

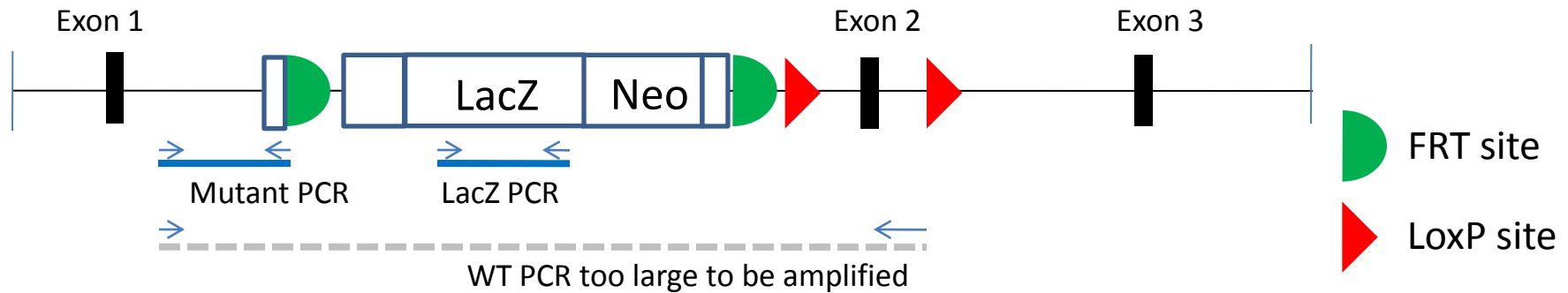
Genotyping conditional knockout colonies after Flp treatment of Tm1a alleles

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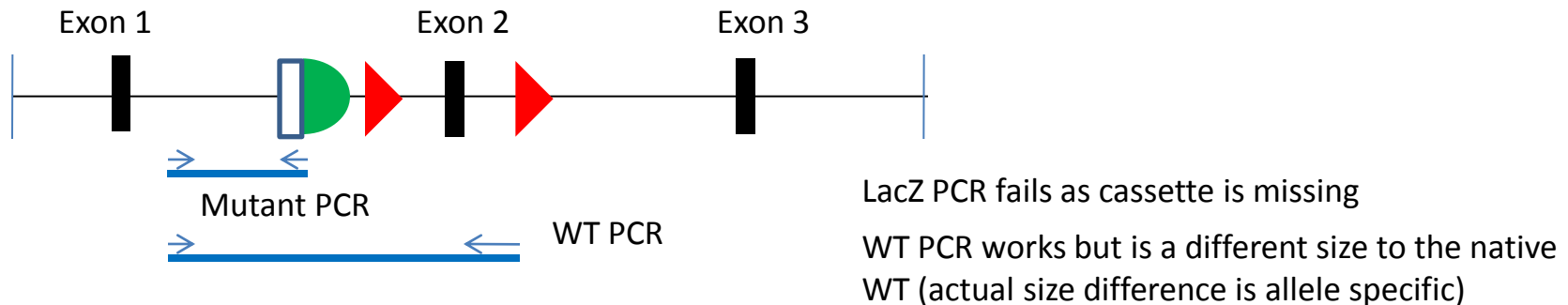
2nd February 2011

Genotyping flipped out knockout-first alleles

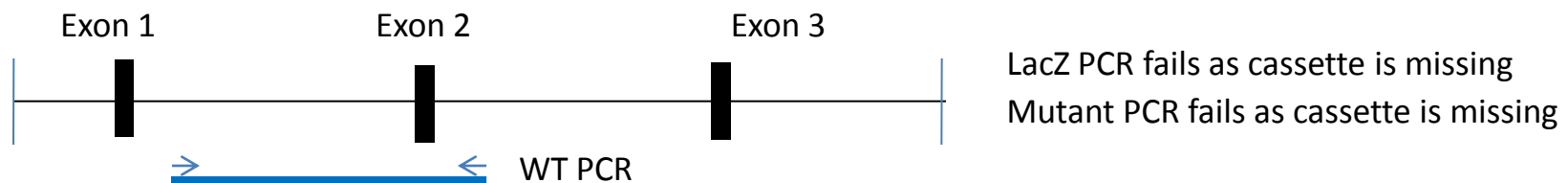
Knockout-first mutant allele (Tm1a) (cassette not to scale)



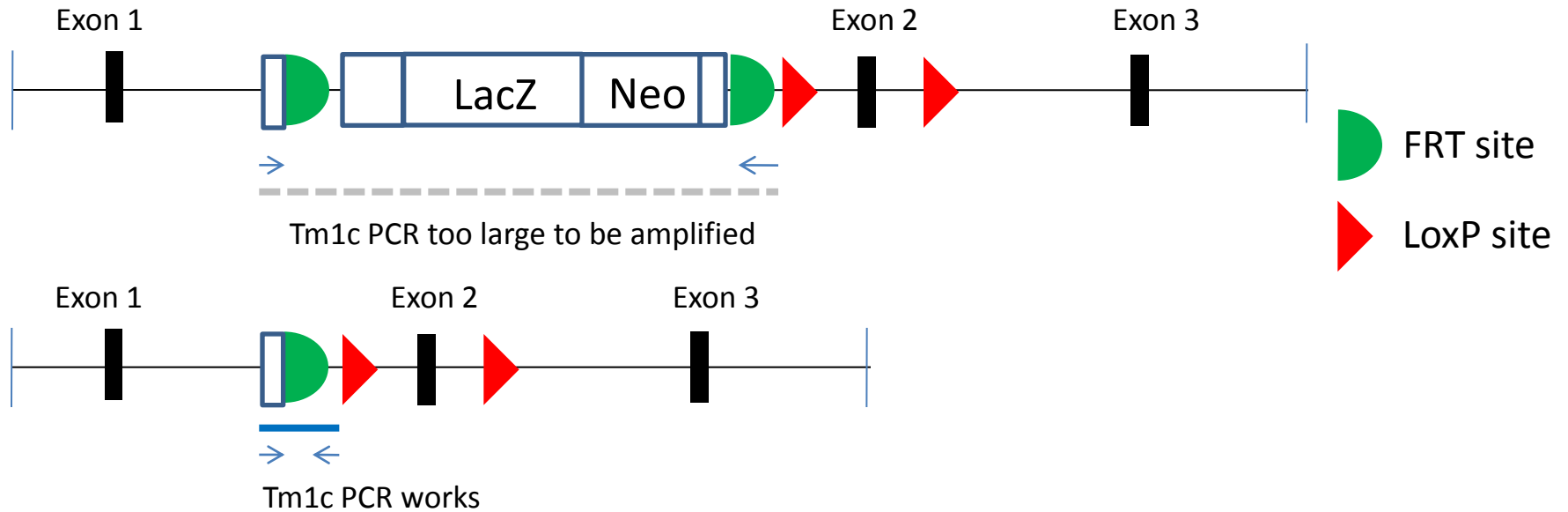
Flipped out mutant allele (Tm1c) – WT function restored. One FRT site remains



WT allele

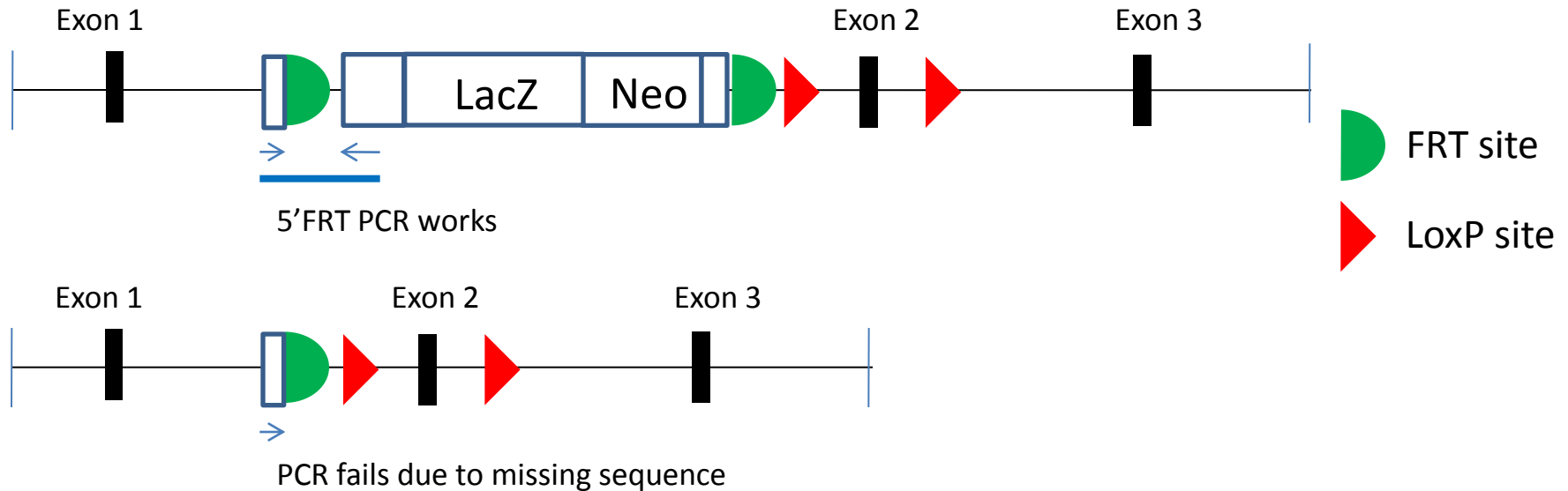


Tm1c PCR



- Presence of band calls the Tm1c allele

5'FRT PCR



- Absence of band calls the Tm1c allele
- 5'FRT PCR can also be used as a 5' element integrity check in Tm1a alleles

Expected results and assay conditions

	WT assay	Mut assay	LacZ assay	Tm1c assay	5'FRT assay
Cassette removed (Tm1c)	pass (size different to native WT)	pass	fail	pass	fail
Cassette not removed (Tm1a)	fail	pass	pass	fail	pass

gene_id	f_primer	r_primer	exp_size	pcr_type	pcr_prog
5' FRT	5FRT_F	5FRT_R	204	Cassette	TA58
Tm1c	tm1c_F	tm1c_R	218	Tm1c allele	TA58
LacZ	LacZ_2_small_F	LacZ_2_small_R	108	LacZ	TA58

primer_name	sequence
5FRT_F	AGGCGCATAACGATACCACGAT
5FRT_R	CCACAACGGGTTCTTCTGTT
tm1c_F	aaggcgcataacgataccac
tm1c_R	ccgcctactgcgactatagaga
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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
H2O	15.2
DNA	1
Total	20

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

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