L15 Directed Evolution

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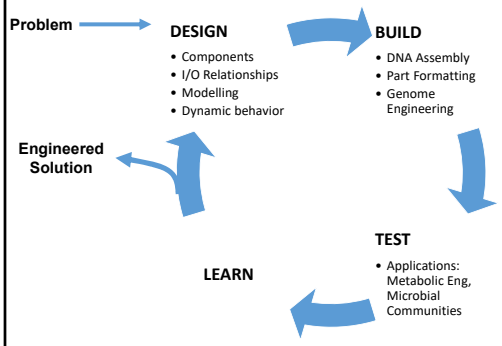
Fall 2018

Recall…



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This lecture

- Approaches to tune system properties
- Creating diverse libraries
- Library screening

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How do we tune the biochemical properties of a protein? *How do we change* β, or K?

1. **Change expression circuit design**
   E.g. a gene cascade amplifies (multiplies) β, plasmid copy number

2. **Rational engineering of the protein/part itself**
   e.g. promoter expression strength controlled by the -10 and -35 regions

http://parts.igem.org/Promoters/Catalog/Anderson

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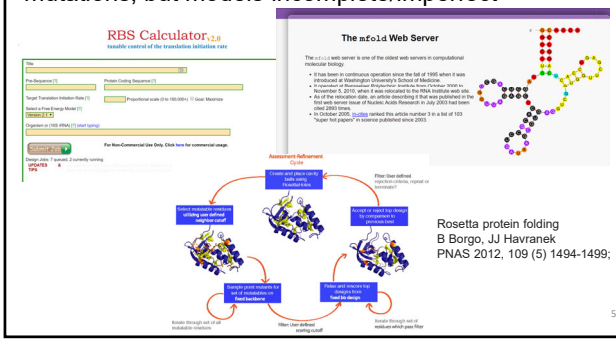
Rational engineering introduces point mutations based on insight

Computational tools exist to predict effect of point mutations, but models incomplete/imperfect

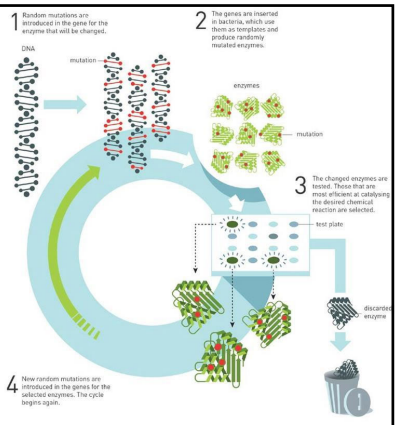


Rosetta protein folding
B Borgo, JJ Havranek
PNAS 2012, 109 (5) 1494-1499;

Directed evolution can also engineer properties ***without*** knowledge of underlying biochemistry

Directed evolution mimics Darwinian evolution in a lab on much shorter timescale

Step 3 works best if property of interest is 'selectable'



https://www.chemistryworld.com/news/what-is-directed-evolution-and-why-did-it-win-the-chemistry-nobel-prize/3009584.article

How do we create variants?

1. Point mutations
   - Can be targeted to key spots (e.g. -10 region, catalytic residues of an enzyme).

How would you generate and assemble these mutants?

Can use site-directed mutagenesis to create specific mutations

- Will frequently iterate through all possible amino acids → <u>saturation mutagenesis</u>

How many mutants would you need to make to mutate <u>every</u> amino acid position in a given protein (~ 100 aas)?

How do we create variants?

2. Random mutagenesis
   - Create untargeted random mutations to quickly iterate through sequence space

<u>Methods</u>

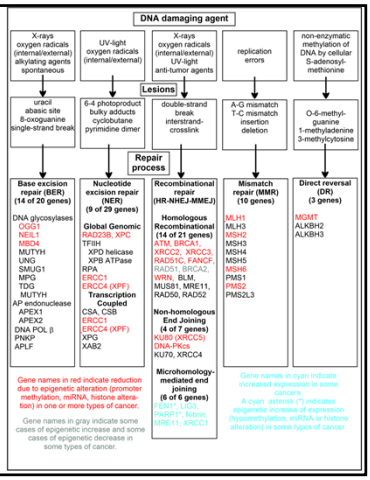
A. Error-prone PCR (ep PCR)
   - run PCR under more permissive conditions to accumulate errors
     - Use non-proof reading enzymes (e.g Taq)
     - ↑ $MgCl_2$
     - Use $MnCl_2$ rather than $MgCl_2$
     - Unbalanced nucleotide concentrations

     Each method will bias the library in some way (e.g. A→G, rather than A→G, C, or T)

Methods of random mutagenesis

B. Mutator strains

- Cells have complex pathways to repair DNA mutations that arise from environmental triggers
- Mutants in these pathways accumulate mutations faster



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

Methods of random mutagenesis

C. Chemical mutagenesis

- chemical mutagens make specific base pair errors (e.g. nitrous acid: C→ U)

	Pros	Cons
epPCR	• Easy to do	• Library bias • Assembly required
Mutator strains	• No cloning required	• Lack of DNA repair means host slowly accumulates lethal mutations
Chemical mutagenesis	• No cloning required	• Library bias

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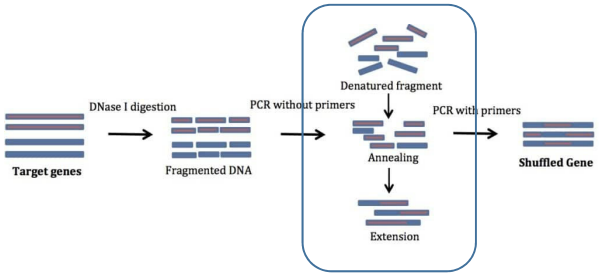
DNA shuffling can help retain function in mutants

DNA shuffling combines mutations from various mutants to maximize exploration of sequence space



https://bitesizebio.com/29721/dna-shuffling-like-pro/

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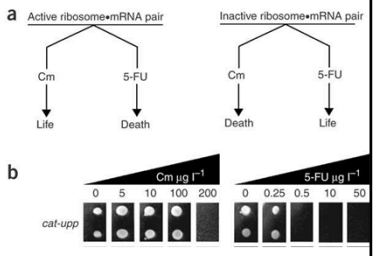
How do we screen mutants?

Selection screens are critical to identify more fit mutants

1. Survival assays
   - Improved function biases population and naturally enriches

   e.g. orthogonal ribosomes used to express antibiotic resistance



O Rackham J W Chin *Nature Chem Biol* **1**, 159-166 (2005)

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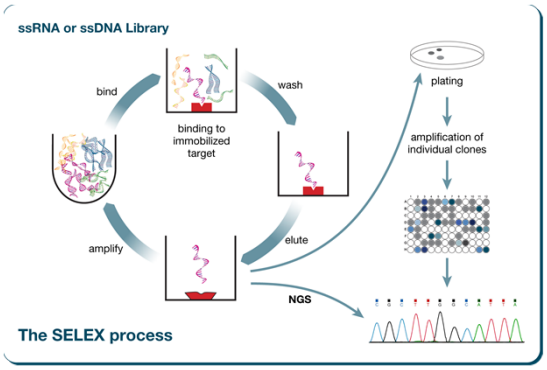
Selection & Directed Evolution can be used to make RNA parts that bind (SELEX – systematic evolution of ligands by exponential enrichment)



https://ngsdataanalysis.com/technology/selexaptamer/

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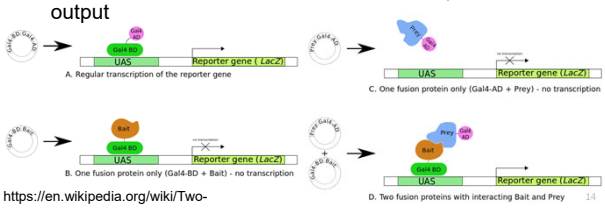
How do we screen mutants?

2. Colorimetric
   - Yields a clear signal such as fluorescence/color (e.g. lycopene production)

3. Complementation
   - Functional mutants interact with other components for output



https://en.wikipedia.org/wiki/Two-hybrid_screening

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The sky is the limit for screening…

Next time:
- Directed evolution journal club
- Sequencing crash course

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