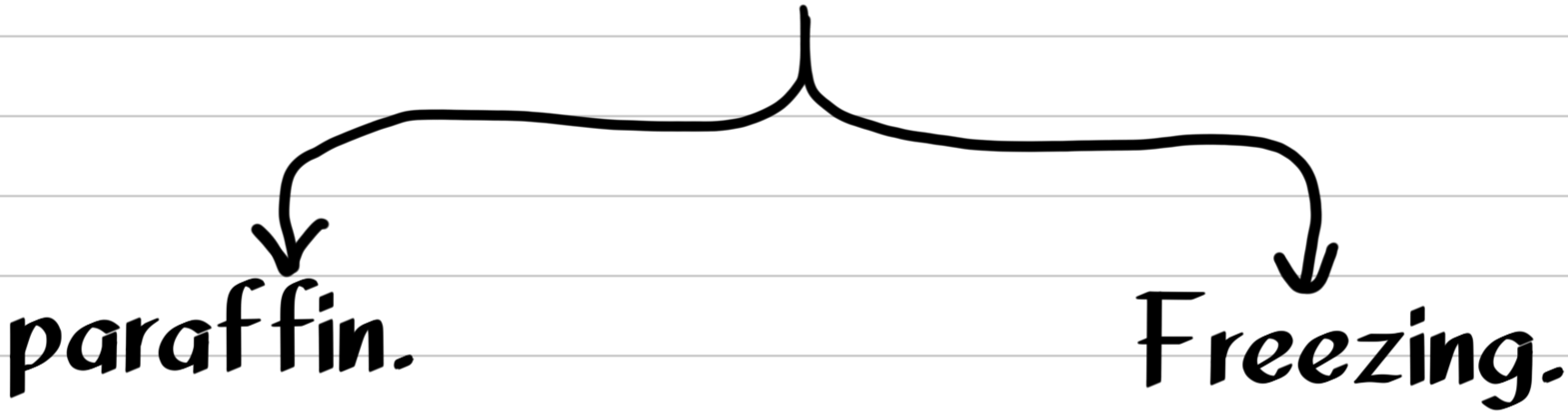
Cell Biology

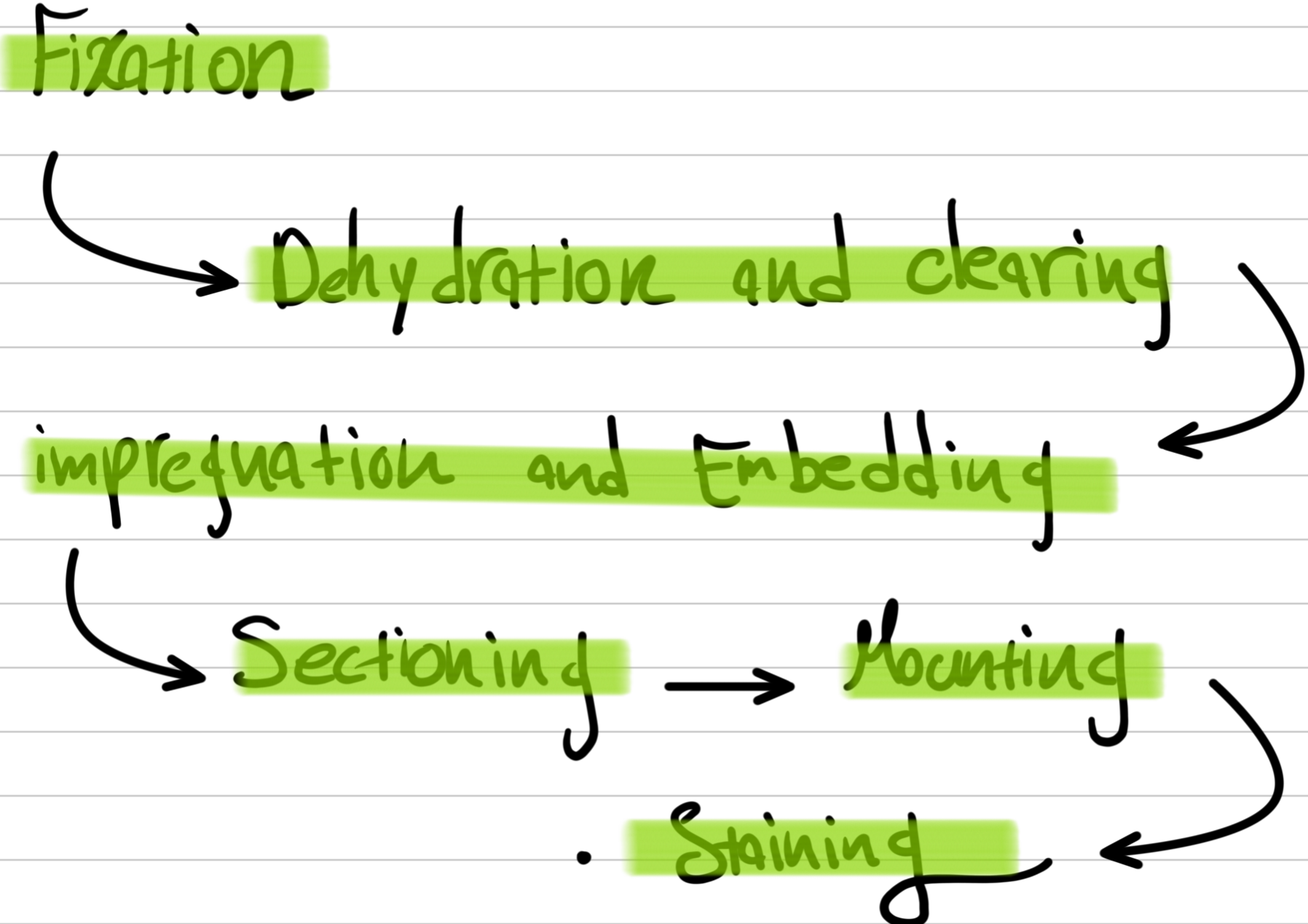
Lecture 2

Micro techniques

For LM



«Poraffin technique»



Fixation

1 - upon removal from the body the sample immediately placed in a fixative solution.

→ to preserve the structure of the tissue as in the life state and to prevent autolysis and to preserve the morphology.

↳ LM → Formol saline.

↳ EM → Glutaraldehyde.
Osmium tetroxide.

Advantage of the fixation step.

- Hardens the tissue by coagulating its protein.
  - ↳ Facilitate the process of cutting and staining and examination.

- prevent putrefaction and stop autolytic changes
  by killing any bacteria.

- preserves the molecular and morphological structure of the tissue.

Dehydration and Clearing

Dehydration → Is done by treating the specimen with ascending concentration of alcohol.

- 50% → 70% → 100%.

Gradual removal of water from the specimen.

Clearing → tissue become translucent

to allow the light to penetrate the specimen.

the tissue is treated with

xylol or benzol

to remove the alcohol.

Impregnation and Embedding.

impregnation

- Soft paraffin wax.
- The wax infiltrates the tissue and occupies all the spaces that were originally occupied with water.

Embedding

- hard paraffin wax.
- The tissue is placed in the center of the paraffin, which hardens as it cools → paraffin block.

Sectioning

Microtome is a mechanical device used to cut extremely thin slices a fixed tissue block.

→ sharp metal knife that used to cut the block into thin sections. (3-10 microns) as it moves up and down.

mounting.

↳ Tissue sections are placed on glass slides smeared with egg albumin. then warmed on a hot plate to dry.

→ The sections are now ready to be stained.

«Freezing technique»

Fresh frozen tissues are cut using

→ cryostat (Freezing microtome).

The specimens placed in cold fluid called

→ Isopentane.

→ liquid nitrogen. (-50C)

Advantages.

- Rapid technique for diagnosis in operation rooms (tumors).

- No fixation, No dehydration, No chemicals are used.

Disadvantages.

- Non serial and Fragmented sections

- Cannot be preserved for a long time.

Applications of the freezing technique :-

- intraoperative diagnosis.
- immunohisto chemistry
- Enzyme histochemistry.
- lipid demonstratration.

«Staining»

- Routine stains.
- Special Stains.

Routine Stains

Hemotoxyline(H)

- blue basic dye (+ve charged)
- Stains acidic (anionic -ve).
  - like ribosomes(r-RNA) nucleus.

Basophilic structure = blue

Eosin(E)

- red acidic dye (-ve charged)
- Stains basic (cationic +ve).
  - like mitochondria cytoplasm, muscles.

Acidophilic structure = red

special stains

Vital stain.

- Staining phagocytic cells (macrophages) with Trypan blue and Indian ink.

Leishman Stain.

(methylene blue and Eosin)

↳ to stain blood films to demonstrate

white and red blood cells.

- malaria parasite.

Metachromatic stain i-

Stain gives the tissue new color different form that of the original stain.

- Toluidine blue when stains Mast cells gives purple color.

- phenomenon called metachromasia.

Trichrome Stoins: (3stains)

↳ Collagen fibers stained blue.

Ocrein stains :-

↳ Stains elastic fibers brown.
(wall of aorta).

Sliver (Ag) stains :-

↳ nerve cell brown , reticular fibers black.

Histochemical stains't

used selectively identify and demonstrate the distribution of chemical substances or enzymes within between the cells.

— mucine or alkaline phosphatase enzyme.

purpose :-

- Detect glycogen, lipids.
- Detect enzyme activity.
- Detect abnormal accumulation.

- Assist in disease diagnosis (storage disorders, liver diseases).

Immano-histochemical (IHC) stoinsi-

↳ using specific antibodies to check for these Antigens (proteins) in tissue samples.

- Antibodies are usually linked to an enzyme or a florescence dye (markers) (Labeled antibodies).

Used :-

1 - diagnosis of cancers (markers).

2- the differentiation between different types of cancers.

Direct. or Indirect.

labeled 1ry antibody.

unlabeled 1ry + labeled 2ry antibody.

methods for study tissues

vivo

→ within the living body.

→ animal model based testing.

→ study of tissues after doing any experiment inside the living body.

vitro

→ outside the body.

→ cell based testing.

→ study of tissues outside their normal biological context.

Cell and Tissue Calture

1- At defined temperature (37C°) using an incubator.

2- Supplemented with a medium containing cell nutrients.

3- Growth factors (like animal serum).

Different types of cells can grow in cultures:-

- White blood cells, fibroblasts.
- skeletal and cardiac muscle.

- epithelial tissue (liver, breast, skin, kidney).
- many different types of tumor cells.

Medical uses of tissue culture :-

1- Studying chromosomal patterns of individuals karyotyping, gene therapy.
2- Used in researches of cancer.
3- Used in cultivation of bacteria, viruses. in order to prepare different vaccination.
4- Study the effects of new drugs.

cell culture.

→ cell can be isolated from the body for in vitro cultures.

Using enzymes → collagenase and trypsin which break down the ECM.

Confluence.

→ percentage of surface area in a culture vessel that covered by adherent cells. (1ry or 2ry cultures). (25%, 50%, 100%).

Primary cultures :-

↳ Refer to the cells that are cultured directly from a tissue (parent cells).

Secondary cultures :-

↳ Once the parent cells reach confluence they have to be sub-cultured. by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

cell line

- population of cells derived from a single cell that has been cultured in vitro.

- limited life span.

- Origin :- tissue

tumor or 1ry cells.

(Hela :- cancer research).

immortalized cell line

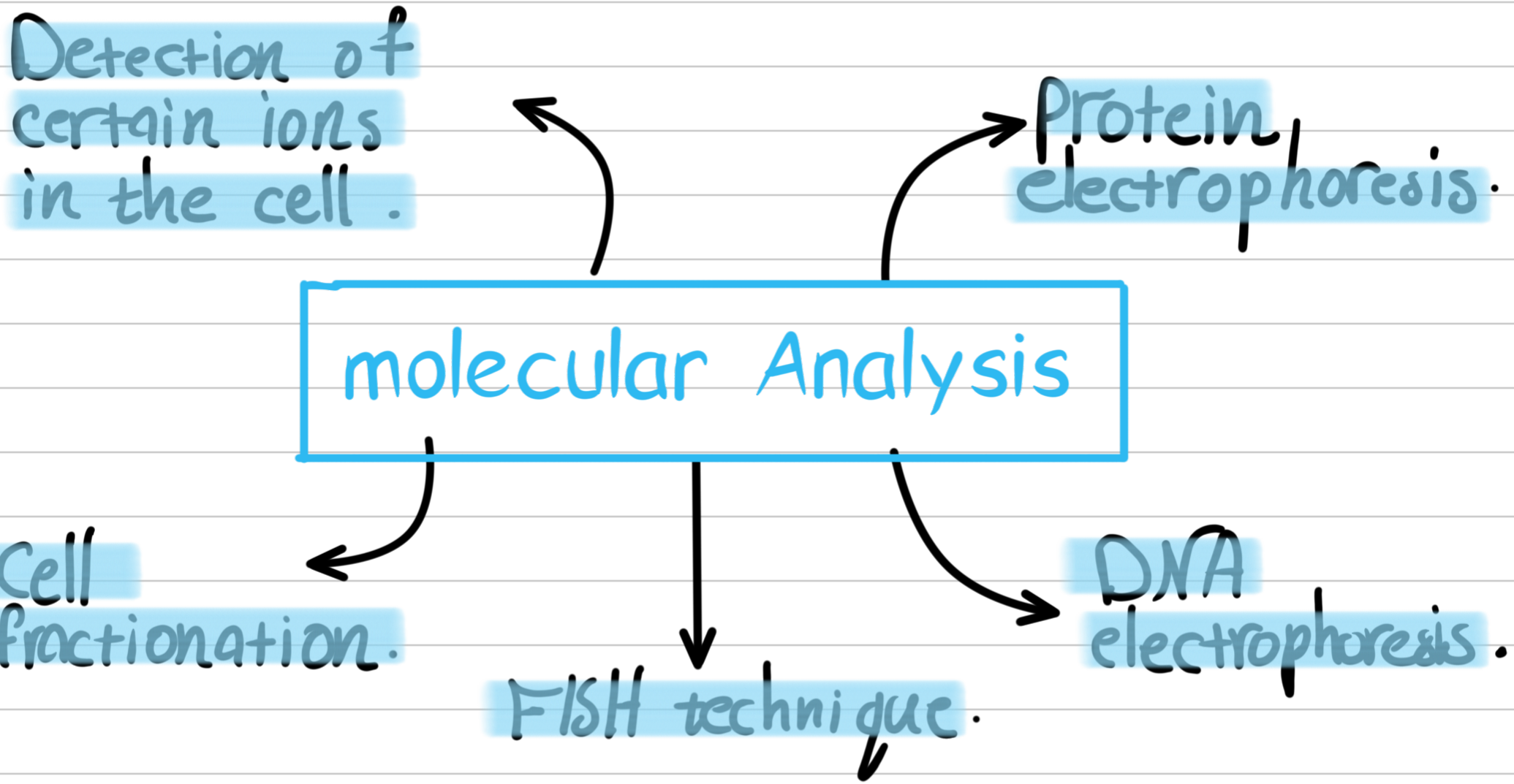
- proliferate indefinitely.

- It is obtained from subcultures of the primary culture.

- Stem cells :- share with IM cells :-

long lived and self renew cells.

- Cancer cells.


Detection of certain ions in the cell.
Protein electrophoresis.
molecular Analysis
Cell fractionation.
FISH technique.
DNA electrophoresis.

« protin_electrophoresis »

- proteins will be separated according to their charge and molecular weight.

  (albumin, Globulin).

multiple mylona → increased gamma globulin protein in urin (M protein).

« DNA-electrophoresis »

- DNA fragment are -ve charged.
- 'is this case separation is based on length of the base pair.

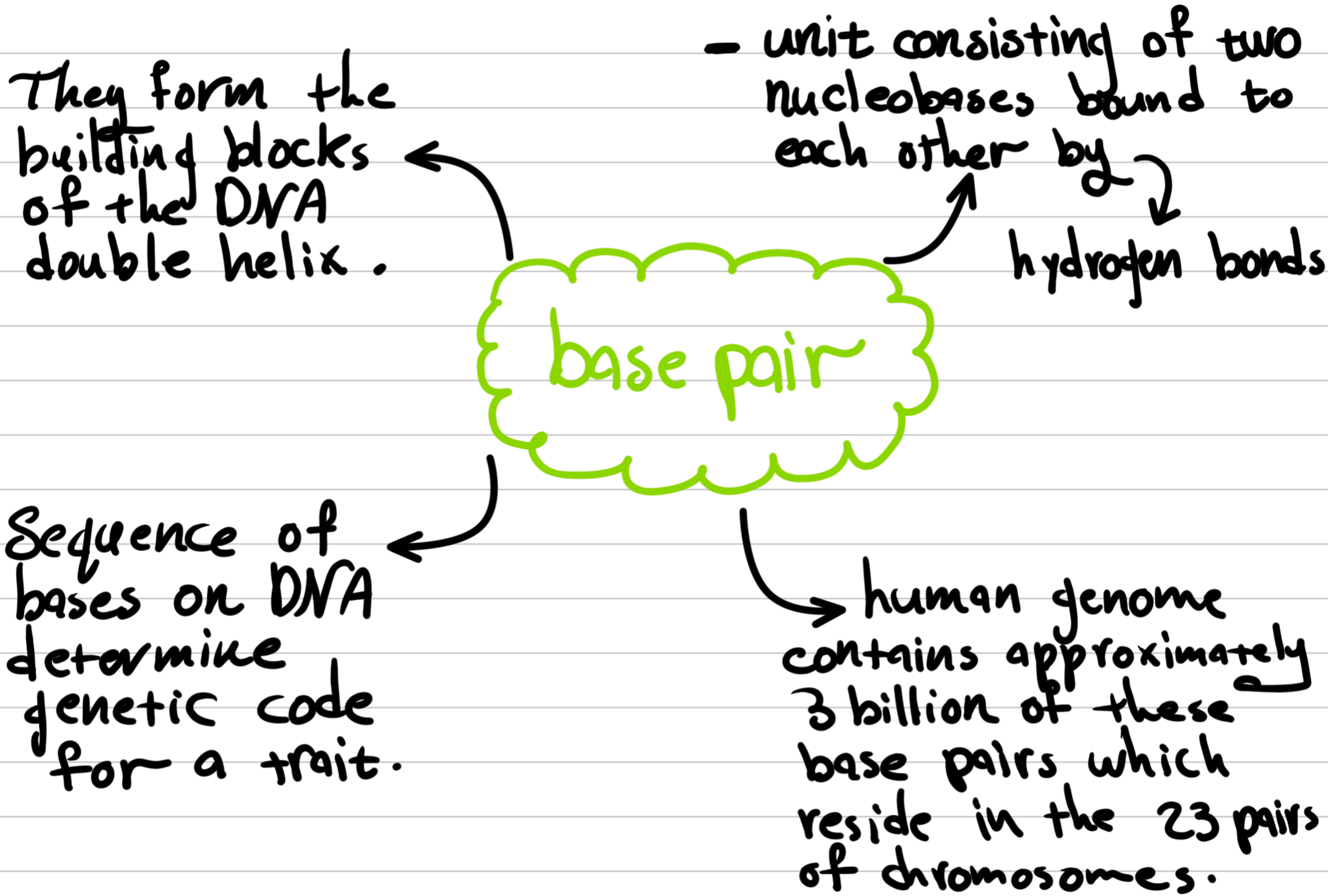
- Samples are loaded into wells of an agarose or acrylamide gel then subjected to an electric field.

  ↳ causing the negatively charged nucleic acids to move toward the positive electrode.

- small fragments will move faster than the large ones.

(DNA fingerpint, gene isolation, disputed paternity).

DNA ladder → DNA fragments of known lengths used to estimate the size of unknown DNA


base pair
They form the building blocks of the DNA double helix.
- unit consisting of two nucleobases bound to each other by hydrogen bonds
Sequence of bases on DNA determine genetic code for a trait.
human genome contains approximately 3 billion of these base pairs which reside in the 23 pairs of chromosomes.

«FISH technique »

- used to visualize and map the genetic material
- use to localize the sits of specific genes / DNA sequence on chromosomes using a fluorescent probe
  - DNA or RNA of designed to be complementary to a specific DNA sequence of interest and radioactively labled.
  - useful in detect chromosomal abnormalities.

« cell fractionation »

↳ isolation of the cell components (nucleus and organelles). while preserving its individual function to study the features of each.

— done by the use of (centrifugation).

nuclei → mitochondria → microsomes → ribosomes.

pellet → sediment at the bottom of the tube.

supernatant → less dense component at the top.