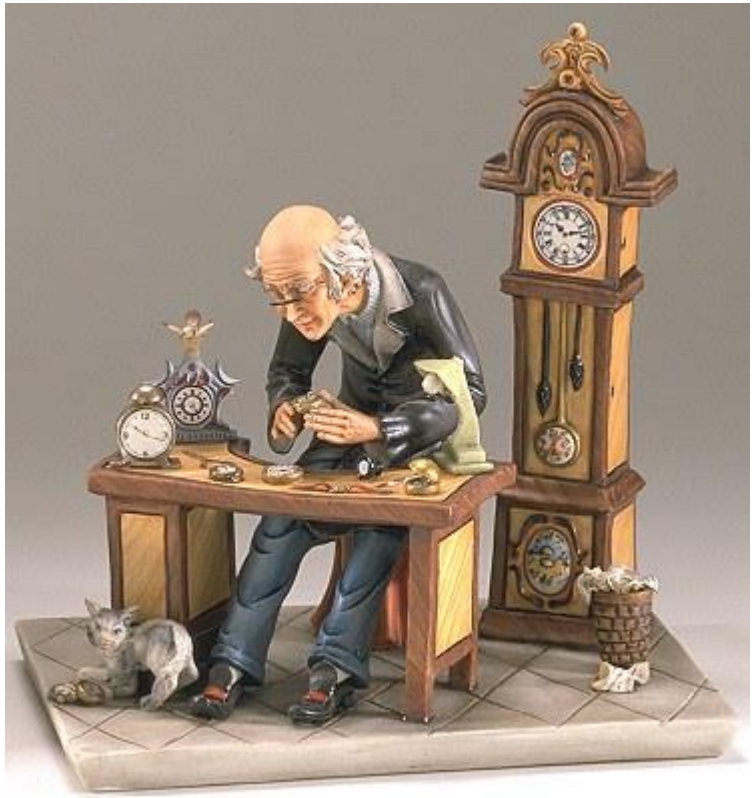
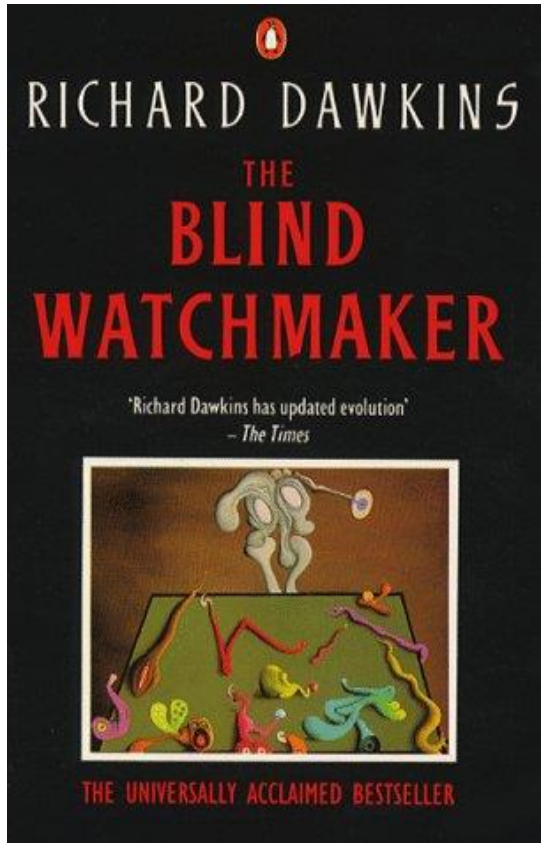
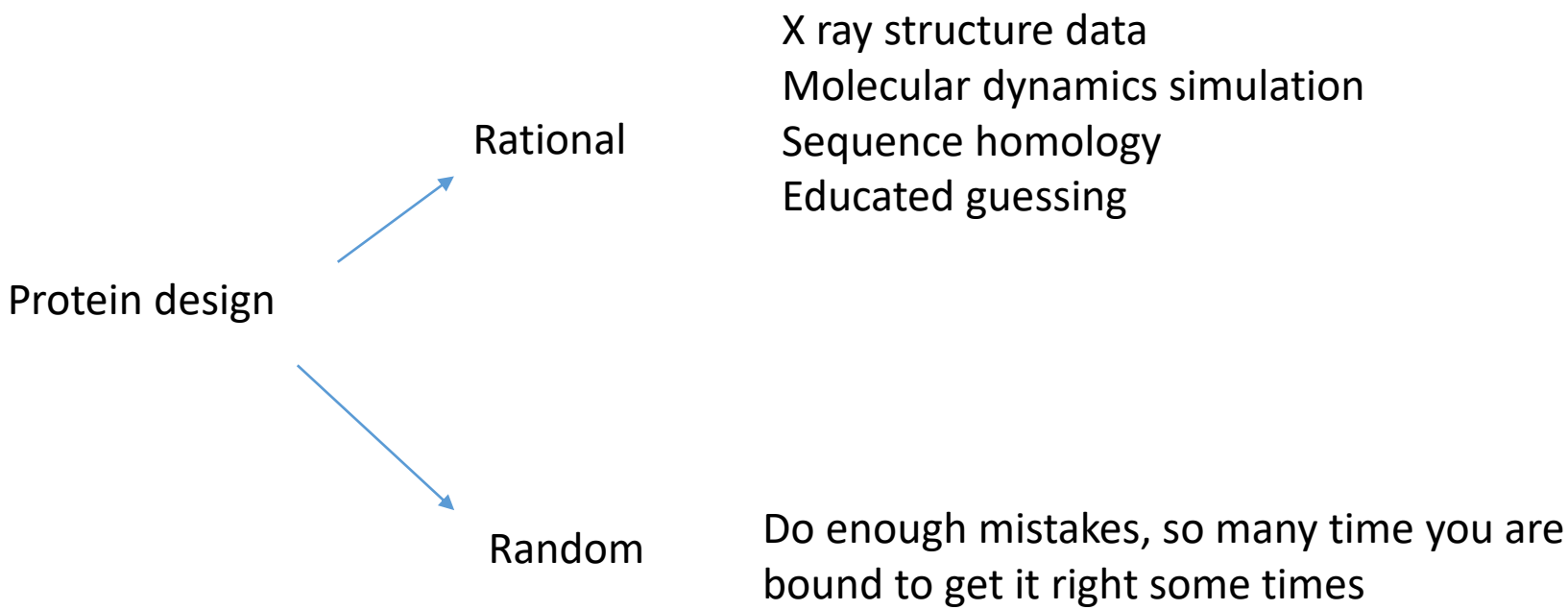
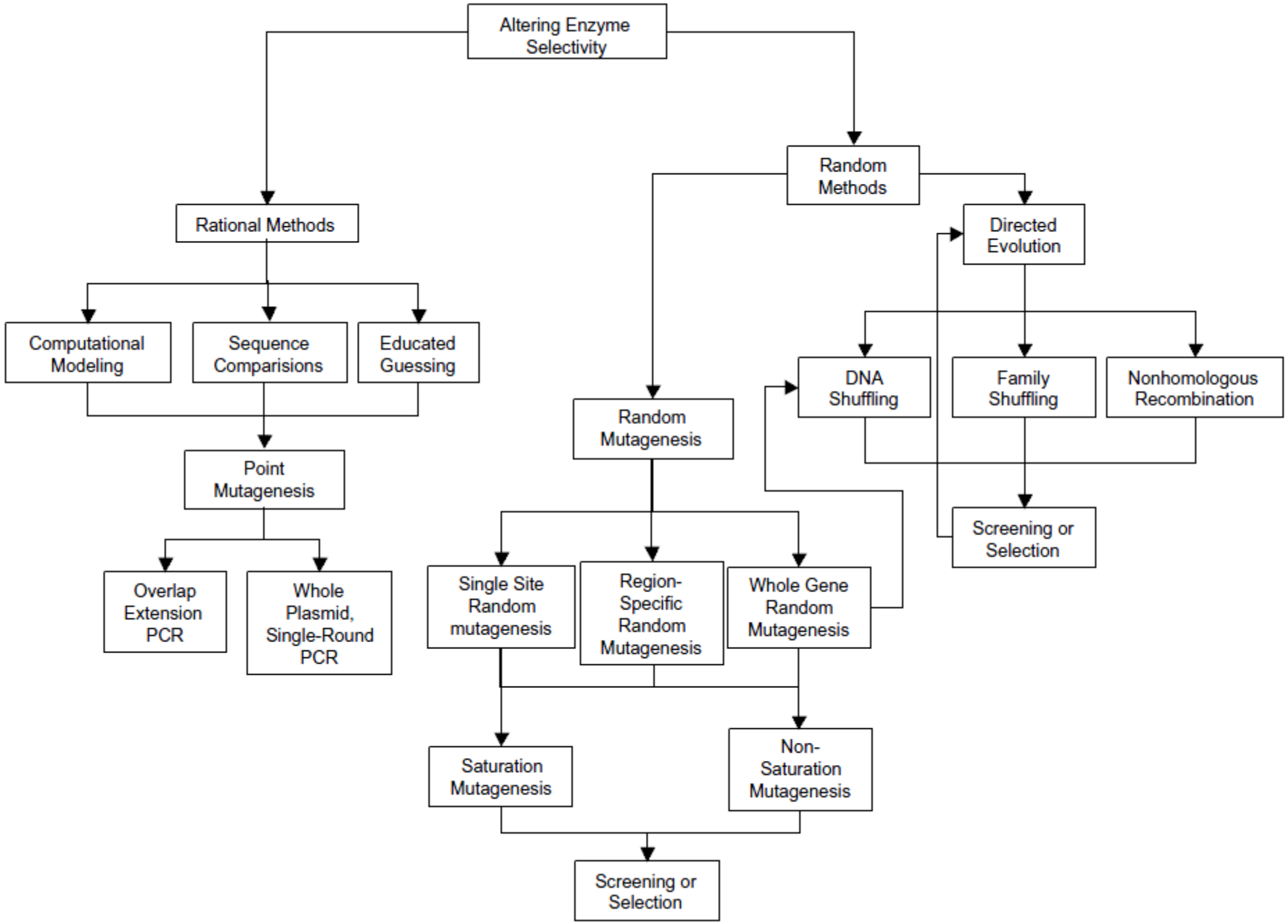
# Biocatalyst Design






Protein design
Rational
X ray structure data
Molecular dynamics simulation
Sequence homology
Educated guessing
Random
Do enough mistakes, so many time you are bound to get it right some times


Altering Enzyme Selectivity
Rational Methods
Random Methods
Computational Modeling
Sequence Comparisions
Educated Guessing
Point Mutagenesis
Overlap Extension PCR
Whole Plasmid, Single-Round PCR
Random Mutagenesis
Directed Evolution
DNA Shuffling
Family Shuffling
Nonhomologous Recombination
Screening or Selection
Single Site Random mutagenesis
Region-Specific Random Mutagenesis
Whole Gene Random Mutagenesis
Saturation Mutagenesis
Non-Saturation Mutagenesis
Screening or Selection

Overlap extension primer

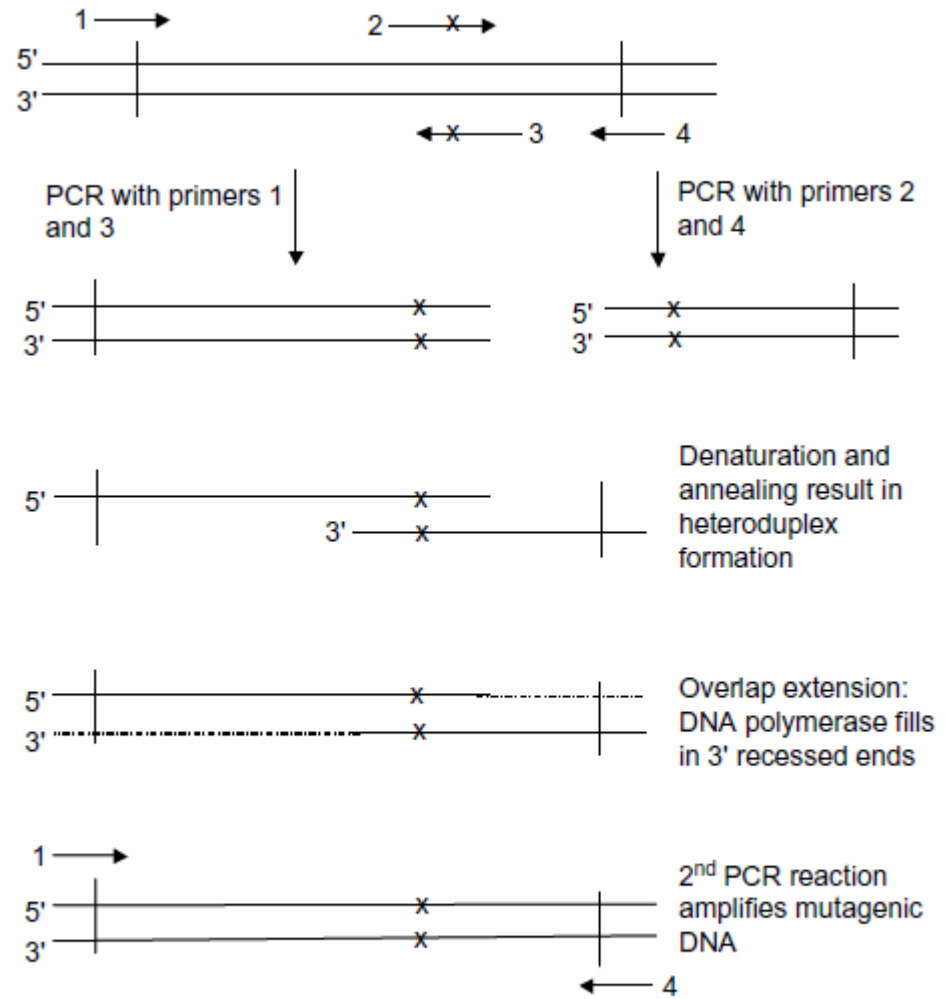
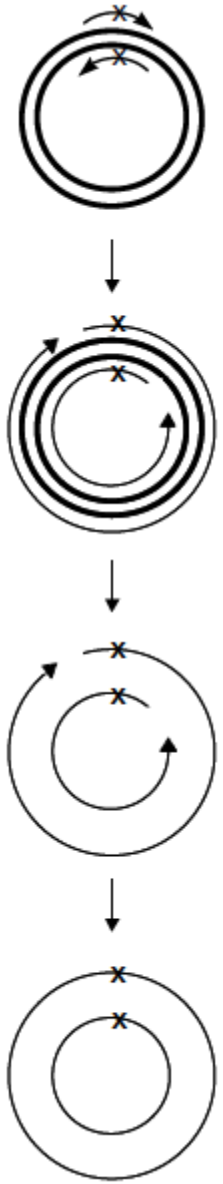



**Figure 2.** Overlap extension PCR method: → represents a primer, and × represents a mutagenic codon.

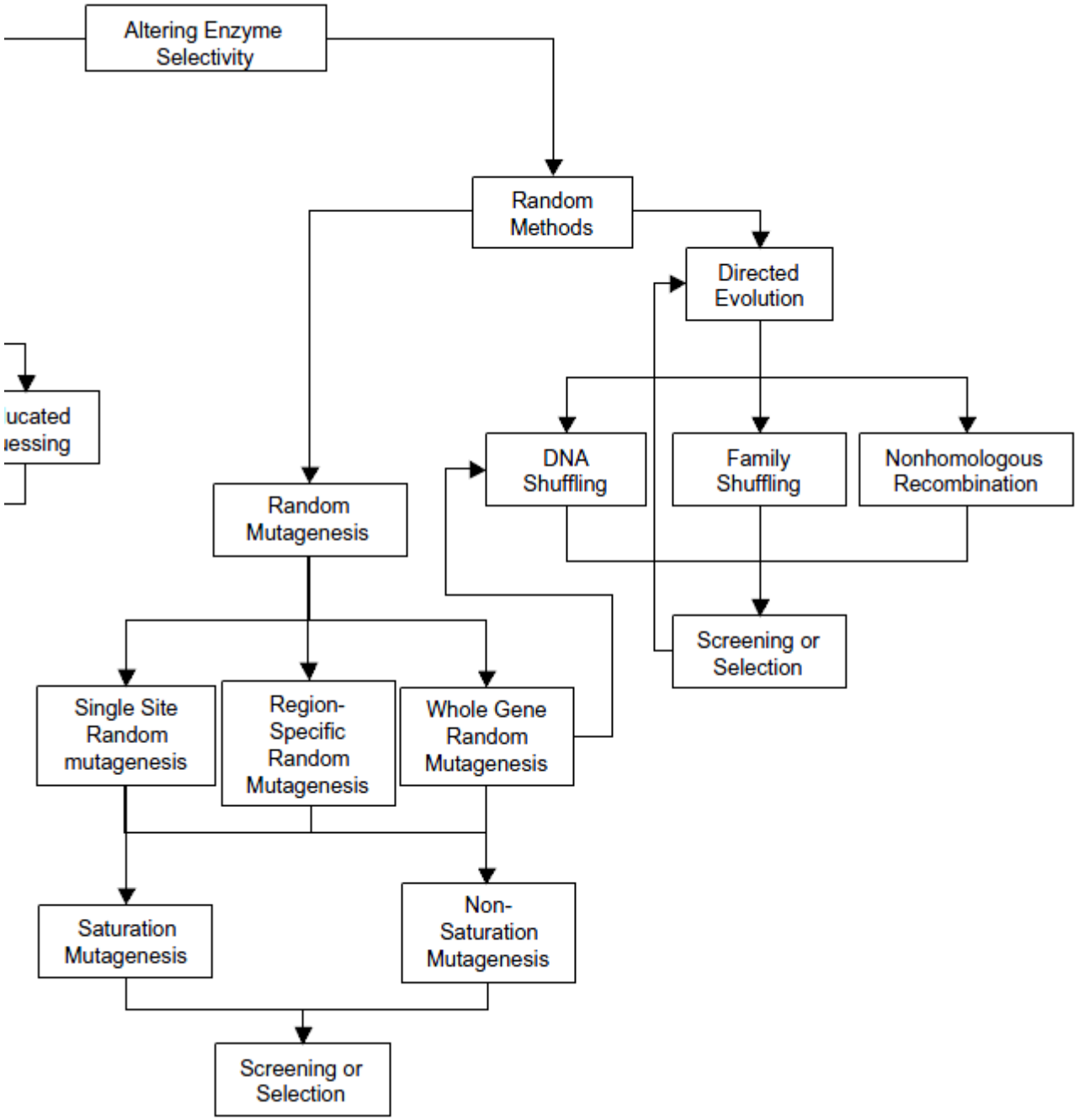
# Whole plasmid single round PCR



Two oligonucleotide primers containing the desired mutation(s), X, are used with a DNA polymerase to extend the original plasmid in single PCR reaction, resulting in a nicked circular strand.

DpnI methylase digests the methylated, nonmutated parental DNA template.

The circular, nicked dsDNA is transformed into competent cells. The competent cells repair the nicks in the mutated plasmids.


Altering Enzyme Selectivity
Random Methods
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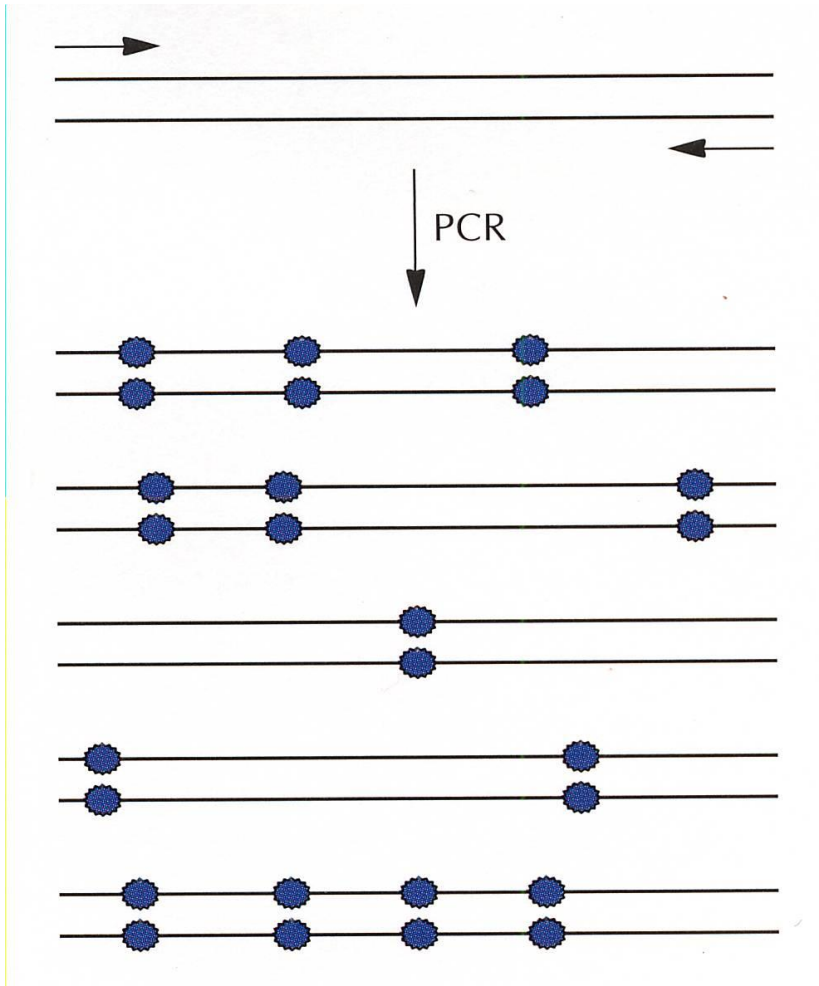
Random Mutagenesis
Screening or Selection
Single Site Random mutagenesis
Region-Specific Random Mutagenesis
Whole Gene Random Mutagenesis
Saturation Mutagenesis
Non-Saturation Mutagenesis
Screening or Selection

Whole Gene: mutation

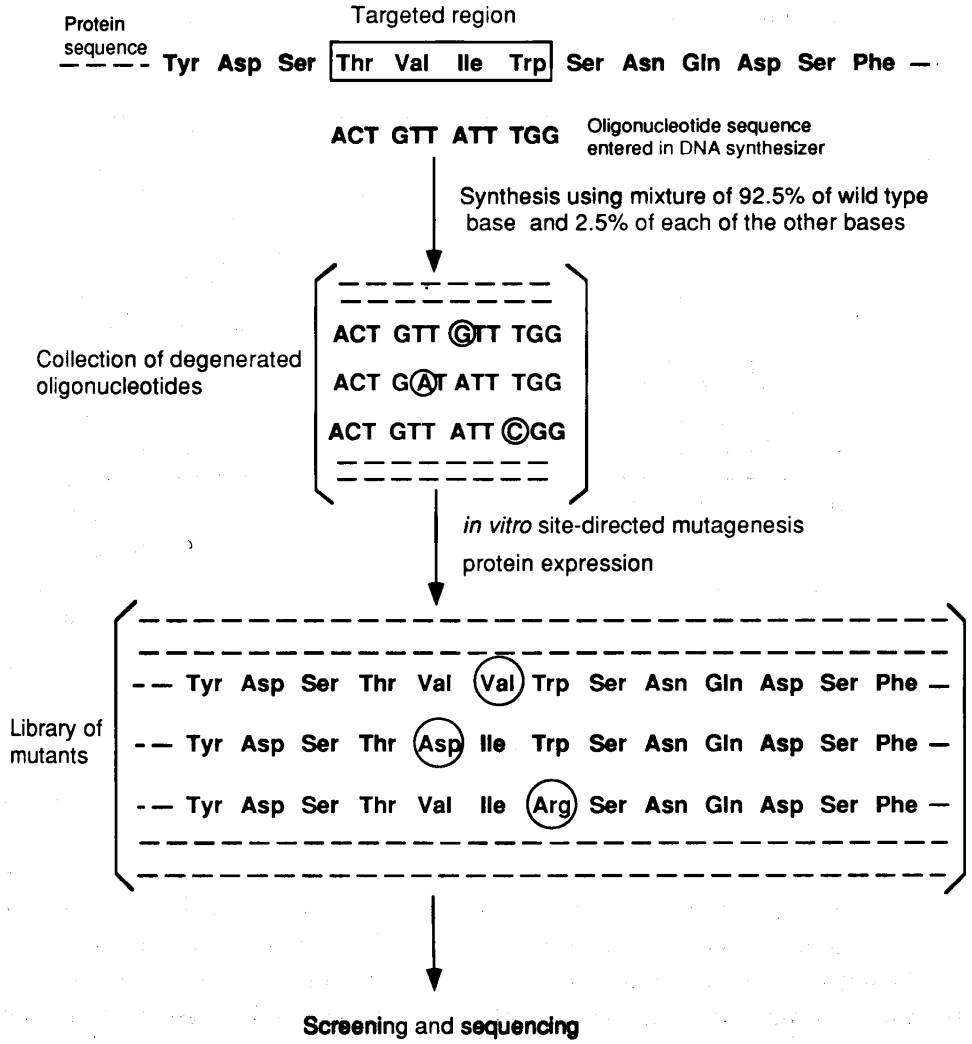


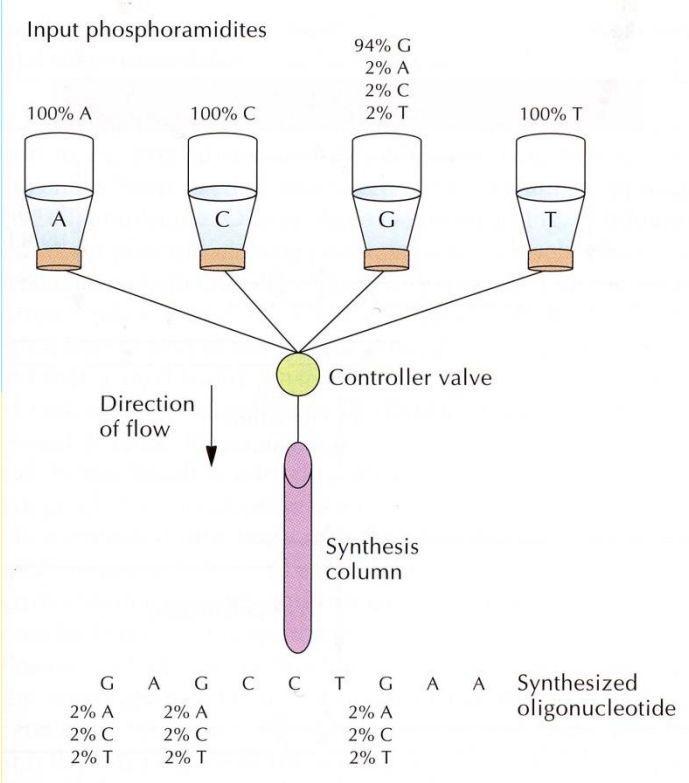

-> PCR with low fidelity !!!

Achieved by:

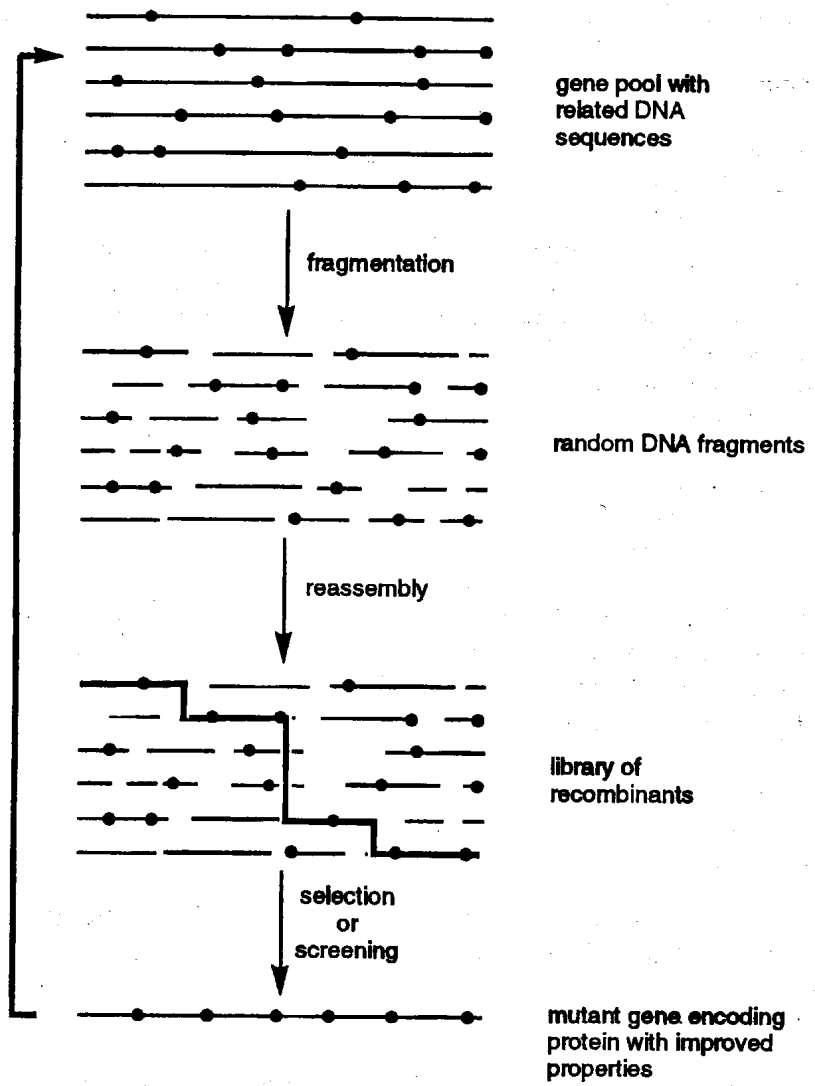
- Increased Mg2+ concentration
- Addition of Mn2+
- Not equal concentration of the four dNTPs
- Use of dITP
- Increasing amount of Taq polymerase (Polymerase with NO proof reading function)

# Single AA/site mutation: degenerative primers



DNA shuffling

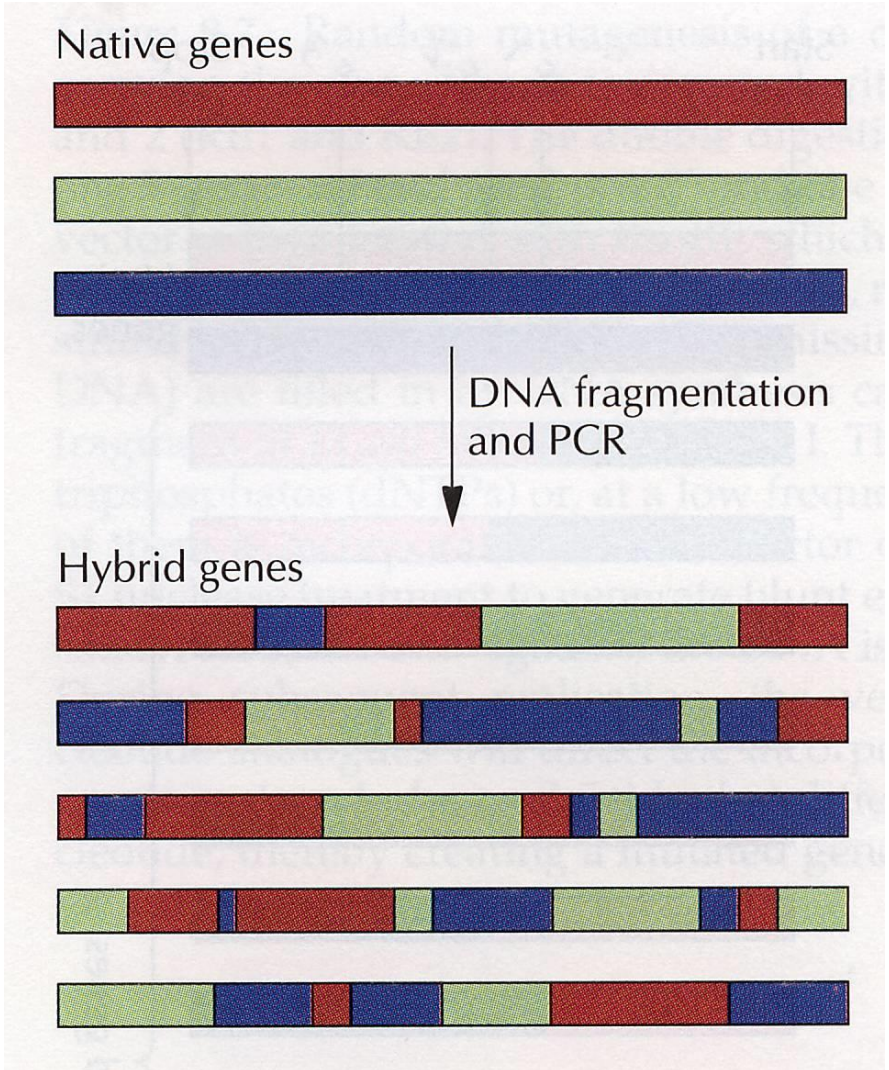



DNase I treatment (Fragmentation, 10-50 bp, $Mn^{2+}$)

Reassembly (PCR without primers, Extension and Recombination)

PCR amplification

Family shuffling

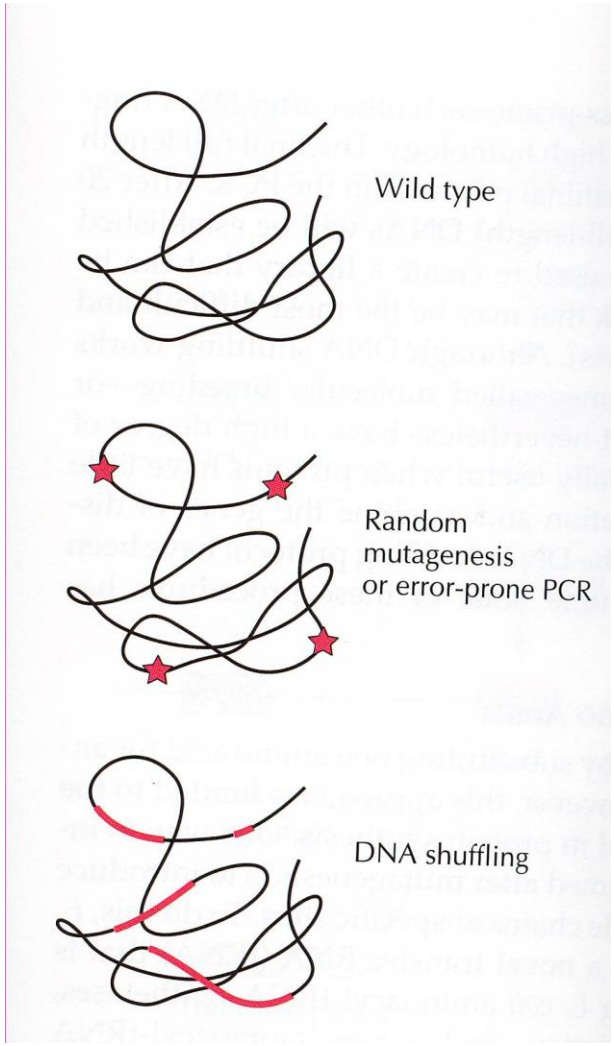



Genes coming from the same gene family -> highly homologous

-> Family shuffling

# Directed Evolution

## Difference between non-recombinative and recombinative methods



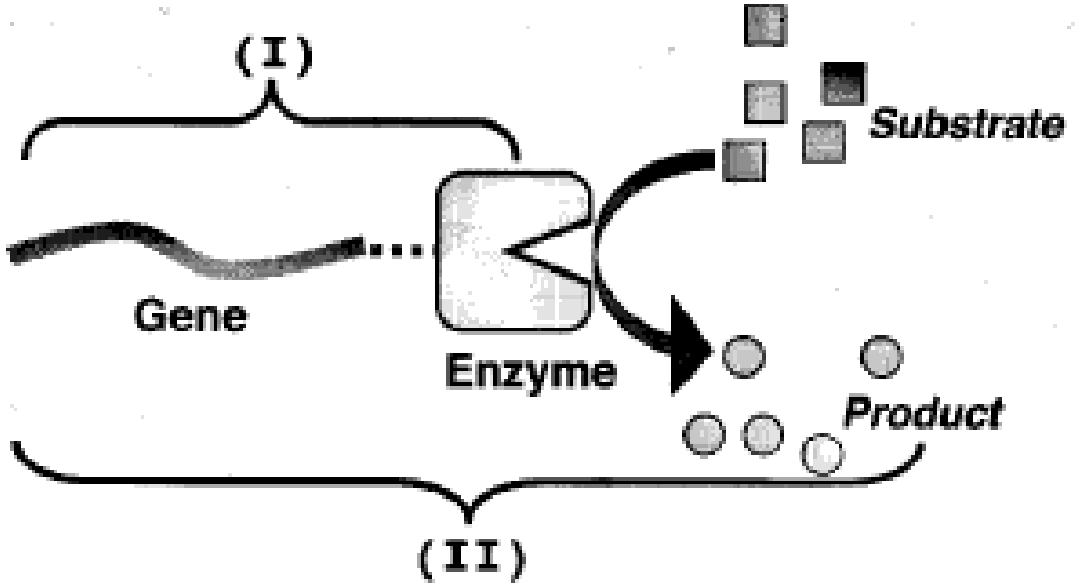

Non-recombinative methods

recombinative methods ->
hybrids (chimeric proteins)

# Screening:
# Basis for all screening & selection methods

Expression Libraries

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




*Kilde: Reymond, Chapter 6 (HTS screening and selection of Enzyme-encoded genes)*

# Low-medium throughput screens

-> Detection of enzymatic activity of colonies on agar plates or ”crude cell lysates” -> production of fluorophor or chromophor or halos

-> Screen up to $10^4$ colonies

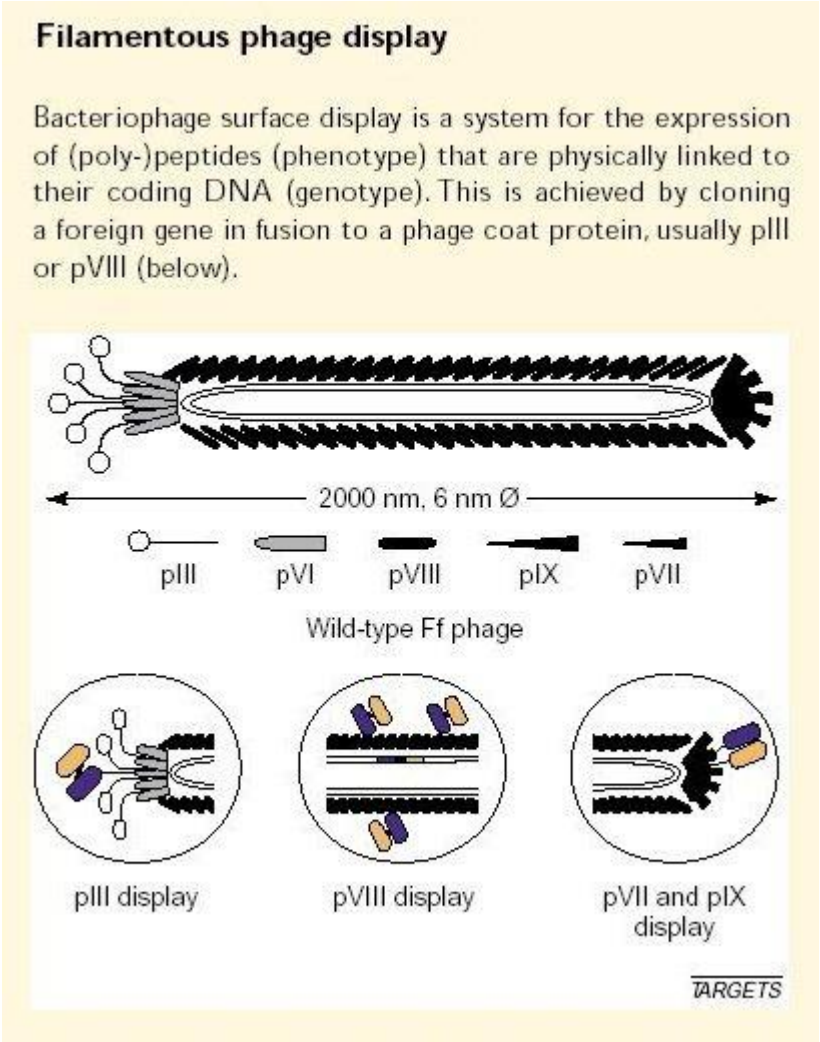
-> effective for isolation of enzymes with improved properties

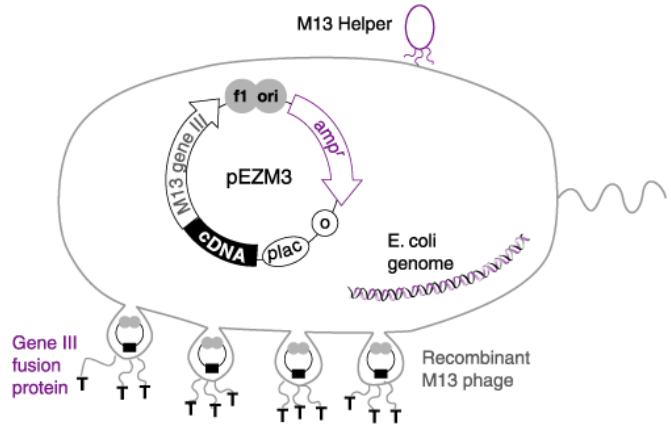
-> not so effective for isolation of variants with dramatic changes of phenotype

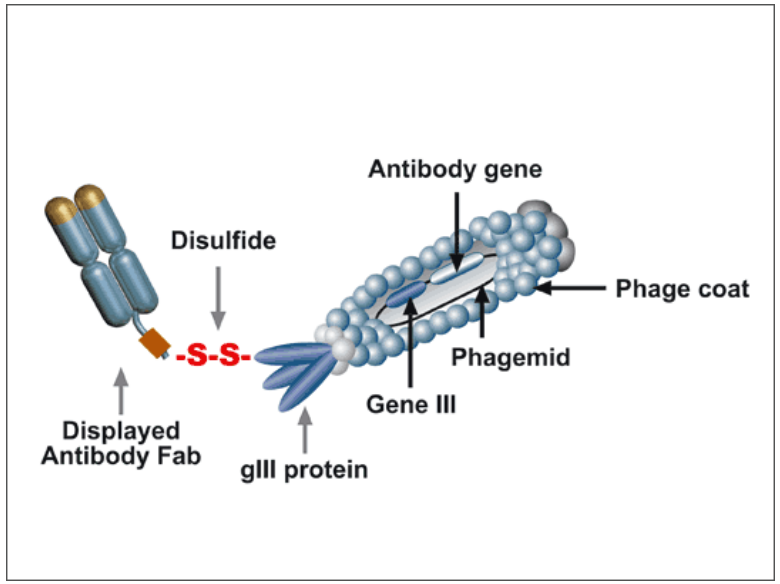
Lipase: variants on Olive oil plates
With pH indicator (brilliant green)

*Kilde: Reymond, Chapter 6 (HTS screening and selection of Enzyme-encoded genes)*

# Phage display screening



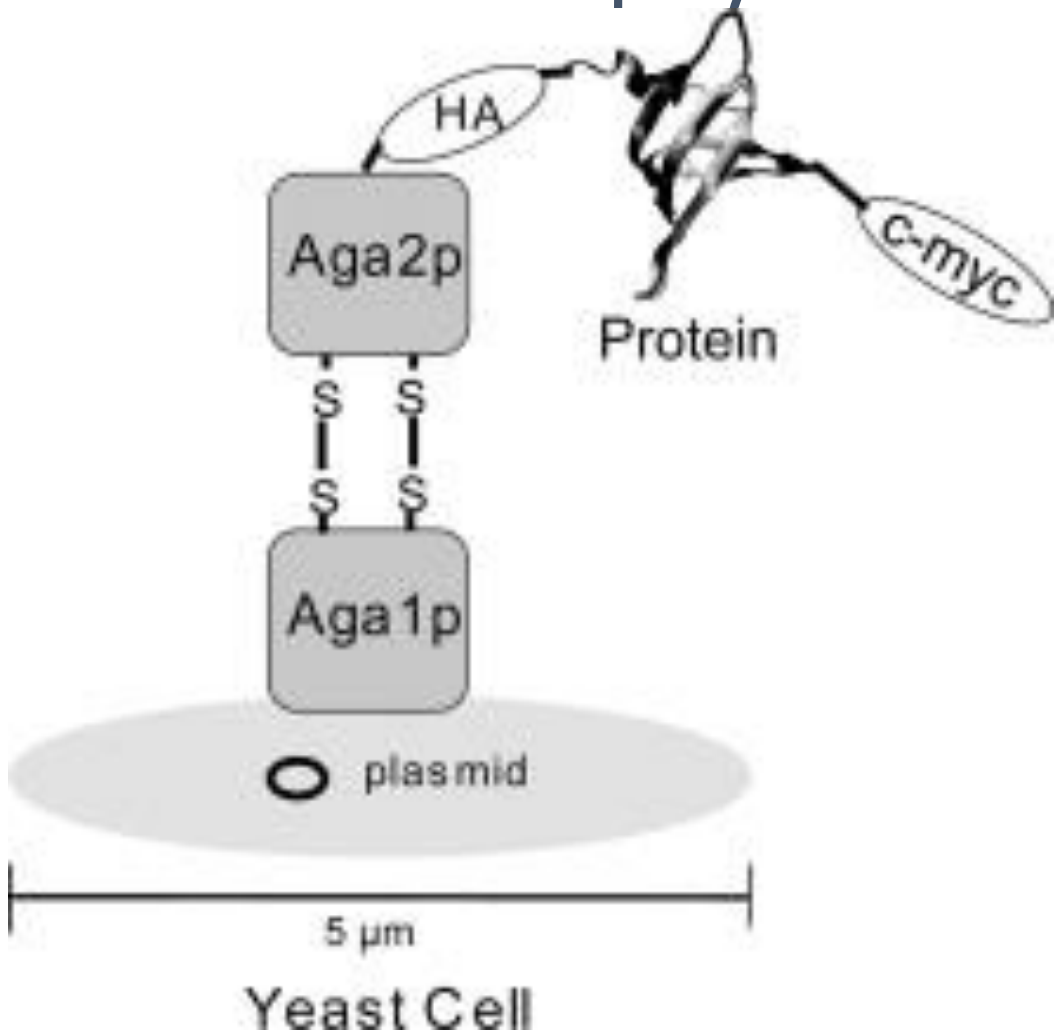



# HTS of enzymes with Cell Display

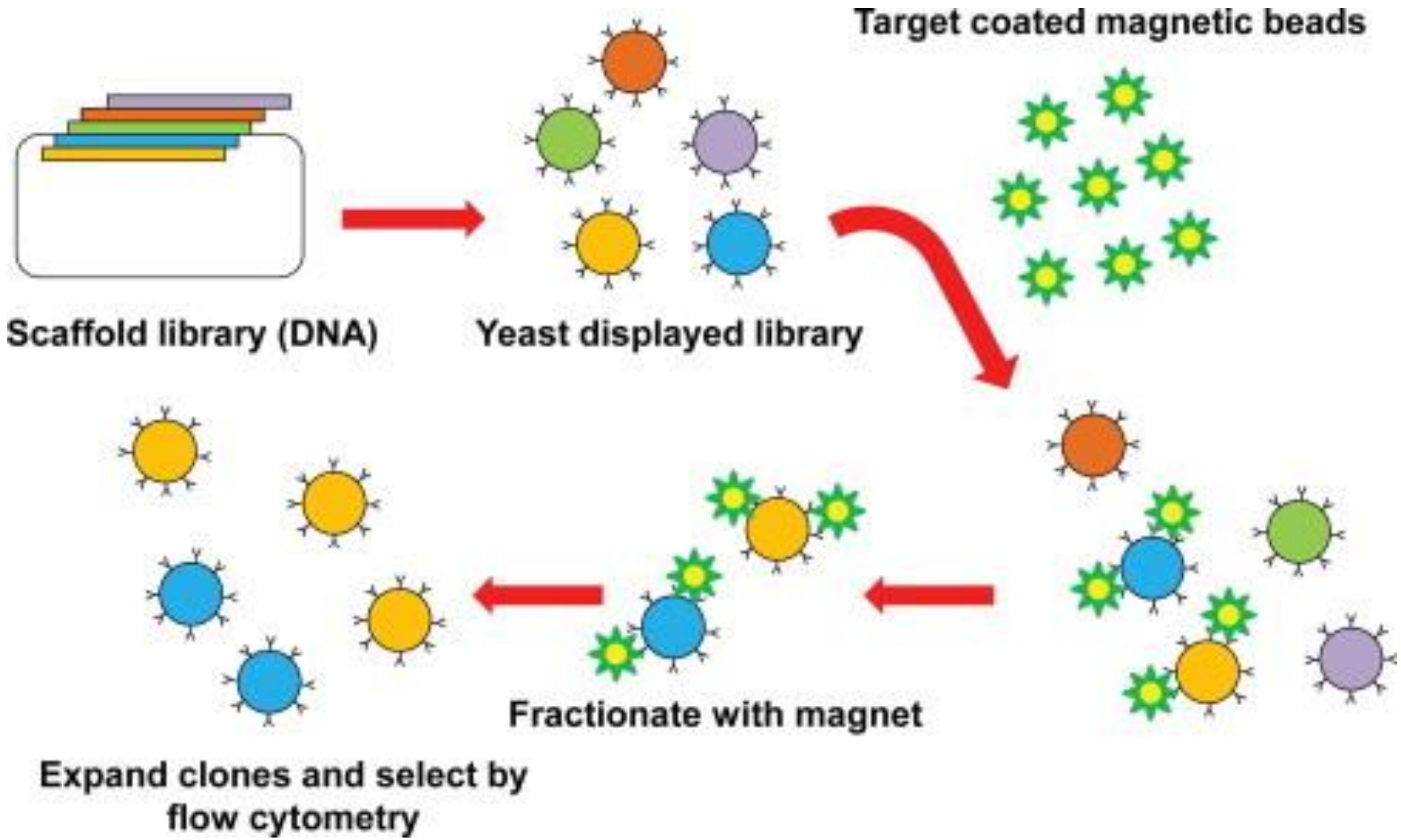
- **Yeast display** of antibody scFv fragments

  -> Expression of scFv on yeast cells is monitored using either the HA or c-myc epitope tags.

  -> Binding of the target antigen, HIV-1 gp120, is visualized using a biotinylated mAb to a noncompetitive epitope on gp120 and fluorescent streptavidin. Gp120-binding scFvs are selected by fluorescence activated cell sorting (FACS) of the yeast cells.

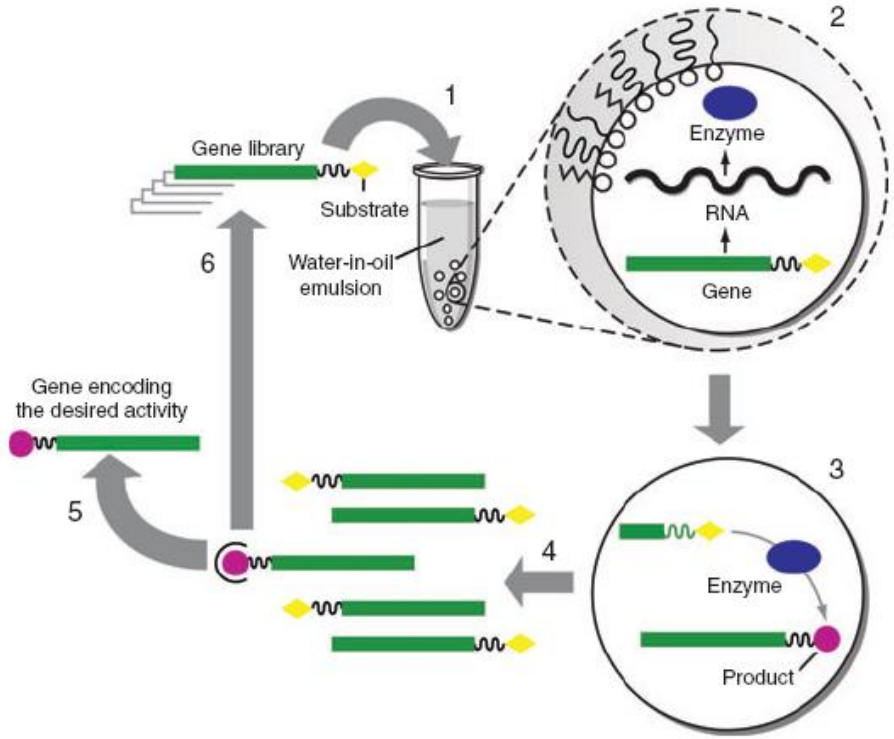



*Kilder: Reymond, Chapter 6 (HTS screening and selection of Enzyme-encoded genes), S. Becker et al., "Ultra-high-throughput screening based on cell-surface display and fluorescence-activated cell sorting for the identification of novel biocatalysts", Current Oppinion in Biotechnology 2004, 15:323-329*


Target coated magnetic beads
Scaffold library (DNA)
Yeast displayed library
Fractionate with magnet
Expand clones and select by
flow cytometry

# In vitro compartmentalization (IVC) or Cell free systems

- Water-in-oil emulsion -> make microscopic compartments (droplet volume ~5 fL) -> no diffusion between compartments.
- Each compartment contains in general one single gene and acts as an artificial cell (invitro transcription and translation)
- -> Gene is linked to substrate
- -> if protein is active substrate will be converted into product -> signal -> fishing out
- -> direct access to gene



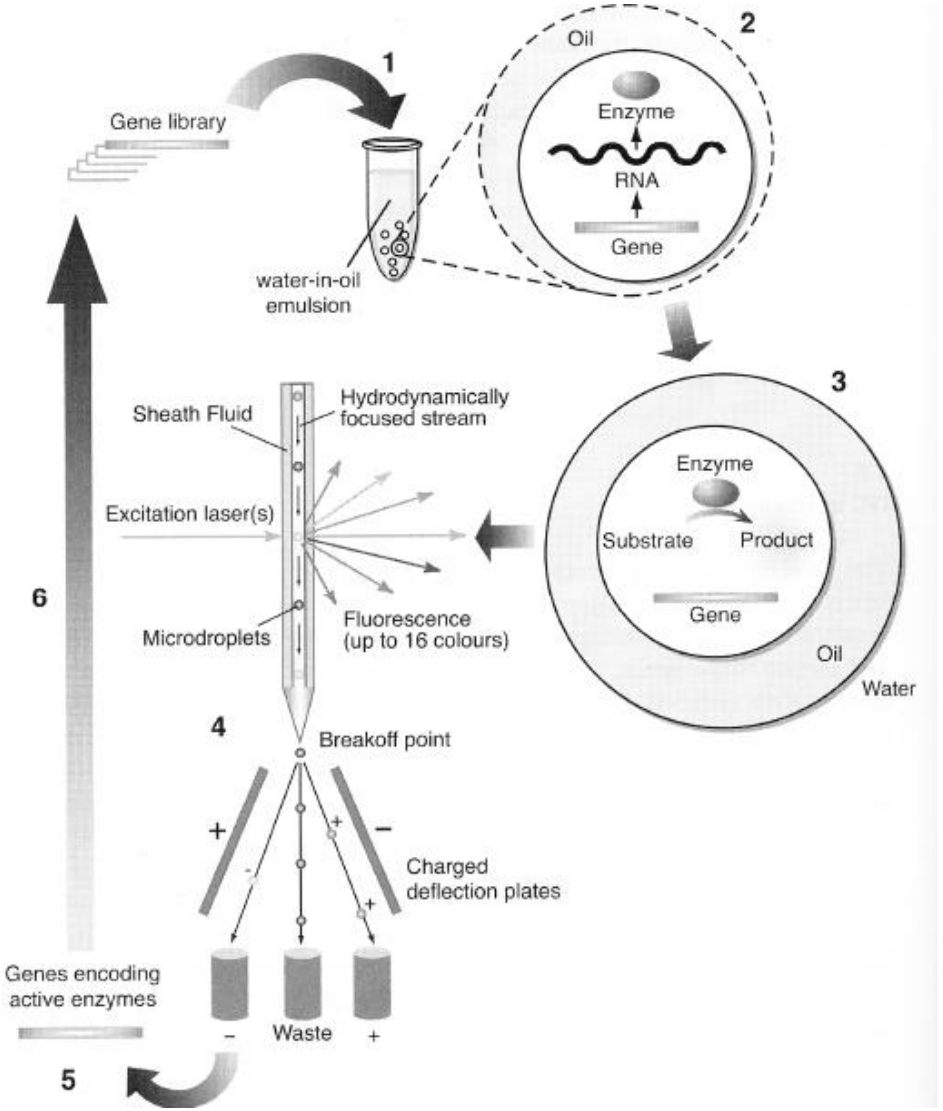

*Kilder: Reymond, Chapter 6 (HTS screening and selection of Enzyme-encoded genes), O.J. Miller, "Directed evolution by in vitro compartmentalization", Nature Methods, vol.3, no. 7, 561-570, 2006*

# In vitro compartmentalization (IVC)

-> w/o/w -> water in oil in water emulsion  (Vesicle)

Avantage:

- No direct link between product and gene necessary -> keep the compartment
- Can be directly analysed with FACS

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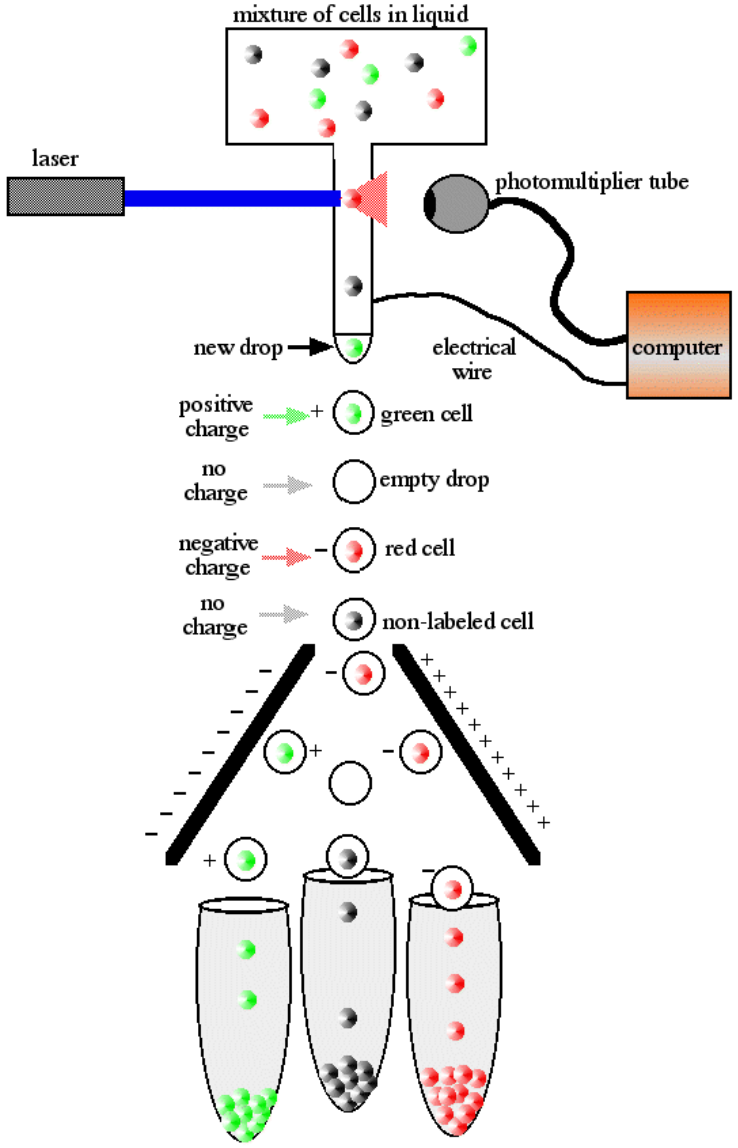
# FACS (Fluorescens-activated cell sorter)




Capacity > $10^7$ per hour

- The cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid.
- A vibrating mechanism causes the stream of cells to break into individual droplets.
- Just before the stream breaks into droplets, the flow passes through a fluorescence measuring station where the fluorescent character of interest of each cell is measured.
- An electrical charging ring is placed just at the point where the stream breaks into droplets. A charge is placed on the ring based on the immediately-prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream. The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge.

*http://www.bio.davidson.edu/COURSES/GENOMICS/method/FACS.html*

Lab on a Chip

PAPER



Cite this: *Lab Chip*, 2014, **14**, 806

# High-throughput screening for industrial enzyme production hosts by droplet microfluidics†

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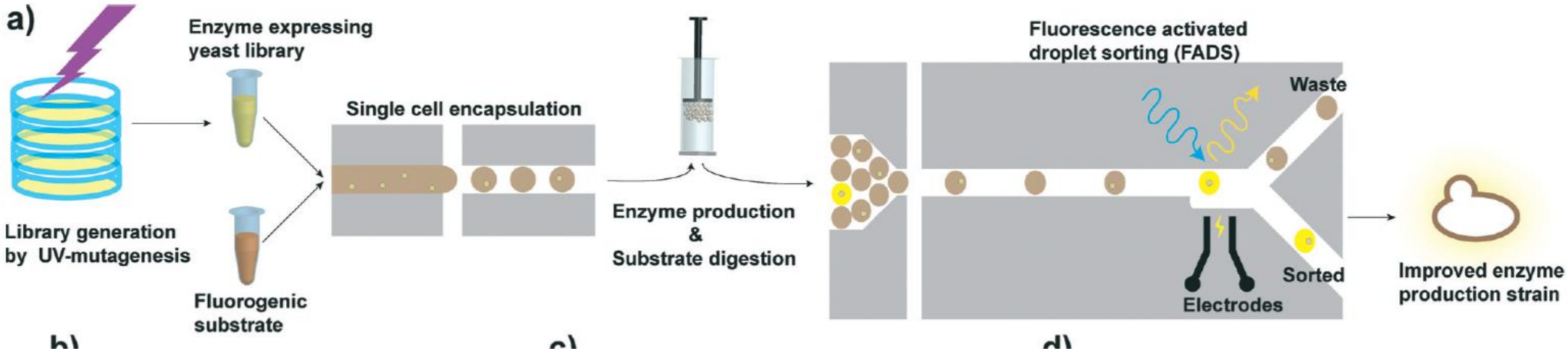
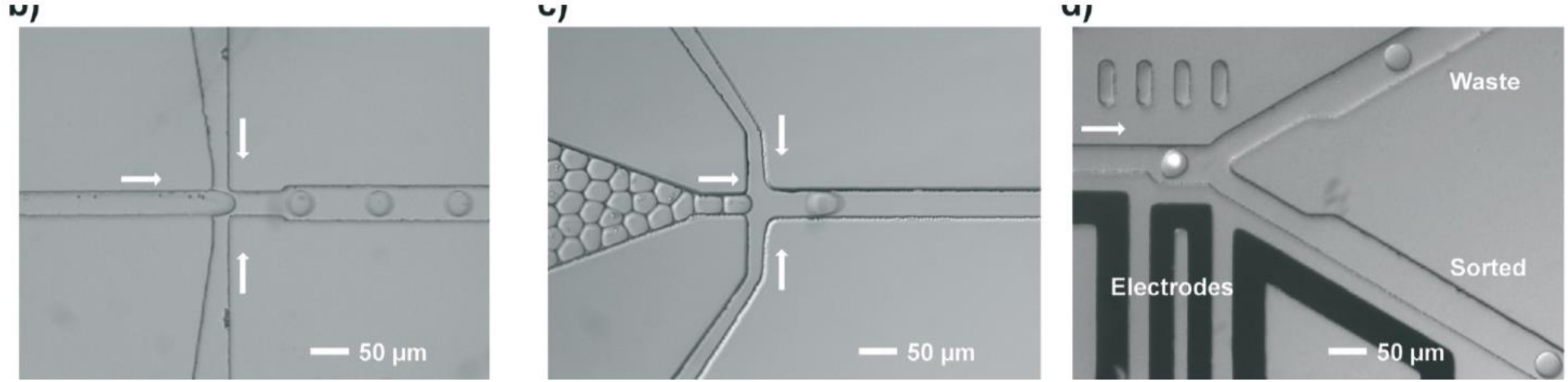
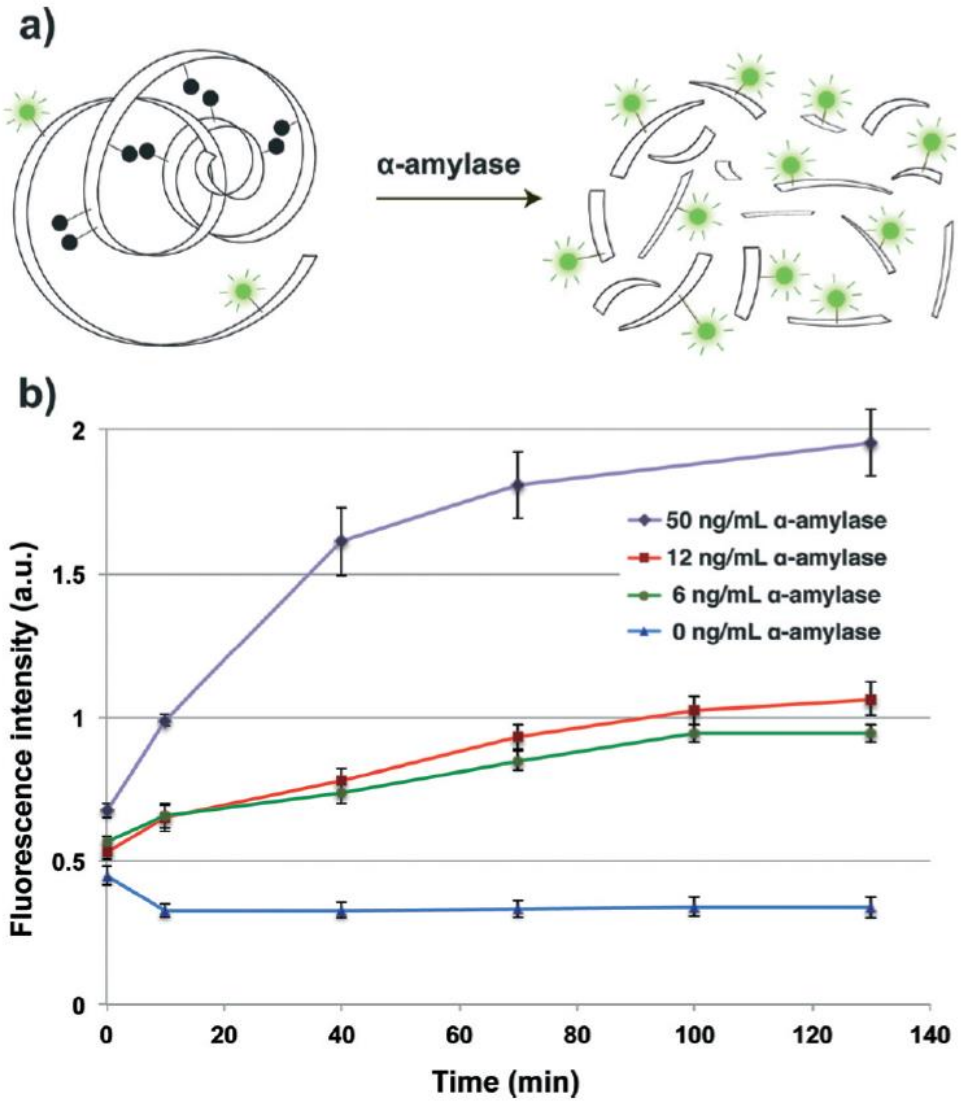
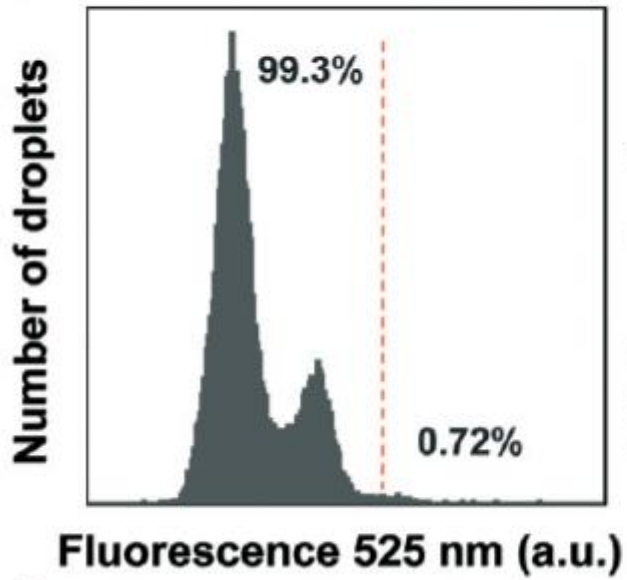

a)
Enzyme expressing
yeast library
Library generation
by UV-mutagenesis
Fluorogenic
substrate
Single cell encapsulation
Enzyme production
&
Substrate digestion
Fluorescence activated
droplet sorting (FADS)
Waste
Sorted
Electrodes
Improved enzyme
production strain

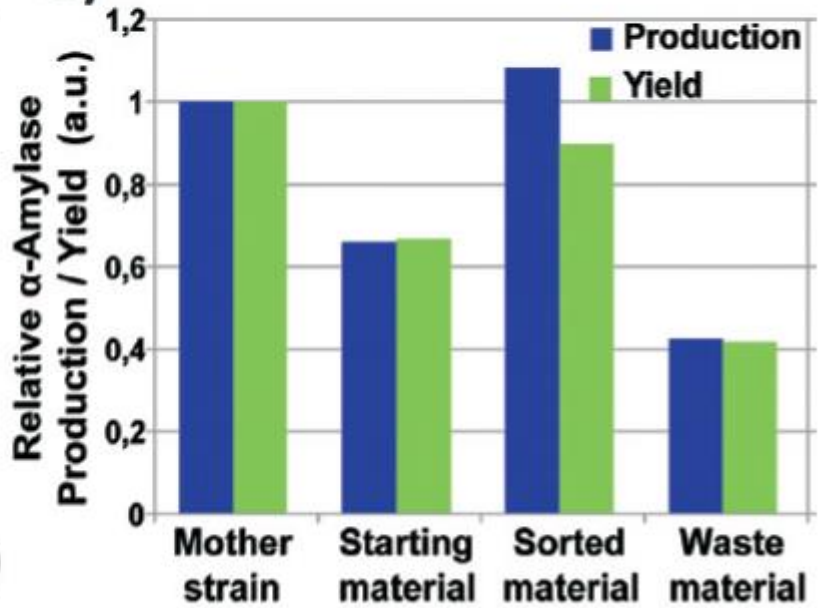

b)
50 µm
c)
50 µm
d)
Waste
Sorted
Electrodes
50 µm


a)
α-amylase
b)
Fluorescence intensity (a.u.)
Time (min)
50 ng/mL α-amylase
12 ng/mL α-amylase
6 ng/mL α-amylase
0 ng/mL α-amylase
0
0.5
1
1.5
2
0
20
40
60
80
100
120
140

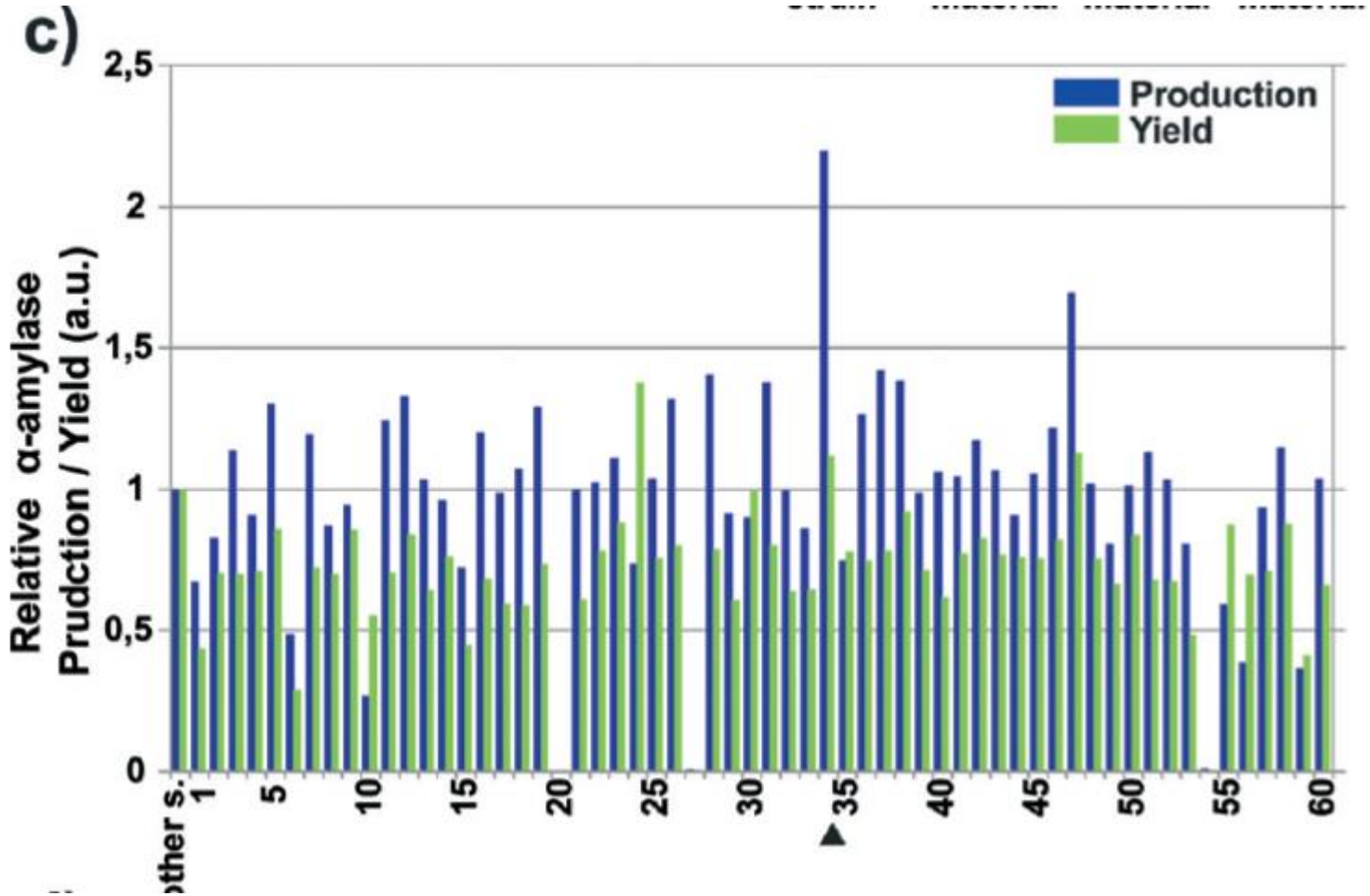
a)

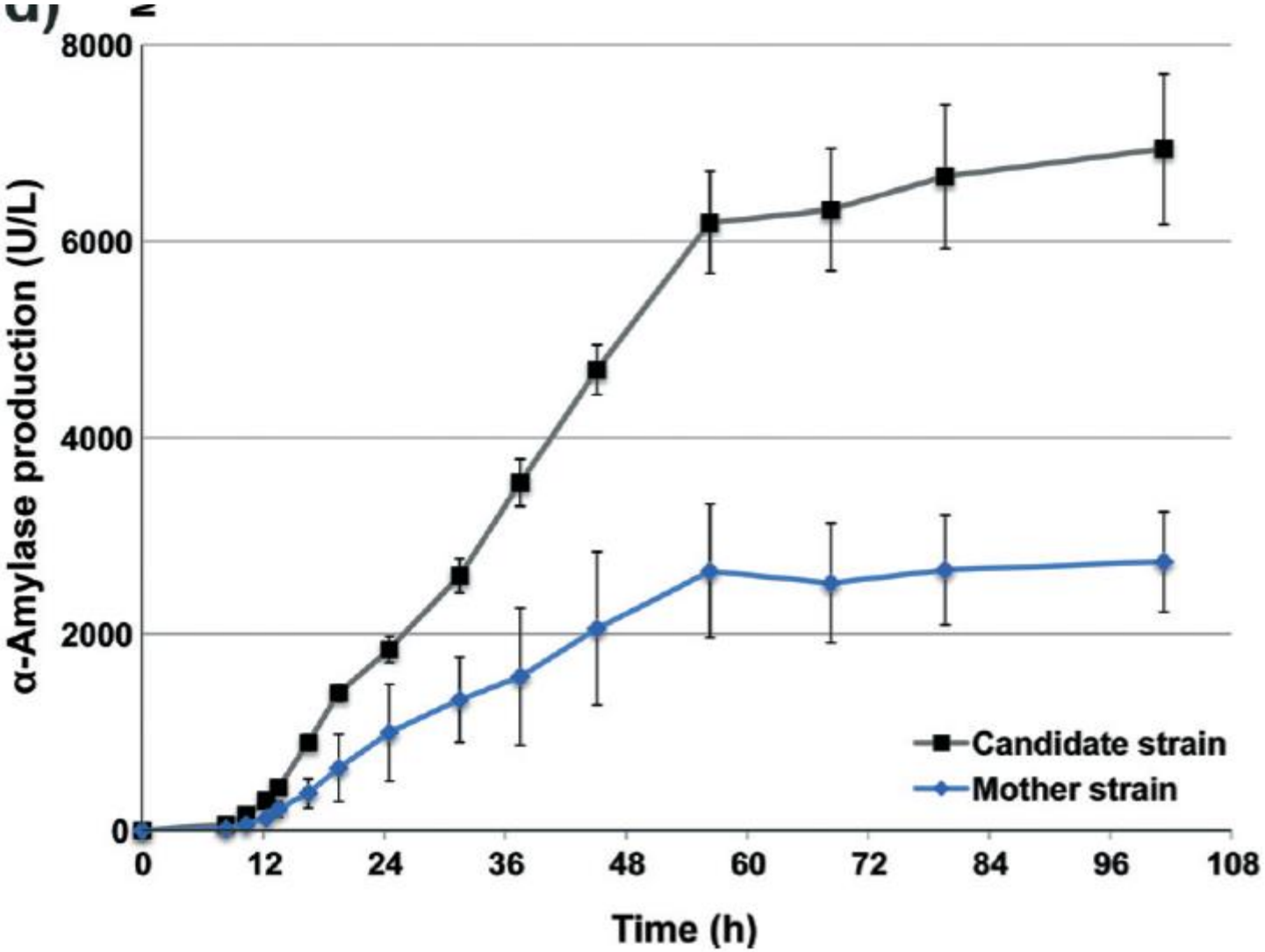

Number of droplets
99.3%
0.72%
Fluorescence 525 nm (a.u.)

b)


Relative α-Amylase Production / Yield (a.u.)
1,2
1
0,8
0,6
0,4
0,2
0
Production
Yield
Mother strain
Starting material
Sorted material
Waste material

c)


c)
Production
Yield
Relative α-amylase
Prudction / Yield (a.u.)
2,5
2
1,5
1
0,5
0
other s.
1
5
10
15
20
25
30
35
40
45
50
55
60


8000
6000
4000
2000
0
α-Amylase production (U/L)
0
12
24
36
48
60
72
84
96
108
Time (h)
Candidate strain
Mother strain