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Chromatin immunoprecipitation V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol can be ued for chromatin immunoprecipitation of RNAPII and associated factors, as well as histones. The settings are given for HeLa cells and should be adapted for other cell types.

Guidelines

The protocol given is suitable for a 15 cm^2 dish of HeLa cells at 80 % confluence per IP. DNA shearing conditions are suitable for HeLa cells and should be tested for other cell types. Antibody concentrations used for IP should also be tested for the specific antibodies to be used. Use molecular biology grade reagents and water.

Materials

MATERIALS

- protein A/G plus agarose **Santa Cruz Biotechnology Catalog #**sc-2003
- Complete protease inhibitor cocktail **Roche Catalog #**05052489001
- normal goat IgG **Santa Cruz Biotechnology Catalog #**sc-2028

Before start

Prepare the following buffers and store at +4°C:

	10 % Sodium deoxycholate: **10 g /100 ml water**			
	Lysis buffer: **Tris-HCl, pH 7.5** **KCl** **EDTA** **NP40 (Igepal)** **SDS** **Water** **<u>Just before use add:</u>** **Protease inhibitor cocktail** **NaF**	**Stock** **1 M** **2 M** **0.5 M** **10 %** **10 %** **50 x** **1 M**	**Final Conc** **50 mM** **150 mM** **5 mM** **1 %** **0.1 %** **1 x** **50 mM**	**Vol (for 2 ml)** **100 µl** **150 µl** **20 µl** **200 µl** **20 µl** **1370 µl** **40 µl** **100 µl**
	RIPA Buffer (Stock Solution): **Tris-HCl, pH 7.5** **KCl** **NP40 (Igepal)** **Water**	**Stock** **1 M** **3 M** **10 %**	**Final Conc** **50 mM** **150 mM** **1 %**	**Vol (for 30 ml)** **1.5 ml** **1.5 ml** **3 ml** **24 ml**
	RIPA Buffer: **RIPA Buffer (Stock Solution)** **for bead washing:** **Water OR** **for IPs:** **Protease inhibitor cocktail** **NaF** **water** **(add just before use)**	**Stock** **50 x** **1 M**	**Final Conc** **1 x** **50 mM**	**Vol (for 3 ml)** **2.7 ml** **300 µl OR** **60 µl** **150 µl** **90 µl**
	Resuspension Buffer: **Tris-HCl, pH 7.5** **EDTA** **DTT** **SDS** **Water**	**Stock** **1 M** **0.5 M** **100 mM** **10 %**	**Final Conc** **50 mM** **5 mM** **10 mM** **1 %**	**Vol (for 1 ml)** **50 µl** **50 µl** **100 µl** **100 µl** **750 µl**
	RIPA Wash Buffer: **Tris-HCl, pH 7.5** **KCl** **NP40 (Igepal)** **Sodium deoxycholate** **Water**	**Stock** **1 M** **2 M** **10 %** **10 %**	**Final Conc** **50 mM** **150 mM** **0.1 %** **0.25 %**	**Vol (for 100 ml)** **5 ml** **7.5 ml** **1 ml** **2.5 ml** **84 ml**
	TE 1 x Wash Buffer **Tris-HCl, pH 7** **EDTA** **Water**	**Stock** **1 M** **0.5 M**	**Final Conc** **10 mM** **1 mM**	**Vol (for 50 ml)** **500 µl** **100 µl** **49.4 ml**

Prepare Protein A/G Beads

1 (Hint: when pipetting beads, cut off ends off of P200 pipette tips to allow the beads to pass through)
For each ChIP, use 160 µl protein A/G agarose beads (equivalent to 40 µl dry bead volume)
Centrifuge beads for 1 min at 6000 rpm
Remove supernatant and add an equal dry bead volume of RIPA buffer (solution for beads) and mix well
Centrifuge 1 min at 2600 rpm, then 10000 rpm for 6 sec
Remove supernatant
Wash 4 times as above with 1 ml RIPA buffer (solution for beads)
Remove supernatant
Add an equal dry bead volume of RIPA buffer (solution for beads), yeast tRNA (200 µg/ml final conc.) and salmon sperm DNA (40 µg/ml final conc.)
Incubate 1 h at room temp on a rotating stand
Centrifuge 1 min at 2600 rpm, then 10000 rpm for 6 sec
Remove supernatant and add 1 dry bead volume RIPA buffer (solution for beads)
Remove 40 µl dry beads using cut-off pipette tip and mix with 160 µl RIPA buffer (solution for beads)
(Keep the remaining bead slurry for pre-clearing of chromatin at step 3)
Add 3 µg of specific antibody or IgG (optimal amount should be determined for each antibody)
Incubate tubes on a rotating stand at +4°C for 4 h

Prepare chromatin extract

2 Perform cross link by adding 1% formaldehyde (final conc.) directly to cell culture medium
Incubate 10 min at RT
Block with glycine pH7.5 (250 mM final conc.) for 5 min at RT
Wash twice with 10 ml PBS
Scrape cells in 1 ml PBS, split into 2 tubes and centrifuge at 3000 rpm for 5 min
Remove supernatant
(Note: it is possible to flash freeze cell pellets in liquid N2 at this stage)
Resuspend each pellet in 600 µl RIPA lysis buffer, on ice
Shear DNA in a Bioruptor nano (Diagenode) by 8 cycles, high setting, 30 sec ON, 30 sec OFF (conditions to be determined for other cell types)
Add 450 µl RIPA buffer (solution for IP) to each tube
Centrifuge at 15000 rpm for 10 min at +4°C to remove cell debris
Pool chromatin extracts
(do not freeze at this stage, proceed directly to step 3)

Immunoprecipitation

3

Pre-clear chromatin extract from step 2 with bead slurry saved from step 1 together with 3 µg normal goat IgG, for 1 h at 4°C
Centrifuge at 10000 rpm for 1 min
Collect supernatant (pre-cleared chromatin extract), remove 100 µl for input, and add the remainder to beads/antibody pre-mix from step 1.
Place tubes on a rotating stand overnight at 4°C.

Washes and Elution

4 Wash beads 4 times in 1 ml RIPA wash buffer (spin 30 sec at 2500 rpm, then a few seconds at 10,000 rpm to compact the beads, discard supernatant)
Wash once in TE wash buffer
Resuspend beads in 100 µl RIPA resuspension buffer
Reverse cross-link samples at 65°C overnight (IP and input) with agitation (thermomixer)
Spin tubes at 5000 rpm for 1 min and recover supernatant (discard beads)
Add 1 µg Proteinase K to samples and incubate at 45°C for 1 h
Purify DNA from samples using QIAquick PCR purification kit
Elute DNA in 50 µl water