Synthetic biology: Engineering new functions, cells, and even life?

BCH394P/364C Systems Biology / Bioinformatics
Edward Marcotte, Univ of Texas at Austin

**Synthetic Biology** = design and engineering of biological systems that aren’t found in nature

**Why would we want to do this?**
- Want to understand natural systems. One of the best ways to understand a system is to change it or make new, related ones

- To fully “understand” a system, we should be able to predict the outcome when we change the system

- For molecular biology, this means:
  - designing new gene circuits and networks
  - modeling the designed systems & predicting their properties
  - making & testing the designs
  - updating our understanding from the model/test agreement
**The Repressilator** = engineered genetic circuit designed to make bacteria glow in an oscillatory fashion = “repressor” + “oscillator”

Transcriptional repressors

\[
\begin{align*}
\text{TetR} \\
\lambda \text{ cl} \\
\text{LacI}
\end{align*}
\]


**The Repressilator** = engineered genetic circuit designed to make bacteria glow in an oscillatory fashion

Repressilator

\[
\begin{align*}
\text{amp}^R \\
\text{tetR-lite} \\
\text{P}_{\text{Lac01}} \\
\lambda \text{ cl-lite} \\
\lambda \text{ P}_{\text{R}} \\
\text{lacI-lite} \\
\text{P}_{\text{Ltet01}}
\end{align*}
\]

Reporter

\[
\begin{align*}
\text{kan}^R \\
\text{gfp-aav} \\
\text{P}_{\text{Ltet01}} \\
\text{ColE1}
\end{align*}
\]

The repressilator in action...


**iGEM: A synthetic biology contest**

Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

Or is biology too complicated to be engineered in this way?

**Broader goals include:**
- Enable systematic engineering of biology
- Promote open & transparent development of tools for engineering biology
- Help construct a society that can productively apply biological technology

(from iGEM's web site)
IGEM: A synthetic biology contest

2004: MIT, UT, Princeton, Boston University, Cornell
2005: 13 teams (the above + UK, Germany, more...)
2006: 32 teams, incl. Japan, Latin America, Korea, India, more Europe

54 teams in 2007, 112 in 2009, 165 in 2011, and 245 in 2013...
...now roughly 6,000 entrants / year

A little local history to illustrate the field:
UT’s 2004/2005 iGEM project – build bacterial edge detector

Adapted from Zack Simpson
Bacterial photography


“Light cannon” developed by Aaron Chevalier, UT undergraduate

The first bacterial photograph (coliroid?)...


Escherichia darwinia

Image: Aaron Chevalier
On to the edge detector...

Tabor et al., Cell 137(7):1272-1281 (2009)

It works!

Tabor et al., Cell 137(7):1272-1281 (2009)
Who needs nature? 
*Made-to-order, designer organisms*

We can now manufacture a complete genome from commodity chemicals

Therefore, we can program whatever changes we want, assuming we can get it into cells...

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**Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome**

Daniel G. Gibson,1 John I. Glass,1 Carole Larngue,2 Vladimir N. Noskov,1 Ray-Yuen Chuang,3 Mikkel A. Algire,2 Gwynned A. Benders,2 Michael G. Montague,3 Li Ma,1 Monzla M. Moodie,1 Chuck Merryman,1 Sanjay Vashis,3 Radha Krishnakumar,1 Nacyra Assol-Garcia,1 Cynthia Andrews-Pfannkoch,1 Eugeniya A. Denisova,1 Lei Young,1 Zhi-Qing Qi,1 Thomas H. Segall-Shapiro,4 Christopher H. Calvey,3 Prashanth P. Parmar,2 Clyde A. Hutchison III,2 Hamilton O. Smith,2 J. Craig Venter1,2

“We report the design, synthesis, and assembly of the 1.08-mega-base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome.”

2 JULY 2010 VOL 329 SCIENCE
“Rebooting” bacteria with synthetic genomes

But, wait! They only changed DNA, not the rest of the cell!

However...

In biology, *software encodes the hardware*. Most (all?) of the cell is specified by the DNA.

It’s as though you bought an old Blackberry...

installed the Android operating system...

& your phone physically morphed into a Galaxy S10...
In 2016, Hutchison, Chuang, et al. reported making living mycoplasma after cutting the genome by ½ the genes. JCVI-syn3.0 now makes for a remarkably compact, engineerable, free living cell “chassis” to study.
...which now has a rich metabolic reconstruction...

...and highly defined composition, to the extent one can write its biomass reaction equation:

Protein
- L-glutamate
- L-lysine
- L-ornithine
- L-proline
- L-serine
- L-tyrosine
- L-lysine
- L-glutamine
- L-cysteine
- L-phenylalanine
- L-histidine
- L-alanine
- L-glutamine
- L-cysteine
- ATP (hydrolyzed)

DNA
- dGMP
- dGTP
- dGDP
- dTDP

Amino acids
- L-lysine (5.75×10^-3)
- L-arginine (8.85×10^-3)
- L-aspartate (5.44×10^-4)
- L-asparagine (9.02×10^-4)
- L-glutamine (3.03×10^-1)
- L-phenylalanine (2.29×10^-3)
- L-lysine (6.77×10^-3)
- L-tryptophan (1.31×10^-3)

RNA
- ATP (hydrolyzed)

Nucleotides
- ATP (2.98×10^-2)
- UTP (2.19×10^-2)
- GTP (1.10×10^-2)
- CTP (8.16×10^-3)

Lipids
- Cholesterol (3.94×10^-1)
- Phosphatidylglycerol (3.03×10^-1)
- Cardiolipin (2.10×10^-1)
- Phosphatidylcholine (2.09×10^-1)
- Fatty acids (1.73×10^-1)
- GSH (6.46×10^-2)

Cofactors
- AMP (3.4×10^-5)
- Spermine (9.1×10^-4)
- Acetyl-CoA (7.09×10^-4)
- Glutathione (9.1×10^-4)

Ions
- K⁺ (8.4×10^-4)
- Na⁺ (5.75×10^-3)
- Cl⁻ (5.93×10^-3)
- SO₄²⁻ (5.91×10^-3)
- HPO₄²⁻ (5.84×10^-3)
- Mg²⁺ (5.04×10^-3)
- Ca²⁺ (4.88×10^-4)

Breuer et al., eLife 2019;8:e36842 DOI: 10.7554/ELIFE.36842
...and even engineered motility

Spiroplasma actin operon

JCVI-syn3.0B

Kiyama et al., bioRxiv, doi:10.1101/2021.11.16.468548

...and now a 3D whole cell model

https://doi.org/10.1515/jib-2022-0013
David S. Goodsell, Integrative illustration of a JCVI-syn3A minimal cell
**E. coli** has also been completely synthesized & “rebooted”

→ Recoded 18,214 codons to make a 61 codon genome: 59 for the 20 amino acids + 2 stops

“…demonstrates that life can operate with a reduced number of synonymous sense codons”


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**How about eukaryotes? “rebooting” yeast with synthetic chromosomes**

**Turns out chromosomes can be synthesized and replaced for yeast too...**

The Sc2.0 consortium’s goal is a complete synthetic yeast genome
In 2017, the Synthetic Yeast Genome Project (Sc2.0) reported on five newly constructed synthetic yeast chromosomes:

![Cover of Science magazine](http://science.sciencemag.org/content/355/6329/eaan1126)

How the cover was made: [http://science.sciencemag.org/content/355/6329/eaan1126](http://science.sciencemag.org/content/355/6329/eaan1126)

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**Design of a synthetic yeast genome**

Sarah M. Richardson, 1,*, 4 Leslie A. Mitchell, 1,*, 3 Giovanni Stranquadama, 1,*, 3
Kim Yang, 1,*, 4 Jessica S. Dyson, 6, 5 James E. D’Carlo, 1,*, 2 Dongwu Lee, 1,*, 2
Cheng Lai Victor Huang, 1,*, 3 Kittiratwana Chandramangalam, 1,*, 4 Vinod C. 1,*, 2
Jeff D. Boecker, 1,*, 3 Joel S. Baden 1,*, 2

We describe complete design of a synthetic yeaceous genome, Sc2.0, a highly modified Saccharomyces cerevisiae genome reduced in size by nearly 8%, with 1.1 megabases of the synthetic genome deleted, inserted, or altered. Sc2.0 chromosome design was implemented with BioStudio, an open-source framework developed for yeaceous genome design, which coordinates design modifications from nucleotide to genome scales and enhances version control to systematically track edits. To achieve complete Sc2.0 genome synthesis.
The consortium constructed all pairwise combinations of 7 synthetic chromosomes—the vast majority show no growth defects at 30C, modest at 37C & moved all 275 tRNAs to a separate chromosome.

![Image of tRNAs]

 Zhao et al., 2022, Cell 186, 6220-6236
  https://doi.org/10.1016/j.cell.2022.09.025
Inevitably (!), the human synthetic genome project:

First major project: Recoding human codons (~200K edits?) to create virus-resistant cells

“…What I cannot create, I do not understand…”

Richard Feynman

While the sequence of the human genome has been known for nearly 20 years, many mysteries in life’s recipe book remain to be solved. That’s why one group of researchers as well as ethicists and communicators is proposing to move from passively reading genomes to actively writing them.

https://en.wikipedia.org/wiki/Genome_Project-Write
https://engineeringbiologycenter.org/

Let’s end the lectures on a fun note, with some speculative near-future synthetic biology experiments

Science fiction? or not? You be the judge!
“De-extincting” extinct species

What if the cells being cloned came from an extinct animal and were put into a surrogate mother? Would that resurrect the species?

Remember Dolly, the cloned sheep?

This was tried in 2009 for the Pyrenean ibex, and almost worked...

Cloned goat dies after attempt to bring species back from extinction

But now there’s another way!

- We can sequence a genome in a few days for a few $K
- We can synthesize or alter big pieces of the DNA
- We can (almost) “reboot” cells with this DNA
- We can convert cells to stem cells to embryos
- We can in vitro fertilize animals

So why not just “edit” the genomes of the closest living animals to be like their extinct relatives?

Sound familiar?
Besides the genome engineering, this hinges on iPS:

From embryonic stem cells, we can grow an entire organism or any cells/tissues in it

& thanks to Yamanaka, we can convert skin cells back into stem cells

Shinya Yamanaka
Nobel Prize, 2012

There’s a serious proposal to resurrect the woolly mammoth. Here’s the process:

✓ Mammoth genome sequence
➢ Make ~100K DNA changes in elephant skin cells to convert elephant skin cells → mammoth skin cells
✓ Convert skin cells to stem cells
✓ Convert stem cells to embryos
➢ In vitro fertilize elephants

This might be a hard step.
As of April 2015...

Using a DNA editing tool called CRISPR, the scientists spliced genes for the mammoths' small ears, subcutaneous fat, and hair length and color into the DNA of elephant skin cells. The tissue cultures represent the first time woolly mammoth genes have been functional since the species went extinct around 4,000 years ago.

The research has not yet been peer-reviewed or published in a scientific journal "because there is more work to do," Church told the U.K.'s *Sunday Times*, "but we plan to do so."

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**A New Company With a Wild Mission: Bring Back the Woolly Mammoth**

With $15 million in private funding, Colossal aims to bring thousands of woolly mammoths back to Siberia. Some scientists are deeply skeptical that will happen.

A team of scientists and entrepreneurs announced on Monday that they have started a new company to genetically resurrect the woolly mammoth.

The company, named Colossal, aims to place thousands of these magnificent beasts back on the Siberian tundra, thousands of years after they went extinct.

Sept. 13, 2021

Colossal grabs $60 million Series A for moonshot mammoth project

The De-Extinction of the Woolly Mammoth Is a Legal and Regulatory Nightmare

A biotech firm wants to resurrect the Pleistocene mammal in Alaska—and it's not clear the U.S. government can stop them.

Andy Lamey
December 15, 2022

A species via biotechnology. The region of Siberia Colossal had in mind, Sakha, has a thriving underground trade in mammoth tusks. Specimens preserved in ice and riverbeds can be passed off as elephant ivory. One find can generate enough income for a hunter to feed his family for a year. So George Church, a Harvard geneticist and co-founder of Colossal, told CNN that in order to avoid its creations being poached, Colossal was considering bringing them back without tusks.
Which animal would you resurrect?

The dodo?
The quagga?
Sabertoothed tiger?

In principle, only need the DNA sequence (so, no dinosaurs)

Aurochs?

I vote for some crazy Australasian animals:

The 12’ tall moa

& of, course, the marsupial Tasmanian tiger

>90° !!!

The moa-eating Haast’s eagle

Actual scale!
And sure enough!

What about neanderthal?
It’s achievable. But should we do it?

- Human and neanderthal genome sequence
- Edit DNA in human skin cells to convert
  convert human skin cells → neanderthal skin cells
  → I give this step 10 years max before we can do this
- Convert skin cells to stem cells
- Convert stem cells to embryos
- In vitro fertilize
  a surrogate mother

So many ethical questions!
Where to start?