

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	32829
MMRRC Strain Name	B6.129- <i>Ift172</i> ^{<i>tm1.1Rama</i>} /Mmnc
Gene Name(s)	intraflagellar transport 172 homolog (Chlamydomonas) (<i>ift172</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	7/22/13

MMRRC #32829 PCR Reaction

Thermal Cycler:

- Step 1: 95°C for 5 min
- Step 2: 95°C for 30 sec
- Step 3: 66°C for 30 sec
- Step 4: 72°C for 30 sec
- Step 5: 30x from step 2 to step 4
- Step 6: 72°C for 10 min

	<u>1X</u>
ddH ₂ O	13.5
5X Buffer	5.0
25mM MgCl ₂	1.5
10mM dNTPs	0.5
10 μM Primer Forward	1.0
10 μM Primer Reverse	1.0
Apex Taq	0.5
DNA	2.0

Taq: Apex and Chromataq 5X Buffer

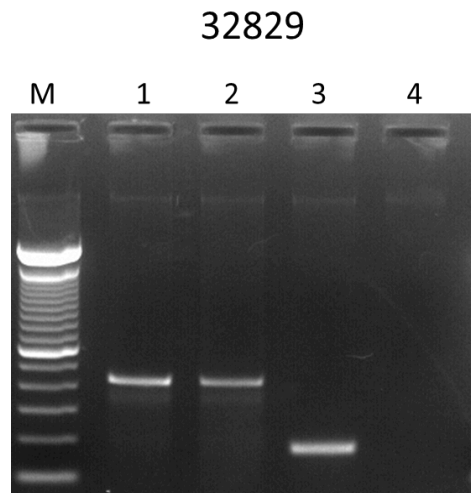
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

IFT172 -Set1-A (32829): TTGGTGCCAGGCATGAGAAATGCC

IFT172 -Set1-B (32829): ACTGGCCTTCCAATCATTCTCCA

Bands expected: MUTANT: 380 bp
 WT: 176 bp

Run on a 2% agarose gel.



Lane 1, 2: Hom; Lane 3: WT; Lane 4: H₂O; M: 100 bp DNA ladder