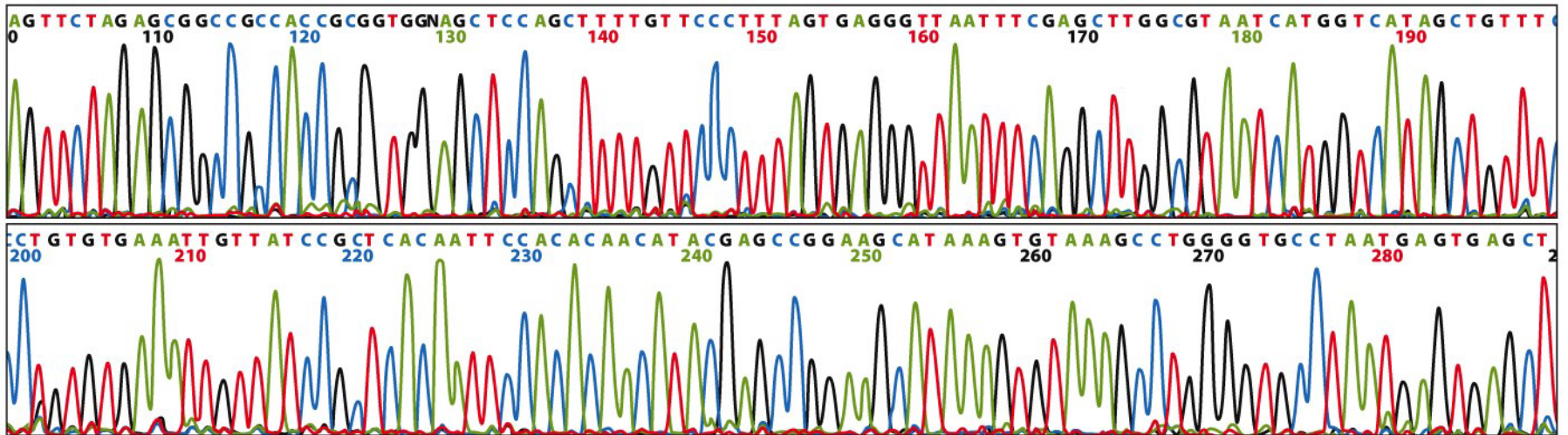


Three Generations of DNA Sequencing

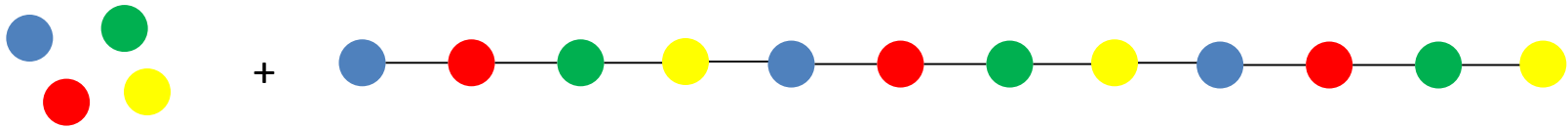


DNA Sequencing

Polymerase Chain Reaction (PCR) revolutionized the fields of biology and biochemistry

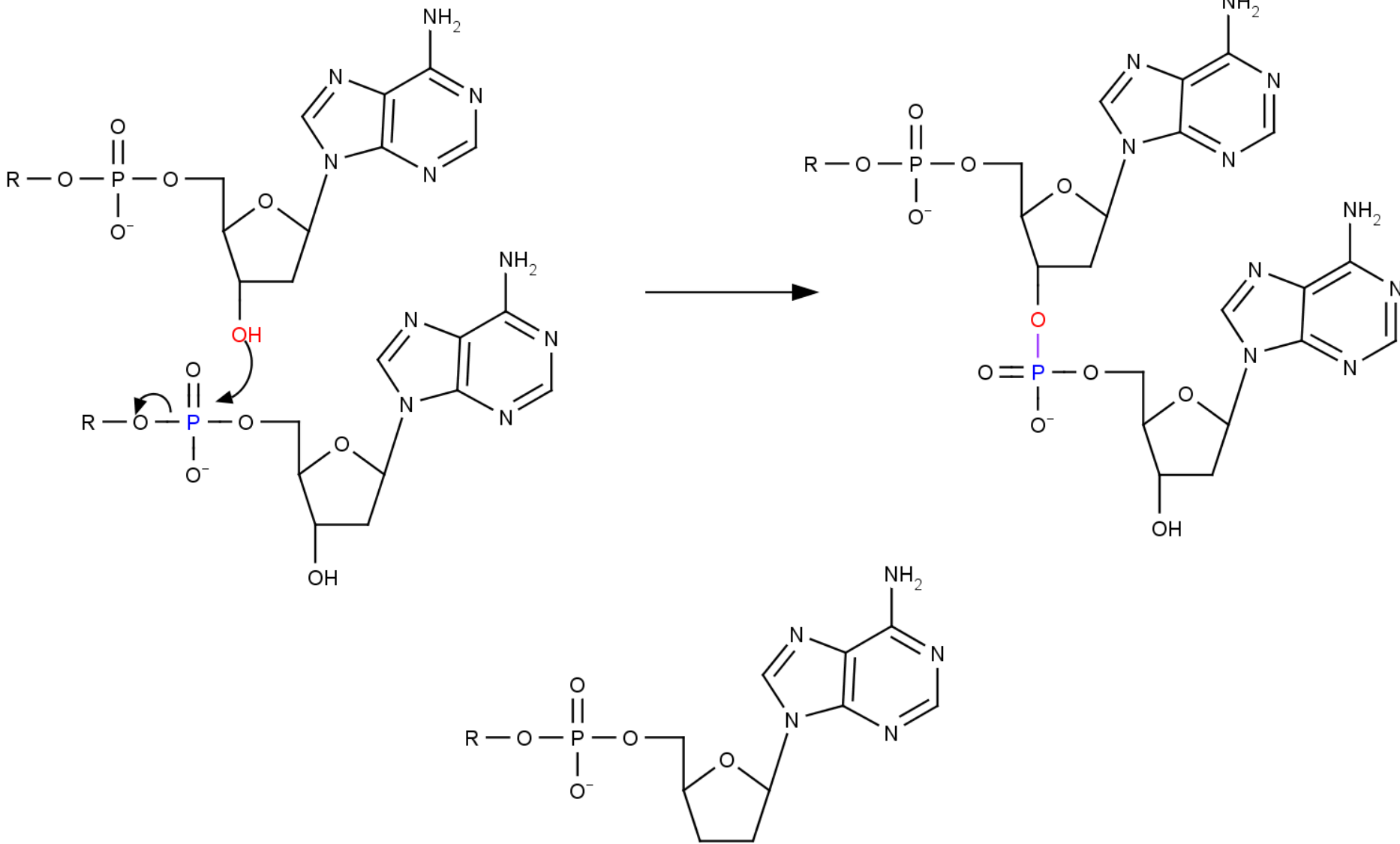
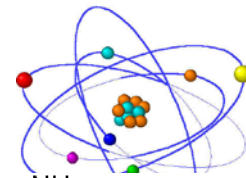


What we need for PCR Reaction:



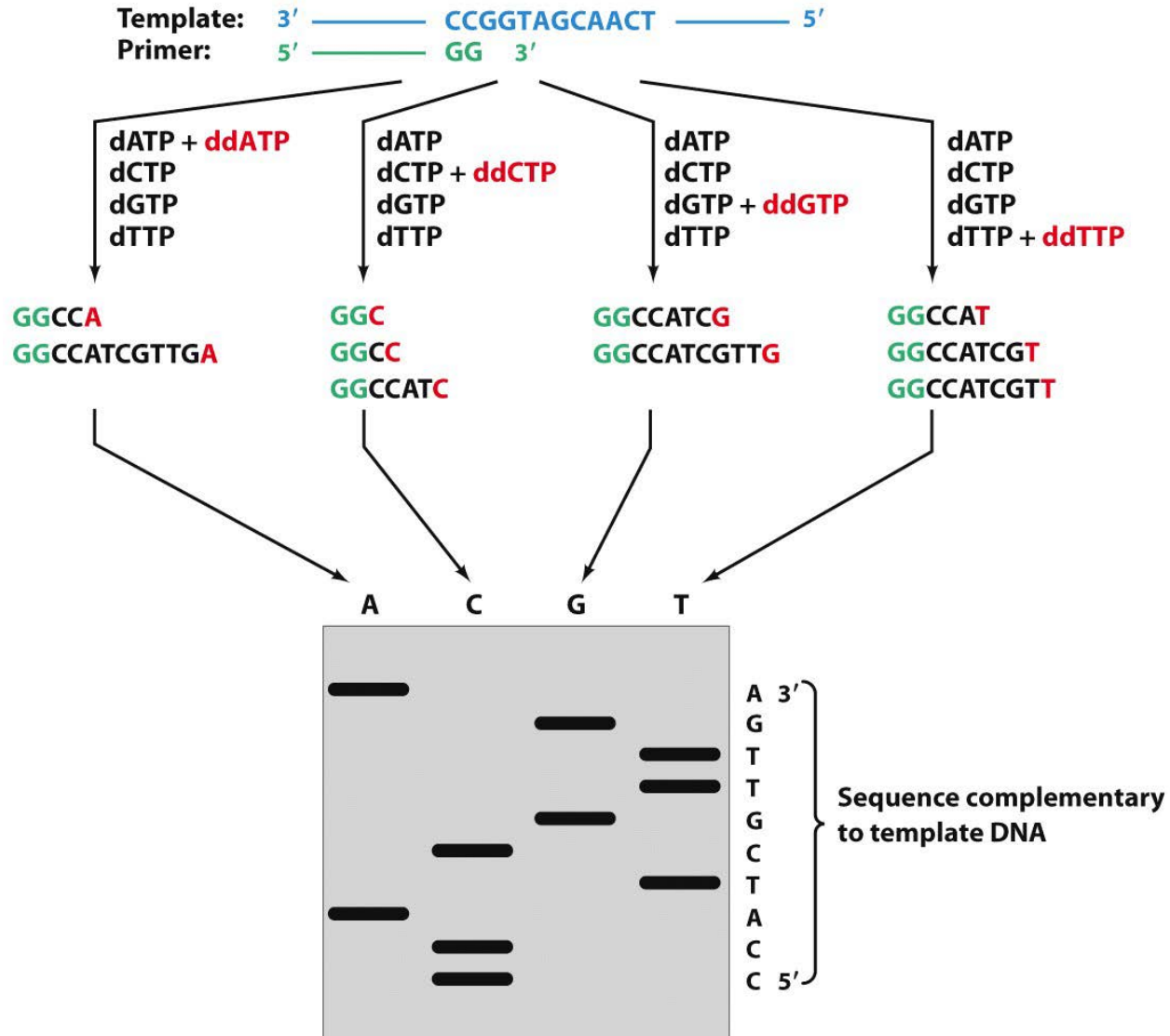
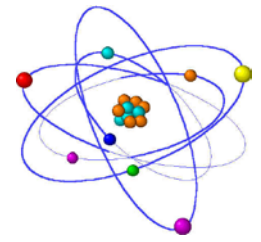
Primers and DNA Polymerase

PCR Termination

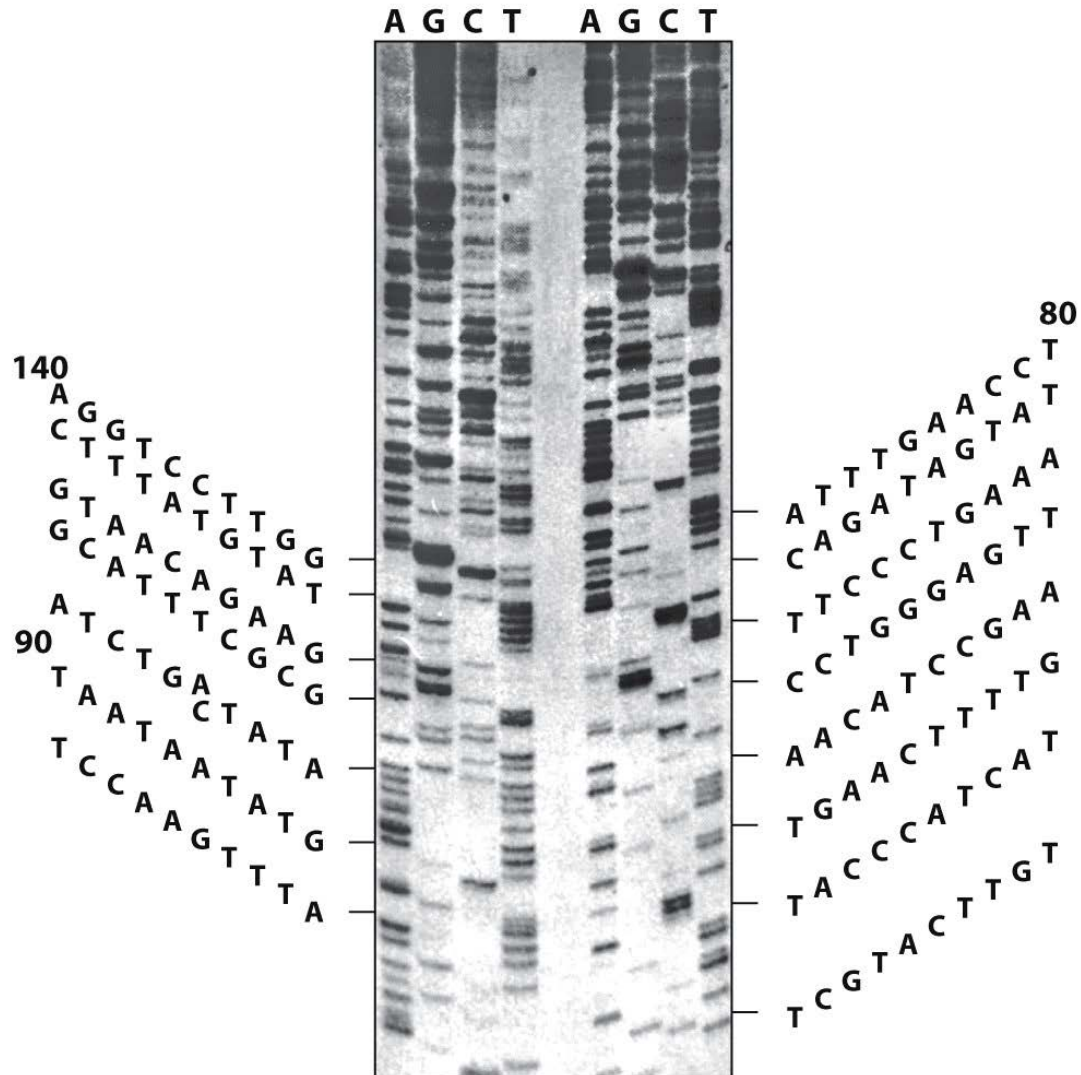
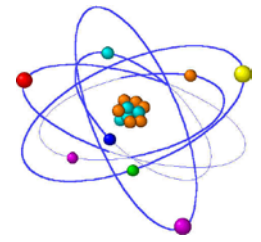


2',3'-dideoxyATP

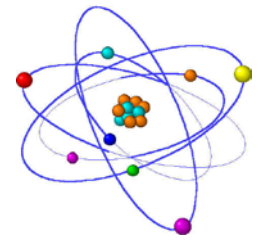
DNA Sequencing



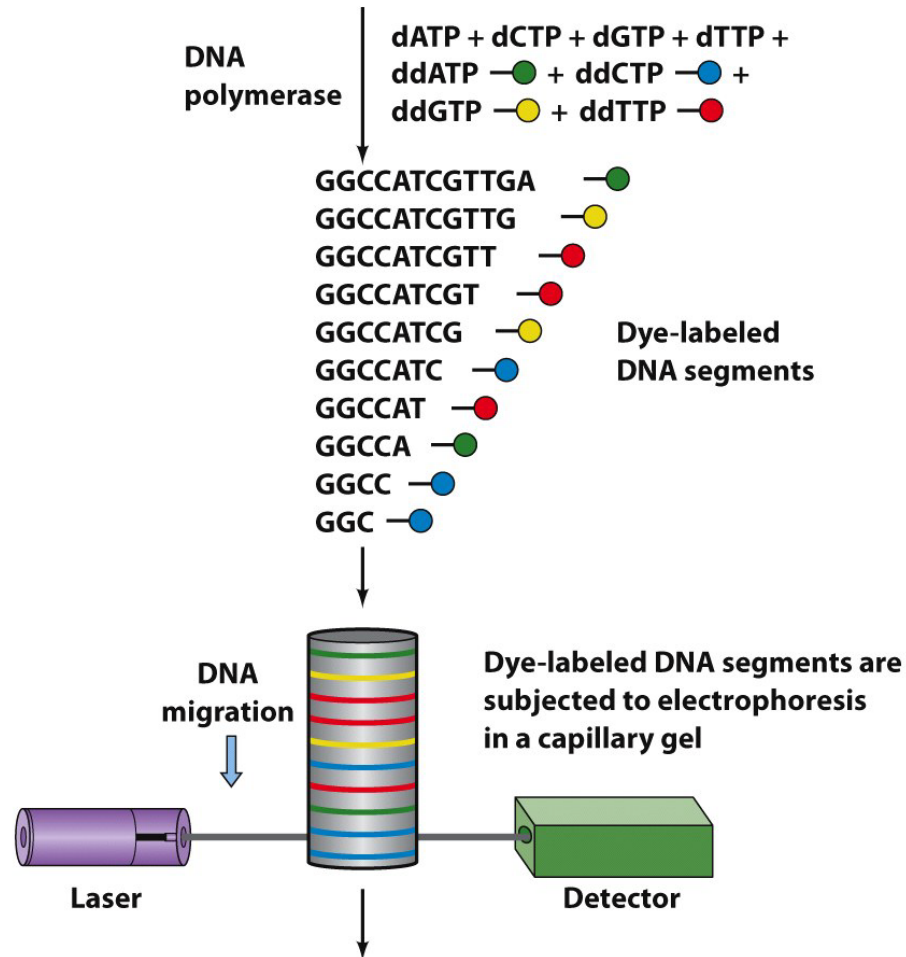
DNA Sequencing



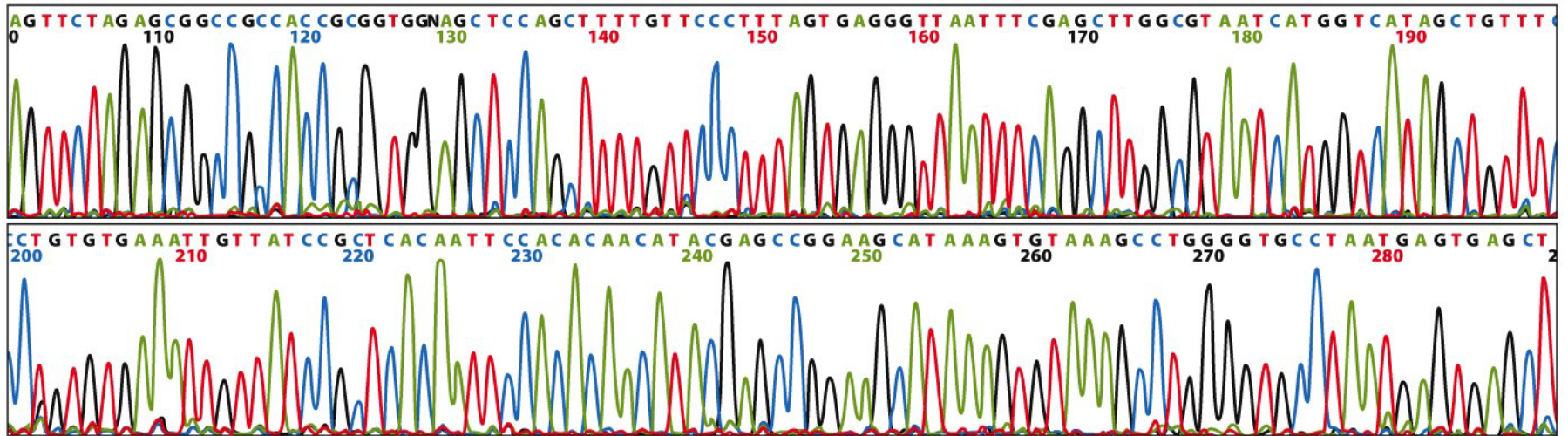
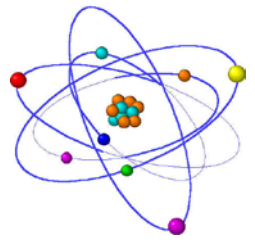
DNA Sequencing



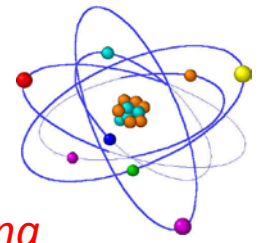
Template: 3' ——— CCGTAGCAACT ——— 5'
Primer: 5' ——— GG 3'



Electropherograms



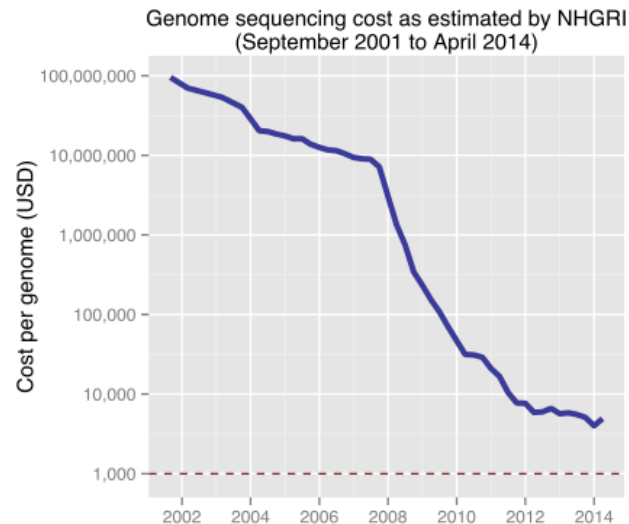
Next Generation Sequencing



Recent advances in sequencing methods has made genome sequencing very fast and cheap!

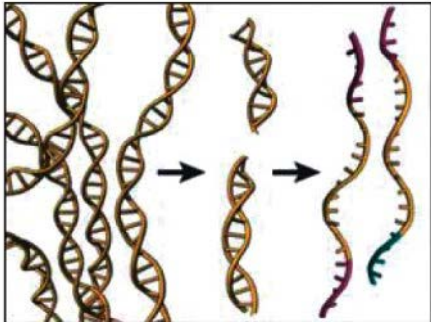
Human Genome Project (used chain termination method) took 10 years to complete and over \$300 million!

Next-Gen techniques allowed the genome of James Watson (of Watson and Crick fame) – the 3rd sequenced human genome) to be sequenced in 2 months for less than \$1M.



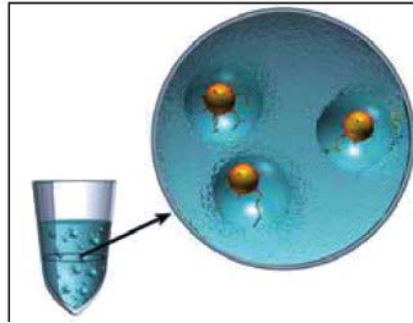
Next Generation Sequencing - Pyrosequencing

DNA is randomly sheared by sonication
300-500 bp fragments produced



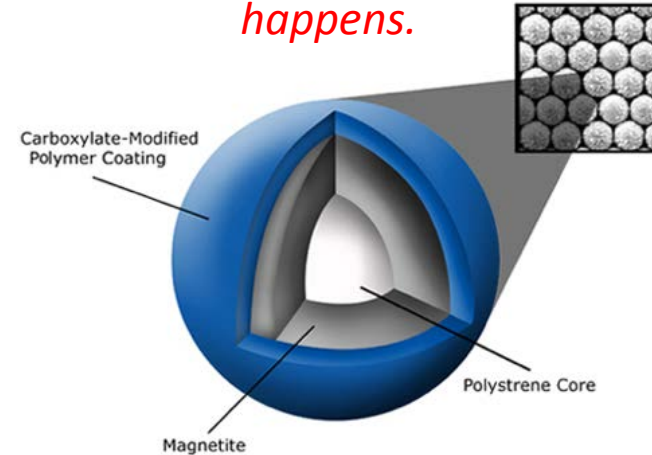
dsDNA is melted apart to form single strands.

ssDNA are bound to DNA "capture" Beads
SPRI Beads = solid phase reversible immobilization



This capture reaction happens under REALLY dilute conditions (< 1 DNA molecule per bead)

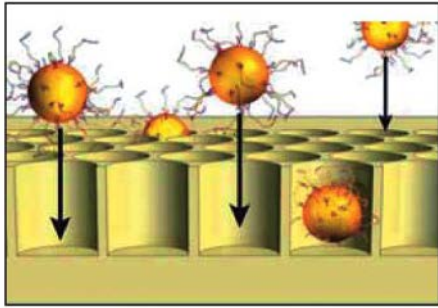
Under high salt conditions (2.5 M NaCl) and PEG, DNA binds to the carboxylate. Believe it, it happens.



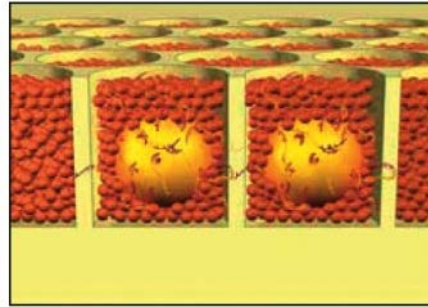
DNA is amplified on the bead by PCR until ~10 million copies are present

Next Generation Sequencing - Pyrosequencing

Beads are transferred to a fiber optic slide with one bead per well.



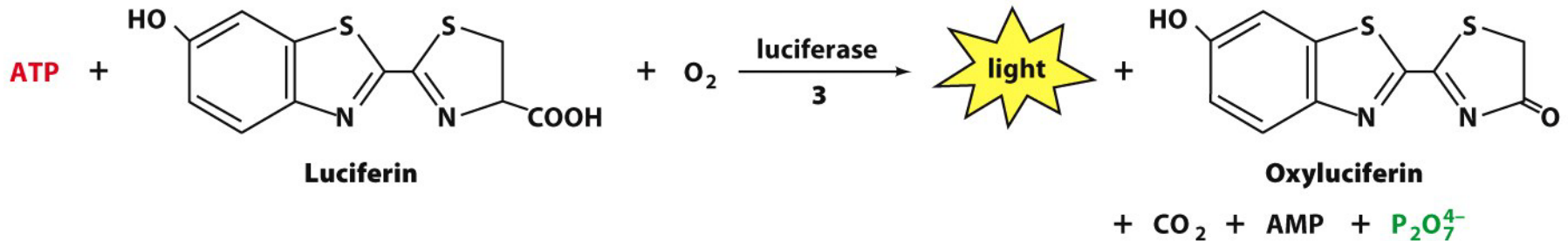
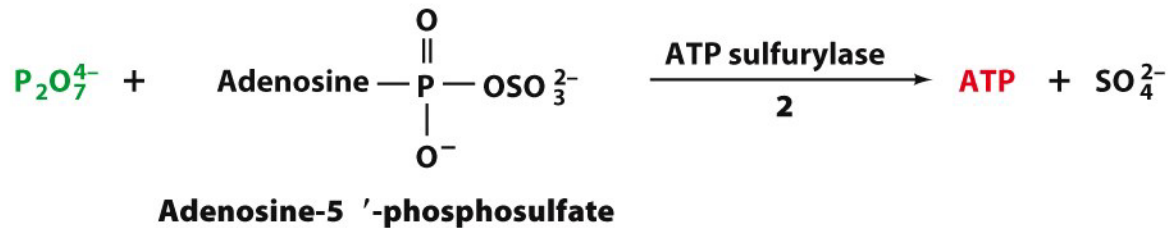
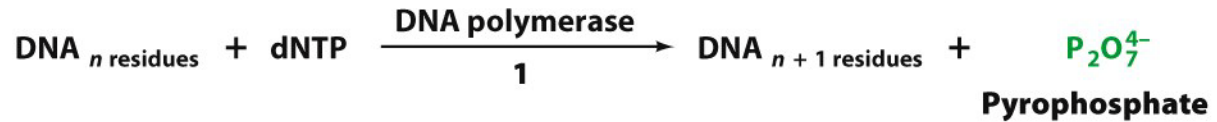
Each well is **75 picoliters** and one slide contains **1.6 million wells**



Pyrosequencing reactions occur in these wells.

1. One nucleotide (let's say dGTP) is added to the slide.
2. A 'burst' of light is seen (sensed through the fiber optic cable) if the dNTP is complementary.
3. Wash off residual dGTP
4. Add dATP – burst – wash
5. Add dCTP – burst – wash
6. Add dTTP – burst – wash
7. Back to 1st step

Next Generation Sequencing - Pyrosequencing



**Once sequencing reactions are done, genome sequence is pieced back together
(shotgun approach)**