Whole Mount Colon staining and Light Sheet Fluorescence Imaging

by Ali Ata TUZ, AG Gunzer
provided for Weaver Lab
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3hrs 7.5mg each

1. Inject 5µg Ly6G-AF647 and 5µg CD31-AF790 i.v. and wait 10min.
2. Sacrifice the mouse, take the gut to a petri dish with 5ml PBS, put on ice.
3. Isolate the colon as a single piece. 4cm colon.
4. Transfer colon to 4% PFA in 15ml Falcon and incubate for 4hours at 4°C (can be extended to o/n. Not more than 24h!).
    a. The Falcon should stay horizontally so that the colon can be fixed as a single line. The foldings at this step might cause imaging disturbances lateron.
5. Wash the fixed colon with PBS and gently clean the fecal content.
    a. 23G or thicker long needles can be used with 2ml syringe to clean. Tip of the needle should be cut, low pressure with high volume should be preffered.
6. Transfer the colon to 15ml Falcon with PBS.
    a. Protocol can be stopped at this step to send to our lab.
7. Colons should be divided into 2cm pieces and put on separate fresh brown vials (with respective labelling on tubes) containing **permeabilization buffer** (2 ml per piece) for LSFM and incubate **O/N** (continuous shaking 50 - 60 rpm) // Separation of colon from liquids via a 100 µm cell sieve
8. Use fresh vials! Transfer the colon into **blocking buffer** (2 ml per lobe) containing 6% rat serum and incubate **O/N** (Continuous shaking 50 - 60 rpm) // Separation of lobe from liquids via a 100 µm cell sieve

9. Use fresh 2 ml Eppendorff tubes! Transfer the colon into blocking buffer containing 3% rat serum, add the antibodies in step 6a and 6b into same solution without changing.
    a. add Fc block-CD16/32 (1 ul per 100 ul solution), incubate colon for 30 minutes on o rotator/shaker.
    b. add the **primary antibodies** 1-3 days at 4°C (500µl per piece) on a rotator/shaker
    Separation of colon from liquids via a 100 µm cell sieve. *Hint: Be careful with fluorochromes when staining multiple cell types in a single sample.*

10. Use fresh vials! Wash colon in PBS with 5 ml PBS for 1 hour and then **O/N** (continuous shaking 50 - 60 rpm) // Separation of lung from liquids via a 100 µm cell sieve
11. Embed the stained colon in 1.5% low melting agarose in a cryomold, then put on ice for 15min. *Hint: Melted agarose temperature should be 37-38C before adding to the tissues*
12. Use fresh vials! Transfer the colon to 20% EtOH-water solution (5 ml pH=9 adjusted) for dehydration and incubate for 2 h (Continuous shaking 50 - 60 rpm)
13. Transfer the lobe into 40% EtOH-water solution (5 ml pH=9 adjusted) for further dehydration and incubate for 2 h (Continuous shaking 50 - 60 rpm)
14. Transfer the blocks into 60% EtOH-water solution (5 ml pH=9 adjusted) for further dehydration and incubate for 2 h (Continuous shaking 50 - 60 rpm)
15. Transfer the blocks into 80% EtOH-water solution (5 ml pH=9 adjusted) for further dehydration and incubate for 2 h (Continuous shaking 50 - 60 rpm)
16. Transfer the blocks into 100% EtOH in a new vial (5ml) for further dehydration and incubate for 2 h (Continuous shaking 50 - 60 rpm)

17. Transfer the blocks into fresh 100% EtOH (10ml) for further dehydration and incubate for O/N (Continuous shaking 50 - 60 rpm)
18. Use fresh vials! Transfer the blocks to ethyl cinnamate (ECI) (7 ml) and wait 2 hours
19. Samples are ready for Imaging at LSFM

Recipes for the solutions used

Permeabilization Buffer

Reagent	*Final concentration*	*For 1 liter*	*For 100 ml*
1 X PBS		*Add upto 1 L*	*Add upto 100 ml*
DMSO	*20 %*	*200 mL*	*20 mL*
Triton X 100	*1 %*	*10 mL*	*1 mL*
Glycine	*2,3 g / 100 ml*	*23 g*	*2.3 g*

Blocking Buffer

Reagent	*Final concentration*	*For 1 liter*	*For 100 ml*
1 X PBS		*Add upto 1 L*	*Add upto 100 ml*
Saponin	*0,1 % w/v*	*1 g*	*100 mg*
Triton X 100	*0,1 % v/v*	*1 mL*	*100 ul*
Sodiumazide	*0,02 % v/v*	*0,2 mL*	*20 ul*

Check stock concentrations for each antibody.

Use concentrations are usually 5 ug/ml.

Low Melting Agarose 1.5% should be prepared in ddH2O, heated until boiling degree in microvawe. Before putting on the sample, Agarose should be maximum 40°C to avoid tissue damage. Do NOT use/reheat the low melting agarose solution more than 3 times.

Low melting agarose: LowMelt Agarose, Biobudget, Cat. 10-36-1020
Cryomold: TT Cryomold® Standard, square (25x20x5mm), 4557, 100 pieces, SA62534-25 **(please check dimensions to fit colon, bigger cryomolds could be necessary)**