

David B. Fankhauser, Ph.D.

7 October 1992, latest revision 31 Dec '95, 14 Jan. '97, 5 Jan '98, 11 Jan 00, 4 Jan 02, 2 Jan 03, 10Jan05 01Jan06, 29Dec09

[http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Root\\_tip\\_chromosomes/Root\\_Tip\\_Chromosomes.html](http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Root_tip_chromosomes/Root_Tip_Chromosomes.html)

See related protocols on *Sprouting Seeds* and *Use of Oil Immersion Lens*.

Chromosomes were first seen by C. Nägeli in 1842, and named in 1888 by W. Waldeyer. Walther Flemming studied and documented the behavior of chromosomes during cell division, a process he termed mitosis. We will perform experiments similar to these early scientists.

Cell division is especially rapid in the growing root tips of sprouting seeds. We can see the chromosomes in the dividing cells of these tissues if we perform the following operations on these root tips: sprouting seeds or bulbs, harvesting, fixing, acid digestion, staining and squashing, and viewing under a microscope. The chromosomes viewed in our lab, best to least, have been: onion, wheat, lentils, barley, rye and alfalfa (mung stains poorly).

**EQUIPMENT**

- cutting boards (at least four)
- 13 x 100 mm tubes, 2/student with:
  - a) 1-2 mL Carnoy's fixative
  - b) corks for 13 x 100 mm tubes
- test tube rack for 13 x 100 mm tubes with numbered slots
- 10 tweezers
- 10 Pasteur pipettes, long tip
- 10 bulbs for Pasteur pipets

**SUPPLIES**

- students' sprouts
- 40 prepared labels
- 10 razor blades
- Carnoy's fixative

**HARVESTING OF ROOT TIPS:**

(29Dec09)

1. Make labels for your root tips with this information: **Seat number, initials, root type, date.**
2. Apply the labels to clean prepared 13 x 100 mm tubes (Wasserman tubes) with Carnoy's fixative)
3. Select five perfect and healthy sprouts whose roots are not too long or broken.
4. With a razor blade, cut off the terminal 1/4 inch portion of the root tip  
**CAUTION:** *monocots* like wheat and rye have hypocotyls which *look* like thick stubby roots. They will not show mitosis. Harvest their roots which look like a cluster of thick hairs.
5. Pick up the tip with tweezers or a Pasteur pipet, deposit in the labeled test tube.
6. Push/wash the tips to the bottom of the tube so they are immersed in Carnoy's fixative.
7. Secure the cork to seal the tube, and set in a rack according to your seat number.
8. Fix the root tips (only about 1/4th inch long) at room temperature for at least 24 hours in Carnoy's fixative.

*cut here*.....

**PREPARATION OF FEULGEN STAIN**

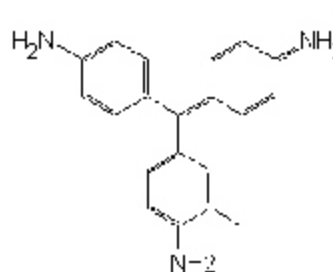
David B. Fankhauser, PhD

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[http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Root\\_tip\\_chromosomes/Feulgen\\_Stain.htm](http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Root_tip_chromosomes/Feulgen_Stain.htm)

This stain contains fuchsin or magenta I, a decolorized dye which has a strong affinity for DNA, producing a red color in its presence. Also called "rosaniline," is an aniline dye which consists of three aniline rings, one meta-methylated, para-attached to a central carbon. Its positively charged nitrogen is attracted to and stains the negatively charged PO<sub>4</sub> groups in the DNA backbone.

It is nearly colorless until it reacts with the PO<sub>4</sub> which turns it red.



For **40 mL** of the stain:

Stage: \_\_\_\_\_ Operation: \_\_\_\_\_

1. Weigh: Weigh out **200 mg of Basic Fuchsin** into 250 mL beaker
2. Dissolve: Pour **40 mL boiling dH<sub>2</sub>O** over the Basic Fuchsin, swirl to dissolve
3. Cool and filter: Cool to 50°C, filter through **Whatman No. 1** paper
4. Acidify: Add **6.0 mL 1N HCl**, mix. (*Caution:* corrosive acid)
5. Decolorize: Dissolve **0.60 g K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>** (potassium metabisulfite) to decolorize the solution. Set in dark for 24 hours to bleach.
6. Filter: Decant or filter through activated charcoal (e.g., Norit) to further clarify (if desired).
7. Storage: Store in light-proof bottle in refrigerator. (*I.e.* wrap in aluminum foil to exclude light.)

[Fuchsin(e) Acid is related, but meta-sulfonated on all three aniline rings.]

**CARNOY'S FIXATIVE:** 60 mL Ethanol, 20 mL glacial acetic acid. Seal in glass stoppered bottle. (Some recipes call for Ethanol, chloroform, acetic acid at the ratio of 6:3:1.)

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students cut root tips too long.

Students cut hypocotyls instead of roots.

Knocked over the 1N HCl...

Each desk needed pasteur pipet with bulb and razor blade.

**Mung beans did not stain at all.**

GOOD things...

Onion was perfect.

Alfalfa and wheat worked, tho not as well.

