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

Sorting of chicken $CD3^{+}CD4^{+}CD8\beta^{-}$ and $CD3^{+}CD4^{-}CD8\beta^{+}$

INRA

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Sorting of chicken lymphocytes

Required reagents
- polystyrene 5 ml FACS tubes (12 x 75 mm, BD Falcon Cat#352058)
- anti-CD4 (mouse anti-chicken CD4-FITC, clone CT-4, IgG1, Southern Biotech Cat#8210-02)
- anti-CD8β (mouse anti-chicken CD8b-PE, clone EP42, IgG2a, Southern Biotech Cat#8280-09)
- anti-CD3 (mouse anti-chicken CD3-SRPD, clone CT-43, IgG1κ, Southern Biotech Cat#8200-13)
- mouse IgG2a-PE (Southern Biotech Cat#0103-09)
- mouse IgG1-FITC (clone 15H6, Southern Biotech Cat#0102-02)
- mouse IgG1κ-SRPD (Southern Biotech Cat#0102-13)
- cell strainer cap, 60µm and 30µm (BD Falcon Cat#352235)
- RPMI
- Fetal Calf Serum (FCS), heat inactivated

Before starting:
Prepare RPMI with 2% heat inactivated FCS.

Important note:
Splenocytes must be prepared and stored before cell staining and sorting according to "INRA_SOP_chicken_splenocytes_sampling_20160721" available for download at ftp://ftp.faang.ebi.ac.uk/ftp/protocols/samples/. Samples must have very few red blood cells.

Preparation of cells

1. Splenocytes are thawed and transferred in 15 ml polypropylene tube qsp 10ml RPMI 2% FCS and washed twice in RPMI 2% FCS (centrifugation at 400g for 10min at 4°C). Supernatant is then discarded. Cell viability is assessed and cell concentration adjusted from $2.5x10^6$ cells/ml for isotype controls and single labelling and $2.5x10^7$ to $1x10^8$ cells/ml for triple labelling (CD3;CD4 and CD8β) in RPMI 2%FCS.

2. Splenocytes are centrifuged to pellet the cells (400g for 10min at 4°C). For triple labelling, cells were incubated 1hour over crushed ice in the dark in 200µl of RPMI 2% FCS with primary antibodies: anti-CD3-SRPD(1/50, final concentration 2µg/ml, clone CT-43, mouse IgG1κ), anti-CD4-FITC (1/250, final concentration: 2µg/ml, clone CT-4, mouse IgG1) and anti-CD8β-PE (1/66, final concentration: 1.5µg/ml clone EP42, mouse IgG2a). Each antibody and isotypic controls are also evaluated individually, at the same concentration, for Cell Sorter calibration. In this case, cell staining was performed in similar condition as above but with $2.5\ 10^6$ using 50µl of working solution.

3. Cells are then centrifuged at 400g for 2min at room temperature and washed twice using RPMI 2%FCS followed by a last wash in RPMI without serum. Cells are centrifuged at 400g for 2min at room temperature. Supernatant is then discarded by inverting the tube.

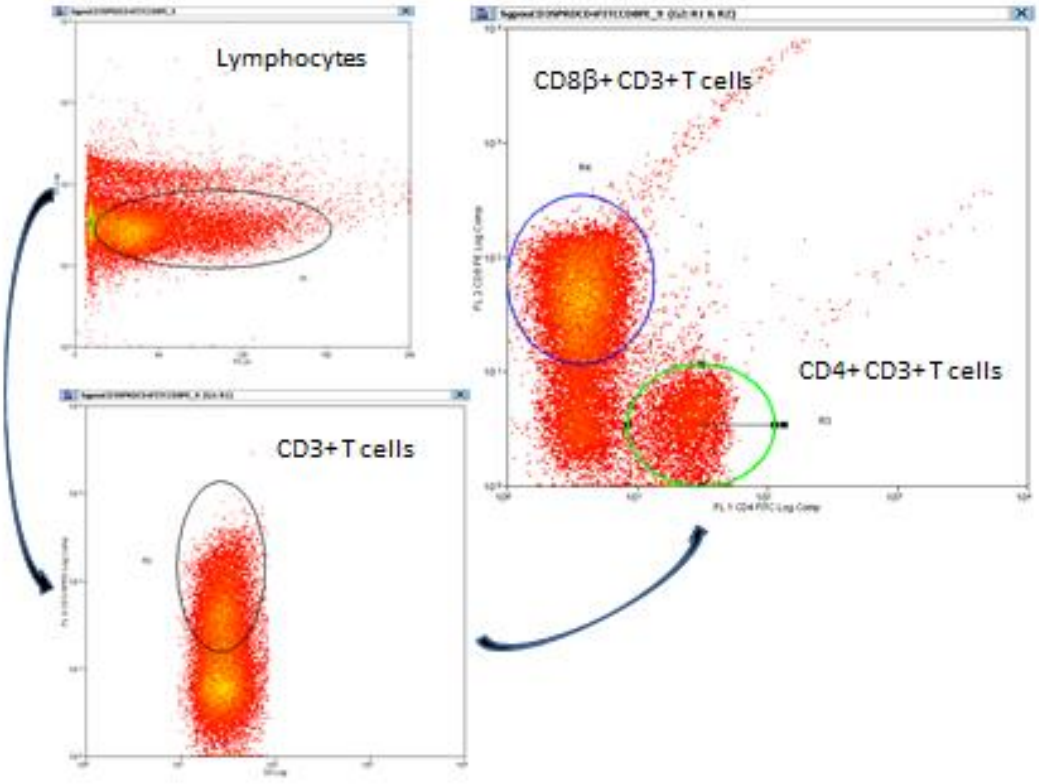
4. The pellet is finally suspended in PBS containing 0.5% BSA and 2mM EDTA at 10^7 cells/ml. Cells are successively filtered with cell strainer cap, 60µm and 30µm.

Flow cytometry acquisition and sorting of cells

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

6. $CD3^+\ CD4^+\ CD8\beta^-$ and $CD3^+\ CD4^-\ CD8\beta^+$ cells are sorted on a MoFlo™ Legacy Cell Sorter, Beckman Coulter.

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


Lymphocytes
CD8β+CD3+T cells
CD4+CD3+T cells
CD3+T cells