ADVANCED ENZYME TECHNOLOGY

PROTEIN ENGINEERING (PART 2)

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Chapter Description

- Expected Outcomes
  - Describe about the general principal of protein engineering
  - Discuss about the strategies of protein engineering
  - Identify the differences between each strategies

CONTENT

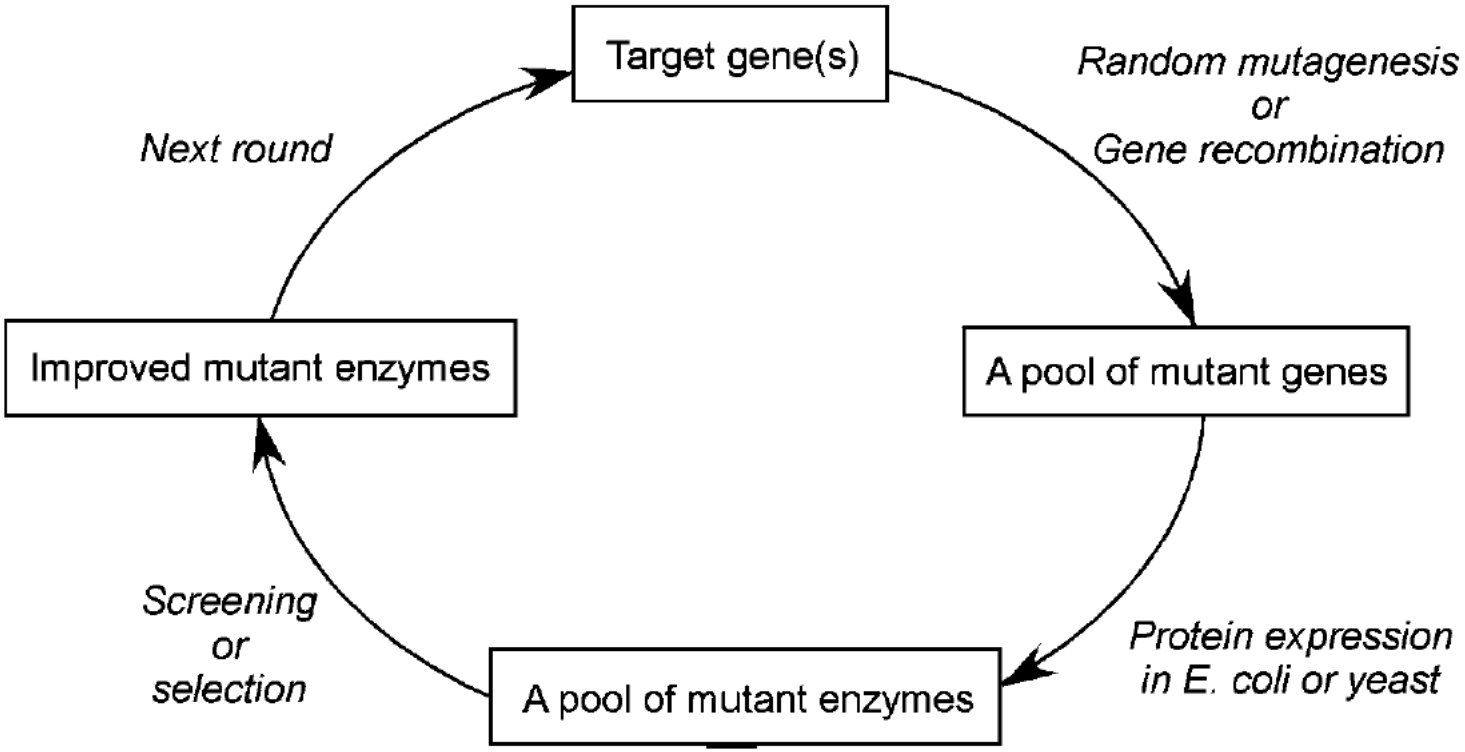
- General introduction
- Strategies of protein engineering
  - *De novo* design
  - Rational design
    - Site directed mutagenesis
      - Overlap extension PCR mutagenesis
  - Directed evolution
    - Random mutagenesis
      - Error-prone PCR

Directed Evolution

- Although **rational design** strategy has been reported as
  - one of an effective protein design strategy, **percentage of successful findings is still considered as low** owing to the **inadequate understanding regarding protein folding, structure, function, and dynamics**.
  - In addition, this technique relatively **time-consuming** due to the requirement of a high-quality 3D protein structure as an experimental guidance.

- In contrast, **directed evolution** does not rely on any predetermined notions about what is important
  - only **depend on a very simple algorithm**: **diversification together with selection**.
  - In principle, directed evolution resembles natural Darwinian evolution implemented in a laboratory environment.

The general scheme of directed evolution



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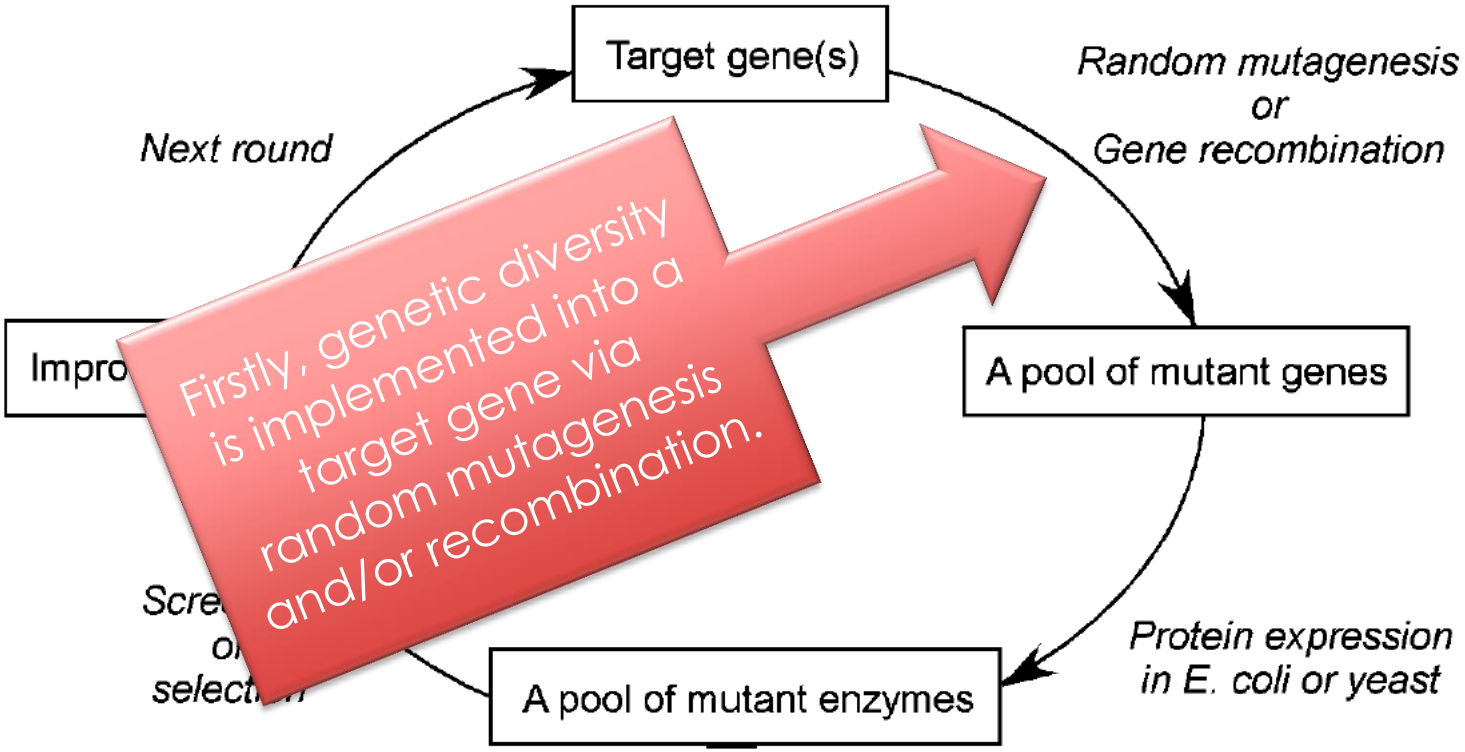

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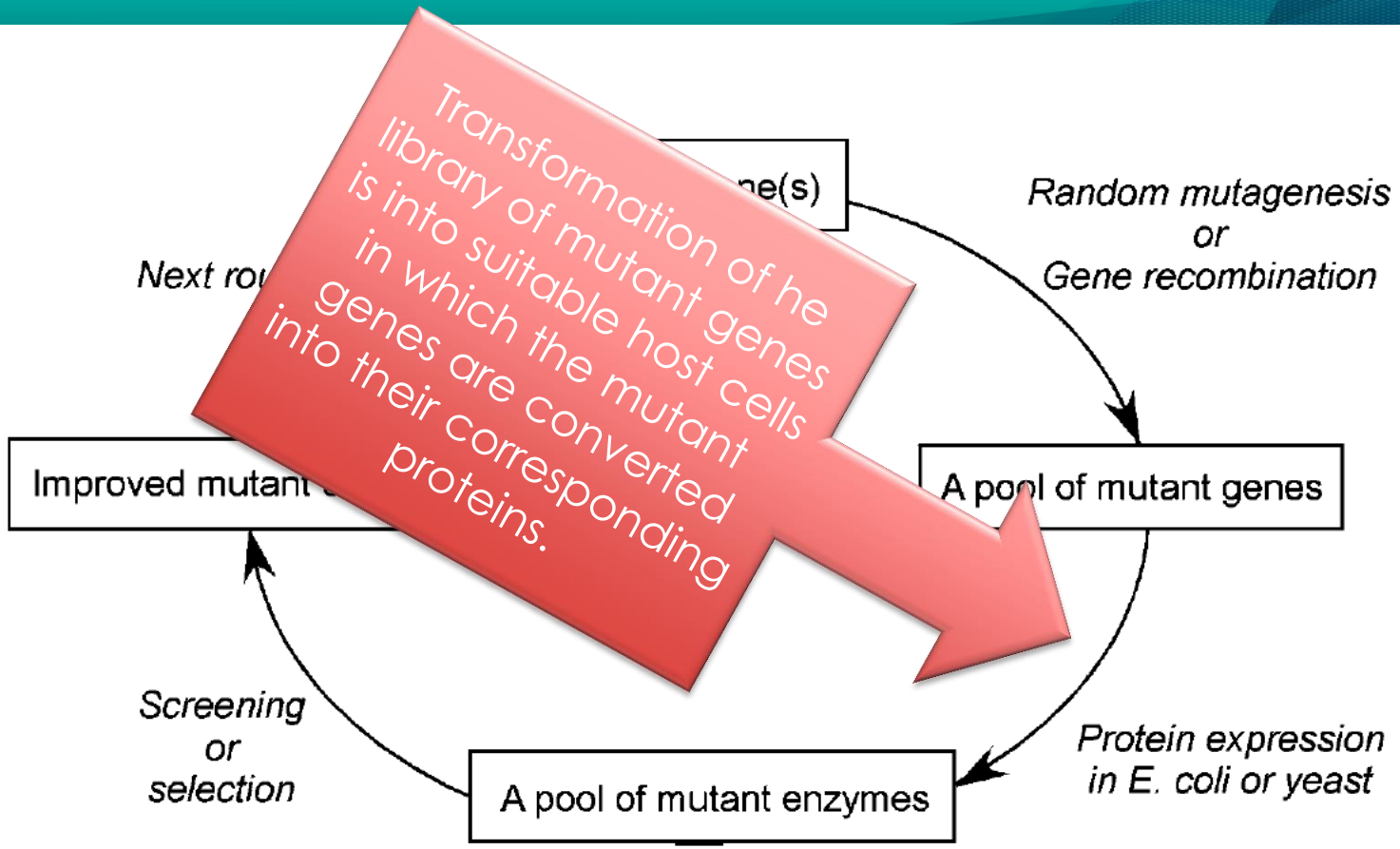

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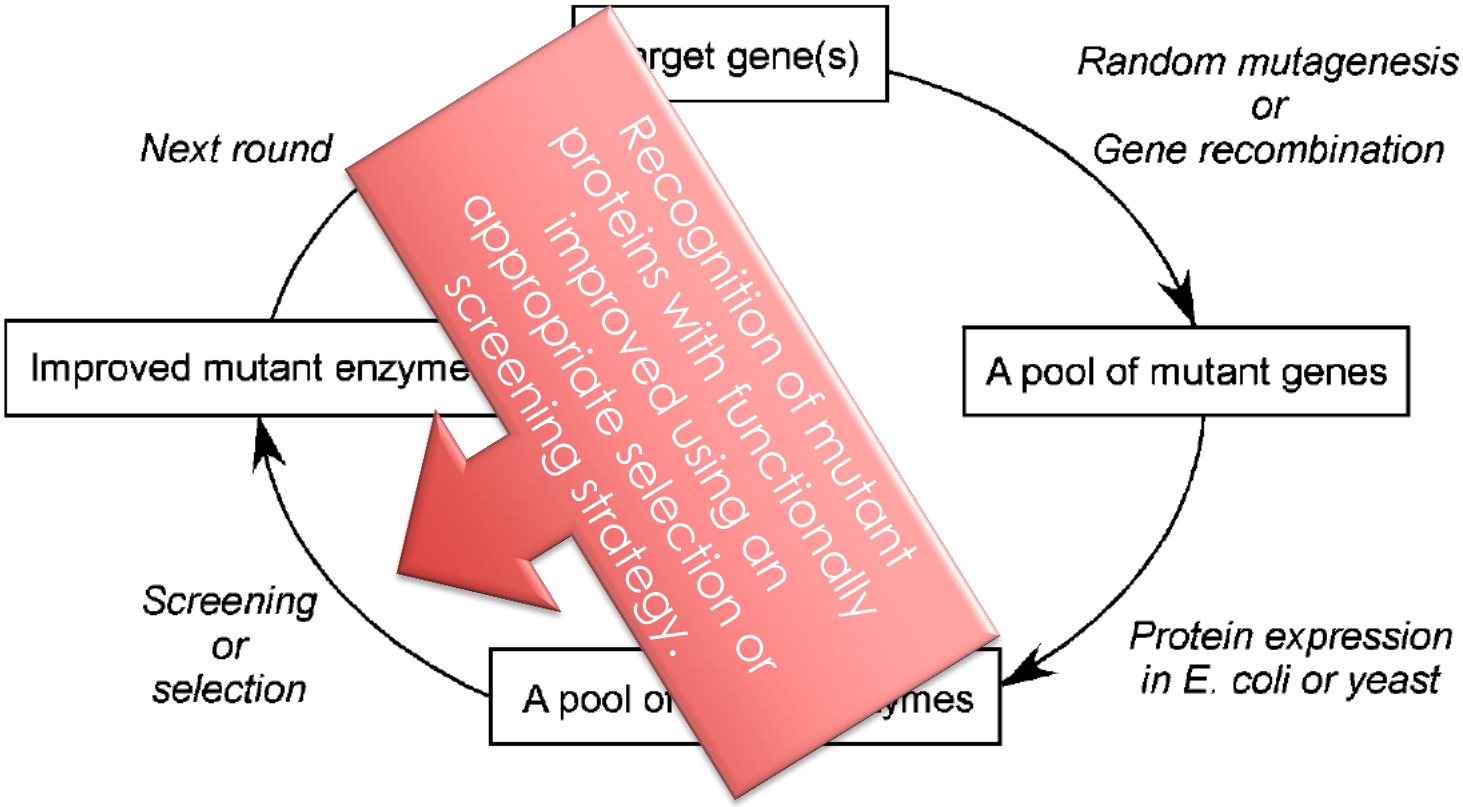

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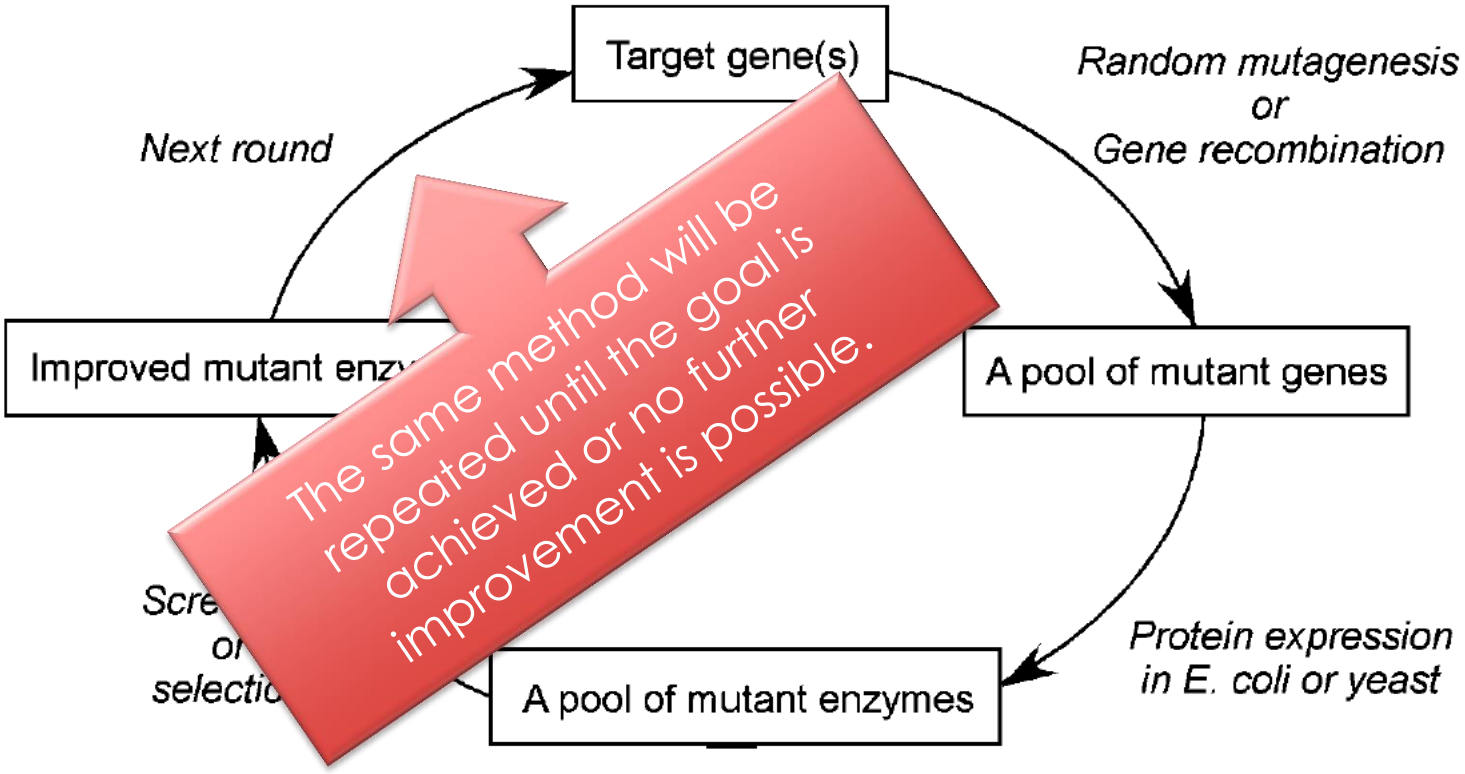


- **NO requirement for structural knowledge**

- **NO requirement for information of mechanism**

Involves 2 General approaches (key component):

1. creating **genetic diversity** via random mutagenesis or gene recombination methods.

2. **Screening and seletion** method development to identify the successful variants.

- Two main categories of genetic diversity:
  - **Random mutagenesis** methods and
  - **Gene recombination** methods .

Differences between random mutagenesis and gene recombination methods.

- **Random mutagenesis**
  - generate a library of variants which contain point mutations (substitution, deletion or insertion) and come from a single parental gene.
  - Example of representative methods such as Error prone PCR, Mutator strains, chemical mutagens and scanning saturation mutagenesis.

- **gene recombination**
  - generally begins by creating blockwise sequence information exchange within the parental genes which can be either single or a pool of homologous genes.
  - Involve breaking of DNA fragments followed by rejoining to form a new DNA combination.
  - Example of representative methods such as DNA shuffling, family shuffling, StEP, ITCHY, SHIPREC

Random Mutagenesis

- Point mutations introduced in all areas within DNA of interest
- Resulted into a library of variant containing both wild-type and random mutated DNA.
- Generally for a real library -> contain many variants -> screening !!!
- If methods efficient -> mostly mutated DNA will be obtained

General strategy for random mutagenesis

Requirements:

- DNA of interest (gene or promoter) must be cloned

- Expression system must be available -> for testing phenotypic change

Error-prone PCR

- The most recognized method of random mutagenesis – simple and efficient!
- Involve a series of the standard PCR which their reaction conditions have been slightly modified.
- Generally, In a standard procedure of DNA amplification via PCR, the possibility of Taq DNA polymerase to introduce incorrect nucleotides into the progeny genes is very limited .

- However, a very minor error rate can be intensely enlarged by altering the reaction buffer that during amplification process will influence the Taq DNA polymerase to introduce more incorrect nucleotides.

- The modification will consist of:
  1. consumption of imbalanced concentrations of dNTPs;
  2. Usage of MnCl2 in addition to MgCl2; and
  3. Increase the concentration of Mg2Cl2 (up to 7 mM).

- The major difficulty of error prone PCR is that it
  - can only get into six amino acid substitutions approximately at a given residue position due to the degeneracy of the genetic code, thus reducing the potential diversity significantly.
  - Mutations occurred are not totally random. For instance, a typical preference of error-prone PCR is the high exchange of AG nucleotide.

DNA shuffling

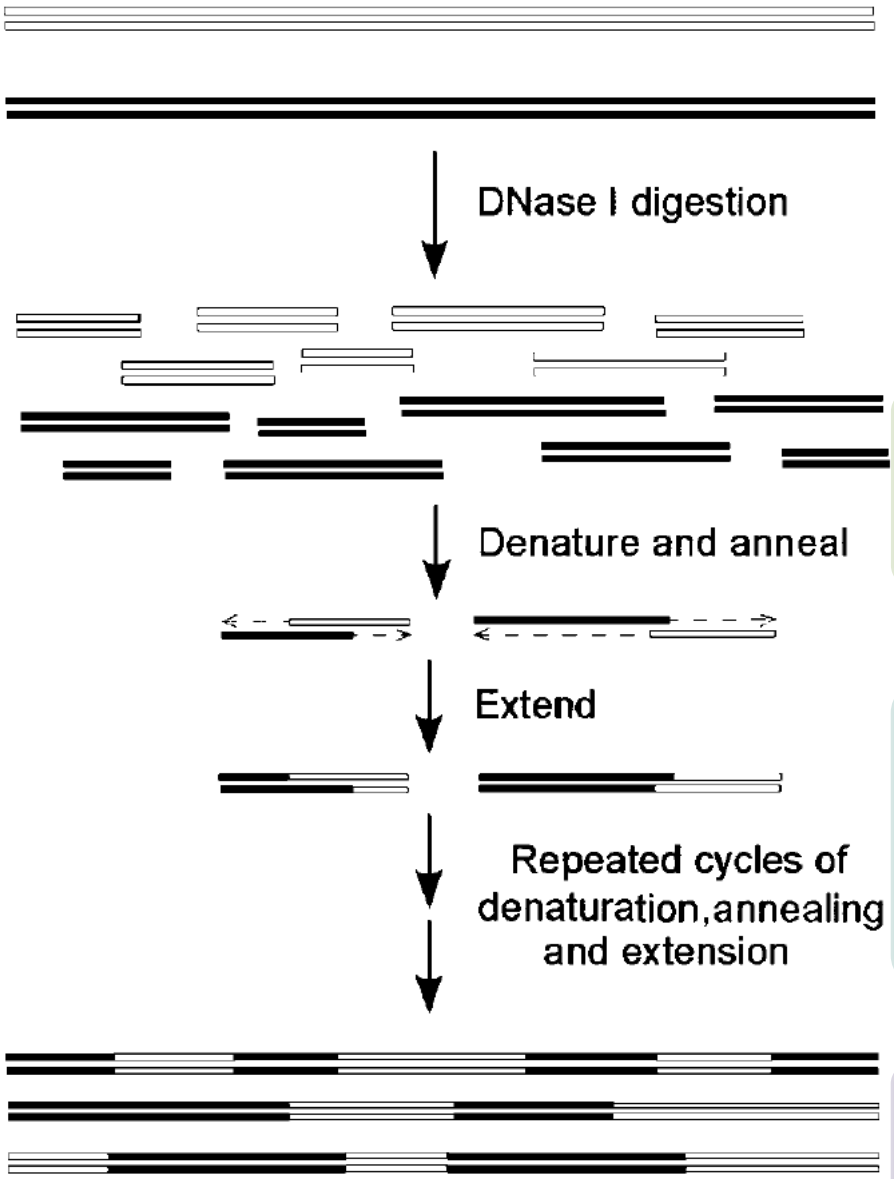
- Also known as "sexual PCR"
- The first and most established method of gene recombination method
- Begin with the formation of a small double-stranded DNA fragments with the read length between 20 to 50 bp. In this case, DNase I will be used as an enzyme to digest randomly a pool of closely related genes containing point mutations.
- A PCR-like reaction without any primers will be performed to purify and reassembled a small double-stranded DNA fragments produced into a full-length gene.

DNA shuffling and family shuffling

- Fragments originated from dissimilar parental genes will prime one another in a process of Recombinogenic events.

- A standard PCR reaction will then be implemented in which reassembly mixture is used as a template with primers flanking the gene of interest.

- The final amplified product will consist of a library of full-length genes containing recombined mutations from different parental genes.



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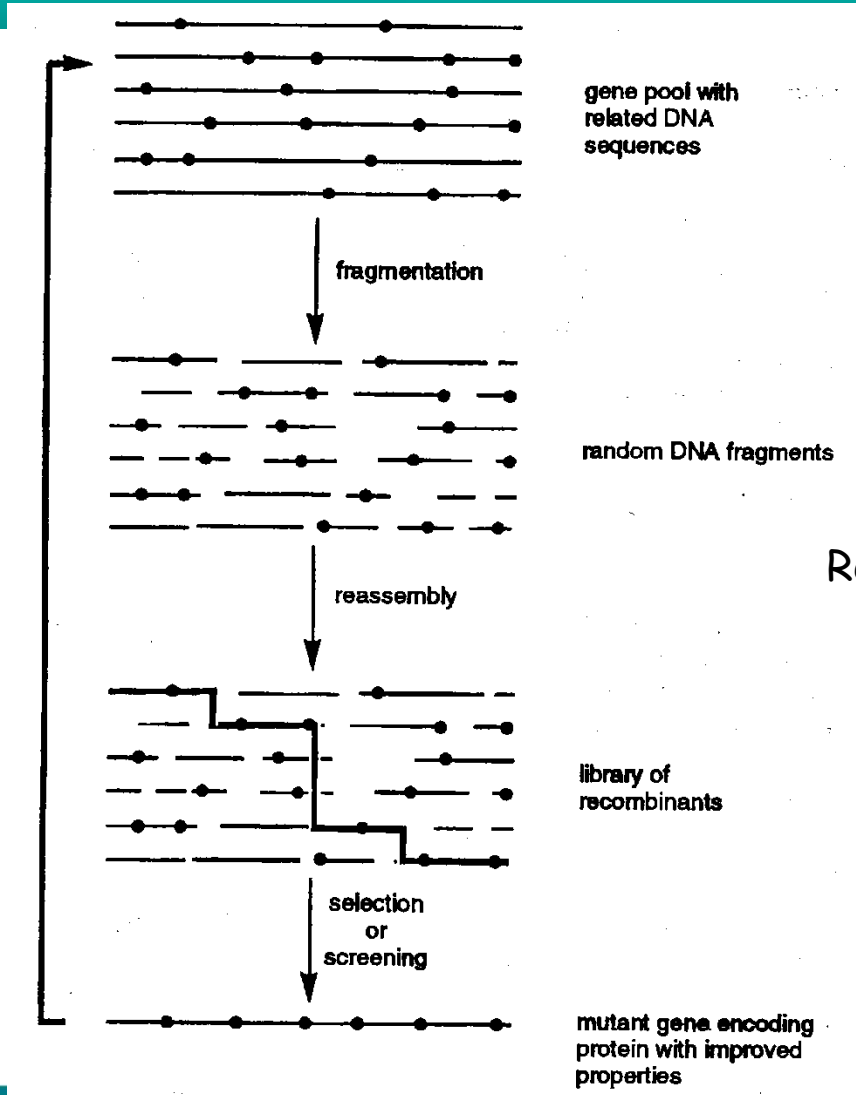
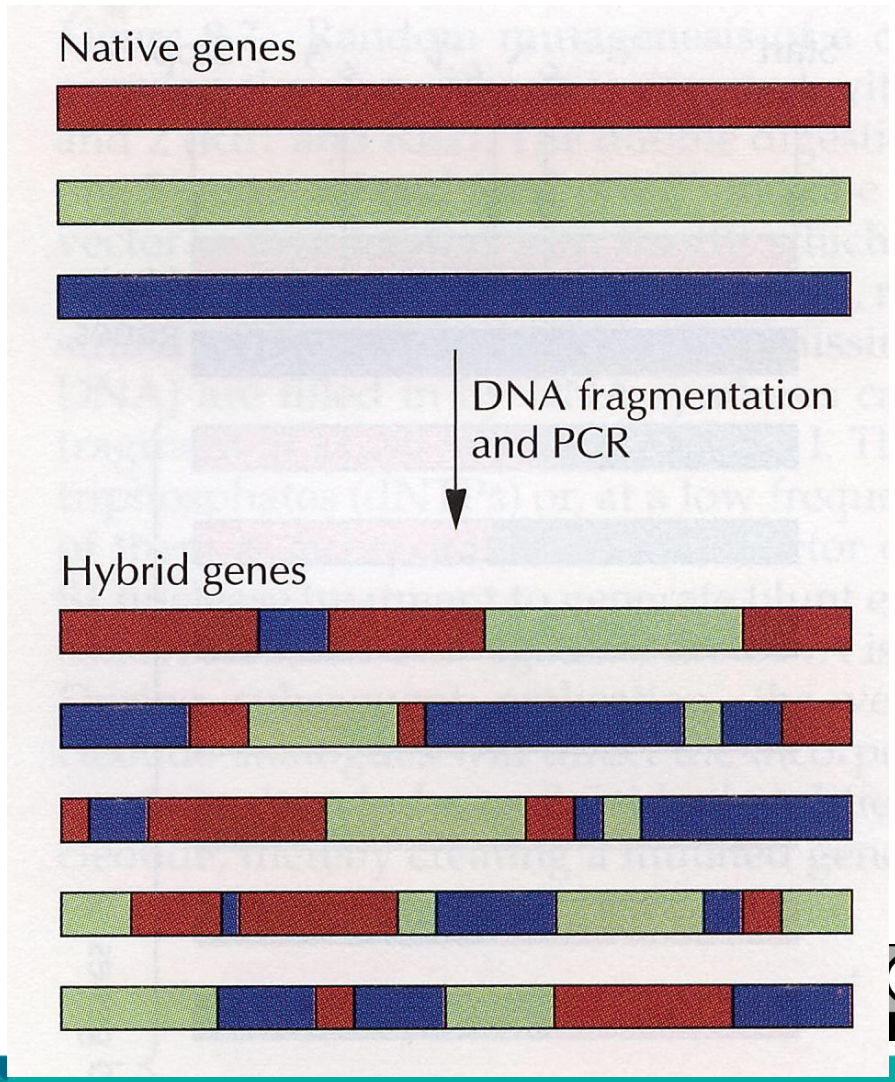


DNase I treatment (Fragmentation, 10-50 bp, Mn^{2+})

Reassembly (PCR without primers, Extension and Recombination)

PCR amplification

Family Shuffling



Several parental genes involves which come from the similar family (highly homologous) -> Family shuffling

Limitation of Directed Evolution

To be succeed:

1. Evolutionary track must be available
2. Screening method must be accessible