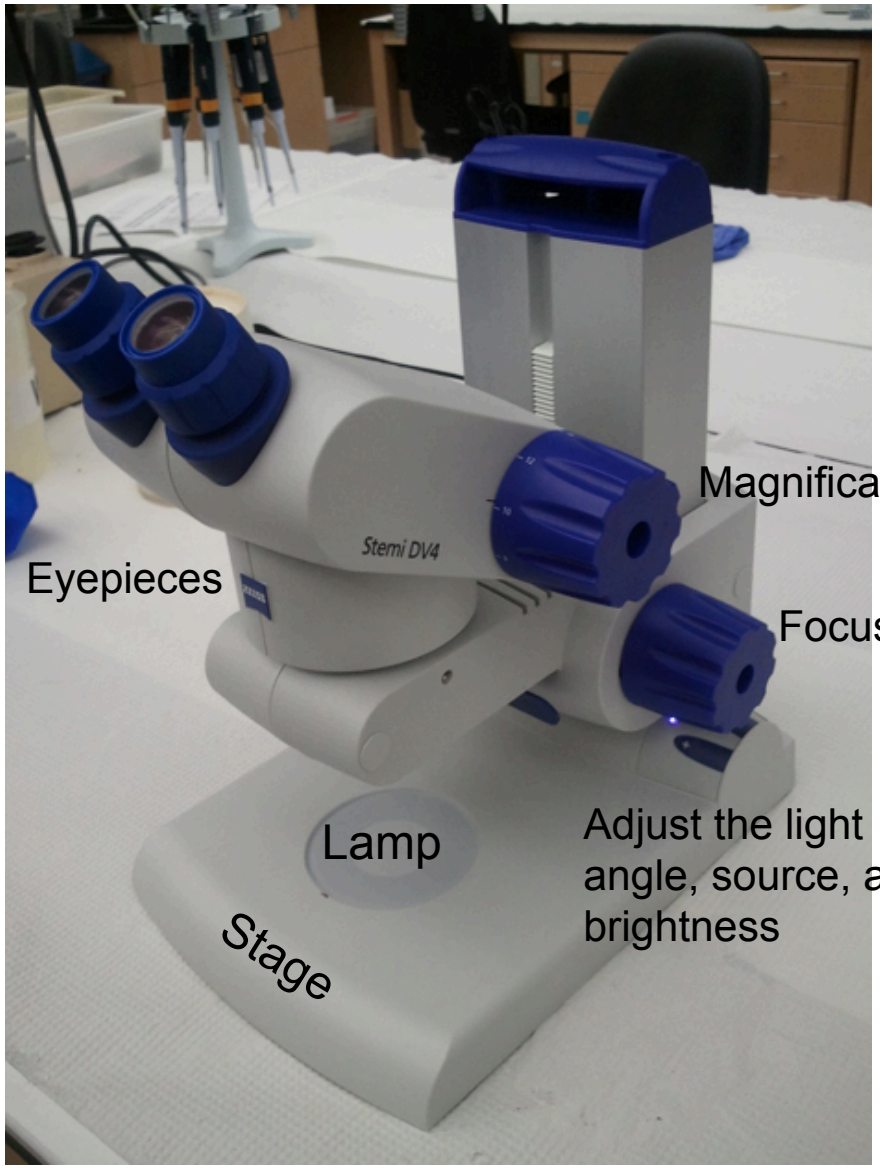
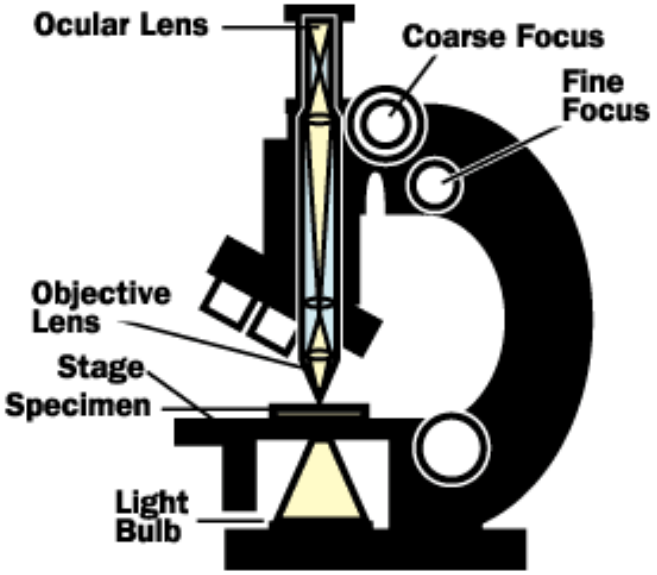
Light Microscopy

HC70AL
August 19, 2014

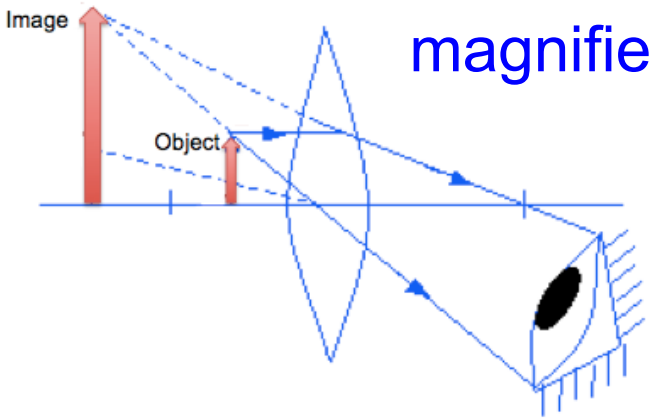
Michael Lyons

Light Microscopy

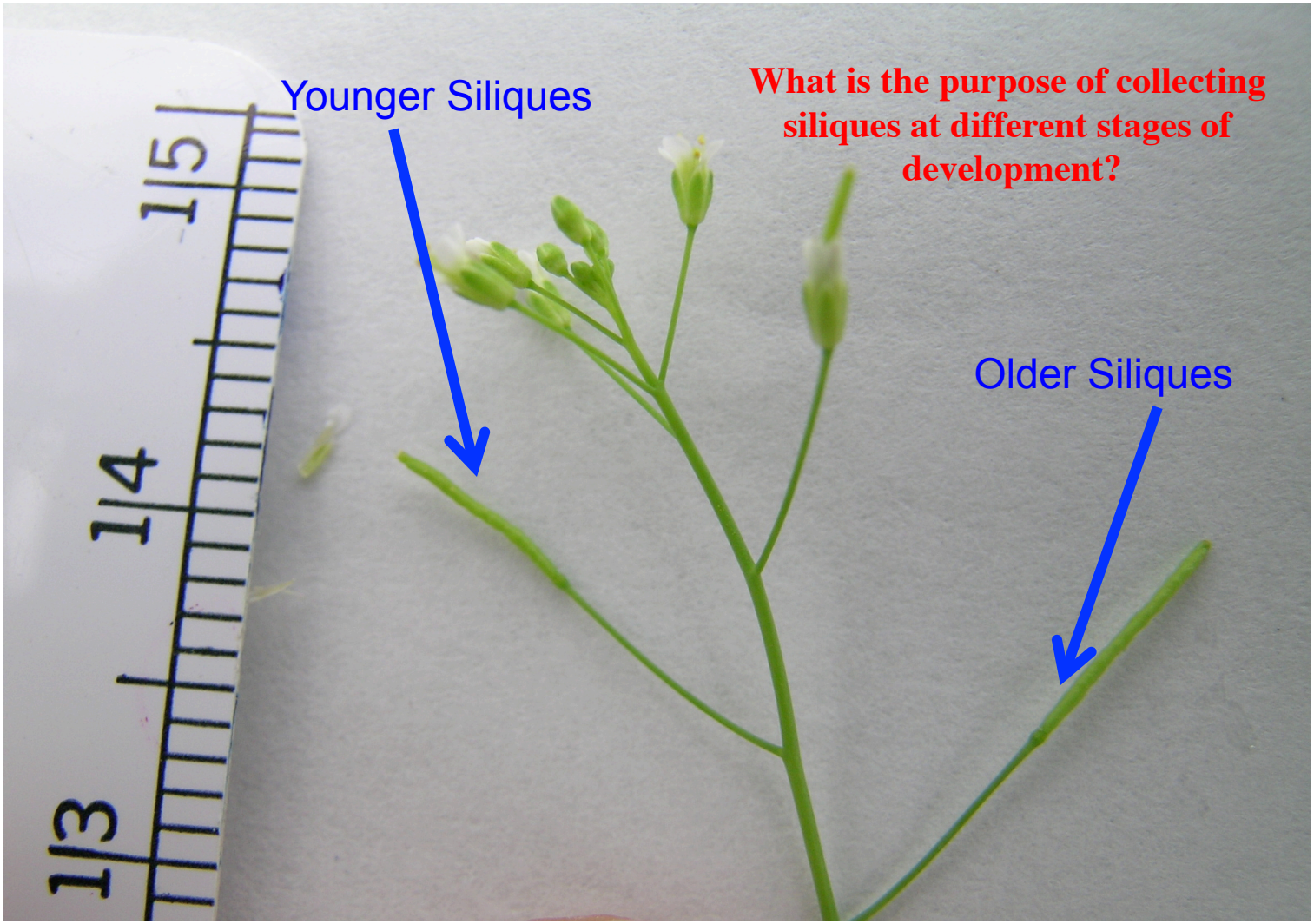




How a lens magnifies



How Do We Collect Seeds In The Desired Stage?



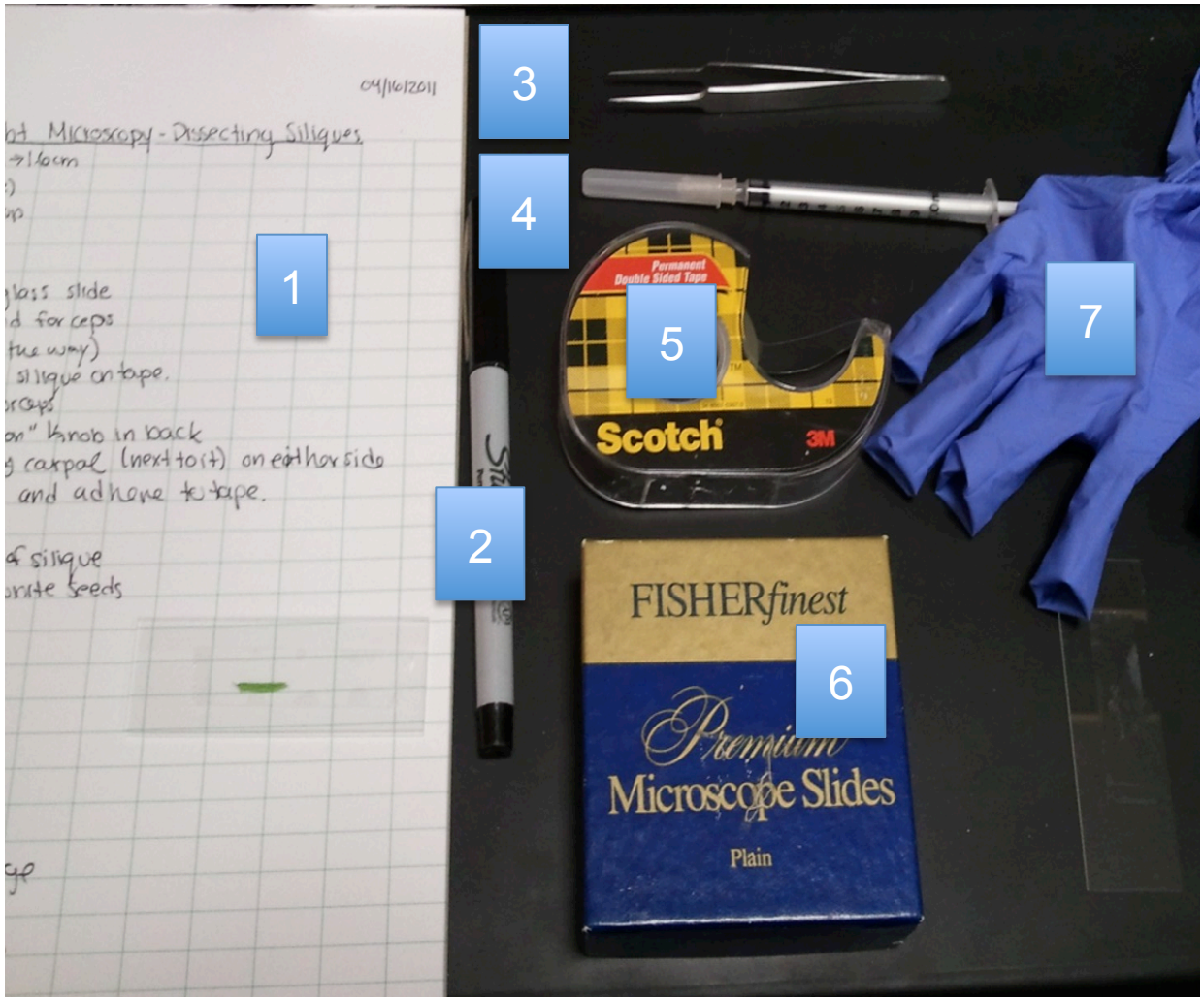
Measure the silique length

Collect siliques and store in 1.5 mL microcentrifuge tube on ice.

What will you use as a control for your mutant siliques?

What Materials Do You Need?

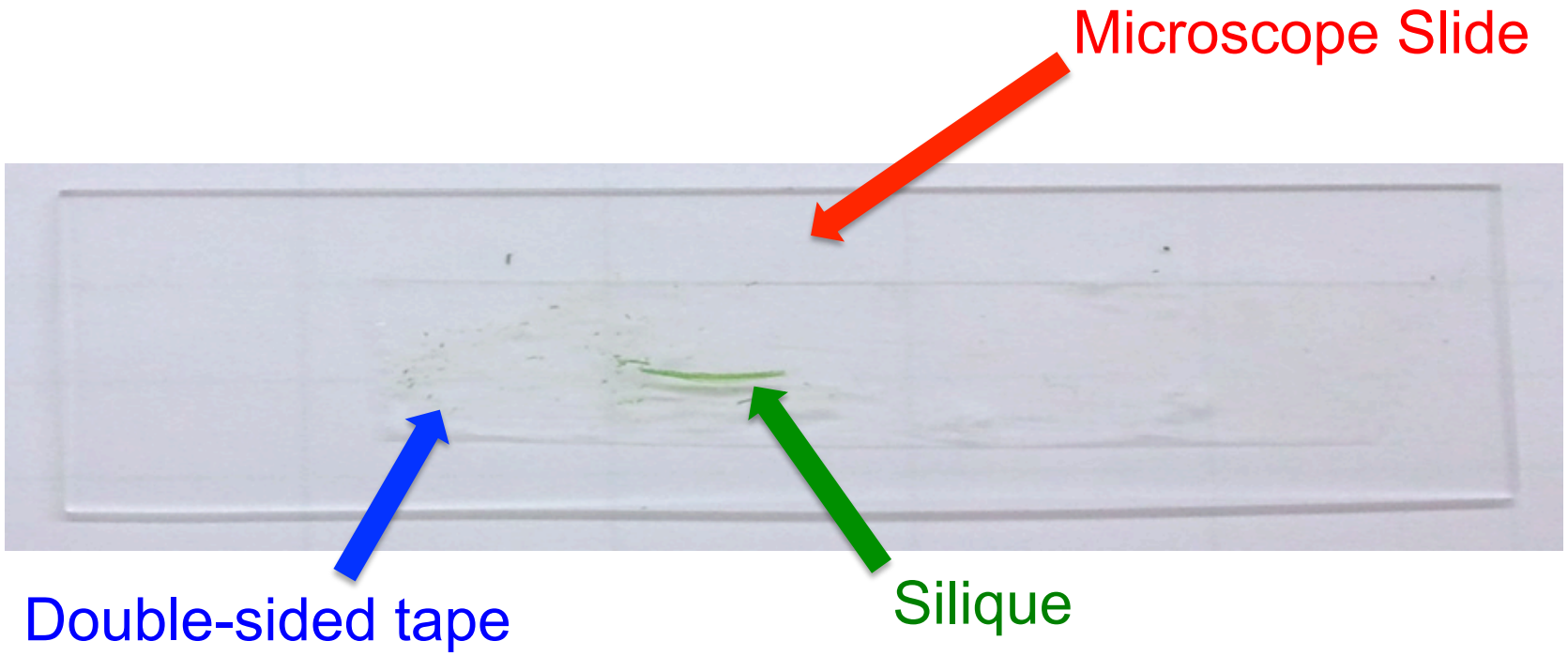
After you collect your siliques from the Plant Growth Center, you need…



1. Lab Notebook
2. Pen
3. Forceps
4. Syringe
5. Double-Sided Tape
6. Microscope Slides
7. Gloves
8. Ice Bucket
9. Ruler
10. Dissecting Microscope

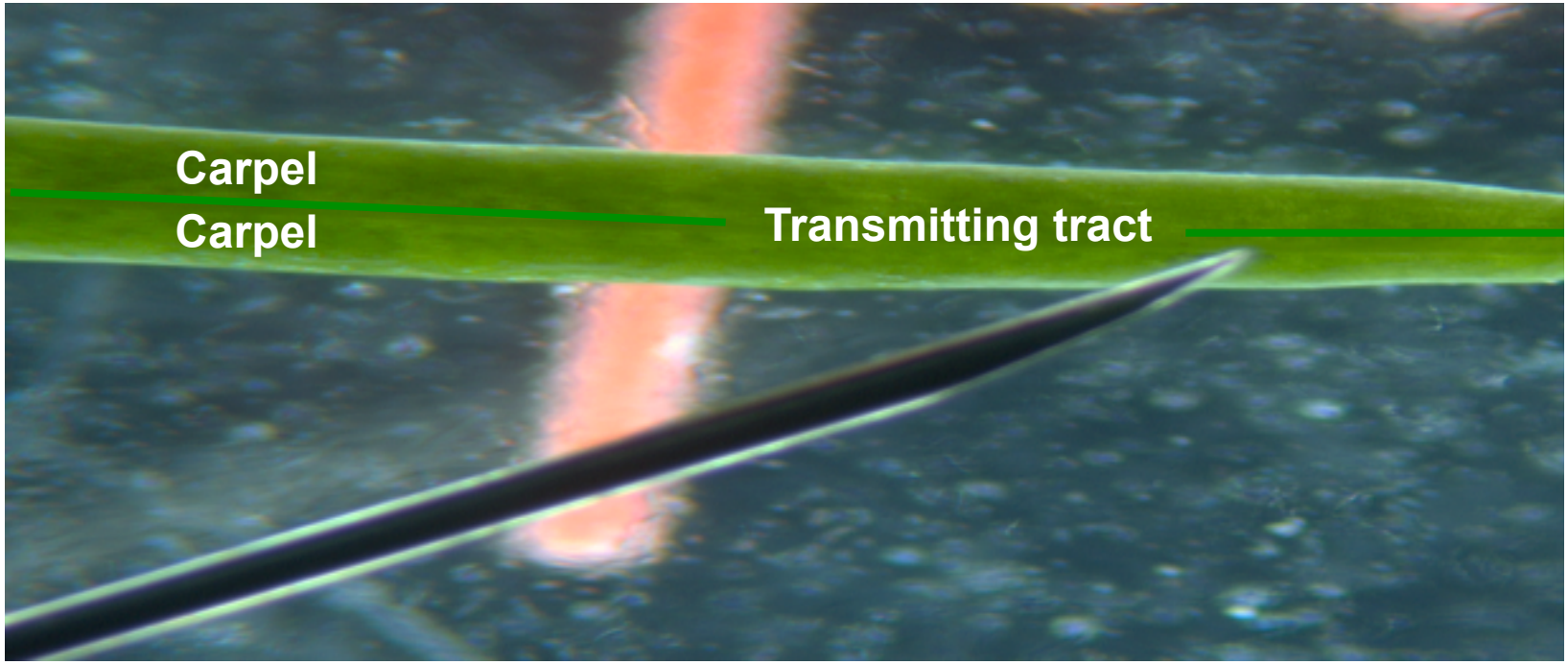
How Do We Prepare Microscope Slides?

1. Keep tubes of siliques on ice
2. Place a piece of double-sided tape onto a microscope slide
3. Take out one silique with forceps and place onto tape



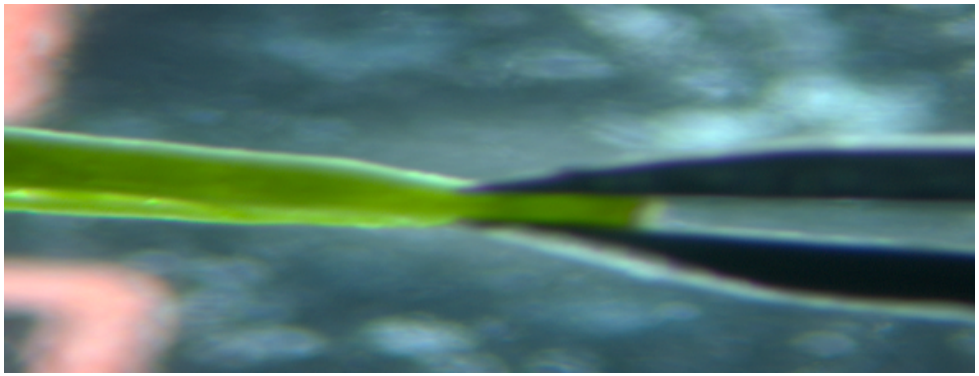
Where Should You Cut?

Slowly cut along either side of the transmitting tract



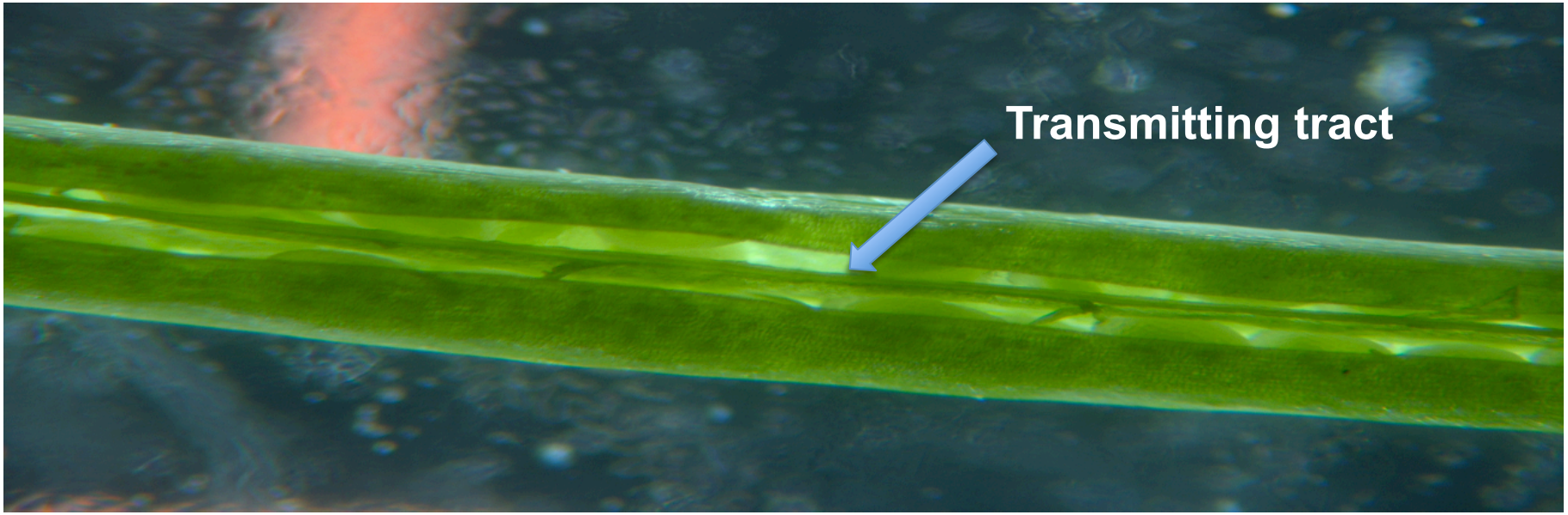
Be careful to not cut too deep. The seeds are just below the outer surface and you do not want to puncture them.

How Should You Cut The Silique Open?



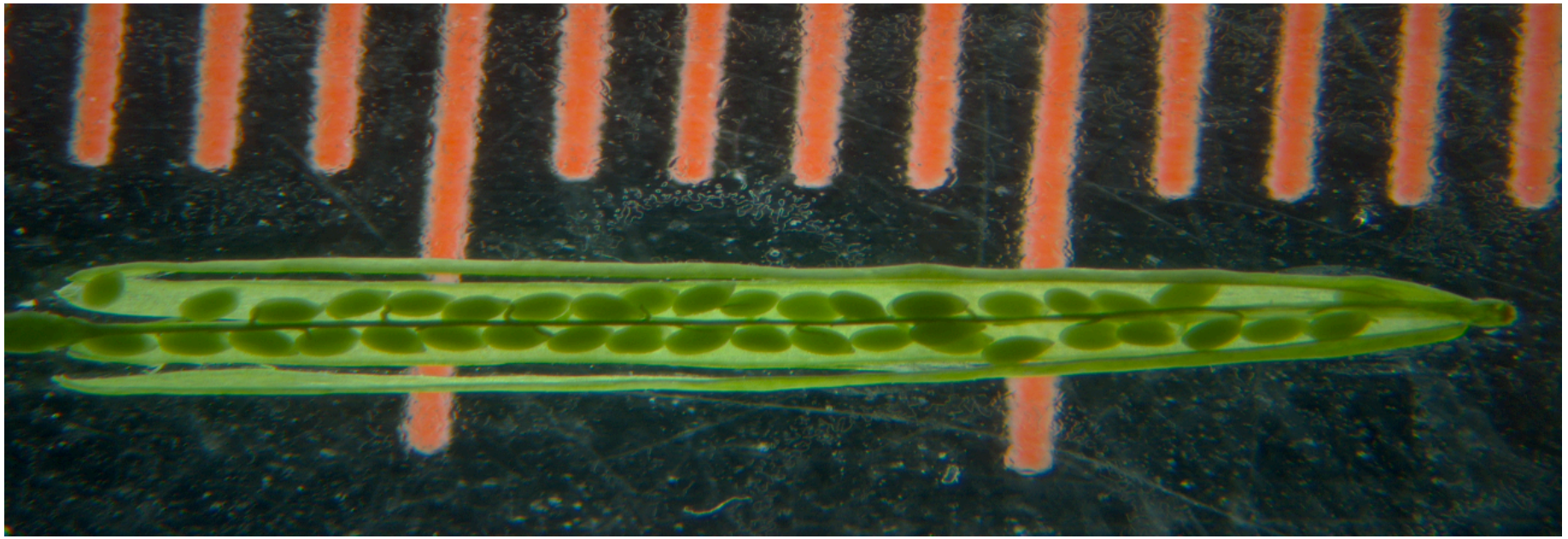
While holding the stem with forceps to steady the silique…

…use the tip of the syringe to cut through the first layer of the silique on both sides of the transmitting tract



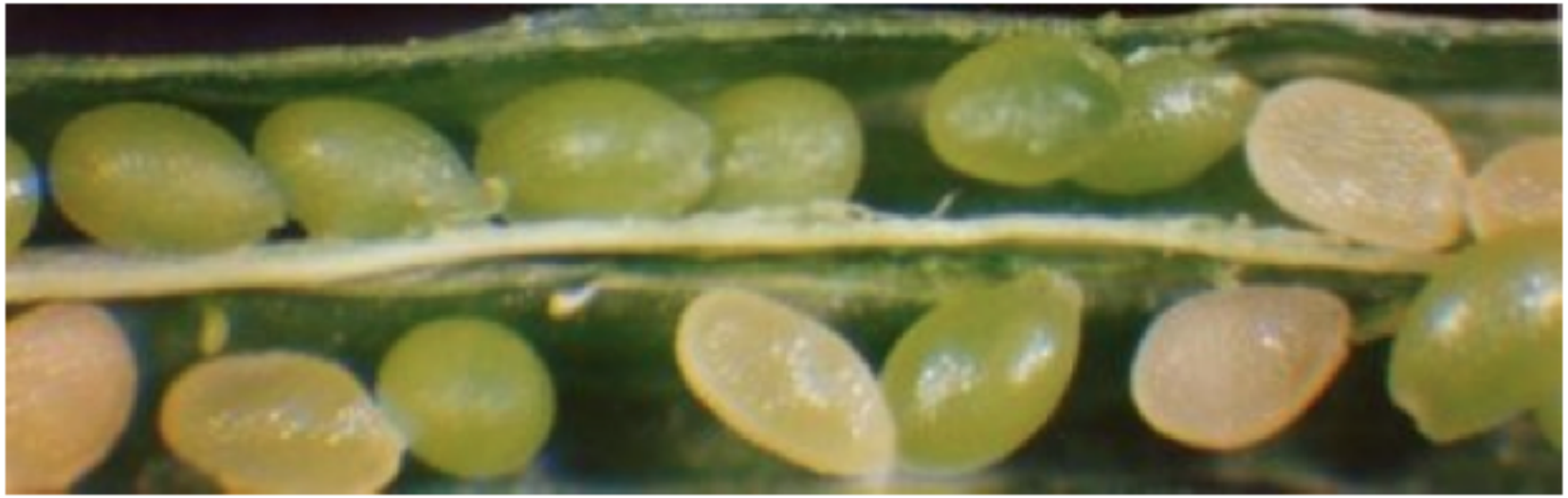
Slowly cut the silique open gently. It does not take a lot of force to get through the outer layer.

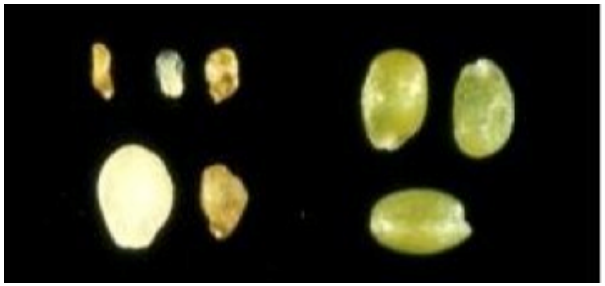
How Should You Analyze the Silique and Seeds?



1. Using the syringe, peel back the sides of the silique and gently press the sides to the tape
2. Fill out the Screening Siliques Chart for each silique
3. Record the length of the silique, not including the stem
4. Label your slide with white tape: AGI #, Plant #, Plant Genotype, Silique #
5. Give the slide to a TA to take a picture
6. Peel silique slowly off of the tape and place into 1 mL of 10% acetic acid, 90% ethanol to fix the siliques for Nomarski microscopy

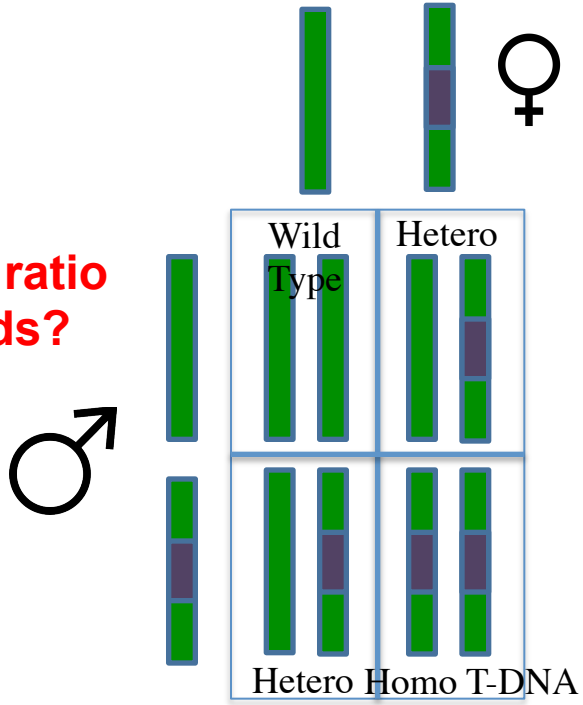
What Phenotype Do You Expect If The Mutation Is Lethal?





Mutant Seeds **Wild Type Seeds**

What is the expected ratio of green:white seeds?



How can we determine this phenotype is due solely to the T-DNA insertion?

Can we be positive that there is not another factor leading to mortality of our seeds?

What is the Chi-Square test?

$$X^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{(\text{Expected})}$$

The Chi-Square Test is used to test a null hypothesis to determine if there is significant difference between our observed values and expected values.

What is a hypothesis? What is a null hypothesis?

How does our X^2 value tell us if our results are significant?

What is a degree of freedom defined as?

What does our P-value tell us?

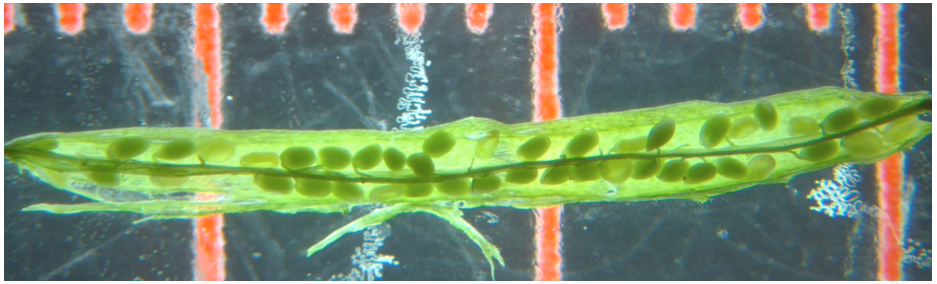
P-Values →

Degrees of freedom ↓

	0.5	0.1	0.05	0.02	0.01	0.001
1	0.455	2.706	3.841	5.412	6.635	10.827
2	1.386	4.605	5.991	7.824	9.210	13.815
3	2.366	6.251	7.815	9.837	11.345	16.268
4	3.357	7.779	9.488	11.668	13.277	18.465
5	4.351	9.235	11.070	13.388	15.086	20.517

X^2 Values

If our P-value is under 0.05, is it likely that our deviations are caused by chance and chance alone?



Observation: 44 total seeds, 12 white and 32 green

What is our null hypothesis?

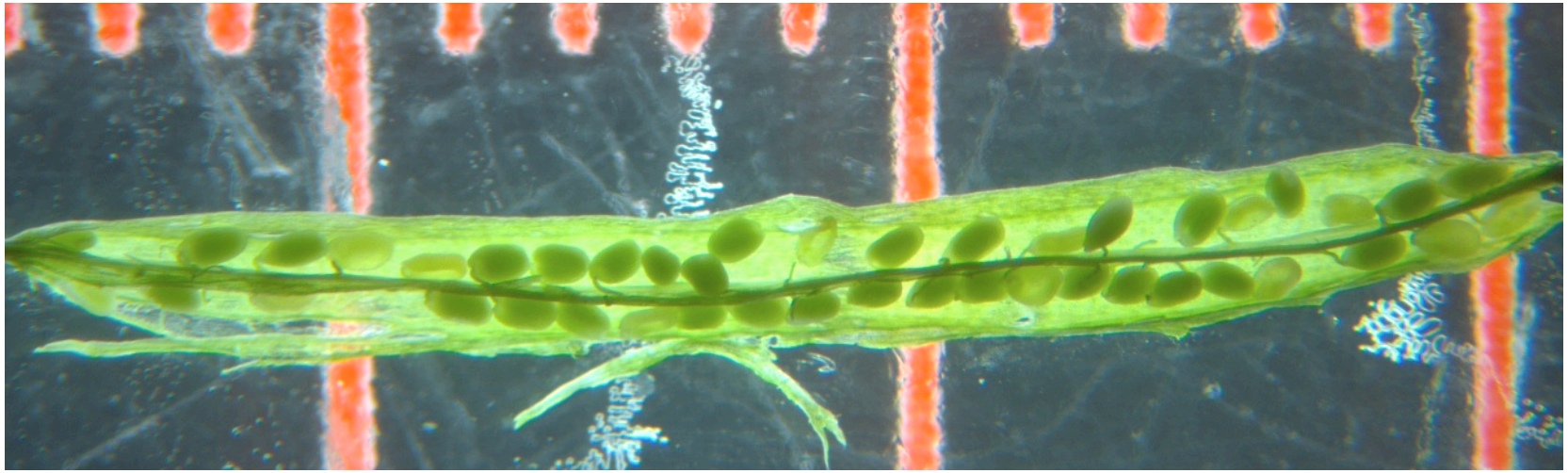
$$X^2 = \frac{(\text{Observed green} - \text{Expected green})^2}{\text{Expected green}} + \frac{(\text{Observed white} - \text{Expected white})^2}{\text{Expected white}}$$

$$= \frac{(32-33)^2}{33} + \frac{(12-11)^2}{11} = 0.1212$$

Does our P-value reject our null hypothesis?

Can we observe where in development the seed was stopped?

By using light microscopy, can we see if any specific tissues have a mutant phenotype?



What can we do in order to observe these phenotypes?