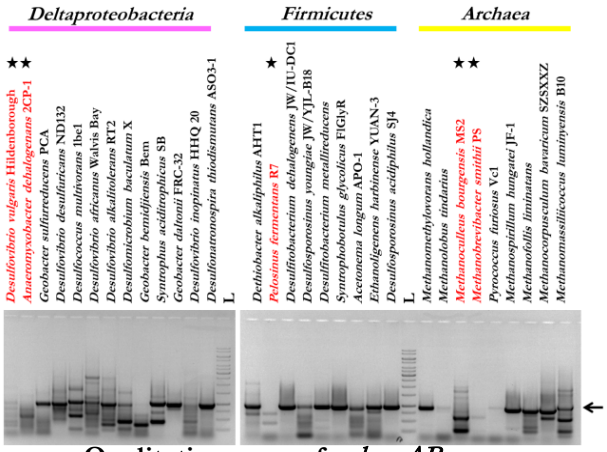
Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment.

- **Challenge:**
  - Design and optimize protocols to determine the presence and abundance of mercury methylating microorganisms.
  - Validate protocol on genomic DNA isolated from pure cultures and environmental samples.



Qualitative screen for *hgcAB*
(arrow denotes expected band)

- **Approach and Results:**
  - Designed unique primer sets from 80+ *hgcAB*$^{+}$ genomes.
  - Developed a qualitative screening (via PCR) to determine the presence and diversity of *hgcAB* microorganisms (94% confirmation rate of 31 microorganisms).
  - Developed a quantitative approach (via qPCR) to determine abundance of *hgcA* from three dominant mercury methylating clades (*Deltaproteobacteria, Firmicutes,* and *methanogenic Archaea*).
  - Validated protocols with an environmental proof-of-principle study using samples collected from the Oak Ridge East Fork Poplar Creek mercury contaminated site.
  - *hgcAB*$^{+}$ microorganisms were determined to be present, in agreement with known mercury methylation activity and previous ORNL Hg SFA publications.

- **Significance and Impact:**
  - Simple, quick and cost-effective protocol developed that can be used in any molecular laboratory.
  - Procedure can be used to determine Hg-methylation potential for risk assessment.

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Reference: *Christensen et al.* 2016. *Applied and Environmental Microbiology.* DOI:10.1128/ AEM.01271-16