Genotyping for Conditional knockout mice-BoZhang lab-20190809

1: Cre PCR

ZB1031 CAC CCT GTT ACG TAT AGC CG

ZB1032 GAG TCA TCC TTA GAC CCG TA

PCR protocol: 94 °C 5 min; (94 °C 30sec, 60 °C 45 sec, 65 °C 1 min) x39; 65 °C 5 min, 12 °C forever

2: Nlg3 floxed mice

ZB09399 TCT TAA AGG AAC AGG CCT GAA ATC TCG G

ZB09401 GAT CAC AGA CAC TGC AAT ATT CAC TGG CAG

(Expected products, WT = 400 bp, floxed = 600 bp)

PCR protocol: 95 °C 5 min; (94 °C 40sec, 62 °C 30 sec, 72 °C 35 sec) x35; 72 °C 2 min, 12 °C forever

If Does not work, try

PCR protocol: 95 °C 3 min; (95 °C 1 min, 61 °C 1min, 72 °C 1 min) x39; 72 °C 5 min, 12 °C forever

3: tdTomato Allele (007908 Jackson lab):

tdT-WF: AAG GGA GCT GCA GTG GAG TA;

tdT-WR: CCG AAA ATC TGT GGG AAG TC

tdT-MF: CTG TTC CTG TAC GGC ATG G;

tdT-MR: GGC ATT AAA GCA GCG TAT CC

(Expected products, WT = 297 bp, and tdT Allele = 196 bp);

PCR protocol: 94 °C 5 min; (94 °C 30sec, 60 °C 45 sec, 65 °C 1 min) x39; 65 °C 5 min, 12 °C forever

4: Cerebellin-1 primers:

Cbln1 forward: TAG GGT GGA CAG AGA AAA GG,

Cbln1 reverse: TGT GGT CAG GAC AGA TTA GAA G,

Cbln1 recombine: TAG ATT CCT TCC TGC CTT TAG A

(Expected products, WT = 342 bp, floxed = 437 bp, and KO = 544 bp)

PCR protocol: 94 °C 3 min; (94 °C 30sec, 55 °C 30 sec, 72 °C 1 min) x35; 72 °C 10 min, 12 °C forever

5: Cerebellin-2 primers

Cbln2 forward: TAAAAGACAGTCCAGAGTTTTAGTC,

Cbln2 reverse: TGTGCTTACTCCTCTCTATTTGA,

Cbln2 recombine: CTATTGGAGTCC TTCAAGGAAA

(Expected products, WT = 225 bp, floxed = 320 bp, and KO = 420 bp)

PCR protocol: 94 °C 3 min; (94 °C 30sec, 55 °C 30 sec, 72 °C 1 min) x35; 72 °C 10 min, 4 °C forever

6: GPR88-cKO mice

GPR88 Forward: GTC CTA GGT GTG GAT ATG ACC TTA G

GPR88 Forward: GAA GAG TGA AAC CAC AGG TGT GTA CAC

GPR88 Reverse: GTT TGT TTC CTC ACT GGC TGA GAG TC

(Expected products, WT = 132 bp, floxed = 242 bp, and KO = 216 bp)

PCR protocol: 94 °C 5 min; (94 °C 1 min, 64 °C 1 min, 72 °C 1 min) x 35; 72 °C 10 min, 12 °C forever