Epithelial Cell Isolation from Mouse Small Intestine (enrich for crypts)

Grace Burgin,Noga Rogel,Moshe Biton

Abstract

To isolate epithelial cells for sorting from the mouse small intestine

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Guidelines

Work quickly and on ice. Keep PBS on ice throughout protocol to maintain cold. Keep extra PBS at +4.

Before start

Put PBS into fridge for use. Only use cold PBS.

Materials

1 Liter PBS [10X] (Phosphate Buffered Saline) (80mM Na2HPO4, 1.5M NaCl, 20mM KH2PO4, 30mM KCl, pH 7.4) R027 by G-Biosciences

EZ-LINE Cell strainer, 70um Nylon PP, 50 per case SP104181.SIZE.1CS by Bio Basic Inc.

✓ 25mM EDTA by Contributed by users

✓ TryplE by Contributed by users

Protocol

Isolate from Mouse

Step 1.

Sacrifice mouse. Open abdomen and move reproductive organs out of the way. Find the colon (a white tube-like structure with poop in it) and cut as low down as possible. Find the stomach (which is located

below the liver) and cut where it connects to the small intestine. Pull the length of the gut (include small intestine, cecum, and colon) from the mouse and place into a 50mL conical with 20mL **cold** PBS.

Clean Tissue

Step 2.

Pour the tissue and PBS into a large petri dish. Use two pairs of forceps to remove lymphatic and connective tissue and fat that runs along the outside of the intestines-- grip the end of the colon with forceps in one hand and pull connective tissue using forceps in the other hand.

Clean Tissue

Step 3.

Remove the cecum and large intestine by cutting just above the cecum. Discard.

Clean Tissue

Step 4.

Use a ball tip syringe to flush the small intestine. Attach a blunt end ball tip needle to a syringe and suck up 3-5 mL of PBS. Insert the ball tip syringe to the top of the small intestine (closest to stomach). Flush through completely, usually 2 times.

Clean Tissue

Step 5.

Once the intestine is clean, insert the needle all the way into the small intestine so that the small intestine is bunched completely on the needle.
Prepare a clean petri dish with a small amount of **cold** PBS.
Pull downwards on the end of the intestine so that it tears ine one line against the needle. In the clean dish of PBS, open the intestine flat.

Clean Tissue

Step 6.

Grip with one finger such that the intestine opens flat. Work with one finger of the other hand to gently slide down the tissue, removing mucus and other debris. Discard dirty PBS and add new PBS. Repeat 2-3 times.

Clean Tissue

Step 7.

Add a small amount of fresh cold PBS. Using scissors, cut intestine into small pieces. Transfer pieces into a 50mL conical. If a 20mL pipette can't be used, the tissue needs to be cut into smaller pieces. Allow tissue to settle and pour off the PBS with fat and debris. Add 25mL clean PBS, swirl tissue, allow tissue to settle, and pour off the PBS. Repeat 2 more times, for a total of 3 washes.

Clean Tissue

Step 8.

Repeat washing step 2 times with 25mM EDTA.

Incubate for Fraction 1

Step 9.

Incubate in 25mM EDTA on ice for 1 hour.

Take Fraction 1

Step 10.

Shake tissue in 50mL conical vigorously and with complete circular motion, 20 times. Remove supernatant using a 10mL pipette tip and put into a new 50mL conical. Add 35mL cold 25mM EDTA to the conical with the tissue for Fraction 2.

Add 1mL of fraction 1 to a well of a 24 well plate. Check under microscope to see the status of your fraction.

EXPECTED RESULTS

Fraction 1 should be primarily villi with fewer crypts. As more fractions are taken, the concetration of crypts should increase, and you should see fewer villi.

Incubate for Fraction 2

Step 11.

Incubate tissue in 25mM EDTA for 30 minutes.

Take Fraction 2

Step 12.

Shake tissue in 50mL conical vigorously and with complete circular motion, 20 times. Remove supernatant using a 10mL pipette tip and put into a new 50mL conical. Add 35mL cold 25mM EDTA to the conical with the tissue for Fraction 3.

Add 1mL of fraction 1 to a well of a 24 well plate. Check under microscope to see the status of your fraction.

Incubate for Fraction 3

Step 13.

Incubate tissue in 25mM EDTA for 30 minutes.

Take Fraction 3

Step 14.

Shake tissue in 50mL conical vigorously and with complete circular motion, 20 times. Remove supernatant using a 10mL pipette tip and put into a new 50mL conical. Add 35mL cold 25mM EDTA to the conical with the tissue for Fraction 4.

Add 1mL of fraction 1 to a well of a 24 well plate. Check under microscope to see the status of your fraction.

EXPECTED RESULTS

Usually 3-5 fractions is enough. You will know that you've taken enough fractions when there are few to no villi in the fraction and it is highly enriched for crypts. Another fraction might be needed. If worried about yield, combine two fractions before filtering.

Prepare Single Cell Suspension

Step 15.

Filter final fraction using 70um filter.

Prepare Single Cell Suspension

Step 16.

Spin down cells for 3 min at 330g.

Prepare Single Cell Suspension

Step 17.

Pour off supernatant. Add trypsin and put in warm water bath for 1min. Add cold PBS to quench the reaction.

NOTES

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Adjust volume of trypsin based on the size of the pellet.

Prepare Single Cell Suspension

Step 18.

Spin down for 3min at 330g.

Prepare Single Cell Suspension

Step 19.

Pour off supernatant. Transfer pellet from 50mL conical to 1.5mL eppendorf tube using SMEM.

Prepare Single Cell Suspension

Step 20.

Spin down using the pulse function for about 9 seconds.

Prepare for Sorting

Step 21.

Remove supernatant.

Add desired antibody mix. Incubate for 10-30min.

Wash once again in SMEM.

Resuspend in SMEM with 7AAD (or other live/dead marker). Filter using a sorting tube with filter top. Dilute based on size of pellet.