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ChIP-Seq.

Like all ChIP experiments,the specificity of the antibody and the ability to immunoprecipitate reasonable amounts of DNA is essential to the outcome. Depending whether the antibody has been used for ChIP before and the availability of suitable protocols, the shearing and immunoprecipitation should be optimized. Most people cross-link their chromatin and shear their DNA to the 500-1000bp range (We have a Covaris S220 available for that). Amounts of antibody, binding buffers and amounts of cross-linked material is antibody and tissue specific. As part of the IP optimization the IP'd DNA can be quantitated using Qubit (or similar) and oligonucleotides to known or predicted genes can be used in qPCR. For qPCR we recommend using controls such as input and IgG controls to verify IP. Where antibodies are specific to factors part of complexes it is important to be aware that they may only bind to larger pieces of DNA and/or epitopes could be masked by other protein interactions.

Once the IP has been optimized it is important to carefully plan your experiment including how you are going to analyse your sequencing results. If you are comparing two different targets or similar targets after undergoing multiple treatments the samples are essentially controls for each other. If you are identifying what a single target is binding you will need an 'input' control to ensure that the sequences identified are not due to some experimental artifact. In most cases IgG controls do not IP DNA, so it is not usually included for sequencing.

As for most biological experiments 3 replicates is considered the absolute minimum for statistical robustness. The more diverse the samples the more replicates that are required. Many bioinformaticians prefer 5 or more biological replicates.

qPCR is less sensitive to contaminating material than the enzymes required to generate ChIP-Seq sequencing libraries. Thus post-ChIP we recommend cleaning up your sample with the Diagenode iPure kit as we get the best results from this.

When you submit your samples please let us the concentration (and how you did this), how you purified post-capture and the expected size of the IP'd DNA. We currently require at least 2ng (by our quantitation methods) plus some for sample QC before we can begin. Normally we re-shear samples with larger fragments down to 200-300bp for preparation of sequencing libraries.

For the sequencing we normally do 50bp Single reads for ChIP; providing that the material has a good reference sequence. For antibodies to specific transcription factors 10-15 million reads per sample is sufficient for analysis; but where lots of different regions are bound and the samples are more complex it might be necessary to increase the numbers of reads per sample. For example current recommendations are 20-30 million for methylated histones etc; and 50 million for MeDIP studies.

Basic ChIP protocol.

Cells (~1×10^6) are cross-linked for eight minutes by dropwise addition of formaldehyde to a final concentration of 1%. After 8 minutes mixture is neutralized with $1/10^{th}$ volume 1.25 M glycine. The cells are then rinsed with PBS and lysed in 130ul Lysis buffer (below; 5min on ice).

Chromatin was sheared to ~500 bp fragments using the Covaris S220 sonicator under the following conditions; Duty Factor 2%, peak incident power 200 watts, cycles/burst 105, time 10 min, temperature 4°C.

Following shearing IP was performed overnight by combining 1ml IP buffer (below) with the sheared lysate, 20ul pre-absorbed Protein A-coated Dynabeads and 7 µg of the specific antibodies (e.g.H3K4me3 (Diagenode pAb-003-050); H3K27ac (Ab4729, Abcam, Cambridge, UK). (Rotating wheel at 4oC)

Beads were then washed with Wash buffer x 3 followed by Final wash buffer x1. After removal of final wash buffer the DNA was eluted and purified using the iPure DNA purification kit (AL-100-0100, Diagenode, Liège, Belgium) according to manufacturer's instructions.

To prepare libraries, 2ng of ChIP DNA was diluted to 50ul in low TE and sheared a second time using the Covaris sonicator (Duty Factor 10%, peak incidence power 175 watts, cycles/burst 200, time 2 minutes, temperature 4°C) to generate 200-300bp fragments. Libraries were then prepared using the Nugen Ovation Ultralow system V2 (as per Protocol M01379v1; 2014).
Libraries are verified by bioanalyzer (size) and qPCR (quantity) prior to being denatured and clustered at 12pM on the Illumina HiSeq.
15-20 million 50bp reads are then generated from each sample for analysis.

Lysis buffer
50 mM Tris-HCl, pH8
10 mM EDTA, pH8
1% SDS
1 mM PMSF (add just before use)
1x complete protease inhibitor cocktail (Roche Cat# 04 693 124 001, add just before use)
20 mM NaBu (if required, add just before use)

	IP Buffer	**Wash Buffer 1**	**Final Wash Buffer**
SDS		0.1%	0.1%
TritonX100	1%	1%	1%
EDTA pH8.0	2mM	2mM	2mM
Tris-HCl pH8.0	20mM	20mM	20mM
NaCl	150mM	150mM	500mM
Inhibitors	1x		