Genome engineering and evolution

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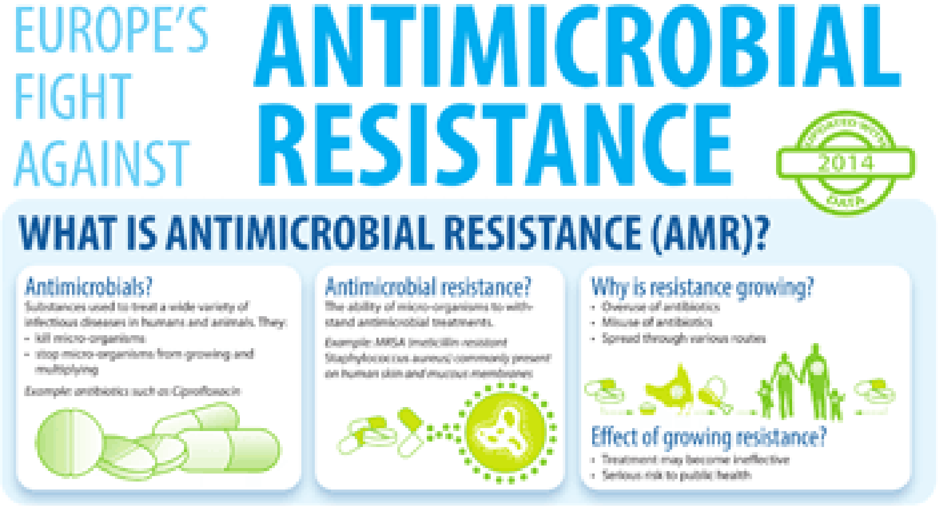
Evolution rapidly transforms our environment



EUROPE'S FIGHT AGAINST **ANTIMICROBIAL RESISTANCE**

2014

WHAT IS ANTIMICROBIAL RESISTANCE (AMR)?

Antimicrobials?

Substances used to treat a wide variety of infectious diseases in humans and animals. They:

- kill micro-organisms
- stop micro-organisms from growing and multiplying

Example: antibiotics such as Ciprofloxacin

Antimicrobial resistance?

The ability of micro-organisms to withstand antimicrobial treatments.

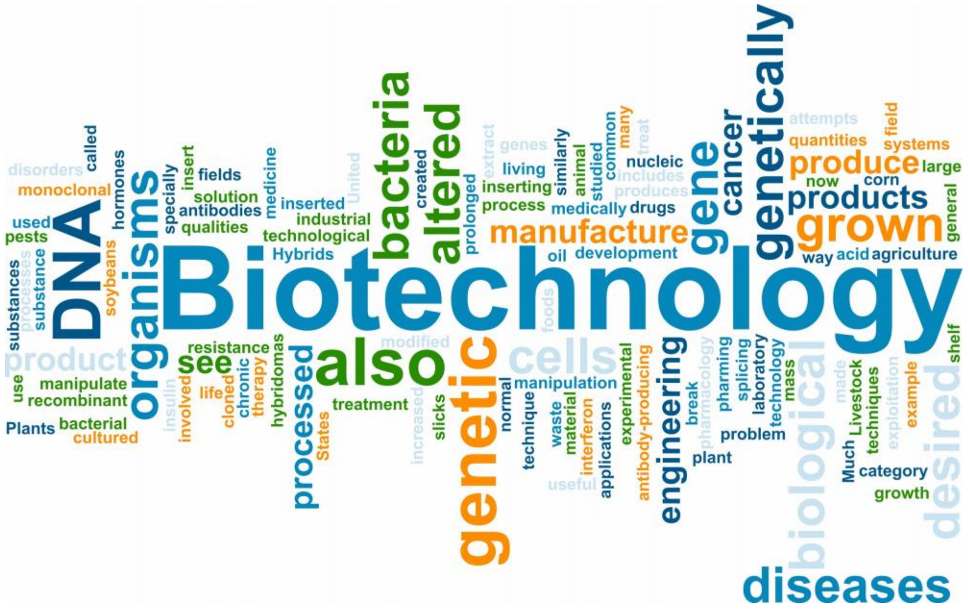
Example: MRSA (meticillin resistant Staphylococcus aureus) commonly present on human skin and mucous membranes

Why is resistance growing?

- Overuse of antibiotics
- Misuse of antibiotics
- Spread through various routes

Effect of growing resistance?

- Treatment may become ineffective
- Serious risk to public health



How to study evolution in real time?

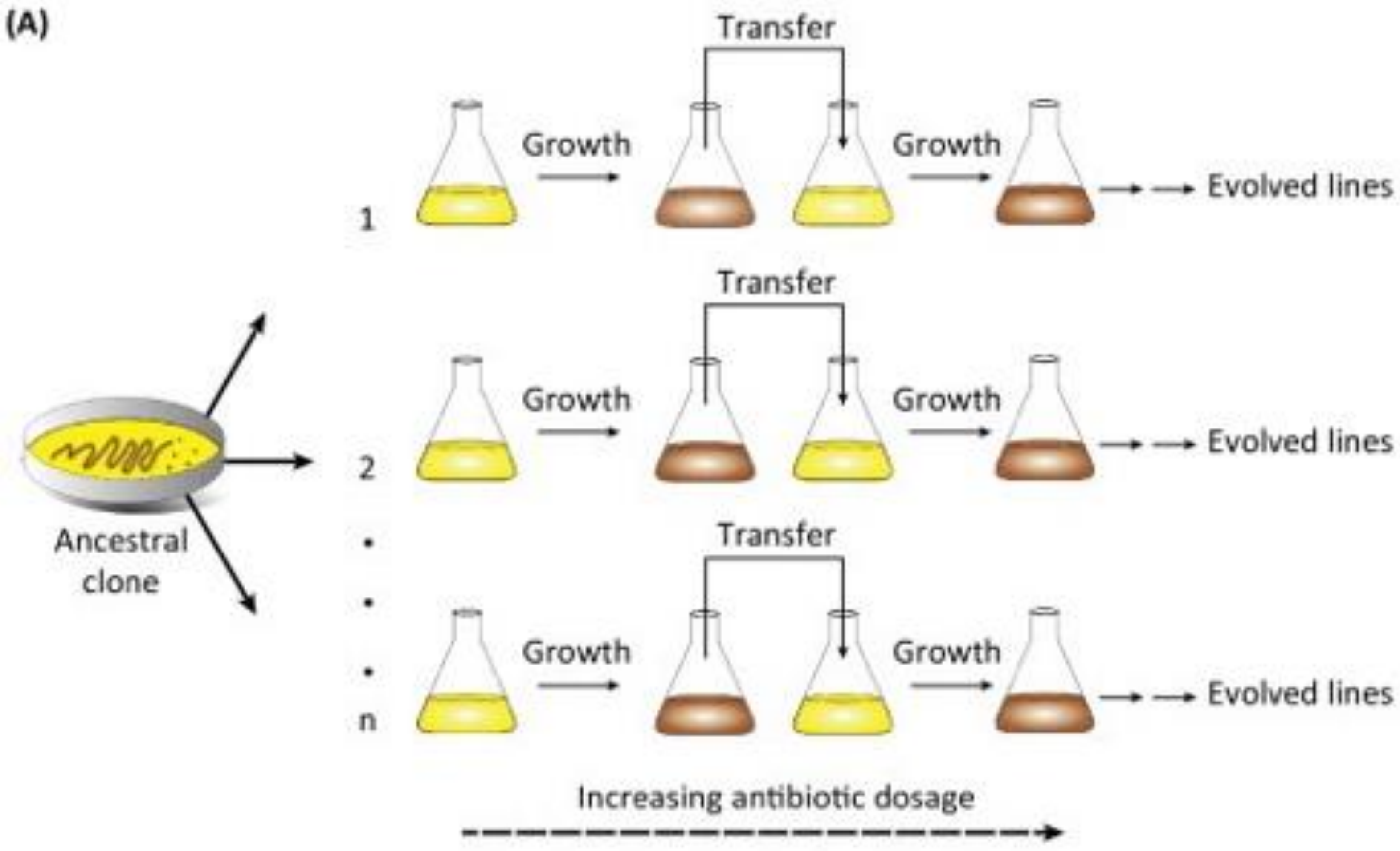
Laboratory evolution with microbes

Rapid cellular division

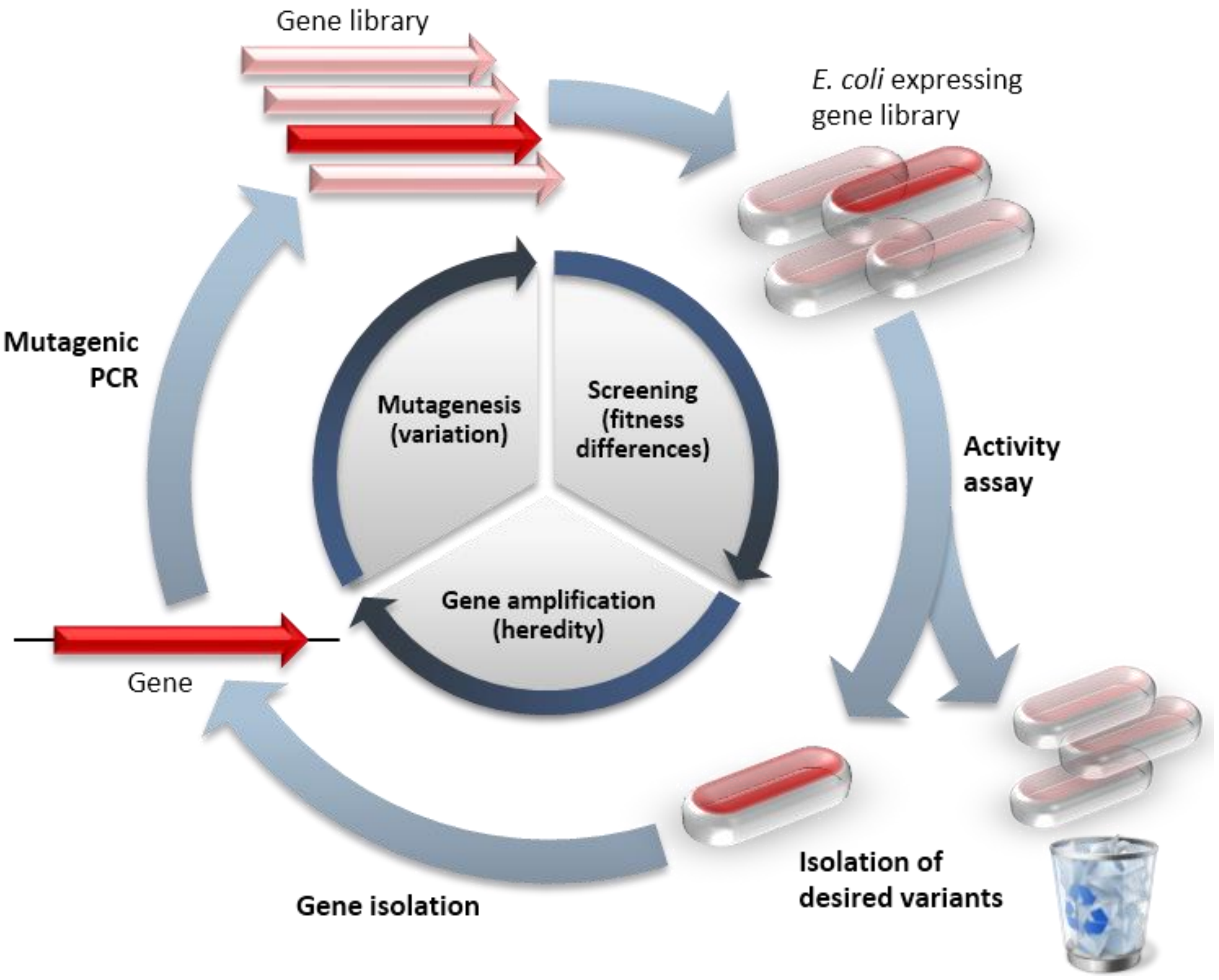
Large population

Freeze

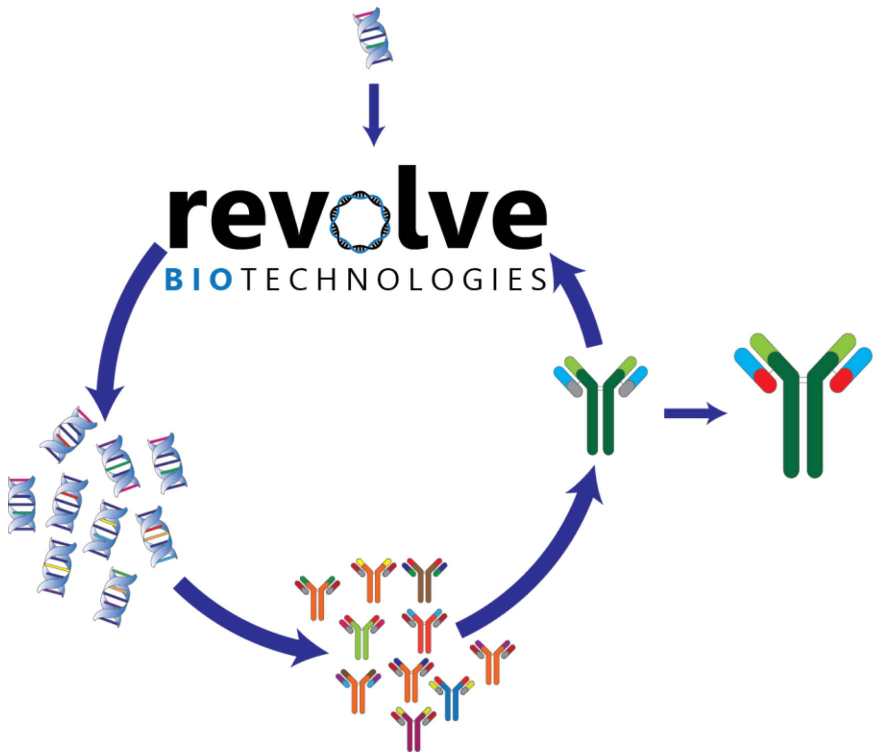
Laboratory evolution with microbes



Directed protein evolution



Biotech and industrial applications of directed evolution



Creative Biolabs

ThermoFisher
SCIENTIFIC

KAPABIOSYSTEMS
evolving better science

Pros and contras

Microbial evolution in the lab:

Changes across the whole genome

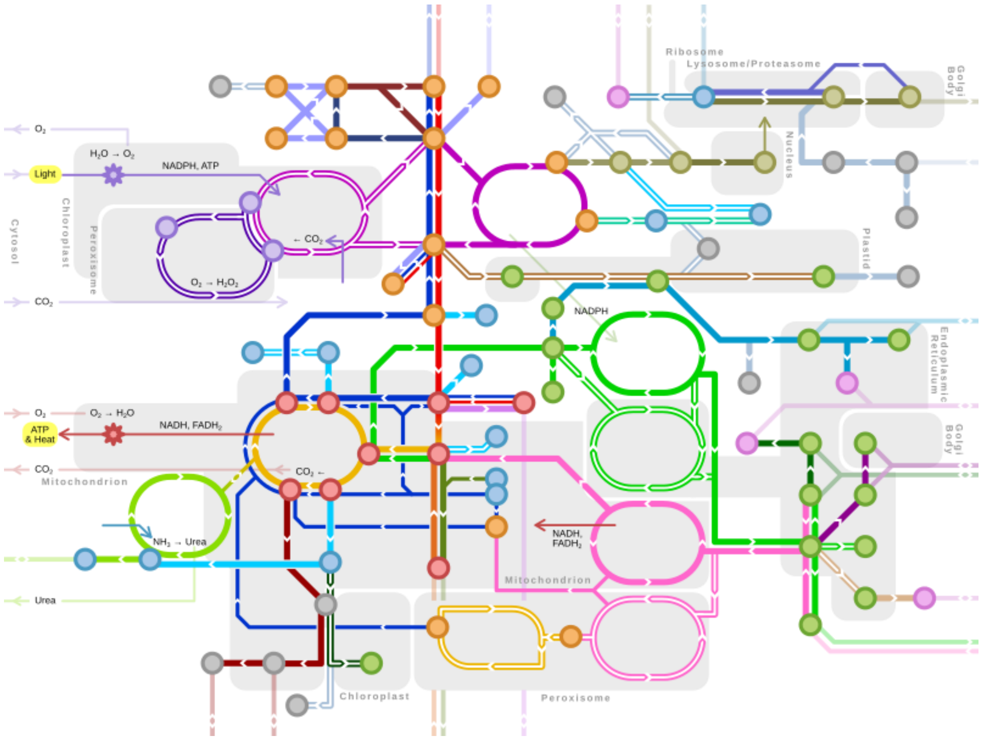
Slow

Directed protein evolution:

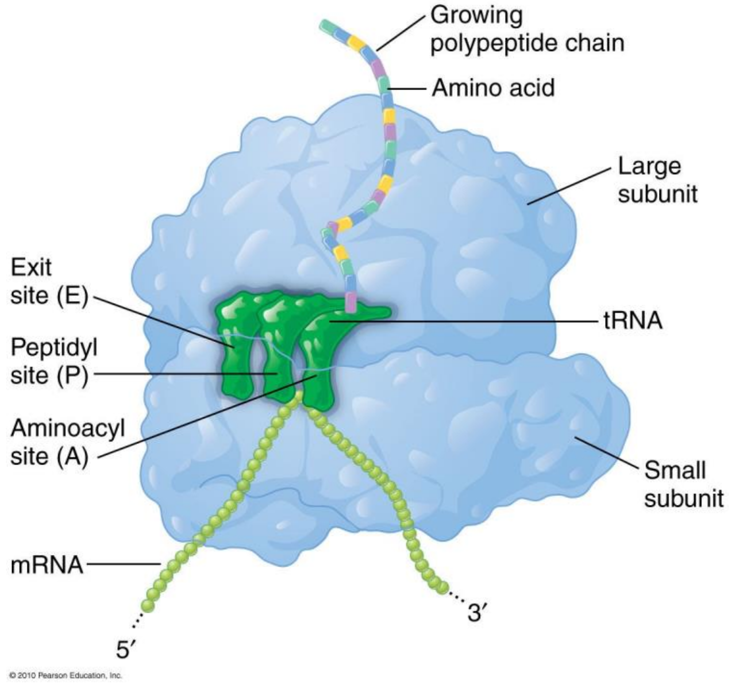
Typically a single protein

Rapid

How to study evolution of multi gene systems?

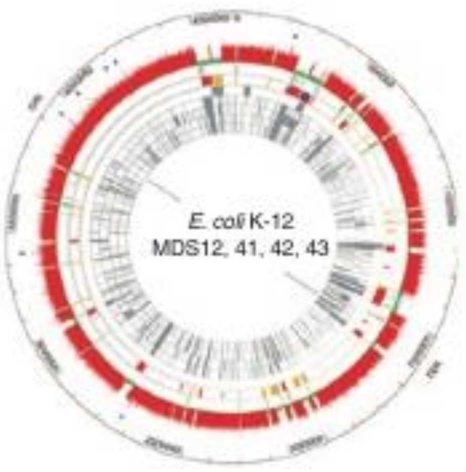


Gene networks

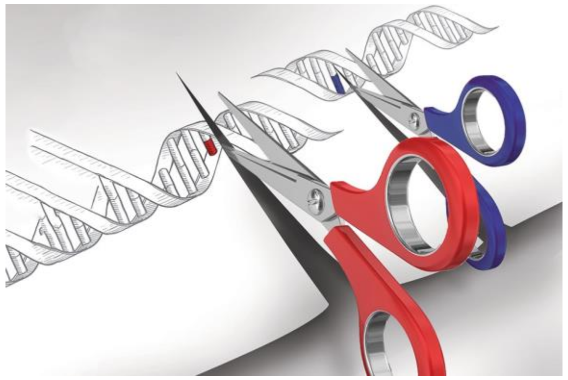


Protein complexes

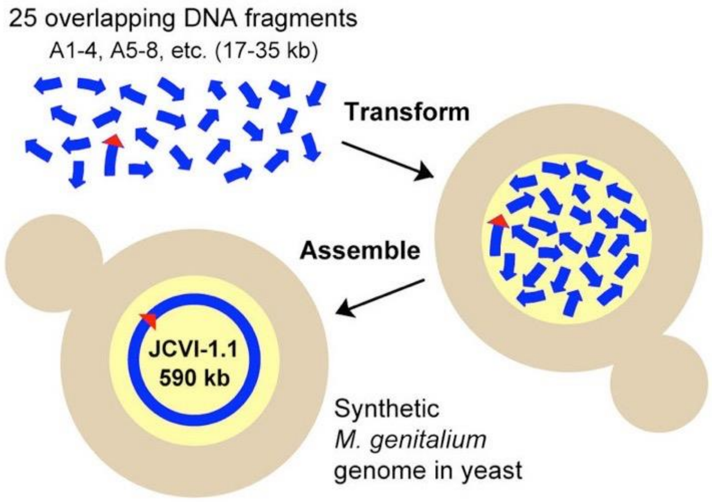
(Bacterial) genome engineering



Minimization



Editing



Synthesis

Goals

New biofuels, chemicals and drugs

Microbiome engineering

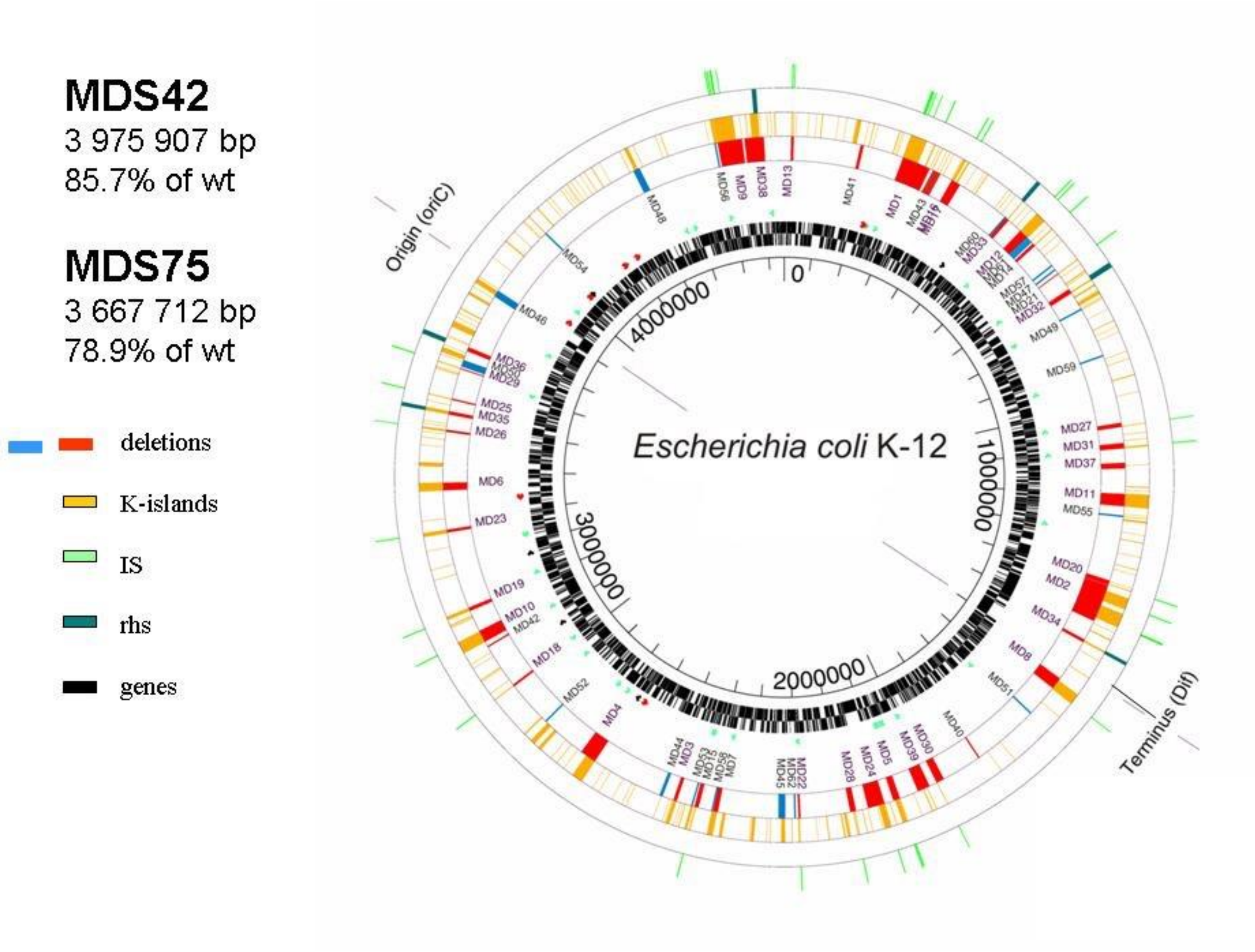
Novel strategies against antibiotic resistant pathogens

Rewiring of the genetic code

Genome reduction

György Pósfai

Genome reduction



Transposons

Horizontally acquired genes

Non-essential cellular processes

↓

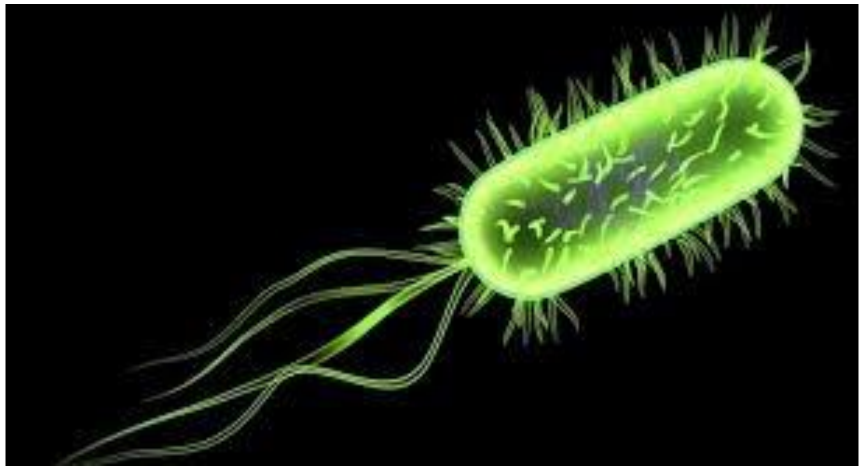
More stable genome, slow evolution
useful for biotechnology

Loss of 21% of the genes and still viable. Why?

Redundant elements

Function in diverse conditions

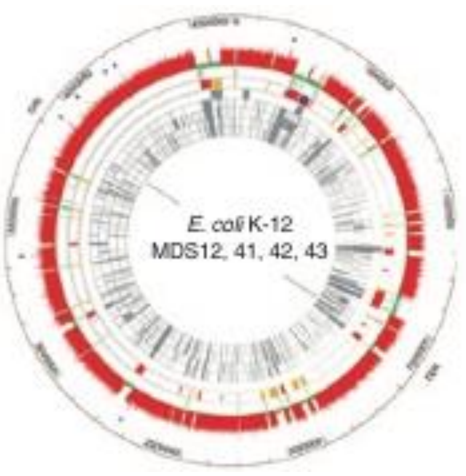
Large variation in genome size across bacterial species



Escherichia coli: ~4000 genes

Mycoplasma sp ~500-600 genes

Bacterial genome engineering



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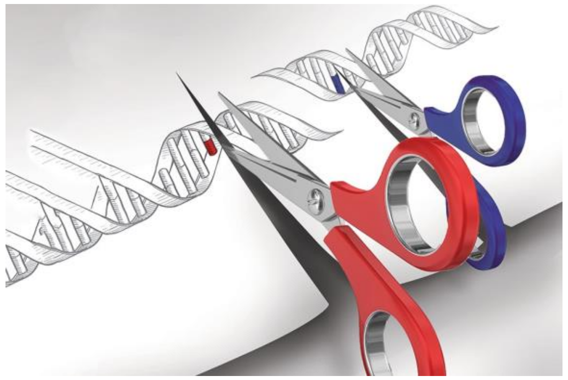

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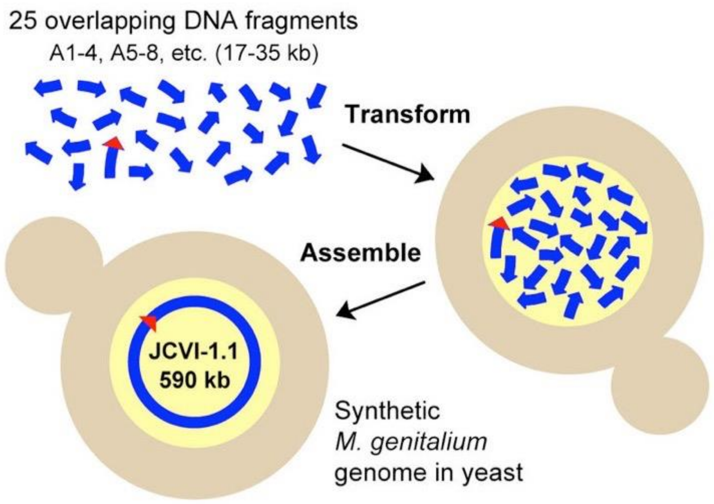
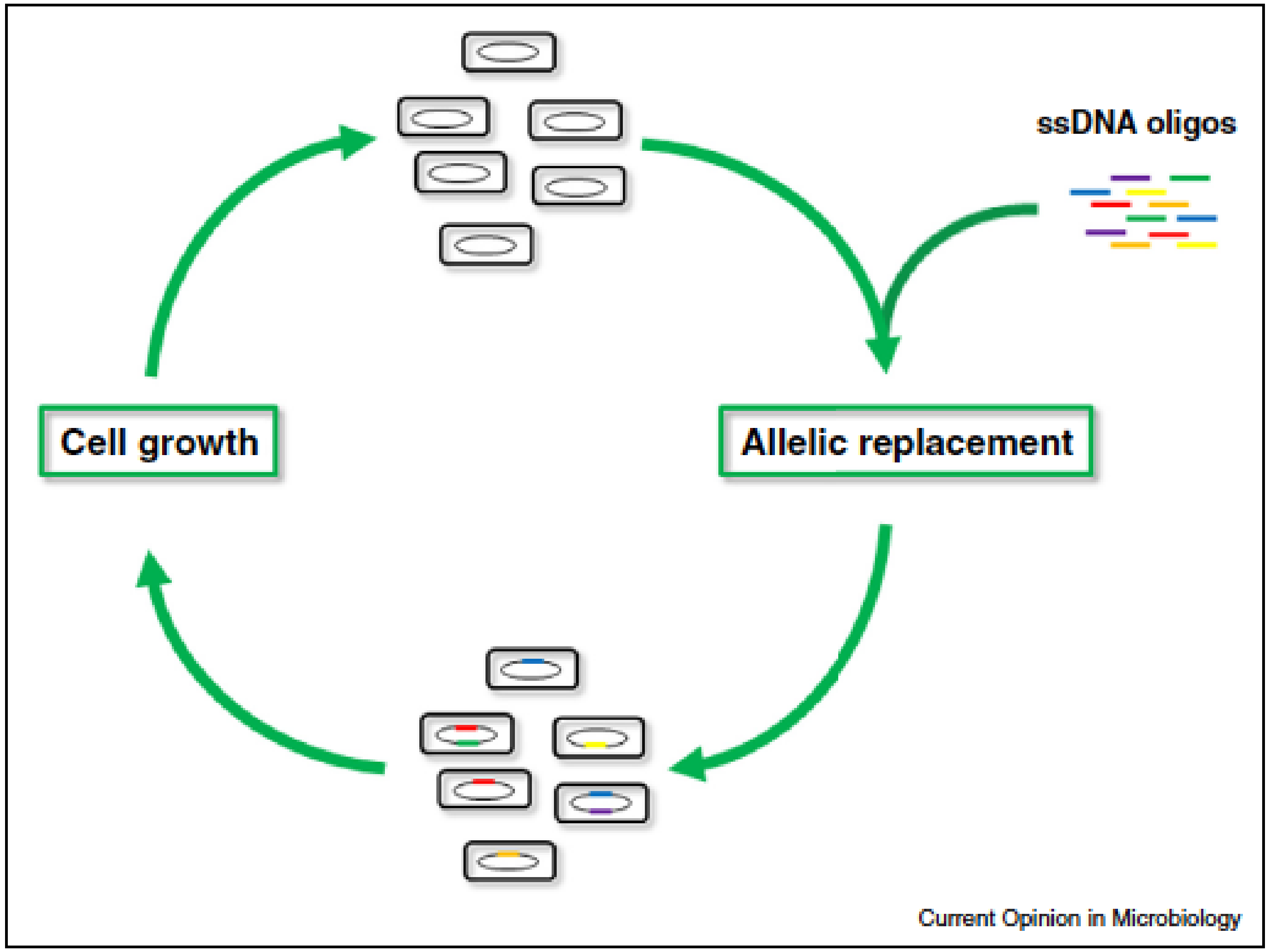


Synthesis

Multiplex automated genome engineering



Current Opinion in Microbiology

Design: DNA oligos encode the desired modifications

Electroporation: Bacterial cells are targeted with the DNA oligo pool

Cellular growth

Large, genetically diverse population at multiple loci

Geo Church and colleagues

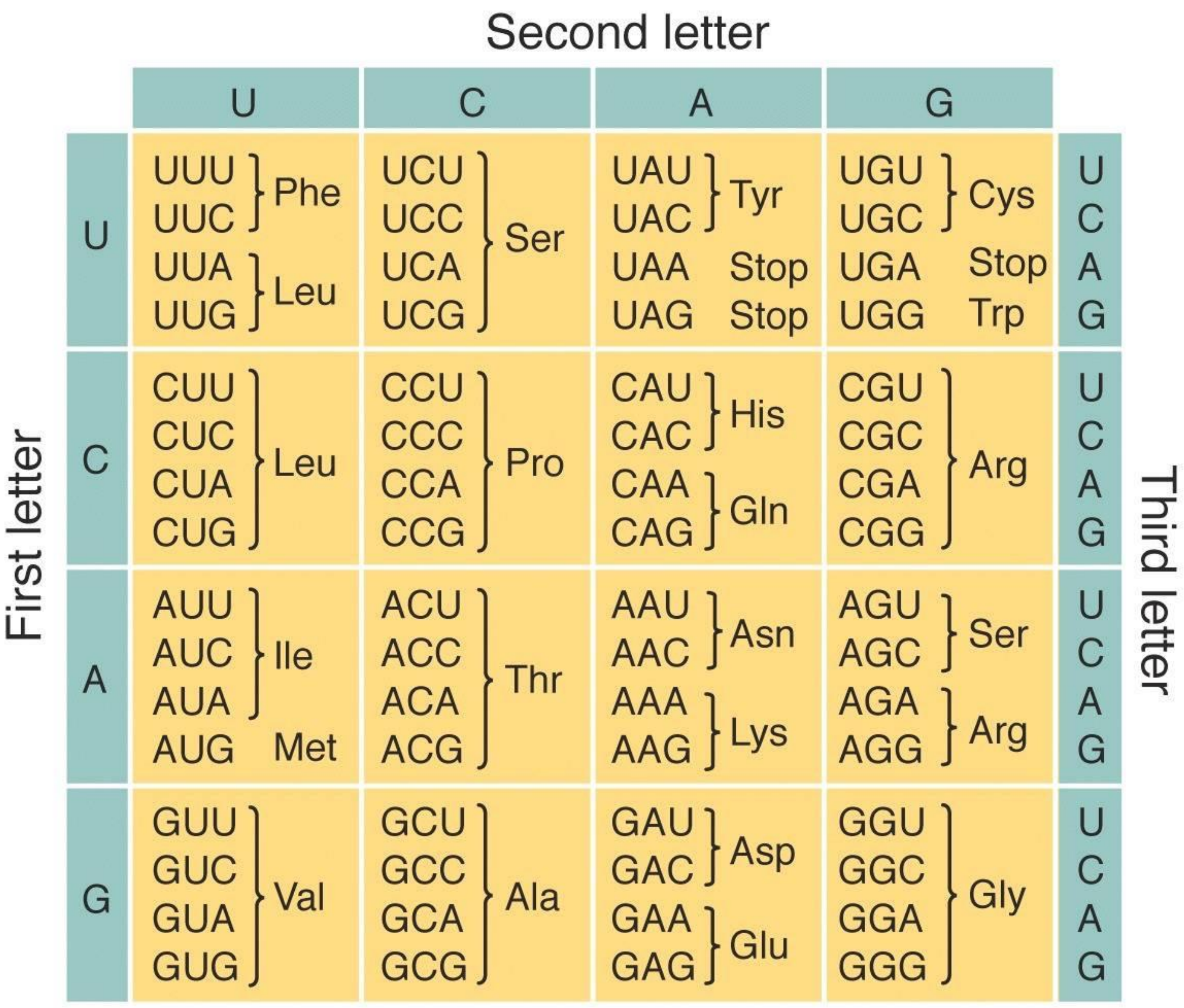
Metabolic engineering: Lycopene production in E. coli

~40 genetic modifications

3 days

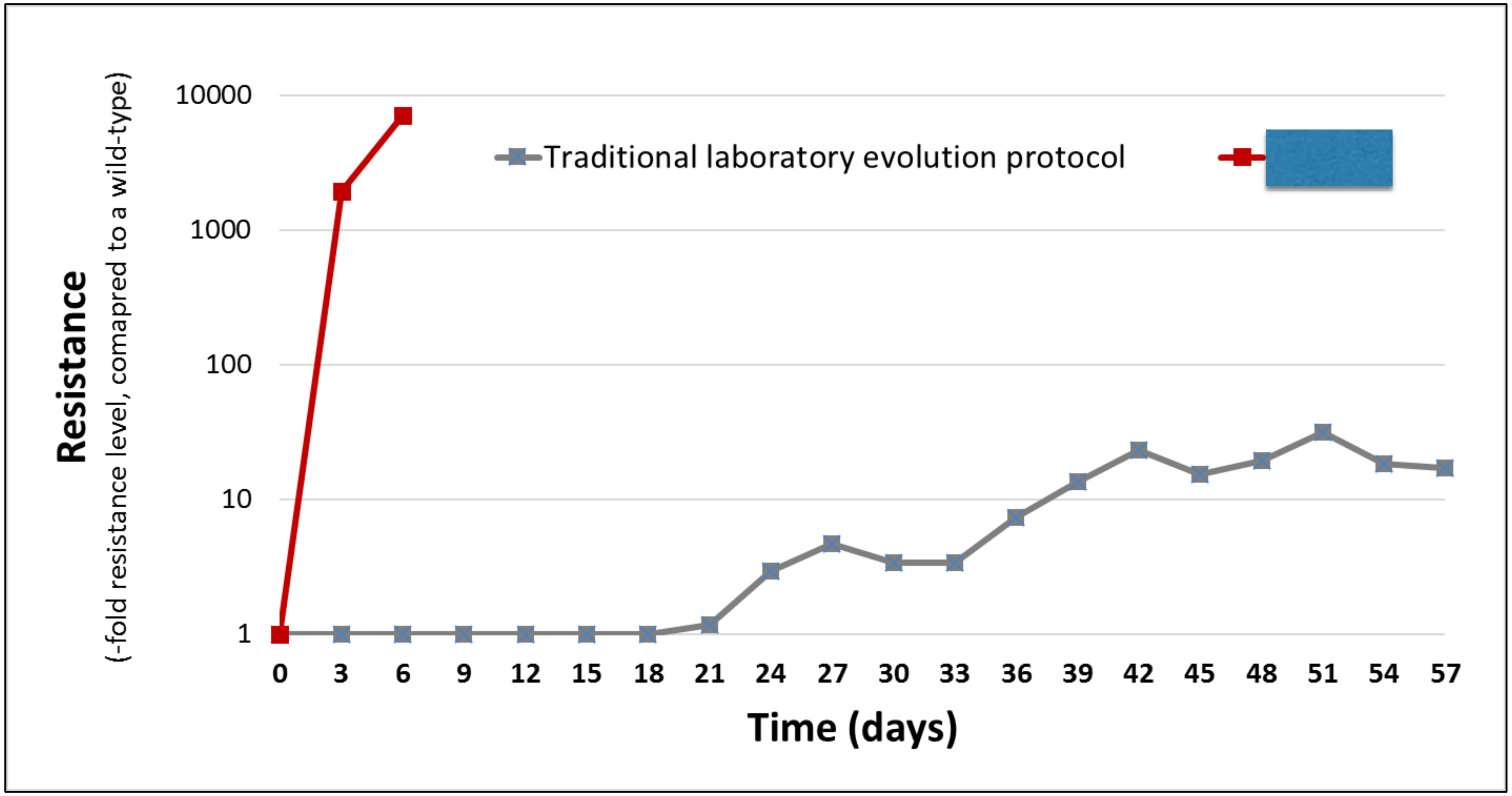
1000 $

Recoding the genetic code



First letter	Second letter								Third letter
	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

Accelerated evolution



Multiplex automated genome engineering

Advantages:

Target multiple loci simultaneously, rapid and cost-effective

Limitations:

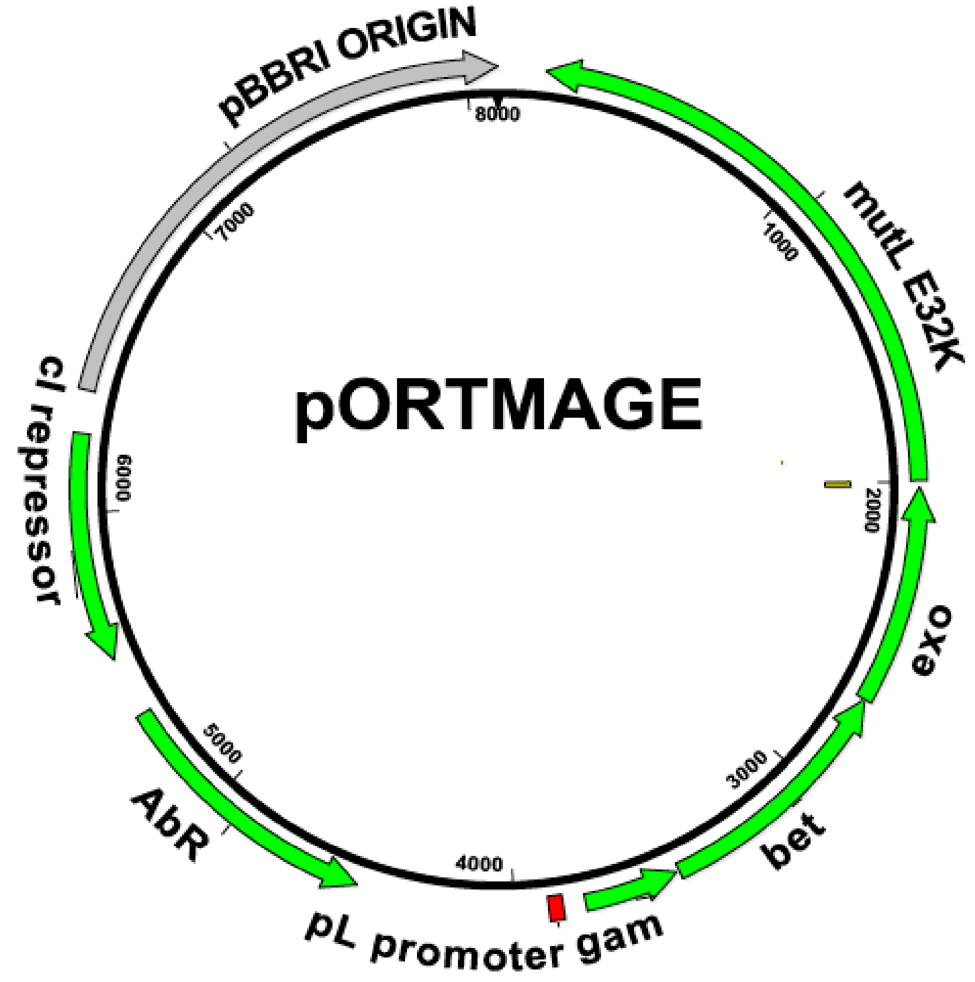
Substantial off-target mutagenesis

Extensive modifications of the host needed prior to genome editing

Applicable to a few model organisms only

A highly precise and portable genome engineering method allows comparison of mutational effects across bacterial species

Ákos Nyerges[a,1], Bálint Csörgő[a,1,2], István Nagy[b,c], Balázs Bálint[c], Péter Bihari[c], Viktória Lázár[a], Gábor Apjok[a], Kinga Umenhoffer[a], Balázs Bogos[a,3], György Pósfai[a], and Csaba Pál[a,2]

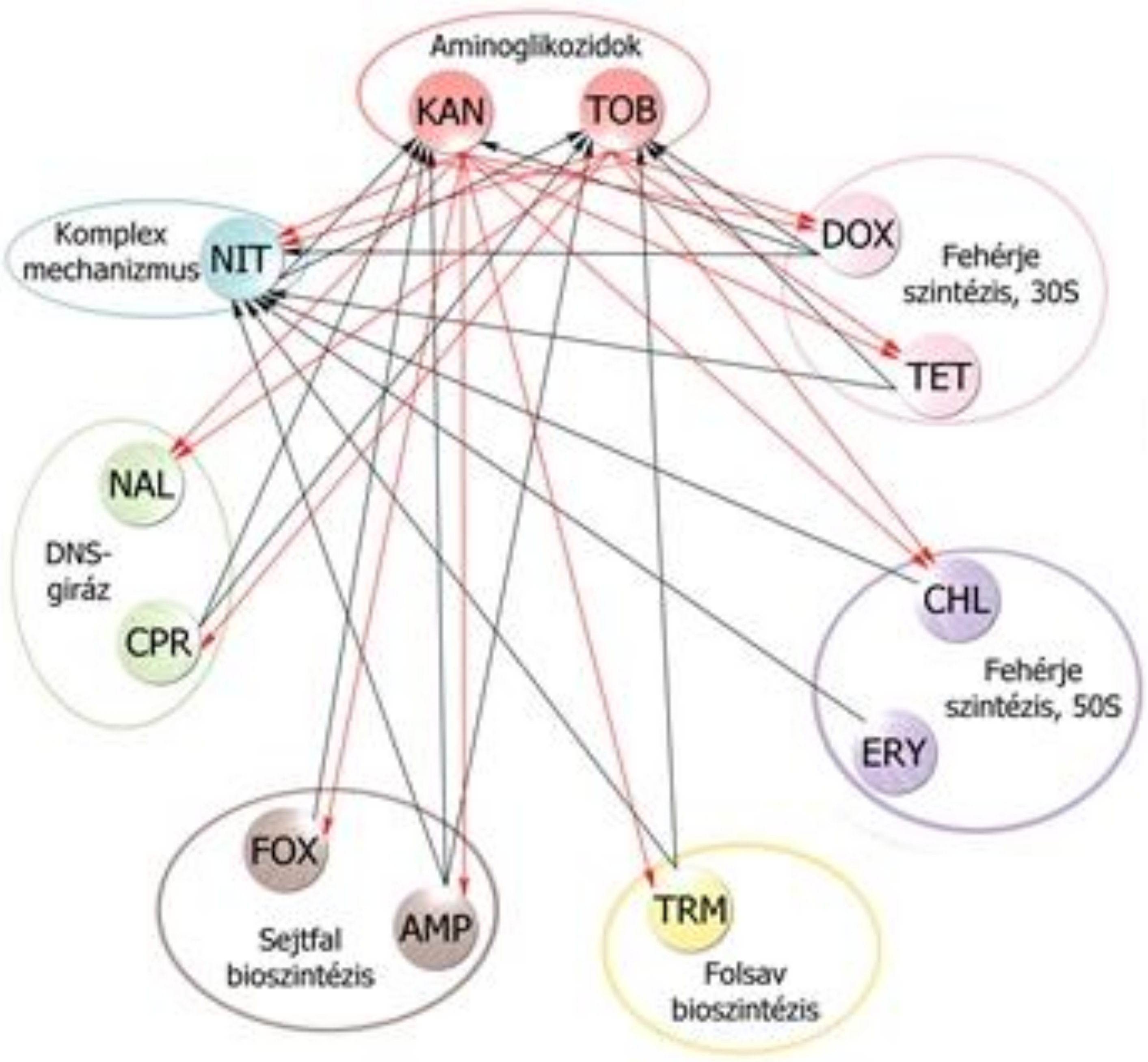


Why important?

1) More precise genome modifications
2) Biotechnological applications on new species
3) Study key issues in antibiotic resistance

effects of resistance mutations conserved across pathogenic sp

Map of collateral sensitivity



Nodes: Drug

Links: evolution of resistance to drug A *increases* susceptibility to drug B

Efficient antibiotic combinations

ARTICLE

PUBLISHED ONLINE: 14 SEPTEMBER 2015 | DOI: 10.1038/NCHEMBIO.1911

Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA

Patrick R Gonzales[1], Mitchell W Pesesky[1], Renee Bouley[2], Anna Ballard[1], Brent A Biddy[1], Mark A Suckow[3,4], William R Wolter[3,4], Valerie A Schroeder[3,4], Carey-Ann D Burnham[5,6], Shahriar Mobashery[2], Mayland Chang[2] & Gautam Dantas[1,5,7]*

Certain mutations cause collateral sensitivity in pathogen A.

Same holds in pathogen B and C?

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OPINION

The dawn of evolutionary genome engineering

Csaba Pál, Balázs Papp and György Pósfai