

## Protocol

### Cell painting protocol using PhenoVue Kit on Droplet Microarray

Materials and reagents:

DMA with 672 spots (catalogue number G-np-102 available at Aquarray)

Humidifying pad (catalogue number AQP-0003 available at Aquarray)

I-DOT PURE L Wells 100 (catalogue number D16110021817 available at Dispensix)

PhenoVue Kit (catalogue number PING11 available at Perkin Elmer)

SecureSeal™ Hybridization Chamber (21,5x71,5x0,8 mm; catalogue number 621507 available at Grace Bio-Labs)

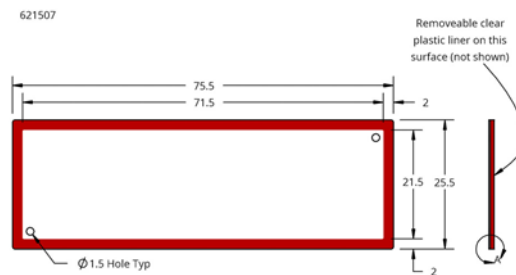
Petri dish

Perkin Elmer's PhenoVue protocol has been adapted from Bray et al.\*

Reconstitution of reagents was conducted according to the manufacturer's instructions. To prepare 3mL of staining solution 2,4 mL ddH<sub>2</sub>O and 0,6 mL PhenoVueDye Diluent A (5x) were mixed.

Preparation of:

- 1) *Staining Solution I*: 2.25 µL of PhenoVue 641 Mitochondrial stain (3x) were added to 1.5 mL PhenoVueDye Diluent A (1x).
  - 2) *Staining Solution II*: 15 µL Triton X-100, 15 µL PhenoVue Fluor55-WGA, 3.75µL PhenoVue Fluor488-Concavalin A, 1.88 µL PhenoVue Fluor 568 Phalloidin, 1.5 µL PhenoVue Hoechst 3342 Nuclear Stain and 1.8 µL of PhenoVue Fluor 512 Nucleic Acid Stain were added to 1.475 mL of PhenoVueDye Diluent A (1x).
1. Dispense 50 nL of staining solution 1 per spot on a Droplet Microarray with 672 spots of 1 mm size containing cells with a limited dosing energy of 75 mbar/ms.
  2. Transfer into a humidity chamber and incubate at 37 °C, 5% CO<sub>2</sub> for 30 min.
  3. Incubate DMA in a 50 mL tube containing 4% Formalin at RT for 20 min in the dark.
  4. Wash DMA two times with 1x HBSS in a 50 mL tube.
  5. Discard HBSS.
  6. Dispense 150 nL per spot with a limited dosing energy of 75 mbar/ms.
  7. Wash DMA three times with 1x HBSS in a 50 mL tube.
  8. Embed
    - a. in Mowiol using a coverslip.
    - b. or remove excess HBSS and add a SecureSeal chamber ("sticky chamber").



(image taken from Grace Biolabs)

### Preparation of “sticky chamber”

1. Cut two small squares of a transparent adhesive tape to close the two holes ( $\varnothing$  1,5mm) on the left lower and right upper part.
2. Prepare a sticky chamber by removing the seal and place on the non-sticking side.
3. Remove the DMA carefully from the humidifying chamber and put the sticky chamber quickly on the DMA to avoid drying of the cells/droplets.
4. Ensure that the sticky chamber is not out of the slide and well fixed on the DMA, otherwise you cannot align it for imaging.
5. Transfer to the microscope for imaging.