

## 129S-*Trp53*<sup>tm2Tyj/J</sup>

Stock No: 008652

Protocol 30721: Probe Assay - *Trp53*<tm2Tyj> 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  $\Delta$ 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= 105 bp

Wild Type = 132 bp

>[chr11:69398179+69398310](#) 132bp CAGCCTGCCTAGCTTCCTC AGAAAATGTTGGCTGGGAAAG

### Sequence

#### Wt Sequence:

```
tcaatagcagcctgcttagcttcctcaggatcaaatgagatgagcccctgagaaga
gcaaggcccgctggcctggaaggccagccctggtgtactcaaacCTCTCG
Agtctattgccttccagccaacatttctacacatccagcctctgtggatactgtgacc
ctctgatctggttctgtgaaaagttcatattggcaact
```

#### Mutant Sequence:

```
agctagccaccatGGCTTGAGTAAGTCTGCAGGTCGAGGGACC
TAATAACTTCGTATAGCATACATTATACGAAGTTATgtcGAG
TCTATTGCCTTTCCAGCCAACATTTTCTTACACATCCAG
CC
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
25929		CCA TGG CTT GAG TAA GTC TGC A		Mutant Forward	A	
35283		AGA AAA TGT TGG CTG GGA AAG		Common	A	
35284		CAG CCT GCC TAG CTT CCT C		Wild type Forward	A	
35285	Fluorophore-1	TCG AGG GAC CTA ATA ACT TCG TAT AGC A	Quencher-1	MUT Probe		
35286	Fluorophore-2	CCT GGT TGT ACT CAA ACC TCT CGA GTC	Quencher-2	WT Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
25929	0.40 uM
35283	0.40 uM
35284	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.

