PCR Reaction Condition Guidelines

Primer Concentration	▸ Final primer concentration should be 100 - 500 nM, which is equivalent to ~100 to 250 ng of an 18- to 25-mer oligonucleotide primer in a 100-µl reaction volume. ▸ Primers should be salt-free and gel-purified.
Template Concentration	▸ When the concentration is known, the reaction should contain at least 10 DNA template molecules. ▸ Template A260/A280 ratios should be between 1.8 to 2.0. ▸ The recommended amount of DNA template is 25 to 100 ng per 100-µl reaction volume (*)pfu* polymerase may need ~250 ng genomic DNA), for amplifying single-copy chromosomal targets. Excessively high concentrations of starting DNA can inhibit amplification reactions (> 500-1000 ng). ▸ When amplifying lambda or plasmid PCR targets and multi-copy chromosomal genes, less DNA can be used. For example: 10 to 100 ng of DNA template per 100-µl reaction volume is generally recommended. ▸ For higher GC content, 1% to 10% DMSO may be added to relax secondary structures but should only be used when necessary. DMSO may increase error rate at higher concentrations (enzyme blends are more affected). ▸ Glycerol from 5% to 20% can also be used in high GC reactions. Glycerol has also been found to act as an enzyme stabilizer.
General Tips	▸ Avoid prolonged denaturing times as these can damage DNA templates and dNTPs, as well negatively affect certain PCR polymerases. For example: 2 to 5 minutes should be sufficient for an initial denaturing step in most applications. ▸ Denaturing temperatures should be between 92° to 95°C for most targets. ▸ Use the appropriate buffer and cycling parameters recommended by Stratagene for your particular polymerase. ▸ Polymerases are sensitive to Mg2+ concentration. ▸ Please follow guidelines in your user manual specific to your PCR enzyme regarding Mg2+.