

Visualization of Gut Microbiota-host Interactions via Fluorescence *In Situ* Hybridization, Lectin Staining, and Imaging

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jove.com/video/62646

Materials

Name	Company	Catalog Number	Comments
Aluminum foil			
Chloroform	Fisher	C298-500	Toxic
Coplin jar	Fisher	08-813E	
Cover glass, 22 × 22 mm, no #1	VWR	CA48366-227-1	
DAPI	Sigma-Aldrich	D9542	Resuspended to 5 mg/mL
Empty pipette tip box(es)			To melt paraffin
Ethanol, anhydrous, molecular grade	--	--	
FISH probes			
FISH hybridization solution	--	--	20 mM Tris-HCl pH 7.4, 0.9 M NaCl, 0.01% (w/v) SDS in nuclease-free water. Optional addition of 5–50% (v/v) formamide
FISH washing buffer	--	--	20 mM Tris-HCl pH 7.4, 0.9 M NaCl
Fluorescently-labeled Ulex Europaeus Agglutinin (UEA-1) lectin	Vector Laboratories	FL-1061 (Fluorescein-labeled) or RL-1062-2 (Rhodamine-labeled)	UEA-1 labels fucosylated glycans, so it is not appropriate for germ-free mice or for samples from fucosyltransferase 2 (FUT2)-deficient hosts.
Forceps			
Formamide	Sigma-Aldrich	F9037	
Fumehood			
Glacial Acetic Acid	Fisher	A38-212	Corrosive and flammable
HybriSlip flexible plastic cover slips, 22 × 22 mm × 0.25 mm	Sigma Aldrich	GBL722222	for FISH hybridization steps, can substitute glass coverslips
ImmEdge Hydrophobic Barrier PAP Pen	Vector Laboratories	H-4000	
Kimwipes			
Methanol	Fisher	A412	Toxic and flammable

Microtome			for sectioning
Multipurpose Specimen Storage Containers, 473 mL	Fisher	14-955-117A	Can substitute with a glass or polyethylene container of your choice. Methacarn is not compatible with polystyrene or metal.
Nail Polish	Electron Microscopy Sciences	72180	Any nail polish should work
Oven			Necessary range: 45-60 °C
Paraffin: Leica Paraplast	Leica	39601006	
PBS, Phosphate-buffered Saline (10x solution)	Fisher	BP3991	Dilute to 1x for protocol
Sodium chloride	Fisher	S271	
Sodium Dodecyl Sulfate (SDS), 20% Solution, RNase-free	Invitrogen	AM9820	
Tissue-Loc HistoScreen Cassettes	Thermo Scientific	CA83009-999	
Trizma hydrochloride solution, 1M Tris-HCl, pH 7.4	Sigma-Aldrich	T2663-1L	
Vectashield	Vector Laboratories	H-1000	
Water, nuclease-free	Fisher	BP2484100	
Xylenes	Fisher	X3P-1GAL	Toxic and flammable