

Genotyping Protocol: **MMRRC 43681**

**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 200ul of AE buffer once.

**Strain Description:** Two *loxP* sites were inserted flanking Exons 4 and 5 of the *Stk25* gene. These *loxP* sites are located in the upstream and downstream introns. Details can be found in Matsuki *et al.* (2013) Neural Development 8:21.

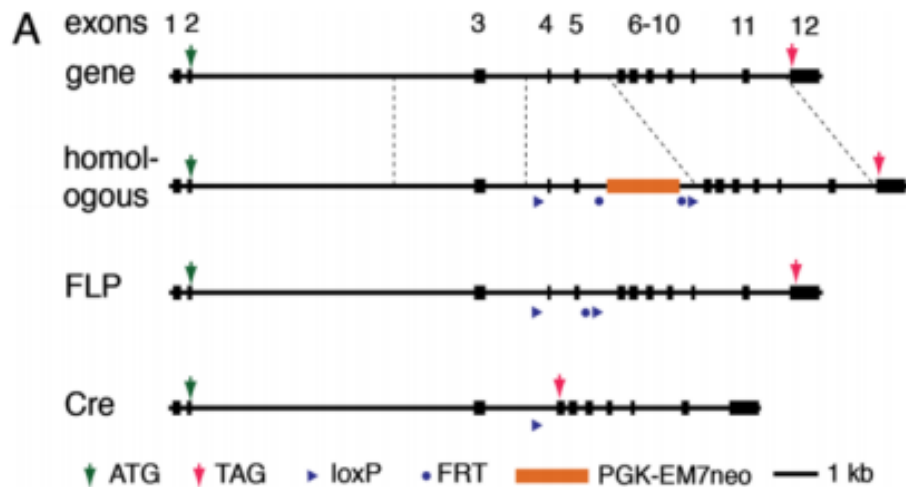


Image from Matsuki *et al.* (2013) Neural Development 8:21.

**Primer Information:**

- 1) Name: M43681 cKO F  
2) Name: M43681 cKO R

Sequence: 5'- TCA GAG AGG CCT TTT CTC CA-3'  
Sequence: 5'- GCC AGC CTG GTC TAC AGA TT-3'

**Primer location:** M43681 cKO F and R are located on either side of the *loxP* site in Intron 3 of *Stk25*.

**Assay name:** *Stk25* flox PCR

**PCR Master Mix Components:**

component	manufacturer	concentration	μl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M43681 cKO F	Sigma or IDT	25 μM	0.3
M43681 cKO R	Sigma or IDT	25 μM	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)

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   | 4 minutes                      |
| 2) | 94°C   | 1 minute                       |
| 3) | 67°C   | 1 minute                       |
| 4) | 72°C   | 1 minute                       |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles |                                |
| 6) | 72°C   | 7 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/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

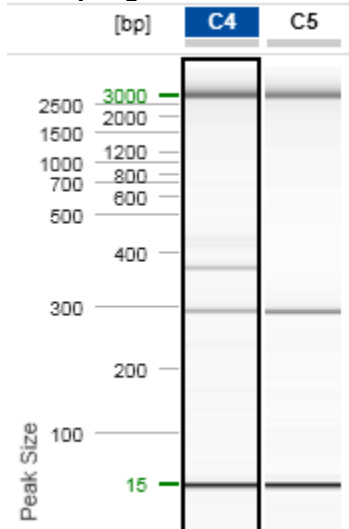
**Expected products:**

Wild-type: 293bp

Homozygous mutant: 375bp

Heterozygous: 293bp, 375bp

**Example gel:**



Lane C4 displays a heterozygous sample (293bp product)  
Lane C5 displays a wild-type sample (293bp and 375bp products)

\*Heterozygotes occasionally display a non-specific doublet above the 375bp band.

\*Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products\*