CMAE SCIENCE MODULES:

EXPERIMENTAL PROCEDURES

Common Techniques Applicable for:

CH 227 (General Chemistry I Lab)
CH 228 (General Chemistry II Lab)
CH 229 (General Chemistry III Lab)

CH 337 (Organic Chemistry I Lab)
CH 338 (Organic Chemistry II Lab)

BI 211 (General Biology I Lab)
BI 212 (General Biology II Lab)
BI 214 (General Biology IV Lab)

CH 360 (Physiological Biochemistry)
CH 467 (Biochemistry Laboratory)

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CONTENTS:

1. **Solubility-based: product is a solid dissolved in solvent → takes advantage of *"like-dissolves-like"***

   a. **Extraction: separate liquid from liquid**

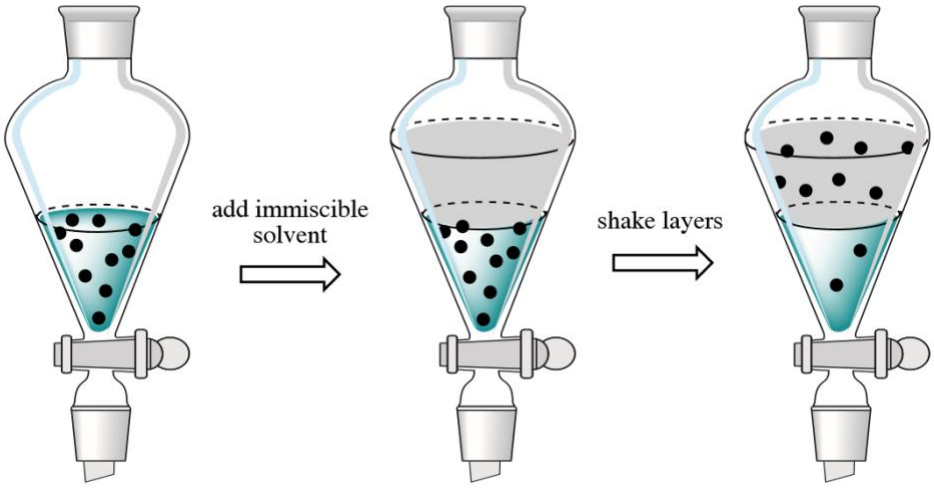
      i. Two immiscible solvents (do not mix).

      ii. Add water → separate aqueous vs. organic phase using a **separatory funnel**. The aqueous layer will dissolve into water and is either removed (if it is denser) or remains in the funnel (if it is less dense).

      iii. The denser layer sinks to the bottom and is removed.

      iv. Multiple extractions with water is more effective than a single extraction with a larger volume of water.

      v. Once desired product has been isolated → evaporate the solvent using a **rotovap** and collect the solid product.



   b. **Filtration: separate solid from liquid**

      i. Pour liquid-solid mixture onto a paper filter → allows only solvent to pass

         1. Residue on top of paper filter = **solids**
         2. Flask full of liquid = **filtrate**

      ii. Gravity filtration: product of interest is in the product in the filtrate

         1. Let the solvent's own weight pulls it through the paper filter
         2. Hot solvent ONLY → keep the product dissolved in the filtrate → if solvent is cool, desired product in the filtrate might precipitate out and remain "stuck" in the solids layer

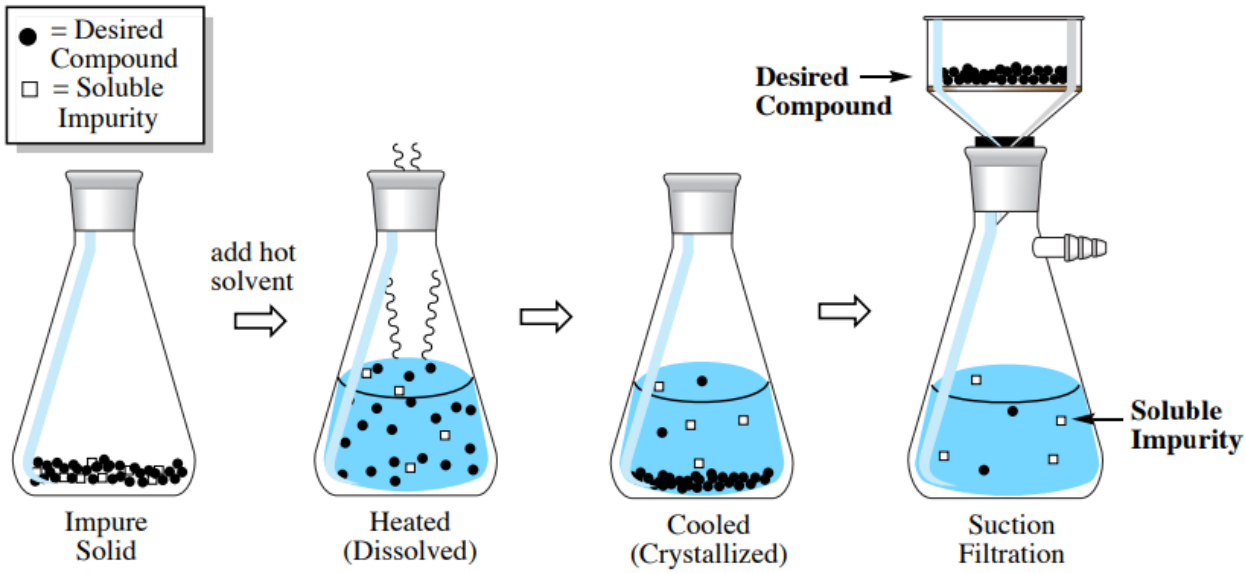
      iii. Vacuum filtration: product of interest is in the solids

         1. Solvent is "sucked" through the filter into the flask by a vacuum

c. Recrystallization: further purifying crystals in solution

i. Desired product dissolves in solvent at HIGH temperatures only

ii. Let solution cools → only the desired product will recrystallize out of solution (impurities remained soluble in cool solution)



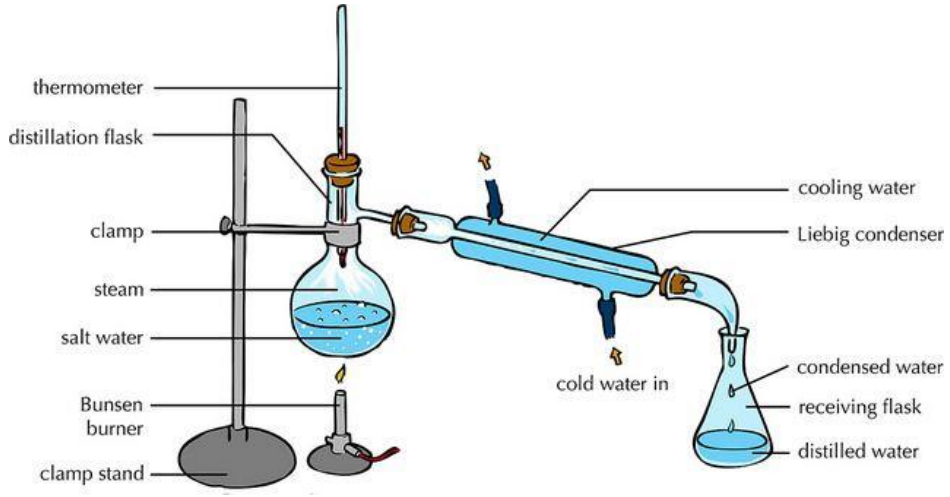
2. Distillation: product is a liquid dissolved in solvent → take advantage of *differences in boiling point (BP)*

Liquid with lower BP vaporizes, condense in a water-cooled condenser **(condensate)**, then drips down into a flask **(distillate).** Liquid with higher BP will NOT boil, condense, or distill into the flask with the distillate (heating temp is kept low) → remains liquid in the initial container.

a. Simple distillation:

i. Separate liquids that boil **below 150°C** and have **at least a 25°C difference in BP**.
   Distilling flask = contains the mixed liquid solution
   Distillation column = contains thermometer + condenser
   Receiving flask = collect the distillate

ii. Prevent superheating (when liquids won't evaporate even above its BP) → add boiling chips to break the surface tension.



b. Vacuum distillation:

i. Separate liquids with **BP above 150°C.**

ii. Using a vacuum → help liquid escape into gaseous phase easier by:
   1. Lowering the ambient pressure.
   2. Decreasing the T that liquid must reach to have sufficient vapor P.

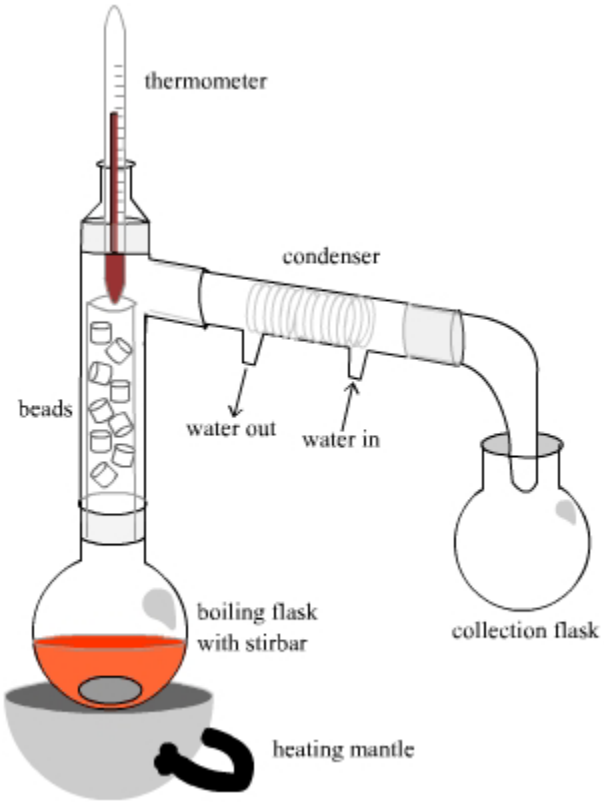
iii. Prevent compounds from degrading at high temperatures.

c. Fractional distillation:

i. Separate liquids with similar BP (**less than 25°C apart**).

ii. Fractional column → surface area increases by glass beads or steel wool.

1. The vapor evaporates, rises up, condenses on the beads, and refluxes back down into the solution.
2. Rising heat causes it to evaporate again, and then condenses again higher in the column → vapor consists of a higher % of the desired product (lower BP) each time.
3. Only the desired product makes it to the top of the column → drips down as distillate into the receiving flask.

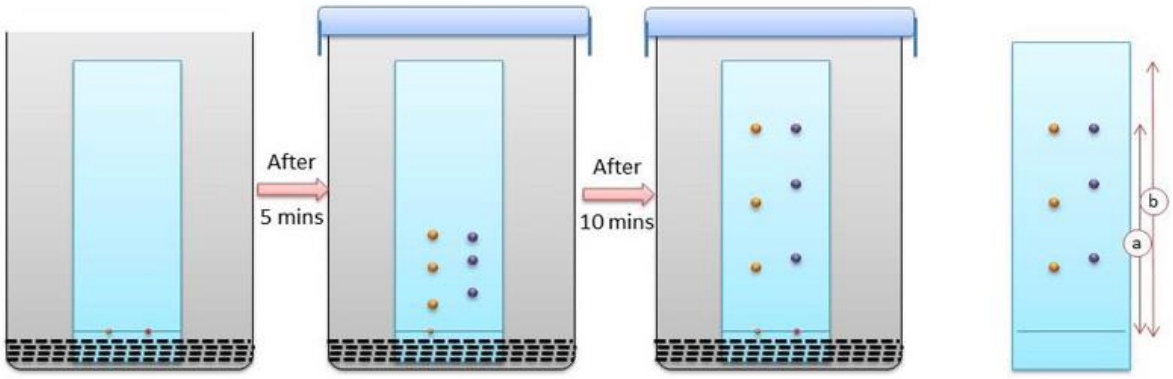


3. Chromatography: Separate a mixture of chemical substances into its individual components, based on "like-dissolves-like"

Stationary phase (absorbent) = solid medium ("s"tationary = "s"olid)
Mobile phase = liquid or gas (gas chromatography) that runs through the stationary phase
Different components → different partitioning coefficients → elute at different rates.

a. TLC or paper chromatography:

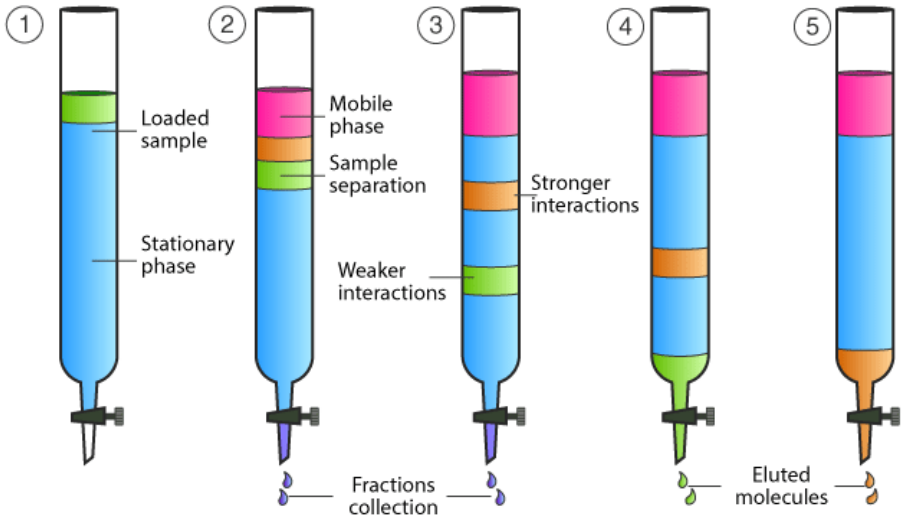
i. Stationary phase (absorbent):
   1. TLC = silica gel (polar)
   2. Paper = cellulose (polar)



$$Rf = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}} = \frac{a}{b}$$

b. Column chromatography:

i. Absorbent = silica or aluminum beads

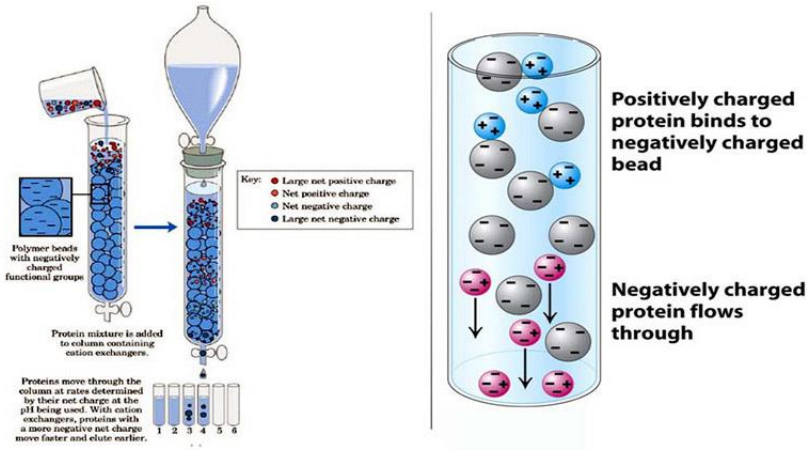


c. Ion-exchange chromatography:

i. Separates protein based on their NET charge

ii. Absorbent = beads that are coated with charged substances

1. Opposite charges "stick" → travel slow
2. Like charges "repel" → elute out first



d. Size-exclusion chromatography:

i. Separates protein based on their sizes

ii. Absorbent = beads that contain tiny pores that allow small proteins to enter; larger proteins don't fit into the pores

1. Small proteins → enter the beads → "stick" and travel slow
2. Large proteins → can't fit into the beads → elute out first

e. Affinity chromatography:

i. Absorbent = beads that have a certain molecule (Ex: glucose) that can bind to the active site of the protein.
ii. Add extra glucose in the solution so that the protein will be released from the beads and bind to the glucose in the solution.

f. Gas chromatography (GC):

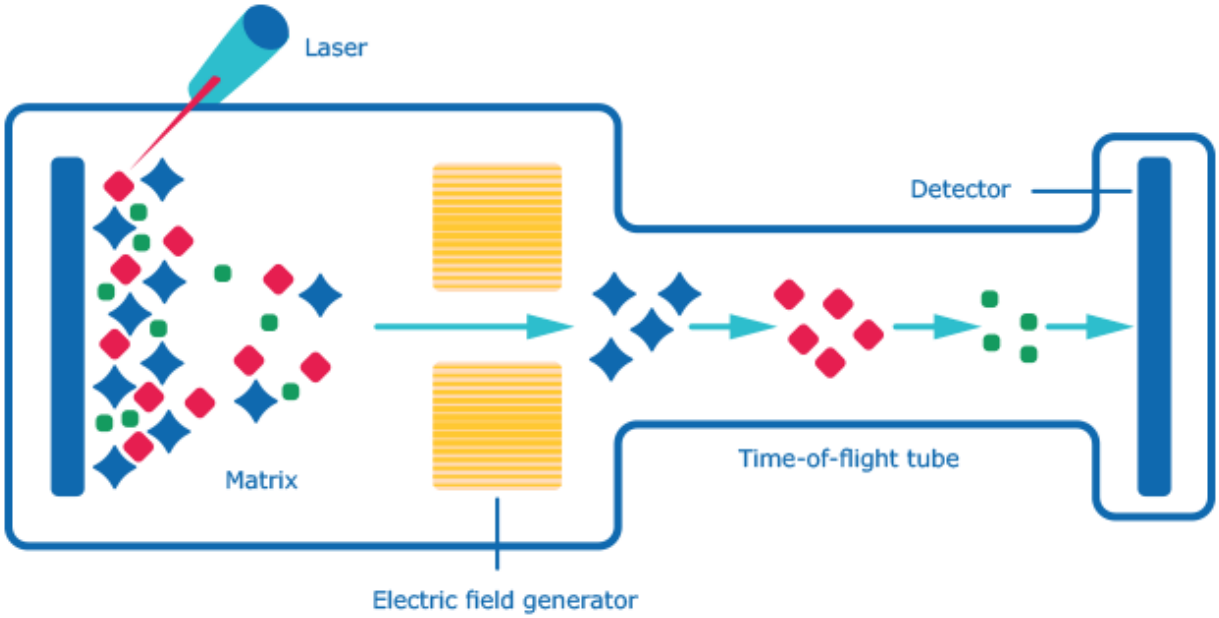
i. Absorbent = crushed metal or polymer inside a 30ft column
ii. Eluent = gas (He or N_2) instead of liquid
iii. Gaseous compounds reach the end of the column at different rates based on how well they adhere to the absorbent.
iv. Mass spectrometry → molecular weight determination → separates compounds by m/z ratio and by running them through a magnetic field

g. High-performance liquid chromatography (HPLC):

i. Similar to GC, but eluent is liquid under pressure instead of gas
ii. The entire process is computerized → more precise and efficient than regular column chromatography

4. Mass spectrometry:

a. Measures the mass-to-charge ratio (m/z or m/q) of ions, presented as a mass spectrum (plot of intensity as a function of the mass-to-charge ratio).
b. Ex: distinguish between isotopes: ^{235}U and ^{238}U
   i. Same Z (atomic number), same C (charge), the only difference is the number of neutrons → different A (mass number), hence difference mass since A closely represents the weight of ions.
c. A sample is injected into the mass spectrometer, and the individual molecules obtain a charge. The charged particle is then shot into a magnetic field and will undergo circular motion, depending upon its mass and charge.
d. Increased mass (m) = increased radius (r) → so depending on where the circular trajectory lands (radius), we can find tell which isotope has a greater mass.
   i. Ex: ^{238}U will have a greater m → greater r → lands farther than ^{235}U
e. https://www.youtube.com/watch?v=-YfemQNTkvA



5. Spectroscopy:

a. NMR spectroscopy:

i. Proton (^{1}H) NMR:

ii. Ranges:

1. TMS peak: 0 ppm (right-most)
2. Aromatic H: 6 - 8.5 ppm
3. Aldehydic H: 9 - 10 ppm
4. Carboxylic acid H: 10.5 - 12 ppm

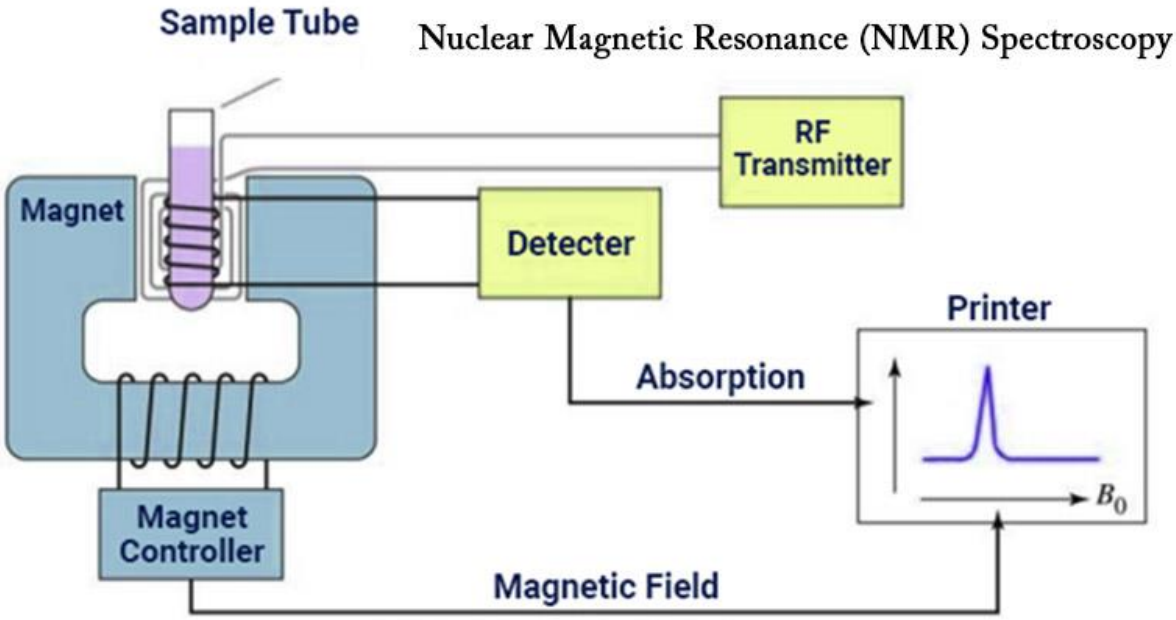
iii. Deshielding e- = Downfield

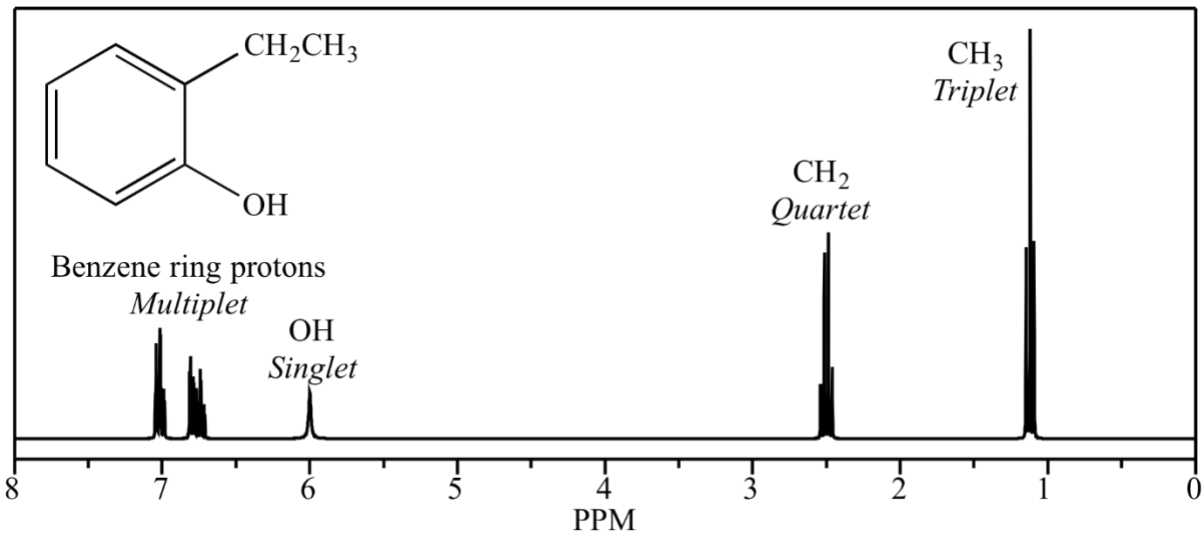
1. Electronegative groups:
    a. Withdraw e- → less e- shielding H+ from magnetic field
    b. Deshielding → peak moves more downfield

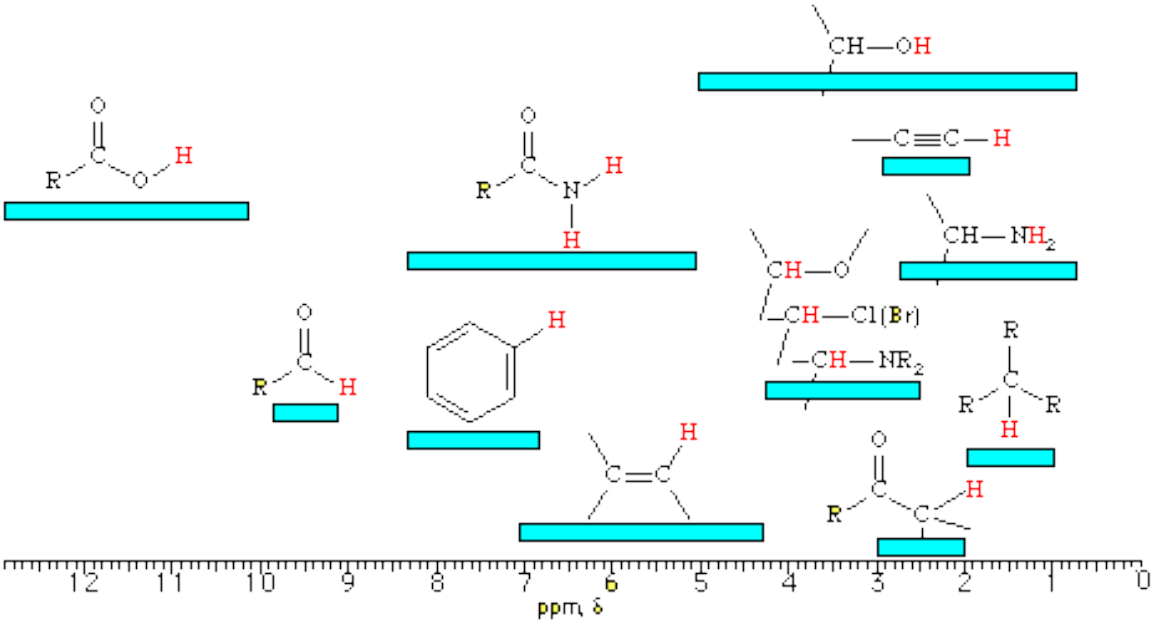
2. Electron-donating groups:
    a. More e- around to shield H+ from magnetic field
    b. Shielding → peak moves more upfield (near TMS)

iv. ^{12}C: no magnetic moment → not useful for NMR

v. Splitting of spectral lines → coupling of H+ on adjacent carbon atoms
    1. N+1 rule: H+ peak is slit into ***n+1*** subpeaks, where ***n is the number of H+*** that are ***three bonds away*** from the H+ of interest
    2. Don't include H+ attached to O or N
    3. Coupling constant (J): a measure of the degree of splitting caused by other atoms in the molecule





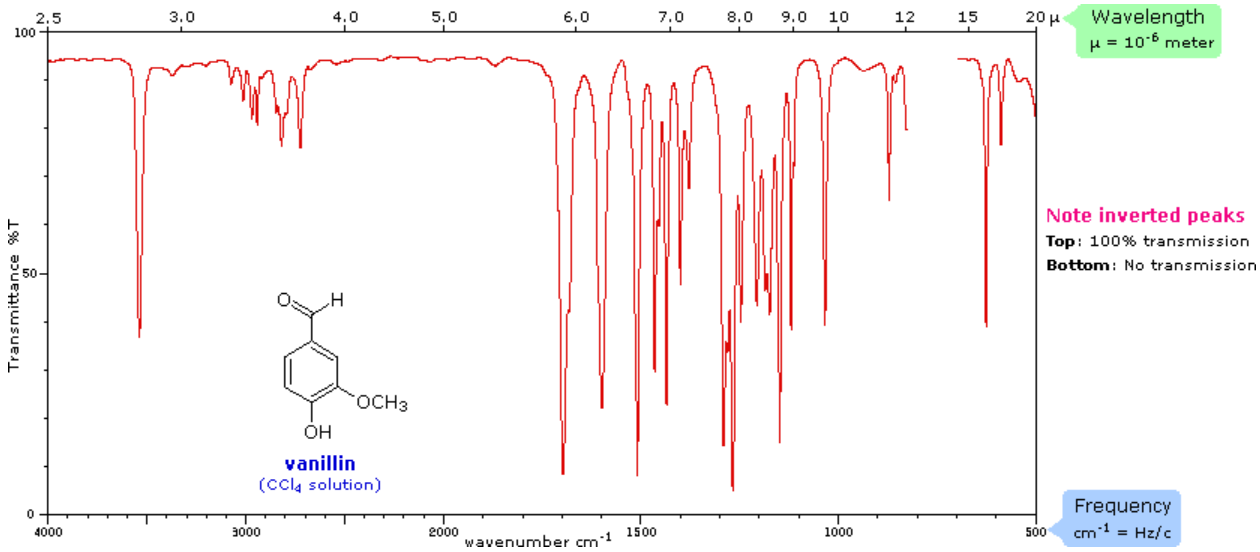


b. **UV-Vis spectroscopy:**

i. Used to detect conjugated alkenes
   1. Double bonds (p orbitals)
   2. Heteroatoms with lone pair (N:)

ii. Must have small enough E difference between HOMO and LUMO to permit an e- to move from one orbital to another
   1. Smaller the difference = longer absorption wavelength
   2. Before absorbing a UV H+, e- can be found in HOMO only

c. **IR spectroscopy:**



i. Used to determine FG, double bond, triple bond

ii. Based on molecular vibrations that induce a change in dipole moment
   1. 1750 cm^{-1}: C=O (strong absorption)
   2. 3100-3500 cm^{-1}: OH or NH (broad absorption)

iii. Wavenumber ∝ Frequency

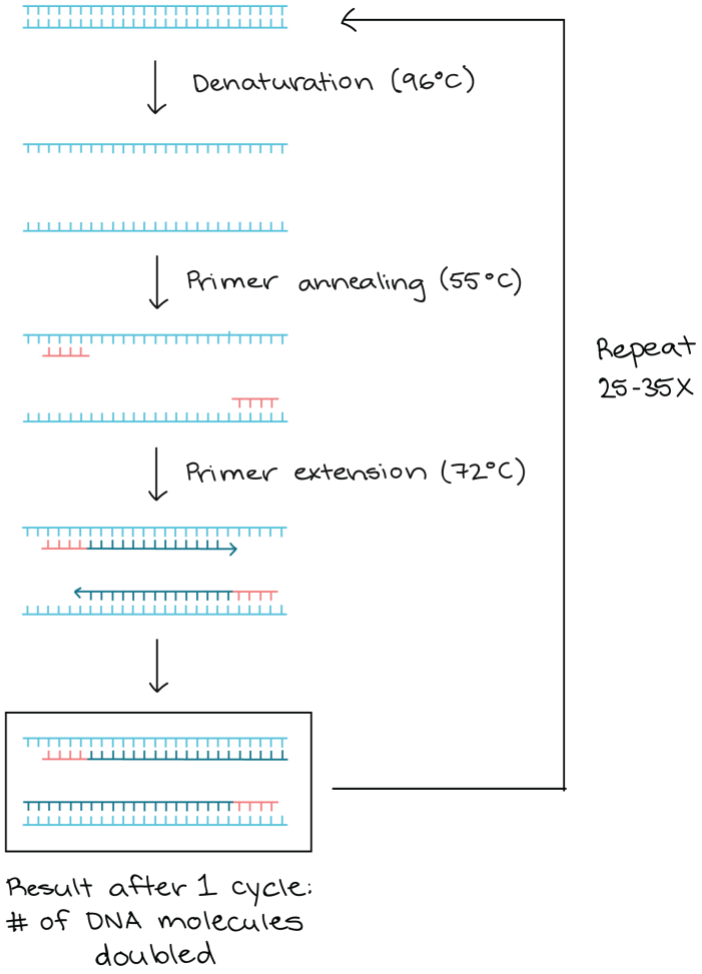
iv. Extended conjugation shifts the absorption band of C=O to lower wavenumber (lower than its usual 1750 cm^{-1}) because delocalization causes some C=O bands to lose double bond characteristic → shift closer to C-O stretching frequency

v. Enantiomers: identical IR spectra because they have same FG
   1. IR mainly detects FG!!

6. PCR: make copies of DNA fragments (in vitro method of DNA Replication)

a. Polymerase chain reaction, or PCR, is a technique to **make many copies of a specific DNA region** in vitro (in a test tube rather than an organism).
b. PCR relies on a thermostable DNA polymerase (an enzyme that works at high temperature): Taq polymerase, and requires DNA primers designed specifically for the DNA region of interest.
    i. DNA polymerase = enzyme that makes new strands of DNA, using existing strands as templates
        1. Taq polymerase: most active at 70°C → ideal for PCR since high temperature is needed to denature the template DNA → separate its strands.
    ii. Primers = short sequence of nucleotides (10-20 bp) that provides a starting point for DNA synthesis
        1. After two DNA strands are separated by heat → primers bind to the template by complementary base pairing.

c. **STEPS OF PCR:**



i. Add into solution (usually water and salt):
   1. DNA fragments
   2. Nucleotides (for Taq to add bp to ssDNA)
   3. Taq polymerase
   4. Primers

ii. Round 1:

iii. **Denaturation (96°C)**: Heat the solution to separate/ denature the dsDNA. This provides a single-stranded template for the next step.

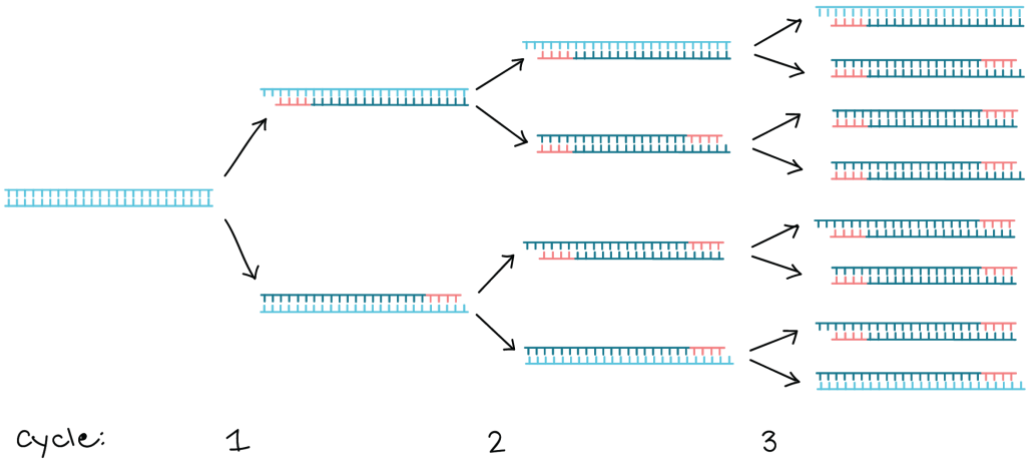
iv. **Annealing (55°C)**: Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.

Q: There are no enzymes to keep the templates separated as ssDNA (SSBP) in the solution, how does the DNA remain separated after denaturation?

A: As soon as heat is removed as annealing starts, the primers present in the solution will immediately bind and “lock” the fragments into ssDNA, awaiting Taq

v. **Extension (72°C)**: Raise the reaction to efficient temperatures → Taq polymerase extends the primers, synthesizing new strands of DNA using nucleotides added into solution initially.

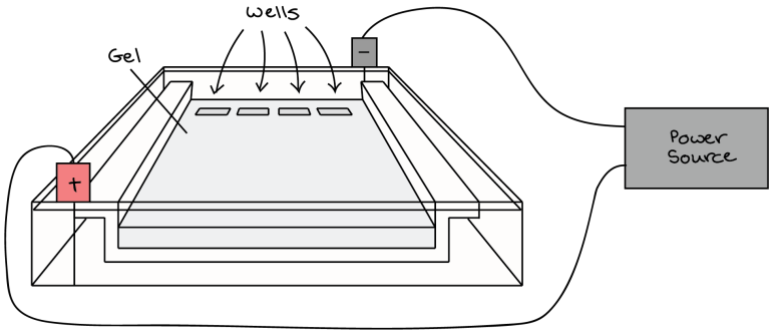
vi. New DNA that's made in round 1 can serve as a template in the next round of DNA synthesis



vii. PCR reactions are usually visualized using gel electrophoresis. A **DNA ladder** is included so that the size of the fragments in the PCR sample can be determined → added in well 1.

viii. https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/v/the-polymerase-chain-reaction-pcr

7. Gel Electrophoresis: separate DNA fragments by size (not good for detecting a specific DNA)



a. "Gel" = gel, "electro" = electric current, "phoresis" = migration
   i. DNA samples are loaded into wells (indentations) at one end of a gel, and an electric current is applied to pull them through the gel.

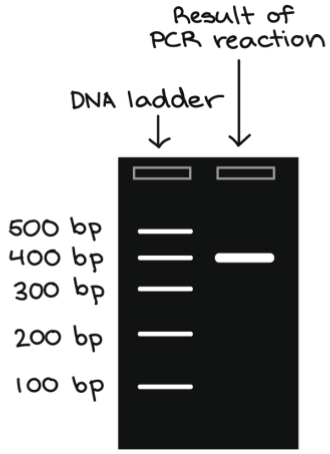
ii. **DNA fragments are negatively charged** → move towards the positive electrode. Since all are (-) charged, they are separated by size only.

1. Negative charge → near the wells
2. Positive charge → on the opposite end of the wells
3. (-) DNA fragments repel and want to get away from the (-) charge → move further down the well towards the (+) side.

iii. Because all DNA fragments have the **same amount of *charge per mass (all negative charge)***, small fragments move through the gel faster than large ones. The shortest of the molecules travel farthest down the gel.

1. 5000 bp → travel slow (has to move the most mass)
2. 150 bp → travel faster (less mass)
3. Gel = usually agarose, a polysaccharide → lots of mesh and glycosidic branches → it makes sense that the smallest/ shortest DNA fragments will move through the fastest

iv. When a gel is stained with a DNA-binding dye, the DNA fragments can be seen as bands, each representing a **group** of same-sized fragments.



b. https://www.youtube.com/watch?v=_EYsykjsfiY

8. cDNA libraries: infer DNA sequence from protein (protein → mRNA → DNA)

a. cDNA Libraries:
   i. cDNA = complementary DNA, single-stranded at first (add DNA Polymerase and DNA Ligase to form a ds-cDNA)
   ii. Includes only transcribed portions of DNA → NO introns

iii. **Reverse transcriptase** → uses an mRNA template to "reverse transcribe" from ssRNA to dsDNA. Contains 3 enzymatic activities:

1. **RNA-dependent DNA polymerase** → synthesizes ssDNA strand complementary to the ssRNA template.
2. **RNase H** → removes ssRNA strand from the RNA–DNA hybrid double helix.
3. **DNA-dependent DNA polymerase** → synthesizes complementary DNA strand to complete the dsDNA.

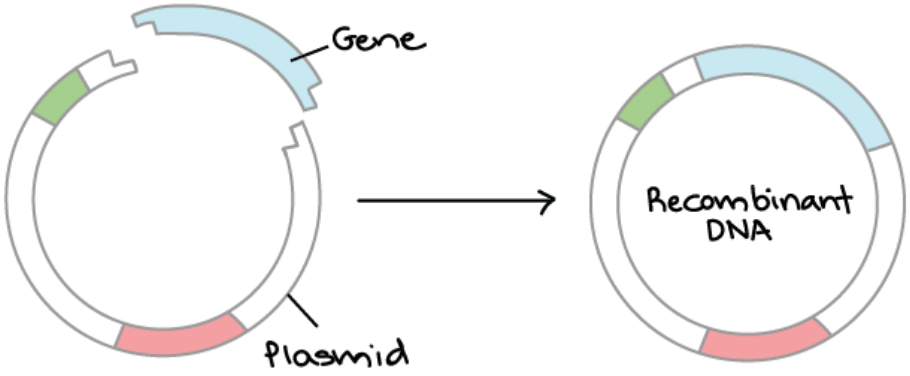
iv. Steps:

1. **AA → mRNA (ssRNA) → dsDNA**
    a.
2. dsDNA → inject into a cloning vector (virus, bacteria) — (amplification)→ sequence of dsDNA → cDNA library

b. Genomic DNA Libraries:

i. Includes the whole-genome, with introns and exons

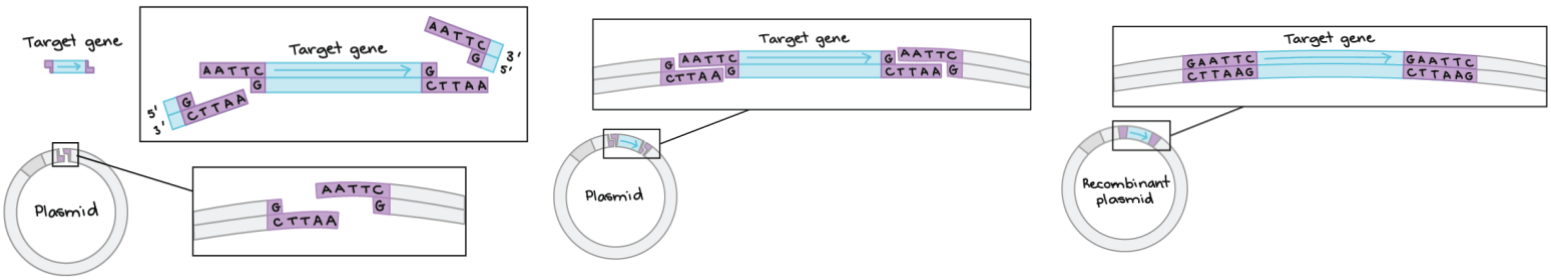
9. DNA cloning: use plasmids to “factory” many identical copies of gene



(Overview) DNA cloning

a. A target gene is inserted into a plasmid. The plasmid is inserted into bacteria via a process called transformation, and bacteria carrying the plasmid are selected using antibiotics.
b. The insertion is done using enzymes that “cut and paste” DNA, and it produces a molecule of **recombinant DNA** (DNA assembled out of fragments from multiple sources). Bacteria with the correct plasmid are used to make more plasmid DNA or, induced to express the gene and make protein.
    i. Ex: human insulin gene is expressed in E. coli bacteria to make insulin
    ii. Green region = promoter to drive target gene expression
    iii. Light blue region = target gene for insulin or GH
    iv. Red region = gene for antibiotics resistance, so bacterias that actually take up the plasmids can survive on plates with antibiotics

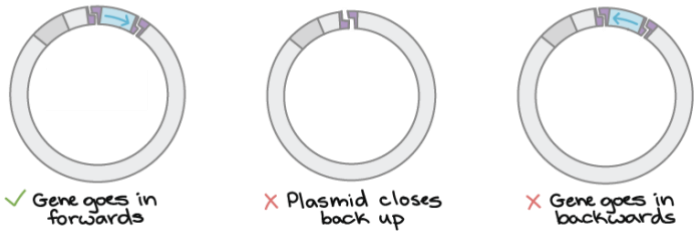
c. **STEPS OF DNA CLONING:**
    i. **Step 1:** Cut open the plasmid and "paste" in the gene. Relies on:
        1. ***Restriction enzymes*** (which cut DNA): produce “sticky ends” with short, single-stranded overhangs. If two molecules have matching overhangs, they can base-pair and stick together.



        2. ***DNA ligase*** (which joins DNA): used to join Okazaki fragments

ii. **Step 2:** Insert the plasmid into bacteria using ***transformation (2.1)*** (bacterial cells are given a heat shock that encourages them to take up foreign DNA.) Use ***antibiotic selection (2.2)*** to identify the bacteria that took up the plasmid.

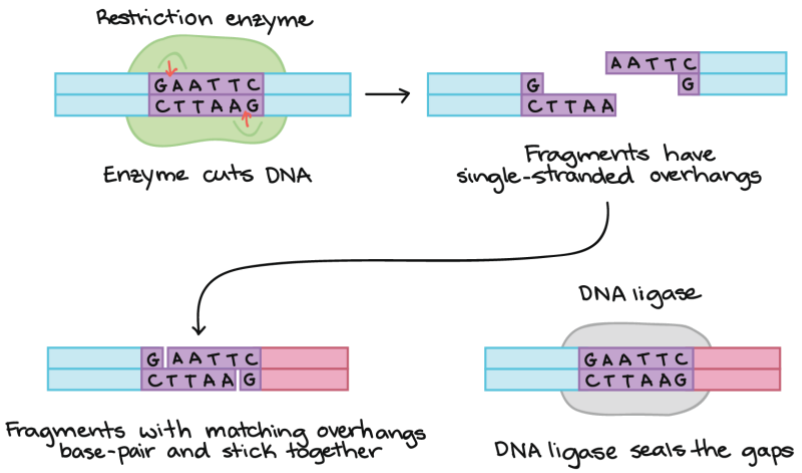
1. Heat shock changes membrane fluidity or causes pores to form → easier for DNA to enter the bacteria.
2. Plasmids contain an antibiotic *resistance* gene that allows bacteria to survive in the presence of antibiotics → bacteria that took up the plasmid will survive and can be selected on nutrient plates containing the antibiotic.
3. Use restriction enzyme digestion or PCR to check the plasmids in case DNA fragments don't get "pasted" correctly by DNA ligase.



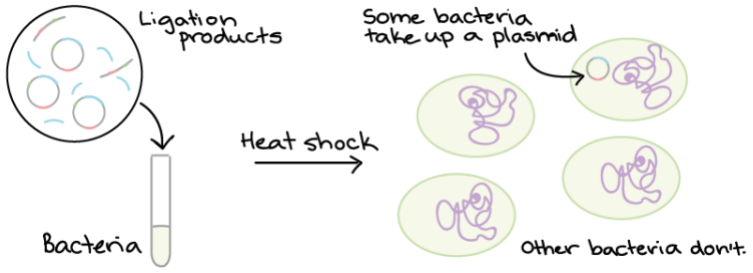
iii. **Step 3:** Grow lots of plasmid-carrying bacteria and use them as "factories" to make the protein. Harvest the protein and purify it.

1. Give the bacteria a chemical signal that instructs them to make the target protein.
2. Once the protein has been produced, the bacterial cells can be split open to release it. The target protein must then be purified (separate from the other contents of the bacterial cells).

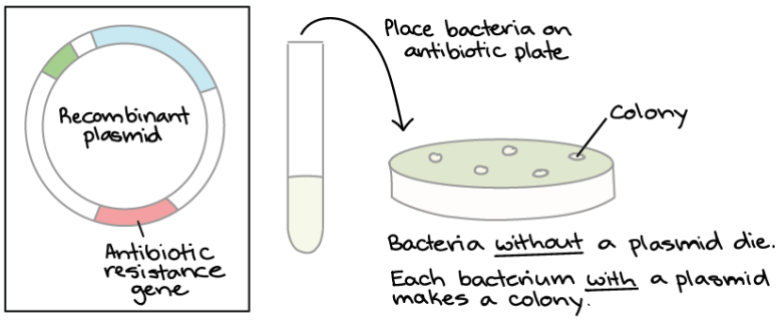
DNA CLONING OVERVIEW:



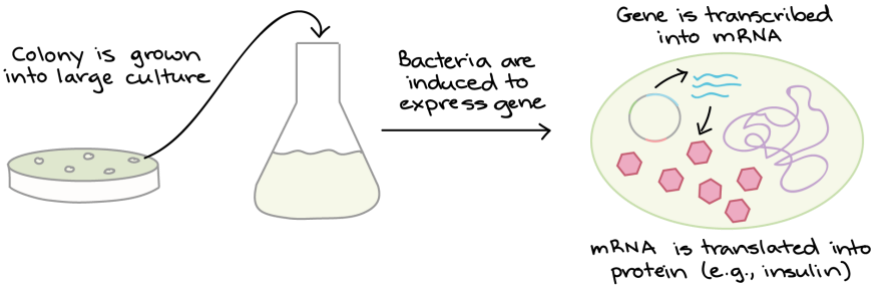
(1) Cut and paste DNA



(2.1) Transformation



(2.2) Antibiotic selection



(3) Protein production

10. DNA sequencing: sequence the nucleotides (A, T, G, C) in a DNA fragment

a. **Sanger sequencing (chain termination method):** target DNA is copied many times, making fragments of different lengths. Fluorescent "chain terminator" ***dideoxynucleotides (ddNTP)*** mark the ends of the fragments and allow the sequence to be determined. Used to sequence individual fragments of DNA, such as target genes on bacteria plasmids (DNA cloning) or DNA copied in PCR.

i. **Essentially the same as PCR, but ddNTP with fluorescent labels are also added** into the PCR solution along with normal, non-fluorescent/ non-chain terminating deoxynucleotide (dNTP).

ii. Ingredients:

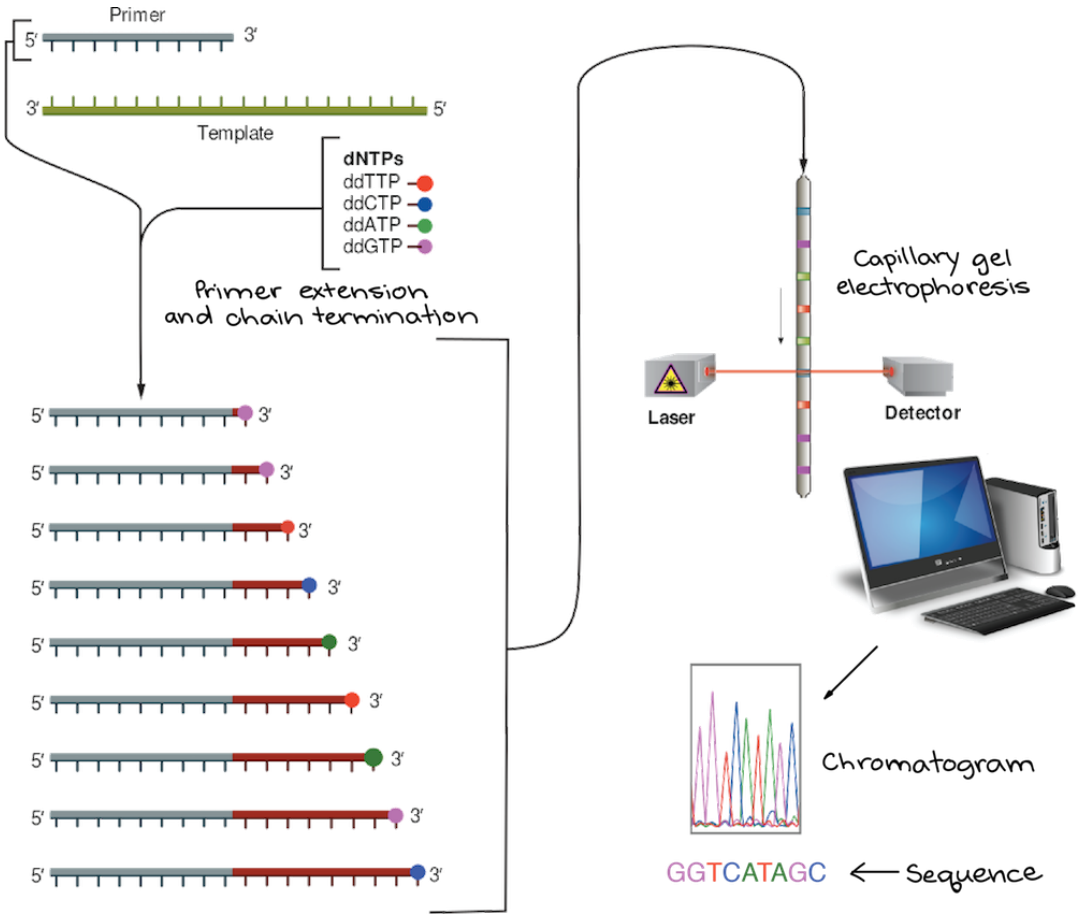
1. DNA polymerase
2. Primer
3. Template DNA (dsDNA) to be sequenced
4. The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)
5. Dideoxy, or chain-terminating, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye
    a. ddNTP lacks an OH group on the 3' carbon of the sugar. The 3' OH group normally acts as a "hook," allowing a new nucleotide to be added to an existing chain.
    b. Once a ddNTP has been added to the chain, there is no 3'-OH available and no further nucleotides can be added. The chain ends with the ddNTP marked with a different color depending on the base (A, T, C, G) that it carries.
    c. The dye molecule on a dideoxy nucleotide is linked to the nitrogenous base.

iii. STEPS OF SANGER SEQUENCING:

1. All ingredients are added to a solution and run like a normal PCR. The four dye-labeled, chain-terminating ddNTP are added in much

smaller amounts than the ordinary DNA nucleotides (dATP, dTTP, dGTP, and dCTP).

2. **PCR:** The mixture is first heated to denature the dsDNA (separate the strands), then cooled so that the primer can bind to the ssDNA template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer.
3. **DNA polymerase will continue adding dNTP to the chain until it happens to add a ddNTP instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the fluorescent ddNTP.**
4. Repeated in a number of cycles → guaranteed that a ddNTP will have been incorporated at every single position of the target DNA in at least one reaction. The tube will contain fragments of different lengths, the ends of the fragments will be labeled with dyes that indicate their final nucleotide.
5. Q: [Will all the fragments be labeled?] A: No. Some fragments consists only of normal, unlabeled dNTP; ends when polymerase falls of the template, not due to ddNTP. **Unlabeled DNA do not interfere with the sequencing reaction, as they are "invisible"** in the detection step due to their lack of a dye label.
6. **Gel electrophoresis:** After PCR is done, the fragments are run through *capillary gel electrophoresis.*
    a. The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer). Long fragments move more slowly
    b. Colors of dyes can be registered depending on the base (A, T, C, G) that it carries, one after another → **the complementary sequence of DNA** can be built up one nucleotide at a time from 5- → 3' → must reverse to find the template DNA sequence!!

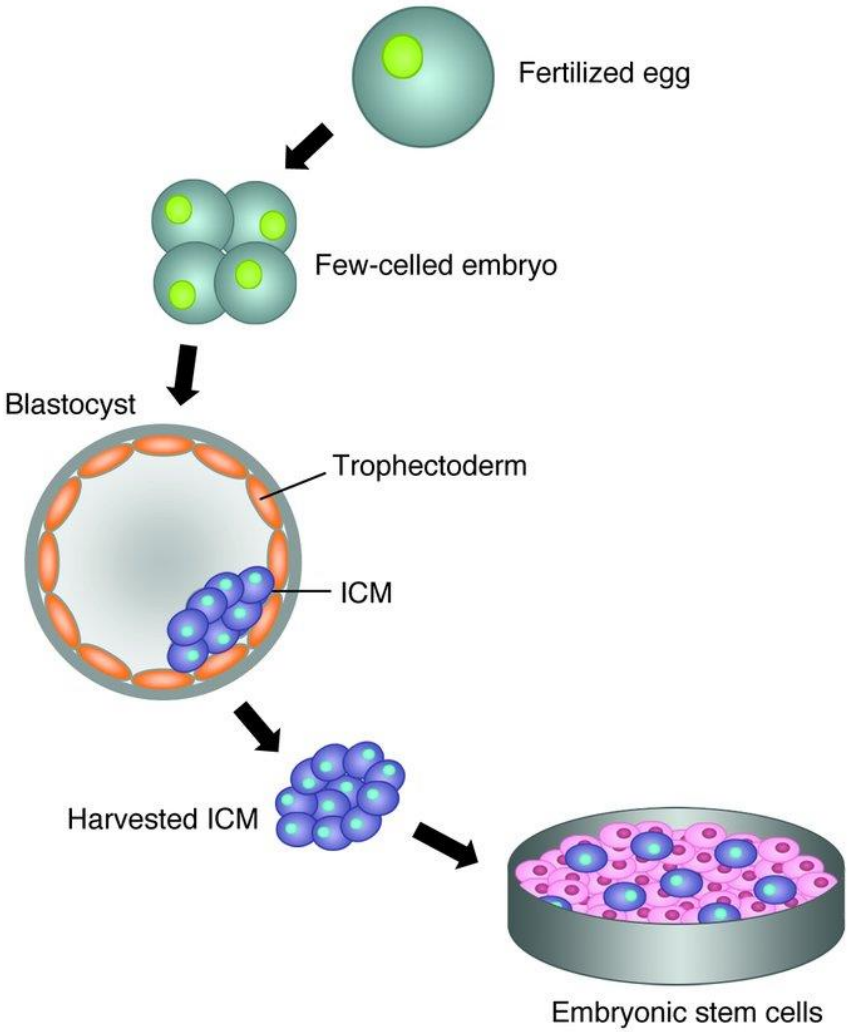


Sanger sequencing

iv. https://www.khanacademy.org/test-prep/mcat/biomolecules/dna-technology/v/dna-sequencing

11. Embryonic Stem Cells:

a. Stem cells derived from the *undifferentiated* inner mass cells of a human embryo.
   i. **Pluripotent**, has the potential to grow (differentiate) into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm.
   ii. Derived from the inner cell mass of a blastocyst
b. Totipotency: can form all the cell types in a body, the extraembryonic, placenta.
   i. Embryonic cells within the first couple of cell divisions after fertilization.
c. Pluripotency: can give rise to all of the cell types that make up the body.
   i. Embryonic stem cells are considered pluripotent.
d. Multipotency: can develop into more than one cell type, but are more limited
   i. Adult stem cells (somatic stem cells) and blood stem cells
e. https://www.khanacademy.org/science/biology/biotech-dna-technology/stem-cells/v/embryonic-stem-cells



12. DNA Hybridization: compare the genomic similarities between different DNA strands.

a. The procedure involves denaturing double stranded DNA (dsDNA) from two different samples, and then allowing the single strands to re-anneal into a dsDNA such that *one strand is from one sample* and *the other strand is from the other sample*, producing a **"hybrid" dsDNA**.
b. The stronger the hybrid strands are able to hydrogen bond together, the more similar the original DNA samples were to each other (stronger binding = more complementary base pairs = more similar sequences).

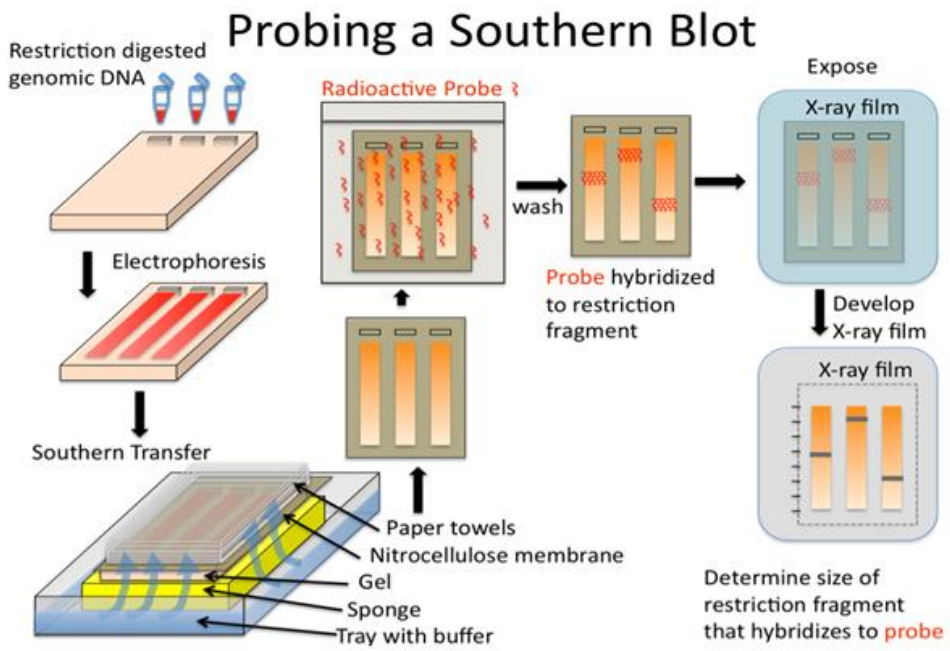
13. DNA Microarray: detect the expression of thousands of genes.

a. A laboratory tool used to detect the expression of thousands of genes at the same time. DNA microarrays are chips with microscopic wells, each containing a known cDNA fragment.
b. 2 cell types (Ex: cancer vs. normal), each labeled with a different fluorescent color. Adding the cDNA of the cells into the well can help determine which cell type predominates, cancer or normal, depending on the color of the well.
    i. Gene A (upregulation):
        1. Cancer gene labeled yellow vs. normal gene labeled green)
        2. If the well fluorescent yellow → there are more of cancer gene A in the cell → overexpression of tumors.
    ii. Gene B (downregulation):
        1. Cancer gene labeled yellow vs. normal gene labeled green)
        2. If the well fluorescent green → there are more of normal gene B in the cell → no downregulation in this cell → divide rapidly, form tumors with no checkpoints.
c. https://www.khanacademy.org/test-prep/mcat/biomolecules/dna-technology/v/hybridization-microarray

14. Southern Blot: detect a specific DNA (ds) in a long DNA strand.

a. Example: interested in finding whether *gene A* is present in a long DNA strand.
    i. Probe: complementary DNA to gene of interest
    ii. Label: **Radio-label** or enzyme
    iii. Gel: Agarose gel
b. STEPS:
    i. Step 1: Cleve DNA using restriction enzymes → get DNA fragments
    ii. Step 2: Run gel electrophoresis → separate DNA fragments by size

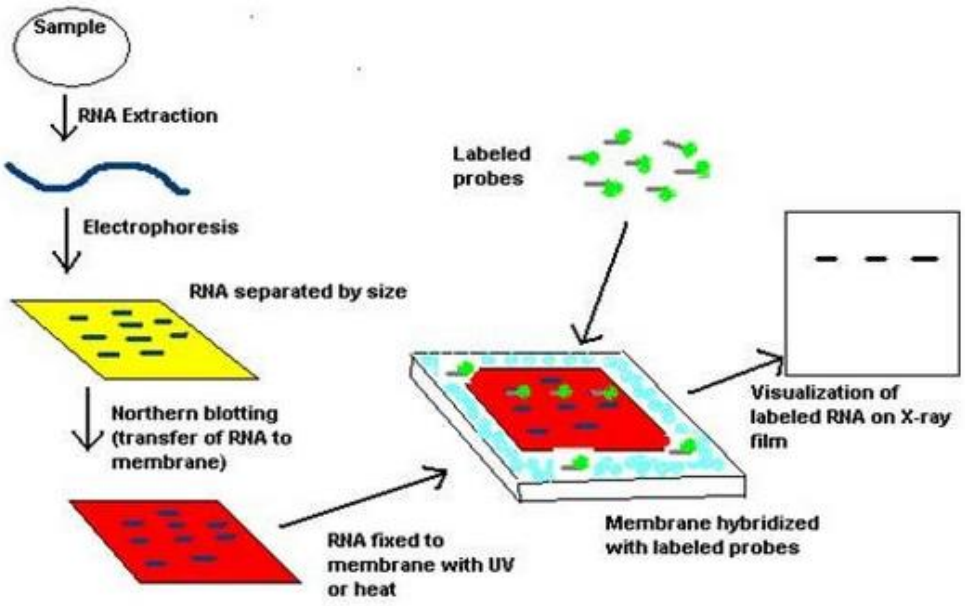
iii. Step 3: Denature the DNA fragments in the gel (make it single-stranded) by making the pH basic.
iv. Step 4: Transfer the gel into a **filter** (sturdier, easier to visualize)
v. Step 5: Expose filter to radio-labeled DNA
    1. **Radio-labeled DNA** = *complementary* to gene of interest (*gene A*)
    2. If gene A was present, the complementary radiolabeled probe will anneal (stick) to gene A on the filter.
    3. The radiation in the complementary probe exposes the film, resulting in a signal detection where the probe has bound.



(Southern Blot)

15. Northern Blot: detect a specific RNA in a long RNA strand.

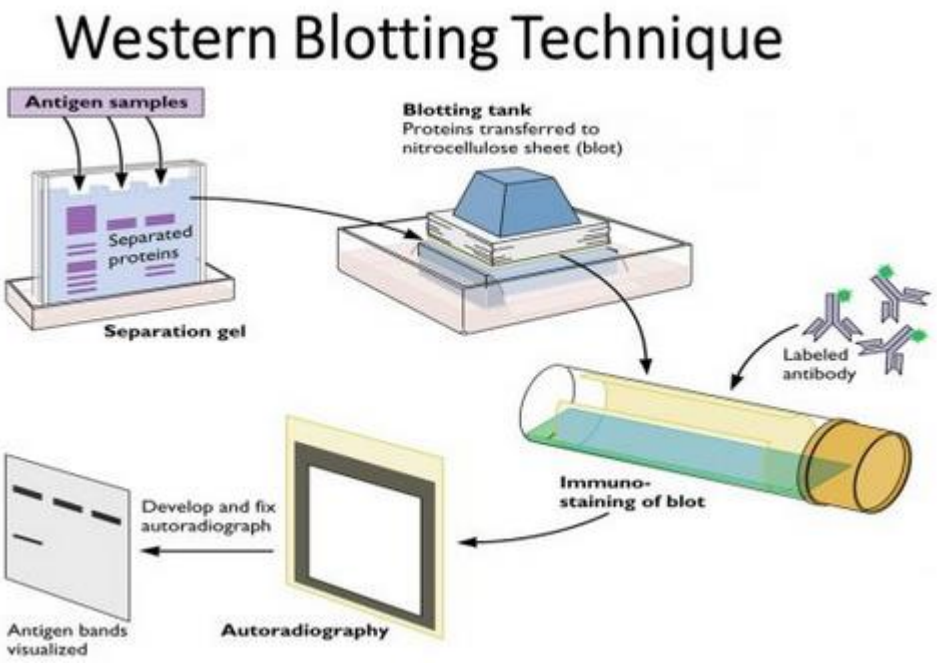
a. Example: interested in finding whether *gene A* is present in a long RNA strand.
    i. Probe: RNA, DNA, or oligo-dNTD fragments
    ii. Label: **Radio-label** or enzyme
    iii. Gel: Formaldehyde agarose gel
b. Similar steps to Southern blotting, except replace DNA with RNA



(Northern Blot)

16. Western (Immune-blotting): detect a specific protein in a sample of protein.

a. Example: interested in finding whether *protein A* is present.
   i. Probe: Antibody
   ii. Label: Enzyme
   iii. Gel: Polyacrylamide gel
b. Similar steps to Southern and Northern blotting



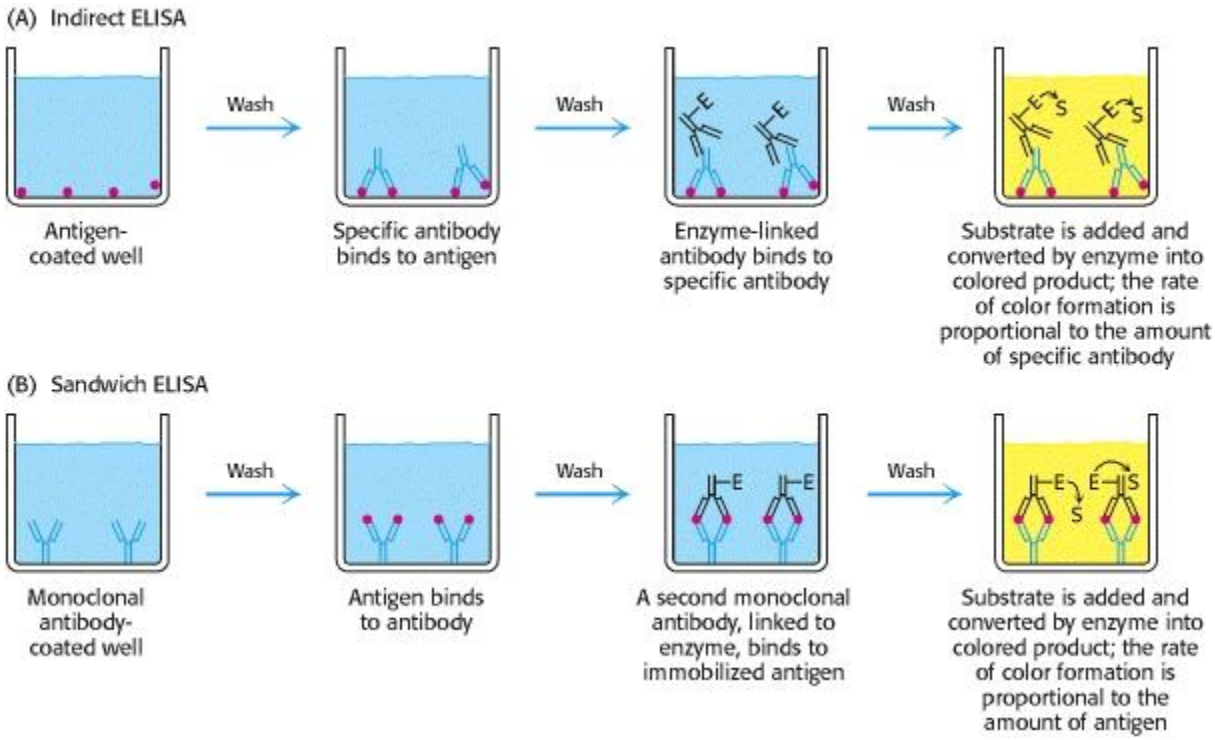
(Western/ Immune Blot)

17. Edman degradation: cleave peptide bond to determine AA sequence.

a. Amino-terminal residues are labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues.

18. ELISA: detect the concentration of antibody/ substrate visually.

a. Enzyme-linked immunosorbent assay (ELISA)
    i. Detect the presence and concentration of an antibody in a sample
b. Affinity chromatography vs ELISA:
    i. Affinity chromatography: used to **separate** compounds out of a mixture based on their affinity to specific interactions. Examples:
        1. Antibody-antigen
        2. Enzyme-substrate (ELISA)
        3. Receptor-ligand
    ii. ELISA: used to just **detect** the concentration of an antibody in a mixture.
        1. Indirect ELISA
        2. Sandwich ELISA



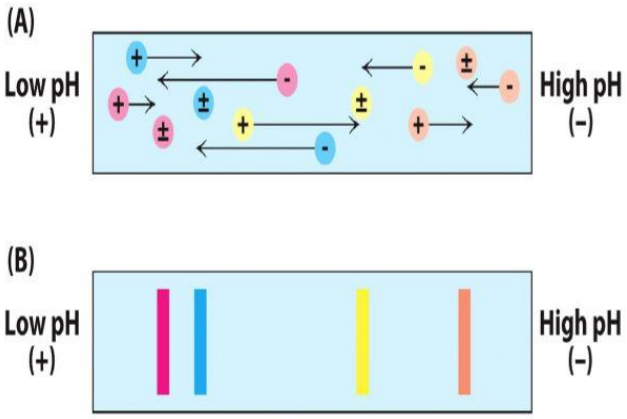
(Indirect vs. Sandwich ELISA)

19. Isoelectric focusing: separating different molecules by differences in their isoelectric point (pI).

a. **Separates** compounds on a gel based on their pI (determined by their relative amounts of acidic and basic amino acid side chains).

b. A mixture a proteins are placed in a gel with a pH gradient and an electrical field is applied. Proteins will migrate until they reach their isoelectric point (pI), the pH at which they have no net charge

   i. After reaching the position of the gel where pH = pI → substance stops migrating, and we can separate them by their pI.



(Isoelectric focusing)

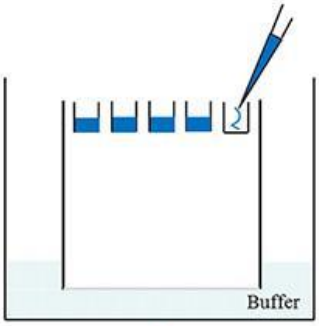
20. SDS-PAGE: ***denaturing*** gel electrophoresis, used for ***proteins*** separation based exclusively on ***mass*** (doesn't convey much about 3D structure)

a. **SDS-PAGE:** sodium dodecyl sulfate-polyacrylamide gel electrophoresis

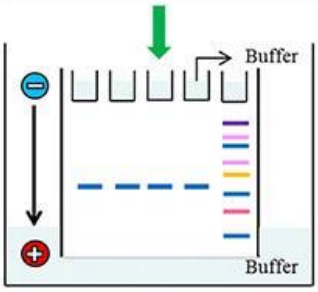
i. High-resolution gel electrophoresis used to separate **proteins** based on **mass exclusively** (protein has been denatured).
    1. Agarose gels are generally used for DNA = low resolution
    2. Polyacrylamide gel (PAGE) = higher resolution
ii. Always runs **vertically** (gel electrophoresis runs horizontally or vertically).
iii. Always **denatures** proteins prior to separation, while Native-PAGE and gel electrophoresis do NOT denature proteins:
    1. One SDS molecule will bind to every 2 amino acids → denatures protein and **gives it a negative charge** → all fragments now have the same mass-to-charge ratio, so they migrate only on the basis of mass.
    2. Denatures protein → doesn't give much info on 3D structure.

b. **Reducing SDS-PAGE:** cleaves disulfide bonds between Cys(C) residues
    i. Denatures protein AND cleaves disulfide bridges
        1. **Disulfide bonds hold difference subunits together** → so this tells us whether the protein is composed of multiple subunits.
            a. Subunits held by disulfide bridges will come apart under reducing SDS-PAGE but not under non-reducing SDS-PAGE and Native-PAGE.
        2. However!! Disulfides can exist independently of subunits (intra-unit and function to make one subunit more compact)

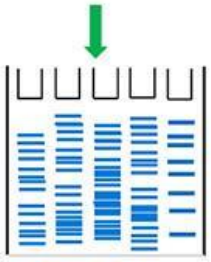
c. **Non-reducing SDS-PAGE:** does NOT cleave disulfide bonds
    i. Still denatures the protein, but leaves the disulfide bridges INTACT
        1. Protein will be mostly denatured and if it has disulfides, those will convey some 3D structure (minimal compared to Native-PAGE, which is the main method used to deduce 3D structures).
        2. Example:
            a. Run reducing SDS-PAGE: migrate at same rate
            b. Run non-reducing SDS-PAGE: one protein migrates further → can conclude that it has disulfide bridges (cleaved by reducing SDS) to hold it into a more compact structure than the other → migrates further.



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

(SDS-PAGE)

21. Native PAGE: ***non-denaturing*** gel electrophoresis, separate proteins and DNA based on ***mass-to-charge ratio***, gives info on ***3D structures***

a. Native-PAGE: Non-denaturing, runs without SDS → **3D shape** is important in determining how the protein migrates, gives more info on protein structure
   i. Example 1:
      1. Proteins: tightly folded → migrate further on gel
      2. DNA: supercoiled → migrate further than regular DNA (less friction)
   ii. Example 2:
      1. Run SDS-PAGE → suggests 2 proteins have similar masses
      2. Run Native-PAGE → if one migrates further, we can deduce that it is more "compact" than the other.

b. No SDS to denature: **mobility depends on both the protein's charge and size** (unlike SDS-PAGE where the electrophoretic mobility depends on mass only).
   i. All proteins exhibit different charges (may be overall (+) or (-), depending on how many acidic/ basic AA side chains there are) → migrate based on m/z ratio towards the (+) end of gel.
   ii. DNA migrates based on mass only since all are (-) charged.