

Dec 12, 2024

🌐 Postmortem Mouse Tissue Processing (Necropsy)

📖 [BMC Methods](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgg3z53lk5/v1>

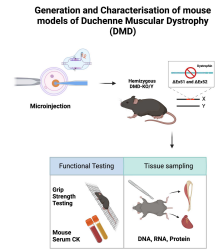
Jayshen Arudkumar^{1,2}, Yu Chinn Joshua Chey^{1,2}, Sandra Piltz^{1,2}, Paul Quinton Thomas^{1,2}, Fatwa Adikusuma^{1,2}

¹University of Adelaide; ²SAHMRI



Jayshen Arudkumar

University of Adelaide, South Australian Health and Medical ...



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.36wgg3z53lk5/v1>

External link: <https://doi.org/10.1186/s44330-024-00019-y>

Protocol Citation: Jayshen Arudkumar, Yu Chinn Joshua Chey, Sandra Piltz, Paul Quinton Thomas, Fatwa Adikusuma 2024. Postmortem Mouse Tissue Processing (Necropsy). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgg3z53lk5/v1>

**Manuscript citation:**

Arudkumar, J., Chey, Y.C.J., Piltz, S.G. *et al.* CRISPR-mediated generation and comprehensive phenotyping of Duchenne Muscular Dystrophy mouse models. *BMC Methods* 1, 19 (2024). <https://doi.org/10.1186/s44330-024-00019-y>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: January 22, 2024

Last Modified: December 12, 2024

Protocol Integer ID: 93942

Keywords: Dystrophin, DMD, Necropsy, Dissection, Mouse, Model, murine models of duchenne muscular dystrophy, duchenne muscular dystrophy, crispr microinjection of embryo, crispr microinjection, molecular analyses of heart, postmortem mouse tissue processing, skeletal muscle tissue, crispr, broader landscape of genetic disorder investigation, cas9 gene, molecular testing at the dna, genetic disorder investigation, molecular testing, serum creatine kinase, animal ethics committee, molecular analysis, generation of diverse animal model, using mouse model, murine model

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: A template for the steps preceding downstream molecular analyses of heart and skeletal muscle tissues. All procedures should be conducted in accordance with the ethical guidelines outlined by your animal ethics committee in your local institution.

Image Attribution

BioRender was used to generate figures for this manuscript.



Materials

- CO₂ chamber.
- Liquid nitrogen (N₂).
- Isopentane, isopentane container.
- 70% ethanol.
- Dissection tools (i.e., scalpels, scissors, tweezers, large forceps).
- Cork disc for cryostat use (20 mm x 3 mm), cork holder.
- Gum tragacanth (Sigma-Aldrich)

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.

Tissue Harvest and Freezing of Muscle Tissues

- 1 Euthanise the mouse using an AEC-approved method. Sacrifice mouse by CO₂ exposure followed by cervical dislocation
- 2 From each mouse, we usually isolate the quadriceps, triceps, heart and tibialis anterior.
- 3 For downstream molecular analysis (DNA, RNA and Protein), cut a small portion of each isolated tissue while the remaining tissues will be subsequently frozen in liquid N₂-cooled isopentane for cryo-sectioning.

Note

Ensure the muscle is in its normal physiological orientation without stretching.

- 4 Prepare the gum tragacanth mixture by mixing 10% (w/v) into water in a small beaker. Continue stirring and leave to set until a slurry forms.
- 5 Pour isopentane in a metal container and place within a larger container containing liquid nitrogen. Chill isopentane in liquid nitrogen until white solid precipitate is visible at the bottom of the container.

Note

The optimal temperature range for isopentane is around -150°C. Once within this temperature range, solid white pebbles of frozen isopentane will form at the bottom. Avoid freezing artifacts by ensuring freezing occurs after this stage. In the event that the isopentane freezes solid, allow it to thaw and then re-chill to freezing temperatures before using it again.

- 6 Place a conservative amount of slurry on the cork pads suitable for tissue mounting. Mount the tissue sections on to the cork to ensure at least 2/3rds of the section remain upright and oriented for a transverse section.
- 7 Using pre-chilled forceps, lower the specimen and cork into the isopentane and suspend for the appropriate time (20s for smaller tissues, 30s for heart and quad), depending on muscle size.



- 8 Transfer specimen immediately to dry ice and label to store in a -80°C freezer until sectioning.

Prepare Tissue Block for Cutting

- 9 Transport tissues on dry ice to the cryostat to prevent thawing.
- 10 Set the cryostat chamber to -20°C to -24°C and let frozen samples equilibrate for 30 min.
- 11 Apply a uniform thin layer of OCT to the specimen disc. With the tissue freeze mounted on the cork, place the sample at the correct orientation.

Sectioning of Tissue

- 12 Mount the specimen onto the specimen head in the cryostat, ensuring good contact.
- 13 Set the chamber temperature to -21°C to -24°C and the chamber head temperature to -15°C.
- 14 Adjust thickness settings (7-10 µm) and trim the block as needed.
- 15 Cut sections perpendicular to the direction of myofiber orientation.
- 16 Collect sections on pre-warmed positively charged microscope slides by pressing them onto the sections.
- 17 Inventory and archive slides at -20°C for long-term storage or proceed to staining.