

# MST 'Fly-Over'

## Terms and acronyms you'll see

- Bacteroides, coliform, E. coli, fecal coliform, fecal indicator bacteria
- Microbial source tracking (MST)
- Primer, polymerase chain reaction (PCR)
- Library dependent vs independent
- e-DNA, next generation sequencing (NGS)

## Why Microbial Source Tracking?

- Fecal bacteria in a water body can represent risk to public health and cause economic losses
- Hard to distinguish between sources of FC pollution with much certainty
- **MST -- a family of methods developed to help identify the origin(s) of fecal pollution found in samples collected from various water bodies**
- Usually means distinguishing between fecal bacteria derived from different mammals – humans, ungulates (cows and horses), dogs

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## Microbial Source Tracking

- Strategically-collect water samples and analyze them for FIB (Pollution Identification and Control programs)
- Analyze water samples for certain chemical 'tracers' - compounds whose use is linked to one or a few source organisms
- **DNA-based MST**
  - **Analyze DNA and/or RNA in water samples, especially segments or genes unique to specific organisms or groups**
  - **Analyze whole genome - identify 'community'**
- **'Library dependent' versus 'library independent'**
- **Advantages and disadvantages to each method**

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## DNA-based Microbial Source Tracking

- EPA guidance 2005; Ecology review/guidance (2011)
- Ecology/EPA R-10 recommendations for MST projects (2012)
- SoCal Coastal Water Research Program (SCCWRP) 'state of science' review (2013)
- **Studies with poor design and/or inadequate QC samples → equivocal results**
- **Few methods truly 'quantitative'**
- **Science *rapidly evolving* - new methods emerging and new publications improving knowledge of existing ones**

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## DNA-based Microbial Source Tracking

- **DNA-based approach/method depends on study objectives**
  - Identify all the crayons ('species')?
  - Identify family of colors?
  - Identify three similar colors?
  - Identify a single unique color?

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## Purpose of DNA-based MST

- Identify entire genome or specific sources?
  - Method will look at all DNA/RNA, all species present
  - Method will identify portions unique to one or a few spp
- For the latter – PCR / qPCR (p/a or relative abundances)
- PCR technology - copy machine/system
- Steps: filter water sample to collect DNA, denature = split into RNA strands, add primers and polymerase to replicate segments, repeat 30 X = 1 billion copies
  - <https://youtu.be/2KoLnlwoZKU>
- Measure presence/absence or relative quantities using known primers and local source samples

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## Needs

- Update Ecology / regional guidance on MST study design, methods, quality control
  - Study objectives and design
  - Choice of MST methods - which are most likely to be successful given objectives
  - Case studies with lessons learned
- Follow with substantial effort to raise awareness of public/stakeholders as well as different regulators

Questions?