Frequently Asked Questions

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, Hi-C, and FAIRE. 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.
Q3. What data are returned by NISC?

A3. Typically, NISC returns to the investigator a file containing basecall 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. Typically, read lengths are 75 bases. This length should be sufficient for mapping of most reads to the reference genome. Some investigators are exploring the utility of longer reads and paired-end reads for advanced analyses.

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

A5. NISC usually targets 20 million single-end reads or read-pairs per library. ChIP-Seq libraries are constructed with indexed adapters, which allow many libraries to be pooled together for sequencing. Greater efficiency is achieved when a pool of 16 libraries are run on a NextSeq 550 instrument [3].

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