**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  batteries
  - (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 an oscillatory fashion

= “repressor” + “oscillator”

**Transcriptional repressors**

![Diagram of TetR, λ cl, LacI proteins per cell over time](image)


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

![Diagram of Repressilator and Reporter circuits](image)

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
```

Adapted from Zack Simpson
Bacterial photography


Cph1/EnvZ Mask

“Light cannon” developed by Aaron Chevalier, UT undergraduate

The first bacterial photograph (coliroid?)...


Escherichia darwinia

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

Projected Mask | Photo strain | Edge detector strain

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 α-Demethylation Operon from Pseudomonas putida CBB85

Erik M. Quandt, Michael J. Hammerling, Hyun Joung Sonnenschein, Peter R. Ornelas, Ben Storz, Kevin N. Ashford, Aparna Durgapal, James L. Richmon, Mun Y. Subramanian, and Jeffrey L. Barick
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...

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

“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.”
“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 S9...

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 reassessed by global transposon mutagenesis. (B) Comparison of JCVI-syn3.0 (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).

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

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

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,"1,5 Leslie A. Mitchell,"1,5 Giovanni Strathmann,"1,5,6
Kun Yang,"7 Jessica S. Staudt,7 James E. DCarlo,7 Desgovic Lee,7
Cheng-Eat Victor Hsuang,7 Brahma J. Chaudhary,8,9 Fei Cai,7
Jef D. Boeke,7,9 Joel S. Badar9

We describe complete design of a synthetic eukaryotic genome, Sc2.0, a highly modified
S. cerevisiae genome reduced in size by nearly 8%, with 1.1 megaribosomes
of the synthetic genome deleted, inserted, or altered. Sc2.0 chromosome design was implemented
with iGEMtools, an open-source framework developed for eukaryotic genome design,
which coordinates design modifications from nucleotides 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>
<tbody>
<tr>
<td>Subtelomeric repeats of varying copy number on multiple chromosomes</td>
<td>Delete and monitor for phenotypes as chromosomes are combined. Exception: villar genes retain one copy.</td>
</tr>
<tr>
<td>Dispersed repeated genes of high copy number, as well as high-copy COS and teraporn genes</td>
<td>Delete and monitor for phenotypes as chromosomes are combined.</td>
</tr>
<tr>
<td>loxP/loxP sites &lt;500 bp apart when inserted algorithmically (not especially useful and more difficult to synthesize)</td>
<td>loxP/loxP thinning to eliminate the loxP/loxP site closer to the centromere.</td>
</tr>
<tr>
<td>Stop codon over a second CDS, insertion of loxP/loxP site would disrupt second CDS, also TAG recoding to TAA could disrupt CDS</td>
<td>Favor preservation of &quot;verified ORFs&quot; over &quot;dubious ORFs&quot; and &quot;uncharacterized ORFs&quot;; always add loxP/loxP site to a verified ORF in this case.</td>
</tr>
<tr>
<td>Tandem repeats inside CDSs (34)</td>
<td>Use GeneDesign’s RepeatSmoother module to recode such genes to minimize DNA level repetitiveness, making DNA easier to synthesize and assemble.</td>
</tr>
<tr>
<td>Homopolymer tracts, including frequent A and T tracts, are difficult to synthesize</td>
<td>In synthesis phase, permit 10% length variation for homopolymer tracts &gt;10 bp provided they are in a noncoding region.</td>
</tr>
<tr>
<td>Intron</td>
<td>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 unfixed protein response, was not deleted (5). Delete all hPNA introns precisely.</td>
</tr>
<tr>
<td>Intrinsically embedded snoRNAs</td>
<td>These are individually recessive and were deleted with their host introns. They could be &quot;refactored&quot; by insertion into the array of smallRNAs on chr II.</td>
</tr>
</tbody>
</table>
"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 recoded</th>
<th>bp of RE sites recoded</th>
<th>No. of tRNA deleted</th>
<th>bp of tRNA deleted</th>
<th>bp of repeats deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr01</td>
<td>230208</td>
<td>19</td>
<td>62</td>
<td>3535</td>
<td>210</td>
<td>4</td>
<td>372</td>
<td>3987</td>
</tr>
<tr>
<td>chr02</td>
<td>813184</td>
<td>770035</td>
<td>93</td>
<td>271</td>
<td>13651</td>
<td>1215</td>
<td>13</td>
<td>993</td>
</tr>
<tr>
<td>chr03</td>
<td>316617</td>
<td>272915</td>
<td>44</td>
<td>100</td>
<td>5272</td>
<td>250</td>
<td>10</td>
<td>794</td>
</tr>
<tr>
<td>chr04</td>
<td>1531933</td>
<td>1454671</td>
<td>183</td>
<td>479</td>
<td>25398</td>
<td>2298</td>
<td>28</td>
<td>2261</td>
</tr>
<tr>
<td>chr05</td>
<td>576874</td>
<td>536024</td>
<td>61</td>
<td>174</td>
<td>8760</td>
<td>813</td>
<td>20</td>
<td>1471</td>
</tr>
<tr>
<td>chr06</td>
<td>270148</td>
<td>242745</td>
<td>30</td>
<td>69</td>
<td>4553</td>
<td>369</td>
<td>10</td>
<td>835</td>
</tr>
<tr>
<td>chr07</td>
<td>1090940</td>
<td>1028952</td>
<td>126</td>
<td>380</td>
<td>17910</td>
<td>1572</td>
<td>36</td>
<td>2887</td>
</tr>
<tr>
<td>chr08</td>
<td>562643</td>
<td>506705</td>
<td>61</td>
<td>186</td>
<td>9980</td>
<td>714</td>
<td>11</td>
<td>878</td>
</tr>
<tr>
<td>chr09</td>
<td>438865</td>
<td>405613</td>
<td>54</td>
<td>142</td>
<td>7943</td>
<td>436</td>
<td>10</td>
<td>736</td>
</tr>
<tr>
<td>chr10</td>
<td>745751</td>
<td>707459</td>
<td>85</td>
<td>249</td>
<td>12582</td>
<td>1102</td>
<td>24</td>
<td>1853</td>
</tr>
<tr>
<td>chr11</td>
<td>666816</td>
<td>659617</td>
<td>68</td>
<td>199</td>
<td>11769</td>
<td>1017</td>
<td>15</td>
<td>1243</td>
</tr>
<tr>
<td>chr12</td>
<td>1078177</td>
<td>999406</td>
<td>122</td>
<td>291</td>
<td>15129</td>
<td>1539</td>
<td>19</td>
<td>1646</td>
</tr>
<tr>
<td>chr13</td>
<td>924431</td>
<td>883749</td>
<td>100</td>
<td>337</td>
<td>15911</td>
<td>0</td>
<td>21</td>
<td>1691</td>
</tr>
<tr>
<td>chr14</td>
<td>784333</td>
<td>753096</td>
<td>96</td>
<td>260</td>
<td>13329</td>
<td>1113</td>
<td>14</td>
<td>1152</td>
</tr>
<tr>
<td>chr15</td>
<td>1081291</td>
<td>1048343</td>
<td>147</td>
<td>399</td>
<td>18015</td>
<td>2058</td>
<td>20</td>
<td>1612</td>
</tr>
<tr>
<td>chr16</td>
<td>948066</td>
<td>902994</td>
<td>127</td>
<td>334</td>
<td>15493</td>
<td>1374</td>
<td>17</td>
<td>1338</td>
</tr>
<tr>
<td>Total</td>
<td>12071297</td>
<td>11352534</td>
<td>1416</td>
<td>3932</td>
<td>199230</td>
<td>16080</td>
<td>272</td>
<td>21762</td>
</tr>
</tbody>
</table>
Synthesis, cyclization, and characterization of synV

A

Let’s end the lectures on a fun note, with some speculative near-future synthetic biology experiments

B

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...

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
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 to 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 GLOVES ANYTIME SOON

By Sarah Ferris


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?
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
- The moa-eating Haast’s eagle
- & of course, the marsupial Tasmanian tiger

>90° !!!

Actual scale!

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

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

Svante Pääbo