SHORT TERM SCIENTIFIC MISSION (STSM) ï SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: CA16212
STSM title: 3D FiSH in plants with DNA probes
STSM start and end date: 17/04/2018 to 24/04/2018
Grantee name: Sophie Desset
Collaboration with: Stefanie Rosa

PURPOSE OF THE STSM/

(max.500 words)

The objectives of this project were the following::

- Adapt Single Molecule RNA FiSH protocol for detection of DNA targets with plants and probes from Stefanie Rosaôs Research model
- Discuss about the protocol to adapt this technique to Sophie Dessetôs research model: How to design probe, to adapt to DNA detection, potential trouble shooting.
- Decide the material organization, agenda and pedagogic aims of COST Training school according to the other 3D FISH techniques (whole mount with LNA probes and whole mounting acrylamide gel): which material (plant, probes, microscope,..), which steps to be improved by applicants and what are the expected results.

This STSM project will therefore support the INDEPTH COST-Action in achieving the specific objectives listed below:

- Objective 2: Generate standardized protocols in 3D imaging of the nucleus (WG1),
- Objective 3: Collect plant data sets in 3D imaging of the nucleus (WG1),
- Objective 11: Foster interdisciplinary training and career development for Early Career Investigators (ECIs) through their involvement in the organization of project activities and their anticipation in training events (TS and STSMs).

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Stefanie Rosa and Sophie Desset implemented 5 sets of experiments with wild-type Arabidopsis plants and a probe mix from Stefanie Rosaôs lab to detect PP2A nucleic targets:

1. A set of RNA smFiSH to detect PP2A transcripts in root squash cells.
2. A set of RNA smFiSH to detect PP2A transcripts in whole mount seedlings.
3. Two sets of DNA FiSH to detect PP2A gene in root squash cells using different hybridization

conditions (2 different buffers and 2 different denaturing conditions).
4. A set of DNA FiSH to detect PP2A gene in whole mount seedlings.

Training School :

- A set of root squash cells were prepared to test the protocol in Clermont-Ferrand and to test the imaging of FiSH by the different GReD labôs microscopes for the training school.
- Discussions for the organization and preparation of a potential time table.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

1. RNA smFiSH technique was successfully realised. We tested the new Stellaris buffers (Washing Buffer A and B, Hybridization buffer): the background is more homogenous but the signal is the same.
2. Whole mount RNA smFish did not work in green tissues. In roots, the cell density is too high, introducing background that slightly masks the signals. However, signals from transcripts were clearly visible especially at the epidermal layers.
3. The first set of DNA FiSH was tested in 10% of formamide and 50%, with or without an RNAse treatment. We observed RNA spots only in 10% of formamide without RNAse. The denaturation (85°C 1ô) maybe affects the RNA because the signal is weaker when compared with RNA FISH protocol. However, the denaturation conditions applied were not sufficient to open the DNA because no signals were detected on the RNAse treated samples. In the second set we used 50% formamide for hybridization, like in LNA FiSH experiments, and we changed the denaturation conditions (90°C 3ô). This last experiment was successful (spots were detected) but the obtained signals are very weak. This DNA FiSH detection is promising but needs to be optimised.
4. The whole mount DNA FiSH was performed like the LNA FiSH experiments in Sophie Dessetôs lab, with a denaturation step at 90°C 4ôand 50% formamide for hybridization. No signal was observed in cotyledons. In roots, we observed a DNA staining like an intercalant staining and in a small part of the root, some nuclei with only 2-6 weak spots. The washing steps were maybe not enough stringent and homogenous. We will have to perform some controls, such as a sample without probe, to conclude that this spots are the DNA PP2A loci and to find the right wash conditions.

FUTURE COLLABORATIONS (if applicable)

(max.500 words)

A training school will be organised by the Sophie Dessetôs lab in Clermont-Ferrand with Stefanie Rosa in october. A first draft of the Training school has been written but will have to be discussed with Dr Hank Bass another trainer involved in the training school.