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Protocol status: Working

Genotyping the ADAMTS5-P (Pfizer) mouse strain

PLOS One

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ABSTRACT

ADAMTS5, the main aggrecanase, is a protein that seems to play a functional role in the development of brown and white adipose tissue (WAT) and browning of WAT. These published observations were made using the ADAMTS5-P mice originally generated by Pfizer, in collaboration with Lexicon Genetics (The Woodlands, TX, USA).

GUIDELINES

-Tails digested in lysis buffer containing proteinase K can be stored at -20°C before continuing the protocol.

Created: Oct 14, 2017

MATERIALS

Last Modified: Feb 21, 2018

PROTOCOL integer ID:
8244

STEP MATERIALS

100 mg Proteinase K Merck MilliporeSigma (Sigma-Aldrich) Catalog #3115879001
Illustra PuReTaq Ready-To-Go PCR Beads GE Healthcare Catalog #27-9557-02
Molecular Biology Grade Agarose Contributed by users Catalog #EP-0010-05
Midori Green Advanced DNA stain Contributed by users Catalog #NG MG04
Orange DNA loading dye Thermo Fisher Scientific Catalog #R0631
TriDye 100 bp DNA Ladder - 125 gel lanes New England Biolabs Catalog #N3271S
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TriDye 100 bp DNA Ladder - 125 gel lanes New England Biolabs Catalog #N3271S

SAFETY WARNINGS

-Dispose of Midori Green contaminated buffer and gels according to the institutional safety guidelines.

- 1 Cut a very small piece of the ear
- 2 Collect them in an labeled eppendorf tube filled with 800 µl lysis buffer supplemented with 8µl 10 mg/ml proteinase K solution

* Lysis buffer (1L):
 - 40 ml 5M NaCl (0.2 M)
 - 20 ml 10% SDS (0.02%)
 - 100 ml 1M Tris HCl pH 8 (0.1 M)

-10 ml 0.5M EDTA (5 mM)
-830 ml ultra-pure (MilliQ) water

*10 mg /ml Proteinase K solution: dissolve 100 mg in 5 ml MilliQ water and 5 ml 100% ethanol.

100 mg Proteinase K **Merck MilliporeSigma (Sigma-Aldrich) Catalog #3115879001**

- 3 Incubate samples overnight at 55°C.
- 4 Vortex the samples.
- 5 Centrifuge samples for 10 min at 13000 rpm at room temperature.
- 6 Transfer the supernatants with DNA in tubes filled with 600 µl isopropanol. Leave until all tubes are transferred.
- 7 Mix one tube slowly at a time by inversion, DNA fibers will appear in the tube.
- 8 Collect DNA fibers with pipette tips (put your finger on the end of the tip to avoid as much as possible that liquid enters in the tip)
- 9 Transfer pipett tips in new labeled tubes containing 200 µl TE buffer (allow rehydration of DNA to occur during 15 min before removing the tips)

*TE buffer (1L):
-10 ml 1 M Tris-HCl pH 7.5 (10 mM)
-2 ml 0.5 M EDTA (1 mM)

-988 ml MilliQ water

-autoclave

- 10** Incubate 1-2 hours at 55°C and vortex to dissolve the DNA. If genotyping is postponed, store samples at -20°C, otherwise proceed.

- 11** Prepare samples by combining PCR beads with 5µl of forward primer 5'-TTT GAA TTT GTC TTT GGA AGG CCT C-3' (10 µM work concentration; 2 µM final concentration) and 5µl of reverse primer 5'-TAT CCC CGG ATG AGT CAA CAC TGT C-3' (10 µM work concentration; 2 µM final concentration), 14µl MilliQ water and 1µl DNA.

Include negative control (no DNA).

Include DNA samples of a previous genotyped ADAMTS5-P wild-type (WT), heterozygous (HE) and homozygous (knockout, KO) animal as positive controls.

Primer sequences were provided by the group of Prof. J. Sandy (Rush University Medical Center, Chicago, IL, USA).

Illustra PuReTaq Ready-To-Go PCR Beads **GE Healthcare Catalog #27-9557-02**

- 12** Put PCR samples in 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA)

- 13** Cycling conditions are:-94°C 2 min-40 cycles of 94°C 30 sec; 63°C 30 sec; 68°C 95 sec-72°C 5 min

- 14** Pour a 2% (= 2g/100 ml) agarose gel in 1x TAE buffer and immerse gel in 0.5x TAE supplemented with Midori Green Advanced DNA Stain (5µl/100 ml buffer)

* 50x TAE (1L):

-242g Tris base

-57.1 ml glacial acetic acid

-100 ml 500 mM EDTA pH 8.0 (50 mM)

-Add MilliQ water until 800 ml

-Stir

-Bring final volume to 1L with MilliQ water

-Autoclave

*1x TAE (1L):

-20 ml 50x TAE

-980 ml MilliQ water

Contains 40 mM Tris, 20 mM glacial acetic acid and 1 mM EDTA.

*0.5x TAE (1L):

-10 ml 50x TAE

-990 ml MilliQ water

Contains 20 mM Tris, 10 mM glacial acetic acid and 0.5 mM EDTA.

Molecular Biology Grade Agarose **Contributed by users Catalog #EP-0010-05**

Midori Green Advanced DNA stain **Contributed by users Catalog #NG MG04**

- 15** Prepare samples: add 5µl Orange DNA loading dye to 25µl PCR mix

Orange DNA loading dye **Thermo Fisher Scientific Catalog #R0631**

- 16** Load samples (18µl) together with 18µl TriDye 100 bp DNA ladder

TriDye 100 bp DNA Ladder - 125 gel lanes **New England Biolabs Catalog #N3271S**

- 17** Run samples for 30 min with 135V on a Mupid-One electrophoresis System Eurogentec SA

- 18** Image gel using the Biorad Molecular Imager Gel Doc XR+ Image System, take picture and digitally save the image.

- 19** Identify ADAMTS5 WT samples as samples exhibiting a single band of 650 bp, ADAMTS5 KO samples as samples showing a single band of 380 bp, while the ADAMTS5 HE samples show both bands.