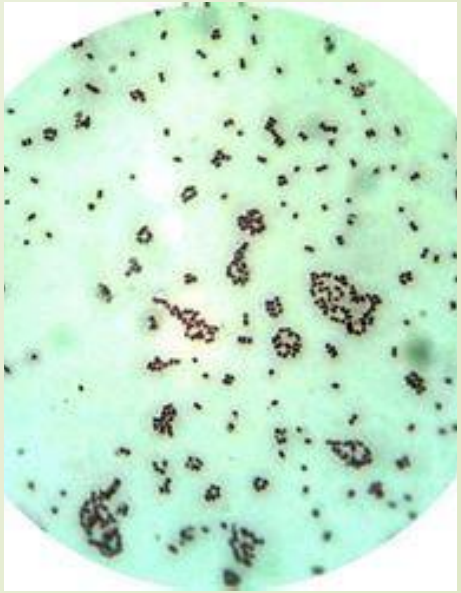
Cell Division

Objectives

- Use a compound microscope
- Describe the phases of mitosis and determine the length of each phase in onion roots

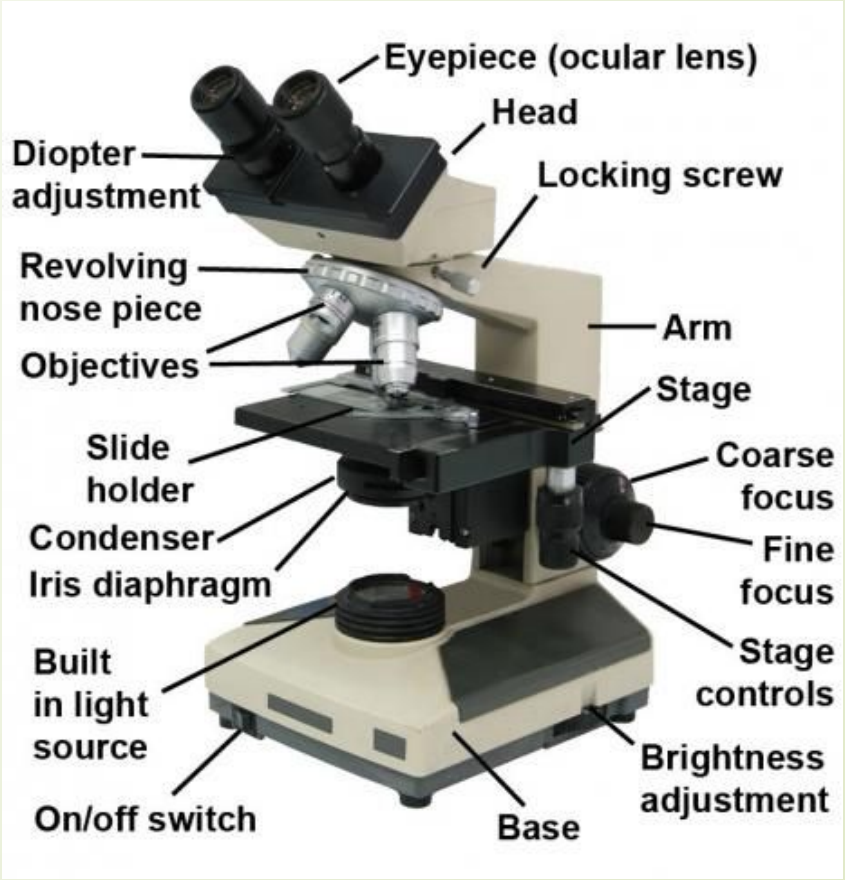
Microscopy

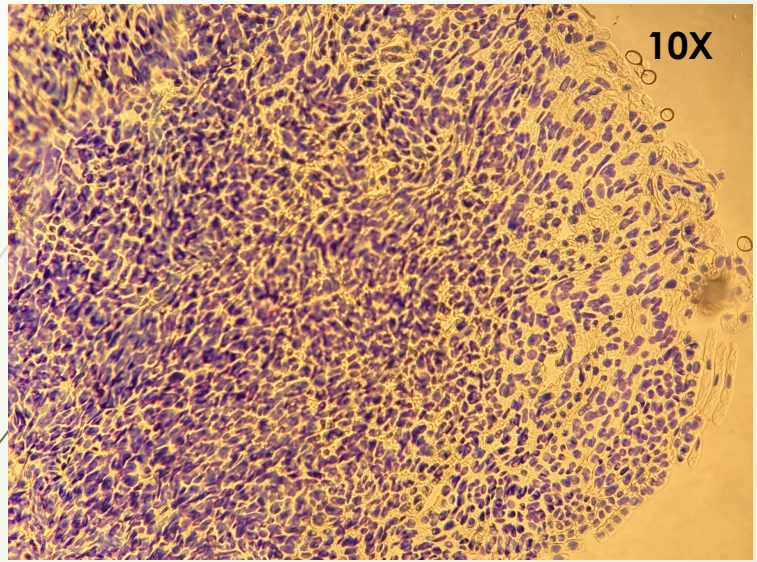
- Compound microscopes allow us to view organisms/molecules that are normally invisible to the naked eye
- This is done through magnification
- Microscope slide staining is the process of adding colored dye to a slide. Staining creates a visual contrast between the object or organism you are viewing and its surroundings. Subjects like bacteria can be seen in greater detail after being dyed, allowing for a more in-depth study.

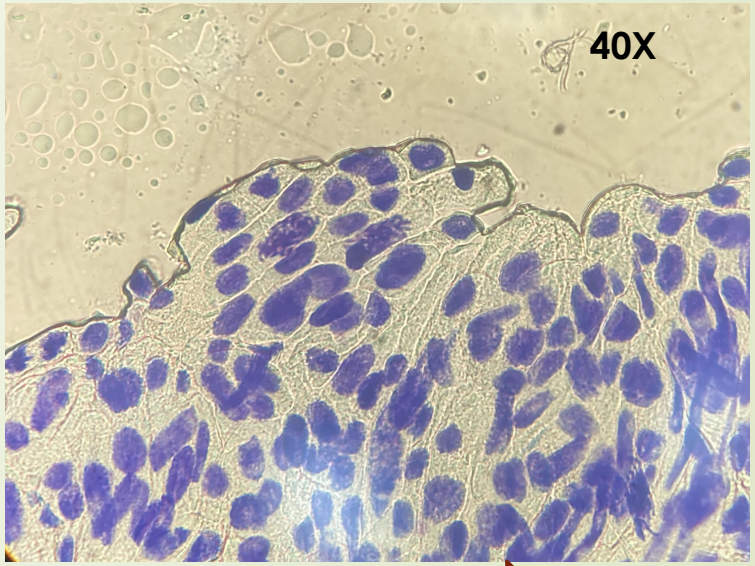


Compound Microscope Anatomy

- Notes:
  - Never slide a microscope across the table
  - Never select the oil immersion objective (only go between 4x, 10x, & 40x)
  - Only use the coarse knob for the 4x & 10x objective







Field of view reduced when you go from 4X to 40X
Magnification increase when you go from 4X to 40X

Total magnification = magnification in ocular lens X magnification of objective lens

magnification in ocular lens = 10X (this does not change)

magnification of objective lens = 4X / 10X / 40X

Activity 1 – Simple Microscopy

- Remove microscope from cabinet, plug in
- Place your first slide on the stage. Make sure that the slide is secure under the clip.
- Turn on light source.
- Make sure that the 4x objective is over the sample and look through the oculars.
- Adjust first the coarse focus using the outer knob, then the fine focus (inner knob) to bring the specimen into focus.
- Turn the objectives so that the 10x objective is over that sample and adjust the focus using the fine focus knob.
- Turn to the 40x objectives so that the 40x objective is over the sample and adjust the focus using ONLY the fine focus. Using the coarse focus could damage the microscope and/or the specimen.
  - Sketch what you see.

The structure and function of a cell's genetic information

- A cell's DNA, packaged as a double-stranded DNA molecule
- Each species of eukaryotes has a characteristic number of chromosomes, or structures within the nucleus that are made up of DNA, in the nuclei of its cells.
- Human body (somatic) cells have 46 chromosomes and include bone cells,muscle cells and nerve cells, while human **gametes** (sperm or eggs) have 23 chromosomes each.

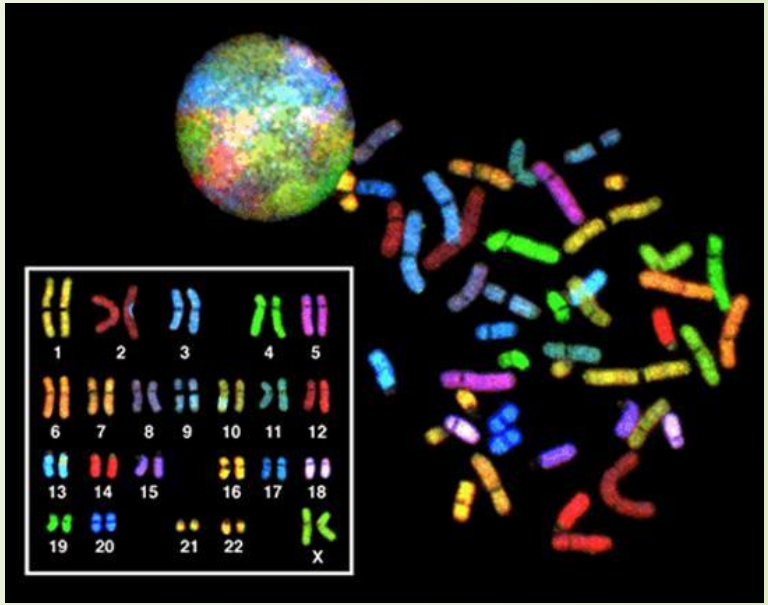


Figure. There are 23 pairs of homologous chromosomes in a female human somatic cell.
Chromosomes are viewed within the nucleus (top), removed from a cell during mitosis and spread out on a slide (right).

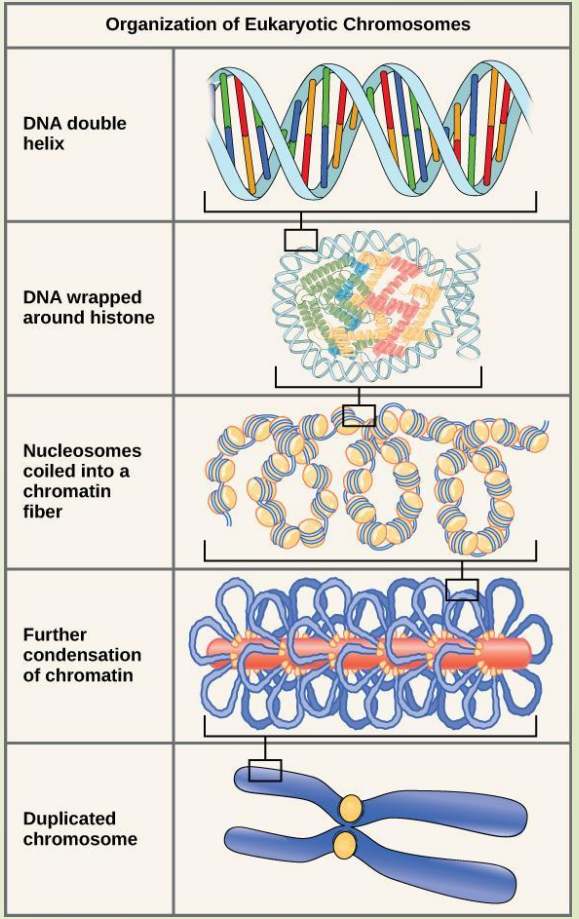
- A typical body cell contains two matched or homologous sets of chromosomes (one set from each biological parent)—a configuration known as **diploid**
  - (Note: The letter *n* is used to represent a single set of chromosomes; therefore, a diploid organism is designated 2*n*.)

- Human cells that contain one set of chromosomes are called gametes, or sex cells; these are eggs and sperm, and are designated *1n*, or **haploid**.

- Upon fertilization, each gamete contributes one set of chromosomes, creating a diploid cell containing matched pairs of chromosomes called **homologous** ("same knowledge") **chromosomes**.

Eukaryotic Chromosomal Structure and Compaction

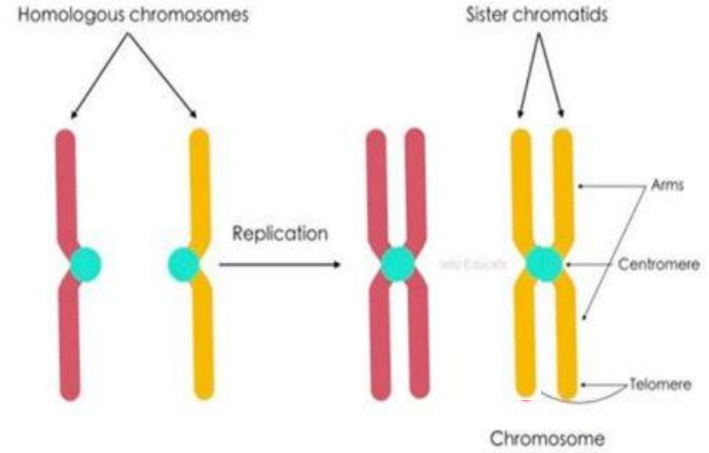
- **Chromatin-** DNA double helix wrap around a core of eight histone proteins at regular intervals along the entire length of the chromosome
- **Nucleosome** – bead-like histone DNA complex. A DNA molecule in this form is about seven times shorter than the double helix without the histones
- The second level of compaction occurs as the **nucleosomes coil into a chromatin fiber**. This coiling further *condenses* the chromosome so that it is now about 50 times shorter than the extended form.
- In the third level of compaction, **a variety of *fibrous proteins* is used to "pack the chromatin."**
- DNA replicates in the S phase of interphase. After replication, the chromosomes are composed of two linked sister **chromatids**. The connection between the sister chromatids is closest in a region called the **centromere**.



Eukaryotic Compaction

Chromosome Counting

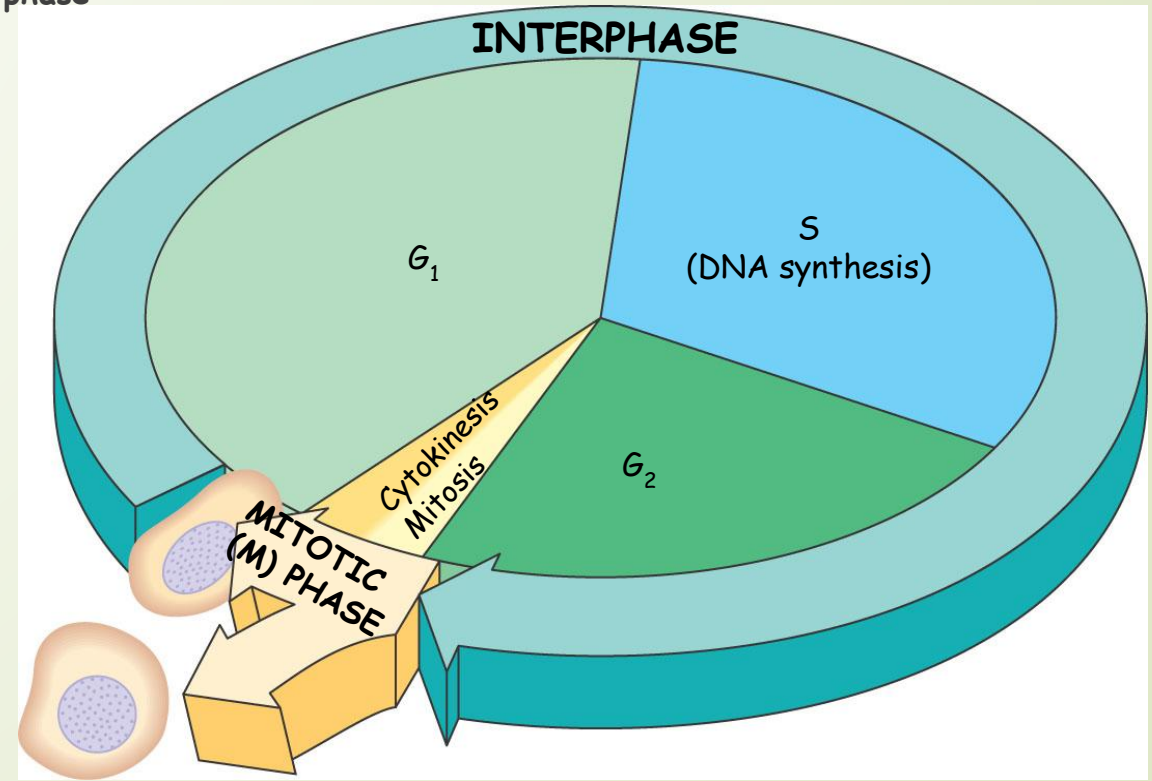
- You can count the number of chromosomes by counting the number of centromeres
- Chromatids are identical copies of the same chromosome

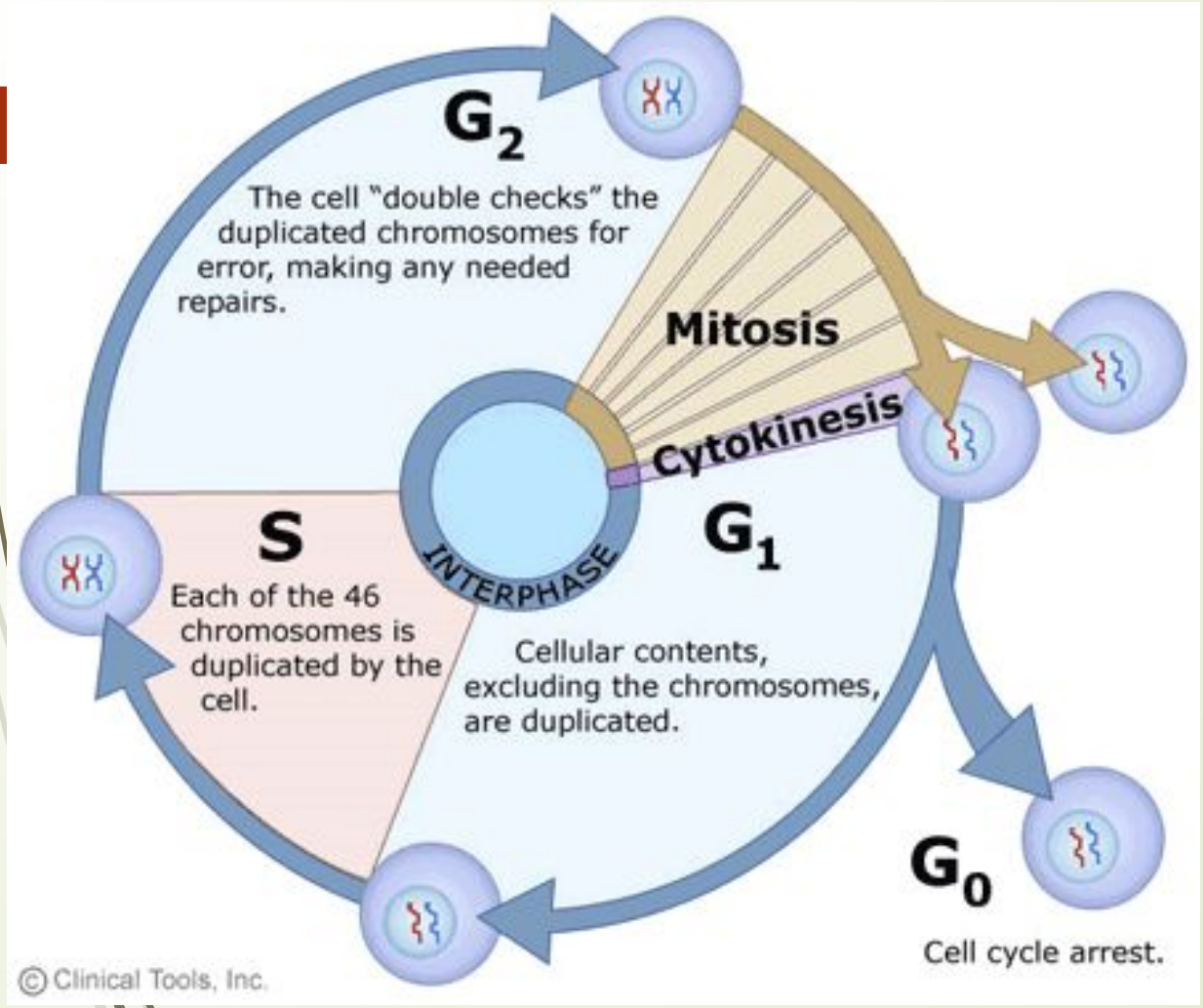


Phases of the Cell Cycle

? The cell cycle consists of

1. **The Mitotic phase**
2. **Interphase**





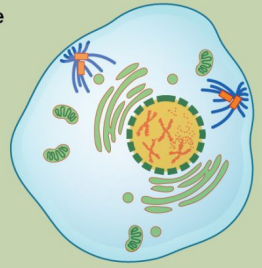
The size of the wedges in proportional to the amount of time a cell stays in that part of the cycle.

If our cells were constantly undergoing mitosis, we wouldn't stop growing!

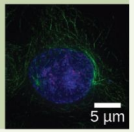
Interphase

- G1 Phase (First Gap)- getting ready by prepping for replicating each chromosome in the nucleus. Includes proteins and energy reserves.
- S Phase (Synthesis of DNA)- results in the formation of identical pairs of DNA molecules—sister chromatids—that are firmly attached to the centromeric region. Centrosomes also duplicate.
- G2 Phase (Second Gap)- Increases energy stores and synthesizes proteins necessary for chromosome manipulation and movement. Additional growth.
- G0 Phase (resting phase) - The G0 phase, also known as the resting phase, is the time when the cell is neither dividing nor preparing to divide.

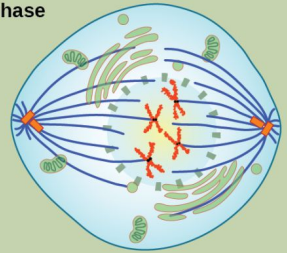
Prophase



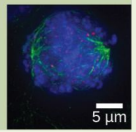
- Chromosomes condense and become visible
- Spindle fibers emerge from the centrosomes
- Nuclear envelope breaks down
- Nucleolus disappears



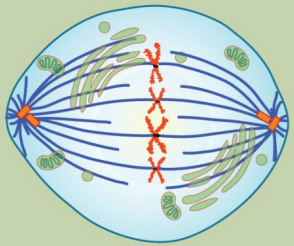
Prometaphase



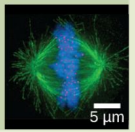
- Chromosomes continue to condense
- Kinetochores appear at the centromeres
- Mitotic spindle microtubules attach to kinetochores
- Centrosomes move toward opposite poles



Metaphase

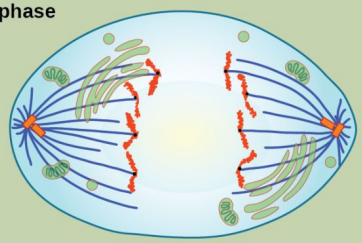


- Mitotic spindle is fully developed, centrosomes are at opposite poles of the cell
- Chromosomes are lined up at the metaphase plate
- Each sister chromatid is attached to a spindle fiber originating from opposite poles

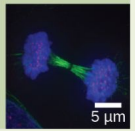


MITOSIS

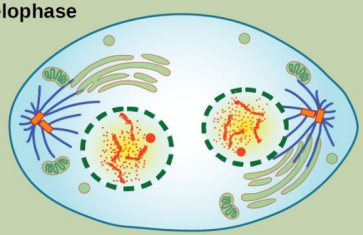
Anaphase



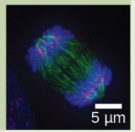
- Cohesin proteins binding the sister chromatids together break down
- Sister chromatids (now called chromosomes) are pulled toward opposite poles
- Non-kinetochore spindle fibers lengthen, elongating the cell



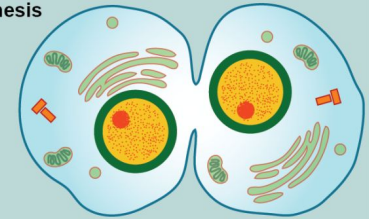
Telophase



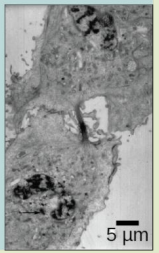
- Chromosomes arrive at opposite poles and begin to decondense
- Nuclear envelope material surrounds each set of chromosomes
- The mitotic spindle breaks down

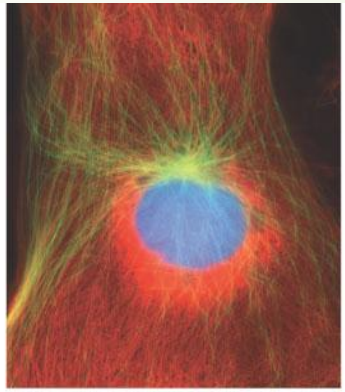


Cytokinesis

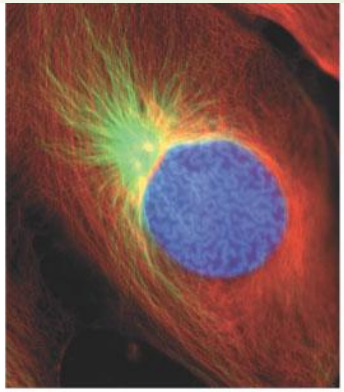


- Animal cells: a cleavage furrow separates the daughter cells
- Plant cells: a cell plate separates the daughter cells

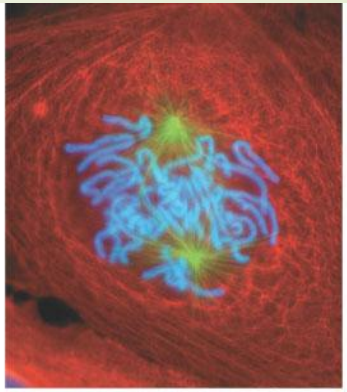




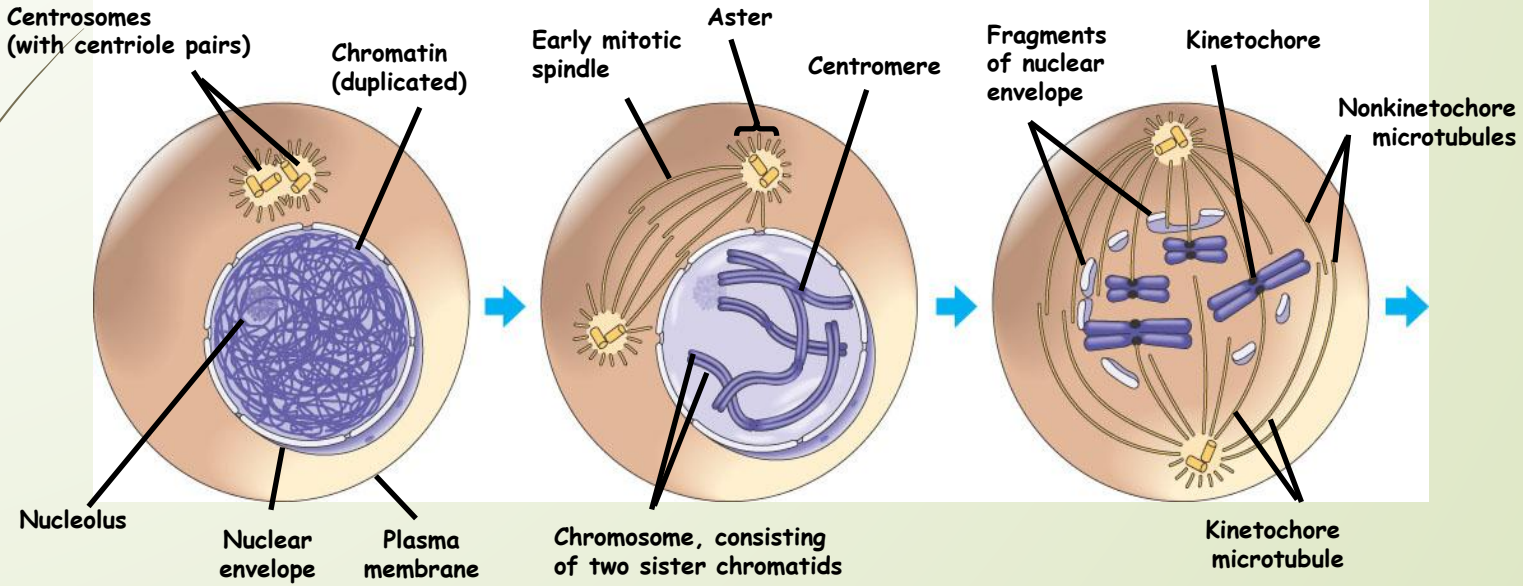
G_2 OF INTERPHASE

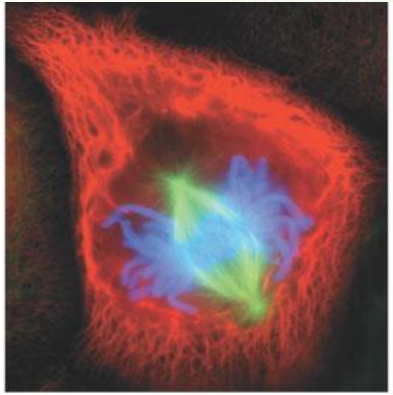


PROPHASE

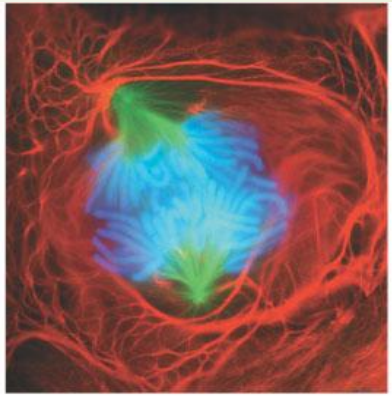


PROMETAPHASE

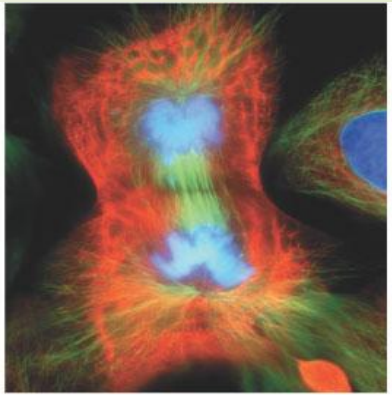




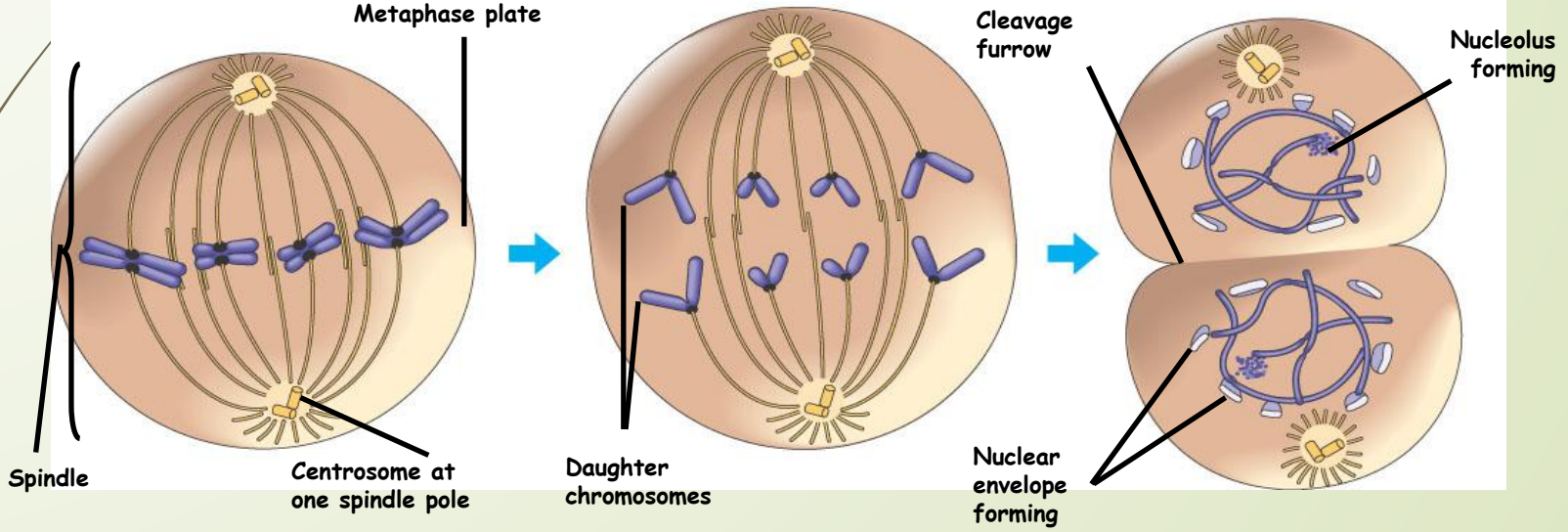
METAPHASE



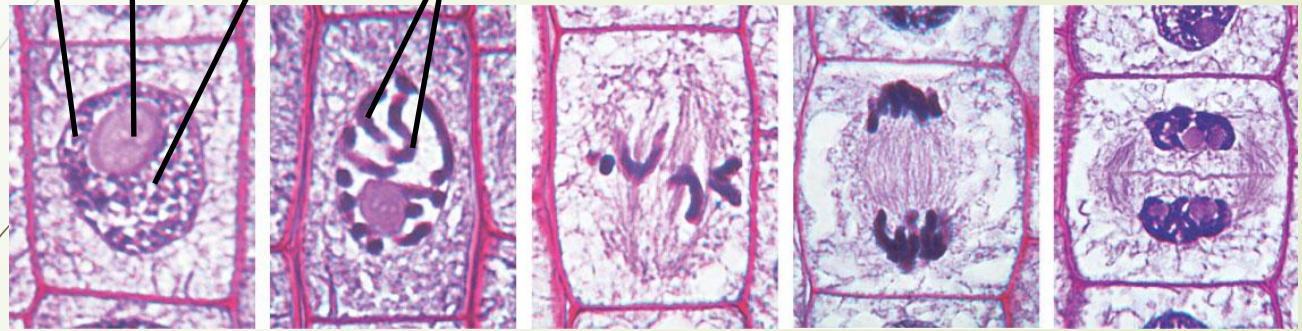
ANAPHASE



TELOPHASE AND CYTOKINESIS



Mitosis in a plant cell



1 **Prophase.** The chromatin is condensing. The nucleolus is beginning to disappear. Although not yet visible in the micrograph, the mitotic spindle is staring to from.

2 **Prometaphase.** We now see discrete chromosomes; each consists of two identical sister chromatids. Later in prometaphase, the nuclear envelop will fragment.

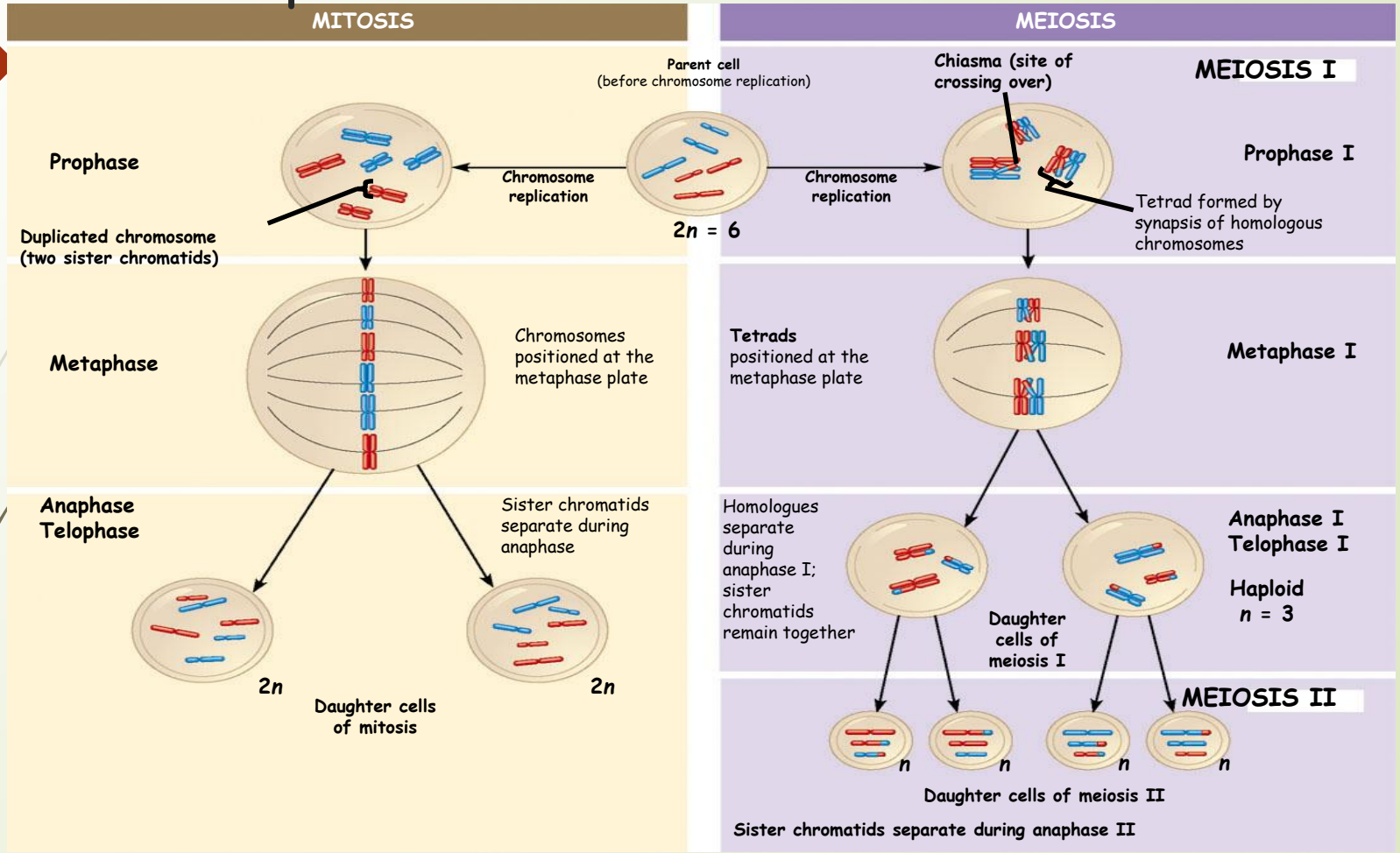
3 **Metaphase.** The spindle is complete, and the chromosomes, attached to microtubules at their kinetochores, are all at the metaphase plate.

4 **Anaphase.** The chromatids of each chromosome have separated, and the daughter chromosomes are moving to the ends of cell as their kinetochore microtubles shorten.

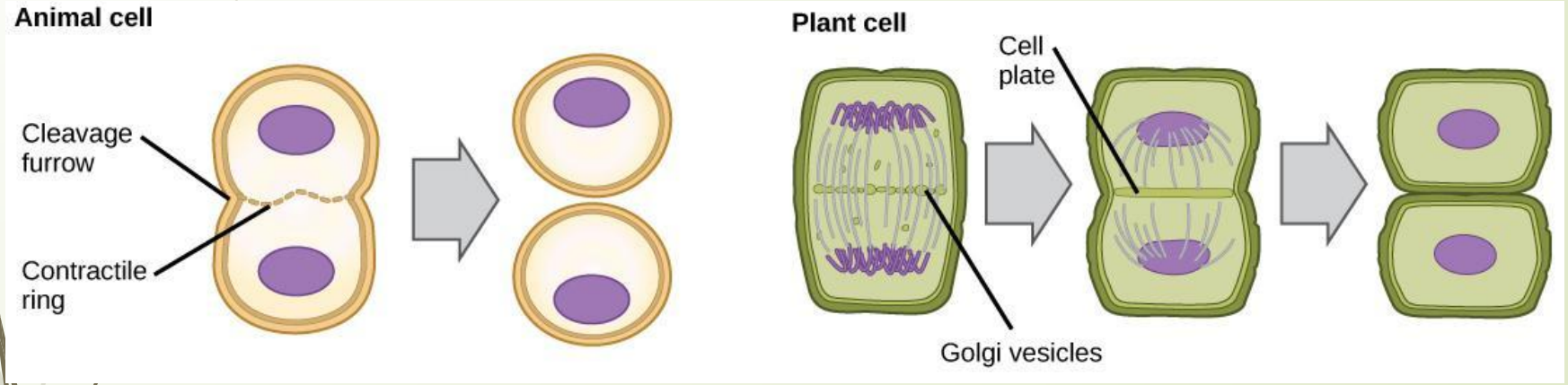
5 **Telophase.** Daughter nuclei are forming. Meanwhile, cytokinesis has started: The cell plate, which will divided the cytoplasm in two, is growing toward the perimeter of the parent cell.

Prophase for lab

A comparison of mitosis and meiosis



Animal cells vs plant cells



Putting it all together

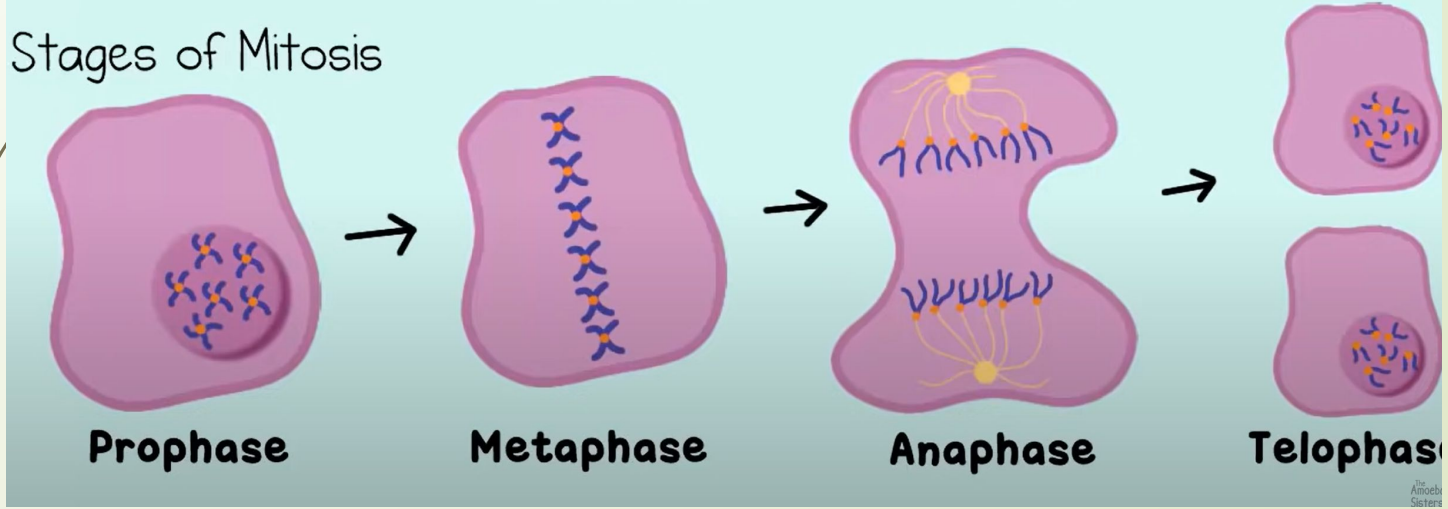
Petunia can only draw a limited number of chromosomes in these small pictures.

Mitosis:

(per daughter cell)

	Before Interphase	After Interphase	During Mitosis*	After Mitosis
Chromosomes	46	46	46	46
Chromatids	46	92	92	46

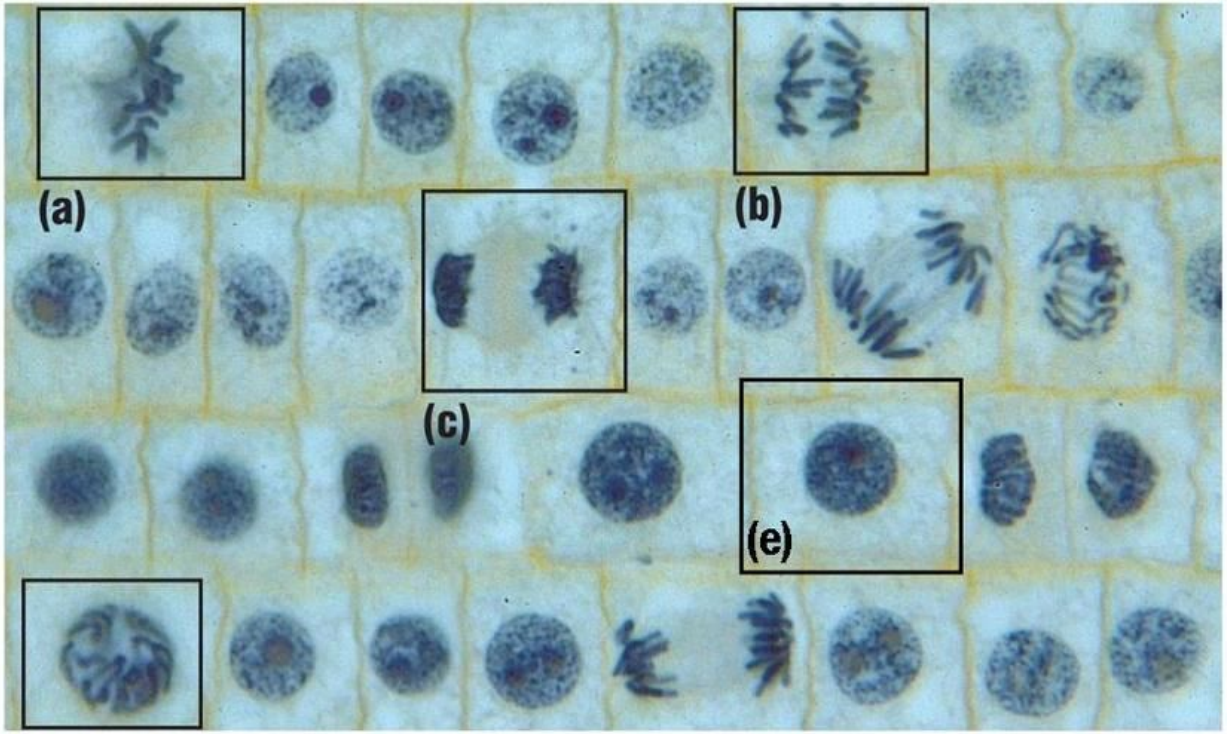
Stages of Mitosis



From: Amoeba Sisters

Unnumbered Figure, Pg. 142

Onion (*Allium*) root tip mitosis



a. Metaphase
b. Anaphase
c. Telophase
d. Prophase
e. Interphase

Safety

- **Gloves, Goggles, Closed-toe shoes**
- All pipet tips should be ejected into the tip waste bin on your bench.
- Acid waste should be disposed of in the Acid Waste beaker.
- Toluidine Blue waste should be disposed of in the Toluidine Blue waste beaker.
- Glass slides and coverslips should be disposed of in the glass waste box.

Activity 2: Mitosis

- Fixing and staining:
  - Completely remove 2 fresh onion roots, trim to 0.5-1 cm and keep the tips. Place all in a labeled 1.5 mL microcentrifuge tube.
  - Cover the root tips in your tube with 500 µL of 1M HCl. Cap the tube and place the tube in the rack in the water bath set to 55˚C. Incubate for 10 min
    - Get a ~100 mL beaker of fresh water ready for the washes below.
  - After incubation, use a P1000 to remove the acid. Discard the acid in a waste beaker.
- Wash the roots 3 times with 1 mL water to remove the acid:
  - Add water
  - Remove water and discard in waste beaker, empty your waste beaker in the acid waste container.
    - There is no need to change tips during this step.

Activity 2 continued …

- Using a new pipette tip, add 500 µL Toluidine Blue and incubate at room temp for 4 min.
  - Remove the stain by micropipetting and dispose of the stain in the disposal beaker on your bench.
- Wash the root tips with 1 mL water 5 to 6 times (5 to 6 mL total volume) and dispose of the washings in the disposal beaker on your bench. Leave the root tips in the tube in water at the end of your final wash

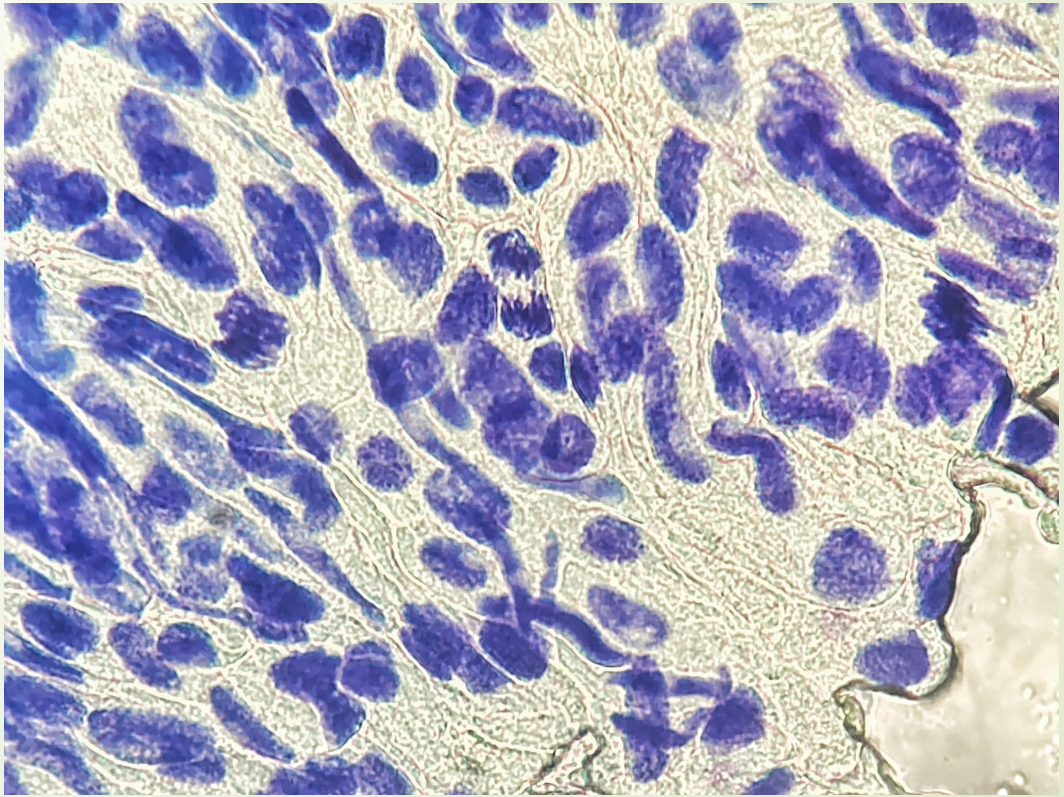
Activity 2 continued …

- Mounting slides:
  - Remove 1 onion root to a clean slide and trim tip to ~5 mm if not there already. Keep the very tip, discard the rest of the root.
  - Place a drop of water over the root tip tissue.
  - Carefully place a coverslip over the root tip and cover with a Kimwipe.
  - Gently press straight down on the coverslip with the eraser end of a pencil.
    - See protocol tip if cover slip breaks
  - Remove excess water with kimwipe

Activity 2 continued …

- Analyzing the cell cycle:
  - Transfer slide to microscope. Count only the cells that are still in the cell cycle (small, not elongated).
    - Distinguish between interphase, prophase, metaphase, anaphase, telophase/cytokinesis
    - Use table 1 to record number of cells in each phase
      - Count approx. 50-75 cells
  - Calculate percent of total cells counted that each phase represents
  - Calculate the time (hours) for each phase (see activity 2, step 4)

Identify the stages



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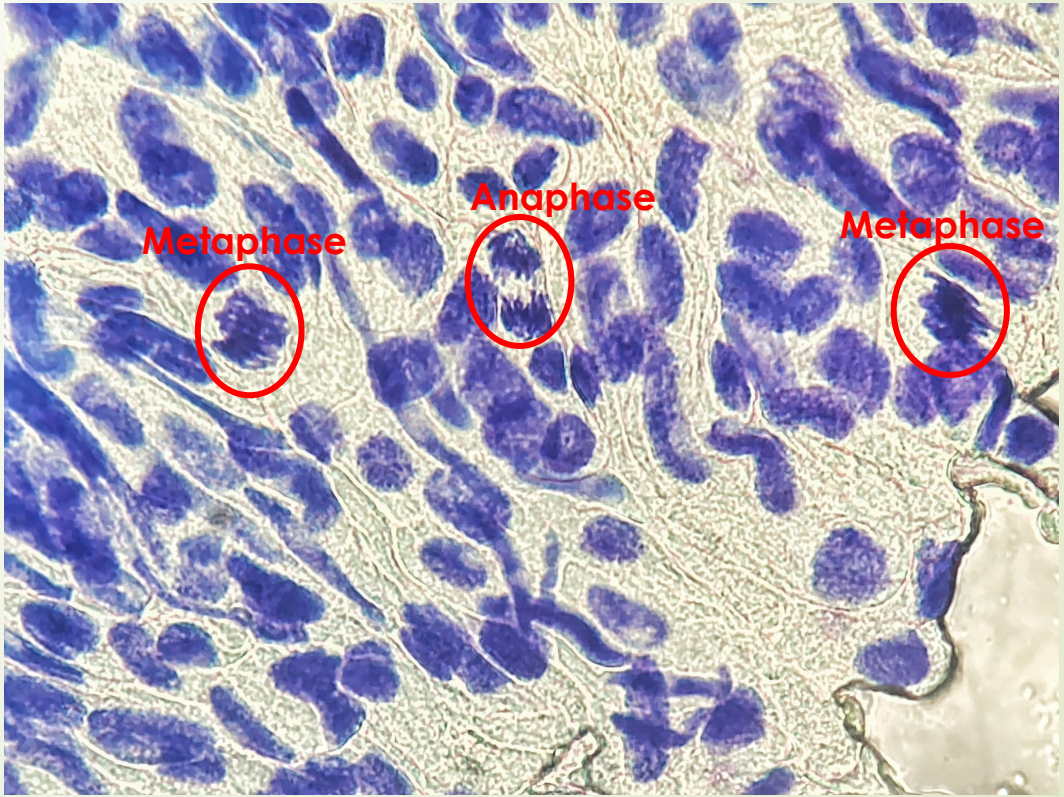

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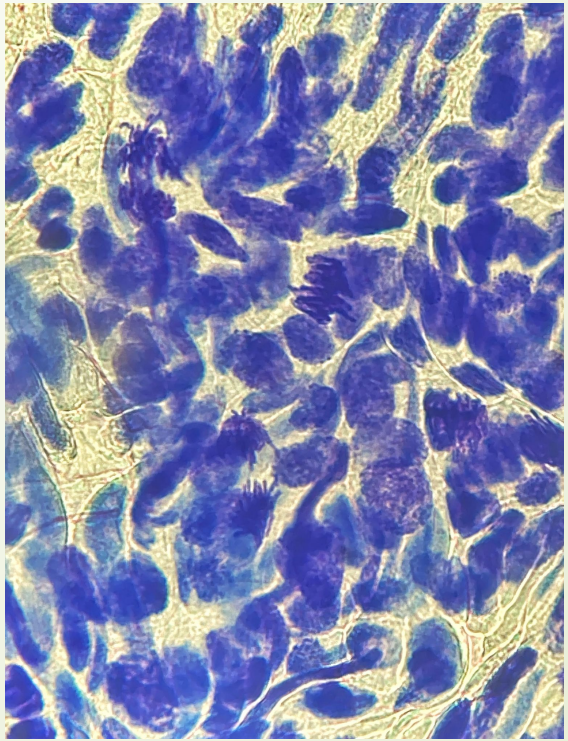

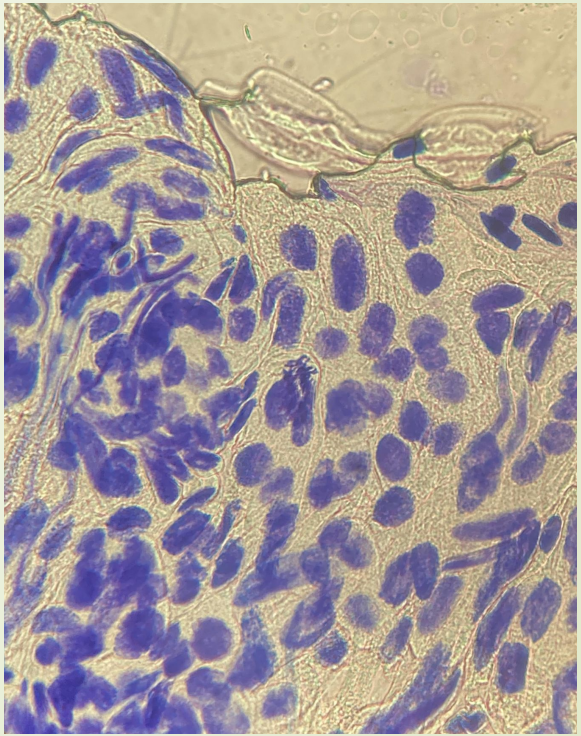

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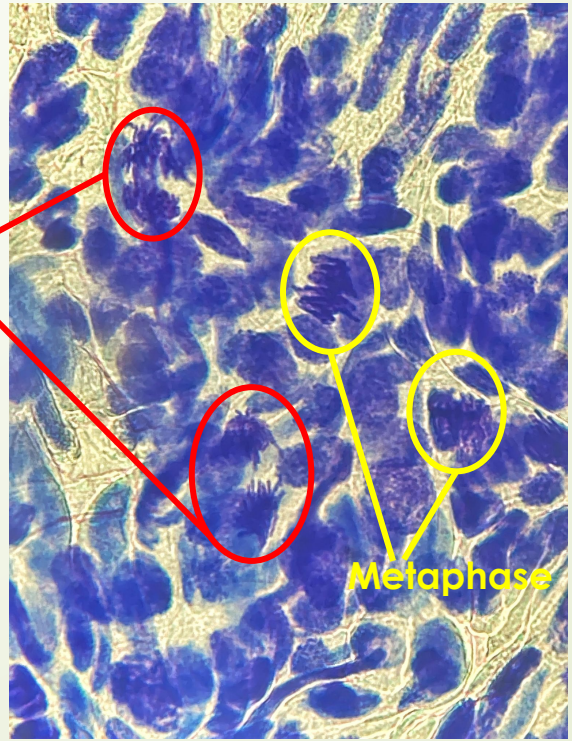

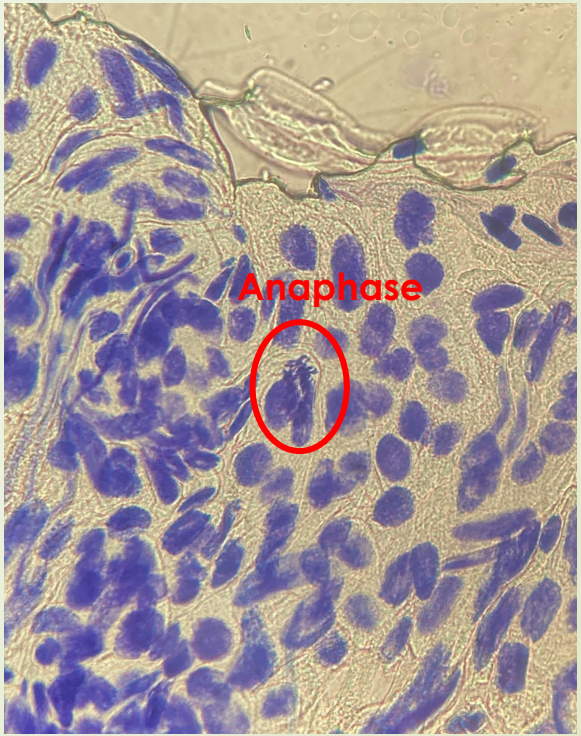

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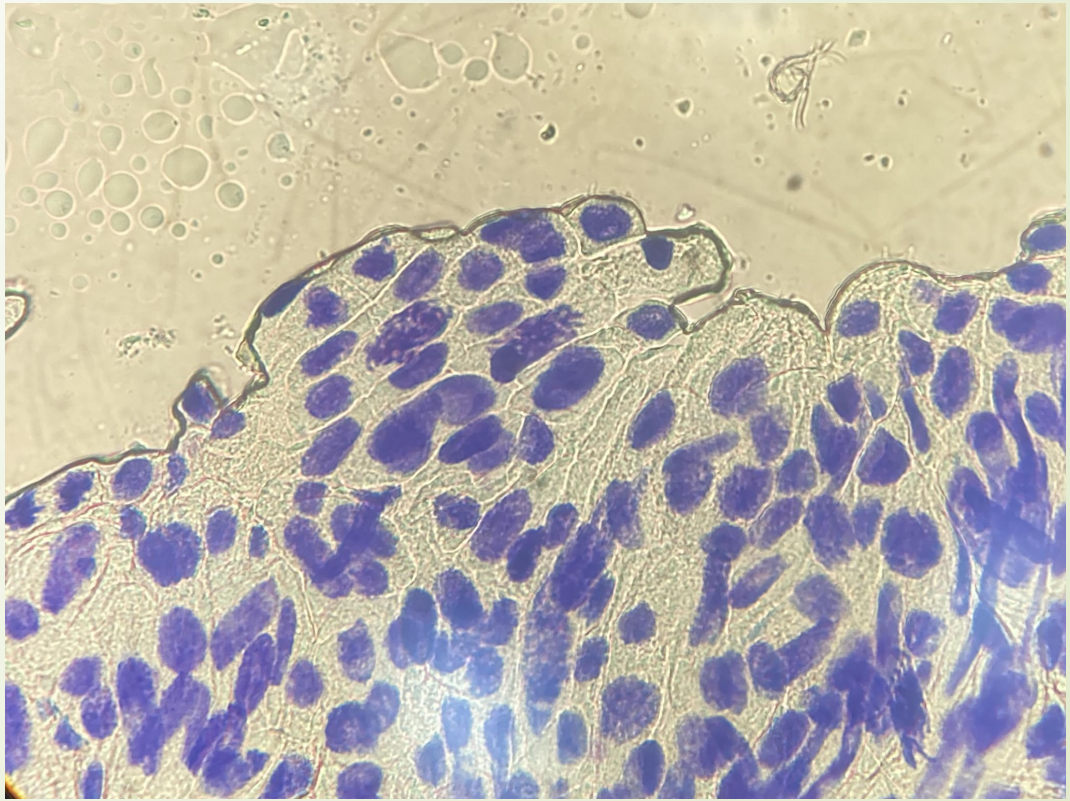
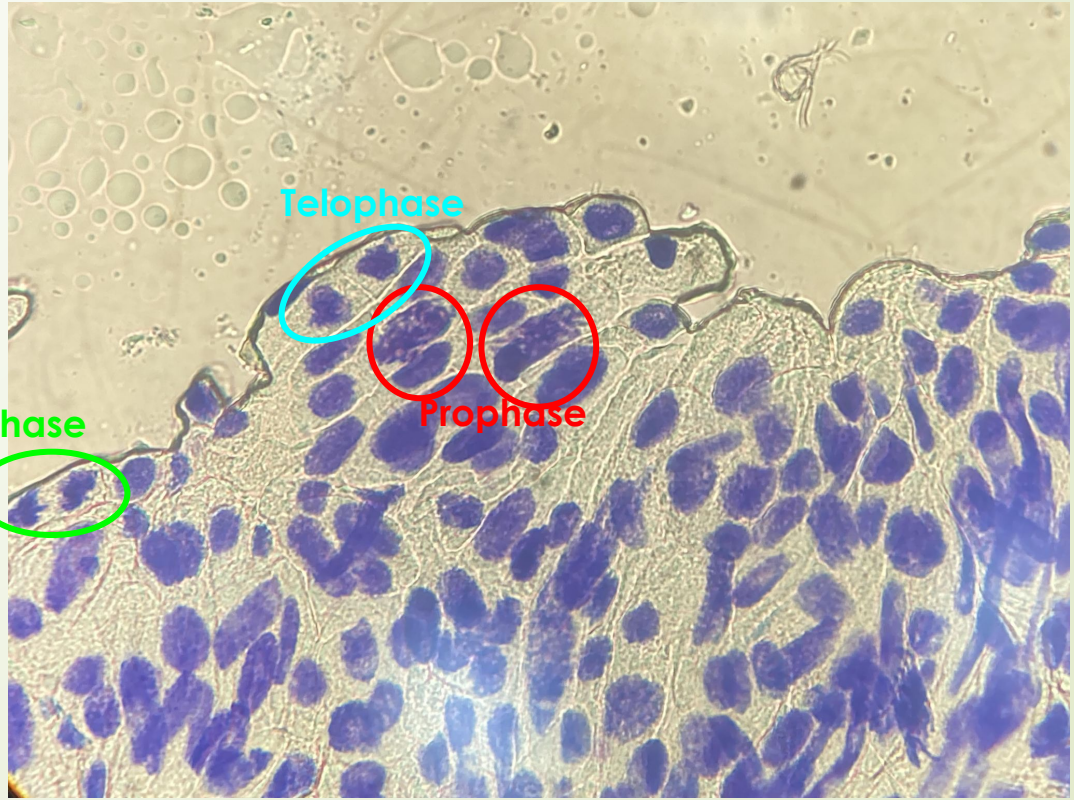


Identify the stages



This is a Dummy table, note that the data here are not from the class data

Phase or Stage	Class Totals	Percent	Time in hours
Interphase	200	71.43	17.14285714
Prophase	10	3.57	0.857142857
Metaphase	15	5.36	1.285714286
Anaphase	30	10.71	2.571428571
Telophase	15	5.36	1.285714286
Cytokinesis	10	3.57	0.857142857
Totals	280	100%	24 hrs

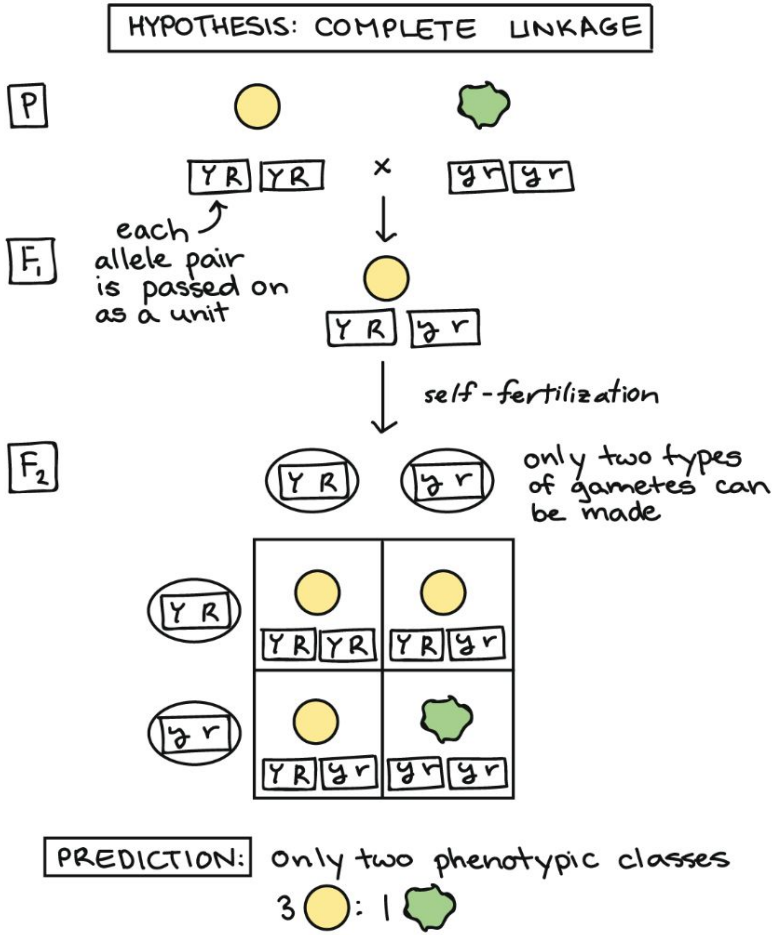
For Interphase, to get the percentage use the class totals

200/280 * 100 = 71.43%

Time in hours (cell cycle stages is equivalent to 24hrs)
71.43% = 71.43/100 = 0.714
0.714 x 24= 17.14 hrs
Calculate for the other stages and use the class data

Monohybrid cross

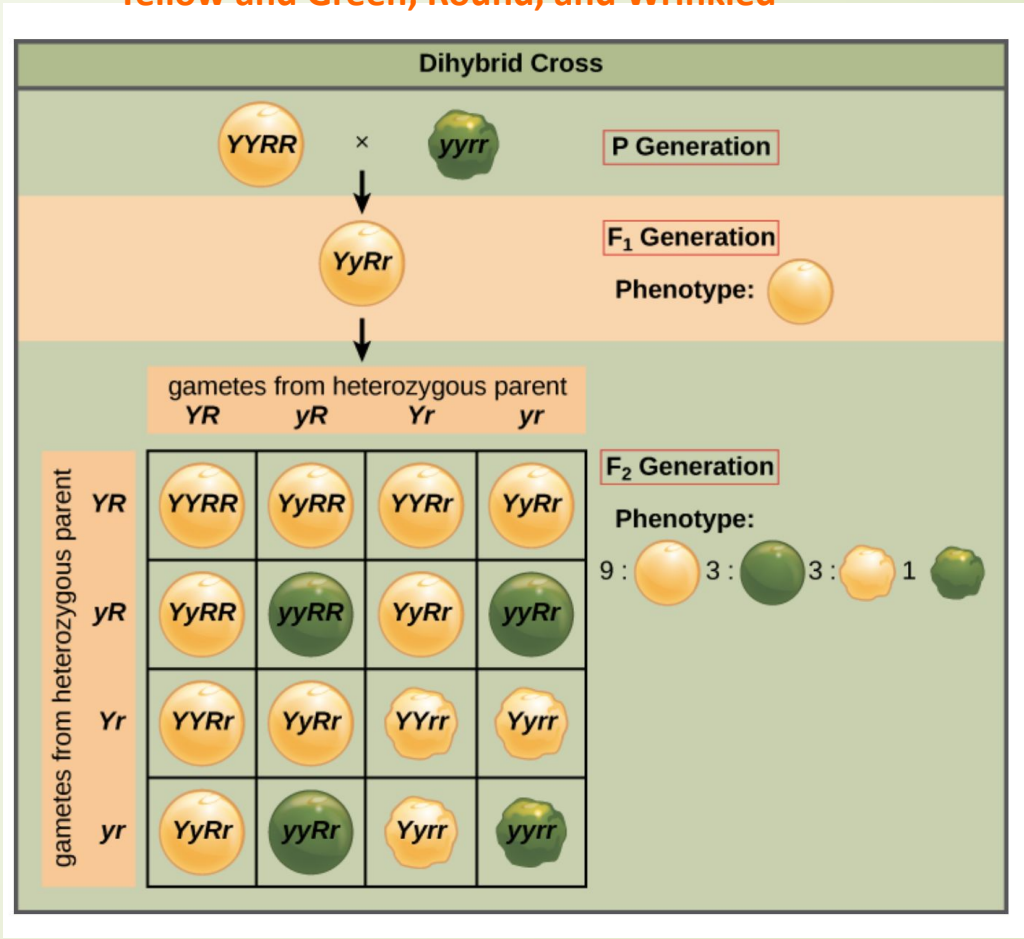
? A cross of two individuals that are homozygous genotypes which result in the opposite phenotype for a certain genetic trait.



Dihybrid Cross

- Describes the mating between two organisms that are a hybrid for the two traits
  - A hybrid organism is one that is heterozygous (meaning carries two different alleles at a particular genetic position or locus)
  - Phenotypic ratio: 9:3:3:1

Yellow and Green, Round, and Wrinkled

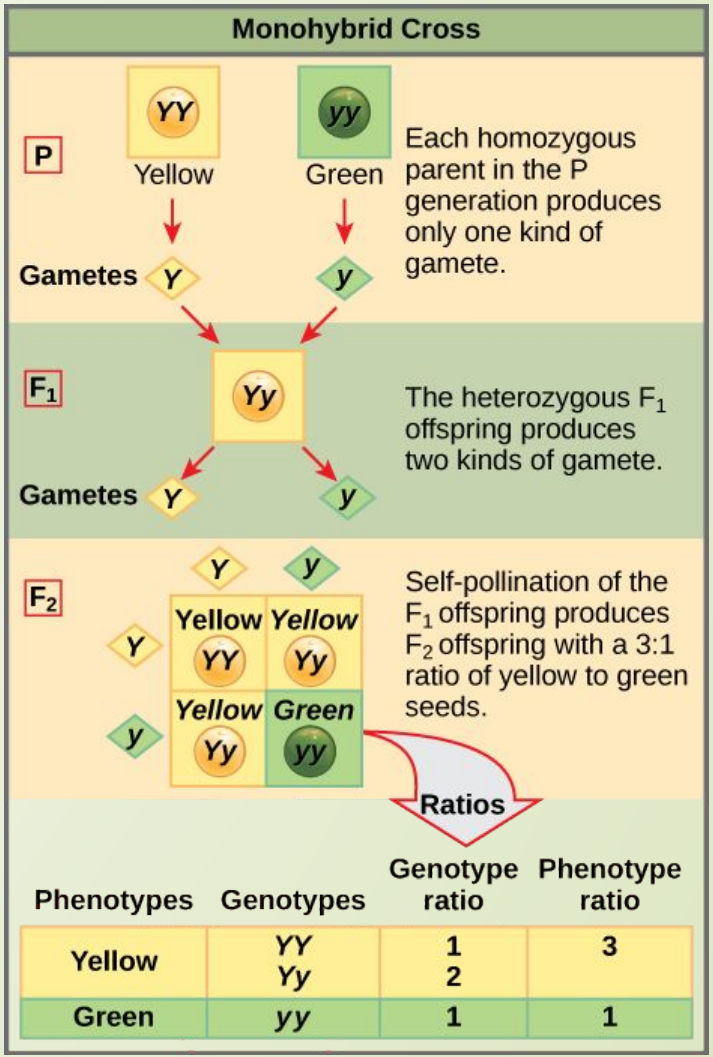


Terms

- **Dominant traits** are those that are inherited unchanged in a hybridization (F1).
- **Recessive traits** become latent, or disappear, in the offspring of a hybridization (F1).
- The recessive trait does, however, reappear in the progeny of the hybrid offspring (F2).

Punnett square

- All possible combinations of the parental alleles are listed along the top (for one parent) and side (for the other parent) of a grid, representing their meiotic segregation into haploid gametes.
- Combinations of egg and sperm are made in the boxes in the table to show which alleles are combining.
- Each box represents the diploid genotype of a zygote, or fertilized egg, that could result from this mating.



In lab today

- You will be observing the plant *Arabidopsis thaliana*
- The parental lines are
  - **gl1**= gl1gl1/gfp2/gfp2 this phenotype is bald with no gfp expression (not glowy)
  - **Gfp1**=gl2gl2/gfp1/gfp1 this phenotype is hairy and expresses gfp (glowy)
- You will be observing pictures of the parental lines
- You will then cross the parental lines to get the F1 generation
  - Gl1gl2/gfp1gfp2 this phenotype will be glowing and hairy
- You will then conduct a dihybrid cross of the F1 generation in a punnett square
  - This will be the expected outcome
- Then, each table will be assigned to seedling groups (A, B, C or D) (working in groups of 4
  - There are 25 seedlings that you will look at to determine their genotypes based on their phenotype
  - This is considered the actual outcome- you are comparing the expected to actual outcome for genetic ratios (9:3:3:1)

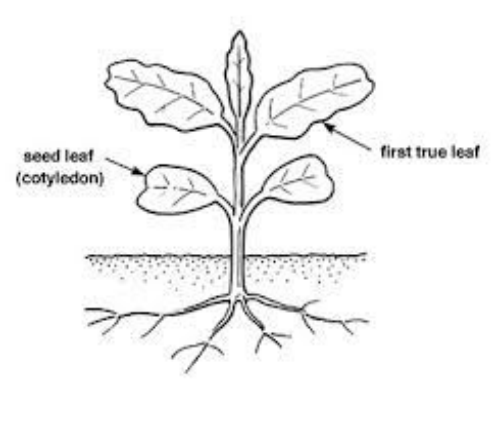
Heredity I: Observe the plants Note What's different

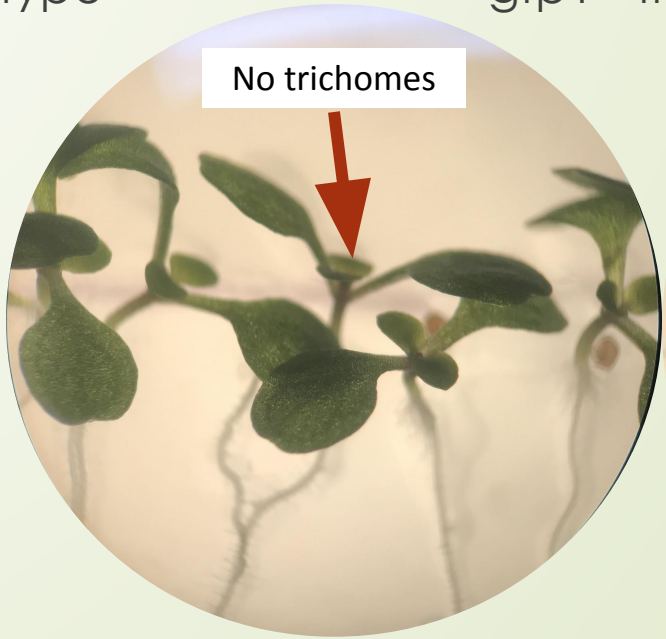
? *gl1* – glabrous (bald) phenotype

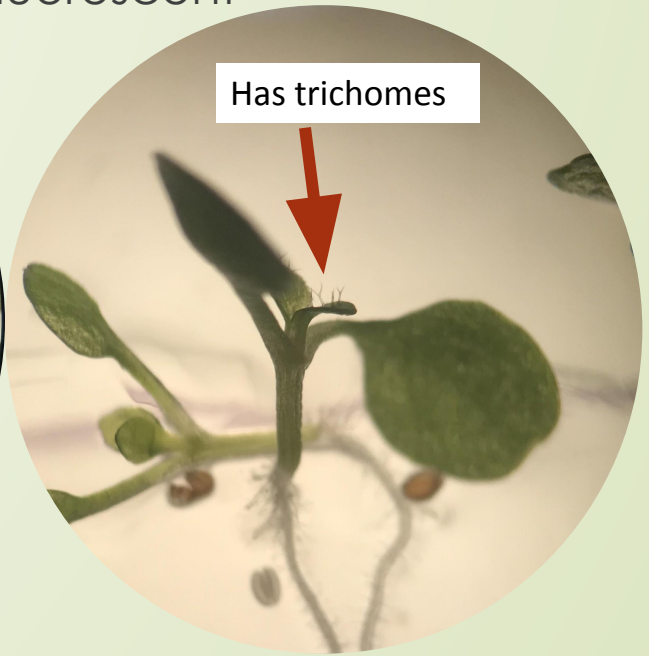
? *gl2* – trichomes phenotype

gfp2 - non-fluorescent

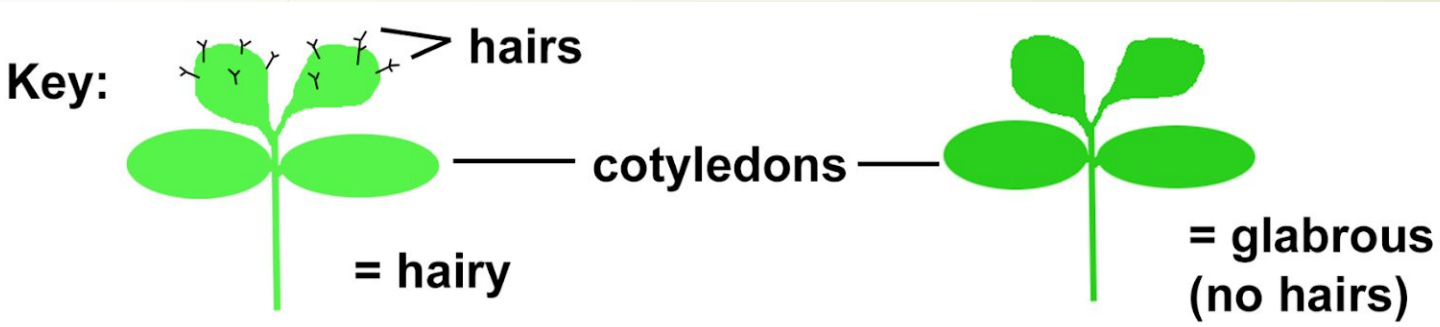
gfp1 - fluorescent

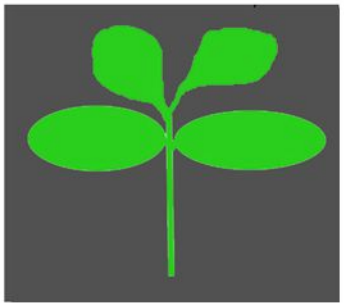




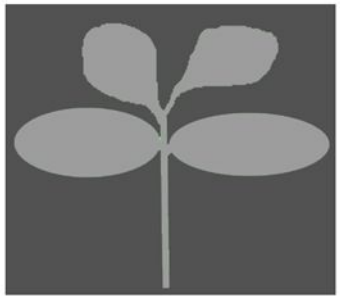


Phenotypes



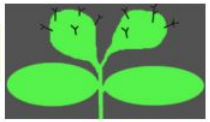


plants expressing gfp glow green under blue light, whether or not they are hairy



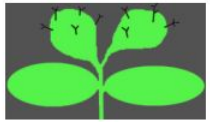
Plants without gfp appear as dim gray shapes under blue light

gl2,gl2/gfp1,gfp1



gl1,gl1/gfp2,gfp2

P Generation



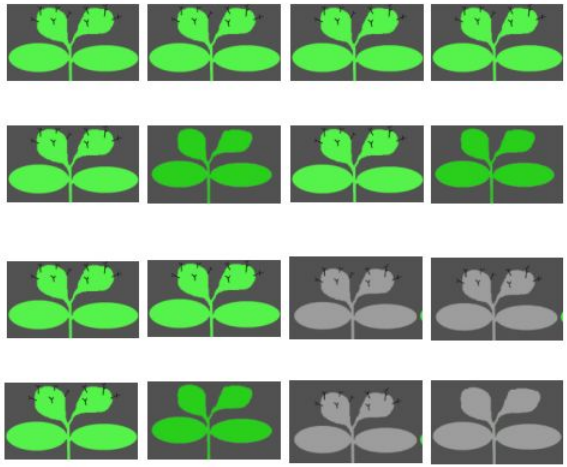
gl1,gl2/gfp1,gfp2

Glowing and Hairy

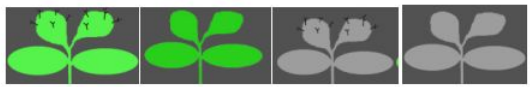
F1 Generation

Gametes from heterozygous parents
gl2/gfp1 | gl1/gfp1 | gl2/gfp2 | gl1/gfp2

Gametes from heterozygous parents
gl1/gfp2 | gl2/gfp2 | gl1/gfp1 | gl2/gfp1



F2 Generation
Phenotypes:



9 : 3 : 3 : 1

F1 generation

gl1 gl1/gfp2 gfp2 X gl2gl2/gfp1gfp1

gl1 gfp2, gl1 gfp2, gl2gfp1, gl2gfp1

Homozygote F2 Generation

Dominant gene

Parental	gl1 gfp2	gl1 gfp2
gl2gfp1	gl1 gl2 gfp1 gfp2	gl1 gl2gfp1gfp2
gl2gfp1	gl1 gl2 gfp1 gfp2	gl1 gl2 gfp1 gfp2

F2 generation

Heterozygous

F1 gl1 gl2 gfp1 gfp2 X F1 gl1 gl2 gfp1 gfp2

gl1gfp1, gl2 gfp2, X gl1gfp2, gl2gfp1 (Horizontal)

gl1gfp1, gl2 gfp2, X gl1gfp2, gl2gfp1 (Vertical)

Heterozygote F2 Generation

F1/F1	gl1 gfp1	gl1 gfp2	gl2 gfp1	gl2 gfp2
gl1 gfp1	gl1 gl1 gfp1 gfp1 (no trichomes/glow)	gl1 gl1 gfp2 gfp1 (no trichomes/glow)	gl2 gl1 gfp1 gfp1 (trichomes/glow)	gl2 gl1 gfp2 gfp1 (trichomes/glow)
gl1 gfp2	gl1 gl1 gfp1 gfp2 (no trichomes/glow	gl1 gl1 gfp2 gfp2 (no trichomes/no glow)	gl2 gl1 gfp1 gfp2 (trichomes/glow)	gl2 gl1 gfp2 gfp2 (trichomes/no glow)
gl2 gfp1	gl1 gl2 gfp1 gfp1 (trichomes/glow)	gl1 gl2 gfp2 gfp1 (trichomes/glow)	gl2 gl2 gfp1 gfp1 (trichomes/glow)	gl2 gl2 gfp2 gfp1 (trichomes/ glow)
gl2 gfp2	gl1 gl2 gfp1 gfp2 (trichomes/glow)	gl1 gl2 gfp2 gfp2 (trichomes/no glow)	gl2 gl2 gfp1 gfp2 (trichomes/glow)	gl2 gl2 gfp2 gfp2 (trichomes/no glow)

- Deliverables:
  - 10X and 40X sketches—take pictures of sketches and paste in word doc.
  - Completed Tables 1 & 2
  - Responses to questions 1-4.
  - **Cell Division is DUE the following class.**

  - **Heredity I & II will be due November 29th at 11:59pm**