Design, synthesis, and testing toward a 57-codon genome

Celine Chang and Archana Kikla

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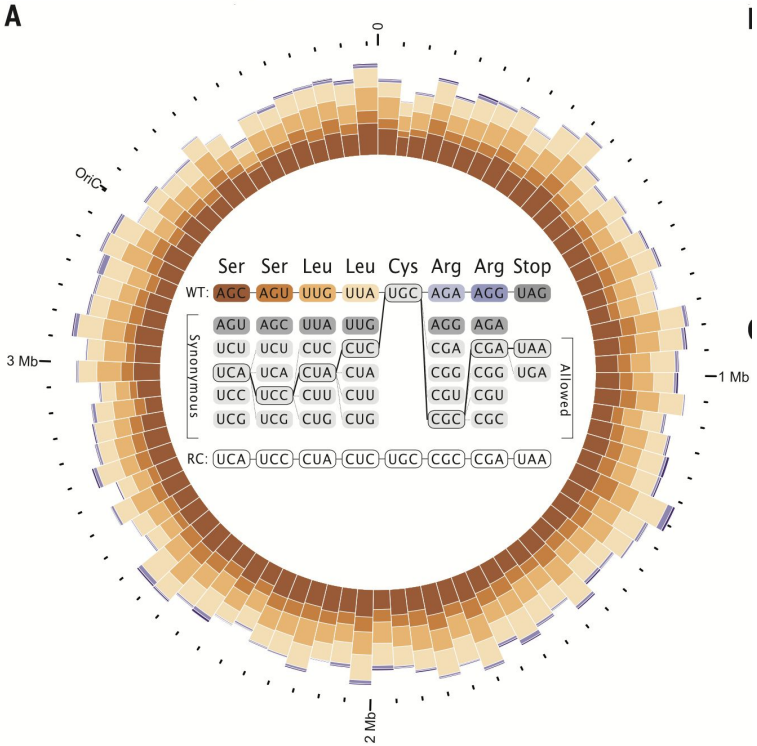
Objectives

- Create a virus-resistant biocontained bacterium for industrial applications
- Make over 60,000 genome changes to replace seven codons at once
- Create functional *E. coli* genome using only 57 codons instead of 64

Why?

- Phage resistance
- Virus resistance
- Basic Science Research
  - Large-scale codon exchange
- Biocontainment

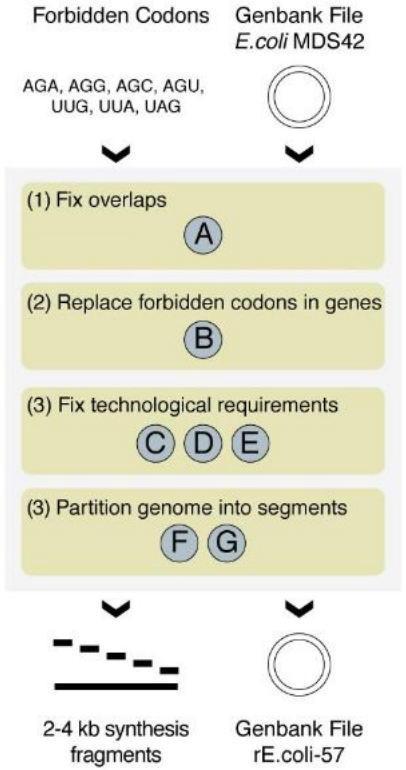
What?



	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

How codons were selected for recoding

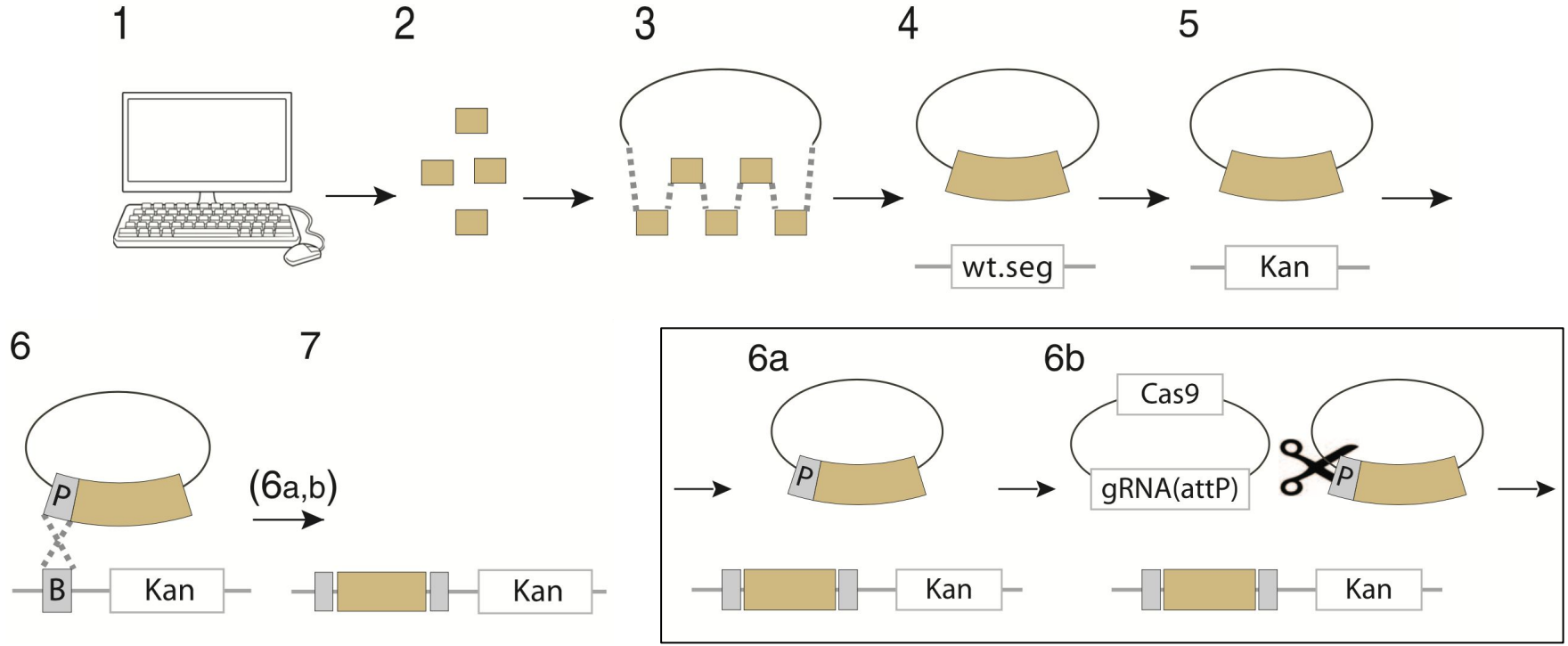
- UAG (stop)
  - Previously replaced genome-wide
- AGG and AGA
  - Rarest codons
- AGC, AGU, UUG, UUA
  - Anticodon is not recognized as a tRNA identity element by endogenous aminoacyl-tRNA synthetases upon codon reassignment



Codon Selection Criteria

- Minimize disruption of biological motifs (RBS, mRNA secondary structure)
- Conserve relative codon usage to meet translational demand
- If synonymous codon not found - relax constraints until alternative found
    - Iterative process

Recoding Methods

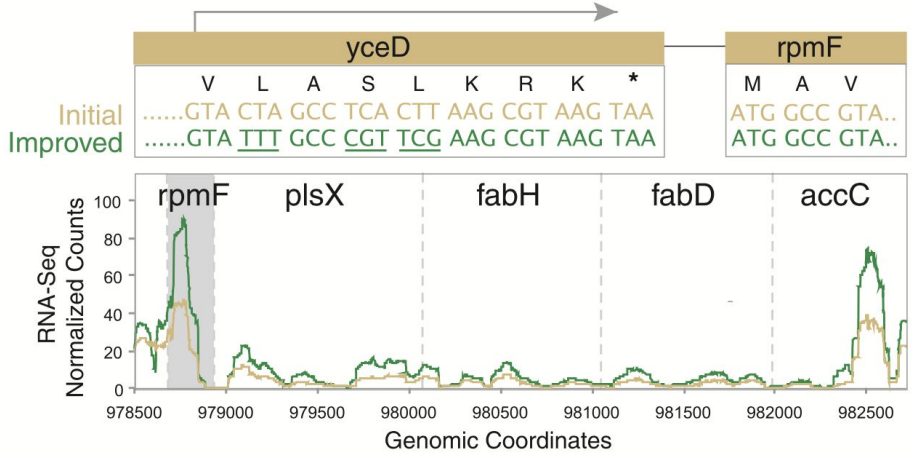


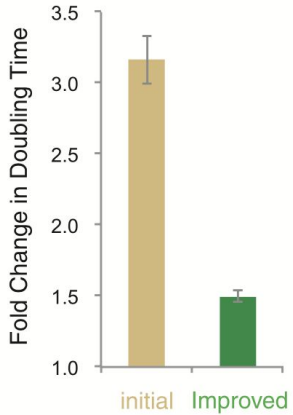
Recoding Methods Cont.

- Single editing codon strategies
- Multiple alleles
  - MAGE
  - Cas9
- Advantages
  - Modular process
  - Cost
- Limitations
  - Methylation machinery of *E. coli* MDS42 still intact, could influence gene expression

Reduced fitness in recoding genes

- Segment 21
  - Insufficient expression of recoded fatty acid biosynthesis operon *rpmF-accC*
  - Codon changes in upstream *yceD* disrupted operon promoter
- Segment 84
  - Three genes caused fitness impairment
  - Including recoded gene *ytfP* (large deletion)





Experimental Conclusions

- Successfully replaced 7 codons (62,214 instances) throughout genome
- Only found problems with 13 of 2,229 genes tested
- 90% of tested essential genes retained functionality with limited fitness effect
    - Currently tested 2/3 of genome
- Can change genome at cost of $1 million