Immunohistochemistry

Materials

70% EtOH
HBSS
Hoechst or DAPI
LB agar plates with Carbenicillin
LB liquid medium with Carbenicillin
OCT compound
Penicilin Stretomycin (5000 Units/mL Penicilin, 5000 µg/mL Streptomycin)
4% PFA
Primary antibody
Secondary antibody
Sterile gavage needle
30% Sucrose solution
Triton X-100

Methods

Preparation of the mice

1. 6- to 8-week-old C57BL/6 WT mice (females or males) were purchased.
2. Fasting mice for 4 hours.
3. Gavage each mouse with 100 µL of the Penicilin Stretomycin. After 2 hours, give the mice food and water.
4. The following day, fasting mice for 4 hours.
5. Gavage each mouse with 100 µL of the overnight bacterial inoculum.

Preparation of the PAK-JΔ9 Pseudomonas aeruginosa Inoculum

1. Obtain viable PAK-JΔ9 Pseudomonas aeruginosa from a frozen glycerol stock and streak onto LB agar plate with Carbenicillin using a sterile inoculating loop or pipette tip. Incubate at 37°C overnight.
2. Inoculate 3 mL of sterile LB liquid medium with Carbenicillin in a falcon culture tube with colonies from the LB plate using an inoculating loop or pipette tip. Preparation of the inoculum should be done using aseptic techniques.
3. Incubate the Pseudomonas aeruginosa culture at 37°C overnight in a benchtop incubation shaker at 200 rpm.
4. Add a certain volume of bacteria (when OD600 value = 1, add 1 mL of the bacteria) to new 1.5 mL centrifuge tube. Centrifugate at 16000 × g for 1 minute.
5. Decant the supernatant without disturbing the pellet. Resuspend the pellet with 100

μL of HBSS.

Procedure

1. After giving the gavage of mice with bacterial inoculum for 4 hours, dissect the intestine for Immunohistochemistry (IHC).
2. Euthanize the mouse using cervical dislocation without prior anesthesia in accordance with ethical approval. Place mouse in a supine position and wet the fur using 70% EtOH, open the intra-peritoneal cavity longitudinally along the midline using a scissors.
3. Secure the stomach with a forceps and sever the connection to the esophagus. Remove 1-2 cm of the small intestine next to the stomach by gently pulling on the stomach.
4. Once the intestines have been isolated remove the stomach. Flush intestines with 1x PBS using a syringe with a blunt ended pipette tip.
   - Each intestine should be immediately processed for one of the downstream applications.
5. Flush intestines with 4% PFA using a syringe with a blunt ended pipette tip.
6. Embed the tissue in fresh new 4% PFA in a dish. Incubate for 1 hour on ice with gentle agitation on a rocking platform. Wash the samples twice with PBS for 5 minutes on ice on a rocking platform.
7. Discard PBS. Dehydrate tissue using 30% sucrose solution at 4°C overnight.
8. Pick up the intestine sections using a forceps, put on absorbent paper to absorb moisture.
9. Properly label the plastic molds and fill a third of the volume of the mold with OCT compound, maintained at RT. Place one fragment of tissue per mold and cover it with OCT compound.
10. Store the samples at -80 °C for 2 hours until tissue sections are performed.
11. For staining, cut 10-μm thick sections using cryostat. Place the section tissue side up on a plastic microscope slide within the cryostat.
12. Remove the plastic slide from the cryostat and allow the OCT compound to melt such that the section adheres to the surface of the slide.
13. Repeat sectioning for serial sections or other regions of interest. Leave at room temperature for 45 minutes.
14. Use the pap pen to circle the tissue on the surface the slides.
15. Incubate sections for 2 hours with 0.5% Donkey Serum in PBST (PBS with 0.1% Triton X-100) at RT to block unspecific binding of the antibodies.
    - Step 15 – Step 18, put the slides in wet box to keep moist and protect from light.
16. Remove the blocking buffer. Incubate with primary antibody diluted in blocking

solution at 4°C overnight.

- Incubate samples in the dark and cover whenever possible.

17. Wash sections with PBST for 5 minutes x 3. Incubate with fluorescent-tagged secondary antibody at the appropriate concentration for 1 hour at RT.
18. Wash slides after secondary antibody treatment with PBST for 5 minutes x 3. Stain nuclei with Hoechst or DAPI.
19. Mount slides under a coverslip using a commercial mounting media.
20. Leave at room temperature for 5 minutes to dry.
21. Acquire pictures of each fluorescent label (color channel) individually.

Reference

Parry, L., Young, M., El Marjou, F., Clarke, A. R. Protocols for Analyzing the Role of Paneth Cells in Regenerating the Murine Intestine using Conditional Cre-lox Mouse Models. J. Vis. Exp. (105), e53429, doi:10.3791/53429 (2015).