

STOCK *Kras^{tm4Tyj} Trp53^{tm1Brn} Tg(Pdx1-cre/Esr1*)#Dam/J*

Stock No: 032429

Protocol 21155: Probe Assay - Trp53<tm1Brn> Probe

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 Δ Ct 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

Mutant= 145 bp

Wild Type = 63 bp

Sequence

Wt

Sequence:tcgatggattgctgtattggaatcaaacagaaatctatgtcattcacagc
agtaacctcctgggaatactcaagagacggagaaagggcgactgactgtgccctc
cgtccttttcgcaatcctttattCTGTTCGAAcataattgt
ttcctgagacagggtttctctgtgtagtcctggctgcctgggactcccctctgtagacca
ggctggcctccaactgtctctgcctcctatgtgctgggatcaaaggtgctgcccaccac
cgctggcttcttagagttgg

Mutant

Sequence:GAGACGGAGAAAGGGCGACTGACTGTGCCCTC
CGTCCTTTTTCGCAATCCTTTATTCTGTTCGAtaagctgatatcg
aatcataactcgtataatgtatgctatacgaagttatctgcagcccg
ggggatctgatataTCGAATCATAATTTGTTTTCTGAGACAGG
GTTTCTCTGTGTAGTCCTGGCTGTCTGGGACTCCCCTCT
GTAGACCAG

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
31852		CGT CCT TTT TCG CAA TCC T		Forward	A	
31853		GAA ACC CTG TCT CAG GAA AAC		Reverse	A	
31854	Fluorophore-1	CAA TCC TTT ATT CTG TTC GAA TCA T	Quencher-1	WT Probe		
31855	Fluorophore-2	TGC TAT ACG AAG TTA TCT GCA GCC	Quencher-2	MUT Probe		

Reaction A

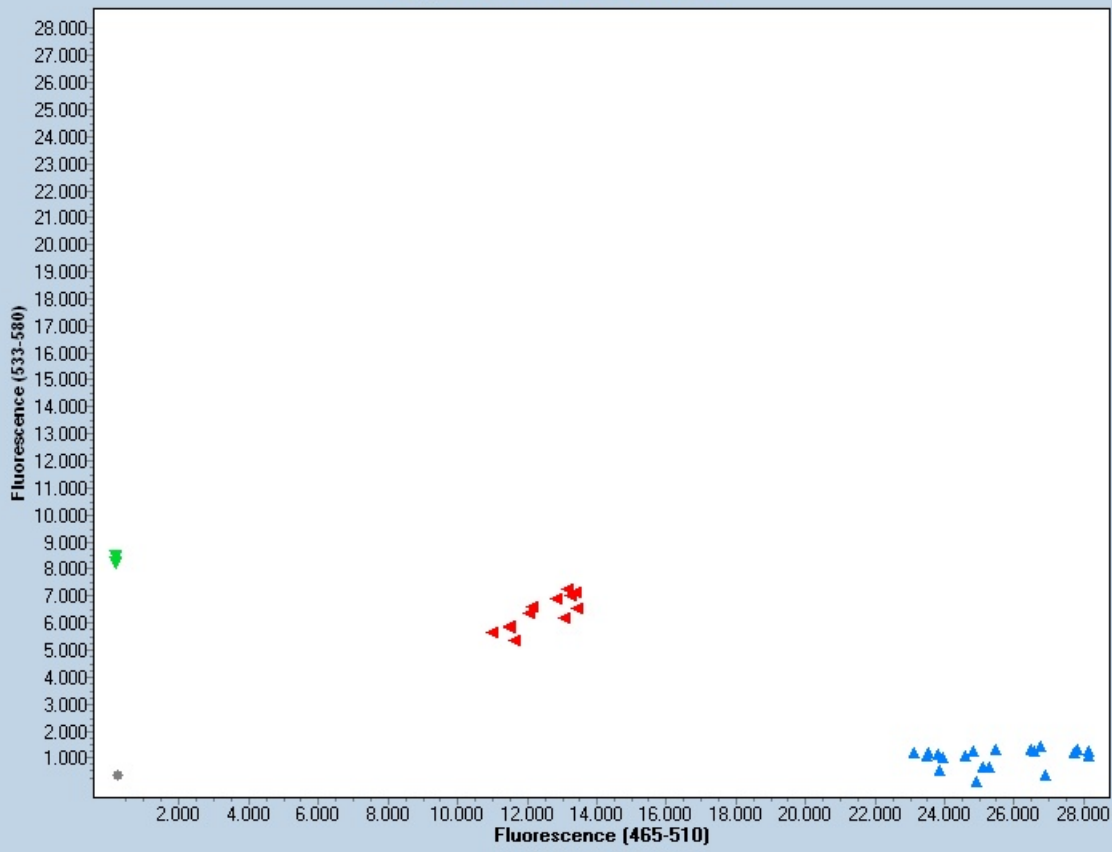
COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
31852	0.40 uM
31853	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

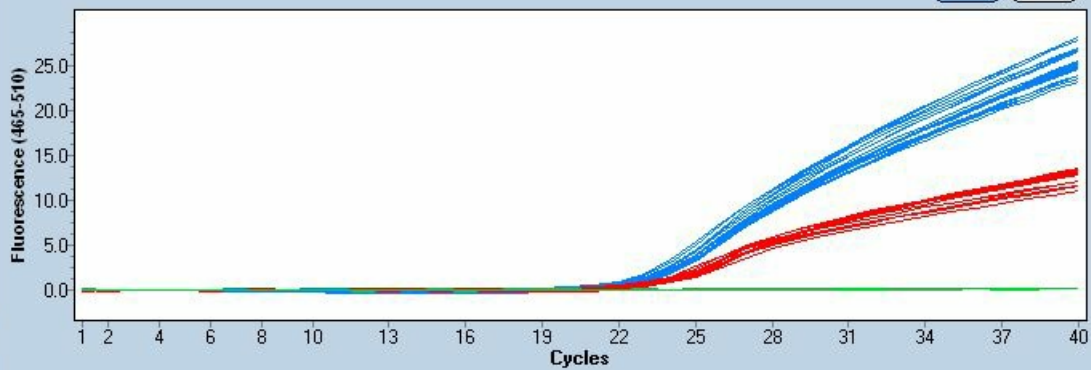
JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

Endpoint Fluorescence Scatter Plot



Fluorescence History

Select Zoom



Fluorescence History

Select Zoom

