Session 2: Plasmids & Competent Cell Preparation

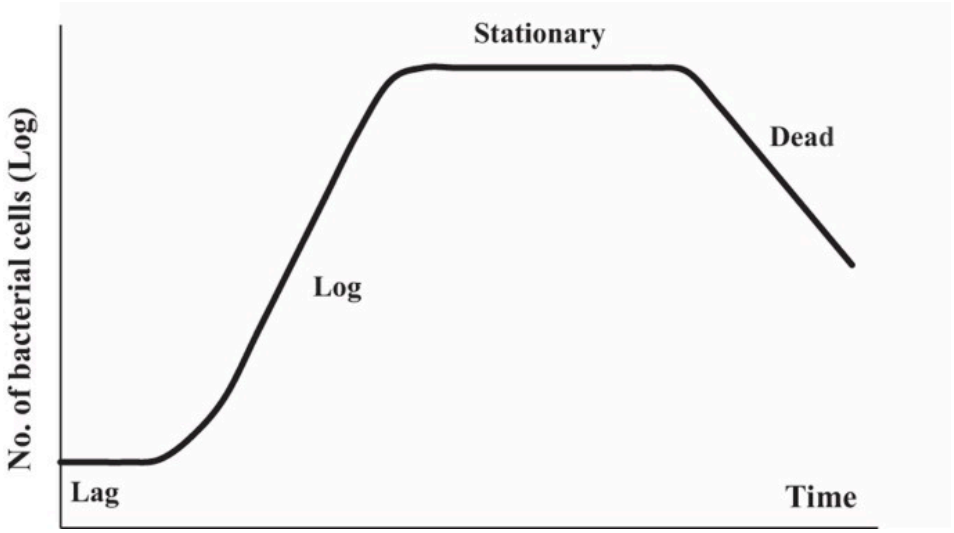
Learning Goals:

- Learn how to prepare competent cells for bacterial transformations
- Learn how to use common biotechnology software, e.g. Benchling
- Learn how to read a plasmid map and identify relevant genetic components

Background:

What are competent cells?

Bacterial cells that are able to take up foreign DNA from their environment are referred to as *competent.* When we introduce engineered DNA into competent cells in the lab, we call this a *transformation.* Competent cells are prepared during the *exponential or "log" growth phase* of the *E. coli* growth curve, meaning they are growing and dividing rapidly, making them more susceptible to taking in foreign DNA. In this lab, we will be using chemically competent cells. Chemically competent *E. coli* cells are treated with salts to weaken the cell membrane, creating temporary pores that allow DNA to enter the cell. We will learn more about how transformations work next week!



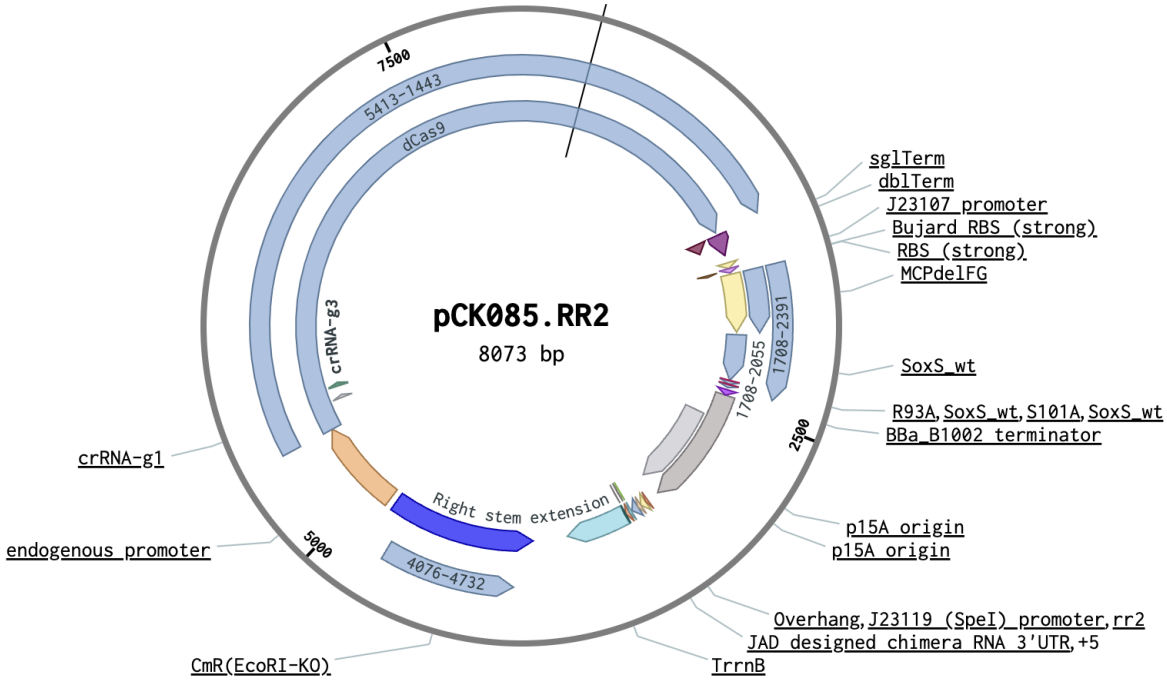
Next week, we will be doing transformations to introduce the genes encoding the CRISPR programs into *E. coli* cells. This week, we will learn how to prepare our own competent cells. We will also learn how to use the biotechnology software Benchling, that allows scientists to keep track of their synthetic DNA sequences. Beyond just looking at a string of 'A', T', 'G', and 'C's, these softwares allow scientists to annotate the DNA encoding different genetic elements.

Lab Tasks:

1. Inoculate subculture of competent cells from overnight culture.
    a. In a 50ml tube, add 10ml of LB media. Inoculate 1:100 (100uL) using a pipette to transfer from your overnight culture to the fresh LB. Then place the tube in the incubator and wait until the OD600 (optical density, used as a measure of cell growth) reaches between .3 - .7.
2. This will take ~1.5-2 hours. While we wait, log onto Benchling.com and create an account.

Plasmid map on Benchling

3. On the dashboard, you can upload DNA sequences by clicking the "+" along the left hand bar. Make sure you have selected a project within the "Projects" tab (Example Project) to upload your sequence into. Under DNA/RNA sequence, select "Import DNA/RNA sequences". Along the dialogue box top, click "Upload Files". You can download this sequence and upload it to your Benchling account.



4. This plasmid, pCK085.RR2, is one of the plasmids we will be using for our experiment during this course. Today, we will learn a little bit about the parts that

make up this plasmid. Here is a list of a few of the genetic elements of this plasmid - see if you can find the length in DNA bases of these parts.

Genetic element	**Purpose**	**Length (DNA bp)**
dCas9	Forms the CRISPR system	
Guide RNA (gRNA): RR2	Guides the CRISPR system to its target DNA	
p15a	Origin of replication	
CmR	Antibiotic resistance	

5. Beyond providing annotations for the DNA sequences of different proteins, Benchling also can show us information about different genetic components that influence how much mRNA and protein will be expressed - components including *promoters, RBSs,* and *terminators.*

Competent cell preparation

6. Check the OD600 of your competent cell culture after 1.5 hours. Move 1ml of your culture to a cuvette [stored where?], and 1ml of clean LB to a different cuvette to use as our “blank”. Using the nanodrop computer, you can measure cell cultures. Make sure “Use cuvette” is checked. Start by measuring the blank, then measure your culture. Record the OD600 here:

Sample	Time	OD600

7. If your OD is below .3, put the culture back in the incubator. The doubling time of *E. coli* cells is ~20 minutes, so you can calculate roughly how long it will take to reach the appropriate OD.
8. Once your OD is at the appropriate level, centrifuge the cells for 5 minutes at 5,000rpm

   a. **After this point, all steps should be done on ice!**
9. Resuspend your cells in 500 uL of Transformation Storage Solution (TSS). Label your tubes with your initials so you will be able to identify them in the future. Store your competent cells in the -80C freezer until ready to use for transformations!

Resources:

1. Introduction to competent cells | GoldBio
   https://goldbio.com/articles/article/Introduction-to-Competent-Cells

2. The Basics of Benchling
   https://help.benchling.com/hc/en-us/articles/9684234496013-The-Basics-of-Benchling

3. OD600 Basics
   https://www.implen.de/od600-diluphotometer/od600/