



MSAR Watershed Pathogen TMDL Workgroup

Bacteroides Monitoring Update

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Presentation Topics

- **Urban Source Evaluation Plan (USEP)**
- **Molecular methods used to identify specific markers (human, dog and cow)**
- **Review preliminary data**



Bacteroides Monitoring of USEP Sites: sample analysis

- Approximately 67 surface water samples have been analyzed
- *Bacteroides thetaiotaomicron* is used to identify the presence of the human and dog marker in surface water samples
- *Prevotella ruminicola* is used to identify the presence of the cow marker in surface water samples
- When a positive PCR signal is observed, approximate concentrations are determined
- Preliminary data is presented; some concentrations may be adjusted as samples are re-analyzed



Detection of specific organisms using genus-specific primers

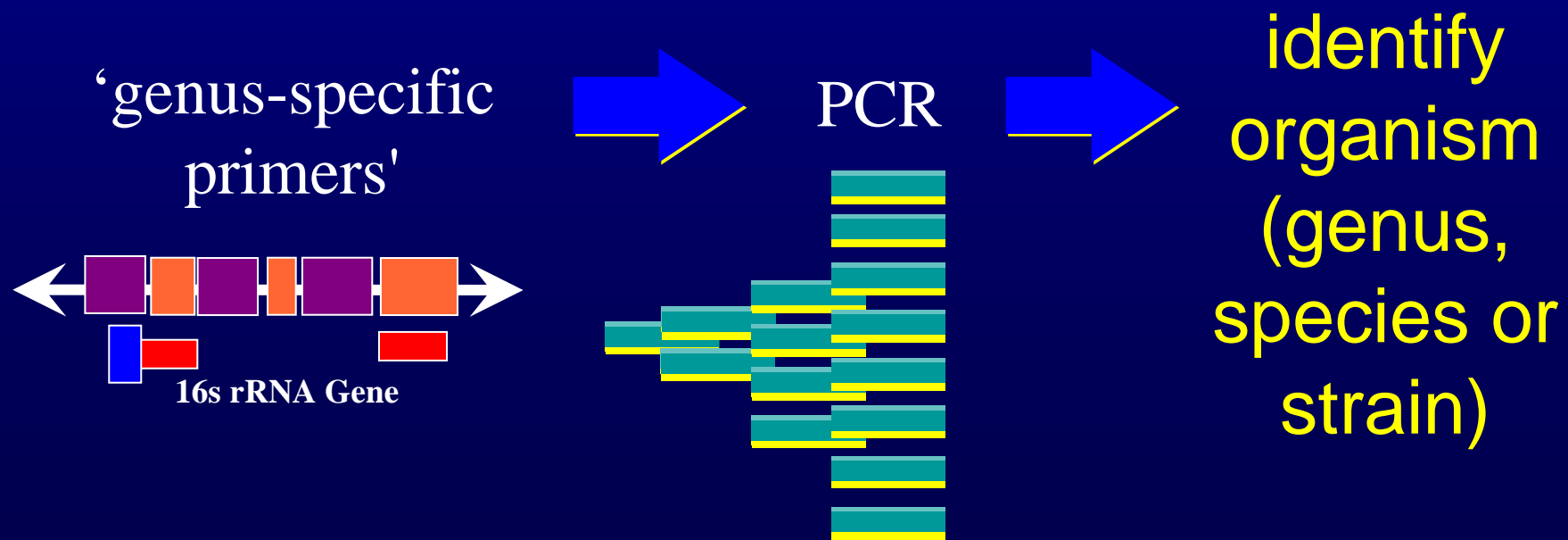
The organisms associated with fecal contamination are detected using a combination of PCR amplification of a segment of the 16S rRNA gene sequence coupled with TRFLP analysis.



Molecular methods used for microbial detection and identification

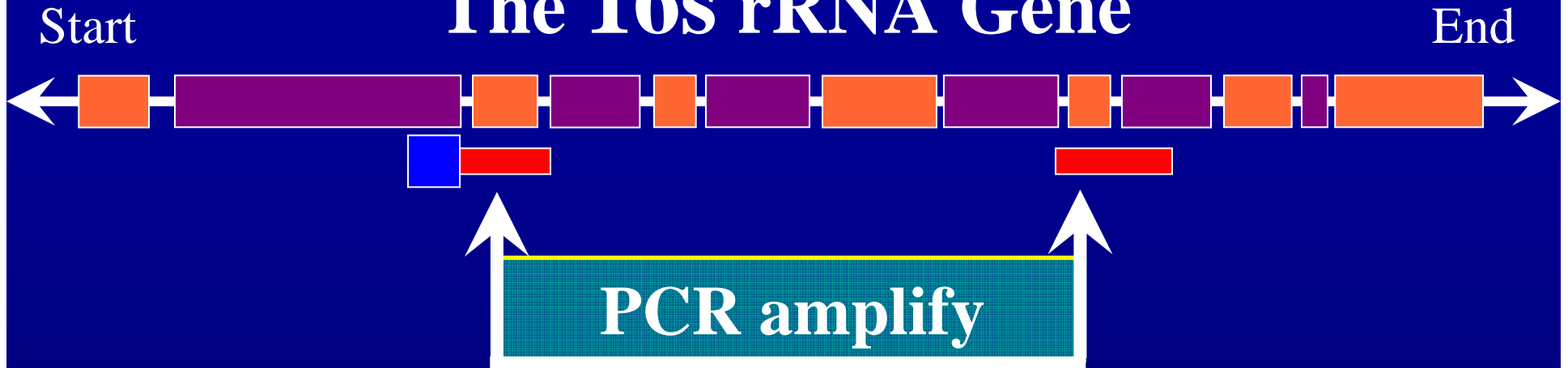


DNA extracted from sample

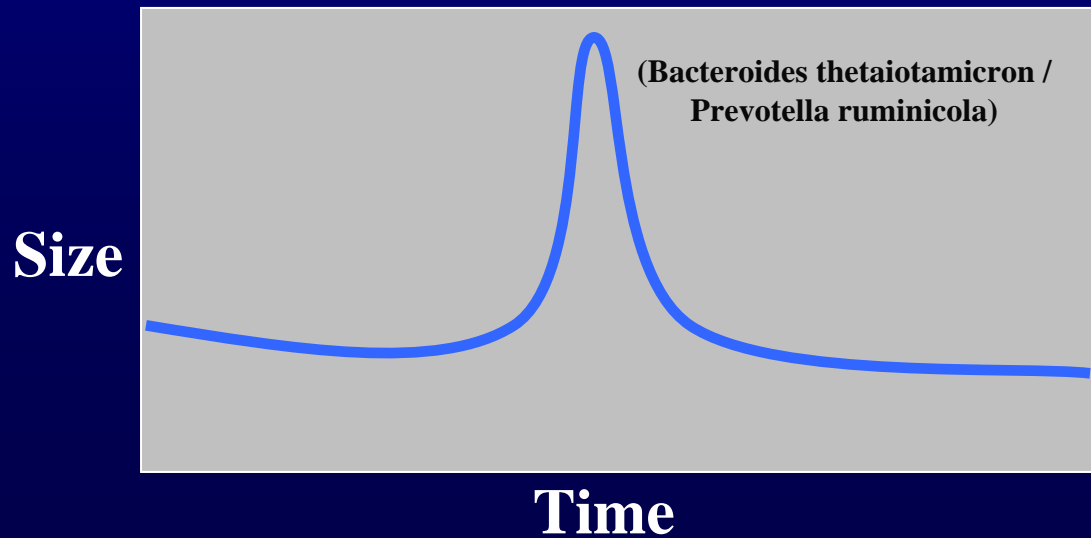




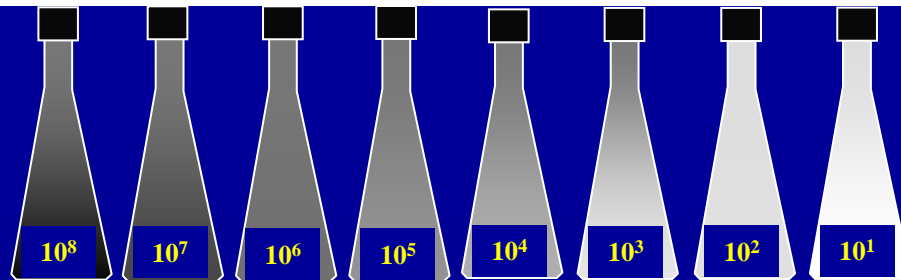
The 16s rRNA Gene



Unique PCR Fragment Size



TRFLP
analysis
(??)

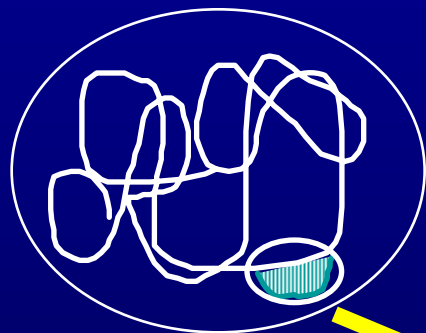


Diluted cells

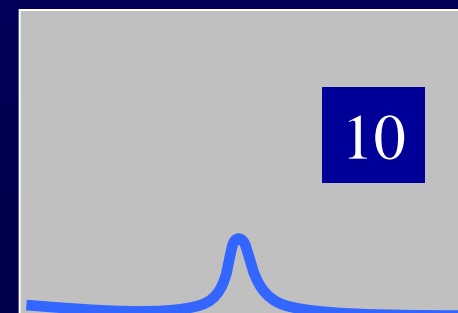
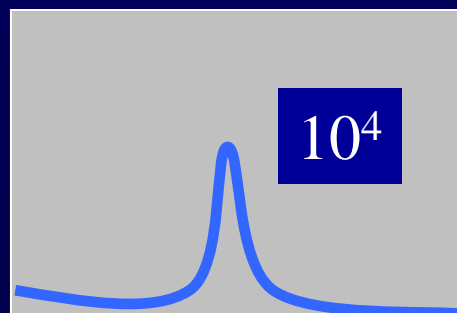
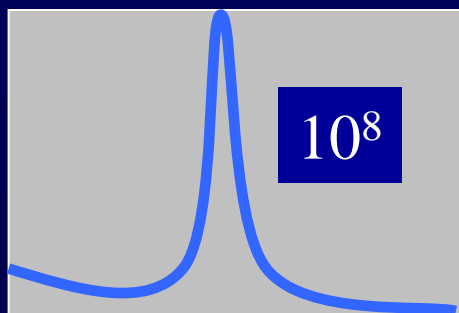
Concentration of cells confirmed using cell counter



Extract DNA from cells



PCR amplify using genus-specific primers





USEP Monitoring Sites





Detection of Human Marker*

Sample Date	Site (relative concentration of marker; Cells/mL)
7/24-25	Box Spring Channel @ Tesquequite Ave. (2.70×10^4)
7/31-8/1	Box Spring Channel @ Tesquequite Ave. (1.00×10^3)
8/7-8	Box Spring Channel @ Tesquequite Ave. (2.00×10^4)
9/18-19	Box Spring Channel @ Tesquequite Ave. (2.70×10^4)
9/25-26	Box Spring Channel @ Tesquequite Ave. (2.70×10^4)

*Human specific marker: *Bacteroides thetaiotamicron*



Detection of Dog Marker*

Sample Date	Site (relative concentration of marker; Cells/mL)				
	US-BXP	US - CHRIS	US - CLCH	US - CCCH	US - DAY
7/24-25	(low conc.)	(1X10 ³)	(1X10 ³)	BDL	NS
7/31-8/01	BDL	(1X10 ³)	(1X10 ³)	(1X10 ²)	(1X10 ³)
8/07-08	BDL	(1X10 ³)	(1X10 ³)	BDL	BDL
9/18-19	BDL	(1X10 ²)	NS	BDL	BDL
9/25-26	BDL	(low conc.)	NS	(1X10 ³)	(1X10 ³)

*Human-specific marker: Bacteroides thetaiotamicron

US-BXP = Box Spring Channel @ Tesquequite Ave.

US - CHRIS = Chris Basin Outflow-Lower Deer Crk.

US - CLCH = County-Line Channel near conf. with Cuc. Crk

US - CCCH = Carbon Canyon Creek Channel @ Pipeline Ave.

US -DAY = Day Crk @ Lucretia Ave.

BDL = below detection limit following 35 PCR cycles

NS = Not sampled



Detection of Bovine Marker*

Sample Date	Site (relative concentration of marker, Cell/mL)					
	US-BXP	US - CYP	US - SACH	US - ANZA	US-SNCH	US - SSCH
7/24-25	(low conc.)	(1X10 ³)	(1X10 ²)	(1X10 ³)	BDL	(1X10 ²)
7/31-8/01	BDL	(1X10 ³)	(1X10 ²)	(1X10 ²)	(1X10 ³)	BDL
8/07-08	(low conc.)	(1X10 ³)	(1X10 ²)	(1X10 ³)	BDL	(1X10 ³)
9/18-19	BDL	(1X10 ³)	(1X10 ³)	(1X10 ³)	BDL	(1X10 ³)
9/25-26	BDL	(1X10 ³)	(1X10 ²)	(low conc.)	BDL	BDL

*Bovine-specific marker: *Prevotella ruminicola*

US-BXP = Box Spring Channel @ Tesquequite Ave.

US - CYP = Cypress Channel @ Kimball Ave..

US - SACH = San Antonio Channel @ Riverside Dr.

US - ANZA = Anza Drain near confluence with Riverside Effluent Channel

US -SNCH = Sunnyslope Channel near confluence with SAR

US-SSCH = San Sevaine Channel in Riverside near confluence with SAR

BDL = below detection limit following 35 PCR cycles



Factors to Consider in the Study

- The study area is affected by multiple sources of non-point microbial contamination
- The complex nature of the study area may obscure the signal from any particular source.



Preliminary data suggests....

- Possible persistent source of human fecal pollution at Box Spring Channel during sampling events
- Highest concentration of dog marker was detected at Lower Deer Crk. and County Line Channel
- Highest concentration of the bovine marker was detected at Cypress and San Antonio Channels and at Anza Drain