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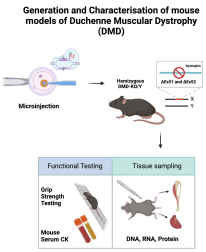
Scientific Protocol for H&E staining in Heart and Skeletal Muscle



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Protocol status: Working
We use this protocol and it's working

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Keywords: H&E, Histology, CRISPR, DMD, Muscle, Heart, murine models of duchenne muscular dystrophy, duchenne muscular dystrophy, crispr microinjection of embryo, crispr microinjection, mouse tissue sample, mounted mouse tissue sample, crispr, broader landscape of genetic disorder investigation, skeletal muscle paper, cas9 gene, molecular testing at the dna, tissue sample, genetic disorder investigation, molecular testing, serum creatine kinase, using mouse model, murine model, generation of diverse animal model, permanent modifications to dna

Disclaimer

These protocols are for research purposes only.

Abstract

Paper abstract: CRISPR-Cas9 gene-editing technology has revolutionised the creation of precise and permanent modifications to DNA, enabling the generation of diverse animal models for investigating potential treatments. Here, we provide a protocol for the use of CRISPR-Cas9 to create murine models of Duchenne Muscular Dystrophy (DMD) along with a step-by-step guide for their phenotypic and molecular characterisation. The experimental procedures include CRISPR microinjection of embryos, molecular testing at the DNA, RNA, and protein levels, forelimb grip strength testing, immunostaining and serum creatine kinase (CK) testing. We further provide suggestions for analysis and interpretation of the generated data, as well as the limitations of our approach. These protocols are designed for researchers who intend on generating and using mouse models to study DMD as well as those seeking a detailed framework of phenotyping to contribute to the broader landscape of genetic disorder investigations.

Protocol summary: The following protocol describes H&E staining of snap frozen cork-mounted mouse tissue samples. These include sections from the heart, tibialis anterior (TA), triceps and quadriceps.

Image Attribution

BioRender was used to generate figures for this manuscript.

Materials

- Lillie Mayer's Haematoxylin (0.5%)
- Tap water
- 0.1% Eosin
- Coverslip
- DPX mountant (Sigma-Aldrich)
- Slide staining tray, rack (ProSci Tech)

Safety warnings

Wear proper PPE (gloves, safety goggles, enclosed shoes and lab coat) and prepare solvents in a chemical fume hood. Dispose used solvents or waste material in an appropriate biohazard waste containers.

Ethics statement

Animal work described in this manuscript has been approved and conducted under the oversight of the Animal Ethics Committee of South Australian Health and Medical Research Institute (SAHMRI) and The University of Adelaide.

Preparation of Slides

1 Allow the slides to air dry for 5 minutes to eliminate any residual moisture.

Haematoxylin & Eosin Staining

2 Immerse the slide in the following reagents order:

2.1 Haematoxylin for 5 min. Drain the excess of the hematoxylin by tapping the slide tray on paper towels

2.2 Place slide staining tray into a container and run through tap water for 2-minutes

2.3 Eosin stain solution for 3 min. Drain the excess eosin by tapping the slide tray on paper towels

Dehydration Steps

2.4 Dip slide tray twice into 70% ethanol

2.5 Dip slide tray four times into 95% ethanol

2.6 100% ethanol for 1 min and repeat in clean 100% ethanol for 1 min.

2.7 Xylene for 1 min and repeat in clean xylenes for 1 min. Drain the excess xylenes by tapping the slide on paper towels.

Mounting and Imaging

3 Add one drop of DPX mounting medium to the tissue section and cover with an appropriate-sized coverslip. Drain any excess mounting media. Allow the slide to air dry in a fume hood.

4 Visualise the stained tissue under a light microscope using bright-field illumination.