## PCR (12/23/08)

**For a 50 ul Taq PCR:**

| | | |
|---|---|---|
| 40 | ul | $H_2O$ |
| 5 | ul | 10 x Taq buffer |
| 1.5 | ul | dNTPs (10 uM each dNTP) |
| 1 | ul | Primer 1 (usually 20 – 40 uM) |
| 1 | ul | Primer 2 (usually 20 – 40 uM) |
| 1 | ul | Template |
| 0.5 | ul | Taq Polymerase |
| 50 | ul | TOTAL |

**Thermal Cycler Protocol:**

| | | | |
|---|---|---|---|
| 1 | 96° C | 2 min | *Hot Start* |
| 2 | 96° C | 30 s | *Denaturing* |
| | 50° C | 30 s | *Annealing* |
| | 72° C | 2 min | *Extension* |
| | cycle 30 x | | |
| 3 | 72° C | 5 min | *Final extension* |

***Variations on the thermal cycler protocol:*** For any given PCR, I try the standard protocol first. Typically, I will troubleshoot a failed PCR by modifying the annealing temperature or the extension time. The rule of thumb for the annealing temperature is the Tm – 5° C. The rule of thumb for the extension time is 1 min for every kilobase to be amplified.

**10 x Taq Buffer**

500 mM KCl
10 mM Tris-Cl, pH 8.4
1 mg/ml Gelatin
25 mM $MgCl_2$
0.5% Tween 20