

Reduced Representation Bisulfite Sequencing (RRBS)

**Turn Around
Time:**

6 – 8 weeks

TEST DESCRIPTION

Sequence the methylation status of particular CpG rich regions of the genome.

SAMPLE REQUIREMENTS

Isolated DNA: 150-300ng.

Cells or Fresh Frozen Tissue *** available with consultation:* DNA extraction methods may be available for your cell or tissue type. 150-300ng.

FFPE: 150-300ng of intact DNA *** size selection may be available to remove some fixation induced breaks.*

Shipping Conditions: -20°C, frozen; overnight.

TEST METHODOLOGY

DNA Methylation Sequencing. DNA (150-300ng) will be digested at CpG motifs with MspI (10 Units, R0106L; New England Biolabs). Digested fragments will undergo end repair and A-tailing with Klenow fragment polymerase (5Units, M0212S; New England Biolabs) and overnight ligation with TruSeq adapters (Illumina) containing next generation barcode sequences. DNA cleanup and fragment size selection will be completed using Agencourt AMPure beads (Beckman Coulter) at 2x beads to sample volume. Samples then will be processed twice for bisulfite conversion using the EpiTect Bisulfite conversion kit (Qiagen). DNA up clean will be completed followed by relative abundance (SYBR green qPCR, Bio-Rad) and PCR amplification of the products performed (Pfu Turbo, Agilent). Libraries will be pooled in equimolar ratios and undergo size selection using 1.2x and 0.7x Agencourt AMPure beads to enrich for 150-500bp products. Final assessment and quantification of the RRBS libraries will be completed with qPCR, fluorescence measurements and Bioanalyzer 2100 DNA High Sensitivity Assay (Agilent). Next Generation Sequencing will be completed at the GSPMC Facility on the Illumina HiSeq 2500. Paired end, 125 base pair reads will be generated at 10-20 million reads per sample.

LIMITATIONS

Coverage is only a part of the genome.

Contact & Submission

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