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,

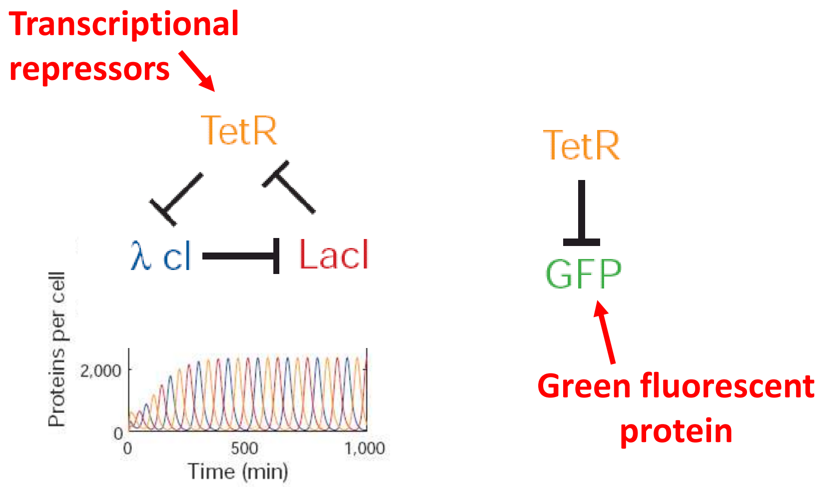
fluorescent proteins	light bulbs or LEDs
transcription factors	transistors or logic gates
repressors	NOT gates
activators	OR/AND gates
polymerases (transcriptional machinery)	batteries

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
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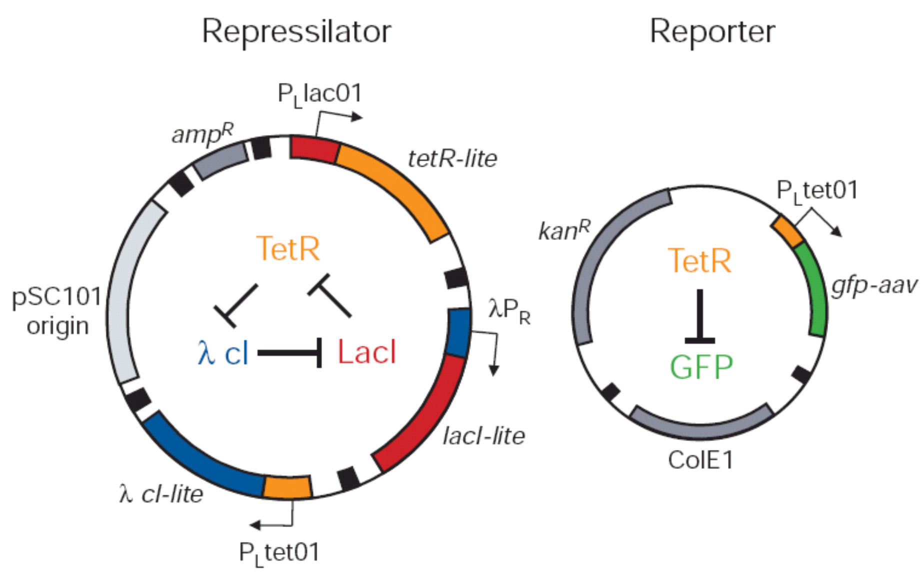
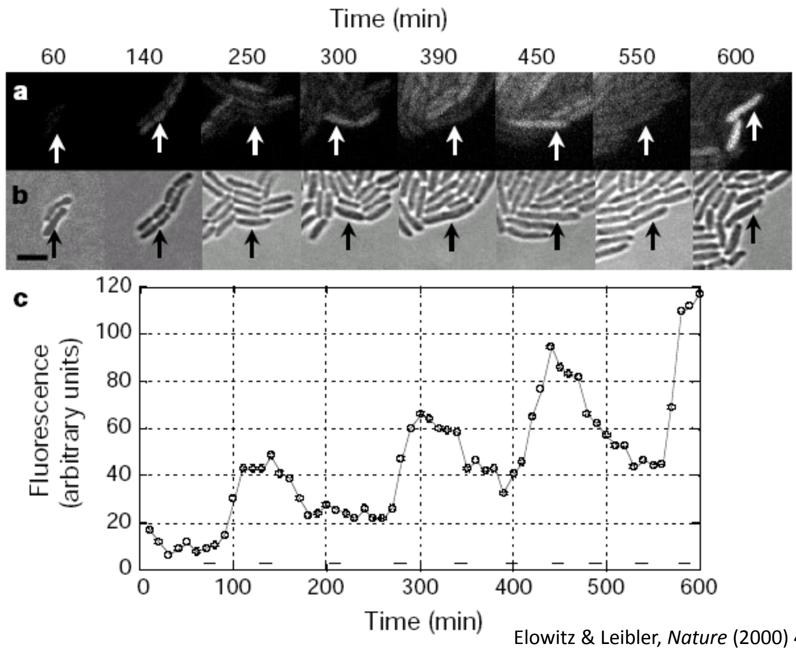


Elowitz & Leibler, *Nature* (2000) 403:335-8

The repressilator in action...



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

iGEM: A synthetic biology contest

(from iGEM's web site)

Can simple biological systems be built from standard, interchangeable parts and operated in living cells? Or is biology simply too complicated to be engineered in this way?

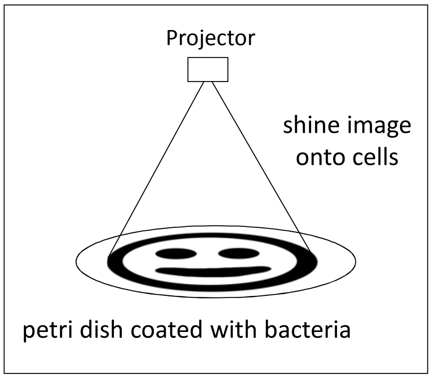
iGEM's broader goals include:

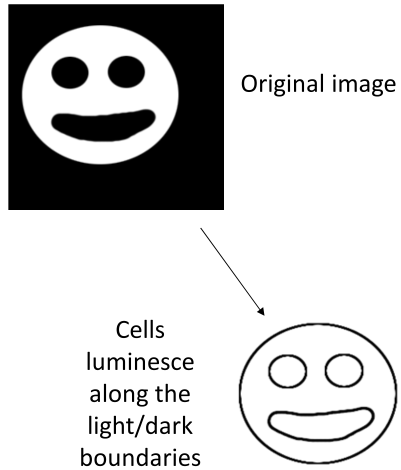
- To enable systematic engineering of biology
- To promote open & transparent development of tools for engineering biology
- To help construct a society that can productively apply biological technology

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, 84 teams in 2008, 112 teams in 2009, 130 teams in 2010, 165 teams in 2011, and 245 teams in 2012 and 2013…

UT’s 2004/2005 iGEM project – build bacterial edge detector

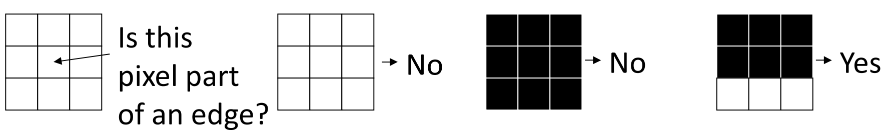


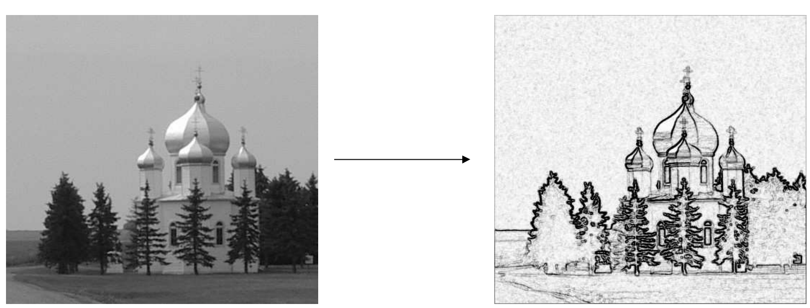


Adapted from Zack Simpson

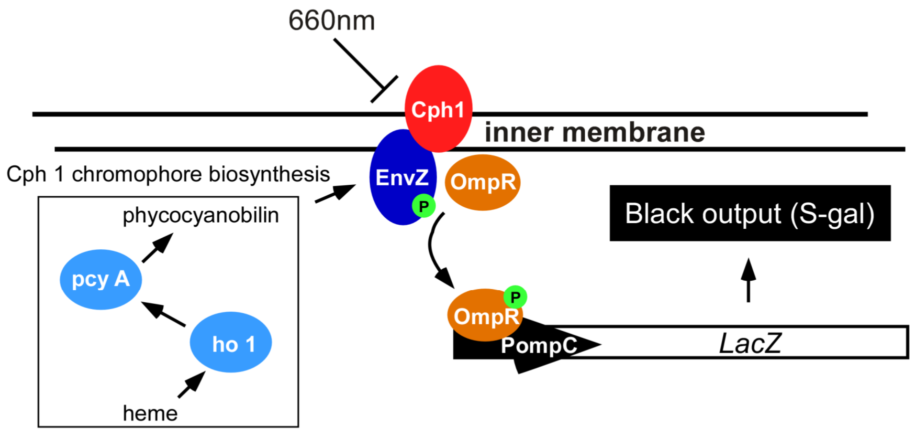
How does edge detection work in principle?

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.



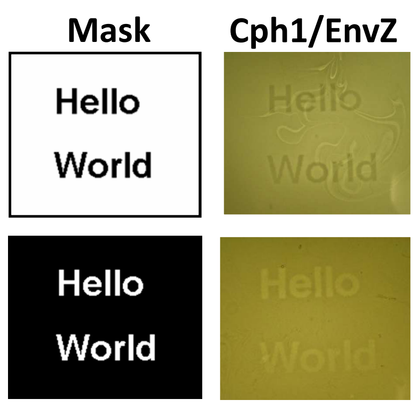


Bacterial photography



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)

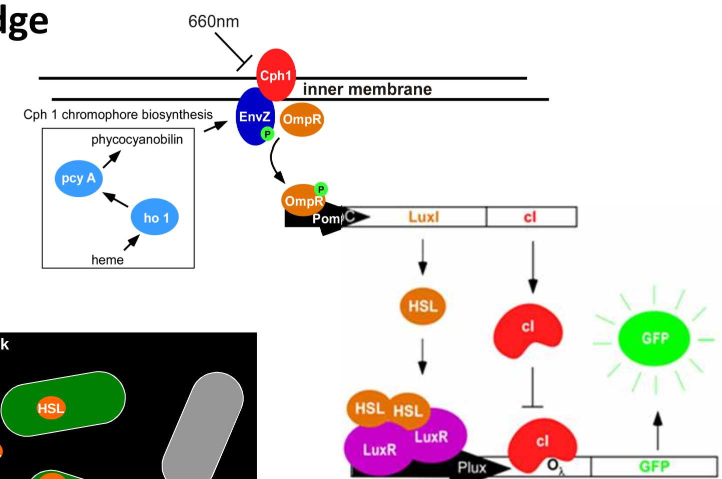
The first bacterial photograph (coliroid?)...

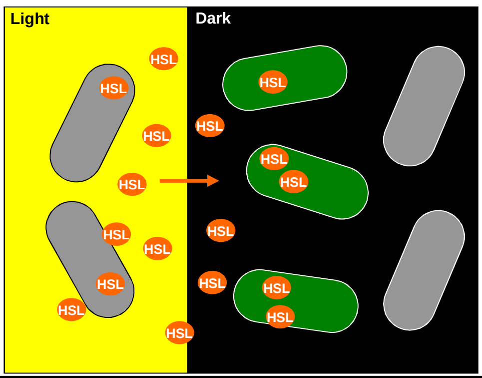
Levskaya *et al. Nature*, 438(7067):441-2 (2005)

Escherichia darwinia

Image: Aaron Chevalier

On to the edge detector…





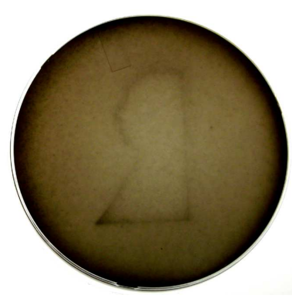
Tabor *et al.*, *Cell* 137(7):1272-1281 (2009)

It works!

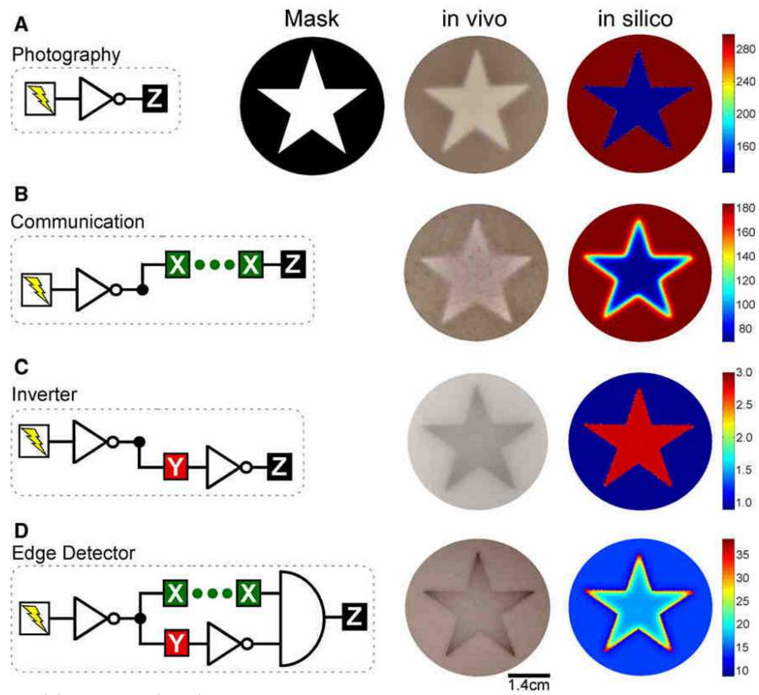
Projected Mask

Photo strain

Edge detector strain



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



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

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

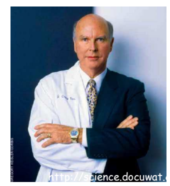
We can now <u>manufacture</u> a complete genome from commodity chemicals

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

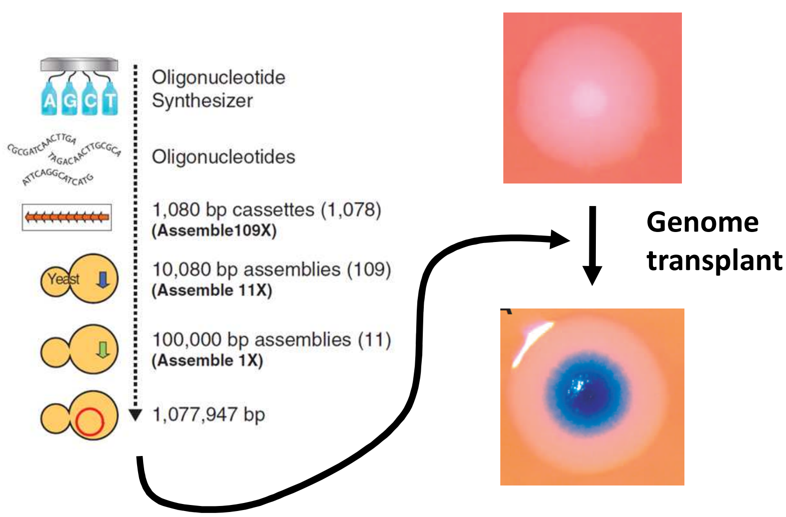
Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1]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



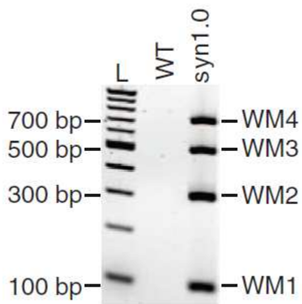
2 JULY 2010 VOL 329 SCIENCE

"Rebooting" bacteria with synthetic genomes



2 JULY 2010 VOL 329 SCIENCE

"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…

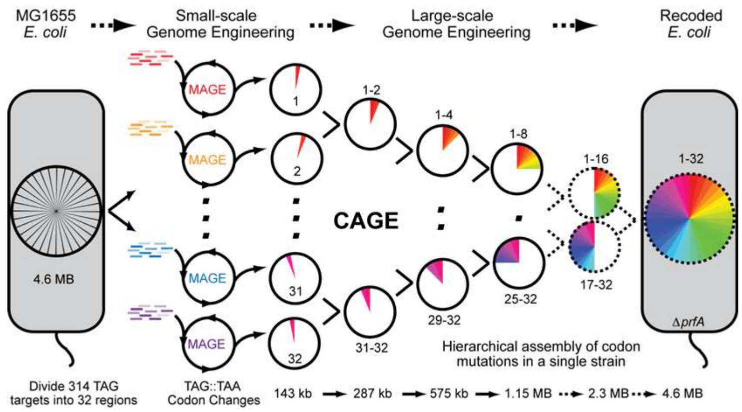
In biology, *software encodes the hardware*.
Most (all?) of the cell is specified by the DNA.

It's as though you bought a Blackberry…

installed the Android operating system…

& your phone physically morphed into a Galaxy S4…

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

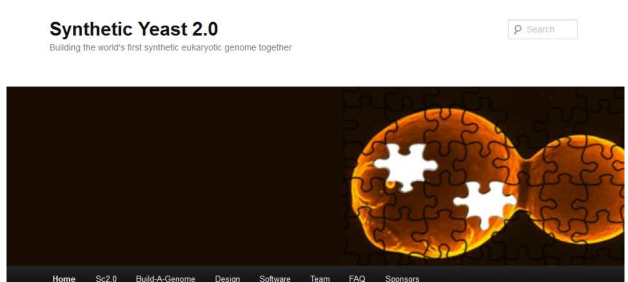
Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[1,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6]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

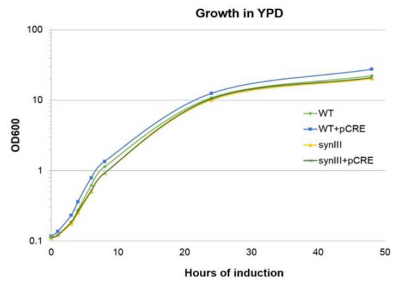
- Recoded all amber (TAG) stop codons to ochre (TAA)
- Introduced 98 Cre/Lox recombination sites
- Introduced unique sequences for PCR and new restriction enzyme sites
- Standardized telomeres

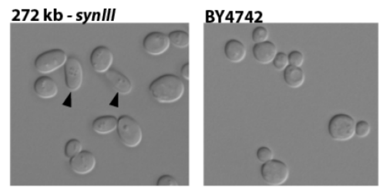
<u>Reduced size from 316,617 bp to 272,871 bp (~14% reduction)</u>

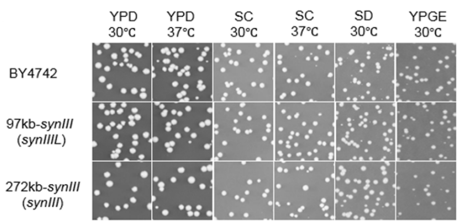
- Deleted 10 tRNA genes, 21 Ty elements/LTRs, silent mating loci
  (only one tRNA was essential, moved to a plasmid)
- Removed leucine biosynthesis gene LEU2 to be an auxotrophic marker
- Deleted all introns (affected 7 genes)
- Deleted subtelomeric DNA

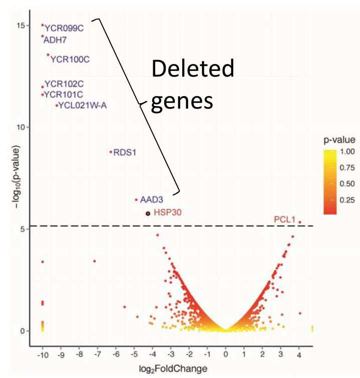
Only 10 errors in assembly: 9 single base changes and 1 lost recombinase site

No significant fitness difference between wt and synIII strain
Only 2 genes are differentially expressed (HSP30 & PCL1)









RNAseq of wt vs synIII

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

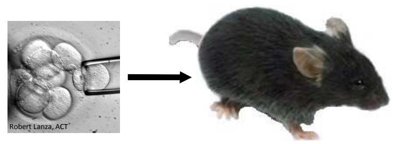
So why not just “edit” the genomes of the closest living animals to be like their extinct relatives?

JURASSIC PARK

Sound familiar?

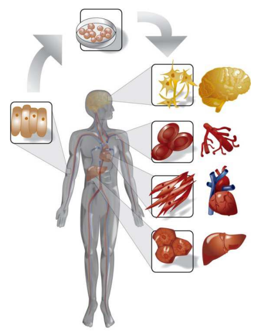
Besides the genome engineering, this hinges on iPS:

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.

Actual frozen mammoth!

nationalgeographic.com

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 g…

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."

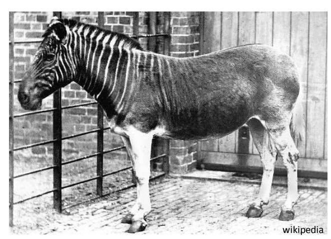
http://www.popsci.com/woolly-mammoth-dna-brought-life-elephant-cells

Which animal would you resurrect?

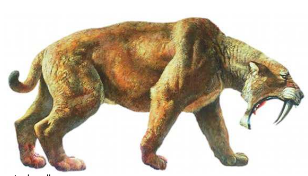
The dodo?

© 2010 Encyclopædia Britannica, Inc.

The quagga?

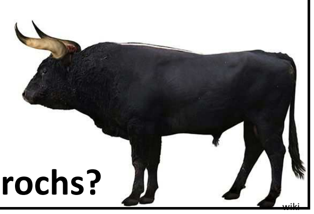


wikipedia



techandle.com

Saber-toothed tiger?



wiki

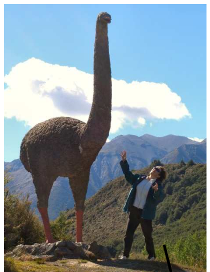
Aurochs?

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

Edward Marcotte/Univ. of Texas/BCH364C-391J/Spring 2015

I vote for some crazy Australasian animals:

The 12' tall moa



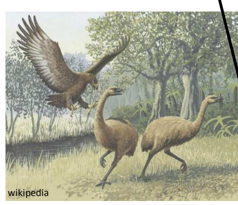
http://www.sandianet.com/kiwi/moabarb.jpg

& of, course, the marsupial Tasmanian tiger

wikipedia

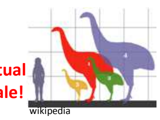
>90° !!!

The moa-eating Haast's eagle



wikipedia

Actual scale!



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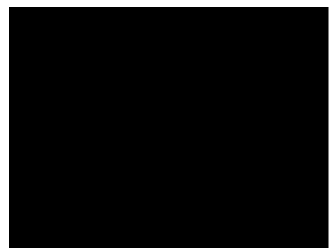

What about neanderthal? Should we do it?

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

Action Press/Rex Features