Interagency Workshop on Synthetic Biology
10/16/2019

Unmet basic science needs

John Glass

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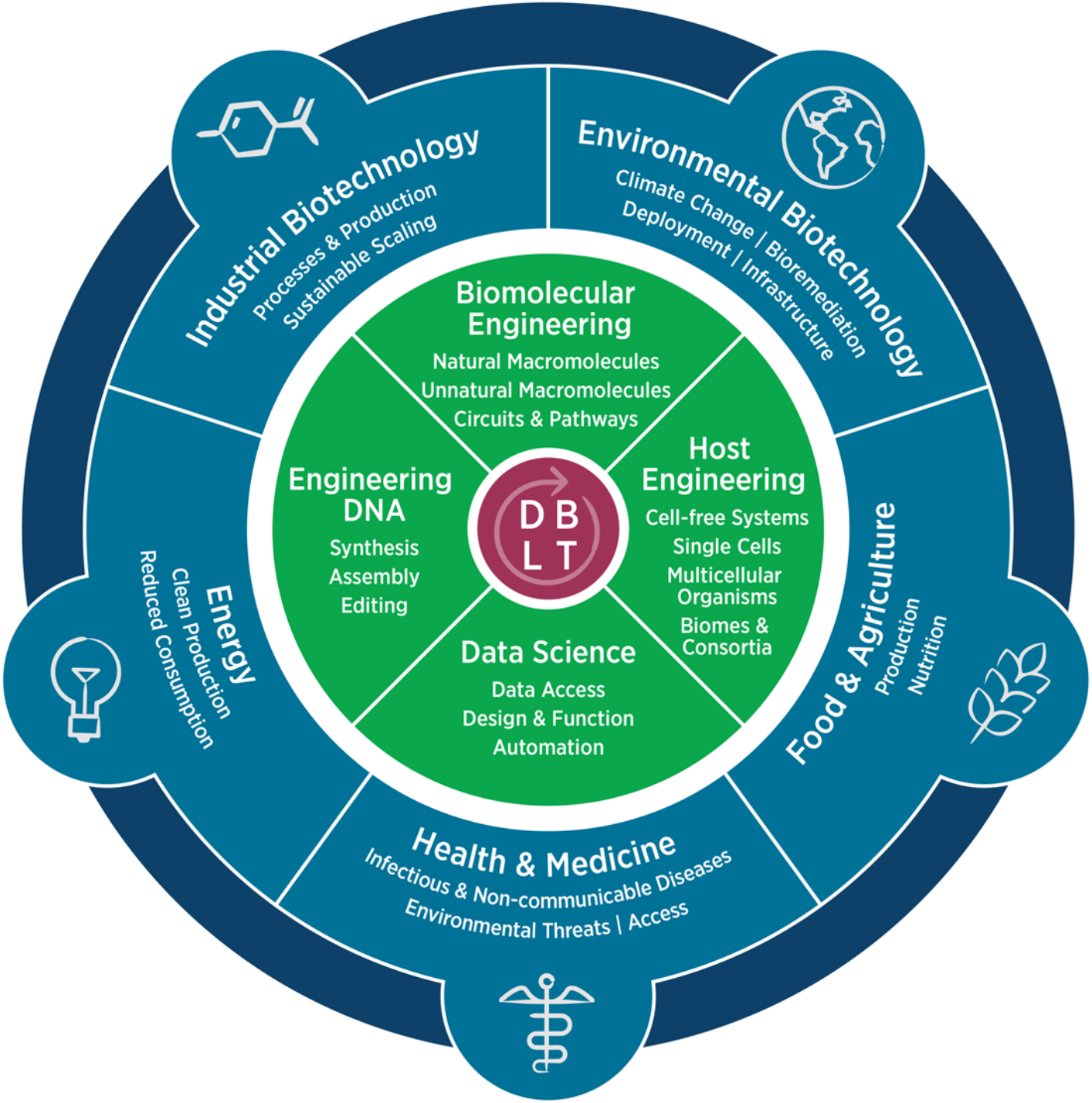
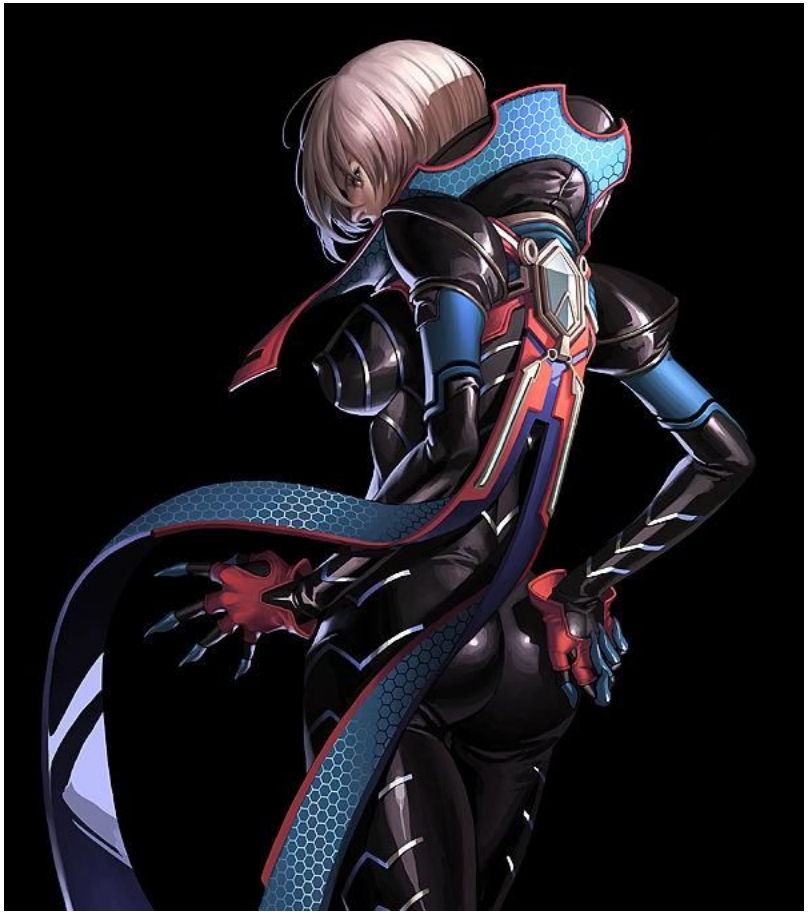
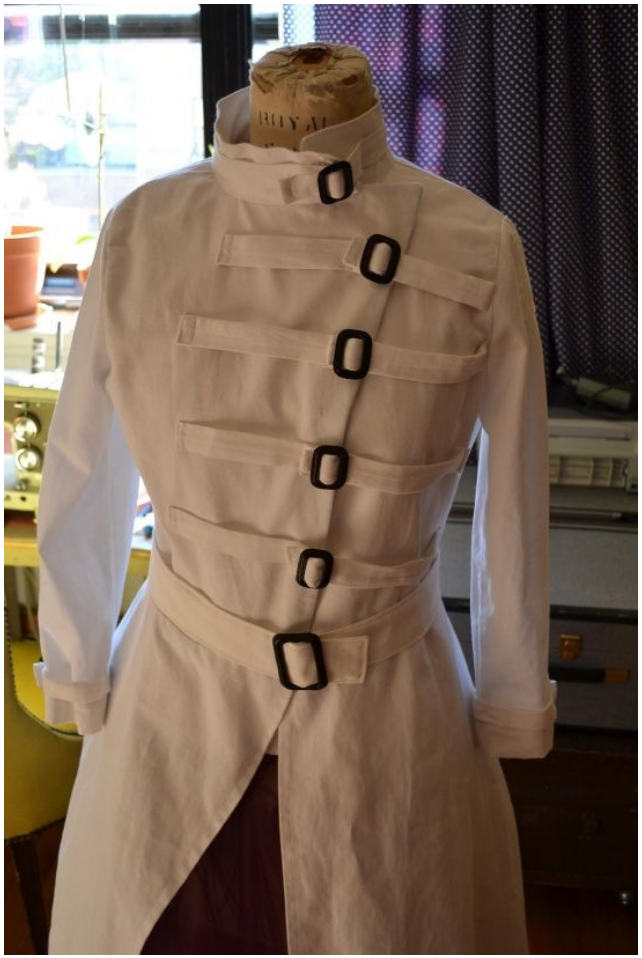
Rockville, MD and San Diego, CA,USA


Industrial Biotechnology
Processes & Production
Sustainable Scaling
Environmental Biotechnology
Climate Change | Bioremediation
Deployment | Infrastructure
Biomolecular Engineering
Natural Macromolecules
Unnatural Macromolecules
Circuits & Pathways
Engineering DNA
Synthesis
Assembly
Editing
D B
L T
Host Engineering
Cell-free Systems
Single Cells
Multicellular Organisms
Biomes & Consortia
Data Science
Data Access
Design & Function
Automation
Energy
Clean Production
Reduced Consumption
Food & Agriculture
Production
Nutrition
Health & Medicine
Infectious & Non-communicable Diseases
Environmental Threats | Access

Uniforms

Uniforms





Uniforms

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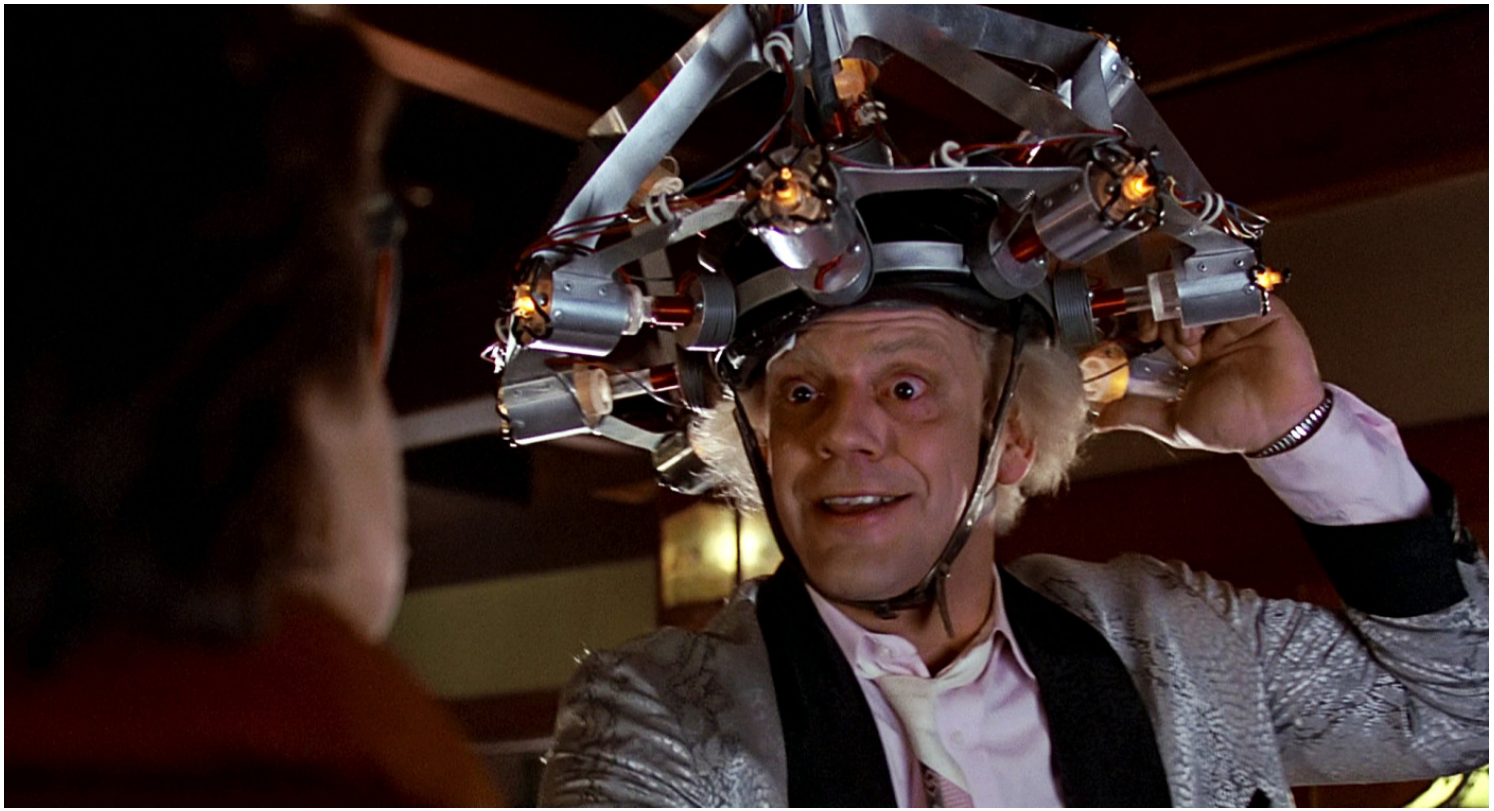
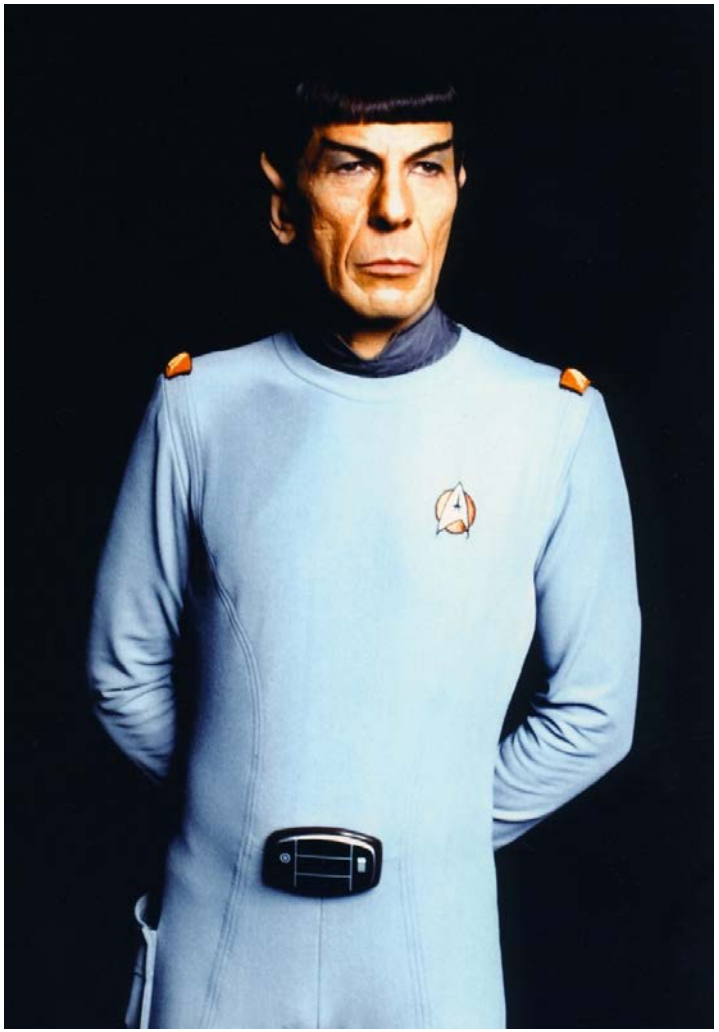
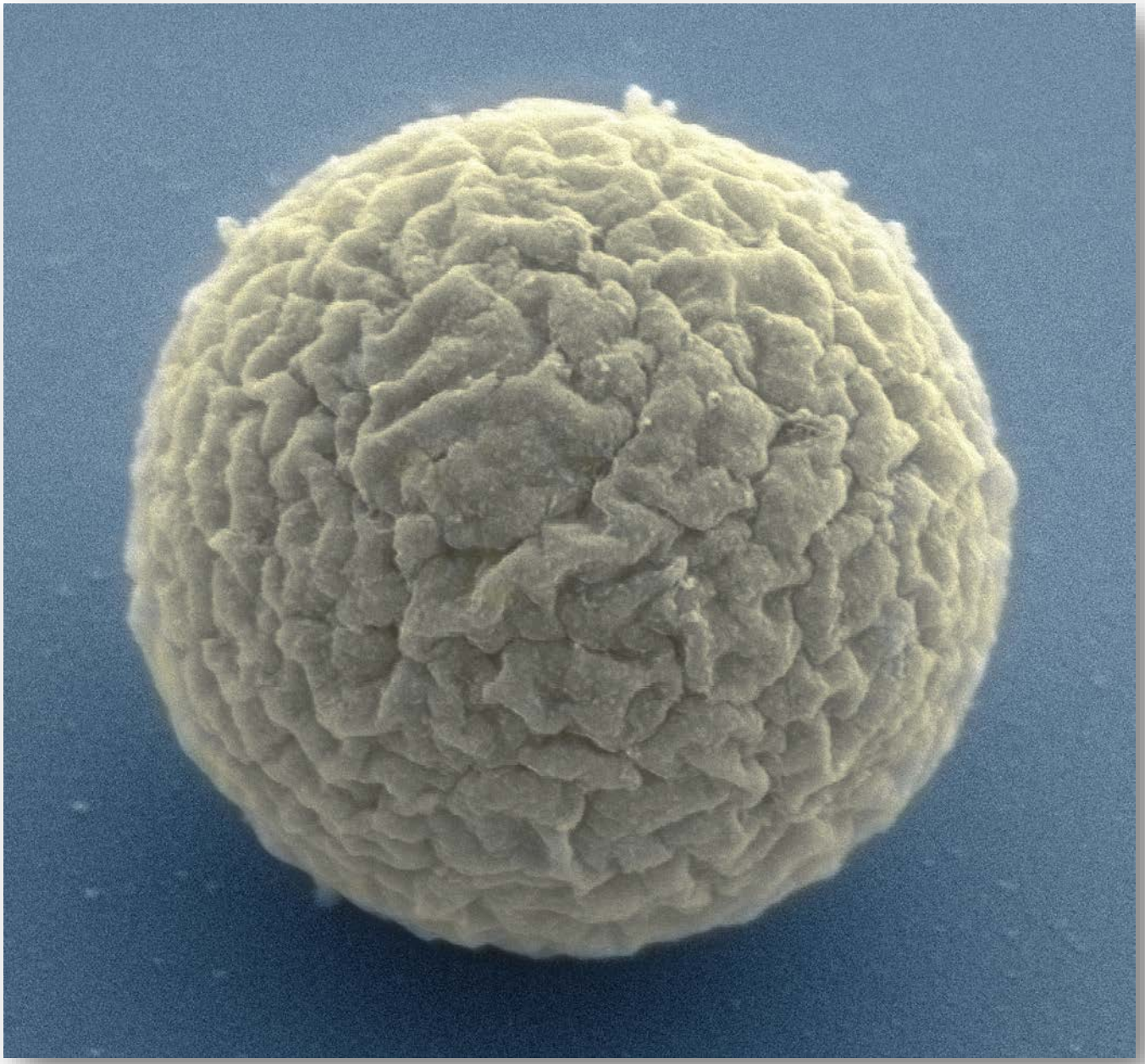


Uniforms





Scanning EM by Tom Deerinck UCSD NCMIR

Engineering Biology Research Consortium – Engineering Biology Roadmap 2019

GENE EDITING, SYNTHESIS, AND ASSEMBLY

- Manufacture thousands of very long oligonucleotides with high fidelity.
- Many-fragment DNA assembly with simultaneous, high-fidelity sequence validation.
- Precision genome editing at multiple sites simultaneously with no off-target effects.

BIOMOLECULE, PATHWAY, AND CIRCUIT ENGINEERING

- Holistic, integrated design of multi-part genetic systems (i.e., circuits and pathways).
- Integrated design of RNA-based regulatory systems for cellular control and information processing.

Engineering Biology Research Consortium – Engineering Biology Roadmap 2019

DATA INTEGRATION, MODELING, AND AUTOMATION

- Establish a computational infrastructure where easy access to data supports the DBTL process for biology.
- Establish functional prediction through biological engineering design at the biomolecular, cellular, and consortium scale.
- Establish optimal manufacturing processes from the unit-operation to the integrated-screening scale.

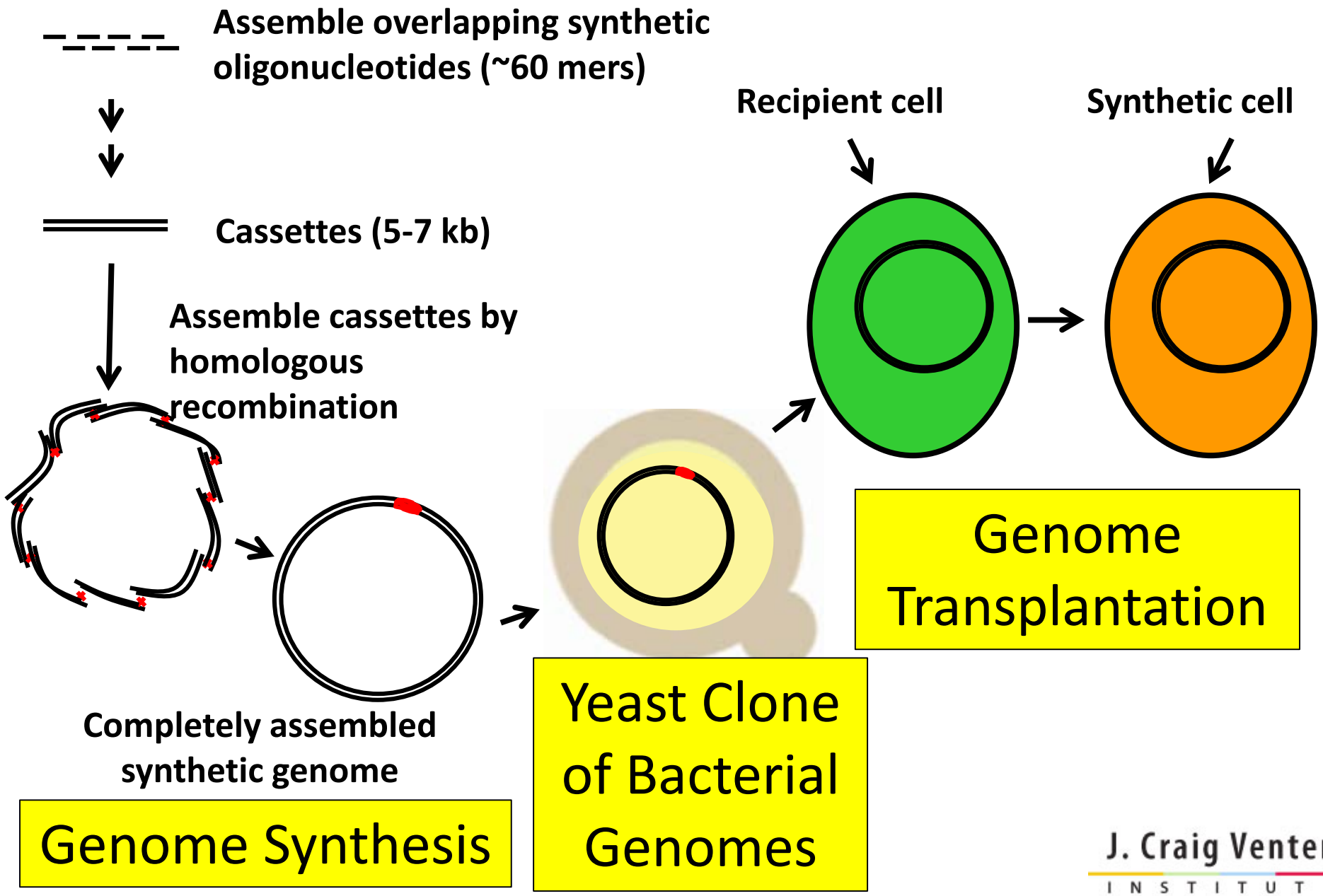
Engineering Biology Research Consortium – Engineering Biology Roadmap 2019

HOST AND CONSORTIA ENGINEERING

- Cell-free systems capable of natural and/or non-natural reactions.
- On-demand production of single-cell hosts capable of natural and non-natural biochemistry
- On-demand fabrication and modification of multicellular organisms
- Generations of biomes and consortia with desired functions and ecologies

This is the area that will be the most difficult

JCVI approach used to build a Synthetic Bacterial Cell



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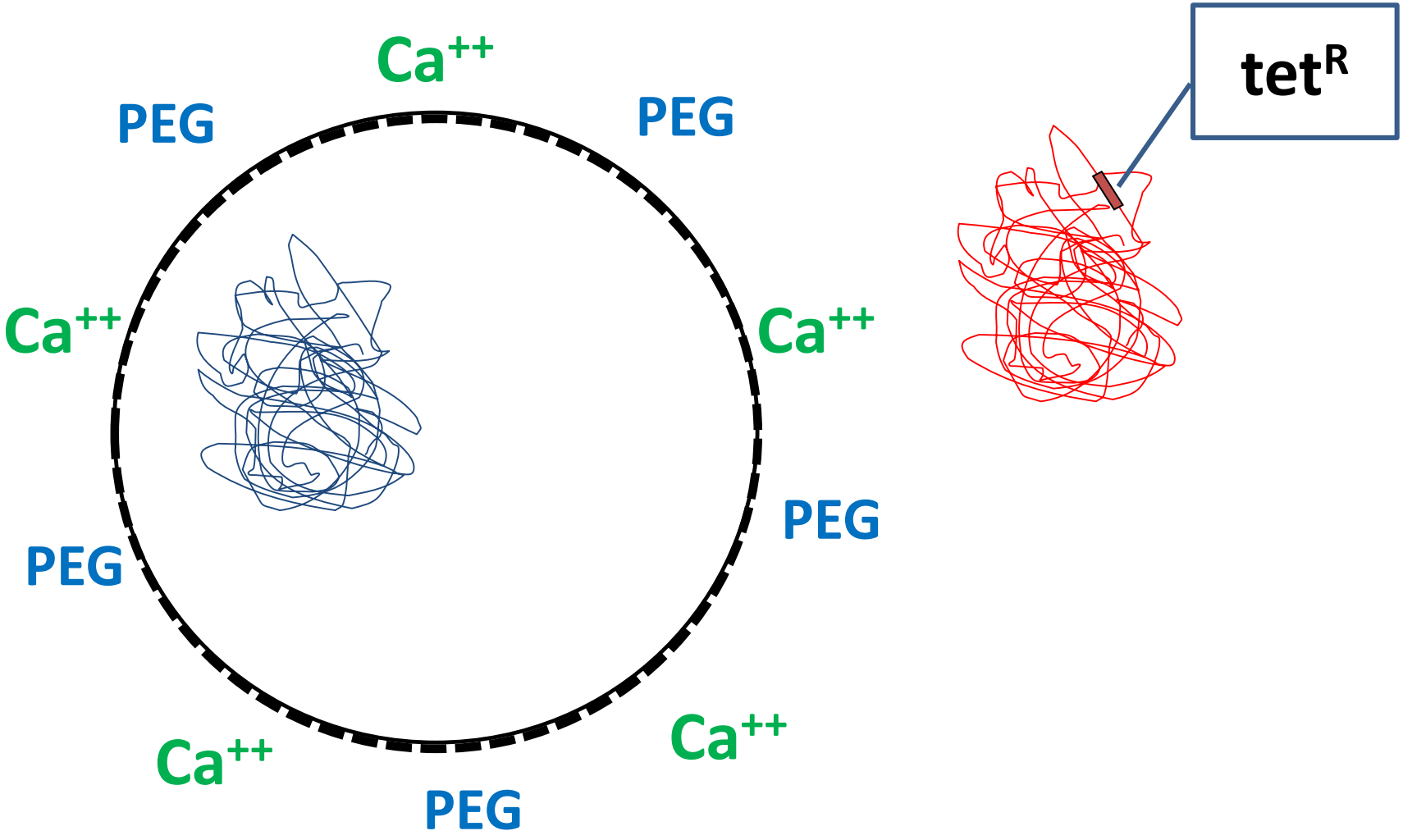
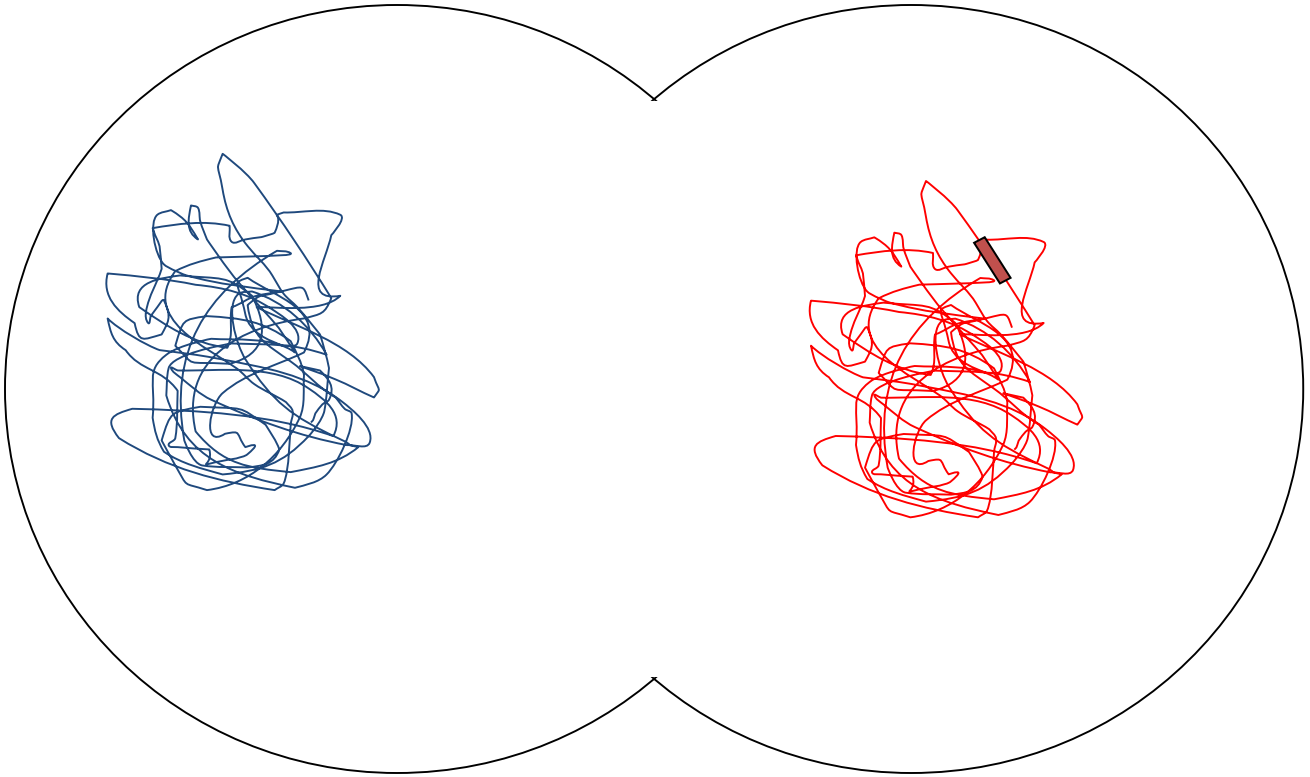
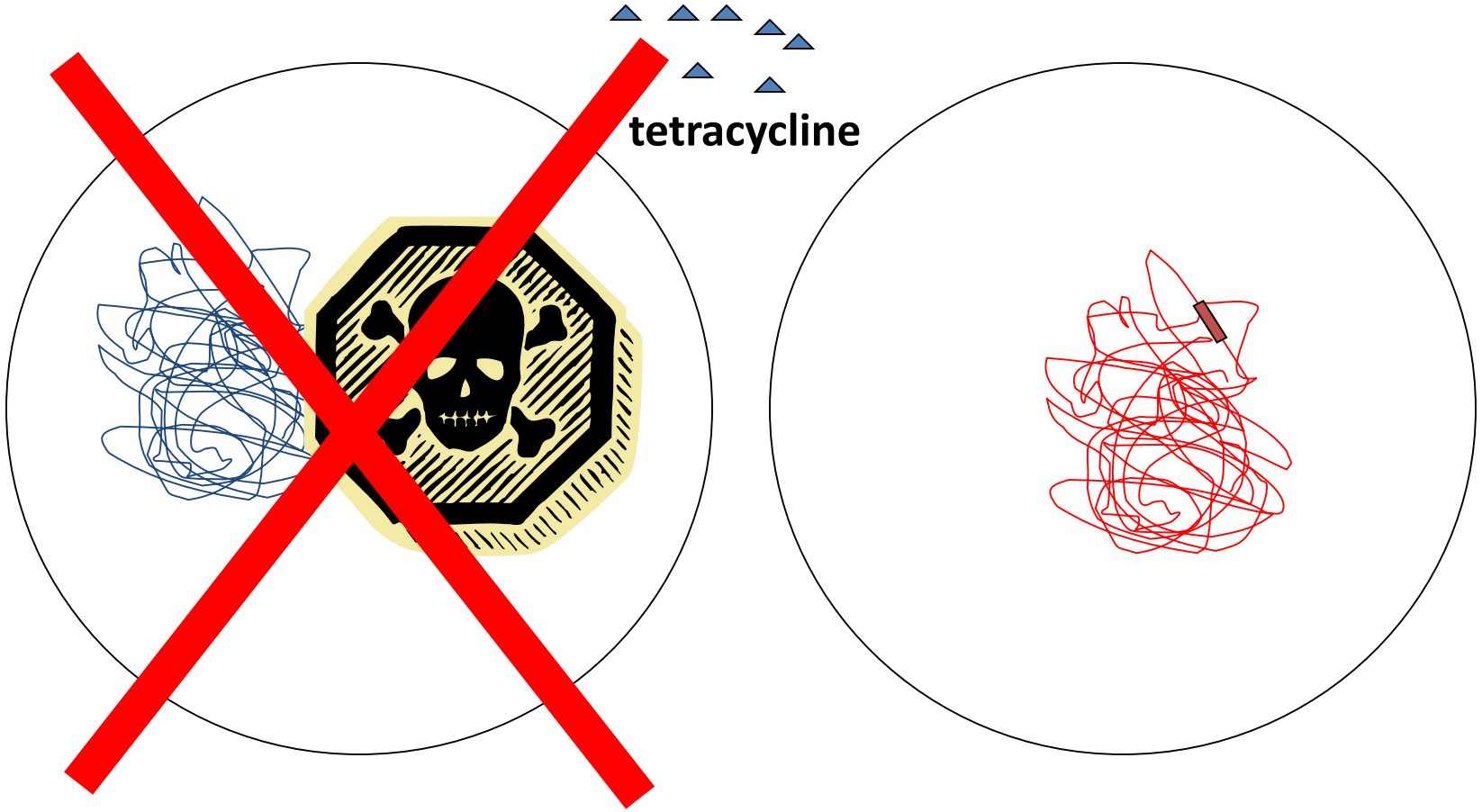
Our naïve starting model for transplantation of tet^R donor genomes into recipient cells



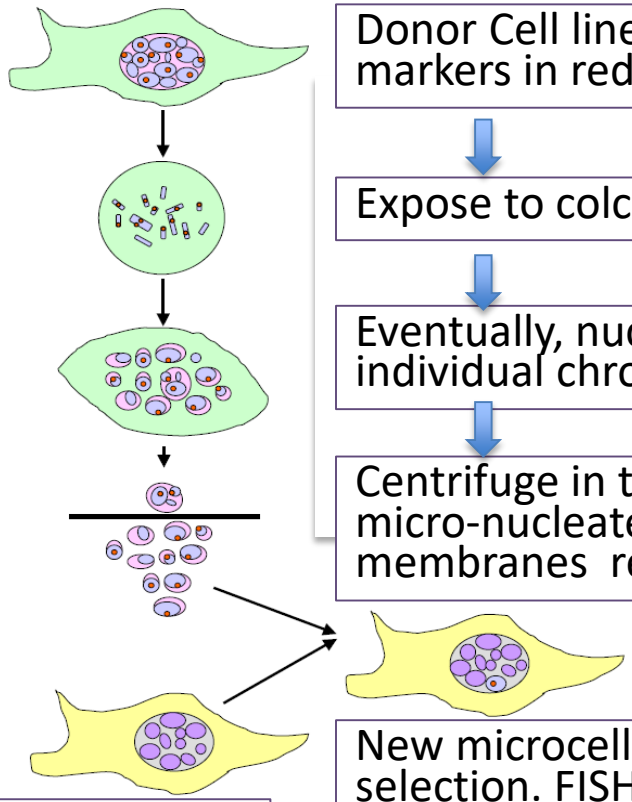
Cell growth and division leads to daughter cells with different genomes



Transplanted genome has a tetracycline resistance gene. Only cells with that marker will grow in the presence of tetracycline.



Microcell-mediated chromosome transfer protocol



Donor Cell line, transfected w/ selectable dominant markers in red. Eg., *neo* or *bsr* resistance

Expose to colcemid to block cells in mitosis

Eventually, nuclear envelope forms around individual chromosomes

Centrifuge in the presence of cytochalasin B collecting micro-nucleated cells. Microcells filtered through membranes removing microcells w/ >1 chromosome

New microcell hybrid cell lines grown under antibiotic selection. FISH analysis done on resulting clonal cell lines.

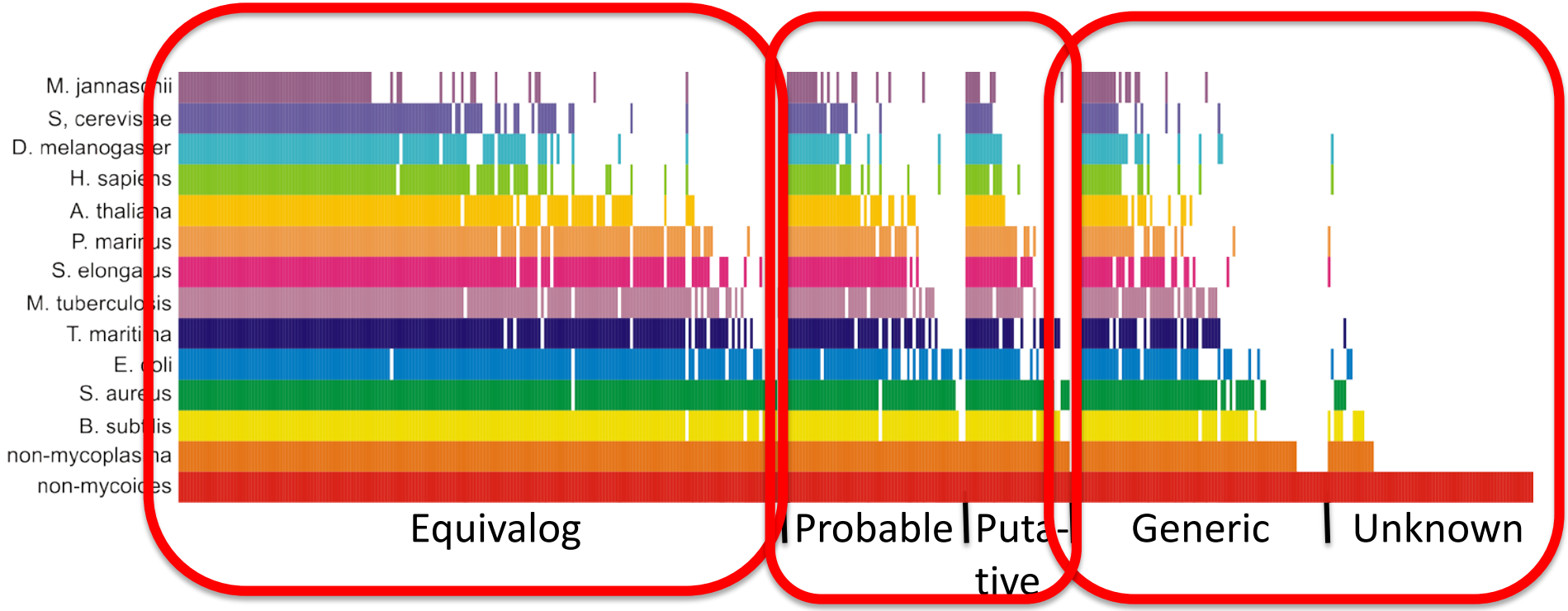
Recipient mammalian cell line

Microcell Mediated Chromosome Transfer (or MMCT) from *The manipulation of chromosomes by mankind: the uses of microcell-mediated chromosome transfer*. KJ Meaburn, CN Parris, JM Bridger. 2005. **Chromosoma**.

Installing large DNA molecules in cells is very uncertain at present

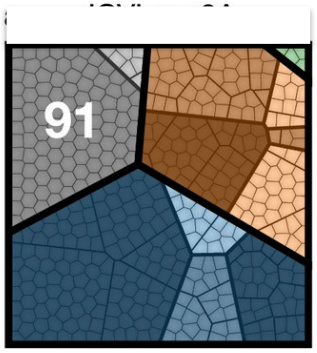
This is true from bacteria to higher eukaryotes

What are the functions of the 149 essential genes we currently do not understand?

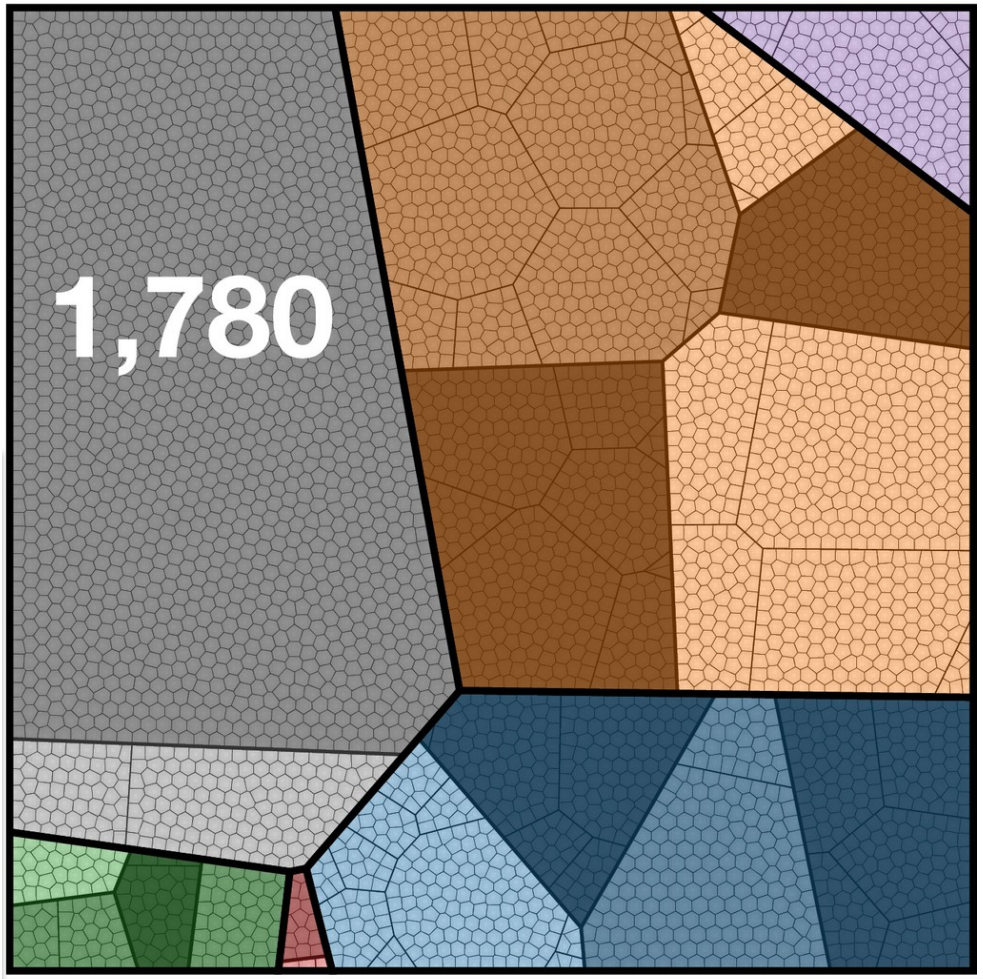


BLASTp searches using all syn3.0 protein coding genes against 14 organisms ranging from mycoplasmas to humans. $1e^{-5}$ is the similarity cutoff. Functional classifications proceed left to right from nearly complete certainty about a gene activity (equivalog), to no functional information (unknown). White space indicates no homologs to a given syn3.0 gene in that organism.

Proteins of unknown function in the genomes of minimal bacterial cell JCVI-syn3A & *E. coli*



JCVI-sy3A



E. coli

A 35-year romance with a seafaring microbe *p. 1004*

Social ties and policy reforms in China's S&T system *pp. 1019 & 1022*

Designing zeolites to react *pp. 1026 & 1032*

Science

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SYNTHETIC CHROMOSOMES

Remodeling the yeast genome piece by piece *p. 1038*

13 Mb yeast chromosome portends large artificial chromosomes that could be transferred to other cells by fusion

ARTICLE

https://doi.org/10.1038/s41586-018-0382-x

Creating a functional single-chromosome yeast

Yangyang Shao[1,2], Ning Lu[1,2], Zhenfang Wu[3], Chen Cai[2,3], Shanshan Wang[3], Ling-Li Zhang[2,3], Fan Zhou[4], Shijun Xiao[4], Lin Liu[4], Xiaofei Zeng[4], Huajun Zheng[5], Chen Yang[1], Zhihu Zhao[6], Guoping Zhao[1,5,7,8]*, Jin-Qiu Zhou[3]*, Xiaoli Xue[1]* & Zhongjun Qin[1]*

Eukaryotic genomes are generally organized in multiple chromosomes. Here we have created a functional single-chromosome yeast from a *Saccharomyces cerevisiae* haploid cell containing sixteen linear chromosomes, by successive end-to-end chromosome fusions and centromere deletions. The fusion of sixteen native linear chromosomes into a single chromosome results in marked changes to the global three-dimensional structure of the chromosome due to the loss of all centromere-associated inter-chromosomal interactions, most telomere-associated inter-chromosomal interactions and 67.4% of intra-chromosomal interactions. However, the single-chromosome and wild-type yeast cells have nearly identical transcriptome and similar phenome profiles. The giant single chromosome can support cell life, although this strain shows reduced growth across environments, competitiveness, gamete production and viability. This synthetic biology study demonstrates an approach to exploration of eukaryote evolution with respect to chromosome structure and function.

John's major unmet basic research needs

1. Installation of large DNA into cells is a lagging sector of synthetic biology
2. There is still a large fraction of the genes in the best characterized cells that we do not understand -- suggesting that there are essential cellular processes we do not understand
3. Standardization of parts and techniques for gene therapy is a major issue

Field of Dreams