Class core values

1. Be **respect**ful to yourself and others
2. Be **confident** and believe in yourself
3. Always do your **best**
4. Be **cooperative**
5. Be **creative**
6. Have **fun**
7. Be **patient** with yourself while you learn
8. Don’t be shy to **ask “stupid” questions**

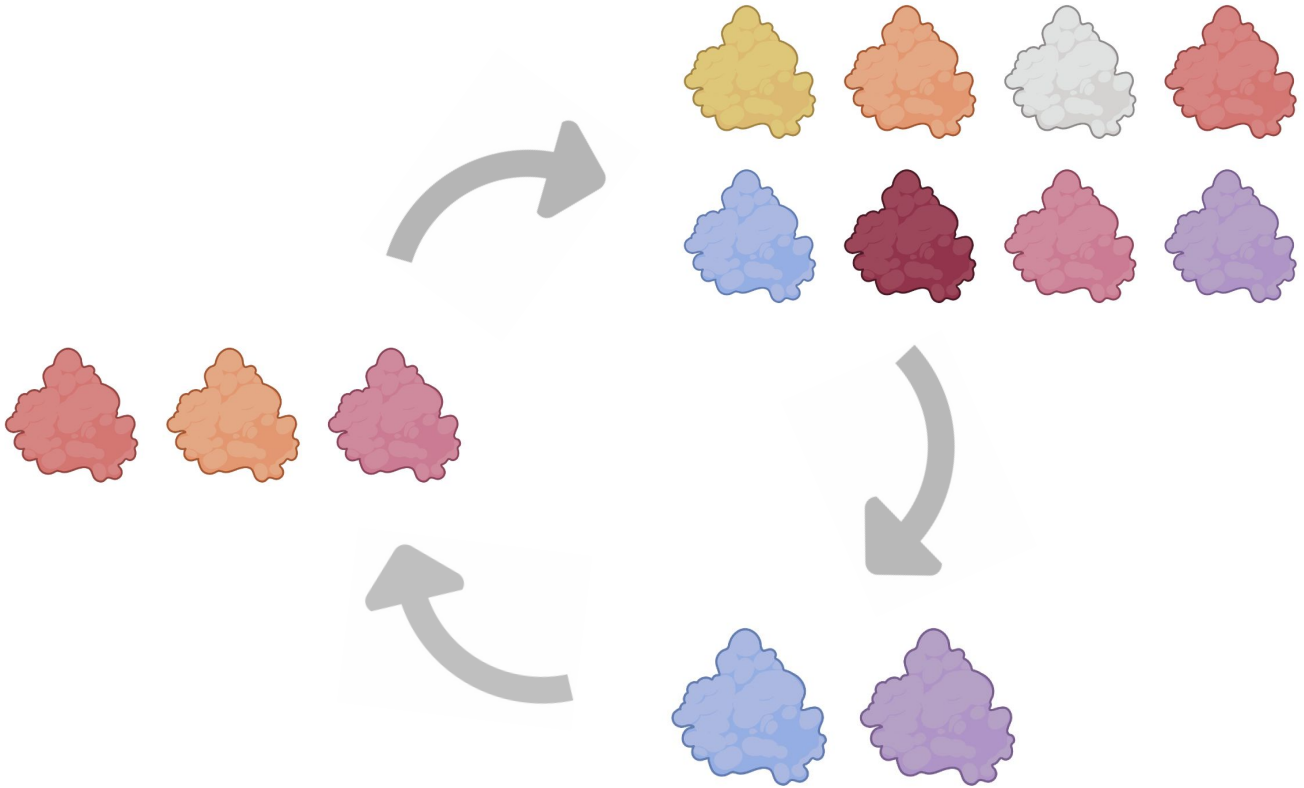
Week 4, Lecture 1

You get what you select for …

Learning Objectives

1. Describe the concepts of selection and screening
2. Identify the main methods for evolving binders
3. Critically evaluate the use of screening/selection methods for a given application
4. Identify methods for linking phenotype and genotype

The overall process of directed evolution



A key part of directed evolution is choosing the best variants

Screening is the process of going through all variants and picking up the best one

Fluorescence can be used for screening

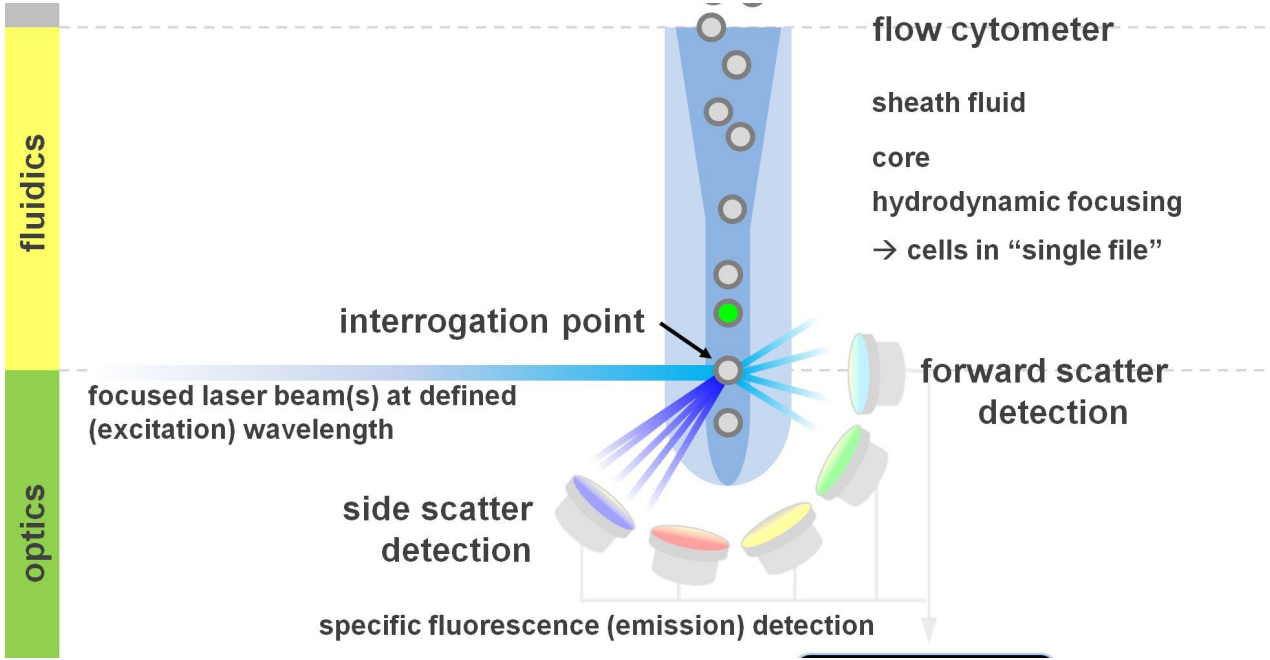
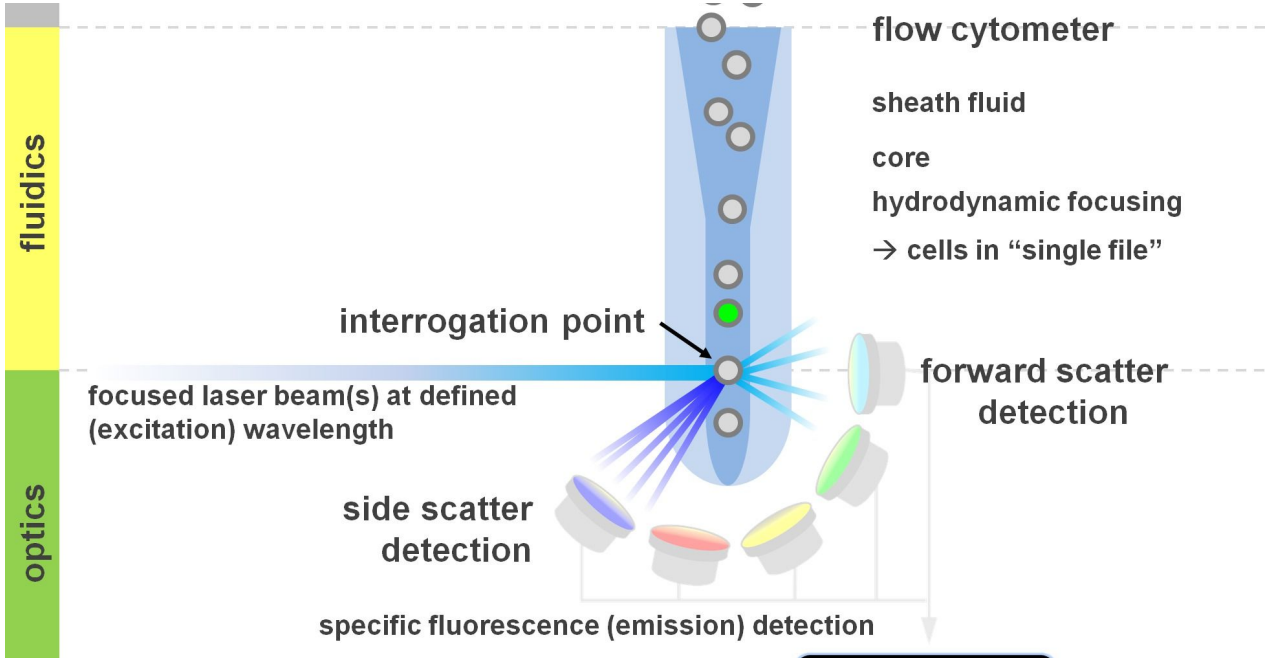


Image from JoVE journal, December 18, 2014

Fluorescence can be used for screening



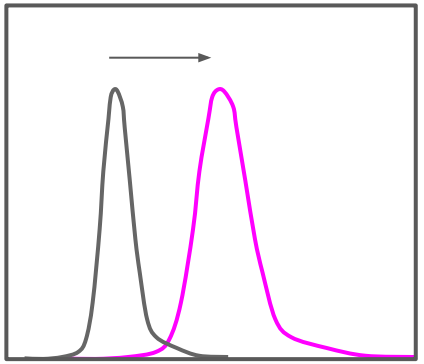


Image from JoVE journal, December 18, 2014

Many enzymatic assays are screening-based

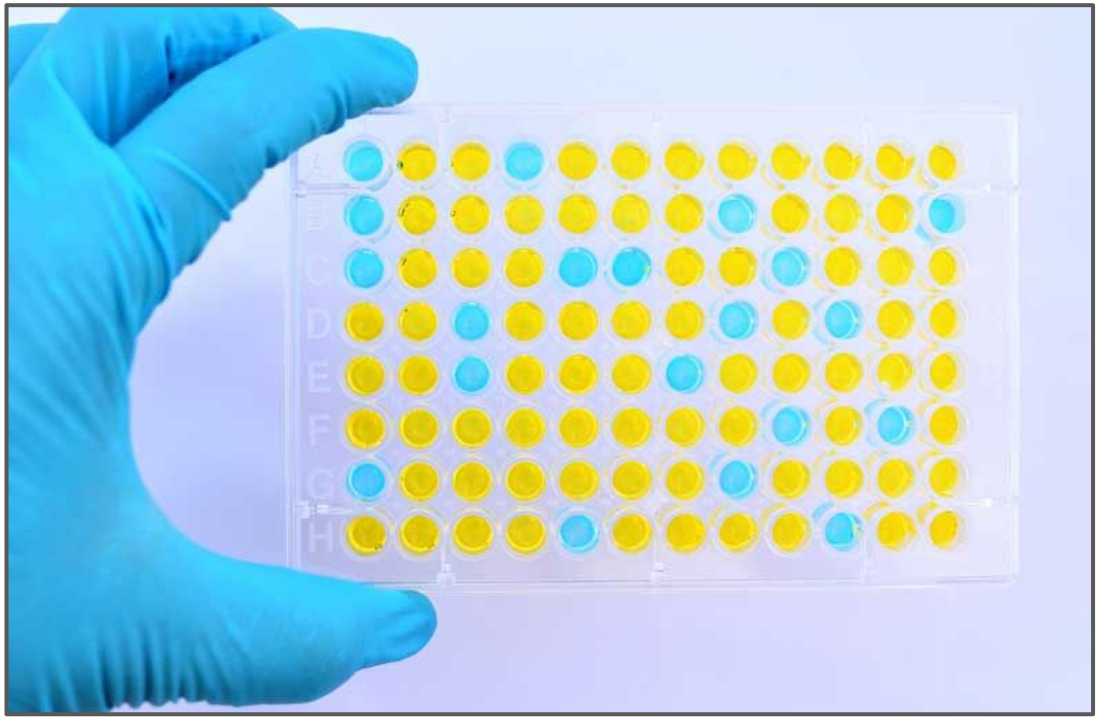
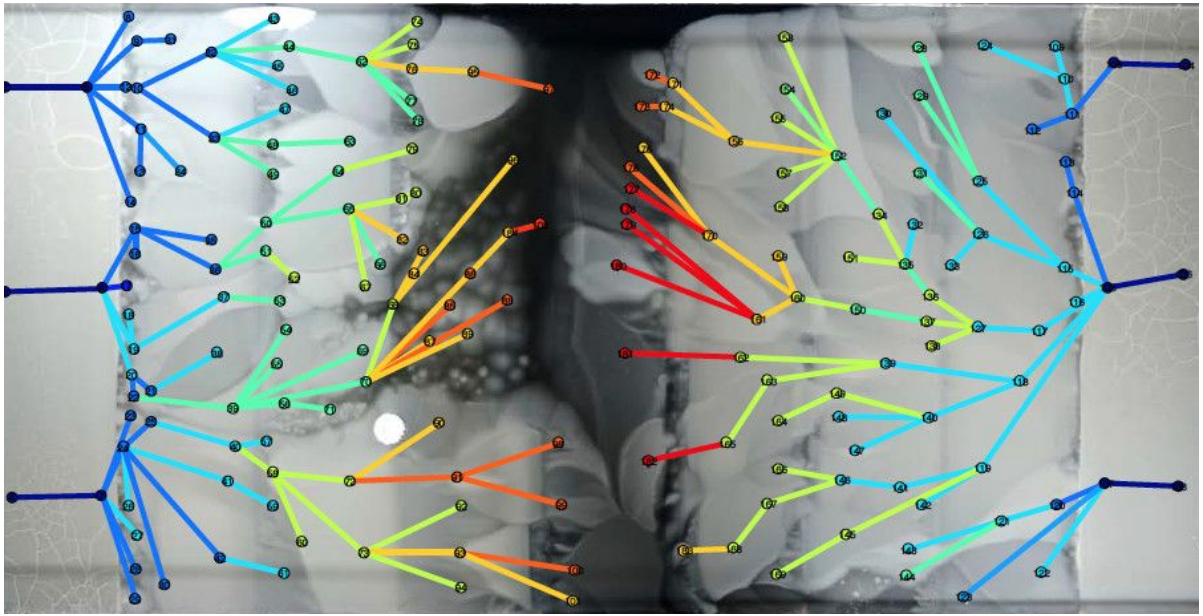


Image from Terpene and testing magazin

When you perform **selection**, only the fit variants survive

Antibiotic plates are the most common method for selection



The molecular ecologist: “Visualizing the evolution of bacterial resistance”

The outcome of evolution studies heavily depend on your selection/screening method

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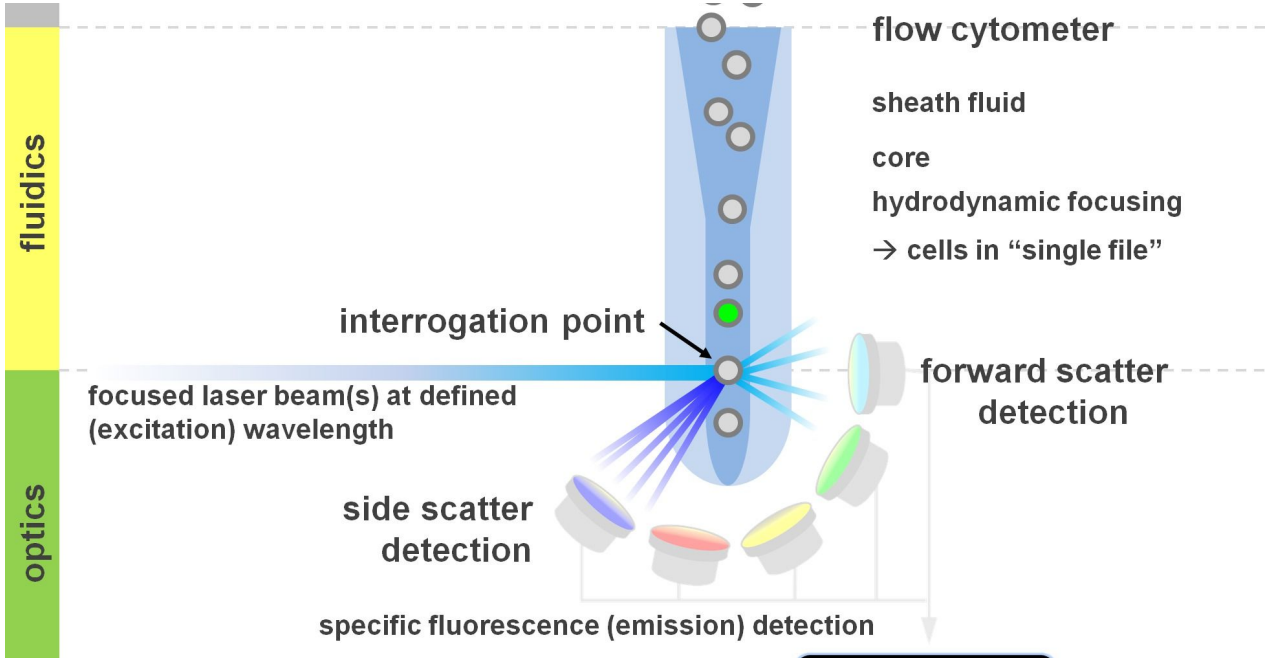
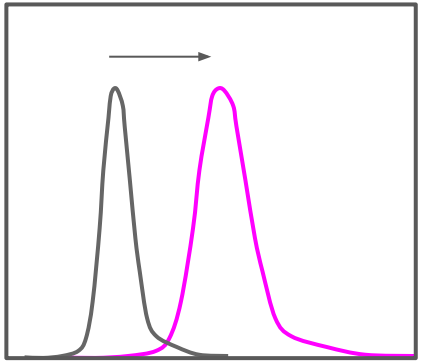
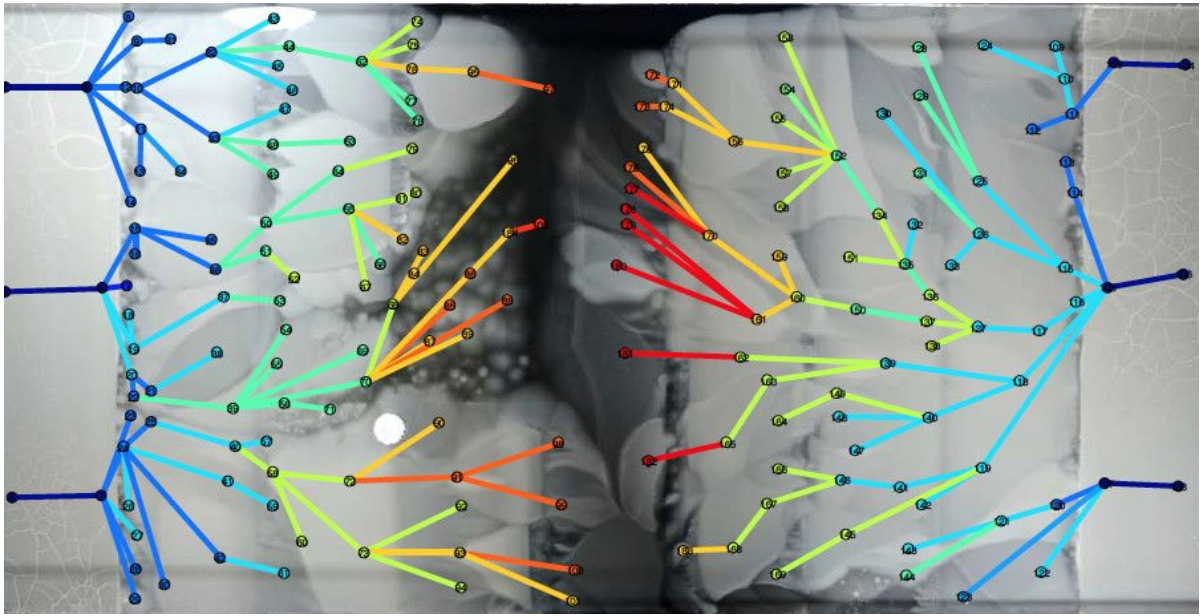
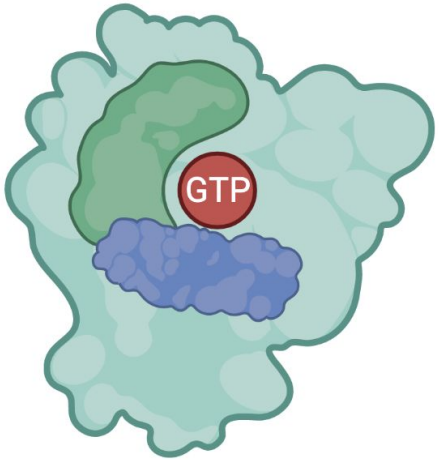




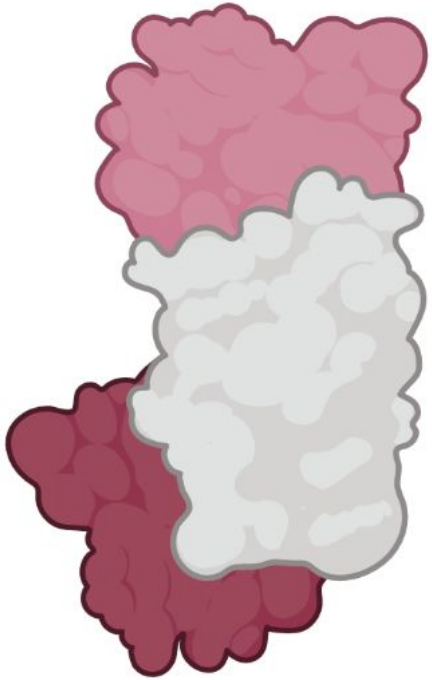
The outcome of evolution studies heavily depend on your selection/screening method



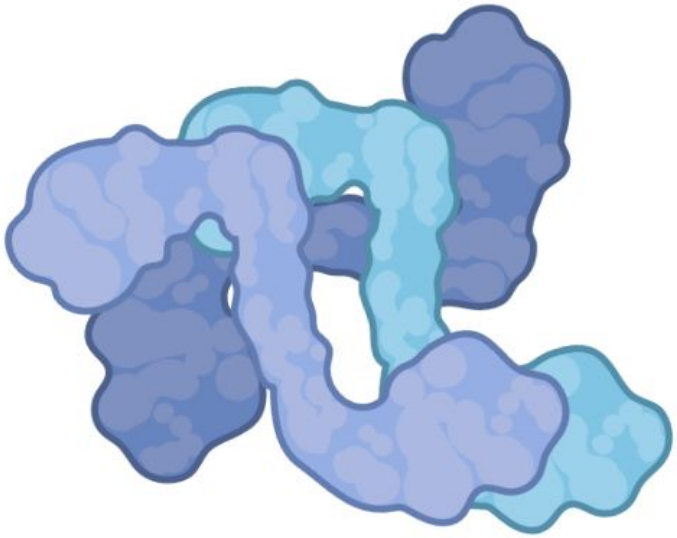
In-class activity: You get what you select for …



Scenario 1

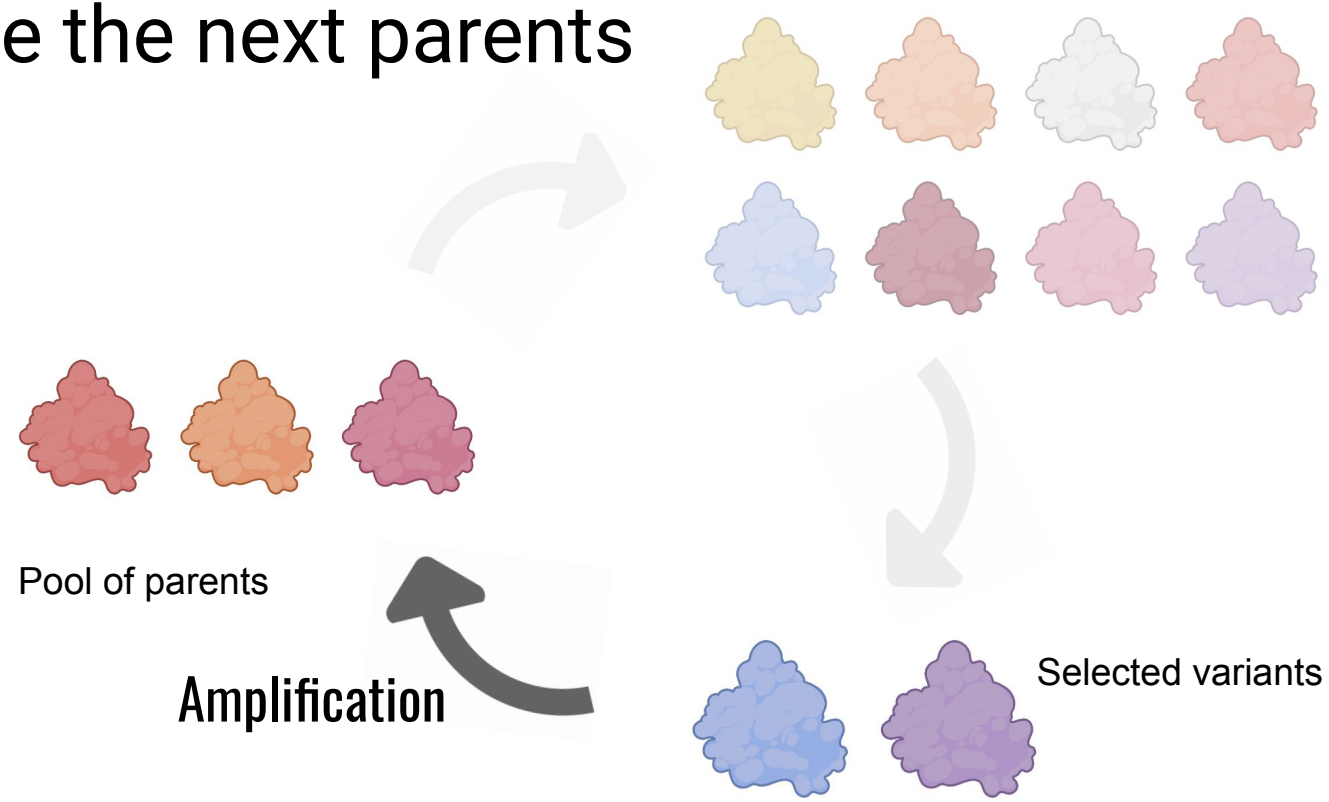


Scenario 2



Scenario 3

The selected variants need to be amplified to create the next parents



Selection happens at the protein level

Selection happens at the protein level, but **amplification** is at the DNA level

Selection happens at the protein level, but **amplification** is at the DNA level

We need a way to link the phenotype (protein) to genotype (DNA)

In low-throughput assays …

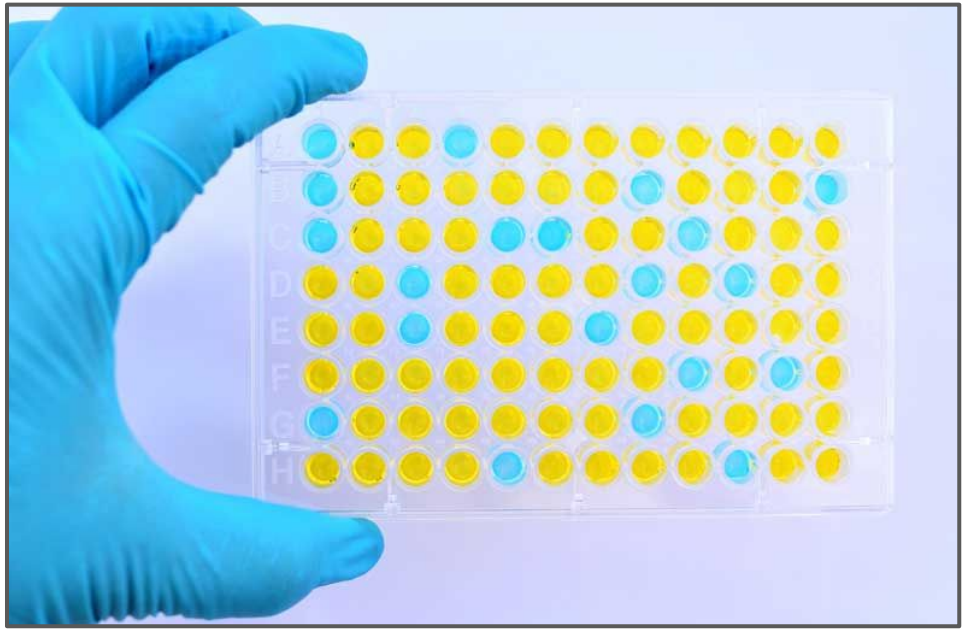
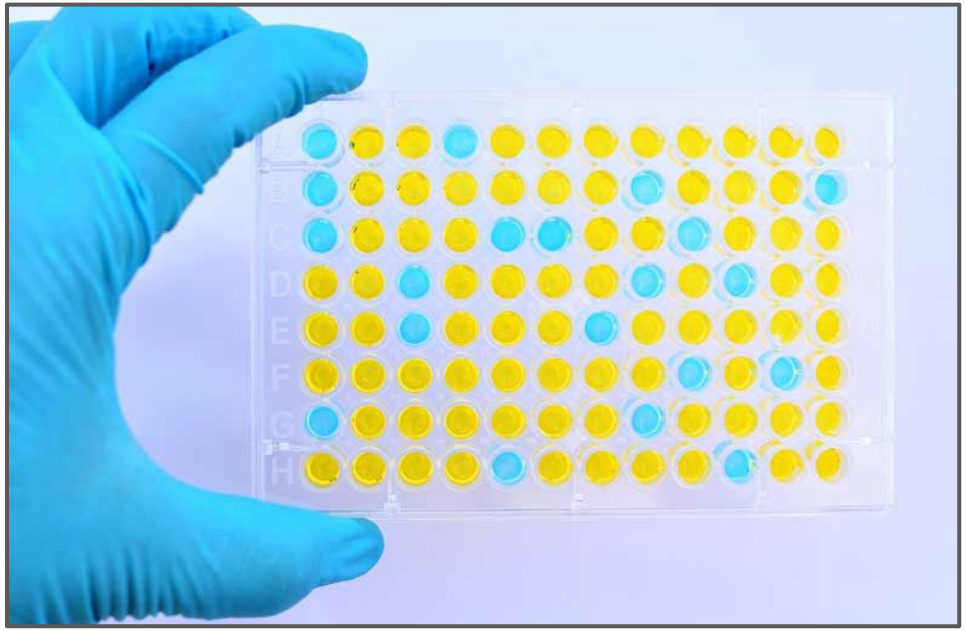


Image from Terpene and testing magazin

In low-throughput assays, you can take note of the phenotype-genotype link

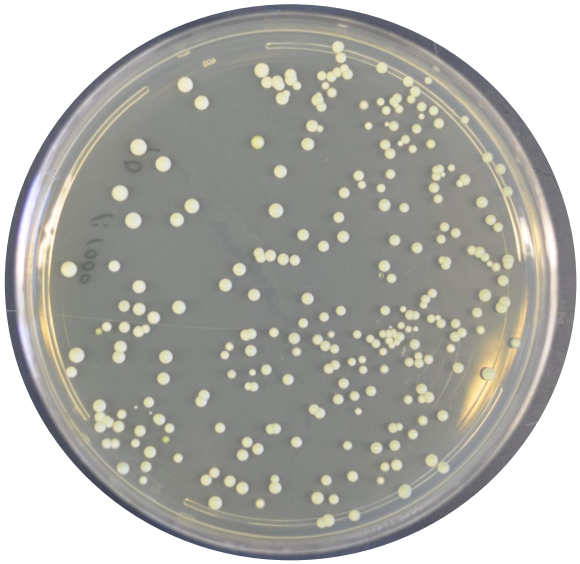


Row ID	Activity	Sequence
A1	90%	LKNMGFTHILKDFSA
A2	25%	LKQMGFSHILKDWSA
A3	40%	IRNMGYTHIVKDFSA
…	…	…
H12	35%	LRNCGWTHIIKDFTV

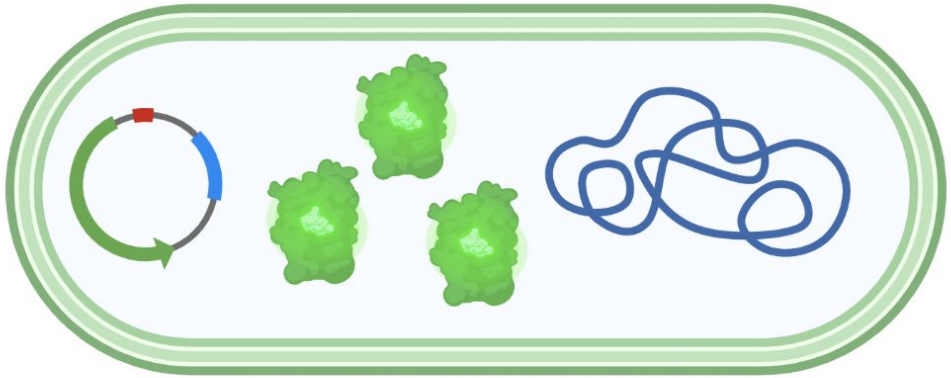
Image from Terpene and testing magazin

Containment: Keeping the activity inside the cell where the DNA is!

Antibiotic resistance is an example of contained activities



Fluorescent protein signals are contained within the cytosol



Oil-water emulsions can be used to contain the phenotype and genotype within the same droplet

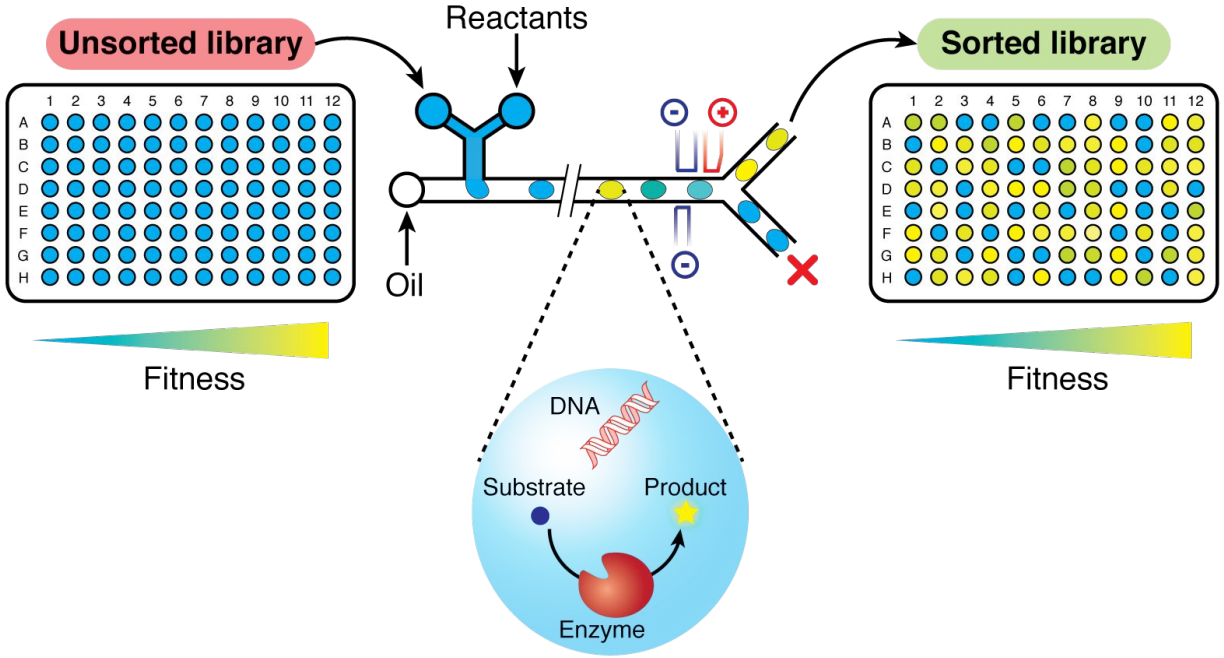


Image from Hilvert/Kats group webpage, ETH

Display: Covalently linking the protein to the DNA source

Phage display is one the most commonly used display methods

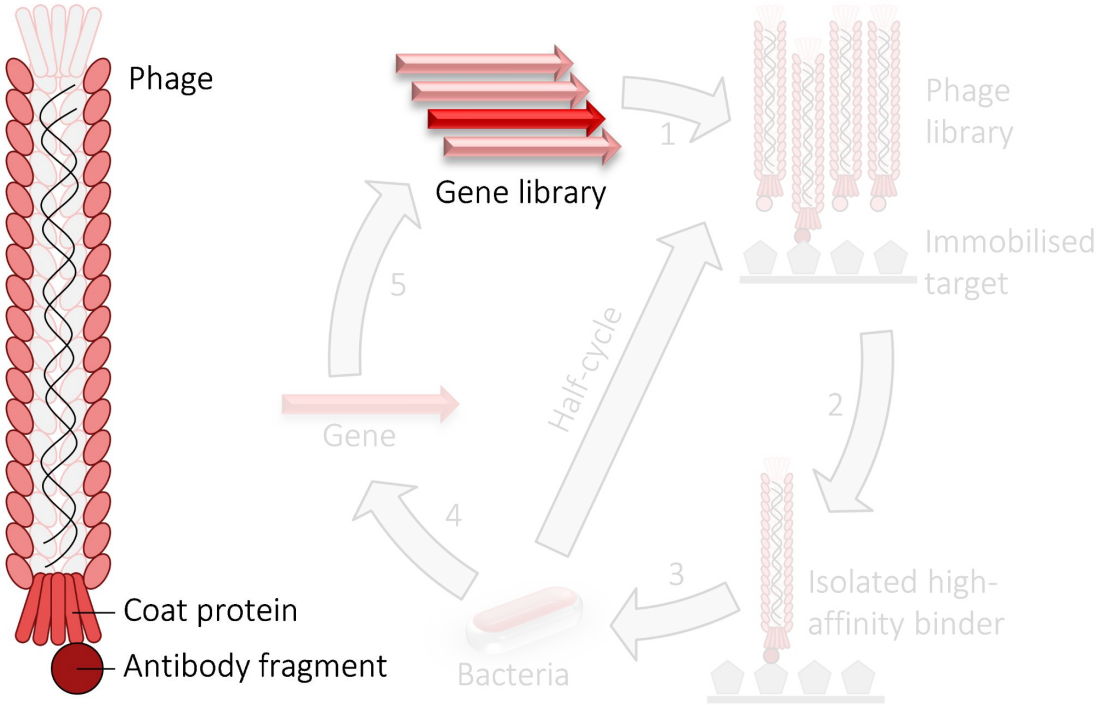


Image from Wikipedia, Phage display

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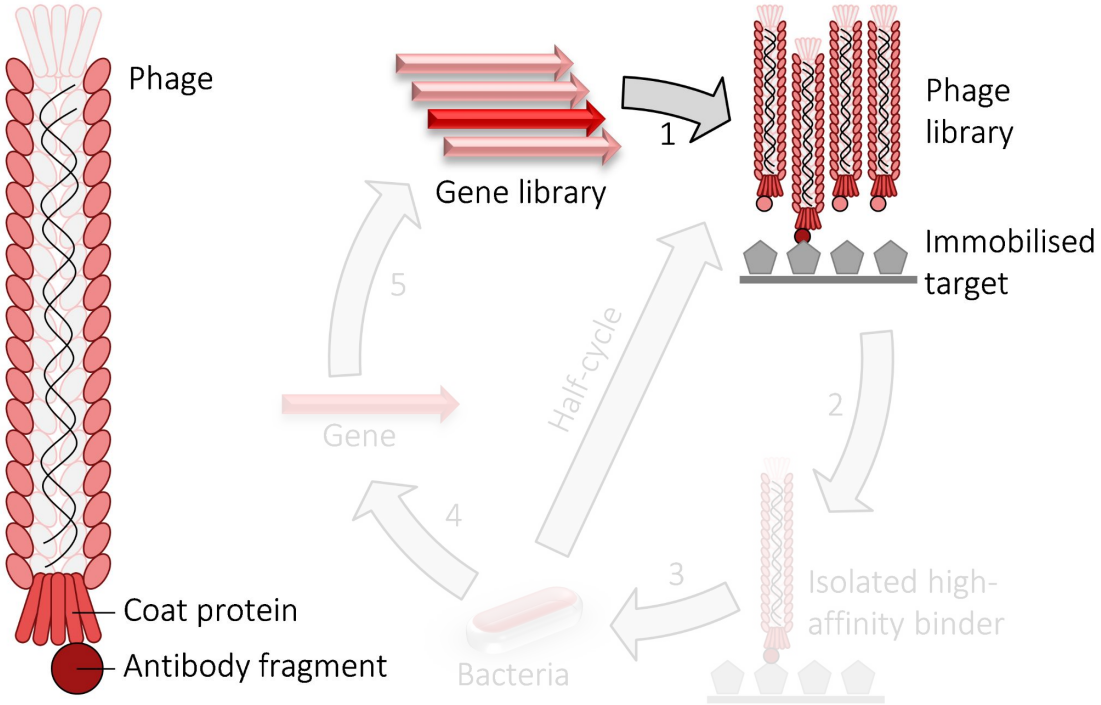

Image from Wikipedia, Phage display

Phage display is one the most commonly used display methods

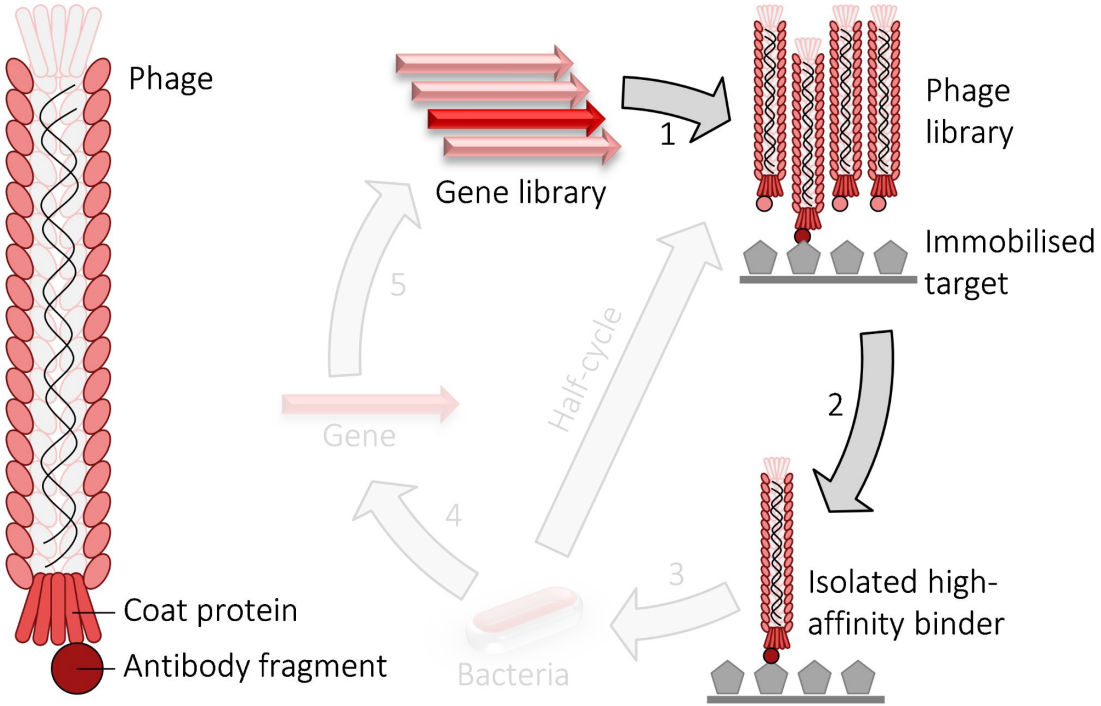

Image from Wikipedia, Phage display

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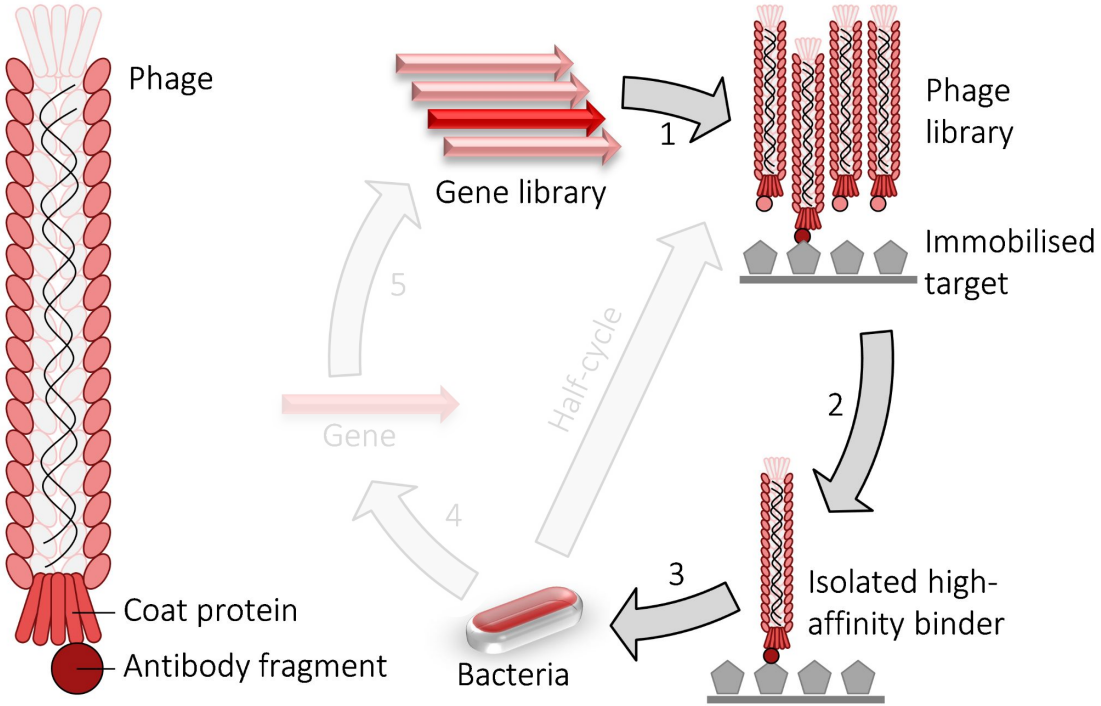

Image from Wikipedia, Phage display

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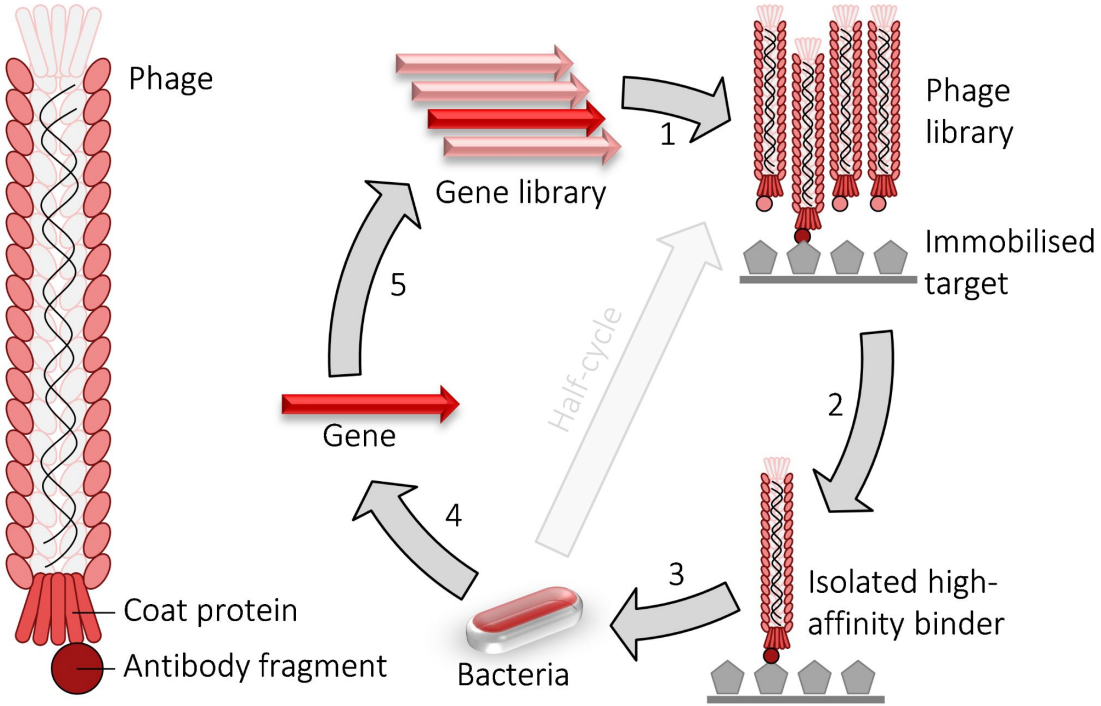

Image from Wikipedia, Phage display

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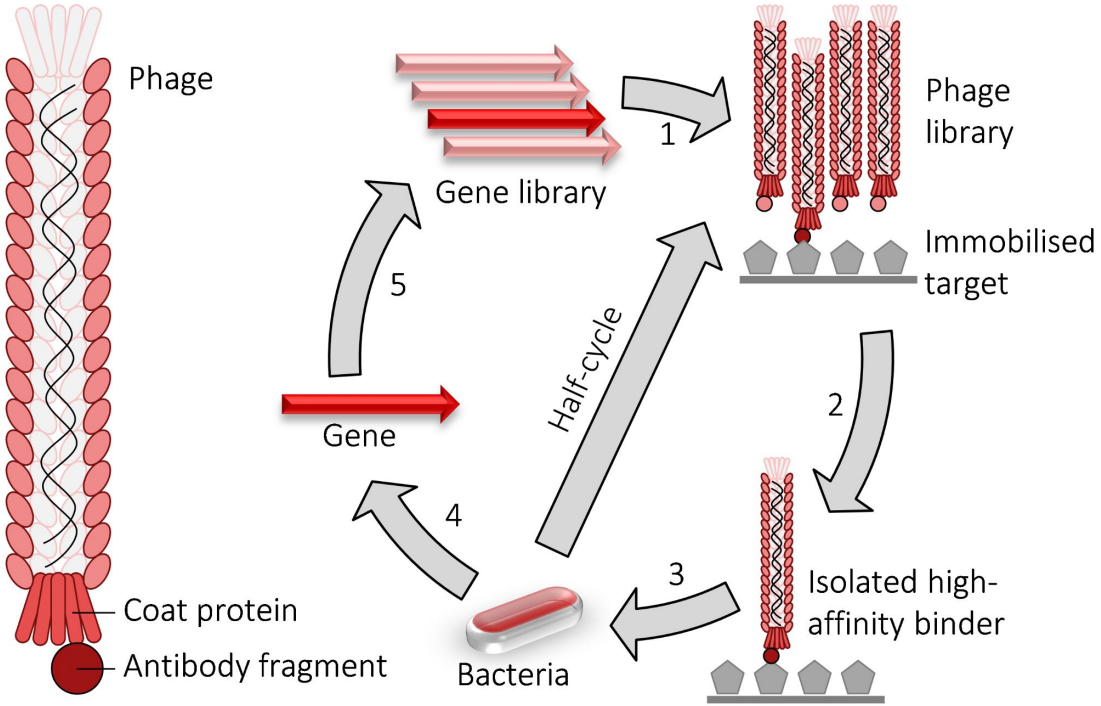

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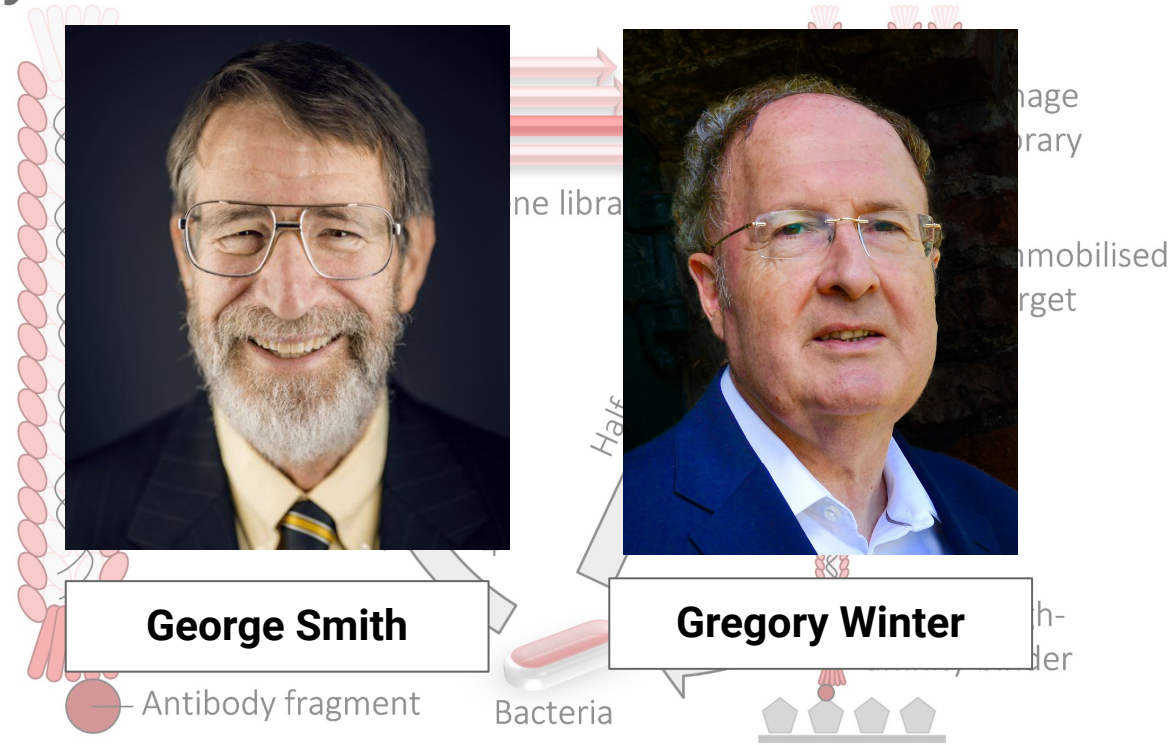

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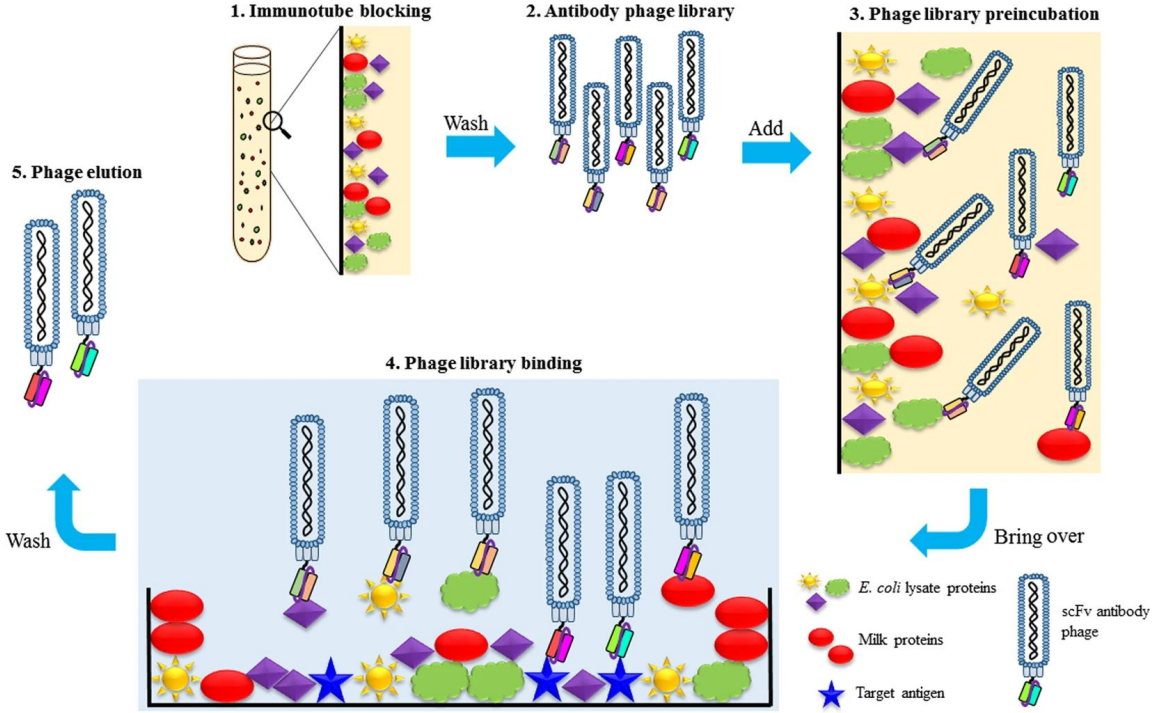
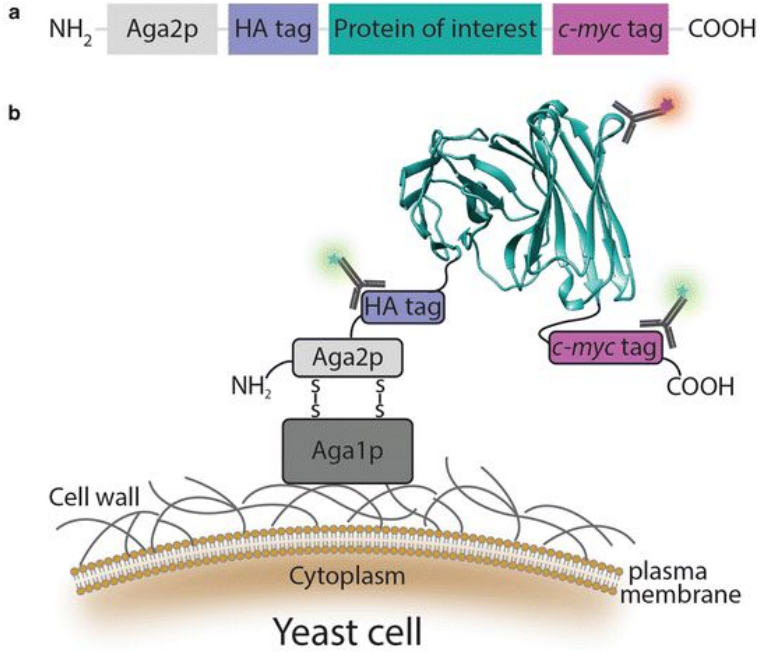


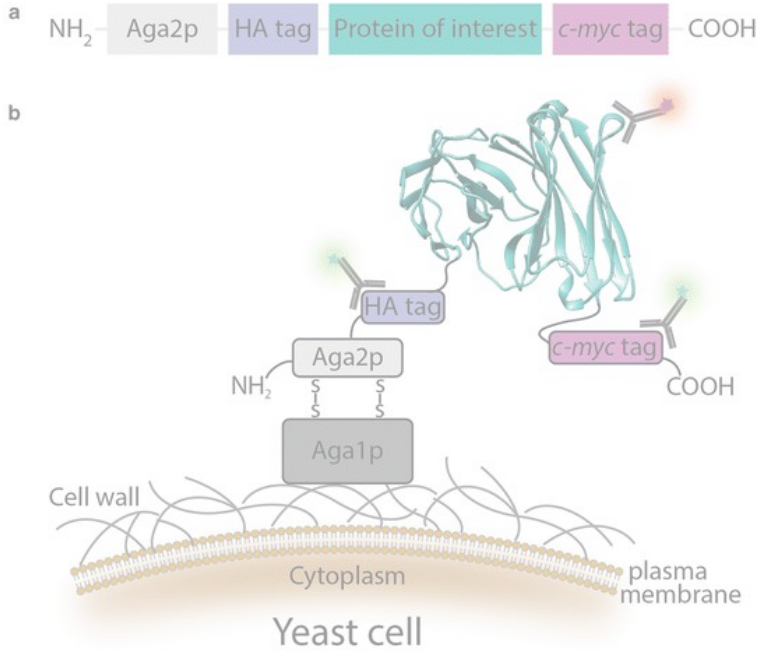
MERS-CoV nucleoprotein antibodies: *Sci Report* 2019, **9** (6088)

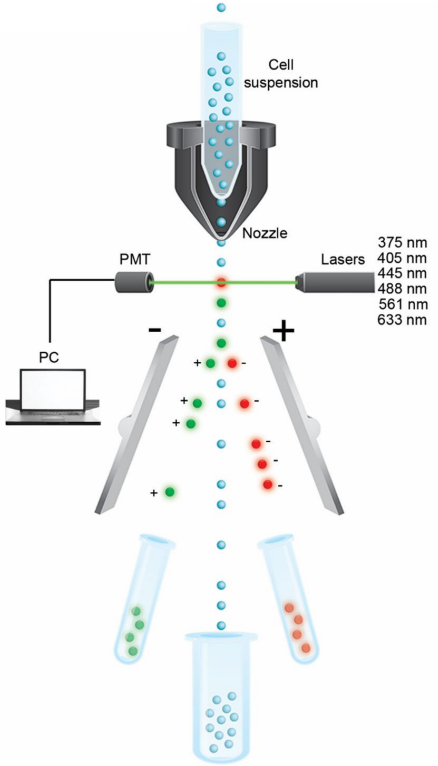
Yeast surface display is the method of choice for most protein engineering approaches



From Yeast Surface Display, pp 155-175

Yeast surface display can be linked with FACS to obtain best binders





From Yeast Surface Display, pp 155-175
Image from *Int J Mol Sci.* 2015 Nov; 16(11): 26832–26849

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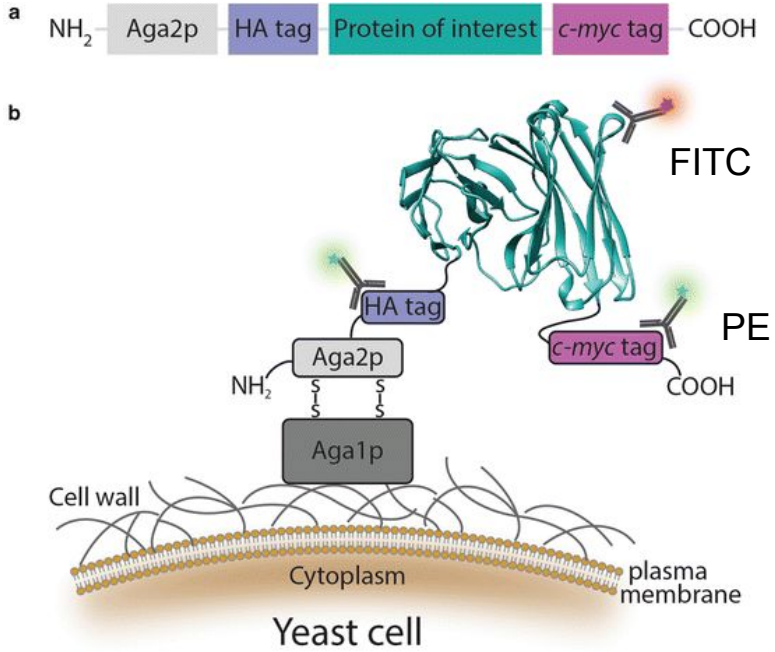

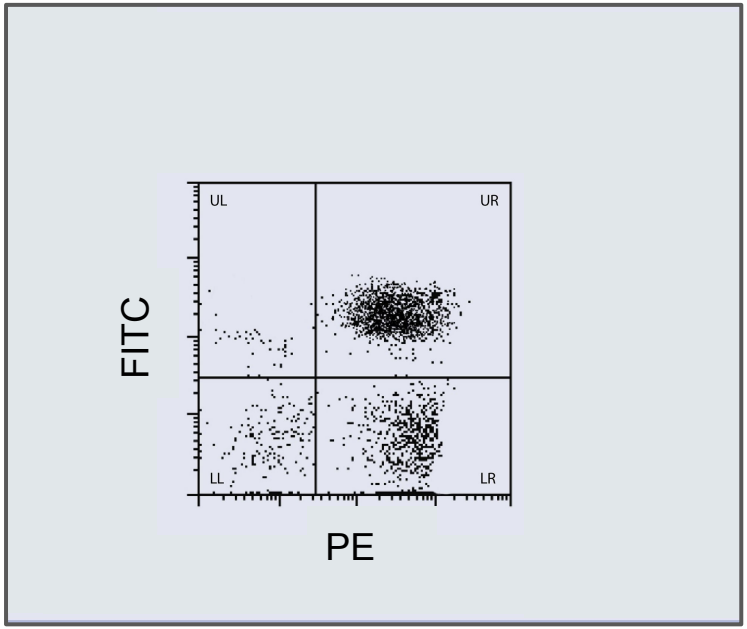

From Yeast Surface Display, pp 155-175
Image from Miltenyi Biotec

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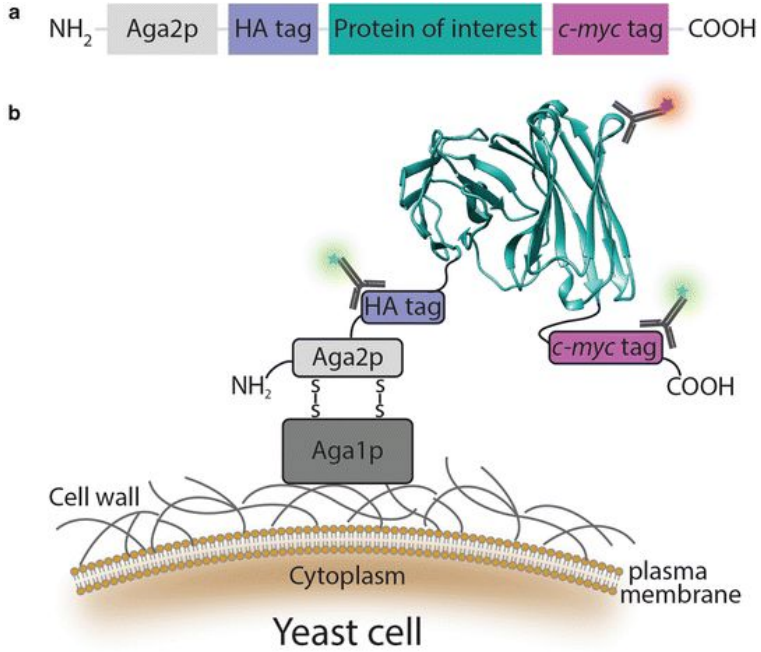

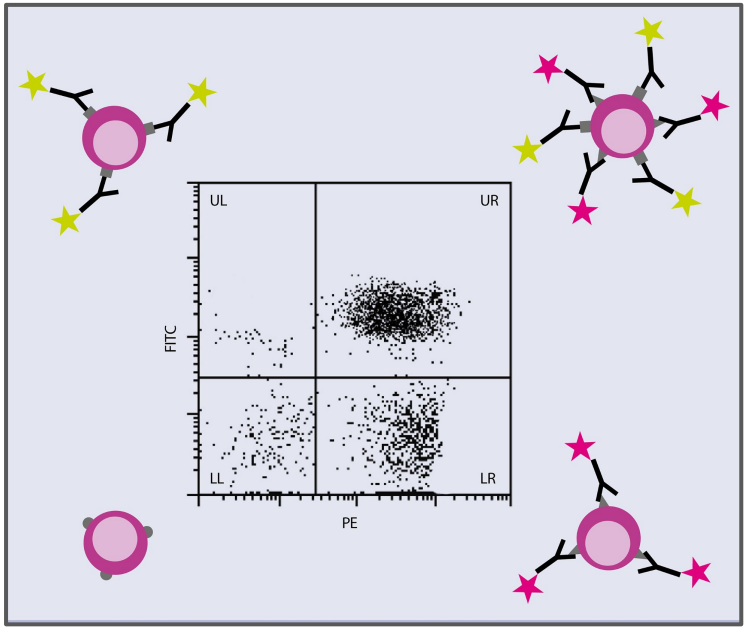

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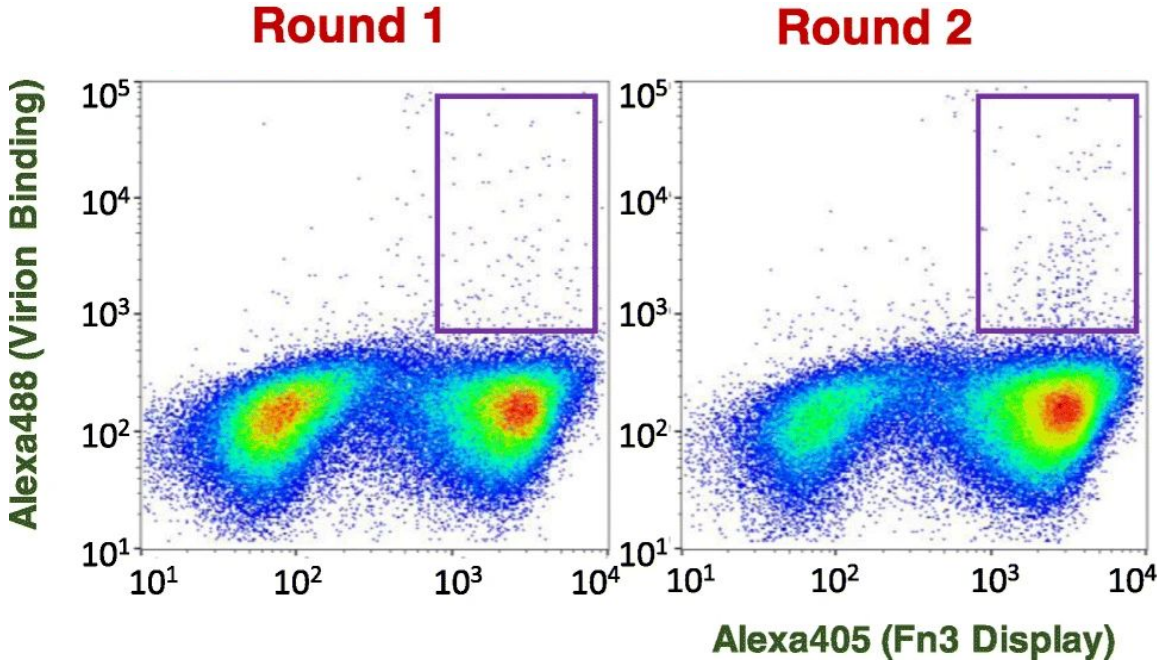
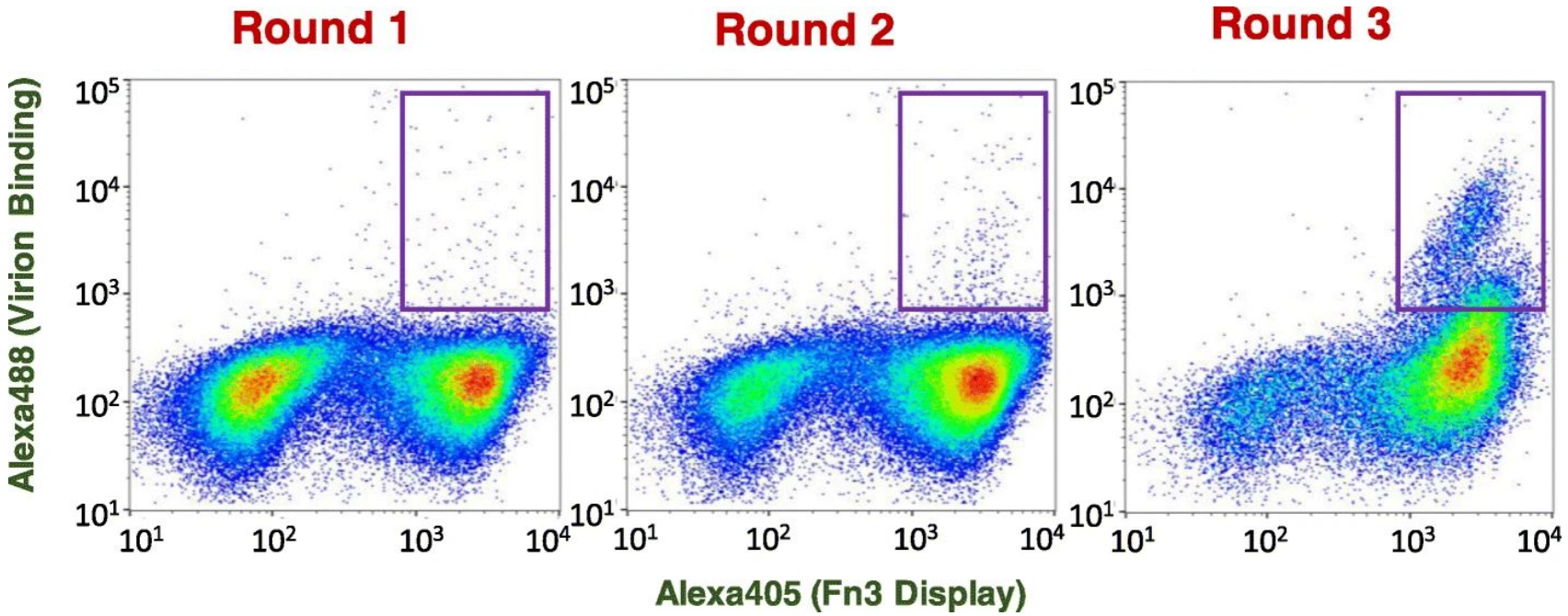
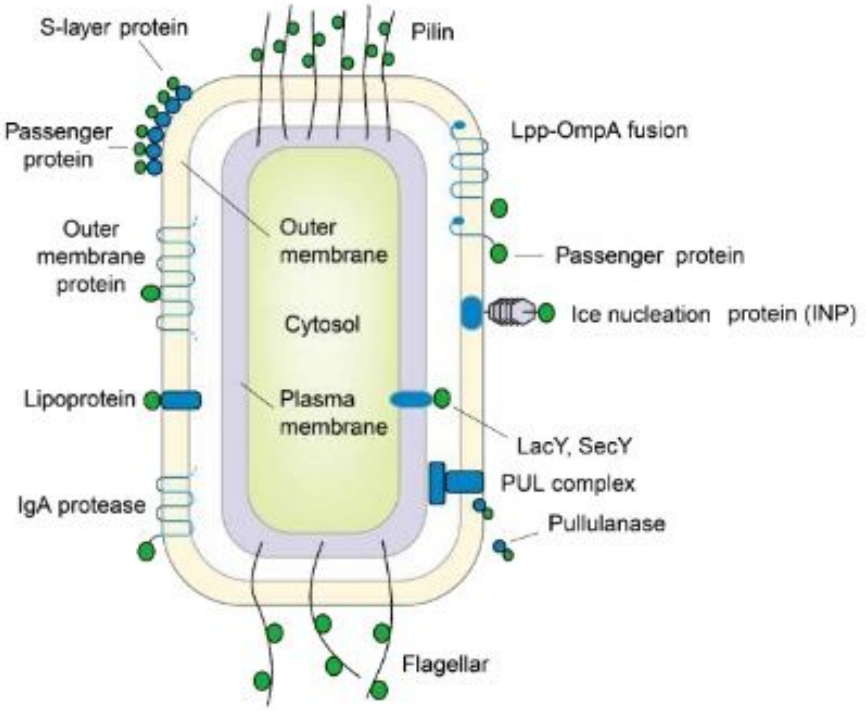


Yeast surface display can be linked with FACS to obtain best binders



Virion binding fibronectin domains: *J Biol Eng* 2019, **13** (76)

Proteins can be displayed on the surface of *bacteria*



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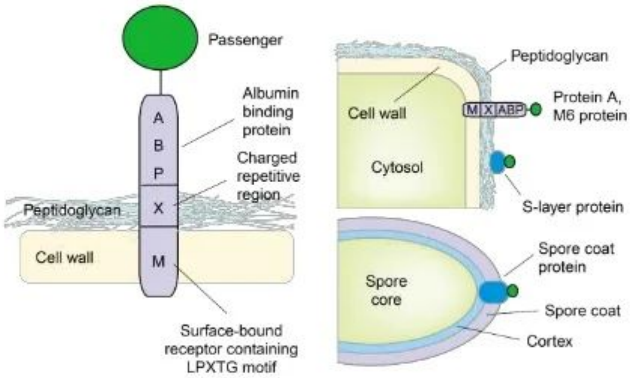
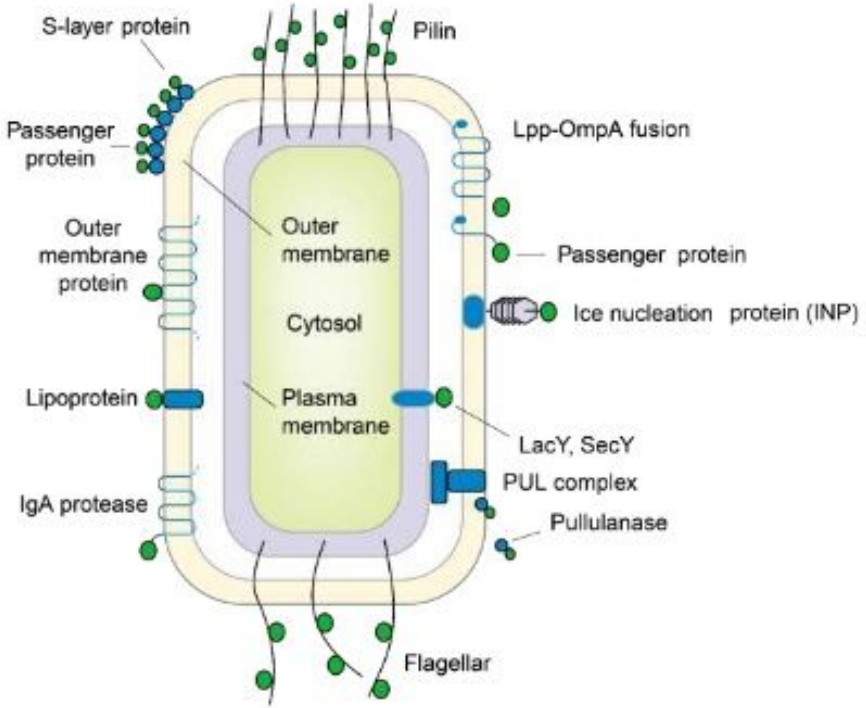
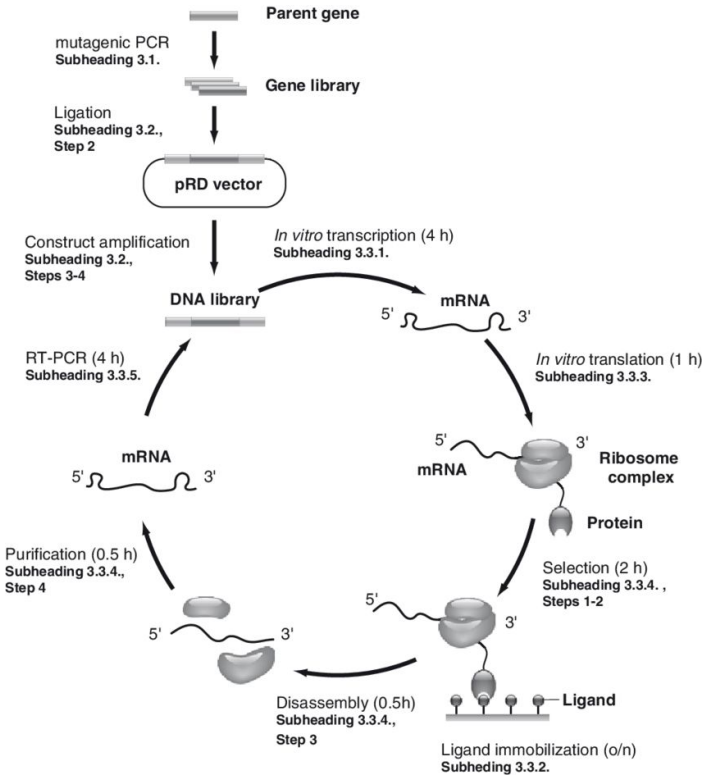
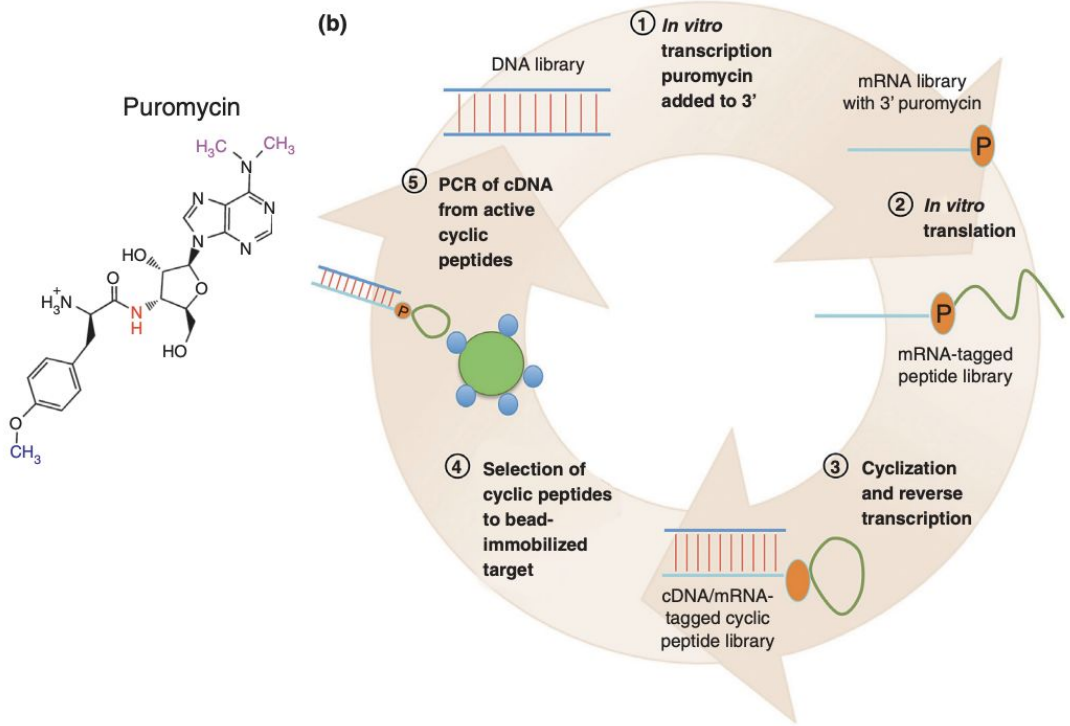




Ribosome display can also be used for displaying proteins



mRNA display is attracting attentions as a method for selecting peptide binders



Each method has its limitations/applications

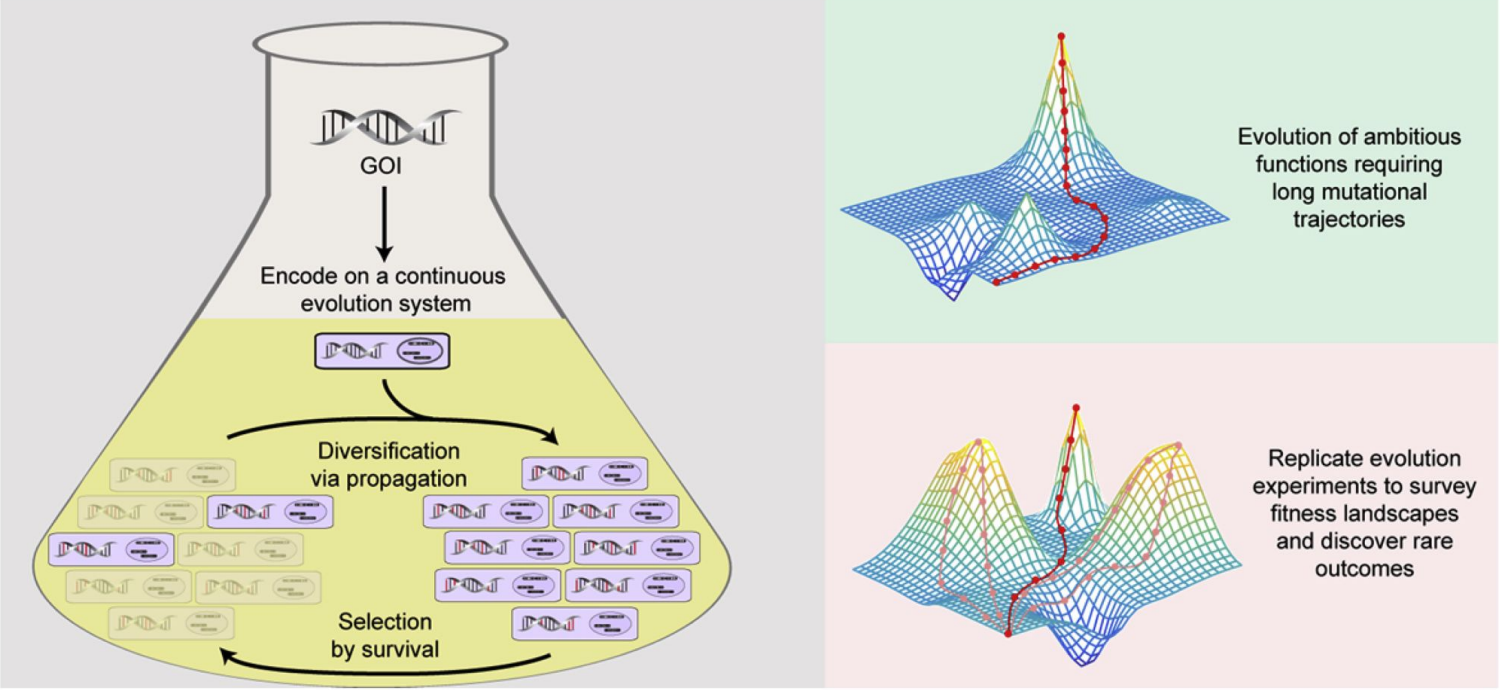
	Phage display	Eukaryotic display	Prokaryotic display	Ribosome display	mRNA/cDNA display
Host organism	Filamentous phages, M13, T4, T7, lambda, phagemid	*S. cerevisiae*, *P. pastoris*	*E. coli*, *B. subtilis*, *L. bacillus*, *S. camosus*	*In vitro*	*In vitro*
Library size					
Highest affinity K_d (M)[a]					
Typical enrichment factor per round					
Nucleic acid selected					
Transformation required					
Library form					
Proteins to be displayed					
Covalent link					
Surface anchorage					
Post translational machinery					

Each method has its limitations/applications

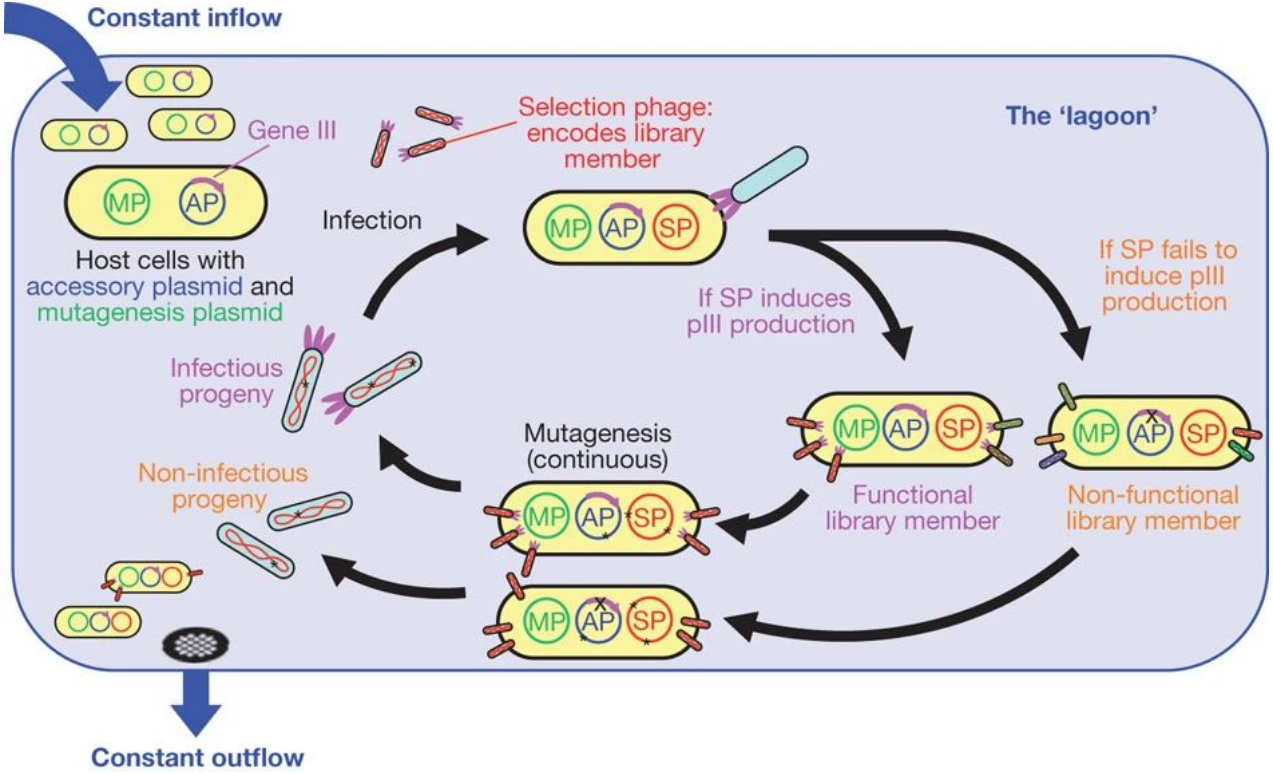
	Phage display	Eukaryotic display	Prokaryotic display	Ribosome display	mRNA/cDNA display
Host organism	Filamentous phages, M13, T4, T7, lambda, phagemid	*S. cerevisiae*, *P. pastoris*	*E. coli*, *B. subtilis*, *L. bacillus*, *S. camosus*	*In vitro*	*In vitro*
Library size	10^{9a}	10^{7}	10^{8-10}	10^{13-14}	10^{13-14}
Highest affinity K_d (M)[a]	10^{-11}	10^{-14}	10^{-13}	10^{-12}	10^{-10}
Typical enrichment factor per round	10^{2-4}	10^{2-3}	10^{2-3}	10^{1-3}	10^{1-3}
Nucleic acid selected	DNA	DNA	DNA	mRNA	mRNA/cDNA
Transformation required	Yes	Yes	Yes	No	No
Library form	Plasmid	Plasmid	Plasmid	PCR fragment or mRNA	mRNA/cDNA, plasmid
Proteins to be displayed	Soluble, nontoxic, compatible with crossing membranes	Soluble and membrane, nontoxic, compatible with crossing membranes	Soluble and membrane, nontoxic, compatible with crossing membranes	Most proteins including cytotoxic, chemically modified and membrane proteins	Soluble, including cytotoxic, chemically modified
Covalent link	No	No	No	No	Yes (synthetic)
Surface anchorage	Capsid proteins	Agglutination proteins, flocculation proteins	Lpp-OmpA, autotransporter proteins, ice nucleation proteins	Ribosome	*In vitro*
Post translational machinery	Simple	Sophisticated	Moderate	Moderate	Simple

Molecular Biosystems 2016, **12**, 2342-2358

Continuous evolution methods combine diversification, selection and amplification



Phage-Assisted Continuous Evolution (PACE)



Nature (2011), **472**, 499-503

For the next lecture:

1. Pre-class assessment for the next lecture
   Needs to be done before the start of class, will be available after this class

2. Post-class assignment
   Write up questions for our panelists

3. Second journal: Will be discussed next week

Next lecture:

The challenging case of enzymes

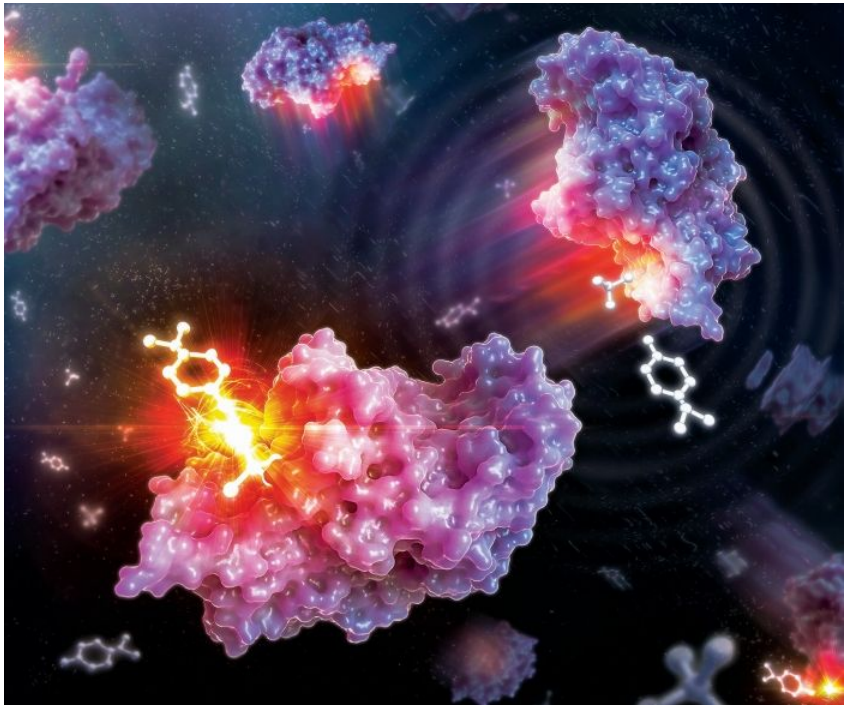


Image from: C&EN, Enzyme catalysis is a moving experience