Precise Manipulation of Chromosomes in Vivo Enables Genome-Wide Codon Replacement

Isaacs, Farren J. et al.

Science, Vol 333, 2011

Presented by: PJ Velez

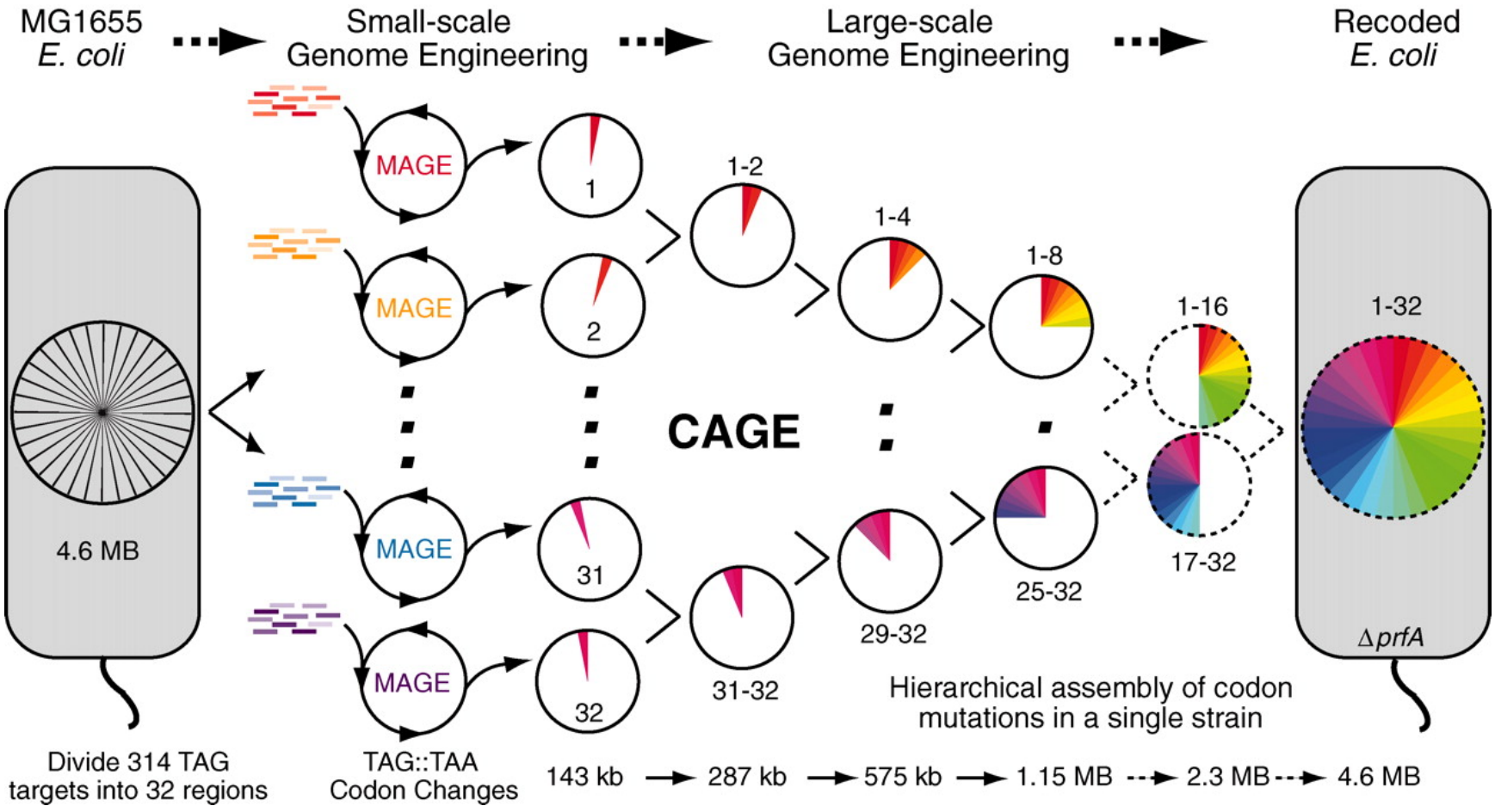
Useful Definitions

- Conjugative Assembly genome engineering (CAGE) Method - Large Scale Engineering
- Multiplex Automated Genome Engineering (MAGE) Method – Small Scale Engineering
- λ Red Protein- Proteins that promote recombination and are mutagenic.
- RF1/RF2 – Release factors in *E. Coli* that recognize stop codons

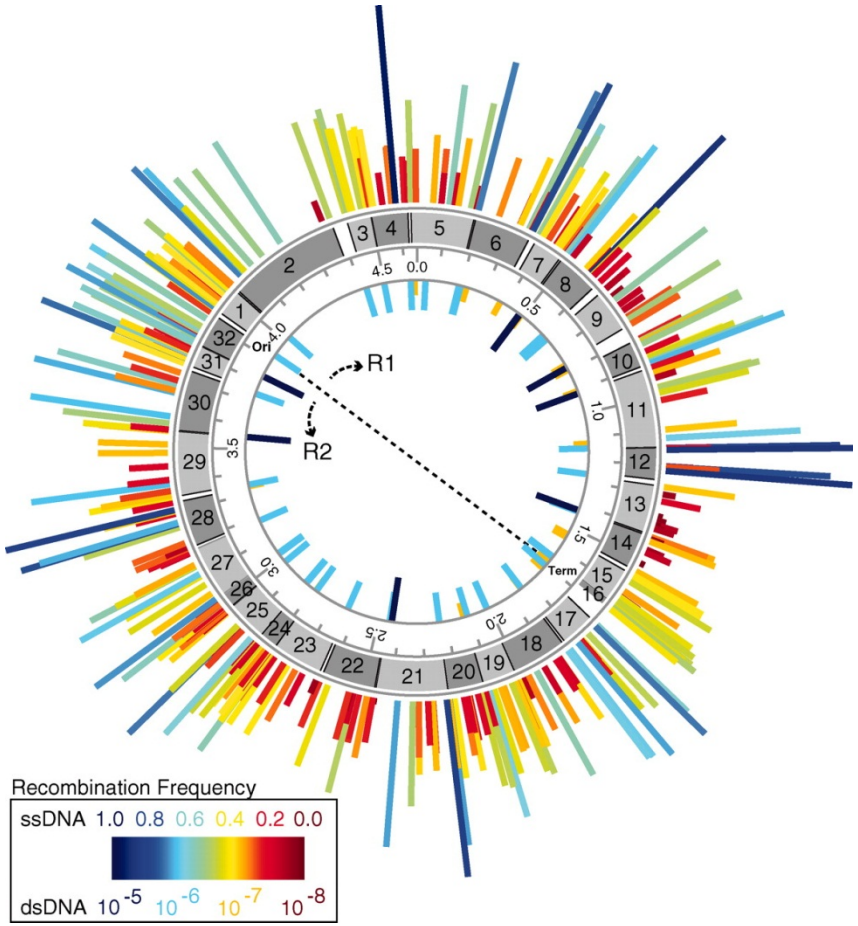
Goals and Motivation

- Long Term Goal: Successfully modify genetic code
- Long Term Impact:
  - Novel Biological System Properties
  - Easier incorporation of unnatural Amino Acids
- Short Term Approach:
  - Replace all TAG stop codons with TAA in viable *E. Coli*
    - RF1 deletion mutant still viable

Overall Strategy

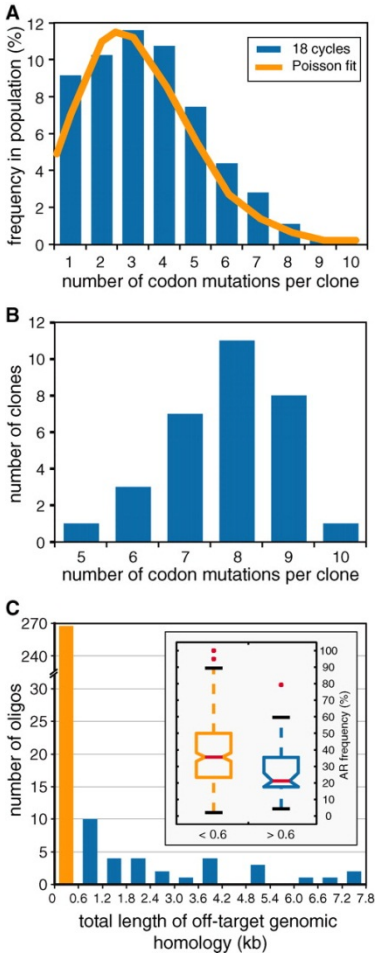


MAGE



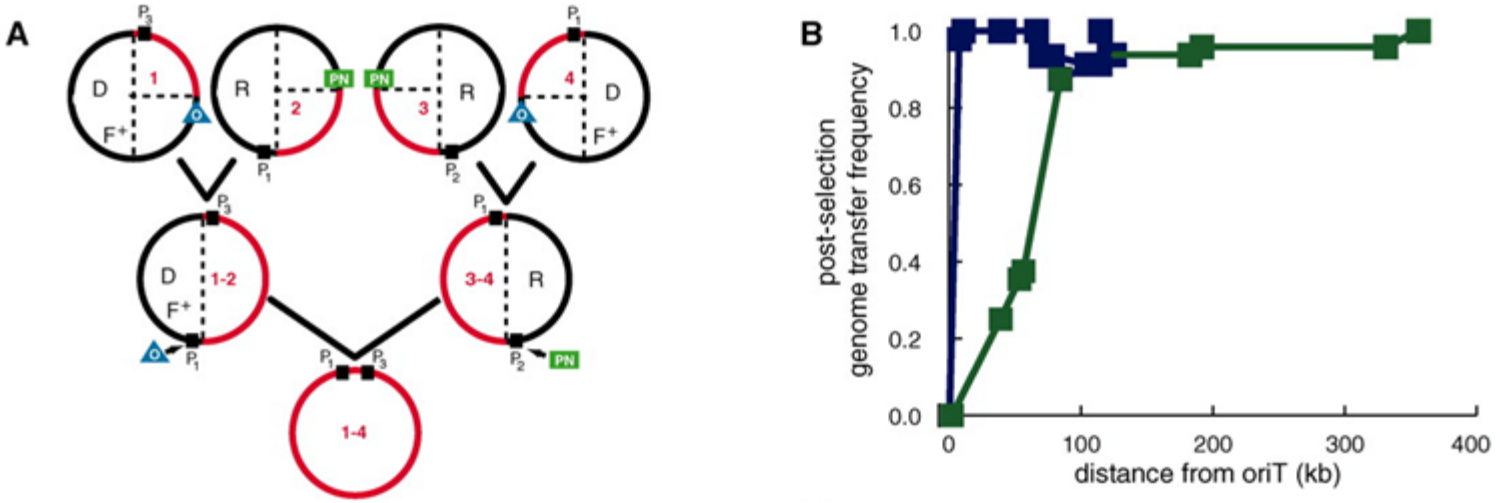
- Genome split into 32 regions of <10 genes with TAA codon
- 18 MAGE Cycles
- Assays to identify greatest number of codon conversion and measure frequencies

MAGE



- Some Cells more susceptible to mutations
- Top clone found for each region
- Also looked at potential unintended mutations
  - BLAST
  - Sanger Sequencing

CAGE



- 32 clones put into pairs
  - Donor Strand
    - *oriT-kan* and positive selectable marker
  - Recipient Strand
    - Positive-Negative selectable marker and different positive marker

Final Experiments

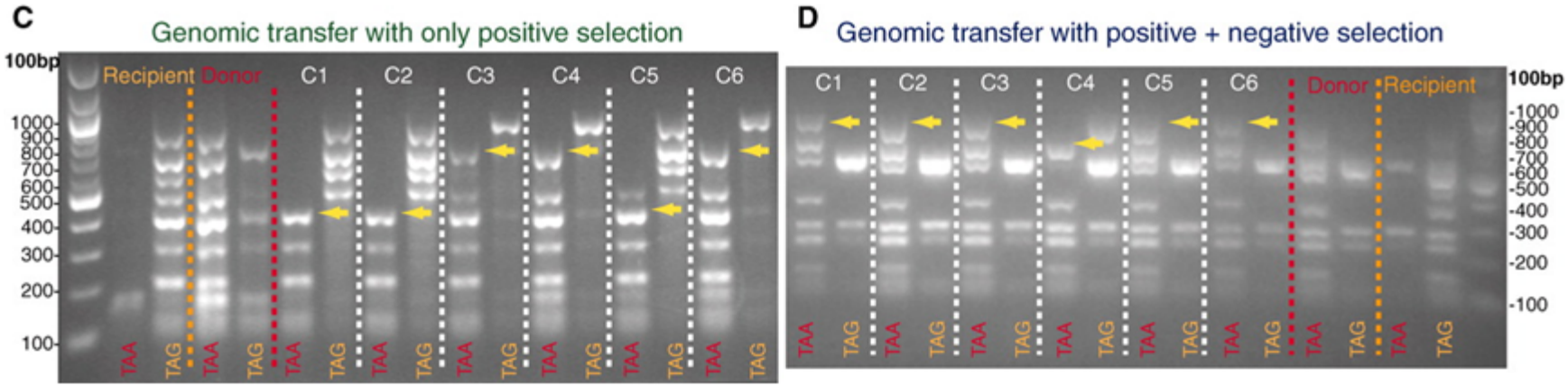
- Performed genome sequencing on two dysfunctional strains and a control after MAGE/CAGE
  - 1 error per genome per 9 replications
- Hypergeometric distribution
  - Determine enrichment level across three strains
- Problem: No figures or data shown in paper
  - Buried in 90 pages of supplement

Conclusions and Future Directions

- Successfully replaced all TAG occurrences with TAA codons
- Improve future genome engineering efforts
  - Dynamic method to introduce change in cell
- Help refine existing genome annotation
- Already been cited four times
  - Came out last July

Supplementary Slides for Discussion

Issues with Final Figure

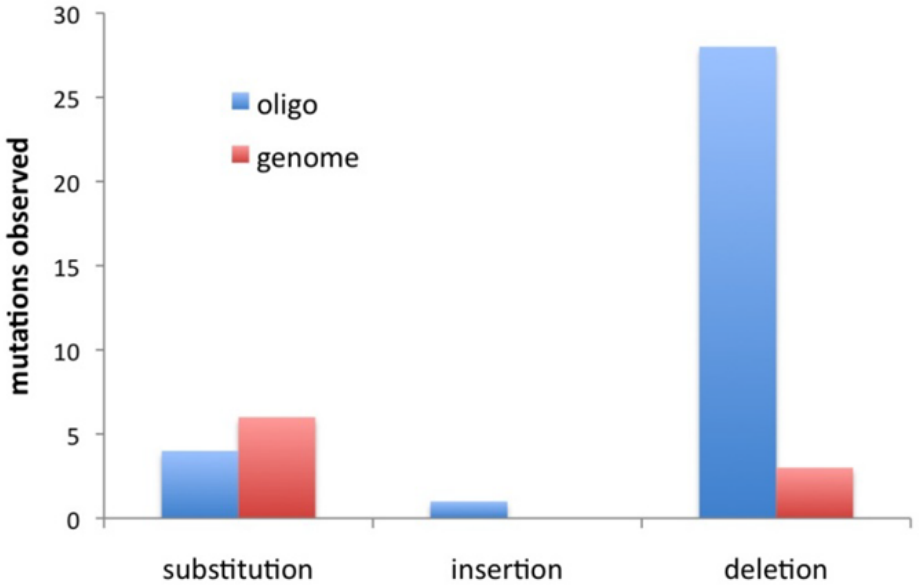


- Overall layout not really specified
  - Media?
  - Order of Rows?
  - Yellow Arrows?

Other points for Discussion

- Successfully show what they set to?
- Final Experiments worthy of being published?

MAGE



- Sanger Sequencing verified presence of conversion and secondary mutations
- Look at 300 bp surrounding replaced site