**Topics of Discussion**

* Low depth – sequencing approaches
* Future of project
* Papers
  + 1: cheaper/more efficient lab method (Manny?)
  + 2: Mada fruit bat epi clocks (what sites work, do they work with other pteropodids) (Sophia)

**Low Depth**

Our data

CpG site position in probe %methyl #reads methyl #reads unmethyl

Table

Description automatically generated

-How does this compare between sub and nonsub? Need to known fold-enrichment. Shouldn’t

Treat like single-end reads (mapping data to probe capture fasta will only work for end of read that does match—other end somewhere else in genome, not going to map),

-supposed to be deep enough

What depth sequencing was done? What depth sequencing will need to be done?

-10gigs per sample for probe capture and non-probe capture

-should be high enough with probe capture

-could be issue with wet lab, sequencing, or bioinformatics

Wilkinson data – going into clock

Table

Description automatically generated

**Future of Project**

Our goal: cheapest, easiest way to reliably age bats in perpetuity, preferably able to be done in Madagascar?

1. Processing Wilkinson samples - Manny
   1. Validate our method on known clock sites
2. Processing known-age Madagascar bats (probe capture method) - Sophia
   1. Build species-specific clocks, Mada pteropodid clock
   2. Rank sites by correlation with age and identify fewest # of sites needed to build clock
3. Develop PCR assay to target these few sites – Brook Lab
   1. Easy, cheap diagnostic to age bats sampled longitudinally into the future
   2. \*could be doing now to validate (not multiplex!!!)
   3. Molecular inversion probes?

Probe Capture

* we don’t know how well it works quantitatively
  + capture vs whole
* need Wilkinson samples to resolve **biases** between bead capture(W) and probe capture(us) – methods paper
  + (vs whole genome)
* Any paper needs to show bead vs probe vs whole genome (we need to do this too)
  + Identify biases (will always be)
  + Subset of samples to clock foundation – Wilkinson, ours

Questions

* Can we build a reliable clock based on dentition?
  + Use recaps also to validate dentition
  + Methylome over time with recaptures
* What is your vision of the long-term goal for this project?

Peter comments

* Using data to age bats paper
* Methods paper
* Natural next step: forget capture altogether, focus on low coverage only
  + Moving to a different kind of clock, probabilistic
  + Smattering of sites, but over whole genome
  + Easily explored, downsampling the 20-50x coverage
  + More we can remove intermediate steps the better
* <https://www.biorxiv.org/content/10.1101/2021.10.25.465725v3>
  + Griffin paper
  + Probabilistic clock
* Probe could work best
* Benefit of low coverage whole genome
* Do you have the training data to make it work?

Next Steps

* Need to figure out funky data
  + Did we lose information or did we
* Sequencing samples we processed
  + Make sure we did get
* 1.Poke around at sequencing data we have
* 2.See if we should proceed with sample we have here
* Keep Peter abreast of clock
  + Continue to touch base
  + Interest for this to be working
* 3.wilkinson samples
  + Use as anchor for experiments
  + Two ways
    - 1. Put through exact same pipeline (hold off until pipeline is set)
  + Wilkinson samples processed after the pipeline is determined
  + Sending Wilkinson samples

**DNA Methylation Clocks and Their Predictive Capacity for Aging Phenotypes and Health span**

Bergsma and Rogaeva

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7376380/

Text

Description automatically generated

Notes

* Blue Pippin
* Practice with beads

Key Takeaways

1. We need to figure out what is going wrong with our samples, in two areas
   1. Why is the data low coverage?
      1. Is there a bioinformatics issue?
   2. Why/when are we losing so much

BUDGET

Whole genome & probe capture on all known age and recaps

Subset for PCR validation

Subset for clock foundation (~$100 each)

Wilkinson whole genome & probe

Computational exercise:

Look at known age bats, do we have tooth data for any recaps? Do we have any recaps that first capture was at juvenile?

Aka profile more deeply the known-age bats