OneTaq (NEB)

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

Add to a sterile thin-walled PCR tube:

Component	50 µl (our lab)	50 µl reaction	Final Concentration
5X One*Taq* Standard Reaction Buffer*	10 µl	10 µl	1X
10 mM dNTPs (#N0447)	1 µl	1 µl	200 µM
10 µM Forward Primer	2 µl	1 µl	0.2 µM
10 µM Reverse Primer	2 µl	1 µl	0.2 µM
One*Taq* DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR**
Bodo	0.125 µl		
Template DNA	variable	variable	< 1,000 ng
Nuclease-free water	to 50 µl	to 50 µl	

*One*Taq* GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons

**For amplicons between 3–6 kb, use 2.5–5 units/50 µl rxn

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute per kb
Final Extension	68°C	5 minutes

Hold	4-10°C	