

B6.129S-*Idua*^{tm1.1Kmke}/J

Stock No: 017681

Protocol 30655: Probe Assay - *Idua*<tm1.1Kmke> 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= 95 bp

Wild Type = 113 bp

>[chr5:109110273+109110385](#) 113bp CATCCTGTGGGCTGAACA AGAGGGCTGAGGCTGTTG

Sequence

Wt

Sequence: tccaaatttctacctctcatctcctccatcctgtgggctgaacagtata
acagactcccaGTATACaaatggtgggagctagatattgggtaggaagccag
atgctaggtatgagagagccaacagcctcagccctctgcttgcttatagATGGA
GAACAACCTctggGCAGAGGTCTCAAAG

Mutant Sequence:

catctcctcccaTCCTGTGGGCTGAACAGTATAACAGACTCCCA
GTAgggctgcaggaattcgatatcaagcttatcgataccgtcgaggacctaATA
ACTTCGTATAGCATACATTATACGAAGTTatattaagggtattgaat
atgatcggaattcctcgagcggccTACAAATGGTGGGAGCTAGATAT
TAGGGTAGGAAGCCAGATGCTAGGTATGAGAGAGCCAAC
AGCCTCAGCCCTCTGCTTGGCTTATAGATGGAGAACAAC
CTAGGCAGAGGTCTCAAAGGCTGGGGCTGTGTTGGACAG

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
35127		CAT CCT GTG GGC TGA ACA		Common	A	
35128		AGA GGG CTG AGG CTG TTG		Wild type Reverse	A	
35129	Fluorophore-1	CCC AGT AGG GCT GCA GGA A	Quencher-1	MUT Probe		
35130	Fluorophore-2	ACT CCC AGT ATA CAA ATG GTG GGA	Quencher-2	WT Probe		
oIMR7882		CGA AGT TAT TAG GTC CCT CGA C		Mutant Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
35127	0.40 uM
35128	0.40 uM
oIMR7882	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.

