# Reactions - Cycle Sequencing

1. 3.1 µl $ddH_2O$
2. 1.9 µl "5x replacement buffer"
3. 2.0 µl 2µM primer1
4. 1.0 µl sequencing mix (ABI BigDye)

Mix and add 7.8 µl of master mix to each reaction. Add 2 µl purified DNA (approximately 20 ng/µl for standard 500-2000 kb pieces).

1. 96°C for 10 sec. (denature)
2. 42°C for 10 sec. (anneal)
3. 60°C for 240 sec. (extend)

Repeat the first three parameters 35 times (I have found 35 cycles to give better resolution for longer sequencing reactions with greater number of extensions). The 42°C annealing temperature works consistently on all primers, although many primers can be done at higher temperatures. Basically lower annealing temperatures do not matter as long as you do not have alternate annealing sites.

**Cycle sequencing 5x replacement buffer (1 liter)**

400 ml 1M Tris-HCl pH 9.0
10 ml 1M $MgCl_2$
590 ml $dH_20$

Stir for minimum of 1 hour. Take and record conductivity. Filter into 1 L disposable filter unit. Aliquot into 15ml conical tubes. Store at room temperature.