Programming Cells by Multiplex Genome Engineering and Accelerated Evolution

By Harris H. Wang, Farren J. Issacs, Peter A. Carr, Zachary Z. Sun, George Xu, craig R. Forest & George M. Church.

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Hypothesis

- Multiplex Automated Genome Engineering (MAGE) allows parallel and continuous accelerated evolution
  - Designed to target many cellular locations on chromosomes across a population of cells
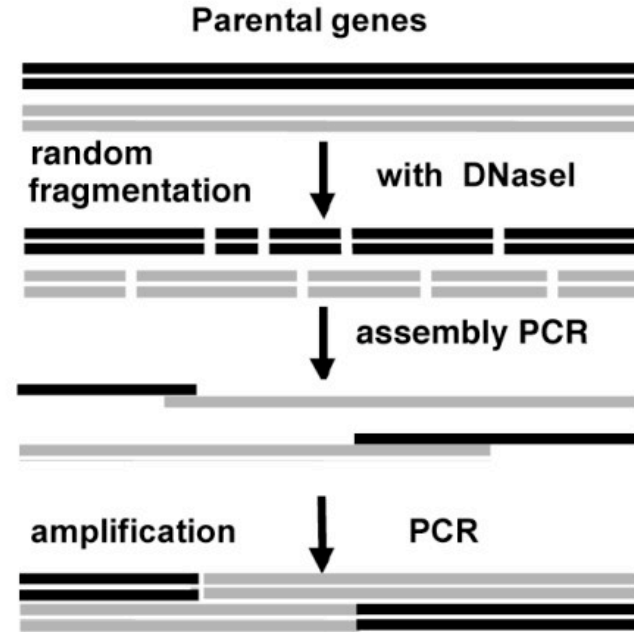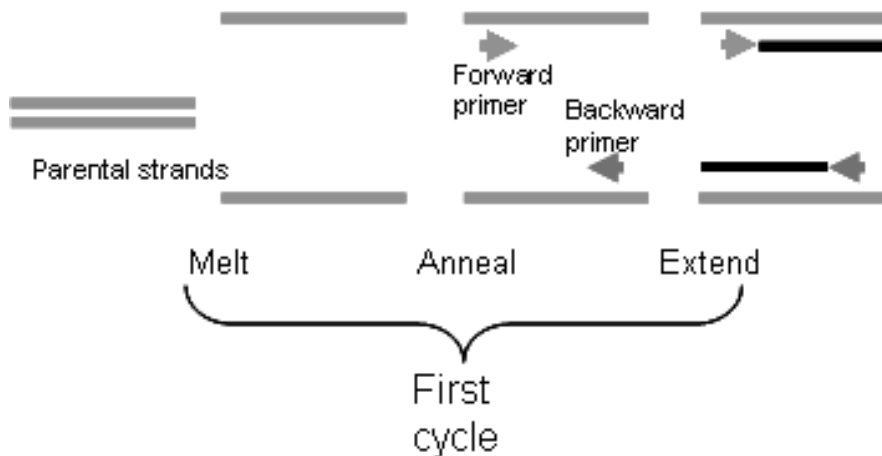 - Cyclical and scalable

Methods in Directed Evolution

Currently:

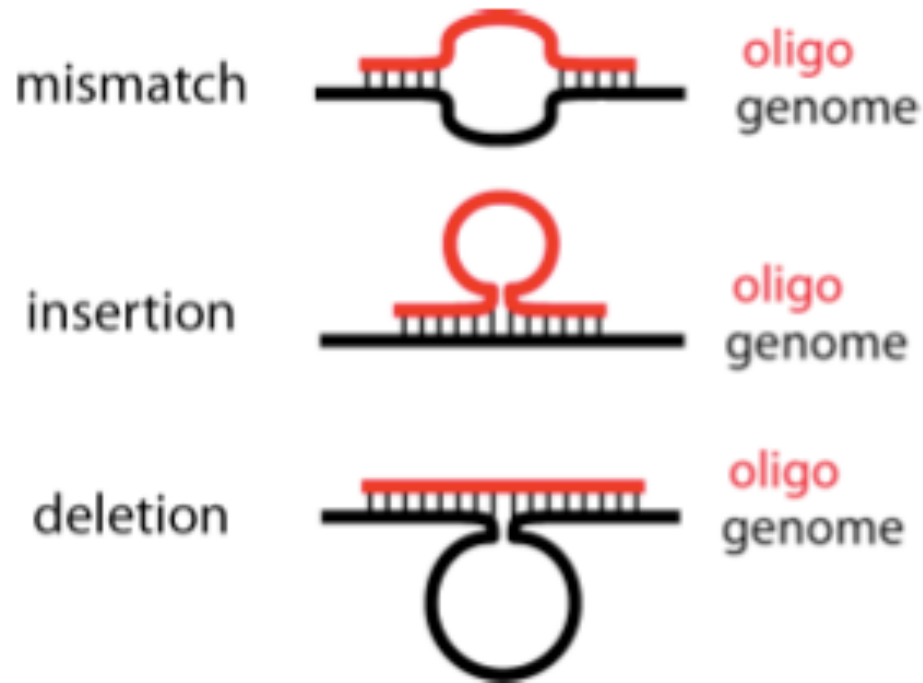Gene mutation → Selection →Amplification
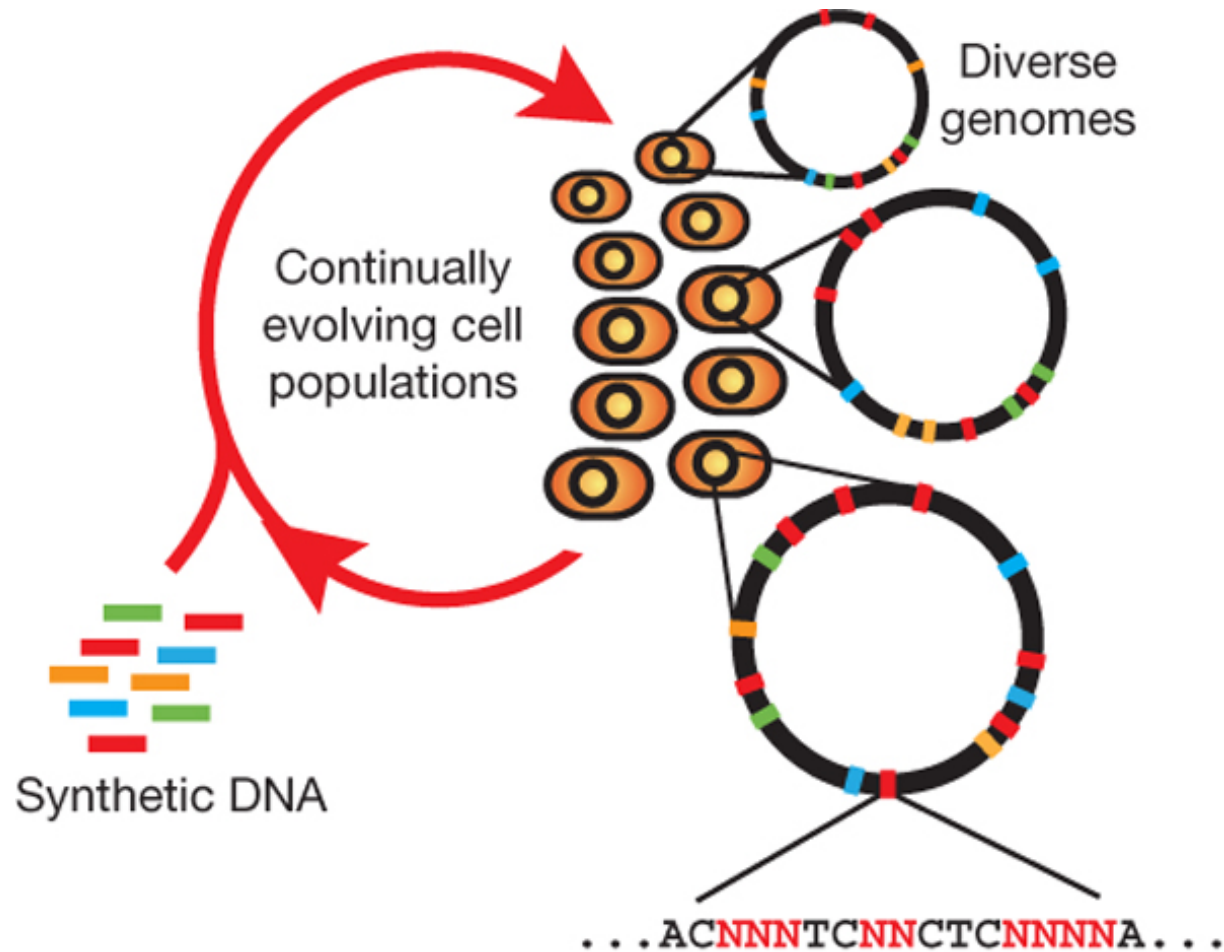
PCR

DNA Shuffling
(Sexual PCR)
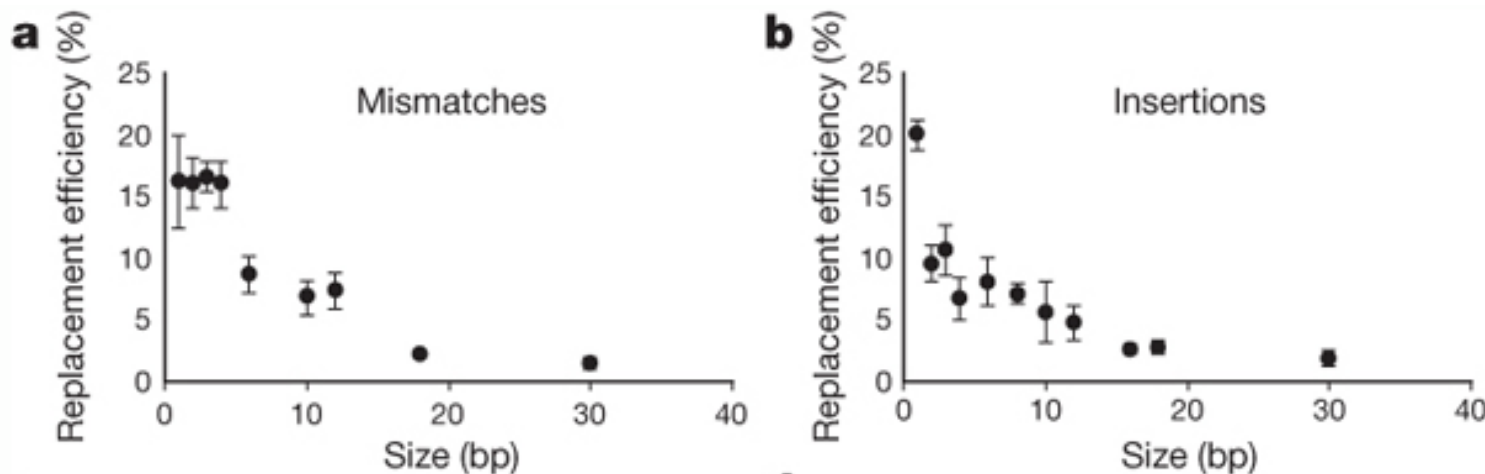
MAGE Directed Evolution

Allows for Rapid and Continuous
Generation of Genetic Changes

MAGE - Multiplex Automated Genome Engineering

MAGE Efficiency

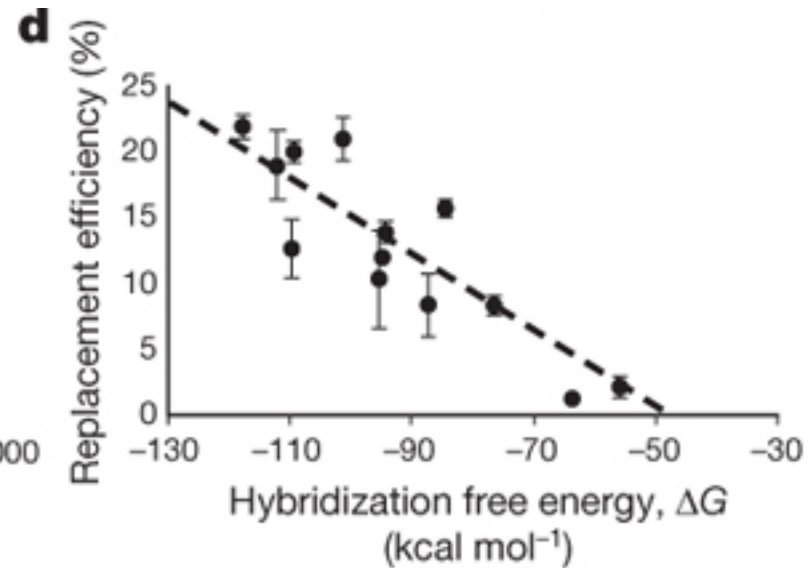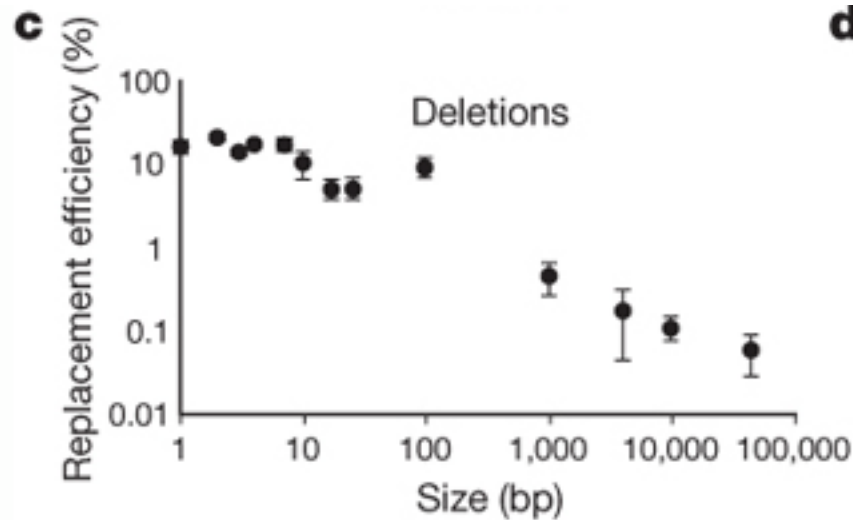## Over 30% genetic modification per cell every 2-2.5 hours

Mismatch and Insertion Efficiency

∝

Amount of homologous Sequence

MAGE Efficiency

Deletion Probability Sequer $\propto$

Size of the Deletion

ΔG between Oligo and Chromosome

$\propto$

Replacement Efficiency

Sequence Diversity over Multiple MAGE cycles

Increased divergence from wild type demonstrated with increased MAGE cycles
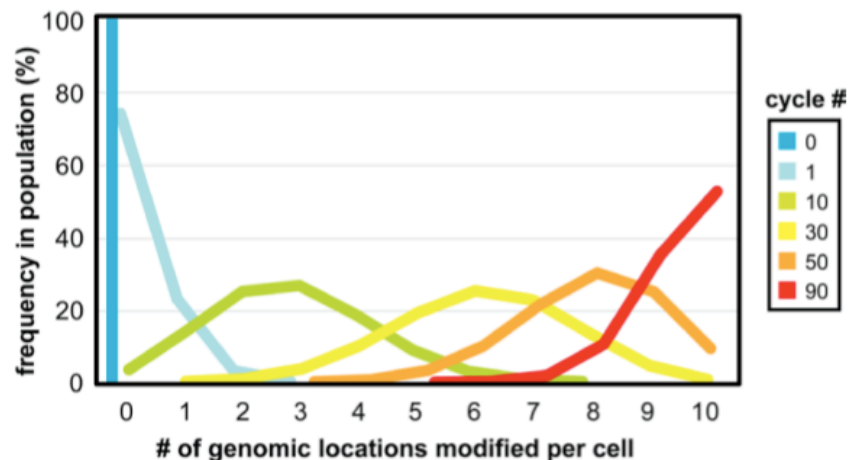
MAGE Diversity

- Degree of Sequence Variation:
    - The number of loci
    - The number of MAGE cycles
- The frequency locus modification can be demonstrated through a binomial distribution:

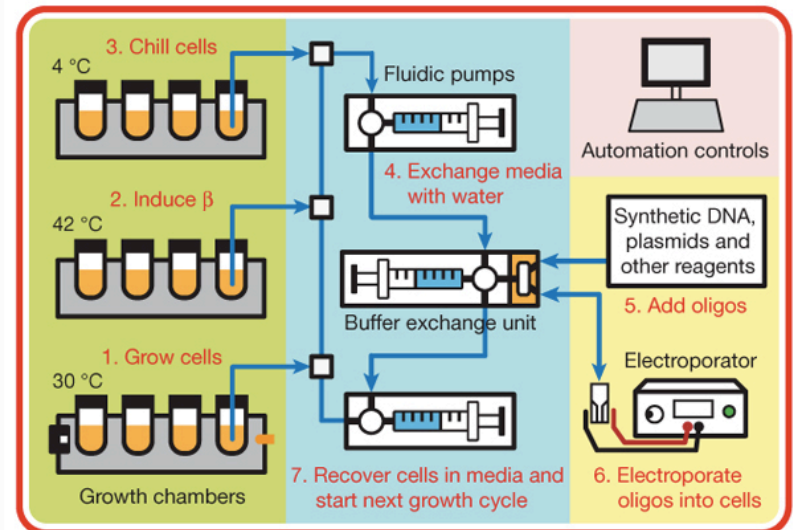$$P(K,N) = \sum_{j=0}^{K} \binom{K}{j} \left((1-M)^N\right)^{(K-j)} \left(1-(1-M)^N\right)^j,$$
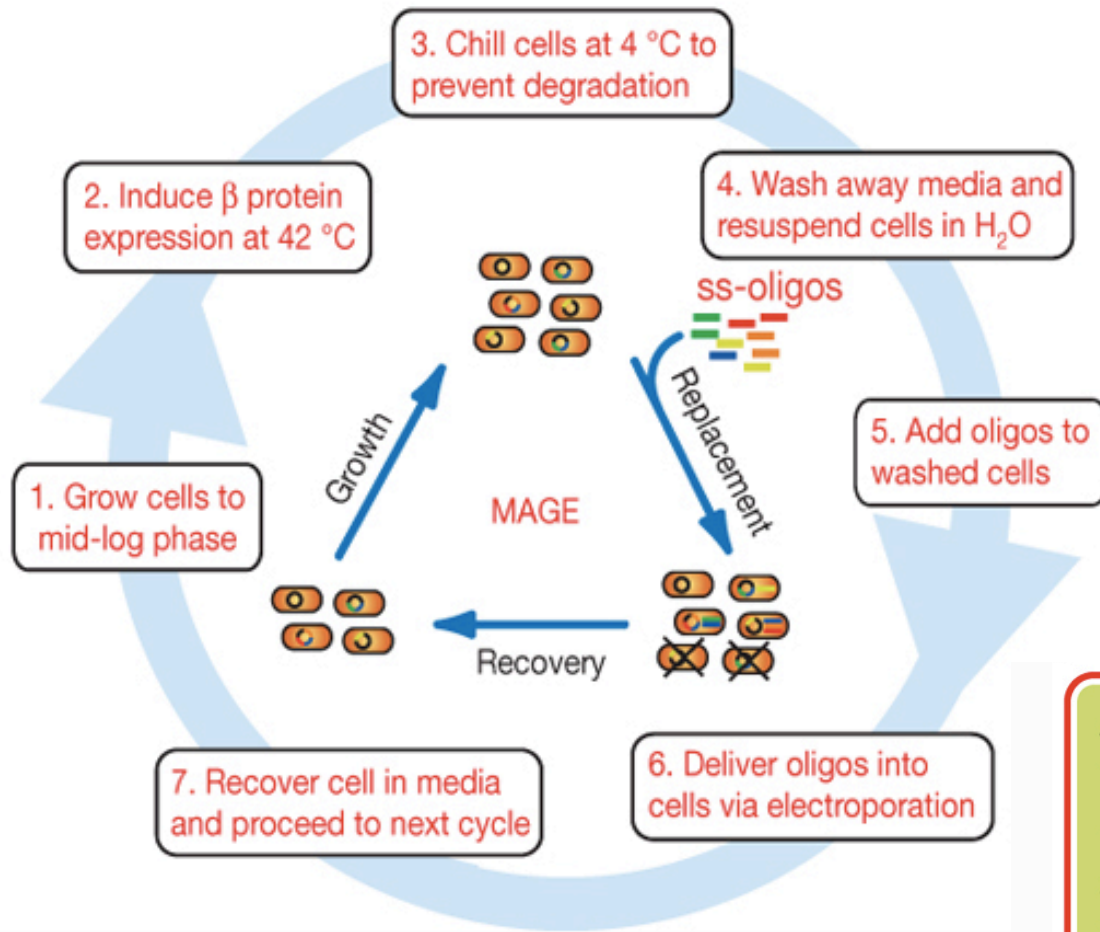
M - Mutation Rate

N - MAGE Cycles

K - Targeted Loci

Automated MAGE

Ex. Lycopene Production

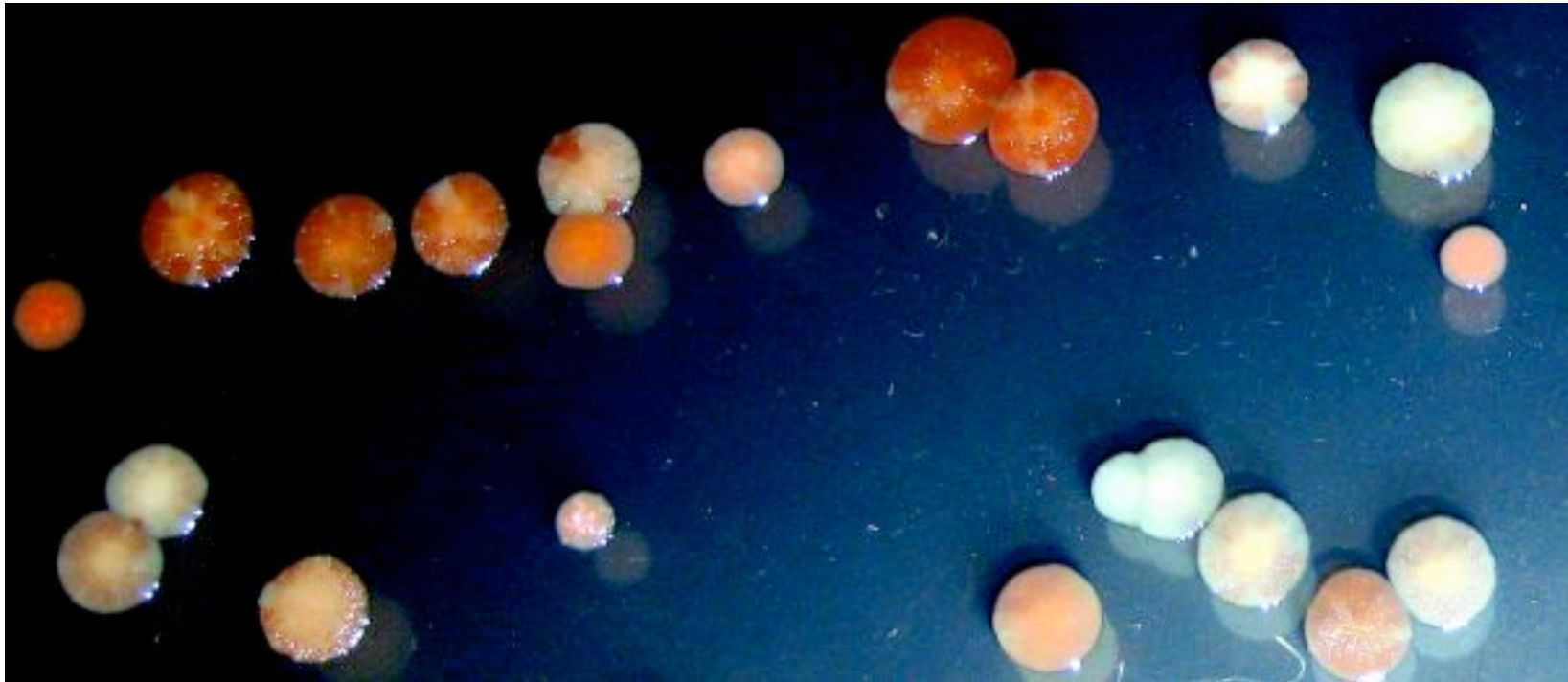**Tune expression**

Gene knockouts

24 genomic targets
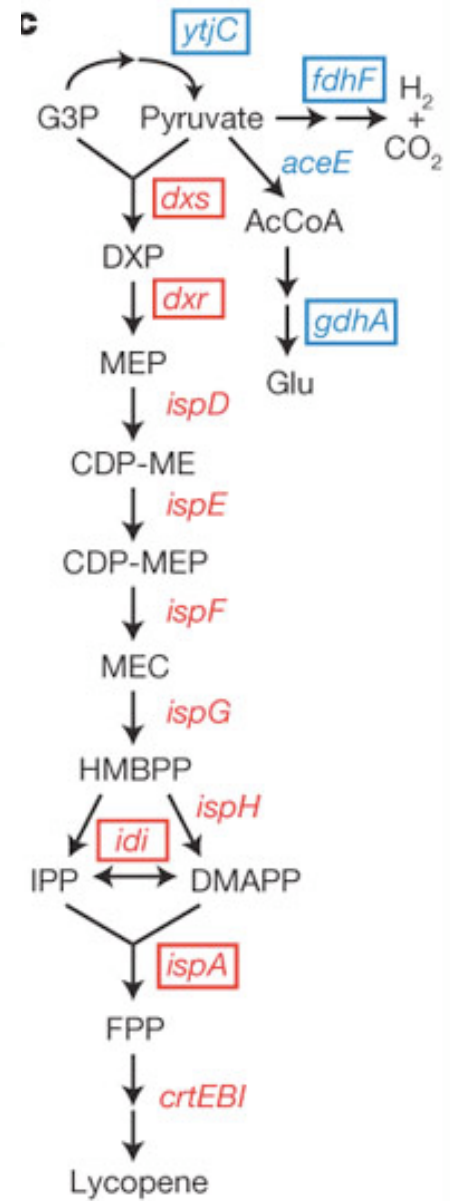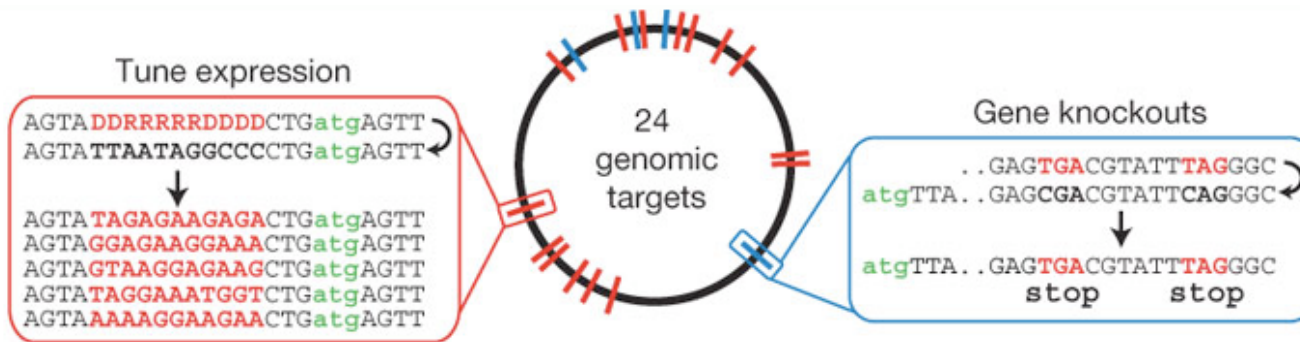
DXP Biosynthesis Pathway:

- 20 Genes known to increase production (RED)

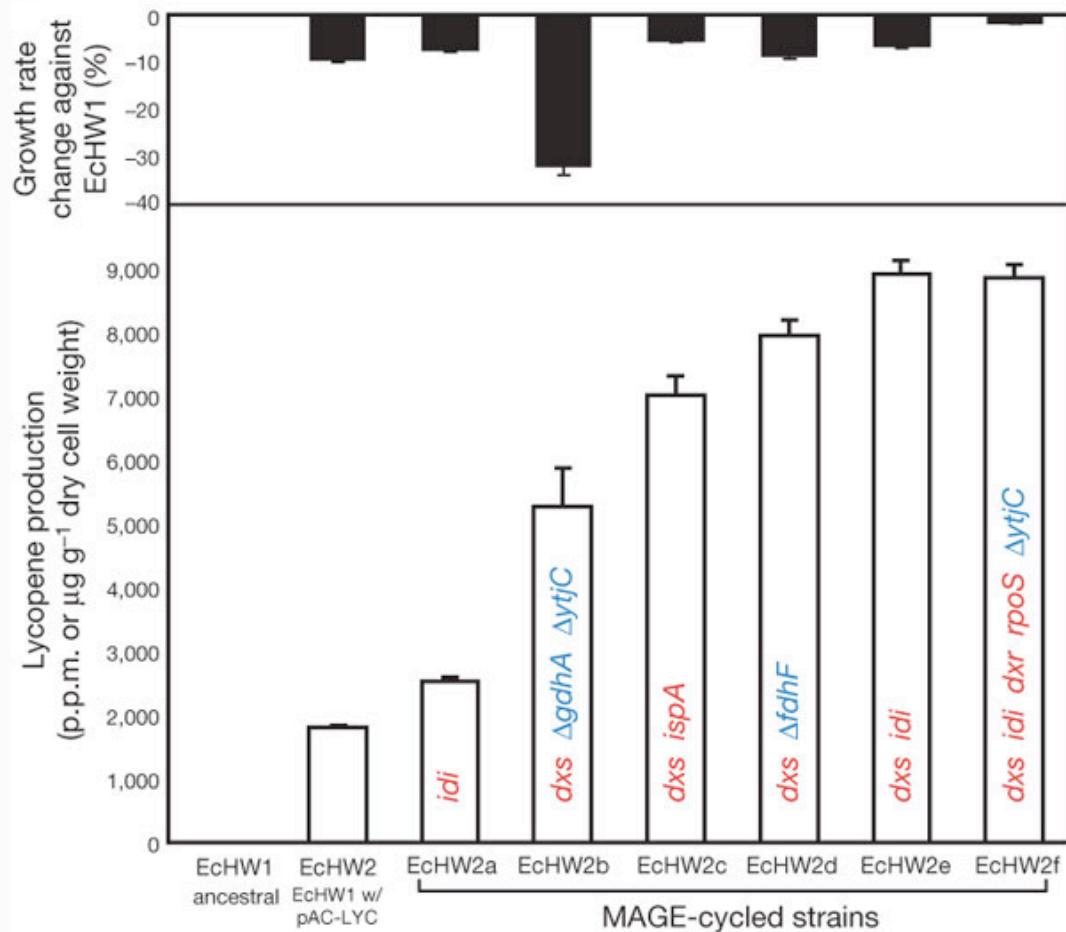- 4 genes known to inhibit production (BLUE)

Total: 15 billion variants

Ex. Lycopene Production

Red - Lycopene production
White - Little Production

Ex. Lycopene Production

1. Characterize isolate mutants to to asses different tuning pathways.

2. Observe the effect of multiple mutations.

Can use MAGE to specifically target specific genes through the use of oligos with defined sequence

MAGE Possibilities

- Modify and Sample Different Strength RBSs.

- Inactivate Protein Coding Sequences.

- Enhance incorporation of non native amino acids.

 - Construct safer multi-virus strains.

Looking Ahead

- Ethically how will the MAGE technique be used in higher organisms and medicine?

- Could this create a highly mutating strain? What if this escaped into the wild?
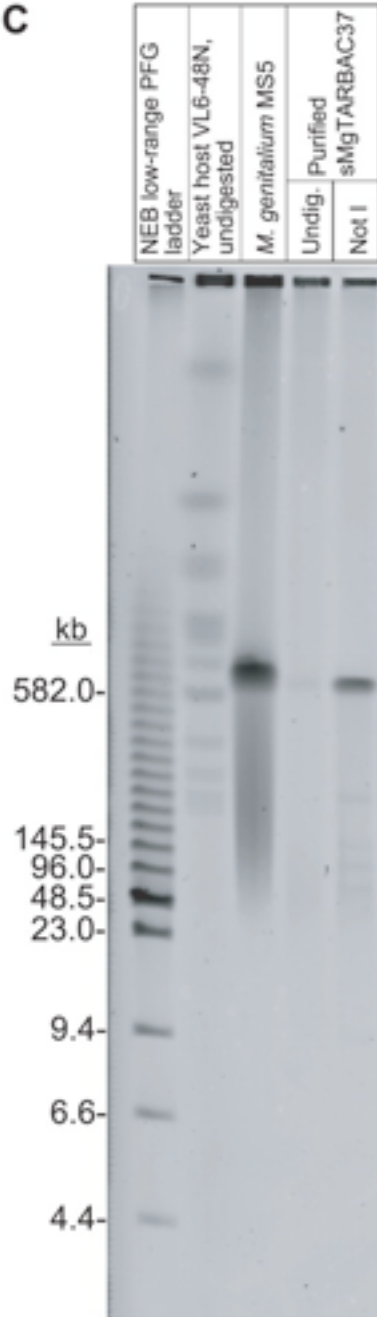
Complete Chemical Synthesis, assembly, and Cloning of a *Mycoplasma genitalium* Genome

By Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C, Young L, Noskov VN, Glass JI, Venter JC, Hutchison CA 3rd, Smith HO.

Genome Assembly
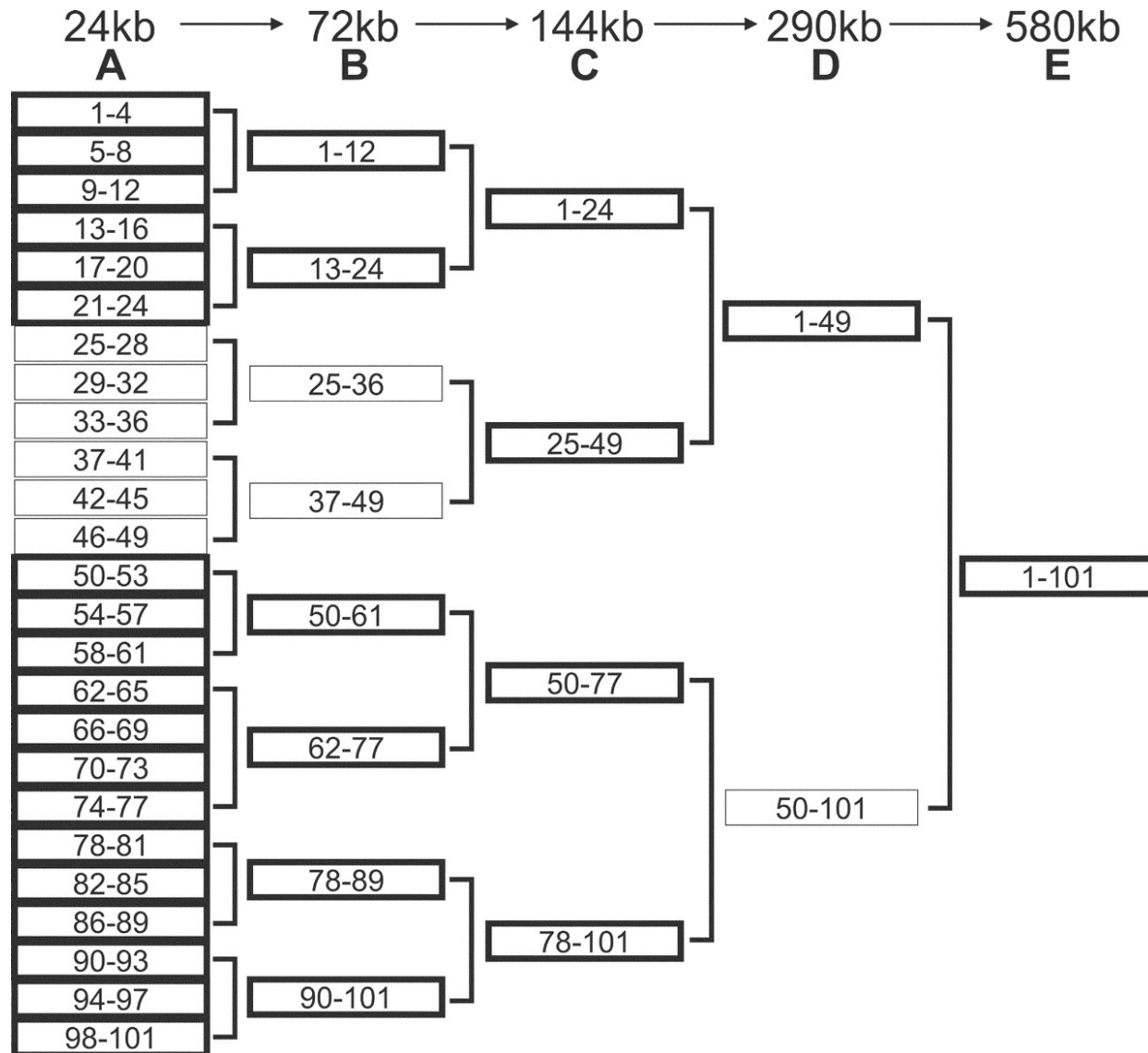
Confirmation of 580kb Genome through CHEF Gel Analysis.