

eDNA monitoring for the Asian Swamp Eel

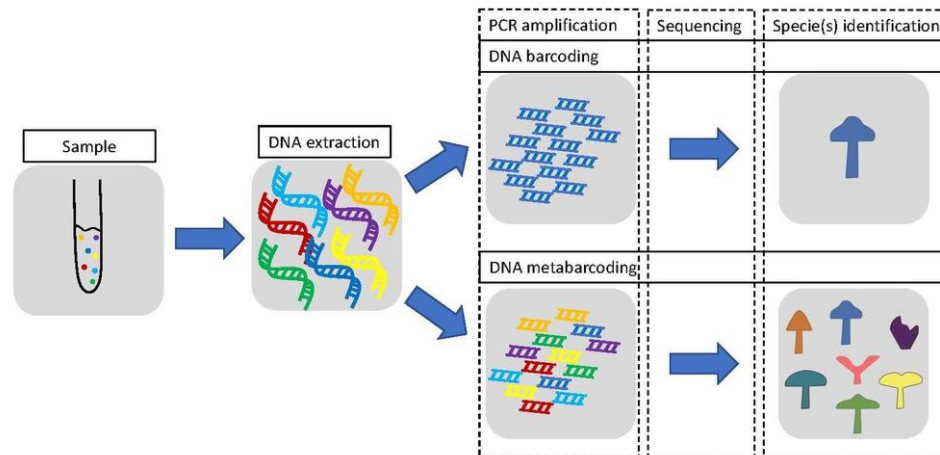


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Brian Bahder

eDNA Monitoring

- Generally, there are two major approaches
 - Metabarcoding (sequence all COI (usually this gene) within a sample)
 - Targeted amplification by digital PCR (dPCR)

Metabarcoding



dPCR

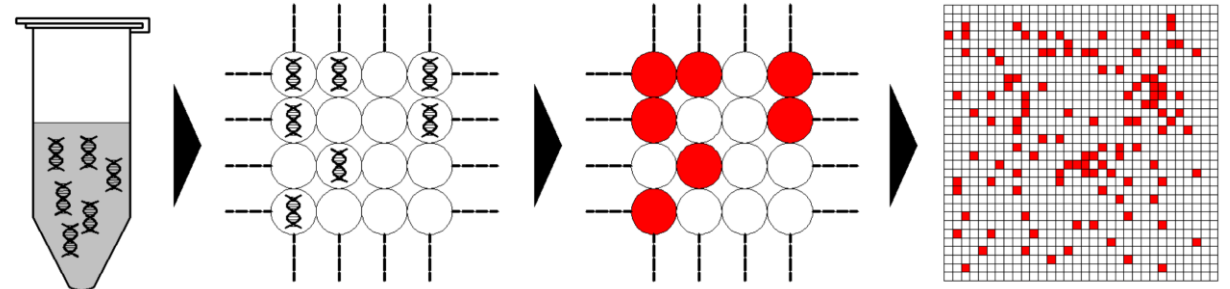


Preparation

Partitioning

Amplification

Analysis



Current Assay

Asian swamp eel (ASE)

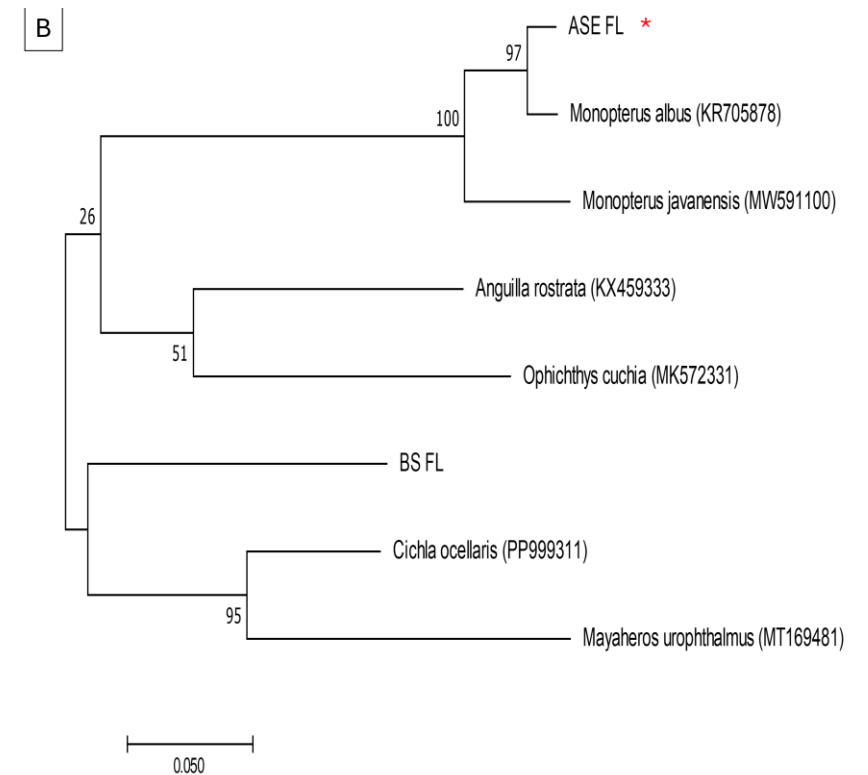
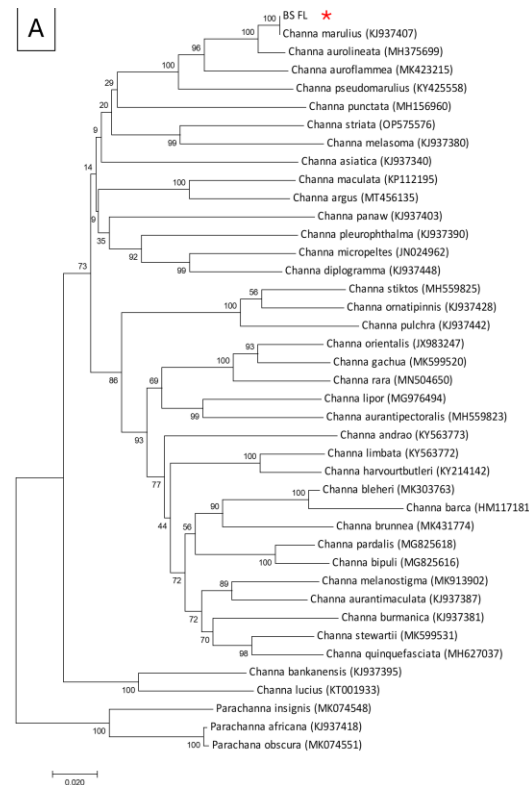


Monopterus albus

Bullseye snakehead (BS)



Channa marulius



dPCR Assay Design

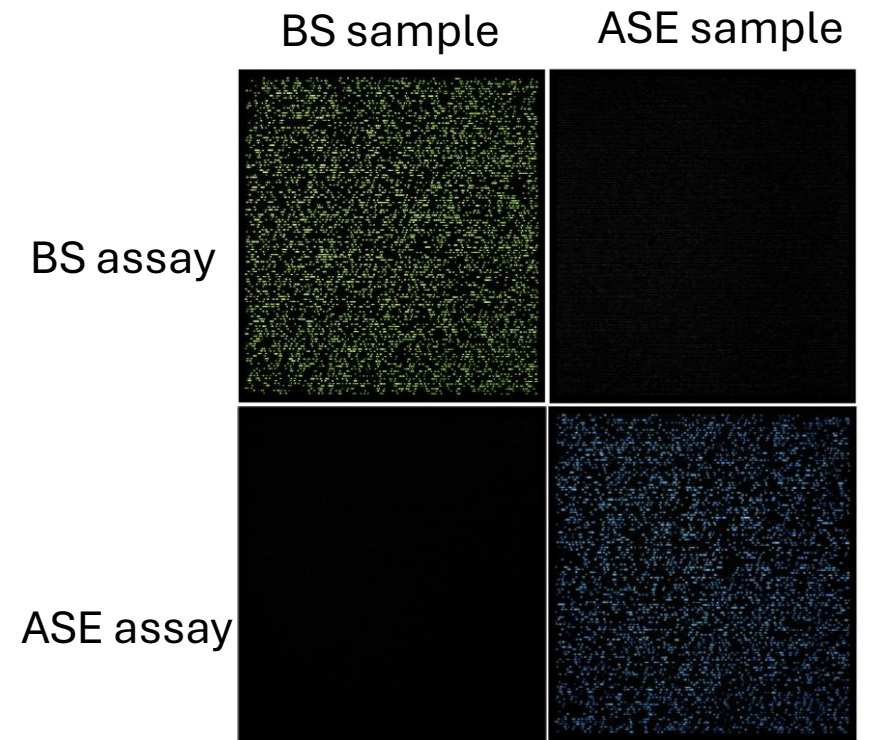
- Suitable region needs to be identified with adequate single nucleotide polymorphisms (SNPs).
- Thermal cycling parameters optimized.

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ASE_FL      ACAGCCCTAAGCCTATTAATCCGAGCAGAGCTCTGCCAACCTGGATCCCTCTTGGGGACGACCAAATCTATAATGTATCGTAACCGCTCAGCCTTC[99]
BS_FL
Monopterus_javanensis_(MW591100) ..C....C....CC....T..G.....A..AA.....C..CG....A..A.....T.....C.....A..T...S..A..A..T....T[99]
Anguilla_rostrata_(KX459333)      ..T..A..G.....TC.....T..C..AT..AA..T....A..CG....TC..T..A..T.....T..C.....C.....A..G..T....T[99]
Ophichthys_cuchia_(MK572331)     ..T..TT.....C..T..T.....A..AA..T....A..TG..T..C..A..A..T.....T.....T..A..C..T..T....T[99]
Cichla_ocellaris_(PP999311)      ..T..A.....C..T.....T.....A..AA.....A..C..T..C..C.....T.....T..C..C.....T..T..A..T....T[99]
Mayaheros_urophthalmus_(MT169481) ..C..AT.....CC..G.....A..TA.....A..C..T..C..T..A.....T.....T.....C..A.....T..A.....T....T[99]

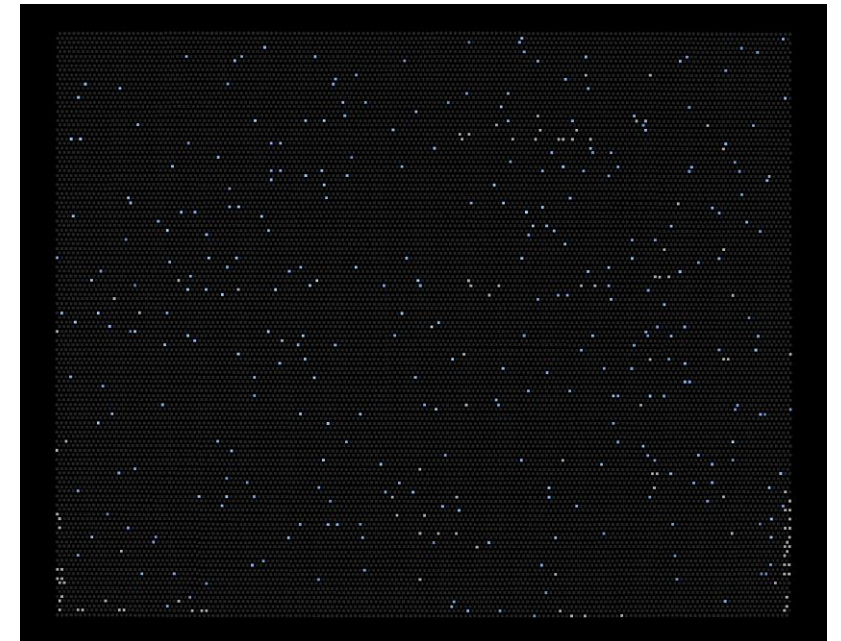
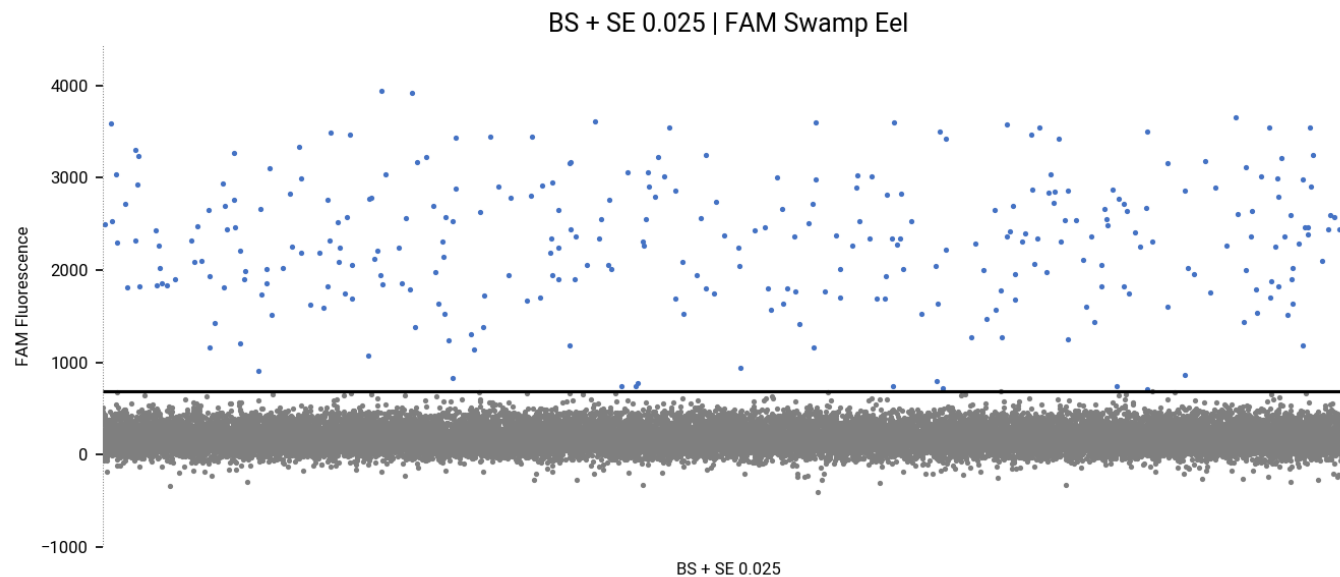
ASE_FL      ATTATATCTCTTTTAGTAATGCCTATTATAATCGGGGCTTCGGAAATTGATTAGTCCCTTAATAATCGGAGCCCCGGACATGGCCTTCCCCCGA[198]
BS_FL
Monopterus_javanensis_(MW591100) G.A....T....C..G..C..A..A..A..G..T..A....T.....C.....T..AC.....T..C.....T.....A..A.....C[198]
Anguilla_rostrata_(KX459333)      G.A....T.....C.....C.....A.....T.....C.....T.....A.....T.....T.....T.....T.....T[198]
Ophichthys_cuchia_(MK572331)     G.A....T.....AG..A.....A..A..T..C..C..C..T..G..A.....C..T..A.....A..G.....T[198]
Cichla_ocellaris_(PP999311)      G.A....T.....A..A..C.....T..A..T..C..C.....T..TC.....T.....T..A..A.....T..T[198]
Mayaheros_urophthalmus_(MT169481) G.A....T.....T..A..A..C..G..T..A.....T.....C..C..A..T..AC.....G..T..C.....C.....A.....T.....[198]

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Determine Optimal Concentration

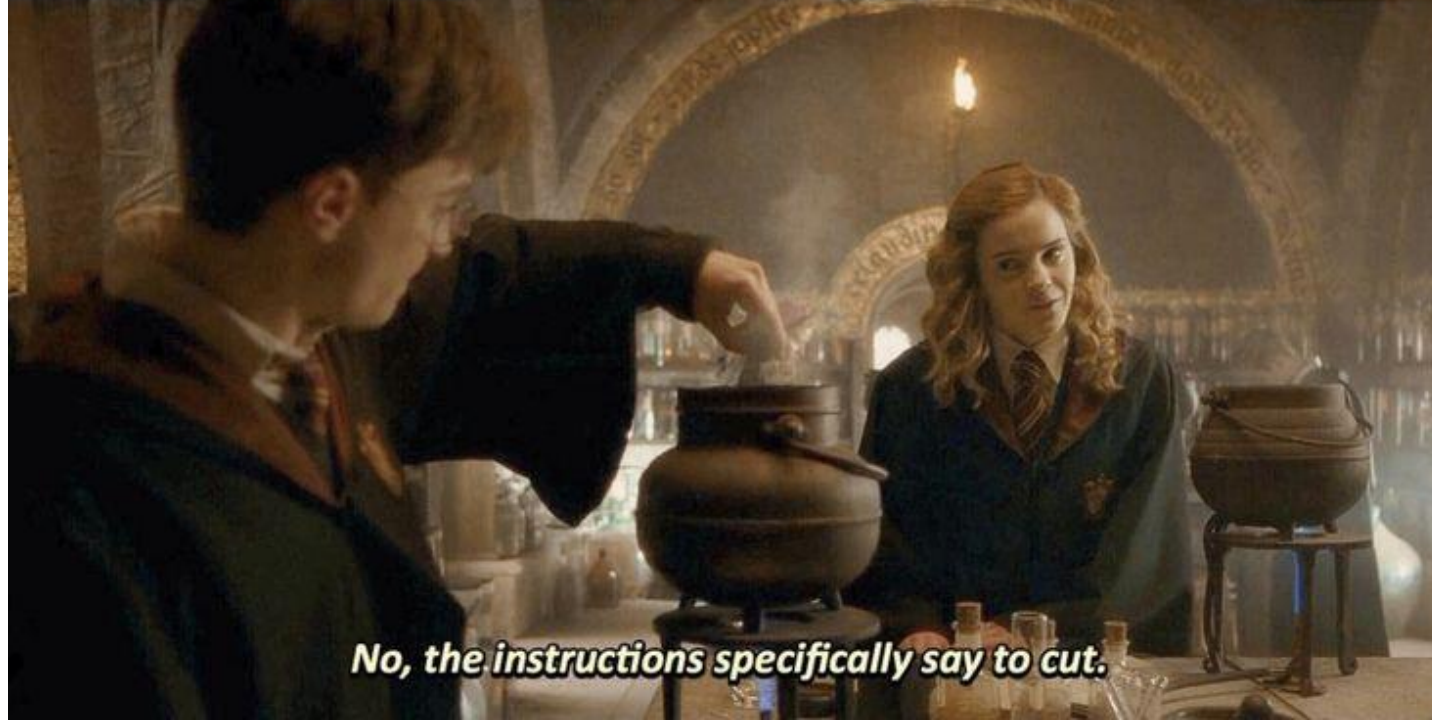
- Generally, this is 0.025 ng/ μ l in final eluate from extraction of tissue.
- 10^3 copies/ μ l for plasmid standard.



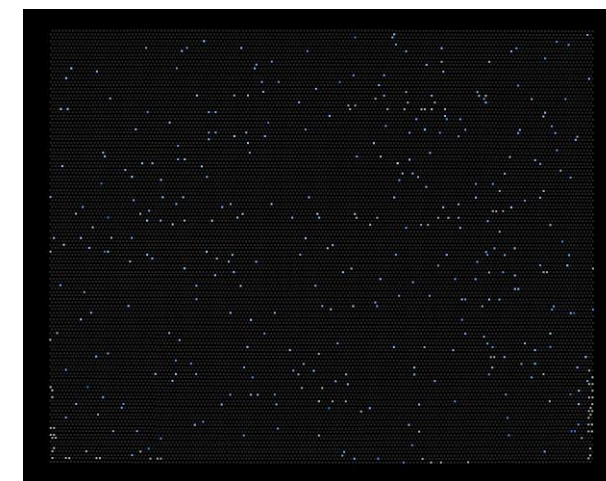
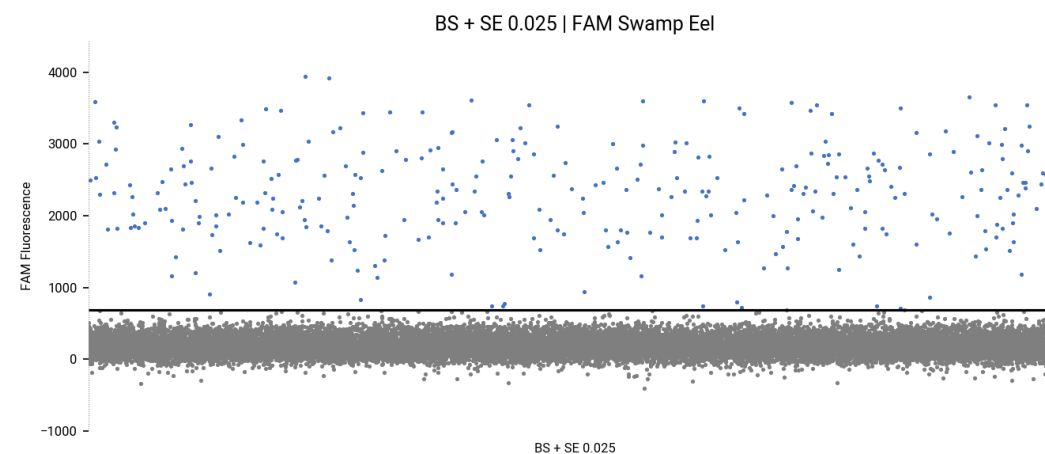
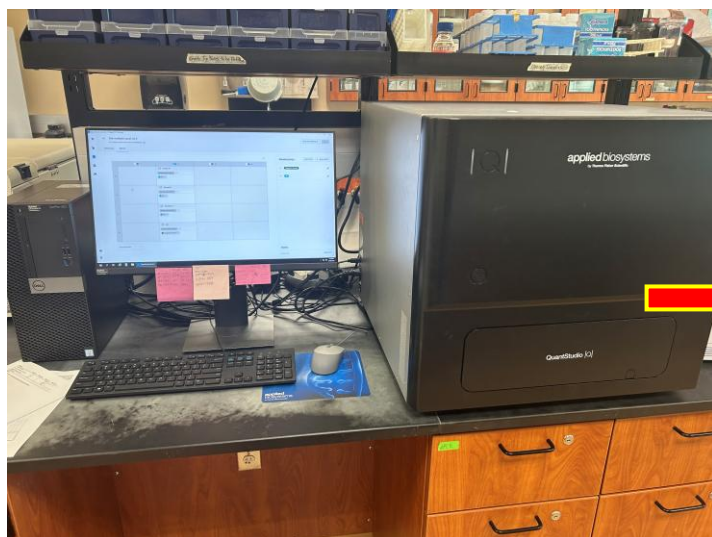
Optimizing our
protocol



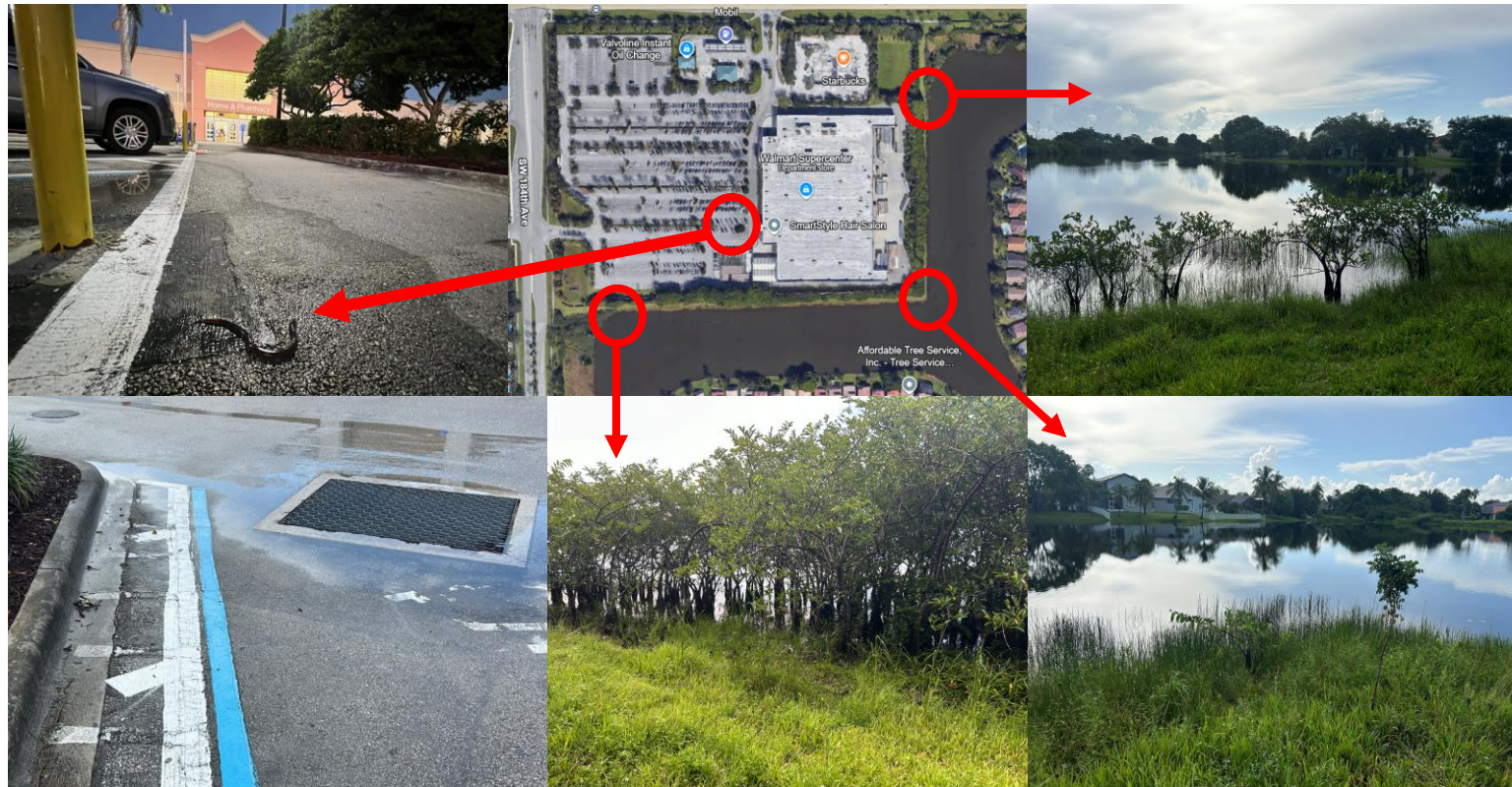
Peer-reviewers



Workflow for eDNA Samples



eDNA in real world scenarios

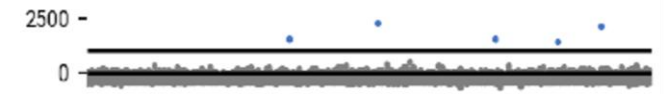


SE corner with *M. albus*

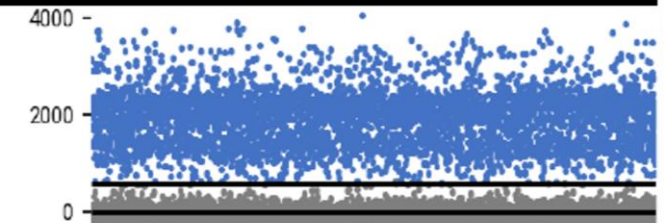
BS-VIC



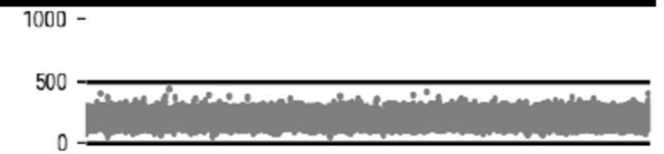
ASE-FAM



(+) control



(-) control:
BS-VIC

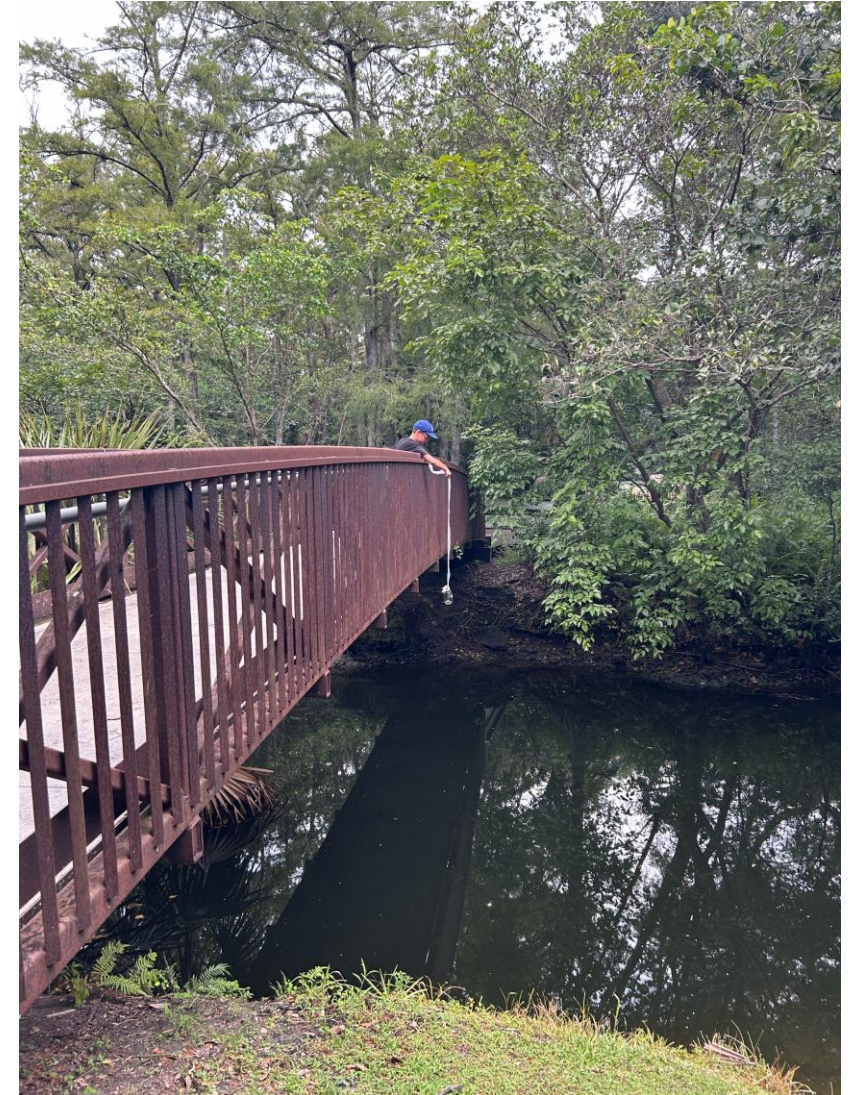


(-) control:
ASE-FAM



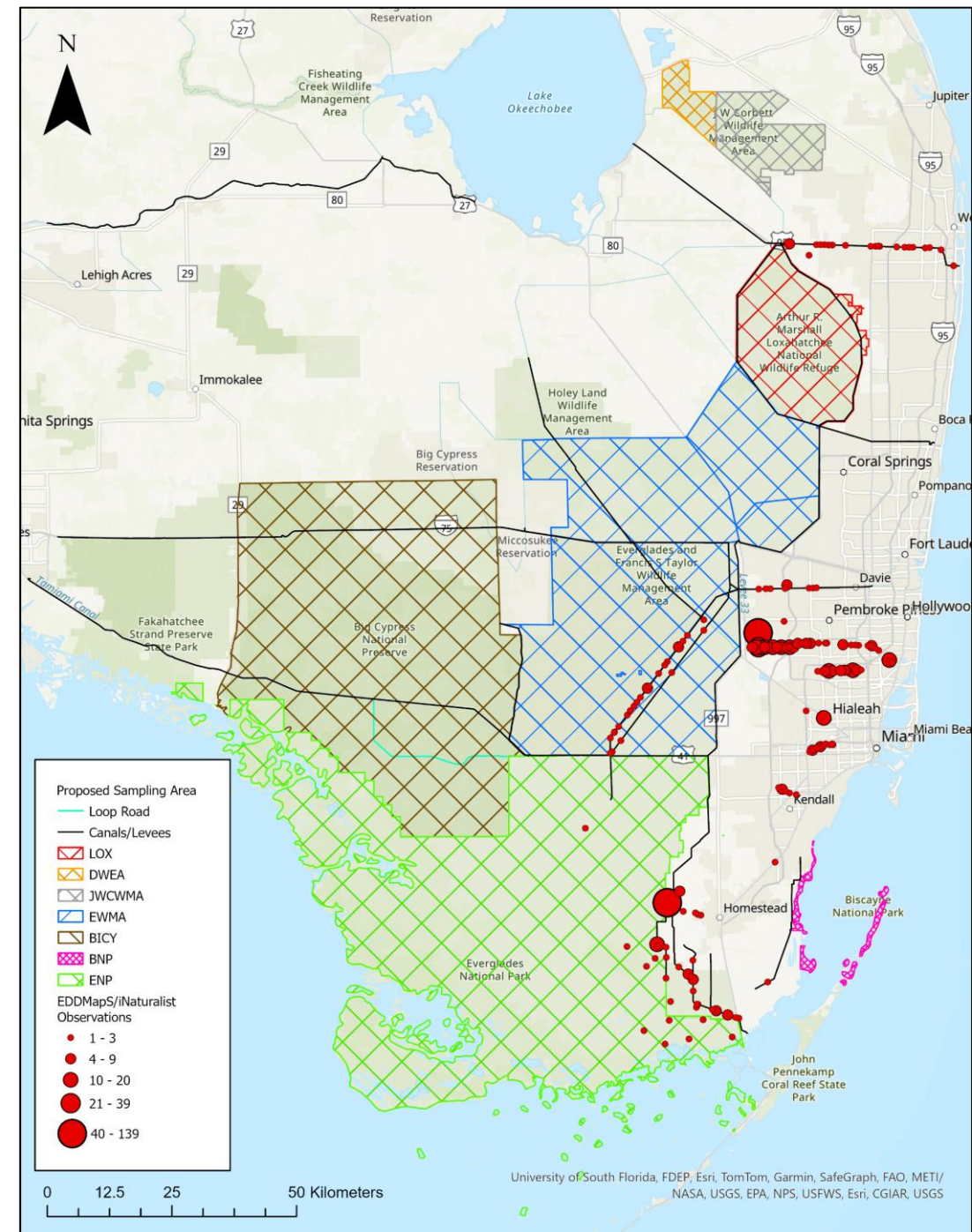
Future Efforts

- Optimize sampling for habitats with established populations of ASE.
 - Depression marshes
 - Sawgrass marsh
 - Alligator holes
 - Ponds
 - Canals
 - Ditches
 - Lakes
- Controlled lab/field experiments.
 - Place organism and sample at different time intervals



Survey Target Areas

- State and Federal public lands where status is unknown/unverified.
 - Arthur R. Marshall Loxahatchee National Wildlife Refuge
 - John G. and Susan H. DuPuis, Jr. Wildlife and Environmental Area
 - J.W. Corbett Wildlife Management Area
 - Everglades and Francis S. Taylor Wildlife Management Area
 - Big Cypress National Preserve
 - Biscayne National Park
 - Everglades National Park
- Canals adjacent to these areas.



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- South Florida Water Management District
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Thank you! Questions?



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