

## **Sequencing with Agencourt**

Note: you need special bar-coded tubes with a septum lid. Agencourt provides two suppliers. See the additional pages.

### **Preparing Samples**

1. Plasmid DNA for sequencing needs to be at 15-25 ng/μl concentration, minimum volume 40 μl (10 μl/rxn for high copy plasmids, 15 μl/rxn for low copy plasmids). Primers should be at 3.2 pmol/μl, minimum volume 20 μl (2 μl/rxn). Pipette samples directly into the special bar-coded tubes (push pipette tip through rubber septum, inject, and remove tip – no need to open tube).
2. Write the sample name and/or primer name (something that is short to write because the tubes are small) on the tubes. Remember what you wrote on the tubes when you go to the computer to register your samples.

My group suggests:

On the tube, write a usually abbreviated form of the sample name, put the same abbreviation under "Sample Tube or Well ID" on the Agencourt form, and write the entire sample name under "Sample Name" on the form. If sending them primers, put again an abbreviated primer name on the side of the primer tube and then specify on the form which primer is needed for a sequencing reaction. All the important information (which samples to sequence with which primer) is on the form, and what's written on the tube is enough so they know which tubes to use for which reaction. (For example, for the following construct, pYFG1, pYFG2, pYFG3, we put "Y1", "Y2", and "Y3" on the tubes, and "p3" and "p6" for the primers [prYFG-3, prYFG-6].) You can also shorten it further and put 1, 2, 3, etc.

3. Put the tubes in a small Ziploc bag with the printout that you make in the computer (see below).

Agencourt provides the following universal primers:

Universal T7  
M13-FWD  
M13-REV  
SP6  
Universal T3  
KBR/TJ  
2BP Clamp  
SeqL-A  
SeqL-B

### **Online Sample Form**

Log in at [www.agencourt.com/sample/](http://www.agencourt.com/sample/)

login: your labs username  
password: your labs password

Here, you can view current projects, submitted projects, and access results. To create a new project, click the aptly named Create a New Project button. Name the project and select the QuickLane radio button under Individual Sample Sequencing. Continue.

Under Template and Primer Association, click the Sample Info in Excel Format and save the excel file. Fill it out accordingly with the names you have listed on the tubes and upload it by clicking the Upload button.

[NB: Do not fill out the form directly on the webpage. Agencourt tends to completely erase anything you've written when submitting, prompting you to refill the form again.]

Under Data Delivery, enter your email address (Agencourt will notify both the lab worker and the professor when sequences are ready). Select the data formats you want to receive: I recommend having both the chromatogram (ab1) and the sequence text (seq).

Then enter the P.O. Number.

Once submitted, print out the page (in duplicate if you want a copy for yourself). Put this along with your bag o' tubes into the plastic bag and take to the fancy dropbox in the Koehler Lab. Then the Koehler lab will see that samples are sent out on a daily basis with UPS at approximately 3:00 every day.

Turnaround time is usually a day.

Access sequences at:

<https://cust.agencourt.com>

and enter login and password when prompted. Sequences are in tar.gz compressed format – use a compression software such as WinZIP or WinRAR to extract the files.