Genotyping AagTg$^{+/-}$ Mice from Ear/Tail

Reagents:

Lysis Buffer
Isopropanol
Proteinase K
TE

Lysis Buffer:

100 mL of 1 M Tris Cl pH 8.0
10 mL of 0.5 M EDTA
10 mL of 20% SDS
40 mL of 5 M NaCl
840 mL of diH_2O

DNA Extraction Procedure:

Per sample:	Lysis Buffer	500 µL
	Proteinase K	3.2 µL

1. Mix lysis buffer and proteinase K to make digestion master mix.
2. Add 500 µL of digestion master mix to each ear/tail sample.
3. Incubate at 55°C overnight.
4. The next day, remove tubes from the incubator and vortex for 15 seconds.
5. Spin samples for 10 minutes at max RPM.
6. Label and fill new Eppendorf tubes with 500 µL of isopropanol.
7. Remove samples from centrifuge and pour supernatant into the isopropanol, being careful not to dislodge the pellet.
8. Invert tubes 15-20 times to precipitate out the DNA.
9. Spin samples for 15 minutes at max RPM.
10. Remove samples from centrifuge. Pour off the supernatant and let the open tubes dry inverted on a paper towel for 20 minutes.
11. Add 100 µL of TE to each tube and let sit for 20 minutes.
12. Vortex briefly before use. Keep at 4°C, store at -20°C long term.

Primers:

AagTg P1	GAC CGT GAA CTC TGT AGT GGT
AagTg P2	TGT CCC CAT AAT TTT TGG CAG AGG

PCR Procedure (per sample):

H_2O	13.5 µL
10x buffer	2.5 µL
50 mM $MgCl_2$	0.25 µL
10 µM AagTg P1	2.0 µL
10 µM AagTg P2	2.0 µL
2 mM dNTPs	2.5 µL
5 U/µL Taq	0.25 µL
DNA	2.0 µL
Total	25.0 µL

PCR Settings:

1. 94°C 5 min
2. 94°C 30 sec
3. 59°C 30 sec
4. 72°C 30 sec
5. Repeat steps 2-4 (29x)
6. 72°C 10 min
7. 10°C forever

Band Size:

AagTg –	No band
AagTg +	~430 bp