Connor Smith

Shannon McWeeney

BMI 559, F17

10 November 2017

HW4: Methyl-Seq Protocol

1. Key questions/considerations
   1. Will we be doing a targeted analysis or whole-genome bisulfite sequencing?
      1. Whole Genome – TruSeq® (conversion of nonmethylated Cs to U) [[1]](#footnote-1)
   2. What sites are we focusing on?
      1. CpG islands (MethylC-Seq, RRBS)
      2. CpG Shores (MethylC-Seq)
      3. Promoters (MethylC-Seq, Infinium)
   3. Presence of hmC?
      1. MethylC-Seq has issues[[2]](#footnote-2)
      2. Use Tab-Seq to differentiate between mC and hmC sites[[3]](#footnote-3)
2. Best Practices
   1. Quality control of raw reads, adapter trimmed reads[[4]](#footnote-4)
   2. Removal of duplicate reads (common with other methods)
   3. Analysis
      1. Illumina apps[[5]](#footnote-5)
         1. MethylSeq – whole genome and targeted, calculates alignment scores and variant calling using PUMA
         2. methylKit – R package adapted to Illumina app, allows for customization of methods and custom reference, annotation for hg19
   4. MethyCap-Seq – pipeline has been developed for rapid processing and deployment[[6]](#footnote-6)
3. Initial Steps
   1. Identify available resources (machines, funding, etc.)
      1. Our exact steps will depend on our question (including those listed above)
   2. Identify best practices for sample collection/preservation
   3. Identify analysis plan based on method for data collection/generation and the parameters involved
      1. I would use various resources to “practice” the steps needed and get an understanding of the following:
         1. What kind of QC do we need to do and where (consult other papers/pipelines, some listed below)
         2. What will the data look like going in and coming out (how to analyze)
   4. Develop cost estimates for doing this analysis to advise lab

1. “Field Guide to Methylation Methods”. Illumina Sequencing Methods. [↑](#footnote-ref-1)
2. http://lsl.sinica.edu.tw/Services/Class/files/20151118475\_3.pdf [↑](#footnote-ref-2)
3. “Field Guide to Methylation Methods”. Illumina Sequencing Methods. [↑](#footnote-ref-3)
4. http://lsl.sinica.edu.tw/Services/Class/files/20151118475\_3.pdf [↑](#footnote-ref-4)
5. Methylation Sequencing. (n.d.). Retrieved November 10, 2017, from https://www.illumina.com/techniques/sequencing/methylation-sequencing.html [↑](#footnote-ref-5)
6. Rodriguez, B. A., Frankhouser, D., Murphy, M., Trimarchi, M., Tam, H., Curfman, J., . . . Bundschuh, R. (2012). Methods for high-throughput MethylCap-Seq data analysis. *BMC Genomics,* *13*(Suppl 6). doi:10.1186/1471-2164-13-s6-s14 [↑](#footnote-ref-6)