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

BCH394P/364C Systems Biology / Bioinformatics

Edward Marcotte, Univ of Texas at Austin

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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

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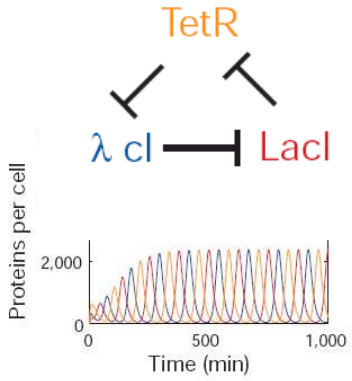
The Repressilator = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion = "repressor" + "oscillator"

Transcriptional repressors

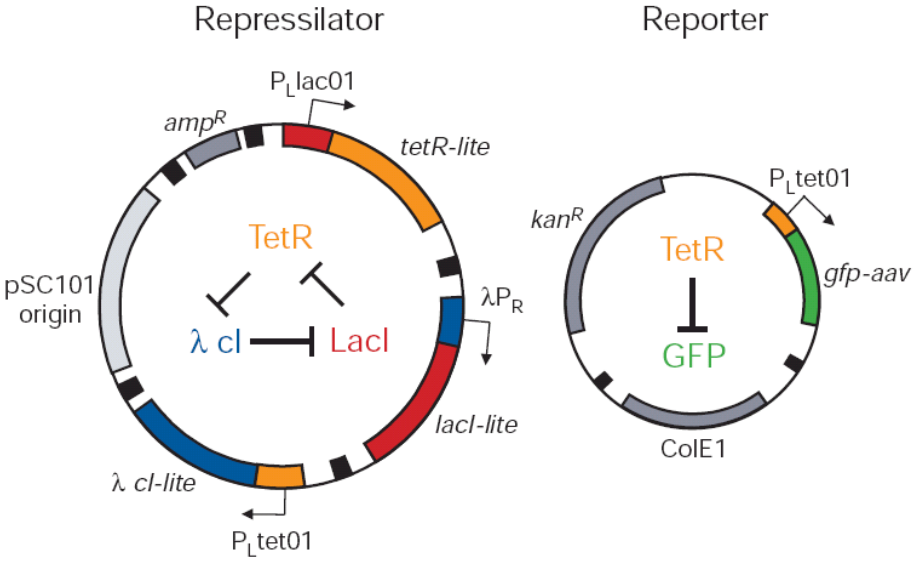


Green fluorescent protein

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

3

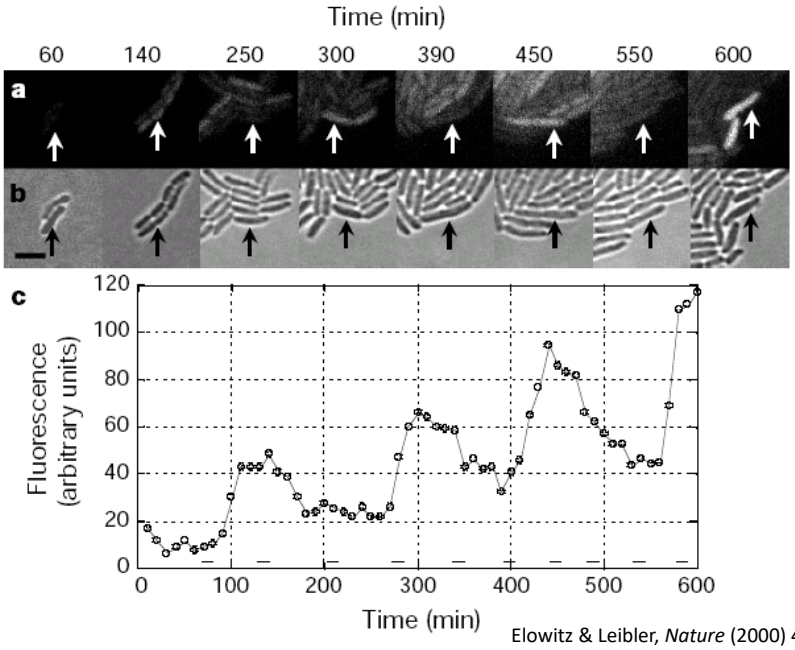
The Repressilator = engineered genetic circuit designed to make bacteria glow in a oscillatory fashion



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

4

The repressilator in action...



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

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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)

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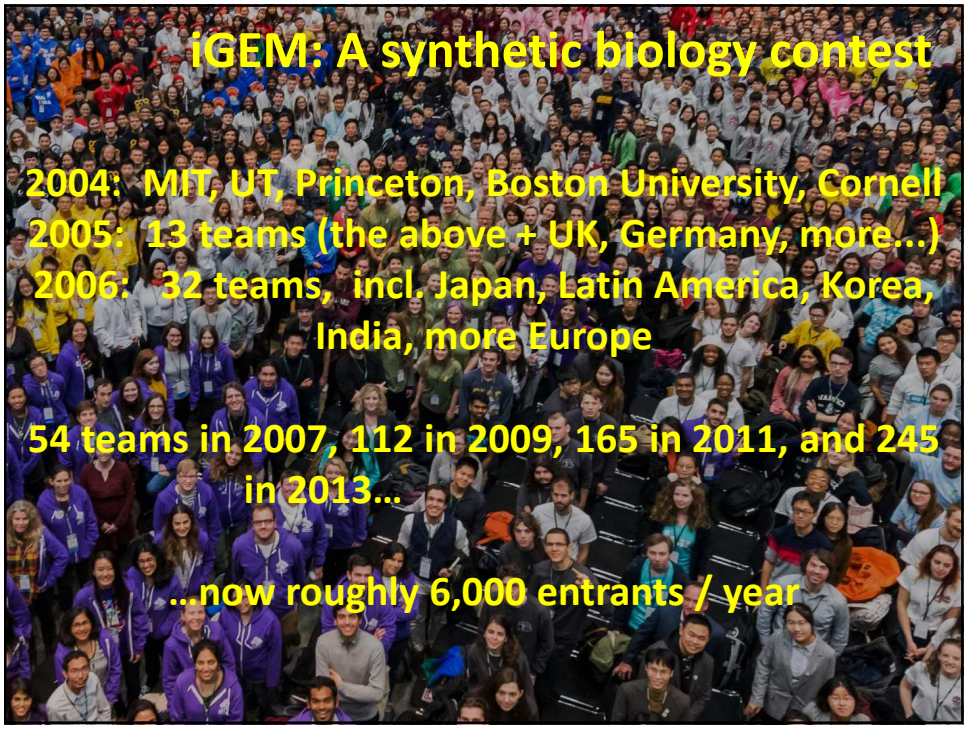

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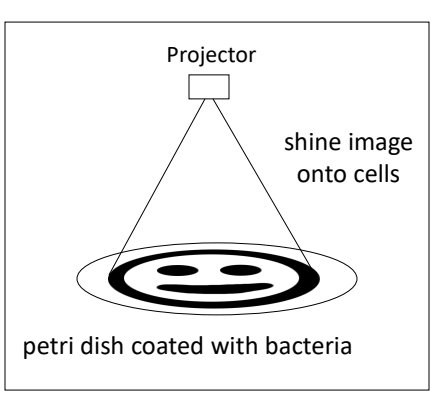
A little local history to illustrate the field:
UT's 2004/2005 iGEM project – build bacterial edge detector



Original image

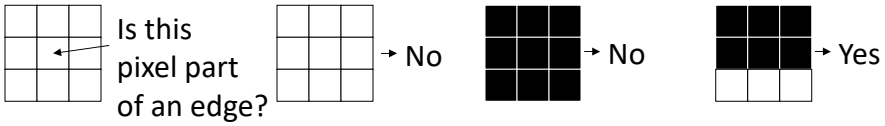
Cells luminesce along the light/dark boundaries

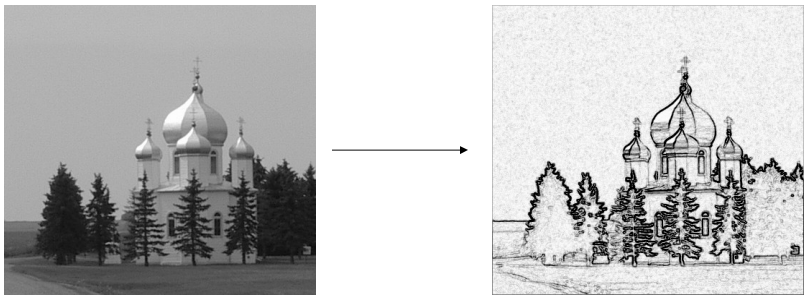
Adapted from Zack Simpson

8

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.





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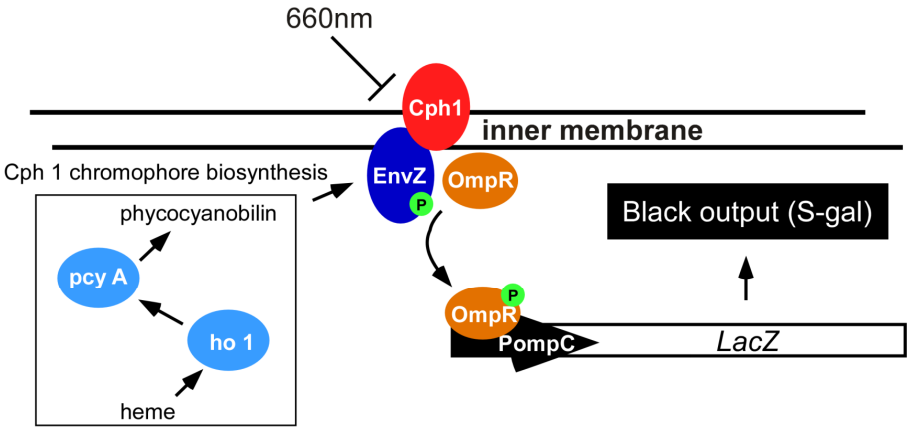
Bacterial photography



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

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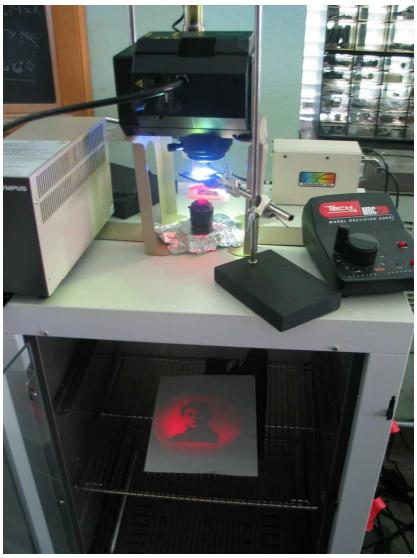
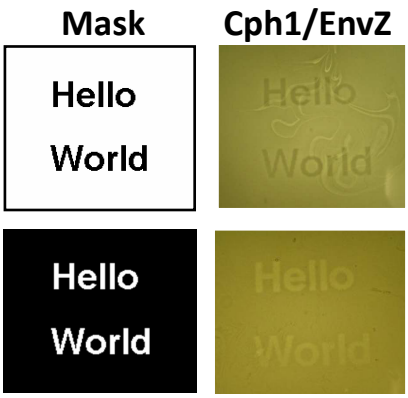


“Light cannon” developed by Aaron Chevalier, UT undergraduate



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

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The first bacterial photograph (coliroid?)...

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

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Escherichia darwinia

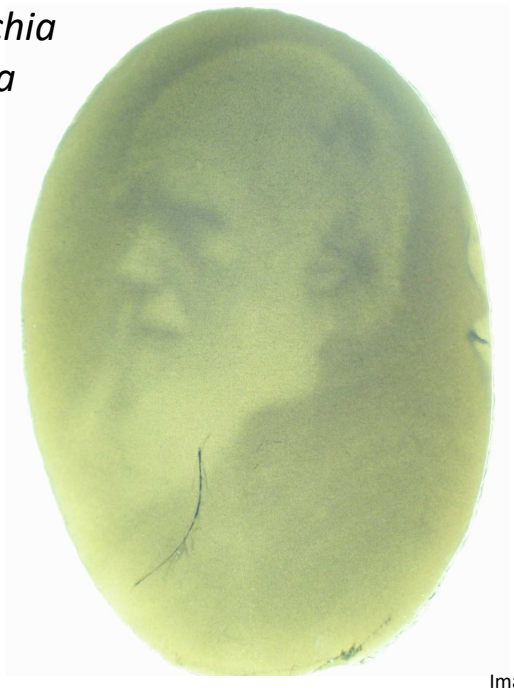

Image: Aaron Chevalier

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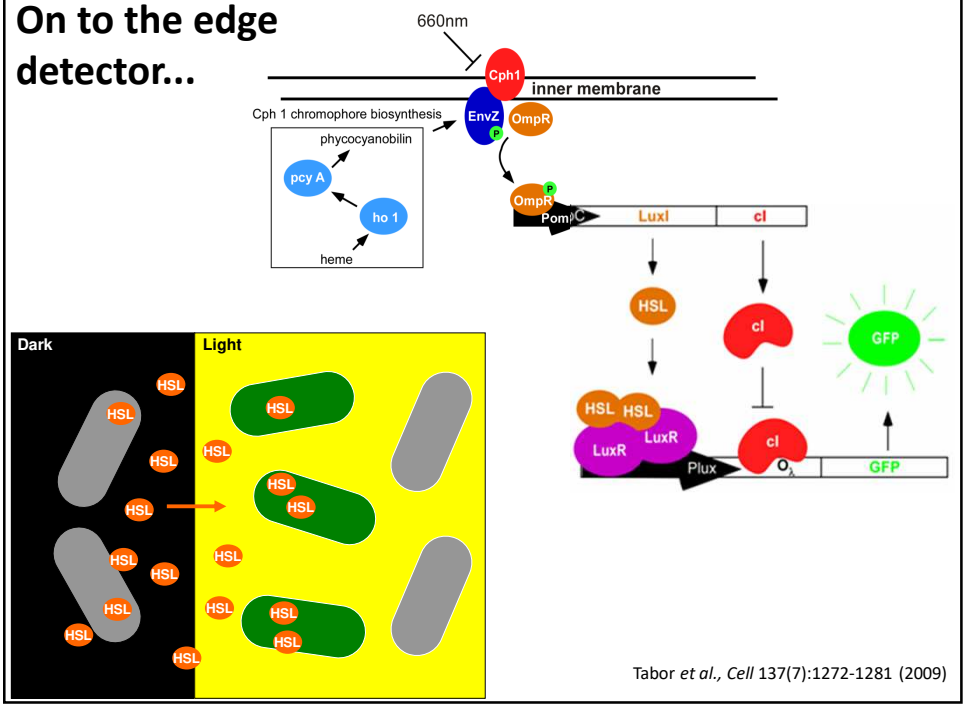
On to the edge detector...



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

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It works!

Projected Mask

Photo strain

Edge detector strain

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

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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…

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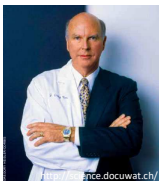
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

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."



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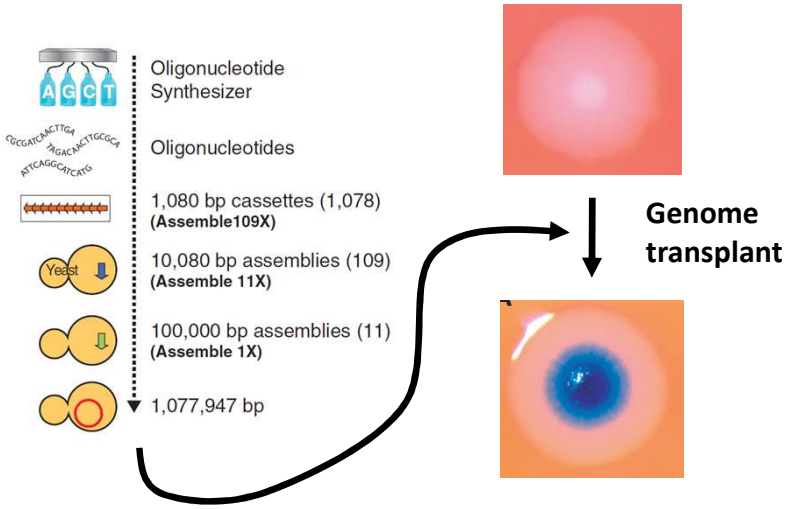
"Rebooting" bacteria with synthetic genomes



Genome transplant

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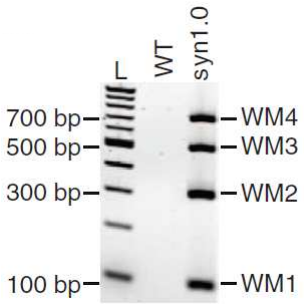
"The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication."



PCR of 4 engineered "watermarks"

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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…

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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."

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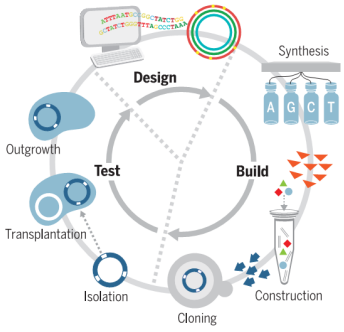
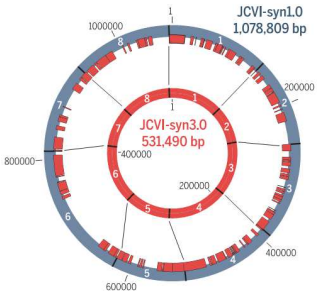
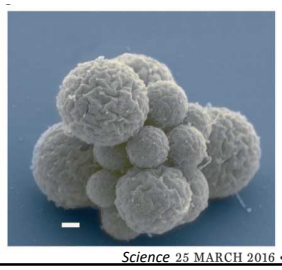
In 2016, Hutchison, Chuang, *et al.* reported making living mycoplasma after cutting the genome by ½ the genes





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

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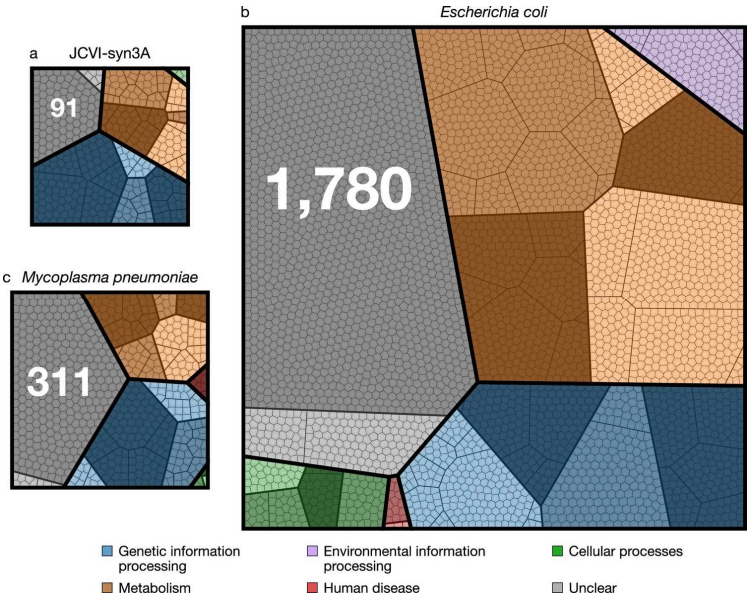
JCVI-syn3.0 now makes for a remarkably compact, engineerable, free living cell "chassis" to study



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

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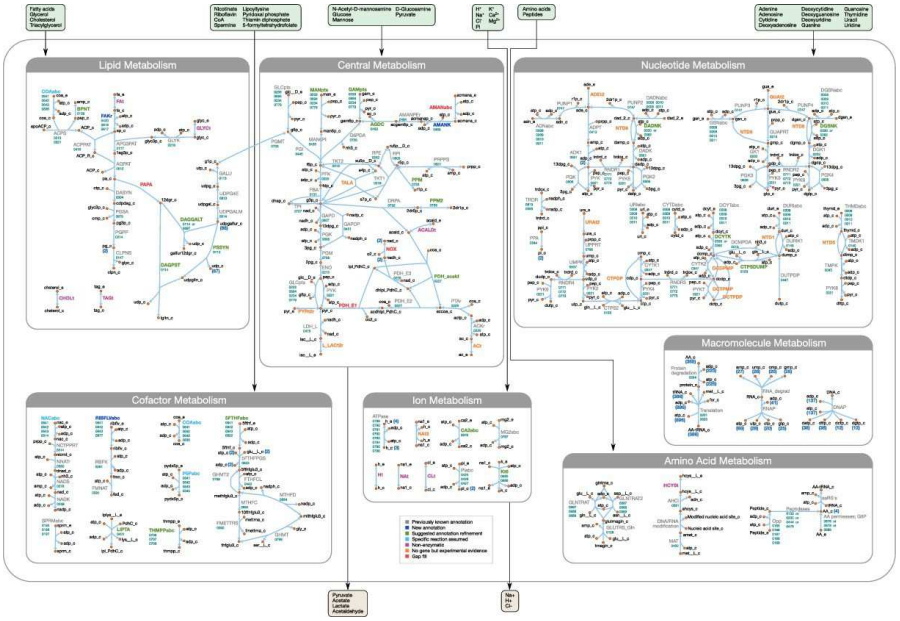
…which now has a rich metabolic reconstruction…



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

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…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$(Glu)_3$
(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

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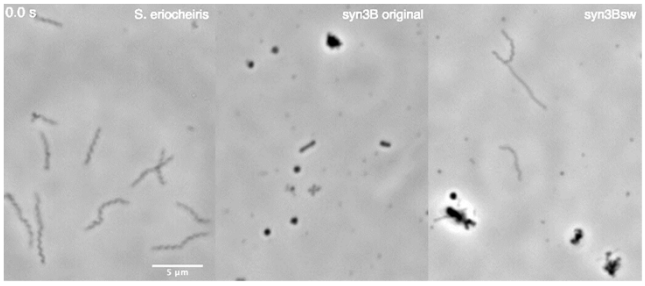
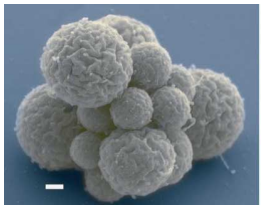
…and most recently, engineered motility

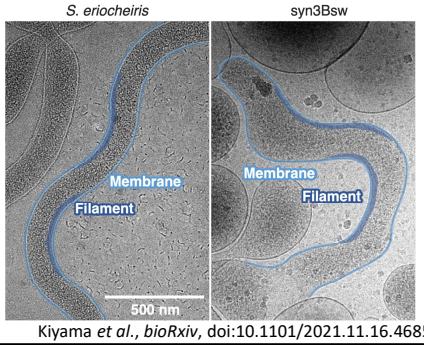


***Spiroplasma* actin operon**

+



JCVI-syn3.0B



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

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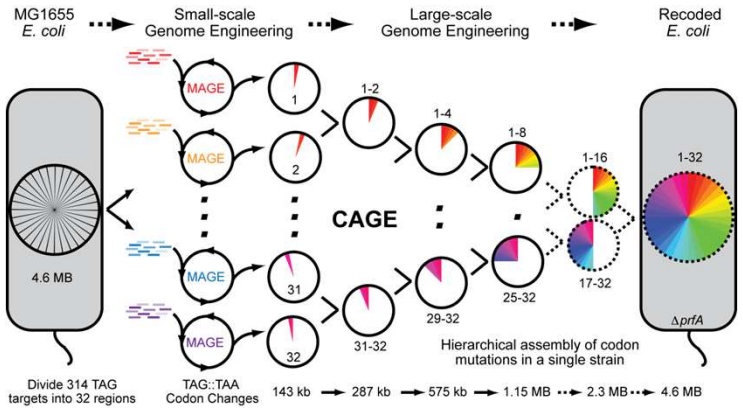
In parallel, methods were developed to edit genomes at many locations in parallel, e.g. reassigning all amber (TAG) stop codons in *E. coli* to ochre (TAA)



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

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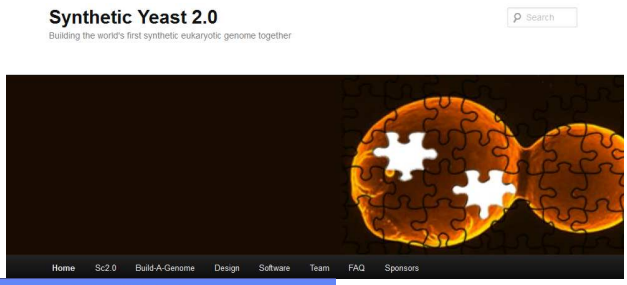
& now, "rebooting" yeast with synthetic chromosomes

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



& China is pushing for a completely synthetic yeast genome…

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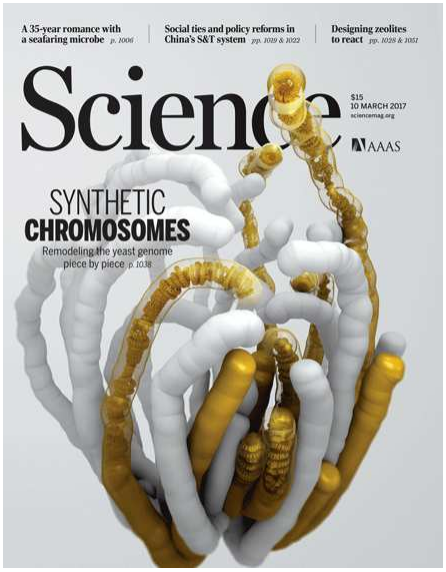
In 2017, the Synthetic Yeast Genome Project (Sc2.0) reported on five newly constructed synthetic yeast chromosomes:



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

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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,

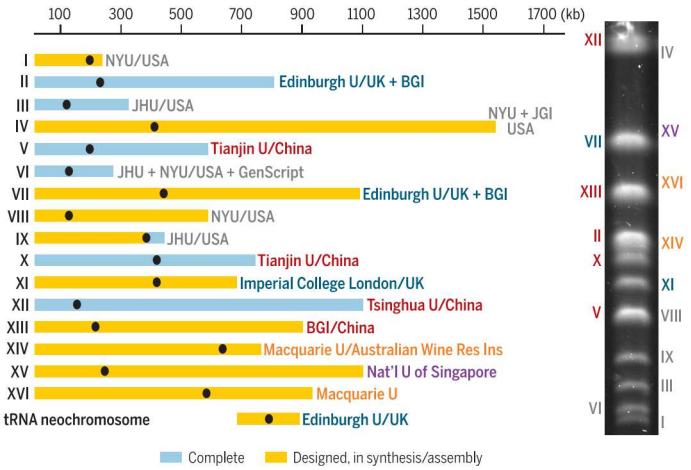

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Table 1. Design challenges and policies adopted. CDS, gene coding sequence; snoRNA, small nucleolar RNA.

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.

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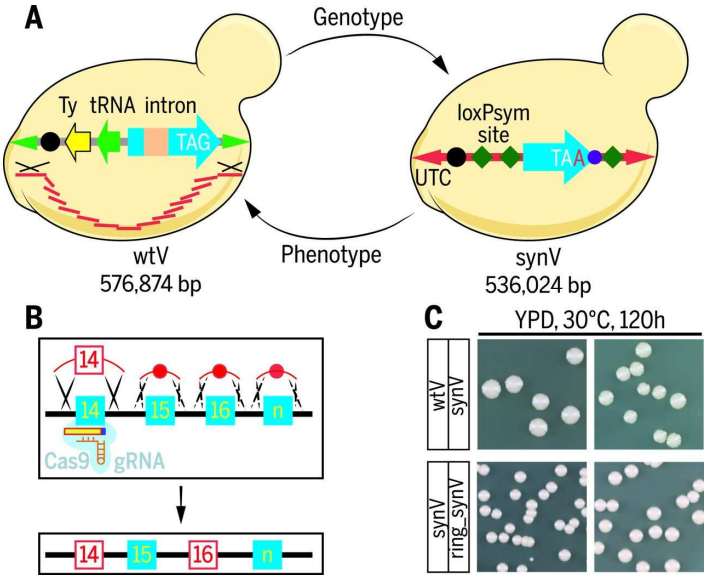
Synthesis, cyclization, and characterization of synV



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

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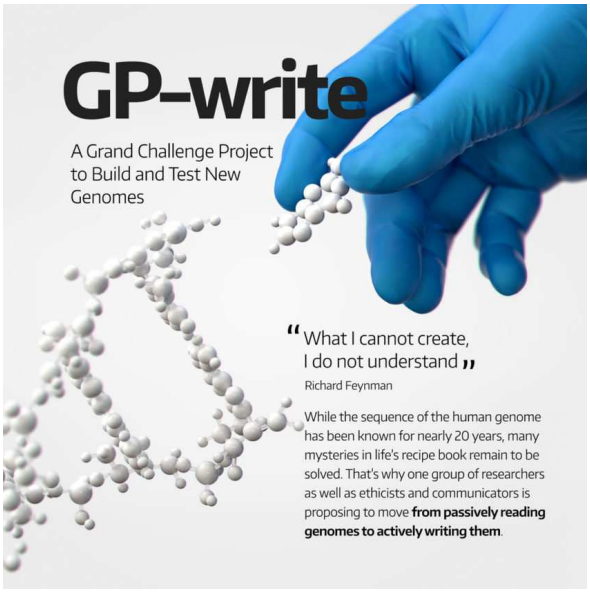
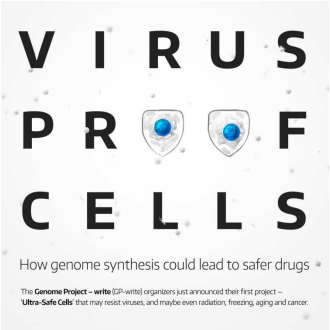
Inevitably (!), the human synthetic genome project:



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



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

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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!

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"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

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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

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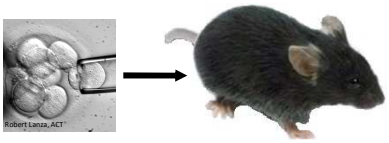
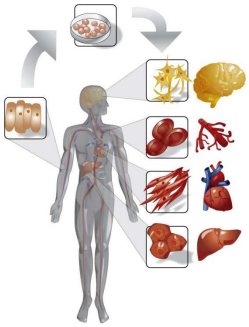
Besides the genome engineering, this hinges on iPS:

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



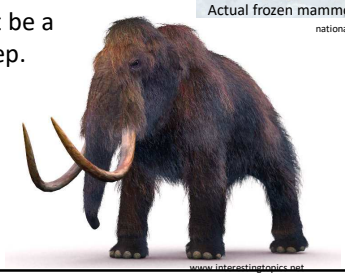
www.regenexx.com

41

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

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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

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In fact, they're hiring!

Woolly Mammoth Revival Fellowship Announcement

Description of the Opportunity

Revive & Restore is pleased to announce a post-doctoral fellowship opportunity for well-qualified individuals interested in a full-time appointment researching the science underlying Woolly Mammoth de-extinction in the laboratory of Dr. George Church at Harvard Medical School. This fellowship is fully supported by Revive & Restore, the leading non-profit organization working to bring biotechnologies to wildlife conservation. The fellowship will

…

The Woolly Mammoth has emerged as a leading candidate for this work. It can be attempted because a close relative of the mammoth is still living—the Asian elephant. Thanks to the similarity of their genomes, the genes of woolly mammoth traits can be edited into the Asian elephant genome, and the combination brought to life as an elephant cousin, once again adapted to the conditions of the far north.

The ultimate goal of Woolly Mammoth Revival is to bring back this extinct species so that healthy herds may one-day re-populate vast tracts of tundra and boreal forest in Eurasia and North America. The intent is not to make perfect copies of extinct Woolly Mammoths, but to focus on the mammoth adaptations needed for Asian elephants to thrive in the cold climate of the Arctic. The milestones along the way range from developing elephant tissue cultures to genome editing and most importantly, developing insights that help with Asian elephant conservation.

https://genetics.hms.harvard.edu/about-us/departmental-employment-opportunities

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Although the race is on as other groups try to resurrect frozen mammoth cells:

SCIENTIFIC REPORTS

OPEN

Signs of biological activities of 28,000-year-old mammoth nuclei in mouse oocytes visualized by live-cell imaging

Received: 12 October 2018
Accepted: 19 February 2019
Published online: 11 March 2019

Kazuo Yamagata[1], Kouhei Nagai[1], Hiroshi Miyamoto[1], Masayuki Anzai[2], Hiromi Kato[2], Kei Miyamoto[1], Satoshi Kurosaka[2], Rika Azuma[1], Igor I. Kolodeznikov[3], Albert V. Protopopov[3], Valerii V. Plotnikov[3], Hisato Kobayashi[4], Ryouka Kawahara-Miki[4], Tomohiro Kono[4,5], Masao Uchida[6], Yasuyuki Shibata[6], Tetsuya Handa[7], Hiroshi Kimura[7], Yoshihiko Hosoi[1], Tasuku Mitani[1], Kazuya Matsumoto[1] & Akira Iritani[2]

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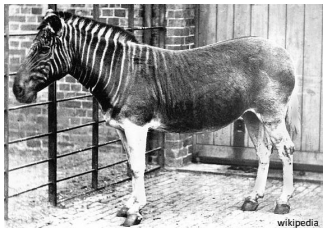
Which animal would you resurrect?

The dodo?

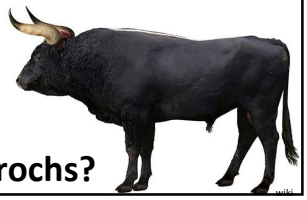
The quagga?



wikipedia

techandle.com

Saber-toothed tiger?



wiki

Aurochs?

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

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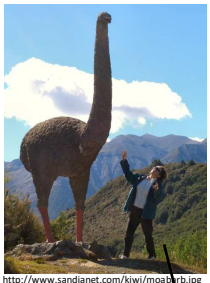
I vote for some crazy Australasian animals:

The 12' tall moa



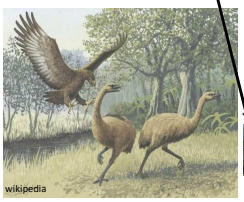
http://www.sandianet.com/kiwi/moabirb.jpg

& of, course, the marsupial Tasmanian tiger

wikipedia

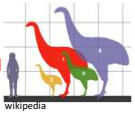
>90° !!!

The moa-eating Haast's eagle



wikipedia

Actual scale!



wikipedia

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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

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