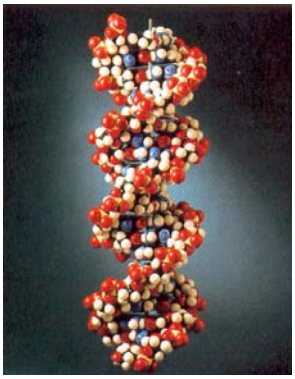
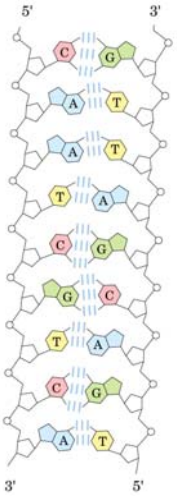
Directed Evolution

Enzyme Engineering

DNA structure

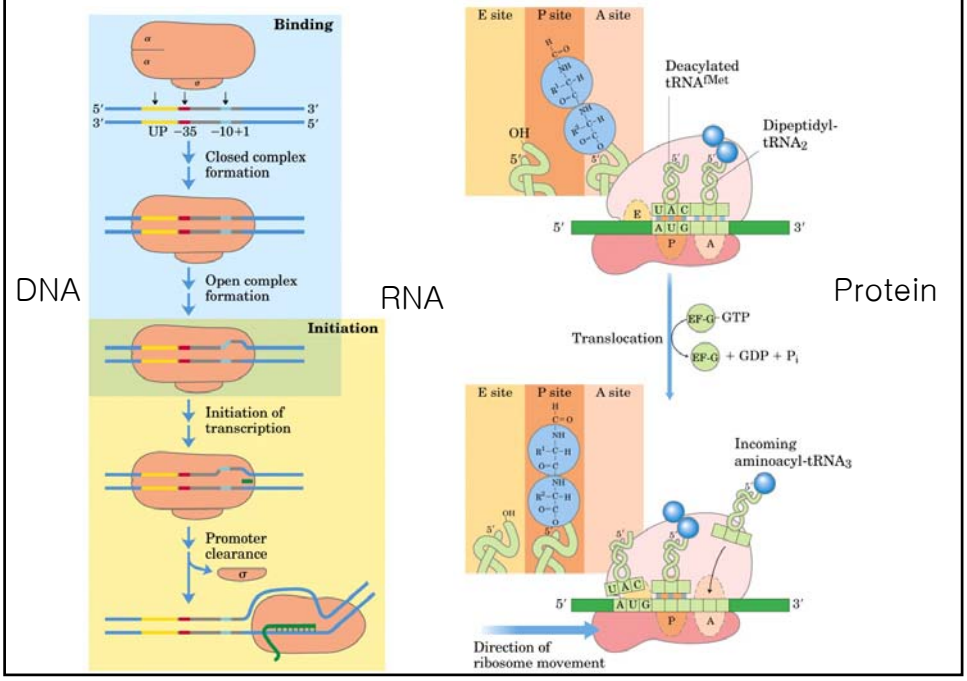




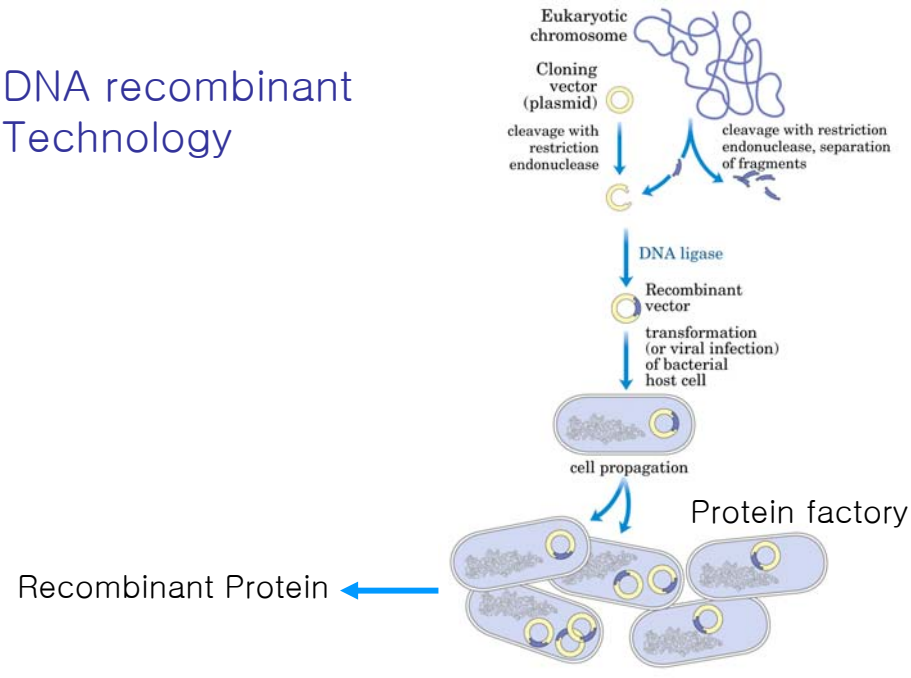
The simplest living organisms ~ 2 X 10^6 base pairs

Human cells ~ 3.2 X 10^9 base pairs

Transcription – Translation

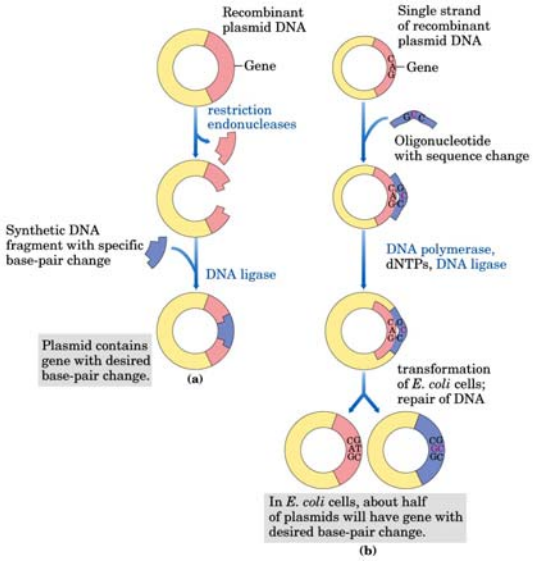


DNA recombinant Technology

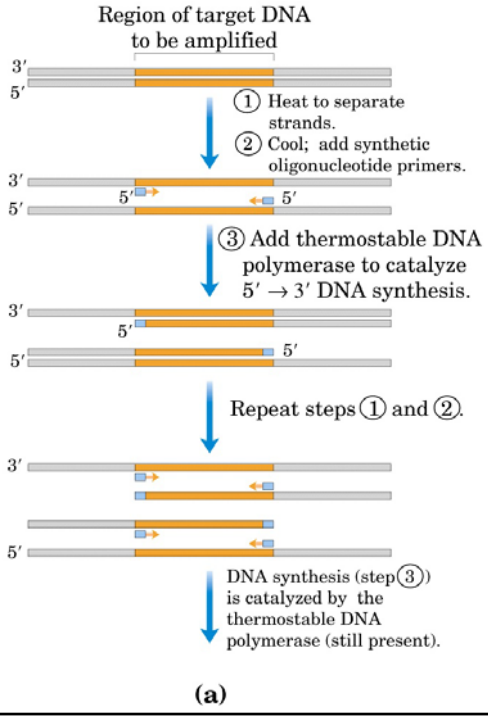


Protein Engineering

- By changing gene sequence, a new protein can be synthesized



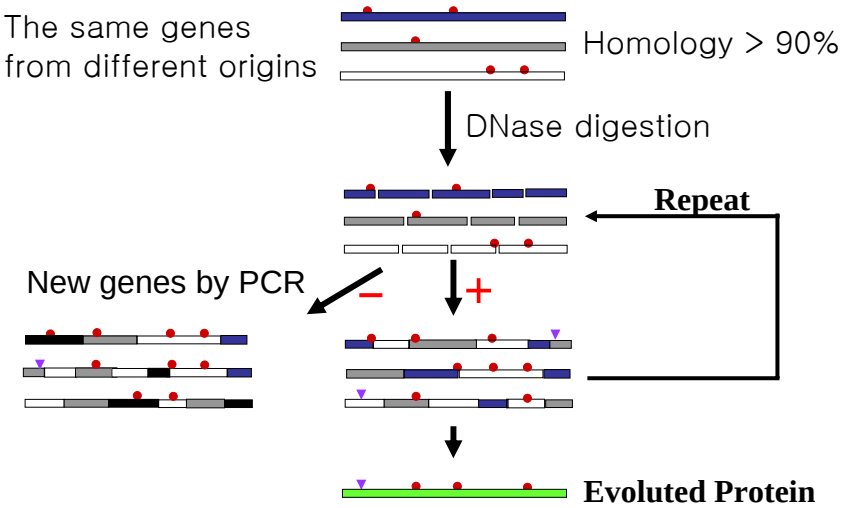
PCR



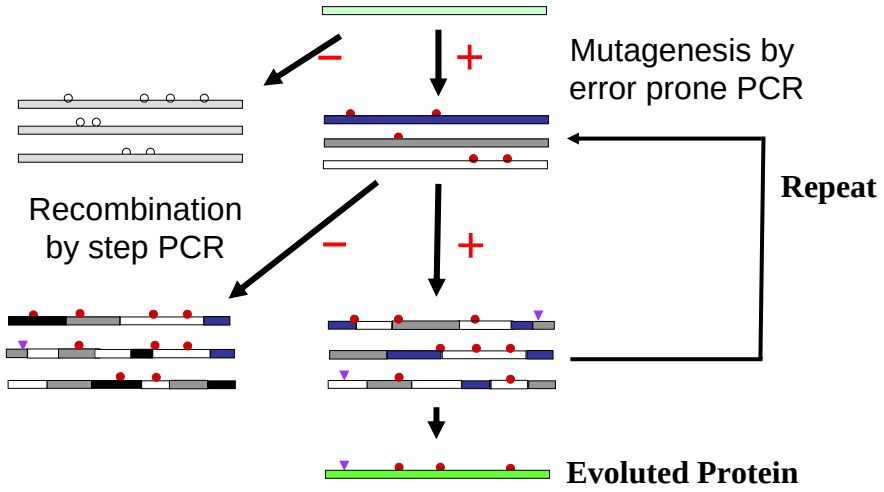
Directed Evolution

- The process of natural selection can be harnessed by human
- Protein was not evolved for artificial condition, so it can be more evolved using natural selection process
- Introducing a lot of random mutations →
  Selecting or screening beneficial mutations
  Recombining the mutations

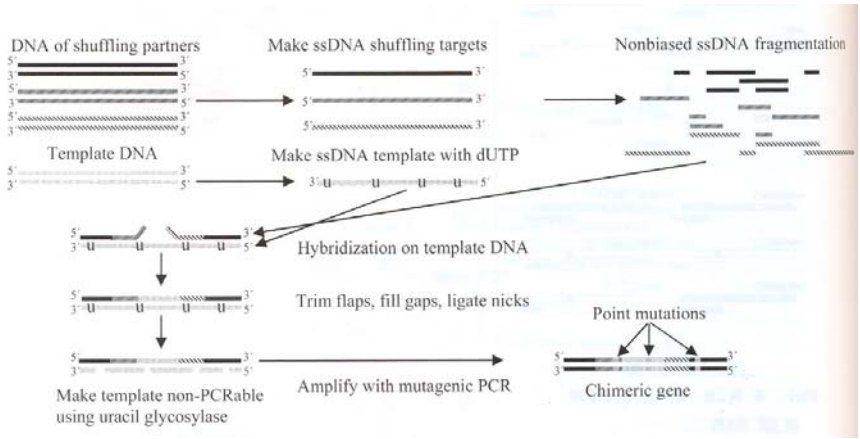
Creating diversity by Family Shuffling (Stammer, 1994)



Creating diversity by StEP (Staggered extension process, 1998, Arnold)



Creating diversity by RACHITT (2001, Gibbs)

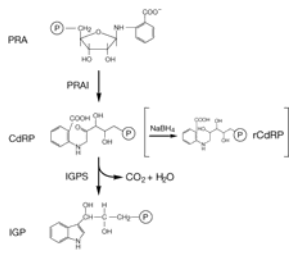


Checking for Hits : Selecting

- Selecting living cells or fast growing cells
- Example 1 : Antibiotics (Nature, 1998. 391:288)
  - Using four cepharosporinase C from Enterobacter etc.
  - Family shuffling and selecting more active enzyme with increased concentration of β-lactam antibiotics
  - 50,000 colonies tested during four cycles
  - 270- to 540-fold increase of enzyme activity
  - 33 amino acids substitution and 7 crossover
- Example 2. Resistance to a new antibiotics (Nature, 1994. 370:389)
  - 32,000-fold increase in β-lactamase resistance toward cefataxime

Checking for Hits : Selecting

- Example 3. Indole-3-glycerol-phosphate synthase (Nature, 2000. 403:617)
  - Changing IGPS to PRAI
  - Acquiring new catalytic activity



- Example 4, Chorismate mutase (Science, 1998. 279:1958)
  - Chorismate mutase is the first enzyme to make Phe and Tyr and dimeric enzyme
  - *Methanococcus jannaschii* CM is mutated to produce inactive and monomeric enzyme
  - Monomeric chorismate mutase was screened on Phe^- Tyr^- agar

Checking for Hits : Screening

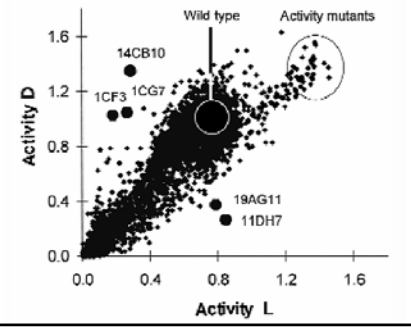
- Inexpensive substrate, high dynamic range, and low variability are required → Not always available
- High throughput analysis is essential
  - Absorbance or fluorimeter assay using microplate reader allows 10^3–10^4 per day
  - Colony imaging can be performed on the plate : 10^3 colonies per plate → 10^5 per day
  - Flow cytometry or solid phase capture → 10^6–10^9 per day

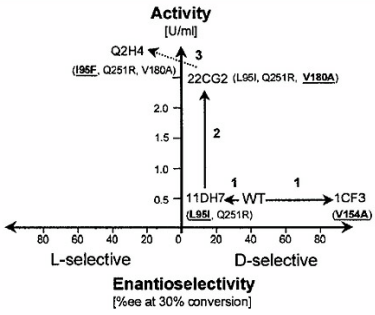
Checking for Hits : Screening

- Example 1. Active subtilisin in organic solvent DMF (Protein Sci. 1996)
  - Screening with colony imaging using casein-containing plates with 60% DMF
  - 500-fold increase of activity was achieved with 10 000 colonies screened (12 amino acid substitutions)
- Exampe 2. Increasing thermal stability (PNAS 1998)
  - *Bacillus subtilis p*-nitrobenzyl esterase (pNBE)
  - DNA shuffling was performed and the activities of enzymes were tested on 96-well microplate reader
  - T_m (54°C) increased more than 14°C without changing the activities

Checking for Hits : Screening

- Example 3. Inversion of enantioselectivity (Nat. Biotech. 2000. 18:317)
  - Wild type hydantoinase from *Arthrobacter* sp. DSM9771 is insufficiently enantioselective for producing L-amino acids
  - D-specific enzyme and 5-times active L-specific enzyme were screened
  - Screening 10000 enzymes using pH changes





Checking for Hits : Screening

- Example 4. Changing substrate selectivity (Nat. Biotech. 2000. 18:317)