

02.16.10 MS
 07.30.10 HB updated
 03.11.11 MS updated
 09.11.17 MLS updated

Genotyping Protocol: **MMRRC 29995**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: Exons 3-22 of the piwi-like homolog 1 gene (*Piwil1*) were replaced with a loxP-flanked PGK-neo cassette preceded by a GFP reporter gene. This results in a GFP fusion product after the first 9 amino acids of the *Piwil1* gene. Details can be found in Deng, W and Lin, H (2002) *Developmental Cell* 2:819-830.

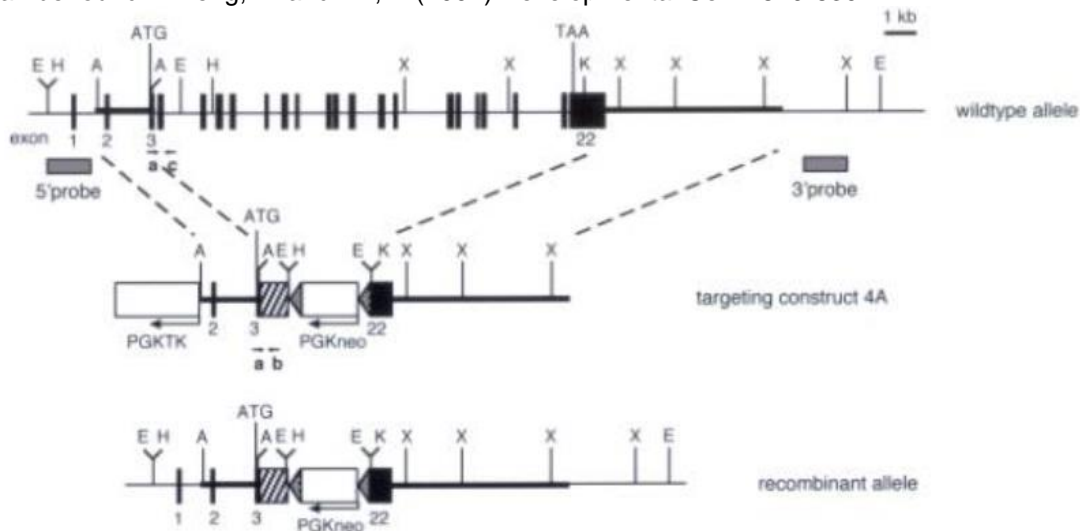


Image from Supplemental Material from Deng, W and Lin, H (2002) *Developmental Cell* 2:819-830.

Primer Information:

- | | |
|---------------------|---|
| 1) Name: Piwil1.F | Sequence: 5'- TGA TTT GGG GAC TTA TTT TAG AGC -3' |
| 2) Name: Piwil1.R | Sequence: 5'- ACT TAC CTT GTG ACT TGG ATG TG -3' |
| 3) Name: PiwilMut.R | Sequence: 5'- TTG AAA AGC ATT GAA CAC CAT AAG -3' |

Primer location: Piwil1.F is located directly before exon 3 and Piwil1.R is located in exon 4 of the *Piwil1* gene on Chromosome 5. Piwil1Mut.R is located in the targeting construct.

Assay name: **Piwil1 KO PCR**

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
10X Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat#U1515)	1.25mM	3.2
Piwil1.F	Sigma or IDT	25µM	0.3
Piwil1.R	Sigma or IDT	25µM	0.3
PiwilMut.R	Sigma or IDT	25µM	0.3
Faststart <i>Taq</i>	Roche(Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 61°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)

Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)

Method: AM320 Injection: 10s at 5KV

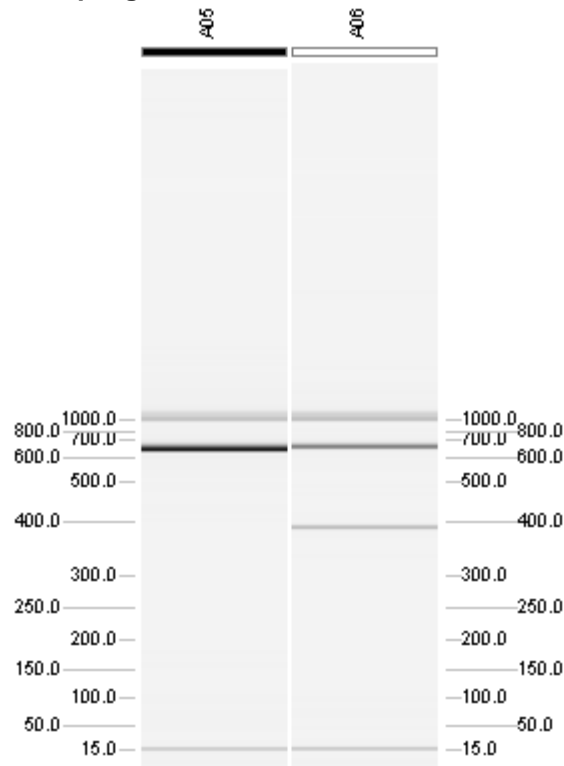
Separation: 320s at 6KV

Expected products:

WT: 643bp

Mut: 390bp

Example gel:



Lane A05 displays a WT sample (643bp band).
Lane A06 displays a heterozygous sample (643bp and 390bp bands).

Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel method and do not represent expected products.