INDIRECT IMMUNOFLUORESCENT STAINING OF CELL SURFACE PROTEIN

Before you consider to start this experiment to characterize a Biobrick, please take the time to think what you can use as a positive control and what you can use as a negative control.

This protocol requires an antibody that is tagged with biotin, so you can use fluorescent avidin to make it visible.

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 biotin (100x dilution of concentration xxy)
- Microscopy slide and coverslip
- Cuvette to measure the OD
- IPTG if your protein is under the control of a lac promoter

PROCEDURE:

□ Inoculate 1 colony in 3 ml LB containing the appropriate antibiotic

Add 1mM IPTG if your protein is under the control of a lac promoter.

□ 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 till 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 the Eppendorf Rotor

□ Wash 3 times with PBS

□ Add 2 µl of Avidin daylight labeled Thermo specific #22845 1 mg/ml

□ Incubate for 1 h on the rotating thing (rapped in aluminium foil)

□ Wash 3 times with PBS

□ Pipett 2 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