

Congo red staining of pollen tube growth in Arabidopsis pistils:

(Originally developed by Dr. Anna Edlund in Daphne Preuss lab and later modified by Dr. Tatsuya Tsukamoto in Ravi Palanivelu lab).

(For images taken using this protocol, see references: Palanivelu et al (2003), Cell: 114:47-591 and Kasahara et al (2005), Plant Cell, 17: 2981-2992).

1. Prepare the slide first in which pistils will be mounted. Apply vacuum grease and make a hollow square, with each side about ~10mm or twice the length of a pollinated Arabidopsis pistil. Do not complete the square and leave a small gap on the top right hand corner. (This small gap allows trapped air bubbles to escape at the time of applying the cover slip on the pistil). Apply 0.1 % congo red solution both within and outside the square. (Sigma, Catlog #: C6277-25G; Prepare solution in water; 1% stock solution remains usable for a long time even when stored at room temperature).
2. Locate a stage 15/16 flower (as per Christensen et al 1997, Sexual Plant Reproduction 10: 49-64). The right stage flower is usually not more than a day older than the flower that is undergoing anthesis.
3. Remove the flower from the plant and do not forget to retain as much stalk as possible. Remove all other flower parts except the pistil. Place the pistil on a double-sided sticky tape (Scotch, 3M). If fresh, the tape has too much adhesive; in such instances, gently rub the tape with hand before placing the pistil. Place the pistil in such a way that the transmitting tract is in the middle and the two chambers are on the side.
4. Using a 27.5" gauge needle, cut a slit, perpendicular to the orientation of the pistil on the tape, on the top and bottom of both ovary chambers (total = 4 cuts).
5. Starting from the top slit, reach the bottom slit, by making a slit, on the ovary on each side of the replum (ie. parallel to the transmitting tract).
6. Once cut, flip the peeled ovary wall over and make them stick to the tape. This exposes the ovules within each chamber. Then, very carefully, slide the needle under the ovules (without disrupting them!) and reach the ovary wall that is stuck on the tape. Make a slit as described in 5, starting from the top and reaching the bottom. Repeat these two actions on the second ovary chamber.
7. Use a forceps to hold the stalk and lift the pistil off the tape. Place the pistil in the congo red solution taking care to do the following:
 - a.) place the pistil so that one ovary is facing up and the second one is below (ie. this position is similar to the one you get when you rotate the pistil 90° from where the pistil is in 3).
 - b.) anchor the pistil on the grease in such a way that much of the stalk and may be even a row or two of ovules are pushed well into the grease.

8. Ensure the pistil is submerged in the congo red solution. Lay the cover slip over the pistil, gently push the cover slip against the pistil (but not too much!) taking care not to disturb the orientation of the pistil. If the cover slip is hurriedly laid, the pistil tends to rotate and you do not get good images. In fact, both chambers were exposed in 6, so that the pistil movement and rotation are kept to a minimum when mounting.

9. After mounting, place the slides in a humid chamber (a box with wet kimwipes) and the slides can be imaged after 1 hour.

10. Observed the pistils in a confocal microscope using excitation wavelength (543 nm) and observe after allowing the emitted light to pass through red filters (633nm).