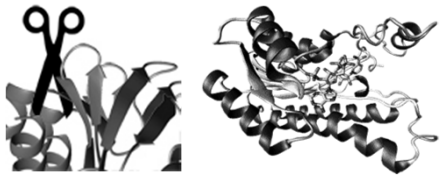
Protein engineering



By
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Protein engineering

- Protein engineering is the design of new enzymes or proteins with new or desirable functions.
- It is based on the use of recombinant DNA technology to change amino acid sequences.

Protein engineering

-> Mutagenesis used for modifying proteins
Replacements on protein level -> mutations on DNA level

Assumption : Natural sequence can be modified to improve a certain function of protein

What can be engineered in Proteins ?

-> 1. **Folding (+Structure):**

1. **Thermodynamic Stability**
(Equilibrium between: Native ⇔ Unfolded state)

2. **Thermal and Environmental Stability**
(Temperature, pH, Solvent, Detergents, Salt)

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What can be engineered in Proteins ?

->2. **Function:**

1. **Binding ;**
   : Interaction of a protein with its surroundings.

2. **Catalysis ;**
   : Catalysis is the chemical reaction brought about by a catalyst.

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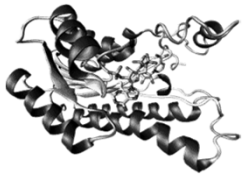
Protein engineering methods

Protein engineering

- Irrational design
  - random mutagenesis or Directed evolution
- Rational design
  - Site-directed mutagenesis

Random mutagenesis or Directed evolution



- Random mutagenesis is a powerful tool for generating enzymes, proteins, entire metabolic pathways, or even entire genomes with desired or improved properties.
- This technology is used to evolve genes in vitro through an iterative process consisting of recombinant generation.
- Coupled with the development of powerful high-throughput screening or selection methods, this technique has been successfully used to solve problems in protein engineering.

Random mutagenesis – Directed Evolution

-> based on the process of natural evolution

- NO structural information required

- NO understanding of the mechanism required

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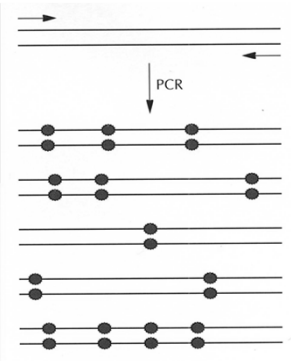
Random Mutagenesis (PCR based) Error –prone PCR



-> PCR with low fidelity !!!

Achieved by:

- Increased Mg2+ concentration
- Addition of Mn2+
- Not equal concentration of the four dNTPs
- Use of dITP
- Increasing amount of Taq polymerase (Polymerase with NO proof reading function)

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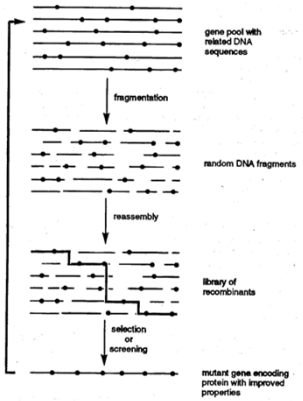
Random Mutagenesis (PCR based) DNA Shuffling



DNase I treatment (Fragmentation, 10-50 bp, Mn^{2+})

Reassembly (PCR without primers, Extension and Recombination)

PCR amplification

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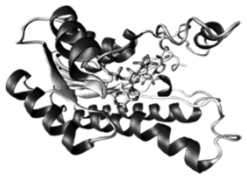
Site-directed mutagenesis



Requirements ⇒ Site –directed mutagenesis

-> Knowledge of sequence and preferable Structure (active site,coennzyme binding)

-> Understanding of mechanism (knowledge about structure – function relationship)

-> Identification of cofactors.

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Directed mutagenesis (Point mutations):

1. Substitution :
   change of one nucleotide (i.e. A-> C)
2. Insertion :
   gaining one additional nucleotide
3. Deletion :
   loss of one nucleotide

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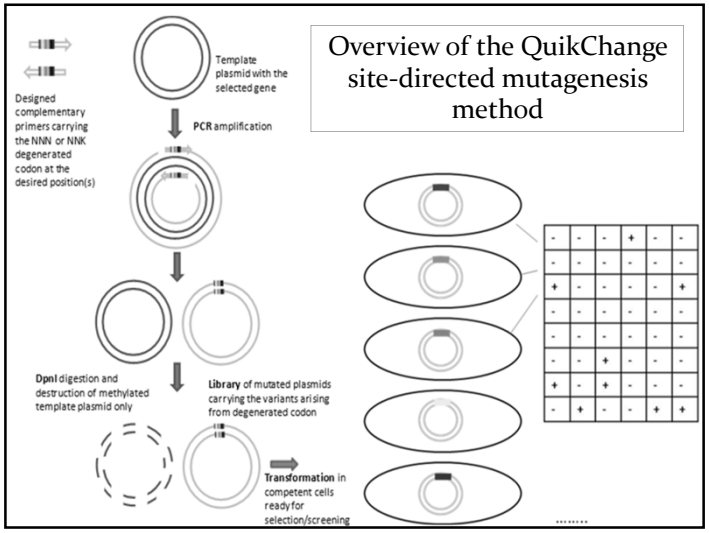
QuikChange site-directed mutagenesis

The Quik-Change site-directed mutagenesis kit is used to ….

- make point mutations
- replace amino acids

-and delete or insert single or multiple adjacent amino acids



Screening:
Basis for all screening & selection methods

Expression Libraries
->link gene with encoded product which is responsible for enzymatic activity

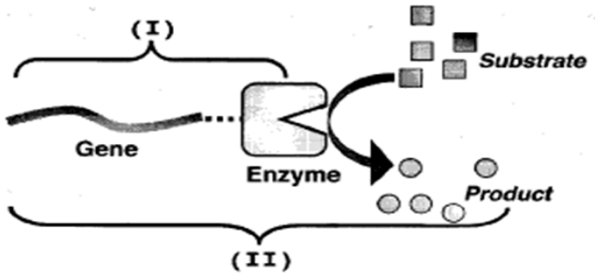


Table 8.2 Properties of T4 lysozyme and six engineered variants

Enzyme	Amino acid at position: 3	9	21	54	97	142	164	No. of -S-S-	% Activity	T_m (°C)
wt	Ile	Ile	Thr	Cys	Cys	Thr	Leu	0	100	41.9
pwt	Ile	Ile	Thr	Thr	Ala	Thr	Leu	0	100	41.9
A	Cys	Ile	Thr	Thr	Cys	Thr	Leu	1	96	46.7
B	Ile	Cys	Thr	Thr	Ala	Thr	Cys	1	106	48.3
C	Ile	Ile	Cys	Thr	Ala	Cys	Leu	1	0	52.9
D	Cys	Cys	Thr	Thr	Cys	Thr	Cys	2	95	57.6
E	Ile	Cys	Cys	Thr	Ala	Cys	Cys	2	0	58.9
F	Cys	Cys	Cys	Thr	Cys	Cys	Cys	3	0	65.5

Adapted from Matsumura et al., *Nature* **342**:291–293, 1989.

wt, wild-type T4 lysozyme; pwt, pseudo-wild-type enzyme; A through F, six engineered cysteine variants; -S-S-, disulfide bonds; T_m, "melting" temperature (a measure of thermostability).

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Table 8.4 Aminoacylation activity of native (Thr-51) and modified (Ala-51 and Pro-51) tyrosyl-tRNA synthetases

Enzyme	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (s^{-1} M^{-1})
Thr-51	4.7	2.5	1,860
Ala-51	4.0	1.2	3,200
Pro-51	1.8	0.019	95,800

Adapted from Wilkinson et al., *Nature* **307**:187–188, 1984.

The units for K_m, the binding constant of the enzyme for ATP, are millimolar units (mM); the units for k_{cat}, the catalytic rate constant, are reciprocal seconds (s^{-1}); and the units for k_{cat}/K_m, the catalytic efficiency, are s^{-1} M^{-1}.

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Table 8.3 Stability at 100°C of the yeast enzyme triosephosphate isomerase and its engineered derivatives

Enzyme	Amino acid at position: 14	78	Half-life (min)
Wild type	Asn	Asn	13
Variant A	Asn	Thr	17
Variant B	Asn	Ile	16
Variant C	Thr	Ile	25
Variant D	Asp	Asn	11

Adapted from Ahern et al., *Proc. Natl. Acad. Sci. USA* **84**:675–679, 1987.

Enzyme stability is expressed as the half-life, or rate of enzyme inactivation, at 100°C. A longer half-life indicates a more stable enzyme.

Approaches for……

-> site-directed mutagenesis
-> point mutations in particular known area

result -> library of wild-type and mutated DNA (site-specific)
not really a library -> just 2 species

-> random mutagenesis
-> point mutations in all areas within DNA of interest

result -> library of wild-type and mutated DNA (random)
a real library -> many variants.

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Protein Engineering - Applications

• **Site-directed mutagenesis** -> used to alter a single property.

Problem : changing one property -> disrupts another characteristics.

• **Random mutagenesis** (Molecular breeding) -> alteration of multiple properties.

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Protein Engineering - Applications

• A variety of protein engineering applications have been reported in the literature.
• These applications range from biocatalysis for food and industry to environmental, medical and nanobiotechnology applications.

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Food and detergent industry applications

- Protein engineering methods to design new enzymes for enzyme biotechnological industries.
- Those properties include thermostability, specificity and catalytic efficiency.
- Additionally, the design and production of new enzymes for food industry by using protein engineering was discussed to produce new food ingredients.

Environmental applications

-Recently genetic methods and strategies for designing microorganisms to eliminate environmental pollutants.
- Those methods and strategies included gene expression regulation to provide high catalytic activity under environmental stress conditions.
- such as the presence of a toxic compound, rational changes introduced in regulatory proteins that control catabolic activities, creation of new metabolic routes and combinations thereof.

Medical applications

- Medical use of protein engineering for cancer treatment.
- The use of novel antibodies as anticancer agents and protein engineering methods are used to modify antibodies to target cancer cells for clinical applications.
- Additionally, multifunctional and smart drug vehicles can be produced at the nanoscale, by protein engineering