**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”

Transcriptional repressors


---

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

The repressilator in action...


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

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.

Is this pixel part of an edge?  

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

→ No  → No  → Yes

Cells luminesce along the light/dark boundaries.
Bacterial photography

Cph1 chromophore biosynthesis

phycocyanobilin

pcy A

ho 1

heme

660nm

inner membrane

Cph1

EnvZ

OmpR

OmpR

PomC

LacZ

Black output (S-gal)

“Light cannon” developed by Aaron Chevalier, UT undergraduate

The first bacterial photograph (coliroid?)...

Escherichia darwinia


Image: Aaron Chevalier
On to the edge detector...

It works!

Tabor et al., Cell 137(7):1272-1281 (2009)
UT’s 2012 iGEM project – build caffeine biosensor

Basic idea
Block de novo guanine synthesis
Convert caffeine to xanthine
Addict E. coli bacteria to caffeine

Decaffeination and Measurement of Caffeine Content by Addicted Escherichia coli with a Refactored N-Demethylation Operon from Pseudomonas putida CB89
Erik M. Quick,1 Michael J. Hammerling,1 Ryan M. Sontag,1 Peter B. Choquet,2 Ben Stone,1
Kean N. Abinante,1 Ayushi Dhodapkar,1 James E. Rutenbar,1 Meni Y. Scherfeld,1
and Jeffrey L. Barick1

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

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.”
**Who needs nature?**

*Made-to-order, designer organisms*

We can now manufacture a complete genome from commodity chemicals

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

"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

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

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).
& now, “rebooting” yeast with synthetic chromosomes

It turns out chromosomes can be synthesized and replaced for yeast too...

& China is pushing for a completely synthetic yeast genome...

“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

Last year, 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](http://science.sciencemag.org/content/355/6329/eaan1126)
Design of a synthetic yeast genome

Sarah M. Richardson,1,4 Leslie A. Mitchell,2,3 Giovanni Strasemann,3,4-
Kun Yang,1,3 Jessica S. Dineen,7 James E. Darnell,7 Deungsoo Lee,7
Cheng Kai-Victor Hsiao,2,3 Krishnan Chandaarangana,2,3 Vishal Chal,2,3
Jae D. Bokke,1,3 Joel S. Badar1,3

We describe complete design of a synthetic eukaryotic genome, Sc2.0, a highly modified
Saccharomyces cerevisiae genome reduced in size by nearly 4%, with 1.1 megabytes of
the synthetic genome deleted, inserted, or altered. Sc2.0 chromosome design was implemented
with tools that is an open-source framework developed for synthetic genome design,
which coordinates design modifications from nucleobase 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.

<table>
<thead>
<tr>
<th>Design challenge or amendment</th>
<th>Policy adopted by design team</th>
</tr>
</thead>
</table>
| Subtelomeric repeats
  of varying copy number
  on multiple chromosomes | Delete and monitor for phenotypes
  as chromosomes are combined. Exception:
  vlinera inhibitorsynthesis genes retain one copy |
| Dispersed repeated genes of high copy number, as well as high-copy CDS and
  sniiptron genes | Delete and monitor for phenotypes as
  chromosomes are combined. |
| loxP sites ≤500 bp apart when
  inserted algorithmically (not especially
  useful and more difficult to synthesize) | loxP sites →eliminate the loxP site
  closer to the centromere. |
| Stop codon over a second CDS,
  insertion of loxP site would disrupt
  second CDS; also TAG recoding to TAA |
  could disrupt CDS | Favor preservation of “verified ORFs”
  over “dubious ORPs” and “uncharacterized ORPs”;
  always add loxP site to a verified ORF in this case |
| Tandem repeats inside CDSs (34) | Use GeneDesign’s RepeatSmasher
  module to locate 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 plan, permit 10% length
  variation for homopolymer
  tracts >10bp provided they are
  in a nontarget region. |
| Introns | Delete pre-mRNA introns precisely, except from genes
  with evidence of a fitness defect caused by intron
  deletion (35, 36). The HAC intron, which uses separate
  splicing machinery and is known to play a critical
  role in regulation of the unfolded protein response,
  was not deleted (3). Delete all HPR introns precisely |
| Intrinsically embedded snoRNAs | These are individually nonessential and
  were deleted with their host introns.
  They could be “refactorized” by
  insertion into the array of smallRNAs on chr II. |
“Mega-chunk” assembly

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

<table>
<thead>
<tr>
<th>WT size</th>
<th>SYN size</th>
<th>No. of stop codon swaps</th>
<th>No. of loxP sites added</th>
<th>bp of PCRTag recorded</th>
<th>bp of RE sites recorded</th>
<th>No. of trRNA deleted</th>
<th>bp of trRNA deleted</th>
<th>bp of repeats deleted</th>
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</table>
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

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

Remember Dolly, the cloned sheep?

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

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 \textit{in vitro} fertilize animals

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

Sound familiar?

http://jurassicpark.wikia.com
Besides the genome engineering, this hinges on iPS:

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

& thanks to Yamanaka, we can convert skin cells back into stem cells

Shinya Yamanaka
Nobel Prize, 2012

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 into mammoth skin cells
✓ Convert skin cells to stem cells
✓ Convert stem cells to embryos
  ➢ In vitro fertilize elephants

This might be a hard step.
As of April 2015...

WOOLLY MAMMOTH DNA SUCCESSFULLY SPliced INTO ELEPHANT CELLS
BUT DON'T EXPECT MAMMOTH SLOTHES ANYTIME SOON
By Sarah Zielinski
Published: March 04, 2015

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."


Which animal would you resurrect?

The dodo?

The quagga?

Sabertoothed tiger?

Aurochs?

In principle, only need the DNA sequence (so, no dinosaurs)
I vote for some crazy Australasian animals:

The 12’ tall moa

& of, course, the marsupial Tasmanian tiger

The moa-eating Haast’s eagle

Actual scale!

What about neanderthal? Should we do it?

✓ 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