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

Separation of PBMCs from swine blood samples protocol

INRA
Division of Animal genetics

Written by :
Silvia Vincent-Naulleau (silvia.vincentn@cea.fr)

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Required reagents
- RPMI 1640 without L-glutamine (Eurobio, ref: CM1RPM00-01 500ml)
- PBS without Ca2+ /mg2+ (Euroio, ref CSPBS01-01, 500ml)
- MSL: Lymphocyte Separation Medium sterile filtered, density 1.077+/-0.001 (MSL Eurobio ref CMSMSL01-0U, 100 ml)
- FCS sterile filtered (Eurobio, ref CVFSVF06-01 500ml)
- NH4Cl 1X prepared from NH4Cl 10X: 74.7 g de NH4Cl, 85 ml Tris HCl ph:7.5, qsp H2O 1L

Before starting:
Put the NALGENE Mr Frosty at 2-4°C overnight (the isopropanol level must be correct and must be completely replaced after the fifth freeze-thaw cycle).
Prepare a 20% DMSO/FCS mixture and allow cooling at 2-4°C for 1 day.
Thaw an aliquot of FCS and allow cooling at 2-4°C.

1. Under a laminar flow cabinet Class II, transfer blood from blood collection tubes into one or two, 50 mL tubes.
2. Dilute blood with PBS (1:1 dilution).
3. Add MSL in sufficient 15 mL (or 50mL) tubes to use all the blood volume (respect 1 volume of diluted blood on ½ volume of MSL).
4. Carefully layer diluted whole blood over MSL medium; the diluted blood is added to the gradient by gently pipetting onto the separation medium with the tubes held at an angle.
5. Spin at 818 xg (2000 rpm with the centrifuge) for 30 min, at 20°C, acceleration 9, no brake (braking rate 0).
6. Carefully remove the tubes from the centrifuge while not disturbing the layering.
7. Carefully remove the PBMC layer from the tube and transfer to new 15 mL conical tubes (or 50 mL when pooling of 2 PBMC layers) containing 10 ml of RPMI+10%FCS. Avoid aspirating MSL. Add PBS qsp 15mL (or 50mL).
8. Spin at around 650 xg (1900 rpm) for 10 min, acceleration 9, deceleration 9.
9. Decant the supernatant and add 5ml of NH4CL on each pellet for red blood cells lysis . Incubate during 15-20 minutes. Stop the reaction by adding 5ml of RPMI+10%FCS. Pool all the PBMC of each animal. Add PBS qsp 15mL (or 50mL).
10. Spin at 1300 rpm for 10 min, acceleration 9, deceleration 9.
11. If necessary, make a new step of red blood cells lysis. Incubation during 10 minutes.
12. Decant supernatant and resuspend the cells in 10 ml of PBS.
13. Count the cells
- ✓ manually with a Malassez cell and determine the cell viability with trypan blue by mixing a small volume (50 µL) of the PBMC with trypan blue solution 1:1. Load the Malassez with the cell mixture and wait for at least 30 seconds before counting
- ✓ or with a GUAVA cytometer. In a 96 wells microplate, add in 2 wells for each PBMC 180µl of Viacount (Millipore). Add 20µl of cells in the first well (dilution 1/10) and 20µl of the 1/10 dilution in the second well (1/100 dilution). Load the application VIACOUNT on the cytometer and launch the analysis.

14. Spin at 1300 rpm for 10 min, acceleration 9, deceleration 9.
15. If PBMC are prepared for long term storage, hand-label cryotubes and put them on ice. Generally we store PBMC at 10-30 10^6 cells in 1 ml of FCS/10%DMSO per cryotube.
16. Decant supernatant and resuspend PBMC in half necessary volume of cold FCS; first, add 0.5 or 1mL FCS, mix/detach the cells by gentle pipetting and then add the remaining volume.
17. Add the same volume of cold FCS/ 20%DMSO slowly drop by drop. Mix gently two times and aliquot 1mL of cell suspension to each cryotube (1.8 mL).
18. Put the tubes into the NALGENE Mr Frosty.
19. Place the box immediately at -80°C for 24hr and put tubes in liquid nitrogen for long term storage.