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SOP –UNITS-3.0 Author: **E. Campaner, A. Zannini** Approved: **G. Del Sal** Issued: **12/04/2019** Revised:

MOUSE MAMMARY ORGANOID CHARACTERIZATION – IMMUNOFLUORESCENCE AFTER CYTOSPINNING

Purpose

The SOP-UNITS-3.0 was issued to describe how to perform molecular characterization of mouse mammary organoids.

Scope

SOP 3.0 is intended to cover all resources, personnel and equipment needed to perform molecular characterization of mouse mammary organoids.

Introduction

Molecular characterization of mouse organoids can be performed using different techniques. Here the procedure for immunofluorescence analysis of mouse mammary organoids using anti-α-smooth muscle actin (α-SMA) and anti-cytokeratin 18 antibodies is described. In the breast tissue, α-SMA is specifically expressed in myoepithelial cells whereas cytokeratin 18 is specifically expressed in luminal cells.

1. Reagents and solutions

- PBS 1X (Life Technologies, cat. no. 14190-094)
- Cell recovery solution (Corning, cat. no. 354253)
- Paraformaldehyde ((Sigma-Aldrich, cat. no. 158127)
  !CAUTION Paraformaldehyde contains formaldehyde, which can cause cancer; handle it using appropriate safety gear.
- FBS (Euroclone, cat. no. ECS0180L)
- Triton-X100 (Sigma-Aldrich, cat. no.T8787)
- Hoechst 33342 (Life Technologies, cat. no. H3570)
- dH_2O
- Prolong gold antifade reagent (Life Technologies, cat. no. P36930)

- Nail polish
- Anti-α-SMA, anti-cytokeratin 18 antibodies
- Goat anti-mouse IgG Alexa Fluor 568 (Thermo Fisher Scientific, cat. no. A-11004)
- Goat anti-rabbit IgG, Alexa Fluor 488 (Thermo Fisher Scientific, cat. no. A-11008)

2. Equipment

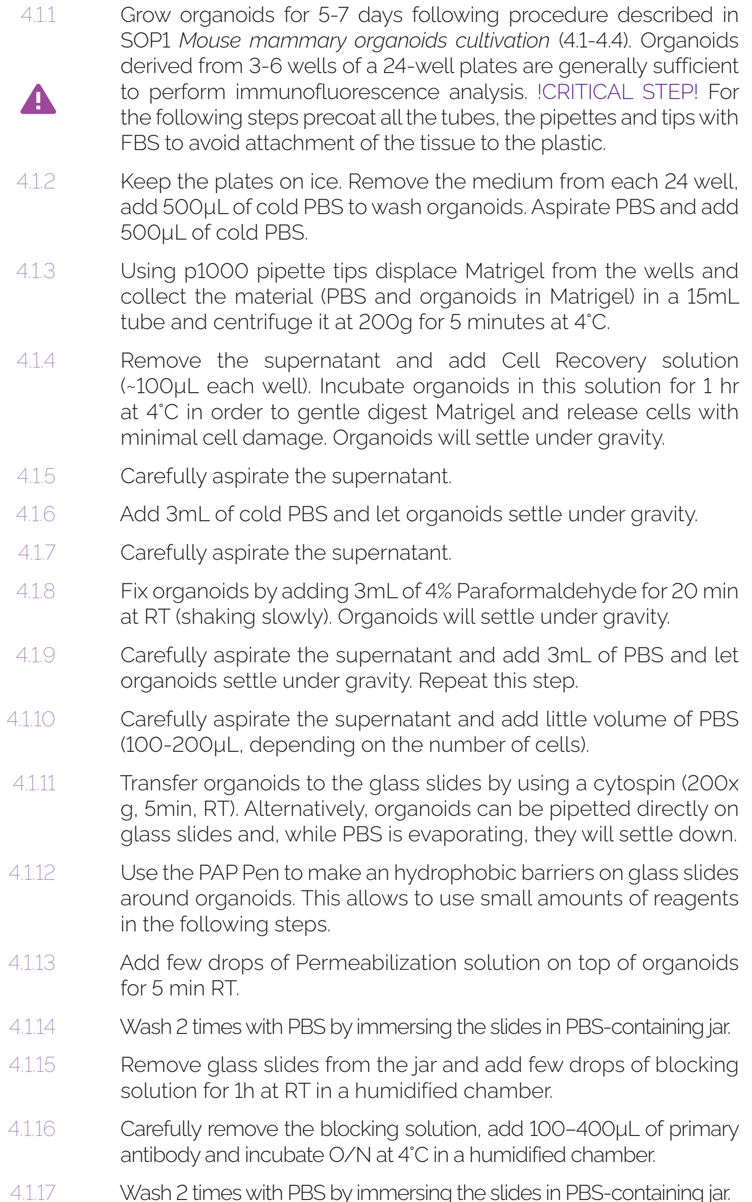
- High-speed centrifuge (Eppendorf centrifuge 5810R)
- Pipette aid, serological pipettes (Euroclone, cat. no. EPS05N; EPS10N)
- Pipettes
- Pipette tips
- Conical centrifuge tubes (Euroclone, cat. no. ET5015B; ET5050B)
- Cytospin
- Microscopes slides Superfrost® Plus (Thermo Scientific, cat. no. J1800AMNZ)
- Microcentrifuge tubes (Euroclone, cat. no. ET3415)
- Staining jars
- PAP Pen for immunostaining (Sigma-Aldrich, cat. no. Z377821)
- Cover glasses circles (Life Technologies, cat no. 12-545-80)
- Confocal fluorescence microscope

3. Reagent setup

- Permeabilization solution: 1% Triton X-100 in PBS
- Blocking solution: 10% FBS, 0.3% Triton X-100 in PBS
- Primary antibodies solution: dilute primary antibodies at an assay depending concentration in blocking solution
- Secondary antibodies solution: dilute secondary antibodies 1:500 in blocking solution
- Hoechst solution: dilute Hoechst reagent 1:1000 in PBS

4. Procedure

4.1 Staining organoids



4.1.1 Grow organoids for 5-7 days following procedure described in SOP1 *Mouse mammary organoids cultivation* (4.1-4.4). Organoids derived from 3-6 wells of a 24-well plates are generally sufficient to perform immunofluorescence analysis. !CRITICAL STEP! For the following steps precoat all the tubes, the pipettes and tips with FBS to avoid attachment of the tissue to the plastic.

4.1.2 Keep the plates on ice. Remove the medium from each 24 well, add 500µL of cold PBS to wash organoids. Aspirate PBS and add 500µL of cold PBS.

4.1.3 Using p1000 pipette tips displace Matrigel from the wells and collect the material (PBS and organoids in Matrigel) in a 15mL tube and centrifuge it at 200g for 5 minutes at 4°C.

4.1.4 Remove the supernatant and add Cell Recovery solution (~100µL each well). Incubate organoids in this solution for 1 hr at 4°C in order to gentle digest Matrigel and release cells with minimal cell damage. Organoids will settle under gravity.

4.1.5 Carefully aspirate the supernatant.

4.1.6 Add 3mL of cold PBS and let organoids settle under gravity.

4.1.7 Carefully aspirate the supernatant.

4.1.8 Fix organoids by adding 3mL of 4% Paraformaldehyde for 20 min at RT (shaking slowly). Organoids will settle under gravity.

4.1.9 Carefully aspirate the supernatant and add 3mL of PBS and let organoids settle under gravity. Repeat this step.

4.1.10 Carefully aspirate the supernatant and add little volume of PBS (100-200µL, depending on the number of cells).

4.1.11 Transfer organoids to the glass slides by using a cytospin (200x g, 5min, RT). Alternatively, organoids can be pipetted directly on glass slides and, while PBS is evaporating, they will settle down.

4.1.12 Use the PAP Pen to make an hydrophobic barriers on glass slides around organoids. This allows to use small amounts of reagents in the following steps.

4.1.13 Add few drops of Permeabilization solution on top of organoids for 5 min RT.

4.1.14 Wash 2 times with PBS by immersing the slides in PBS-containing jar.

4.1.15 Remove glass slides from the jar and add few drops of blocking solution for 1h at RT in a humidified chamber.

4.1.16 Carefully remove the blocking solution, add 100–400µL of primary antibody and incubate O/N at 4°C in a humidified chamber.

4.1.17 Wash 2 times with PBS by immersing the slides in PBS-containing jar.

4.1.18 Remove glass slides from the jar, add 100–400µL of secondary antibody and incubate 2 hrs at RT in a humidified chamber.

4.1.19 Wash 2 times with PBS by immersing the slides in PBS-containing jar.

4.1.20 Remove glass slides from the jar, add few drops of Hoechst solution and incubate for 5 min at RT.

4.1.21 Wash 2 times with PBS by immersing the slides in PBS-containing jar.

4.1.22 Wash 1 time with H_2O by immersing the slides in H_2O-containing jar.

4.1.23 Remove glass slides from the jar and let them dry for 1-2 minutes at RT.

4.1.24 Add a drop of Prolong anti-fade reagent and cover organoids with a coverslip.

4.1.25 Block the coverslip using nail polish and check organoids on a confocal fluorescence microscope (***Figure1***).

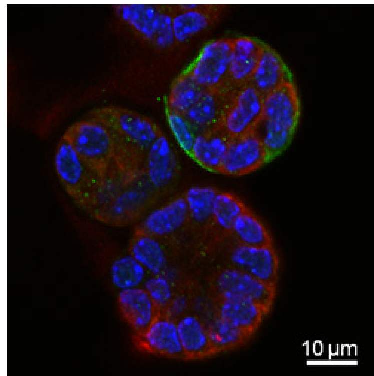


Figure 1. Mouse mammary organoids contain myoepithelial and luminal cells. Representative picture of mouse mammary organoids stained with anti-α-SMA (green) and anti-cytokeratin 18 (red) antibodies. Nuclei are stained with Hoechst (blue). Scale bar is indicated.

5. Applicable references to SOPs

SOP-UNITS-1.0

6. References

- Jaimeson et al., Derivation of a robust mouse mammary organoid system for studying tissue dynamics. Development (2017)