

Introduction

In an ideal world, each genetic part, device and system would come with a specification sheet. Since experimental variations can affect a part's performance, specification sheets would include information about the part's "operating conditions," e.g. the optimal strain, media, temperature, and growth state, or perhaps in a few years, the community will recognize a set of conditions as "standard." It would be wonderful if, knowing these conditions and the parts specifications, a user could predict the behavior of a genetic element, anticipating the amount of DNA, RNA and protein generated rather than taking the current approach, namely to try the experiment and find out. There are many methods to quantify DNA copy number, RNA levels and a protein's concentration, activity, and interactions, so it would be ideal if the specification sheet reported values that held true no matter which measurement technique was used. And while we're dreaming, why not also include information to help combine genetic elements in sensible ways, detailing a part's incompatibilities or the level of crosstalk between parts at a minimum.

The challenge in characterization is to decide what aspects of a biological response need to be known and then to decide how best to measure them. Different measurement techniques are sensitive to different things. Since no description of engineering efforts would be complete without a car analogy, consider the problem of "noise" in biological responses in terms of what you'd see at a traffic light on a busy road that's 10 lanes wide. At the red light the cars will wait, all poised to go forward. As the light turns green, one of the cars may race ahead, another may move ahead slowly. If a driver is distracted with the radio or a cell phone call (or lost in thought about the 20.109 "for next time" assignment), that car may not move until a nearby car moves or honks. Thus, even if all The cars get the same green light signal at the same time, they don't perform identically. So what can be predicted about the reaction of cars (and their drivers) to a green light? As a population they move forward, individually they do so with different rates and if you were to look at just one car over time you might observe a different response than if you looked at another.

Biological responses are similarly noisy and inherent fluctuations lead to the heterogeneity of a cell population. As an example, consider again the "repressilator" plasmid that was introduced earlier ([Elowitz and Leibler, Nature, 2000](#)). The design, which mimics an electronic oscillator, should generate changing concentrations of three proteins (cI, tetR, and lacI), and flash GFP as a reporter for the tetR concentration. Simulations showed distinct peaks of the three proteins, temporally oscillating as intended, when modeled with a half dozen, coupled first-order differential equations. Periodicity was seen even when the system was modeled with more complex terms to account for confounding factors like cooperative binding, leaky promoters and different rates of mRNA decay.

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[Elowitz and Leibler, Nature, 2000](#)

When the cells were built and tested, the GFP production fluctuated as the simulations predicted, but, perhaps surprisingly, not in every cell and not in unison. GFP oscillation was observed in ~ 40% of the cells (leaving ~60% of the cells not behaving as predicted), and for those that did flash, the period was measured as 160 minutes +/- 40. This observed period is longer than predicted by modeling, and showed a large standard deviation, with sibling cells exhibiting the most highly correlated behavior but only for a few cell divisions. So while this work provides an important demonstration of “cells built to order” it also reveals some inherent limitations.

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[Elowitz and Leibler, Nature, 2000](#)

As you consider the measurements you are making in lab, it's important to know the strengths and limits of the techniques you've chosen. Noise is most evident when observing the behavior of individual cells within a population, like the microscopic observations detailed above. However, single-cell measurements are generally time and labor intensive and there are limited ways to count individual molecules in a single cell. More common are population measurements, for example beta-galactosidase activity data, however these bulk measurements cannot differentiate hetero- from homogeneous expression. 10% of the cells expressing LacZ at 100% efficiency will give the same activity measurement as 100% of the cells expressing LacZ at 10% efficiency. Distributions within a population can be measured with techniques like FACS and flow cytometry, but it is difficult to apply these techniques to study individual cells over time.

Without a genie to grant us all our wishes, we are left with a somewhat limited menu of choices for system and parts characterization. Nevertheless, it's worth considering what a perfect characterization would look like, informing this description with user experiences. Collectively this experience can help define some uniformly satisfying standards for parts and systems characterization. Today you will be generating some useful data on the robustness of the bacterial photography system, measuring its protein and RNA output under different operating conditions. You will be completing the experimental work on this characterization next time. The data analysis and documentation of your work will be done in the final lab session of this experimental module.