MEDICAL COLLEGE OF WISCONSIN

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

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

Test Description

Chromatin Immunoprecipitation Sequencing (ChIP Seq) is an epigenetic method to understand the intersection of proteins (transcription factors or histones with regulatory marks) with specific regions of the human genome. Understanding and mapping these interactions can reveal common binding sites and delineate structural changes that can regulate the ultimate ability of the cell to express arious genes. By adding a layer of epigenetic regulation to the transcriptional alterations occurring in cells and tissues, investigators can bring additional mechanistic understanding to tissue development, disease pathology, and translational research. To reduce input needed and background signals, cut&run or cut&tag technologies may also be utilized during the immunoprecipitation protocol.

Sample Types and Requirements

Consultation with the lab is requested, cells and tissues have the potential to be processed.

Recommended Sequencing Depth

5 to 10 million unique fragments, Dependent on transcription factor or histone mark being interrogated and methodology utilized; paired end, 2 x 50bp or more

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

DNA quantity and quality controls (PDF)
Peak calling
BAM, fastq.gz files
All annotated NGS data files will be delivered via Mellowes Center portal

Test Methodology

Cells are lysed and nuclei isolated before chromatin shearing by 10-30 cycles on a Diagenode sonicator and optional enzymatic digestion with DNase. Shearing is checked to ensure DNA size is amenable to downstream applications. Immunoprecipitation (IP) proceeds with specific antibodies, and enrichment confirmed by qPCR. ChIP libraires are prepared using a low input DNA protocol (Diagenode) and enrichment again confirmed by qPCR. The quality and quantity of the final 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

ChIP seq report includes:

- Quality control and sequencing metrics
- Correlation and coverage plots among conditions
- Identification and correlation with genomic location of enriched peaks
- Additional analysis can identify loci where peaks are differently called among conditions and may correlate to upregulated or repressed expression of transcripts

Contact & Submission

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