Procedure for CPX Mouse Genotyping

I. Mouse Tail DNA Extraction

1. Cut the mouse tail ~0.5 cm, put it into 1.5 ml centrifuge tube, cool on ice
2. Each tail is dissolved in 200 µl Tail Buffer with 0.1 mg Proteinase K, put the tube that contain tail on a nutator in 55^{o}C overnight;
3. Centrifuge samples for 5min, 14,000rpm (if do with new born mice, this step and step 4 are not necessary)
4. Transfer the supernatant solution to another centrifuge tube (this step might lose around 30 µl solution, so we have only 170 µl transferred)
5. Add 1 / 2 volume 8 M NH_4OAc into the solution (170 * 1 / 2 = 85 µl, now we have 170 + 85 = 255 v)
6. Add 2 X volume 100% ethanol to the solution (255 * 2 = 510 µl, now we have 255 + 510 = 765 µl)
7. Vortex the tubes for 1 min to precipitate the DNA (you should see the white fibers made by DNA fibers)
8. Centrifuge samples for 5min, 14,000rpm
9. Decant the supernatant and wash the pellet with 70% ethanol 3 times (be careful not to lose the pellets);
10. Put the tubes on the paper towel upside down to allow samples to dry ~3 hrs;
11. Add enough TE-4 to redissolve the pellet (here around 100 µl)

II. Genotyping by PCR

Melting Temperature (Tm): Tm (^{o}C) = 4 * [G+C] + 2*[A+T]

	Complexin I	Complexin II
MQ H_2O	15.38	13.78
10 X Buffer	2	2
Mg^{2+} (50 mM)	0.6	1.2
dNTP (10 mM of each)	0.4	1.6
Taq DNA Polymerase (5u/ml)	0.12	0.12
Primer (10 pmol/ul)	0.5	0.5
Sample	1	2

* MQ H_2O is autoclaved double distilled water

Primer	Program			Fragment Size
CPX I /WT	3' 94 ^{o}C	30'' 94 ^{o}C 30'' 52 ^{o}C 1' 72 ^{o}C 30 X	7' 72 ^{o}C ∞ 4 ^{o}C	332 bp
CPX I /KO	3' 94 ^{o}C	30'' 94 ^{o}C 30'' 58 ^{o}C 1' 72 ^{o}C 30 X ^{o}C	7' 72 ^{o}C ∞ 4 ^{o}C	426 bp
CPX II/WT	3' 94 ^{o}C	30'' 94 ^{o}C 30'' 53 ^{o}C 1' 72 ^{o}C 30 X ^{o}C	7' 72 ^{o}C ∞ 4 ^{o}C	400 bp
CPX II/KO	3' 94 ^{o}C	30'' 94 ^{o}C 30'' 57 ^{o}C 1' 72 ^{o}C 35 X ^{o}C	7' 72 ^{o}C ∞ 4 ^{o}C	378 bp

III. Agarose Gel Electrophoresis

1. Prepare 1 X TAE Buffer from 50 X TAE Buffer
2. Prepare 1.5% Agarose Gel:
   For 100 ml system, in microwave oven for ~50 sec, 2 times, until clear; cool down (60 ^{o}C, not feel hot with hand), add 1.5 µl of EtBr (10mg/ml); pour into plate to make gel
3. Loading sample:
   add 2 µl sample loading buffer (10X) to PCR product tube;
   load 5 µl DNA ladder (100 bp, 50 ng/ul);
   load 14 ~15 ul sample per well;
   80 ~ 100 V for 30 to 45 min
4. Take photo of the Gel

IV. Solutions

Tail Buffer:

50 mM Tris, PH8.0	25 ml 1 M Tris, PH8.0
100 mM EDTA	100 ml 0.5 M EDTA
0.5% SDS	25 ml 10%
autoclave	Add dH_2O to 500 ml

When use, add 0.1 mg Proteinase K / 200 µl Tail Buffer

8 M NH_4OAc F.W. 77.08 Sterilize by Filtration

Final Volume (ml)	Amount (g)
100	61.664
200	123.328
500	308.32

TE-4 Buffer:

10 mM Tris, PH 8.0	1 ml 1 M Tris, PH8.0
0.1 mM EDTA	20 µl 0.5 M EDTA
autoclave	Add dH_2O to 100 ml

50 X TAE Buffer:

242 g	Tris base
57.1 ml	Glacial Acetic Acid
100 ml	0.5 M EDTA (PH 8.0)

V. Resouces

	Company	Cat. No.	Comments
Proteinase K	Roche	1000144	
Taq DNA Poly	Invitrogen	18038-042	
NH_4OAc			