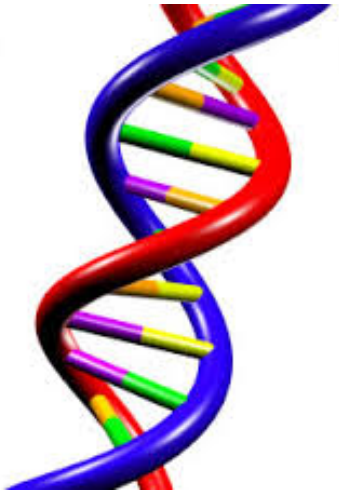
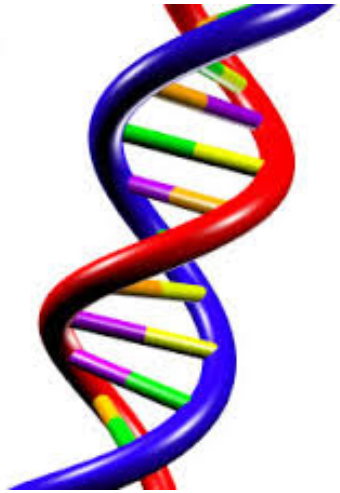
DIYBio in the Triangle



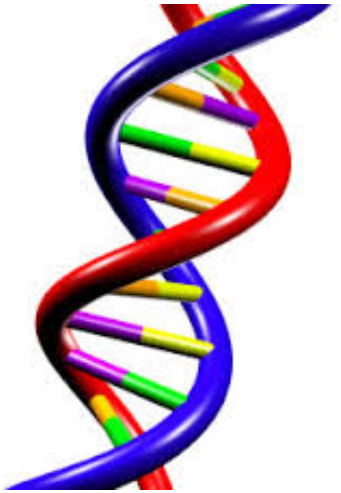
STaRS, 2017, NIEHS

Tom Randall
tarandall@gmail.com



On behalf of Triangle DIY Biology

DIYBio in the Triangle



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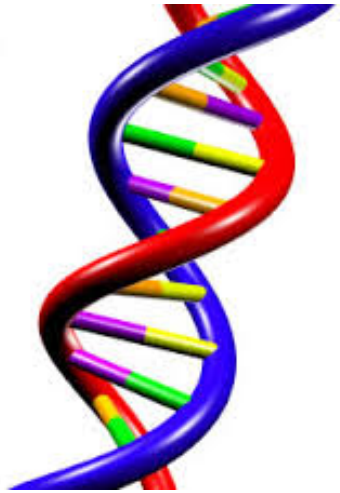


On behalf of Triangle DIY Biology

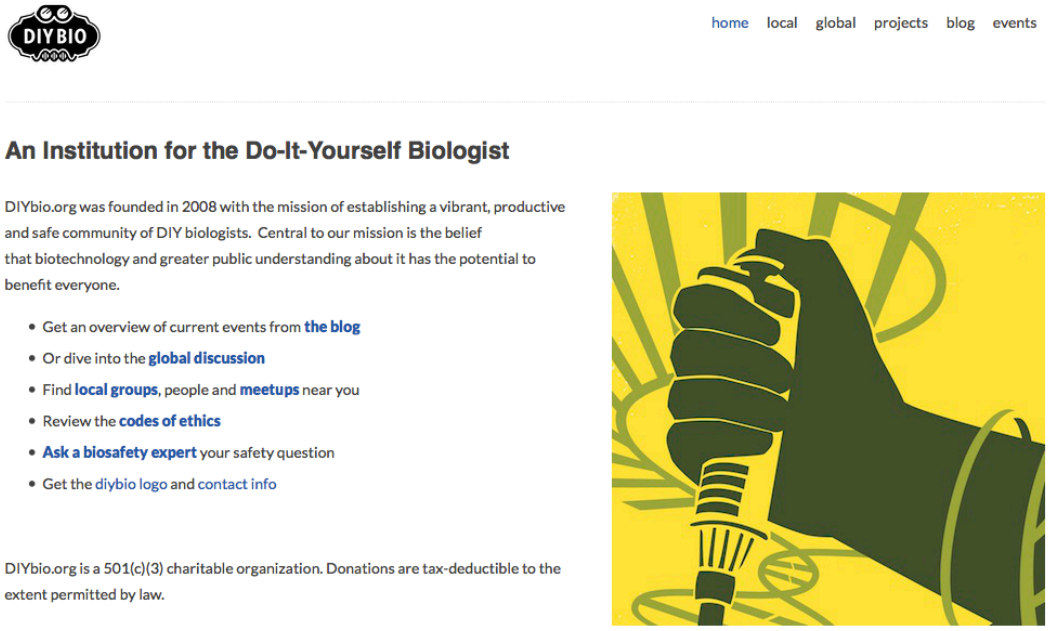
BS, Univ. of Michigan, Biology, Microbiology, Molecular and Cellular Biology
PhD, Michigan State Univ., Microbiology and Molecular Genetics
Univ. of Wisconsin, Biomolecular Chemistry
Univ. of Washington, Seattle, Genetics (now Genome Sciences)
Univ. of California, Riverside, Plant Pathology

@North Carolina

Syngenta Biotechnology Inc. (1999-2003)
UNC Chapel Hill, Center for Bioinformatics (2004-2010)
NIEHS, Integrative Bioinformatics, Contractor (2010-?)

Outline

- What is DIYBio?
- Various DIYBio activities in the Triangle
- Safety, sourcing equipment & reagents
- Building/running an agar gel box (live)



http://diybio.org/

Founded 2008 by Jason Bobe and Mackenzie Cowell

Website and google group

>4800 members worldwide

In reality…

DIYbio is:

Community based labs

Home based labs

Doing their own thing, independently and with no governing oversight

Basic scientific research

Building low cost equipment

Developing low cost reagants/strains

Outreach/education to public/schools

CHARLOTTESVILLE
Open Bio Labs

Ronin Genetics

It's A World-Wide Thing

DIYBIO

local projects hardware blog weekly news events

Local

Local Groups

NORTH AMERICA

Baltimore	MD	http://www.bugssonline.org/
Berkeley	CA	http://berkeleybiolabs.com/
Bethesda	MD	http://www.meetup.com/CapitalAreaBioSpace/
Boston	MA	http://bosslab.org/
Brooklyn	NY	http://genspace.org/
Cambridge	MA	http://openwetware.org/wiki/MIT_DIYbio
Carlsbad	CA	http://biotechnbeyond.com/
Charlottesville	VA	http://openbiolabs.org
Chicago	IL	https://groups.google.com/forum/#!forum/diybio-chicago
Columbus	OH	https://www.facebook.com/diybiocolumbus
Denver	CO	http://denverbiolabs.com
Durham	NC	http://www.roningenetics.org/
Guanajuato	MX	https://www.facebook.com/groups/DIYbioMexico/
Houston	TX	http://www.brightworkcoresearch.com/
Jackson	MS	http://www.diyneurotech.com/
La Jolla	CA	http://lajollalibrary.org/your-library/bio-lab/
Los Alamos	NM	http://biodidact.net/
Los Angeles	CA	http://www.biohackers.la/
Montreal	QC	http://bricobio.org/
New York City	NY	http://www.meetup.com/Biohackers-NYC/
New York City	NY	http://harlembiospace.com/
Norfolk	VA	http://www.biologiklabs.org/
Oakland	CA	http://counterculturelabs.org/
Orlando	FL	https://familab.org/
Portland	OR	???
Research Triangle Park	NC	http://www.tridiybio.org/

Sunday, March 27	URL
Bethesda, MD, USA	http://www.meetup.com/CapitalAreaBioSpace/events/229284421/
Oakland, CA, USA	http://www.meetup.com/Counter-Culture-Labs/events/229769788/
Monday, March 28	
Austin, TX, USA	http://www.meetup.com/prophase/events/229666020/
Sunnyvale, CA, USA	http://www.meetup.com/BioCurious/events/229380979/
Tuesday, March 29	
Amsterdam, NLD	http://www.meetup.com/Dutch-DIY-Bio/events/229250969/
Austin, TX, USA	http://www.meetup.com/prophase/events/229856959/
Minneapolis, MN, USA	http://www.meetup.com/MN-diyBio/events/229427392/
Mountain View, CA, USA	http://www.meetup.com/Silicon-Valley-Computational-Biology-Meetup/events/229415019/
Somerville, MA, USA	http://www.meetup.com/BosLab/events/229557316/
Wednesday March 30	
Brooklyn, NY, USA	https://www.eventbrite.com/e/crispr-workshop-beyond-the-hype-tickets-22712426479
Hertogenbosch, NTL	https://www.eventbrite.com/e/tickets-lagerhuisdebat-law-and-new-materials-22173199637?aff=eac2
Vancouver, BC, USA	http://www.meetup.com/open-science-network/events/229404794/
Thursday, March 31	
Brooklyn, NY, USA	http://www.meetup.com/Brooklyn-Biohackers/events/229845604/
London, GBR	http://www.meetup.com/Bioinformatics-London/events/228853443/
Melbourne, VIC, AUS	http://www.meetup.com/Melbourne-BioHack/events/229781364/
Oakland, CA, USA	http://www.meetup.com/Counter-Culture-Labs/events/229394888/
Oakland, CA, USA	http://www.meetup.com/Counter-Culture-Labs/events/229464856/
Sunnyvale, CA, USA	http://www.meetup.com/BioCurious/events/229527963/
Friday, April 1	
Montreal, QC, CAN	http://www.meetup.com/DIYBio-Montreal/events/229849965/
Saturday, April 2	
Austin, TX, USA	http://www.meetup.com/prophase/events/229857616/
Brooklyn, NY, USA	https://www.eventbrite.com/e/your-body-electric-diy-electromyography-workshop-tickets-22654993696?aff=mcivte&mc_eid=65a7191140&mc_cid=226babc13e
Cambridge, GBR	http://www.meetup.com/Cambridge-Synthetic-Biology-Meetup/events/227359402/
San Diego, CA, USA	http://www.meetup.com/The-Wet-Lab-a-DIYBio-maker-community-for-algae-enthusiasts/events/wfqqflyvgbdb/

DIY Biology
vs
Citizen Science

DIY Biology - self directed

Citizen Science - collaboration w/academics
non-experts collect data, analysis by experts

DIY Biology
vs
Citizen Science

DIY Biology - self directed

Citizen Science - collaboration w/academics
non-experts collect data, analysis by experts

Illumina genotyping
@ > 500K sites
GWAS: genome wide association study

42 GWAS studies in all

Shared genetic variants suggest common pathways in allergy and autoimmune diseases
Journal of Allegy and Clinical Immunology
A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci
Nature Genetics
Genome-Wide Analysis Points to Roles for Extracellular Matrix Remodeling, the Visual Cycle, and Neuronal Development in Myopia
PLOS Genetics
Genome-wide meta-analysis of cognitive empathy: heritability, and correlates with sex, neuropsychiatric conditions and cognition
Molecular Psychiatry
Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants
Nature Communications
Identification of genetic loci shared between schizophrenia and the Big Five personality traits
Scientific Reports
Meta-analysis identifies novel risk loci and yields systematic insights into the biology of male-pattern baldness
Nature Communications
GWAS of self-reported mosquito bite size, itch intensity and attractiveness to mosquitoes implicates immune-related predisposition loci
Hum Mol Genet

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (NIH GUIDELINES)

March 2013

- **BSL 1 – biosafety level 1**
- **No pathogens (animal, plant, human)**
- **No animal or human research**
- **No radionucleotides, ^{32}P ^{14}C, ^{3}H, etc.**

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

****

Visit the OBA Web site at:
http://oba.od.nih.gov
For current information on Guidelines, Protocols, Principal Investigators, Meetings, and information about upcoming Gene Therapy Policy Conferences

These *NIH Guidelines* shall be in effect on March 5, 2013 and on that date, shall supersede all earlier versions until further notice.

****

Antonie van Leeuwenhoek

1632–1723

draper, politician, surveyor, … first DIY microbiologist?

"...animalcules were in such enormous numbers, that all the water...seemed to be alive." — van Leeuwenhoek (1683)

DIY CRISPR kit

Overview | Reviews | Similar Products

PRODUCT DESCRIPTION

Want to really know what this whole CRISPR thing is about? Why it could revolutionize genetic engineering? This kit includes everything you need to make precision genome edits in bacteria at home including Cas9, tracrRNA, crRNA and Template DNA template for an example experiment.

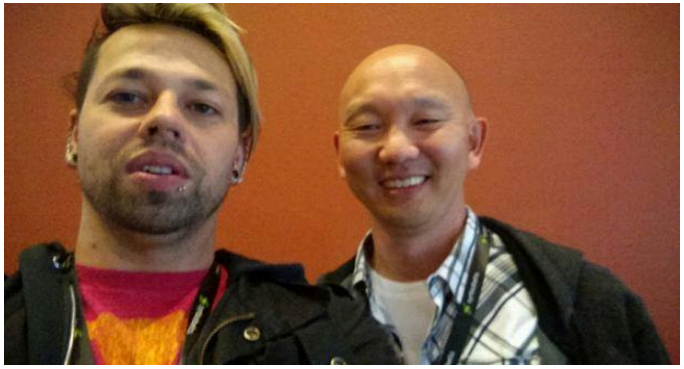
Includes example experiment to make a genome mutation(K43T) to the rpsL gene changing the 43rd amino acid, a Lysine(K) to a Threonine(T) thereby allowing the bacteria to survive on Strep media which would normal prevent its growth.

Kit contains enough materials for around 5 experiments or more

- Protocol Guide
- LB Agar
- LB Strep/Kan Agar
- Glass bottle for pouring plates
- *E. coli* HME63 strain
- Inoculation Loops
- 10-100uL variable volume adjustable pipette(1uL increments)
- Box of 96 Pipette Tips
- 14 Petri Plates
- Microcentrifuge tube rack
- 10 Pairs Nitrile Gloves
- 50 microcentrifuge tubes
- Bacterial transformation buffer 25mM CaCl2, 10% PEG 8000 5% DMSO
- LB Media for transformation recovery
- Cas9 and tracrRNA plasmid
- crRNA plasmid
- Template DNA

CRISPR-CAS9 – gene editing
Evolved as a bacterial defense against phages
Widely applicable in many eukaryotic systems
Highly efficient
Precise targeting at the bp level

http://www.the-odin.com/diy-bacterial-crispr-kit/



Josiah Zayner (ODIN; left) who is distributing CRISPR gene editing kits to the public through an Indiegogo funded project, and Edward You, Special Agent, FBI.

Ronin Genetics

Home Page
Protocols
Sequencing
Genotyping
Readings

An independent molecular genetics lab

established as non-profit in 2005

any questions, contact tarandall at gmail.com or
tarandall at roningenetics.org

"night science": a stumbling, wandering exploration of the natural world that relies on intuition as much as it does on the cold, orderly logic of "day science." In today's vastly expanded scientific enterprise, obsessed with impact factors and competition, we will need much more night science to unveil the many mysteries that remain about the workings of organisms.

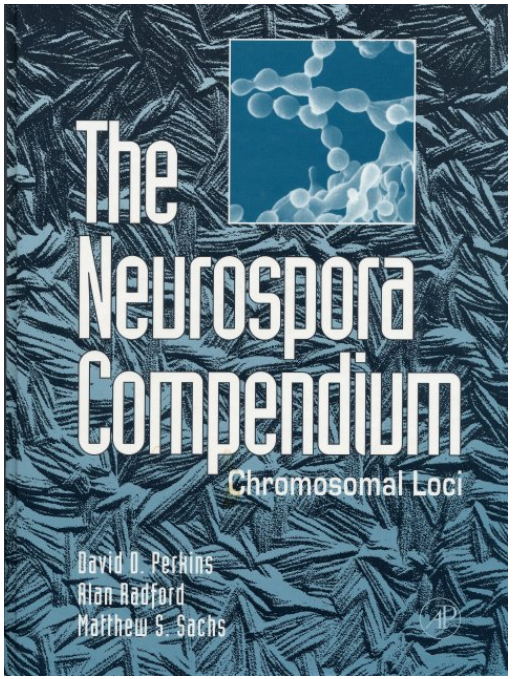
Francois Jacob, Science 332: 767

Recent events

SciTech Expo 2016 w/TriDIYbio
http://www.tridiybio.org/home.html
click on link under "Recent News"

Interview with Ernie Hood of Radio In vivo
https://radioinvivo.org/2016/04/27/diy-biology/

http://www.roningenetics.org/



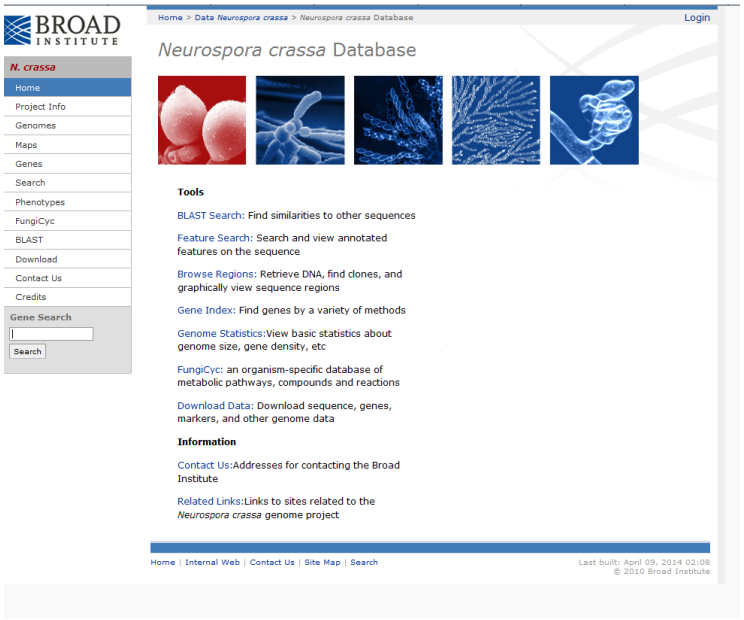
Neurospora crassa

~12,000 genes, 40 Mb genome
haploid, seven chromosomes
>1000 genetic loci mapped
KO project > 6000 knockouts available

A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors

Hildur V. Colot*†, Gyungsoon Park†‡, Gloria E. Turner§, Carol Ringelberg*, Christopher M. Crew‡¶, Liubov Litvinkova‡, Richard L. Weiss§, Katherine A. Borkovich‡, and Jay C. Dunlap*‖

PNAS 103:10352



www.fgsc.net

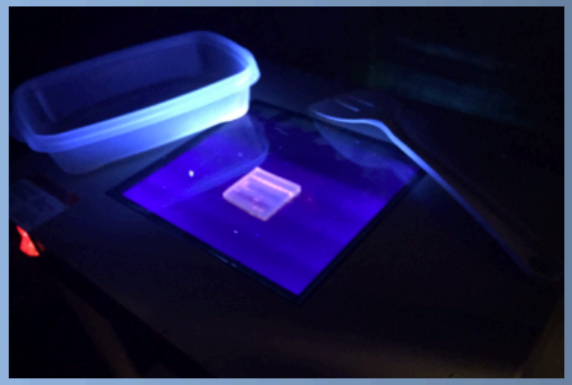
So we started a group:

Triangle DIY Biology

Our Goals are to:

- Bring together and connect anyone interested in DIYBio and citizen science in the local Raleigh, Durham, Chapel Hill area
- Provide a space for group projects, exploration, and experimentation
- Allow anyone to learn (or practice) their lab skills while doing real, hands-on projects
- Promote a broader understanding of science and biotechnology as it continues to apply more and more to our everyday lives

Triangle DIY Biology: Community Citizen Science and DIYBio Group of the NC Triangle



Home | About Us | Contact Us | Events/Outreach | Safety | Resources | Workshops | Archives

Upcoming Events:

- July 3rd Sunday TriDIYBio meeting at SplatSpace: Sign up via our Meetup here.
- July 14 at STaRS (Summer Teachers at Research Summer @NIEHS) TriDIYBio will present "DIY Biology in the Triangle"

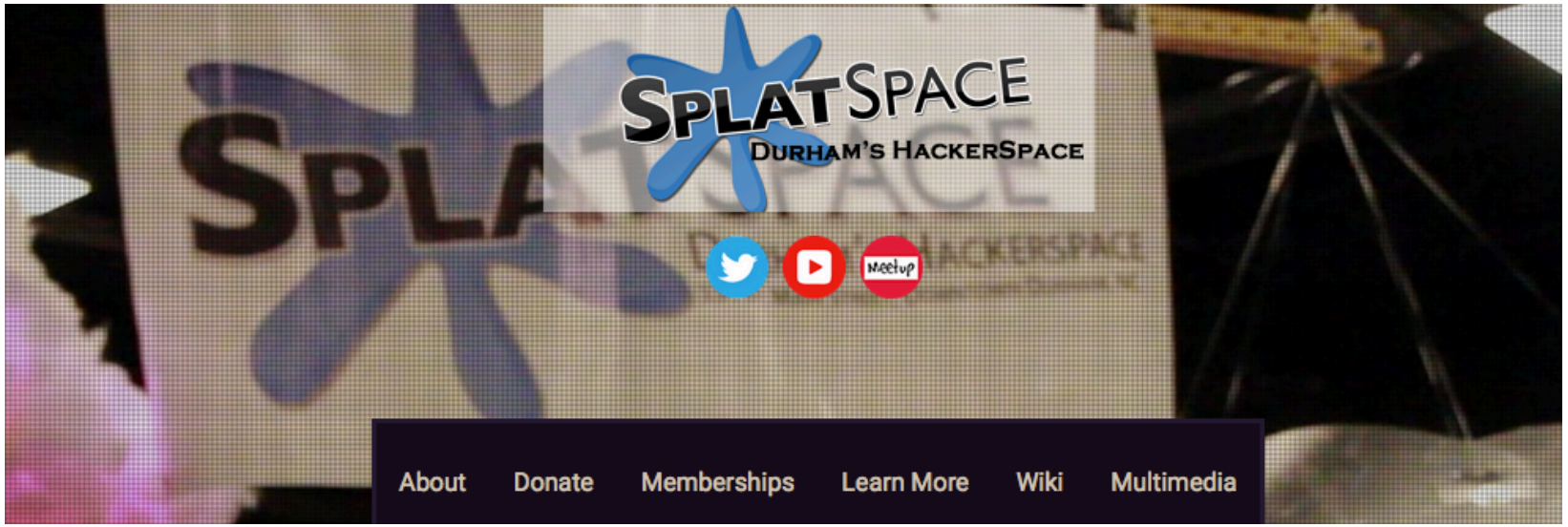
Interesting Science News:

- Radio In Vivo Local Science News; new interviews every month or so. Past guests include Dr. Rodolphe Barrangou, NCSU CRISPR expert and us at TriDIYBio

Spring-summer 2015

tridiybio.org

8-10 core members currently
60+ communicate via Slack
Monthly meetings at **SplatSpace**



http://splatspace.org/ Old 5 Points area of Durham

Member supported community hacker/maker space
Collection of expensive tools available to a range of people to use and/or learn
Usually some members with expertise in various fields collaborating on projects

3D printing
Metalwork
Woodwork
Laser cutting
Programming/computers/arduinos
etc…

Since June 2016 - TriDIYBio

TriDIYBio Outreach Events

SciTech Expo @ Museum of Natural Science, Raleigh, 2016
SciTech Expo @ Museum of Natural Science, Raleigh, 2017
Building with Biology @ Museum of Life Sciences, Durham
March for Science, Raleigh, Apr 2017

- Microbiome sampling
- GFP painting
- Smartphone microscope
- Agar gel electrophoresis
- Mudwatt battery
- DIY equipment

TriDIYBio Outreach Events

SciTech Expo @ Museum of Natural Science, Raleigh, 2016
SciTech Expo @ Museum of Natural Science, Raleigh, 2017
Building with Biology @ Museum of Life Sciences, Durham
March for Science, Raleigh, Apr 2017

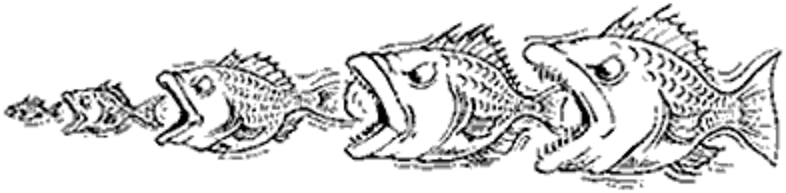
- Microbiome sampling
- GFP painting
- Smartphone microscope
- Agar gel electrophoresis
- Mudwatt battery
- DIY equipment

Workshops @ SplatSpace

- Using pipettemen
- Running agarose gels
- Microbiological Techniques

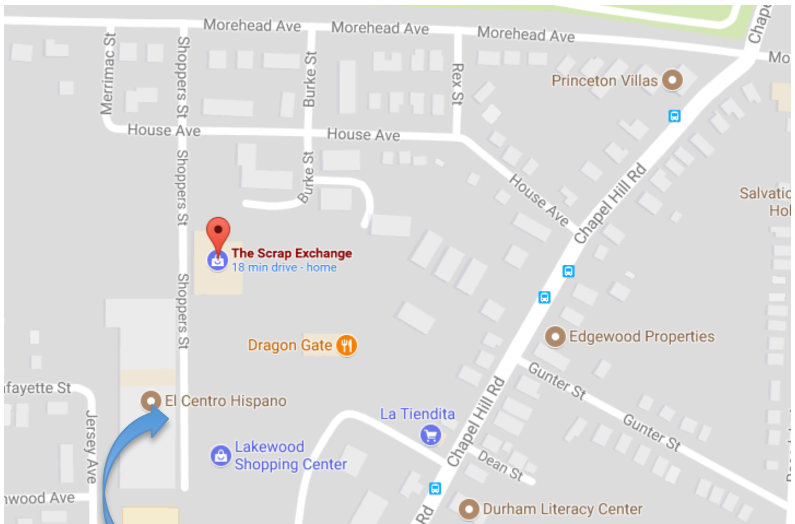
Seminars @ SplatSpace

- Current Issues In Agricultural Biotechnology, Edward Richards
- Gene editing with CRISPR: What is CRISPR and why is it important?, Tom Randall
- Deep Coalbed Biosphere off Shimokita, Elizabeth Trembath-Reichert



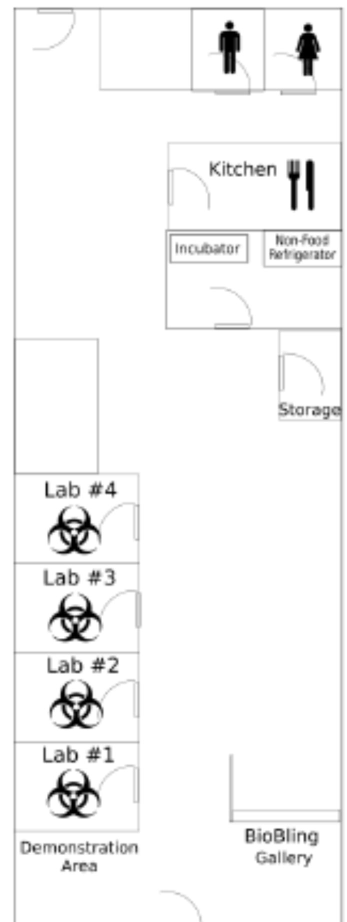
TriDIYBio > SplatSpace > Scrap Exchange

THE SCRAP EXCHANGE 1991 26 YEARS 2017 CREATIVE REUSE ARTS CENTER / DURHAM NC



RADLab @ Lakewood

Reuse Arts District: Suite 25



TriDIYBIO
RADLab

Suite 25

2020 Chapel Hill Road

Evolution as a Tool

A new technology

for protein engineering

Peter Reintjes

Innatrix Inc.

SPLATSPACE
DURHAM'S HACKERSPACE

TriDIYBio

INNATRIX INC.

Dr. Marshall Hall Edgell, PI
Professor Emeritus UNC-CH, CEO of Innatrix

Peter Reintjes
Dr. Martha Collier

RTP Headquarters – 12 Davis Drive Research Triangle Park

Use a bacterial virus (phage) to evolve a custom protein

PACE - **P**hage **A**ssisted **C**ontinuous **E**volution

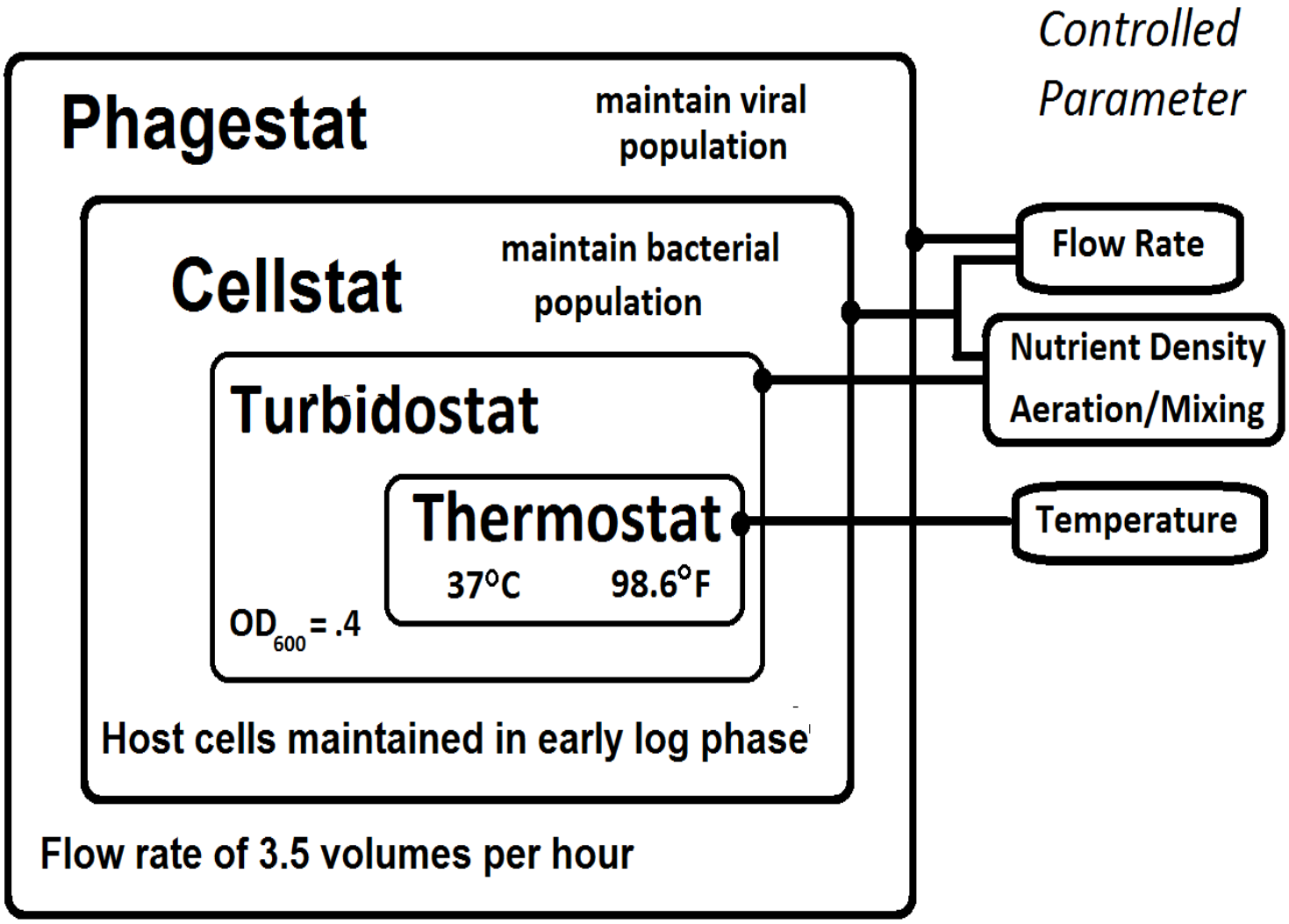
Esvelt et al. A system for the continuous directed Evolution of biomolecules
Nature 472, 499, April 2011

*You need to **build a Phagestat** to maintain a population of evolving virus (Husimi 1989)*

Off-the-shelf, manually-operated hardware for about $30,000

- or -

DIY hardware + open source ~$1000


Controlled Parameter
Phagestat
maintain viral population
Cellstat
maintain bacterial population
Turbidostat
Thermostat
37°C
98.6°F
OD600 = .4
Host cells maintained in early log phase
Flow rate of 3.5 volumes per hour
Flow Rate
Nutrient Density
Aeration/Mixing
Temperature

Motivation

- Shigella kills 1,000,000 people – mostly children in the developing world – every year
- Shigella without the extracellular proteases **Pic** and **SepA** is harmless
- *Hypothesis*: We can evolve protease inhibitors with strong binding to **Pic** and **SepA** to diminish Shigella's virulence
- *Hypothesis*: These engineered proteins produced by a probiotic *(lactobacillus)* could provide inexpensive, long-term immunity

Potential applications of PACE:

Protein-based pharmaceuticals

- Specificity: reduced side-effect potential
- Proteins are easily metabolized
- Environmentally friendly: Proteins degrade quickly in the waste stream
- Binding affinity is the principal characteristic of metabolic processes and pharmaceuticals
- Increased binding affinity lowers dosage; sub nM binding affinity possible

PACE: Phage Assisted Continuous Evolution

Evolution = Variation + *Selection*

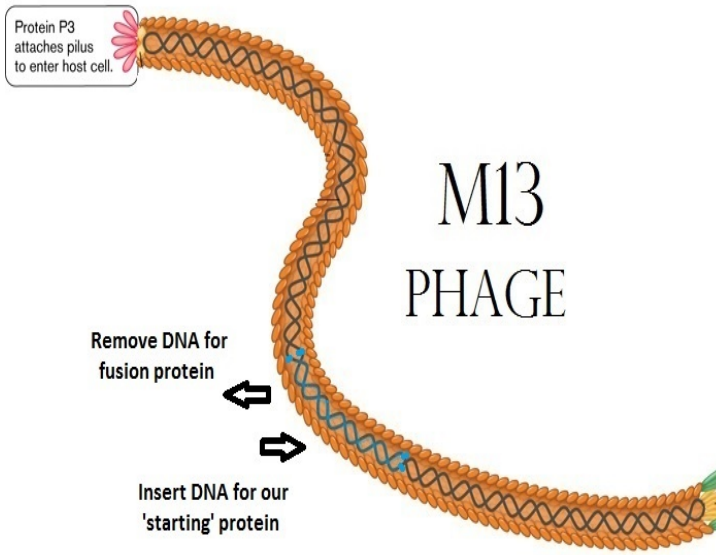
1) Phage with the sequence to evolve replacing its fusion gene

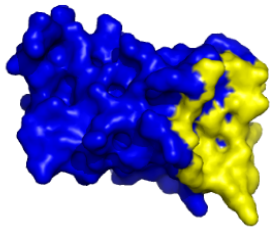
2) *E. coli* with two extra plasmids

Variation: Error-prone DNA polymerase within *E. coli*

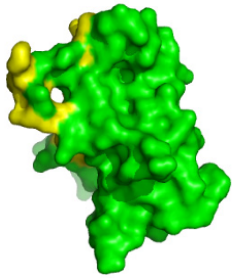
Selection: Create fusion protein when the evolving protein binds to a target

(tighter binding causes more fusion protein to be made)





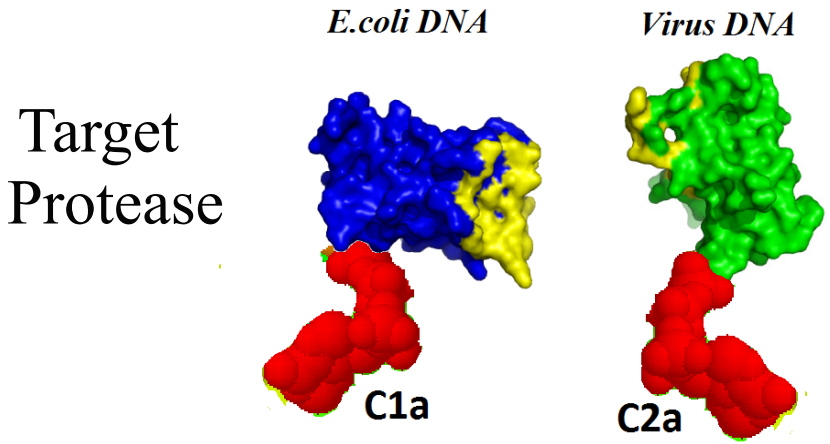
Target Protease



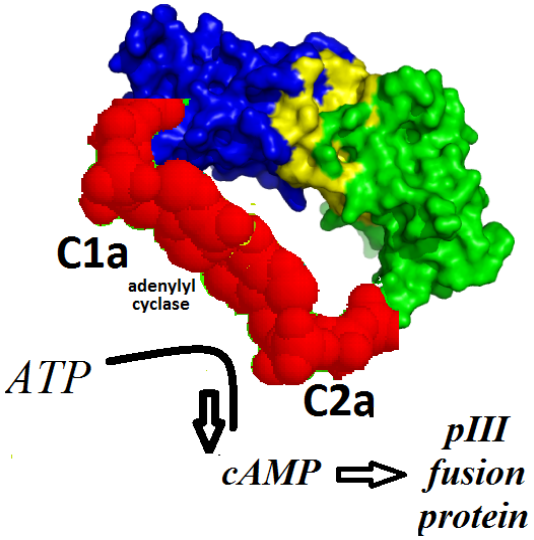
Weak Protease Inhibitor (e.g. Ecotin)

Put fusion protein (pIII) in *E.coli*

Two-hybrid System



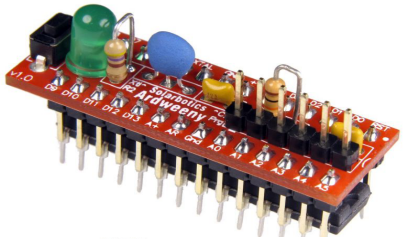
C1a, C2a; subdomains of adenylate cyclase

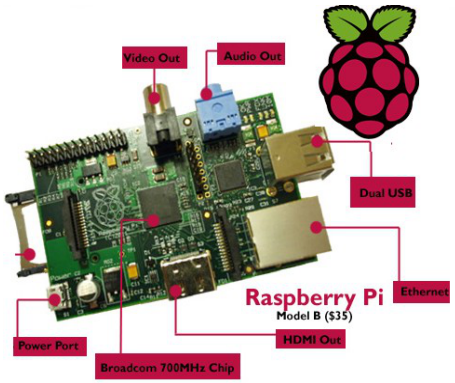


- Production of cAMP inducible pIII fusion protein essential for M13 infection
- Bacteria makes mistakes when copying the viral DNA
- Viruses are released in proportion to how tightly the evolving protein binds with the target

M13 generation time 15 min; many rounds of evolution possible quickly

Raw Materials





Arduino micro-controller

Raspberry Pi / Linux

Webcam

Python programming language

OpenCV image processing software

PIR (Passive InfraRed) temp sensor

LEDs, resistors, motors, magnets

Discarded flatbed scanner

Styrofoam shipping containers

PVC plumbing hardware

3D printer

THE SCRAP EXCHANGE 1991 25 YEARS 2016 CREATIVE REUSE ARTS CENTER / DURHAM NC

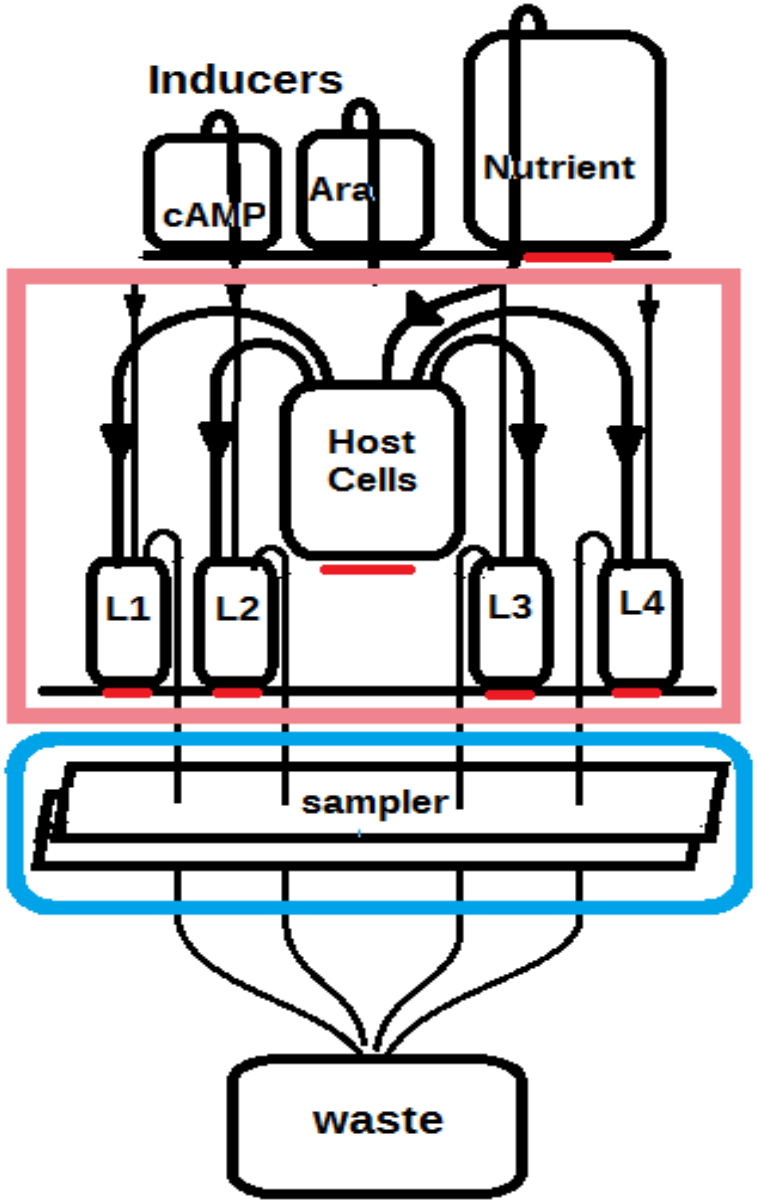
http://scrapexchange.org/

Material Costs for a phagestat

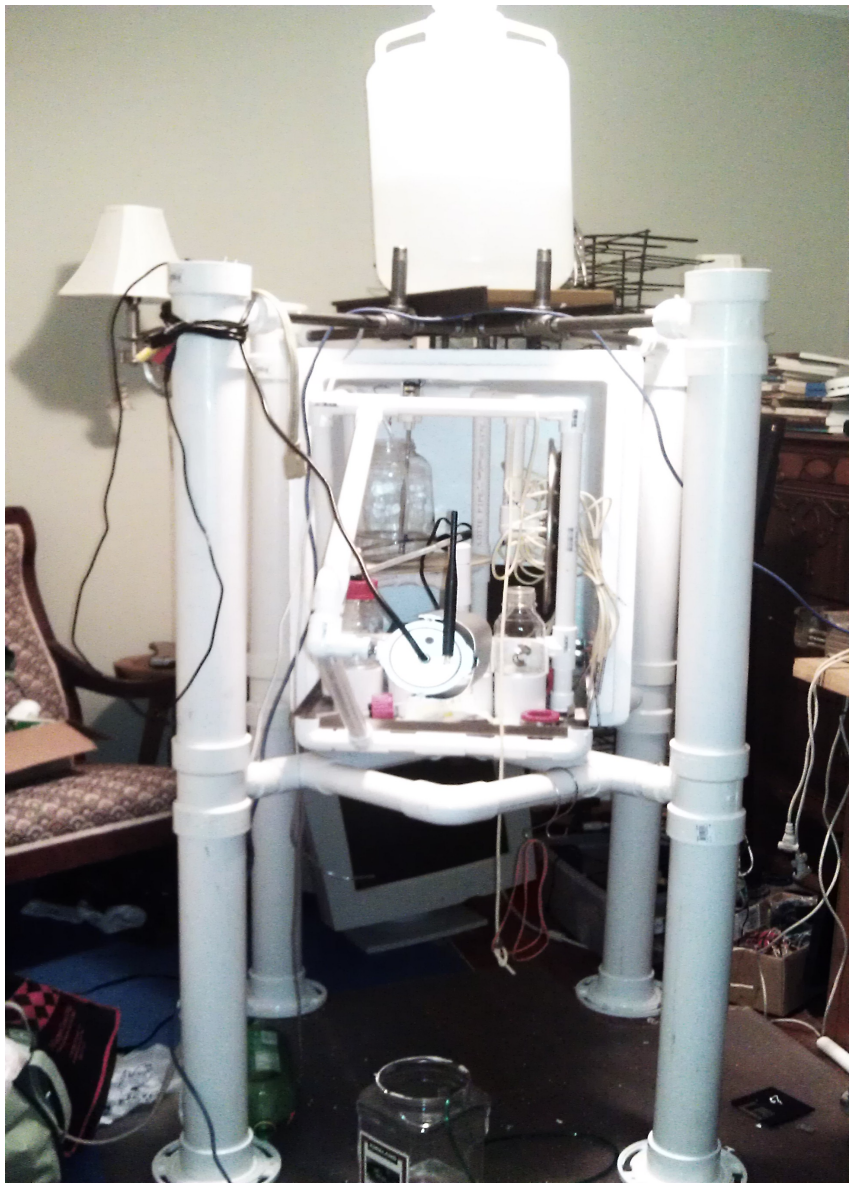
- Raspberry PI main computer + SD card ($40)
- Wide-angle USB Camera ($40)
- 6X Arduino + Bluetooth ($36)
- PIR (passive infra-red) temperature sensor ($35)
- Laser, LEDs, Photo transistor ($20)
- Styrofoam boxes ($20 X 2)
- Heating Element ($25)
- Stirring Motors w/magnets ($5 X 5)
- Aquarium air pump ($35)
- Valves ($50)
- Miscellaneous Hardware-PVC ($180)
- 5- 12-V Power Supply ($30)
- Glassware ($200)
- Tubing + Nutrient (operating cost)

< $800

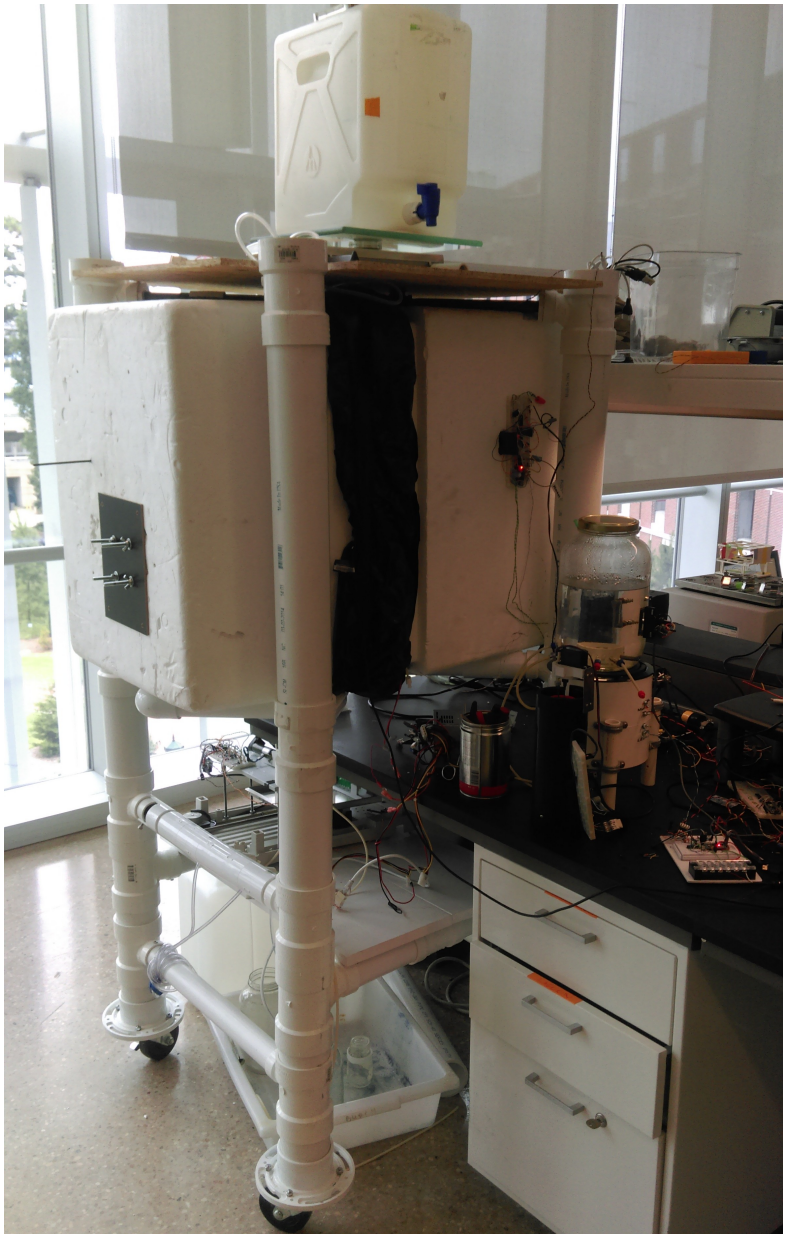
schematic



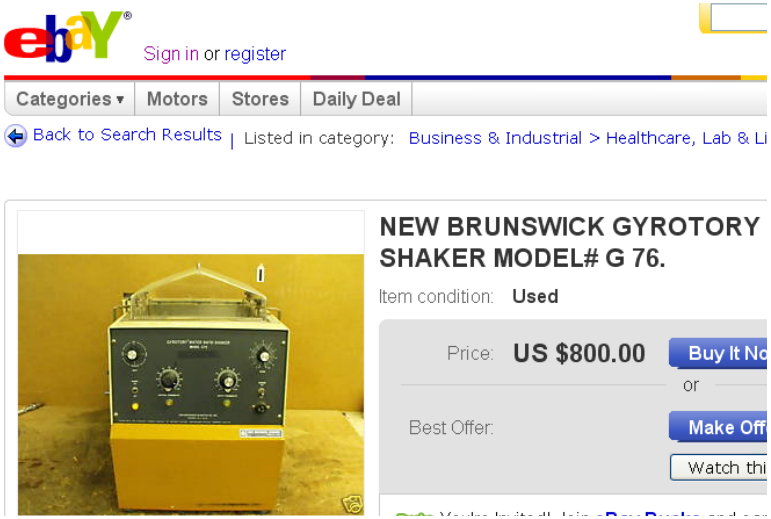
version 1.0

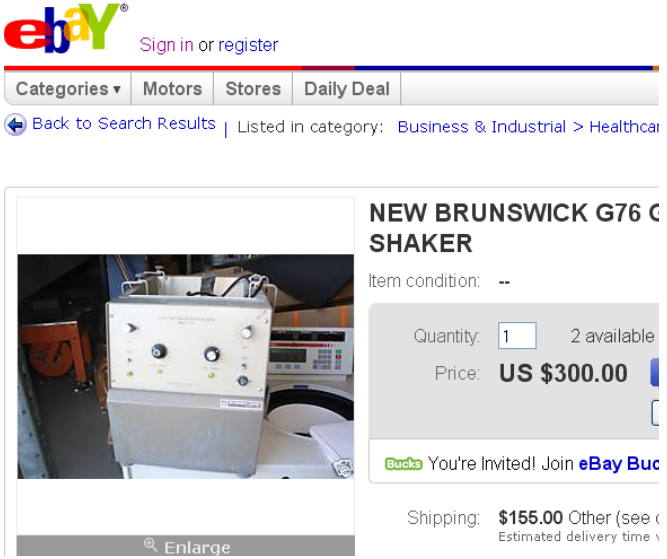


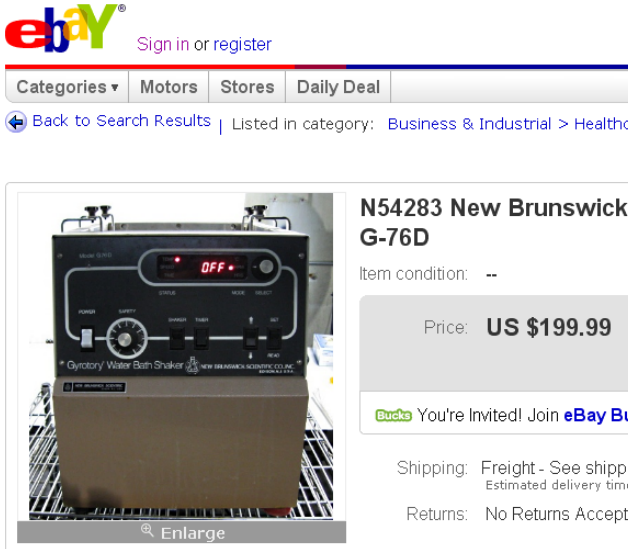
Phagestat v2.0



Shaken, not stirred

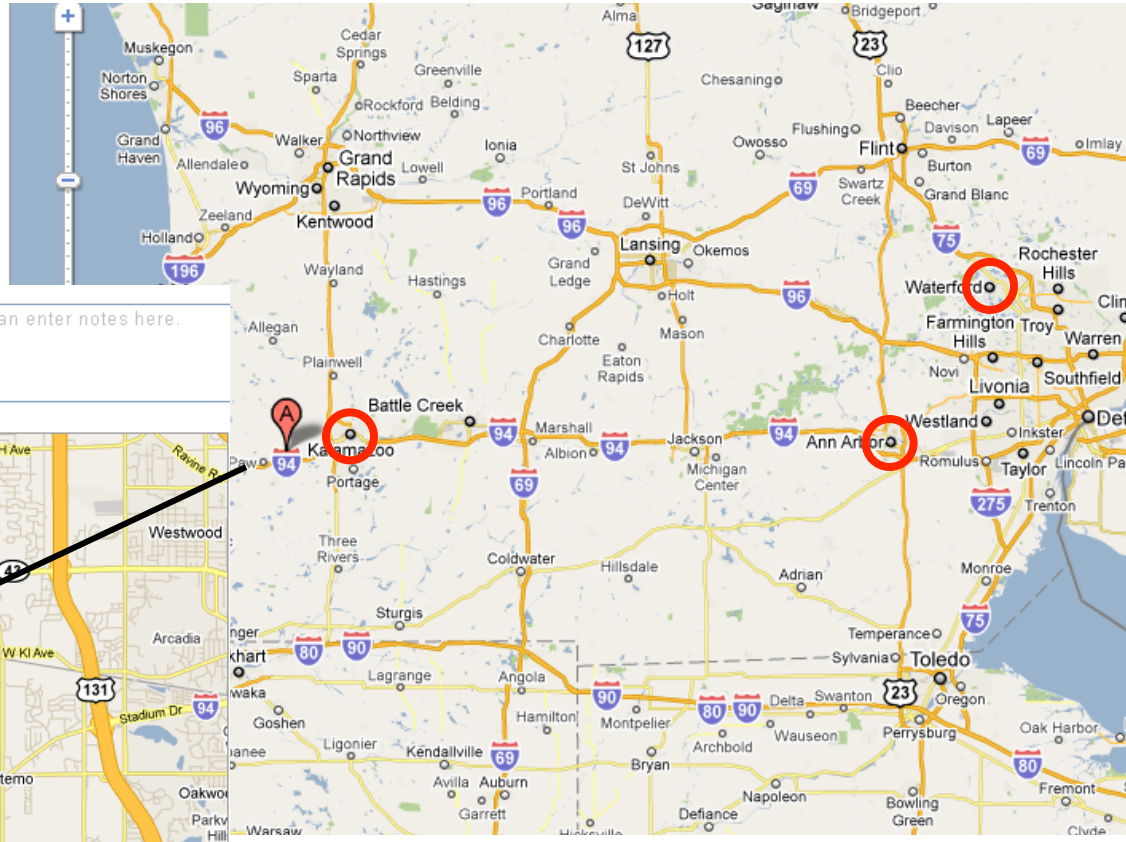






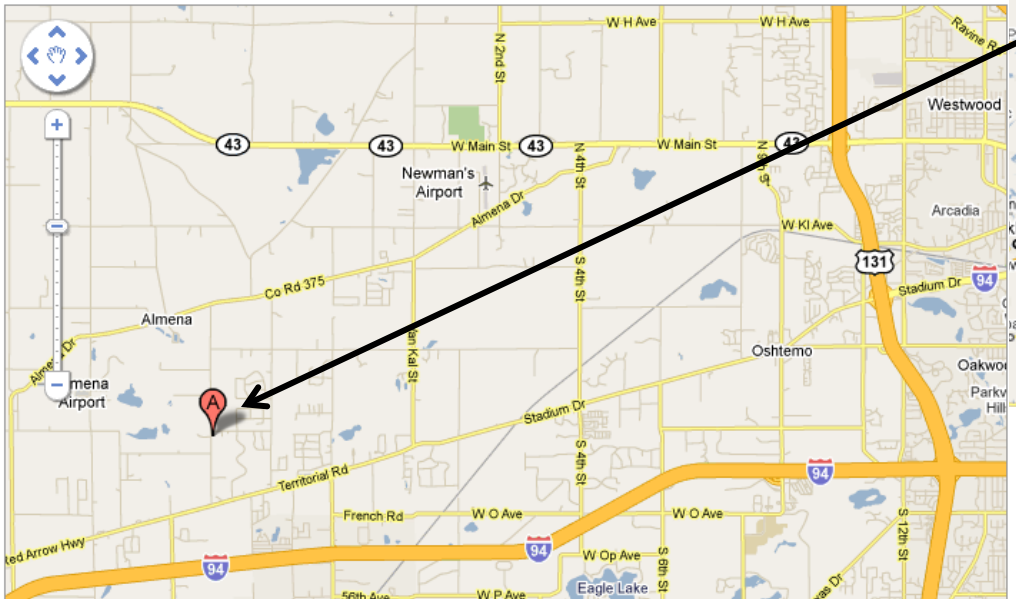
Road Trip

UpJohn (Kalamazoo) > Pharmacia > Pfizer > Inventory Reduction > Joe's garage in Mattawan, MI In July 2009



Google maps Address **48430 27th St Mattawan, MI 49071**

Notes You can enter notes here.



Star Trek: The Arena

“the planet's surface has sufficient raw materials to build a weapon”

Black powder: sulfur, charcoal, KNO_3 (saltpeter)

Borax
$Na_2B_4O_7 \cdot 10H_2O$

(MiraLAX)
Polyethylene glycol 3350

Epsom salts
$MgSO_4$

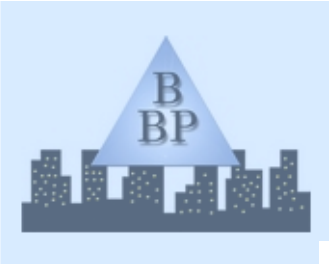
GNC
LIVE WELL

Rubbing alcohol
91% Isopropanol

White vinegar
6 % acetic acid

95% EtOH

Agar agar
Bacto-agar subsitute



Boston BioProducts

Reagents for Biosciences

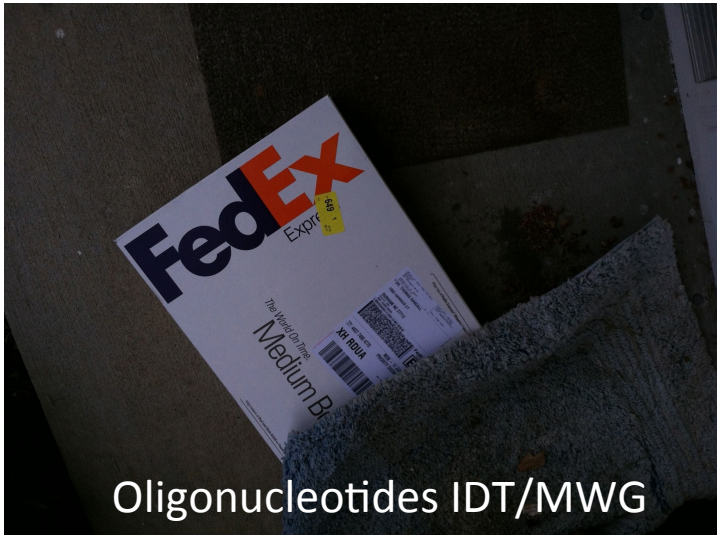
Chemsavers

CAROLINA®

Home delivery

DNA 2.0

DNA synthesis



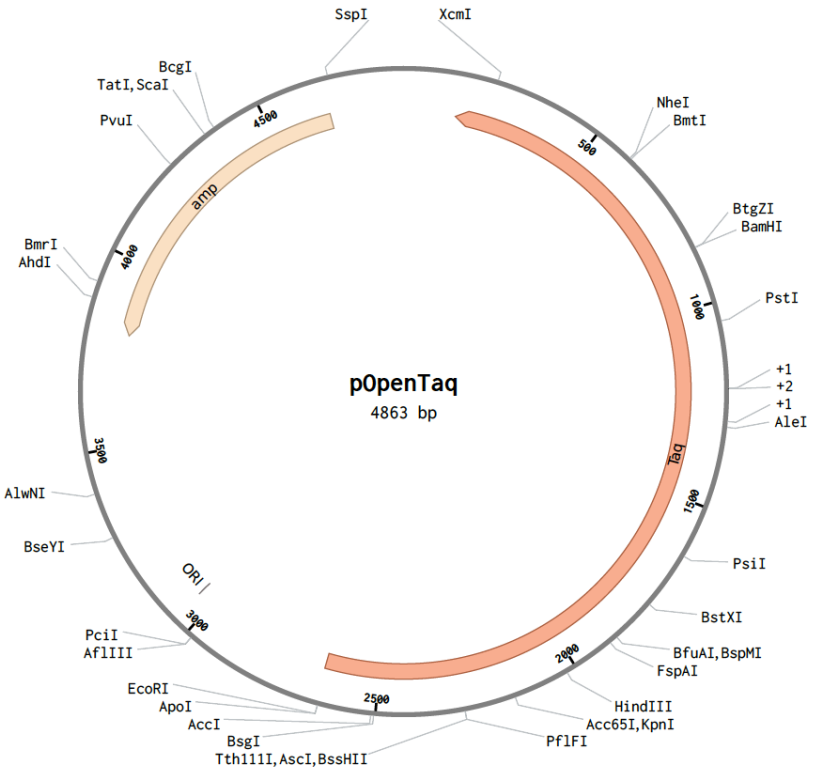
Oligonucleotides IDT/MWG

Make your own Taq polymerase

For PCR (polymerase chain reaction)

https://www.geneandcell.com/products/taq-polymerase-plasmid

pOpenTaq (4863 bp)



Expression

Transform E. coli BL21(DE3) with pOpenTaq
Any standard method is fine, such as chemical or electrotransformation.

Transfer the transformed cell mixture into LB containing 100 mg/L ampicillin
It is not necessary to select individual clones.

Grow the culture overnight.
At 37C under shaking. This is the starter culture.

Transfer 20 ml of the starter culture into fresh LB/amp for each 1 l of expression culture.
The precise amounts may vary.

Grow the expression culture until its OD600 reaches 70% of the starter culture.
Grow under heavy shaking. Good aeration is important in this step to make the healthiest cells you can have. Measure the OD using any spectrophotometric device. The precise wavelength is unimportant. Any visible wavelength will work, as long as you use the same wavelength on both expression culture and starter culture. The precise induction point has some room for error. Anything 50% to 90% of the starter culture will work almost equally well. It should take 3-5h to reach the 70% value.

Induce the culture overnight with 1 mM IPTG.
Time your day so that this overnight step will go for 8-16 hours. We have not found any differences in this time frame, and have not tried other times. If you are short on IPTG, it's OK to use less. We have successfully expressed the polymerase with as little as 0.05 mM IPTG final concentration (This will not generally work on other proteins! pOpenTaq seems to be somehow special in this regard. We don't know why).

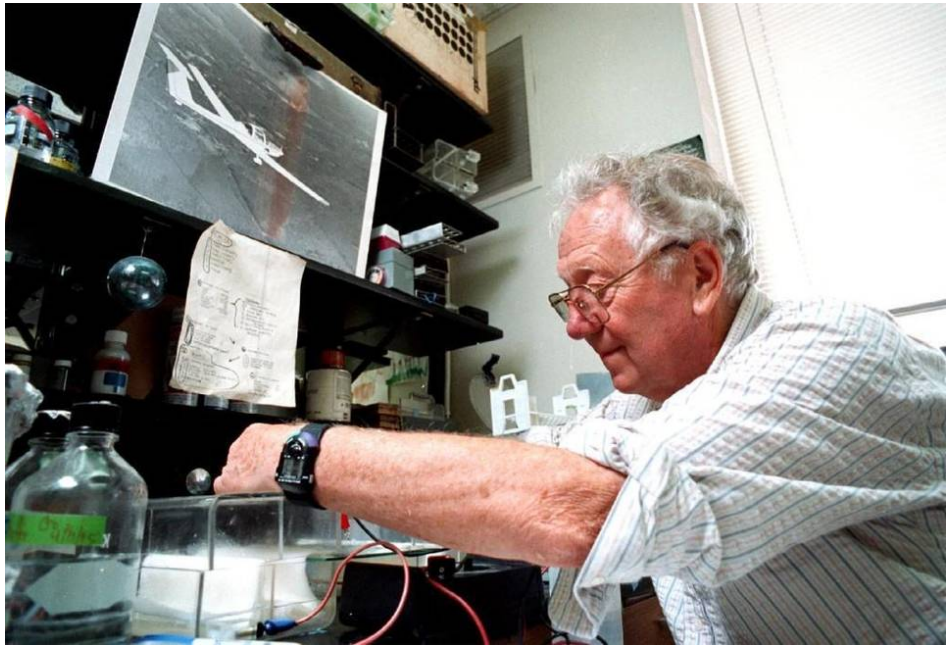
NIEHS “ReUse” Center

Starch gel electrophoresis

Separation of proteins by size

Resolution not so good

Smithies, O., Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults (1955) Biochem J 61: 629



Oliver Smithies

The Nobel Prize in Physiology or Medicine 2007
Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies

Share this: 10

Oliver Smithies - Facts

Photo: U. Montan

Oliver Smithies

Born: 23 June 1925, Halifax, United Kingdom

Died: 10 January 2017, Chapel Hill, NC, USA

Affiliation at the time of the award: University of North Carolina, Chapel Hill, NC, USA

Prize motivation: "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Field: genetics

Prize share: 1/3

Teacher Make & Take: Gel Electrophoresis

Are gel electrophoresis labs too expensive to do with your students? Come to the Micro World Investigate Lab and learn how to make a DIY gel electrophoresis unit out of used tip boxes, 9 volt batteries and paperclips. Also explore alternatives to using agarose for the separation matrix. Materials will be provided so that each participant takes a working electrophoresis unit back to their classroom.

From workshop done by Christy Flint, Nov 2015

Nature Research Center
NC Museum of Natural Science

MICRO WORLD ILAB

HOME > LEARN > INVESTIGATION > **MICRO WORLD INVESTIGATION LABORATORY**

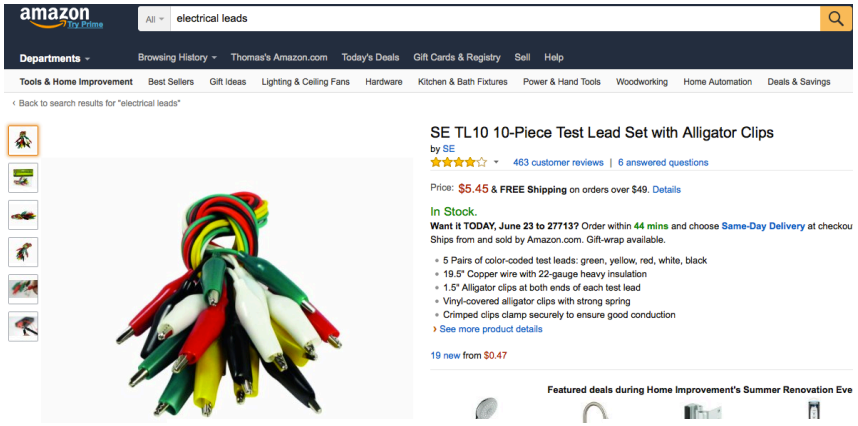
ON A MICROSCOPIC SCALE

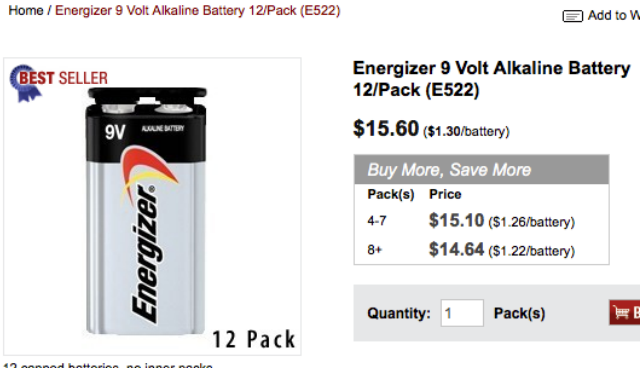
The Micro World Investigate Lab is a hands-on science education lab where the public is encouraged to discover nature on a microscopic scale. Using state-of-the-art scientific tools and techniques, visitors explore topics ranging from cellular processes such as photosynthesis and bioluminescence to how researchers isolate and analyze molecules essential for life such as DNA and proteins. Up to seven different hands-on activities are available during public hours. While the topics presented in the lab are targeted at middle school students and up, adults with younger children are encouraged to work with and make connections for their budding scientists.

Public Hours
Sunday, 1–4 pm
Monday, 10 am–1 pm
Tuesday–Saturday, 10 am–4 pm

Please note: The Micro World Investigate Lab closes to the public during registration-based programs, therefore the hours listed above are subject to change without notice. Before your visit to the lab, contact the Micro World Investigate Lab at 919.707.8090 for updated information.

Building your own gel electrophoresis box





or