



**VAN ANDEL**  
RESEARCH INSTITUTE

## ChIP-Seq Submission Recommendations

ChIP-seq is one of the most technically complicated next-generation sequencing projects to undertake and is entirely dependent upon the quality of sample submitted for library preparation. If at any time, you would like us to give an opinion on your samples, please email us at [genomics@vai.org](mailto:genomics@vai.org) and we'll be happy to help.

### IP/Experimental Guidelines

#### **Chromatin sizing: IP'd fragments must be 200-500 bp in length**

Fragments greater than 1000 bp are selected against during the library preparation process and cluster/sequence poorly on the Next Generation instruments. If sonication is proceeding for >60 min and a significant portion of the sample is still >1000 bp, overcrosslinking is the likely culprit, and you run the risk of destroying your epitope with more sonication. Crosslinking time should be titrated for each cell type in each lab, and the minimum crosslinking time during which the epitope is maintained (as measured by western blot) should be used for IP experiments.

#### **Controls**

1. **Input:** In order to properly normalize an IP experiment, an input control should be submitted to sequence along each IP. An input control is defined as sheared, reverse crosslinked DNA that has been pulled aside prior to immunoprecipitation. Input controls are important because it gives a picture of exactly what chromatin is available for immunoprecipitation. We assume that when you shear DNA to a certain size range that it's all there. In practice, this is not true—there are hot spots and cold spots that are created due to shearing that are unique to each sample. Not controlling for these regions can often lead to false positives and negatives.
2. **IgG:** IgG controls can give you the noise of your assay, anything that is non-specifically pulled down. As such, it can be a useful control for weeding out false positives. However, if you have a very specific assay, then your IgG control may not produce enough DNA to create an effective Illumina library, meaning that certain regions may become over-amplified during the PCR step and actually confound your sequencing results. As such, it may be better to save this control for post sequencing qPCR validation on targets of interest.

## Replication

As with all “counting” based sequencing experiments (ChIP-seq, RNA-seq), biological replication is necessary for a robust statistical analysis. As a general rule, we suggest a minimum of 3 biological replicates in order to determine experimental error.

A great review that discusses these considerations and more:

<http://www.nature.com/ni/journal/v12/n10/abs/ni.2117.html>

## Submission Guidelines

1. Submit 100ng of each input and the entirety of each IP sample. Most IP samples are too dilute to quantify accurately using spectrophotometers/nanodrop, so don't waste your precious sample using one. Intercalating dyes such as PicoGreen (Life Tech.) or QuantusOne (Promega) are better options. If you don't have access to these dyes, please submit IPs without quantifying; we do it as part of our initial QC and will send you the results. We will use up to 30ng of each IP sample to create your libraries, normalizing between samples if possible.
2. Each submitted sample should be sheared between 200-500 bp. We will assess this by running each input sample on Agilent Bioanalyzer.
3. Each Input and IP sample should be reverse crosslinked and the DNA precipitated and resuspended in either water or 10mM Tris pH 7-8.

## Shipping

1. A filled out submission form must accompany all shipments.
2. Samples <12 should be submitted in either a 0.5ml or 1.5ml nuclease-free microfuge tube with the sample name clearly written on the cap- NO more than 6-8 letters/numbers- and the PI's name on the side. If >12 samples submitted, the samples should be in a nuclease-free PCR plate sealed appropriately with the PI name on the side of the plate skirt (spreadsheet with plate layout should be e-mailed to [genomics@vai.org](mailto:genomics@vai.org) and should also accompany plate). All items shipped should be sealed in a plastic bag.
3. Sample should be shipped on dry ice overnight to

**Van Andel Institute**

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