This protocol was kindly provided to me by Toshiyuki Mori and please quote his paper as a reference if you want cite this procedure in your paper:

**Mori T., Kuroiwa H., Higashiyama, T., and Kuroiwa T. (2006). Generative Cell Specific 1 is essential for angiosperm fertilization.** ***Nature Cell Biology*** **8(1): 64-71.**

# Anilin blue staining of pollen tube in the Arabidopsis pistil

## Recipe

Fixative

Acetic acid/EtOH (1:3) solution

EtOH series

70%, 50% and 30% EtOH

Alkaline treatment solution (ATS)

8 M NaOH

Decolorized(*) aniline blue solution (DABS)

0.1% (w/v) aniline blue in 108 mM K3PO4 (pH ~11)

* Decolorization

After preparation of the solution above, store it in the fridge at 4C overnight. Prepare a funnel with filter paper and add a teaspoonful active carbon powder, then filter the solution through the powder on the following day. Add glycerol to the filtrate so that its final concentration becomes 2% (v/v). Store it in the fridge at 4C.

## Procedures

1. Fixation

   Collect Arabidopsis pistils from one- or two-day-old flowers after flowering and put them in a plastic tube of the fixative. Aspirate the air in the capped tube using a 50 ml-syringe with 18 gauge until the specimen does not release any bubbles. Leave the tube for at least 2 hr at room temperature (RT).

2. Rehydration

   Exchange the fixative to 70% EtOH and leave for 10 min at RT. After that, do the same treatment using 50, 30% EtOH and DW.

3. Alkaline treatment

   Move the specimen into the small petri dish of ATS carefully. Leave the dish with its lid overnight at RT.

4. Washing

   Exchange ATS to DW carefully because each pistil must be very softened. At this time, you can observe the specimen clearing. Leave the dish for 10 min at RT.

5. DABS staining

   Exchange DW to DABS carefully and leave for at least 2 hr under dark condition using a piece of

aluminum foil at RT. You do not have to wash the specimen after this treatment.

6. Observation

Put each pistil with extra DABS on the slide glass, and then put a cover slip on it carefully from the end of pistil with avoiding bubble contamination. At this time, you can see ovary walls are split by the weight of cover slip. After that, you only have to observe pistils with a microscope under UV irradiation condition. If you would not like to disperse pollen tubes so randomly, you had better increase the glycerol concentration in DABS.