Overnight culture with one antibiotic

DO NOT FORGET to perform a glycerol stock from the performed overnight culture! Se bottom of the page for instructions

Procedure:

The amount of LB should be “6X+2” ml, where X is the number of the samples with the given antibiotic resistance, and 1 ml for the negative control

- Label “6X+2” number of 50 ml Falcon tubes with the proper sample name, clone number and negative control
- Put “6X+2” ml of LB into the tube of negative control
- Add antibiotic into the LB of the negative control
    - Since we have the stock with the right, add “6X+2” µl of antibiotic will be good
    - Mix the antibiotic with LB by inverting the Falcon tube several times
- Take up 1 ml of LB with antibiotic into each sample tube
- Pick one colony of each sample clone with pipette tip, and transfer into the LB solution
- Make sure everything is sterile and CHANGE pipette tip between sample. You can leave the pipette tip in the O/N culture.
- Grow the samples and the negative control overnight in 37°C in a shaker

Expected result: no growth in negative control, full growth in all the sample tubes

Repeat the process for every other antibiotic needed.

Glycerol stock

Take the overnight culture from the previous description, transfer 600 µl of culture sample into 2 ml tubes. Add 400 µl of 50% glycerol to obtain a final volume of 1 ml and 20% glycerol concentration.

Store the labeled 2 ml tubes in -80°C freezer indefinitely.