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MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Phf6 cKO

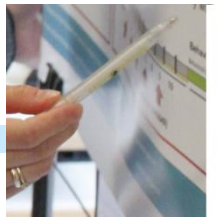
Project code: Kos8114 / IR8144

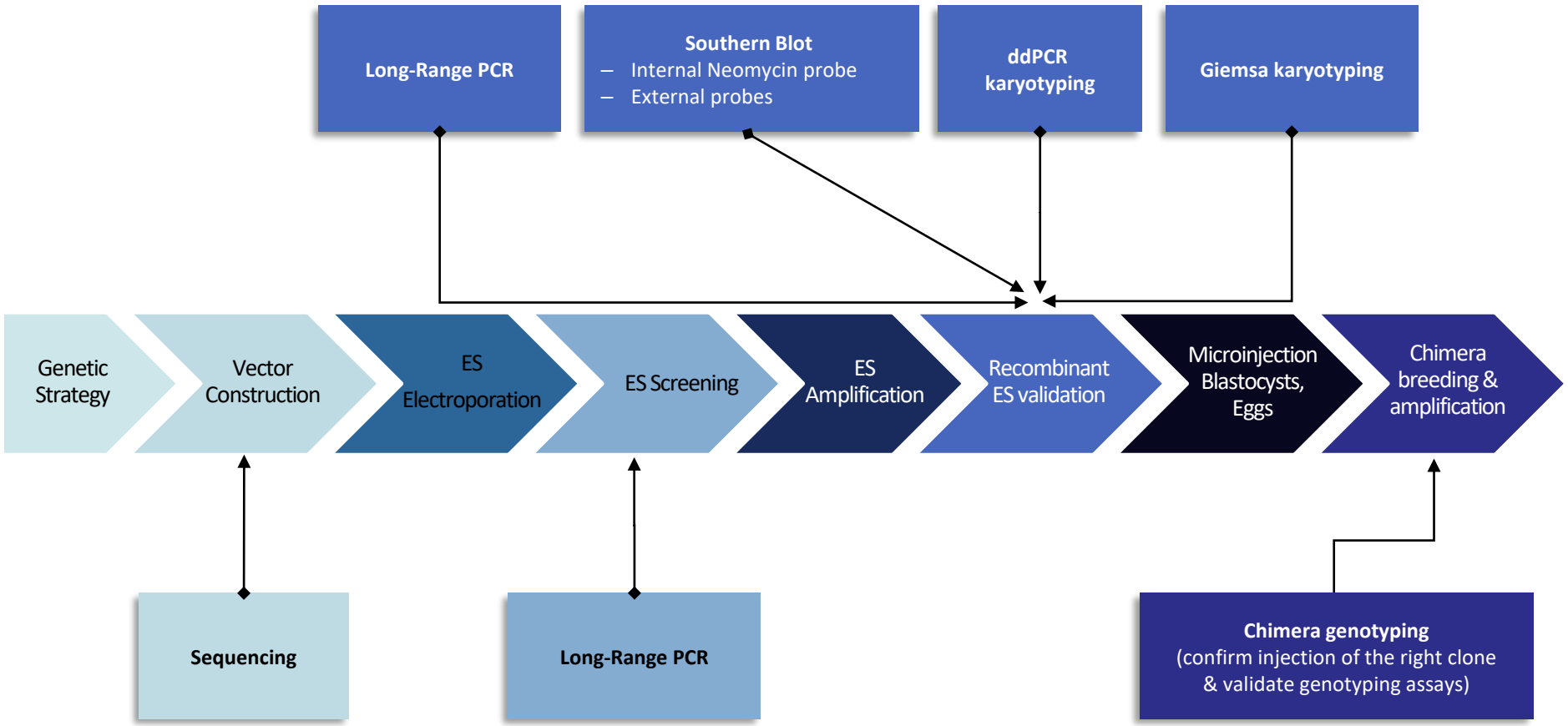
Report finalized: 2023/10/16

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

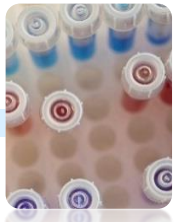
MODEL GENERATION TECHNICAL REPORT

PROJECT PROCESS & QUALITY CONTROLS



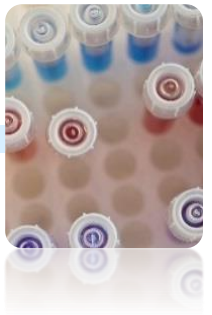


2 GENETIC STRATEGY

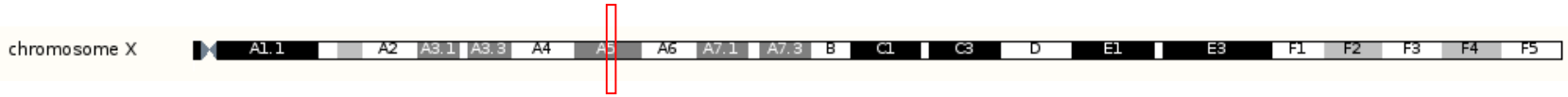


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

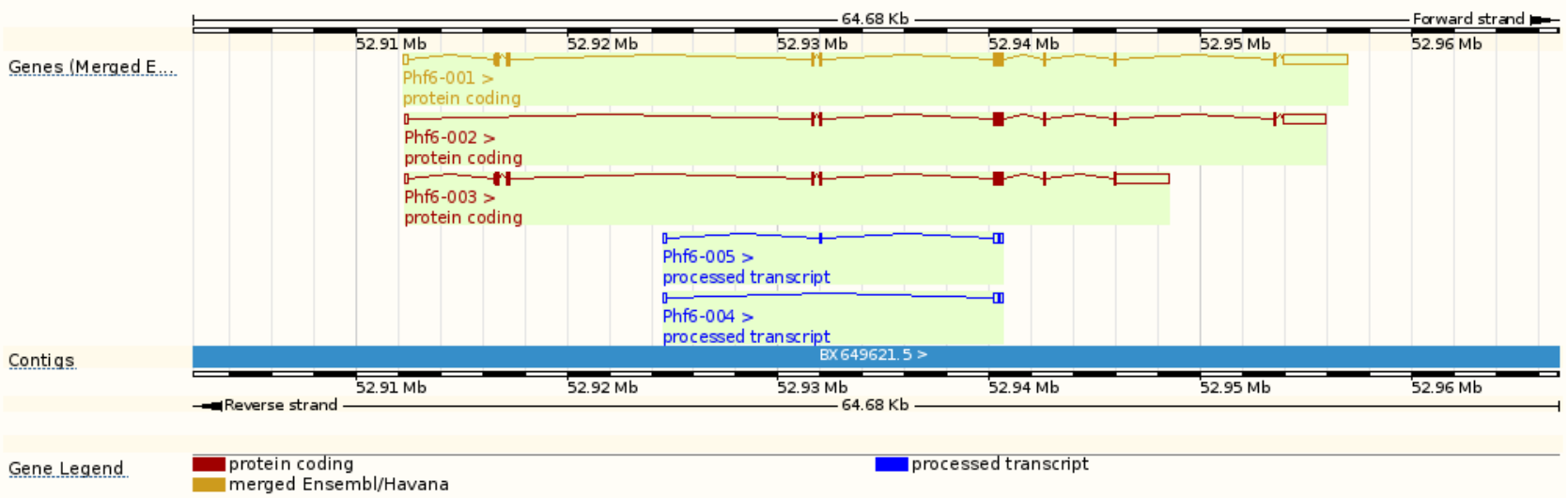
Phf6 mouse genomic locus – structure



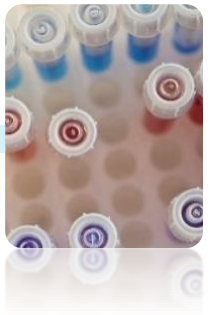
Location:



Ensembl Gene ID: Phf6 ENSMUSG00000025626

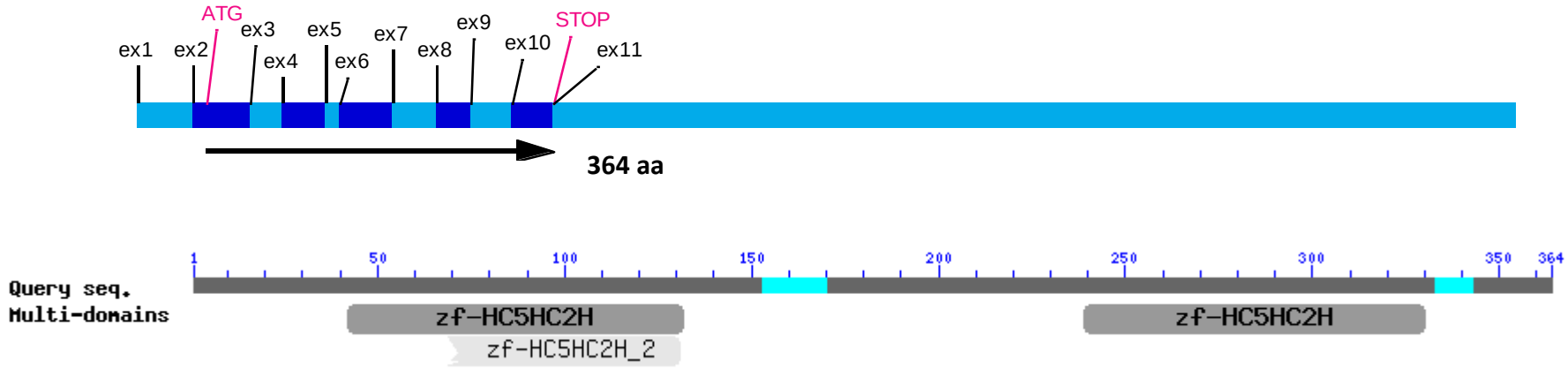


Phf6 mRNAs and proteins

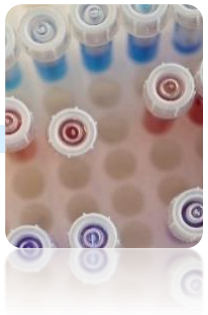


Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
Phf6-001	ENSMUST00000078944	4350	ENSMUSP00000077971	364	Protein coding	CCDS30127
Phf6-002	ENSMUST00000154864	3057	ENSMUSP00000130358	284	Protein coding	-
Phf6-003	ENSMUST00000101587	3639	ENSMUSP00000110497	323	Protein coding	-
Phf6-004	ENSMUST00000179014	377	No protein product	-	Processed transcript	-
Phf6-005	ENSMUST00000177780	404	No protein product	-	Processed transcript	-

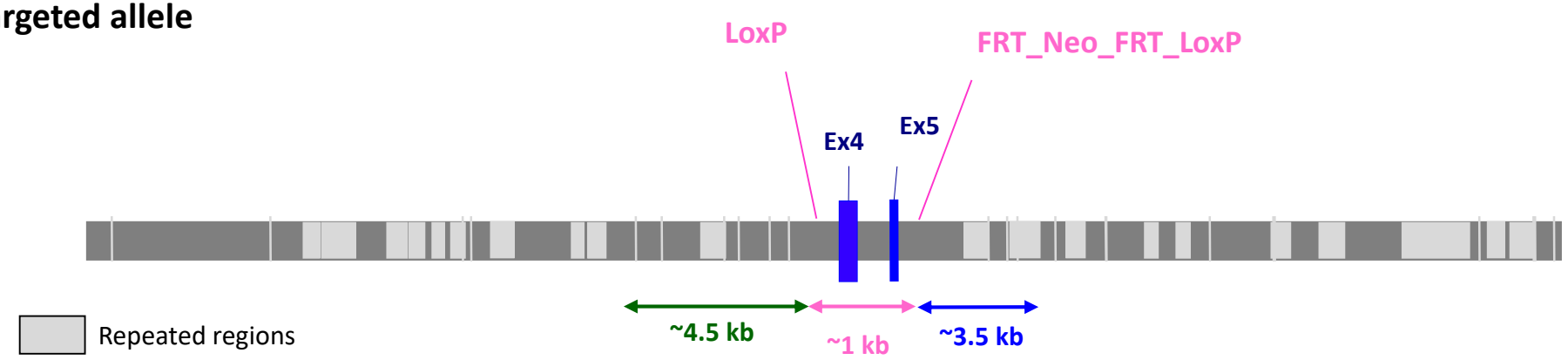
Phf6-001 ENSMUST0000007894



Approach selected: flox exons 4 and 5

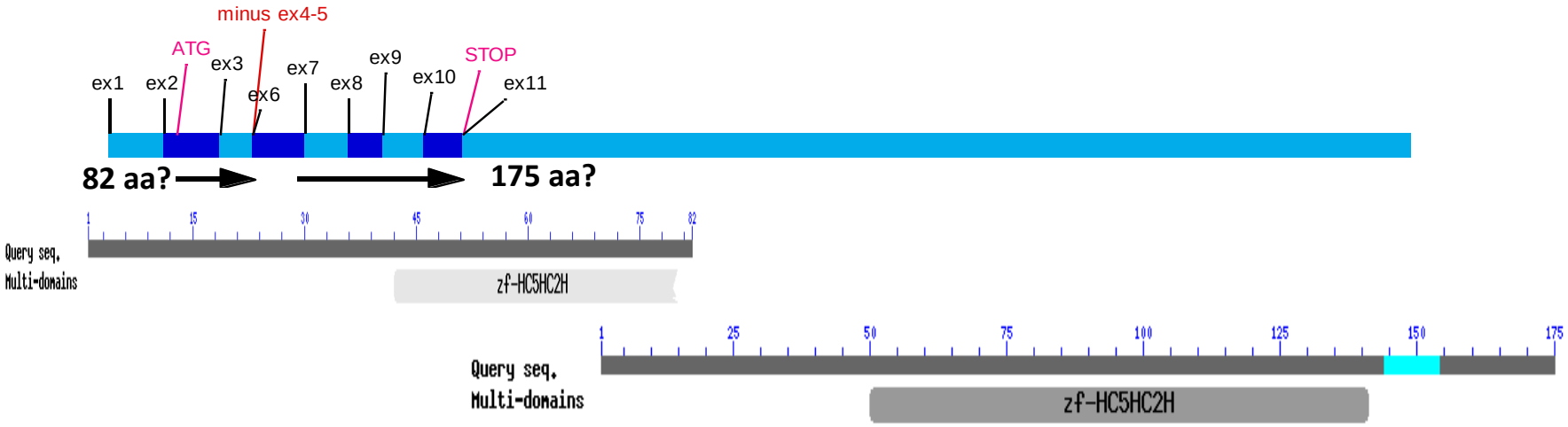


Targeted allele



Exons Ensembl ID:
Ex4: ENSMUSE00001342134
Ex5: ENSMUSE00001258800

mRNA and protein obtained after Cre mediated excision (Phf6-001)



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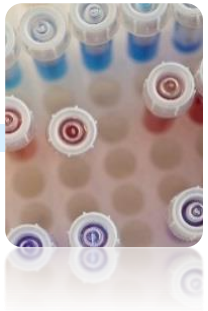
PROs& CONs evaluation of the strategy



Pros

- Appropriate size of the floxed fragment

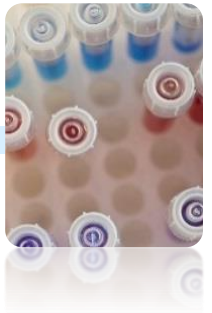
Cons

- A protein of 82 aa might be expressed after Cre mediated excision if RNA decay does not occur
- Presence of repeated regions (in light) in both homology arms (green and blue arrows) might render PCR amplification and/or homologous recombination at the locus difficult

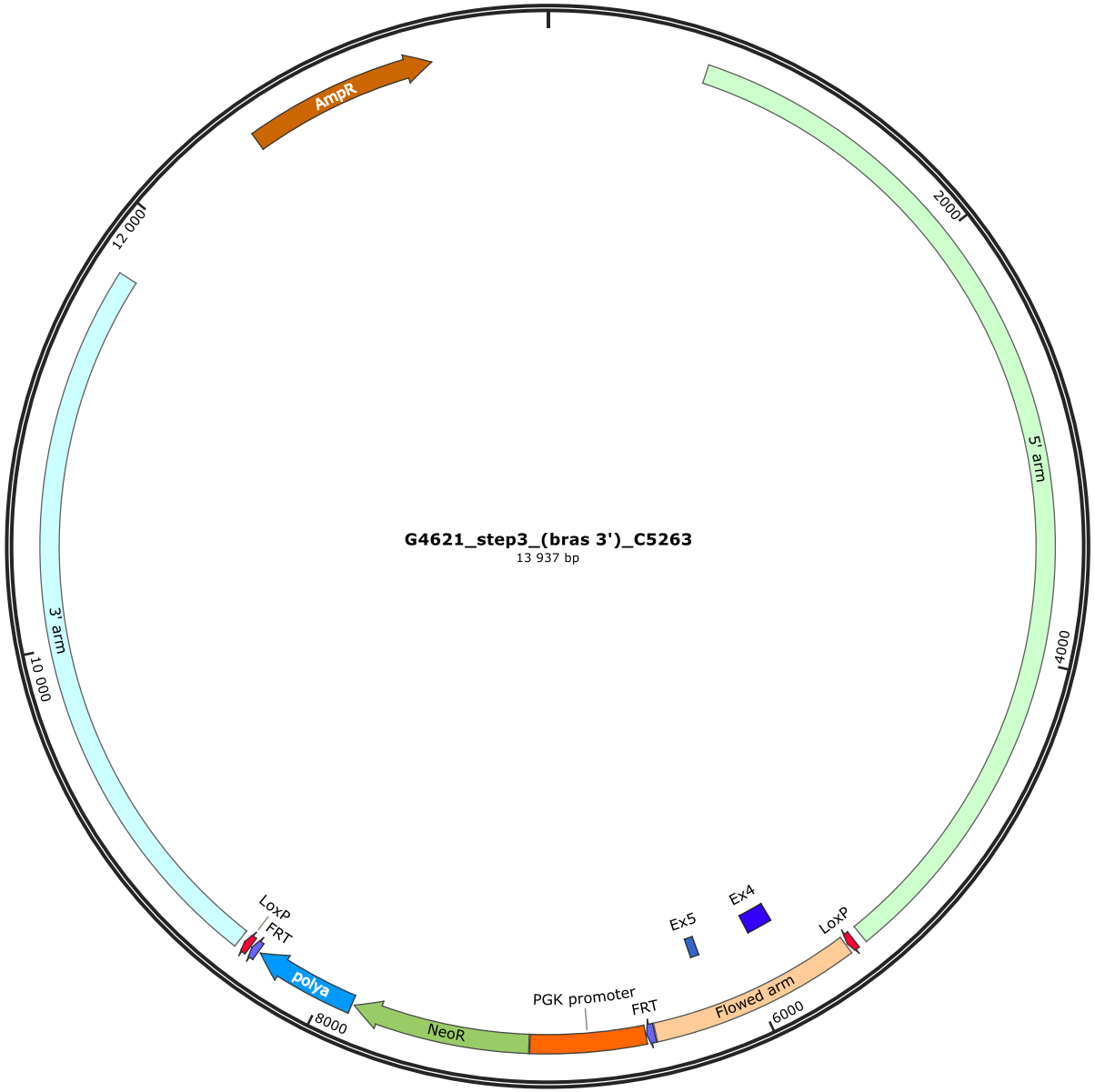
The selection cassette (FRT-Neo-FRT) will be removed by breeding male chimera with a flp deleter line which shows maternal contribution (*Birling et al.*, 2012)

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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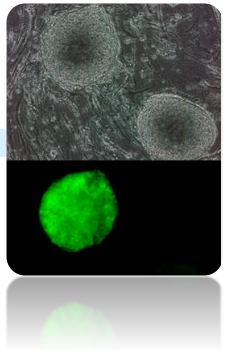
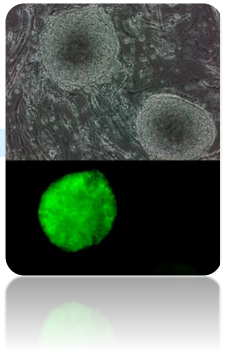
4 ES cell electroporation & Screening of recombinant clones



- 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



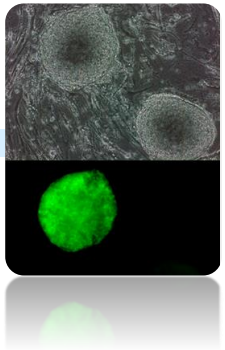
The targeting vector was electroporated in the proprietary C57BL/6NTac TB1 ES cell line.

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.

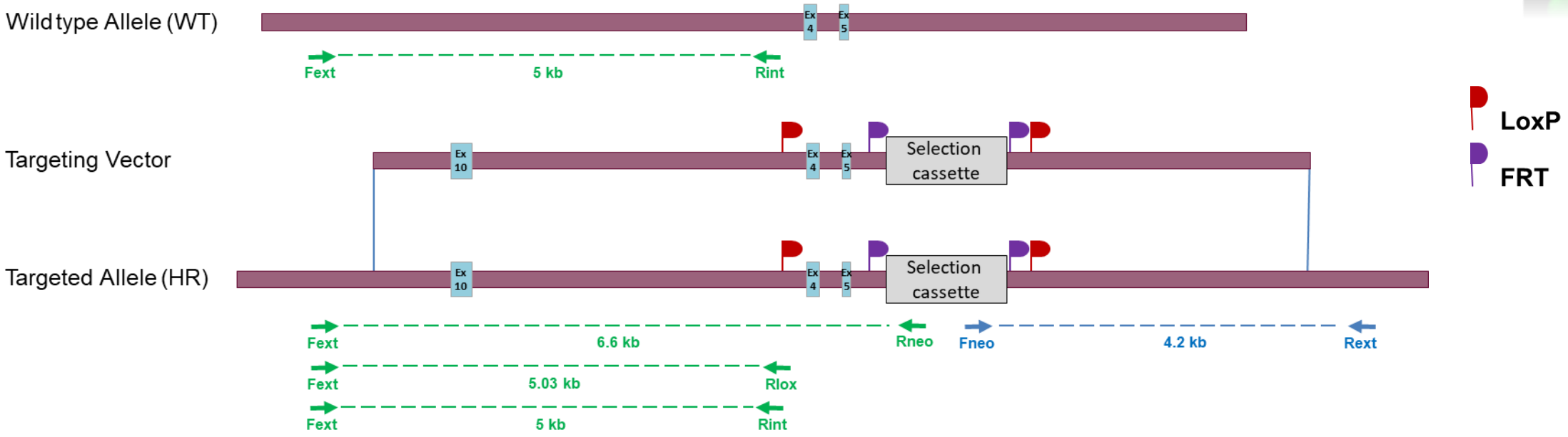
Screening process steps:

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

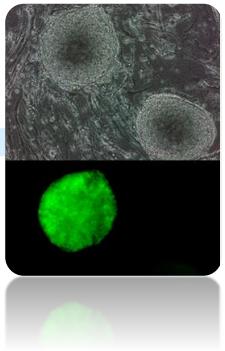


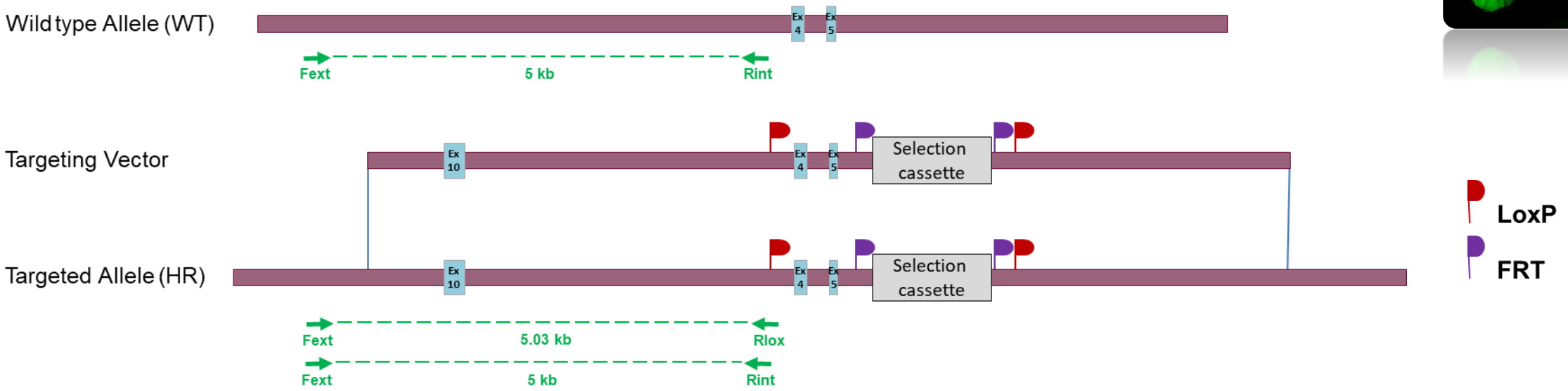
Schematic 5’ and 3’ PCR screening strategy



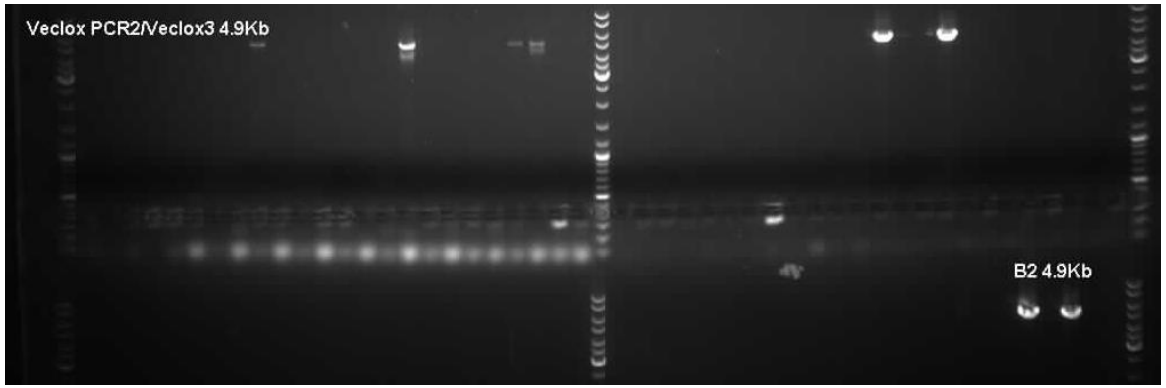
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	GAGATGATGTTATTACAGCCAGAAG	6.6 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
5’ PCR	Fext	GAGATGATGTTATTACAGCCAGAAG	5.03 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	GAGATGATGTTATTACAGCCAGAAG	5 kb
	Rint	AATATGGCCGGCCGTGGTCAGGAGAACATTTGAAATGC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.2 kb
	Rext	AGTGCTTCATAACACTTCATGTTGC	

Long-Range 5’ PCR screening – results

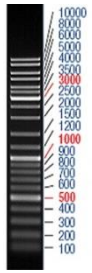




PCR Fext – Rlox : 5.03 kb



B2 : Control DNA



Ladder pattern

Pcr Fext – Rint : 5 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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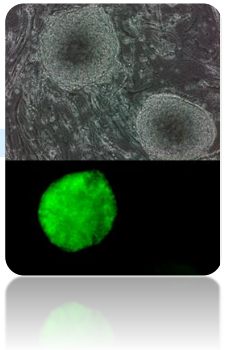
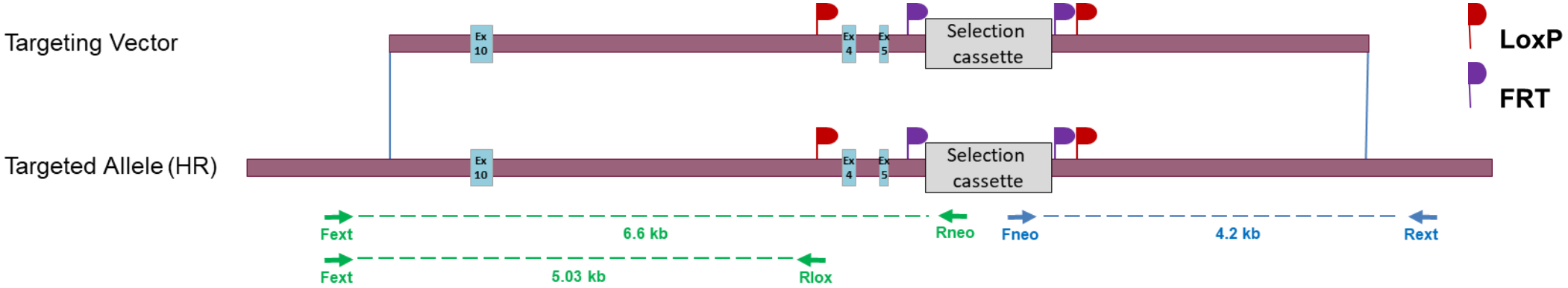
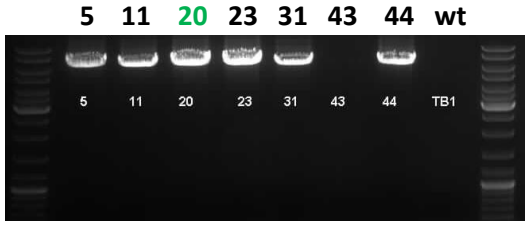
Recombinant ES validation by Long Range PCR



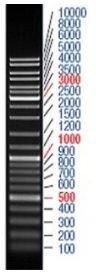
Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs



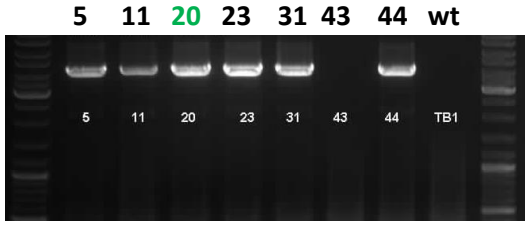


PCR Fext – Rneo : 6.3 kb

PCR Fext – Rlox : 4.2 kb



Ladder pattern



PCR Fneo – Rext : 4.2 kb

Seven candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Six clones (clones #5, #11, #20, #23, #31 and 44) were confirmed.

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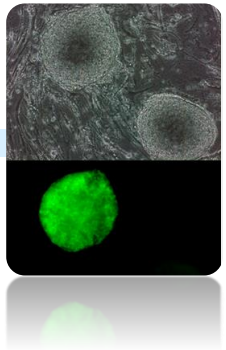
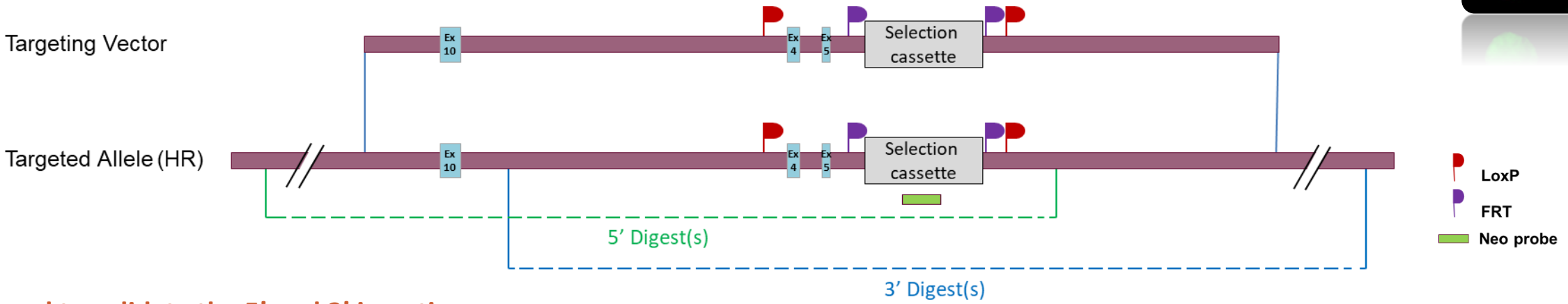
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

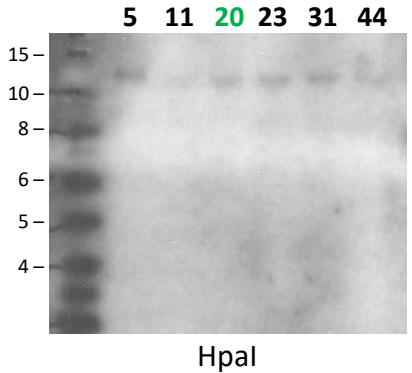
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



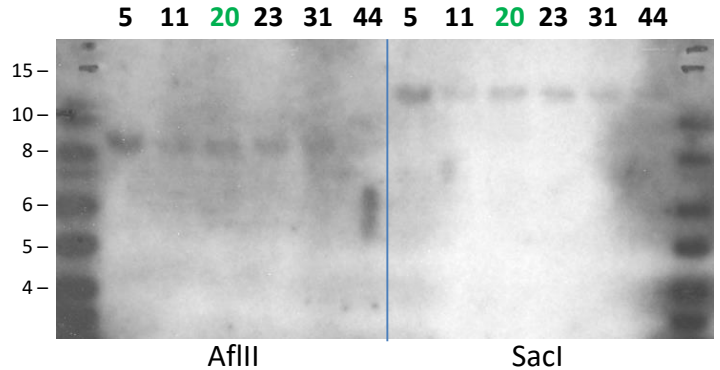
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	SbfI	11.6
	3' digest	AflII	9.1
		SacI	13.1

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAG
GGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATG
CAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATG
GAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCAT
GCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCG
ACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCT
GACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGA
TCCGCTGTAAGTCT

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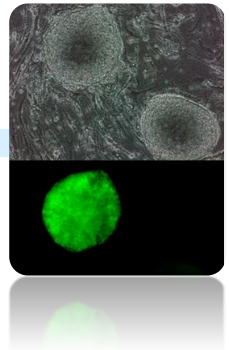
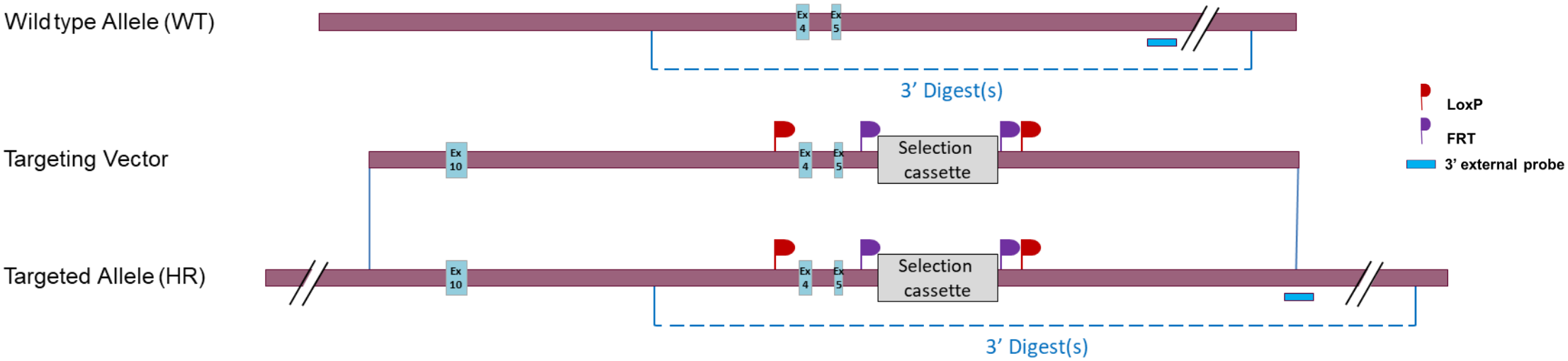
Recombinant ES clones validation by Southern Blot – External probe



Schematic Southern Blot validation strategy

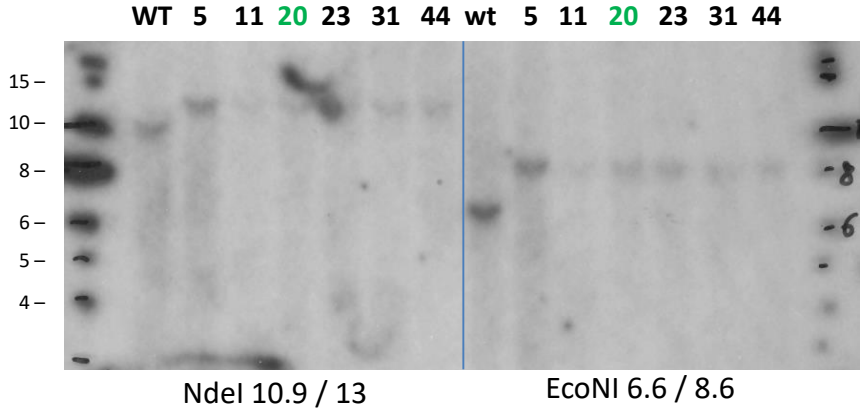
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
3' external probe	3' first digest	NdeI	10.9	13
	3' second digest	EcoNI	6.6	8.6

Southern blot – 3' probe



3' probe sequence

AGACAGACAGCTCTCCAGCCCCAGTGTTGGCATTATTTATATTTAGAGAATTTGGCTTTTTAG
CTCAAATTTATTTTTTATTGAGTACTTTGTCAGAAACAGCTAAGATTTGTTCTGATGGGAAAT
GTGTTCTACTTTATGATGGTAATTTATGATGGGGAGTTAAAGGATTTTATTTCAGGCATTCTC
CAAGAACTATACTTCATTGGTCTCTAGGGCAAAGGACTTGATACCTGTGACAGTTTCTTTAGC
ATTTACTATCTGTAGCTCAATATTTCTGATACTAATCTGTCATGTTAGCCTTATGCAACATGA
AGTGTTATGAAGCACT

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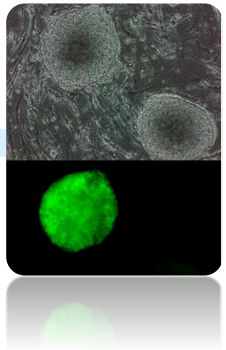
Aneuploidy screening in ES recombinant clones



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

Results of aneuploidy analysis are presented in the table below.

Clone ID	Giemsa
#5	Not done
#11	Not done
#20	**Pass**
#23	Not done
#31	Not done
#44	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 #20 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
#20	9	5	5	19

Breeding to F1 generation

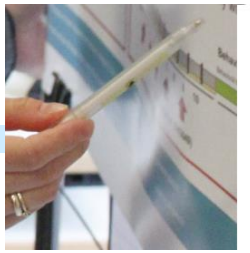
- Eight highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with inbred C57BL/6NCrl Flp deleter females showing maternal contribution* (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to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

- Germ line transmission was obtained the : 07/05/2014

- Allele nomenclature (following MGI guidelines) : **Phf6$^{tm1.1Ics}$**

***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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TGAGATATTTTTCCTAATTACTGGTATCCAGCAAGTTCTGGATTGCAAAAGAGATCTAGGTTTTTATAGTGTCAGTTGTATCTAGCTCAGCTCCATGATTTATAGCATTTCAAATGTTCTCCTGA
CCACGGCCGGCCAAGCTTCTCGAGCTTAAGGTCGACCTGCAG**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**TTAATTAAGTAGACCATCACACATTTATAGCATCCTACATATTTTGTC
AGTAAAATAGAAAGTACCATCAGAATAATTTTATTCAATTTTTTTCAGGAGACAAAATAAGGAAAATAACATGAATTAGACTTTTTTCTATAACCAGTTTGCTTTTCCTGACAGAAATTGGAA
TGTCCTAGCCTGTTTGGGACAGTATTAGCAGAATTACATTTGAATATTGTTTTGTTGTTTTTCCCTAGATGTGTTCTCTTTGTCATTGTCCTGGAGCAACCATTGGCTGTGATGTGAAAACCT
GCCACAGGACATACCACTACCACTGTGCATTGCATGATAAAGCTCAGATCCGAGAGAAACCTTCGCAAGGGATTTACATGTAACTGGCTCATTTTGCTTGGTTTTAAAGCAGCCCCGCTGT
CCTTCAGGCTTTTCCATTTTCCTCCATTCAAAGCATTTCACCGTCACTATGAAGGCTTTTTTGATATGTATGATATGTAATAGTTAAAGCAATGATTTAGGAAGTGTCACACTGAGGTTTATAA
CATAATTCCAGTTTATGAATAAGGTGGACTGTGCTGCTCATTTTTGTAATTTGAAAATGAATTGAATTTAGTTTTATATACTTCACATTTTGTATTTTTCCCCTTCAGGGTTTATTGTCGAAAAC
ACAAGAAAACTGCACATAACTCGGAAGGTATGTTTCATTAGCCAGTTTTATGTTTCAAGAGAAAATTAGAGTAAAATATAAATGAGTAGTCCTCTACTAAGTTTTTCCTTATGATAGGTCTTA
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ATATTAGGAAGAAACTGTGGTAGAGGTGACATGTTTCTCACCG**GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCG**CGGCCGG**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GG
ATCCATCGACCCCCTGCAGGCCACAGGTGGTTATTGGATTCTTGCTCCCAATAGCTATTACAGTGTGAGCCTAGGAATCCTAGTGCACTTTGGATCAGCGTGCACTCTGGTGAAATGTCAAC
ACCCCATCTCTGCTTTTGCTTTTCATGGTTCACTTATTCCATGGTTCAGCTCATTGCATCATGAGCTGTCATGATCCAAAAATGGAAGTTTCCAAAAAGAAACAAGTTTCAAATCAGTTTGAT
TATAATATATTATGCTTTATTGATGTCTTACTGTGTCCAATTTATAAGTCAGGCTTTATAATGTGTAGGCATGTGTAGGGAAAACCACATTTAGTATTATTCATGATTTTGTGTATCTACTGGGAA
TGTAAAAACAAATCCCCCACTGAGAAATAAGTGGAGACATACTATGTTTTTTTTCTAGAAAGCAAAAGTTTGTAAGTAACTAAATTTAAAATAGAAGCTTATCAAGTTATTTTATAGTTTGTT
TCCACTTAAATTTATAAAACTAGTTT

LoxP FRT Exons 4 & 5

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/10/16

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EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Phf6

(PHENOMIN-ICS reference IR00004621 / G4621)

This report has been **prepared** by: David MOULAERT

This report has been **validated** by: Sylvie Jacquot, PhD
Head of Genotyping Service

The first version of this report was finalized the: 21 Sep 2016
The last update of this report was done the: 21 Sep 2016

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PHENOMIN-ICS
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Web site: http://www.ics-mci.fr/

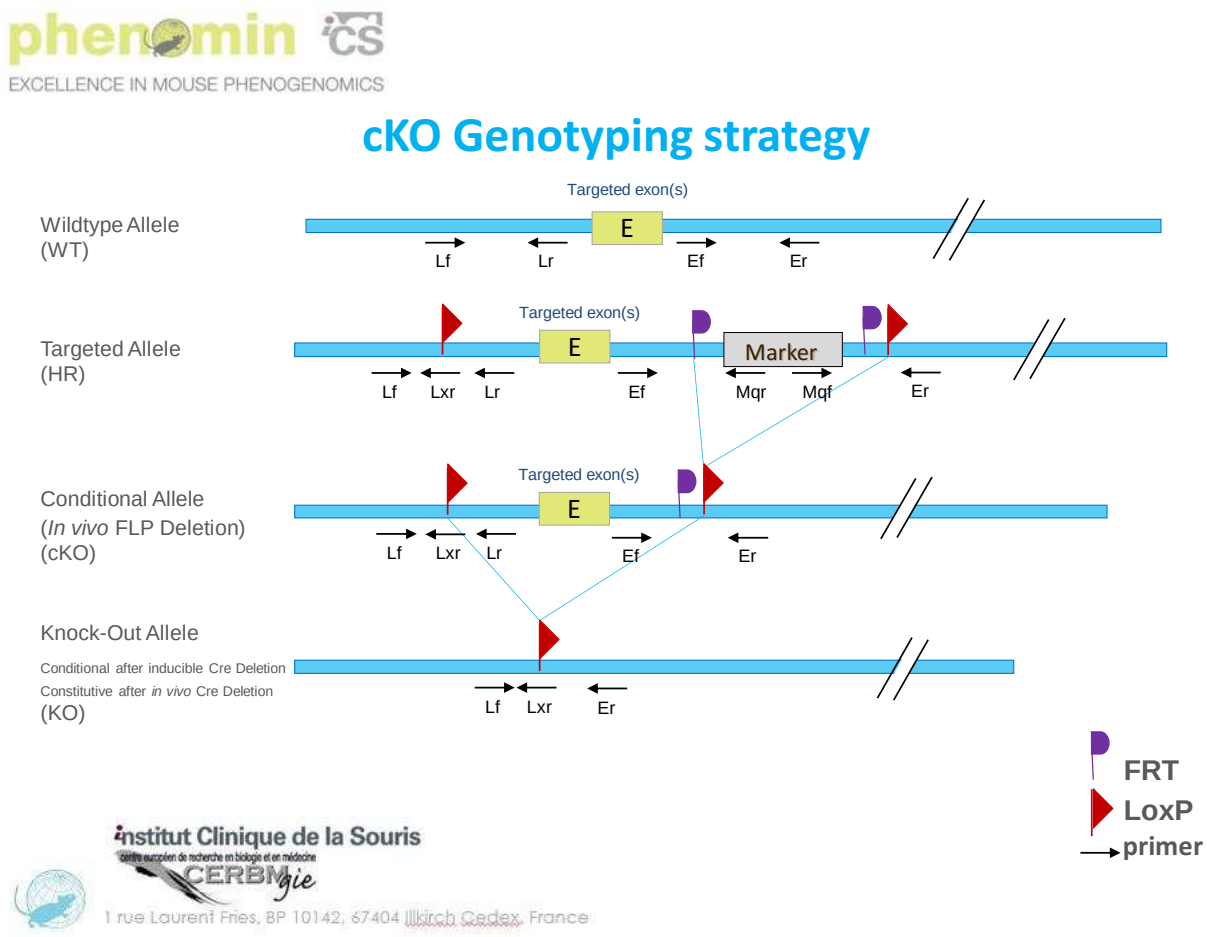
Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Phf6** Conditional Knockout (cKO) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	7822	GGAGATGTTATTACATCAGTCCAATG
Er	7821	GCAGAGATGGGGTGTTGACATTTCAC
Lf	7819	TTTTCCTAATTACTGGTATCCAGCAAG
Lf[2]	7818	TCTTTGCCGATGATATTAGGACTGGTG
Lr	7820	TTCTGCTAATACTGTCCCAAACAGGCT
Lxr	5049	CATACATTATACGAAGTTATCTGCAG
Mq1f	1219	CAGCTCATTCCTCCCACTCATGATC
Mq1r	3721	GTAGAAGGTGGCGCGAAGGGGC

[2]: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	cKO allele	KO allele	WildType allele
Presence of the distal loxP	7819-7820	Lf / Lr	397	397	---	318
Excision of the selection marker	7822-7821	Ef / Er	2212*	359	---	256
5' part of the selection marker	7822-3721	Ef / Mq1r	361	---	---	---
3' part of the selection marker	1219-7821	Mq1f / Er	433	---	---	---
LoxP specific PCR	7818-5049	Lf[2] / Lxr	281	281	281	---
Excision of the floxed exon(s), i.e. knock out	7818-7821	Lf[2] / Er	3315*	1462*	432**	1280*

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: no Amplicon should be obtained

1.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

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. Epub 2012 Mar 20.