Validation Of Antibodies For ChIP

I. Purpose

The purpose of this work instruction is to outline the protocols to validate an antibody, once it is received, for ChIP (Chromatin Immunoprecipitation).

II. References

Document Title	Document Number
N/A	N/A

III. Related Documents

Document Title	Document Number
Western Blot	LIBPR.0076
Native ChIP Using 100,000 Cells	LIBPR.0138
MODified Histone Peptide Array	LIBPR.0089
Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing	LIBPR.0137
qPCR of Native ChIP Libraries	LIBPR.0144

IV. Procedure

1. When to Order An Antibody

1.1. Follow the guidelines below to determine when to place an order for an antibody:

a. Less than 50 immunoprecipitations (IPs) worth of antibody remaining in production stock. This is referring to the diluted and one time working aliquots.

b. Request by supervisor.

1.2. Discuss with supervisor before placing an order. Request the current production lot of the antibody when entering the information on the ordering sheet. The ordering sheet can be found in geneexplab\Library Core\Lab Operations\Ordering. The Purchasing Group will contact the vendor/manufacturer before placing the order, to determine if the current production lot is available. If it is not, the supervisor will contact the manufacturer and order 1 vial of the new lot for in-house validation.

2. Receiving/Storing and Barcoding the Antibody(ies)

2.1. Vial(s) of antibody are shipped on either dry ice, wet ice or at room temperature. The containers are placed in the 6th floor Library Core receiving area once received by the Purchasing team.

2.2. Remove the vial and the data sheet from the shipping container. Quickly check the data sheet for the lot# of the antibody. If the lot # is not listed, write the lot#, which is on the vial, on the data sheet. Also note the receiving date on the data sheet.

2.3. Pooling Antibodies:

2.3.1. If more than one vial of same antibody type is received; the vials of antibodies are pooled by lot# if they are shipped within the same order. Follow the criteria listed below to determine whether or not the vials should be pooled:

a. Same order, same shipment arrival, same Lot# - POOL
b. Same order, different shipment arrival, same Lot# - DO NOT POOL
c. Same order, same/different shipment arrival, different Lots# - DO NOT POOL

2.3.2. Make an aliquot for validation to avoid repeated freeze / thaw cycle.

2.4. Transfer the vial to the -20°C freezer or 4°C fridge, depending on the manufacturer's recommendation for optimal storage conditions and place the vial in the 'Antibodies to be Validated' box.

2.5. Your supervisor will generate solution IDs (sol#) in LIMS for the vial or pooled vial of antibody and the aliquot with the information from the data sheet. Antibody info such as concentration, clonality, special note, antibody volume etc. will also be entered by supervisor. This information will be tracked in 'Antibody Inventory Tracking' page in LIMS.

2.6. Place the sol# barcode on the antibody vial and the aliquot for validation accordingly.

2.7. Scan the antibodies into the 'Pending Validation' box (rac116433) located in the -20°C freezer or '4C Fridge' box (rac27643).

2.8. Note the solution ID (stock) on the data sheet and keep it in the Antibodies Binder as a reference.

This is a non-controlled version.
****Note: controlled versions of this document are subject to change without notice.***

3. Processes to Complete to Validate the Antibody

3.1. The following processes are to be completed to validate new antibody received:

3.1.1. Western Blot Analysis (WB) using document LIBPR.0076- Antibody Validation Western Blot.

3.1.2. Histone Peptide Array (PA) using document LIBPR.0089- MODified Histone Peptide Array.

3.1.3. ChIP in HL60 cells using document LIBPR.0138- Native ChIP Using 100,000 Cells.

3.1.4. Library Construction and qPCR using documents LIBPR.0137- Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing and LIBPR.0144- qPCR of Native ChIP Libraries.

3.2. The following criteria, shown in Figure 1, should be followed when a shipment of an antibody arrives when determining method of validation (Pooled Vials or Singlets).

Antibody	Western	Peptide Array	Native ChIP	LC including qPCR	Sequencing
Lot same as production	N	N	Y	Y	N
Lot different than production	Y	Y	Y	Y	Y
Monoclonal (same OR different lot as production)	N	N	Y	Y	N

Figure 1

4. Send out Results and Confirm Results With Supervisors

4.1. Summarize the results of the processes listed in Step 3 in a powerpoint presentation. Include all pertinent details such as type and amount of chromatin used to validate the antibody, gel images, Agilent profiles, and any technical issues encountered during the processes. If unsure, consult supervisor.

4.2. Send the PowerPoint document via email to supervisors. Upon confirmation that the antibody has been passed and is suitable for production, proceed to the following step.

5. Making Antibody Aliquots for Long and Short Term Storage.

5.1. For antibodies bought in bulk, follow Steps 2-4 upon arrival.

5.2. After completion of validation of the antibody, most of the antibody will be aliquotted and stored for long term storage to be located in a -80°C freezer (in 50µg aliquots). However, a portion of the antibody (10µg) will also be aliquotted for short term storage and stored at -20°C.

5.3. Create aliquots in LIMS and affix barcodes on both the long term and short term stock aliquots.

Note: The volume of each aliquot is in ml not µl.

6. Dilute and Making Working Aliquots for Native ChIP for Production Use

6.1. Dilutions are made based on the concentration of the antibody and the amount of the antibody that will be used in the IPs in the Native ChIP process.

6.2. Since the concentration of the stock antibody (ies) are high, they generally require dilution for use in LIBPR.0138- Native ChIP Using 100,000 Cells.

6.3. On ice, dilute the stock antibody to an appropriate working concentration. Dilute the antibody in the same buffer the stock it is stored in. The storage buffer components are listed on the data sheet provided by the supplier.

6.3.1. However, if antibodies are obtained from the Hiroshi lab, they are to be diluted fresh just before use, diluting the antibody with the same storage buffer as used by antibodies from Diagenode.

Note: Working aliquots will be stored either at -20°C or 4°C according to the manufacturer's recommendation.

6.4. Aliquot the appropriate volume of diluted antibody into a 0.5mL non-stick tubes for 1 time use (working aliquot), ensuring that there is enough dead volume, so you do not run short of the antibody for the ChIP experiment.

6.5. Create aliquots in LIMS.

6.6. Put the barcodes on all the working aliquot tubes and store these aliquots in the -20°C or the 4°C in the current antibody production boxes.