Library Preparation and DNA Sequencing:

Up to 30 ng of post-Immunoprecipitation dsDNA as measured by Invitrogen™ Qubit™ high-sensitivity spectrofluorometric measurement was made into Illumina-compatible NGS libraries using the ThruPLEX® DNA-Seq kit from Takara Bio Inc. Each created library was dual-indexed with 8-base molecular barcodes for high level multiplexing. After 13 cycles of PCR amplification, completed libraries were sequenced on an Illumina NovaSeq™ model (6000 or X Plus) sequencer, generating 20 million or more high quality, 100 base length read pairs per sample.

ChIP-Seq Analysis:

A quality control check on the generated fastq files was performed using FastQC. Upon passing basic quality metrics, the sequence reads were used to enter a ChIP-Seq specific alignment and analysis pipeline.

Alignment and Peak Calling Analysis:

The reads were mapped to a reference genome using Bowtie2[1] (version 2.4.2, command line: "`bowtie2 -p2 -x <index> --local -1 <fastq_1> -2 <fastq_2> -S <output sam>`"), and peaks were called with MACS2[2] (version 2.1.4, command line: "`macs2 callpeak -t <input bam> -f AUTO`").

1. Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. *Nature Methods*. 2012, 9:357-359.

2. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS. Model-based Analysis of ChIP-Seq (MACS). *Genome Biology*. 2008, 9:R137

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