

## STOCK *Hba*<sup>tm1Paz</sup> *Hbb*<sup>tm1Tow</sup> Tg(HBA-HBBs)41Paz/J

Stock No: 003342

Protocol 21979: End Point Analysis Assay - Hbb&lt;tm1Tow&gt;-PROBE

Version 4.0

### 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

MUT = 164bp

WT = 98bp

### Sequence

Mutant sequence, including the junction:

```
(gagactagtgagacgtgctacttccattgtcacgtcctgcacgacgcgagctgcgg
ggcgggggggaactcctgactaggg
gaggagtagaaggtggcgcaaggggcccaccaaagaacggagccggtggcg
cctaccggtggatgtggaatgtgtcgag
gccagaggccactgtgtagcgaagtgcccagcggggctgctaaagcgcagtc
tccagactgcctgggaaaagcgctc
ccctaccggtagaattcctgcagcccggg)GGATCCTGAGAACTTCAG
GGTGAGTCTGATGGGCACCTCC
TGGGTTTCCTTCCCCTGGCTATTCTGCTCAACCTTCCTATC
AGAAGGAAAGGGGAAGCGATTCTAGGGAGCA
GTCTCCATGACTGTGTGTGGAGTGTGACAAGAGTTTGA
TATTTTATTC TCTACTCAGAAT
CGCTGCTCCCCCTCACTCTGTTCTGTGTTGTCAT
```

WT Hbb, including junction:

```
(ctgctggtgtctacccttgaccagcggtaacttgatagcttggagacctatcctctgcctctgctatc
atgggtaatgccaaagtgaaggccatggcaagaa
agtgataactgccttaacgatggcctgaatcactggacagcctcaagggcaccttggcagcctcagt
gagctccactgtgacaagctgcatgt)GGATCC
TGAGAACTTCAGGGTGAGTCTGATGGGCACCTCCTGGGTTTCCTTCCCCT
GGCTATTCTGCTCAACC
TTCTATCAGAAGGAAAGGGGAAGCGATTCTAGGGAGCAGTCTCCATGA
CTGTGTGTGGAGTGTG
ACAAGAGTTTGGATATTTTATTCTCTACTCAGAATCGCTGCTCCCCCTCACT
CTGTTCTGTGTTGTCAT
T
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
19726		CAG ACT CAC CCT GAA GTT CTC		Common	A	
19727		CGG TGG ATG TGG AAT GTG T		Mutant Reverse	A	
19728	Fluorophore-1	CTT GTG TAG CGC CAA GTG CCC	Quencher-1	MUT Probe		
19729		TGA ATC ACT TGG ACA GCC TC		Wild type Forward	A	
19730	Fluorophore-2	ACC TTT GCC AGC CTC AGT GAG C	Quencher-2	WT Probe		

### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
19726	0.40 uM
19727	0.40 uM
19729	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.

