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MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Wdr62 conditional Knock-out

Project code: G15 / IR00003729

Report finalized: 07/09/2023

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Université de Strasbourg
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MODEL GENERATION TECHNICAL REPORT

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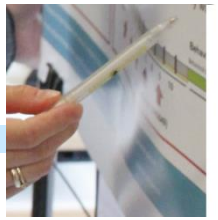
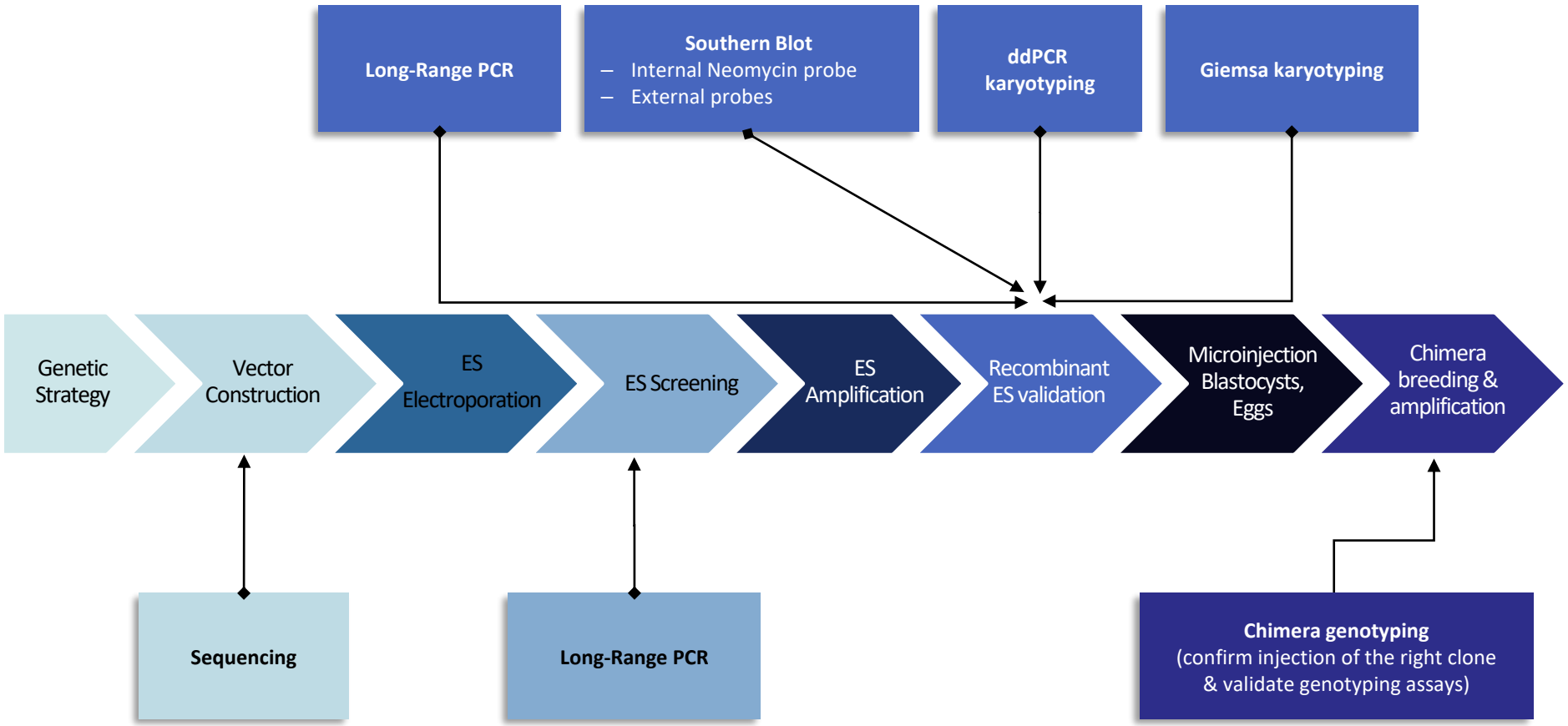
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

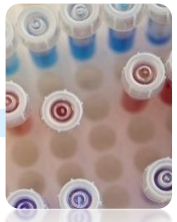
6 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROLS



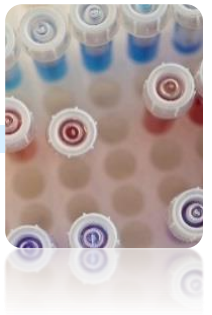


2 GENETIC STRATEGY (done 13/09/2011)

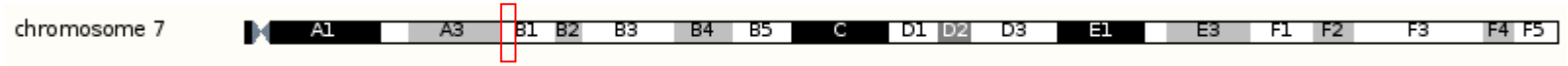


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

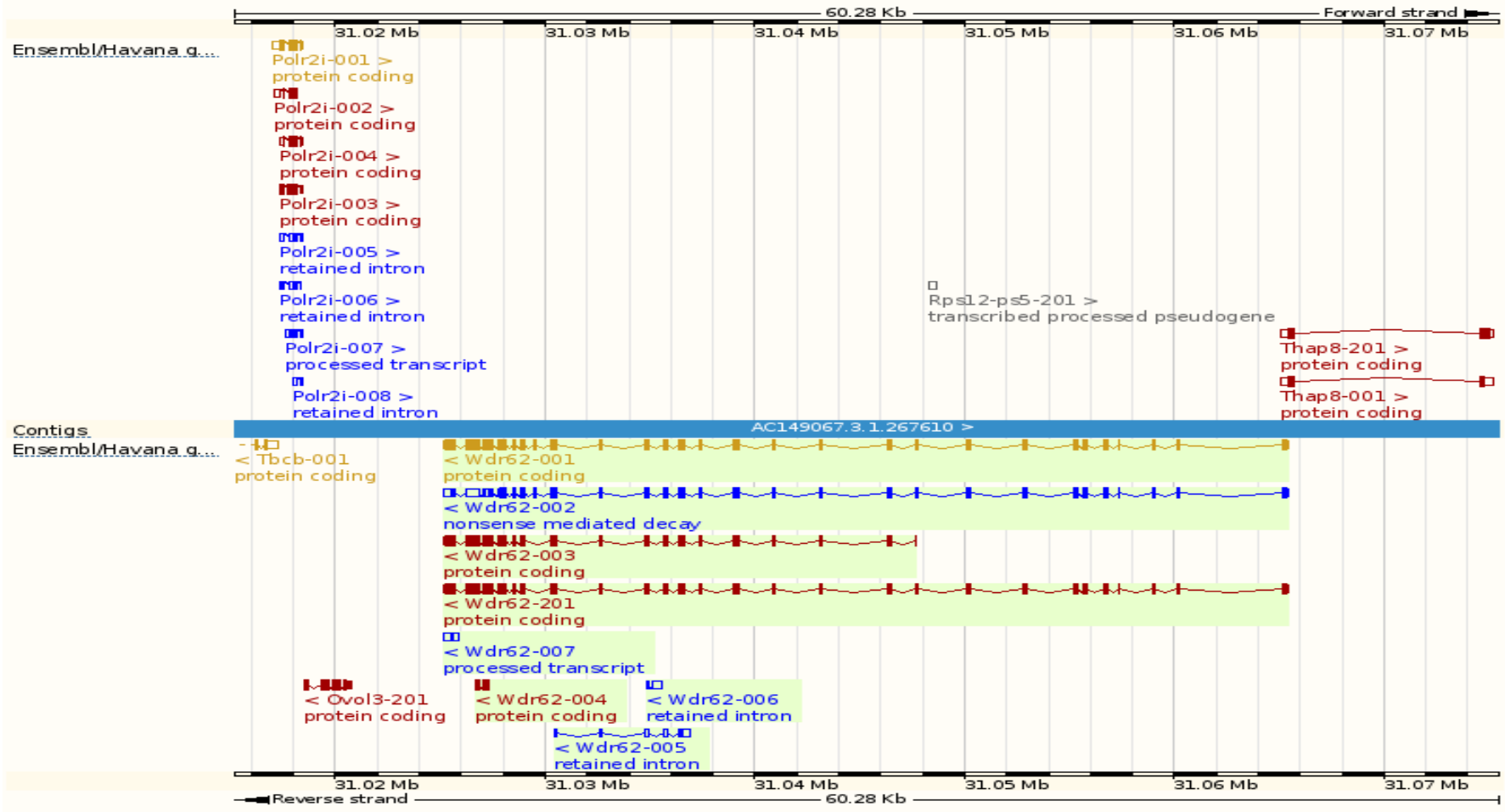
Wdr62 mouse genomic locus – structure



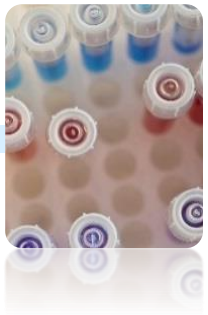
Location:



Wdr62 (ENSMUSG00000037020)



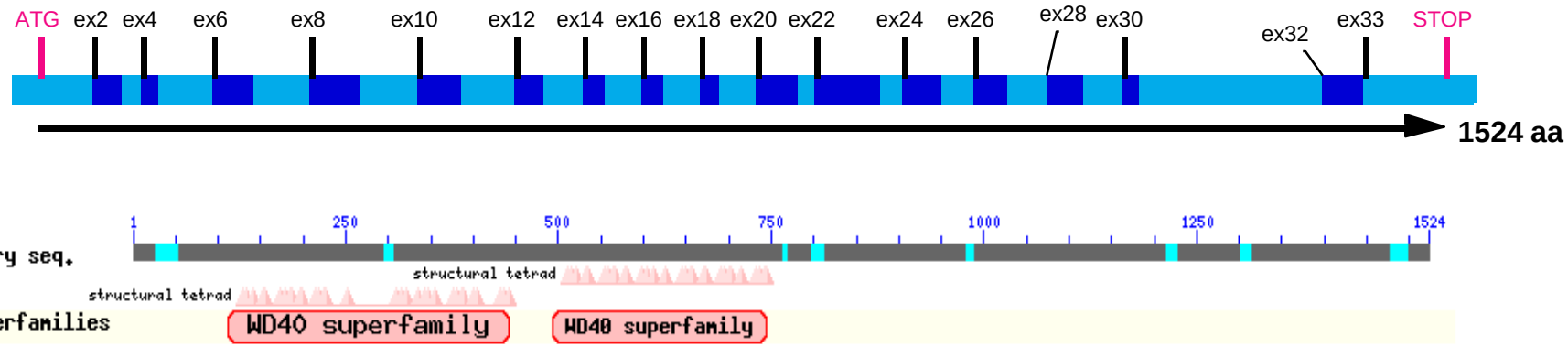
Wdr62 mRNA(s) and protein(s)



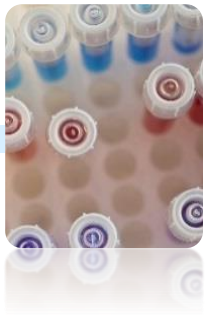
No Sanger design for Wdr67: 33 exons

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)
Wdr62-201	ENSMUST00000085760	4642	ENSMUSP00000082912	1498
Wdr62-001	ENSMUST00000108190	4742	ENSMUSP00000103825	1524
Wdr62-004	ENSMUST00000133347	374	ENSMUSP00000115768	125
Wdr62-003	ENSMUST00000134570	3245	ENSMUSP00000116139	1053
Wdr62-002	ENSMUST00000145027	4768	ENSMUSP00000116772	1076
Wdr62-007	ENSMUST00000152543	648	No protein product	-
Wdr62-005	ENSMUST00000132483	791	No protein product	-
Wdr62-006	ENSMUST00000152234	573	No protein product	-

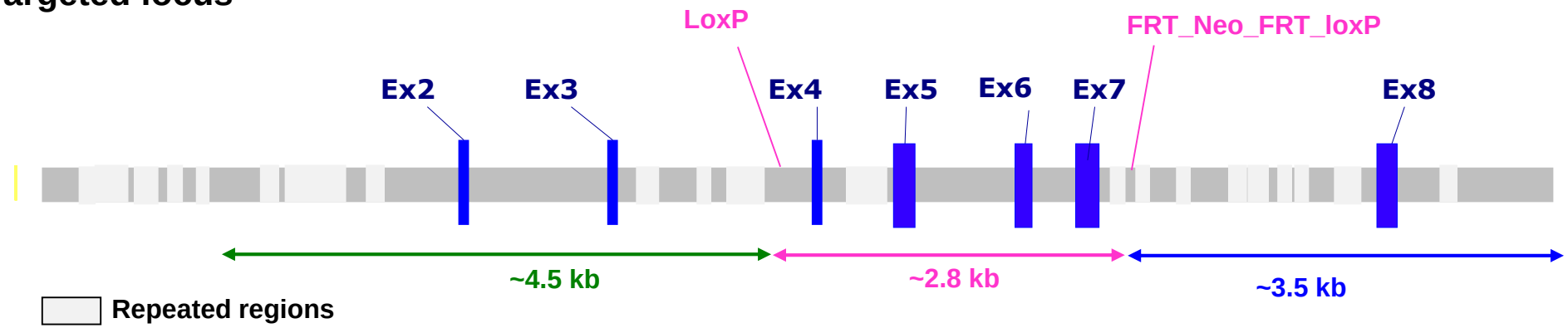
Wdr62-001



Approach selected: flox exons 4 to 7

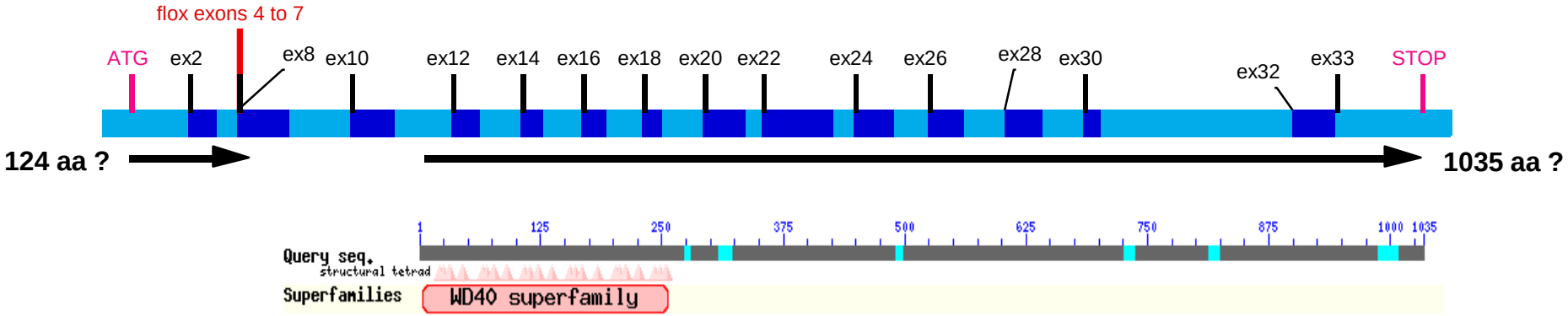


Targeted locus



Ex4: ENSMUSE00000246782
Ex5: ENSMUSE00000534618
Ex6: ENSMUSE00000246769
Ex7: ENSMUSE00000246761

mRNA and protein expected after Cre mediated excision



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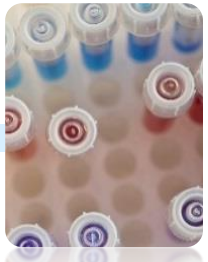
PROs& CONs evaluation of the strategy



Pros

- The WD40 domain will be disturbed

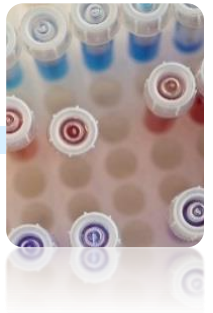
Cons

- A protein of 124 aa might be expressed (if RNA decay does not occur) corresponding to the 111 N-terminus aa of Wdr62 plus 13 out of frame aa
- A protein of at most 1024 aa might be expressed is reinitiation does occur at one of the in frame ATG present in exon 11 (or further exons)
- Presence of repeated regions in both homology arms (light grey sequences) might render PCR amplification or PCR screening difficult

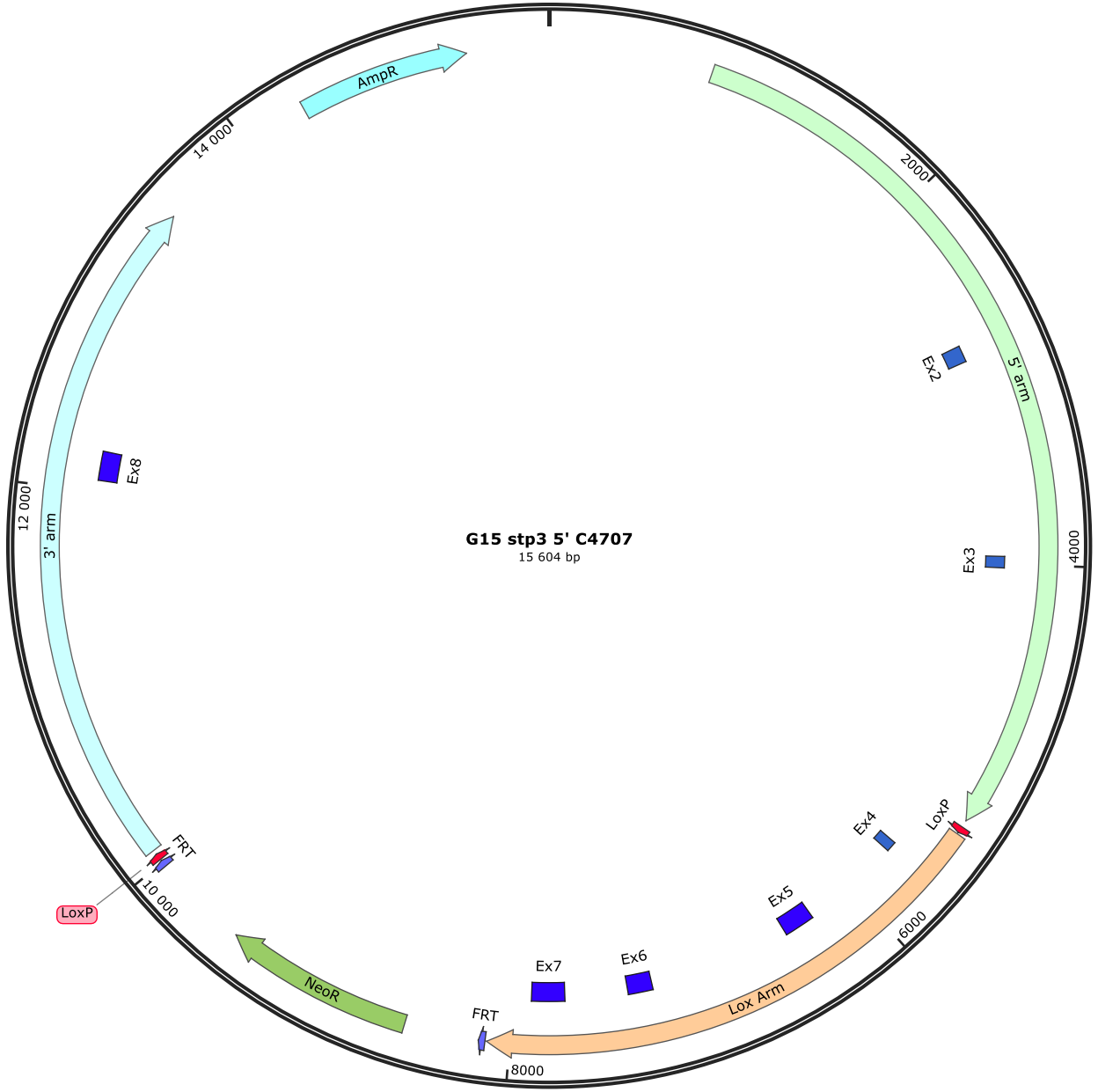
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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



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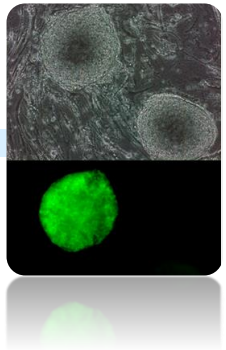
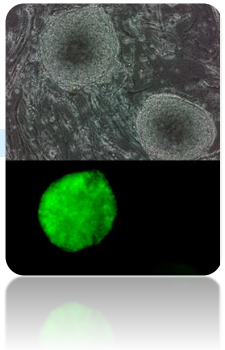
4 ES cell electroporation & Screening of recombinant clones



- 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



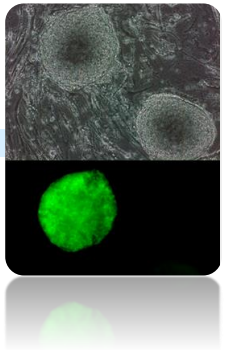
The targeting vector was electroporated in the proprietary C57BL/6N BD10 cell line.

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.

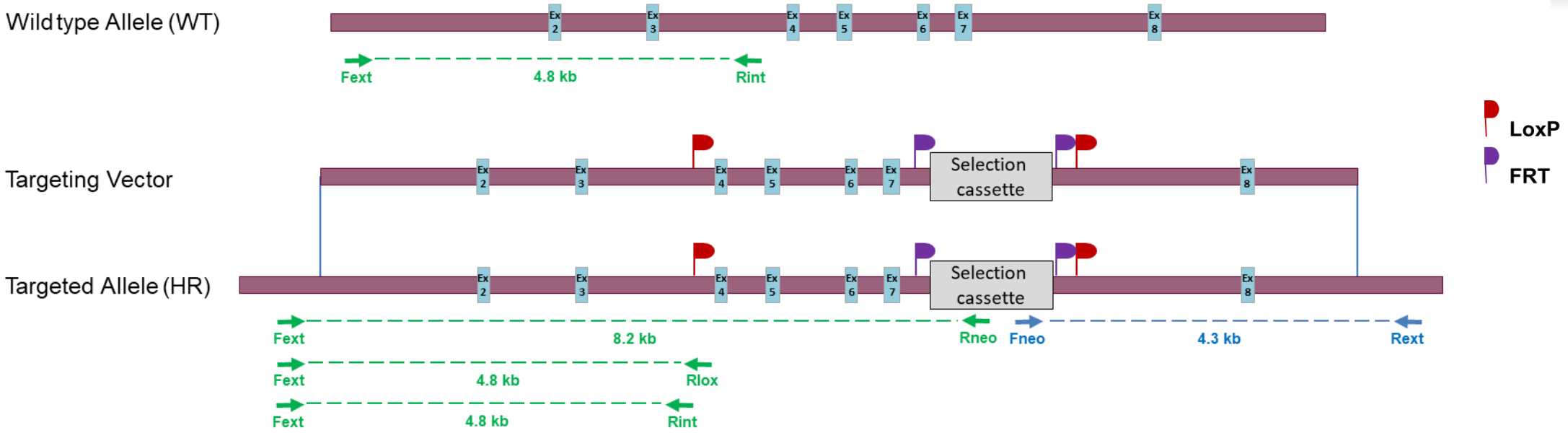
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Seven 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

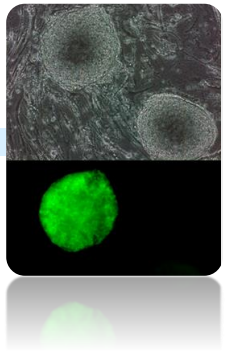


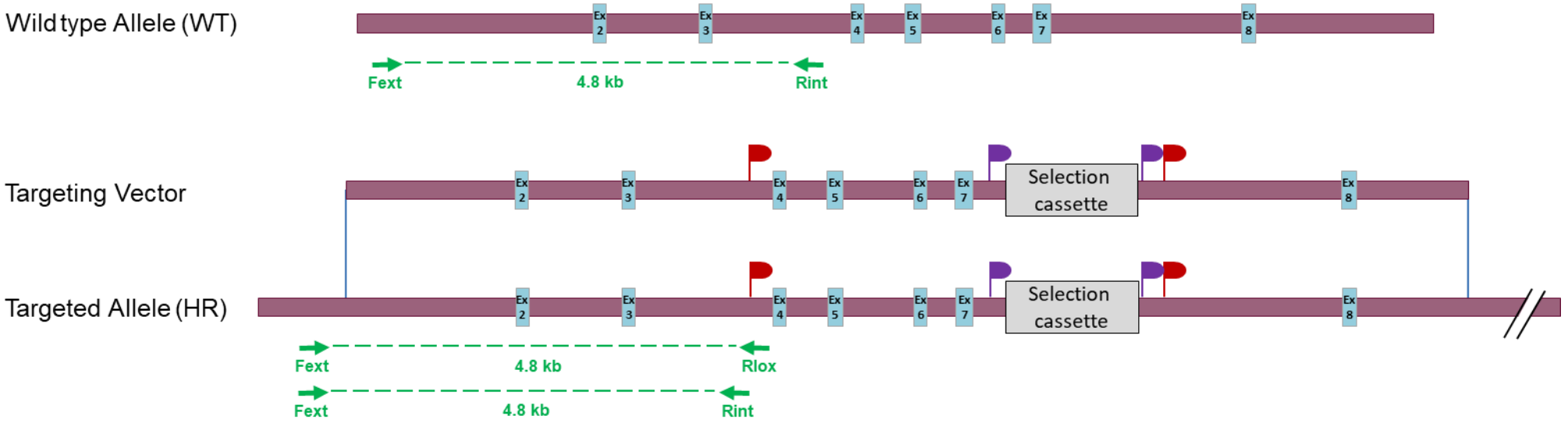
Schematic 5’ and 3’ PCR screening strategy



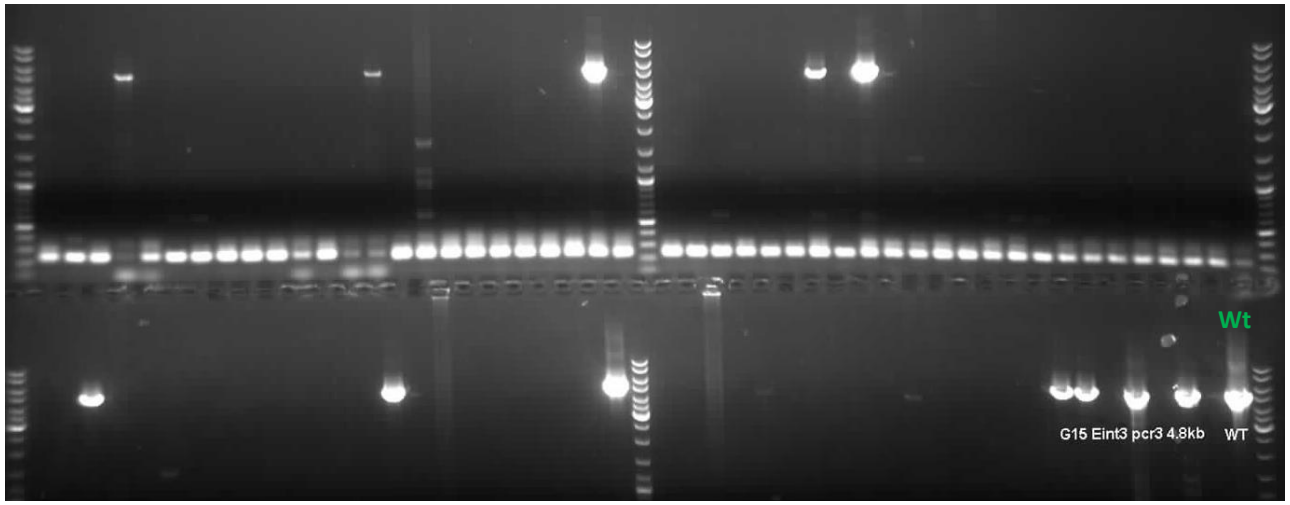
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	ATGATGTAGAATCCCCCACAACATA	4.8 kb
	Rint	CAGTGTGGAGTGAGCTTTCCAGG	
5’ PCR	Fext	ATGATGTAGAATCCCCCACAACATA	4.8 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	ATGATGTAGAATCCCCCACAACATA	8.2 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.3 kb
	Rext	GCCACAATGCCTGGCTGAATTACTG	

Long-Range 5’ PCR screening – results

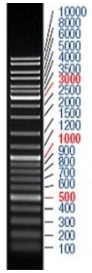




PCR Fext – Rlox: 4.8 kb



Wt : Control DNA



Ladder pattern

PCR Fext – Rint : 4.8 kb

Seven candidate clones out of the 7 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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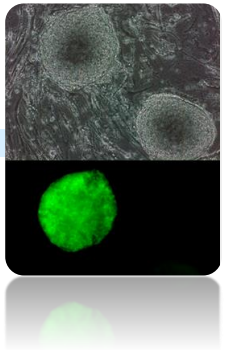
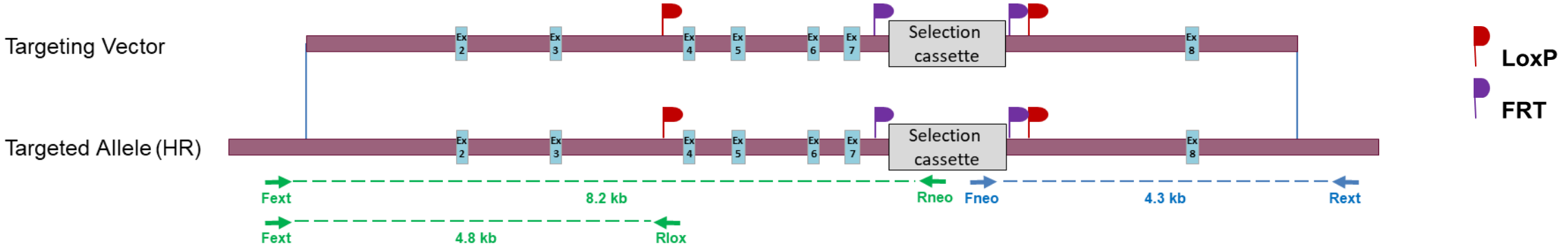
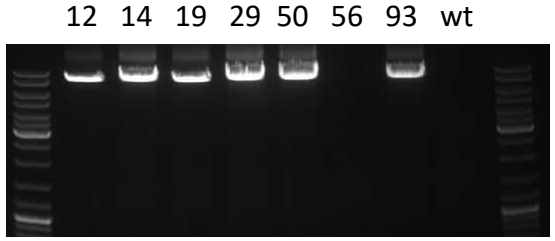
Recombinant ES validation by Long Range PCR

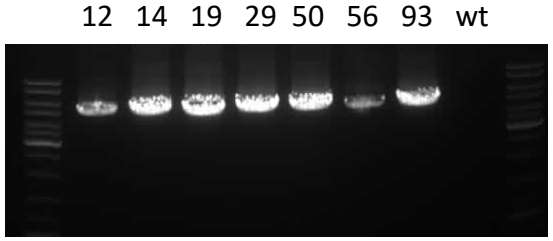


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs



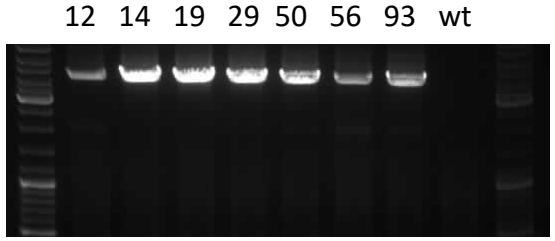


PCR Fext – Rneo : 8.2 kb



PCR Fext – Rlox : 4.8 kb

Ladder pattern



PCR Fneo – Rext : 4.3 kb

Seven candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Six clones (clones #12, #14, #19, #29, #50, and #93) were confirmed.

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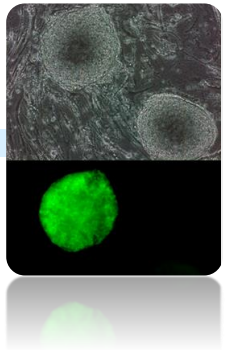
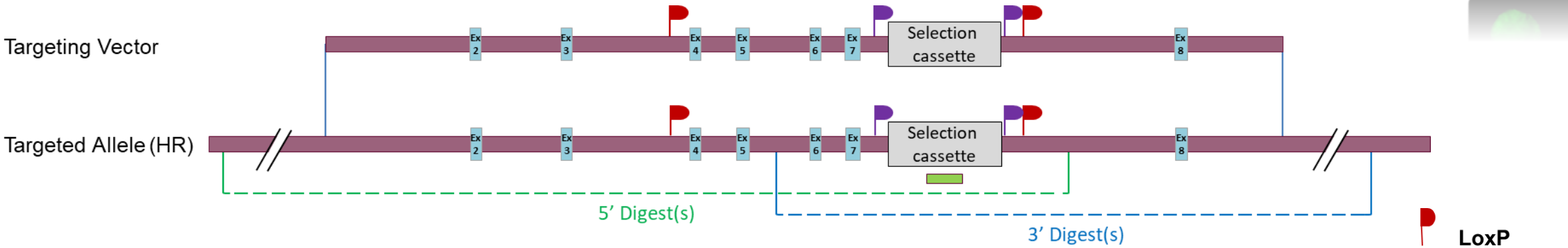
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

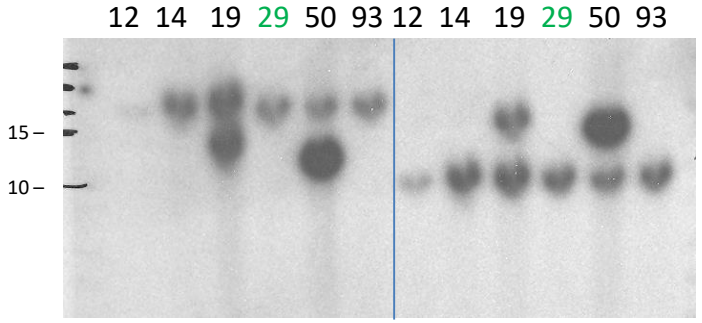
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



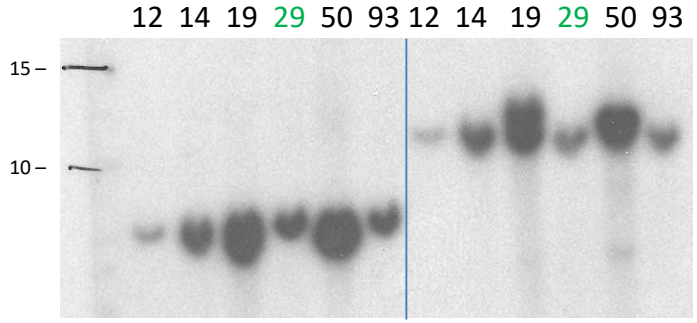
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	EcoRV	18
		BamHI	9.5
	3' digest	KpnI	6.2
		SpeI	12

Southern blot - Neo 5'



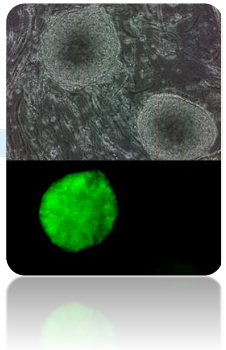
Southern blot - Neo 3'



Neo probe sequence

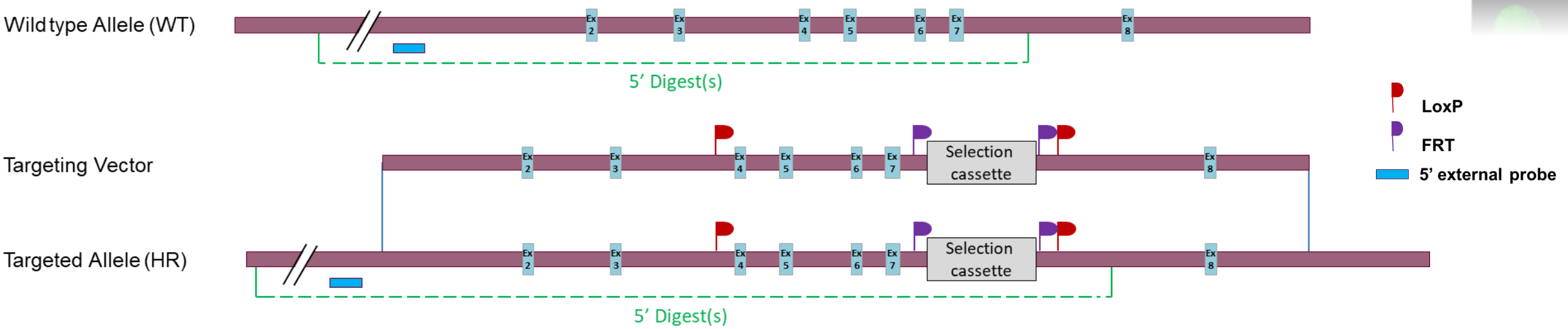
CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
CTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGC

Recombinant ES clones validation by Southern Blot – External probe

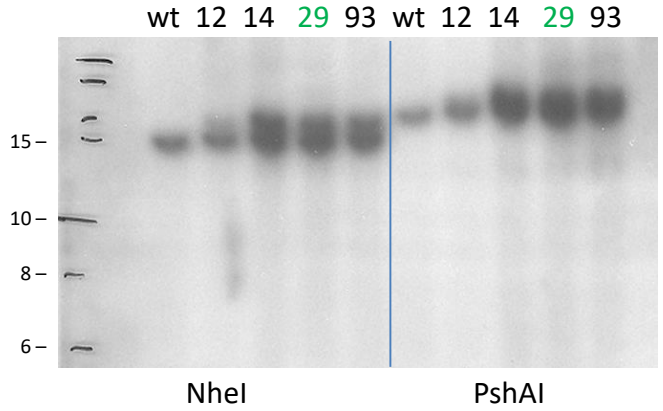


Schematic Southern Blot validation strategy

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

GGATGCAGTAAGCTGGTTTGAAGCATAGACTCCACACTCCACTGCCTG
AACTCAAATATTCATTCTGTTTCTTGTAAGCAAGTTAGGAACTTCTCC
CTGCCTCAGTTATCCTAGCTGTAAAACAAAGATAATGATGTAGAATCC
CCCACAACATACATCTTATTATAATCCAAACTGCCCTTAAACTTCGCT
CCTTCTGTTTCCCAAGTGCTGGGATCACAGATGTGTGATACCTTACCA
GGTCTCCCTCTTAGGGTACCTGTGAGATTCTGCGGTTTCCTATATCTT
AAGCCTCAGAACAACACC

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	NheI	15.5	17.6
	5' second digest	PshAI	18.6	20.7

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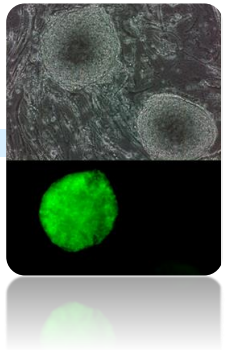
Aneuploidy screening in ES recombinant clones



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

Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#12	Not done
#14	**Pass**
#29	**Pass**
#93	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 #14 and #29 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
#14	3	6	3	12
#29	2	0	6	8

Breeding to F1 generation

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

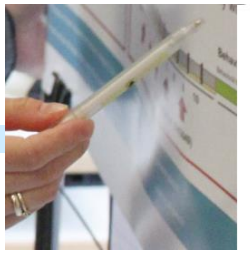
- Germ line transmission was obtained the : 09/05/2012

- The line issued from clone #29 was cryopreserved

- Allele nomenclature (following MGI guidelines) : **Wdr62$^{tm1.1Ics}$**

***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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CACATGCATTCACGCACACACAGAGTAATAGAAACATTTCAGTTGTTGGGGTTTCTAGAACACTCAATGAGCTATATCTGTGCCCTGTCTACCAGGGTCCACCCATGAGCATAGCTCTTCTTTAGTTAGGCCAAGCCTGTACC
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AATTAAGGAAGGAAGCAAAAACCTCGATTCTGGGTCTGCAGTGTTTGGGTTTGGTATTGAGGGAGGAGGAGACCTCTGCTTTCTTGTTCCATTCCCTGAGTGCCATGCTGGGCACACAGGCTACAGTGGCTGTGTAGGAACTG
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TTTTTTTGTCAAA

LoxP FRT Exons 4, 5, 6 and 7

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/09/07

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