

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	29873
MMRRC Strain Name	B6.129P2- <i>Darc</i> ^{tm1Scp} /Mmnc
Gene Name(s)	Duffy blood group, chemokine receptor (Darc)
Breeding Protocol(s)	Random Intra-Strain Mating
Protocol Date	5/3/13

MMRRC #29873 PCR Reaction

2,2 Primer reaction: WT-F + Com-R for wildtype
and Mut-F + Com-R for mutant reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 63°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 35

Step 6: 72°C for 7 min

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	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: ~650bp

WT: 397bp

Primer sequences 5' to 3':

WT-F(29873): 5'-CCCTTTTCTCCAAACCCAAATGCC-3'

Com-R(29873): 5'-TGCCCACTGCTAACTCTGCCAGAA-3'

Mut-F(29873): 5'-AGCCGATTGTCTGTTGTGCCAGT-3'

Run on 2% agarose gel in TAE.