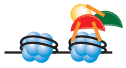
X-ChIP

Map proteins/histone modifications to genomic loci

Chromatin and associated proteins.

Cross-link

Start with 2 X 150 cm^2 dishes of confluent cells
(1 X 10^7 - 5 X 10^7 cells per dish)
Add formaldehyde to a final conc of 0.75%
Incubate 2 - 30 min RT
Add glycine to a final conc of 125 mM
Shake gently for 5 min



Cross-linking fixes proteins to DNA.

Cell collection

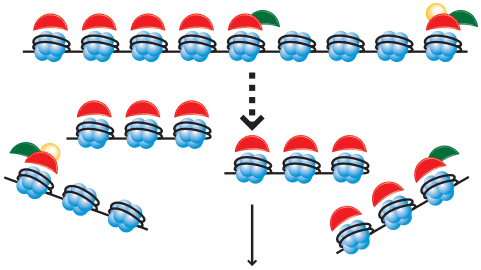
Wash cells with ice cold PBS
Scrape and collect cells into 5 ml ice cold PBS and transfer to a new tube
Wash dishes with 3 ml PBS to ensure all cells collected

Cell lysis

Centrifuge 5 min 1 000 g and remove supernatant
Add FA lysis buffer to cell pellet (750 µl per 1 X 10^7 cells)

Sonication

Sonicate to give a fragment size of 500 - 1000 bp



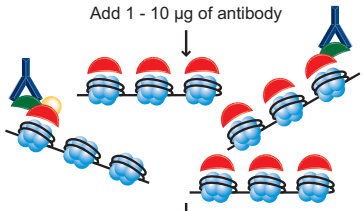
Sonication generates sheared, soluble chromatin. Optimize by performing a time course and purify DNA. Analyse fragment size on 1.5 % agarose gel.

Centrifuge 30 sec 4˚C 8 000 g to pellet cell debris
Transfer supernatant containing chromatin to a new tube

Remove 50 µl (INPUT) and purify DNA to calculate the DNA concentration

Immunoprecipitation

Use an amount of chromatin equivalent to approximately 25 µg of DNA per IP
Dilute 1:10 with RIPA buffer
Add 1 - 10 µg of antibody

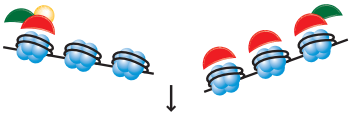


Antibody binds to target and associated DNA is isolated. DNA fragments not associated are removed during washes.

Add 20 µl of protein A/G beads
Rotate overnight 4˚C
Centrifuge 1 min 2 000 g
Wash 3 x with wash buffer
Wash 1 x with final wash buffer.

Elute protein / DNA complex

Add 120 µl of Elution Buffer to the protein A/G beads
Rotate 15 min 30˚C
Centrifuge 1 min 2 000 g
Transfer supernatant containing protein/DNA complex to a new tube



Reverse cross-links and purify DNA

EITHER

PCR purification kit

Add 2 µl RNase A (0.5 mg/ml)
Heat 65˚C 4 - 5 hr

Purify DNA using PCR purification kit
according to manufacturer's instructions

Phenol:chloroform extraction

Add 5 µl proteinase K (20 mg/ml)
Heat 65˚C 4 - 5 hr

Extract DNA using phenol:chloroform
Precipitate with ethanol and 10 µl glycogen (5 mg/ml)
Resuspend pellet in 100 µl H_2O

DNA Analysis

Analyze DNA isolated by:
quantitative PCR, sequencing (ChIP-seq), microarray (ChIP-chip).