## PCR Genotyping Protocol for Eif5a-cKO mice

**Detection of floxed alleles in which the neomycin-resistant gene sequence has been removed.**

The 3' side loxP is located downstream of exon 5 (i.e., intron 5) of the Eif5a gene.

**<Primer sequences>**

| | | |
|---|---|---|
| Forward | located in intron 4 | TGATCCTTAATTCGAAGTCTTTCAG |
| Reverse | located in intron 5 downstream of loxP. | TTGGGGGCTTCATATTAAACTTGTA |

**Length of the PCR products**

Wild-Type Allele: 851 bp

Floxed Allele (Neomycin gene removed): 1005 bp

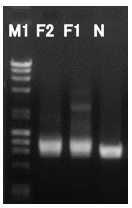
**<PCR conditions>**

Thermal cycles:

| | | | |
|---|---|---|---|
| 94°C | 2 min | | |
| 98°C | 10 sec | } 35 cycles | |
| 64°C | 3 min | } | |
| 68°C | 3 min | | |
| 4°C | store | | |

Components of PCR reaction solution

| | |
|---|---|
| $H_2O$ | 1.2 μL |
| 2xPCR Buffer for KOD FX | 7.5 μL |
| 2.0 mM dNTP | 3.0 μL |
| 10 μM Forward primer | 0.5 μL |
| 10 μM Reverse primer | 0.5 μL |
| Template Genome DNA | 2.0 μL |
| DNA polymerase | 0.3 μL |
| | 15.0 μL |




Floxed allele (1005 bp)
Wild-type allele (851 bp)

1% Agarose gel
4 μL apply

| | |
|---|---|
| **DNA polymerase** | **KOD FX (TOYOBO)** |
| **PCR reaction volume** | **15 μL** |
| **Template Genome DNA** | **30 ng** |
| **Marker (M1)** | **λ*Hin* d III Digest + ϕX174 *Hae* III Digest Mix Marker** |