Bat Aging Meeting Karen, Manny, Cara, Sophia

June 27, 2023

* Loose agenda – protocol development
* From the most recent bat aging group meeting, we are the ones that are actively engaged or interested in being actively engaged in the lab work
* Most everyone else wanted to use it as a tool once developed

Update on that lab work:

* Morselli et al protocol from 2021
  + Karen, you know about this?
* Two outstanding hurdles
  + 1. Optimize for bats (Karen, sounded like you had done before?)
  + 2. Likely will need to be modified for low DNA input
    - We have restrictions on wing-punch sampling, so others will too
* Protocol development and optimization
  + Out of my wheel house
* Karen, you mentioned interest/ability in lab methods?
* Manny, you talked about working on transposase optimization?
* Get an understanding of your interest and potential timeline

Alternatives for those who just want to get the data now:

* Clock foundation all
* Clock foundation some – PCR the rest
* Whole genome low coverage

NOTES

* Lenient with concentrations
* Bat clock
* 35uL, concentrations 50-100ng/uL
* Successfully down to in the 20’s
* Karen has colony of Rousettus
  + Can extract DNA – 30-40 known aged individuals not chip
  + 30 each hipposideros and rhinolophids already aged on Horvath chip
    - Mormupids, molossids (Belize and Trinidad)
* Transposease : bioanalyzer backward optimization
  + Allows low-input methods
  + Needs different indexes for sequencing
  + Need to pool down biotin samples because things get biotinylated
    - Getting rid of parent molecule but none of PCR duplicated
    - Extra couple of steps
    - Not a loss step
* Low coverage whole genome
  + Approach done Peter Griffin (David Sinclair)
  + Issue: need genome sequence to do that method
    - Can be absolute garbage but so long as nucleotide’s of interest are present (doesn’t have to be fully annotated, just assembled)
    - We have genomes at DNA Zoo
    - DNA for DNA seq
* Library prep method: sonication vs transposease

We have two problems

* Historical samples that allow us to calibrate clock are a complete dataset
* Forward, method to continue to age bats

Moving forward:

* Happy to be a part of a proposal for future
* For historical subset
  + Should we send to clock foundation?

Comments

* Paired down sample, what do I really need to do?
  + Pick a species?
* Clock is increments of 12
* Can keep working on methods at the same time

?maybe I don’t need the data for my thesis? We can wait until the methods are more fully set? I can take a different project?

* Can clock age a few indls and see how the clock estimate compares to our dentition
  + How much can we trust dentition?
  + How many and exactly what to send to clock foundation?
* Connect directly to Matteo to discuss a proposal
  + For future work
  + Is low coverage as effective/simpler?
    - Depth is different
    - Will eventually turn stone
  + NSF for proposal – NSF EEID section

How many samples would we need to send to the clock foundation in order to get a paper on the reliability of dentition to train clocks?

* Should we even do this, or should we just wait to get all the data later on
* Should we meet with matteo?
* No matter what, will need to optimize low input and low coverage protocols

Aging estimates from environmental DNA with super low input

This is turning into a big giant collaborative thing, there are people interested in the bioinformatics part