# SeqAmp™ DNA Polymerase Protocol-At-A-Glance

**SeqAmp DNA Polymerase** (Cat. Nos. 638504 and 638509) is a high fidelity PCR enzyme with hot start capabilities that is well-suited for use with specific SMARTer® kits for next-generation sequencing (NGS). This optimized PCR enzyme has been shown to perform well even with challenging templates containing GC-rich and AT-rich regions. When using SeqAmp DNA Polymerase with a SMARTer kit, follow the protocol in the particular SMARTer kit user manual. For other applications use the protocol below.

1. Set up the reaction by adding each component indicated in Table 1. To determine how much template to use, refer to Table 2. After adding all the components, mix by tapping the bottom of the tube, then centrifuge briefly.

**NOTE:** Keep enzymes and reagents on ice.

**Table 1. Recommended Reaction Set-Up**

| Reagent | Volume | Final Conc. |
|---|---|---|
| 2X SeqAmp PCR Buffer (includes $Mg^{2+}$, dNTPs) | 25 µl | 1X[1] |
| Primer 1 | 10–15 pmol | 0.2–0.3 µM[2] |
| Primer 2 | 10–15 pmol | 0.2–0.3 µM[2] |
| Template | < 500 ng | |
| SeqAmp DNA Polymerase | 1 µl | 1.25 U/50 µl |
| Sterilized distilled water | up to 50 µl | |
| **Total volume per reaction** | **50.0 µl** | |

[1] $Mg^{2+}$ (1 mM); dNTPs (200 µM each).

[2] When amplifying fragments ≥10 kb, use 0.2 µM.

**Table 2. Recommended Amounts of Purified DNA and cDNA Templates**

| DNA Source | Recommended Amount (standard) | Recommended Amount (long targets) |
|---|---|---|
| Human genomic DNA | 5–500 ng | 100–500 ng |
| *E. coli* genomic DNA | 100 pg–200 ng | 10–200 ng |
| Plasmid DNA | 10 pg–10 ng | 1–10 ng |
| cDNA | 25–750 ng | 250–750 ng |

**NOTE:** This product is incompatible with bisulfite-treated DNA or other uracil-containing templates.

2. Program your thermal cycler with one of the following sets of cycling conditions:

**a. 3-Step PCR**

| | | |
|---|---|---|
| 94°C | 1 min[1] | |
| 98°C | 10 sec | 30 cycles |
| 55 or 60°C[2] | 15 sec | |
| 68°C[3] | 30 sec/kb[4] | |

**b. 2-Step PCR**

| | | |
|---|---|---|
| 94°C | 1 min[1] | |
| 98°C | 10 sec | 30 cycles |
| 68°C | 30 sec/kb[4] | |

[1] When amplifying a GC-rich region or a long target, perform the initial denaturation at 94°C for 1 min.

[2] Set the annealing temperature to 60°C for primers with a $T_m$ (determined by the following equation) > 55°C, and to 55°C for primers with a $T_m \leq 55$°C. **NOTE:** $T_m$ (°C) = [ (nA + nT) x 2] + [(nC + nG) x 4 ] − 5

[3] For 3-step PCR, set the extension temperature to 68°C.

[4] When amplifying from crude samples, set the extension time to 1 min/kb.

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