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

Tissue sampling protocol 3b

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This protocol describes the preparation and storage of isolated alveolar macrophages from the lung of mammals. It has been applied to cattle, goat and pig.

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Code de champ modifié

See related references: PMIDs: 19794892, 24021155,

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Objective:
Obtain a suspension of bronchio-alveolar cells for long term storage in liquid nitrogen (note : this will be an heterogeneous preparation containing around 70% macrophages that will adhere to a culture plate after defrosting+ non adherent macrophages, lymphocytes and epithelial cells)

Reagents:

- PBS without Ca2+ /Mg2+ (Eurobio, ref CSPBS01-01, 500ml)
- L-Glutamine 200mM ((Fisher Scientific, ref 11500626)
- Penicillin, streptomycin (Fisher Scientific, ref 11548876)
- 0.5 M EDTA
- NH_4Cl 1X (140 mM) (prepared from NH_4Cl 10X: 74.7 g NH_4Cl, 85 ml 2M Tris HCl ph:7.5, qsp H_2O 1L)
- Fetalcalf serum, sterile filtered (Eurobio, ref CVFSVF06-01 500ml)
- Dimethylsulfoxide (DMSO)

Materials:

- Ice
- Falcon tubes (50ml)
- Cryotubes
- A big beaker
- A funnel
- 2ml, 5ml, 10ml pipets
- Pipet-Aid
- Centrifugation machine for 50ml falcon tubes – refrigerated
- Thermo Scientific™ *Mr. Frosty*™ Freezing Container.

Procedure

Before start:
Put the NALGENE Mr Frosty at 2-4°C overnight (the isopropanol level must be checked and must be completely replaced after the fifth freeze-thaw cycle).

Prepare a 20% DMSO/FCS solution and allow cooling at 2-4°C for 1 day.

Prepare buffer A = PBS 1X, 2mM EDTA, 100 U/ml penicillin, 100 µg/ml streptomycin), 2 mM L-Glutamine.

1. Using a funnel, infuse into one lung 250 ml of cold buffer A via the trachea.
2. Apply gentle massaging to the lung.
3. Pour the liquid suspension into a beaker placed on ice.
4. Repeat twice steps 1-3 using the same beaker.
5. Split cell suspension collected from the lung washes into falcon tubes of 50 ml.
6. Centrifuge 300g (1700 rpm) for 7 minutes, at 4°C.
7. Decant the supernatant and wash pellet with 50 ml of cold buffer A

8. Centrifuge 300g (1700 rpm) for 7 minutes, at 4°C
9. Decant the supernatant and add 10ml of NH_4CL 1X on each pellet for the lysis of red blood cells. Incubate 10 minutes on ice. Stop the reaction by adding 40 ml of PBS/2mM EDTA.
10. Centrifuge 300g (1700 rpm) for 7 minutes, at 4°C. Decant the supernatant and resuspend the cells in 10 ml of PBS.
11. Count cells using a haemocytometer *(it is not necessary to evaluate quality at this stage; this will be done upon thawing).*
12. Centrifuge 300g (1700 rpm) for 7 minutes, at 4°C and resuspend in 1 ml of FCS/10%DMSO per cryotube:
    - Spin at 1700 rpm for 7 min
    - Decant supernatant and resuspend cells in half necessary volume of cold FCS
    - Add the same volume of cold FCS/ 20%DMSO slowly drop by drop.
    - Mix gently and aliquot 1mL of cell suspension to each cryotube. Not exceed 50 $x10^6$ cells/ml
13. -Put the tubes into the NALGENE Mr Frosty.
14. - Place the box immediately at -80°C for 24hr and put tubes in liquid nitrogen for long term storage.

It is possible to continue directly for ATAC-Seq preparation, without freezing.

See protocol Fr-AgEncode_sampling_protocol_5, step II.