DIYBio in the Triangle

STaRS, 2017, NIEHS

Tom Randall
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On behalf of Triangle DIY Biology
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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

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)

DIYbio.org was founded in 2008 with the mission of establishing a vibrant, productive and safe community of DIY biologists. Central to our mission is the belief that biotechnology and greater public understanding about it has the potential to benefit everyone.

- Get an overview of current events from the blog
- Or dive into the global discussion
- Find local groups, people and meetups near you
- Review the codes of ethics
- Ask a biosafety expert your safety question
- Get the diybio logo and contact info

DIYbio.org is a 501(c)(3) charitable organization. Donations are tax-deductible to the extent permitted by law.

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 reagents/strains

Outreach/education to public/schools
It's A World-Wide Thing

Local Groups

NORTH AMERICA
Baltimore  MD  http://www.bugsontline.org/
Berkley  CA  http://berkeleybiolabs.com/
Bethesda  MD  http://www.meetup.com/CapitolAreaBioSpace/
Boston  MA  http://boslab.org/
Brooklyn  NY  http://gensepace.org/
Cambridge  MA  http://openwetware.org/wiki/MIT_DIYbio
Carlsbad  CA  http://biotechbeyond.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.roningenerics.org/
Guadajato  MX  https://www.facebook.com/groups/DIYbioMexico/
Houston  TX  http://www.brightworkresearch.com/
Jackson  MS  http://www.divineurotech.com/
La Jolla  CA  http://sajillilibrary.org/your-library/bio-lab/
Los Alamos  NM  http://biolab.net/
Los Angeles  CA  http://www.biohackers-la/
Montreal  QC  http://biocaps.ca/
New York City  NY  http://www.meetup.com/biobockers-NYC/
New York City  NY  http://harlequinbio.com/
Norfolk  VA  http://www.biologixlabs.org/
Oakland  CA  http://counterculturelabs.org/
Orlando  FL  http://familab.org/
Portland  OR  ???
Research Triangle Park  NC  http://www.tridylbio.org/

Sunday, March 27

Baltimore, MD, USA  http://www.meetup.com/CapitolAreaBioSpace/events/229236421/

Oakland, CA, USA  http://www.meetup.com/Counter-Culture-Labs/events/229763788/

Monday, March 28

Asilomar, CA, USA  http://www.meetup.com/Prophase/events/229656020/

San Vicente, CA, USA  http://www.meetup.com/BioCurious/events/229330970/

Tuesday, March 29

Amsterdam, ND, USA  http://www.meetup.com/Dutch-DIY-Bio/events/229350968/

Asilomar, CA, USA  http://www.meetup.com/Prophase/events/229856995/

Minneapolis, MN, USA  http://www.meetup.com/MN-diyBio/events/229427535/

Wednesday, March 30

Mountain View, CA, USA  http://www.meetup.com/Silicon-Valley-Computational-Biology-Meetup/events/229415019/

Savannah, GA, USA  http://www.meetup.com/iolab/events/229557316/

Thursday, March 31

Brooklyn, NY, USA  https://www.eventbrite.com/e/crispr-workshop-beyond-the-hype-tickets-22712426479

Heritage Park, NJ  http://www.eventbrite.com/e/tickets-agr锈t Meadow tickets-22713996378

Vancouver, BC, Canada  http://www.eventbrite.com/e/open-science-network/events/229802679/

Friday, April 1

Brooklyn, NY, USA  http://www.meetup.com/nybiobrooklyn/events/229895607/


Melbourne, VIC, Australia  http://www.meetup.com/Melbourne-Biotech/events/229783364/

Oakland, CA, USA  http://www.meetup.com/Counter-Culture-Labs/events/229551488/

San Vicente, CA, USA  http://www.meetup.com/Prophase/events/229537961/

Saturday, April 2

Asilomar, CA, USA  http://www.meetup.com/Prophase/events/229837616/

Brooklyn, NY, USA  https://www.eventbrite.com/e/your-body-electric-day-emmy-worshops-tickets-22659993696


San Diego, CA, USA  http://www.eventbrite.com/e/the-wet-labs-at-cybio-maker-community-for-algae-enthusiasts-tickets-22610590118
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

42 GWAS studies in all

- Shared genetic variants suggest common pathways in allergy and autoimmune diseases
  Journal of Allergy 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

Illumina genotyping
@ > 500K sites
GWAS: genome wide association study
• BSL 1 – biosafety level 1
• No pathogens (animal, plant, human)
• No animal or human research
• No radionucleotides, $^{32}$P, $^{14}$C, $^{3}$H, etc.
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)
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.
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


PNAS 103:10352
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
Spring-summer 2015

triدييبيو.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

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
Evolution as a Tool
A new technology for protein engineering

Peter Reintjes
Innatrix Inc.

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 Drive Research Triangle Park
Use a bacterial virus (phage) to evolve a custom protein

PACE - Phage Assisted Continuous Evolution

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
Phagestat

Cellstat

Turbidostat

Thermostat

$\text{OD}_{600} = 0.4$

37°C  98.6°F

Host cells maintained in early log phase

Flow rate of 3.5 volumes per hour

Controlled Parameter

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)

Put fusion protein (pIII) in *E. coli*
Two-hybrid System

Target Protease
Evolving Protein

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

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

Inducers
- cAMP
- Ara
- Nutrient

Host Cells

L1, L2, L3, L4

Sampler

Waste
Shaken, not stirred
Road Trip

UpJohn (Kalamazoo) > Pharmacia > Pfizer > Inventory Reduction > Joe’s garage in Mattawan, MI
In July 2009
Star Trek: The Arena

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

Black powder: sulfur, charcoal, KNO$_3$ (saltpeter)
Borax
$\text{Na}_2\text{B}_4\text{O}_7\cdot10\text{H}_2\text{O}$

(MiraLAX)
Polyethylene glycol 3350

Epsom salts
$\text{MgSO}_4$

Rubbing alcohol
91% Isopropanol

White vinegar
6% acetic acid

95% EtOH

Agar agar
Bacto-agar substitute
Home delivery

DNA synthesis

Oligonucleotides IDT/MWG
Make your own Taq polymerase
For PCR (polymerase chain reaction)
https://www.geneandcell.com/products/taq-polymerase-plasmid

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

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 37°C 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


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"
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

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.

Nature Research Center
NC Museum of Natural Science
Building your own gel electrophoresis box

or