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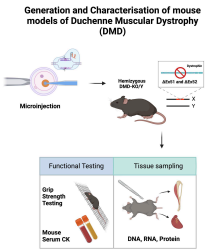
Lateral Tail Vein Bleeding for Blood Collection in Mice



BMC Methods

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

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Keywords: Serum, Mouse, Creatine Kinase, Phenotyping, DMD, mouse model of duchenne muscular dystrophy, lateral tail vein of mice, crispr microinjection of embryo, crispr microinjection, serum creatine kinase, broader landscape of genetic disorder investigation, blood collection in mice paper, murine models of duchenne muscular dystrophy, molecular testing at the dna, crispr, tail vein bleeding, cas9 gene, duchenne muscular dystrophy, genetic disorder investigation, molecular testing, elevated serum ck levels in the dmd model, lateral tail vein, sampling of blood, using mouse model, serum for downstream assessment, mouse model, blood, protein level

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: Here we demonstrate the sampling of blood from the lateral tail vein of mice. Tail vein bleeding is necessary to isolate serum for downstream assessment of serum creatine kinase (CK) levels in our mouse model of Duchenne Muscular Dystrophy (DMD). We anticipate elevated serum CK levels in the DMD model compared to its wildtype counterparts, serving as a key indicator of the pathological changes associated with DMD.

Image Attribution

BioRender was used to generate figures for this manuscript.

Materials

- Individually ventilated cages (IVC)
- PPE (Personal Protective Equipment) - gloves, scrubs, mop cap, and unit shoes
- Empty IVC cage with bedding
- 70% ethanol, F10 SC Veterinary Disinfectant
- Scalpel blade
- Non-treated Microvette® CB300 Blood collection System (Kent Scientific)
- EMLA cream (5%)
- Tissues
- Eppendorf 1.5 or 2 ml tubes
- Box (or Esky) and ice
- Marker pen
- Small gauze or swaddle

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.

Before start

Ensure there is suitable approval from your local Animal Ethics Committee for the maximum allowable blood volume to be taken per mice and that any operator is properly trained by their institution prior to commencing this protocol.

Preparation

1 Label microvette tubes with animal ID

2 Prepare ice and transport facilities

3 Arrange microvettes for easy access during bleeding

4 Prepare the empty IVC cage with bedding

Skin Numbing and Thermacage Preparation

5 Take the cage of animals and check to see if each mice present no health concerns.

6 Apply EMLA cream to the tail's incision area and leave mice to sit for 5-min.

7 Use Thermacage heat box and ensure it is warmed to 37°C.

8 Place the animal in the Thermacage and let them sit for no longer than 10-min.

9 Gently place the animal in the restrainer – holding on to its tail.

Tail Bleeding and Collection

10 Make a small incision over the lateral tail vein.

11 Encourage blood flow and collect into the Microvette tube.

12 Stop bleeding by applying gentle pressure using gauze and return animal to the empty IVC cage to sit for 5-min

13 If vein stops bleeding early, nick opposite vein if more blood required to be collected

14 Return the animal to its home cage and keep samples at room temperature

Serum Isolation

15 Ensure blood samples are left for 15-30-min from their last collection

16 Spin samples at 2000 RCF for 10-min at 4°C

17 Take colourless serum off the blood clot and place in microcentrifuge tube.

18 Freeze serum in -80°C.