Genotyping protocol

Frmpd4 (MGI gene code: 3042378) conditional knock-out mouse

(Internal reference: IR00003732b / G18b)

For any question, please contact:

PHENOMIN-ICS

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

The first version of this report was finalized the: Friday, February 14, 2025

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 ***Frmpd4-201*** transcript are indicated and numbered as in ENSEMBL exons table.

Wild-type allele (WT) Transcript: ENSMUST00000112145

Conditional allele (tm.1 / cKO allele)

The selection cassette is removed.
The shipped animals carry this allele.

Allele obtained after removal of the selection cassette using a Flp deleter

KO allele (tm.2 / KO allele)

Allele obtained after removal of exon 3 (in ***Frmpd4-201***) using a Cre deleter

Genotyping using PCR

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.

Primer’s sequence

Primers	Sequences
7079	CTGTGGGTCAGACACAGTTCTAG
7080	TTTCTCTCTCCACCAAGGAACCACTTA
7081	TTGTCAAACCCAGTGAGAATGACTTCA
7082	CTGACCCAAGGATATGATGGATTTGG
7083	TCTCCTTTCAGAAATGATGCAATGGTT
5049	CATACATTATACGAAGTTATCTGCAG

Genotyping using PCR

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PCR fragments size

				PCR fragments expected size (bp)		
PCR	Region analyzed	PCR adjuvant	Primers used	tm.1 / cKO allele	tm.2/ KO allele	WT allele
1	***tm.1 and WT PCR (5' loxP region)***	None	7079 / 7080	332	---	256
2	***tm.1 and WT PCR (3' loxP region)***	None	7081 / 7082	447	---	341
3	***tm.1 or tm1.2 detection PCR***	None	7079 / 5049	150	150	---
4	***tm1.2 PCR***	None	7079 / 7083	**(1359)**	310	**(1177)**

---: 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.

Genotyping using PCR

Reaction mix

If mentioned in "PCR fragments size" table, add corresponding adjuvant to the reaction mix

Reagent	Volume (µl)
FastStart PCR Master 2x (Roche)	3
Each primer (100µM)	0.024
DNA (50ng/µl)	1.5
Sterile H2O	up to 6

Cycling conditions

Temperature (°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	---	---

NB: these PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale or to your own laboratory conditions may be required.

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).

Relevant publications & information

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