

STRAIN NAME :

**JAS3-Rosa26-Zip14(L438R)**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- Primers 30µM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX :

PCR1: Flox/WT allele

MIX	Quantity / well
Water	6,80 µl
2X Kapa Buffer	10,00 µl
ZIP14WTF	0,60 µl
ZIP14FloxR	0,30 µl
ZIP14WTR	0,30 µl
DNA	2,00 µl
Final Quantity	20,00 µl

PCR2: KI (=deflox) allele :

MIX	Quantity / well
Water	7,40 µl
2X Kapa Buffer	10,00 µl
ZIP14KIF	0,30 µl
ZIP14KIR	0,30 µl
DNA	2,00 µl
Final Quantity	20,00 µl

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	35 cycles
15 sec	60°C	
30 sec	72°C	
5 min	72°C	

MIGRATION :

Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

PCR1 :

- ZIP14WTF 5' CAATACCTTTCTGGGAGTTCTCTGC 3'
- ZIP14FloxR 5' GATGAATGAAGTCTGCCAGGAGG 3'
- ZIP14WTR 5' CTGCATAAAACCCAGATGACTACC 3'

PCR2 :

- ZIP14KIF 5' AGAGGTTCTGGGTCCTTCCTTGG 3'
- ZIP14KIR 5' GGGCAACGTGCTGGTTATTGTGC 3'

EXPECTED AMPLIFICATIONS

PCR1 :

WT allele : 304 bp  
Floxed or KI allele: 998 bp

PCR2 :

KI allele : 410 bp

*Perfection date* : 26/02/2018

*Update* :