

Future After the First Synthetic Cell...

Presenter | Sijie Li

2010
JCVI-Syn1.0

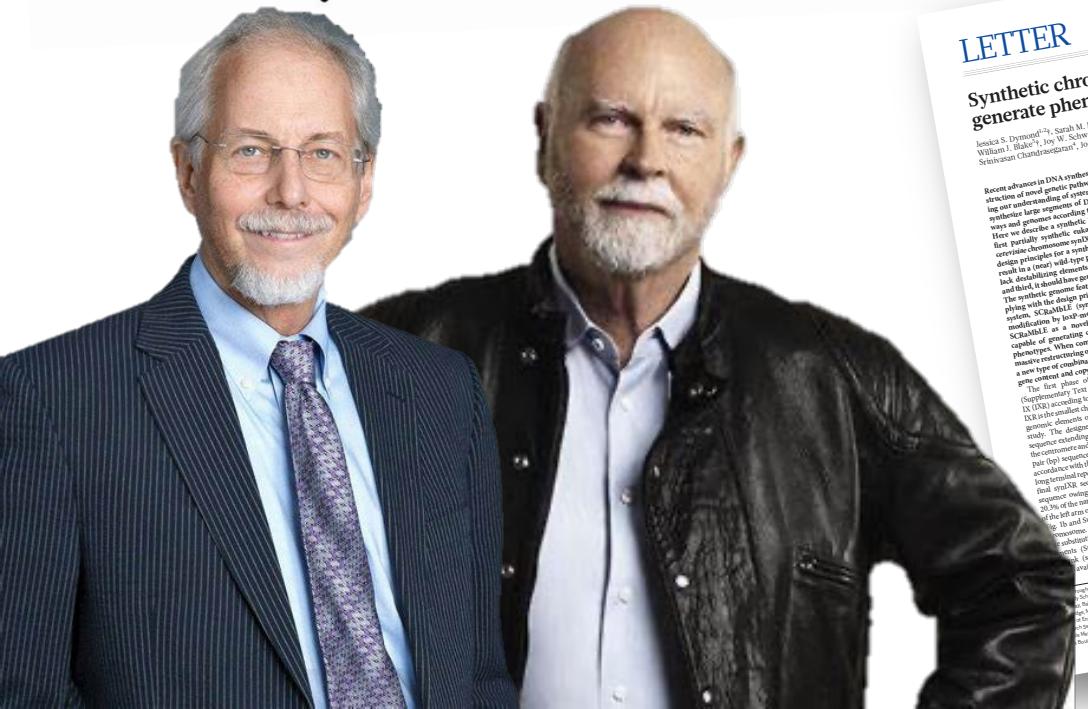
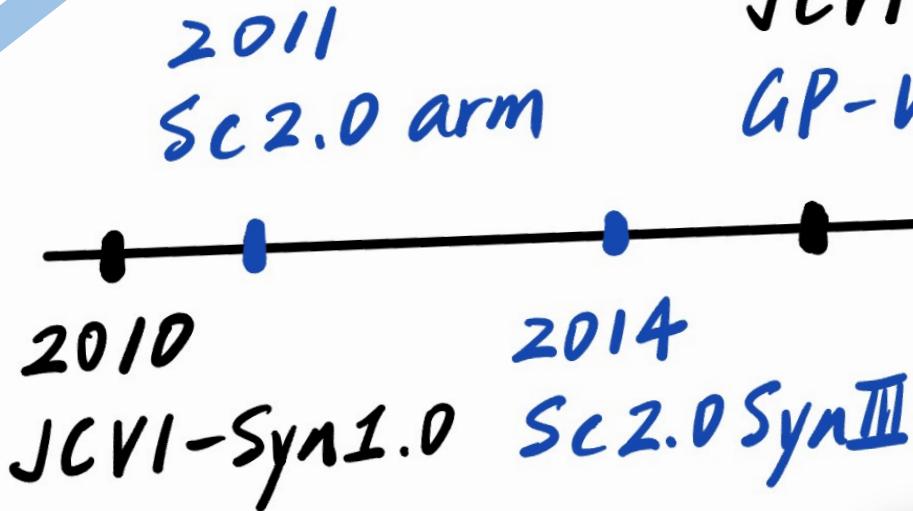
2011
Sc2.0 arm

2014
Sc2.0 SynIII

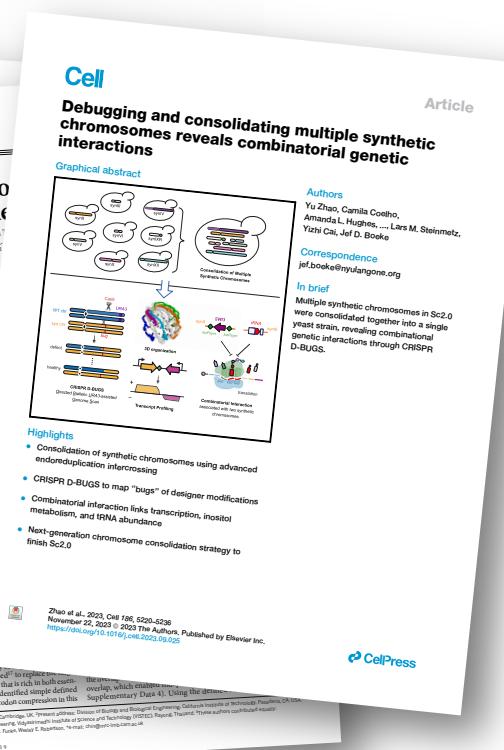
2023
Sc2.0 6.5+1

Questions I Want to Know

- **Design:** size? Donor & recipient? Modification design? Automatic? Generalized principles?...
- **Synthesis:** accuracy? Cost (price and time)? Modification synthesis?...
- **Assembly and transplantation:** Accuracy? Automatic? Complexity?...
- **Validation:** viability under different conditions?
- **Ethics:** firewall design? Regulations establishment?
- **Application:** therapy? Industrial? Biological mechanism?



Prokaryotype and eukaryotype Reduce and scale up



The authors of this article are grateful to the many individuals who have contributed to the success of the Sc2.0 project, including the members of the Boeke and Gibson laboratories, and the many other researchers who have contributed to the field of synthetic biology.

Received January 10, 2014; revised June 28, 2016; accepted June 28, 2016; published online July 1, 2016. This work was supported by grants from the National Institutes of Health (NIH) (J.D.B.) and the National Science Foundation (NSF) (G.G.). We thank the members of the Boeke and Gibson laboratories for their contributions to this work.

Correspondence to: Georges González-Chamazares (e-mail: ggonzalez@nyulangone.org).

DOI: 10.1016/j.cell.2023.01.026



POLICY FORUM

The Genome Project-Write

We need technology and an ethical framework for genome-scale engineering

By Jeff D. Boeke,* George Church,* Andrew Anelli,†,‡,§,¶,|| Kelley A. Amo,|| John Vazquez,|| Rob Carlson,|| Michael Chakravorti,|| Virginia W. Corrath,|| Liam Holt,|| Farren J. Isaacs,|| Todd Kuhnen,|| Max Lajoie,|| Tracy Lesser,|| Jennifer Malmstrom,|| Daniel R. Mills,|| Leslie A. Mitchell,|| Jasper Rine,|| Susan Kosier,|| Neville E. Sanjana,|| Pamela A. Silver,|| David Viale,|| Harris Wang,|| Jeffrey C. Way,|| Luhuan Yang,||

The Human Genome Project ("HGP-read"), prominently completed in 2004, dramatically reduced the cost and time to sequence the human genome, and to improve the technology, cost, and quality of DNA sequencing (*J. D. Boeke et al.*, *Science*, **355**, eaau0001, 2017). It was the first genome-scale project of its kind, and its success was controversial by some. Now, it is recognized as one of the great feats of exploration that has revolutionized science and medicine.

Although we have made significant strides in advancing DNA technology at a breakthrough pace, the capability for constructing DNA sequences in cells is presently limited to a small set of large segments. This limits our ability to manipulate and understand biological systems. Further understanding of genetic biology requires a goal of building entire large genomes, (GB)-sized animal and plant genomes, including the human genome, which would, in turn, drive development of tools and methods to facilitate this goal.

This goal is necessarily ambitious, because building a human genome at today's prices would require more than HGP-read (90 cell lines) or even HGP-write (10 cell lines) within 10 years. This will include whole-genome engineering of human cell lines and model organisms, and medical applications. Human genome editing in particular has raised intense moral debate (*i.e.*, gene regulation, genetic diseases, and evolution).

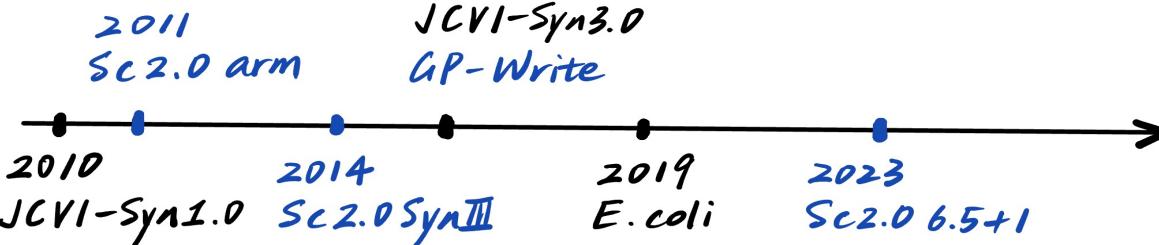
We believe that the public discourse on HGP-write; having such conversations well in advance of project implementation will guide emerging capabilities in science and contribute to societal decision-making.

The list of author affiliations is available in the supplemental material. *These authors contributed equally to this work. Email: jeff.boeke@nyu.edu

126 | 8 JULY 2016 | VOL 353 ISSUE 6286

Published by AAAS

science.org SCIENCE



2016

HGP-Write

From HGP-read to
25 years' later
Human Genome
Project-Write

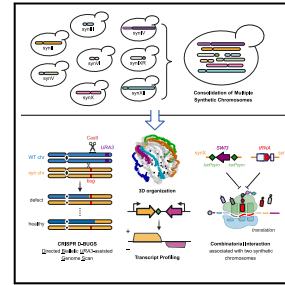
\$100 million in
committed
support

Cell

Article

Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions

Graphical abstract



Highlights

- Consolidation of synthetic chromosomes using advanced endoreduplication intercrossing
- CRISPR D-BUGS to map "bugs" of designer modifications
- Combinatorial interaction links transcription, inositol metabolism, and tRNA abundance
- Next-generation chromosome consolidation strategy to finish Sc2.0

Zhao et al., 2023, *Cell* 196, 5220–5236
November 22, 2023 © 2023 The Authors. Published by Elsevier Inc.
<https://doi.org/10.1016/j.cell.2023.09.025>

CellPress

2023

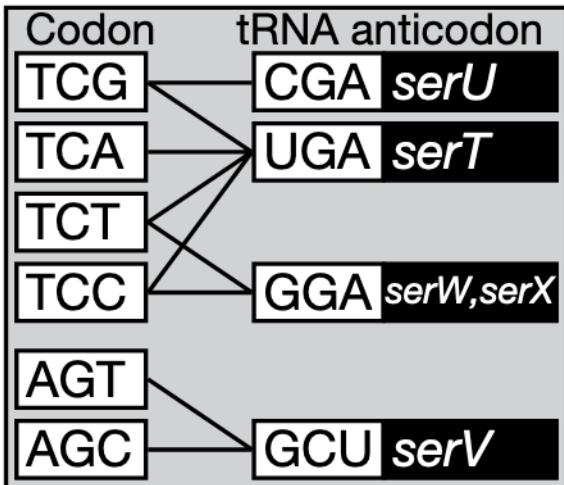
Consolidate 6.5 + 1 chr (16 ✓) CRISPR D-BUG

>50% synthetic chromosome

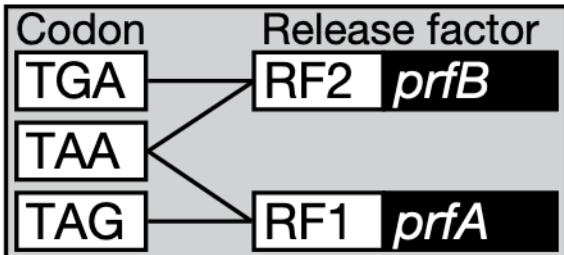
- Advanced Endoreduplication intercrossing
- chromosome substitution (largest chr synIV)



Serine

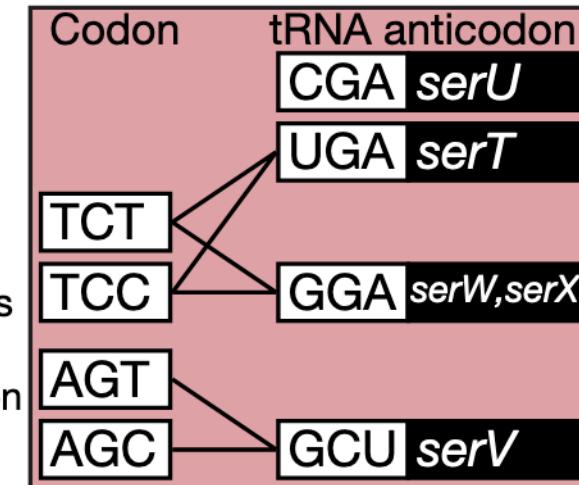


STOP

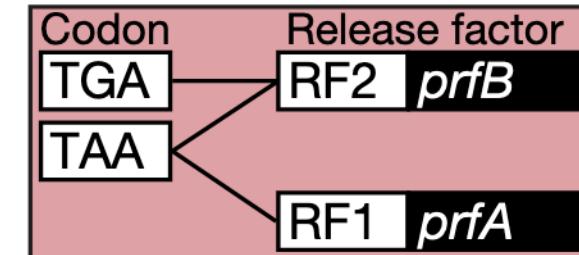


Wild-type genome

Serine

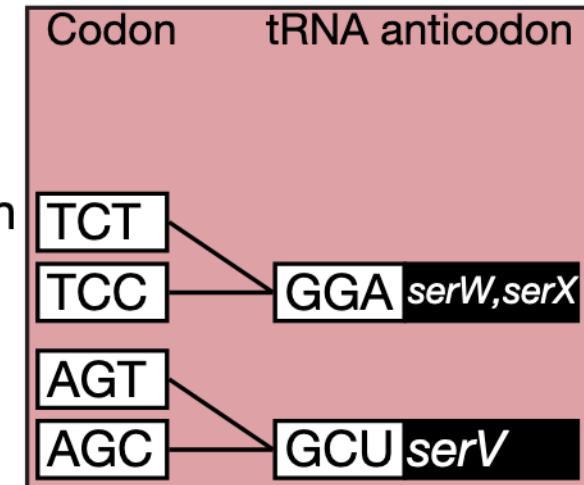


STOP

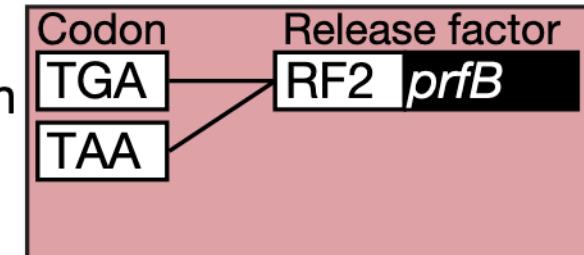


Recoded genome

Serine



STOP



Recoded genome

Sc2.0 (Yeast 2.0)

nature communications

[View all journals](#) [Search](#) [Log in](#)

[Explore content](#) [About the journal](#) [Publish with us](#)

[Sign up for alerts](#) [RSS feed](#)

[nature > nature communications > collection](#)

Collection | 22 May 2018

Yeast 2.0

Synthetic biology aims to redesign and reconstruct biological systems for new, useful end goals. One of the ambitious projects currently underway is Sc2.0: the design and synthesis of a complete eukaryotic genome - *Saccharomyces cerevisiae*.

This collection highlights the experimental work published in *Nature Communications* on redesigning the *S. cerevisiae* genome along with commentary from the community about the potential applications and implications of this work for synthetic biology, biotechnology and our understanding of the genome.

CellPress

[Submit](#) [Log in](#) [Register](#) [Subscribe](#) [Claim](#)

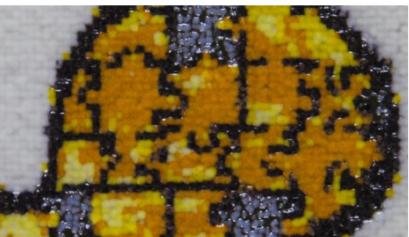
[Journals](#) [Publish](#) [News & events](#) [About Cell Press](#)

Search for... [Advanced search](#)

Synthetic Yeast Genome Project (Sc2.0) consortium

The Synthetic Yeast Genome Project (Sc2.0) is a global consortium working to develop the first synthetic eukaryote genome from scratch. The eventual goal of the project is to assemble a fully synthetic yeast organism and to facilitate synthetic biology and engineering research in eukaryotes. This will pave the way for engineering more complex synthetic multi-cellular organisms and provide a toolkit for designing and implementing fully synthetic genomes in the future.

Cell Press is proud to present the latest research from the Sc2.0 consortium in *Cell*, *Cell Genomics*, and *Molecular Cell*. The papers featured showcase the design and optimization of “debugged” synthetic chromosomes in *Saccharomyces cerevisiae*. In a cluster of papers dedicated to individual synthetic chromosomes, the Sc2.0 consortium describes new findings on aneuploidy, extrachromosomal DNA regulation, chromosome fusion, and many other aspects of yeast genome biology. Highlights in this collection include the creation of a yeast strain containing seven synthetic chromosomes that functions similarly to wild-type yeast, a strain with all tRNA genes re-located to an entirely synthetic tRNA neochromosome, and the re-design and 3D structural genomic characterization of the largest yeast chromosome.



INSIGHTS | PERSPECTIVES

for large-scale energy applications. The nonperiodic layered nanophotonic structure showed good performance (6), but its required nanometer precision control of the thin films is still a challenge for scaling up to the size of meters, which is needed for even a small (kilowatt-scale) cooling system.

The unprecedented properties of a material such as negative refraction and superlensing originates from its internal structures instead of its chemical constituents (7). Because its structural unit cell is often smaller than the wavelength of interest, periodic structures of optical metamaterials have always been limited (8). Zhai *et al.* devised a glass-polymer metamaterial in which a set of glass microspheres were randomly and uniformly dispersed in a visibly transparent polymer matrix. Because of the surface phonon-polariton Mie resonance excited at room temperature on the glass surface, this amorphous metamaterial has a maximal broadband emissivity—near the blackbody limit across the entire atmospheric window—that results in cooling of the material itself (8). Both the polymer and glass are transparent to the full solar spectrum, so the hybrid metamaterial minimally absorbs and reflects incoming energy when backed with a thin silver mirror (see the figure).

Zhai *et al.* demonstrated an average radiative cooling flux greater than 110 W m^{-2} in a continuous 3-day field test. This en-

ables metamaterials, detailed thermal design will be important to maximize the cooling rate for the substrate side, and effective heat exchange strategies therefore must be developed. In addition, the IR radiation transport inside metamaterials caused by volumetric multiple scattering among the random Mie resonating glass spheres should be carefully studied so as to further maximize the total emissive power. Other issues should also be carefully investigated, such as how weather conditions negate cooling performance and how the polymer-based metamaterial maintains its performance during long-term outdoor exposure.

Although extraction of the 110 W m^{-2} heat flux is a relatively low cooling rate, these designed metamaterials should find promising application for cooling large systems such as buildings in warm climates (9). Presently, air conditioning uses ~6% of the electricity produced in the United States, and as a result, more than 100 million metric tons of carbon dioxide are released into the atmosphere each year. The impact of such a passive radiative cooling without use of electricity for building applications alone can be immense. The broad use of radiative cooling technology not only leads to energy savings but also reduces fluxes of greenhouse gases from refrigerants used in conventional air conditioners, thus improving air quality. At higher temperatures, the passive radiative cooling can be drastically enhanced because the outgoing radiative flux is proportional to T^4 according to the Stefan-Boltzmann law. This scalably manufactured metamaterial may enable transformative cooling farms for power plants and data centers, which consume unsustainable amounts of water and electricity.

Although radiative cooling is promising, the better use of this waste energy can be more desirable. For example, the waste heat could be converted into electricity by photovoltaic solar cell materials, but with the great advantage of running both day and night. More impressively, the key roadblock for large area deployment of radiative cooling was removed. Because the material is amorphous and flexible, the authors developed a glass-polymer hybrid manufacturing technique to produce the microstructured metamaterial, which can be made as films several meters in length in a continuous roll-to-roll manner. Using such a scalable metamaterial, they demonstrated passive water cooling by nearly 10 Celsius degrees below ambient temperature without use of electricity.

There are still challenges yet to be addressed for the implementation of radiative cooling metamaterials into applications. Given that the cooling occurs on both sides

SYNTHETIC BIOLOGY

Yeast genome, by design

Scientists are inching closer to generating a synthetic eukaryotic cell

By Krishna Kamani¹ and Daniel G. Gibson^{1,2}

A core theme in synthetic biology, “understanding by creating” inspired the effort to generate the first synthetic cell, JCVI-Syn1.0 (1). The project Sc2.0 is elevating this concept by attempting to create a synthetic version of a more evolved organism, *Saccharomyces cerevisiae*, a eukaryotic single-celled yeast. In a set of papers in this issue (2–8), scientists of the Sc2.0 project who previously constructed a single yeast chromosome (9) now report constituting five additional chromosomes (10). The third of the entire genome (see the photo). Using a variety of phenotypic assays and structural and functional genomics techniques, the researchers observed that the synthetic chromosomes drive biological processes just like the natural, native chromosomes.

The quintessential first step toward creating a synthetic organism is the careful design of the genomic material, which ultimately controls every physiological process in the cell. Project Sc2.0 built a software framework, BioStudio, to generate chromosomal designs (2). A set of rules were applied while designing each chromosome, including removal of repetitive regions and insertion of TAA (allowing TAG to be reprogrammed), and the relocation of transfer RNA genes into a neochromosome. In addition, sites (loxPwym) were introduced throughout the chromosome some at the 3' ends of nonessential genes for chemically-inducible genome rearrangements (through Cre-recombinases). This allowed the selection of desired phenotypes and the examination of corresponding genotypes (synthetic chromosome rearrangement and modification by loxP-mediated evolution, or SCRaMBLE). Despite the many variations (thousands) introduced during the construc-

REFERENCES

1. V. D. Sazonov. *Nature* **355**, 1062 (2001).
2. F. D. Stoyan, P. M. Davis, *Physics of the Earth* (Wiley, 1977).
3. R. Hillerbrand, T. Tauber, F. Keilmann, *Nature* **418**, 159 (2002).
4. C. Lee *et al.*, *Renew Sustain Energy Rev* **65**, 1079 (2016).
5. E. Rephaeli, A. Raman, *Sci Nano Lett* **13**, 1457 (2013).
6. A. Raman *et al.*, *Nature* **515**, 540 (2014).
7. Y. Liu, X. Zhang, *Chemical Rev* **40**, 2494 (2011).
8. J. L. S. Hwang, T. Tachibana, M. L. Brongersma, *Phys Rev Lett* **99**, 107401 (2007).
9. N. Fernandez, W. Wang, K. Ahn, S. Katagiri, *Pacific Northwest National Laboratory Report no. PNNL-2494*, Richland, WA (2015).

10.1126/science.aam8566

Synthetic Genomics, Inc., 11149 North Torrey Pines Road, La Jolla, CA 92037 USA; *J. Craig Venter Institute*, 4210 Capricorn Lane, La Jolla, CA 92037 USA; Email: dgibson@synthgenomics.com

sciencemag.org SCIENCE

Published by AAAS

Downloaded from https://www.science.org/AAAS/Smithsonian Institute of Science and Technology on March 23, 2018

Main Principles

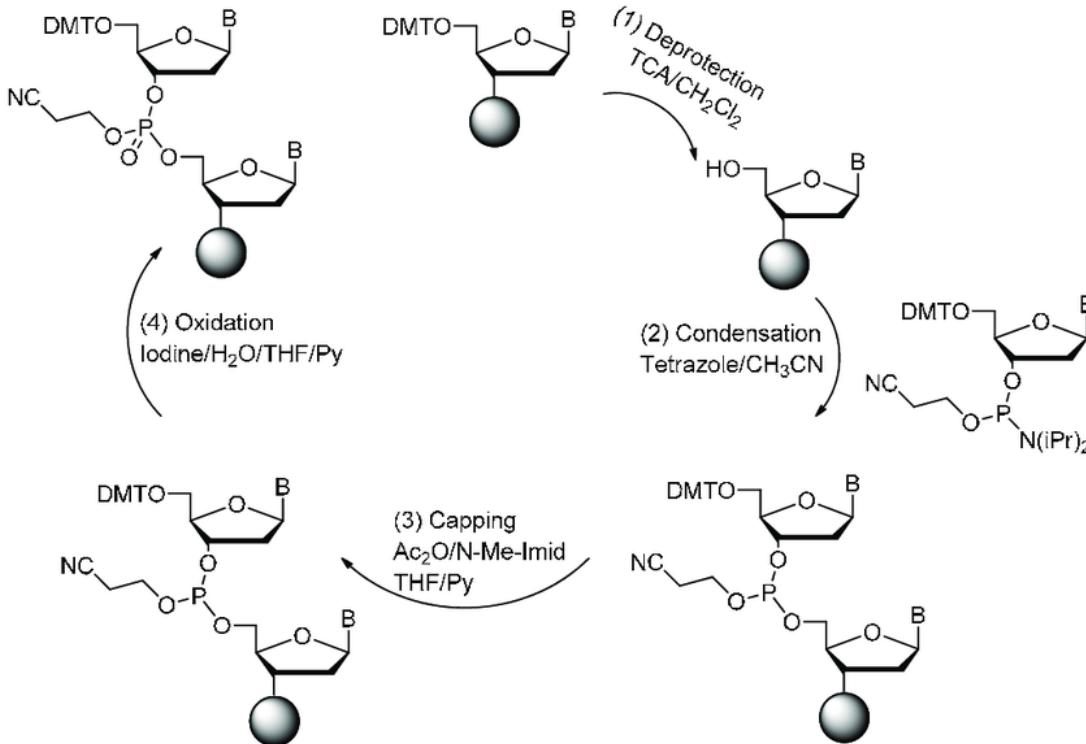
- **Delete** repeat, intron, transposon, unstable
- **Codon redesign**
 - modulate translation efficiency
 - firewall
- **tRNA relocate** tRNA array, neochromosome
 - Easy to manage
 - Match codon abundance
 - Aggregate to avoid damage due to disperse while deletion (naturally, near unstable region)

Answers and Thinkings...

Size? Donor & recipient? Modification design? Automatic? Generalized principles?...

- **Trend:** Pro-euk-mice-human!
- **Podcast wishes:** *M.capricolum* as a **generalized donor** without R-M system
- “**so few bacterial and eukaryotic synthetic genomics efforts**” (Venter et al., 2022)—cost and need!

Phosphoramidite Nucleoside Method

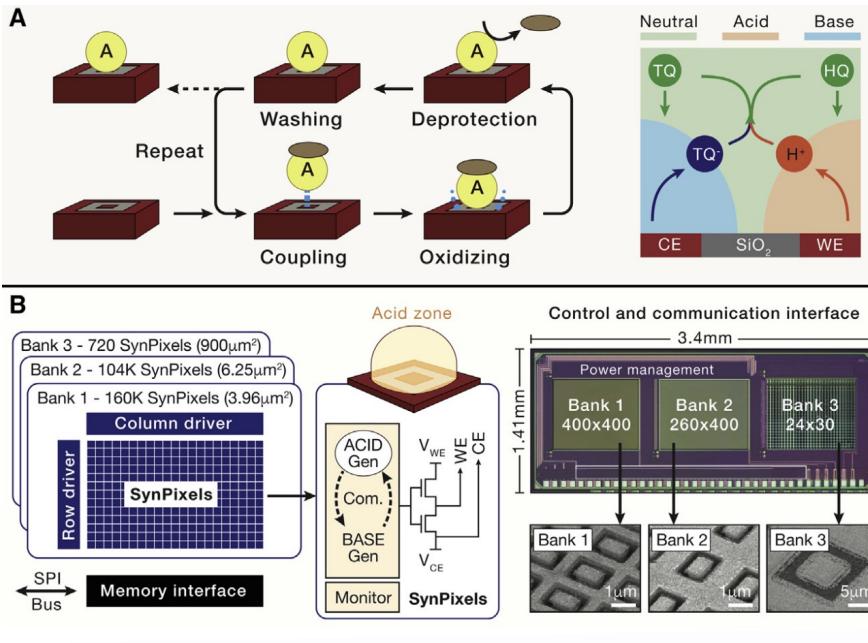


chemical synthesis
(for decades)

- **Complex steps:** protection, deprotection, capsulation, condition controlling...
- maximum effective synthesis of only ~200 base oligonucleotides
- produce **hazardous wastes**

Ni, Shuaijian & Yao, Houzong & Wang, Lili & Lu, Jun & Jiang, Feng & Lu, Aiping & Zhang, Ge. (2017). Chemical Modifications of Nucleic Acid Aptamers for Therapeutic Purposes. International Journal of Molecular Sciences. 18. 1683. 10.3390/ijms18081683.

Emerging Approach: CMOS Chip



25\$/chip

>100,000,000 sites × ~100,000 copies × <100-mers

$$\begin{aligned} &\sim 2.5 \times 10^{-15} \$/\text{bp} \xrightarrow{\times 8} \sim 2 \times 10^{-14} \text{¥}/\text{bp} \quad 10^{16} \text{ bp} \\ &\sim 2.5 \times 10^{-10} \$/\text{bp} (\text{unique}) \xrightarrow{\times 8} \sim 2 \times 10^{-9} \text{¥}/\text{bp} \quad 10^{10} \text{ bp} \end{aligned}$$

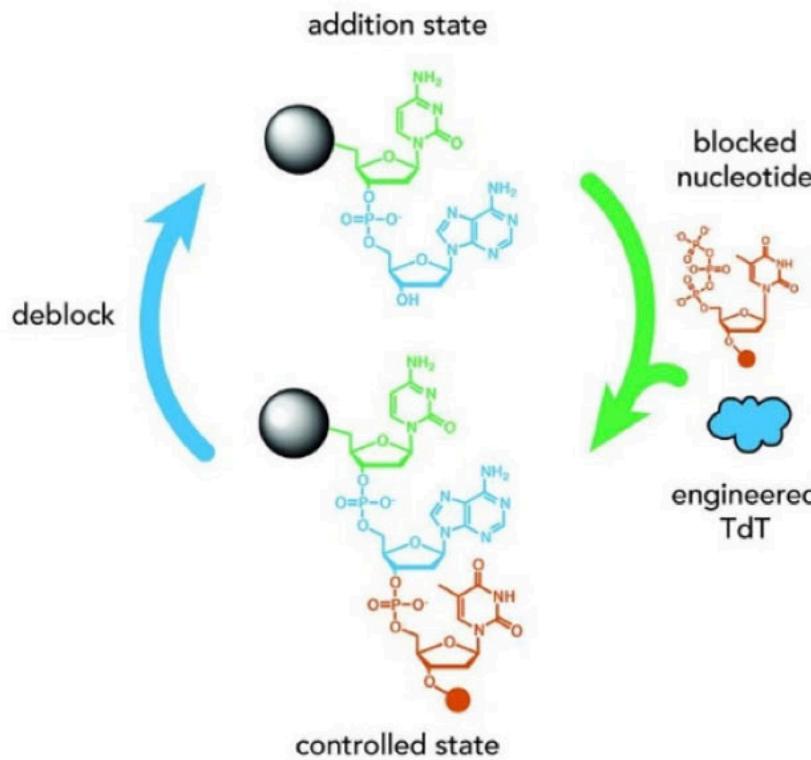
AT A TIME!

scalable semiconductor chip

- Base on DNA synthesis with electrochemical reactions (Egeland and Southern, 2005) – condition controlling
- JCVI + Avery Digital Data: synthesize a complete eukaryote genome

"personal communication with B. Merriman of Avery Digital Data" (Venter et al., 2022)

Enzymatic Synthesis



Non-templated enzymatic synthesis of oligonucleotides

- **terminal deoxynucleotidyl transferase (TdT)**
- **Longer oligonucleotides:** enzyme specificity, mild biological conditions (aqueous) → less damage
- **NO hazardous wastes**
- inability to synthesize **structural DNA** (e.g. small hairpin) → improving ...

Synthesis

Accuracy? Cost (price and time)? Modification synthesis?...

Enzymetic synthesis “**seem plausible for the first time in decades and may eventually lead to enzymatic oligonucleotide synthesizers**” (Venter et al., 2022)

OPC 纯化	≤ 35 mer	2 OD	1.5 元 / base	2 ~ 3 个工作日
		5 OD	2 元 / base	
	≤ 40 mer	10 OD	3 元 / base	
		2 OD	2.5 元 / base	
	41 ~ 60 mer (含 60)	5 OD	3 元 / base	
		10 OD	5 元 / base	
	61 ~ 80 mer (含 80)	2 OD	3 元 / base	4 个工作日
		5 OD	4.5 元 / base	
	81 ~ 100 mer (含 100)	10 OD	6 元 / base	
		2 OD	5 元 / base	
	101 ~ 120 mer (含 120)	5 OD	7 元 / base	5 ~ 7 个工作日
		10 OD	10 元 / base	
	121 ~ 135 mer (含 135)	2 OD	6 元 / base	
		5 OD	8 元 / base	
	136 ~ 150 mer (含 150)	10 OD	12 元 / base	7 ~ 10 个工作日
		2 OD	7 元 / base	
	151 ~ 160 mer (含 160)	5 OD	11 元 / base	
		10 OD	20 元 / base	
	161 ~ 170 mer (含 170)	2 OD	12 元 / base	请咨询客服
		5 OD	18 元 / base	
	环状 DNA	10 OD	30 元 / base	
		2 OD	12 元 / base	
	HPLC 纯化	5 mer ~ 40 mer	24 元 / base	3 ~ 4 个工作日
		2 OD	15 元 / base	
		5 OD	19 元 / base	请咨询客服
		10 OD	33 元 / base	

≤ 35 mer 1.5 ¥/base
VS
2E-9 ¥/base

Assembly and Transplantation

Accuracy (complex QC is needed)? Automatic? Complexity?

→ **Venter's team keeps on improving the techniques...**

2010 Chemical **synthesis** of the **mouse** mitochondrial genome

2012 **Assembly** of large, high **G+C** bacterial DNA fragments in yeast

2013 Direct **transfer** of whole genomes from bacteria to yeast

2013 **Assembly** of eukaryotic algal chromosomes in yeast

2014 two **facile transposon-mediated** approaches for **introducing genes** into the synthetic cell based on *M. mycoides*

2014 **Transferring** whole genomes from bacteria to yeast spheroplasts using entire bacterial cells to reduce DNA shearing (**R-M**)

2019 Direct **Transfer** of a Mycoplasma mycoides Genome to Yeast Is Enhanced by Removal of the Mycoides Glycerol Uptake Factor Gene *glpF* (**other genetic factors**)

...

Utilize model organisms

e.g. yeast for its ability to homologous recombination, mycoplasma for its growing speed ...

Improve the techniques to **manipulate and interact** with them

Validation

Viability under different conditions?

- Definition of minimal cell “**depend on the environment in which the cell is grown**” (Hutchison et al., 2016)
- So far “supplies virtually **all the small molecules required for life**” in the medium
- **Less permissive** conditions, **additional genes** may be required (E.g. lac operon)

Ethics

Firewall design? Regulations establishment?

Design

- **Screen** sequences against lists of pathogen agents
- **Genome-wide codon system redesign** (codon and tRNA) → orthogonal!
(NOT YET)

Brainstorming: difference enables specificity

- **Water mark** – distinguish and trace responsibility
- Engineer to need **metabolites** not found in nature

Synthesis

- **Machine detects and blocks** alteration / non-approved assembly

Application

Therapy? Industrial? Biological mechanism?

Technique

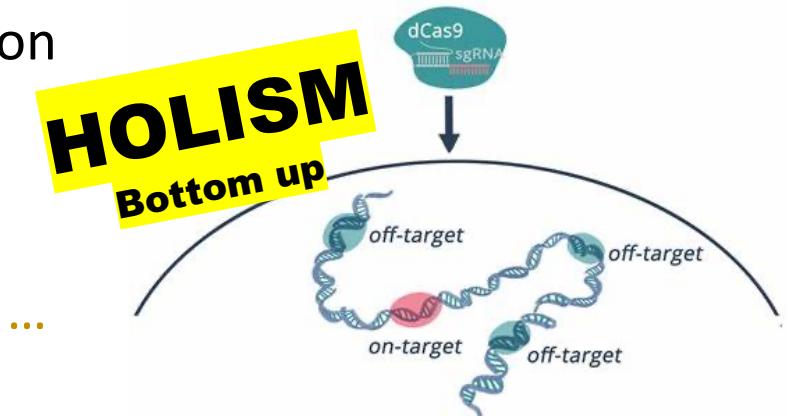
- **SCRaMbLE** (Sc2.0, produce high diversity from a single genotype) ...

Synthetic genomics (compare to conventional gene editing)

- **Understand cell:** “redundant genes for essential functions are scored as nonessential in these studies” (Gibson & Venter, 2014)
- **More flexible:** non-natural cassette with whole new function
- **Larger scale design or modify:** off-target effect of CRISPR

Virus vaccine, organ xenotransplantation (10 genes edited)

cell-based anticancer therapeutics, new metabolic pathways ...



Prospect

- **Accumulations:** techniques, mechanism/annotation discoveries
- **Collaboration:** among laboratories and fields

“franchise-like approach has greatly reduced the time taken to construct all 16 chromosomes and improved the efficiencies of scale for such a large project” (Schindler et al., 2023)

- **“The future of this field will clearly be exciting to see unfold.”** (Venter et al., 2022)



WE...

Reference List

1. Annaluru, N., et al. "Total Synthesis of a Functional Designer Eukaryotic Chromosome." *Science* 344, no. 6179 (2014): 55–58. <https://doi.org/10.1126/science.1249252>.
2. Boeke, J. D., et al. "The Genome Project-Write." *Science* 353, no. 6295 (2016): 126–27. <https://doi.org/10.1126/science.aaf6850>.
3. Dymond, J., et al. "Synthetic chromosome arms function in yeast and generate phenotypic diversity by design." *Nature* 477, no. 7365 (2011): 471–76. <https://doi.org/10.1038/nature10403>.
4. Fredens, J., et al. "Total Synthesis of Escherichia coli with a Recoded Genome." *Nature* 569, no. 7757 (2019): 514–18. <https://doi.org/10.1038/s41586-019-1192-5>.
5. Gibson, D. G., and J. C. Venter. "Construction of a Yeast Chromosome." *Nature* 509, no. 7499 (2014): 168–69. <https://doi.org/10.1038/509168a>.
6. Hutchison, C. A., et al. "Design and Synthesis of a Minimal Bacterial Genome." *Science* 351, no. 6280 (2016): aad6253. <https://doi.org/10.1126/science.aad6253>.
7. Kannan, K., and D. G. Gibson. "Yeast genome, by design." *Science* 355, no. 6329 (2017): 1024–25. <https://doi.org/10.1126/science.aam9739>.
8. Karas, B. J., et al. "Assembly of eukaryotic algal chromosomes in yeast." *J Biol Eng* 7, no. 1 (2013): 30. <https://doi.org/10.1186/1754-1611-7-30>.
9. Karas, B. J., et al. "Direct transfer of whole genomes from bacteria to yeast." *Nat Methods* 10, no. 5 (2013): 410–12. <https://doi.org/10.1038/nmeth.2433>.
10. Karas, B. J., et al. "Rescue of mutant fitness defects using in vitro reconstituted designer transposons in *Mycoplasma mycoides*." *Front Microbiol* 5 (2014): 369. <https://doi.org/10.3389/fmicb.2014.00369>.
11. Karas, B. J., et al. "Transferring whole genomes from bacteria to yeast spheroplasts using entire bacterial cells to reduce DNA shearing." *Nat Protoc* 9, no. 4 (2014): 743–50. <https://doi.org/10.1038/nprot.2014.045>.
12. Karas, B. J., et al. "Direct Transfer of a *Mycoplasma mycoides* Genome to Yeast Is Enhanced by Removal of the *Mycoplasma* Glycerol Uptake Factor Gene *glpF*." *ACS Synth Biol* 8, no. 2 (2019): 239–44. <https://doi.org/10.1021/acssynbio.8b00449>.
13. Annaluru, N., et al. "Total Synthesis of a Functional Designer Eukaryotic Chromosome." *Science* 344, 55–58 (2014). DOI:10.1126/science.1249252.
14. Noskov, V. N., et al. "Assembly of large, high G+C bacterial DNA fragments in yeast." *ACS Synth Biol* 1, no. 7 (2012): 267–73. <https://doi.org/10.1021/sb3000404>.
15. Schindler, D., et al. "Methodological advances enabled by the construction of a synthetic yeast genome." *Cell Rep Methods* 4, no. 4 (2024): 100761. <https://doi.org/10.1016/j.crmeth.2024.100761>.
16. Venter, J. C., et al. "Synthetic Chromosomes, Genomes, Viruses, and Cells." *Cell* 185, no. 15 (2022): 2708–24. <https://doi.org/10.1016/j.cell.2022.06.046>.
17. Zhao, Y., et al. "Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions." *Cell* 186, no. 24 (2023): 5220–36.e16. <https://doi.org/10.1016/j.cell.2023.10.025>.