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

Engineers often look at biological systems & think that the systems are equivalent to electronic circuits

e.g,

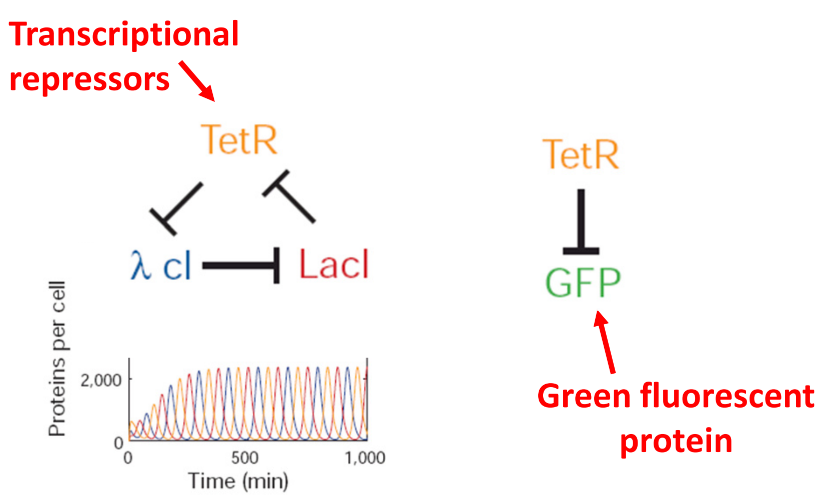
fluorescent proteins	light bulbs or LEDs
transcription factors	transistors or logic gates
repressors	NOT gates
activators	OR/AND gates
polymerases (transcriptional machinery)	batteries

and so on...

Are they right?

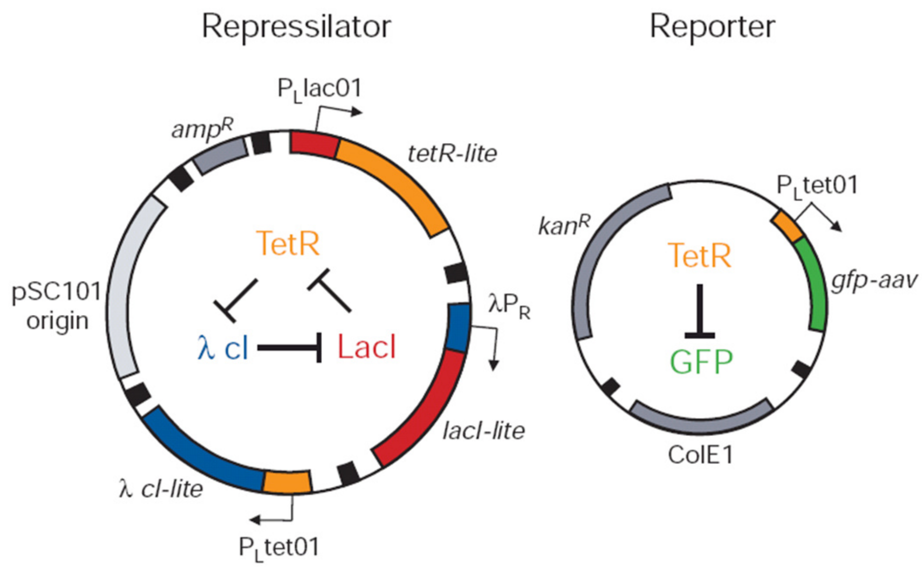
→ raises the possibility that biological parts (genes, proteins, etc.) could be combined using the rules established for analog/digital circuits

The Repressilator = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion = “repressor” + “oscillator”



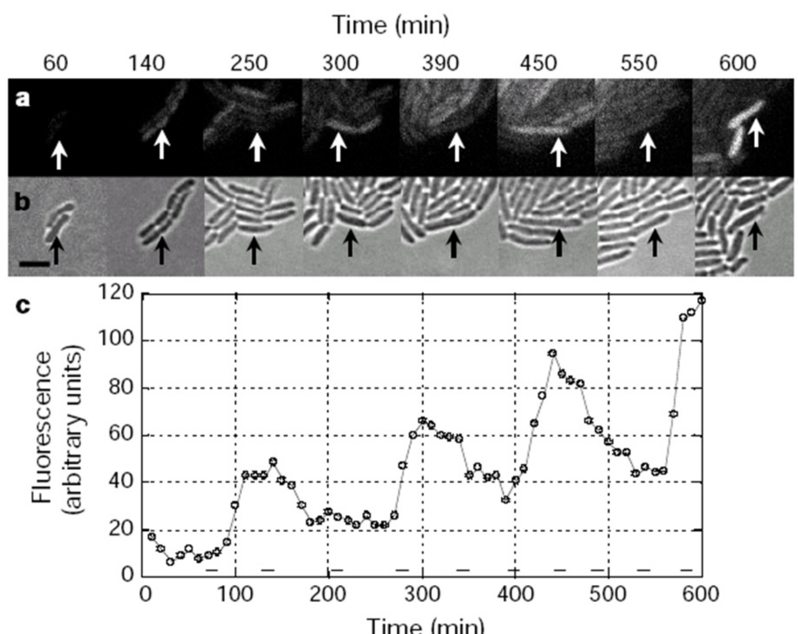
Elowitz & Leibler, *Nature* (2000) 403:335-8

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

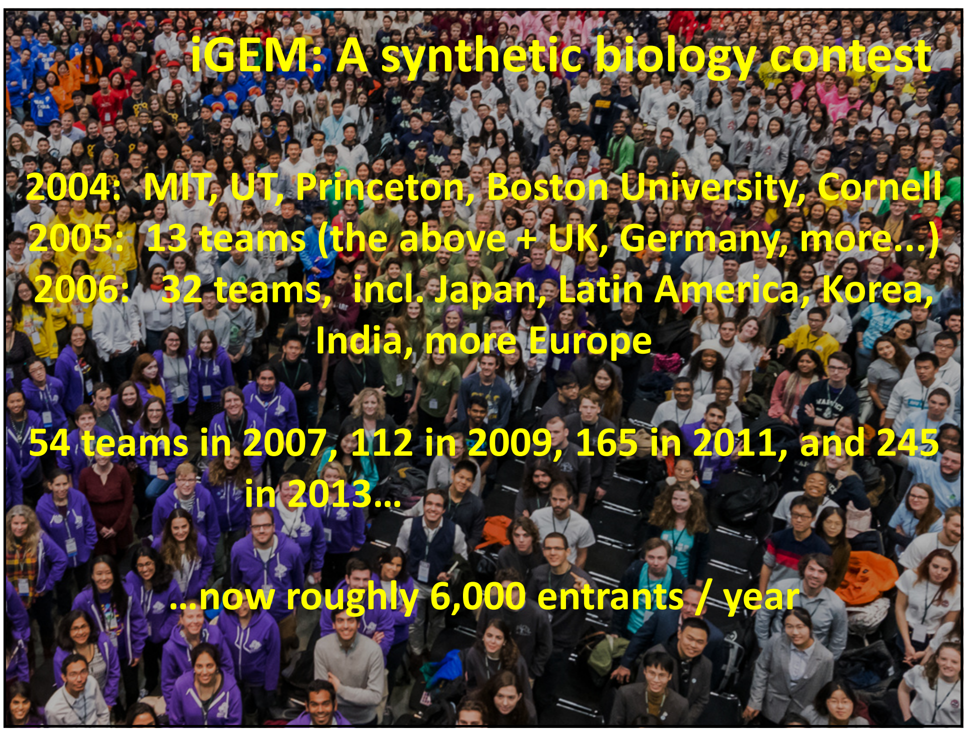
Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

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

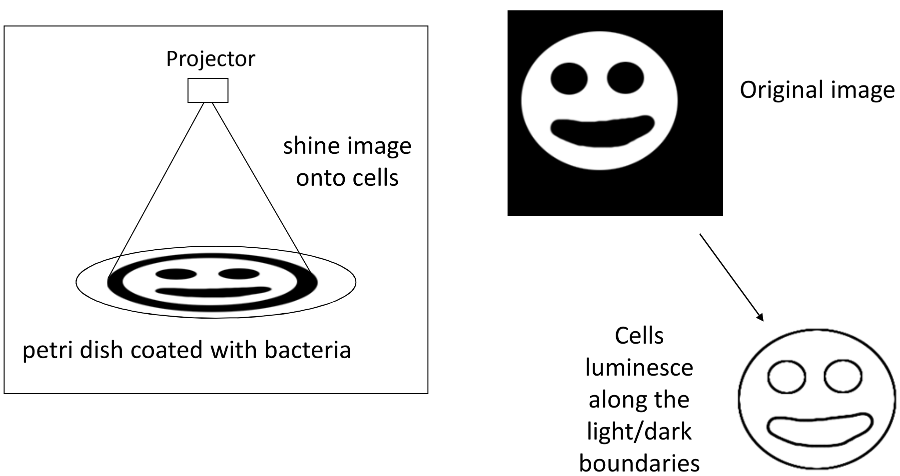
Broader goals include:

- **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)



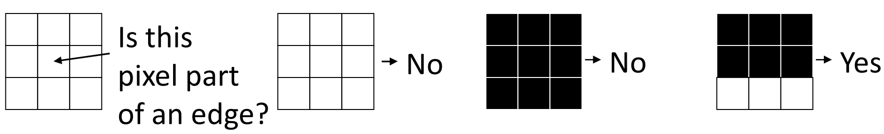
A little local history to illustrate the field: UT's 2004/2005 iGEM project – build bacterial edge detector

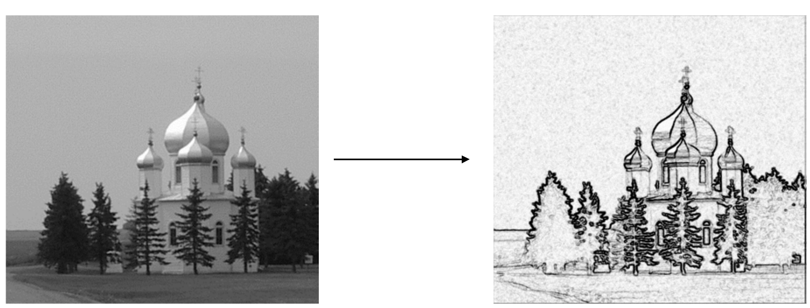


Adapted from Zack Simpson

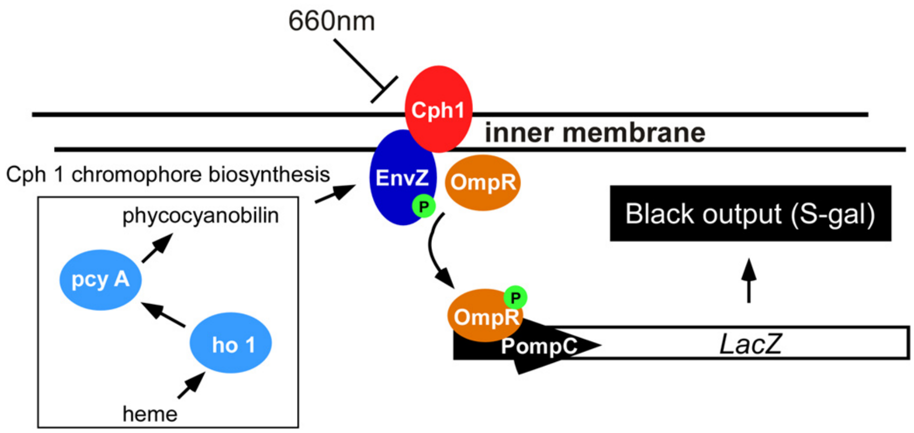
How does edge detection work in principle?

A computer might visit each pixel in turn, and check to see if it is bordered by both black & white pixels. If yes, highlight the pixel.

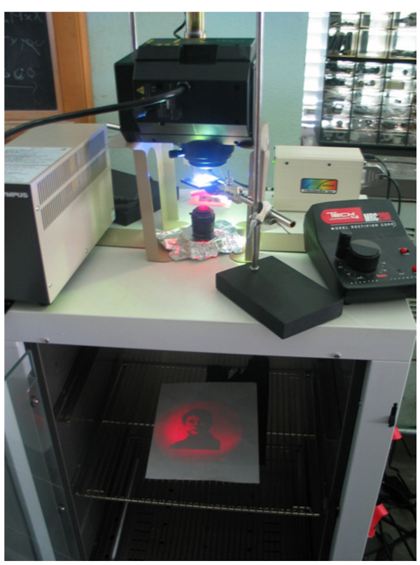




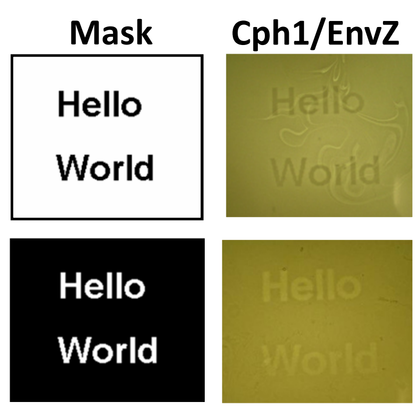
Bacterial photography



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)

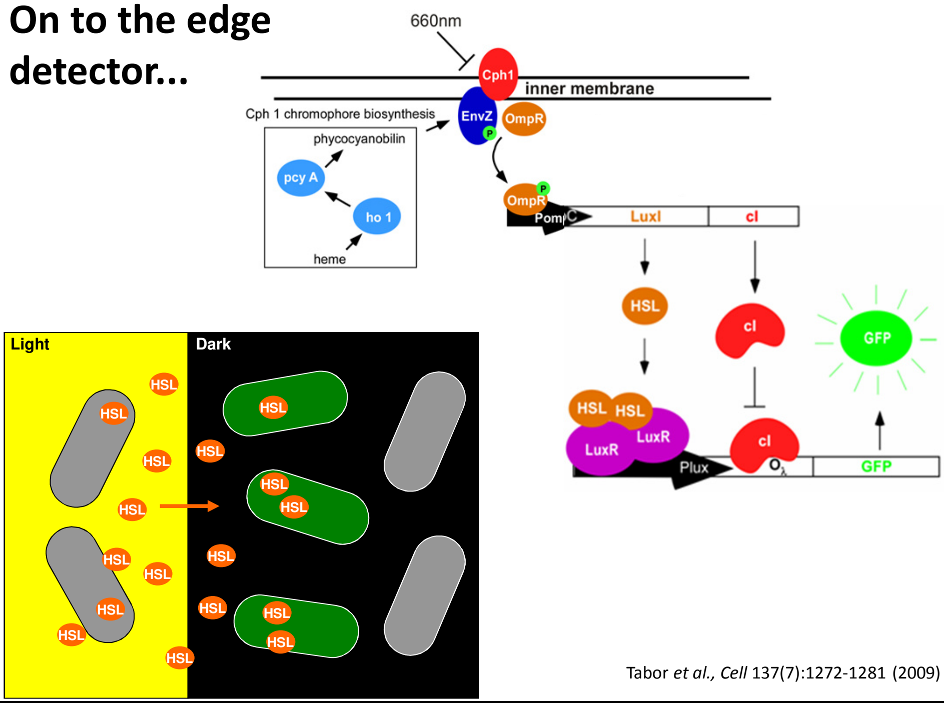
The first bacterial photograph (coliroid?)...

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)

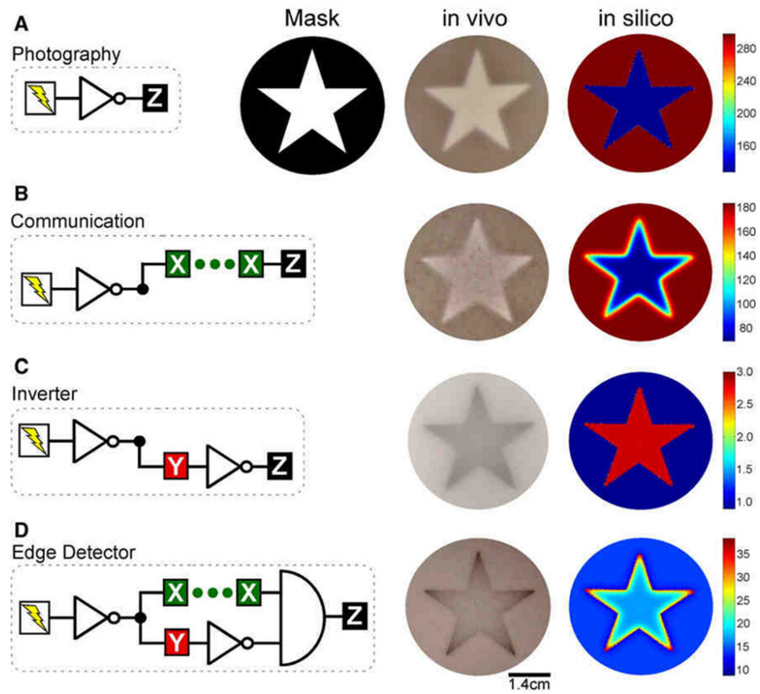
It works!

Projected Mask

Photo strain

Edge detector strain

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



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

UT's 2012 iGEM project – build caffeine biosensor

ACS Synthetic Biology — Letter — pubs.acs.org/synthbio

Decaffeination and Measurement of Caffeine Content by Addicted *Escherichia coli* with a Refactored *N*-Demethylation Operon from *Pseudomonas putida* CBB5

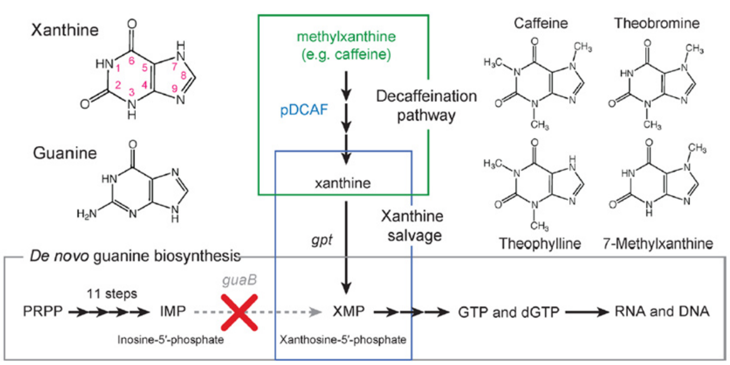
Erik M. Quandt,[†] Michael J. Hammerling,[†] Ryan M. Summers,[‡] Peter B. Otoupal,[†] Ben Slater,[†] Razan N. Alnahhas,[†] Aurko Dasgupta,[†] James L. Bachman,[†] Mani V. Subramanian,[‡] and Jeffrey E. Barrick[*,†]

<u>Basic idea</u>

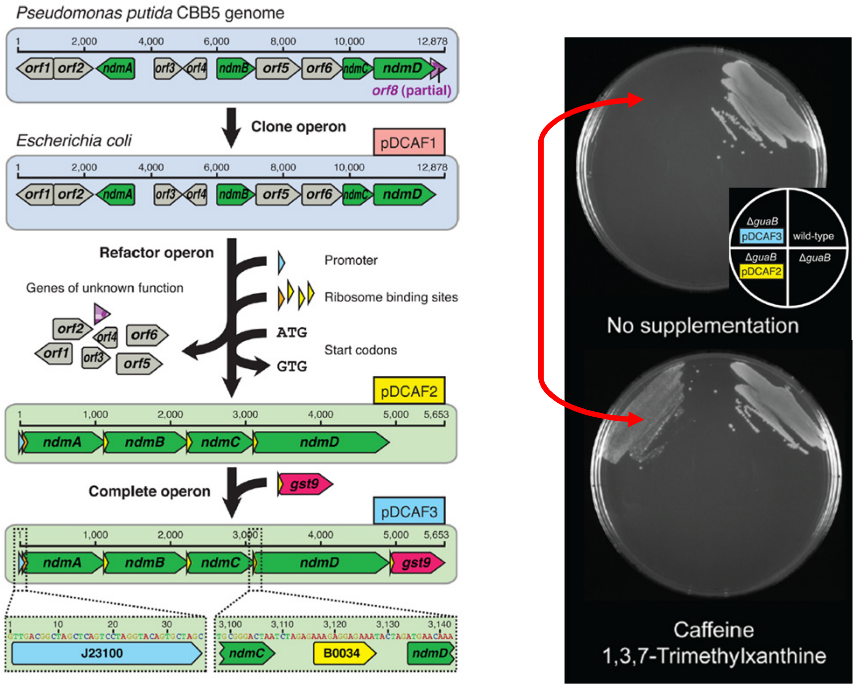
Block *de novo* guanine synthesis

Convert caffeine to xanthine

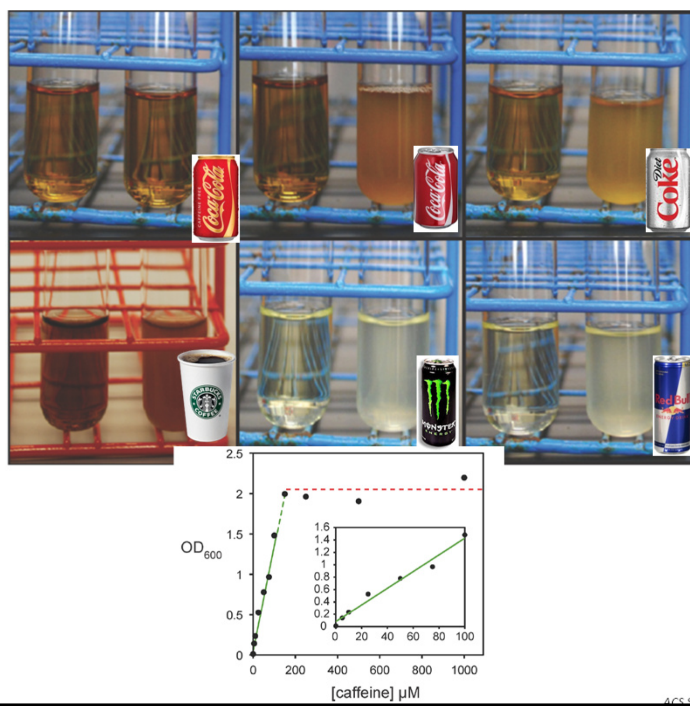
Addict *E. coli* bacteria to caffeine



ACS Synth. Biol. 2013, 2, 301−307



ACS Synth. Biol. 2013, 2, 301−307

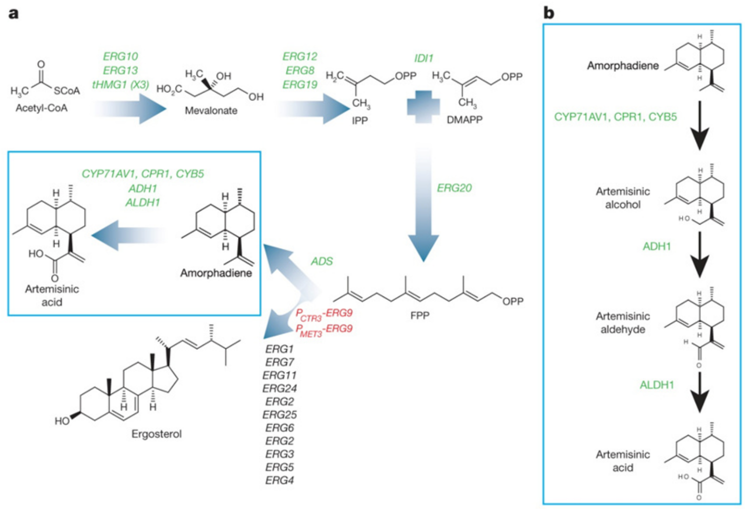


ACS Synth. Biol. 2013, 2, 301−307

One major success of synthetic biology is the engineering of the Artemisinic acid production pathway from wormwood into yeast:

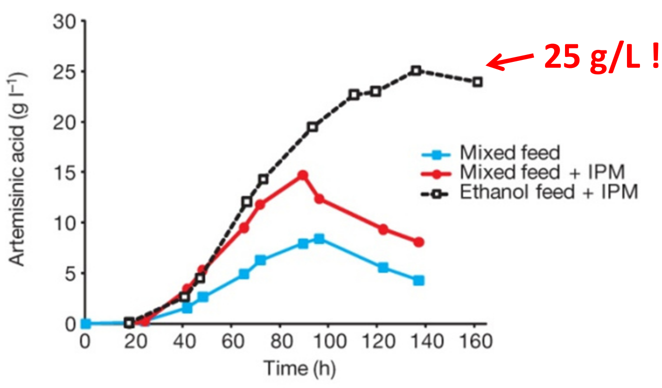
Normal source = sweet wormwood

wikipedia



CJ Paddon *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12051

Increasing production of artemisinic acid by strain engineering and addition of IPM to cultures.



“These key developments in yeast strain engineering, fermentation, and artemisinin synthetic chemistry pave the way for an industrial process capable of supplementing the world supply of artemisinin from a second source independent of the uncertainties associated with botanical production.”

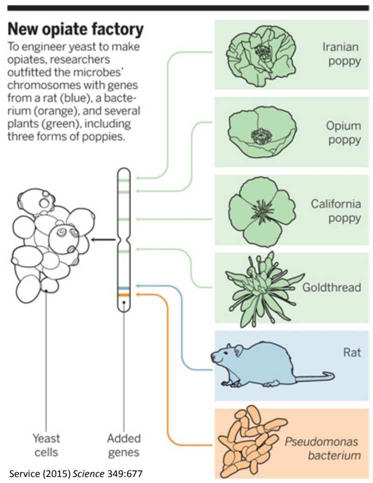
CJ Paddon *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12051

A couple of other recent successes in this space:

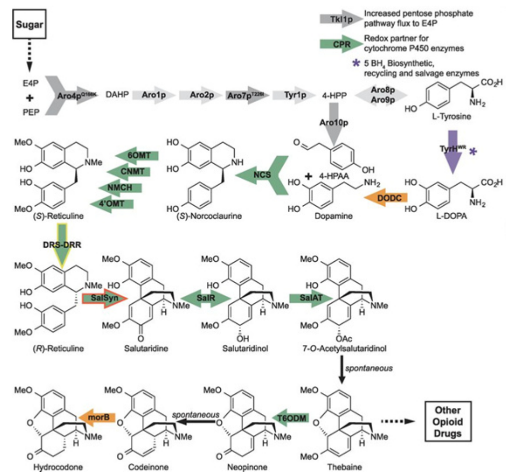
SYNTHETIC BIOLOGY

Complete biosynthesis of opioids in yeast

Stephanie Galanie,[1] Kate Thodey,[2] Isis J. Trenchard,[2] Maria Filsinger Interrante,[2] Christina D. Smolke[2*]



Service (2015) *Science* 349:677

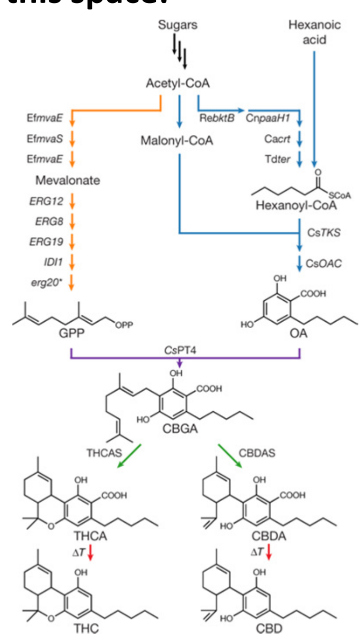


Galanie *et al.* (2015) *Science* 349:1095-100

A couple of other recent successes in this space:

Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

Xiaozhou Luo[1,15], Michael A. Reiter[1,2,15], Leo d'Espaux[3,12], Jeff Wong[3,12], Charles M. Denby[1,13], Anna Lechner[4,5,14], Yunfeng Zhang[1,6], Adrian T. Grzybowski[1], Simon Harth[3], Weiyin Lin[3], Hyunsu Lee[3,7], Changhua Yu[3,5], John Shin[3,4], Kai Deng[8,9], Veronica T. Benites[3], George Wang[3], Edward E. K. Baidoo[3], Yan Chen[3], Ishaan Dev[3,4], Christopher J. Petzold[3] & Jay D. Keasling[1,3,4,5,10,11*]



Luo *et al.* (2019) *Nature* 567:123-126

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

www.genscript.com

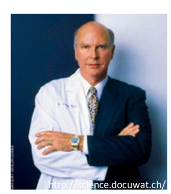
We can now <u>manufacture</u> a complete genome from commodity chemicals

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

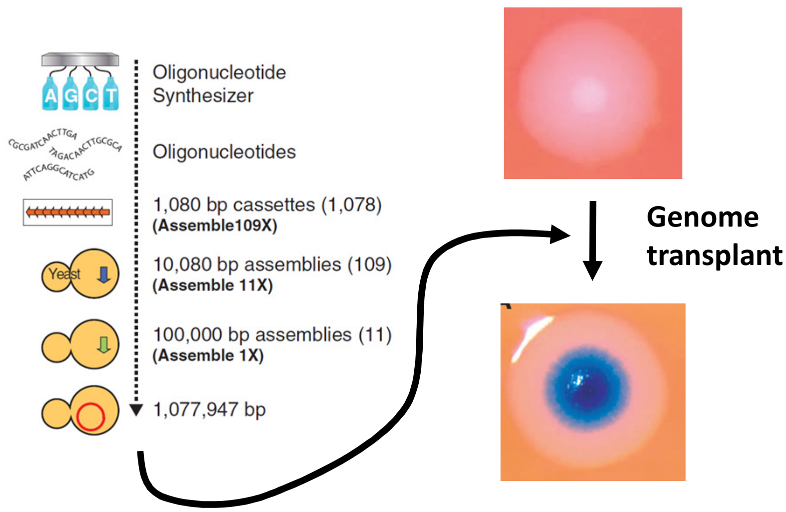
Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] 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."



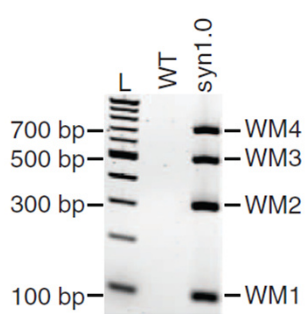
2 JULY 2010 VOL 329 SCIENCE

“Rebooting” bacteria with synthetic genomes



2 JULY 2010 VOL 329 SCIENCE

“The only DNA in the cells is the designed synthetic DNA sequence, including “watermark” sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.”



PCR of 4 engineered “watermarks”

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.

www.cellphones.ca

It's as though you bought a Blackberry…

installed the Android operating system…

& your phone physically morphed
into a Galaxy S9…

www.pinterest.com

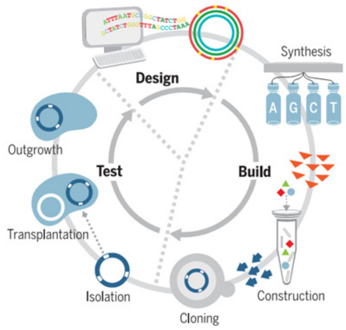
Some good quotes from the paper:

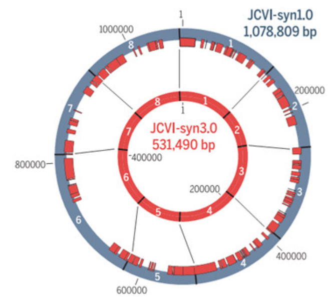
"If the methods described here can be generalized, design, synthesis, assembly, and transplantation of synthetic chromosomes will no longer be a barrier to the progress of synthetic biology."

"We expect that the cost of DNA synthesis will follow what has happened with DNA sequencing and continue to exponentially decrease. Lower synthesis costs combined with automation will enable broad applications for synthetic genomics."

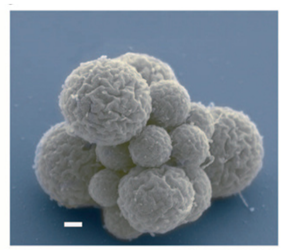
"As synthetic genomic applications expand, we anticipate that this work will continue to raise philosophical issues that have broad societal and ethical implications."

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



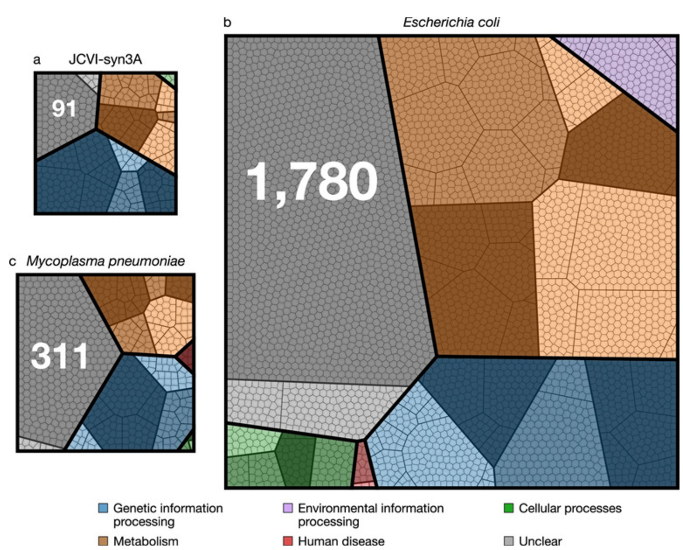


Four design-build-test cycles produced JCVI-syn3.0. (**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (**B**) Comparison 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).



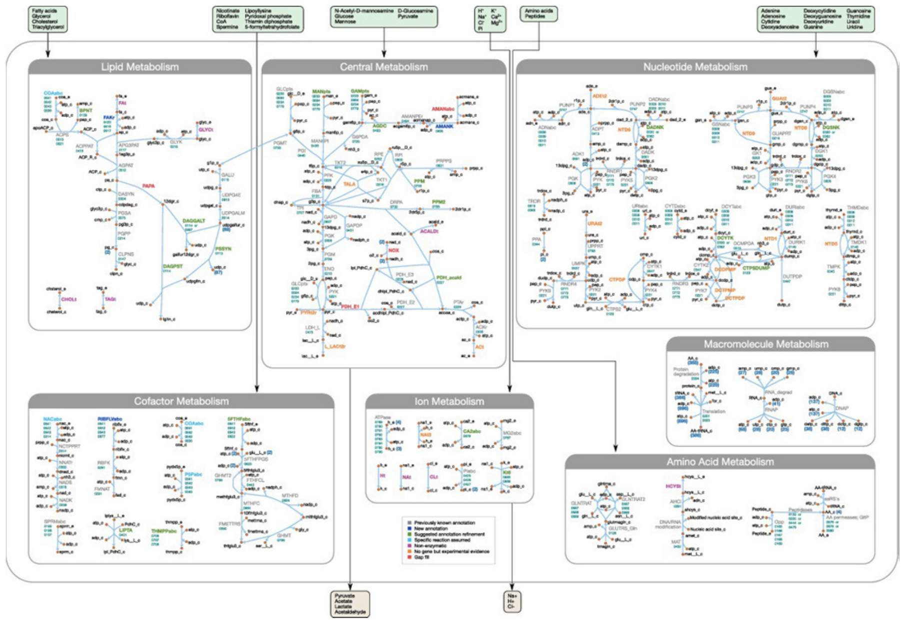
Science 25 MARCH 2016 • VOL 351 ISSUE 6280

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



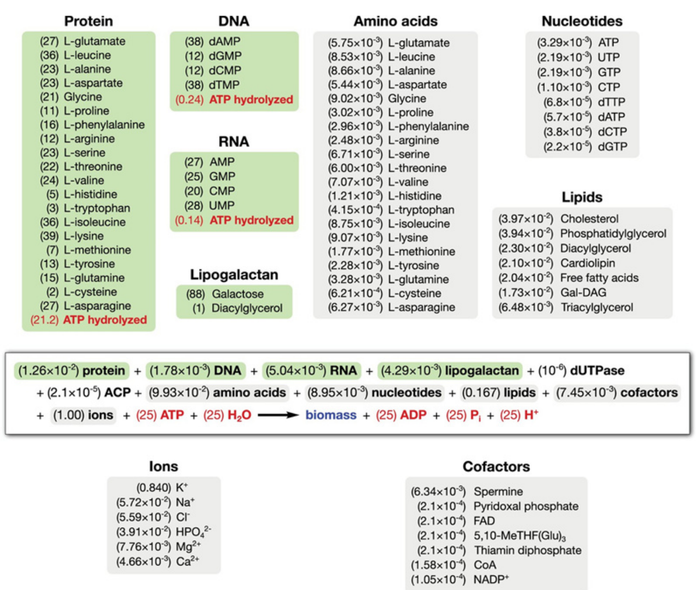
Breuer *et al.*, *eLife* 2019;8:e36842 DOI: 10.7554/ELIFE.36842

…which now has a rich metabolic reconstruction…



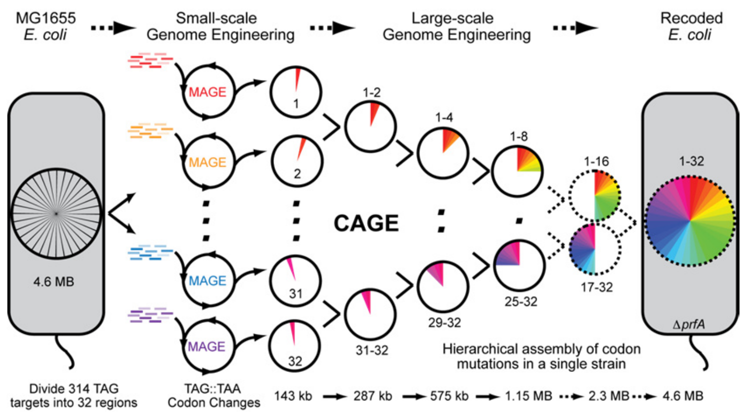
Breuer *et al.*, *eLife* 2019;8:e36842 DOI: 10.7554/ELIFE.36842

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



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

In parallel, methods were developed to edit genomes at many locations in parallel, e.g. reassigning all amber (TAG) stop codons in *E. coli* to ochre (TAA)



Genomically Recoded Organisms Expand Biological Functions

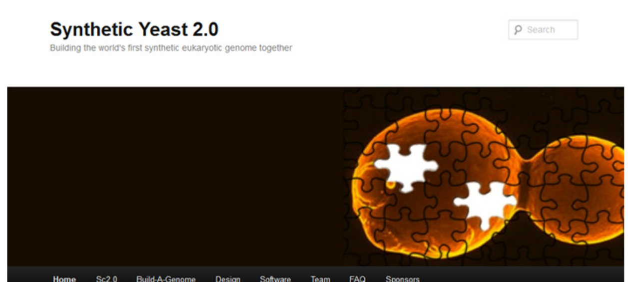
Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[1,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6] George M. Church,[1,13*] Farren J. Isaacs[3,4*]

SCIENCE VOL 342 18 OCTOBER 2013

http://isaacs.commons.yale.edu/files/2012/07/rE.coli_.Fig1_.png

& now, "rebooting" yeast with synthetic chromosomes

Turns out chromosomes can be synthesized and replaced for yeast too…



& China is pushing for a completely synthetic yeast genome…

Science April 4, 2014: Vol. 344 no. 6179 pp. 55-58

Total Synthesis of a Functional Designer Eukaryotic Chromosome

Narayana Annaluru,[1*] Héloïse Muller,[1,2,3,4*] Leslie A. Mitchell,[2,5] Sivaprakash Ramalingam,[1] Giovanni Stracquadanio,[2,6] Sarah M. Richardson,[6] Jessica S. Dymond,[2,7] Zheng Kuang,[2] Lisa Z. Scheifele,[2,8] Eric M. Cooper,[2] Yizhi Cai,[2,9] Karen Zeller,[2] Neta Agmon,[2,5] Jeffrey S. Han,[10] Michalis Hadjithomas,[11] Jennifer Tullman,[6] Katrina Caravelli,[2,12] Kimberly Cirelli,[1,12] Zheyuan Guo,[1,13] Viktoriya London,[1,13] Apurva Yeluru,[1,13] Sindurathy Murugan,[6] Karthikeyan Kandavelou,[1,14] Nicolas Agier,[15,16] Gilles Fischer,[15,16] Kun Yang,[2,6] J. Andrew Martin,[2,6] Murat Bilgel,[13] Pavlo Bohutskyi,[13] Kristin M. Boulier,[12] Brian J. Capaldo,[13] Joy Chang,[13] Kristie Charoen,[13] Woo Jin Choi,[13] Peter Deng,[11] James E. DiCarlo,[13] Judy Doong,[13] Jessilyn Dunn,[13] Jason I. Feinberg,[12] Christopher Fernandez,[12] Charlotte E. Floria,[12] David Gladowski,[12] Pasha Hadidi,[13] Isabel Ishizuka,[12] Javaneh Jabbari,[12] Calvin Y. L. Lau,[13] Pablo A. Lee,[13] Sean Li,[13] Denise Lin,[12] Matthias E. Linder,[12] Jonathan Ling,[13] Jaime Liu,[13] Jonathan Liu,[13] Mariya London,[12] Henry Ma,[13] Jessica Mao,[13] Jessica E. McDade,[13] Alexandra McMillan,[12] Aaron M. Moore,[12] Won Chan Oh,[13] Yu Ouyang,[13] Ruchi Patel,[13] Marina Paul,[12] Laura C. Paulsen,[13] Judy Qiu,[13] Alex Rhee,[13] Matthew G. Rubashkin,[13] Ina Y. Soh,[12] Nathaniel E. Sotuyo,[12] Venkatesh Srinivas,[13] Allison Suarez,[13] Andy Wong,[13] Remus Wong,[13] Wei Rose Xie,[12] Yijie Xu,[13] Allen T. Yu,[12] Romain Koszul,[3,4] Joel S. Bader,[2,6] Jef D. Boeke,[2,11,5]† Srinivasan Chandrasegaran[1]†

"Here, we report the synthesis of a functional 272,871–base pair designer eukaryotic chromosome, synIII, which is based on the 316,617–base pair native *Saccharomyces cerevisiae* chromosome III. Changes to synIII include TAG/TAA stop-codon replacements, deletion of subtelomeric regions, introns, transfer RNAs, transposons, and silent mating loci as well as insertion of loxPsym sites to enable genome scrambling."

Changes engineered into chromosome III

<u>~2.5% of sequence changed</u>

- Recoded all amber (TAG) stop codons to ochre (TAA)
- Introduced 98 Cre/Lox recombination sites
- Introduced unique sequences for PCR and new restriction enzyme sites
- Standardized telomeres

<u>Reduced size from 316,617 bp to 272,871 bp (~14% reduction)</u>

- Deleted 10 tRNA genes, 21 Ty elements/LTRs, silent mating loci
  (only one tRNA was essential, moved to a plasmid)
- Removed leucine biosynthesis gene LEU2 to be an auxotrophic marker
- Deleted all introns (affected 7 genes)
- Deleted subtelomeric DNA

Only 10 errors in assembly: 9 single base changes and 1 lost recombinase site

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,

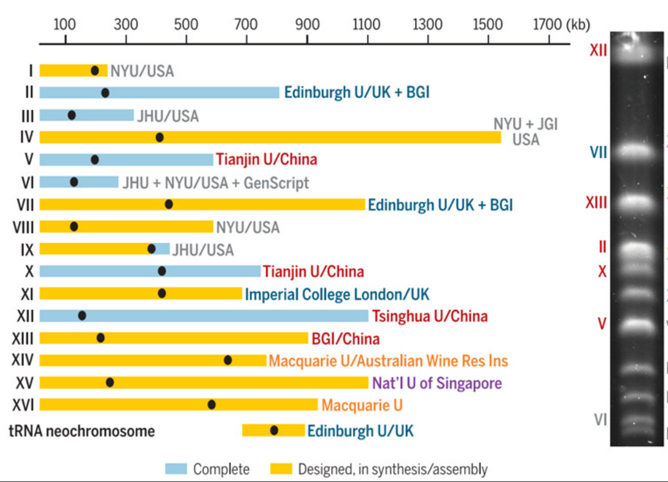


Table 1. Design challenges and policies adopted. CDS, gene coding sequence; snoRNA, small nucleolar RNA.

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.

"Mega-chunk" assembly

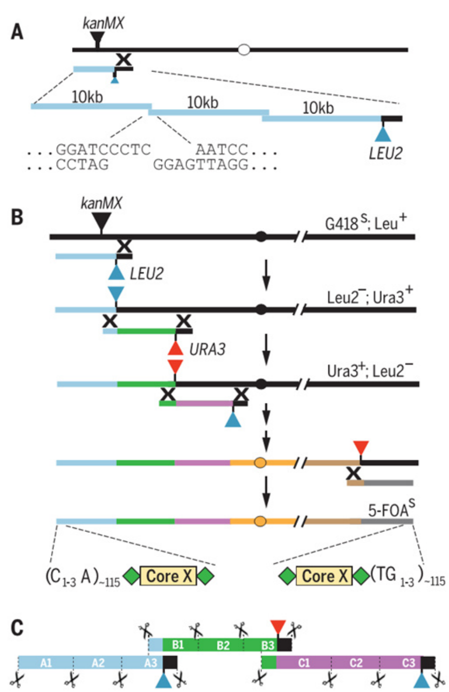
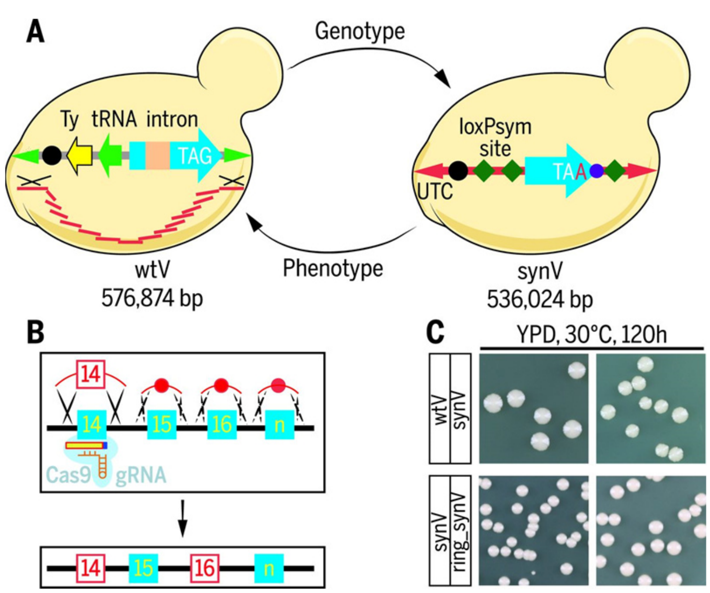


Table 3. Summary statistics for design of Sc2.0. WT, wild type; SYN, synthetic.

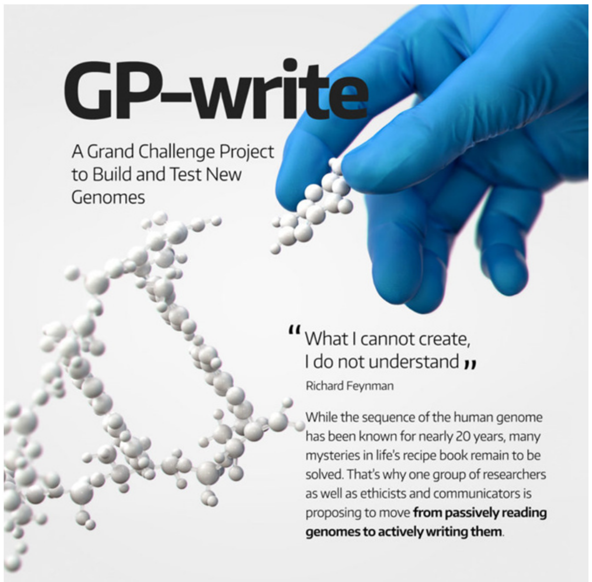
	WT size	SYN size	No. of stop codon swaps	No. of loxP sites added	bp of PCRTag recoded	bp of RE sites recoded	No. of tRNA deleted	bp of tRNA deleted	bp of repeats deleted
chr01	230208	181030	19	62	3535	210	4	372	3987
chr02	813184	770035	93	271	13651	1215	13	993	7030
chr03	316617	272195	44	100	5272	250	10	794	7358
chr04	1531933	1454671	183	479	25398	2298	28	2261	11674
chr05	576874	536024	61	174	8760	813	20	1471	11181
chr06	270148	242745	30	69	4553	369	10	835	9297
chr07	1090940	1028952	126	380	17910	1572	36	2887	13284
chr08	562643	506705	61	186	9980	714	11	878	19019
chr09	439885	405513	54	142	7943	436	10	736	11632
chr10	745751	707459	85	249	12582	1102	24	1853	7523
chr11	666816	659617	68	199	11769	1017	15	1243	4214
chr12	1078177	999406	122	291	15129	1539	19	1646	10843
chr13	924431	883749	100	337	15911	0	21	1691	7673
chr14	784333	753096	96	260	13329	1113	14	1152	5115
chr15	1091291	1048343	147	399	18015	2058	20	1612	9542
chr16	948066	902994	127	334	15493	1374	17	1338	10048
Total	12071297	11352534	1416	3932	199230	16080	272	21762	149420

Synthesis, cyclization, and characterization of synV



Ze-Xiong Xie et al. *Science* 2017;355:eaaf4704

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…

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?

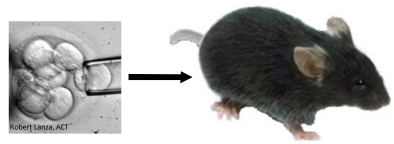
JURASSIC PARK

Sound familiar?

http://jurassicpark.wikia.com

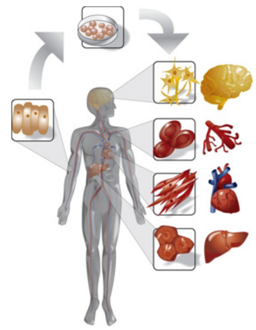
Besides the genome engineering, this hinges on iPS:

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



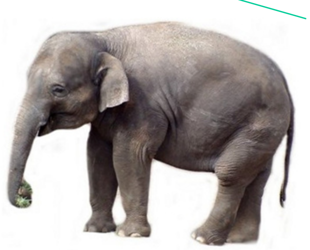
www.regenexx.com

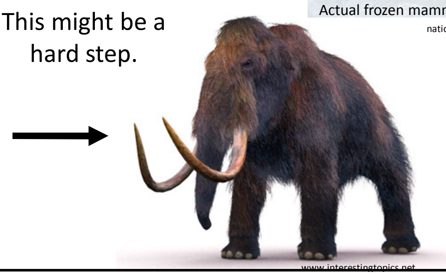
There's a serious proposal to resurrect the woolly mammoth. Here's the process:

- ✓ **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**

Actual frozen mammoth!
nationalgeographic.com

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

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.

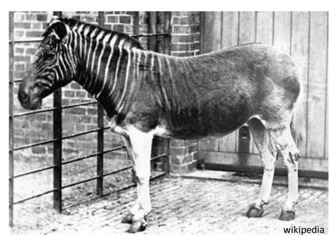
The research has not yet been peer-reviewed or published in a scientific journal "because there is more work to do," Church told the U.K.'s *Sunday Times*, "but we plan to do so."

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

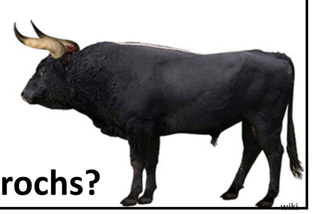
Which animal would you resurrect?

The dodo?

The quagga?



Saber-toothed tiger?

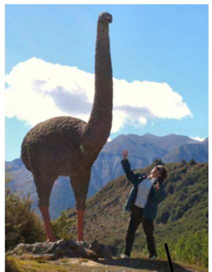


Aurochs?

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

I vote for some crazy Australasian animals:

The 12' tall moa



http://www.sandianet.com/kiwi/moabarb.jpg

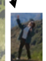
& of, course, the marsupial Tasmanian tiger

wikipedia

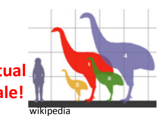
>90° !!!

The moa-eating Haast's eagle

wikipedia



Actual scale!



wikipedia

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?

Action Press/Rex Features