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Genotyping the ADAMTS5-J (Deltagen) mouse strain

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Protocol status: Working

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 observations were made using the ADAMTS5-P mice originally generated by Pfizer, in collaboration with Lexicon Genetics (The Woodlands, TX, USA). ADAMTS5-J mice were generated by Deltagen Inc. and are now commercially available through The Jackson Laboratory (Bar Harbor, USA). The targeting construct, containing an IRES-lacZ-neomycin cassette, substituted 134 nucleotides of exon 2.

GUIDELINES

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

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MATERIALS

Last Modified: Feb 21, 2018

PROTOCOL integer ID:
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STEP MATERIALS

Proteinase K 100 mg 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 lanesNew England Biolabs Catalog #N3271S
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SAFETY WARNINGS

- Dispose of Midori Green contaminated buffer and gels according to the institutional safety guidelines.
- Work with β -mercaptoethanol in a fume hood equipped with a filter for this product.

- 1 Cut a very small piece of the ear
- 2 Collect them in an labeled eppendorf tube filled with 800 μ l lysis buffer supplemented with 8 μ l 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.

Proteinase K 100 mg 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 pipette 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 1 µl of forward primer 5'-GGG CCA GCT CAT TCC TCC CAC TAC T-3' recognizing the neo cassette (10 µM work concentration; 400 nM final concentration), 2 µl of forward primer 5'-GCA TAC CAC TCC AAA CTT AGA GAG G-3' recognizing the endogenous ADAMTS5 DNA sequence (10 µM work concentration; 800 nM final concentration), 2 µl of reverse primer 5'-CGC AGC TGA CTG CTC TTG TGC TTG-3' recognizing the endogenous ADAMTS5 DNA sequence (10 µM work concentration; 800 nM final concentration), 19 µl MilliQ water and 1 µl DNA.
- Include negative control (no DNA).
- Include DNA samples of a previous genotyped ADAMTS5-J wild-type (WT), heterozygous (HE) and homozygous (knockout, KO) animal as positive controls.
- Illustra PuReTaq Ready-To-Go PCR Beads GE Healthcare Catalog #27-9557-02
- 12** Put PCR samples in 2720 Thermal Cycler (Applied Biosystems).
- 13** Cycling conditions are:-95°C 2 min-28 cycles of 95°C 30 sec; 60°C 30 sec; 72°C 30 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 (5 µl/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 #NGMG04

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 40 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 271 bp, ADAMTS5 KO samples as samples showing a single band of 424 bp, while the ADAMTS5 HE samples show

both bands.