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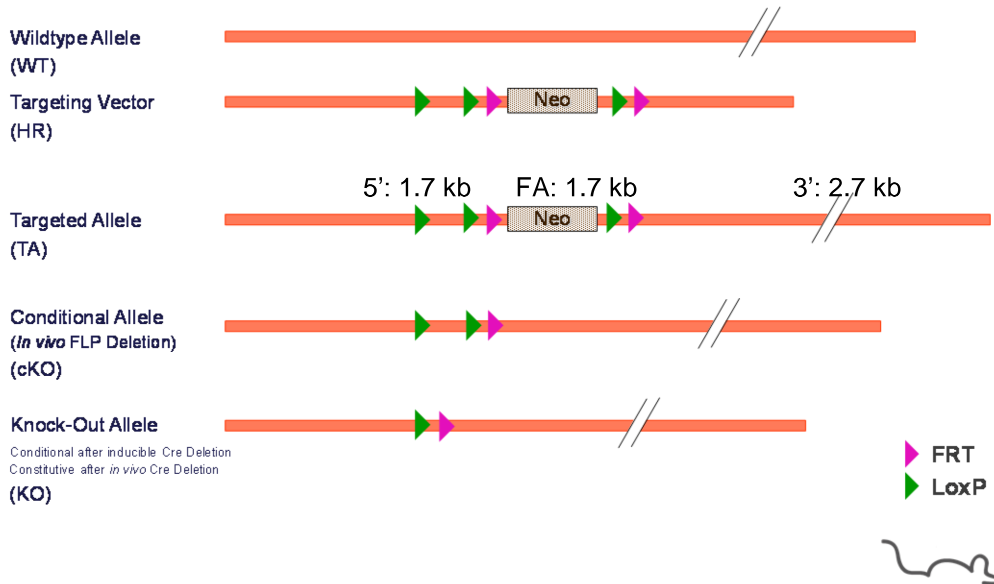
This protocol has been prepared by Loic Lindner, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

### 1.1. Overview

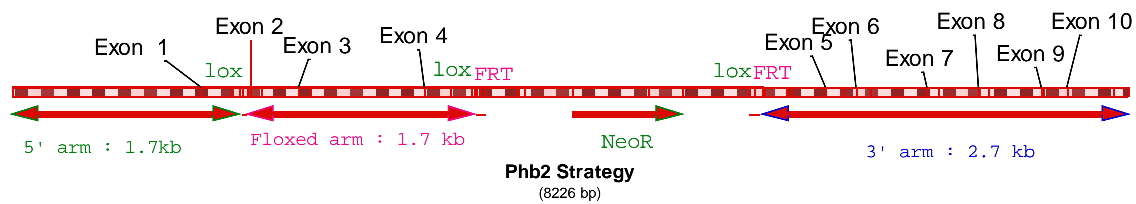



Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

### 1.2. Strategy chosen: flox of exons 2-3-4

Phb2 gene (also named REA) 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=36145

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

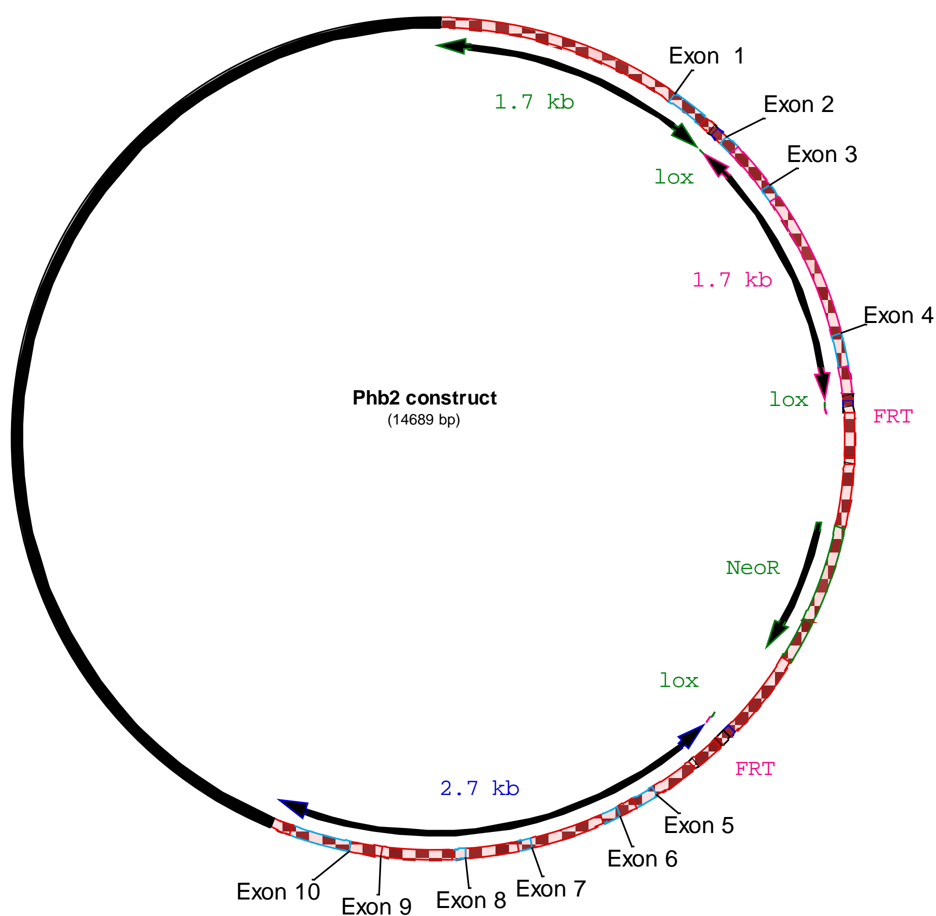


2. Construct used for homologous recombination in ES cells: Phb2 project

## 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)

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### 2.4. Floxed fragment (1.7 kb)

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### 2.6. 3' homology arm (2.7 kb)

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### 2.7. Vector backbone sequence

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tcatcaccgaaacgcgcgaggcagctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgc
ctgttcatccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcgggccatgt
taagggcggttttttcctgtttggtcactgatgcctccgtgtaagggggatttctgttcatgggggtaatgata
ccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaacgttgtga
gggtaaacaactggcggtatggatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgtta
atacagatgtaggtgttccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggc
gctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcaga
cgttttgcagcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaacccc
gccagcctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgag
atgcgccgcgtgcggctgctggagatggcggacgcgatggatatgttctgccaagtcagcgtttaaacttaatt
aagtcgacggccggccctcgaggcc
```

## 3. ES cell lines targeted and validation data:

### 3.1. ES cell lines targeted

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

Number of clones screened: 400
Number of positives: 1

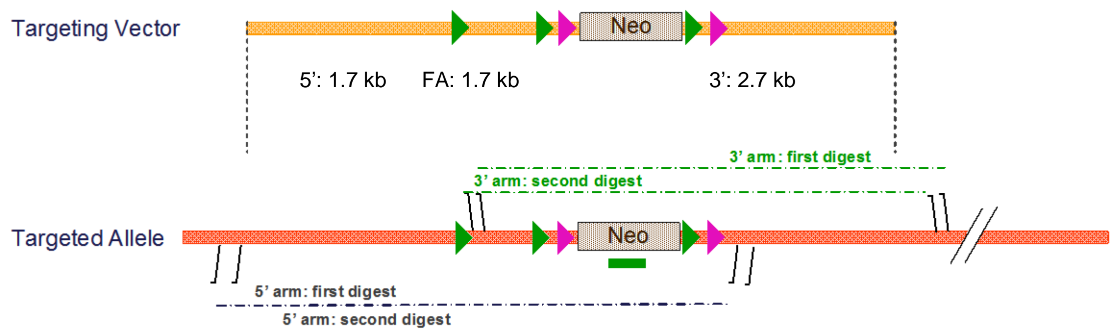
Reference of clone used to generate the mouse line:

- clone **DG10-60**

### 3.2. Southern data on positive clone

#### 3.2.1. Neo Southern strategy

Southern Screening Strategy



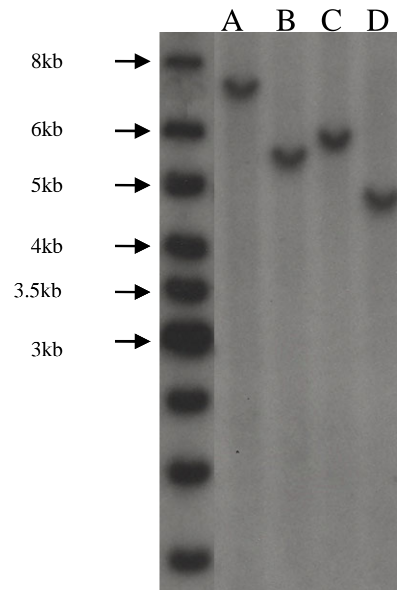

**Digestions used to validate the 5' and 3' insertion**

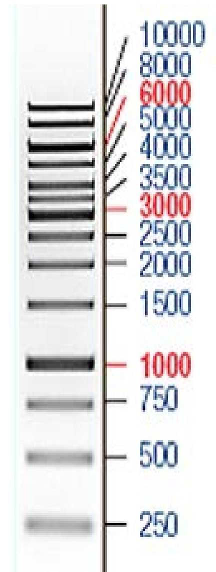
| Probe | Name | Genomic DNA digest | WT allele (kb) | Targeted Allele (kb) |
|---|---|---|---|---|
| Neo | 5' arm first digest | AflIII | / | 7.8 |
| | 5' second digest | BglII | / | 5.9 |
| | 3' arm first digest | NheI | / | 6.3 |
| | 3' arm second digest | SexAI | / | 5.1 |

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



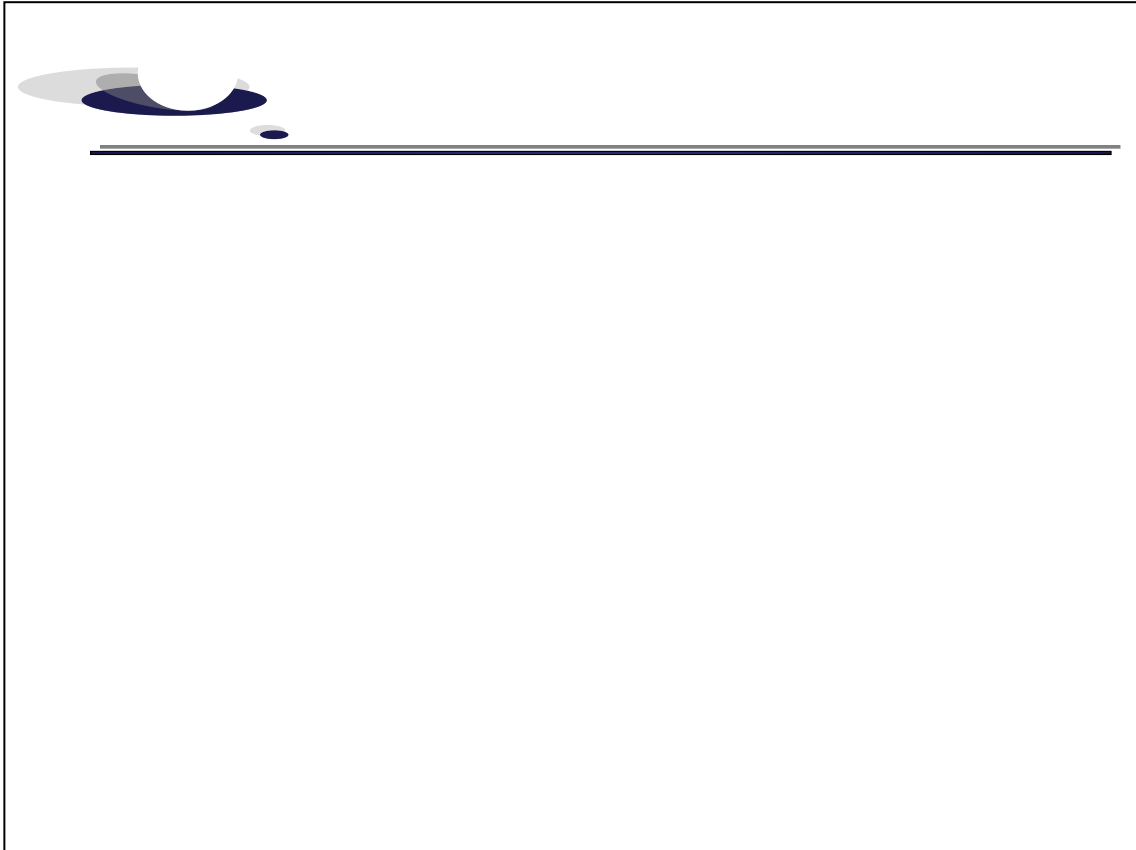

**A**: AflIII 7.8kb
**B**: BglII 5.9kb
**C**: NheI 6.3kb
**D**: SexAI 5.1kb

## 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

| Position | Primers | Sequence |
|---|---|---|
| Lf | 35 | TCCGCGAATCCGTGTTCACC |
| Lr | 36 | AGCAGCTCCGAATTGTTAAGTCTGG |
| Ef | 37 | TTCAATGCCTCGCAGCTGATCACC |
| Er | 38 | AGTTTCCAGTTAACGGAGGAAGACC |

PCR fragments expected size (bp):

| Region analyzed | **Primers used** | Position on the primer (see the map above) | Conditional allele (L2) | Knock-Out allele (L-) | WT allele (WT) |
|---|---|---|---|---|---|
| Presence of the distal loxP | 35-36 | Lf / Lr | 446 | --- | 323 |
| Excision of the selection marker | 37-38 | Ef / Er | 575 | --- | 441 |
| Excision of the floxed exon(s), i.e. knock out | 35-38 | Lf / Er | 2219* | 446 | 2031* |

*: 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: |
|---|---|
| - FastStart PCR Master (Roche) | 7.5µl |
| - DNA (50ng/µl) | 1.5µl |
| - 5' primer (100 □M) | 0.06µl |
| - 3' primer (100 □M) | 0.06µl |
| - Sterile $H_2O$ | up to 15 µl |

Cycling conditions:

| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 34 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 20°C | 5 min | 1 |

**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

Representative genotyping picture