

## B6.129P2-Lgr6<sup>tm2.1(cre/ERT2)Cle</sup>/J

Stock No: 016934

Protocol 32131: Separated PCR Assay - Lgr6<sup>tm2.1(cre/ERT2)Cle</sup>

Version 4.0

### Notes

This assay does not work well without the use of a Hotstart Taq polymerase.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Mutant = ~210 bp

Heterozygote = 257 bp and ~210 bp

Wild type = 257 bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
12775		CTG TGG CTT TGC GCT GTG		Wild type Forward	A	
12776		AAG GGC ACC AAA CGA GTG T		Wild type Reverse	A	
13020		GCC CAC CGA CGG CGC AGC CC		Mutant Forward	B	
9528		GCT GAA CTT GTG GCC GTT TA		Mutant Reverse	B	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTPS-kapa	0.26 mM
12775	0.50 uM
12776	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

### Reaction B

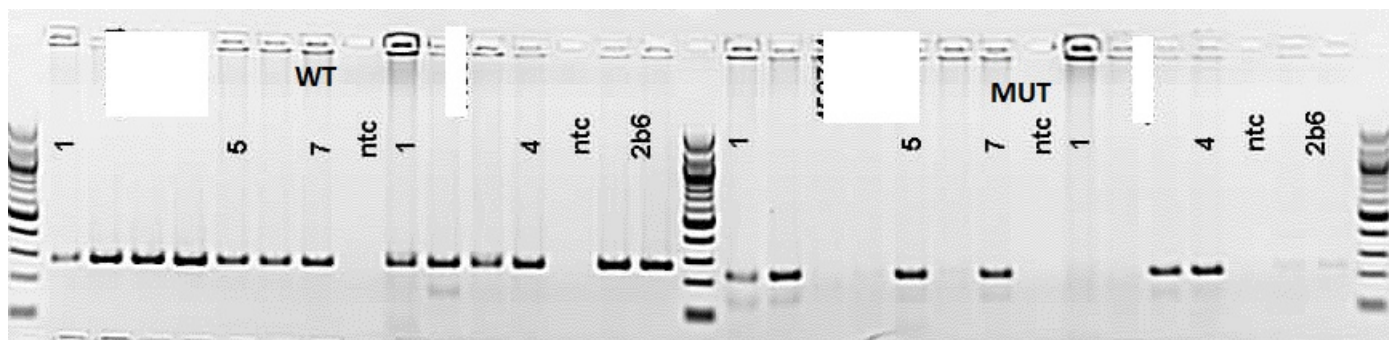
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
13020	0.50 uM
9528	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
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9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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**Lgr6 tm2.1**  
**JR-16934**