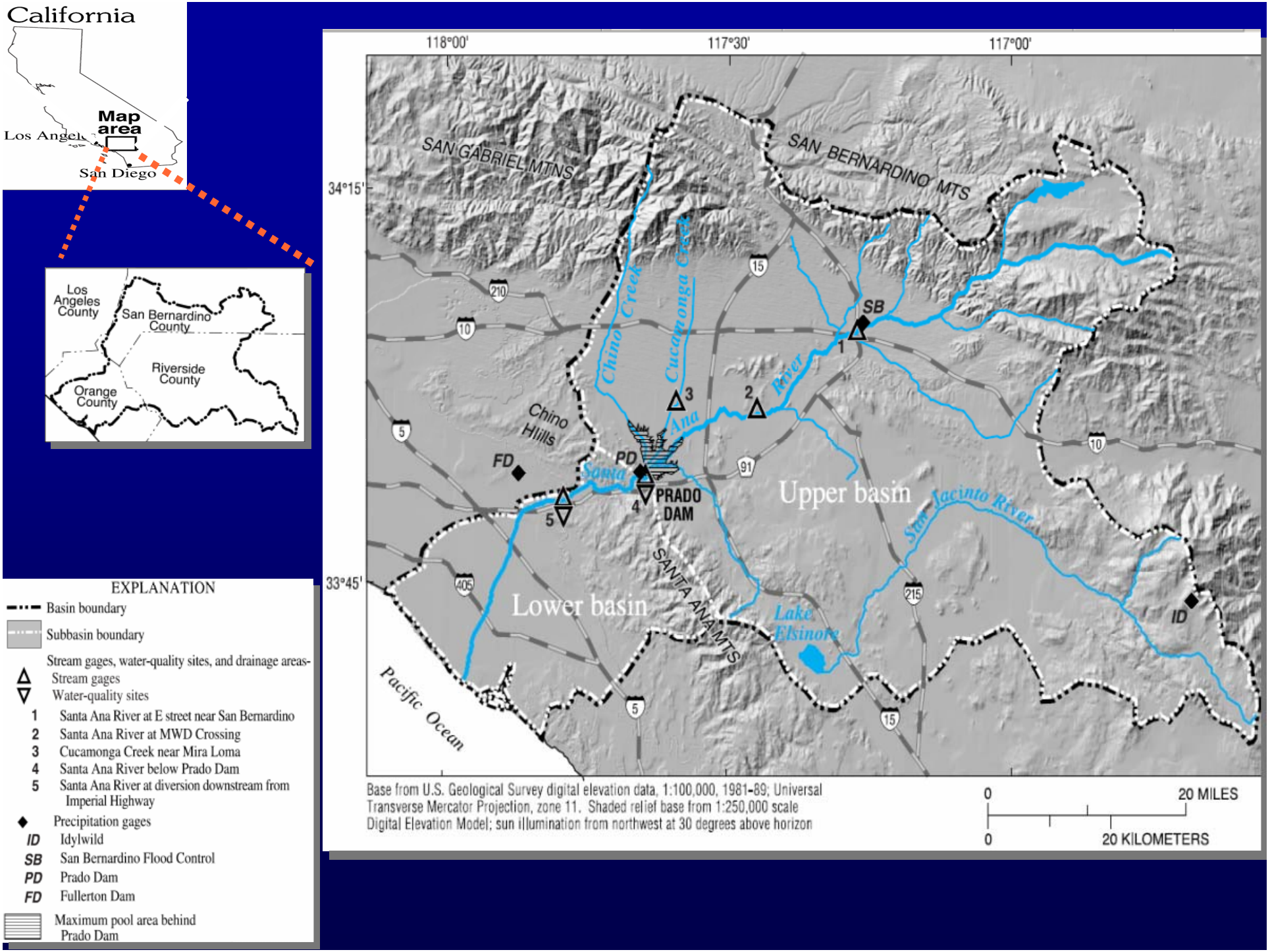
Chino Creek Pathogen Source Evaluation Study

Menu B. Leddy, Greg Woodside

and Nira Yamachika

Orange County Water District

12/06/05


California
Map area
Los Angeles
San Diego
Los Angeles County
San Bernardino County
Riverside County
Orange County
118°00'
117°30'
117°00'
34°15'
33°45'
SAN GABRIEL MTNS
SAN BERNARDINO MTS
Chino Creek
Cucamonga Creek
Santa Ana River
Chino Hills
SB
FD
PD
PRADO DAM
Upper basin
Lower basin
San Jacinto River
Lake Elsinore
SANTA ANA MTS
ID
Pacific Ocean
EXPLANATION
Basin boundary
Subbasin boundary
Stream gages, water-quality sites, and drainage areas-
Stream gages
Water-quality sites
1 Santa Ana River at E street near San Bernardino
2 Santa Ana River at MWD Crossing
3 Cucamonga Creek near Mira Loma
4 Santa Ana River below Prado Dam
5 Santa Ana River at diversion downstream from Imperial Highway
Precipitation gages
ID Idylwild
SB San Bernardino Flood Control
PD Prado Dam
FD Fullerton Dam
Maximum pool area behind Prado Dam
Base from U.S. Geological Survey digital elevation data, 1:100,000, 1981-89; Universal Transverse Mercator Projection, zone 11. Shaded relief base from 1:250,000 scale Digital Elevation Model; sun illumination from northwest at 30 degrees above horizon
0 20 MILES
0 20 KILOMETERS

Collaborating Agencies

- Orange County Water District
- Inland Empire Utilities Agency
- Regional Water Quality Control Board
- Orange County Public Health Agency

Sources of Funding

- State Water Resources Control Board
  Nonpoint Source Pollution Control Programs
  Proposition 13 Grant
- Inland Empire Utilities Agency
- Orange County Water District

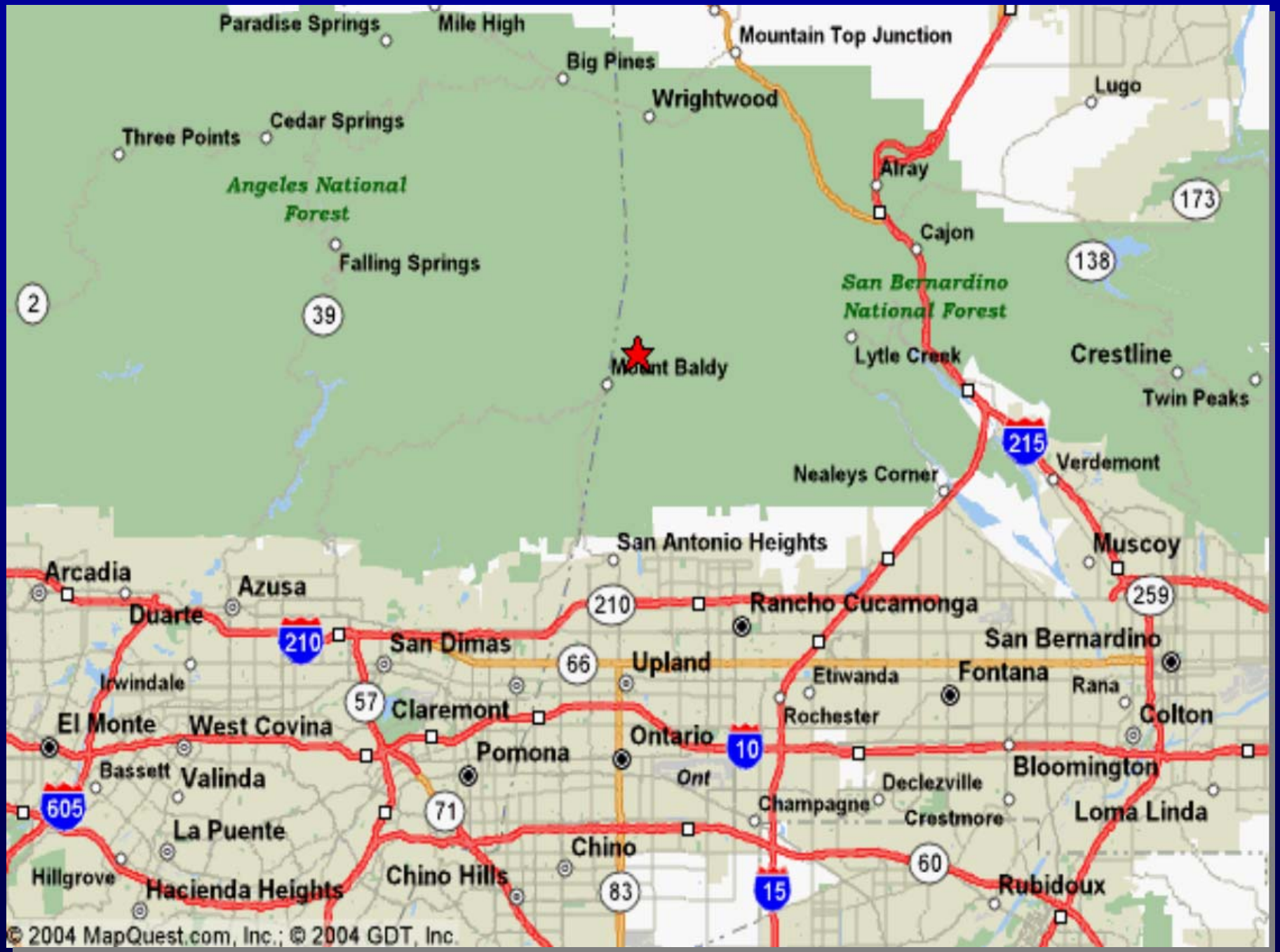
Project Duration: 10/2004-03/2006

Study Goals

- Monitor changes in the microbial & chemical composition along Chino Creek during base flow and storm conditions to determine seasonal and temporal variations.
- Determine the pathway of microbial transport as it relates to water quality within the Chino Creek watershed.

Analysis Parameters

- Fecal indicator bacteria: Total & Fecal Coliforms, E.coli, Enterococcus, total bacteria (viable & non-viable)
- Chemical analyses: turbidity, EC, temp. pH, DO, flow rate (where available).
- PCR based analysis of total microbial communities, Enterococcus communities and Bacteroides spp. (human-specific marker).


Paradise Springs
Mile High
Mountain Top Junction
Big Pines
Wrightwood
Lugo
Three Points
Cedar Springs
Angeles National Forest
Alray
173
Cajon
138
San Bernardino National Forest
Falling Springs
2
39
Mount Baldy
Lytle Creek
Crestline
Twin Peaks
215
Verdemont
Nealeys Corner
San Antonio Heights
Muscoy
Arcadia
Azusa
259
Duarte
210
Rancho Cucamonga
San Dimas
San Bernardino
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Upland
Etiwanda
Fontana
Rana
Irwindale
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Claremont
Rochester
Colton
El Monte
West Covina
Ontario
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Pomona
Bassett
Valinda
Ont
Declezville
Bloomington
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71
Champagne
Crestmore
Loma Linda
La Puente
Chino
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Hillgrove
Chino Hills
Hacienda Heights
83
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Rubidoux
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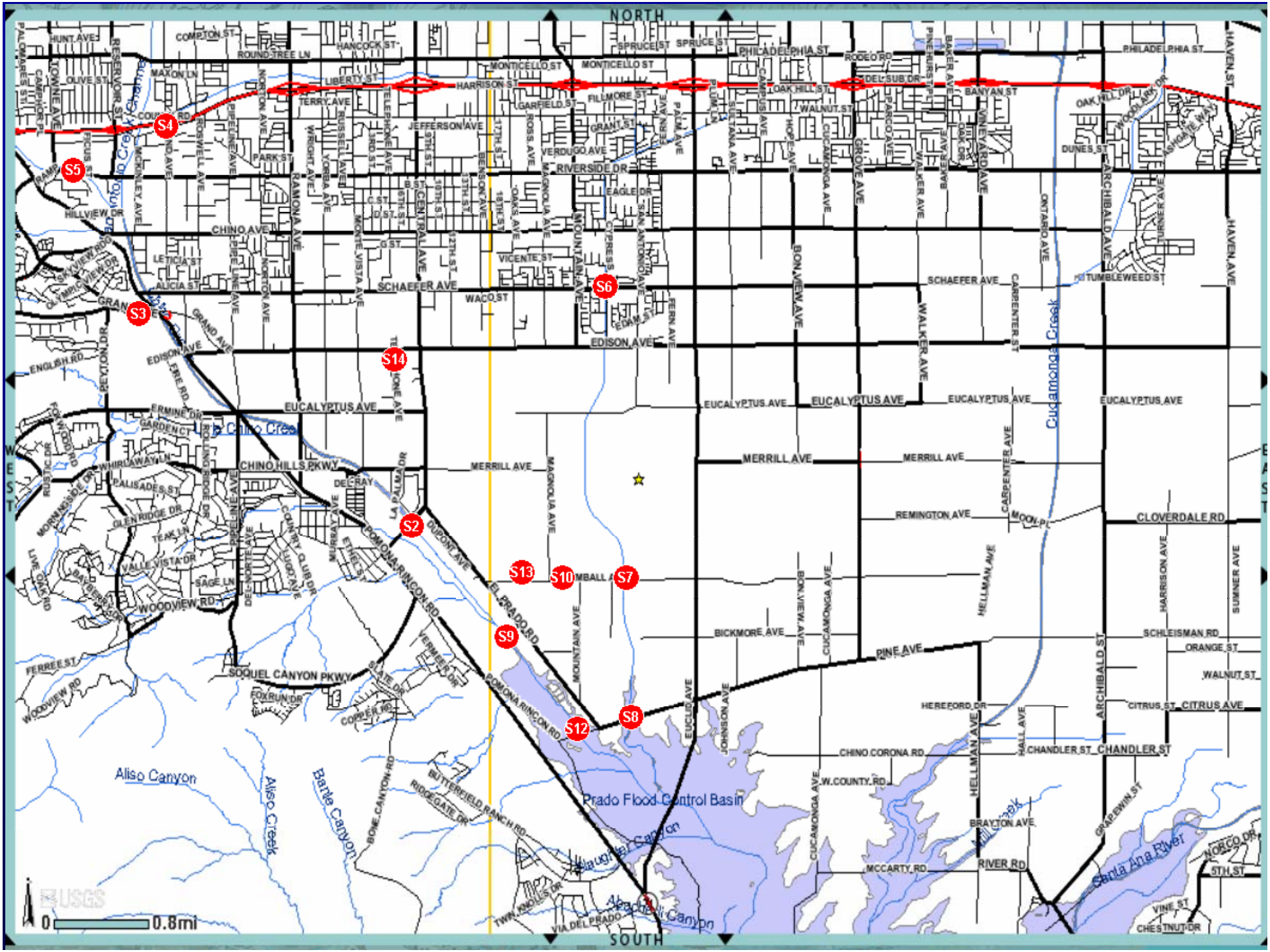
NORTH
SOUTH
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S6
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S8
S9
S10
S12
S13
S14
Cucamonga Creek
Chino Creek
Aliso Canyon
Aliso Creek
Bane Canyon
Prado Flood Control Basin
Slaughter Canyon
Santa Ana River
CHINO AVE
SCHAEFER AVE
EDISON AVE
EUCALYPTUS AVE
MERRILL AVE
CHINO HILLS PKWY
EL PRADO RD
POMONA RINCON RD
SOQUEL CANYON PKWY
WOODVIEW RD
RIVERSIDE DR
HARRISON ST
PHILADELPHIA ST
CLOVERDALE RD
PINE AVE
CHINO CORONA RD
CHANDLER ST
CITRUS AVE
BRAYTON AVE
RIVER RD
MCCARTY RD
0
0.8mi
USGS

Sampling Sites

Location	Land use
Ice House Canyon	Open Space
Chino Ck. @ Central Ave.	Urban runoff
Chino Ck. @ Shaeffer Ave.	Urban runoff
San Antonio Wash @ County Dr. & East End Ave.	Urban runoff + commerical wash out
Chino Crk. @ Riverside Drive	Urban runoff
Cypress Channel @ Shaffer Ave.	Urban runoff
Cypress Channel @ Kimball Ave	Urban runoff+agricultural
Cypress Channel @ golf course	Urban runoff
Storm drain south of 'Big League Dreams'	Urban runoff
Dirt channel on Kimball Ave	Urban runoff+ agricultural
Chino Creek @ Pine Ave.	Urban runoff+ wastewater
IEUA Regional Water Recycling Plant #5	Effluent from wastewater treatment plant
IEUA Carbon Canyon Waste Reclamation Facility (CCWRF)	Effluent from wastewater treatment plant

All sites will be sampled quarterly and more frequently during storm events.

Sampling sites

Ice House Canyon in Mt. Baldy (S1)

Chino Creek @ Central Ave (S2)

Chino Creek @ Schaffer Ave (S3)

San Antonio Wash @ County Drive & East End (S4)

Chino Creek @ Riverside Drive (S5)

Cypress Channel @ Shaffer Ave (S6)

Sampling sites, cont.

Cypress Channel @ Kimball (S7)

Cypress Channel @ golf course (S8)

Storm drain south of 'Big League Dreams' (S9)

Dirt channel on Kimball Ave (S10)

Chino Creek @ Pine Ave (S12)

IEUA RP- 5 plant (S13)

Sampling site

IEUA Carbon Canyon Waste Reclamation Facility (S14)


Paradise Springs
Mile High
Mountain Top Junction
Big Pines
Wrightwood
Lugo
Three Points
Cedar Springs
Angeles National Forest
Alray
173
Cajon
138
San Bernardino National Forest
2
1000000
100000
10000
1000
100
10
1
Open space
Dec-04
Feb-05
Mount Baldy
Lytle Creek
Crestline
Twin Peaks
S1
Ice House Canyon
215
Nealeys Corner
Verdemont
San Antonio Heights
Muscoy
Total coliform (CFU/100mL)
sample date: 12/28/04 & 2/11/05
210
Rancho Cucamonga
259
Range on all graphs
0 - 1000,000 CFU/100ml
66
Upland
San Bernardino
Etiwanda
Fontana
Rana
57
Claremont
Colton
El Monte
West Covina
Rochester
Ontario
10
Pomona
Bassett
Valinda
Ont
Bloomington
Declezville
605
71
Champagne
Crestmore
Loma Linda
La Puente
Chino
60
Hillgrove
Hacienda Heights
Chino Hills
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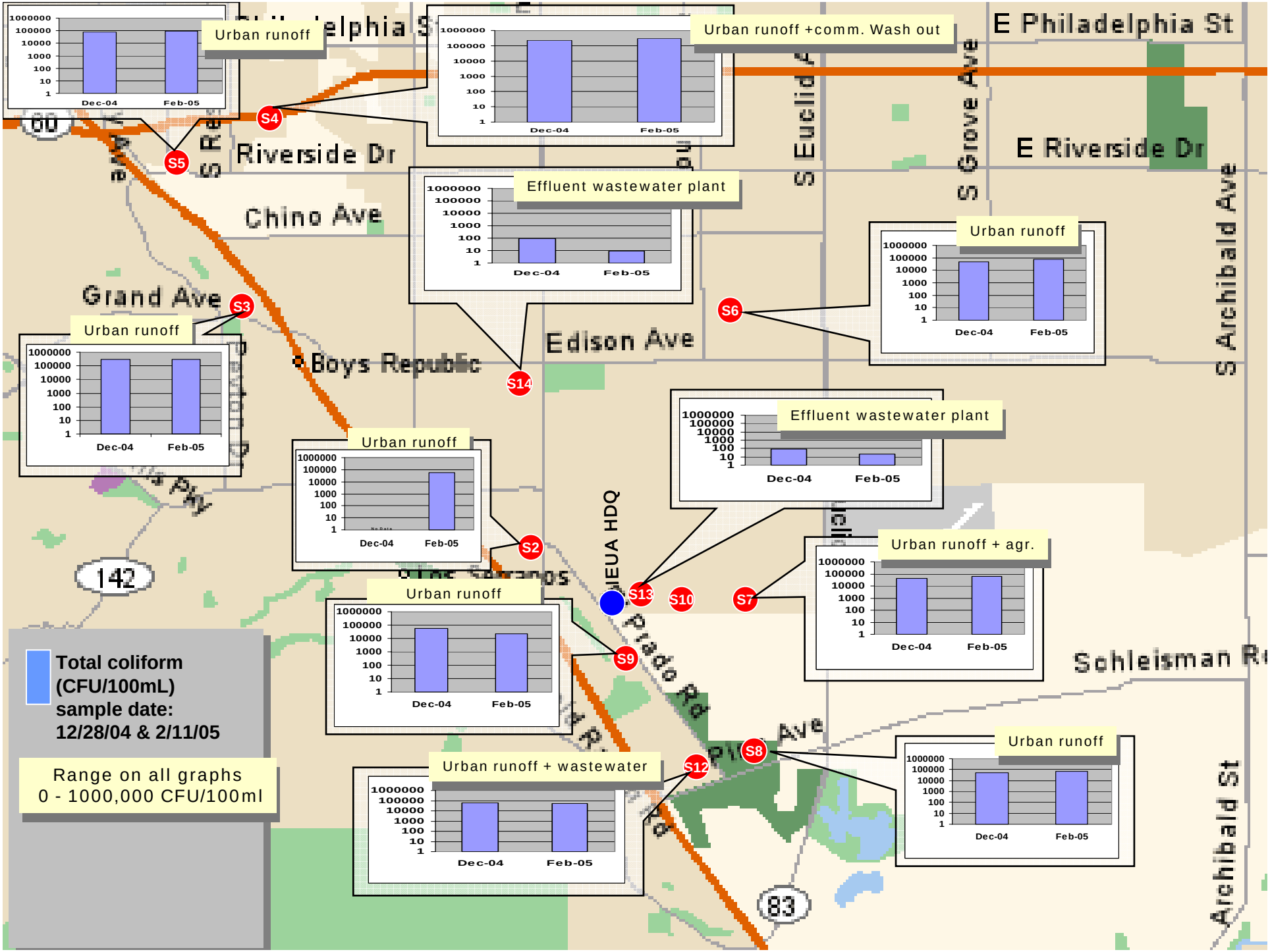
Urban runoff
Urban runoff +comm. Wash out
Effluent wastewater plant
Urban runoff
Urban runoff
Effluent wastewater plant
Urban runoff
Urban runoff + agr.
Urban runoff
Urban runoff
Urban runoff + wastewater
Dec-04
Feb-05
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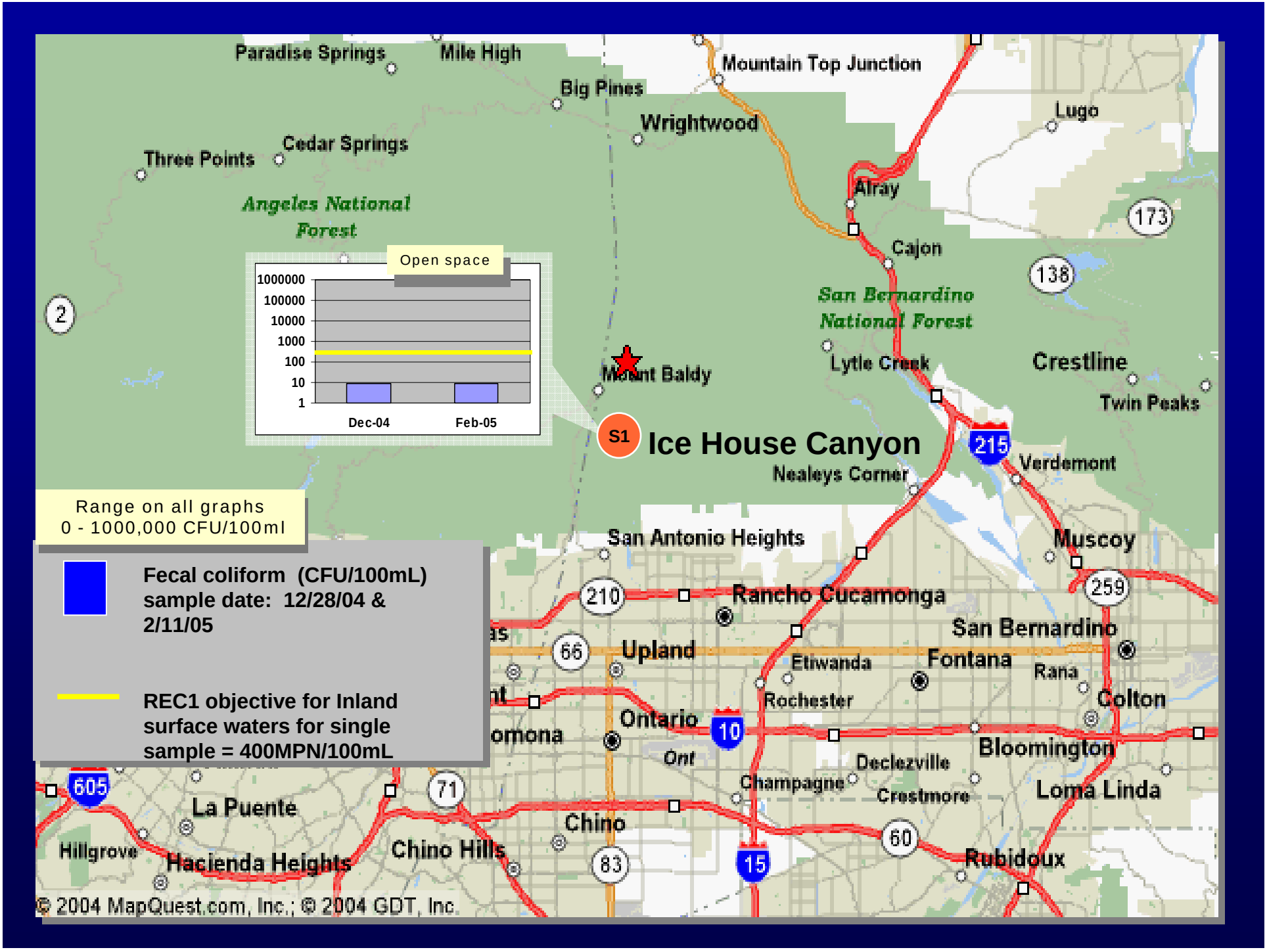
E Philadelphia St
E Riverside Dr
Riverside Dr
Chino Ave
Grand Ave
Edison Ave
S Euclid Ave
S Grove Ave
S Archibald Ave
Archibald St
Schleisman Rd
Prado Rd
Boys Republic
IEUA HDQ
142
83
Total coliform (CFU/100mL) sample date: 12/28/04 & 2/11/05
Range on all graphs 0 - 1000,000 CFU/100ml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Feb-05
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Range on all graphs
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San Antonio Heights
Muscoy
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Etiwanda
Fontana
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Colton
REC1 objective for Inland surface waters for single sample = 400MPN/100mL
Rochester
Ontario
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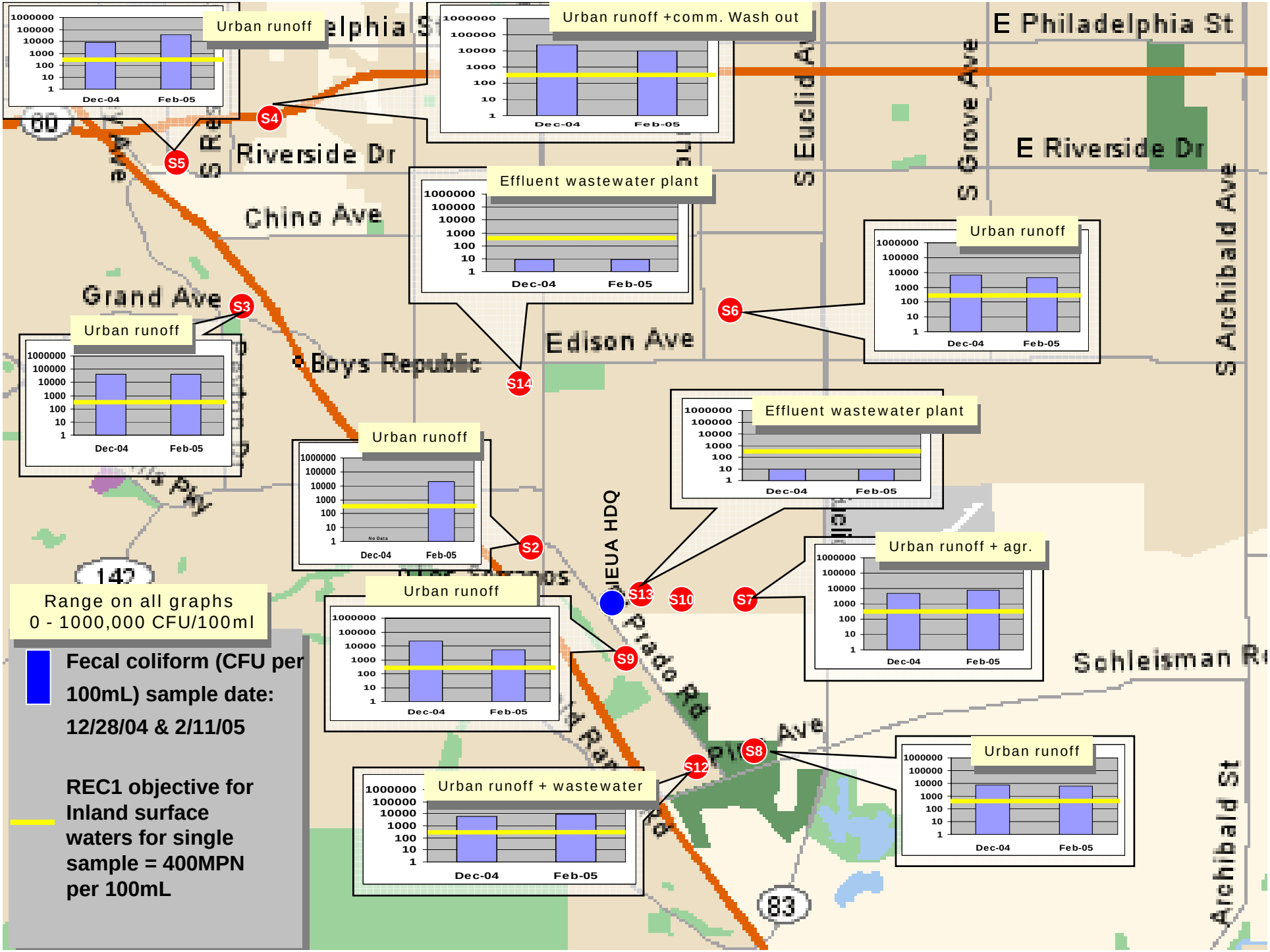
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Urban runoff + wastewater
Dec-04
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Prado Rd
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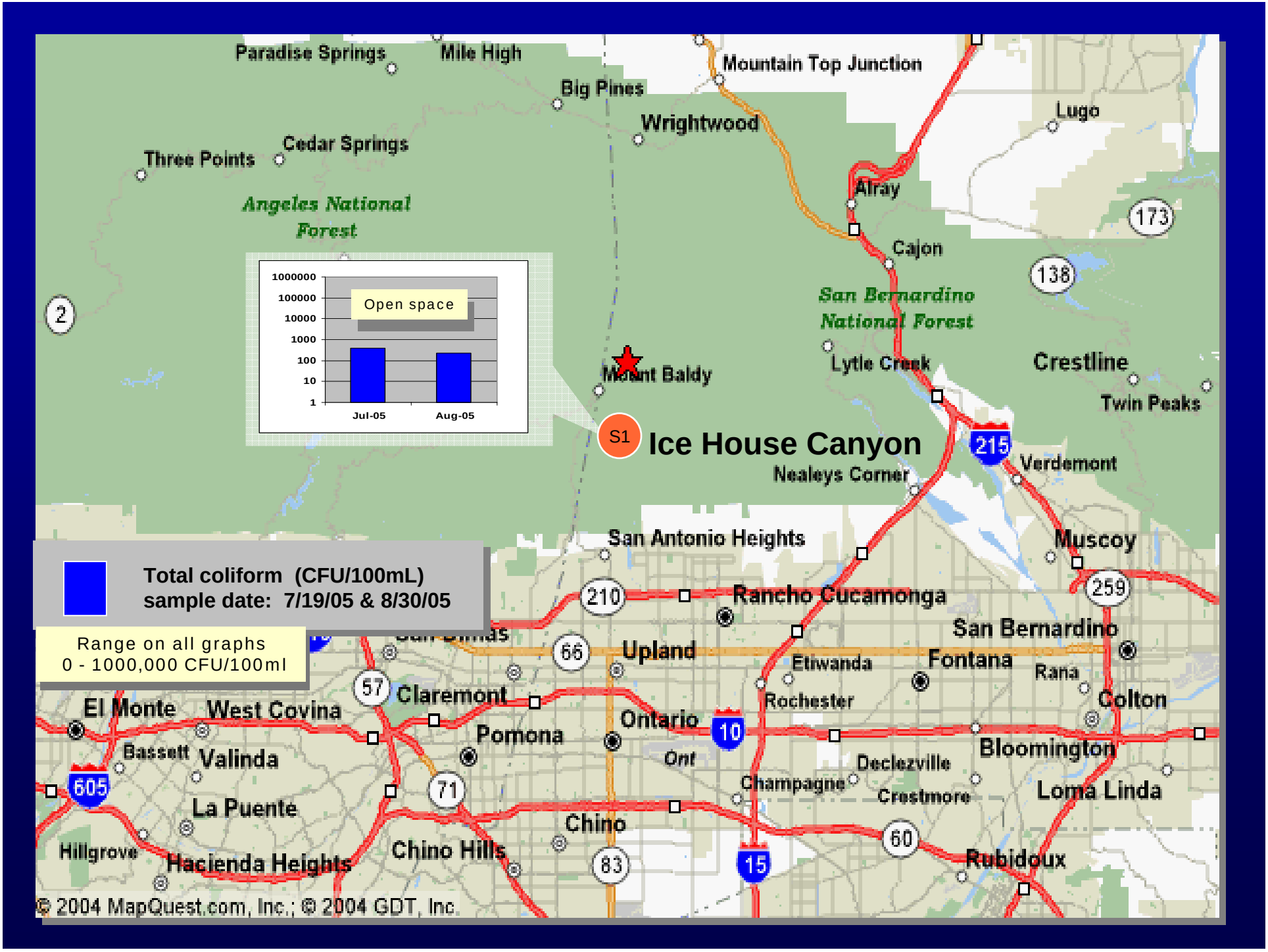
Range on all graphs
0 - 1000,000 CFU/100ml
Fecal coliform (CFU per 100mL) sample date: 12/28/04 & 2/11/05
REC1 objective for Inland surface waters for single sample = 400MPN per 100mL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Jul-05
Aug-05
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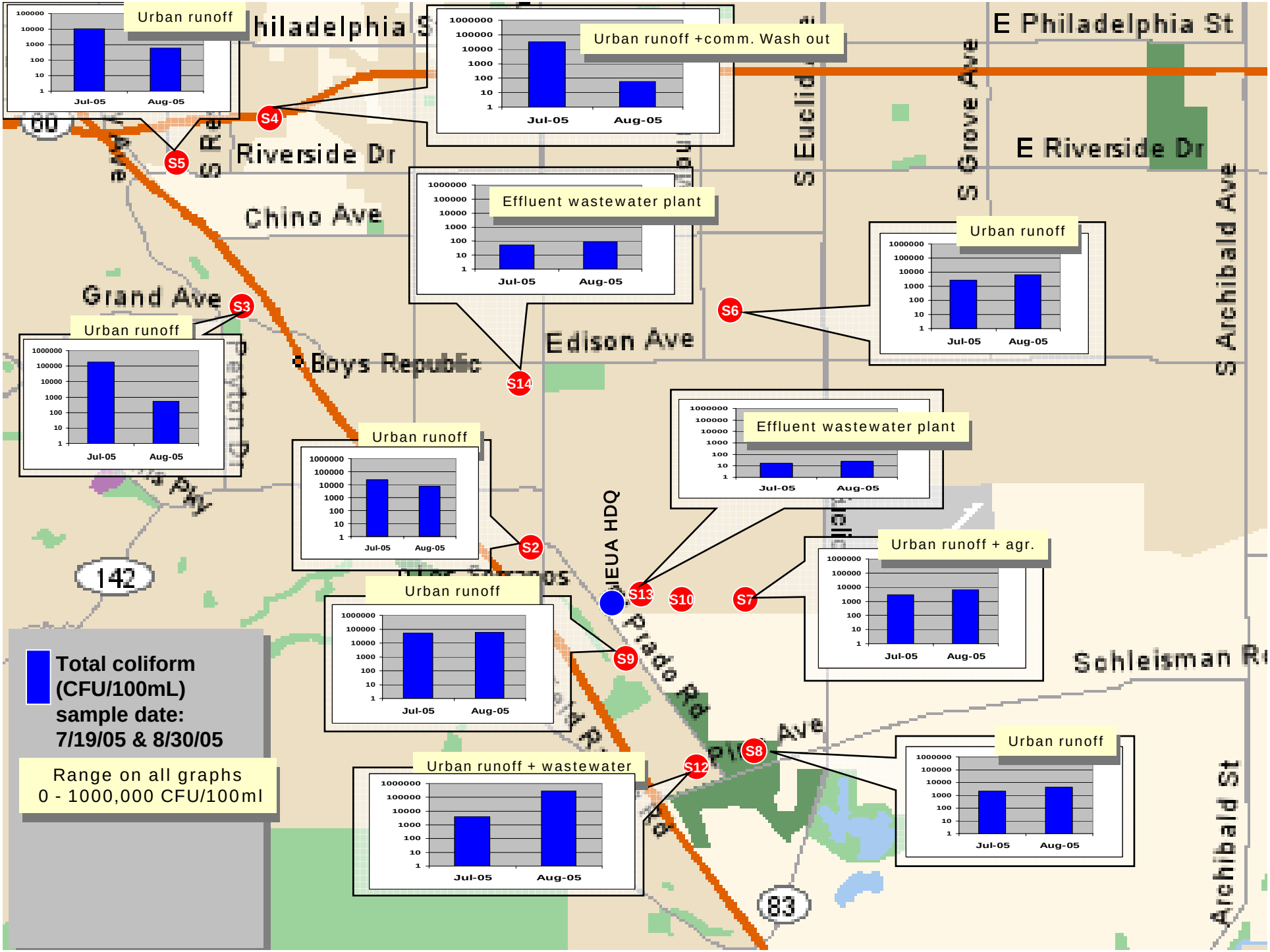
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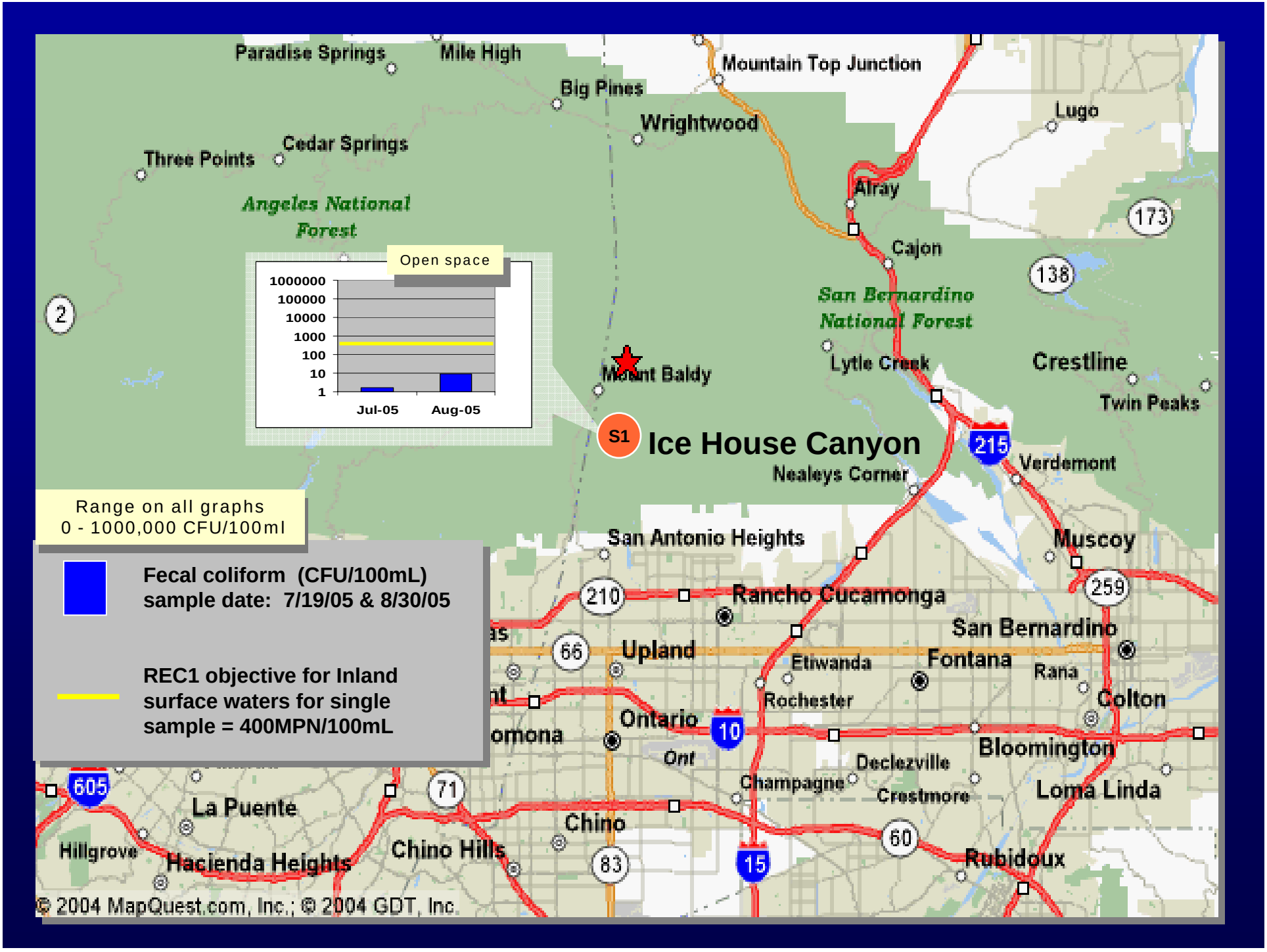
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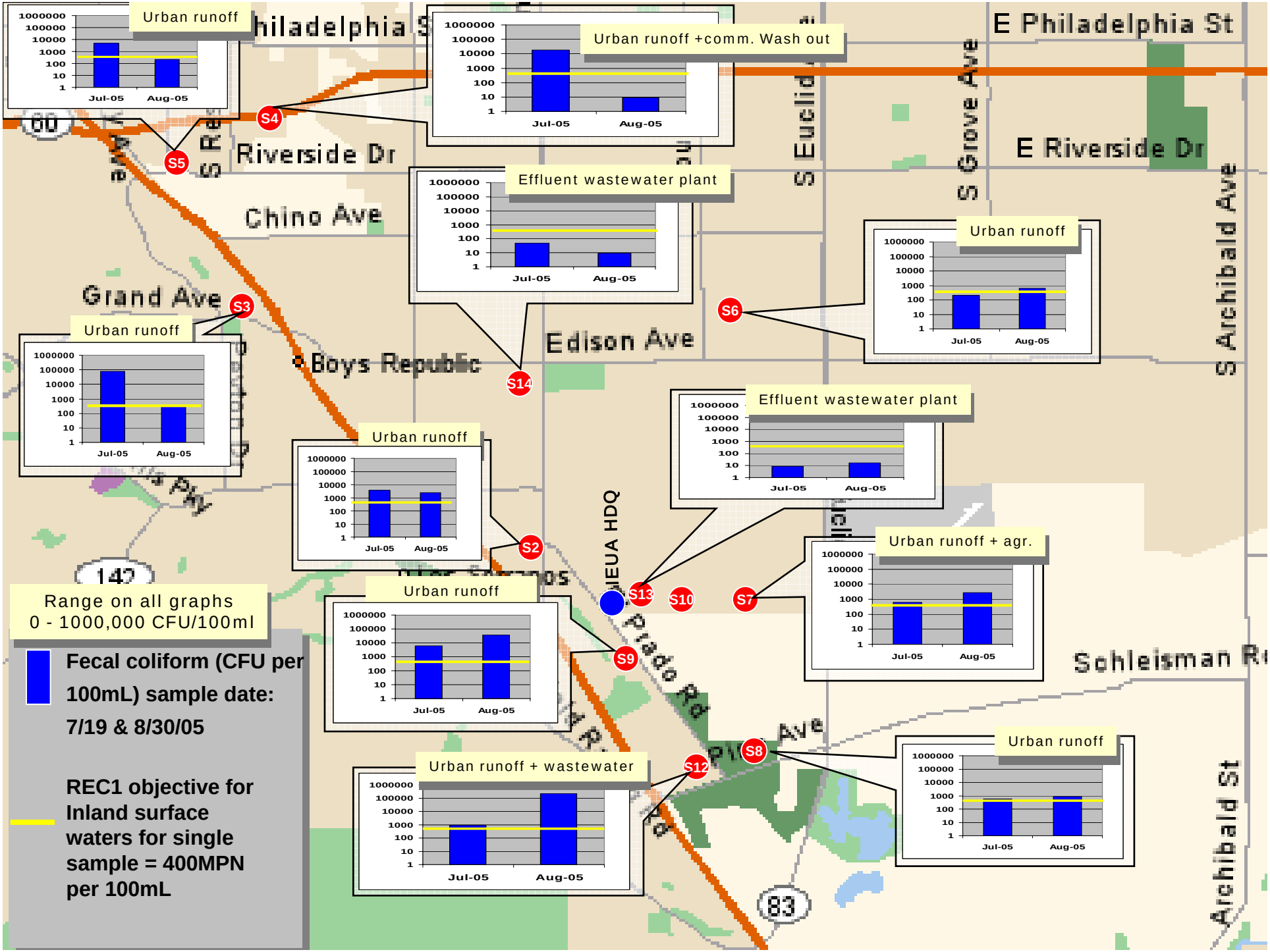
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Jul-05
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Relevance of the Study

Monitoring microbial communities may allow:

- detection/tracking of significant water quality changes
- identification of possible sources of pathogens
- identification of pathways of microbial transport
- identification of appropriate indicator organisms
- characterize microbial population dynamics
- provide baseline data for future water quality assessments (e.g., impact of future development)

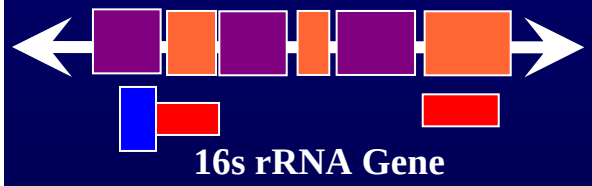
Genetic Methods Used for Microbial Analysis

- Microbial Community Structure (MCS) Analysis using the 16s rRNA gene.
- Detect specific organisms (i.e., Bacteroides and Enterococcus spp.) using genus-specific PCR primers.
- Correlate with field parameters (i.e., pH,DO & temp.) and other bacterial indicators (i.e., total coliform & Enterococci).

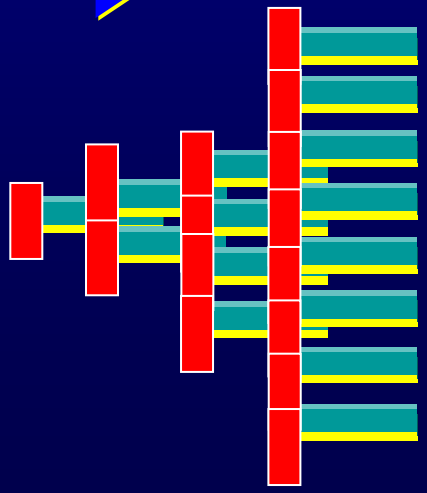
Current method used to analyze microbial communities

DNA extracted
from water sample

'Universal primers'



PCR



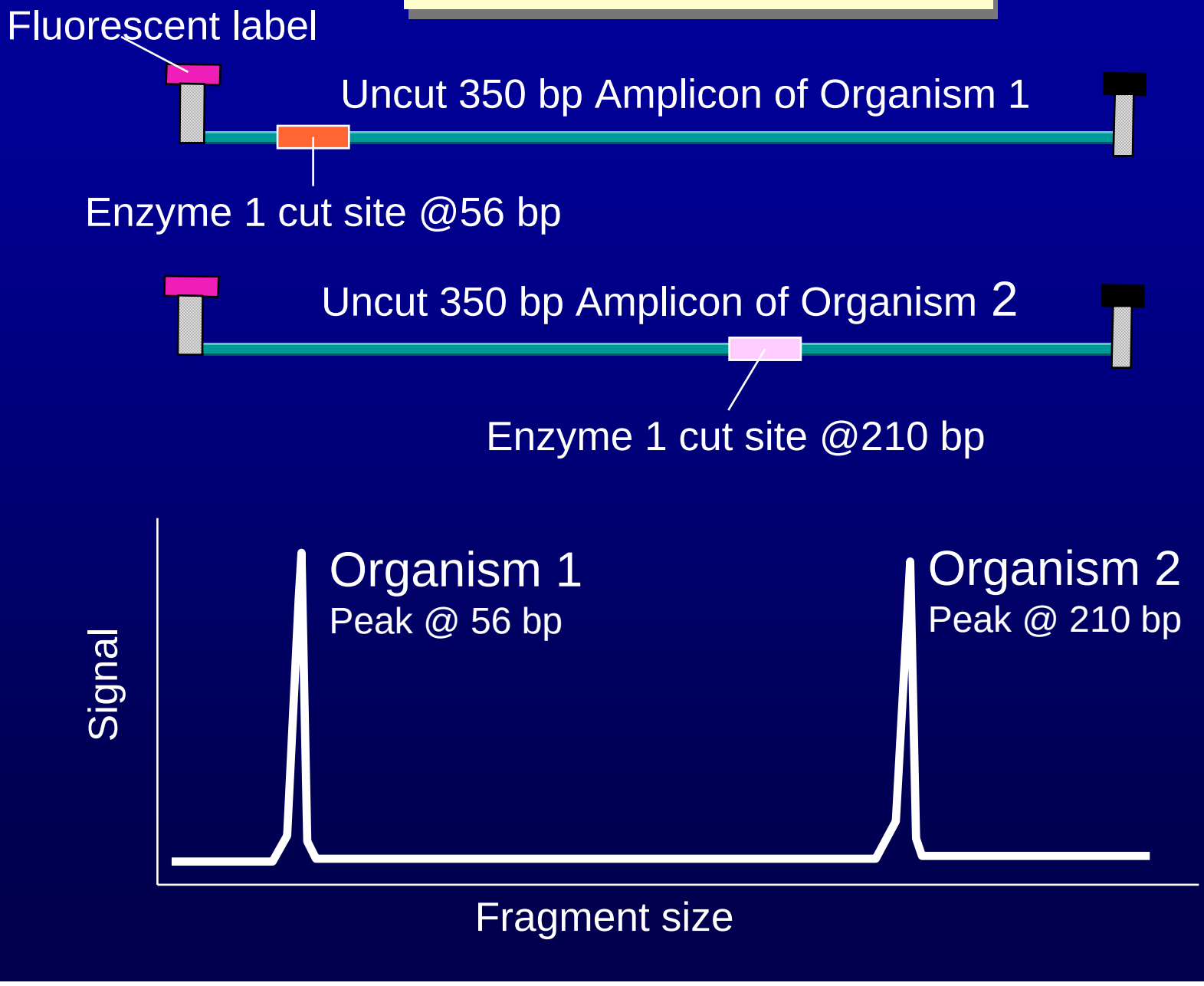
Capillary electrophoresis to separate fragments

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Used to confirm the identity of organisms by the location of a specific cut sites in the the PCR fragment using chemical scissors.

Separates PCR fragments of different sizes.

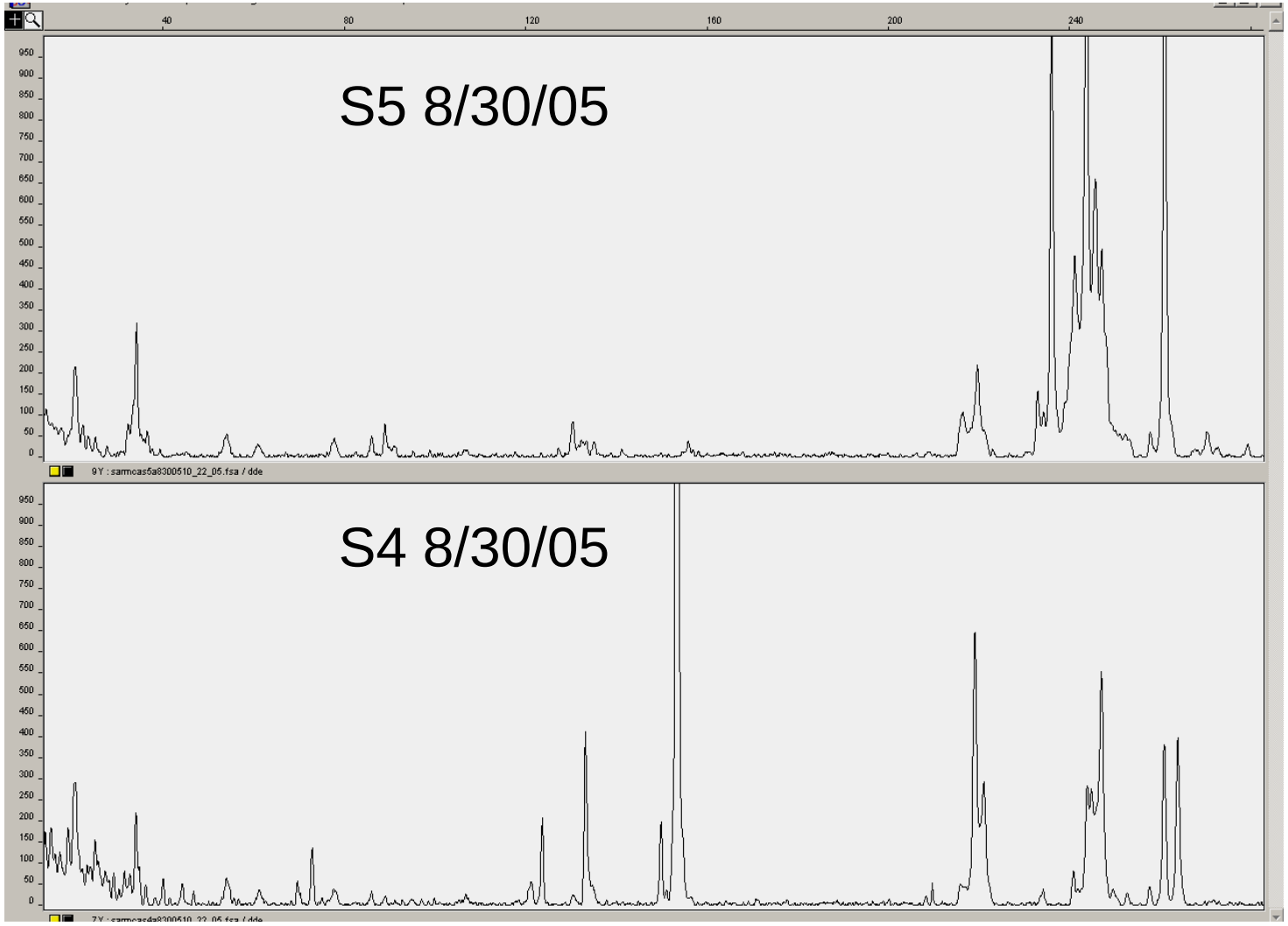
T-RFLP analysis



Community Profile Using TRFLP

Size

Amplitude



Size	S5	S4
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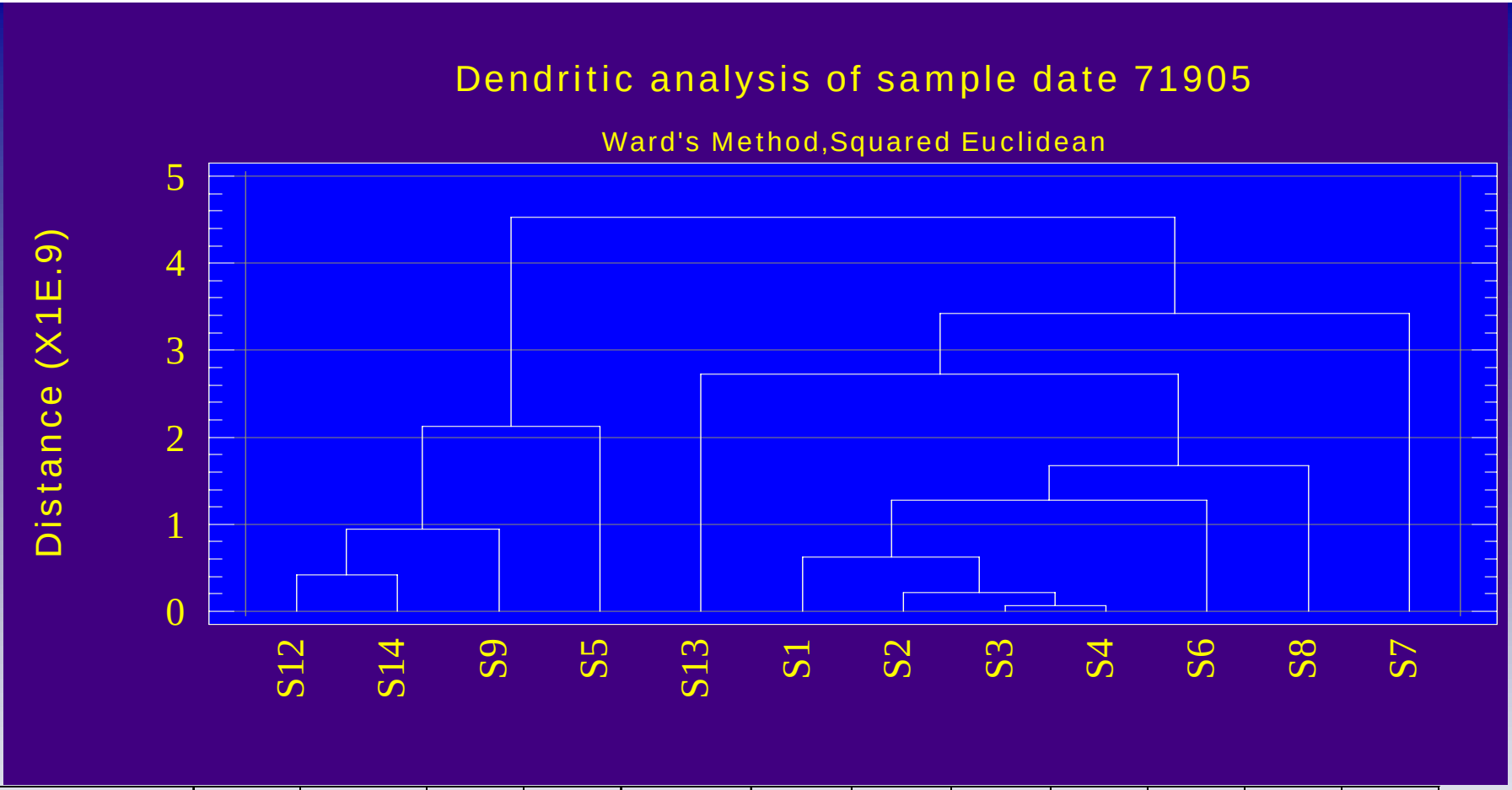
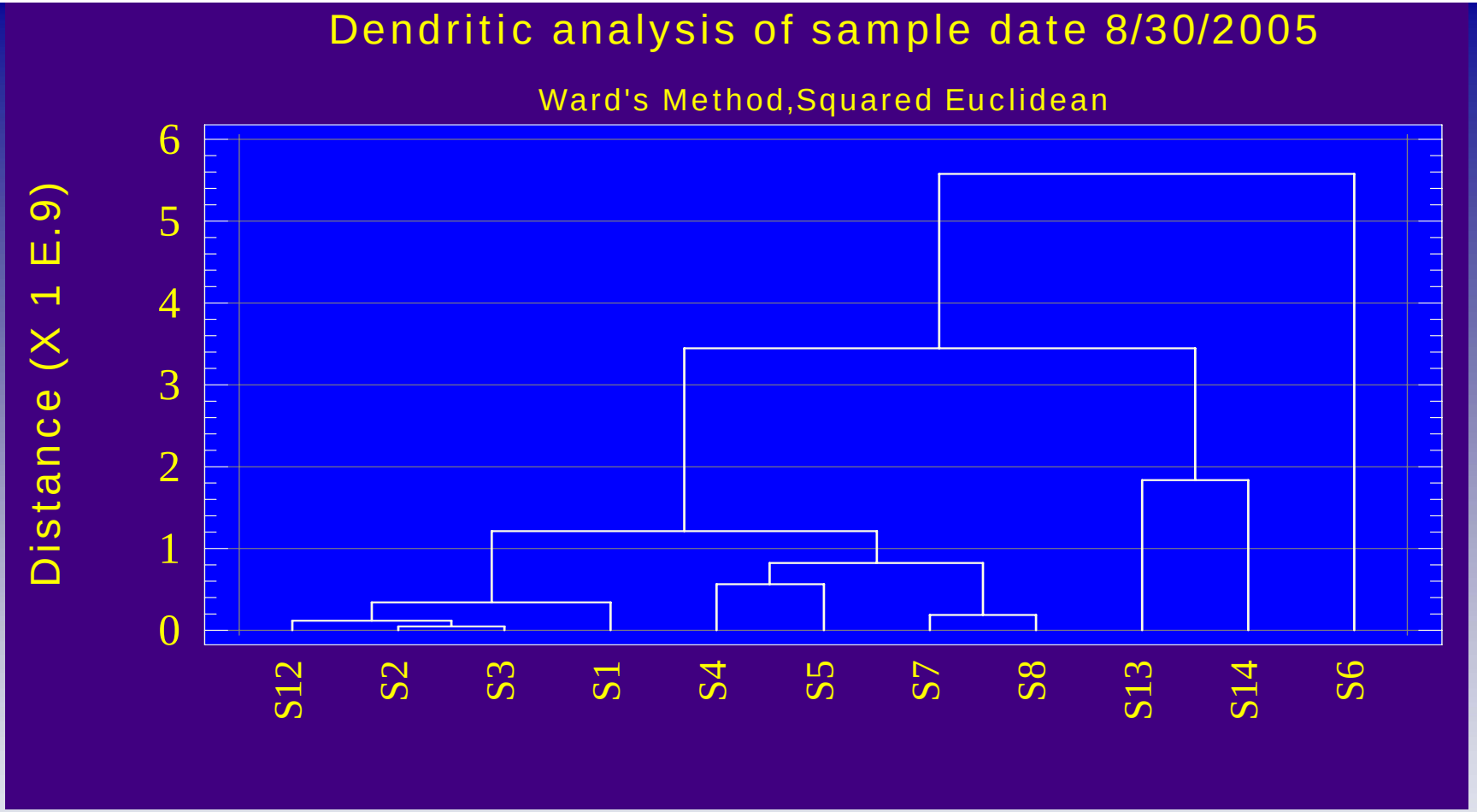


	S12	S14	S9	S5	S13	S1	S2	S3	S4	S6	S8	S7
Analysis Parameters												
land use	waste+runoff	treatment plant	runoff	runoff	treatment plant	open space	runofff	runoff	runoff	runofff	runoff	runoff+Ag.
lined	no	yes	no	yes	yes	no	no	yes	yes	yes	no	no
site is downstream of :	S9	none	S2	none	none	none	S3	S4	none	none	S7	S6
Avg. total bac.counts (epi)	5.02E+05	4.53E+05	6.61E+06	1.21E+06	3.54E+07	3.02E+06	8.53E+07	4.45E+08	2.19E+07	1.16E+08	1.02E+09	1.20E+09
Avg. total coliform	4.00E+03	4.00E+01	4.85E+04	1.09E+04	1.95E+01	4.85E+04	2.30E+04	1.85E+05	3.30E+04	2.80E+03	2.10E+03	3.00E+03
Avg. fecal coliform	8.60E+02	5.00E+01	6.00E+03	5.00E+03	9	2.00E+00	3.55E+03	8.20E+04	1.75E+04	2.30E+02	5.95E+02	6.40E+02
Avg. E.coli	6.05E+02	3.50E+01	4.30E+03	1.80E+03	1.45E+01	2.50E+00	1.10E+03	4.25E+03	3.10E+02	9.00E+00	6.15E+02	5.90E+02
Avg. Enterococcus	3.10E+03	9.00E+00	1.77E+04	1.06E+03	9.00E+00	1.65E+01	3.10E+04	1.11E+04	2.50E+03	1.28E+03	9.60E+02	1.06E+03
Avg. turbidity	1.69	0.75	11.15	1.69	0.96	0.41	1.40	4.34	1.22	3.07	4.21	5.63
Weather (dry, storm, rec. flow)	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry

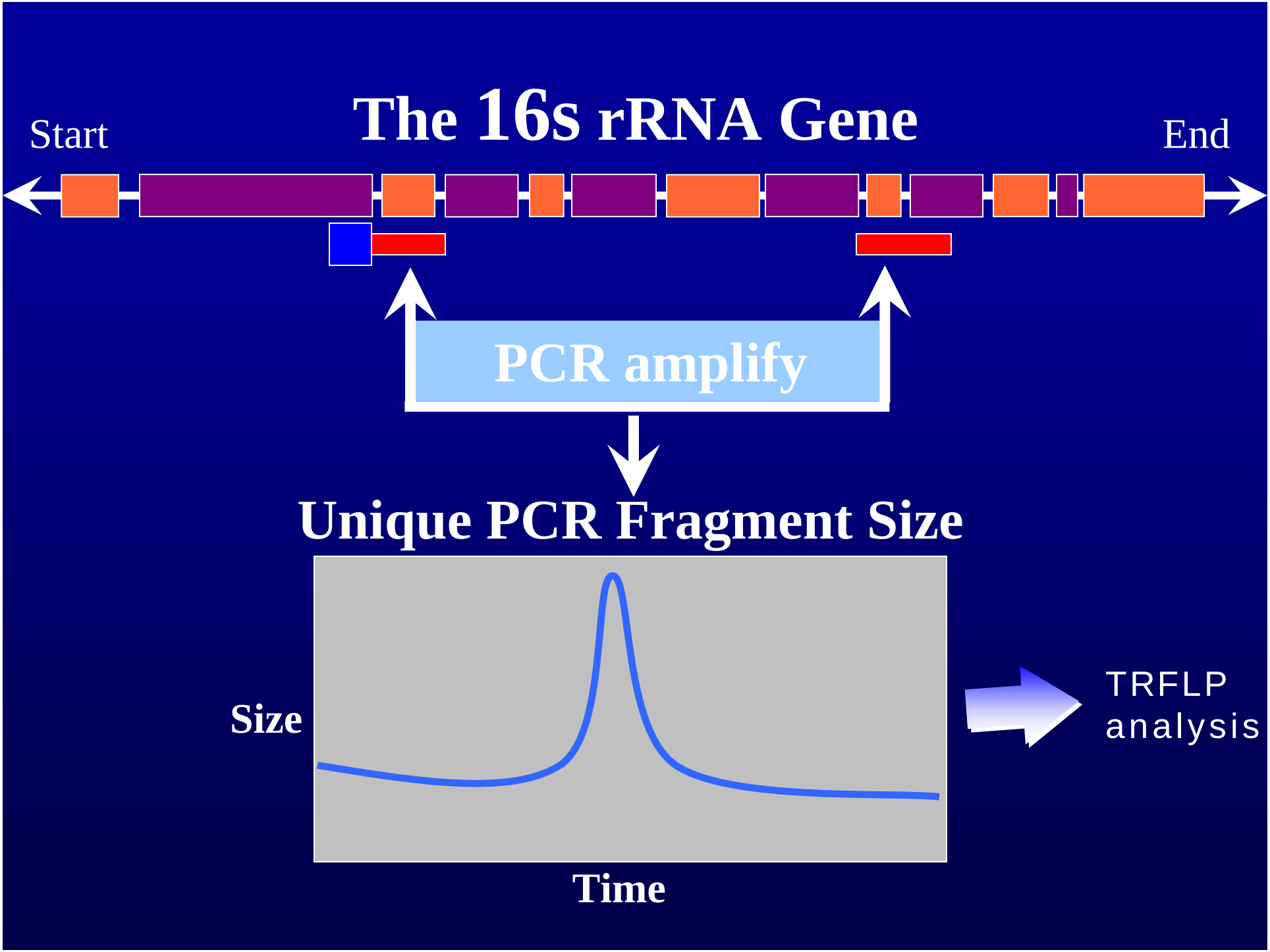


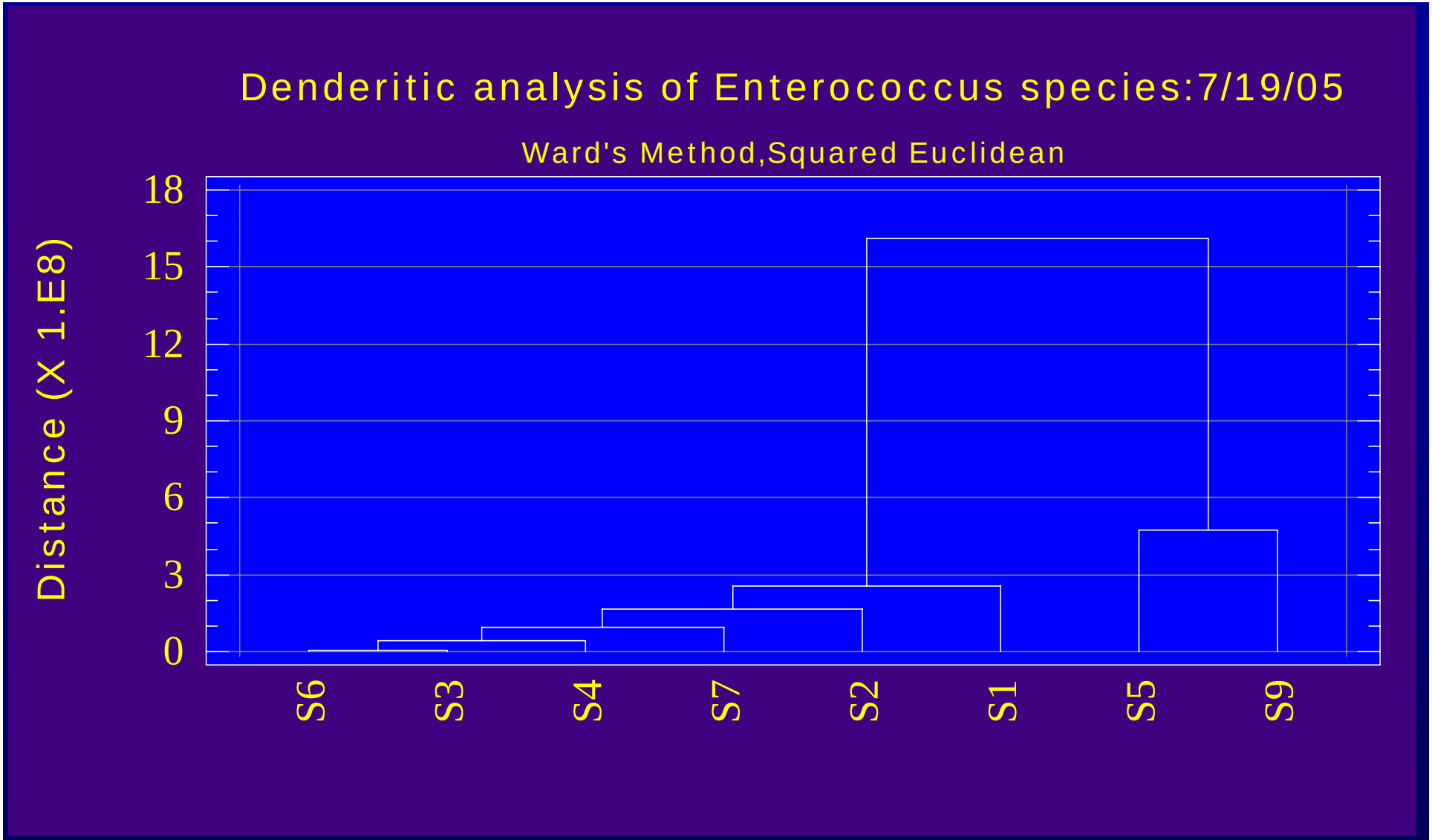
	S12	S2	S3	S1	S4	S5	S7	S8	S13	S14	S6
Analysis Parameters											
land use	waste+runoff	runoff	runoff	open space	urban+comm.	runoff	runoff+Ag.	runoff	treatment plant	treatment plant	runoff
lined	no	no	yes	no	yes	yes	no	no	yes	yes	yes
site is downstream of :	S9	S3	S4/S5	none	none	none	S6	S7	none	none	none
Avg. total bac.counts (epi)	9.30E+05	2.24E+06	3.74E+06	1.36E+05	3.20E+06	2.40E+06	1.79E+07	1.57E+07	7.09E+05	1.90E+06	2.94E+06
Avg. total coliform	3.05E+05	7.35E+03	5.50E+02	2.30E+02	6.00E+01	6.00E+02	6.65E+03	4.35E+03	2.50E+01	9.00E+01	6.45E+03
Avg. fecal coliform	2.25E+02	2.55E+03	3.25E+02	9.00E+00	9.00E+00	2.25E+02	2.85E+03	8.70E+02	1.95E+01	9.00E+00	6.85E+02
Avg. E.coli	4.50E+02	2.25E+02	1.40E+02	2.50E+01	9.00E+00	4.50E+01	1.75E+03	7.05E+02	9.00E+00	9.00E+00	1.45E+01
Avg. Enterococcus	2.65E+03	8.15E+03	3.70E+02	1.00E+02	5.10E+02	3.50E+02	6.70E+02	3.15E+03	1.45E+01	3.50E+01	2.45E+03
Avg. turbidity	1.62	3.36	6.22	0.39	6.14	1.18	23.75	5.58	0.77	0.94	10.08
Weather (dry, storm, rec. flow)	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry

Detection of specific organisms using genus-specific primers

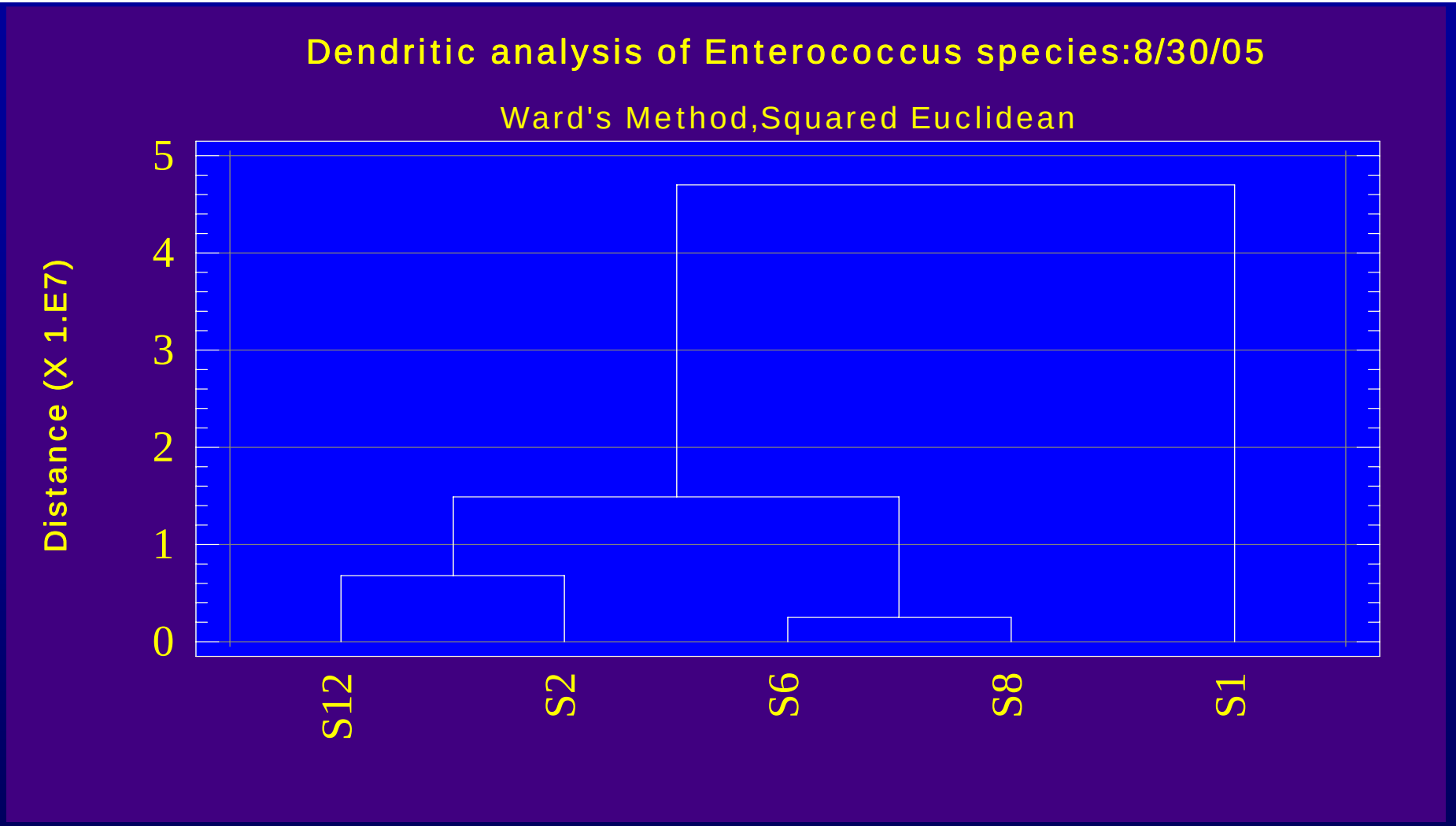
The organisms associated with fecal contamination are detected using a combination of PCR amplification of a segment of the 16S rRNA gene sequence coupled with TRFLP analysis.

OCWD-designed software has been used to identify organism-specific PCR primers and TRFLP patterns to differentiate between organisms, often to the strain level.


The 16s rRNA Gene
Start
End
PCR amplify
Unique PCR Fragment Size
Size
Time
TRFLP
analysis



Cluster 1 with S5 and S9 are related: geographically and land use.
Cluster 2 with S6,S3,S4,S7,S2 and S1are some what related: indigenous Entro. population but that population is impacted by either addition or subtraction of Entro. species.



Cluster 1 with S1: land use and geographically distinct from rest.
Cluster 2 with S6 and S8 are related: geographically (Cypress channel), land use and microbial data (total and fecal coliforms).
Cluster 3 with S12 and S2 are related: geographically and total bacteria counts (epi).

Identification of Human-specific *Bacteroides* marker

	Jul-05		August-05	
Site No.	Bacteroides	F.C. > 400 *	Bacteroides	F.C. > 400*
S1	-	-	-	-
S2	+	+	+	+
S3	-	+	-	-
S4	+	+	-	-
S5	+	+	-	-
S7	-	+	-	+
S9	+	+	-	+
S12	-	-	-	+
S13	+	-	+	-
S14	+	-	+	-

***REC1 objective for Inland surface waters for single sample = 400 MPN per 100mL.**

Relevance of using standard and genetic-based methods

- Comprehensively monitor changes in the microbial communities along Chino Creek and Cypress channel using both genetic and microbial analysis.
- Characterize the impact of runoff (baseflow / storm flow) in Chino Creek.
- Possibly identify microbial transport relationships.
- Identify relationships between fecal indicator bacteria & other organisms/communities (e.g. Enterococcus & Bacteroides).
- Possibly identify appropriate indicator organisms.

Factor to Consider in the Study

- Some of the methods that are employed have previously been used in controlled field conditions (see references on last slide).

- Chino Creek study area is affected by multiple sources of non-point microbial contamination.

- The complex nature of the Chino Creek site may obscure the signal from any particular source.

- Additional data collection beyond one year of data collection as outlined, may be required to completely understand temporal variations in the system and the effectiveness of potential control strategies (and the impact of wet versus dry conditions).

Conclusions

- Microbial, chemical and genetic methods should be employed to comprehensively monitor microbial communities in areas impacted by urban runoff.

- Microbial and genetic methods can possibly be used to identify transport relationships based on geographic locations and land use.

- Total bacti counts (epi) can be used in addition to standard methods to comprehensively monitor changes in microbial populations especially in areas impacted by urban runoff.

- Increase frequency of sampling at specific sites to determine the stability of microbial relationships.

References

1. Izbicki, J.A., M.I. Pimentel and M.B. Leddy. Microbial and Dissolved Organic Carbon Characterization of Stormflow in the Santa Ana River at Imperial Highway, Southern California, 1999-2002. U.S. Geological Survey, Scientific Investigation Report 2004-5116.

2. Baby Beach Bacteriological Special Studies Report Dana Point Harbor, California, June 2003. Prepared by: Orange County Public Health Laboratory, Science Applications International Corporation, County of Orange Public Facilities & Resources Department (PFRD) Watershed and Coastal Resources Division.

3. La Montagne, M.G., J. P. Schimel and P. A. Holden. 2003. Comparison of Subsurface and surface soil bacterial communities in California grassland as Assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16s rDNA. Microbial Ecology 46:216-217.

Thank You

Questions????