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MODEL GENERATION TECHNICAL REPORT

G6pc1 conditional overexpression driven by the Rosa26 promoter

Project code: K483 / IR2157

Report finalized: 2024/11/27

cnrs
Université de Strasbourg
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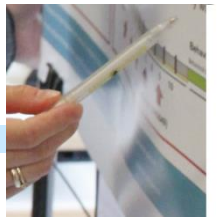
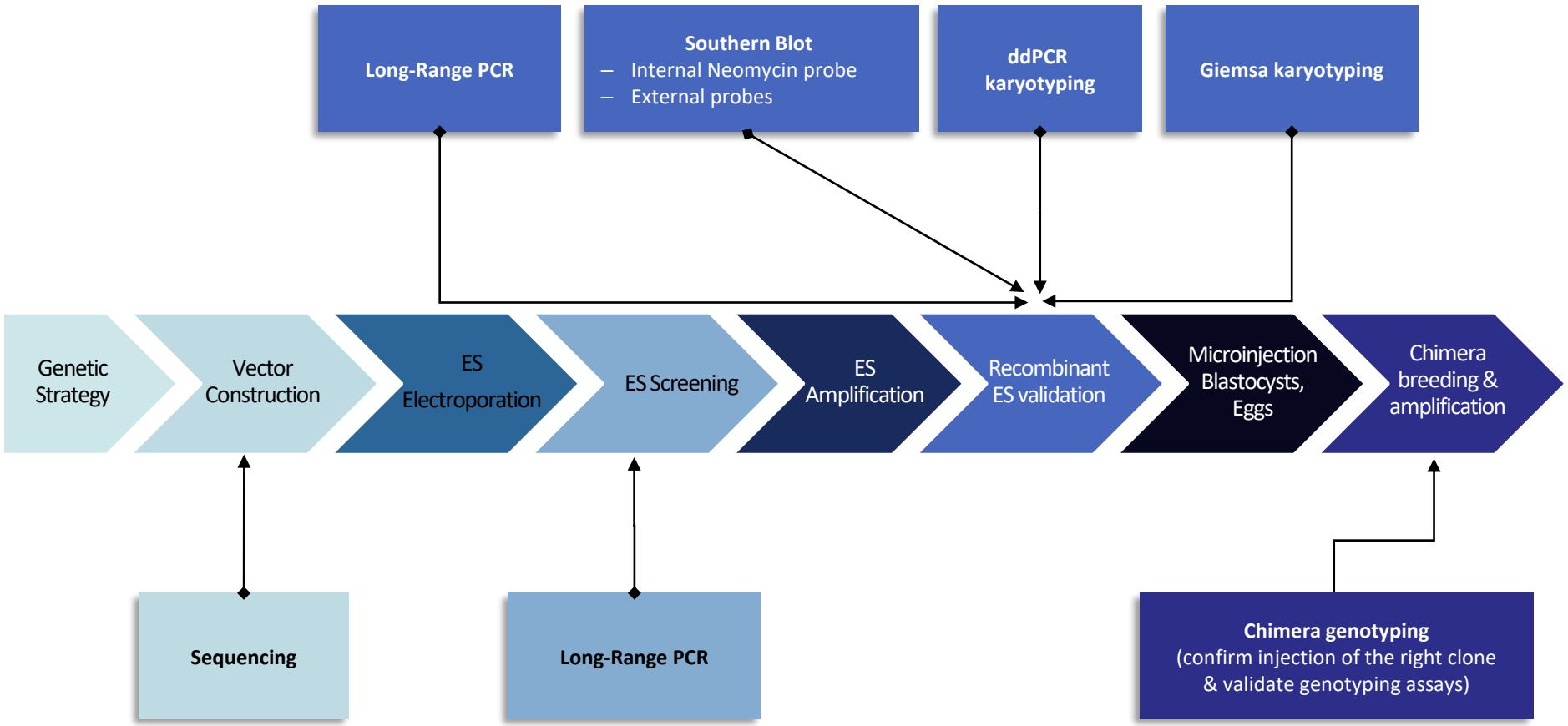
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

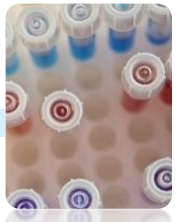
MODEL GENERATION TECHNICAL REPORT

PROJECT PROCESS & QUALITY CONTROLS



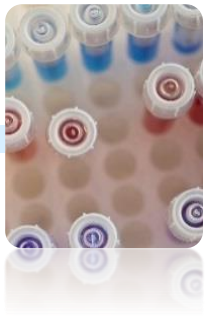


2 GENETIC STRATEGY

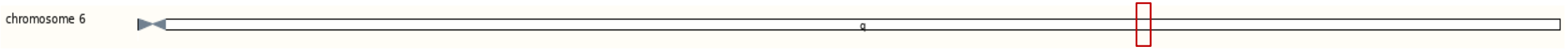


- Target locus structure
- mRNA(s) and protein(s)

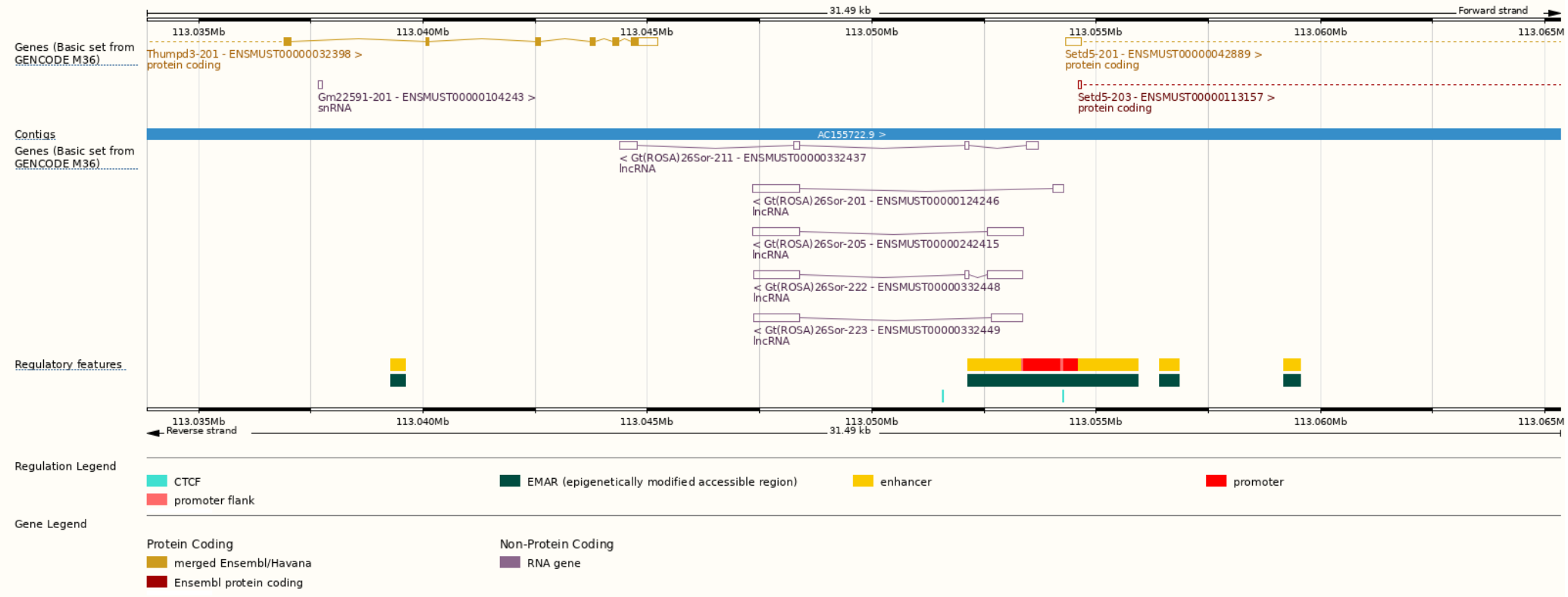
Rosa26 mouse genomic locus – structure (GRCm39)



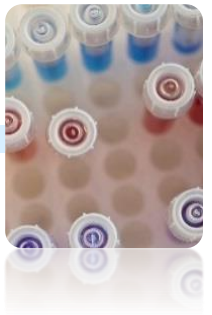
Location : Chromosome 6: 113,043,843-113,055,336



Gene: Gt(ROSA)26Sor ENSMUSG00000086429



G6pc1 mRNAs and proteins



(GRCm39)

ENSMUST00000019469.3	G6pc1-201	2414	357aa	Protein coding	CCDS25466	P35576

MEEGMNILHDFGIQSTRYLQVNYQDSQDWFILVSVIADLRNAFYVLFPIWFHLKETVGINLLWVAVVGDWFNLVFKWIL
FGQRPYWWVLDTDYYSNSSVPIIKQFPVTCETGPGSPSGHAMGAAGVYYVMVTSTLAIFRGKKKPTYGFRCLNVILWLG
FWAVQLNVCLSRIYLAAHFPHQVVAGVLSGIAVAETFSHIRGIYNASLRKYCLITIFLFGFALGFYLLLKGLGVDLLWTLEKAK
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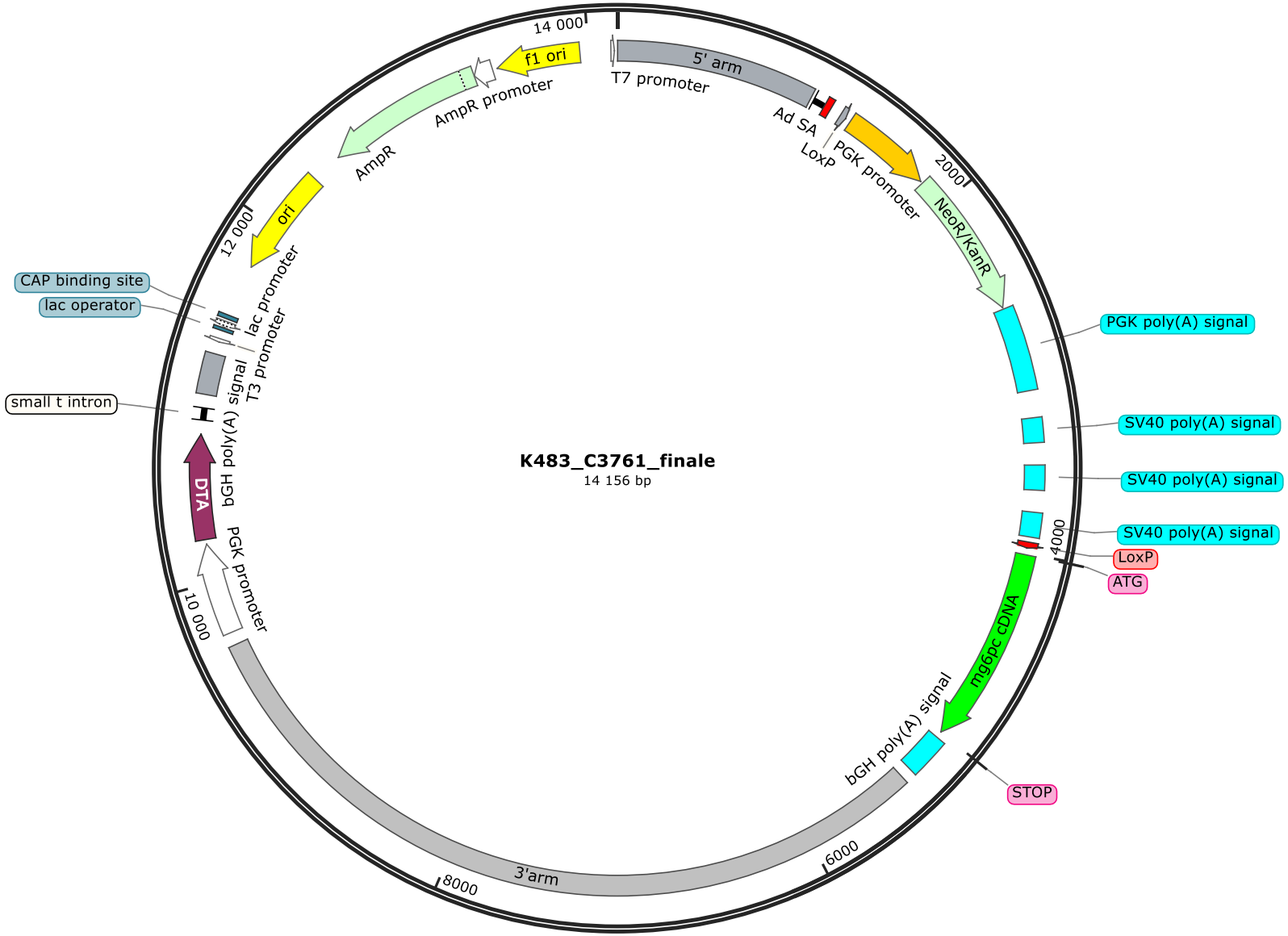
FCKSATVPFASVSLIPYCLARILGQTHKKSL

3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



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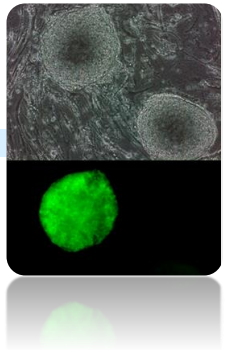
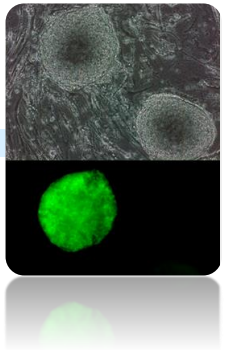
4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Long-Range 5’ PCR screening – results
- Recombinant ES validation by Long Range PCR
- Recombinant ES clones validation by Southern Blot – internal probe
- Recombinant ES clones validation by Southern Blot – External probe
- Aneuploidy screening in ES recombinant clones

Electroporation and screening process



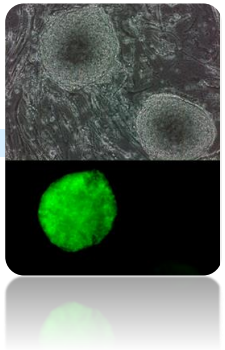
The targeting vector was electroporated in the proprietary 129SV/Pas (P1) embryonic stem cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 372 resistant ES clones were isolated. The clones were then submitted to the screening process allowing secured identification of those harbouring the expected recombination events at both ends of targeting vector.

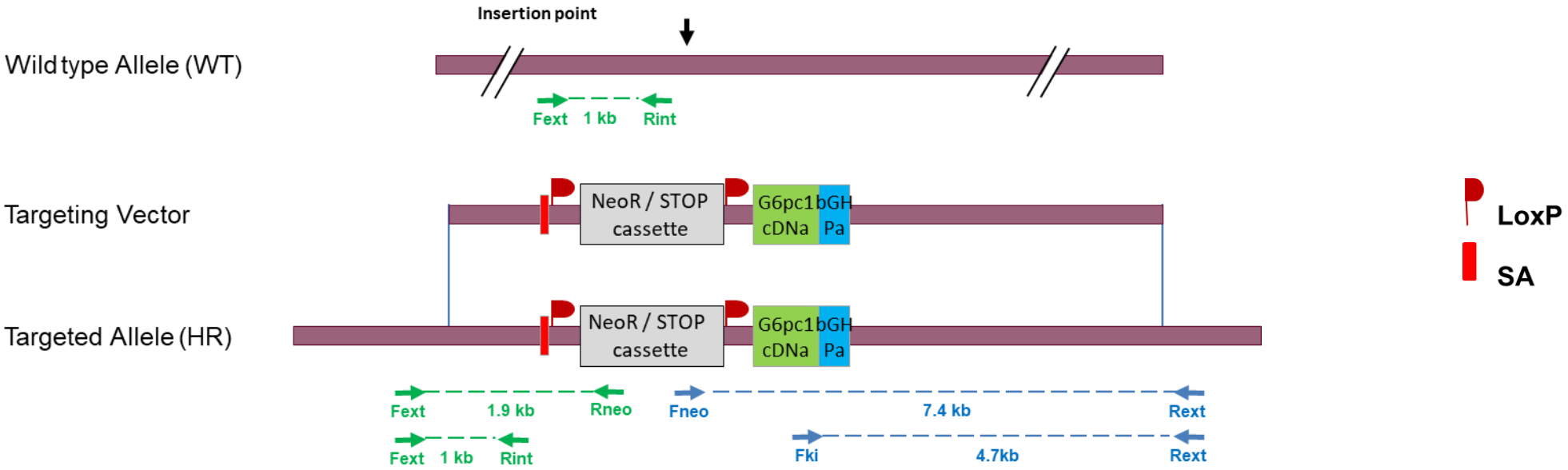
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Ten of 5’ PCR positive clones are confirmed for 3’ recombination event by Long-Range PCR
3. Positive clones in step2 are further validated by Southern blot analysis using internal and external probes
4. The karyotype of at least 2 validated clones is verified using Giemsa staining

Long range PCR screening – strategy

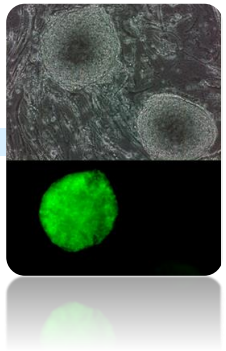


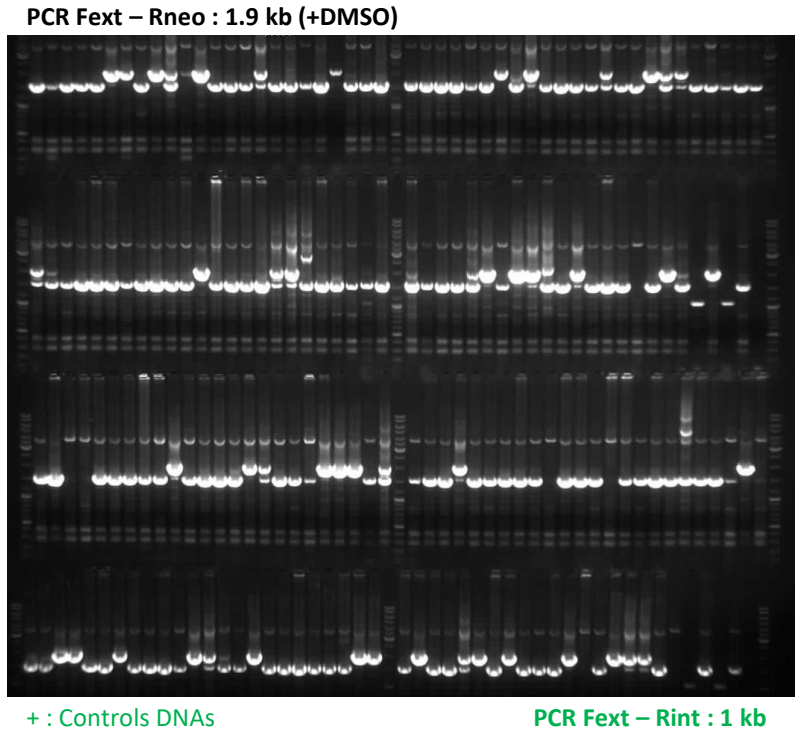
Schematic 5’ and 3’ PCR screening strategy



PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GGTAGGGGATCGGGACTCTGGCGGG	1.9 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
5’ PCR	Fext	GGTAGGGGATCGGGACTCTGGCGGG	1 kb
	Rint	CTCCCAGAAAGGTATTGCAACACTC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	7.4 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	
3’ PCR	Fki	GACACACAAGAAGTCTTTGTAAGGC	4.7 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	

Long-Range 5’ PCR screening – results

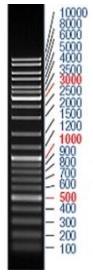




PCR Fext – Rneo : 1.9 kb (+DMSO)

+ : Controls DNAs

PCR Fext – Rint : 1 kb



Ladder pattern

Ten candidate clones out of the positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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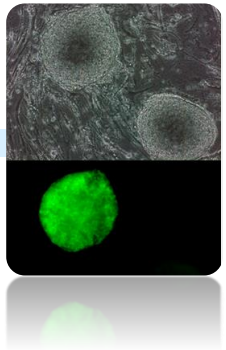
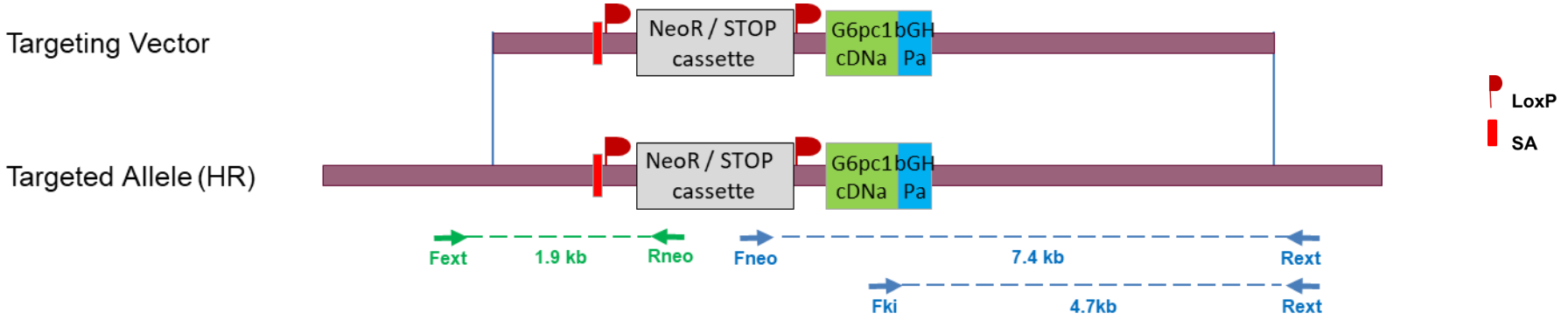
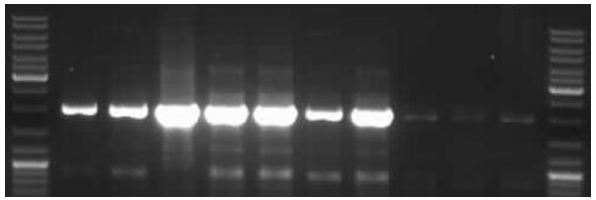
Recombinant ES validation by Long Range PCR

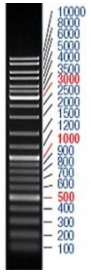


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

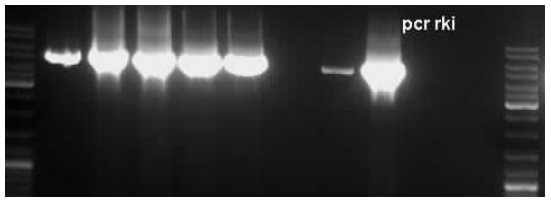




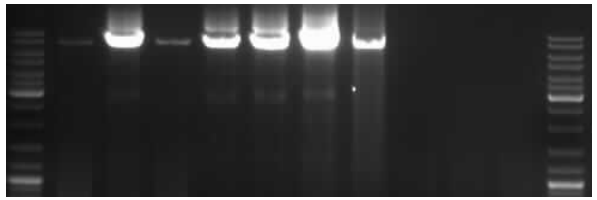
PCR Fext – Rneo : 1.9 kb



Ladder pattern



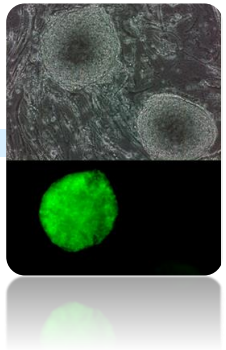
PCR Fki – Rext : 4.7 kb



PCR Fneo – Rext : 7.4 kb

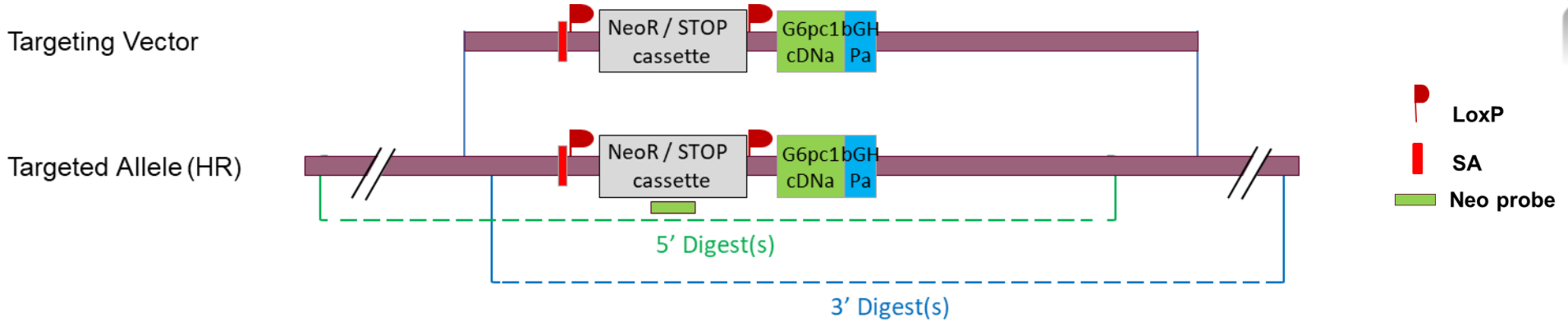
Ten candidate clones identified by 5’ PCR screening were further analysed by 3’ Long-Range PCR screening.
Six clones (clones #203, #209, #215, #228, #230 and #254) were further analysed.

Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

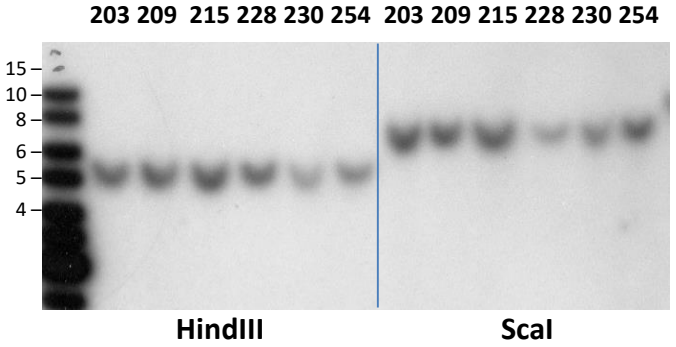
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



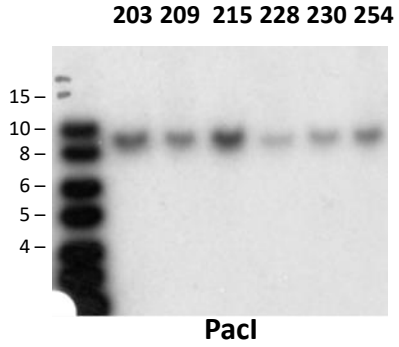
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	HindIII	5.7
		ScaI	7.6
	3' digest	PacI	8.8

Southern blot - Neo 5'



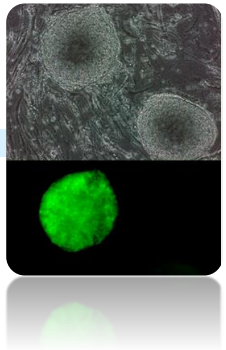
Southern blot - Neo 3'



Neo probe sequence

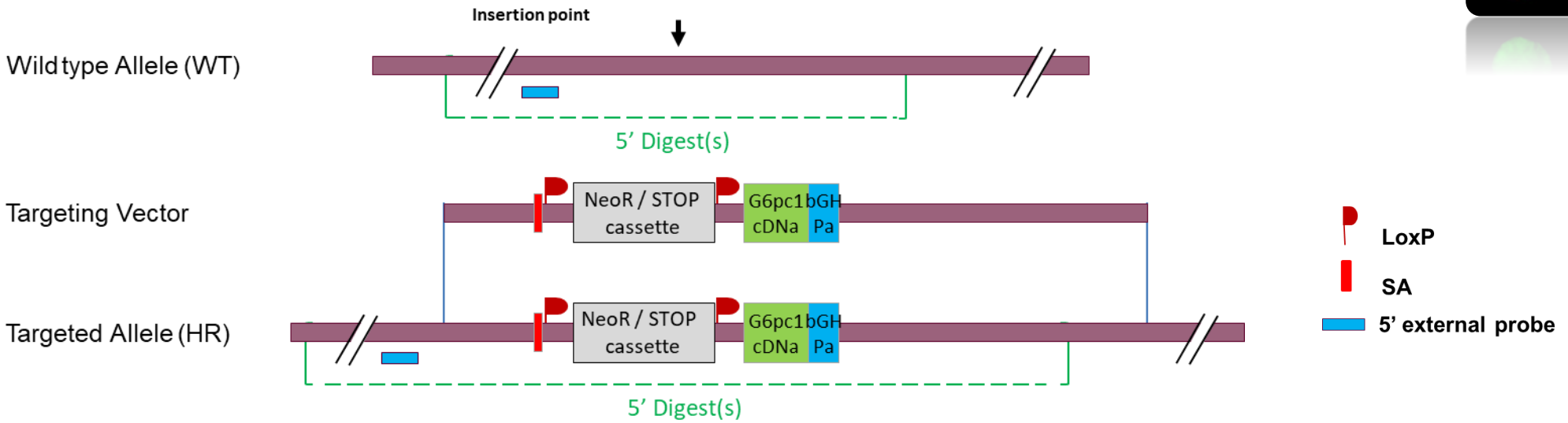
CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
CTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCCGCTG
TAAGTCTG

Recombinant ES clones validation by Southern Blot – External probe

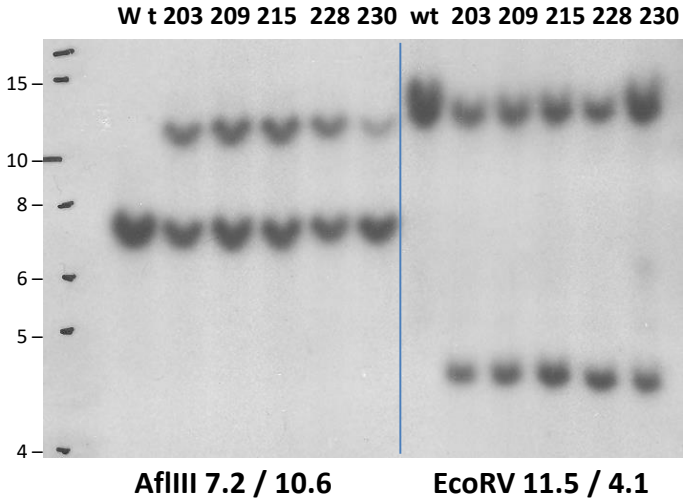


Schematic Southern Blot validation strategy

Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



Southern blot – 5' probe



5' probe sequence

TATGTGTATTTTGAGAGCAGGGTTGGGAGGCCTCTCCTG
AAAAGGGTATAAACGTGGAGTAGGCAATACCCAGGCAAA
AAGGGGAGACCAGAGTAGGGGGAGGGGAAGAGTCCTGAC
CCAGGGAAGACATTAAAAAGGTAGTGGGGTCGACTAGAT
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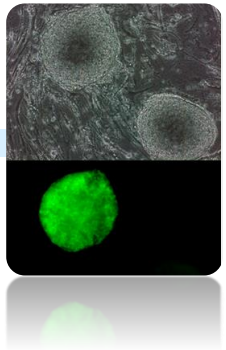
GTGTGTAAAGGTAGCTGAGAAGACGAAAAGGGCAAGCAT
CTTCCTGCTACCAGGCTGGGGAGGCCCAGGCCCACGACC
CCGAGGAGAGGGAACGCAGGGAGACTGAGGTGACCCTTC
TTTCCCCCGGGGCCCGGTCGTGTGGTTCGGTGTCTCTTT
TCTGTTGGACCCTTACCTTGACCCAGGC

Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
5' external probe	5' first digest	AflIII	7.2	10.6
	5' second digest	EcoRV	11.5	4.1

Aneuploidy screening in ES recombinant clones



Selected recombinant ES cells clones were karyotyped by Giemsa metaphase staining.

Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#203	**Pass**
#209	**Pass**
#215	Not done
#228	Not done

5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a 129SV/Pas (P1) mouse strain (which have agouti coat colour). These cells were injected into blastocysts derived from an C57BL/6N strain, which have a black coat colour. The resulting offspring are thus chimeras of two different cell types (ES cell-derived cells and host blastocyst-derived cells) and the degree of chimerism was monitored by the percentage of light and dark patches on these animals.
- Recipient blastocysts were isolated from mated C57BL/6N females (Health status SPF Specific Pathogens Free).
- Recombinant ES clones #203 and #209 validated in previous project phase were injected into blastocysts to generate chimeric males. The results are presented in the table below.

Clone ID	Number of chimeric males identified according to chimerism rate (Number of chimeric males bred to F1 generation)			
	5 - 40%	45% - 55%	60-100%	Total
#203	1	1	2	4
#209	0	1	1	2

Breeding to F1 generation

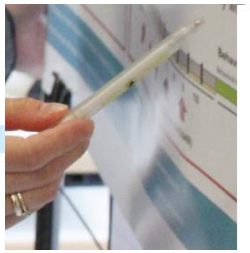
- Three highly chimeric males generated in the previous phase by blastocyst injection of the ES clone #203 were mated with wild-type C57BL/6NTac females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 18/02/2009

- Allele nomenclature (following MGI guidelines) : Gt(ROSA)26Sor$^{tm14(G6pc1)Ics}$

- MGI allele ID: MGI:7788416

6 SEQUENCE OF THE DELIVERED ALLELE



ISO 9001·NFX 50-900

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LoxP Splice Acceptor Neo/STOP cassette Mg6pc cDNA bGHpA

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REPORT REDACTION & VALIDATION
Protocol finalized on 2024/11/27
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ROSA 26 (IR00002157 / K483 ICS internal reference) mouse line genotyping protocol

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For any question, please contact:

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Email: mutagenesis@igbmc.fr

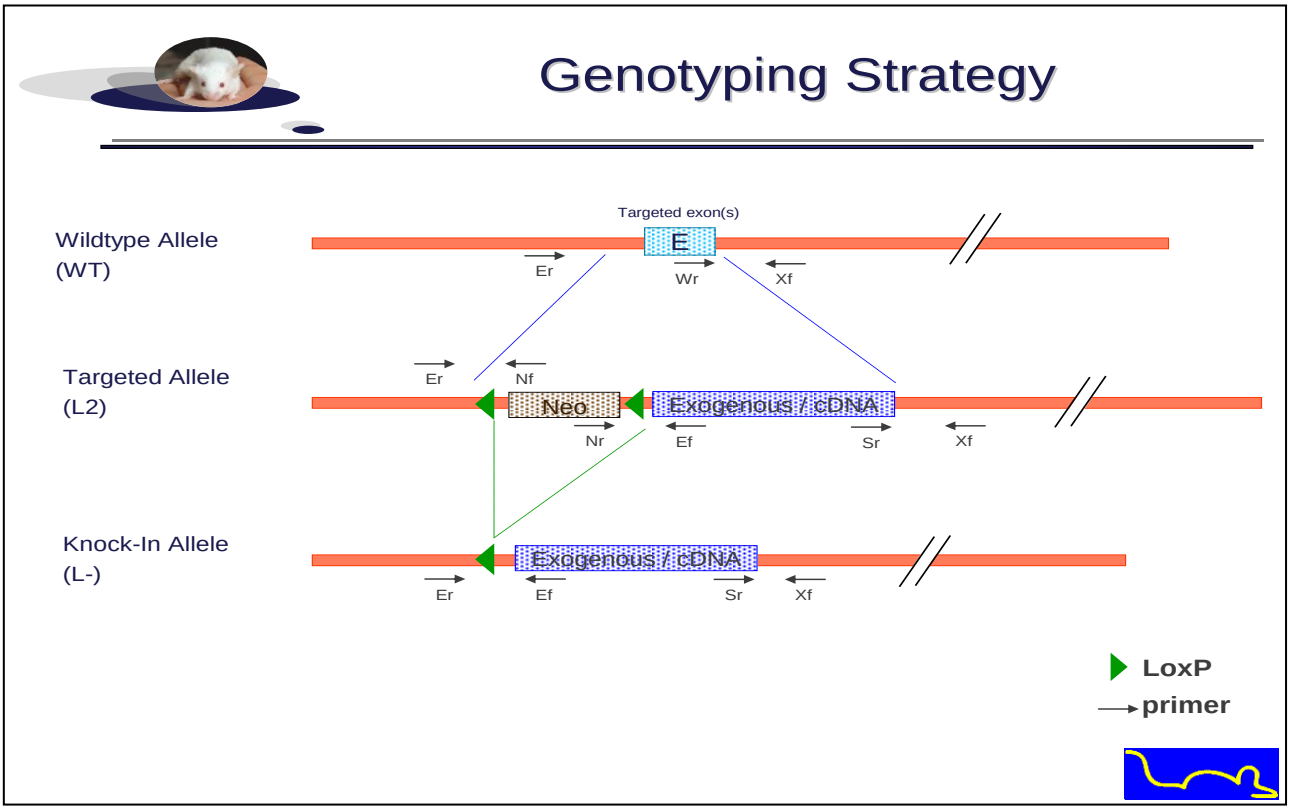
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **ROSA 26** Knockin (KI) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping

Position	Primers	Sequence
Ef	4137	ATCACAGACACAAGGATGAACC
Er	4138	AGTGTTGCAATACCTTTCTGG
Nf	265	TGCTAAAGCGCATGCTCCAGACTGC
Nr	4139	GGATCCCCATCAAGCTGATCCG
Sr	4142	CTCCCACTGTCCTTTCCTAATA
Wr	4141	AGTGTTGCAATACCTTTCTGG
Xf	4140	AAGTCTTGTCCCTCCAATTTTA

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L2)	KI allele (L-)	WildType allele (WT)
WildType allele specific PCR (5' part of the targeted locus)	4140-4141	Xf / Wr	---	---	221
Excision of the selection marker	4137-4138	Ef / Er	3149*	480**	---
5' part of the selection marker	4137-4139	Ef / Nr	210	---	---
3' part of the selection marker	265-4138	Nf / Er	416	---	---
Exogenous/cDNA specific PCR	4140-4142	Xf / Sr	313	313	---

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
** This PCR is only verified if mice are generated
--- No Amplicon should be obtained

1.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
-10x Buffer (Roche)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Roche)	0.2µl
-DNA (50ng/µl)	3µl
-5' primer (100 µM)	0.125µl
-3' primer (100 µM)	0.125µl
-Sterile H2O	up to 25 µl

Cycling conditions:

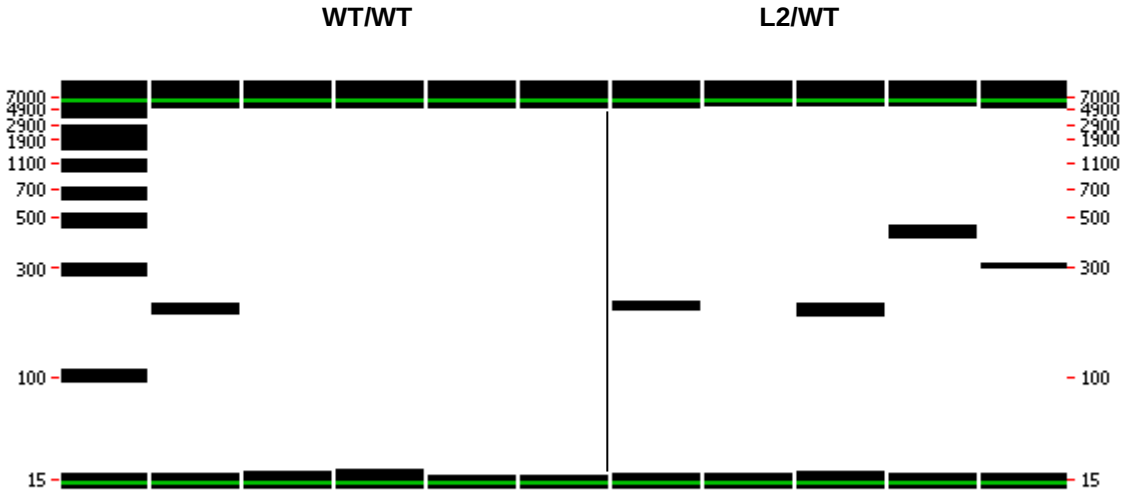
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



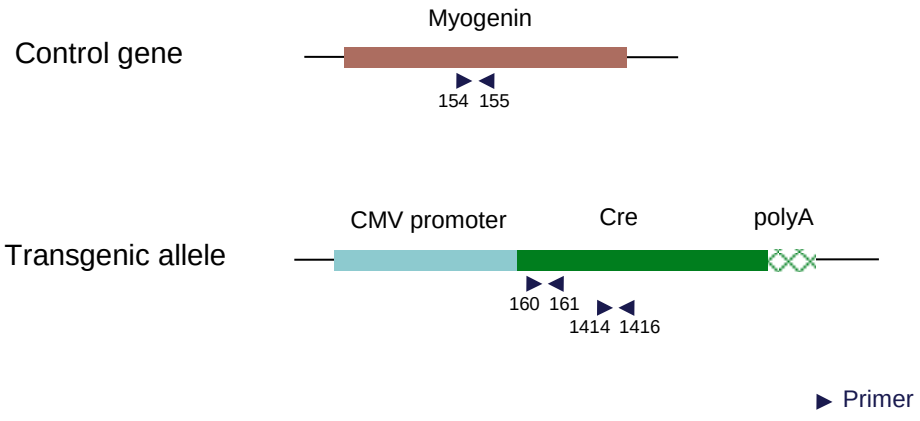
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.

2.1. Cre genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
154	ACTCCCTTACGTCCATCGTG
155	ACCCAGCCTGACAGACAATC
160	GAACCTGATGGACATGTTCAGG
161	AGTGCGTTCGAACGCTAGAGCCTGT
1414	CGTACTGACGGTGGGAGAAT
1416	CCCGGCAAAACAGGTAGTTA

PCR fragments expected size (bp):

Primer pair	160-161	1414-1416	154-155
Region analyzed	5' part of Cre transgene	Middle of Cre transgene	Myogenin control gene
Control gene	/	/	99
Tg allele	345	165	/

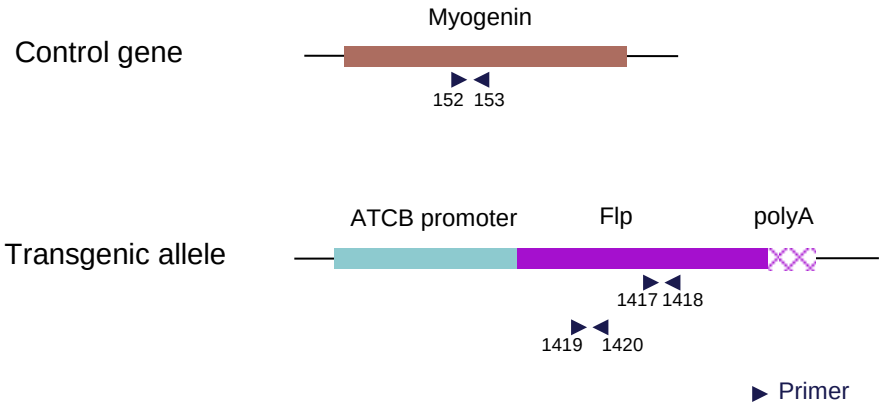
Cycling conditions:

Temp	Time	#Cycles
95°C	3min	1
95°C	10s	
62°C	20s	35
72°C	20s	
95°C	5s	
62°C	30s	1 (melting curve generation)
72°C	72s	
37°C	30s	1
4°C	∞	

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2.2. Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
152	TTACGTCCATCGTGGACAGC
153	TGGGCTGGGTGTTAGCCTTA
1417	TTCTTTAGCGCAAGGGGTAG
1418	GCTCCAATTTCCCACAACAT
1419	TGGGAAATTGGAGCGATAAG
1420	CTGCCACTCCTCAATTGGAT

PCR fragments expected size (bp):

Primer pair	1417-1418	1419-1420	152-153
Region analyzed	Middle part of Flp transgene	5' of Flp transgene	Myogenin control gene
Control gene	/	/	245
Tg allele	299	175	/

PCR protocol and cycling conditions are identical to those described in chapter 1.2