

## B6.129P2-Aim2<sup>Gt(CSG445)Byg/J</sup>

Stock No: 013144

Protocol 31940: QPCR Assay - Generic LacZ QPCR Alternate 1

Version 8.0

### Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing  $\Delta C_t$  values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

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

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
37301		GTT TAT GCA GCA ACG AGA CG		Transgene Reverse	A	
37302	Fluorophore-1	CAG GAT ATG TGG CGG ATG AGC	Quencher-1	Tg Probe		
mom0095		TGA CGG CAG TTA TCT GGA AG		Transgene Forward	A	
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
TmoIMR0105	Fluorophore-2	CCA ATG GTC GGG CAC TGC TCA A	Quencher-2	IC Probe		

#### Reaction A

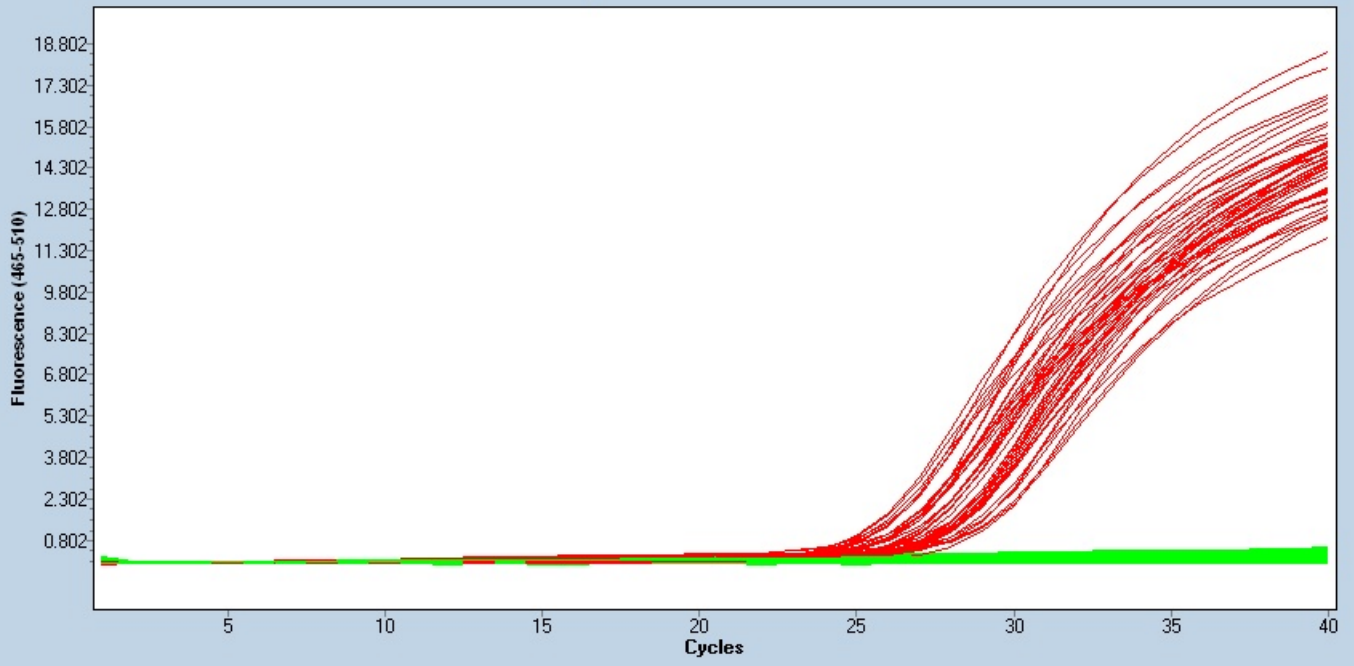
COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa Probe Fast QPCR	1.00 X
37301	0.40 uM
mom0095	0.40 uM
oIMR1544	0.40 uM
oIMR3580	0.40 uM
Tg Probe	0.15 uM
IC Probe	0.15 uM
DNA	

#### Cycling

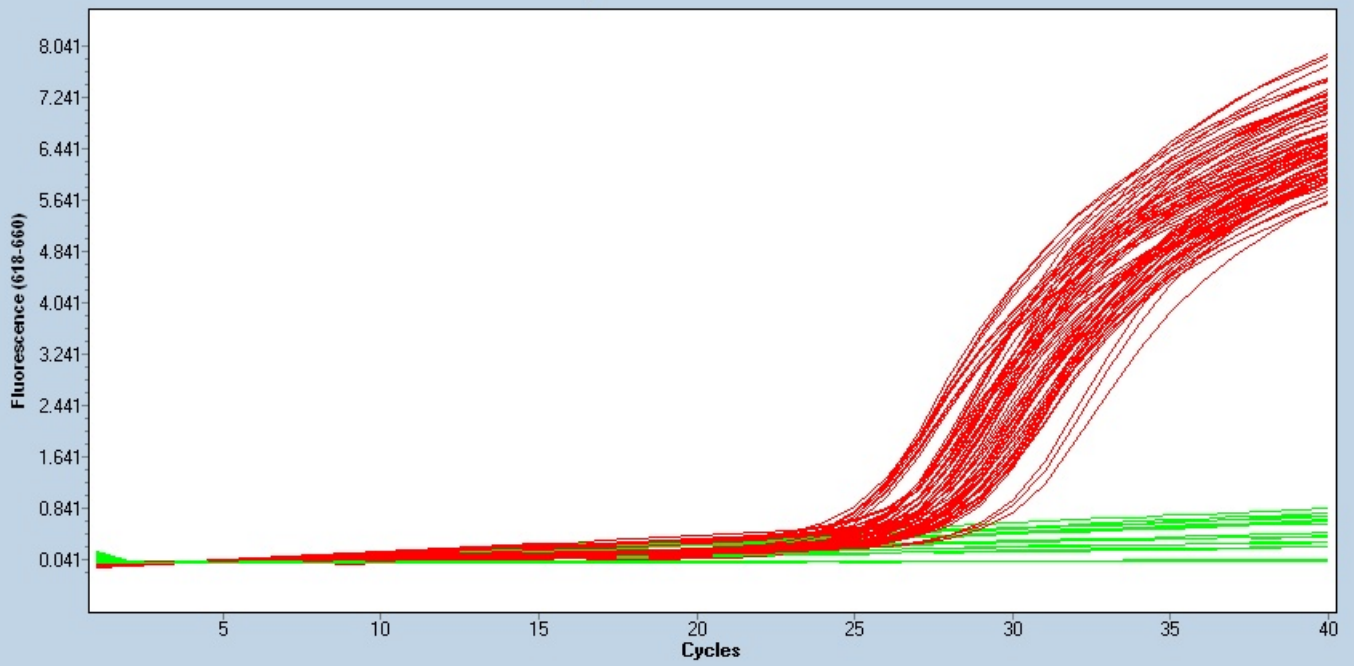
STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	repeat steps 2-3 for 40 cycles

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

**Amplification Curves**



**Amplification Curves**



### Median Delta Cycle Threshold

