

C57BL/6J-*Tbk1*^{em3Lutz}/1J

Stock No: 027080

Protocol 22082: End Point Analysis Assay - Smcx/Smcy-PROBE

Version 2.0

Notes

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

Male = X/Y (Het)

Female = X/X (Wt)

For Standard PCR: Omit probes. Primers will produce the following:

WT (X Chromosome) = 123 bp

Mut (Y Chromosome) = 78 bp

Males will appear "het"

Females will appear "wt"

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21115		AAA GGA ATT CTG GAG GCT GG		Mutant Forward	A	
21116		TCT TTG GAG TGA ATG TCT GCT C		Mutant Reverse	A	
21117	Fluorophore-1	AGC AGC ATT GAG GAG GAT GTG ACA G	Quencher-1	MUT Probe		
21118	Fluorophore-2	GAC TAG GTT CAT AGG CAC TGG	Quencher-2	Wild type Forward	A	
21119	Fluorophore-3	CCG CCA AAA CTC CTT CTC TAC	Quencher-3	Wild type Reverse	A	
21120	Fluorophore-4	CCC CAG ATG GTA CCC ACA GAA CTT G	Quencher-4	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
21115	0.40 uM
21116	0.40 uM
21118	0.40 uM
21119	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

