

Gene: FLPeR

Colony prefix: FLPT

Allele: *Gt(ROSA)26Sor^{tm1(FLP1)Dym}*

Allele type: *Targeted (Recombinase)*

Allele information:

An enhanced flip cassette containing a splice acceptor site was ligated to a downstream PGK-neo cassette and inserted at the endogenous locus. Efficient frt-specific recombination in transgenic mice carrying a reporter gene was reported to reflect constitutive expression of the *Gt(ROSA)26Sor* from preimplantation onward. ([J:66893](#), [J:92442](#))

Genotyping Information

Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the cassette, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: cassette positive, mutant positive, wild type positive = heterozygous

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wildtype	FLPeR_F	FLPeR_wt_R	250
Standard PCR	Mutant	FLPeR_F	FLPeR_mut_R	603

Primer sequences

Primer	Primer Sequence (5' > 3')
FLPeR_F	AAAGTCGCTCTGAGTTGTTAT
FLPeR_wt_	GGAGCGGGAGAAATGGATAT
FLPeR_mut	GCGAAGAGTTTGTCCCTCAACC

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Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl ₂ (50 mM)	0.6
Platinum Taq (Invitrogen)	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 µM)	0.4
Primer 2 (10 µM)	0.4
ddH ₂ O	15.2
Total	20

Amplification conditions

Step	Conditions	Time
1	94°C	5 min
2	94°C	30 sec
3	58°C	30 sec
4	72°C	45 sec
5	Go to '2' + 34 cycles	-
6	72°C	5 min
7	12°C	forever

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Genotyping using universal copy number qPCR assays designed to the selection cassette

The cassette qPCR assays use a hydrolysis probe assay (eg Applied Biosystems TaqMan technology) to determine genotype via the copy number of the selection cassette in a sample. Homozygotes will possess two copies, heterozygotes one copy and wild type mice will show no amplification when compared to known homozygote controls.

These FAM®-labeled assays are multiplexed with a VIC® labeled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366).

Please note that these assays are not gene-specific – other information should be used in conjunction with the universal cassette assays (for example the mutant-specific srPCR) when confirming the gene identity.

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Cassette	Flp-E	CATCTGGGAGATCACTGAGAAAATA CT	TGAAAGTAGCTAGGAAGAGGAA TTGGT	AAGTTTTGTTTTGTAAATCTC

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems ViiazTM with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpressTM buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress TM buffer	5
20x target assay	0.5
ddH ₂ O	3
Tfrc endogenous 20x assay	0.5
DNA	1

Amplification conditions

Step	Conditions	Time
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34 cycles	-

Genesis. 2000 Nov-Dec;28(3-4):106-10. Links

Widespread recombinase expression using FLP_{eR} (flipper) mice. Farley FW, Soriano P, Steffen LS, Dymecki SM.

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