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.

**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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