

ZAP GENOTYPING PROTOCOL

GENE NAME: Z/AP (LacZ/hPLAP Reporter)

PCR PROTOCOL NAME: ZAP3 (NOT Zp3)

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

PRIMERS: ZAP3 Forward (20uM Standard Working Concentration)
 ZAP3 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	68.6
dATP	1.2	200.0 μM	114.4
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	200.000 nM	6.9
Reverse Primer	20.0000	200.000 nM	6.9
Polymerase	5.0000	0.3120 U	1.6
MgCl ₂	0.0000	0.0000 mM	0.0
Template (Vol)	1.0000	-	26.4
	0.0	0.0000 %	0.0
	0.0000	0.0000 U	0.0
	0.0000	0.0000 nM	0.0
	0.0000	0.0000 U	0.0
Distilled Water	-	-	461.6
Totals:	26.0		686.4

Misc Parameters

Single Rxn Volume (μL):

Total Reactions:

Pipetting Excess (%):

Protocols

Protocol:

Protocol Name:

Comments:

NEB buffer

Update Calculations
Generate Summary
Done

THERMOCYCLER CONDITIONS:

95 C for 3 min

95 C for 30 sec
60 C for 30 sec 40 cycles
72 C for 2 min

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 Xylene dye per PCR reaction
Use 100 bp Ladder (5ul)
Load maximum volume per well
Run 80 V for 0.5 hour
Score absence (-) or presence (+) of a ~300bp product

COMMENTS: