MEDICAL COLLEGE OF WISCONSIN

Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine

Reduced Representation Bisulfite Sequencing (RRBS)

Test Description

DNA methylation is an epigenetic mechanism whereby tissues and cells can regulate gene expression, specifically hypermethylation of promoters and intergenic regions typically leads to repressed gene expression. These modifications have been linked to and play a critical role in cancer initiation, progression, tissue development, and many other cellular processes. To focus on the methylation status of a small portion of the genome (1-5%), enzymes that target CpG regions of the genome first fragment the DNA. Those regions then undergo bisulfite conversion to convert unmethylated cytosines to uracils, thus allowing differential identification of the methylated bases.

Sample Types and Requirements

300-500ng of genomic DNA, consultation with the lab is requested

Recommended Sequencing Depth

10-20 million total reads; paired end, 2 x 100bp sequencing

Submission Requirements

Sample Intake Form and iLabs request. Contact lab for drop off or shipping requirements.

Turnaround Time

6-8 weeks for fastq files only
3+ additional weeks for bioinformatics analysis

Deliverables

RRBS report with identification of methylated base pairs and differentially methylated regions among sample cohorts
Annotation of nearby promoters, genes or genomic features
BAM, fastq.gz files
All annotated NGS data files will be delivered via Mellowes Center portal

Test Methodology

Total DNA is isolated and fluorescently quantified using the BioTek Synergy LX or Qubit. To focus fragments to CpG regions, DNA will be digested with MspI, ends repaired, and ligation completed with Illumina adapters. Samples are processed twice through bisulfite conversion (Qiagen) before library amplification and clean up. The quality and quantity of the DNA library is checked by fragment analysis and qPCR respectively. Libraries are then pooled and distribution confirmed on the Illumina MiSeq before sequencing is completed on the Illumina NovaSeq.

Bioinformatic Core Analysis

RRBS report includes:

- Quality control and sequencing metrics
- Basic CpG coverage and distribution of methylation mapped on chromosomes
- Sample comparison by Principle component analysis (PCA), dendogram, heat maps, and volcano plots of differentially methylated regions (DMRs)
- Identification of genes and pathways that are candidates for regulation by the differential methylation
- Additional analysis available upon request

Contact & Submission

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