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

Sorting of bovine NKp46^{-} CD4^{+} CD8^{-} and NKp46^{-} CD4^{-} CD8^{+} protocol

INRA

Written by :
Fabrice Laurent, Françoise Drouet, Sonia Lacroix-Lamandé and Yves Le Vern

December 2017

Sorting of bovine NKp46^{-} CD4^{+} CD8^{-} and NKp46^{-} CD4^{-} CD8^{+} cells

Required reagents
- polystyrene 5 ml FACS tubes (12 x 75 mm, BD Falcon Cat#352058)
- anti-NKp46 (clone AKS1, IgG1, Biorad Cat#MCA2365GA)
- anti-CD4 (clone GC50A, IgM, King Fisher Cat#WS0563B-100)
- anti-CD8a (clone 38.65, IgG2a, Biorad Catt#MCA2216GA)
- mouse IgM (Dako Cat#X0492)
- mouse IgG2a (Dako Cat#X0943)
- mouse IgG1 (Dako Cat#X0931)
- goat anti-mouse IgM-AF488 (Fluoprobes Cat#FP-SA4030)
- goat anti-mouse IgG1-TC (ThermoFisher Scientific Cat#M32006). Alternatively rat anti-mouse IgG1-PECy7 can be used (Biolegend Cat#406613).
- goat anti-mouse IgG2a-PE (ThermoFisher Scientific Cat#M32204)
- cell strainer cap, 35 µm (BD Falcon Cat#352235)
- PBS without Ca2+ /mg2+ (Eurobio, Cat#CSPBS01-01)
- Bovine Serum Albumin (BSA)
- Fetal Calf Serum (FCS)
- EDTA

Before starting:
Prepare PBS containing 0.5% BSA and 2mM EDTA.

Important note:
PBMCs must be prepared and stored before cell staining and sorting according to "INRA_SOP_PBMC_purification_cattle_caprine_20160504" available for download at ftp://ftp.faang.ebi.ac.uk/ftp/protocols/samples/. Samples must have very few red blood cells. If necessary, another step of red blood cells lysis can be performed by incubating cells during 2 minutes with 5ml of lysis buffer (NH4Cl 155mM + KHCO3 10mM + EDTA 0.2 mM). Stop the reaction by adding 45ml of RPMI+10%FCS. Spin at 400g for 10 min at 4°C.

Preparation of cells

1. PBMCs from each blood samples are thawed are transferred in 15 ml polypropylene tube qsp 10ml RPMI 10% FCS and centrifuged at 400g for 10min at 4°C. Supernatant is then discarded by inverting the tube. Cell viability is assessed and cell concentration adjusted to $5x10^7$ living cells/ml in RPMI 10%FCS.

2. PBMCs are incubated 1hour over crushed ice with primary antibodies: anti-NKp46 (1/200, clone AKS1, mouse IgG1), anti-CD4 (1/500, clone GC50A, mouse IgM) and anti-CD8a (1/5000, clone 3865, mouse IgG2a). NB: Each antibody is also evaluated individually for Cell Sorter calibration. In this case, cell staining was performed in similar condition as above but with 2.5 10^6 in 50ul and in a 96 well microplate.

3. Cells are centrifuged at 400g for 5min at 4°C and washed twice by adding 3mL of PBS containing 0.5% BSA and 2mM EDTA (200ul in microplate wells) followed by centrifugation at 400g for 5min at 4°C. Supernatant is then discarded by inverting the tube (or the microplate).

4. The pellet is suspended at $5x10^7$ living cells/ml in RPMI 10%FCS with secondary antibodies: goat anti-mouse IgG1TC (1/600), goat anti-mouse IgG2a-PE (1/200) and goat anti-mouse IgM-AF488 (1/1000) and incubated 1 hour over crushed ice.

5. Cells are centrifuged at 400g for 5min at 4°C and washed twice as described previously. Supernatant is then discarded by inverting the tube or the plate.

6. The pellet is finally suspended in PBS containing 0.5% BSA and 2mM EDTA at 10^7 cells/mL. Cells are filtered with cell strainer cap, 35µm.

Flow cytometry acquisition and sorting of cells

7. Compensation matrix is realized with single stainings used as controls.

8. NKp46$^-$ CD4$^+$ CD8$^-$ and NKp46$^-$ CD4$^-$ CD8$^+$ cells are sorted on a MoFlo™, Beckman Coulter.

9. Cells were then snap-frozen for RNA-seq and HiC experiments or frozen in 10% DMSO-FCS for ATAC-seq experiments.