Creating genomic resources for environmental DNA assay development for threatened and endangered turtle species

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Background

- Habitat destruction and fragmentation has led to the drastic decrease in freshwater turtle populations.[1]
  - The Bog Turtle (*Glyptemys muhlenbergii*), Wood Turtle (*Glyptemys insculpta*), and Spotted Turtle (*Clemmys guttata*) are critically endangered.[2,3,4]
  - These species are difficult to monitor and manage because they are evasive and live in small and isolated populations.
- Genetic techniques, such as environmental DNA (eDNA) assays, are being used to survey populations.
  - eDNA assays are used to make accurate estimates in distribution and relative abundance.[5]
  - eDNA assays would greatly contribute to conservation efforts associated with freshwater turtle species.
- The three endangered species (above) lack fully sequenced mitochondrial genomes (mitomes), which are needed for eDNA assay development.
- Dr. Rosenbaum has a large collection of turtle blood samples
  - Samples comprise eight different turtle species, including the endangered species (above).
  - Extracted DNAs will used in the development of eDNA assays that could help with freshwater turtle conservation efforts.

Objectives

- Sequence the extracted turtle DNAs and assemble the mitomes of five turtle species.
- Use mitomes as a resource for eDNA assay development for the future surveys to detect and estimate relative abundance of threatened and endangered turtles.

General methods

- Compile samples into a database so individuals can be identified.
- Extract DNA from turtle blood of individuals using a DNEasy Qiagen kit for blood and tissue samples.
- Prepare extract DNAs for sequencing using NEBNext Illumina kit.
- Have prepared DNA sequenced using an Illumina HighSeq 4000 offsite.
  - Use results to assemble mitomes using assemblers optimized for bacterial genomes- e.g., SPAdes.[6]

Planned research

1) Several turtle species sampled across New York State

- Locations are shown ambiguously to ensure limited harm for the threatened and endangered species.

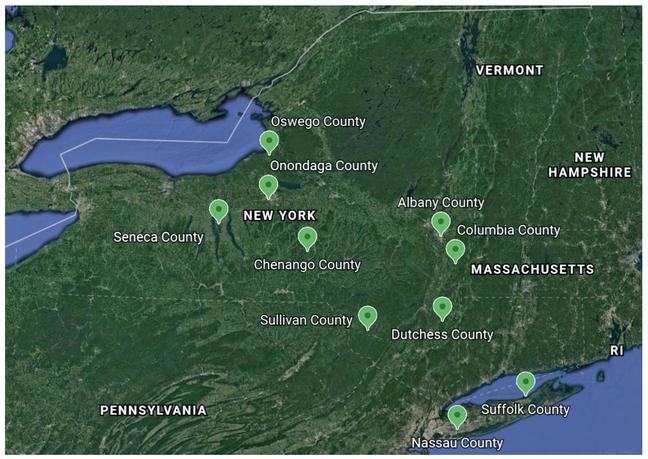
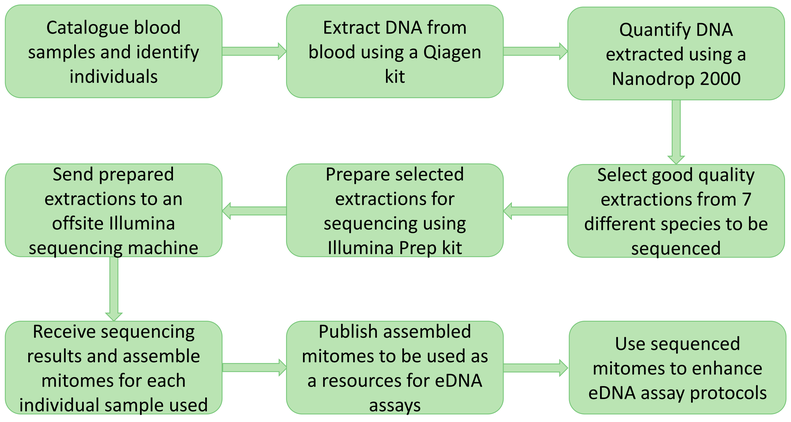


Figure 1. Map of New York State with marked counties representing regions where turtles have been sampled.

3) Schematic of proposed approach

- The steps leading to the sequencing process and intended use of sequenced mitomes.



2) Full mitome sequences are lacking

- Several of the turtle species in Dr. Rosenbaum's collection do not have their mitomes sequenced.

Table 1. List of the common and scientific names of the eight species in Dr. Rosenbaum's collection of turtle blood samples, as well as how many complete mitomes are published for each species on GenBank and how many sequences were published at the 12S, 16S, and COI loci.

Common name	Scientific name	Mitomes	12S	16S	COI
Blanding's Turtle	*Emydoidea blandingii*	0	0	0	8
Bog Turtle	*Glyptemys muhlenbergii*	0	0	0	4
Box Turtle	*Terrapene carolina*	0	1	3	29
Musk Turtle	*Sternotherus odoratus*	0	3	5	12
Painted Turtle	*Chrysemys picta*	4	1	4	12
Snapping Turtle	*Chelydra serpentina*	3	9	5	13
Spotted Turtle	*Clemmys guttata*	0	0	0	7
Wood Turtle	*Glyptemys insculpta*	0	1	3	4

Discussion and Future work

- A total of 80 Bog Turtles have extracted DNAs.
  - Future work will extract DNAs from individuals from other species.
  - High quality DNAs will be selected as individuals to be prepared and sent for sequencing at Novogene.
- Mean DNA concentration thus far is 64 $\pm$ 30 ng/µl (1 standard deviation).
  - Maximum and minimum values ranged from 120 to 6 ng/µl.
- Results suggest that most samples have high DNA concentrations.
- Mean A260/A280 ratio was 2.0 $\pm$ 0.1, suggesting that DNAs were generally of high quality.
- Given that the year blood samples were collected varied over two decades, we sought to test if DNA concentration was correlated with preservation period.
  - Results suggest a weak correlation (R^2 = 0.28).
- Once libraries are prepared and sequenced, we will assemble the mitomes.
- Mitomes assembled for species with already published mitomes can be used to compare to those we assemble to assess our *de novo* assembly approach.
- Mitomes will be used as a genetic resource to develop eDNA assays to detect a range of turtle species.

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