PCR Reactions

I. Standard PCR

Per reaction (25 µl)
20.4 µl dH_2O
2.5 µl 10X buffer
0.5 µl dNTP mix (10 mM each)
0.25 µl each primer (25 µM)
0.1 µl Taq polymerase

1 µl template DNA (1-5 ng/µl)

Prepare a master mix including everything but template DNA; mix well and aliquot before adding template.

Parameters that can be varied: Annealing temperature (45-60°C), primer concentration (this one is on the low end), Mg^{++} concentration (standard is 1.5 mM; reduce for higher stringency or increase for lower stringency), template concentration (1 ng/µl is enough for standard Taq, but high-fidelity polymerases usually require more).

Standard PCR program: 94°C/4 min., 25-35X[94°C/30 s, 55°C/30 s, 72°C/1 min/kb], 72°C 10 min.

II. PCR for sequencing

Per reaction (10 µl)
6.18 µl dH_2O
0.5 µl BigDye v3.1
2 µl 5X sequencing buffer (includes enzyme)
0.32 µl primer (10 µM)

1 µl template DNA (50-100 ng/µl)

Prepare a master mix including everything but template DNA; mix well and aliquot before adding template.

Sequencing program: 96°C/4 min., 30X[96°C/10 s, 50°C/5 s, 60°C/4 min]