# PCR Optimization: PCR Steps

There are three major steps in a PCR, which are repeated for 30-35 cycles.

1. **Denaturation.** Use a temperature appropriate for polymerase of choice (95°C standard, 92°C for long targets).
2. **Annealing.** Use appropriate temperature based on the calculated TM of primers, usually 5°C below Tm.
3. **Extension.** Typically at 72°C.

- At 70°C-72°C, the activity is optimal for many polymerases. 30 seconds is sufficient for reliable amplification of 1 kb sequences.
- Longer products require longer times: 1 minute per 1 kb
- Longer times may also be helpful in later cycles when product concentration exceeds enzyme concentration and when dNTP and/or primer depletion may become limiting.
- Proofreaders like Pfu typically require 1-2 mintues/kb.