MODEL GENERATION TECHNICAL REPORT

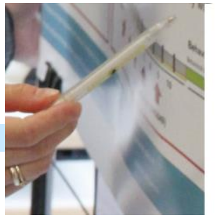
Conditional cKO of Wdr5

Project code: Kos580 / IR2982

Report finalized: 26/01/2024

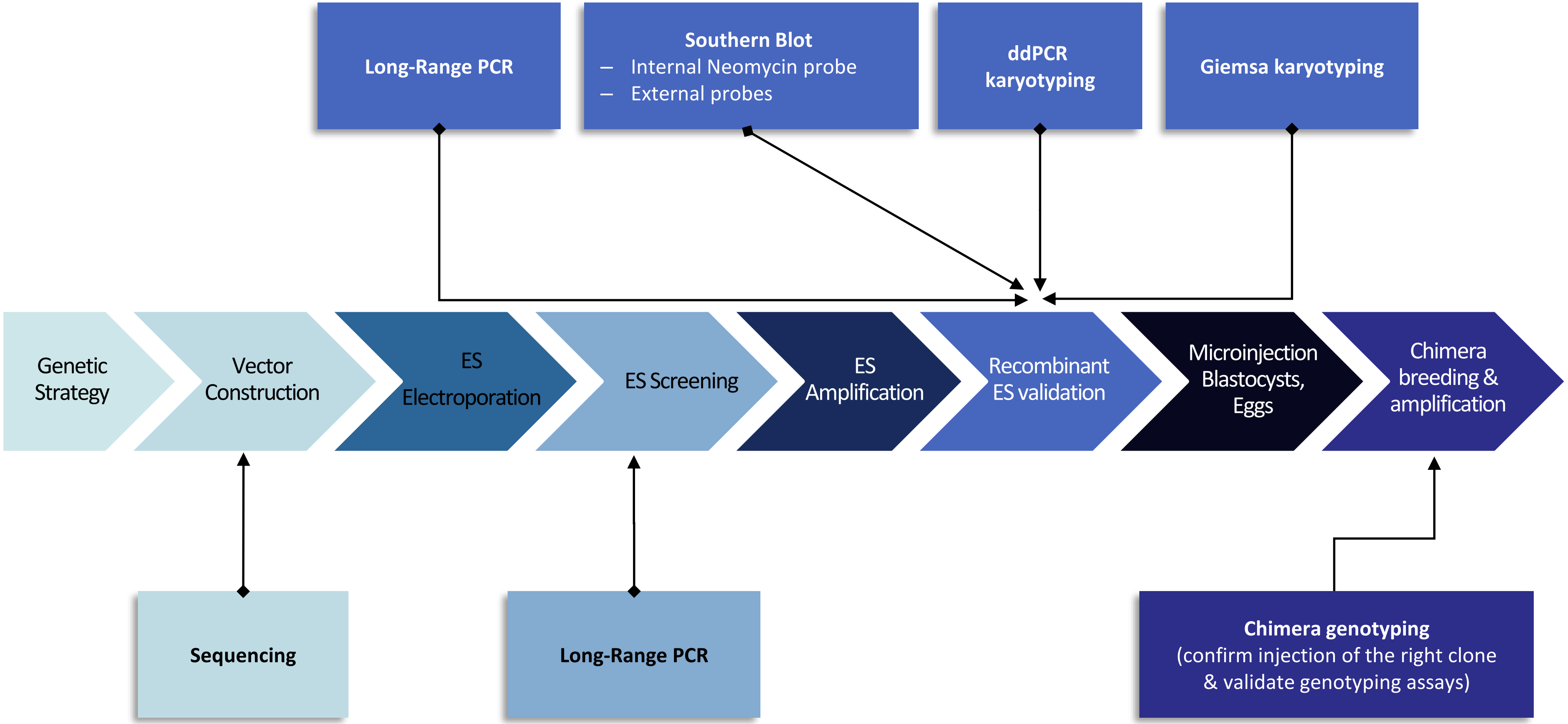
MODEL GENERATION TECHNICAL REPORT

PROJECT PROCESS & QUALITY CONTROLS



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

INVESTIR L'AVENIR



Long-Range PCR

Southern Blot
- Internal Neomycin probe
- External probes

ddPCR karyotyping

Giemsa karyotyping

Genetic Strategy

Vector Construction

ES Electroporation

ES Screening

ES Amplification

Recombinant ES validation

Microinjection Blastocysts, Eggs

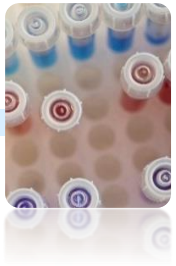
Chimera breeding & amplification

Sequencing

Long-Range PCR

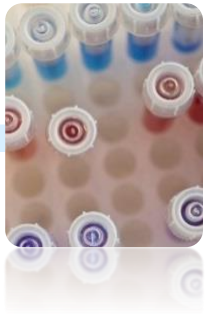
Chimera genotyping
(confirm injection of the right clone & validate genotyping assays)

2 GENETIC STRATEGY



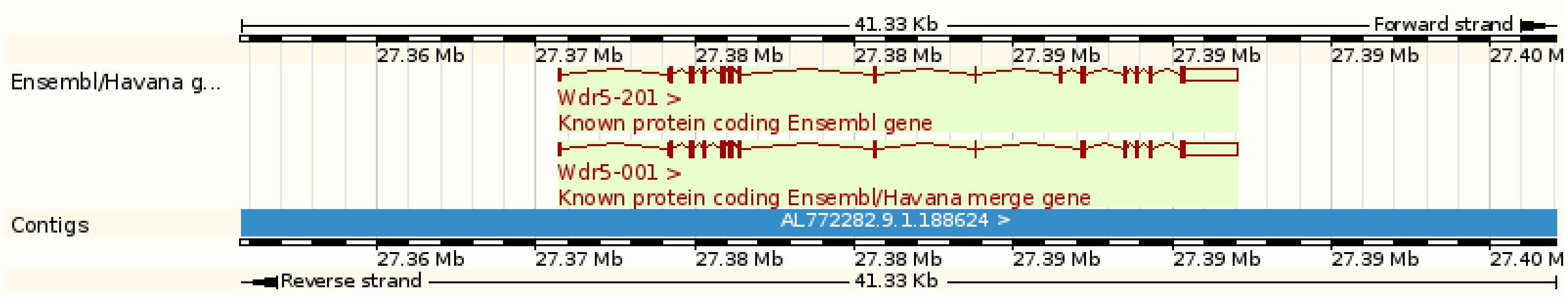
- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

Wdr5 mouse genomic locus – structure

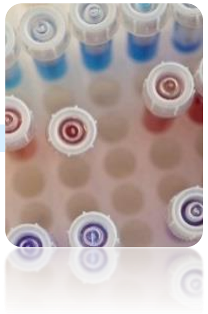


Location: Chromosome 2

Ensembl Gene ID: ENSMUSG00000026917

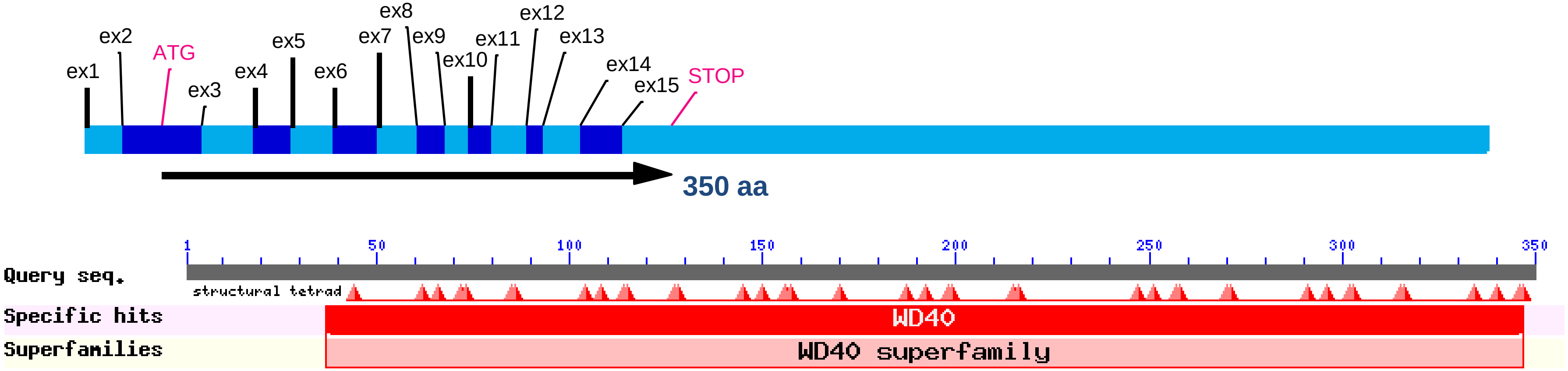


Wdr5 mRNAs and proteins



Name	Transcript ID	Protein ID
Wdr5-001	ENSMUST00000113952	ENSMUSP00000109585
Wdr5-201	ENSMUST00000113953	ENSMUSP00000109586

Wdr5-201



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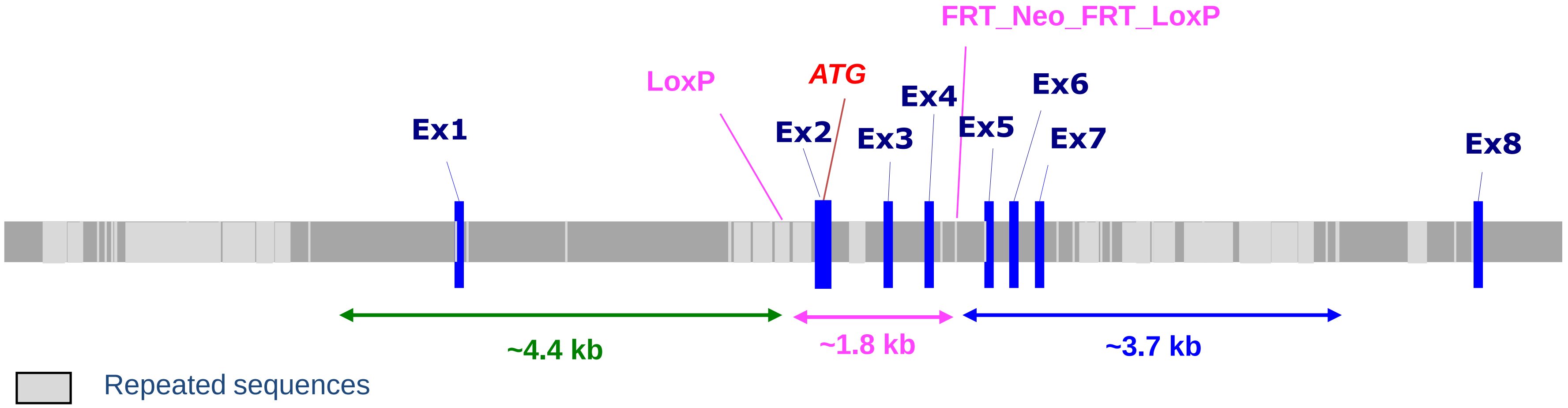
Approach undertaken: flox exons 2 to 4

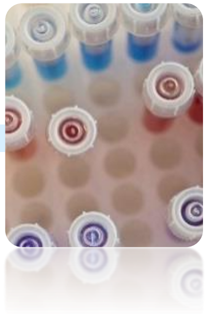
Targeted locus



mRNA and protein expected after Cre mediated excision

No protein

PROs & CONs evaluation of the strategy



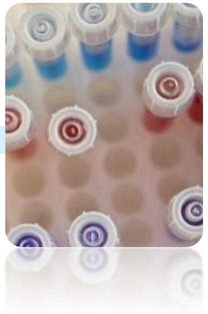
Pros

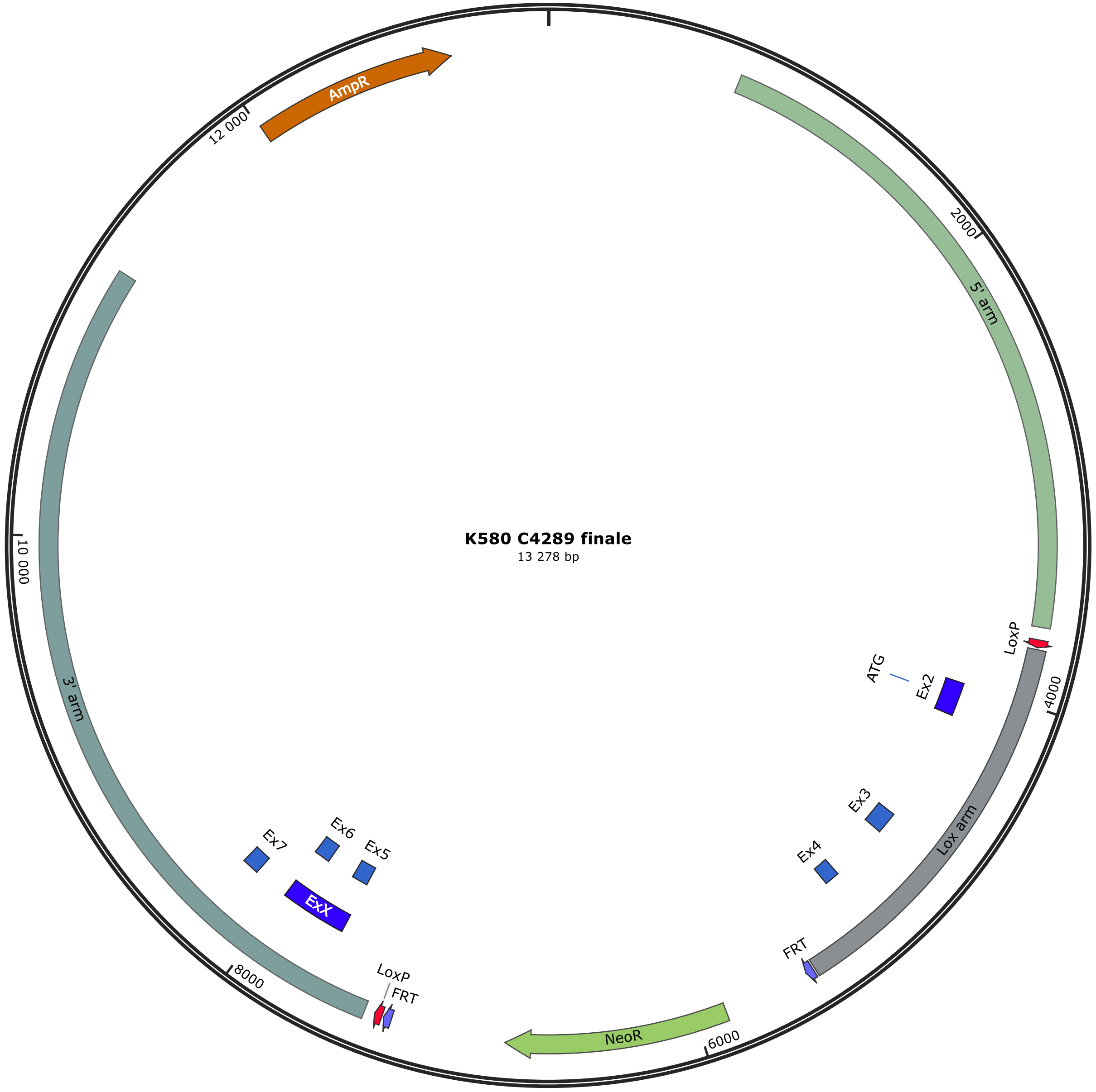
- ✓ No protein expressed after Cre mediated excision even if the RNA is stable

Cons

- ✓ Insertion of the 5’ LoxP into intron 1 (could disturb expression of the conditional allele)
- ✓ Presence of mumerous repeated regions in the 3’ homologous arm (blue arrow)

3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION





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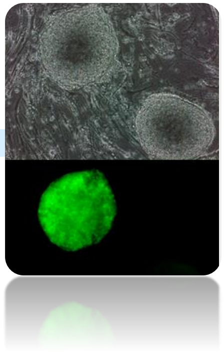
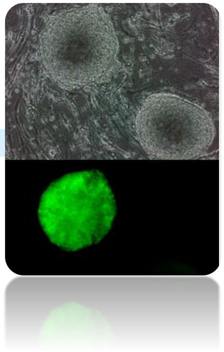
4 ES cell electroporation & Screening of recombinant clones



- 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



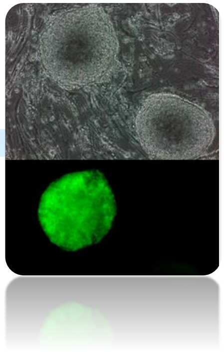
The targeting vector was electroporated in the ICS proprietary C57BL/6NTac ESC line TB1.

Transfected ES clones were submitted to neomycin selection (G418) and 186 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.

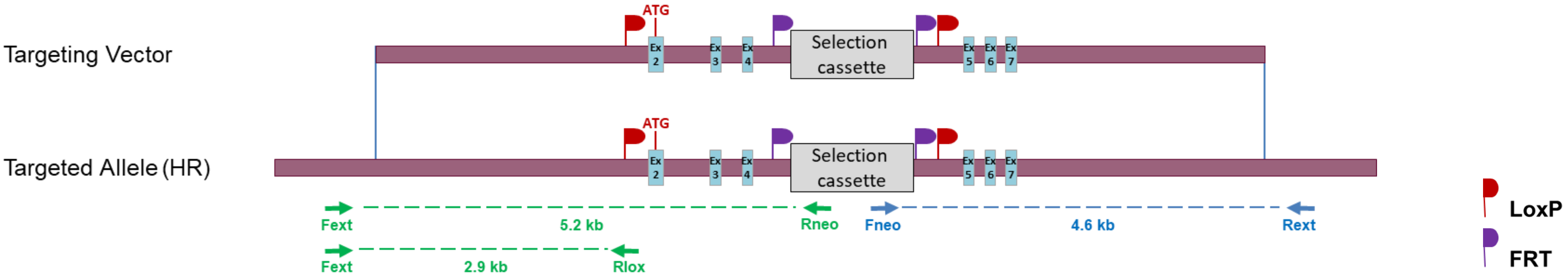
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. Six 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	TCACTGGGAGTGGAATTCTCTG	5.2 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
5’ PCR	Fext	TCACTGGGAGTGGAATTCTCTG	2.9 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.6 kb
	Rext	GAGACACAGTGAACGGCTGAGGGGA	

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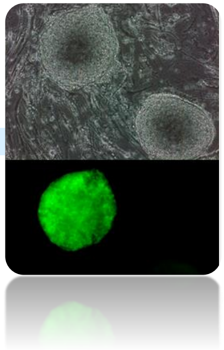
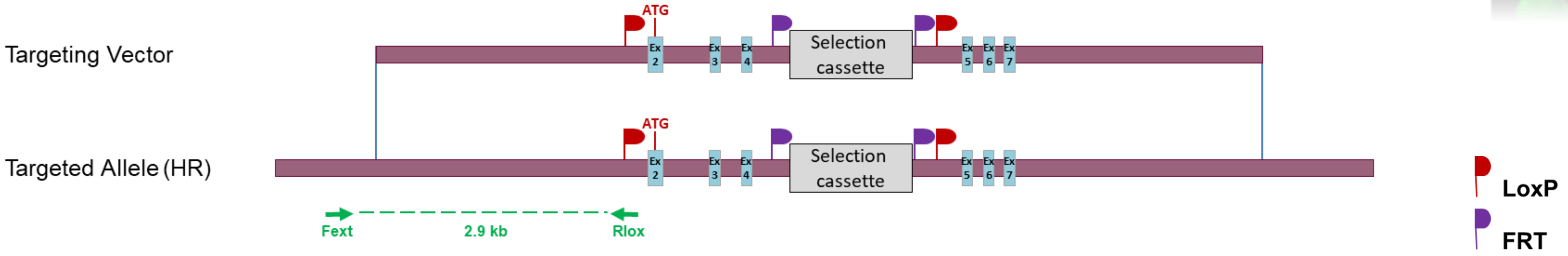
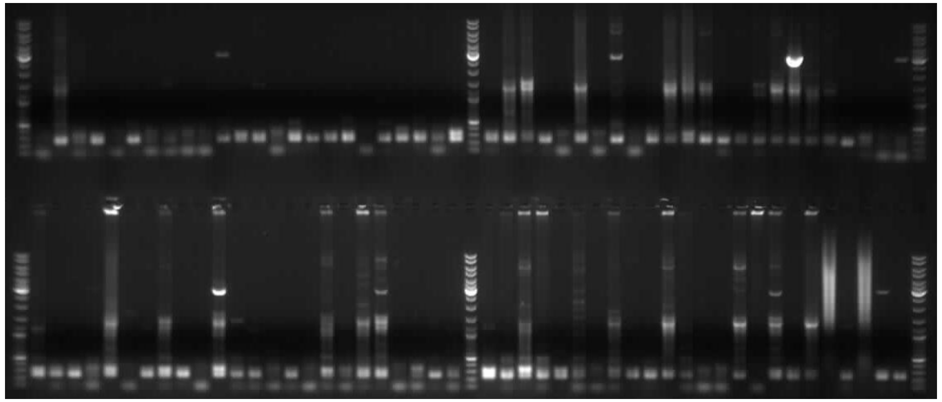
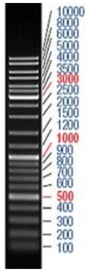
Long-Range 5’ PCR screening – results





PCR Fext – Rlox : 2.9 kb

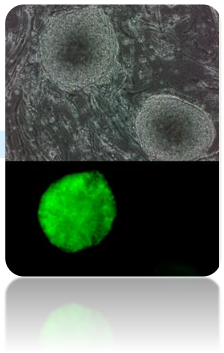




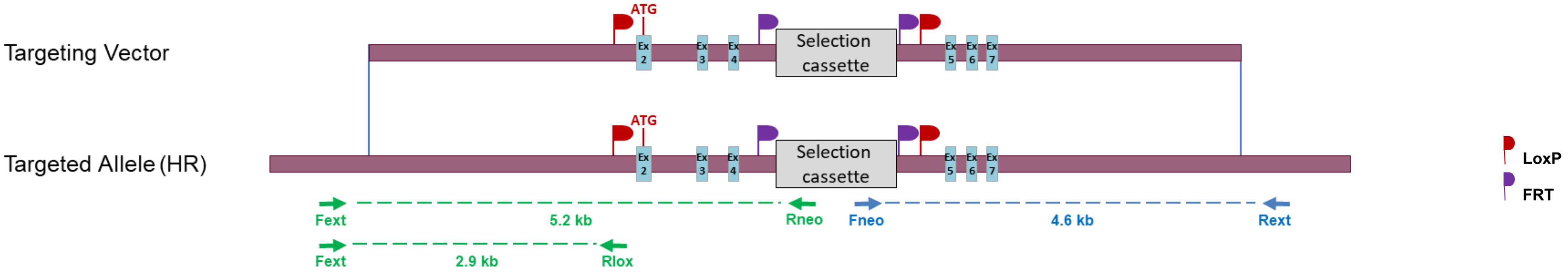
Ladder pattern

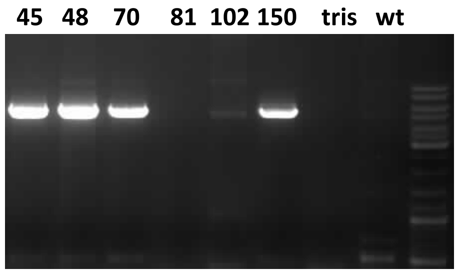
Six candidate clones out of the six positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

Recombinant ES validation by Long Range PCR

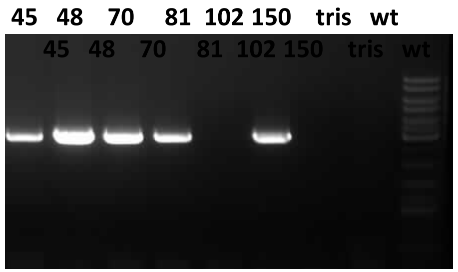


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

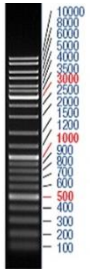




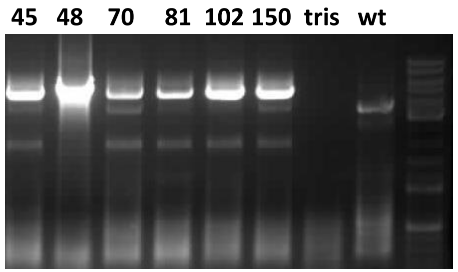
PCR Fext – Rneo : 5.2 kb



PCR Fext – Rlox : 2.9 kb



Ladder pattern



PCR Fneo – Rext : 4.6 kb

Six candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Five clones (clones #45, #48, ,#70, #81 and #150) were confirmed.

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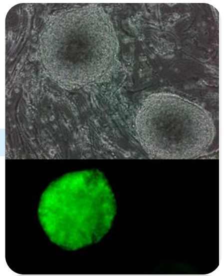
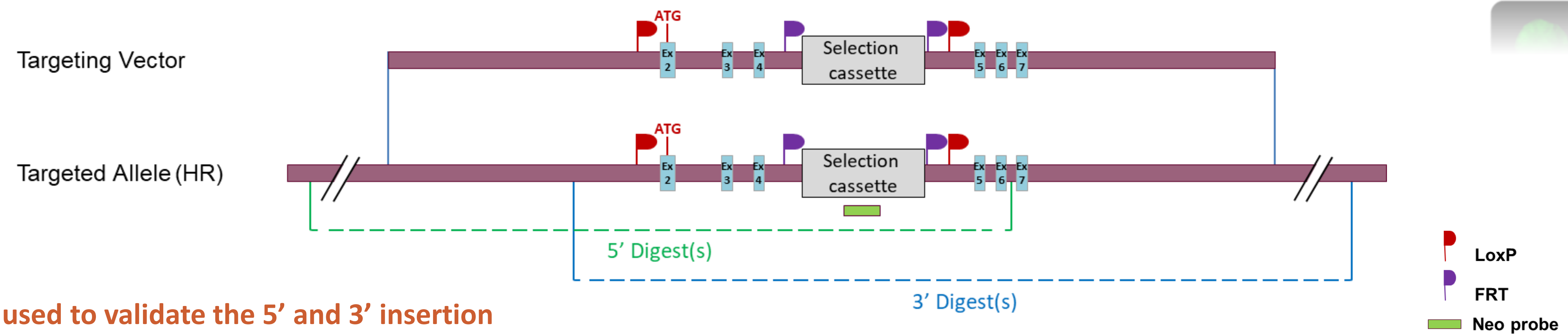
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

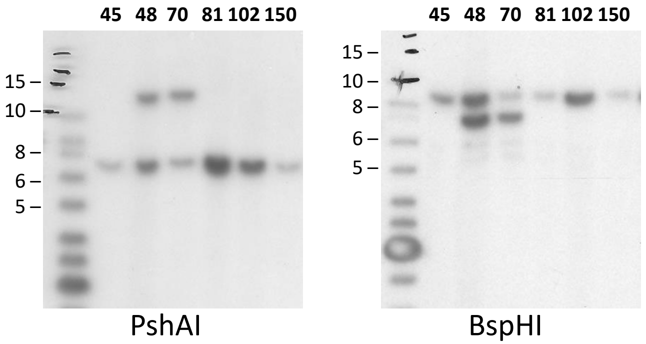
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



Digestions used to validate the 5' and 3' insertion

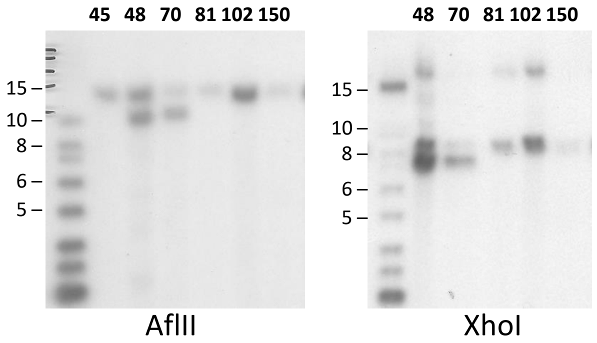
Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	PshAI	7
		BspHI	8.9
	3' digest	AflII	15.1
		XhoI	8.6

Southern blot - Neo 5'



PshAI BspHI

Southern blot - Neo 3'



AflII XhoI

Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
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ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCCGCTG
TAAGTCT

=> Clones #48 and #70 are not correct (extra band observed)

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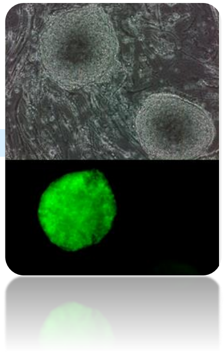
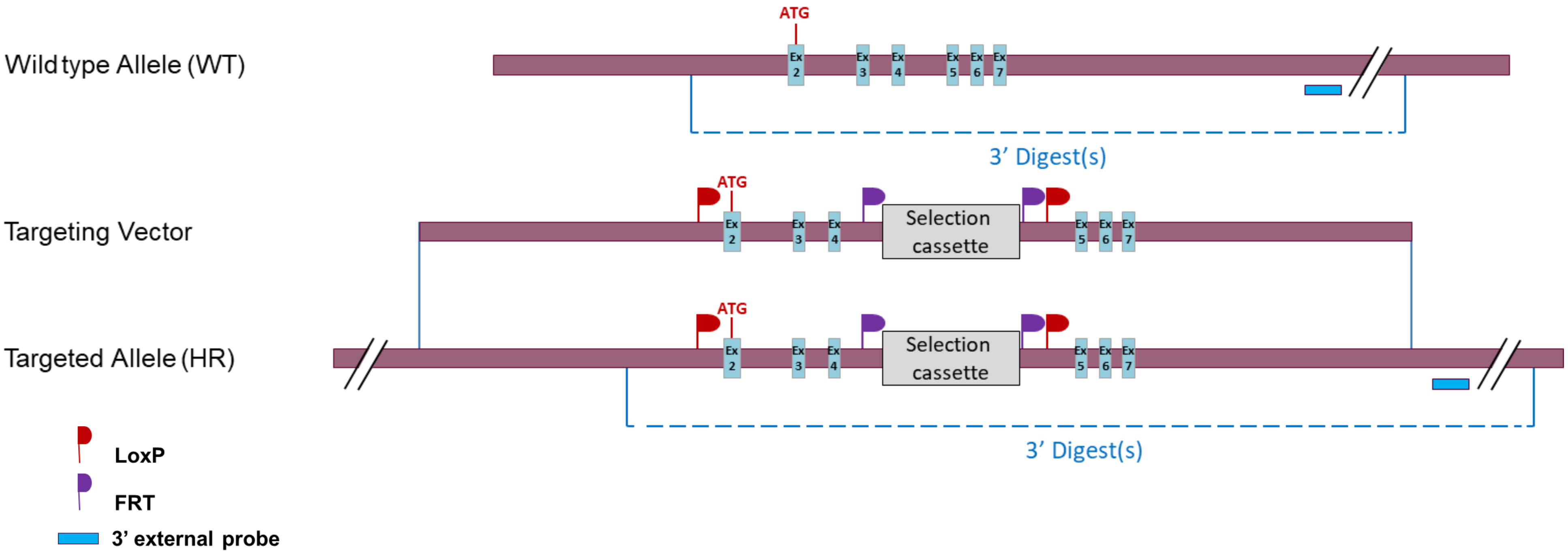
Recombinant ES clones validation by Southern Blot – External probe



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.



5' probe sequence

GGCCTCTGGTAAGTGAGAGTCTTCATGTAGT
CGGTGACCTGTTGTTTATGGCCTGCTATGTT
GTTCCAGCTCTGTGGTGCATCAAAGTGGGGG
CCTGTGCCTCCTCATCCTCCTGAGGCCTGCG
CCCCACTGGGTGCAGAGTTCAGGGGATGAAA
AGCTATGAAGGGAAAGGTAGATGATAGGTGA
GGGCACCCAGGGCAGAGTGAGAGCTGGGAGC
TCCTTGCATGCCCCTGAGTGTACCCTTTCAA
AGTTGTAGAAAGTGAGTTAAAGGTCTGAGCC
CTTGTAAGTGCAGTGGTGCTGTTCACAGATC
CCATGAGCTCTGGGCAGGTGAACCACCAGAT
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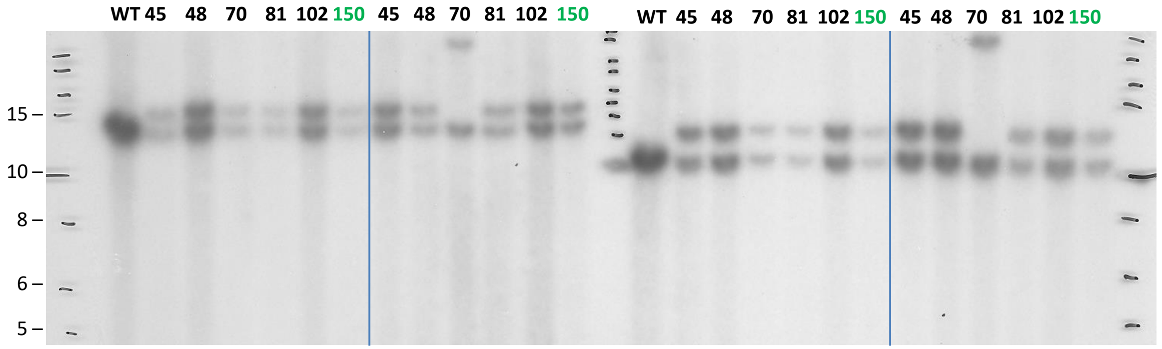
CACAAAAGGCAGGCTTGGGAGTACAGTGCTA
TGTACTTGC

Southern blot – 3' probe



NheI 13.5 / 15.3

SexAI 10.4 / 12.5

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	NheI	13.5	15.3
	3' second digest	SexAI	10.4	12.5

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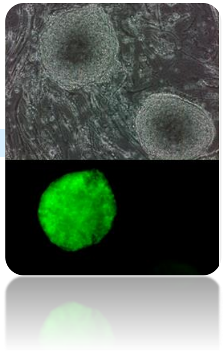
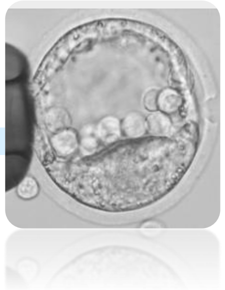
Aneuploidy screening in ES recombinant clones



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.

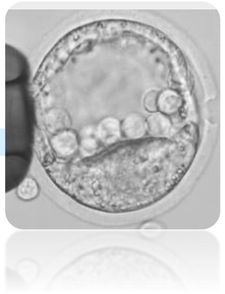
Clone ID	Giemsa
#45	Not done
#81	**Pass**
#102	Not done
#150	**Pass**

5 MICROINJECTION & BREEDING



- Microinjection
- Breeding to F1 generation

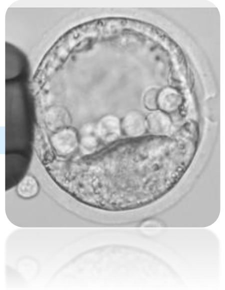
Microinjection



- 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 #81 and #150 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
#81	7	0	0	7
#150	**5**	**3**	**0**	**8**

Breeding to F1 generation

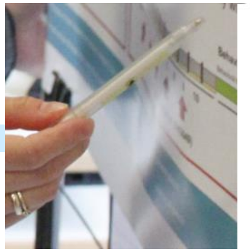


- Four highly chimeric males generated in the previous phase by blastocyst injection of ES clone #150 were mated with C57BL/6N Flp deleter females (health status SPF – Specific Pathogen Free) to investigate whether the recombined ES cells have contributed to the germ layer.

- Germ line transmission was obtained the : 08/06/2011

- Allele nomenclature (following MGI guidelines) : $\mathbf{Wdr5}^{\mathbf{tm1.1Ics}}$

6 SEQUENCE OF THE DELIVERED ALLELE



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LoxP FRT Exons

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REPORT REDACTION & VALIDATION
Protocol finalized on 2024/01/26
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

WDR5 (IR00002982 / K580 ICS internal reference) mouse line genotyping protocol

Table of contents

For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)
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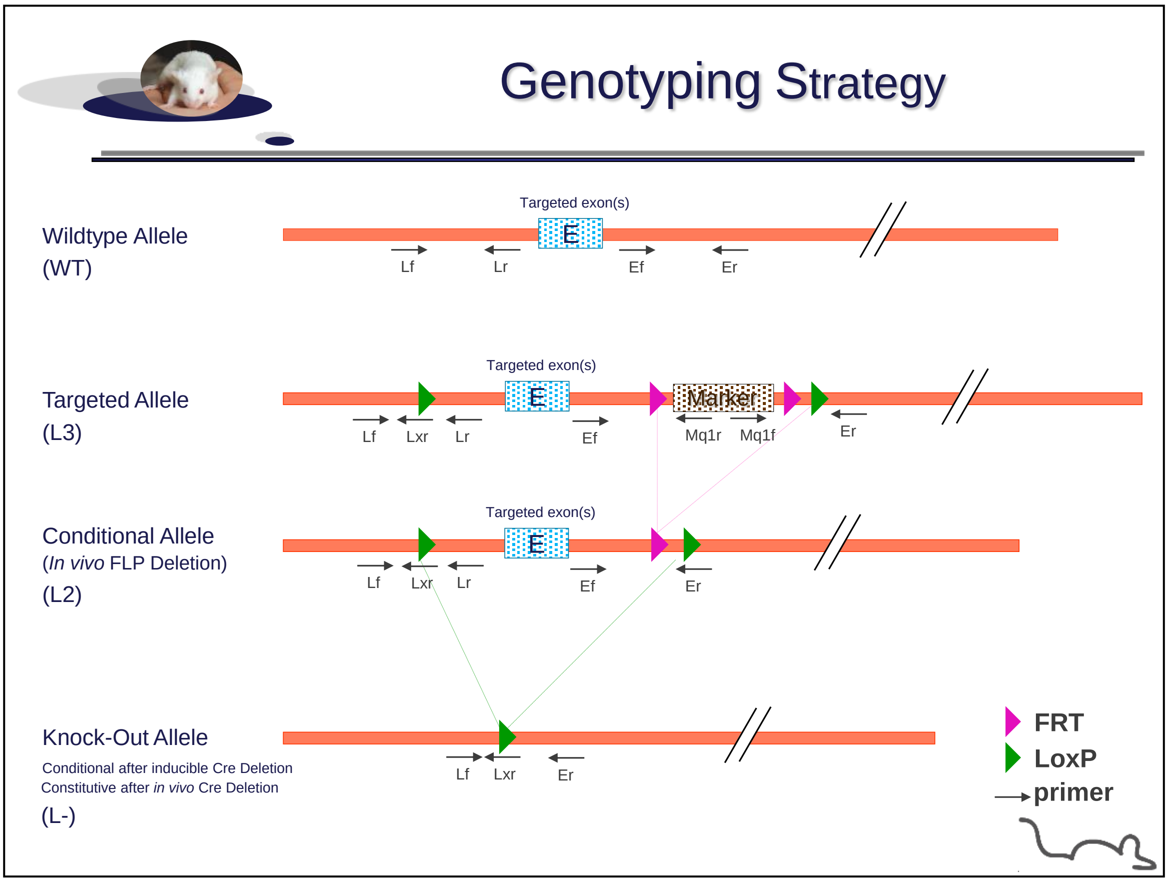
This protocol has been validated by Pauline Cayrou.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **WDR5** Conditional Knockout (cKO) project.

1.1. Genotyping strategy

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


Genotyping Strategy

Sequence of primers used for genotyping

Position	Primers	Sequence
Ef	5492	CTTACCAAGTAGTCCAATTTGGTCTG
Er1	5493	CTGTGGAGGCTCAAAGTGCTTGTG
Er2	5494	CAGTGTTAGCACCACAAAACACCTGG
Lf1	5489	CCTGGAACTCAGAAGTCTGCCTG
Lf2	5490	CTCTGTGTAGCTCTGACTGTCCTG
Lr	5491	CTGTCAGCTGAACTCTATAGTAAGG
Lxr	4724	CGAAGTTATCTGCAGGTCGACCTTAAG
Mq1f	1219	CAGCTCATTCCTCCCACTCATGATC
Mq1r	238	TGACTAGGGGAGGAGTAGAAGGTG

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	5489-5491	Lf1 / Lr	428	428	---	332
Excision of the selection marker	5492-5494	Ef / Er2	2182*	328	---	203
5' part of the selection marker	5492-238	Ef / Mq1r	303	---	---	---
3' part of the selection marker	1219-5493	Mq1f / Er1	394	---	---	---
Excision of the floxed exon(s), i.e. knock out	5490-5494	Lf2 / Er2	4019*	2165*	386**	1944*
LoxP specific PCR	5490-4724	Lf2 / Lxr	182	182	182	---

* 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:
- 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:

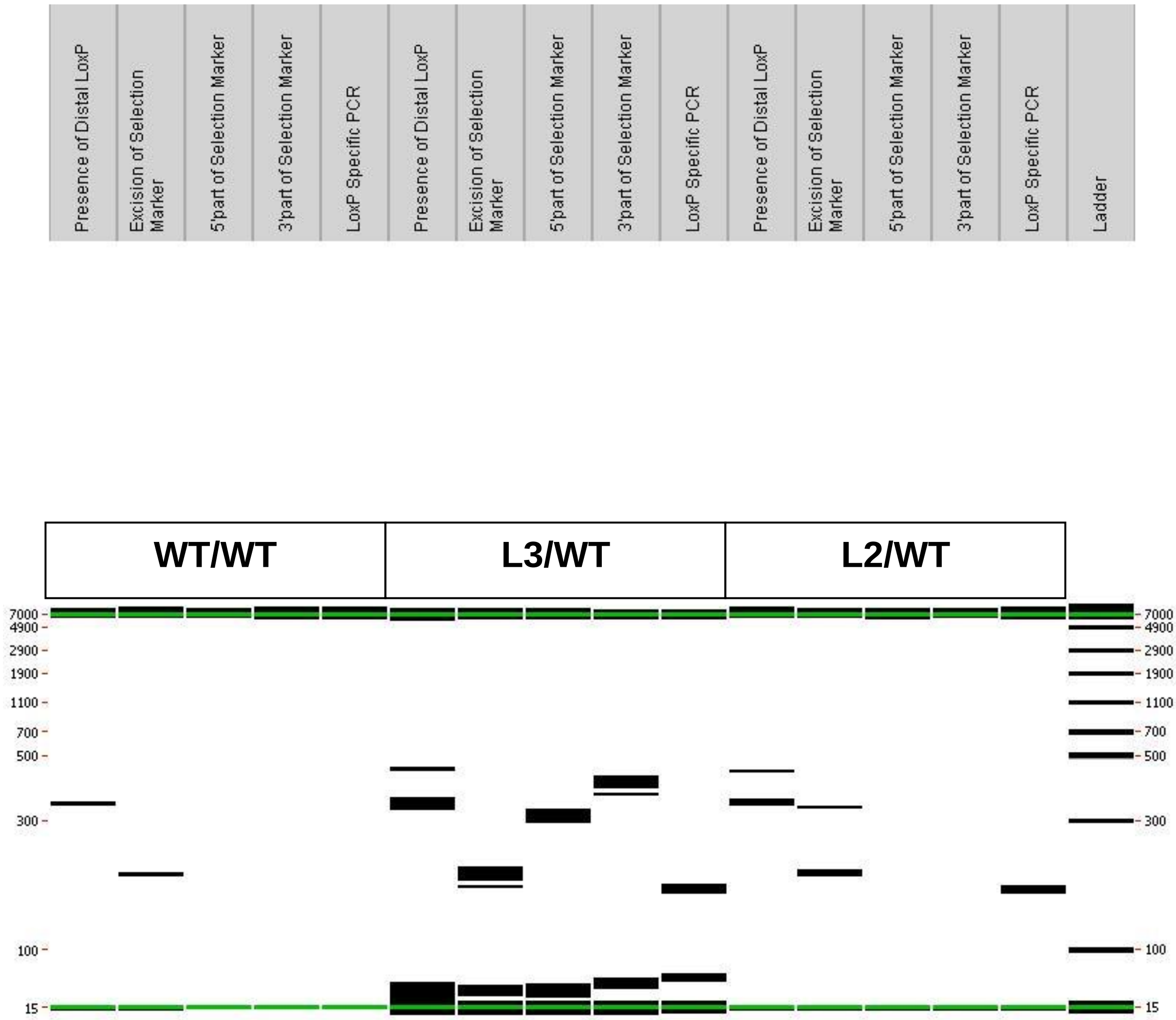
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5 min	1

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



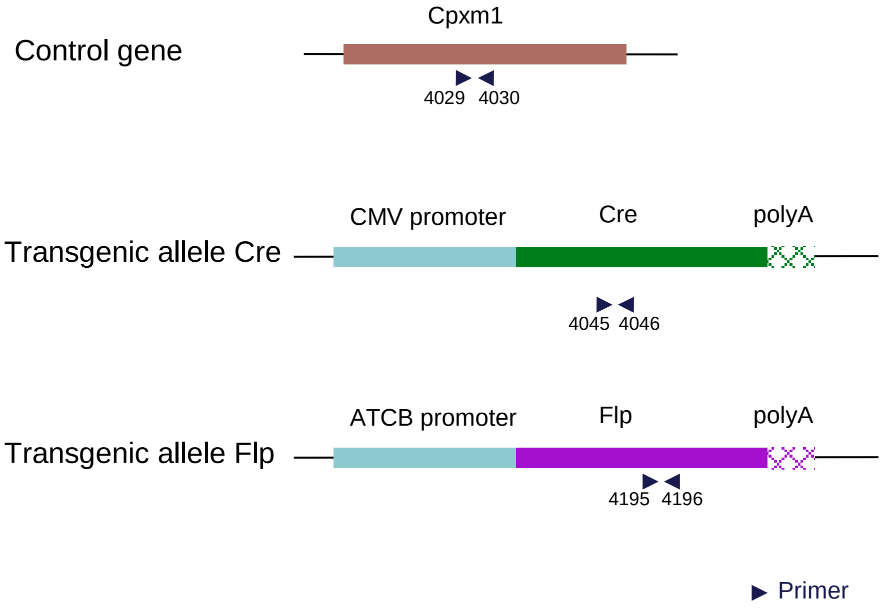
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

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

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