Print version

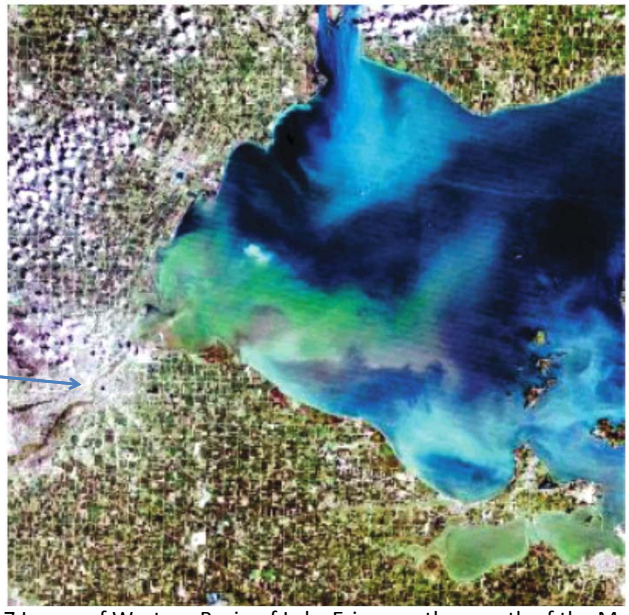
CEE 697z

Organic Compounds in Water and Wastewater

Cyanotoxins
qPCR Method
Prepared and presented by Kristie Stauch-White

Lecture #29

qPCR Quantification of Microcystis during Lake Erie Blooms in 2003 and 2004



LANDSAT 7 Image of Western Basin of Lake Erie near the mouth of the Maumee River

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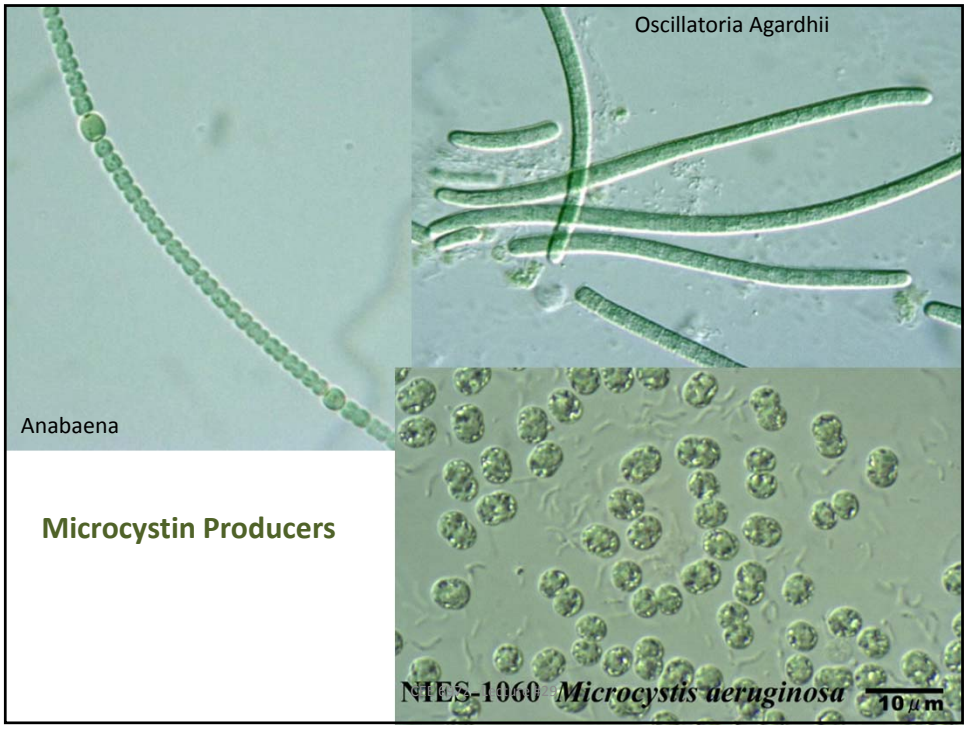
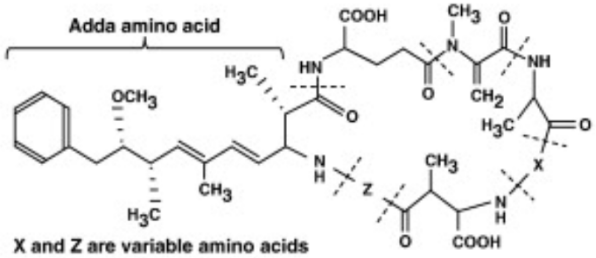


Microcystin Producers

Cyanobacterial Hepatotoxins

Microcystin



Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E. and Thomas, O. (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment International 59, 303-327.

BIOASSAYS TO DETECT CYANOTOXINS

- Animal Bioassays
- Immunoassays:
  - ELISA
  - PPIA
- qPCR

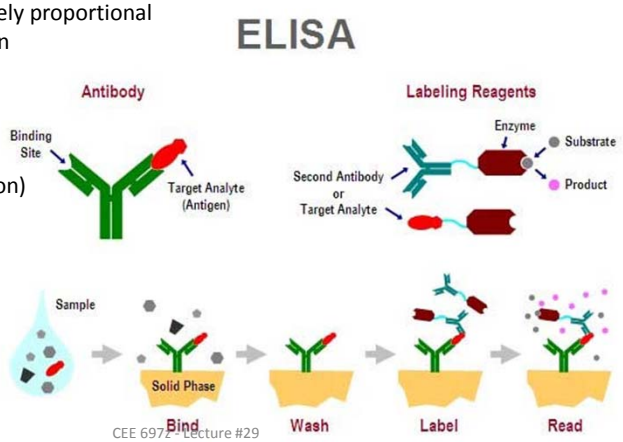
ELISA - Enzyme Linked Immunosorbent Assay

- Lowest Detection Limit .05 ppb to .5 ppb

Direct Competitive ELISA using Polyclonal Antibody

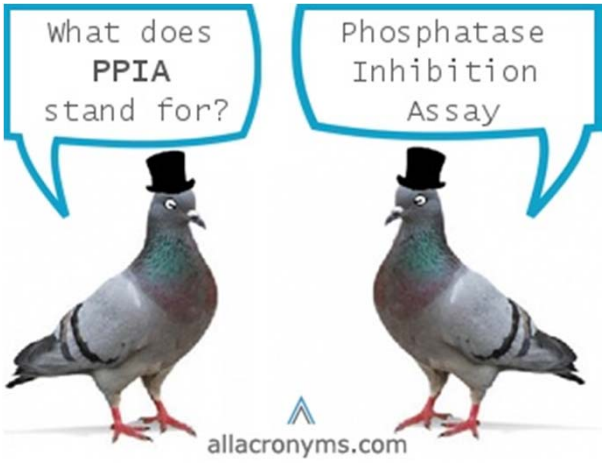
- Anti-Microcystin antibody attached to a high binding capacity microtiter plate
- MCYST-LR is used as a standard
- Microcystin-LR-peroxidase used to compete with MCYST-LR for the binding site of the antibody on the microtiter plate.
- Color developed inversely proportional to MCYST concentration

(lighter color = higher MCYST concentration)



http://www.jove.com/science-education/5061/the-elisa-method

PPIA



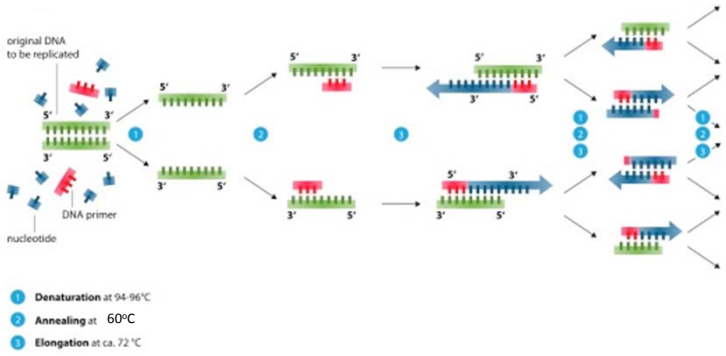
PPIA (Phosphatase Inhibition Assay) – How it works:

- MCYSTs and NODLNs – naturally potent inhibitors of protein serine and threonin phosphatases (PP1 and PP2A)
- Protein phosphatases dephosphorylate p-nitrophenylphosphate (pNPP) – commonly used substrate for alkaline phosphatases
- Colorimetric
- Detection limit similar to ELISA (1 – 20 µg/L)
- Advantage over ELISA – ability to detect bioactivity in MCYSTs and NODLNs – therefore based on functional activity rather than structure

Primers:

mcyB 2959F	TGGGAAGATGTTCTTCAGGTATCCAA	26 bp each,
mcyB 3278R	AGAGTGGAAACAATATGATAAGCTAC	PCR product 60 – 1600 bp

Polymerase Chain Reaction - PCR



Wikipedia

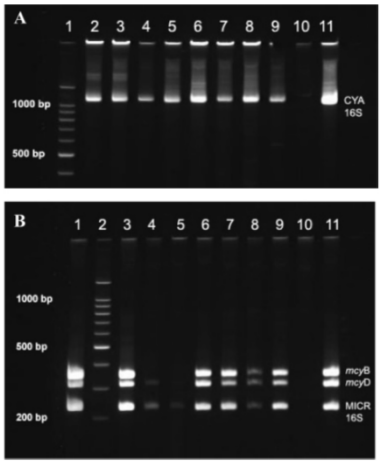


FIGURE 3. Gel image of multiplex PCR results. Gel A: detection of cyanobacterial 16S rDNA fragment. Lane 1: 100 bp molecular weight marker; lane 2: site 1a; lane 3: site 1b; lane 4: site 2; lane 5: site 3; lane 6: site 4; lane 7: site 5; lane 8: site 6; lane 9: site 7; lane 10: negative control, no template DNA; lane 11: positive control (*M. aeruginosa* LE-3 genomic DNA). Gel B: detection of *Microcystis* spp. 16S rDNA fragment and microcystin synthetase genes *mcy*B and *mcy*D. Lane 1: site 1a; lane 2: 100 bp molecular weight marker; lane 3: site 1b; lane 4: site 2; lane 5: site 3; lane 6: site 4; lane 7: site 5; lane 8: site 6; lane 9: site 7; lane 10: negative control, no template DNA; and lane 11: positive control (*M. aeruginosa* LE-3 genomic DNA).

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TABLE 3. Chlorophyll *a* and Toxin Concentrations in Samples Collected in the Western Basin of Lake Erie in August 2003 (Sample Stations Numbered 1–7) and 2004 (Sampling Stations Numbered with 3 or 4 Digits)[a]

sampling station	chlorophyll *a* (μg L^{-1})	microcystin (μg of microcystin LR equiv L^{-1})
1	40.0	15.4
2	5.2	<0.3
*3	6.4	0.3
4	4.0	<0.3
5	15.3	0.4
6	26.0	1.8
7	6.5	<0.3
493	8.8	0.1
311	14.1	0.3
1163	20.1	2.6
885	19.1	0.1
496	21.7	0.4
495	15.5	0.4
494	12.8	0.3
357	7.4	0.1
974	7.8	1.0
882	8.3	0.04

[a] Microcystin concentrations are in microcystin–LR activity equivalents per liter. Detection limits are controlled by the volume of water filtered (2003, ~1 L; 2004, ~20 L).

*Toxin detected, but toxin-producing genes not detected at this site

TABLE 4. Initial Screening of Water Samples using Multiplex PCR Assays[a]

sampling site	Cyan 16S	Micr 16S	*mcy*B	*mcy*D
1	+	+	+	+
2	+	+	–	+
*3	+	+	–	–
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
493	+	+	+	+
311	+	+	+	+
1163	+	+	+	+
885	+	+	+	+
496	+	+	+	+
495	+	+	+	+
494	+	+	+	+
357	+	+	+	+
974	+	+	+	+
882	+	+	+	+

[a] The columns are labeled with the PCR primers used for the analysis. The presence or absence of a visible band in the gel after staining with SYBR green I is indicated by + or –.

* Neither toxin producing gene found at this site, but microcystin detected (see Table 3)

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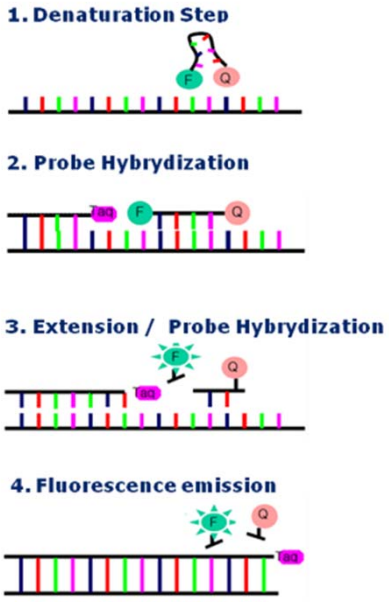
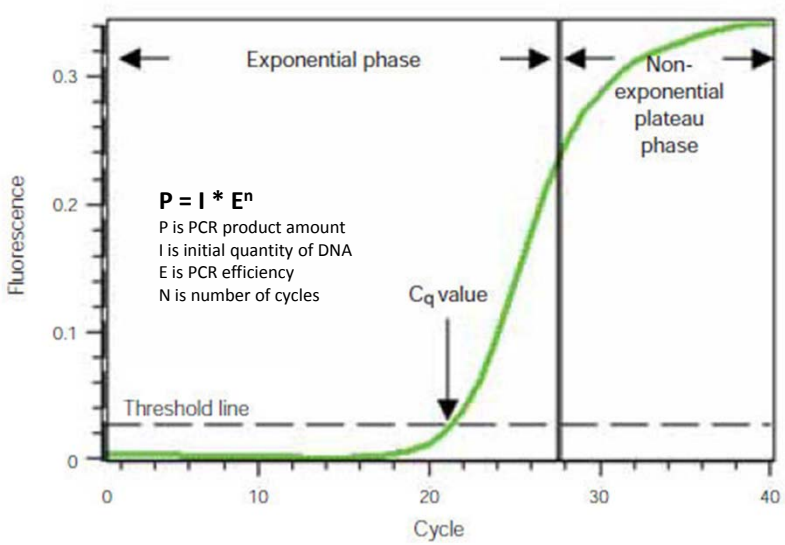
qPCR Fluorescence Emission



Applied Biosystems


Exponential phase
Non-exponential plateau phase
P = I * E^n
P is PCR product amount
I is initial quantity of DNA
E is PCR efficiency
N is number of cycles
Cq value
Threshold line
Fluorescence
Cycle
0
0.1
0.2
0.3
0
10
20
30
40

Bio-Rad

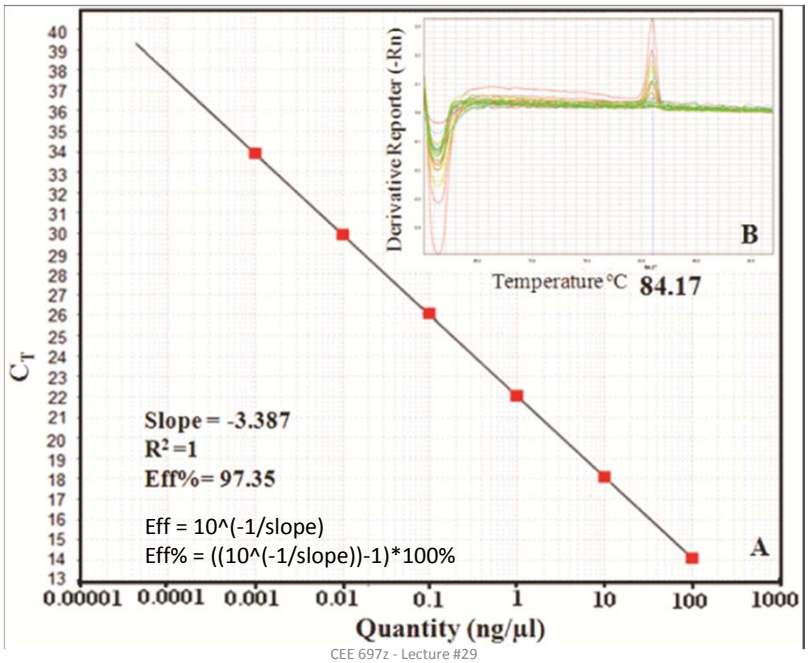

Derivative Reporter (-Rn)
B
Temperature °C 84.17
Slope = -3.387
R² =1
Eff%= 97.35
Eff = 10^(-1/slope)
Eff% = ((10^(-1/slope))-1)*100%
A
C_T
Quantity (ng/µl)
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000

TABLE 5. Real-Time PCR-Based Quantification of Abundances of Three Target Genes in Water Samples and Cell Abundance as LE-3 Equivalents of Total Cyanobacteria, Total *Microcystis*, and Toxic *Microcystis* (Cells Carrying *mcyD* Gene) in Samples Collected in August 2003 (Sampling Sites Numbered 1−7) and August 2004[a]

sampling site	Cyan 16S copies L^{-1}	Micr 16S copies L^{-1}	*mcyD* copies L^{-1}	total cyanobacteria (LE-3 equiv) L^{-1}	total *Microcystis* (LE-3 equiv) L^{-1}	toxic *Microcystis* (LE-3 equiv) L^{-1}
1	$3.9 (\pm 3.8) \times 10^{10}$	$3.4 (\pm 0.5) \times 10^{10}$	$3.2 (\pm 0.6) \times 10^{8}$	$9.9 (\pm 1.1) \times 10^{8}$	$3.9 (\pm 1.1) \times 10^{8}$	$1.1 (\pm 0.3) \times 10^{6}$
2	$1.7 (\pm 0.5) \times 10^{7}$	$6.2 (\pm 1.8) \times 10^{4}$	BQL	$3.2 (\pm 0.6) \times 10^{5}$	$1.8 (\pm 0.5) \times 10^{3}$	BQL
3	$1.5 (\pm 0.1) \times 10^{9}$	BQL	ND	$3.1 (\pm 0.3) \times 10^{7}$	BQL	ND
4	$2.1 (\pm 0.6) \times 10^{8}$	$1.1 (\pm 0.7) \times 10^{7}$	$2.8 (\pm 0.6) \times 10^{6}$	$4.7 (\pm 0.5) \times 10^{6}$	$6.6 (\pm 4.3) \times 10^{4}$	$9.0 (\pm 4.0) \times 10^{4}$
5	$1.0 (\pm 0.1) \times 10^{8}$	$1.7 (\pm 0.0) \times 10^{7}$	$7.0 (\pm 4.2) \times 10^{5}$	$1.9 (\pm 0.1) \times 10^{6}$	$1.0 (\pm 0.0) \times 10^{5}$	$3.4 (\pm 2.0) \times 10^{4}$
6	$2.9 (\pm 0.3) \times 10^{8}$	BQL	BQL	$5.0 (\pm 0.6) \times 10^{6}$	BQL	$4.2 (\pm 1.2) \times 10^{3}$
7	$8.7 (\pm 0.2) \times 10^{7}$	$2.4 (\pm 6.9) \times 10^{5}$	$7.7 (\pm 2.7) \times 10^{4}$	$1.6 (\pm 0.1) \times 10^{6}$	$7.5 (\pm 0.5) \times 10^{3}$	$8.6 (\pm 2.8) \times 10^{3}$
493	$4.3 (\pm 1.0) \times 10^{8}$	$5.1 (\pm 0.8) \times 10^{7}$	$4.2 (\pm 2.8) \times 10^{5}$	$5.5 (\pm 1.4) \times 10^{6}$	$7.0 (\pm 1.1) \times 10^{5}$	$2.0 (\pm 1.3) \times 10^{4}$
311	$6.6 (\pm 0.3) \times 10^{8}$	$1.3 (\pm 0.7) \times 10^{8}$	$3.9 (\pm 0.5) \times 10^{6}$	$8.7 (\pm 0.4) \times 10^{5}$	$1.8 (\pm 1.0) \times 10^{6}$	$1.8 (\pm 0.2) \times 10^{5}$
1163	$5.6 (\pm 0.4) \times 10^{9}$	$5.6 (\pm 0.4) \times 10^{7}$	$1.5 (\pm 1.0) \times 10^{6}$	$7.9 (\pm 0.2) \times 10^{7}$	$7.4 (\pm 0.6) \times 10^{5}$	$6.8 (\pm 4.5) \times 10^{4}$
885	$2.8 (\pm 0.3) \times 10^{8}$	$4.0 (\pm 0.8) \times 10^{7}$	$3.5 (\pm 0.3) \times 10^{6}$	$3.9 (\pm 0.4) \times 10^{6}$	$5.4 (\pm 1.1) \times 10^{5}$	$1.6 (\pm 1.6) \times 10^{5}$
496	$8.0 (\pm 2.2) \times 10^{8}$	$1.7 (\pm 0.1) \times 10^{8}$	$6.0 (\pm 0.3) \times 10^{6}$	$1.6 (\pm 7.8) \times 10^{7}$	$3.2 (\pm 1.5) \times 10^{6}$	$2.8 (\pm 0.1) \times 10^{5}$
495	$1.2 (\pm 0.4) \times 10^{8}$	$8.3 (\pm 0.3) \times 10^{6}$	$1.5 (\pm 0.7) \times 10^{5}$	$1.3 (\pm 0.8) \times 10^{6}$	$8.4 (\pm 2.9) \times 10^{4}$	$7.1 (\pm 3.4) \times 10^{3}$
494	$2.3 (\pm 0.7) \times 10^{8}$	$1.6 (\pm 0.2) \times 10^{7}$	$1.4 (\pm 1.0) \times 10^{6}$	$3.3 (\pm 1.1) \times 10^{6}$	$2.3 (\pm 0.3) \times 10^{5}$	$6.6 (\pm 4.7) \times 10^{4}$
357	$2.5 (\pm 1.3) \times 10^{7}$	$0.8 (\pm 1.1) \times 10^{7}$	$3.5 (\pm 0.3) \times 10^{4}$	$2.7 (\pm 1.9) \times 10^{5}$	$5.4 (\pm 6.4) \times 10^{4}$	$1.7 (\pm 0.2) \times 10^{3}$
974	$1.2 (\pm 0.05) \times 10^{7}$	$4.0 (\pm 1.3) \times 10^{5}$	$1.5 (\pm 1.0) \times 10^{4}$	$1.4 (\pm 0.1) \times 10^{5}$	$4.6 (\pm 1.5) \times 10^{3}$	$8.0 (\pm \text{ND}) \times 10^{2}$
882	$6.2 (\pm 1.0) \times 10^{7}$	$3.5 (\pm 0.1) \times 10^{7}$	$7.4 (\pm 1.5) \times 10^{5}$	$7.5 (\pm 1.3) \times 10^{5}$	$4.8 (\pm 0.2) \times 10^{5}$	$3.4 (\pm 0.7) \times 10^{4}$

[a] Description for all samples $n = 3$ (±standard deviation) except sampling site 1, where $n = 5$ (±standard deviation) and sampling site 974 toxic *Microcystis*, where $n = 2$. ND = not detected, and BQL = detected but below quantifiable limit.

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- To next lecture