PCR Amplification

1. **PCR Reaction Set Up**:
   - 2.0 µl MgCl₂, 25 mM stock solution
   - 2.5 µl Taq polymerase 10X buffer
   - 2.5 µl dNTP mixture = 18 µl ddH₂O (Store dNTP mixture at -20°C for 1 week.)
     - 0.5 µl dATP (100 mM stock, stored at -70°C)
     - 0.5 µl dCTP ("")
     - 0.5 µl dGTP ("")
     - 0.5 µl dTTP ("")
   - 1 µl Primer 1 (12.5 pmol/µl) (final concentration = 0.5 µM = 0.5 pmol/µl.)
   - 1 µl Primer 2 (12.5 pmol/µl)
   - 1 µl Template DNA (2.5-25 ng; for pure template such as plasmid DNA, use 0.1-1 ng)
   - 15 µl Sterile ddH₂O
   - 25 µl Total volume
   - 0.25 µl Taq polymerase (5 units/µl)
   - 50 µl Mineral oil on top (if not hot top)

2. **Generic Thermal Cycling Parameters**:
   - Denaturation = 94°C.
   - Extension = 68 - 72°C.
   - Aim for annealing temperature = 45 - 55°C.
   - Consider hot start, which can be done by adding polymerase after tubes are at 94°C.
   - Final extension for 5-10 minutes to complete PCR.
   - Hold reaction at 4°C at end.

3. **Optimization**:
   - MgCl₂ is critical, try final [MgCl₂] = 1.0 - 3.0 mM.
   - Template DNA is typically added at 0.1-1 ng/µl. Can try more for genomic DNA.
   - Annealing temperature is critical. Try a range of temperatures with 1-2°C differences.
   - As an alternative, Taq may be added after the reaction reaches 94°C (hot start).
   - DMSO lowers melting temperatures. Try 1-2% (10% can inhibit Taq).
   - Betaine lowers melting temperatures of GC rich regions.
   - Reaction pH can be varied. Try adding small amounts of Tris acid or Tris base.
   - Also, try 0.1-1.0% of gelatin, glycerol, Tween-20, or Triton X-100.

   There are an infinite number of conditions to try. Only a few are listed here. Usually, the parameters mentioned here have a significant influence on PCR amplification.