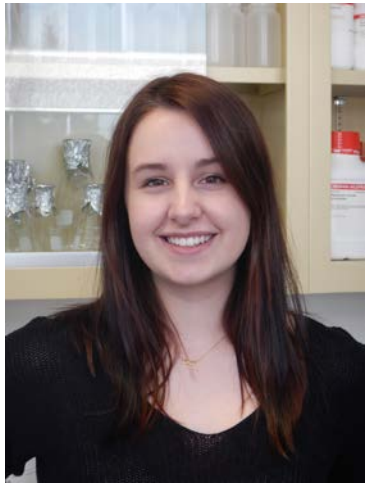
Multi-parameter assessment of natural recreational waters in Alberta using point of contact molecular tests



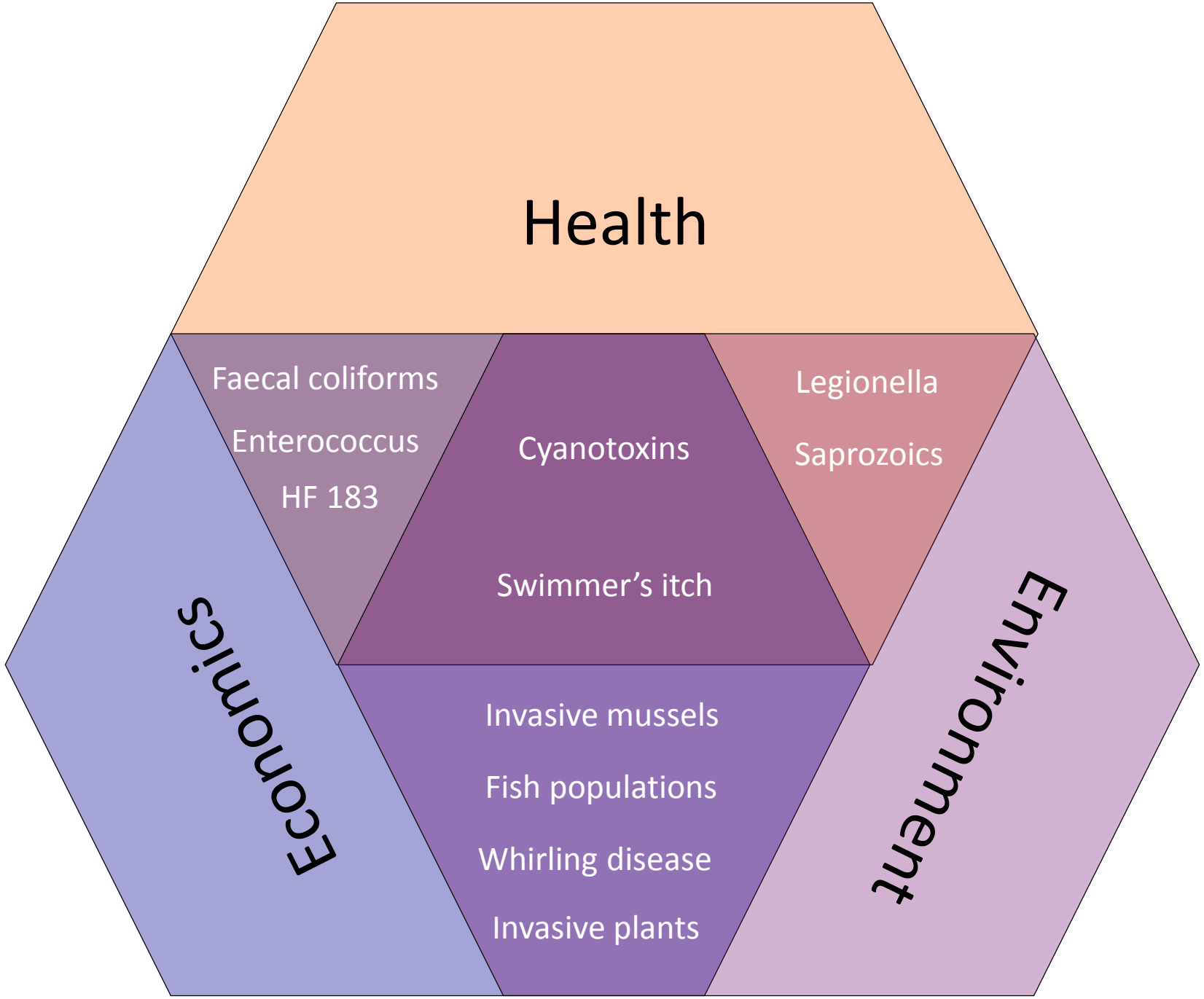
Patrick C. Hanington
University of Alberta

UNIVERSITY OF ALBERTA
SCHOOL OF PUBLIC HEALTH

ALBERTA INNOVATES

Alberta Environment and Parks

NSERC CREATE
Host-Parasite Interactions
Calgary
Alberta
Lethbridge

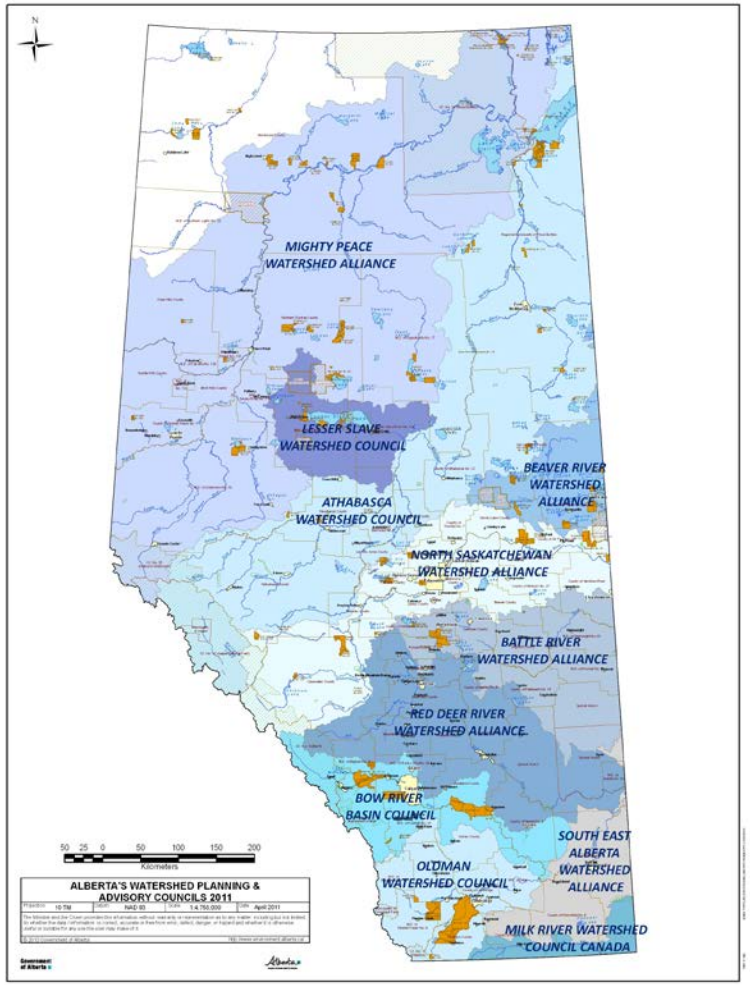

Health
Faecal coliforms
Enterococcus
HF 183
Cyanotoxins
Legionella
Saprozoics
Swimmer's itch
Economics
Invasive mussels
Fish populations
Whirling disease
Invasive plants
Environment

WASTE WATER	STORM WATER	RECREATIONAL WATER	DRINKING WATER

Waste water and drinking water are often monitored very vigilantly, other water types are poorly monitored, infrequently monitored or not monitored at all

ISSUES

- Alberta is large, with hundreds of named lakes and thousands of storm water catchment areas
- There are many types of targets to monitor for, and many have unique tests to detect them
- While some targets have associated policy for exposure limits, many do not, or do not cause health issues
- Water is dynamic and can change quickly, altering the targets that must be monitored



OUR SOLUTION

- Unify testing methodologies whenever possible
- Decentralize lab testing for primary monitoring purposes
- Train and trust citizen scientists
- Democratize water monitoring to vastly expand the scope, frequency and number of areas monitored

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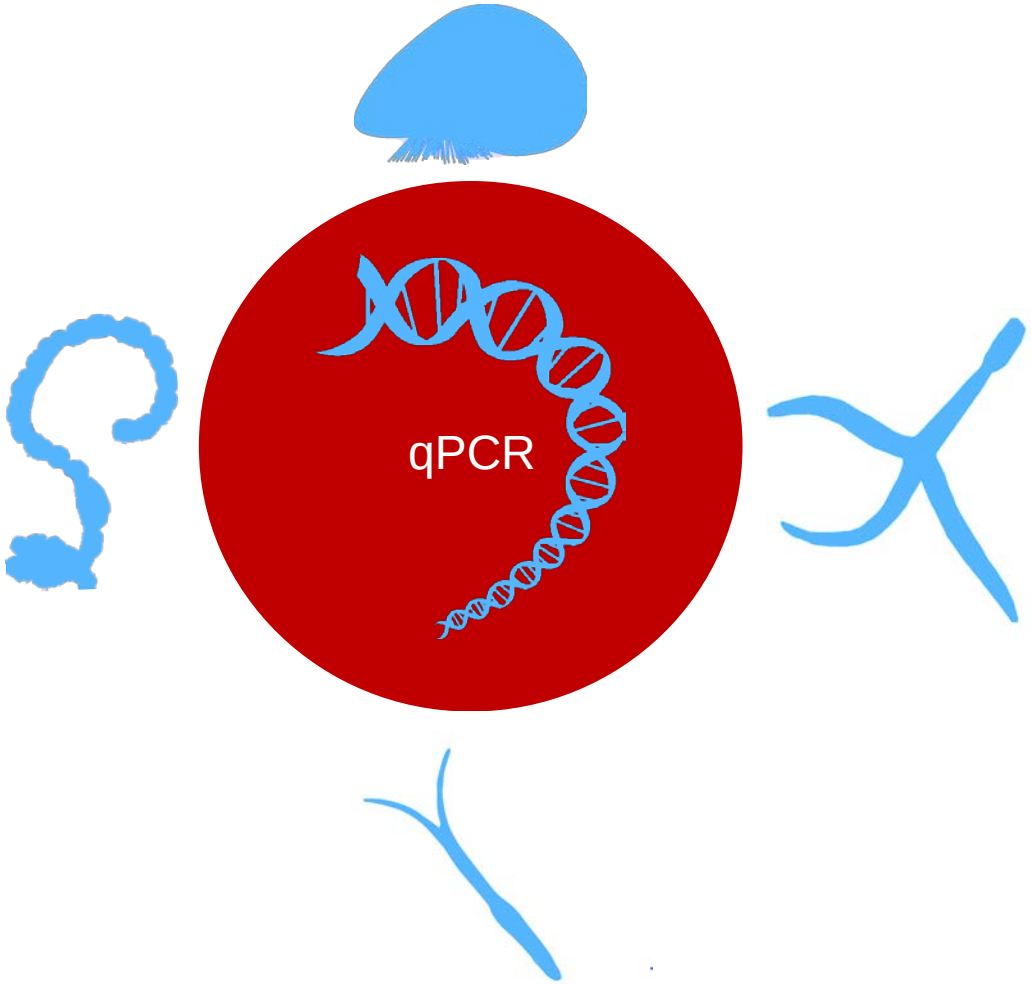

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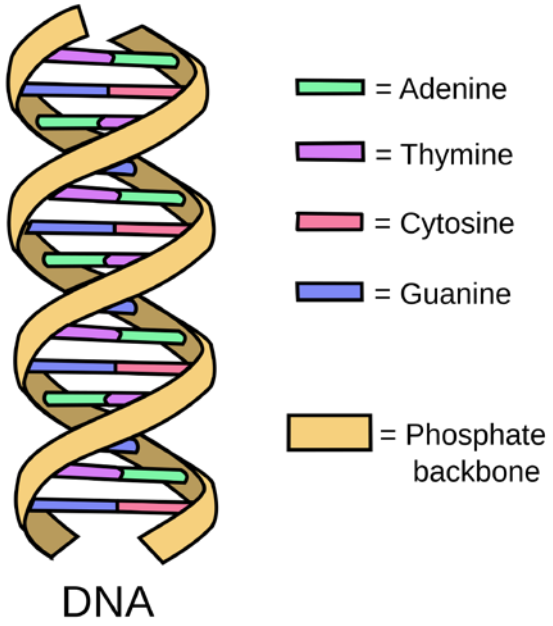
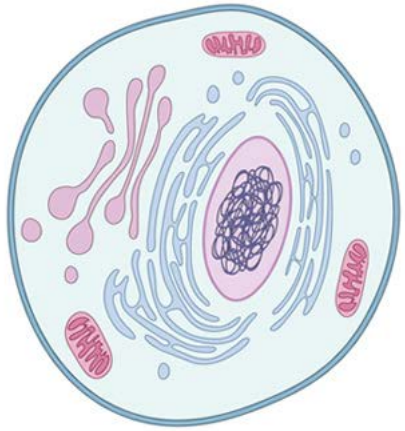
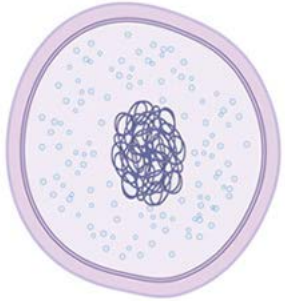
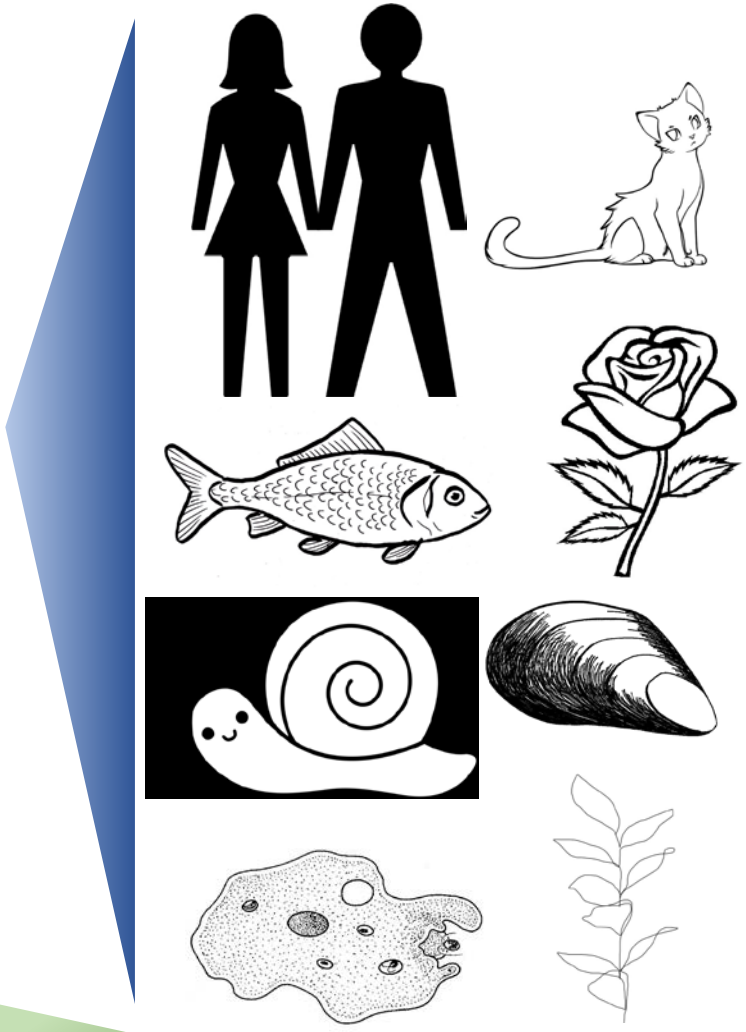
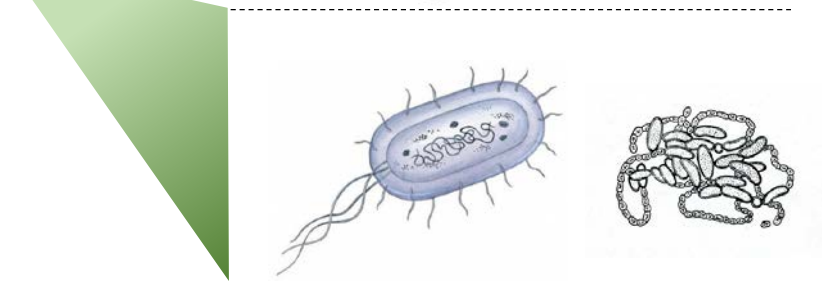
DNA – everybody's got it







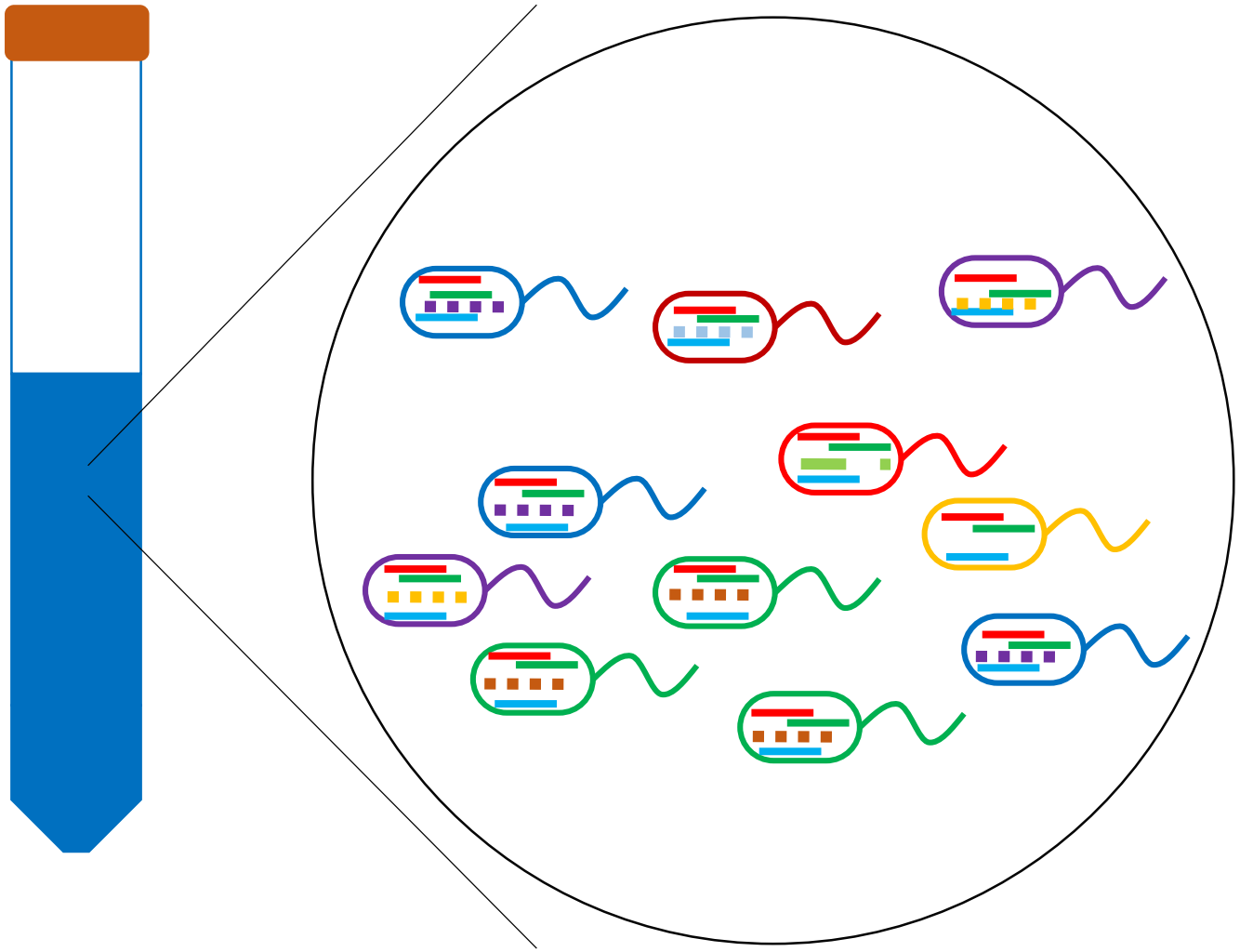




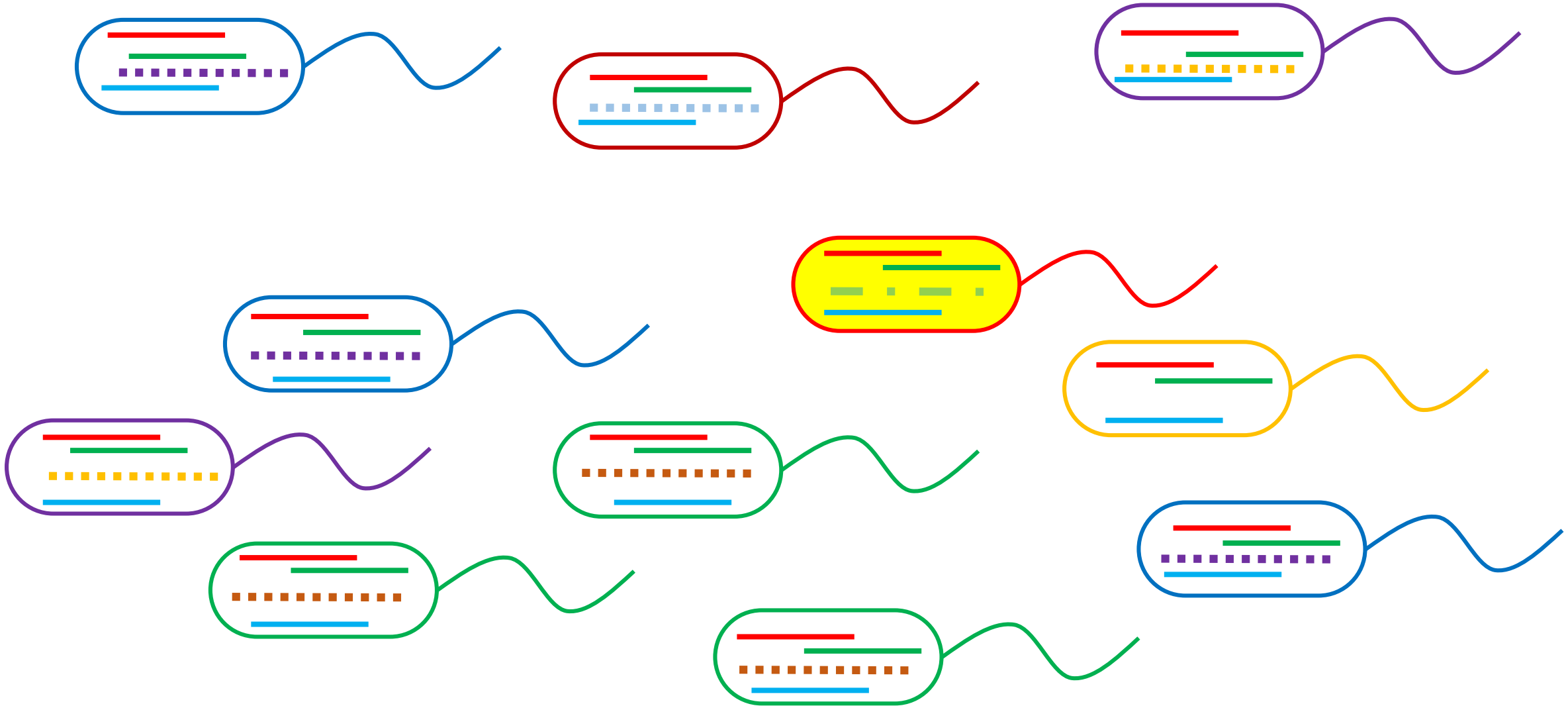
Some genes are very similar between different species and some are very different

If we know the sequences of those different genes, we can design ways to detect them using qPCR

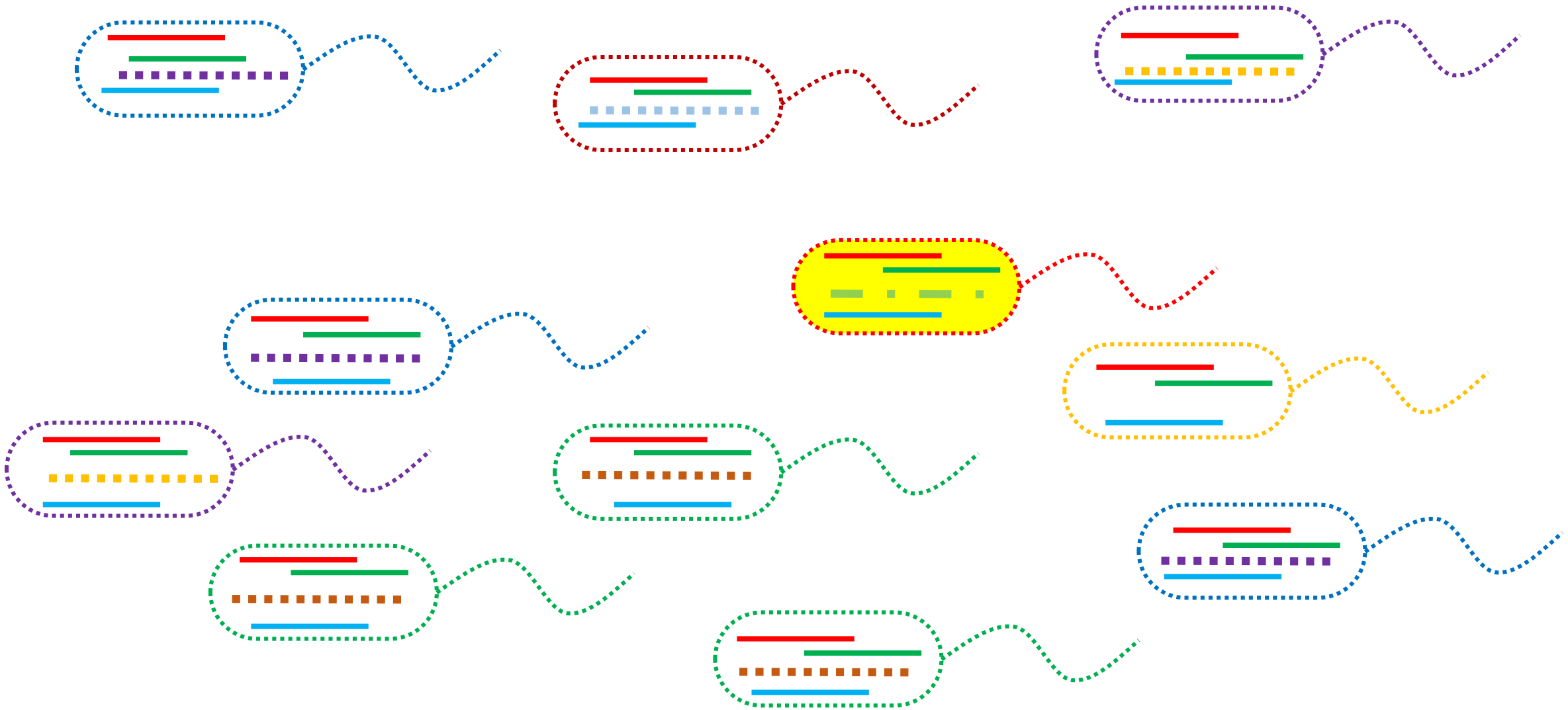
If you collect appropriately, you can catch the organisms you want to detect



Some DNA is shared between different organisms and some is unique



Breaking the cells open releases the DNA and allows us to access it



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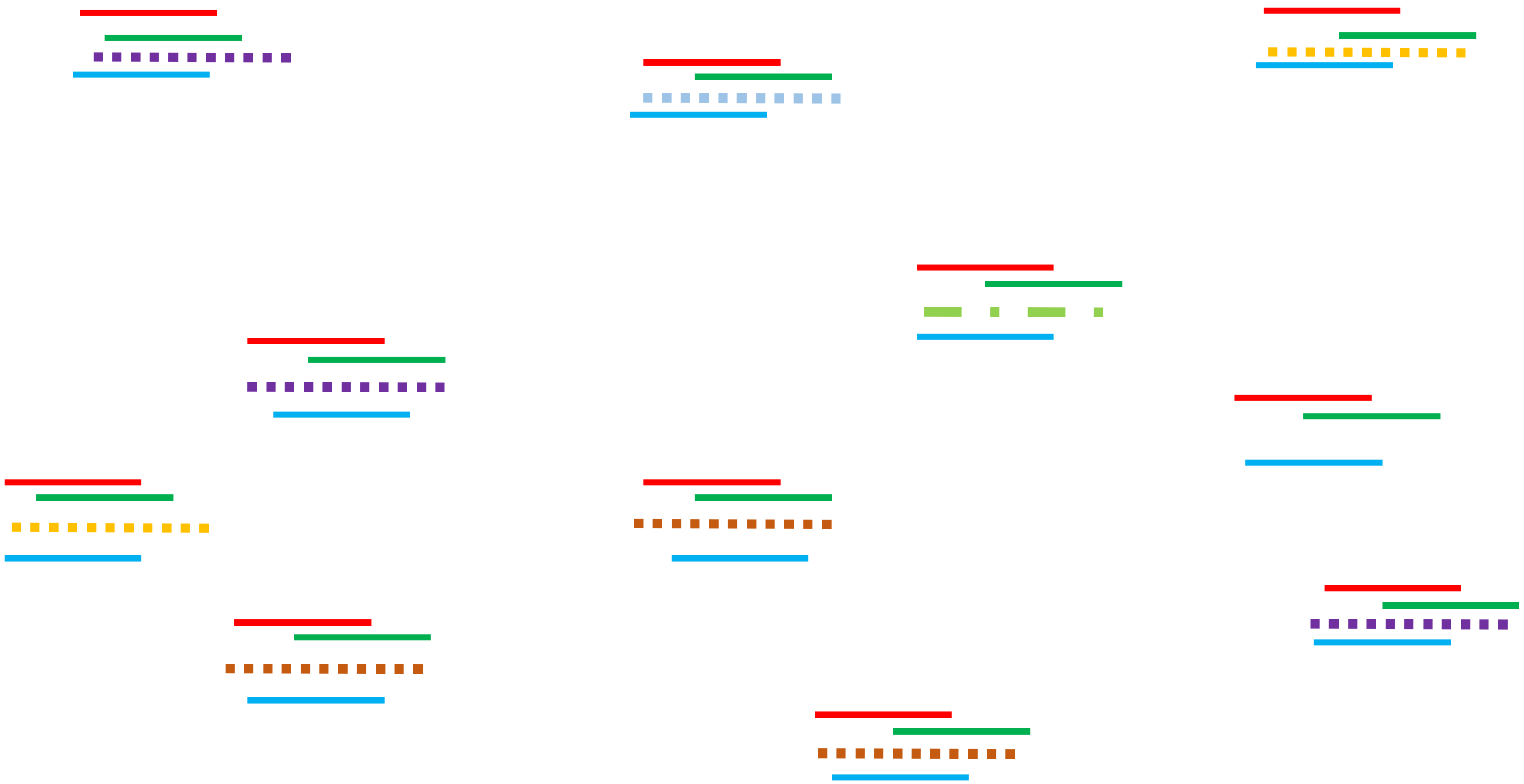
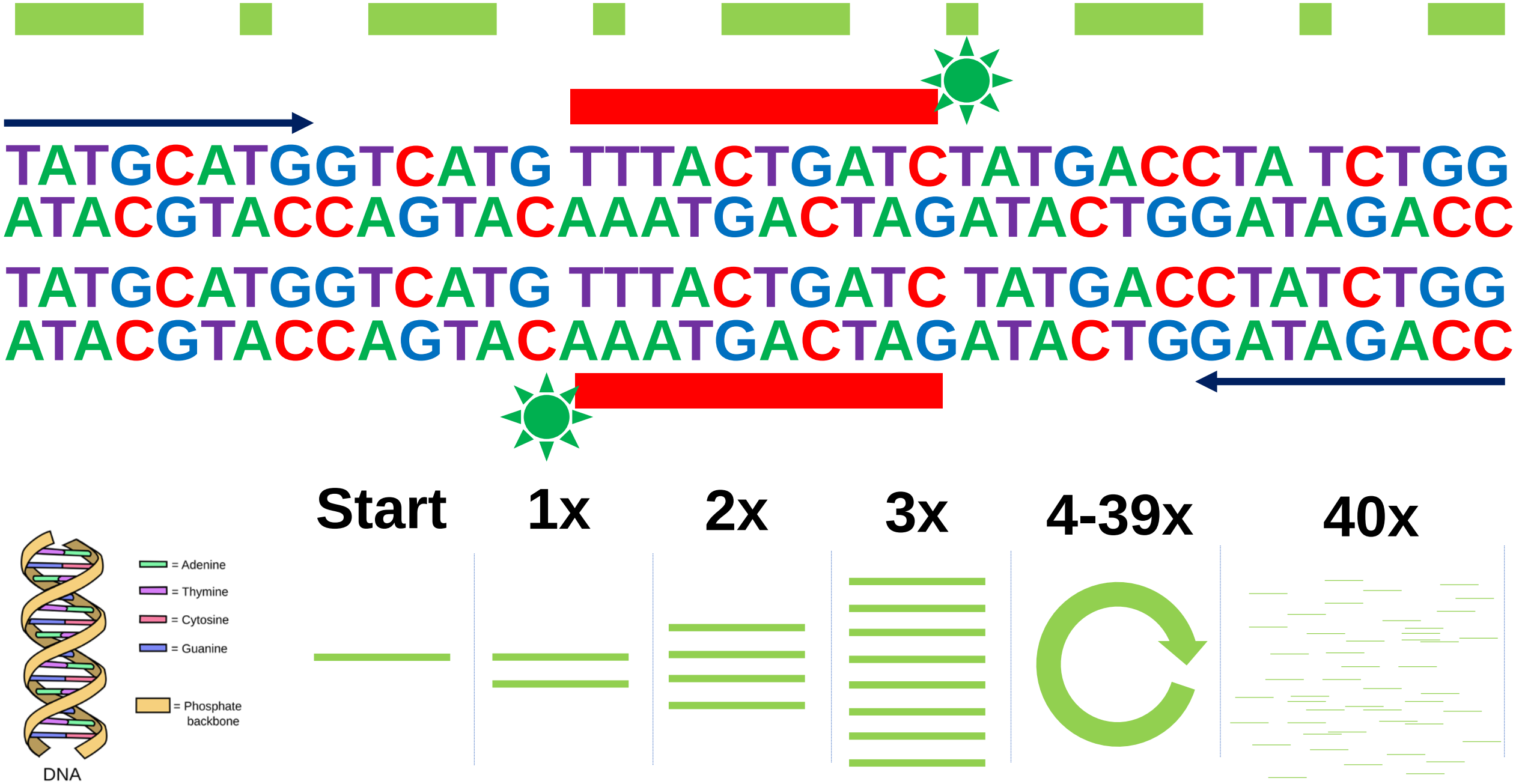
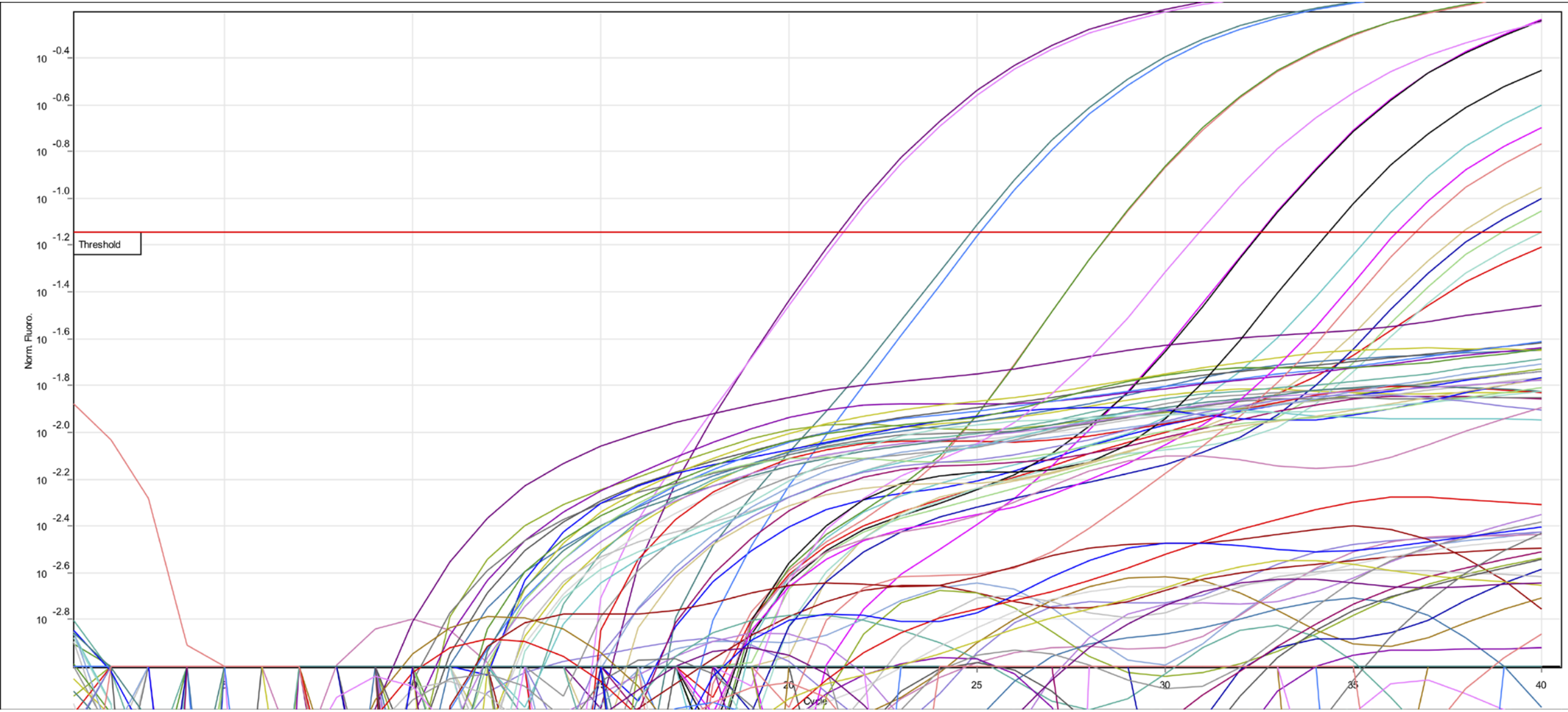
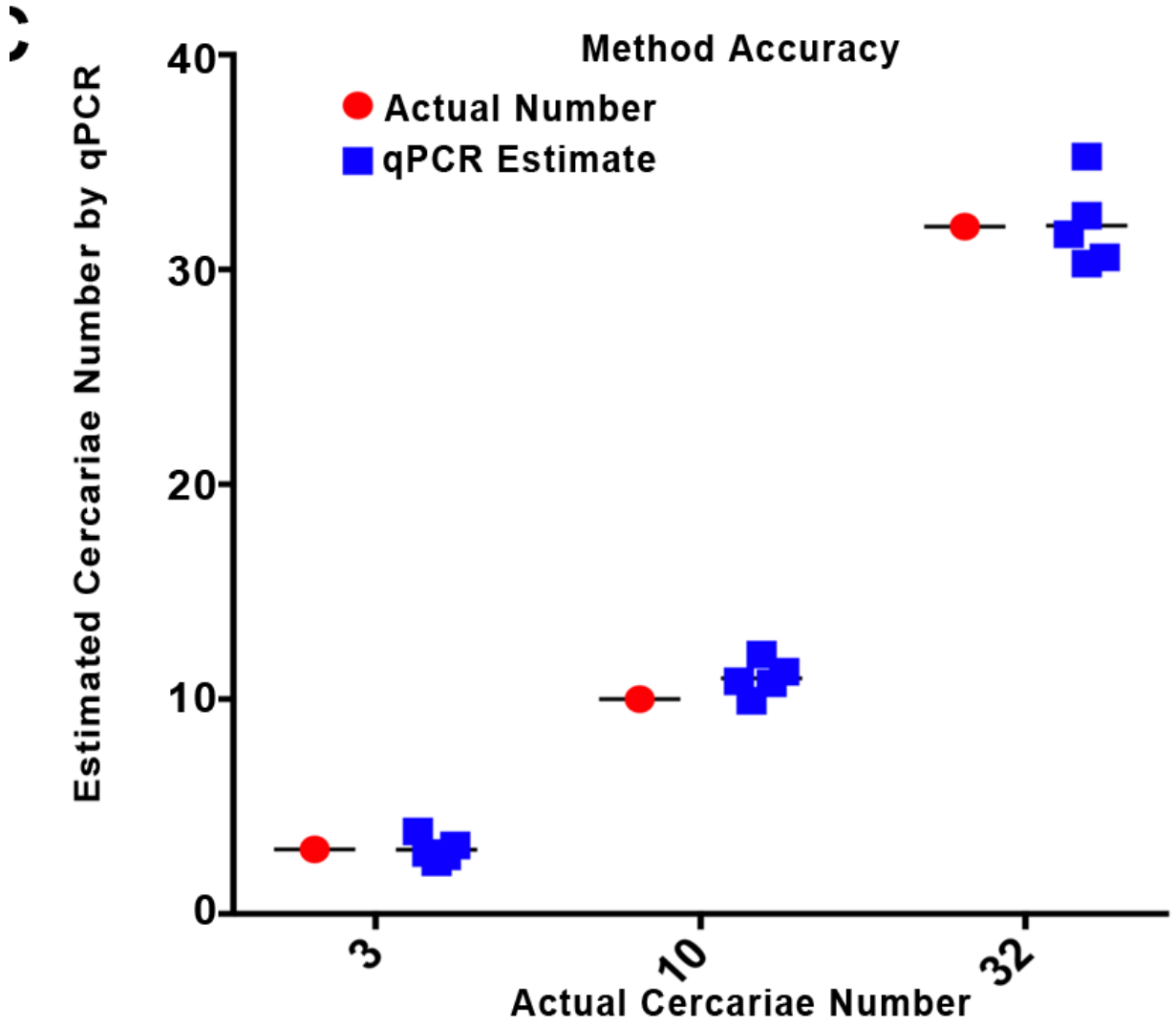



TATGCATGGTCATG TTTACTGATCTATGACCTA TCTGG
ATACGTACCAGTACAAATGACTAGATACTGGATAGACC
TATGCATGGTCATG TTTACTGATC TATGACCTATCTGG
ATACGTACCAGTACAAATGACTAGATACTGGATAGACC
Start
1x
2x
3x
4-39x
40x
= Adenine
= Thymine
= Cytosine
= Guanine
= Phosphate backbone
DNA


Threshold
Norm. Fluoro.
10 -0.4
10 -0.6
10 -0.8
10 -1.0
10 -1.2
10 -1.4
10 -1.6
10 -1.8
10 -2.0
10 -2.2
10 -2.4
10 -2.6
10 -2.8
20
25
30
35
40
Cycle

Blind validation to confirm quantification



- How many copies of the target gene are in the organism?
- Are there variations in the organism that can influence copy number?
- What is the extraction efficiency from the collected matrix?
- Are PCR inhibitors present in the matrix?
- Are there other organisms in the sample that could generate false-positive results?

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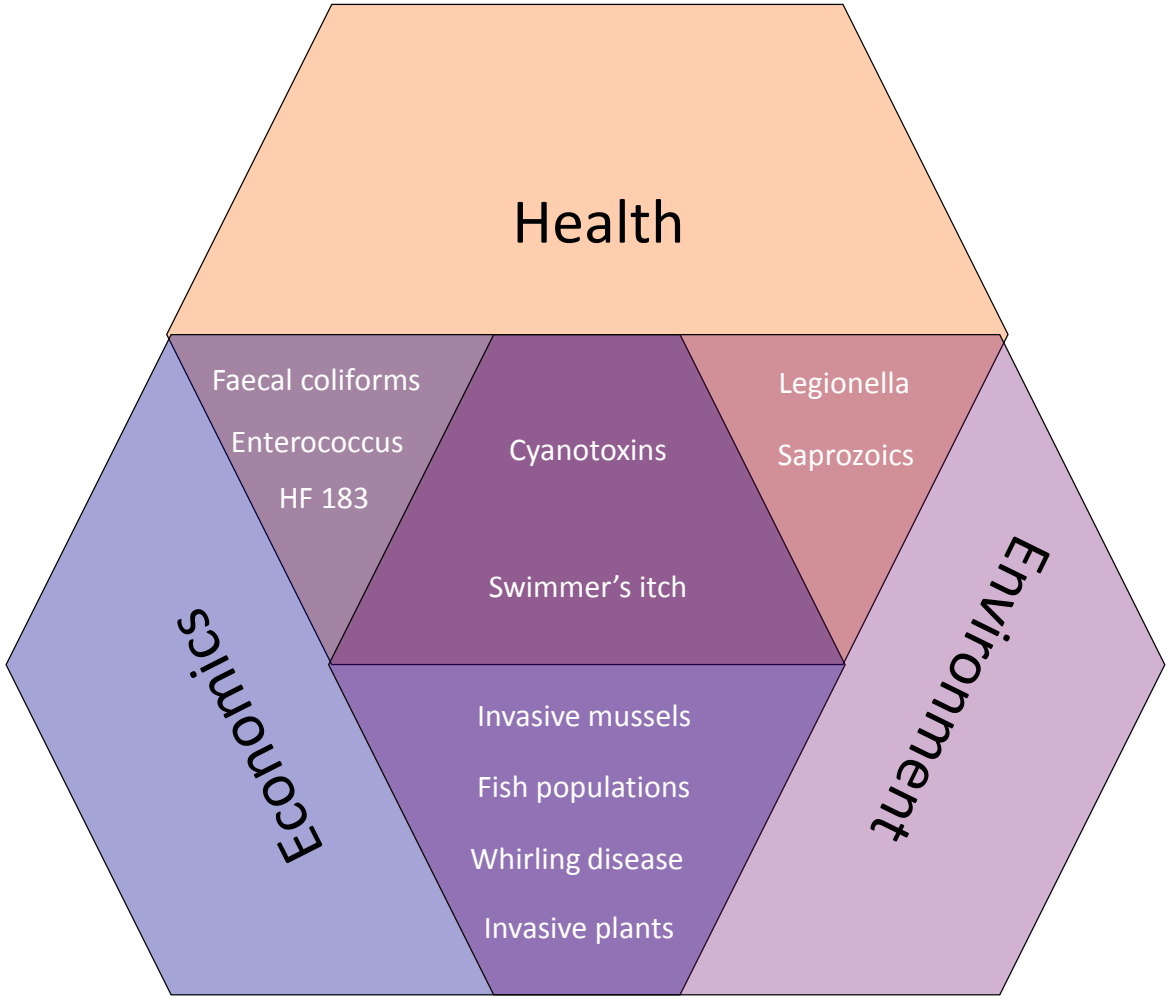

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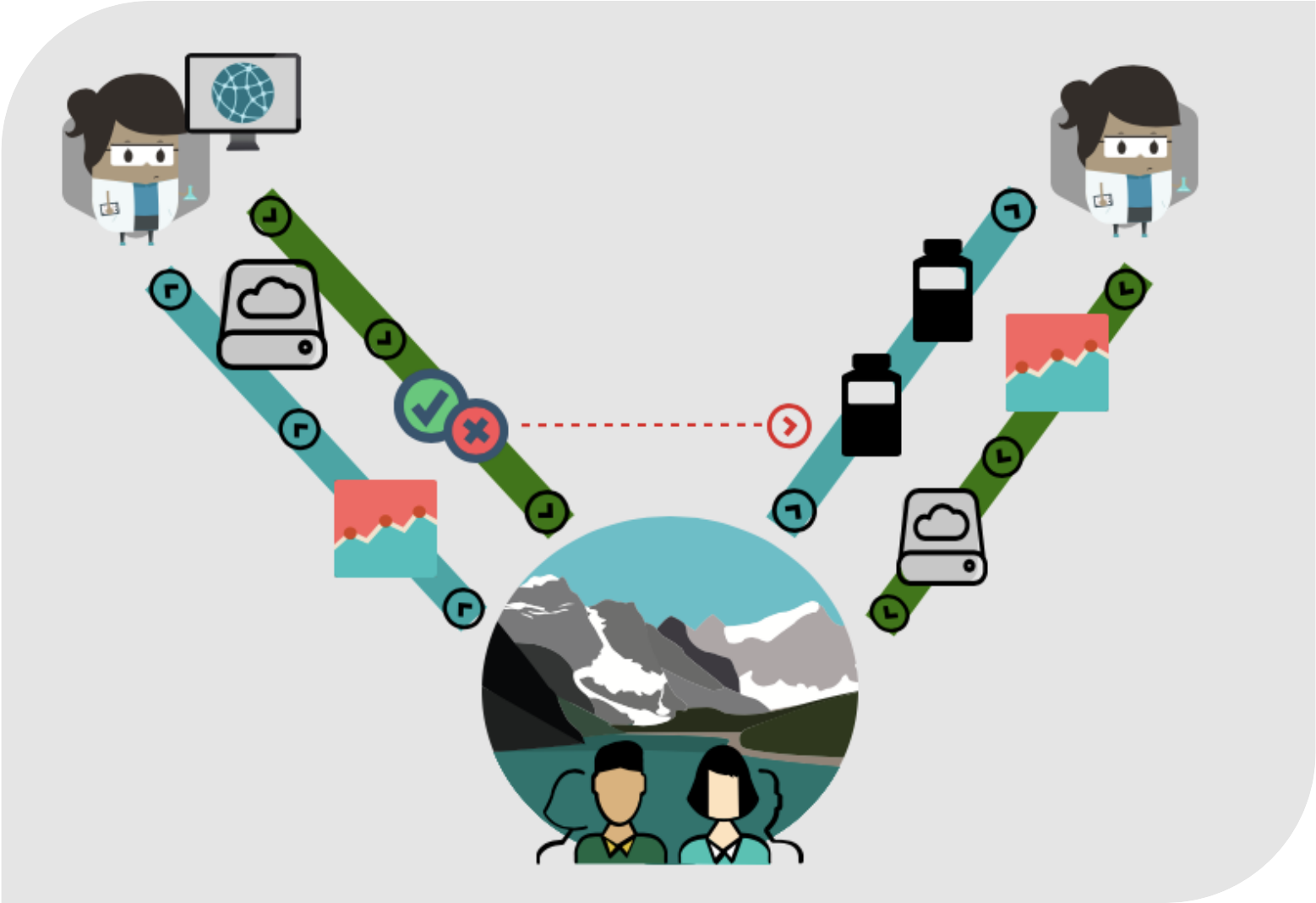
Changing the way we think about monitoring water

WASTE WATER | STORM WATER | RECREATIONAL WATER | DRINKING WATER



Adapting lab methods for remote use

Water collection?

DNA extraction?

Raw water?

Hydragel system?

Lyophilized qPCR mastermix?

Liquid master mix?

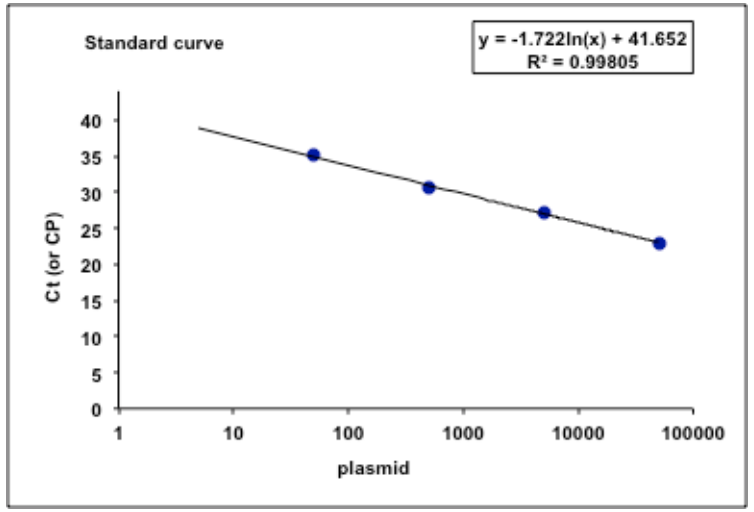
Thermocycler

Cloud reporting?

Does portable equipment perform comparably to lab equipment? What are the differences/weaknesses?

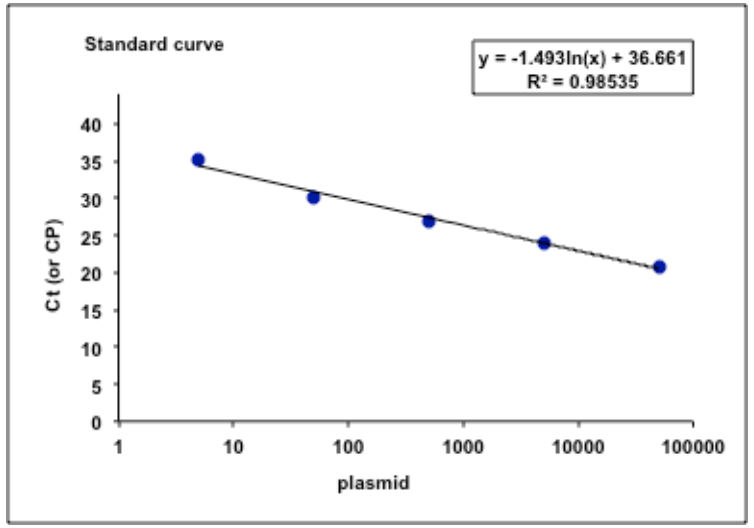
Chai - Portable

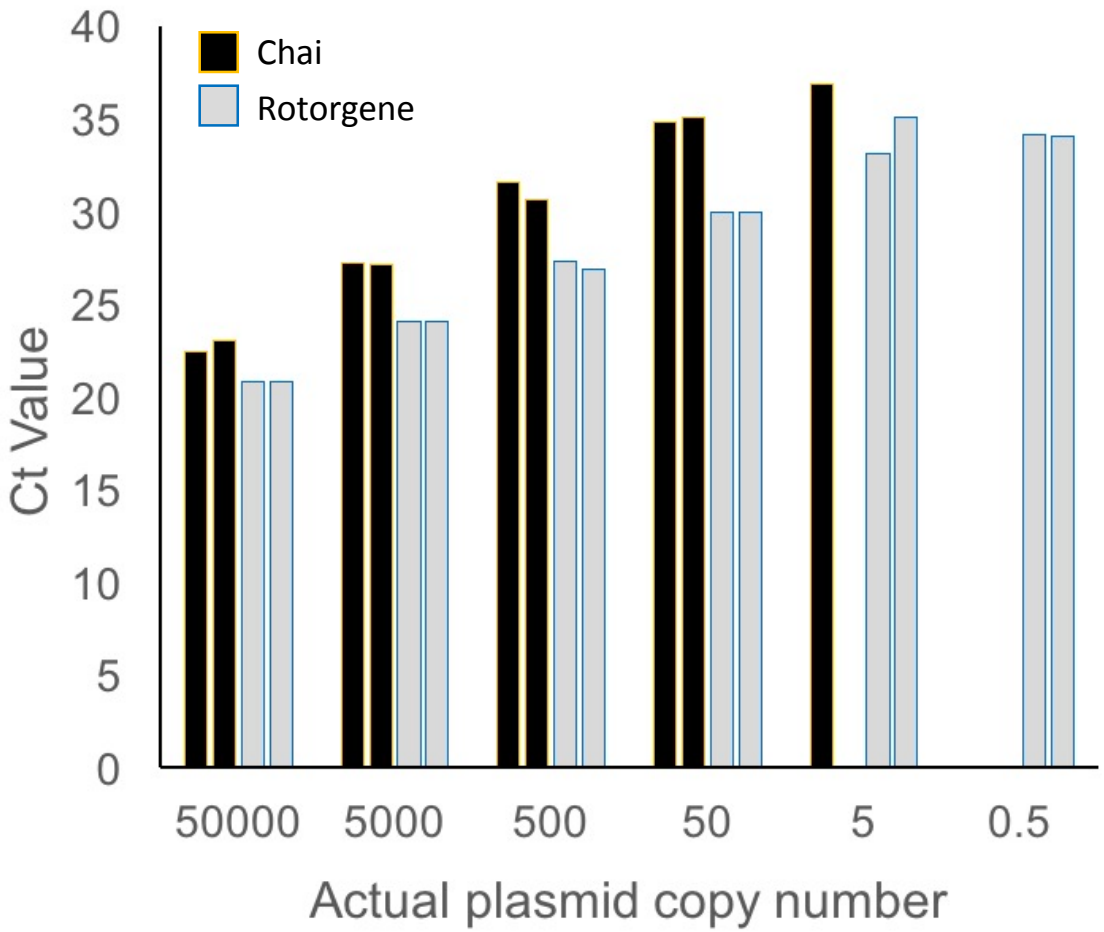
R2= 0.998
Eff= 1.6-1.7 (ideally 2)
LOD95: 50 copies

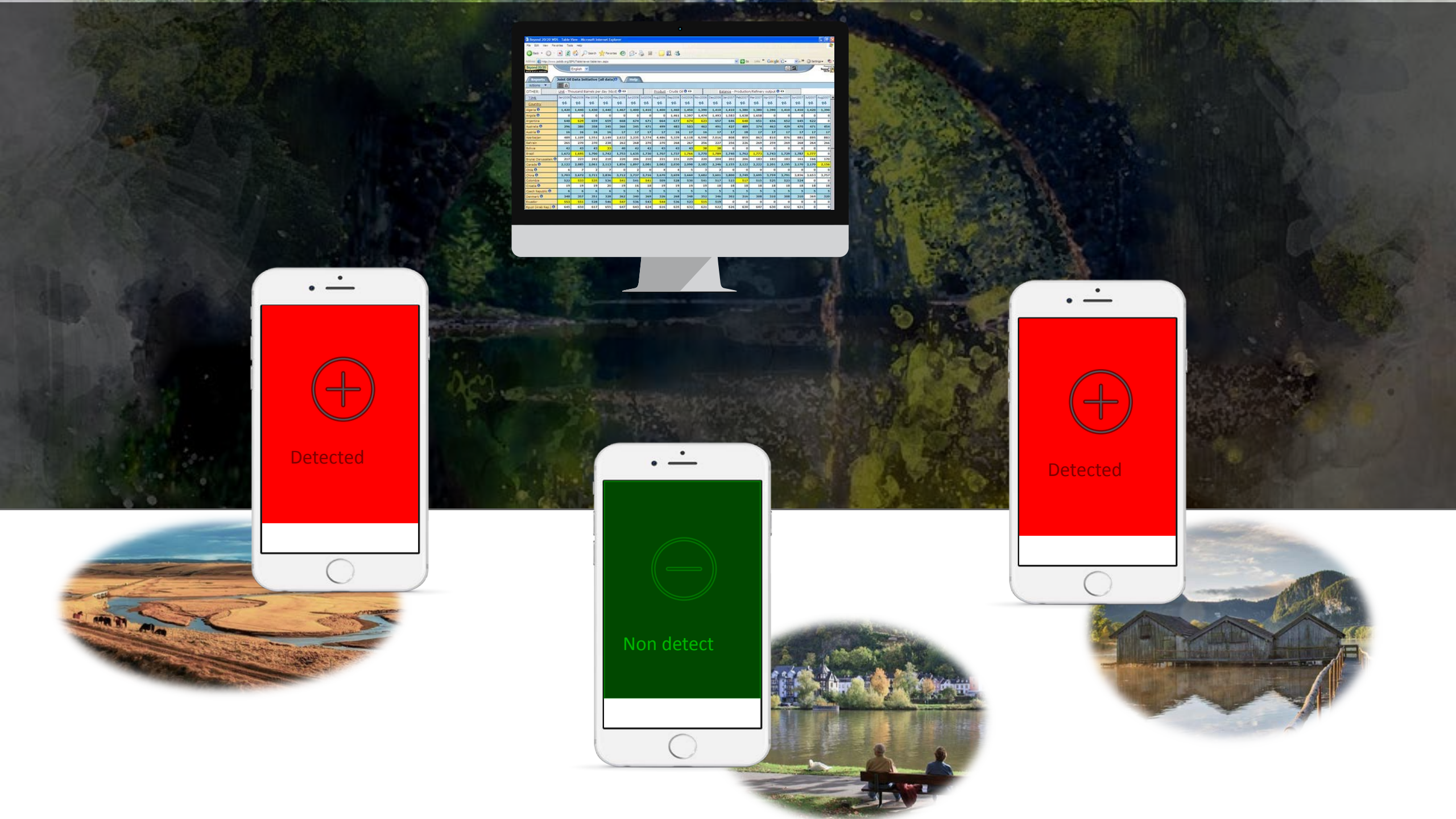


Rotorgene - Lab

R2= 0.985
Eff= 1.9 (ideally 2)
LOD95: 2 copies






Detected
Non detect
Detected

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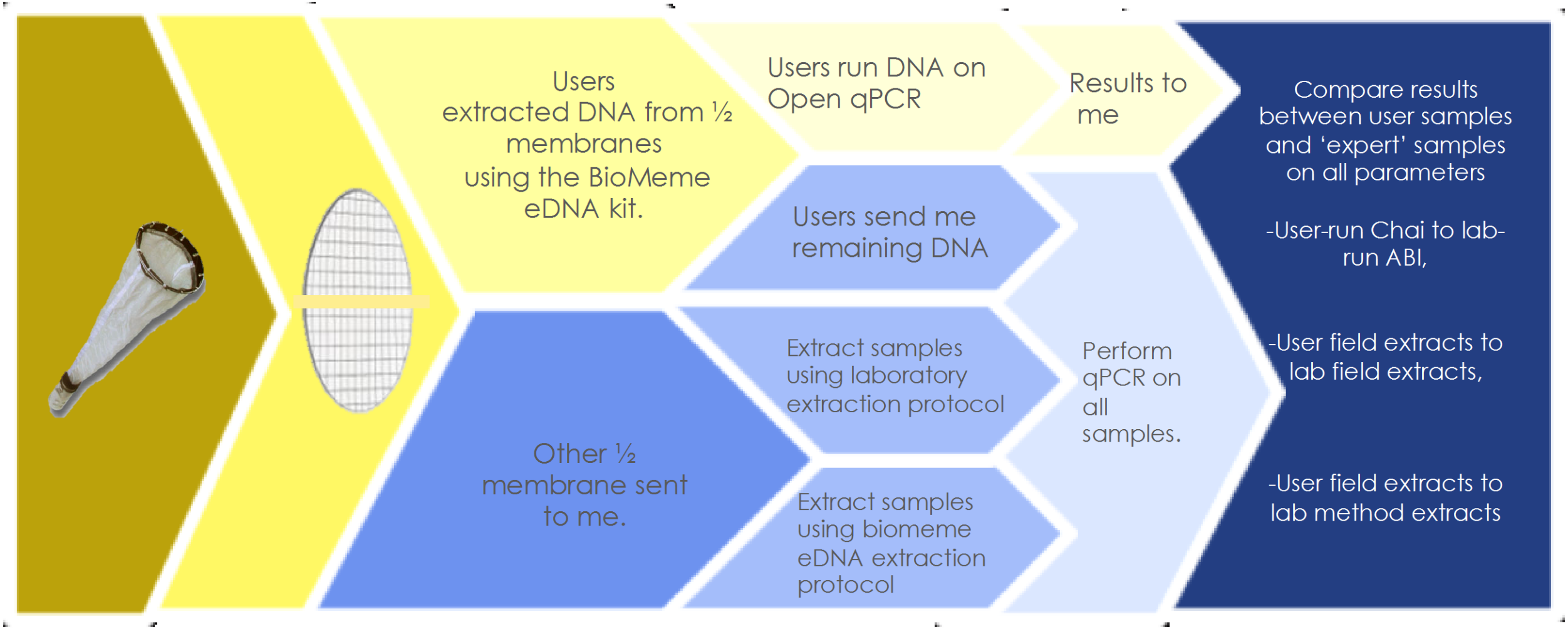
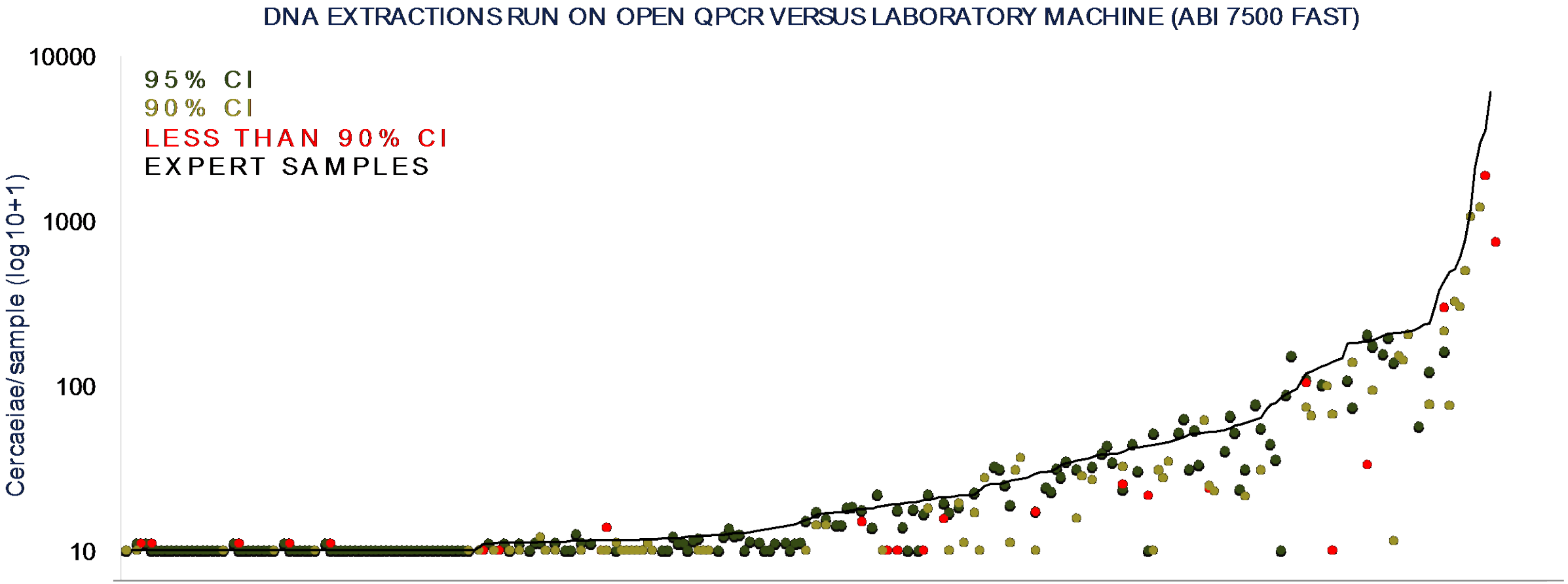
Establishing an experimental design that allows us to measure inexperienced user success (or failure)




On the North Wall of the Nave
Stanford University Chapel

Comparing experienced and inexperienced qPCR user results



User's DNA extractions were run in duplicate using the lab reagents and equipment. Data was used to estimate 95, 90% confidence intervals. Users cercariae numbers were then compared to these confidence intervals.

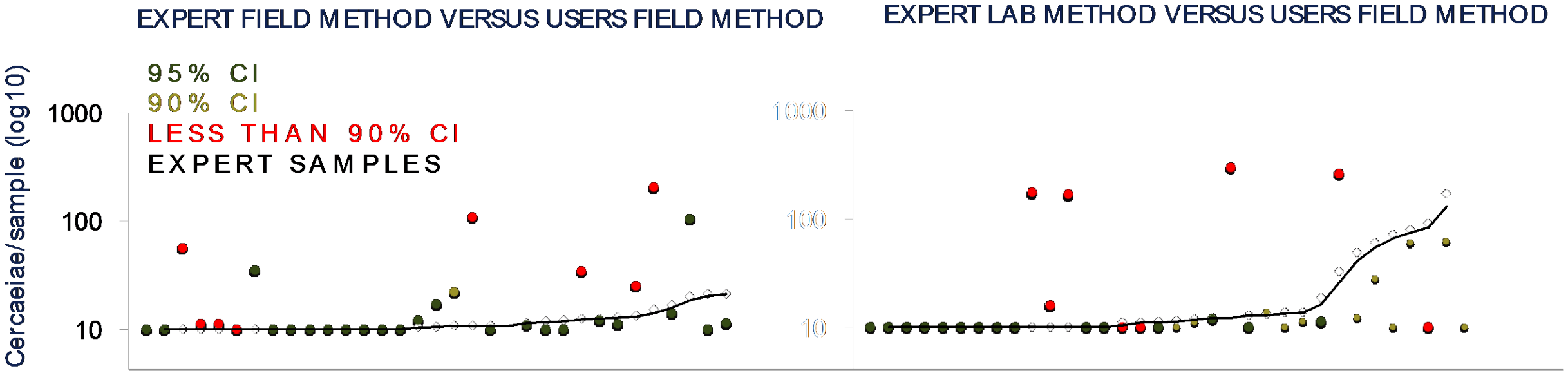
355 SAMPLES

64.8% within 95% CI

91.8 % within 90% CI

0 50 100 150 200 250

Identifying where the error is: comparing DNA extraction between methods and users



68.8% Within 95% CI

78.1 % within 90% CI

User's DNA water samples were extracted and analyzed using the field method, and the lab method. Data was used to estimate 95, 90% confidence intervals. Users cercariae numbers were then compared to these confidence intervals.

42.9% within 95% CI

76.5% within 90% CI

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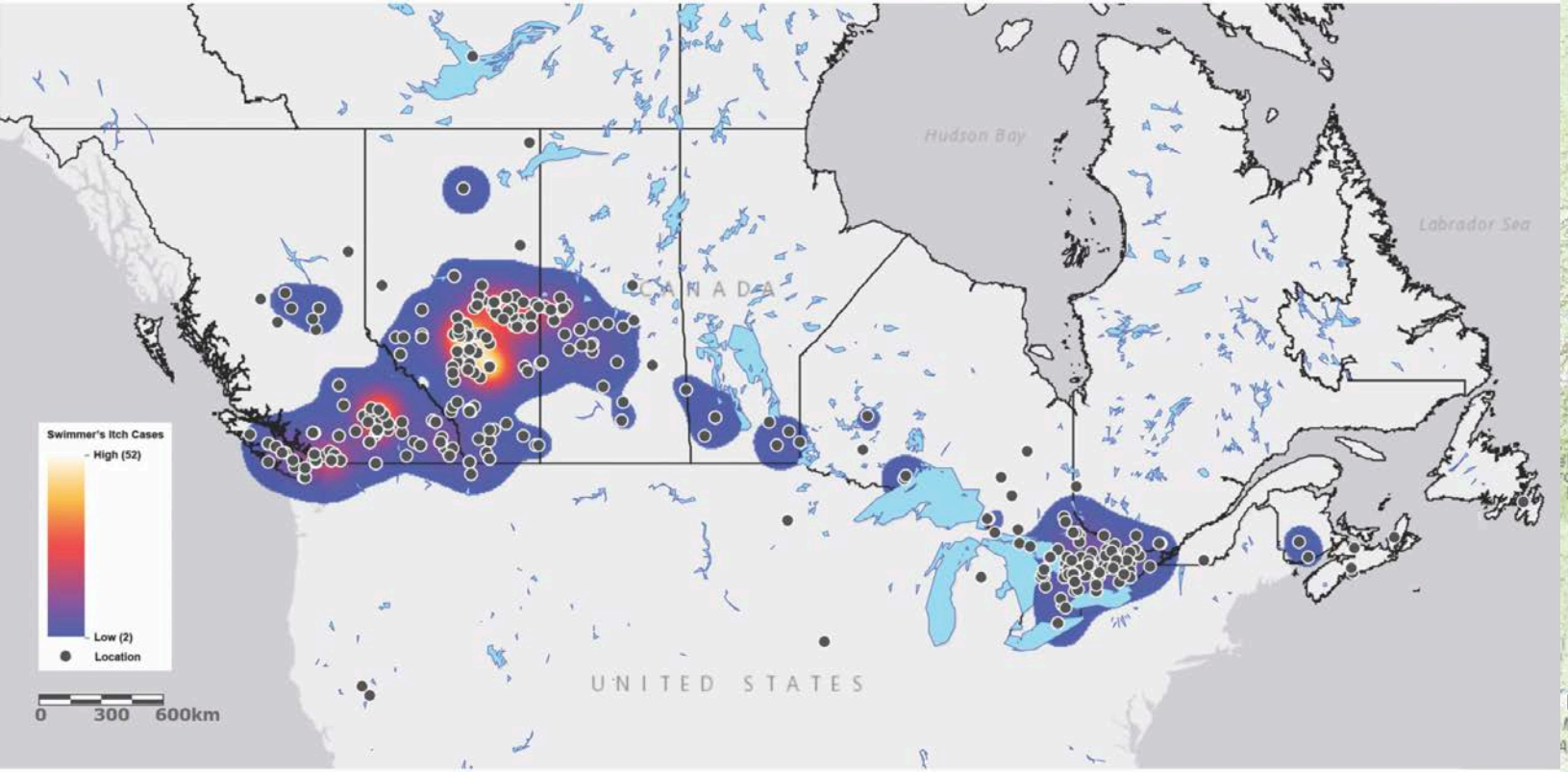
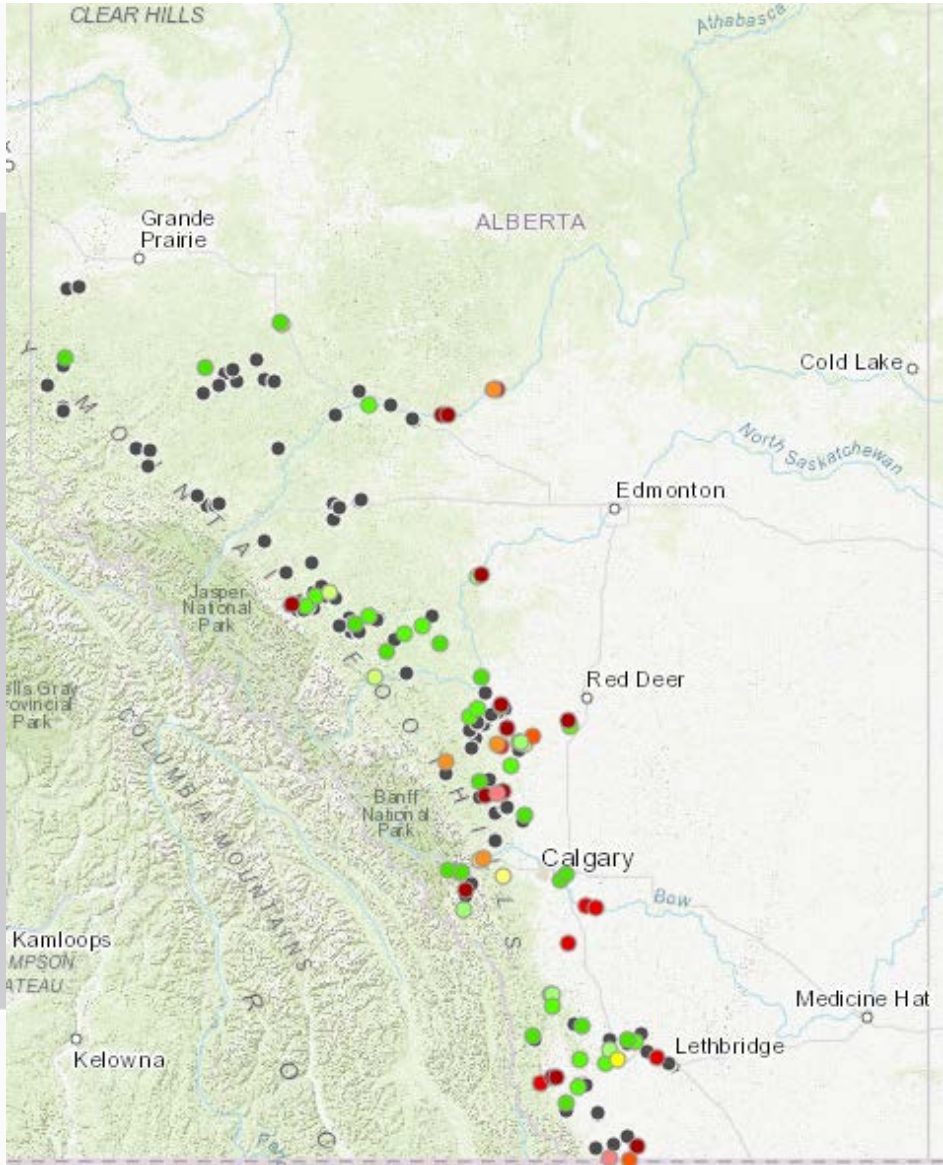
Some examples of how we've advanced our understanding of water-based organisms





Acknowledgments

Michelle Gordy | Sydney Rudko | Danielle Barry

Emmanuel Pila | Jacob Hambrook| Dr. Hongyu Li | Dr. Nick Ashbolt | Dr. Norm Neumann | Dr. Lilly Pang | Dr. Ron Zurawell | Ron Reimink | Marie Veillard | Clayton James | Bev Larson | Peter Giamberardino | Dr. Heather Proctor | Dr. Mike Pauldon | Dr. Simon Otto | Bradley Peter | Laura Redmond | Arin MacFarlane Dyer | Kathryn Wagner | Inside Education | Jay White

Mahmoud Tarrabain Lisa Kish Valerie Phillips Cerina Lee Emily Buss Alyssa Turnbull
Arnika Oddy van-Oploo Leah Brummelhuis

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ALMS Alberta Lake Management Society

NSERC CRSNG

Biomeme