Print version

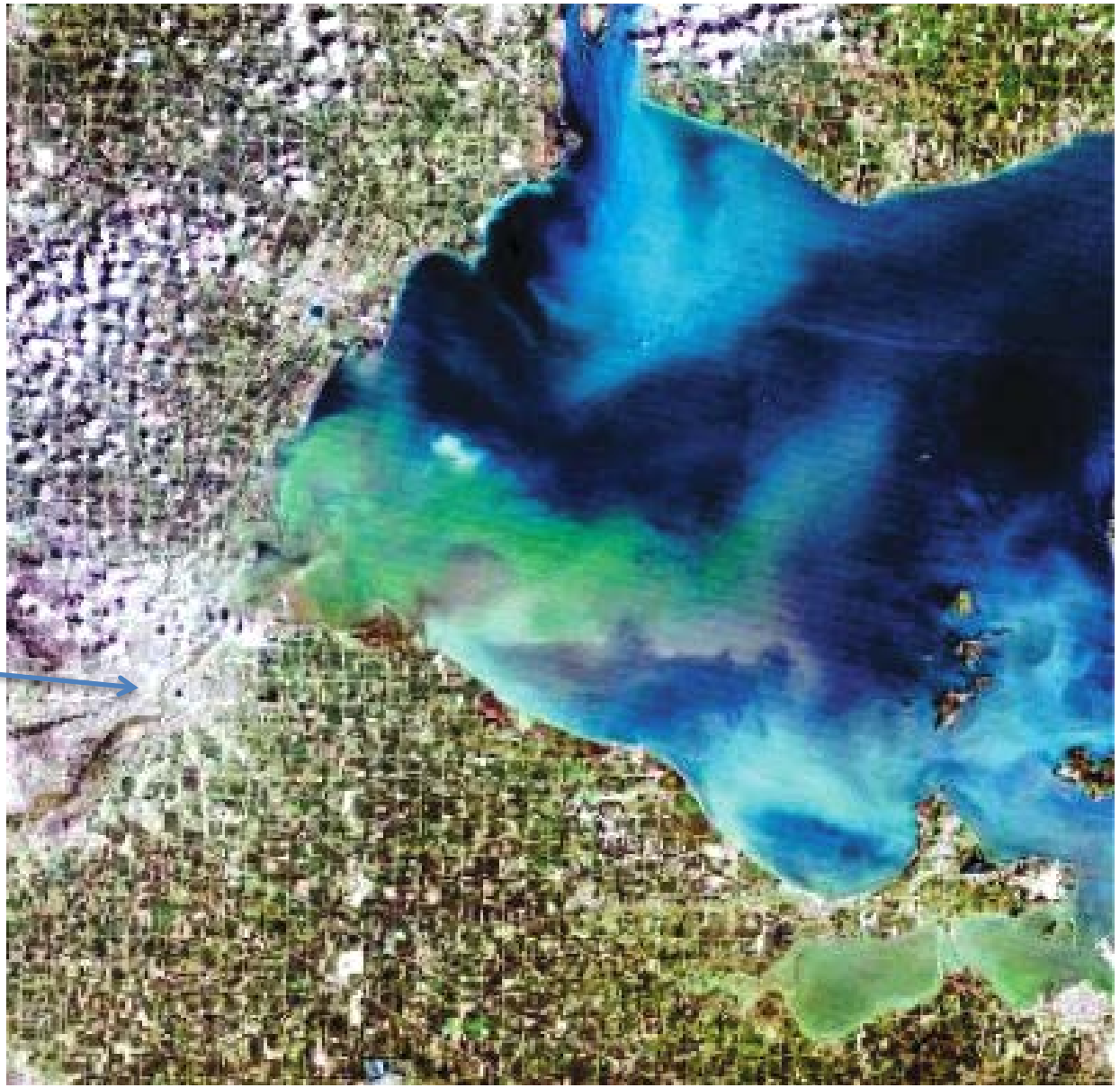
CEE 697z

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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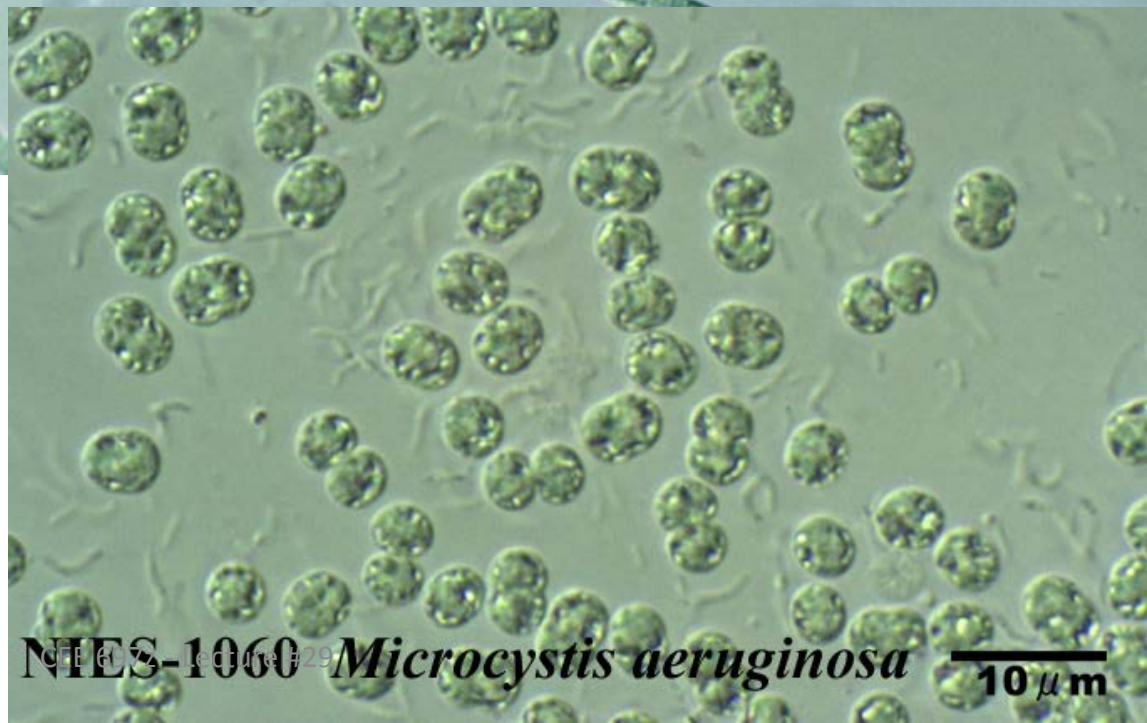
Anabaena

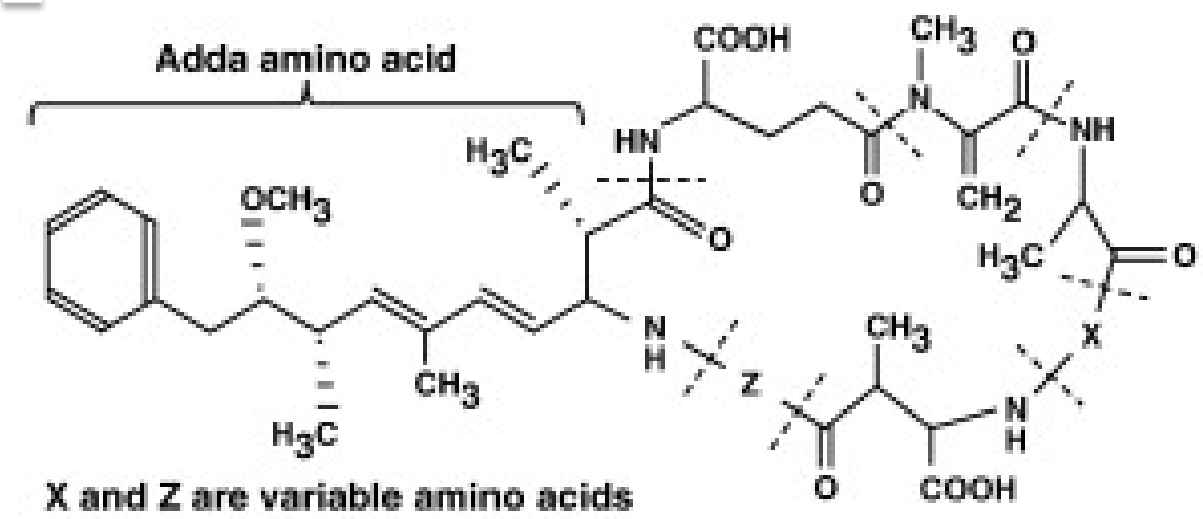
Oscillatoria Agardhii



Microcystin Producers

Cyanobacterial Hepatotoxins

Microcystin



X and Z are variable amino acids

otoxins. Environment International 59, 303-327.

BIOASSAYS TO DETECT CYANOTOXINS

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

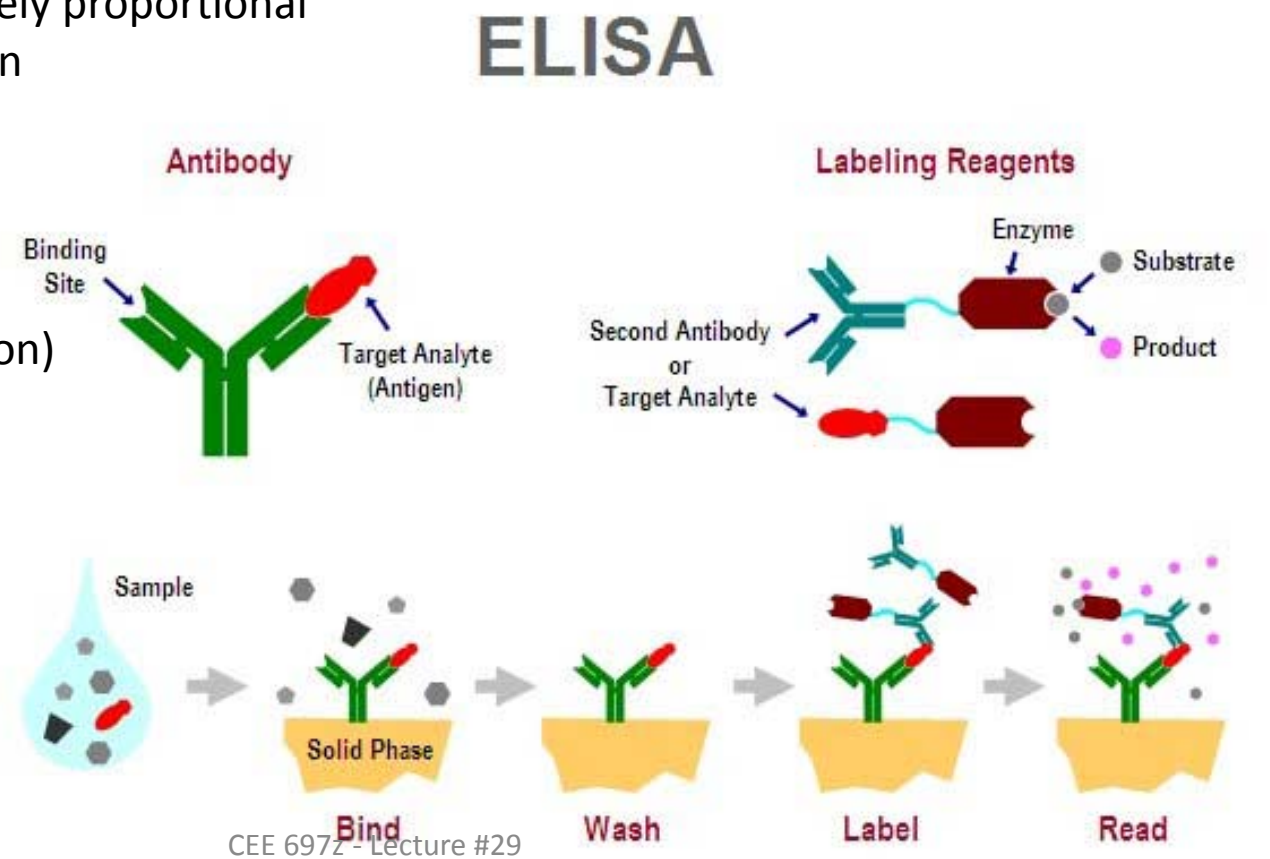
ELISA - Enzyme Linked Immunosorbent Assay

- Lowest Detection Limit .05 ppb to .5 ppb

Direct Competitive ELISA using Polyclonal Antibody

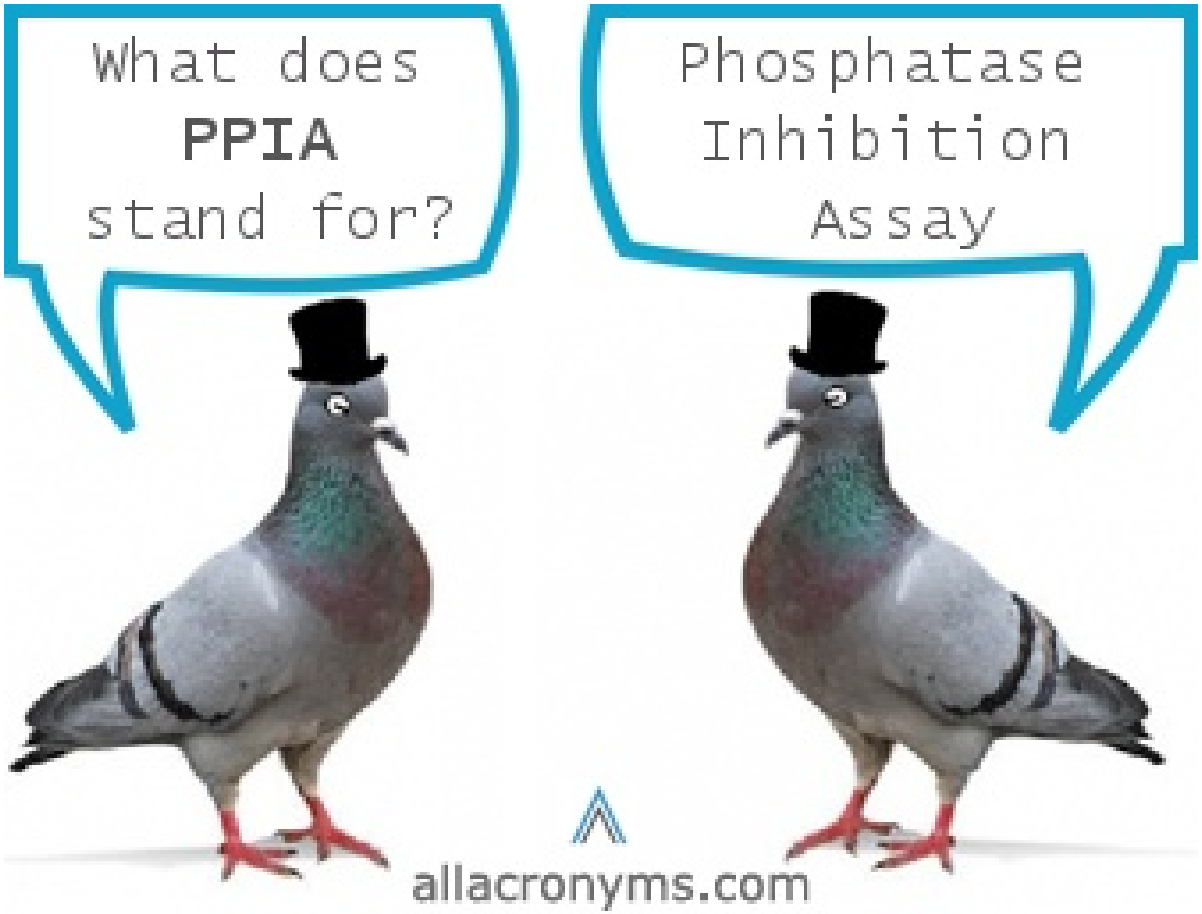
- Anti-Microcystin antibody attached to a high binding capacity microtiter plate
- MCYST-LR is used as a standard
- Microcystin-LR-peroxidase used to compete with MCYST-LR for the binding site of the antibody on the microtiter plate.
- Color developed inversely proportional to MCYST concentration

(lighter color = higher MCYST concentration)



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

PPIA


What does
PPIA
stand for?
Phosphatase
Inhibition
Assay
allacronyms.com

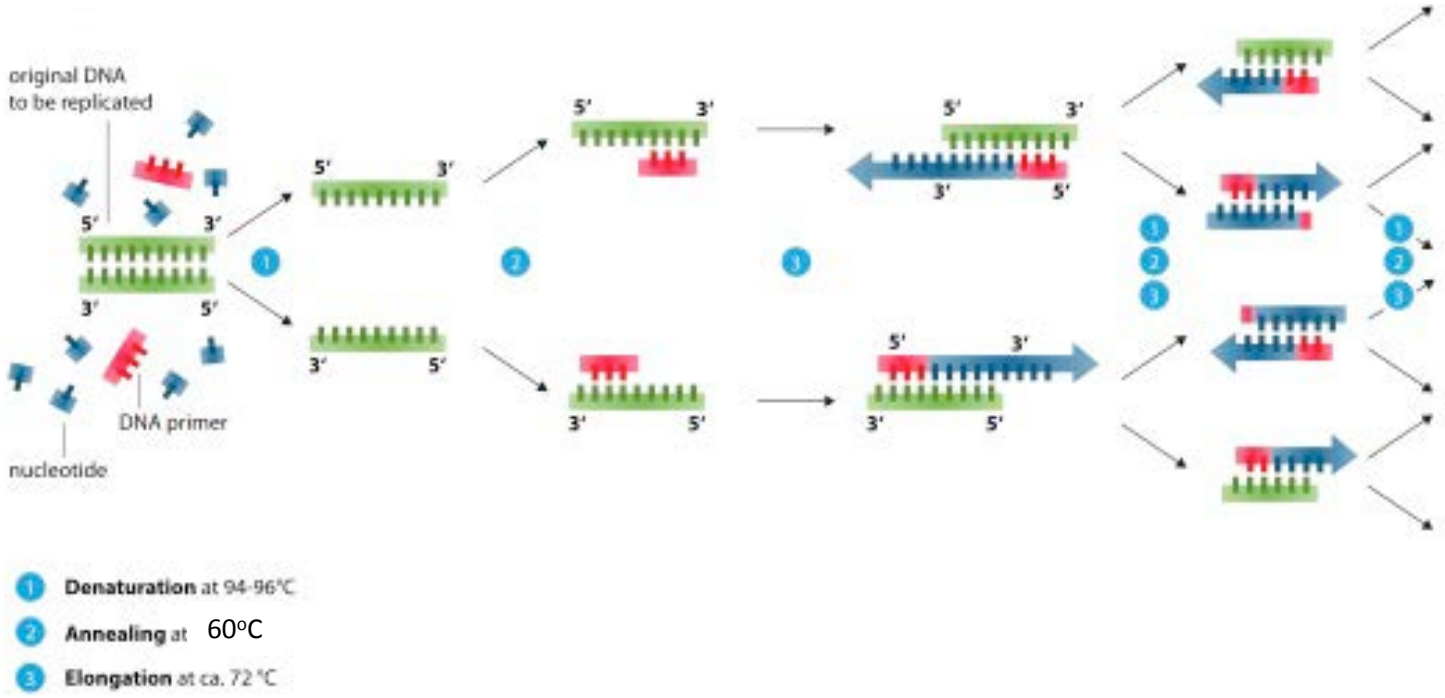
PPIA (Phosphatase Inhibition Assay) – How it works:

- 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

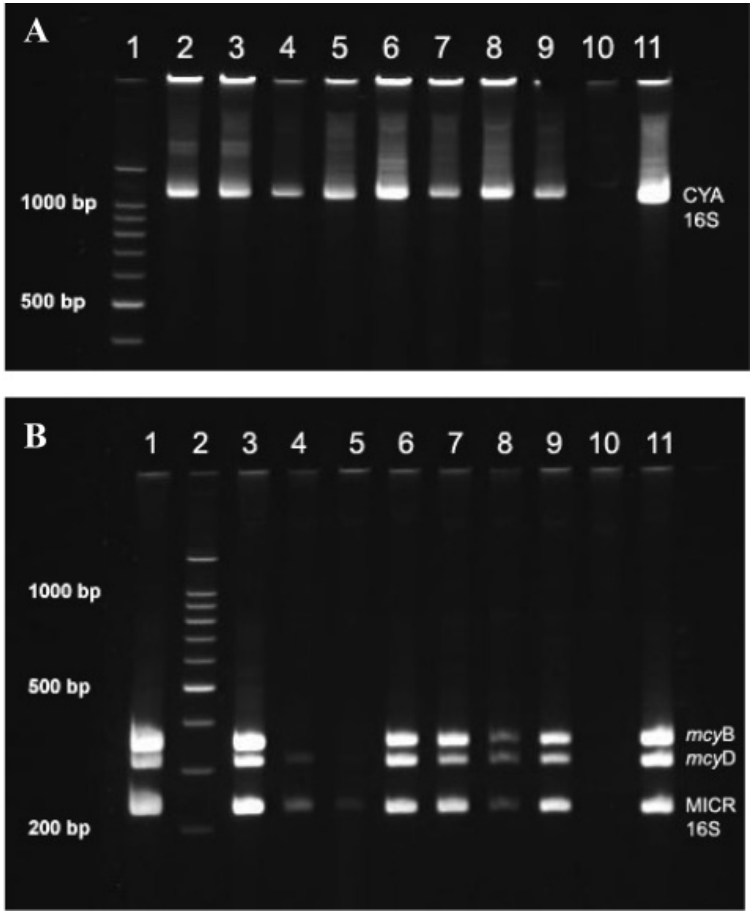


FIGURE 3. Gel image of multiplex PCR results. Gel A: detection of cyanobacterial 16S rDNA fragment. Lane 1: 100 bp molecular weight marker; lane 2: site 1a; lane 3: site 1b; lane 4: site 2; lane 5: site 3; lane 6: site 4; lane 7: site 5; lane 8: site 6; lane 9: site 7; lane 10: negative control, no template 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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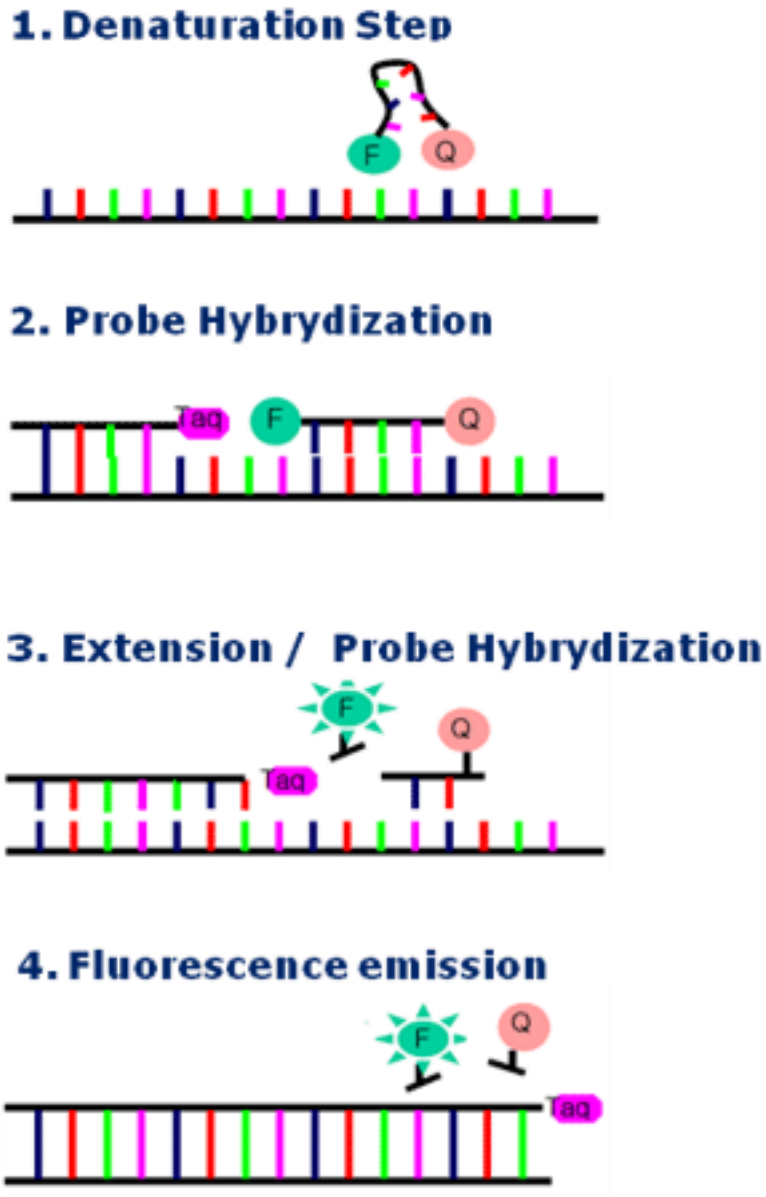
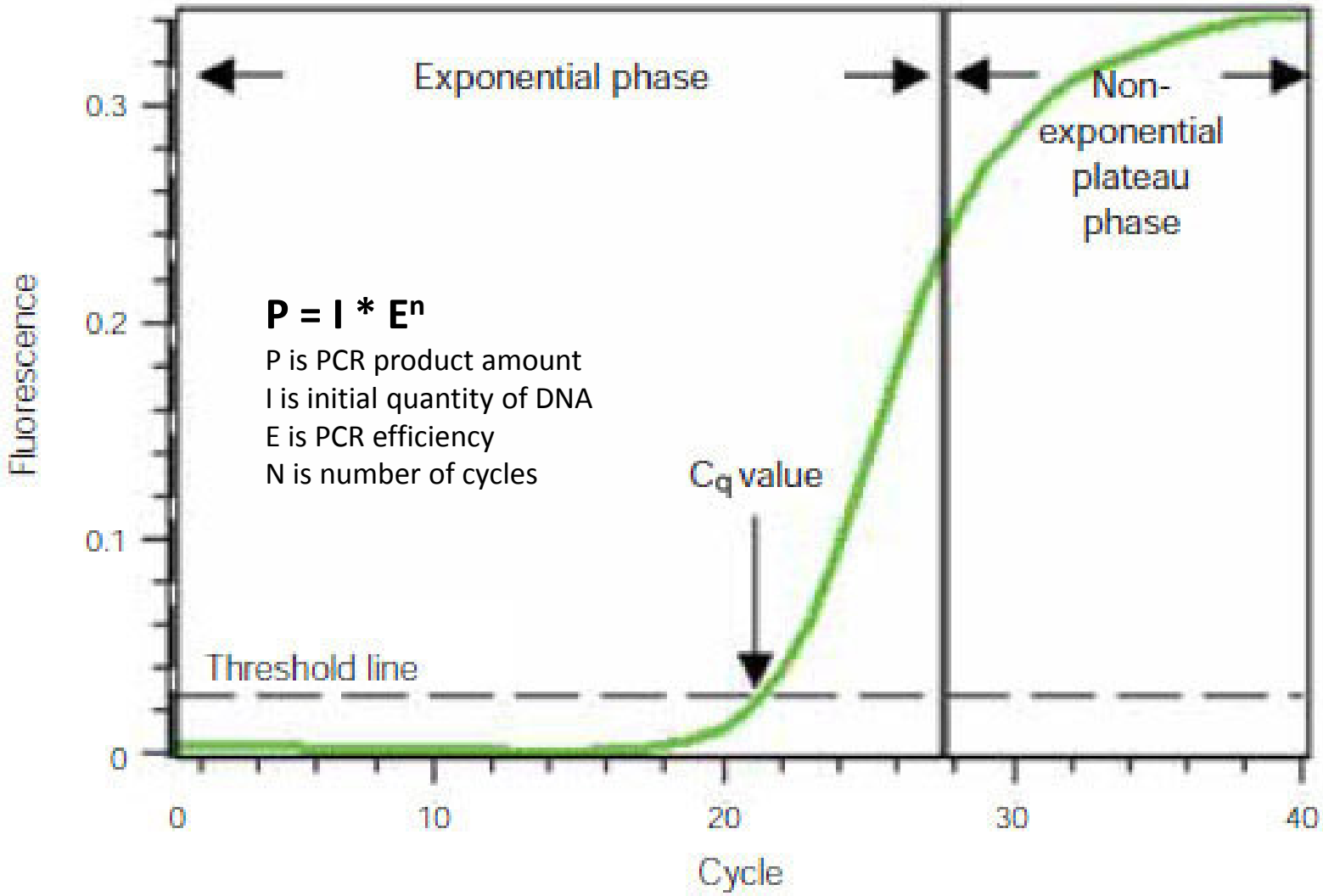
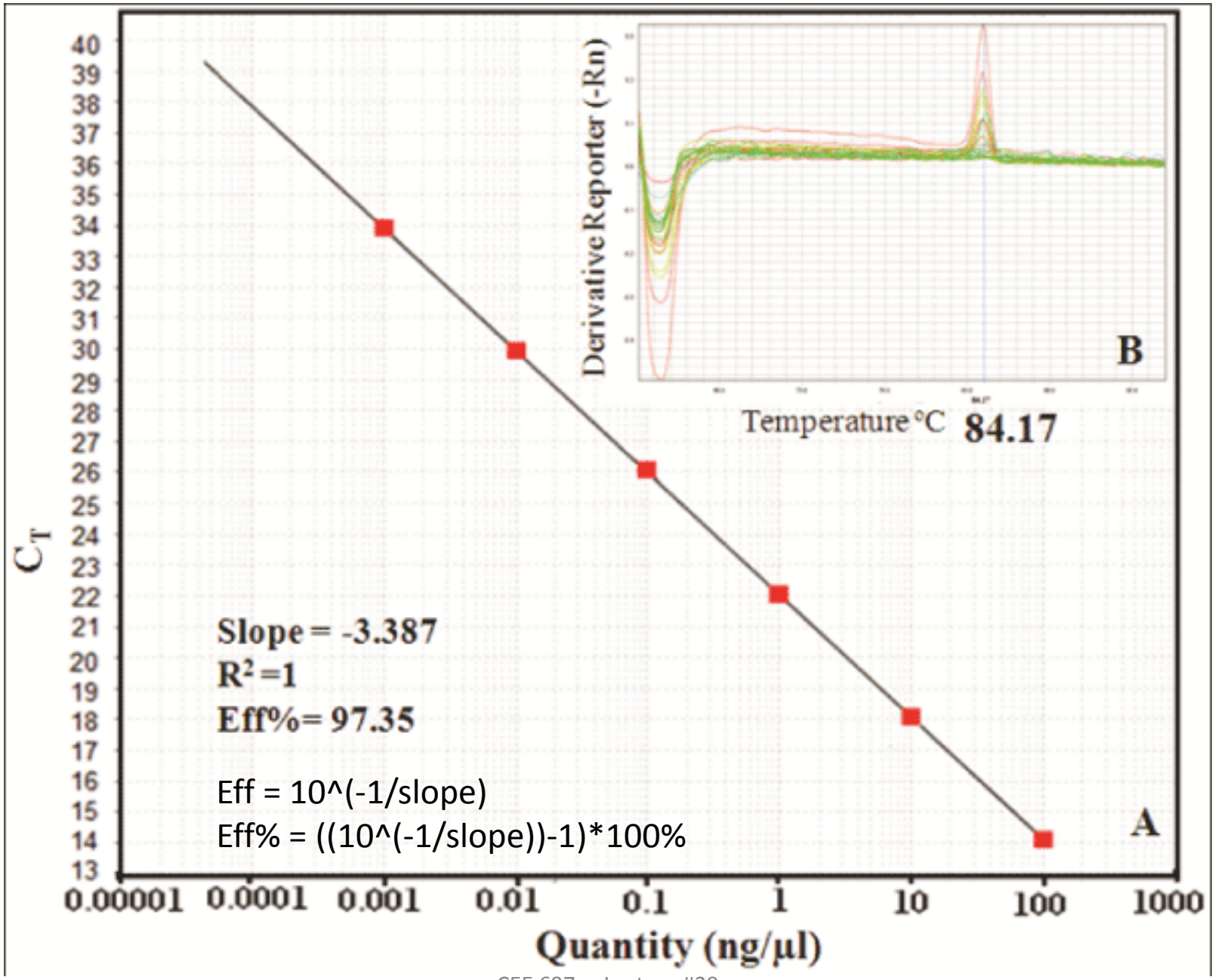
qPCR Fluorescence Emission



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
C_q value
Threshold line
Fluorescence
0.3
0.2
0.1
0
0
10
20
30
40
Cycle

Bio-Rad


40
39
38
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31
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29
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26
25
24
23
22
21
20
19
18
17
16
15
14
13
C_T
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Quantity (ng/µl)
Slope = -3.387
R^2 =1
Eff%= 97.35
Eff = 10^(-1/slope)
Eff% = ((10^(-1/slope))-1)*100%
A
Derivative Reporter (-Rn)
Temperature °C
84.17
B

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}	*mcy*D 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