Detection of the 5' insertion of the ROSA targeting cassette into the ROSA26 locus.

The group (V.T. Chu and R. Kuhn) published a paper entitled "*Efficient generation of Rosa26 knock-in mice using CRISPR/Cas9 in C57Bl/6 zygotes* in BMC Biotechnology (2016). The primer pairs used to detect insertion of the Rosa cassette into the Rosa26 locus are described.

1. **Rosa HR detection**:

The primers are:

Rosa26 Fw Out 1.1HA	5' -TAGGGGATCGGGACTCTGGC-3'
Rosa26 Rev CAG	5'- GGGCTATGAACTAATGACCCCG-3'
Rosa26-Fw bGH	5'- GGAAATTGCATCGCATTGTCTGA-3'
Rosa26-Rev Out 4.3HA	5'- ACCATTCTCAGTGGCTCAACAACA-3'

PCR conditions:

Input-

Genomic DNA	2.0ul
2x GC buffer I	25ul
dNTPs (2.5mM)	8ul
Primer F (100uM)	.25ul
Primer R (100uM)	.25ul
LA Taq	0.5ul
H_2O	14.0ul
total volume	50.0ul

Program:

94°C 1 min
94°C 30 sec
60°C 30 sec — 30 cycles
72°C 2 min

72°C 7 min
4 Hold

2. **Amp^r random insertion detection**:

The integration of the Rosa targeting cassette may have occurred randomly, into the desired location in the *Rosa26* locus or you may have both types of integration events in the same genome. To identify random integrations, you can screen for the presence of the Amp^r cassette as the vector DNA would have integrated along with the targeting cassette. Use the same LA Taq DNA polymerase (Takara # RR02AG) for the analysis and use Amp^r specific primers.

Amp^r Primers:

Primer	Sequence	Product
Amp^r (A) Forward	5’-AAGATGCTGAAGATCAGTTG-3’	>143bp
Amp^r (B) Reverse	5’-ATAATACCGCGCCACATAGC-3’	
Amp^r (C) Forward	5’-AATTAATAGACTGGATGGAG-3’	>199bp
Amp^r (D) Reverse	5’-TTCATCCATAGTTGCCTGACTC-3’	

Program:

Temperature	Time	
$95^{o}C$	10 min	
$94^{o}C$	1 min	22 cycles
$53^{o}C$	1 min	
$72^{o}C$	1 min	
$72^{o}C$	10 min	
$4^{o}C$	HOLD	

PCR #_390__

PCR DETECTION OF TRANSGENES (Genotyping)

Date_10/11/2022___

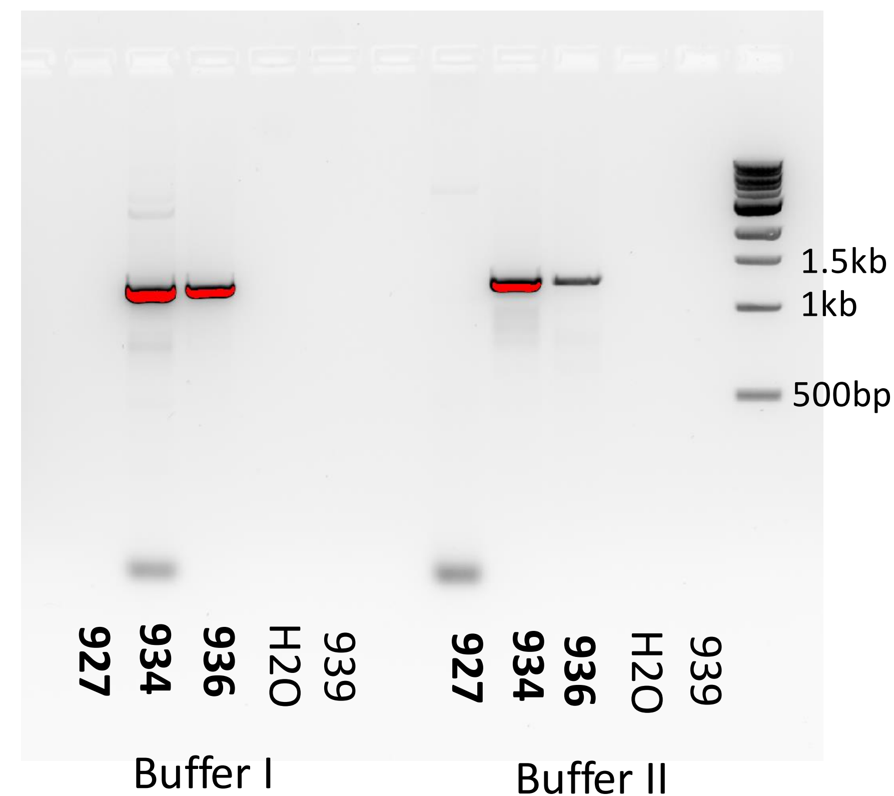
I.Insertion: Rosa26-Color Switch -- To test specific insertion in Rs26

II. Primers: *Fw1 OUT Rs26* (5'- *taggggatcgggactctggc* -3')
Rev1 CAG (5'-*gggctatgaactaatgaccccg*..3')

III. Results:
WT band: N/A
KI band (R26 Specific): 1248bp

IV. MIX: **LA Taq DNA w/ GC buffer** master mix 48uL each + 2uL Sample

	x 1 Sample	x5 Samples
Primer 1 (100uM)	*0.25*uL	1.25
Primer 2 (100uM)	0.25uL	1.25
LA Taq	0.5uL	2.5
2x GC buffer I or II	25uL	125
dNTP (2.5mM)	8uL	40uL
H2O	*15*uL	75uL



V. PCR program:

94°C 1min
94°C 30sec
60°C 30sec
72°C 2min
72° 5min
4 Hold

(94°C 30sec – 72°C 2min: 30cycles)

VI. Samples:

1.__927___
2.__934_
3.__936___
4.__H2O_
5.__939 (WT)_______
6._____

Buffer I: Fragments longer than 5Kb
Buffer II: Fragments 2-3kb in length

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II. Primers: *Fw1 OUT Rs26* (5'- *taggggatcgggactctggc* -3')
Rev1 CAG (5'-*gggctatgaactaatgaccccg*..3')

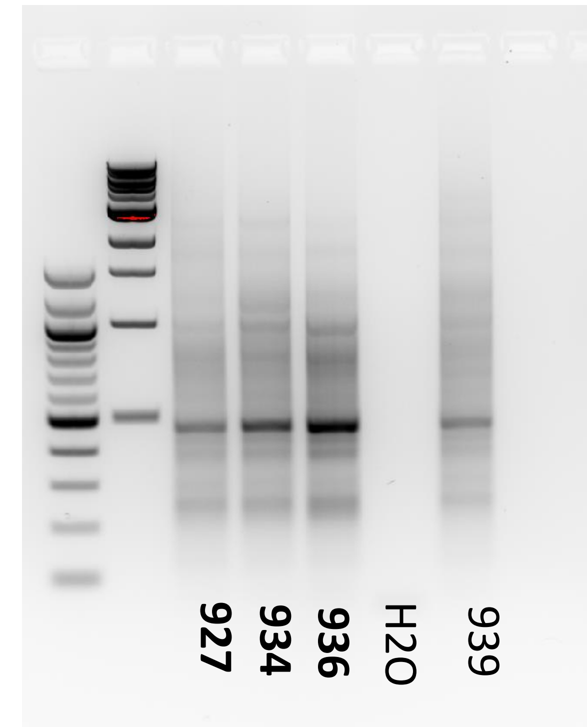
III. Results:
WT band: N/A
KI band (R26 Specific): 1248bp

IV. MIX: **KOD hot start** master mix 18uLeach + 2uL Sample

	x 1 Sample		x5 Samples
(100uM)	*Primer 1*	*0.25*uL	1.25
(100uM)	*Primer 2*	0.25uL	1.25
	MM	10uL	50
	H2O	*8.5*uL	42.5

V. PCR program:

95°C 2min
95°C 20sec
60°C 10sec
70°C 1.15min
(30cycles)
70° 2min
4 Hold



VI. Samples:

1.__927___
2.__934_
3.__936___
4.___H2O_
5.__939 (WT)_______
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