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Myosin Heavy Chain (MyHC) 2a & 2b stain of mouse intrinsic tongue

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We use this protocol and it's working

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Abstract

This protocol describes a method used to section and stain the intrinsic tongue muscles of the Ts65Dn mouse model of Down syndrome and euploid sibling controls. Unfixed, thin tissue sections are labeled through immunofluorescence staining with antibody detection of laminin to reveal myofiber borders and other extracellular structures. Antibodies to Myosin Heavy Chain isoforms (MyHC) are used to reveal different populations of myofibers. Analysis of stained intrinsic tongue sections contributes to a larger study determining how neuromuscular maturation of the intrinsic tongue is impacted by age and genotype in this mouse model.

Materials

- Animal: Ts65Dn mouse model (The Jackson Laboratory, stock number: 005252, RRID: IMSR_JAX:005252)
- Tissue-Tek O.C.T. Compound (Sakura, catalog number: 4583)
- Hydrophobic pen (Invignome's GnomePen, catalog number: GPF-VPSAc-R)
- Bovine Serum Albumin (BSA)
- Triton X-100 (Sigma-Aldrich, catalog number: T8787)
- PBS (Sigma-Aldrich, catalog number: P-3813)
- Normal Goat Serum (Equitech-Bio, catalog number: SG30)
- ProLong Gold antifade mounting media (Invitrogen, catalog number: P36930)

Primary Antibody:

- Anti-Laminin antibody produced in rabbit (Sigma-Aldrich, catalog number: L9393, RRID: AB_477163)
- Myosin Heavy Chain Type IIB antibody - Schiaffino,S.; Universita degli Studi di Padova (DSHB, catalog number: BF-F3, RRID: AB_2266724)
- Myosin Heavy Chain Type IIA antibody - Schiaffino, S.; University of Padova (DSHB, catalog number: SC-71, RRID: AB_2147165)

Secondary Antibody:

- Goat anti-Mouse IgM Cross-Adsorbed Secondary Antibody AF350 (Thermo Fisher Scientific, catalog number: A-31552, RRID: AB_2536169)
- Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody AF568 (Thermo Fisher Scientific, catalog number: A-21124, RRID: AB_2535766)
- Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody AF633 (Thermo Fisher Scientific, catalog number: A-21071, RRID: AB_2535732)

Microscope:

- Olympus BX53 Fluorescence Microscope

Software:

- Olympus Cellsens Dimension 4.1

Before Starting

1 The mouse model used for this experiment is from Jackson Laboratories, Ts65Dn stock number: 005252 (RRID:IMSR_JAX:005252). After euthanasia, muscles are promptly isolated, carefully positioned in a plastic cassette containing O.C.T., and flash frozen through immersion in freezing isopentane within a metal cup surrounded by liquid nitrogen.

2 Prepare slides with fresh frozen, unfixed 10 µm muscle sections. Each experimental iteration should include experimental intrinsic tongue slides, one technical negative control slide of intrinsic tongue tissue from which the primary antibody will be omitted, and biological positive and negative control slides comprised of extensor digitorum longus (EDL) muscle, which is positive for Myosin Heavy Chain (MyHC) 2b, and soleus muscle which is mostly negative for MyHC 2b.

3 Once tissue is sectioned onto slides, draw a border along the perimeter of the slide surrounding the tissue using a hydrophobic pen. After sectioning, slides should be air dried for at least 15 minutes, after which they can be stored in -80ºC for up to two weeks prior to staining.

Staining Day One

45m

4 Blocking Step

4.1 Prepare a blocking solution of 1% Bovine Serum Albumin (BSA) and .1% Triton X-100 (Sigma-Aldrich catalog number: T8787) in PBS. Vortex thoroughly.

4.2 Take slides out from storage. Allow brief thaw, place slides into a slide staining container with a few drops of water on the bottom.

4.3 Apply blocking solution to all slides. Incubate for 45 minutes at room temperature. 45m

5 Primary Antibody

5.1 Prepare a primary antibody mixture of 1:200 laminin (Sigma), 1:3 BF-F3 (DSHB), 1:3 SC-71 (DSHB), 1:3 PBS. Vortex to mix.

	A	B	C	D	E	F	G	H	I
	Name	**RRID**	**Source or reference**	**Catalog number**	**Antibody type**	**Target**	**Raised in**	**Clonality**	**Dilution used**
	Anti-Laminin antibody produced in rabbit	RRID:AB_477163	Sigma-Aldrich	L9393	primary	Laminin	rabbit	polyclonal	1:200
	Myosin Heavy Chain Type IIB antibody - Schiaffino, S.; Universita degli Studi di Padova	RRID:AB_2266724	DSHB	BF-F3	primary	Myosin Heavy Chain Type IIB	mouse	monoclonal	1:3
	Myosin Heavy Chain Type IIA antibody - Schiaffino, S.; University of Padova	RRID:AB_2147165	DSHB	SC-71	primary	Myosin Heavy Chain Type IIA	mouse	monoclonal	1:3

5.2 If needed, centrifuge primary antibody cocktail for 3 minutes ≥ 10,000 rpm to remove antibody aggregates. Using a pipette, remove the top 95% (supernatant) into a new 2 mL tube.

5.3 Remove Block from slides by tapping slide edges on paper towel.

5.4 Apply primary antibodies. Do not add to the technical negative control slide. Add PBS to the negative control slide.

5.5 Close staining container, place in 4°C fridge overnight.

Staining Day Two

1h 30m

6 Secondary Antibody

6.1 Prepare a secondary antibody cocktail of the following in 10% normal goat serum (NGS) (Equitech-Bio catalog number: SG30) in PBS. Vortex to mix.

	A	B	C	D	E	F	G	H	I
	Name	**RRID**	**Source or reference**	**Catalog number**	**Antibody type**	**Target**	**Raised in**	**Clonality**	**Dilution used**
	Goat anti-Mouse IgM Cross-Adsorbed Secondary Antibody AF350	RRID:AB_2536169	Thermo Fisher Scientific	A-31552	secondary	Mouse IgM (Heavy chain)	goat	polyclonal	1:50
	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody AF568	RRID:AB_2535766	Thermo Fisher Scientific	A-21124	secondary	Mouse IgG1	goat	polyclonal	1:300
	Goat anti-Rabbit IgG	RRID:AB_2535732	Thermo Fisher	A-21071	secondary	Rabbit IgG (H+L)	goat	polyclonal	1:250

	A	B	C	D	E	F	G	H	I
	(H+L) Highly Cross-Adsorbed Secondary Antibody AF633		Scientific						

6.2 If needed, centrifuge secondary antibody cocktail for 3 minutes ≥ 10,000 rpm to remove antibody aggregates. Using a pipette, remove the top 95% (supernatant) into a new 2 mL tube.

6.3 Remove slides from 4°C.

6.4 Remove primary antibodies/PBS from slides by tapping slide edges on paper towel.

6.5 PBS Wash: Apply PBS to all slides. Allow PBS to sit for 5 minutes. 5m

6.6 Repeat wash for a total of three times. 10m

6.7 Add secondary antibodies to all slides. Can add to technical negative control slide too, provided the primary antibody has been omitted.

6.8 Allow secondary antibodies to incubate on the slides for one hour at room temperature. 1h

6.9 Remove secondary antibodies by tapping slide edges on paper towel.

6.10 PBS Wash: Apply PBS to all slides. Allow PBS to sit for 5 minutes. 5m

6.11 Repeat wash for a total of three times. 10m

6.12 Add 2-3 drops of ProLong Gold antifade mounting media (Invitrogen catalog number: P36930) to each slide.

6.13 Add a cover slip to cover slide, remove bubbles by tapping slide edges on paper towel.

6.14 Store in a dark, dry, environment at 4°C. Image within two weeks.

Microscopy

7 The microscope used for this experiment is the Olympus BX53 fluorescence microscope with xyz motorized stage control. The software used is Olympus CellSens Dimension 4.1. Images are obtained with the UPLSAPO 20x objective, N.A. 0.75 (Olympus).

8 Prior to image acquisition of experimental tissue sections, use the positive control and negative control slides to validate the image acquisition settings for each staining run.

8.1 Use the stained EDL muscle (positive for MyHC 2b and MyHC 2a) and stained soleus muscle (negative for MyHC 2b and positive for MyHC 2a) to select exposure settings for MyHC 2b and MyHC 2a such that positive fibers are detected and negative fibers are not detected. Use the technical negative control slide (from which primary antibodies were omitted) to verify that signal is not detected in the negative control at the selected exposure settings for all channels (DAPI, TRITC, Cy5). Use these parameters to guide exposure settings for image acquisition of the experimental intrinsic tongue tissue sections.

9 Photograph each intrinsic tongue section in the x,y plane in its entirety, at 20x.

9.1 Create a multi-point focus map to manually select the optimal focus at multiple locations across the x,y plane of each tissue section. For midsagittal intrinsic tongue sections, focus map points should be located primarily within the transverse/verticalis muscles of the intrinsic tongue, such that, at minimum, the anterior, middle, and posterior intrinsic tongue regions each have an optimal focal plane selected.