

BXSB/Mpj

Stock No: 000740

Protocol 35972: QPCR Assay - X chrom qPCR

Version 1.0

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 ΔC_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

Tg= 123 bp

IPC = 74 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21118	Fluorophore-1	GAC TAG GTT CAT AGG CAC TGG	Quencher-1	Forward	A	
21119	Fluorophore-2	CCG CCA AAA CTC CTT CTC TAC	Quencher-2	Reverse	A	
21120	Fluorophore-3	CCC CAG ATG GTA CCC ACA GAA CTT G	Quencher-3			
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-4	CCA ATG GTC GGG CAC TGC TCA A	Quencher-4	IC Probe		

Reaction A

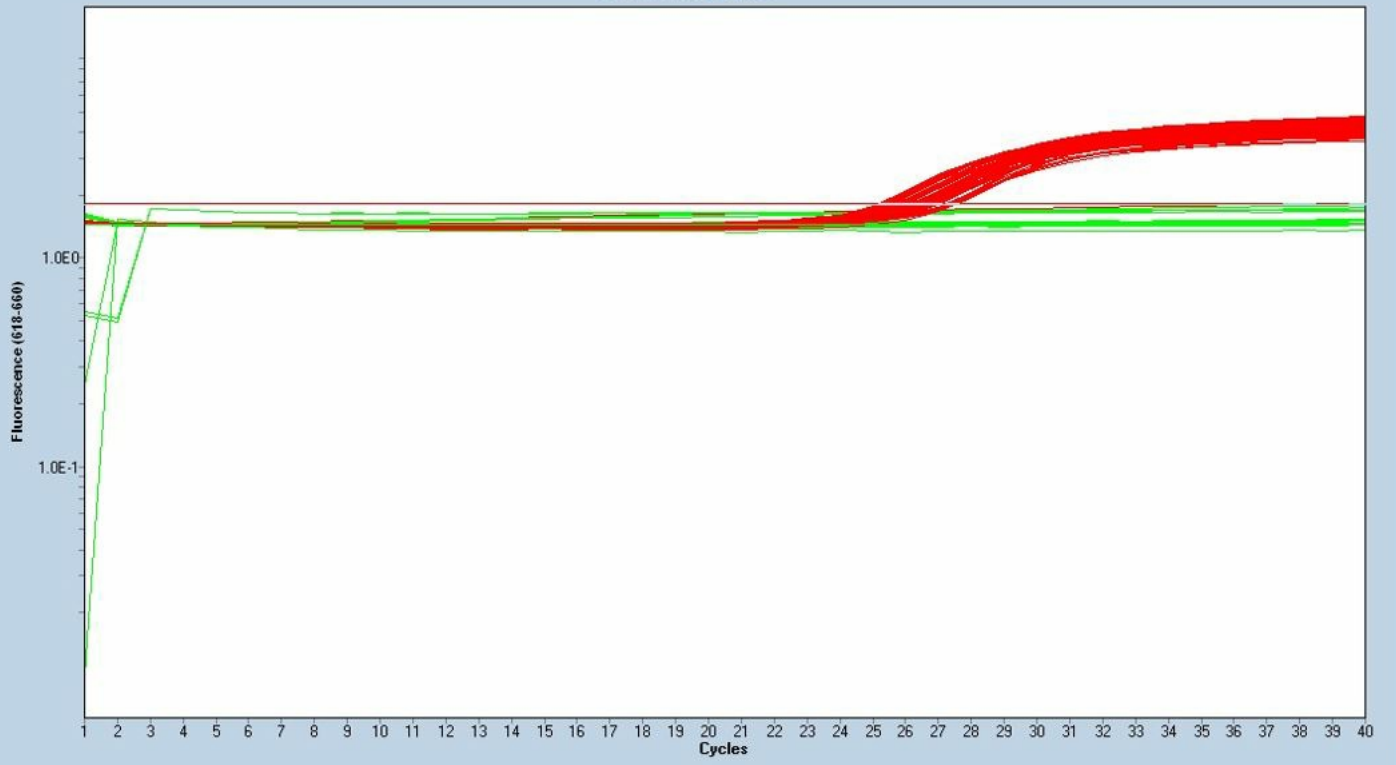
COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH ₂ O	
21118	0.40 uM
21119	0.40 uM
oIMR1544	0.40 uM
oIMR3580	0.40 uM
Tg Probe	0.15 uM
IC Probe	0.15 uM
DNA	

Cycling

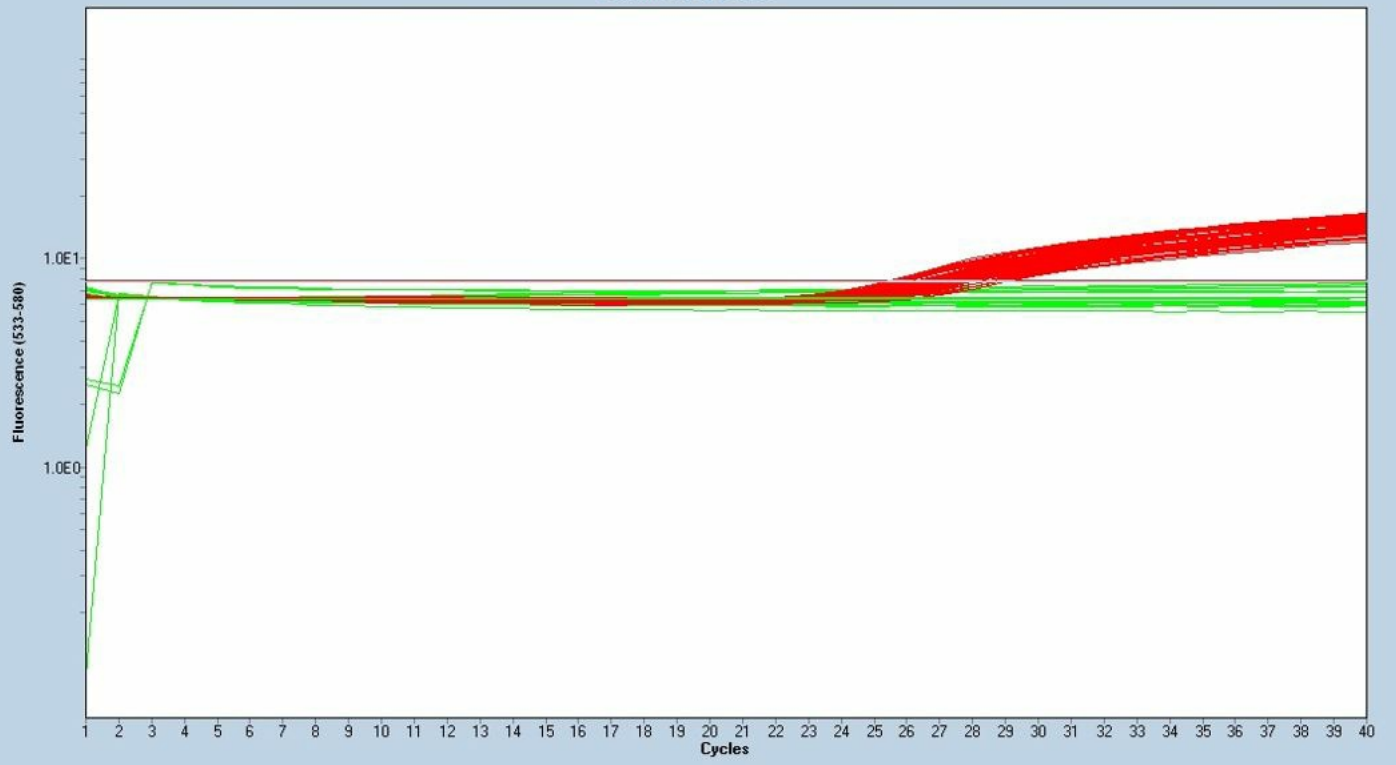
STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	repeat steps 2-3 for 40 cycles

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

