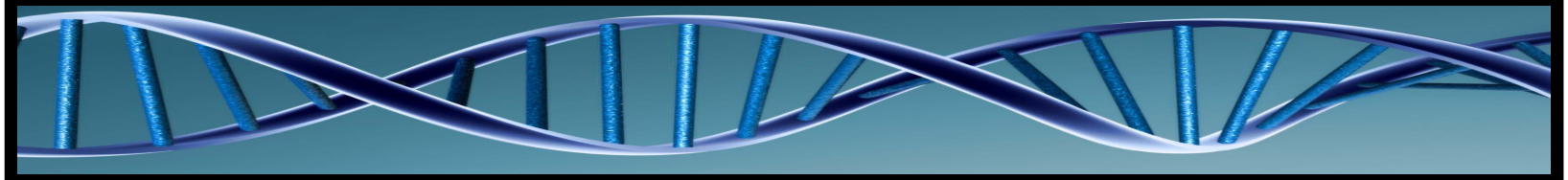
Application of molecular tools for routine water quality monitoring



MWQI Technical Meeting - May 27, 2015

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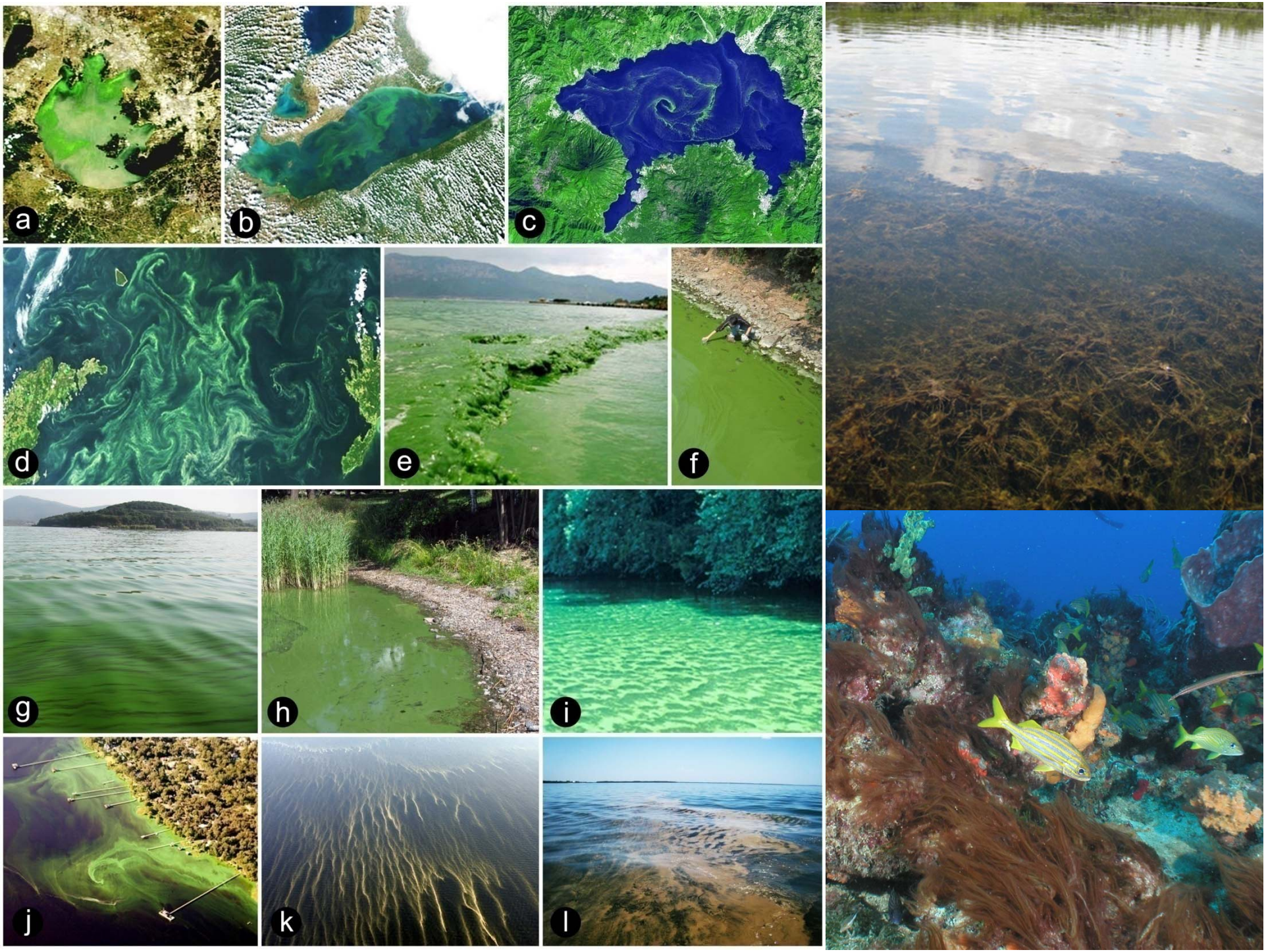
Overview of presentation contents

- Brief background on CyanoHABs, sample collection/processing and genetic tools for their study
- Application of each method (case studies):
  - Comparison of QPCR vs. microscopy for routine monitoring
  - Metagenomics as a roadmap for lake monitoring
    - Elucidate microbial community structure & function
    - Development and validation of a geosmin QPCR assay
- Goal is to be able to use this information to more effectively manage our aquatic resources and to protect human and environmental health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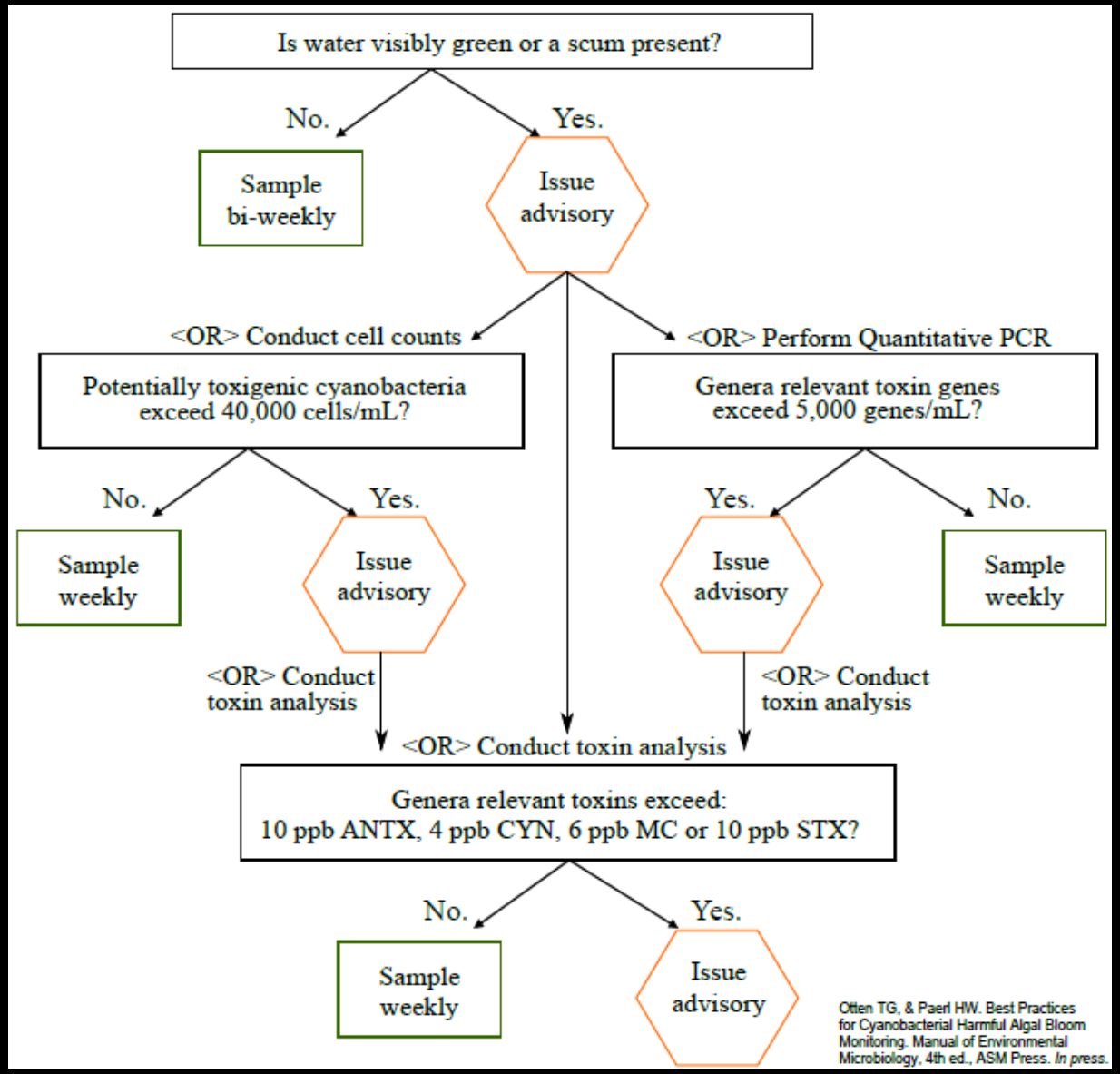
Sample collection

- Collection method varies by need
  - Public health - collect scum from most impacted site
  - Environmental - off-shore, depth integrated (1-2 SD)
  - Collect 0.5L in sterile plastic bottles (HDPE-2 is best)
  - Store on wet ice in the dark until processing
    - Don't want it to freeze which will lyse the cells
- Goal is to identify harmful/nuisance organisms before they reach problematic concentrations
- Adaptive management guides sampling frequency

Development of a tiered management approach to CyanoHAB monitoring

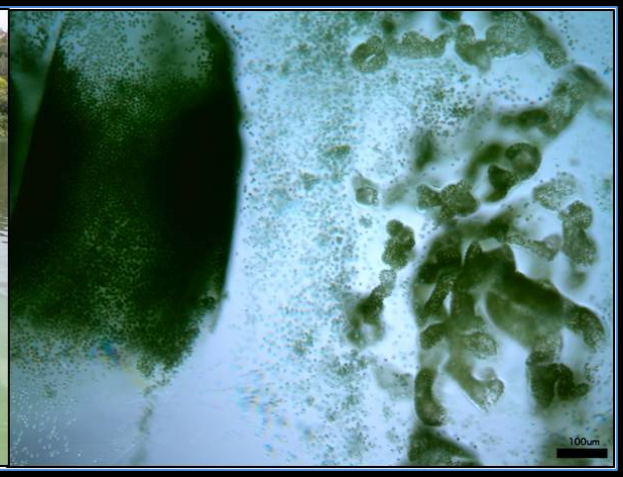
WHO guidelines

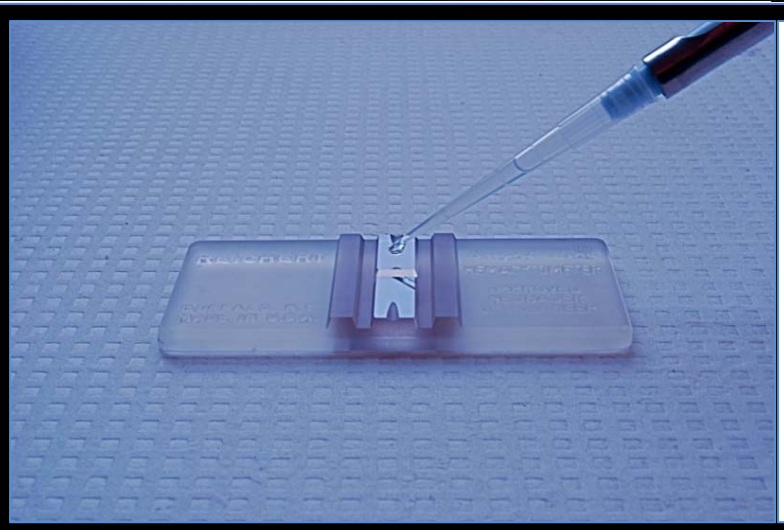


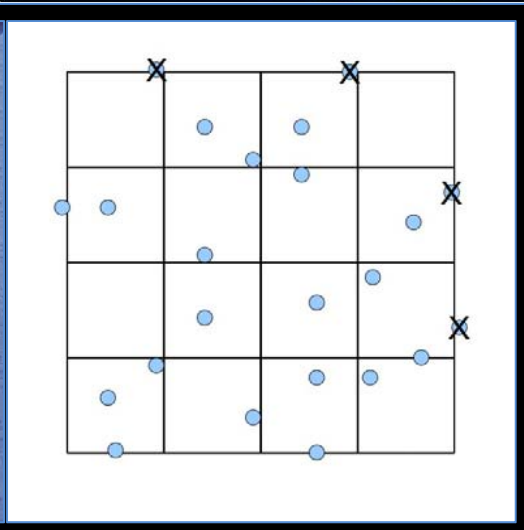
Experimentally derived

Varies by state

Phytoplankton enumeration by microscopy







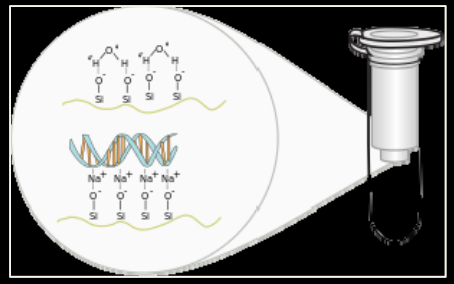
Pros: Can inventory all phytoplankton, equipment/materials costs are low

Cons: Hard to accurately count amorphous colonies, cannot distinguish toxic from nontoxic cells, relatively expensive and slow

~$125-$350/sample

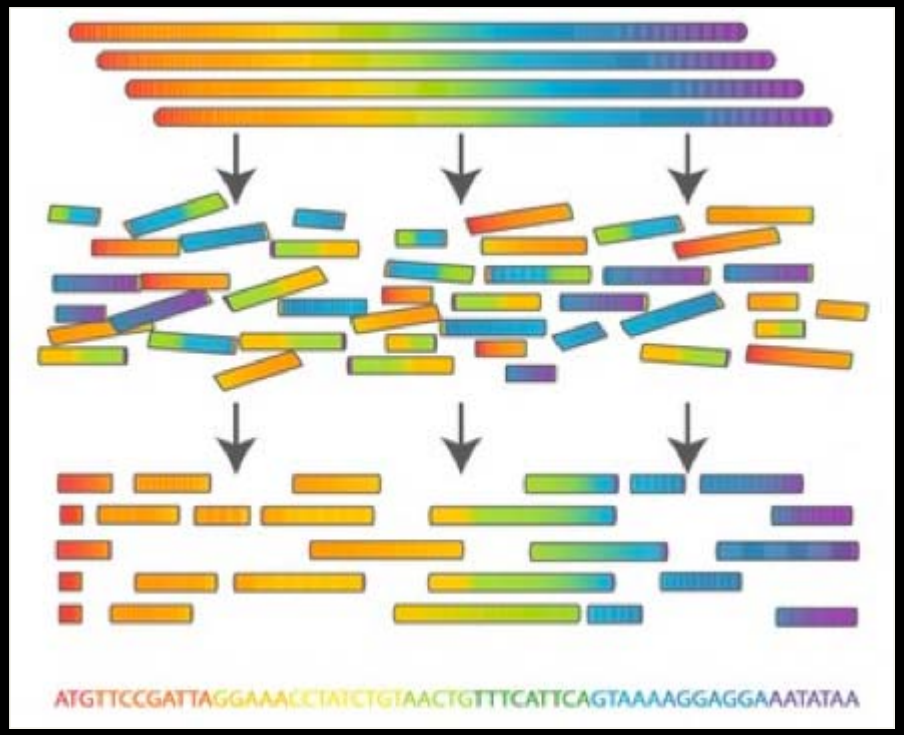
DNA extraction and processing

1. Concentrate sample



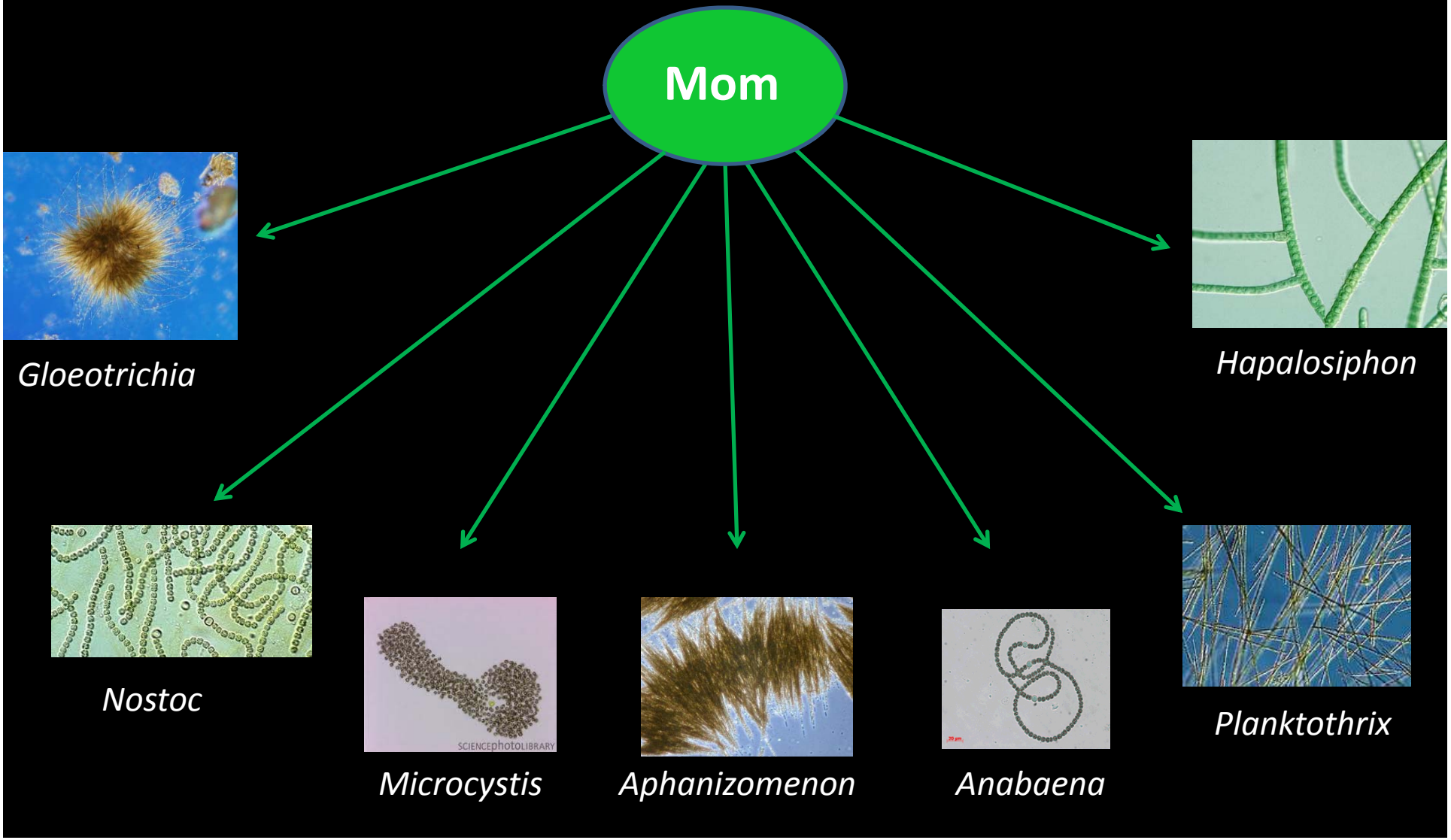
2. Extract DNA

3. DNA is now ready for downstream applications such as PCR/QPCR or sequencing

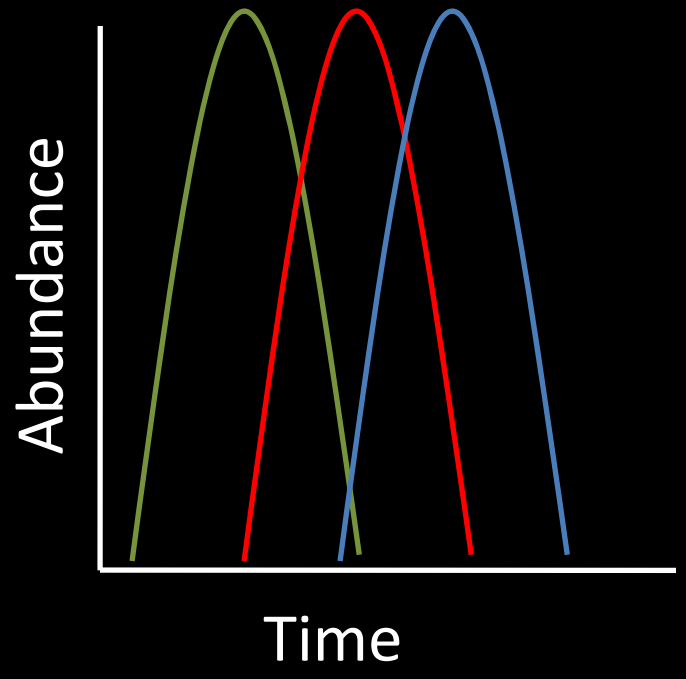


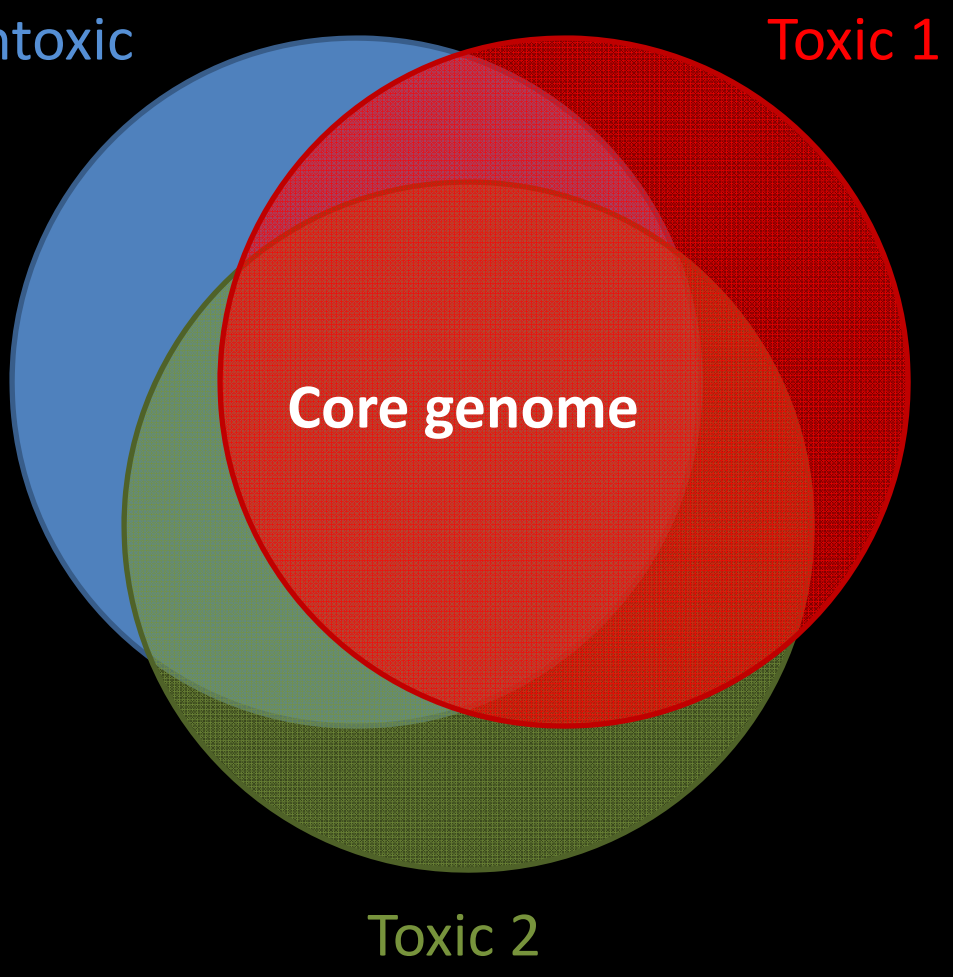
*Store filters at ≤ -20°C until DNA extraction

Descent from common *mcy*$^{+}$ ancestor

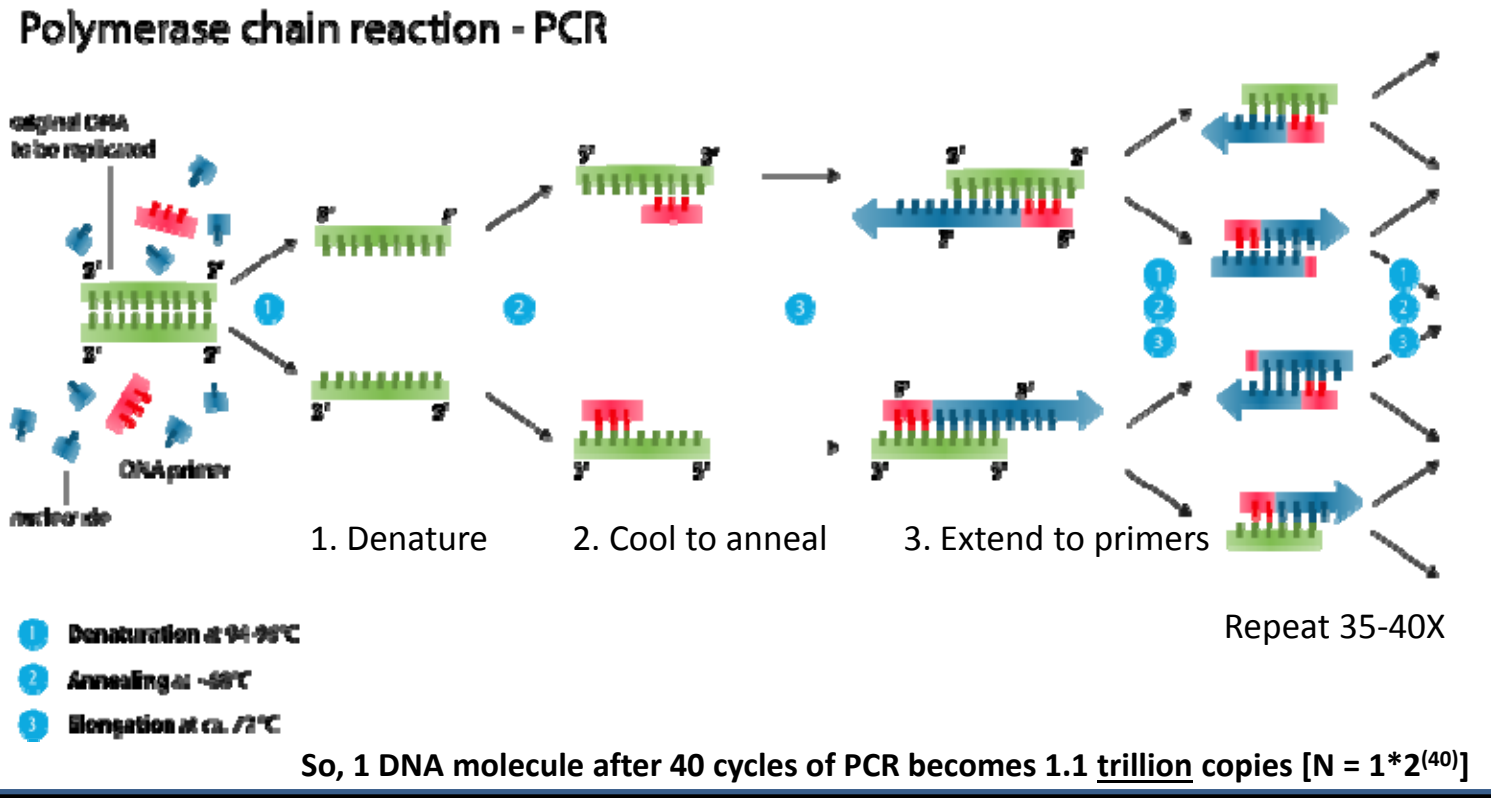


Like many genes, toxicity and T&O are strain specific traits (not genus/species)

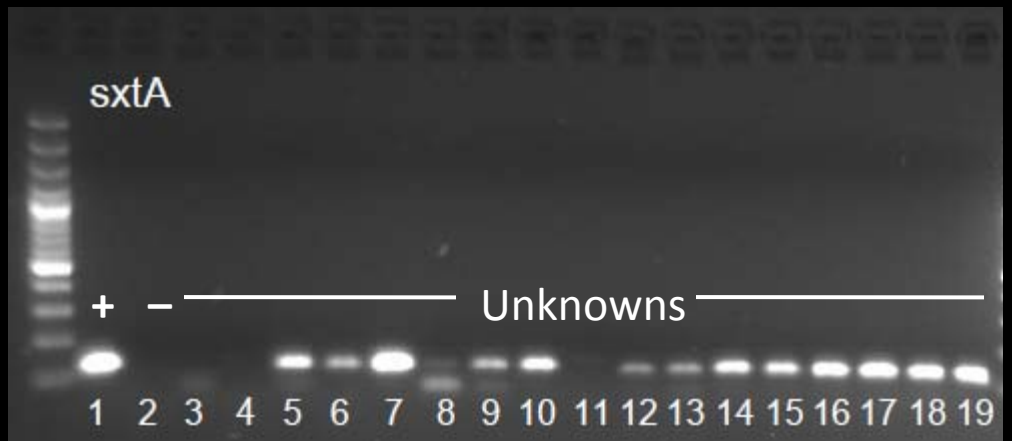




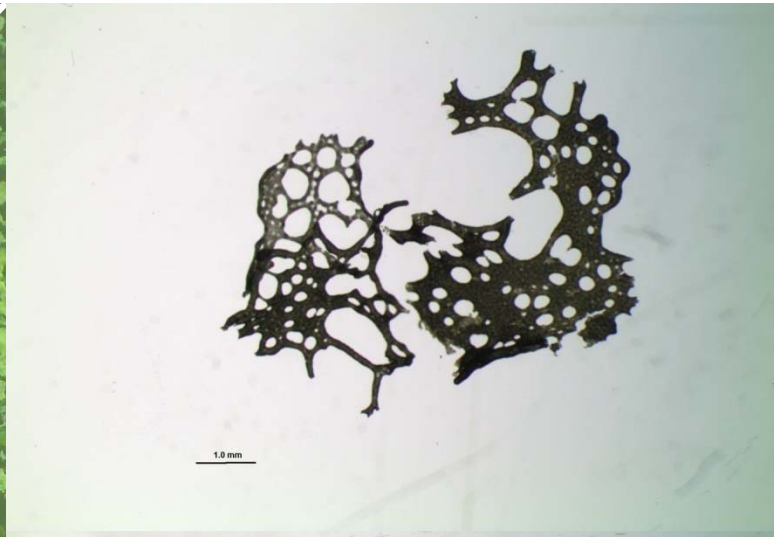
Polymerase Chain Reacion (PCR)



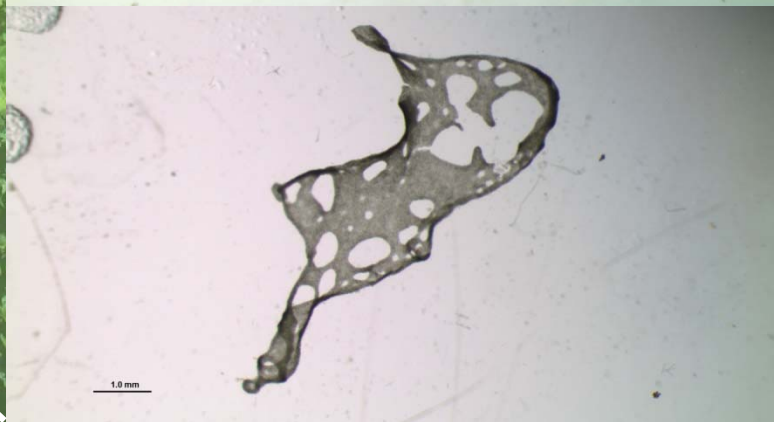
So, 1 DNA molecule after 40 cycles of PCR becomes 1.1 <u>trillion</u> copies [N = $1*2^{(40)}$]



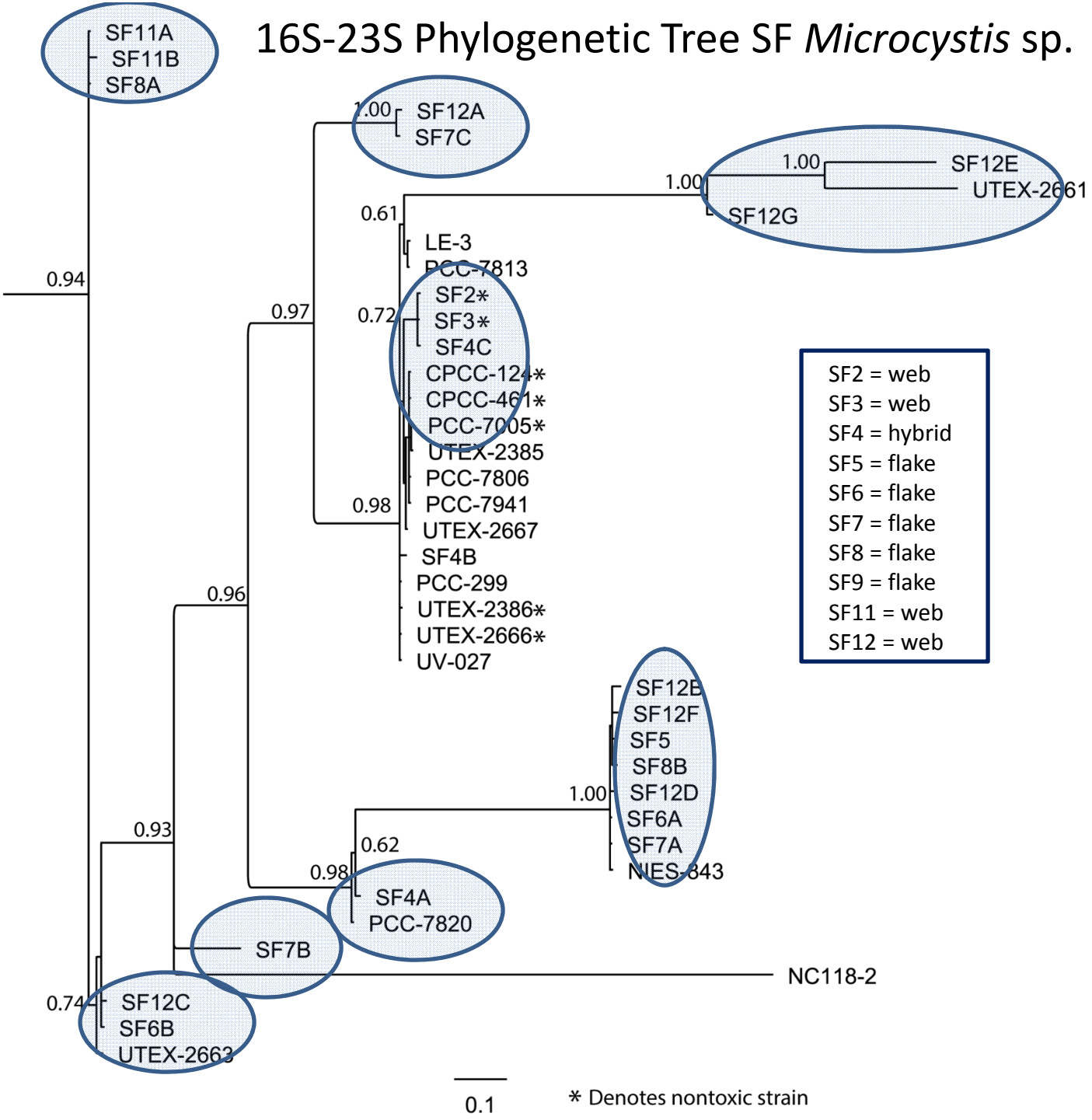
Amplification products can be visualized under UV light after gel electrophoresis


1.0 mm

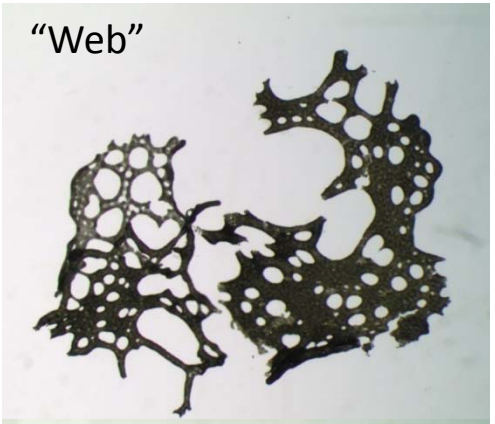

1.0 mm

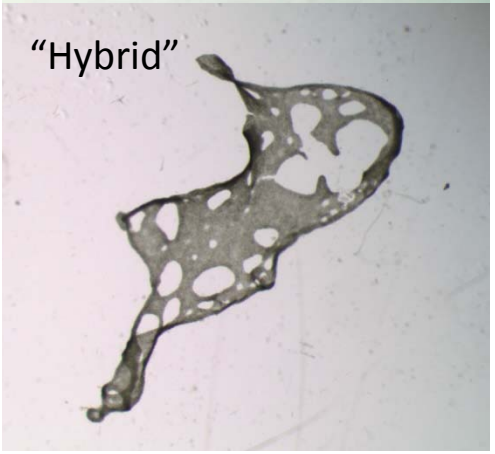

1.0 mm

16S-23S Phylogenetic Tree SF *Microcystis* sp.

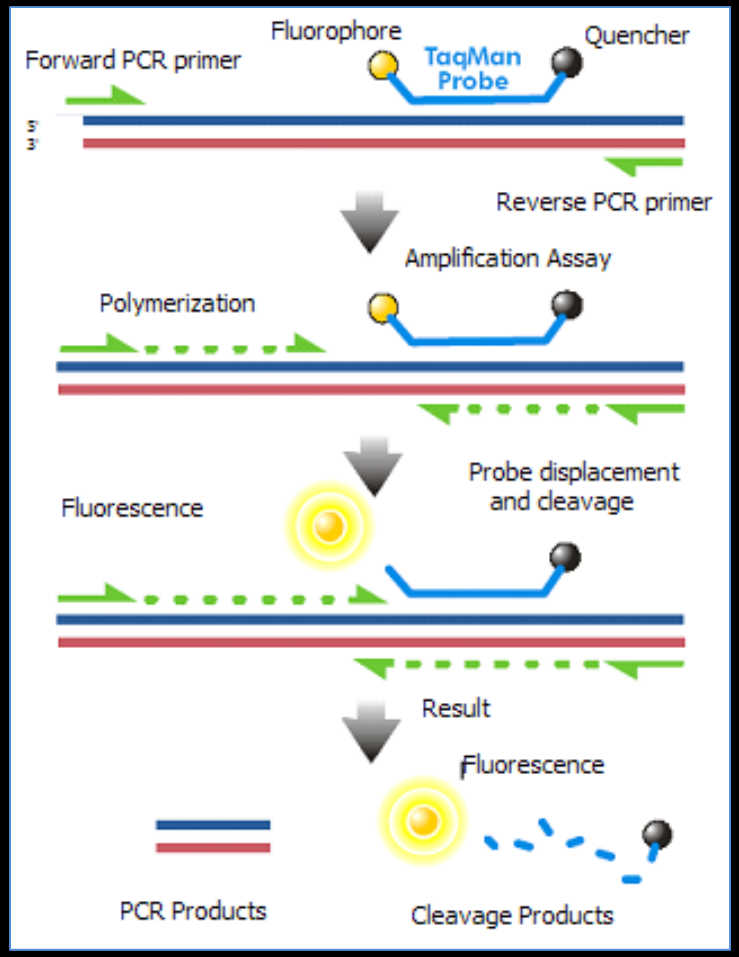


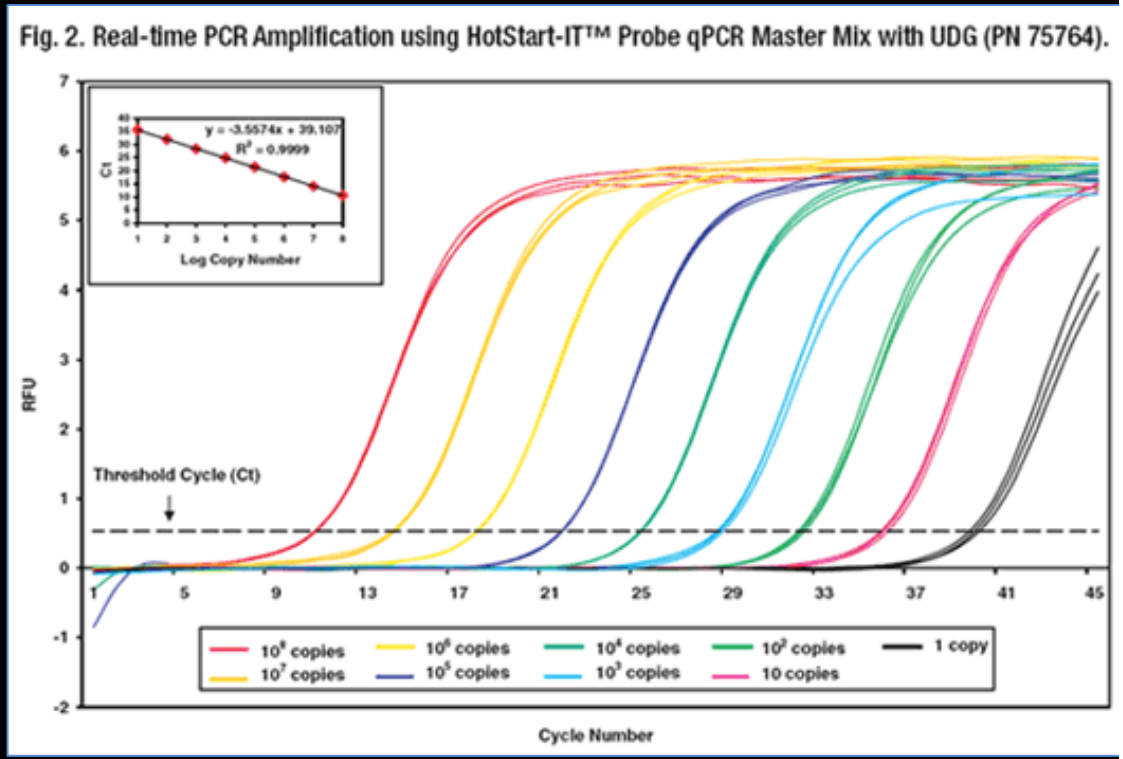
SF2 = web
SF3 = web
SF4 = hybrid
SF5 = flake
SF6 = flake
SF7 = flake
SF8 = flake
SF9 = flake
SF11 = web
SF12 = web





Overview of real-time quantitative PCR (QPCR)

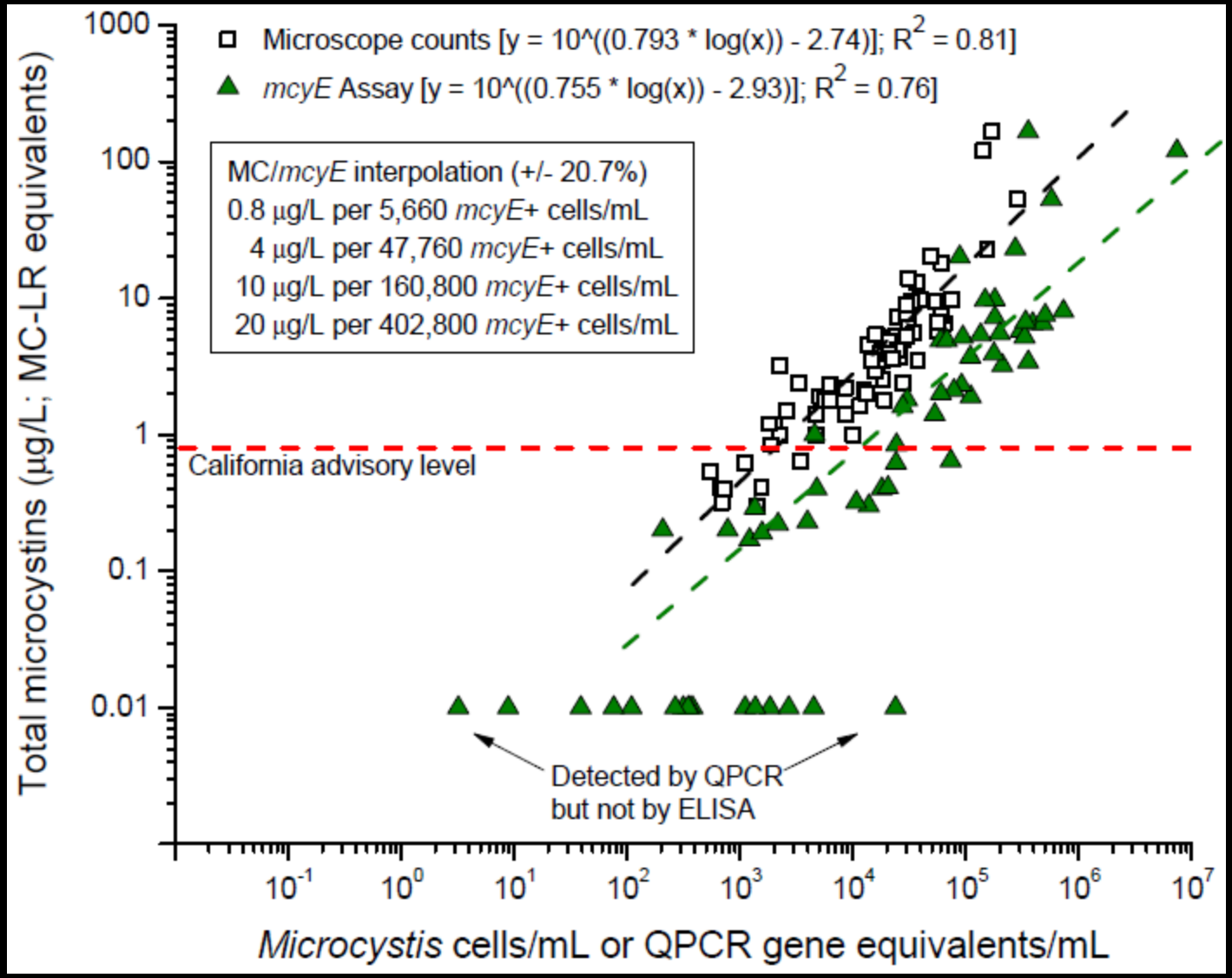




DNA extraction = $20
QPCR/sample/assay = $30

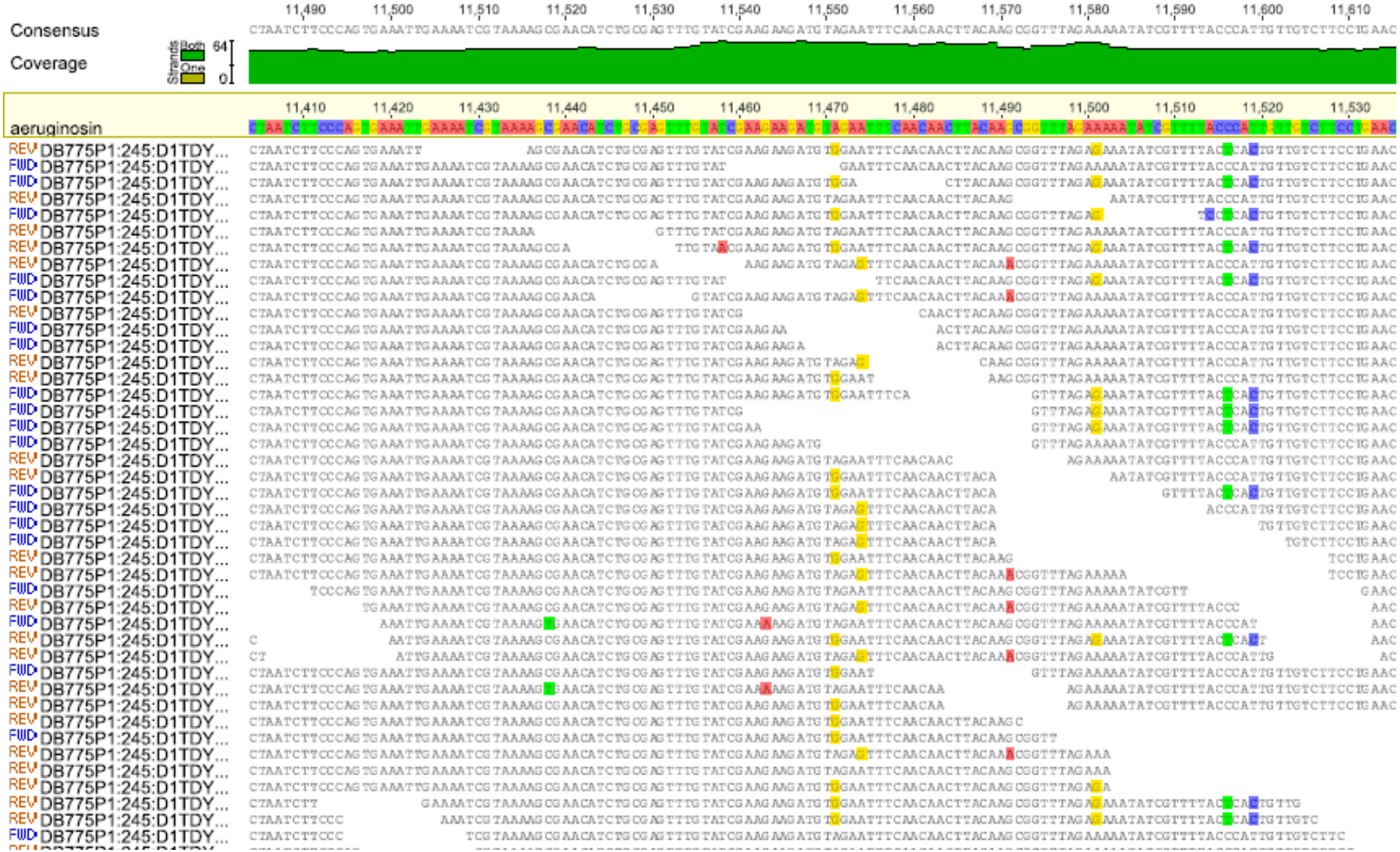
Pros: Provides earlier warning detection of nuisance organisms than direct measurements of toxins or cell counting, robust predictor of toxicity and T&O, no user objectivity as in cell counting and facilitates high throughput sample processing.
Cons: Relatively expensive equipment and relatively technical to conduct

Comparison of microscopy and QPCR for estimating cyanotoxin risks

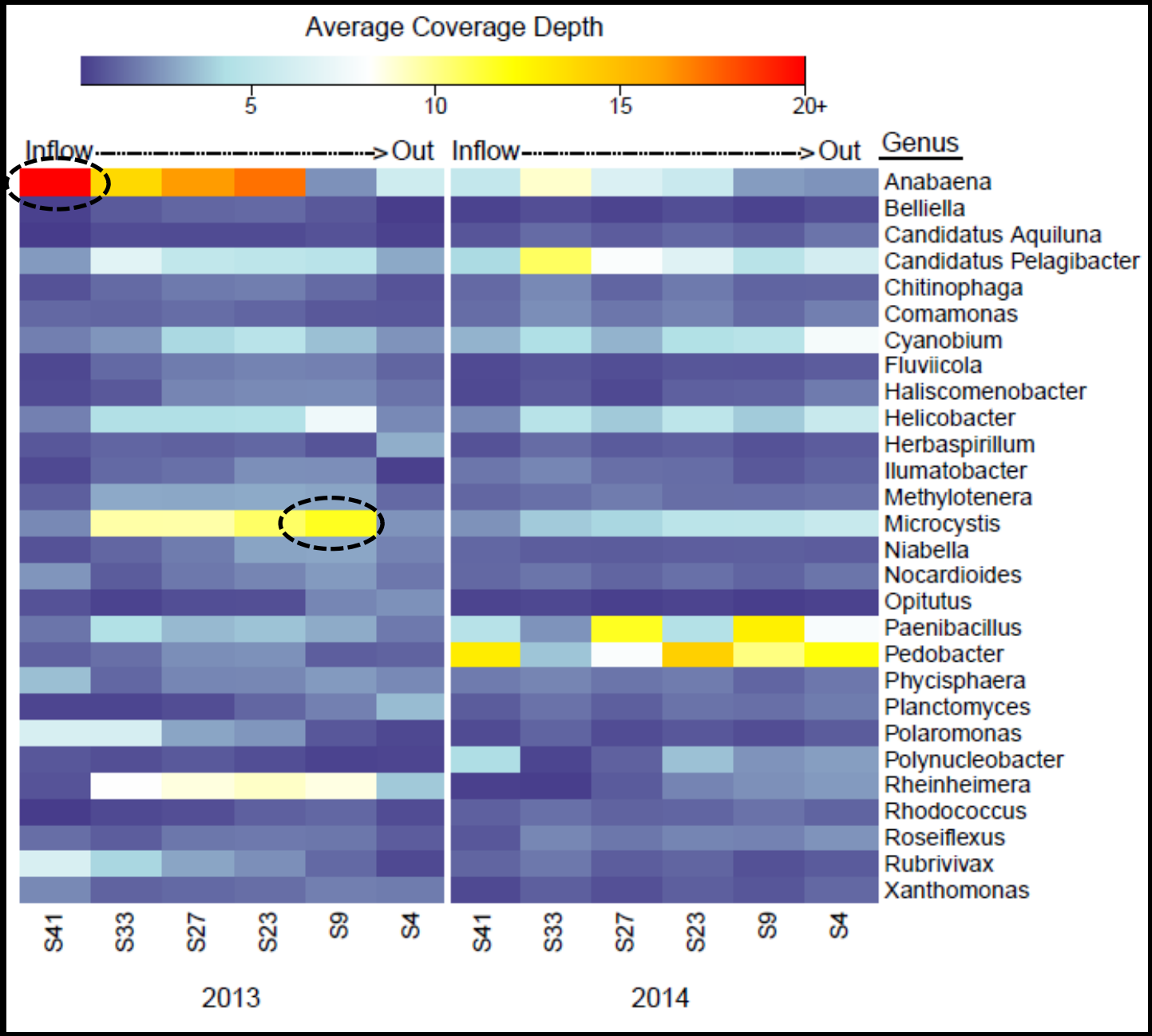


Otten et al., 2015. Harmful Algae, *In press*.

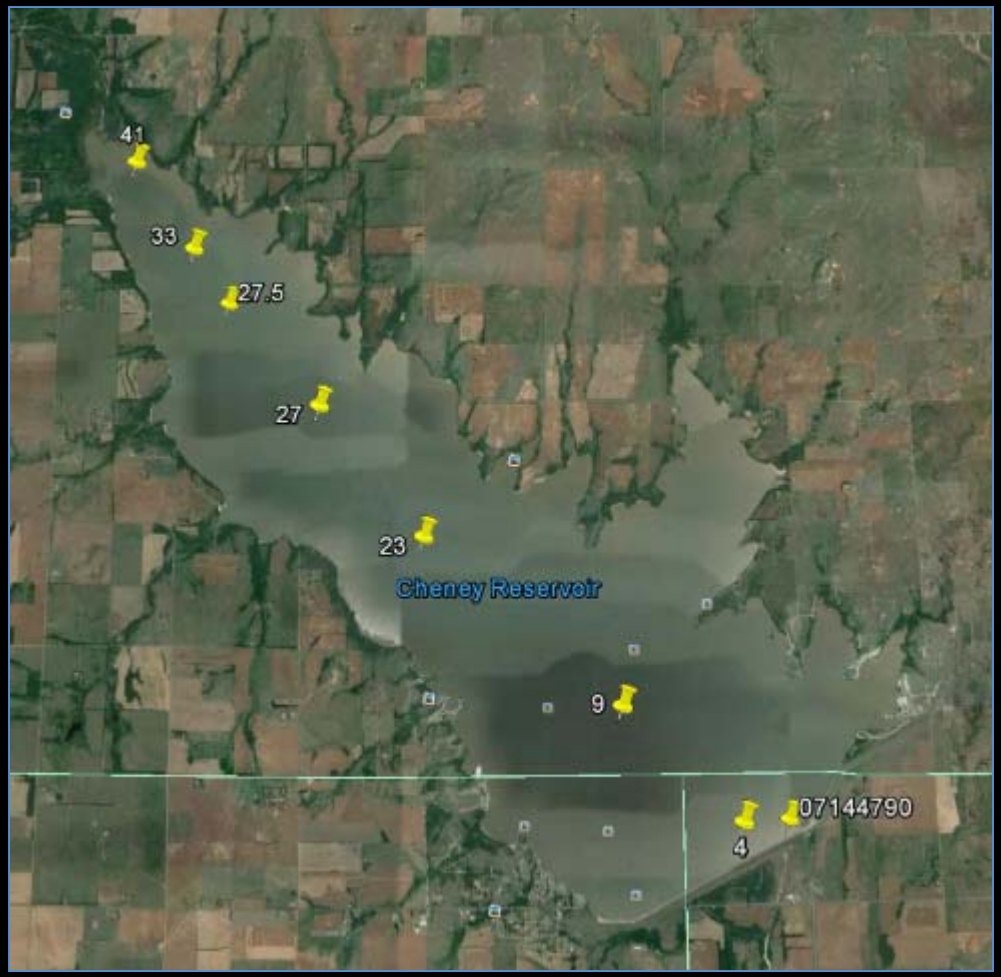
Shotgun metagenomics for assessing microbial community structure and physiological potential



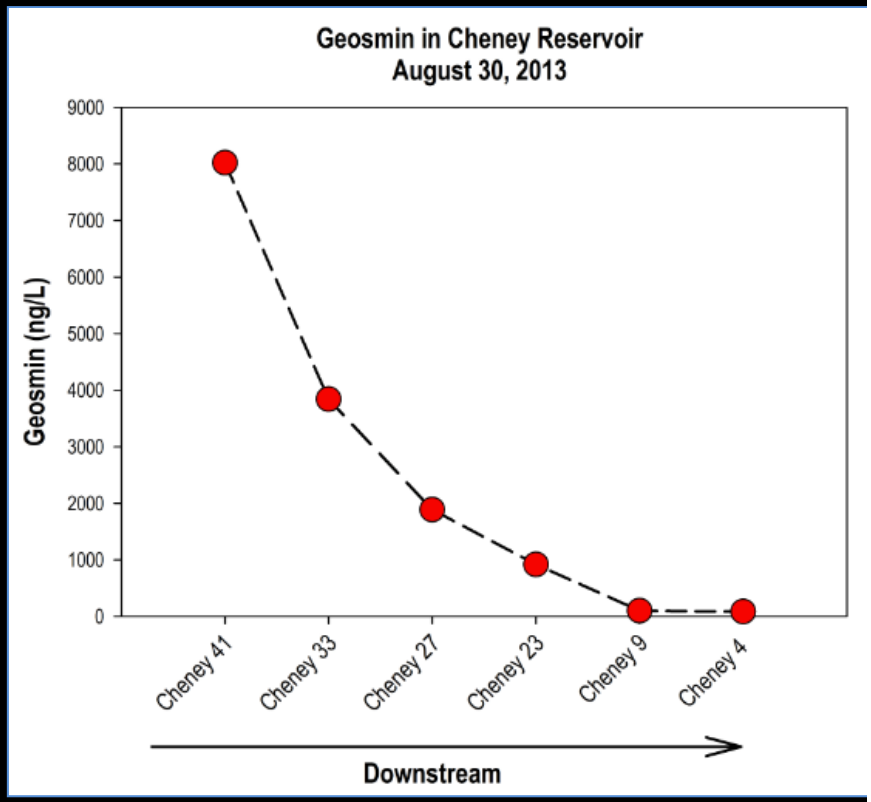
Assessment of microbial diversity and abundance



2013 transect caught a major geosmin event

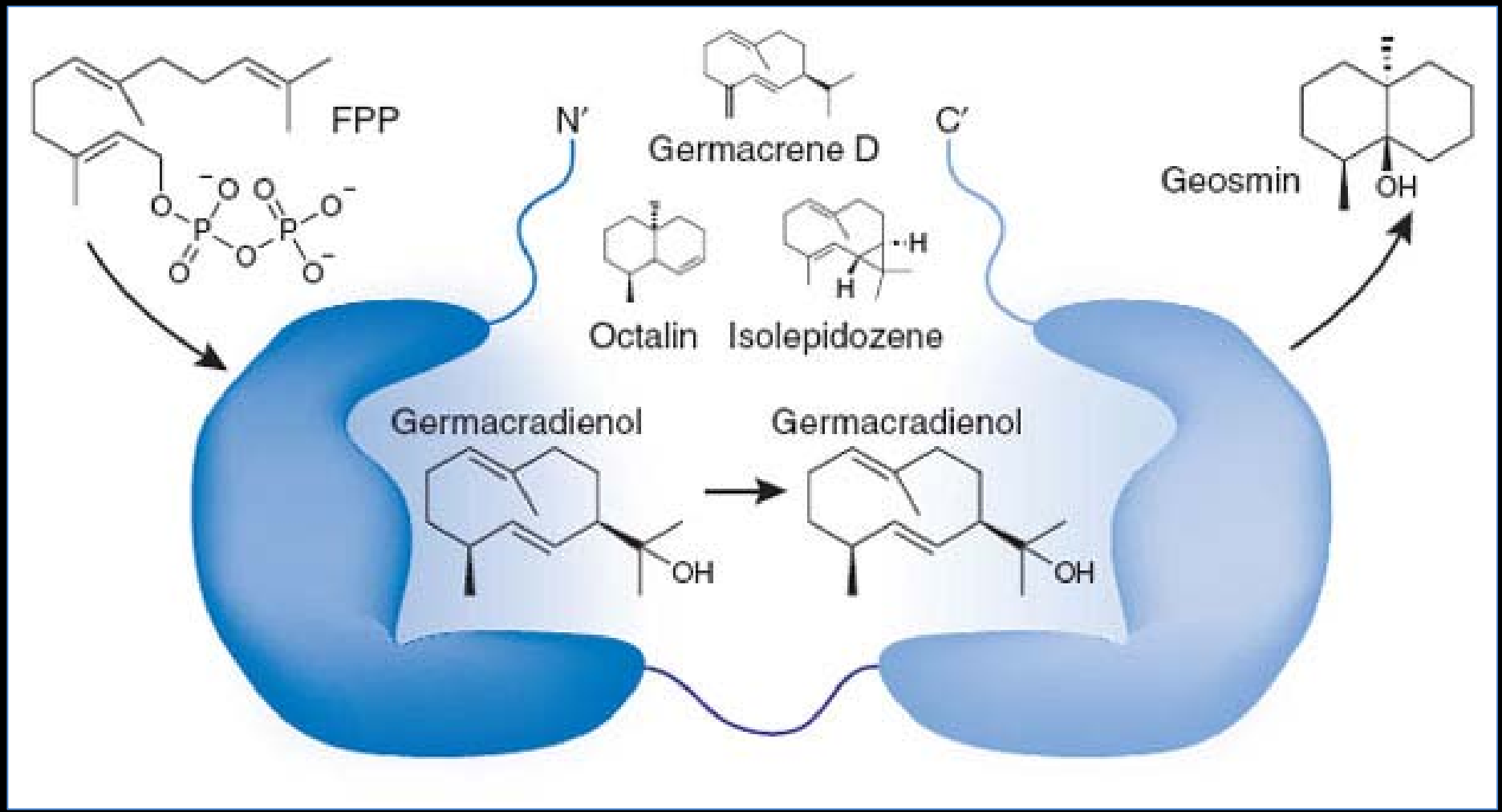


Cheney Reservoir: Wichita, Kansas

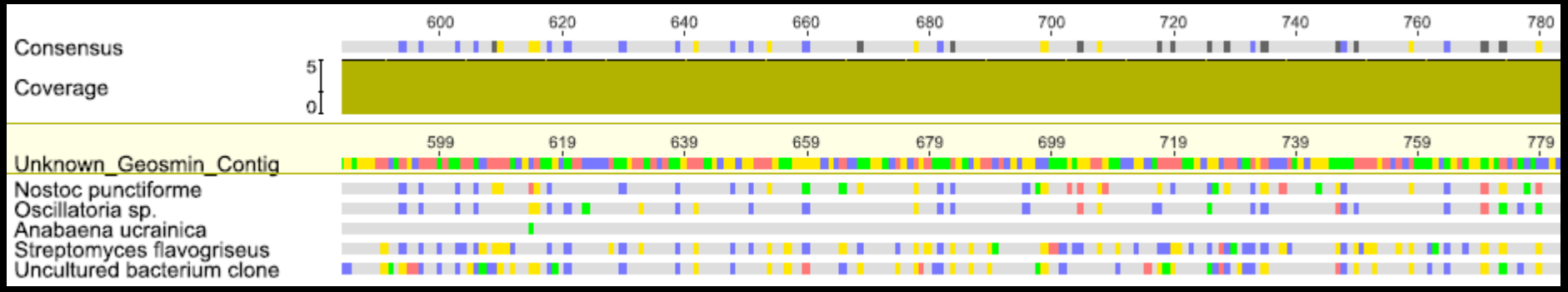


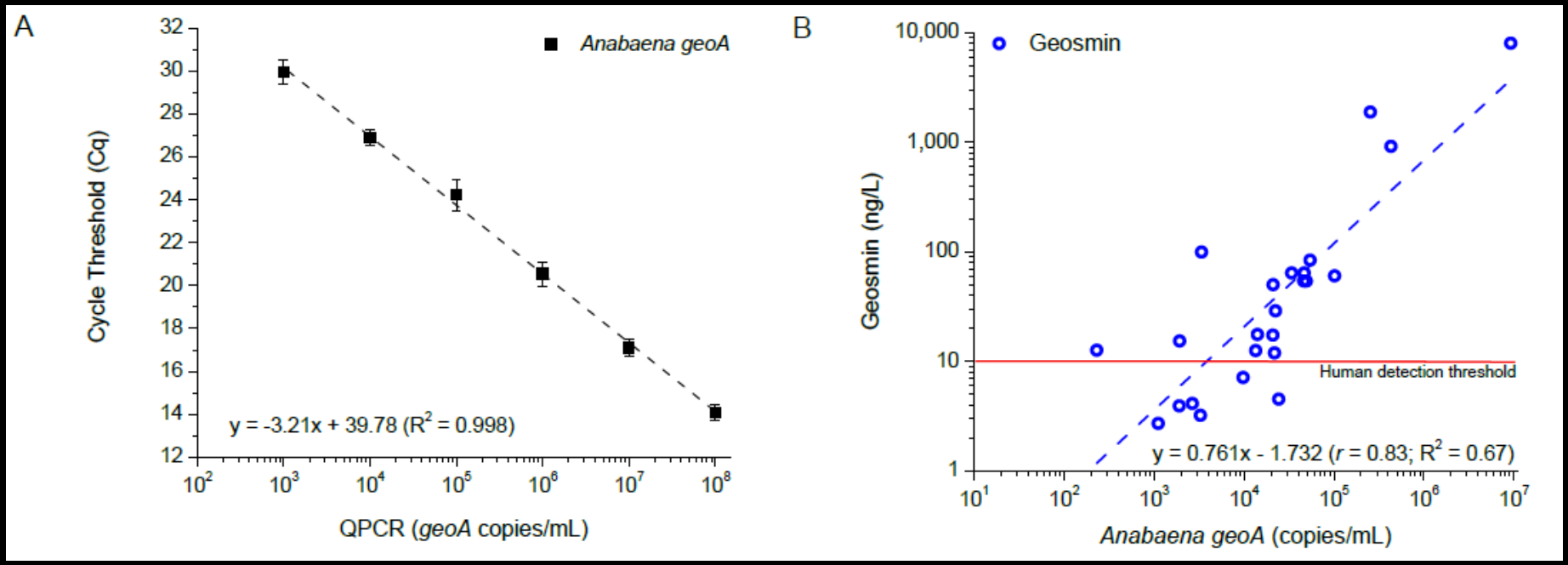
Human perception of geosmin is 4-10 ng/L

Geosmin synthase



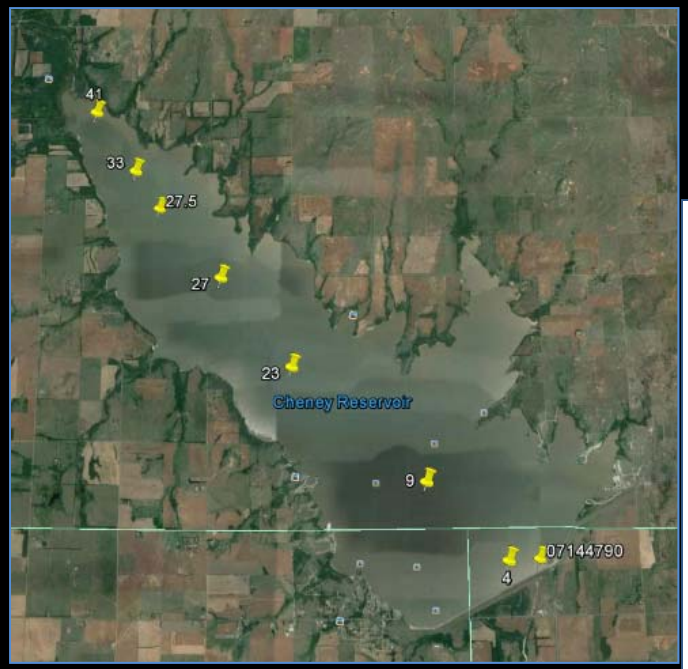
Development of a QPCR assay for geosmin detection

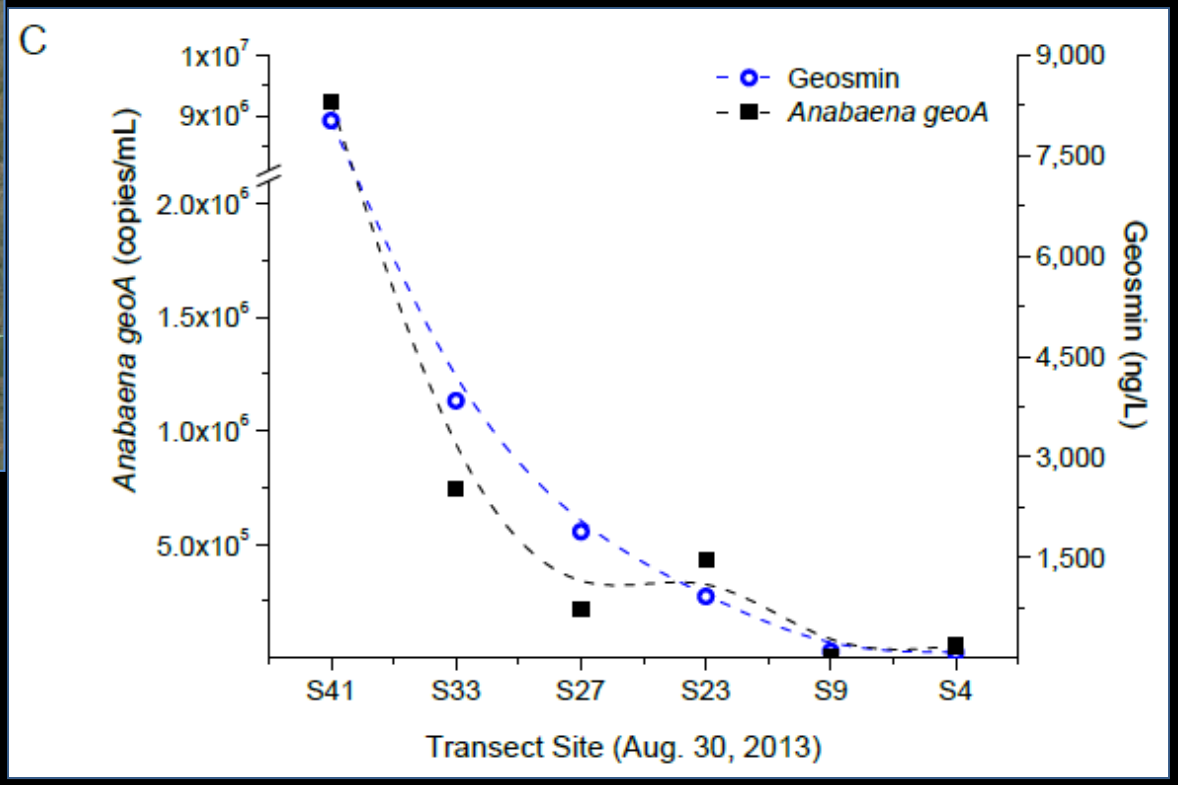




Otten et al., *In prep*.

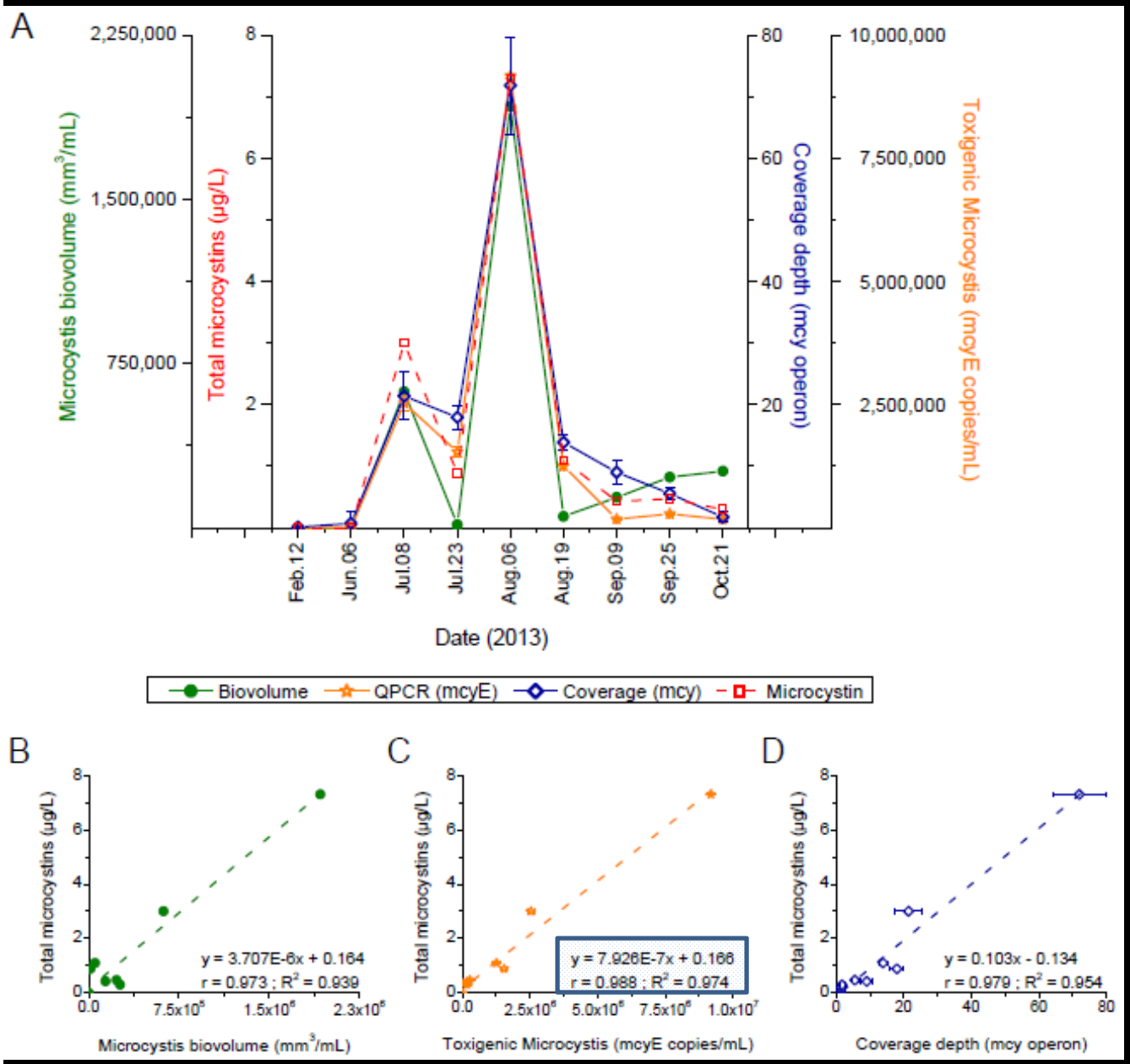
Application of the new QPCR assay to the 2013 geosmin event





Otten et al., *In prep*.

Inference of MCs by microscopy, QPCR and metagenomics

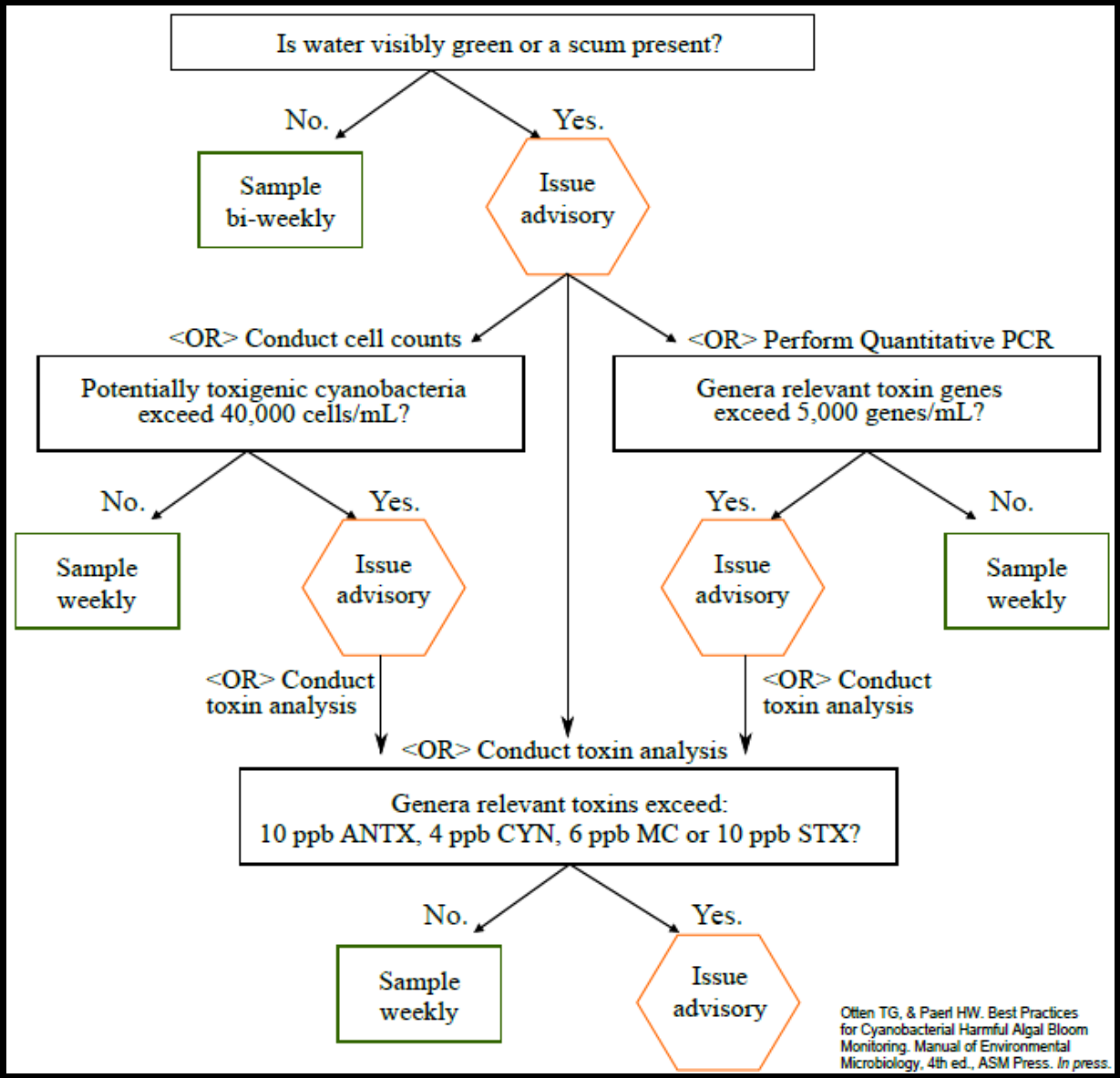


A) Time-series of a Midwestern reservoir showing microcystin (MC-LR eq.), *Microcystis* biovolume, toxigenic *Microcystis* by QPCR and shotgun metagenomics.

B-D) Linear regressions for each method relative to total microcystin.

Otten et al., *In prep*.

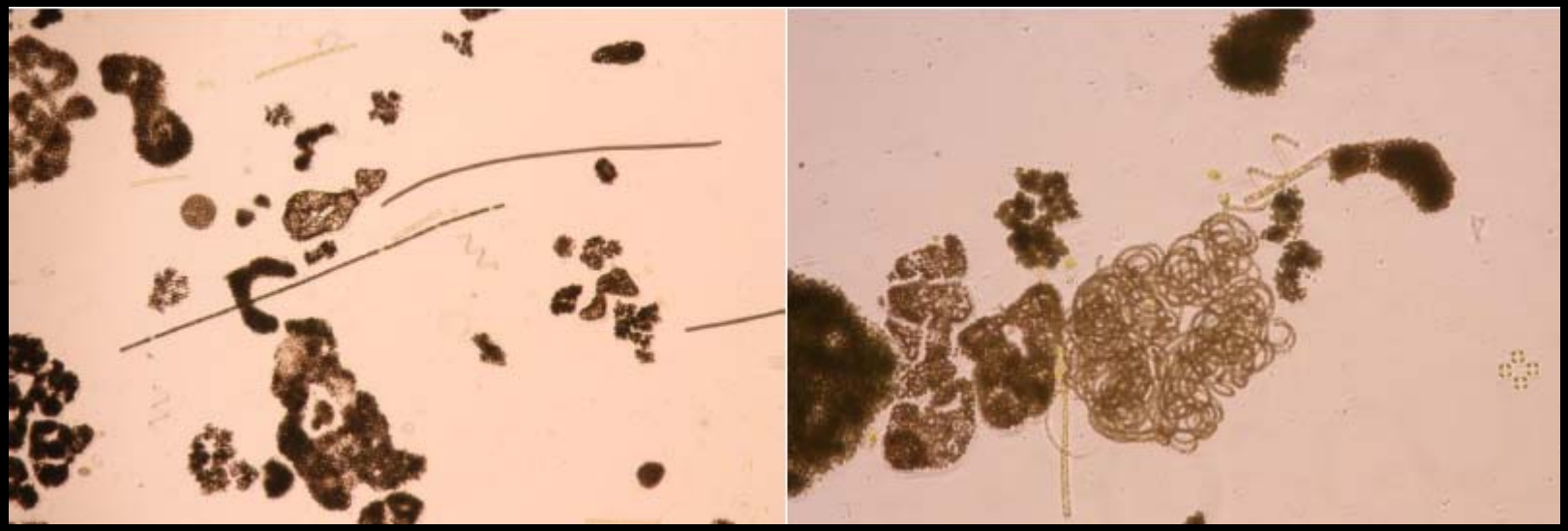
Proposed method for CyanoHAB monitoring



1. Conduct bi-weekly visual assessments of water.
2. Collect samples when algae becomes visible or bi-weekly during peak months (e.g., May – Oct)
3. Use QPCR to quantify potential toxin producers, if these > ~5,000 toxin genes/mL conduct appropriate ELISA

Rationale: DNA based methods are cheaper ($50/sample/assay) and higher throughput than direct measurements of toxins ($75/sample/assay) and because most samples may contain low/no toxicity.

Thanks for your attention!



Please feel free to contact me with any questions.

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