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Mouse Olfactory Horizontal Basal Cell Culture

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We use this protocol and it's working

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Abstract

This protocol describes isolation and culture of basal cells from the olfactory epithelium of mice. The process involves serial enzymatic treatment of dissected olfactory epithelia and subsequent plating in commercial airway medium with dual SMAD inhibitors. The resulting cultures express the horizontal basal cell markers CK5 and CK14.

Materials

Dissection medium:

- Pneumacult-Ex medium
- 1x B27 supplement without vitamin A
- 1x N2 supplement
- 1% penicillin/streptomycin

HBC medium:

- Pneumacult-Ex medium
- 1x Glutamax
- 1x B27 supplement without vitamin A
- 1x N2 supplement
- 1% pen/strep
- 10ng/mL TGF alpha
- 1 uM A-83-01

- 1uM DMH-1

1 Dissect olfactory mucosa from nasal septum On ice .
Induce anesthesia via intraperitoneal injection of 0.15 mL euthanyl, followed by decapitation. Peel off skin and excise the eyes and the jaw. Disinfect the skull with 70% ethanol and carefully bisect along the sagittal plane, maintaining a 1 mm distance from the interfrontal suture. Transfer the larger segment of the skull under a dissection microscope, dissect a 1 mm distal section from the nasal bone. Carefully remove the nasal bones and collect the olfactory epithelium surrounding the septal cartilage into Hank's Balanced Salt Solution.

2 Mince the tissue in 2.7 mL dissection medium, add 300 µL 10x Collagenase/Hyaluronidase and transfer to a 15 mL falcon.

3 Briefly vortex falcon and rotate at 37 °C for 1h.

4 Spin down at 80 x g for 30s and discard supernatant.

5 Triturate pellet in 5 mL TrypLE for 2 min and add 10 mL cold HBSS.

6 Spin down at 350 x g for 5 min and resuspend cells in 1 mL warmed dispase and 50 uL DNase 1. Triturate for 2 min.

7 Add 10 mL cold HBSS and filter through 40 um cell strainer.

8 Spin down at 350 x g for 5 min, discard supernatant, resuspend in HBC medium with [M] 10 micromolar (µM) ROCK inhibitor Y27632.

9 Plate on PDL and laminin coated wells, one mouse septum on 1.9 cm2 (1 well in a 24-well plate)

10 10. Refresh medium the next day without ROCK inhibitor.

11 11. Feed every 2 days. Passage with accutase and do not use any other cell dissociation products such as TryplE of trypsin. Cultures are homogenously CK5+ after 3 passages.