Detection of the 5' and 3' insertion of the ROSA targeting cassette into the ROSA26 locus.

The group (V.T. Chu and R. Kuehn) 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.

***Please note the difference in the 5' and 3' PCR Taq kits

For 5' Detection use Takara LA Taq with GC Buffer (#RR02AG)

The 5' primers are:

Rosa26 Fw Out 1.1HA	5' -TAGGGGATCGGGACTCTGGC-3'
Rosa26 Rev CMV	5'- GGGCTATGAACTAATGACCCCG-3'

5' PCR conditions:

Input-		
Genomic DNA	2.0ul	
2x GC buffer I or II	25ul	
dNTPs (2.5mM)	8ul	
Primer F (100uM)	.25ul	(or 5ul @10pmol/ul)
Primer R (100uM)	.25ul	(or 5ul @10pmol/ul)
LA Taq	0.5ul	
H_2O	14.0ul	
total volume	50.0ul	

Program:

94°C	1 min	
98°C	30 sec	⎫
68°C	30 sec	⎬ 30 cycles
72°C	2 min	⎭
72°C	7 min	
4	Hold	

For 3' Detection use Takara LA Taq DNA Polymerase (#RR002A)

The 3' primers are:

Rosa26-Fw bGH	5'- GGAAATTGCATCGCATTGTCTGA-3'
Rosa26-Rev Out 4.3HA	5'- ACCATTCTCAGTGGCTCAACAACA-3'

3' PCR conditions:

Input-		
Genomic DNA	2.0ul	
10x LA Buffer II	5ul	
25mM $MgCl_2$	5ul	
dNTPs (2.5mM)	8ul	
Primer F (100uM)	.25ul	(or 5ul @10pmol/ul)
Primer R (100uM)	.25ul	(or 5ul @10pmol/ul)
LA Taq	0.5ul	
H_2O	29.0ul	
total volume	50.0ul	

Program:

94°C 1 min

98°C 10 sec
68°C 15 min] 30 cycles

72°C 7 min
4 Hold

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 cassette as the vector DNA would have integrated along with the targeting cassette. Use the same LA Taq with GC Buffer (Takara # RR02AG) for the analysis and use AMP specific primers.

Amp^r Primers:

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:

95^{o}C	10 min	
94^{o}C	1 min	22
53^{o}C	1 min	
72^{o}C	1 min	
72^{o}C	10 min	
4^{o}C	HOLD	

PCR DETECTION OF TRANSGENES II

Date: 11/25/25

I. Transgene: Test for 5' Rosa HR insertion

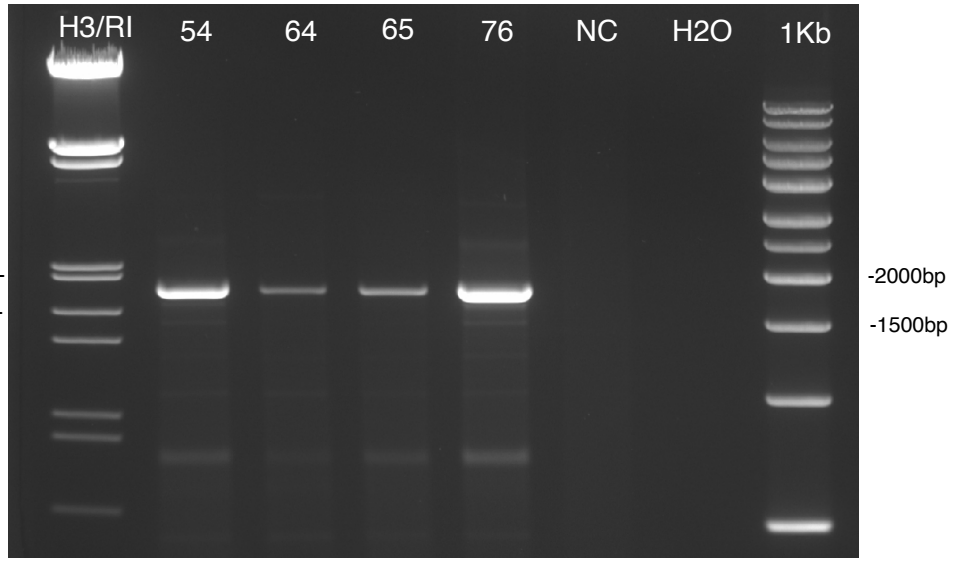
Primers: R26 FW Out 1.1HA, R26 REV-CMV

II. PCR Rxn. (Takara LA Taq w/ GC Buffer) 8 X

DNA	_1_ µl	
2x GC Buffer II	12.5 µl	100
dNTPs	4.0 µl	32
Oligo A F	2.5 µl	20
Oligo B R	2.5 µl	20
Oligo C	µl	
Oligo D	µl	
Ster. H_20	2.25 µl	18
Taq. Pol	0.25 µl	2

(25 µl / rxn)

V. Results: photo 1.4% gel



III. PCR program: Standard Biometra #3,5

Other: 60°C (30s ,30s, 2min)

30 cycles

IV. Samples:

Lane										
1.	H3/RI	8.	1Kb Direct Load	15.		22.		29.		36.	
2.	54	9.		16.		23.		30.		37.	
3.	64	10.		17.		24.		31.		38.	
4.	65	11.		18.		25.		32.		39.	
5.	76	12.		19.		26.		33.		40.	
6.	NC	13.		20.		27.		34.			
7.	H2O	14.		21.		28.		35.			

PCR DETECTION OF TRANSGENES II

Date: 11/19/2025

I. Transgene: Test for 3' Rosa HR insertion

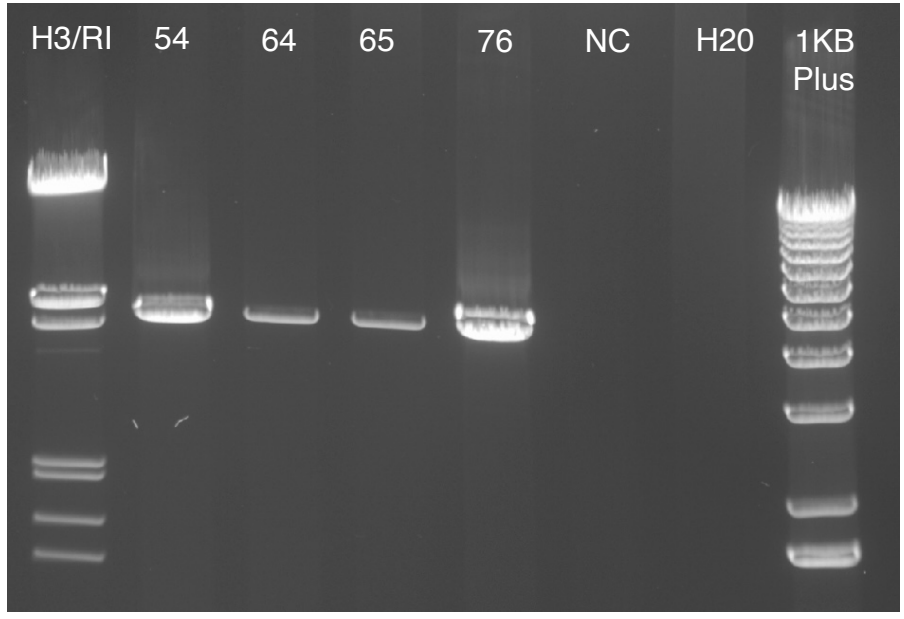
Primers: R26 bGH-F, R26 Out 4.3HA-R

II. PCR Rxn. (Takara LA Taq DNA Poly) 8 X

DNA	1 µl	
10x LA Buffer II	2.5 µl	20
25mM $MgCl_2$	5 µl	20
dNTPs	0.5 µl	32
Oligo A F	2.5 µl	20
Oligo B R	2.5 µl	20
Oligo C	µl	
Oligo D	µl	
Ster. H_20	9.75 µl	78
Taq. Pol	0.25 µl	2

(25 µl / rxn)

V. Results: photo



III. PCR program: Standard Biometra #3,10

Other: 68°C (10sec, 15min)

30 cycles

3'Rosa HR 4662bp

IV. Samples:

Lane1. H3/RI	8. 1Kb Plus	15.	22.	29.	36.
2. 54	9.	16.	23.	30.	37.
3. 64	10.	17.	24.	31.	38.
4. 65	11.	18.	25.	32.	39.
5. 76	12.	19.	26.	33.	40.
6. NC	13.	20.	27.	34.	
7. H20	14.	21.	28.	35.	

PCR DETECTION OF TRANSGENES II

Date: 1/6/25

I. Transgene: Amp Detection for Rosa 26

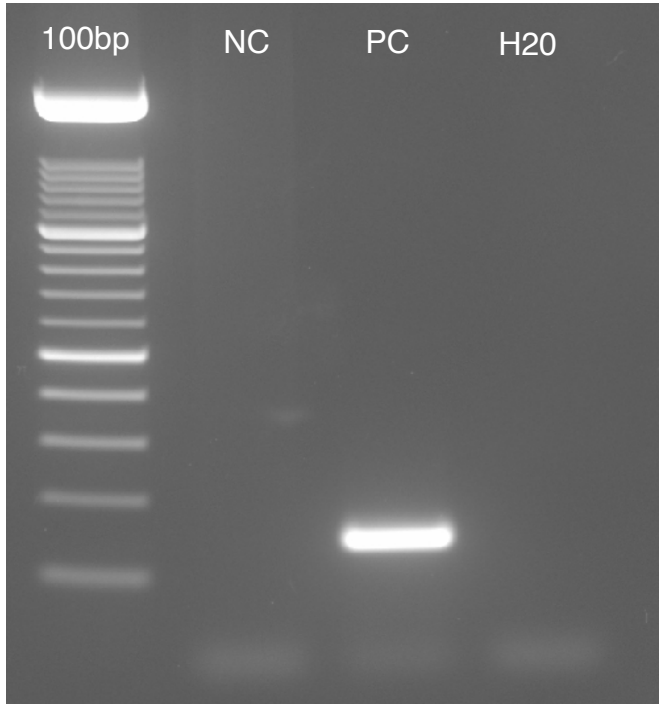
Primers: Amp^r -A (Forward), B (Reverse)

II. PCR Rxn. (Takara LA Taq w/ GC Buffer) 4 X

DNA	_1_ µl		
2x GCBuffer II	12.5 µl		50
dNTPs	4.0 µl		16
Oligo A A	2.5	µl	10
Oligo B B	2.5	µl	10
Oligo C		µl	
Oligo D		µl	
Ster. H_20	2.25	µl	9
Taq. Pol	0.25 µl		1

(25 µl / rxn)

V. Results: photo 2% gel



III. PCR program: Standard Biometra #64

Other: 53°C (1,1,1)

22 cycles

143bp

IV. Samples:

Lane					
1. 100bp	8.	15.	22.	29.	36.
2. NC (C57Bl6)	9.	16.	23.	30.	37.
3. PC (1ng/ul pR26)	10.	17.	24.	31.	38.
4. H20	11.	18.	25.	32.	39.
5.	12.	19.	26.	33.	40.
6.	13.	20.	27.	34.	
7.	14.	21.	28.	35.	