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MODEL GENERATION TECHNICAL REPORT

Targeted transgenesis of pCAG-Cre-2A-Flpo-2A-eGFP in HPRT

Project code: R5 / IR3160

Report updated: 07/02/2023

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MODEL GENERATION TECHNICAL REPORT

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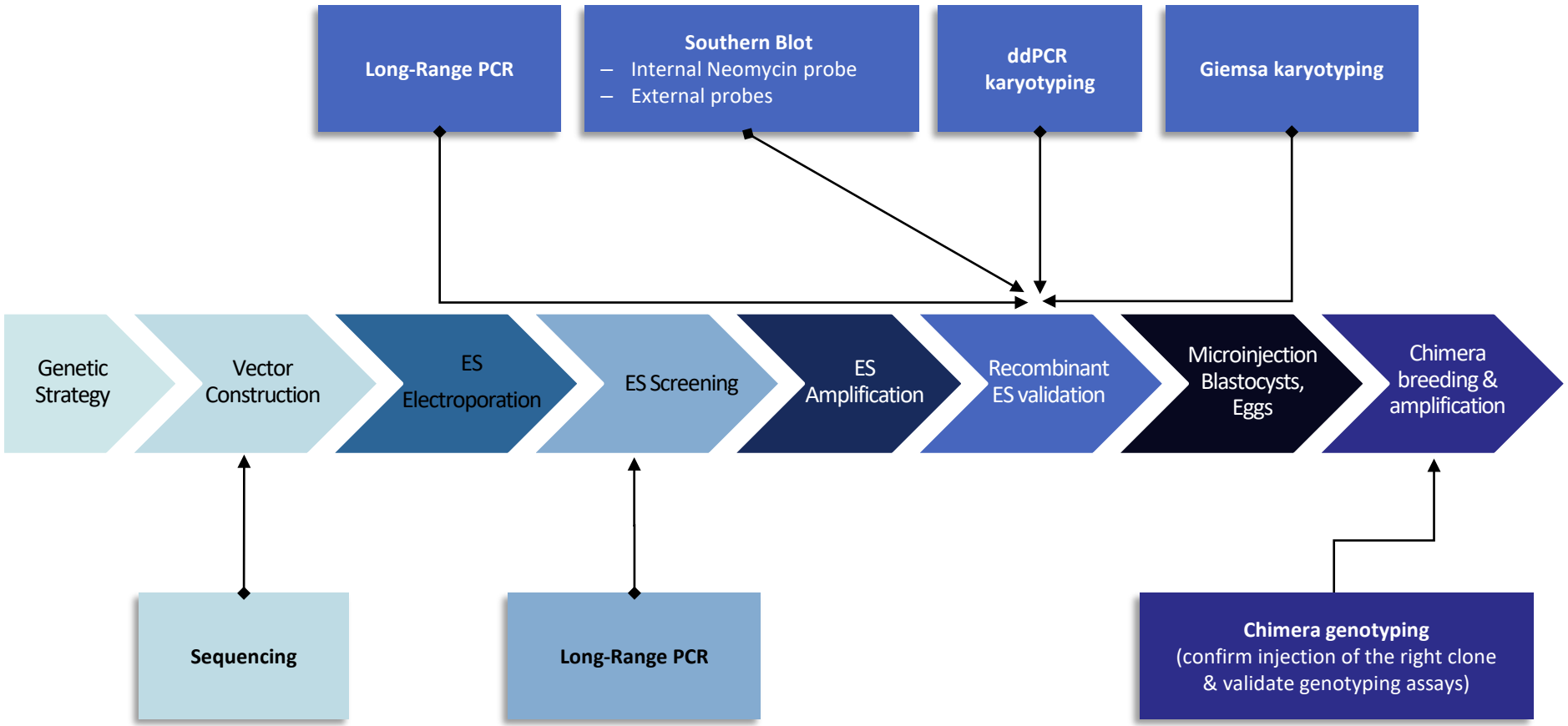
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

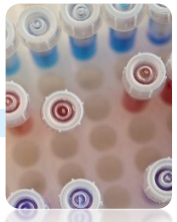
5 MICROINJECTION & BREEDING

6 SEQUENCE OF THE DELIVERED ALLELE

Project process & quality controls

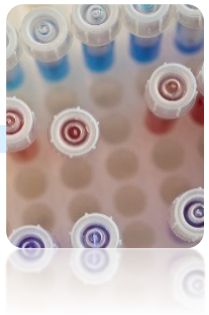


2 GENETIC STRATEGY



- Target locus structure
- Genetic strategy

HPRT mouse genomic locus – structure



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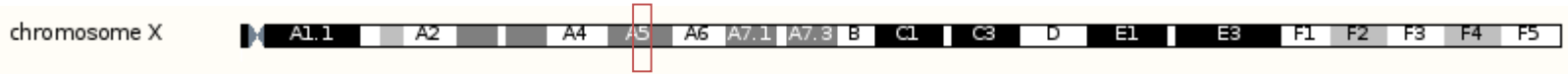

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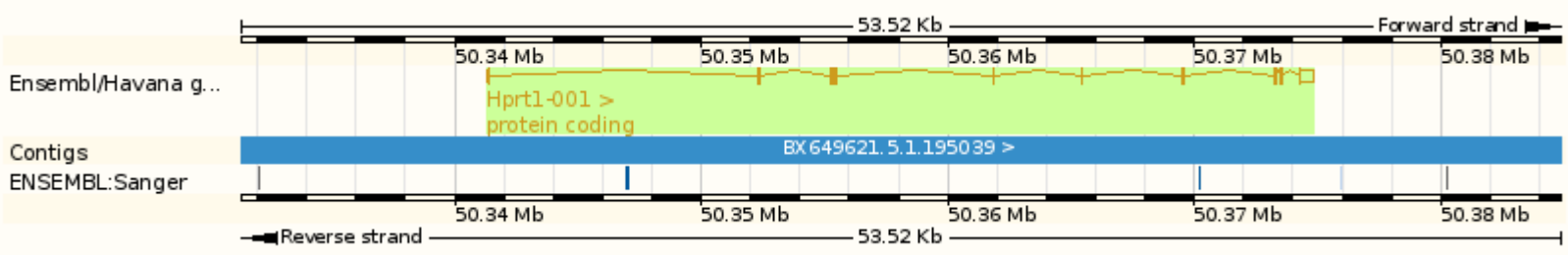
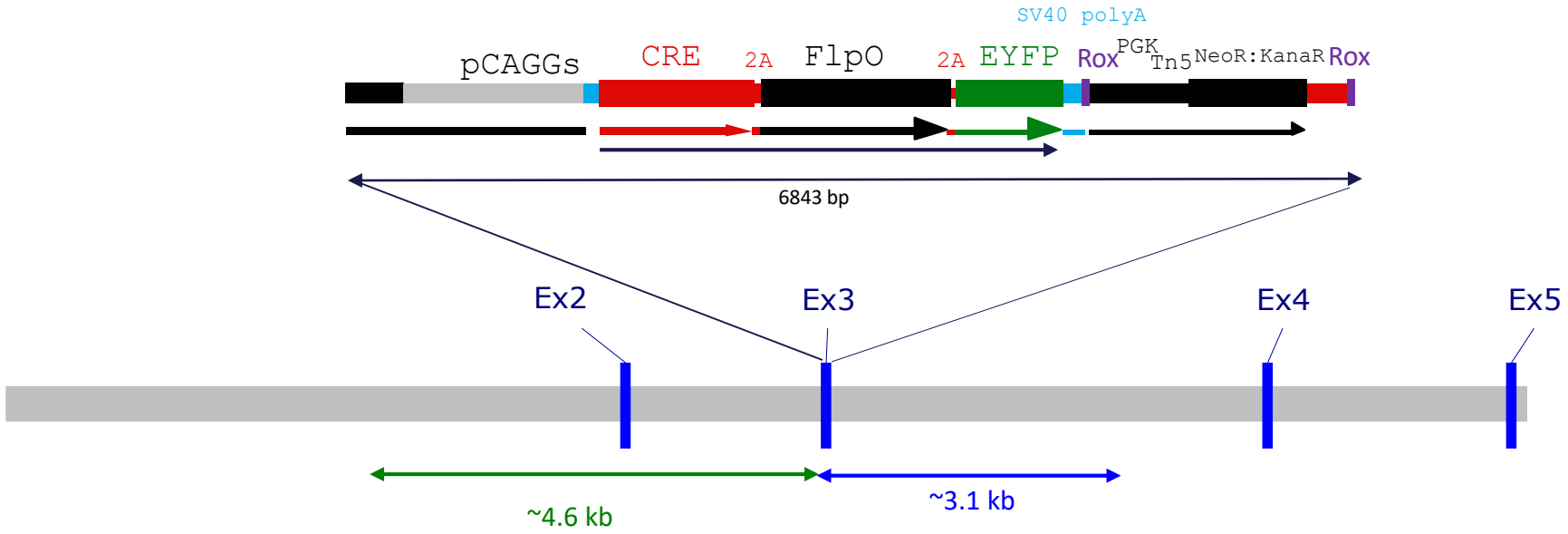
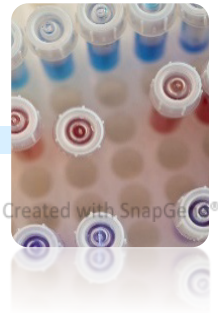
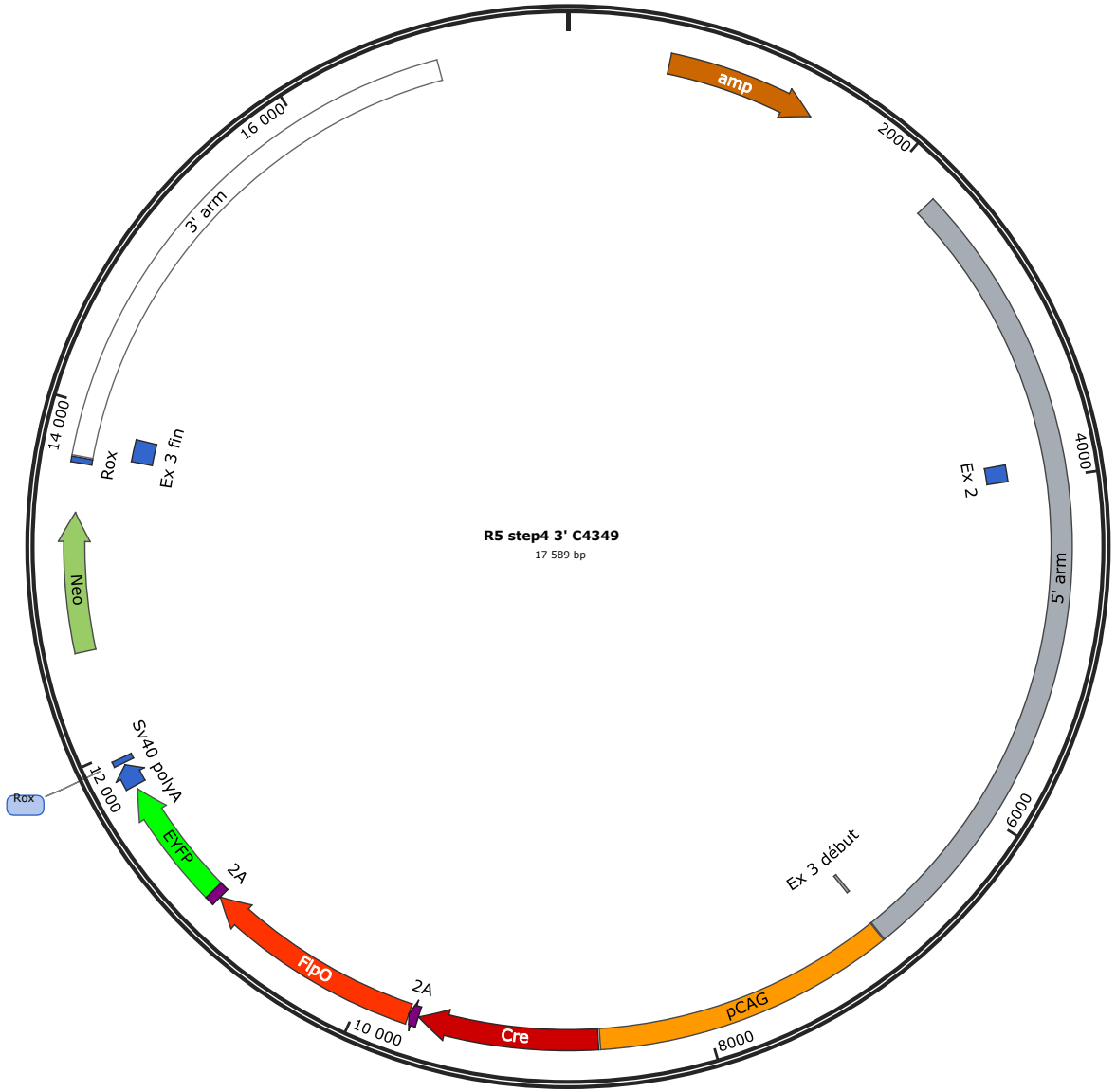


Strategy: targeted transgenesis of pCAG-CRE-2A-Flpo-2A-eYFP in HPRT



3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION





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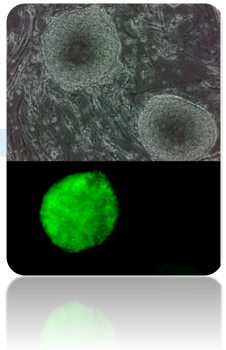
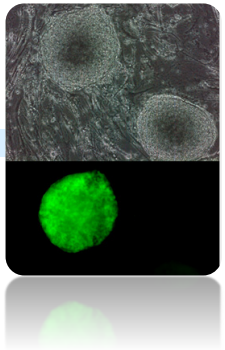
4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- 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



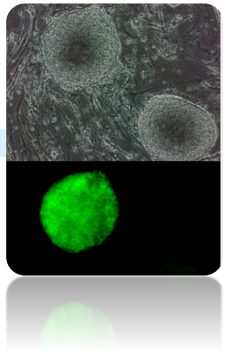
The targeting vector was electroporated in the proprietary BALB/N F122 embryonic stem cell (ESCs) line.

Transfected ES clones were submitted to neomycin selection (G418) and 47 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.

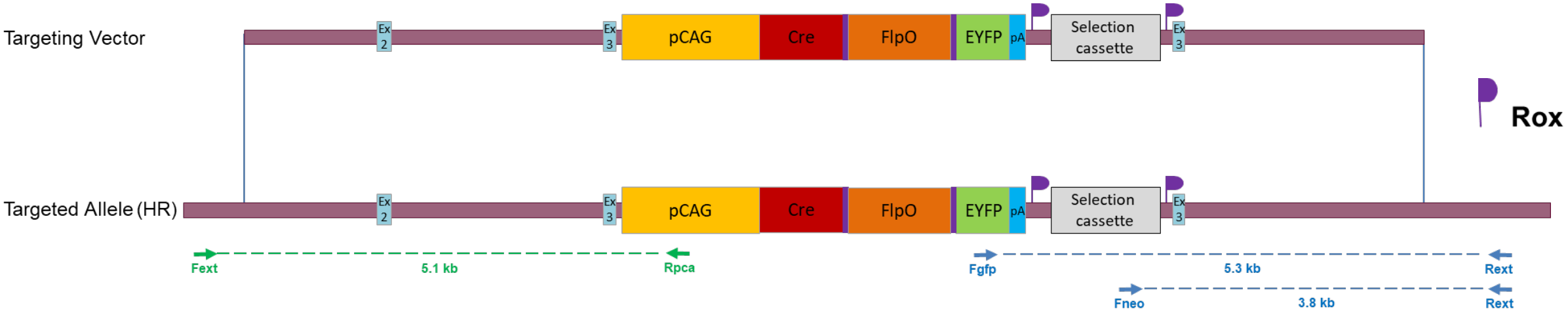
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Two of 3’ PCR positive clones were confirmed for 5’ recombination event by Long-Range PCR
3. Positive clones in step 2 were further validated by Southern blot analysis using internal and external probes
4. The karyotype of the validated clone was verified using Giemsa staining

Long range PCR screening – strategy

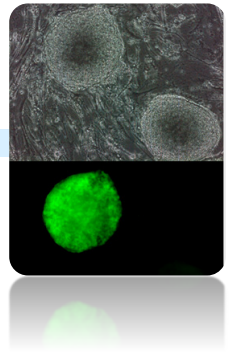


Schematic 5’ and 3’ PCR screening strategy

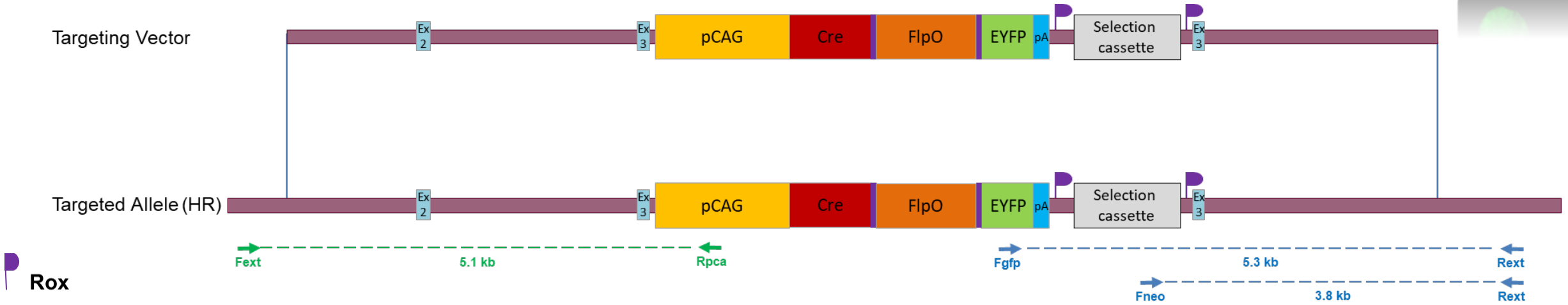


PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GAGTTTTAGGCCAGTTTAGGATCCA	5.1 kb
	Rpca	GCAGAACGTGGGGCTCACCTCGACC	
3’ PCR	Fgfp	CCCGTGCTGCTGCCCGACAACCACT	5.3 kb
	Rext	AATATGGCCGGCCTCTAAATAGAAACAAGAGCTCAGG	
3’ PCR	ADB296	AGGGGCTCGCGCCAGCCGAACTGTT	3.8 kb
	Rext	AATATGGCCGGCCTCTAAATAGAAACAAGAGCTCAGG	

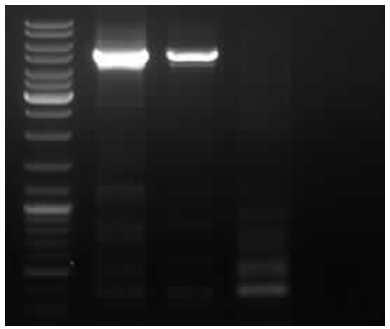
Recombinant ES validation by Long Range PCR



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



46 47 wt tris



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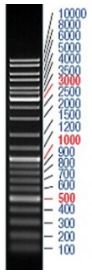
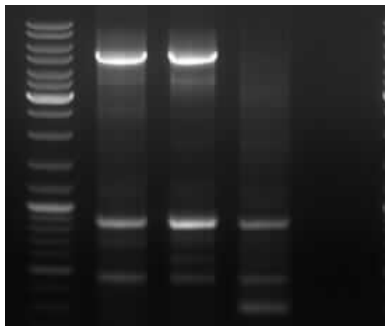


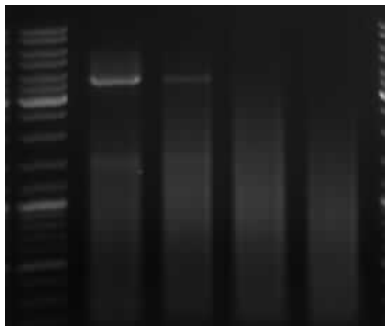
Ladder pattern

46 47 wt tris



Pcr Fgfp – Rext : 5.3 kb

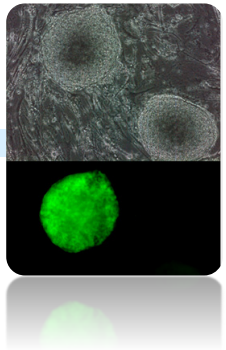
46 47 wt tris



Pcr Fneo – Rext : 3.8 kb

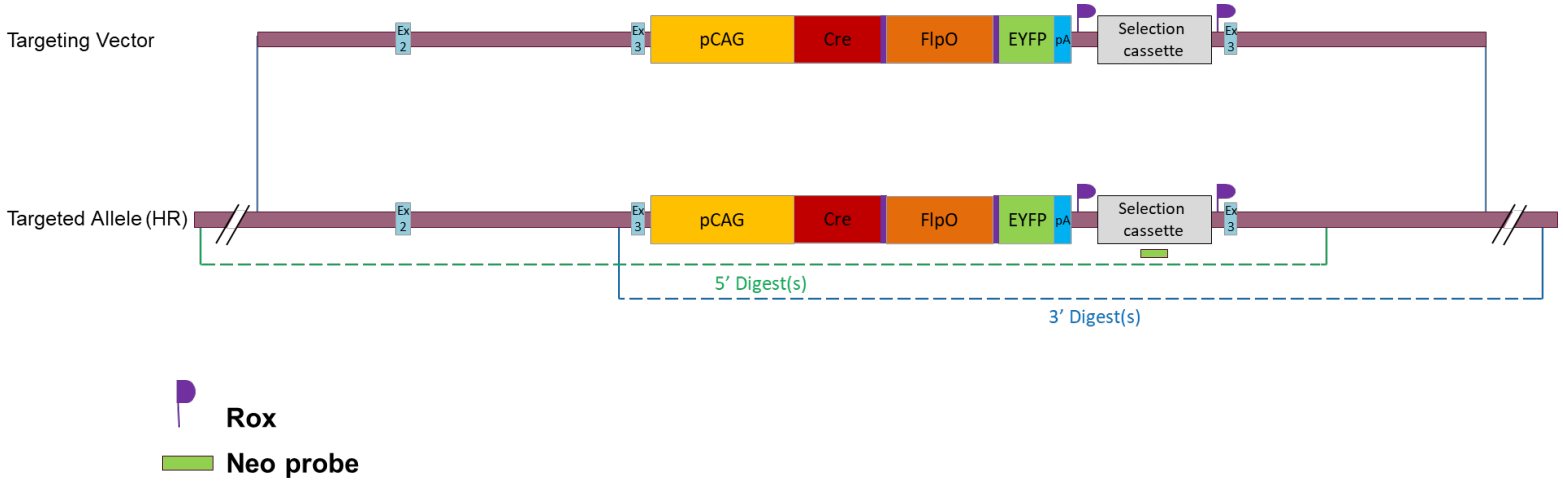
Two candidate clones were analysed by 5’ and 3’PCRs screening.
Two clones (clones #46 and #47) were confirmed.

Recombinant ES clones validation by Southern Blot– Internal probe



Schematic Southern Blot validation strategy

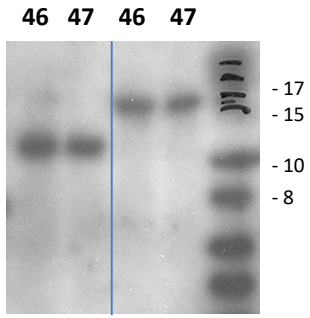
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



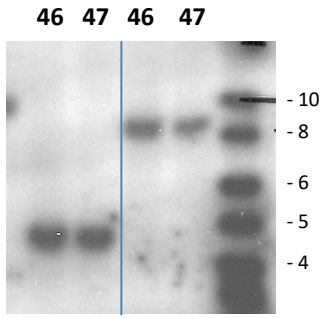
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	SacI	12
		AvrII / KpnI	18
	3' digest	BglII	4.9
		XmnI	8.9

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

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ACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCT
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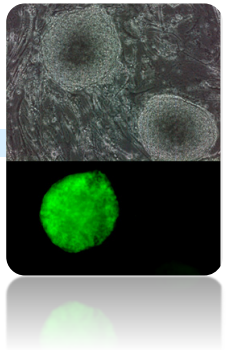
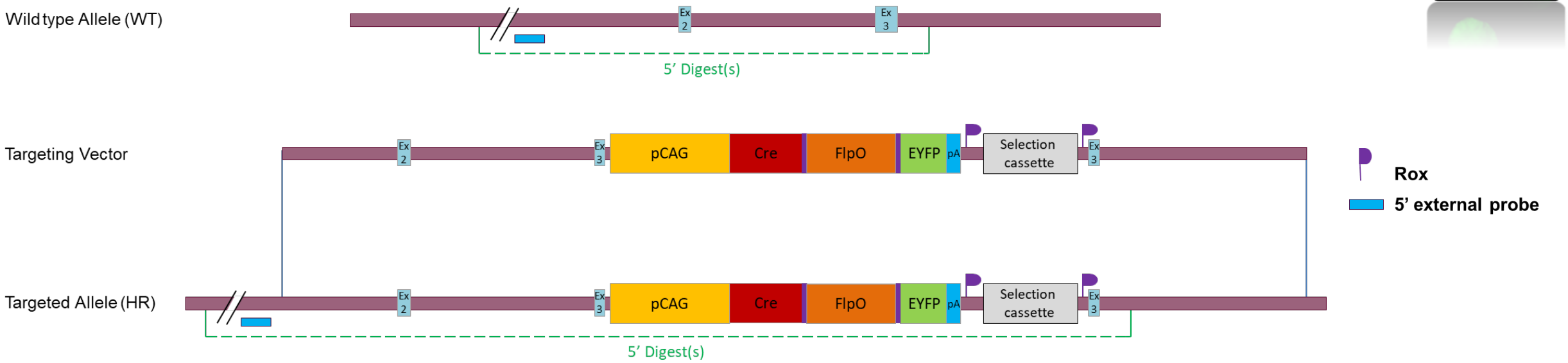
Recombinant ES clones validation by Southern Blot– External probe

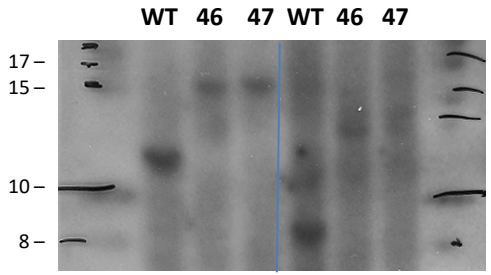


Schematic Southern Blot validation strategy

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



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	EcoNI	12	18.8
	5' second digest	ApaLI	7.1	13.9

5' PROBE SEQUENCE

GGCAGAGTTCACATTGGATTTGTCTTTAATTGAAACAGTCTTTGAAGTTCTTAG
GTAGGTTACCTGTAGAACCTGTAACCAGGCATTGAGCTATTTTAGTAGGTGCAG
TAGTCTGATGAAACCTGGGTGTGATAGGCTTAAGGCAGAAACAGAGTGAGTGGG
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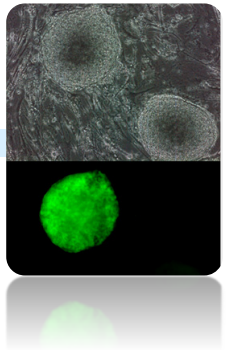
Aneuploidy screening in ES recombinant clones



Selected recombinant ES cells clones were karyotyped by ddPCR as described in Codner *et al.* [1] and by Giemsa metaphase staining. Results of aneuploidy analysis are presented in the table below.

Clone ID	ddPCR	Giemsa
#46	**Pass**	**Pass**
#47	Pass	Pass

[1] Codner, G.F., Lindner, L., Caulder, A., Wattenhofer-Donzé, M., Radage, A., Mertz, A., Eisenmann, B., Mianné, J., Evans, E.P., Beechey, C.V., Fray, M.D., Birling, M.-C., Hérault, Y., Pavlovic, G., Teboul, L
Aneuploidy screening of embryonic stem cell clones by metaphase karyotyping and droplet digital polymerase chain reaction.
BMC Cell Biology 2016 **doi:10.1186/s12860-016-0108-6**

5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a BALB/cN mouse strain (which have white 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 #46 and #47 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
#46	0	1	2	3
#47	0	0	1	1

Breeding to F1 generation

- Three highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with wild-type BALB/cN 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: 25/12/2012

- Allele nomenclature (following MGI guidelines) : **BALB/cN Hprt$^{tm1(pCAG-Cre-Flpo-EYFP)Ics}$**

6 SEQUENCE OF THE DELIVERED ALLELE

LLOYD'S REGISTER · LRQA
ISO 9001 · NFX 50-900

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AGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCAT
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pCAG Cre 2A FlpO EYPF Rox Sv40 polyA

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