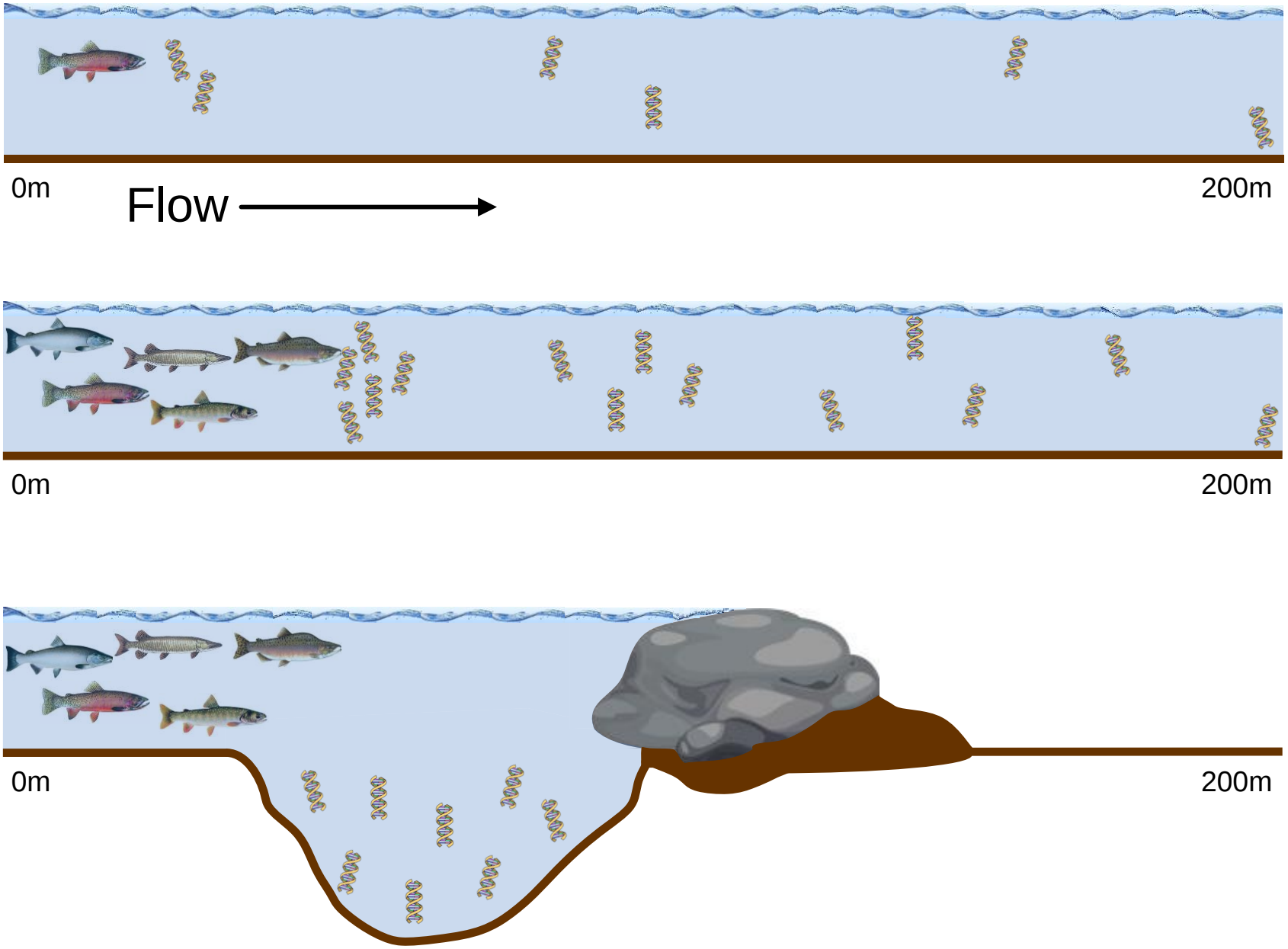
ENVIRONMENTAL DNA DETECTION OF CRYPTIC, RARE OR PROTECTED AQUATIC SPECIES

CRAMER FISH SCIENCES
An employee-owned company

GENIDAQS℠
Aquatic Species Sampling Simplified

WHAT IS EDNA?

eDNA comes from biological material shed into the environment in the form of tissues, cells, feces, and naked DNA.


0m
Flow
200m
0m
200m
0m
200m

eDNA APPLICATIONS ARE VAST & DIVERSE

- Desert / terrestrial
- Wetland interface / semi – aquatic
- Estuary / Delta
- Riverine
- Lacustrine
- Alpine
- Coastal forest

OUR PHILOSOPHY

01

Where are they?

02

How many are there?

03

How are they doing?

PRESENTATION OVERVIEW

- Sample/Survey design
  - **Probabiltiy of Detection (PoD)?**
  - Life history
- Sampling methodology
  - Water filtration
    - Pore size
    - Volume
    - Replicates
- Sample storage/preservation/transport
- DNA extraction
- DNA analysis (**qPCR assay validation, MIQE**)
- **Data interpretation**

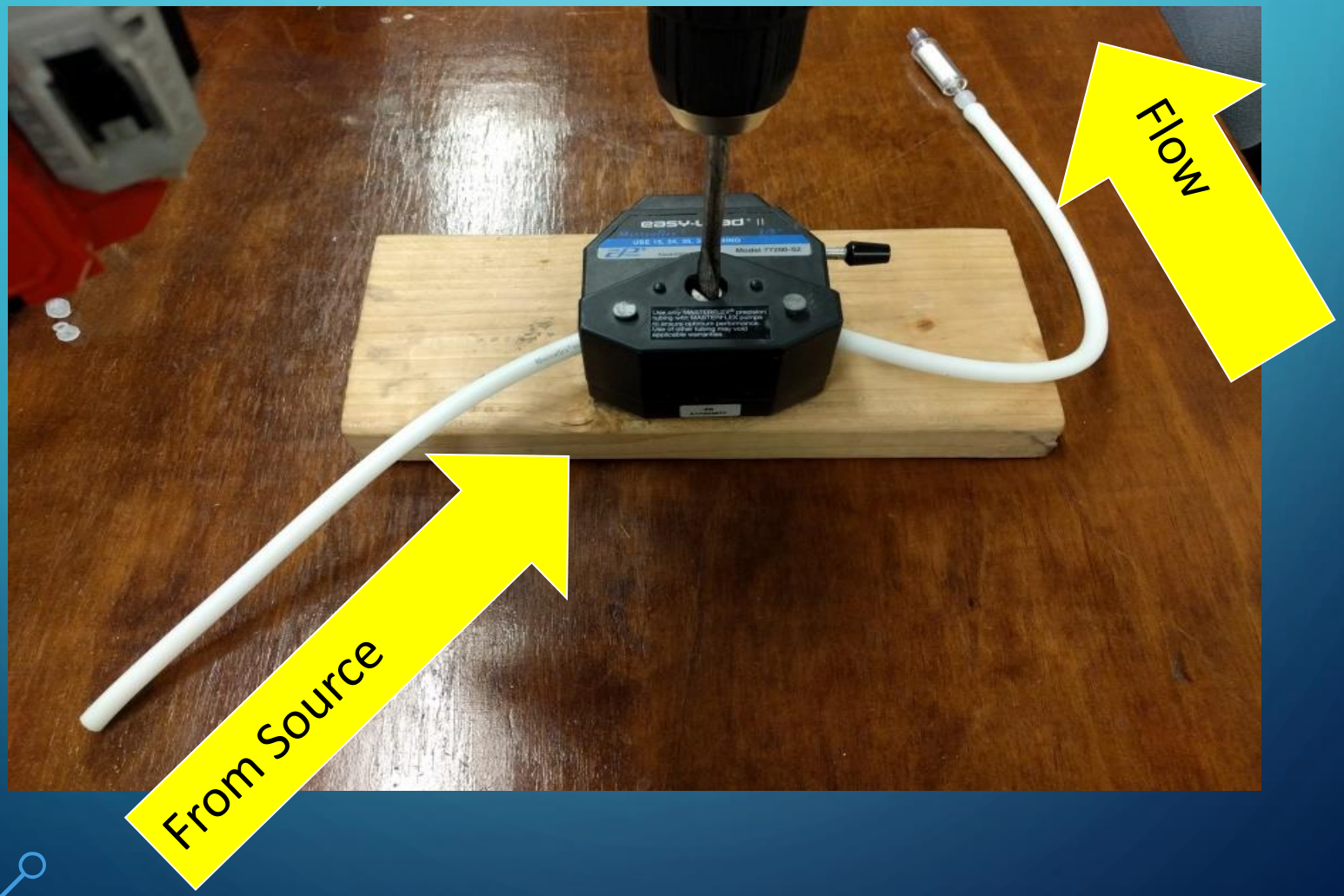
Methods in Ecology and Evolution
BRITISH ECOLOGICAL SOCIETY

Critical considerations for the application of environmental DNA methods to detect aquatic species

Caren Goldberg, et al.

May 2016

COMPLETE APPARATUS



SAMPLING/SURVEY DESIGN

- Sampling apparatus
- Probability of Detection (PoD)
  - Habitat
  - Filter type
  - Volume
  - Replication
  - Frequency
  - Interval
  - Life History
- Budget
- Expectations?

Probability of detecting (PoD) eDNA

Determining a PoD

- Volume
- Filtering and filter type
  - Disc vs Sterivex
- Distance from source
- Biomass
  - Live vs Dead

<u>Laboratory: DNA extraction and qPCR or Metabarcoding</u>

Determining PoD of Delta Smelt at CVP

Sample direction

N=20-100

Volume = 50ml - 300ml/ filter

Live

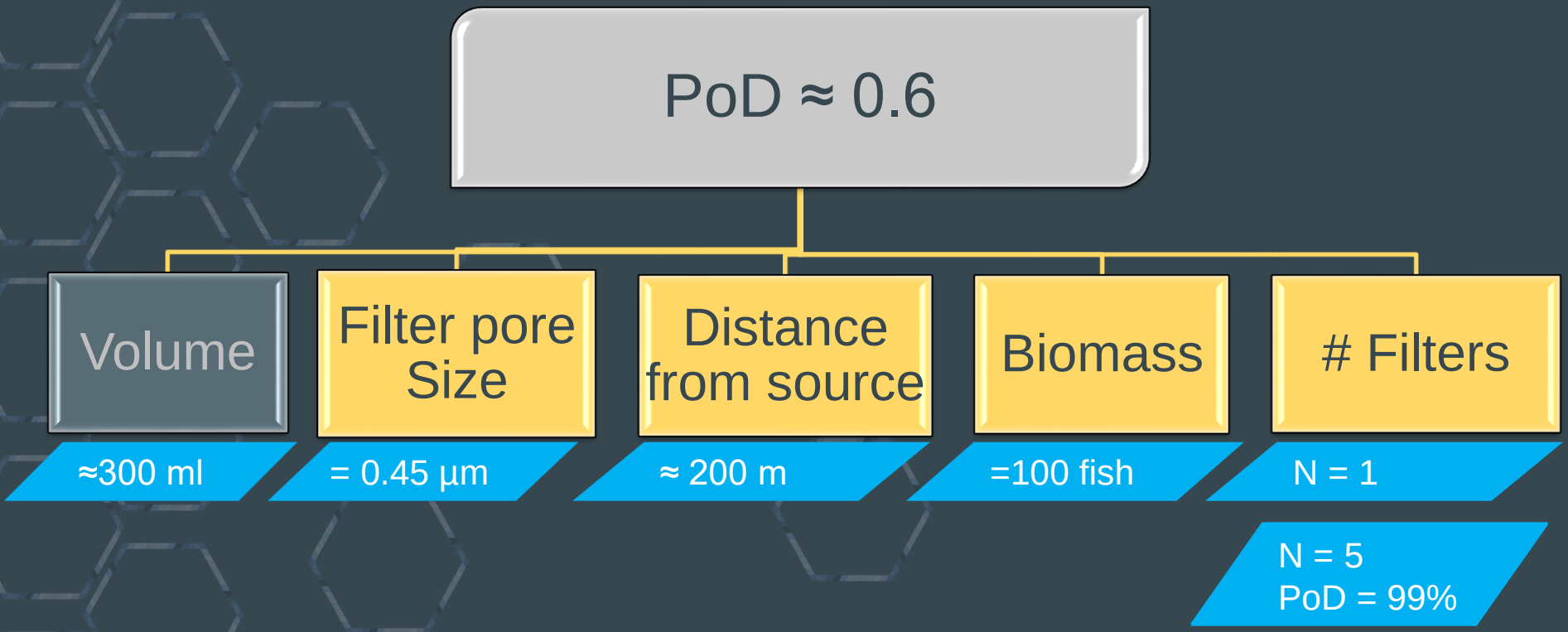
Live

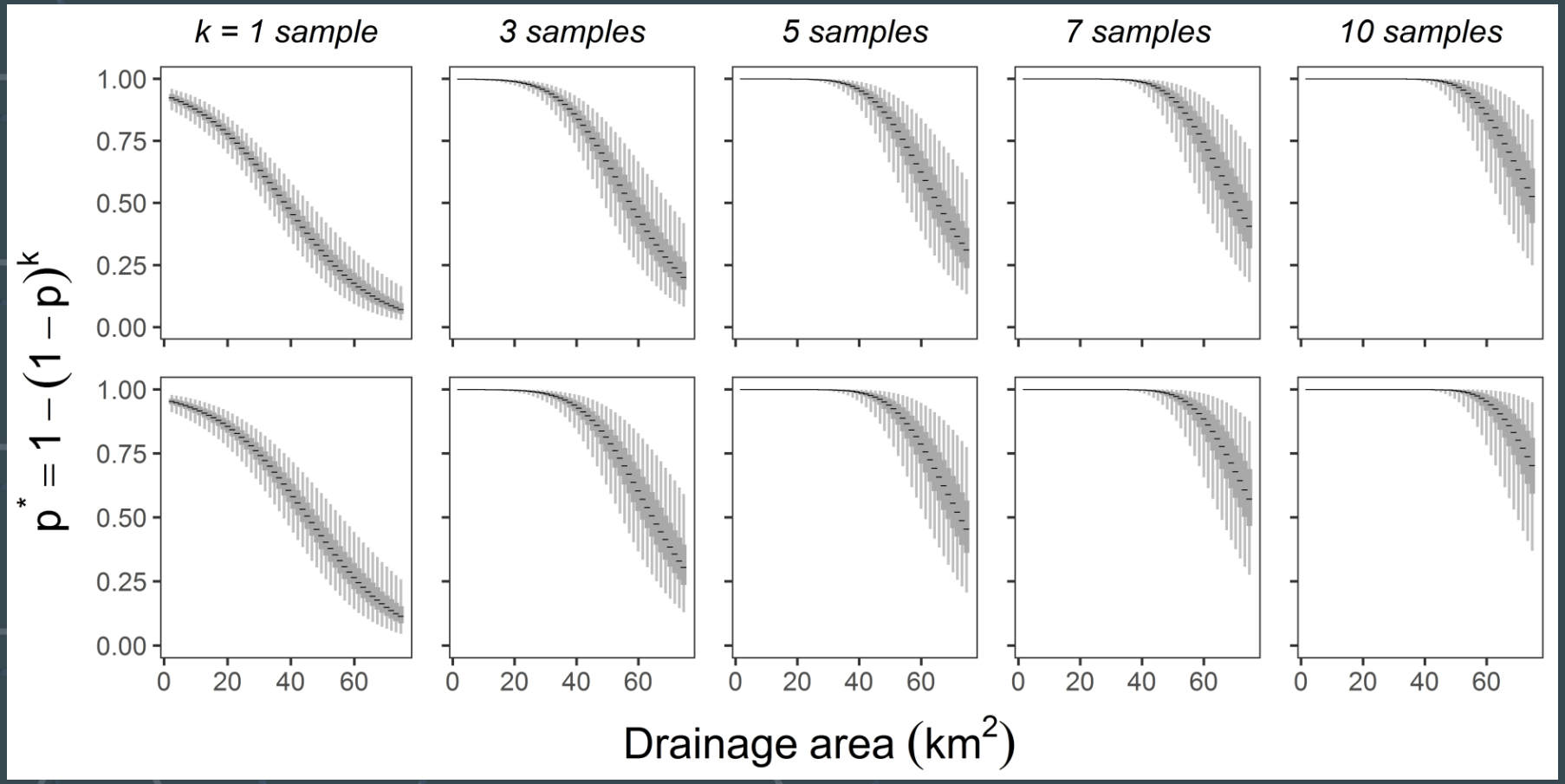
Replicate = 2 filters/ point

Distance	10	20	30	40	50	200	300	400	500	600	700	800	900	1000

This distance configuration can be adjusted based on access/safety

What is Delta Smelt PoD at CVP?





* Eric Waits and Roy Martin
US EPA

ASSAY VALIDATION QUANTITATIVE POLYMERASE CHAIN REACTION (QPCR)

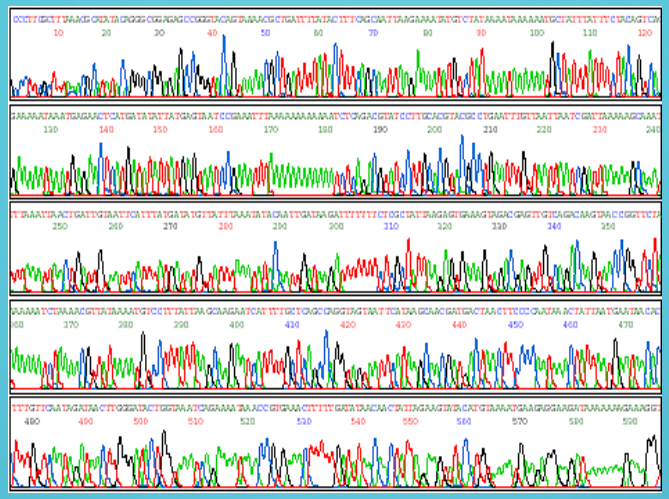
ASSAY DOCUMENTATION

The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.

Bustin et al. 2009 Clinical Chemistry 55(4)

DNA Extraction

DNA Sequencing



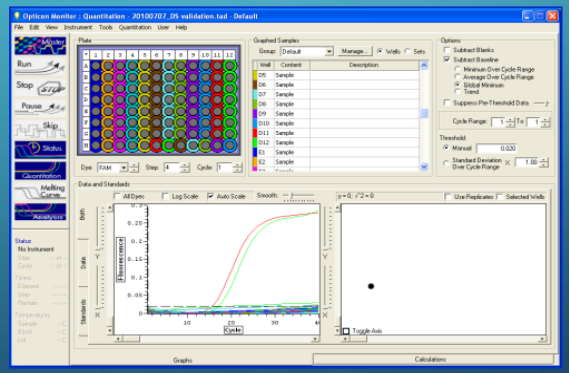
Barcodinig DNA Data Analysis

1.Find Conserved DNA Sequence

TTAATTCAACTACAAGAACCC
TAATGGCCAACCTTCGGAAAT
TAATTCAACTACAAGAACCC
TAATGGCCAACCTTCGGAAA

2."In Silico" Validation: Nucleotide BLAST
http://blast.ncbi.nlm.nih.gov/Blast.cgi

3. Assay Design
TTAATTCAACTACAAGAACCC
TAATGGCCAACCTTCGGAAA
Primer Express Software v2.0 ™
Applied Biosystems



Assay Optimization/Validation (Sensitivity, Specificity)

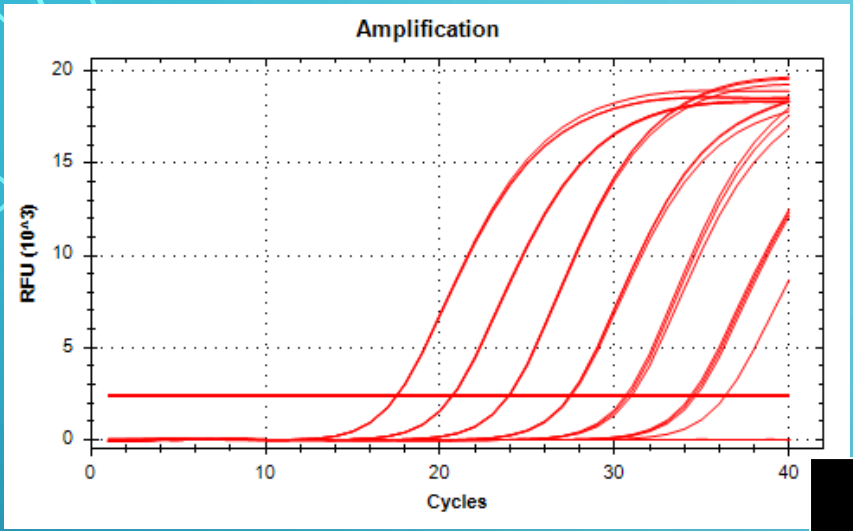
Unknowns? Test on Field Samples

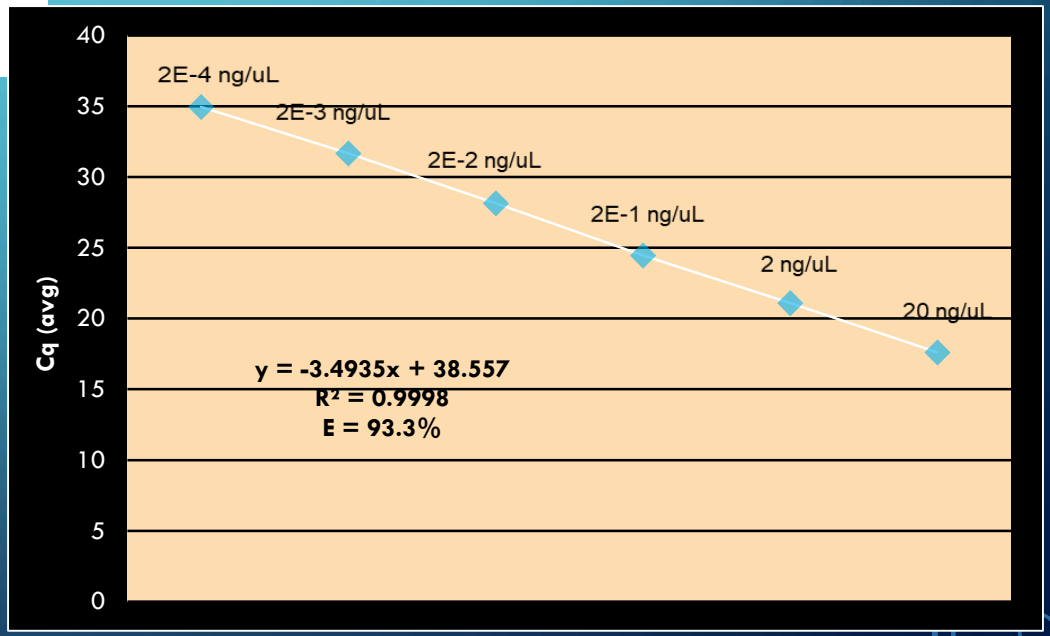
Vouchered Specimen

qPCR Assay Development 16s, CytB

ASSAY VALIDATION

- Assay sensitivity and limit of detection





- PCR Efficiency (E) = 93.3% (slope -3.4935)
- Y-intercept = 38.557
- $R^2 = 0.9998$
- 40% amplification of 2.0×10^{-4} ng μL^{-1}

DATA

eDNA is a presence absence dataset

- eDNA is either there or not there?
  - C(q): strength of detection
  - Strength of detection in relation to other sites
- Where are they?
  - When are they there?

- Occupancy Model (ESA)
- Eradication or containment

Where are they?

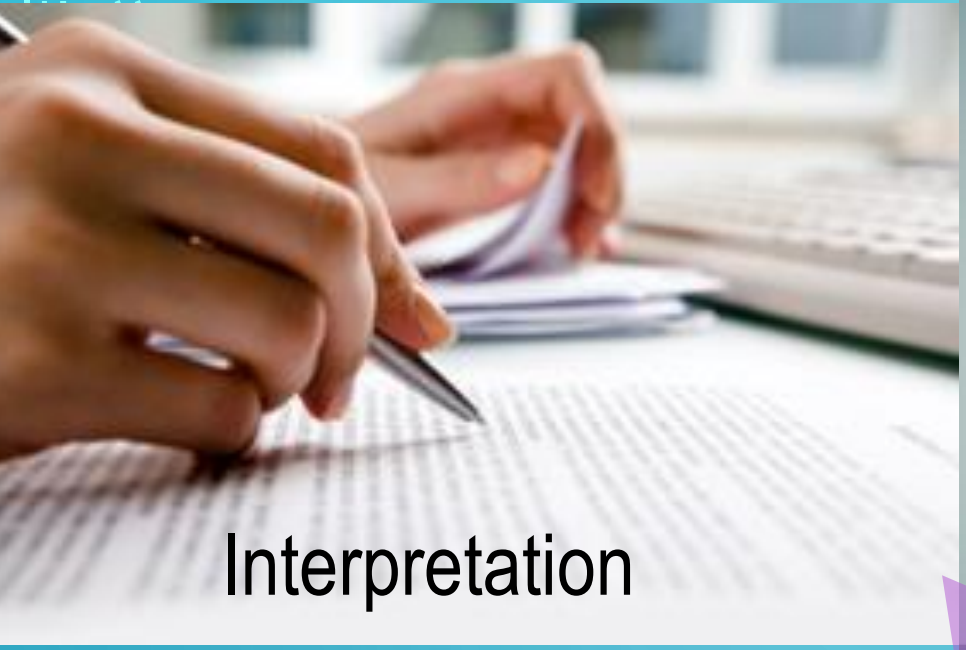
How many are there?

N=?

How are they doing?

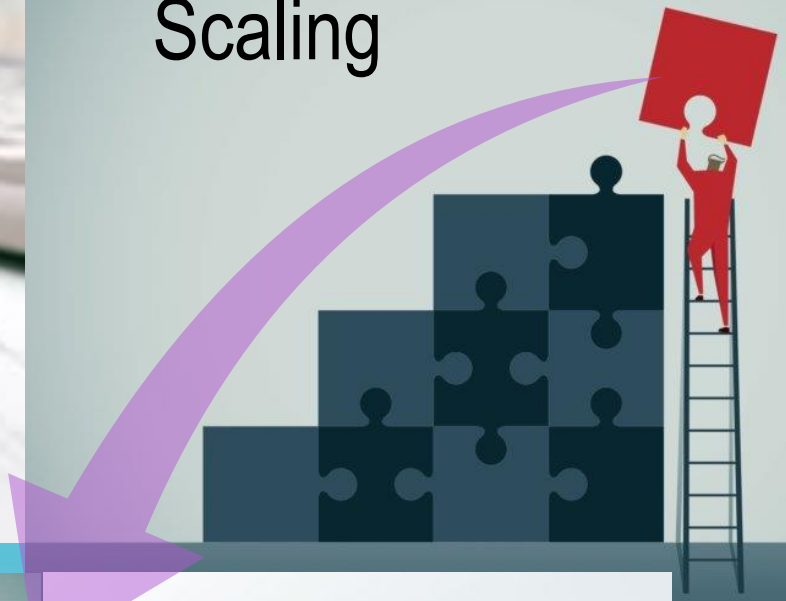
Our ability to generate data has out paced our ability to confidently analyze, interpret, and compare across labs.

- Bioinformatics
- Publically available DNA reference databases
- Publically available qPCR assay database



Interpretation

Scaling



With caution, cooperation, and continued effort toward a greater understanding of methodology and an adherence to minimum reporting requirements the potential for eDNA will be realized.