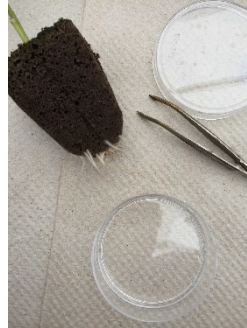


## CHROMOSOME COUNT PROTOCOL

We grow seedlings in 3" clay pots until they are ready to transplant and then transplant into Q-plugs (see transplanting protocol for full instructions)



Just a few days after transplanting, the first vigorous roots will emerge on the side or bottom of the Q-plug.



About an inch of the most vigorous roots are collected early in the morning.



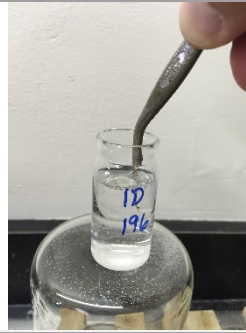
The collected roots are put into 5 ml of 0.3% colchicine.



They are shaken in the 0.3% colchicine for 6-7 hours in a dark cold (40F) spot - we put the shaker in our tuber storage cooler.



Roots are then put into a 3:1 fixative (ethanol:glacial acetic acid) at room temp for about 24 hours and then refrigerated. You can keep them there indefinitely.



When ready to microscope, transfer the root tips into 1 N HCl at 60C and cook for 10-12 minutes. Take care not to undercook, because undercooked roots do not allow the central cells with the division figures to be mashed and presented on the slide. Overcooked cells may become damaged and chromosomes may dissociate from the cell.



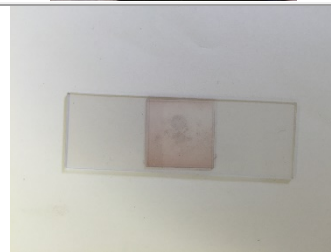
Transfer the root tips out of the HCL and into water. Chromosomes should be counted on same day of cooking.



Cut off just the very tip (about 1 mm) of the cooked root tip. You can do 2-3 tips on the same slide. Put a drop of acetocarmine stain on the roots and macerate. You might also add a tiny speck of iron from a saturated iron acetate solution. This iron seems to improve contrast, as does, gentle heating. If cells stain too dark, chromosomes will be very difficult to locate.



Put on a cover slide, very carefully press it down. It may help to distribute the cells if you gently tap the cover glass with the eraser end of a pencil before pressing.



Put slide under scope. With luck you should be able to locate many cells at 40 Power where chromosomes are well distributed for accurate counts. Locate a few good cells, jot down locations, and do evaluations at 100 Power.

