**FISH Procedure**

**Fixation**

Prepared by Jason He/Lars Angenent

1. Centrifuge 2 ml sample at 12,205 x g for 5 minutes, then decant the supernatant
2. Add 2 ml of 4% ice-cold paraformaldehyde (PFA) fixative and hold the sample at 4 ºC for 30 minutes up to 12 hours.
3. Centrifuge and remove paraformaldehyde
4. Add 1 ml PBS, and mix or vortex until pellet is released.
5. Centrifuge the sample and remove PBS
6. *This wash process can be repeated for several times if necessary*
7. Add 1 ml PBS and 1 ml ice-cold 100% ethanol, and mix or vortex.
8. Store the sample at –20 ºC.

**Hybridization**

1. Take the fixed samples and mix or vortex.
2. Transfer 2 ml of sample to slide (in circle well area), spread the sample in the well, and write down the order and number of wells in your lab journal. Write in **pencil!!!** on slide the date and sample identification.
3. Dry the slide in microbial hood for about 5 minutes.
4. Dehydrate the slide in 50%, 80%, and 100% of ethanol for 3 min respectively.
5. Dry the slide in microbial hood for about 5 minutes
6. Preparation of hybridization buffer in a 2-ml microcentrifuge tube:
   - 360 µl of 5M NaCl
   - 40 µl of Tris-HCl, pH7
   - 400 µl of formamide (20%)
   - 1198 µl of deionized water
   - 2 µl of 10% SDS or 1 µl of 20% SDS
7. Add 8 µl of hybridization buffer to a centrifuge vial and add 1 µl of each fluorescently labeled probe (50 ng/µl).
8. Place the folded paper towel into a 50-ml polypropylene tube (hybridization chamber) and pour remaining hybridization buffer onto the paper towel
9. Apply the hybridization buffer plus probes to the well of the slide, spread the buffer out without touching the glass surface, and place the slide into the 50-ml tube. Cap it and place horizontally into hybridization oven at 46 ºC for 1-2 h.
10. Preparation of wash buffer in 50-ml tube:
    - 2150 µl of 5M NaCl (20% formamide)
    - 1 ml of 1M Tris-HCl, pH7
    - 500 µl of 0.5 M EDTA
    - Fill deionized water up to 50 ml
    - 50 µl of 10% SDS
    - Warm the tube in 48 ºC in a water bath for 1-2 h.
11. Rinse both sides of slide with warm wash buffer, and then place the slide into the pre-heated wash buffer tube (48 ºC) for 15 minutes. After washing, put the slide into distilled water: wash the slide and dry it with air in the microbial hood.
12. Add one drop of DAPI on each well, and hold for 2-5 minutes. Rinse it with ice-cold water and rapidly dry the slide with air.
13. Add 3 drops of cityflour on well, and then place a glass cover (avoid any bubbles).