Achieving phenotypic fitness using rational and evolutionary engineering strategies

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May 20, 2021

What is this talk about?

While synthetic biology uses an approach that is information-rich, laboratory evolution explores an information-poor space.

This talk will briefly discuss a few evolutionary engineering approaches that have been successfully employed to obtain phenotypes of interest.

‘A cell is not just a bag of enzymes’

A cell is an exquisitely engineered entity that can respond to perturbations in its environment within set bounds.

The mechanisms whereby such responses arise are well-studied (in some cases), allowing us to rationally engineer cells to behave in a predictable fashion.

Some complex phenomena are yet to be understood in their entirety, making it difficult to reliably engineer cells to achieve a specific goal.

Rational engineering strategies

- Recombinant DNA technology
- Metabolic engineering
- Systems biology
- Synthetic biology
- . . .

The synthetic biology paradigm

Synthetic biology relies on standardized parts and protocols to engineer cells.

Insistence on idempotency in assembly of genetic parts, for example, strives to make synthetic biology predictable and well-defined.

DBT cycle

Design $\rightarrow$ Build $\rightarrow$ Test $\rightarrow$ Learn

Evolution can give rise to new traits

As cells divide and multiply, they accumulate mutations that may be fixed in the population if they confer an advantage to the cell.

Mutations that can arise in a population cannot be predicted a priori, though they can be selected for.

Directed evolution

Adaptive or directed evolution is a technique used to rapidly select mutated cells exhibiting a trait of interest.

Cells are serially grown in a selective medium until rapidly dividing cells arise, a task that is commonly accomplished over the course of a few months.

It has been hypothesized that the mutation rate can be modulated by the selection strategy employed.[1]

[1] Nature 485, 95–98 (2012)

Evolutionary engineering

Synthetic biology tools can be used to increase mutation rates, introduce phenotypic perturbations and accelerate evolution.

Approaches for evolutionary engineering

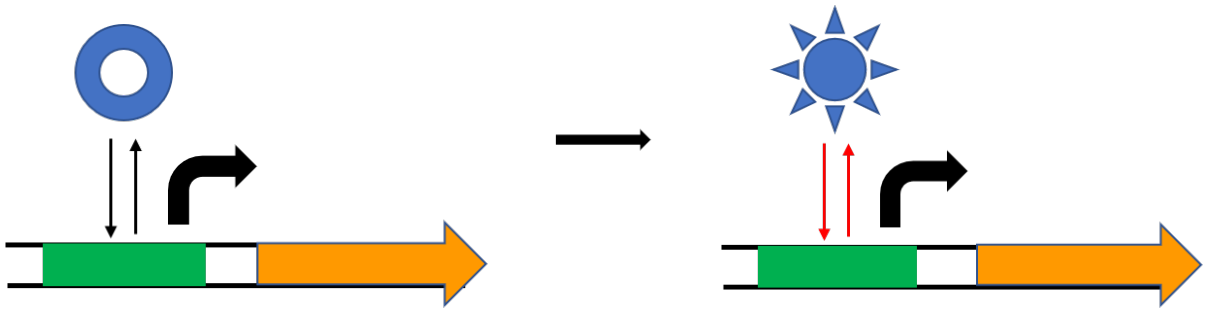
A variety of approaches have been described for evolutionary engineering of cells. These include

- Altering global transcription patterns
- Tuning target gene expression
- Genome-scale recombination
- Continuous directed evolution

Altering transcription patterns

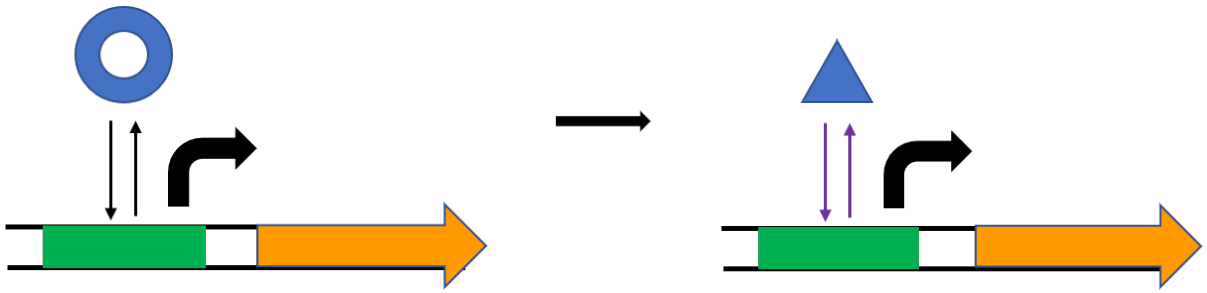
Changing transcription rate and pattern of gene expression can lead to diverse and novel phenotypes, using an approach called 'global transcription machinery engineering' (gTME).

The phenotype of interest can then be selected for using appropriate methods.



Using a mutant library of the transcription factor Spt15p, *Saccharomyces cerevisiae* cells displaying increased ethanol tolerance and more efficient glucose to ethanol conversion were isolated.[2]

[2] Science 314, 1565–1568 (2006)



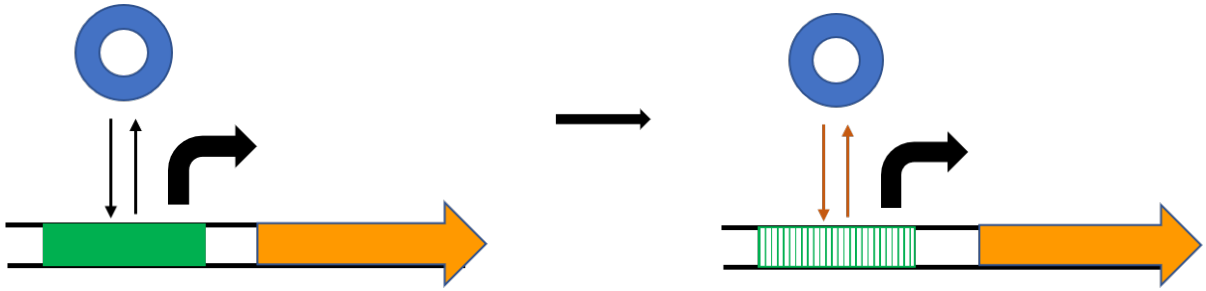
Expression of an evolved exogenous global regulator (IrrE) from *Deinococcus radiodurans* enhanced ethanol, butanol and acetate stress tolerance in *Escherichia coli*.[3]

[3] PLoS ONE 6(1), e16228 (2011)

Tuning target gene expression

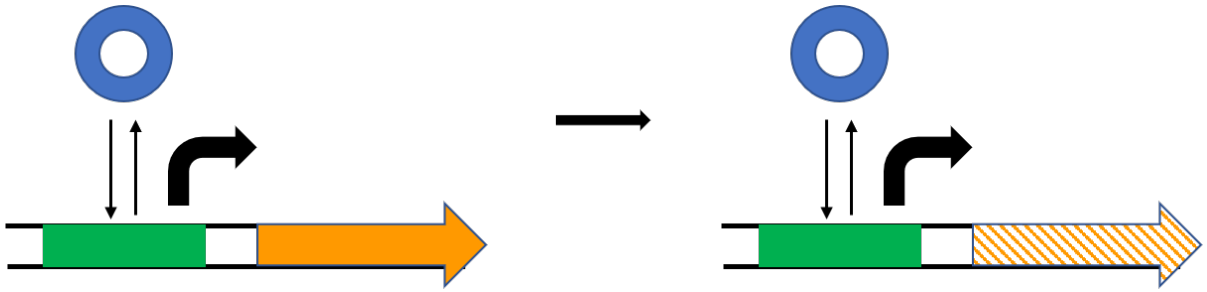
Expression of multiple genes can be varied over a wide range in a high throughput fashion to identify optimal transcription levels.

The phenotype of interest can be selected using appropriate methods.



Exchanging promoters of multiple genes with a library of synthetic promoters of varying strengths is another strategy that can be explored for isolating evolved strains.[4]

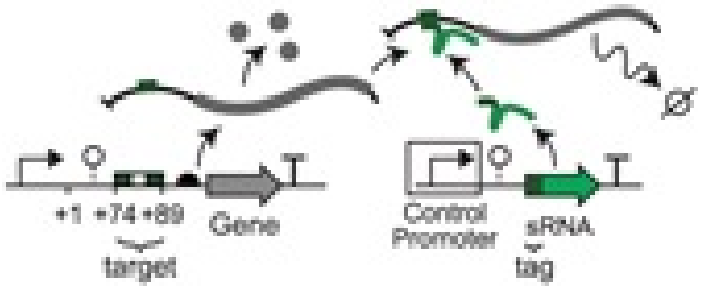
[4] Biotechnol Biofuels 12, 113 (2019)



Multiplex automated genome engineering (MAGE) uses ssDNA to introduce sequence diversity in targeted genomic regions by allelic replacement. Under optimized conditions, genetic modifications were introduced in >30% of the cell population.[5]

Synchronous gene editing using CRISPR has been demonstrated in mammalian cells to obtain mutant alleles of the *TP53* and *EGFR* loci, leading to modulation of their expression levels.[6]

[5] Nature 460, 894–898 (2009)
[6] Sci Rep 8, 17499 (2018)



Using a library of synthetic small regulatory RNA (sRNA) expressed using promoters of different strengths (yielding $\sim 10^7$ combinations), transcription of multiple genes was modulated to optimize a metabolic pathway in *E. coli*.[7]

[7] Nucleic Acids Res 45, 8116–8127 (2017)

Genome-scale recombination

Genome-scale recombination is a powerful technique for evolving new phenotypes at the whole cell level.

Genome shuffling is a widely-employed technique to achieve genome-scale recombination that can be applied to a variety of host cells.

Genome shuffling involves whole-genome recombination between cells with diverse genotypes to create progeny with improved fitness.[8]

[8] Nature 415, 644–646 (2002)

SCRaMbLE (synthetic chromosome rearrangement and modification by loxP-mediated evolution) was originally described for generating phenotypic diversity in yeasts.[9]

This technique has also been reported to be used for synthetic metabolic pathway construction by strategic positioning of loxPsym sites.[10]

[9] Nature 477, 471–476 (2011)
[10] Nat Commun 9, 1936 (2018)

Continuous directed evolution

The evolutionary engineering approaches discussed till now utilize 'discrete' mutagenesis events that occur in a specified time period.

Once genome diversity is generated, phenotypes of interest can be selected for and isolated. Such evolved strains can be iteratively subjected to further mutagenesis rounds until an evolved strain with desired characteristics is obtained.

The continuous directed evolution approach mimics natural evolution, such that mutations are dynamically generated using an in vivo integrated mutator mechanism.[11]

[11] Nat Chem Biol 16, 610–619 (2020)

Approaches for continuous directed evolution

- Expression of error-prone DNA polymerase I[12]
- Expression of bacterial retroelements ('retrons') using mutagenic T7 RNA polymerase[13]
- Expression of error-prone DNA polymerase III subunit DnaQ926 in combination with genes involved in replication fidelity such as proofreading, MMR and translesion synthesis[14]
- Producing ssDNA in vivo from retrons to incorporate edits at multiple genomic locations[15]

[12] Proc Natl Acad Sci USA 100, 9727–9732 (2003)
[13] ACS Synth Biol 7, 2600–2611 (2018)
[14] Nat Commun 6, 8425 (2015)
[15] Proc Natl Acad Sci USA 118, e2018181118 (2021)

Conclusion

Synthetic biology-enabled evolutionary engineering has given rise to possibilities that traditional adaptive evolution and directed evolution approaches cannot achieve.

Evolutionary engineering approaches, be they random or targeted, discrete or dynamic, enable genomic pertubation and acquisition of novel phenotypes.

Advances in genome sequencing techniques allow mapping of genotype to phenotype and aid rational engineering of organisms for diverse applications in industry and medicine.

Thank you!

Questions?