Genotyping the ADAMTS5-P (Pfizer) mouse strain

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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).

Citation: Bianca Hemmeryckx,Dries Bauters,H. Roger Lijnen Genotyping the ADAMTS5-P (Pfizer) mouse strain. **protocols.io**
dx.doi.org/10.17504/protocols.io.kaucsew
Published: 14 Oct 2017

Guidelines

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

Protocol

Step 1.
Cut a very small piece of the ear

Step 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.

REAGENTS

100 mg Proteinase K 3115879001 by Sigma-aldrich

Step 3.
Incubate samples overnight at 55°C.

Step 4.
Vortex the samples.

Step 5.
Centrifuge samples for 10 min at 13000 rpm at room temperature.

Step 6.
Transfer the supernatants with DNA in tubes filled with 600 µl isopropanol. Leave until all tubes are transferred.

Step 7.
Mix one tube slowly at a time by inversion, DNA fibers will appear in the tube.

Step 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)

Step 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

Step 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.

Step 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 controles.

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

REAGENTS

Illustra PuReTaq Ready-To-Go PCR Beads 27-9557-02 by Ge Healthcare

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

Step 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

Step 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.

REAGENTS

- Molecular Biology Grade Agarose EP-0010-05 by Contributed by users
- Midori Green Advanced DNA stain NG MG04 by Contributed by users

Step 15.

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

REAGENTS

Orange DNA loading dye R0631 by Thermo Fisher Scientific

Step 16.

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

REAGENTS

- TriDye 100 bp DNA Ladder - 125 gel lanes N3271S by New England Biolabs

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

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

Step 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.

Warnings

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