phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Larp7 cKO

Project code: Kos8114 / IR8144

Report finalized: 2023/10/16

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

2 GENETIC STRATEGY

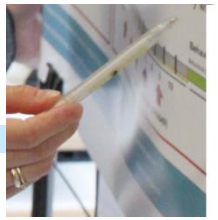
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

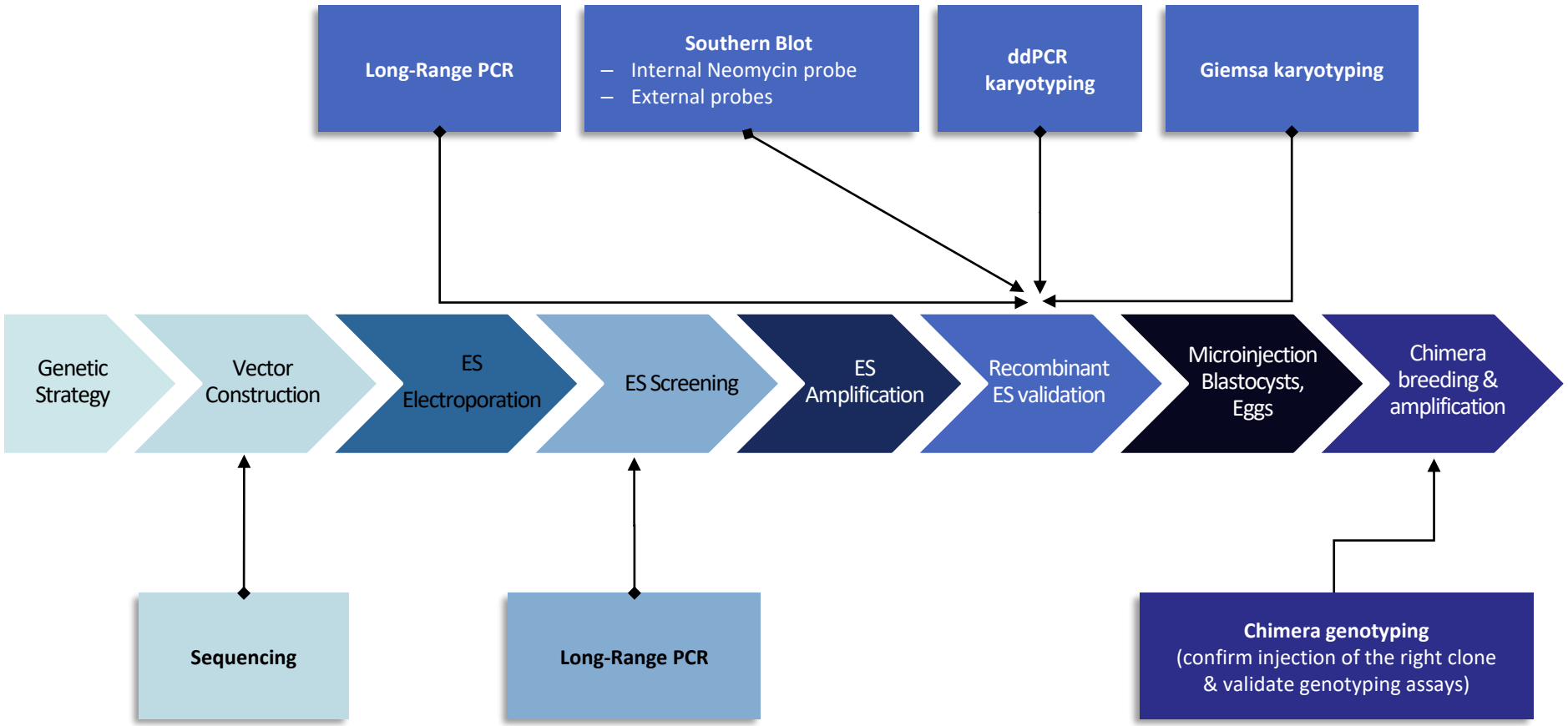
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

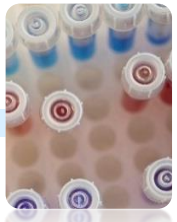
6 SEQUENCE OF THE DELIVERED ALLELE

PROJECT PROCESS & QUALITY CONTROLS



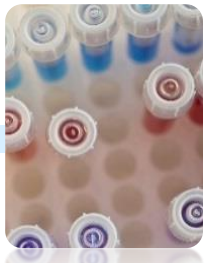


2 GENETIC STRATEGY

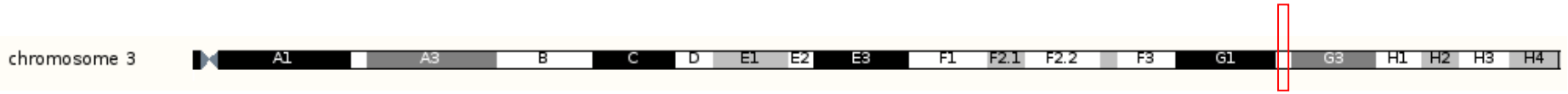


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

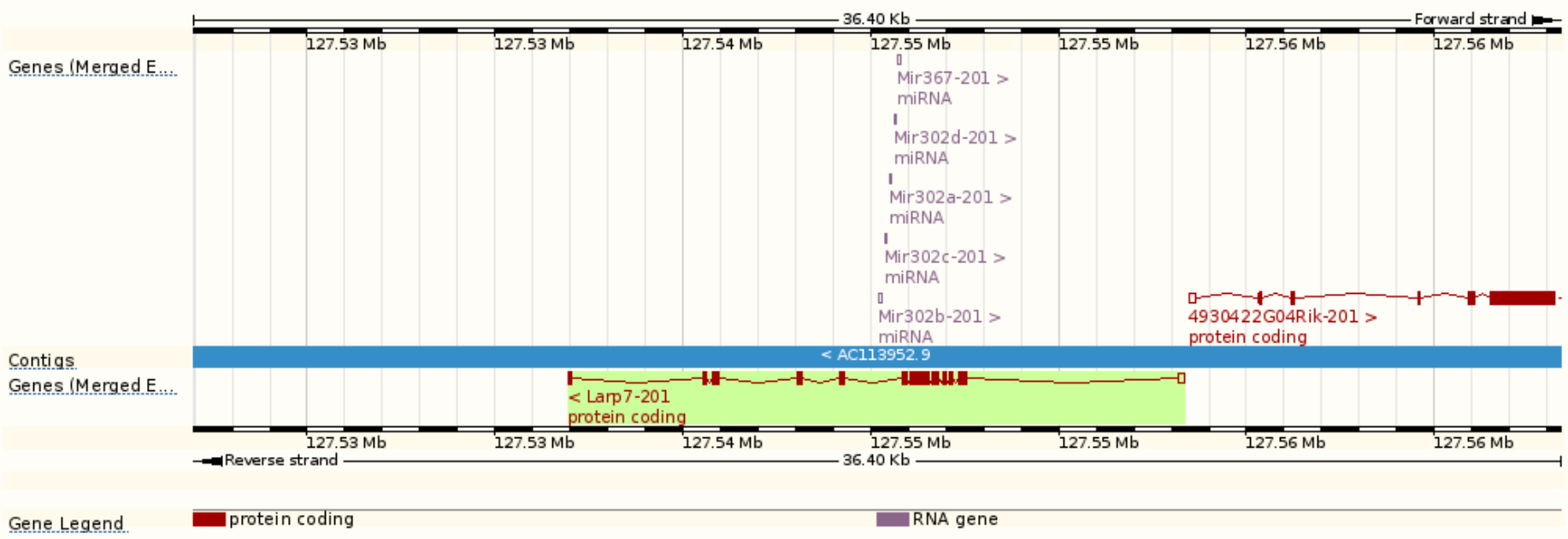
Larp7 mouse genomic locus – structure



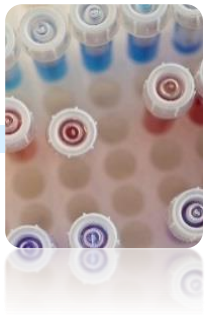
Location:



Ensembl Gene ID: Larp7 ENSMUSG00000027968



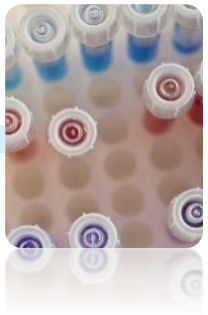
Larp7 mRNA(s) and protein(s)



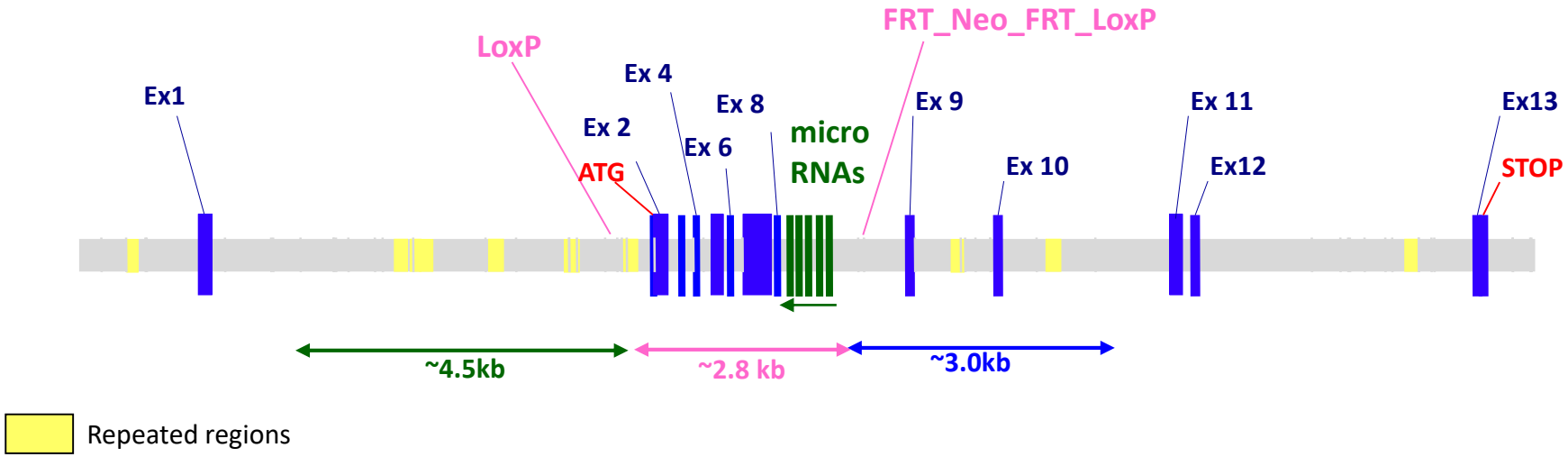
Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
Larp7-201	ENSMUST00000029588	1880	ENSMUSP00000029588	570	Protein coding	CCDS38626

Larp7-201 ENSMUST0000029588

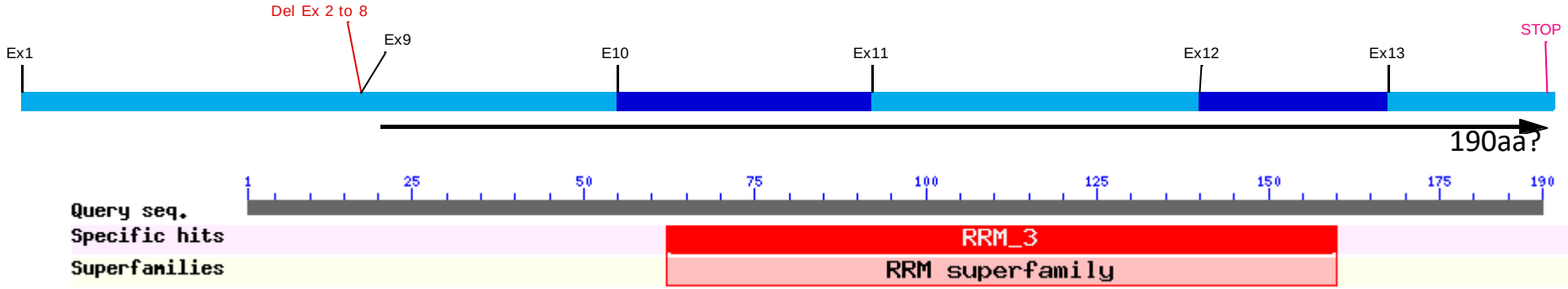
Approach selected: flox exons 2 to 8



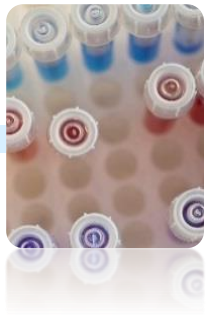
Targeted allele



mRNA and protein expressed after Cre mediated excision



PROs& CONs evaluation of the strategy



Pros

- The start codon containing exon will be removed after Cre mediated excision

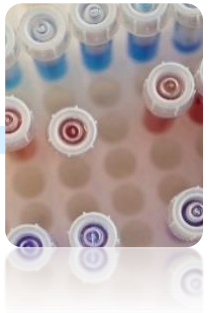
Cons

- The floxed region will also contain microRNAs 367 and 302a-d. The phenotype observed after Cre mediated excision might be due to the lack of these microRNAs.
- A protein of 190 aa (at most) might be expressed after Cre mediated excision if initiation occurs at the next in frame ATG.
- The 5’ LoxP site has a high chance to be lost after homologous recombination as recombination might occur in the floxed fragment instead of the 5’ HR arm (in blue)
- Presence of repeated regions might render PCR amplification and screening difficult.

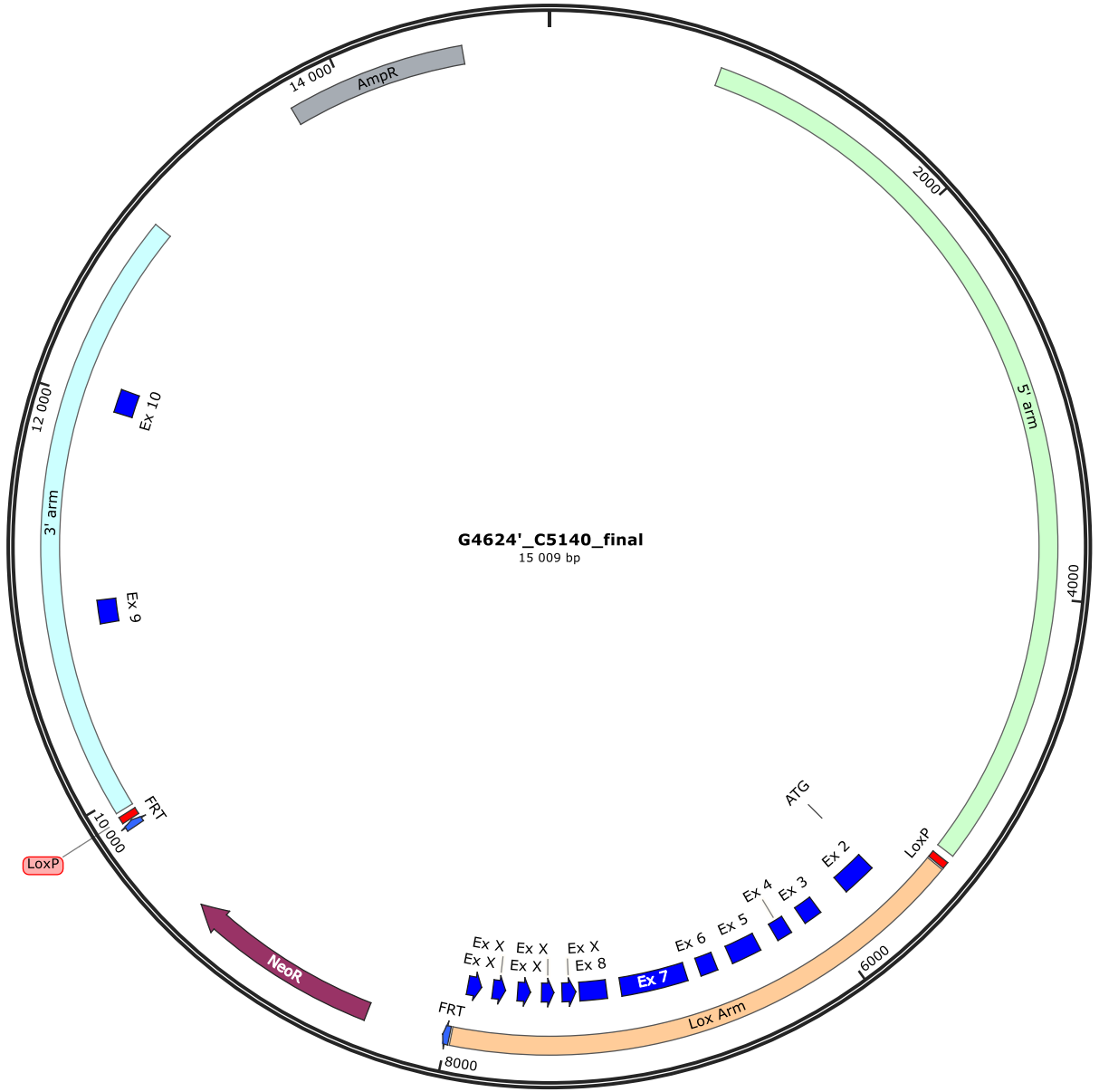
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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

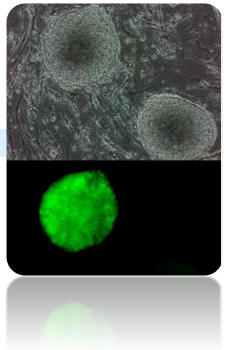


Created by SnapGene



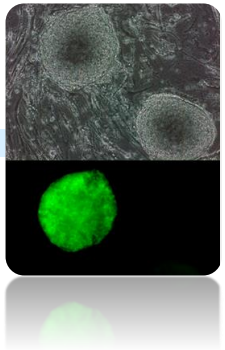
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

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



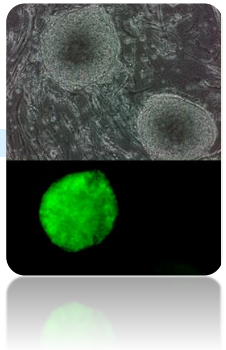
The targeting vector was electroporated in the proprietary C57BL/6NTac TB1 cell line.

Transfected ES clones were submitted to neomycin selection (G418) and 93 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.

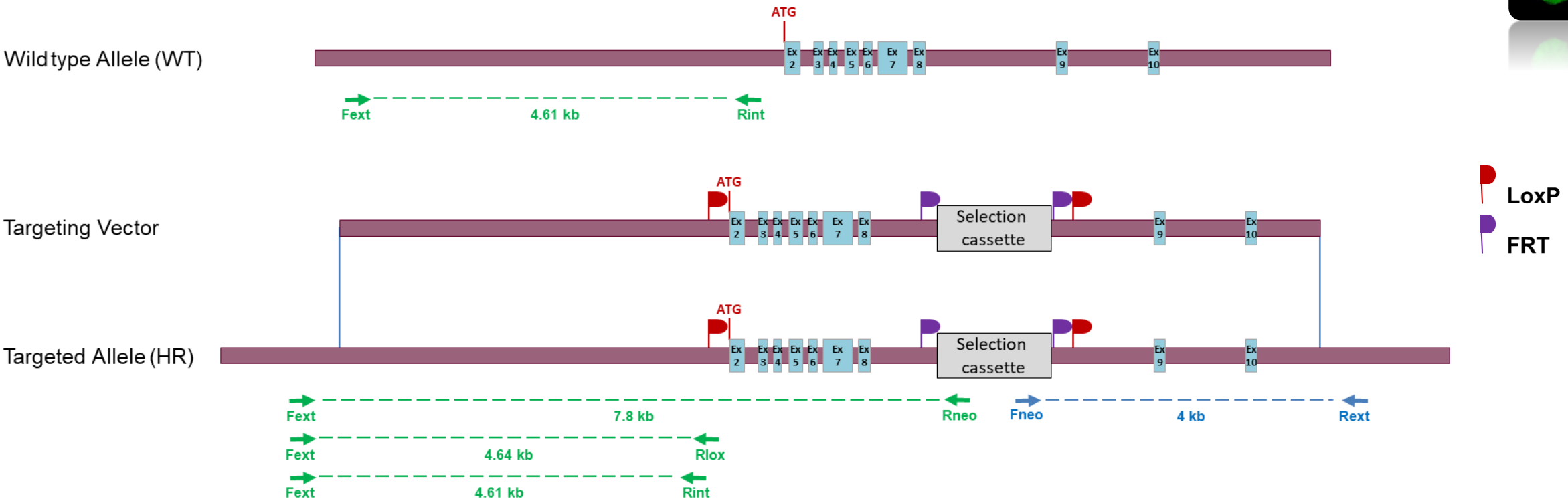
Screening process steps:

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

Long range PCR screening – strategy

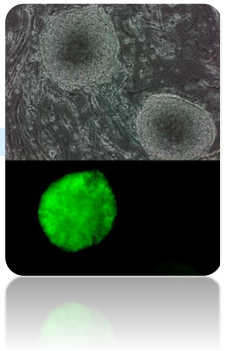


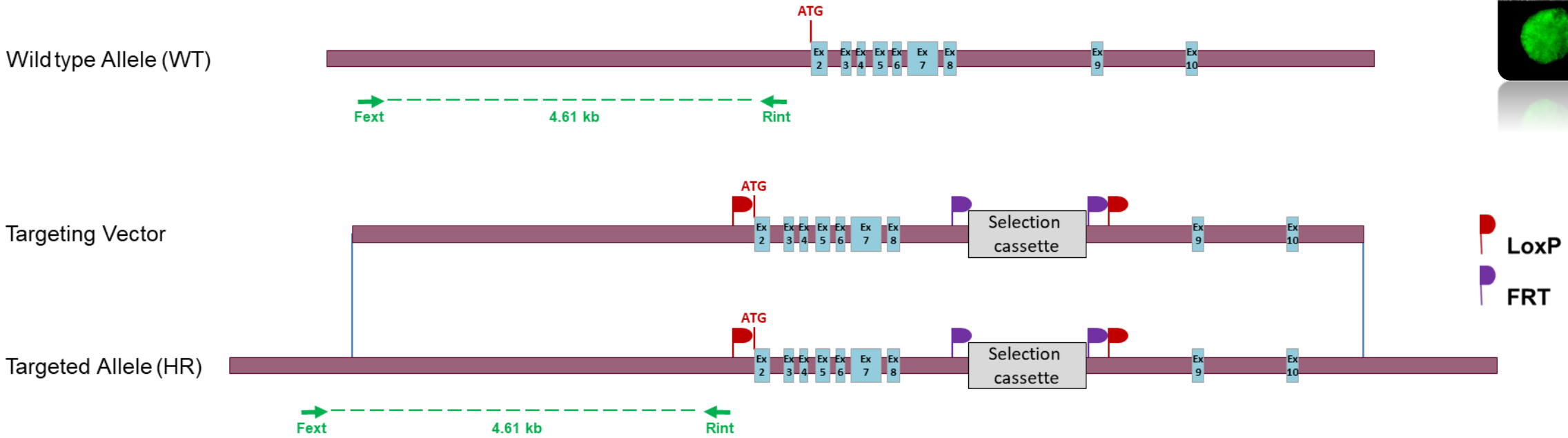
Schematic 5’ and 3’ PCR screening strategy



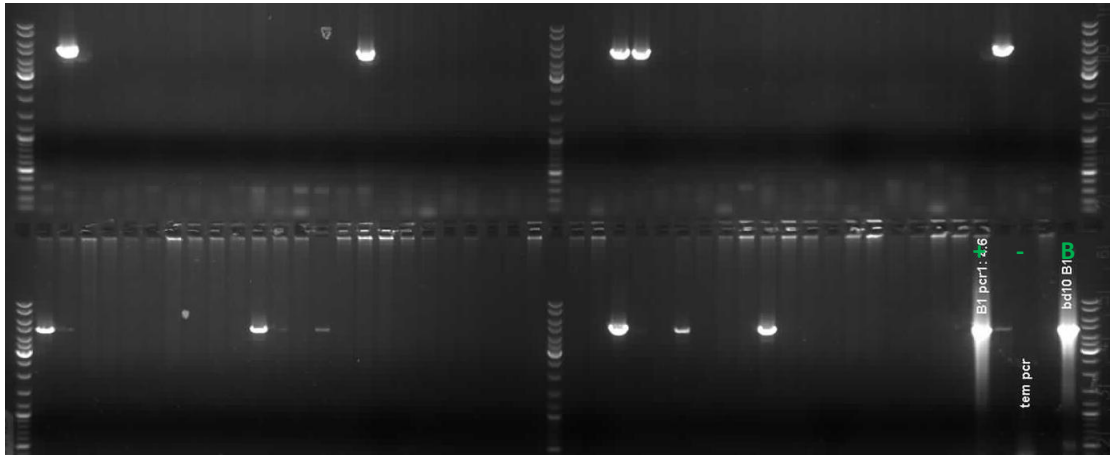
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GCTTTACATCTAGCACAGCTTACTT	7.8 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
5’ PCR	Fext	GCTTTACATCTAGCACAGCTTACTT	4.64 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	GCTTTACATCTAGCACAGCTTACTT	4.61 kb
	Rint	AATATGGCCGGCCCCTTTGGACTTGTAAACTTTATATG	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4 kb
	Rext	CCATTAAAAGTCAGGCTGATCTCC	

Long-Range 5’ PCR screening – results

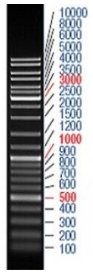




PCR Fext – Rlox : 4.64 kb



+ / - / B : Controls DNAs



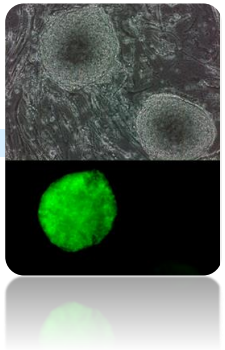
Ladder pattern

PCR Fext – Rint : 4.61 kb

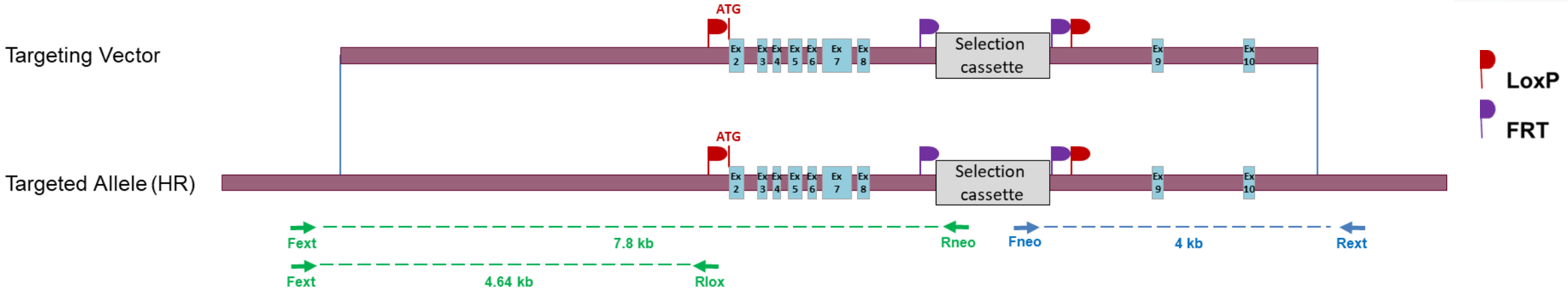
Five candidate clones out of the 12 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

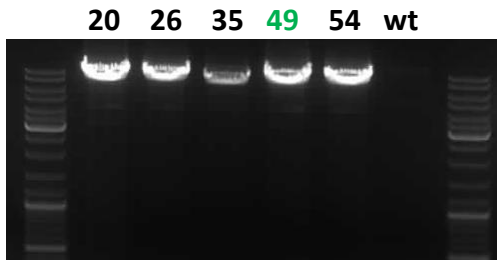
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES validation by Long Range PCR

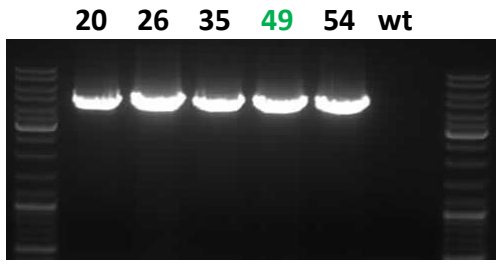


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

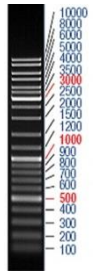




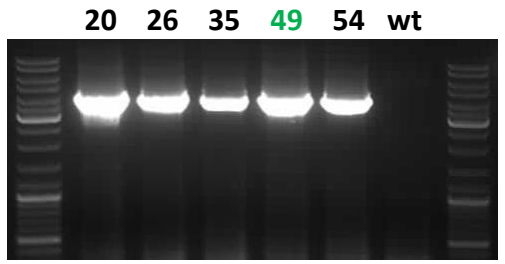
PCR Fext – Rneo : 7.8 kb



PCR Fext – Rlox : 4.64 kb



Ladder pattern

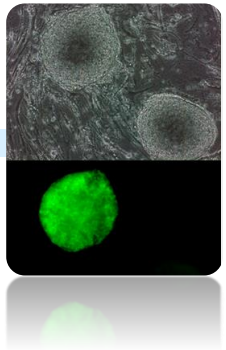


PCR Fneo – Rext : 4 kb

Five candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Five clones (clones #20, #26, #35, #49 and #54) were confirmed.

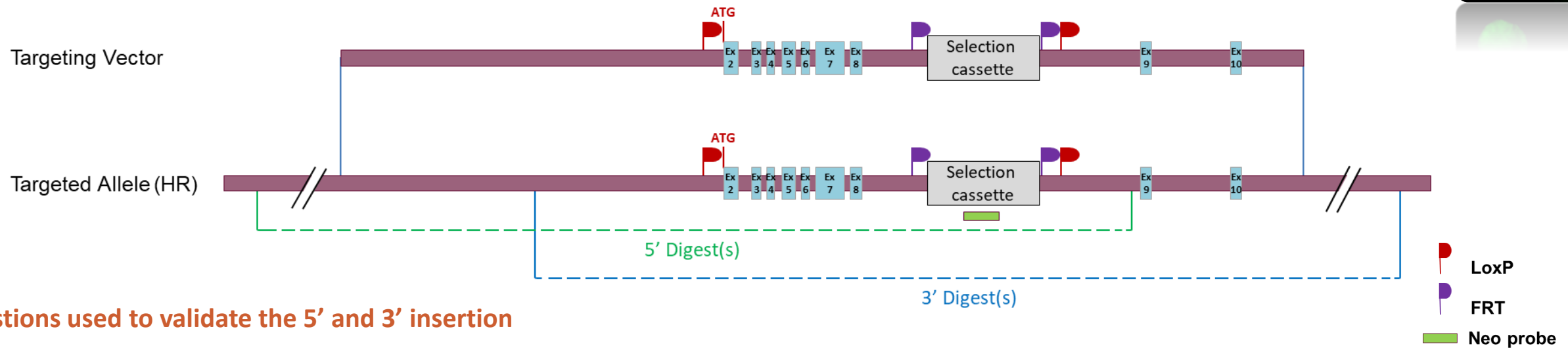
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

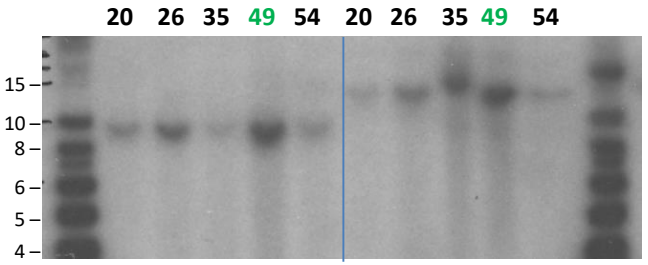
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



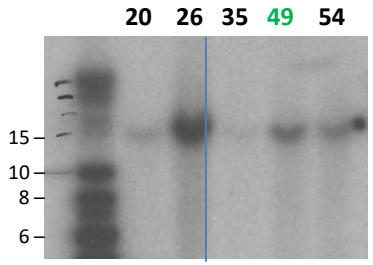
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	SspI	10
		SacII + SexAI	13.5
	3' digest	DrdI	15.9

Southern blot - Neo 5'



Southern blot - Neo 3'

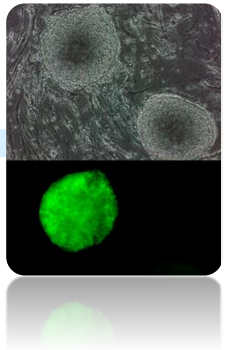


Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
CTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCCGCTG
TAAGTCT

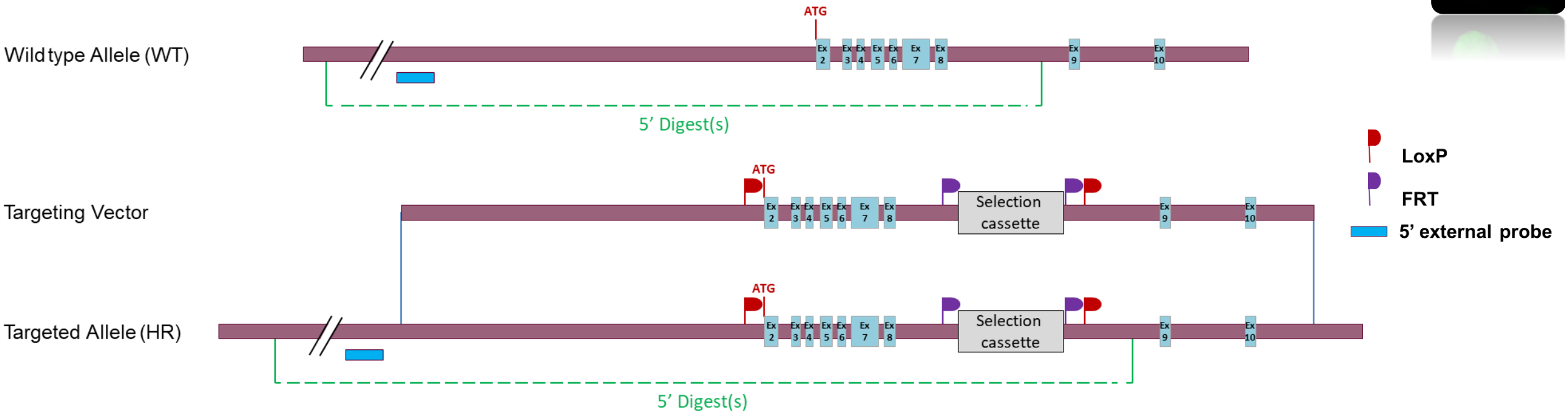
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – External probe

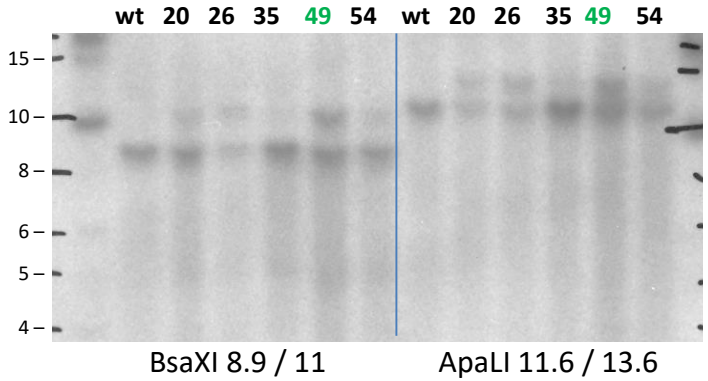


Schematic Southern Blot validation strategy

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



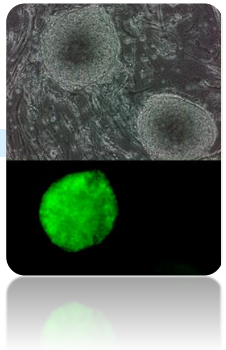
5' probe sequence

GGAAGTAGGGTTGCTCAGAGGAACTGTGGGTGTTAGGCAGGTT
GTCTTCCCAAAGGACATCAGTTGTGAGAACTTGCATTCTAAAG
TTCAGTCTTTTCTTTTTGCTAGTCCCTCTATAGAAAAAGCCGT
TGTAGAAGGGGCTAACAGTAATGAATTTAAGTTCTGAGTGCGT
TTATGAGAAATTAAGGAGAAATATGAGTGGAGAGTTCATTGAG
AATGGAAATCCTCAAGAAGTAAGACTGCCTTTTAGTAGTCTTT
CGAAGCCGGGCGGTGGTGGGAAACCGAATGACTTCATAAAACA
TTCACACTAAAAGATTATTTGGAGCAAAGTTCCAGACCG

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	BsaXI	8.9	11
	5' second digest	ApaLI	11.6	13.6

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	qPCR	Giemsa
#26	Pass	Not done
#35	Pass	Limit
#49	**Pass**	**Pass**
#54	Pass	Not done

[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 C57BL/6NTac 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 #49 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
#49	1	3	1	5

Breeding to F1 generation

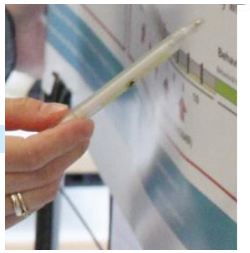
- Four highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with C57BL/6NCrl Flp deleter females that show maternal contribution*(health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ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

- Germ line transmission was obtained the : 27/11/2013

- Allele nomenclature (following MGI guidelines) : **Larp7$^{tm1.1dlcs}$**

***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



AAGCCAGATGAAGAGGTCAGGTAACTGTTGGCATTAGGCAAACACTGAGCTCATTGGGGTTCCGCTTAAATGGTAGGAATGAAAGCCTGGAAGGATACGGTGAGTTGAAAACCTGAGATGAGATCCAGTATTCAC
CTATTTTTTAAAGACAAGTACACTGTAGCTGTCTTCAGACACGCCAGAAGAGGGCGGCAGATCCCCTTACAGATTATAGATGGTTGTGAGGCACCATGTGGTTGCTGGGAATTGAACTCAGGAGCAGTTCATCTCTC
CAGCCCTCCCCCCCCCCCCCATAGGGCTACAATGTACTTAATTAGTAATTATCAGGTAGAGGGTTTTTTTTTCCTCTTTTTTTTAATTAGGTATTTTCCTCGTTTACATTTCCAATGCTATCCCAAAAGTCCCCCATACCCAC
CCCCCCAATCCCCTACCCACCCACTCCCCCTTTTTGGCCTTGGCGTTCCCCTGTACTGGGGCATATAAAGTTTACAAGTCCAAAGGGGCCGGCCAAGCTTCTCGAGCTTAAGGTCGACCTGCAG**ATAACTTCGTATAA**
TGTATGCTATACGAAGTTATTTAATTAAGCCTCTCTTTCCAGTGGTGGCTGACTAGGCCATCTTTTGAGGTAGAGGTTTTACTACTTGAATTTACTACAGAATTTGCACTTGCCTGACAGACCAGCTTTAAAAACATCC
TTACTGATGTCAGTGAGATGGCGTCCTGCAGCAGGAATTCCTGCAGCAGGAAGGTTCAGGTGACAGGGGCATTTAGGCACTTTACAGTTTGGCATTTTTGTTTTTTCCCCTCTCAGAATGGAAACTGAAAACCAAA
AAACTATGGAAGAAAGCACTAAGAGAAAAGAAGAAAAAAAGAAGCGCTCCCGGGTTAAACAGGTGCTTGCAGATATTGCTAAGCAGGTGGACTTCTGGTTTGGAGATGCAAACCTTCACAAGGACAAGTTTCTG
CGAGAGCAAATTGAAAAGTCTAGAGATGGATGTAAGTTTTTTGTCTTTGTTGGAATTTCCAACTCACATTTATATGGGGCCAGAGCCTCCTGAGAGCTCAGTACTGTATATGTTCATGACACACAACTGCATGTTGAA
CTCGTGCTTGTTTGCACACATGTATATATTCCAGTGTTTAACATCCCACTATCCTATTTACTAGATGTGGACATTTCTCTCCTGGTGTCTTTTAACAAAATGAAGAAGCTGACAACTGATGGGAAGCTAATAGCCAGAGC
ATTGAAAAGCTCATCTGTTGTAGAGGTAAAAAACTTACATTTCAAGTCACCCACATCCAATTAACTACTGACTTTATTTAGCTTTCCTTTTTTTTTCCCCCCTCATAAAGTTGGACTTAGAAGGGACCAGAATCAGGAG
GAAAAAACCCCTAGGTGAGAGACCAAAGGACGAGGAGGAACGCACGGTATATGTGGTAAGAAATGACACAAATTTTAATTCTAGTTCATTTATTTATAATTCATTTATTGGTAAAAATGTAGGAACTTTAAAATGTTC
ATACCATGATTTATTACAGGAGTTGCTCCCCAAAAATGTTACTCATAGCTGGATTGAGAGAGTATTTGGGAAATGTGGCAATGTGGTTTACATAAGTATTCCACATTACAAGTCTACTGGGGATCCTAAGGGATTTGCC
TTTGTGGAATTTGAAACAAAAGAGCAAGCAGCAAAAGCCATTGAGGTACGTACTTCTGTGGGACCCTGAAAAGTAAAGAAAAAGCACAGGGTTAACAAGAGTAACAAATTCTGCAATCAATCTTTTCAGTTTCTG
AACAACCCACCAGAAGAAGCACCTAGAAAGCCTGGTATATTTCCCAAGACAGTGAAAAACAAGCCCATCCCTTCCTTACGAGTAGCAGGTGAGTGCTTACTTAGAGTGGCCAAGTAAACAATAAACTATTATGACT
GTAAATAATAAAATCTTGTTACAGAAGAGAAGAAAAAGAAGAAAAAGAAAAAAGGCAGAATAAAGAAGGAAGAGAGTGTGCAGGCCAAAGAGTCGGCCGTGGACAGCAGCAGCTCGGGCGTGTGTAAAGCC
ACGAAGAGACCAAGGACGGCCTCTGAGGGCTCCGAAGCAGAAACCCCGGAAGCTCCAAAGCAACCTGCAAAGAAAAAGAAGAAGCGGGACAAGGTGGAAGCATCCAGCTTACCTGAAGCCAGGGCAGGGA
AGAGGGAGAGGTGCAGCGCCGAGGACGAAGACTGCCTCCCCCCAAGGCCAAAAGCTAAGAAAAGGGCTCAGAAGGATGGCGTCGGCCAAGCAGCTTCCGAGGTCTCCAAGGAGAGCAGAGGTACCGTGGCA
GTAGGGTTACAAATGTTTTAACCAGTTTAACCACAACAACGGACTTACGGAAATCTTTTTTCTAGACTTAGAATTCTGCTCTACTGAGGAGGAGAAGGAGACAGACAGAAAAGGTGACTCACTCTCAAAGGTGAA
GAGGAAGCATAAGAAGAAACACAAGGAGAGACATAAGATGGGCGAGGAGGTTATACCATTGAGAGTGCTGTCCAAGTAAGTGGCCACTGACACAGGTCCATCACCATTGCTAAAGTGCAATTCCAATTTCTATTC
AACAGAGTTGCATGTTAGCAACAGTGACGGCCTTGGTCTATCTATATGCAGCACAATGAAGATTAAGTAGGAGCCACCACACTCAAACATGGAAGCACTTATTTTTAAATGCACAGCAAGTGCCTCCATGTTAAAGT
AAAGGAGGCCCCTTAAGCAGACTTGAGTGGTCTTTACAAAGGCATGAATATAAAGGAAAACATACCATCACCAAAACATGGAAGCACTTACTTTCTTAGGTCTAAAGCAAGTACAACCACGTTTAAGTGGTGGGAA
GCCCAGTCAATAACTCCCTTAACCTACAGCATCTTAGAGAAAATGTTGAACAGTTTAAGACACCCCCACTGAAACATGGAAGCACTTGCTTTTGTTTAACACAGCAGGTAACCCCATGTTAAAGCAGAGGGGACTCC
CATCAGACTCTGAATTAAAAGGCTAGCTTTCTTATTCCAGAGTTTCTTGAATTCACTTCTACTAAAACATGGAAGCACTTACTCTTCGATGAGACAGAAAGCATTCCCATGTTAAAGTTGAAGGGAACCCACCCACAA
GGCAACTAGTTAAAACAGAAGAGCACACCGGTG**GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC**GCGGCCGG**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GGATCCATCGACCCCCTGCAGGAACCC
ACAGGAAAGTAAAGTGACCCGGTTACAGACATAAGCTTTACCTCCTTTACCTTCGTGACCGCCTCCCAAAGAGTCCTGTTCTGTCCTCGGAGGGTGAAAGACGCCGACTTTAGACCACTTGTATTTATACTACTCCTA
ACTCCTGAGTTCCTTTTGGCTTTCTATCTCTGAGAATCATTACAAGACCCTTTGTTATTTTAATGTATCAGCTCTGGGGAAAGTAGGTCCTGGGGACCTGGGTAGGTCTGGATTTTTTTTTTTTTTTTCCTTTTTTGAAG
TAAACAGATGTCAATGTTAACTGCTAAAACCATTACCACAAGATATCCTCCTCTTTAAAAAAAATCTCCTATCTGCCCGTTTGATGGCACAGATTAGGGTAACAATGAGCGCTCCCACAGAGCCAGGAGATGCATTGT
GCTTCTCTGGAGGAGCCCTAGCTGTTTGAAGATACATCTACATGTTAAATGTATGTAAATAAATAAATTGCTTCCATGG

LoxP FRT Exon 2 to Exon 8

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/10/16

Prepared by Romain LORENTZ, IE

Verified and finalized 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