Genotyping protocol

Mir124a-2 (MGI gene code: 3618700) knock-out mouse

(Internal reference: IR00004505 / G4505)

For any question, please contact:

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

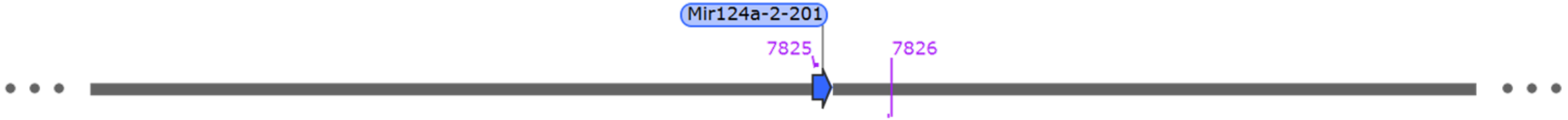
The first version of this report was finalized the: Friday, February 14, 2025

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.

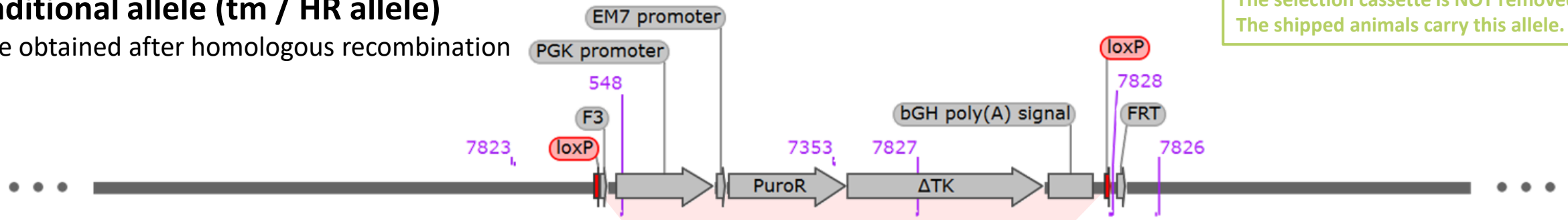
Wild-type allele (WT) Transcript: ENSMUST00000175332



Conditional allele (tm / HR allele)

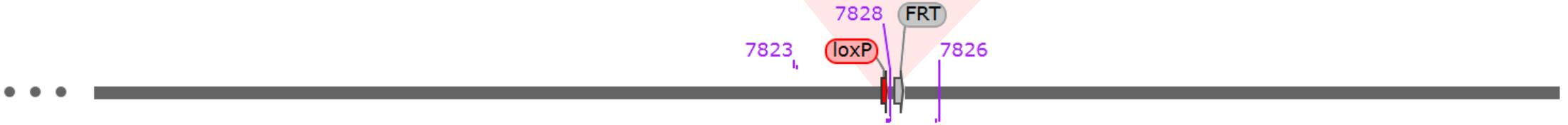
Allele obtained after homologous recombination

The selection cassette is NOT removed.
The shipped animals carry this allele.



KO allele (tm.1 / KO allele)

Allele obtained after removal of the selection cassette 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
7353	GCTTCACCGTCACCGCCGA
7827	ATGGCGGTCGAAGATGAGGGTG
7823	AAATGTCTTAGGACGGCATTCAGGG
548	CCAGACTGCCTTGGGAAAAG
7826	GTTTTCCCAGTTCGACCCAGAATTT
7828	GAGGGACCTAATAACTTCGTATAG
7825	CAGAGACTCTGCTCTCCGTGTTCACA
7824	TTTTCTCCCTGCCCTTCACCCT

Genotyping using PCR

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PCR fragments size

PCR fragments expected size (bp)

PCR	Region analyzed	PCR adjuvant	Primers used	tm / HR allele	tm.1/ KO allele	WT allele
1	***internal selection cassette marker PCR***	None	7353 / 7827	437	---	---
2	***5' selection cassette junction PCR***	None	7823 / 548	565	---	---
3	***WT PCR (3' region)***	None	7825 / 7826	---	---	393
4	***tm1.2 PCR***	None	7823 / 7828	**(3062)**	460	---

---: 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