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MODEL GENERATION TECHNICAL REPORT

Generation of mouse model : Dync1h1 K3334N point mutation-conditional KO

Project code: G4564/ IR4564

Report finalized: 21/09/2023

MODEL GENERATION TECHNICAL REPORT

1 PROJECT PROCESS & QUALITY CONTROLS

2 GENETIC STRATEGY

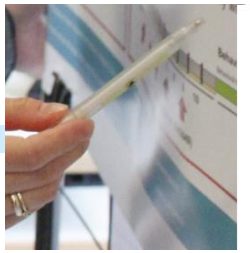
3 HOMOLOGOUS RECOMBINATION VECTOR CONSTRUCTION

4 ES ELECTROPORATION & SCREENING OF RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

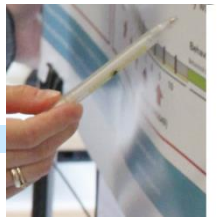
6 SEQUENCE OF THE DELIVERED ALLELE

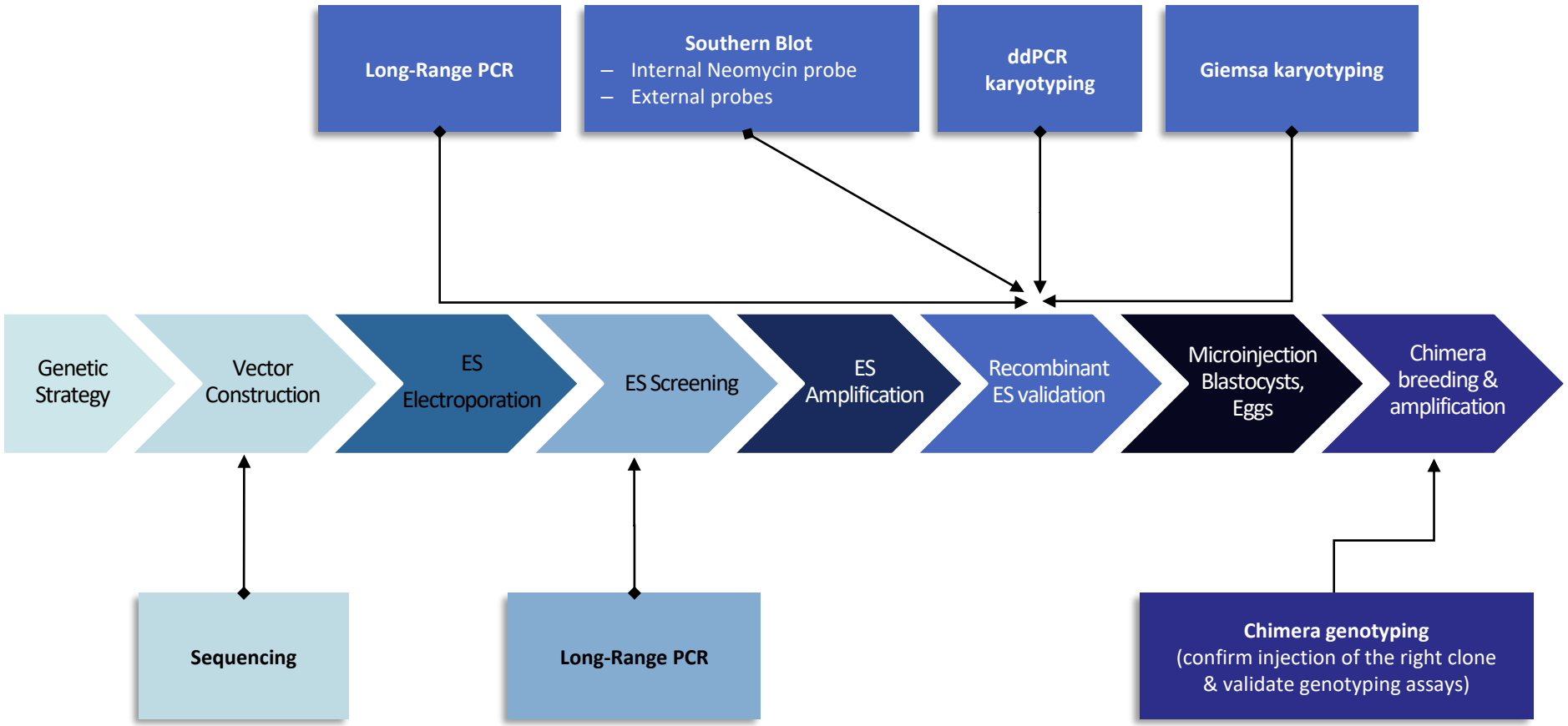
1 PROJECT DESCRIPTION



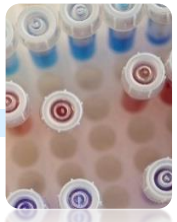
- Aim
- Project process & quality controls

PROJECT PROCESS & QUALITY CONTROLS



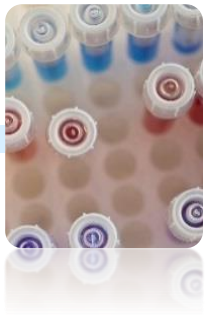


2 GENETIC STRATEGY

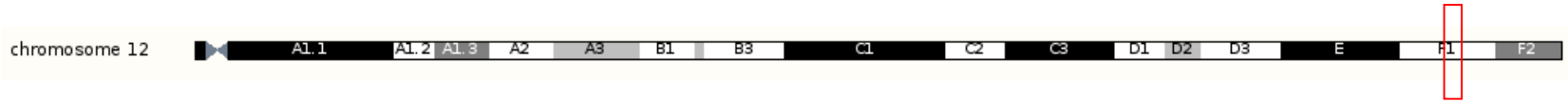


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

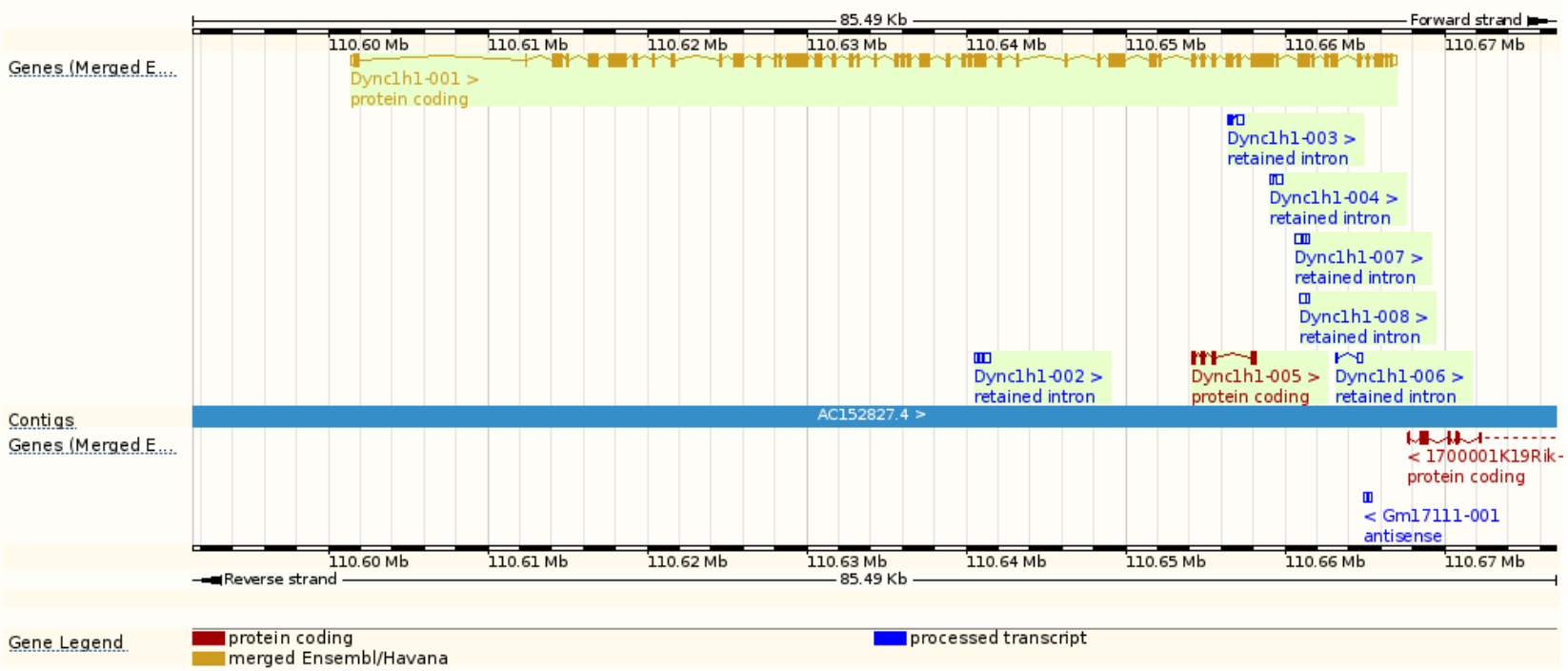
Dync1h1 mouse genomic locus – structure



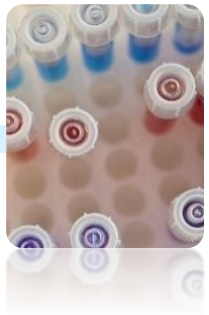
Location:



Dync1h1 ENSMUSG00000018707

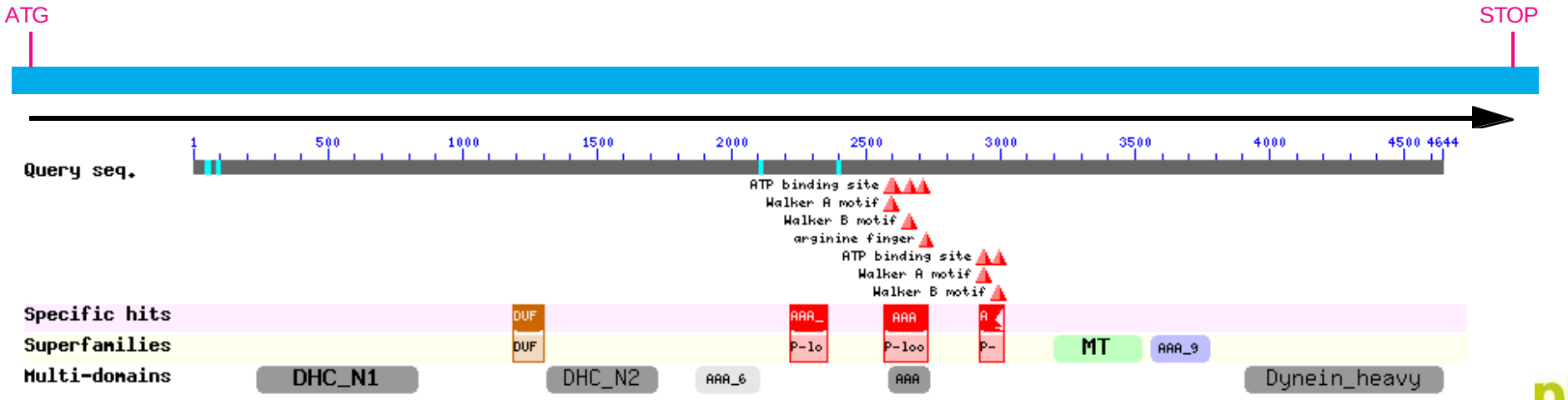


Dync1h1 mRNAs and proteins

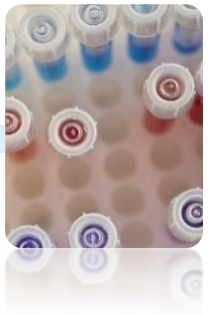


Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CDS incomplete	CCDS
Dync1h1-001	ENSMUST00000018851	14342	ENSMUSP00000018851	4644	Protein coding	-	CCDS36559
Dync1h1-002	ENSMUST00000172218	851	No protein product	-	Retained intron	-	-
Dync1h1-003	ENSMUST00000166185	522	No protein product	-	Retained intron	-	-
Dync1h1-004	ENSMUST00000107660	577	No protein product	-	Retained intron	-	-
Dync1h1-005	ENSMUST00000167395	756	ENSMUSP00000126117	252	Protein coding	5' and 3'	-
Dync1h1-006	ENSMUST00000166323	427	No protein product	-	Retained intron	-	-
Dync1h1-007	ENSMUST00000171535	621	No protein product	-	Retained intron	-	-
Dync1h1-008	ENSMUST00000170024	479	No protein product	-	Retained intron	-	-

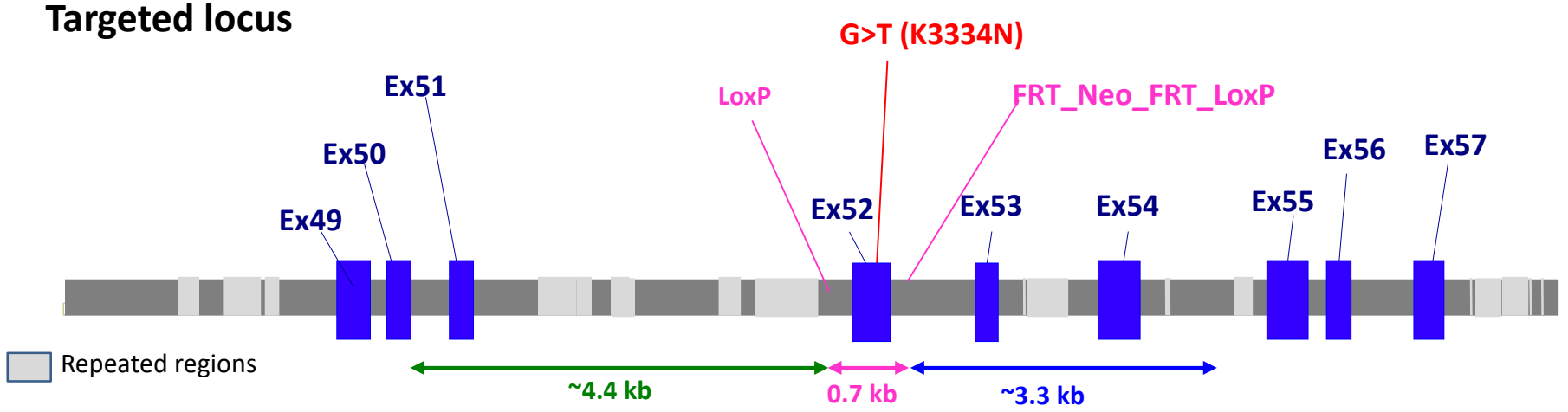
Dync1h1-001 ENSMUST00000018851



Proposal: introduction of the K3334N point mutation and flox exon 52 (Cre inducible KO)

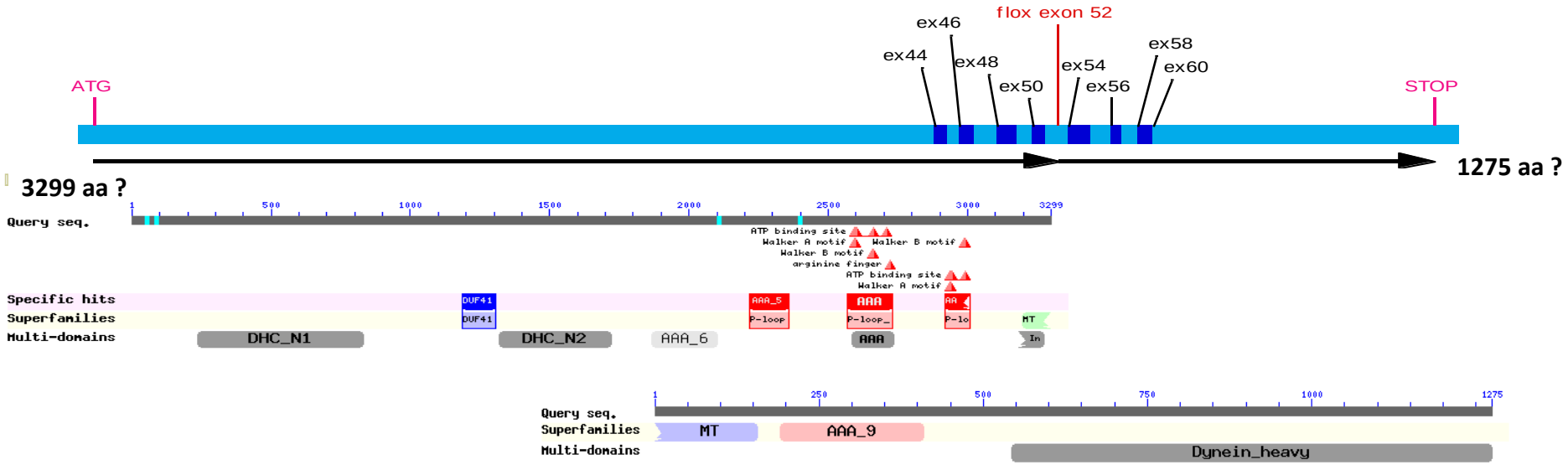


Targeted locus



Exon 52 Ensembl ID: ENSMUSE00000116238

mRNA and protein expressed after Cre mediated excision



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Strategy

Partial cDNA sequence (please check mutation)

ex50

GTG GAA GAA CTG CGC CGT GAC CTG AGG ATC AAG AGC CAA GAG CTG GAG GTC AAG AAC GCA GCG GCC AAT GAC AAG CTG
V E E L R R D L R I K S Q E L E V K N A A A N D K L

AAG AAA ATG GTG AAA GAC CAG CAA GAG GCT GAG AAG AAG AAG GTC ATG AGC CAA GAA ATC CAG GAG CAG CTA CAC AAA
K K M V K D Q Q E A E K K K V M S Q E I Q E Q L H K

CAG CAG GAA GTG ATC GCA GAC AAG CAG ATG AGT GTC AAG GAG GAC CTG GAT AAA GTG GAG CCT GCT GTC ATC GAG GCC
Q Q E V I A D K Q M S V K E D L D K V E P A V I E A

ex52

CAG AAC GCT GTG AAG TCC ATC AAG AAG CAG CAC CTG GTG GAG GTG AGG TCC ATG GCC AAC CCT CCT GCA GCT GTG AAG
Q N A V K S I K K Q H L V E V R S M A N P P A A V K

G>T (K3334N)

CTG GCT CTG GAG TCC ATC TGC CTG CTG CTT GGT GAG AGC ACC ACG GAC TGG AAT CAG ATC CGC TCC ATC ATC ATG AGA
L A L E S I C L L L G E S T T D W **N** Q I R S I I M R

GAG AAC TTC ATC CCC ACC ATC GTC AAC TTC TCG GCT GAG GAG ATC AGT GAT GCC ATA CGA GAG AAG ATG AAG AAA AAC
E N F I P T I V N F S A E E I S D A I R E K M K K N

TAC ATG TCC AAC CCC AGT TAC AAC TAT GAG ATC GTC AAC CGG GCT TCC CTG GCT TGT GGT CCT ATG GTG AAG TGG GCG
Y M S N P S Y N Y E I V N R A S L A C G P M V K W A

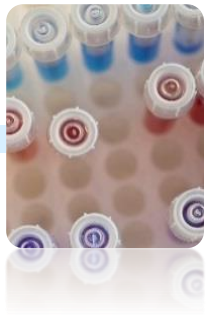
ex54

ATT GCA CAG CTC AAT TAT GCA GAC ATG TTA AAG CGA GTG GAG CCC CTG AGG AAT GAG CTG CAG AAG CTG GAA GAT GAC
I A Q L N Y A D M L K R V E P L R N E L Q K L E D D

GCC AAG GAC AAC CAG CAG AAA GCC AAT GAG GTG GAG CAG ATG ATC AGG GAC CTG GAA GCC AGC ATT GCC CGC TAC AAG
A K D N Q Q K A N E V E Q M I R D L E A S I A R Y K

GAG GAG TAC GCT GTC CTC ATC TCT GAG GCC CAG GCC ATC AAG GCA GAC CTG GCA GCT GTG GAA GCA AAG
E E Y A V L I S E A Q A I K A D L A A V E A K

PROs& CONs evaluation of the strategy



I. Pros

- K3334N mutation introduced

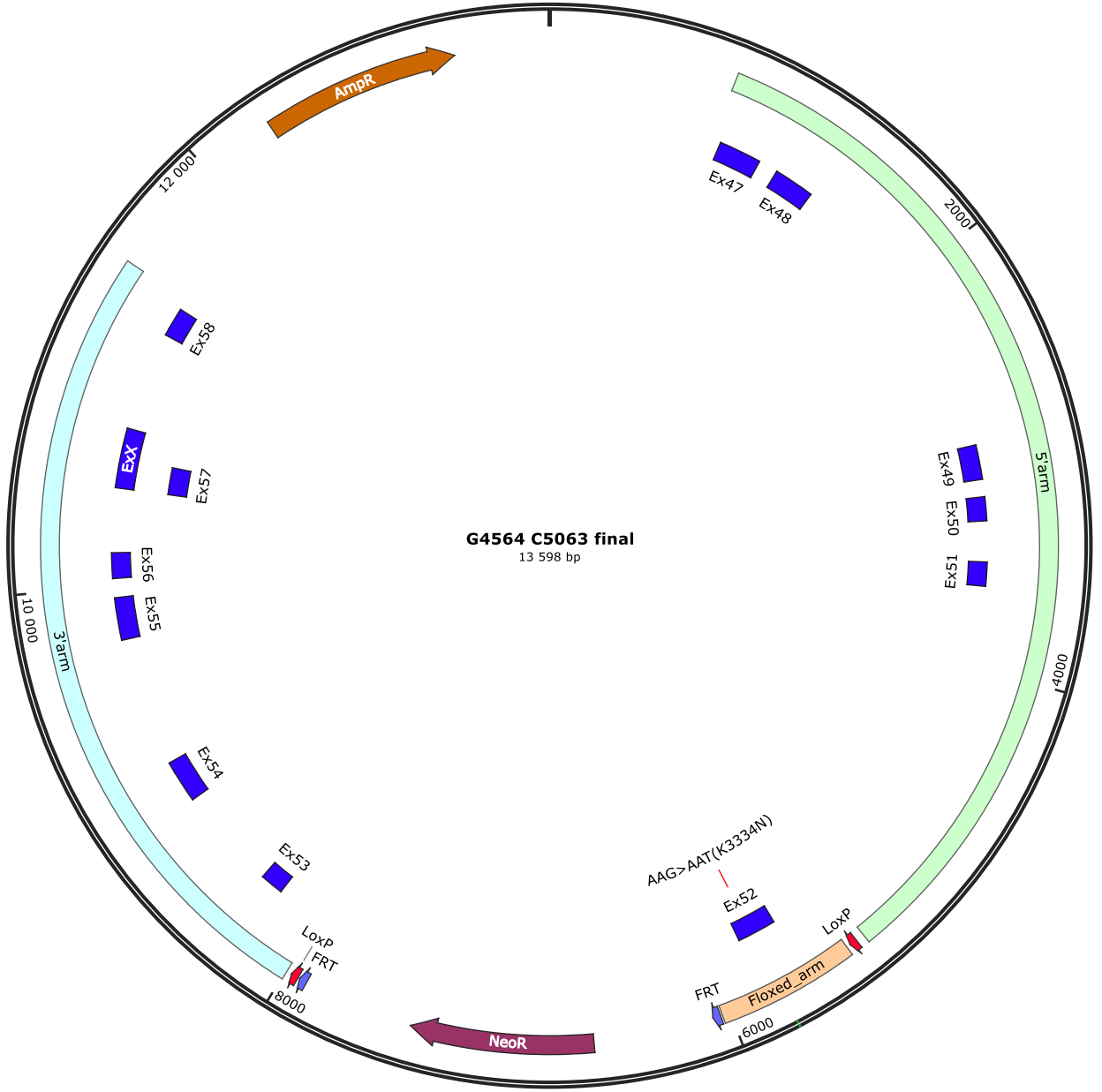
I. Cons

- A protein of 3299 aa might be expressed after Cre mediated excision if RNA decay does not occur.
- A protein if at most 1274 aa might be expressed after Cre mediated excision if reinitiation occurs at one of the 'in frame' ATG present in exon 53 or further exons (if RNA decay does not occur).
- Presence of repeated sequences might render PCR amplification and/or 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



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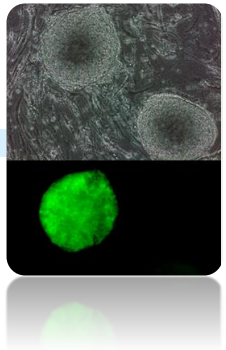
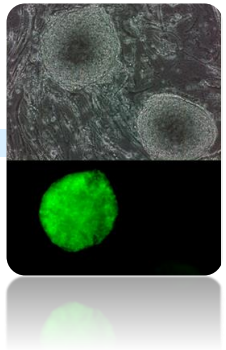
4 ES cell electroporation & Screening of recombinant clones



- 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



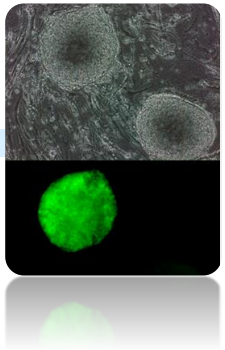
The targeting vector was electroporated in the proprietary C57BL/6N TB1 cell line.

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.

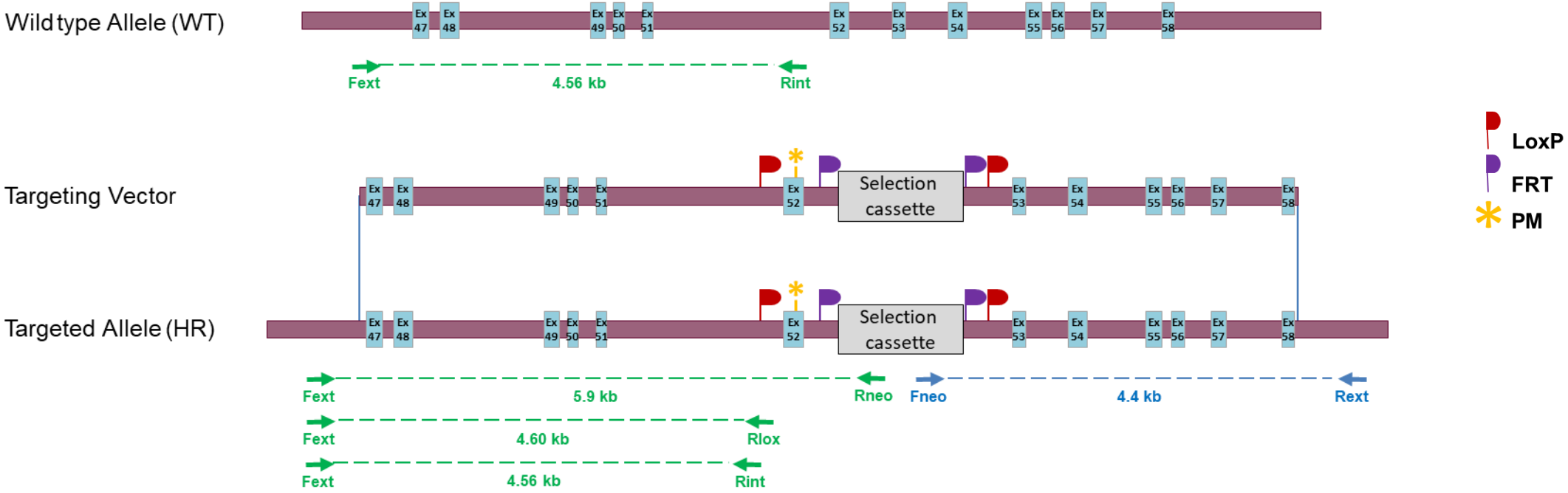
Screening process steps:

1. Identification of candidate recombinant clones by initial 5’ Long-Range PCR
2. 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 ddPCR aneuploidy screening and Giemsa staining

Long range PCR screening – strategy

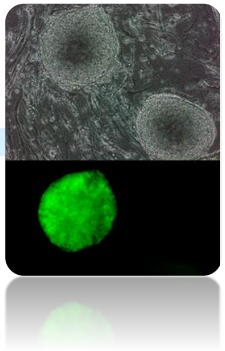


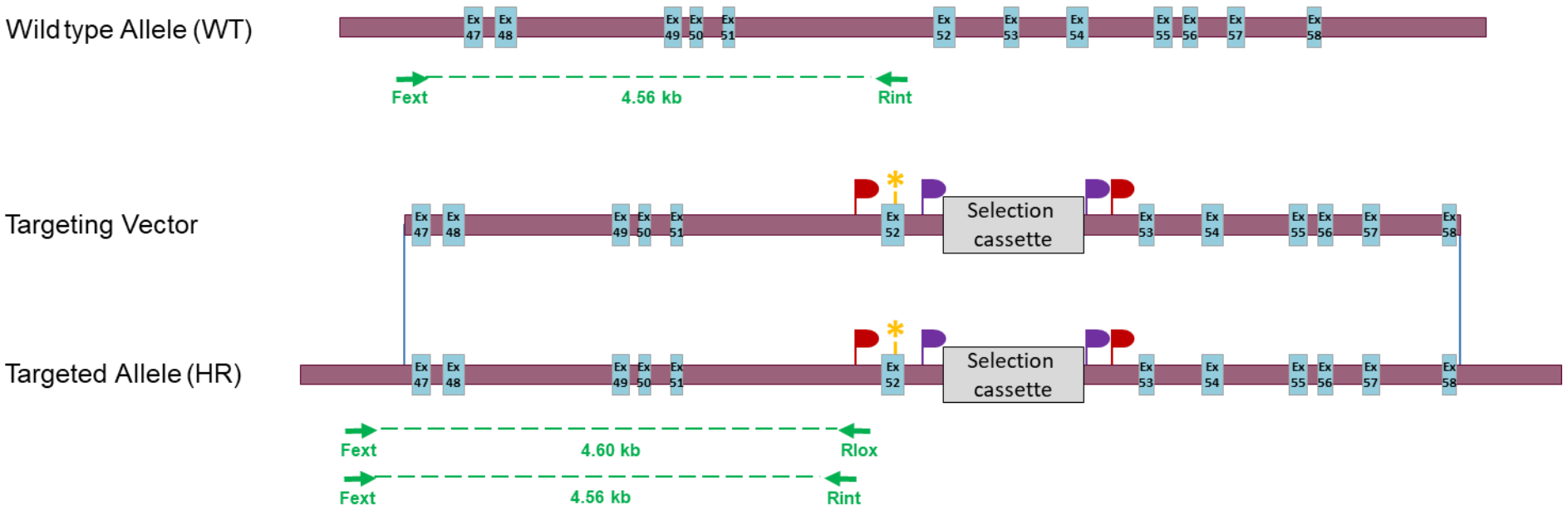
Schematic 5’ and 3’ PCR screening strategy



PCR	Primer Name	Primer sequences	PCR product size
5' PCR	Fext	CTAATGGCGAGGTAAGGGAGCTTGC	4.60 kb
	Rlox	GTTATCTGCAGGTCGACCTTAAGCT	
5’ PCR	Fext	CTAATGGCGAGGTAAGGGAGCTTGC	4.56 kb
	Rint	CACTGGTGATGGAACGGTTTCTGTC	
5’ PCR	Fext	CTAATGGCGAGGTAAGGGAGCTTGC	5.9 kb
	Rneo	GCGGCCGGAGAACCTGCGTGCAATC	
3’ PCR	Fneo	AGGGGCTCGCGCCAGCCGAACTGTT	4.4 kb
	Rext	GTCTGGTGGGAACTCCACCTGTCAG	

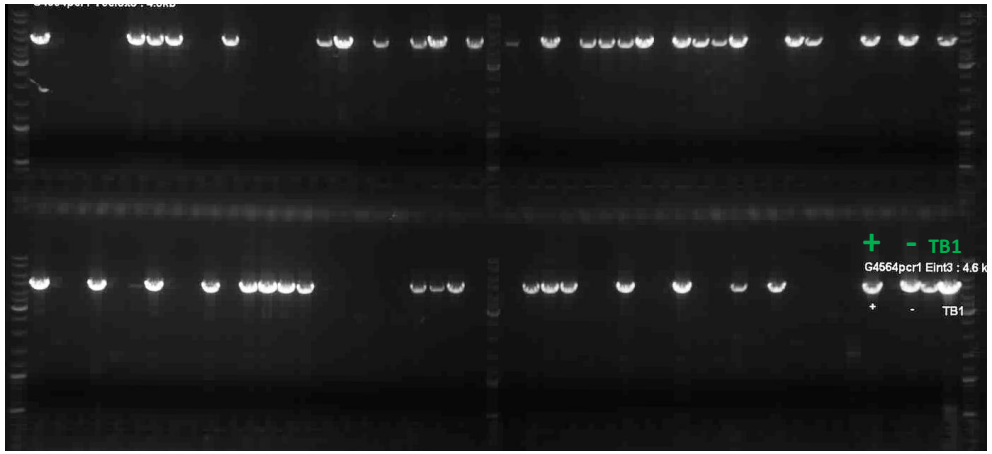
Long-Range 5’ PCR screening – results



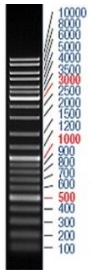


LoxP
FRT
PM

PCR Fext – Rlox : 4.6 kb



+ / - / TB1 : Controls DNAs



Ladder pattern

PCR Fext – Rint : 4.56 kb

Six candidate clones out of the 45 positive clones were selected for 3’ Long-Range PCR and Southern blot validation.

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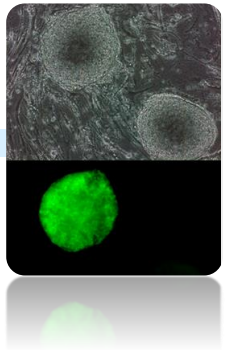
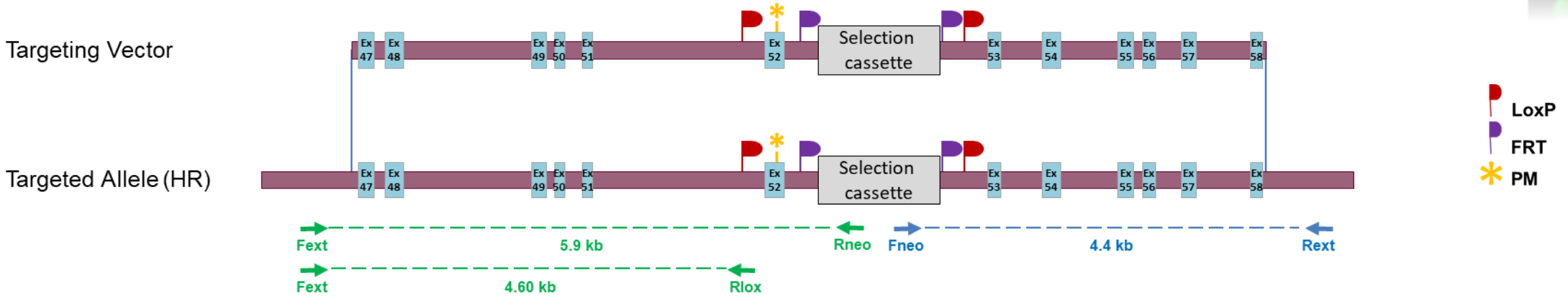
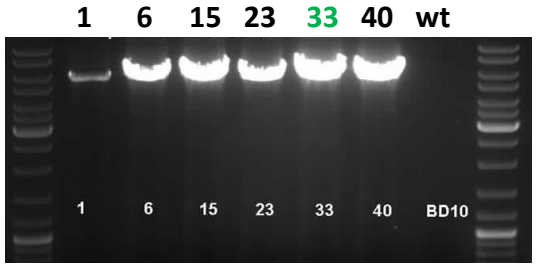
Recombinant ES validation by Long Range PCR

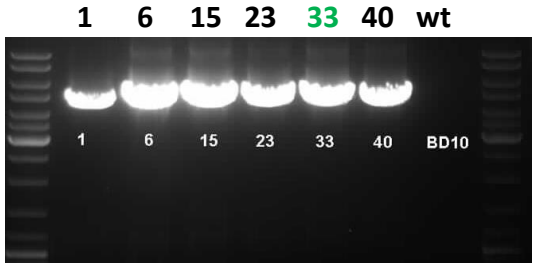


Confirmation and Validation of candidate recombinant ES clones by 5’ and 3’ PCRs

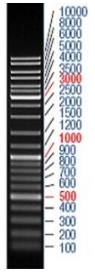




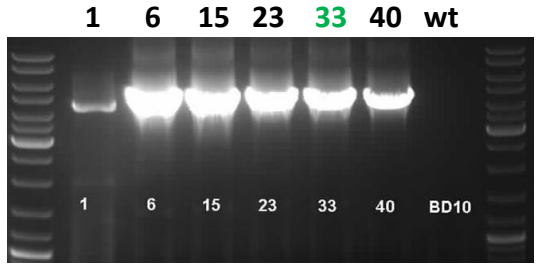
PCR Fext – Rneo : 5.9 kb



PCR Fext – Rlox : 4.60 kb



Ladder pattern



PCR Fneo – Rext : 4.4 kb

Six candidate clones identified by 5’ PCR screening were further analysed by 3’ PCR screening.
Six clones (clones #1, #6, #15, #23, #33 and #40) were confirmed.

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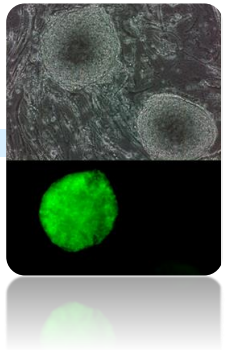
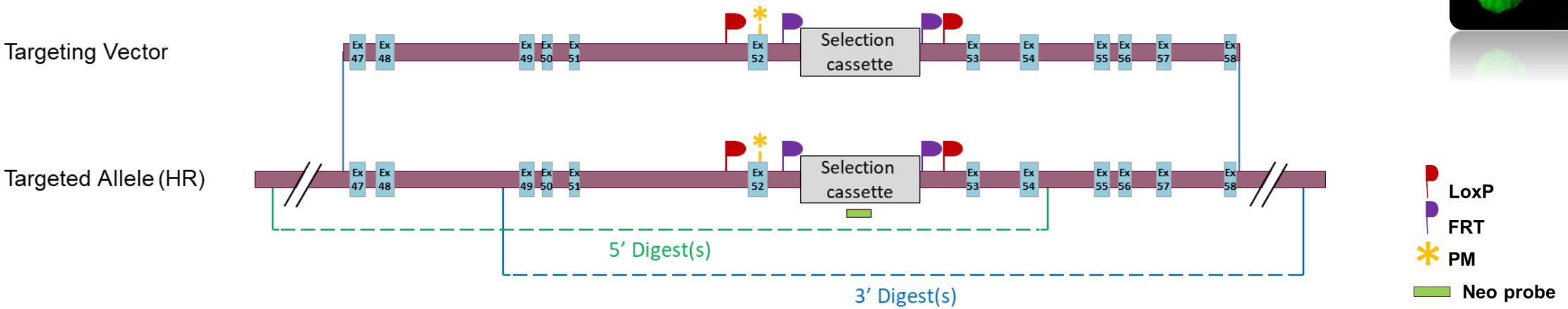
Recombinant ES clones validation by Southern Blot – Internal probe



Schematic Southern Blot validation strategy

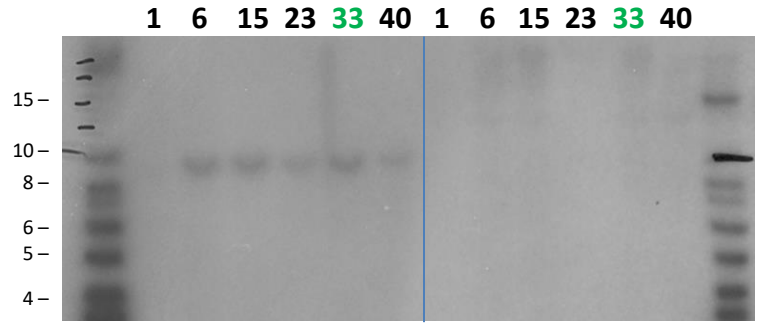
Digests on the scheme illustrate the position of the chosen restriction sites relative to the probe. They don't show the exact position of the restriction sites.



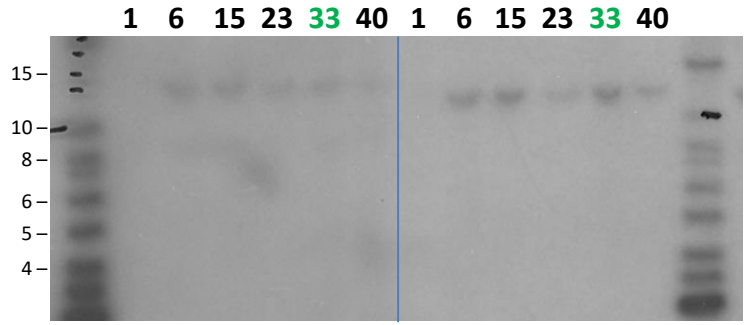
Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	AseI	9.9
		NsiI	14
	3' digest	DrdI	9.1
		PacI	13

Southern blot - Neo 5'



Southern blot - Neo 3'



Neo probe sequence

CTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG
CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGG
CTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
GCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGG
CTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTC
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC
ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
ATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCT
CCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCCGCTG
TAAGTCT

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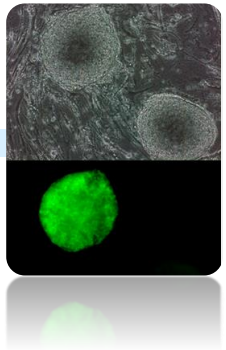
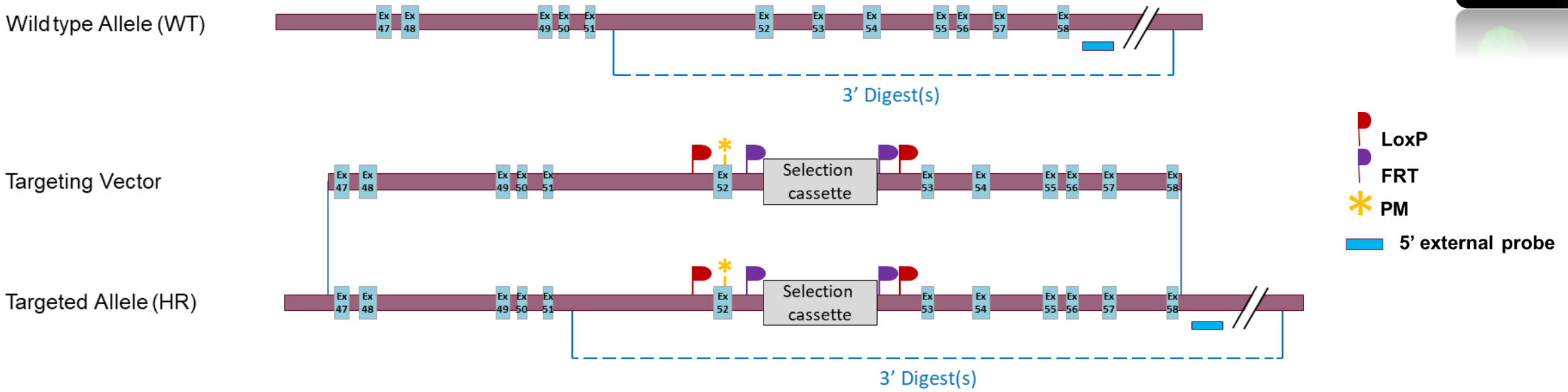
Recombinant ES clones validation by Southern Blot – External probe

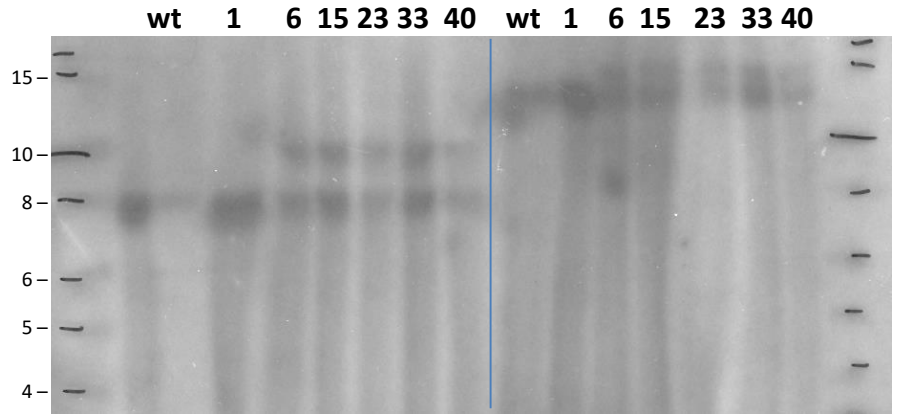


Schematic Southern Blot validation strategy

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.



Southern blot – 3' probe



HindIII 7.9 / 9.7 SspI 12.9 / 14.9

3' probe sequence

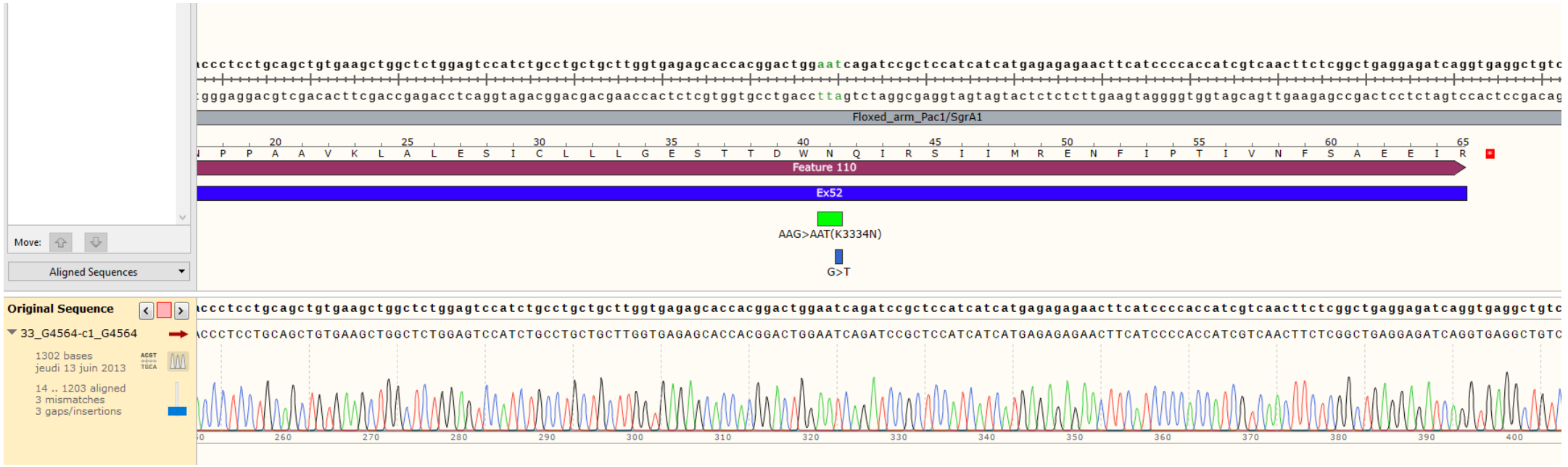
GGTCTGTCTTGTGTCCTGACAGGTGGAGTTC
CCACCAGACCTCTGCTCCCGGGTGACCTTTG
TGAACTTCACCGTCACCCGCAGCAGTTTACA
GAGCCAGTGTCTCAATGAAGTGCTTAAAGCA
GAAAGGCCAGACGTGGACGAGAAGCGCTCCG
ACCTCCTGAAACTGCAAGGTGTGCCTCTGGT
CCCTGGCTTCCCAACCGAGTTGGGTAGCAGG
CTGAGCTCCGGAGGGTGAGGGTGACACTGCT
CCCCAGTGGCAGAGTGACACAAATCTGTCTT
GTCACAGGGGAGTTCCAACTCCGTTTACGG

Digestions used to validate the 5' and 3' insertion

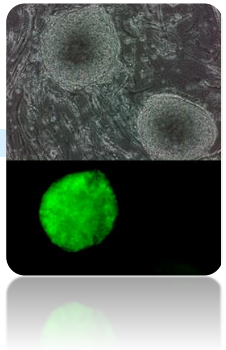
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
3' external probe	3' first digest	HindIII	7.9	9.7
	3' second digest	SspI	12.9	14.9

Recombinant ES clones validation– Sanger sequencing

Clone #33



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
#6	Pass	Pass
#15	Pass	Not done
#23	Pass	Not done
#33	**Pass**	**Pass**
#40	Failed	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/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 #33 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
#33	**7**	**3**	**4**	**14**

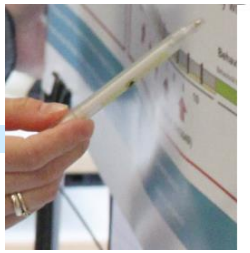
Breeding to F1 generation

- Six highly chimeric males generated in the previous phase by blastocyst injection of the ES clones were mated with wild-type C57BL/6NCrl 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 : 13/11/2013

- Allele nomenclature (following MGI guidelines) :**Dync1h1$^{tm1.1(K3334N)Ics}$**

6 SEQUENCE OF THE DELIVERED ALLELE



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LoxP FRT Exon 52 AAG>AAT(K3334N)

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EXCELLENCE IN MOUSE PHENOGENOMICS

REPORT REDACTION & VALIDATION

Protocol finalized on 2023/21/2023

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