

## B6.129-Ucp1<sup>tm1Kz</sup>/J

Stock No: 003124

Protocol 22204: QPCR Assay - Generic Neo Quantitative PCR-QPCR- 1.2

Version 8.0

### Notes

Taqman qPCR protocols are run on an ABI 7500, 7700, 7900 or the Roche LightCycler 480. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing  $\Delta C_t$  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

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
13741	Fluorophore-1	CAT CGC ATC GAG CGA GCA CGT ACT	Quencher-1	Tg Probe		
oIMR1080		CGG CTG CAT ACG CTT GAT C		Transgene Forward	A	
oIMR1081		CGA CAA GAC CGG CTT CCA T		Transgene Reverse	A	
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-2	CCA ATG GTC GGG CAC TGC TCA A	Quencher-2	IC Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH <sub>2</sub> O	
oIMR1080	0.40 uM
oIMR1081	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.