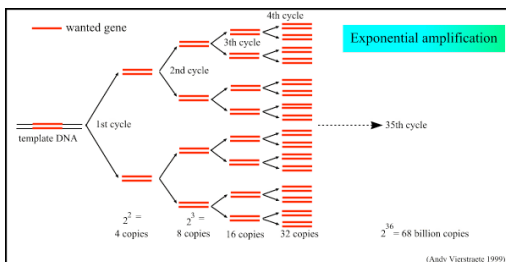
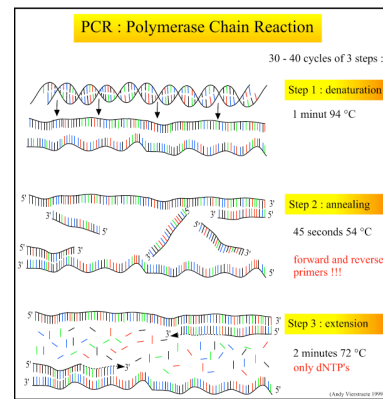
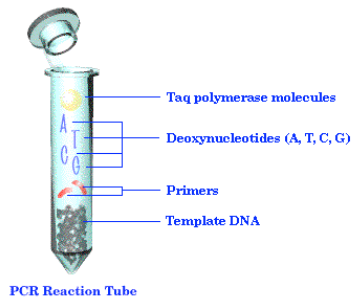


# PCR, gel electrophoresis and DNA sequencing

November 11, 2004

## Polymerase chain reaction (PCR)

- PCR makes huge numbers of copies of a region of interest (usually a gene) on template DNA molecules (usually genomic DNA)
  - Three major steps which are repeated 30-40 times
    - **Denaturation** at 94°C: Double-stranded DNA melts into single strands
    - **Annealing** at 37-60°C (usually 55°C): Lower temperature allows hydrogen bonds to form between complementary bases on primer and template DNA
    - **Extension** at 72°C: At optimal temperature, DNA polymerase replicates DNA strand, adding bases that are complementary to the template strand



## Gel electrophoresis

- Separates molecules in a gel matrix based on their size:
  - An electric field applied to the gel moves the molecules through the gel based on their charge
  - Smaller molecules travel more quickly through the gel matrix than larger molecules
  - Molecules of the same size travel as a group, forming a "band" in the gel that can be visualized when stained
  - Bands of unknown size are compared to molecular size standards to determine their length/weight

