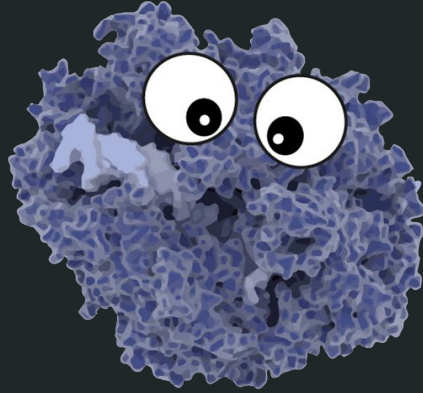


# Friendzymes



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Let's Democratize  
The Means of Biotechnological Production

Isaac Larkin

**We need a massive economic,  
political and technological  
mobilization to combat the climate  
crisis and build a good future.**

# **We Need to Accelerate the Democratization and Distribution of Biological Technologies **Now****

**We need to build a just, resilient and carbon negative civilization in 10-30 years.**

**Biological technologies, everywhere and at every scale, will be required.**

**Right now, we don't have anywhere near enough biotechnological practitioners or productive capacity to achieve this transformation.**

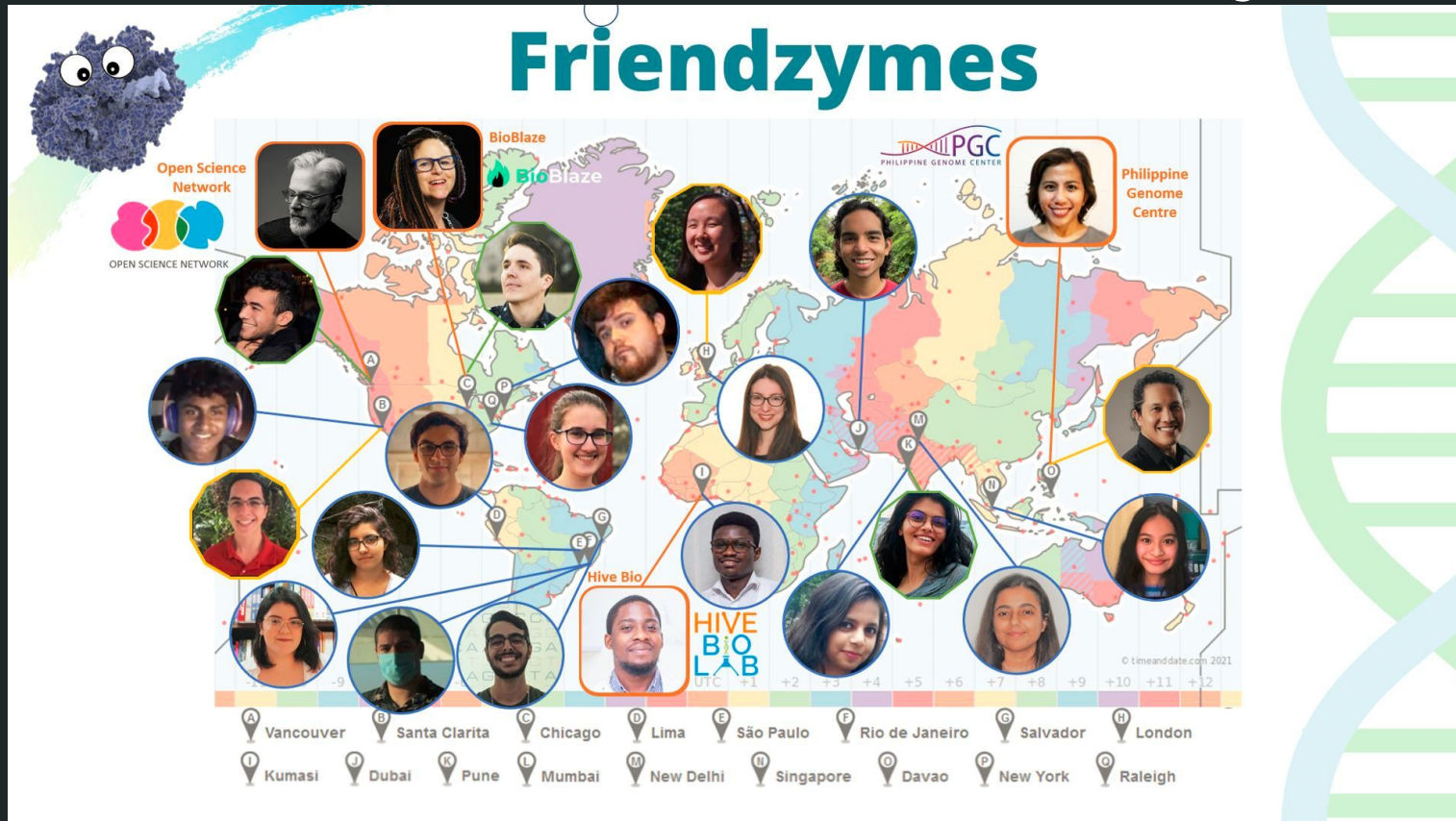
# **Bioengineering and Synthetic Biology Have Been Difficult and Expensive**

- **Cost of Equipment**
- **Cost of Reagents**
  - Especially synthetic DNA and enzymes
- **Cost (money & time) of licensing patents  
and material transfer agreements**
- **Cost (money & time) of developing skills**

# **How Do We Make Biotechnological Production Scalable, Open, and Frugal?**

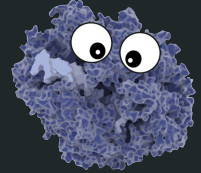
- **Capable of going from benchtop tinkering to large-scale supply of a good or service**
- **Public domain, open-source, shareable hardware, software, wetware, protocols**
- **As cheap and easy as possible**

# Friendzymes: An International Team and Project



# Friendzymes: An International Team and Project

- **Goal:** Frugal, open, scalable manufacturing of useful proteins
  - Starting with enzymes required for biological engineering
- **Goal:** Frugal, high capacity, (mostly) automated, open biofoundries for high-throughput biological design-build-test-learn-scale cycles



# What Enables Friendzymes?

## OpenMTA

### An Open-Source License for Biotechnology



**Gives recipient permission to copy, modify, redistribute, and commercialize biotechnological materials**

**Enables free and easy sharing of biological material between academia, industry, and everyone else**



**Led by Linda Kahl**



# What Enables Friendzymes?

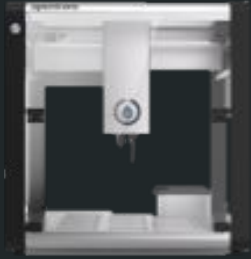
## The FreeGenes Project

### GNU/Linux for Biotechnology

- Libraries of off-patent/IP-free synthetic genetic parts, distributed under the OpenMTA for free
- Anyone can contribute new free and open wetware libraries
- Re-synthesizing the iGEM registry for 2022 for distribution under the OpenMTA

# What Enables Friendzymes?

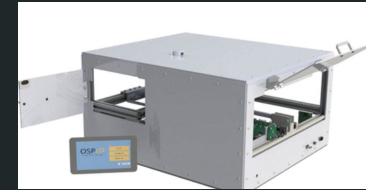
## Cheap and/or Open-Source Lab Hardware



**OpenTrons OT-2  
Liquid Handler**  
~\$5,000-\$10,000



**Oxford Nanopore MinION  
DNA sequencer**  
\$1,000



**\$3500**

### **An Open-Source Plate Reader**

Karol P. Szymula, Michael S. Magaraci, Michael Patterson, Andrew Clark, Seville G. Mannickarottu, and Brian Y. Chow\*

[Cite this: \*Biochemistry\* 2019, 58, 6, 468–473](#)

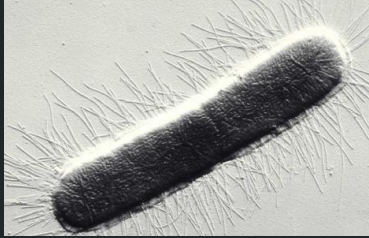
[Article Views](#)

[Altmetric](#)

[Citations](#)

# Challenge: E. coli Requires Expensive Equipment to Extract Recombinant Protein

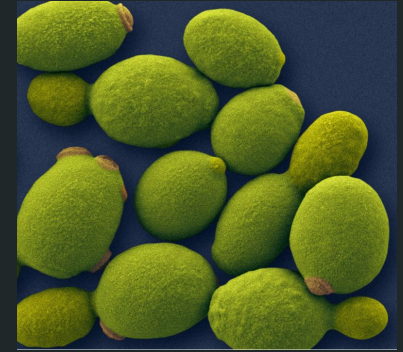
E. coli



Our Approach



Bacillus subtilis



Pichia  
(Komagataella)  
pastoris

## 1. Sonicator/French Press

a. \$1000s



## 2. Refrigerated Centrifuges

a. \$1000s-\$10,000s



- **Secrete enzymes**

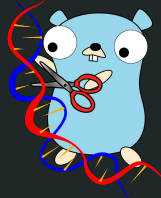
- Secretion as purification
- Avoid expensive equipment for popping, pelleting cells

# Wetware Design: *B. subtilis* and *P. pastoris*

## Open Wetware Toolkits

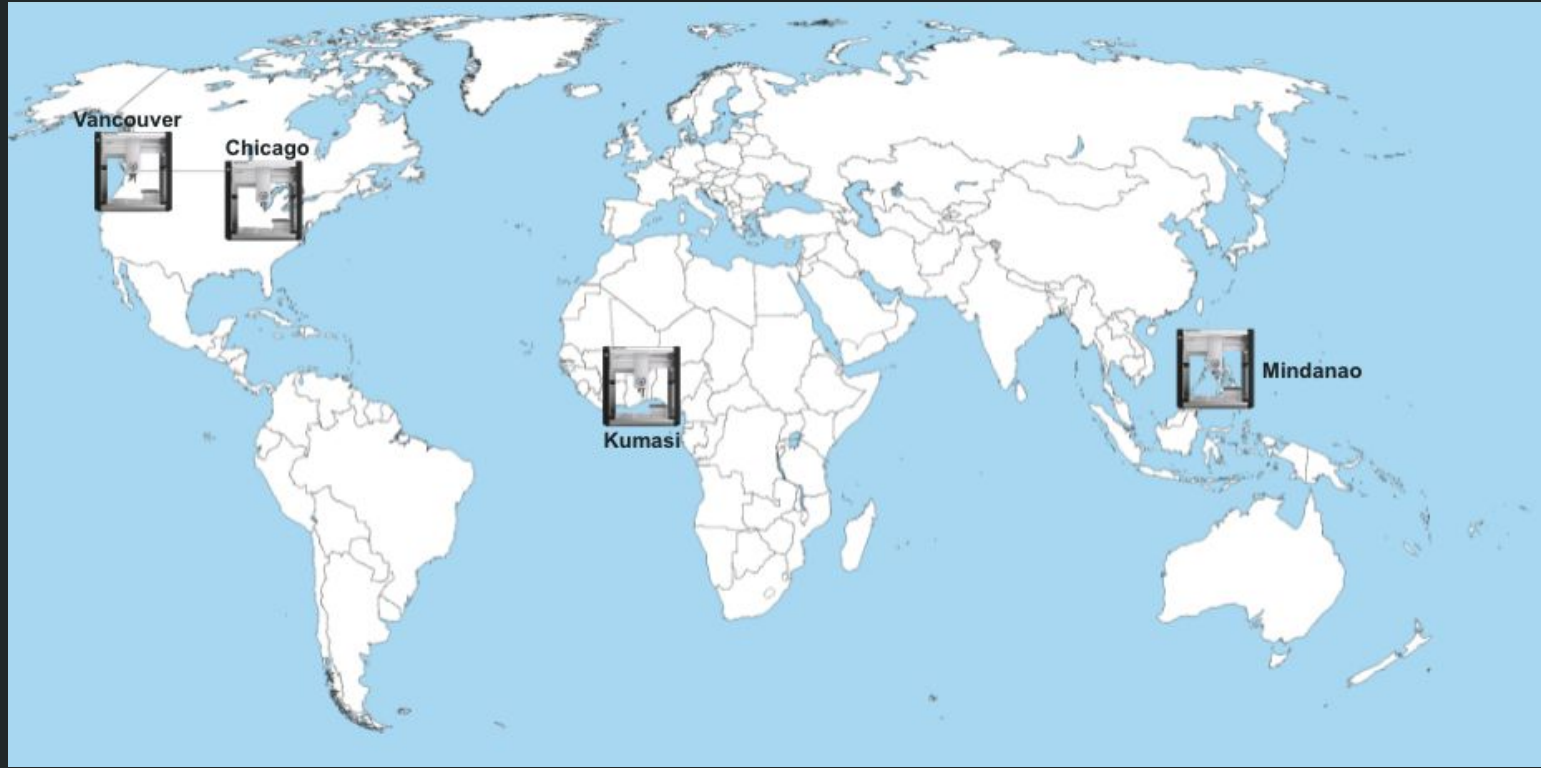
- Promoters, selection markers, terminators, homology arms for genomic integration
- Library Plasmids with ~150 different *B. subtilis* secretion tags
- Strategies for both replicating vectors, and multi-copy recombinant gene integration in the genome, while avoiding genetic instability
- Expansions of the Modular Cloning (MoClo) assembly standard
  - (v1.0) BaClo: Assembly standard to build plasmid/vector backbones capable of shuttling from *E. coli* to *B. subtilis* or *P. pastoris*, including genomic integration or replication and multigene cassettes
  - (v1.0) ProClo: Assembly standard to build multi-tagged genes for expressing, secreting, purifying and quantifying proteins of interest
  - (In the pipeline) FiveClo and ThreeClo: Assembly standards for building multi-part 5' and 3' untranslated sequence regions for sophisticated control of gene expression
  - (In the pipeline) Part type switching in the assembly standard via *BsaI*/*BtgZI* cutting (SwitchClo) and/or methylation (MetClo)

# Friendzymes/Poly Partnership: Easy, Open-Source Biodesign



- Find and fix problematic subsequences
  - Homopolymers, restriction sites, low/high GC regions, sequence repeats, stable secondary structures
- Calculate gene synthesis complexity (IDT API)
- Customize codon optimization
- Add Golden Gate overhangs and cut sites
- Simulate Golden Gate assembly
- Jupyter/Colab notebook tutorials for Poly
- Friendzymes GitHub Repo showing how we optimize the sequences of our genetic parts:
  - <https://github.com/friendzymes/friendzymes-toolkit>
- One of Isaac G.'s Colab notebooks showing how to remove repetitive sequences from a CDS with Poly:
  - <https://drive.google.com/file/d/1NGDMQi-OGV5-XABOgz87JWtSc4l2hHyh/view?usp=sharing>

# Opentrons Sponsorship of Friendzymes



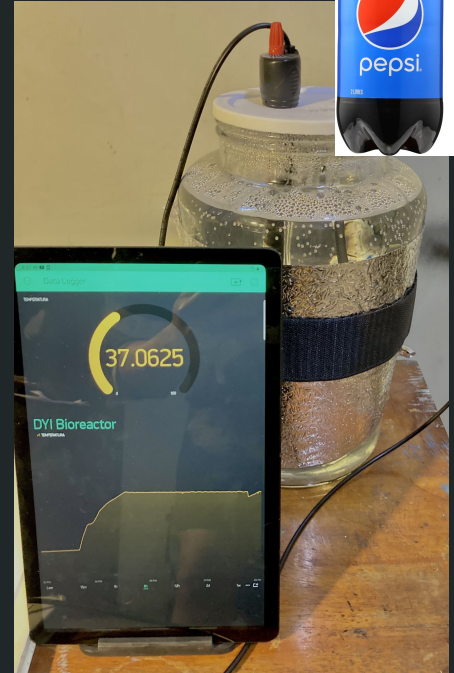
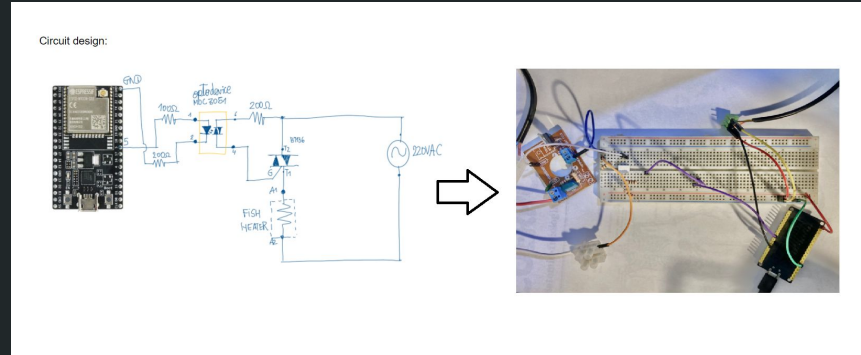
# Frugal Bioreactor Build in Progress:

## Diego Muñoz in Peru

## Sarah Ware at BioBlaze

- Frugal Bioreactor

- Design from Sebastian Cocioba
- 4L reactor volume
- ~\$180 in components
- Mixes and oxygenates with an aquarium pump





# Open Plate Reader build just starting at **Hive Biolab** in Ghana (Thanks to all your donations!)

<https://gofund.me/251b6e38>

## Help Team Friendzymes Democratize Biotechnology



**\$1,106** USD raised of \$10,700 goal

10 donors 38 shares 14 followers



Karl Schmieder  
\$166 · 4 d

Anonymous  
\$50 · 4 d

Arye Lipman  
\$100 · 10 d



Team Friendzymes is organizing this fundraiser to benefit Gathering for Open Science Hardware Inc. ✓




**\$5000 Flash Grant Awarded**

TABLE \$1. OSP component list and costs. The total instrument cost is ~\$3,500. Laser cut and 3D-printed costs are reflected in material costs.  
Key: Blue = optics (O). Gray = Frame (F). Orange = Electronics (E). Green = Laser Cut (L). Pink = 3D Print (P).

#	Vendor	Part Description	Part / Model Number	Unit cost	Number	Line subtotal
01	Thorlabs	Universal Base Plate, 2.5" x 2.5" x 3/8"	UBP2	\$36.47	2	\$72.94
02	Thorlabs	Cage Assembly Rod, 4" Long, Ø6 mm	ER4	\$7.10	1	\$7.10
03	Thorlabs	Cage Assembly Rod, 3" Long, Ø6 mm	ER3	\$6.60	1	\$6.60
04	Thorlabs	SM1-Threaded 30 mm Cage Plate, 6 mm Thick	CP6S	\$19.16	2	\$38.32
05	Thorlabs	Aspheric Condenser Lens, Ø12 mm, f=10.5 mm, NA=0.54, Uncoated	ACL1210U	\$18.05	2	\$36.10
06	Thorlabs	SM05 Lens Tube, 0.50" Thread Depth, One Retaining Ring Included	SM05L05	\$14.10	3	\$42.30
07	Thorlabs	Adapter with External SM1 Threads and Internal SM05 Threads, Knurled Edge	SM1A6FW	\$19.99	2	\$39.98
08	Thorlabs	SM1 Graduated Ring-Actuated Iris Diaphragm (Ø1 - Ø12 mm)	SM1D12C	\$103.02	1	\$103.02
09	Thorlabs	SM1 Lens Tube, 2" Thread Depth, One Retaining Ring Included	SM1L20	\$16.50	1	\$16.50
010	Thorlabs	LED Socket	8060-2	\$9.89	5	\$49.45
011	Thorlabs	SM05-Threaded Mount for TO-18, TO-39, TO-46, or T-1 3/4 LEDs	S05LEDM	\$32.64	1	\$32.64
012	Thorlabs	SMA Fiber Adapter Plate with External SM1 (1.035"-40) Thread	SM1SMA	\$29.58	2	\$59.16
013	Thorlabs	SM1-Threaded Aluminum Mount for TO-5 Laser Diodes	S1LM05	\$36.47	1	\$36.47
014	Thorlabs	LED with a Glass Lens, 430 nm, 8 mW, TO-18	LED430L	\$11.73	1	\$11.73
015	Thorlabs	Epoxy-Encased White Light LED, 13.0 mW, 7.5" Half Viewing Angle, Qty. of 5	LEDWE-15	\$9.38	1	\$9.38
016	Thorlabs	543 nm, f = 7.86 mm, NA = 0.51 SMA905 Fiber Collimation Pkg.	F240SMA-A	\$150.96	1	\$150.96
017	Thorlabs	1/4"-20 Low-Profile Channel Screws (100 Screws/Box)	SH25LP38	\$23.66	1	\$23.66
018	Thorlabs	Drop-In T-Nut, 1/4"-20 Tapped Hole	XE25T1	\$29.33	1	\$29.33
019	Thorlabs	Si Photodiode, 10 ns Rise Time, 350 - 1100 nm, 3.6 mm x 3.6 mm Active Area	FDS100	\$14.08	1	\$14.08
020	Thorlabs	SM05 Lens Tube, 2" Thread Depth, One Retaining Ring Included	SM05L20	\$20.60	1	\$20.60
021	Thorlabs	OEM Flange to Internal SM1 Adapter, 10 mm Thread Depth	SM1F1	\$19.18	1	\$19.18
022	LED Supply	5mm LED - Blue 470nm 15 Degree Viewing Angle	L1-0-B5TH15-1	\$0.47	1	\$0.47
023	LED Supply	5mm LED - Orange 610nm 30 Degree Viewing Angle	L4-0-G5TH30-1	\$0.60	1	\$0.60
024	LED Supply	5mm LED - Green 525nm 15 Degree Viewing Angle	L1-0-G5TH15-1	\$0.49	1	\$0.49
025	Ocean Optics	STS-VIS Spec (Included fiber)	STS-VIS	\$1,000.00	1	\$1,000.00
F1	80/20	1.00" X 1.00" T-Slotted Profile - Four Open T-Slots (19 inch, 2x End Tap)	1010	\$10.22	5	\$51.10
F2	80/20	1.00" X 1.00" T-Slotted Profile - Four Open T-Slots (19 inch, Counter-bore)	1010	\$15.32	3	\$45.96
F3	80/20	1.00" X 1.00" T-Slotted Profile - Four Open T-Slots (22 inch, 2x End Tap)	1010	\$10.91	6	\$65.46
F4	80/20	1.00" X 1.00" T-Slotted Profile - Four Open T-Slots (10 inch, 2x End Tap)	1010	\$4.25	4	\$17.00
F5	80/20	1.00" X 1.00" T-Slotted Profile - Four Open T-Slots (8 inch, 2x End Tap)	1010	\$3.79	2	\$7.58
F6	80/20	10 Series 3 Way - Squared Corner Connector	4042-Black	\$19.21	8	\$153.68



# Friendzymes-JOGL Hackathon Collaboration




## PUMP UP RECYCLING

The project and frugal Biofoundry proposition aims to provide an easy solution for the contamination from organic materials of solid wastes like...

Skills: Big data, data science, devop

+15

5 Members, 0 Needs, 0 Posts




## THE AMAZON BIOFOUNDRY

Climate change and non-sustainable extractivism are leading to the destruction of the Amazon Rainforest, a place that holds 10% of...

Skills: Data scientist, Genome mining, Molecular cloning

+2

4 Members, 1 Need, 0 Posts




## EXTREMOPHILES-DERIVED ENZYME AND COMPOUND...

Indonesia has enormous amount of diverse extremophiles. However, the utilization of these extremophiles are very minimal. Here we propose...

Skills: Biotechnology, Electrical engineering, Engineer software

+2

11 Members, 0 Needs, 0 Posts




## THE PHAB PROJECT

PhAB (Philippine Accessible Biotech): Democratizing biotechnology research and innovation in the Philippines

Skills: Molecular biology, Synthetic biology, microbiology

+6

2 Members, 0 Needs, 0 Posts




## INAVATION - ANTIMICROBIAL DRUG DISCOVERY FRUGAL...

We plan to create biofoundry to extract and test natural products on microbes with aim to support the biotechnology research ecosystem i...

Skills: Biotechnology, Biofoundry, Natural product

+5

9 Members, 0 Needs, 0 Posts




## PARAMETRIC MODELLER

An application that uses various open-source applications to design and simulate a bioreactor. It allows anyone to design a basic batch...

Skills: Open science, Bioreactor, Upscaling

1 Member, 0 Needs, 0 Posts




## CURUPIRA BIOFOUNDRY

The Curupira Biofoundry aims to speed up the domestication of new strains from Brazilian biodiversity. Our goal is to combine the industrial...

Skills: Microalgae expertise, Biofoundry, Open hardware

6 Members, 0 Needs, 0 Posts




## MICROSAFE

With the boom in synthetic biology, transportation and sharing of biological samples will increase greatly. But are the current methods...

Skills: optic, Electronic, microbiology

+2

3 Members, 0 Needs, 0 Posts



## OPENPETASE

Setup bio foundries in 3 regions to serve bio enthusiasts, and eventually general citizens to degrade PET plastics faster and collectively.

Skills: Logistic, Microbiology, Engineering design

+1

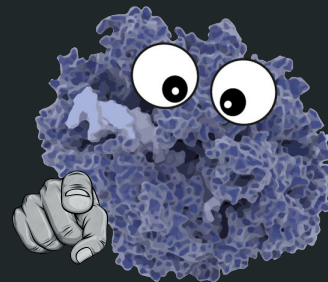
9 Members, 0 Needs, 0 Posts

9 project ideas that came about from our JOGL-Friendzymes Hackathon event (2-3 Oct 2021)

# Join Us!

- Learn and help to design, build and test expression, secretion and purification of useful enzymes
  - Pfu-Ss07d polymerase, Bsal/Btgzi restriction enzymes, T4 ligases and much more
- Learn and help to design & validate a new and powerful genetic assembly standard
- Learn and help to design OpenTrons protocols to automate the bioengineering design, build, test cycle in frugal biofoundries
- Learn and help to do advanced biodesign with Poly
- Learn and help to build open source lab equipment
  - Plate readers, bioreactors, chromatography systems, flow cytometers
- Learn and help to sequence many plasmids cheaply on Nanopore devices

I need YOU



To help democratize  
the means of  
biotechnological  
production

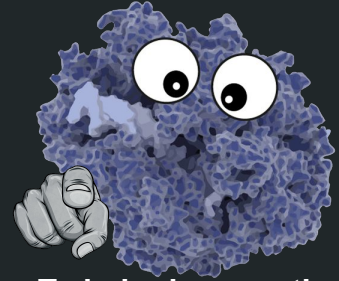
- <https://bit.ly/friendzymes-signup>
- [twitter.com/friendzymes](https://twitter.com/friendzymes)

**Anyone, anywhere,  
any age, any skill  
level, is welcome.**

# Join Us!

- **Help us build and kickstart a 'FreeCells' project**
  - Useful, public domain platform cell strains available under OpenMTA
    - E. coli expressing ccdA
    - E. coli expressing a Bsal-silencing methyltransferase
    - Open Pichia strains
    - B. subtilis with strict inducible control of sporulation
    - Desiccation-resistant E. coli and P. pastoris
- **Help us to help design the iGEM Parts Registry 2.0**

I need YOU



To help democratize  
the means of  
biotechnological  
production

- <https://bit.ly/friendzymes-signup>
- [twitter.com/friendzymes](https://twitter.com/friendzymes)

**Anyone, anywhere,  
any age, any skill  
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