## Whole Mount 3D DNA FisH

## Protocols are adapted from:

**Whole mount:**

* **Bauwens, S., Katsanis, K., Van Montagu, M., Van Oostveldt, P., and Engler, G. (1994).** Procedure for whole mount fluorescence in situ hybridization of interphase nuclei on Arabidopsis thaliana. The Plant Journal 6, 123–131.
* **Till David Bey, Maria Koini, and Paul Fransz (2018).** Fluorescence In Situ Hybridization (FISH) and Immunolabeling on 3D Preserved Nuclei. Methods Mol Biol.1675: 467- 480
* **Fransz, P., de Jong, J.H., Lysak, M., Castiglione, M.R., Schubert, I., (2002).** Interphase chromosomes in Arabidopsis are organized as well defined chromocenters from which euchromatin loops emanate. Proceedings of the National Academy of Sciences 99, 14584 –14589.
* **Poulet A, Duc C, Voisin M, Desset S, Tutois S, Vanrobays E, Benoit M, Evans DE, Probst AV, Tatout C. (2017).** The LINC complex contributes to heterochromatin organisation and transcriptional gene silencing in plants. J Cell Sci. Feb 1;130 (3):590-601

## Dissection, fixation & dehydration

Material:

* Ethanol, methanol
* Fixation butter (provided): formaldehyde (1%), DMSO (10%) in PBS-EGTA 1x
* Plants grown 9 days at 23°C in Sanyo on ATG medium.
* Basket tubes: cut-off eppendorf tube and sealed with mesh filter - Sefar Nitex 03-50 / 37
* One 24 well box
* forceps, micro- scissors,
* Vacuum chamber, binocular microscope

Step procedures:

1. Distribute 6 cotyledons per basket tube and place these tubes in a 24 well box with water.

2. Add 1 ml of fixation buffer in each basket. Check that cotyledons are covered with fixation buffer and can move, not stuck to the wall. Fix specimens for 25 min at RT and 5 min under vacuum.

3. Remove the fixative and rinse 2x 1 ml methanol, 5-10 min each time, and then 2x 1 ml ethanol then store at 4 °C o/n. You can leave them a few days at 4°C before to continue.

## Rehydration, pre-hybridization

Material:

* 1x PBT (1xPBS, tween20 0.1%)
* 20xSSC
* HB50 provided (50% desionised formamide, 50 mM Sodium Phosphate pH7.0, 2xSSC)
* 24 wells box and fixed plants in basket.
* Needles, forceps, binocular microscope
* Ø60 mm Petri dish
* Probe solution: LNA-180bp-TYE563 1µM in HB50 (provided)
* 37°C chamber

Step procedures:

1. Don’t forget to finish step 3 of previous session.
2. Replace ethanol by 1 mL of 1xPBT 3x 5min.
3. Replace 1xPBT by 1 mL of 2xSSC 3x 5 min.
4. Replace by 1mL of 2xSSC 2x 5 min.
5. Replace by 1 mL of 2xSSC:HB50 30 min.
6. Replace by 1 mL of HB50. Incubate 30 min at RT.
7. Transfer the specimen to a Ø60 mm Petri dish. With 2 needles or a fine forceps, through a binocular microscope, put 1 to 2 pair of cotyledons without liquid (you can gently absorbe liquid on absorbent paper like 3M Whatmann) and transfer to a 1.5 ml microtube containing 30 µL of probe mix.
8. Cover all cotyledons and incubate for at least 1 hr at 37°C.

## Denaturation, hybridization

Material:

* Thermomixer at 90°C
* Ice
* 37°C chamber

Step procedures:

1. Place tubes containing probe and cotyledons at 90°C for 4 min in the thermomixer.
2. Place the tube on ice for 3 min.
3. Hybridize o/n at 37°C.

## Washing, mounting

Material:

• SF50 provided: formamide: 4xSSC 1:1 (v/v)

• 2xSSC

• 1xPBS

• DAPI 0.1 mg/ml

• Glycerol: 1xPBS 80:20 (v/v) provided

• Slides and 24x32 **1.5** coverslip

• Needles, forceps

• Ø60 mm dish

• Picodent or varnish

Step procedures:

1. Remove the hybridization solution and wash the cotyledons with 2xSSC.

2. Stringent wash with SF50 1/2 hr at 42°C 2x.

3. Wash the cotyledons in 2xSSC at RT.

4. Wash in 1x PBS for 10 min.

5. Replace by 100 μl DAPI 1/100 (1 μg/ml final) in 1xPBS 15 min at RT (or o/n at 4°C).

6. Wash 3x in 1 ml 1xPBS.

7. Transfer cotyledons to small dish. Remove PBS (you can adsorb it on absorbent paper).

8. Cover each pair of cotyledons with 50 μl of PBS: glycerol (20:80) to equilibrate.

9. Transfer separate cotyledons on a coverslip (with help of a binocular microscope). Be careful not to wet the other side of coverslip. Cover them with 30 µL of PBS: glycerol (20:80).

10. Cover with a slide, let the liquid spread out and then turn over the slide: the coverslip with the cotyledons are transferred.

11. Leave the slides flat and protected from light.

12. Possibly complete the liquid between slide and coverslip before putting varnish or Picodent.