

PCR: BACKGROUND

1. Denature so primer and template will **dissociate** = **95°C**.
2. **Anneal** primer and template: **Typically** = **60°C**
Choose appropriate temperature based on primers:
(4°C per GC's) + (2°C per AT's)
3. **Extend** with Taq Polymerase: optimal temperature **72°C**
 - Begin w/ **one minute** extension time for **≤ 1Kb**.
 - For each add'n 1Kb, add **15s** to extension time.

Sample cycle: 1 = 95°C for 1:00

2 = 95°C for 1:00

3 = 60°C for 0:45

4 = 72°C for 1:00

5 = go to Step 2 for add'n 30 cycles

6 = 72°C for 7:00

7 = 4°C indefinitely

Note: The above program runs @ 2hr.