

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29172
MMRRC Strain Name	129- <i>Xist</i> ^{tm2Jae} /Mmnc
Gene Name(s)	Inactive X specific transcripts (<i>Xist</i>)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	12/9/13

MMRRC# 29172 PCR Reaction

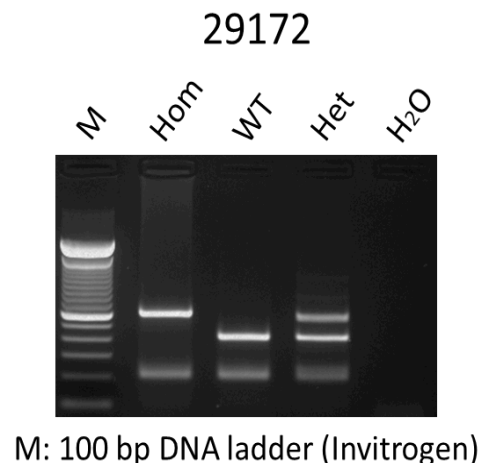
	<u>1X</u>	<u>8X</u>	<u>10X</u>	<u>15X</u>	<u>20X</u>	<u>25X</u>
DNA	1.0	8.0	10.0	15.0	20.0	25.0
10X Buffer (w/out Mg)	5.0	40.0	50.0	75.0	100.0	125.0
25 mM MgCl ₂	2.0	16.0	20.0	30.0	40.0	50.0
10mM dNTPs	0.5	4.0	5.0	7.5	10.0	12.5
10 uM Primer 1	0.5	4.0	5.0	7.5	10.0	12.5
10 uM Primer 2	0.5	4.0	5.0	7.5	10.0	12.5
10 uM Primer 3	0.5	4.0	5.0	7.5	10.0	12.5
Taq	0.5	4.0	5.0	7.5	8.0	12.5
ddH ₂ O	<u>15.0</u>	<u>120.0</u>	<u>150.0</u>	<u>225.0</u>	<u>300.0</u>	<u>375.0</u>
	25.0	200.0	250.0	375.0	500.0	625.0

Denaturing: 94°C for 45 sec
 Annealing Temp: 55°C for 45 sec
 Extension: 72°C for 1min
 Cycles: 35
 Taq: **Apex and Denville Buffer**

Bands: *Xist2lox*-specific = 613bp
 Xist1lox-specific = 513 bp
 Wild-type-specific = 429 bp

Primer sequences 5' to 3':

Xint3R: CACTGGCAAGGTGAATAGCA
XpromL: TTTCTGGTCTTTGAGGGCAC
5'LoxR: ACCCTTGCCTTTTCCATTTT



Run on 2% agarose gel.