C57BL/6J-*Ghrhr*^{lit}/J

Stock No: 000533
Protocol 18775: Sanger sequencing Assay - Ghrhr<lit>-SEQ
Version 1.0

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

WT = A/A
Het = A/G
Mut = G/G

Sequence

tcagaatctcctccctcataggttaccccatgtctcctccttctccacctagGATGCC
CTGGGACCTGGGa/gTGGGCTGCTGTGCTGGCCCCCGAC
AGGCTCTGGCCAGTGGGTCTCTCTCCCCTGCCCTGAATT
CTTCTCTCACTTCGGCTCAGACACAGgt

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
27611		ATG TCC CTG GTC CTG ACA TC		Forward	A	
27612		ACC ACC TCC CTT TCT CCA GT		Reverse	A	

Reaction A

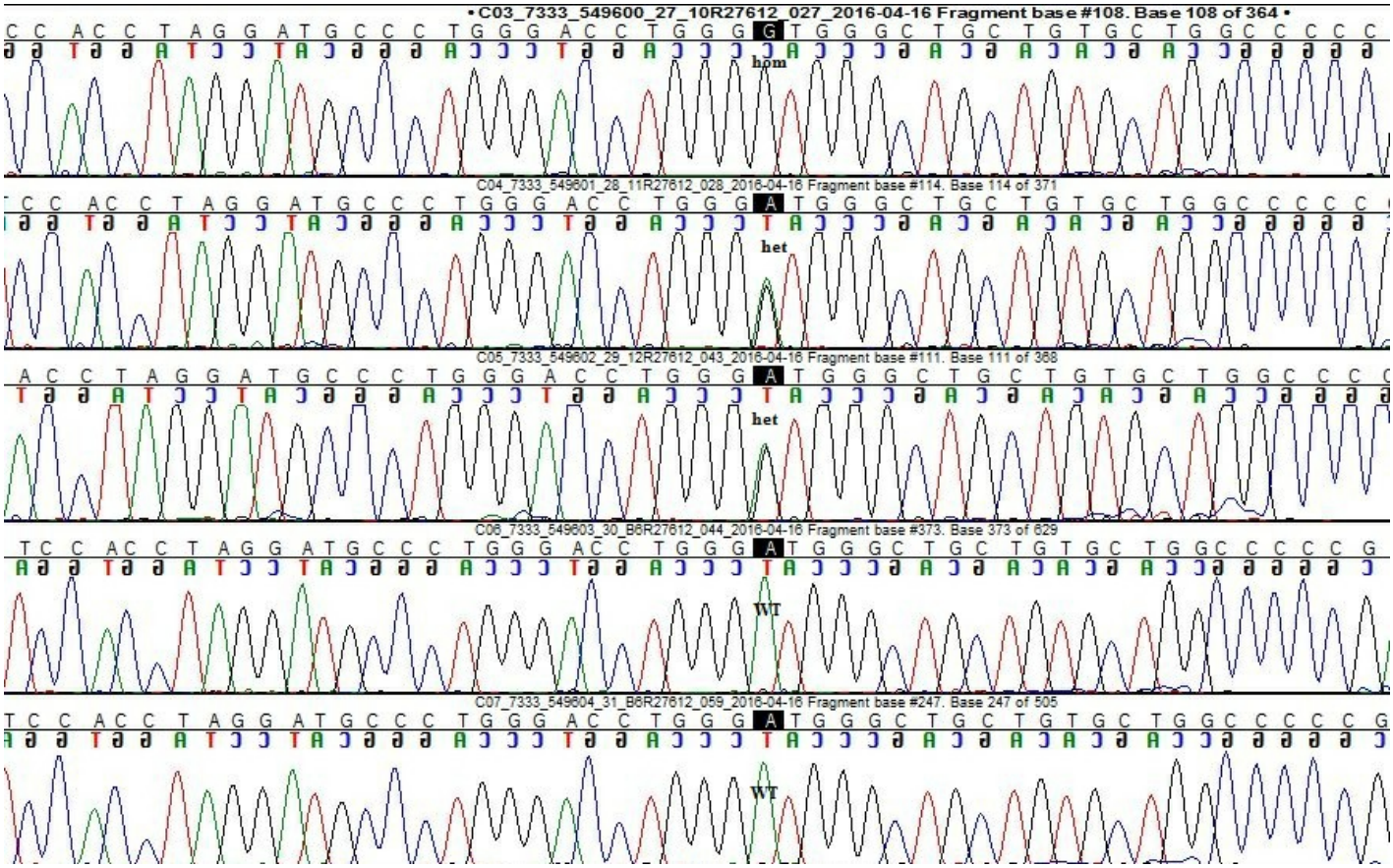
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
27611	0.50 uM
27612	0.50 uM
Glycerol	6.50 %
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.


• C03_7333_549600_27_10R27612_027_2016-04-16 Fragment base #108. Base 108 of 364 •
hom
C04_7333_549601_28_11R27612_028_2016-04-16 Fragment base #114. Base 114 of 371
het
C05_7333_549602_29_12R27612_043_2016-04-16 Fragment base #111. Base 111 of 368
het
C06_7333_549603_30_B6R27612_044_2016-04-16 Fragment base #373. Base 373 of 629
WT
C07_7333_549604_31_B6R27612_059_2016-04-16 Fragment base #247. Base 247 of 505
WT