Recap:

We have two outstanding issues with our data:

1. Coverage appears to be low in bismark files
   1. Sophia - bioinformatics
2. Final bioanalyzer demonstrates signific ant loss of product
   1. Manny – PCR
   2. PCR didn’t work, might be bisulfite conversion step? Could do it shorter. Before, look into supernatant from previous steps to see when lost. (could also use a different method of bisulfite conversion)
   3. Sophia – send over quants from previous steps- try to use it to identify loss step
   4. Maybe just start over instead of trying to troubleshoot—do just 1 sample before scaling back up (be very strict with bisulfite step—start ending 10 mins early)

**Low Depth**

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Coverage of CpGs **(site level, not probe level)**

**M. evotis B1S1-3**

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\*more breadth, but slightly less depth

**M. californicus B1S1-4**

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\*more breadth, but slightly less depth

**SUMMARY POINTS**

Our coverage is actually not problematic, and it is to be expected. This is based on the number of sites (>2,000, not totally sure how many actually), samples, and gigs of data sampled. So there doesn’t appear to be an issue with sequencing or in the bioinformatics pipeline. We also know that we will lose sample for two reasons, 1. Paired end sequencing (half the reads) and 2. Deduplication (losses vary but in one sample it was ~1/3 reads).

PCR did not work, however, on the samples that had low product. We don’t know why this is. Manny suspects it is likely to be due to the methylation step, which we need to be very strict on the end timing with. Instead of trying to troubleshoot randomly, Sophia is going to try and re-do the entire pipeline. She may have to optimize fragmentation and bisulfite conversion.

**TO-DO**

1. Re-do entire pipeline with a single sample, a DNA control (from what species?) and E.coli methyl and unmethyl spike (Sophia)
   1. Quant as I go, save samples for bioanalyzer
2. Investigate capture efficiency (Sophia)
   1. Map sub files to whole genome (Myotis yumanensis) to see which hits are on target (in probes) vs off target (not in probes)
3. Re-sequence to a higher depth the original samples (Manny)
   1. To get higher coverage

To Order

<https://www.diagenode.com/en/p/dna-methylation-control-package-V2-48-rxns>

All reagents for DNA methylation pipeline