Molecular Biology Data
Nr3b2 conditional knock out model
ICS references:

Table of contents

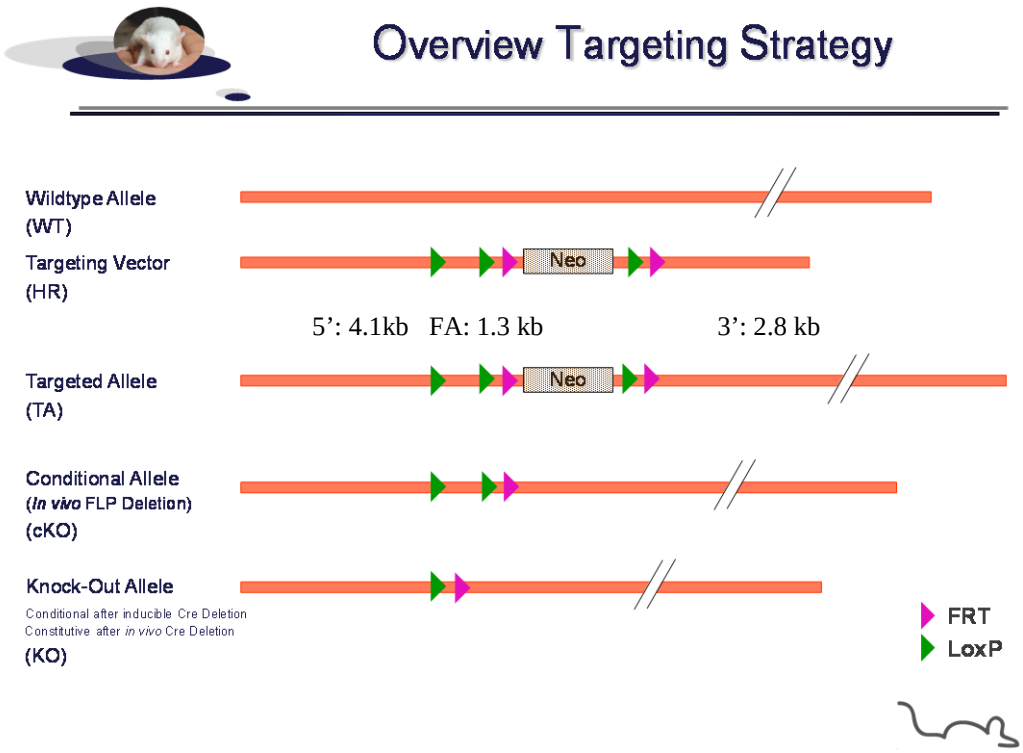
For any question, please contact:

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

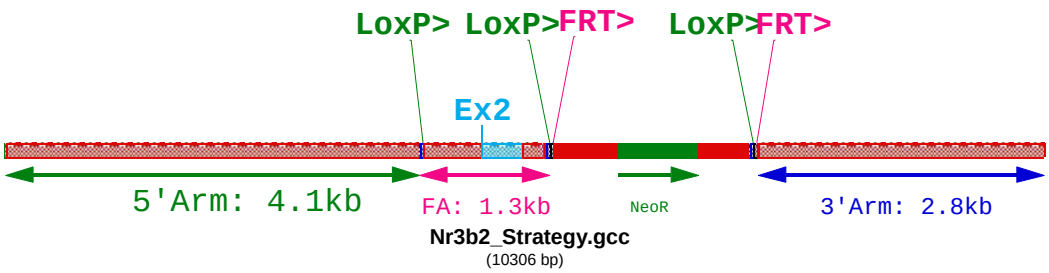


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exon 2

Nr3b2 gene (also named Esrrb) 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=43303

Strategy used to generate the conditional knock out model

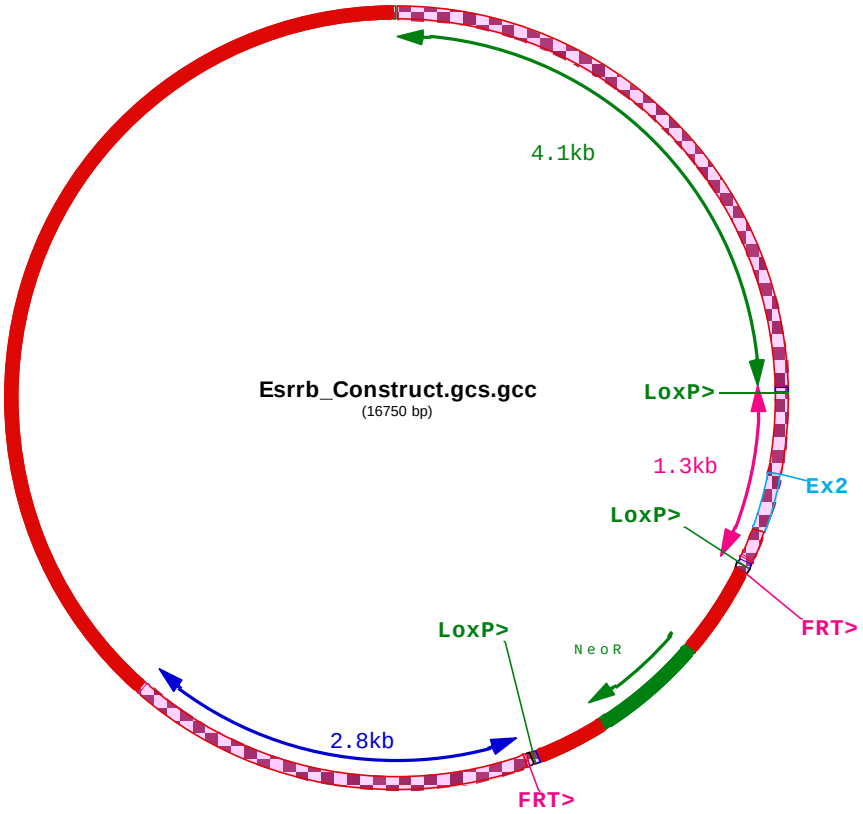


2. Construct used for homologous recombination in ES cells: Nr3b2 project

2.1. 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 fully sequenced. Regions sequenced are indicated in bolds.

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (4.1 kb)

GCAAGCACTCTACCAGCTAAGCTATATGCCCCACCTCTCCCCTACCAGCTAGCTATATGCCCCACCTCTCCCCTA
CCAGCTAGCTATATGCCCCACCTCTCCCCTACCAGCTAAGCTATATGCCCACCTCTCCCCTACCAGCTAAGCTAT
ATGCCCCACCTCTCCCCTACCAGCTAAGCTATATGTCCCACCTCTCCCCTACCAGCTAAGCTATATGCCCCACCT
CTCCCTTCCCTTCTTAATATCAGTAACTGACAGAAAGCGCAGTAGACTCTAATCTGAAAATCTGAGATCAAAATT
TGCCCCCAAATCTGAATTCTTGTTTTGTTTTGTTGAGTCCAAGGTATCATACACGGCTTTCACCTGAATTGTATC
CGAAGTAGTAGGCAAGTATTTCTAATAATTTAAAACTTGTAGCCATTAAGTCATTAGACTTGTAGCATCAGGATA
GTTCTCCAAAAGTTTGGGATTTGGGGGCTTGCTCCTGGGTTTCATCCCCAGAATTAAAAAAAAAAAAAGTATAAA
ATTACTTTCTGGCAGATTGATAGCCTTTGGAGAAAATAAATCCCTTGCATGTTTAGACTTTCCCATCTTCCAGGT
CATATTATTTGTATTCAAATATTCCAAAATCTAAACAAACAAACAAACAAAAACAAAAAACCTCAAATCCAAAAT
ACTTTTTGGTCTGAGGTATTGCATTACGAACTACTCAATCAGTGATCTGCAGTCTGTGTTGAGAGAAATGGGGAG
GAAGATTTAGTGGGGCGGAGCTAAATTAGTGGTCCCGGGAAGAACTGAATTGCTTGGGGAAGACTGGAAAGTATG
GACTGTTATTCTTAAATACCTTAAATTCTTTCCATTCTCAGTGAATGGCCTCTCATCCCAGATGAGACTCACACC
AGTCCAAAGTGCATCCTGTCCCCACACCCTGCCTTGTGTGTAAGCAGGTGCTGCTGACAGCTGGTAAAGAAGGCA
CTTCATTGGACCCCCCTCCCTTACCCTCCCCCCATCTTACTCCGCAGCCAAATTCCGAAGAATTGTCTGCTTCAT
TCCCTCTGGCCATTGCAACACCACATCCATCCCCACCATAAAGGTGAGCCCGCTCTGGAGCCAGGCCACCTCCTA
ATGACTAAAGGAACAGAGGACCAGCCCCTTCTTCCTCGGTTTCACCTTGTGGCCCCATCTAATGTGCTTCCAGAC
AGCTCTGACCATTGCATTTACAGCAGGCTCTTTTTAGTCTTTTCAGTGTGCATGTGTTTGAAGAGGGGGTGCCTG
TATGTTTATGGGGGCTAGAGGTTGACATTGGGTATCTTAATTATAATACACTTTTTTTGTTTGTTTTGAGACAGG
GTCTCAGCCAGAGGAGGTGGCACACACCTTTGATCTCAGTACTCAGGAGGCAGAGACAGGTGGATCTCTTGAGTT
TGAGGCCAGCCTAGTCCACAGACTAGTGAGTTCCAAGACAGCCAAGAATATATAGATCCTGTCTCAAACAAACAA
ACAAACAAACAAACAAACAAACAAATACTTGCCTACTACTTCGGATACAATTCAGGTGAAAGCCGTGTATGATAC
CTTGGACTCAACAAAACAAAACAAAACAAAACGGGCATGGTGGGTCACGCCTTTAATCCCAGCACTCGAGAGGCA
GAGGCAAGCAGATTTCTGAGTTGGAGGCCAGCCTGTTCTACAAAGTAAGTTCTAGGACAGCCAGGGCTATACAGA
GAAACCCTGTCTCGAGACAAACAAACAAACAAACAAACAAACAAAAAACACTAAAGAAATAAATGCAAAGAGAGA
AAGAGAGAGAGGGACAGAGGAAGGGAAGAGGAGGGAGGGGTAGGTGTGGGAGGAAAGTAAATAATGACATCGTTA
AATCATATGCCCCTCTACTGGGATACAGATACTAAATTTCTGGCTGCTTATCCTACACCAACTCTGACTGAGACC
AGGATCCAATGAAGGTTTCTGAATCAAGACACAAGGCTGGAGAGGAAGCCAGGGCTTGGGAGCACAACCTGACCC
CTCGAGTGTCTTTCAGAGCAGGCTGAGTTTCAAAACTGGGAAGGCAGTGGAAAATTGTGGCCCATACCCTCCCAG
GAGAAGCTGCAGCCTCCCCTCCCCTCTCCCTCCTGGAAAATGCTTCTTGTGCCTCAGAGATGAAGGGGAGCAGGG
GCTCATAGGGCACACTCAACAGAAGCTGCTACTGAGACTTCCTGTGTCCGGTGTAAATTGGGAAATCTCTGGTGC
TCTCAGAGCCTTTGGAAAGTTGGGTGGAATTACAGGATTTGTGAGTTCTGGTTCCCCCAGGCCTTCACACTGAGC
CCAAGGTGCTGTGGAGAGGAACACAGCCCAAAGCAGTCAACAGGGAAAGCTAGAGAGGGTCAGACATTCAGTCGA
GAGGAGTCAGCTGCCCAGCAGAGGAGGACAACGCTTTAGGCCGTGTGCTCAGTCAGACTCCCCTCCAGATGGTGC
CCTGCTATTCCCTCAGGATACCTTGTTGGTGGTTAGACAAGGTAGCCATACCCAGGAAGATCACAAGGCACCAGC
CAGAGATGCAGAAAGGATCCAGAGGAGACCCTTAACTGGGGACCCGCCGCTTATGATGCAGAGAATGGGGGGTGG
GGGTTCGGAAGGAGAGGATTCCCAGGGTTAAAGGAACATTCCATCTAGAACAGTAGGGACCTGGGGACCCAGTCT
TTACCAACTACGAATGTGGGCTTGTAAGCTGGAGTGCTGGGCCTTTAGTGGGCCTTTCATTCCAATACCTGAAGC
TCGCCCTCTCTAGACAAGTTCATGTGCAGGTGAGTGAGTGACACCCTCAGGAGGTGTGCTGAGTGTCATTGCACT
TTAGTGAGACCAAAAGCCCAGTCACATTACCAACTCCGTGGTCTGGGTTTGAGGCTAGACCAGGAATTGCTATGT
GGGTGCAATGATAAAGGCTCCGTCCCCATTCCCTCCGGAGTCACCCTTTTTTGTGGGCACTTTACTAAAACGAGG
AACTCACAAGCAAGTTACCTGCAAGTGGCACAGGAGGAACAGACCGCCACCCACCCCCGCAGCTCTGAGGTCTTC
CTTCCTACCCAGGAGTCTTTCTTTCTGTGTTGTTTCCCCTGCTCTTTTCCTTGTTCTGGGTGCCTAAGCCAGTAC
GGACTCTCTCCACTGAGGCAGGCCTGGGATCGAGGCTACTTTCAGGGATCATACCTCTAGACAGTTTTGTTTCTT
GGAGACAGGGTCTCTCTTTGCAGTCCTGGCTGTCCTGGGATACTTTGTGTAGACCAGGCTGGCCCCAAACTCGAG
ATCTTTCTGTCTCTGCCTCTGACGTACCACAATATTTGAGACCATCTACTTAGGCCTCCTGCCTCAGACCCAAAT
CCTAACTGAACATGTCGAACAACCAGGTTCCCCTGAACGGTCCTTCAATCCAGTCCCCTTTACGTGAACTTGGCT
GGAGAGACAGAACTCCCAGTAATGGGAAAGGCATGGTTATCCTACAGTTTTCAGGGGTCCCAGATGATCTGCCTC
ATTGGTGAGGCCCCAAAGCATTCCAGAAGGTTCTGGGCTAACAAAACAAAACAAGGTGAAAG**GATGATAGTAGGT**
GATGAGCAGAGTGCTTTTGAGCTTTATGTGAGTTAGGGGTGGGTGGGTGTTTAATCTCTAAAAGAAAAAAACAAC
TCTGGCTAAGTATTACATGGTGTCATGCCAGGCTTTGAGGAGCATGCCTTTAGTCCCAGCACTCAGGTGGCAGAG
GCAGGCAGATCTCTGTGAGTCTGAGCCTAGCCTGGTCTACATAGTCAGTTCTAGGCCAGCCAGGGCTACACAGAG
CGACTCTGTAAAGTTACACGGTGGTGCAGACCTGTAGTCCCAGTTTGTTCCTGGCTGAGGCAGTAGGATACAGAG
TAGGATGGGGGGGGGGGCTTCATGCGTTCTCATGGAGACAGCCTTTGGGGACTGGGGGTGCTGGATGCAAGCTGG
ATTATCTCAGAGCTAAGAAGGAAAAATAAAAGCCAGAGCAGATCCCCCCTCCCCGGGGCAGGGGCAGAGACTGGG
GGATCTCTGAGTTTGAGGCCACTCTGTTCTACAGAGCAAGATCTTGGTGG

2.4. Floxed fragment (1.3 kb)

ggccgggatggccataacttcgtataatgtatgctatacgaagttatCCAGAACTACACAAAGGAACCCTGCCTC
GAGAAACAAAACAAAATAAACAAAAAAGCAGAAAAAGAGAAGACAGGATCAGGAATCATACTTTAGATGGGACCG
CCATTATCCAGCCCGGACCACGGGGAAAAACTCACTTGGTGCCTTTTGGTTAAACGGTCTCTCTCCGTCCAGTTA
GAAAACTTGGCAGTCTTGGTGACTGTGAAGTCCTATTGAAGCAAATCCCAAGCTTGGACTTCACAGCTCTTTGGG
ACAGGCACACGGTCAGCTTCCACTTTTTGTGAGGTAGGCAGGTTGGCCAAATATTTAGGAGGGACAGCTAACATC
TTGCCATCCTTAGGCCAGCAATTTGCAAAAGACAGGGTCTCTGATTTGAAGTTTACGAATTCAAAGTTTCTTAGA
ACTCTGAAGTCGCAGACTTTTCTGGAAAGCAGCTACAGACCTGGCCACCCTCGCAGCTCTCCTACGTCATACCCG
TAGACACGCCCATGTGGCCCGCCCACTAGGCCATAGCCCCGCCCAACCTCTCACCTCACTTAGAACTGACTCCCG
CGTTCTGTGTTCGCAGGCTGCTGAACCGAATGTCGTCCGAAGACAGGCACCTGGGCTCTAGTTGCGGCTCCTTCA
TCAAGACGGAGCCATCCAGCCCGTCCTCGGGCATTGATGCCCTCAGCCACCACAGCCCCAGCGGCTCGTCGGACG
CCAGTGGTGGCTTTGGCATTGCCCTGAGCACCCACGCCAACGGTCTGGACTCGCCGCCTATGTTCGCAGGTGCGG
GGCTGGGAGGCAACCCGTGCCGCAAGAGCTACGAGGACTGTACTAGTGGTATCATGGAGGACTCCGCCATCAAAT
GCGAGTACATGCTTAACGCCATCCCCAAGCGCCTGTGCCTCGTGTGCGGGGACATTGCCTCTGGCTACCACTACG
GAGTGGCCTCCTGCGAGGCTTGCAAGGCGTTCTTCAAGAGAACCATTCAAGGTGCGTGGTGGGCAGCTCAAGACC
TGGATTCAGGCTTTGGGTGGGGGCCTTGGTCTACAGGTCTAGTTGGGTTATTAATTCCAAGTCTCGTTTCCTGGG
GGCAGAGGTGCTCTAGAAGTCTCCTGATCTGTGTCTCTGGGATCCTAGACAAGGATTTCAACTCTAAGACTCATA
GATATACCATGTTTCTTCCTGCTTGGTCCGGCCTGTGTGGCCATCGCGGACTAATGGCCataacttcgtataatg
tatgctatacgaagttat

2.5. PGK-Neo region

gaagttcctattctctagaaagtataggaacttcgctagctcataaaaaatttatttgctttcaggaaaatttttt
ctgtataatagattcataaatttgagagaggaggcgcgccgaattcctgcaggattcgagggcccctgcaggtca
attctaccgggtaggggaggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttg
gcgctacacaagtggcctctggcctcgcacacattccacatccaccggtagcgccaaccggctccgttctttggt
ggcccccttcgcgccaccttatactcctcccctagtcaggaagttcccccccgccccgcagctcgcgtcgtgcagg
acgtgacaaatggaagtagcacgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggta
ggcctttggggcagcggccaatagcagctttgctccttcgctttctgggctcagaggctgggaaggggtgggtcc
gggggcgggctcaggggcgggttcaggggcggggcgggcgcgaaggtcctattgtgagcgctcacaatcccggca
ttctcgcaagcttcaaaagcgcacgtctgccgcgctattgtgagcgctcacaattccgggcctttcgagaaggag
ccaatatgggatcggccattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattcg
gctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccgg
ttctttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctgg
ccacgacgggcgttccttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggcg
aagtgccggggcaggatctcctgtcatctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgc
ggcggctgcatacgcttgatccggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcacgta
ctcggatggaagccggtcttgtcgatcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactgt
tcgccaggctcaaggcgcgcatgcccgacggcgatgatctcgtcgtgacccatggcgatgcctgcttgccgaata
tcatggtggaaaatggccgcttttctggattcatcgactgtggccggctgggtgtggcggaccgctatcaggaca
tagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccgcttcctcgtgctttacggta
tcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgaggggatcgatccgtcc
tgtaagtctgcagaaattgatgatctattaaacaataaagatgtccactaaaatggaagtttttcctgtcatact
ttgttaagaagggtgagaacagagtacctacattttgaatggaaggattggagctacgggggtgggggtggggtg
ggattagataaatgcctgctctttactgaaggctctttactattgctttatgataatgtttcatagttggatatc
ataatttaaacaagcaaaaccaaattaagggccagctcattcctcccactcatgatctatagatctatagatctc
tcgtgggatcattgtttttctcttgattcccactttgtggttctaagtactgtggtttccaaatgtgtcagtttc
atagcctgaagaacgagatcagcagcctctgttccacatacacttcattctcagtattgttttgccaagttctaa
ttccatcagaagctgactctagatctggatccataacttcgtataatgtatgctatacgaagttatctcgaggaa
gttcctattctctagaaagtataggaacttcgagctggccagctaggcc

2.6. 3’ homology arm (2.8 kb)

CTGAAACGCCGTAGAAAGAGCCACAGGAATACCTGCTCTTGGACAGCTCTCAACACGCATGCTGTCCACTGCATG
AACCCTCCCTCCAAAAGCCTATTTGTGTGTGTGTGTGGGGTGGGGTGGGAGGTTGAGGGGAGGAATAGGTGCTAC
TATTATTTGACTCACTATCTCATACTTTTGGCTGTCTTTTTGCTTCTATCTTAATCACACCTGTAGAAAGGTAGG
CCCGATTTCACGTGGGCAGGAGCTGACTGGGATCCTGGAAGCTCTGGGCTATTTTTCCATACATTTGCATATCAC
TAGGGTCACATGCTGATAGCCCTGCGGTATTCAGCTCTTTCATCTCCATGAATGTGTTTGGGAGTCCTGGAGCCT
CCCTCTGTGCAGGTGAGTGGAGGATAATAATGGTGGATCCAGTCCTCACTGGGCGATATTCTTTCTGGCCCTGTG

GGGCTGGGAACGAGACTGCTTATGCTTCAACTTAGTGGTGAACACTTAACTGACAGCTGTTGGTCAGTCAAAACA
TCTGGCGAGCACCTCCTAAGCACCATCTATGCAGGTAACCTACAAGGGCCAATTCCTCCAGCCTGGGCGGACTCA
CCGCCATACCCAAAGATGCTGGCCATTCCTCCCATTCTGGCTCCCCACCCTCACCCCCCACCTACATCCCACTTA
CCTCCCCACCCTCCCATCCCCTACTCCCCCTCCACCCTACCCCACCCTCTTGTCCTGTCTTTTGGCTTCAACTTC
ATTCTGTTCAACTAGTCTCACATGACCACTCTGGAAGGCATTGGCTAAGCTTCCCTAGGGTGATTCTGTTTATTC
AACAAGCTTATGTTTGTTAATCTCATTGGATAATGATCTATTAGACCACTTGAGGGCATTAAATGTTGGGTATAT
TTACAGTTCTACTTAACCATTTGGAGGCTGGGCTAACCCACCAGGCTTCTGGGTCAGACTGACACTTGAACCAGC
TCTTACCTGTGAGCTCAAACGTGGGTGTGCTGCCTCAGTTCCCTTATGTGCTTTACAGAGTGCGTTTTATTTTTA
GGGTTTTATGTAGCCCTCTGTAGGCTCAATTTACTGTGTCAGCAAGACTGGTCTCCGATCGCCGTCTTTACCTTC
CAAATTTCCATGTGCAGGGACTATGGGATGGTCTATCACCACCACACCTAGATTTTCCTTCCTCTTCTTCCTTTC
CTCCTTTTCTTTTTTCCCTATACACTATTGAGAATTGAACCTAGGAACTGATTGTCTGTGCTTATCAAGGCATGC
TTGTAGAAGTCAGAGGACAACTTGCTGGAGCAGGTCCTTCCTTTAACACGTGGGCAAACGCGTATCAGGAGCCTT
GGCAGTAAGTGTCTCTCGCCACTGAGCCATCATGCTGGCCCCTAGCTTAATGTTCATTGCTATTTTTACTTTTAA
CAATATCACTTACCGTACACTTTTACTATAACATCAAGTTTCATTGTTCAGAACAAGCTTTGTCATAGGTGTATA
TTTAAATAAAGTAGTATAATTACTTTGATCTAATAAAATTTAGATTACTAAACTATTTTAGGGCCTCTGAGATTG
CCTAGTGTAAATATGCCCGTCACCAAGCCTAATGACCTTTCGGTCCCAGTGACCCACGTGGTGGGTAACTTATGA
CCTCACATGCACACACACGTGCACACAAAATAACTAAACTACATTTAGTCCATTGTGGTCATGAATGATATTTAT
TTGGTGTTTTGTGACAGGATCTTTCTGTATAGTCCTAGCTCAGCCAAAACACATGAGACCCACCTGCCTCAGTTT
CCTGAGTACTGGGATTAAAGGAATACACTAGCCGGGCAGTGGTGGCGCATGCCTTTAATCCCAGCACTTGGAAGG
CAGAGGCAGGTGGATTTCTGAGTTCGAGGCCAGCCTGGTCTACAGAGTGAGTTCCAGGACAGCCAGGGCTACACA
GAGAAACCCTGTCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAGGACTACACTAACAGGCCTGGCCACAA
ATGATTCTCCCCTTCCTACAGAGTTTTGCTCTGTAGCCCTTGCTGTCCTGGAACTTGCTGTGTAGACCAGGCTGG
CCTCAAACTCAAAGACCTCTGTTTGCCCCTGCTTCCCGAGTGCTGGGACTTCAGGCCTGTGTCACCACAGTGAGA
TATGAACAAAATCTCCCTTAGAATCTTTTTGCC**GAGCCAGCCTGCCTATGTGTAGGCTGAACACATAGGGCCATC**
TTGTTGTATACTAGGAGCAGAGGCAGGCAGTAAATGCCCCCTGGGCTGCTCCTGCCTTGTAGCCCCCTTTTGTTG
CTGGGGTGTGACTCAGGATATGGCACCTGGGGCTTTTGCTGCTCTATACCCCCACCCTTGGGTGGAGGGGACCTC
AAGGTGAAATGAATGGTATGGCAGACAGTTGTGTCTCTTGCTTCAGCCGGTGCCTAGAGCTTACATTGAGCAAGT
GCTGGCCACGTATGTTTGAAGCATGGCATGCCACCGAAACACGGGCTTTTCCTGGTTAAATTGGGAAGTGACCTT
GCAGGTTTGTTTTATACTCCTTAAAATAATTTTTTTTTTTGAAAATGGGATTTTAGAAGACAGTCACGTTTTCTCC
TTTCCGTGGGTTTCTTTAAATGGGGCAGAGATATTCTAGATCCGGTTTTTCTAGAAACCAAGGCTGGTATGTATA
GACCCAGCTCCTCTGCTACCCCCTTTCCCAGGGACCTAGGGAAGATCTGTAAACAGTATTAATTACGTGCTACCG
TGAAAAGGATACAGATCTTCTTCCTAGAAGTTGGCTGAATACCGTGG

2.7. Vector backbone sequence

ggcctcagtggccactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccg
cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgac
gtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggaggagtatttacggtaaactgccca
cttggcagtacatcaagtgtatcatatgccaagtacgcccctattgacgtcaatgacggtaaatggcccgcctg
gcattatgcccagtacatgaccttacgggactttcctacttggcagtacatctacgtattagtcatcgctattac
catggttcgaggtgagccccacgttctgcttcactctcccatctccccccctcccacccccaattttgtatt
tatttatttttaattattttgtgcagcgatgggggcgggggggggggggcgcgccaggcggggcggggcgg
ggcgaggggcggggcggggcgaggcggagaggtgcggcggcagccaatcagagcggcgcgctccgaaagtttcct
tttatggcgaggcggcggcggcggcccctataaaaagcgaagcgcgcggcgggcgggagtcgctgcgttgcct
tcgccccgtgccccgctccgcgccgcctcgcgccgcccgccccggctctgactgaccgcgttactcccacaggtg
agcgggcgggacggcccttctcctccgggctgtaattagcgcttggtttaatgacggctcgtttcttttctgtgg
ctgcgtgaaagccttaaagggctccgggagggccctttgtgcgggggggagcggctcgggggtgcgtgcgtgtg
tgtgtgcgtggggagcgccgcgtgcggcccgcgctgcccggcggctgtgagcgctgcgggcgcggcgcggggctt
tgtgcgctccgcgtgtgcgcgaggggagcgcggccgggggcggtgccccgcggtgcgggggggctgcgaggggaa
caaaggctgcgtgcggggtgtgtgcgtgggggggtgagcaggggggtgtgggcgcggcggtcgggctgtaaccccc
ccctgcacccccctccccgagttgctgagcacggcccggcttcgggtgcggggctccgtgcggggcgtggcgcgg
ggctcgccgtgccgggcgggggggtggcggcaggtgggggtgccgggcggggcggggccgcctcgggccggggagg
gctcgggggaggggcgcggcggcccccggagcgccggcggctgtcgaggcgcggcgagccgcagccattgccttttt
atggtaatcgtgcgagagggcgcagggacttcctttgtcccaaatctggcggagccgaaatctgggaggcgccgc
cgcaccccctctagcgggcgcgggcgaagcggtgcggcgccggcaggaaggaaatgggcggggagggccttcgtg
cgtcgccgcgccgccgtcccctttctccatctccagcctcggggctgccgcaggggacggctgccttcgggggggg
acggggcagggcggggttcggcttctggcgtgtgaccggcggctctagagcctctgctaaccatgttcatgcctt
cttcttttcctacagctcctgggcaacgtgctggttgttgtgctgtctcatcattttggcaaagaattcacctg
ccagaccatgccaaaaaagaagagaaaggtcatgaaaccagtaacgttatacgatgtcgcagagtatgccggtgt

ctcttatcagaccgtttcccgcgtggtgaaccaggccagccacgtttctgcgaaaacgcgggaaaaagtggaagc
ggcgatggcggagctgaattacattcccaaccgcgtggcacaacaactggcgggcaaacagtcgttgctgattgg
cgttgccacctccagtctggccctgcacgcgccgtcgcaaattgtcgcggcgattaaatctcgcgccgatcaact
gggtgccagcgtggtggtgtcgatggtagaacgaagcggcgtcgaagcctgtaaagcggcggtgcacaatcttct
cgcgcaacgcgtcagtgggctgatcattaactatccgctggatgaccaggatgccattgctgtggaagctgcctg
cactaatgttccggcgttatttcttgatgtctctgaccagacacccatcaacagtattattttctcccatgaaga
cggtacgcgactgggcgtggagcatctggtcgcattgggtcaccagcaaatcgcgctgttagcgggcccattaag
ttctgtctcggcgcgtctgcgtctggctggctggcataaatatctcactcgcaatcaaattcagccgatagcgga
acgggaaggcgactggagtgccatgtccggttttcaacaaaccatgcaaatgctgaatgagggcatcgttcccac
tgcgatgctggttgccaacgatcagatggcgctgggcgcaatgcgcgccattaccgagtccgggctgcgcgttgg
tgcggatatctcggtagtgggatacgacgataccgaagacagctcatgttatatcccgccgtcaaccaccatcaa
acaggattttcgcctgctggggcaaaccagcgtggaccgcttgctgcaactctctcagggccaggcggtgaaggg
caatcagctgttgcccgtctcactggtgaaaagaaaaaccaccctggcgcccaatacgcaaaccgcctctccccg
cgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagaattcactcc
tcaggtgcaggctgcctatcagaaggtggtggctggtgtggccaatgccctggctcacaaataccactgagatct
ttttccctctgccaaaaattatggggacatcatgaagccccttgagcatctgacttctggctaataaaggaaatt
tattttcattgcaatagtgtgttggaatttttgtgtctctcactcggaaggacatatgggagggcaaatcattt
aaaacatcagaatgagtatttggtttagagtttggcaacatatgccatatgctggctgccatgaacaaaggtggc
tataaagaggtcatcagtatatgaaacagccccctgctgtccattccttattccatagaaaagccttgacttgag
gttagatttttttatatttgttttgtgttatttttttctttaacatccctaaaatttccttacatgttttac
tagccagatttttcctcctctcctgactactcccagtcatagctgtccctcttctcttatgaagatccctcgacc
cgagaattcggcttaaagcttatcgatgataagctgtcaaacatgagaattcttgaagacgaaagggcctcgtga
tacgcctatttttataggttaatgtcatgataataatggtttcttagacgtcaggtggcacttttcggggaaatg
tgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgat
aaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttg
cggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtg
cacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttc
caatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtgttgacgccgggcaagagcaactcg
gtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggca
tgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacga
tcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaac
cggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaatggcaacaacgttgcgca
aactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttg
caggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggt
ctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtc
aggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcag
accaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcc
tttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaaga
tcaaaggatcttcttgagatccttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccag
cggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagatac
caaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcg
ctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgat
agttaccggataaggcgcagcggtcgggctgaacgggggggttcgtgcacacagcccagcttggagcgaacgacct
acaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggt
atccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttata
gtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatgga
aaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgt
tatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccg
agcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgcggta
tttcacaccgcatatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgct
atcgctacgtgactgggtcatggctgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgtct
gctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatc
accgaaacgcgcgaggcagctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttc
atccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcgggccatgttaagggc
ggtttttttcctgtttggtcactgatgcctccgtgtaagggggatttctgttcatgggggtaatgataccgatgaa
acgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaacgttgtgagggtaaaca
actggcggtatggatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgttaatacagatgt
aggtgttccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccg
cgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgcagca

gcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccagcctagccg
ggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgagatgcgccgcgtgcg
gctgctggagatggcggacgcgatggatatgttctgccaaggcgtttaaacttaattaagtcgacggccggccct
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]

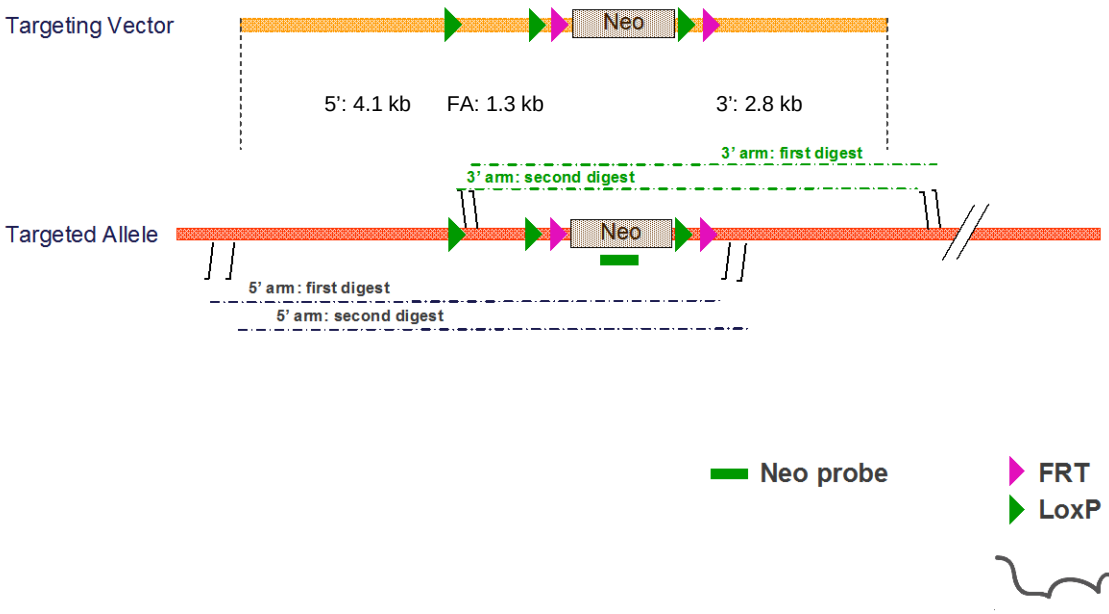
Number of clones screened: 744
Number of positives: 3

Reference of clone used to generate the mouse line:

- clone **K176P1-251**

3.2. Southern data on positive clone

3.2.1.Neo Southern strategy



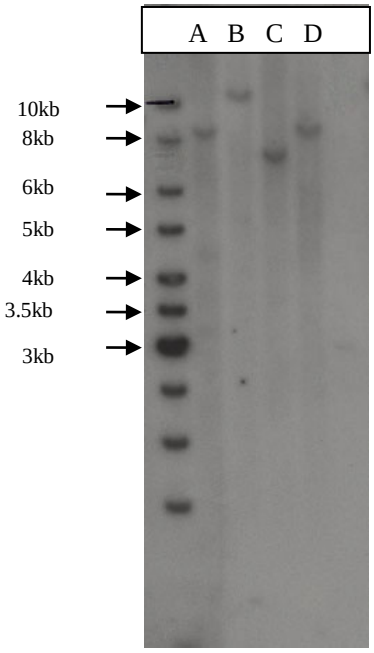
Digestions used to validate the 5' and 3' insertion

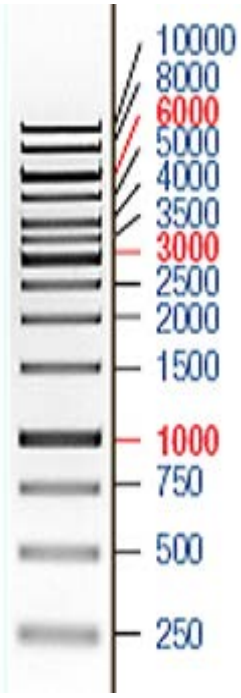
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	AvrII	/	8.6
	5' second digest	SacI	/	10.9
	3' arm first digest	SexAI	/	7.6
	3' arm second digest	HincII	/	9.0

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: AvrII 8.6Kb
B: SacI 10.9Kb
C: SexAI 7.6Kb
D: HincII 9.0Kb

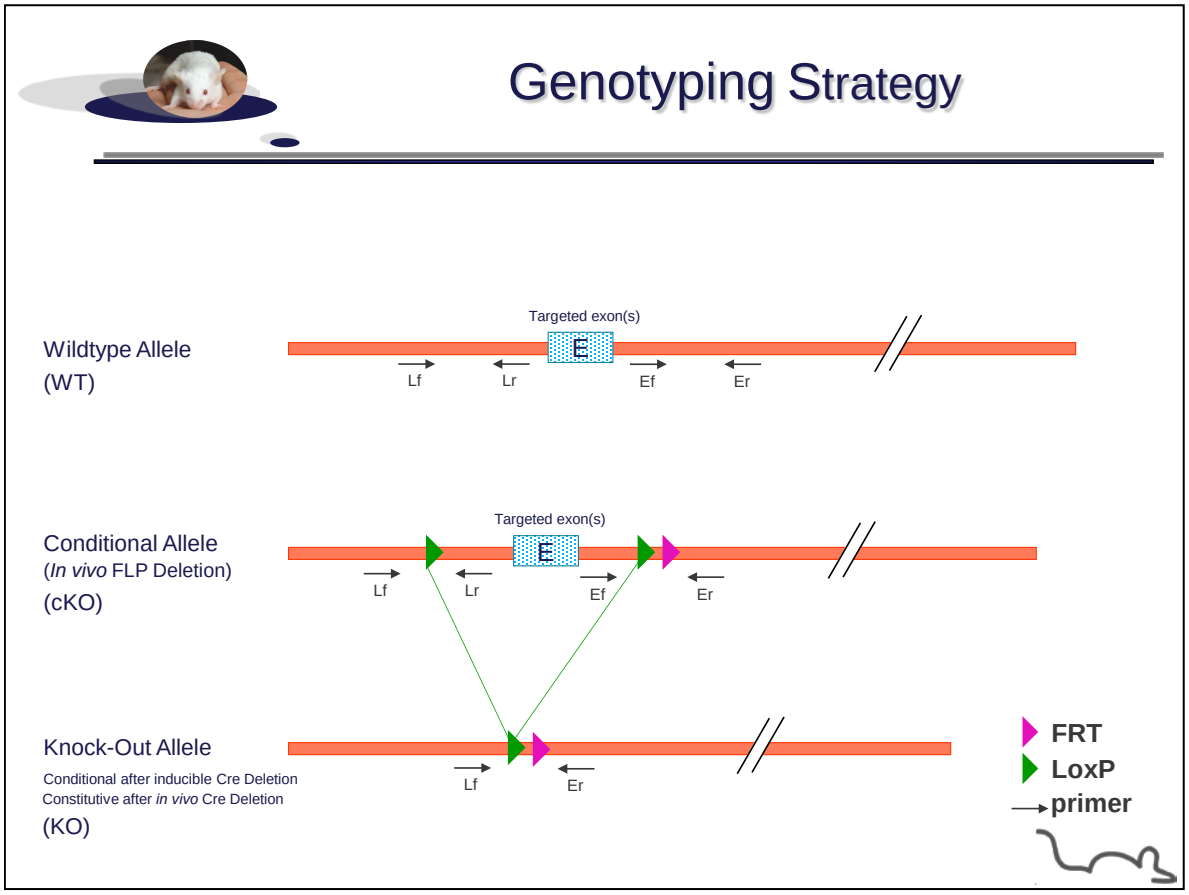
4. Data on conditional and knock-out animals

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

4.1. Genotyping protocol and data

4.1.1. 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
Lf	840	GCAAGCTGGATTATCTCAGAGCTAAG
Lr	841	GGCGGTCCCATCTAAAGTATGATTCC
Ef	617	AATTCCAAGTCTCGTTTCCTGGGGG
Er	619	ACAGCATGCGTGTTGAGAGCTGTC

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	840-841	Lf / Lr	285	---	238
Excision of the selection marker	617-619	Ef / Er	308	---	191
Total Excision (excision of the floxed exon(s), i.e. knock out)	840-619	Lf / Er	1544*	297	1381*

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
--- No Amplicon should be obtained

4.1.2.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:

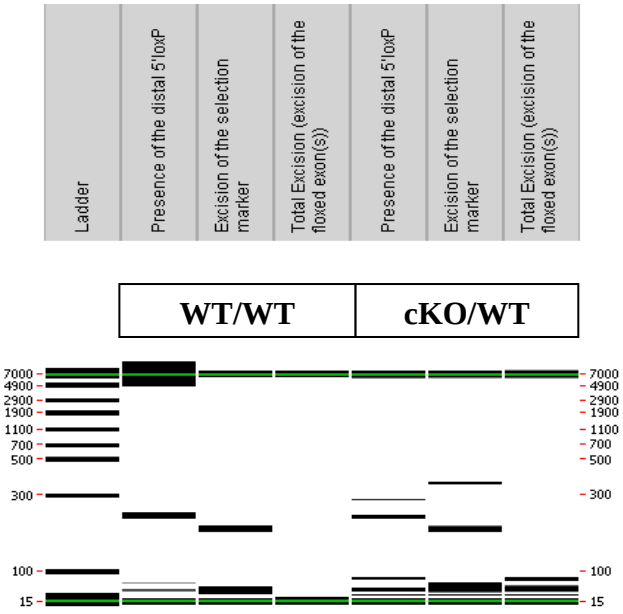
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

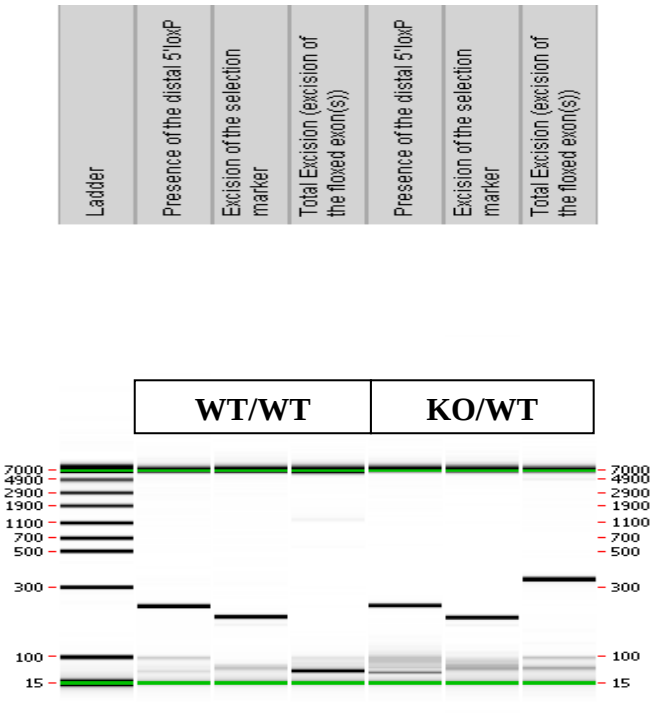
4.1.3.Picture of genotyping with various alleles

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.

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



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



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

4.2. Evaluation of lethality of homozygote KO (KO/KO)

Males knock-out heterozygotes (KO/WT) were crossed with females knock-out heterozygotes (KO/WT). Offspring was genotyped to evaluate the ratio of the different genotypes. Results are provided in the table below.

Genotype	WT/WT	KO/Wt	KO/KO	**Total**
Number of pups obtained	16	49	0	**65**
Experimental Ratio	25%	75%	0%	**100%**
Theoretical Ratio	25%	50%	25%	**100%**
Theoretical Ratio if KO/KO are not viable	33%	66%	0%	**100%**

The Esrrb knock-out homozygotes are not viable.

Legend:

- >13% Homozygous = Viable
- >0% and ≤13% = Subviable
- 0% = Lethal