

Sex GENOTYPING PROTOCOL

GENE NAME:

PCR PROTOCOL NAME: Sex1

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

PRIMERS: Sex1 Forward (20uM Standard Working Concentration)
 Sex1 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 (μL)	Final Concentration	Total Volume (μL)
Buffer Stock	10.0	1X	66.0
dATP	1.2	4.2 μM	110.0
dCTP	0.0	0.0 μM	0.0
dGTP	0.0	0.0 μM	0.0
dTTP/dUTP	0.0	0.0 μM	0.0
Forward Primer	20.0000	0.6 μM	16.5
Reverse Primer	20.0000	0.6 μM	16.5
Polymerase	5.0000	0.1 U/μL	1.6
MgCl ₂	0.0000	0.0 mM	0.0
Template (Vol)	1.0000	-	26.4
	0.0	0.0 %	0.0
	0.0000	0.0 U/μL	0.0
	0.0000	0.0 μM	0.0
	0.0000	0.0 U/μL	0.0
Distilled Water	-	-	423.0
Totals:	25.0		660.0

Misc Parameters

Single Rxn Volume (μL):

Total Reactions:

Pipetting Excess (%):

Protocols

Protocol:

Protocol Name:

Comments:

NEB: 95/3min, 35x 95/30s-60/1m-72/3m, 72/5m

THERMOCYCLER CONDITIONS: Sex (or Sex1)

95 C for 3 min

95 C for 30 sec
60 C for 1 min 35 cycles
72 C for 3 min

72 C for 5 min
4 C forever

ELECTROPHORESIS CONDITIONS:

2% 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 0.5 hour
Score Females (340 bp) or Males (340bp & 301 bp)

COMMENTS: