

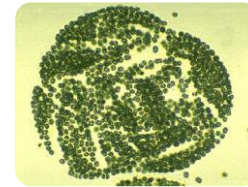
Novel diagnostic tools

A/Prof Aaron Jex

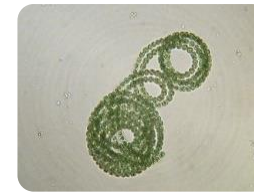
Population Health and Immunity Division

Walter and Eliza Hall Institute of Medical Research

Cyanobacteria ID and taxonomy



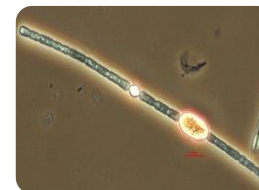
Microcystis sp.



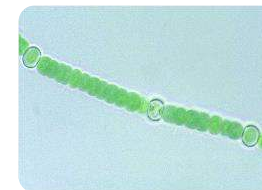
D. circinale



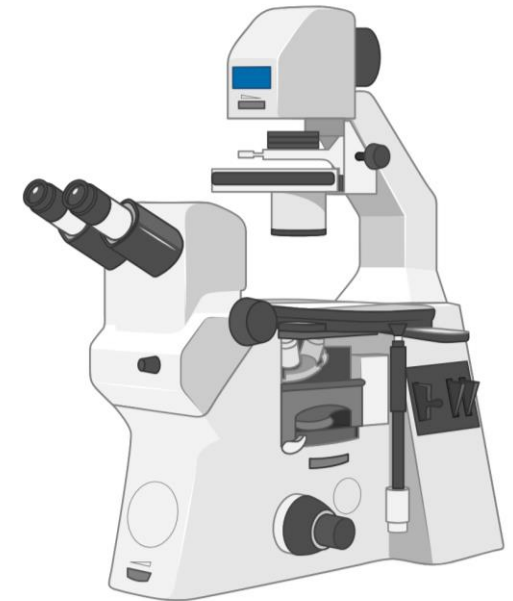
C. raciborskii



C. ovalisporum



N. spumigena

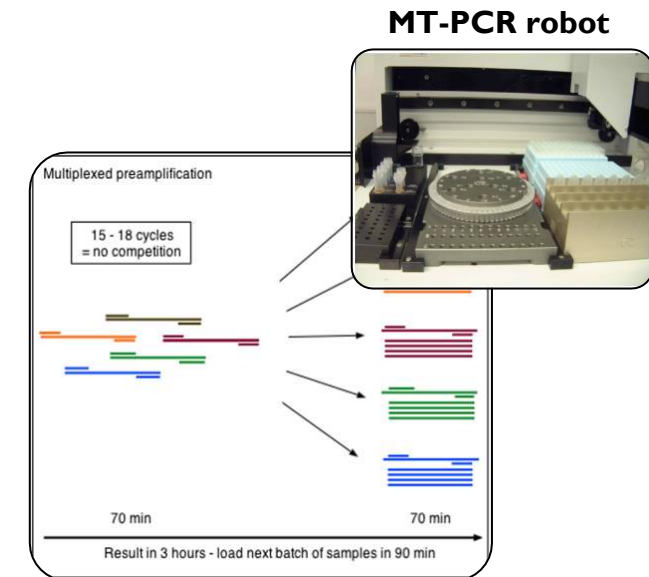
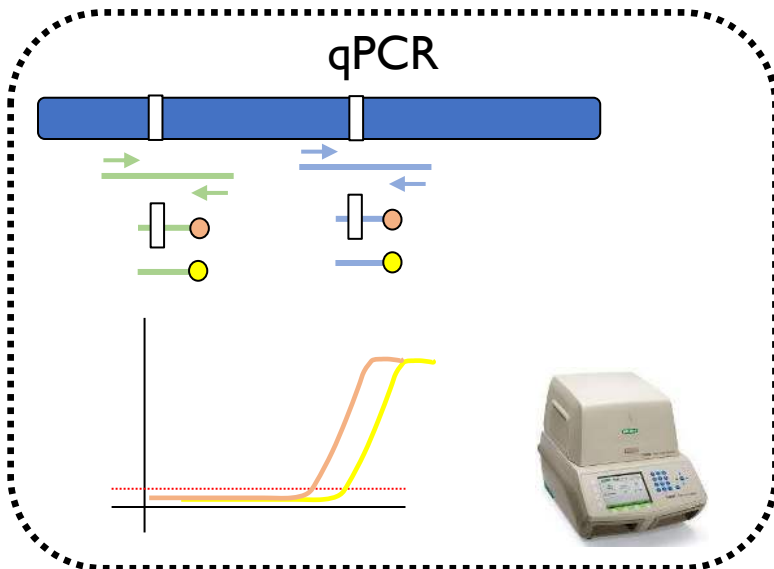


Toxin and T/O compound diagnostics

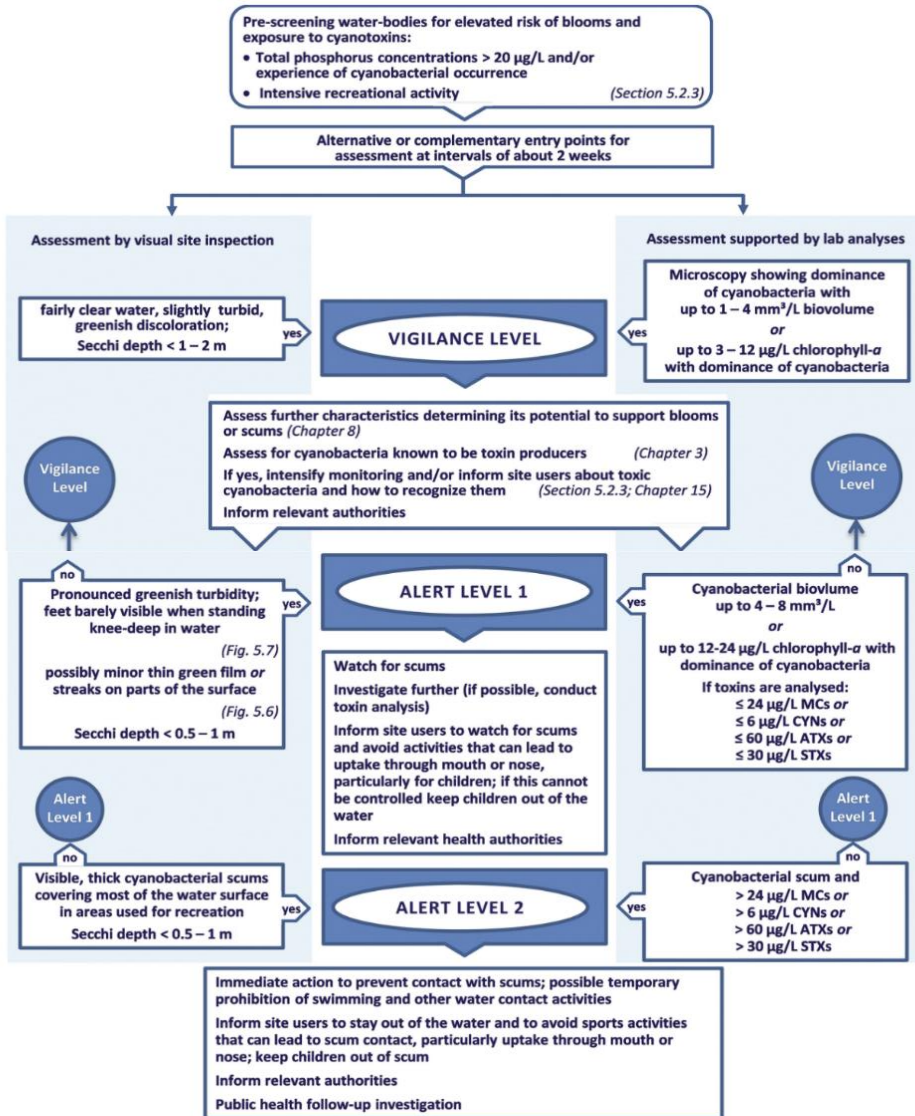
Cyanotoxins	Toxicity	Genera/Species	Guideline values
Microcystins	Hepatotoxic	<i>Microcystis</i> sp., <i>Dolichospermum circinale</i>	1.3 µg/L or 6500 cells/mL
Nodularins	Hepatotoxic	<i>Nodularia spumigena</i>	1.3 µg/L or 40,000 cells/mL
Saxitoxins	Neurotoxic	<i>Dolichospermum circinale</i> , <i>Cylindrospermopsis raciborskii</i>	3 µg/L or 20,000 cells/mL
Cylindrospermopsins	Neurotoxic/ Hepatotoxic	<i>C. raciborskii</i> , <i>Chrysochlorum ovalisporum</i>	1 µg/L or 15,000 cells/mL
Anatoxins	Neurotoxic	<i>Cuspidothrix issatschenkoj</i> , <i>Dolichospermum</i> sp., <i>Aphanizomenon</i> sp.	None
Geosmin	Taste/Odor	<i>Dolichospermum</i> sp., <i>Aphanizomenon</i> sp., <i>Oscillatoria</i> sp., many others	10 ng/L*
MIB	Taste/Odor	Many producers	~2-20 ng/L*
BMAA	Uncertain	<i>Nostoc</i> but many others	No advisory

Current gene testing approaches

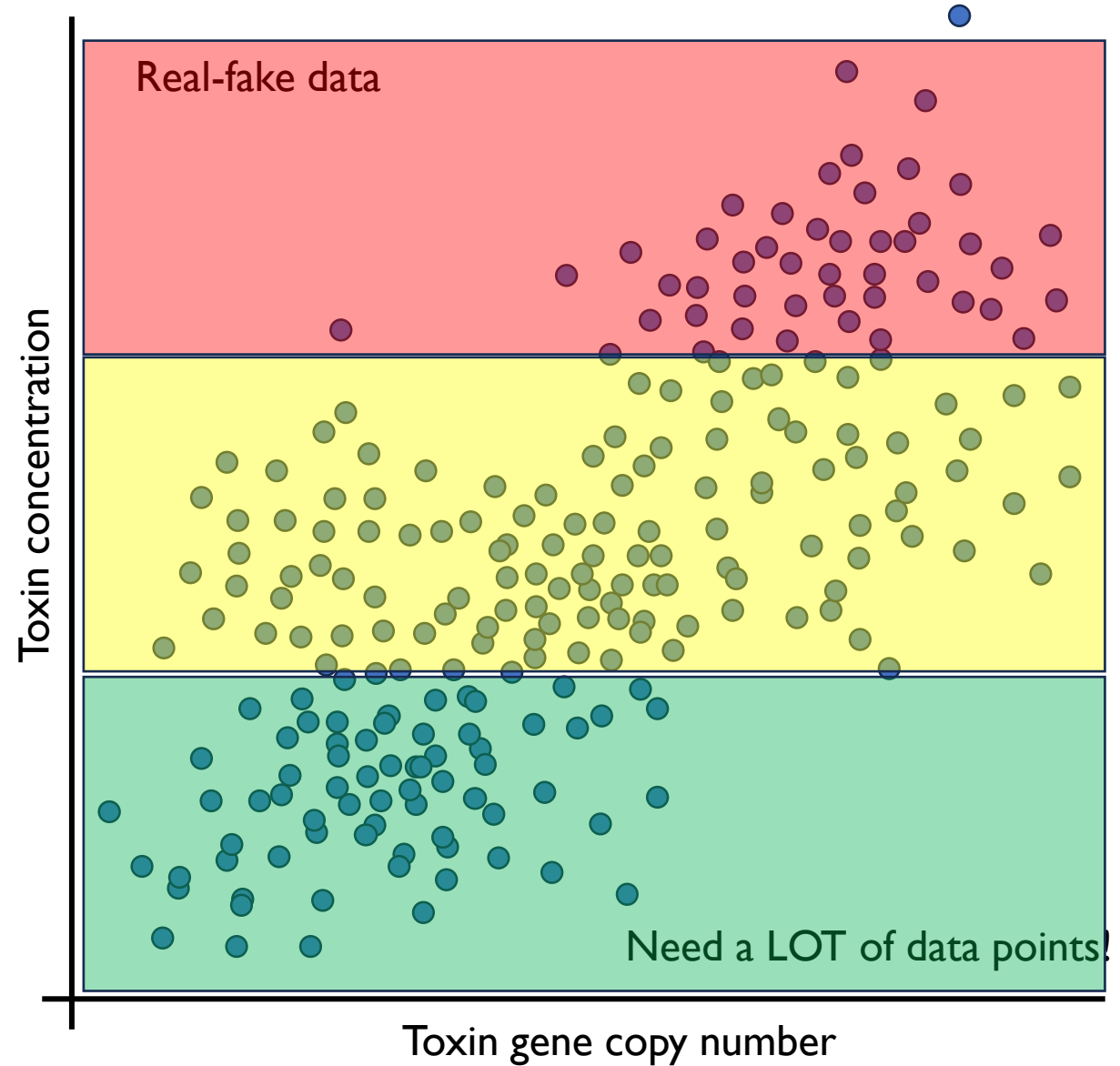
Cyanotoxins	Toxicity	Common Genera/Species	Common diagnostic gene
Microcystins	Hepatotoxic	<i>Microcystis</i> spp*., <i>Dolichospermum circinale</i> , <i>Planktothrix</i> sp., <i>Phormidium</i> sp., <i>Nostoc</i> sp.	<i>mcyE</i>
Nodularins	Hepatotoxic	<i>Nodularia spumigena</i> *, <i>Iningainema pulvinus</i> *, <i>Nostoc</i> sp.	<i>ndaF</i>
Saxitoxins	Neurotoxic	<i>D. circinale</i> *, <i>Raphidiopsis raciborskii</i>	<i>sxtI</i>
Cylindrospermopsins	Neurotoxic/ Hepatotoxic	<i>R. raciborskii</i> *, <i>Chrysochlorum ovalisporum</i> , <i>Lyngbya wollei</i> *	<i>cyrC</i>
Anatoxins	Neurotoxic	<i>Cuspidothrix issatschenkoi</i> , <i>Dolichospermum</i> sp., <i>Aphanizomenon</i> sp., <i>Phormidium</i> sp.	<i>anaC</i>



Applying toxin gene levels to toxin safety thresholds

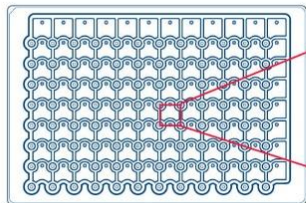
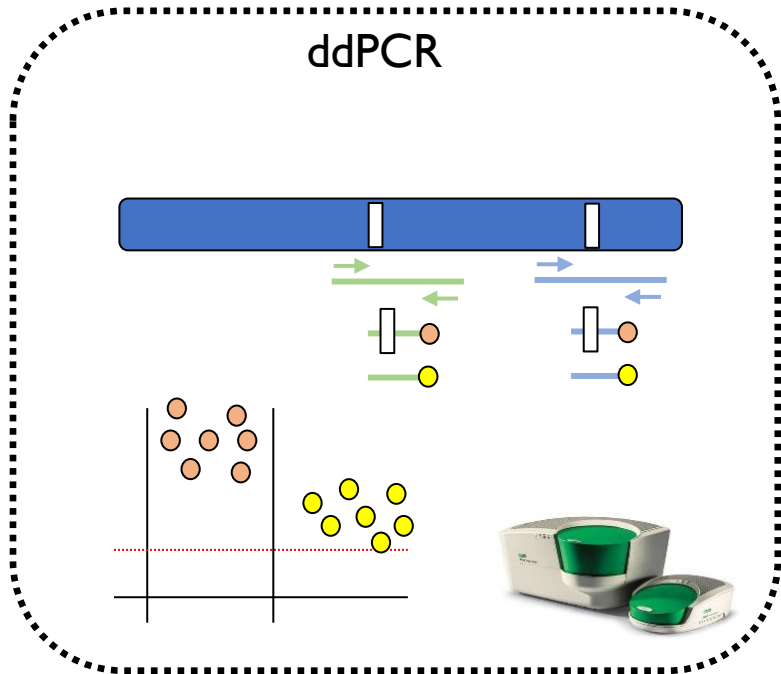


Source: (Chorus & Welker, 2021).

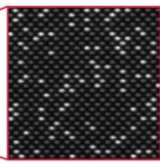


Improved quantitation, portability or T/O coverage

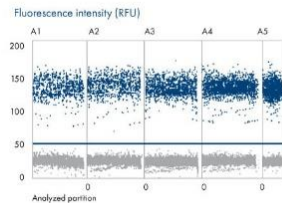
ddPCR



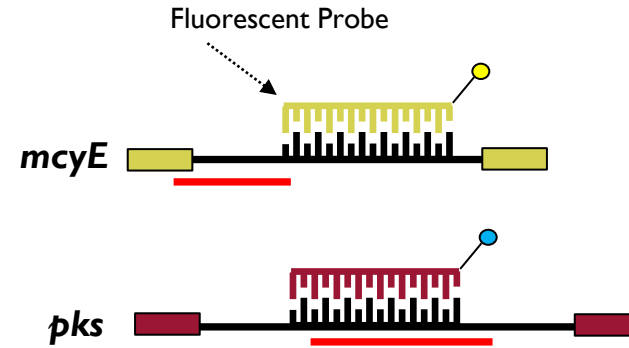
dPCR nanoplate with 96 wells



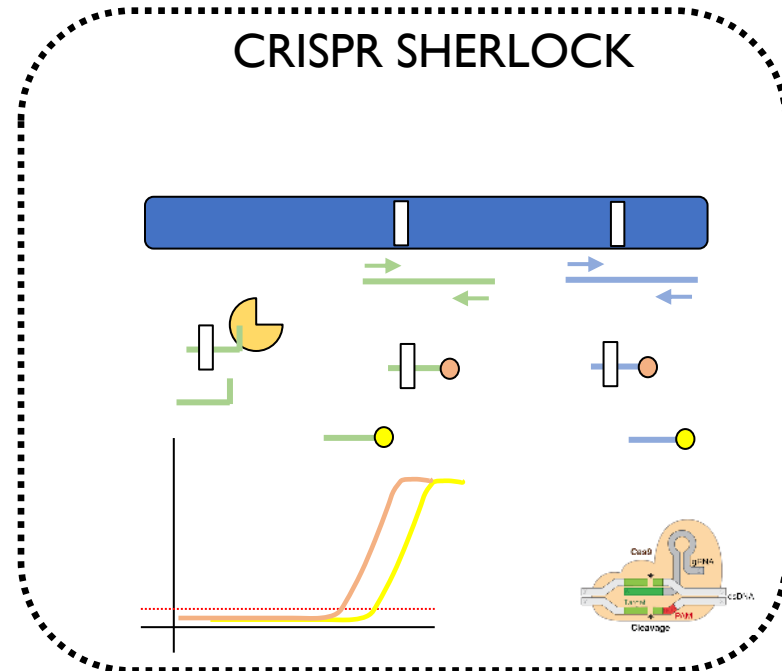
Single well detail



Analysis view (Representative 1D Scatterplot)



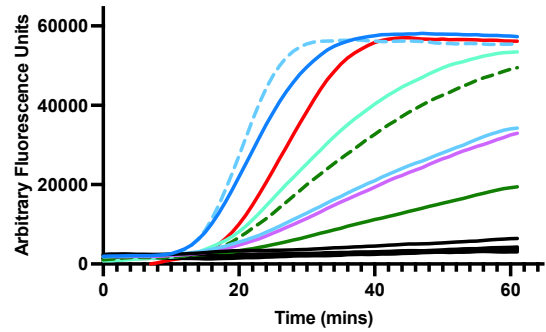
CRISPR SHERLOCK



Lateral flow visual readout

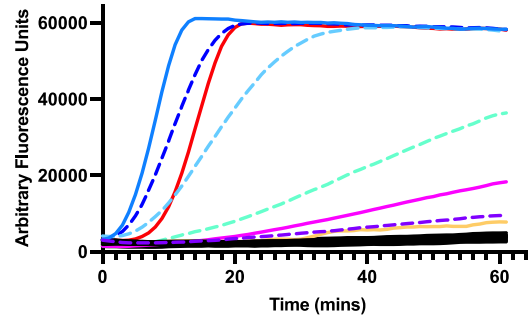
RPA + CRISPR assay of cyanotoxin genes

RPA+CRISPR_mcyE_Bloom Samples



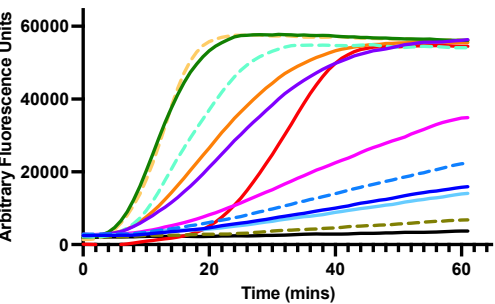
- MR3
- MR4
- MR5
- MR9
- MR11
- MR13
- MR17
- MR20
- MR22
- MR27
- MR13_repeat_10uL
- MR27_repeat_10uL
- Pos. ctrl_200 copies
- NTC

RPA+CRISPR_anaC_Bloom Samples



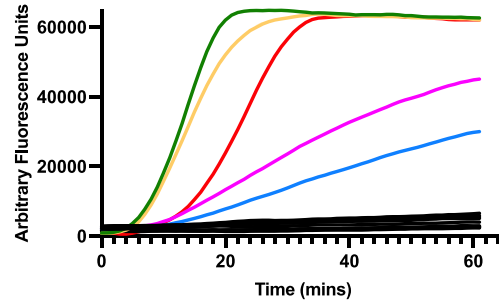
- MR14
- MR46
- MR48
- MR89
- MR92
- MR700
- MR702
- MR704
- MR710
- MR720
- MR700_repeat_3 uL
- MR702_repeat_3uL
- MR704_repeat_3uL
- MR720_repeat_3uL
- MR86_10 uL
- MR88_10 uL
- MR93_10 uL
- MR720
- MR96_10 uL
- MR109_10uL
- MR720_repeat_10 uL
- Pos. ctrl_200 copies
- NTC

RPA+CRISPR_sxtI_Bloom Samples

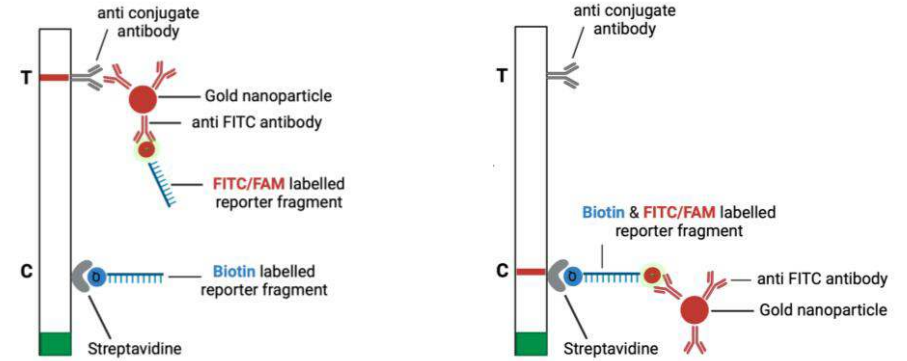


- MR5
- MR11
- MR15
- MR16
- MR19
- MR20
- MR28
- MR37
- MR69
- MR70
- Pos. ctrl_200 copies
- NTC

RPA+CRISPR_cyrC_Bloom Samples



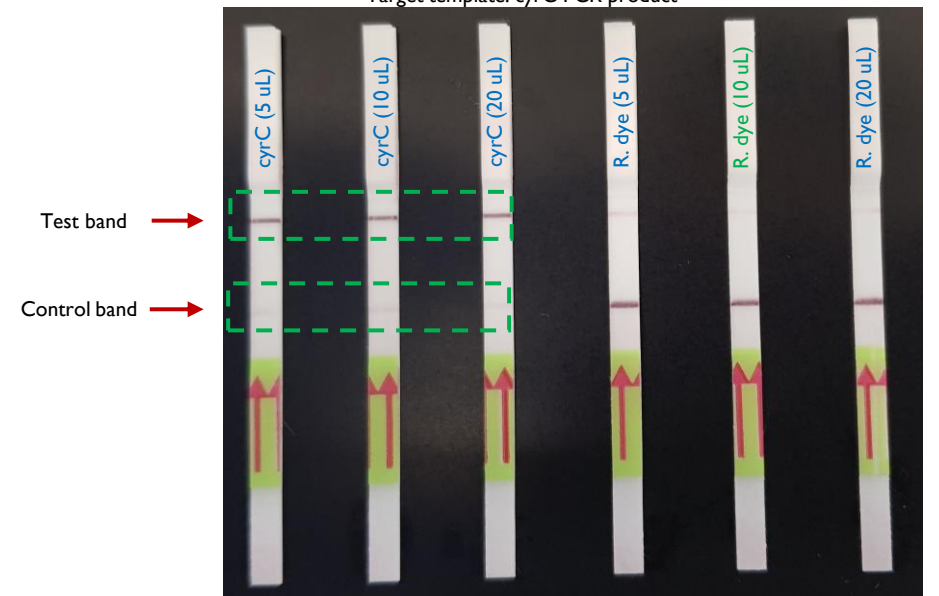
- MR3
- MR4
- MR5
- MR9
- MR11
- MR15
- MR16
- MR19
- MR28
- MR31
- MR3_repeat_10uL
- MR5_repeat_10uL
- MR15_repeat_10uL
- MR31_repeat_10uL
- Pos. ctrl_200 copies
- NTC



Positive result

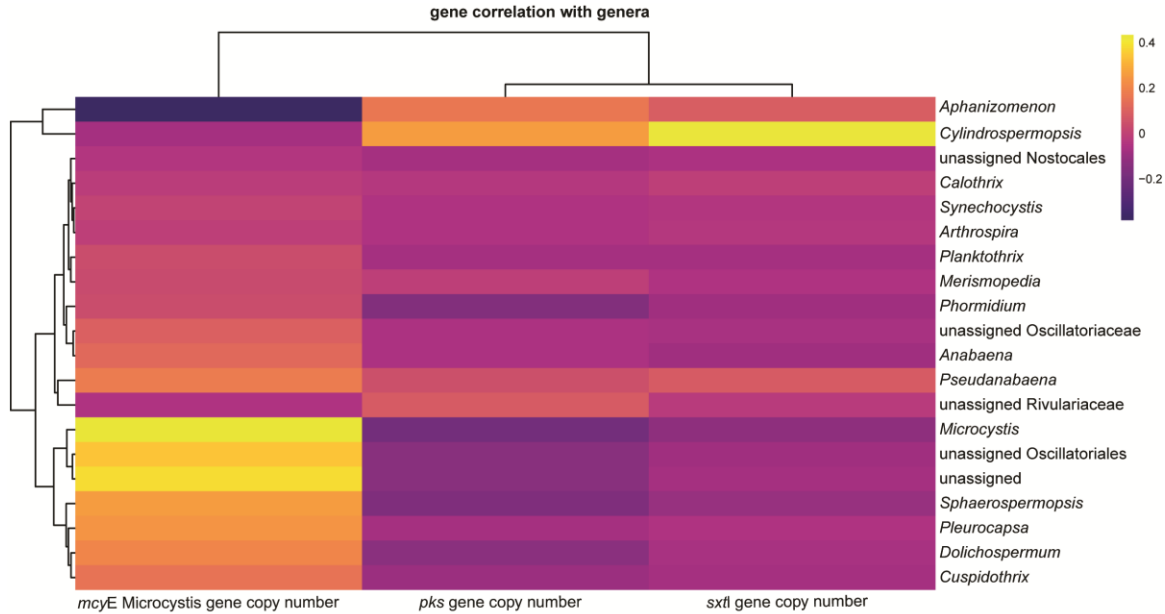
Negative result

Reporter dye: 0.1 pmol
Target template: cyrC PCR product

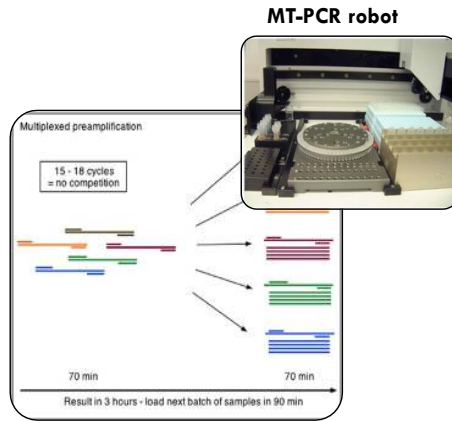
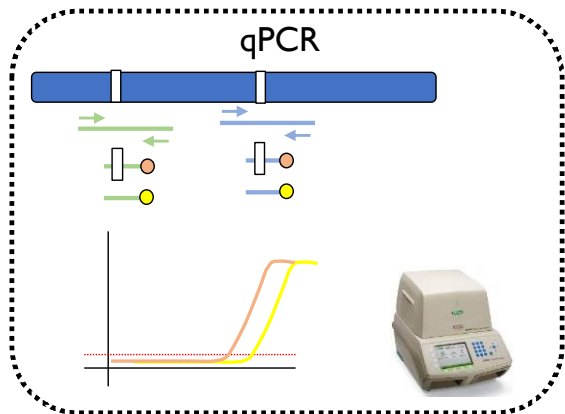
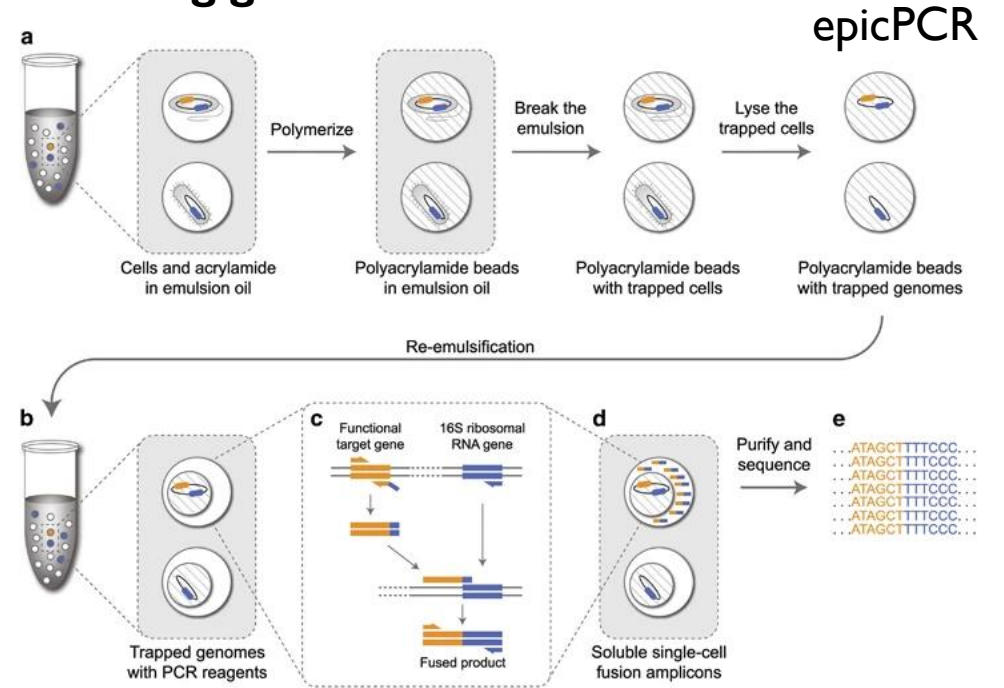


Attributing toxin genes to cyanobacterial species

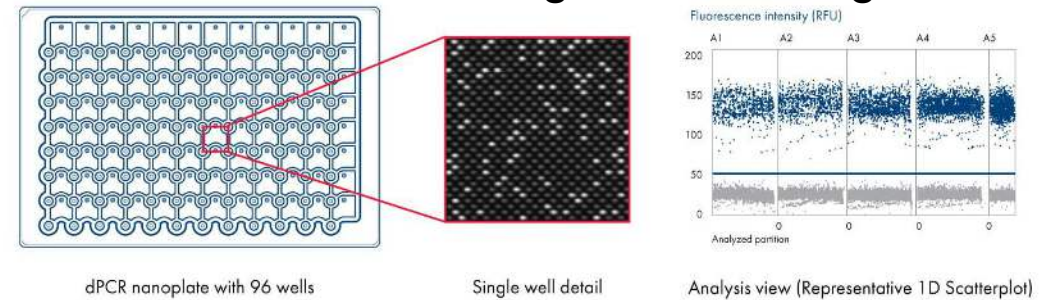
Correlating toxins with taxonomic markers



Colocalising genes in cells



single-cell PCR or genomics



We need genomic resources and reference strains!



Hydrobiologia
<https://doi.org/10.1007/s10750-023-05356-7>

MICROALGAL FUNCTIONAL TRAITS



Review Paper

Welcome to the jungle!: An overview of modern taxonomy of cyanobacteria

Jan Kaštovský

Eur. J. Phycol. (2016), 51: 346–353



A polyphasic approach for the taxonomy of cyanobacteria: principles and applications

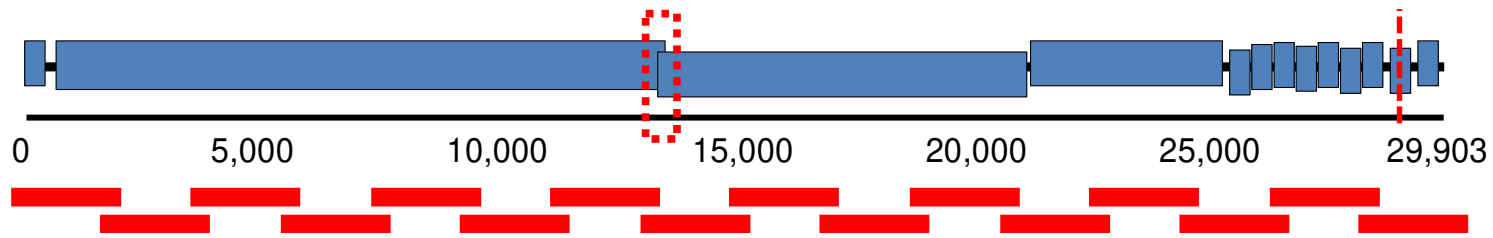
JIRÍ KOMÁREK

recognition of past misidentifications. It has been estimated that about half of the cyanobacterial strains in culture collections have been misidentified (Komárek and Anagnostidis, 1989). Several studies have cautioned against the over-emphasis on morphological characters for identification and taxonomic classification (Beltran and Neilan, 2000; Rajaniemi et al., 2005a,b; Zapomělová et al., 2007, 2010b).

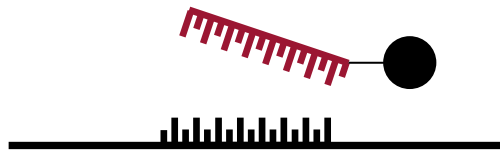
Li et al., 2016; *Harmful Algae*

Targeted sequencing strategies

Tiled amplicons



Bait capture



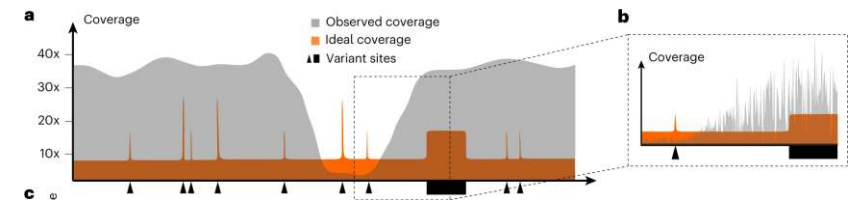
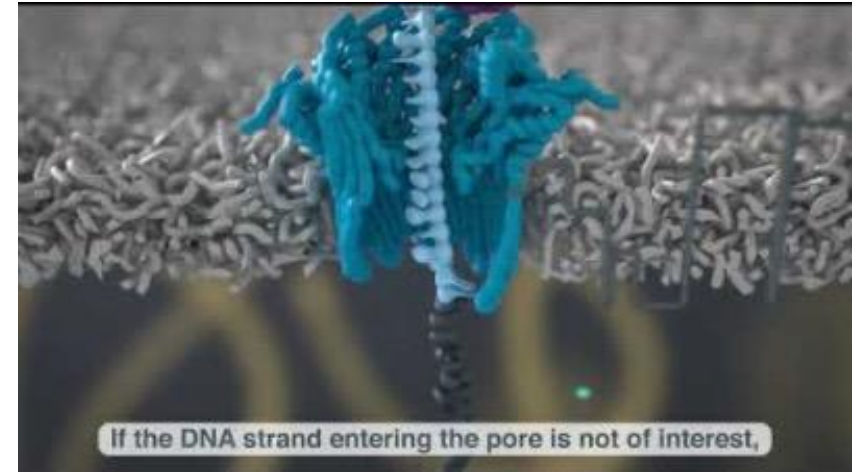
Illumina
MiSeq



Illumina
HiSeq



Nanopore



Concluding summary

- Broad range of qPCR methods for quantifying toxin genes
- Some genetic assays for taste and odor genes, not BMAA
- Correlating toxin genes with toxin levels or health thresholds remains challenging (need more data!)
- May be assisted with truly quantitative dPCR
- CRISPR diagnostics can provide low-cost, rapid and semiquantitative field-based assay
- Need methods to link toxins to taxa
- Need genomic resources and reference strains
- This can be greatly enhanced with targeted sequencing methods





WEHI
brighter together

Thank you

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