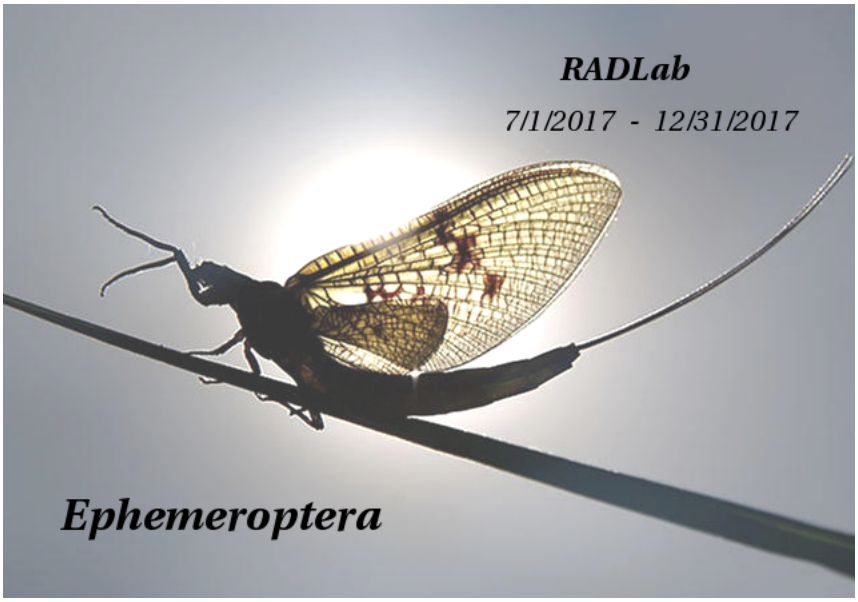


RADLab: A Community Laboratory for Teaching Biotechnology and Supporting Citizen Science Experimentation

Peter Reintjes[1,2,3]

1. SplatSpace, Durham's Makerspace
2. Triangle Do-it-yourself Biology Initiative (TriDIYBio)
3. Innatrix, Inc.

Inspired by Ephemeroptera (the Mayfly), we wish for the moment to ignore the difficulties and politics of securing a source of long-term funding for this important community resource. We choose instead to create a community laboratory with a lifespan of **six months** *using salvaged equipment, under-utilized space at the Scrap Exchange, and volunteer manpower. Rather than making powerpoint presentations about this idea to potential funding sources, we choose to use our own resources to simply make the laboratory real. We expect that the expiration date (12/31/2017), will encourage people to investigate and take advantage of this resource as we carry out its educational and research aspirations.*

Introduction

Biotechnology startups in the Research Triangle have a number of alternatives to rent laboratory space. A small company can be fully operational by renting bench space in a facility with shared infrastructure in the form of parking, secure access, restrooms, common areas, housekeeping and facilities operations, purified water, autoclaves for sterilization and waste disposal, and Internet access.

Developments that may lower the cost of lab space include GlaxoSmithKline selling its 20-building campus to Parmer Innovation Partners and the availability of space at the original Syngenta facility, 190,000 square feet of office and laboratory space, plus greenhouses.

There are a number of organizations in the Triangle with such incubators for biotechnology startups. BioLabs, based in Cambridge, Massachusetts, has a facility in the recently renovated Chesterfield building in downtown Durham. The cost of the BioLab space is $1800 per month for six feet of bench space, plus $300 per month per researcher for shared resources including temporary desk space, Internet access, -90 degree freezers, autoclaves, dish washers, centrifuges, and photo-spectometry equipment. Alexandria Real Estate manages several facilities, including one at 6 Davis Drive housing approximately 20 companies at a somewhat lower cost for bench space, but with more office space and less shared infrastructure (e.g., they provide autoclave access, but no freezers, centrifuges, or spectrophotometers for general use). As a practical matter, there are many opportunities for entities within the facility to negotiate their own infrastructure sharing arrangements.

The infrastructure at BioLabs is awe inspiring, but we are consequently inspired to create a more modest laboratory environment at a much lower price point. Our goal is to produce an environment for research and education at approximately one-quarter of the price of BioLabs. We believe this is possible because of the significant breakthroughs occurring in biotechnology. Prices are falling rapidly in PCR DNA amplification and DNA sequencing services, oligonucleotide synthesis, and plasmid assembly. There are open-source tools for plasmid design including ApE (A plasmid Editor), Serial Cloner, and MCDS (Molecular Cloning Design Simulator).

These falling prices are enhanced by interdisciplinary engineering, using inexpensive microcontrollers, open-source software, generic commodity-priced hardware/plumbing, as well as discarded/surplus equipment from the Scrap Exchange, Triangle E-cycling, and J&D Recyclers. Designing and building low-cost automated laboratory equipment provides an outcome-driven teaching platform, which doesn't require biological materials until the equipment is actually being used by biologists. Therefore, most of this engineering/teaching can occur under relaxed (not laboratory grade) space requirements.

RADLab

RADLab will be a community accessible laboratory in the Lakewood Reuse Arts District (RAD). Our goal is to support educational and research activities with an extremely low-cost infrastructure. Although some of us are engaged in research on specific organisms and health related issues, the initial emphasis of the laboratory will be teaching laboratory techniques and the creation of low-cost alternatives to commercial laboratory equipment.

Some of these build-it-yourself alternatives have an important advantage other than cost. They allow for the creation of re-configurable systems to automate many laboratory procedures, thereby increasing the speed, safety, and repeatability of future research programs. It turns out that designing and building the automated laboratory of the future is something that can be accomplished by anyone with a computer, a soldering iron, and access to resources like The Scrap Exchange.

Splatspace / TriDIYBio currently has the following supplies available:

1. Gloves and Safety Glasses
2. Pipetman and pipette tips
3. Centrifuges

4. Scales
5. 4 degree C refrigerator
6. Distilled water
7. Incubator
8. Microscopes
9. Computer systems, electronic parts, valves, pumps, and micro-controllers.

While things that we would like to have include:

1. -20 degree C freezer
2. -70 degree C freezer
3. Autoclave
4. Water purification system

The RADLab facility will be maintained at **Biosafety level 1** (BSL-1) – which is a level of safety and cleanliness achievable in a high-school biology classrooms. An on-line course is available to learn about the different levels of Biosafety. A good primary source is the CDC.

A BSL-1 lab does not require an autoclave, though an on-site autoclave is recommended for a BSL-2 lab and required for higher levels of biosafety. There is still a need to sterile equipment as well as procedures to render biohazards harmless. For this purpose, autoclavable equipment and hazard bags will be made available. A researcher can place materials in an equipment bag and mark it to be autoclaved, and the bag will be autoclaved at the earliest opportunity by a volunteer with access to autoclave facilities (there are several of us). There may be a delay of a few days unless arrangements are made in advance.

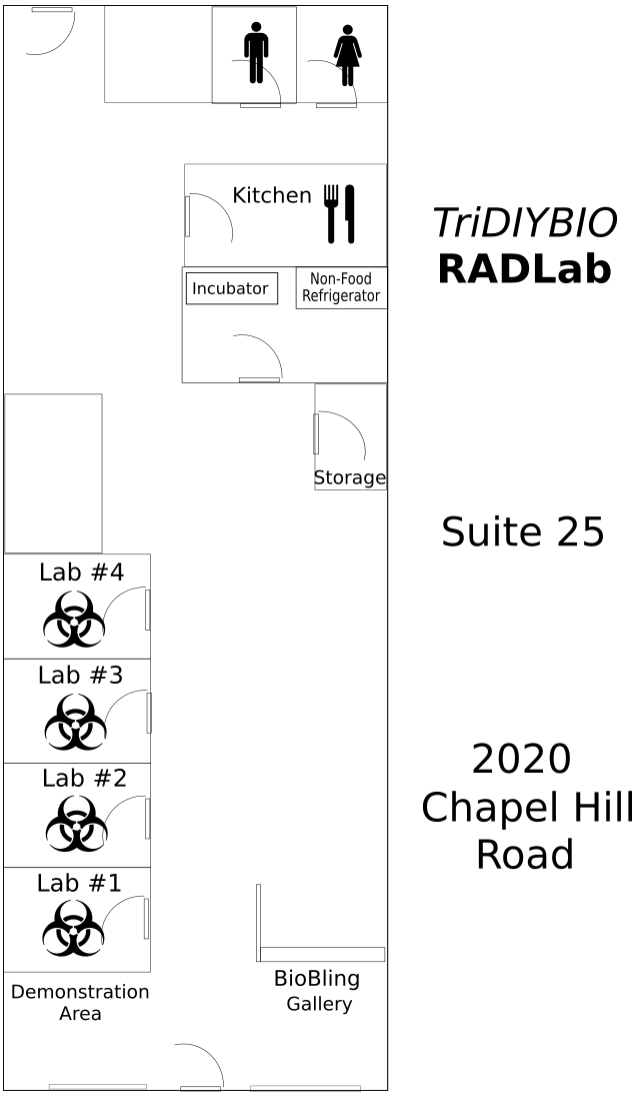
Similarly, the biological waste will be collected from the laboratory on a regular basis and processed by one of our volunteers. We can use this as an opportunity to teach about alternatives, such as chlorine bleach, but also how different methods can be incompatible: *Never autoclave a container that has been contaminated with hypo-chlorite as chlorine will be released by the high pressure and temperature.*

Embracing Ephemera

Inspired by the mayfly, our plan is to create the RADLab as a temporary, six-month installation, in other words: a pop-up laboratory. By giving it a limited lifespan, we hope to focus attention and get more community involvement (use it *and* lose it).

Most members of TriDIYBio are also members of Splatspace (Durham's Makerspace) and as a result, we conduct our outreach activities as representatives of Splatspace. Biological experimentation being just another maker activity of Splatspace. It is very likely that Splatspace will relocate to the Lakewood Resuse Arts (and Sciences) District later this year (December 2017) and so the pop-up laboratory can also function as a staging area to make that move go more smoothly. We plan to have an electronic workbench in the RADLab and could accommodate a 3D printer, or other tools that are duplicated at Splatspace.

Reuse Arts District: Suite 25



RADLab Projects

Laboratory Procedures

Regular workshops have been presented at Splatspace and will be continued at RADLab on sterile techniques, pipetting, plating bacteria (micro-biome sampling), green fluorescent protein (GFP) expression, centrifuge and microscope usage, and running electrophoresis gels.

Bio-Bling Gallery

The Bio-Bling Gallery will be a showcase for the design, construction, and distribution of:

1. Smartphone microscopes

2. Smartphone spectrometers
3. Arduino-based, Bluetooth® devices for monitoring turbidity and bio-luminescence.

Directed Evolution

One of the projects in the RADLab supports engineering novel proteins via continuous evolution of bacteriophage. The main part of this project is the construction of an EvoStat, a computer controlled platform for supporting continuous experiments in viral evolution spanning days or weeks.

An automated system for directed evolution (EvoStat) has been developed by the author. We plan to construct a prototype in the RADLab to demonstrate the accessibility of continuous evolution to researchers with limited funding. An earlier version of this device was in operation at UNC Dept. of Biology (2014-2017), and one is currently in use at Innatrix (6 Davis Dr. RTP, NC).

Directed evolution of bacteriophage has the potential to become a potent protein engineering tool [1] [5]. Continuous evolution rapidly produces a viral genome containing a gene which has undergone many generations of mutation and selection for a particular property. The generality of this approach to protein engineering is limited by our ability to insert an expressable initial gene into the phage and create a selection mechanism for the desired activity.

PACE

Specifically, the Phage Assisted Continuous Evolution (PACE) [1] system requires:

1. A modified viral genome replacing a crucial phage gene with the gene to be evolved.
2. A transformed host with inducible mutagenesis.
3. A host plasmid containing a selection mechanism to provide the crucial phage gene in proportion to the desired activity of the evolving gene.

However, the PACE procedure is patented by Harvard University and so we are investigating alternative selection mechanisms including one called M-Selection.

M-Selection

Extremely high, yet controllable, in vivo mutation rates are now possible [2]. This broad-spectrum mutagenesis with a (claimed) 320,000:1 dynamic range is generated and can be controlled within an individual host cell. I suggest that if this mutagenesis is initiated by infection, say with the phage shock promotor but then reduced by the desired activity, the two principal components of evolution will be satisfied: The desired activity will be selected for as the number of phage progeny of the individual exhibiting that activity will be amplified by more faithful reproduction. I propose calling this mechanism Mutagenesis-selection or M-selection. So far, all PACE-derived procedures induce a uniform mutagenesis in the lagoon while M-selection doesn't even require the small-molecule (Ara) induction mechanism.

We require 2 clones per generation to infect hosts and produce phage to prevent washout of a genotype. Assuming phage production of 100/hour and a lagoon transit time of one hour, we calculate calculate a mutation rate of about 4 mutations per phage genome. The percentage of phage progeny which are exact copies of the parent is given by the Poisson distribution where $\mu = 0$ is the expected number of mutations and λ is

the mutation rate per virion (mutation rate/base * 6kbp/ genome). A mutation rate of $\lambda = 4$ gives a 1.8% probability of zero mutations.

$$\mathrm{P}\left(\mu, \lambda\right) = \frac{e^{-\lambda}\lambda^{\mu}}{\mu!} \rightarrow P(0,4) = \frac{e^{-4}}{1} = .018 \tag{1}$$

So the maximum per-base mutation rate that a genome could tolerate and still stay in the lagoon is $6400/4 = 6.25X10^{-}4$ (See References).

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Bacteriophage M13. The genome size is 6407 bp. The mutational target is 258 bp of an inserted Escherichia coli lacZa sequence. The spontaneous mutant frequency is 6.4×10^{-4} per genome or 7.2×10^{-7} per base pair *Drake, 1991*