X-RAY CRYSTALLOGRAPHY
-xray beam thru protein crystal → collection plate → fast fourier transform→ electron density map
-denisty map
+in 3D
+needs to be high resolution to see alpha carbon backbone and side chains
>this obtained using fourier transformation software

DOING A MOLEULCAR SYNAMIC SIMULATION
-designed around Newtons laws of motion
-atoms test degrees of freedom
-enables protein to 'find' dif transition states
+for the mechanism of function

LEC 8 – POLYMERASE CHAIN REACTION

PCR
-amplify specific DNA sequences
+using oligonucleotide primers
>that're complementary to the 2 strands
>deisnged to allow extension towards eachother by a DNA Pol
-in vitro enzymatic reaction
+primer directed
+produces specific DNA sequence
-primers
+delineate DNA region to be amplified
-thermostable DNA Pol required
-cycles
+20-40 cycles
+denaturation
>one DNA target
>heated to 90-96 degrees
> 15-60 secs (longer for longer fragments)
>separated into single strands
+primer annealing
>50-70 degrees (depends on primer length and the sequence)
>two primers anneal (to the complementary sequences)
>primers define region for amplification
+primer extension (DNA Pol)
> 68-72 degrees (the optimal temp of enzyme)
>DNA synthesis
>DNA Pol (grabs the nucleotides and joins em) makes a copy of the template DNA
-adds nucleotides to ends of primers
-extends primer 5'→3' on both strands
-needs
+target/template DNA
>single or double stranded
> 25 ng to 1 ug
+primers (forwards and reverse)

>single stranded
>18-30 bases
>made in DNA synthesiser
>didstance between them defines length of PCR product

+DNA Pol
>adds nucleotides to 3' end
>needs to be thermostable
>isolated from thermophilic bacteria
>Taq Pol from Thermus aquaticus (half life of 1.6 hours at 95 degrees)
>Pfy Pol from Pyrococcus furiosus (high fidelity)
>Tth Pol from Thermus thermophilus (long range PCR)

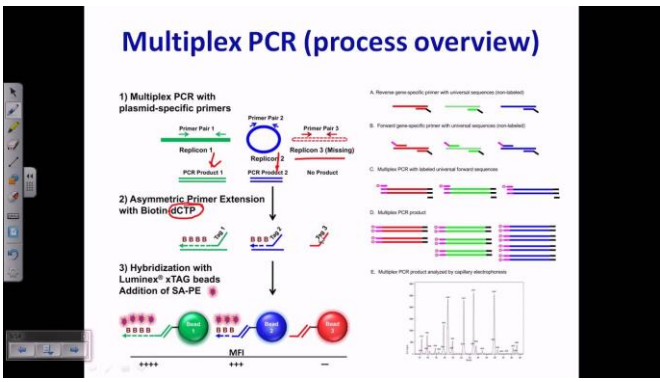
+nucleotides (aka dNTPs)
>A (dATP)
>T (TTP)
>G (dGTP)
>C (dCTP)

+Mg^{2+} ions (via MgCl2 usually)
>amount has effect on primer annealing
>too little = low product
>too much = nonspecific products

PCR PRODUCTS
-aka amplicons
-mispriming
+band above product
+due to nonspecific binding of primers (to non-target sequences)
-primer dimers
+band below product

PCR AUTOMATION
-thermal cyclers
-repeated temp change
-can sometimes have 1536 slots so large amounts can be run

VARIATIONS OF PCR
-multiplex
+add multiple pairs of primers
+amplifies more than one target
+can distinguish b/t products due to dif sizes
+used to test for microorganisms in contaminated food/water
>test for a number of organisms at once



-long range
+normal pcr is 2-3kb
+can do fragments as big at 50kb
+extension step can be 30mins
-reverse transcription (RT-PCR)