

Flow cytometry analysis

1. Make overnight cultures in LB for every strain you want to analyze. Take more than one clone for biological repeats. Don't forget to have controls (i.e. Wildtype and a construct already quantified)!
2. Prepare day cultures in 3 mL LB with 2 μ L from your O/N cultures. Let grow for three hours, at 37 °C, 200 rpm to an OD = 0,1
3. Stain 100-500 μ L of your day cultures by adding 1:100 FM4-64 for 1 h at 37 °C, 200 rpm.
4. Dilute 5 μ L stained culture in 200 μ L PBS
5. Run it at through the FACS

FACS Settings:

Runtime: minutes

Flow rate 10 μ L/minute

Core size 5 μ m

Threshold FSC-H < 11000