ChIP-Seq and Related Protocols

Q1. What is meant by ChIP-Seq ?

A1. From Illumina [1]: "*Chromatin immunoprecipitation (ChIP) is a powerful method to selectively enrich for DNA sequences bound by a particular protein in living cells. ChIP-Seq on Illumina sequencing systems supports virtually unconstrained selection of any ChIP-able protein and/or modification to be studied. These include transcription factors, polymerases and transcriptional machinery, structural proteins, protein modifications, and DNA modifications. ... The ChIP process enriches specific crosslinked DNA protein complexes using an antibody against a protein of interest. Unique oligonucleotide adapters are then added to the small stretches of DNA that are bound to the protein of interest to enable massively parallel sequencing.*" Some applications of this technology include [2]:

- Discovery of transcription factor binding sites
- Identification of genes regulated by known transcription factors and co-regulators
- Analysis of epigenetic events
- Direct comparison of regulatory events in different cell states (i.e. normal vs. disease)
- Investigation of drug effects and other stimuli on regulatory pathways

There are many protocols that, like ChIP-Seq, result in small amounts of fragmented DNA representing selected regions of the genome. NISC has experience in producing libraries from many different ChIP-Seq-like methods, such as ATAC-Seq and Hi-C. These often require careful coordination with investigators to insure handoffs are well understood.

Q2. What material should I send to be analyzed by ChIP-Seq ?

A2. Generally, we start with 10-50 ng of ChIP-enriched DNA. Samples should be submitted in 1.5-1.7 ml microfuge tubes (example: VWR cat. no.89000-028) or 2 ml screw cap tubes (example: Sarstedt cat. no. 72.694.007). Please DO NOT send samples in 0.5 or 0.2 ml tubes. If possible, the sample should be evaluated by the investigator by testing for the relative enrichment of a relevant gene. The best control material is an unprocessed aliquot of the input DNA that went into the ChIP enrichment step. A light sequencing of this sample in parallel can reveal potential false-positives.

Frequently Asked Questions

Q3. What data are returned by NISC ?

A3. Typically, NISC returns to the investigator a file containing basecalls and quality scores (fastq files) for each sample. The investigator is expected to provide data analyses; this is not offered by NISC.

Q4. How long do the reads need to be for ChIP-Seq analysis ?

A4. Our standard read length on the Illumina NovaSeq X Plus is 2 x 150 bases, but for small projects it may be more cost-effective to sequence these libraries with 2 x 50 base reads.

Q5. How many reads are used for a mammalian ChIP-Seq analysis ?

A5. Read counts per sample for this set of experiment types is variable, but at a minimum we recommend 20M pairs of reads and up to 600M for Hi-C type. ChIP-Seq libraries are constructed with indexed adapters, which allow many libraries to be pooled and sequencing together for optimal cost efficiencies.

Reference:

1. Illumina, Inc. (2014): "ChIP-Seq DNA Sample Prep Kit"
   http://www.illumina.com/products/chip-seq_dna_sample_prep_kit.ilmn
2. Illumina, Inc. (2014) "Whole-Genome Chromatin IP Sequencing (ChIP-Seq)"
   http:// http://support.illumina.com/sequencing/literature.ilmn