phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

MODEL GENERATION TECHNICAL REPORT

Conditional cKO of H2az1

Project code: K401(b) / IR1643

Report finalized: 2024/11/19

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 CONTROL

2 GENETIC STRATEGY

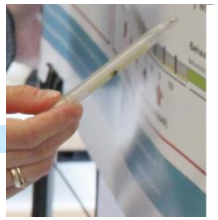
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

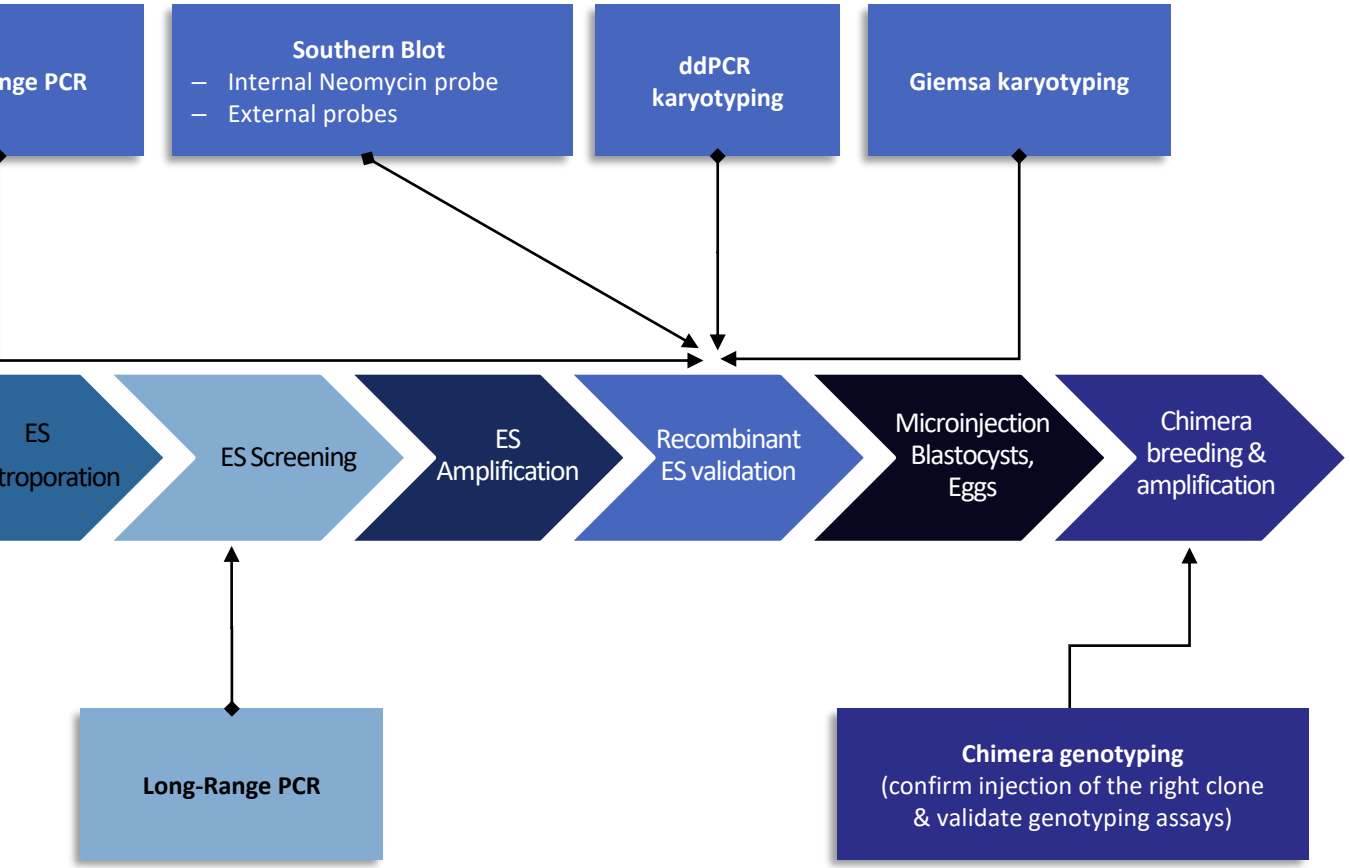
4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

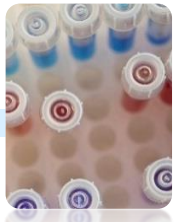
6 SEQUENCE OF THE DELIVERED ALLELE

Project process & quality controls



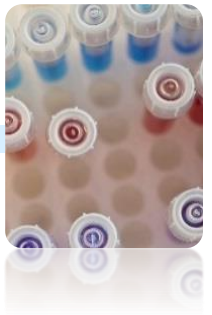


GENETIC STRATEGY

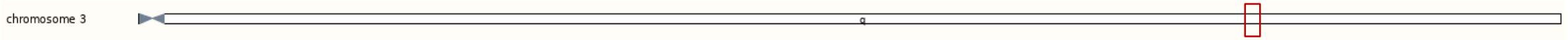


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- Sequence detail
- PRO & CONS evaluation of the strategy

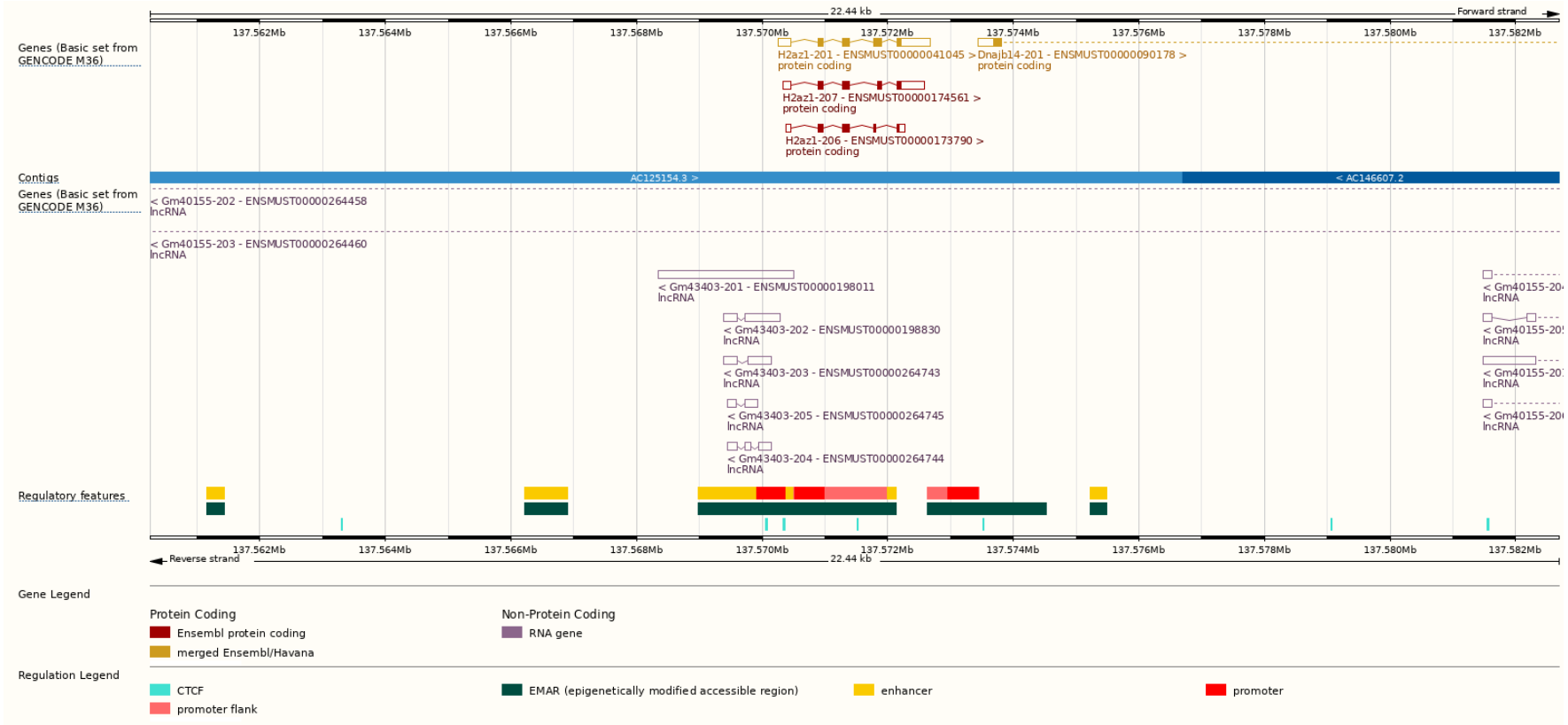
H2az1 mouse genomic locus – structure (GRCm39)



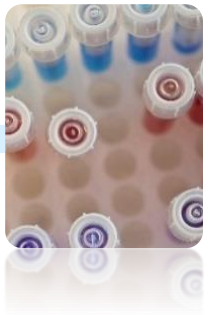
Chromosome 3: 137,570,248-137,572,683



Gene: H2az1 ENSMUSG00000037894

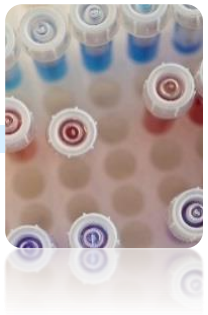


H2az1 mRNAs and proteins

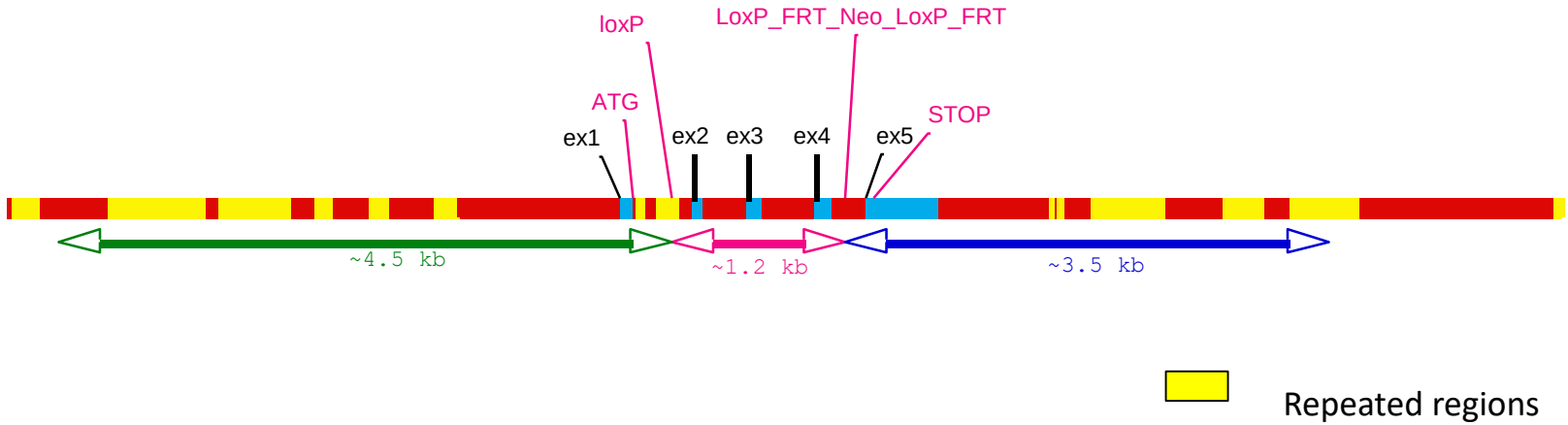


Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match
ENSMUST00000041045.14	H2az1-201	1069	128aa	Protein coding	CCDS38647	P0C0S6
ENSMUST00000174561.8	H2az1-207	810	105aa	Protein coding	CCDS84676	Q3UA95
ENSMUST00000173790.8	H2az1-206	437	87aa	Protein coding		G3UWL7
ENSMUST00000172696.2	H2az1-204	349	54aa	Nonsense mediated decay		G3UX40
ENSMUST00000173666.8	H2az1-205	361	No protein	Protein coding CDS not defined		-
ENSMUST00000125821.2	H2az1-202	882	No protein	Retained intron		-
ENSMUST00000138010.3	H2az1-203	870	No protein	Retained intron		-

Approach undertaken: flox exons 2 to 4 (H2az1-201)

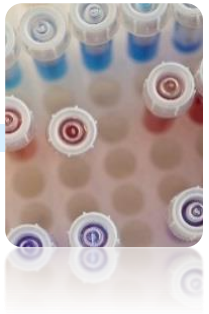


Targeted locus



No expression after cre mediated excision

PROs& CONs evaluation of the strategy



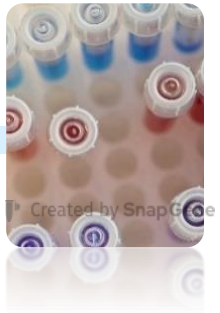
Pros

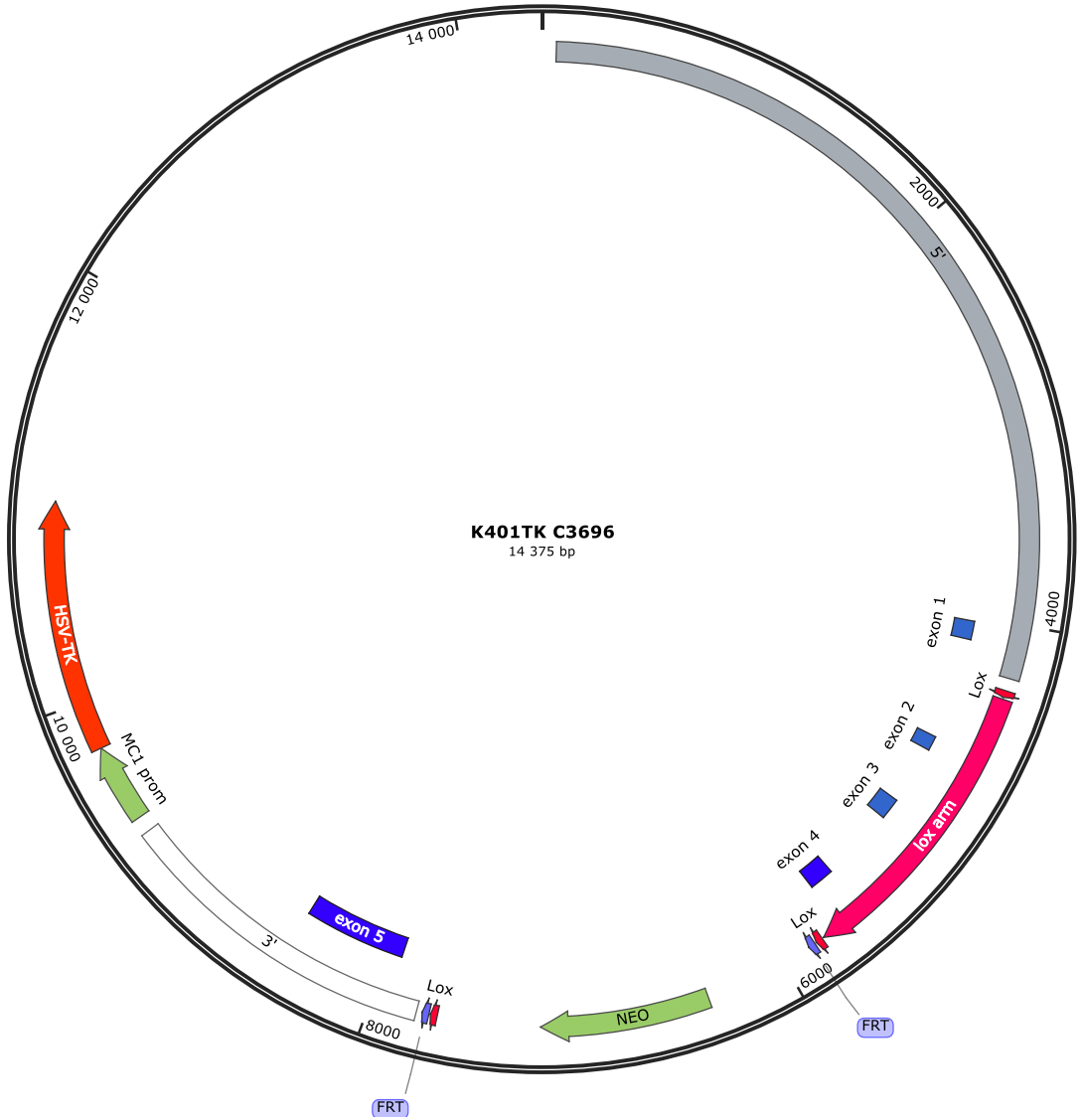
- ✓ No peptide expression after Cre mediated excision.

Cons

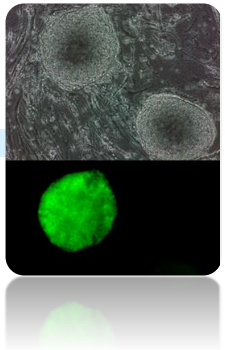
- ✓ Presence of repeated elements in the homologous arms which could render recombination difficult.
- ✓ The 5'LoxP might be lost if recombination occur in the interLoxP region.

3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION



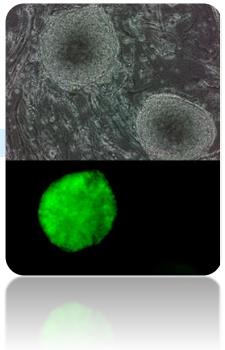


ES cell electroporation & Screening of recombinant clones



- 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



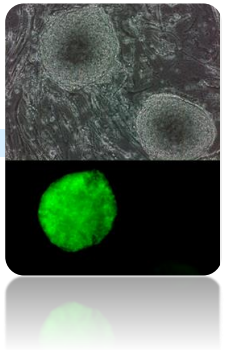
The targeting vector was electroporated in the proprietary 129SV/Pas cell line (P1).

Transfected ES clones were submitted to neomycin selection (G418) and 333 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.

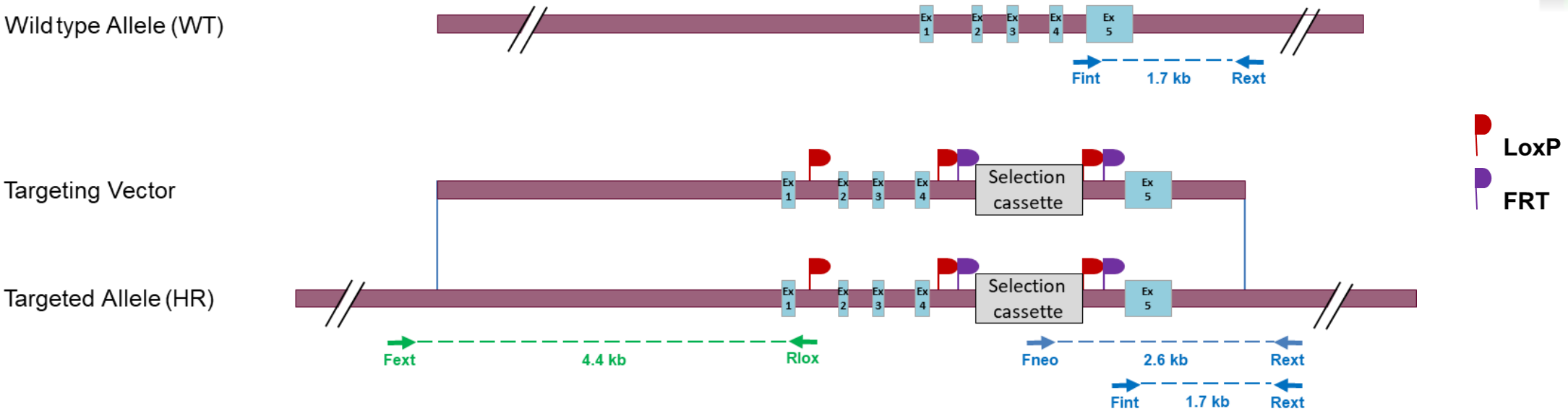
Screening process steps:

1. Identification of candidate recombinant clones by initial 3’ Long-Range PCR
2. Three 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

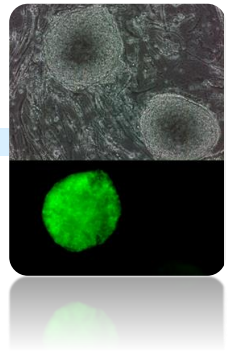


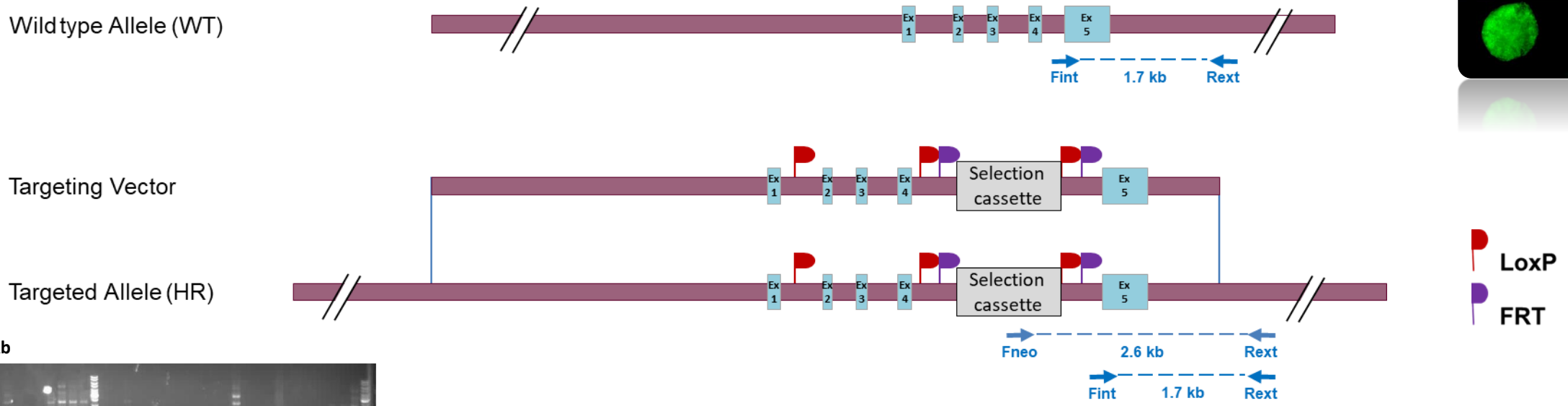
Schematic 5’ and 3’ PCR screening strategy



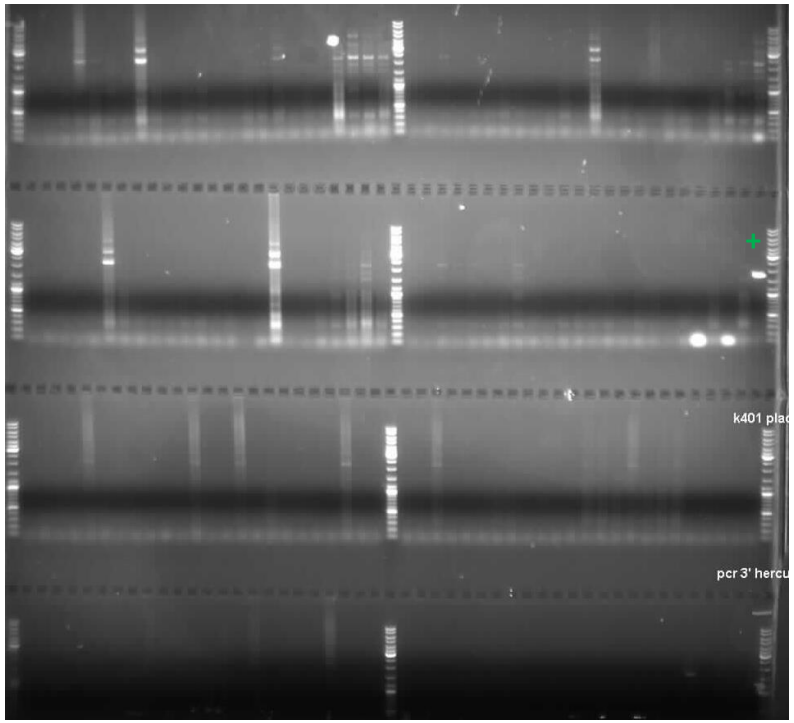
PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	CGTGGTTCCACTCCCATAAAGAAGC	4.4 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	2.6 kb
	Rext	CGAGCGATCTGCACGCATTTCTCCG	
3’ PCR	Fint	TGGCTTTCCACTTAGTTTTTGCTAAG	1.7 kb
	Rext	CGAGCGATCTGCACGCATTTCTCCG	

Long-Range 3’ PCR screening – results



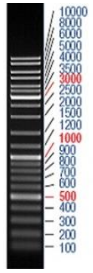


PCR Fneo – Rext : 2.6 kb



+ : Controls DNAs

PCR Fext – Rint : 1.7 kb

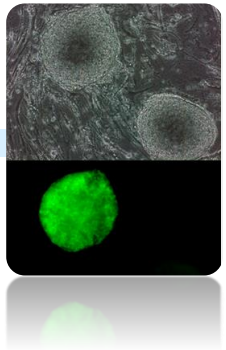


Ladder pattern

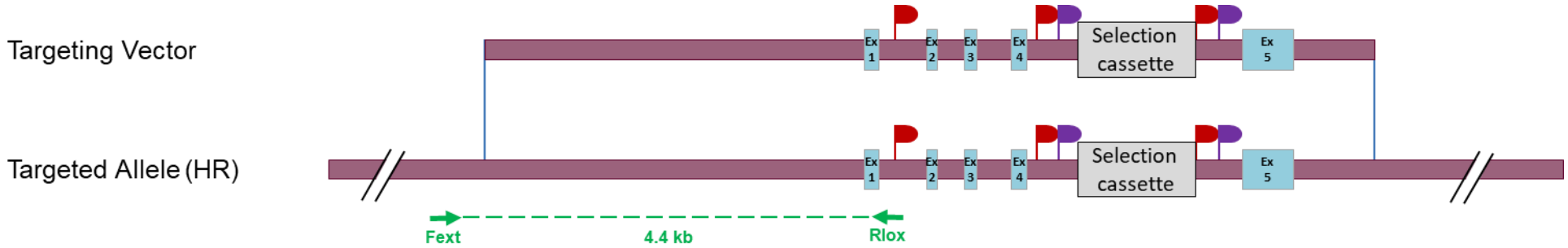
Three candidate clones out of the positive clones were selected for 5’ Long-Range PCR and Southern blot validation.

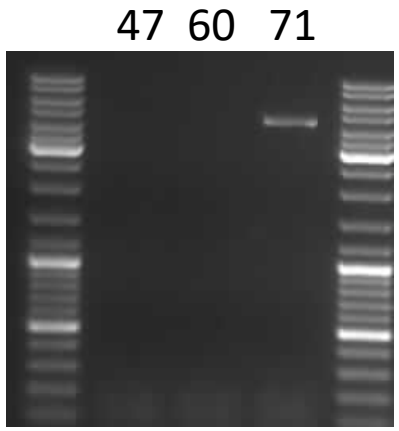
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES validation by Long Range PCR

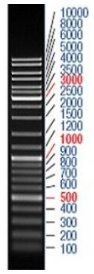


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs





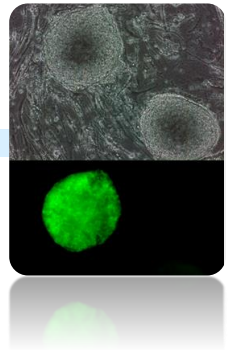
PCR Fext – Rlox : 4.4 kb



Ladder pattern

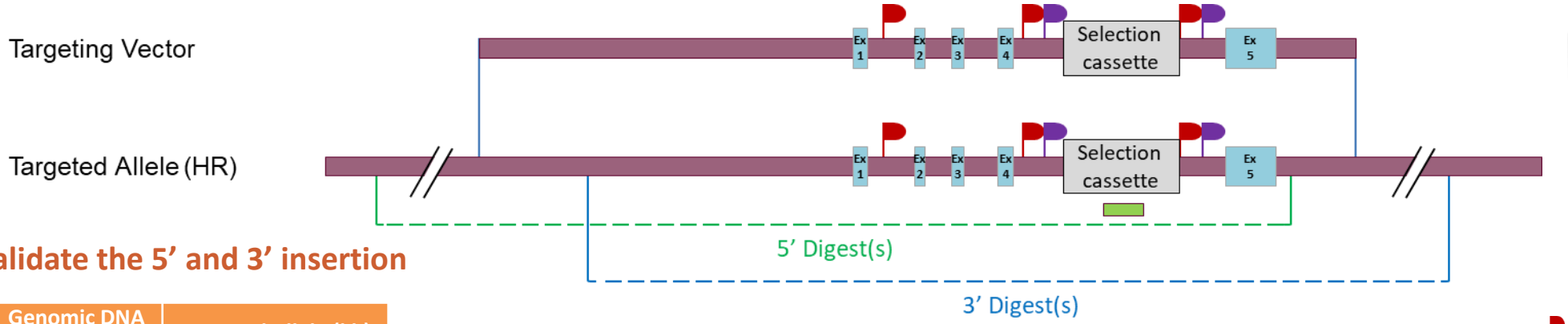
Three candidate clones identified by 3’ PCR screening were further analysed by 5’ Long-Range PCR screening.
Only one clone (clone #71) was further analysed.

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.

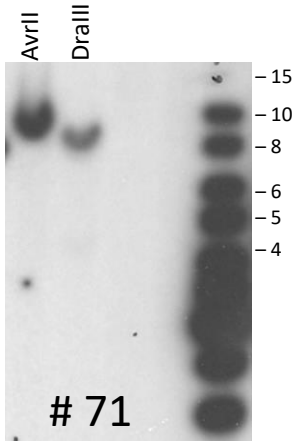


Digestions used to validate the 5' and 3' insertion

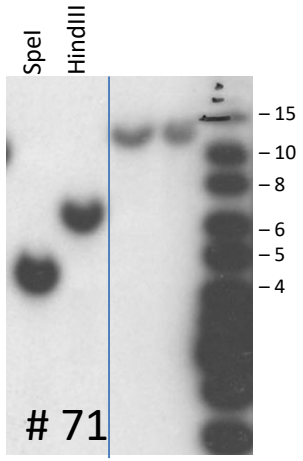
Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	AvrII	10
		DraIII	9.2
	3' digest	SpeI	4.6
		HindIII	6.7

LoxP
FRT
Neo probe

Southern blot - Neo 5'



Southern blot - Neo 3'

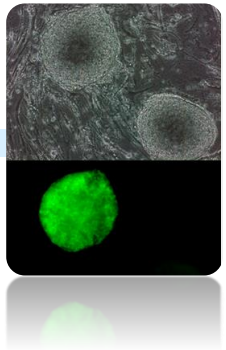


Neo probe sequence

AGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGT
AAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCA
ACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGC
GGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGC
CGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTT
CGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGAT
GTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCAT
CAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCA
CTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAG
GAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAG

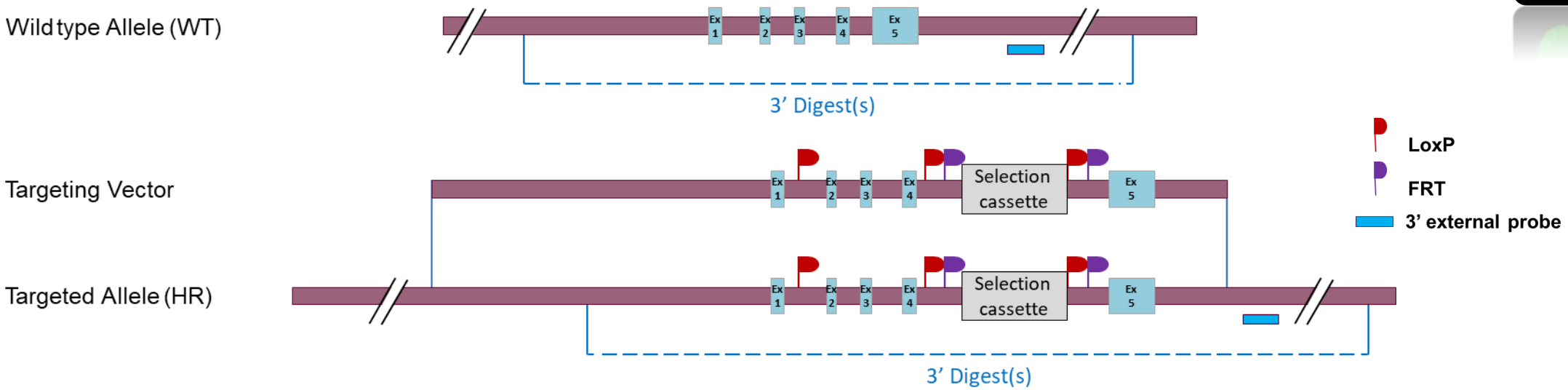
phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

Recombinant ES clones validation by Southern Blot – External probe

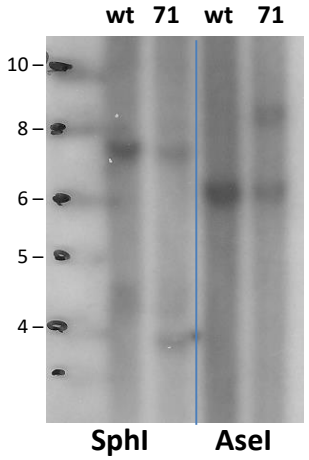


Schematic Southern Blot validation strategy

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 – 3' probe



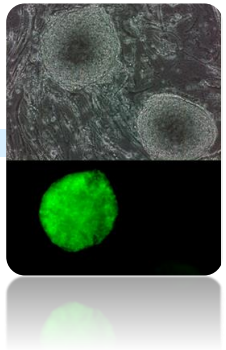
3' probe sequence

AGTACCTCCACCGAATGAACTTCGGATATAATTTGCTTT
TAGTGACCAGACACAGGTGTGAAATGCTAGTCACATCTT
GATTCATGCCAAGCATGTTCCATTATTATACATTGACCC
CTTTCCCCTCCCTGAGCCTTAAGACATGTATCCTAATTC
CTGGAGCAAATCAAACAGAATAGGAGGTGCTTAAGCAGC
CTATGTGGATGAAAGAACTTCCTGCCTTACAGCTGCTTT
AACCCAAAGAGCTGCATTGTACCTGCCAGGTGGCAGGTG
GGTCAGATGTGCCGCTGAGAAGGGCTGGGGAACTGCGGG
GGTTGGGGGTTCCAGGGCTTCCGATCCAAAG

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	SphI	7.3	4
	3' second digest	AseI	6.4	8.5

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	Giemsa
#71	**Pass**

[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**

MICROINJECTION & BREEDING

- Microinjection
- Breeding to F1 generation

Microinjection

- The ES cells used in the injection experiment were originally derived from a 129SV/Ps mouse strain (which have agouti coat colour). These cells were injected into blastocysts derived from a C57BL/6N strain, which have a black 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 C57BL/6N females (Health status SPF Specific Pathogens Free).
- Recombinant ES clones #71 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
#71	2	2	3	7

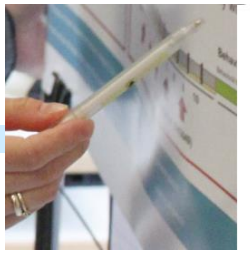
Breeding to F1 generation

- Four highly chimeric males generated in the previous phase by blastocyst injection of ESC clone #71 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 : 03/06/2009

- Allele nomenclature (following MGI guidelines) : **H2az1$^{tm1.1Ics}$**

SEQUENCE OF THE cKO ALLELE



CCGCCGCGGCGCCCCTTCTTCTCCCGGTCCTCGTCCCGCCGCATCTCCTCCCCCCCTAACTCATCCCCCACGCGCCAATCATCGCTCGAGCTCCCGAGCGCCCGCCCCGCCACTCCGCTG
TGCGTTCTCCCATTGGCTGGAGCCTCAAGGACGCGTCCCGAGGGCGGGAGGCAACCATTGGTGGGCCGAACCGGCCGAGTTCCCGGATGAGGGAACATTCTGCAGTATAAAGGGCGCGAG
GAAGGCGGGAGACGGCGCAGTTTGAATCGCGGTCCGACGGAGGAGTGGGCGCTGGGATCTCGCTGAGCGTCCGCCTGGCCTCGTCTCTTCCTCGCTCGTCGGAGCTTCAGCACGGTCCGA
GATGGTAACGTGAAGAGTGACGGCGGGCGGCCGCGGGCTGCAGGCTCCGAGCGGGCGGGGCCGCCGTCCCCGGGGCTGGGAGAGGCGGCGGCGGCGGCGAGTGGGAGCGTGCGGGGACGG
GGTCGGGACGAGCGGAGGGGCCCGTCTGTCCAATCAGGGGCGGCCGGCCAAGCTTCTCGAGCTTAAGGTCGACCTGCAGATAACTTCGTATAATGTATGCTATACGAAGTTATTTAATTA
AAGGTGTTTTCGGAGCCCCGGAGCTCCAGGCGGGAGGCGACGGTTGGGGGGGCGCGCACGGCGCGGCTGGGAGCCGGCGCGGGGGCGCGCAGCCGCGGCGGGCTCGCGGCAGGGGTCGGC
GGGGGAGAAGGGCGGGCGGGGGGCGAGCGGCGGCCCGGGGTCCCCGGCGGGGCGGGCGGCGGGCGGCCGAGCGCGCGCTCGCTCGCCTCCAGGACGCGCTGTGGTGGGGGAGGGGCGCGC
CGCCTTGGTAATTCTATCTTCTCCGTCCCTCCCGGGCTCGCGGGCGTCGGCGGCGCCGCAGGCTGGCGGTAAGGCTGGAAAGGACTCCGGAAAGGCCAAGACAAAGGCGGTTTCCCGCTC
GCAGCGAGCCGGCTTGCAGGTGGGTAGCAACTGCCGCAGTCGGGTGACCGGGCCTTTATGTTTGCACCCCCCTTGCTTGTTTGTCTGCACCCTCCATGGGTGTTCCATCTTAGCATGAGT
GATGTGTCGCGACTTGGCCCTGCGGAGACAGTGAATACTGGGGTTTGGGGAGGGGGGGCGTCGGATCACATGTGTGTTAACTGGCGGGGCGCGTTGGTGCACCACCACCGTCCTTGGCTT
TGGCGCGCACATCTTATCTTAACGAATTTCCTATCTTTGTGCATTTTTTTTCTTGTTTGTTCGTTTGTTTGTGTGCATAAAAATCAAGTTCCCTGTGGGCCGTATTCATCGACACCTGAA
ATCTAGGACAACCAGCCACGGACGTGTGGGCGCGACCGCCGCTGTGTACAGCGCAGCCATCCTGGAGTACCTCACCGCAGAGGTAAGCGGGAGTACGCCTGTGATCTGGAAAAGCTCTGG
GGGGGCGGGGGAAAGTGGCGAGAGAGAAGGAGGAAAGTGATGCAAGAAAACTCGCAGGCCCTAAGCTAAGAGCGGCGCGCAGACCGAGGGAGCGCTAGAGGGAGCTCGCGTTCAGCCGAG
GAGGCTGCTGGGCCAGAGTGTGCTGCTCCTGCAAATGCAATTGCGTGCCCCTTCGGGGTTTTTTGGGTGGTTTTTTTTTTTTTTTTGCATAATTCTGCATAATTTTATCATTCAGGTAGT
AGGGAATGACAACAGTTGGTAAATAACACCATTGAGGTTCAAAGATCTTGATTGAAAGTAAGTTGGAATATTGATCATCAGATTTTTATTTATTTATTTTTTGCCTTTTAGGTACTTGAG
TTGGCAGGAAATGCGTCAAAAGACTTAAAGGTAAAGCGTATCACCCCTCGTCACTTGCAGCTTGCTATACGTGGAGATGAAGAATTGGATTCTCTGATCAAAGCTACCATTGCTGGTGGT
GGTATGTCATCCCTAAACTCCTAACATTCTATTTAAGAAGAAAATTCCTACACTCCCATTGTTTCTAGAAGAGATTTCAGTACAGGGTGCTTTCATGGGGTTCACCGGTGATAACTTCGT
ATAATGTATGCTATACGAAGTTATGCGGCCGGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCCGCGGATCCATCGACCCCCTGCAGGTGGCTTTCCACTTAGTTTTTGCTAAGAT
CATTGTTCCATCCCTCAAGTTTTCACTTTGTATATGAATTTTACCTGCTTTGAGTTCATGTATTGTTTTTTTAAATGTTTATCCAGAAGGTAGACTTAATCTGTGTCCCTCTCCTAGGTG
TCATCCCACACATCCACAAATCGCTGATCGGGAAGAAAGGACAACAGAAGACTGTTTAAGGATGCCTGGATTCCTTATTATCTCAGGACTCTAAATATTCCTAACAGCTGTCCAGTGTTG
GTGATTCCAGTGGACTGTATCTCTGTGAAAAACACAATTTTGCCTTTTTGTAATTCTATTTGAGCAAGTTGGAGGCTTAATTAGCCTTCCAACCAACCAAATTTCTGCATTCGAGTCTTA
ACCATATTTAAGTGTTACTGTGGCTTCAAAGAAGCTATTGATTCTGAAGTAGTGGGTTTTGATTGAGTTGACTGTTTTTAAAAAACTGTTTGGATTTTAATTGTGATGCAGAA

LoxP FRT Exons 2, 3 and 4

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION
Protocol finalized on 2024/11/19
Prepared by Romain LORENTZ, IE
Verified by Marie-Christine BIRLING, PhD

CONTACT US
By email at mutagenesis@igbmc.fr
By phone at +33 (0)3 88 65 57 43

www.phenomin.fr

macroH2A.Z (IR00001643 / K401 ICS internal reference) mouse line genotyping protocol

Table of contents

For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)
ICS genotyping service
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex France
Email: mutagenesis@igbmc.fr

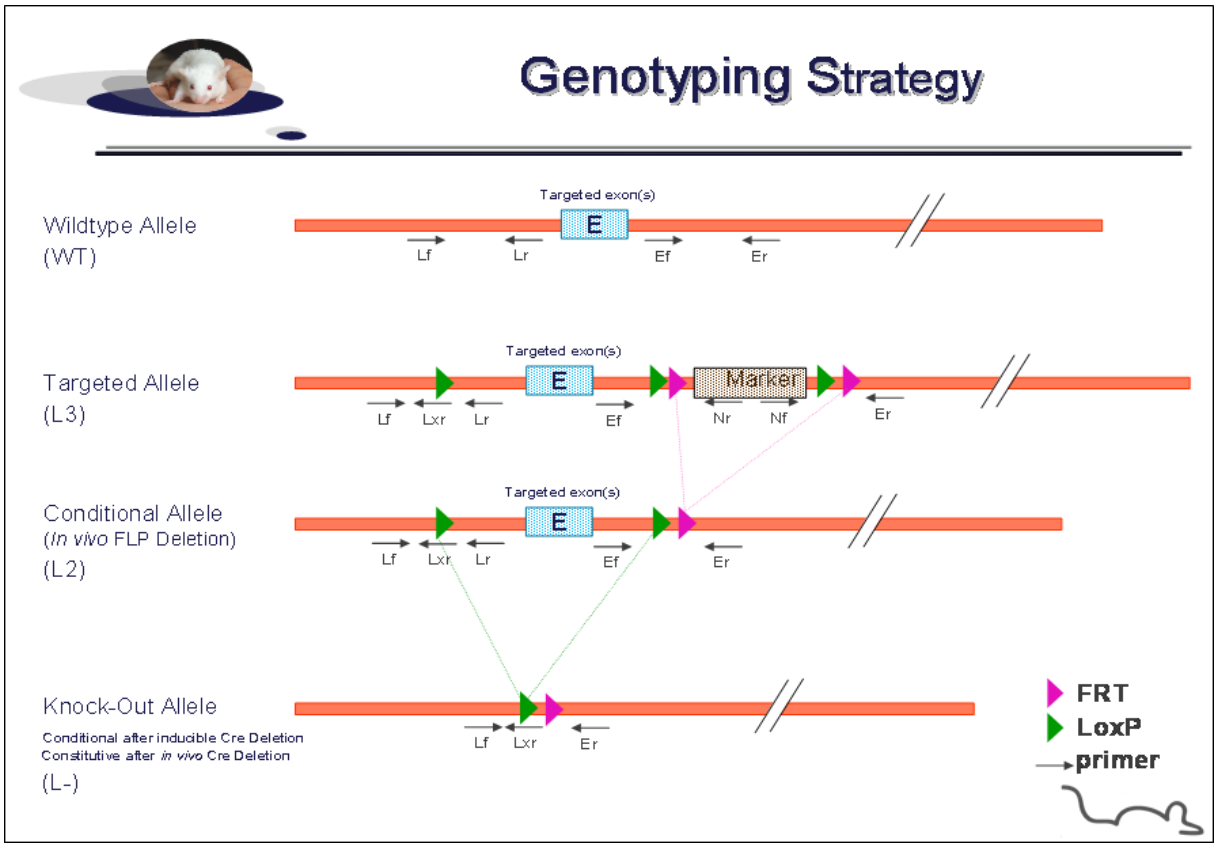
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **macroH2A.Z** 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	4421	GCTACCATTGCTGGTGGTGGTATGTCA
Er	4422	TGTGTGGGATGACACCTAGGAGAGGG
Er	4423	CCCGATCAGCGATTTGTGGATGTGTGG
Lf	4419	CGTCGGAGCTTCAGCACGGTCC
Lf	4424	CCACTCCGCTGTGCGTTCTCCC
Lr	4420	TGGAGCTCCGGGGCTCCGAA
Nf	3720	AGGGCCAGCTCATTCCTCCCACTC
Nr	3721	GTAGAAGGTGGCGCGAAGGGGC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
Presence of the distal loxP	4424-4420	Lf / Lr	519***	519***	---	418***
Excision of the selection marker	4421-4422	Ef / Er	2283*	388	---	278
5' part of the selection marker	4421-3721	Ef / Nr	385	---	---	---
3' part of the selection marker	3720-4423	Nf / Er	505	---	---	---
Excision of the floxed exon(s), i.e. knock out	4419-4422	Lf / Er	3849*	1954*	483**	1731*

***This sequence is GC-rich. Addition of dimethyl sulforide (DMSO) is required to enhance the efficiency of PCR.
* This PCR product will not be observed using our PCR genotyping conditions (see description below)
** This PCR is only verified if mice are generated
--- 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:
-10x Buffer (Roche)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Roche)	0.2µl
-DNA (50ng/µl)	3µl
-5' primer (100 µM)	0.125µl
-3' primer (100 µM)	0.125µl
-Sterile H2O	up to 25 µl

Cycling conditions:

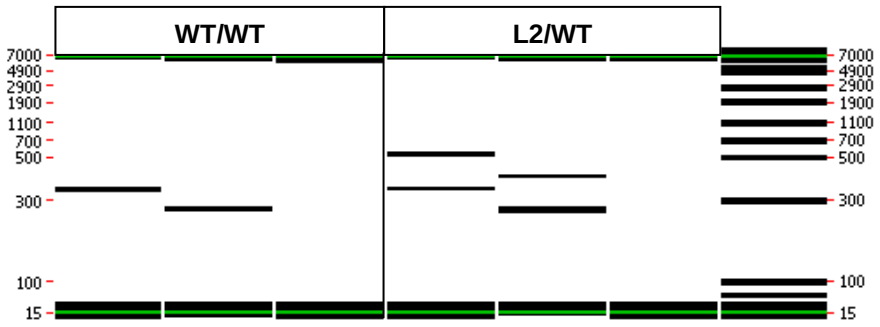
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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



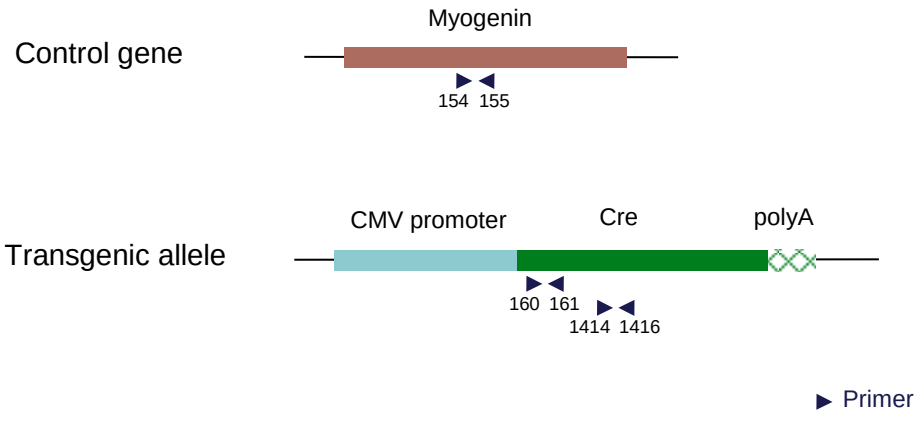
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.

2.1. Cre genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
154	ACTCCCTTACGTCCATCGTG
155	ACCCAGCCTGACAGACAATC
160	GAACCTGATGGACATGTTCAGG
161	AGTGCGTTCGAACGCTAGAGCCTGT
1414	CGTACTGACGGTGGGAGAAT
1416	CCCGGCAAAACAGGTAGTTA

PCR fragments expected size (bp):

Primer pair	160-161	1414-1416	154-155
Region analyzed	5' part of Cre transgene	Middle of Cre transgene	Myogenin control gene
Control gene	/	/	99
Tg allele	345	165	/

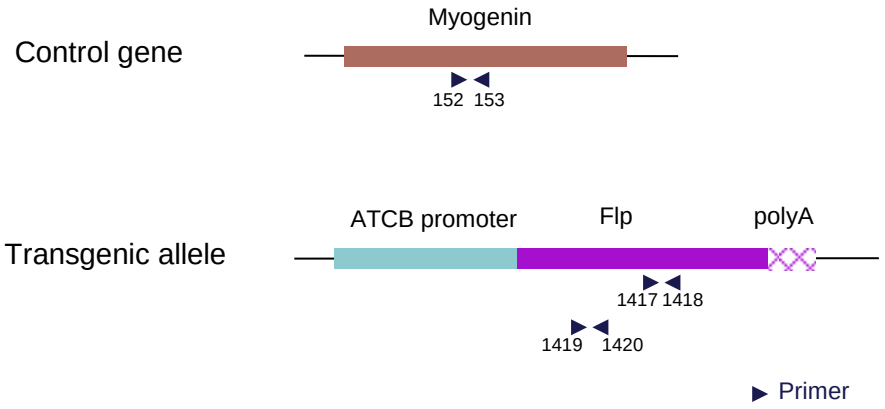
Cycling conditions:

Temp	Time	#Cycles
95°C	3min	1
95°C	10s	
62°C	20s	35
72°C	20s	
95°C	5s	
62°C	30s	1 (melting curve generation)
72°C	72s	
37°C	30s	1
4°C	∞	

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

2.2. Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
152	TTACGTCCATCGTGGACAGC
153	TGGGCTGGGTGTTAGCCTTA
1417	TTCTTTAGCGCAAGGGGTAG
1418	GCTCCAATTTCCCACAACAT
1419	TGGGAAATTGGAGCGATAAG
1420	CTGCCACTCCTCAATTGGAT

PCR fragments expected size (bp):

Primer pair	1417-1418	1419-1420	152-153
Region analyzed	Middle part of Flp transgene	5' of Flp transgene	Myogenin control gene
Control gene	/	/	245
Tg allele	299	175	/

PCR protocol and cycling conditions are identical to those described in chapter 1.2