phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Generation of mouse model :

Kif5c E237V point mutation with conditional KO potential

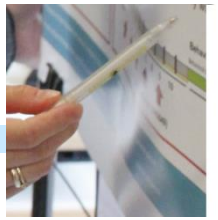
Project code: G4563/ IR4563

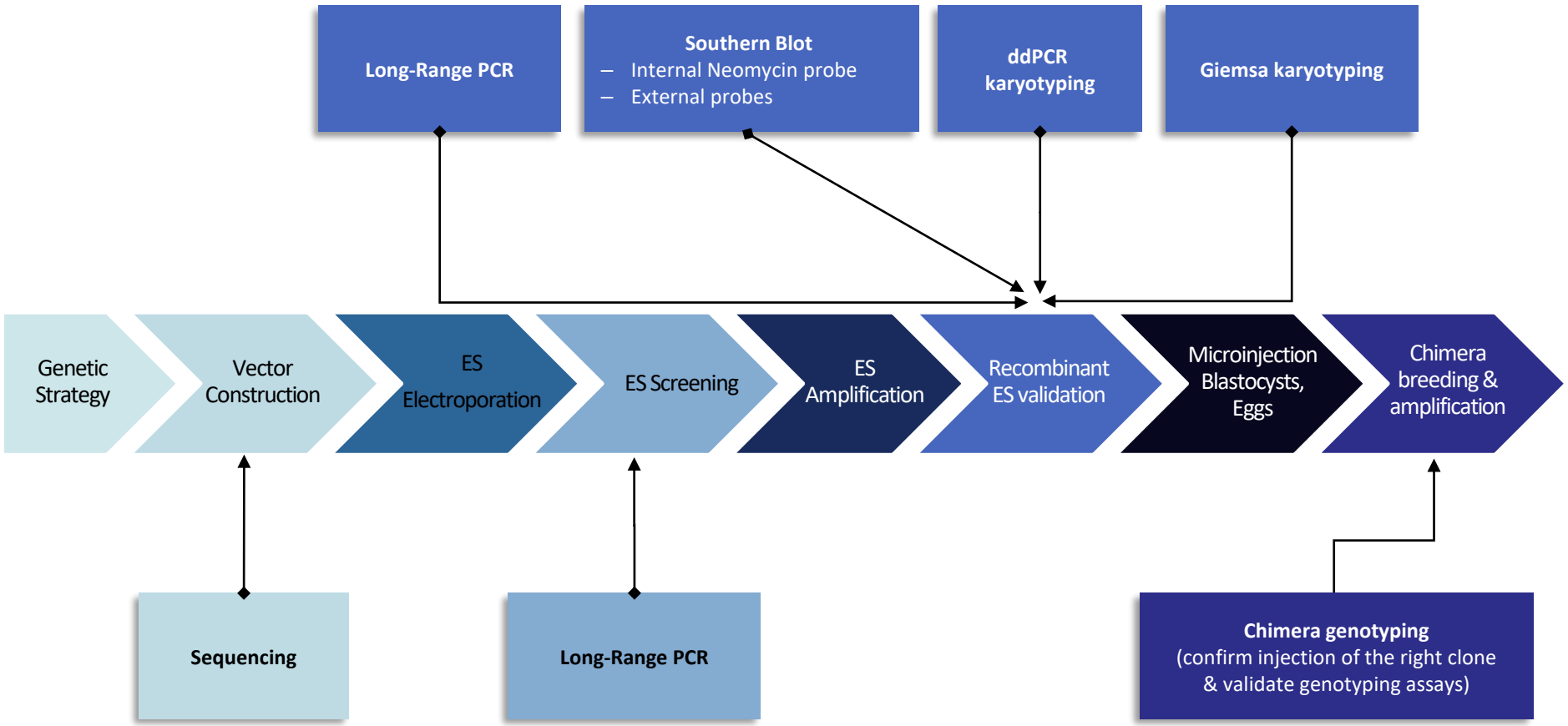
Report finalized: 20/09/2023

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

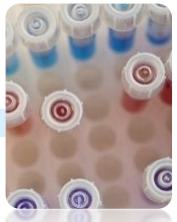
MODEL GENERATION TECHNICAL REPORT

PROJECT PROCESS & QUALITY CONTROLS



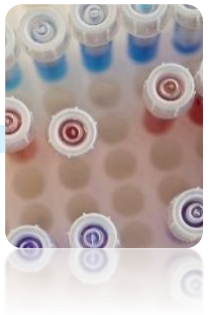


2 GENETIC STRATEGY



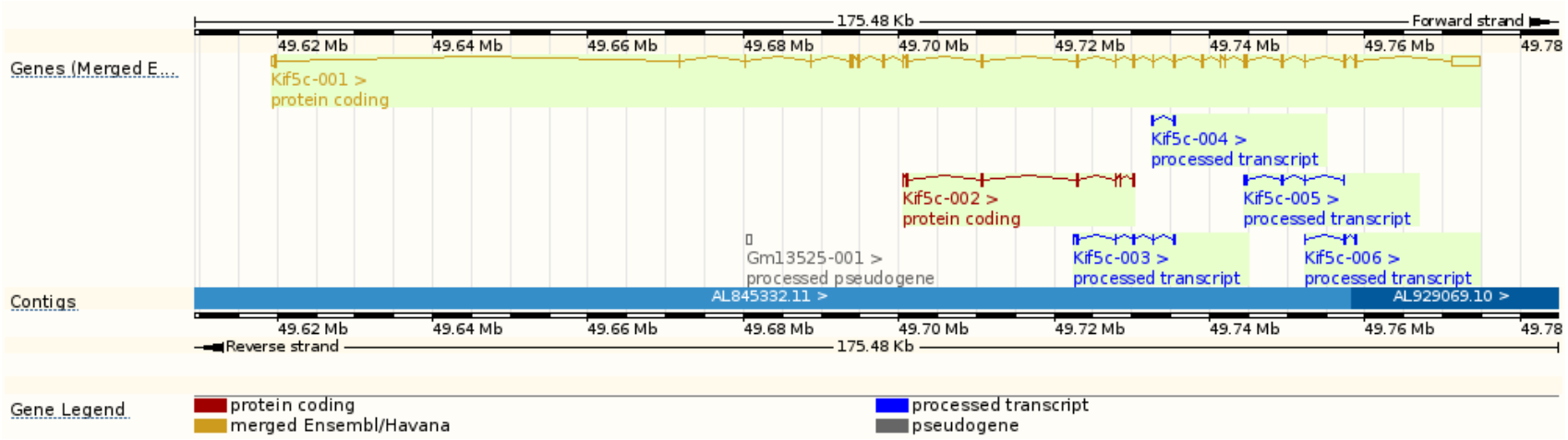
- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

Kif5c mouse genomic locus – structure

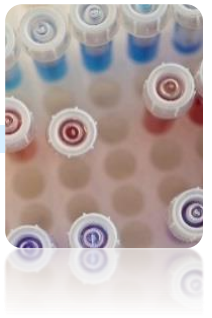


Location :

Ensembl ID: Kif5c ENSMUSG00000026764

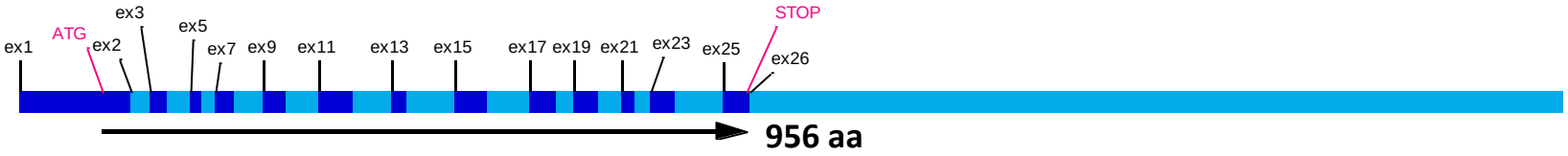


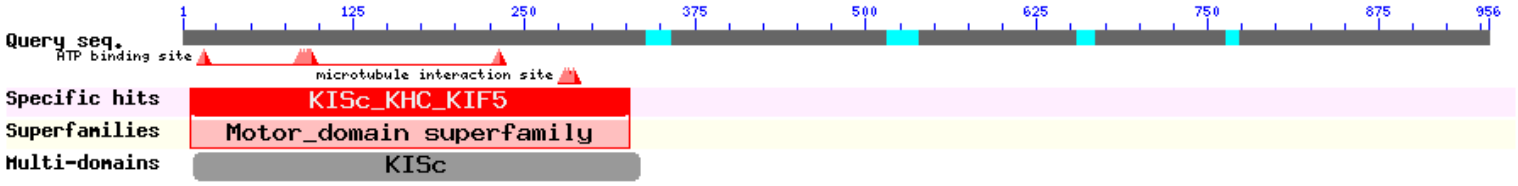
Kif5c mRNAs and proteins



Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CDS incomplete	CCDS
Kif5c-001	ENSMUST00000028102	6856	ENSMUSP00000028102	956	Protein coding	-	CCDS16024
Kif5c-002	ENSMUST00000146247	729	ENSMUSP00000117370	243	Protein coding	5' and 3'	-
Kif5c-003	ENSMUST00000138834	775	No protein product	-	Processed transcript	-	-
Kif5c-004	ENSMUST00000152353	383	No protein product	-	Processed transcript	-	-
Kif5c-005	ENSMUST00000127245	367	No protein product	-	Processed transcript	-	-
Kif5c-006	ENSMUST00000132968	480	No protein product	-	Processed transcript	-	-

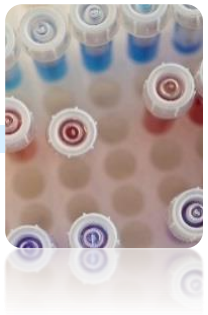
Kif5c-001 ENSMUST00000028102



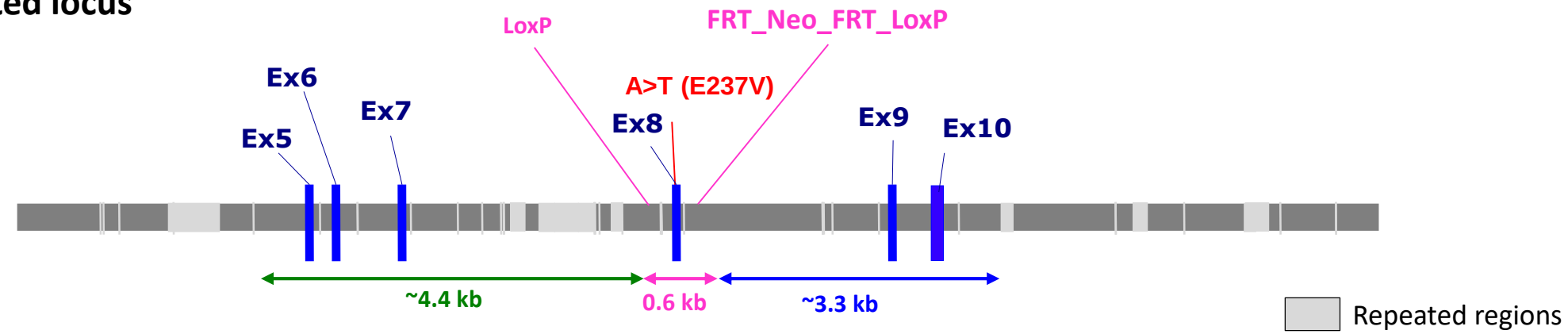


phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

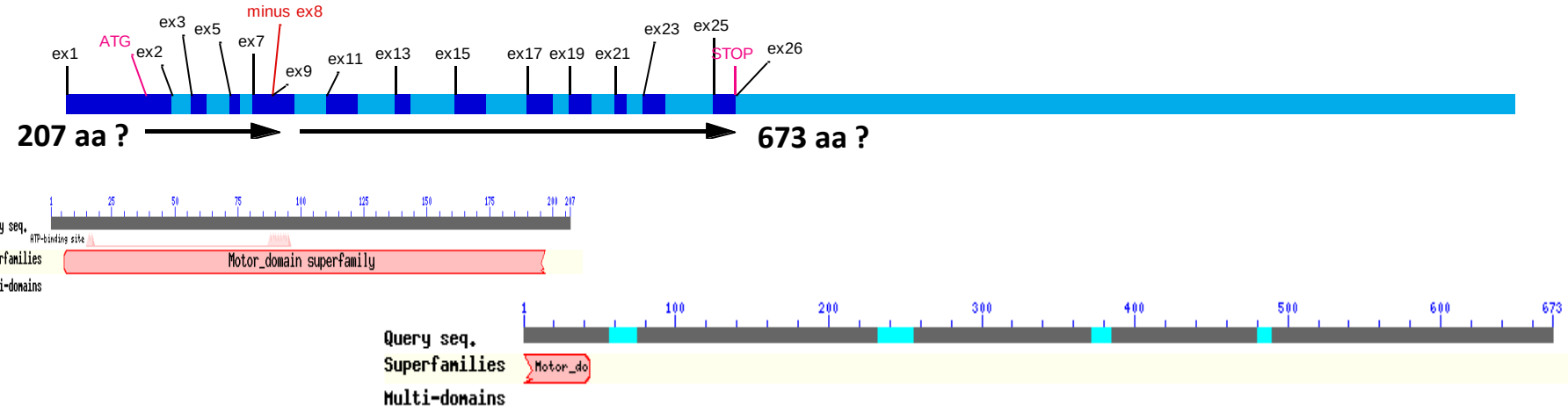
Approach selected: E237V PM with conditional KO potential



Targeted locus



mRNA and protein expressed after Cre mediated excision



phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Partial cDNA sequence

ex7

GGG TGC ACC GAG AGG TTT GTG TCA AGC CCG GAG GAG GTC ATG GAT GTG ATC GAT GAG GGC AAA GCA AAC CGA CAC GTG GCT GTG ACA AAC ATG AAC

G C T E R F V S S P E E V M D V I D E G K A N R H V A V T N M N

GAA CAC AGC TCT AGG AGT CAC AGT ATC TTC CTG ATT AAC ATT AAG CAA GAG AAT GTG GAG ACT GAA AAA AAA CTC AGC GGG AAG CTG TAT TTG GTT

E H S S R S H S I F L I N I K Q E N V E T E K K L S G K L Y L V

A>T (E237V) ex9

GAT TTG GCT GGG AGT GTA AAG GTC AGC AAA ACC GGT GCC GAG GGA GCT GTT CTT GAT GAA GCT AAA AAT ATC AAC AAG TCT TTG TCT GCT CTT GGA

D L A G S V K V S K T G A E G A V L D E A K N I N K S L S A L G

AAT GTG ATT TCT GCC TTG GCA GAA GGG ACA AAA ACA CAT GTA CCG TAC CGG GAC AGC AAG ATG ACT CGG ATT CTC CAG GAC TCT CTG GGT GGG AAC

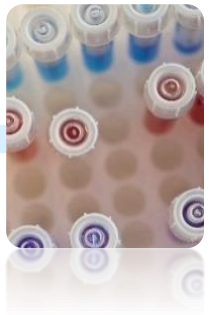
N V I S A L A E G T K T H V P Y R D S K M T R I L Q D S L G G N

ex11

TGT AGG ACC ACC ATT GTC ATT TGC TGT TCT CCT TCA GTC TTC AAT GAA GCC GAG ACC AAG TCC ACG CTG ATG TTT GGA CAG AGA

C R T T I V I C C S P S V F N E A E T K S T L M F G Q R

PROs& CONs evaluation of the strategy



I. Pros

- **E237V mutation introduced**

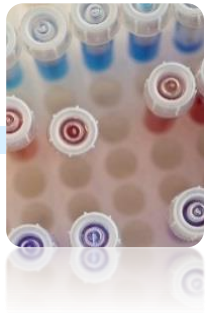
I. Cons

- **A protein of 207 aa might be expressed after Cre mediated excision if RNA decay does not occur.**
- **A protein if at most 673 aa might be expressed after Cre mediated excision if reinitiation occurs at one of the in frame ATG present in exon 10 or further exons (if RNA decay does not occur.**
- **Presence of repeated regions might render PCR amplification and/or PCR screening difficult**

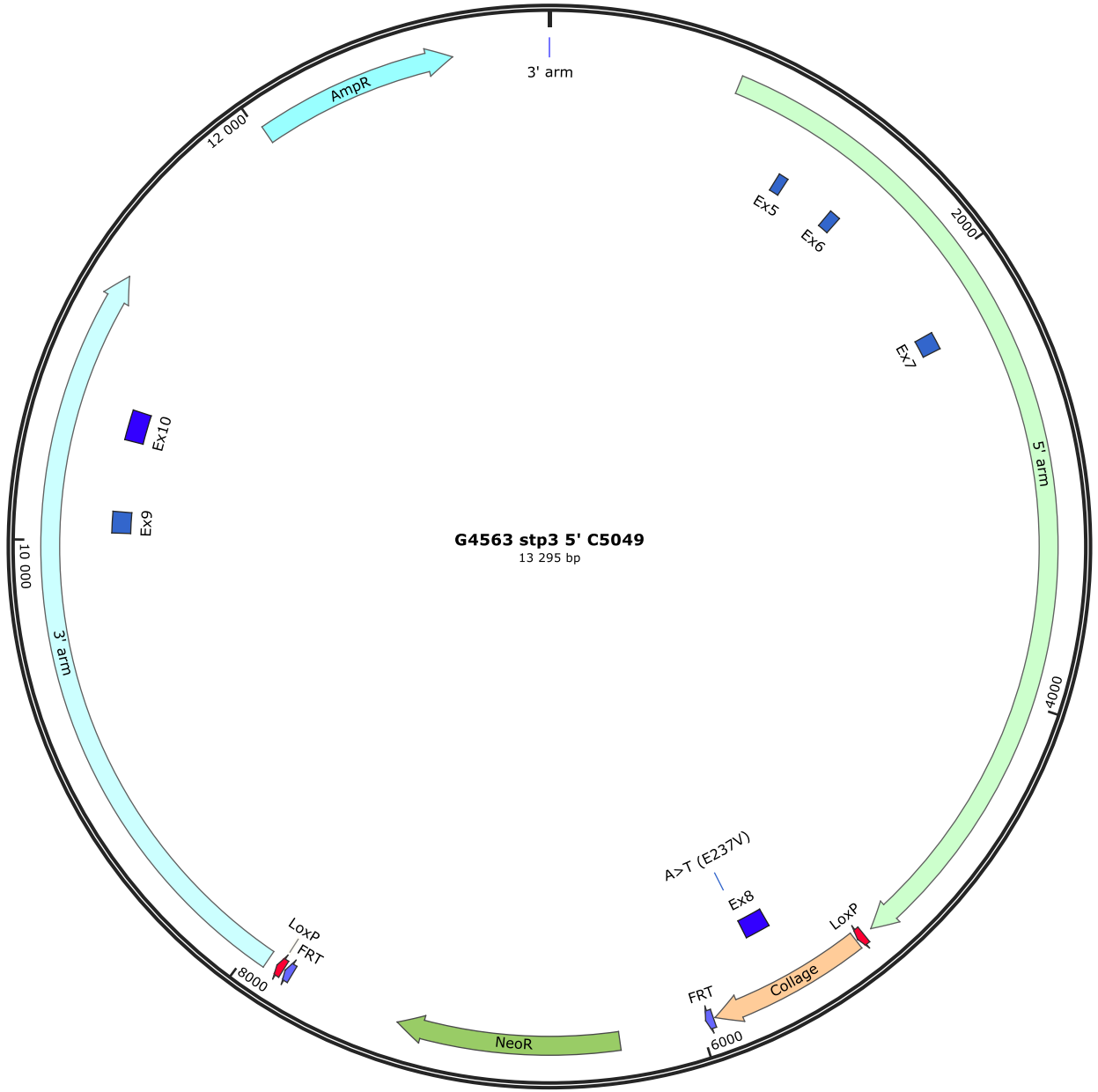
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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

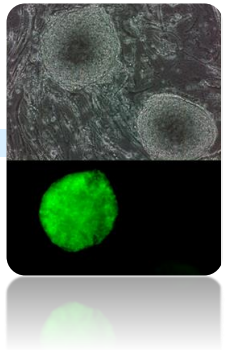


Created by SnapGene



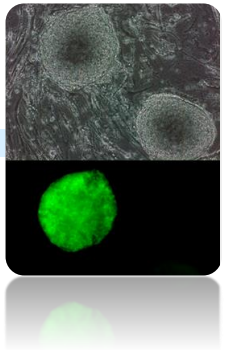
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

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



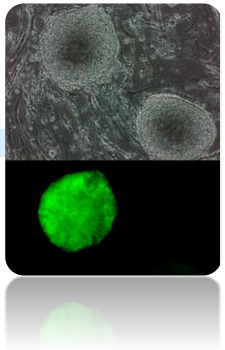
The targeting vector was electroporated in the proprietary C57BL/6NTac BD10 ES cell line.

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.

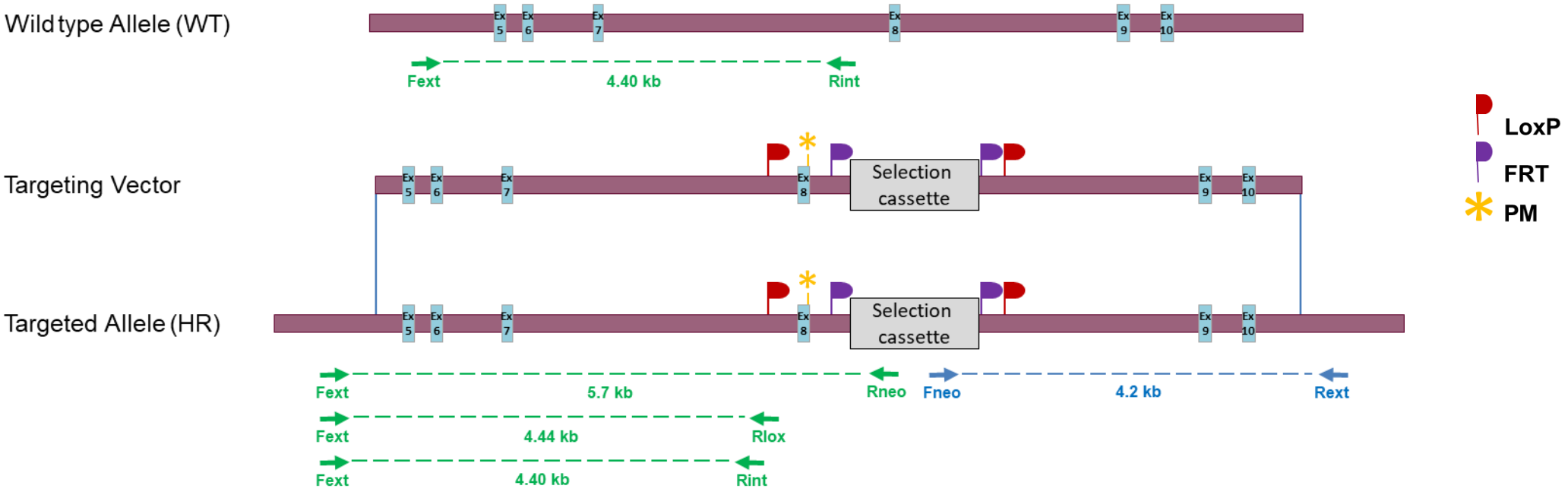
Screening process steps:

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 ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

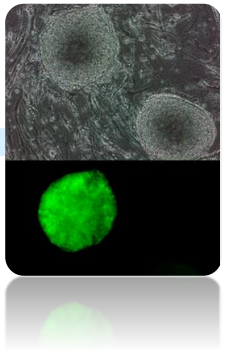


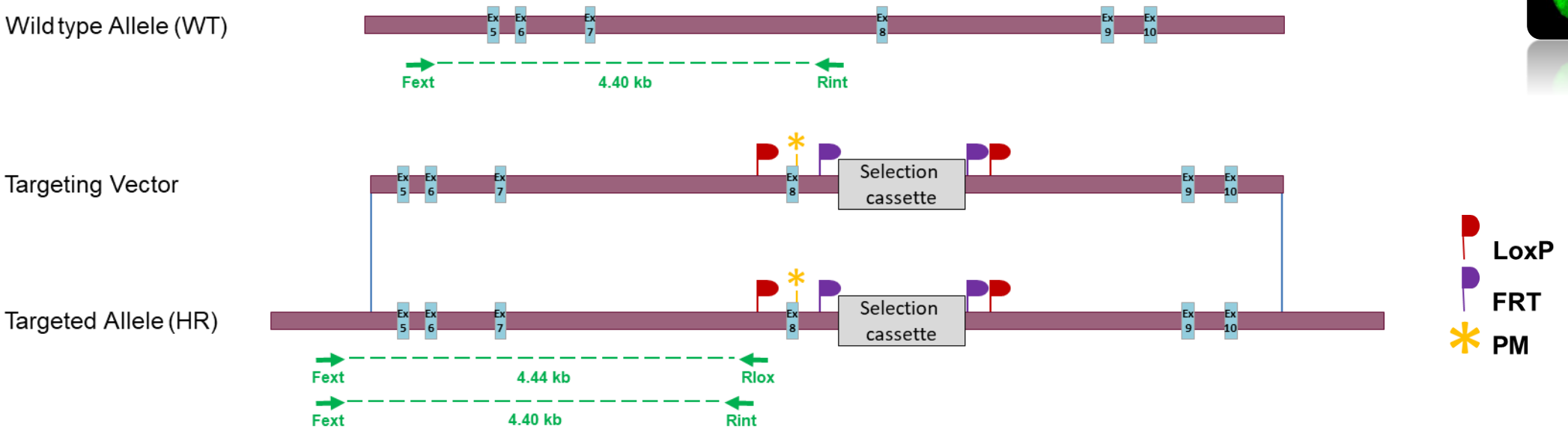
Schematic 5’ and 3’ PCR screening strategy



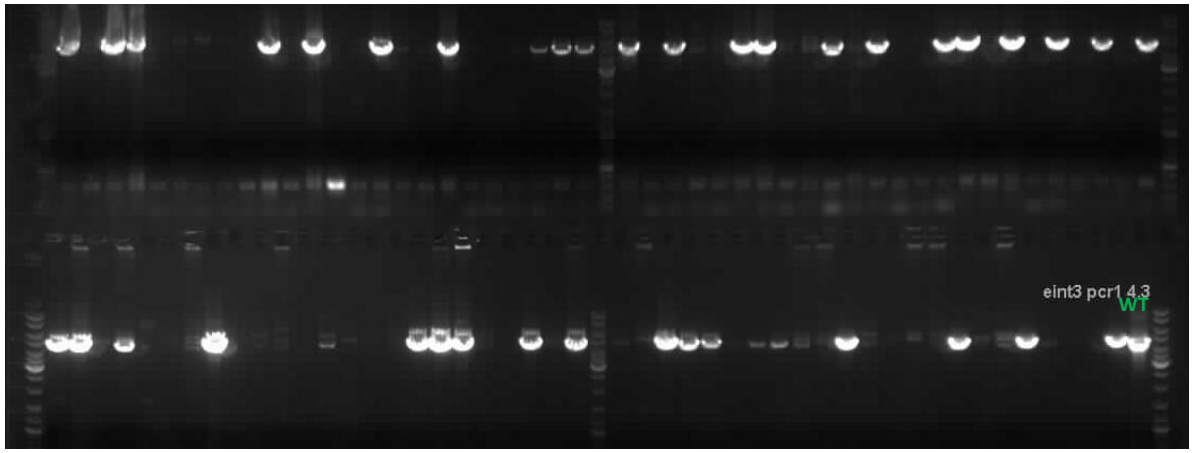
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	CATCATGCAGCTGGGTGAAGTCTGC	4.44 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	CATCATGCAGCTGGGTGAAGTCTGC	4.40 kb
	Rint	TCCATCCCATACCCCACTCTCTCAA	
5’ PCR	Fext	CATCATGCAGCTGGGTGAAGTCTGC	5.7 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.2 kb
	Rext	CTGCTCTAGCTCCTTCATGCTTGCA	

Long-Range 5’ PCR screening – results

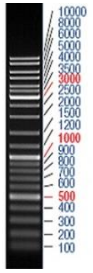




PCR Fext – Rlox : 4.44 kb



WT : Controls DNA



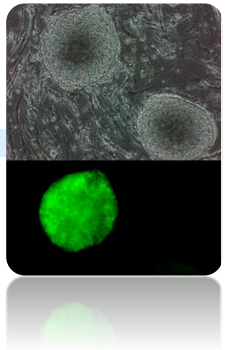
Ladder pattern

PCR Fext – Rint : 4.40 kb

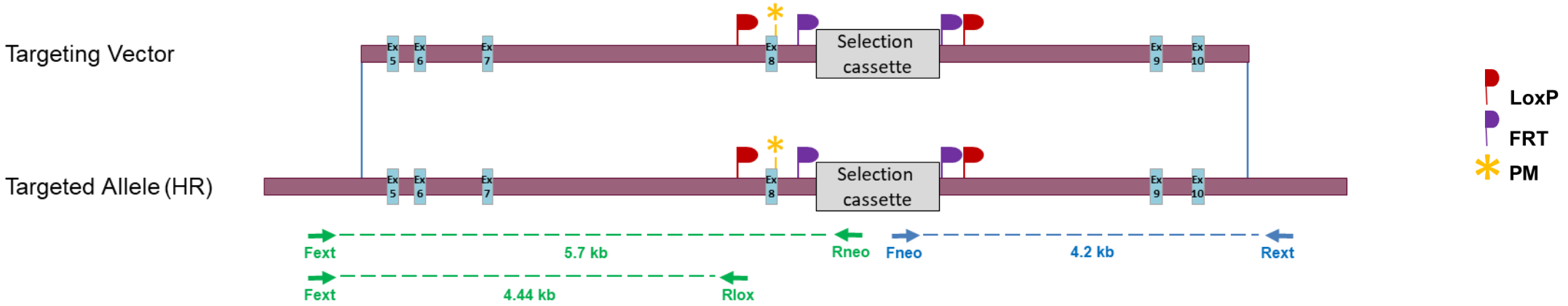
Five candidate clones out of the 38 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

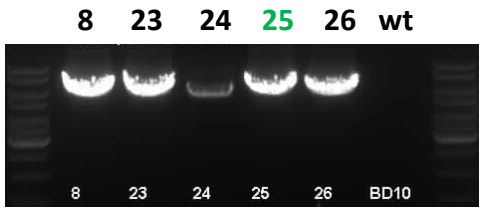
phenomin ics
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES validation by Long Range PCR

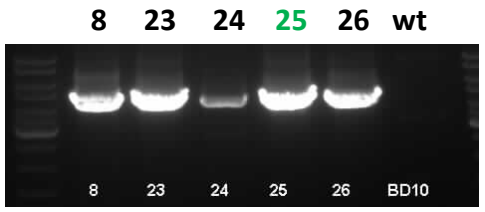


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

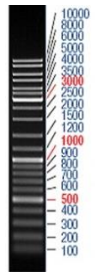




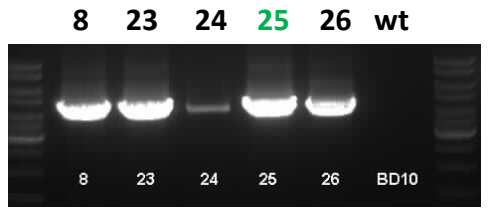
PCR Fext – Rneo : 5.7 kb



PCR Fext – Rlox : 4.44 kb



Ladder pattern

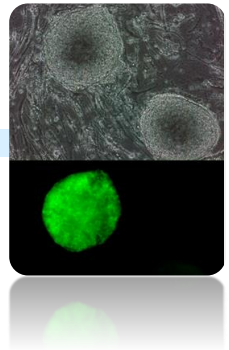


PCR Fneo – Rext : 4.2 kb

Fice candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Fiveclones (clones #8, #23, #24, #25 and #26) were confirmed.

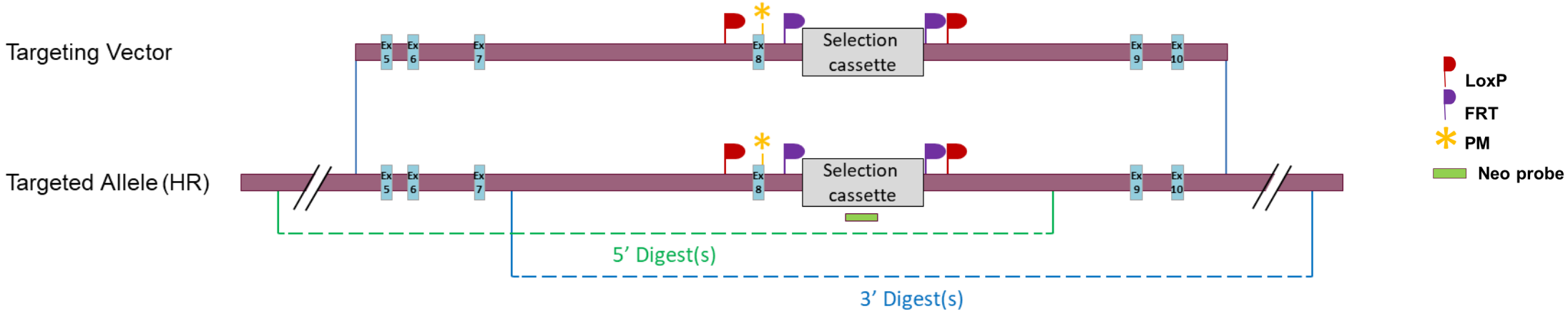
phenomin ics
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

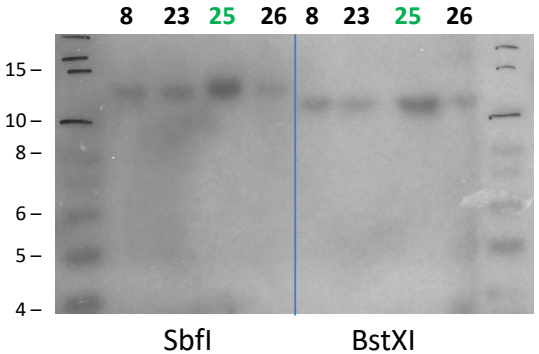
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



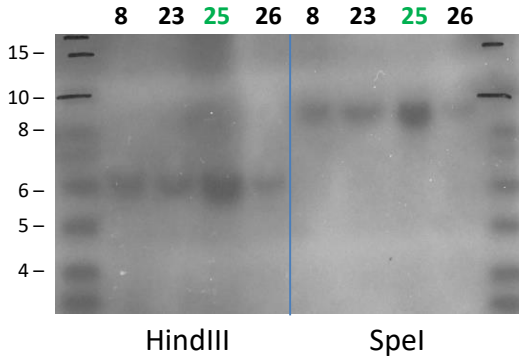
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	SbfI	12.3
		BstXI	11
	3' digest	HindIII	6.4
		SpeI	9.4

Southern blot - Neo 5'



Southern blot - Neo 3'

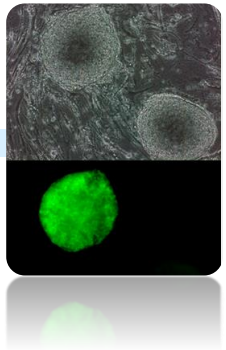


Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA
CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGC
GGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGT
CTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGA
GGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGG
GTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTT
TACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCCGCTGTAAGTCT

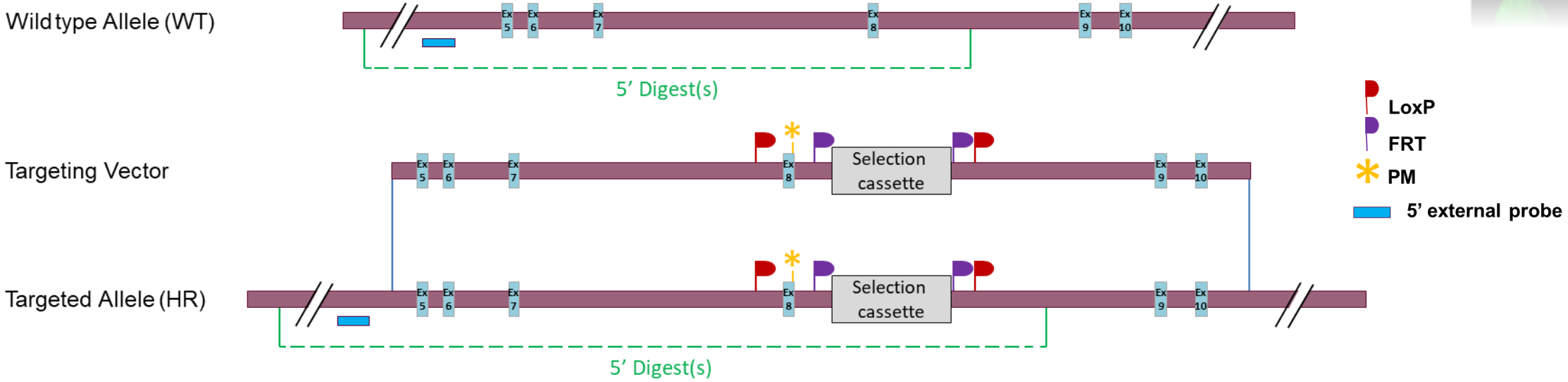
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – External probe

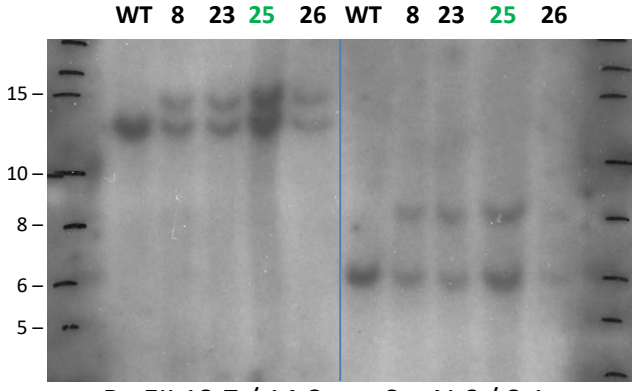


Schematic Southern Blot validation strategy

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

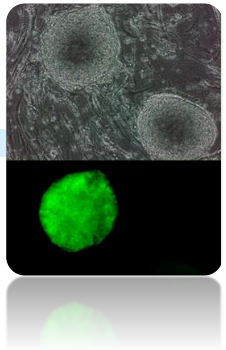
CCAGCTGACCTTAGATTCACAACAACCCTCTCAC
TCAGCCTTCTTATCTGGGACATAGGCATAGCTAC
CACACCTGGCCAATAGTCTTTATTAAACCACATT
TGTTCCAGTCCATCACAGACTCCAAACCTGGTGT
CTTCATCAGTACCTTCTCAGGTACCTGTCTGAAC
TCCTGTTGTTCCAAGCATCCTTAGCCTCTCCGTC
ATGCACTGTTCCTGTGGGTCTGACGAGGCTGTGG
CATCATGCAGCTGG

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	BstEII	12.7	14.8
	5' second digest	SexAI	6	8.1

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

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
#8	Pass	Pass
#23	Failed	Failed
#25	**Pass**	**Pass**
#26	Pass	Limit

[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 #8, #25 and #26 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
#8	2	1	0	3
#25	**3**	**4**	**4**	**11**
#26	4	4	0	8

Breeding to F1 generation

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

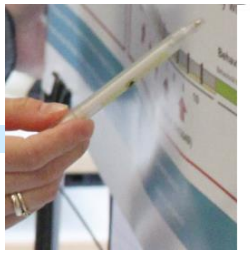
- The flipped NeoR cassette was removed by breeding the male chimeras will Flp deleter females. The KO allele was obtained after and additional breeding of the conditional allele with a Cre deleter line.

- Germ line transmission was obtained the : 19/02/2014

- The line cryopreserved is issued from clone #25.

- Allele nomenclature (following MGI guidelines) : **Kif5c$^{tm1.2Ics}$**

6 SEQUENCE OF THE KO ALLELE



AAAGCTGGGCGTGGTGGCGCACGCTTTTAATCCCAGCACTTGGGAGGCAGAGGCAGGCGGATTTCTGAGTTCGAGGACAGCCTGGTCTACAGAGTGAGTTCCAGGACAGCCAGGGCT
ACACAGAGAAACCCTGTCTCGAACCCCCTCCCCCCCAAAATAAAATGGGATCTTTAGAGGGTTTATTTTTTTTGAACAATCTGGTGAGATGCTTTCTGAGGAATGGTGAAAGTTGCTTGG
GACCATTACTGGGTCTGCTGTAAGGGGAAGCGTTTTTCCTTGGTATCCTGGCCATTTAACCTGGGAGGCCAGTGCGTGTTTCATATGGGTTGTAGAGGAATGTGTCTTAATCTTCCCTGAA
GGAAGCTCCTGGTAGTTCAGGCTACACATGGGCAGGACAAACTGTGTCAGGCACTACACTGTAATAAACTTCAGGGCTTCCACAGGACCAGCAAGGAAGCTGTGAGCTTCTTGGGGGT
TGAGAGAGTGGGGTATGGGATGGAGGCCGGCCAAGCTTCTCGAGCTTAAGGTCGACCTGCAG**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GGATCCATCGACCCCCTGCAGGAT
TTAAATGGCCACTGAGGCCGCAGCTGCAAAAGGTGGGATGGGCGCCCTTAGGATGGAAGGAGCCATCTGATTTCATGGACTCCATACAGTAGGCTCGGGAGGGCTCTCCCCCTGGGAC
ATGGTTGGCCCTGCTTCTGTACCTTTGCCTTGCTGAGGAAAGGTCTGGAACCTGGGCTCTGCCAGTGTTTCCGTTCTCCTGGAAGTGGGTTTGAAGTAACAGGGGACCTTTCATATGGGT
GACCTTGGGTCATCCGGTTGGAAAGGCTTCTTTGGTTTTGTTGTGATTCTCTTTCCACACCTCGCTCTGTTCTATGCCCCCAGGCACCGGCTAAGACCACATCCTCTTCCCATGCCAGCCCC
CTTCCACAGCACCAGGGGATGTTCCCATGGCCATACTCTGGCGGGCACAAGCCCTGTAGACAGCATCATAGAGCCAGCATACATGCACAATTTCTATTTCTGCCATCATCAGTGACATCAG
CTGGTTGTTCTCATCAGAGCAGGTCCTGAAGTACTGTC

LoxP

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/09/20

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