PCR genotyping

Preparation of DNA lysates

1. Cut tail (3~5 mm).
2. Prepare lysis buffer. To each sample add 50 μl of lysis buffer with fresh ProK to a final concentration of 200 μg/ml (1.5 μl ProK each). Incubate at 60°C overnight.
3. Inactivate ProK activity by heating lysats at 100°C for 10 min.
4. Quick spin for 10"

Amount of lysis buffer:	Yolk sac
Tail or toe: 100 μl	E8.5: 15 μl
E7.5 embryos: 15 μl	E9.5-E10.5: 50 μl
E8.5 embryo: 30 μl	>E10.5: 100 μl

PCR reaction

1. Prepare PCR reaction mixture and aliquot 19 μl to each tube.
2. Add 1 μl of DNA lysate as template.
3. Put tubes in PCR machine and initiate designed program.

Note. To have a more robust and cleaner PCR amplification, I routinely use "hot start" PCR. To do this, prepare the reaction on ice, start PCR program and wait until the block reach 94°C to put in tubes quickly.

Run gel to analyze PCR amplified products.

Lysis buffer I

50 mM Tris pH8.8
1mM EDTA (pH8.0)
0.5% Tween 20
Add poteinase K 200ug/ml to the final concentration

Lysis buffer II

50 mM KCl	1M	5 ml
10 mM Tris pH8.3	1M	1 ml
2.5 mM MgCl2	1M	0.25 ml
0.1 mg/ml Gelatin	1 mg/ml	10 ml
0.45% NP40	100%	0.45 ml
0.45% Tween 20	100%	0.45 ml

Add proteinase K to 100 ug/ml. 400 ul for tail, 100ul E8.5 YS, 200 ul E9.5 YS.

PCR reaction

***Gbx2/Neo* PCR**

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	15.2	121.6	243.2	364.8	486.4
Gbx2-R (20 μM)	0.5	4	8	12	16
Gbx2-F (20 μM)	0.5	4	8	12	16
Neo (15-) (1μg/μl)	0.2	1.6	3.2	4.8	6.4
Neo (255-) (1μg/μl)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/GBX2
1. 95°C 3'
2. 95°C 55"
3. 58°C 50"
4. 72°C 1'
5. Go to step 2 and repeat 30 times
6. 4°C for ever
7. End

Neo: 250 bp
Gbx2: 180 bp
Gbx2-flox: 220 bp
Run 2~3% gel

Neo (15-36): GAACAAGATGGATTGCACGCAG
Neo (266-245): TTCAGTGACAACGTCGAGCACA
Gbx2 (1586-1565): TGCTTGGATGTCCACATCTAGG
Gbx2 (1404-1425): CTGTTCACGTTAGCAGGTTCGC

LacZ/RAP PCR

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	15	120	240	360	480
LacZ1 (20 μM)	0.5	4	8	12	16
LacZ2 (20 μM)	0.5	4	8	12	16
RAP1 (20 μM)	0.3	2.4	4.8	7.2	9.6
RAP2 (20 μM)	0.3	2.4	4.8	7.2	9.6
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/LACZ
1. 95°C 3'
2. 95°C 40"
3. 72°C 45"
4. Go to step 2 and repeat 30 times
5. 4°C for ever
6. End

LacZ: 800 bp
RAP: 600 bp
Run 1% gel

Otx1/Otx2 PCR

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	16.2	129.6	259.2	388.8	518.4
Otx2-r (0.5µg/µl)	0.1	0.8	1.6	2.4	3.2
Otx2-f (0.5µg/µl)	0.1	0.8	1.6	2.4	3.2
Otx1-r (1µg/µl	0.1	0.8	1.6	2.4	3.2
Otx1-f (1µg/µl)	0.1	0.8	1.6	2.4	3.2
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/OTX

1. 95°C 3'
2. 95°C 50"
3. 64°C 55"
4. 72°C 1"
5. Go to step 2 and repeat 30 times
6. 4°C for ever
7. End

Otx2: 220 bp
hOtx1: 320 bp
Run 2~3% gel

Gbx2/Neo-Promoter

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	15.4	124	247	370	493
Gbx2-R (20 µM)	0.5	4	8	12	16
Gbx2-F (20 µM)	0.5	4	8	12	16
Neo-PrmR (1µg/µl)	0.2	0.8	1.6	2.4	3.2
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/Gbx2

1. 95°C 3'
2. 95°C 50"
3. 59°C 55"
4. 72°C 1'
5. Go to step 2 and repeat 30 times
6. 4°C for ever
7. End

Gbx2: 180 bp
Neo: 300 bp
Run 2% gel

Flp PCR

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	16.2	129.6	259.2	388.8	518.4
SD41 (20μM)	0.2	1.6	3.2	4.8	6.4
SD42 (20 μM)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/FLP
1. 95°C 3'
2. 94°C 55"
3. 67°C 55"
4. 72°C 1'
5. Go to step 2 and repeat 30 times
6. 4°C for ever

Flp: 750 bp
Run 1% gel
SD41: GTGGATCGATCCTACCCCTTGCG
SD42: GGTCCAACTGCAGCCCAAGCTTCC

Cre PCR

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	16.2	129.6	259.2	388.8	518.4
Cre-f (20 μM)	0.2	1.6	3.2	4.8	6.4
Cre-r (20 μM)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/CRE
1. 95°C 2'
2. 95°C 40"
3. 59°C 1'
4. 72°C 50"
5. Go to step 2 and repeat 29 times
6. 4°C for ever
7. End

Cre: ~300 bp
Run 1~2% gel
CreF: TAAAGATATCTCACGTACTGACGGTG
CreR: TCTCTGACCAGAGTCATCCTTAGC

Gbx2-cDNA PCR

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	16.2	129.6	259.2	388.8	518.4
Gbx2 B2 (20 μM)	0.2	1.6	3.2	4.8	6.4
Gbx2-5a' (20 μM)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Gbx2 cDNA:~400 bp
Run 1~2% gel

Program /JAMES/GBX2
1. 95°C 3'
2. 94°C 45"
3. 55°C 50"
4. 72°C 50"
5. Go to step 2 and repeat 25 times
6. 4°C for ever
7. End

Gbx2 B2:CAGTGGAATTCTTTCTCC
Gbx2-5a': AGACGGCAAAGCCTTCTTGG

Gbx2/Cre **PCR**

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	15.2	121.6	243.2	364.8	486.4
Gbx2-R (20 μM)	0.5	4	8	12	16
Gbx2-F (20 μM)	0.5	4	8	12	16
Cre-f (1μg/μl)	0.2	1.6	3.2	4.8	6.4
Cre-r (1μg/μl)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/CRE

1. 95°C 2'
2. 95°C 40"
3. 59°C 1'
4. 72°C 50"
5. Go to step 2 and repeat 29 times
6. 4°C for ever
7. End

Neo: 250 bp
Gbx2: 180 bp
Gbx2-flox: 220 bp
Cre: 700 bp
Run 2~3% gel

Gbx2-Neo-promoter/Cre

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	15.5	124	248	372	496
Gbx2-R (20 μM)	0.5	4	8	12	16
Neo-PrmR (1μg/μl)	0.2	1.6	3.2	4.8	6.4
Cre-f (1μg/μl)	0.2	1.6	3.2	4.8	6.4
Cre-r (1μg/μl)	0.2	1.6	3.2	4.8	6.4
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/CRE

1. 95°C 2'
2. 95°C 40"
3. 59°C 1'
4. 72°C 50"
5. Go to step 2 and repeat 29 times
6. 4°C for ever
7. End

LacZ: 800 bp
RAP: 600 bp
Run 1% gel

ROSA26 locus

	1X	8X	16X	24X	32X
Buffer	2	16	32	48	64
H_2O	17.3	138.2	276.8	415.2	553.6
RR1/2/3 (1µg/µl)	0.3	2.4	4.8	7.2	9.6
dNTPs (25 mM)	0.2	1.6	3.2	4.8	6.4
Taq	0.2	1.6	3.2	4.8	6.4
Total	**19**	**152**	**304**	**456**	**608**

Program /JAMES/ROSA.

1. 94°C 2'
2. 95°C 1'
3. 61°C 1'
4. 72°C 1'
5. Go to step 2 and repeat 29 times
6. 72°C 10'
7. 4°C for ever
8. End

Wt band: 580 bp
Mutant: 254 bp
Run 1% gel

RR1: AAAGTCGCTCTGAGTTGTTAT
RR2: GCGAAGAGTTTGTCCTCAACC
RR3: GGAGCGGGAGAAATGGATATG