

Cre-Recombinase GENOTYPING PROTOCOL

GENE NAME: Cre-Recombinase (works for Tnap-Cre and Zp3-Cre Recombinases)
 PCR PROTOCOL NAME: Cre

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

PRIMERS: Cre Forward (20uM Standard Working Concentration)
 Cre 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	<input type="text" value="10.0"/> X	2.6	1X	68.6
dATP	<input type="text" value="1.2"/> mM	4.3	<input type="text" value="200.0"/> μM	114.4
dCTP	<input type="text" value="0.0"/> mM	0.0	<input type="text" value="0.0"/> μM	0.0
dGTP	<input type="text" value="0.0"/> mM	0.0	<input type="text" value="0.0"/> μM	0.0
dTTP/dUTP	<input type="text" value="0.0"/> mM	0.0	<input type="text" value="0.0"/> μM	0.0
Forward Primer	<input type="text" value="20.0000"/> μM	0.3	<input type="text" value="200.000"/> nM	6.9
Reverse Primer	<input type="text" value="20.0000"/> μM	0.3	<input type="text" value="200.000"/> nM	6.9
Polymerase	<input type="text" value="5.0000"/> U/μL	0.1	<input type="text" value="0.5000"/> U	2.6
MgCl ₂	<input type="text" value="0.0000"/> mM	0.0	<input type="text" value="0.0000"/> mM	0.0
Template (Vol)	<input type="text" value="1.0000"/> μL	1.0	-	26.4
	<input type="text" value="0.0"/> %	0.0	<input type="text" value="0.0000"/> %	0.0
	<input type="text" value="0.0000"/> U/μL	0.0	<input type="text" value="0.0000"/> U	0.0
	<input type="text" value="0.0000"/> μM	0.0	<input type="text" value="0.0000"/> nM	0.0
	<input type="text" value="0.0000"/> U/μL	0.0	<input type="text" value="0.0000"/> U	0.0
Distilled Water	-	17.4	-	460.6
Totals:		26.0		686.4

Misc Parameters

Single Rxn Volume (μL):

Total Reactions:

Pipetting Excess (%):

Protocols

Protocol:

Protocol Name:

Comments:

THERMOCYCLER CONDITIONS:

95 C for 3 min

95 C for 30 sec
53.5 C for 30 sec 45 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: