INTACT purification of nuclei

Purpose and Background

The INTACT method (for Isolation of Nuclei Tagged in specific Cell Types) allows in vivo affinity labeling and subsequent purification of nuclei from a cell type of interest. This is achieved through cell type-specific expression of a tripartite nuclear tagging fusion protein (NTF) consisting of a nuclear envelope targeting domain, GFP, and the biotin ligase recognition peptide (BLRP). Co-expression of NTF along with the E. coli biotin ligase, BirA, in the cell type of interest results in the production of fluorescently labeled, biotinylated nuclei specifically in that cell type. These labeled nuclei can then be affinity purified from a crude tissue homogenate using streptavidin-coated magnetic beads, thus allowing access to RNA and chromatin from the cell type of interest.

Materials

- 70 µM Cell Strainer (Fisher cat # 22-363-548)
- M-280 Streptavidin Dynabeads (Invitrogen cat #11205D) or NEB Streptavidin beads (cheaper and seems to work better in Brady lab)
- Dynamag 15 magnet (Invitrogen cat #12301D) – or a homemade version of this ☺
- Dynamag 2 magnet (Invitrogen cat #12321D) – or a homemade version of this ☺
- Nutator single-speed orbital mixer (Fisher cat # 14-062)

Solutions

Stock Solutions
0.5M MOPS – filter sterilize, aliquot and store at -20C
1M NaCl – autoclave and store at RT – or shop-bought 5M NaCl (see library buffer stocks)
2M KCl – autoclave and store at RT
0.5M EDTA pH8 – autoclave and store at RT – or shop-bought equivalent (see library buffer stocks)
0.5M EGTA pH8 – autoclave and store at RT
0.2M Spermidine – filter sterilize, aliquot and store at -20C
0.2M Sperimine – filter sterilize, aliquot and store at -20C

Nuclei Purification Buffer base	for 60ml (one sample)	for 1l (~16 samples)
20 mM MOPS (pH 7)	2.4 ml 0.5M MOPS	40ml 0.5M MOPS
40 mM NaCl	2.4 ml **1M** NaCl	8ml **5M** NaCl
90 mM KCl	2.7 ml 2M KCl	45ml 2M KCl
2 mM EDTA	240 ul 0.5M EDTA pH8	4ml 0.5M EDTA pH 8
0.5 mM EGTA	60 ul 0.5M EGTA pH8	1ml 0.5M EGTA pH8

	50ml NPB	**50 ml** NPBt	**300 ml** NPBt	**Stock component**
0.1% Triton X-100	0 ul	500 ul	3000 ul	10% Triton X-100
0.5 mM Spermidine	125 ul	125 ul	750 ul	0.2M Spermidine
0.2 mM Spermine	50 ul	50 ul	300 ul	0.2M Spermine
1x Complete Protease Inhibitors	2000 ul	0 ul	0 ul	25x CPI (2ml/tablet)
For 8 samples:	Prep 2x50ml	Prep 6 x 50ml **or** 1 x 300ml		

Without spermidine, spermine and complete protease inhibitors the solution can be filter sterilized, stored at 4 °C for up to 3 months and used as a stock to prepare each of the other nuclei purification buffers. Add the last components just prior to use and keep at 4C.

NPBt: NPB containing 0.1% Triton X-100

(add Triton X-100 **before** spermidine and sperimine (no CPI), filter sterilize and keep at 4C after preparing this)

Procedure

Note: for using the INTACT material for ChIP, fresh tissue needs to be cross-linked.

(Crosslink proteins to DNA by placing tissue in 30 ml of NPB with 1% formaldehyde, and incubating the tissue under vacuum for 10 min. Add glycine to a final concedntration of 0.125 M, place under vacuum for 5 more min. Wash tissue 3 times with water and blot dry.)

1. **Prepare NPB and** NPBt **buffers** as needed (~15ml NPB and 45ml NBPt per sample).
2. **Grind** frozen tissue (50-200 tomato root tips) in liquid nitrogen with ceramic mortars and pestles until very fine.

   Optional: Pour powder into 13ml Falcon tube and weigh the sample.
3. Pour ~8ml (3 transfer pipettes) of ice-cold **NPB** into a mortar and **resuspend** the powdered tissue by gently grinding it (scale up for larger samples). It is important that **NPB** does not freeze, so either use minimal amounts of liquid nitrogen or move to a fresh mortar for this step.

   Filter the resuspension through the 70 µM cell strainer into a 50ml Falcon tube.
4. **Spin** 1200g for 7 min at 4C. (2,450 rpm on the Eppendorf 5810R centrifuge).
5. **Prepare the beads in 1.5ml tubes**. Wash **50ul of NEB streptavidin beads** (or 25ul of Invitrogen M-280 Streptavidin Dynabeads) for each sample. To wash the beads, pipette well-mixed beads into a 1.5ml tube, place on magnet, remove supernatant, mix with 1ml NPB, place on magnet, remove supernatant, resuspend in NPB of original volume (50ul).
6. *Optional: Mark down the volume of supernatant and set aside 10% of the supernatant for Westerns. ("SN1200" – if you want to check you have not lost any nuclei / biotin signal in your supernatant)*.

   Discard the supernatant (by careful pouring).

   Resuspend the nuclei in 1ml of cold **NPB**.

   Set aside 11ul of the nuclei for quantification and yield calculations (PCR strip works well).

   Optional: set aside 10% of the resuspension "P1200" for Westerns (total amount of signal in the sample).

7. **Add the nuclei to the washed beads** (in 1.5ml tubes) and **rotate** on a nutator at 4° C for 30 min.
8. Dilute the 1 mL of bead-nuclei mixture to 14 ml in a falcon tube with NPBt. Mix gently and place on the nutator for 30 sec. Place tube on the magnet for 15 -20 min.

   Optional: Set aside 10% of the supernatant for later use ("Unbound" – also for Westerns to check if you are losing any of the tagged nuclei in the wash).
9. Carefully remove the supernatant with a pasteur pipette and gently resuspend the beads in 14 ml NPBt. Mix gently and place on the nutator for 30 sec. Place tube on the magnet for 10 min (at 4C).
10. Repeat step 8.
11. Gently remove the supernatant. Resuspend the beads in 1ml of NPBt.

    Set aside 11 ul of the nuclei for quantification and yield calculations (PCR strip works well).

    Optional: Set aside 10% of the resuspended beads for later use ("Bound" – also for Westerns to make sure most of your biotinylated signal is here)
12. Transfer nuclei bound on beads into 1.5ml tube and capture on the magnet.
13. Remove supernatant, resuspend beads in 20ul **NPB.**

    Do not freeze nuclei until after they have been counted and aliquoted.

Counting nuclei with hemocytometer to quantify the efficiency and yield

1. Mix 20ul nuclei (either on beads from step 12 or from the resuspended pellet on step 5) with 0.5ul 1mg/ml Propidium Iodide (PI) stain (keep in fridge, wrap in foil to prevent degradation).
2. Pipette the sample onto a hemocytometer (In-Cyto, #DHC-N01-2).
3. View the samples under fluorescent light, count the nuclei per square. Use this to calculate the number of nuclei in total sample.

   Kaisa calculates 16 squares in the corner of the grid, comprising $1/9^{th}$ of the whole grid. (This area is 1mm x 1mm x 0.1mm, or 0.1 ul, and represents $1/10{,}000^{th}$ of 1ml).

   Multiply the number of nuclei found inside this area by 10,000 to find out total yield.

   Optional calculations & advice:

   Typical yields for 35Spro:NTF is 1,500,000 – 2,300,000 nuclei/gram.

   Efficiency: (35S-IN beads – background) / (35S-IN pellet)

   *Background: (35S-IN pellet) * (M82 beads) / (M82 pellet)*

Aliquoting nuclei for RNA and ATAC

1. Calculate how many ul of nuclei you need out of the final 20ul to get 50,000 nuclei for ATAC. Pipette these onto a PCR strip on ice and start ATAC protocol.
2. Calculate additionally 2.5ul aside to another PCR strip as a back up for ATAC libraries. Store at -80C.
3. Rest of the nuclei freeze for RNA at-80C.

References

1. Deal, R.B. and Henikoff, S. (2010) A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Developmental Cell. 18: 1030-1040.

2. Deal, R.B. and Henikoff, S. (2011) The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. Nature Protocols. 6: 56-68.

3. Ron, M.R., Kajala K., et al., (2014) Hairy root transformation using Agrobacterium rhizogenes as a tool for exploring cell type-specific gene expression and function using tomato as a model. Plant Physiology.