phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of an ubiquitous dual pCAG-Cre-F3-ER^{T2}-F3

Validation of the functionality of Cre-F3-ER^{T2}-F3 (Tam inducible Cre recombinase) and Cre-F3 (constitutive Cre) cassette

Project code: R7b / IR3452

Report updated: 10/02/2023

cnrs
Université de Strasbourg
Instituts thématiques Inserm institut national de la santé et de la recherche médicale
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

MODEL GENERATION TECHNICAL REPORT

1 PROJECT DESCRIPTION

- Aim
- Project process & quality controls

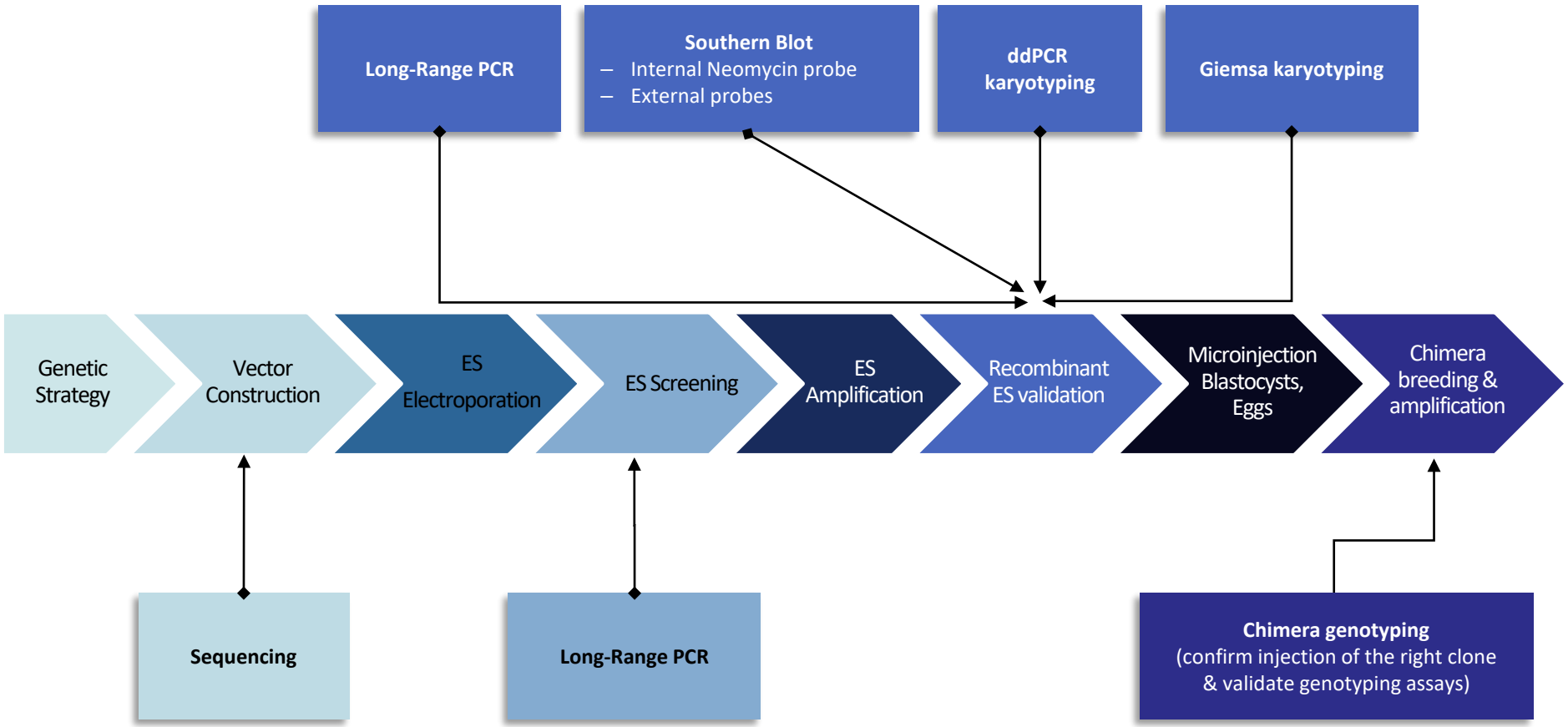
Aim

- The aim of this project was to validated *in vivo* the dual Cre-F3-ER^{T2}-F3 cassette.

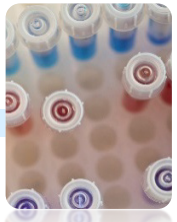
- This cassette was put under a pCAG ubiquitous promoter.

- The generation of a line with dual Cre-F3-ER^{T2}-F3 cassette allows to obtain a 2 in 1 mouse line
  - Before Flp mediated deletion of the F3-ER^{T2}-F3 sequence, the Cre-F3-ER^{T2}-F3 cassette should behave as a CreER^{T2} cassette. The model should be very close to the ubiquitous inducible model generated by Berns' lab (Hameyer e al (2007) Physiol Genomics 31:32-41).
  - After an additional Flp breeding step, the F3-ER^{T2}-F3 sequence will be excised, leaving a single F3 site, this allele should behave like a constitutive Cre line.

Project process & quality controls



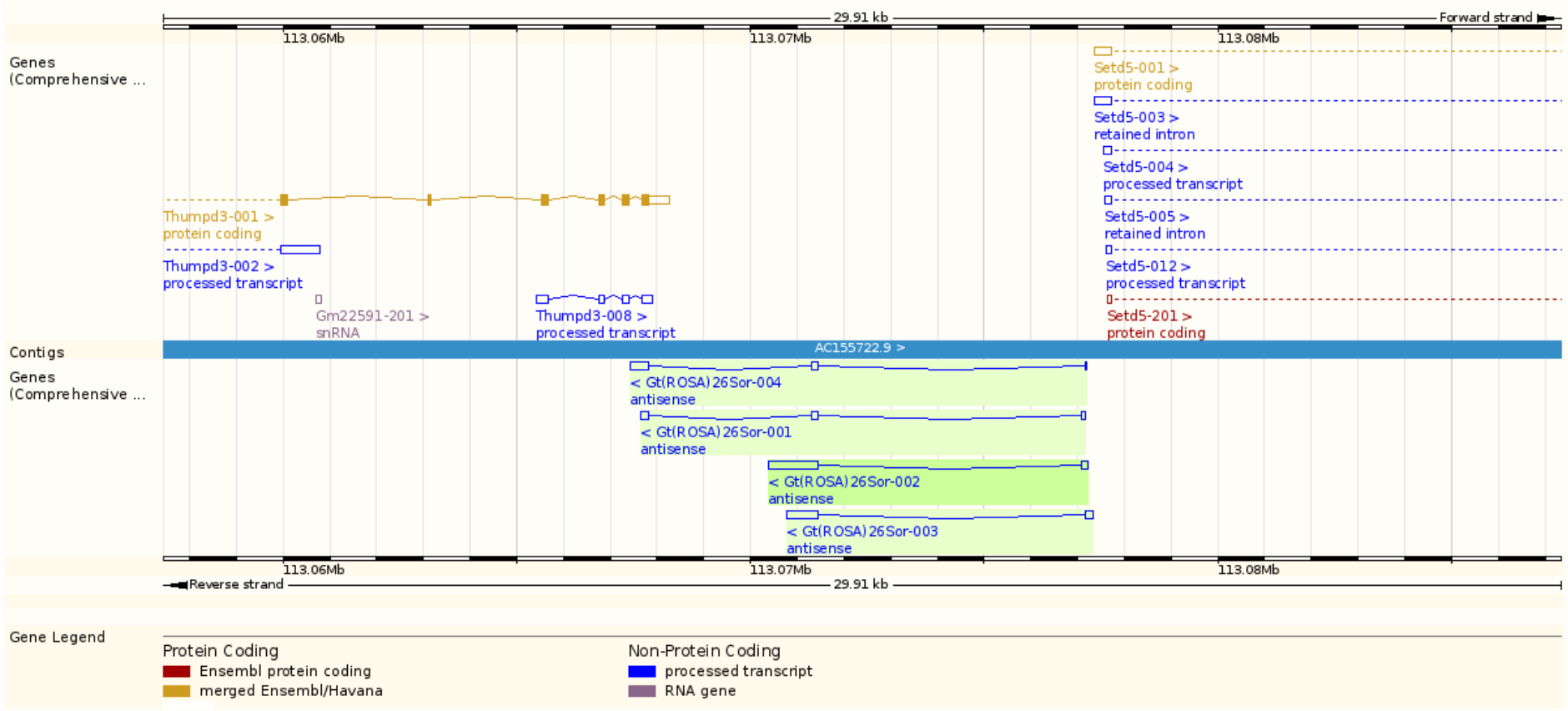
2 GENETIC STRATEGY



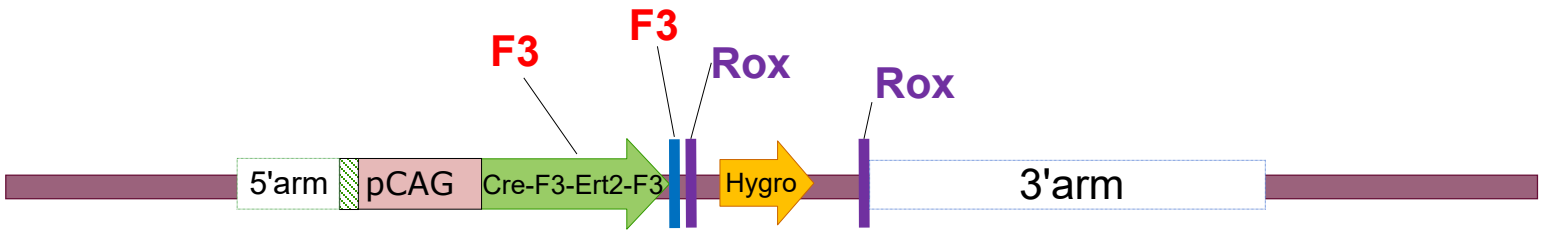
- Target locus structure
- Genetic strategy

mouse genomic locus – structure

Gene: Gt(ROSA)26Sor ENSMUSG00000086429

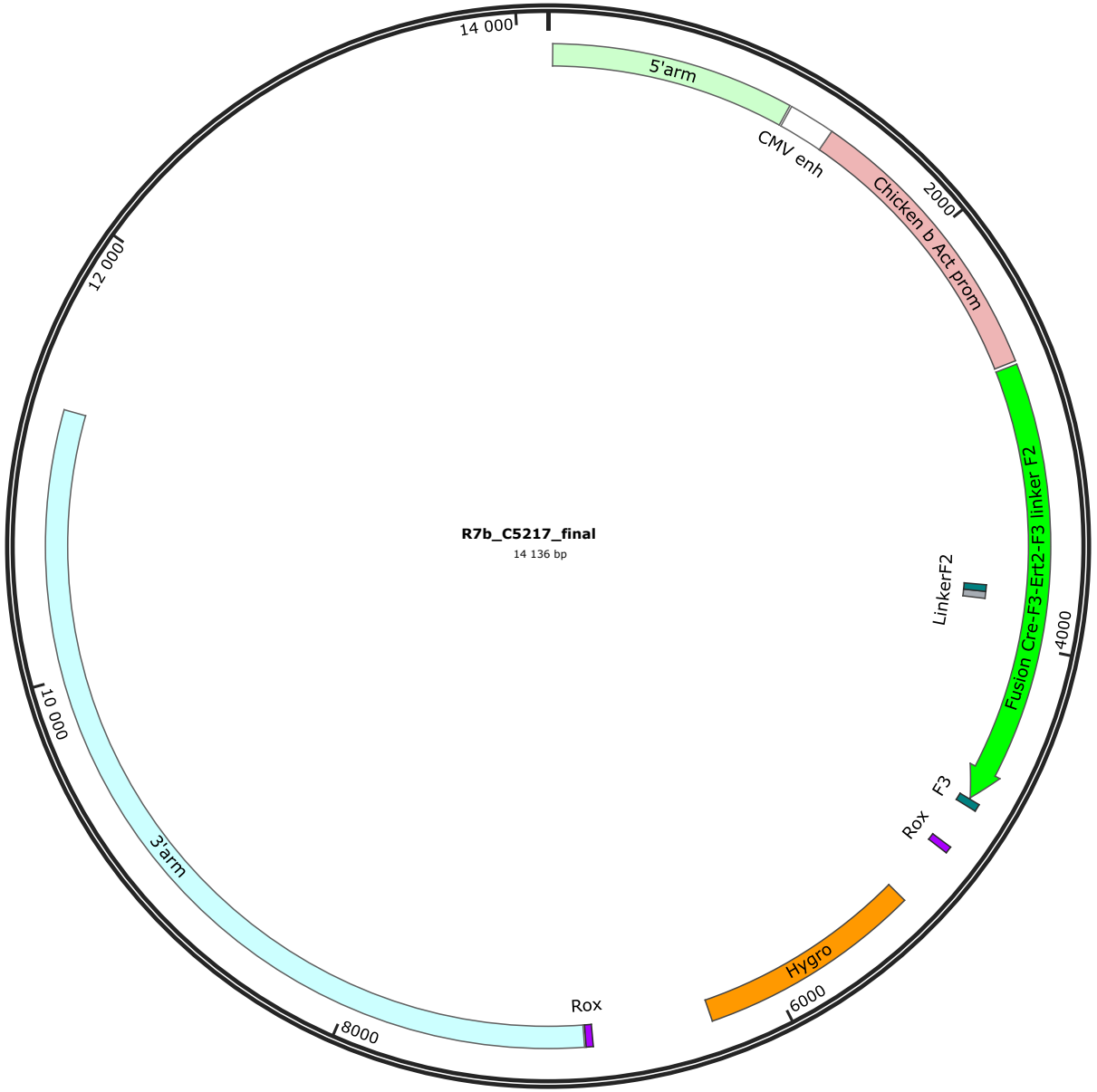


Strategy: knock-in of the pCAG-Cre-F3-ERT2-F3 cassette in Rosa29



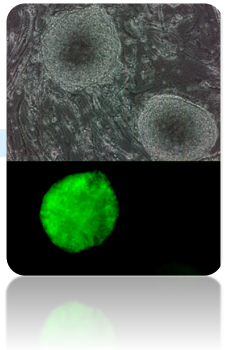
3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION

Created with SnapGene®



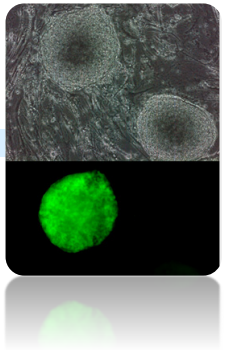
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Recombinant ES validation by Long 5’ and 3’ long range PCRs
- 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



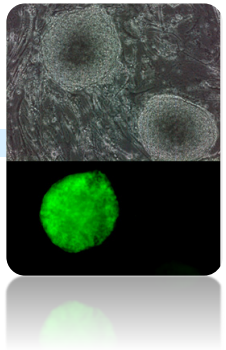
The targeting vector was electroporated in the proprietary BD10 cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 68 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.

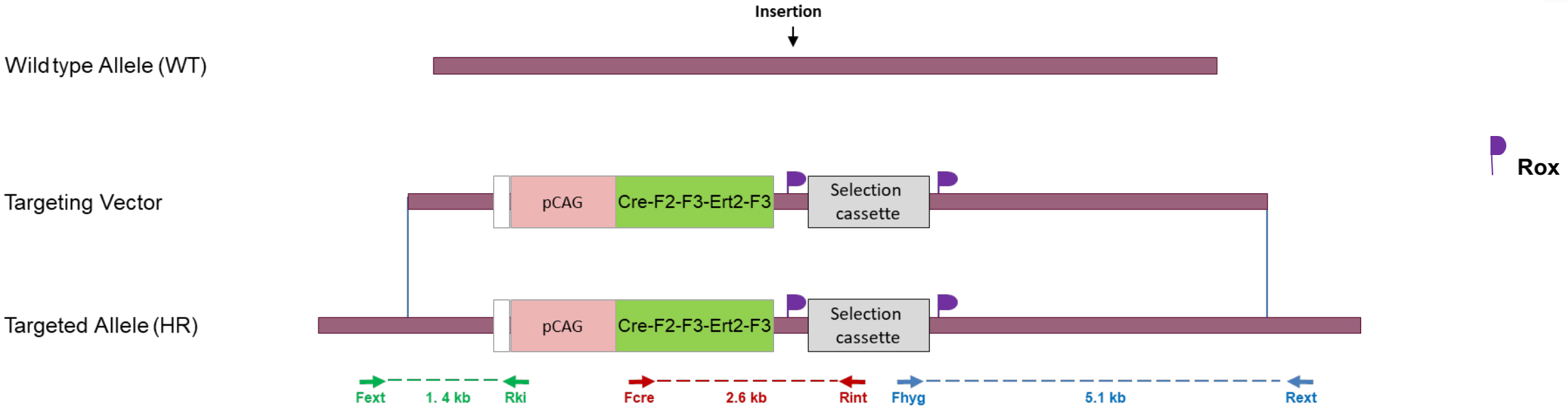
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR (data not shown)
2. Three of 3’ PCR positive clones are confirmed for 5’ 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 ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

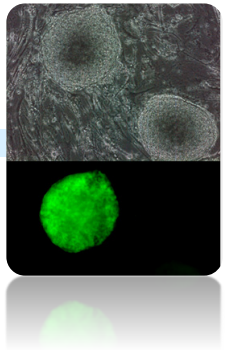


Schematic 5’ and 3’ PCR screening strategy

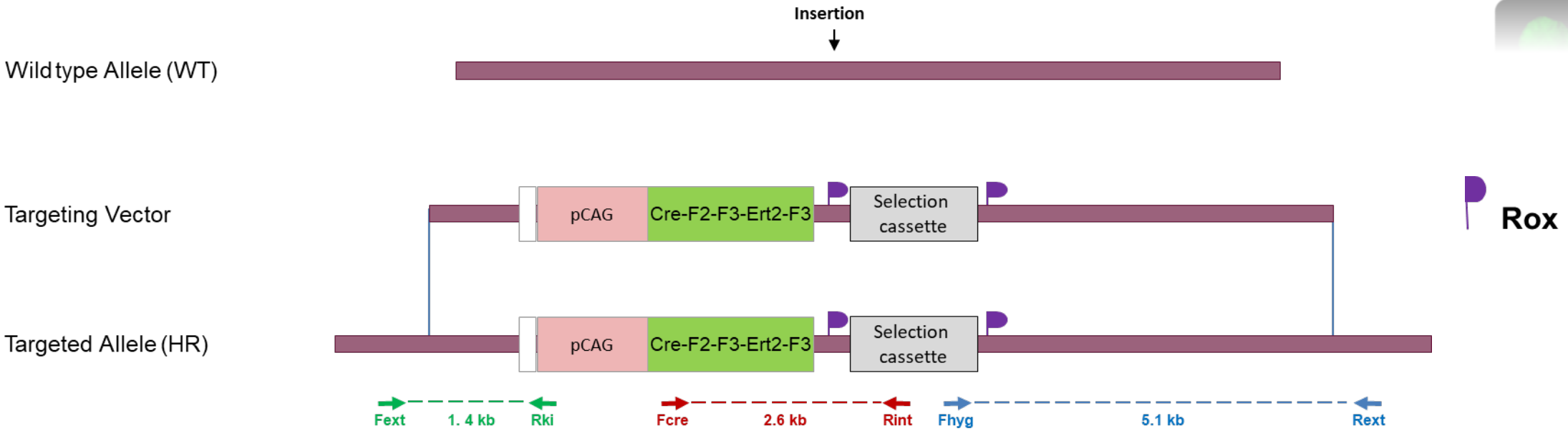


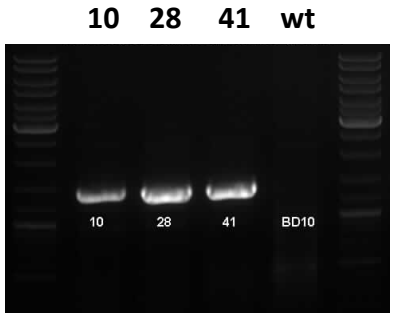
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GGTAGGGGATCGGGACTCTGGCGGG	1.4 kb
	Rki	GGAGAGTGAAGCAGAACGTGGGGCT	
Cre PCR	Fcre	GCCTGCATTACCGGTCGATGCAAC	2.7 kb
	Rint	CGCCGATAGTGGAAACCGACGCCC	
3’ PCR	Fhyg	CTGCATCAGGTCGGAGACGCTGTCG	5.1 kb
	Rext	CTCAGTGGCTCAACAACACTTGGTC	

Recombinant ES validation by Long Range PCR

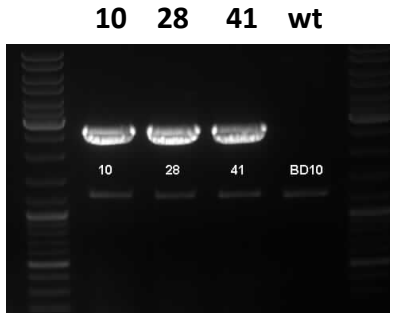


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

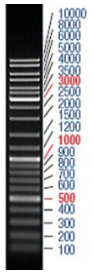




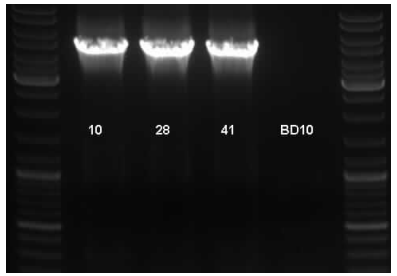
Pcr Fext – Rki : 1.4 kb



Pcr Fcre – Rint : kb



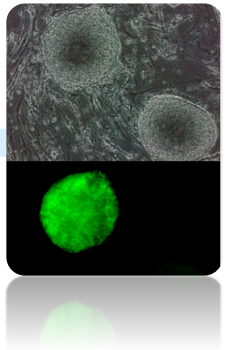
Ladder pattern



Pcr Fhyg – Rext : 5.1 kb

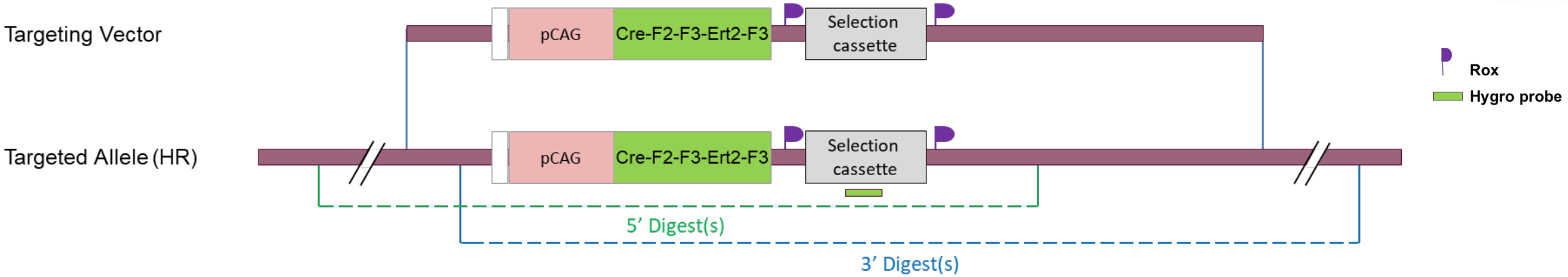
Three candidate clones identified by 3’ screening were further analysed by 3’ and 5’ PCRs screening.
Three clones (clones #10, #28 and #41) were confirmed.

Recombinant ES clones validation by Southern Blot – Internal probe

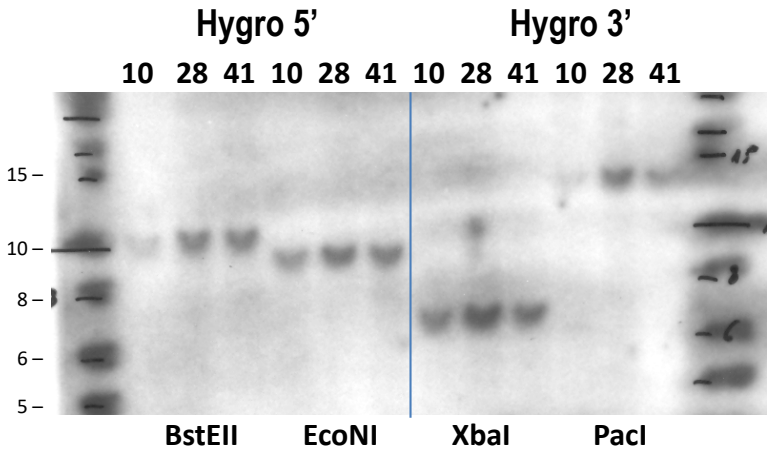


Schematic Southern Blot validation strategy

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.



Southern blot



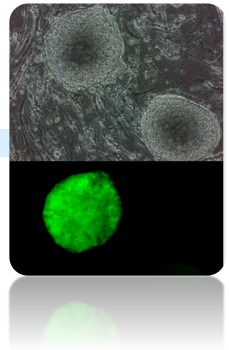
Neo probe sequence

GACCAATGCGGAGCATATACGCCCGGAGCCGCGGCGATCCTGCAAG
CTCCGGATGCCTCCGCTCGAAGTAGCGCGTCTGCTGCTCCATACAA
GCCAACCACGGCCTCCAGAAGAAGATGTTGGCGACCTCGTATTGGG
AATCCCCGAACATCGCCTCGCTCCAGTCAATGACCGCTGTTATGCG
GCCATTGTCCGTCAGGACATTGTTGGAGCCGAAATCCGCGTGCACG
AGGTGCCGGACTTCGGGGCAGTCCTCGGCCCAAAGCATCAGCTCAT
CGAGAGCCTGCGCGACGGACGCACTGACGGTGTCGTCCATCACAGT
TTGCCAGTGATACACATGGGGATCAGCAATCGCGCATATGAAATCA
CGCCATGTAGTGTATTGACCGATTCCTTGCGGTCCGAATGGGCCGA
ACCCGCTCGTCTGGCTAAGATCGGCCGCAGCGATCGCATCCATGGC
CTCCGCGACCGGCTGCAGAACAGCGGGCAGTTCGGTTTCAGGCAGG
TCTTGCAACGTGACACCCTGTGCACGGCGGGAGATGCAATAGG

Digestions used to validate the 5' and 3' insertion

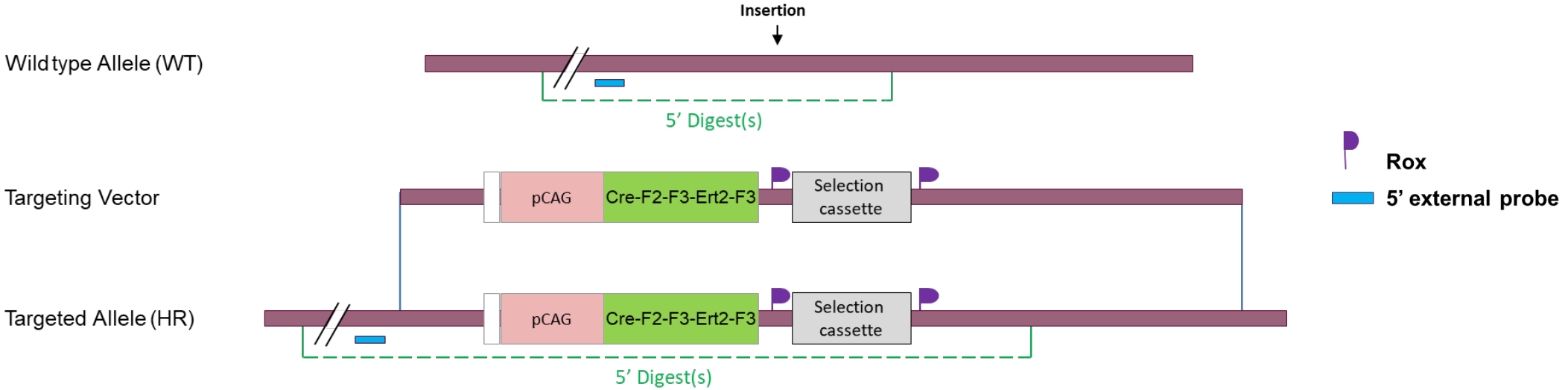
Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	BstEII	10.5
		EcoNI	9.6
	3' digest	XbaI	7.1
		PacI	13.7

Recombinant ES clones validation by Southern Blot – External probe

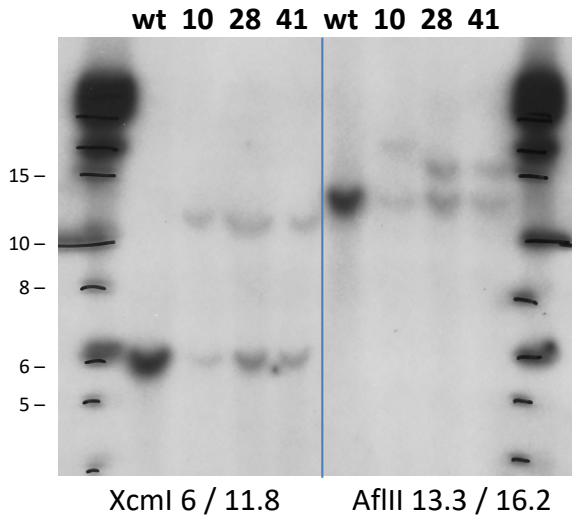


Schematic Southern Blot validation strategy

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



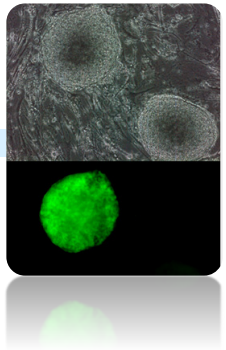
5' probe sequence

TATGTGTATTTTGAGAGCAGGGTTGGGAGG
CCTCTCCTGAAAAGGGTATAAACGTGGAGT
AGGCAATACCCAGGCAAAAAGGGGAGACCA
GAGTAGGGGGAGGGGAAGAGTCCTGACCCA
GGGAAGACATTAAAAAGGTAGTGGGGTCGA
CTAGATGAAGGAGAGCCTTTCTCTCTGGGC
AAGAGCGGTGCAATGGTGTGTAAAGGTAGC
TGAGAAGACGAAAAGGGCAAGCATCTTCCT
GCTACCAGGCTGGGGAGGCCCAGGCCCACG
ACCCCGAGGAGAGGGAACGCAGGGAGACTG
AGGTGACCCTTCTTTCCCCCGGGGCCCGGT
CGTGTGGTTCGGTGTCTCTTTTCTGTTGGA
CCCTTACCTTGACCCAGGC

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	XcmI	6	11.8
	5' second digest	AflII	13.3	16.2

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
#28	Failed
#41	**Pass**

5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a BD10 mouse strain (C57BL/6NTac genetic background, 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 clones #41 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
#41	3	2	1	6

Breeding to F1 generation

- Three highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with C57BL/6NCrl Dre deleter females (MGI: 6467222; with 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 01/10/2013

- Allele nomenclature (following MGI guidelines) : Gt(ROSA)26Sor$^{tm5.1(CAG-cre/ERT2)Ics}$

6 SEQUENCE OF THE DUAL ALLELE (before Flp excision of the F3-ER^{T2}-F3 cassette)

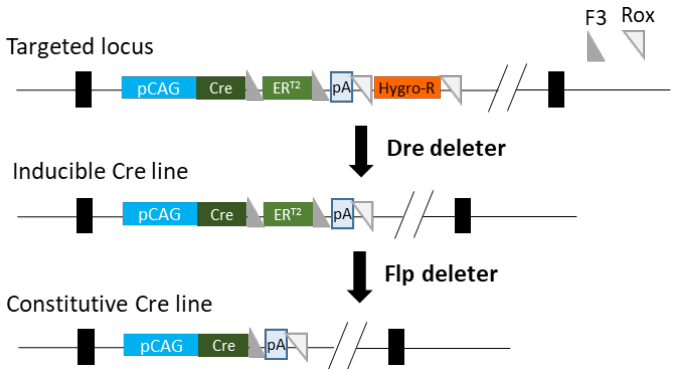
TCCATCCGCCGGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTCCGGCCCTCCCCTCGGCCCCGCGCCGCAGAGTCTGGCCGCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGGGGCGGGGACGGGCAGTAGGGCTGAGCGGCTGCGGGG
CGGGTGCAAGCACGTTTCCGACTTGAGTTGCCTCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAAGGAGCGAGGGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTCTGAGTTGTTAT
CAGTAAGGGAGCTGCAGTGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCCGGAGGGGGGAGGGGAGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGTGAT
CTGCAACTCCAGTCTTTCTGGCCGGCCCGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCAT
TATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGT
GCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGC
CCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGC
GCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGA
GCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGG
CGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCG
GGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC
GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGC
AGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGGCCGGCCGCCGCCACCATGTCCAATTTACTGA
CCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAA
CCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCAC
TGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACGTAATCTGGCATTTCTGGGGATTGCTTATAA
CACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACTTAGCCTGGGGGTAACTAAACTGGTCGAGC
GATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGAT
GACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAA
ACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATCTGAAGTTCCTATACTATTTGAAGAATAGGAACTTCGCATCTCCATCTAATCCAGGTGCTTCTAAGGGATCTTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAAC
GCTCTAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCT
GGTTCACATGATCAACTGGGCGAAGAGGGTGCCAGGCTTTGTGGATTTGACCCTCCATGATCAGGTCCACCTTCTAGAATGTGCCTGGCTAGAGATCCTGATGATTGGTCTCGTCTGGCGCTCCATGGAGCACCCAGTGAAGCTACTGTTTGCTCCTAACTTGC
TCTTGGACAGGAACCAGGGAAAATGTGTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGCTACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGAGGAGTTTGTGTGCCTCAAATCTATTATTTTGCTTAATTCTGGAGTGTACACATTTCTGTCC
AGCACCCTGAAGTCTCTGGAAGAGAAGGACCATATCCACCGAGTCCTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAGGCAGGCCTGACCCTGCAGCAGCAGCACCAGCGGCTGGCCCAGCTCCTCCTCATCCTCTCCCACATCAGGCACATGA
GTAACAAAGGCATGGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCCCTCTATGACCTGCTGCTGGAGGCGGCGGACGCCCACCGCCTACATGCGCCCACTAGCCGTGGAGGGGCATCCGTGGAGGAGACGGACCAAAGCCACTTGGCCACTGC
GGGCTCTACTTCATCGCATTCCTTGCAAAAGTATTACATCACGGGGGAGGCAGAGGGTTTCCCTGCCACAGTCTGATGAGAAGTTCCTATACTATTTGAAGAATAGGAACTTCTGAGCTCCCTGGCGGAATTCGGATCCAGATCTTATTAAAGCAGAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGTCGACACCGGTGCCTGCAGGTAACTTTAAATAATTGGCATTAT
TTTAAAGTTACCTGCAGGAAGATGGGCGGGAGTCTTCTGGGCAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTTGTCCTTAAGGGGAATTGAACAGGTGTAAAATTGGAGGGACAAGACTTCCCACAGATTTTCGGTTTTGTCGGGAAGTTTTTTAATAGGGG
CAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGTTTTATGCAGCAAAACTACAGGTTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCGAGGTAGATTAAAGACATGCTCACCCGAGTTTTATACTCTCCTGCTTGAGATCCTTACTACAGTAT
GAAATTACAGTGTCGCGAGTTAGACTATGTAAGCAGAATTTTAATCATTTTTAAAGAGCCCAGTACTTCATATCCATTTCTCCCGCTCCTTCTGCAGCCTTATCAAAAGGTATTTTAGAACACTCATTTTAGCCCCATTTTCATTTATTATACTGGCTTATCCAACCC
CTAGACAGAGCATTGGCATTT

CMV enh pCAG Cre F3 F2 linker Ert2 Rox

Functional validation of both inducible and non inducible Cre cassette

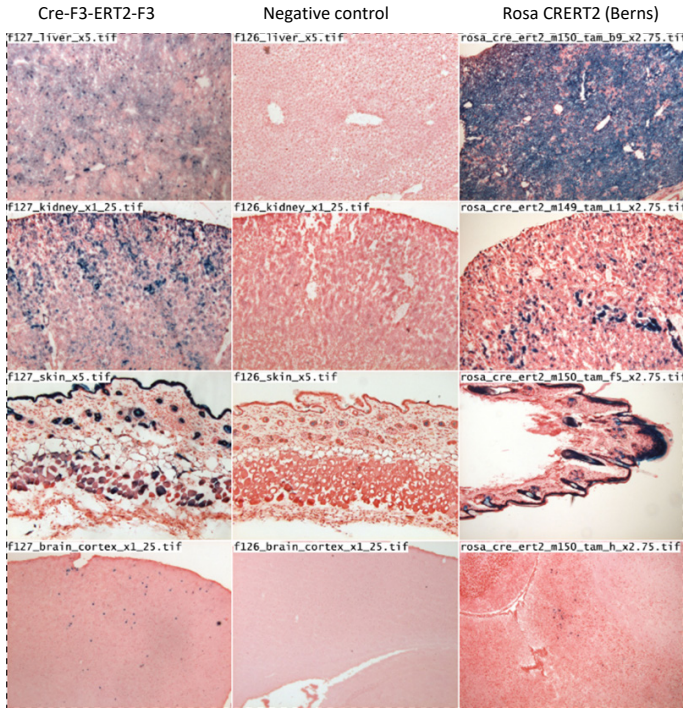
Validation of a CreER^{T2} cassette allowing to obtain two lines in one :

- CreER^{T2} inducible using Tamoxifen
- Constitutive (Cre) after crossing with a deleting Flp line

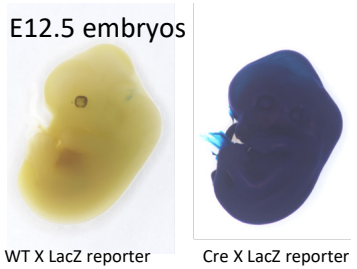


Cre-F3-ERT2-F3 Contrôle négatif Rosa CRERT2 (Berns)

Inducible Cre

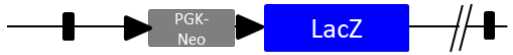


Constitutive Cre



WT X LacZ reporter Cre X LacZ reporter

Reporter from Soriano (1999) **Generalized *lacZ* expression with the ROSA26 Cre reporter strain**
Nat Genetics 21, 70-71



- Same level of efficiency than the CreERT2 line from Berns lab
- Ten existing lines with this cassette
- Including seven lines funded by PHENOMIN

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

phenomin

EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/02/10

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 56 57

www.phenomin.fr