

Hücre fabrikalarının oluşturulması – 1

**"in the search of the
wasteful strain"**

Proteinler

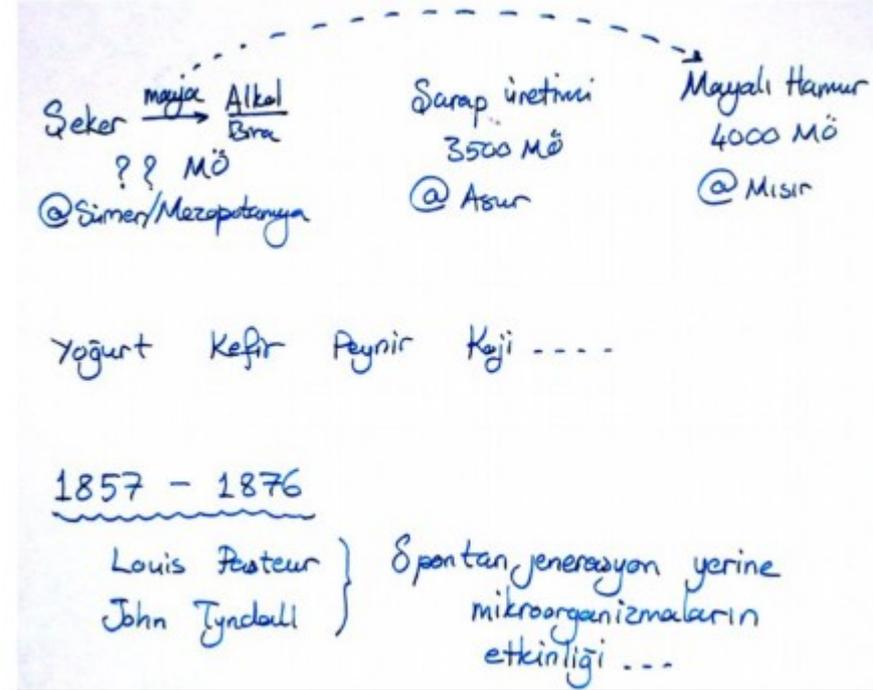
Nükleik asitler

Karbonhidrat polimerleri

Hücreler

Küçük moleküller

(1°/2° metabolitler)



enzim pazarı

t/a	product	enzyme
> 1 000 000	high-fructose corn syrup	glucose isomerase
> 100 000	lactose-free milk	lactase
> 10 000	acrylamide	nitrilase
	cocoa butter	lipase
> 1 000	nicotinamide	nitrilase
	D-pantothenic acid	aldonolactonase
	(S)-chloropropionic acid	lipase
	6-aminopenicillanic acid	penicillin amidase
	7-aminocephalosporanic acid	glutaryl amidase
	aspartame	thermolysin
	L-aspartate	aspartase
	D-phenylglycine	hydantoinase
	D- <i>p</i> -OH-phenylglycine	hydantoinase
> 100	ampicillin	penicillin amidase
	L-methionine, L-valine	aminoacylase
	L-carnitine	dehydrase/ hydroxylase
	L-DOPA	β -tyrosinase
	L-malic acid	fumarase
	(S)-methoxyisopropyl-amine	lipase
	(R)-mandelic acid	nitrilase
	L-alanine	L-aspartate- β -de-carboxylase

geeeel vatandaaaş...

Hedefler:

- Aktivite artışı
- Termostabilite
- Substrat özgüllüğü
- Ürün özgüllüğü
- Enantioseçicilik



Scientific Background on the Nobel Prize in Chemistry 2018

DIRECTED EVOLUTION OF ENZYMES AND BINDING PROTEINS

Controlled optimization by the above procedure really becomes an interesting challenge when genotype and phenotype are different molecules.

Let us therefore expand the procedure as follows -

- 10 PRODUCE A MUTANT SPECTRUM OF SELF-REPRODUCING TEMPLATES
- 20 SEPARATE AND CLONE INDIVIDUAL MUTANTS
- 30 AMPLIFY CLONES
- 40 EXPRESS CLONES
- 50 TEST FOR OPTIMAL PHENOTYPES
- 60 IDENTIFY OPTIMAL GENOTYPES
- 70 RETURN TO 10 WITH A SAMPLE OF OPTIMAL GENOTYPES

Manfred Eigen, 1984

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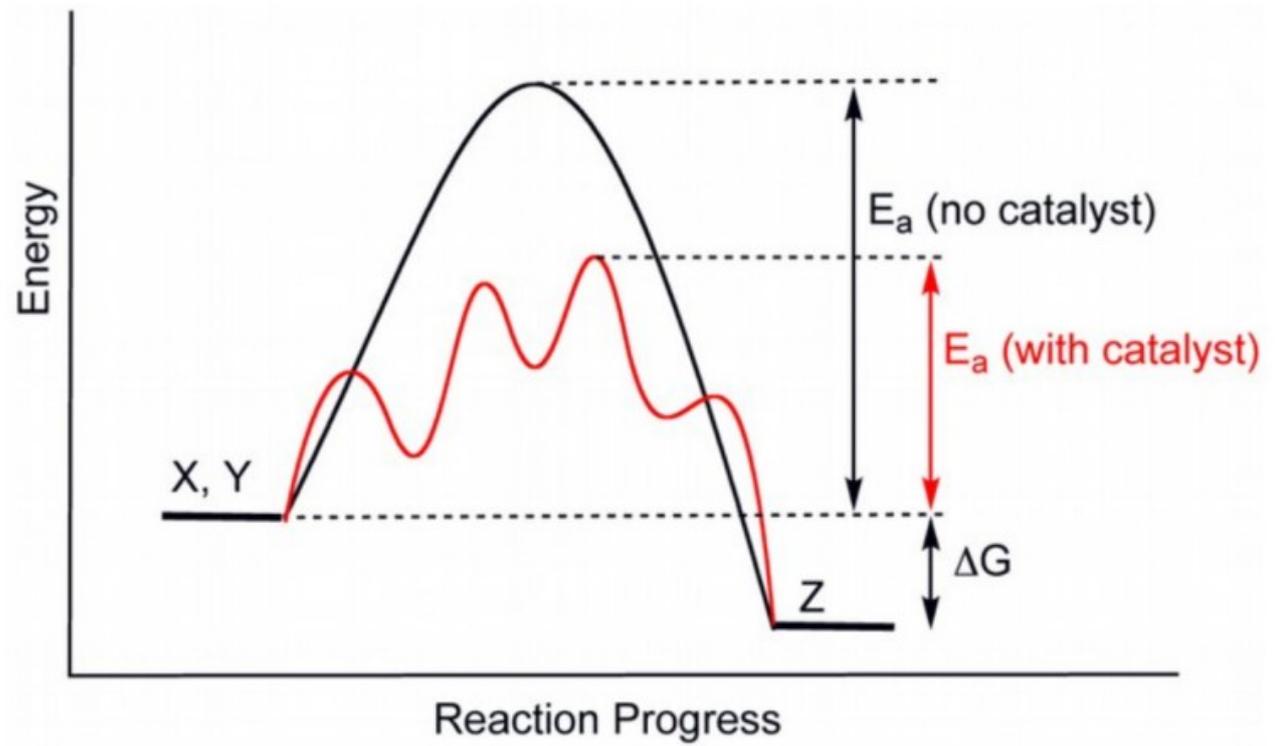
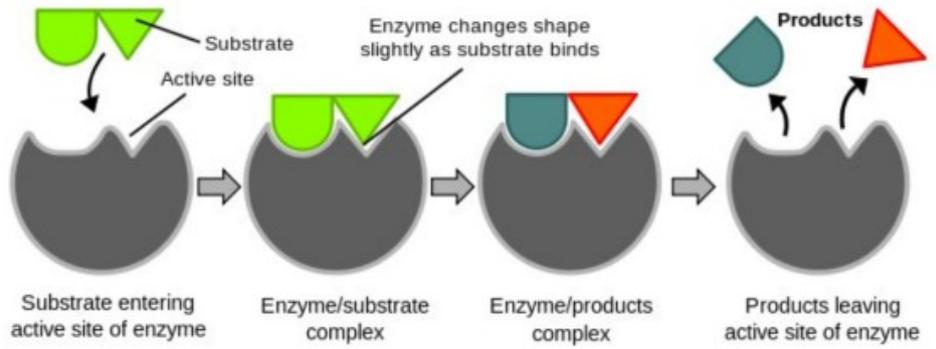


The Comprehensive Enzyme Information System



EC Explorer [\[SEARCH\]](#) [\[BROWSE\]](#)

-  **1 Oxidoreductases** (6951 organisms)   
-  **2 Transferases** (5035 organisms)   
-  **3 Hydrolases** (8314 organisms)   
-  **4 Lyases** (3622 organisms)   
-  **5 Isomerases** (1373 organisms)   
-  **6 Ligases** (1149 organisms)   



> %90 endüstriyel enzim rekombinant olarak üretiliyor → saflık, aktivite
↘ koşullara dayanıklılık!

① Rasyonel tasarım
işisi sanse bırakma
~~~~~  
Kritik noktalara  
hedeflenmiş mutasyonlar

② yönlendirilmiş evrim  
az bilgi — çok şans  
Darwinian optimizasyon

③ Orta Yol

## Rassal ve yarı rasyonel mutajenez için:

"error prone PCR"

baz analogları  
alkilleyiçer ajanlar  
 $Mg^{2+} \rightarrow Mn^{2+}$   
dNTP dengesizliđi  
- - -

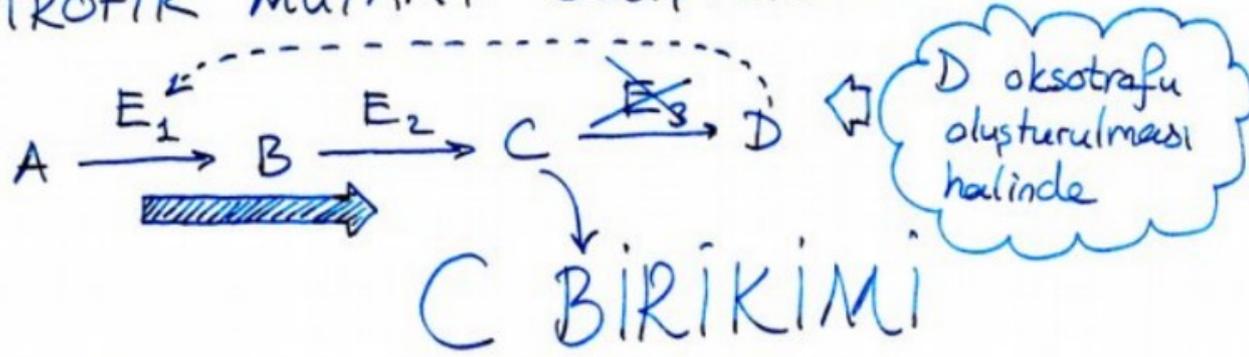
"Sequence Saturation Mutagenesis"

alkaliđe duyarlı  $\alpha$ -fosfatiyonaz  
TdT enzimi

Uzun oligolar

Çooooook büyük kütüphanelerde bir çok anlamsız dizi...  
samanlıkta iđne aramaktan farksız !!!

# OKSOTROFİK MUTANT OLUŞTURULMASI



Nasıl yapılır?

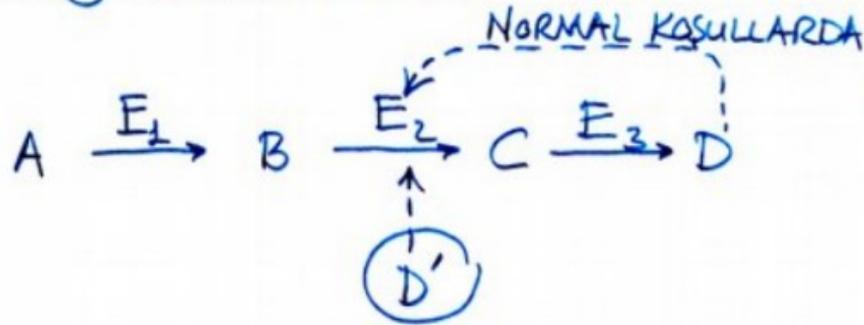
- mutasyona maruz bırak, oksotrafu oluşturulmak istenen substrat bulunmayan besiyerinde inkübe et
- kulture penisilin ekle → Enzim defektif hale gelip oksotraf olan hücreler üremeyecek/çözülemeyecek aktif bölünenler penisilin etkisi ile PARÇALANIR

- Penisilin(-) / Substrat(+) besiyerinde oksotrafı seç!

Örnek:

L-ornitin üretimi için L-Arginin oksotrafı seçilmesi

# Dallanmayan yollarda regülatuar mutantların seçimi



D': amino asit analogu

TOKSİK!

E<sub>2</sub>'yi inhibe eder

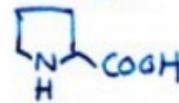
son ürün D

sentezlenemez

- Minimal besiyerinde D' kullanılarak seçim yapılır.
- ~~Yalnızca~~ Yalnızca E<sub>2</sub> regülasyon defekti olan mutantlar D üretebilir ve hayatta kalabilir!

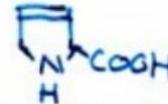
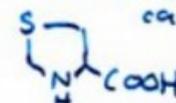
*Serratia marcescens*'de prolin üretimi

Prolin

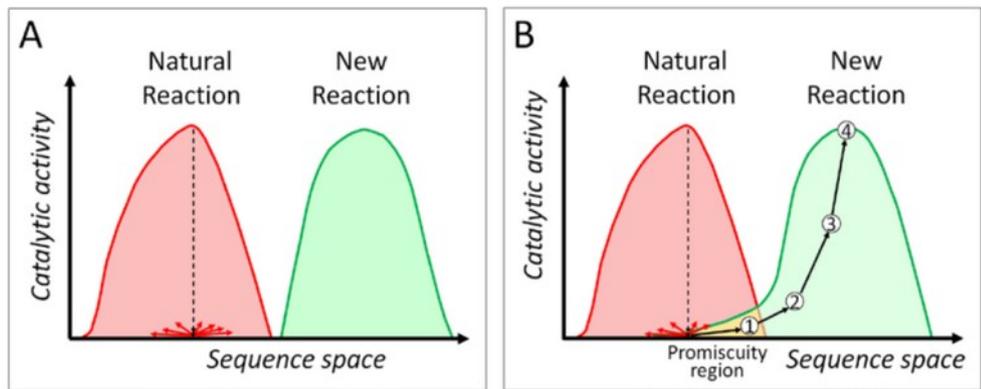
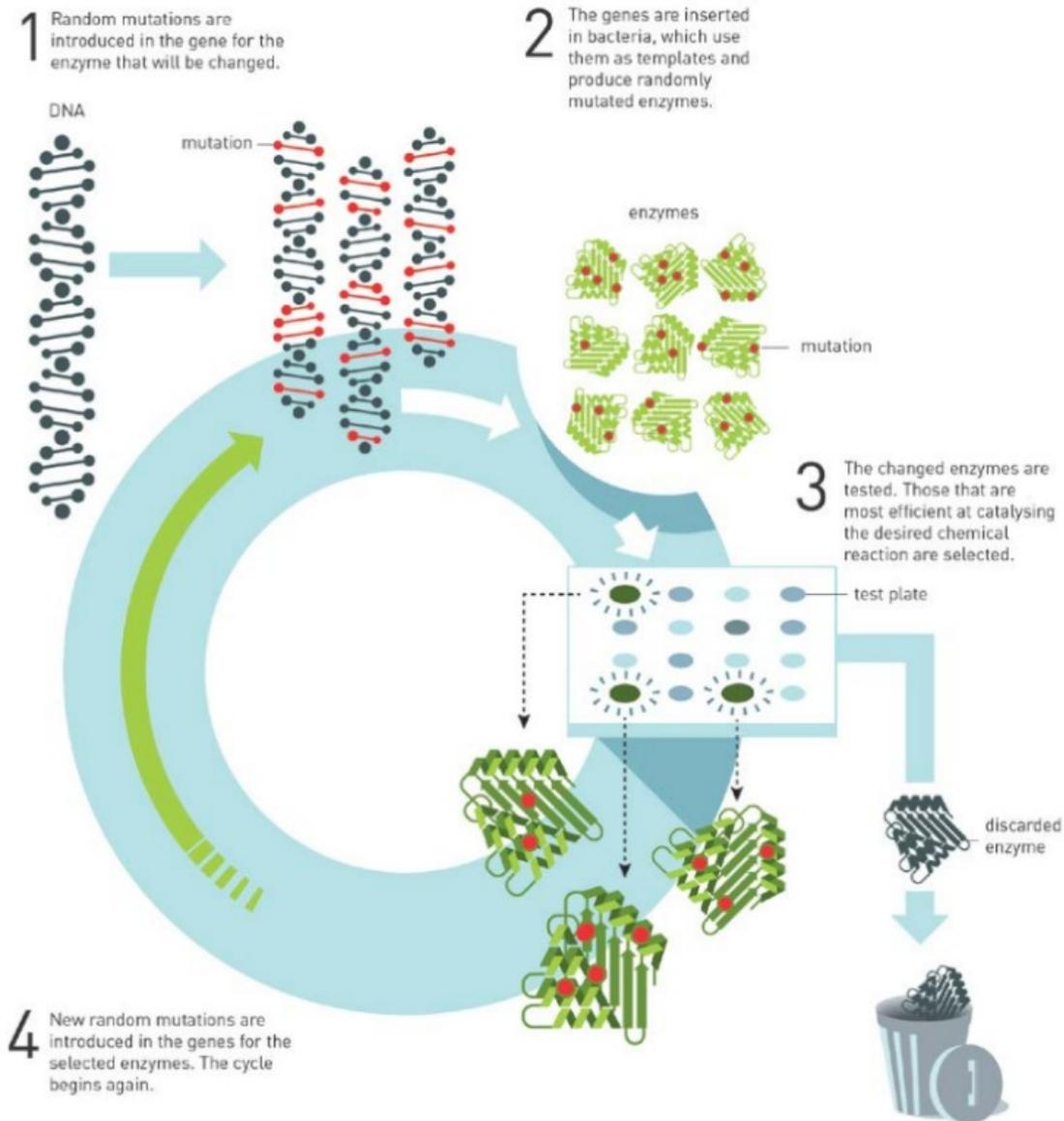


Thiazolidine-4-

carboxylic acid



3;4-Dehydroprolin



**Figure 4. A:** A starting point with no activity for the intended reaction is useless since no sequence variations (red arrows) create the new reactivity. **B:** A promiscuous enzyme with at least low activity for the intended reaction is a suitable starting point. Some combinations of random mutations may improve the new reactivity (black arrow). The first variant (1) serves as a starting state for sequential rounds of variation and screening  $\rightarrow(2)\rightarrow(3)\rightarrow(4)$  for improved variants. Only a small number of cycles and are typically needed to boost up the new reactivity.

