

Protocol

Part 1: Standard Assembly (à la Registry of Standard Biological Parts)

We have defined a “part” as a basic unit of sequence with biological function. The parts included in the Registry of Standard Biological Parts have been modified to accommodate the Registry’s method for standard assembly. Specifically, the parts

- include EcoRI and XbaI restriction sites as their “prefix”
- include SpeI and PstI restriction sites as the “suffix”
- be devoid of all four sites within the part, using site-directed mutagenesis to introduce silent changes to the sequence as needed.

Begin by using the NEB catalog that’s in the lab or use the [online resource](#) to fill in the following table:

Restriction enzyme	Recognition sequence	Overhang	Activity in buffer 1, 2, 3, and 4
EcoRI			
XbaI			
SpeI			
PstI			

With this information, review the figure from the introduction that shows how to join a generic Blue Part to a generic Green Part. In this example, the plasmid containing the Blue Part gets digested with EcoRI and SpeI to generate the fragment for the ligation reaction. In a separate reaction the plasmid containing the Green Part is digested with EcoRI and XbaI to generate the backbone for the ligation reaction. Based on the information you collected about each restriction site and what you know about DNA Engineering from the first experimental module, determine:

- what buffer you should use for generating the Blue Part as a fragment to clone
- what buffer you should use for generating the Green Part as a backbone to clone into
- if the overhangs of fully digested backbone could ligate together
- if the fragment can ligate to the backbone in one orientation or more than one
- which of the four restriction sites will be present in the properly assembled plasmid, i.e. a plasmid bearing the Blue Part followed by the Green Part
- if this resulting plasmid could be reused as the backbone in a third assembly (to add a Red Part for example)
- if this resulting plasmid could be the source of a fragment for a third assembly (into a backbone containing a Red Part for example)

Once you are confident in the steps used for standard assembly, please fill in the following strategy for constructing the protein generator you designed and specified last time. A template is offered for the first few steps of the assembly process but it is likely you will have to extend or modify the template to fully describe your assembly.

Step 1

Digest	with enzymes	in NEB buffer #	size of DNA piece (#bp)
BBa_XXXX			
BBa_XXXX			

Ligate, transform, pick candidates into liquid culture, miniprep. Then check your assembly via digestion.

Digest	with enzymes	in NEB buffer #	look for DNA piece of size (#bp)
BBa_XXXX			

Call the construct BBa_XXXX and enter it into the Registry as an assembly intermediate.

Step 2

Digest	with enzymes	in NEB buffer #	size of DNA piece (#bp)
BBa_XXXX			
BBa_XXXX			

Ligate, transform, pick candidates into liquid culture, miniprep. Then check your assembly via digestion.

Digest	with enzymes	in NEB buffer #	look for DNA piece of size (#bp)
BBa_XXXX			

Call the construct BBa_XXXX and enter it into the Registry as an assembly intermediate.

Once you have finished planning the assembly, consider how long it would take if everything went perfectly at each step. You can assume that the digestion, ligation and transformation can be done in one day, the overnight cultures of candidates requires one day more, and the miniprep/digestion/diagnostic digests takes a third day. You can choose to work weekends or not but be sure to indicate that decision in your answer.

Part 2: Beta-galactosidase assay

The bacterial photography system you are studying [described in reference] modifies a signaling pathway in *E. coli* so light striking the cell can be converted to a detectable output (beta-galactosidase enzyme). A “black box” depiction of the system looks like:



"Black box" depiction of bacterial photography system.

Some of the details within each box are:



Bacterial photography system.

This figure is more detailed but harder to decipher without prior understanding of several aspects of the system. For example, light at 660nm is detected by the input-sensing device, turning it off. Light-sensing requires the combined action of two sets of proteins: Cph8, itself a fusion of a light-sensing protein called Cph1 that comes from an algae attached to a transmembrane signaling protein called EnvZ, and phycobilin producing proteins that generate accessory pigments needed for the light-sensing protein to work. The input sensing device generates a signal within the cell's osmoregulation pathway (OmpR is the signal carrier), changing the activity of an OmpR regulated promoter that is directing transcription of the output: lacZ....whew! The black boxes were a whole lot easier.

You should review the procedure for beta-galactosidase assays that you performed during the Protein Engineering module at assessing beta-galactosidase, then perform assays on your overnight liquid cultures that were grown in the dark and the light. Activity calculations for these samples will be part of your assignment for next time.

Part 3: Black and white photography

Retrieve the Petri dishes you set up last time and compare the appearance of the light and dark grown samples. Because the dark grown cells were in a completely dark box, the difference between the two plates is the greatest contrast you can expect in your bacterial photographs. Media containing S-gal is available for you to supplement with antibiotics and cells as you did last time.

Next decide what image you would like to photograph. Generate a computer file with this image and print it to a transparency. Transparencies will be available in the lab for you to use as masks, taping them to the back of the Petri dish before incubating. The goal is to have each cell growing distinctly in the light or dark, but be sensitive to the fact that light can bounce around and can blur the resulting image. As much as possible you want a setup where light hits the cells then continues through as little agar as possible, then hits black background and dies. In general, it's better to have a dark background and a light image rather than the other way around. To darken the dark parts of your photo, you might want to print it on two transparencies and use them both to mask your Petri dish.