

OCT4-GFP GENOTYPING PROTOCOL

GENE NAME: OG2 or GOF18 or OCT4 or GFP

PCR PROTOCOL NAME: OCT4 or GFP or OCT4-GFP (not COX10)

PCR REAGENTS: 10X NEB THERMOPOL PCR BUFFER
 NEB THERMOPOL TAQ POLYMERASE
 dNTPs (1.25 uM Standard Working Concentration)

PRIMERS: OCT4 Forward (20uM Standard Working Concentration)
 OCT4 Reverse (20uM Standard Working Concentration)

REACTION VOLUME: 26 ul (25 ul + 1ul DNA)
 Add Master Mix to DNA

Master Mix Calculator

Component (Stock Concentration)	Single Rxn Vol (μ L)	Final Concentration	Total Volume (μ L)
Buffer Stock	10.0 X	2.6 1X	68.6
dATP	1.2 mM	4.3 200.0 μ M	114.4
dCTP	0.0 mM	0.0 0.0 μ M	0.0
dGTP	0.0 mM	0.0 0.0 μ M	0.0
dTTP/dUTP	0.0 mM	0.0 0.0 μ M	0.0
Forward Primer	20.0000 μ M	0.4 300.00 nM	10.3
Reverse Primer	20.0000 μ M	0.4 300.00 nM	10.3
Polymerase	5.0000 U/ μ L	0.2 1.2500 U	6.6
MgCl ₂	0.0000 mM	0.0 0.0000 mM	0.0
Template (Vol)	1.0000 μ L	1.0 -	26.4
	0.0 %	0.0 0.0000 %	0.0
	0.0000 U/ μ L	0.0 0.0000 U	0.0
	0.0000 μ M	0.0 0.0000 nM	0.0
	0.0000 U/ μ L	0.0 0.0000 U	0.0
Distilled Water	-	17.0 -	449.8
Totals:		26.0	686.4

Misc Parameters

Single Rxn Volume (μ L):

Total Reactions:

Pipetting Excess (%):

Protocols

Protocol:

Protocol Name:

Comments:

THERMOCYCLER CONDITIONS:

95 C for 3 min

95 C for 30 sec

51 C for 30 sec 40 cycles

72 C for 45 sec

72 C for 2 min

4 C forever

ELECTROPHORESIS CONDITIONS:

1% Agarose Gel (e.g. 1.5 g of Agarose per 150 ml of 0.5X TBE), 5ul EtBr

Use 20-well combs (not 30-well combs): 1 comb/gel

Add 4ul of Bromophenol Blue dye per PCR reaction

Use 100 bp Ladder (5ul)

Load maximum volume per well

Run 80 V for 1 hour

Score presence (+) or absence (-)

COMMENTS: