Gene x generic Cre mice

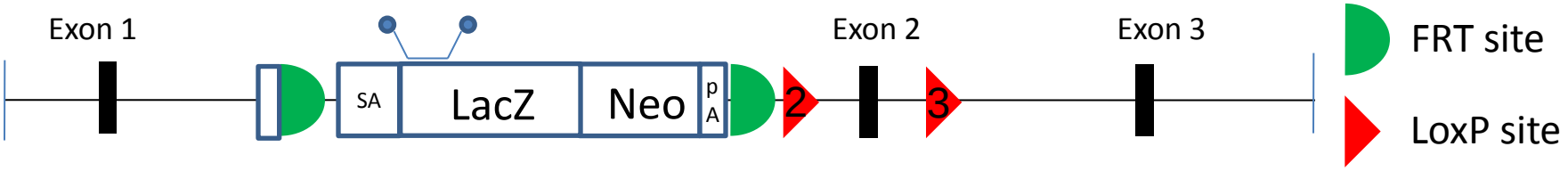
Ed Ryder, 19th May 2011

Version 3.1

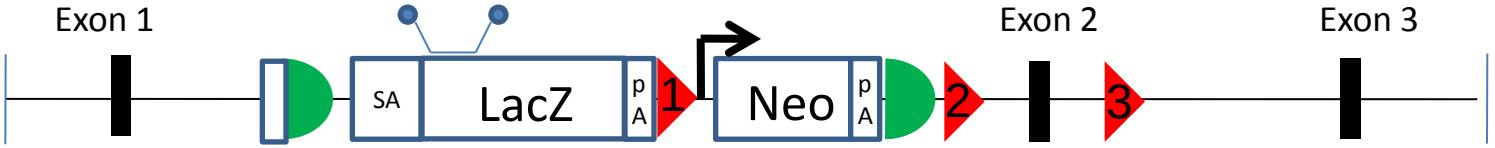
Selection cassettes

- There are two main cassette designs used in the high throughput gene targeting labs
- A LacZ qPCR assay can be used as a high-throughput method of determining the presence of the mutant gene, but it will not discriminate between converted forms of the b allele

Promoterless selection cassette – Tm1a configuration



Promoter-driven selection cassette - – Tm1a configuration

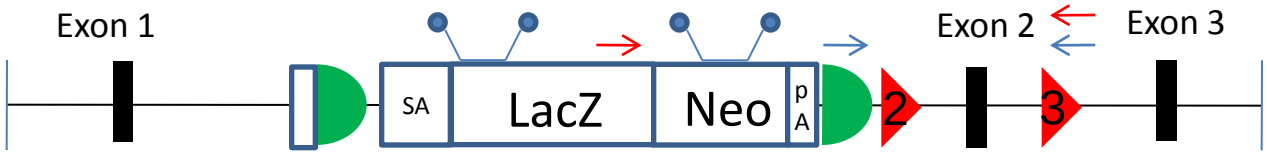


SA: En2 splice acceptor
pA: SV40 polyA initiation site

qPCR assay

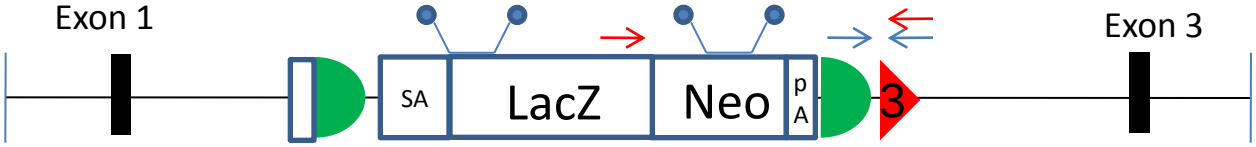
Promoterless – cre recombination

Outcome 1: no recombination event occurs - Tm1a allele



- LoxP PCR size same as tm1a
- Neo count unaltered
- Tm1b PCR ~2.5kb (or fails)

Outcome 1: recombination occurs - Tm1b allele



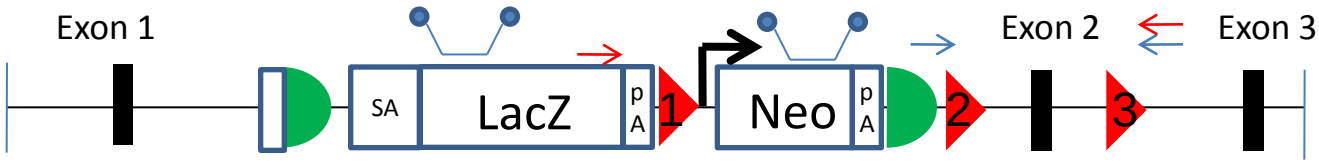
- LoxP PCR size reduced to ~130bp
- Neo count unaltered
- Tm1b PCR ~1.5kb (or fails)

→ LoxP PCR

→ Tm1b PCR

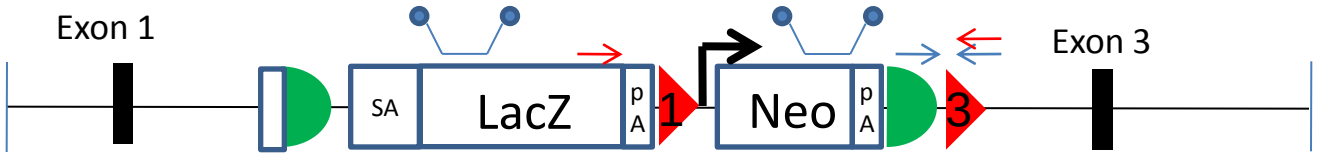
Promoter-driven cre recombination

Outcome 1: no recombination event occurs – Tm1a allele



- LoxP PCR size same as tm1a
- Neo count unaltered
- Tm1b PCR fails

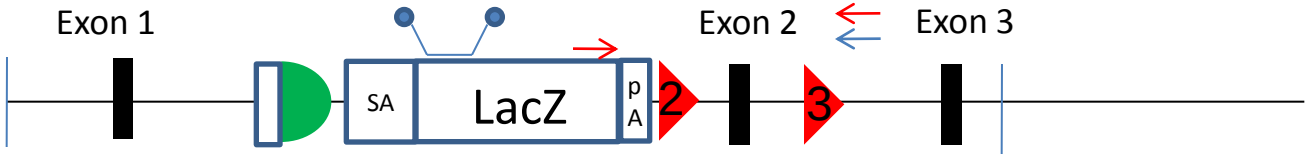
Outcome 2: only critical exon removed – Tm1b.1 allele



- LoxP PCR size reduced to ~130bp
- Neo count unaltered
- Tm1b PCR fails
- Configuration not stable if Cre is still in the mouse

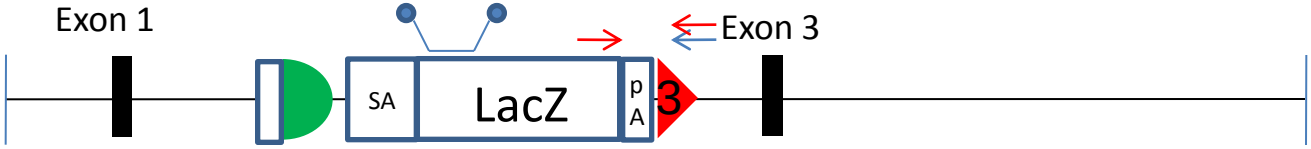
Promoter-driven cre recombination II

Outcome 3: only Neo marker removed – Tm1b.2 allele



- LoxP PCR fails
- Neo count reduced by 1 copy
- Tm1b PCR gives a ~1kb product

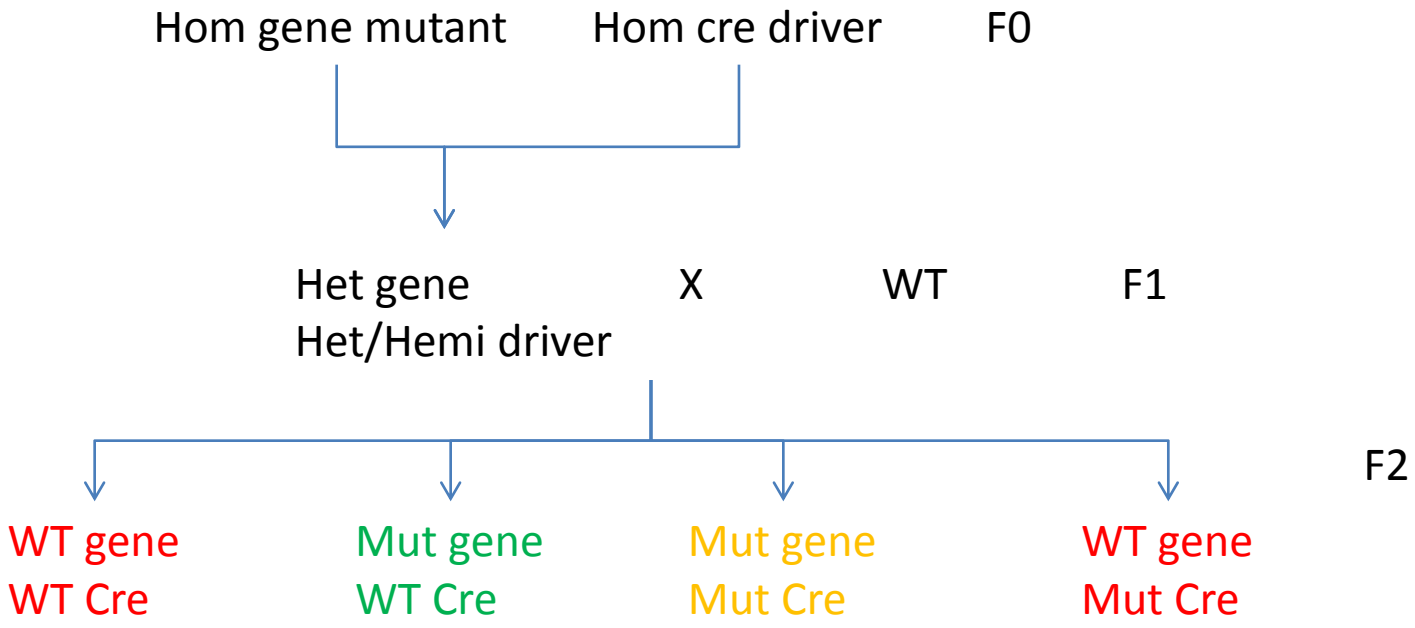
Outcome 4: critical exon and neo marker removed – Tm1b allele



- LoxP PCR fails
- Neo count reduced by 1 copy
- Tm1b PCR works

Outcome 4 is the preferred option

Mouse breeding



- Mut gene / WT Cre with full conversion to Tm1b is most desirable
  - The line is stable at this point and can be genotyped with just the normal gene-specific srPCR primers (although not Neo count qPCR if the line is promoter-driven)

Genotyping and detecting conversion

- Gene and Cre detectors:
  - Gene WT-specific srPCR
  - Gene mutant-specific srPCR
  - Cre-specific srPCR (or qPCR)
  - Neo count qPCR (cannot distinguish between gene and Cre versions if Neo is present in the Cre driver cassette)
  - LacZ count qPCR
- Recombination detection:
  - Neo count qPCR
  - LoxP PCR
  - Tm1b PCR

- *While Neo count qPCR gives extra information it is not critical for correct genotype confirmation*

F2 genotyping outcomes

Promoter- driven lines for further breeding

Gene WT	Gene Mut	Cre	Lox P	Neo Count	Tm1b	Mutant gene present?	Cre driver present?	Tm1a conversion
pass	pass	pass	tm1a size	2	fail	yes	yes	no
pass	pass	pass	200bp	2	fail	yes	yes	Tm1b.1
pass	pass	pass	fail	1	pass	yes	yes	Tm1b
pass	pass	fail	tm1a size	1	fail	yes	no	no
pass	pass	fail	200bp	1	fail	yes	no	Tm1b.1
pass	pass	fail	fail	0	pass	yes	no	Tm1b
pass	pass	pass	fail	1	large	yes	yes	Tm1b.2
pass	pass	fail	fail	0	large	yes	no	Tm1b.2

	breed with WT to produce a stable colony
	breed with WT and selectively remove Cre progeny from next generation
	do not breed or breed with a Cre driver mouse to attempt another allele conversion
	genotype next generation - conversion in germ line may have occured

Promoterless lines for further breeding

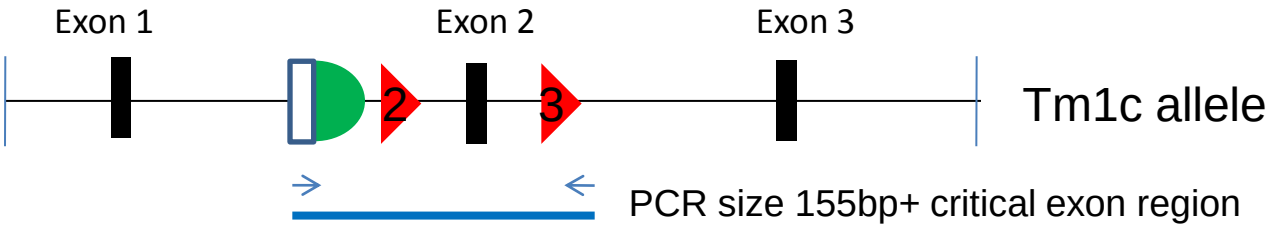
Gene WT	Gene Mut	Cre	Lox P	Neo Count	Tm1b	Mutant gene present?	Cre driver present?	Tm1a conversion
pass	pass	pass	tm1a size	2	fail	yes	yes	no
pass	pass	pass	200bp	2	fail	yes	yes	Tm1b
pass	pass	fail	tm1a size	1	fail	yes	no	no
pass	pass	fail	200bp	1	fail	yes	no	Tm1b

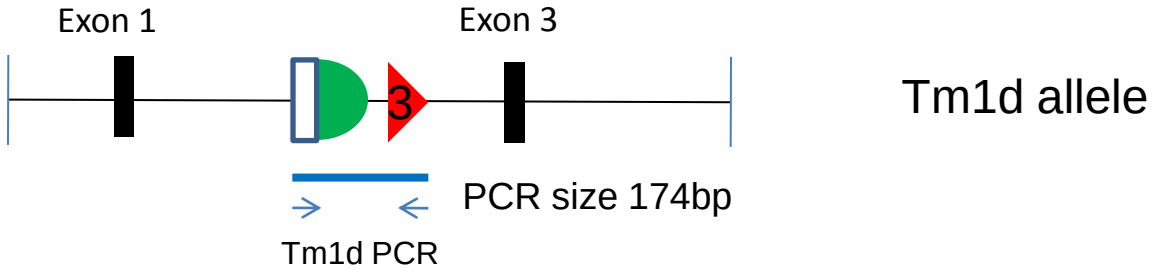
	breed with WT to produce a stable colony
	breed with WT and selectively remove Cre progeny from next generation
	do not breed or breed with a Cre driver mouse to attempt another allele conversion
	genotype next generation - conversion in germ line may have occured

- Gene mutant-specific PCR fail results not shown.
- Please note that Neo count in this example assumes the presence of Neo in the Cre driver cassette.

Genotyping Tm1d alleles

• For lines that have previously undergone cassette excision to produce the Tm1c allele, a different assay is needed.





• If Cre mediated recombination has not occurred the PCR product size will be 155bp plus the size of critical exon region (usually ~900bp to 1.5kb)
• If recombination has occurred this product size will be fixed at 174bp
• If the critical exon region is very large then a gene-specific reverse primer can be designed within it; amplification of a band would strongly suggest that the Tm1d allele has not been produced
• LacZ qPCR assay will fail when the Tm1d form is present

PCR primers and conditions

gene_id	f_primer	r_primer	exp_size	pcr_type	pcr_prog
Cre	Cre_F	Cre_R	233	Cre	TA58
LoxP	Floxed PNF	Floxed LR	800 - 1kb	LoxP	TA58
Tm1b	Tm1b_prom_F	Floxed LR	380	cassette	TA58
Tm1d	Tm1c_F	Floxed LR	174	cassette	TA58

primer_name	sequence
Cre_F	CATTTGGGCCAGCTAAACAT
Cre_R	TAAGCAATCCCCAGAAATGC
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Floxed PNF	ATCCGGGGGTACCGCGTCGAG
Floxed LR	ACTGATGGCGAGCTCAGACC
Tm1b_prom_F	CGGTCGCTACCATTACCAGT
Tm1c_F	AAGGCGCATAACGATACCAC

Reagent	volume (ul)
Primer 1 (10mM)	0.4
Primer 2 (10mM)	0.4
MgCl2 (50mM)	0.6
10x Buffer	2
dNTP (100mM)	0.2
PtTaq*	0.2
H20	15.2
DNA	1
Total	**20**

*Platinum Taq is used in the pcr reaction (Invitrogen)

TA58

1	94 °C	5 min
2	94 °C	30 sec
3	58 °C	30 sec
4	72 °C	45 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever