# Molecular Biology Data
# Nr3c1 conditional knock out model

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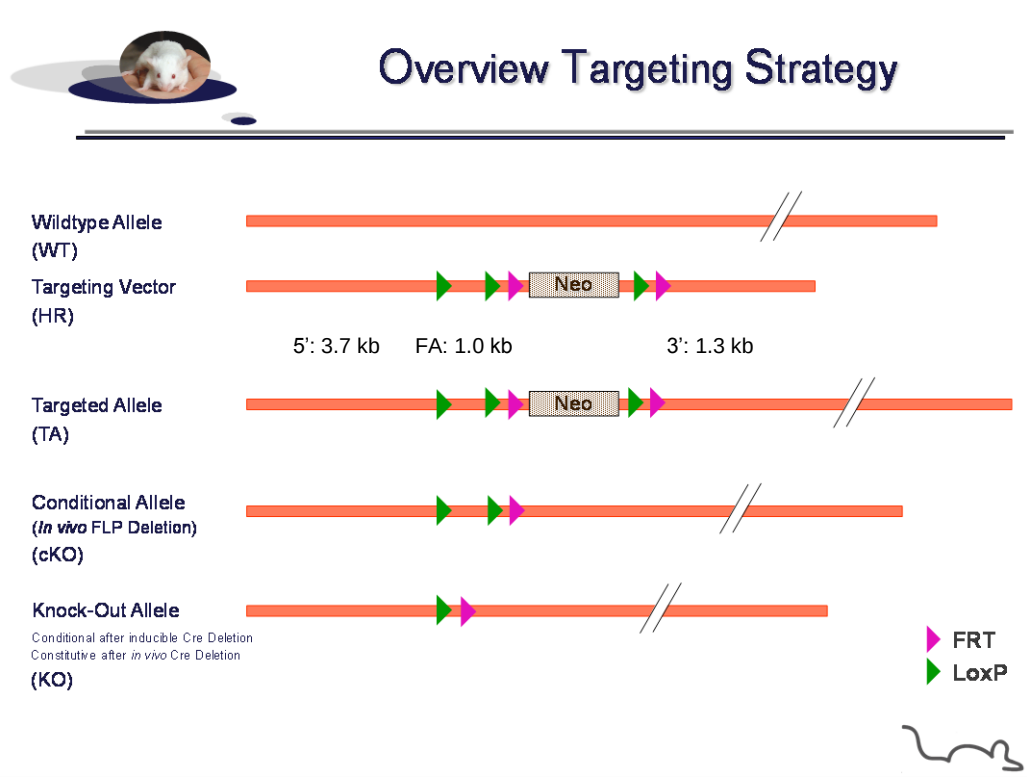
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

### 1.1. Overview

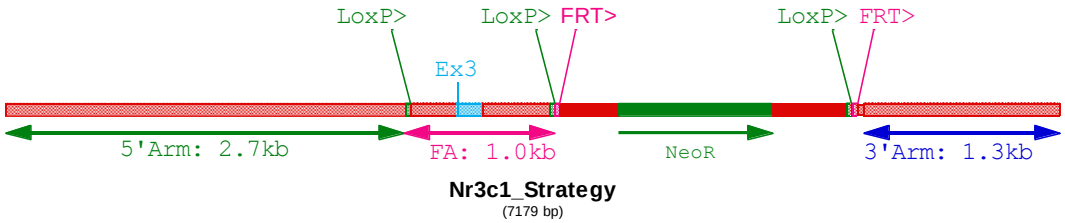



Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

### 1.2. Strategy chosen: flox of exon 3

Nr3c1 gene (also named Grl-1) 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=9277

**Strategy used to generate the conditional knock out model**

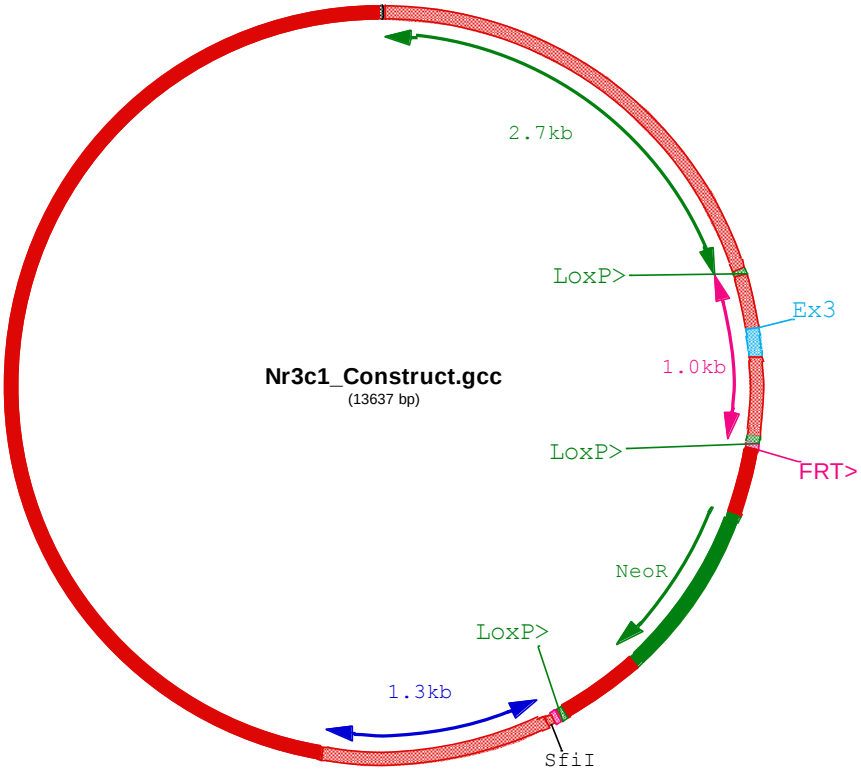


2. Construct used for homologous recombination in ES cells: Nr6a1 project

### 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 (3.7 kb)

**GCCATCTACATAGATCACACCTCAGTGACCTGAACATAAGTTTCCATATATCCTAATTTCTATGTGATCATTTGG**
**GGTCTACTGTCCTAGATTTAAGACTTAAGATTTGTTTCCCTTAAGAAAATAGTGCTGGGCAGTGGTGGCTCACAC**
**CTTTAATCCCAGCACTTTGAAGGCAAAGGCAGGCGGATTTCTGAGTTTGAGCCCAGCCTGGTCTACAGAGTGAGT**
**CTACAGAGAAGCCCTGTCTTGAAAAAAAAAAAAAGAAAAGAAAAAAAGAAAGAAAATAGTGAACTTGTATGTTTC**
**AATTCTTTGCTGCAAGTACTAGACATCTAAACATGTTCACAGGTGACACTTCTCAGCTTCCTGATAAAAACTAGA**
**GCTCATCAGAGCAGATGATTCCCAAAAGCTATAGGACTGTCAAGTGGGACTGCCCAGACCAAAACAAATCAGGAT**
**TCTCCCTGGTCAGCCACGTGCTACTGTTTACATGACCTCAACTAGGGATGGTCGCATCTTATCACAAGGTTGTTC**
**AGAGGATAAATGAGATTCCCTTATGGAAGTGTTCTGAAACCATGATGTACCTAAATACTGTTTAAAAGTCAAATA**
**TATACATATATTTTATATATGTGAATATTATGTTTAAAACAGGCAAACGAAACAATATAGAATT**GACCTTAAAAA
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ACTGTTGTGTGTATGTTTCACATGCATGGGAATATGC**AAATATGTAGAGACCAGAGCAGCACATAGGGCATCTTC**
**TATAGCGCTCCGCCTTTCTGCCTTGAGACAGGCATGAATGGTAACCTCACTGTCTGTTTGTGAGCTCTTCATATC**
**CACCTGACT**

### 2.4. Floxed fragment (1.0 kb)

**ggccgggatggcc**ataacttcgtataatgtatgctatacgaagttat**CTCTTCCTCAATGCTGGGATTATAGGCA**
**TGCACAATTACGGCCTTCTTTTTAAGAAAGTGGTAAAGATTTGAACTTATATCCTCATGCCTGCTAGGCAAATGA**
**TCTTAACCACTGTACCATCTTGTCCATTTAATGCATTTCATATTTCATACAGAAAAATAGTATATTCCAATATAT**
**TGAAATCAGGTATTGGTGCTTGTTAGCACTTAAAAGACAGAATTCAGTGTGAACATGCTGACATGGAATGAGAAG**
**GGGGAAGAAATATATTCTGACATTCCTTTCATAATCATGCCAAGCATAACCCTGTTTATGCTTCCTATAGCCCTG**
**GAATGAGACCAGATGTGAGTTCTCCTCCGTCCAGCTCCTCCACAGCAACGGGACCACCTCCCAAACTCTGCCTGG**
**TGTGCTCCGATGAAGCTTCGGGATGCCATTATGGGGTGCTGACGTGTGGAAGCTGTAAAGTCTTCTTTAAAAGAG**
**CAGTGGAAGGTAGTGTGTGTTTTGAAGAGTTTAATTTTTGTTCCTATTTCTCACAGTAAATGTTTAACTGTTCAT**
**TGTATACAAAGTCCACTAATGATAGACATTAGTTCAGGTGCAAGCATGGTGGCTCTTAAATCCTCCATTGGGAAG**
**GGACAGAAGTAAGGAGAAGAACTAGAATAAGTAATTAAGTTCAGCCTGAGAAAGAACTAAAGCAAGCATTGTAAG**
**GAACGCTGCTGCACGGACTGGCAGTACTCTGAGTCTGGGCAGTCCGAGAATGGTGGCTTTTCATTCCCTGTAAGC**
**AAGCTGGCTTCAAAGCTACCAGCTATACCAGACTGACAAAATCAGTGACCCTGGGGAGGAACAATAAGGGAGGGA**
**CAGCATTCTTTGCTTTACTTTTTTCCTGTTAAATAAATAAAGAGGTCATGTTAAGTACCACAATGGCTGggcctg**
**tgtggccatcgcggactaatggcc**ataacttcgtataatgtatgctatacgaagttat

### 2.5. PGK-Neo region

**gaagttcctattctctagaaagtataggaacttcgctagctcataaaaaatttatttgctttcaggaaaatttttt**
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### 2.6. 3’ homology arm (1.3kb)

**AGGCCAAATTAACAAAGTACAGAGATTATCAGATAGGTGAAGTCTAGAAATATGGTGTTTGAAAGAAGATGACTG**
**CAAGGTGATCATGTGTTGACGGGTTCACATTCCAGAGCAGCCGGTTAATATTAGAGATAGTTTATATACCCTACC**
**CAAAAGTATCTTAATGGAATTAAACCAGAAAATAAAGCTACATTGATCTTTCAATATACTACAATTCACAGCCCA**
**TTGTTCTTATTTAAAATGAACTCATTCCCTTATTTTTATGTAAAAATAAGTATTAGCTATGCTATTTATAAAATG**
**TTTACCATTTAGTTATTTCAGCAAAGCGTGGTGGCTAGATTACATTAGTAAGGAAGCCTTTAGCTGTGTACGGTG**
**TTAATGAGTAAGCCTCTTGGCTCAGCTTCCTCCCTAACTTGAGTTTCAGTGTTCTCAAAGGCACTTTCCAGCTCT**
**AACATTCTGTAAACCCTTGATACTCTAATTCAAGGAAAAAAATTTATCTAGACTTGTAAAATCCATAA**CATAGTT
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**CCAATGACTCTTCACTTGCCGTGTGTGAAGTACATGCATCCAAAATCAATTAGTGGCCCTGTGAACACTTAGATC**
**CTGTTAGGATCTGTGTCTGCCAGACTAGCAAACAGGTTCAGAAAGTTGAAGTAGCCAAGCTGGGATTGCAACAAA**
**GTGGTCTCCAAGTAAGGTCTTATTCTCAACAAAGATCCTCAAAGATGCATCTGACAAGCCCTTGATACCTGCTAG**
**ATTATAATTCAGGTAGATGCTGAAATAAAAGTAATCACAAGAGATTTAACTATTAGCTACTATATACTGAGTAGT**
**TATGGTGTGTTGAGCACAGTATCGTCTGCATAATTACTTCCAAGCTTGGCAAATTTTCTAACAGTTCATAAGAGA**
**ACAAACTCCAAAAAGTTAATGTTTCCAAGATCAGTTTCTTGGCATGCCACTATACCACTGCTACTGAGAACTGCT**
**ATAGAAGCGTAAGCTGATAGTATTAGTTTATACTTAATCTCCAGGTACTCACAGTACAGTCTGTA**

### 2.7. Vector backbone sequence

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cgaggc

## 3. ES cell lines targeted and validation data

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:

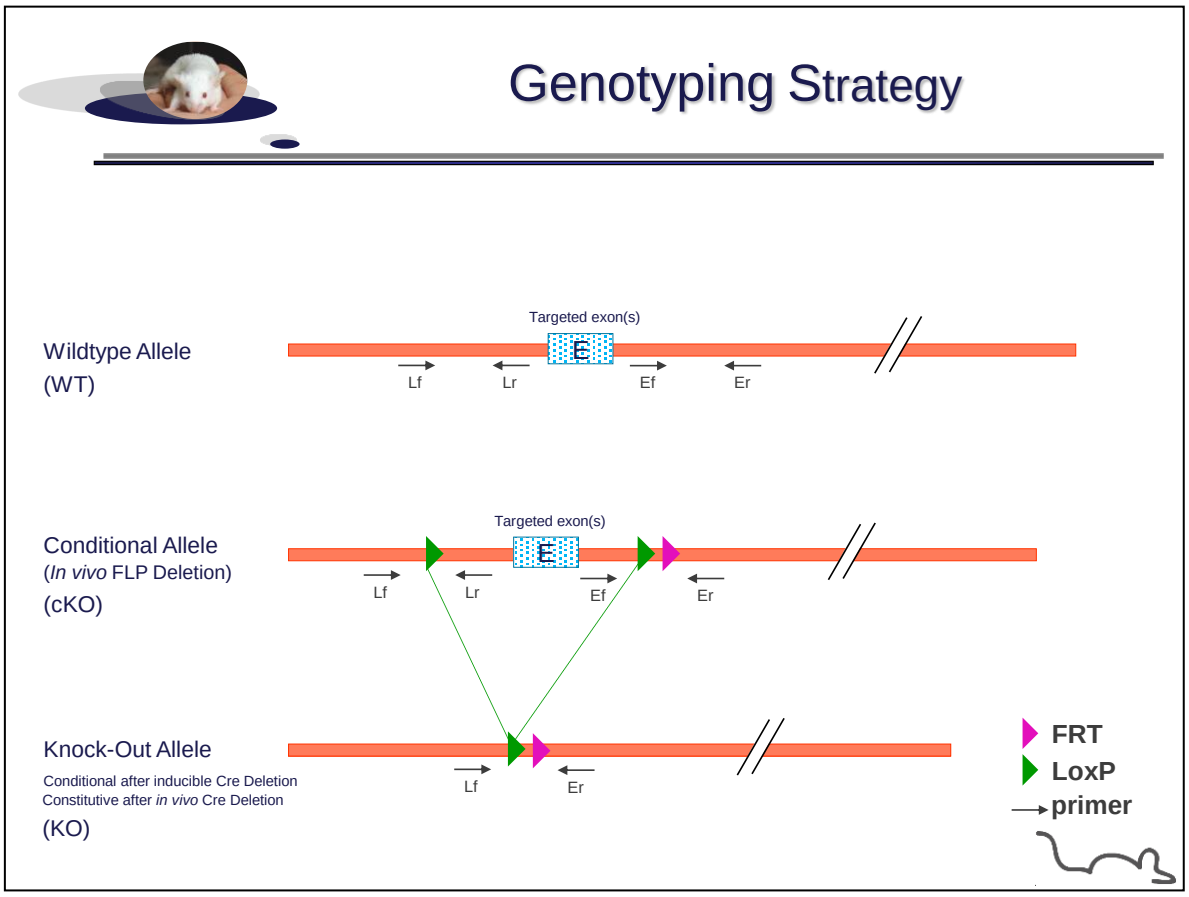
- clone **K165P1-139**

## 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 | 55 | CCAGAGAACTAATTGGCTCTTGCAC |
| Lr | 56 | AGATCATTTGCCTAGCAGGCATGAG |
| Ef | 57 | GACTGACAAAATCAGTGACCCTGGG |
| Er | 58 | GTCAACACATGATCACCTTGCAGTC |

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 | 55-56 | Lf / Lr | 351 | --- | 304 |
| Excision of the selection marker | 57-58 | Ef / Er | 348 | --- | 209 |
| Total Excision (excision of the floxed exon(s), i.e. knock out) | 55-58 | Lf / Er | 1401* | 415 | 1215* |

* 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

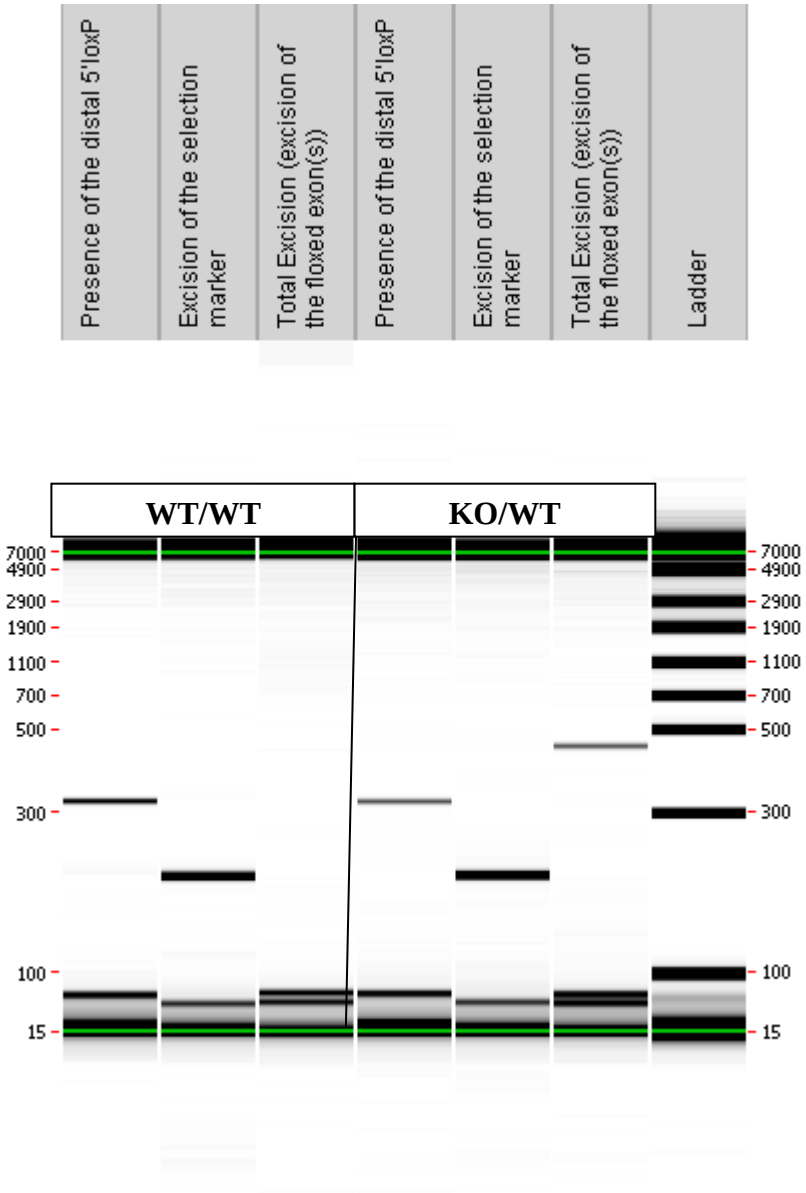
- Picture of genotyping with conditional knock-out (cKO) allele

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.