Probing Arabidopsis protein array for protein-protein interactions

1. Prepare the following buffers fresh prior to use, mix well (do not vortex) and store on ice until use. Amount usage per slide: 30 mL PBST blocking buffer, 100 mL probing buffer. Stocking solution: 30% BSA (stored in -20 ^{o}C), 1 M DTT, 1 M $MgCl_2$.

   PBST Blocking Buffer
   1X PBS
   1% BSA
   0.1% Tween 20

   Probing Buffer
   1X PBS
   5 mM MgCl2
   0.05% Triton X-100
   5% Glycerol
   1% BSA

2. Take out the Arabidopsis protein array slide(s) from -80 ^{o}C, put the slide into a dry 50 mL Corning tube immediately, close the cap tight, and leave in 4 ^{o}C cold room for 15 min. This step will equilibrate the slides at 4 ^{o}C while preventing any moist condensation on the slide surface.

3. **Blocking**: Pour 30 mL blocking buffer into a 50 mL Corning tube, and quickly place the slide inside, close the cap, and place the tube on a rotator in cold room, with the spotting surface of the slide face up. Rotate at 30 rpm for 1 hour. Use 1 tube for every slide.

4. **Probing**: Take out the slide with forceps, drain it by touching the edge of the slide with a Kimwipe, put down the slide on a level flat surface in cold room, with the protein spot side facing up. Pipette the probing protein with V5 tag with ~100 μl of probing buffer onto the surface, cover with a Lifterslip cover slip (Thermo Scientific, 25X60I-2-4789). To maintain high humidity during the probing process, the flat surface with the slides can be put in a tray with cover, with a small amount of water at the bottom. The surface has to stay above the water. Incubate for 1.5 hour.

5. **Wash:** Pour ~10 mL cold probing buffer into one of the wells in a 4-well rectangular dish (nunc 267061). Place the slide in the well, submerged in the solution. Remove the Lifterslip, leave for 1 min. Repeat once by moving the slide to another well with solution for 1 min.

6. **Antibody recognition:** Pick up the slide with forceps, drain the slide again by touching the edge with a Kimwipe tissue. Similar to the probing step, apply ~150 μL Cy3-anti-V5 antibody onto the surface (1:700 in probing buffer, Sigma V4014), cover with Lifterslip, place in cold room for 30 min, avoid light as much as possible.

7. **Wash:** same as step 5, wash twice.

8. **Drying:** Drain the slide, put it into an empty 50 mL corning tube (with a Kimwipe tissue at the bottom of the tube), spin 3 min at 700 rpm, in a table top centrifuge.

9. Scan slide with a GenePix 4000B scanner, at the 532 channel. PMT Gain 650.