PNA-FISH and Life/Dead Staining

By Kasper Nørskov Kragh, University of Copenhagen

PNA-FISH

Deparafination for paraffin embedded formalin fixated specimens
1. 2 x 5 min in Xylene
2. 2 x 3 min in 99.9% EtOH
3. 2 x 3 min in 96.5% EtOH
4. 3 x 3 min in MiliQ

OR

Fixation for experimental cultures
1. Add a drop of fixation liquid (approx. 20 μl) and mix with 10-20 μl of culture
2. Incubate at 65°C for 20 minutes, may vary based on material, until hardened
3. Slide is ready for PNA-FISH protocol

PNA-FISH protocol
1. Turn on workstation (55°C)
2. On the fixed sample squeeze one drop of PNA-FISH probe onto the area of interest and add a small coverslip (22 μm x 22 μm)
3. Incubate the slide on the workstation for 90 minutes (at 55°C)
4. Turn on water bath and set to 55°C
5. Dilute 4 ml 60x wash buffer to 240ml miliQ and preheat in glass tub
6. Once the 90 minutes are up, gently remove the coverslip, and incubate for 30 minutes in the wash buffer
7. Air-dried the samples
8. Cover the whole specimen with 3mM DAPI solution
9. Incubate for 15 min at RT in the dark
10. Gently wash with PBS by dripping 1-2 ml over the specimen
11. Air-dry in the dark
12. Add a drop of ProlongeGold to sample and press out any bobbles add coverslip, and sealed with nail polish.
13. When dry, the slides can be imaged or stored at 5°C almost indefinitely
**Life/Dead staining**

**Stock solutions**
1. Propidium Iodine (PI) should be solute in miliQ water in a ratio of 100 ug of PI powder per 1ml miliQ
2. The SYTO9 are supplied in stock concentration at purchase

**Staining**
Staining must be done before any fixation!

3. In liquid cultures (treated or non-treated) add 1 μl of both PI and SYTO9 stock solution per 1 ml of culture
4. Incubate for 15 min at RT in the dark
5. Small volumes (approx. 10-20 μl) of the stained cultures can now be dropped onto an objective glass and covered by a coverglass and imaged

**Life/dead of surface attached biofilms**
6. For life/dead imaging off plate colonies, microtiter wells or any other attached biofilm a staining solution are needed.
7. The same 1:1000 of the two reagents can be used. Dilute PI stock and SYTO9 stock 1:1000 in the same miliQ water
8. The diluted reagents can be dripped onto whatever biofilm needed stained.
9. The specimen need to be completely soaked for 15 min at RT in the dark before imaging