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For any question, please contact:

Mouse Clinical Institute – Institut Clinique de la Souris (ICS)

1 rue Laurent Fries, BP 10142

67404 Illkirch Cedex France

Email: ics@igbmc.fr

Web site: <http://www.phenomin.fr/en-us/>

This protocol has been prepared by Claudia Caradec, Engineer

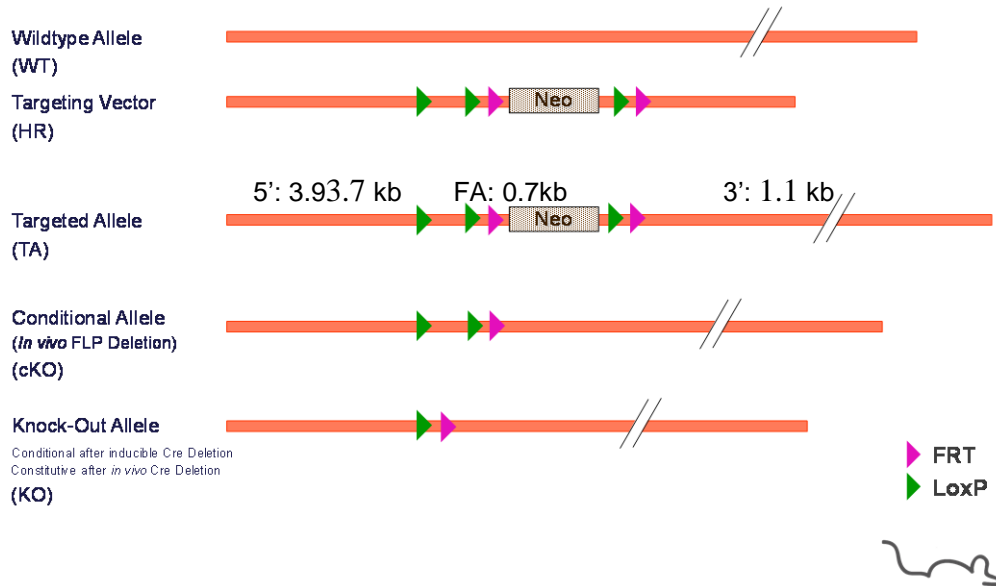
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview



Overview Targeting Strategy



Legend:

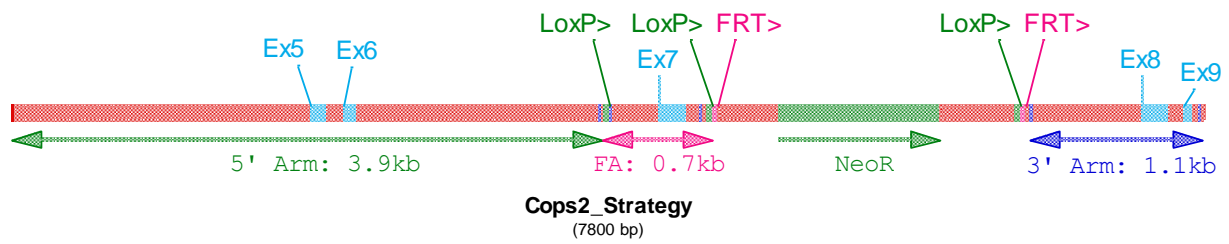
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
 This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 7

Cops2 gene (also named Trip15) is a member of the nuclear receptor family. Additional information on this gene can be accessed at:

<http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=markerDetail&key=39262>

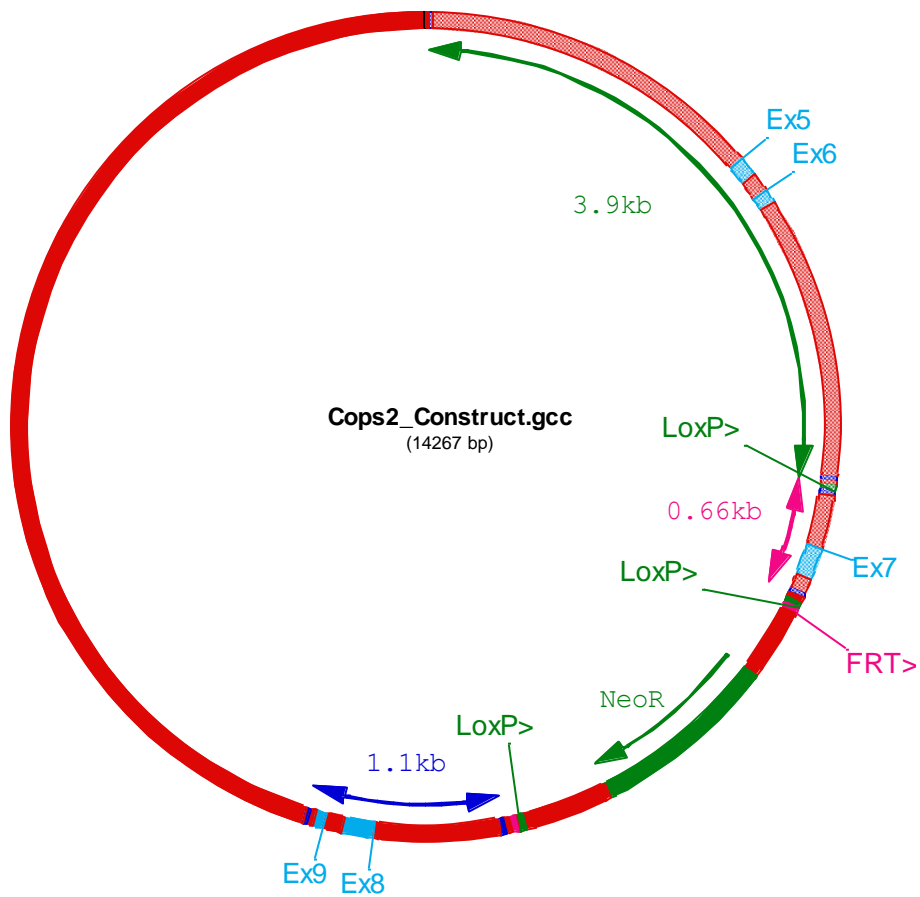
Strategy used to generate the conditional knock out model



2. Construct used for homologous recombination in ES cells: Cops2 project Legend

loxP sites are indicated in green ; FRT sites are indicated in purple; *Mus musculus* sequences are indicated in uppercase ; exogenous sequences are marked in lowercase.
 The targeting vector was generated in 129Sv/Pas and was not sequenced.

2.1. Map of targeting vector plasmid





2.2. 5' homology arm (3.9 kb)

GCCCCAACTAGCTGTGTGACCAAGGGTACCCTGACCTCCCCTTTCTGCCTCCCAAATGCTGATGG
ACAGGTGCCATCATGCCTGCTAAGTCGTCATTTTTATTTTAAAGAAAGGCCAATTTACTAATTACTAGTGTGCTTC
AAAAGTATTTTTATTGATTCTGGTACTAATTAGCATTAAAAAGTAATATATCCAAGTTAATCTCTTTAAATAGTA
TCTTCAGAGACTTAAATACTACCTGTAAATAAAGTGGGCAGACGTTTTTATTTTTTTTTCAGACTCCTTGCACCTGT
TGTTATAGCTGTTTTTGGTTTTTGGTTTTTGGTGGTTTTTTTTTTTTTTTTTTTTTTTTTTTGGATGAAGTTG
TGTCTGCATTTAAACTTGGGGCATTGAGCACTTTAGACAAATGTTTCCATTGAAATTCACGTCACACACACAC
ATTGGGAATGTGAAATGTGAACATGAGTCACAAGTTGTGATGTTAGATTAAAGTCTTGAGAAGAACTTGTCTCTG
TGCCAGACTCAGTAGGTGCTCTATACATGATTCTATCAAGGATGCATGGTGATAGCTGATAAAGGGAAAGAGGAA
AGGGGGAAATGGCTTCTTCTAGAATGTGGTAGCTAACTAACAAGGTAATGATGATTTTATGTATTCATCCTATT
TATTTATTTTTATTTATTTTTAAGCTTTGTTACCCTCTGGTGTGTTGAACACAGAATGTTCTCTGTATCATTTAGTA
TCTGTTTTTGTAAATCCTAACAAGCATTCTCAGAATCAGCCTCATCACACACATATACACACACCCATACTCACCC
ATGTACGCACGCACATATGCACATCTGGGAATAAGGGAAAGGAAACCTTGTCACTTAGTGGCAATAACTCTAGAG
CATCCTCCTGCTGTGTAATGAGTTTGGAGCCAGCCTGGCTGGTGGCACATGTTAACCTTGGGAACAAGGTAAGTT
TGAGACCAGCCTGGACTACATGAGATACTGTATCTAAATAAATAACACTAAATTATGTGGAAACATATTTTCTGT
TATTTGCAAACAAGTATCATAAAAAACATATTTGGTACTGTGTTTATAAATGTTATTTCAAAAATGCTAGGGGACT
AGAATTGAATAATTTTAAACTTGAAGGTTGTATTCTCTGTGTGTTAATTTGAAACTTTTGCCTTTTGAAGAAA
AAGTAGCTATTGTGGGTAGCACTTAACCTCATAACCATCAGGAACCCCTGAGTAAGTAGGCAGCCTTCTTGAGA
GTCTCAGACTCTCCGAGAGTTTCCAGGATCCAGACACCAAAAAACAAGCTACAAATAGAGGAGGATGAGCAGAGACT
CTTGAGATGGTGTAAAACAATGACCTTTATGGGGAAAGTGTCACTCTAATTAGTGATTAACCAACAGCCAAAATC
ACACAAAAAAAAGTAGAACTGTTCTAAAACATACATGGGATGTGGTGGCTTACACCTGTACTCCCAGCACTGAG
GAGGGTAAGATAGACCACCACAAAGAGTTCCAGGCCACTAGCTCTACGGAGTAAGACTGTCTCAAAAATAAAA
GACAAAACCATTTTGTAGGAAGGGTGCATTGCCCCGCCCCGCCCCATTACCCCCCTCCTCTTCTATTTAGT
GAAGGTCCAGCATATCGTTCTGATGATATAAAAATAAGTGGTCATATTGGGAAGGAGCTGACTGTTAGGGCAGTAG
AATTTATGACCATATATAGTTTACATGATAAGATATATTCCTTGGTTTTACACAACAAACAAAAATATCTTGGCA
CTTATAAATGTGATCTCTTAATGTTTTTACTATAGTAGACATTTGGTAAGAAATAAATAGTGTATCTTACATGT
ATTAAGTTCAAAGATGCACCTTTAGTTTGTTCAAATTAACCTTTATTAACAGGTACAAATTTGCTGTTTACAATT
ATAGAATTTCTGATTTTTTATGTCAGATGGATTTACTGCAGGAATTTTATGAAACAACACTGGAAGCTTTGAAAGA
TGCTAAGAATGATAGACTGTGGTTTTAAGACAAACACAAAGGTAACAATTCATTTGTCCTTTGGAAGAGTGGGTG
GGTACCTGGAGTGATATGCTCCAAGTCTATGTACCTCCTCCTAAAGGCTTTTCATCTTAATTAATTTTTTTTTTACA
GCTTGGAAAATTATATTTAGAACGAGAAGAATATGGAAAGCTTCAAAAAATTTTACGACAGTTACATCAGTCTTG
TCAGGTAACATTCCTTACCTTTTCTGAATATAAACATGAAATAATAGTATTCAGCTTACTTAGGCATGTTTGT
GTGAAAGTATCTATCCCAAAAATAAATTTAAAGAAGTAAGTTTTTAAAGGATCATGATCATGAGTTCAAGTCCA
GCCTTTTTTATAAAGTACGCTTTTGGCTTGGAGGTGAGGCTGACTTTCTGAGTCATTTGTTAGATCAGTGGCTCTCA
GCCTTCTTAATGCTGTGACCCCTTAAATATAGTTCTTCTGATGTTGTTGGTGACCCAGCCATAAAAATTTCTTTTAA
AAGATTAATGATTTTTATTCCAGAGTGTCTGCATATATATCTGTGTGTTAGAAGAAGGCACCAGATCTCATCATA
GATGGTTGTAAGCCACCATGTGGTTGCTGGGAATTGAACTCAGGCCCTCTGGAAGAGCAATCAGTGCTCTCCAGC
CCCATACATTTATTTTTATTGCTACTTTGTAAGTGAATTTTGTACTGTTGTGTCATCATAATGTAATAATTTGA
TATGCAGTATTATCTAATAGCAGCCCCTGTGAAACGATTGTTTGCCTCTCAGGGGATTGTGACCCACAACTGA
GAACCACTGGTCAAAATGCTGGATTCAATTCCTTACCATTATGGTATGAGGTGTAGTTGTCAGTGAGCGAATAAG
CCCCTTATAATATGTAGTAAATATGTACTACCTAGAAATAATGCAATATAGAGTTAAGCCTTGGTATTTACTTTAT
TTGCTTTGGGAATATGGGGAATAGTTTTATACATATAAATAACAAAGATACTATATATTTCAATATATGGTATAT
CTTTTTGTATATCATTTGTAGCATTATTCTCAGATAAAAATTTGGAGTGATAGTGTCTATGTCTGCAATCCAATAT
AGCCATGTTAGGTGTTGCATATTTAATATGCGAGGCATTATGTTGAGACATGTGCATGGTAAGCTGGTTPATGAT
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TTTTTAAATATTTATAGCTATGTGGCTATAGGCCATAAACTAACTAAGGCTCAAATTTGCAAGCATATTTCTGGGT
TGCTATGTAATCTTCTGACATAAGAATGGCTGAGATGAGAAAAGTGTAGCATATTTTTATCTCCTTAGGTGCATT
CAGGAAGTGGGTTTTCCATTGGCTAGATGATCTAAACCAATTGTGAAGGAAGAACATTAGTGTACTTCTTAGCTGA
AAATATAAACTGCGTTGATTTTTAAATTACATTTAAGTGTGGGCACGGTATACACACATGTACATAACCACAAAG
TGCATGTTGAGGTGAGAGGACAACCTTGTGGGAATCACTTCTCCTTACACTGTGGAAGATAGATGGCTTGACA
GCAAGGGTCTTCCAACGTCAAATTTTAGTACATGGGACTGGAGAGATGGTTCAGTAAGTAAGAGCACTTTGTTAA
GTGAGTACAACTTAGTTCCACATCACCTACTGGTGACAGTCAGCATGGCCATGCATGTCTGGCCAATGCTGGGT
TGGGGTGTGGCCACCAGGCTAAGTGAATAATCGGTG

2.3. Floxed fragment (0.71.0 kb)

gccgggatggccataacttcgtataatgtagtatacgaagttatCACTTCAACTTCCCTGAGAGTTCTTGCTT
CAAGAGATGAGGCAGACAGTGTTCCTGACCTACACACAGGAATGCACAAGCATAACATATGCAACACACAGAG
GTCTTCTTAATGAATGTCATTTCTTTAAAAGTATTTTATACACAGGAAAACCTTCTAGCCCCCTTTCCCTATCCA
GTTTCTTTGGTCACTCTACAGTCAGCCATGATGCAGTAAAATCTATGATGCATATAACACAGAGATACAAGGCT



cggtggtttggttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagatac
caaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcg
ctctgctaatacctgttaccagtggtgctgctgccagtgccgataagtcgtgtcttaccgggttgactcaagacgat
agttaccggataaaggcgcagcggctcgggctgaacggggggttcgtgcacacagcccagcttgagcgaacgacct
acaccgaactgagatacctacagcgtgagctatgagaaaagcggccacgcttcccgaaggagaaaaggcggacaggt
atccggtaagcggcaggggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttata
gtcctgtcgggtttcgccacctctgacttgagcgtcgatTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGA
AAAACGCCAGCAACCGGGCCTTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCCACATGTTCTTTCTCGT
TATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATAACCGCTCGCCGAGCCGAACGACCG
AGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTA
TTTACACCCGCATATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATAACTCCGCT
ATCGCTACGTGACTGGGTCTGGCTGCGCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCT
GTCCTCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTCATC
ACCGAAAACGCGCGAGGCGAGCTGCGGTAAGCTCATCAGCGTGGTCTGGAAGCGATTACAGATGTCCTGCTGTT
ATCCGCTCCAGCTCGTTGAGTTTTCTCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGC
GGTTTTTCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGATTTCTGTTTATGGGGTAATGATAACCGATGAA
ACGAGAGAGGATGCTCACGATACGGGTACTGATGATGAACATGCCCGTTACTGGAACGTTGTGAGGGTAAACA
ACTGGCGGTATGGATGCGGCGGGAACAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGT
AGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCG
CGTTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCA
GCAGTCGCTTACGTTCTGCTCGCTATCGGTGATTCATTCTGCTAACAGTAAGGCAACCCCGCAGCCTAGCCG
GGTCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCGAGATGCGCCGCTGCG
GCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGCGTTAACTTAATTAAGTCGACGGCCGGCCT
CGAGGCC

3. ES cell lines targeted and validation data:

3.1. ES cell lines targeted

The targeting vector was electroporated in P1 ES cells [MCI-129Sv/Pas background]

Number of clones screened: 372

Number of positives: 2

Reference of clone used to generate the mouse line:

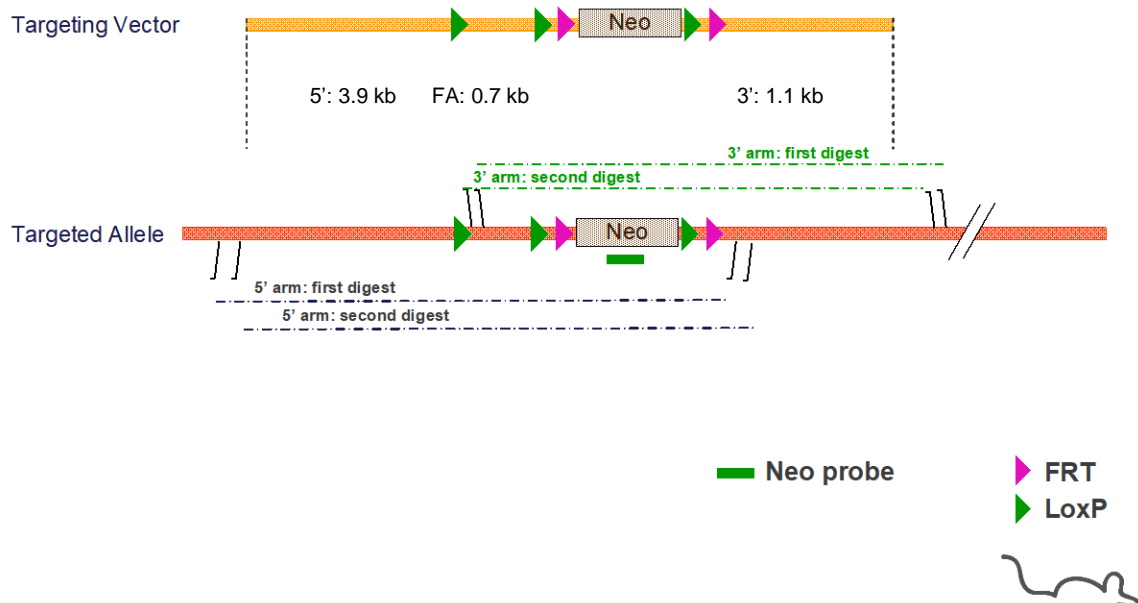
- clone **DG30-184**

3.2. Southern data on positive clone

3.2.1. Neo Southern strategy



Southern Screening Strategy



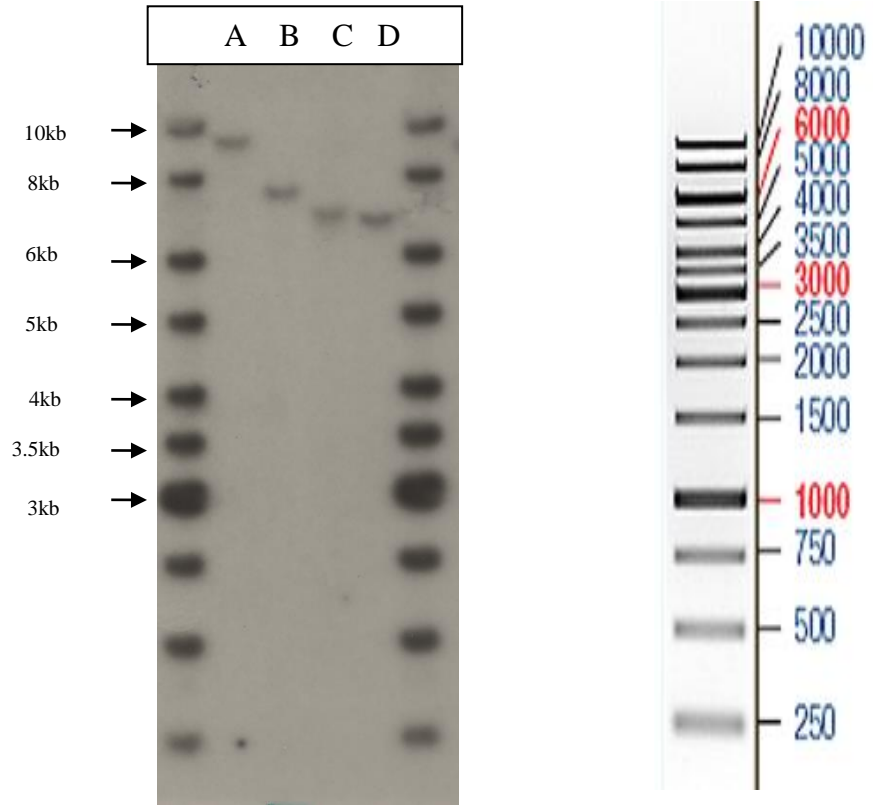
Digestions used to validate the 5' and 3' insertion

| Probe | Name | Genomic DNA digest | WT allele (kb) | Targeted Allele (kb) |
|-------|----------------------|--------------------|----------------|----------------------|
| Neo | 5' arm first digest | AfIII | / | 9.4 |
| | 5' second digest | Scal | / | 7.7 |
| | 3' arm first digest | EcoNI | / | 7.3 |
| | 3' arm second digest | HincII | / | 6.9 |

Four different digests are used to validate correct HR event. Two digests validate the 5' insertion, 2 other digests validate the 3' insertion

3.2.2. Picture of Neo Southern

Neo southern blot: 5' and 3' arm validation ladder



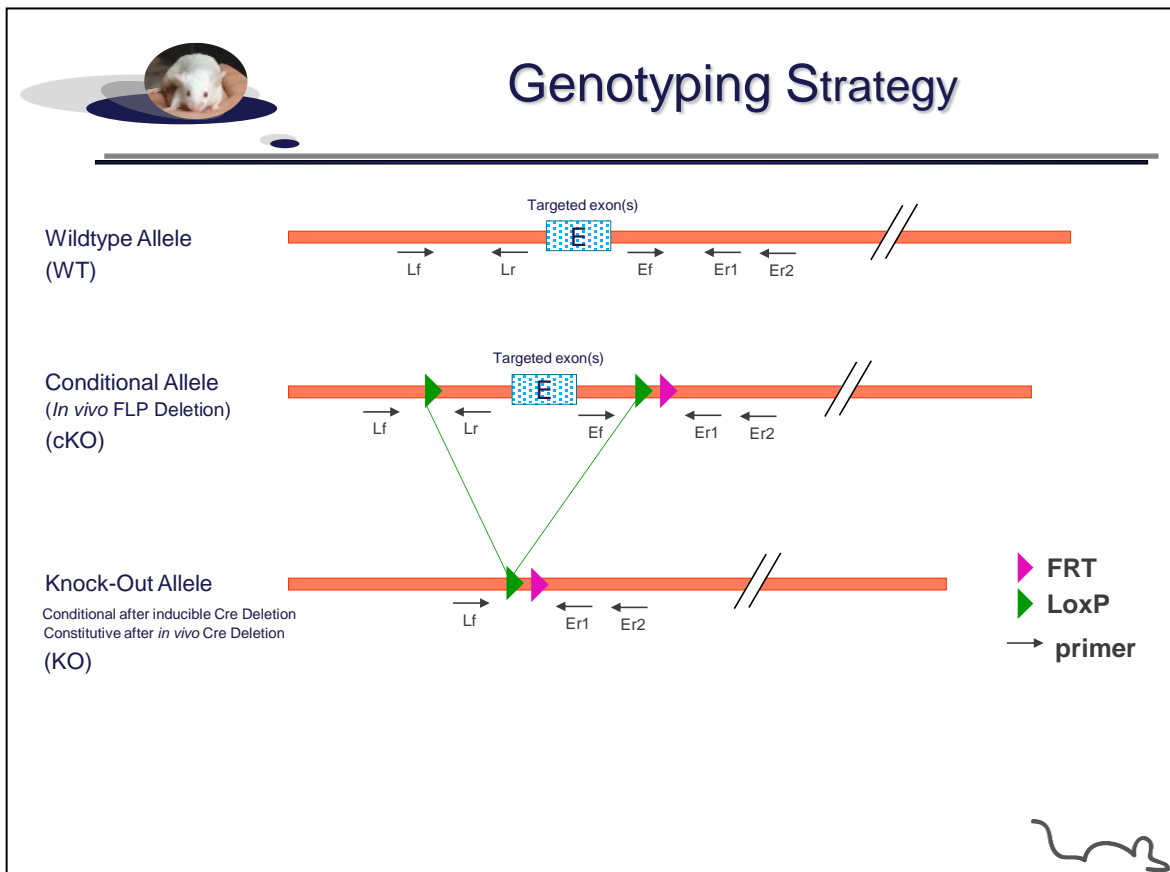
A: AfIII 9.4Kb
B: Scal 7.7Kb
C: EcoNI 7.3Kb
D: HincII 6.9Kb

4. Genotyping protocol and data on conditional and knock-out animals

Both conditional and knock-out mouse models were backcrossed in C57BL/6J background.

4.1. 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 |
|----------|---------|---------------------------|
| Lf | 990 | ACCTACTGGTGACAGTCAGCATGG |
| Lr | 991 | CTTGTGCATTCTGTGTAGGTCAG |
| Ef | 73 | CACTTCACATCAAGTCTGCCATCCC |
| Er | 74 | TTTTTAGTTTTTCGAGACAGGGTTT |
| Er2 | 2625 | CTCGAACTCAGAAATGGGCCTGC |



PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer (see the map above) | Conditional allele (cKO) | Knock-Out allele (KO) | WT allele (WT) |
|---|--------------|--|--------------------------|-----------------------|----------------|
| Presence of the distal 5'loxP | 990-991 | Lf / Lr | 212 | --- | 165 |
| Excision of the selection marker | 73-74 | Ef / Er1 | 464 | --- | 325 |
| Total Excision (excision of the floxed exon(s), i.e. knock out) | 990-2625 | Lf / Er2 | 970* | 295 | 784* |

* This PCR product will not be observed using our PCR genotyping conditions (see description below)

--- No Amplicon should be obtained

4.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:

-10x Buffer (Roche)

-dNTPs 10mM (Amersham Biosciences)

-Taq DNA Polymerase (Roche)

-DNA (50ng/μl)

-5' primer (100 μM)

-3' primer (100 μM)

-Sterile H2O

Volume:

2.5μl

0.5μl

0.2μl

3μl

0.125μl

0.125μl

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 optimized for high-throughput genotyping. Adaptation to small-scale may be required.

4.3. Picture of genotyping with various alleles

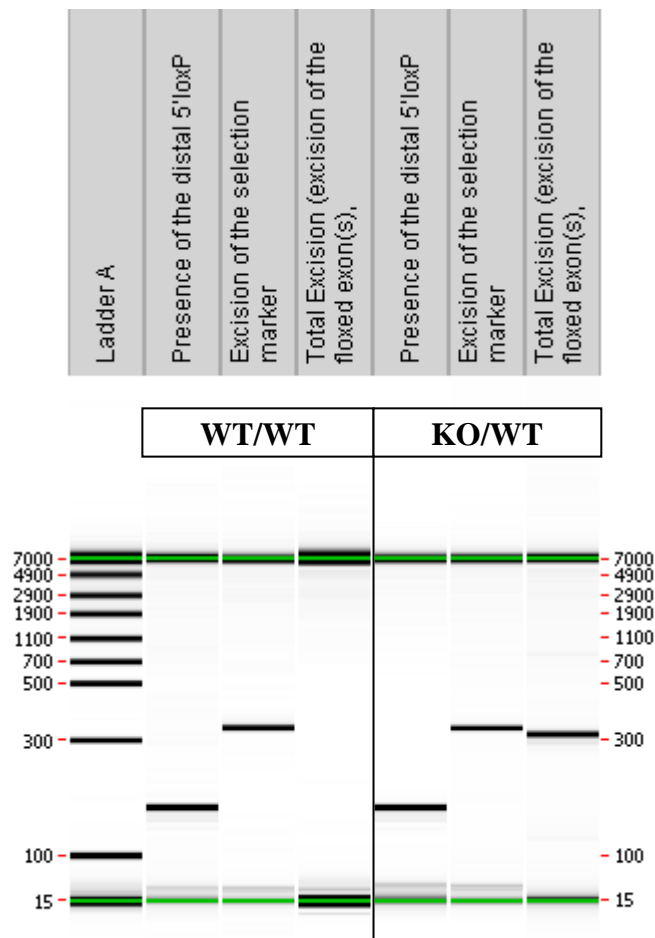
- Picture of genotyping with conditional knock-out (cKO) allele

Data not shown

- Picture of genotyping with knock-out (KO) allele

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.