DIRECT STAINING OF CELL SURFACE PROTEIN

Direct staining is faster, than indirect staining, but requires an antibody that is already fluorescently tagged. When you are looking for an antibody to order, check the known companies used by your university, and check if the antibody was tested for the assay you want to do. Also if you have the choice between different antibody, choose the one that was the most used by other scientists. Sometimes important, ask the company if they have the antibody in stock/ how much time the shipping takes.

MATERIALS

- Agar plate of your bacteria of interest, to pick colony
- Agar plate of the bacteria you want to use as a positive control
- Agar plate of the bacteria you want to use as a negative control
- 3 ml LB for overnight culture, and approximatively 5 ml to make the dilution in the morning
- PBS
- Eppendorf tubes
- Fluorescently tagged antibody against your protein of interest
- Microscopy slide and coverslip
- Cuvette to measure the OD

PROCEDURE

□ Dilute the bacteria solution to an OD of approximatively 0.3

With our bacteria, we had to put 1 ml of bacteria solution in 5 ml of fresh LB, but it depends how fast your bacteria grow.

□ Pellet 500 µl of Bacteria grown in LB with an OD of 0.8

We normally used an overnight culture, diluted it in the morning to an OD of 0.3 and then put it back in the incubator for approximatively 1 h to have an OD of 0.8

□ Remove supernatant

□ Resuspend pellet in 200 µl PBS containing 2% BSA

The BSA needs to be added to avoid unspecific binding

□ Add 10 µl of 1/60 dilution of anti-body 1 mg/ml

□ Incubate for 1 h on an Eppendorf rotor *(wrapped in alufoil)*

□ Wash 3 times with PBS

To wash means to pellet the cells with a tabletop centrifuge, remove the supernatant and resuspend the pellet in 200 µl *PBS.*

□ Pipette 2-10 µl on a slide, put a coverslip on top and do microscopy

We advise you to let an advisor handle the microscope, since normally they are much faster in finding the right focus, which is important because the bacteria start dying on the slide.To analyze images use a program as for example "image J" to make merge images