



Genotyping protocol

Gt(ROSA)26Sortm23(UBC*)^{Ics/Ics}

IR00004024 / K725

(ICS internal reference)

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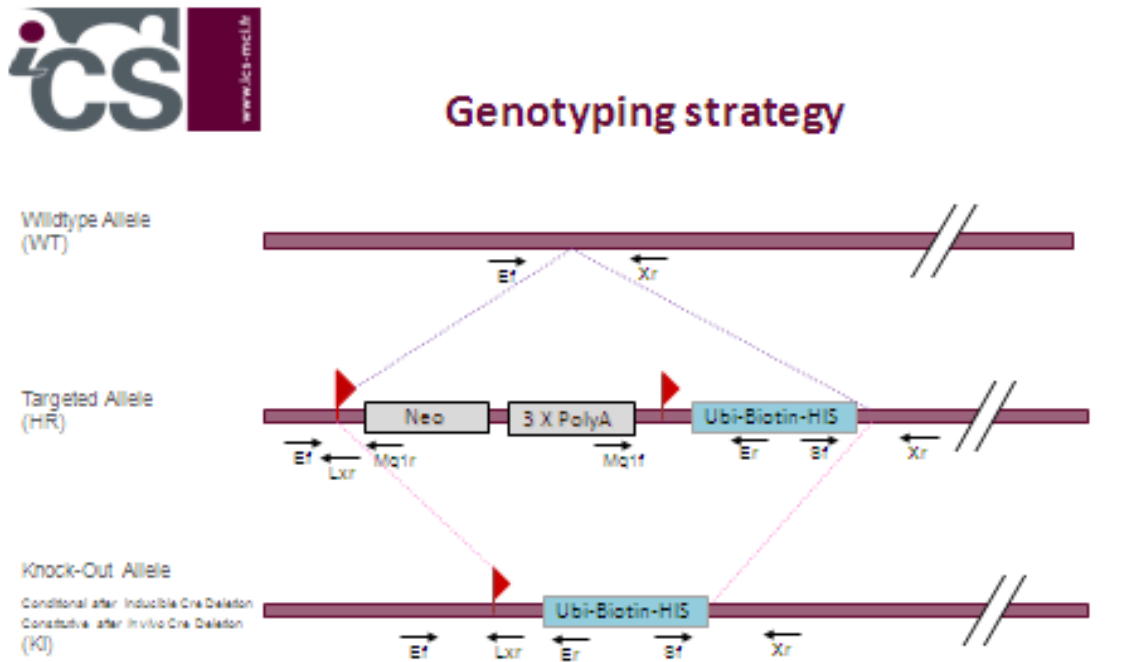
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **rosa26-stop-Ubi-Biotin-HIS** Knockin (KI) project.

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 | Sequence |
|----------|-----------------------------|
| Ef | CTTTCTGGGAGTTCTCTGCTGCCTC |
| Er | GATTTACACCTTCACCGCCTTTCCAG |
| Lxr | GTCGAGGGACCTAATAACTTCGTATAG |
| Mq1f | TCCCCATCAAGCTGATCCGGAACCC |
| Mq1r | TGCTAAAGCGCATGCTCCAGACTGC |
| Sf | CTGGTCTCCGTCTCAGAGGTG |
| Xr1 | CCTTTAAGCCTGCCAGAAG |
| Ef | AAAGTCGCTCTGAGTTGTTAT |
| Xr2 | GACAACGCCACACACCAGGTTAG |

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer (see the map above) | I3 (L3) | Targeted allele (L2) | KI allele (L-) | WildType allele (WT) |
|--|--------------|---|---------|----------------------|----------------|----------------------|
| WildType allele specific PCR (5' part of the targeted locus) | 4036-4035 | Ef / Xr1 | | --- | --- | 239 |
| 5' part of the selection marker | 6509-265 | Ef / Mq1r | | 402 | --- | --- |
| 3' part of the selection marker | 4537-6510 | Mq1f / Er | | 157 | --- | --- |
| cDNA 3' | 6512-6513 | Sf / Xr2 | | 409 | 409 | --- |
| LoxP specific PCR | 6509-6511 | Ef / Lxr | | 336 | 336 | --- |

---: no Amplicon should be obtained

1.2. 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 µM) | 0.06µl |
| - 3' primer (100 µM) | 0.06µl |
| - Sterile H ₂ O | up to 15 µl |

Cycling conditions:

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

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