Standard operating procedure for single cell isolation from fresh bovine lung tissue

modified after the protocol from Rock et al. (2011) (PMID: 22123957)

Research Institute for Farm Animal Biology (FBN)

Institute of Genome Biology, Genome Physiology Unit

Written by Doreen Becker (becker.doreen@fbn-dummerstorf.de)

25th August, 2023

Purpose

Collecting single cells from fresh lung tissue. Appropriate preparation of required materials and professional training of lab technicians involved cell isolation have to be performed in advance.

Materials

- pipettes, pipette tips
- 1X PBS
- 1X PBS + 0.04% BSA
- beakers
- centrifuge, table centrifuge
- 50 ml Falcon tubes
- protease solution (450 U/ml Collagenase type 1; 4 U/ml Elastase, 5 U/ml Pronase, 0.33 U/ml DNase I in DMEM/F12)
- heater shaker
- DMEM/F12 + 10% FBS
- 0.1% trypsin-EDTA
- DNase I
- 100 µm cell strainer
- Red Blood Cell lysis buffer
- AO/PI
- Cellometer Auto2000

Single-cell isolation

1. the animals are killed according to regular slaughtering protocol including stunning and exsanguination by expert staff in an experimental slaughterhouse of the institute

2. lung tissue is collected during slaughter by expert staff and distributed to trained lab technicians for further sample preparation

3. lung tissue is washed with cold 1X PBS and unnecessary tissue (i.e. fat) is removed

4. an approximately coin-sized piece of lung tissue is placed in a beaker containing cold 1X PBS for transport into the laboratory

5. tissue is cut into small pieces (<2 mm2)

6. tissue pieces are placed into a 50 ml Falcon tube

7. Falcon is filled with 5-10 ml protease solution (450 U/ml Collagenase type 1; 4 U/ml Elastase, 5 U/ml Pronase, 0.33 U/ml DNase I in DMEM/F12) and incubated for 25 min at 37°C with constant agitation (shaking)

8. 10 ml DMEM/F12 with 10% FBS is added and the tissue is additionally dissociated by pipetting

9. Falcon is centrifuged with 500 g for 5 min at 4°C and the supernatant is removed

10. Falcon is incubated for 20 min at 37°C in 5 ml 0.1% trypsin-EDTA and 0.325 mg DNase I with constant agitation (shaking)

11. 5 ml DMEM/F12 with 10% FBS is added and the tissue is additionally dissociated by pipetting

12. cell solution is filtered through a 100 µm filter into a new 50 ml Falcon (if necessary: use a plunger of a disposable syringe to support the filtering of the cell suspension by rubbing on the filter)

13. Falcon is centrifuged with 500 g for 5 min at 4°C and the supernatant is removed

14. cells are washed with 5 ml DMEM/F12 with 10% FBS

15. Falcon is centrifuged with 500 g for 5 min at 4°C and the supernatant is removed

16. cells are treated with 2 ml 1X Red Blood Cell lysis buffer for 1.5 min at room temperature

17. Falcon is centrifuged with 500 g for 5 min at 4°C and the supernatant is removed

18. cells are resuspended with 1 ml 1X PBS containing 0.04% BSA

19. cell concentration and viability are measured using AO/PI staining and a Cellometer Auto2000 (Nexcelom)