Genome-scale engineering for systems and synthetic biology

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Part 1
- Introduction.

Part 2
- Review current technologies and methodologies for genome-scale engineering.
- Discuss the prospects for extending efficient genome modification to new hosts.
- Explore the implications of continued advances toward the development of flexibly programmable chasses, and safer organismal and ecological engineering.

Part 3
- Concluding remarks.

Part 1.Introduction

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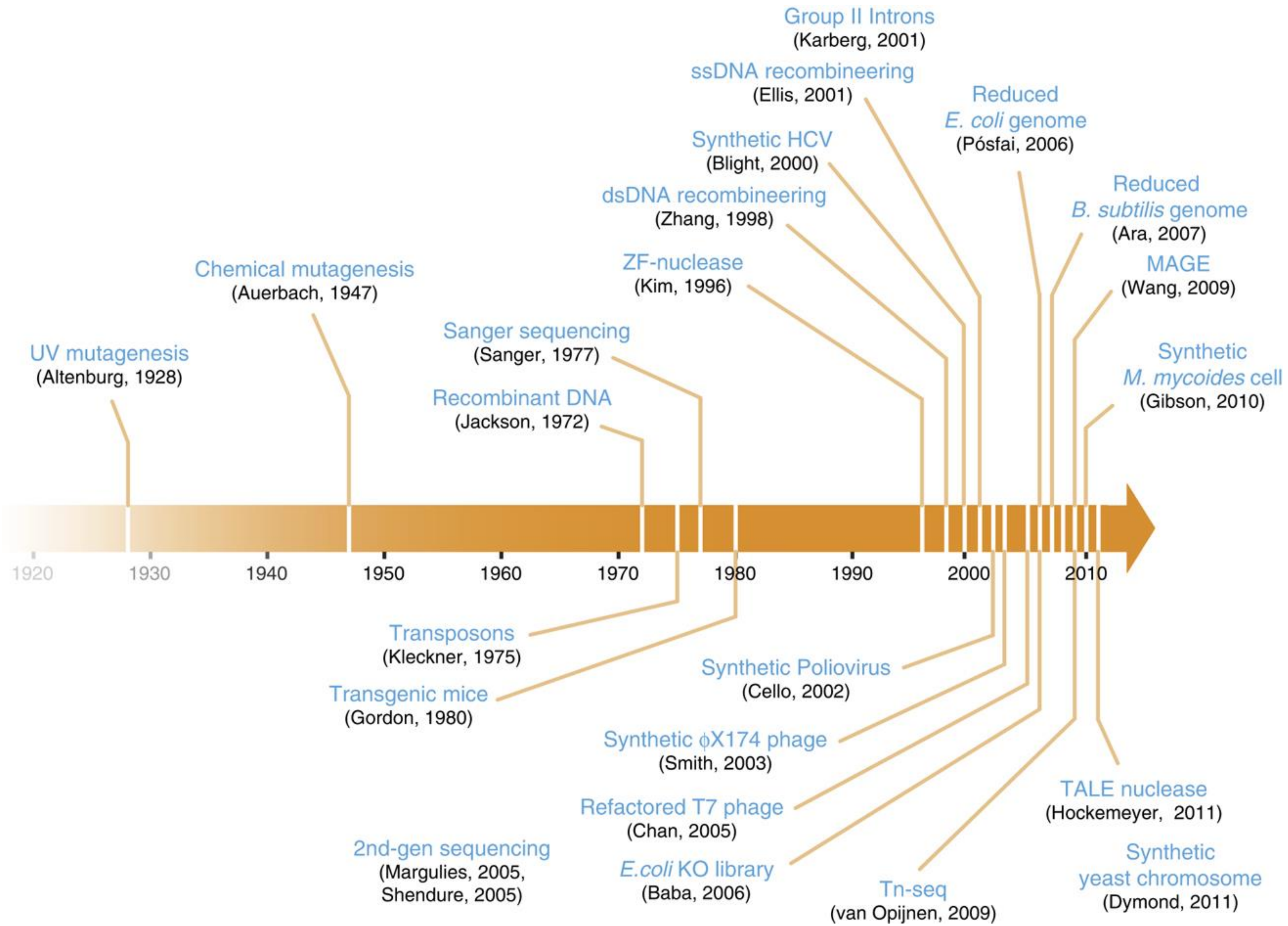
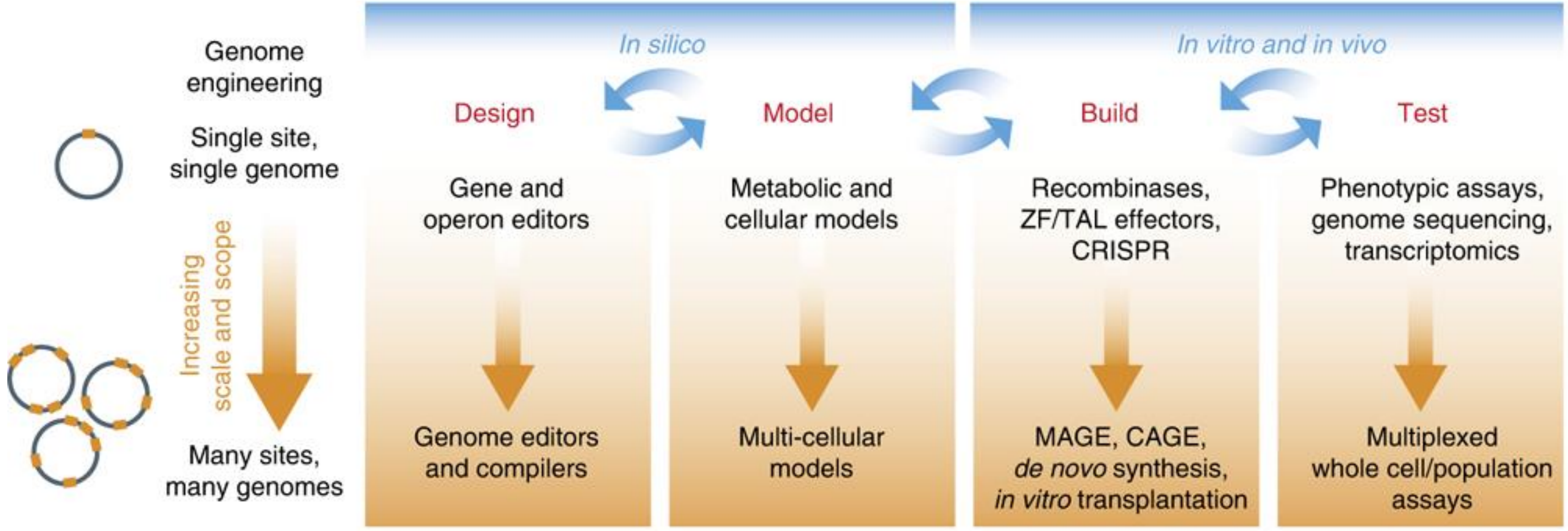


- What is genome-scale engineering?
- It is the art of constructing a genotype that gives rise to a desired phenotype.



Part 2.current technologies and methodologies for genome-scale engineering

- Genome designs and models

Describe the underlying blueprint of living organisms, built upon the information encoded in genes across the genome(Recordkeeping , J5 , Genome Compiler, Clotho).

The complexity of biological systems often renders effective design a challenge.

Fortunately, computational models can provide a useful guiding framework.

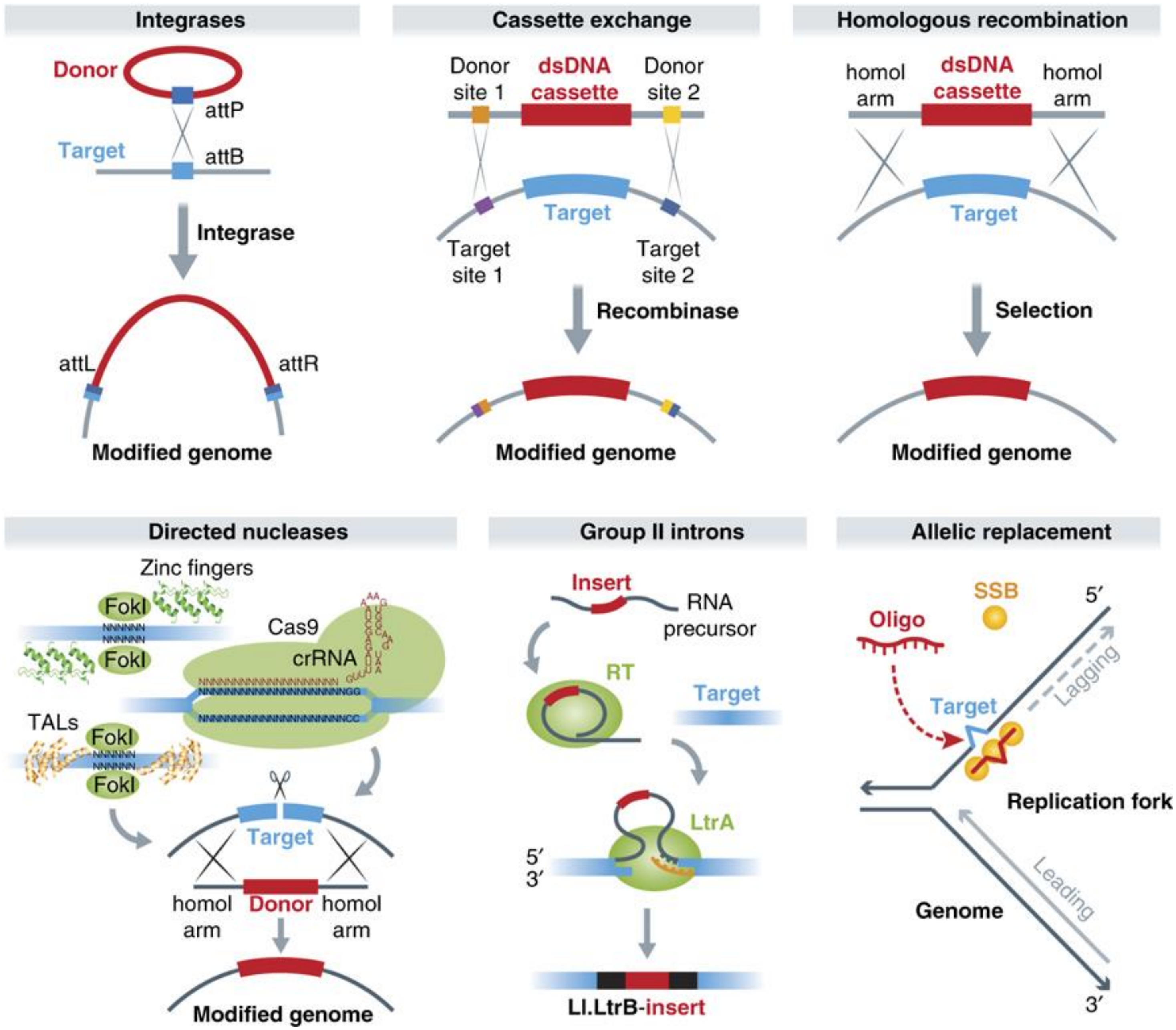
Accurate genotype-to-phenotype predictions of multiple genomic perturbations are still challenging due to biological complexity

Part 2.current technologies and methodologies for genome-scale engineering

- An expanding toolbox for genome construction and manipulation（3 Types）

1. Targeted genome engineering:

(Recombinases , Zinc-finger nucleases and TAL effector nucleases, Group II intron retrotransposition , Recombineering , RNA-guided CRISPR nucleases)

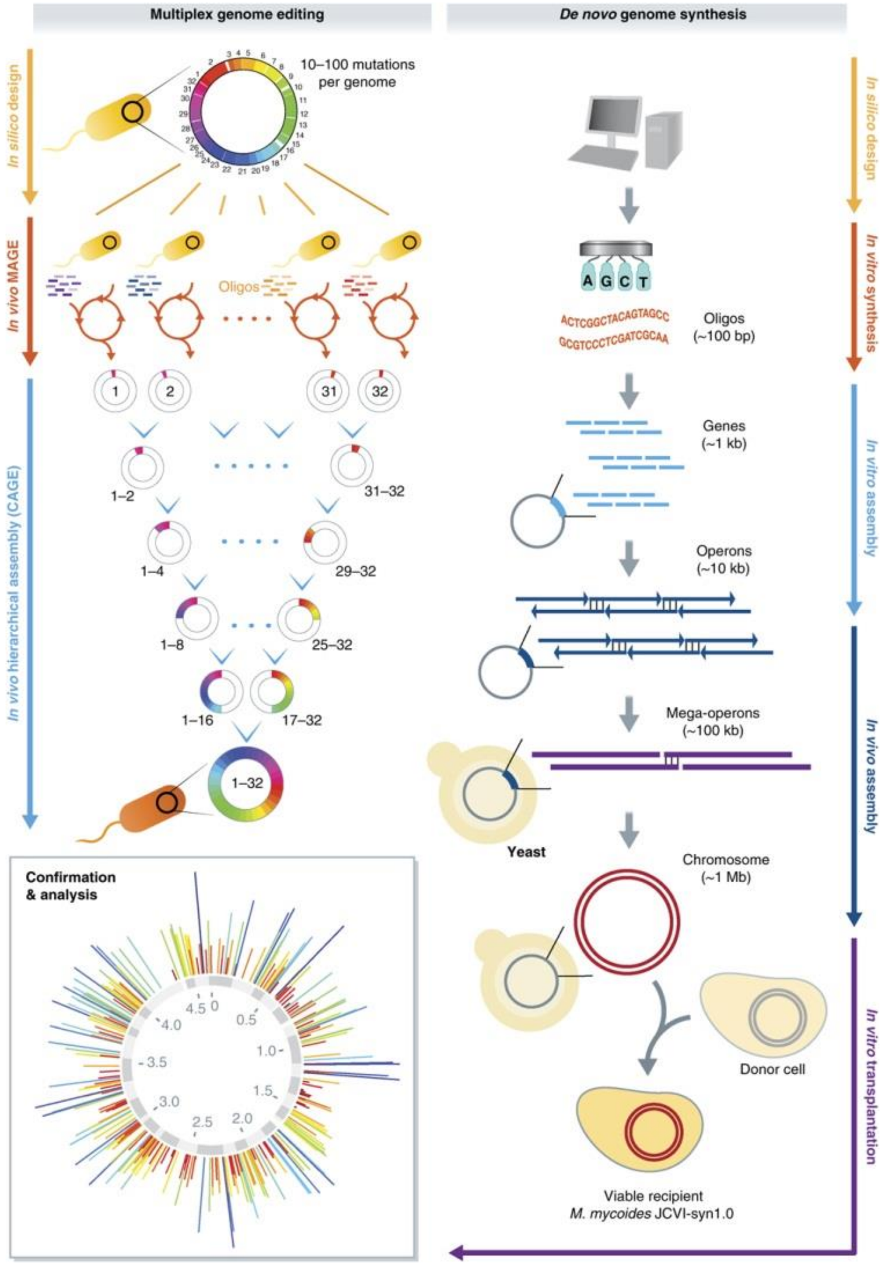

Integrases
Donor
attP
Target
attB
Integrase
attL
attR
Modified genome
Cassette exchange
Donor site 1
dsDNA cassette
Donor site 2
Target
Target site 1
Target site 2
Recombinase
Modified genome
Homologous recombination
homol arm
dsDNA cassette
homol arm
Target
Selection
Modified genome
Directed nucleases
Zinc fingers
FokI
FokI
Cas9
crRNA
TALs
FokI
FokI
Target
homol arm
Donor
homol arm
Modified genome
Group II introns
Insert
RNA precursor
RT
Target
LtrA
5′
3′
Ll.LtrB-insert
Allelic replacement
SSB
Oligo
5′
Target
Lagging
Replication fork
Genome
Leading
3′

2. Multiplexed genome engineering

Techniques that generate DSBs to catalyze homology-directed repair may be difficult to multiplex due to the toxicity of multiple simultaneous breaks and the high rate of NHEJ, which could easily lead to unintended rearrangements.

Multiplex Automated Genome Engineering (MAGE) that utilizes short ssDNA oligonucleotides (oligos) instead of dsDNA cassettes to mediate targeted genome modification.

3. Semi-synthetic and synthetic



3. Semi-synthetic and synthetic

- A genome editing approach may be optimal when generating genomes with a moderate degree of specified.
- A de novo synthesis approach is more likely to be appropriate for largerscale alterations such as codon or refactoring.

- More generally, developments that further combine synthetic, semi-synthetic, and hybrid approaches will lead to deeper understanding of the limits of rational design and optimization for engineered biological systems.

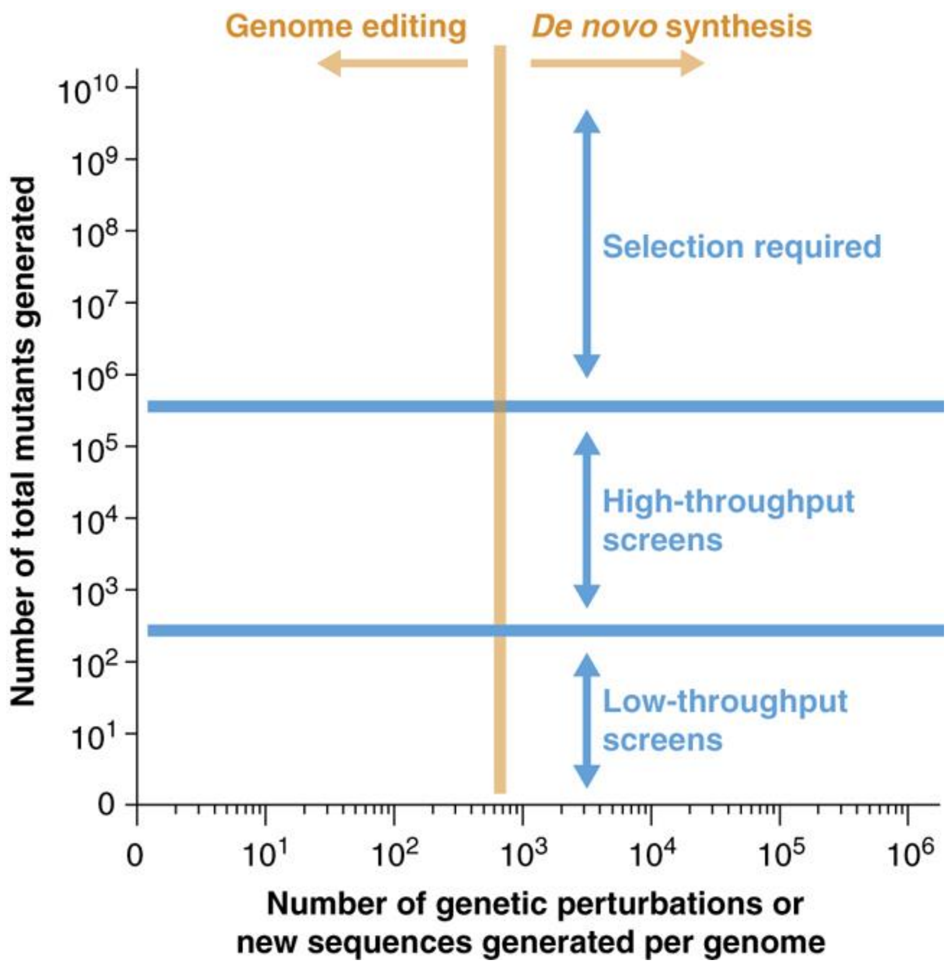
Part 2.current technologies and methodologies for genome-scale engineering

- Testing and validation of engineered genomes

Typical assays can be divided into low throughput screens and high-throughput screens, which identify variants from populations of limited size and high-throughput selections, which enable the isolation of variants from much larger populations.

For example, validating a constructed genome sequence by high-throughput sequencing is a form of low-throughput screen.

A viability assay testing the ability to survive and replicate under specific conditions is a selection.


Genome editing
De novo synthesis
Selection required
High-throughput screens
Low-throughput screens
Number of total mutants generated
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
10^{10}
Number of genetic perturbations or new sequences generated per genome

Part 2.Discuss the prospects for extending efficient genome modification to new hosts.

- Genome-scale metabolic engineering

For example, OptKnock (Burgard et al, 2003), a computational tool that uses bi-level metabolic flux optimization to predict the phenotype of gene knockout combinations, has been used to improve microbial production of lactic acid（乳酸）

The application of genome-scale approaches to metabolic engineering provides an excellent example of an integrated platform

Part 2.Discuss the prospects for extending efficient genome modification to new hosts.

- Organismic genome engineering

- Dairy cows are classic examples of slow-growing, expensive, multicellular organisms that nonetheless have a large industry invested in their improvement.

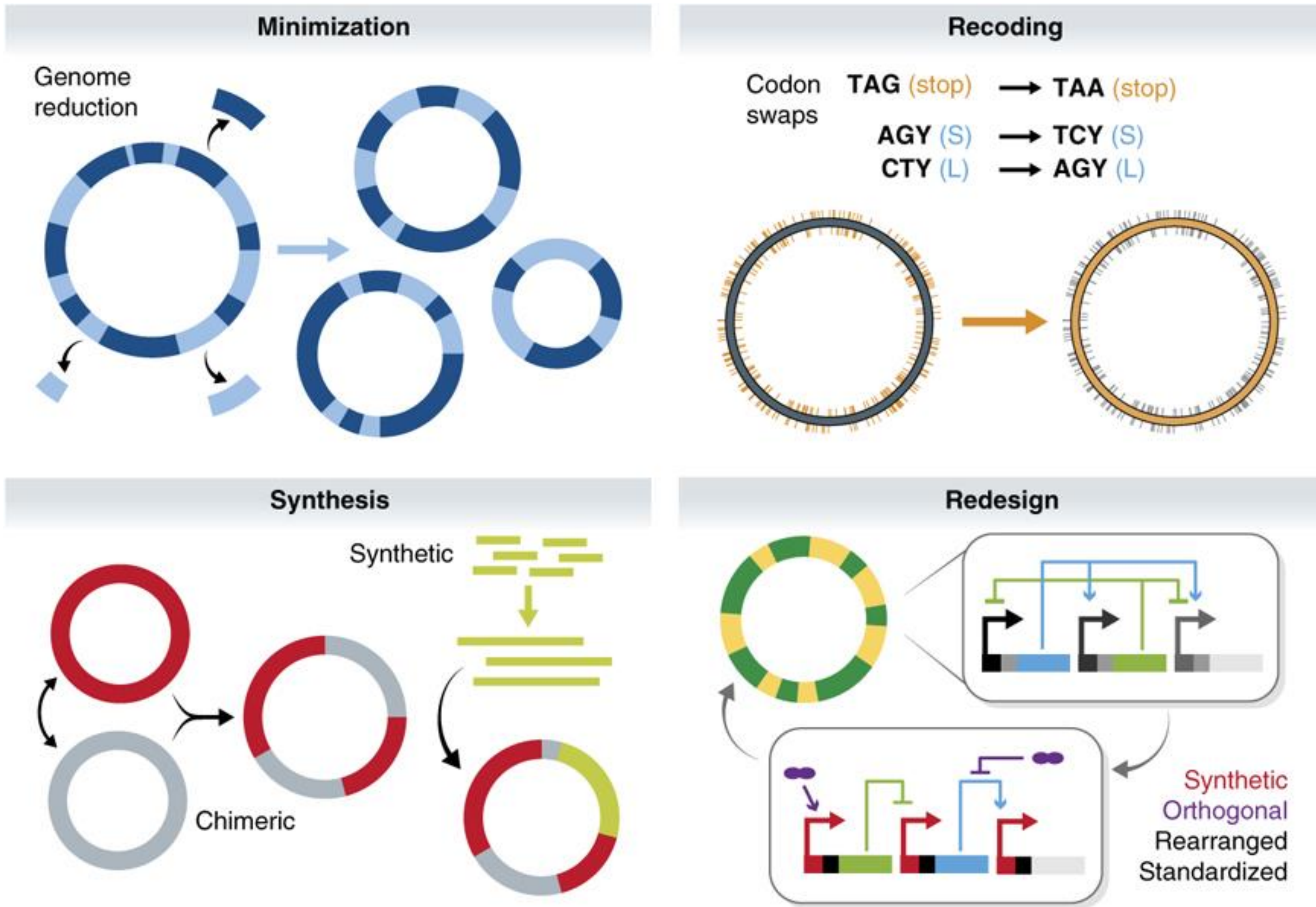
- Future technologies will ideally extend some of the advantages enjoyed by model organisms, enabling more genome engineering endeavors to combine model-driven targeted manipulation with the best growth and selection paradigm available to the target organism.

Part 2.Explore the implications of continued advances

- Toward a flexibly programmable biological chassis

Rational genome design would be greatly facilitated by the construction of an underlying biological 'chassis' that is simple, predictable, and programmable.

1 . Reducing biological complexity
2. Orthogonal information encoding
3. Expanded biochemical repertoire


Minimization
Genome reduction
Recoding
Codon swaps
TAG (stop) → TAA (stop)
AGY (S) → TCY (S)
CTY (L) → AGY (L)
Synthesis
Synthetic
Chimeric
Redesign
Synthetic
Orthogonal
Rearranged
Standardized

Part 2.Explore the implications of continued advances

- Toward engineering the pan-genome

Similar and related techniques might be adapted to modify most or all of the individual genomes that together constitute a single species: the pan-genome.

There are important safety and ecological considerations to assess before attempting any such project.

Part 3.Concluding remarks

- Further enhancements and extension to other organisms and across species will be needed to extend our engineering capabilities to the ecological level.
- Improved *in silico* modeling capabilities are urgently needed to guide rational genome design and synergize productively.
- The construction of a flexibly programmable biological chassis may serve as a foundation and standard for synthetic biology.

Thank you for your attention!