

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36555
<b>MMRRC Strain Name</b>	B6N.129S6-Gapdhs <sup>tm1<sup>Dao</sup></sup> /Mmnc
<b>Gene Name(s)</b>	glyceraldehyde-3-phosphate dehydrogenase, spermatogenic (Gapdhs)
<b>Breeding Protocol(s)</b>	Sib-Mating (heterozygous x heterozygous)
<b>Protocol Date</b>	2/26/13

### MMRRC # 36555 PCR Reaction

**2,2 Primer Reaction: (WT-F + R) for wild-type and (KO-F + R) for mutant reaction**

**Thermal Cycler:**

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 65°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10uM WT Primer	1
10uM Mut Primer	1
Taq	0.5
DNA	2

Taq: **Apex Taq and Chromataq 5X Buffer**

**Bands:** MUTANT: 610bp

WT: 389bp

**Primer sequences 5' to 3':**

R (36555): GAG CCA TTG GAG GTG TCA CTG GGA

KO-F (36555): CGC ATC GCC TTC TAT CGC CTT CTT

WT-F(36555): CAG TGG TGC TTG GAG ACC CTA ACC

Run on 2% agarose gel in TAE.