Introduction to Bioengineering
BIOE/ENGR.80
Stanford University

Spring 2020 Class Slides

Day 23
29 May 2020

Week 8 look ahead

CONCEPT
SKILL

Evolution as algorithm

Evolution as service

Evolution 2.0?

<Evolutionary algorithm sandbox>

Evolution as algorithm

Competition among individuals whose behaviors vary (i.e., phenotypes).

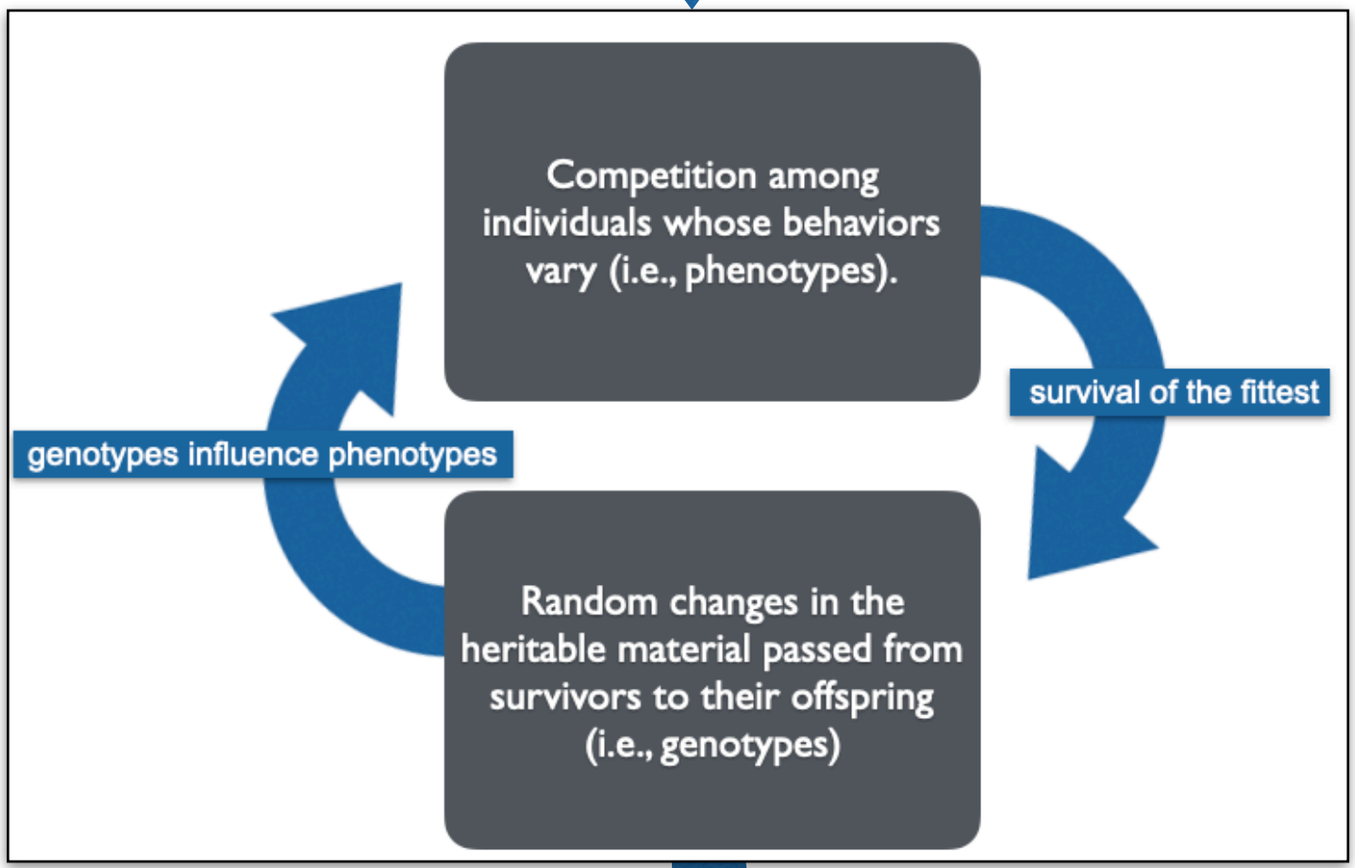
survival of the fittest

Random changes in the heritable material passed from survivors to their offspring (i.e., genotypes)

genotypes influence phenotypes

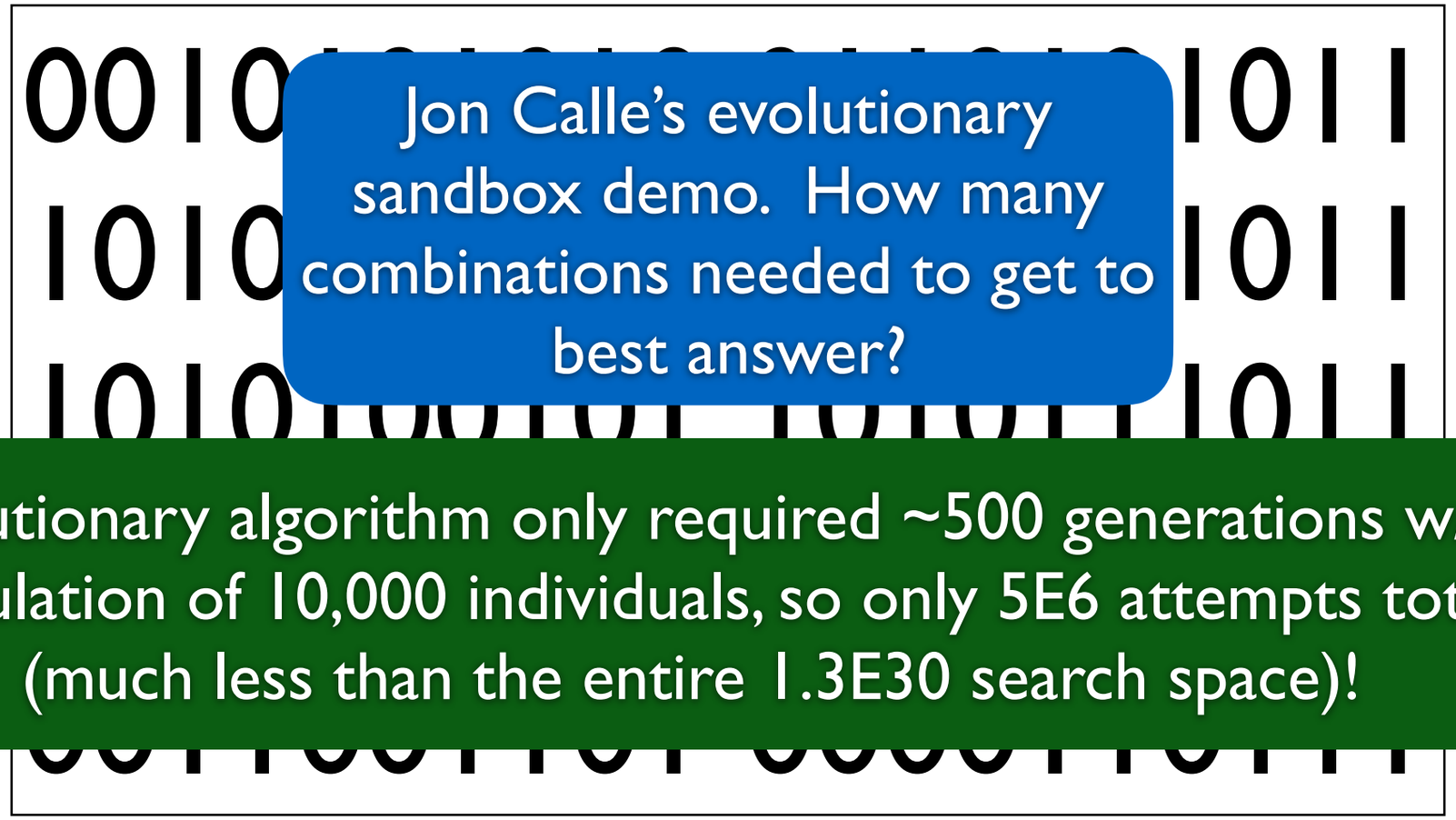
Evolution as a service

E.g., I wish for a string of 100 bits that sum to the highest value



Here you go!

Practical Power of Evolution



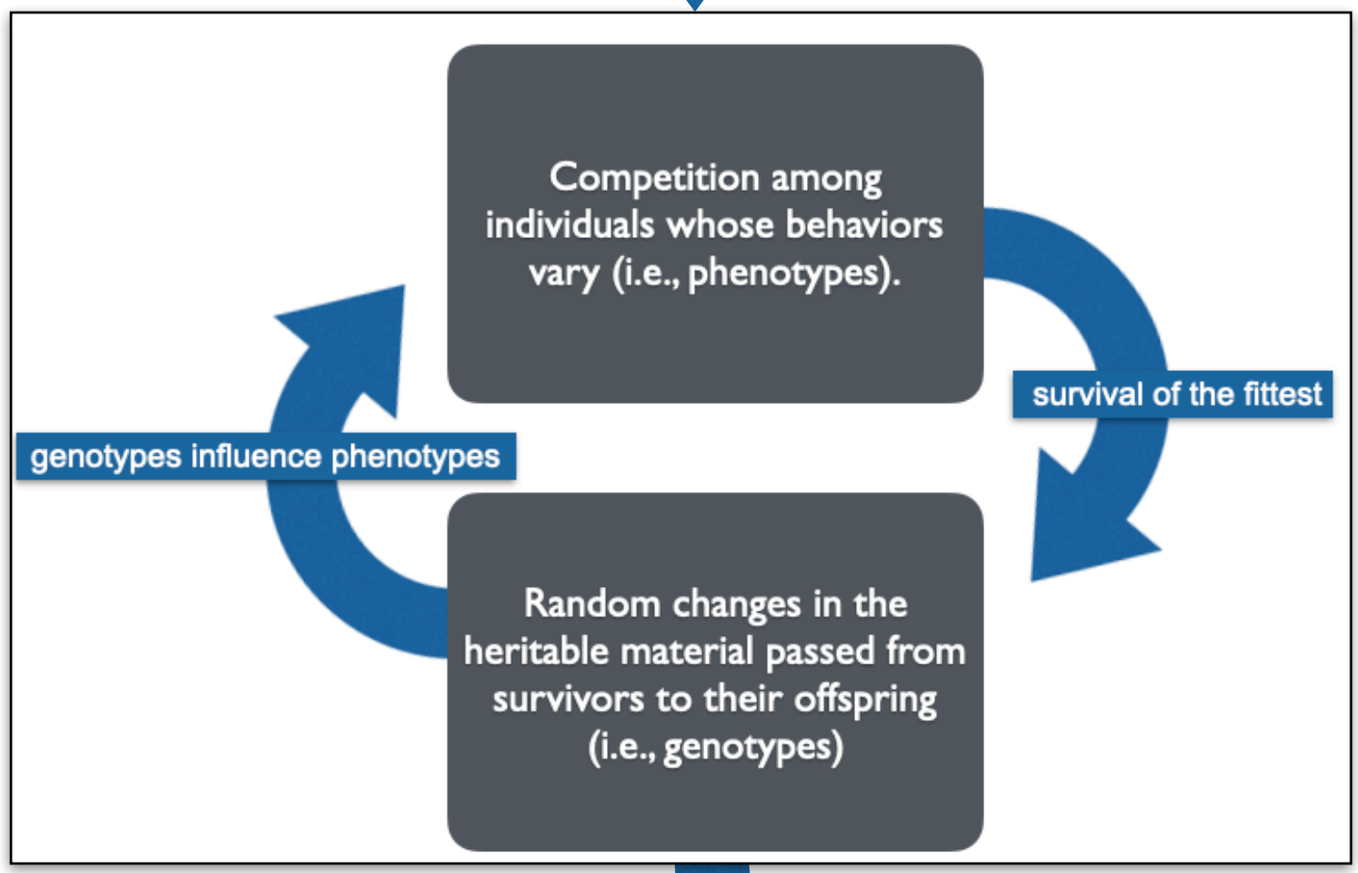
sum of bits = 54

max. sum = 100

2^100 ~ 1.3E30 combinations

Evolution as a service

I wish BIOLOGY did the following for me!



Here you go!

Massive Caveat!

Requires mapping your wish into an evolutionary process

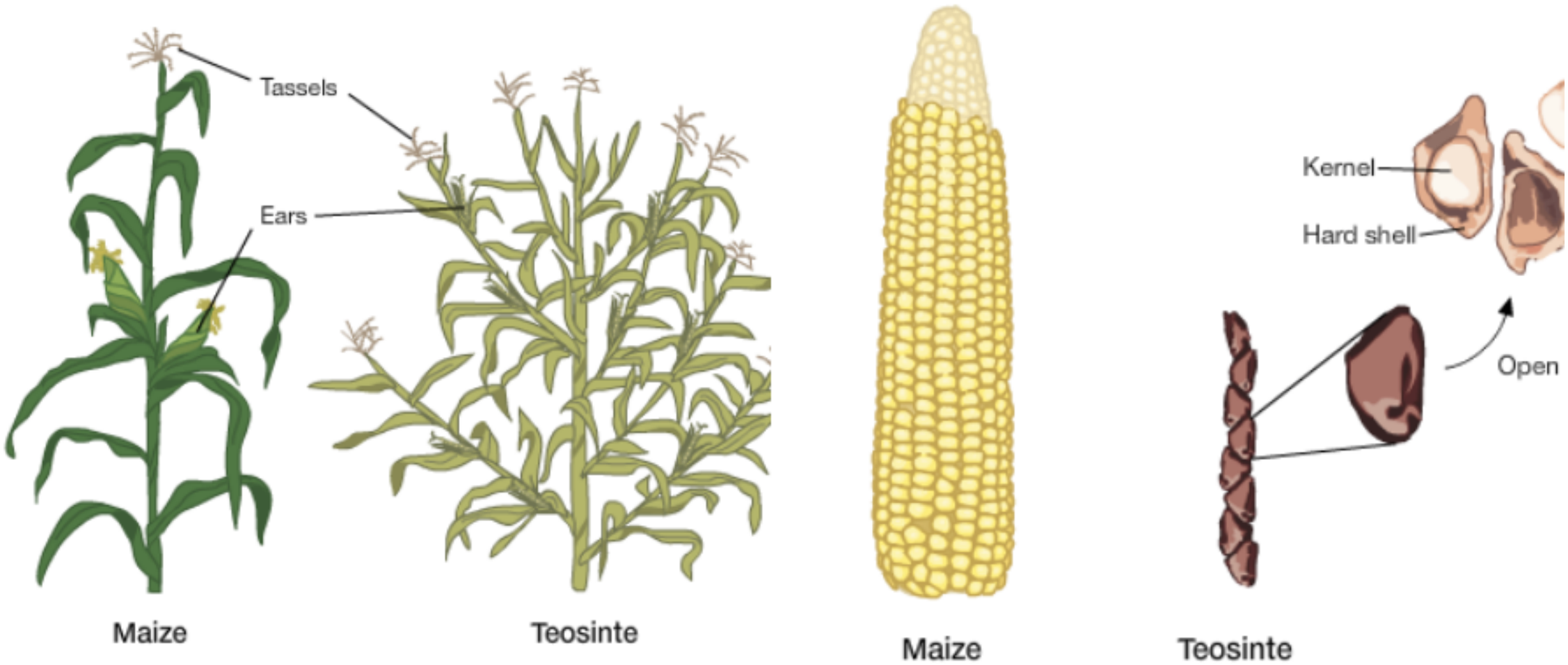
Massive Caveat!

Requires mapping your wish into an evolutionary process

Challenge 1 — linking desired behavior to heritable material that can mutate

Challenge 2 — ensuring that selective pressure (i.e., survival of the fittest) is actually selecting for the specific property you care about

Historically practiced, limited to biological wishes in which both challenges implicitly addressed…



http://learn.genetics.utah.edu/content/selection/corn/

21st-century bioengineering continues in this tradition, but with an significant expansion in scope via "directing" & "sculpting" evolution

Available online at www.sciencedirect.com
ScienceDirect
Biomolecular Engineering 24 (2007) 381–403
Biomolecular Engineering
www.elsevier.com/locate/geneanabioeng

Review

SELEX—A (r)evolutionary method to generate high-affinity nucleic acid ligands

Regina Stoltenburg, Christine Reinemann, Beate Strehlitz *

UFZ, Helmholtz Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

Received 30 April 2007; received in revised form 31 May 2007; accepted 1 June 2007

Abstract

SELEX stands for systematic evolution of ligands by exponential enrichment. This method, described primarily in 1990 [Ellington, A.D., Szostak, J.W., 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346, 818–822; Tuerk, C., Gold, L., 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249, 505–510] aims at the development of aptamers, which are oligonucleotides (RNA or ssDNA) binding to their target with high selectivity and sensitivity because of their three-dimensional shape. Aptamers are all new ligands with a high affinity for considerably differing molecules ranging from large targets as proteins over peptides, complex molecules to drugs and organic small molecules or even metal ions. Aptamers are widely used, including medical and pharmaceutical basic research, drug development, diagnosis, and therapy. Analytical and separation tools bearing aptamers as molecular recognition and binding elements are another big field of application. Moreover, aptamers are used for the investigation of binding phenomena in proteomics. The SELEX method was modified over the years in different ways to become more efficient and less time consuming, to reach higher affinities of the aptamers selected and for automation of the process. This review is focused on the development of aptamers by use of SELEX and gives an overview about technologies, advantages, limitations, and applications of aptamers.

https://www.sciencedirect.com/science/article/pii/S1389034407000664

SELEX implements a general-purpose system by which DNA or RNA sequences can be selected for interactions with other stuff…

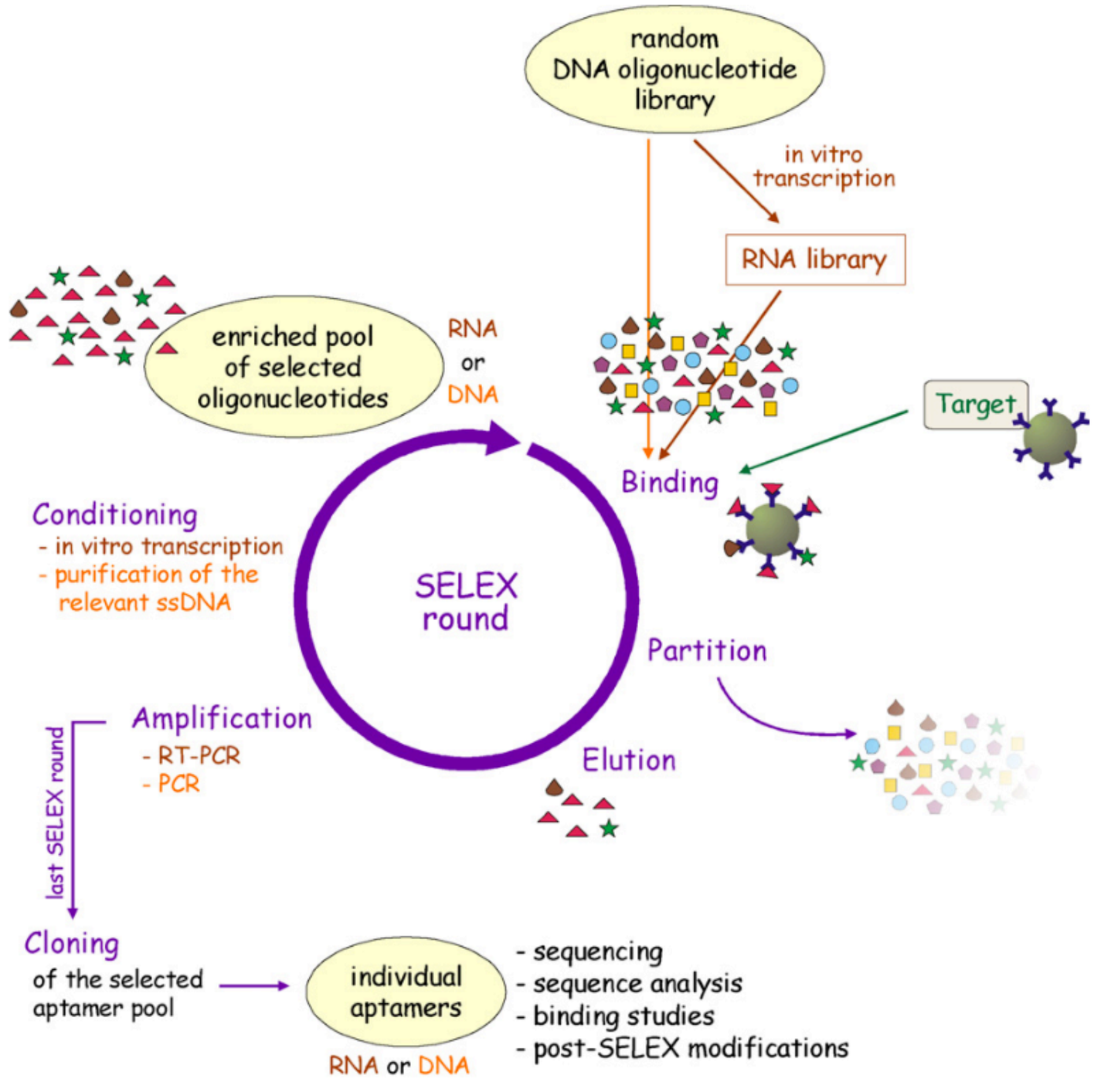


Fig. 2. In vitro selection of target-specific aptamers using SELEX technology. Starting point of each SELEX process is a synthetic random DNA oligonucleotide

https://www.sciencedirect.com/science/article/pii/S1389034407000664

Second case study — Yeast Surface Display…

Applications of Yeast Surface Display for Protein Engineering

Gerald M. Cherf and Jennifer R. Cochran

Abstract

The method of displaying recombinant proteins on the surface of *Saccharomyces cerevisiae* via genetic fusion to an abundant cell wall protein, a technology known as yeast surface display, or simply, yeast display, has become a valuable protein engineering tool for a broad spectrum of biotechnology and biomedical applications. This review focuses on the use of yeast display for engineering protein affinity, stability, and enzymatic activity. Strategies and examples for each protein engineering goal are discussed. Additional applications of yeast display are also briefly presented, including protein epitope mapping, identification of protein-protein interactions, and uses of displayed proteins in industry and medicine.

Key words Yeast surface display, Protein engineering, Random mutagenesis, DNA shuffling, Affinity maturation, Protein stability engineering, Enzyme engineering

https://link.springer.com/protocol/10.1007/978-1-4939-2748-7_8

Create a general-purpose system by which any protein of interest can be made by a yeast cell and presented on outside of the cell…

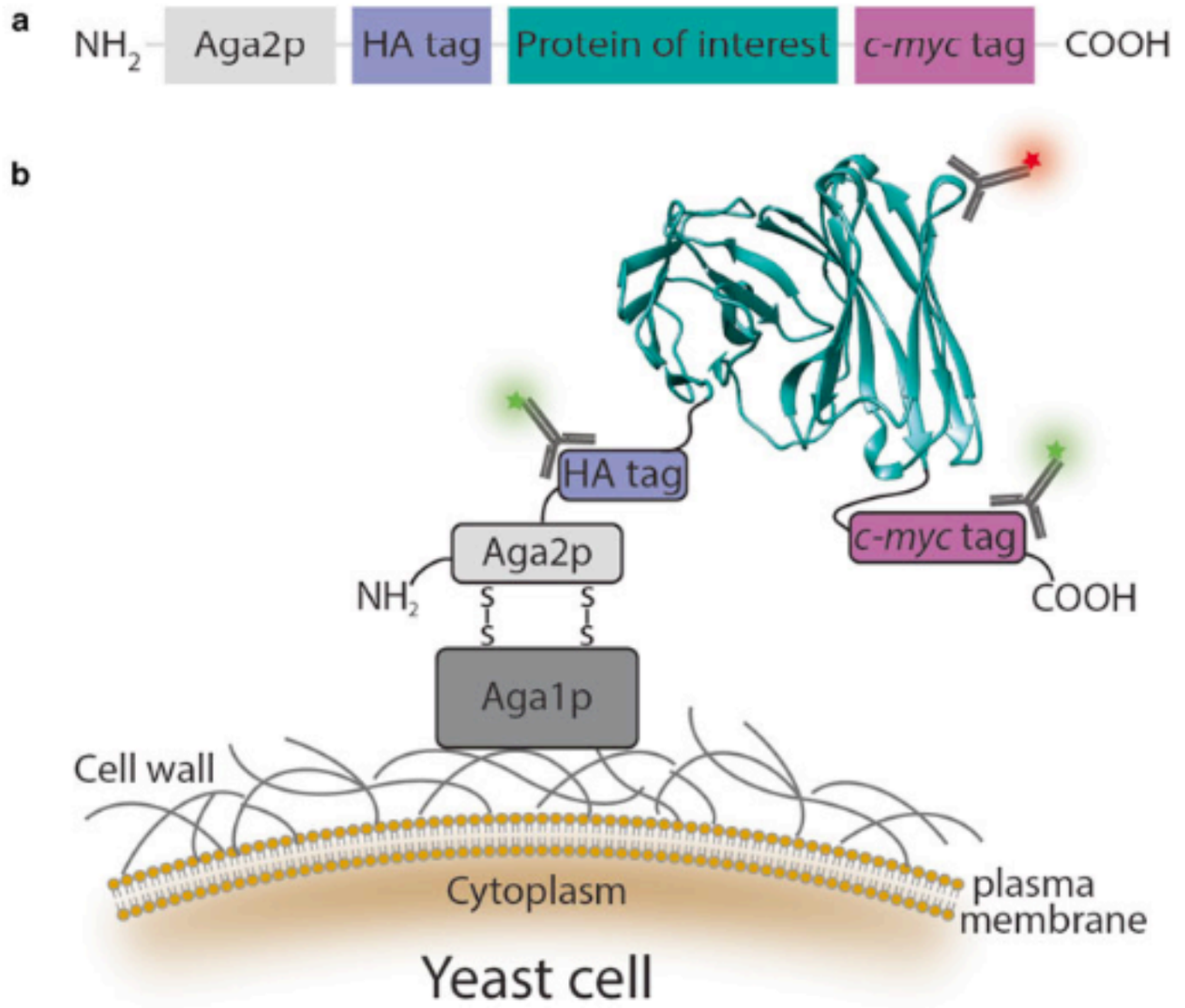


Fig. 1 Schematic representation of yeast surface display. (**a**) The protein of interest is flanked by two epitope tags: a 9-amino acid hemagglutinin antigen (HA) tag and a 10-amino acid *c-myc* tag, and is fused to the C-terminus of the *a*-agglutinin Aga2p subunit. (**b**) Protein display on the yeast cell surface. Following translation,

https://link.springer.com/protocol/10.1007/978-1-4939-2748-7_8

Use to select for any behavior of the protein that you can implement a selection for… (e.g., binding, stability, enzyme activity)

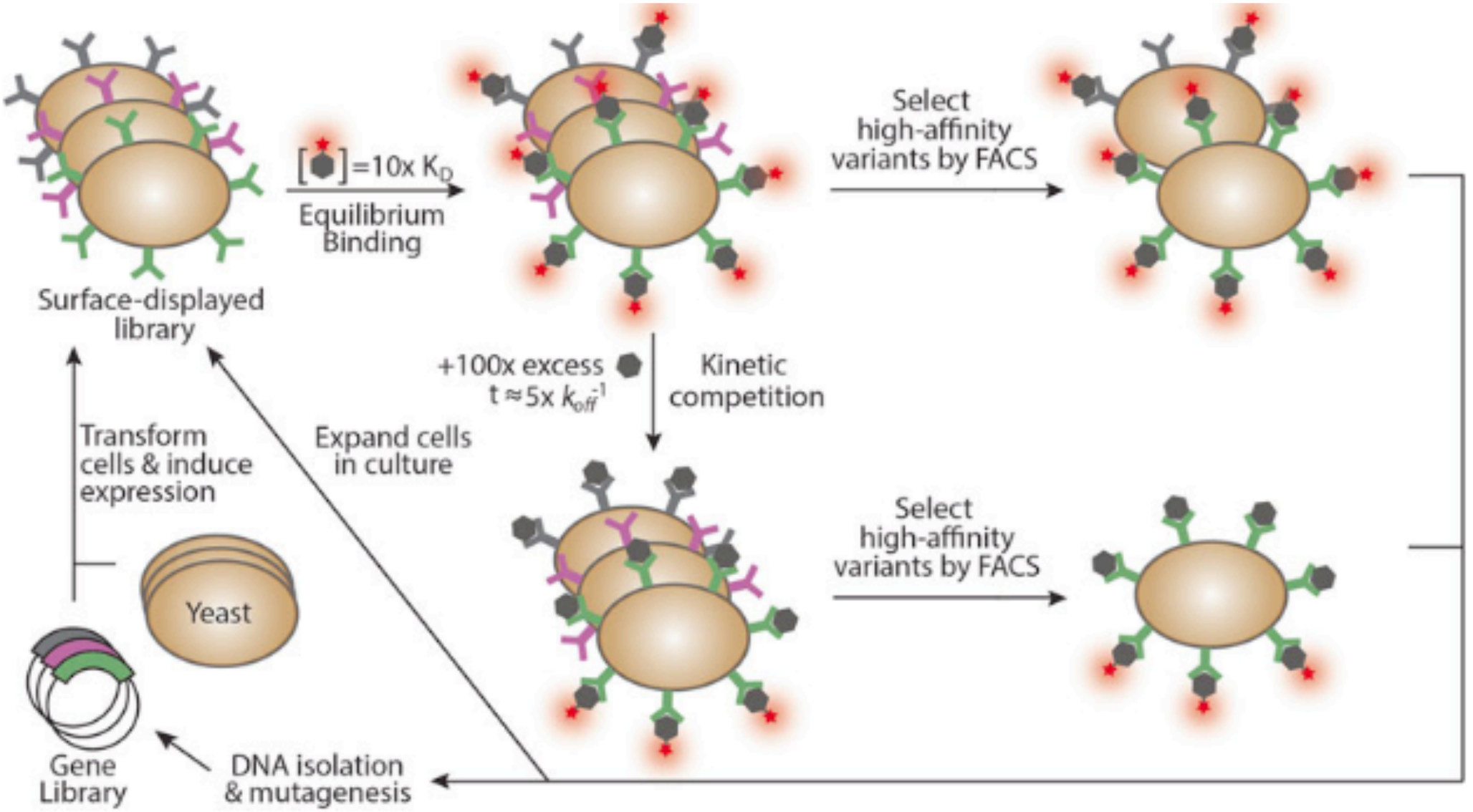


Fig. 2 Isolating high-affinity protein variants from a yeast-displayed library by FACS. Following transformation

https://link.springer.com/protocol/10.1007/978-1-4939-2748-7_8

Third case study — Phage-Assisted Continuous Evolution (PACE)…

Published: 10 April 2011

A system for the continuous directed evolution of biomolecules

Kevin M. Esvelt, Jacob C. Carlson & David R. Liu

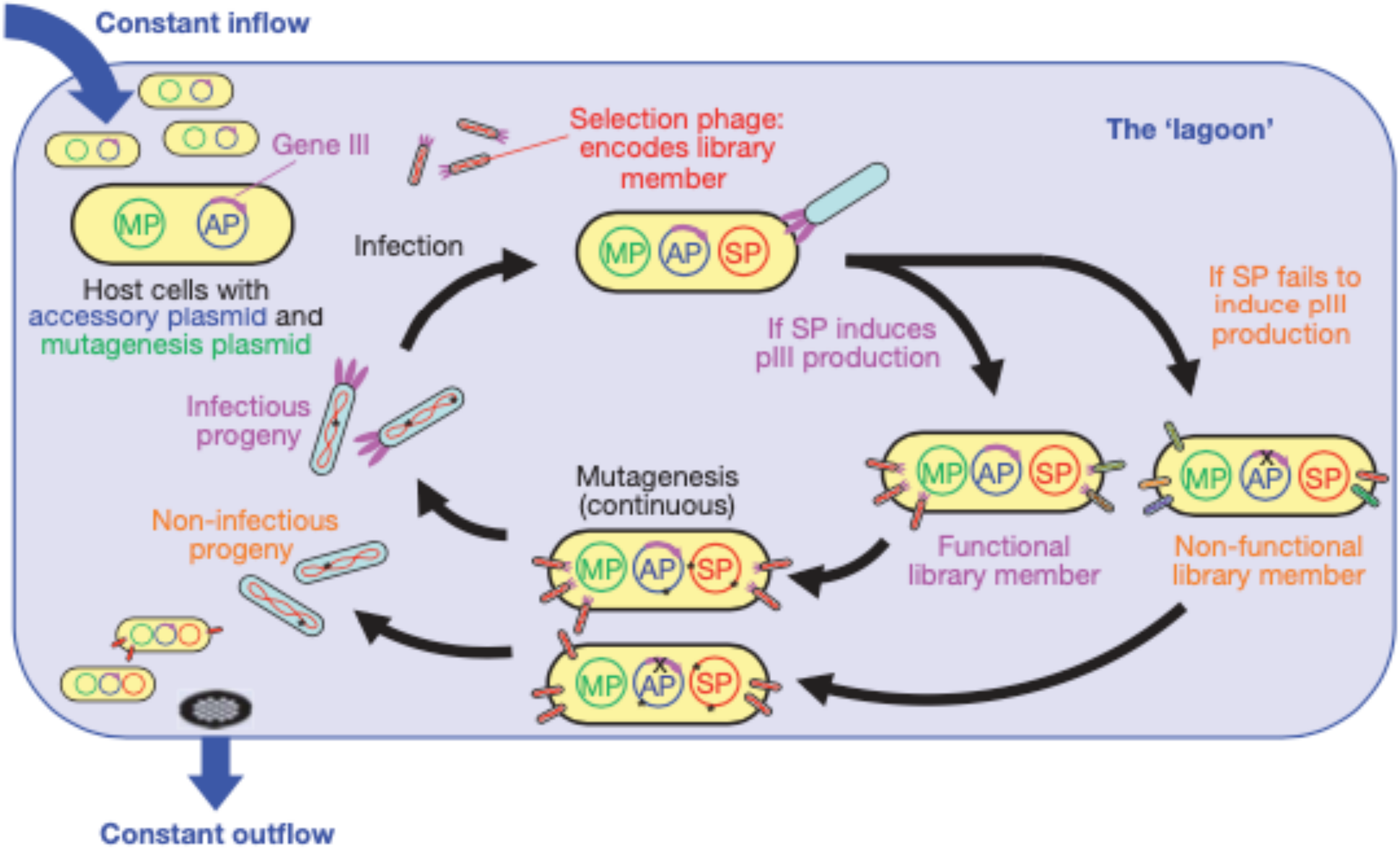
Nature **472**, 499–503(2011) | Cite this article

Abstract

Laboratory evolution has generated many biomolecules with desired properties, but a single round of mutation, gene expression, screening or selection, and replication typically requires days or longer with frequent human intervention[1]. Because evolutionary success is dependent on the total number of rounds performed[2], a means of performing laboratory evolution continuously and rapidly could dramatically enhance its effectiveness[3].

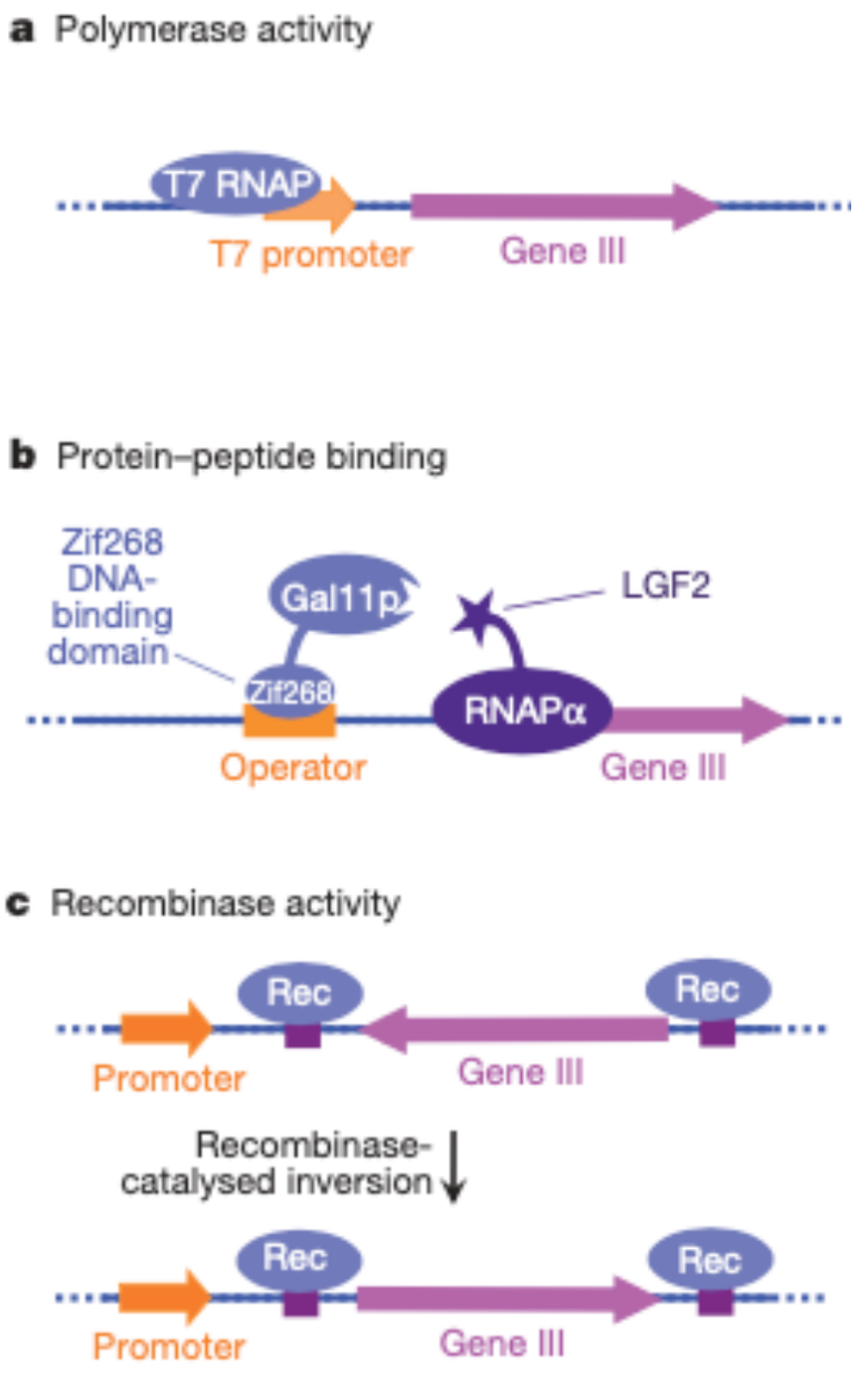
https://www.nature.com/articles/nature09929

PACE can be used to automatically select for anything that can be turned into something to activate gene expression….



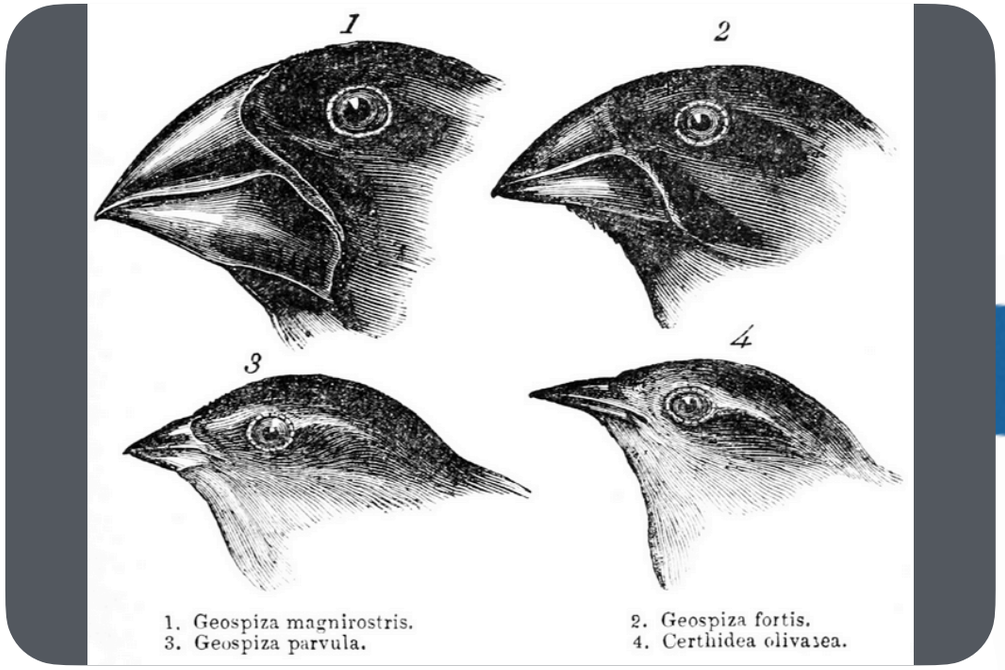
https://www.nature.com/articles/nature09929

PACE can be used to automatically select for anything that can be turned into something to activate gene expression….



https://www.nature.com/articles/nature09929

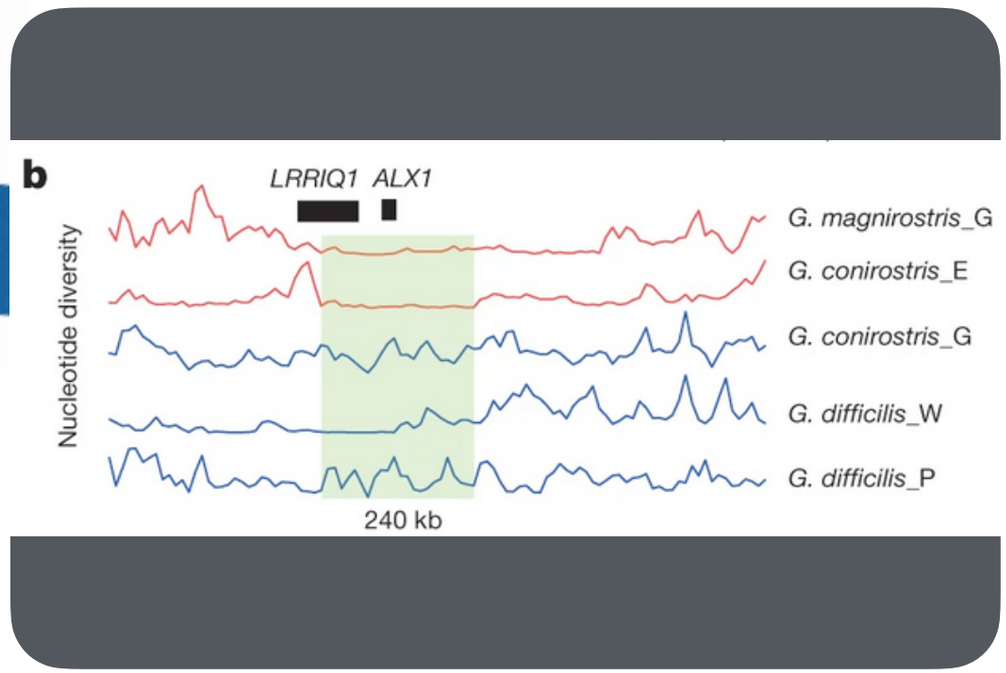
Gene Drive Example…



https://en.wikipedia.org/wiki/Darwin%27s_finches

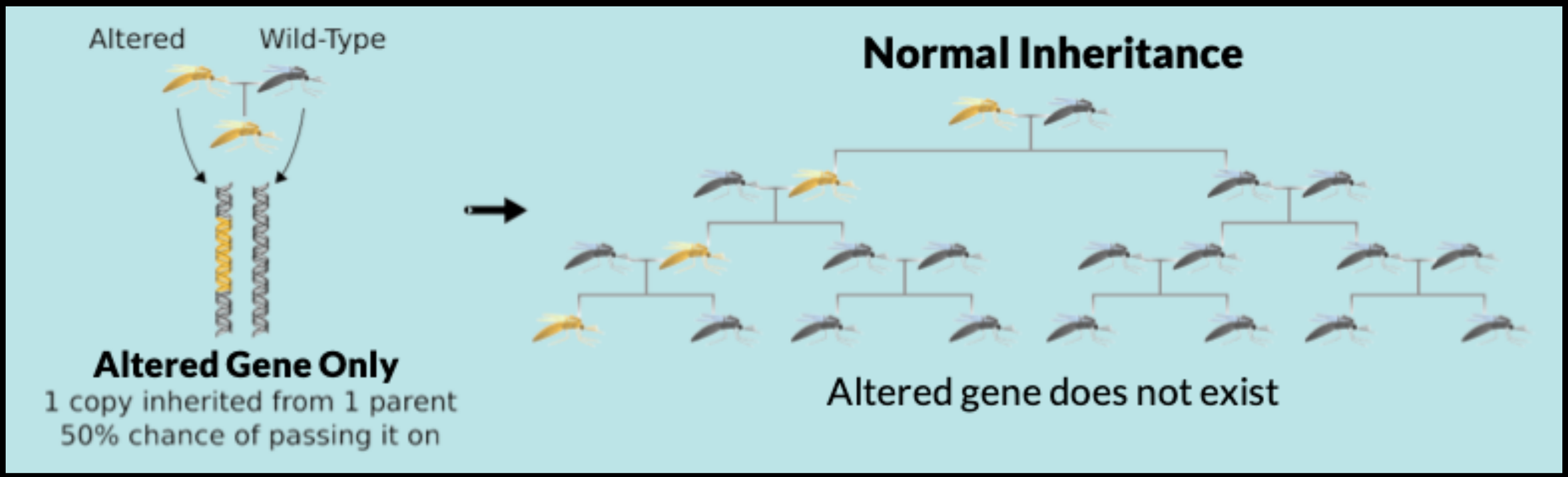
survival of the fittest

genotypes influence phenotypes



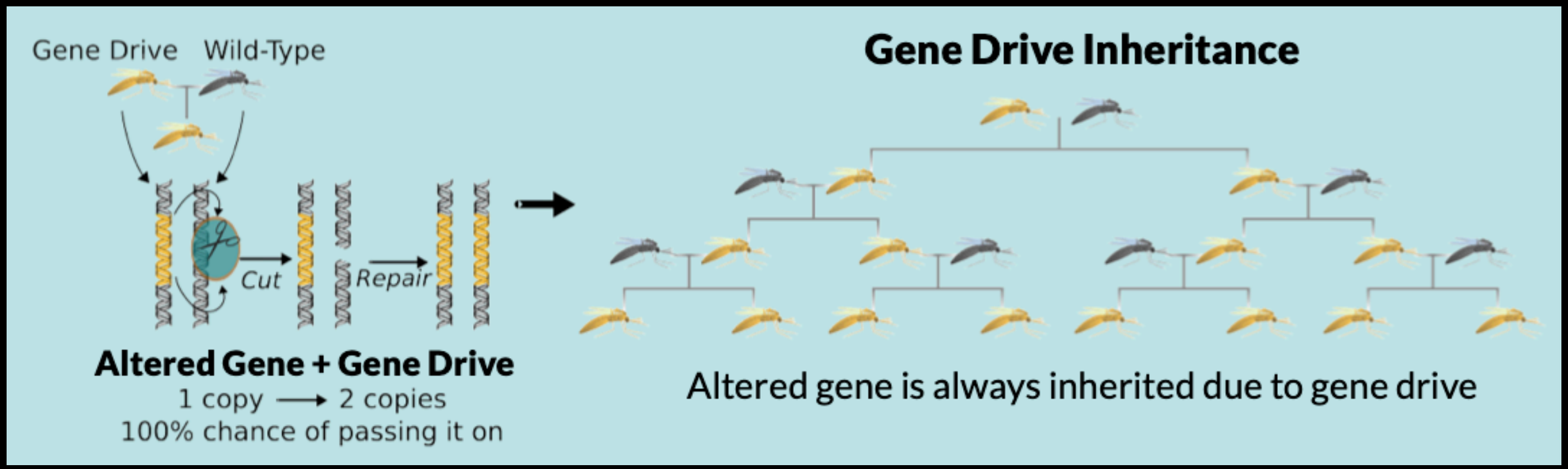
https://www.nature.com/articles/nature14181

Note — all preceding examples maintain randomness of mutations and normal inheritance…



https://en.wikipedia.org/wiki/Gene_drive

What if bioengineers changed these fundamental aspects of the evolutionary process? GENE DRIVES!!!



https://en.wikipedia.org/wiki/Gene_drive

In-progress project assessments

Group #	First assessment	Second assessment
Group-1		
Group-2		
Group-3		
Group-4		
Group-5		
Group-6		
Group-7		
Group-8		
Group-9		
Group-10		
Group-11		
Group-12		
Group-13		
Group-14		
Group-15		
Group-16		
Group-17		
Bioe Baddies