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EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of Dyrk1a cKo mouse model

Project code: G8/ IR00003721

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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 PROCESS & QUALITY CONTROLS

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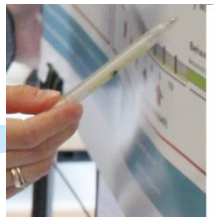
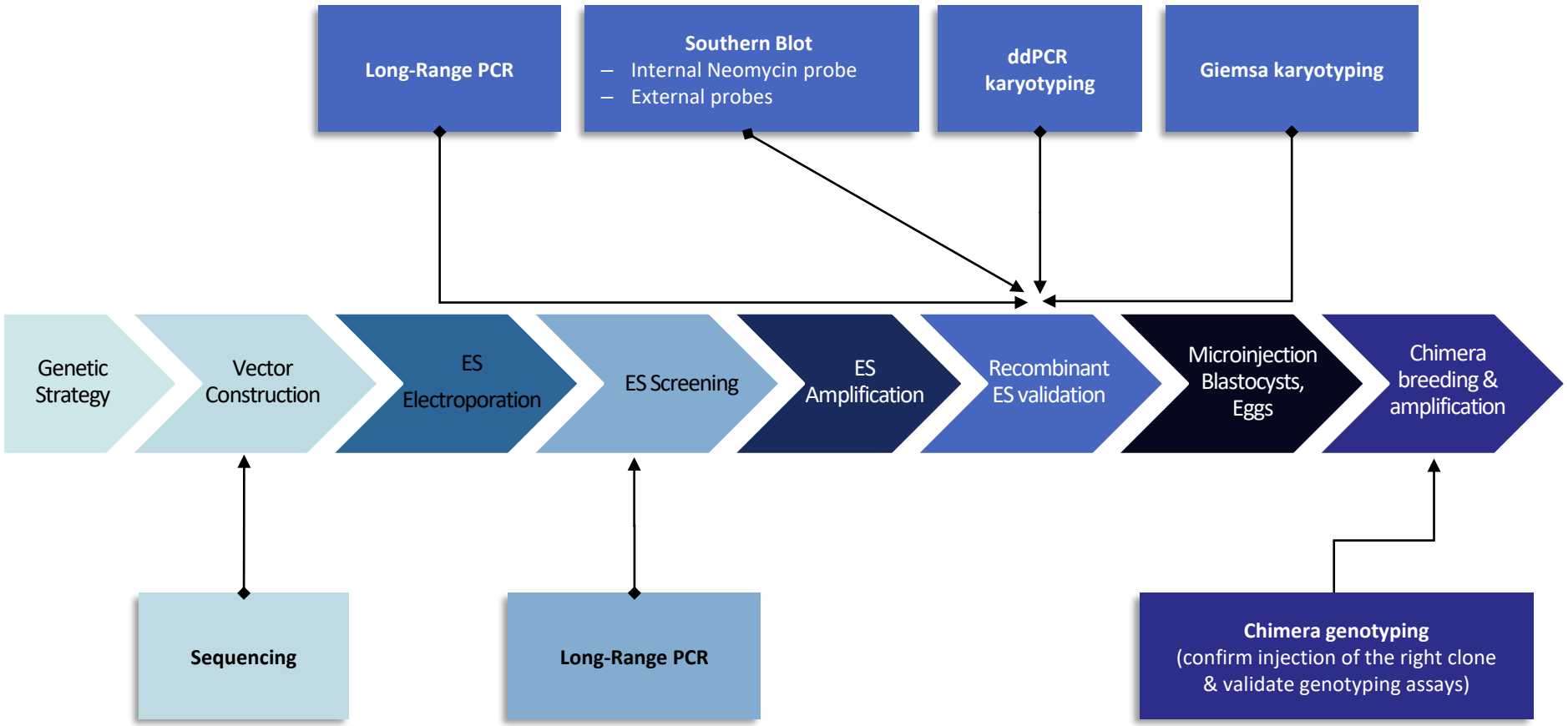
5 MICROINJECTION & BREEDING

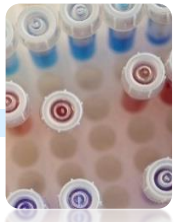
6 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROLS



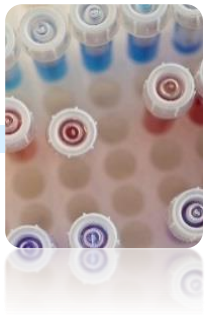


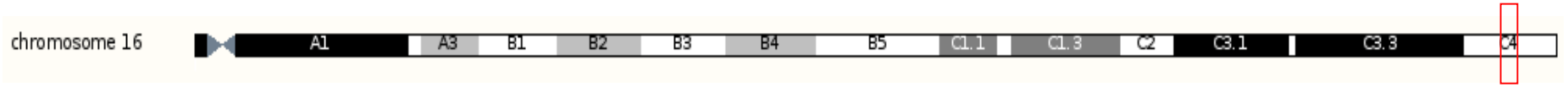
2 GENETIC STRATEGY



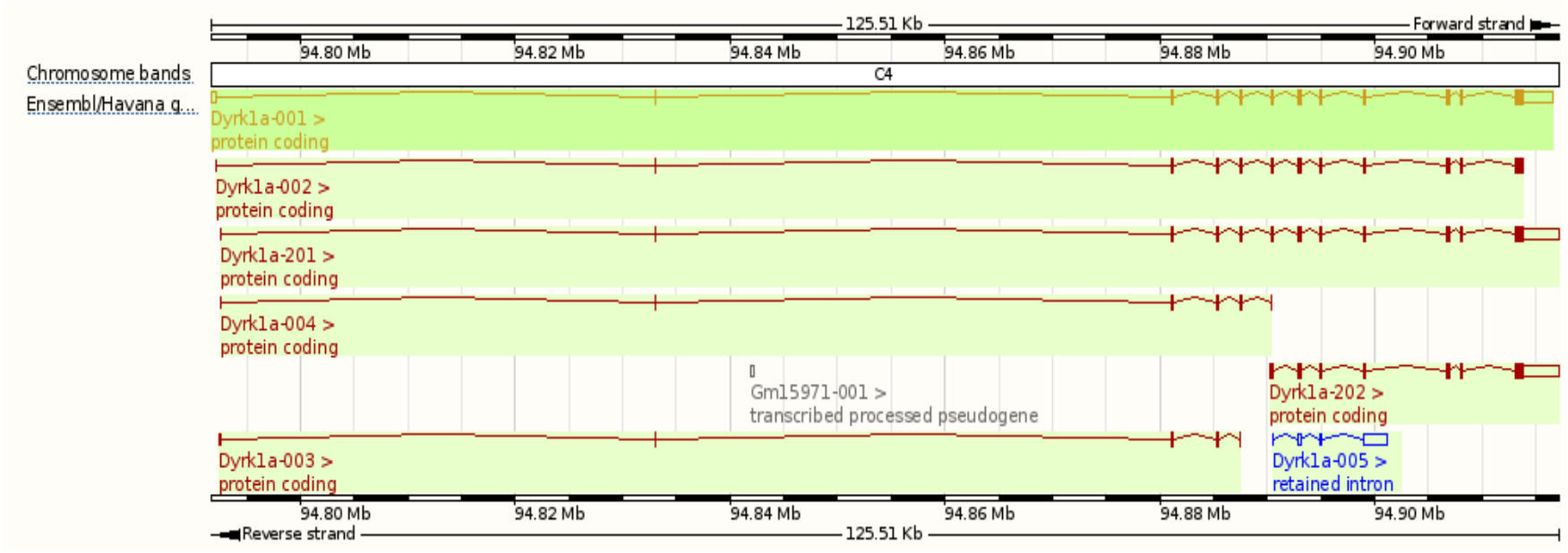
- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

Dyrk1a mouse genomic locus – structure

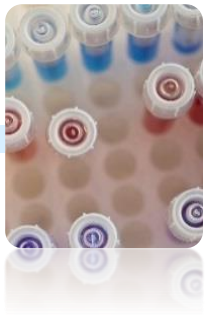




Ensembl Gene ID: ENSMUSG00000022897

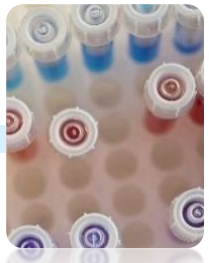


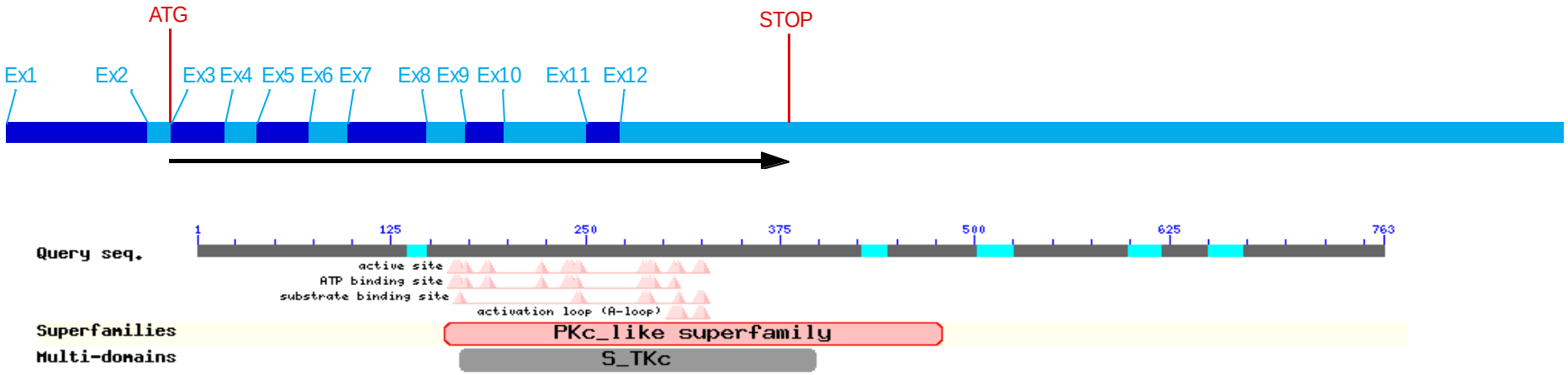
Dyrk1a mRNAs and proteins



Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
Dyrk1a-001	ENSMUST00000119878	5759	ENSMUSP00000113660	763	Protein coding	CCDS28350
Dyrk1a-002	ENSMUST00000122284	2356	ENSMUSP00000112853	754	Protein coding	-
Dyrk1a-003	ENSMUST00000139250	521	ENSMUSP00000120344	110	Protein coding	-
Dyrk1a-004	ENSMUST00000155791	689	ENSMUSP00000119669	181	Protein coding	-
Dyrk1a-201	ENSMUST00000023614	5754	ENSMUSP00000023614	763	Protein coding	CCDS28350
Dyrk1a-202	ENSMUST00000168180	5098	ENSMUSP00000131824	593	Protein coding	-
Dyrk1a-005	ENSMUST00000134383	2561	No protein product	-	Retained intron	-

Dyrk1a-001 (ENSMUST00000119878)

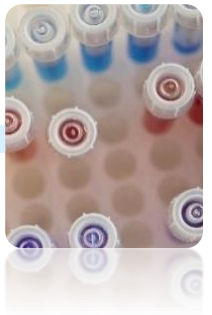




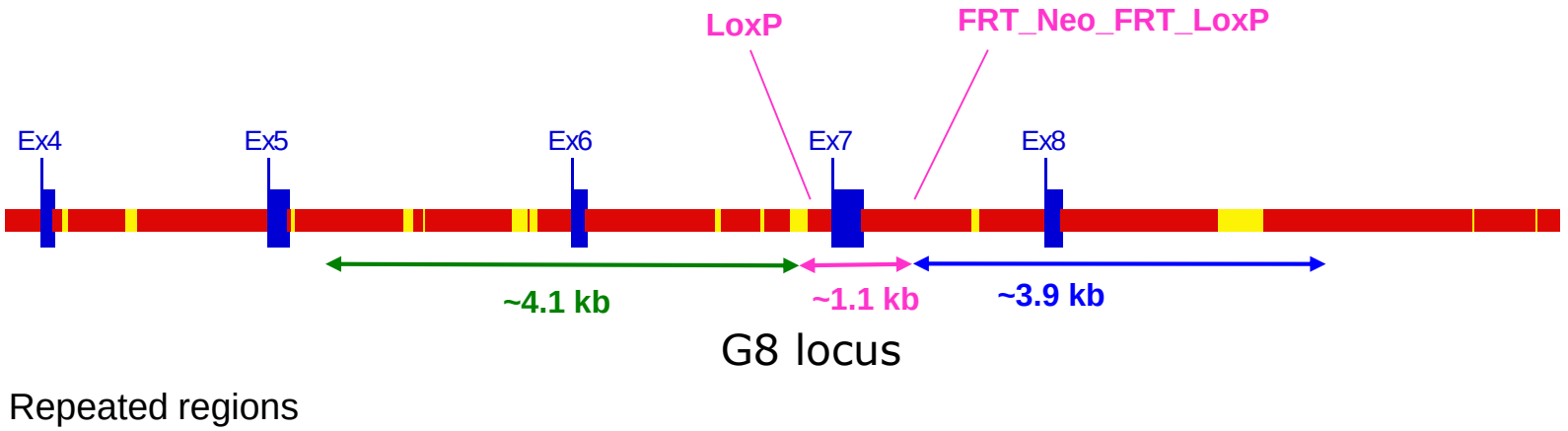
Exon 7	ENSMUSE00000555140	94,892,910	94,893,196	287

TGCATTTGAAACGCCACTTTATGTTTCGAAACCATCTCTGTTTAGTGTT
TGAAATGCTGTCCTATAATCTCTATGATTTGTTGAGAAACACCAACTTC
CGAGGGGTCTCTTTGAACCTAACACGAAAGTTTGCGCAACAGATGTGC
ACAGCATTGCTTTTTCTTGCGACTCCAGAACTTAGTATCATTCACTGTG
ACTTAAAGCCTGAGAACATCCTTCTGTGTAACCCCAAACGGAGTGCAA
TCAAGATTGTTGATTTTGGCAGCTCTTGTCAGTTGGGGCAGAGG

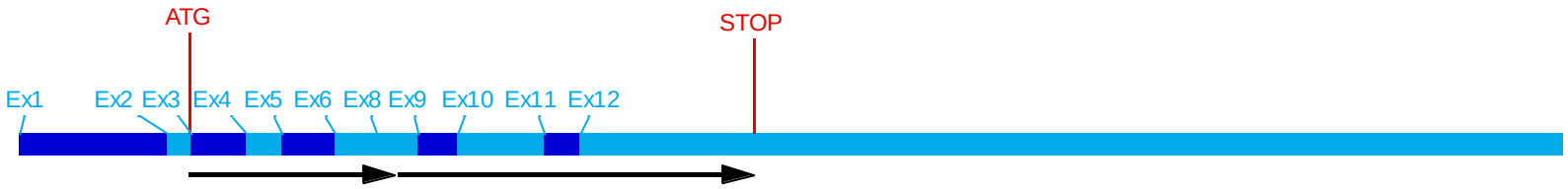
Proposal: flox exon 7 (from isoform -001)

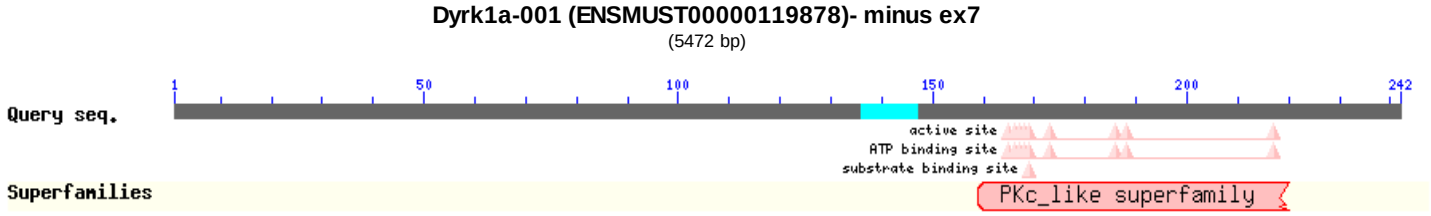


Targeted locus



Example of mRNA and protein expressed after Cre mediated excision (Dyrk1a-001 minus exon 7)





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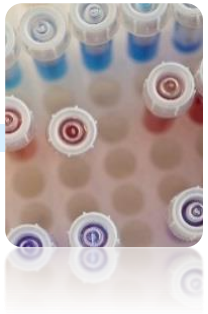
PROs& CONs evaluation of the strategy



Pros

- The Serine/Threonine Protein Kinases Active Site Signature domain will be removed

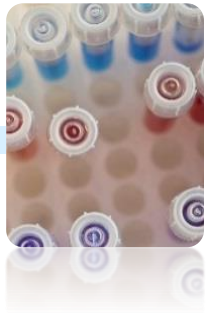
Cons

- A protein of 242 aa might be expressed (if RNA decay does not occur) corresponding to the 221 N-terminus aa of Dyrk1a plus 21 out of frame aa
- A protein of at most 420 aa might be expressed if reinitiation does occur at one of the in frame ATG present in exon 8 (or further exons)
- Presence of repeated regions in both homology arms (blue and green sequences) might render PCR amplification or PCR screening difficult

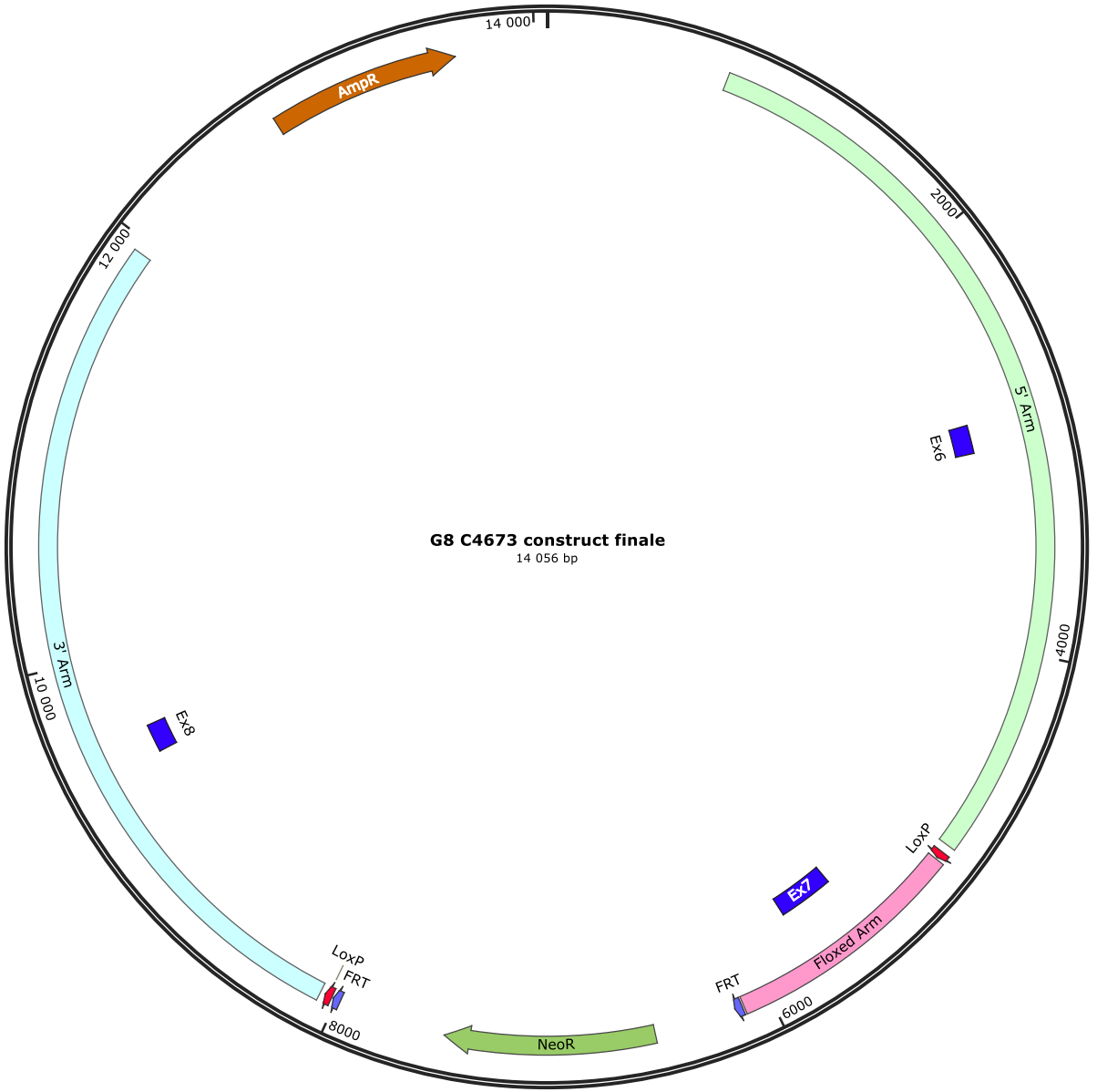
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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.

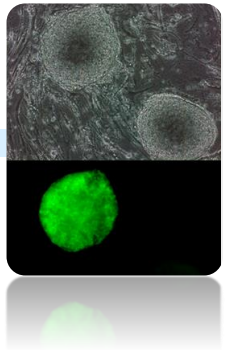
3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



Created by SnapGene

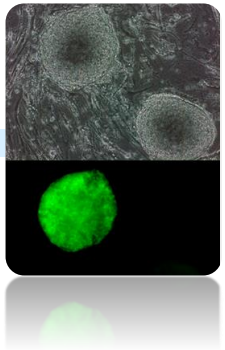


4 ES cell electroporation & Screening of recombinant clones



- 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



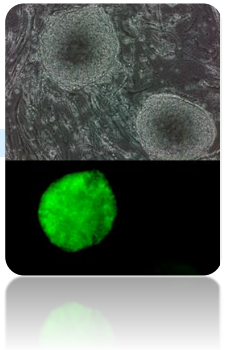
The targeting vector was electroporated in the proprietary C57BL/6N BD10 ES cell line.

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.

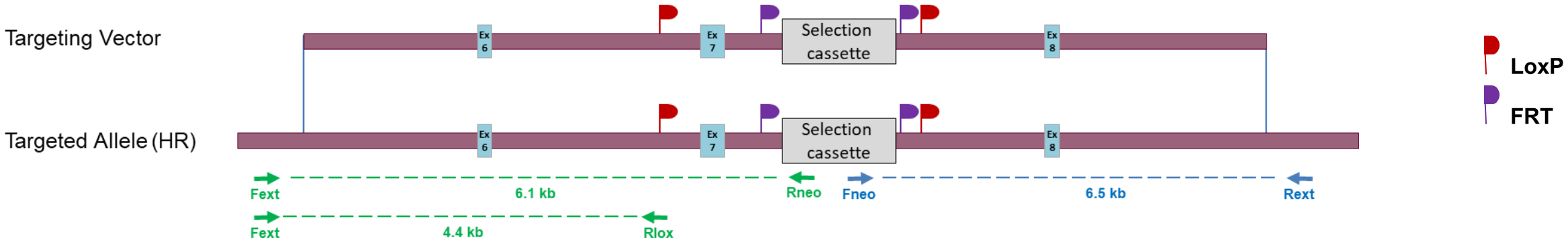
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Six 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 ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

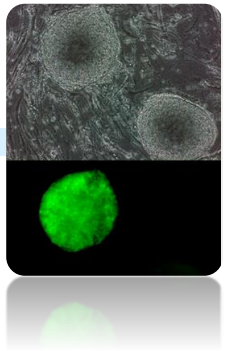


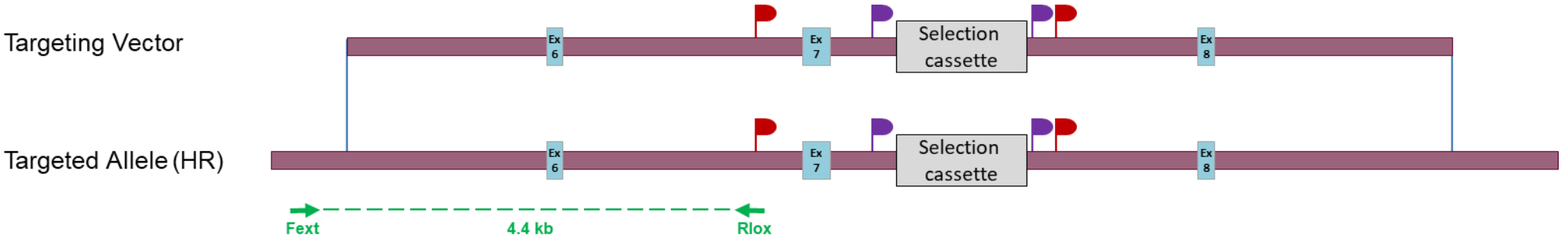
Schematic 5’ and 3’ PCR screening strategy



PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GATTATTGTTACCGCCATTGTTTAG	4.4 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	GATTATTGTTACCGCCATTGTTTAG	6.1 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	6.5 kb
	Rext	CAACTTCTCAAAGAACTTTCTTGCT	

Long-Range 5’ PCR screening – results

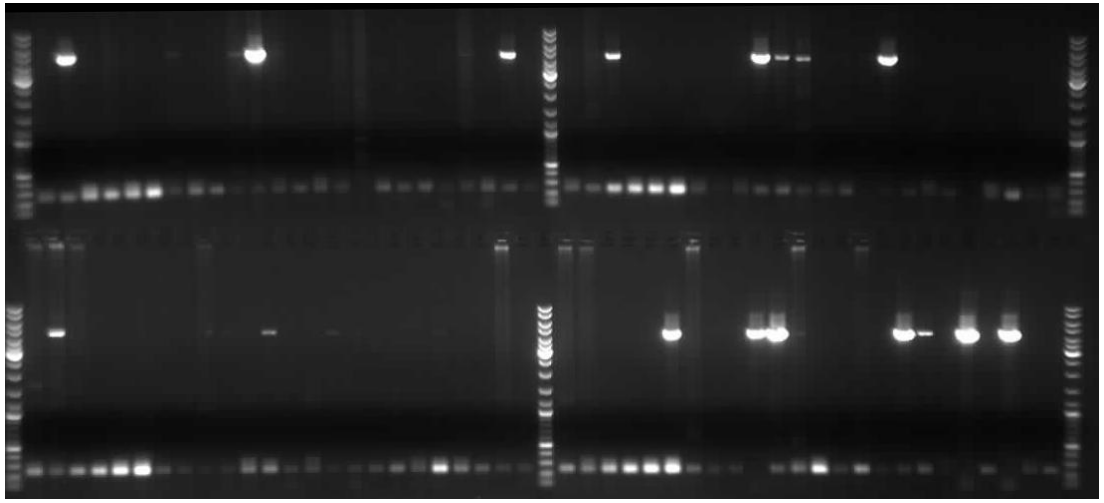


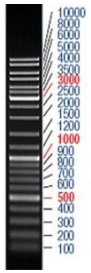


LoxP

FRT

Pcr Fext – Rlox : 4.4 kb





Ladder pattern

Six candidate clones out of the 30 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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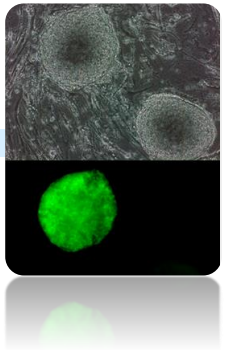
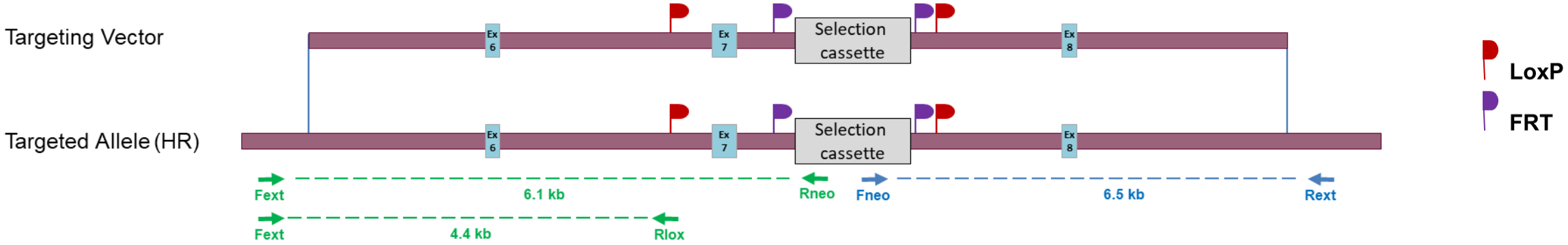
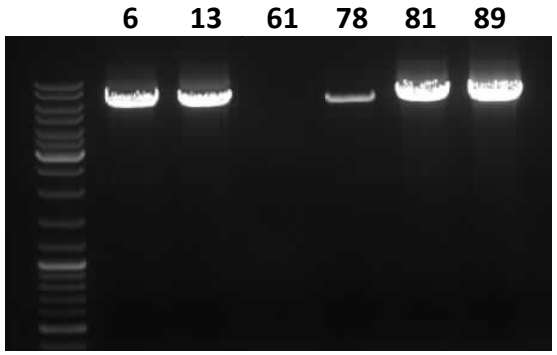
Recombinant ES validation by Long Range PCR

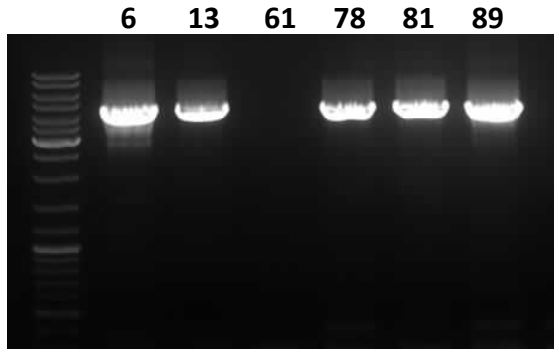


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

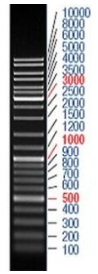




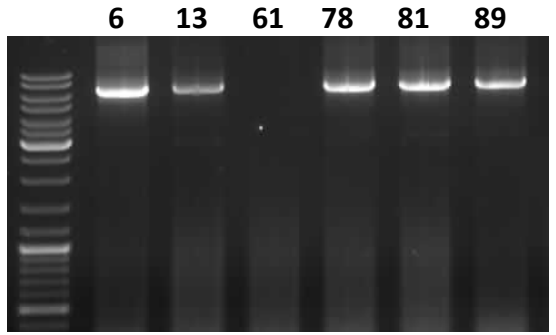
Pcr Fext – Rneo : 6.1 kb



Pcr Fext – Rlox : 4.4 kb



Ladder pattern



Pcr Fneo – Rext : 6.5 kb

Six candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Five clones (clones #6, #13, #78, #81 and #89) were confirmed.

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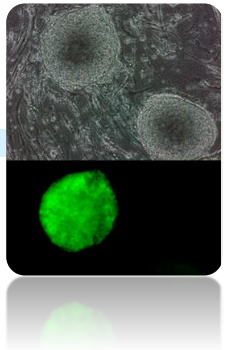
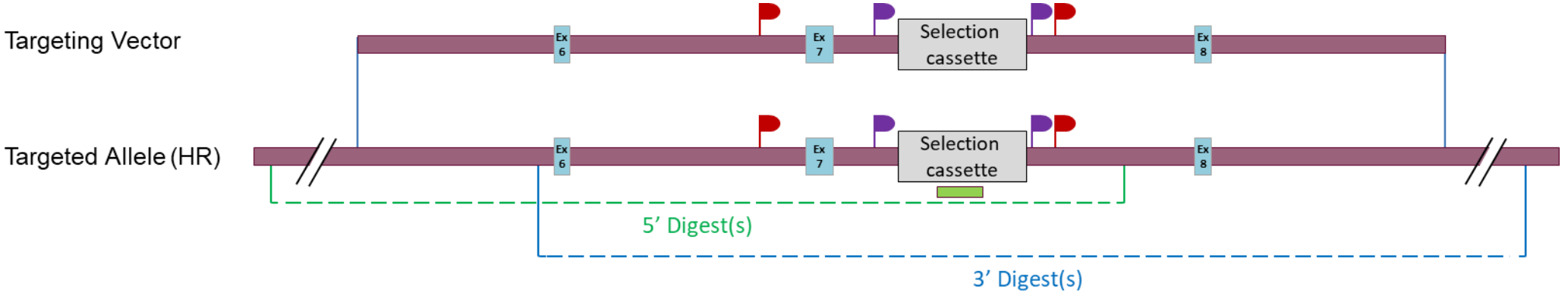
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

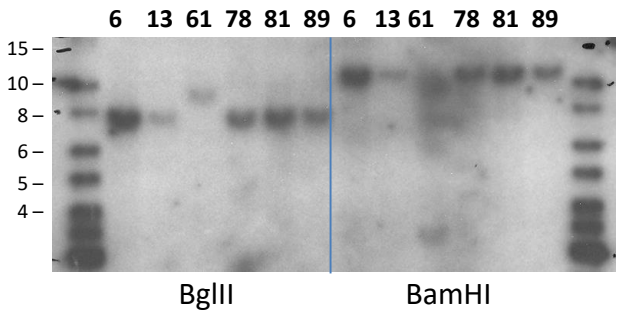
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



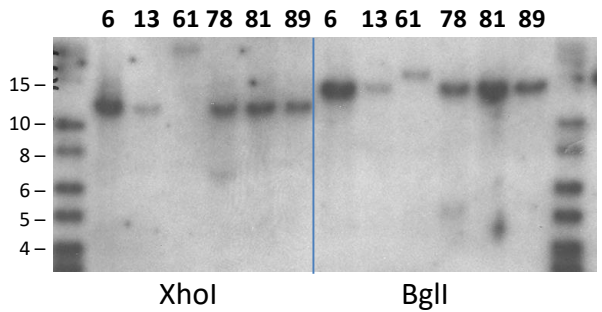
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	BglII	8.1
		BamHI	11.8
	3' digest	XhoI	12.4
		BglI	15.8

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

AGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATAC
CGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGG
TAGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATC
CAGAAAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGA
CGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGA
GCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTAC
GTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCG
TATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAG
ATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAG
TGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCG
CTGCCTCGTCCTGCAG

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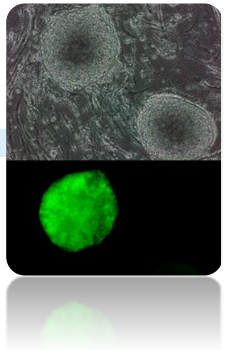
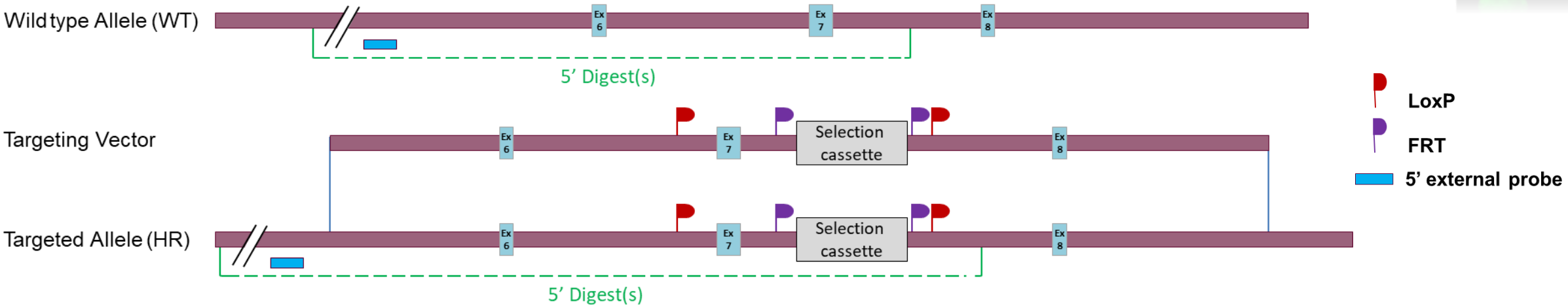
Recombinant ES clones validation by Southern Blot – External probe

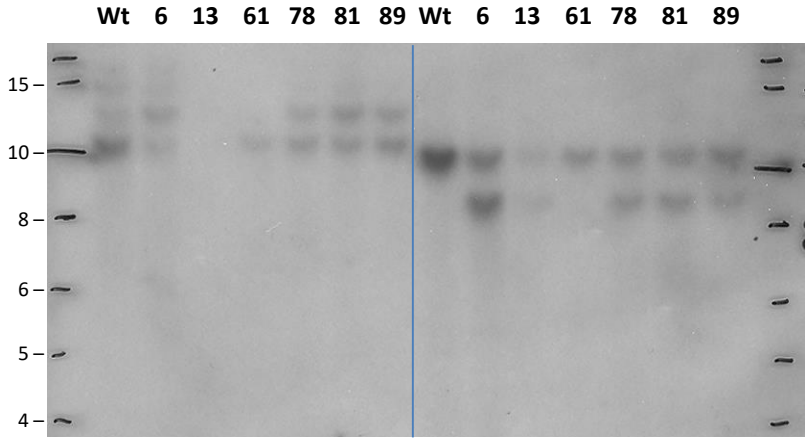


Schematic Southern Blot validation strategy

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



NdeI 11.2 / 13.2 XmnI 10.6 / 8.7

5' probe sequence

CCTGTCTTCATCTAAACACCTAGACTCCGTGCTCTCCC
ACAAGTGCTCATTCCCTCTTTTGTCTCCATTGGAAGCG
GTCTGCACAGGCCTTGTTCCTTCTTCAGCTTTCTTAGT
GTTAGATGGCAGCAAGCTGCCACTTCTTGCACTTGTGT
AAAGGTTTCTGGCTAGTCAGTGCCACCCTGCCCCAGTT
ACTAAGCTGTTGATGTTAGAGGCCTGGATTATTGTTAC
CGCCATTGTTTAGGTAATTACATGTGATCTAAATGTAC
TTCTTACGGAGTACAGTAAAGTGGAGGTTAAAGTGACG
TCTCATGAGGCTCCCCTGTGCTGGTGTCGATCAAAAAC
CAACTGTACAGCTGG

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	NdeI	11.2	13.2
	5' second digest	XmnI	10.6	8.7

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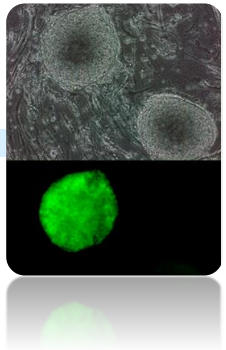
Aneuploidy screening in ES recombinant clones



Selected recombinant ES cells clones were karyotyped by Giemsa metaphase staining. Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#6	**Pass**
#13	Not done
#81	**Pass**
#89	Not done

5 MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a C57BL/6N 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 clones #6 and #81 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
#6	1	1	11	13
#81	5	6	6	17

Breeding to F1 generation

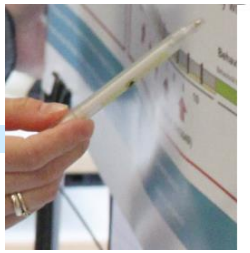
- Four highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with wild-type C57BL/6NCrl Flp deleter females showing maternal contribution* (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 07/03/2012

- Allele nomenclature (following MGI guidelines) : **Dyrk1a$^{tm1.1Ics}$** (MGI:6783454)

***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.

6 SEQUENCE OF THE DELIVERED ALLELE



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LoxP FRT Exon 7

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/08/30

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