



# MODEL GENERATION TECHNICAL REPORT

## Map6d1 (SL21) conditional Knock-out

Project code: K256 / IR554

Project started in 2005

Report finalized: 25/10/24

1 PROJECT PROCESS &  
QUALITY CONTROLS

2 GENETIC STRATEGY

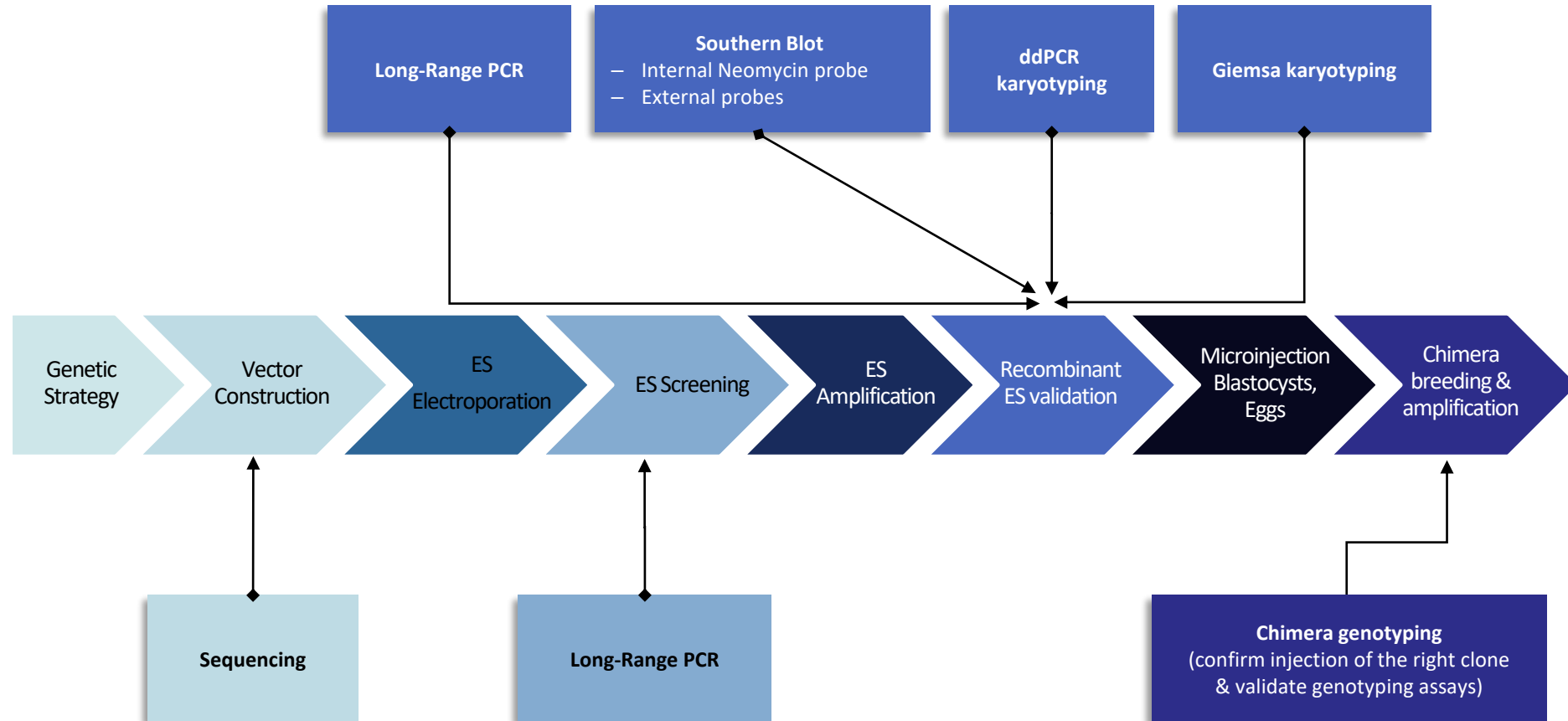
3 HOMOLOGOUS RECOMBINATION  
VECTOR CONSTRUCTION

4 ES ELECTROPORATION & SCREENING OF  
RECOMBINANT CLONES

5 MICROINJECTION & BREEDING

6 SEQUENCE OF THE DELIVERED ALLELE

# PROJECT PROCESS & QUALITY CONTROLS



## 2 GENETIC STRATEGY

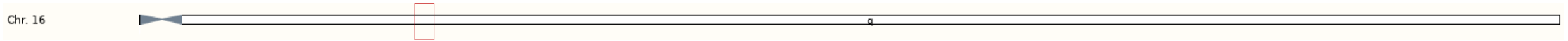


- Target locus structure
- mRNA(s) and protein(s)
- Genetic strategy
- PRO & CONS evaluation of the strategy

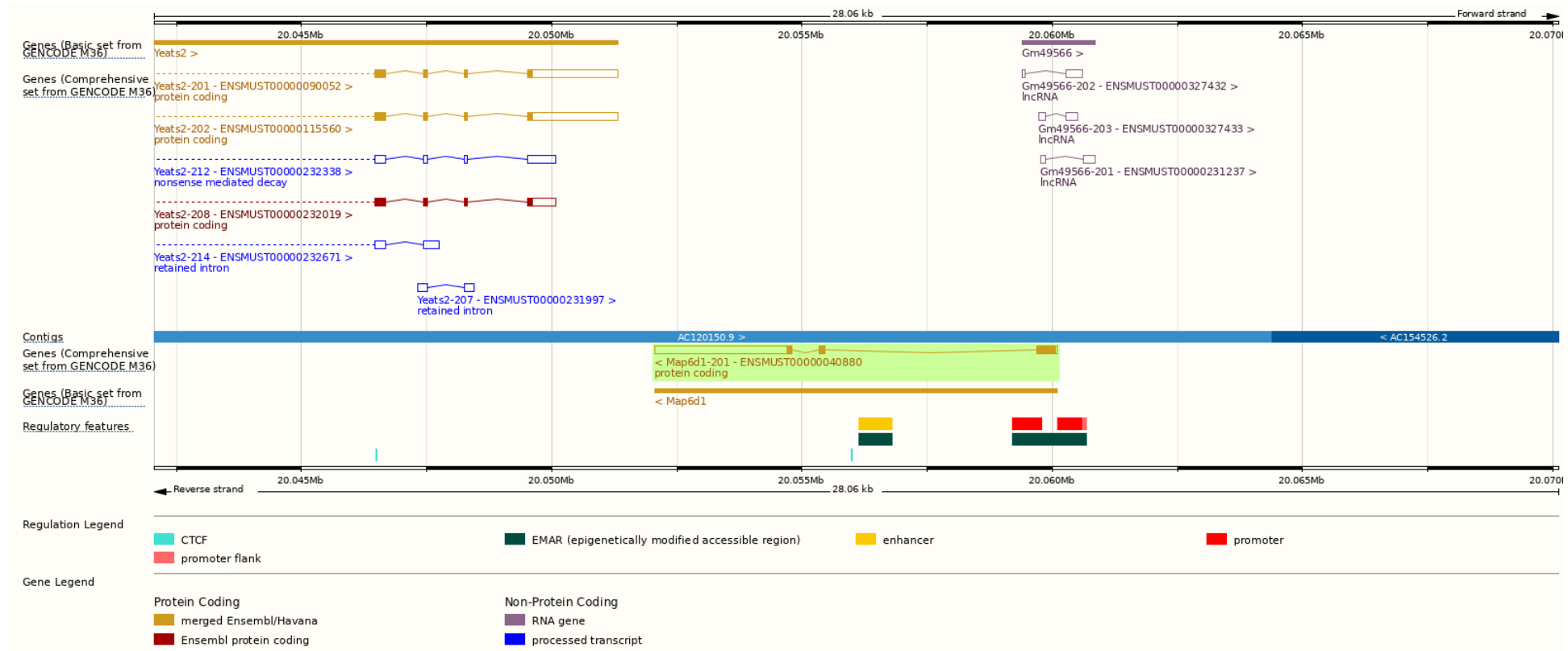
# Map6d1 mouse genomic locus – structure (GRCm39)



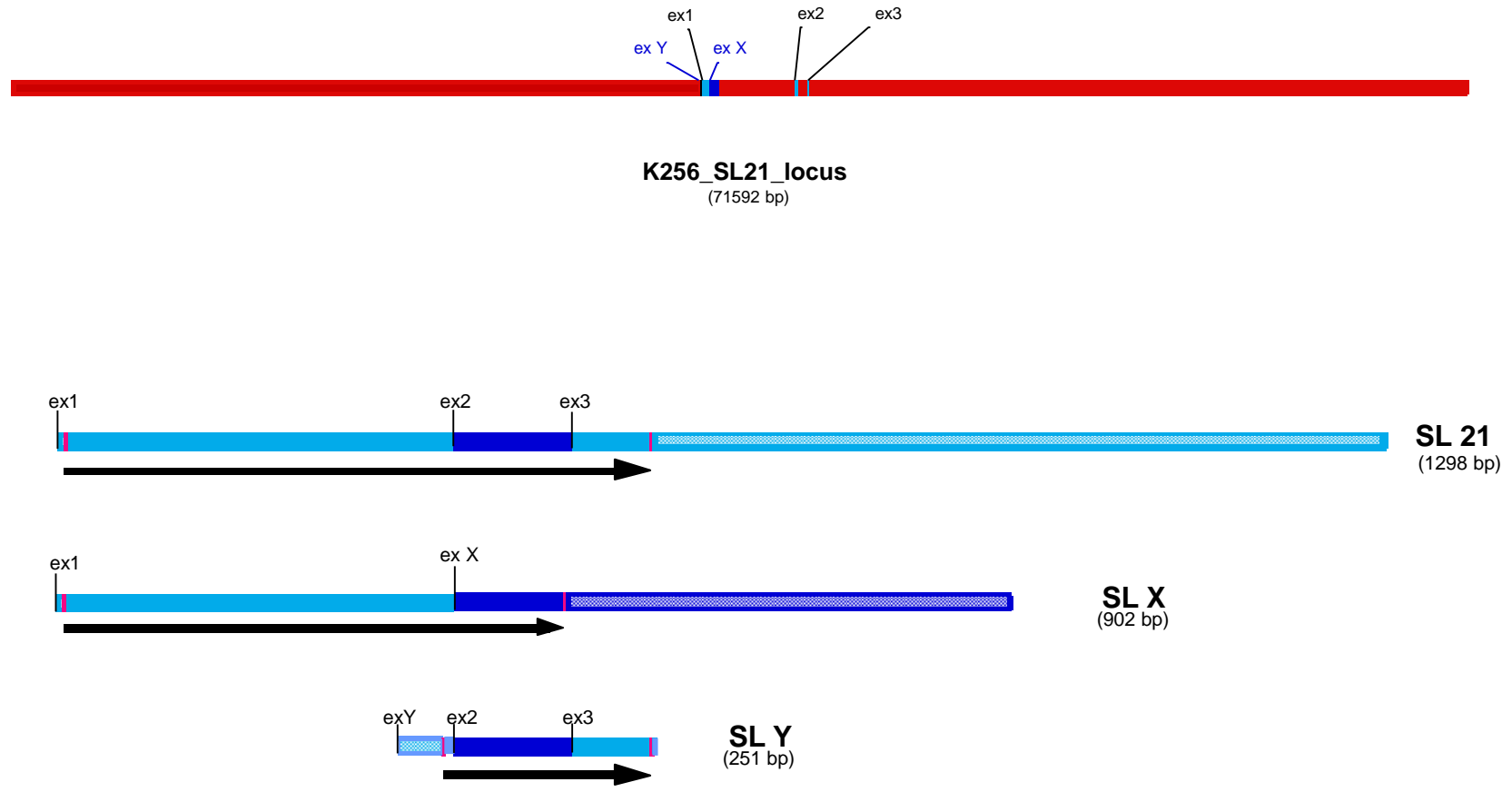
Location : Chromosome 16: 20,052,053-20,060,108



## Ensembl Gene ID: Map6d1 ENSMUSG00000041205



■ 2005 data



# Mapd6d1 mRNA and protein



Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match
<a href="#">ENSMUST00000040880.9</a>	Map6d1-201	3280	<a href="#">191aa</a>	Protein coding	<a href="#">CCDS37286</a>	<a href="#">Q14BB9</a>

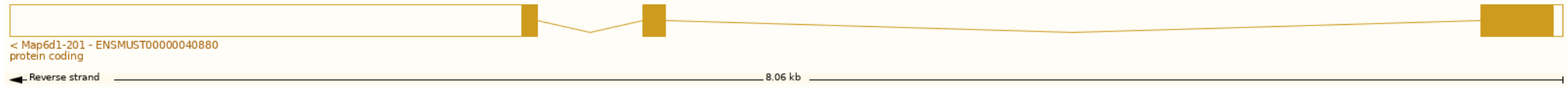


Figure 2 : SL isoforms

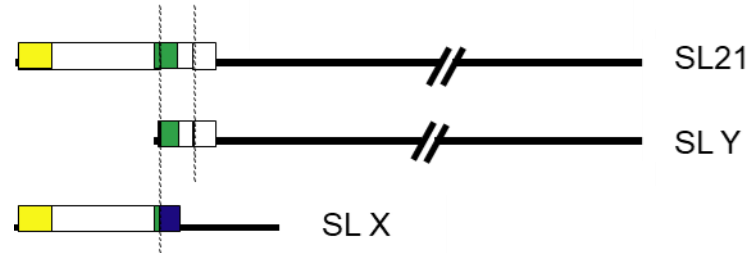
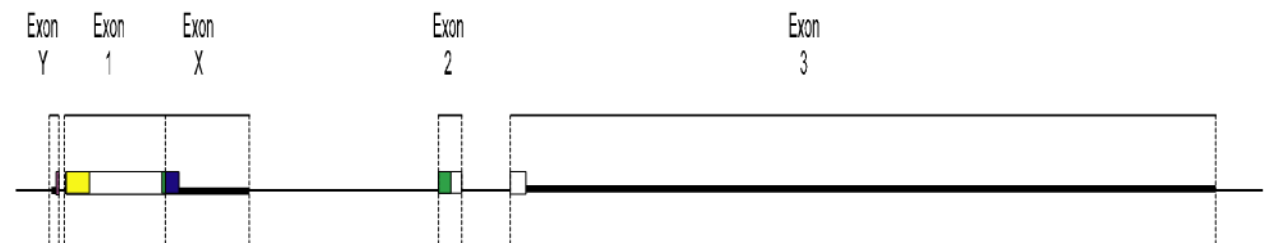
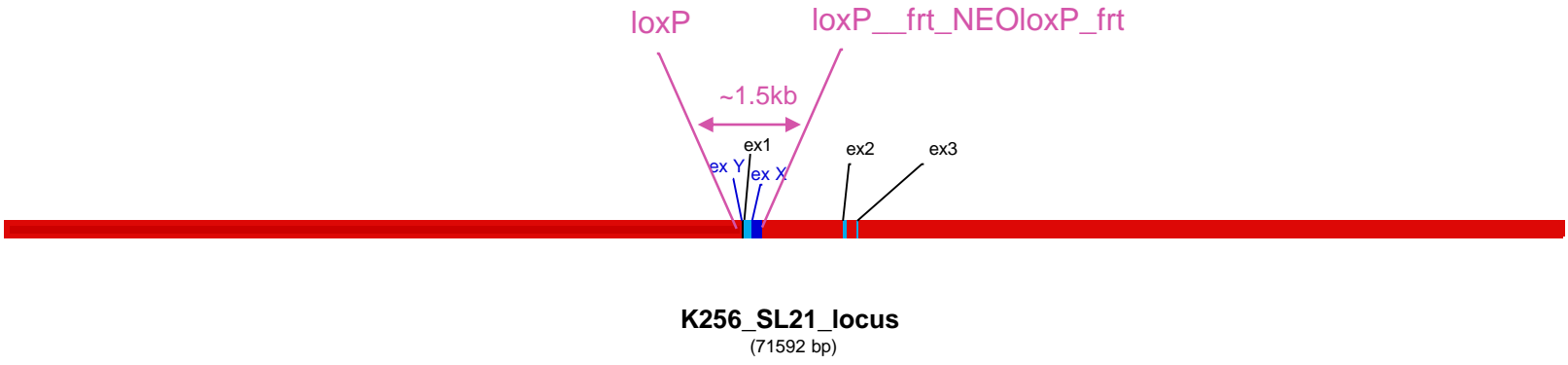


Figure 3 : SL21 gene organisation



# ■ Strategy undertaken in 2005: flox exon 1



~~SL X~~



## ■ PROs& CONs evaluation of the strategy



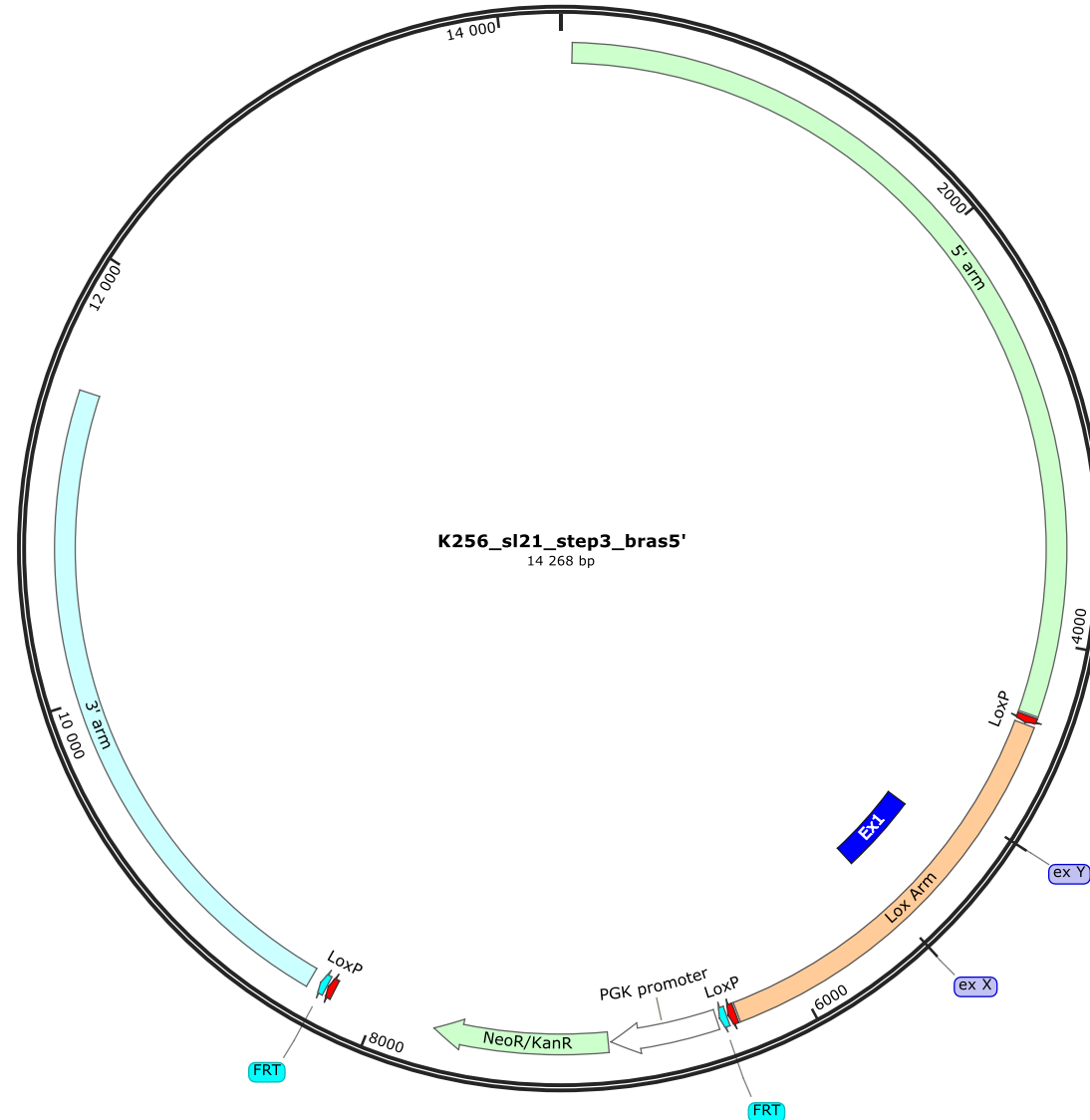
### Pros

- ✓ Reasonable distance between the two loxP sites
- ✓ All isoforms will be affected

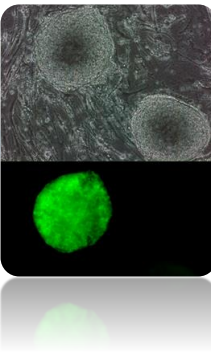
### Cons

- ✓ A lox P site will be inserted in the 5' UTR (~200 bp upstream of the ATG in exon 1) in sequences that show no homology to human sequences
- ✓ The exons structure is based on EST, no real cDNA sequences were found (except for SL21)

# 3 HOMOLOGOUS RECOMBINATION - VECTOR CONSTRUCTION

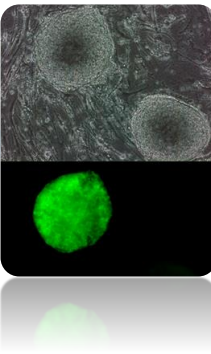


## 4 ES cell electroporation & Screening of recombinant clones



- Electroporation and screening process
- Recombinant ES clones validation by Southern Blot – internal probe
- Aneuploidy screening in ES recombinant clones

## ■ Electroporation and screening process



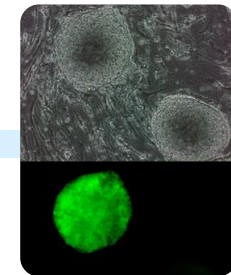
The targeting vector was electroporated in the ICS 129SV/Pas proprietary embryonic cell line P1.

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:

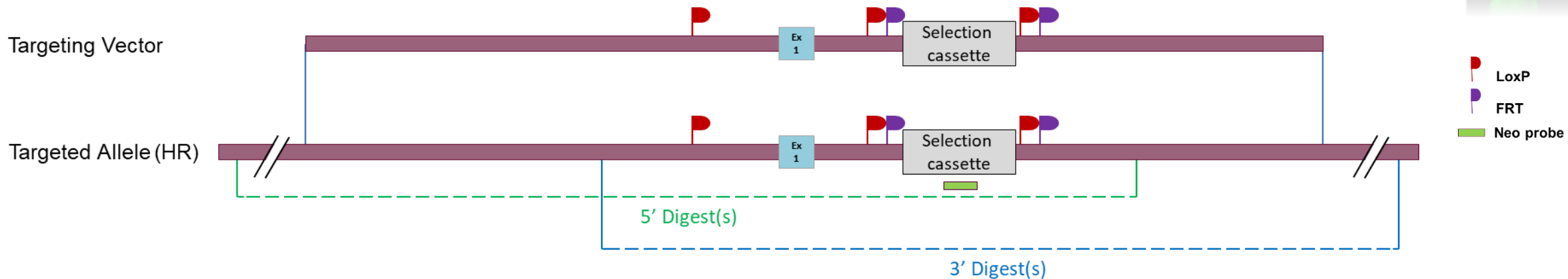
1. Positive clones were identified and confirmed for recombination event by Long-Range PCR (data not shown)
2. Positive clones in step 1 were further validated by Southern blot analysis using internal probe
3. The karyotype of at least 2 validated clones is verified using Giemsa staining

# Recombinant ES clones validation by Southern Blot – Internal probe



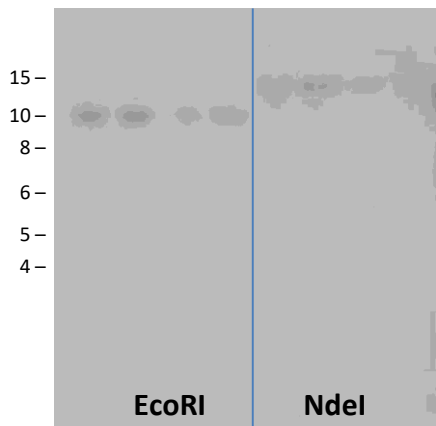
## Schematic Southern Blot validation strategy

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.



## Southern blot - Neo

89 204 211 242 89 204 211 242



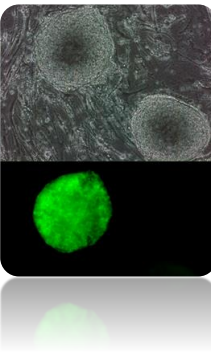
## Neo probe sequence

```
AGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGT
AAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCAAGCTTTCAGCAATATCACGGGTAGCCA
ACGCTATGTCCTGATAGCGGTCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGC
GGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTCGC
CGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTT
CGTCCAGATCATCTGATCGACAAGACCGCTTCCATCCGAGTACGTGCTCGCTCGATGCGAT
GTTTCGCTTGGTGGTGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGATTGCAT
CAGCCATGATGGATACTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCA
CTTCGCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAG
GAACGCCCGTTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAG
```

## Digestions used to validate the 5' and 3' insertion

Probe		Genomic DNA digest	Targeted Allele (kb)
Neo	5' digest	EcoRI	10.5
	3' digest	NdeI	13

## ■ Aneuploidy screening in ES recombinant clones



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

Clone ID	Giemsa
#89	Pass
#204	Pass
#211	Pass
#242	Pass

# 5 MICROINJECTION & BREEDING



- Microinjection
- Breeding to F1 generation

## ■ Microinjection



- The ES cells used in the injection experiment were originally derived from a 129/SvPas mouse strain (which have agouti coat colour). These cells were injected into blastocysts derived from an C57BL/6J 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 agouti and black patches on these animals.
- Recipient blastocysts were isolated from mated C57BL/6J females
- Recombinant ES clone #89 validated in previous project phase was injected into blastocysts to generate chimeric males. The results are presented in the table below.

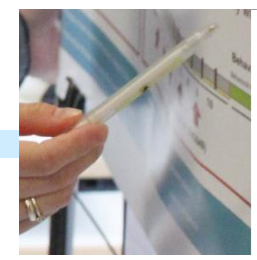
Clone ID	Number of chimeric males identified according to chimerism rate (Number of chimeric males bred to F1 generation)			
	5 - 40%	45% - 55%	60-100%	Total
#89	1	0	2	3

## ■ Breeding to F1 generation



- Two chimeric males generated in the previous phase by blastocyst injection of the ES clone #89 were mated with C57BL/6J Flp deleter females to investigate whether the recombined ES cells have contributed to the germ layer.
- Germ line transmission was obtained the : 10/05/2006
- Allele nomenclature (following MGI guidelines) : **Map6d1<sup>tm1.1lcs</sup>**

# 6 SEQUENCE OF THE TM1.1 ALLELE (cKO)



## Map6d1<sup>tm1.1lcs</sup>

CATTAGGGATGGCTTACAGGTTCAAGTCCATTATCATCAAGGTGGGGCGTGGCAGTGCCCAGGCAGGCATGGTGCAGGCAGAGCTGAGAGTTCTACATCTTCATCTAAAGGCCACTAGGAGAAGA TTGGCTC  
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CCATACCCATGAATGCACCTTCAGTAAAGGTTTTCCCAACTCCAGACTTAACTGTGAGTATGGGGTTCATAGCCAGACCTGGGGTGTAAACCAGCAATGGTATGTCACCTCCAGTACAACCTTGCAGCTAATGAA  
GTTGAGGAAGATGAGCTGACCAATGGGCCAGACTGGGCAGTCTCTAGGTGCAGACTGGATGCTTCTCAGGTTCCAGCCAGAAGTTGTAACCTACCACCCTTCGGCCGGCCATAACTTCGTATAATGTATG  
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ACAGCACAGTAAACAGCAAATGGAGATTCACGAGCTGACATGGCCCTAAGTTCTCACTTGCATCACCTTCCTGGTTGCCCTCCACCCTTCCACTCTGGGTGGTGCCTGAGTCCGCTAGGCACAGTTAAATC  
TATTTTGCAGCCACCCGTGTCCACAAATGCCAACAGAGTTCTCTGGTCTGCCGCCAGAGCCTTCTTGATGGATTGATTGTTCTTAAAAAAGTAGCAGGCTTAGTGACGGT GAGGAAA  
TGCCCTCCTTCGACCTCGCTCTCGATGAAGCCAGGTCTGATTTCTGAGCTCACATTGGGGAACTCTGGGTTTAGGACTGCCCGCAGGCCGTCTTGGCGGGGCTCAGGGTTGCGCGGGGGCAGTGGGGTAG  
TGCGCGCGCGGGCGCAGGCCGCGCGGCCGAGCGGCATGGCGTGGCCCTGCATCAGCCGGCTGTGCTGCCTGGCCGGCGATGGAACCAGCTGGACCGCTCGGACGTAGCGGTGCCGCTGACTCTGCATGGTT  
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CGATTTGATTCTCCTCCTTCGGTCTCAGATTGTTCTTTTTAACTTCTGTGGGGTGGAGCAAAGAGGGCGGTGCACAGCCCCTCATCCCACGCCCCACGCCGTGCCAGGGCCAGTGCCTGCGCTGTTCAAG  
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CCTTAACACCCTCACGCCTCCCCTCCCAACAGATGGATCTTCTAATTCTTAGTATAAATGAATGCATACACATTCAGTTTCTCCACGATCTTATTCTTGCTGTTGCGATGTTTTAAAATATTTAATAT  
ATATTAGTGTGGAGAGGGGGCGAGAGGAGGGGCGAGCAGATCAGAAGACAACCTTTAGAAAGTCACTCCTCTTCCCCTTCTGCCATGGATTCCAGGGATCCATCTCTCGGATTGTCAGGCTCACACATTATA  
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CCTGATATTGGGGATGCACTAGTTATGGGTCTGTCATAGGGTGGGACACTGAAGCCCTGCAGGTACA

LoxP

FRT

Exon 1

# 6 SEQUENCE OF THE TM1.2 ALLELE (KO)



Map6d1<sup>tm1.2lcs</sup>

CATTAGGGATGGCTTACAGGTTCAAGTCCATTATCATCAAGGTGGGGCGTGGCAGTGCCCAGGCAGGCATGGTGCAGGCAGAGCTGAGAGTTCTACATCTTCATCTAAAGGCCACTAGGAGAAGATTGGCTC  
CCACATGGTTAGGAGGAGGGTCTCATTGTCCACCCTGAACAGTGACACACTTCCTCCAACAAGGCCACACCTATTCCAACAAAGTTACACCTCTTAATAGTGGCACTCCCTGGGCCAAACATATTCTAACTA  
CCATACCCATGAATGCACCTTCAGTAAAGGTTTTCCCAACTCCAGACTTAACTGTCAGTATGGGGTCATAGCCAGACCTGGGGTGTTAACCAGCAATGGTATGTCACTCCAGTACAACCTTGCAGCTAATGAA  
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CTATACGAAGTTATGCGGCCGGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC

LoxP

FRT



## REPORT REDACTION & VALIDATION

Protocol finalized on 2024/10/25

Prepared by Romain LORENTZ, IE

Verified by Marie-Christine BIRLING, PhD

## CONTACT US

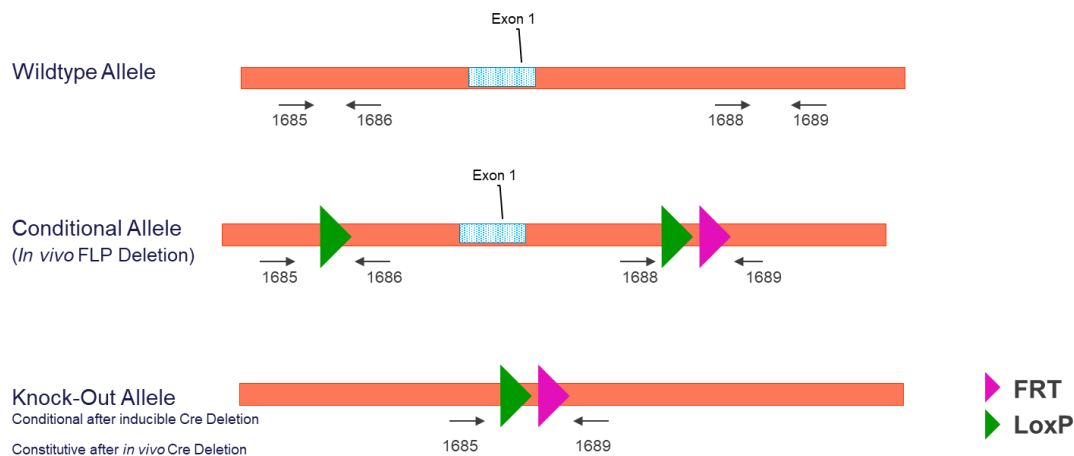
By email at [mutagenesis@igbmc.fr](mailto:mutagenesis@igbmc.fr)

By phone at +33 (0)3 88 65 56 57

[www.phenomin.fr](http://www.phenomin.fr)

## SL21(IR554/ICS internal reference K256) mouse line genotyping protocol

This protocol describes the strategy and the PCR conditions used for SL21 conditional knock out (cKO) line genotyping.



### Primers sequence:

Primers	Sequence 5'-3'
1685	CAGTCCTCCTAGGTGCAGACTG
1686	GTTCCATTGCAGGGCTATAATCCAGC
1688	CATGGATTCCAGGGATCCATCTCTC
1689	GGGGCTTATGCCTGTGGCTATATG

### PCR to be done (please see the scheme above):

- Characterisation of conditional allele animals: PCR 1685/1686; PCR 1688/1689
- Characterisation of conditional allele /Knock-out allele animals (after breeding with a Cre deleter mice<sup>1</sup>): PCR 1685/1686; PCR 1688/1689; PCR 1685/1689
- Characterisation of Knock-out allele animals: PCR 1685/1686; PCR 1688/1689; PCR 1685/1689

<sup>1</sup> Genotype Mosaicism may be observed, ie conditional/Knock-out allele genotype in the same animal from breeding with a Cre deleter mice.



**PCR fragments expected size:**

Primer pair	1685/1686	1688/1689	1685/1689
WT allele	183	171	2014*
Conditional Allele	233	302	2195*
Knock-Out Allele	N/A	N/A	223

N/A: not applicable

\*: no amplification products under standard PCR condition (see description below)

**PCR protocol:**

Reagents:	Volume:
-10x Thermopol Reaction Buffer (Biolabs )	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Sigma can be used)	0.2µl
-DNA (50ng/µl)	2µl
-Primer 1 (100 µM)	0.25µl
-Primer 2 (100 µM)	0.25µl
-Sterile H2O	up to 25 µl

**Cycling conditions:**

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

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