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Los Angeles

Characterization of Levels of Antibiotic Resistance Gene, *erm*(B), and Anthropogenic Influence in Soil at 26 Public Parks in Four Cities and Two Pristine Sites in California

A thesis submitted in partial satisfaction of
the requirements for the Master of Science degree
in Civil Engineering

by

Renjie Li

2016

ABSTRACT OF THE THESIS

Characterization of Levels of Antibiotic Resistance Gene, *erm*(B), and Anthropogenic Influence in Soil at 26 Public Parks in Four Cities and Two Pristine Sites in California

by

Renjie Li

Master of Science in Civil Engineering
University of California, Los Angeles, 2016
Professor Jennifer Ayala Jay, Chair

The goal of this study was to characterize the soil and the presence of antibiotic resistance genes (ARG) *erm*(B) in soils from 26 parks across California to investigate the correlation between the human population density and land use with levels of the antibiotic resistance gene *erm*(B). Los Angeles, San Diego, Bakersfield and Fresno were selected and sampled at six publicly accessible park sites in each city. Two pristine sites, Yosemite National Park and Mount Baldy were selected and sampled as well. DNA was extracted from soil samples, normalized and quantified for the presence of 16S rDNA *erm*(B) using quantitative polymerase chain reaction (qPCR). *Erm*(B) levels were quantified via two analysis approaches, on a per gram of soil basis and normalized to relative

16S gene abundance. Among 26 parks, the highest levels of *erm*(B) occurred in one park located in Yosemite. However, levels of *erm*(B) decreased as population density increased for the 4 urban cities, which thus *erm*(B) abundance seems not clearly related to population density. In addition, agricultural areas showed high detection of gene *erm*(B), which it might be influential to the *erm*(B) detection.

The thesis of Renjie Li is approved.

Michael K. Stenstrom

Keith D. Stolzenbach

Jennifer Ayala Jay, Committee Chair

University of California, Los Angeles

2016

Contents

Introduction

Over the past few decades, there has been growing concern about the proliferation of antibiotic resistance (AR), owing to the wide and increasing usage of antibiotics. Nowadays, the majority of antibiotics are used to treat or avoid animal diseases and more widely to promote animal growth. For instance, 80 percent of all antibiotics sold in the United States are for use on chickens, pigs and cows to enable growth in confined animal feeding operations (CAFOs) and to make growth faster according to Natural Resources Defense Council. In China, over 8000t of antibiotics are used as food additives each year and 33% of total pharmaceutical consumption of antibiotics is on feeding operations of veterinary usage (Ji et.al., 2012). Further, antibiotics excreted in feces and urine excreted were persistent and accumulated in soils after repeated manure application, causing high content of antibiotics detected in soils and also in vegetables (Ji et.al., 2012).

Antibiotics are of concern as environmental contaminants due to the rapidly increasing number of antibiotic resistance genes (ARGs) found in bacteria. ARGs are now considered emerging environmental contaminants and have been measured in several environmental compartments (Pruden et.al., 2006). Resistant bacteria adapt by various mechanisms to the point where antibiotics

are not longer effective on them, according to the 2011 report of World Health Organization. ARGs exist naturally in the chromosomes and plasmids of environmental bacteria and are thus capable of transfers between non-pathogenic and pathogenic bacteria, even distantly related organisms through horizontal gene transfer (HGT). This process involves a transmission of deoxyribonucleic acid (DNA) between different genomes (Ji et.al., 2012) and is a mechanism whereby ARGs can proliferate under selective pressure.

For over the past 60 years, ARGs have been categorized into five classes, which are related to tetracycline (tet), aminoglycoside, macrolide-lincosamide-streptogramin, chloramphenicol and vancomycin, sulfonamide and trimethoprim, and β-lactam, where tet genes are most widely found in the environment (Zhang et.al.,2008). *Erm*(B), which is the focus of this paper, is considered to be the most prevalent gene in environmental microorganisms among the macrolide resistance determinants, easily been transferred from one host to another seeing that they are usually associated with mobile elements like plasmids and transposons (Zhang et.al., 2008). And *erm*(B) indeed is detected in some areas. In Israel, it is reported that the relative abundance of *erm*(B) were detected higher in the freshwater-irrigated soil than in the treated wastewater-irrigation soils,

specifically in Gilat (Negreanu et.al., 2012). *Erm*(B) were detected 100% in all parts of the lettuce tissues in polluted soil at Zhu Jiashan dairy farm of Nanjing, Eastern China (Ye et.al., 2016). Nevertheless, there are not many studies about *erm*(B) detected in the United States.

Therefore, this study builds on work begun by a senior practicum team through the Institute of the Environmental and Sustainable at UCLA in which 26 parks across California were selected, characterized, and tested for *erm*(B) and 16S rDNA genes, six parks in each of four cities, Los Angeles, Fresno, Bakersfield and San Diego were selected along with Yosemite National Park and Mount Baldy as non-urban controls. In a previous study, ARGs in water and soil were quantified significantly increasingly from all classes since 1940 (Knapp et.al., 2010) and many ARGs positively correlated with soil copper leves (Knapp et al., 2011).

Four cities were selected as urban areas including Los Angeles, Fresno, Bakersfield and San Diego and two Site selection was designed to give an overview of whether ARGs were presented in each area and whether the different concentrations of ARGs occurred among each area and between urban cities and pristine areas via the aid of mapping tools and census data. Samples were processed and analyzed in the laboratory with performance of DNA extraction, normalization and qPCR to

determine the presence of the ARGs after sample collection from each area.

To build on the existing work, this study specifically focuses on the detection of *erm*(B), the resistance mediated by rRNA methylases to prevent antimicrobials from binding to ribosomal protein via methylating the adenine residues (Zhang et.al., 2008), and characterize *erm*(B) genes with population density and agricultural involvements.

Material and Methods

Site Selection

To compare and analyze the presence of ARGs, four urban cities located mostly in parks that have large popularities and two pristine cities with minimum popularity densities. Los Angeles, Fresno, Bakersfield, and San Diego were chosen as potential urban areas while Yosemite National Park and Mount Baldy were chosen as pristine sites. Six parks from each site were sampled, of which three parks near the center of the city (urban areas) were sampled while the other three parks were located towards the suburban and rural areas since parks are accessible

public areas to facilitate sampling. Totally, soil samples were collected from 27 parks with the measurements of each park's geographical coordinates (see Appendix). After collecting at each park, samples were carefully taken back to the lab immediately in coolers and were stored properly in assigned refrigerators.

Sample Processing and DNA Extraction

Soils were collected from each site. Three 50mL tubes were used for collecting soil from three 1 square meter of land at each site, as an indicative representation of each site. Then DNA from the samples of these 27 parks was extracted. 0.25 gram of soil were added into previously weighed beaded tubes after each sampled tube was hand mixed well for 15 minutes. Each beaded tube contained 1 gram of 0.7 mm garnet beads. DNA was then extracted using a MoBio PowerFecal DNA extraction kit.

DNA Normalization

In this study, 0.25 ng/μL was used as the working DNA concentration for each sample in order to make sure easier comparison between each other. Initial DNA concentrations were measured

using NanoDrop 2000 for each sample. To ensure the accuracy of each sample, 2μL of each sample was pipetted into the Nanodrop and measured at least running two trials per sample after vortex-mixing each trial. If the difference between two concentration readings were less than or equal 4.0, the concentrations would be recorded and used for further experiments.

Calculations of all DNA concentration measurements were then accomplished an Excel spreadsheet, which averaged the two readings of each sample site. The total volume of molecular grade water added to dilute each sample was determined by conservation of mass to the 0.25ng/μL working DNA concentration for qPCR running. The initial volume of samples was limited between 2μL to 10μL for various raw DNA concentrations. Finally, the calculated molecular grade water was added to a 1.7 mL tube along with the initial sample volume.

Quantitative Polymerase Chain Reaction (qPCR)

qPCR is run after DNA extraction and normalization were performed on the samples. The amplification reactions were performed in 96-well plates with a 25μL volume in each well. Each 25μL volume usually contained 2μL of diluted sample, 12.5μL of SYBR Green I, each 1.25μL of

the 16S forward and reverse primer assay and 8μL of molecular grade water. However, after comparing the results of adding 2μL and 10μL diluted sample and 10μL diluted sample showed better and more positive results, 10μL of diluted sample were added while no more 8μL water were added and the rest of the recipes were constant which still added up to 25μL totally for each well.

Primers were used to detect the target genes with corresponding melting temperatures used during the thermal cycles for running qPCR. The *erm*(B) gene and 16S had the forward and reverse primer sequence (5' to 3') shown in Table 1 (Knapp et.al., 2010).

Gene	Forward Primer	Reverse Primer
erm(B)	AAAACTTACCCGCCATACCA	TTTGGCGTGTTTCATTGCTT
16S	GCGGACGGGTGAGTAATGT	TCATCCTCTCAGACCAGCTA

Table 1: Primers used for qPCR on the genes *erm*(B) and 16S.

The StepOnePlus Real Time PCR System (Applied Biosystems) were used to detect the *erm*(B) gene and 16S gene. Reactions for 25μL volume were performed on 96-well plates. The thermal cycle for *erm*(B) is 95°C (10 min), and then 45 cycles of 94°C (20 sec), annealing temperatures of

60°C for 60 seconds (Charles W. Knapp et.al., 2011). Melting curves were then obtained for further data analysis of ARG.

Standards for *erm*(B) are performed with other qPCR running for data analysis as well. The standards start at a concentration of 10^6 and then dilute as a series of 1:10 until the concentration reaches 10^0 for later usage of analysis data. We did not have access to a standard for the 16S gene. A triplicate of a positive control is included on the plate to make sure the correctness of the qPCR test performance. Extraction blanks for all corresponding sample sites are included to clarify the DNA extraction process and a triplicate of negative control is also included to clarify the master mix by adding molecular grade water. The extra wells included in the plates are to ensure the clarification of the overall process from NDA extraction to qPCR.

Data Analysis

A computer software known as StepOne Real-Time PCR Systems was used for gathering the qPCR data. A graph is visually created to illustrate the amplification of each well of the plate. A threshold line is set at 0.03, ensuring that the ignorant data from the qPCR test is not accounted for. The Cycle threshold (C_T) value is the point where the amplification curve crosses the threshold. The C_T values

were tested from 16S and were calculated in an excel document for relative data analysis for *erm*(B). C_T values for *erm*(B) were converted into quantities in another excel document to calculate averages and standard deviations for error exposure. Averages were normalized to 45 cycles for *erm*(B) by subtracting 45 minus C_T. Gene quantities for *erm*(B) were divided by the normalized 16S C_T values to determine the proportion of bacteria that contained *erm*(B) genes of interests. 16S C_T values were normalized to $100/2^n$, where n is the average of 16S C_T values subtracted by the lowest C_T value of *erm*(B) of all samples in all cities. Bar graphs were created to illustrate the quantities of *erm*(B) genes for each qPCR run of each city.

Results

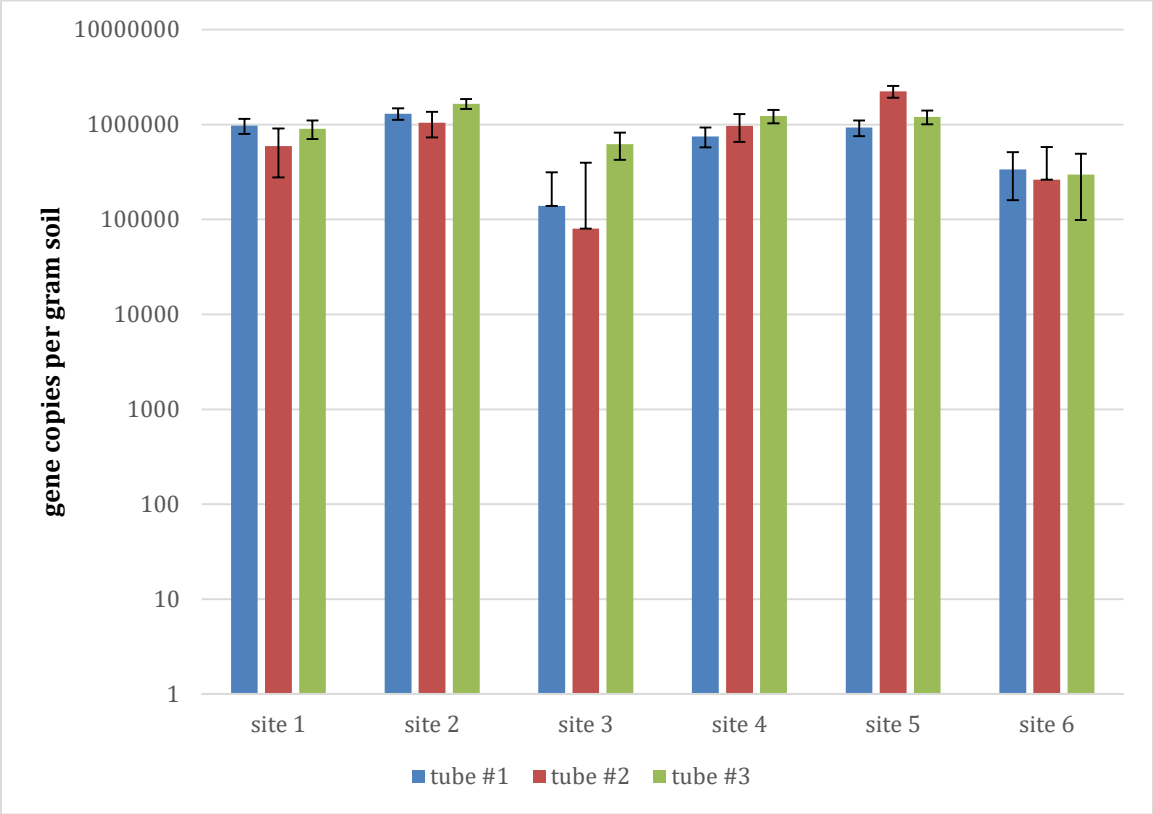


Figure 1 Detected levels of *erm*(B) gene copies per gram soil present in soil samples from city of Los Angeles in each tube (Error bars indicate the standard deviation of each tube.)

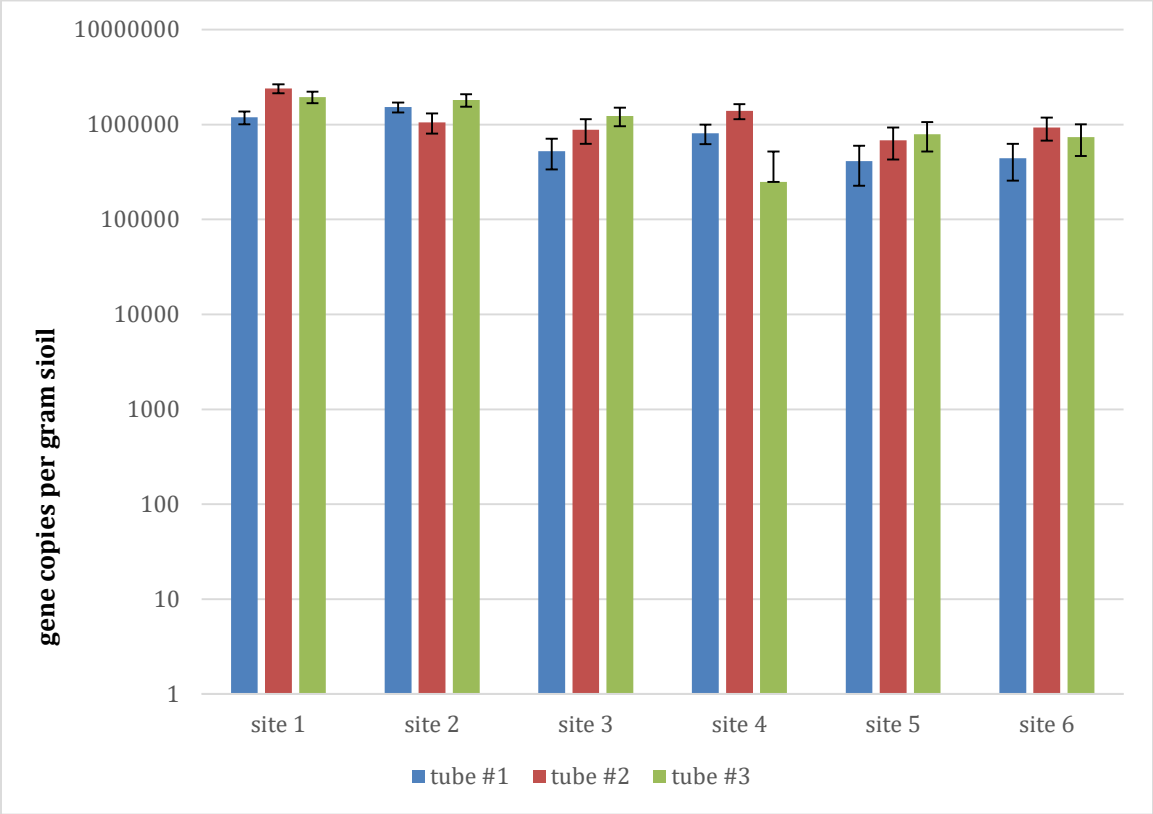


Figure 2 Detected levels of *erm*(B) gene copies per gram soil present in soil samples from city of San Diego in each tube (Error bars indicate the standard deviation of each tube.)

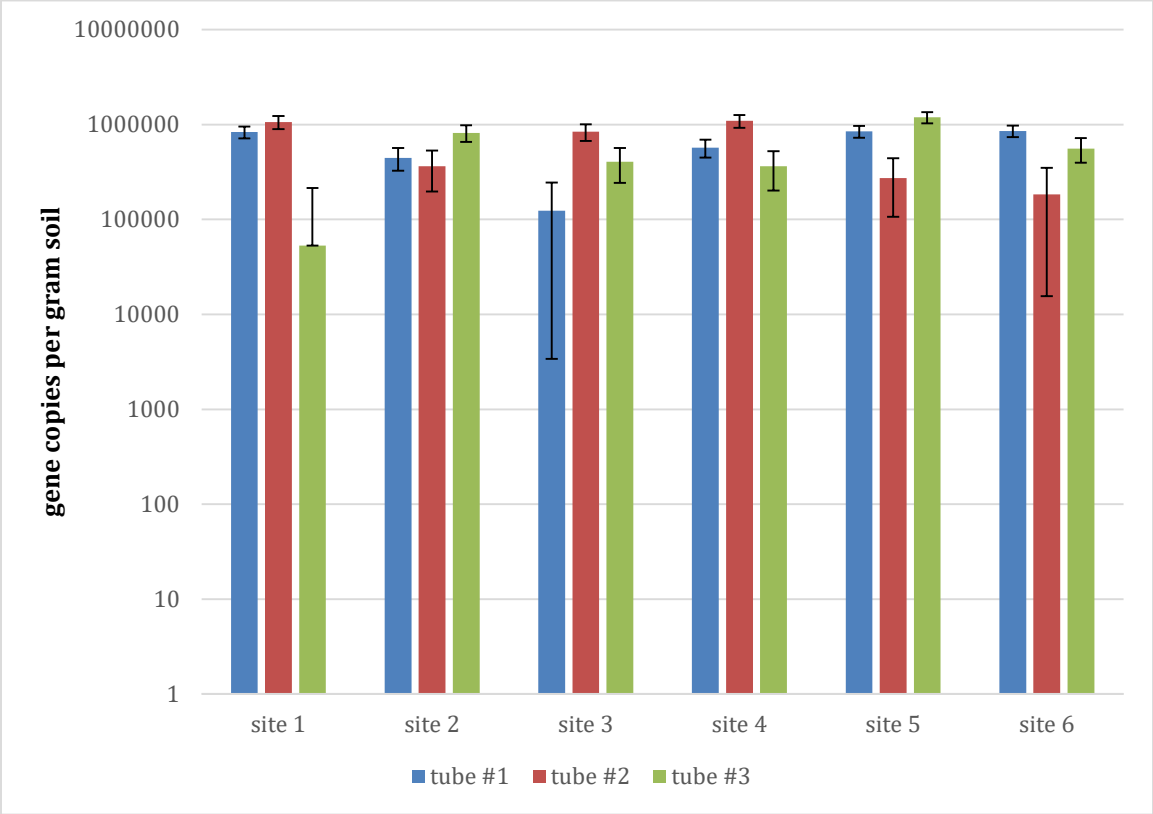


Figure 3 Detected levels of *erm*(B) gene copies per gram soil present in soil samples from city of Bakerfield in each tube (Error bars indicate the standard deviation of each tube.)

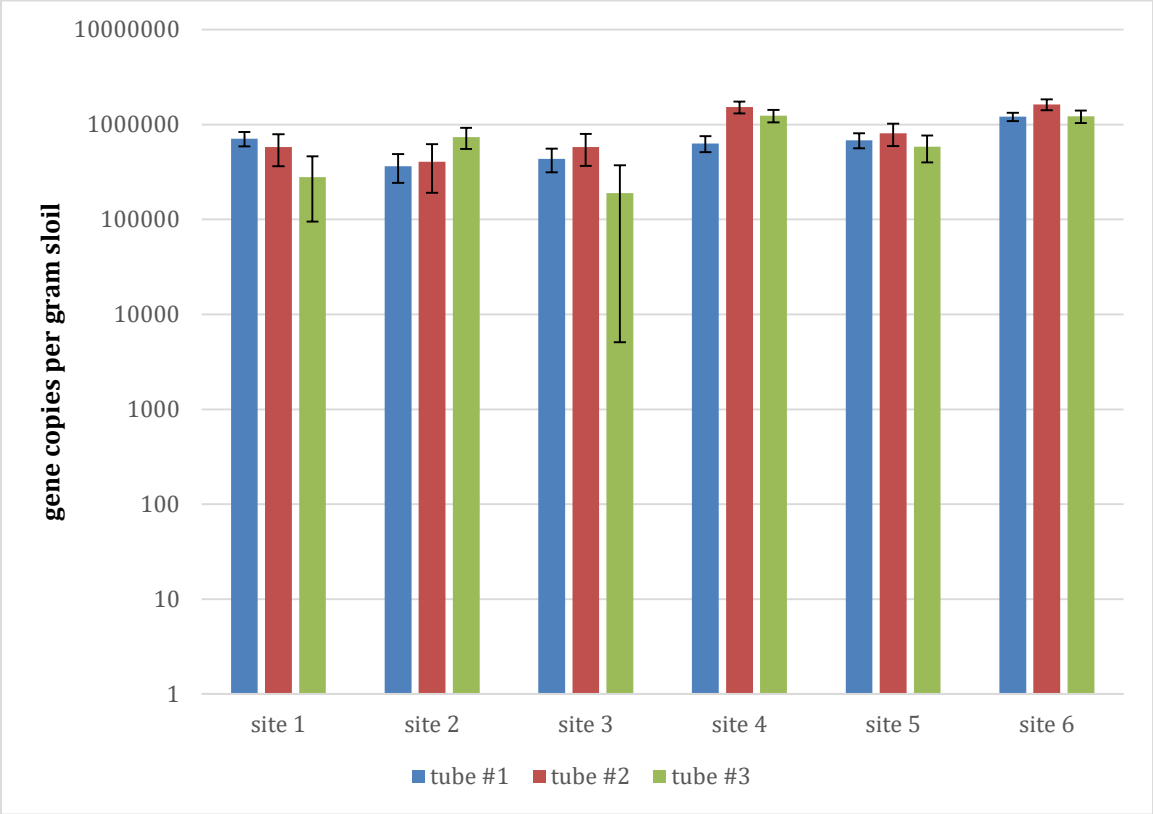


Figure 4 Detected levels of *erm*(B) gene copies per gram soil present in soil samples from city of Fresno in each tube (Error bars indicate the standard deviation of each tube.)

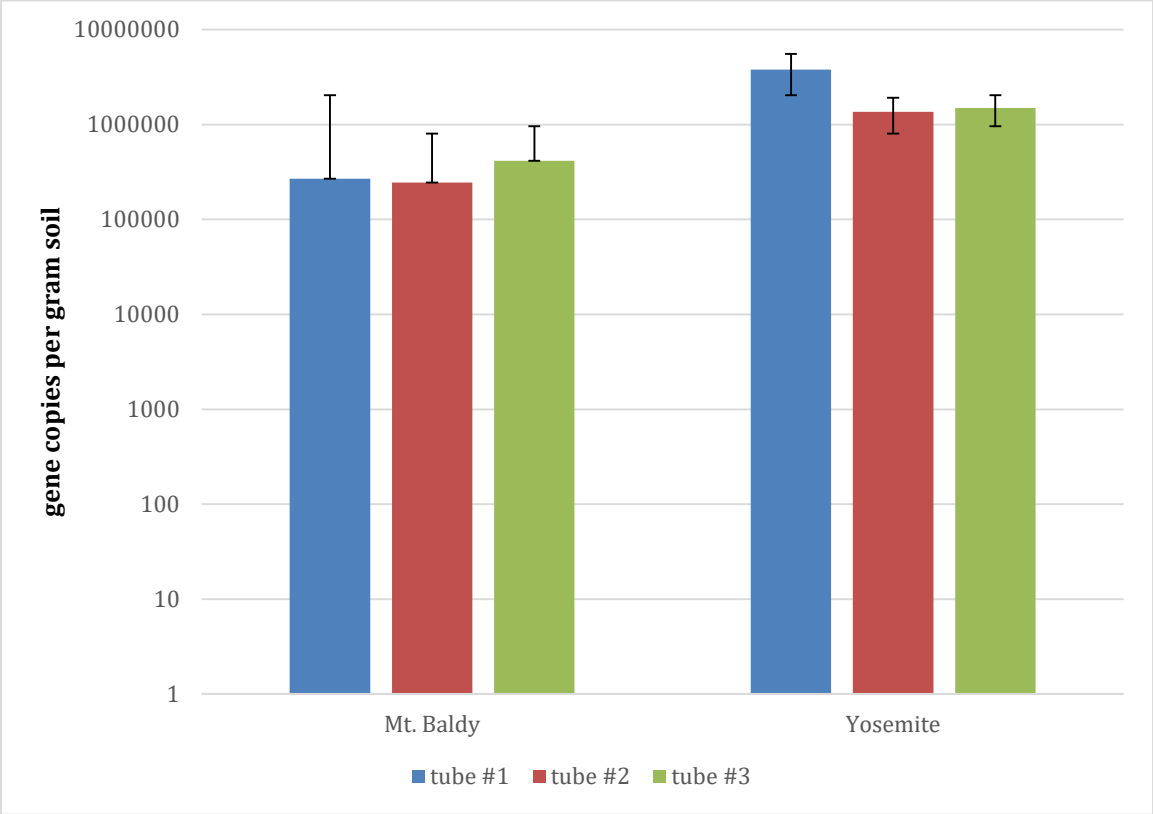


Figure 5 Detected levels of *erm*(B) gene copies per gram soil present in soil samples from Yosemite and Mount Baldy in each tube (Error bars indicate the standard deviation of each tube.)

Results show that the *erm*(B) gene is present in all of the 26 sites (Figure 1). Among the four urban cities, the triplicate of site 5 in city of Los Angeles show the highest levels of *erm*(B) per gram soil while site 3 shows the lowest (Figure 1). In city of San Diego from Figure 2, the levels of *erm*(B) vary almost the same from site 2 to site 6 triplicate, but the third tube of the triplicate in site 1 shows the dramatically lowest comparing with another 2 results. Site 4 and site 6 in Bakersfield shows the highest levels while the triplicates in site 3 are relatively the lowest. Interestingly, results of the triplicates in Mount Baldy and Yosemite, which are defined as pristine

sites, both show certain levels of *erm*(B) gene. More interestingly, the levels of the triplicate in Yosemite are all above 1000000 gene copies per gram soil, and the first tube of the triplicate even shows much higher than any other tubes of all sites.

Discussion

Welch's T-test was applied to determine whether differences in *erm*(B) gene quantities between cities and pristine sites were significantly different via R Studio. In table 3, p values are all larger than 0.05, indicating that while levels appear to have city to city trends, the differences observed

City & Area	Mean	STDEVs
Los Angeles	863563.1	556651.300
San Diego	1055903.7	578792.6994
Bakersfield	605419.5	349992.201
Fresno	768211.6	424350.4463
Mount Baldy	309,832.5	92744.73674
Yosemite	2222063.4	1372428.865
Lumped Urban	823274.5	503767.246
Lumped Pristine	1265948.5	1361563.182

Table 2 Mean and standard deviation of each city and area (Lumped urban cities include Los Angeles, San Diego, Bakersfield and Fresno; lumped pristine sites include Yosemite and Mount Baldy.)

City & Areas	Los Angeles	San Diego	Bakersfield	Fresno	Mt Baldy	Yosemite	Lumped urban
Los Angeles		0.3167	0.1067	0.5674	0.000969	0.2262	
San Diego			0.00861	0.09895	0.00006659	0.2773	
Bakersfield				0.2181	0.009648	0.1769	
Fresno					0.0008897	0.2064	
Mt Baldy						0.1366	
Yosemite							
Lumped pristine							0.4636

Table 3 p value of each City and area (Lumped urban cities include Los Angeles, San Diego, Bakersfield and Fresno

are not significant at the $p<0.05$ level between any of the four urban sites.

acute non-specific urethritis. More interestingly, they are also a useful alterative for people with penicillin and cephalosporin allergy, which are widely and frequently used as alterative medicine in families. Therefore, it is hypothesized that areas with higher populations are inclined to have larger amount of macrolide related resistant genes detected in the soil owing to the more frequent and larger usage of the medicines with this antibiotics.

In order to test the hypothesis, population densities for each site was created in the table 2. In Table 2, populations for each site are chosen from the city where each park belongs to or is

City	Site	Site Name	Population	Area	Population Density (persons/sq. mi.)
Los Angeles	1	Will Rogers State Historic Park	27,000	Pacific Palisades	1182
	2	Kenneth Hahn State Recreation Area	30,123	Baldwin Hills/Crenshaw	10,459
	3	Seoul International Park	124,281	Korean Town	42,609
	4	MacArthur Park	8,270	MacArthur Park	1,501
	5	Grand Park	52,400	Downtown LA	4,770
	6	Griffith Park	36,933	Los Feliz	13,512
San Diego	1	Petco Park	37,832	Downtown San Diego	15,763
	2	Mission Bay	46,910	Pacific Beach	10,669
	3	Boone Park	28,548	Bay Terraces	9,220
	4	Balboa	130,092	Balboa Park	69,382
	5	Torrey Pines	42,808	Village of La Jolla	9,256
	6	Mission Trails	29,387	San Carlos	5,304
Bakersfield	1	Jefferson Park	347,483	Bakersfield	2,420
	2	Deer Peak Park	3,850	Seven Oaks	5,090
	3	Patriots Park	347,483	Bakersfield	2,420
	4	Bear Mountain Park	Agri. Area		
	5	Tule Elk State Reserve Park	Agri. Area		
	6	Buena Vista Park	Agri. Area		
Fresno	1	Kearney Park	Agri. Area		
	2	Orchid Park	509,924	Fresno	4,555

	3	Fresno Regional Sports Complex	509,924	Fresno	4,555
	4	Pasa Tiempo Park	102,189	Clovis	4,390
	5	Carozza Park	509,924	Fresno	4,555
	6	Radio Park	509,924	Fresno	4,555
Yosemite		June Lake	Prist. Site		
Mt Baldy			Prist. Site		

Table 4 Population and population density for areas where the sites of each city are located.

identified between 2008 and 2014. Population densities are calculated with population divided by areas as square miles. For Fresno, due to the lack of reference for site 2 (Orchid Park), site 3 (Fresno Regional Sports Complex), site 5 (Carozza Park) and site 6 (Radio Park), the statistics for the whole city of Fresno was cited instead. Same method is also used for site 1 (Jefferson Park) and site 3 (Patriots Park) of Bakersfield. From site 4 to site 6 of Bakersfield and site 1 of Fresno are identified as agricultural areas because the Fresno County is responsible for conducting regulatory and service functions pertaining to the multi-billion dollar agricultural industry under direction of the California Department of Food and Agriculture (The County of Fresno).

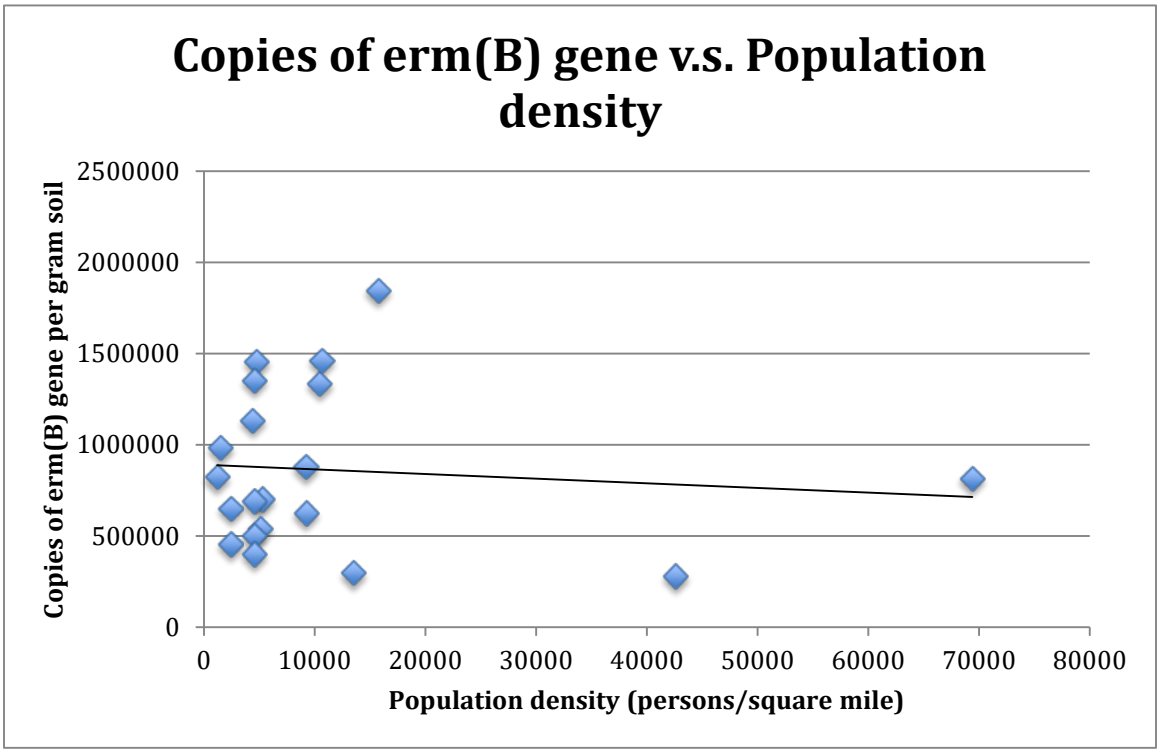


Figure 6 Trend of copies of *erm*(B) v.s. population density in the 4 cities (site 4, 5 and 6 in Bakersfield are not included; site 1 in Fresno is not included.)

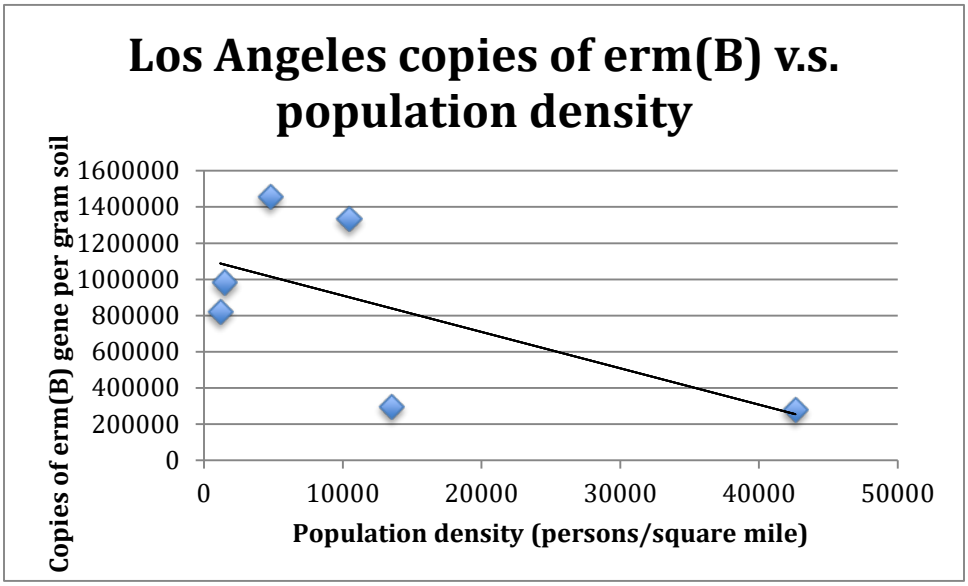


Figure 7 Trend of copies of *erm*(B) v.s. population density in Los Angeles

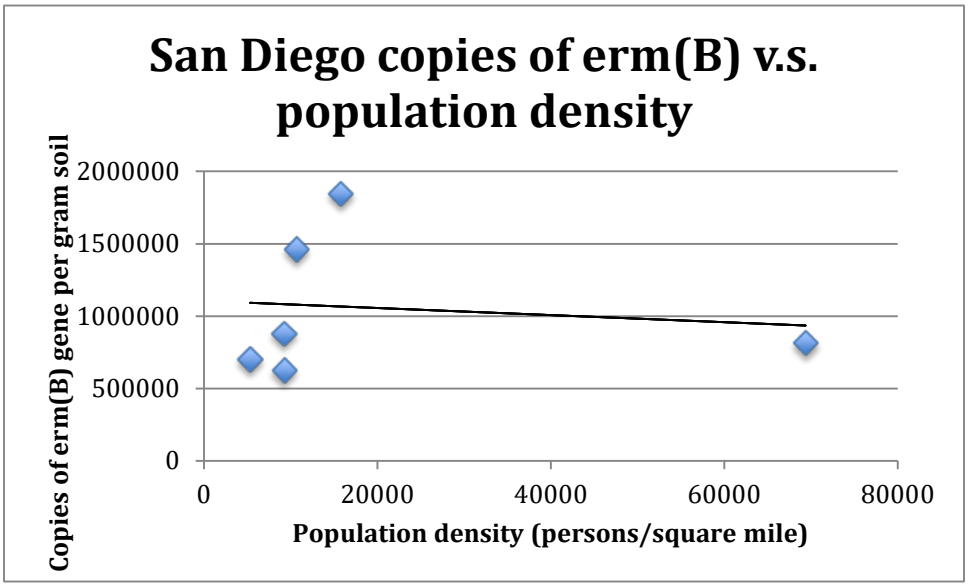


Figure 8 Trend of copies *erm*(B) v.s. population density in San Diego

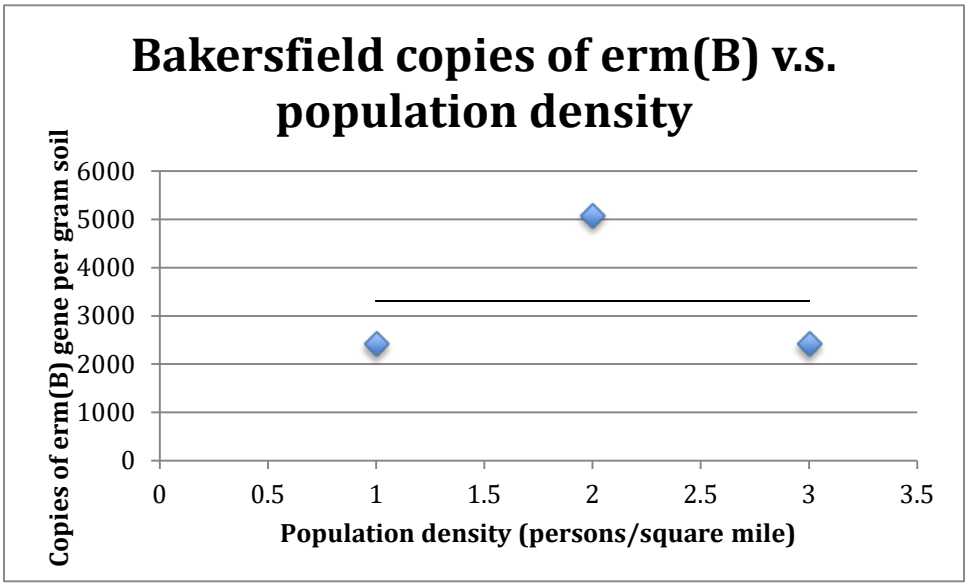


Figure 9 Trend of copies of *erm*(B) v.s. population density in Bakersfield (Site 4, 5, and 6 are not included because they are defined as agricultural areas.)

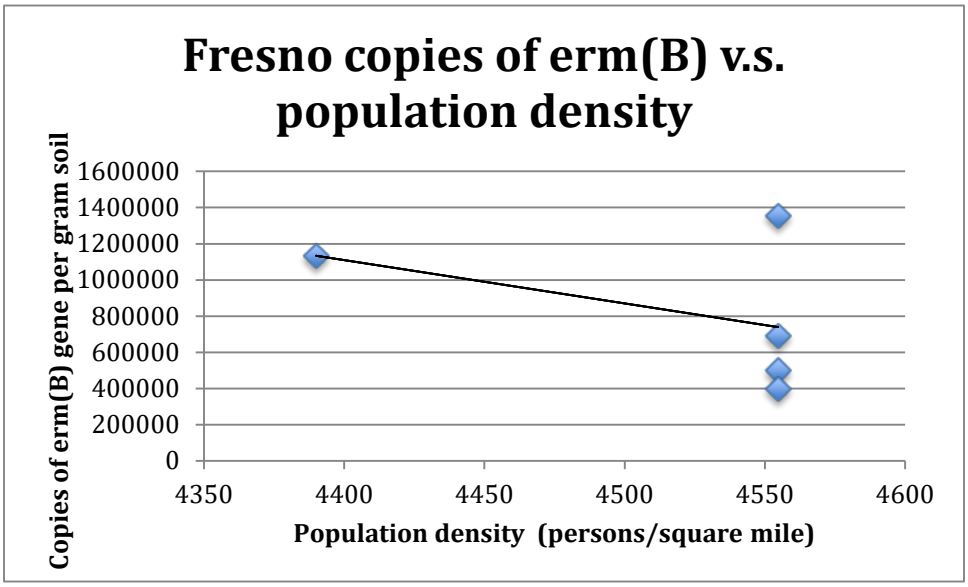


Figure 10 Trend of copies of *erm*(B) v.s. population density in Fresno (Site 1 is not included because it is defined as agricultural area.)

From the Figure 1 and table 4 for *erm*(B) genes copies per gram soil were highest in site 3 (Seoul International Park) among all of the Los Angeles sites, while it is located in Koreantown of Los Angeles with the highest population density among the six parks. Also, Kenneth Hahn State Recreation Area (site 2) in Los Angeles is located in the area with almost 10 times higher population density than that of Will Rogers State Historic Park (site 1), but copies of *erm*(B) genes per gram soil in site 2 were detected almost the same as the copies per gram soil in site 1. This might be because people there use less medicine of macrolides related antibiotics.

Figure 6 shows that copies of *erm*(B) gene per gram soil decrease while population density increases. And the levels are all negatively correlated with population density except in the city of Bakersfield (Figure 7-10). It might be because there are only three sets of data which site 4 to 6 are excluded due to being defined as agricultural areas. However, due to limited data, it is unlikely that levels of *erm*(B) correlate with population density. More study will be needed further. In addition, other factors such as urbanization, soil content, and irrigation systems might influence the results, which also needs to be further studied.

Bakersfield is also defined as agricultural areas, since agricultural commodities export various crops, including almonds, almonds, apricots, beans, cabbage, cantaloupe, carrots, citrus, cotton lint and planting seed, grapes, hay, honeydew, lettuce, nectarines, nursery stock, peaches, plums, rose plants and watermelon, with markets over 85 foreign countries, producing $6,769,855,590, according to the 2013 total value of agricultural commodities (Greater Bakersfield Chamber of Commerce). In Bakersfield, soil sampled in site 4 is very close to agriculture and soil in site 5 is in the middle of agriculture, according to the Appendix. *Erm*(B) were all detected in site 4, 5 and 6 from Figure 3. Also, site 1 in Fresno shows high *erm*(B) detection among the six sites, where it is

identified as agricultural area as well. The results might be caused by the usage of fertilizers with antibiotics or the usage of excrements as fertilizers from animals adding antibiotics in their feed. In China, *erm*(B) were also detected in the soils of agricultural areas adjacent to swine farms (Li et.al., 2013). Joy and Li et.al. found that *erm*(B) were exhibited in swine manure due to adding antibiotics into feed and *erm*(B) were hard to be treated after first order degradation. Their results are consistent with the results of this paper. Therefore, *erm*(B) genes might be widely existed in agricultural areas. But research will be needed for the further study of the correlation of *erm*(B) and agricultural areas.

The hypothesis in this paper, established to some extent that parks in California with a higher population density show higher level of *erm*(B) is contradictory to the results that population density might not be a major factor to the level of *erm*(B). In addition, *erm*(B) might be more abundant in agricultural areas than that in urban areas due to the fertilizer usage.

Due to limited time, *erm*(B) was only assessed and limited sites in each city were included.

Further research is needed for the relationship between the levels of ARGs and the population density as well as agricultural involvements.

Appendix

Sampling sites

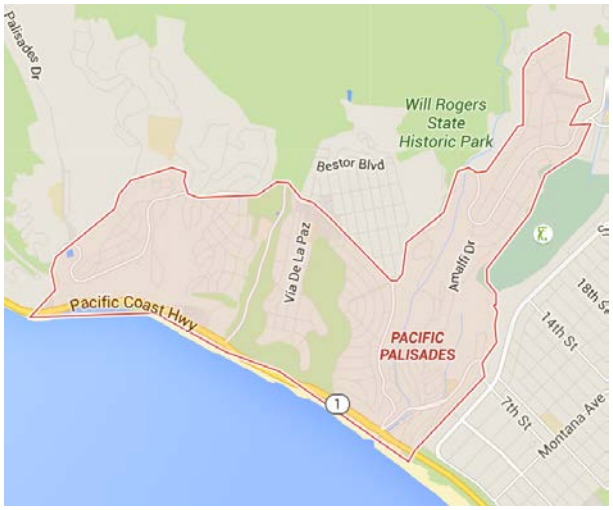
City Site	Name	Latitude	Longitude	Notes
Los Angeles S1	Will Rogers State Historic Park	N34.05489441010547	W118.51119685929129	
Los Angeles S2	Kenneth Hahn State Recreation Area	N34.01068364273199	W118.37023613054153	
Los Angeles S3	Seoul International Park	N34.054009	W118.301012	
Los Angeles S4	MacArthur Park	N34.059710	W118.278300	
Los Angeles S5	Grand Park	N34.05547	W118.244938	
Los Angeles S6	Griffith Park	N34.118113	W118.295020	
San Diego S1	Petco Park	N32.709112	W117.156767	
San Diego S2	Mission Bay	N32.789578	W117.21036	
San Diego S3	Boone Park	N32.6972751	W117.03725	
San Diego S4	Balboa	N32.738572	W117.128649	

San Diego S5	Torrey Pines	N32.9210	W117.2532	
San Diego S6	Mission Trails	N32.8278	W117.0511	
Bakersfield S1	Jefferson Park	N35.389345	W118.986005	3 Brand Cattle Co, 34377 Lerdo Highway, Bakersfield, CA 93308
Bakersfield S2	Deer Peak Park	N35.343669	W119.121428	4 Brand Cattle Co, 34377 Lerdo Highway, Bakersfield, CA 93308
Bakersfield S3	Patriots Park	N35.341474	W119.058111	5 Brand Cattle Co, 34377 Lerdo Highway, Bakersfield, CA 93308
Bakersfield S4	Bear Mountain Park	N35.261390	W118.919701	very close to agriculture

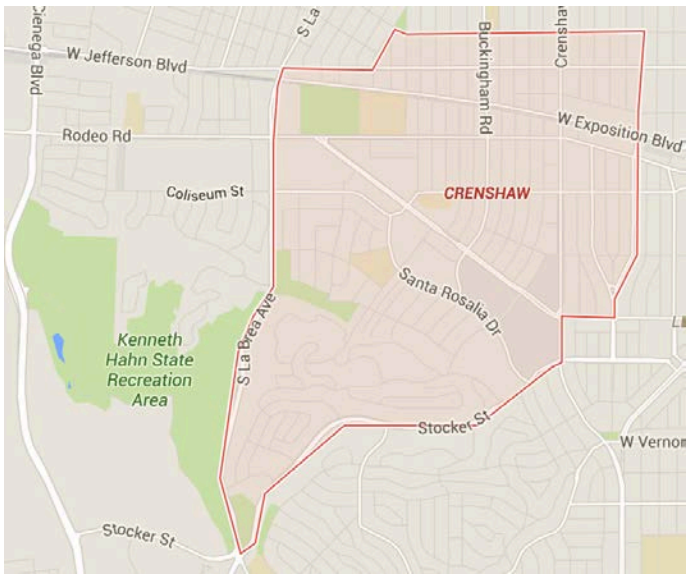
Bakersfield S5	Tule Elk State Reserve Park	N35.332201	W119.363835	in the middle of agricultural fields
Bakersfield S6	Buena Vista Park	N35.23597	W119.328283	
Fresno S1	Kearney Park	N36.727584	W119.92288	
Fresno S2	Orchid Park	N36.839263	W119.852161	
Fresno S3	Fresno Regional Sports Complex	N36.697017	W119.834781	Adjacent to a feedlot
Fresno S4	Pasa Tiempo Park	N36.816133	W119.647037	Fresno State is closest feedlot
Fresno S5	Carozza Park	N36.757641	W119.730777	Fresno State is closest feedlot
Fresno S6	Radio Park	N36.771529	W119.772957	Fresno State is closest feedlot
Yosemite	June Lake	N37.783975	W119.07494	Pristine area
Mt Baldy		N34.17945	W117.6757	Pristine area

Maps

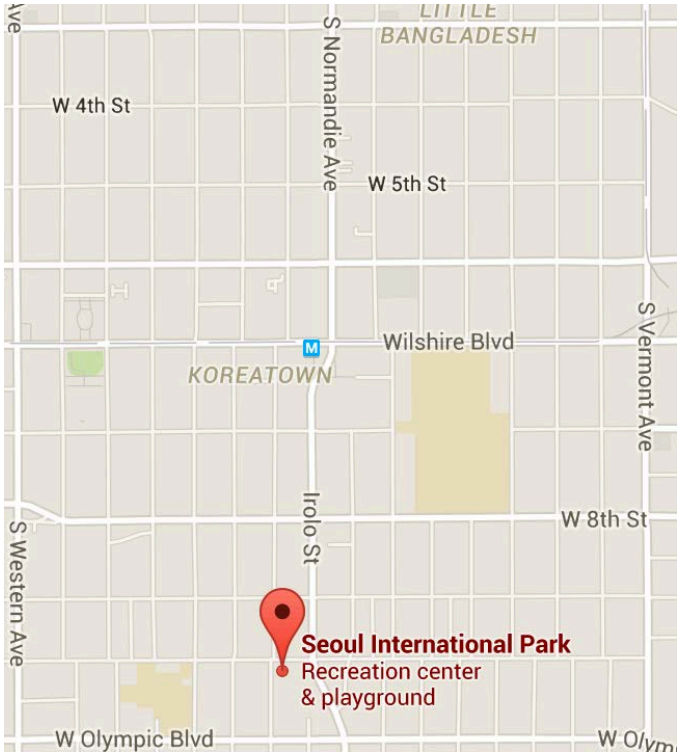
Will Rogers State Historic Park (Pacific Palisades)



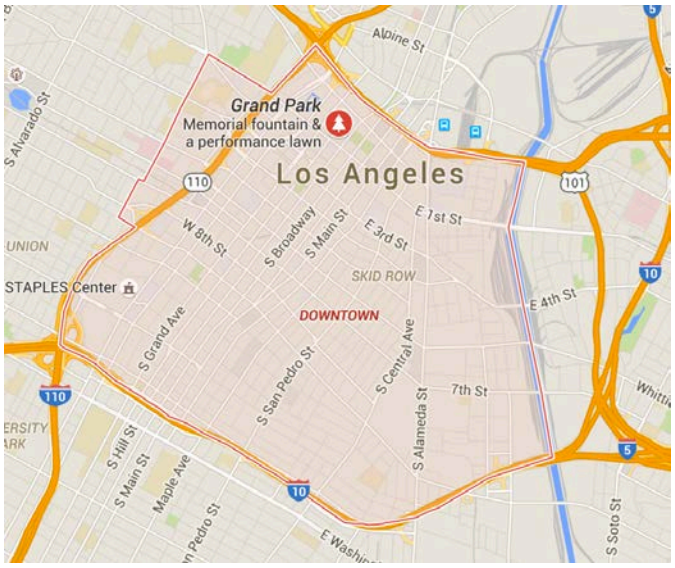
Kenneth Hahn State Recreation Area (Balwin Hills/Crenshaw)



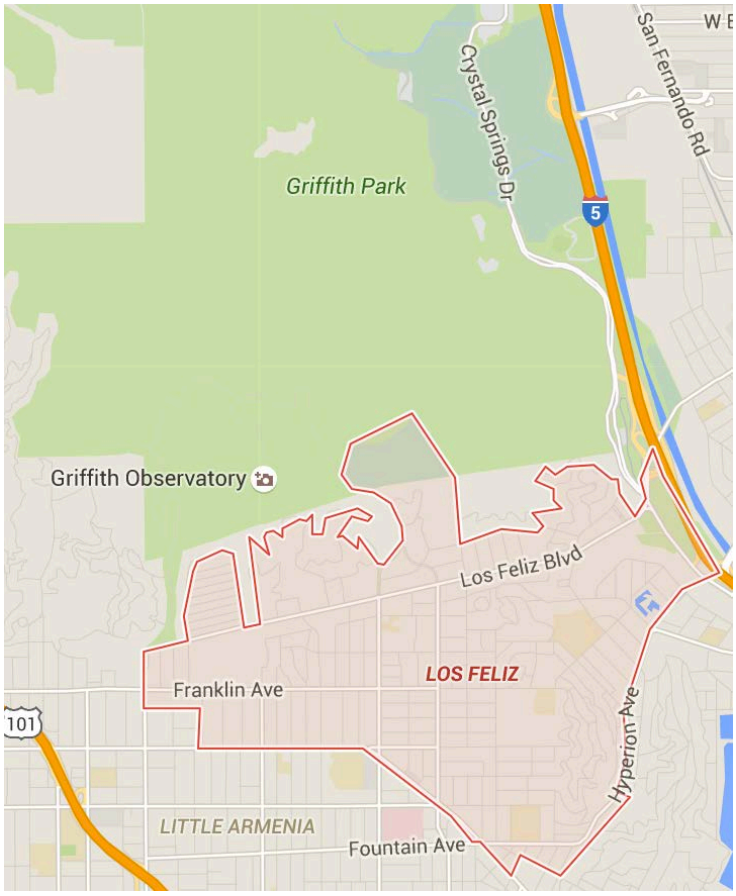
Seoul International Park (Koreantown)



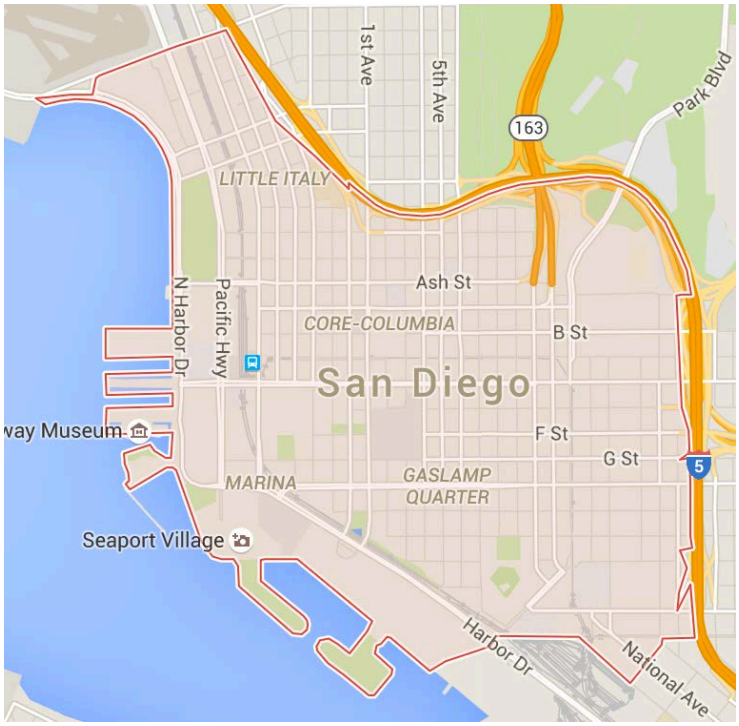
Grand Park (Downtown Los Angeles)



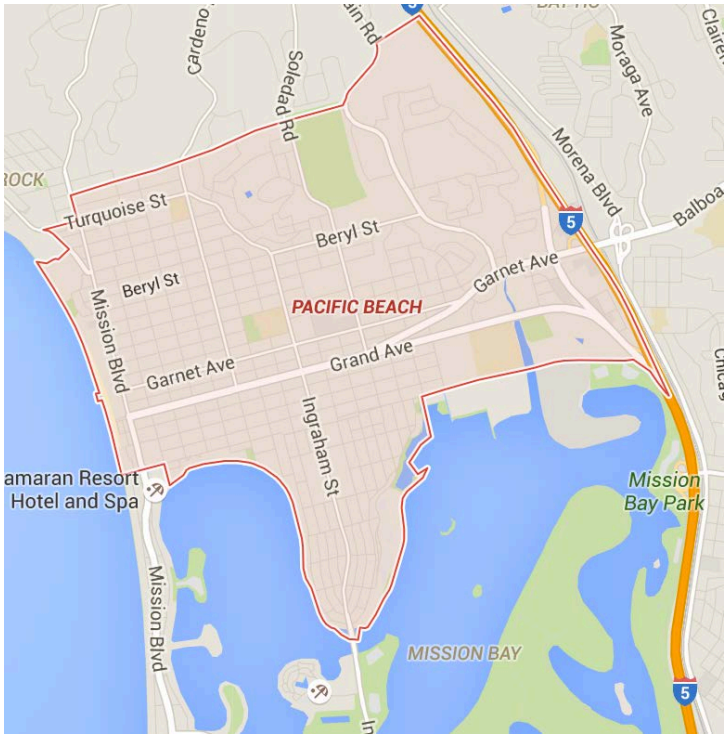
Griffith Park (Los Feliz)



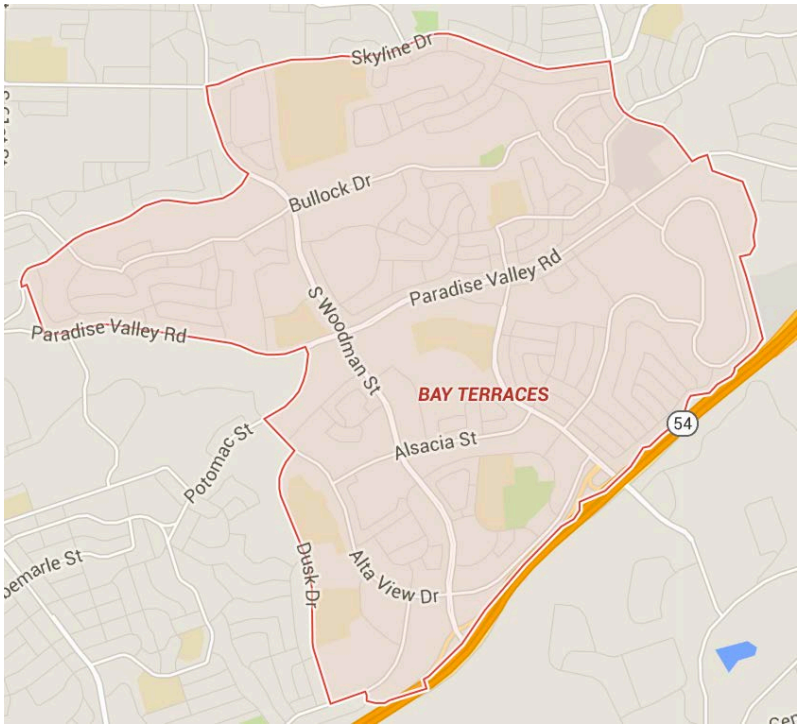
Petco Park (Downtown San Diego)



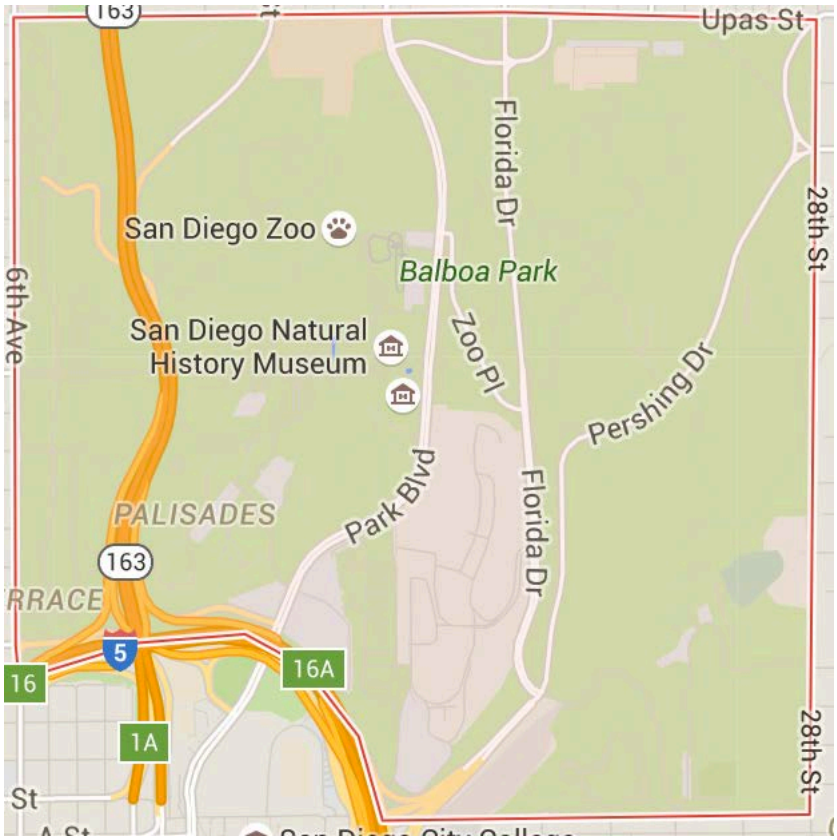
Mission Bay (Pacific Beach)



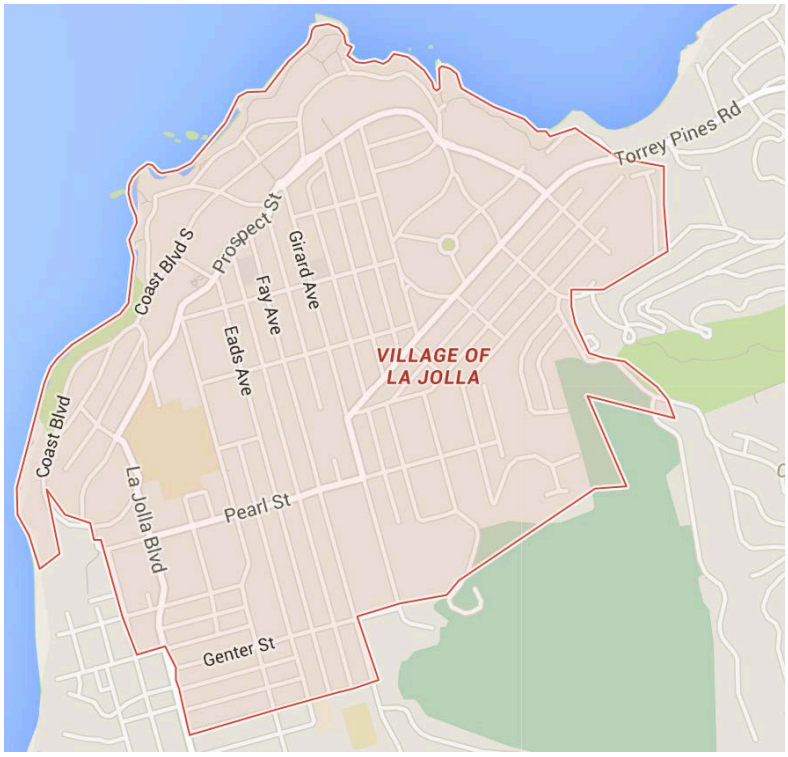
Boone Park (Bay Terraces)



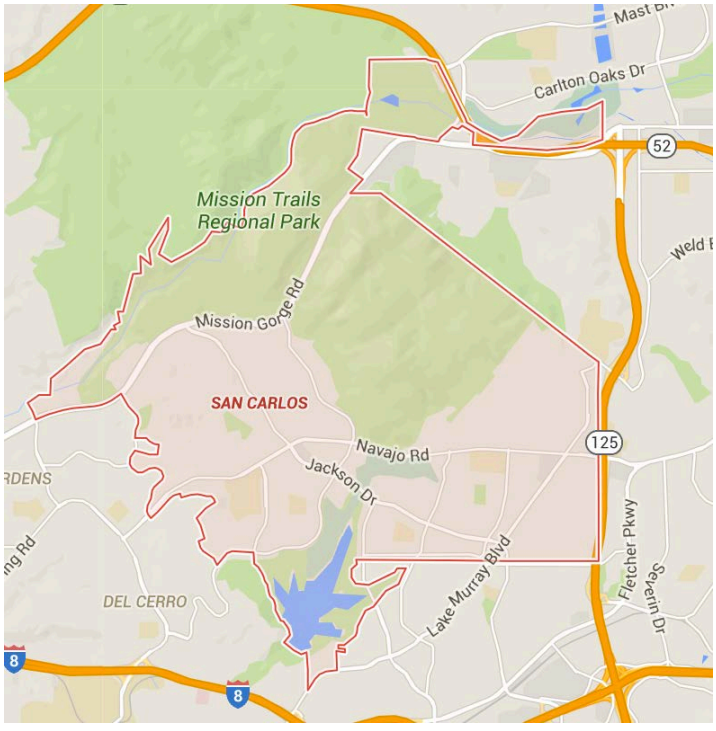
Balboa (Balboa Park)



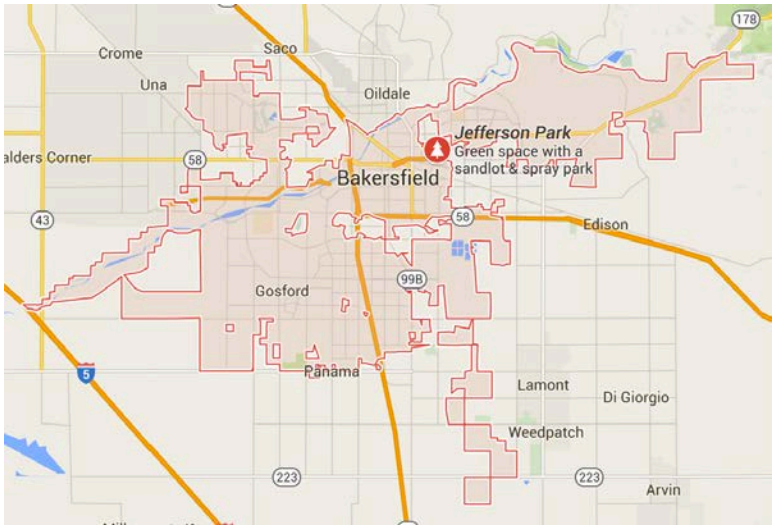
Torrey Pines (Village of La Jolla)



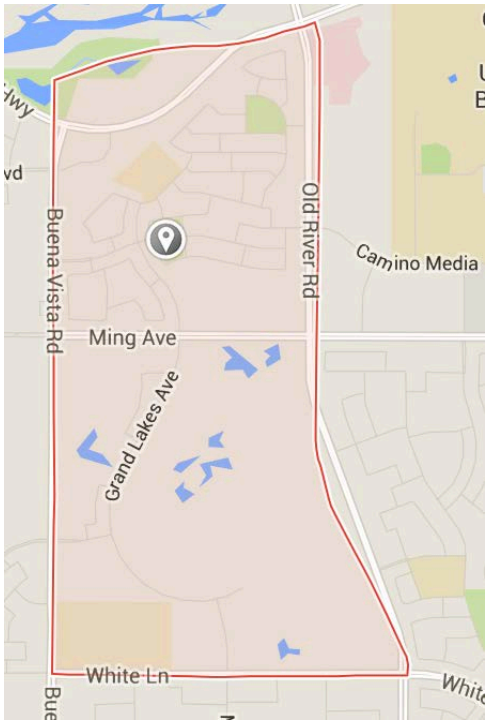
Mission Trails Regional Park (San Carlos)



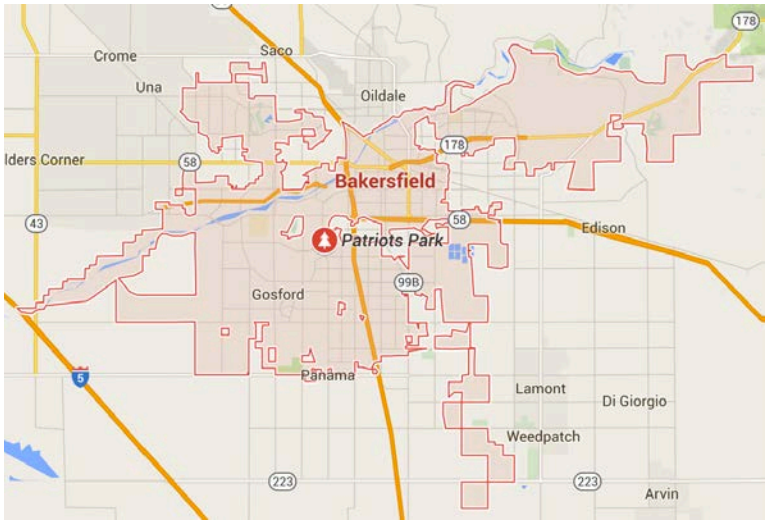
Jefferson Park (Bakersfield)



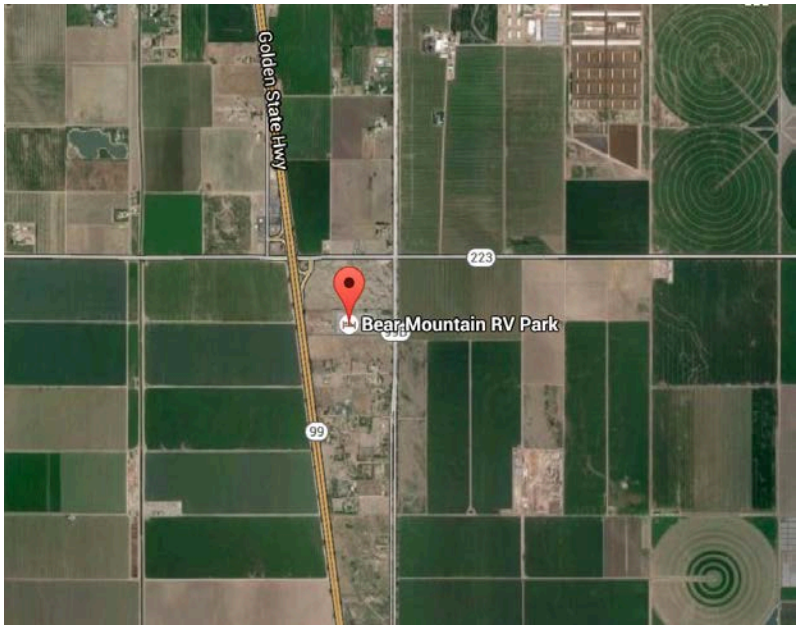
Deer Peak Park (Seven Oaks)



Patriots Park (Bakersfield)



Bear Mountain Reserve Park (Agricultural Area)

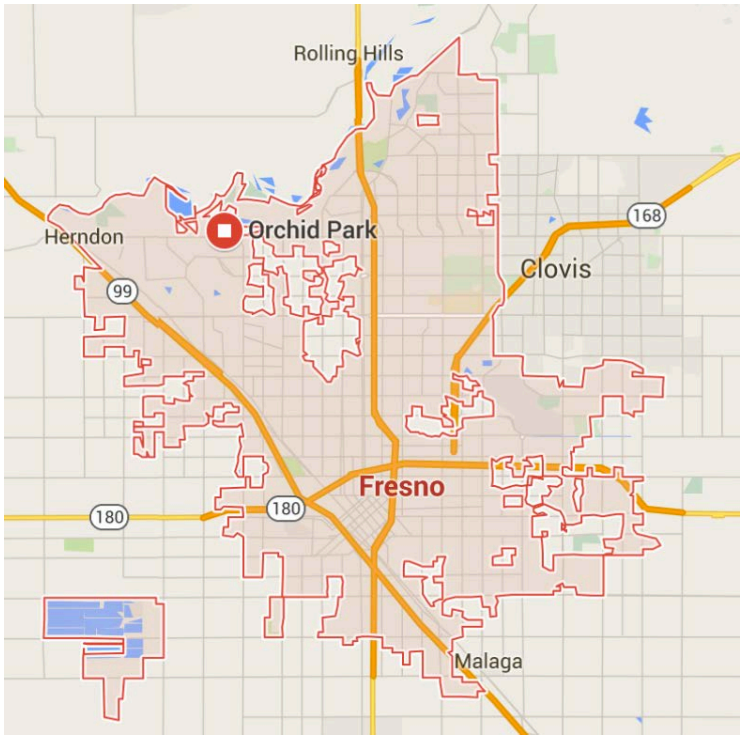


Tule Elk Reserve State Park (Agricultural Area)

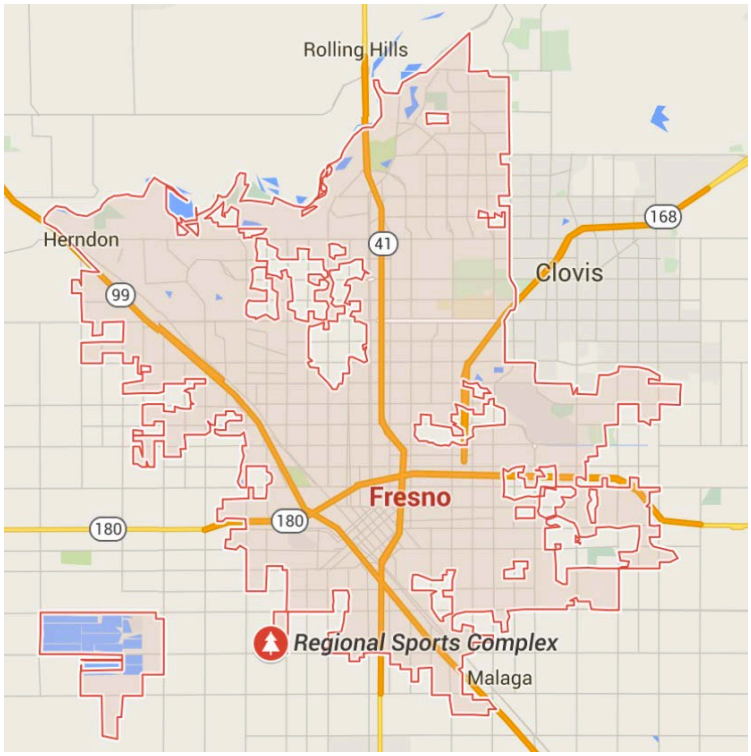
Buena Vista Park (Agricultural Area)

Kearney Park (Agricultural Area)

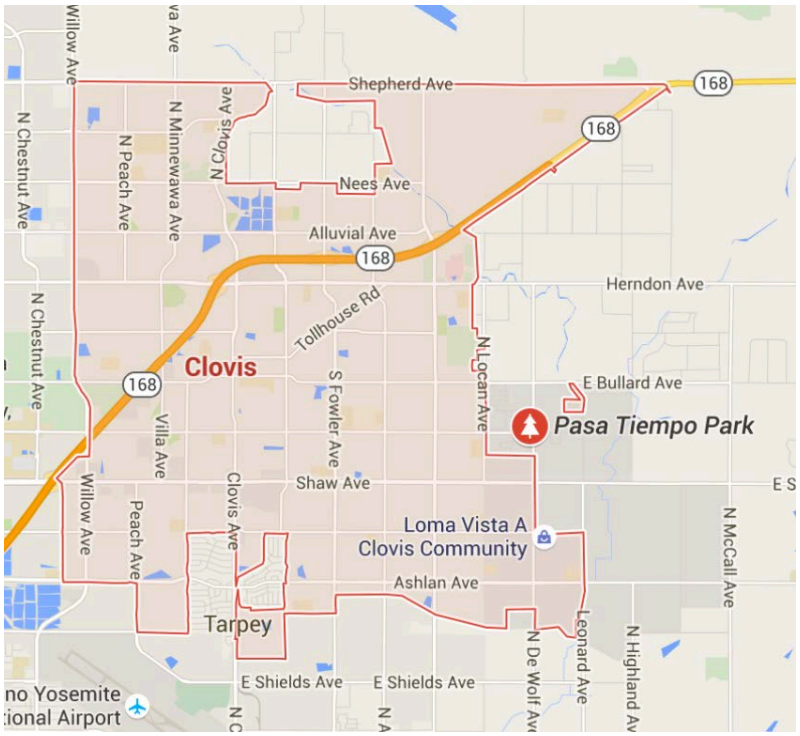
Orchid Park (Fresno)



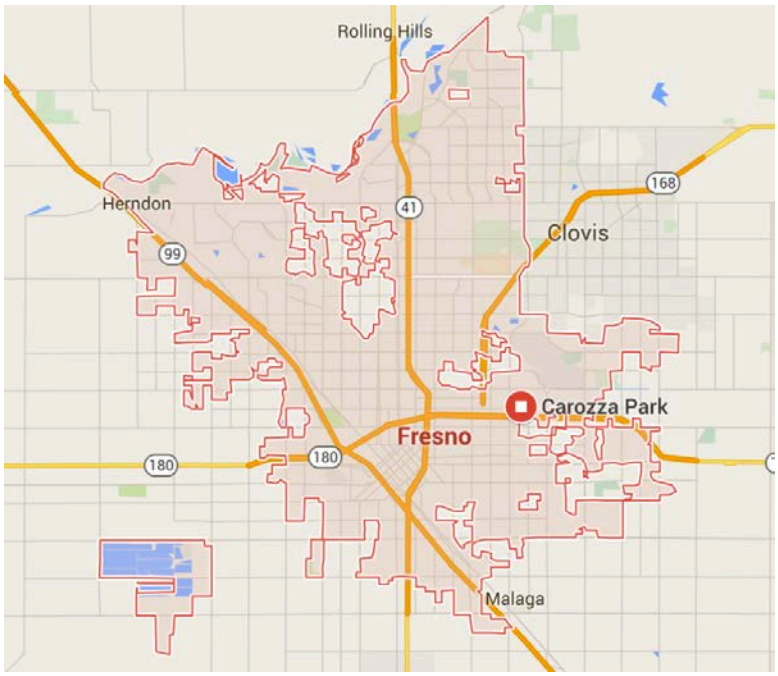
Regional Sports Complex (Fresno)



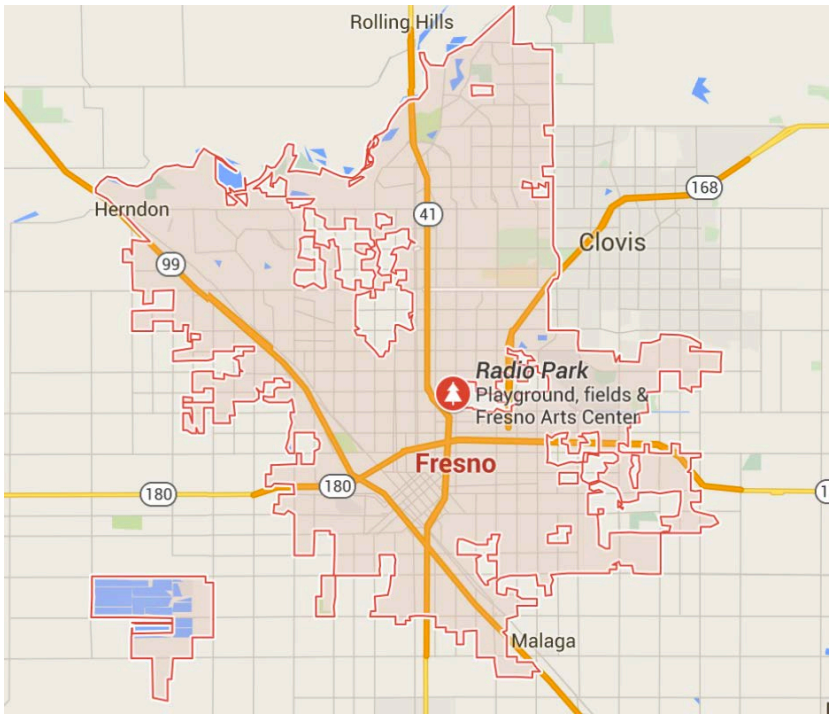
Papa Tiempo Park (Clovis)



Carozza Park (Fresno)



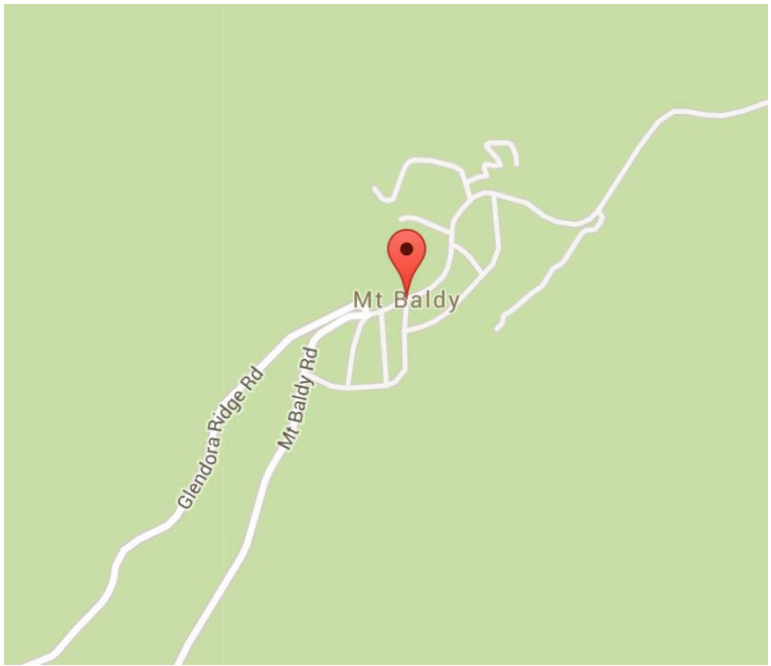
Radio Park (Fresno)



June Lake (Yosemite)


395
167
Lundy
Lee Vining
120
Mt Dana
Mono Mills
120
Whispering Pines
$132
Crestview
Mammoth
Lakes
395
Toms Place

Mt Baldy


Mt Baldy
Glendora Ridge Rd
Mt Baldy Rd

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