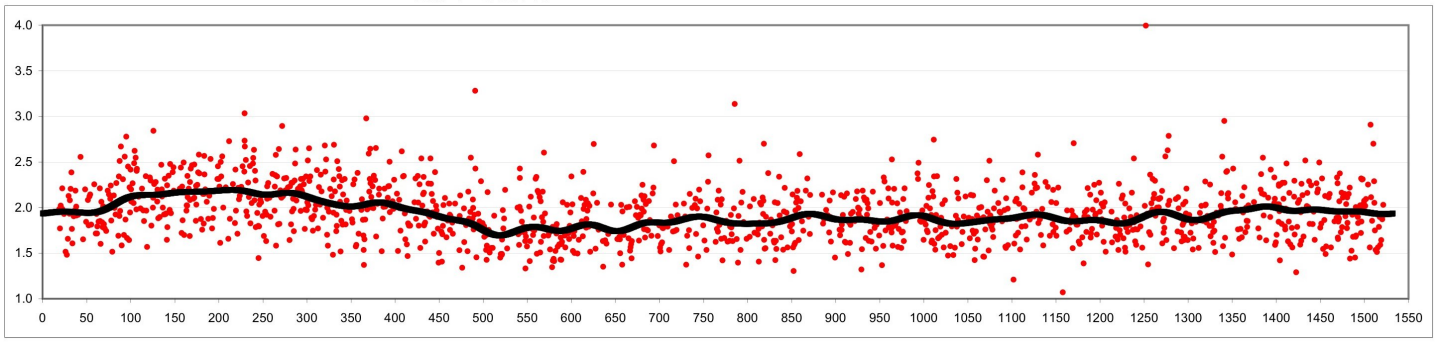
YJL10273 – ARS1238 Linker B



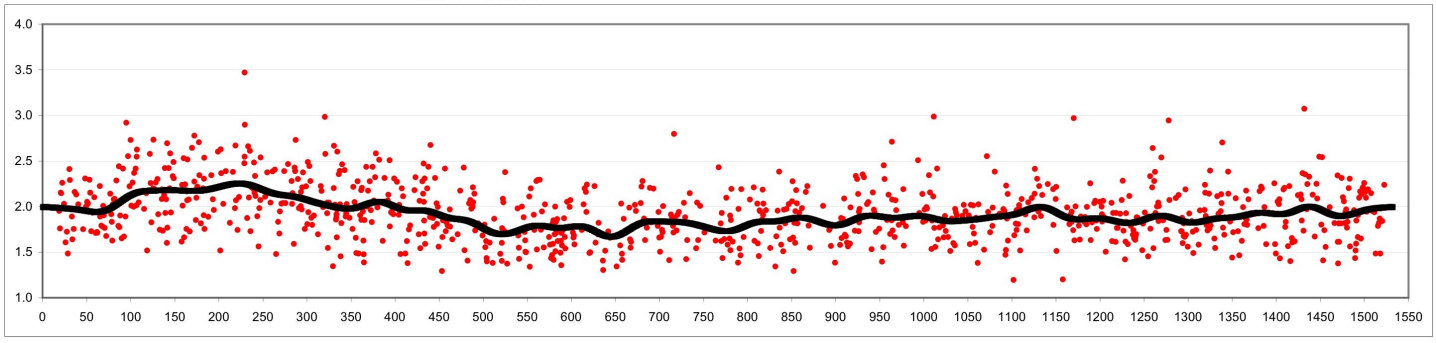
YJL10274 – ARS1238 Linker B



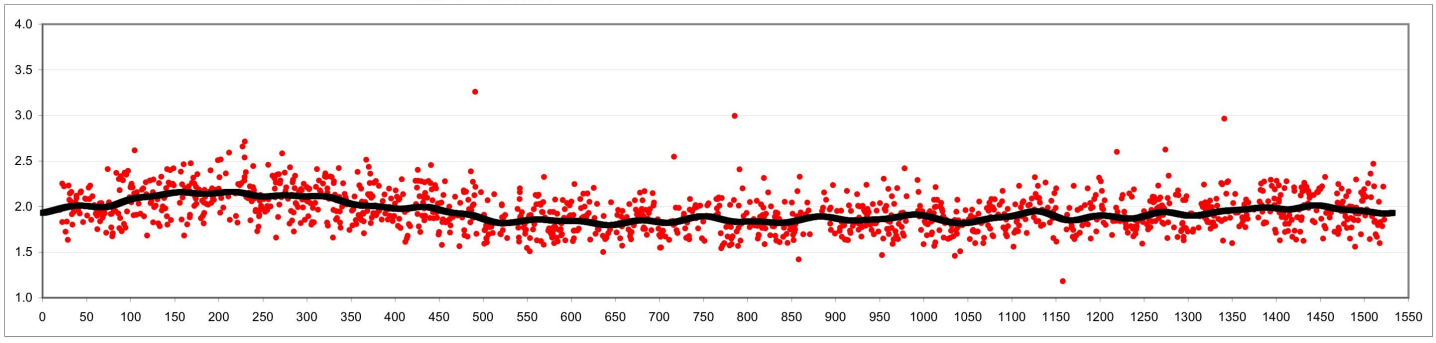
YJL10275 – ARS1238 Linker C



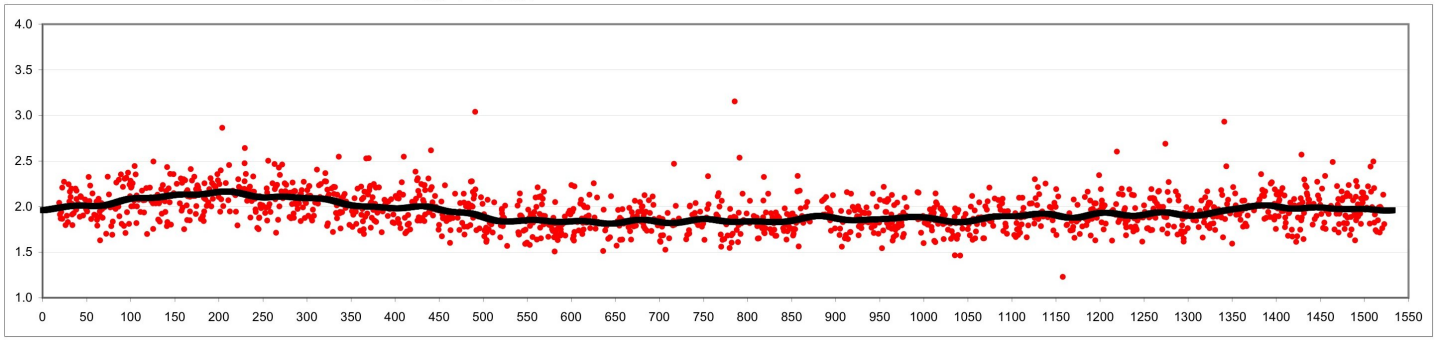
YJL10276 – ARS1238 Linker C



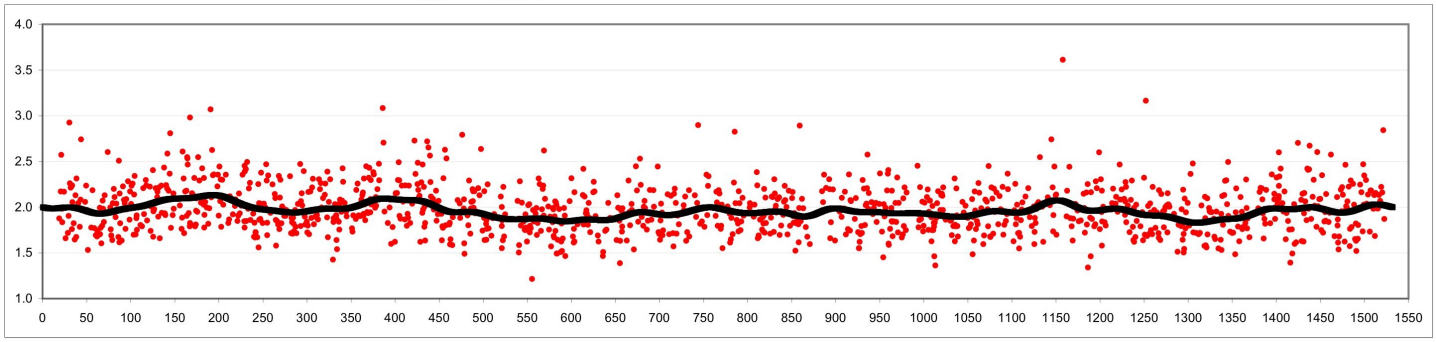
YJL10277 – ARS1238 Linker D



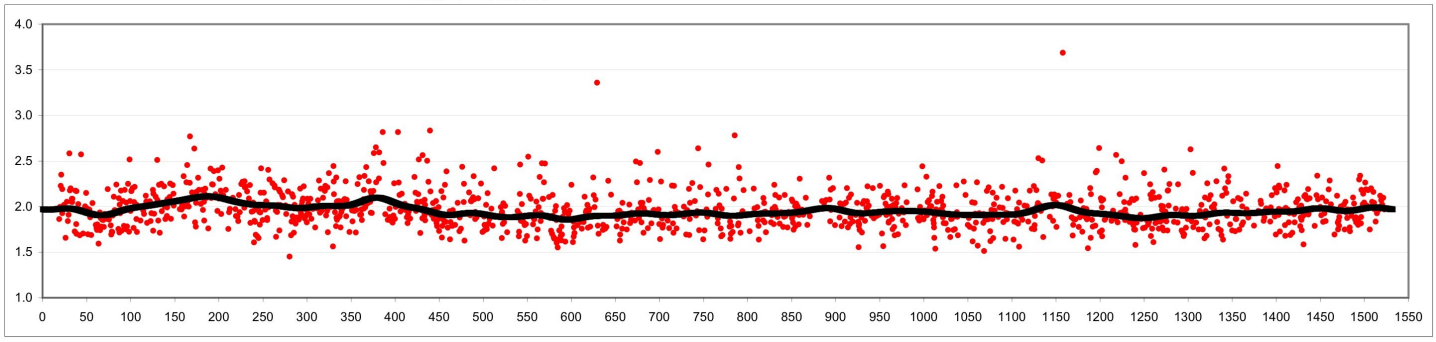
YJL10278 – ARS1238 Linker D



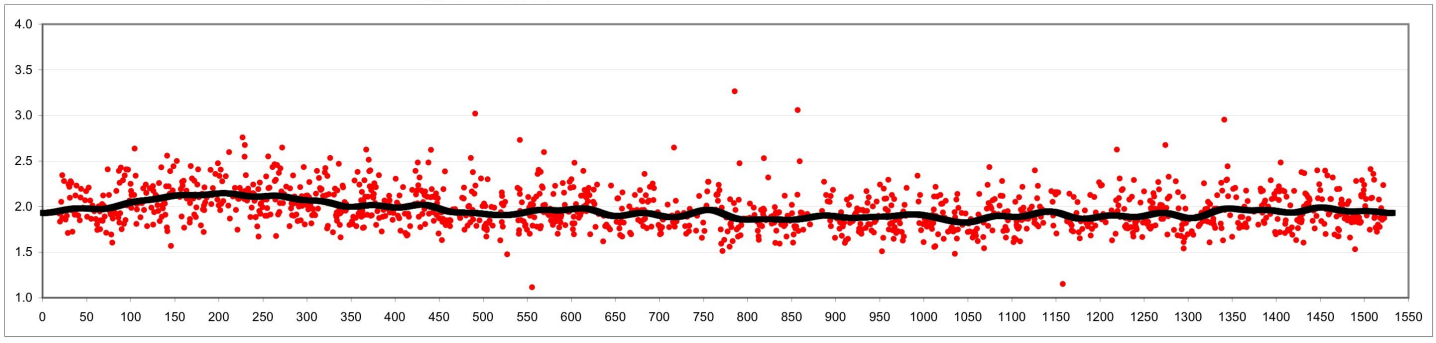
YJL10279 – ARS1238 Linker E



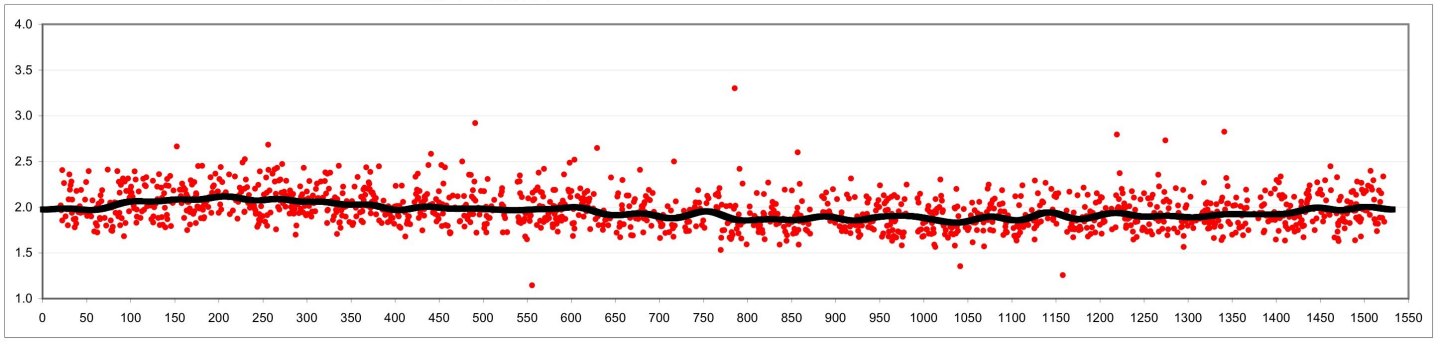
YJL10280 – ARS1238 Linker E



YJL10281 – ARS1238 Linker F



YJL10282 – ARS1238 Linker F



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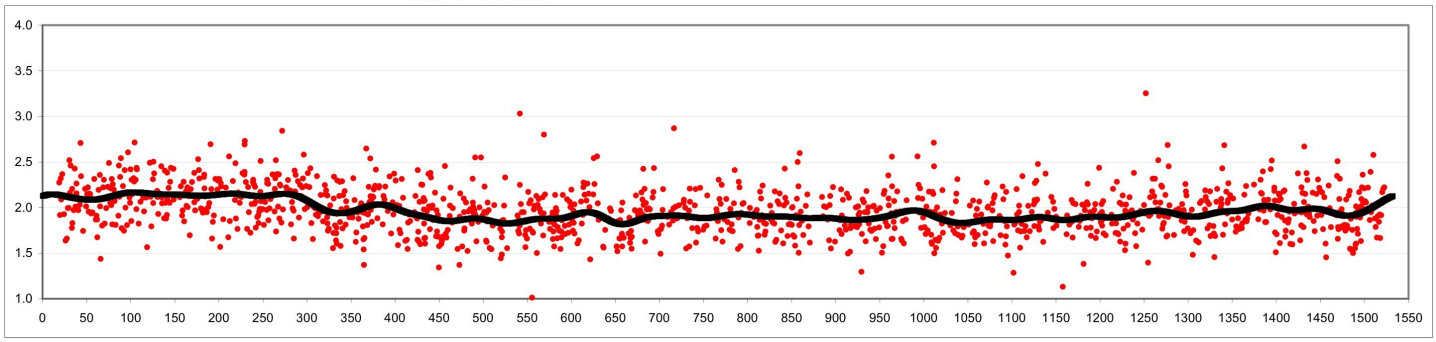

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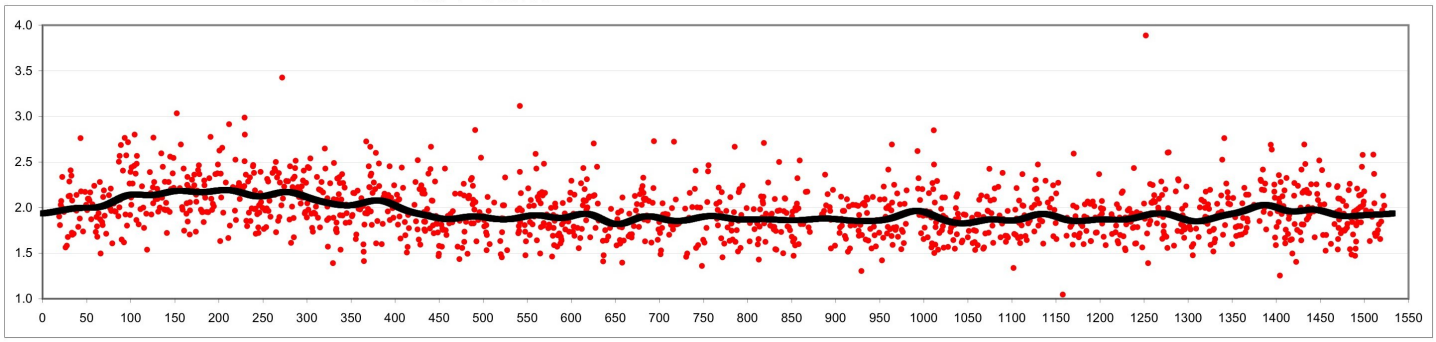
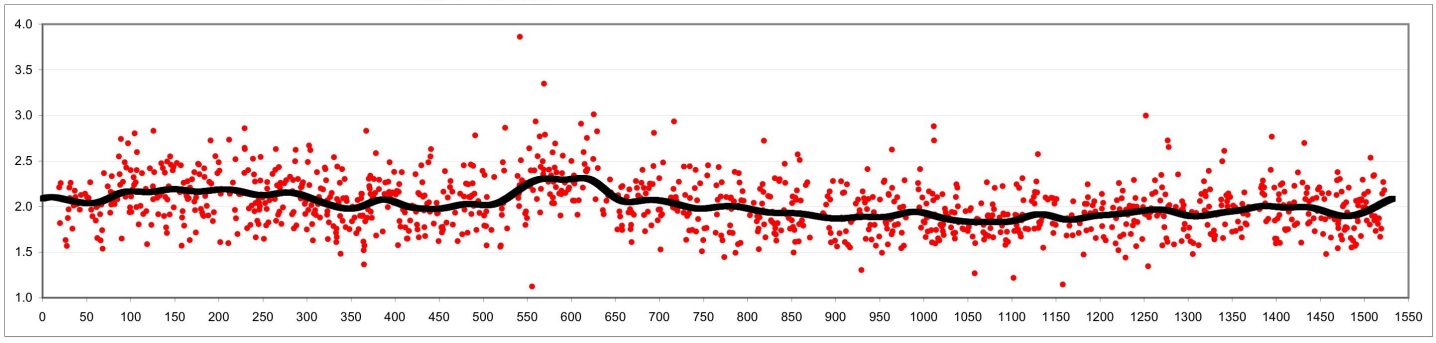


YJL10285 – ARS1238 Linker H



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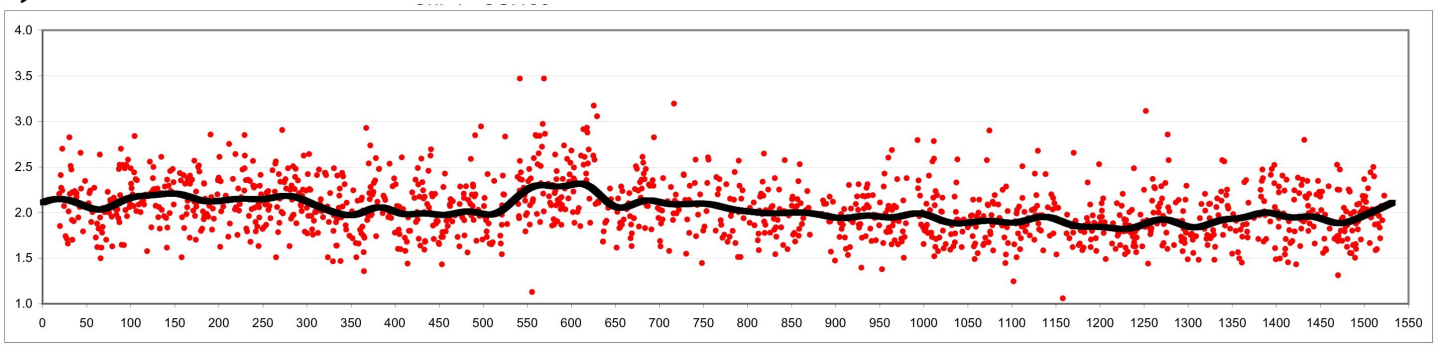
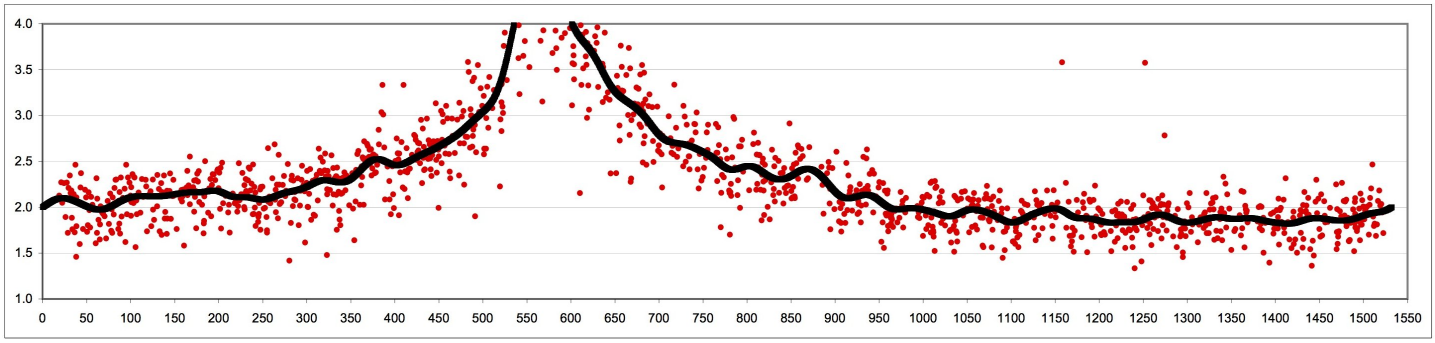
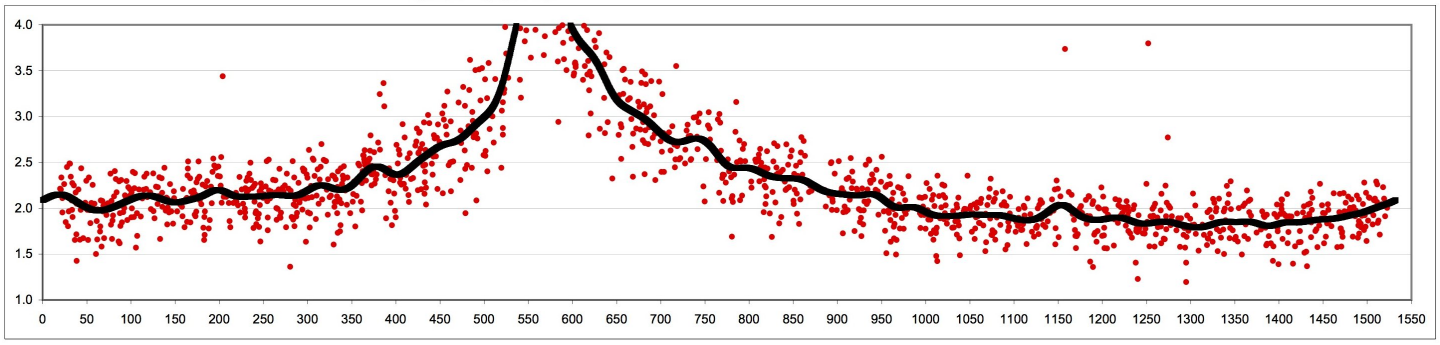


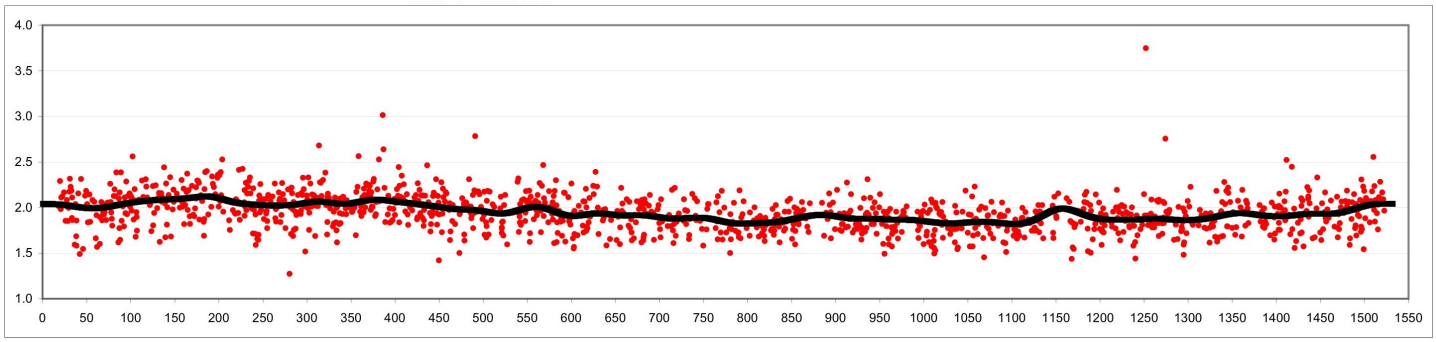
Supplemental Figure 5
YJL8398 – control



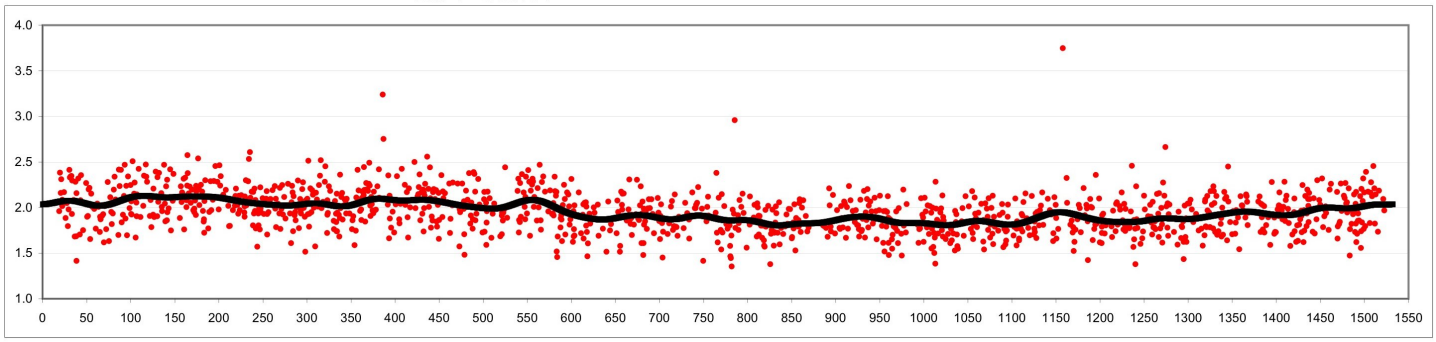
YJL8399 – control



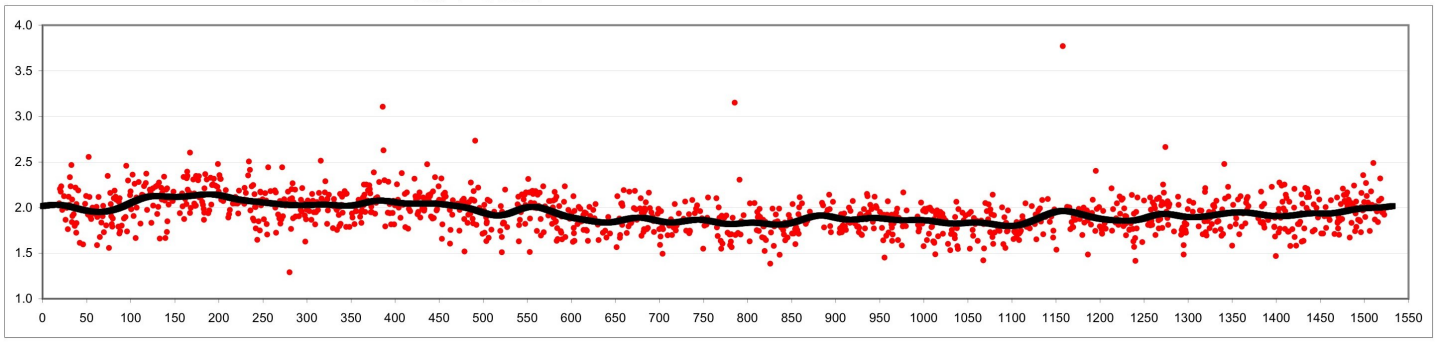
YJL10319 – C-D Reverse



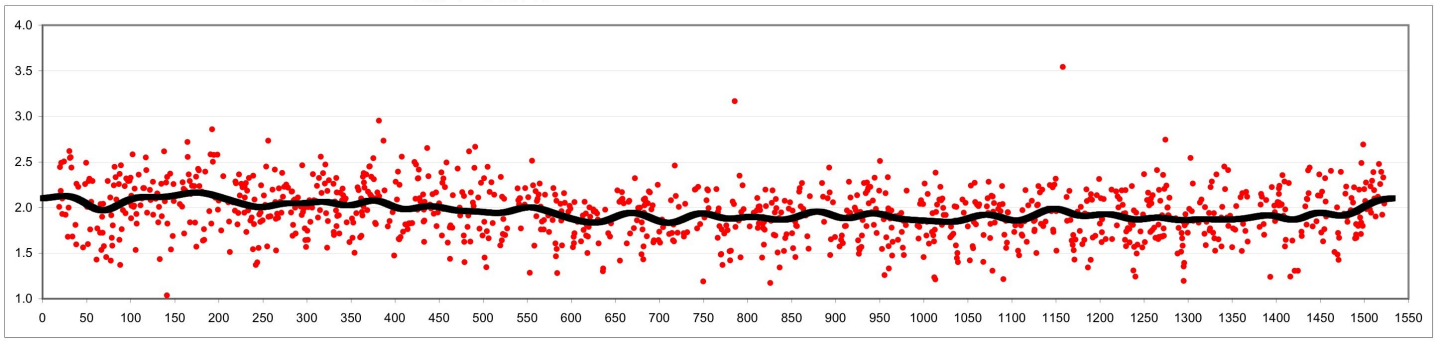
YJL10320 – C-D Reverse



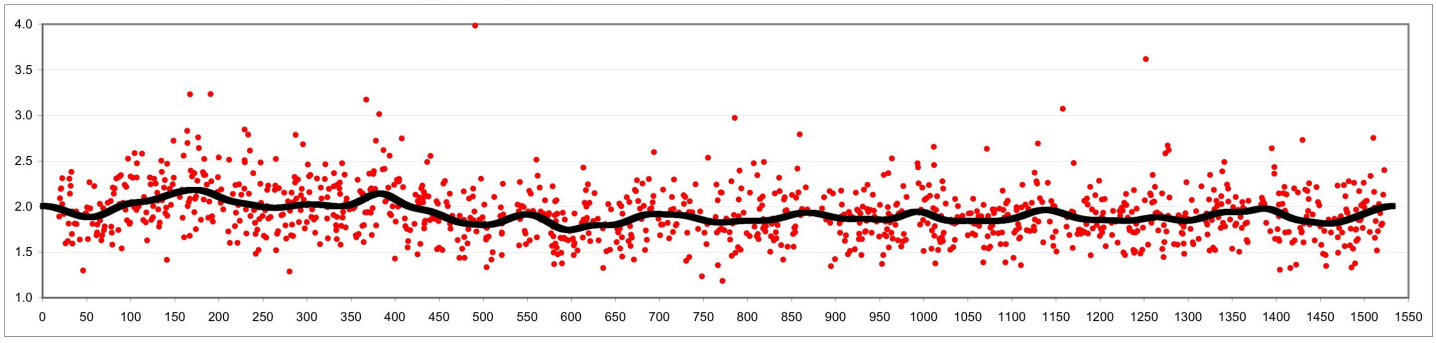
YJL10321 – C-D Transversion



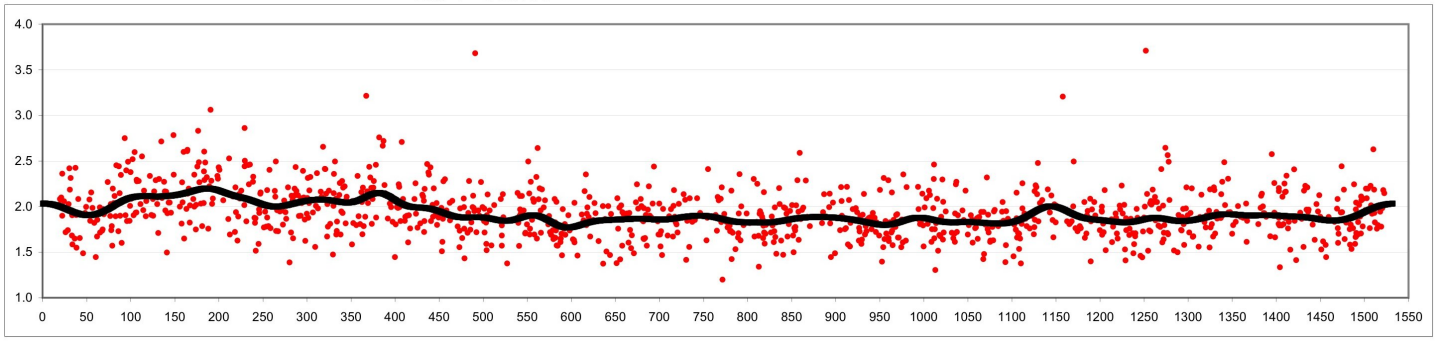
YJL10322 - C-D Transversion



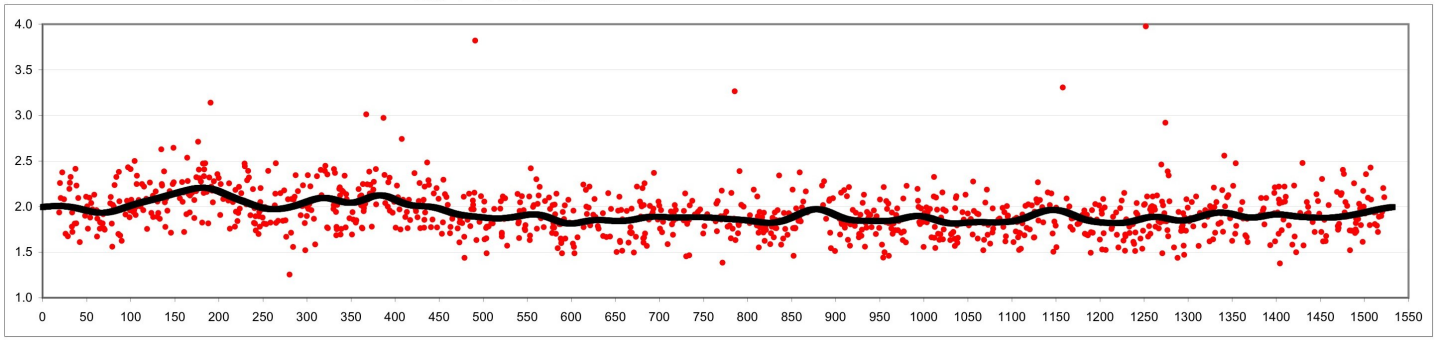
YJL10323 - C-D Scramble



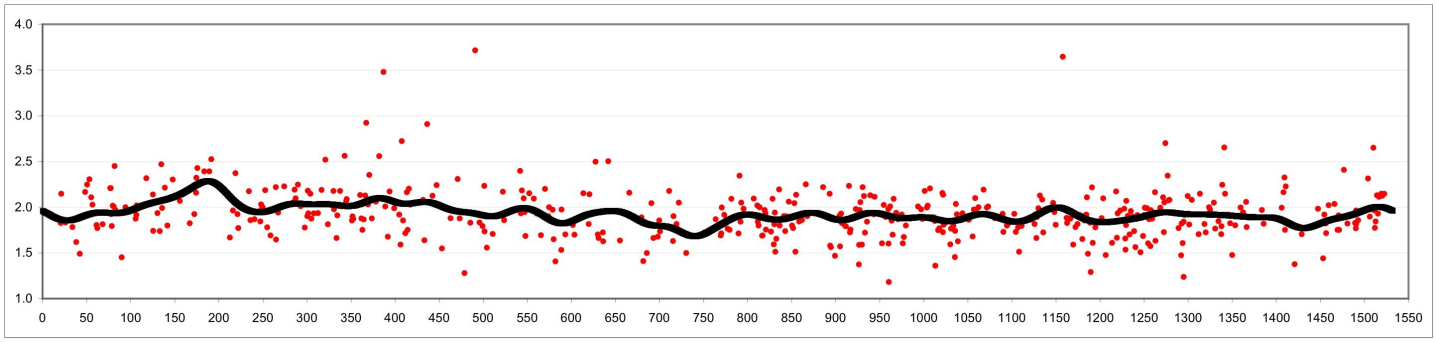
YJL10324 - C-D Scramble



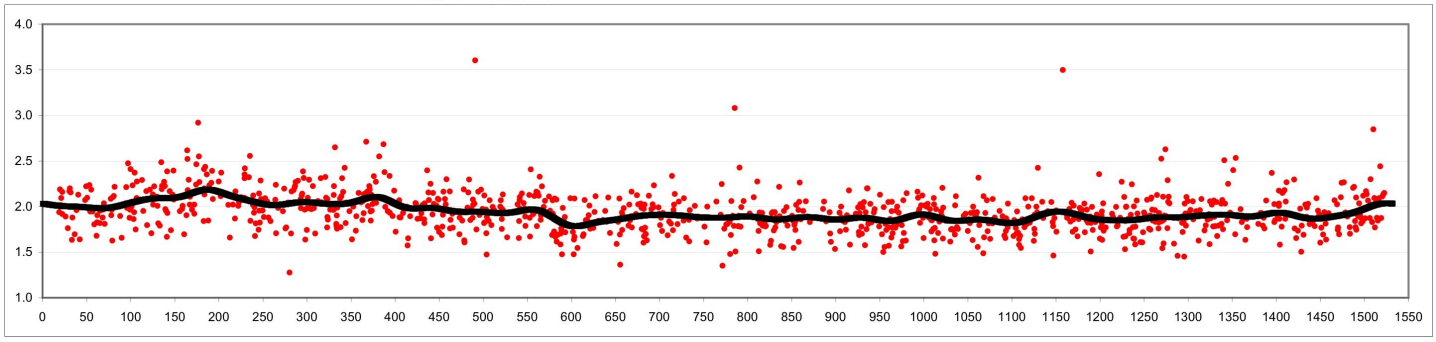
YJL10325 – C Transversion



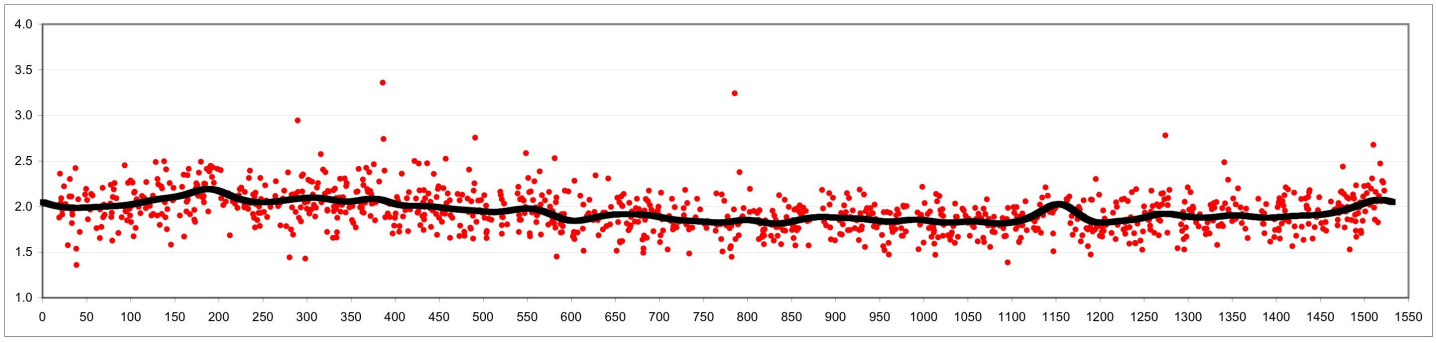
YJL10326 - C Transversion



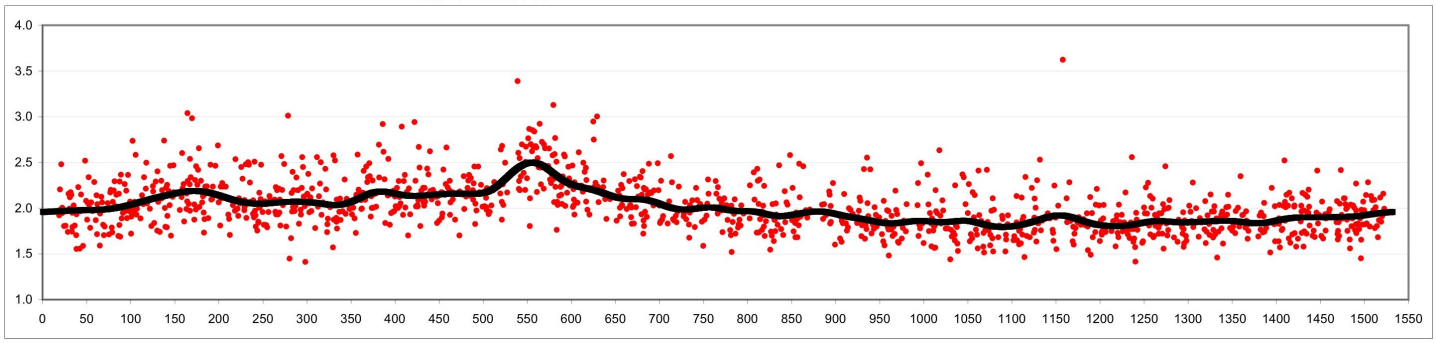
YJL10327 - D Transversion



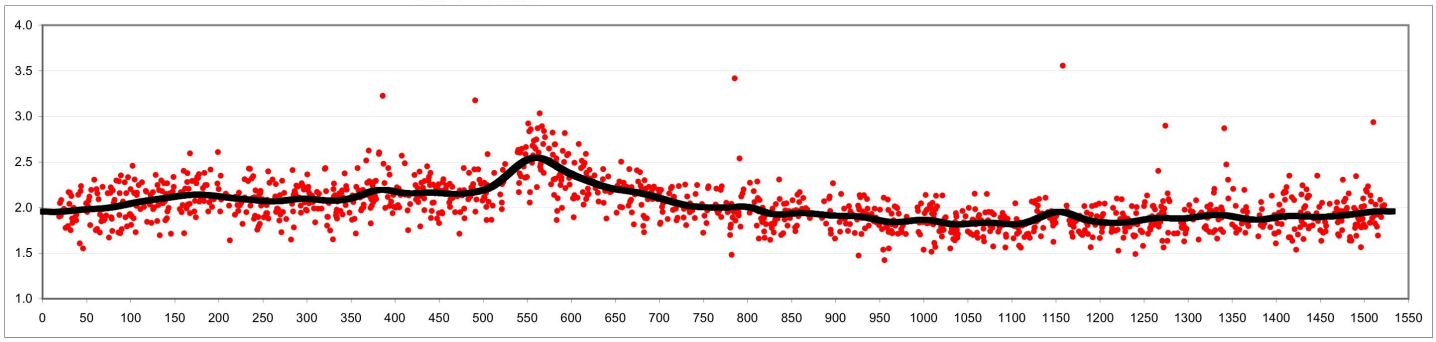
YJL10328 - D Transversion



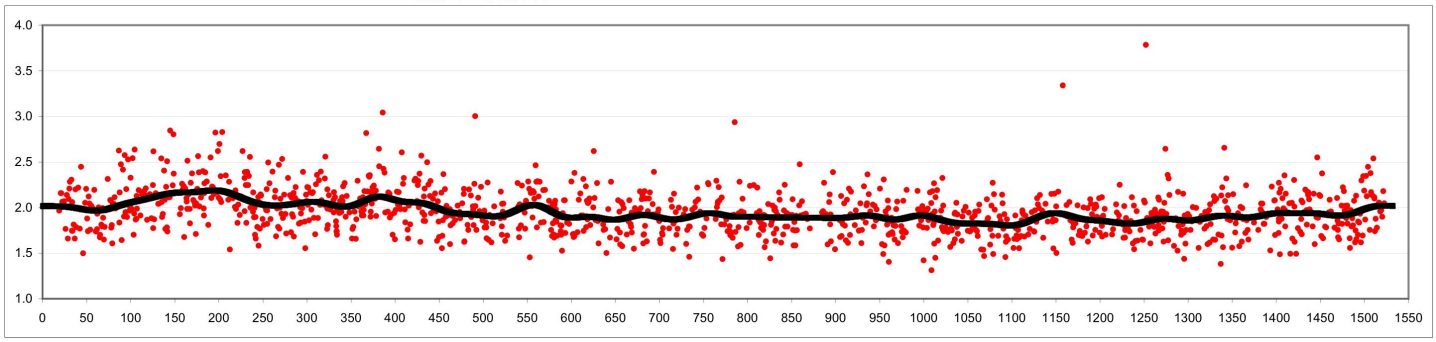
YJL10329 – C1 Transversion



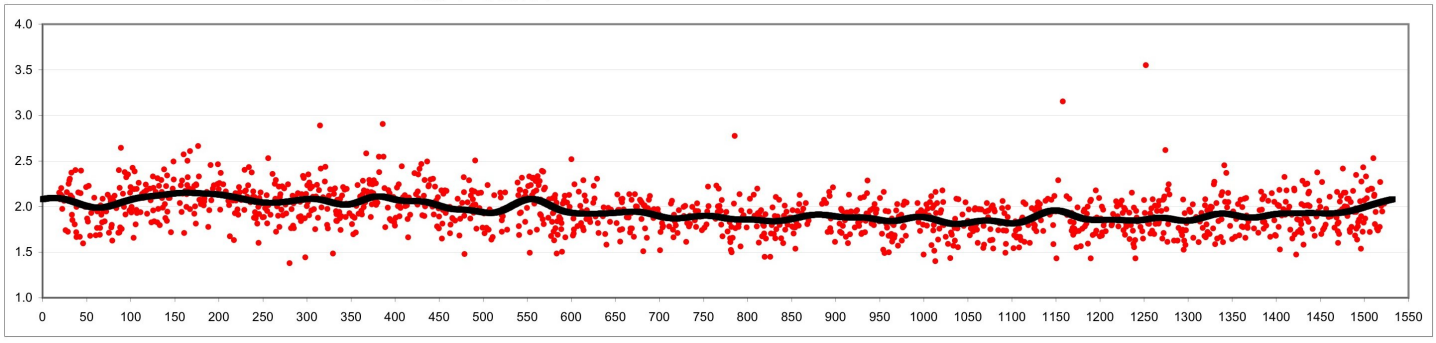
YJL10330 – C1 Transversion



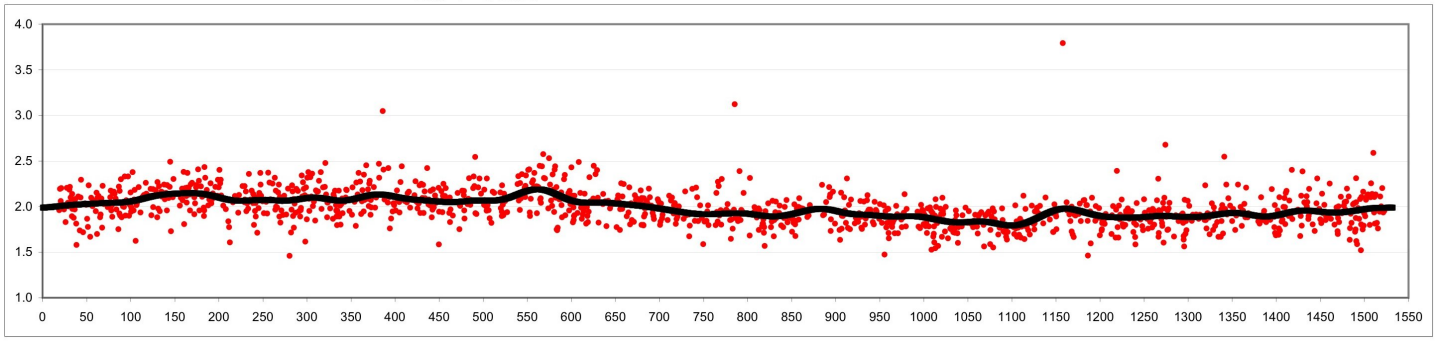
YJL10331 – C2 Transversion



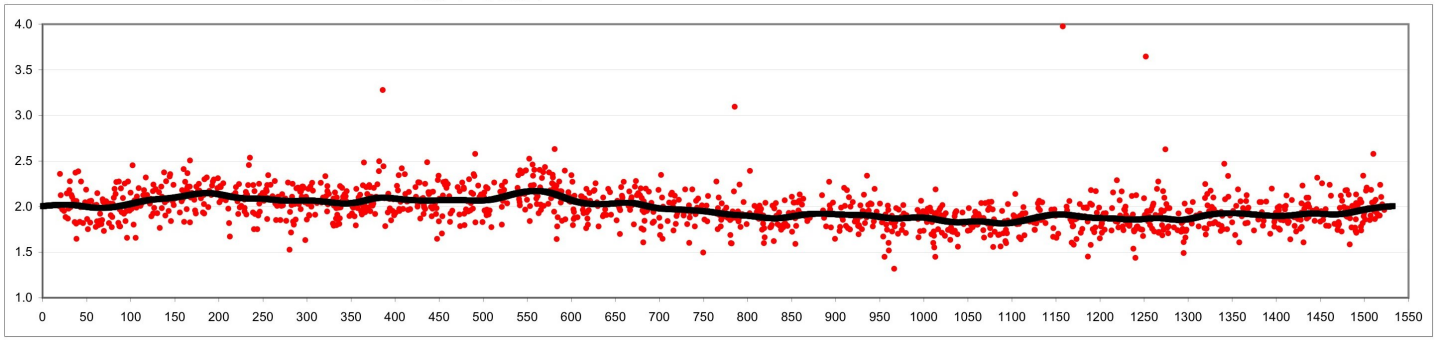
YJL10332 – C2 Transversion



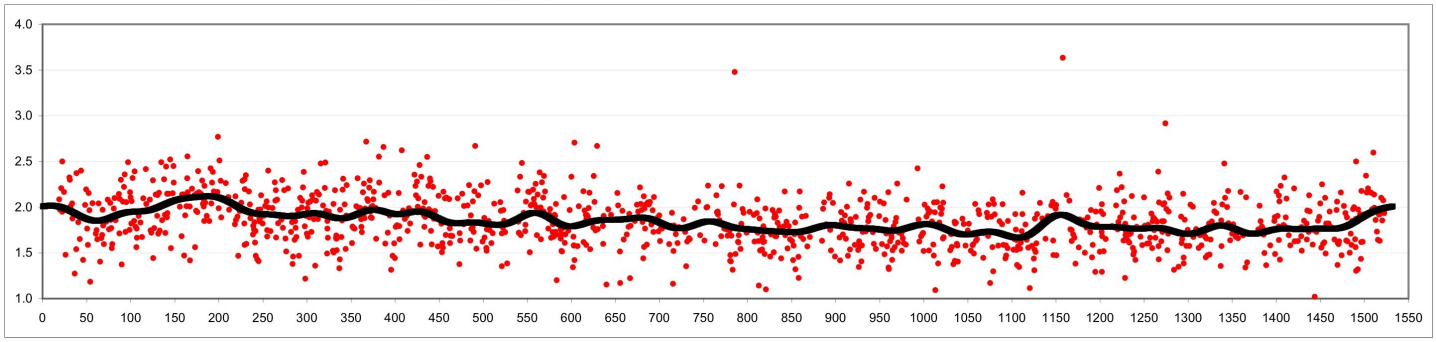
YJL10333 – D1 Transversion



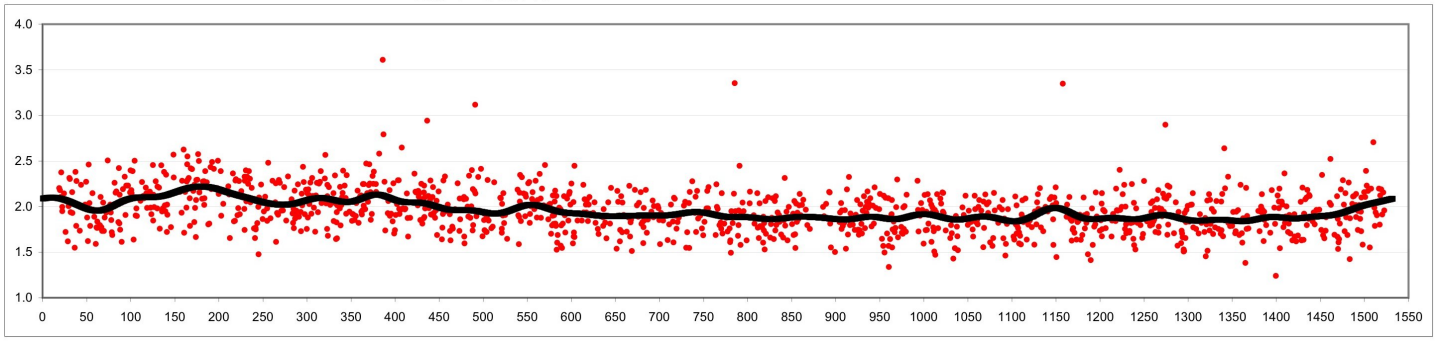
YJL10334 - D1 Transversion



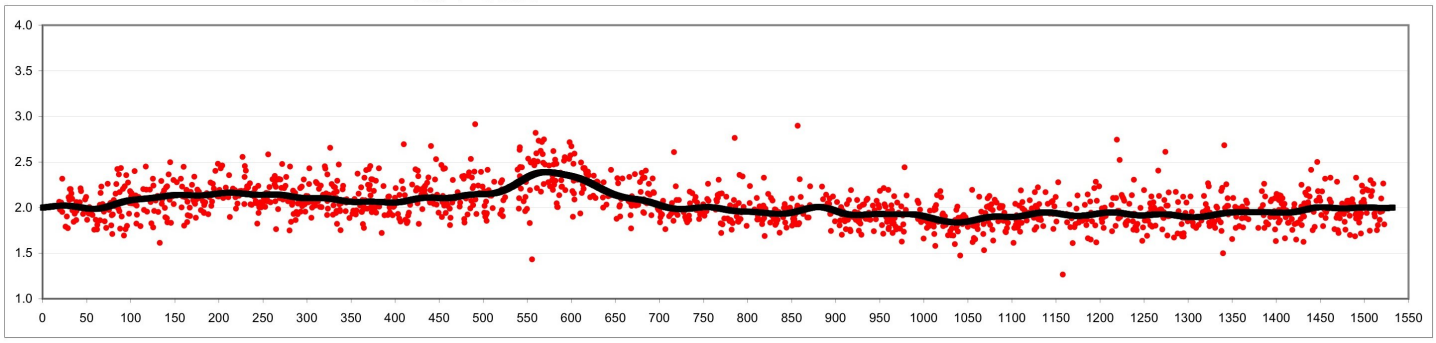
YJL10335 – D2 Transversion



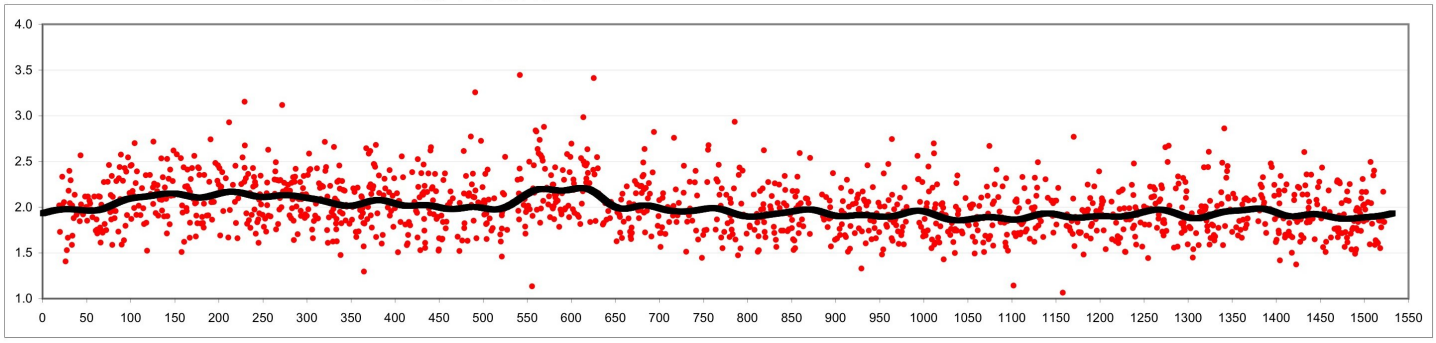
YJL10336 – D2 Transversion



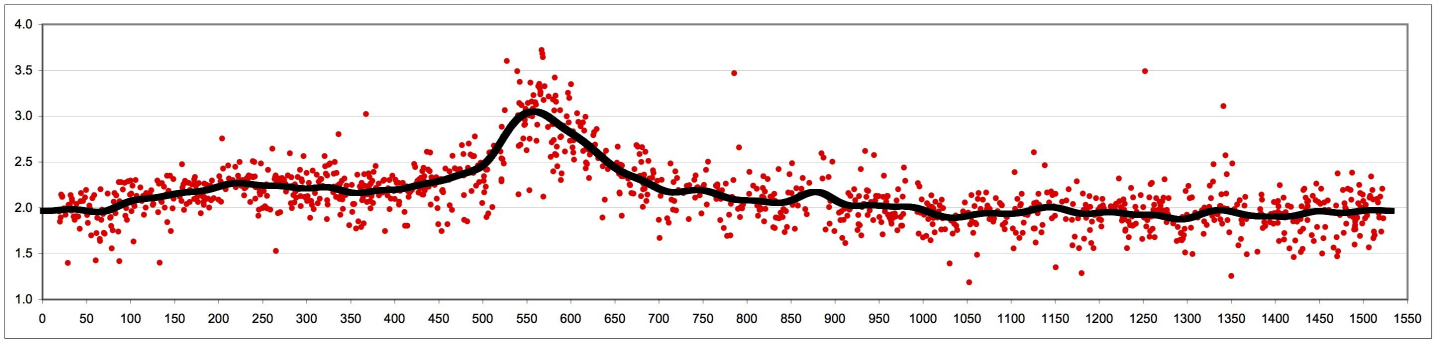
Supplemental Figure 6A
YJL10160



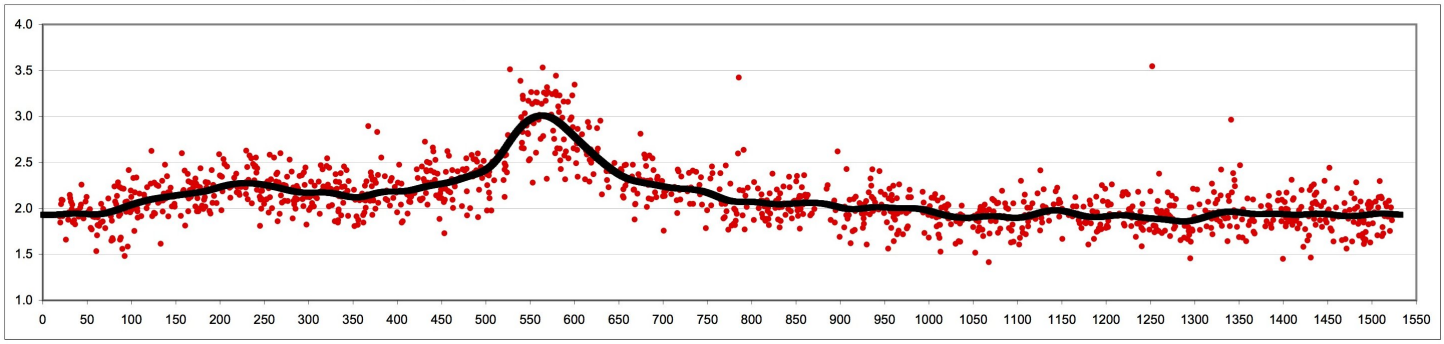
YJL10161



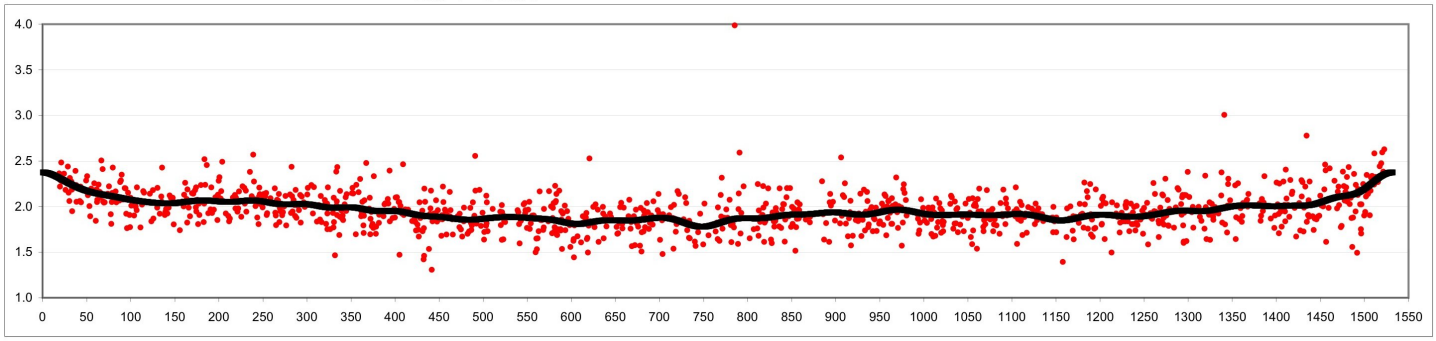
Supplemental Figure 6B
YJL9082 – 6 hour



YJL9082 – 6 hour



YJL9084



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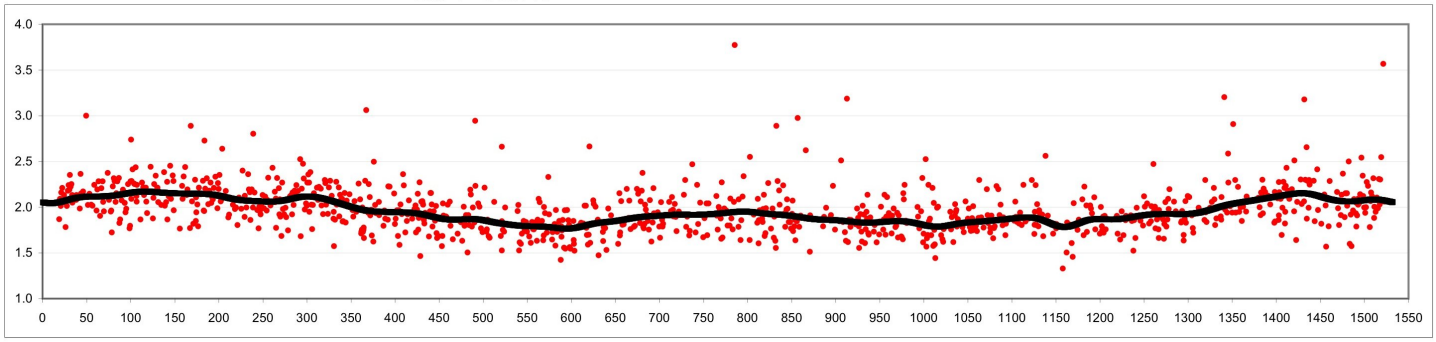
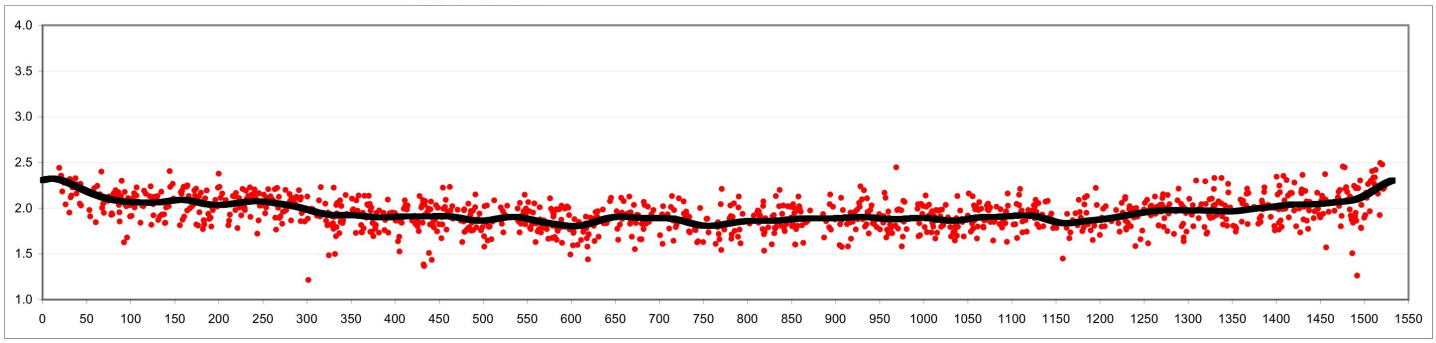


YJL9086



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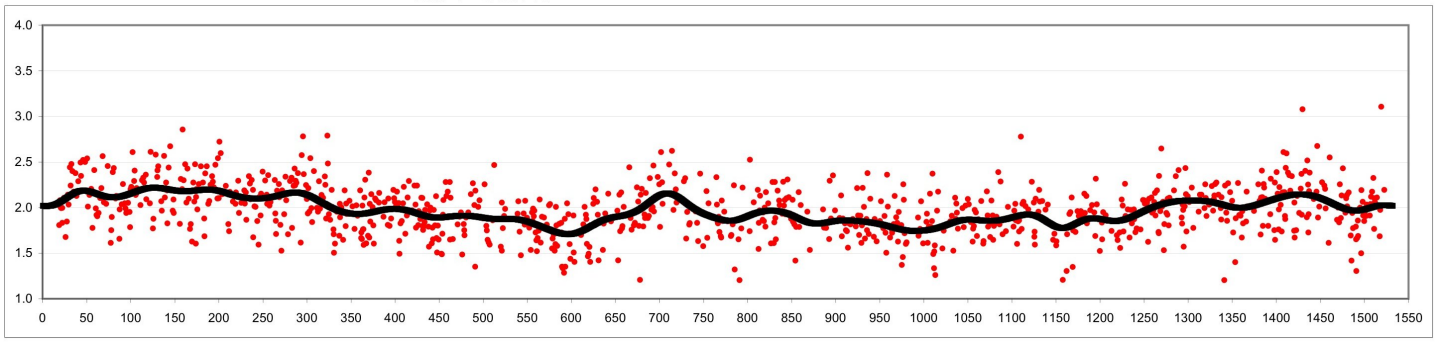
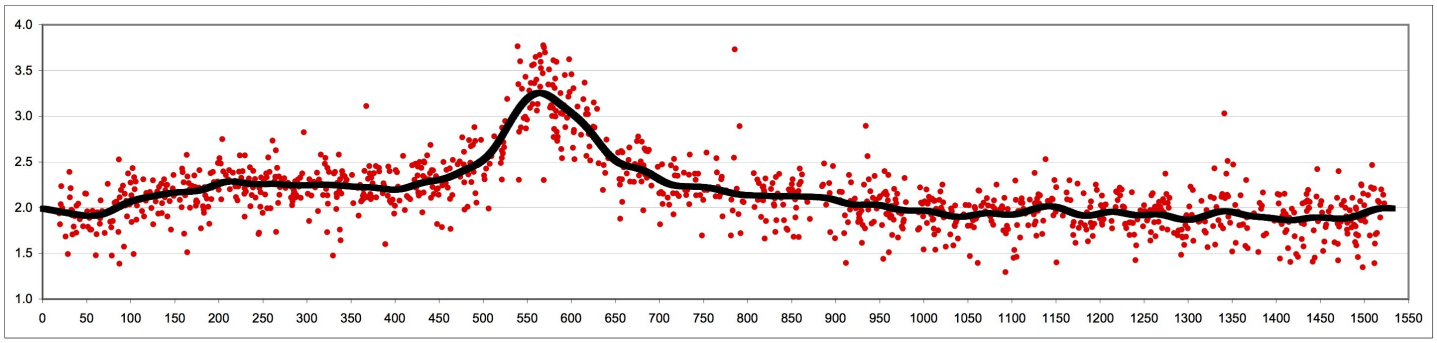


YJL9088 – 6 hour



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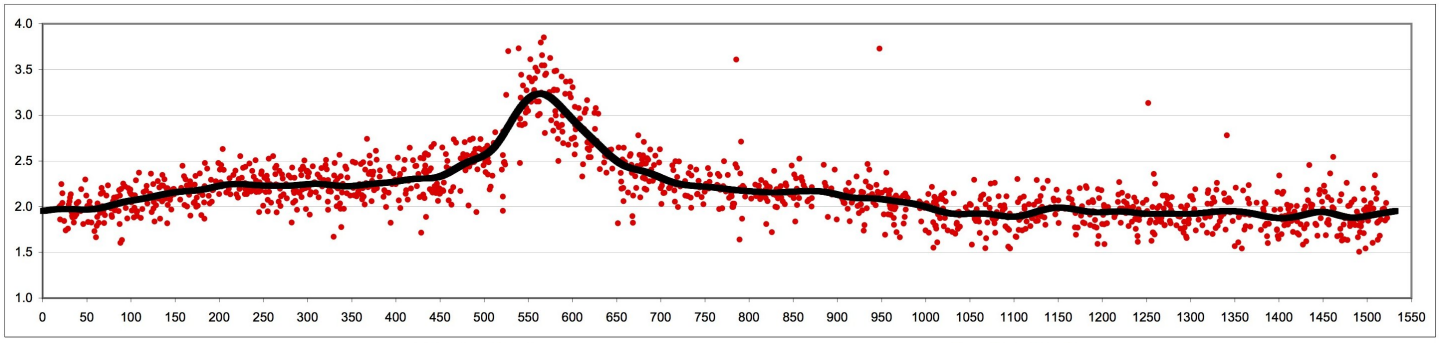

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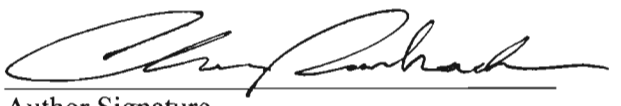

Author Signature

6/12/14

Date