Table of contents

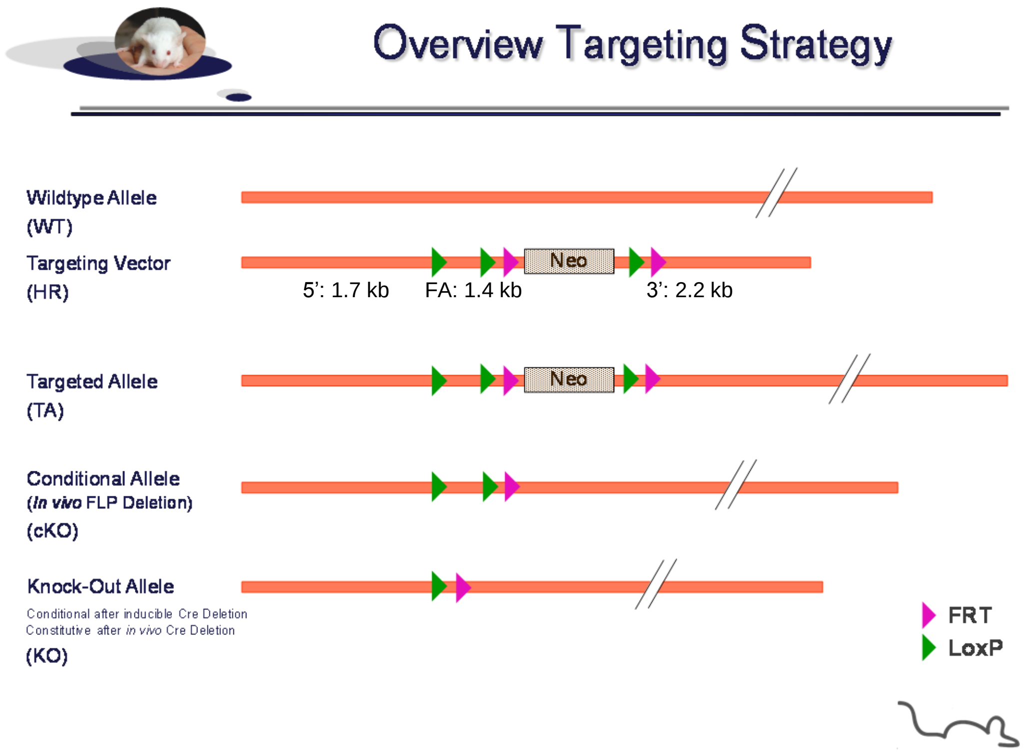
For any question, please contact:

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

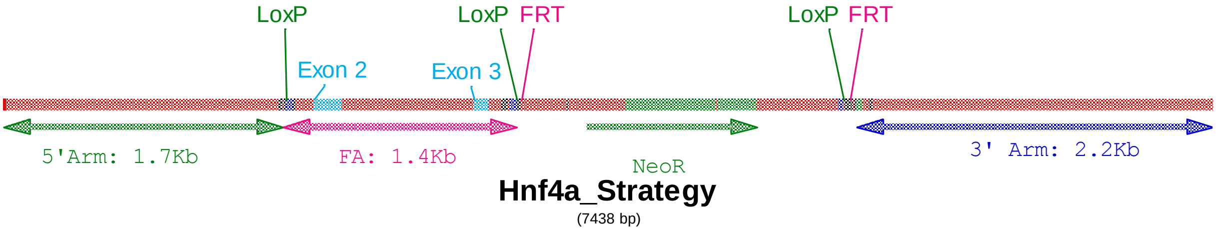


Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 2-3

Nr2a1 gene (also named Hnf4a) 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=35577

Strategy used to generate the conditional knock out model

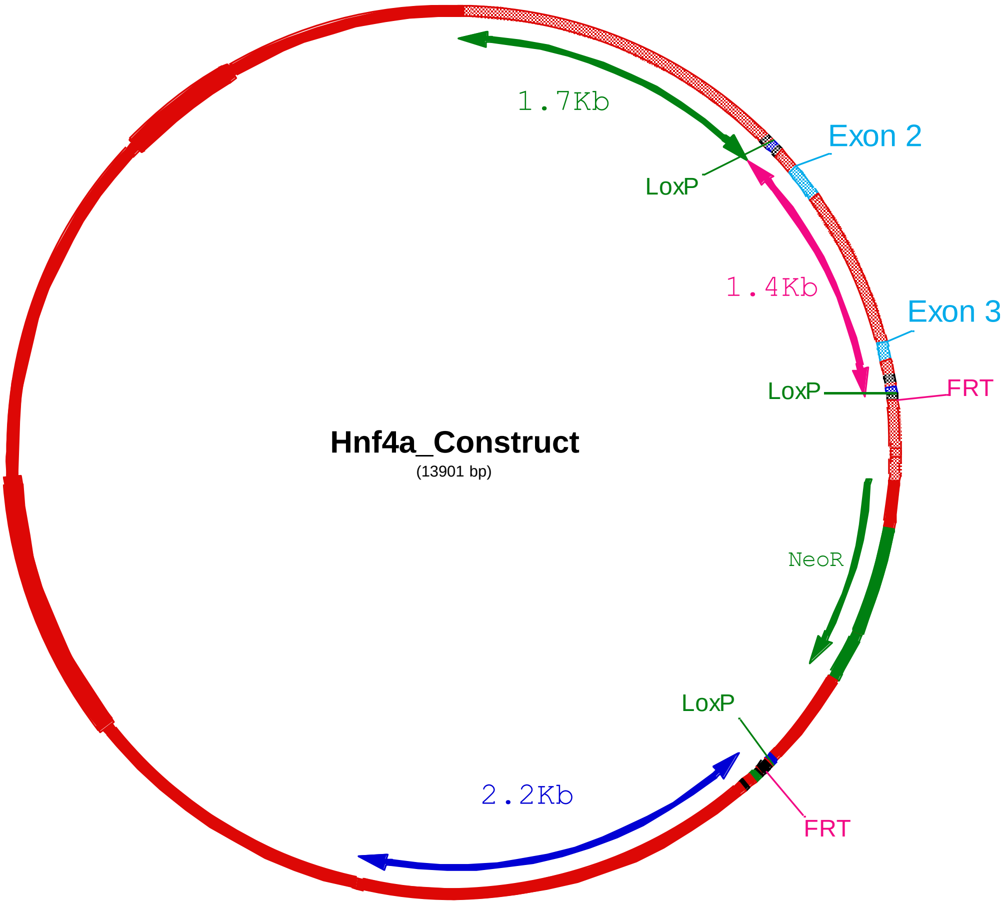


2. Construct used for homologous recombination in ES cells: Nr2a1 project

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

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (1.7 kb)

AAGGGTAACATGGTTAGGGCACAGTATACTAAGCCATAAACCCAGTCCCTCTGCCTGGAAGCAGTGTAACGATGC
TGGACAAGTCCCTTGTTCTCTTTGAGCCTCAGTGTTCTTAACTGGGAAATGGAAGTGGCCTGCTGACCTCAGGGG
GCCTATGCTGCCCAGCAAGGGGACACTTGCTGGCCTCTGCCCAAGTTGCGGGCTCAGCTCTCCCTGGGGGTCAAG
GGAGGAGAGGCTGAGGGAGAAAGAGCCAATGAAGCTTAACAATTATCCCCGCCTTGGGAATAATTCACCAGCCGG
AGGGGGCTCCGCTCTGAACACTCAGAAGTCCTAGGGATGGTTCAGCCAGAAGACAGCAGAAATGAGAAACAGCTG
CCAGACACCTGCTGGGGGAGTTCTCTTCTGCGGAGAGGTGTTTAAATCACAGATAGTTCCAGAACTTACTGTATG
CCAAGCACTTCTGGCTGACCGACTATGTATGGACAGCTCCGGGAGATGATGATGCTCTGTCTGACAGGTGGAGGG
GGGCAACGACAAGAAATTGCCCAGTGCCACAAGAACGGAGGGCATTTGATCCAATTCTATGACGCTTAAGAGTCT
CTGCTTCAGTGTACCACTGCCCTCTCCAGTTTGCAATAGGACATTCCCCTAGGAACCCAAGGGCCAAGTTTCTAT
GGGAAATGCTCCCTCCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATTGCACACATGCATGGACTT
TTGTTTTGGGAGGAATAATTCTAGAGTTCAGTCATCTCTGAAGGGTGGTTTTTGTTCTCTTCCTTCCTTCCTTCC
TTCCTTTCTTCCTTCCTTCCTTCCTTCCTTCCTTTCTTCCTTCCTTCCTTTCTTCCTTCCTTCCTTCCTTCCTTC
CTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTTCAGACAAGGTCTCAGGTAGCCCTGACTGACTTGGAACTTACTA
TTTAGCTAAGTCTGACCTTGAACTCTTTCTTGATCTTCCTGCCTTTACCTTCCAGGTGATGAGATTTTCTAAAGA
GTTTATATGTGGTGGGAGATTTGCTTTTAGAAAAAGTATGATCTTTTGATATATGTGTGTGTTAATATATATATA
TATATATATACACACACACACACACACACACACACACACACACACACACACACACACATATTTCTAAAACGAGCC
AAACCAGTTAAACGGATTCCTTTGCAGTTGAGGAGACTGGGGCTAGGAAACAGACCTGGTCTAAGACCAGAGGTG
AGTCAAGGGTAGCACGAGTCCAAATGCCCAGGGCTTTTGGCACACAAGTTGAAATCCCCTGTGCCAGAGGTTTTG
AGTCTGTCCTCTTCACACTCAGATGGGGAGATGGCTTCTGGAGATGGTGGTAAGAGGGTCCCACATCTGCCCAGA
GGTGGCTGGCAACTCATCCACCCCAGAGGCAAAGAAGGAATGTGCTTAGCTTATATCCTTCCGCTCTCTGGGAGG
TTCTGTGGAGTCTGAAGGCTAAGCTCAGGTCCCTCTGGTCCAGTTTTTCTGATGACAAAGTGGCTGGGTAGTCAC
TGTCCCCATAGCTTCTAACGAGGAGTCTGTAAATGCCTGTGATGATGAGGGCTGCACCCCTTCCTGAAACAAGCC
CTTGCTGGTGCCCCCAGGAGATCAGCAGTACAGTTGCTTCTGATTTATAGACCGGAAACTGAGACTCCAGGCCGG
GATGGCC

2.4. Floxed fragment (1.4 kb)

GACCTGCAGataacttcgtataatgtatgctatacgaagttatGCGGCCGCAAAGCCATCTGACACTGAGAGGAA
CAGGGCATAGCCCCCAGCTCTGGTGGTCTGCTCTGAGTCTCAGGCTTGTTTGTGGTTTGTTTTACCCTCTACCCC
TCCTGGAATCTCATTTGCCCTCTTTTGGTGCAGACACGTCCCCATCTGAAGGTGCCAACCTCAATTCATCCAACA
GCCTGGGCGTCAGTGCCCTGTGCGCCATCTGTGGCGACCGGGCCACCGGCAAACACTACGGAGCCTCGAGCTGTG
ACGGCTGCAAGGGGTTCTTCAGGAGGAGCGTGAGGAAGAACCACATGTACTCCTGCAGGTGAGGAGCCAGCTCGG
GCCTTAACTTTCTCATCTGGGATATGGGTGTTTTGGGATTCATGTCCTCTGAGGGTCCATCTTGAGGCATCTGCC
CTTCCAGACATTCAAGGCCAGTCTGGTTTCTTAGCATCTGCGGATTCAGATGTGAGCAGCTGCTTTTCCAACTGC
CTAACAACCTTAGAGAGGATAGGACCAACCTCCAAGCTGATCCTTTTCTAGGTTCTAACAAGGCATTGGCCCTAG
TTCCTATTTCAGTTGACTGAGCCATTGCTCCATGGCAAGCCTGTACTATAATGTCGTCACCTGCCAATAACCTTC
TGACGTGGTATCCTCGTTTTATGCCTTGGAGGGCTAAGCATTTACCTGAAGCCAATGAATGCTGGAGTCAGTACT
GAACTCCATGCCCCCCCCCCACACTCCCATAACCTTGCCTTGACCTGTTGTGGACCAACTACTGACTCTAGGCC
ATAGCCAGGAGCATGCTAAGGGCTGTGGGCTCTCACAGGATATGGTAGAAAAAGCTGGTACCCCAGACACTAAGG
TTTTCCACTCAGCTCCACTGCCAACCAGCTGAGTGAGGAAAGTCACATGCCACCTTTCTGGGCTTCAGTTTCCTA
TGTCAAGGGGACAAAATGGACTTTCAGACAGTCCACAGACCCCTCTGGCTTCCGATTAACATTAGCACTCCTGGG
GTGCAAACATGGAGCAGGGAGGCTGGGGAGGGGGTGACTGACAGCCAGGACCTGATTCTGTCCTAAGAGAAAGTG
GCTGTTTCCTCCTCCATCCAACCCTCCTATGTCCTCTCCAGGTTTAGCCGACAATGTGTGGTAGACAAAGATAAG
AGGAACCAGTGTCGTTACTGCAGGCTTAAGAAGTGCTTCCGGGCTGGCATGAAGAAGGAAGGTGAGTCCCAGCTC
CCCGCCTGCCCCACCTGCACGCAGGCTCCAACCAACCCCGCATTTACAACTGTAGCCATACTTTATGGCTCTGTG
ACAGGCCCTGGGATGTCGACGCGATCGCGGACTAATGGCCataacttcgtataatgtatgctatacgaagttat

2.5. PGK-Neo region

gaagttcctattctctagaaagtataggaacttcgctagctcataaaaaatttatttgctttcaggaaaatttt
ctgtataatagattcataaatttgagagaggaggcgcgccgaattcctgcaggattcgagggcccctgcaggtca
attctaccgggtaggggaggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttg
gcgctacacaagtggcctctggcctcgcacacattccacatccaccggtaggcgccaaccggctccgttctttgg
tggccccttcgcgccaccttctactcctcccctagtcaggaagttcccccccgccccgcagctcgcgtcgtgcag
gacgtgacaaatggaagtagcacgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggt
aggcctttggggcagcggccaatagcagctttgctccttcgctttctgggctcagaggctgggaaggggtgggtc
cgggggcgggctcaggggcgggttcaggggcggggcgggcgcgaaggtcctattgtgagcgctcacaatcccggc
attctcgcaagcttcaaaagcgcacgtctgccgcgctattgtgagcgctcacaattccgggcctttcgagaagga

gccaatatgggatcggccattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattc
ggctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccg
gttctttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctg
gccacgacgggcgttccttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggc
gaagtgccggggcaggatctcctgtcatctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatg
cggcggctgcatacgcttgatccggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcacgt
actcggatggaagccggtcttgtcgatcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactg
ttcgccaggctcaaggcgcgcatgcccgacggcgaggatctcgtcgtgacccatggcgatgcctgcttgccgaat
atcatggtggaaaatggccgcttttctggattcatcgactgtggccggctgggtgtggcggaccgctatcaggac
atagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccgcttcctcgtgctttacggt
atcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgaggggatcgatccgct
gtaagtctgcagaaattgatgatctattaaacaataaagatgtccactaaaatggaagtttttcctgtcatactt
tgttaagaagggtgagaacagagtacctacattttgaatggaaggattggagctacgggggtgggggtggggtgg
gattagataaatgcctgctctttactgaaggctctttactattgctttatgataatgtttcatagttggatatca
taatttaaacaagcaaaaccaaattaagggccagctcattcctcccactcatgatctatagatctatagatctct
cgtgggatcattgtttttctcttgattcccactttgtggttctaagtactgtggtttccaaatgtgtcagtttca
tagcctgaagaacgagatcagcagcctctgttccacatacacttcattctcagtattgttttgccaagttctaat
tccatcagaagctgactctagatctggatccataacttcgtataatgtatgctatacgaagttatctcgaggaag
ttcctattctctagaaagtataggaacttcaaggtcctcgctctgtgtccgttgagct

2.6. 3’ homology arm (2.2 kb)

GGCCAGCTAGGCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAAGGTCCTCGCTCTGTGTCCGTTGAGCT
GGCCAGCTAGGCCGACTGGCTAATGGCTGAGAGGAGGGAGGGCCTGGAGATCTGACCACAGGGAGTGGCTGGGCT
TGGTCTTGAGAAAGATTCAGAGATAAAGCTCCCACTCACCCACTTCAGCTCCCAGCAACTGCCACTGGGCCTTCC
TGTGCTACCATGAGGCGGCCAAGATGGGCGCTCTTTCTAATGGATACCGTGAGCATTAGGGGAAAAAAAAAATCA
CAGAACAGGACACAGCAAACACCCAAGGCCTGCCACAAAGGCTTCACTGATATGAACAAAGGATCCTACTATGAG
TTTCCTGTCCAAAGACAAGAACCACAATCCCCTTAGTCCTCATCGCTGGGTGTCAGGTCATCTCTAGAAATTTCC
CTCTCCTAACAGTGGACTGAGGCATCTAAGCCAGCCTGGATGGTGGAGATGGAATTACCCAGCACAGTGCCCTTT
AACTGCCCTGGCCAACTTCTGCATCAGACATGGAGCTAGCTCTTCCTTGTGTCCTCTCTGAGAGGCTGGAATGAG
TCCTGTCCCCCTCTATGGCAGAAACAACTGAGGACCCTAGCAGTTAGTCACCTTGCTGAAGGTCTCAATCCAAAC
ACAGTGGTTACTGGAAGTGCAGAGGTCCCAACCCCAGCTTCATGCTGTGTGGGCACCTCAGTGCTGCCGATGCTG
TGACACCCCCCCACCAGGCCTCATTTATGACAGTTTTCAGTAACCAGAGCGGGAGCATGGTAGTAGAGCCATCGC
CTGGCAAACATGCAGAGCCCTGGGCCACTCCCAGCCTACCGGGAGAATAAGAAAGAAAGAGAGAGAGAGAGAGAG
AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGAAAGAAGGAAGAAAAGAAGAAAAGGAAAAAAATATTTTTCCT
CAAGCTCAGAAATAAAAAACAAAACACTTTAAACTTGTTTTTTTCTAAATAACAAATTCATTTTCAAACCTCTCC
CCTTTCTTCTCTTAACTTACTTTTCCCAACTAGCATGTACCCCTCTGGCCAGACTATCAGACACACACATTCTCT
CTTTCTCTCCCTCTCCCTTTCCCTCTCCTCCCTCTCTCTGCCCCCCCCCCCACACACACACTCTGAATCAAGAAC
CTGGGACCCAGCTCATGCTGGGCTGAGAGAGACAGAAGGCAACGTAAAGGTGACAGCCTCCCTGCTCAGTTTCTG
CCCTCCATCCTGAGGTCACTCTTCCTTTCTGTCCCTCAGAAGGGTCCTTACAGGCCTCTGCTTCTCTGACATAGG
GGCCTATGCCCCTCCCCTTCCCCTATGGCTTCCCATGGGCACAGGGCTCCCTGGCTAACCTCGTCTCCTCTTTGA
AGACTCCTTTGTGGAAGGGGAATGTGGTGCATGTGTGAAATATTGGAGACAAGATGAAGGATTCCAGCCTAAGGG
TTTCCTATTGTACGGTTCTAATAGCTGGTGGTTCTGTTATCCCATCCCCTGAGATAATAAGCACTCAGTGATTAC
CCATAAGCGTTGTGTGTGAGAATGAGTCTCTCCTATAGAATTCTCAGCAGGTGGGAGAAAGGCAGGCAGTCCAAT
GGGGCTAGCTAGTAAGTCTGTATGTGGTCATGTGTCTTTGATTAGACAGTTTCTAGAGGGAAACCGAGGGAAGCT
TCCAGCCCCTCTGGGCTCTACTTTTCCAGATGACCCACAAAGCCAAGCAACCCTAGATCCTAGCTCCTGAGAGAA
GAGAAGTCTCACACCATCCAGCCATAGCTCAGCCTTGAAACGAACTGCTTGCTTCTTTGGGCTGAGAAATAAATC
TGCCAGGGGAACAAGAGAGCCACTGAGTCACTGGCAGACAGTGCTGGCCAGTAAACACTCGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTCCATGCACATGTGCATTATTGAGAAATTGTGAGGTACAAGTAGACCAGAAGAGC
AAAGCATTCTGGGAGATGCCTTGGGCAAGCACTAGCAAGGTTATTGCTAGATCCAACTGTCCCCAGAACCCTTCC
TCGGTTTGATGGGAAAATTCCCAAAGCCACTGACCAGGTGACTGGGGACATGTTTTCATTTATTTTCCTGTCAAT
GTCTCCTGCACATGGACCTAACAAACTGGGCCTCAGTGGCC

2.7. Vector backbone sequence

ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT
ACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATA
GTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACAT
CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAG

TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTG
AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAA
TTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGG
CGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCG
GCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCC
GCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG
CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCT
TAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGA
GCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGT
GTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGC
GGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCT
CCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCG
GGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGG
CGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGC
GAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCT
AGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGC
CGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGG
GCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTC
CTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTCACCTGCCAGACCATG
CCAAAAAAGAAGAGAAAGGTCATGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAG
ACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCG
GAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACC
TCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGC
GTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGC
GTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTT
CCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGA
CTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCG
GCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGC
GACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTG
GTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATC
TCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTT
CGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTG
TTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC
GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGAATTCACTCCTCAGGTGCAG
GCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCCCTCT
GCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATT
GCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAG
AATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGA
GGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATT
TTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGA
TTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATCCCTCGACCCGAGAATT
CGGCTTAACTTATCGATGATAAGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATT
TTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAAC
CCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCA
ATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTG
CCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGG
TTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAG
CACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT
ACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG
AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACC
GAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAA
TGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAAC
TGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACT
TCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTAT
CATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTAT
GGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTA
CTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAA
TCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATC
TTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTG
TTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGT

CCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAAT
CCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA
TAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACT
GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAG
CGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG
GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAG
CAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGA
TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGA
GTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCG
CATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGT
GACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCA
TCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGC
GCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCC
AGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCC
TGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGG
ATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTA
TGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCA
CAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGA
CTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTT
CACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAAC
GACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGAGATGCGCCGCGTGCGGCTGCTGGAG
ATGGCGGACGCGATGGATATGTTCTGCCAAGTCAGCGTTTAAACTTAATTAAGTCGACGGCCGGCCCTCGAGGCC

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]

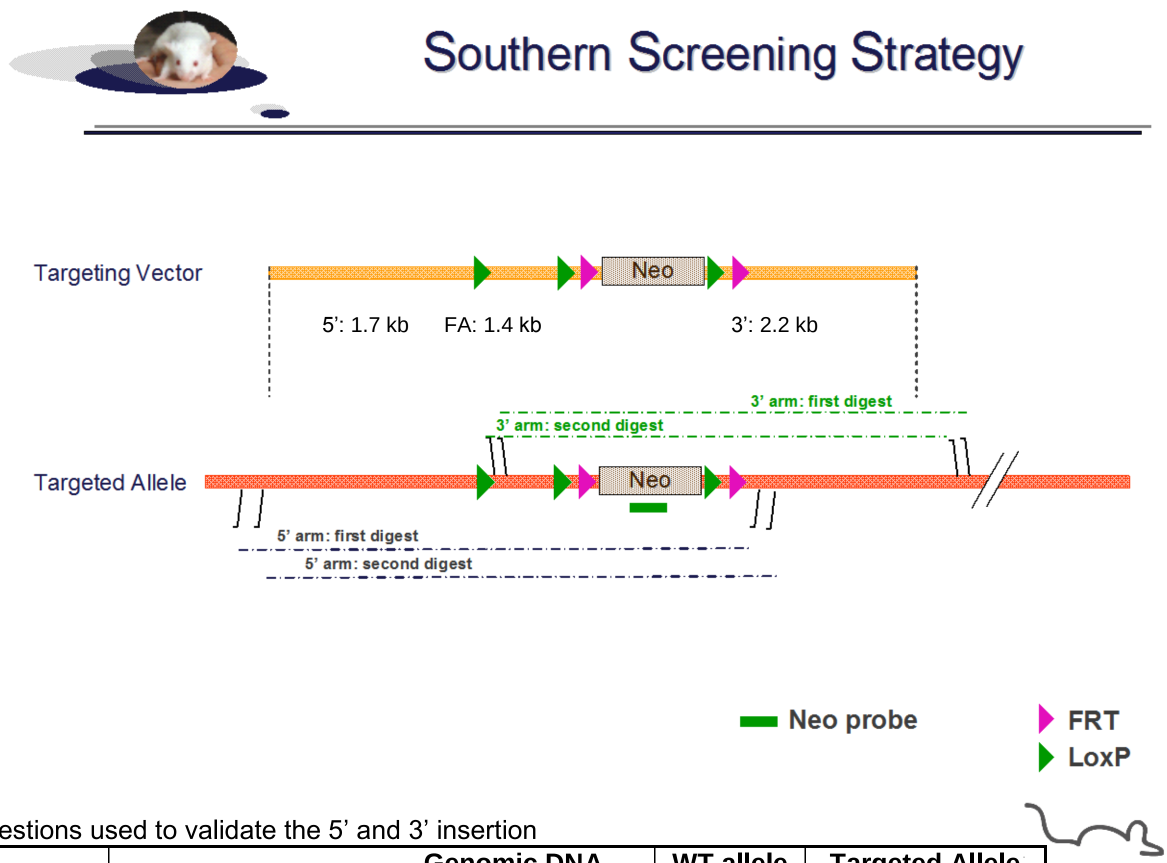
Number of clones screened: ~ 400
Number of positive: 1

Reference of clone used to generate the mouse line:

- clone **DG4-170**

3.2. Southern data on positive clone

3.2.1. Neo Southern strategy



Digestions used to validate the 5' and 3' insertion

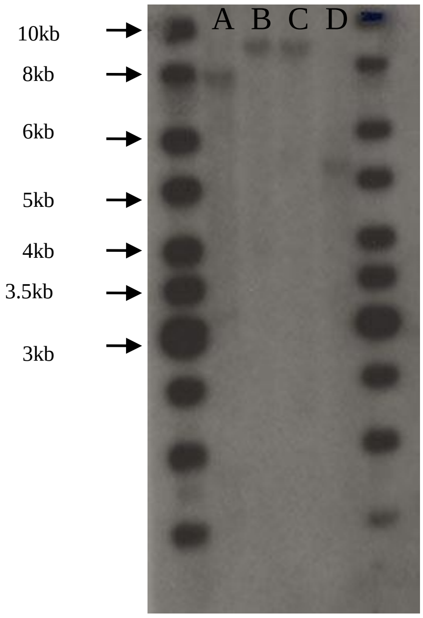
Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	EcoRV	/	8.0kb
	5' second digest	BamHI	/	9.1kb
	3' arm first digest	AflII	/	9.0kb
	3' arm second digest	EcoNI	/	5.4kb

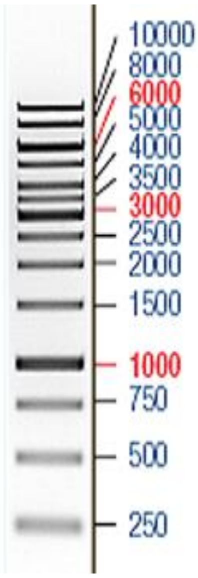
Four different digests are used to validate correct HR event. Two digests validate the 5' insertion, 2 other digests validate the 3' insertion

3.2.2. Picture of Neo Southern

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

A: EcoRV 8.0kb
B: BamHI 9.1kb
C: AflII 9.0kb
D: EcoNI 5.4kb



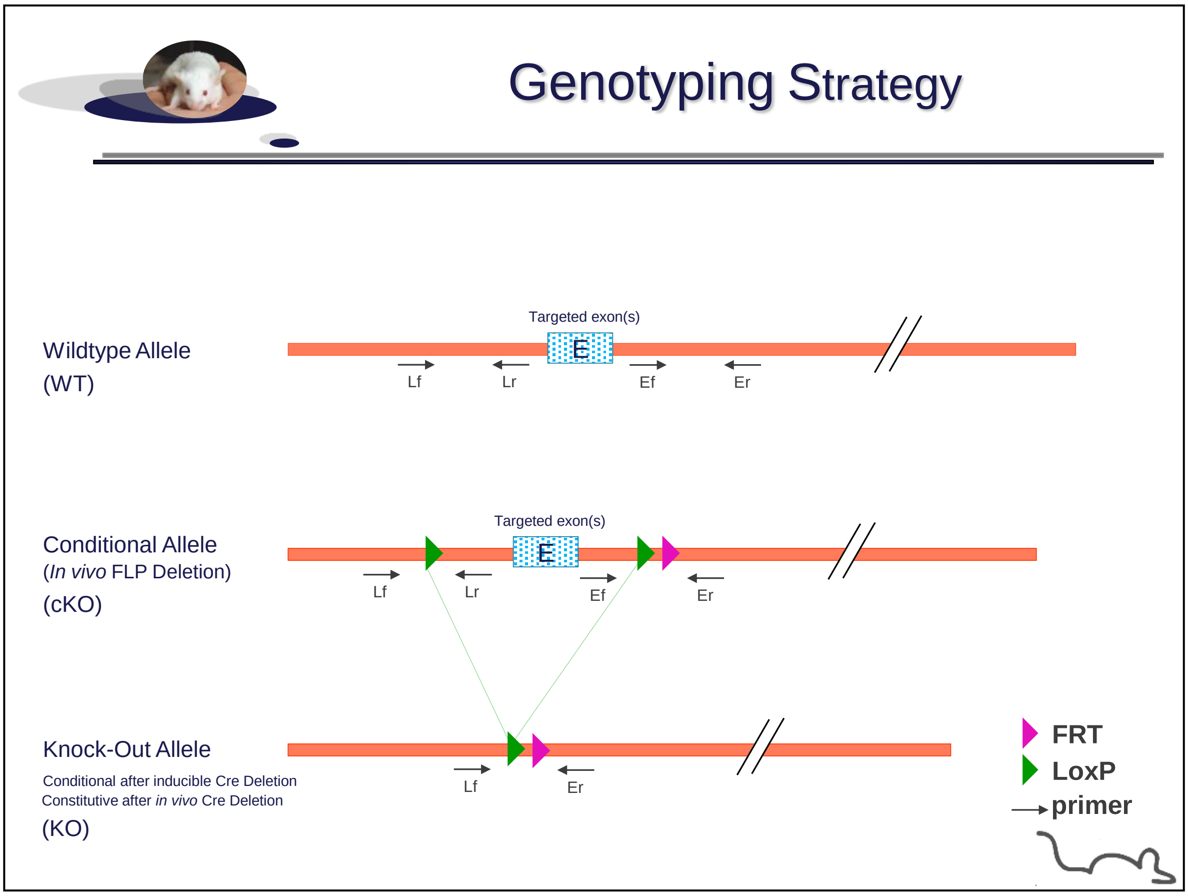


4. Genotyping protocol and data

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

Primers	Sequence
Lf	AGTCTGAAGGCTAAGCTCAGGTCCC
Lr	CACAAACAAGCCTGAGACTCAGAGC
Ef	GGTAGACAAAGATAAGAGGAACCAG
Er	GGACAGGAAACTCATAGTAGGATCC

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	18-19	Lf / Lr	342	---	288
Excision of the selection marker	20-21	Ef / Er	611	---	451
Total Excision (excision of the floxed exon(s), i.e. knock out)	18-21	Lf / Er	2009*	628	1820*

* 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:	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:

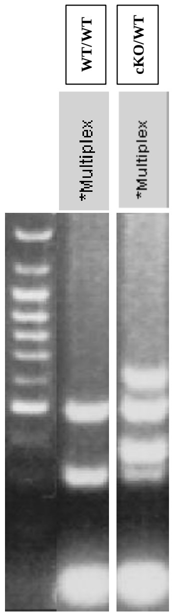
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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

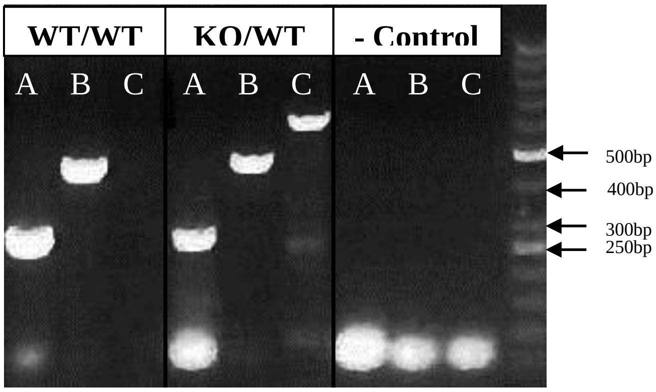
4.3. Picture of genotyping with various alleles

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

Analysis of both WT/WT and cKO/WT mice was done by multiplex: the presence of the distal 5'loxP and the excision of the selection marker were tested simultaneously.



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



A: Presence of the distal 5' loxP
B: Excision of the selection marker
C: Total Excision (excision of the floxed exon(s), i.e. knock out)