Module 3: Bacterial Transformation
Due Feb. 24th at 2pm, submit on Compass

In this module we took an existing plasmid, pGLO, and transformed it into E. coli HB101 K-12. The presence of the transformed plasmid was verified by GFP expression and cell growth on LB agar plates with antibiotic (ampicillin). By picking a colony with pGLO plasmid and growing an overnight culture, we were able to make many more copies of the pGLO plasmid. We then extracted the pGLO plasmid from the cells by a “miniprep”.

Often similar protocols are used to generate larger amounts of plasmid DNA either for storage or experiments. Any time a plasmid is prepared it is advisable to verify the plasmid identity and quality. Plasmid identity can be verified by restriction digest and/or by DNA sequencing. In this module we confirmed the identity of our plasmid preps by a restriction digest. Based on the known sequence of pGLO, we could confirm whether the digest produced the expected DNA fragments. In addition to the restriction digest, we analyzed the concentration and quality of our preps using a spectrophotometer (Nanodrop). Both the concentration and quality of a DNA prep will be important considerations when using plasmid DNA for an experiment.

For this assignment imagine the following scenario:
You are an engineer at a biotech company and prepped pGLO plasmid to store in the company's repository. Your task is to write a quality report about your plasmid prep that will be the record for your prep. The report should include basic information about pGLO (what features does the plasmid contain and what is their purpose) and should include any data you have collected that demonstrates the quality of your prep (concentration, purity, restriction digest, etc). The report should be sufficient for another scientist to use your plasmid prep for their experiment. Think critically about which pieces of data are relevant to share.

You do not need to include detailed methods of how the experiment was done, only how the data was obtained/measured. For example:
"pGLO plasmid was digested by EcoRI and EcoRV enzymes and produced the expected fragments at X and Y base pairs (see Figure #)."

If anything went wrong with your experiment (or the data is not as expected) describe both what is expected and what you believe to be wrong. Explain why you think this is the case based on the data you have and what information is in your ELN, do not simply give a list of possibilities.

What is a quality report?
Quality reports are reports that describe and/or assess the quality of a reagent, device, or other item. Quality engineers ensure that the company produces the
best products by tracking, documenting, and ensuring quality metrics have been
met. Some companies may provide documentation with a product that describes
its quality based on data collected at the company.

Things to consider:
What data do you have to demonstrate how the features of the plasmid function
(ie bla, GFP, araC)?

Did the restriction digest produce the expected fragments based on the known
pGLO sequence?

What data do you have that demonstrates the purity of your prep?

Be sure to annotate your data figures so that they are easily interpretable by the
reader. For example, labeling each lane of your DNA gel and annotating the
band sizes in the DNA ladder. Crop images if necessary.

Calculate the number of cells you started with (using optical density data
collected from your overnight culture) and calculate your miniprep yield (amount
of DNA per starting cells).

Include a map of pGLO that could help the reader understand the features of the
plasmid and how to interpret your restriction digest.

The full sequence for pGLO is available in the ELN as a reference.

There is 2-page limit including figures and references.