

TSA *IN SITU* HYBRIDISATION

1- FISH general principles

2- Methodology

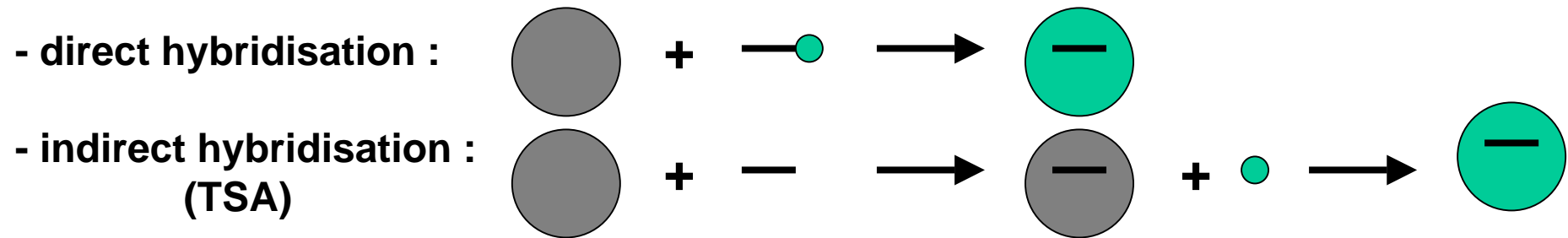
- probe labelling**
- protocol steps**
- practicals (pictures and slide observations)**

3- References

Isabelle Biegala

1- FISH : General principle

FISH = whole cell identification of micro-organisms with the help of fluorescent molecular probes targeting the 16S, the 18S, the 23S or the 28S **ARNr**

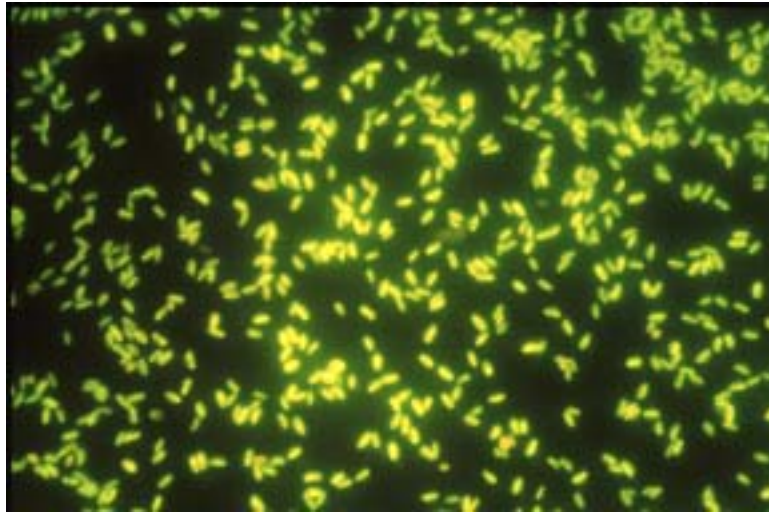


Equipment to visualise fluorescent cells :

- epifluorescent microscopy
- confocal microscopy
- flow cytometry

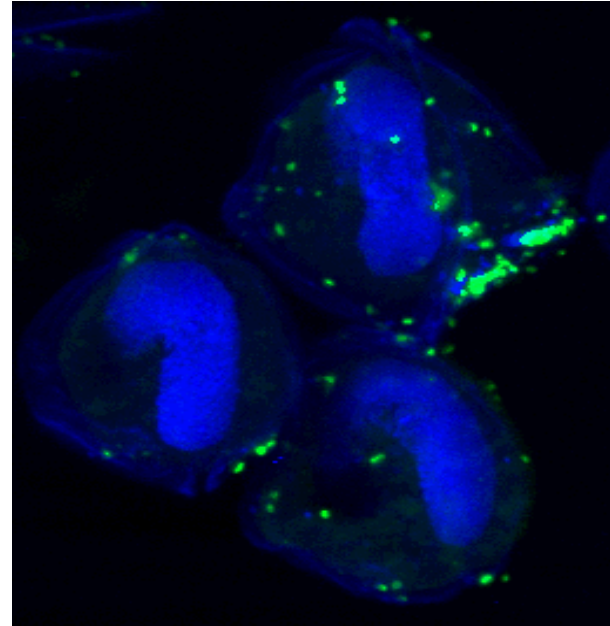
| Dye excitation | | Dye emission |
|----------------|---|----------------|
| UV: 380 nm | → | Bleu : 480 nm |
| Bleu : 488 nm | → | Vert : 550 nm |
| Vert : 568 nm | → | Rouge : 585 nm |

Epifluorescent microscopy



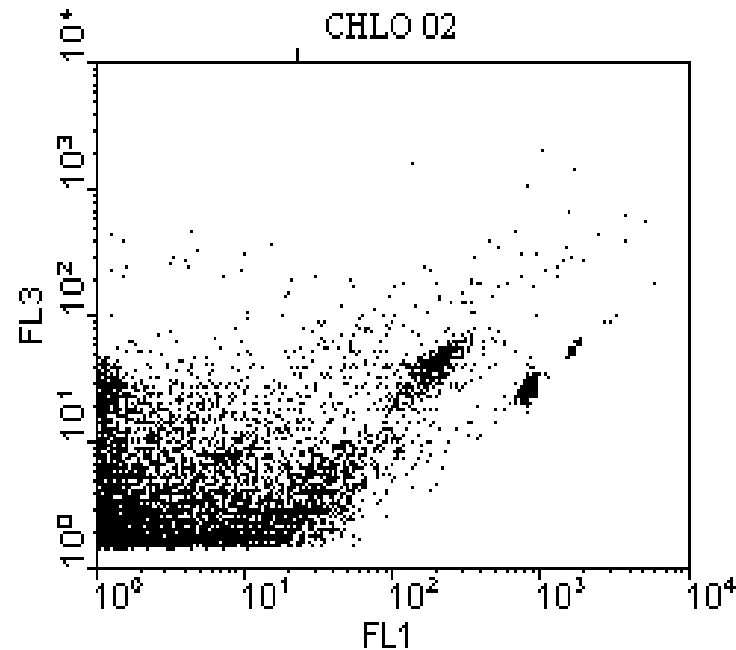
1rst screening / quantification

Confocal microscopy



Visualisation
of cells in 3D/
quantification

Flow cytometry



Quantification made easy

2- FISH : Methodology

FISH : Fluorescent *In Situ* Hybridisation

2.1. Different techniques

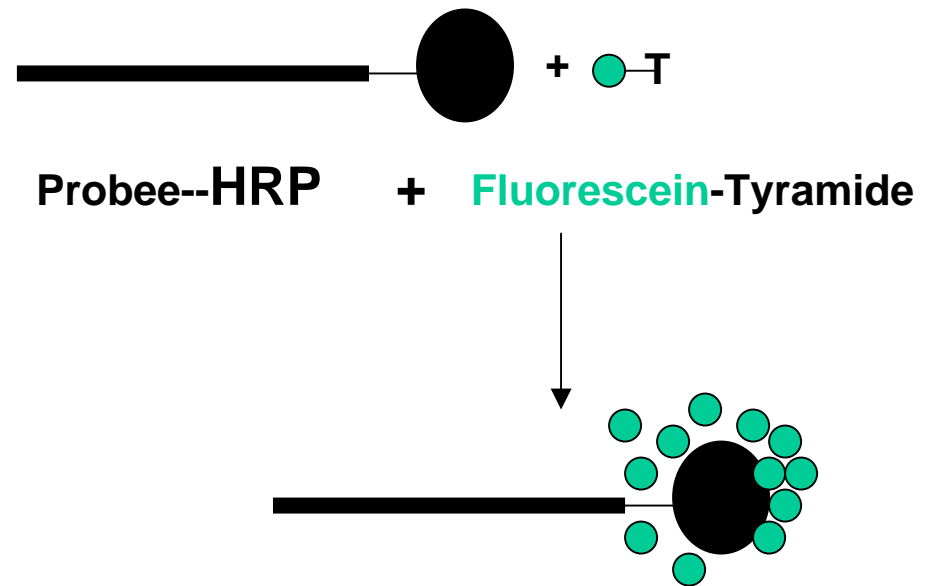
Monolabeled probes

- + quicker
- difficult to observe if important background (organism / environment)

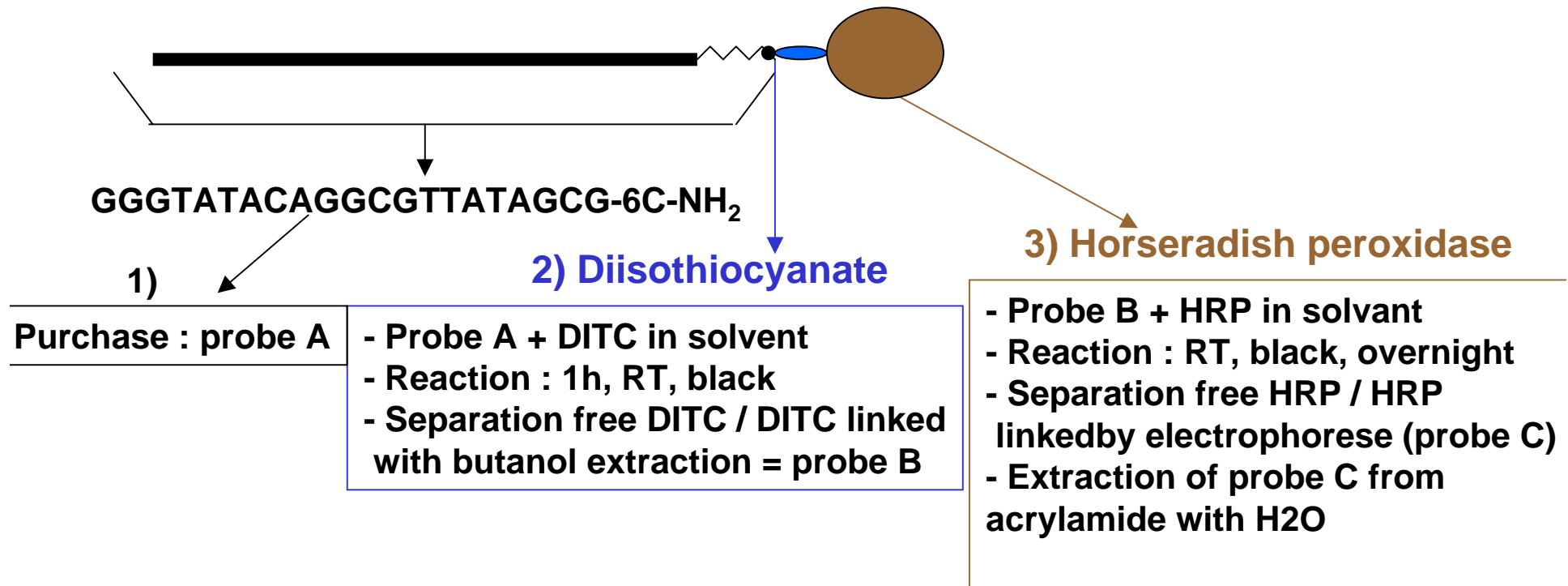


FISH-TSA (Tyramide Signal Amplification)

- longer
- + high signal/background ratio



2.2. HRP labelling of probes



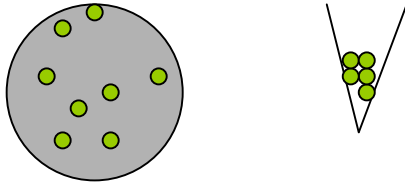
- 4) - Check probe purity and concentration by spectrophotometry
- stock at 50 ng/μl at -80°C

Remarq : 1.5 days of manipulation for 4 probes which last for 3 to 6 months

2.3. FISH-TSA steps

1) Target cells are fixed with PFA

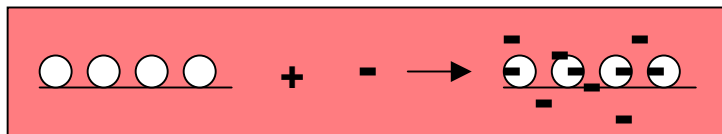
2) Cells are collected



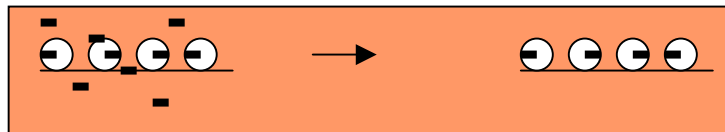
3) Cells are dehydrated with ETOH

4) Cells are stored at - 80 °C

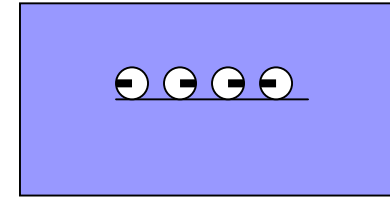
5) Cells are hybridised



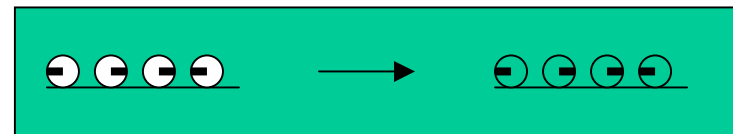
6) Unhybridised probes are washed away



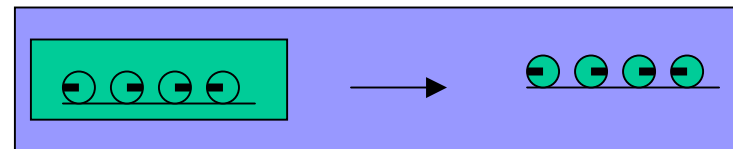
7) Cells are equilibrated in a buffer compatible with the enzymatic reaction (TSA or HRP step)



8) Target cells are stain with fluorochrome = TSA reaction



9) Unfixed tyramide-fluorescein is washed away



10) DNA of Target cells and non target cells is labelled with DAPI or propidium iodide

11) Cells are mounted with antifading

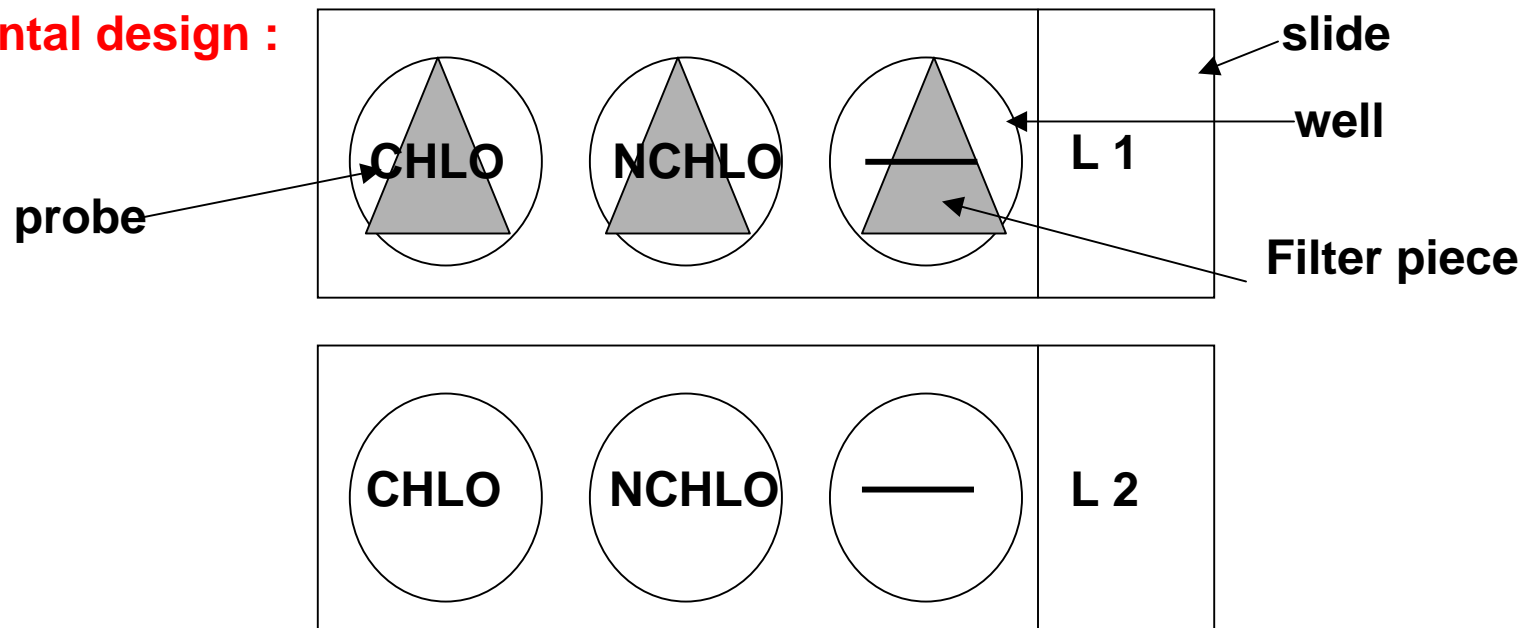
FISH experiment : picoeukaryotes from natural environment

Aims : Quantification of picoeukaryotes belonging to chlorophyta division and non-chlorophyta division with specific probes.

Check the presence of non-specific labelling with a « no probe » control.

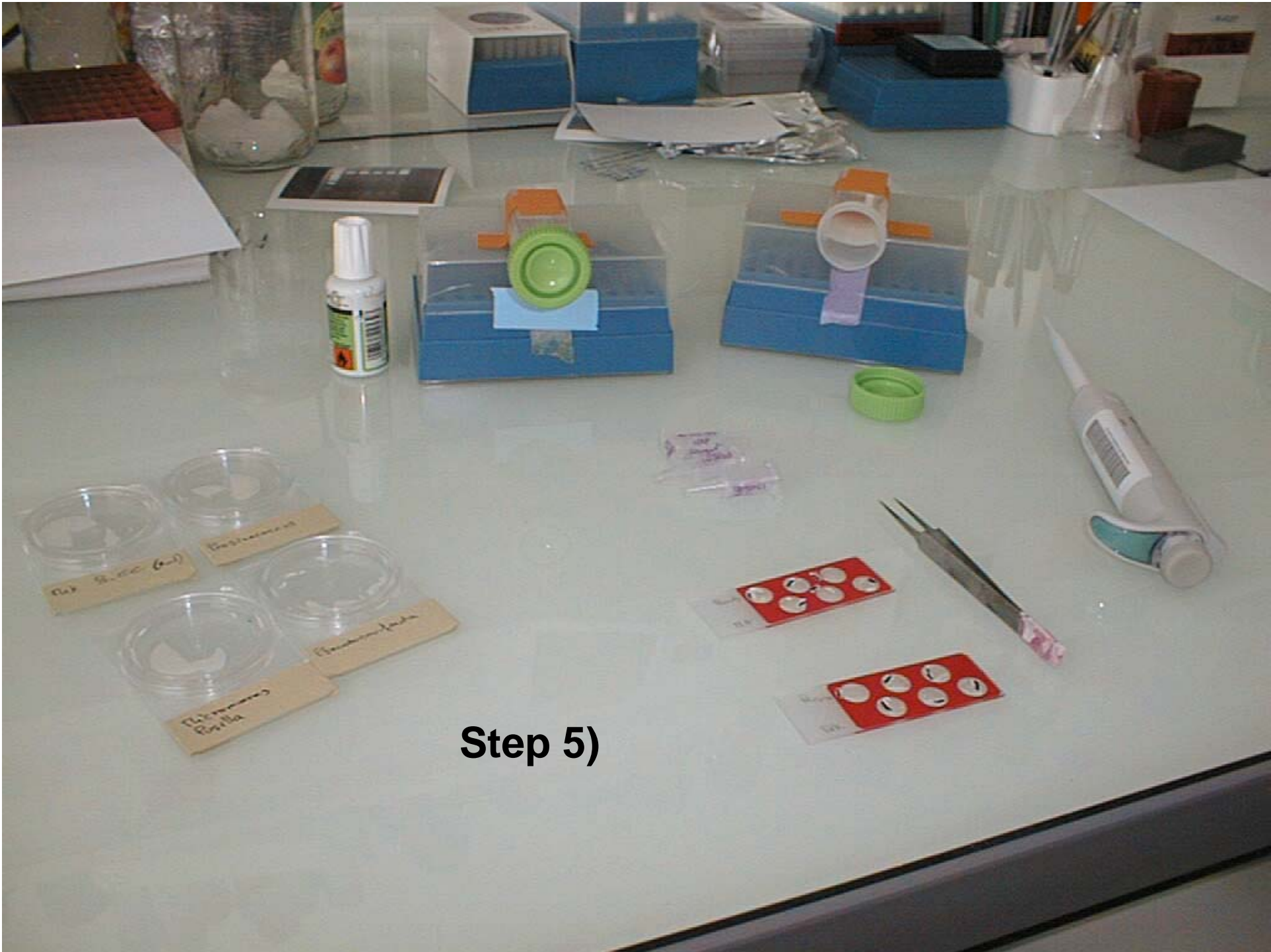
Experiment done on picoeukaryotes collected on filters (L1) or by centrifugation (L2).

Experimental design :



Steps 1) 2) 3)



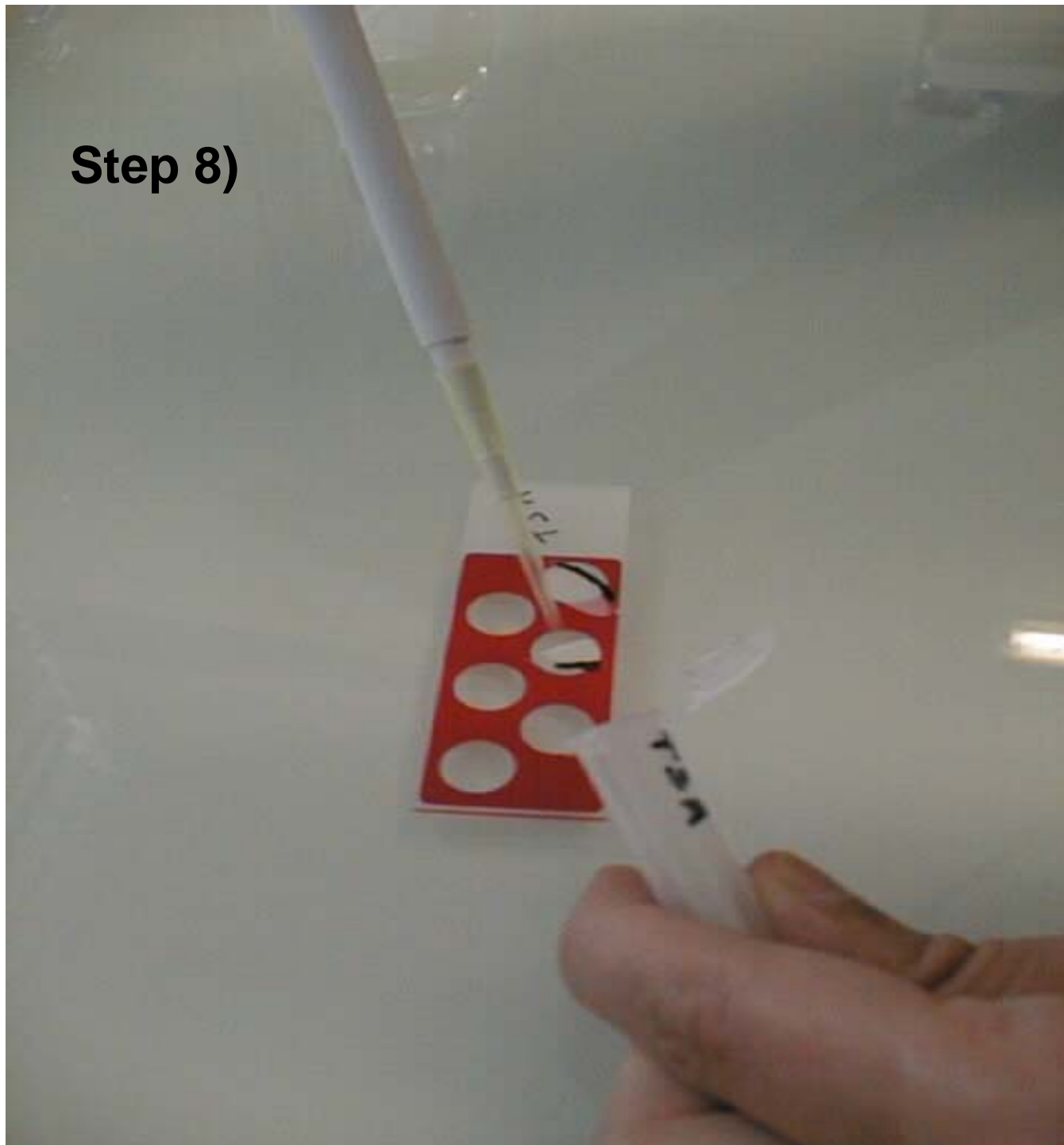


Step 5)

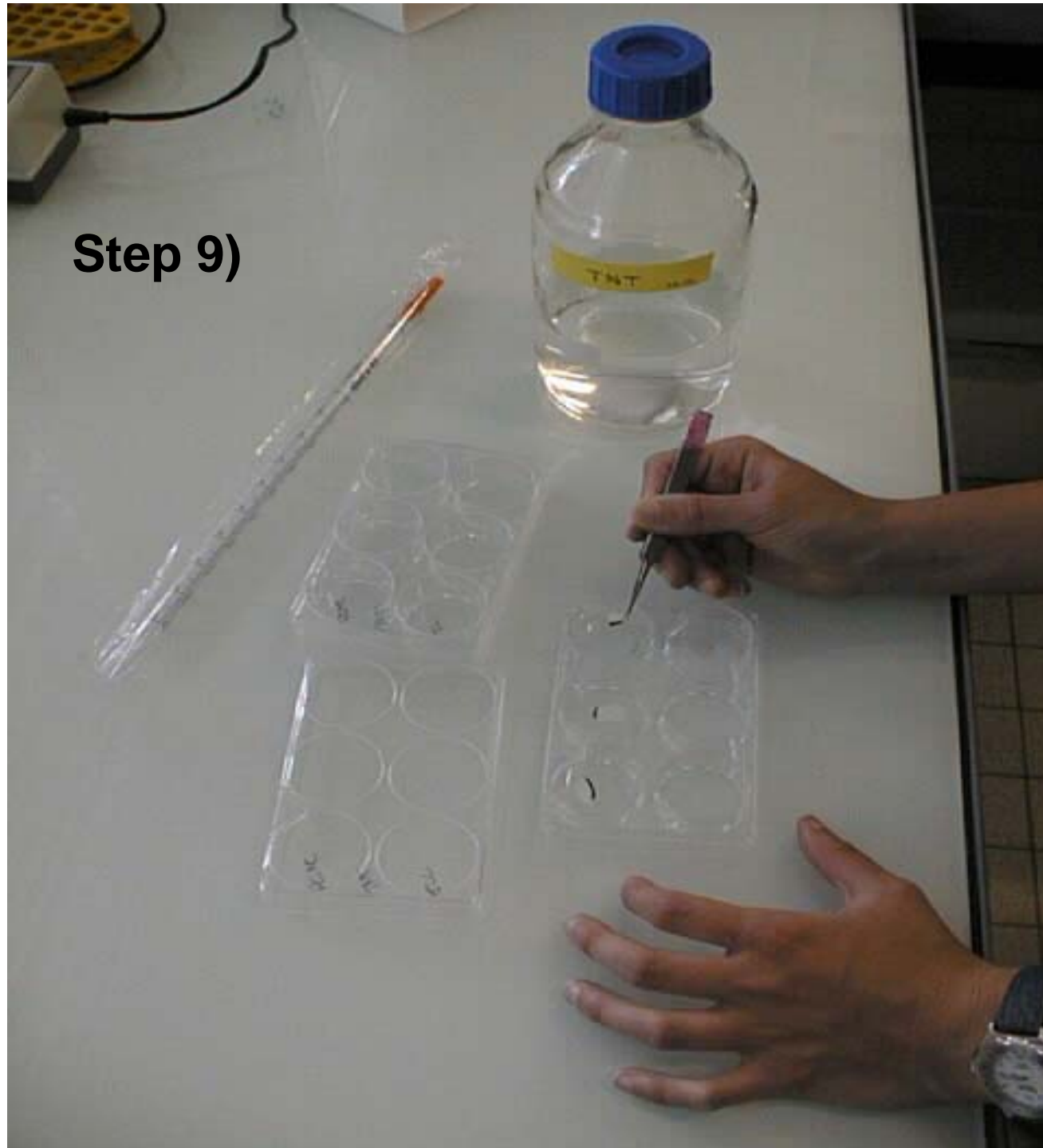
Steps 6) 7)



Step 8)



Step 9)



3- References

Urdea et al. 1988. A comparison of non-radioisotopic hybridization assay methods using fluorescent, chemiluminescent and enzyme labeled synthetic oligodeoxyribonucleotide probes. *Nucleic Acids Res.* 16:4937-4956.

Bobrow et al. 1989. Catalysed reporter deposition, a novel method of signal amplification. Application to immunoassay. *J. Immunol. Methods* 125:279-285.

Amann 1995. *In situ* identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. *Molecular Microbiol Ecology Manual*, 3.3.6 : 1-15.

Schönhuber et al. 1999. In situ identification of Cyanobacteria with horseradish peroxidase-labeled, rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology*, 65 : 1259-1267.

Biegala et al. 2002. Identification of bacteria associated with dinoflagellates (dinophyceae) *Alexandrium* spp. Using tyramide signal amplification-fluorescent *in situ* hybridization and confocal microscopy. *J. Phycol.* 38 : 404-411.