Standard Thermal Cycling: 25 µL Endpoint PCR

1. For all components except CleanAmp™ dNTPs and DNA polymerase, thaw the reaction components, vortex to mix, centrifuge briefly and store on ice.
2. Prepare CleanAmp™ dNTPs:
   a. Thaw at room temperature or on ice.
   b. Vortex and pulse centrifuge to thoroughly mix.
   c. If necessary, remove an aliquot of the stock solution and dilute with water or buffer (pH 8-10.5) to desired working concentration.
3. Prepare a mastermix containing all components except for the DNA template sample. Add each of the components as shown in Table 1 (multiply amounts by the number of reactions needed) in a centrifuge tube, on ice.
4. Mix the mastermix gently to protect the enzyme, by pipetting up and down. (Do not Vortex.) Pulse spin if necessary.
5. Aliquot 20 µL of mastermix into each thin-walled PCR tube.
6. To each 20 µL aliquot of mastermix, add 5 µL of the appropriate template DNA for a final reaction volume of 25 µL.
7. Pulse spin PCR tubes to remove bubbles and collect reaction solution at bottom of tube.
8. Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions for target amplification:
   95°C for 10 min
   [95°C for 40 sec; X°C[1] for 1-30 sec; 72°C for 0.5-2 min]
   35-40 cycles 72°C for 10 min
9. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

Table 1

Component	Final Concentration (25 µL reaction)	Volume per reaction
Forward/Reverse Primer	50-500 nM	Variable
Sterile De-ionized Water	Up to 25 µL	Up to 25 µL
$MgCl_2$ (50 mM)[2]	4.5 mM	2.25 µL
10X PCR Buffer[3]	1X	2.5 µL
CleanAmp™ dUTP Mix[4] (10 mM)	0.2 mM	1 µL
Taq DNA Polymerase (5 units/µL)	0.05 units/µL	0.25 µL
DNA Template	Variable	Variable
Total Volume (µL)	**25 µL**	**25 µL**

[1] The annealing temperature should be chosen for optimal PCR performance. Most primer design software recommends an annealing temperature. The annealing temperature can also be optimized experimentally by using a thermal cycler with gradient functionality or by performing sequential experiments in which the annealing temperature is varied.
[2] For protocols utilizing alternate DNA polymerases, the $MgCl_2$ concentration can be optimized between 3.0 and 5.0 mM.
[3] 10X PCR buffer for Invitrogen's *Taq* DNA polymerase (200 mM Tris-HCl, pH 8.4 at 25ºC; 500 mM KCl)
[4] CleanAmp™ dUTP should be used in a 3-fold excess over dATP, dCTP and dGTP.