

B6.129P2(C)-Mecp2^{tm6.1Bird}/J

Stock No: 026848

Protocol 27284: Standard PCR Assay - Mecp2<tm#Bird>alternate1

Version 1.2

Notes

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 = ~321 bp

Heterozygote = ~321 bp and 547 bp

Wild type = 547 bp

X-linked

This Assay Cannot Distinguish JRs 26762, 26847 and 26848

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
15020		CTG AAC TTG TGG CCG TTT AC		Mutant Reverse	A	EGFP/EYFP
23982		CAG CAG CAT CTG CAA AGA AG		Common	A	
23983		CTG GTC AAC AGC TTG TCT GG		Wild type Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
15020	0.50 uM
23982	0.50 uM
23983	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.



