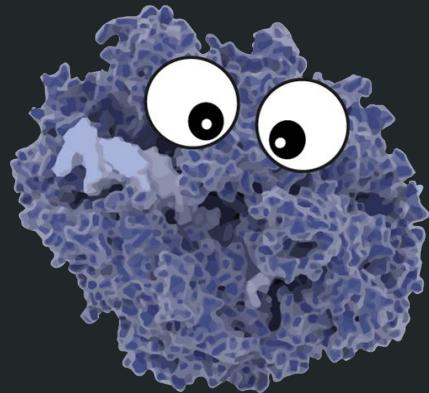


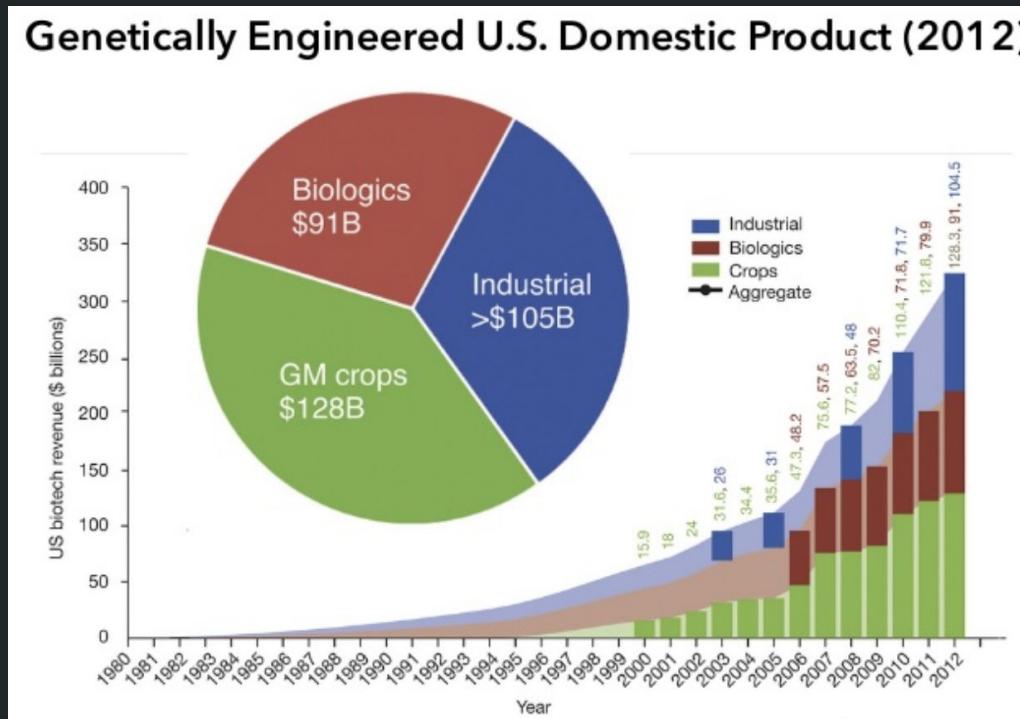
FreeGenes and Friendzymes



Let's Democratize and Globally Distribute
The Means of Biotechnological Production

Isaac Larkin

The Story We Tell About Biotech: Rapid Growth, World-Changing Potential



But We're On A Deadline.

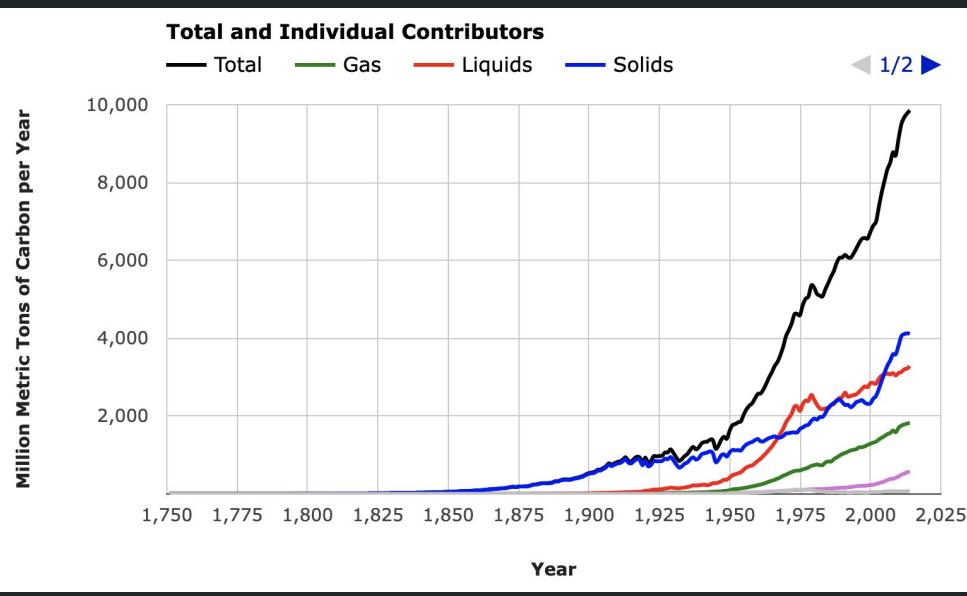
NEWS · 08 OCTOBER 2018

IPCC says limiting global warming to 1.5 °C will require drastic action

Humanity has a limited window in which it can hope to avoid the worst effects of climate change, according to climate report.

The world would have to curb its carbon emissions by at least 49% of 2017 levels by 2030 and then achieve carbon neutrality by 2050 to meet this target, according to a summary of the latest IPCC report, released on 8 October. The report draws on research conducted since nations unveiled the 2015 Paris climate agreement, which seeks to curb greenhouse-gas emissions and limit global temperature increase to between 1.5 and 2 °C.

And We Are Moving In The Wrong Direction.



List of largest companies by revenue [edit]

State-owned enterprise (government owns 50% or more)

Rank	Name	Industry	Revenue (USD millions)
1	Walmart	Retail	▲ \$523,964
2	Sinopec Group	Oil and gas	▼ \$407,009
3	State Grid	Electricity	▼ \$383,906
4	China National Petroleum	Oil and gas	▼ \$379,130
5	Royal Dutch Shell	Oil and gas	▼ \$352,106
6	Saudi Aramco	Oil and gas	▼ \$329,784
7	Volkswagen	Automotive	▲ \$282,760
8	BP	Oil and gas	▼ \$282,610
9	Amazon	Retail	▲ \$280,522
10	Toyota	Automotive	▲ \$275,288
11	ExxonMobil	Oil and gas	▼ \$264,938
12	Apple	Electronics	▼ \$260,174

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

Biological Technologies Can Help

- Replace Animal Agriculture



- Replace the Petrochemical Industry



- Microbial Alternatives to Industrial Processes



- Bio-Based Fuels for Aircraft, Etc.



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.

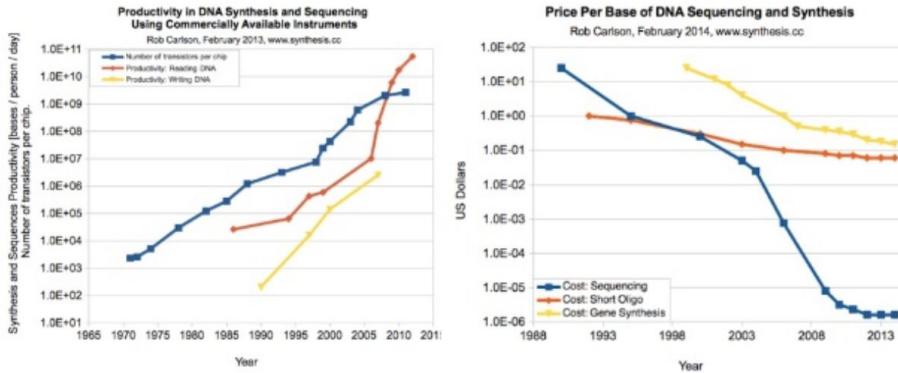
Biological technologies, everywhere and at every scale, will be required.

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 Barrier

DNA sequencing (read) & synthesis (write)
are improving faster than silicon-based fab.



Bioengineering and Synthetic Biology Have Been Difficult and Expensive

Cost Barrier



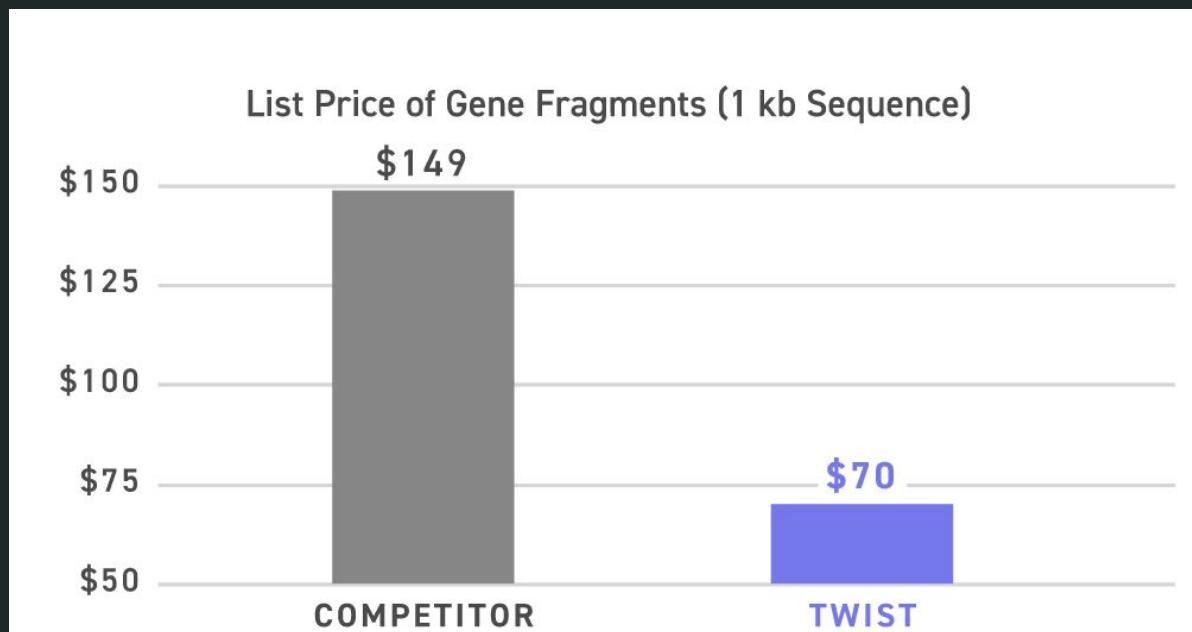
iSeq - \$25,000



NovaSeq - >\$1,000,000

Bioengineering and Synthetic Biology Have Been Difficult and Expensive

Cost Barrier



Similar Price
for an
AddGene
Plasmid

Bioengineering and Synthetic Biology Have Been Difficult and Expensive

IP Barrier

Published: November 2007

Are university researchers at risk for patent infringement?

Amy Yancey & C Neal Stewart Jr

Nature Biotechnology 25, 1225–1228(2007) | [Cite this article](#)

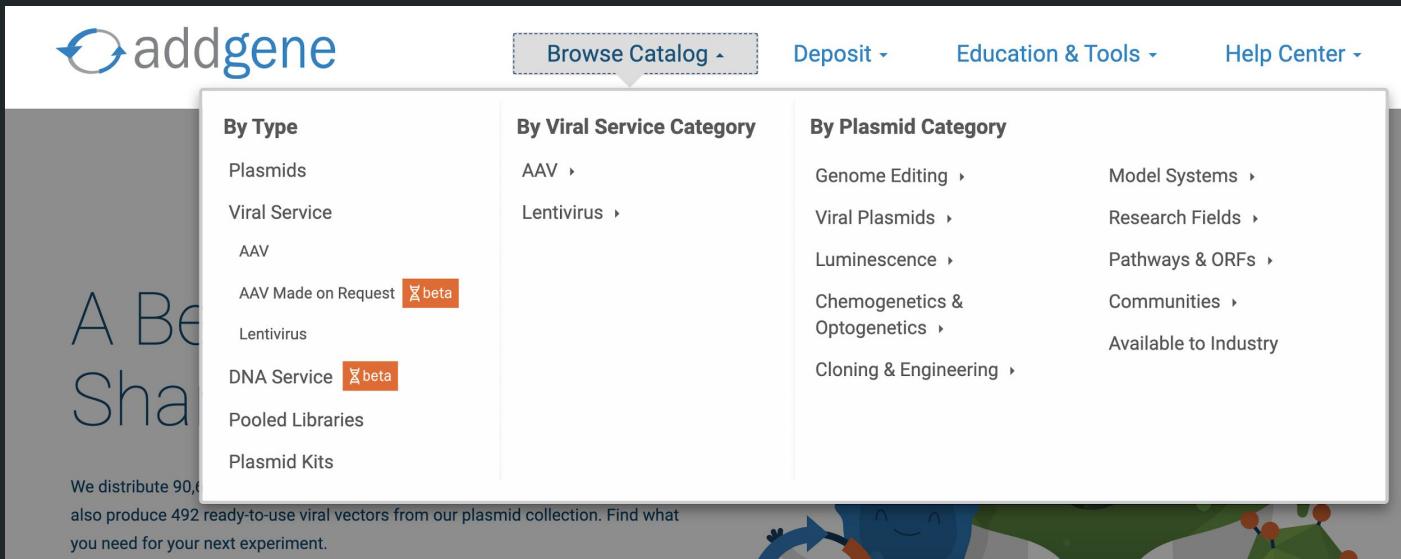
Patent thicket

From Wikipedia, the free encyclopedia

A **patent thicket** is a concept with negative [connotations](#) that has been described as "a dense web of overlapping intellectual property rights that a company must hack its way through in order to actually commercialize new technology",^[1] or, in other words, "an overlapping set of patent rights" which requires innovators to reach licensing deals for multiple patents from multiple sources.^[2]

Bioengineering and Synthetic Biology Have Been Difficult and Expensive

IP Barrier



The screenshot shows the Addgene website's search interface. At the top, there is a navigation bar with the Addgene logo, a search bar containing "A Be Sha", and dropdown menus for "Browse Catalog", "Deposit", "Education & Tools", and "Help Center". Below the search bar, there are three main categories: "By Type", "By Viral Service Category", and "By Plasmid Category".

- By Type:**
 - Plasmids
 - Viral Service
 - AAV
 - Lentivirus
 - AAV Made on Request beta
 - Lentivirus
 - DNA Service beta
 - Pooled Libraries
 - Plasmid Kits
 - By Viral Service Category:**
 - AAV
 - Lentivirus
 - AAV Made on Request beta
 - Lentivirus
 - DNA Service beta
 - By Plasmid Category:**
 - Genome Editing
 - Viral Plasmids
 - Luminescence
 - Chemogenetics & Optogenetics
 - Cloning & Engineering
 - Model Systems
 - Research Fields
 - Pathways & ORFs
 - Communities
 - Available to Industry

We distribute 90,000+ plasmids and also produce 492 ready-to-use viral vectors from our plasmid collection. Find what you need for your next experiment.

Uniform Biological Material Transfer Agreement

Bioengineering and Synthetic Biology Have Been Difficult and Expensive

IP Barrier

The screenshot shows the homepage of the iGEM Registry of Standard Biological Parts. The header includes the iGEM logo and links for tools, catalog, repository, assembly, protocols, help, search, and a search bar containing "BBa_". A folder icon is also present. The main content area features three boxes: "Add and Document Parts" (instructions for documenting parts), "Sample Submissions" (information about sample requirements), and "Update: Distribution Kit" (a note about distribution kit availability due to the pandemic).

Registry of Standard Biological Parts

iGEM tools catalog repository assembly protocols help search BBa_

Add and Document Parts
Start adding and documenting your parts early! Throughout the season you can update your part documentation with new information, modelling data, and/or experimental characterization.

Sample Submissions
iGEM teams do not need to send samples of their parts anymore. We encourage teams to focus on the design, modeling, characterization, and documentation of their parts!

Update: Distribution Kit
As a result of the current pandemic, we will not be able to manufacture and ship our 2020 DNA Distribution Kits to teams and labs this year. We sincerely apologize for any inconvenience this may cause.

No MTA, Legal Limbo
Only safe as long as it stays on biotech's fringes!

Bioengineering and Synthetic Biology Have Been Difficult and Expensive

Expertise Barrier

Takes a lot of time and money to become a proficient bioengineer

Pipetting

Sterile

Time

Sample

Technique

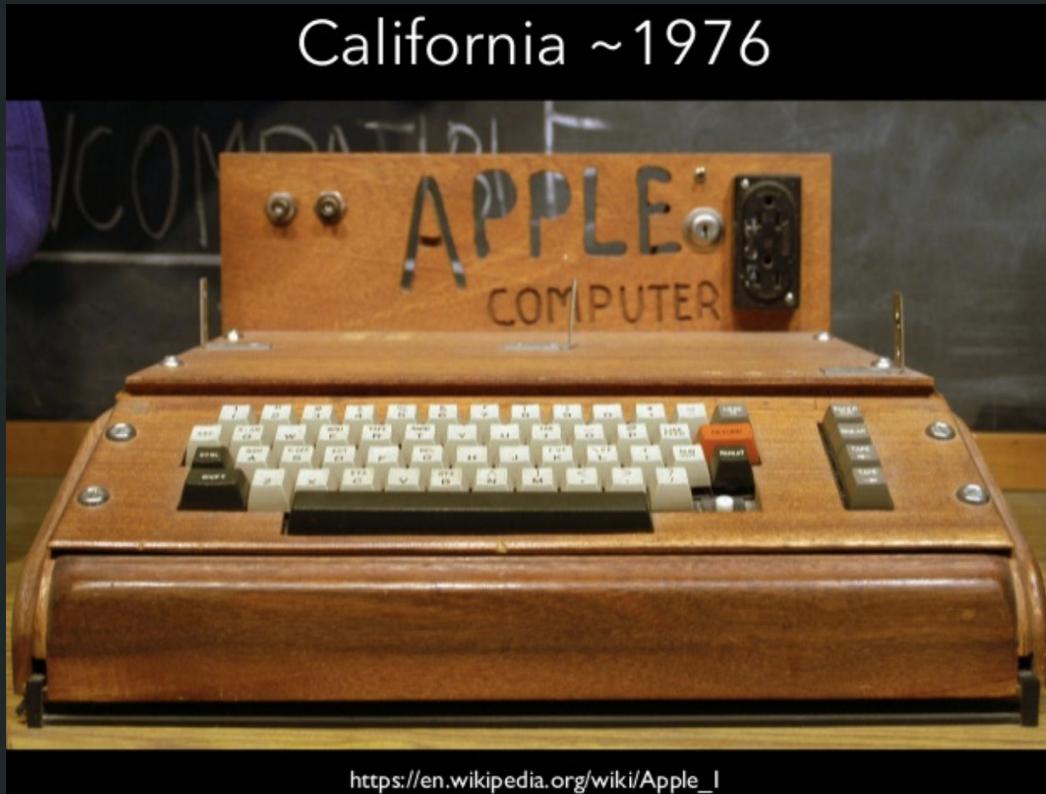
Management

Management

Troubleshooting

PCR, Cloning, Protein Expression + Purification

Making it Cheaper, Faster and Easier For People to do Bio-Computer Engineering



\$3,000 in 2019 dollars

Costs to operate:
electricity

Making it Cheaper, Faster and Easier For People to do Bio-Computer Engineering



Apple II - June 1977

\$5,500-\$11,000 in 2019 dollars

Costs to operate: electricity

Making it Cheaper, Faster and Easier For People to do Bio-Computer Engineering



GNU Operating System



GNU General
Public License



Popular Licenses

The following OSI-approved licenses are popular, widely used

- Apache License 2.0
- BSD 3-Clause "New" or "Revised" license
- BSD 2-Clause "Simplified" or "FreeBSD" license
- GNU General Public License (GPL)
- GNU Library or "Lesser" General Public License (LGPL)
- MIT license
- Mozilla Public License 2.0
- Common Development and Distribution License
- Eclipse Public License version 2.0

Making it Cheaper, Faster and Easier For People to do Bio-Computer Engineering



+



GNU

Linux Kernel

1991

\$0 in any denomination

Cost to operate:
electricity

Linux was originally developed for [personal computers](#) based on the [Intel x86](#) architecture, but has since been [ported](#) to more [platforms](#) than any other operating system.^[20] Because of the dominance of Android on [smartphones](#), Linux also has the [largest installed base](#) of all [general-purpose operating systems](#).^{[21][22]} Although it is used by only around 2.3 percent of [desktop computers](#),^{[23][24]} the [Chromebook](#), which runs the Linux kernel-based [Chrome OS](#), dominates the US [K-12 education market](#) and represents nearly 20 percent of sub-\$300 [notebook](#) sales in the US.^[25] Linux is the leading operating system on [servers](#) (over 96.4% of the top 1 million web servers' operating systems are Linux),^[26] leads other [big iron](#) systems such as [mainframe computers](#), and is the only OS used on [TOP500 supercomputers](#) (since November 2017, having gradually eliminated all competitors).^{[27][28][29]}

**Where is biotech's
GNU Public License?**

Where is biotech's GNU/Linux?

Where is biotech's Apple I/II?

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

The FreeGenes Project: Building GNU/Linux for Biotechnology



At SB7.0 in the summer of 2017, Drew Endy announced that the BioBricks Foundation had received a donation sufficient to fund the synthesis and free distribution (under the OpenMTA) of thousands of useful, off-patent/IP-free synthetic genetic parts.

**Project was
Keoni's idea**



**Name was
my idea**



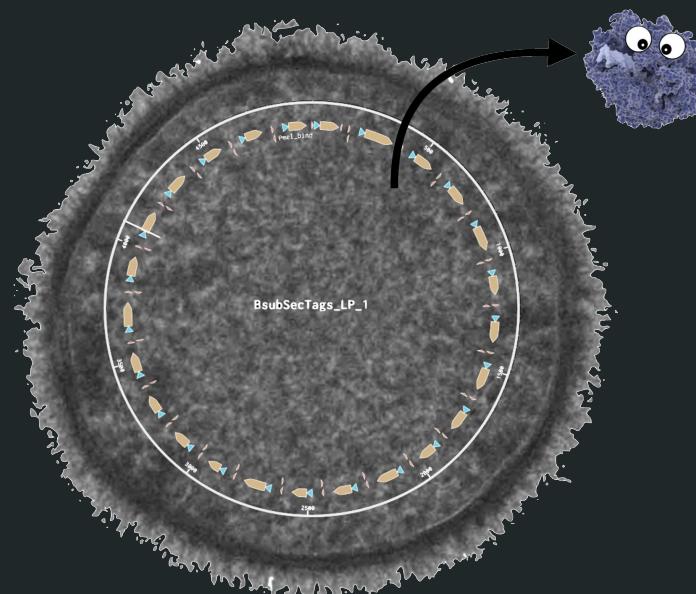
**FREE
GENES**

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

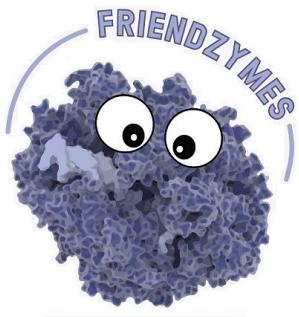
- Capable of going from benchtop tinkering to large-scale supply of a good or service
- Public domain, open-source, shareable hardware, software, wetware, protocols
- As cheap and easy as possible

Wetware Design Goals:

- Make recombinant protein expression, secretion, and purification as cheap and easy as possible
- Make it easy to engineer and optimize strains



Friendzymes: A Team and Project Enabled by Free Genes and the OpenMTA

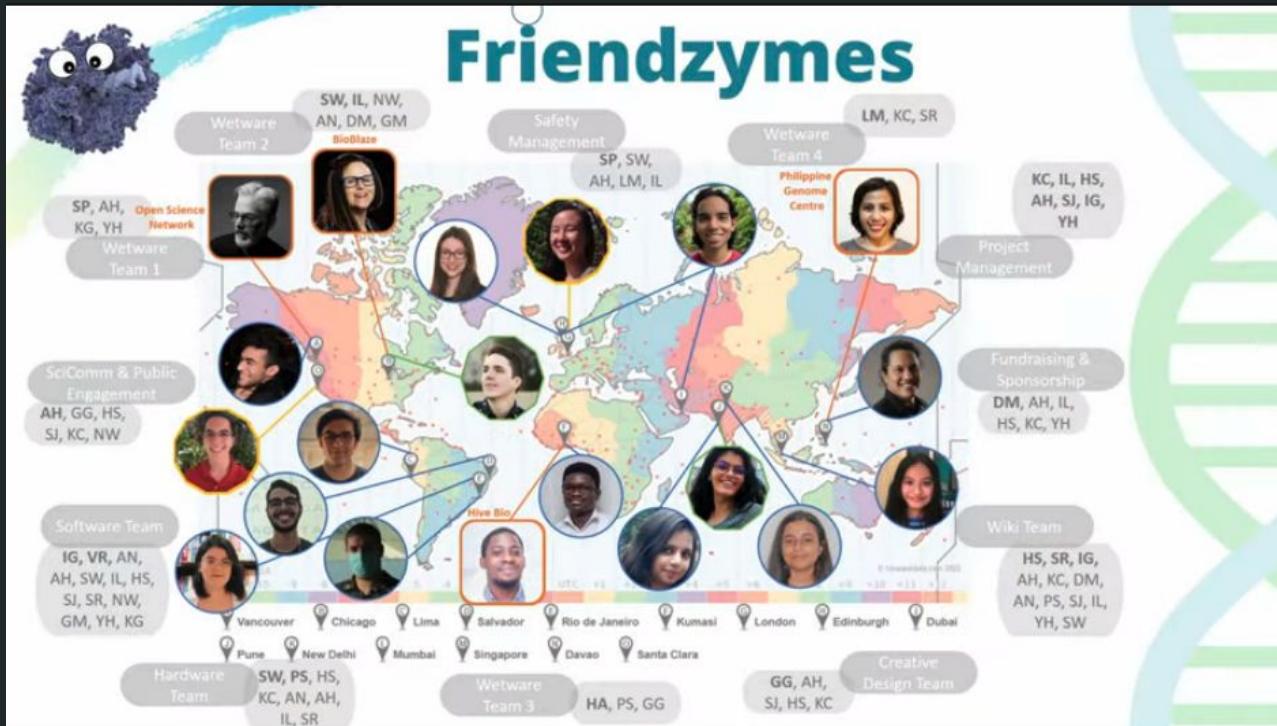


OPEN SCIENCE GLOBAL
iGEM 2021



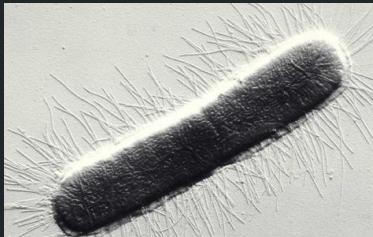
- Goal: Democratize Recombinant Protein Production and Purification
- Team members from 9 countries, 5 continents
- 3 community labs + the Philippine Genome Center

Friendzymes: A Team and Project Enabled by Free Genes and the OpenMTA



- Goal: Democratize Recombinant Protein Production and Purification
- Team members from 9 countries, 5 continents
- 3 community labs + the Philippine Genome Center

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



1. Sonicator/French Press

a. \$1000s

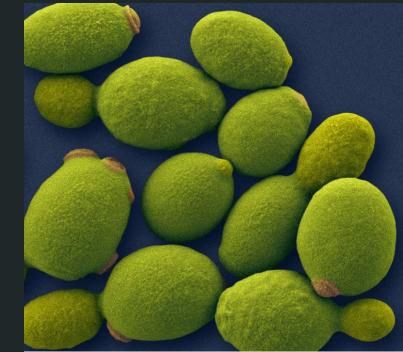


2. Refrigerated Centrifuges

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



Bacillus subtilis

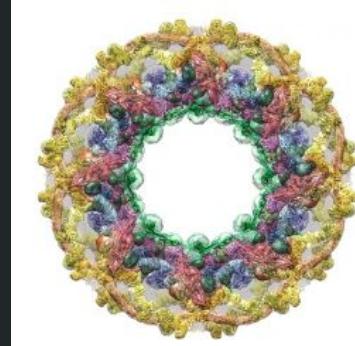
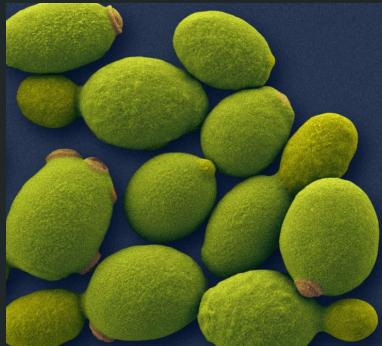


Pichia pastoris

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

Wetware Design: *P. pastoris* Genetic Toolkit

- Promoters, selection markers, terminators, homology arms for genomic integration
- Strategies to achieve multi-copy recombinant gene integration in the genome, while avoiding genetic instability
 - Nearby, identical DNA sequence repeats → genetic instability → mutation
 - Non-repetitive, synonymous gene expression cassettes, and/or:
 - Rapid multi-locus genomic integration



Open Yeast Collection

\$0.00

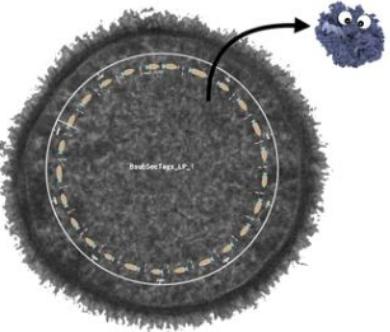
Free Shipping

ADD TO CART

Wetware Design: *B. subtilis* Genetic toolkit

- Promoters, selection markers, terminators, homology arms, and secretion tags for genomic integration





B. subtilis Protein Secretion Toolkit

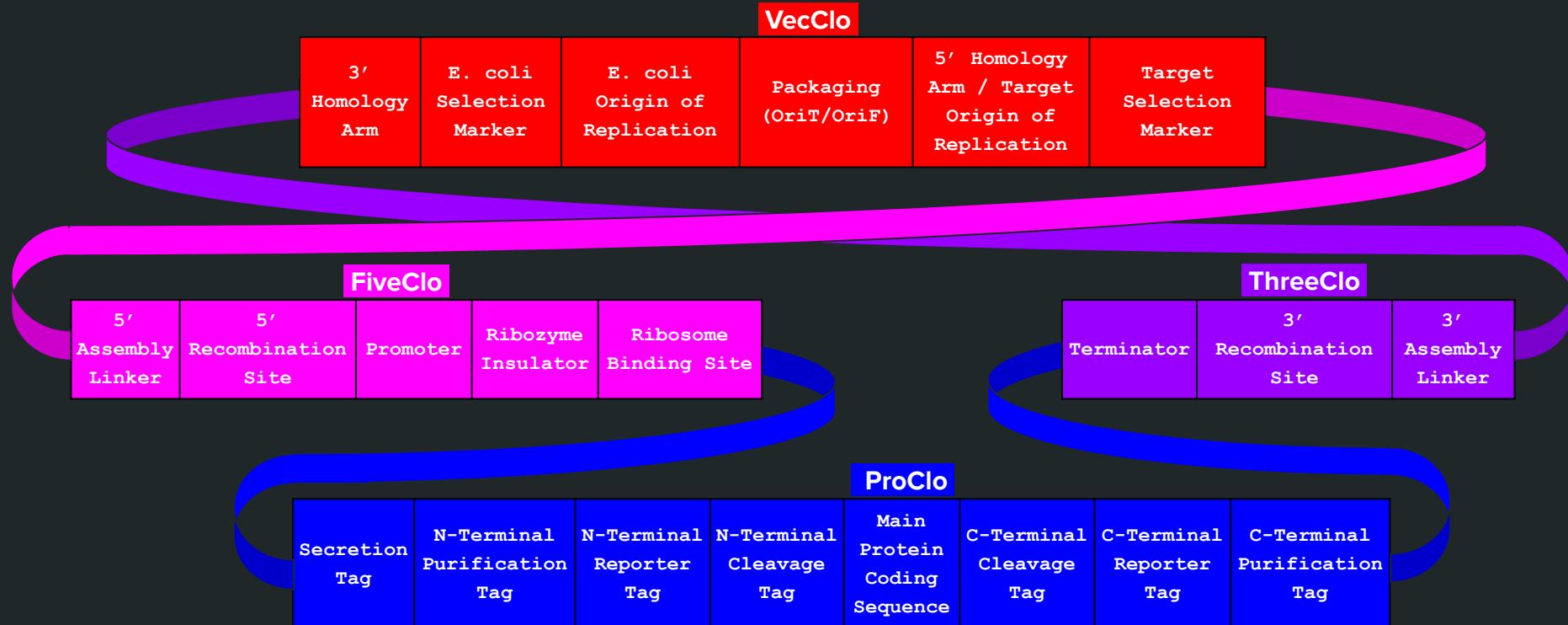
\$0.00
Free Shipping

[ADD TO CART](#)

Description Genes **Bionet** MTA

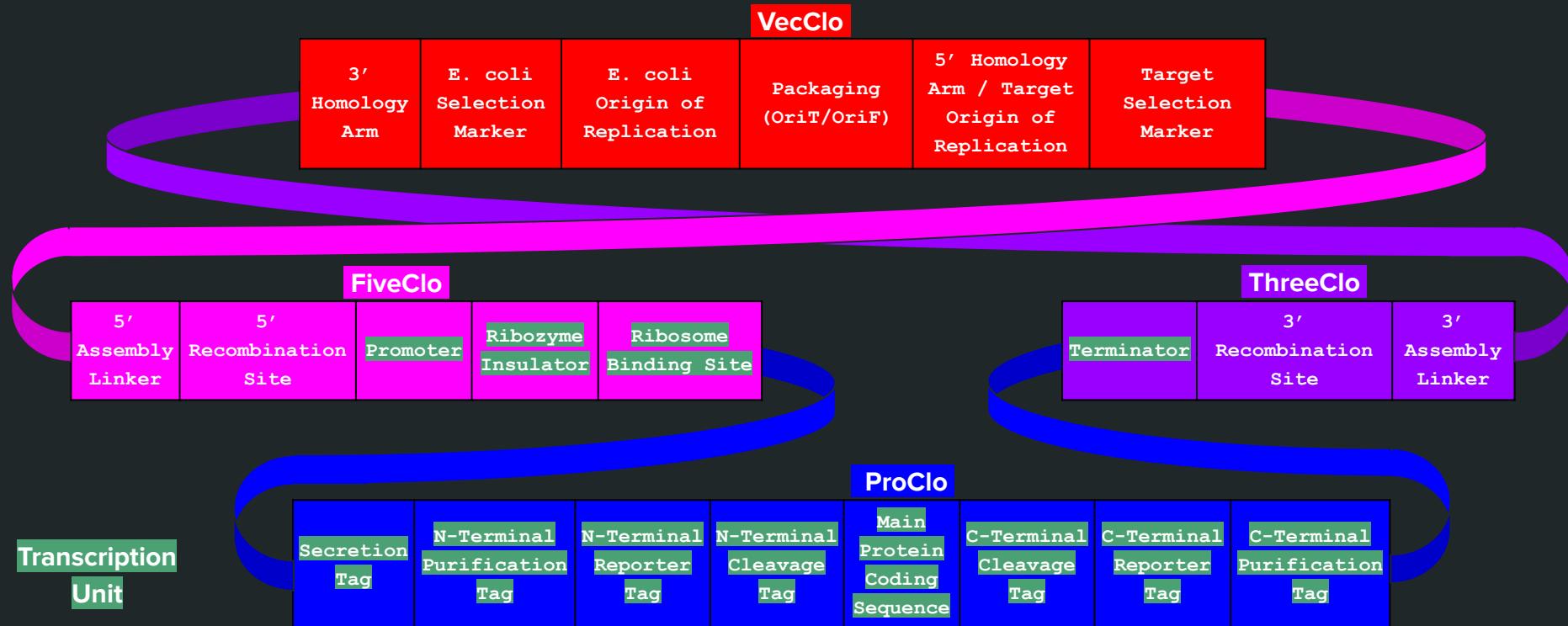
AllClo: Expanded, High Fidelity MoClo/uLoop Assembly Standard

- Enable rapid assembly of libraries of complex genetic devices



AllClo: Expanded, High Fidelity MoClo/uLoop Assembly Standard

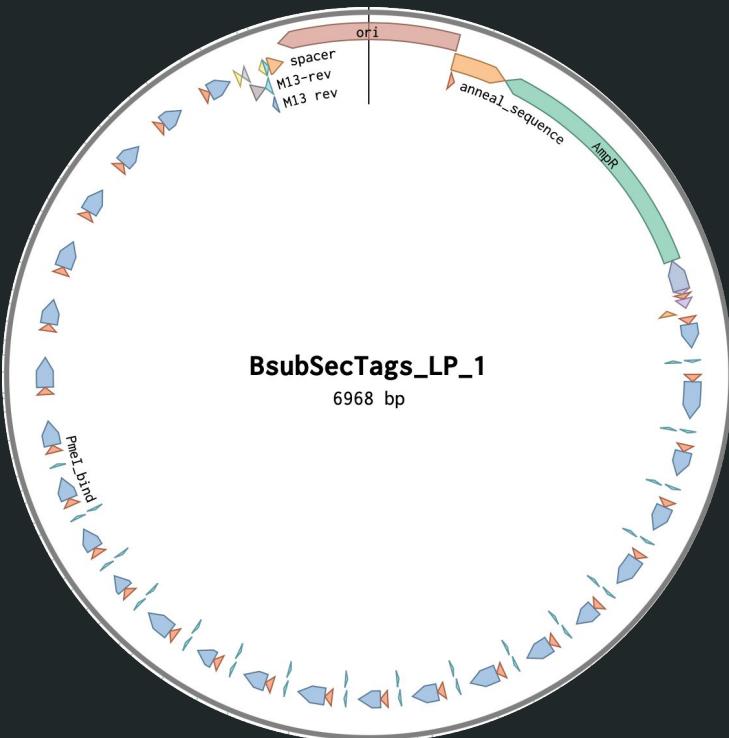
- Enable rapid assembly of libraries of complex genetic devices



Challenge: Selecting the Right *B. subtilis* Secretion Signal Peptide Tag

- *B. subtilis* has >150 secretion tags
- Which secretion tag will efficiently secrete a particular protein is impossible to predict

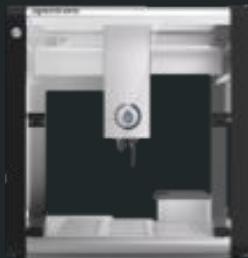
Solution: Library Plasmids



Challenge: High-Throughput Strain Engineering for High-Yield Protein Secretion

Our Approach

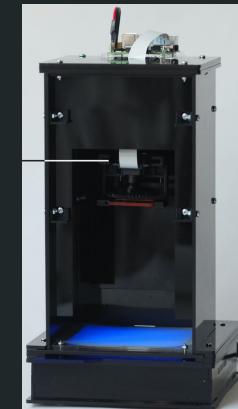
- Frugal biofoundry for high-capacity, semi-automated biological design-build-test cycles on low-cost liquid handling, DNA sequencing, and plate reading instruments



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

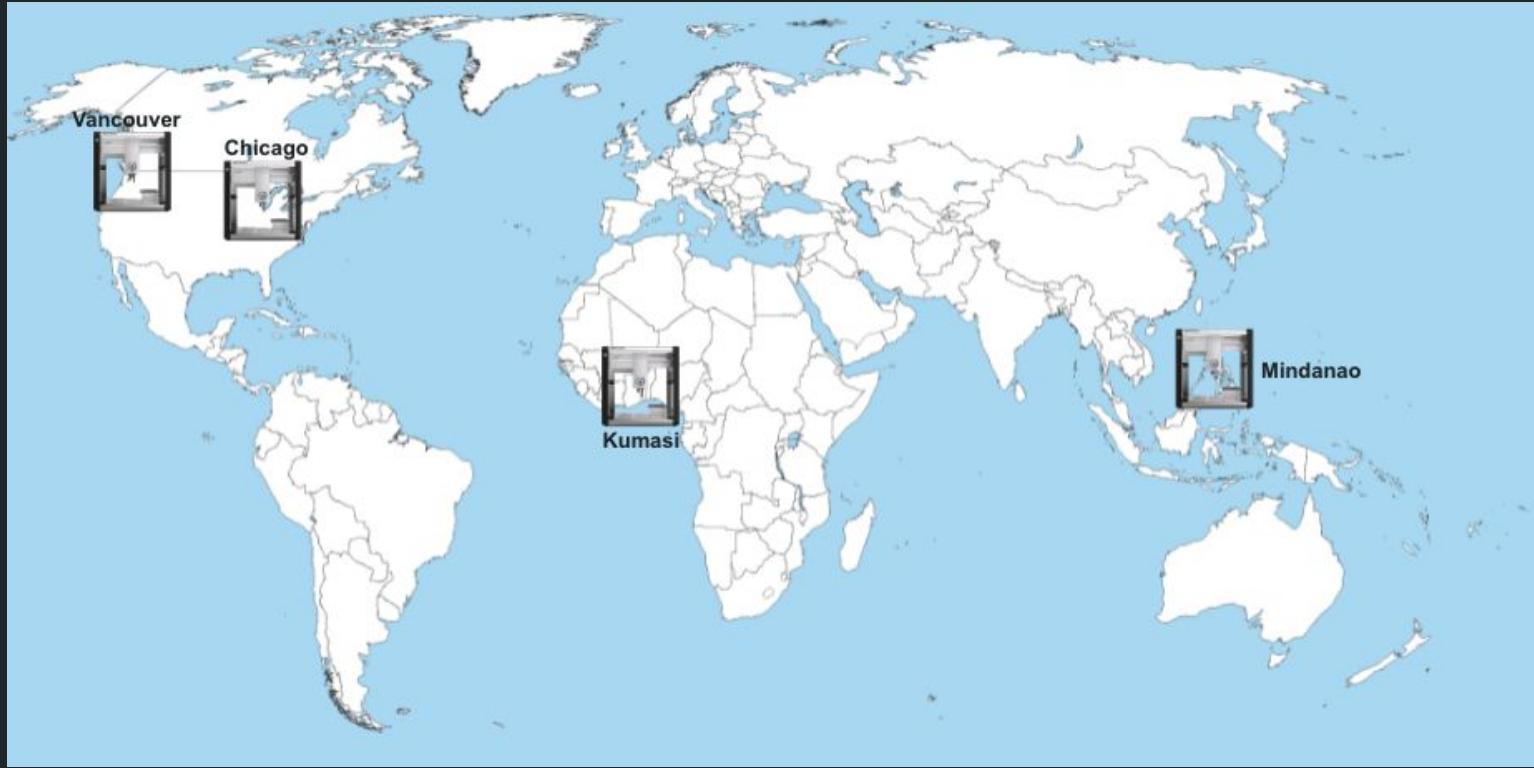


**Oxford Nanopore MinION
DNA sequencer**
\$1,000



**FluoPi Open Source
Fluorescence Imager**
~\$200

Opentrons Sponsors Friendzymes



Shuttleworth Foundation Sponsors Friendzymes



- \$5,000 Flash Grant
- Enough to buy 3 nanopore DNA sequencers and build 4 FluoPi fluorescence imagers

Challenge: Frugal Protein Production/Fermentation

Our Approach

Standard Method

1. Baffled Glass Shaker Flasks

- a. \$10s-\$100s



2. Bioreactors

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



- **Frugal Shaker Flask**

- 2L Pepsi bottle!



- **Frugal Bioreactor**

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



Challenge: Frugal Protein Purification

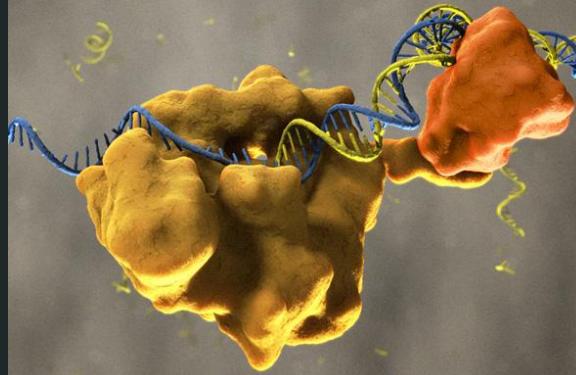
Standard Method

1. **Immobilized Metal Ion Affinity Chromatography (IMAC)**
 - a. \$100s for Nickel IMAC resin
 - b. \$1000s-10,000s for chromatography instrument



Our Approach

1. **For thermostable enzymes, pursue secretion + heat purification**



**Phusion Thermostable
DNA Polymerase**

Challenge: Frugal Protein Purification

Standard Method

1. Immobilized Metal Ion Affinity Chromatography (IMAC)
 - a. \$100s for Nickel IMAC resin
 - b. \$1000s-10,000s for chromatography instrument

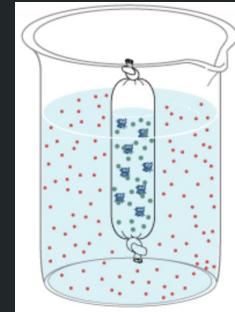


Our Approach

2. For reagent-grade enzymes (~90% purity), pursue secretion + dialysis



\$75 / 6 clips



Potato chip bag clips: \$11 / 38 clips

Challenge: Frugal Protein Purification

Standard Method

1. Immobilized Metal Ion Affinity Chromatography (IMAC)
 - a. \$100s for Nickel IMAC resin
 - b. \$1000s-10,000s for chromatography instrument



Our Approach

3. For high purity applications (>99% purity), pursue secretion + frugal chromatography

- a. Mentor Dushanth Seevaratnam has developed methods for extracting silica from sand and purifying enzymes with it
- b. Elastin-like Polypeptide tags reversibly aggregate in salt, heat
 - i. Off-patent as of 2021



iGEM: The International Genetically Engineered Machines Competition

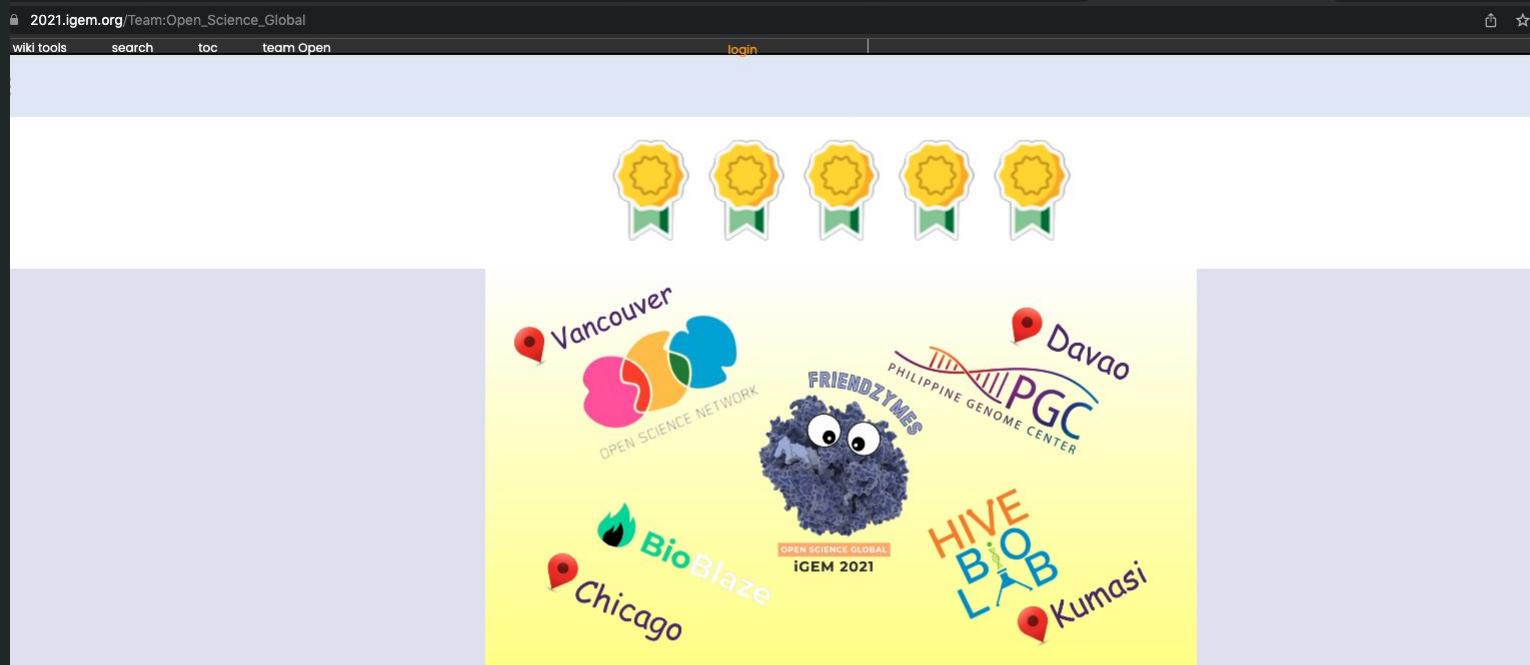
First Precursor to iGEM
MIT 2003



iGEM Giant Jamboree
2016



2021 Friendzymes iGEM Team



ABSTRACT

Biotechnology can change the world or even better, protect the world we have right now. Rapid development and worldwide deployment of advanced biological technologies in various areas can help transition human civilization off of fossil energy and materials and avert a mass extinction. So, we need more experts to tackle world problems using the versatile tools of biotechnology. The barriers(time, money, resources) needed to just contribute using biotechnology is high. When we think of just the bare minimum biofoundry costs - all the biomaterials, chemical reagents, and hardware required, even that is expensive. But the good news is, the barrier is breaking down slowly through various global efforts like the FreeGenes project and OpenMTA. We as Team Friendzymes, an internationally distributed team, want to catalyse those efforts handful of steps at a time to ultimately have a frugal biofoundry in place analogous to the chaply.

FreeGenes, iGEM, and Friendzymes Are Now Collaborating



* iGEM is partnering with the BioBricks Foundation's Free Genes project to coordinate the design and construction of all materials going into the iGEM Distribution 2.0. Doing so will ensure that all parts will be newly synthesized and provided free-and-clear of property-rights encumbrances. The entire distribution will be made available via the Unilateral OpenMTA, a materials transfer agreement that enables free use and sharing of so-provided samples.

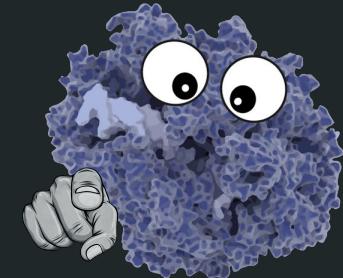
https://2021.igem.org/Competition/Distribution_Kit

A Linux kernel for biotechnology!

Join Us!

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

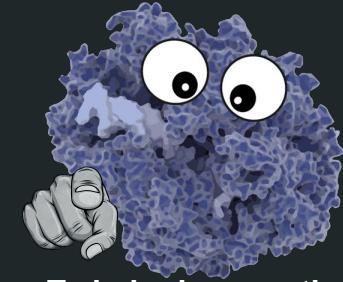
● friendzymes@gmail.com

**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
 - E. coli with a conjugative helper plasmid
 - Open Pichia strains
 - B. subtilis with strict inducible control of sporulation
 - B. subtilis that doesn’t smell like feet
 - 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

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