FR-AgEncode: a French pilot project to enrich the annotation of livestock genomes

Tissue sampling procedure 4a

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This procedure describes the steps to be applied for storing frozen dissociated cells from a solid tissue, in the view of performing Hi-C assay.

It does not describe the anatomical procedure to isolate a specific organ or tissue.

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The objective is to produce a snap-frozen pellet of around 5 million fixed cells per aliquot.
This procedure has been applied to liver, spleen and mammary cells.

Reagents:
Formaldehyde F8775-4x25ML, SIGMA
Protease inhibitors: P8340-1ML SIGMA
Ice cold PBS 1X for Cell Culture
DMEM (SIGMA D0819 or equivalent)
Glycine 1.25M in water: 50046-50G SIGMA
StemPro Accutase (Life technologies): A1110501 **(prewarm at 37°C)**
Before starting: prepare ice cold PBS with protease inhibitors (100ul of protease inhibitors (P8340) for 10ml PBS).
Vectashield+DAPI : H-1200, Vector Labs
Phalloïdin-TRITC: P1951 SIGMA

Materials:
Forceps Dumont
Scalpel blades
Petri dishes (90mm)
Falcon tubes (15ml and 50ml)
5ml, 10ml, 25ml pipettes
Pipet-Aid
10ml sampling bulbs (blue ones)
Cell strainer 70µm (352350 BD Biosciences)
2ml Eppendorf tubes
Liquid nitrogen or dry ice
Centrifugation machine for 50ml and 15ml falcon tubes
Centrifugation machine for 2 ml tubes
Microscope slides and coverslip
Pens

Procedure

A. Cell dissociation (step time: around 1 hour)

1. Dissect the tissue.
2. Wash the tissue biopsy in PBS to remove the circulating blood as much as possible.
3. Dilacerate the tissue with scalpel blades as fine as you can (the finest the best to remove blood and optimize dissociation). A few cm^3 piece is sufficient to produce around 20-40 million of cells

4. Wash the pieces of tissue with PBS to remove blood as much as possible. For that, add PBS to the pieces, agitate well, decant and remove the supernatant, repeat until the supernatant is transparent. Remove the supernatant
5. Add 15ml of StemPro Accutase previously warmed at 37°C and incubate for **10 minutes** with moderate agitation in a water bath at 37°C
6. Using a 10ml pipet and a pipet-aid (or sampling bulbs), pipet up and down 20 times to homogenize the suspension (if it is well done, you won't have problem to do it either with a 25ml pipet or better with a 10ml pipet)
7. Filter through a cell strainer of 70µm into a 50ml tube
8. Centrifuge at 250g for 5 minutes
9. Remove the supernatant (and remaining blood cells covering the pellet) and add 40 ml of DMEM without serum. Evaluate cell quality and count the cells
10. Centrifuge at 250g for 5 minutes
11. Remove the supernatant (and remaining blood cells covering the pellet) and resuspend at a concentration of 5 million/cells per 10ml of DMEM without serum

B. Cell Fixation (step time: around 40 minutes) (volumes are described for 5 million/cells)

1. Add 1% final of freshly opened formaldehyde to the growth medium *(270µl of 37% formaldehyde for 10ml of medium)*.
2. Incubate for 10 minutes at room temperature (around 20°C).
3. Add 0.125M final Glycine to quench the fixative (*1.1ml of glycine 1.25M for 10 ml of medium*)
4. Incubate 5 minutes at RT and cool 5 minutes on ice.
5. Centrifuge the tubes at 250g for 5 minutes to pellet the cells
6. Aspirate the supernatant and wash the cell pellets with 5 ml ice cold PBS (with protease inhibitors)
7. Centrifuge at 250g 5 minutes to pellet the cells
8. Resuspend the cell in 1ml ice cold PBS (with protease inhibitors)
9. Transfer the cell to the storage tube (eppendorf 2ml)
10. 🎧 *Check nuclei integrity: remove 10µl of cell suspension and add 10µl of Vectashield+DAPI +1µl of 10X Phalloidine-TRITC (500µg/ml). Place 10µl over a clean microscope slide and cover with a coverslip. Observe under an epifluorescence microscope.*
11. Centrifuge at 250g 5 minutes to pellet the cells
12. Keep cell pellets on ice and discard the supernatant
13. Flash freeze the pellets in liquid nitrogen and store at -80°C