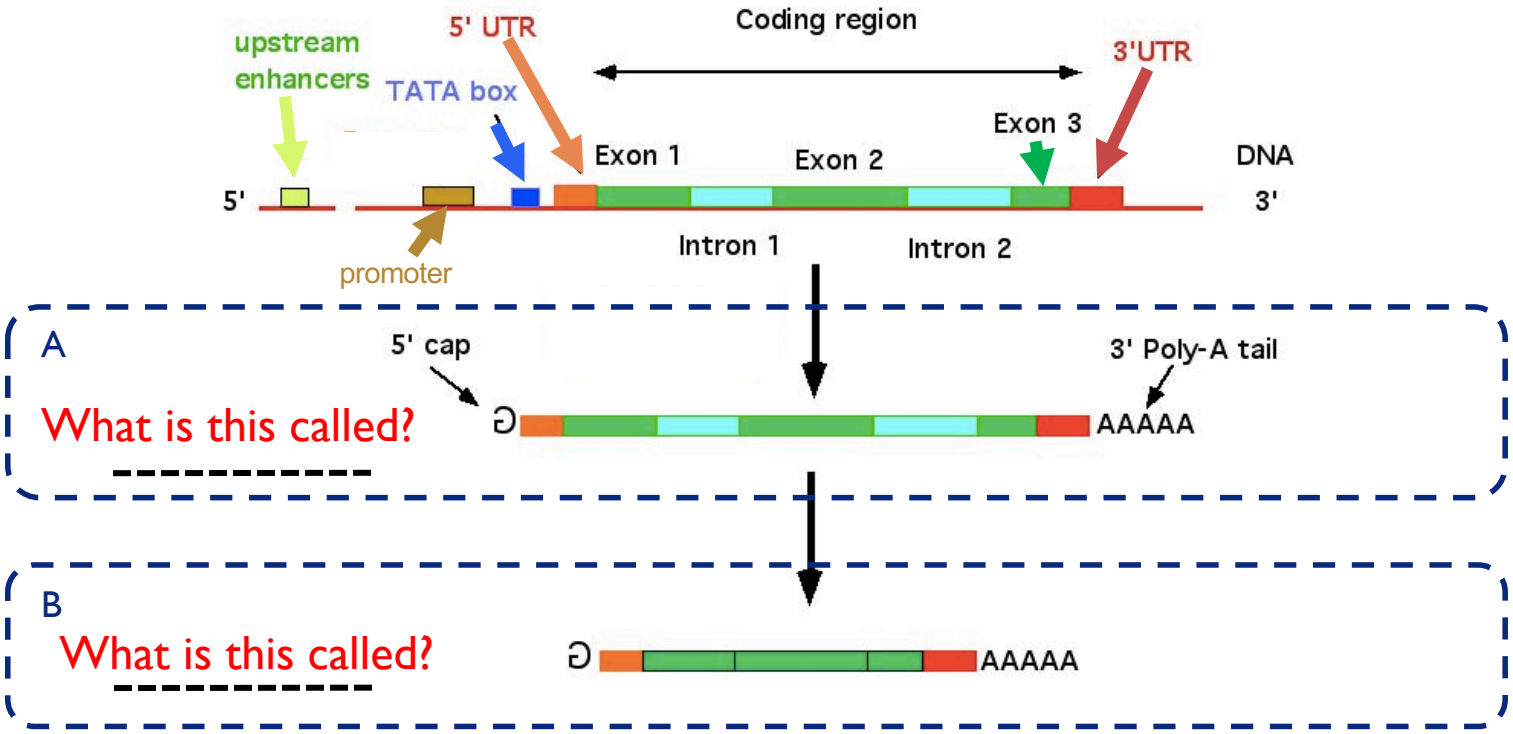
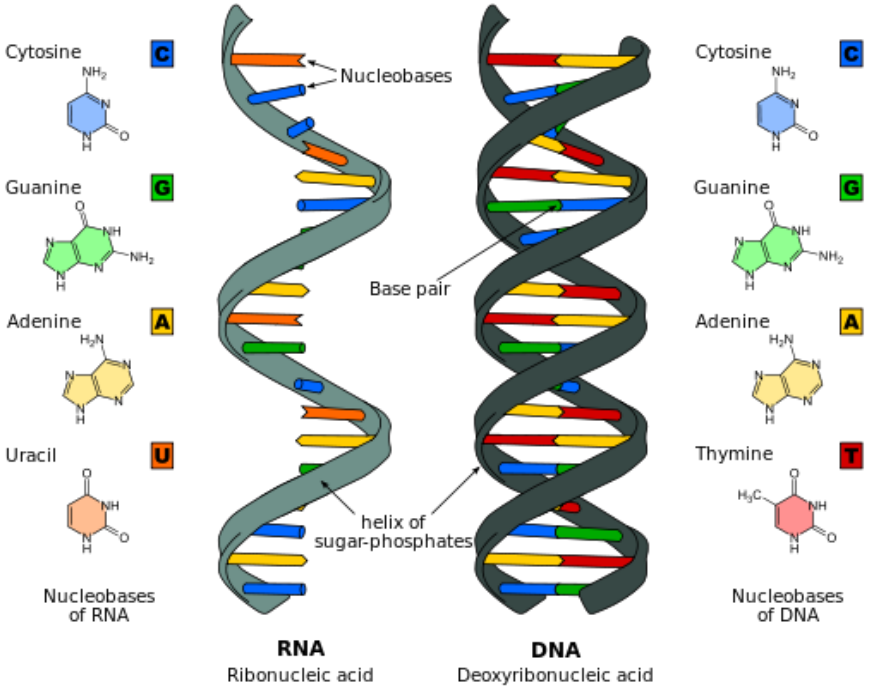
Transcription: a gene in detail



Review: Nucleic acids

	DNA	RNA
Backbone (same or different?) (what is/are the basic units of each made of?)		
Components (same or different?) (what are the components?)		
Chemical Stability		
Macromolecular Structure (most of the time)		
Role in the cell		

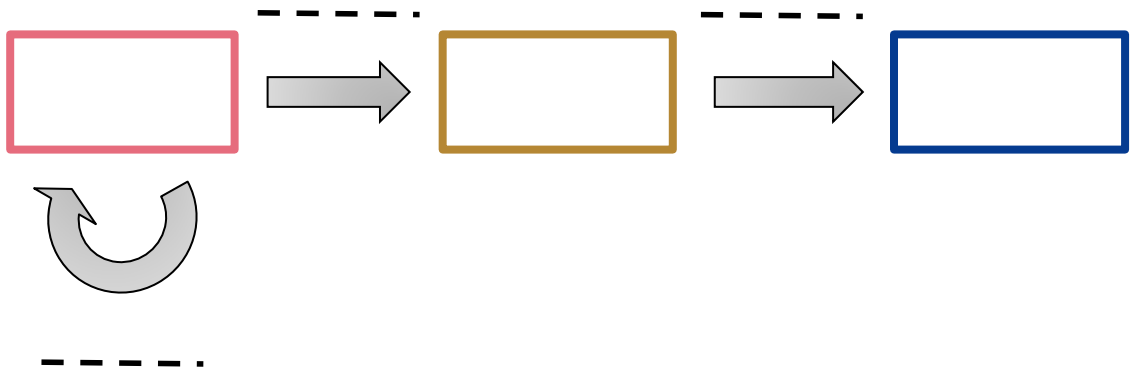
Review: Nucleic acids



By Difference_DNA_RNA-DE.svg: Sponk (talk) translation: Sponk [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0, via Wikimedia Commons

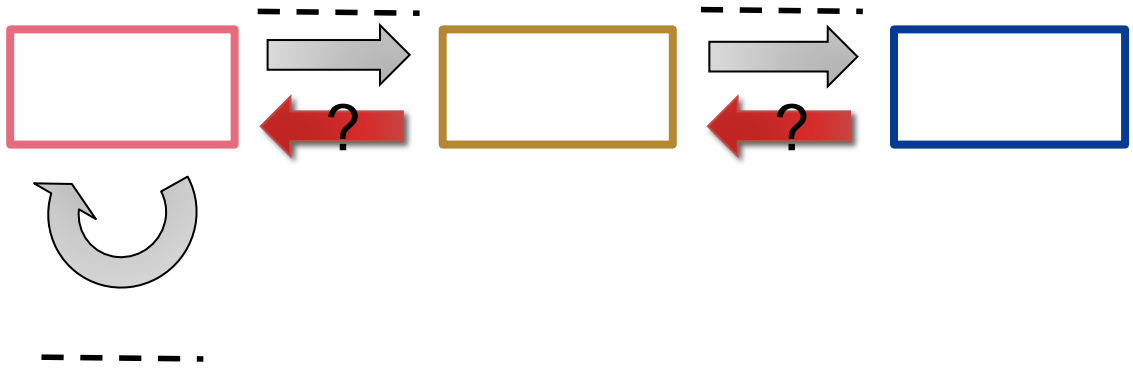
Review: The Central Dogma

- Fill in the blanks:



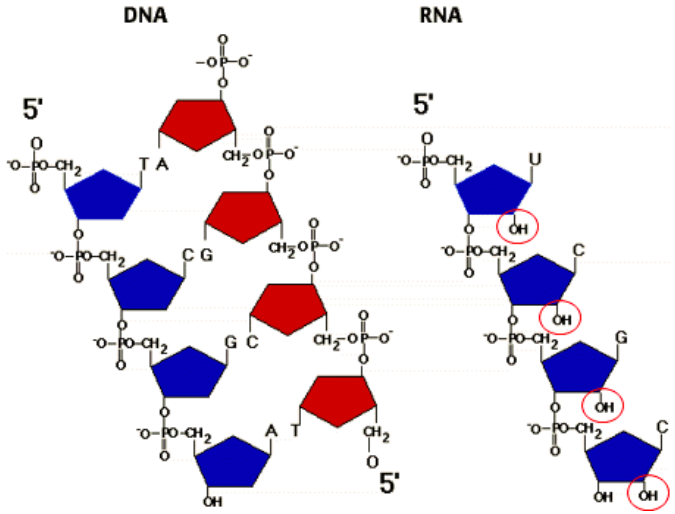
Review: The Central Dogma

- Question:



Review: The Central Dogma

- Question:
  - Why is DNA the medium for "long-term storage" of genetic information and not RNA?



Review: Transcription

- DNA to RNA
- Review: What are the main components involved in transcription?
- Transcription is directional:
  - Enzyme adds dNTP at the 3' end (Why?); this means new RNA is synthesized from the 5' end to the 3' end
  - Question: Relative to the template strand, which direction is the enzyme moving? What about relative to the non-template strand?

Review: Transcription

A. In eukaryotes, what is the name of the molecule that results from transcription? Where in the cell does transcription happen?
B. What happens next to generate molecule A? Where in the cell does this happen?
C. What happens next to generate molecule B? Where in the cell does this happen?
D. What happens next? Where does molecule B go? What is the name of molecule B?

Review: Translation

- Translation is mRNA to protein
- Which direction do you think translation occurs on the mRNA?
- Note on terminology: translation gives PEPTIDES, not proteins. Peptides are long chains of amino acids, but they are not folded yet, and are not proteins, which have secondary structure.

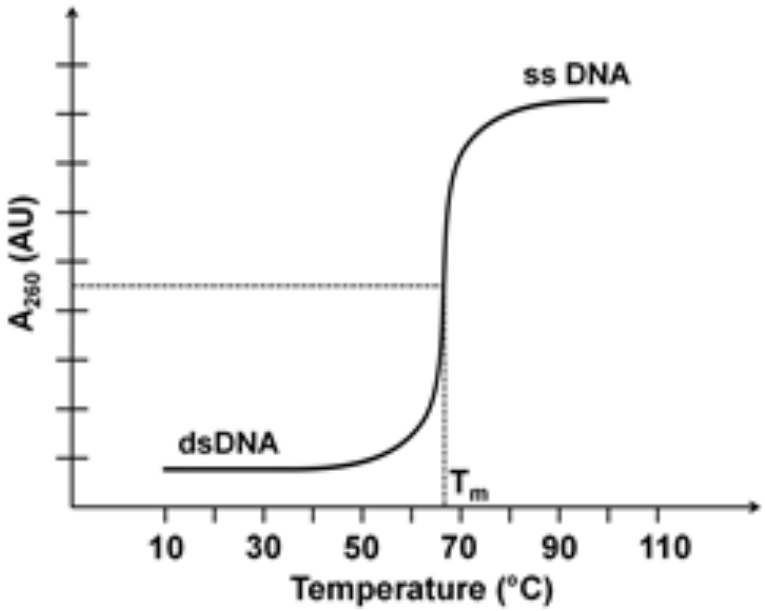
Review: Transcription and translation

- https://youtu.be/-K8Y0ATkkAI?t=35m4s

Review: DNA denaturation

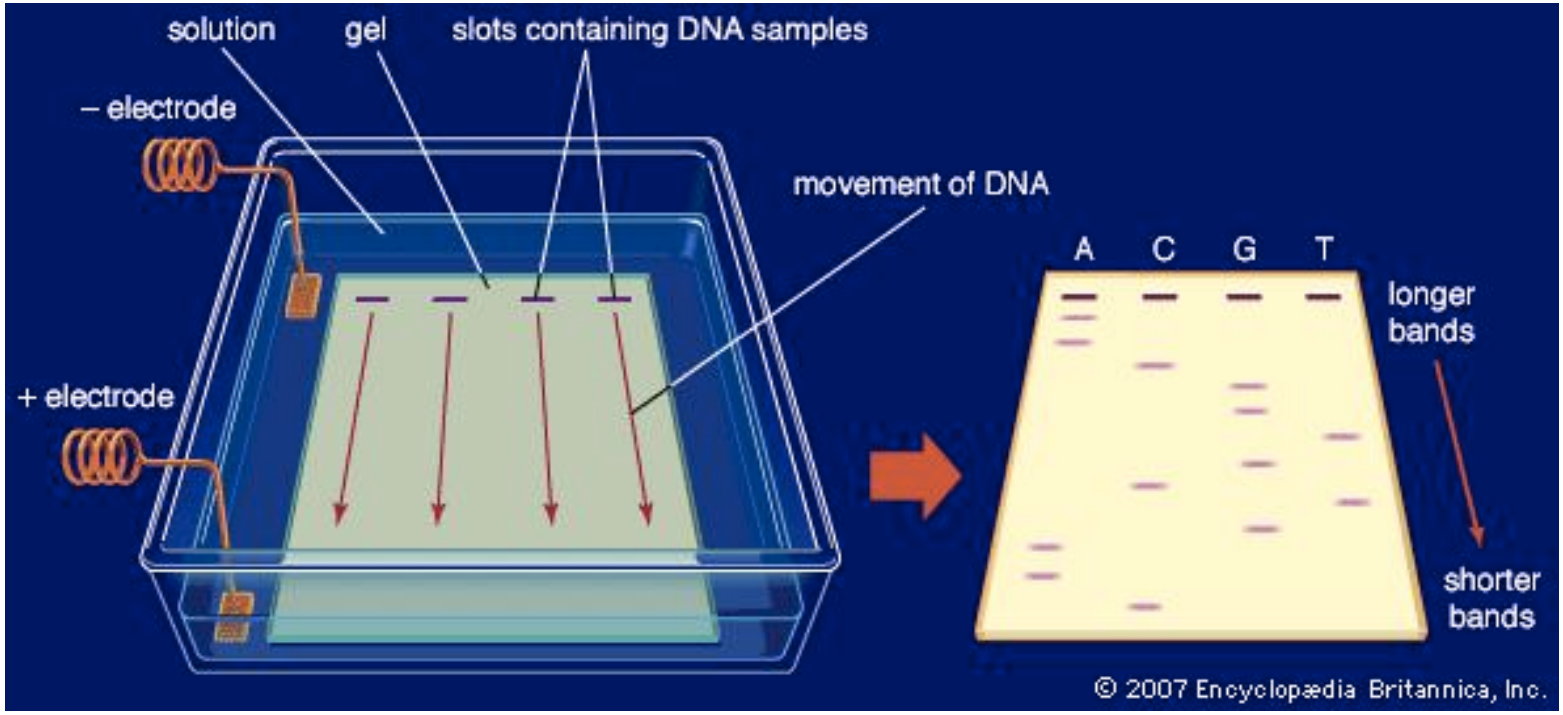
- The temperature where equilibrium state (half dsDNA, half ssDNA) is achieved is called Tm (DNA melting temp)

- Conditions favoring denaturation/melting:
  - **low salt** concentrations: electrostatic repulsion of the negatively charged DNA backbone makes it energetically more favorable to separate the strands
  - **high pH** (basic conditions) also breaks hydrogen bonds
    - High pH = more free OH-
    - More free OH- → deprotonation → disrupt H-bonding



http://nptel.ac.in/courses/102103047/7

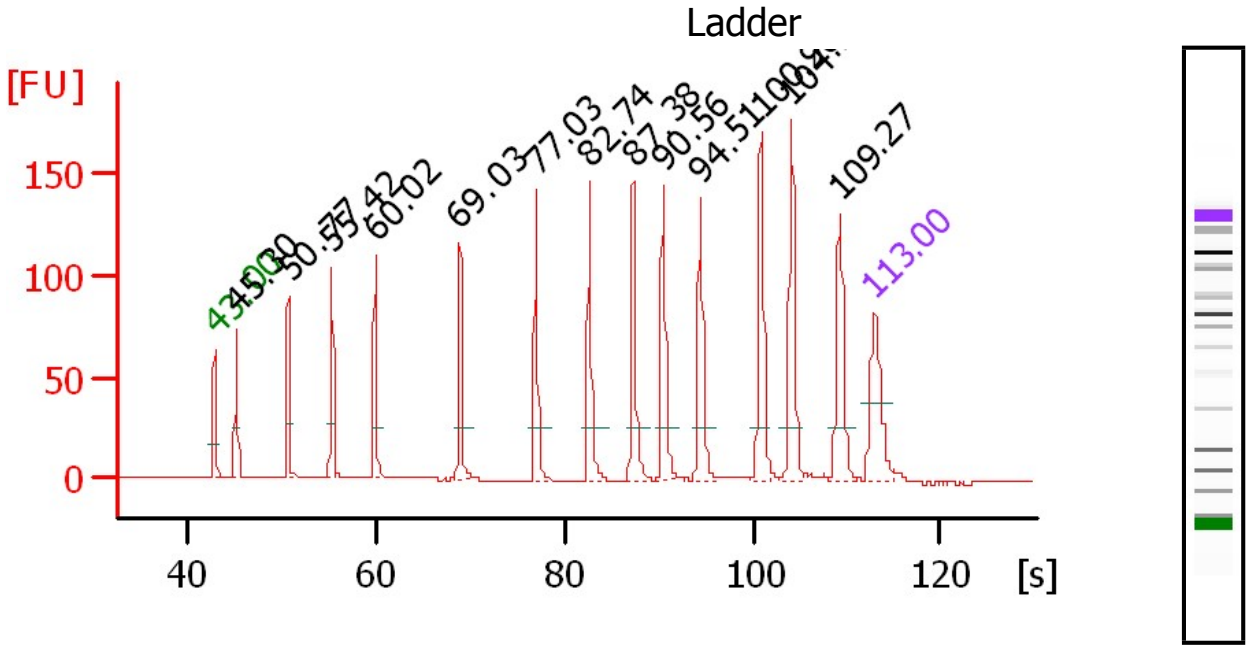
Review: Gel Electrophoresis



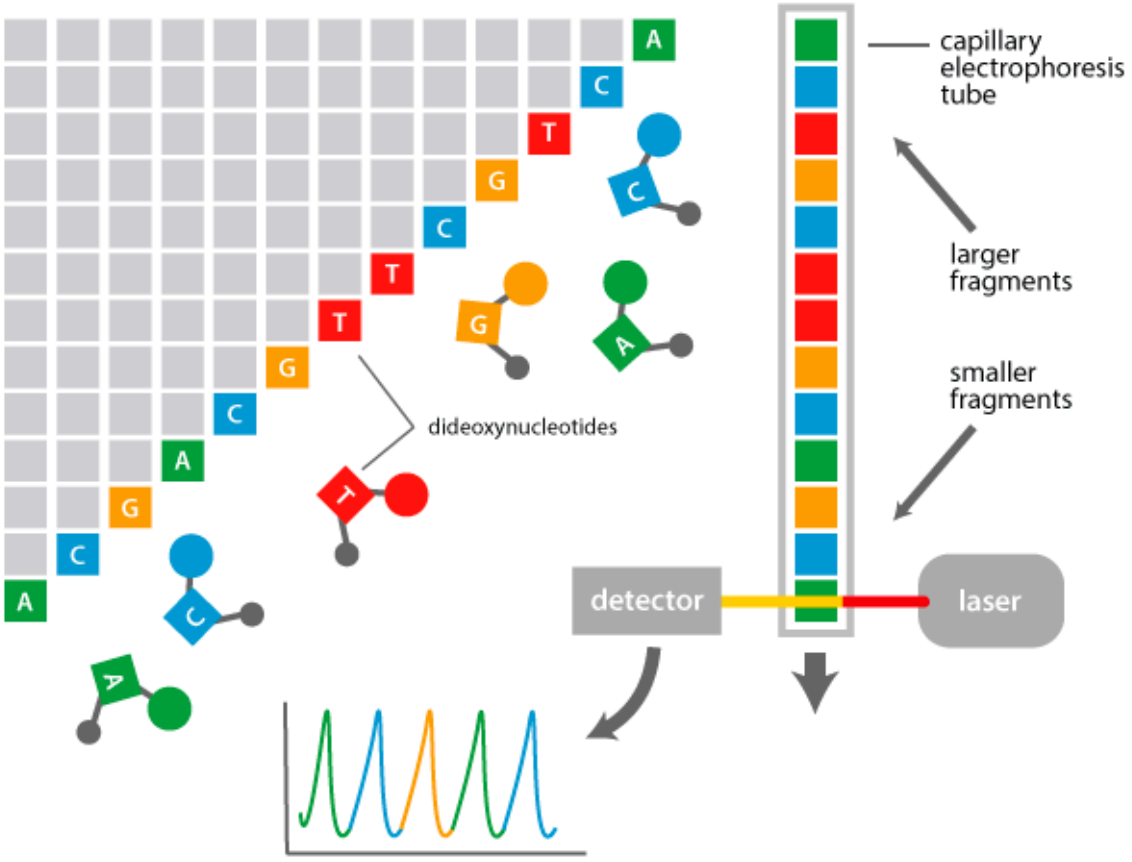
https://global.britannica.com/science/gel-electrophoresis

Question 2 from last time – Capillary Electrophoresis

Based on this raw data from a DNA sizing experiment using capillary electrophoresis technology, can you suggest some possible mechanisms for how capillary electrophoresis works? Try to imagine that you are building a prototype capillary electrophoresis machine – what major components would you need, and what are they for?



Sanger sequencing



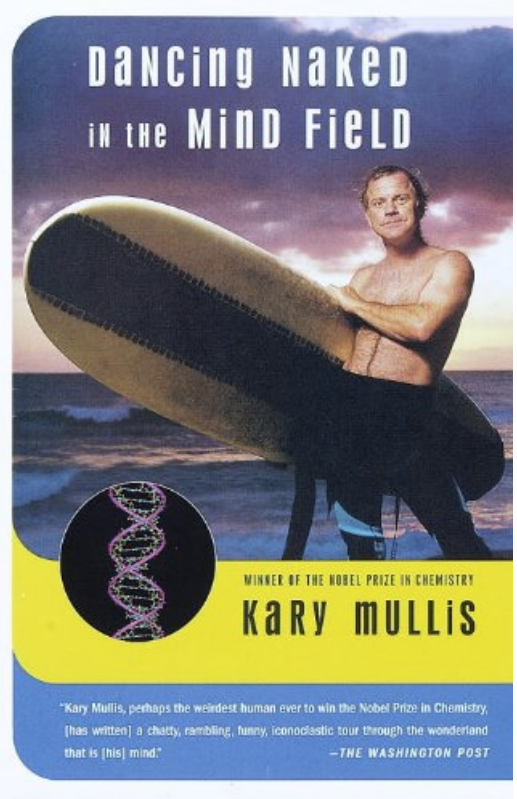
https://www.abmgood.com/marketing/knowledge_base/next_generation_sequencing_introduction.php#sanger

Sanger sequencing

- https://www.youtube.com/watch?v=jFCD8Q6qSTM&list=PL_VcB7OJ1TCAWRXN6vnC5lKbMHjlMtN8P&index=2

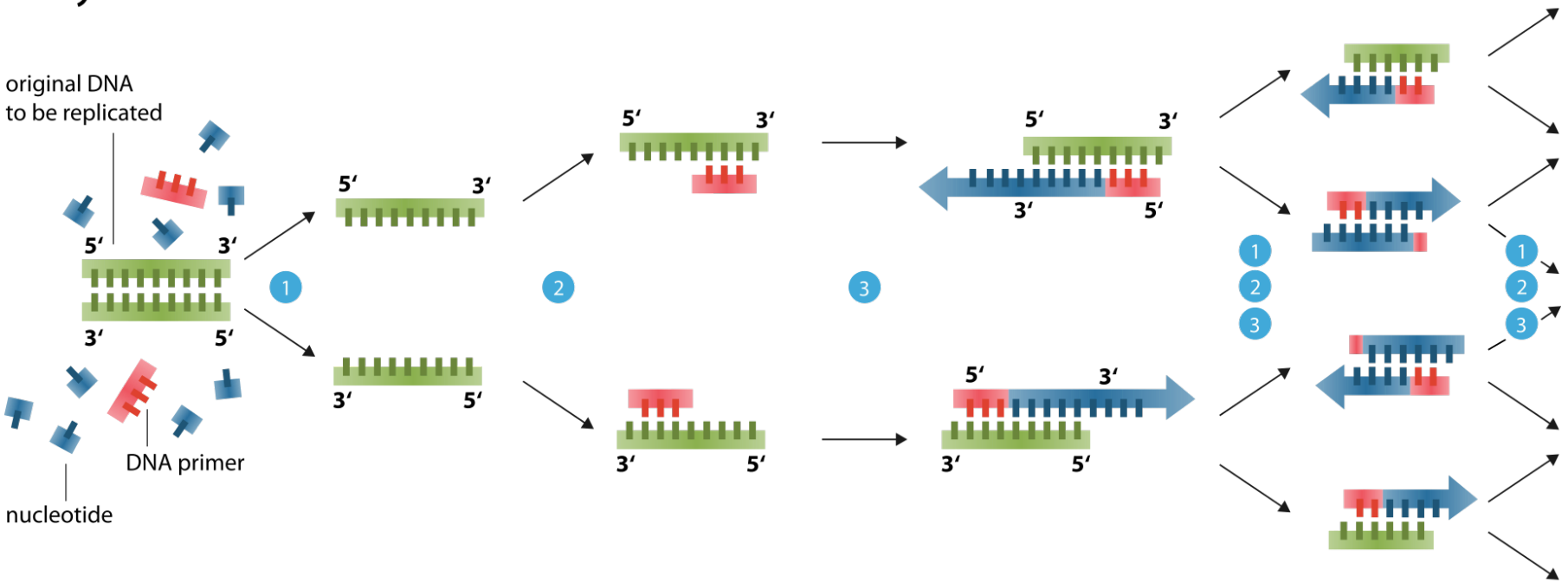
PCR Fun Fact

- Invented by Dr. Kary Mullis, who got the Nobel Prize for Chemistry in 1993
- "Nearly a year after he collected his Nobel, Mullis told California Monthly: "Back in the 1960s and early '70s I took plenty of **LSD**. A lot of people were doing that in Berkeley back then. And I found it to be a mind-opening experience. It was certainly much more important than any courses I ever took." And in 1997, he told the BBC, "What if I had not taken LSD ever; would I have still invented PCR? I don't know. I doubt it. I seriously doubt it.""



PCR

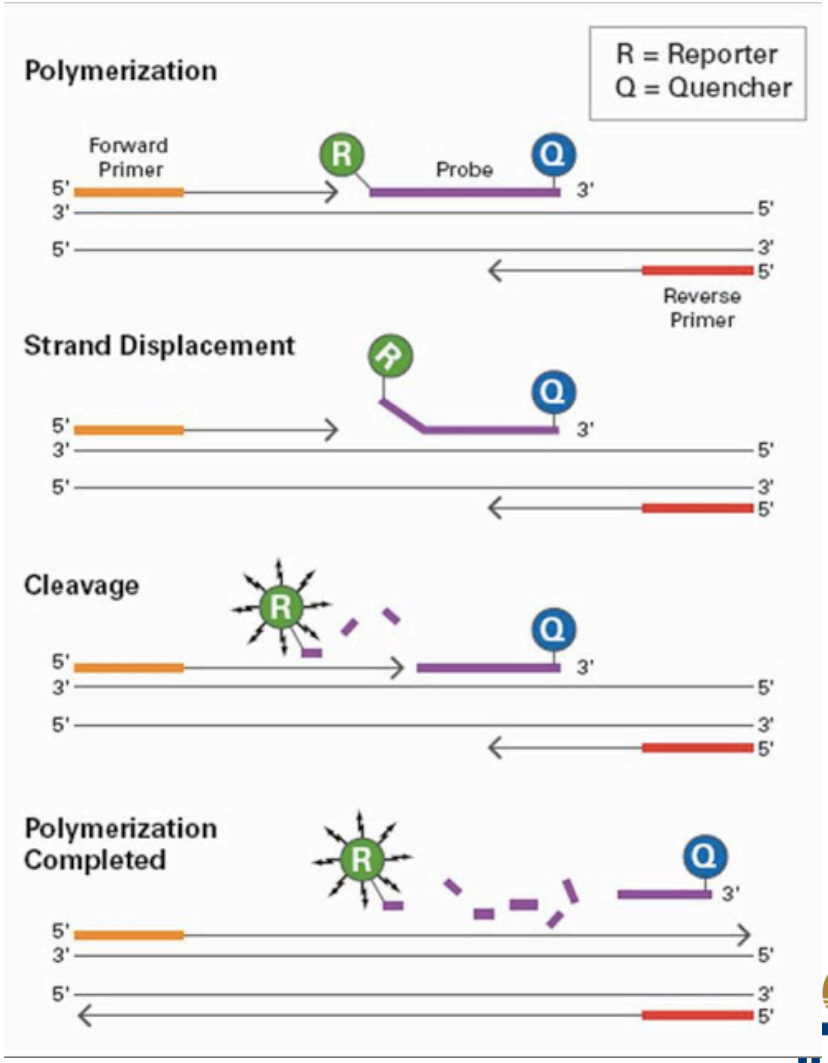
Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

Quantitative PCR

- Using Taqman chemistry:
  - Fluorescent dye and quencher are on the same probe
  - In close proximity, fluorescence is quenched
  - With positive amplification, the polymerase will cleave the probe as it copies template
  - Cleavage releases dye from quencher, results in emission
  - More copies = more dye released = more fluorescent signal
  - Highly specific: probe + primers required for signal



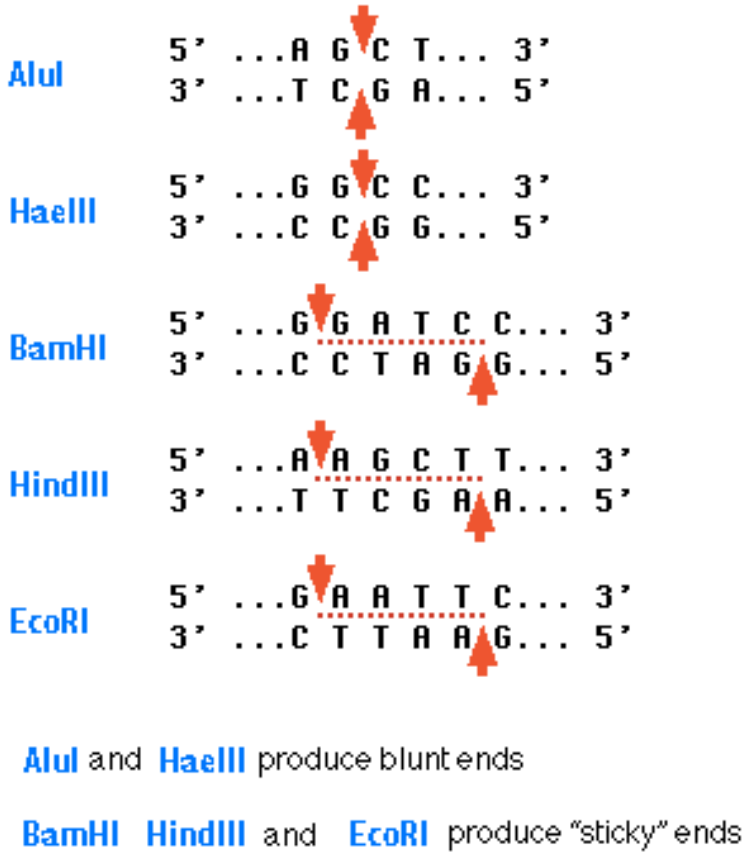
Recombinant DNA

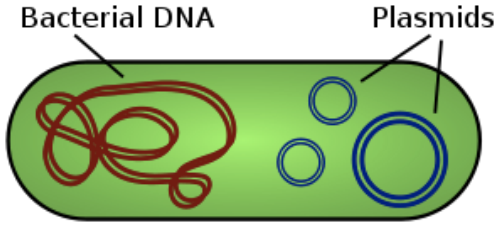
- "DNA molecules formed by laboratory methods of genetic recombination, such as molecular cloning, to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotide sequence within that identical overall structure."
- **Cloning genes of interest**
- **Combining different DNA fragments into one**
- **Specific applications:** fusion proteins; expressing new protein in existing genome; making protein in large quantities (e.g. insulin)

https://en.wikipedia.org/wiki/Recombinant_DNA

Molecular Cloning

- **Restriction enzymes** – enzymes (mostly from bacteria) that make cuts in DNA at specific sequences (palindromic site); these are "Type II"
- **Plasmids** – small DNA separate from chromosomal DNA, and can replicate separately; commonly found in bacteria
- **Origin of Replication (ORI)** – DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins



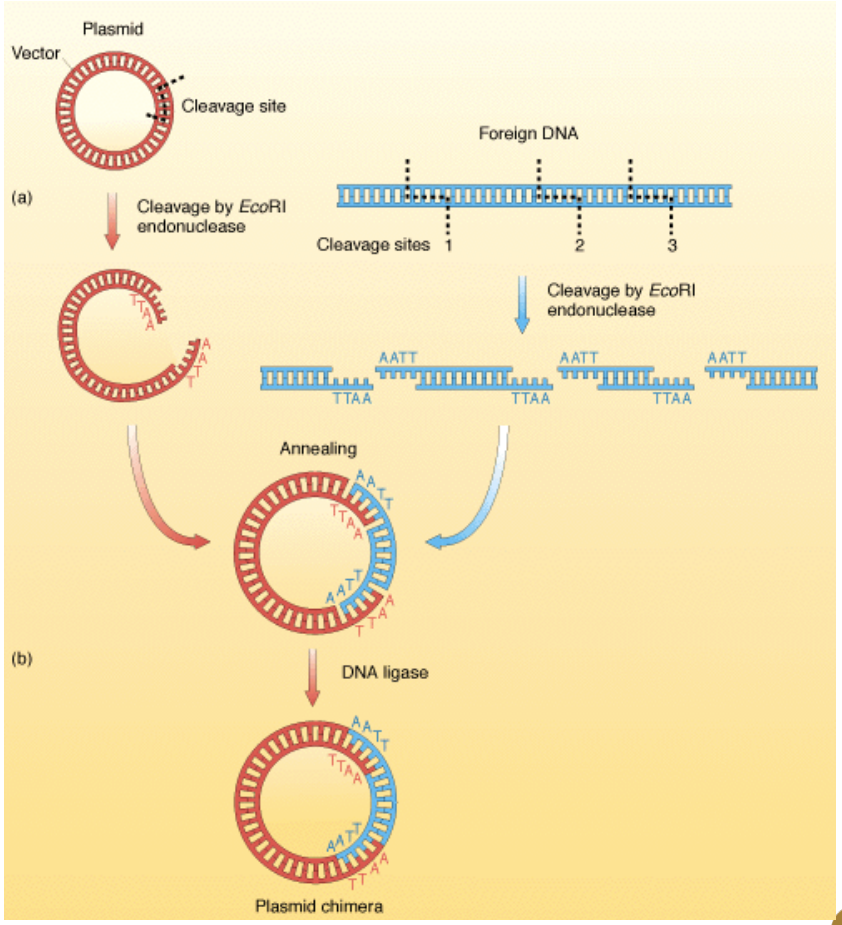


https://en.wikipedia.org/wiki/Recombinant_DNA

http://www.biology-pages.info/R/RestrictionEnzymes.gif
By User:Spaully on English wikipedia (Own work) [CC BY-SA 2.5 (http://creativecommons.org/licenses/by-sa/2.5)], via Wikimedia Commons

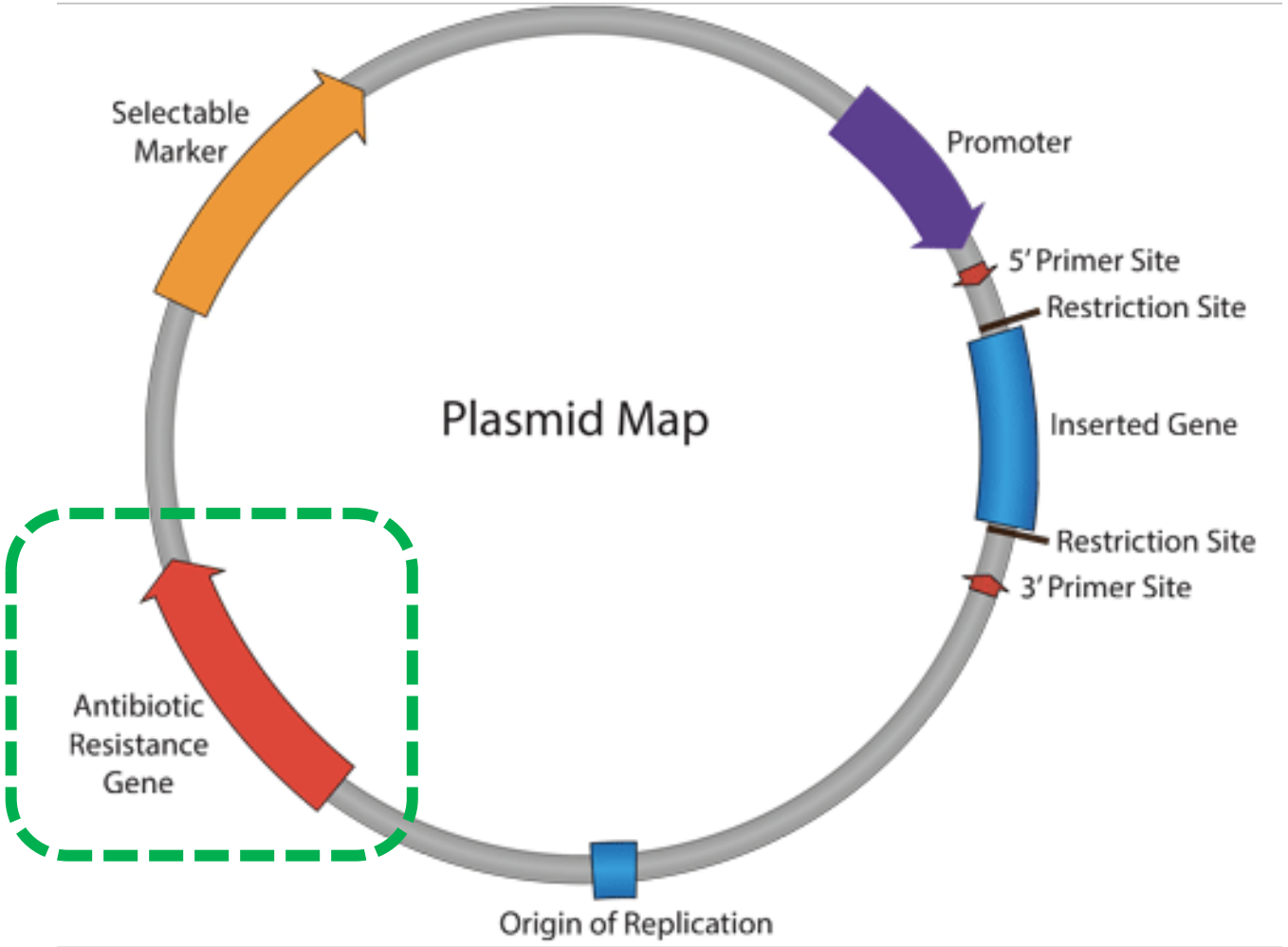
Molecular cloning

- **Vector -** DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated/expressed
- Cut the plasmid vector; Cut the insert sequence using the same restriction enzyme
- Join/"Ligate" the two together



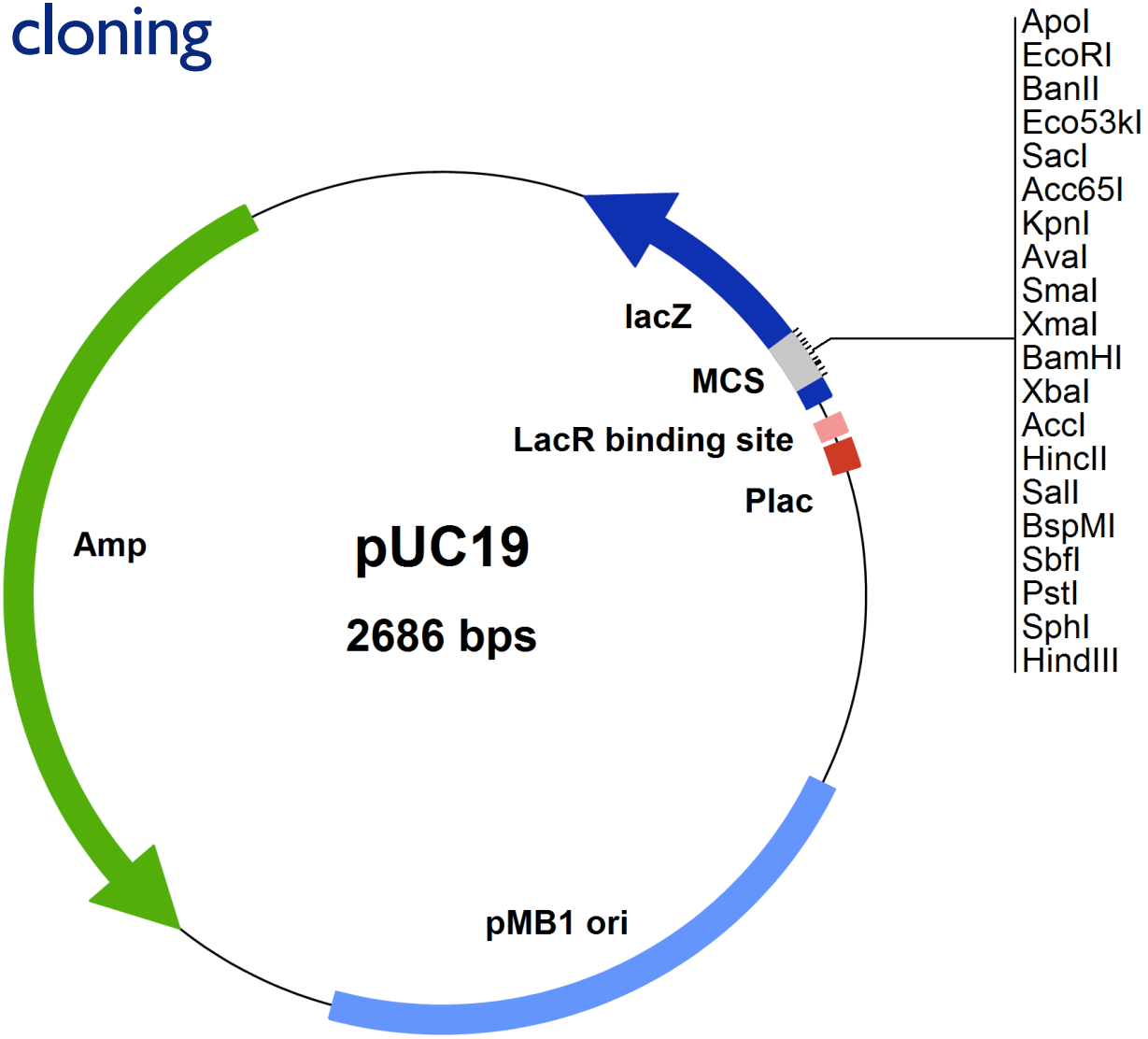
https://en.wikipedia.org/wiki/Recombinant_DNA
http://www.bio.miami.edu/dana/pix/chimericDNA.gif

Molecular cloning



http://blog.addgene.org/hs-fs/hub/306096/file-404153303-png/Plasmid_Map.png?t=1474663191759&width=350&name=Plasmid_Map.png

Molecular cloning

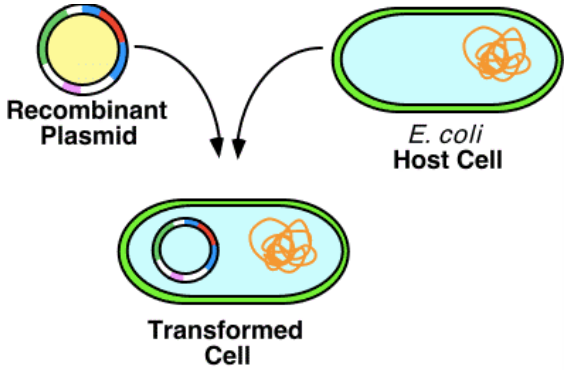


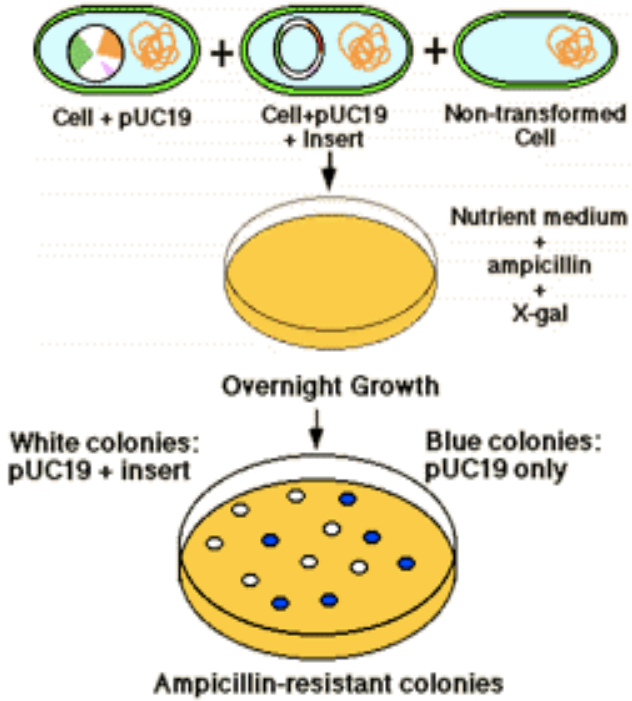
http://www.mobitec.com/cms/bilder/products/vector_sys/pUC19.png

Molecular cloning

- **Transformation** – genetic alteration of a cell resulting from direct uptake and incorporation of exogenous DNA through the cell membrane; typically achieved by heat shock, electroporation, or chemical treatment of cells (DNA precipitation)

- **Selection** – use of a selectable marker or antibiotic resistance gene to distinguish cells that did not take up plasmid, or did not insert the gene in the right place, or took up empty plasmid



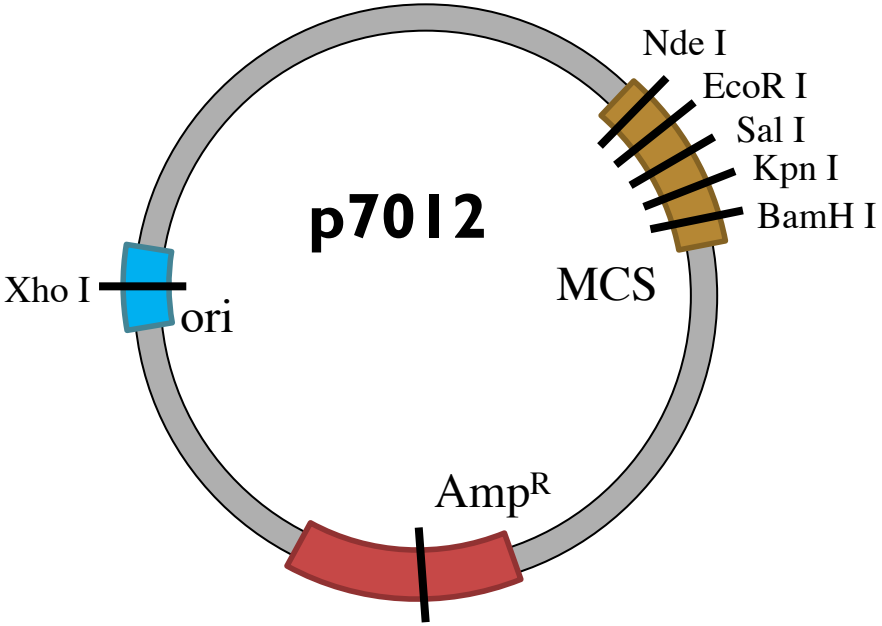


"Homework" Question 3

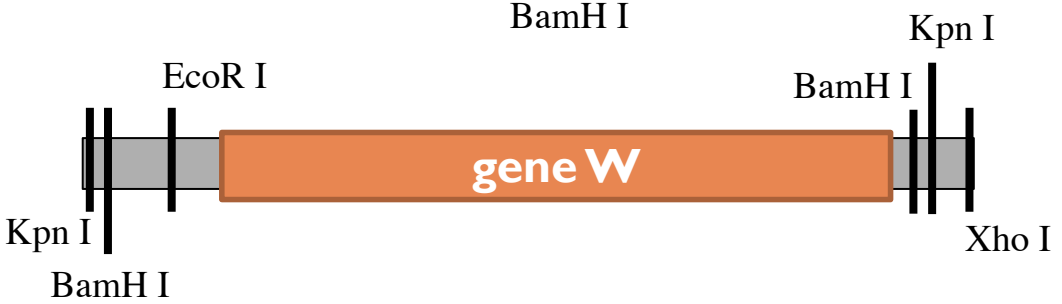
- Here is vector p7012:
- Here are the restriction enzymes:

Nde I:	Sal I:	Kpn I:
5' CATATG	5' GTCGAC	5' GGTACC
3' GTATAC	3' CAGCTG	3' CCATGG

EcoR I:	BamH I:	Xho I:
5' GAATTC	5' GGATCC	5' CTCGAG
3' CTTAAG	3' CCTAGG	3' GAGCTC



- Here is gene W:



a) *What are 3 strategies for cloning gene W into p7012?*

Answers will be posted online next week

b) *In which strategies would gene W be inserted into the vector in only one direction?*

c) *After cloning, you transform and plate bacterial cells using your cloned plasmid. Onto what type of growth medium will you plate your cells in order to distinguish between bacterial cells that obtained the plasmid and those that did not?*

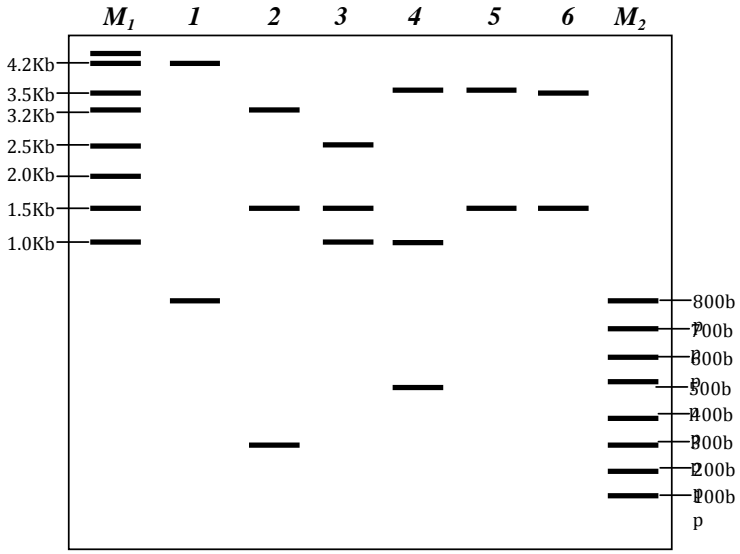
Sample question adapted from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

"Homework" Question 4

You are given a plasmid. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis.

a) *What is the approximate size of the plasmid?*

b) *Add the SmaI, KpnI, BglII sites to plasmid map. On your map give the distances between each of the restriction sites.*



***M1 and M2 are DNA markers.**

Answers will be posted online next week

Lane	*Digest*	*Size of fragments in bp*
1	Bam*HI* and Sma*I*	*4200, 800*
2	Sma*I* and Kpn*I*	*3200, 1500, 300*
3	Kpn*I* and Bgl*II*	*2500, 1500, 1000*
4	Bam*HI* and Kpn*I*	*3500, 1000, 500*
5	Kpn*I*	*3500, 1500*
6	Bgl*II* and Bam*HI*	*3500, 1500*

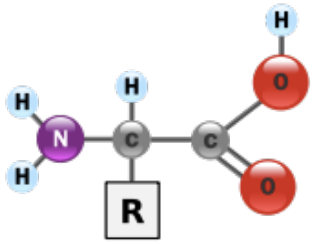
Sample question taken from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

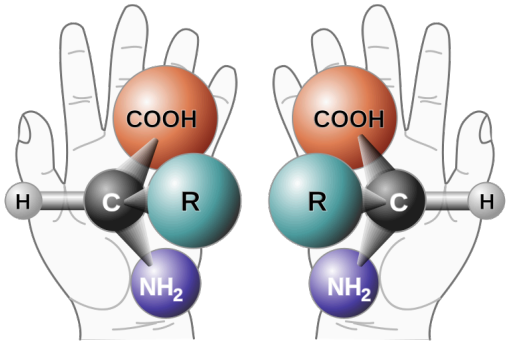
PROTEIN STRUCTURE

Folding in-vivo, and analysis techniques

Amino acids

- Basic component of proteins
  - Amine group
  - Carboxylic acid group
  - Side chain (R)
- They are chiral (handed-ness)
  - The body only uses the L-amino acid

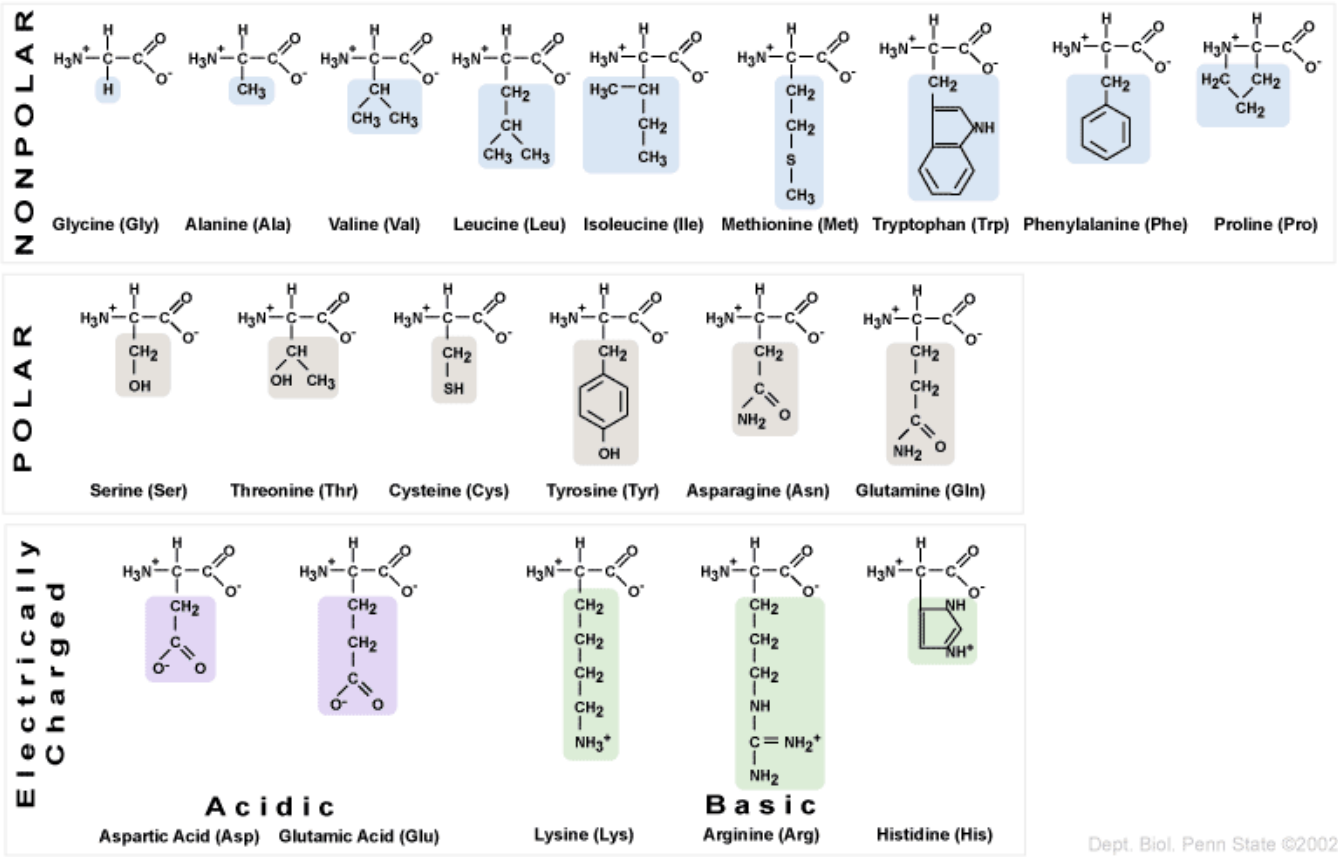




https://upload.wikimedia.org/wikipedia/commons/thumb/e/e8/Chirality_with_hands.svg/765px-Chirality_with_hands.svg.png

Amino acids

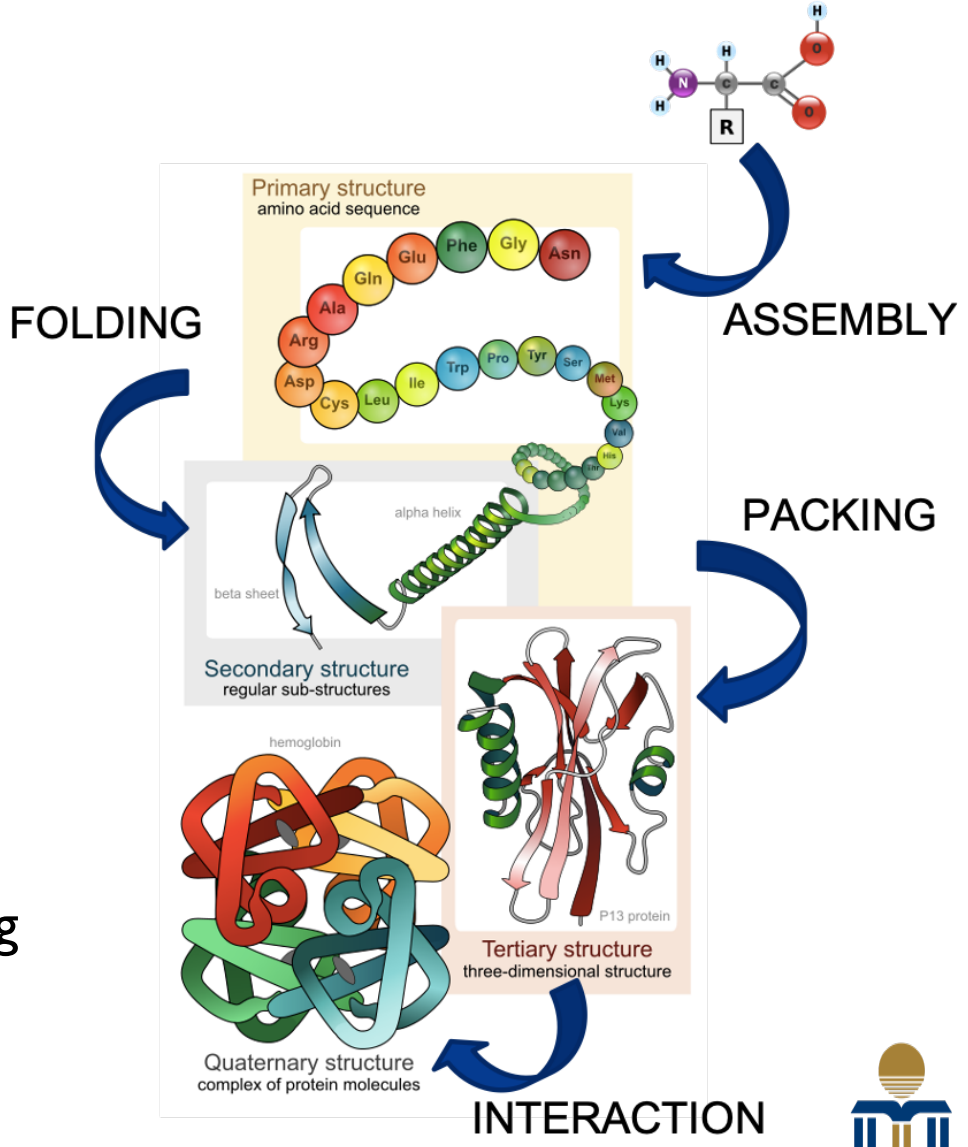
- There are 20 different side-chains



http://www.personal.psu.edu/staff/m/b/mbt102/bisci4online/chemistry/chemistry8.htm

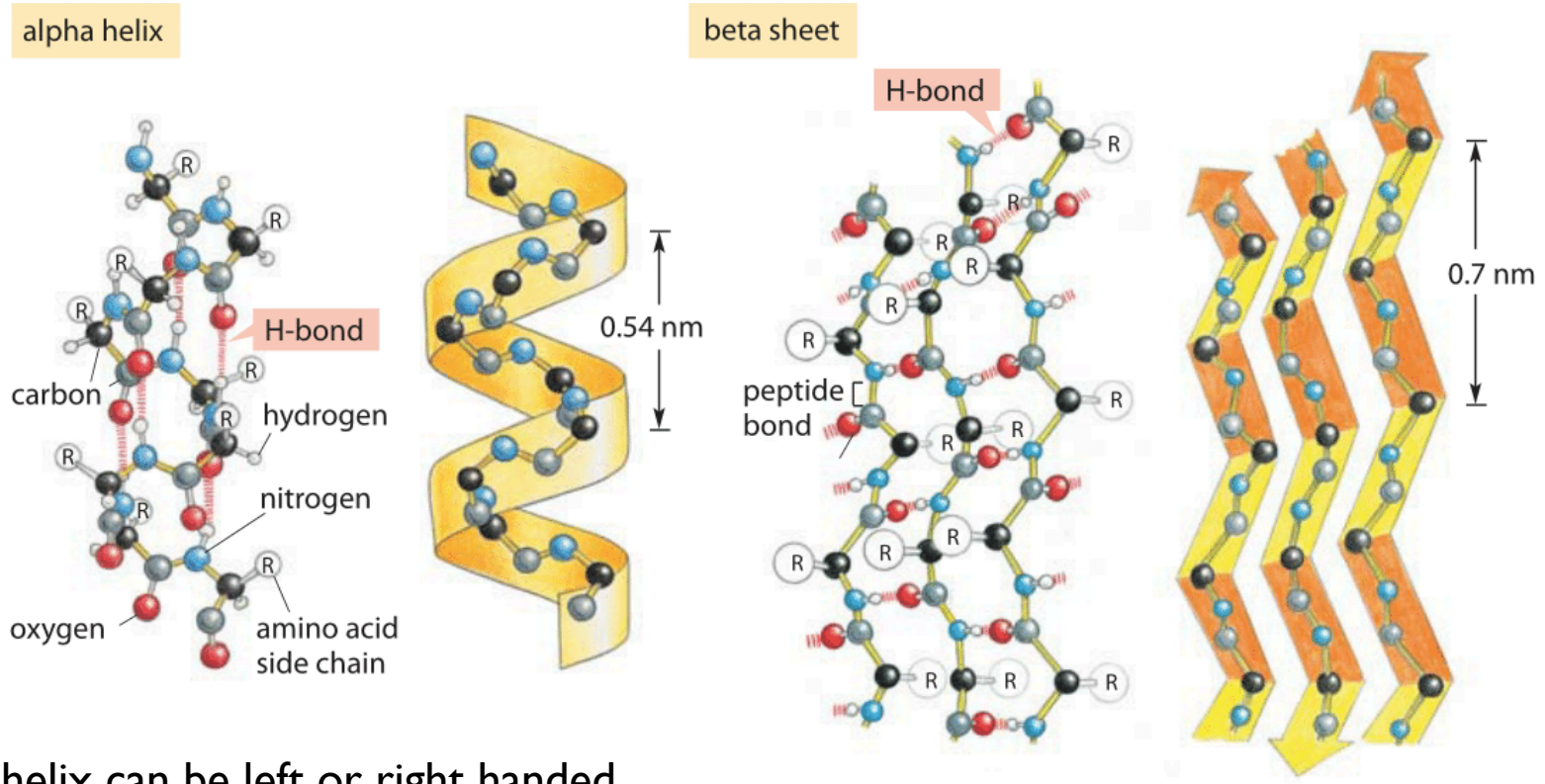
Protein structures

- Amino acids "assemble" into peptides ("peptide assembly")
- Peptides "fold" into secondary structures ("protein folding")
- 2° structures "pack" into 3° structures ("protein packing")
- 3° structures "interact" with each other to form 4° structures ("protein interactions")
- 4° structures are defined as having more than one peptide chain, e.g. Haemoglobin



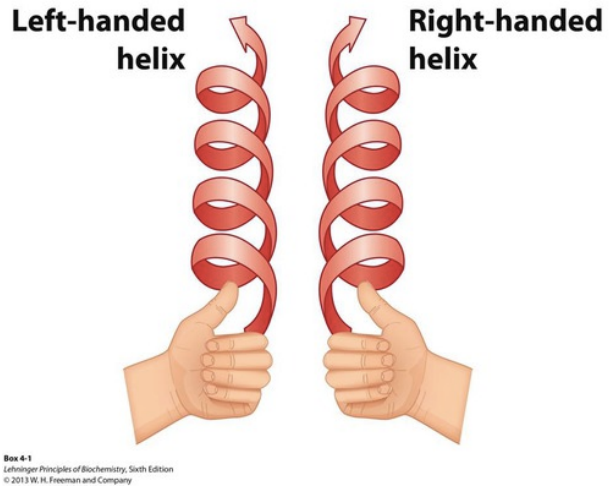
By LadyofHats [Public domain], via Wikimedia Commons

Protein folding – Secondary structures/motifs



- Alpha helix can be left or right handed
- Common in DNA-binding/recognition domains
- Common for lipid-membrane spanning domains
- Common when structure requires elasticity

Alpha helices



Box 4-1
Lehninger Principles of Biochemistry, Sixth Edition
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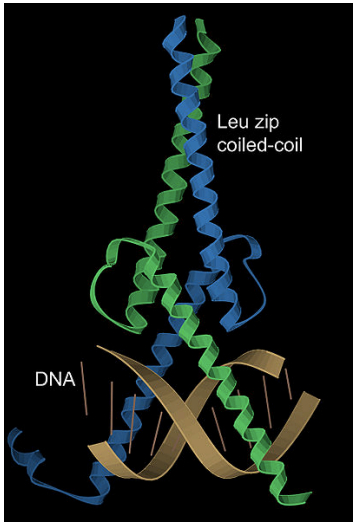
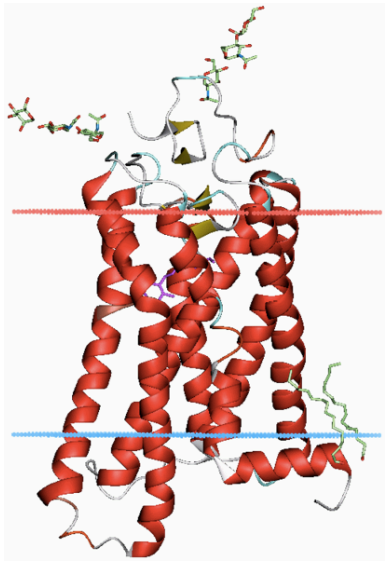
Alpha helix can be left or right handed



Common in DNA-binding/recognition domains



Common for lipid-membrane spanning domains

Common when structure requires elasticity, e.g. keratin (hair), myosin (muscle)

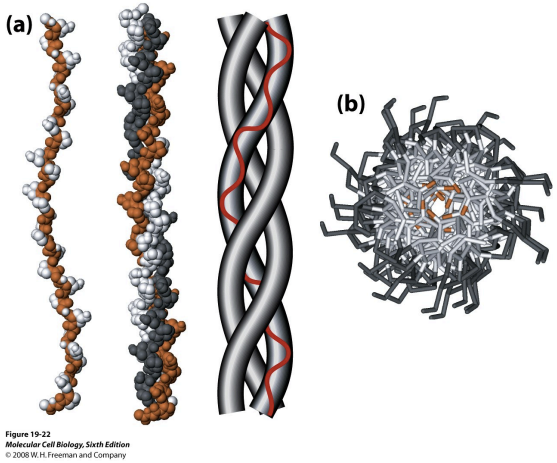


Figure 19-22
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company

Lehninger, Principles of Biochemistry, Sixth edition
Molecular Cell Biology, Sixth edition

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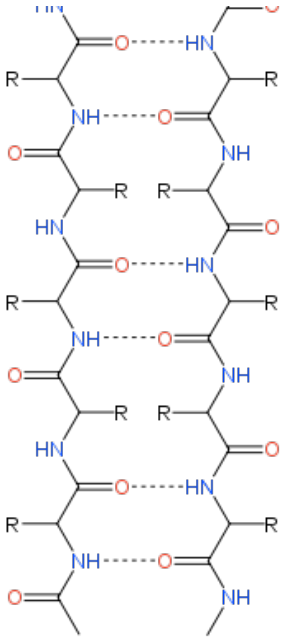
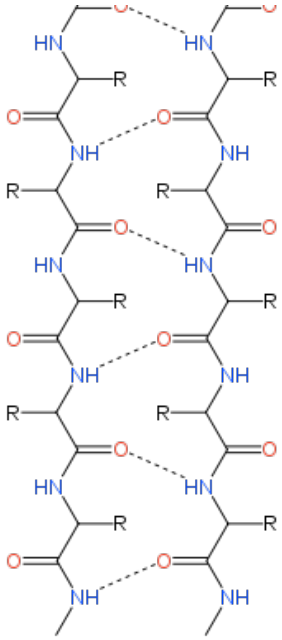
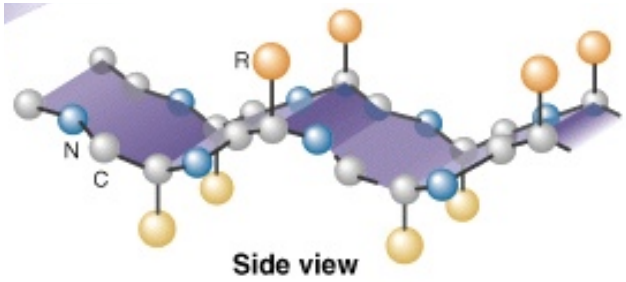
Beta sheets



antiparallel

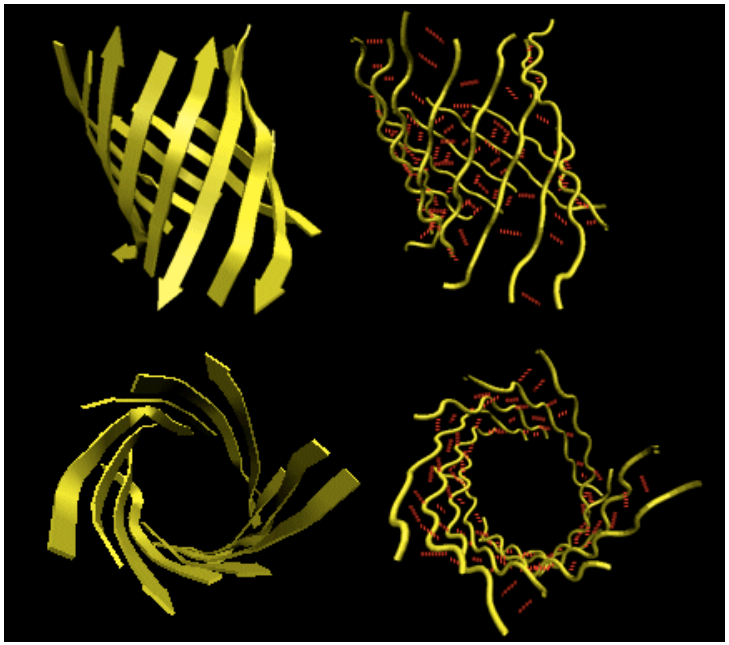


parallel



Structurally open; can stack like sandwiches; resist stretching, but can curve.

Can form beta barrel structure – common in porins that cross lipid membrane



Protein folding - thermodynamics

- Forces at play:
  - H-bonding: A hydrogen bond occurs when two electronegative atoms share the same hydrogen atom
  - **Hydrophobic effect: strong**
  - van der Waals interaction: weak, short in range
  - Steric repulsion: opposite to van der Waals interaction
  - **ENTROPY**

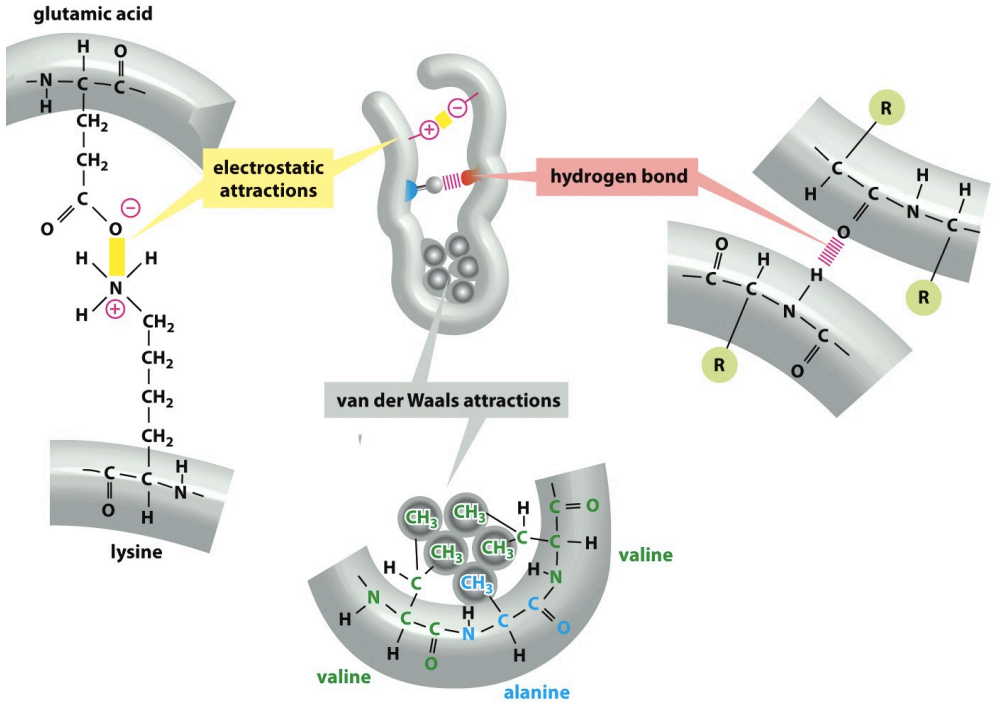
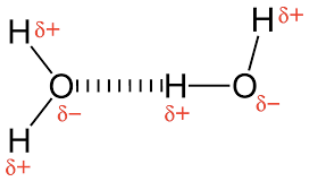


Figure 4-4 Essential Cell Biology 3/e (© Garland Science 2010)

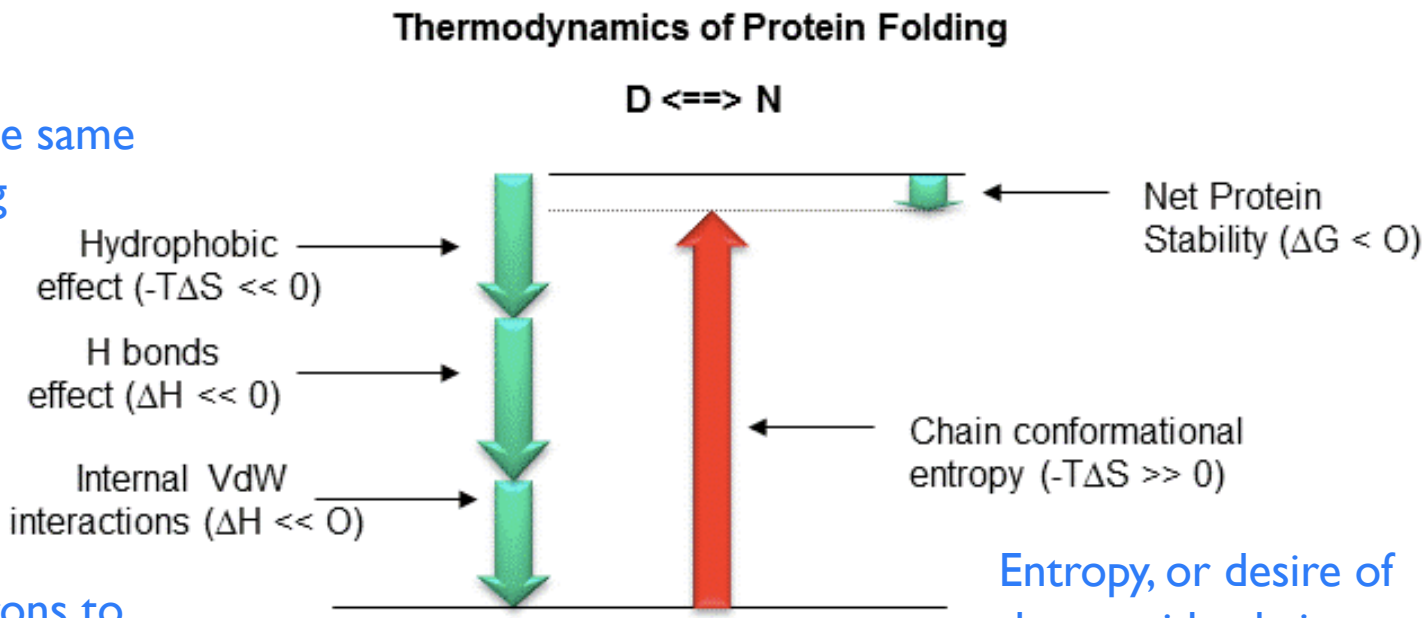
Protein folding - thermodynamics



Essentially the same as H-bonding

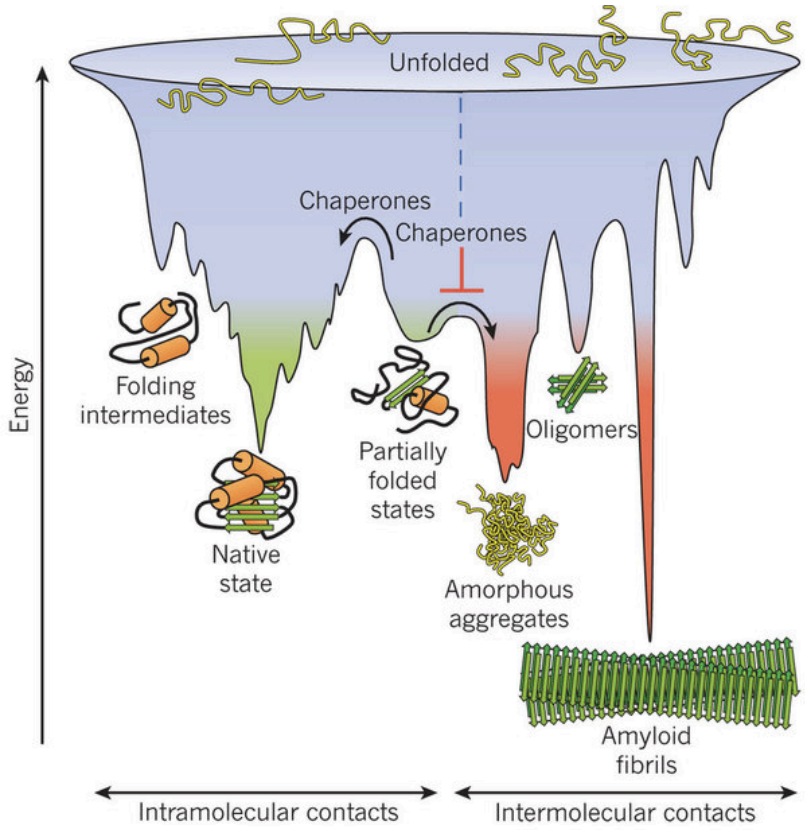
Forming H-bonds reduces enthalpy

Desire of electrons to arrange in a specific way that is electrostatically favorable reduces enthalpy



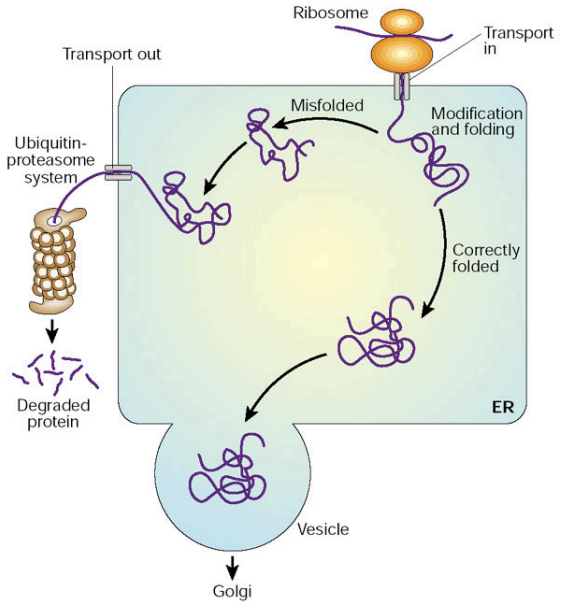
Entropy, or desire of the peptide chain to be free to flop around

Protein folding - thermodynamics



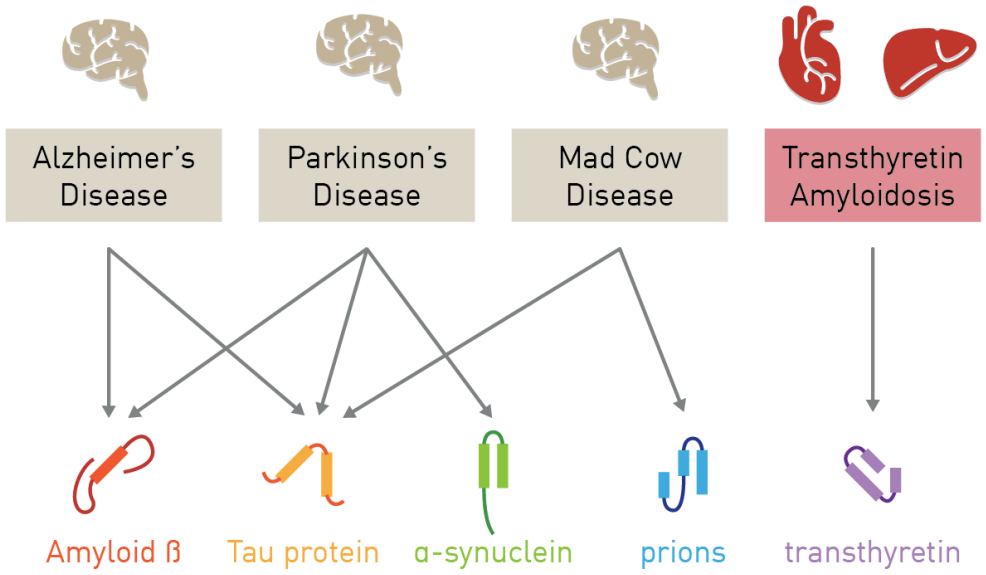
But how does it know to end up in the right conformation?

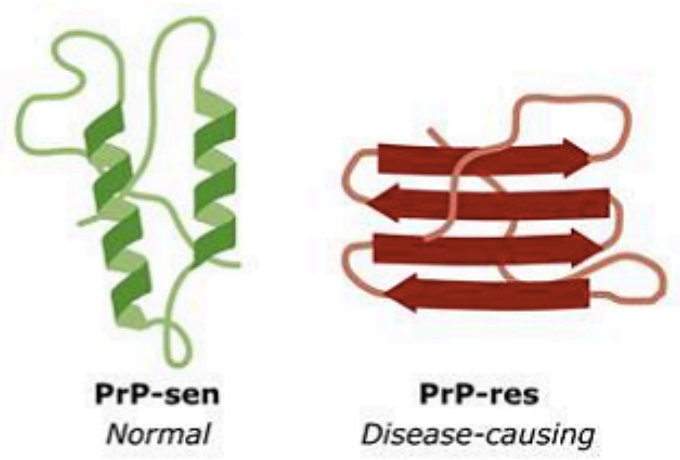
Answer: **CHAPERONES**



Protein degradation as a safeguard

Protein folding - Misfolding





Why do you think the disease-causing fold structure is problematic?

http://neurophage.com/science/protein-misfolding-diseases/

Protein denaturing

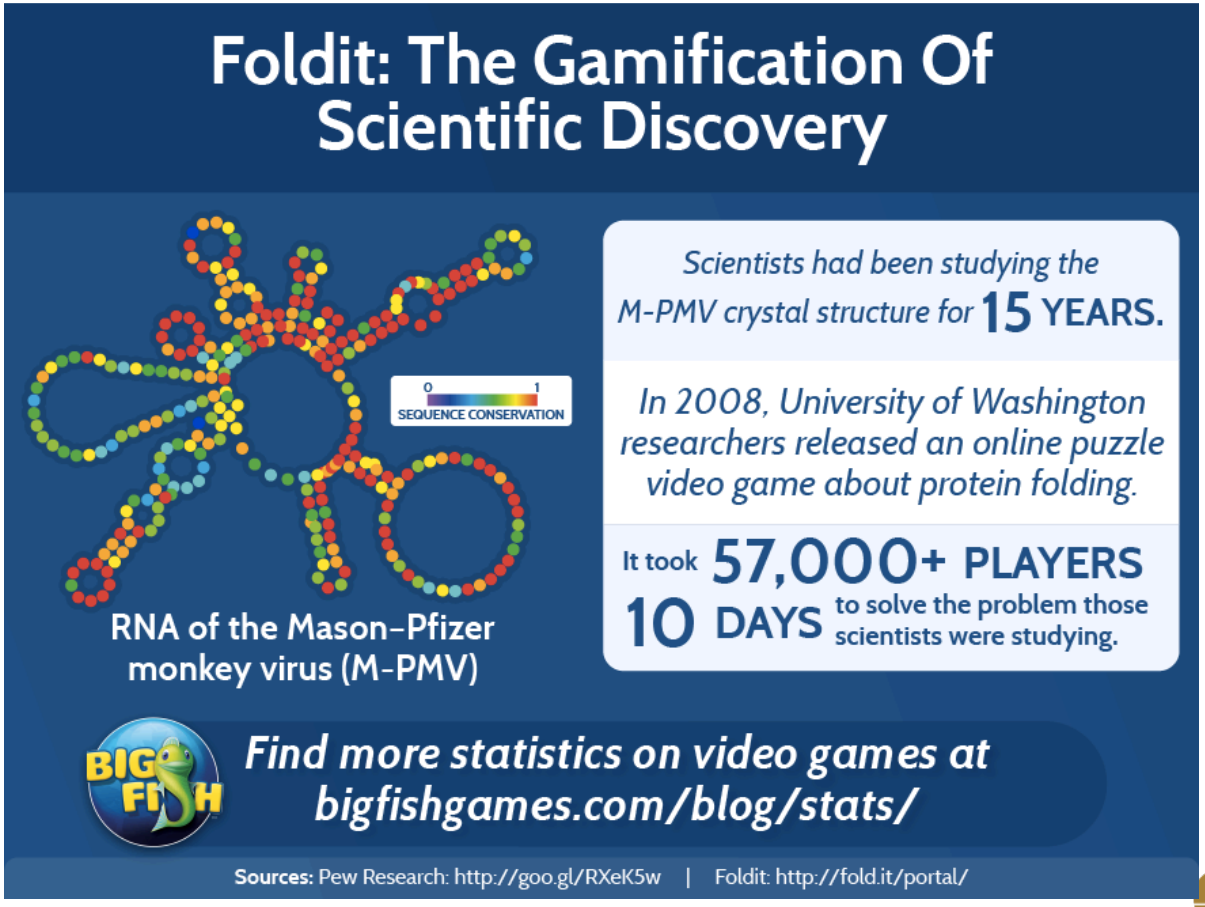
- What conditions do you think would cause a protein to denature (unfold)?

You can have some fun with Foldit!

http://fold.it/

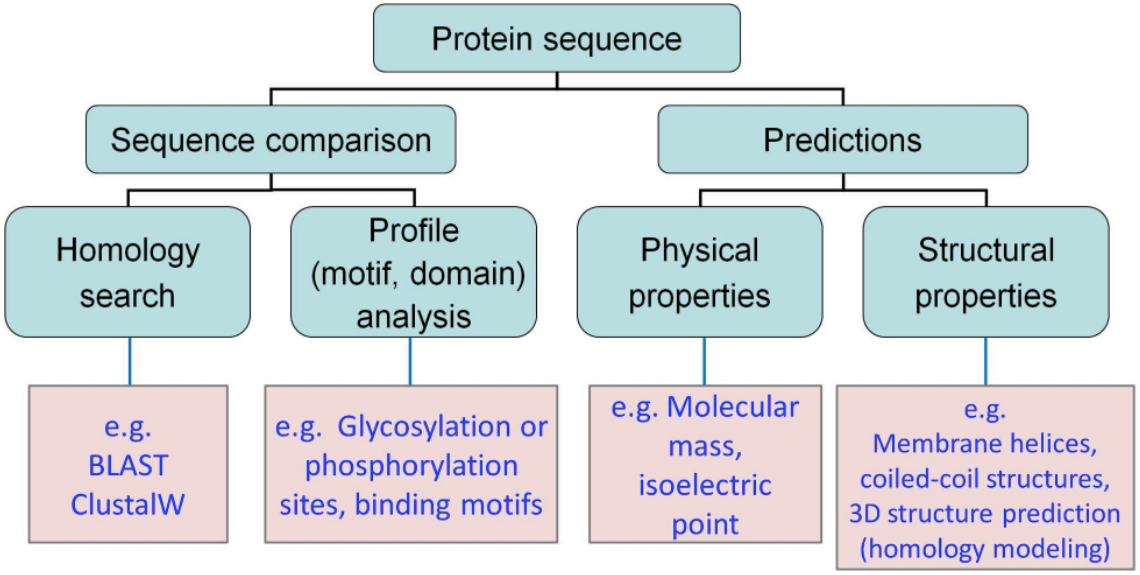
Download

Create an online account



Protein Characterization

- Next lecture you will learn about physical characterization of macromolecules, including proteins, using methods such as:
  - NMR
  - X-ray crystallography
  - Mass spectrometry
- But what about assessment of protein functional properties such as charge, hydrophobicity, binding partners, function, location in the cell, etc.?

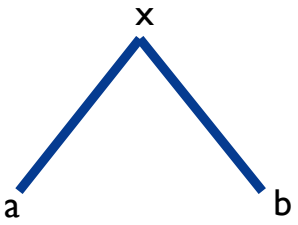


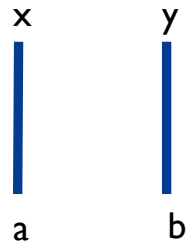
http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch11s03.html

Amino acid sequence analysis

- There are huge databases of genes and proteins online now:
  - Worldwide Protein Database (wwPDB) - http://wwpdb.org/
  - NIH NCBI Protein Database - https://www.ncbi.nlm.nih.gov/protein
  - UniProt - http://www.uniprot.org/
  - UC Santa Cruz (UCSC) Genome browser - https://genome.ucsc.edu/
  - NIH NCBI Gene Database - https://www.ncbi.nlm.nih.gov/gene
- Sequence similarity tells us about **HOMOLOGY**:
  - Homologous protein (or DNA) sequences share a common ancestry
  - Evolutionary hypothesis

a, b are homologous





a, b are NOT homologous

Amino acid sequence analysis

- Sequence similarity gives clues about function:
  - mRNA sequence → Amino acid sequence → protein structure → biochemical function
  - Similar amino acid sequence often means similar function; sometimes if we don't have the AA sequence, starting from mRNA can also be helpful
  - Function of newly discovered protein usually begins with a search for previously identified proteins that are similar in AA sequences

- Similar proteins can be from the same protein family, or even other organisms/species (homology)

- **Similarity DOES NOT automatically mean homology**

Amino acid sequence analysis

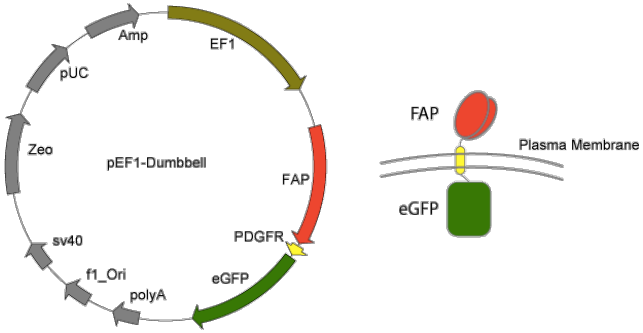
- Tools for comparing sequences:
  - For comparing one sequence to a database of sequences (e.g. novel protein against known) - **BLAST** (http://blast.ncbi.nlm.nih.gov/Blast.cgi)
  - For comparing multiple sequences to each other, or multiple sequence alignments (e.g. comparing a family of proteins, or determining protein homology) - **ClustalOmega** (http://www.ebi.ac.uk/Tools/msa/)

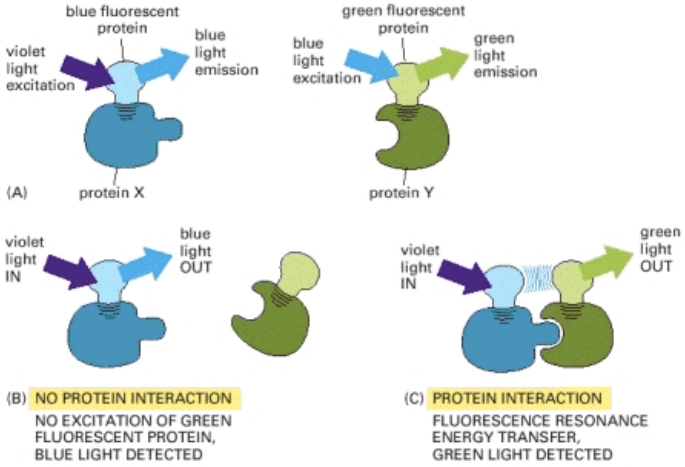
- Try BLAST: https://www.ncbi.nlm.nih.gov/Class/BLAST/blast_course.html

Fusion proteins

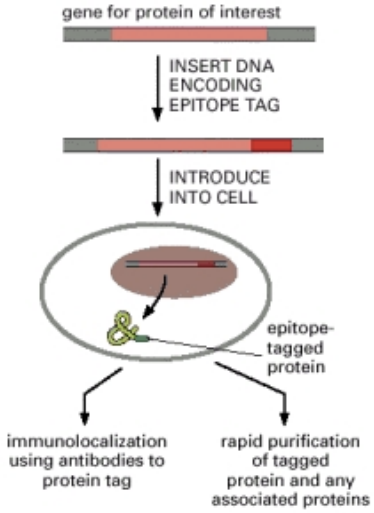
- Analyze protein function
- Track protein in living cells

GFP fusion protein





FRET-based detection of protein interaction



Epitope tagged protein

Use of fusion protein to investigate protein dynamics in live-cell imaging

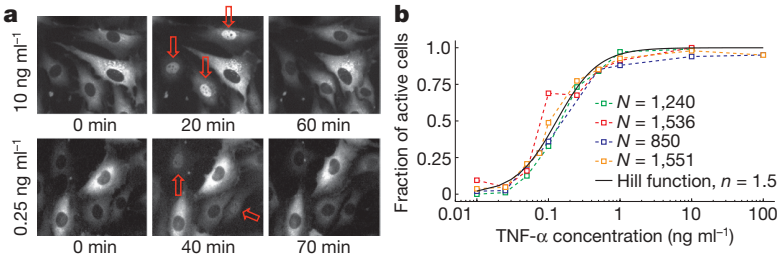
Vol 466|8 July 2010|doi:10.1038/nature09145 nature

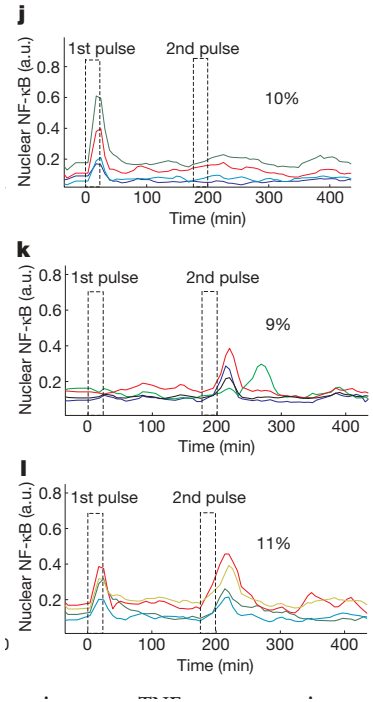
LETTERS

Single-cell NF-κB dynamics reveal digital activation and analogue information processing

Savaş Tay[1,2]*, Jacob J. Hughey[1]*, Timothy K. Lee[1], Tomasz Lipniacki[3], Stephen R. Quake[1,2] & Markus W. Covert[1]

"We used a lentiviral system to create p65$^{-/-}$ mouse fibroblast (3T3) cells expressing the fluorescent fusion protein p65–DsRed under control of the endogenous mouse p65 promoter."

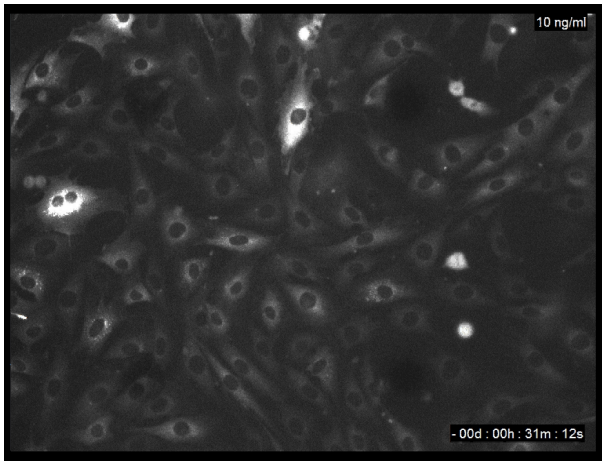


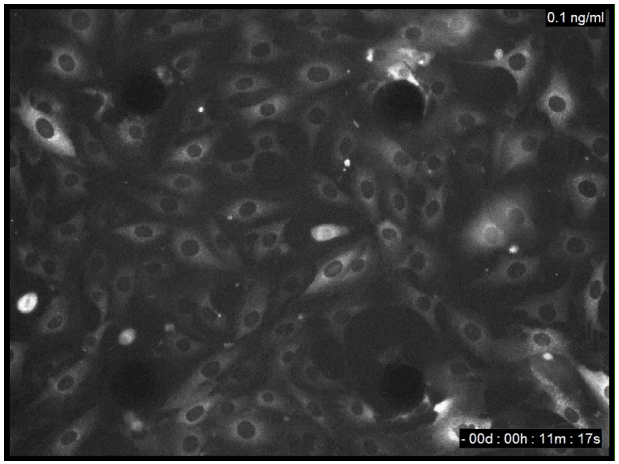


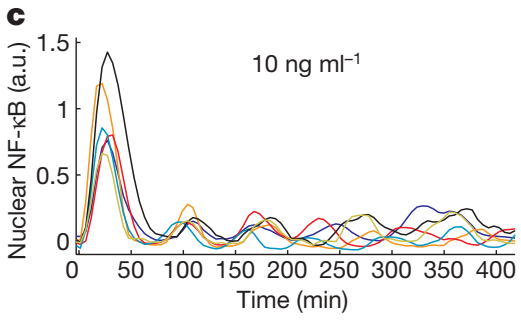
S. Tay et al., Nature, 2010 (http://www.nature.com/nature/journal/v466/n7303/full/nature09145.html)

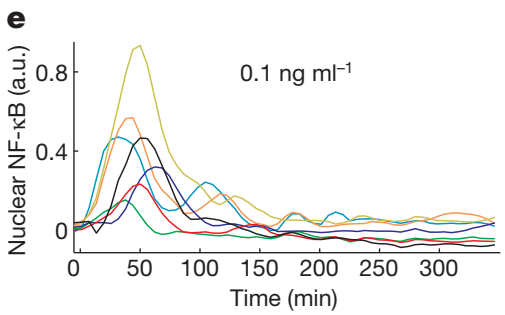
High amount of stimulation:
all cells activate and in sync

Low amount of stimulation:
cells activate stochastically
without synchrony





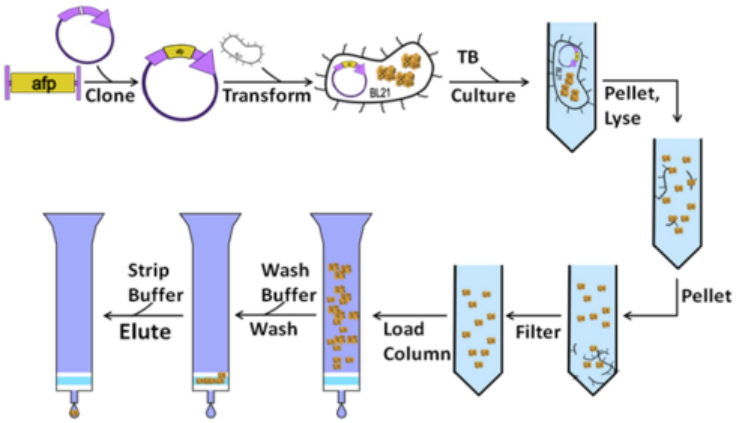




S. Tay et al., Nature, 2010 (http://www.nature.com/nature/journal/v466/n7303/full/nature09145.html)

Protein purification

Affinity chromatography



- Protein purification can separate out proteins based on:
  - Protein properties, such as charge and size
  - Protein binding partner (the **LIGAND**)
  - Fused protein tag

https://youtu.be/pnT587wUGyY

Chromatography

Type of Chromatography	Separates Proteins By	Bind With	Elute With
Affinity	A specific interaction	No competing ligand	Competing ligand (specific); conditions that disrupt protein/protein interactions (non-specific)
Ion Exchange	Net surface charge	Low ionic strength	High ionic strength; Increased (cation exchange) or decreased (anion exchange) pH
Hydrophobic Interaction	Hydrophobicity	High ionic strength	Low ionic strength
Size Exclusion	Hydrodynamic radius		

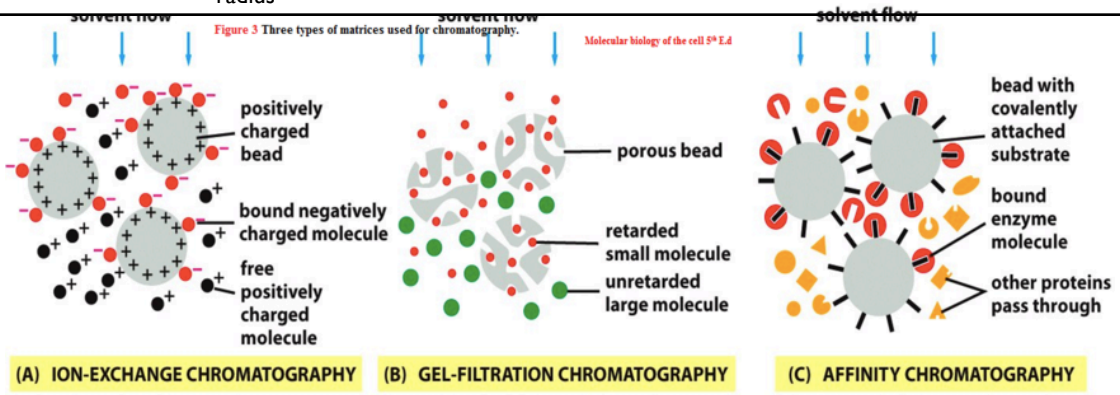
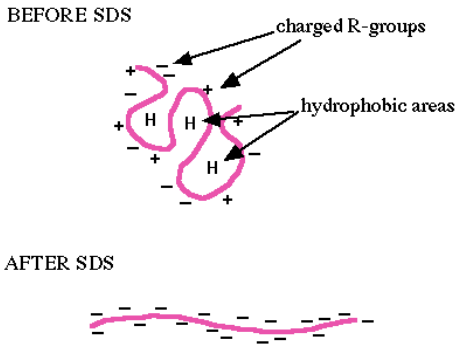


Figure 3 Three types of matrices used for chromatography. Molecular biology of the cell 5th Ed

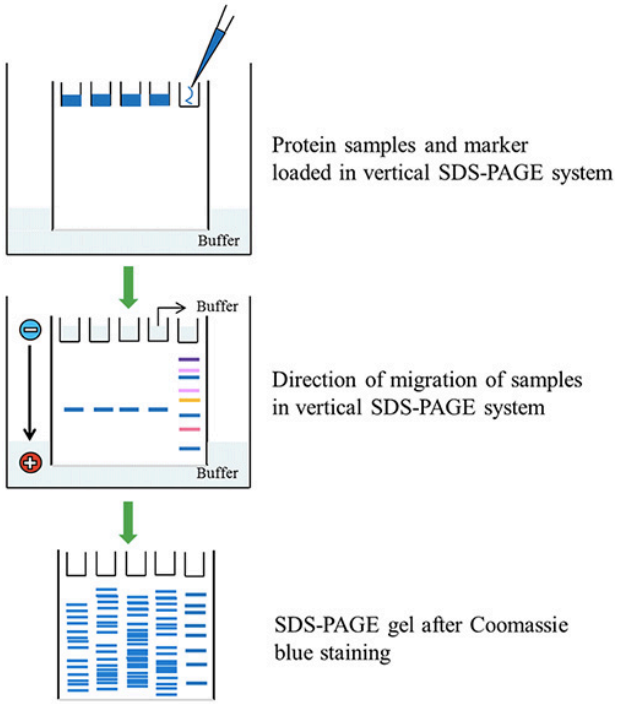
Alberts et al., Molecular Biology of the Cell, 5th Ed.

Protein separation



- **SDS-PAGE**
  - SDS – **S**odium **D**odecyl **S**ulfate
  - PAGE – **P**oly**A**crylamide **G**el **E**lectrophoresis
- SDS unravels the protein into its peptide chain (linearize/denature)
- PAGE separates the proteins based on their mobility in the gel
  - Mobility is determined by size, charge, conformation
  - SDS removes/minimizes charge and conformation contribution, allowing separation by only size
- Idea and setup is similar to DNA gel electrophoresis

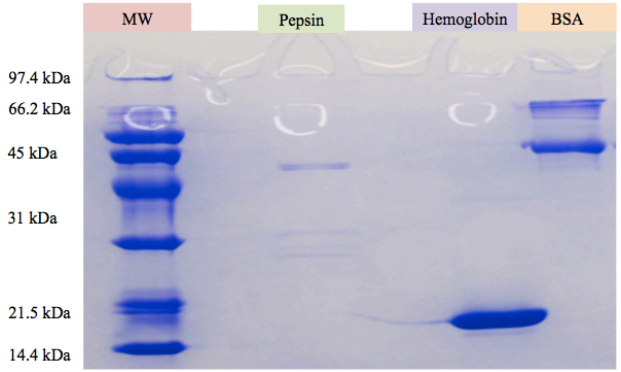
Protein separation



Protein samples and marker loaded in vertical SDS-PAGE system

Direction of migration of samples in vertical SDS-PAGE system

SDS-PAGE gel after Coomassie blue staining

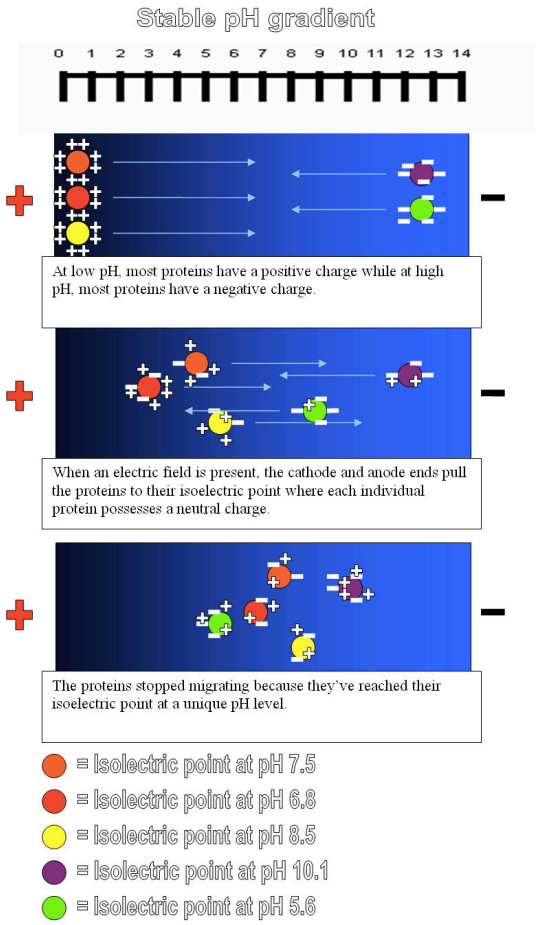


http://www.sigmaaldrich.com/technical-documents/articles/biology/sds-page.html

Protein separation

- **IEF,** or **electrofocusing**
  - **I**so**E**lectric **F**ocusing
- **Isoelectric point (pI)** is the pH at which a particular molecular (i.e. amino acid or protein) carries NO CHARGE
- Different from the SDS-PAGE that just has a charge gradient, IEF requires a pH gradient as well

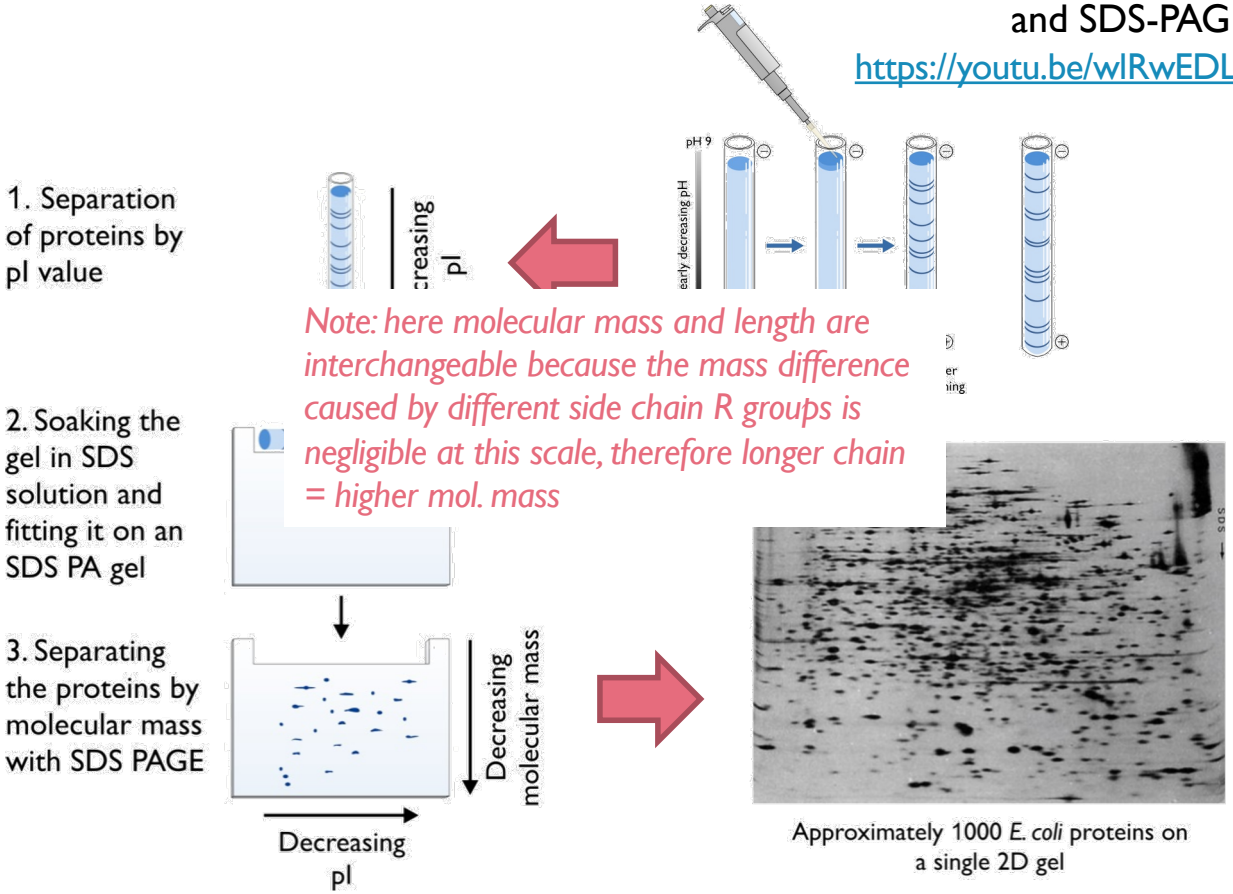
https://youtu.be/9jW8n1Ailic



By Mrbean427 (Own work) [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0) or GFDL (http://www.gnu.org/copyleft/fdl.html)], via Wikimedia Commons

Protein separation

If we do both IEF and SDS-PAGE…
https://youtu.be/wIRwEDLQdGE



Note: here molecular mass and length are interchangeable because the mass difference caused by different side chain R groups is negligible at this scale, therefore longer chain = higher mol. mass

http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch07s03.html