

### **Preparation of competent cells and DNA transformation**

**Goals-** prepare *E. coli* cells to become competent to take up DNA and transform them with plasmid DNA.

You will be working by yourself for this experiment.

#### **Summary of the experimental protocol**

1. Grow up cells - This will be started by instructor.
2. Pellet cells in preparative centrifuge.
3. Resuspend cells in 100 mM CaCl<sub>2</sub>.
4. Pellet cells again in centrifuge.
5. Resuspend again in 100 mM CaCl<sub>2</sub>
6. Mix competent cells and DNA
7. Incubate on ice
8. Heat shock cells
9. Plate cells
10. Incubate plates at 37 degrees overnight.

Before next Thursday or Friday

11. Pick colony and grow up liquid bacterial culture
12. Pellet bacterial cells
13. Freeze bacterial cells in -20°C.

#### **Reagents**

100 mM CaCl<sub>2</sub> - on ice

#### **Equipment and Supplies**

45 degree water bath  
25 and 50 ml sterile graduate cylinders  
sterile 50 ml centrifuge tubes  
sterile pasteur pipettes  
sterile 15 ml centrifuge tubes

## **1. Grow up cells**

This portion will be done by the instructor.

- a) The night before, a colony of *E. coli* strain XI-1 Blue will be put in 5 ml of 1X LB broth, and will shake overnight at 37°C.
- b) The next morning 2ml of the bacterial culture will be diluted into LB broth (200 ml/500 ml flask) at 37°C with shaking.
- c) The growth of the cells will be monitored by measuring OD<sub>600</sub> in a spectrophotometer. This measures light scattering by cells (the more cells the greater the light scattering). Usually a saturated culture is about 1.2-1.4 ODs. Cells are best for transformation in mid-log phase which is 0.3 to 0.8 ODs.  
At an OD<sub>600</sub> of 0.3-0.5 the cells will be rapidly cooled in an ice water bath and kept at 4 degrees.

Now you get to take over:

**Keep the cells cold during this protocol.**

**Handle the cells gently, CaCl<sub>2</sub> shocked cells are fragile.**

**SWAP DOWN YOUR LAB BENCH WITH 70% ETHANOL BEFORE AND AFTER YOUR EXPERIMENT.**

## **2) Pellet cells in a preparative centrifuge.**

You will be given explicit instructions in using the centrifuge by your instructor.

- a) Measure 20 ml of ice cold cells into a sterile 50 ml centrifuge tube. You can use a sterile graduated cylinder to measure the cells. Keep the cells on ice. Label your centrifuge tube with labelling tape.
- b) Using the pan balance, balance your centrifuge tube with another centrifuge tube of someone in the lab.

**Centrifuge tubes must be balanced before being centrifuged or the rotor may explode.**

- c) Carefully place your centrifuge tube in a slot that is **opposite** of its balanced partner.
- d) There are 12 slots for centrifuge tubes in the SA600 rotor. So one rotor will work for the entire class.
- e) Screw the lid on the rotor. Unlike standard threads, this lid is screwed on by turning counter clockwise. It should firmly tightened, but no Herculean effort is needed.
- f) Set the speed to 6000 rpm.

**Always know the limits of speed that rotors can tolerate. The SA600 rotor is good for up to 17,000 rpm. Other rotors can only tolerate speeds up to 8000 rpm.**

**Never use a rotor without knowing its tolerances!**

- g). Set the time for 5 minutes
- h) Start.

**Watch the centrifuge as it comes up to speed. If it starts to wobble violently, there is a serious imbalance problem and you should abort the run by turning time to zero or by hitting the stop button.**

- i) You can leave the centrifuge area when the run is up to speed.
- j). At the end of the centrifuge run, the speed will be at zero. Do not open the centrifuge until rotor stops spinning.

### **3. Resuspend cells in 100 mM CaCl<sub>2</sub>.**

- a) Remove the centrifuge tube from the rotor. Note the pellet of cells at the side of the bottom of the centrifuge tube.

**Put the tube on ice immediately.**

- b) Pour off the supernatant into a waste beaker.  
(The bacterial waste will be collected at end of lab for autoclaving).
- c) Invert tube for on paper towel for 30 seconds.
- c) Carefully wipe the insides of the tube to remove any residual liquid.
- d) Add 4 ml 100 mM CaCl<sub>2</sub> to the cells, (using a 10 ml grad cylinder).
- e). Using a sterile pasteur pipette, resuspend the cells gently.

#### 4. Pellet cells again (very gently)

a) Pellet the resuspended cells again by spinning them at 4000 rpm for 7 minutes. You can use this time to prepare the tubes that you will be using for the transformation.

#### 5. Resuspend cells again in 100 mM CaCl<sub>2</sub>

a) Carefully pour off supernatant into bacterial waste beaker. Invert tube on paper towel for 30 seconds to allow fluid drain away. These cells are loosely packed. *Make sure cells do not slide off from the tube.* Wipe inside of tube again.

b) Gently resuspend cells in 0.8 ml of 100 mM CaCl<sub>2</sub>. (You can use a p200 or p1000).

#### 6. Mix competent cells and DNA

a) Preparing tubes with DNA.

Label 4 sterile 1.5 ml eppendorf tubes as

Tube 1- 10 ng of plasmid DNA

Tube 2- 1 ng of plasmid DNA

Tube 3- 1 μl of ligation mix

Tube 4 - NO DNA

Optimally, you should add no more than 1 μl of DNA per 200 μl of transformation rxn. Aliquot the DNA into tubes 1 and 2.

*Can you accurately aliquot these amounts of DNA at concentration of DNA given?  
If so, how?*

6b. Aliquot competent cells

Add 200 μl of cells into each of the eppendorf tubes for transformation.

#### 7. Incubate on ice

After aliquoting DNAs into the various eppendorf tubes, mix very gently by flicking tube and incubate on ice for 1 hour.

## 8. Preparing LB AMP plates

You will add X-Gal and IPTG to prewarmed LB ampicillin plates during the incubation with DNA.

## 9. Heat shock cells

After an hour on ice, place the eppendorf tubes containing competent cells and DNA into a 45 degree water for 5 minutes. (The heat shock makes the bacterial membranes more fluid and permeable to DNA.)

Afterwards, set tubes in rack at room temp and plate the cells.

## 10. Make cells healthy again

Add 1 ml SOC media and incubate at 37°C for an additional 45 min.

## 11. Plate cells

You will be pipetting the cells onto either LB plates with or without ampicillin and spreading them with a bent glass rod to get them distributed as evenly as possible. You will need to sterilize the glass rod before and after each sample, which is done best by flaming after dipping in ethanol.

*Your instructor will show you how to plate the cells.*

- a) Before plating, be sure all plates are labeled on the bottom with relevant information such as the sample, your initials and the date.
- b) Gently aliquot 200  $\mu$ l of cells onto a plate.
- c) Dip the bent glass rod into a beaker which is 1/5 full of ethanol.
- d) Flame the glass rod. **Watch out!** Sometimes flaming drops of ethanol drip from the rod. Be careful not to catch the beaker of ethanol on fire. (If necessary, such a fire can be suffocated by placing another beaker over it).
- e) Allow the rod to cool for 15 seconds or so, then take the cooled and sterile bent glass rod and spread the cells evenly over the plate.
- g) After spreading, cover the spread plate, dip and flame the glass rod, and proceed to plate the next cell sample on a new plate.
- h) Leave the plates right side up on the lab bench for 15 minutes to allow the liquid to absorb into the agar.

## **12. Incubate the plates at 37 degrees for overnight.**

- a) Place your plates in the incubator upside-down, so drops of condensation don't fall on your colonies and smear them together.
- b) After the bacterial colonies have grown up, the plates will be stored in refrigerator.

## **13. Picking a bacterial colony and growing up liquid bacterial cultures with ampicillin .**

You will need to pick colonies to grow up cells for isolating DNA for the next lab. During this lab, we will show you how to pick colony and place into sterile media and add ampicillin for growing up cells. You will grow up the cells in an shaking incubator set at 37 degrees for 12-16 hours.

You need to pick colonies and grow up bacterial cells by the day before your lab next week, or you will have no cells to isolate DNA from.

- a) Pick one colony and use sterile technique to put it into 5 ml of sterile LB broth (in a 25 ml beaker).
  
- b) Add the appropriate amount of a 50 mg/ml ampicillin stock to make the final ampicillin concentration 100  $\mu\text{g/ml}$ . The ampicillin stock will be in the freezer. The sterile LB broth will be in the back of the room.

## **14. Pelleting Cells**

After the cells have grown up overnight, you will pellet 5 ml of the cells in a 15 ml centrifuge tube. You will use the IEC table top centrifuge in southwest corner of the lab. To pellet the cells you should spin them at 3500 rpm for 5 minutes. Remove the supernatant into the bacterial waste.

## **15. Freezing Bacterial Pellet**

Put your name on some tape on the tube and place the tube in a rack in the -20 freezer. The frozen cells can be stored until we isolate the DNA from them next week.