Supplementary Table 1 – Ordinary one-way ANOVA Tukey's and Bonferroni's multiple comparisons, two-way ANOVA Sidak's comparisons or unpaired t-test as indicated

Ordinary one-way ANOVA					
Tukey's multiple comparison			**Bonferroni's multiple comparison**		
	Comparison	(q,DF), p		Comparison	(t,DF), p
3E	V1+/CGRP- vs. V1+/CGRP-	(4.732,25), 0.0071	**4D**	Veh vs. 2AT	(3.834,203), 0.0005
	V1+/CGRP- vs. CGRP+	(0.2494,25), 0.9830		Veh vs. 2AT	(1.030e-006,203), >0.9999
	V1+/CGRP- vs. CGRP+	(5.224,25), 0.0030		Veh vs. 2AT	(0.5904,203), >0.9999
Two-way ANOVA Sidak's Multiple Comparisons					
Fig	Comparison	(t,DF), p	Fig	Comparison	(t,DF), p
5A	*F2rl1^{flox}Pirt^{Cre} - F2rl1^{flox}Pirt$^{+/+}$*		**7A**	*F2rl1^{flox}Pirt^{Cre} - F2rl1^{flox}Pirt$^{+/+}$*	
	Day 0	(0.567,36), 0.9941		Day 0	(0.8334,36), 0.6579
	Day 1	(3.566,36), 0.0063		Day 1	(2.713,36), 0.0595
	Day 3	(3.451,36), 0.0086		Day 3	(1.924,36), 0.3200
	Day 5	(3.128,36), 0.0207		Day 5	(1.350,36), 0.7081
	Day 24	(2.924,36), 0.0351		Day 24	(2.780,36), 0.0505
	Day 48	(1.968,36), 0.2959		Day 48	(1.860,36), 0.3573
5B	Day 0	(0.0128,36), >0.9999	**7B**	Day 0	(0.6346,36), 0.9892
	Day 1	(2.858,36), 0.0415		Day 1	(0.9149,36), 0.9353
	Day 3	(5.035,36), <0.0001		Day 3	(1.433,36), 0.6503
	Day 5	(4.061,36), 0.0015		Day 5	(0.3080,36), 0.9998
	Day 24	(3.394,36), 0.0101		Day 24	(0.4505,36), 0.9983
	Day 48	(0.1576,36), >0.9999		Day 48	(0.6950,72), >0.9999
5C	Day 0	(0.4197,72), 0.9988	**7C**	Day 0	(0.7067,72), 0.9807
	Day 1	(4.567,72), 0.0001		Day 1	(1.779,72), 0.3914
	Day 3	(3.246,72), 0.0106		Day 3	(0.4273,72), 0.9987
	Day 5	(5.836,72), <0.0001		Day 5	(0.2667,72), >0.9999
	Day 24	(2.230,72), 0.1612		Day 24	(0.09526,72), >0.9999
	Day 48	(0.4778,72), 0.9976		Day 48	(0.6950,72), 0.9823
6A	Day 0	(0.000,36), >0.9999	**8A**	Day 0	(0.2688,36), >0.9999
	Day 1	(3.550,36), 0.0066		Day 1	(0.8489,36), 0.9541
	Day 3	(5.071,36), <0.0001		Day 3	(1.542,36), 0.5717
	Day 5	(5.071,36), <0.0001		Day 5	(1.316,36), 0.7310
	Day 24	(1.521,36), 0.5867		Day 24	(0.2830,36), 0.9999
	Day 48	(1.014,36), 0.8987		Day 48	(0.5235,36), 0.9961
6B	Day 0	(0.000,36), >0.9999	**8B**	Day 0	(0.4783,36), 0.9974
	Day 1	(4.914,36), 0.0001		Day 1	(2.935,36), 0.0342
	Day 3	(6.047,36), <0.0001		Day 3	(3.560,36), 0.0064
	Day 5	(3.024,36), 0.0272		Day 5	(3.168,36), 0.0186
	Day 24	(1.512,36), 0.5935		Day 24	(1.388,36), 0.6818
	Day 48	(0.3780,36), 0.9994		Day 48	(0.1483,36), >0.9999
6C	Day 0	(0.2657,72), >0.9999	**8C**	Day 0	(0.06628,72), >0.9999
	Day 1	(5.048,72), <0.0001		Day 1	(0.3125,72), 0.9998
	Day 3	(9.830,72), <0.0001		Day 3	(2.992,72), 0.0225
	Day 5	(3.720,72), 0.0024		Day 5	(2.178,72), 0.1808
	Day 24	(1.860,72), 0.3404		Day 24	(0.06628,72.), >0.9999
	Day 48	(0.2657,72), >0.9999		Day 48	(0.3125,72), 0.9998
Two-way ANOVA Bonferroni's multiple comparisons					
Fig	Comparison	(t,DF), p	Fig	Comparison	(t,DF), p
9A	IL-31 (19 pmol)	(4.092,21), 0.0026	**9B**	IL-31 (19 pmol)	(4.083,20), 0.0029
	2AT (30 pmol)	(0.9546,21), >0.9999		2AT (30 pmol)	(4.168,20), 0.0024
	2AT (100 pmol)	(0.7710,21), >0.9999		2AT (100 pmol)	(4.644,20), 0.0008
	2AT (10 nmol)	(7.330,21), <0.0001		2AT (10 nmol)	(1.750,20), 0.3944
	2AT (10 nmol) *F2rl1$^{-/-}$*	(2.644,21), 0.0760		2AT (10 nmol) *F2rl1$^{-/-}$*	(7.566,20), <0.0001
Unpaired t-test					
Fig	(t,DF), p		Fig	(t,DF), p	
5A	(4.081,6), 0.0065		**7A**	(3.004,6), 0.0239	
5B	(8.638,6), 0.0001		**7B**	(0.1899,6), 0.8556	
5C	(5.919,12), <0.0001		**7C**	(0.3496,12), 0.7327	
6A	(9.798,6), <0.0001		**8A**	(0.2170,6), 0.8354	
6B	(4.494,6), 0.0041		**8B**	(2.541,6), 0.0440	
6C	(6.545,12), <0.0001		**8C**	(4.023,12), 0.0017	

Supplementary Table 2 - Two-way ANOVA Dunnett's comparisons

Fig	Comparison	(q,DF), p	Comparison	(q,DF), p
	F2rl1^{flox}*Pirt*^{Cre}		*F2rl1*^{flox}*Pirt*$^{+/+}$	
5A	Day 0 vs. Day 1	(2.732,30), 0.0422	Day 0 vs. Day 1	(5.802,30), <0.0001
	Day 0 vs. Day 3	(1.750,30), 0.2975	Day 0 vs. Day 3	(4.702,30), 0.0003
	Day 0 vs. Day 5	(2.017,30), 0.1863	Day 0 vs. Day 5	(4.639,30), 0.0003
	Day 0 vs. Day 24	(1.974,30), 0.2018	Day 0 vs. Day 24	(4.387,30), 0.0006
	Day 0 vs. Day 48	(0.02667,30), >0.9999	Day 0 vs. Day 48	(1.408,30), 0.4959
5B	Day 0 vs. Day 1	(1.865,30), 0.2247	Day 0 vs. Day 1	(4.650,30), 0.0003
	Day 0 vs. Day 3	(2.543,30), 0.0644	Day 0 vs. Day 3	(7.440,30), <0.0001
	Day 0 vs. Day 5	(2.023,30), 0.1843	Day 0 vs. Day 5	(5.976,30), <0.0001
	Day 0 vs. Day 24	(0.07999,30), 0.9999	Day 0 vs. Day 24	(3.225,30), 0.0130
	Day 0 vs. Day 48	(0.3381,30), 0.9966	Day 0 vs. Day 48	(0.5035,30), 0.9815
5C	Day 0 vs. Day 1	(5.233,60), <0.0001	Day 0 vs. Day 1	(9.373,60), <0.0001
	Day 0 vs. Day 3	(3.790,30), 0.0017	Day 0 vs. Day 3	(6.605,60), <0.0001
	Day 0 vs. Day 5	(1.083,60), 0.7181	Day 0 vs. Day 5	(6.477,60), <0.0001
	Day 0 vs. Day 24	(1.186,60), 0.6454	Day 0 vs. Day 24	(2.989,60), 0.0176
	Day 0 vs. Day 48	(0.9787,60), 0.7879	Day 0 vs. Day 48	(0.08473,60), 0.9999
6A	Day 0 vs. Day 1	(0.000,30), >0.9999	Day 0 vs. Day 1	(3.337,30), 0.0098
	Day 0 vs. Day 3	(0.476,30), 0.9854	Day 0 vs. Day 3	(5.244,30), <0.0001
	Day 0 vs. Day 5	(0.476,30), 0.9854	Day 0 vs. Day 5	(5.244,30), <0.0001
	Day 0 vs. Day 24	(1.059e-015,30), >0.9999	Day 0 vs. Day 24	(1.430,30), 0.4812
	Day 0 vs. Day 48	(0.000,30), >0.9999	Day 0 vs. Day 48	(0.9535,30), 0.8035
6B	Day 0 vs. Day 1	(1.348,30), 0.5361	Day 0 vs. Day 1	(7.187,30), <0.0001
	Day 0 vs. Day 3	(0.8984,30), 0.8362	Day 0 vs. Day 3	(8.086,30), <0.0001
	Day 0 vs. Day 5	(0.8984,30), 0.8362	Day 0 vs. Day 5	(4.492,30), 0.0005
	Day 0 vs. Day 24	(4.987e-016,30), >0.9999	Day 0 vs. Day 24	(1.797,30), 0.2751
	Day 0 vs. Day 48	(9.975e-016,30), >0.9999	Day 0 vs. Day 48	(0.4492,3), 00.9888
6C	Day 0 vs. Day 1	(0.8478,60), 0.8653	Day 0 vs. Day 1	(6.500,60), <0.0001
	Day 0 vs. Day 3	(0.5652,60), 0.9709	Day 0 vs. Day 3	(11.30,60), <0.0001
	Day 0 vs. Day 5	(0.5652,60), 0.9709	Day 0 vs. Day 5	(4.804,60), <0.0001
	Day 0 vs. Day 24	(1.098e-015,60>0.9999	Day 0 vs. Day 24	(2.261,60), 0.1051
	Day 0 vs. Day 48	(0.2826,60), 0.9984	Day 0 vs. Day 48	(0.2826,60), 0.9984
7A	Day 0 vs. Day 1	(0.5954,30), 09630	Day 0 vs. Day 1	(2.471,30), 0.0752
	Day 0 vs. Day 3	(1.415,30), 0.4911	Day 0 vs. Day 3	(2.503,30), 0.0701
	Day 0 vs. Day 5	(0.06533,30), >0.9999	Day 0 vs. Day 5	(0.5806,30), 0.9665
	Day 0 vs. Day 24	(1.512,30), 0.4296	Day 0 vs. Day 24	(3.454,30), 0.0073
	Day 0 vs. Day 48	(0.1470,30), 0.9998	Day 0 vs. Day 48	(1.172,30), 0.6582
7B	Day 0 vs. Day 1	(2.212,30), 0.1281	Day 0 vs. Day 1	(2.515,30), 0.0684
	Day 0 vs. Day 3	(1.758,30), 0.2936	Day 0 vs. Day 3	(2.618,30), 0.0545
	Day 0 vs. Day 5	(2.981,30), 0.0236	Day 0 vs. Day 5	(1.964,30), 0.2054
	Day 0 vs. Day 24	(2.537,30), 0.0652	Day 0 vs. Day 24	(2.339,30), 0.0992
	Day 0 vs. Day 48	(2.997,30), 0.0227	Day 0 vs. Day 48	(2.442,30), 0.0799
7C	Day 0 vs. Day 1	(3.373,60), 0.0059	Day 0 vs. Day 1	(4.518,60), 0.0001
	Day 0 vs. Day 3	(4.138,60), 0.0006	Day 0 vs. Day 3	(2.928,60), 0.0207
	Day 0 vs. Day 5	(3.691,60), 0.0023	Day 0 vs. Day 5	(3.221,60), 0.0092
	Day 0 vs. Day 24	(3.757,60), 0.0018	Day 0 vs. Day 24	(3.104,60), 0.0128
	Day 0 vs. Day 48	(3.877,60), 0.0013	Day 0 vs. Day 48	(3.864,60), 0.0013
8A	Day 0 vs. Day 1	(0.3268,30), 0.9970	Day 0 vs. Day 1	(0.2558,30), 0.9987
	Day 0 vs. Day 3	(1.080,30), 0.7213	Day 0 vs. Day 3	(0.1989,30), 0.9997
	Day 0 vs. Day 5	(0.04263,30), >0.9999	Day 0 vs. Day 5	(1.549,30), 0.4070
	Day 0 vs. Day 24	(0.3268,30), 0.9970	Day 0 vs. Day 24	(0.3126,30), 0.9977
	Day 0 vs. Day 48	(0.2984,30), 0.9982	Day 0 vs. Day 48	(0.04263,30), >0.9999
8B	Day 0 vs. Day 1	(0.5990,30), 0.9620	Day 0 vs. Day 1	(2.409,30), 0.0857
	Day 0 vs. Day 3	(0.3777,30), 0.9949	Day 0 vs. Day 3	(3.399,30), 0.0084
	Day 0 vs. Day 5	(0.8595,30), 0.8578	Day 0 vs. Day 5	(2.4350,30), 0.0811
	Day 0 vs. Day 24	(2.513,30,) 0.0686	Day 0 vs. Day 24	(3.620,30), 0.0048
	Day 0 vs. Day 48	(0.3386,30), 0.9966	Day 0 vs. Day 48	(0.4428,30), 0.9895
8C	Day 0 vs. Day 1	(2.463,60), 0.0666	Day 0 vs. Day 1	(2.821,60), 0.0274
	Day 0 vs. Day 3	(1.495,60), 0.4315	Day 0 vs. Day 3	(4.338,60), 0.0002
	Day 0 vs. Day 5	(1.352,60), 0.5273	Day 0 vs. Day 5	(3.474,60), 0.0044
	Day 0 vs. Day 24	(0.9581,60), 0.8010	Day 0 vs. Day 24	(1.084,60), 0.7177
	Day 0 vs. Day 48	(0.2776,60), 0.9985	Day 0 vs. Day 48	(0.08059,60), 0.9999

cyagen
We help you discover life

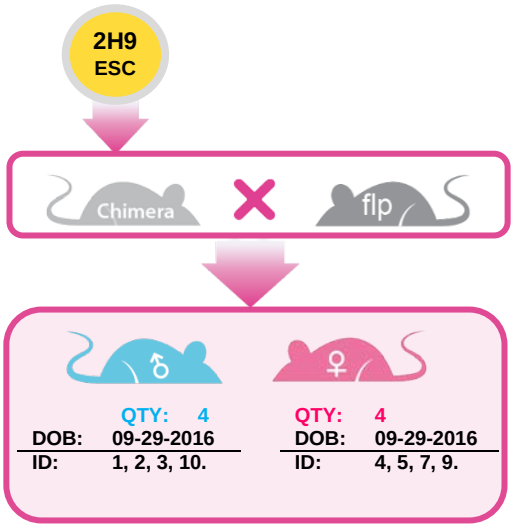
Animal Report

Quote TKC-150921-ARR-01

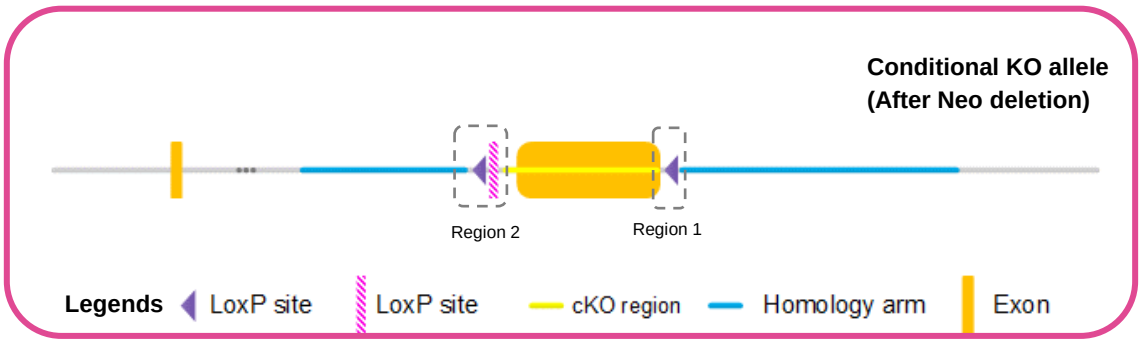
Gene mF2rl1

Method After confirming correctly targeted ES clones via Southern Blotting, we selected some clones for blastocyst microinjection, followed by chimera production. Founders were confirmed as germline-transmitted via crossbreeding with Flp-deleter. In the end, 4 male and 4 female F1 heterozygous mutant mice were confirmed as the final deliverables for this project.

Diagram Breeding Outcome



Genotyping Strategy



Primers for Region1 (Annealing Temperature 60.0 ºC):

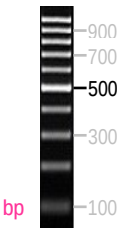
```
Forward: AGAACGGTCAGCTGAAAAGGTGTGT
Reverse: TGAATGCCTCCAGTGGTCAGAGAG
```

Primers for Region2 (Annealing Temperature 60.0 ºC):

```
Forward: CTAGGTTGAATCTTGGCTTAAGGC
Reverse: CACCAGCAGTCTTTGATTGGTCAG
```

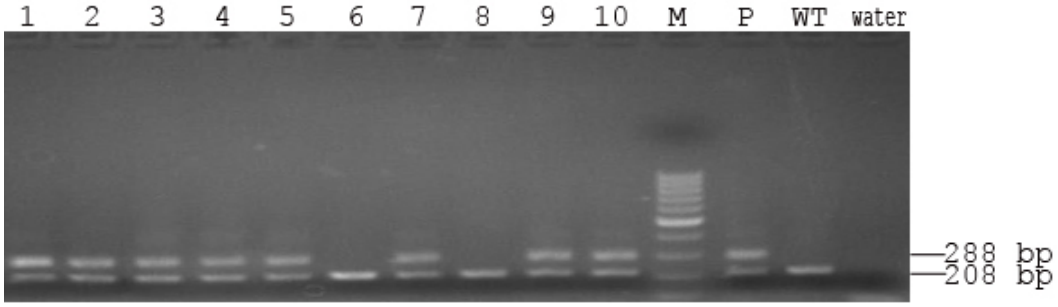
Result 8 pups from clone 2H9 were identified positive by PCR screening for region1 and the positive pups were reconfirmed by PCR screening for region2.

Marker



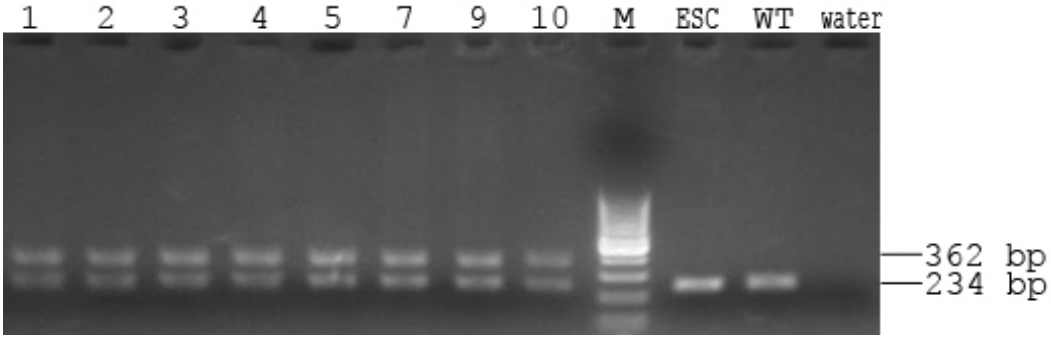
Germline F1 from Clone 2H9

Region 1 (WT: 208 bp; MT: 288 bp)



Germline F1 from Clone 2H9

Region 2 (WT: 234 bp; MT: 362 bp)



ES Cell Report

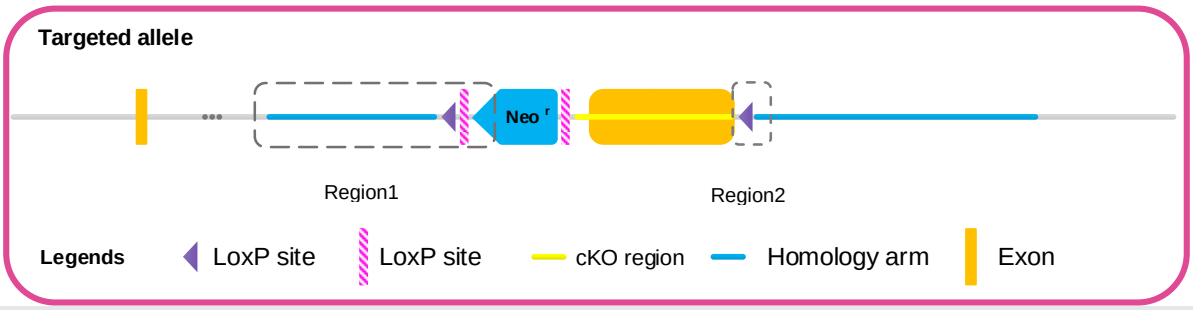
Quote TKC-150921-ARR-01

Gene mF2rl1

Method The linearized vector was subsequently delivered to ES cells (C57BL/6) via electroporation, followed by drug selection, PCR screening, and Southern Blot confirmation.

After gaining 184 drug-resistant clones, we have confirmed 10 potentially targeted clones, 6 of which were expanded for Southern Blotting.

Diagram 1 Regions in the following diagram were selected for PCR screening



Primers for Region 1 (Annealing Temperature 60.0 ºC):

Forward: CTCACACTCAAGTTAGCCATGTTG

Reverse: GCTGACCGCTTCCTCGTGCTTTA

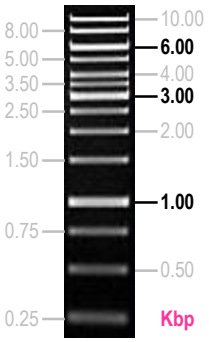
Primers for Region 2 (Annealing Temperature 60.0 ºC):

Forward: AGAACGGTCAGCTGAAAAGGTGTGT

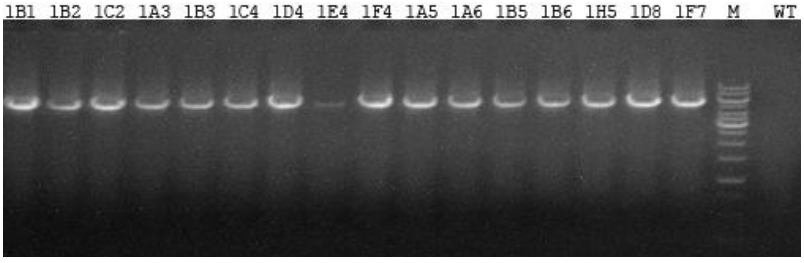
Reverse: TGAATGCCTCCAGTGGTCAGAGAG

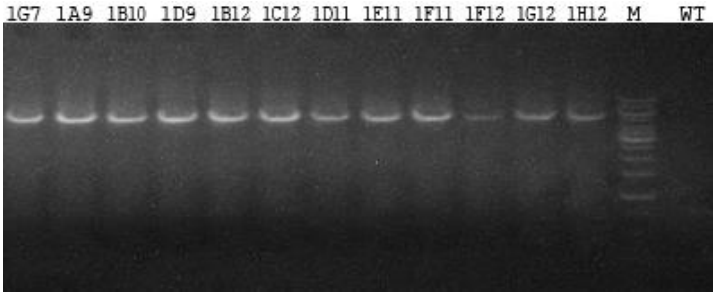
Result Samples 1B2, 1H5, 1F7, 1B10, 1H12, 2C2, 2H1, 2E8, 2B10 and 2H9 have been confirmed as potentially targeted ES clones.

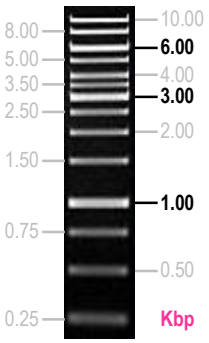
Marker 1

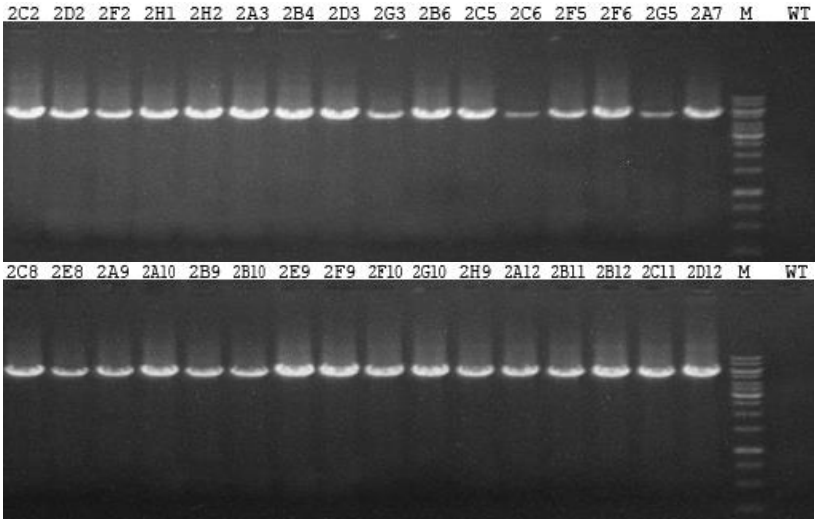


Region 1 (WT: N.A.; MT: 5.7 Kbp)



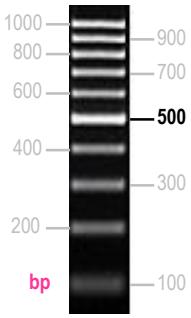






Marker 2

Region 2 (WT: 208 bp; MT: 288 bp)



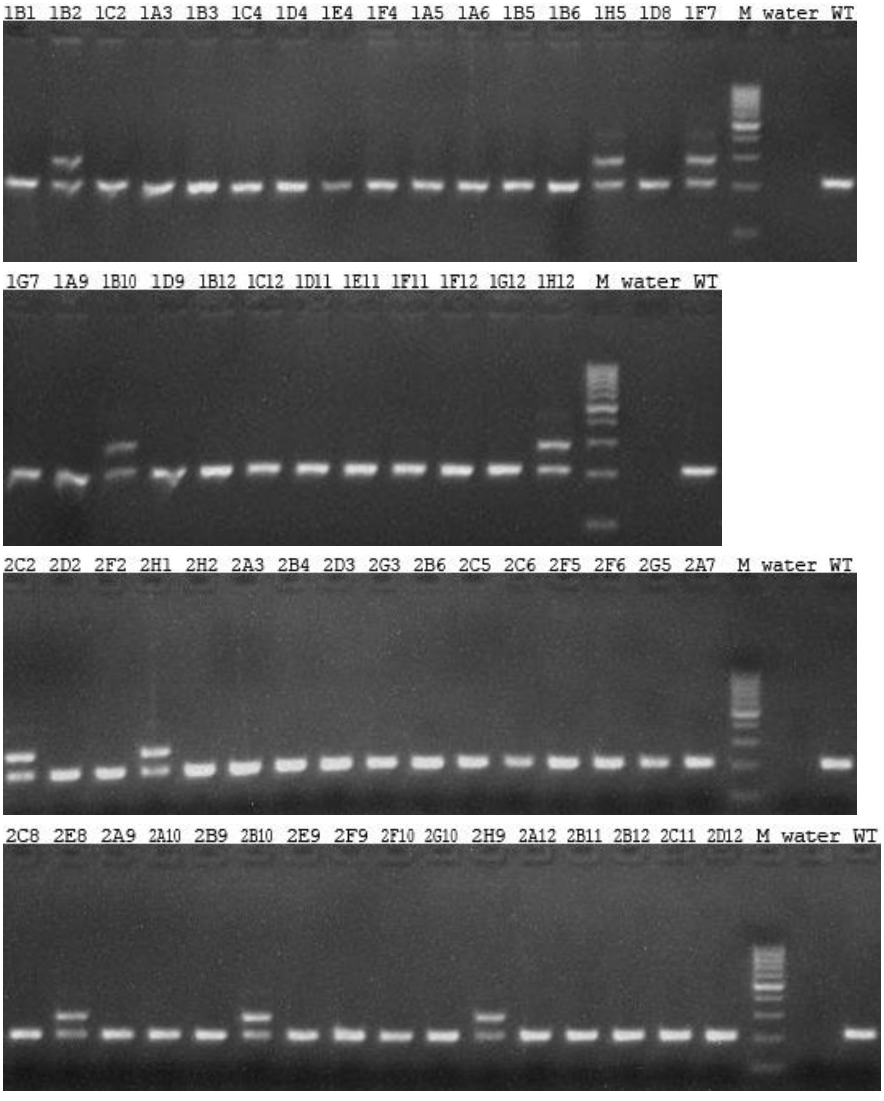
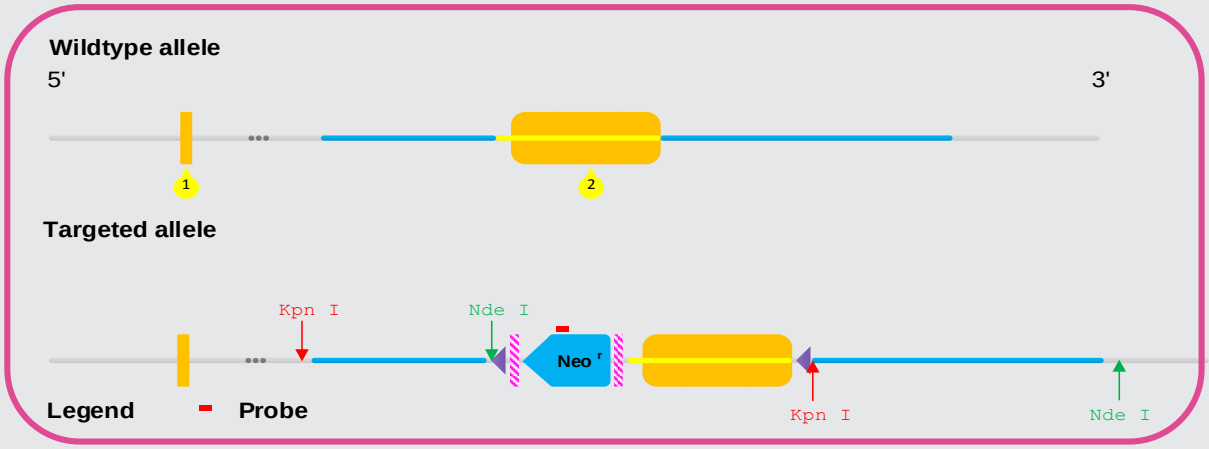


Diagram 2 Regions in the following diagram were selected for Southern Blotting

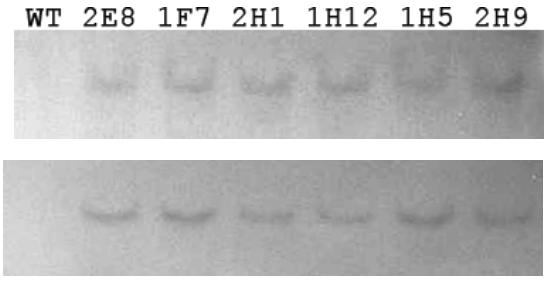


Expected Fragment Sizes for Southern Blotting:

Neo Probe (containing 5' HA) – 14.0 Kbp – Kpn I

Neo Probe (containing 3' HA) – 14.4 Kbp – Nde I

Result All of the six ES clones (2E8, 1F7, 2H1, 1H12, 1H5 and 2H9) were confirmed correct by southern blot analysis.



5'-HA (14.0 Kbp; Kpn I)

3'-HA (14.4 Kbp; Nde I)