Video Script:

Introduction:

"This video includes a demonstration on using hand pollinations to make crosses in common bean, Phaseolus vulgaris. Due to flower structure in legumes these methods are broadly applicable to other legumes including runner bean, pea, sweet pea, fava bean, peanut, and others."

Identifying flowers:

"To begin you'll need to identify flowers at the appropriate stages for pollination. The paternal parent will be an open flower, which is indicative of pollen shed. The maternal parent will be an unopened flower that is within a day or so of opening.

If you look at these examples, you can see both maternal and paternal parents that are at varying stages for successful cross-pollination. It is important to note that the maternal and paternal flowers will be at different morphological stages and due to this you will need to have beans at varying stages of flowering to begin making cross pollinations.

Once you have determined the presence of both paternal and maternal flowers, you can move forward to the emasculation of the maternal flower."

Maternal Flower Emasculation:

"The emasculation of the maternal flower is done prior to flower opening to ensure that pollen shed has not begun, and that self-pollination will not occur. It's best to do pollination early in the morning to find flowers at these stages.

There are 6 stages of the emasculation process. The separation of banner petals, the folding back of banner petals, the removal of wing petals, the removal of the keel petal, the removal of the 10 anthers, and a confirmation of no remaining anthers on the flower.

You'll begin by opening the outer petals, known as the banner petals. You can do this by identifying the line that divides these two petals along the base of the flower bud and separating them with forceps.

You will then gently fold the banner petals back and away from the remaining petals. When folding back the banners be careful not to damage them, as you'll want to keep them for future use.

Once the banners have been folded back you will identify the wing petals, which are tucked directly inside of the two banner petals. You will remove both wing petals. The wing pedals are

attached to the keel petal, which surrounds the reproductive organs. You will want to leave this portion of the flower undisturbed until the wing petals have been removed.

Then using the forceps, you will begin opening the keel pedal. To do this gently grasp the petal near the base, being careful not to grab any tissue beyond the petal. Slowly begin to unfurl the keel being very cautious to not break the stigma, which is coiled inside of the keel petal. This stage can be the most difficult, as the stigma is small and fragile. If broken, you will need to begin the process on a new flower.

Once the keel is removed you should be able to identify the 10 male organs, or stamen, and the one female organ, the stigma. Since this flower will be the maternal parent you will remove all 10 anthers from the stamen and keep the stigma intact. While removing the anthers be sure to count them to ensure that the emasculation is complete. If an anther is left behind it could begin to shed pollen in the coming days and interfere with your desired cross pollination.

At this point the emasculation process is complete and it is time to prepare the paternal flower."

Paternal flower preparation and cross-pollination:

"Prior to beginning the emasculation process you should have identified a flower at the appropriate stage for pollen donation. There are 6 steps in this part of the hand-pollination process: pinching the wing petals, removing the pollen-covered stigma, brushing or hooking the pollen-donor stigma onto the maternal stigma, closing the banner petals, taping the petals shut, and final steps to ensure successful pod set.

Bring this flower close to the maternal parent and begin to extract the stigma from the paternal flower by pressing on the wing petals. This should cause the stigma to protrude out of the keel petal.

While holding the wing petals remove the stigma from the paternal flower. It should be covered in pollen from its own anthers. Here you can see a close-up of the pollen on the paternal flower's stigma.

Carry out the cross pollination by sticking the two stigmas together. You can try to hook the paternal stigma onto the maternal stigma, leaving it behind when you close the outer petals, or simply brush them together to distribute the pollen. Once you have finished pollinating, carefully close the banner petals to retain high humidity within the flower, and gently pinch the banner petals with a piece of tape.

In the final step of hand pollinations, you will want to remove all other flower buds that are growing on the same flower stalk as your hand-pollinated bud. Additionally, it is important to make note of the cross that was made. We use the notation of maternal parent name crossed by paternal parent name. You may also include other relevant information on the label, such as the date of the cross pollination or the name of whoever made the cross." Closing comments:

"By following these steps, and practicing your technique, you should be ready to successfully make crosses through hand-pollination of common bean. Tracking other metrics such as temperature or time of day when cross was made may help you to further refine your process.

We hope that you found this tutorial helpful.

This video was put together by a team of researchers at Oregon State University.

On a final note, we would like to acknowledge our funding sources that allowed us to put time into creating these videos.

Thank you."