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5.36 Biochemistry Laboratory  
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## SESSION 11 (lab open 1-5 pm)

Today you will isolate your mutant DNA from the transformed cells. You will then design primers (or use T7 primers) to sequence your mutant DNA and prepare samples for sequencing.

### 1.) DNA isolation of mutant plasmid DNA

Isolate the DNA from your three selected colonies. A general miniprep procedure can be found in Appendix A3 and is included below. Note that this is the same procedure that you carried out for isolation of the wt Abl(229-511)-encoding plasmid DNA in session 2. Based on your skillful lab work and a little luck, at least one of these plasmids should contain the desired mutation.

#### **DNA isolation from a bacterial pellet (miniprep)**

[http://www1.qiagen.com/literature/handbooks/PDF/PlasmidDNAPurification/PLS\\_QP\\_Miniprep/1043788\\_HB\\_QIAprep\\_122006.pdf](http://www1.qiagen.com/literature/handbooks/PDF/PlasmidDNAPurification/PLS_QP_Miniprep/1043788_HB_QIAprep_122006.pdf)

- a.) Harvest the bacterial cells from your 3-mL overnight culture by transferring 1.5 mL into a 1.5 mL eppendorf tube and spinning down the cells in a microcentrifuge for 3 minutes. Discard the supernatant and add the remaining 1.5 mL of cell culture to the tube. Repeat the centrifugation and discard the supernatant. You should have a small bacterial pellet at the bottom of the tube.
- b.) Check that RNaseA has been added to Buffer P1. Add 250  $\mu$ L of Buffer P1 to the cell pellet and completely resuspend the pellet by vortexing.
- c.) Add 250  $\mu$ L of Buffer P2 and mix by inverting the tube 4-6 times. Do not vortex, since that can cause shearing of the DNA. If you have properly lysed the cells, the cell suspension will turn blue after the addition of the P2 buffer. If there are colorless regions or brown clumps in the cells, continue mixing until a homogenous blue solution appears.
- d.) Add 350  $\mu$ L of Buffer N3 and mix immediately by inverting the tube 4-6 times. The solution should become colorless and cloudy.
- e.) Centrifuge the tube for 10 min at 13,000 rpm in you bench top microcentrifuge. A compact white pellet should form.
- f.) Apply the resulting supernatant (which contains the plasmid DNA) to a QIAprep spin column by decanting or pipetting.
- g.) Centrifuge for 30-60 s. Discard the flow through.
- h.) Wash the spin column by adding 0.75 mL Buffer PE and centrifuge for 30-60 s. Discard the flow through.
- i.) Centrifuge for an additional 1 min to remove residual wash buffer. This is essential for the success of any future enzymatic reactions.
- j.) Place the QIAprep spin column into a clean and labeled 1.5-mL microcentrifuge tube. To elute DNA, add 50  $\mu$ L of Buffer EB (10 mM TrisCl, pH 8.5) to the center of the spin column, let stand for 1 min, and centrifuge for 1 min.

For long term storage, keep purified DNA at -20  $^{\circ}$ C.

## 2.) Quantification of DNA concentration

Calculate the concentration of each of your three miniprep elutions. For each sample, dissolve  $x$   $\mu\text{L}$  (typically, 1-3  $\mu\text{L}$ ) in 100  $\mu\text{L}$  of water. Measure the absorbance at 260 nm in a quartz cuvette. See Section **Appendix A2** for UV/Vis spectrophotometer instructions. To calculate the concentration in  $\mu\text{g}/\mu\text{L}$  of your double stranded (ds) DNA, multiply the Abs<sub>260</sub> by  $(0.05)(100/x)$ . Typical concentrations of miniprep DNA are 0.1-2  $\mu\text{g}/\mu\text{L}$ .

Calculate the concentration of each of your three miniprep elutions, as was done previously for your wt Abl(229-511)-containing plamid. See Appendix A4 for instructions on measuring and calculating DNA concentration. You will use some of the miniprep DNA for sequencing and should store the remaining DNA, labeled with the desired mutation and numbered 1-3 at  $-20$   $^{\circ}\text{C}$ .

## 3.) Preparation of DNA sequencing samples

You will send the plasmid DNA isolated from the three selected colonies for DNA sequencing to check for the desired mutation. For each submission, combine the following in a DNA sequencing tube:

- 3.2 pmol of your sequencing primer
- 200-500 ng of the plasmid DNA
- ddH<sub>2</sub>O to a final volume of 12  $\mu\text{L}$

Label each tube with a 3 letter name that is distinguishable from all other samples in the class, and fill in the information on a numbered sequencing sheet for the class. Make note of the numbers of your samples as well as the names, since the sequencing can come back with numbers only.