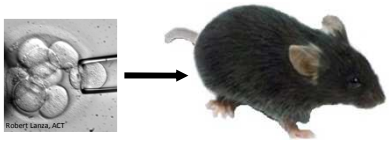
A few advances in biology are really opening up new territories, especially…

We can <u>sequence</u> a genome for a few $K in a few days

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

Amazing advances in <u>cloning</u>



<u>Stem cells</u>!

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

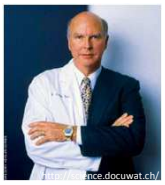
We can now <u>manufacture</u> a complete genome from commodity chemicals

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

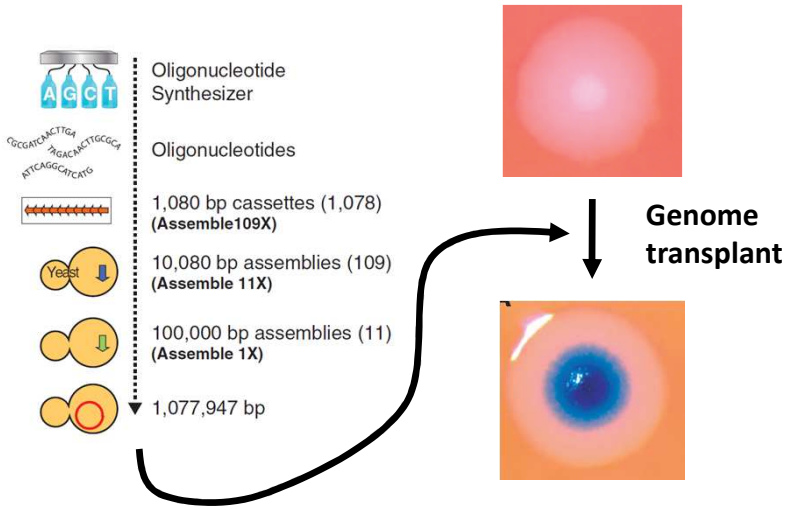
Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1]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



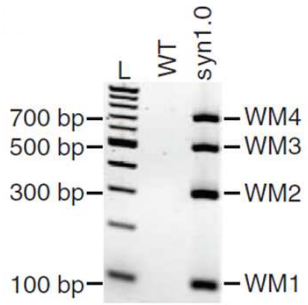
2 JULY 2010 VOL 329 SCIENCE

"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…

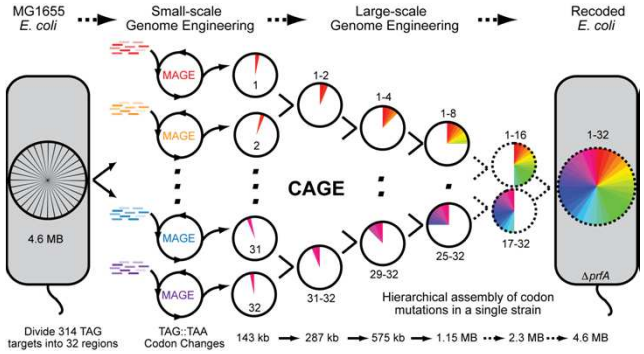
Some good quotes from the paper:

"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 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

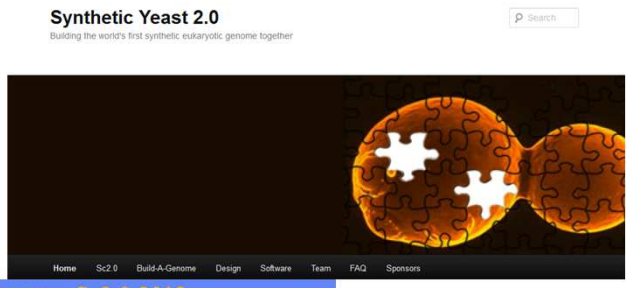
Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[3,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6]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

SCIENCE VOL 342 18 OCTOBER 2013

http://isaacs.commons.yale.edu/files/2012/07/cE.coli_Fig1_.png

& now, "rebooting" yeast with synthetic chromosomes

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



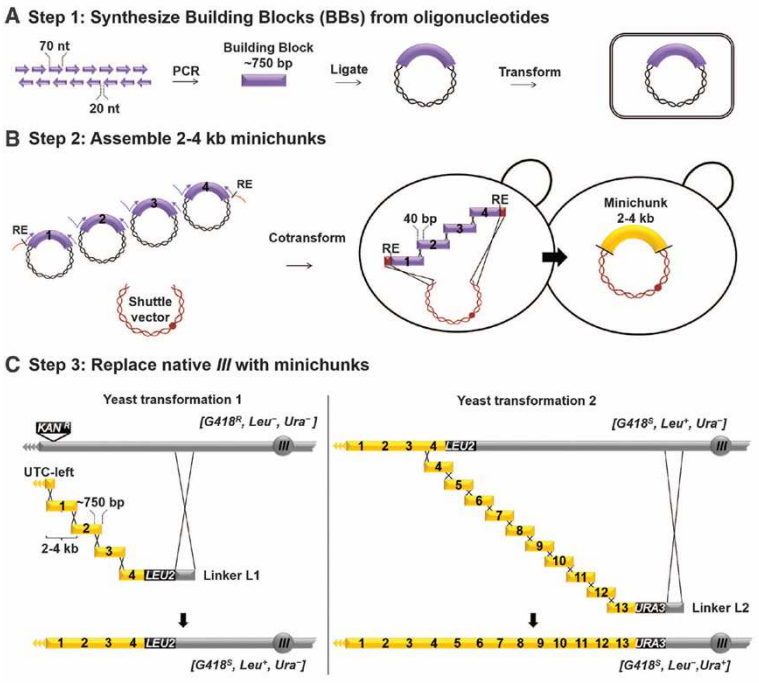
& China is pushing for a completely synthetic yeast genome…

Just published! *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

~2.5% of sequence changed

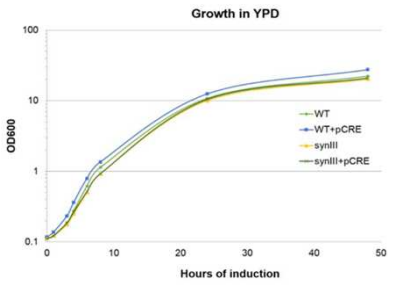
- Recoded all amber (TAG) stop codons to ochre (TAA)
- Introduced 98 Cre/Lox recombination sites
- Introduced unique sequences for PCR and new restriction enzyme sites
- Standardized telomeres

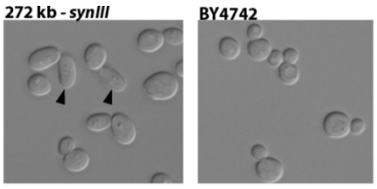
Reduced size from 316,617 bp to 272,871 bp (~14% reduction)

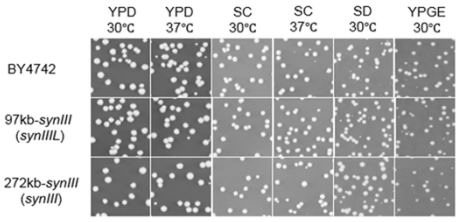
- Deleted 10 tRNA genes, 21 Ty elements/LTRs, silent mating loci
  (only one tRNA was essential, moved to a plasmid)
- Removed leucine biosynthesis gene LEU2 to be an auxotrophic marker
- Deleted all introns (affected 7 genes)
- Deleted subtelomeric DNA

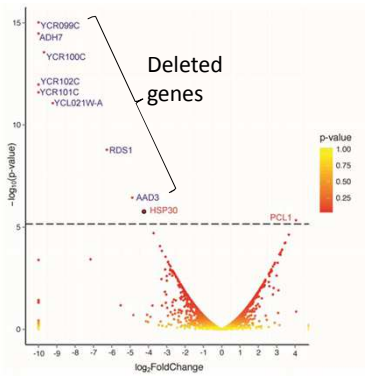
Only 10 errors in assembly: 9 single base changes and 1 lost recombinase site

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

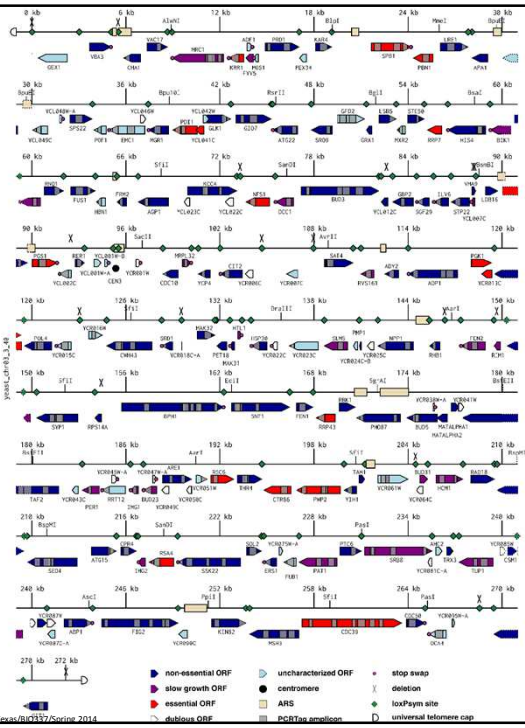








RNAseq of wt vs synIII



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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!

"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?

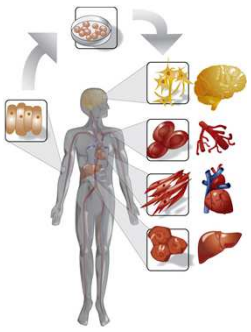
Sound familiar?

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



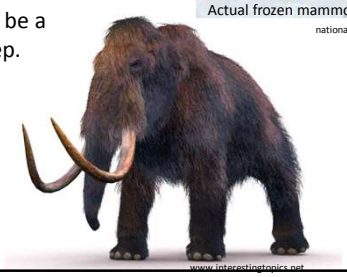
www.regenexx.com

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

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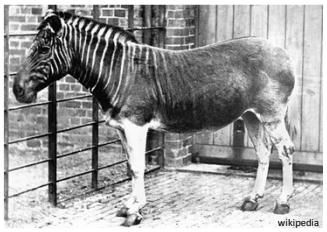
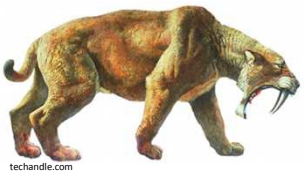
Which animal would you resurrect?

The dodo?

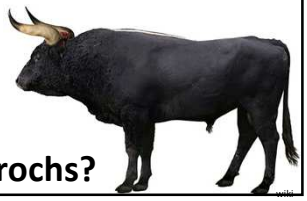

© 2010 Encyclopaedia Britannica, Inc.

The quagga?


wikipedia


techandle.com

Saber-toothed tiger?


wiki

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

Aurochs?

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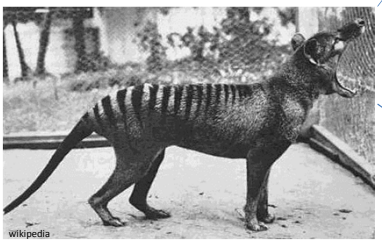
I vote for some crazy Australasian animals:

The 12' tall moa

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

& of, course, the marsupial Tasmanian tiger



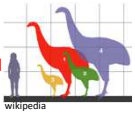
wikipedia

>90° !!!

The moa-eating Haast's eagle

wikipedia

Actual scale!



wikipedia

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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

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