* Crude DNA sample preparation from tail biopsy

Youxi Ai (July 19, 2006)

1. Put tails (~2mm) in 1.5ml tubes;

2. Add 300μl of 50mM NaOH and put in steamer (pre-heated 10') for 1 hr or 95°C heat block for 50 min;

3. Neutralize with 30μl of 1M Tris (pH 8.0) after cooled down and invert the tubes (**Do Not Vortex!**);

4. Quick spin for 5' at 13,000 rpm (then take 1.5μl sup. for PCR).

Reaction :

1.5 uL DNA
1.5 uL 10x PCR Buffer (NEB with $MgCl_2$)
3.0 uL 1.25mM dNTPs,
0.25 uL For Primer (10 uM)
0.25 uL Rev Primer (10 uM)
0.15 uL NEB Taq (part # M0273S, 5U/ul)
8.35 uL PCR water
total : 15 ul

PCR program,

1. 95C 5 min
2. 94C 1 min
3. 55C 30 sec
4. 72C 1 min
5. go back to step 2, repeat for 36 cycles
6. 72C 7 min
7. 12C forever

Primers

***HuR** (Loxp 385; WT 290; Δ 507)**:**
F: 5'-AGGCAGATGAGCACATGTGA-3' (3455-84)
R: 5'-AGGCTCTGGGATGAAACCTA-3' (3821-40)
ΔR2: 5'-TACTGAGATGTTCTGGGAGG-3' (17657-17676)

SK1: (WT 300; KO 350) **annealing temp 60C**
F: 5'-AGAAGGCACTGGCTCCTCCAG-3'
R: 5'-TGTCACCCATGAACCTGCTGT-3'
Neo 5'-TCGTGCTTTACGGTATCGCCGCTCCC-3'

SK2 (KO 300; WT 700 ?**)**

F: 5'-GCACCCAGTGTGAATCGAGC-3'
R: 5'-TCTGGAGACGGGCTGCTTTA-3'
Neo 5'-CGCTATCAGGACATAGCGTT-3'

SPK1 f/f : (KO 600, flox 250, WT 120) **annealing temp 60C**

lox F: 5'**-**GGA CCT GGC TAT GGA ACC -3'
lox R1: 5' -ATG TTT CTT TCG AGT GAC CC -3'
lox R2: 5'- AAT GCC TAC TGC TTA CAA TAC C -3'

Edg-1: (WT ~400; lacZ 250)

F: 5'-CCATCCTCTGCAGGATCT-3'
R: 5'-TGCTGCGGCTAAATTCCATG-3'
Neo 5'-TCGCCTTCTTGACGAGTTCTTCTGAG-3'

S1P1/Loxp (Loxp 250, WT 200, deleted Δ/Δ 200)**:**

loxP1: 5'- GAG CGG AGG AAG TTA AAA GTG – 3'
l**oxP2**: 5' – CCT CCT AAG AGA TTG CAG CAA -3'
loxP3: 5' – GAT CCT AAG GCA ATG TCC TAG AAT GGG ACA -3'

S1P2 $^{-/-}$ (- 200; + 150)**:**

For 5'-GCAGTGACAAAAGCTGCCGAATGCTGATG-3'
Rev 5'-AGATGGTGACCACGCAGAGCACGTAGTG-3'
Neo 5'-TGACCGCTTCCTCGTGCTTTACGGTATCG-3'

S1P3 : (KO 380; WT 130)

F: 5'-TCAGTATCTTCACCGCCATT-3'
R: 5'-AATTCACTACGGTCCGCAGAA-3'
Neo 5'-GTGCAATCCATCTTGTTCAAT-3'

S1P2 f/f (fl/fl 444 ; WT 349; deleted Δ/Δ 216)

loxF1 5' CAA GCT CTC TAC CAA GTG AGT TAC 3' (3478-3501)
loxR1 5' TGACCCTTCATGTGACAGGCAGAA 3' (3921-3898)
loxR2 5' GGC CAA TTA CAA CCA CCA TAG A 3' (7284-7263)

ApoM : (WT 154 ; KO 154)

mApoM 51:CACCCAGCAACTCATCCTTT
mApoM 31: GCAGCCATGTTGAAGACAAT

Neo-51:GTAGCCGGATCAAGCGTATG
Neo-31:CTGTGCTCGACGTTGTCACT

ApoM Tg: (403)
hApoM-51: GGGACTTGAATTCCTCCACA
hApoM-31: TGAAGGGAGCACAGATCTCA

S1P1 f/s/f : (700)
209bF: 5'-CACTGCATTCTAGTTGTGGT
208bR: 5'- GTGTAGTTTCATCTTCAGCA

ApoE (WT 155 ; KO 245)
180 : 5' GCC TAG CCG AGG GAG AGC CG-3' Common
181 : 5' TGT GAC TTG GGA GCT CTG CAG C 3' WT Reverse
182 : 5' GCC GCC CCG ACT GCA TCT 3' Mutant Reverse

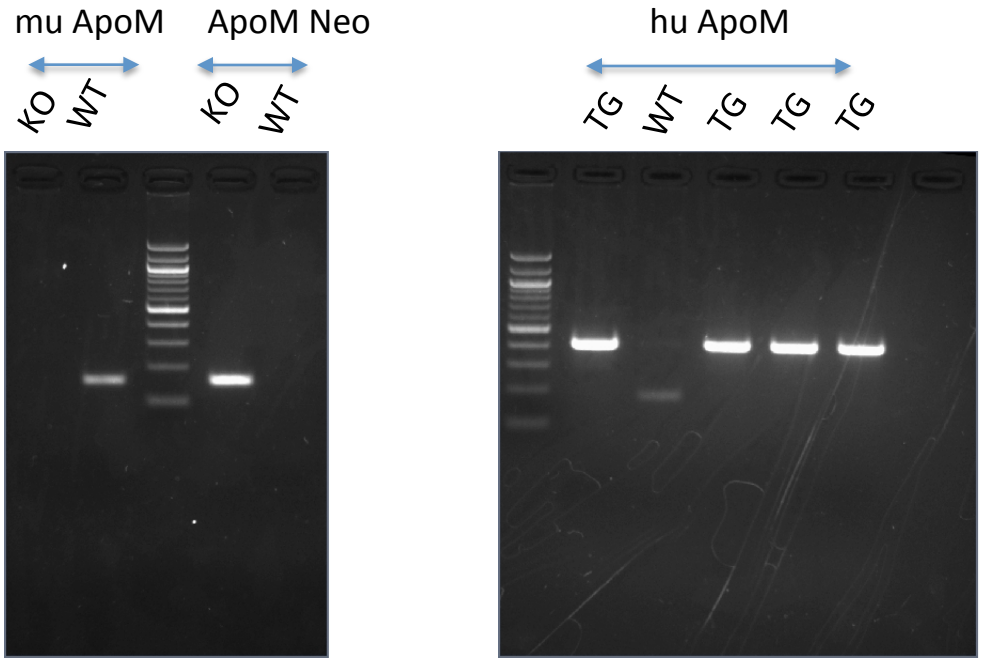
S5A (WT 400; KO 300)
S5a Fwd ; 5'-AAG TGC AGT GAG GCC AAA CA-3'
S5a Rev: 5'-GCA AGC ATG AGG CAA GRC TA-3'

gCre (300)
F: GATATCTCACGTACTGACGG
R: TGACCAGAGTCATCCTTAGC

Rosa Cre (WT 550; Cre 350):
F: 5'-AAAGTCGCTCTGAGTTGTTAT-3'
R1: 5'-GCGAAGAGTTTGTCCTCAACC-3'
R2: 5'-GGAGCGGGAGAAATGGATATG-3'

****LyzsM Cre** (WT 350; Cre 700) **annealing temp 56C**
For 5'-CCCAGAAATGCCAGATTACG-3'
Rev1 5'-CTTGGGCTGCCAGAATTTCTC-3
Rev2 5'-TTACAGTCGGCCAGGCTGAC-3'

ApoM KO and Tg genotyping



mu ApoM WT : 154 bp
Neo: : 154 bp

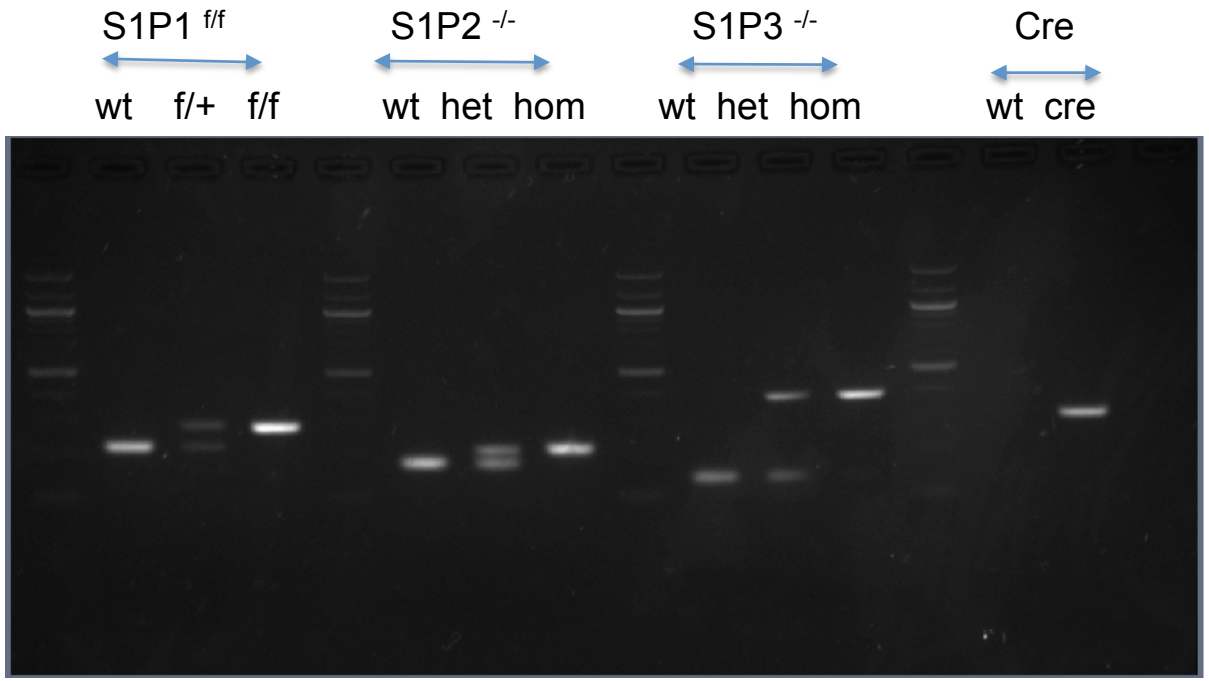
hu ApoM : 403 bp

mApoM : WT
mApoM (in1-2)-51:CACCCAGCAACTCATCCTTT
mApoM (ex2 target)-31: GCAGCCATGTTGAAGACAAT

ApoM Neo
Neo-51:GTAGCCGGATCAAGCGTATG
Neo-31:CTGTGCTCGACGTTGTCACT

Hu ApoM
hAPoM-51: GGGACTTGAATTCCTCCACA
hApoM-31: TGAAGGGAGCACAGATCTCA

S1P receptors genotyping



WT: 200 bp fl/fl: 250 bp	WT: 170 bp KO: 220 bp	WT: 130 bp (For+Rev) KO: 380 bp (Neo+Rev)	Cre: 300bp

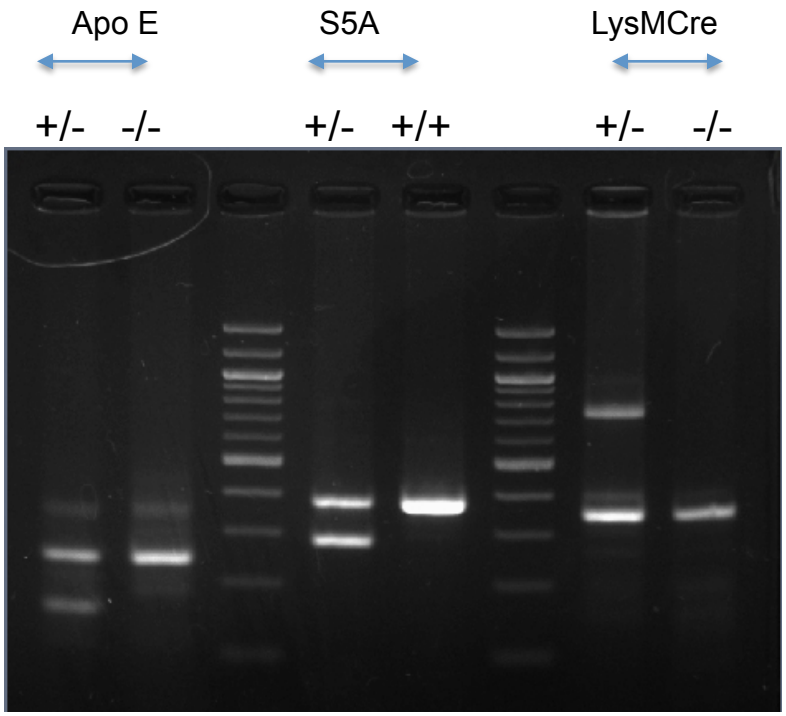
S1P1 f/f
S1P1flox-P1: 5' GAG CGG AGG AAG TTA AAA GTG 3'
S1P1 flox -P2: 5' CCT CCT AAG AGA TTG CAG CAA 3'
S1P1 flox -P3: 5' GAT CCT AAG GCA ATG TCC TAG AAT GGG ACA 3' (for deleted allele)

S1P3-/-
S1P3 For : 5'-TCAGTATCTTCACCGCCATT-3'
S1P3 Rev: 5'-AATTCACTACGGTCCGCAGAA-3'
S1P3 Neo: 5'-GTGCAATCCATCTTGTTCAAT-3'

S1P2 -/-
For 5'-GCAGTGACAAAAGCTGCCGAATGCTGATG-3'
Rev 5'-AGATGGTGACCACGCAGAGCACGTAGTG-3'
Neo 5'-TGACCGCTTCCTCGTGCTTTACGGTATCG-3'

Generic Cre-ERT2
For: 5'-GAT ATC TCA CGT ACT GAC GG- 3'
Rev: 5'-TGA CCA GAG TCA TCC TTA GC-3'

ApoE, S5A and LysM Cre



WT: 155 bp
KO: 245 bp

WT: 400 bp
KO: 300 bp

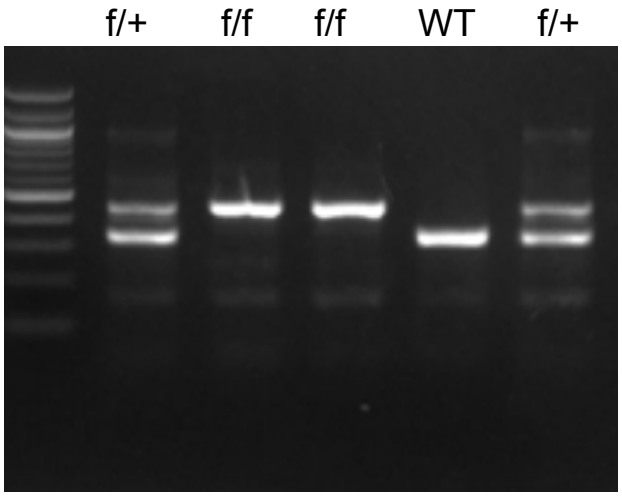
Mutant: 700 bp
WT: 300 bp

S5A
S5a Fwd ; 5'-AAG TGC AGT GAG GCC AAA CA-3'
S5a Rev: 5'-GCA AGC ATG AGG CAA GRC TA-3'

LysMCre
For 5'-CCCAGAAATGCCAGATTACG-3'
Rev1 5'-CTTGGGCTGCCAGAATTTCTC-3
Rev2 5'-TTACAGTCGGCCAGGCTGAC-3'

ApoE
180 : 5' GCC TAG CCG AGG GAG AGC CG-3' Common
181 : 5' TGT GAC TTG GGA GCT CTG CAG C 3' WT Reverse
182 : 5' GCC GCC CCG ACT GCA TCT 3' Mutant Reverse

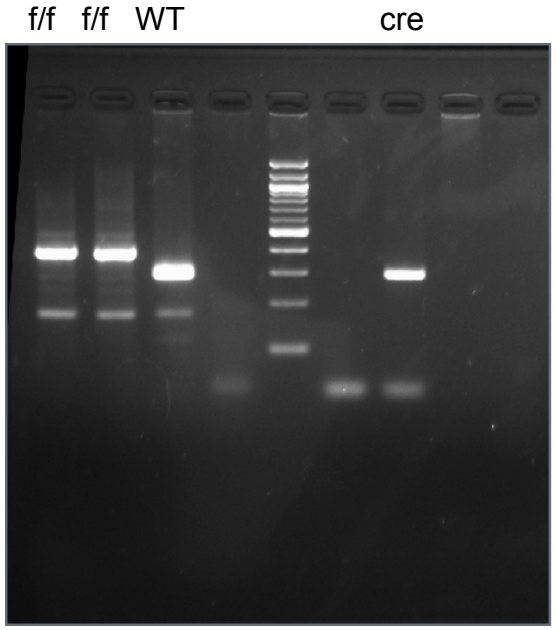
S1P2 $^{f/f}$ genotyping



WT: 349 bp
fl/fl : 444 bp

S1P2_F1 5' CAA GCT CTC TAC CAA GTG AGT TAC 3' (3478-3501)
S1P2_R1 5' TGACCCTTCATGTGACAGGCAGAA 3' (3921-3898)
ΔR2 5' GGC CAA TTA CAA CCA CCA TAG A 3' (7284-7263) Δ/Δ=213bp

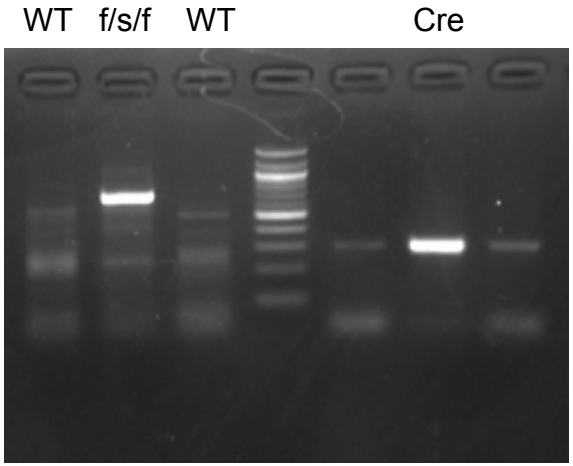
HuR genotyping



fl/fl : 385 bp
WT: 290 bp

F: 5’-AGGCAGATGAGCACATGTGA-3’ (3455-84)
R: 5’-AGGCTCTGGGATGAAACCTA-3’ (3821-40)
ΔR2: 5’-TACTGAGATGTTCTGGGAGG-3’ (17657-17676)

S1P1 $^{f/s/f}$ genotyping



Target: 700bp

209bF: 5’-CACTGCATTCTAGTTGTGGT
208bR: 5’- GTGTAGTTTCATCTTCAGCA