Synthetic Cells
a J. Craig Venter Institute Perspective

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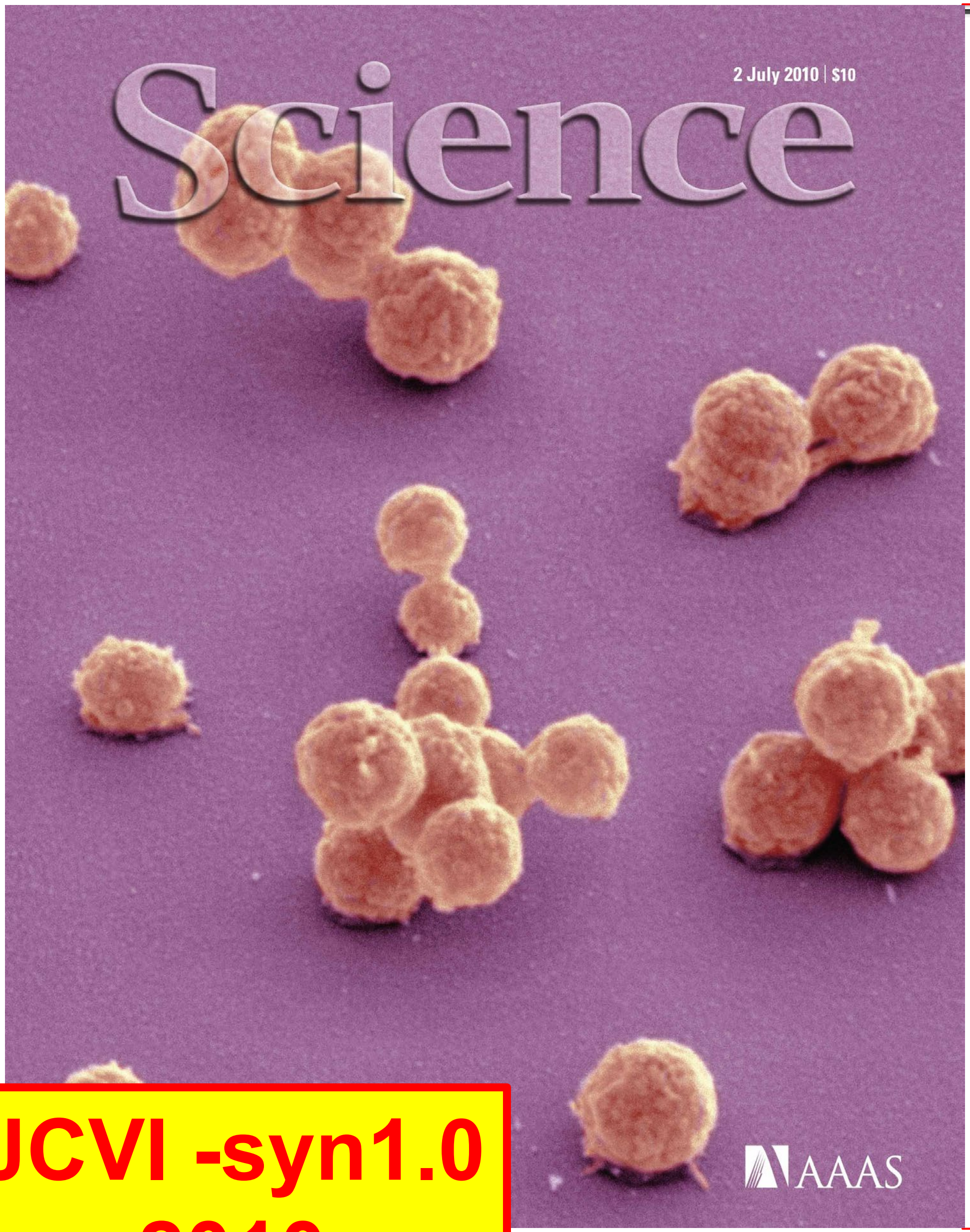
Life by Intelligent Design

JCVI



RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

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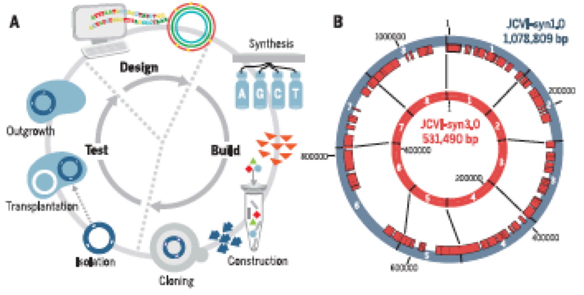
INTRODUCTION: In 1984, the simplest cells capable of autonomous growth, the mycoplasmas, were proposed as models for understanding the basic principles of life. In 1995, we reported the first complete cellular genome sequences (*Haemophilus influenza*, 1815 genes, and *Mycoplasma genitalium*, 525 genes). Comparison of these sequences revealed a conserved core of about 250 essential genes, much smaller than either genome. In 1999, we introduced the method of global transposon mutagenesis and experimentally demonstrated that *M. genitalium* contains many genes that are nonessential for growth in the laboratory, even though it has the smallest genome known for an autonomously replicating cell found in nature. This implied that it should be possible to produce a minimal cell that is simpler than any natural one. Whole genomes can now be built from chemically synthesized oligonucleotides and brought to life by installation into a receptive cellular environment. We have applied whole-genome design and synthesis to the problem of minimizing a cellular genome.

RATIONALE: Since the first genome sequences, there has been much work in many bacterial models to identify nonessential genes and define core sets of conserved genetic functions, using the methods of comparative genomics. Often, more than one gene product can perform a particular essential function. In such cases, neither gene will be essential, and neither will necessarily be conserved. Consequently, these approaches cannot, by themselves, identify a set of genes that is sufficient to constitute a viable genome. We set out to define a minimal cellular genome experimentally by designing and building one, then testing it for viability. Our goal is a cell so simple that we can determine the molecular and biological function of every gene.

RESULTS: Whole-genome design and synthesis were used to minimize the 1079–kilobase pair (kbp) synthetic genome of *M. mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology in combination with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three more cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kbp, 473 genes). Its genome is smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 has a doubling time of ~180 min, produces colonies that are morphologically similar to those of JCVI-syn1.0, and appears to be polymorphic when examined microscopically.

CONCLUSION: The minimal cell concept appears simple at first glance but becomes more complex upon close inspection. In addition to essential and nonessential genes, there are many quasi-essential genes, which are not absolutely critical for viability but are nevertheless required for robust growth. Consequently, during the process of genome minimization, there is a trade-off between genome size and growth rate. JCVI-syn3.0 is a working approximation of a minimal cellular genome, a compromise between small genome size and a workable growth rate for an experimental organism. It retains almost all the genes that are involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions, suggesting the presence of undiscovered functions that are essential for life. JCVI-syn3.0 is a versatile plat-

ON OUR WEB SITE
Read the full article at http://dx.doi.org/10.1126/science.aad6253



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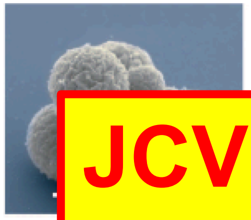


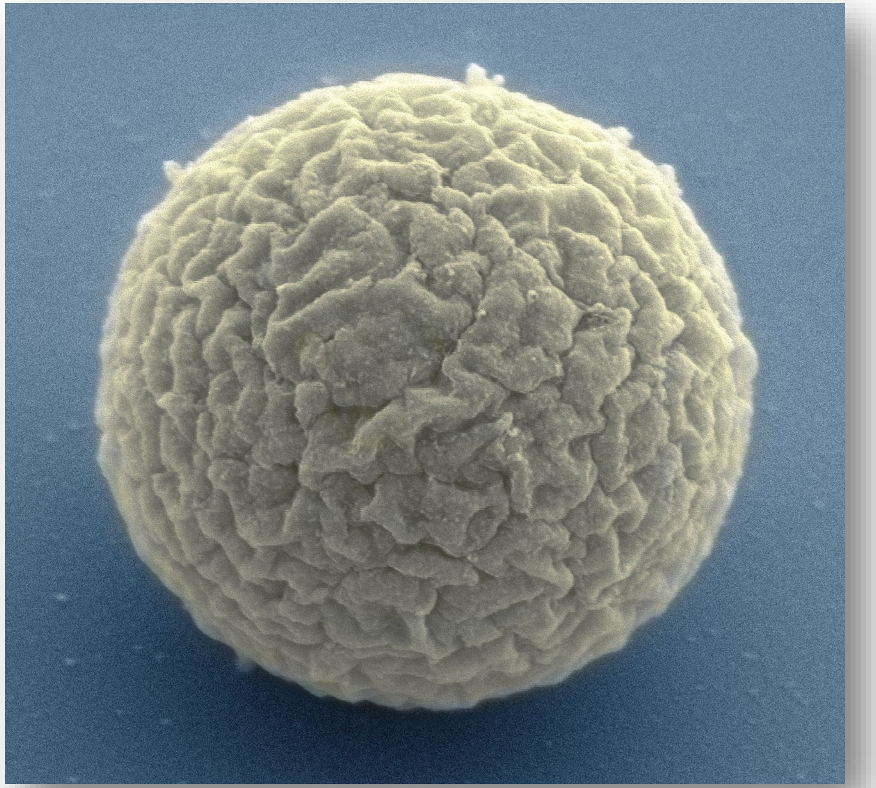
Four design-build-test cycles produced JCVI-syn3.0. (**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (**B**) Comparison of JCVI-syn1.0 (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).

JCVI -syn1.0
2010

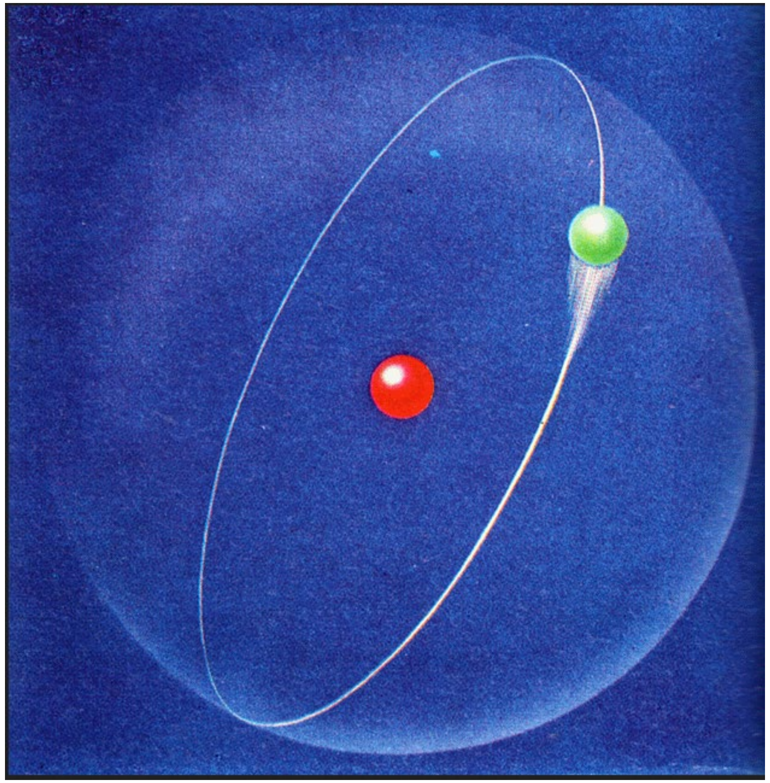
JCVI –syn3.0
2016

JCVI

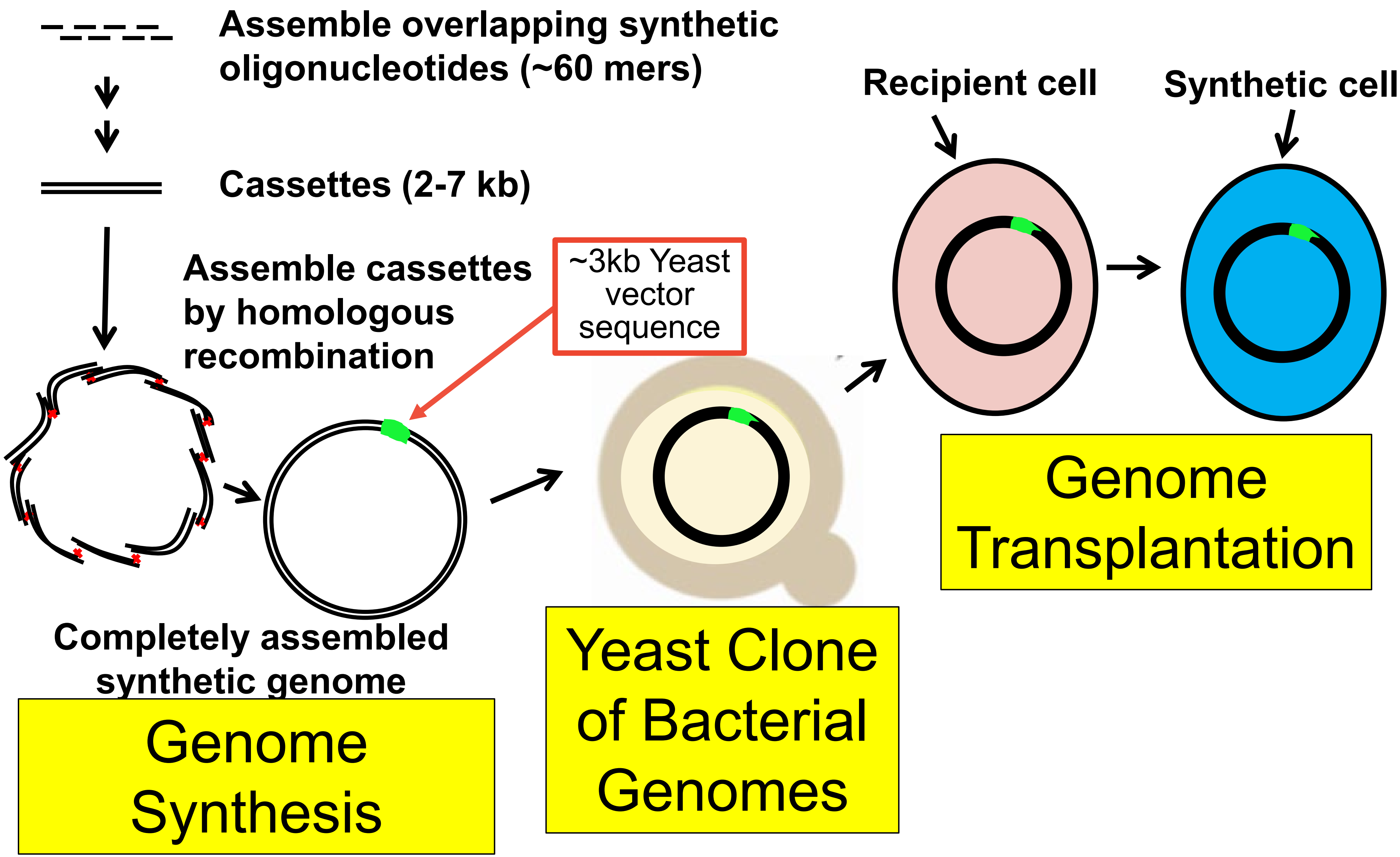
A minimal cell would be the hydrogen atom of biology



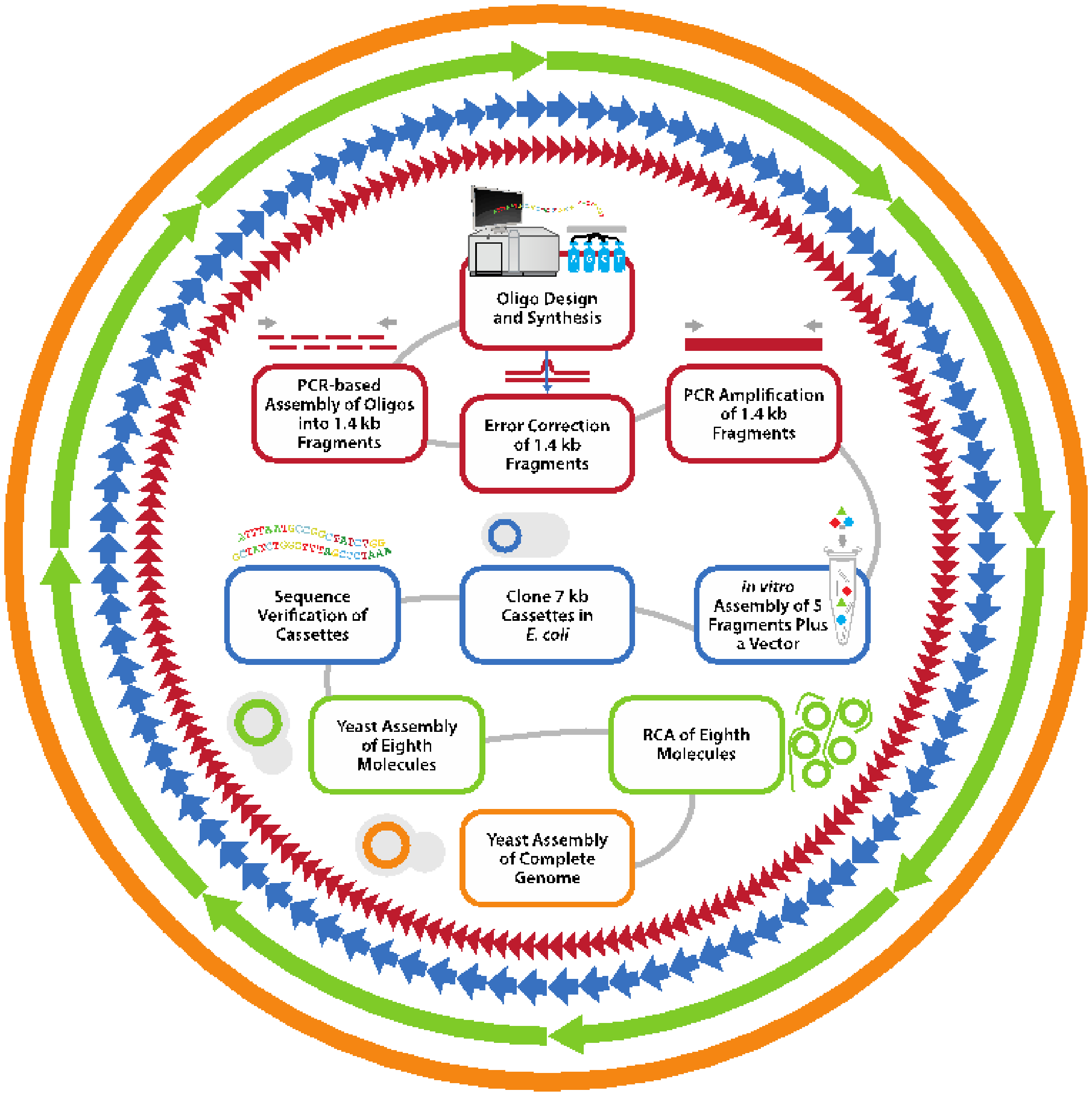
Minimal bacterial cell JCVI-syn3.0

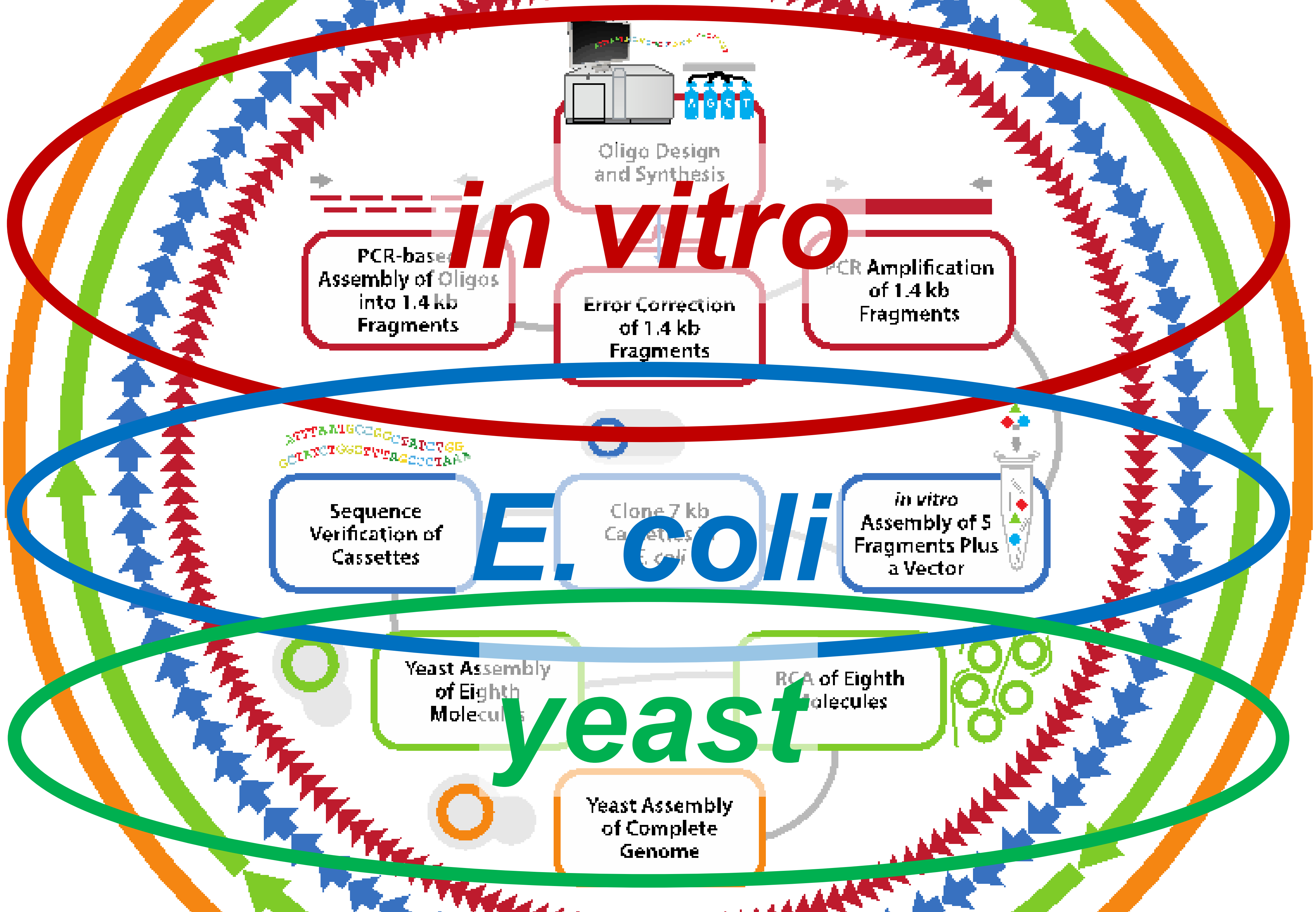


Approach Used to Build a Synthetic Bacterial Cell

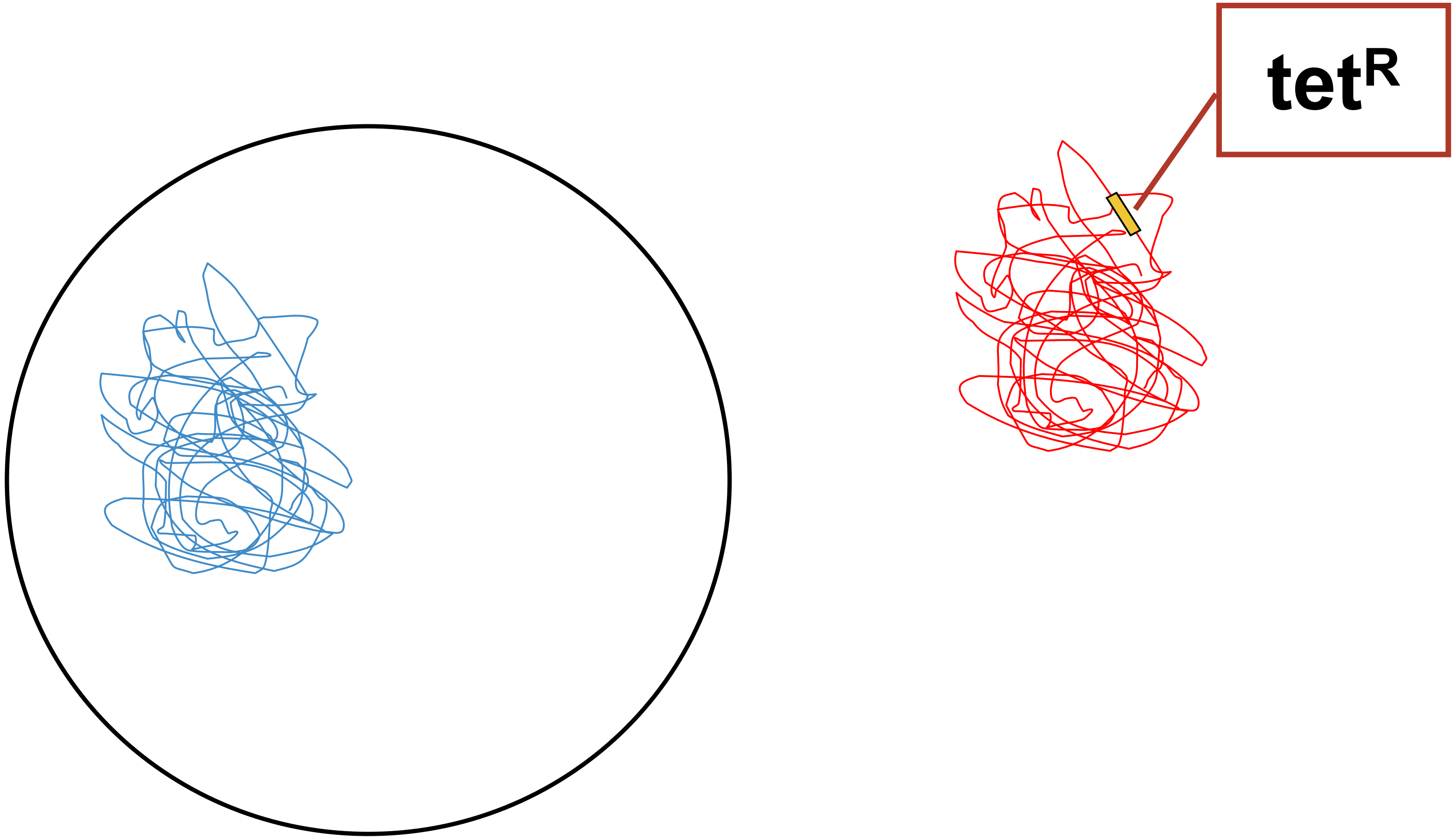


Strategy for whole genome synthesis




in vitro
E. coli
yeast
Oligo Design and Synthesis
PCR-based Assembly of Oligos into 1.4 kb Fragments
Error Correction of 1.4 kb Fragments
PCR Amplification of 1.4 kb Fragments
Sequence Verification of Cassettes
in vitro Assembly of 5 Fragments Plus a Vector
Yeast Assembly of Eighth Molecules
RCA of Eighth Molecules
Yeast Assembly of Complete Genome

Booting up a synthetic genome using whole genome transplantation



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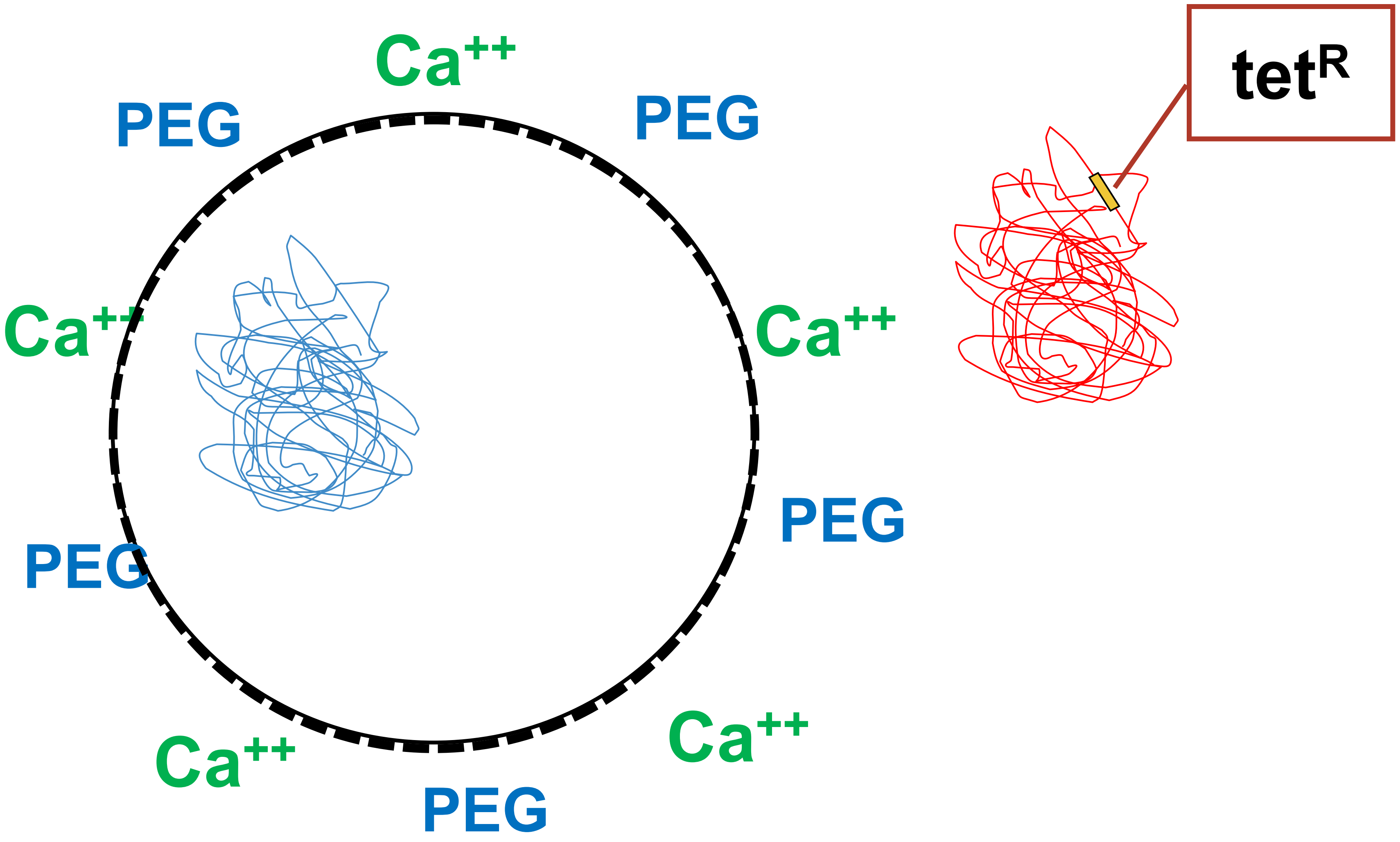
Our naïve starting model for transplantation of tet^R donor genomes into recipient cells



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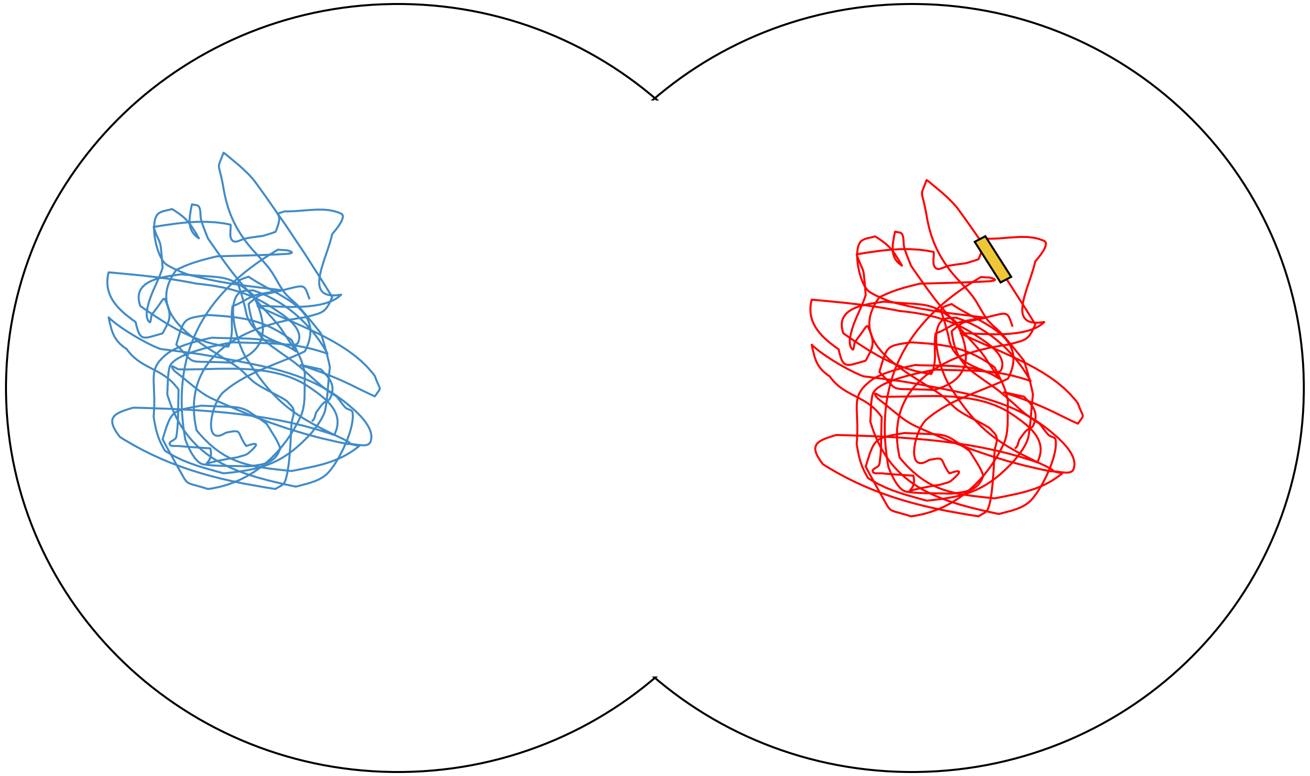
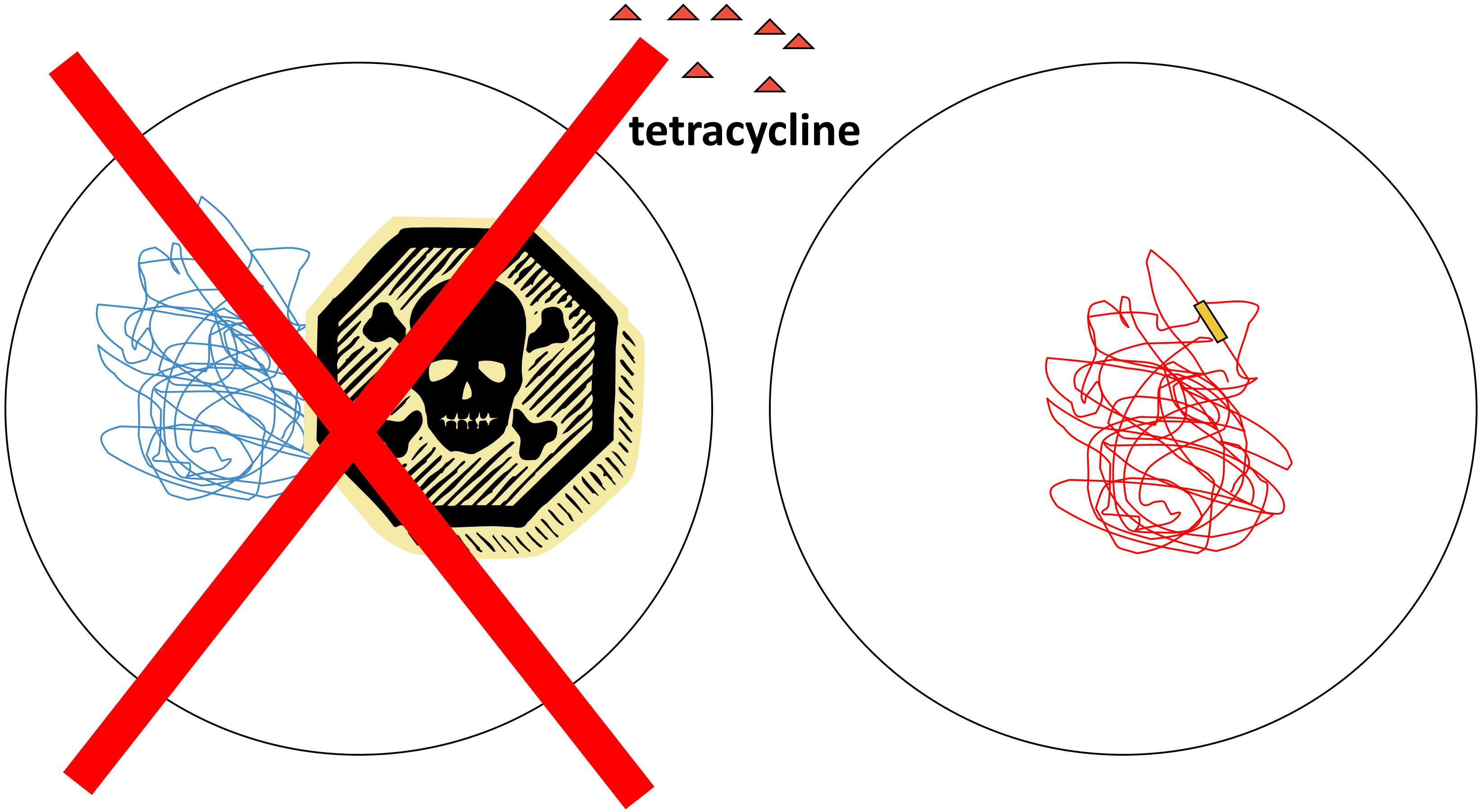
Cell growth and division leads to daughter cells with different genomes



Transplanted genome has a tetracycline resistance gene.

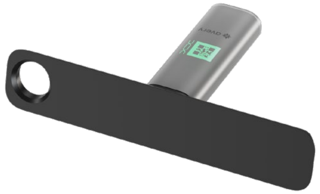
Only cells with that marker grow in the presence of tetracycline.



We are still at the dawn of making living “synthetic cells”

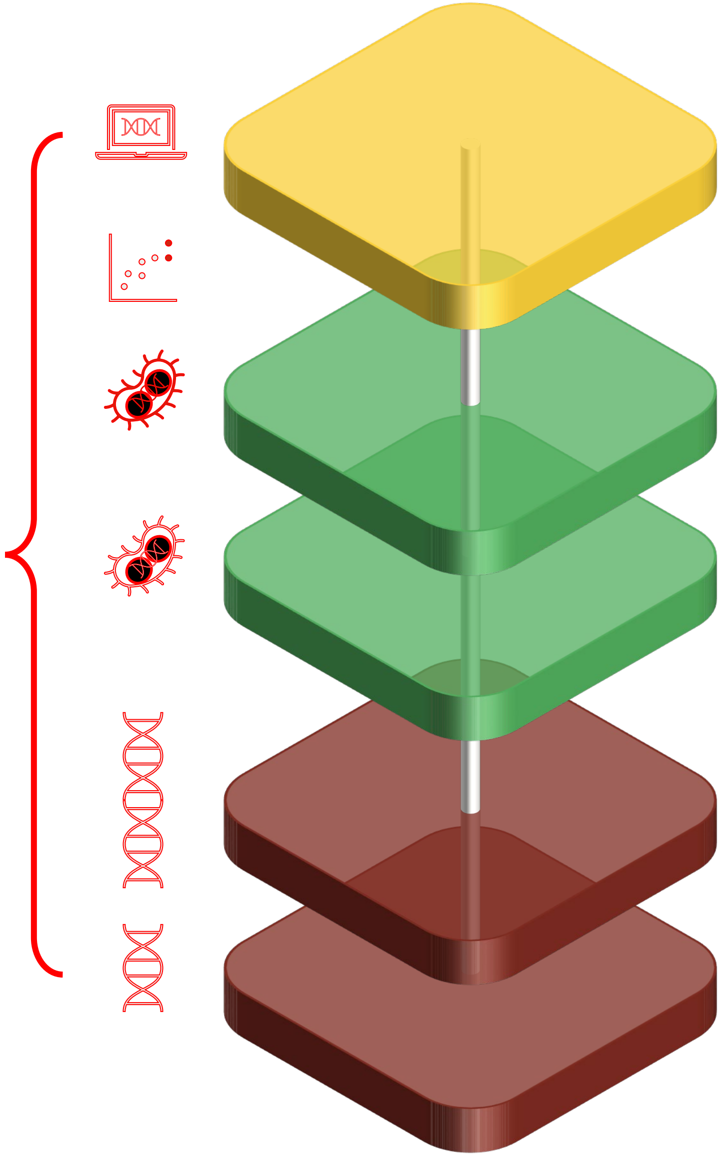
- Genome synthesis is solved: we can synthesize any bacterial genome needed as a yeast artificial chromosome
  - Genome synthesis may become fast and cheap

Avery Digital is scaling biological experimentation on nanofluidic chips



Bio Processing Unit

Fully Integrated Chipset,
Full Data Traceability
& Observability



AI-Assisted Design
Data Collected from Every Variant Tested

Functional Testing
Optical Screening of Fluorescence Assays

Cell & Cell-free Packaging
Multiplexed DNA Payload Packaging into Expression Systems

Kilobase to Megabase DNA Assembly
Millions of Kilobase to Thousands of Megabase Variants

DNA Synthesis
Hundreds of Millions of Oligonucleotide Variants

We are still at the dawn of making living "synthetic cells"

- Genome synthesis is solved: we can synthesize any bacterial genome needed as a yeast artificial chromosome
  - Genome synthesis may become fast and cheap
- Booting up the genomes is much harder
  - Whole genome transplantation only works for mycoplasmas
- We still do not know much about how cells function
- One cellular chassis for all synthetic cell needs and universal interchangeable parts are unlikely

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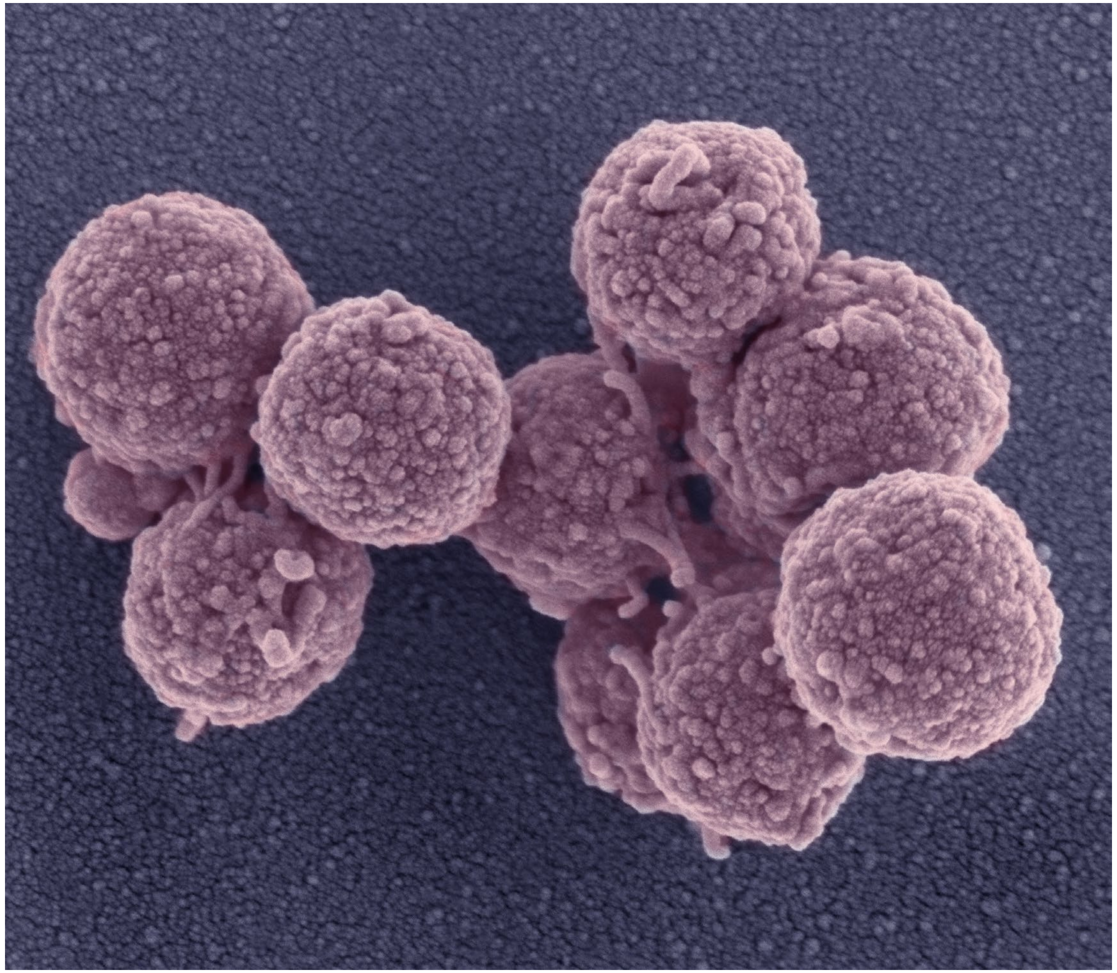
PIONEERING GENOMICS TO POSITIVELY IMPACT LIFE

In 2010 the scientific community called JCVI-syn1.0 a "synthetic cell"



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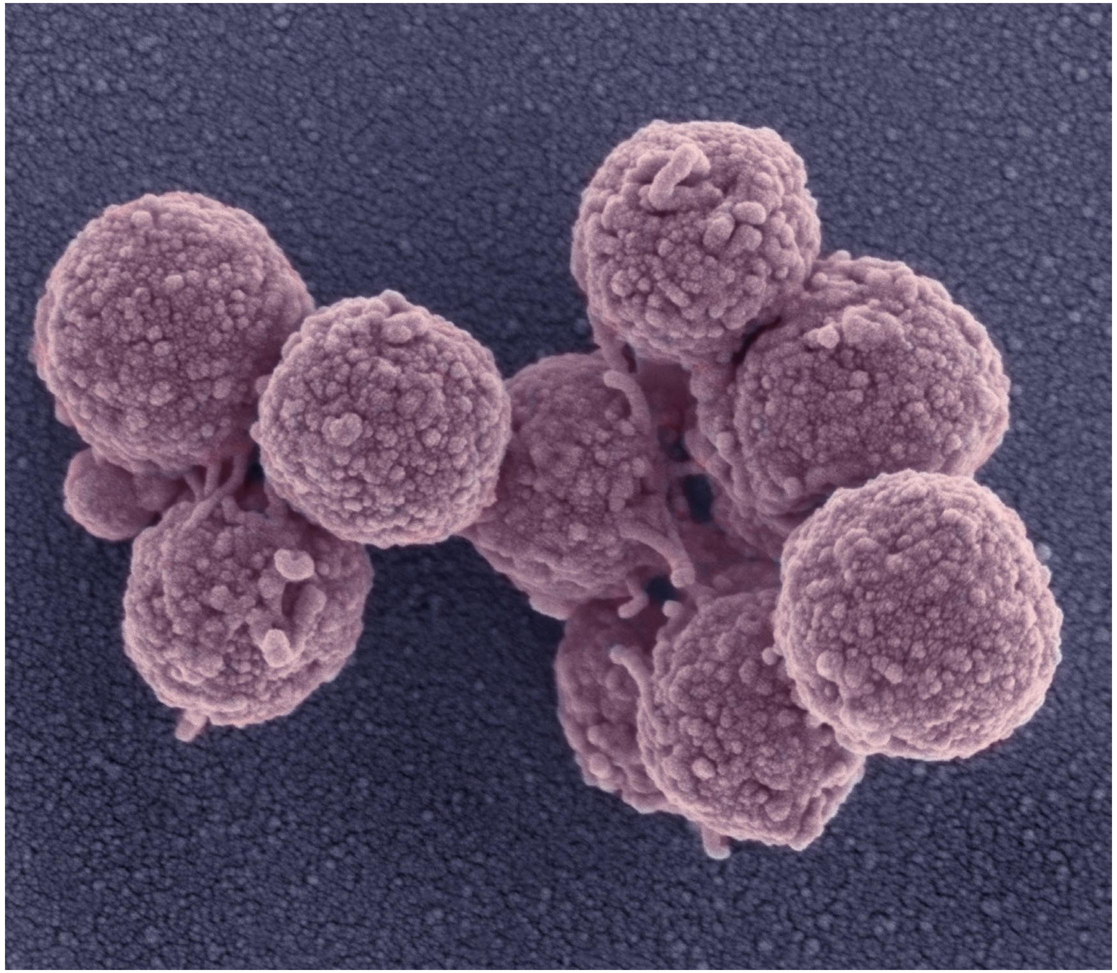
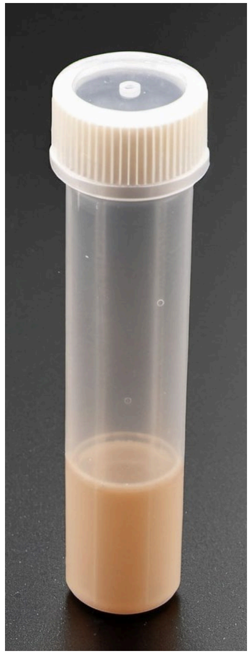
Today, it is not a synthetic cell, but rather a cell with a synthetic genone

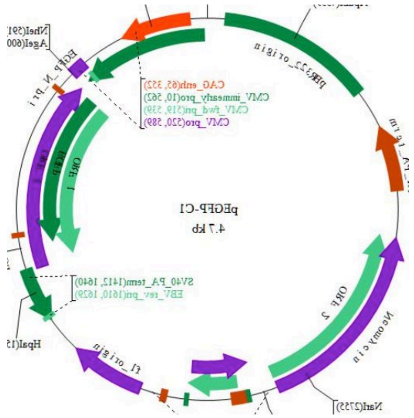


Building a living synthetic cell from non-living parts

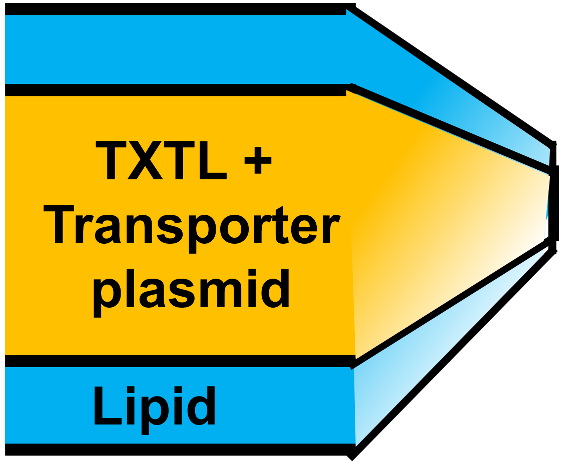
Mycoplasma culture



cytoplasmic extract TXTL system



multi-transporter plasmid



0.5-2.0 µm vesicles

nozzle for extrusion of mycoplasma-like TXTL extract filled vesicles

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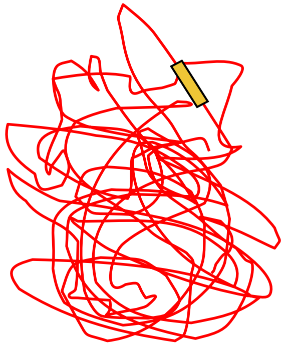
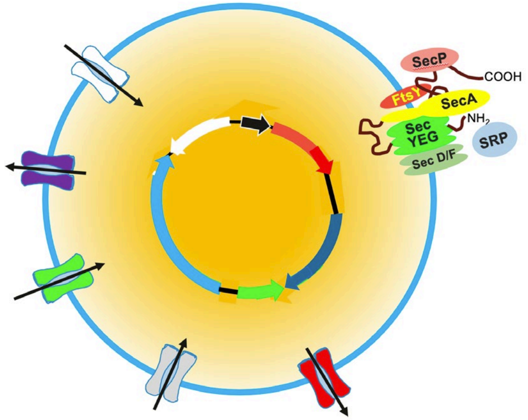
Building a living synthetic cell from non-living parts





Genome Transplantation to install genome

Synthetic cells as intracellular bacterial endosymbionts of eukaryotes

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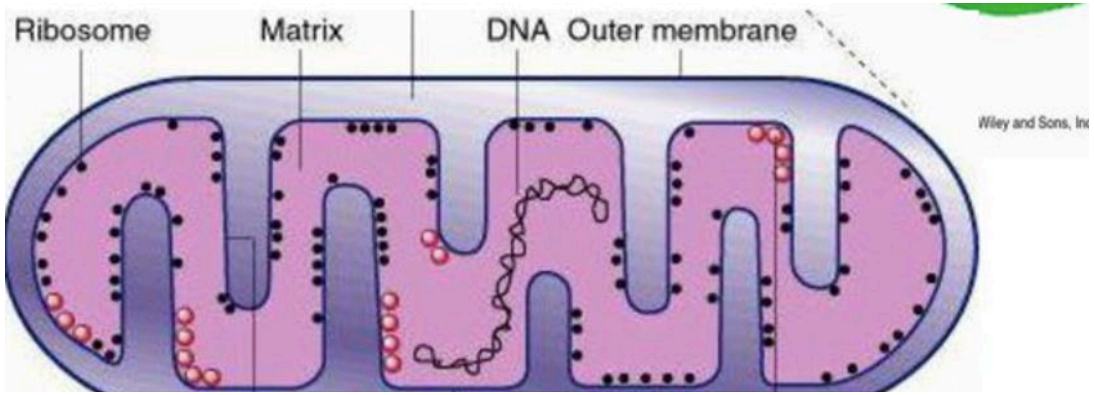
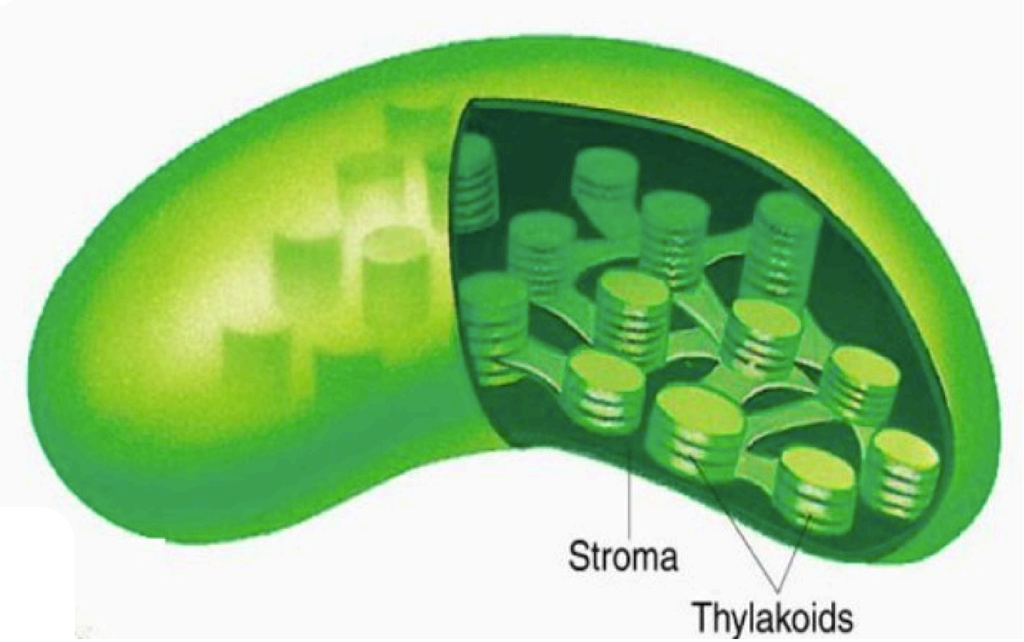
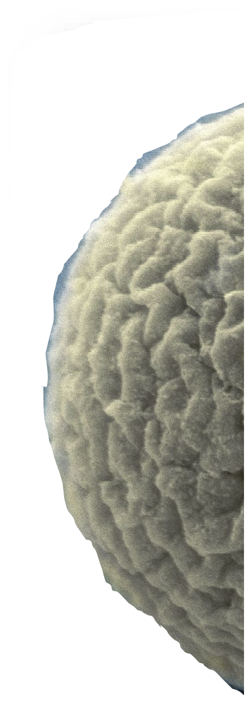
PIONEERING GENOMICS TO POSITIVELY IMPACT LIFE

Mitochondria & Chloroplasts evolved from intracellular bacteria

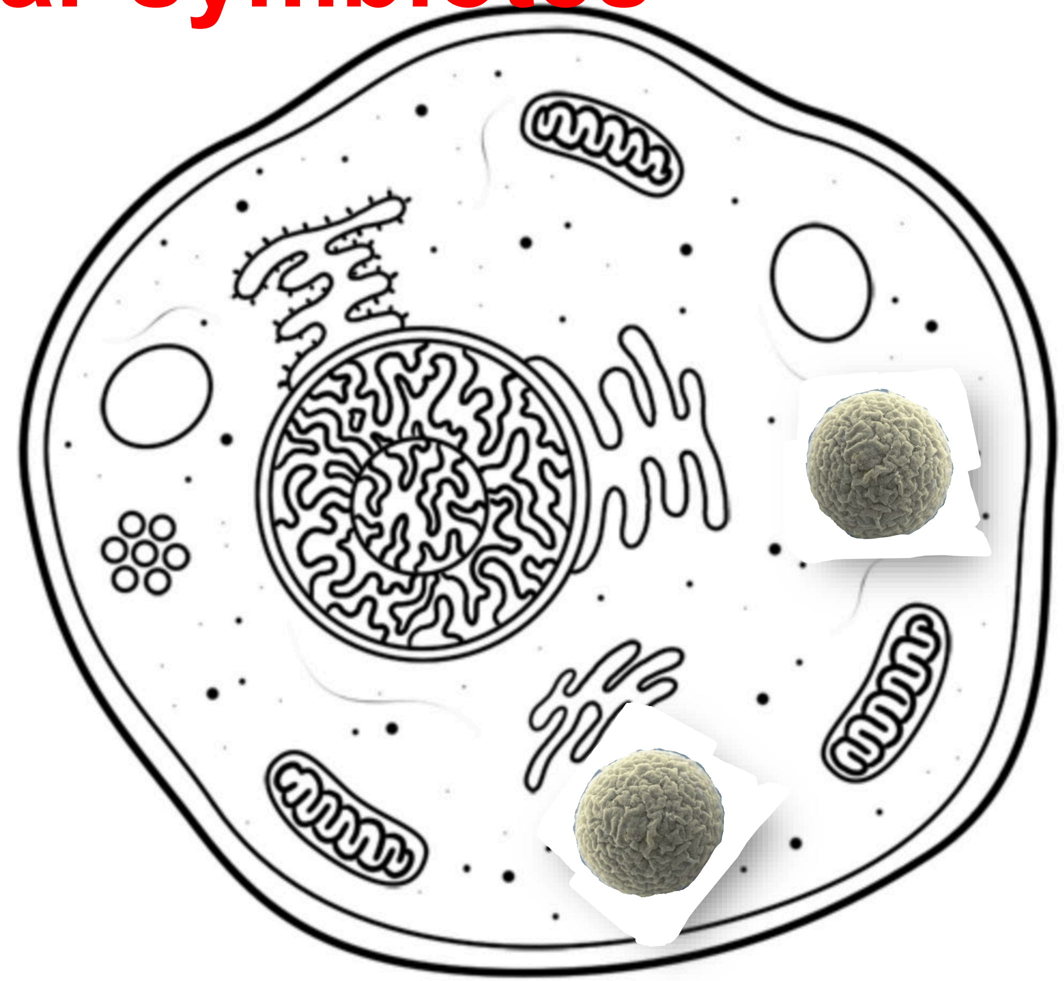




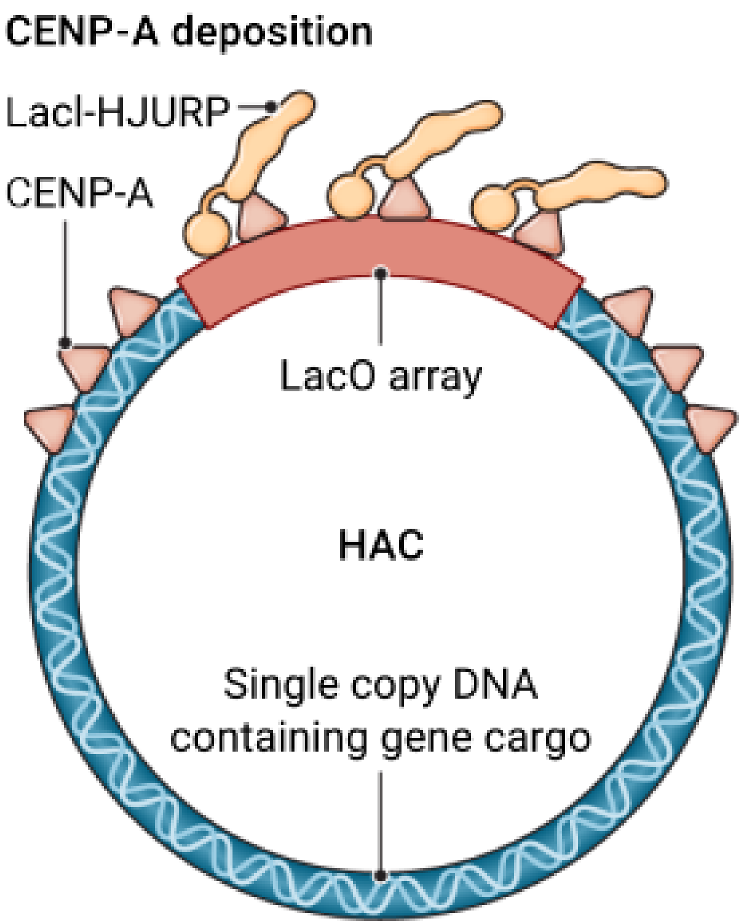
JCVI cells with synthetic genomes are being engineered to function as intracellular symbiotes

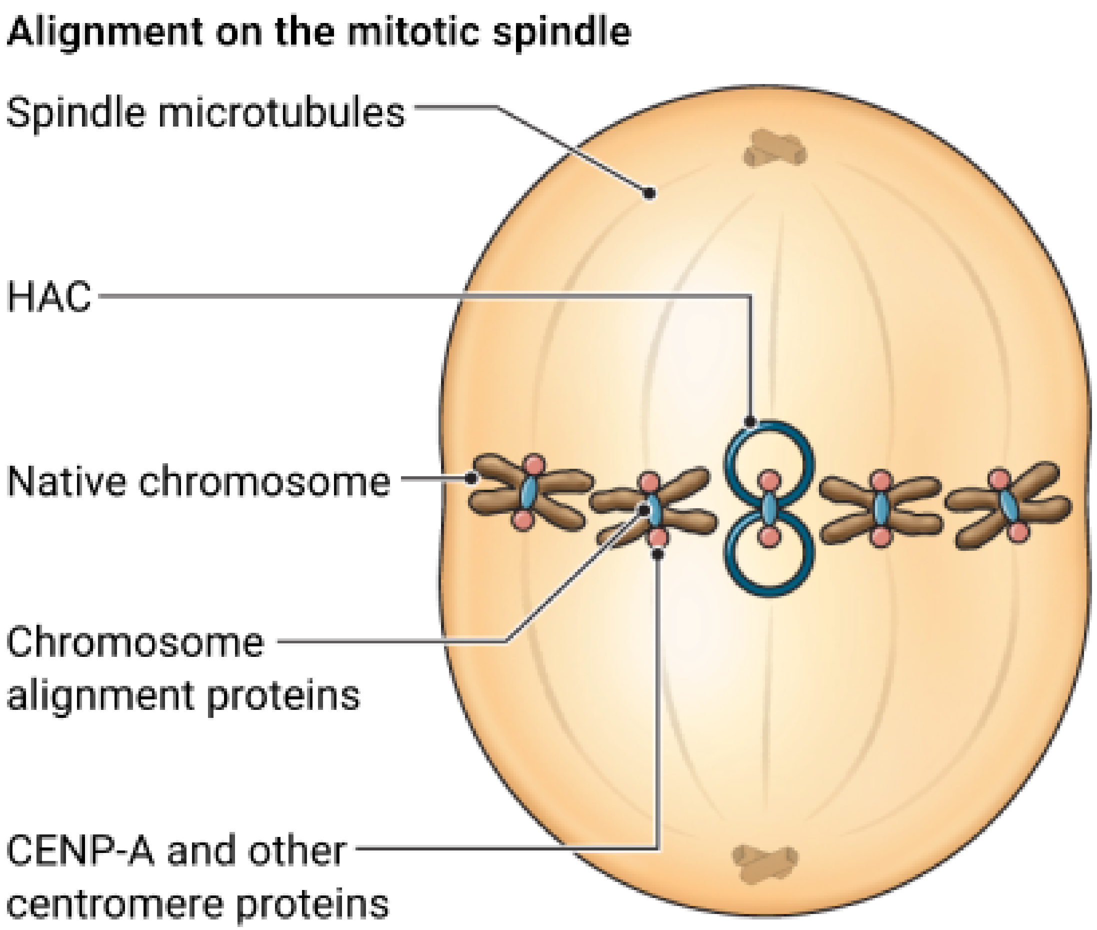


Mycoplasma cells are not pathogenic & might be engineered to solve metabolic problems



Human Artificial Chromosomes (HACs)





Science | AAAS

Efficient formation of single-copy human artificial chromosomes, *Science*, 03/21/24
Engineering better artificial chromosomes, *Science*, 03/21/24

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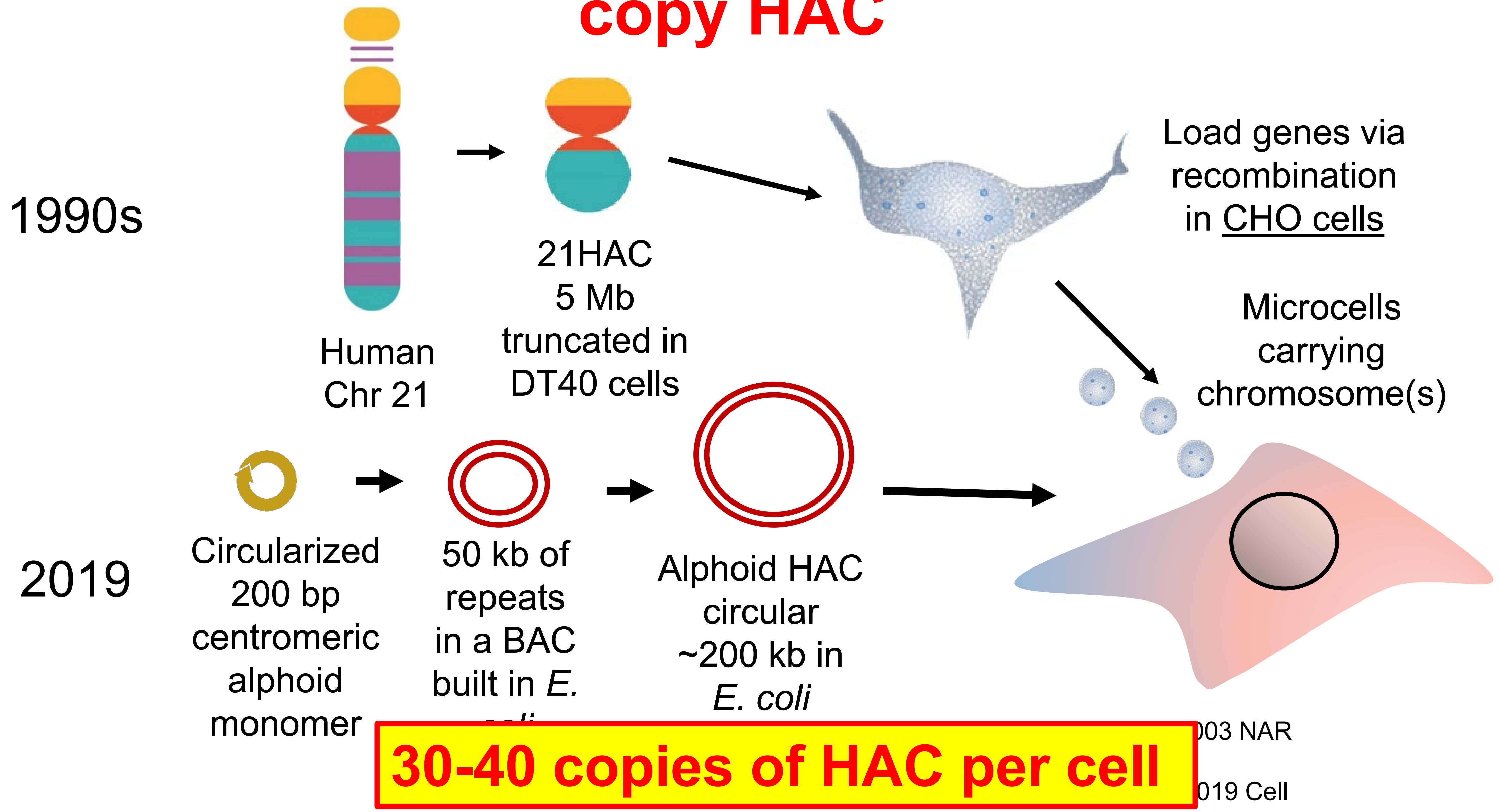
Multi-million bp HACs may let us…

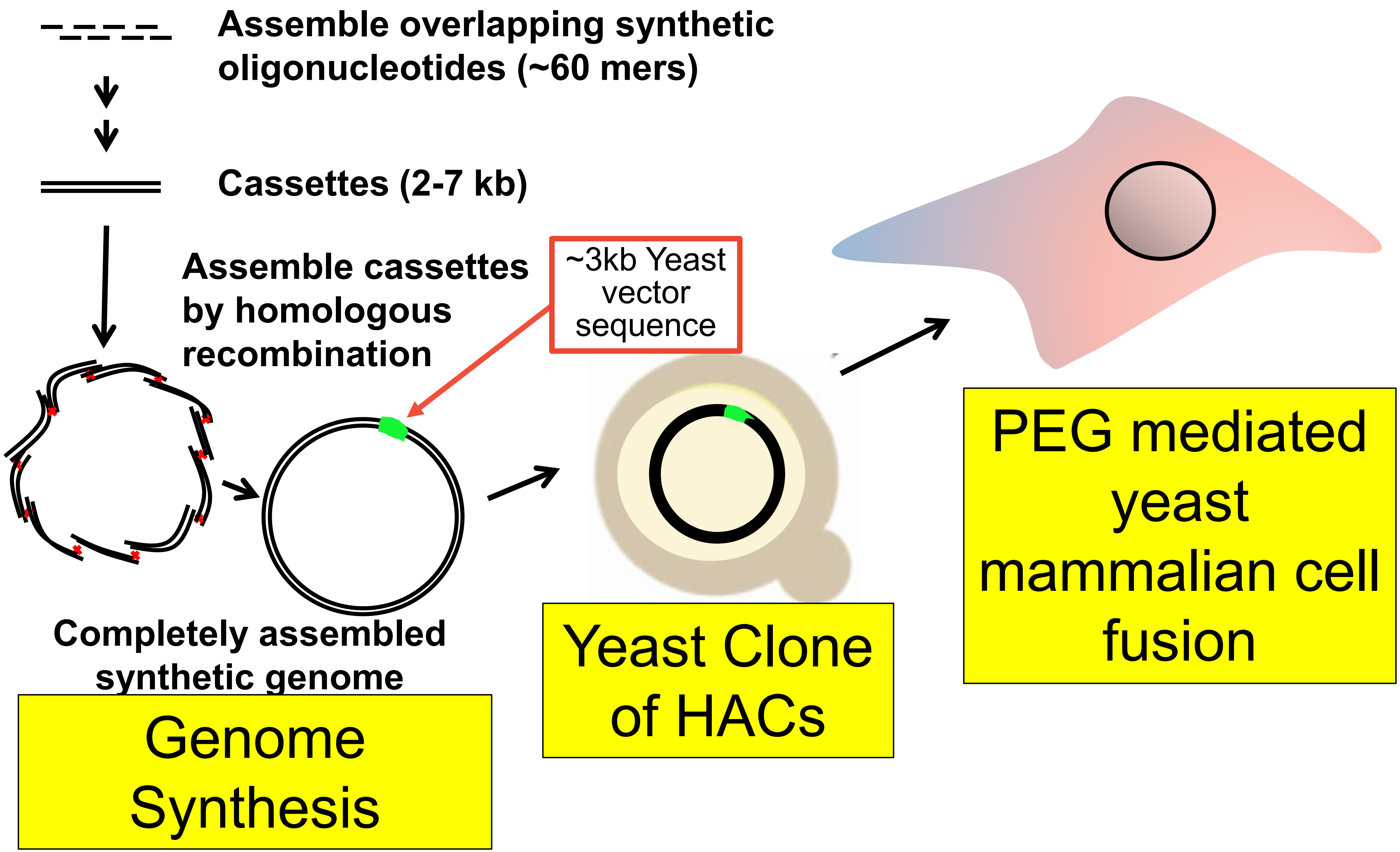
- Anti-cancer therapies better than current CAR-T approaches
- Gene therapy using whole genes (introns & exons) to reduce gene silencing characteristic of some approaches
- Build cells with designer antibody repertoires to protect against multiple infectious diseases
- Modify human cells for therapeutic purposes that need multiple new genes expressed
- Transgenic animals with large sets of human genes for replacement organs
- Plant artificial chromosomes

HAC technology prior to Penn-JCVI single copy HAC

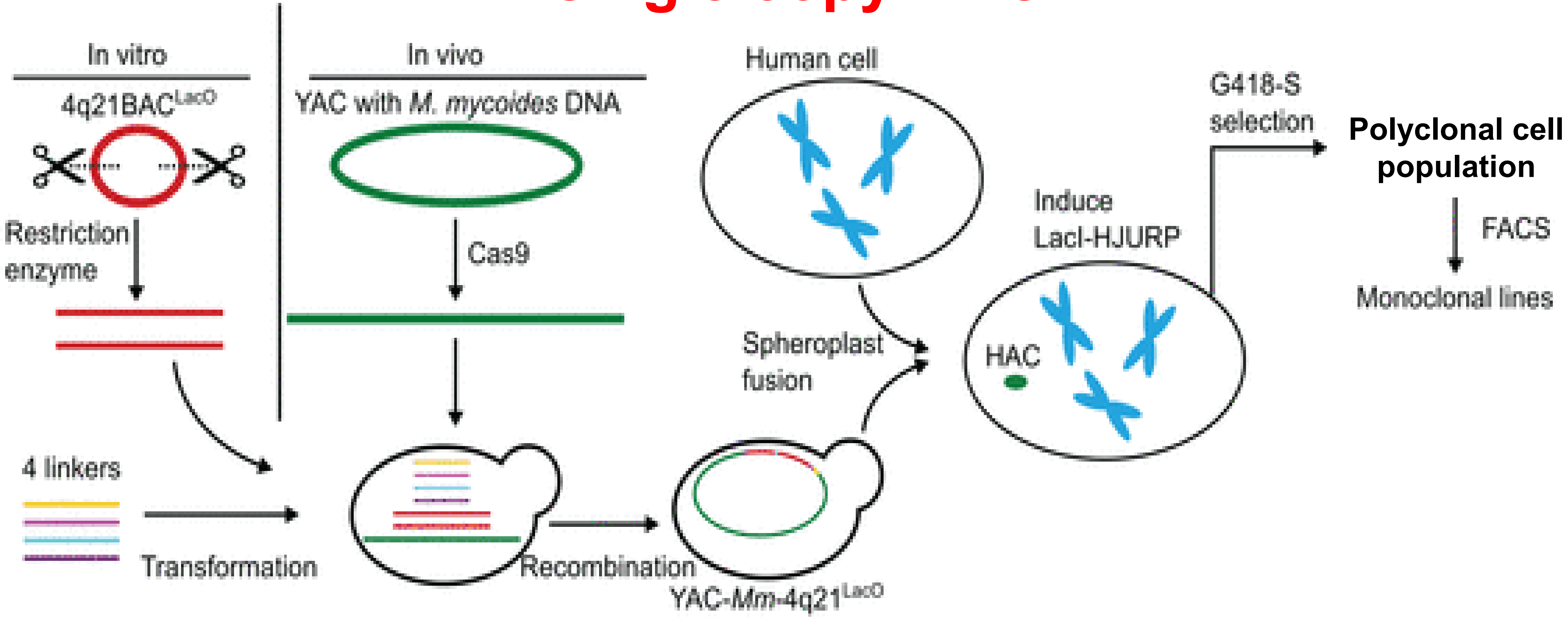


30-40 copies of HAC per cell

Approach Used to Build a HAC



Construction & delivery of Penn-JCVI single copy HAC



Efficient formation of single-copy human artificial chromosomes, *Science,* 3/21/24,


Mirror
Life

Science

December 12, 2024

POLICY FORUM

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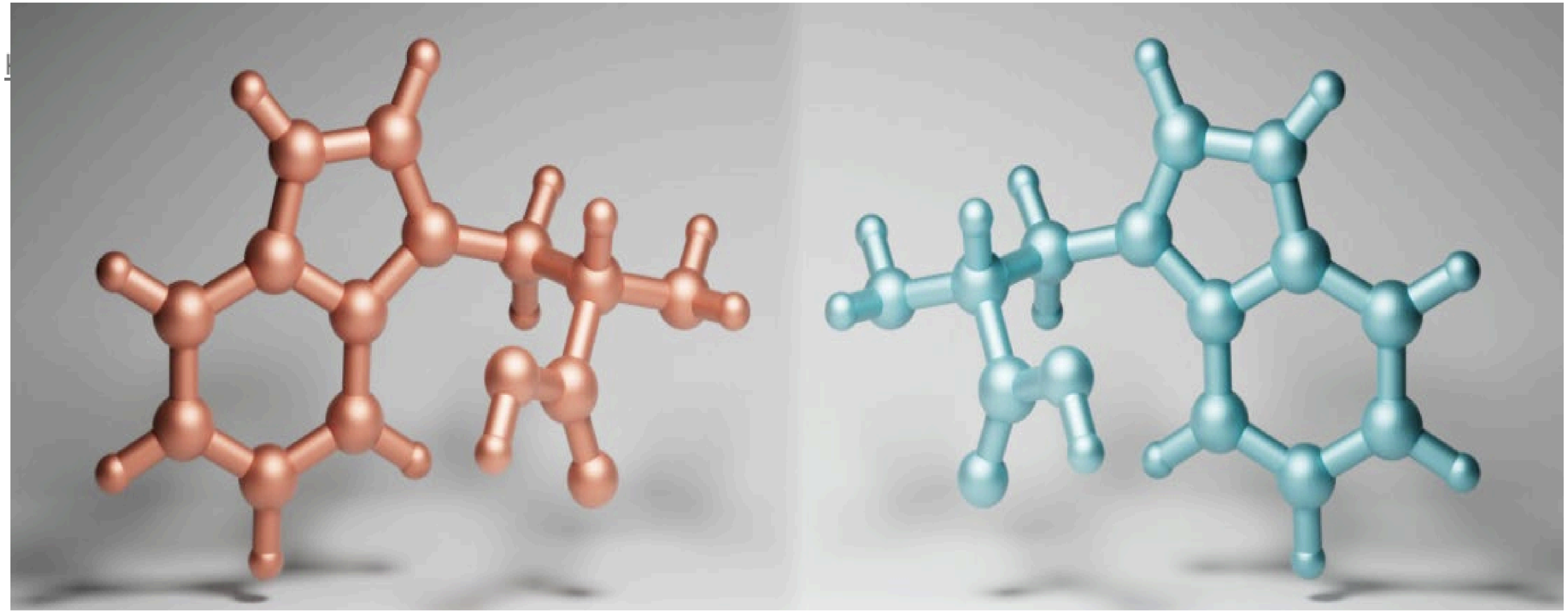
Confronting risks of mirror life

Broad discussion is needed to chart a path forward



A chemical structure model of a naturally occurring amino acid, L-tryptophan (left), is shown with it mirror image (right).

It Takes a Village to Create a Cell

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- Mikkel Algire
- Nina Alperovich
- Nacyra Assad-Garcia
- Gwyn Benders
- Daniella Bittencourt
- David Brown
- Ray-Yuan Chuang
- Andras Cook
- Evgenia Denisova
- Ian Erhlenreich
- Marcelo Freire
- Dan Gibson
- John Glass
- Sage Glass
- Tyler Goshia
- Clyde Hutchison
- Shige Kakizawa
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- Carole Lartigue
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- Vladimir Noskov
- Cindi Pfannkoch
- Ronald Rodriguez
- Zumra Seidel
- Ham Smith
- Lijie Sun
- Yo Suzuki
- Sanjay Vashee
- Craig Venter
- Kim Wise
- Feilun Wu

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- Nataliya Safronova

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- Andreas Mershin
- Neil Gershenfeld

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- Jiwoong Kwon
- Pam Silver
- Jeff Way

Penn

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- Gabe Birchak
- Ben Black

SGI.

- Dan Gibson
- John Gill
- Krishna Kannan
- Billyana Tsvetanova
- Craig Venter

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- Fatema-Zahra Rashid

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