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

modified after the protocol from Ramachandran P. et al. (2019) (PMID: 31597160)

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Purpose

Collecting single cells from fresh bovine liver 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
- 1X HBSS
- enzyme cocktail (5 mg/ml Pronase, 2.93 mg/ml Collagenase B and 1.9 mg/ml DNase I)
- heater shaker
- 50 ml Falcon tubes
- 100 µm cell strainer
- PEB buffer (1X PBS, 1 mg/ml BSA, 2 mM EDTA)
- DNase I
- Red Blood Cell lysis buffer
- centrifuge, table centrifuge
- 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. liver tissue is collected during slaughter by expert staff and distributed to trained lab technicians for further sample preparation

3. liver tissue is washed with cold 1X PBS

4. a walnut-sized piece of liver is cut from the tissue (without capsule) and placed in a beaker with cold 1X HBSS (for transport to the laboratory)

5. tissue is minced into small pieces (1-2mm2).

6. tissue pieces are incubated in a 50 ml Falcon in 10 ml enzyme cocktail of 5 mg/ml Pronase, 2.93 mg/ml Collagenase B and 1.9 mg/ml DNase I at 37°C for 30 min with gentle shaking (200 rpm)

7. remaining tissue pieces are removed

8. a 100 µm cell filter is placed on a 50 ml Falcon and the cell suspension is filtered using PEB buffer (1X PBS, 1 mg/ml BSA, 2 mM EDTA) and 0.02 mg/ml DNase I (if necessary: use a plunger of a disposable syringe to support the filtering of the cell suspension by rubbing on the filter)

9. Falcon is centrifuged with 400 g for 7 min at 4°C and the supernatant is removed

10. cells are resuspended in 20 ml cold (1X) Red Blood Cells lysis solution and the Falcon is inverted several times

11. cells are incubated in 1X RBC lysis solution for 5 min at room temperature

12. Falson is centrifuged with 400 g for 7 min at 4°C and the supernatant is removed

13. cells are resuspended in 15 ml PEB buffer with 0.02 mg/ml DNase

14. cells are centrifuged with 400 g for 7 min at 4°C and the supernatant is removed

15. cells are resuspended in 5 ml 1X PBS + 0.04% BSA

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