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SUBJECT Zoo501

SMESTER Second

GROUP "C"

DURATION 31st May-18 June 2021

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Practical 1 :-

Preperation of different Stock solutions used in molecular biology.

A solution is a mixture of homogenous mixture of one or more solutes

Stock solution:-

It can be best described as solutions of known, accurate concentrations that will be diluted for future laboratory.

WORKING SOLUTIONS:-

These are any dillutions that are made using the stock, generally into aqueous solutions.

2x GEL LOADING DYE:-

2% Bromophenol blue 0.25 ml
2% Xylene Cyanol 0.25 ml
Glycol 7ml
H_2O 10ml
store at room temperature

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⇒ 6M SODIUM CHLORIDE

Sodium Chloride 351g
H_2O 600ml
Raise the volume to 1L using distilled water.
Store at room temperature.

⇒ 50x TAE ELECTROPHORESIS

Tris Base 242 g
Acetic Acid 57.1 ml
0.5 MEDTA pH 8.0
Raise the volume to 1L using distilled water.
Store at room temperature.

⇒ PRACTICAL 2:-

Isolation of DNA by organic method.

=> SOURCES OF DNA:-

Purified DNA is required for a variety of molecular biology applications. DNA can be purified from any living thing.

=> ORIGIN OF SAMPLES:-

1: Blood
2: Hair
3: Human Tissues.
4: Bacteria
5: Fungi
6: Leaf
7: Soil
8: Rodent Tissue
9: Spores.
10: Forensic Samples.
11: Insects.

=> DNA EXTRACTION IS USED:-

To isolate:-

Types of DNA
Mitochondrial DNA
Genomic DNA
Plasmid DNA.

=> LAB EQUIPMENT NEEDED:-

* Pippetes.
* Microcentrifuge
* Racks
* Vortex
* Freezer
* Centrifuge

=> STEPS IN DNA EXTRACTION:-

* Lysis of Red blood cells.
* Digestion steps.
* Phase seperation steps.
* DNA precipitation.
* Washing with ice cold Ethanol.
* Dilution.

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=> PRACTICAL 3:- Quantification of DNA and RNA

The Spectrometer is an instrument which measures the amount of light intensity of a sample absorbs. It works by passing a light beam through a sample to measure the light intensity of sample.

=> PRINCIPLE:-

Readings should be taken at wave length of 260 nm and 280 nm. The reading at 260 nm allows calculations of concentration of Nucleic Acid in sample.

=> EQUIPMENT REQUIRED:-

* UV/VIS Spectrophotometer.
* 1 ml quartz cuvette
* DNA/RNA samples.
* TE Buffer.

⇒ PROCEDURE:-

* Turn on the spectrophotometer at power strip
* Enter the wavelength as 260nm.
* Open the sample compartment lid. Add TE Buffer Solution. Record the absorbtion in memory.
* Press "read". and calibration is complete when "scan" is displayed

⇒ ADVANTAGES:-

* It is an easier and cost-effective method with reliable results if performed adequately.
* Protein contamination cannot be reliably assessed.

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PCR Using prepared 2x Mix

Re agent	Volume
2x PCR Master Mix	25 µl
Forward Primer	2.5 µl
Reverse Primer	2.5 µl
Sterile Distilled H_2O	17 µl
Total	47 µl.

⇒ PRACTICAL 4:-

DNA amplification through Polymerase Chain Reaction.

PCR is the Vitro amplification of specific sequences of Nucleic Acids.

The process of PCR and enzyme DNA polymerase were named by science Magazine as the 1989 "Molecule of the year" as they were likely to have the greatest influence on history.

⇒ PRINCIPLE:-

The basic component of PCR reaction include a DNA tempelate, primer, nucleotides, DNA polymerase and a buffer.

It envolves 3 major steps:-

Denaturation
Annealing
Extension.

It is an in vitro method for expontial amplification.

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=> REAGENTS REQUIRED:-

* PCR master mix.
* $dNTP_3$
* $MgCl_2$
* PCR buffer.
* PCR primers.
* PCR grid water.

=> EQUIPMENT REQUIRED:-

* Thermal Cycler with analysis
* Vortex mixture
* Micro centrifuge.
* Pippets.
* PCR Safety Cabinet.

=> PROCEDURE:-

* Label the PCR tubes for samples and controls.

* Thaw the PCR reagents and prepare mix.
* Aliquot the reaction mix in PCR.
* Open PCR machine software and turn off

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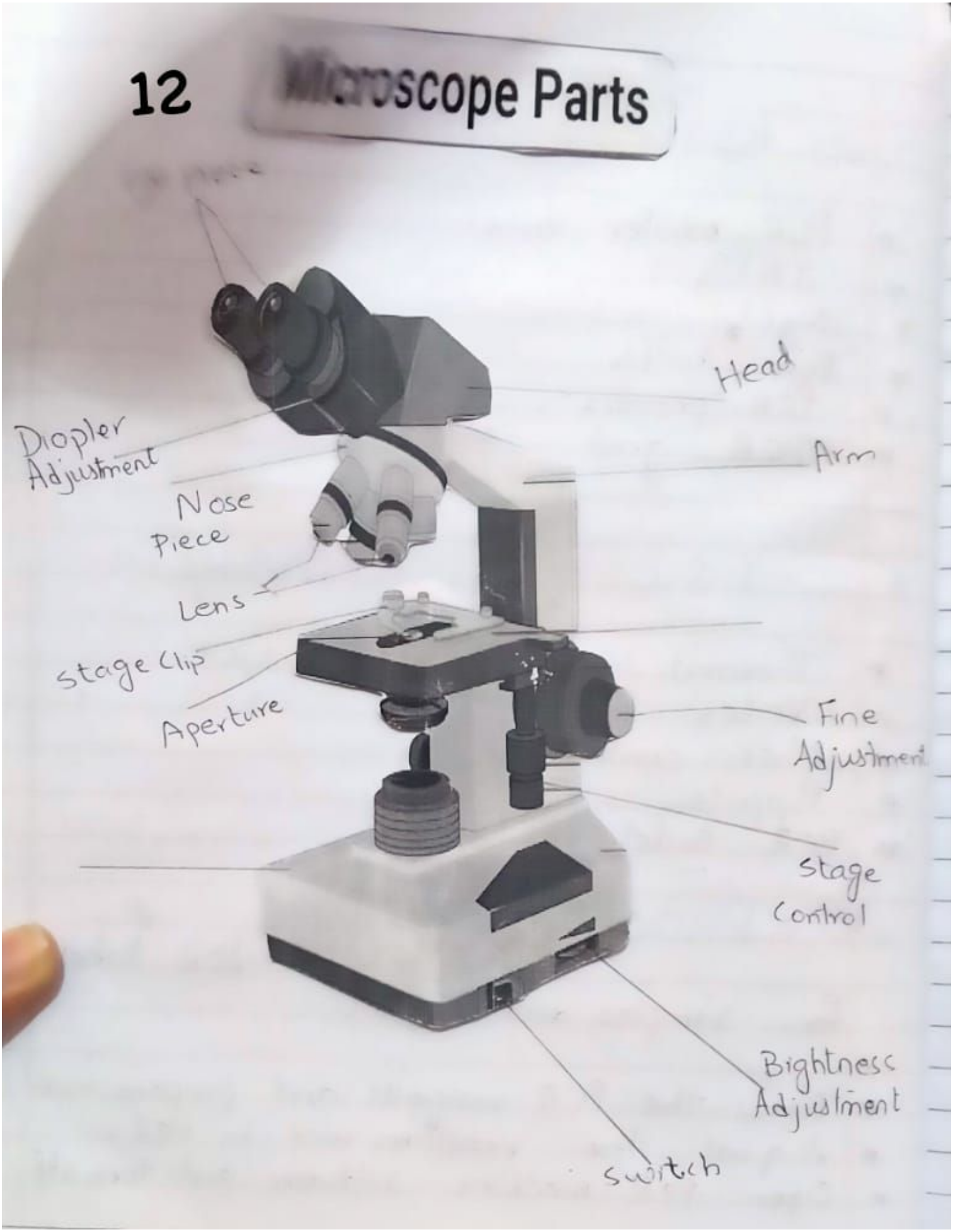
Microscope Parts



⇒ PRACTICAL 5:-

Microscopy

A cell is the living unit of life. They are so small that they cannot be seen through naked eyes. It is studied through a microscope.

Various types of microscope are:-

Simple microscope.
compound microscope.
Interference microscope.
Electron microscope.
Atomic force microscope.

⇒ TUBE:-
It connects the eyepiece to the object lenses.

⇒ RESOLVING NOSE PIECE:-
It is the turret

=> FINE ADJUSTMENT NOBE:-
Used to focus on oil. It moves the body for high lens.

=> ARM:-
It supports the tube of microscope and connects to the base.

=> STAGE:-
A flat platform used for placing slides under observation.

=> STAGE CLIP:-
It holds the slide in proper place.

=> CONDENSOR:-
The main function of condenser is focusing the light on specimen under observation.

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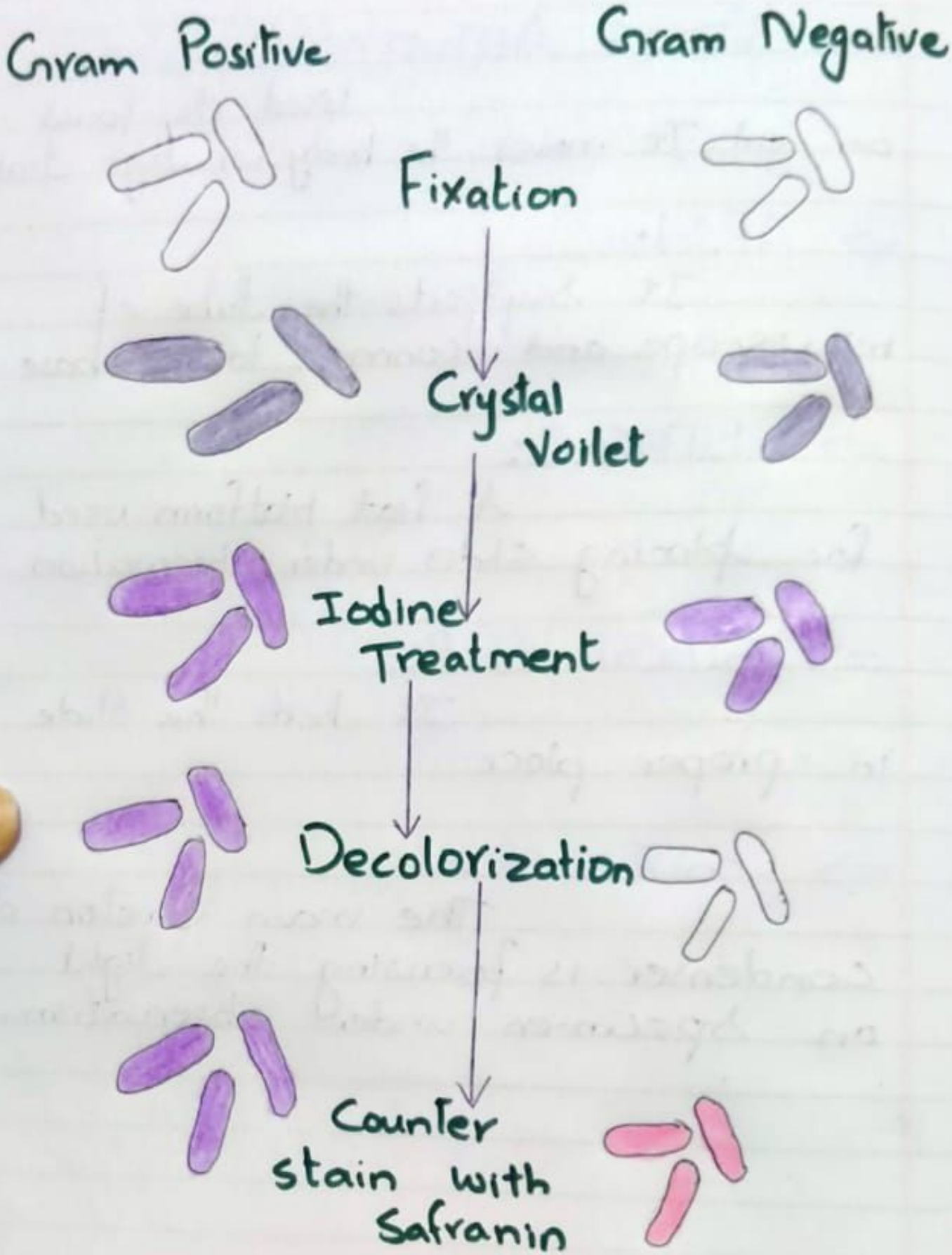
Gram Positive
Gram Negative
Fixation
Crystal Voilet
Iodine Treatment
Decolorization
Counter stain with Safranin

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=> PRACTICAL 6:-

"Staining Techniques"

=> Staining:-

It is a technique which is used to define and examine different types of microbes.

=> TYPES:-

There are two types of Staining:-

Simple staining techniques
Differential staining techniques.

=> MATERIALS REQUIRED:-

Clean Glass Slide, Inoculating loop, Bunsen burner, Microscope, lens paper, lens cleaner, distilled water, Ethyl Alcohol.

⟹ PROCEDURE:-

Smear preperation
A small loop of microorganisms is placed on a slide and permitted to dry air.

Flood the slide with crystal vailet leave for 30 seconds.

Wash the ethyl alcohol wing slide It act as decolorizing agent.

Observe Under microscope.

Gram positive bacteria stains purple.

Gram Negative bacteria strains pink.

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=> PRACTICAL 7:-

Preperation of temperary whole mount.

Observing human samples to under a microscope is a simple way to quickly view a human cell structure

You can replicate observational experiment at home with any standard microscope.

=> MATERIAL REQUIRED:-

Tooth pick,
slide
Cover slip
microscope.
Methylene blue.
distilled wader.
Paper towel.

=> PROCEDURE:-

Swab the inside of your

cheek with a nonsharp end of toothpick

Place the swabbed end of tooth pick onto the middle of microscope slide.

Add single drop of water squeezed from plastic pippet.

Check for tiny bubbles under the cover slip.

Place the edge of paper towel on any solution outside the cover slip to absorbe excess water.

=> RESULTS:-

Note down the obsorbed results and draw a set of tables on notebook.

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=> PRACTICAL 11:-

Preperation of permanent whole mount.

A permanent slide is a slide that can be fixed and locked in one place. while temperary slides usually have a top and mottom with an object secured. in between, permanent slides have top and bottom that are glued or sealed together

=> MATERIAL REQUIRED:-

Fresh water,
Sample of Algae.
Formaldehyde.
paper
Slide
Cover Slip
Oven
Microscope
Glycerine.

=> PROCEDURE:-

Wash the slide and cover slip properly.

Put a drop of fresh water on slide

Wash the Algae.

Place the Algae on slide.

Add drop of formalin

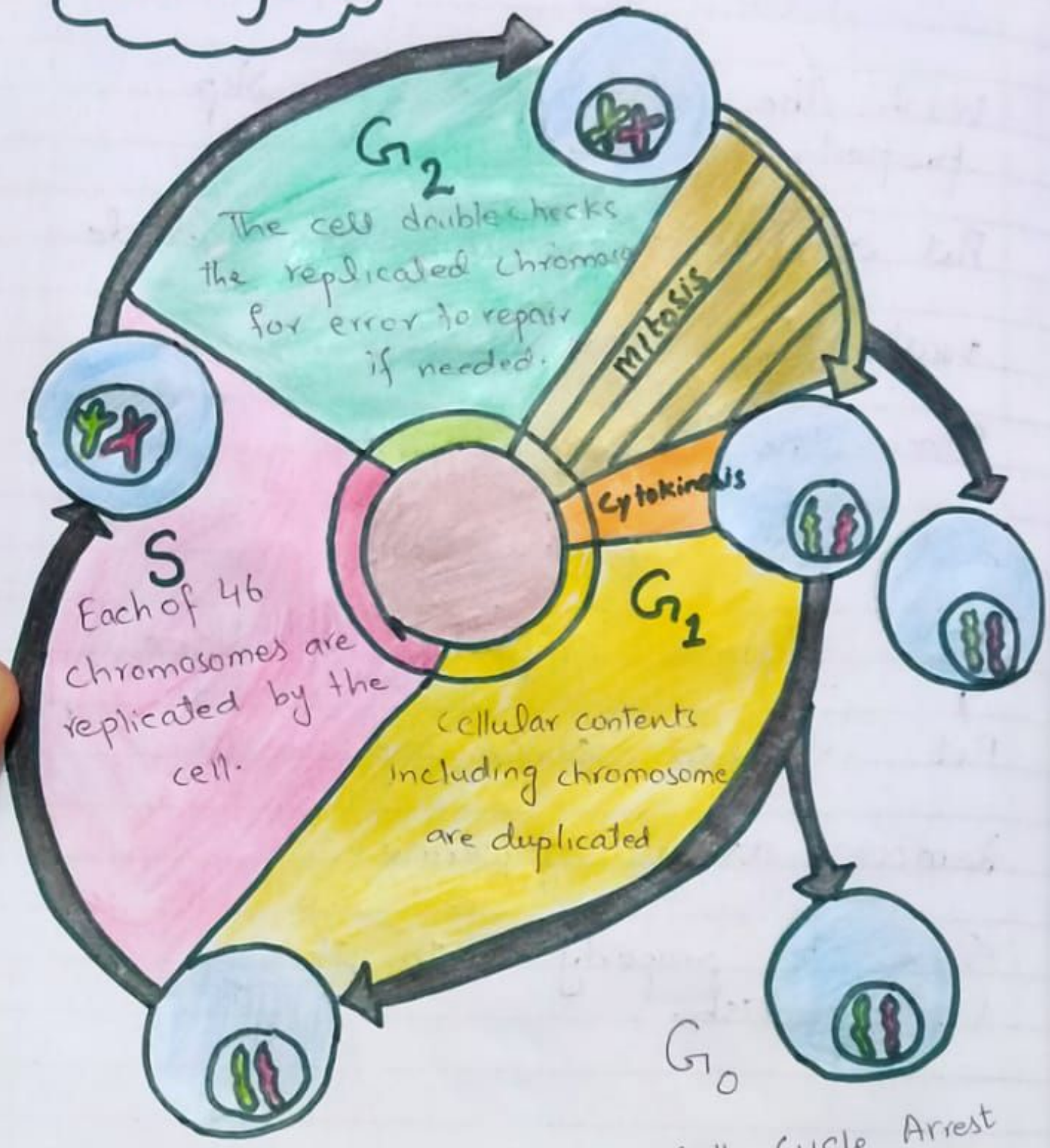
Put some glycerine on Algae

Put washed cover slip on it.

Remove axcess glycerine.

Seal it properly with glue or nail polish.

Cell cycle


G2
The cell double checks the replicated chromosome for error to repair if needed.
Mitosis
Cytokinesis
S
Each of 46 chromosomes are replicated by the cell.
G1
Cellular contents including chromosome are duplicated
G0
Cell Cycle Arrest

⇒ PRACTICAL 12:-

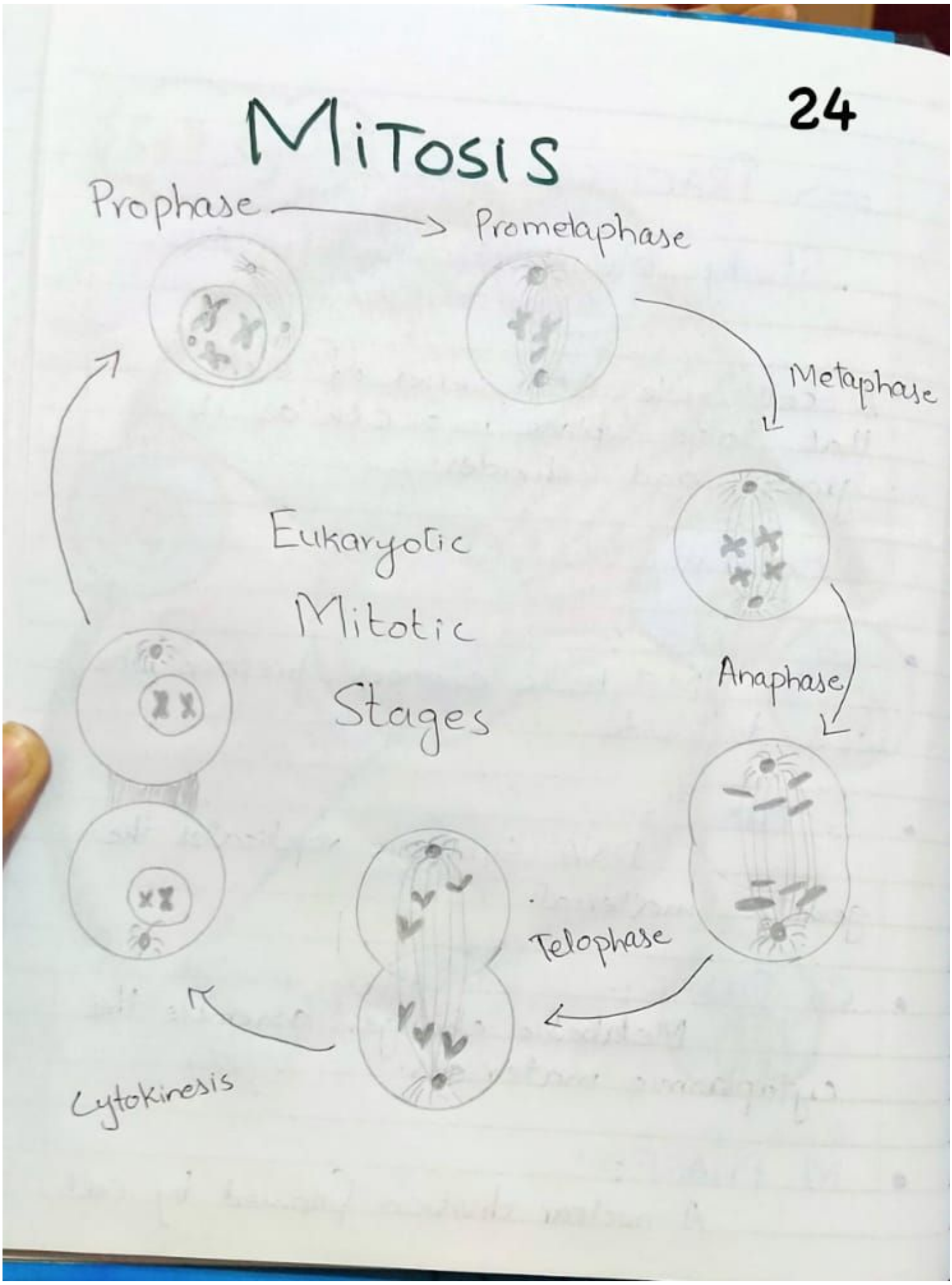
Study of mitotic and meiotic stages.

A cell cycle is a series of events that takes place in a cell as it grows and divides.

⇒ The CELL CYCLE:-

- G_1 PHASE:-
  Metabolic changes prepare the cell to divide.
- S PHASE:-
  DNA synthesis replicates the genetic material.
- G_2 PHASE:-
  Metabolic changes assemble the cytoplasmic materials.
- M PHASE:-
  A nuclear division followed by cell.

Mitosis


Prophase
Prometaphase
Metaphase
Anaphase
Telophase
Cytokinesis
Eukaryotic
Mitotic
Stages

⇒ WHAT IS MITOSIS:-

Mitosis is a vegetative cell division in Eukaryotes which divides parent cell's replicated genome between two daughter cells.

The two cells are genetically identical bearing an aproximately equal number of organelles and cytoplasm.

The mitotic phase is called the M phase of cell cycle. Eukaryotes have large number of chromosomes.

These chromosomes are replicated during S phase of interphase of cell cycle.

Two types of mitotis can be identified among angiosperms.