



Research Topics and Seminar (501)

Presentation and Discussion of the Paper:

Design and synthesis of a minimal bacterial genome

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Outline

- The paper
- Introduction
- Aim
- Methods
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The Paper

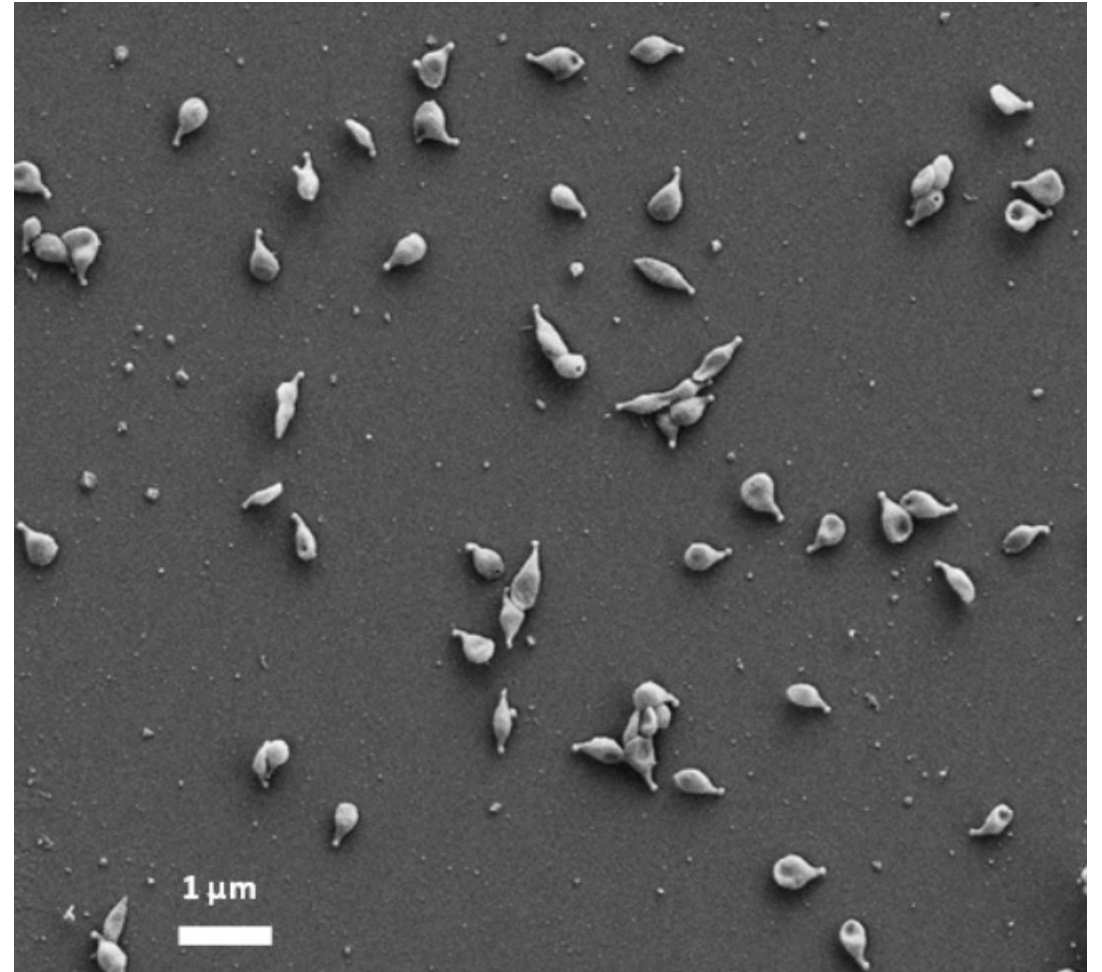
Design and synthesis of a minimal bacterial genome

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We used whole-genome design and complete chemical synthesis to minimize the 1079–kilobase pair synthetic genome of *Mycoplasma mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology combined 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 cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kilobase pairs, 473 genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design.

Introduction

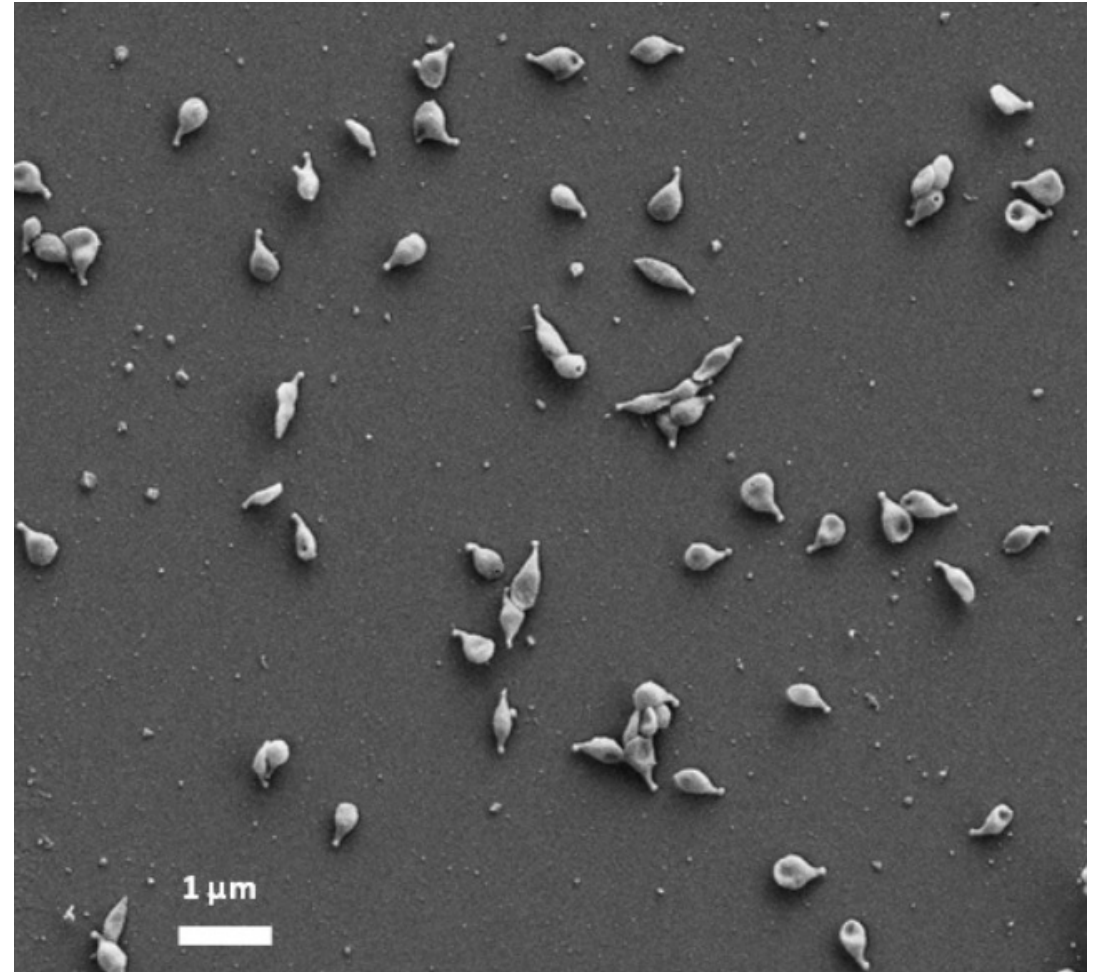
- Cells are the fundamental units of life.
- The genome being as its operating system. It contains the genes; which will code for a specific function in the cell.



<https://www.usomycoplasmology.org/single-post/2015/09/04/how-to-see-mycoplasmas>

Introduction

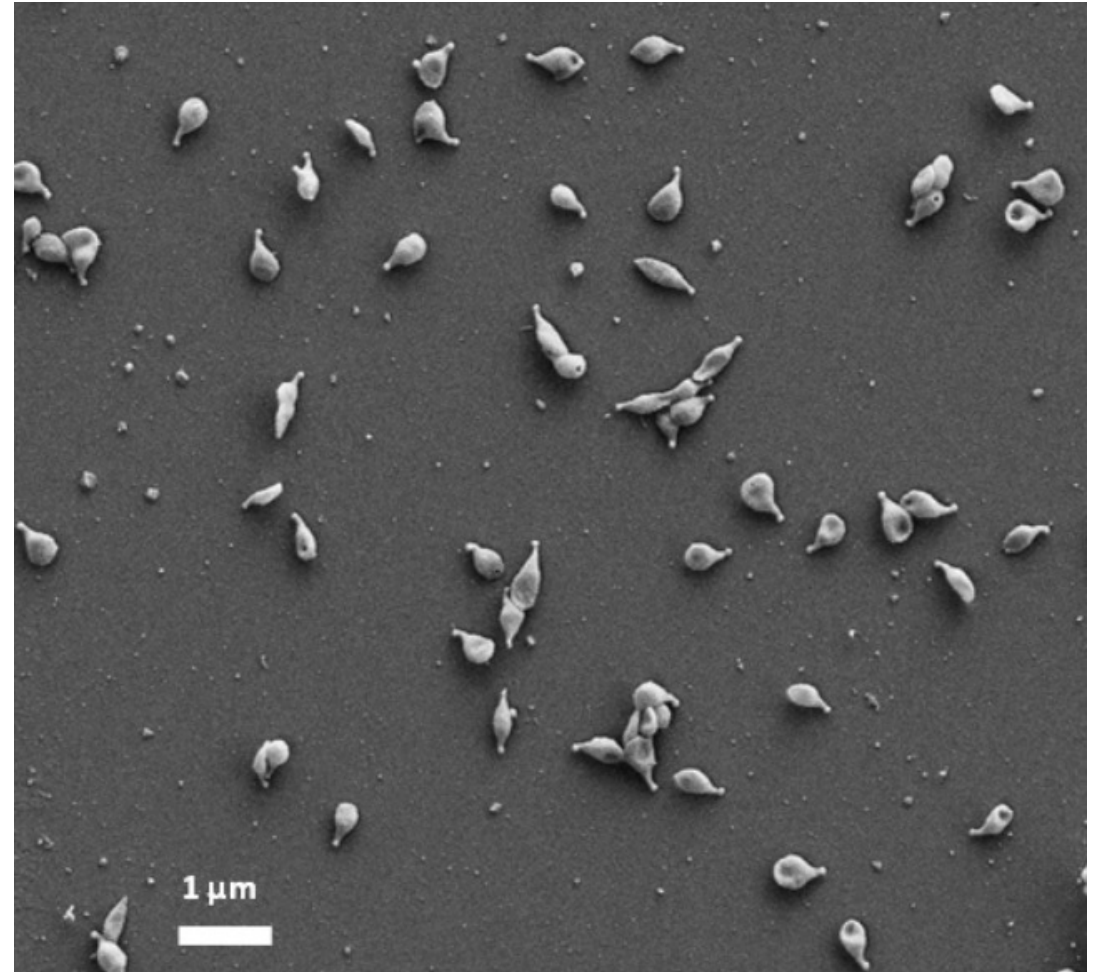
- Some genes are essential for a cell to live and replicate.
- While other genes not as much necessary.



<https://www.usomycoplasmology.org/single-post/2015/09/04/how-to-see-mycoplasmas>

Introduction

- Mycoplasmas, a cell wall free bacteria, has been proposed to be the simplest cell capable of growth.
- It has the smallest genome of any autonomously replicating cells.

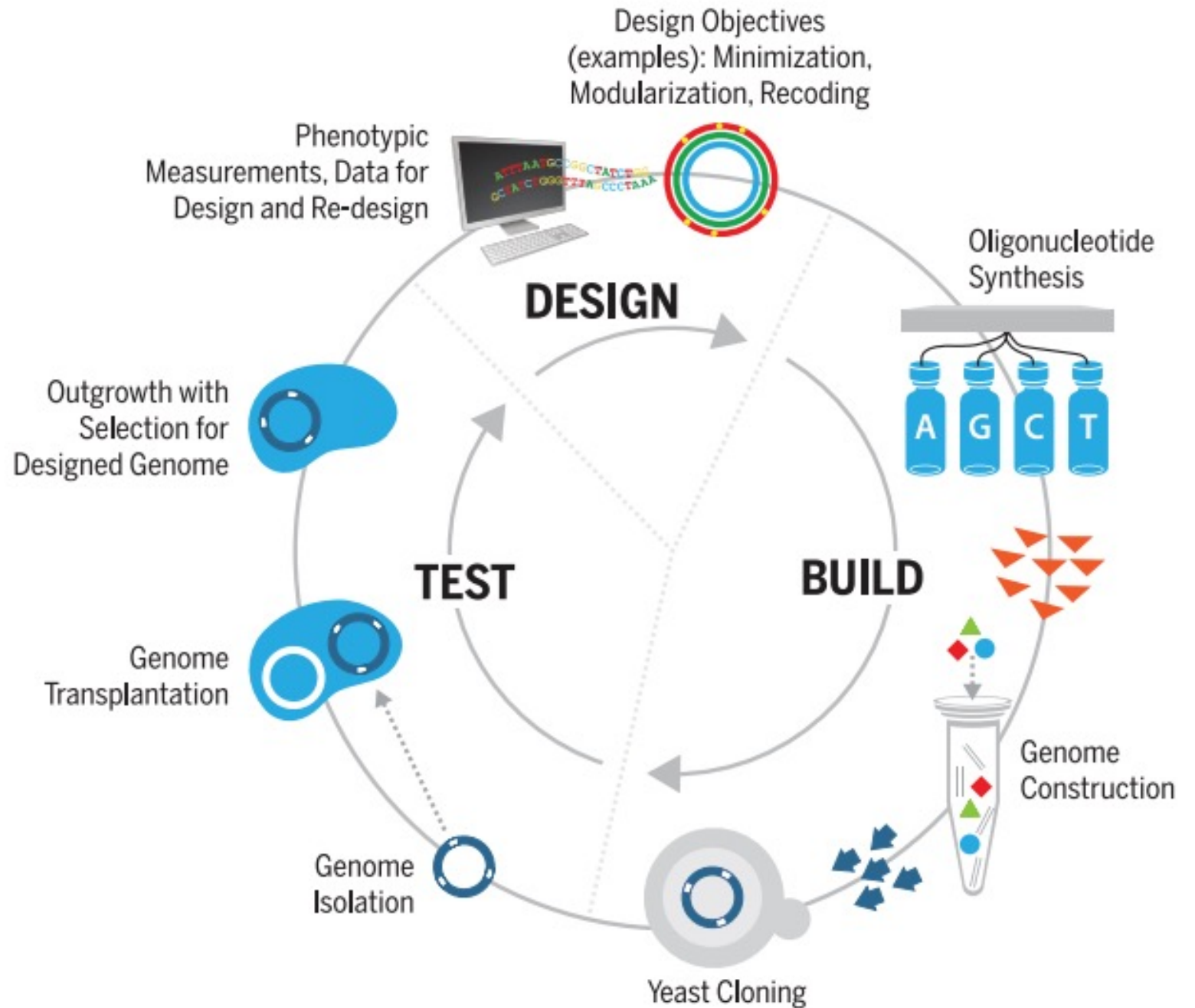


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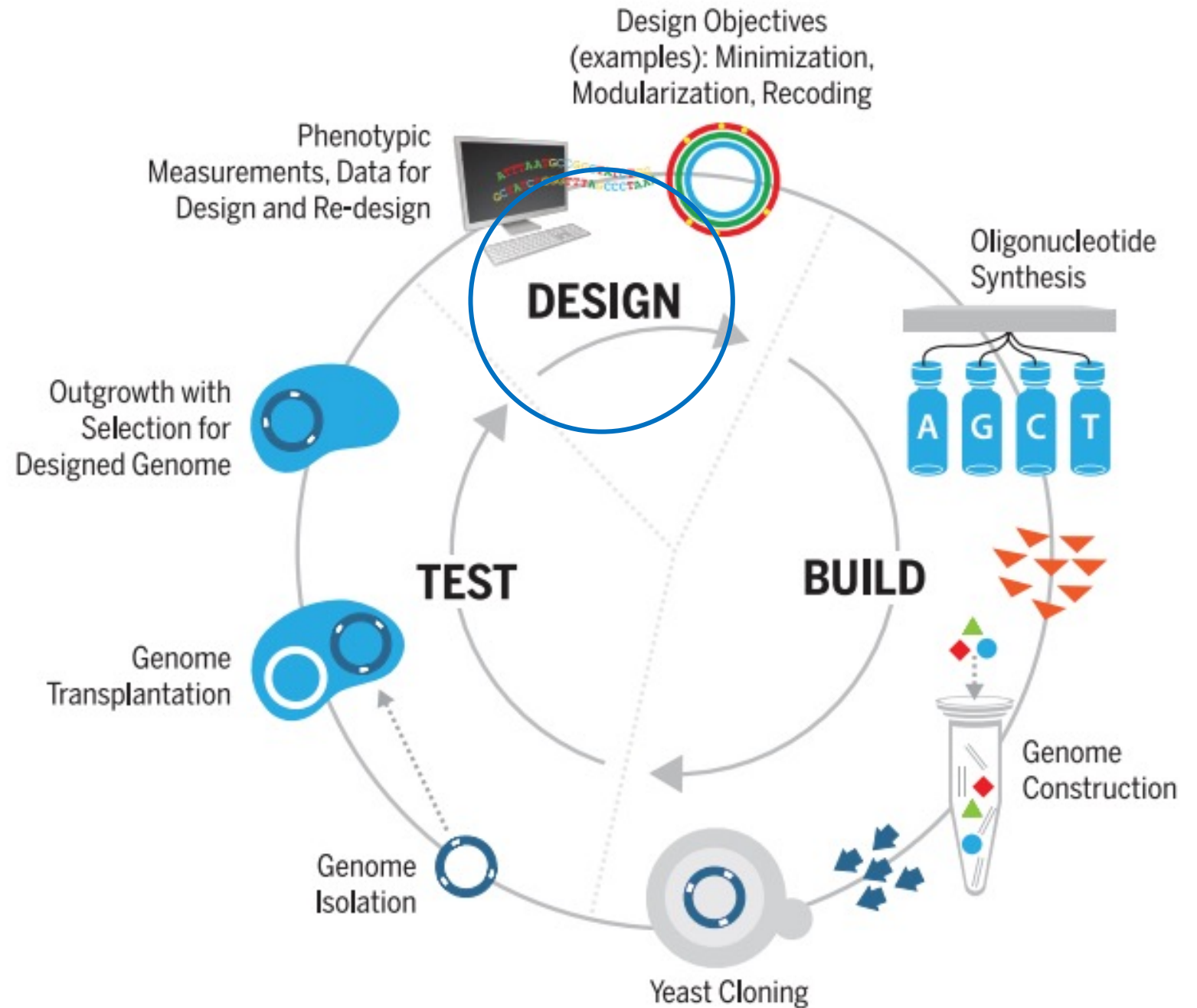
Aim

- To define a minimal cellular genome experimentally.
- To design a cell so simple that we can determine the molecular and biological function of every gene.

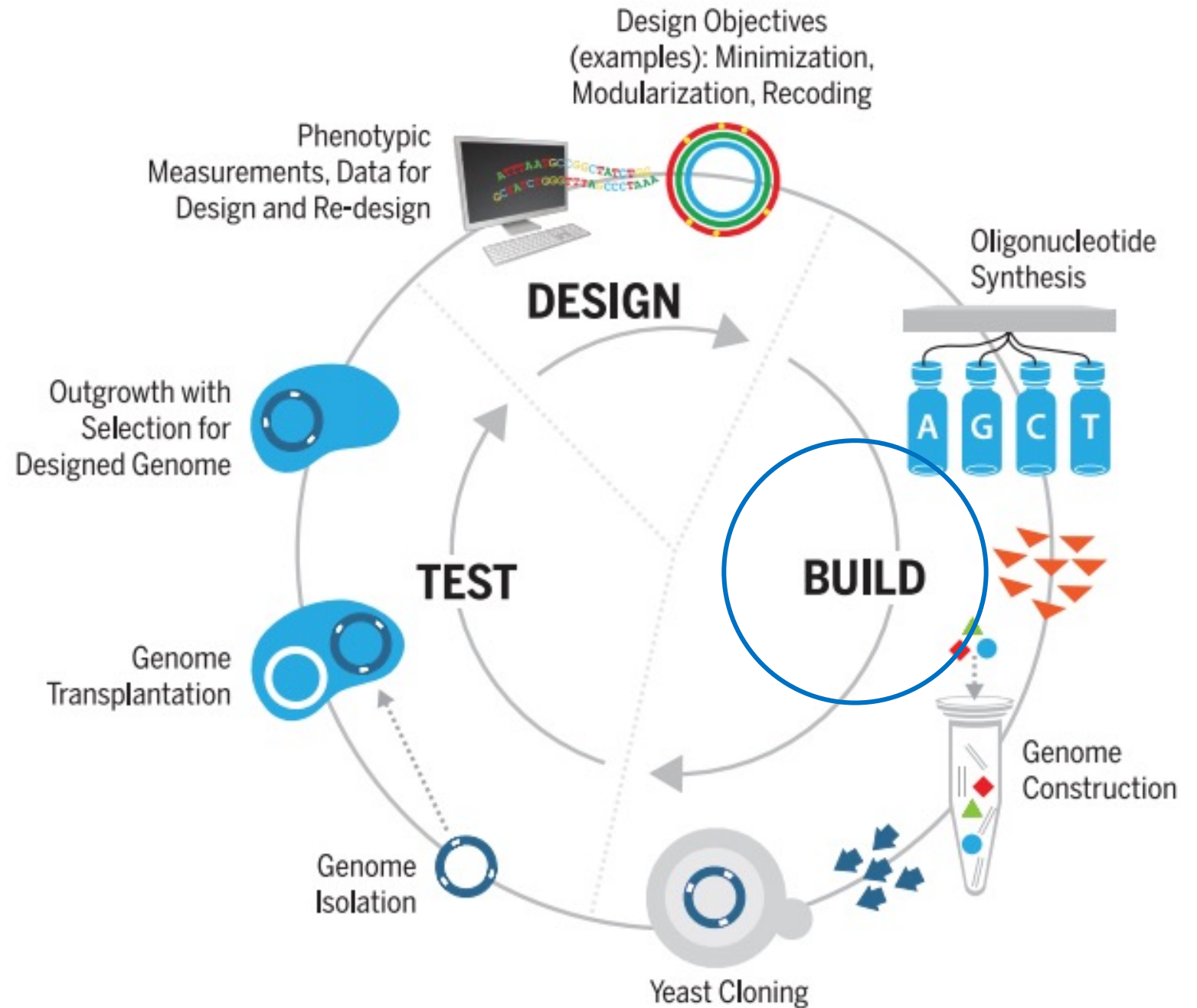
Methodology



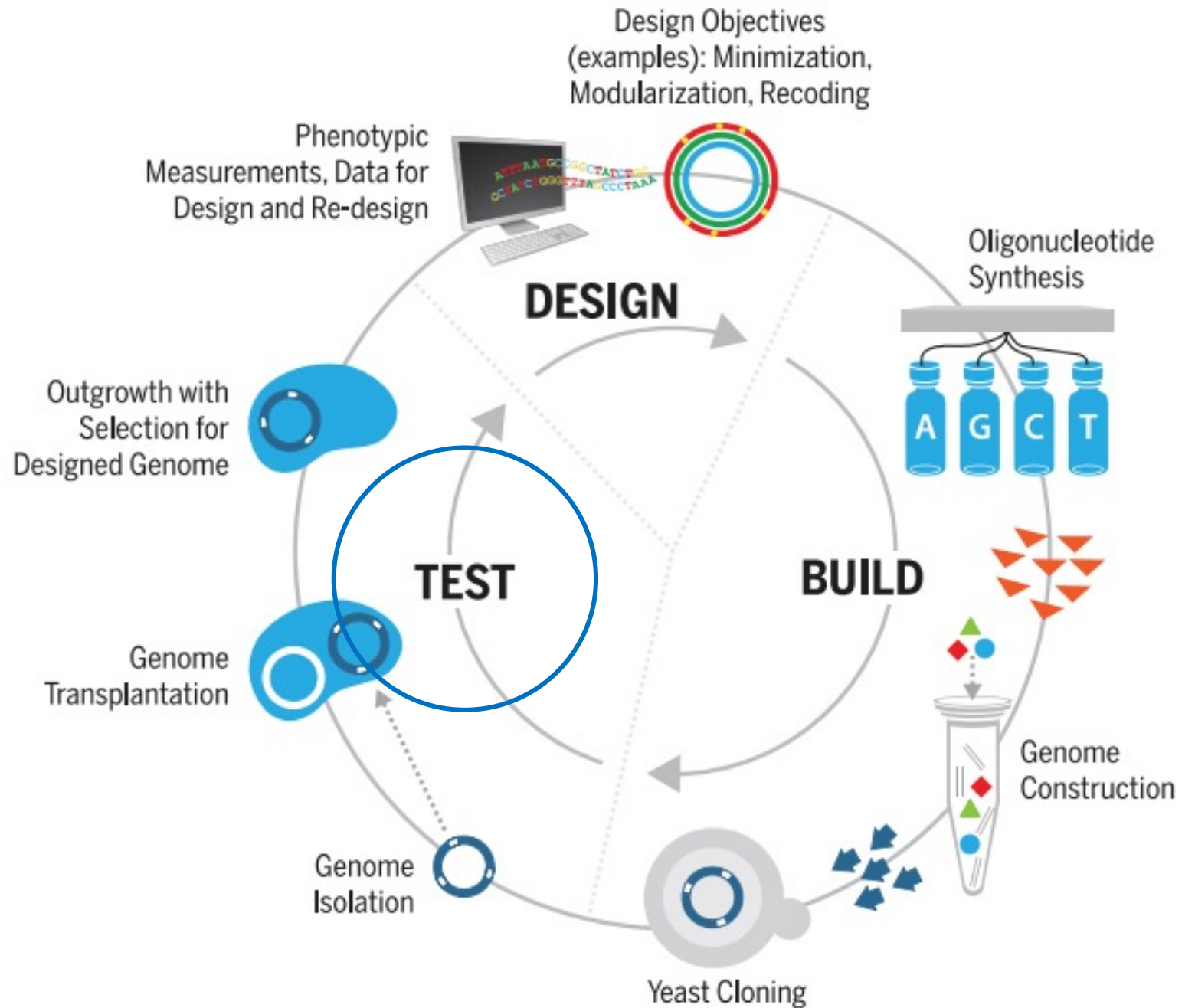
Methodology



Methodology



Methodology



Methodology

- The bacteria used was *Mycoplasma mycoides*.
- Identifying essential, quasi-essential, and non-essential genes using Tn5 mutagenesis.
- Designing the genome to be built as 8 segments.

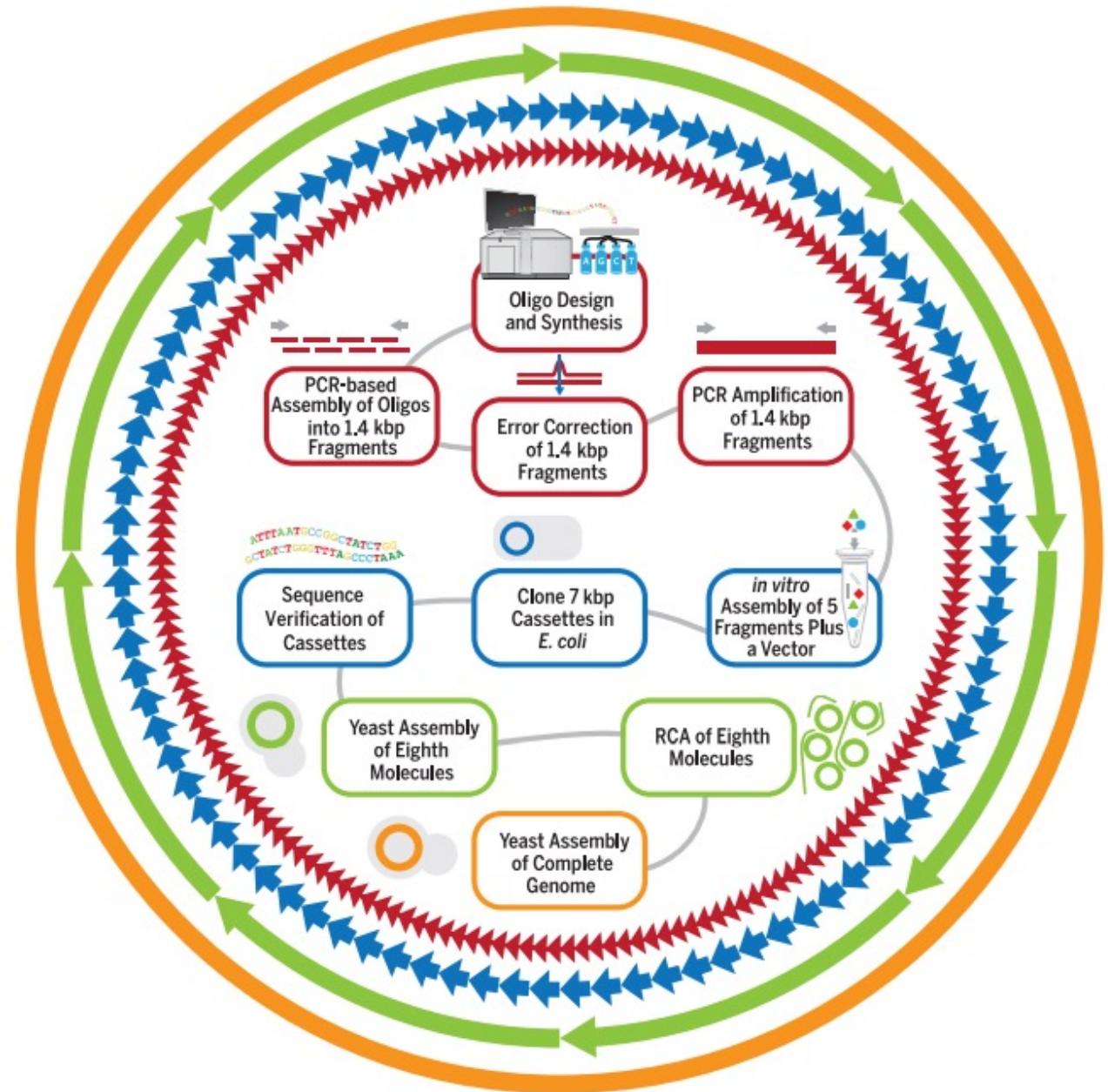
Methodology

- Synthesizing oligonucleotides.
- Assembly of 5 fragments into a vector.
- Cloning it into yeast.

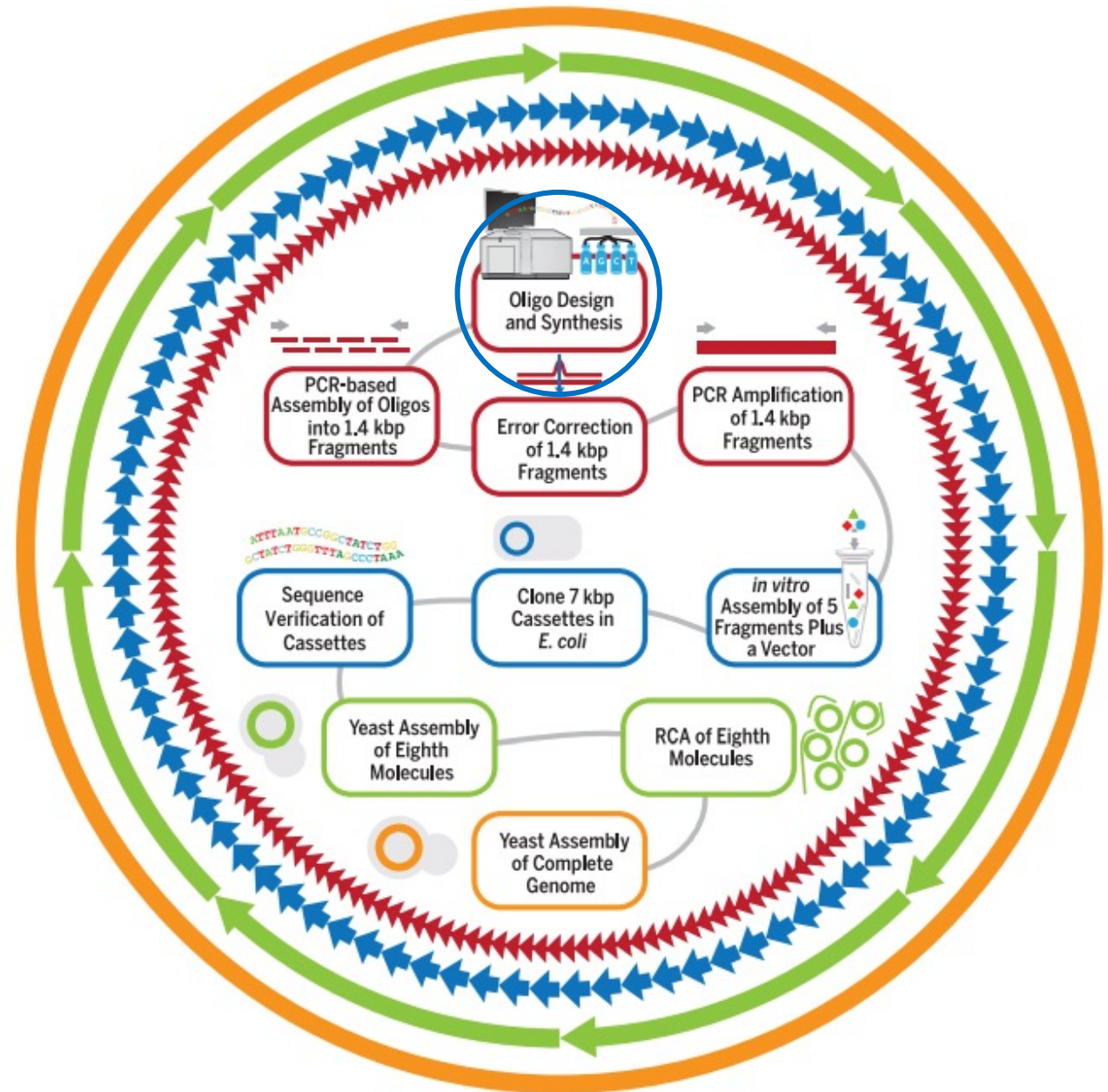
Methodology

- Genome isolation.
- Genome transplantation.
- Selection of the designed genome.

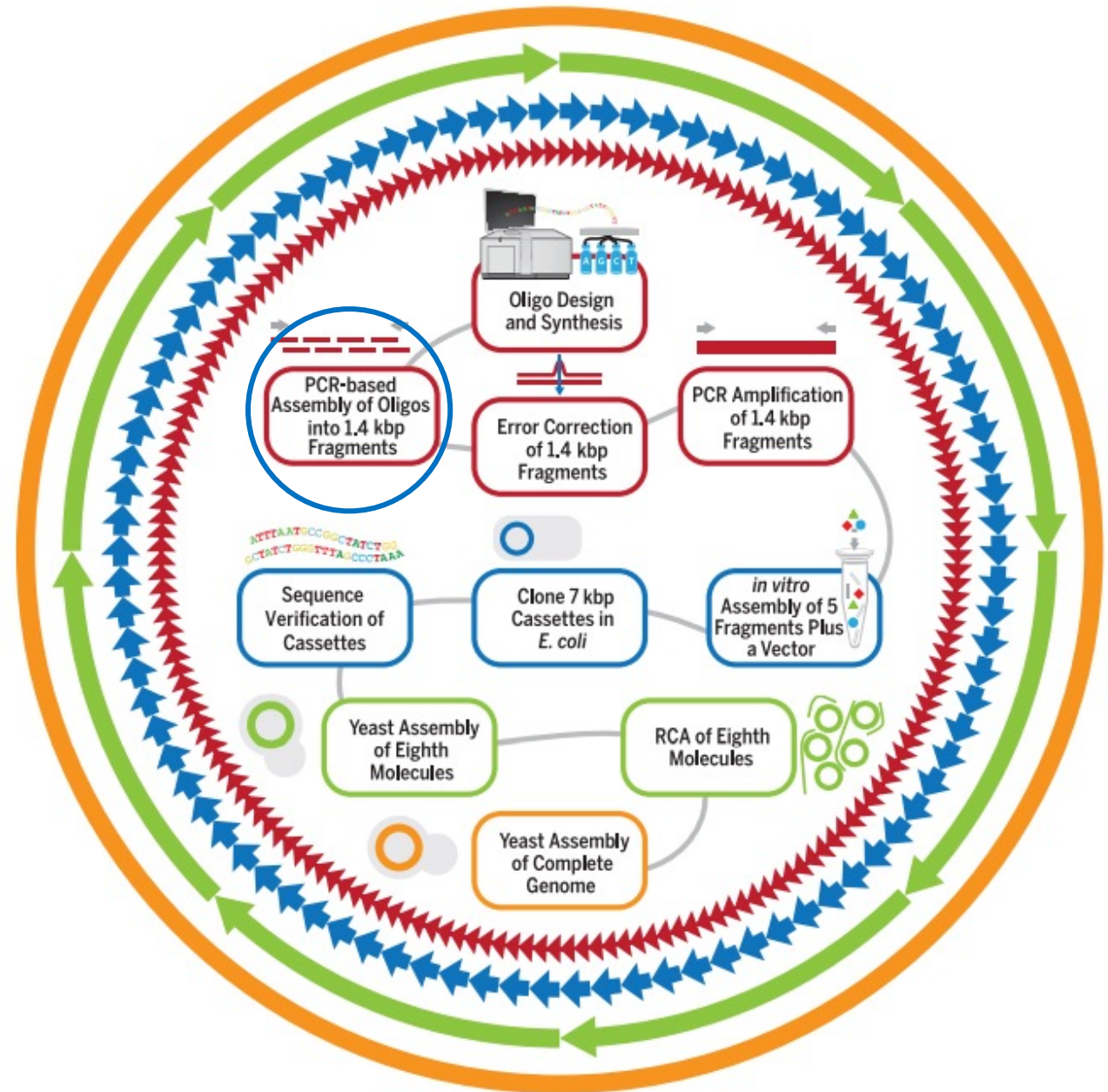
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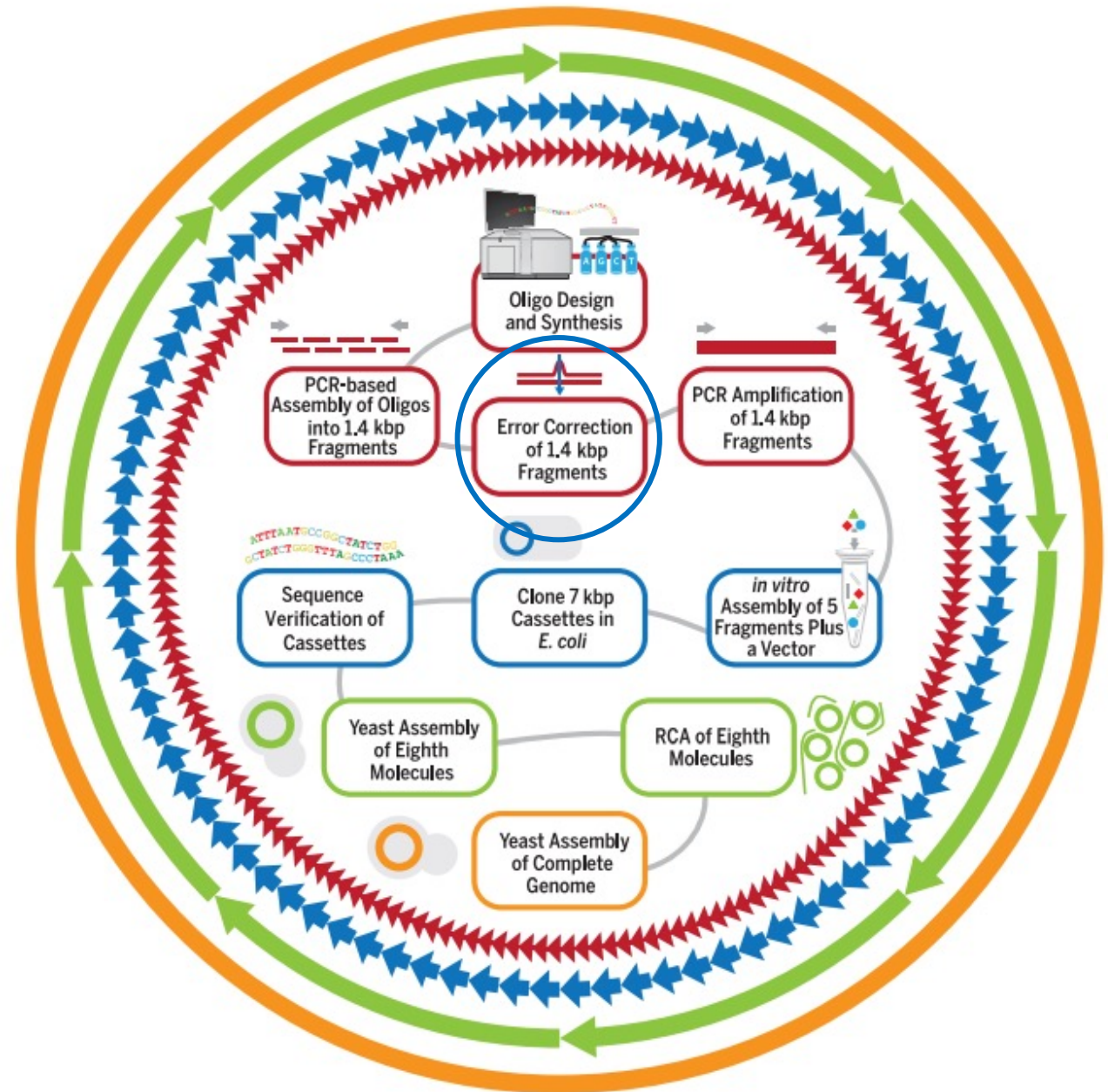
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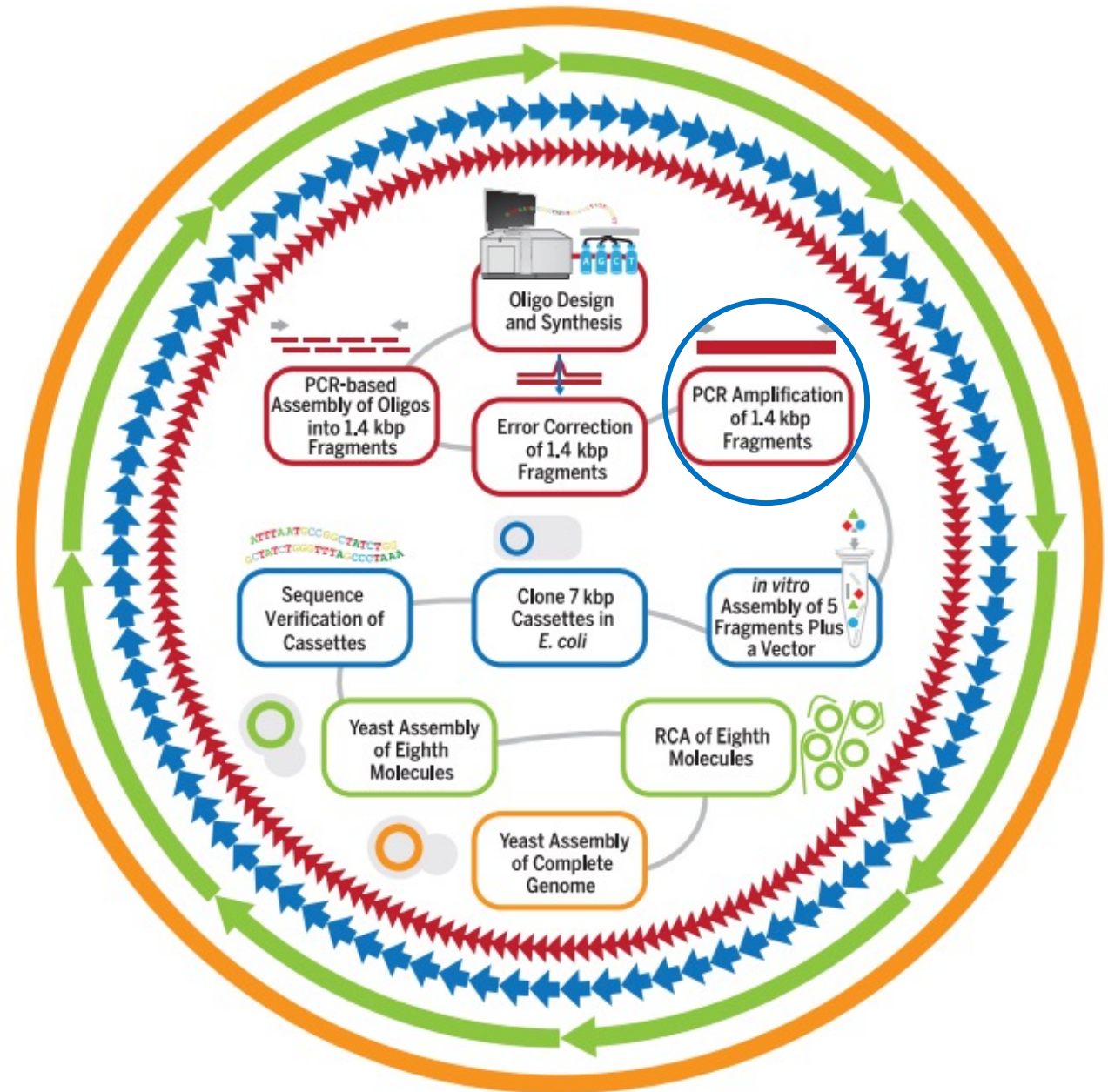
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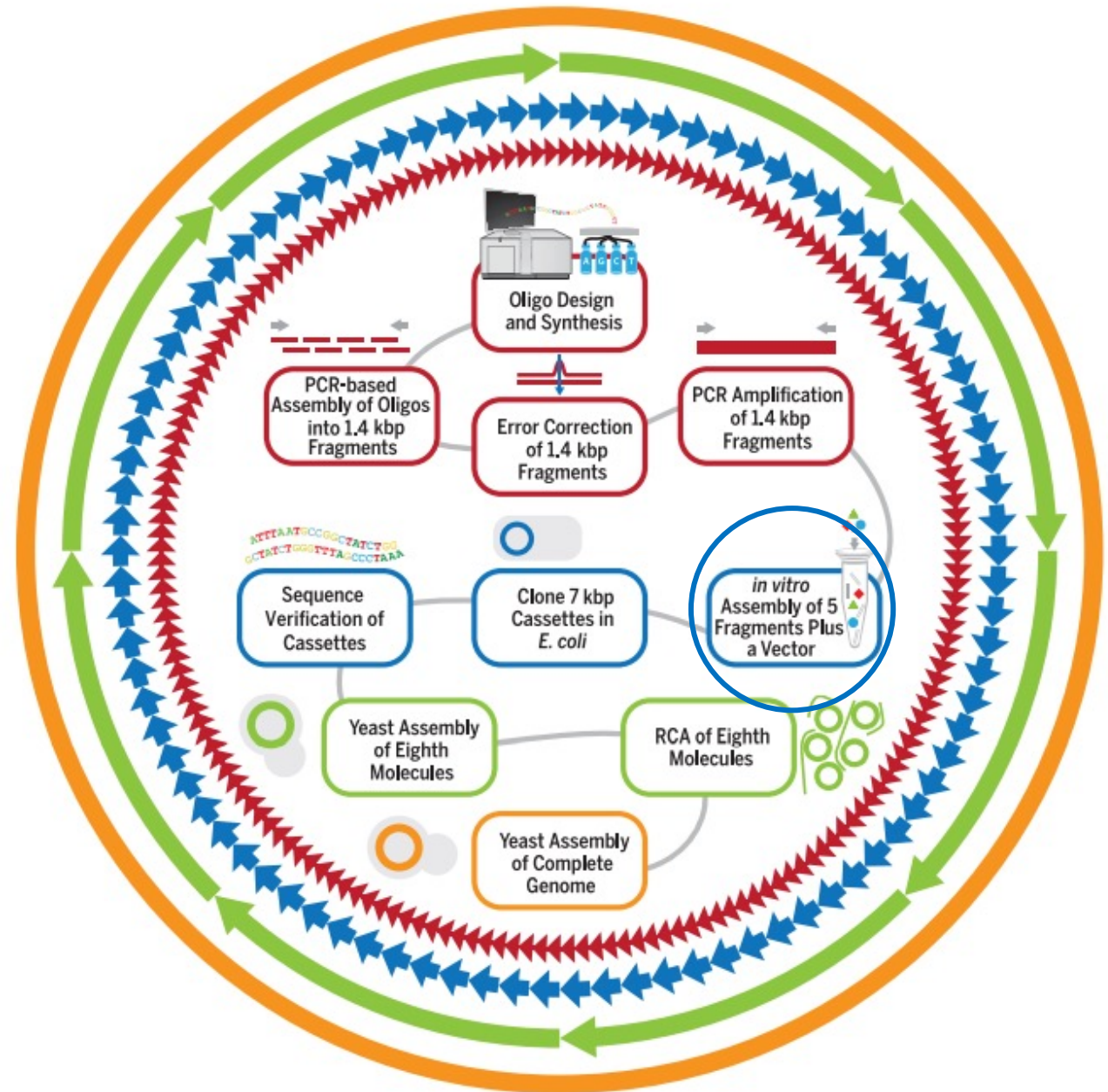
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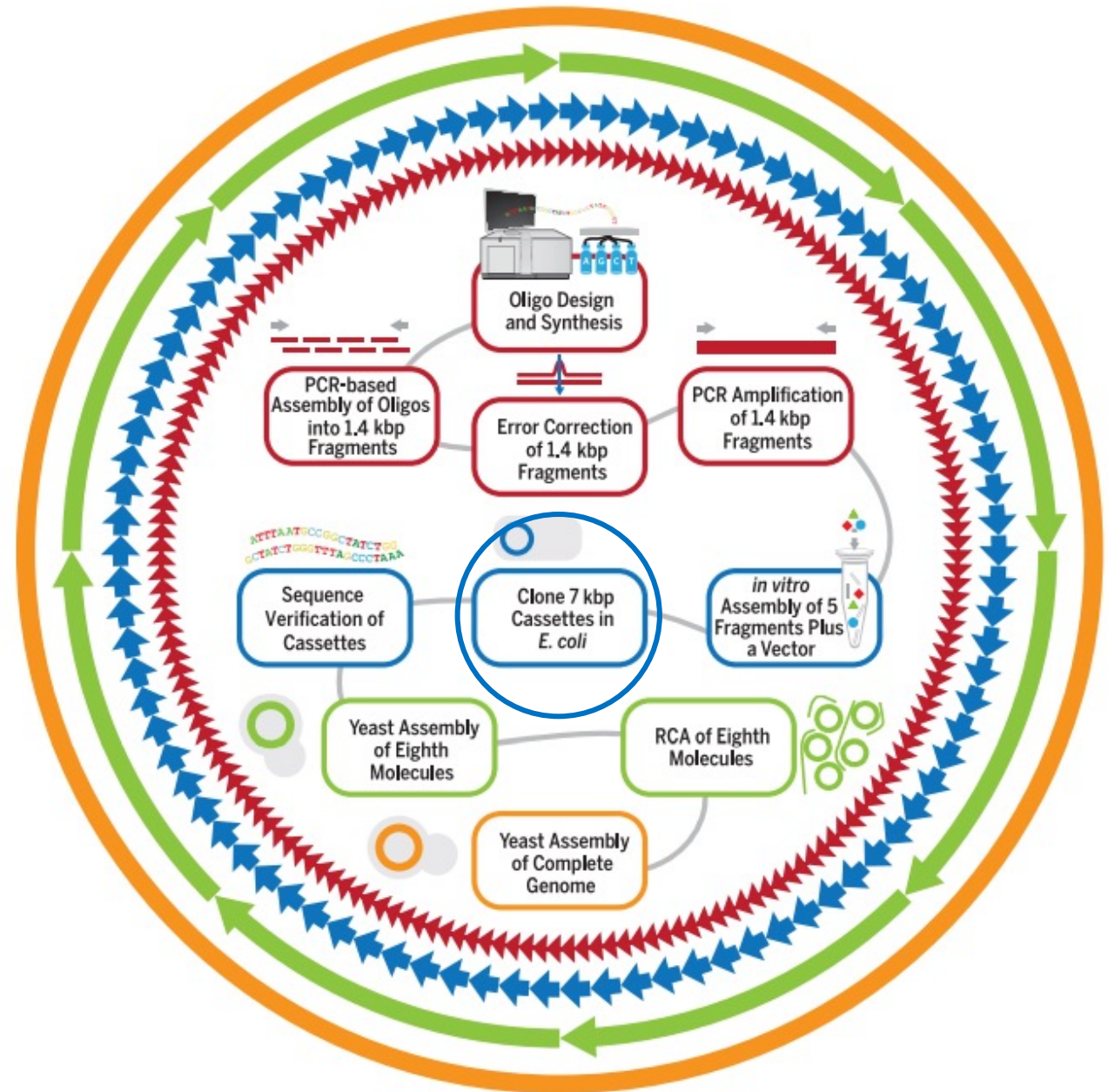
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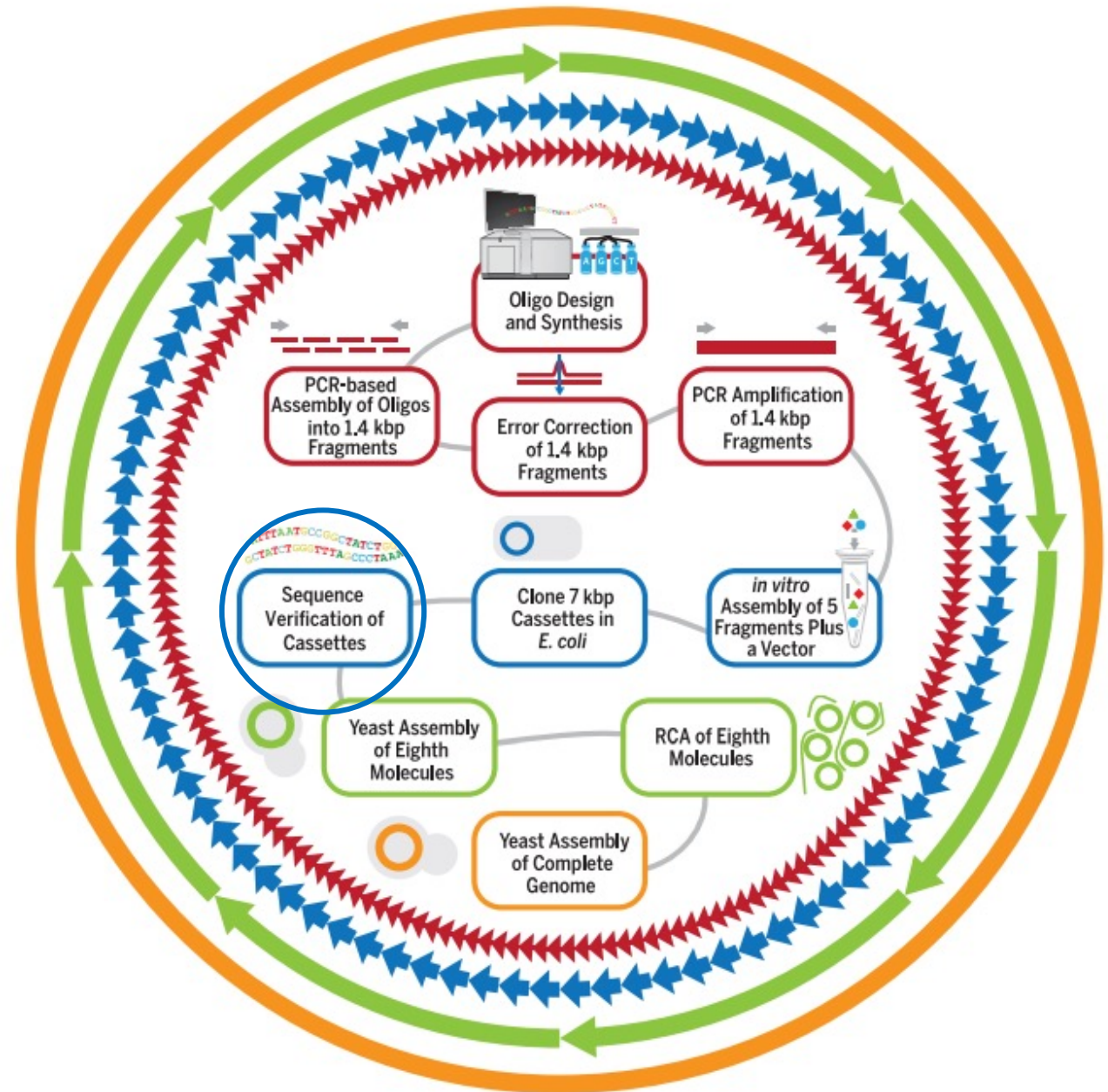
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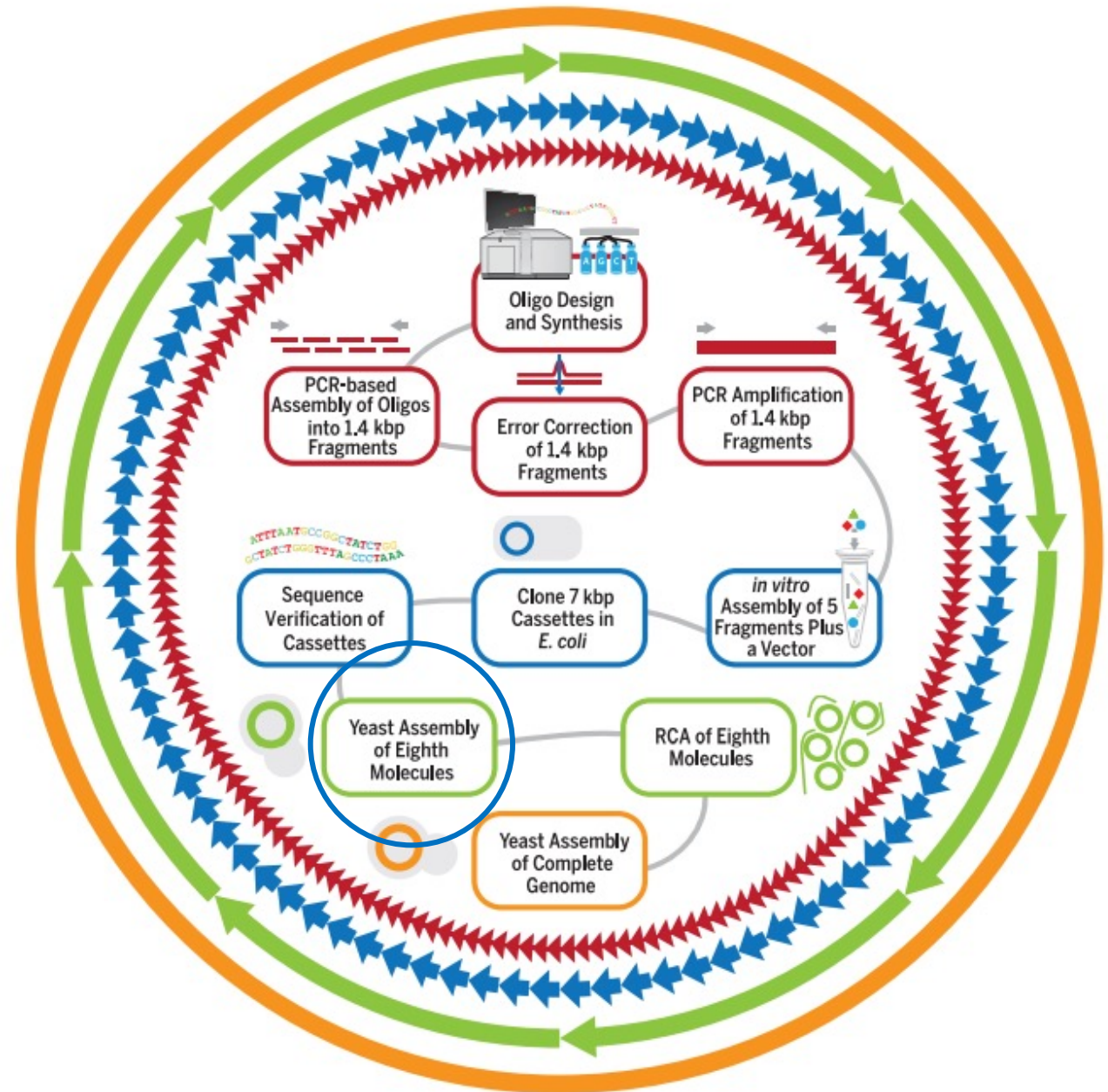
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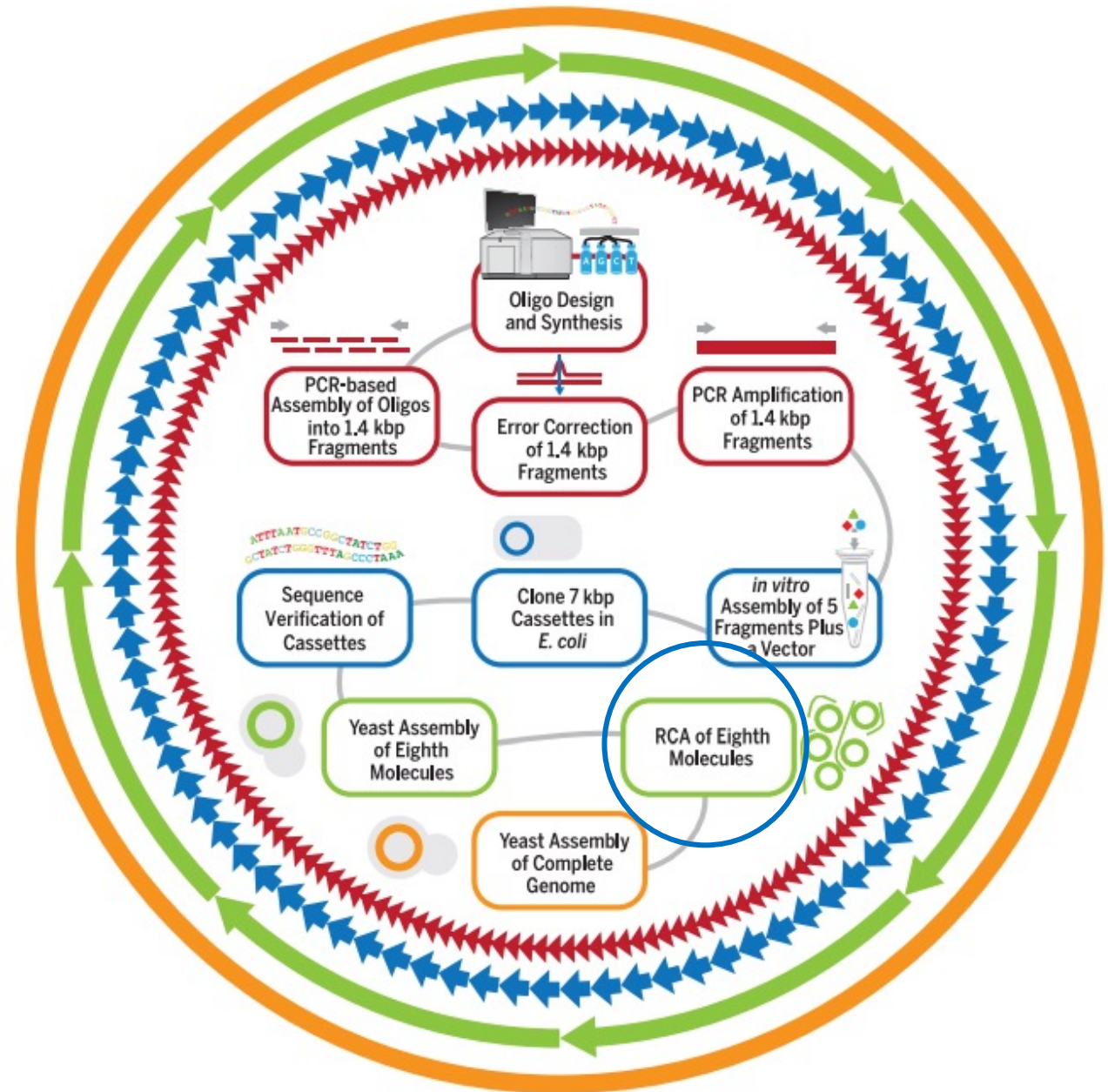
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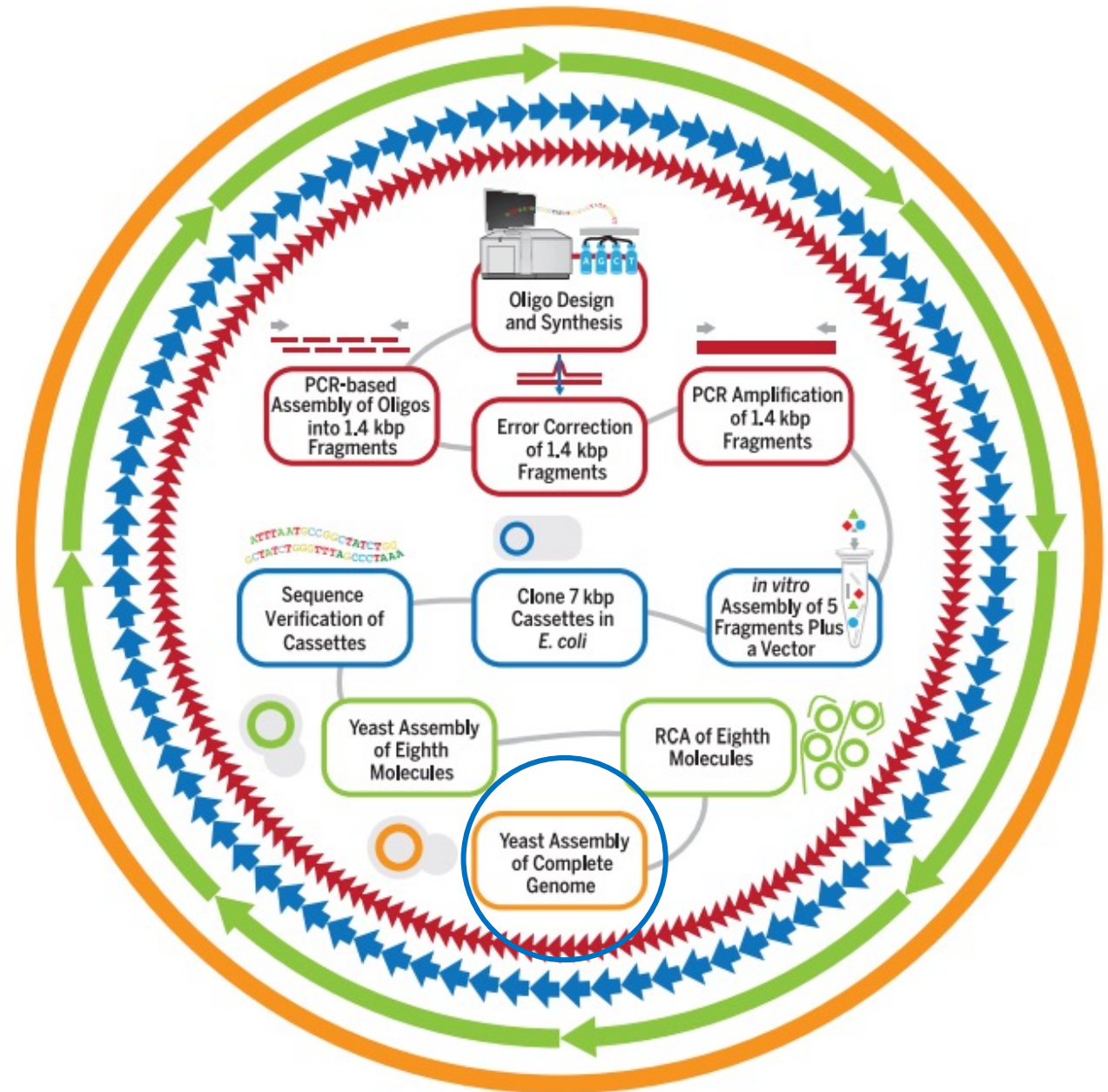
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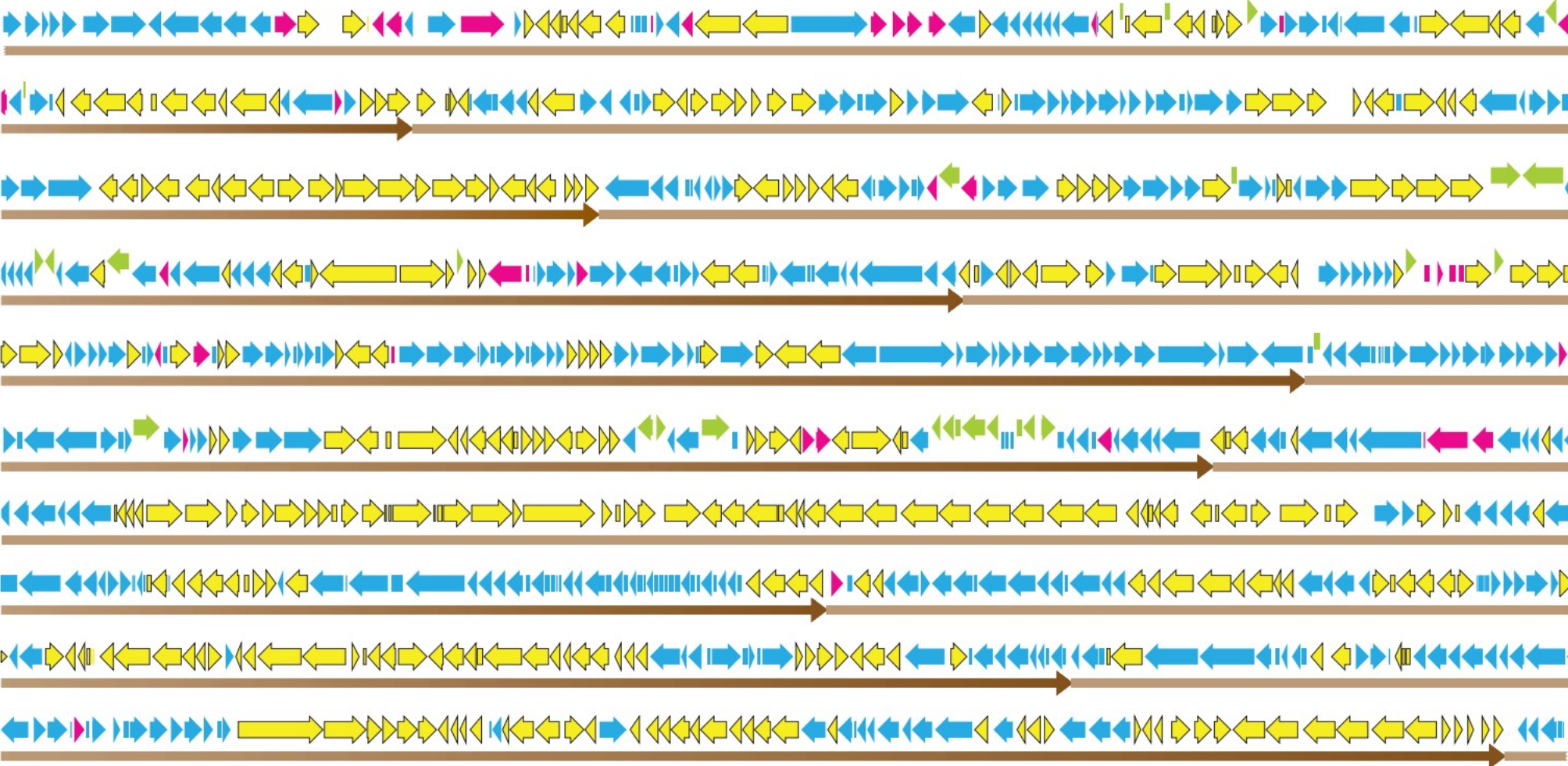
Methodology



Methodology



Results



Results

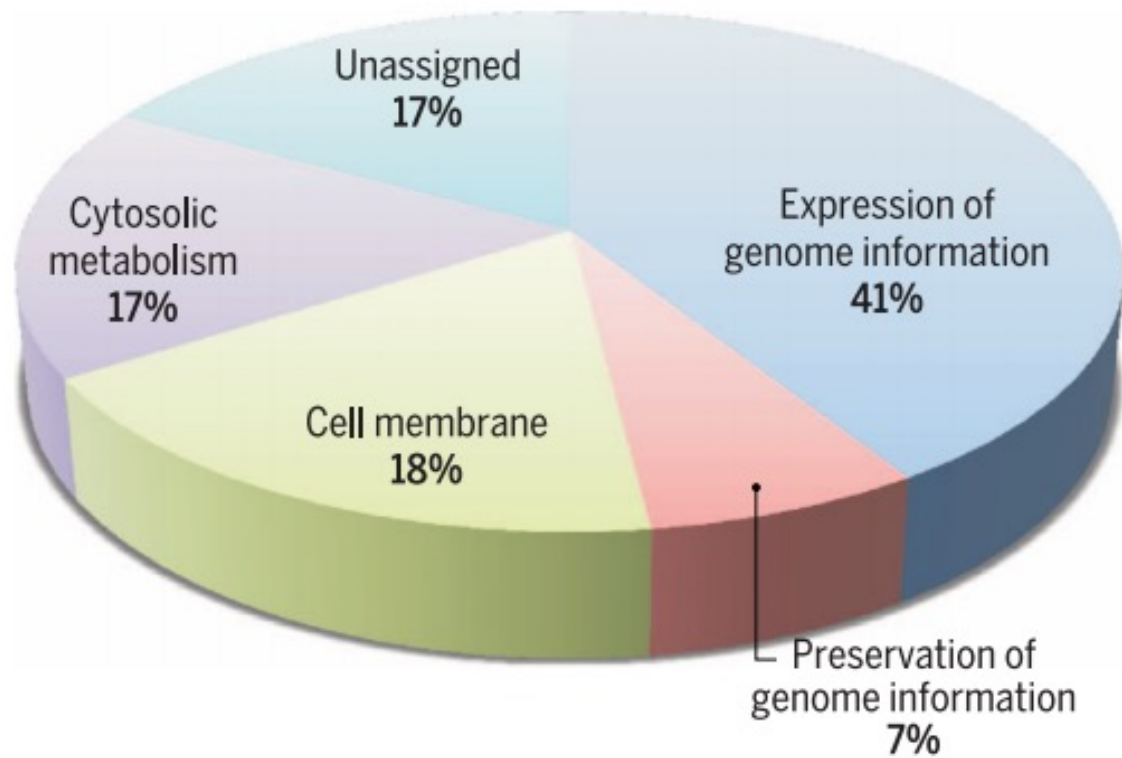
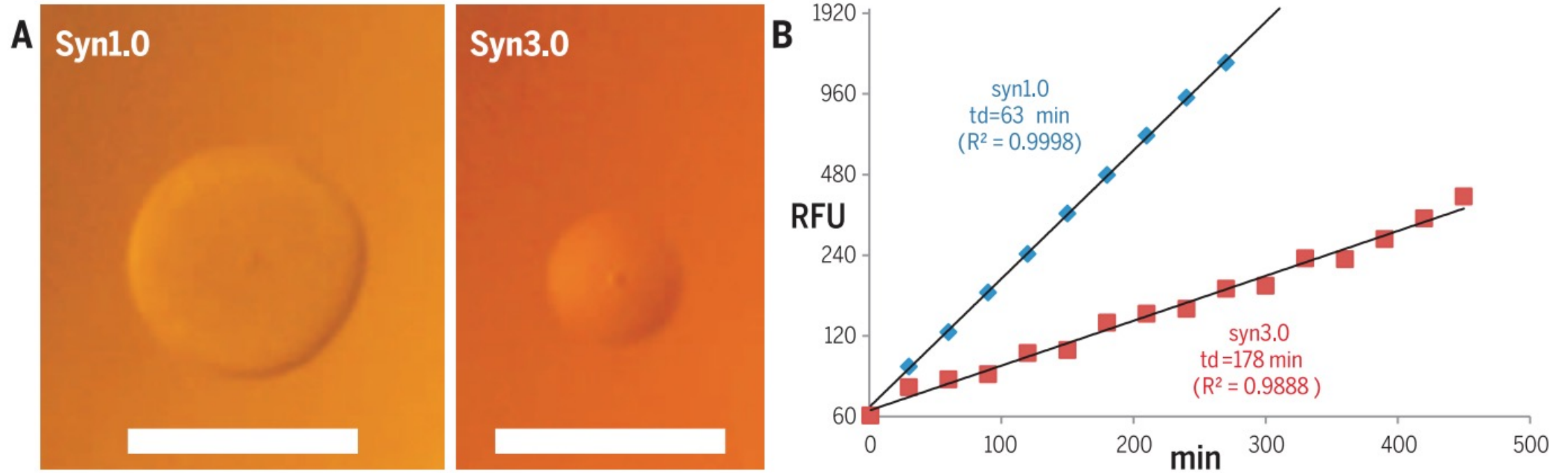


Table 1. Syn1.0 genes listed by functional category and whether they were kept or deleted in syn3.0. Categories with asterisks are mostly kept in syn3.0, whereas those without are depleted in syn3.0. Vector sequences, for selection of the genome and for propagation in other hosts, are not included in these gene tallies.

Functional category	Kept	Deleted
Glucose transport and glycolysis*	15	0
Ribosome biogenesis*	14	1
Protein export*	10	0
Transcription*	9	0
RNA metabolism*	7	0
DNA topology*	5	0
Chromosome segregation*	3	0
DNA metabolism*	3	0
Protein folding*	3	0
Translation*	89	2
RNA (rRNAs, tRNAs, small RNAs)*	35	4
DNA replication*	16	2
Lipid salvage and biogenesis*	21	4
Cofactor transport and salvage*	21	4
rRNA modification*	12	3
tRNA modification*	17	2
Efflux*	7	3
Nucleotide salvage	19	8
DNA repair	6	8
Metabolic processes	10	10
Membrane transport	31	32
Redox homeostasis	4	4
Proteolysis	10	11
Regulation	9	10
Unassigned	79	134
Cell division	1	3
Lipoprotein	15	72
Transport and catabolism of nonglucose carbon sources	2	34
Acylglycerol breakdown	0	4
Mobile elements and DNA restriction	0	73
Total	473	428

Results



Discussion

- While a minimal cell is usually defined as a cell which all its genes are essential, this definition is considered incomplete because the genetic requirements for survival depends on the environment in which the cells are grown.
- While the hypothetical minimal genome proved to be non-viable, one of its eight segments (segment 2) was functional when tested in the context of the other seven syn1.0 segments.

Conclusion

- Successful development of a fully synthesized minimal genome in *Mycoplasma mycoides* (Syn3.0) with a 531,560 base pair genome that contains a total of 473 genes.
- Surprisingly, it contains 149 essential genes with a yet to be known function. Thus, making it a versatile for further investigation.

Thank you