phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

H2afv targeted trapped LacZ with conditional potential (corresponding to the IMPC tm1a allele)

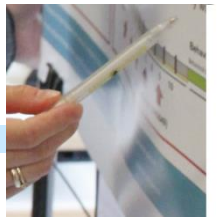
Project code: K578b/IR2876

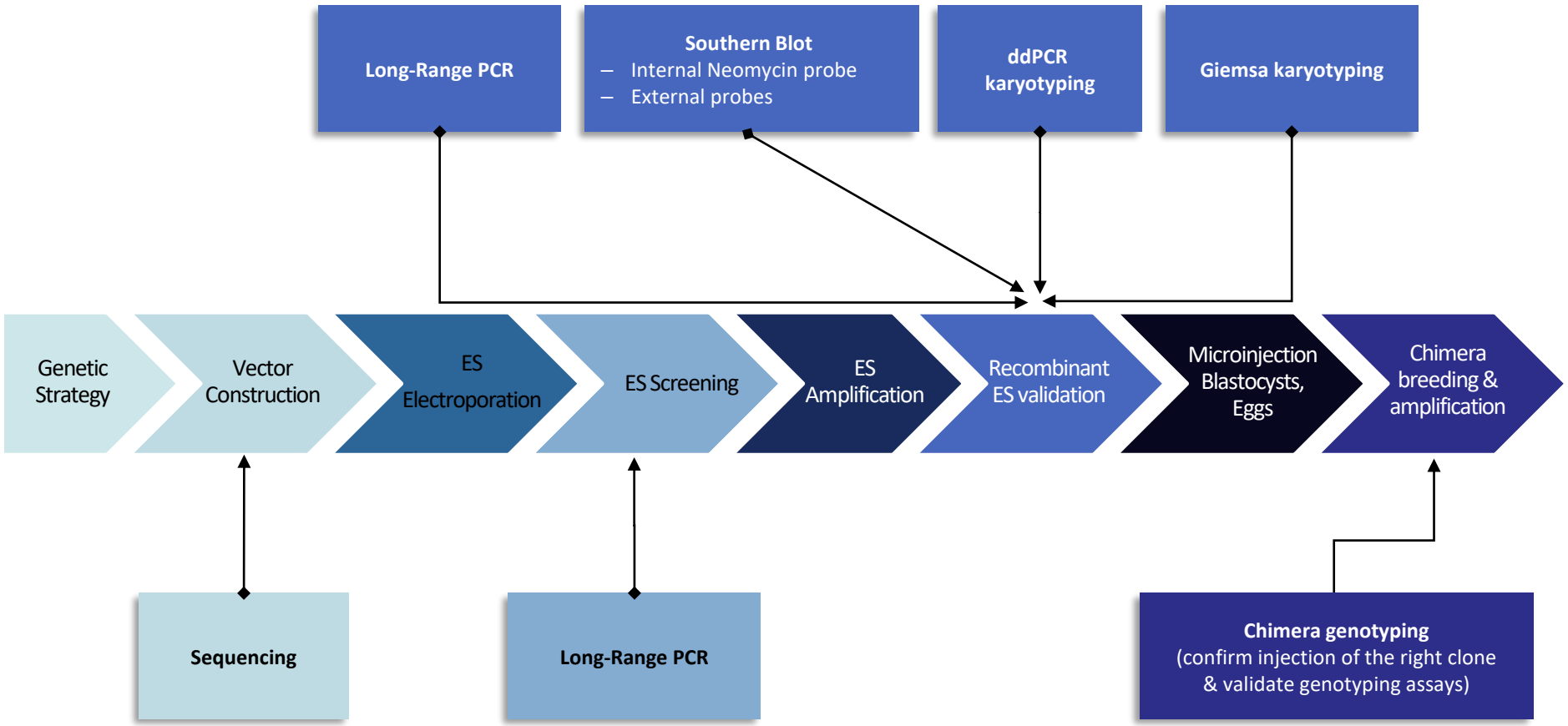
Report updated: 2024/10/09

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

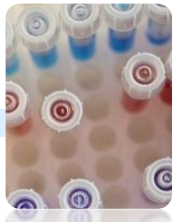
MODEL GENERATION TECHNICAL REPORT

PROJECT PROCESS & QUALITY CONTROLS



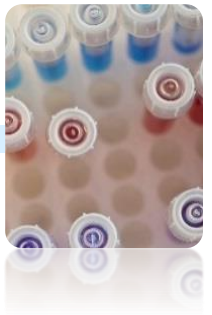


2 GENETIC STRATEGY

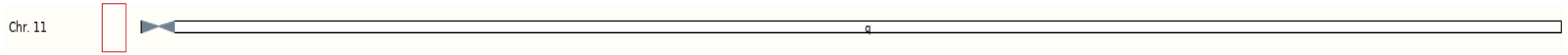


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy

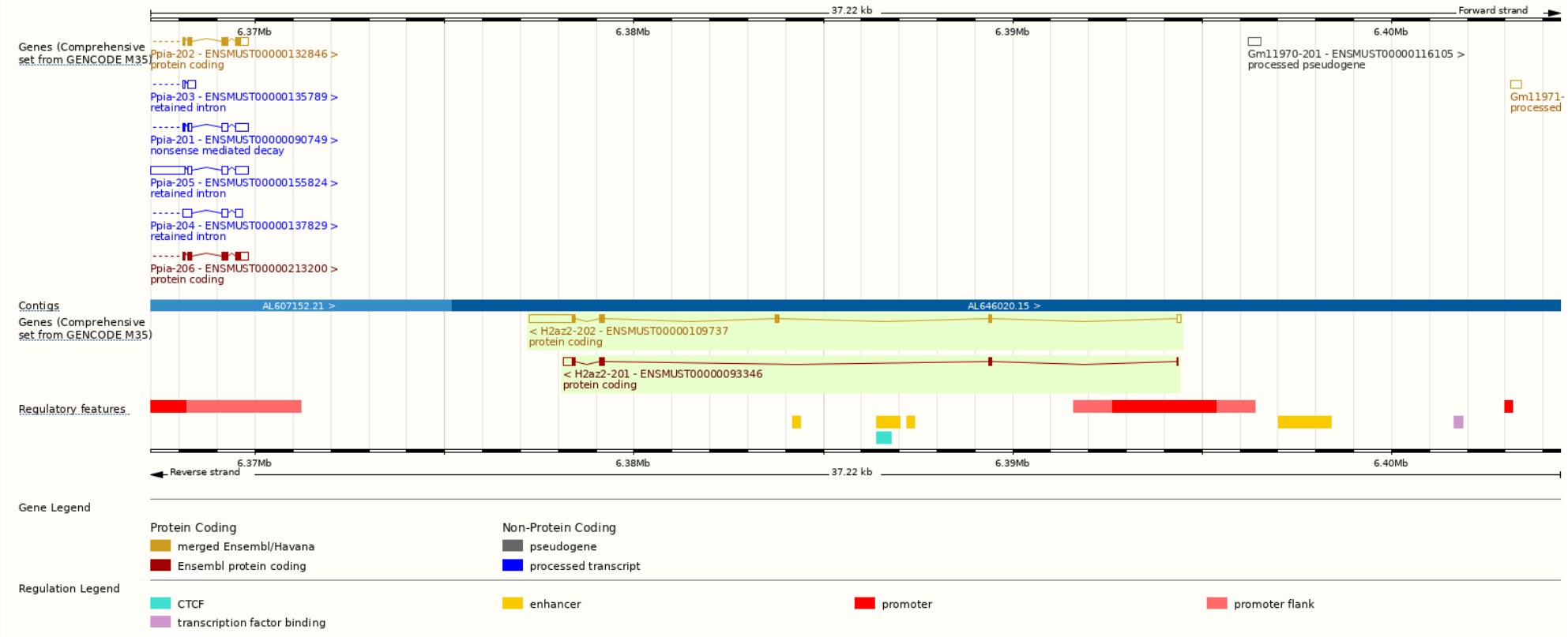
H2az2 mouse genomic locus – structure



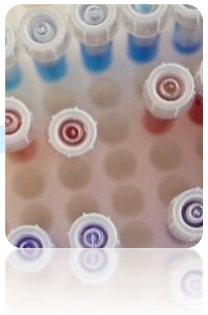
Location : Chromosome 11: 6,377,229-6,394,443



Ensembl Gene ID: H2az2 ENSMUSG00000041126

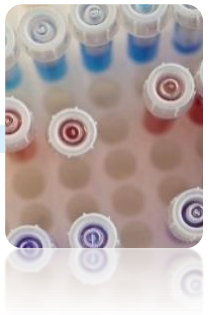


H2az2 mRNAs and proteins

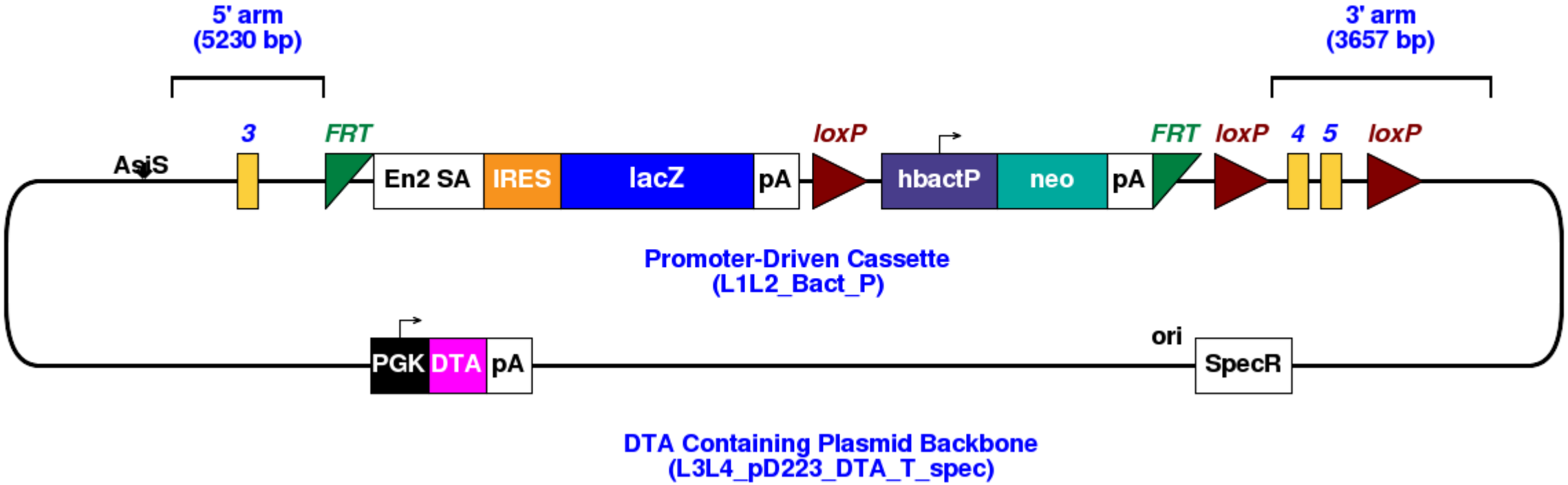


ENSMUST00000109737.9	H2az2-202	1633	128aa	Protein coding	CCDS48750	B2RVP5 Q3THW5
ENSMUST00000093346.6	H2az2-201	519	90aa	Protein coding	CCDS83779	Q8R029

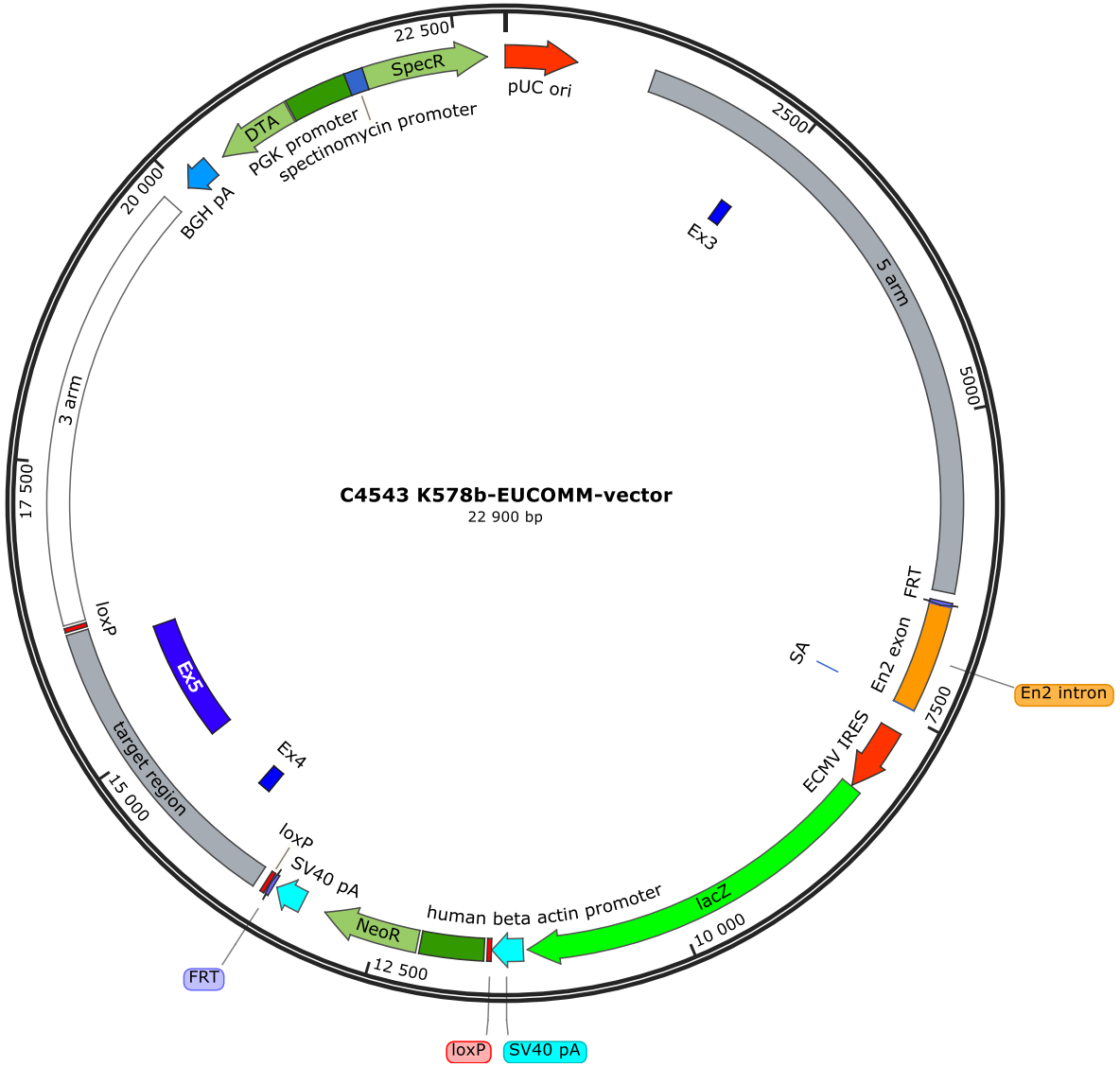
EUCOMM Strategy



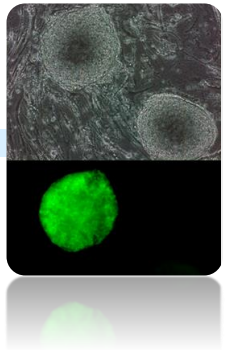
Targeting Vector obtained from EUMMRC: HTGR04010_Z_2_A03



3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION

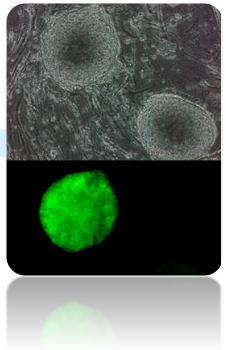


4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Long range PCR screening – strategy
- Long-Range 3’ 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



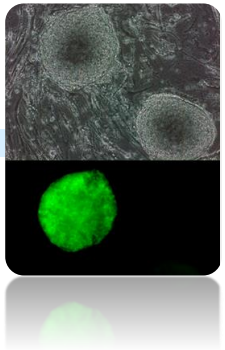
The targeting vector was electroporated in the proprietary C57BL/6NTac BD10 cell line.

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.

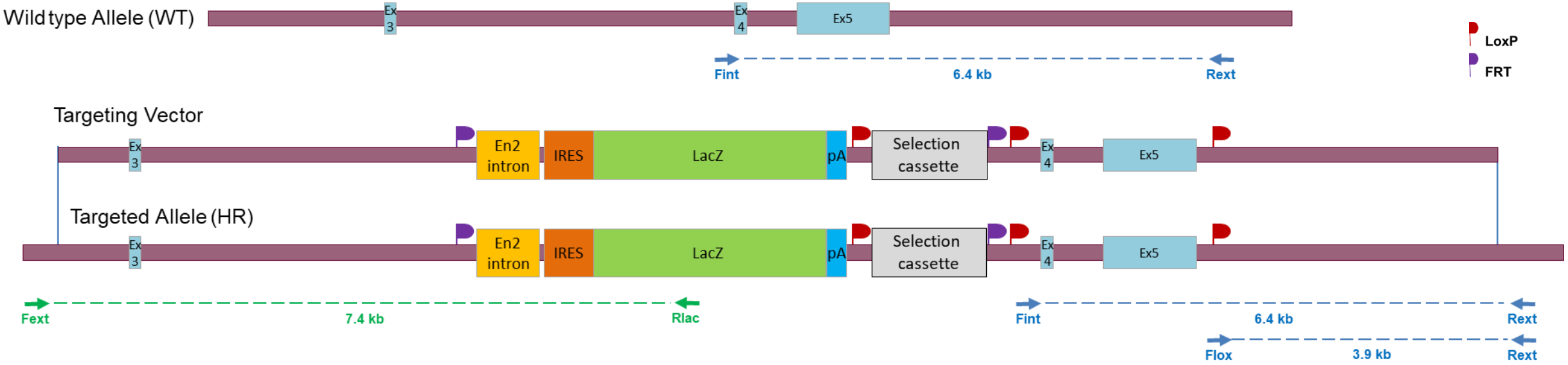
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Ten of 3’ PCR positive clones are confirmed for 5’ 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

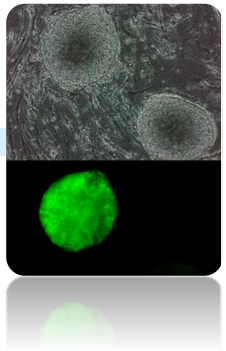


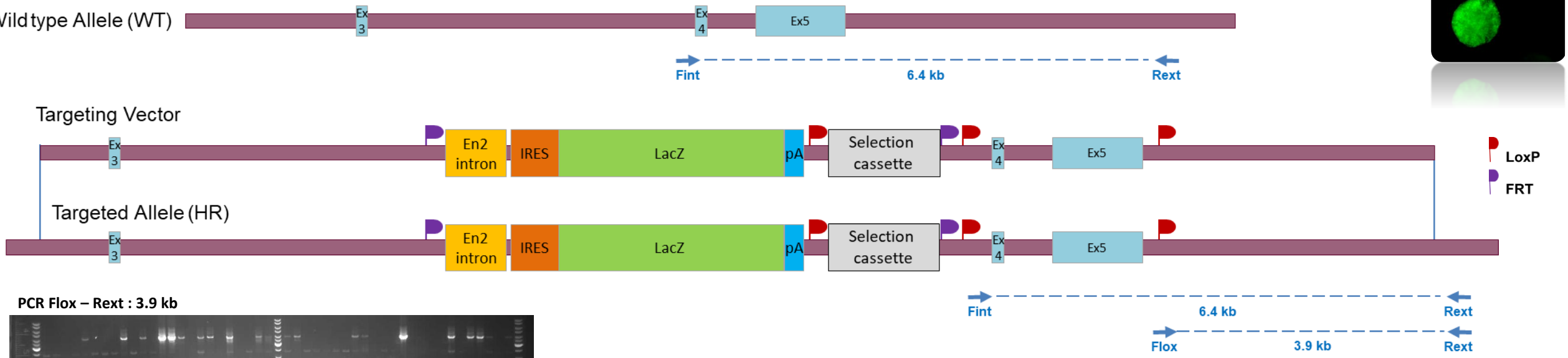
Schematic 5’ and 3’ PCR screening strategy



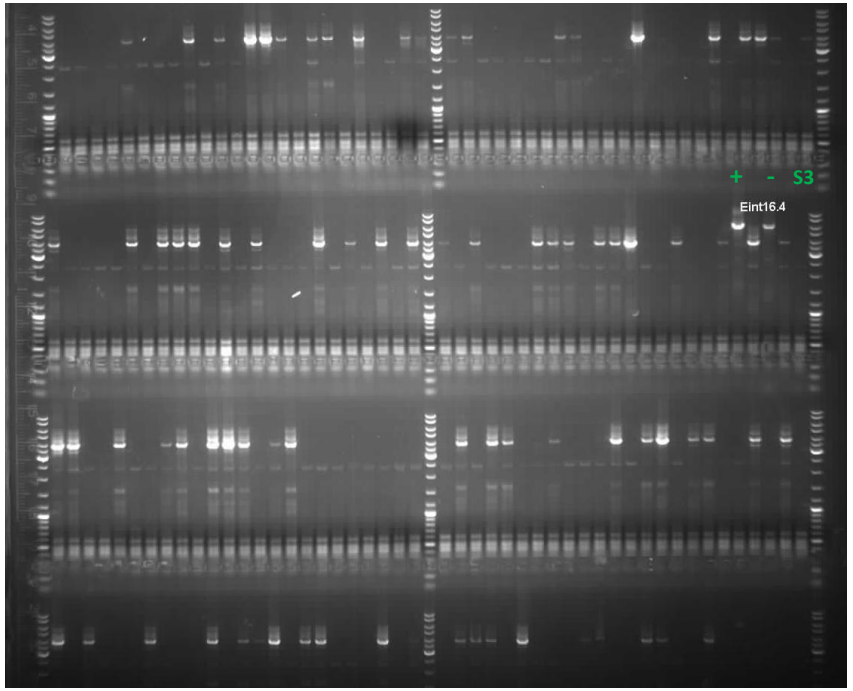
PCR	Primer Name	Primer sequences	PCR product size
5’ PCR	Fext	GGCTCACGTGTTGTGTATCCATACA	7.4 kb
	Rlac	CCACAGATGAAACGCCGAGTTAACGCCA	
3’ PCR	Flox	TTATGGTCTGAGCTCGCCATCAGTT	3.9 kb
	Rext	GGCAGTCTTTAGGTTCCTGGGCAGT	
3’ PCR	Fint	GCTCACACTTAGTATCTTGGCTGAT	6.4 kb
	Rext	GGCAGTCTTTAGGTTCCTGGGCAGT	

Long-Range 3’ PCR screening – results

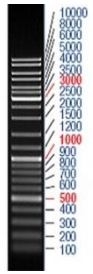




PCR Flox – Rext : 3.9 kb



+ / - / S3 : Controls DNAs



Ladder pattern

PCR Fint – Rext : 6.4 kb

Ten candidate clones out of the positive clones were selected for 5’ Long-Range PCR and Southern blot validation.

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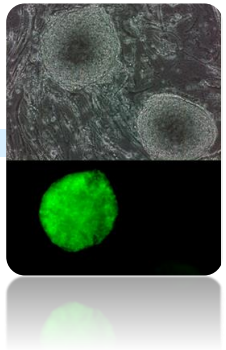
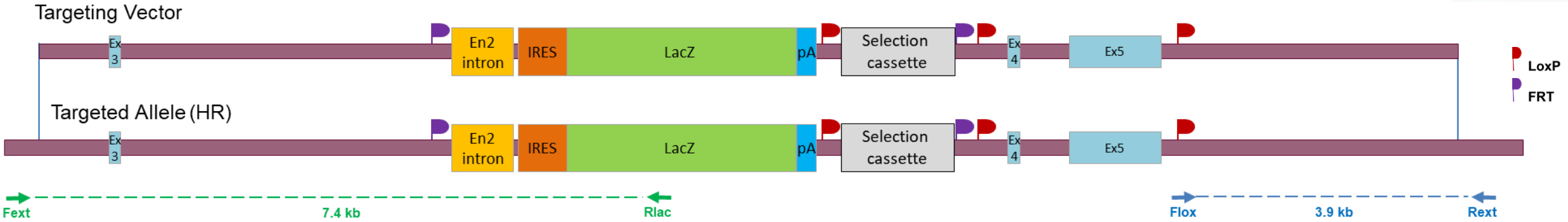
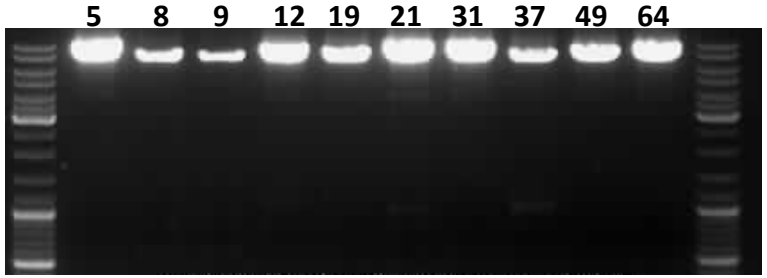
Recombinant ES validation by Long Range PCR

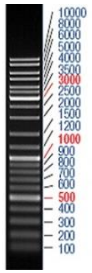


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

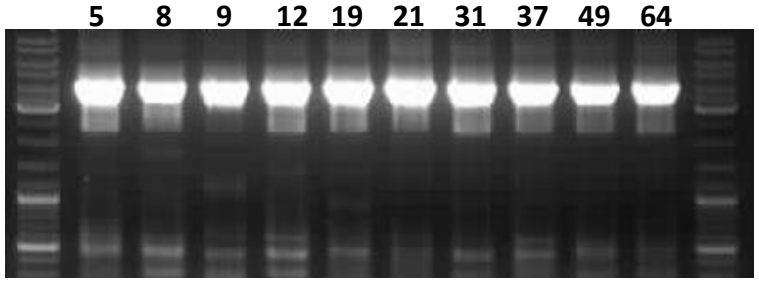




PCR Fext – Rlac : 7.4 kb



Ladder pattern



PCR Flox – Rext : 3.9 kb

Ten candidate clones identified by 3’ PCR screening were further analysed by 5’ Long-Range PCR screening.
Six clones (clones #5, #12, #19, #21, #31 and #64) were further analysed.

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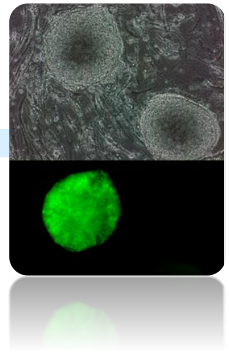
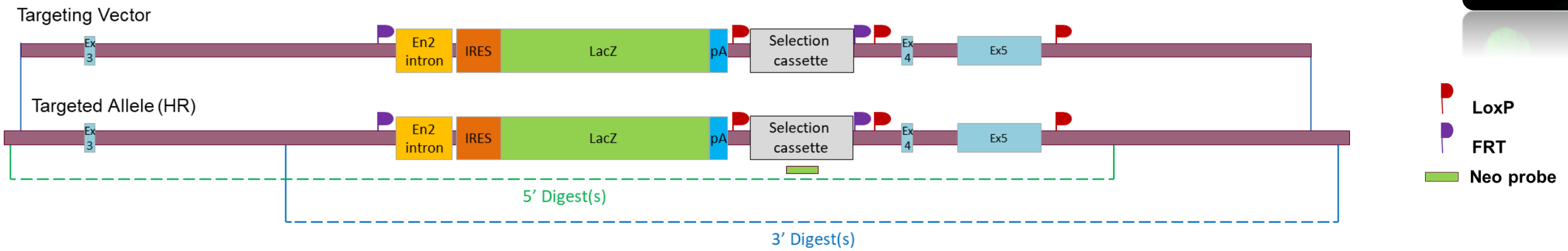
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

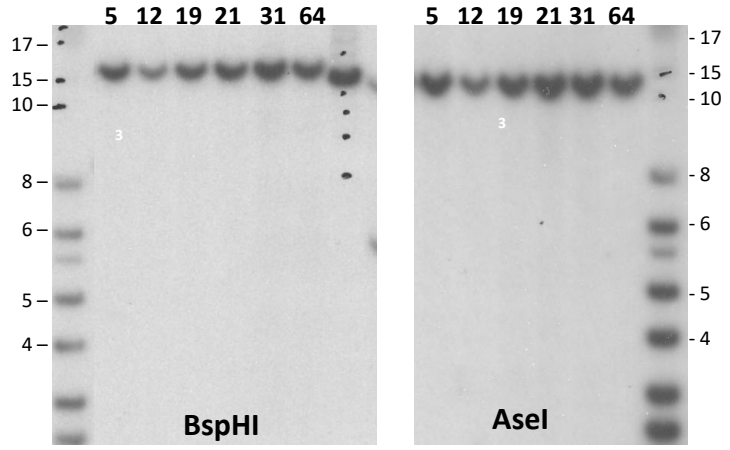
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



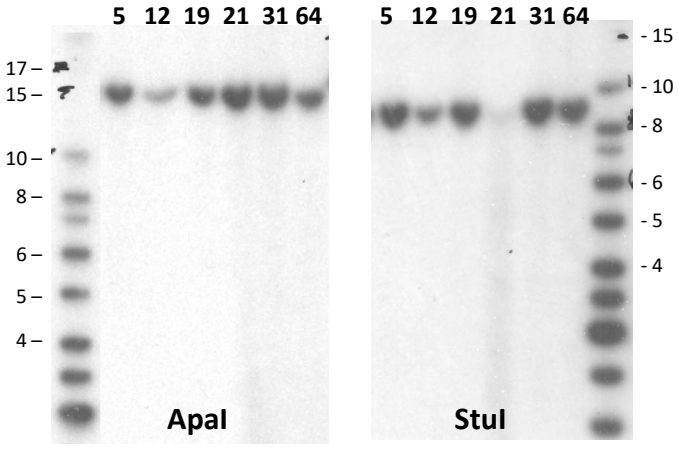
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	BspHI	19
		AseI	16.7
	3' digest	ApaI	15
		StuI	9

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

AGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAG
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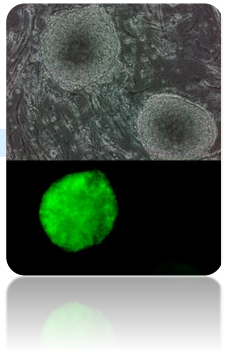
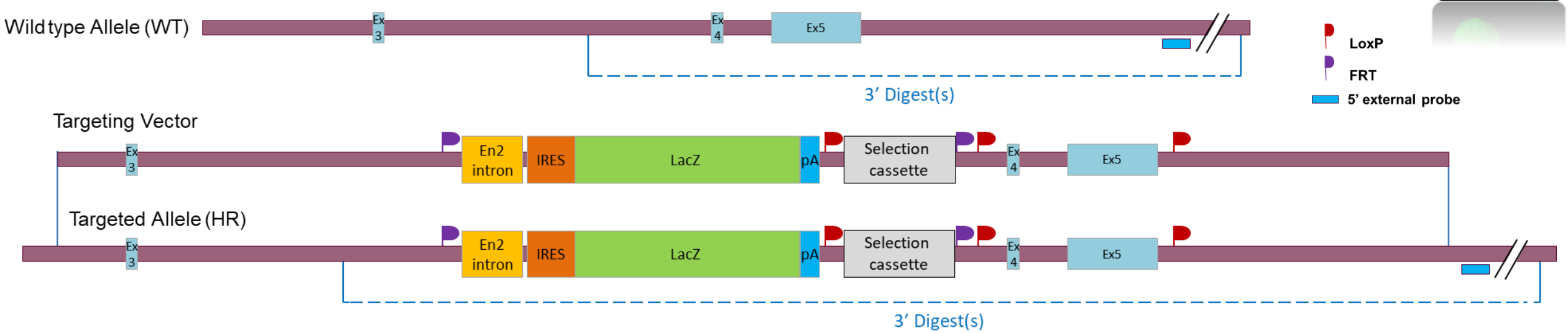
CGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGA
CAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCG
TATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCA
CTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCC
ACGATAGCCGCGCTGCCTCGTCCTGCAG

Recombinant ES clones validation by Southern Blot – External probe

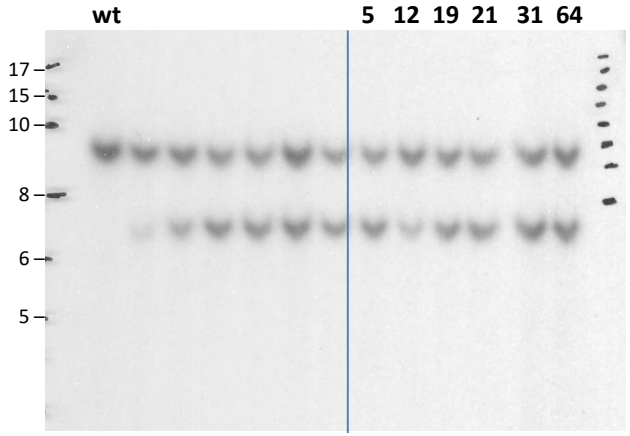


Schematic Southern Blot validation strategy

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 – 3' probe



StuI : 13.1 / 9

3' probe sequence

GGTGGGTGTGTCTGTTAGATACAGTTCCTAGAGGCAGAGAG
ACTGGGACTTTATTCTTGATATGGAGCTGAAAATGCATGGC
TTTCATAAGTGTCCAGTTGACTGCGCTCTACAGAACTGAGC
ACACTGGGCTGCAATACCCCAGCATTTCTAAGAATGGTCAG
TGGGAAAGCAGCACACTGCAAACTGCCCAGGAACCTAAAGA
CTGCCCACGTAAGTGTGGATGTGGATATGGTCCATGAACCG
CCTCAGCCTCAGCCGTATCCATTGTATGGAATTAGCAACAT
CACCTCGGGATCTTGTTTTTAGTGAAGCCGCTTGCTCTTCC
GCCACGACTCACAATGTGCCATTCTCTTGAGTGGCTGCTAT
CAGCTTCTCAATCACACGTTCTCTCTCC

Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
3' external probe	3' digest	StuI	13.1	9

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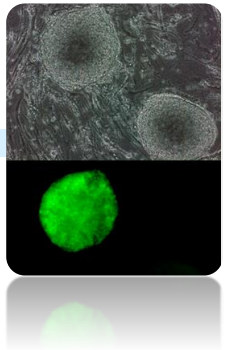
Aneuploidy screening in ES recombinant clones



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
#12	Not done
#19	**Pass**
#21	Not done
#31	**Pass**
#64	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 #19 and #31 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
#31	0	2	6	8
#19	**7**	**4**	**3**	**14**

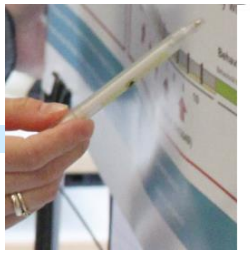
Breeding to F1 generation

- Four highly chimeric males generated in the previous phase by blastocyst injection of the ES clone #19 were mated with C57BL/6N Flp deleter females (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 : 31/08/2011

- Allele nomenclature: H2az2tm1Ics

6 SEQUENCE OF THE ALLELE (KO first with conditional potential)



LLOYD'S REGISTER · LRQA
ISO 9001 · NFX 50-900

Allele nomenclature (following MGI guidelines) : **H2az2^{tm1Ics}**

GAGTGGTGGTATAGCCTGTAATTCTTGCTCTTGGAGACAGGAGGGGGTTAGAAAGCAGATGCTTGTCATCCCTGGCTACATAGCAAATTCAAGGCCAGCCTAGGAAAACAACAGAAAAAAGAAAAAAAGTGTTCTACTATAGAATAAAAGGCTTCTGGCTTATTGTTTATAGCTCTTAGGGCTCACCTTTGGCCAGAAGGTCAAGGCCATCCTTCCTTGCACAGCCCTTTTGTTTTTCTG
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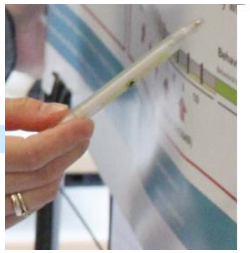
EXCELLENCE IN MOUSE PHENOGENOMICS

6 SEQUENCE OF THE cKO ALLELE (after Flp excision



Allele nomenclature (following MGI guidelines) : **H2az2$^{tm1.1Ics}$**

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2024/10/09

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Verified by Marie-Christine BIRLING, PhD

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Genotyping protocol

H2afv

IR00002876 / K578b

(ICS internal reference)

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The first version of this report was finalized the: 07 Feb 2012

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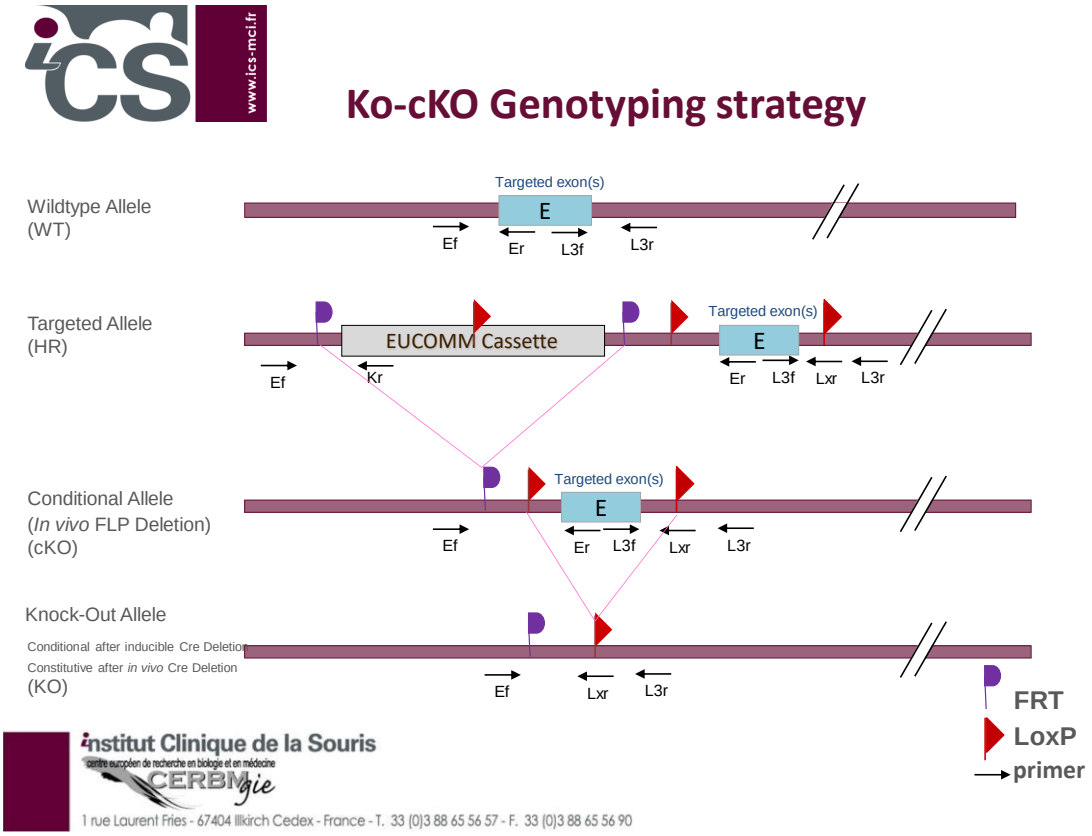
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **H2afv** Constitutive Knockout / Conditional Knockout (KO-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	5978	CCACGTAATGAGATCCAGTGCCCT
Er	5981	CATGCACCTGCCATTATGTCTGGTA
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	5980	TTTATGGTCAATTTTAGCCGTGTCCCC
L3r	5979	TGTCAACTTGGGCTACAGATATGGCTC
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	5978-3278	Ef / Kr	327	---	---	---
Presence of the distal loxP	5980-5979	L3f / L3r	366	366	---	348
Distal loxP specific PCR	5980-3255	L3f / Lxr	208	208	---	---
Excision of the selection marker	5978-5981	Ef / Er	7337*	433	---	257

*: 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:

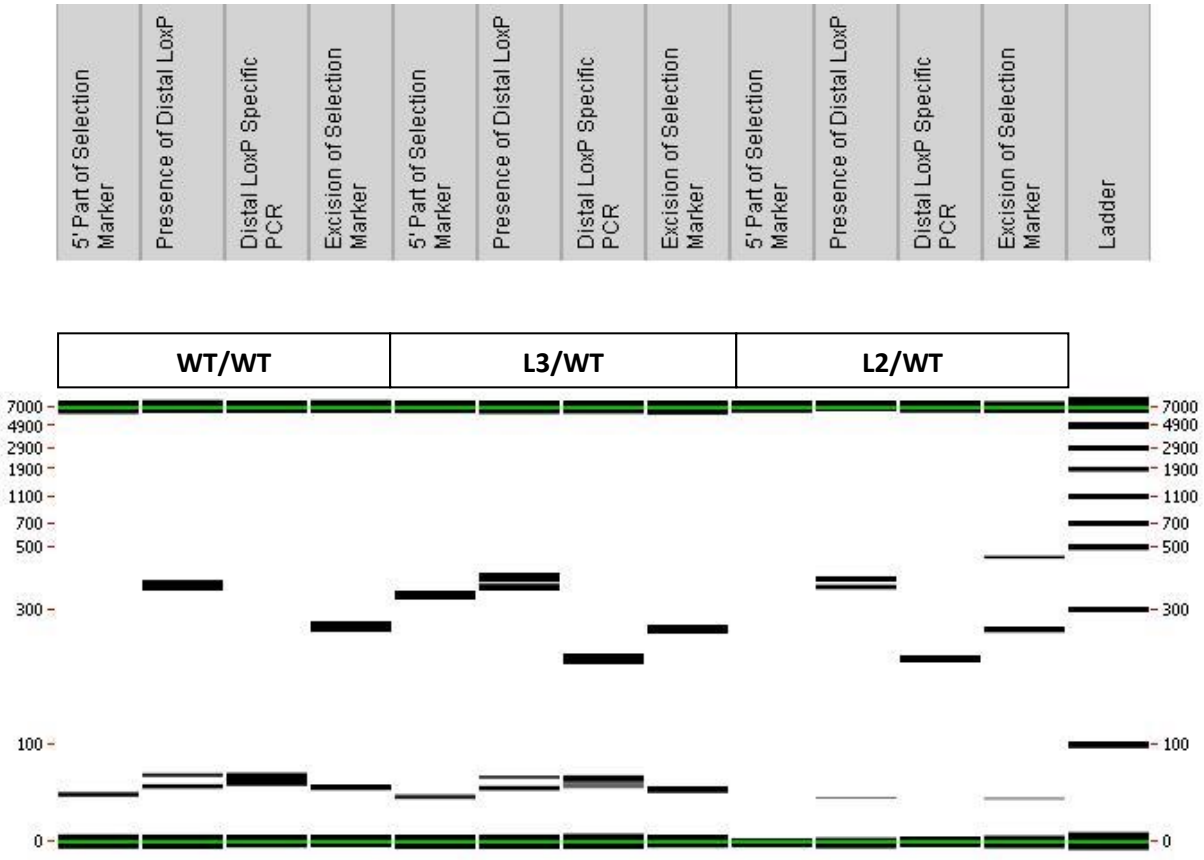
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



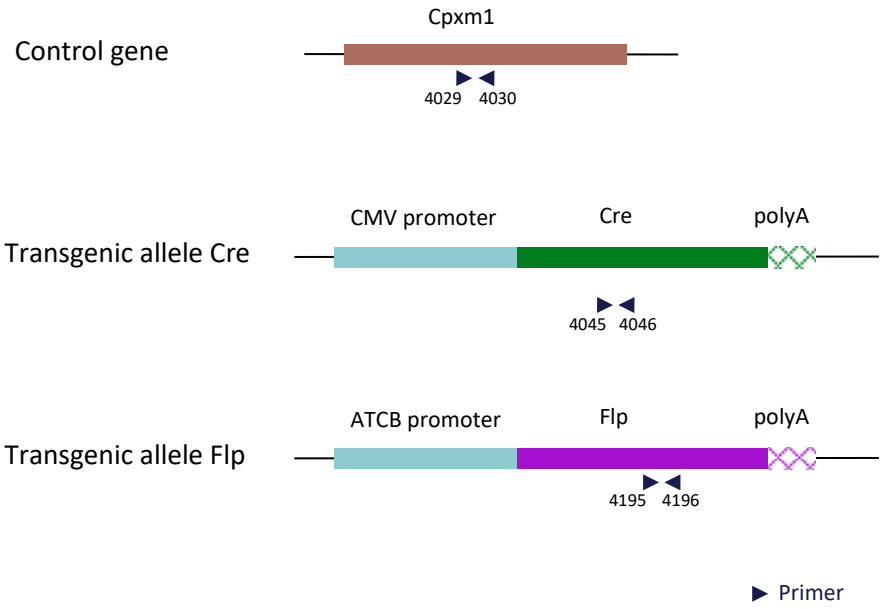
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.
Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTCACC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	446
Tg allele	281	328	/

2.2. 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)	0.05µl
3' primer (100 µM)	0.05µl
Sterile H_2O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2