

## TNAP GENOTYPING PROTOCOL

GENE NAME: TNAP-Cre Recombinase

PCR PROTOCOL NAME: TNAP

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

PRIMERS: TNAP-Cre Forward (20uM Standard Working Concentration)  
 TNAP-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	10.0	X	2.6
dATP	1.2	mM	4.3
dCTP	0.0	mM	0.0
dGTP	0.0	mM	0.0
dTTP/dUTP	0.0	mM	0.0
Forward Primer	20.0000	μM	0.3
Reverse Primer	20.0000	μM	0.3
Polymerase	5.0000	U/μL	0.3
MgCl <sub>2</sub>	0.0000	mM	0.0
Template (Vol)	1.0000	μL	1.0
	0.0	%	0.0
	0.0000	U/μL	0.0
	0.0000	μM	0.0
	0.0000	U/μL	0.0
Distilled Water	-		17.3
Totals:			26.0

  

Misc Parameters	
Single Rxn Volume (μL):	26.0
Total Reactions:	24
Pipetting Excess (%):	10.0

  

Protocols	
Protocol	TNAP PCR
Protocol Name	TNAP PCR
Comments	NEB

Save
Delete

Update Calculations
Generate Summary
Done

THERMOCYCLER CONDITIONS:

95 C for 3 min

95 C for 30 sec

56 C for 45 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 0.5 hour

Score absence (-) or presence (+) of a ~300bp product

COMMENTS: