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?

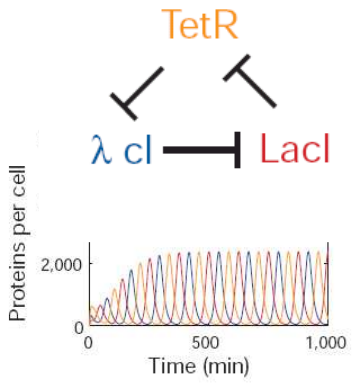
- Want to understand natural systems. One of the best ways to understand a system is to change it or make new, related ones

- 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 a oscillatory fashion = "repressor" + "oscillator"

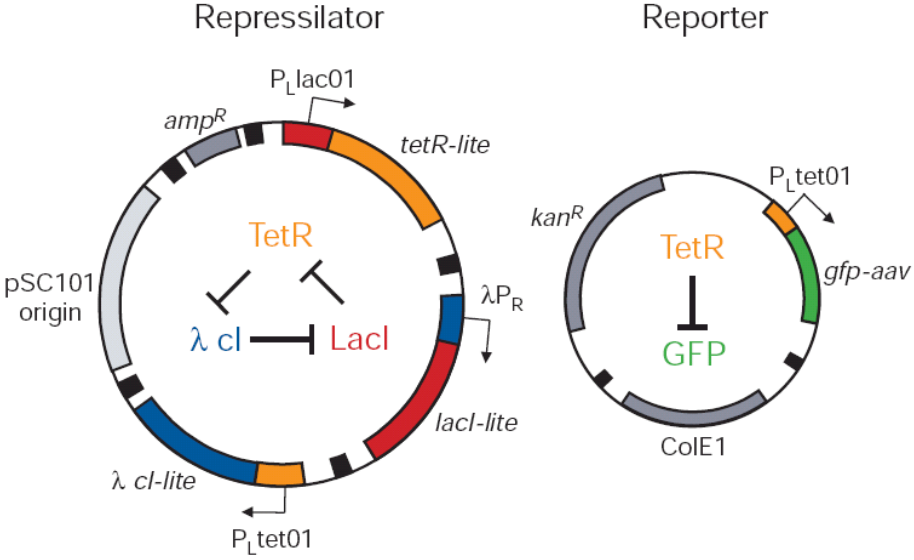
Transcriptional repressors



Green fluorescent protein

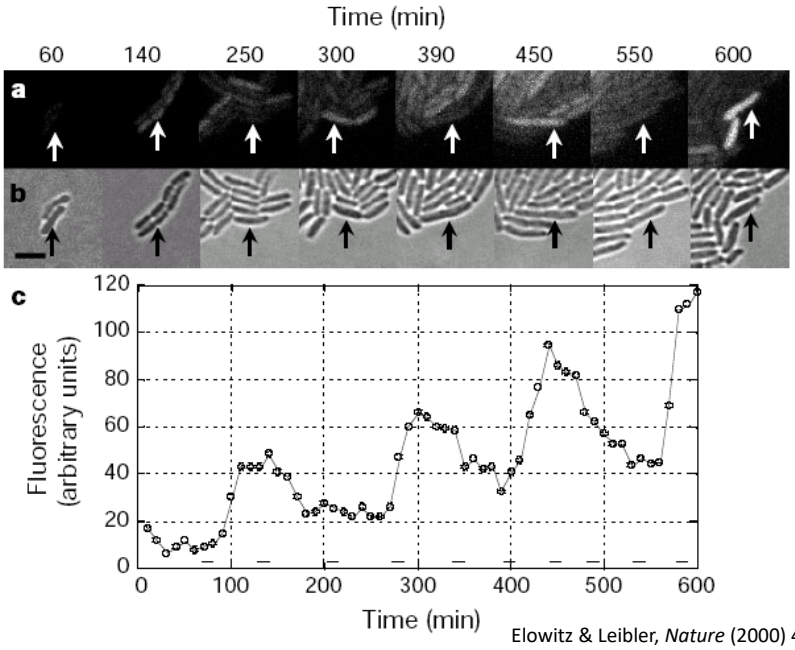
Elowitz & Leibler, *Nature* (2000) 403:335-8

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



Elowitz & Leibler, *Nature* (2000) 403:335-8

The repressilator in action...



Elowitz & Leibler, *Nature* (2000) 403:335-8

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?

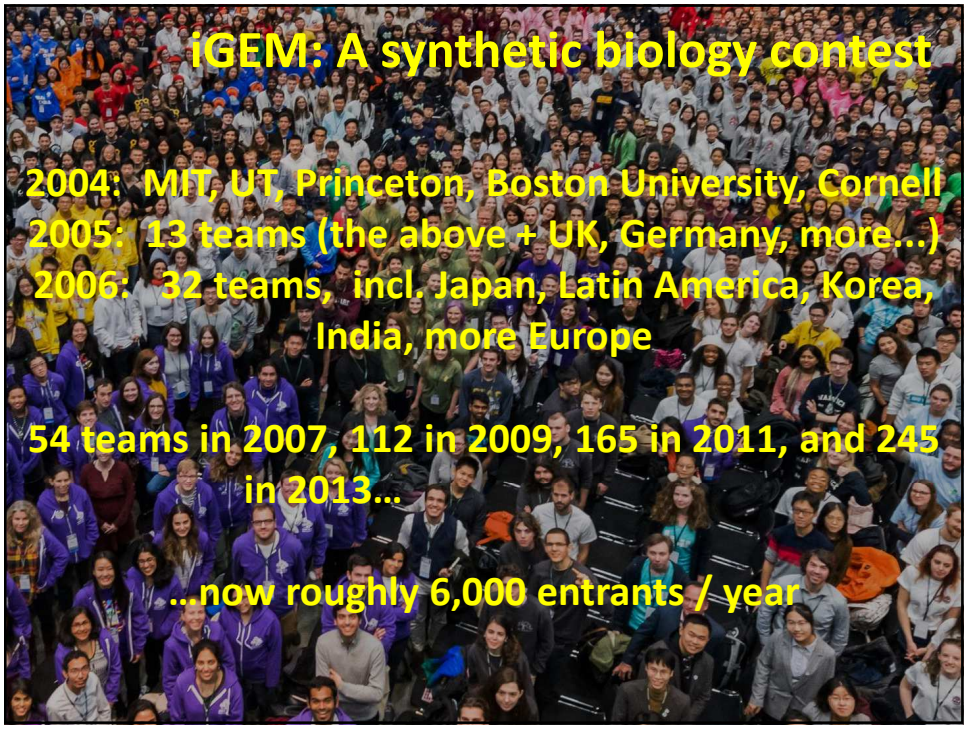
<u>Broader goals include:</u>

- Enable systematic engineering of biology

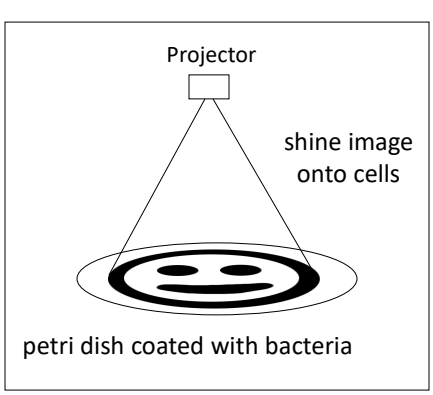
- Promote open & transparent development of tools for engineering biology

- Help construct a society that can productively apply biological technology

(from iGEM's web site)



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

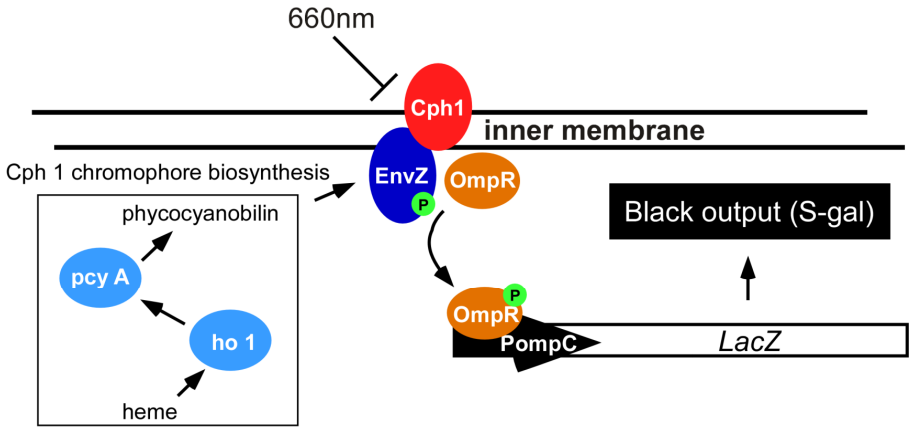


Original image

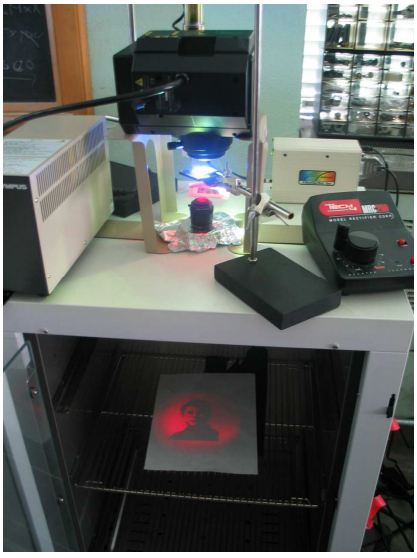
Cells luminesce along the light/dark boundaries

Adapted from Zack Simpson

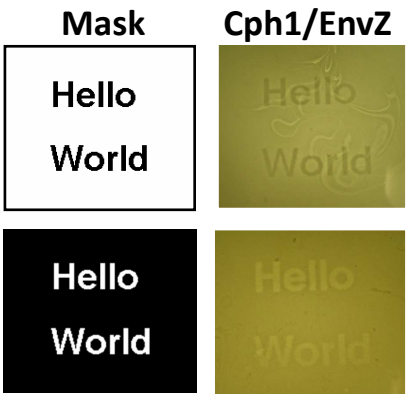
Bacterial photography



Levskaya *et al. Nature*, 438(7067):441-2 (2005)



"Light cannon" developed by Aaron Chevalier, UT undergraduate



Levskaya *et al. Nature*, 438(7067):441-2 (2005)

The first bacterial photograph (coliroid?)...

Levskaya *et al. Nature*, 438(7067):441-2 (2005)

Escherichia darwinia

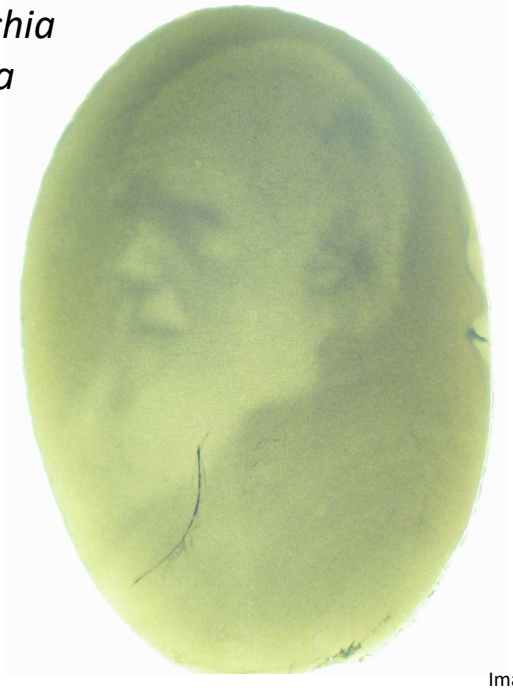
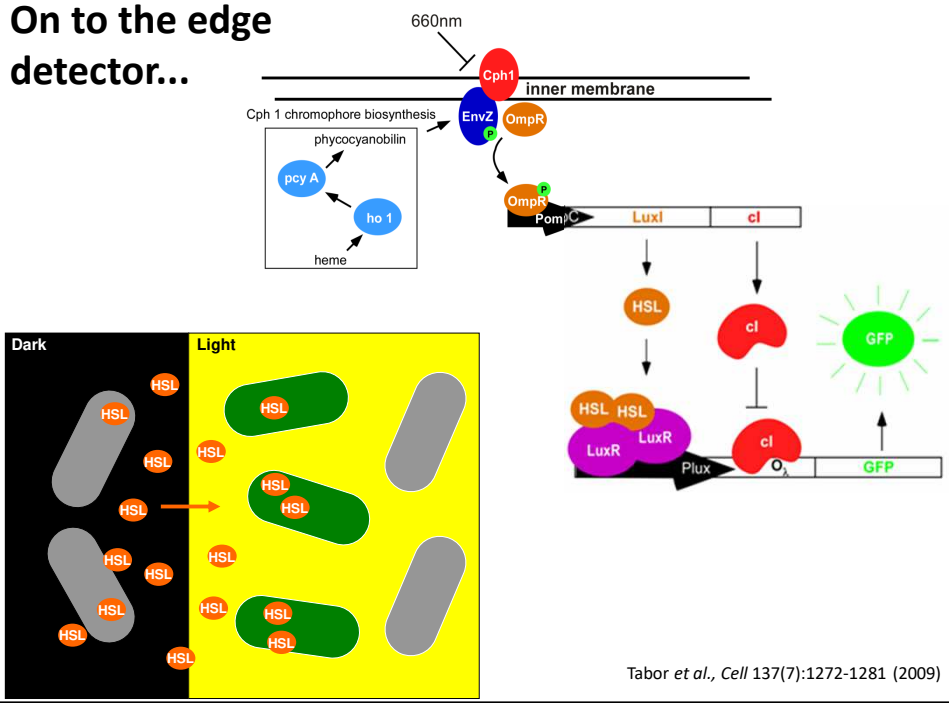


Image: Aaron Chevalier

On to the edge detector...



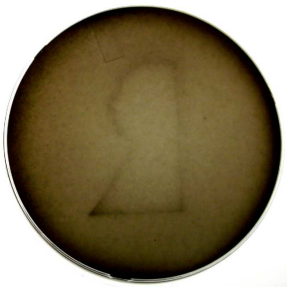
Tabor *et al.*, *Cell* 137(7):1272-1281 (2009)

It works!

Projected Mask

Photo strain

Edge detector strain



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

Who needs nature?
Made-to-order, designer organisms

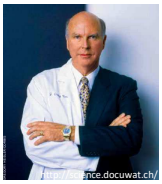
We can now <u>manufacture</u> a complete genome from commodity chemicals

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

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

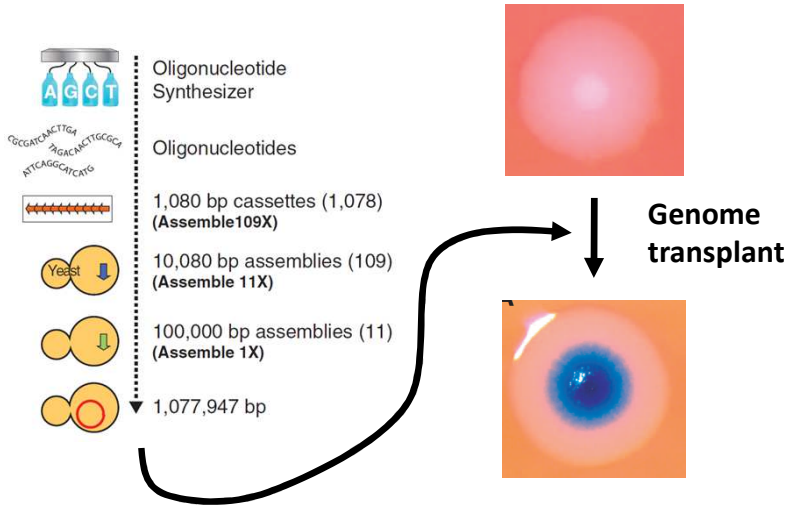
Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

"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



2 JULY 2010 VOL 329 SCIENCE

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

However…

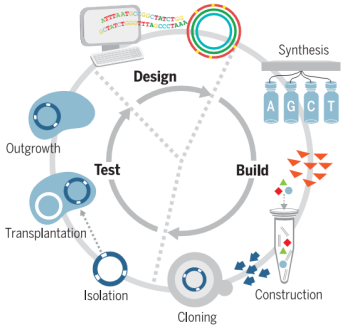
In biology, *<u>software encodes the hardware</u>*.
Most (all?) of the cell is specified by the DNA.

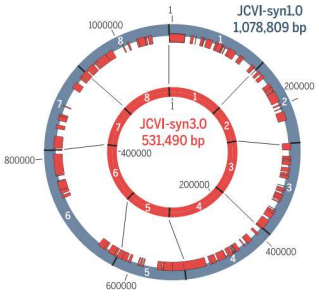
It's as though you bought a old Blackberry…

installed the Android operating system…

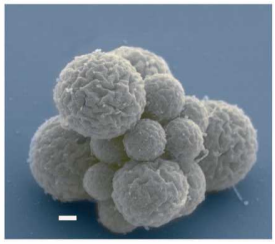
& your phone physically morphed into a Galaxy S10…

In 2016, Hutchison, Chuang, *et al.* reported making living mycoplasma after cutting the genome by ½ the genes



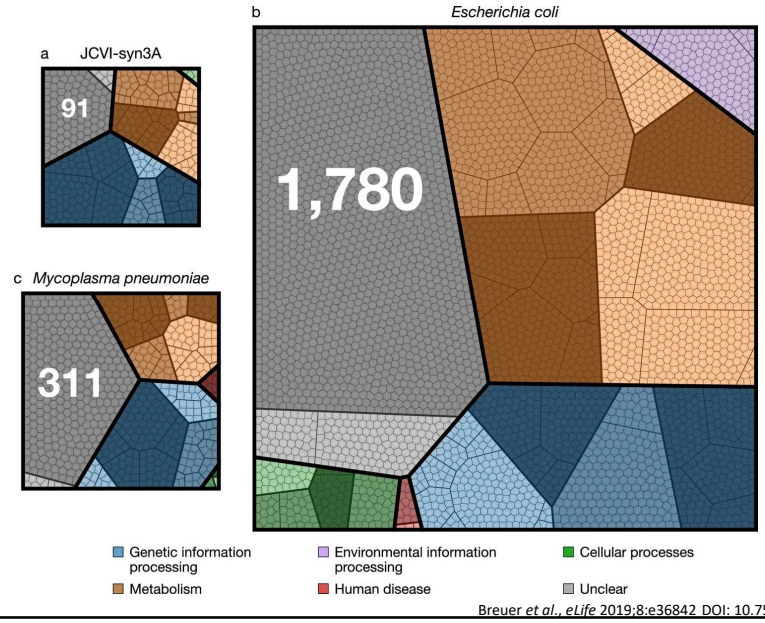


Four design-build-test cycles produced JCVI-syn3.0. (**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (**B**) Comparison of JCVI-syn1.0 (outer blue circle) with JCVI-syn3.0 (inner red circle), showing the division of each into eight segments. The red bars inside the outer circle indicate regions that are retained in JCVI-syn3.0. (**C**) A cluster of JCVI-syn3.0 cells, showing spherical structures of varying sizes (scale bar, 200 nm).



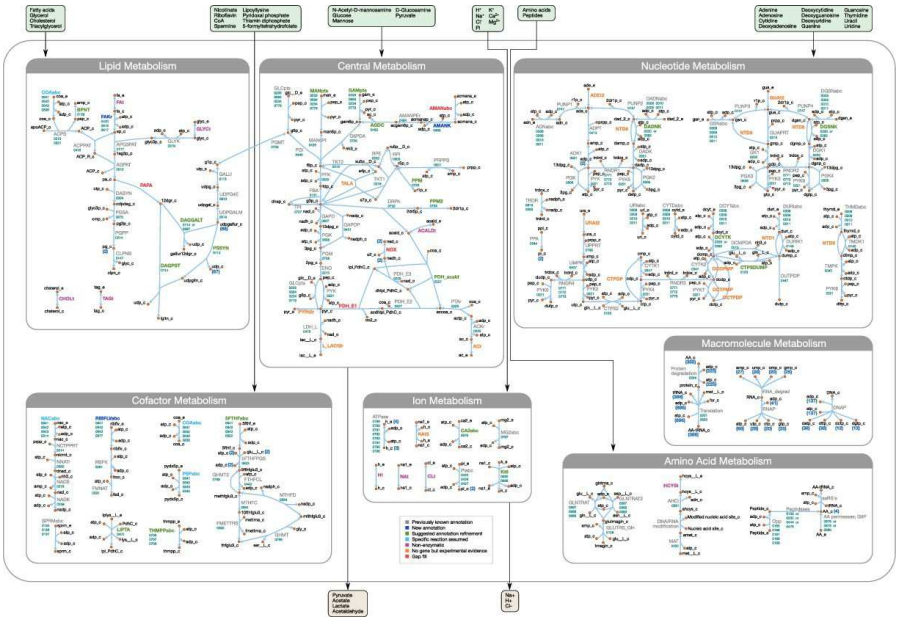
Science 25 MARCH 2016 • VOL 351 ISSUE 6280

JCVI-syn3.0 now makes for a remarkably compact, engineerable, free living cell "chassis" to study



Breuer *et al.*, *eLife* 2019;8:e36842 DOI: 10.7554/ELIFE.36842

…which now has a rich metabolic reconstruction…



Breuer *et al.*, *eLife* 2019;8:e36842 DOI: 10.7554/ELIFE.36842

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

Protein

(27)	L-glutamate
(36)	L-leucine
(23)	L-alanine
(23)	L-aspartate
(21)	Glycine
(11)	L-proline
(16)	L-phenylalanine
(12)	L-arginine
(23)	L-serine
(22)	L-threonine
(24)	L-valine
(5)	L-histidine
(3)	L-tryptophan
(36)	L-isoleucine
(39)	L-lysine
(7)	L-methionine
(13)	L-tyrosine
(15)	L-glutamine
(2)	L-cysteine
(27)	L-asparagine
(21.2)	**ATP hydrolyzed**

DNA

(38)	dAMP
(12)	dGMP
(12)	dCMP
(38)	dTMP
(0.24)	**ATP hydrolyzed**

RNA

(27)	AMP
(25)	GMP
(20)	CMP
(28)	UMP
(0.14)	**ATP hydrolyzed**

Lipogalactan

(88)	Galactose
(1)	Diacylglycerol

Amino acids

(5.75×10^{-3})	L-glutamate
(8.53×10^{-3})	L-leucine
(8.66×10^{-3})	L-alanine
(5.44×10^{-3})	L-aspartate
(9.02×10^{-3})	Glycine
(3.02×10^{-3})	L-proline
(2.96×10^{-3})	L-phenylalanine
(2.48×10^{-3})	L-arginine
(6.71×10^{-3})	L-serine
(6.00×10^{-3})	L-threonine
(7.07×10^{-3})	L-valine
(1.21×10^{-3})	L-histidine
(4.15×10^{-4})	L-tryptophan
(8.75×10^{-3})	L-isoleucine
(9.07×10^{-3})	L-lysine
(1.77×10^{-3})	L-methionine
(2.28×10^{-3})	L-tyrosine
(3.28×10^{-3})	L-glutamine
(6.21×10^{-4})	L-cysteine
(6.27×10^{-3})	L-asparagine

Nucleotides

(3.29×10^{-3})	ATP
(2.19×10^{-3})	UTP
(2.19×10^{-3})	GTP
(1.10×10^{-3})	CTP
(6.8×10^{-5})	dTTP
(5.7×10^{-5})	dATP
(3.8×10^{-5})	dCTP
(2.2×10^{-5})	dGTP

Lipids

(3.97×10^{-2})	Cholesterol
(3.94×10^{-2})	Phosphatidylglycerol
(2.30×10^{-2})	Diacylglycerol
(2.10×10^{-2})	Cardiolipin
(2.04×10^{-2})	Free fatty acids
(1.73×10^{-2})	Gal-DAG
(6.48×10^{-3})	Triacylglycerol

(1.26×10^{-2}) **protein** + (1.78×10^{-3}) **DNA** + (5.04×10^{-3}) **RNA** + (4.29×10^{-3}) **lipogalactan** + (10^{-6}) **dUTPase** + (2.1×10^{-5}) **ACP** + (9.93×10^{-2}) **amino acids** + (8.95×10^{-3}) **nucleotides** + (0.167) **lipids** + (7.45×10^{-3}) **cofactors** + (1.00) **ions** + (25) **ATP** + (25) H_2O ⟶ **biomass** + (25) **ADP** + (25) P_i + (25) H^+

Ions

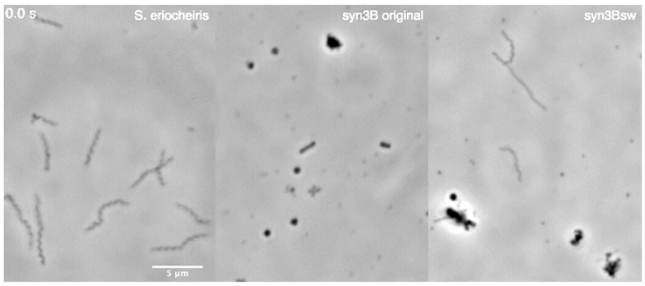
(0.840)	K^+
(5.72×10^{-2})	Na^+
(5.59×10^{-2})	Cl^-
(3.91×10^{-2})	HPO_4^{2-}
(7.76×10^{-3})	Mg^{2+}
(4.66×10^{-3})	Ca^{2+}

Cofactors

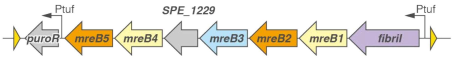
(6.34×10^{-3})	Spermine
(2.1×10^{-4})	Pyridoxal phosphate
(2.1×10^{-4})	FAD
(2.1×10^{-4})	5,10-MeTHF($Glu)_5$
(2.1×10^{-4})	Thiamin diphosphate
(1.58×10^{-4})	CoA
(1.05×10^{-4})	$NADP^+$

Breuer *et al.*, *eLife* 2019;8:e36842 DOI: 10.7554/ELIFE.36842

…and even engineered motility



***Spiroplasma* actin operon**



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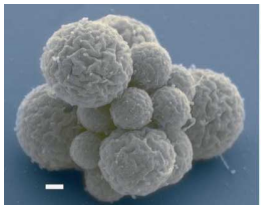
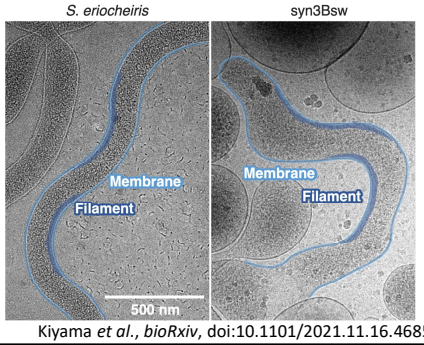
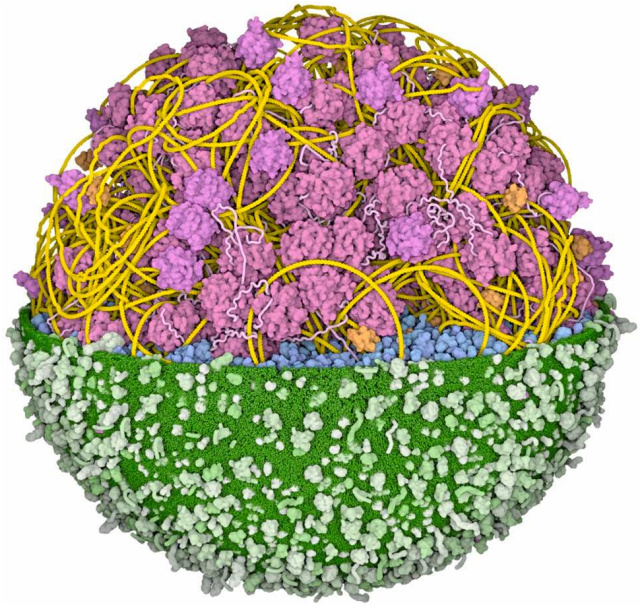


JCVI-syn3.0B



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

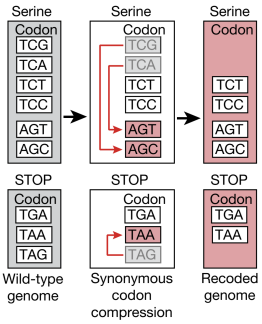
…and now a 3D whole cell model



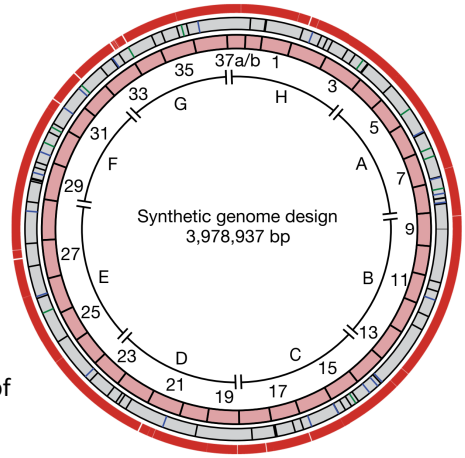
https://doi.org/10.1515/jib-2022-0013
David S. Goodsell, Integrative illustration of a JCVI-syn3A minimal cell

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



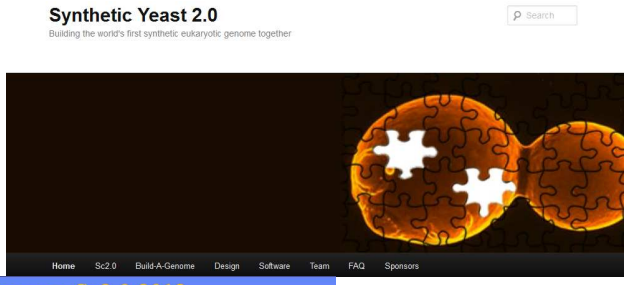
"…demonstrates that life can operate with a reduced number of synonymous sense codons"



Nature 569:514 (2019)

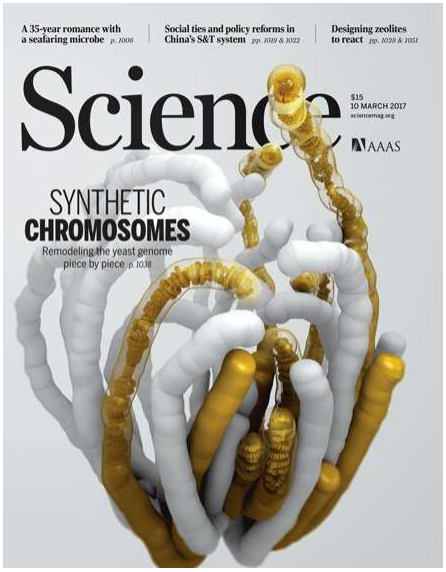
How about eukaryotes?
"rebooting" yeast with synthetic chromosomes

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:



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

Design of a synthetic yeast genome

Sarah M. Richardson,[1,2]*‖ Leslie A. Mitchell,[2,3]‖ Giovanni Stracquadanio,[1,2,4]‖ Kun Yang,[1,2]‖ Jessica S. Dymond,[2]† James E. DiCarlo,[2]‡ Dongwon Lee,[1]§ Cheng Lai Victor Huang,[2] Srinivasan Chandrasegaran,[5] Yizhi Cai,[2,6] Jef D. Boeke,[2,3]# Joel S. Bader[1,2]#

We describe complete design of a synthetic eukaryotic 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 eukaryotic genome design, which coordinates design modifications from nucleotide to genome scales and enforces version control to systematically track edits. To achieve complete Sc2.0 genome synthesis,

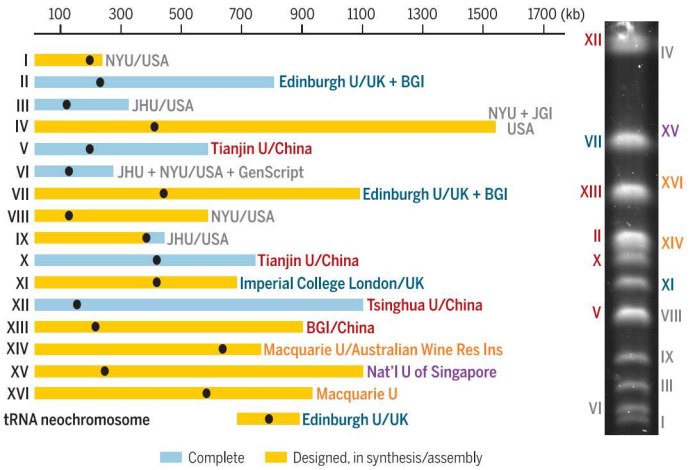
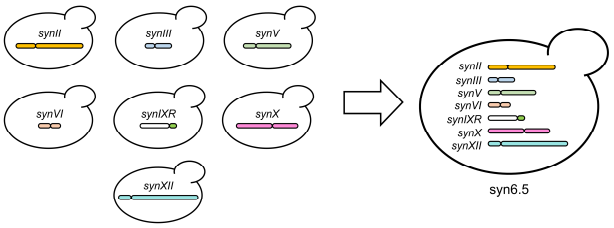


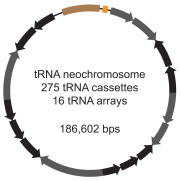
Table 1. Design challenges and policies adopted. CDS, gene coding sequence; snoRNA, small nucleolar RNA.

Design challenge or amendment	Policy adopted by design team
Subtelomeric repeats of varying copy number on multiple chromosomes	Delete and monitor for phenotypes as chromosomes are combined. Exception: vitamin biosynthesis genes retain one copy.
Dispersed repeated genes of high copy number, as well as high-copy COS and seripauperin genes	Delete and monitor for phenotypes as chromosomes are combined.
loxPsym sites <300 bp apart when inserted algorithmically (not especially useful and more difficult to synthesize)	loxPsym thinning to eliminate the loxPsym site closer to the centromere.
Stop codon overlaps a second CDS; insertion of loxPsym site would disrupt second CDS; also TAG recoding to TAA could disrupt CDS	Favor preservation of "verified ORFs" over "dubious ORFs" and "uncharacterized ORFs"; always add loxPsym site to a verified ORF in this case
Tandem repeats inside CDSs (*34*)	Use GeneDesign's RepeatSmasher module to recode such genes to minimize DNA level repetitiveness, making DNA easier to synthesize and assemble.
Homopolymer tracts, including frequent A and T tracts, are difficult to synthesize	In synthesis phase, permit 10% length variation for homopolymer tracts >10 bp provided they are in a noncoding region.
Introns	Delete pre-mRNA introns precisely, except from genes with evidence of a fitness defect caused by intron deletion (*35*, *36*). The *HAC1* intron, which uses separate splicing machinery and is known to play a critical role in regulation of the unfolded protein response, was not deleted (*9*). Delete all tRNA introns precisely.
Intronically embedded snoRNAs	These are individually nonessential and were deleted with their host introns. They could be "refactored" by insertion into the array of snoRNAs on chr II.

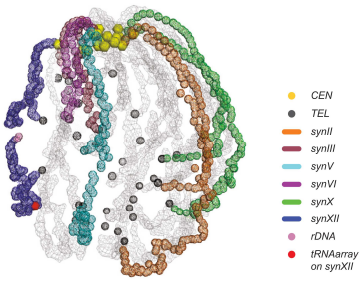
The consortium constructed all pairwise combinations of 7 synthetic chromosomes→the vast majority show no growth defects at 30C, modest at 37C

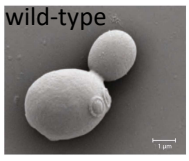


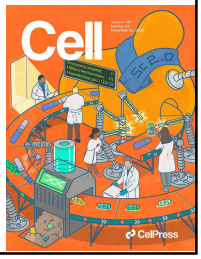
& moved all 275 tRNAs to a separate chromosome



Cell *186*, 5237–5253, November 22, 2023

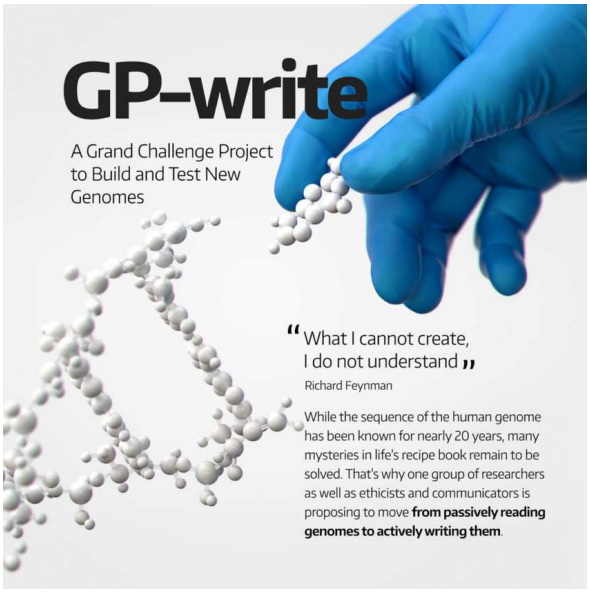




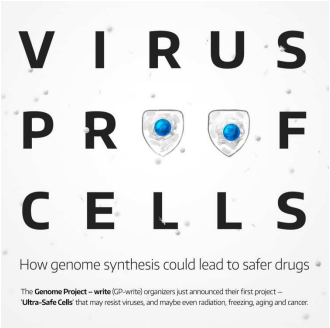


Zhao et al., 2023, Cell *186*, 5220–5236
https://doi.org/10.1016/j.cell.2023.09.025

Inevitably (!), the human synthetic genome project:



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



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

Remember Dolly, the cloned sheep?

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

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

wikipedia

Cloned goat dies after attempt to bring species back from extinction

Groundbreaking experiment fails, but scientists pave way for 'return' of other creatures

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

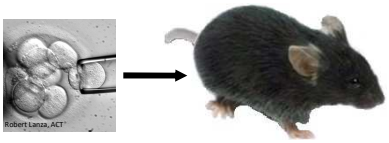
So why not just "edit" the genomes of the closest living animals to be like their extinct relatives?

Sound familiar?

http://jurassicpark.wikia.com

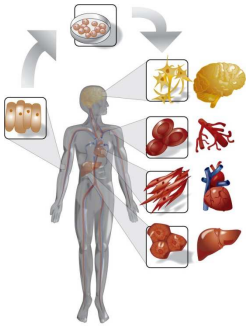
Besides the genome engineering, this hinges on iPS:

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



Shinya Yamanaka
Nobel Prize, 2012

& thanks to Yamanaka, we can convert <u>skin</u> cells back into <u>stem</u> cells

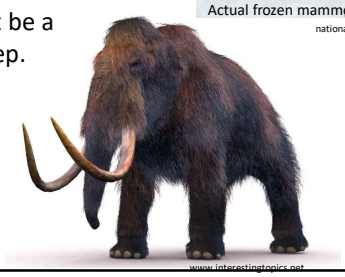


www.regenexx.com

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.



www.interestingtopics.net

As of April 2015…

ANIMALS

WOOLLY MAMMOTH DNA SUCCESSFULLY SPLICED INTO ELEPHANT CELLS

BUT DON'T EXPECT MAMMOTH CLONES ANYTIME SOON

By Sarah Fecht Posted March 24, 2015

347 Shares

Woolly Mammoth Museum

A group of researchers are p

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."

http://www.popsci.com/woolly-mammoth-dna-brought-life-elephant-cells

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.

Give this article 669

The biologist George Church unearthing woolly mammoth remains in Siberia. Eriona Hysolli

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

https://www.nytimes.com/2021/09/13/science/colossal-woolly-mammoth-DNA.html

https://colossal.com/mammoth/

Colossal grabs $60 million Series A for moonshot mammoth project

THE CIA JUST INVESTED IN WOOLLY MAMMOTH RESURRECTION TECHNOLOGY

While skeptics doubt the prospects for de-extinction, the CIA's venture capital firm deems powerful genetic manipulation tools worth the money.

COLOSSAL PROBLEM

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

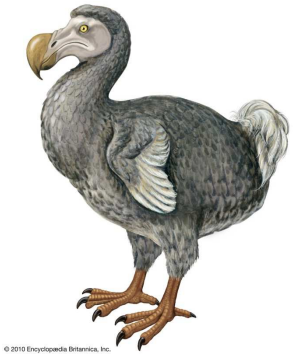
A biotech firm wants to resurrect the Pleistocene mammal in Alaska—and it's not clear the U.S. government can stop them.

Andy Lamey
December 15, 2022

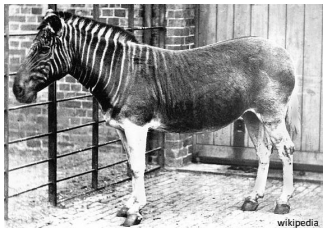
species via biotechnology. The region of Siberia Colossal had in mind, Sakha, has a thriving underground trade in mammoth tusks. Specimens preserved in ice and riverbeds can be passed off as elephant ivory: One find can generate enough income for a hunter to feed his family for a year. So George Church, 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.

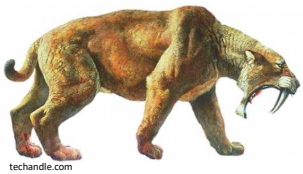
Which animal would you resurrect?

The dodo?



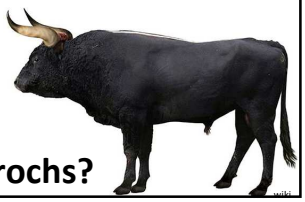
The quagga?





Saber-toothed tiger?

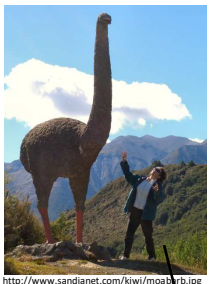
In principle, only need the DNA sequence (so, no dinosaurs)



Aurochs?

I vote for some crazy Australasian animals:

The 12' tall moa

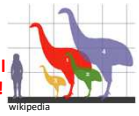


& of, course, the marsupial Tasmanian tiger

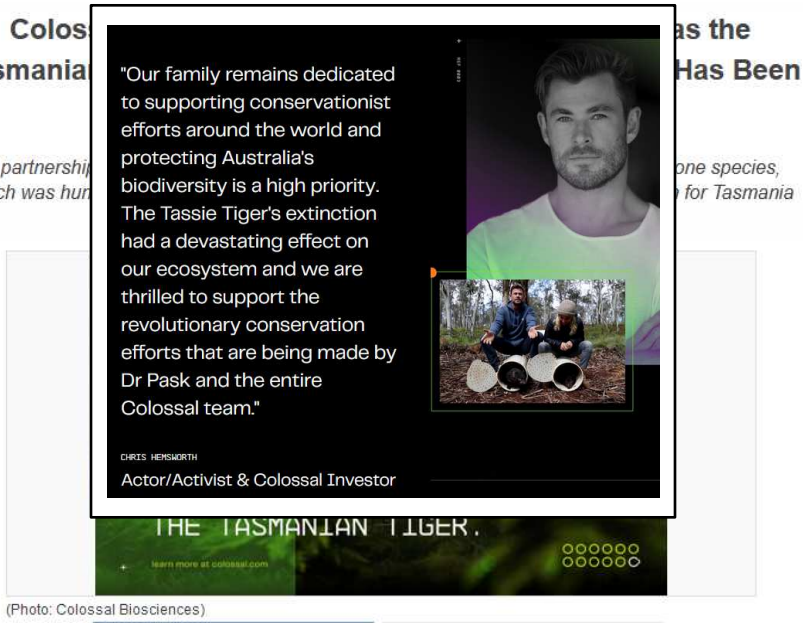
>90° !!!

The moa-eating Haast's eagle

Actual scale!



And sure enough!



(Photo: Colossal Biosciences)

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

Svante Pääbo

- ✓ **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?