Purification of nuclei from specific plant cell types using the INTACT method

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Overview:

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.

The protocol presented here has been optimized for purification of either root epidermal non-hair cell or trichome nuclei from a transgenic *Arabidopsis thaliana* line in which NTF labels the nuclei in both of these cell types (GL2p:NTF/ACT2p:BirA line), as well as nuclei from the root epidermal hair cell type using the ADF8p:NTF/ACT2p:BirA transgenic line.

Method 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.

Materials:

- 70 μM Cell Strainer (Fisher cat # 22-363-548)
- M-280 Streptavidin Dynabeads (Invitrogen cat #11205D)
- Dynamag 15 magnet (Invitrogen cat #12301D)
- Dynamag 2 magnet (Invitrogen cat #12321D)
- Nutator single-speed orbital mixer (Fisher cat # 14-062)

Solutions:

Nuclei purification buffer (NPB)
20 mM MOPS (pH 7), 40 mM NaCl, 90 mM KCl, 2 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine, 0.2 mM spermine, 1X Complete protease inhibitors. Spermidine, spermine, and Complete protease inhibitors are added just before use and the solution is kept on ice.

Procedure:

1) Grind 3g of roots or 0.5g of leaves in liquid N2 and resuspend tissue powder in 10 mL of cold NPB buffer. Filter this extract through 70 μM nylon mesh and spin down the nuclei at 1200g for 7 min at 4° C.

2) Gently resuspend the nuclei in 1 mL of cold NPB and move to a 1.5 ml tube.

3) Wash the appropriate amount of Invitrogen M-280 Streptavidin Dynabead suspension (25 uL for each 3 g of roots or 10 uL for each 0.5 g of leaves) with 1 mL of NPB and then resuspend the beads with NPB to their original volume. Add the bead suspension to the nuclei from Step 2 and rotate at 4° C for 30 min.

4) Dilute the 1 mL of bead-nuclei mixture to 14 mL in a falcon tube with NPB containing 0.1% Triton X-100 (NPBt). Mix gently and place on the nutator for 30 sec. Place the tube in the DynaMag 15 magnet for 2 minutes.

5) Carefully remove the supernatant with a serological pipette and gently resuspend the beads in 14 mL of NPBt. Mix gently and place on nutator for 30 sec. Place the tube in the DynaMag 15 magnet for 2 minutes.

6) Repeat step 5.

7) Gently remove the supernatant with a serological pipette and resuspend the beads in 1 mL of NPBt. Remove a 25 uL sample for counting of the captured nuclei on a hemocytometer.

8) Transfer the resuspended beads to a 1.5 mL tube and capture on a Dynamag 2 magnet.

9) Remove the supernatant, resuspend beads in 20 uL of NPB, and proceed with downstream processing (RNA isolation or ChIP). Alternatively, nuclei/beads can be stored at -80° C until further use.

10) To view purified nuclei under the microscope, add 1 uL of 0.2 ug/uL DAPI to each 25 uL sample (taken at step 7) and place on ice for 5 min. Count nuclei using a hemocytometer.

Notes:

A) The actual yield of purified nuclei is generally around 50% of the theoretical yield for the cell types we have examined, so it is recommended to begin with an amount of tissue that will yield at least 1 x 10^5 nuclei. This number of nuclei should also yield 100-200 ng of total RNA when purified using the Qiagen RNeasy Micro kit.

B) For crosslinked chromatin immunoprecipitation experiments, starting tissue can be treated with formaldehyde, quenched, washed, and used directly in the above protocol without any alterations.

C) Use of this protocol with other types or amounts of tissue may require optimization. The most important parameters seem to be the amount of beads used per amount of tissue and the volume of solution used for capturing the beads after nuclei binding. For larger scale purifications, the Dynamag 50 magnet can be used to capture the beads in a 40 ml volume.