Arabidopsis is an excellent genetic model plant, however, its small size makes crossing experiments a bit of a challenge. If you are right-handed, you should have less than two left hands, abandon extensive nightlife and control your coffee consumption ☺.

For most efficient crossings, choose mother plants at a stage when they have developed 5-6 inflorescences (they have the largest buds), and father plants that have started to form siliques (this indicates that the pollen is o.k.). Emasculation is not a nice thing at all, so try to be at least gentle with the poor plants ☺. If you work with transgenic plants in classroom make sure that the waste is properly collected and disposed in bags suitable for autoclaving later on.

1. From the inflorescence of the mother plant, remove mature siliques as well as open flowers and buds that have already a white tip, with fine scissors or forceps.

2. Fix the inflorescence gently (e.g. test tube clamp with a crepe rubber cushion) under a binocular with ca. 10-20 x magnification.

3. Clean the tips of two pairs of fine forceps (e.g. SS Inox, Dumont, Switzerland) with 96% ethanol and wipe them dry with a tissue. Do not destroy either the delicate tips of the forceps or yourself!

4. Remove the meristem with those buds that are too small: usually 3-5 flower buds have the right size and should remain.

5. Open one flower bud by inserting the tip of one pair of forceps between petals and sepals. Sometimes, pressing a little bit on the top of the bud makes this job easier. Control if all anthers are still closed (if not, remove this bud and clean forceps again), and remove all immature anthers with the other pair of forceps. The regular number is 6, but there are buds with only 4 or 5 anthers. A piece of tissue paper in one hand helps to get rid of the removed anthers.

6. Repeat this for all remaining buds of the inflorescence.

7. Mark the emasculated inflorescence with a piece of thread around its stem. Double knots are recommended, but do not strangle the plant.

8. Let the plant grow for 2-3 days.

9. Fix the emasculated inflorescence again under the binocular. The stigmata have by now developed a rough, sticky surface. If you have missed an anther the other day, you will see pollen and swelling of the gynoecium. Such a flower needs to be removed.

10. Take an open, mature flower (but not yet yellow) from the father plant with one pair of forceps and bring it under the binocular. With the other forceps, take hold of the filament of an anther with visible pollen shedding. Tap the anther on the stigma and cover it with pollen grains as much as possible. Repeat for all stigmata. Take a second male flower if necessary.

11. Mark the pollinated inflorescence with a colored thread and document the cross (mother, father, date, color code, number of pollinated flowers).

12. Depending on growth conditions, siliques with the hybrid seeds will be mature after 15-25 days. They are harvested by cutting them into a paper bag when the siliques are a little yellow, but prior to opening. They should be kept for a couple of days at room temperature for further maturation. Some ecotypes need a cold treatment (4°C for 7 days) for optimal germination.

continued
Further remarks:
Arabidopsis is very efficient in self-pollination, therefore, care has to be taken to remove anthers in time. Also, cleaning of the forceps between different crosses is essential. Accidentally damaged or removed petals or sepals are no tragedy, but in principal it is better to keep them as during the interval between emasculation and pollination they protect the stigma against unwanted pollen grains. The delicate tips of the forceps can be protected during work by inserting them in a tube filled with EtOH and with a bottom of crepe rubber. If the tips are damaged they can be corrected with the help of a grindstone or sandpaper.