

MIT OpenCourseWare
<http://ocw.mit.edu>

5.36 Biochemistry Laboratory
Spring 2009

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.

SESSION 1:

Session 1 will begin with a safety lecture and lab check in. During laboratory Sessions 1 and 2 you will express the H396P Abl kinase domain. You will also isolate wild type (wt) Abl plasmid DNA for subsequent mutagenesis.

1) Preparation of LB media (the details in grey may be prepared by your TA)

LB (Luria-Bertani) media must be prepared and sterilized for use in the bacterial growth and protein expression you will carry out in the next few sessions. The media should contain a final concentration of 1% bacto-tryptone, 0.5% yeast extract and 1.0% NaCl in water. Using the premixed powder, add 25 g of LB powder/ L of water to achieve the desired concentrations. Prepare a 500-mL solution of LB in a 1-L flask and a separate 100-mL solution in a 200-mL container. For sterilization, cover the containers with aluminum foil and make sure the cap is not screwed onto the 200-mL container. Stick a piece of autoclave indicator tape on the side of each flask. The tape will turn color upon reaching a sufficient sterilization temperature. Your TA will autoclave the media for 20 min. Once the sterilized LB media has cooled enough for you to hold the flask comfortably, use sterile technique to add kanamycin (kan) and streptomycin (strep) (antibiotics) to a final concentration of 50 µg/mL each to the 500-mL solution. The TA's will provide you with a 50 mg/ mL stock of strep and a 50 mg/ mL stock of kan, so you should add 500 µL of strep and 500 µL of kan to the 500 mL of LB. Do not add antibiotics to the 200-mL bottle, and screw the cap on tightly once the solution has cooled completely.

Using sterile technique demonstrated by your TA, transfer two 6-mL and one 10-mL aliquots of LB from the 200-mL bottle into three cell culture tubes. Tomorrow you will check to confirm that your tubes have remained clear, indicating that you have used good sterile technique. If your media becomes cloudy (other than after intentional inoculation), it means the solution has been contaminated, and you must prepare fresh media.

SESSION 1B (lab open 1-2 pm)

1) Inoculation of LB/ kan with *E. coli* containing a plasmid encoding the wt Abl kinase domain

Confirm that your LB media is contamination-free by checking that the solutions are clear. Add 6 µL of the **kan** 50 mg/mL stock solution to each of the culture tubes containing 6 mL of LB to give a final concentration of 50 µg/mL kan. Using a sterile pipette tip, transfer half of a colony from a freshly-transformed bacterial plate (supplied by your TA) to a culture tube. You should drop the entire pipette tip into the solution. With a second pipette tip, transfer the other half of the colony to the second tube. The colonies on the plate are DH5- α *E. coli* cells, which are ideal bacterial storage cells to hold a vector of interest (in this case an Abl(229-511)-encoding plasmid). Place the culture tubes in a 37 °C shaker overnight. In session 2 you will isolate the Abl kinase domain plasmid DNA from the bacteria that grows up overnight.

2) Inoculation of LB/ kan/ strep with *E. coli* containing plasmids encoding the H396P Abl kinase domain and the Yop phosphatase

Add 10 μL of the 50 mg/mL **kan** stock solution and 10 μL of the 50 mg/mL **strep** stock solution to the culture tube containing 10 mL of LB to give a final concentration of 50 $\mu\text{g}/\text{mL}$ of each antibiotic. Using a sterile pipet tip, transfer a colony of H396P Abl(229-511)/ Yop-containing bacteria from a plate (supplied by your TA) to the culture tube. The colonies on the plate are called BL21-DE3 cells, which are a cell-line commonly used for protein expression. Place the culture tube in a 37 °C shaker overnight. In session 2 you will use this “starter culture” to inoculate your 500-mL LB solution to express the H396P Abl(229-511) and Yop proteins.