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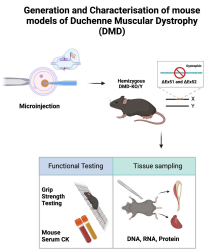
# Forelimb Grip Strength testing

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

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**Keywords:** CRISPR, Grip strength, DMD, Phenotyping, Mice, murine models of duchenne muscular dystrophy, duchenne muscular dystrophy, crispr microinjection, forelimb grip strength testing, crispr microinjection of embryo, forelimb grip strength test, grip strength testing, crispr, forelimb grip strength, molecular testing at the dna, molecular testing, cas9 gene, broader landscape of genetic disorder investigation, evaluation of the muscle integrity, muscle integrity, using mouse model, mouse model, serum creatine kinase, genetic disorder investigation, generation of diverse animal model, diverse animal model, protein level, permanent modifications to dna, protein, murine model, testing

## 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:** We use the forelimb grip strength test to determine the maximum force applied by the mouse onto a grid, as an evaluation of the muscle integrity in the DMD models. The test is based on the tendency of a mouse to instinctively grasp a grid when suspended by the tail, where there is a measure of the maximal peak force generated from the combined front paws (1, 2). The protocol listed here was adapted from the TREAT-NMD standard operating procedure SOP DMD_M.2.2.001 for grip strength testing.

## Image Attribution

BioRender was used to generate figures for this manuscript.

## Materials

- Weighing scale
- Andilog Force Gauge Centor Easy 250N
- Metal grid (SDR Scientific)
- F10 SC Veterinary Disinfectant

## Troubleshooting

## 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.

## Apparatus Setup

1 We attached the grasping grid to the force gauge, using a hooked connector that extends from the base end of the gauge. The grasping grid accessory is a stainless-steel attachment that allows the mouse to grip with both front paws. With regards to the measurement settings, make sure the setting is on MAX and that the units of force is adjusted to grams-of-force.

## Testing

2 Prior to the test, weigh each mouse and return to their cage

3 Gently lower the mouse by the base of its tail and ensure that the mouse grasps the grid tightly with both front paws

4 Pull the mouse away from the grid so that its grasp is broken; the highest force applied to the grid will be shown on the force gauge's display. Manually record this value.

> Note
>
> Only take pulls into account in which the mouse shows resistance to the experimenter. Reject measures in which only one forepaw, or the hindlimbs were used and in which the mouse turned during the pull.

5 Reset the meter at the start of each recording

6 Let the mouse pull the grid three times in a row and then return it in the cage for a resting period of at least one minute.

> Note
>
> Between series of pulls a resting period is necessary for the mouse to recover and avoid habit formation.

7 Then let the mouse perform four series of pulls, each followed by a short 1-min resting period. In this way the mouse has pulled a total of 12x (3 pulls x 4 times = 12 total pulls).

8 Determine the maximum grip strength and normalize for body weight by taking the average of the three highest values out of the 12 values collected.

## Protocol references

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2. Aartsma-Rus A, van Putten M. Assessing functional performance in the mdx mouse model. *Journal of Visualised Experiments: JOVE*. 2014;27;(85):51303. doi: 10.3791/51303.