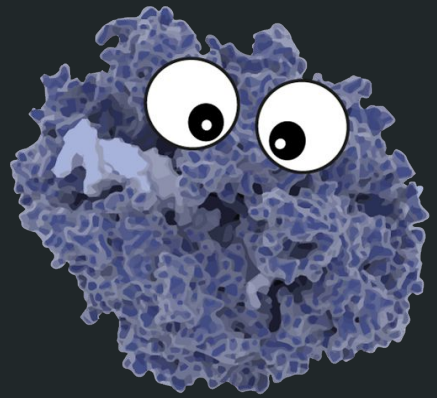
Friendzymes



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

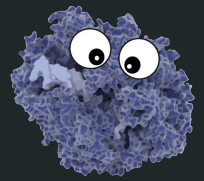
How Do We Make Biotechnological Production Scalable, Open, and Frugal?

- 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

Open MTA

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

BioBricks FOUNDATION

Biotechnology in the public interest

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

BioBricks FOUNDATION

Biotechnology in the public interest

FREE GENES

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

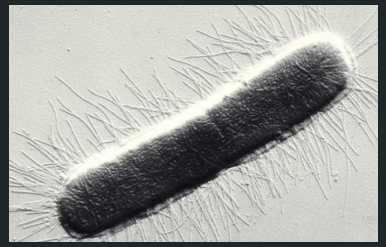
An Open-Source Plate Reader

Karol P. Szymula, Michael S. Magaraci, Michael Patterson, Andrew Clark, Sevile 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



1. Sonicator/French Press
   a. $1000s

2. Refrigerated Centrifuges
   a. $1000s-$10,000s

Our Approach

Bacillus subtilis

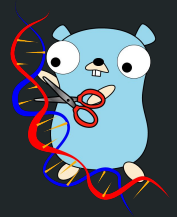
Pichia (Komagataella) pastoris

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

Wetware Design: B. subtilis and P. pastoris Open Wetware Toolkits

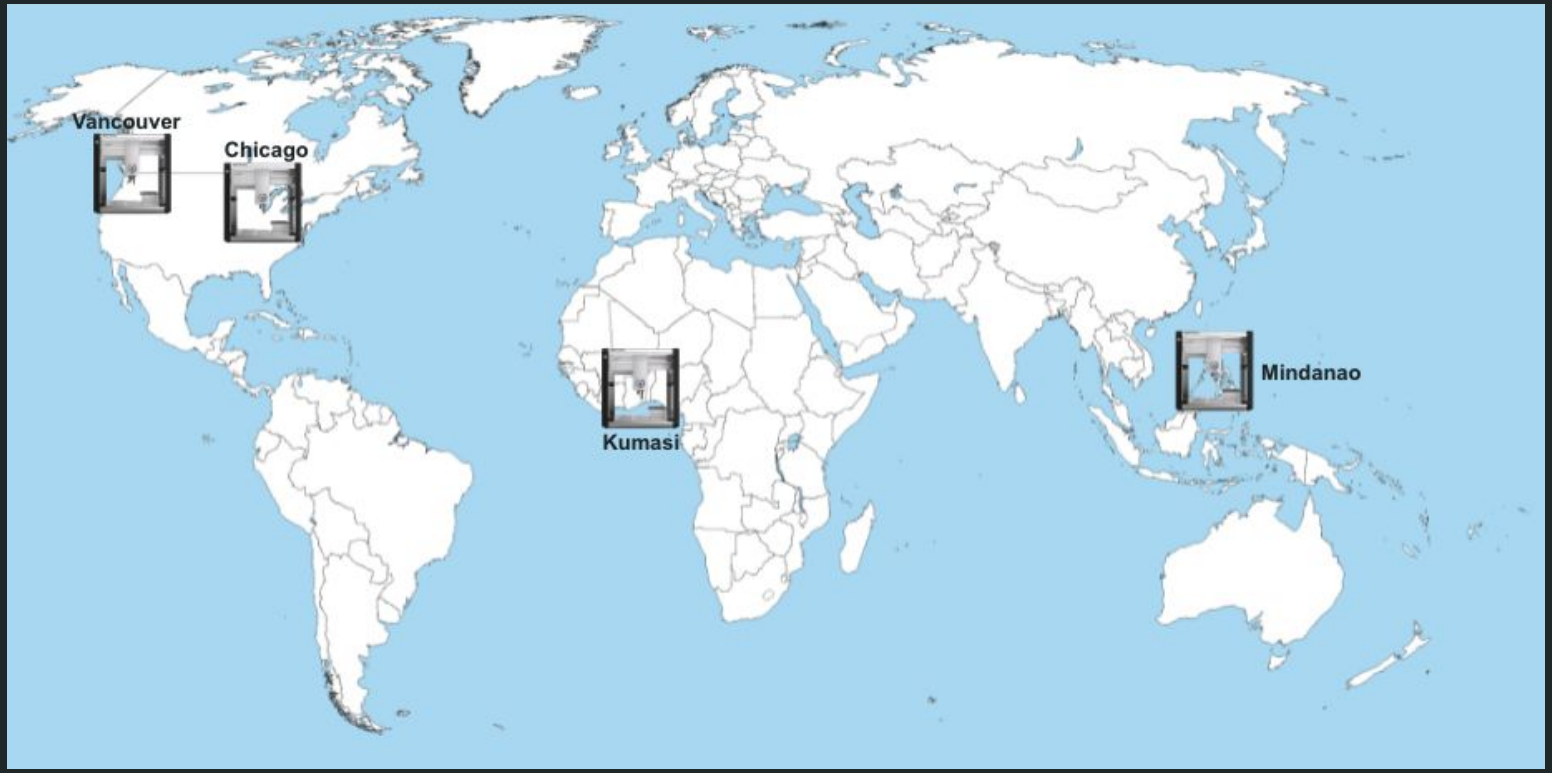
- Promoters, selection markers, terminators, homology arms for genomic integration
- Library Plasmids with ~150 different B. subtilis secretion tags
- Strategies for both replicating vectors, and multi-copy recombinant gene integration in the genome, while avoiding genetic instability
- Expansions of 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



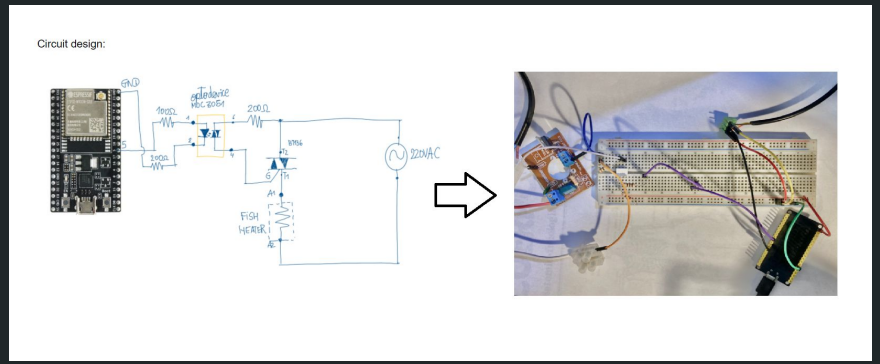
- Find and fix problematic subsequences
  - Homopolymers, restriction sites, low/high GC regions, sequence repeats, stable secondary structures
- Calculate gene synthesis complexity (IDT API)
- Customize codon optimization
- Add Golden Gate overhangs and cut sites
- Simulate Golden Gate assembly
- Jupyter/Colab 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-XAbOgz87JWtSc4I2hHyh/view?usp=sharing

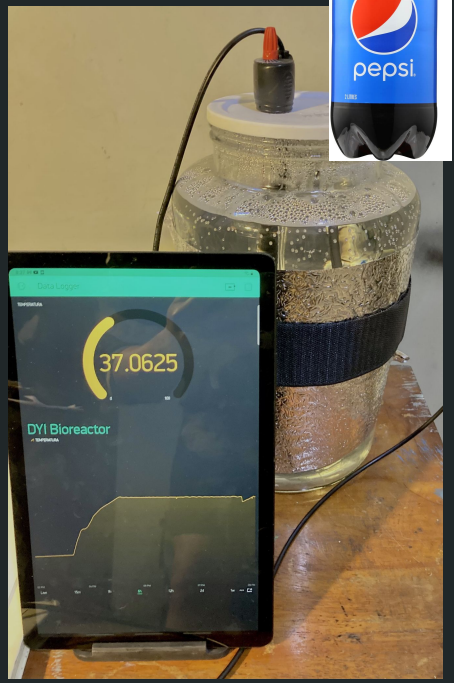
Opentrons Sponsorship of Friendzymes



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

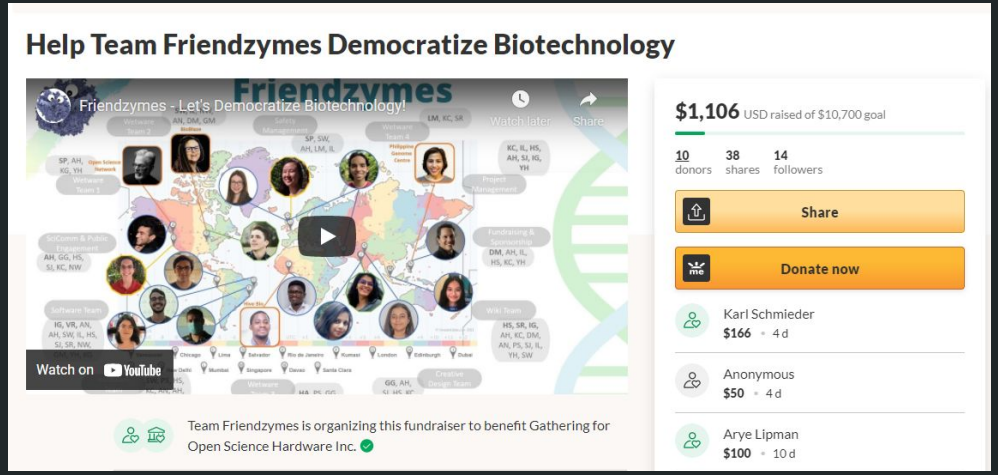


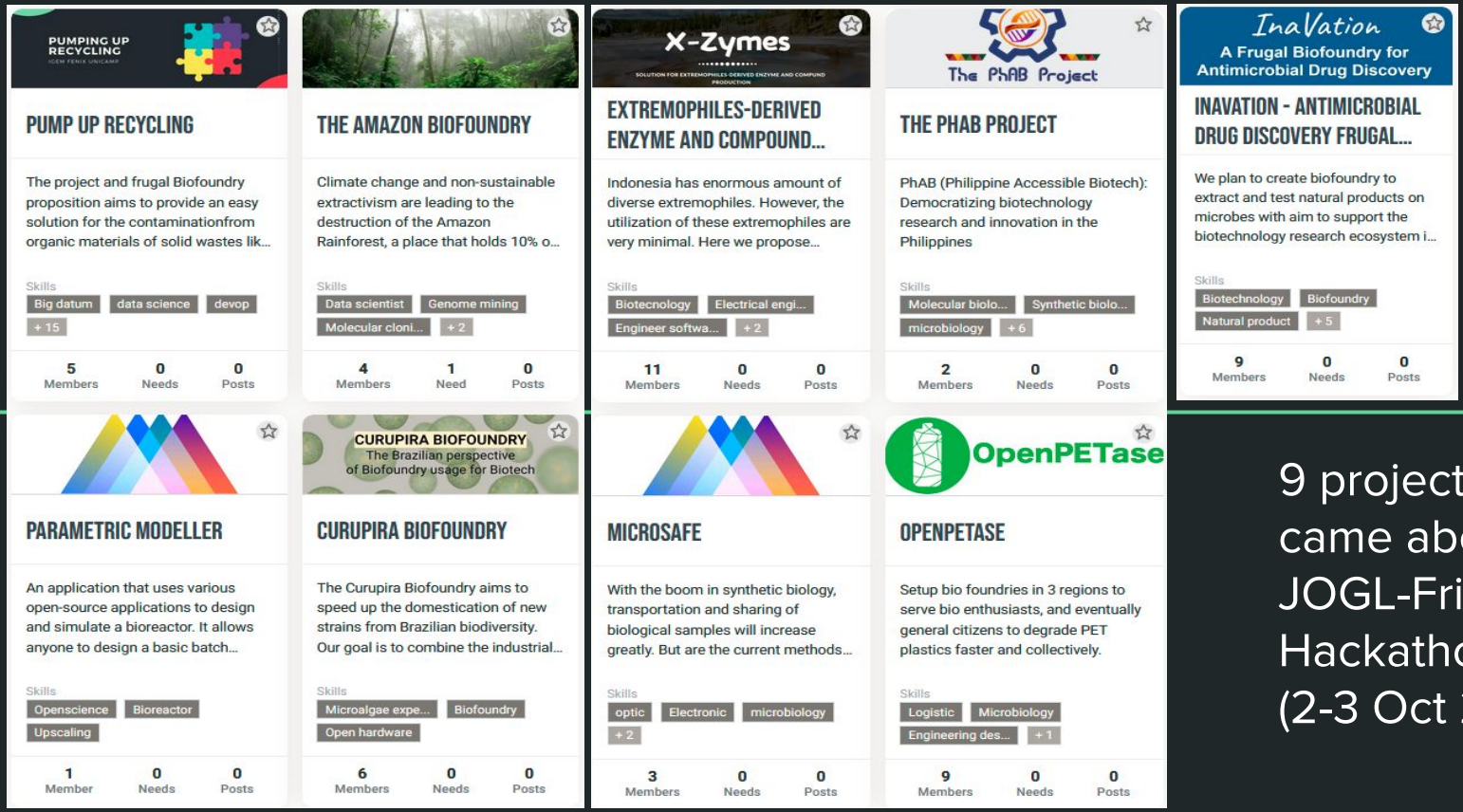
TABLE S1. OSP component list and costs. The total instrument cost is <$3,500. Laser cut and 3D-printed costs are reflected in material costs.
Key: Blue = optics (O). Gray = Frame (F). Orange = Electronics (E). Green = Laser Cut (L). Pink = 3D Print (P).

#	Vendor	Part Description	Part / Model Number	Unit cost	Number	Line subtotal
O1	Thorlabs	Universal Base Plate, 2.5" x 2.5" x 3/8"	UBP2	$36.47	2	$72.94
O2	Thorlabs	Cage Assembly Rod, 4" Long, Ø6 mm	ER4	$7.10	1	$7.10
O3	Thorlabs	Cage Assembly Rod, 3" Long, Ø6 mm	ER3	$6.60	1	$6.60
O4	Thorlabs	SM1-Threaded 30 mm Cage Plate, 6 mm Thick	CP6S	$19.16	2	$38.32
O5	Thorlabs	Aspheric Condenser Lens, Ø12 mm, f=10.5 mm, NA=0.54, Uncoated	ACL1210U	$18.05	2	$36.10
O6	Thorlabs	SM05 Lens Tube, 0.50" Thread Depth, One Retaining Ring Included	SM05L05	$14.10	3	$42.30
O7	Thorlabs	Adapter with External SM1 Threads and Internal SM05 Threads, Knurled Edge	SM1A6FW	$19.99	2	$39.98
O8	Thorlabs	SM1 Graduated Ring-Actuated Iris Diaphragm (Ø1 - Ø12 mm)	SM1D12C	$103.02	1	$103.02
O9	Thorlabs	SM1 Lens Tube, 2" Thread Depth, One Retaining Ring Included	SM1L20	$16.50	1	$16.50
O10	Thorlabs	LED Socket	8060-2	$9.89	5	$49.45
O11	Thorlabs	SM05-Threaded Mount for TO-18, TO-39, TO-46, or T-1 3/4 LEDs	S05LEDM	$32.64	1	$32.64
O12	Thorlabs	SMA Fiber Adapter Plate with External SM1 (1.035"-40) Thread	SM1SMA	$29.58	2	$59.16
O13	Thorlabs	SM1-Threaded Aluminum Mount for TO-5 Laser Diodes	S1LM05	$36.47	1	$36.47
O14	Thorlabs	LED with a Glass Lens, 430 nm, 8 mW, TO-18	LED430L	$11.73	1	$11.73
O15	Thorlabs	Epoxy-Encased White Light LED, 13.0 mW, 7.5° Half Viewing Angle, Qty. of 5	LEDWE-15	$9.38	1	$9.38
O16	Thorlabs	543 nm, f = 7.86 mm, NA = 0.51 SMA905 Fiber Collimation Pkg.	F240SMA-A	$150.96	1	$150.96
O17	Thorlabs	1/4"-20 Low-Profile Channel Screws (100 Screws/Box)	SH25LP38	$23.66	1	$23.66
O18	Thorlabs	Drop-In T-Nut, 1/4"-20 Tapped Hole	XE25T1	$29.33	1	$29.33
O19	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
O20	Thorlabs	SM05 Lens Tube, 2" Thread Depth, One Retaining Ring Included	SM05L20	$20.60	1	$20.60
O21	Thorlabs	OEM Flange to Internal SM1 Adapter, 10 mm Thread Depth	SM1F1	$19.18	1	$19.18
O22	LED Supply	5mm LED - Blue 470nm 15 Degree Viewing Angle	L1-0-B5TH15-1	$0.47	1	$0.47
O23	LED Supply	5mm LED - Orange 610nm 30 Degree Viewing Angle	L4-0-O5TH30-1	$0.60	1	$0.60
O24	LED Supply	5mm LED - Green 525nm 15 Degree Viewing Angle	L1-0-G5TH15-1	$0.49	1	$0.49
O25	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

SHUTTLEWORTH FOUNDATION

$5000 Flash Grant Awarded

Friendzymes-JOGL Hackathon Collaboration

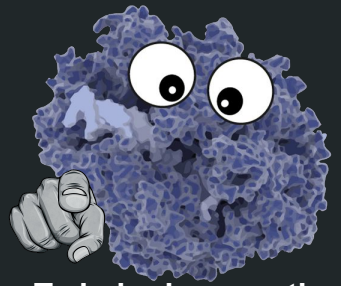


9 project ideas that came about from our JOGL-Friendzymes Hackathon event (2-3 Oct 2021)

Join Us!

- Learn and help to design, build and test expression, secretion and purification of useful enzymes
  - Pfu-Ss07d polymerase, BsaI/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

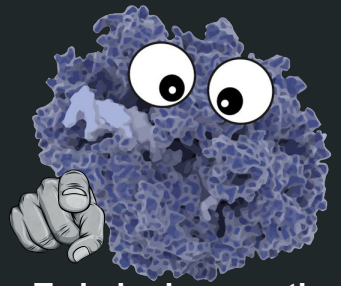
- https://bit.ly/friendzymes-signup
- twitter.com/friendzymes

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 BsaI-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



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