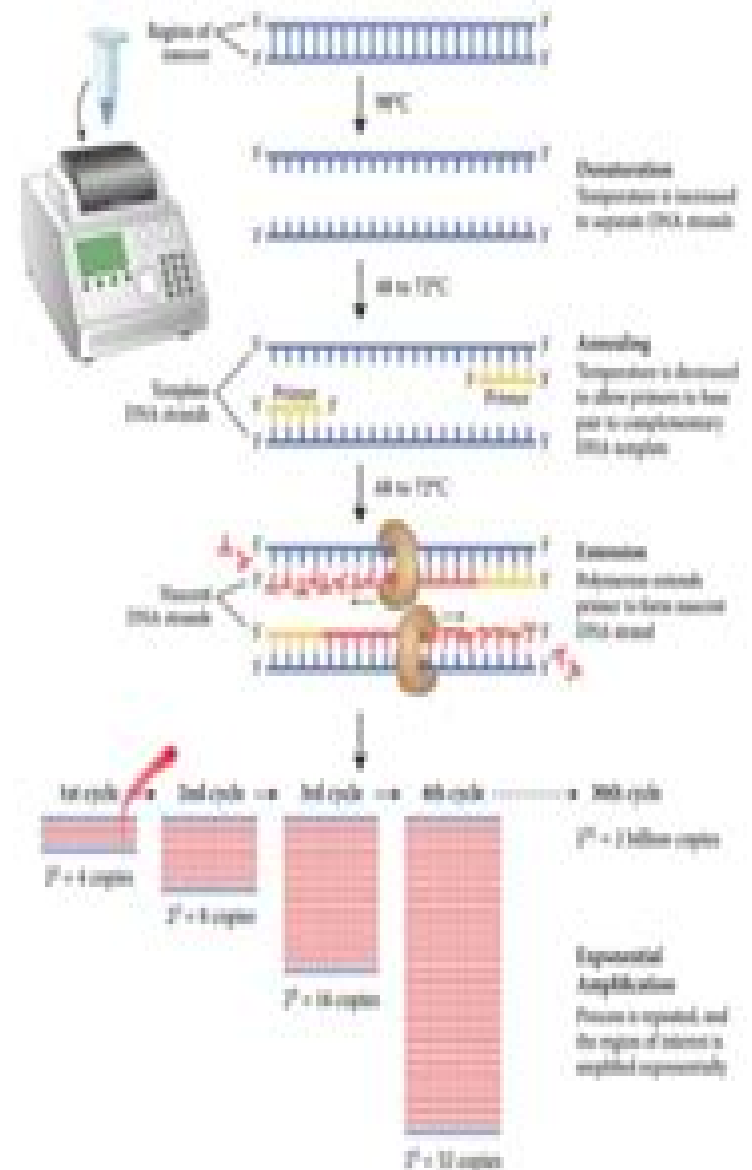


Polymerase Chain Reaction

- DNA extracted from sample and stored at -20.
- PCR requires DNA, primers flanking gene of interest (conferring sequence specificity), heat resistant polymerase (Taq), and nucleotides (dNTPs).
- Taq and dNTPs in buffer (called master mix) are incubated with forward and reverse primers and sample DNA and placed into thermocycler.
 - Strands melted at ~95C
 - When temperature decreases, primers anneal with complementary DNA.
 - Taq polymerase extends primer and replicates target sequence.
- 30 cycles later you have 1 billion copies!
- Sources of error include
 - Mispriming - Primers bind to off target sequence and prime replication.
 - Secondary DNA structure can obstruct primer recognition.
 - Primers dimerize and amplified (primer dimer).



Basic Methods In Molecular Biology

RM Cervero



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