



Genotyping protocol

Introduction of conditional N855S point mutation in Trpa1 mouse gene using FleX2 approach

(Internal reference: IR00008468 / Kos8468)

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This report has been **validated** by: Guillaume PAVLOVIC

The first version of this report was finalized the: Wednesday, September 11, 2024

The last update of this report was done the: **NA**

Position of the primers on the different alleles

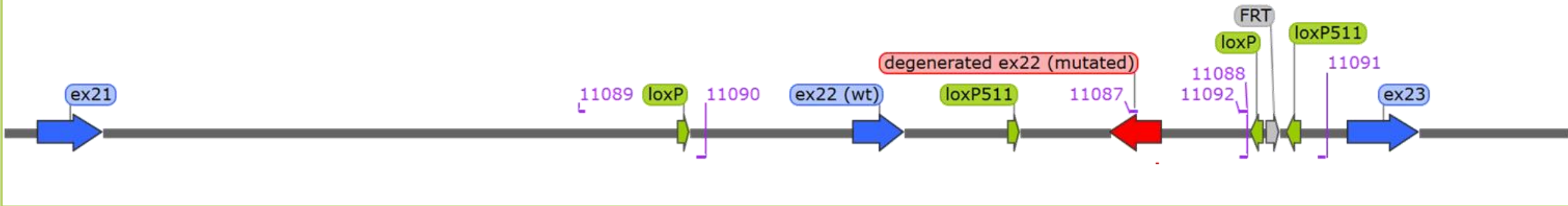


The different primers are indicated in purple. Only exons from *Trpa1-201* transcript are indicated and numbered as in ENSEMBL exons table.

Conditional allele (tm.1 / cKO allele)

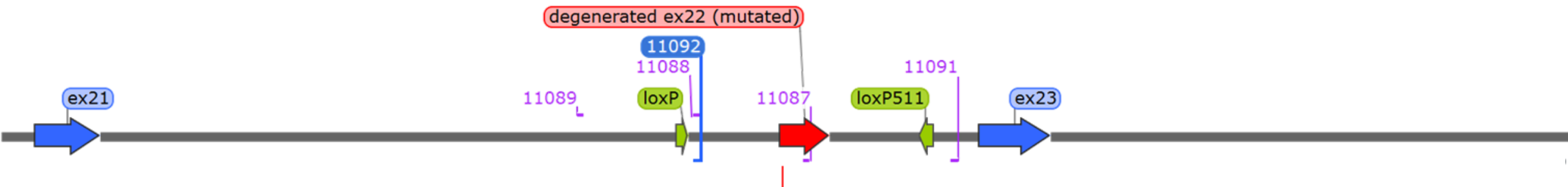
Allele obtained after removal of the selection cassettes by Flp recombination

The selection cassettes are removed.
The shipped animals carry the cKO allele.



Point mutation allele (tm.2 / KI allele)

Allele obtained after Cre recombination



Position of the primers on the different alleles



Wild-type allele (WT) Transcript: ENSMUST00000041447



Genotyping using PCR



Primers sequence

| Primers | Sequences |
|---------|---------------------------|
| 11088 | CTGGAATTCGAGCTCGCTAGCG |
| 11091 | GGGACTTCCTGGTGGTCAAATTATG |
| 11089 | CAGGTGGGTCACAAGGAGGG |
| 11092 | CGCTAGCGAGCTCGAATTCCAG |
| 11090 | GGTCTCTGTGTAACCCTGAATAGAG |
| 11087 | AATGAAGACCCCTGTTGACCG |

PCR fragments size

PCR fragments expected size (bp)

| PCR | Region analyzed | PCR adjuvant | Primers used | tm.1 / cKO allele | tm.2 / KI allele | WT allele |
|-----|--|--------------|---------------|-------------------|------------------|-----------|
| 1 | Presence of the degenerated ex 22 (expressed or not) | Betaine | 11087 / 11088 | 301 | 301 | --- |
| 2 | tm.1 specific PCR | None | 11092 / 11091 | 276 | --- | --- |
| 3 | tm.1 & WT PCR | Betaine | 11089 / 11090 | 328 | --- | 278 |
| 4 | tm.2 specific PCR | Not tested | 11089 / 11092 | --- | 318 | --- |
| 5 | tm.2 specific PCR | Not tested | 11088 / 11091 | --- | 681 | --- |

---: no amplicon should be obtained.

(size): amplicons larger than 700 bp will not be amplified under our genotyping conditions.

This primer pair was not tested as this allele was not generated.

Reaction mix

If indicated in the PCR fragment size table, add 5% of the appropriate adjuvant to the reaction mix.

| Reagent | Volume (μ l) |
|---------------------------------|-------------------|
| FastStart PCR Master 2x (Roche) | 3 |
| Each primer (100 μ M) | 0.024 |
| DNA (50ng/ μ l) | 1.5 |
| Sterile H ₂ O | up to 6 |

Cycling conditions

| Temperature ($^{\circ}$ C) | Time | #Cycles |
|-----------------------------|---------|---------|
| 95 | 0:04:00 | x1 |
| 94 | 0:00:30 | x35 |
| 62 | 0:00:30 | |
| 72 | 0:01:00 | |
| 72 | 0:07:00 | x1 |
| 14 | --- | --- |



These PCR conditions have been optimized for high-throughput genotyping. Our expertise and our robotic platform allow us to perform analyses on very low volumes in 384-well plates. Adaptation to your own laboratory conditions may be necessary.

In case you have difficulties genotyping this line following this protocol, we have published a detailed step-by-step protocol that also contains our recommendations for optimizing genotyping (see last slide, doi: 10.1002/cpmo.65).



Cre and Flp genotyping method

[Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.](#)

Birling MC, Dierich A, Jacquot S, Hérault Y, Pavlovic G.

Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.

Tips and tricks for optimizing your PCR genotyping procedures

[Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.](#)

Jacquot S, Chartoire N, Piguet F, Hérault Y, Pavlovic G. (2019).

[Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65](#)

Free copy of this paper can be accessed online though this link <http://bit.ly/2sxxWvO>