phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

RMCE cassette (FRT-pCAG-Flpe-HygroR-F3) into the Rosa26 locus

Project code: K500 / IR2365

Report finalized: 2024/12/03

MODEL GENERATION TECHNICAL REPORT

1 PROJECT PROCESS & QUALITY CONTROL

2 GENETIC STRATEGY

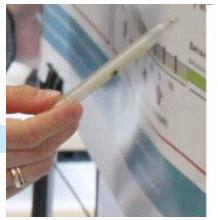
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

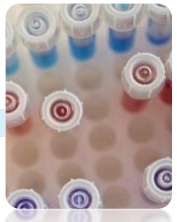
5 MICROINJECTION & BREEDING

6 SEQUENCE OF THE DELIVERED ALLELE

Project process & quality controls

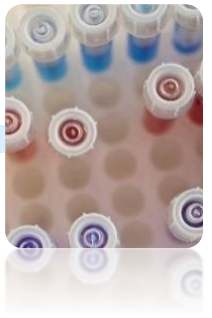


2 GENETIC STRATEGY

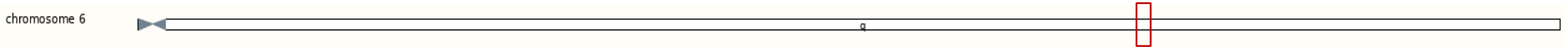


- Target locus structure

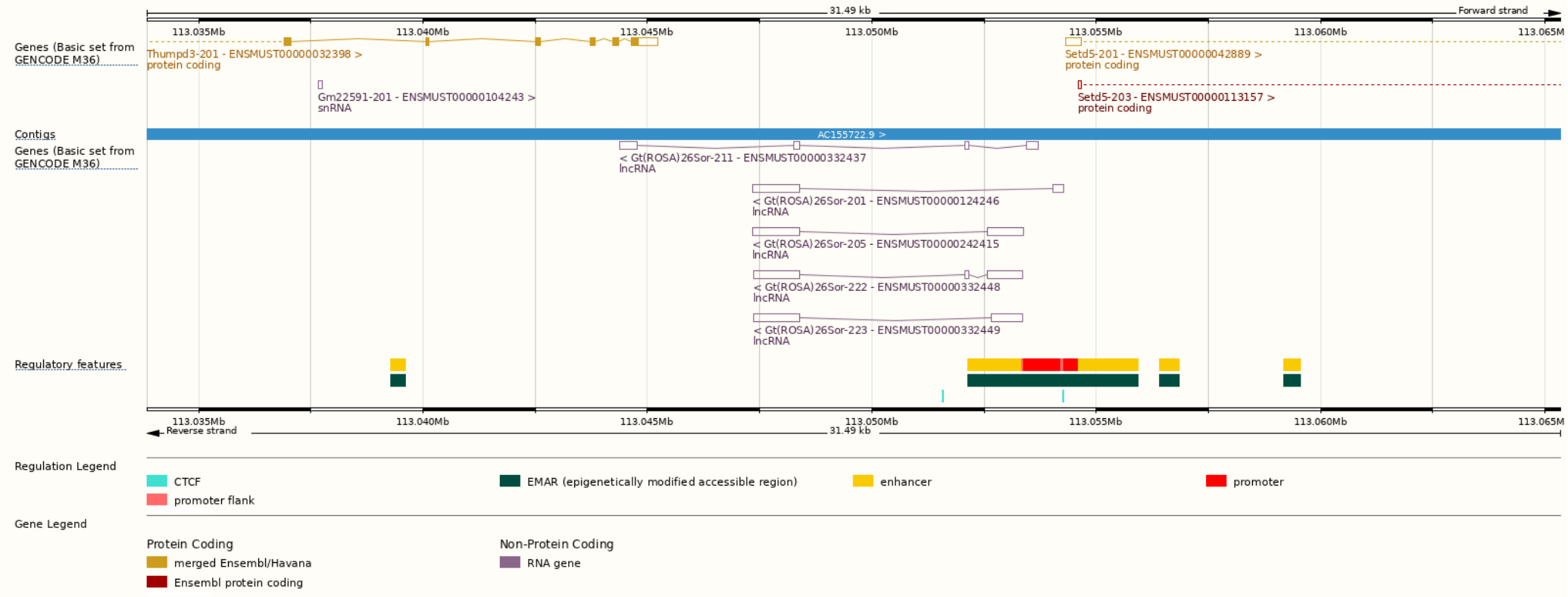
mouse genomic locus – structure (GRCm39)



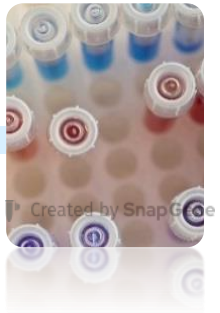
Location : Chromosome 6: 113,043,843-113,055,336

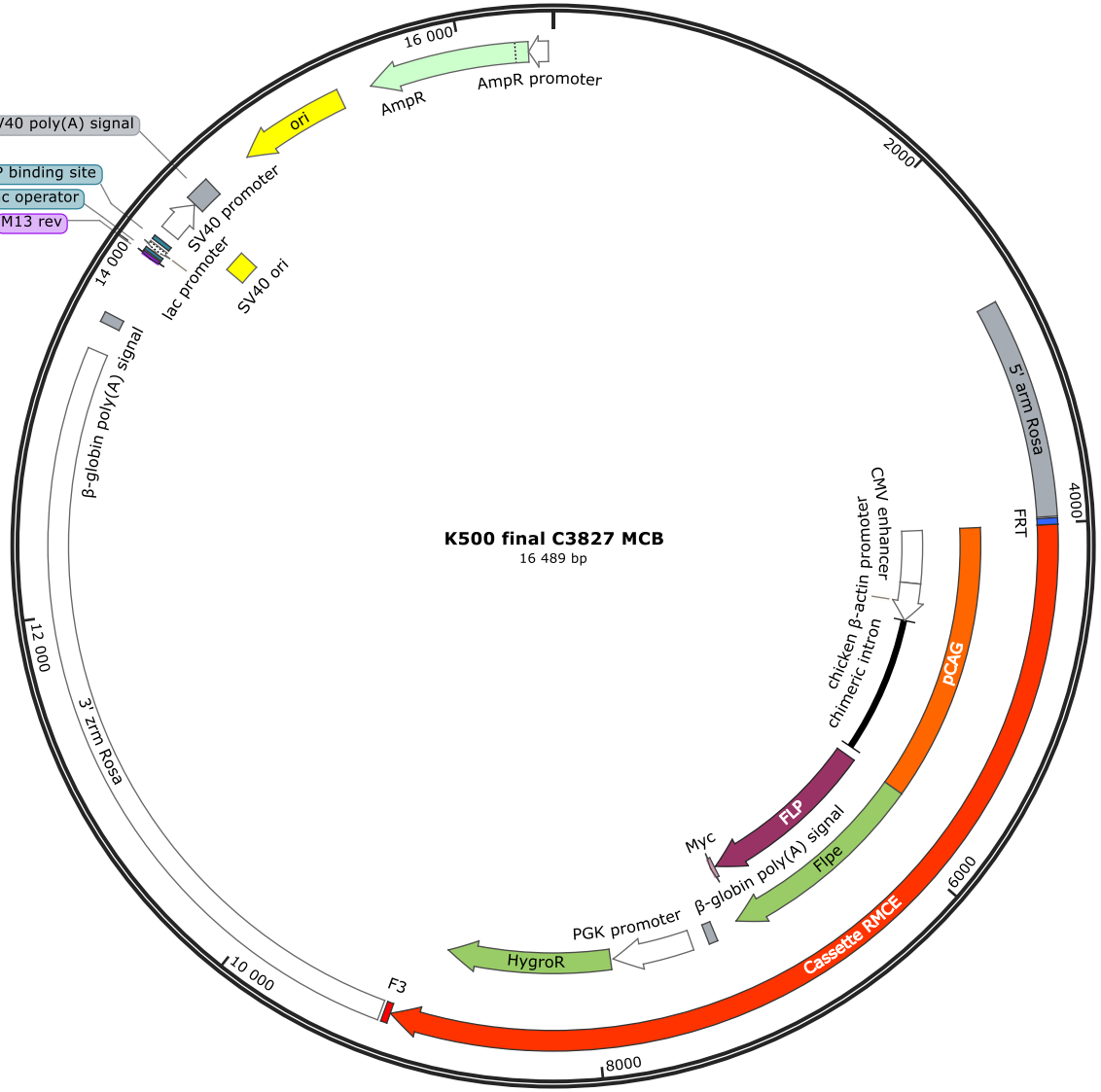


Gene: Gt(ROSA)26Sor ENSMUSG00000086429



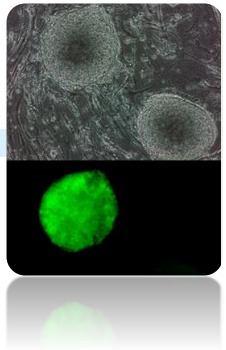
3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION





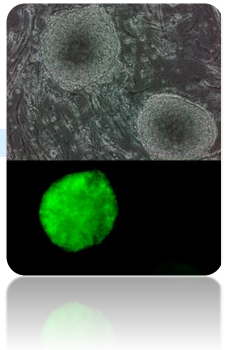
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Long-Range 3’ 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



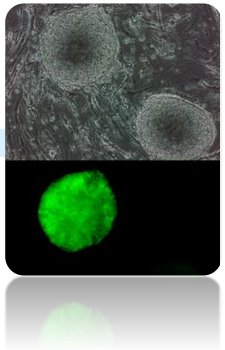
The targeting vector was electroporated in the proprietary C57BL/6NTac (BD10) embryonic stem cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 186 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.

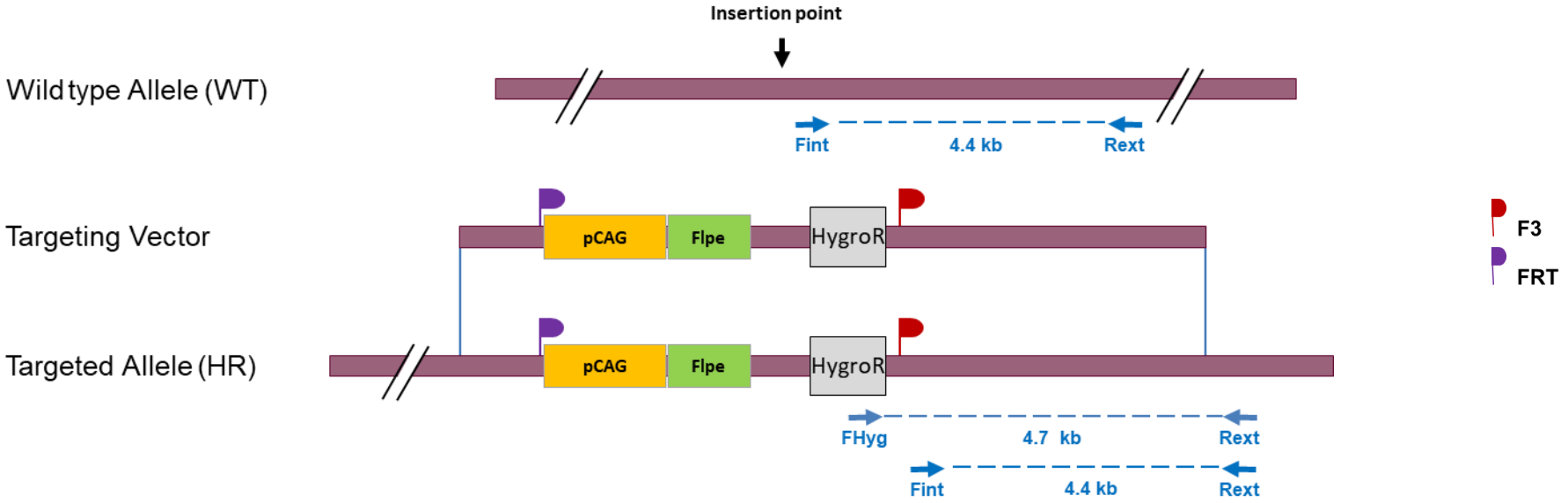
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Three of 3’ 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

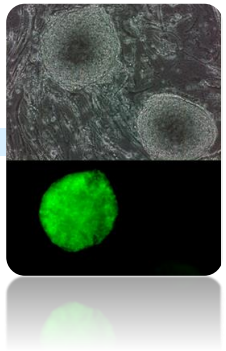


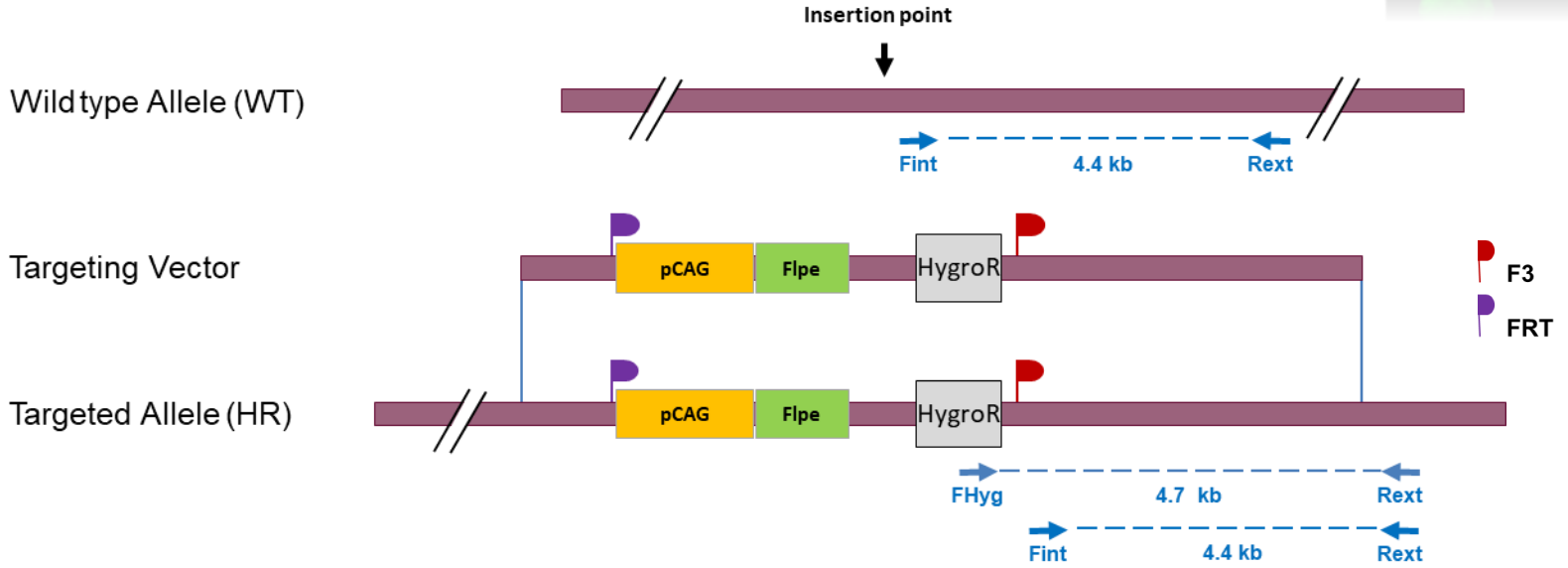
Schematic 5’ and 3’ PCR screening strategy



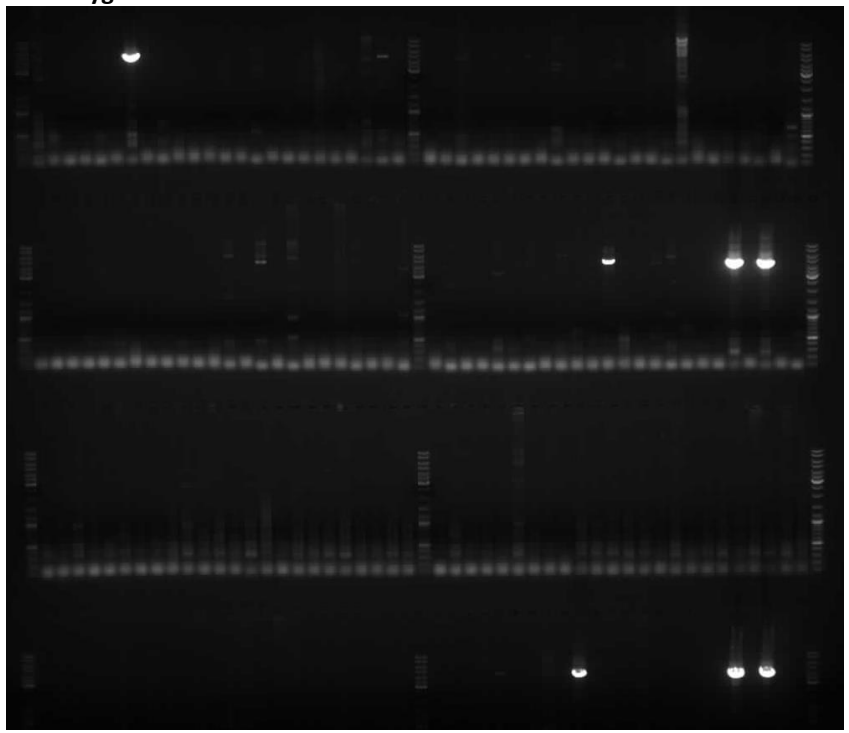
PCR	Primer Name	Primer sequences	PCR product size
3’ PCR	Fhyg	CGCCGATAGTGGAAACCGACGCCC	4.7 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	
3’ PCR	Fint	CTGGTGTGTGGGCGTTGTCCTGCAG	4.4 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	

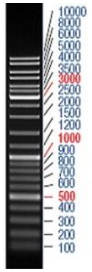
Long-Range 3’ PCR screening – results





PCRFhyg – Rext : 4.7 kb





Ladder pattern

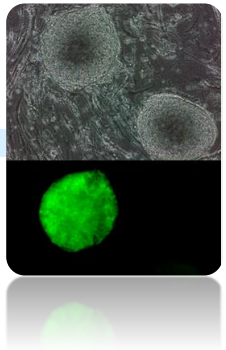
\+ : Controls DNAs

PCR Fint – Rext : 4.4 kb

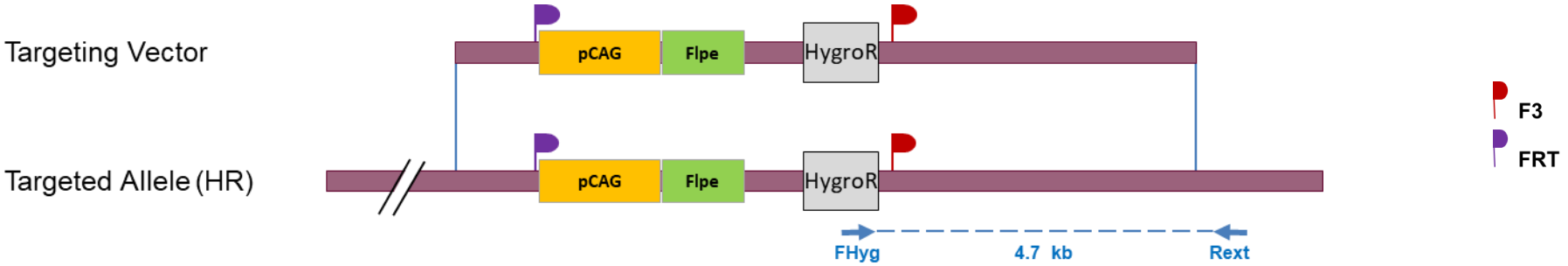
Three candidate clones out of the positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

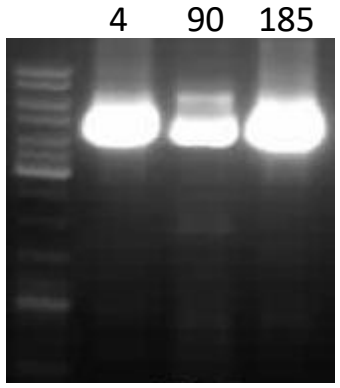
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES validation by Long Range PCR

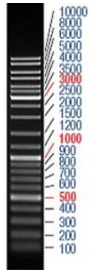


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs





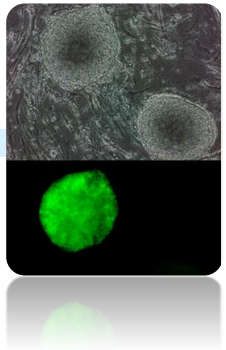
PCR Fhyg – Rext : 4.7 kb



Ladder pattern

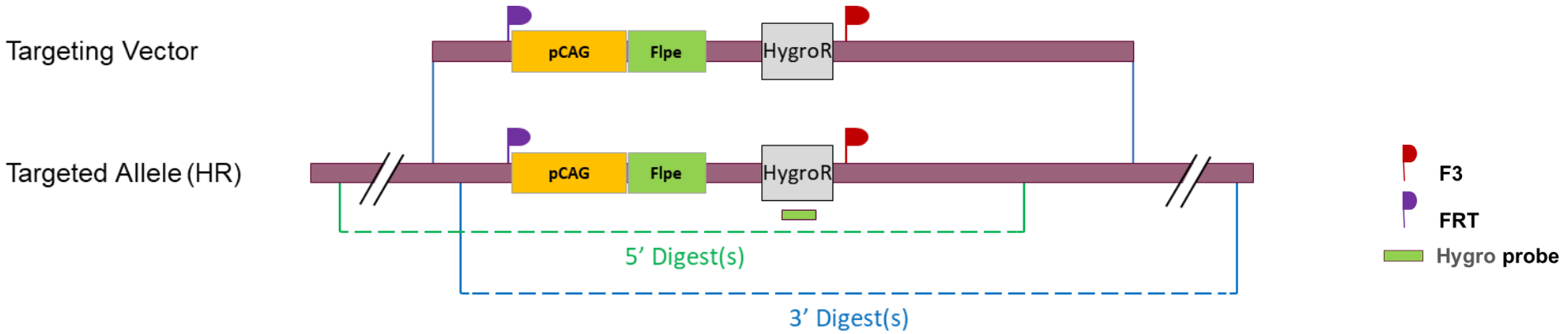
Three candidate clones (clones #4, #90, and #185) identified by 3’ PCR screening were further analysed by 3’ Long-Range PCR and Southern blot .

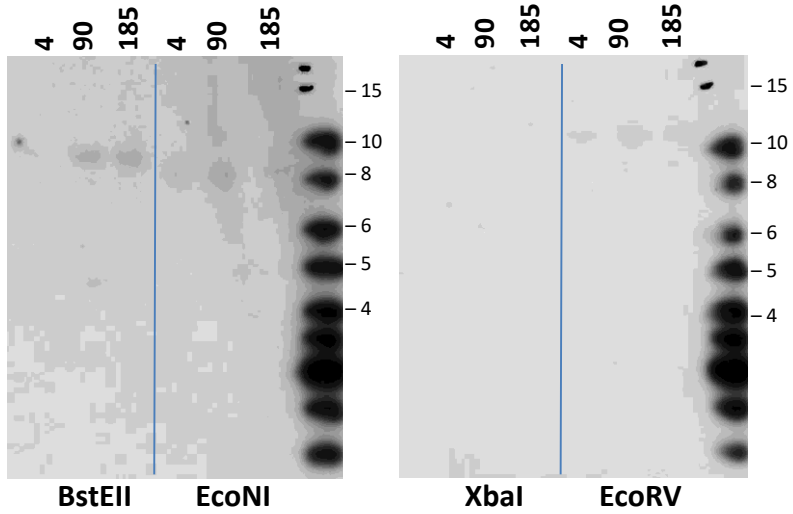
Recombinant ES clones validation by Southern Blot – Internal probe



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.





Hygro probe sequence

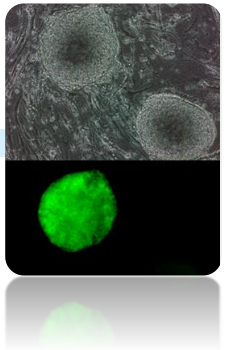
CCTATTGCATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGAC
CTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCGCGGA
GGCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGCG
GGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACA
TGGCGTGATTTCATATGCGCGATTGCTGATCCCCATGTGTATCA
CTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCGC
AGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAA
GTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCT
GACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGG
CGATGTTCGGGGATTCCCAATACGAGGTCGCCAACATCTTCTTC
TGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTT
CGAGCGGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGG
CGTATATGCTCCGCATTGGTC

Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Hygro	5' digest	BstEII	9.9
		EcoNI	9
	3' digest	XbaI	7.8
		EcoRV	12.2

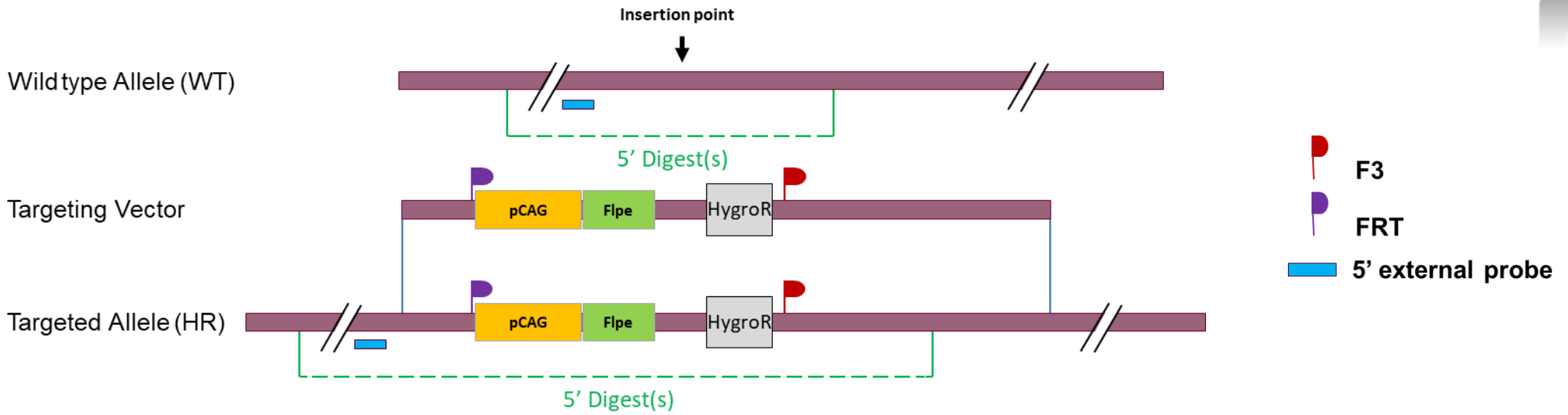
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – External probe

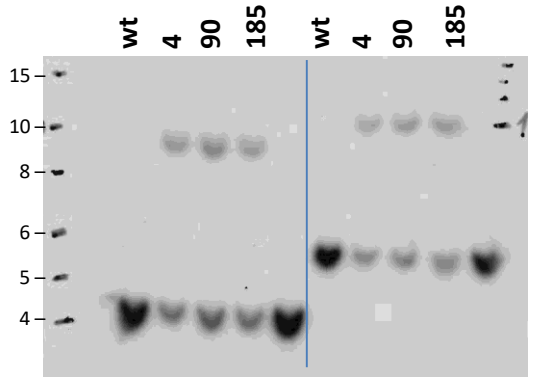


Schematic Southern Blot validation strategy

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



SspI 4.1 / 9.3 AvrII 5.2 / 10.5

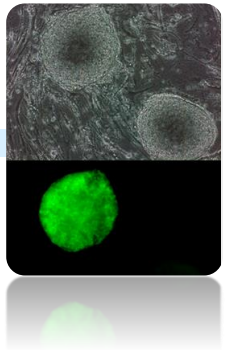
5' probe sequence

TATGTGTATTTTGAGAGCAGGGTTGGGAGGCCTCTCCT
GAAAAGGGTATAAACGTGGAGTAGGCAATACCCAGGCA
AAAAGGGGAGACCAGAGTAGGGGGAGGGGAAGAGTCCT
GACCCAGGGAAGACATTAAAAAGGTAGTGGGGTCGACT
AGATGAAGGAGAGCCTTTCTCTCTGGGCAAGAGCGGTG
CAATGGTGTGTAAAGGTAGCTGAGAAGACGAAAAGGGC
AAGCATCTTCCTGCTACCAGGCTGGGGAGGCCCAGGCC
CACGACCCCGAGGAGAGGGAACGCAGGGAGACTGAGGT
GACCCTTCTTTCCCCCGGGGCCCGGTCGTGTGGTTCGG
TGTCTCTTTTCTGTTGGACCCTTACCTTGACCCAGGC

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	AvrII	5.2	10.5
	5' second digest	SspI	4.1	9.3

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
#4	Pass
#90	**Pass**
#185	Failed

5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a C57BL/6Tac (BD10) mouse strain (which have black coat colour). These cells were injected into blastocysts derived from an BALB/cN strain, which have a white 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 BALB/cN females (Health status SPF Specific Pathogens Free).
- Recombinant ES clone #90 validated in previous project phase was 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
#90	1	0	10	11

Breeding to F1 generation

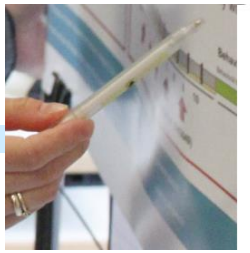
- Four highly chimeric males generated in the previous phase by blastocyst injection of the ES clone #90 were mated with wild-type C57BL/6NTac females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 26/08/2009

- MGI allele ID: MGI:7788436

- Allele nomenclature (following MGI guidelines) : **Gt(ROSA)26Sor$^{tm16(CAG-flpe)Ics}$**

6 SEQUENCE OF THE DELIVERED ALLELE



CTTGGTGCGTTTGCGGGGATGGGCGGCCGCGGCAGGCCCTCCGAGCGTGGTGGAGCCGTTCTGTGAGACAGCCGGGTACGAGTCGTGACGCTGGAAGGGGCAAGCGGGTGGTGGGCAGGAATGCGGTCCGCCCTGCAGCAACCGGAGGGGGAGGGAGAAGGGAGCGGAAAAGTCTCCACCGGACGCGGC
CATGGCTCGGGGGGGGGGGGGCAGCGGAGGAGCGCTTCCGGCCGACGTCTCGTCGCTGATTGGCTTCTTTTCCTCCCGCCGTGTGTGAAAACACAAATGGCGTGTTTTGGTTGGCGTAAGGCGCCTGTCAGTTAACGGCAGCCGGAGTGCGCAGCCGCCGGCAGCCTCGCTCTGCCCACTGGGTGGGGC
GGGAGGTAGGTGGGGTGAGGCGAGCTGGACGTGCGGGCGCGGTCGGCCTCTGGCGGGGCGGGGGAGGGGAGGGAGGGTCAGCGAAAGTAGCTCGCGCGCGAGCGGCCGCCCACCCTCCCCTTCCTCTGGGGGAGTCGTTTTACCCGCCGCCGGCCGGGCCTCGTCGTCTGATTGGCTCTCGGGGCCCAG
AAAACTGGCCCTTGCCATTGGCTCGTGTTCGTCAAGTTGAGTCCATCCGCCGGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTCCGGCCCTCCCCTCGGCCCCGCGCCGCAGAGTCTGGCCGCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGGGGCGGGGACGGGCAGTAGGGCTGAG
CGGCTGCGGGGCGGGTGCAAGCACGTTTCCGACTTGAGTTGCCTCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAAGGAGCGAGGGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTCTGAGTTGTTATCAGTAAGGGAGCTGCAG
TGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCCGGAGGGGGGAGGGGAGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGTGATCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGGAGTCTTCTGGG
CAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTTGTGGCGCGCC**GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC**GTCGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG
CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGG
CGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCT
TCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTG
TGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAA
AGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGC
CGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACC
CCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTC
TAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTCTGAGCCGCCACCATGAGCCAATTTGATATATTATGTAAAACACCACCTAAGGTCCTGGTTCGTCAGTTTGTGGAAAGGTTTGAAAGACC
TTCAGGGGAAAAAATAGCATCATGTGCTGCTGAACTAACCTATTTATGTTGGATGATTACTCATAACGGAACAGCAATCAAGAGAGCCACATTCATGAGCTATAATACTATCATAAGCAATTCGCTGAGTTTCGATATTGTCAACAAATCACTCCAGTTTAAATACAAGACGCAAAAAGCAACAATTCT
GGAAGCCTCATTAAAGAAATTAATTCCTGCTTGGGAATTTACAATTATTCCTTACAATGGACAAAAACATCAATCTGATATCACTGATATTGTAAGTAGTTTGCAATTACAGTTCGAATCATCGGAAGAAGCAGATAAGGGAAATAGCCACAGTAAAAAAATGCTTAAAGCACTTCTAAGTGAGGGTGA
AAGCATCTGGGAGATCACTGAGAAAATACTAAATTCGTTTGAGTATACCTCGAGATTTACAAAAACAAAAACTTTATACCAATTCCTCTTCCTAGCTACTTTCATCAATTGTGGAAGATTCAGCGATATTAAGAACGTTGATCCGAAATCATTTAAATTAGTCCAAAATAAGTATCTGGGAGTAATAAT
CCAGTGTTTAGTGACAGAGACAAAGACAAGCGTTAGTAGGCACATATACTTCTTTAGCGCAAGGGGTAGGATCGATCCACTTGTATATTTGGATGAATTTTTGAGGAACTCTGAACCAGTCCTAAAACGAGTAAATAGGACCGGCAATTCTTCAAGCAACAAACAGGAATACCAATTATTAAAAGATAA
CTTAGTCAGATCGTACAACAAGGCTTTGAAGAAAAATGCGCCTTATCCAATCTTTGCTATAAAGAATGGCCCAAAATCTCACATTGGAAGACATTTGATGACCTCATTTCTGTCAATGAAGGGCCTAACGGAGTTGACTAATGTTGTGGGAAATTGGAGCGATAAGCGTGCTTCTGCCGTGGCCAGGAC
AACGTATACTCATCAGATAACAGCAATACCTGATCACTACTTCGCACTAGTTTCTCGGTACTATGCATATGATCCAATATCAAAGGAAATGATAGCATTGAAGGATGAGACTAATCCAATTGAGGAGTGGCAGCATATAGAACAGCTAAAGGGTAGTGCTGAAGGAAGCATACGATACCCCGCATGGAA
TGGGATAATATCACAGGAGGTACTAGACTACCTTTCATCCTACATAAATAGACGCATAGGACCGGTGGAACAAAAACTTATTTCTGAAGAAGATCTG**TGATACCGGCCGCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATC**
TTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCT<u>AATAAA</u>GGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCA**CTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAA**
CATATGCCATATGCGGCCGGCCGAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCC
ACCTTCTACTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCA
GAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCGATCCAGCCGCCACC**ATGAAAAAGCCTGAACT**
CACCGCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCATC
GGCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGGAATTCAGCGAGAGCCTGACCTATTGCATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGCGG
GTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCACCTCGT
GCACGCGGATTTCGGCTCCAACAATGTCCTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCCCAATACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGCTTGCAGG
ATCGCCGCGGCTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTG
TGTAGAAGTACTCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAGTCGAGAAATTGATGATCTATTAAAC<u>AATAAA</u>GATGTCCACTAAAATGGAAGTTTTTCCTGTCATACTTTGTTAAGAAGGGTGAGAACAGAGTACCTACATTTTGAATGGAAGGATTGGAGCTACG
GGGGTGGGGGTGGGGTGGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGAT**GAAGTTCCTATACTATTTGAAGAATAGGAACTTC**ATCAAGCTTCCTGCAGGGGAATTGAACAGGTGTAAAATTGGAGGGACAAGACTTCCCACAGATTTT
CGGTTTTGTCGGGAAGTTTTTTAATAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGTTTTATGCAGCAAAACTACAGGTTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCGAGGTAGATTAAAGACATGCTCACCCGAGTTTTATACTCTCCTGCTTGAGATCCTTACTA
CAGTATGAAATTACAGTGTCGCGAGTTAGACTATGTAAGCAGAATTTTAATCATTTTTAAAGAGCCCAGTACTTCATATCCATTTCTCCCGCTCCTTCTGCAGCCTTATCAAAAGGTATTTTAGAACACTCATTTTAGCCCCATTTTCATTTATTATACTGGCTTATCCAACCCCTAGACAGAGCATTG
GCATTTTCCCTTTCCTGATCTTAGAAGTCTGATGACTCATGAAACCAGACAGATTAGTTACATACACCACAAA

FRT PCAG Flpe Beta globin pA HygroR F3

EXCELLENCE IN MOUSE PHENOGENOMICS

phenomin

EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2024/12/03

Prepared by Romain LORENTZ, IE

Verified by Marie-Christine BIRLING, PhD

CONTACT US

By email at mutagenesis@igbmc.fr

By phone at +33 (0)3 88 65 57 43

www.phenomin.fr

Genotyping protocol

FRT-pCAG-Flpe-HygroR-F3 RMCE cassette into Rosa

IR00002365 / K500

(ICS internal reference)

This report has been prepared by: **Christelle Roth**
genotyping@igbmc.fr

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
33 (0)3 88 65 57 44
genotyping @igbmc.fr

The first version of this report was generated the: 07 Aug 2014

For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: mutagenesis@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

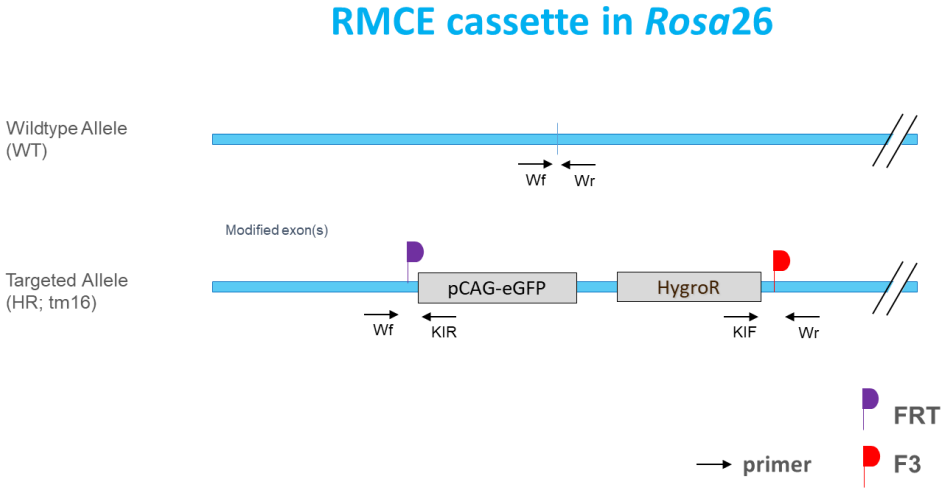
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **FRT-F3 RMCE cassette Rosa** 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	Sequence
Wf	TAAGGGAGCTGCAGTGGAGTAGGCG
Wr	TAGTAAGGATCTCAAGCAGGAGAG
KIF	CCTTATTCCAAGCGGCTTCGGC
KIR	TCCATATATGGGCTATGAACTAATG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	WildType allele
WildType allele specific	Xf / Wr	5684*	484
PCR_5' KI	Xf / KIR	353	---
PCR_3' KI	KIF / Wr	447	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.
Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.