

## STAINING ROOT TIPS TO SEE CHROMOSOMES

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[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).

### SUPPLIES:

- 1) Fixed root tips from freshly sprouted onion or seeds (rye, wheat, lentils, alfalfa, onion, etc), 2-3 cm long, fixed 24 hrs in Carnoy's fixative.
- 2) 1N HCl
- 3) Feulgen stain
- 4) 45% HOAc

### EQUIPMENT for each student:

- Wasserman tubes (13 x 100 mm test tubes)  
microscope slides  
Each desk:  
Pasteur pipets with bulbs  
razor blades  
cover slips  
single Constant temp. "hot block", 60°C

**THE DAY PREVIOUS** (see previous protocol *Root Tip Harvest for Seeing Chromosomes*.)

1. **Fix the root tips** (only about 1/4th inch long) for at least 24 hours in Carnoy's fixative.

### DIGEST, STAIN AND SQUASH THE FIXED ROOT TIPS:

2. **Label a clean 13 x 100 mm tube** "digest tube," your seat number, and root tip type.
3. **Select root tips** which have a firm rounded tip, with a Pasteur pipet, slide two out of the Carnoy's fixative tube onto a clean slide. Draw off xs fluid.
4. **Add 1-2 mL 1N HCl** to the digest tube, ensure the roots are fully immersed.
5. **Digest at 60°C:** Place digest tubes in a 60°C constant temp. "hot block." for 12 minutes.
6. **Stain with Feulgen stain:** Remove HCl with Pasteur pipet, discard in running cold water. Cover tips with about 1 mL Feulgen stain. **CAUTION:** this stain does not *look* brightly colored, but stains strongly. Do not get on clothes, fingers, books, etc.) Let sit at R.T. for 10 minutes until the rounded very tip of the root shows *distinct dark coloring*.
7. **Prepare the squash:** Put a drop of 45% HOAc on a clean microscope slide. Remove the Feulgen stain with a Pasteur pipet, save for a second staining, or discard into the drain. Place the soft root tip in the HOAc on the slide. With a scalpel or razor blade, remove *all but* the red-stained *very tip* of root. Cover with a cover slip, place on white paper and tap gently *straight down* with a pencil eraser until the stained tip is squashed out to a faint purple monolayer. Do not smear side-wise, it shears chromosomes.
8. **Examine under the microscope** at low power to ensure that the cells are adequately spread to a monolayer. If so, examine under higher power. Locate mitotic figures (nearest the tip end), and examine under oil immersion (1000x). (CLEAN UP WHEN DONE.)
9. **Illustrate examples of each mitotic stage** (pro-, meta-, ana- and telophase). Prepare a second squash with a different species, and illustrate its mitotic stages, noting any differences observed between the two species.
10. **Show chromosomes** of two species of plants to the instructor for 5 points each.