酶工程

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中国科学院合成生物学重点实验室

2020年12月10日

主要经历

1991-1995	浙江大学化工系生物化学工程	本科
1995-2000	中国科学院上海生物化学研究所 (酶工程组)	研究生
2000-2020	中国科学院上海生命科学研究院	助研，副研，研究员
2020-	中国科学院分子植物科学卓越创新中心	研究员

研究方向：合成生物（学）　催化剂工程

酶工程课程简介

酶工程

锁定

百度百科

本词条由"科普中国"百科科学词条编写与应用工作项目 审核 。

酶工程（英语：Enzyme engineering）又称蛋白质工程学，是指工业上有目的的设置一定的反应器和反应条件，利用酶的催化功能，在一定条件下催化化学反应，生产人类需要的产品或服务于其它目的的一门应用技术。

Google enzyme engineering

Protein engineering

From Wikipedia, the free encyclopedia

(Redirected from Enzyme engineering)

Protein engineering is the process of developing useful or valuable proteins. It is a young discipline, with much research taking place into the understanding of protein folding and recognition for protein design principles. It is also a product and services market, with an estimated value of $168 billion by 2017.[1]

酶工程课程简介

“酶”

有催化活力的蛋白质

“工程”

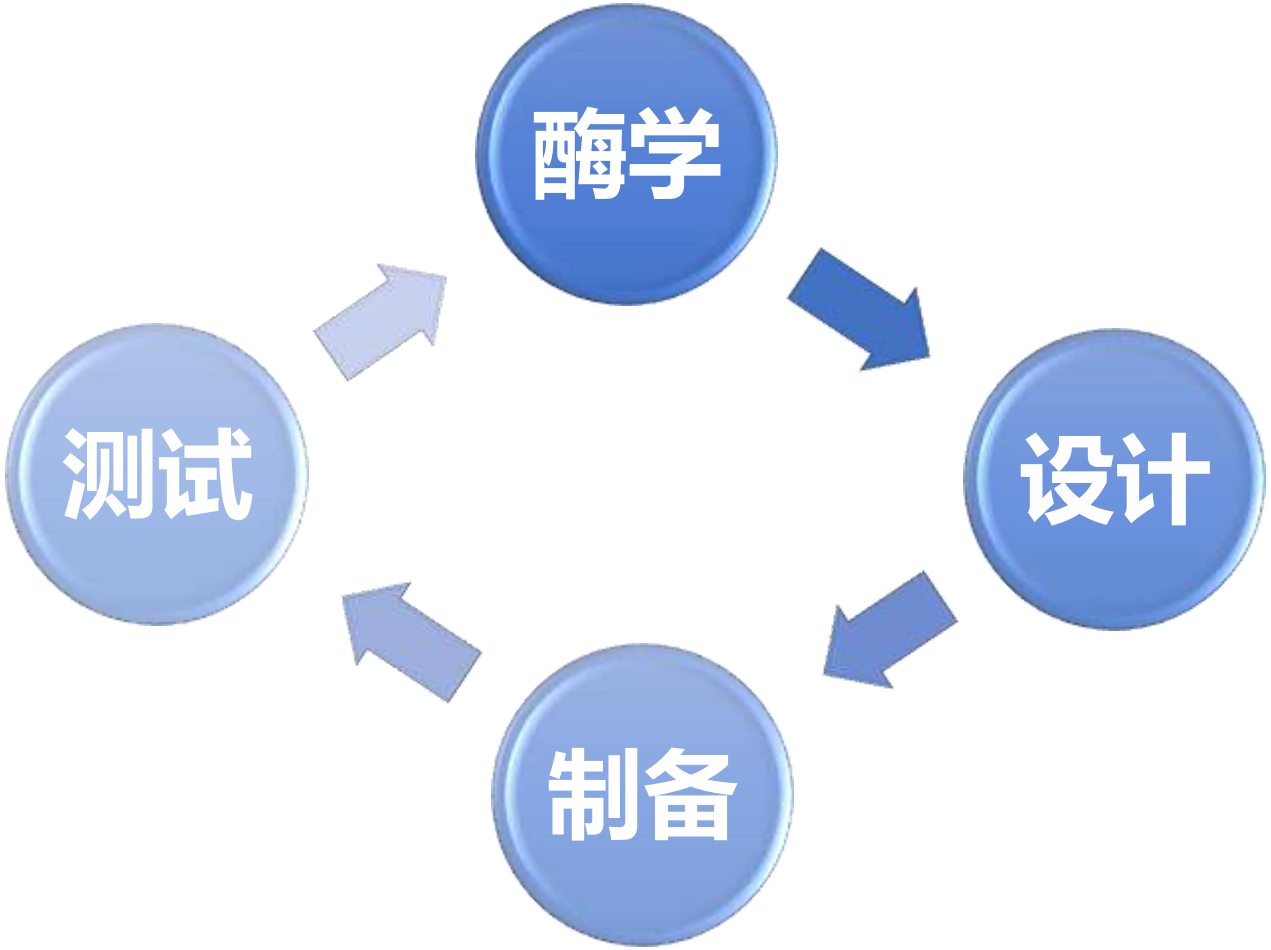
超越常规实践技巧运用数学或系统性知识设计有实用价值的复杂体系

“酶工程”

运用酶学知识设计具目标催化活性的蛋白质制剂及其应用条件

酶工程课程简介

运用酶学知识设计具目标催化活性的蛋白质制剂及其应用条件



教学目标：

在具备生物化学（酶学）与分子（微）生物学等基本知识的前提下

- 了解酶重组**表达**和**改造**的基本概念与方法。
- **独立设计**酶重组表达和改造实验方案。

课程内容安排 (约40分钟休息10分钟)

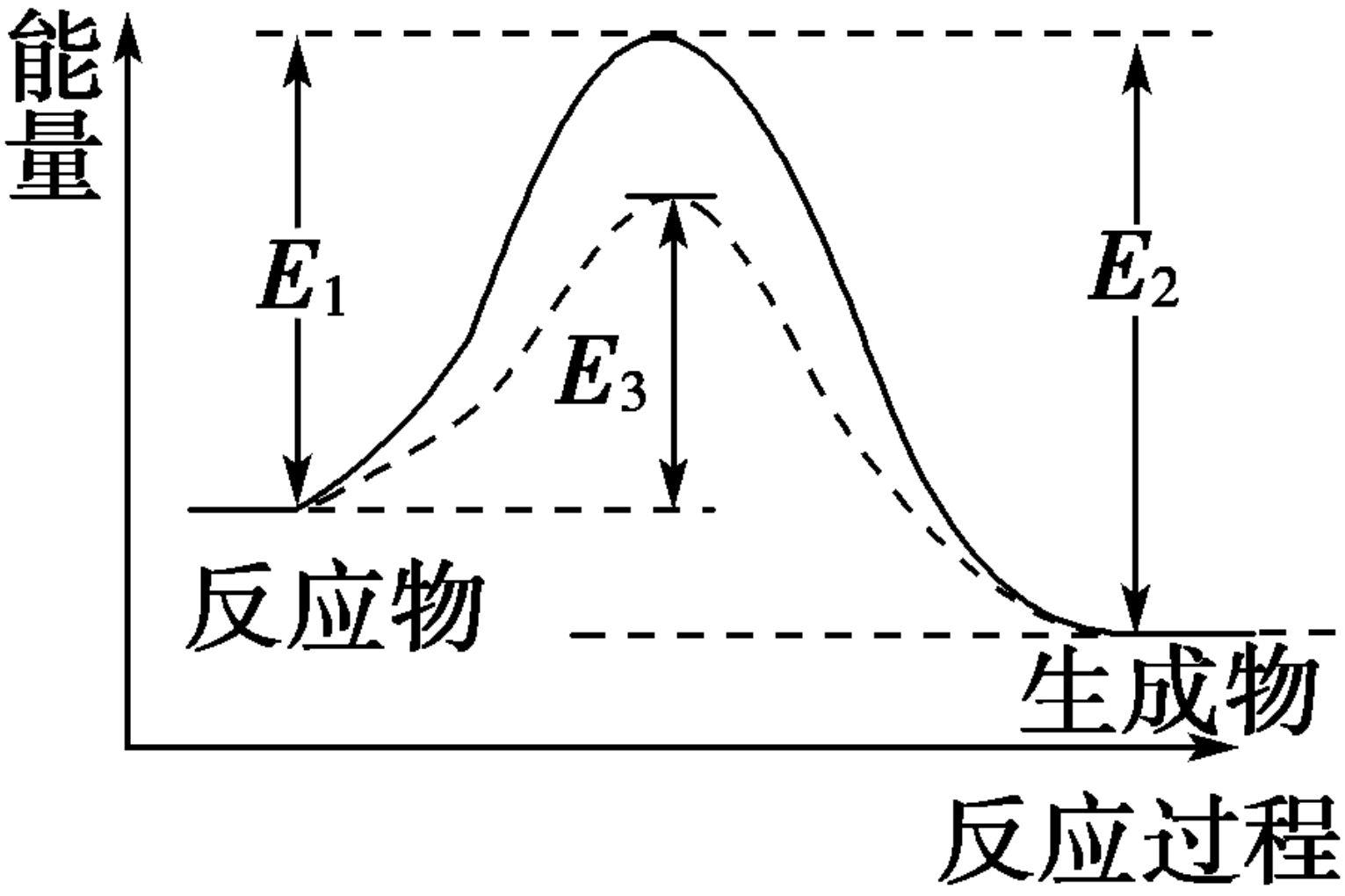
酶学知识复习

王镜岩 《生物化学》 第3版
第8章 酶通论

酶是什么?

- 有催化活力的蛋白质
  - 蛋白质 → 一般不稳定, 反应条件温和
  - 催化能力 → 专一性
    - 化学选择性，区域选择性，立体选择性

催化原理　改变反应路径



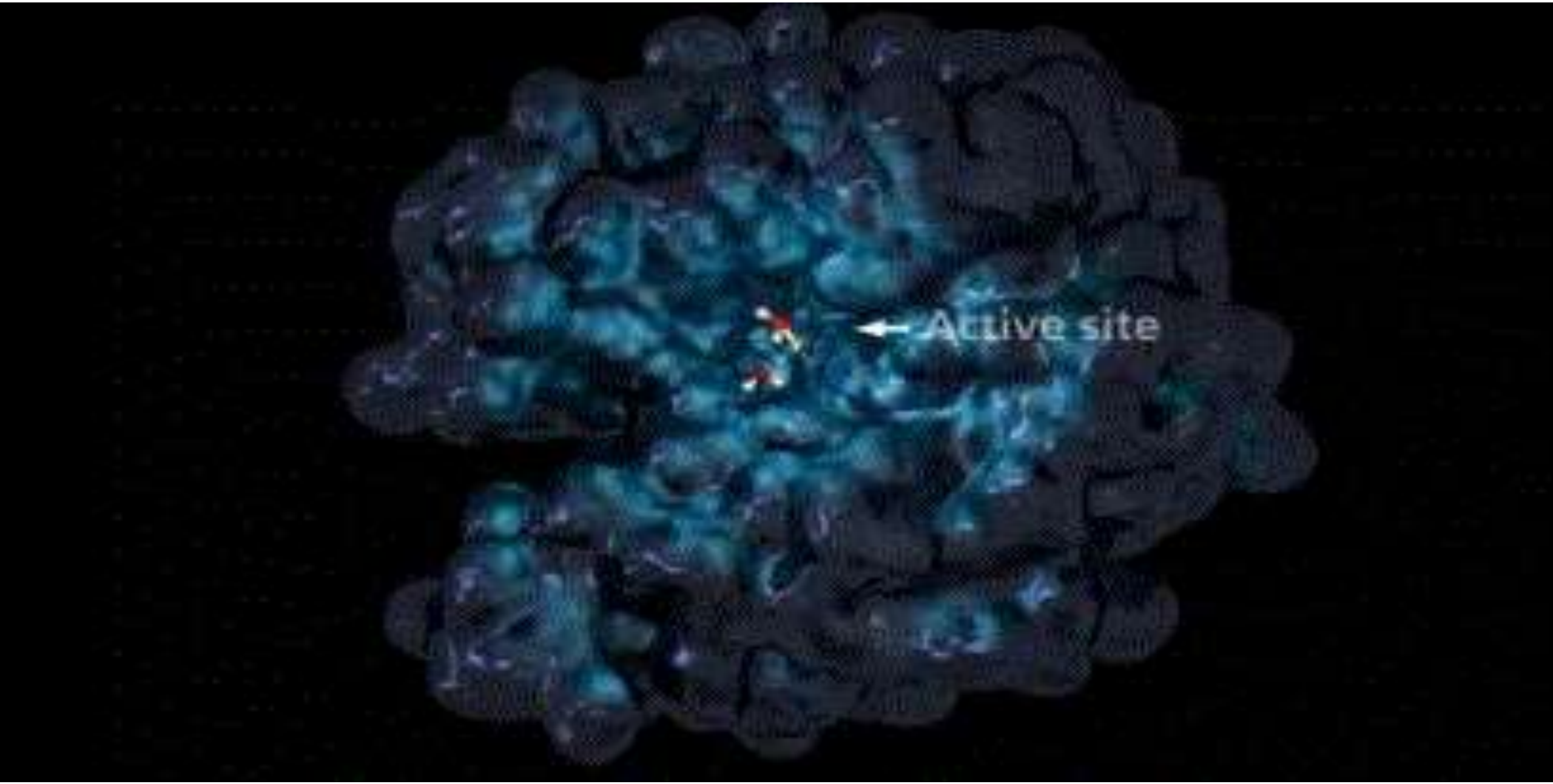
反应速率 $k=A\cdot\exp(-Ea/RT)$

酶的5类催化机制

① 酸/碱催化	RNA酶A
② 共价催化	亲核催化
③ 金属离子催化	碳酸酐酶
④ 邻近和朝向效应	提升速率高达10^7倍
⑤ 优先结合过渡态复合物	提升过渡态浓度

https://chemistryscore.com/types-of-enzyme-catalysis

碳酸酐酶反应模拟动画



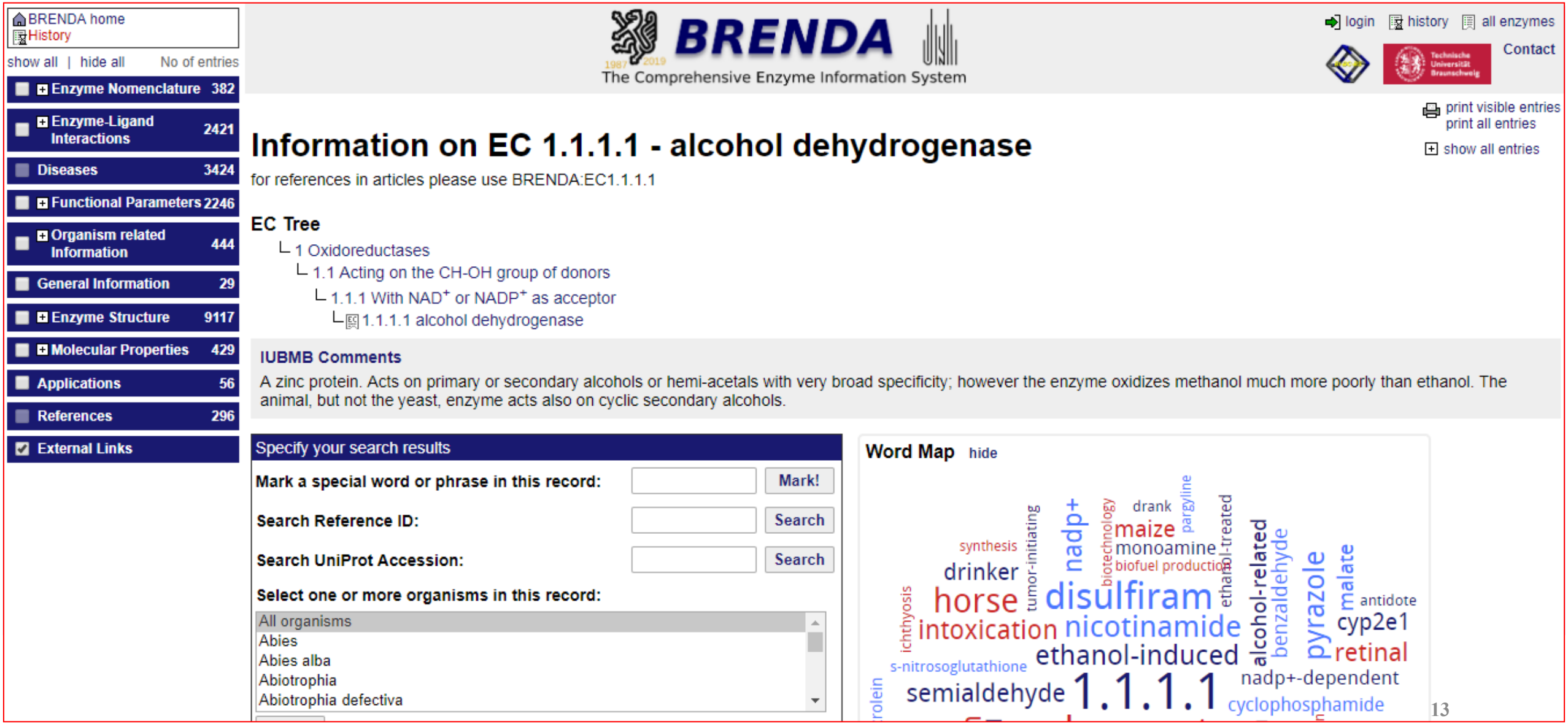
一个活性中心每秒将60万个二氧化碳转换为碳酸氢根离子

国际酶学委员会酶编号规则

第一位	第二位-亚类	第三位—亚亚类
1.氧还酶	氧化反应的供体是 H+ 还是 e-	受体类型
2.转移酶	发生转移的基团种类	发生转移的基团细分
3.水解酶	水解的化学键种类	底物类型
4.裂合酶	断裂的化学键种类	脱去的基团种类
5.异构酶	同分异构体的种类	底物类型
6.连接酶	形成的化学键种类	底物类型
7.易位酶	易位的离子/分子种类	易位驱动反应类型

Enzyme Commission (EC) number 第四位按照具体反应过程在亚亚类中发现的时间排序

http://www.brenda-enzymes.info/



酶知识复习小结

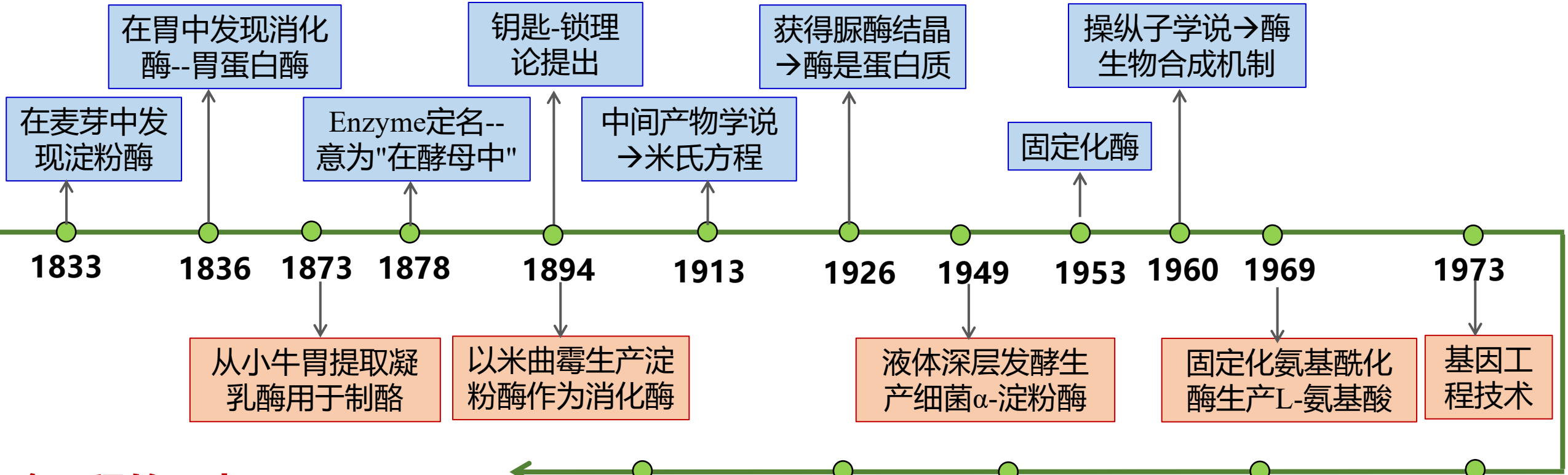

◆酶是有催化活力的蛋白质
◆酶通过改变反应途径加速反应
◆酶分为7大类，酶的命名可以在
http://www.brenda-enzymes.info上查询

基础篇

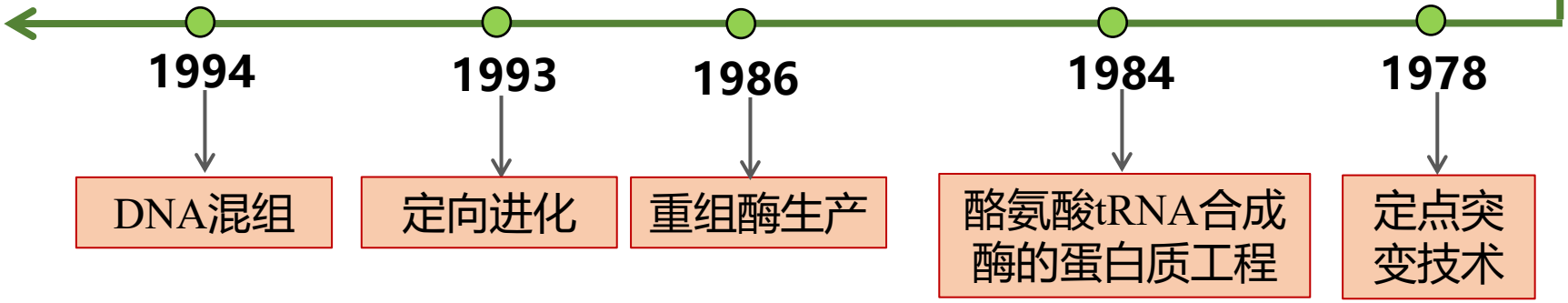
酶的生产

酶及酶工程的历史

酶的历史



酶工程的历史



酶的应用

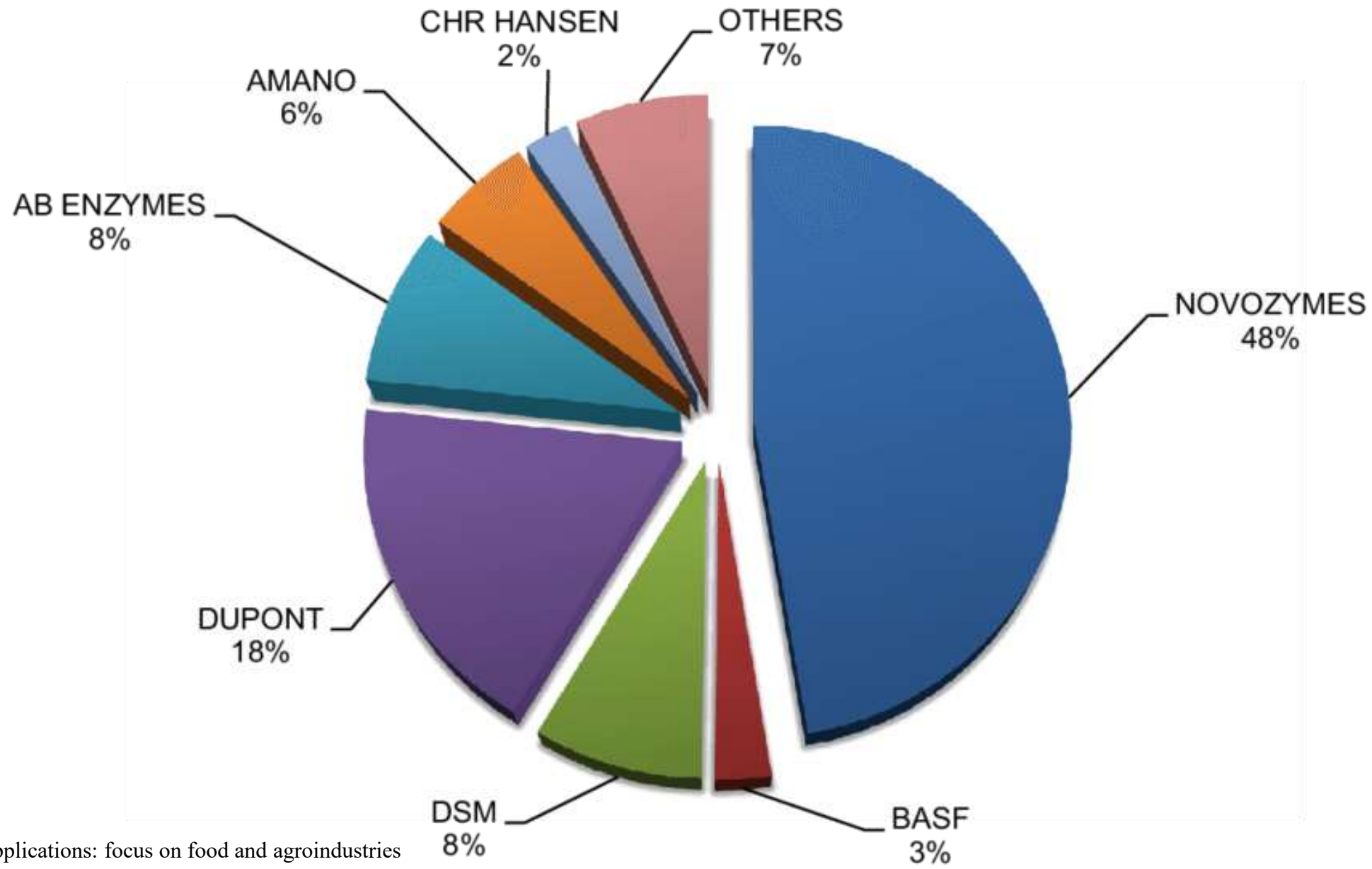
食品饮料、洗涤、生物燃料、饲料、

其他（药用、生物催化、诊断、生物技术研究用酶）

市场规模40亿美元

图片与数据来自Novozymes与DuPont

酶制剂市场份额


CHR HANSEN
2%
OTHERS
7%
AMANO
6%
AB ENZYMES
8%
NOVOZYMES
48%
DUPONT
18%
DSM
8%
BASF
3%

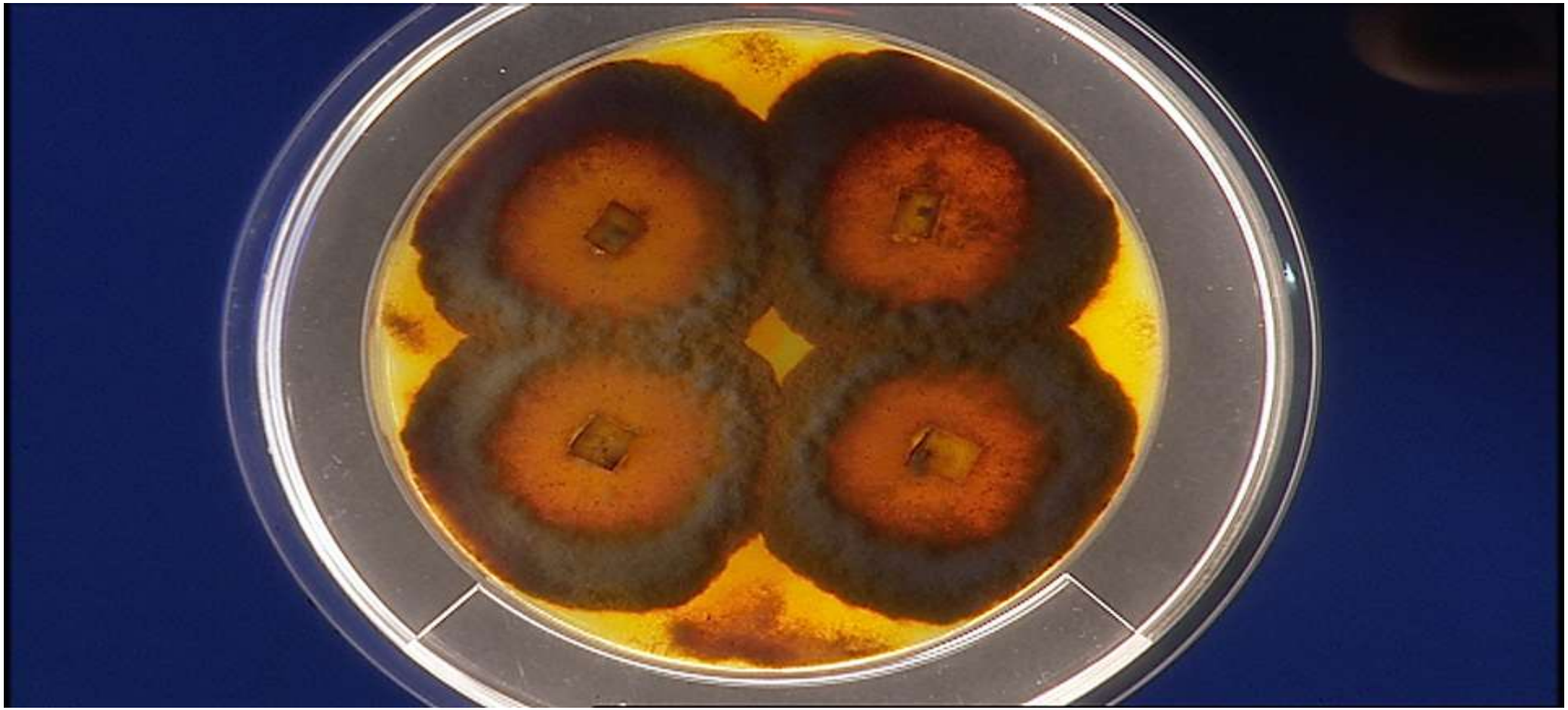
微生物酶生产率提高的手段

◆诱变选育 - - 海量筛选

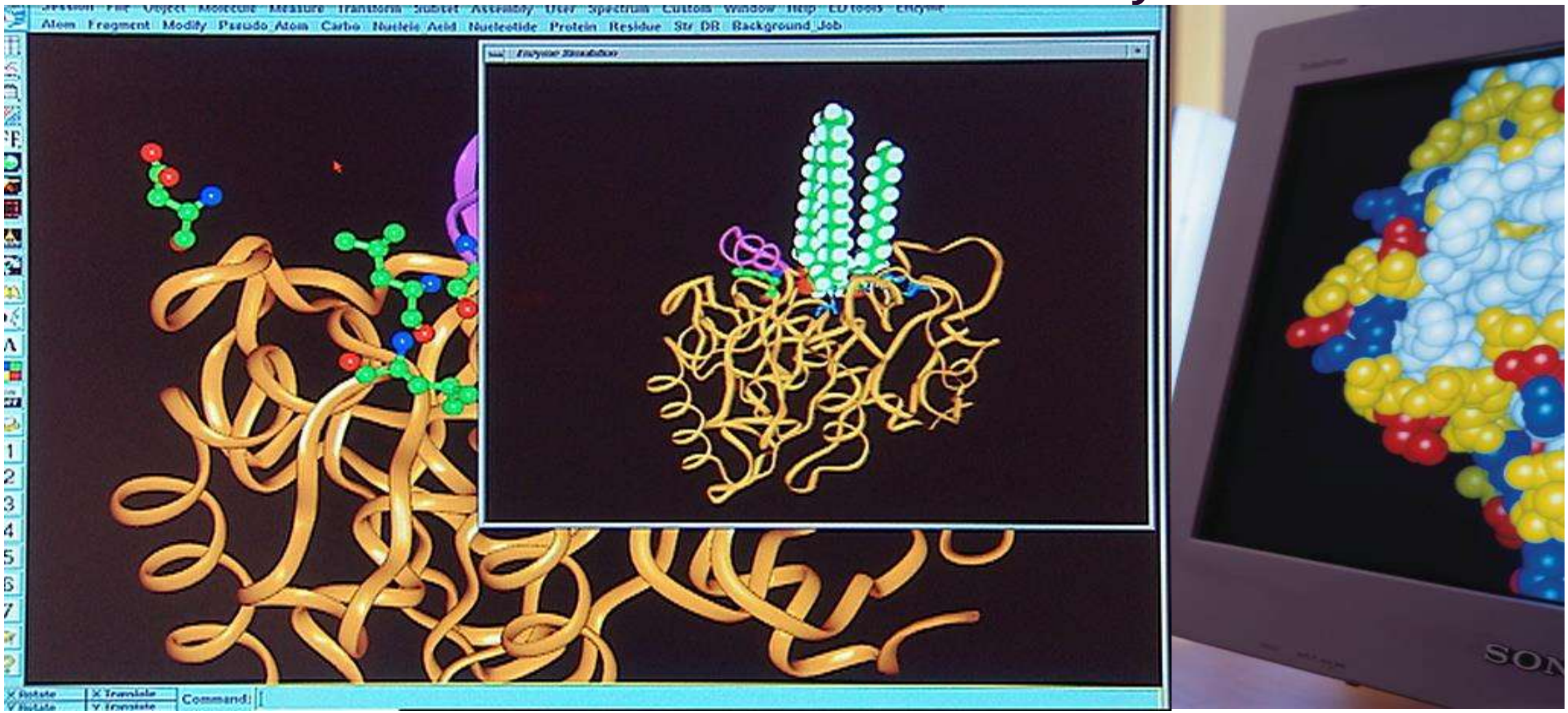
◆工艺优化 - - 过程工程

◆重组表达 - - 基因工程

Found in nature



Refined in the laboratory



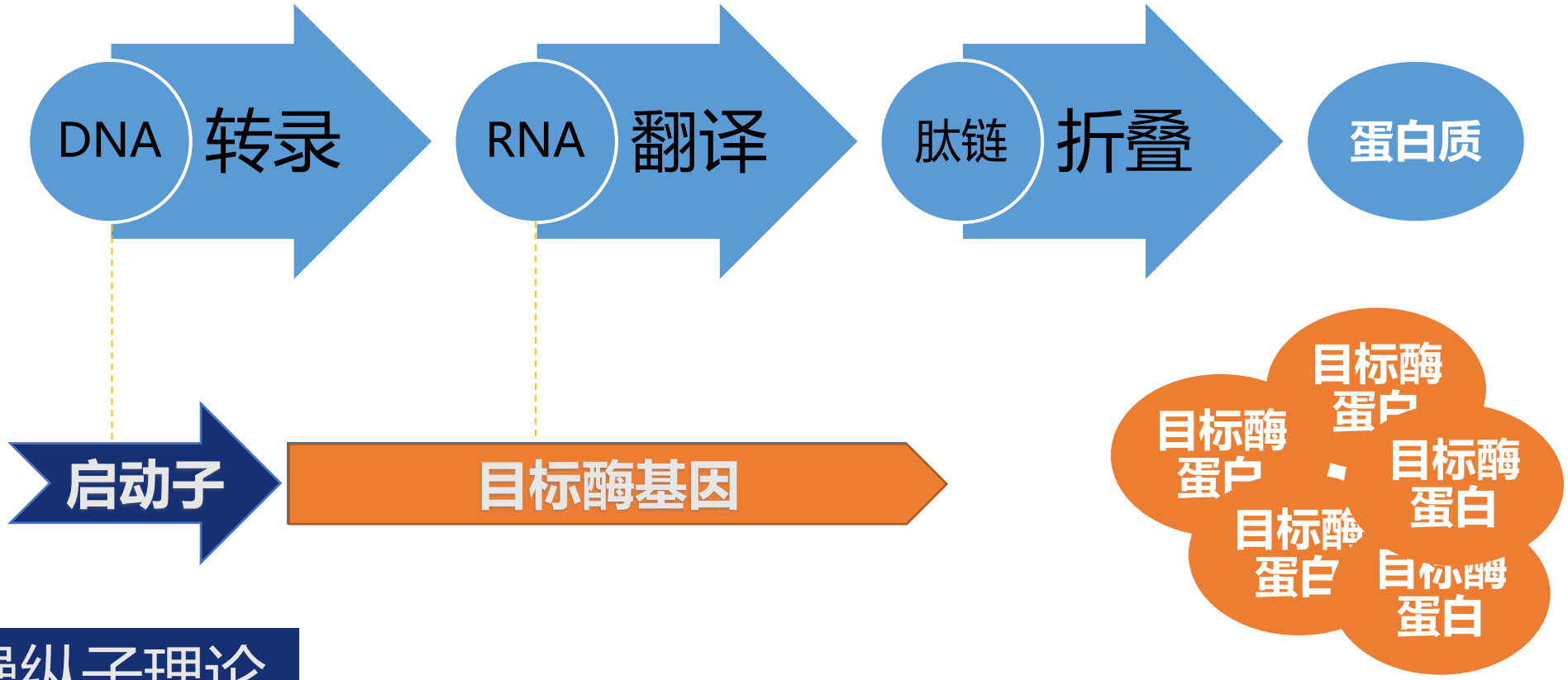
Produced in large quantities

基因工程知识复习

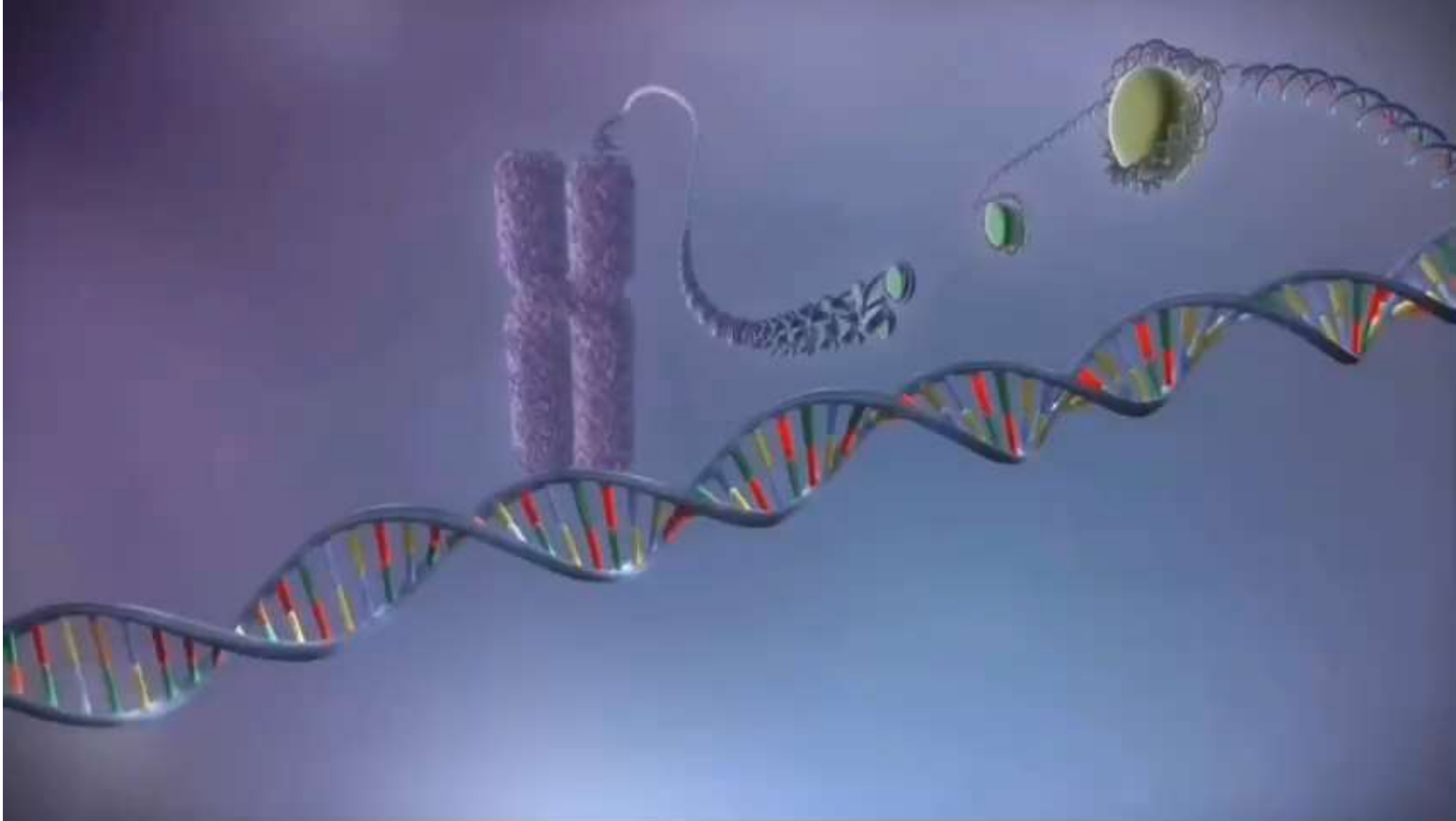
- **理论**
  - 中心法则
  - 操纵子理论
- **工具**
  - PCR
  - 工具酶

基因工程知识复习

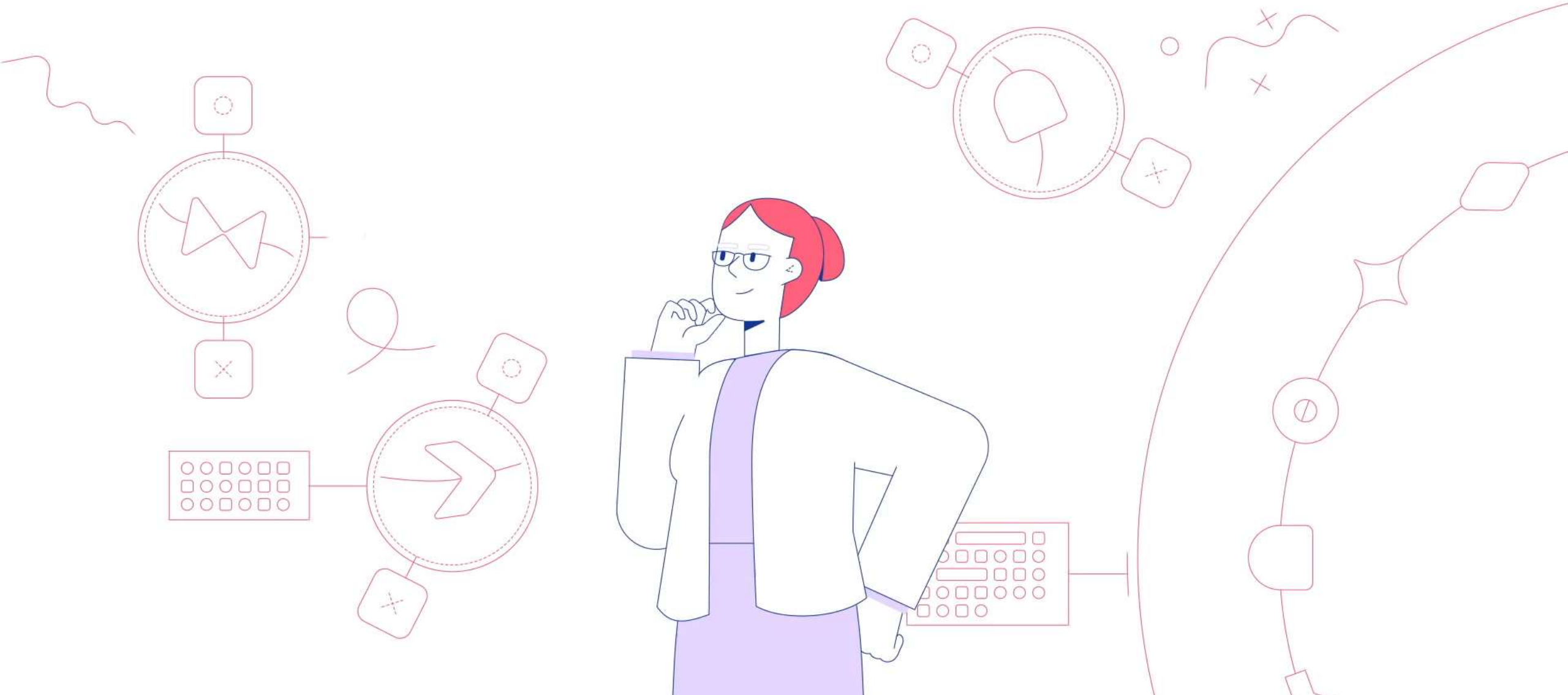
中心法则



操纵子理论



休息10分钟



表达系统选择

重组表达系统

大肠杆菌 (T7, tac/trc, T5, ara …)

芽胞杆菌

巴斯德毕赤酵母 (aox1, gap)

黑曲霉

……

昆虫细胞

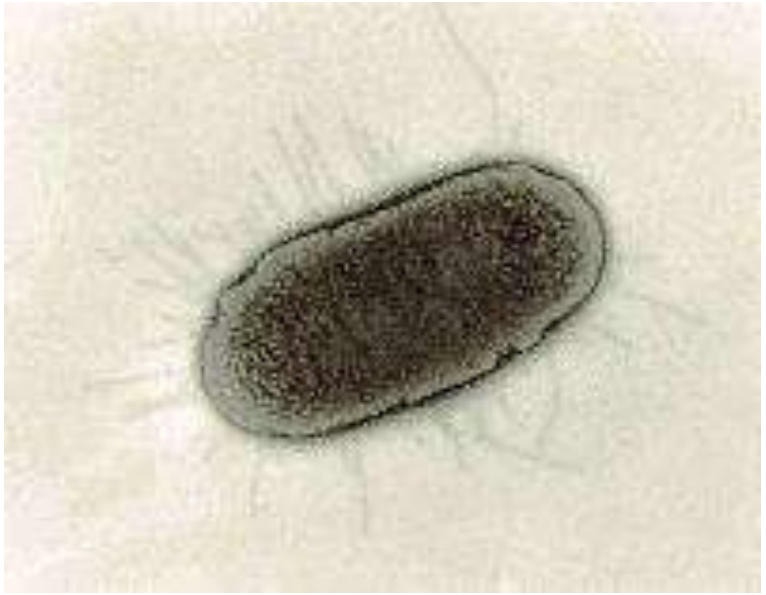
中国仓鼠卵巢细胞

体外翻译系统

考虑因素

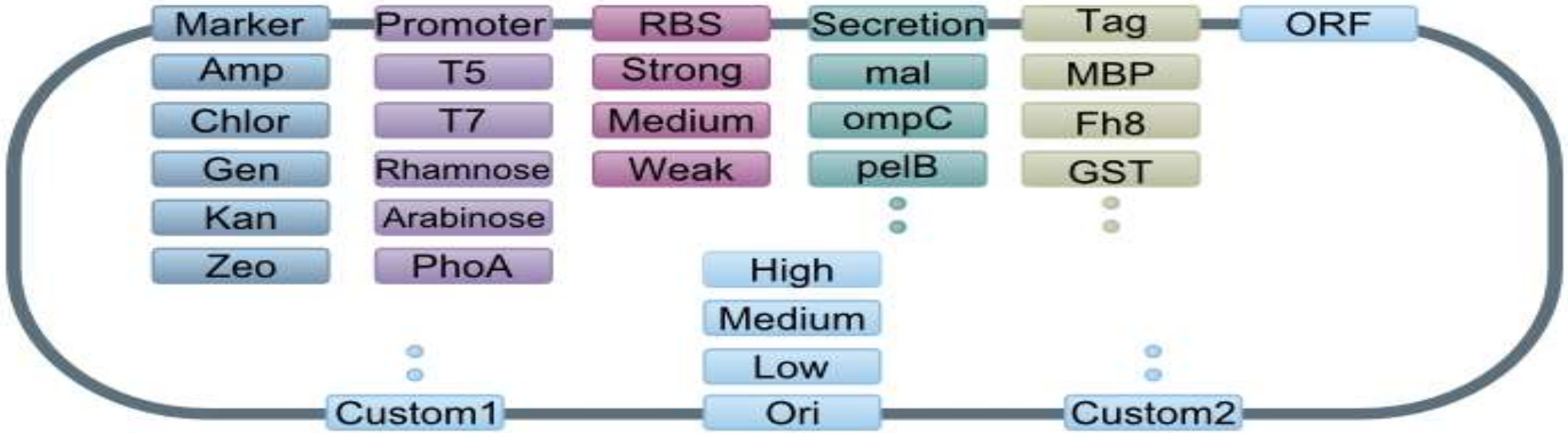
- 蛋白质产量
- 翻译后修饰加工，折叠 (二硫键)和糖基化
- 蛋白质纯化
- 经济性
- 可用设备

首选重组蛋白质表达系统 - - 大肠杆菌



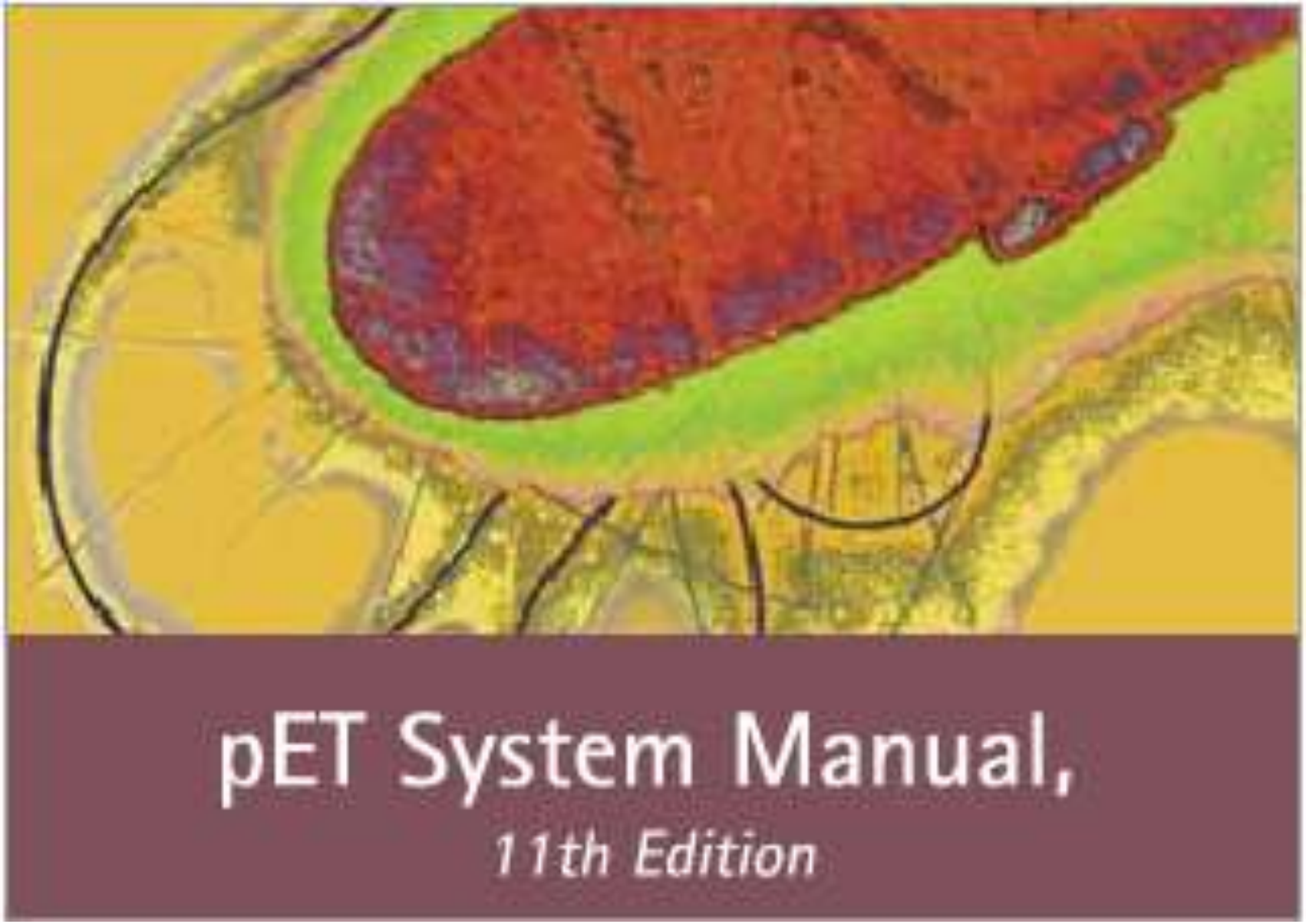
- ✓生长极快
- ✓实验操作最方便
- ✓可高密度发酵
- ✓遗传背景清楚
- ✓容易获得各种载体和宿主

大肠杆菌表达载体

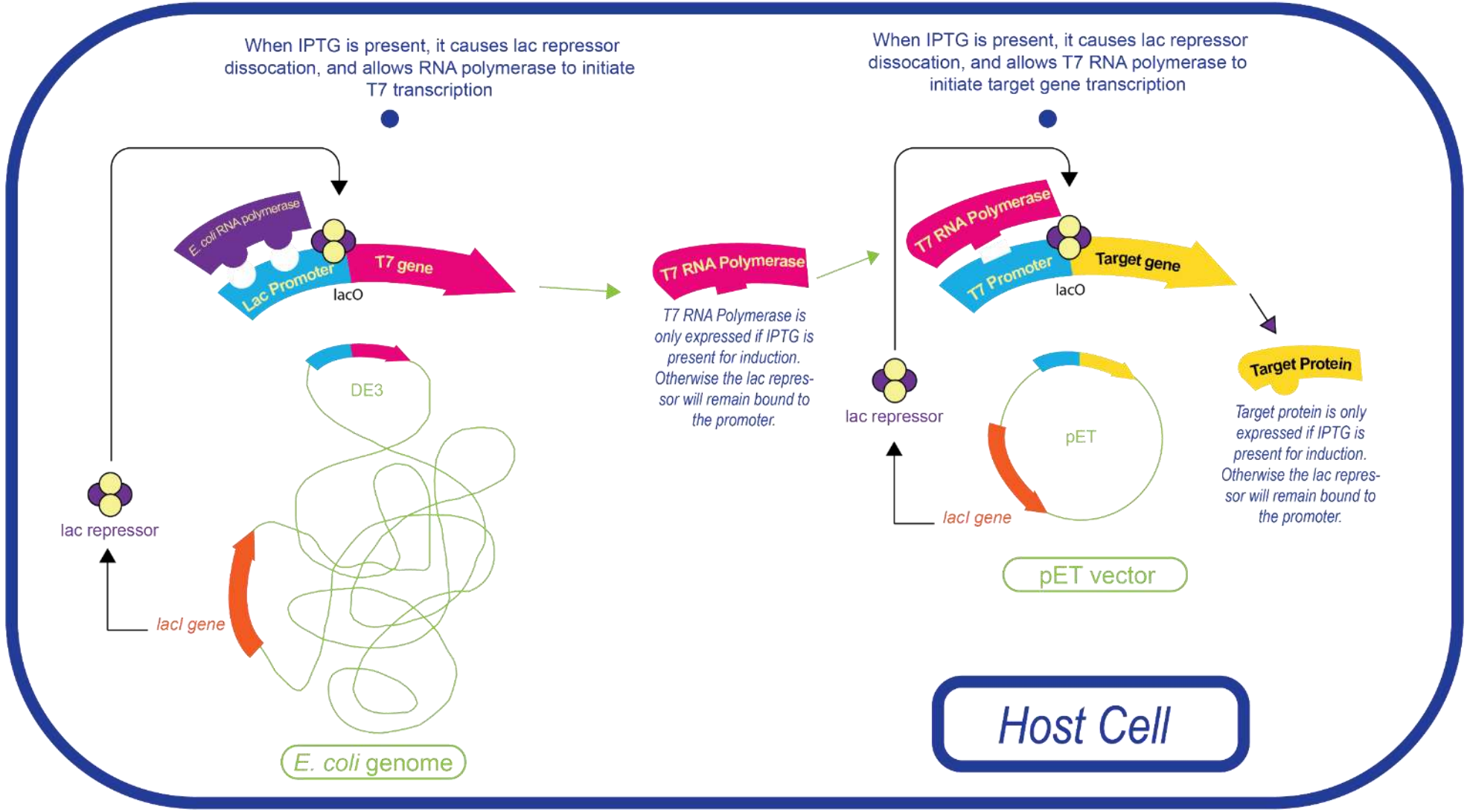


	T5	T7	Rhamnose	Arabinose	PhoA
Expression	Inducible	Inducible	Inducible and Titratable	Inducible and Titratable	Inducible
Expression Tuning	RBS choice	RBS choice	[Rha] and RBS choice	[Ara] and [IPTG]	RBS choice
Localization	Intracellular or Secreted	Intracellular	Intracellular or Secreted	Intracellular	Intracellular
IP	IP-Free	IP-Free	IP-Free	IP-Free	IP-Free

首选中的首选-- pET系统

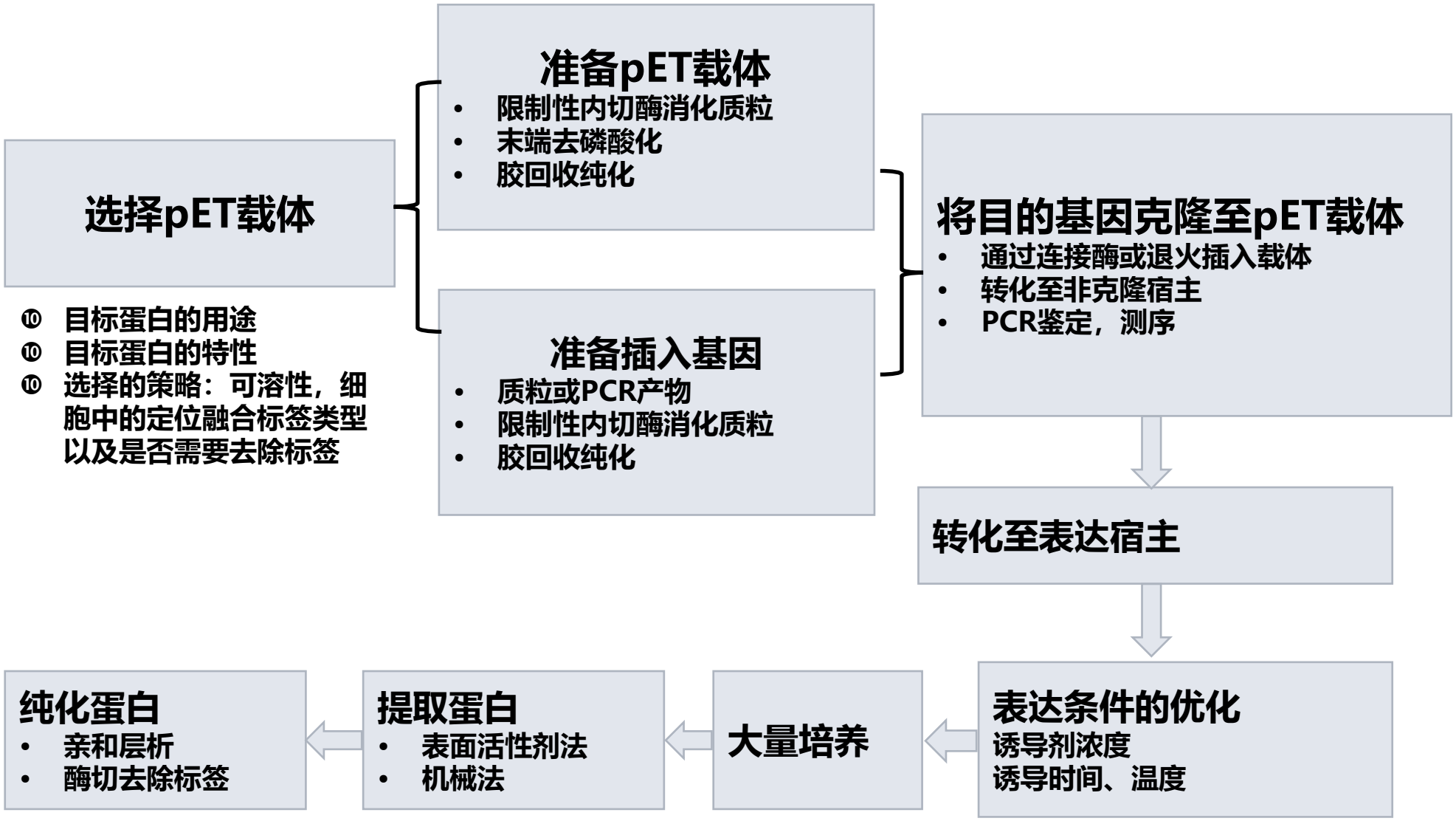


T7表达系统工作原理



https://www.goldbio.com/articles/article/a-deep-dive-into-iptg-induction

pET表达流程

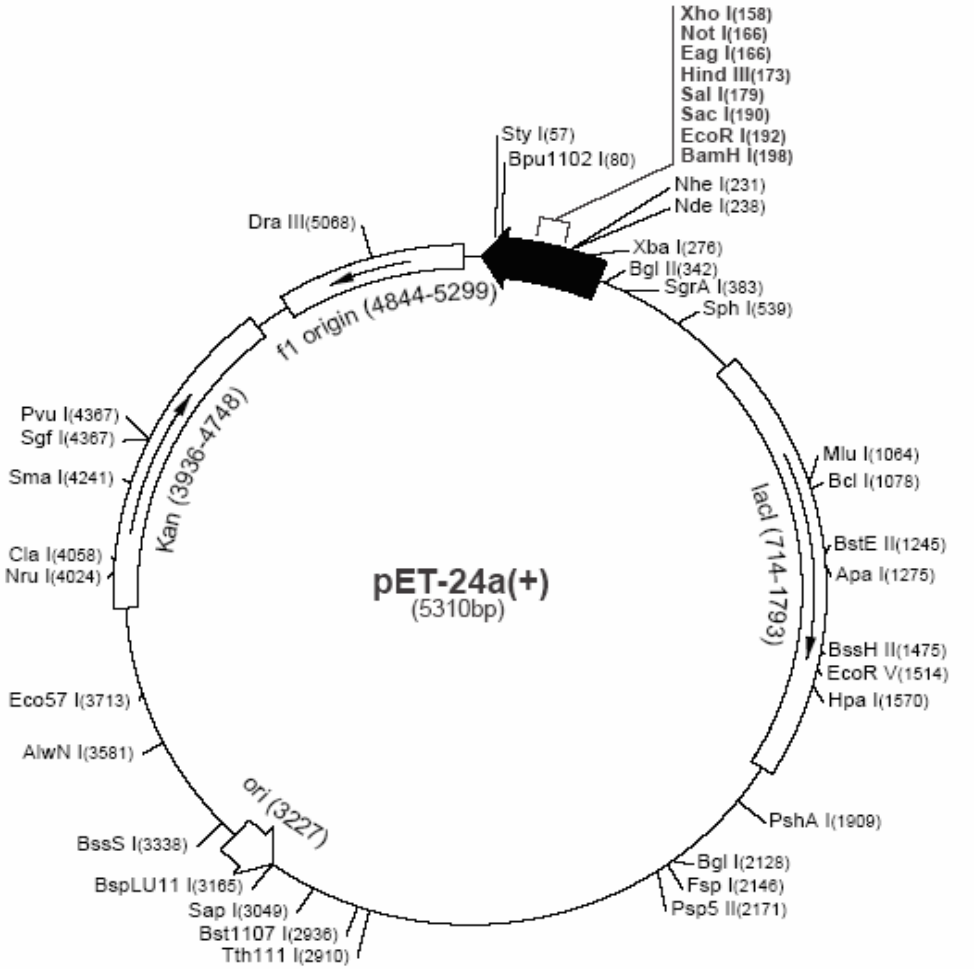

选择pET载体
⑩ 目标蛋白的用途
⑩ 目标蛋白的特性
⑩ 选择的策略：可溶性，细胞中的定位融合标签类型以及是否需要去除标签
准备pET载体
• 限制性内切酶消化质粒
• 末端去磷酸化
• 胶回收纯化
准备插入基因
• 质粒或PCR产物
• 限制性内切酶消化质粒
• 胶回收纯化
将目的基因克隆至pET载体
• 通过连接酶或退火插入载体
• 转化至非克隆宿主
• PCR鉴定，测序
转化至表达宿主
表达条件的优化
诱导剂浓度
诱导时间、温度
大量培养
提取蛋白
• 表面活性剂法
• 机械法
纯化蛋白
• 亲和层析
• 酶切去除标签

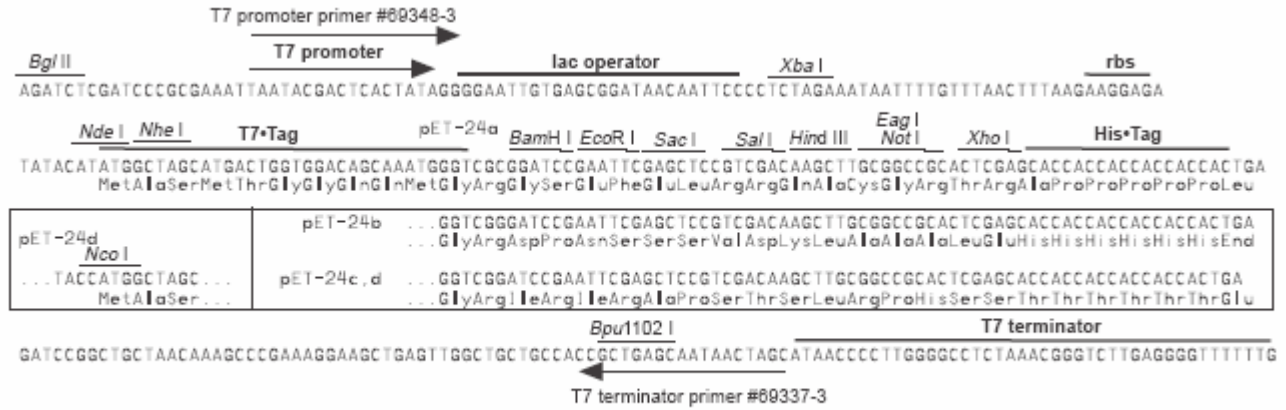
pET 系列载体特性列表

Vector	amp[R]	kan[R]	T7	T7/lac	His•Tag®	T7•Tag®	S•Tag™	Trx•Tag™	KSI	HSV•Tag®	PKA	Dsb•Tag™	GST•Tag™	Nus•Tag™	Strep•Tag® II	protease	signal seq.
pET-3a–d	●		●			N											
pET-9a–d		●	●			N											
pET-11a–d	●			●		N											
pET-14b	●		●		N											T	
pET-15b	●			●	N											T	
pET-16b	●			●	N											X	
pET-17b	●		●			N											
pET-19b	●			●	N											E	
pET-20b(+)	●		●		C												●
pET-21a–d(+)	●			●	C	N											
pET-22b(+)	●			●	C												●
pET-23a–d(+)	●		●		C	N											
pET-24a–d(+)		●		●	C	N											
pET-25b(+)	●			●	C					C							●
pET-26b(+)		●		●	C												●
pET-27b(+)		●		●	C					C							●
pET-28a–c(+)		●		●	N,C	I										T	
pET-29a–c(+)		●		●	C		N									T	
pET-30a–c(+)		●		●	N,C		I									T, E	
pET-30 Ek/LIC		●		●	N,C		I									T, E	
pET-30 Xa/LIC		●		●	N,C		I									T, X	
pET-31b(+)	●			●	C				N								
pET-32a–c(+)	●			●	I,C		I	N								T, E	
pET-32 Ek/LIC	●			●	I,C		I	N								T, E	
pET-32 Xa/LIC	●			●	I,C		I	N								T, X	
pET-33b(+)		●		●	N,C	I					I					T	
pET-39b(+)		●		●	I,C		I					N				T, E	●
pET-40b(+)		●		●	I,C		I					N				T, E	●
pET-41a–c(+)		●		●	I,C		I						N			T, E	
pET-41 Ek/LIC		●		●	I,C		I						N			T, E	
pET-42a–c(+)		●		●	I,C		I						N			T, X	
pET-43.1a–c(+)	●			●	I,C		I			C				N		T, E	
pET-43.1 Ek/LIC	●			●	I,C		I			C				N		T, E	
pET-44a–c(+)	●			●	N,I,C		I			C				I		T, E	
pET-45b(+)	●			●	N		C									E	
pET-46 Ek/LIC	●			●	N		C									E	
pET-47b(+)		●		●	N		C									H, T	
pET-48b(+)		●		●	I		C	N								H, T	
pET-49b(+)		●		●	I		C						N			H, T	
pET-50b(+)		●		●	N,I		C							I		H, T	
pET-51b(+)	●			●	C										N	E	
pET-51 Ek/LIC	●			●	C										N	E	
pET-52b(+)	●			●	C										N	H, T	
pET-52 3C/LIC	●			●	C										N	H, T	

Notes:
C: optional C-terminal tag E: enterokinase H: HRV 3C I: internal tag UC: ligation-independent cloning N: N-terminal tag
signal seq.: signal sequence for potential periplasmic localization T: thrombin X: Factor Xa

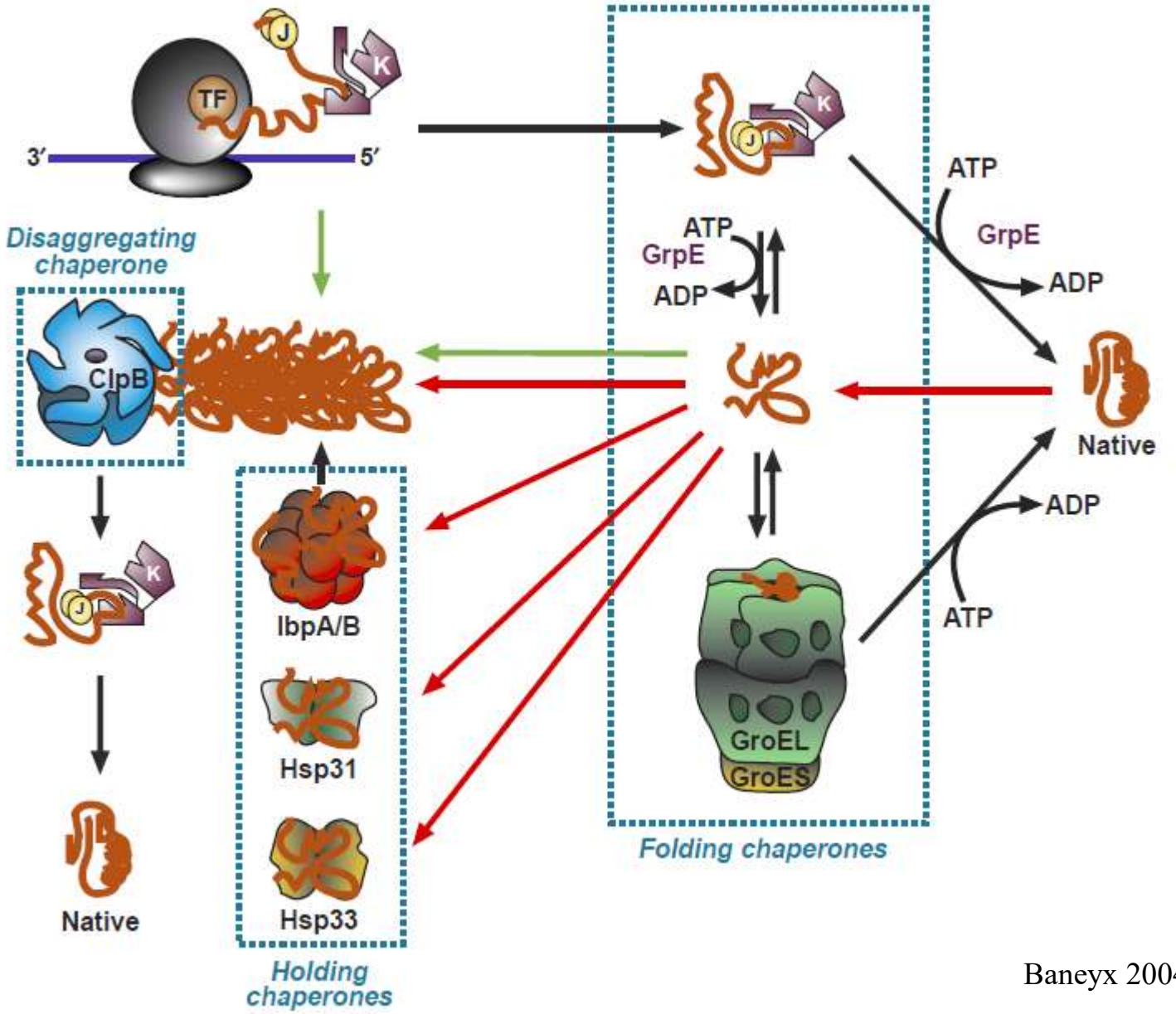
以pET24开始





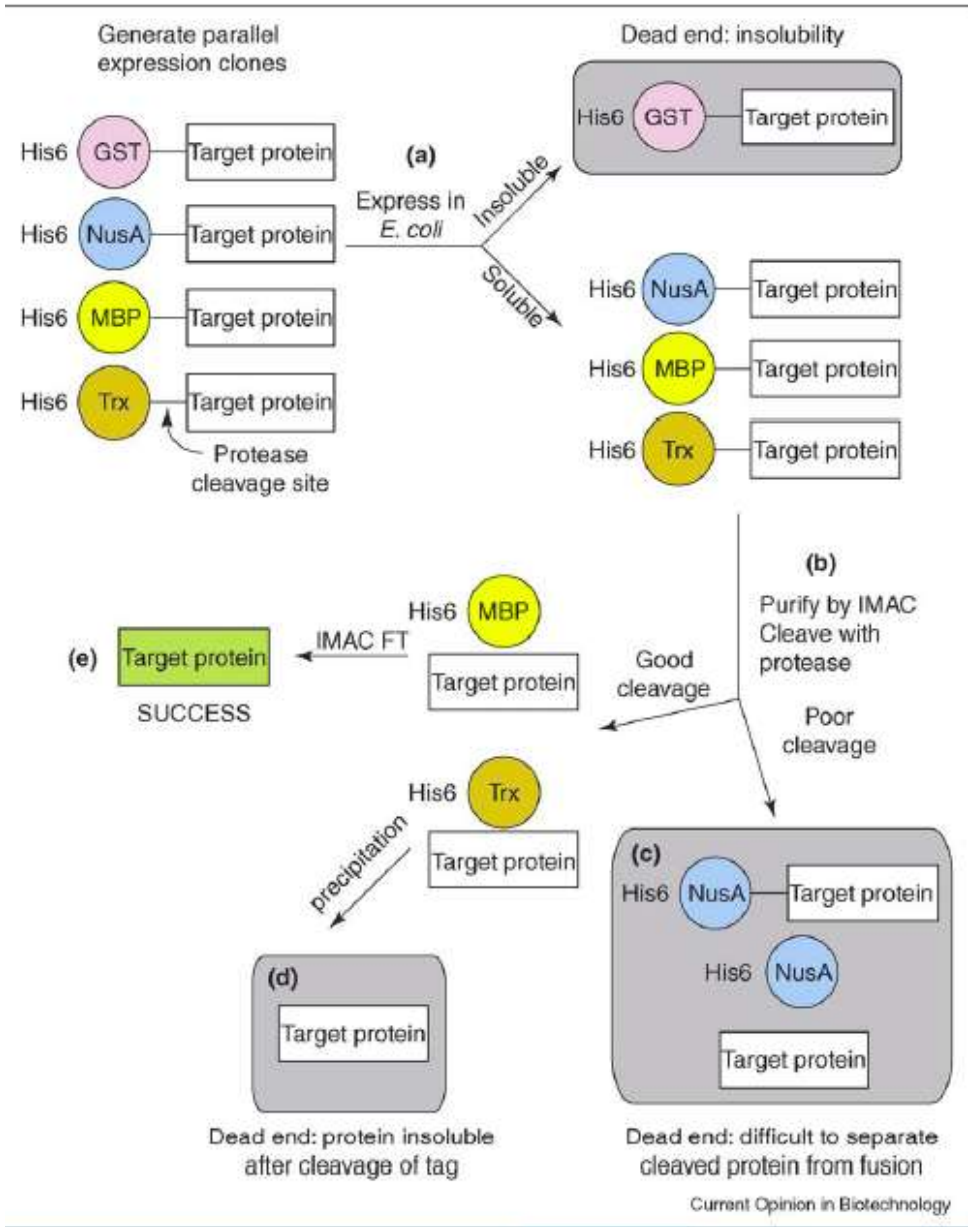
pET-24a-d(+) cloning/expression region

新生肽链的折叠与聚集



Baneyx 2004 Nat Biotechnol

利用增溶标签进行蛋白质重组表达和纯化



Esposito, 2006, Current Opinion in Biotechnology

MBP作为增溶标签的优越性

1. Kataeva I, Chang J, Xu H, Luan CH, Zhou J, Uversky VN, Lin D, Horanyi P, Liu ZJ, Ljungdahl LG et al.: Improving solubility of Shewanella oneidensis MR-1 and Clostridium thermocellum JW-20 proteins expressed into Escherichia coli. J Proteome Res 2005, 4:1942-1951.

   Results from a high-throughput structural genomics project comparing MBP, GST, NusA and His6 solubility tags. The study of 152 proteins from mesophilic and thermophilic bacteria suggested that **MBP was the best tag** for making soluble fusions, and also showed the positive effect of lowered expression temperature on solubility. The authors discuss algorithmsfor predicting the solubility of proteins.

2. Dyson MR, Shadbolt SP, Vincent KJ, Perera RL, McCafferty J: Production of soluble mammalian proteins in Escherichia coli: identification of protein features that correlate with successful expression. BMC Biotechnol 2004, 4:32.

   The authors chose 30 diverse human genes and compared expression with six different N-terminal and eight different C-terminal solubility fusions. Results showed that **MBP and Trx were the best tags** and that MBP also worked as a C-terminal fusion, contrary to previous results. The authors nicely highlight the protein features that seem to correlate with solubility: molecular weight, number of hydrophobic residues, and low complexity regions.

3. Busso D, Delagoutte-Busso B, Moras D: Construction of a set gateway-based destination vectors for high-throughput cloning and expression screening in Escherichia coli. Anal Biochem 2005, 343:313-321.

   This article describes the construction of 10 recombinational cloning vectors and their use for comparing expression and solubility of a small number of genes from Bacillus subtilis. Methods for generating and testing new vectors are clearly discussed and the results of the solubility analysis suggest that **MBP and NusA are much better than GST or Trx** in the case of these partner proteins.

截短表达设计

- **1. Conserved domain search: (for rough boundaries of conserved domains)**
  http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (incorporated in NCBI-blast searches)
  http://www.sanger.ac.uk/Software/Pfam/
  http://smart.embl-heidelberg.de/
- **2. 3D structure prediction from structures of homologous proteins in the PDB:**
  http://www.rcsb.org/pdb/
  http://bioinf.cs.ucl.ac.uk/psipred/ (mGenTHREADER)
- **3. Secondary structure prediction (if there is no structural homologue):**
  http://bioinf.cs.ucl.ac.uk/psipred/ (PSIPRED)
  http://www.compbio.dundee.ac.uk/~www-jpred/submit.html
- **4. Transmembrane helix prediction:**
  http://www.ch.embnet.org/software/TMPRED_form.html
- **5. Predicting unfolded regions:**
  http://bioinf.cs.ucl.ac.uk/disopred/
  http://bioportal.weizmann.ac.il/fldbin/findex
  http://www.strubi.ox.ac.uk/RONN

大肠杆菌表达实验策略（学术版）

✓**首选pET24或pET28（如果需要纯化）与BL21(DE3)**

✓**TB培养基37°生长到1-1.5OD，18 ℃生长1小时到3OD ，0.5mM IPTG诱导19小时至OD达10**

- **高表达低可溶的解救措施→**

1. 降温低至15℃
2. 换培养基为2xYT或ZYP5052(自诱导)，换表达宿主
3. 截短N端和/或C端2-10个氨基酸残基
4. 与MBP等高可溶性蛋白融合表达
5. 化学诱导分子伴侣、共表达分子伴侣/作用蛋白或提供配体

Graslund et al 2008 Nature Methods

大肠杆菌表达系统小结

优点: 最方便，最有效

缺点: 分泌表达能力弱

二硫键形成困难

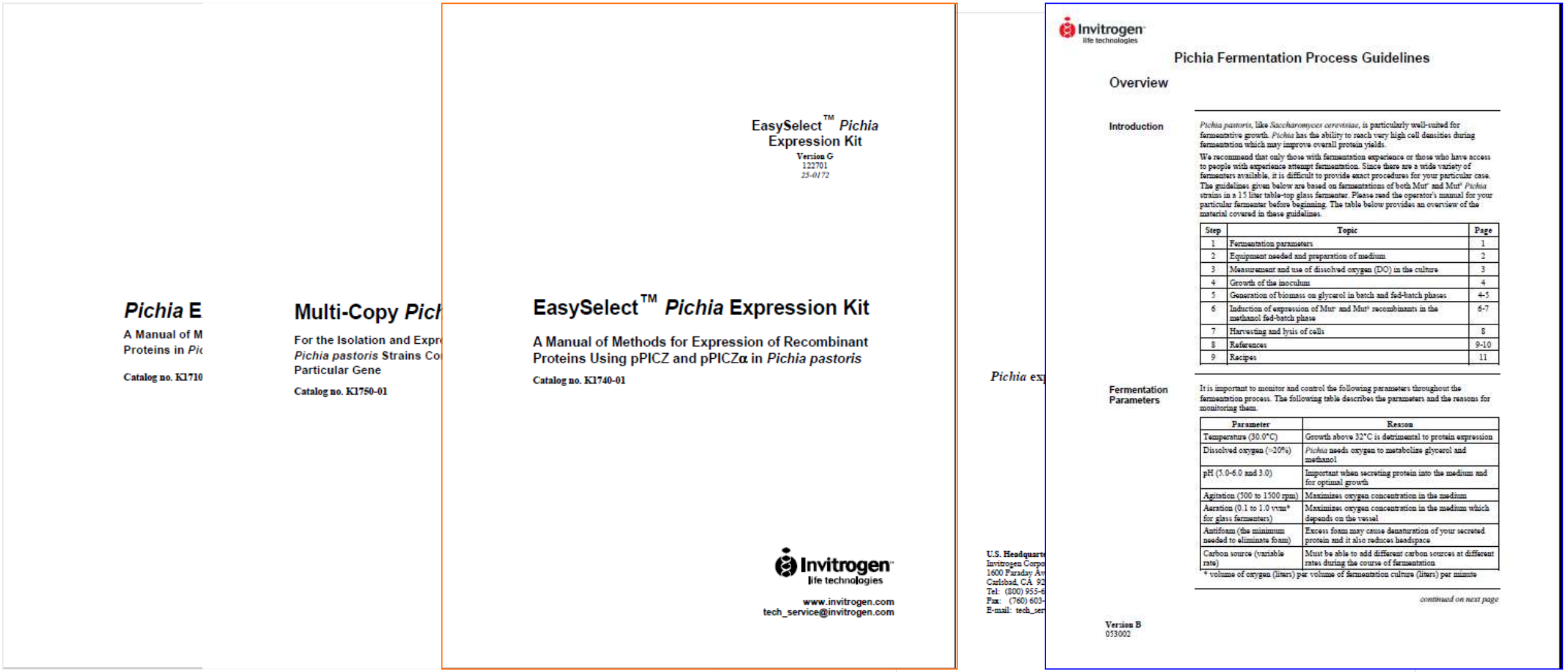
无翻译后修饰

酵母表达系统 (Invitrogen)

Table 1 - Yeast Expression Systems

System/ Vector	Host	Secretion Signal	Fusion Partner: Position	Fusion Partner: Purif.	Fusion Partner: Epitope	Selectable Marker	Promoter	Inducer	Advantage
Pichia Expression System	*P. pastoris*	α-factor or *PHO*	C-term.	6xHis	*c-myc*	*HIS4*, Zeocin™, or Blasticidin	*AOX1* or *GAP*	methanol (*GAP* is constitutive)	High-level expression, copy number control, suitable for industrial-scale protein production
	P. methanolica	α-factor	C-term.	6xHis	V5	*ADE2*	*AUG1*	methanol	High-level expression
YES™ Vector Collection	*S. cerevisiae*	—	N-term. C-term.	6xHis	Xpress™ V5	*URA3* or blasticidin	*GAL1*	galactose	High- or low-copy episomal expression
pTEF1/Zeo pTEF1/Bsd	*S. cerevisiae* *P. pastoris* *P. methanolica*	—	—	—	—	Zeocin™ *or* Blasticidin	*TEF1*	—	Simplified construction of Zeocin™- or Blasticidin-resistant vectors in yeast
SpECTRA™ *S. pombe* Expression System	*S. pombe*	—	C-term.	6xHis	V5	*LEU2*	*nmt1* *nmt41* *nmt81*	thiamine	Flexible control of expression levels in *S. pombe*
pYD1	*S. cerevisiae*	*AGA2*	N-term. C-term.	6xHis	Xpress™ V5	*TRP1*	*GAL1*	galactose	Displays protein on cell surface

Pichia Expression Kits



Pichia E

A Manual of M
Proteins in *Pic*

Catalog no. K1710

Multi-Copy *Pich*

For the Isolation and Expr
Pichia pastoris Strains Co
Particular Gene

Catalog no. K1750-01

EasySelect™ *Pichia*
Expression Kit
Version G
122701
25-0172

EasySelect™ *Pichia* Expression Kit

A Manual of Methods for Expression of Recombinant Proteins Using pPICZ and pPICZα in *Pichia pastoris*

Catalog no. K1740-01

Invitrogen
life technologies

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Pichia ex

U.S. Headquart
Invitrogen Corpo
1600 Faraday Av
Carlsbad, CA 92
Tel: (800) 955-6
Fax: (760) 603-
E-mail: tech_ser

Invitrogen
life technologies

Pichia Fermentation Process Guidelines

Overview

Introduction

Pichia pastoris, like *Saccharomyces cerevisiae*, is particularly well-suited for fermentative growth. *Pichia* has the ability to reach very high cell densities during fermentation which may improve overall protein yields.

We recommend that only those with fermentation experience or those who have access to people with experience attempt fermentation. Since there are a wide variety of fermenters available, it is difficult to provide exact procedures for your particular case. The guidelines given below are based on fermentations of both Mut⁺ and Mut⁸ *Pichia* strains in a 15 liter table-top glass fermenter. Please read the operator's manual for your particular fermenter before beginning. The table below provides an overview of the material covered in these guidelines.

Fermentation Parameters

It is important to monitor and control the following parameters throughout the fermentation process. The following table describes the parameters and the reasons for monitoring them.

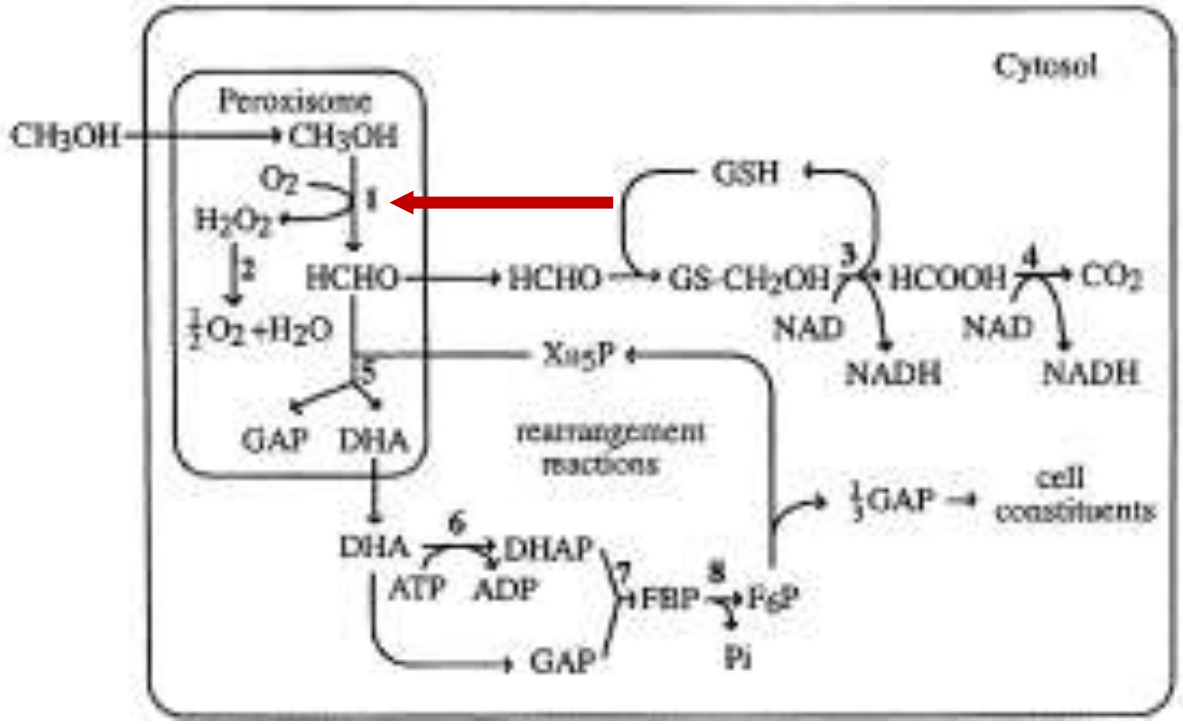
Parameter	Reason
Temperature (30.0°C)	Growth above 32°C is detrimental to protein expression
Dissolved oxygen (>20%)	*Pichia* needs oxygen to metabolize glycerol and methanol
pH (5.0-6.0 and 3.0)	Important when secreting protein into the medium and for optimal growth
Agitation (300 to 1500 rpm)	Maximizes oxygen concentration in the medium
Aeration (0.1 to 1.0 vvm* for glass fermenters)	Maximizes oxygen concentration in the medium which depends on the vessel
Antifoam (the minimum needed to eliminate foam)	Excess foam may cause denaturation of your secreted protein and it also reduces headspace
Carbon source (variable rate)	Must be able to add different carbon sources at different rates during the course of fermentation

* volume of oxygen (liters) per volume of fermentation culture (liters) per minute

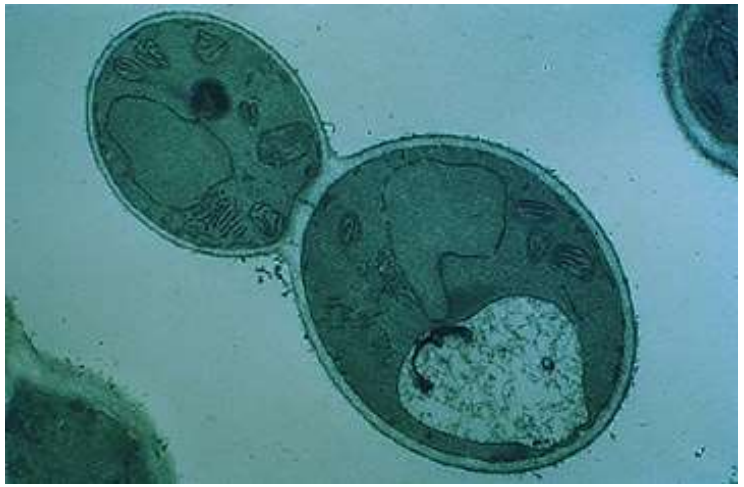
continued on next page

Version B
053002

Pichia pastoris 简介



无快速碳源而含有甲醇，产生醇氧化酶能占到细胞总蛋白的30%



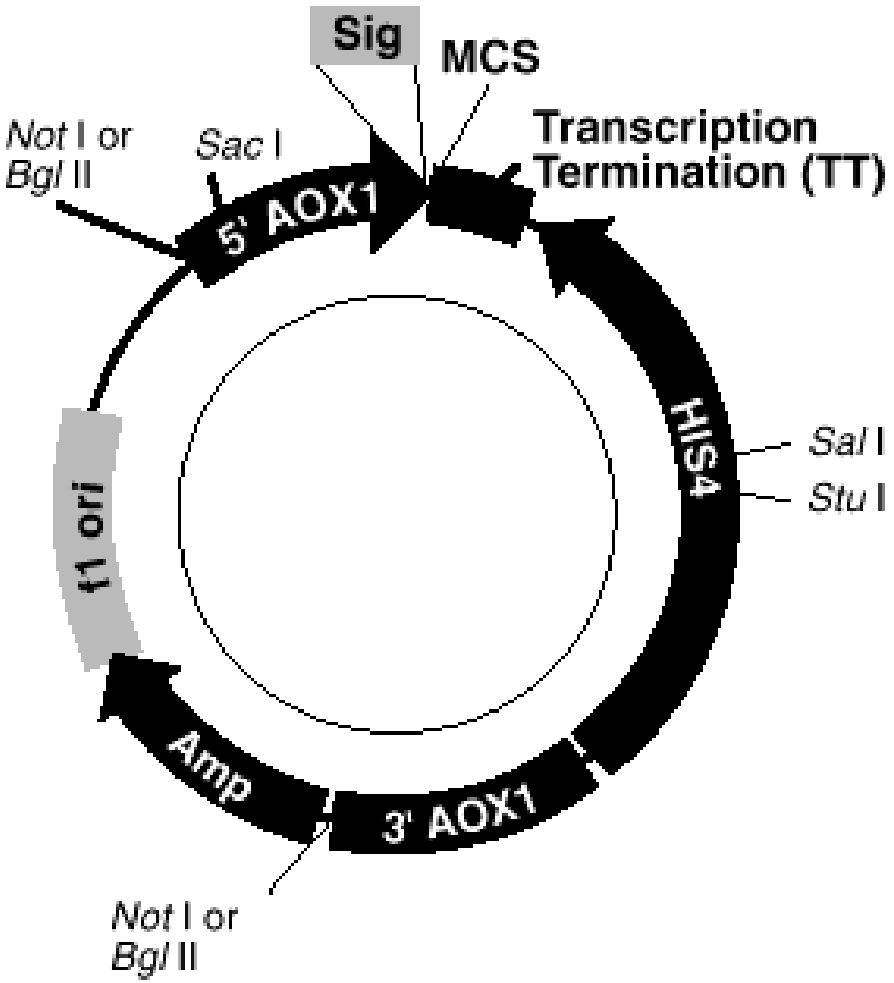
醇氧化酶的催化场所——过氧化物酶体可占到细胞总体积的80%

Budding *Pichia pastoris*

*Pichia pastoris*表达体系的优点

- 外源基因**稳定**整合
- 醇氧化酶基因的启动子强，且可用甲醇**严格调控**表达
- 重组蛋白质可以以**胞内或胞外**形式表达
- 含有真核表达系统共有的**翻译后修饰**功能
- 有**商品化**宿主/载体，操作简便
- 放大方便，发酵**密度极高**

Typical *Pichia pastoris* expression vector



- **5'*AOX1*: AOX1 5' region including promoter**
- **MCS: Multiple cloning site**
- **TT: *AOX1* terminator**
- ***HIS4*: histidinol dehydrogenase**
- **3'*AOX1*: AOX1 3' region**
- **Amp: Ampicillin-resistance marker**
- **fi ori: f1 bacteriophage origin.**
- **Sig: signal sequences**
- ***Sac*I, *Sal*I, *Stu*I and *Bgl*II cutting site are used to linearize the vector before transformation**

Promotor

- *aox1*启动子
  - 强启动子
  - 甲醇诱导
  - 葡萄糖，甘油等快速碳源阻遏
  - 表达调控技术较成熟

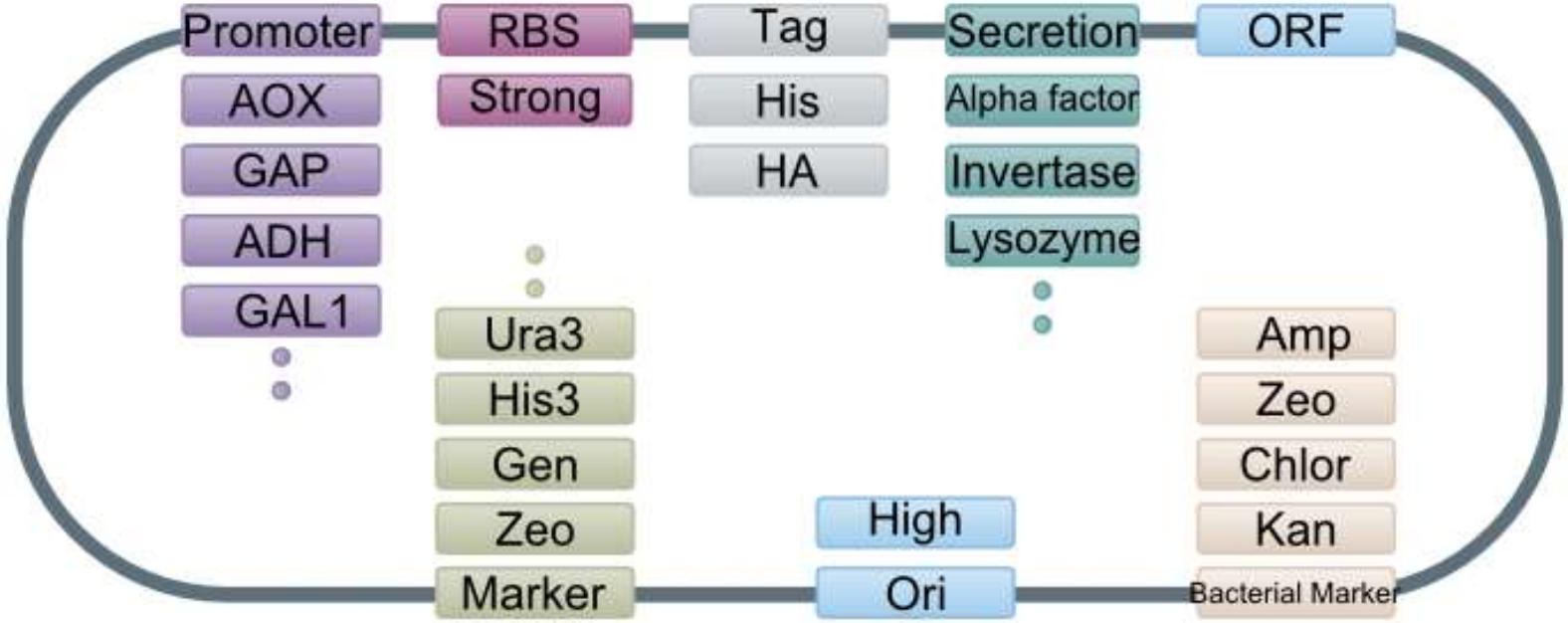
*gap*启动子

- 组成型强启动子
- 葡萄糖　　强启动子
- 甘油　　中等强启动子
- 甲醇　　弱启动子

Pichia pastoris strains

Strain	Genotype	Application
X33	Wild-type	Selection of Zeocin-resistant expression vectors
GS115	*his4*	Selection of *HIS4* expression vectors
KM71	*his4, aox1::ARG4;arg4*	Selection of *HIS4* expression vectors to generate strains with MutS phenotype
KM71H	*aox1::ARG4;arg4*	Selection of Zeocin-resistant expression vectors to generate strains with MutS phenotype
SMD1165	*his4prB1*	Selection of *HIS4* expression vectors to generate strains without protein B activity
SMD1168	*his4pep4*	Selection of *HIS4* expression vectors to generate strains without protease A activity
SMD1163	*his4pep4prB1*	Selection of *HIS4* expression vectors to generate strains without protease A and protease B activity
SMD1168H	*pep4*	Selection of Zeocin-resistant expression vectors to generate strains without protease A

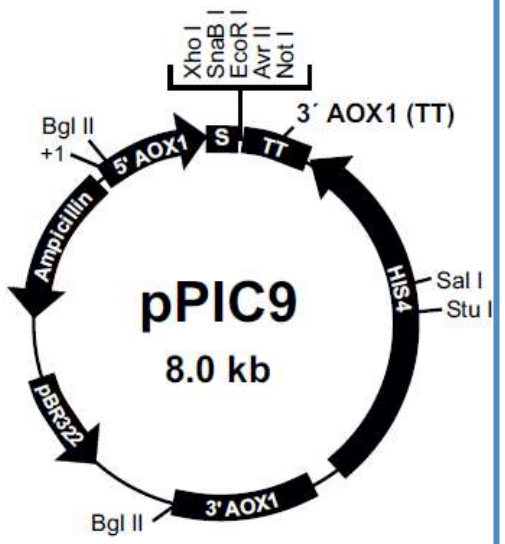
*Pichia*表达载体选择



Function	Pichia	*S. cerevisiae*
Expression	Inducible or Constitutive	Inducible or Constitutive
Propagation	Targeted genomic integration	Yeast self-replicating (2μ) or Targeted integration
Localization	Cytoplasmic or Secreted	Cytoplasmic or Secreted
Integration Protocol	Easy, requires linearization	Easy
Strains	Yes	No
IP	IP-Free	IP-Free

*Pichia pastoris*表达实验流程

外源基因克隆到pPIC9的alpha信号肽序列之后



电转化GS115

初筛
(50ml离心管)

有表达

复筛(250ml摇瓶) 发酵罐

发酵罐

无表达

以pPIC3.5K尝试胞内表达

*Pichia pastoris*表达系统小结

- 优点
  - 有商品化宿主/载体，操作简便
  - 真核表达系统
  - 重组蛋白质可以以胞内或胞外形式表达
  - 高密度发酵方便
  - 有翻译后加工

- 缺点
  - 翻译后加工形式有独特性
  - 存在过度糖基化问题

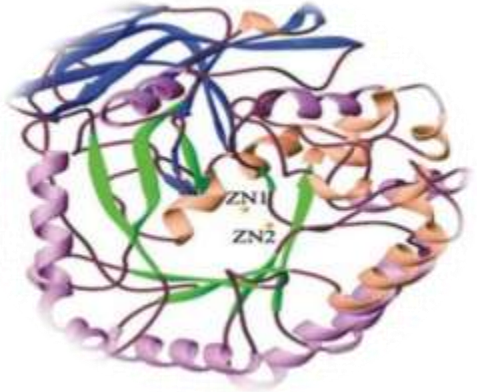
基础篇 （重组酶表达）小结

- 首选文献报道表达系统
- 次选大肠杆菌
- 大肠杆菌中无活性→选用酵母系统
- 根据基因来源定表达系统
  - 芽孢杆菌
  - 放线菌
  - 酵母
  - 霉菌
- 基因加亲和标签便于纯化

获得新酶

新酶

有理



天然

从头

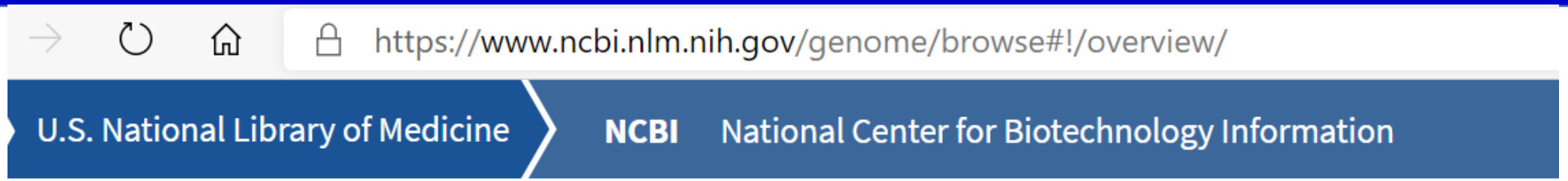
无理

基于功能--生物多样性与新酶的发现

- 嗜热菌（Thermophiles）
- 嗜冷菌（Phyphophiles）
- 嗜酸菌（Acidophiles）
- 嗜碱菌（Alkaliphiles）
- 嗜盐菌（Halophiles）
- 嗜压菌（Barophiles）

极端微生物的特殊生长环境及代谢产物，使酶在“恶劣”环境下能够发挥高效催化作用。

基于序列



Protein Sequence

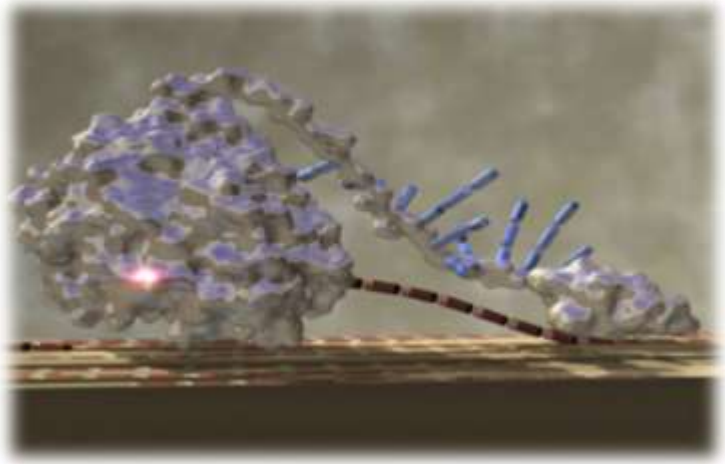
SQETRKKCTEMKKKFKNCEVRCDESNHCVEVRCSDTKYTLC

MSA

MSA: Multiple Sequence Alignment 多重序列联配

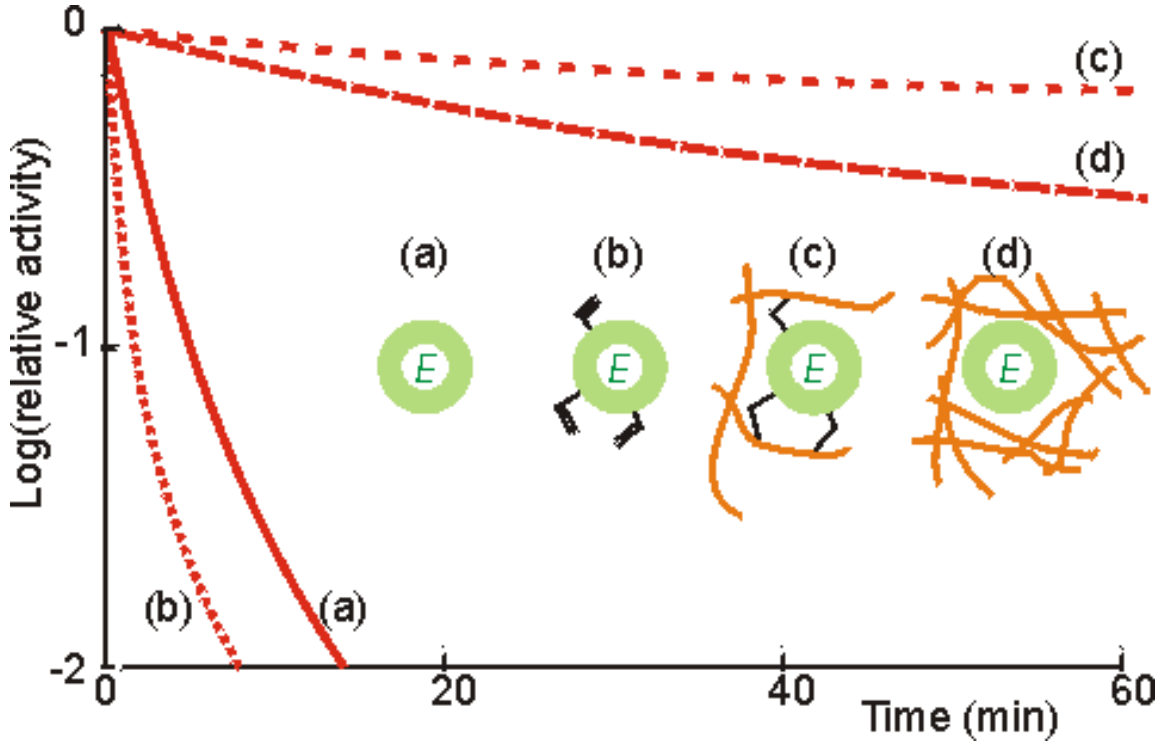
天然酶

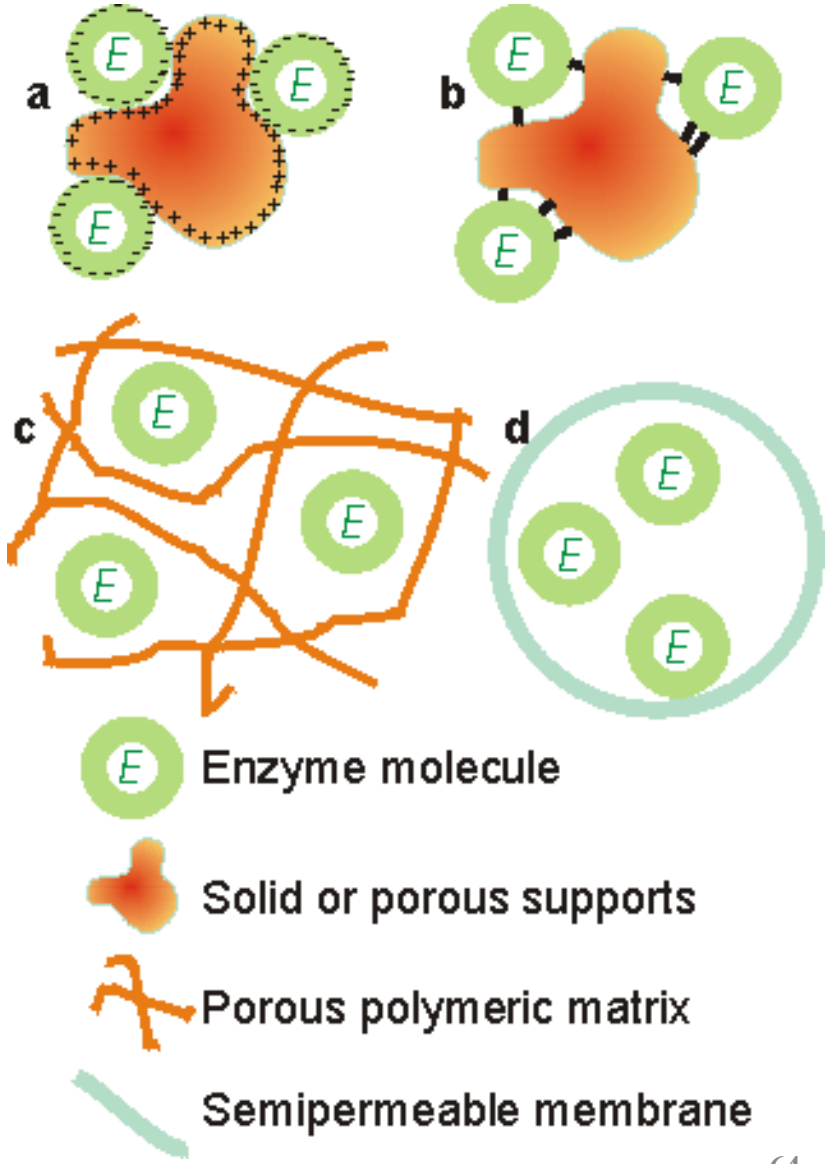
- 天然酶的结构与功能是自然进化结果



- 化学 - 天然底物
- 物理 - 常温
- 生物 - 受调控

酶的改造

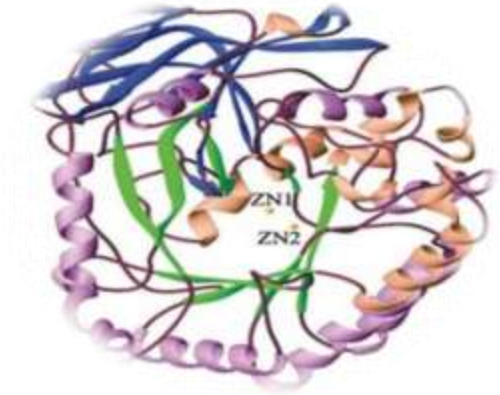

0
-1
-2
Log(relative activity)
0
20
40
60
Time (min)
(a)
(b)
(c)
(d)
E


a
b
c
d
E
Enzyme molecule
Solid or porous supports
Porous polymeric matrix
Semipermeable membrane

酶的改造

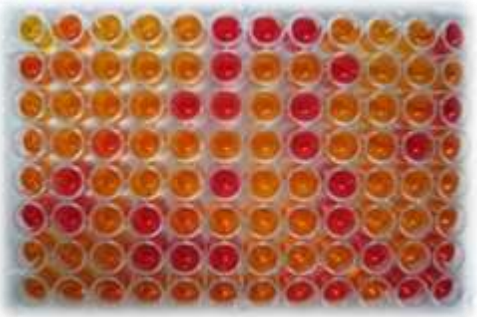
有理设计

- 基于蛋白质结构与功能信息对编码基因改变并重组表达测试



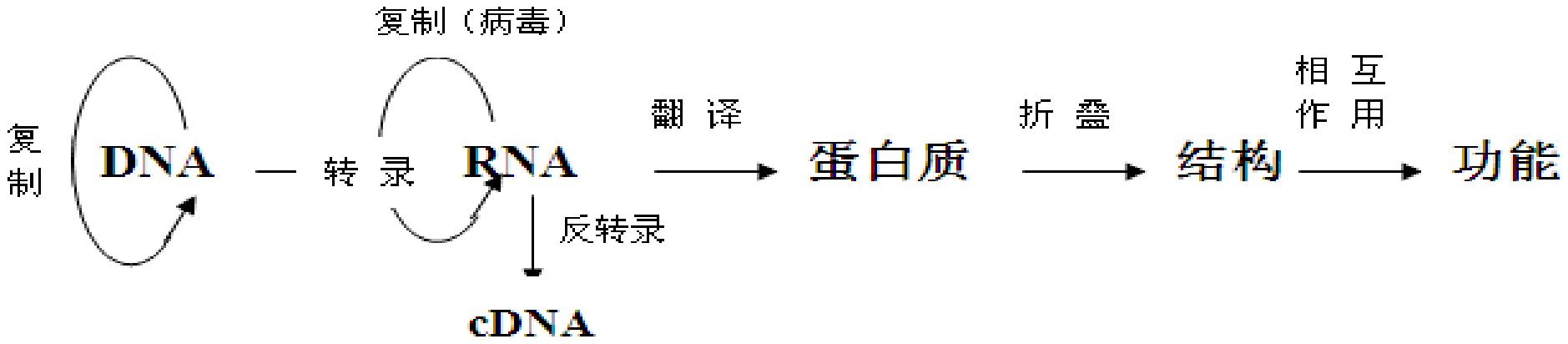
无理设计

- 高频突变或重组编码序列并重组表达并高通量测试



分子生物学知识复习

中心法则



DNA序列→蛋白质序列→立体结构→功能

蛋白质功能 = f(蛋白质序列)

改变序列影响蛋白质功能

蛋白质序列空间

n个氨基酸残基组成的线性长链的序列空间为20^n

$$\sum_{a}^{b} 20^n = 20^a + 20^{a+1} + \ldots + 20^{b-1} + 20^b$$

a表示最小蛋白质的氨基酸残基数，b表示最大蛋白质的氨基酸残基数。

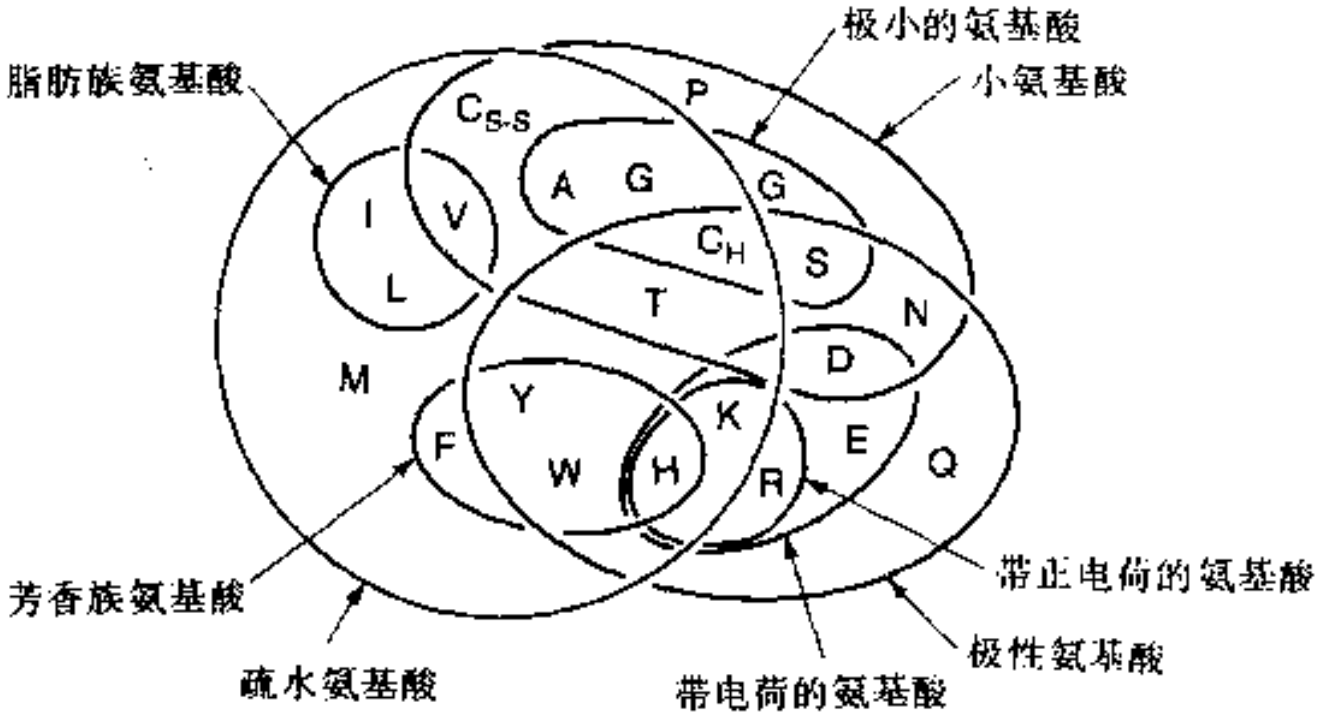
所有蛋白质都可以表示为这个b维空间的唯一一点，比如枯草芽孢杆菌蛋白酶 BPN’坐标为（A,Q,S,V,P,Y,G,V,S,Q,I,K,A,…, A,Q,0,…,0,0,0）。

小至100个氨基酸残基组成的酶的序列空间也大至20^{100}

序列空间→蛋白质特性 序列空间距离近→蛋白质性质接近

PAM250氨基酸相对突变率矩阵和基本氨基酸性质关系venn图

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2																			
R	-2	6																		
N	0	0	2																	
D	0	-1	2	4																
C	-2	-4	-4	-5	4															
Q	0	1	1	2	-5	4														
E	0	-1	1	3	-5	2	4													
G	1	-3	0	1	-3	-1	0	5												
H	-1	2	2	1	-3	3	1	-2	6											
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5										
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6									
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5								
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6							
F	-4	-4	-4	-6	-4	-5	-5	-5	-2	1	2	-5	0	9						
P	1	0	-1	-1	-3	0	-1	-1	0	-2	-3	-1	-2	-5	6					
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	3				
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-2	0	1	3			
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17		
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	2	4



有理设计

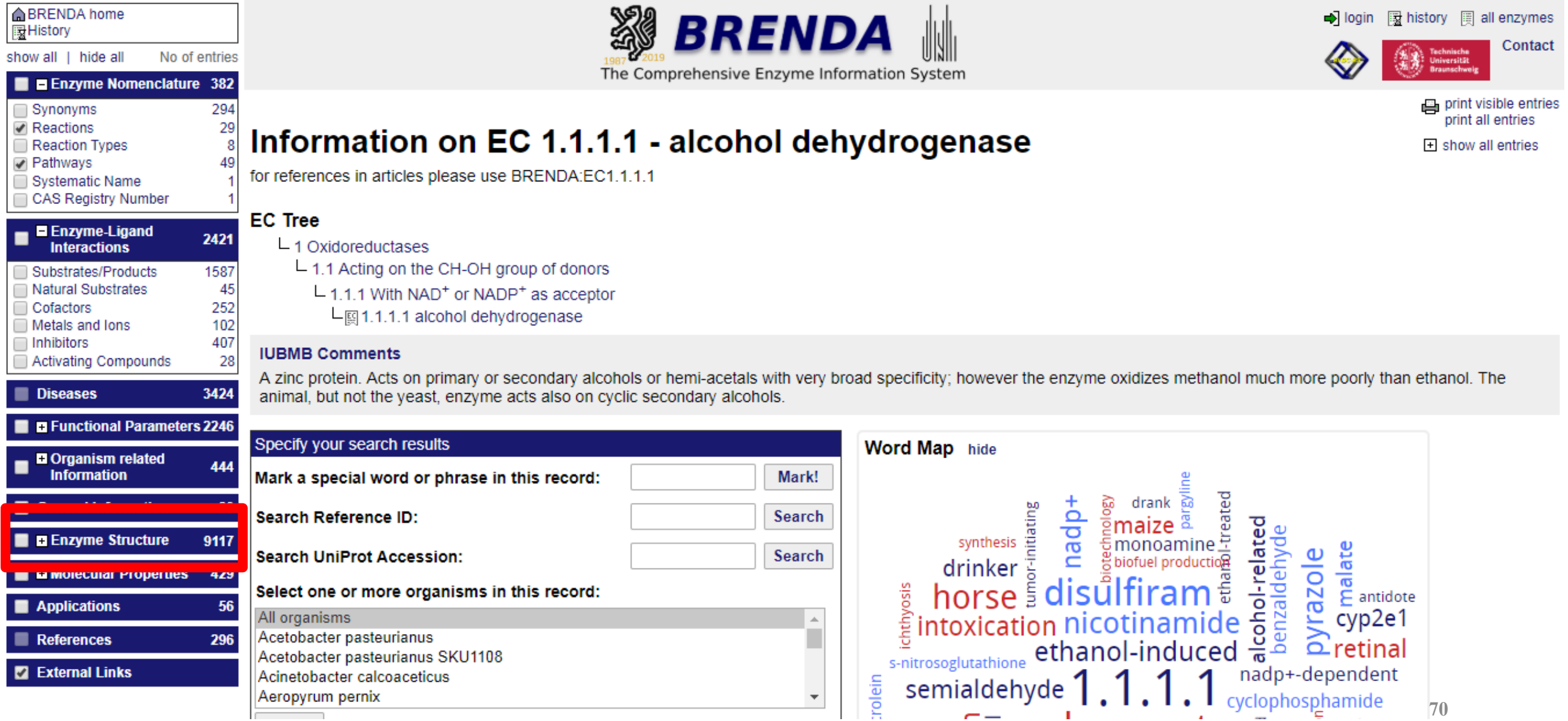
根据详尽的结构功能关系知识

功能	
活性位点	氨基酸残基和辅因子
催化	机理, 特异性和动力学
调节	竞争性抑制和变构控制

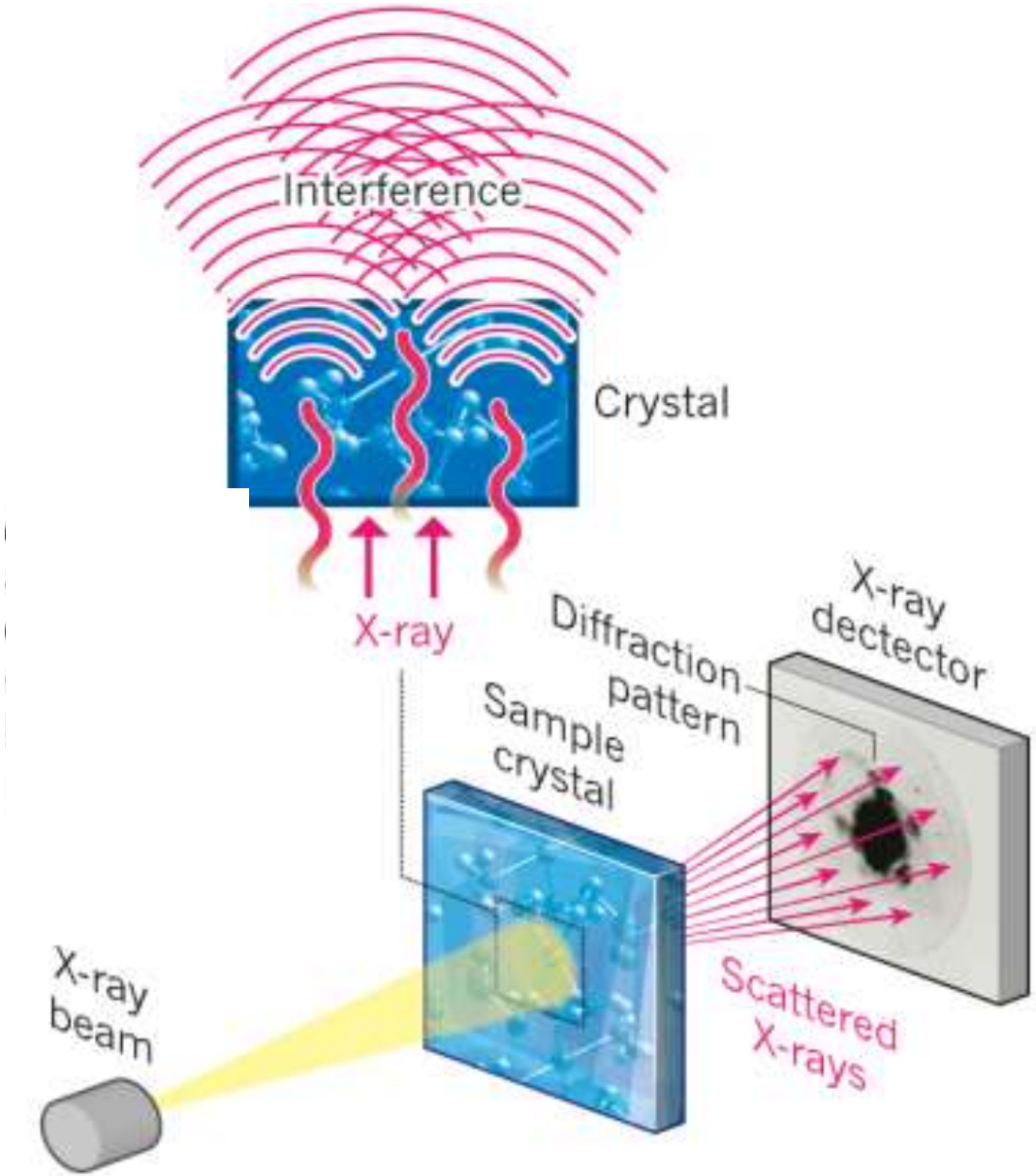
结构	
一级结构	氨基酸顺序
二级结构	a-螺旋, b-折叠, 转角, 无规卷曲
三级结构	原子坐标

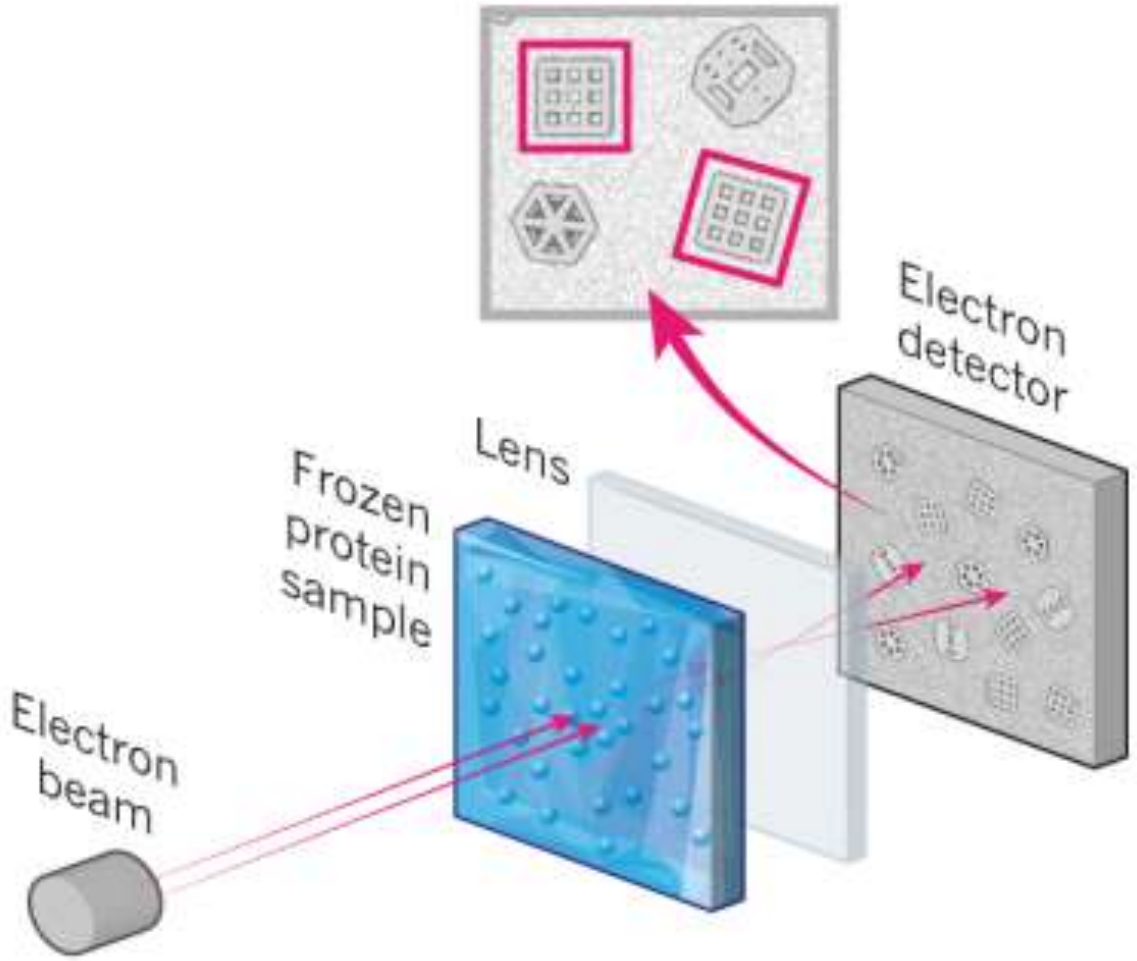
怎么获得这些信息?

BRENDA

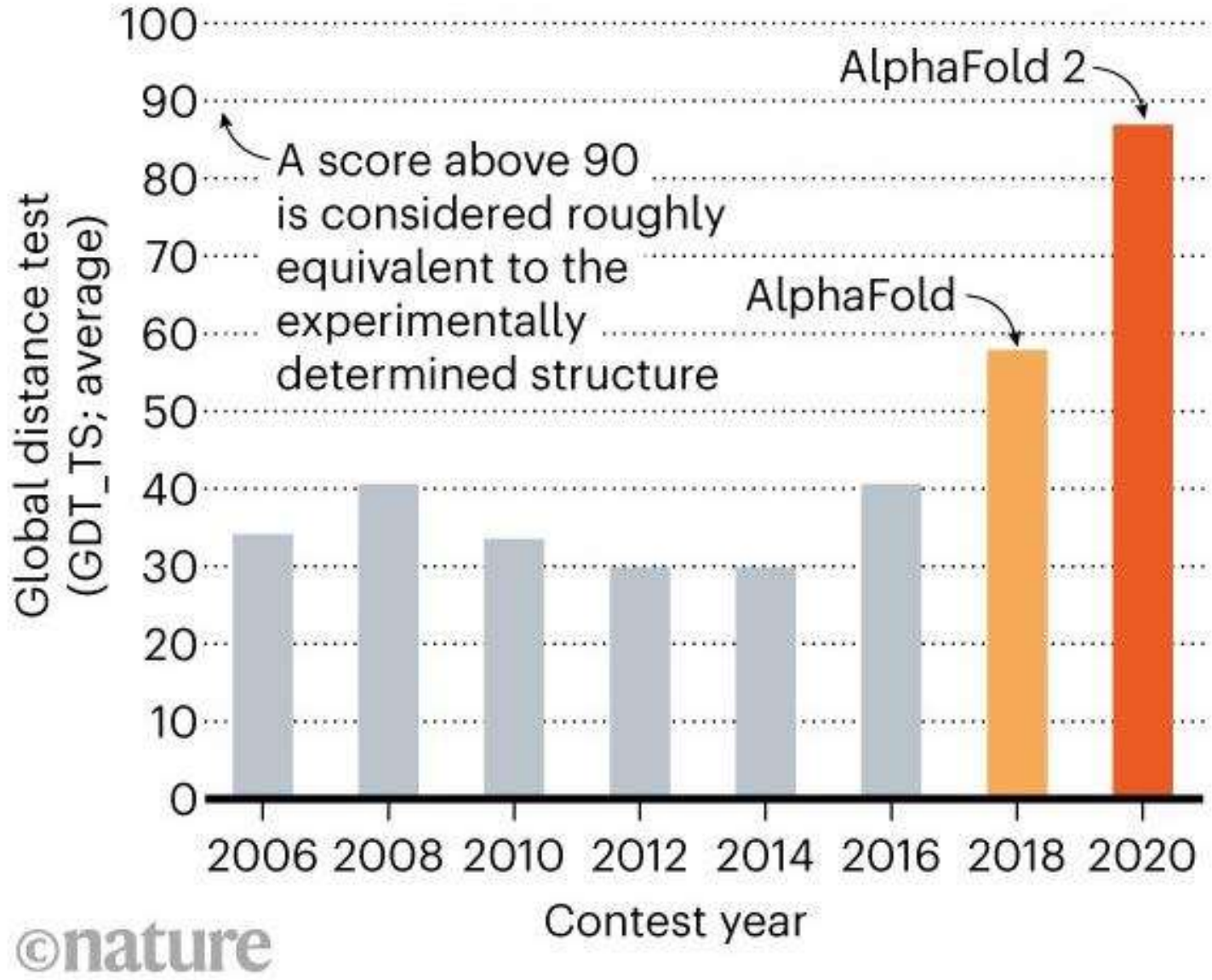

BRENDA home
History
show all | hide all No of entries
Enzyme Nomenclature 382
Synonyms 294
Reactions 29
Reaction Types 8
Pathways 49
Systematic Name 1
CAS Registry Number 1
Enzyme-Ligand Interactions 2421
Substrates/Products 1587
Natural Substrates 45
Cofactors 252
Metals and Ions 102
Inhibitors 407
Activating Compounds 28
Diseases 3424
Functional Parameters 2246
Organism related Information 444
Enzyme Structure 9117
Applications 56
References 296
External Links
BRENDA
1987 2019
The Comprehensive Enzyme Information System
login
history
all enzymes
Contact
print visible entries
print all entries
show all entries
Information on EC 1.1.1.1 - alcohol dehydrogenase
for references in articles please use BRENDA:EC1.1.1.1
EC Tree
1 Oxidoreductases
1.1 Acting on the CH-OH group of donors
1.1.1 With NAD+ or NADP+ as acceptor
1.1.1.1 alcohol dehydrogenase
IUBMB Comments
A zinc protein. Acts on primary or secondary alcohols or hemi-acetals with very broad specificity; however the enzyme oxidizes methanol much more poorly than ethanol. The animal, but not the yeast, enzyme acts also on cyclic secondary alcohols.
Specify your search results
Mark a special word or phrase in this record:
Mark!
Search Reference ID:
Search
Search UniProt Accession:
Search
Select one or more organisms in this record:
All organisms
Acetobacter pasteurianus
Acetobacter pasteurianus SKU1108
Acinetobacter calcoaceticus
Aeropyrum pernix
Word Map
hide
drank
pargyline
synthesis
tumor-initiating
nadp+
biotechnology
maize
monoamine
biofuel production
ethanol-treated
drinker
horse
disulfiram
alcohol-related
benzaldehyde
pyrazole
malate
antidote
ichthyosis
intoxication
nicotinamide
cyp2e1
ethanol-induced
retinal
s-nitrosoglutathione
semialdehyde
1.1.1.1
nadp+-dependent
cyclophosphamide

如何获得结构：X射线晶体衍射→冷冻电镜





蛋白质结构预测突破


100
90
80
70
60
50
40
30
20
10
0
Global distance test
(GDT_TS; average)
AlphaFold 2
A score above 90
is considered roughly
equivalent to the
experimentally
determined structure
AlphaFold
2006
2008
2010
2012
2014
2016
2018
2020
Contest year
©nature

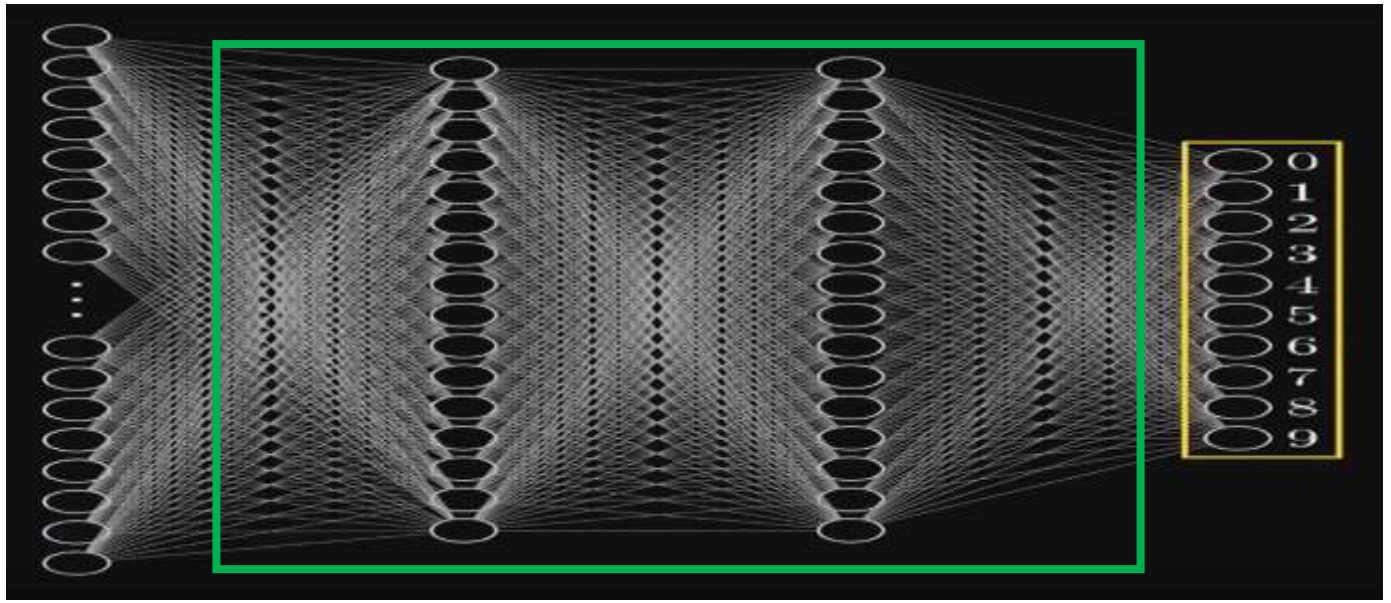
Alphafold2

输入层　　隐含层　　输出层

序列

序列库

MSA



距离

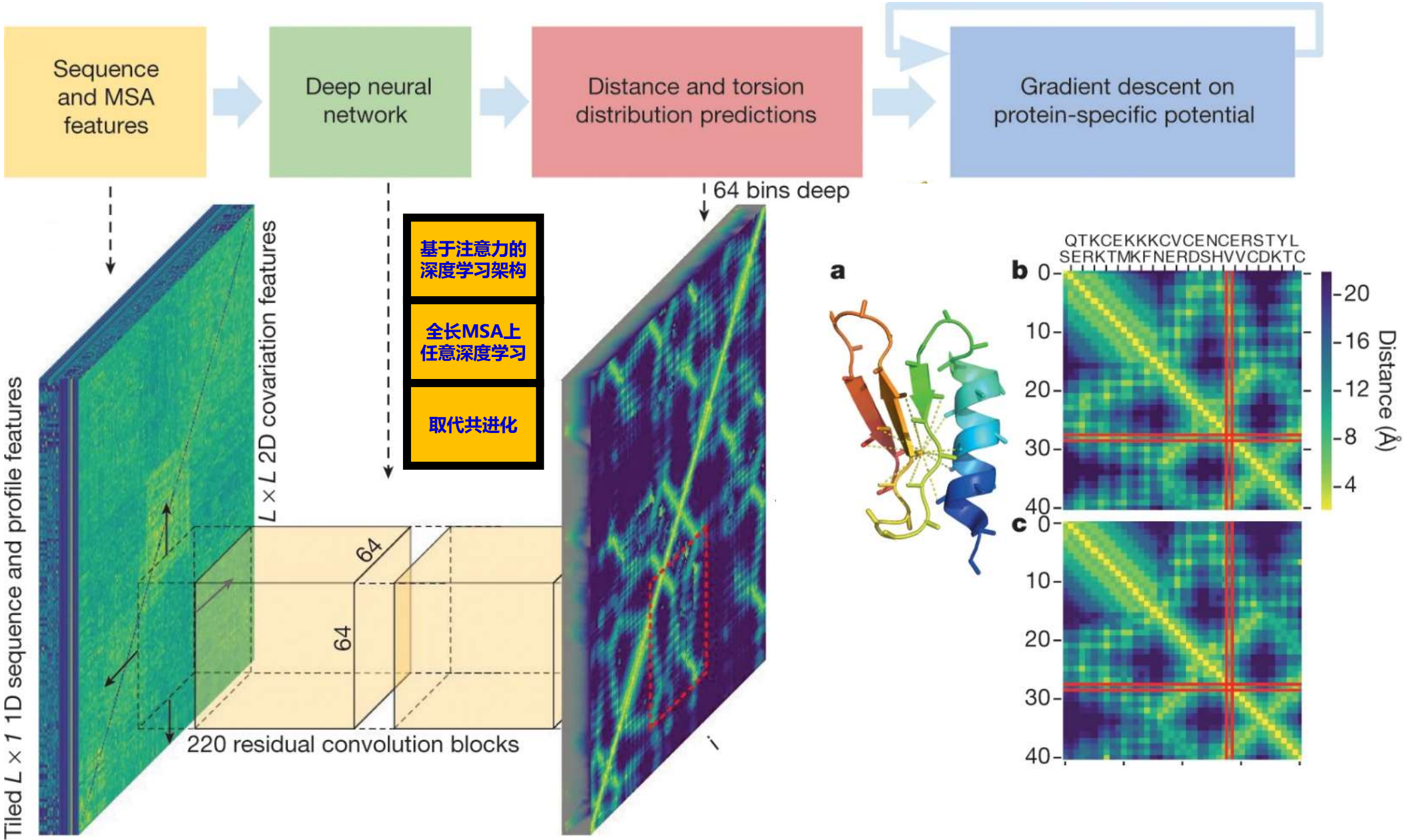
结构库

角度

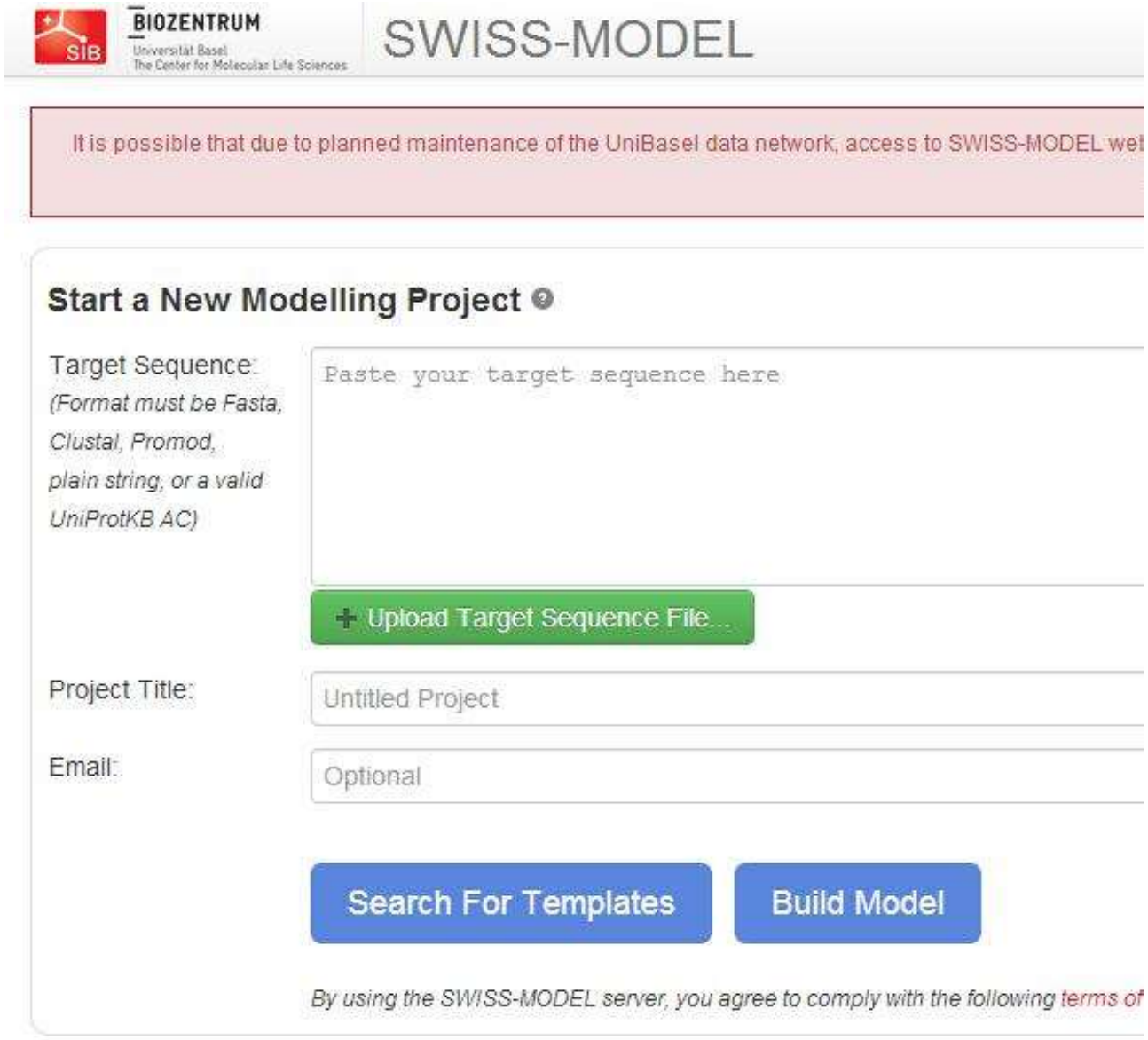
训练深层神经网络

序列→ HHSearch←结构库(Protein Data Bank); MSA: Multiple Sequence Alignment;　距离：氨基酸残基对β碳距离；角度：α碳与肽平面的二面角(ψ和φ)

Alphafold（2） 折叠流程



基于模板建模



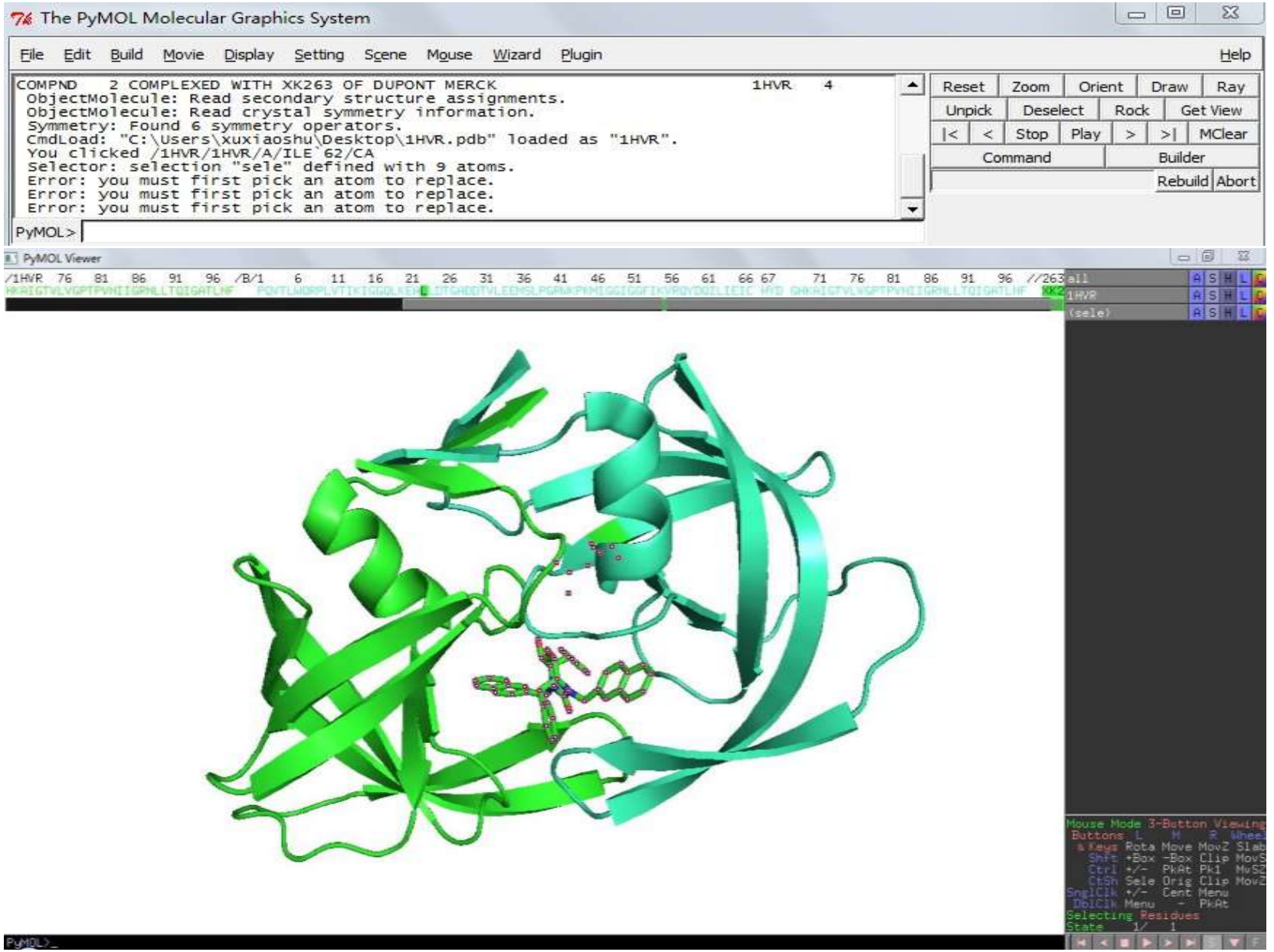
同一性>30%

1. 搜结构模板
2. 联配目标序列与模板
3. 建模
4. 模型质量评估

自由建模

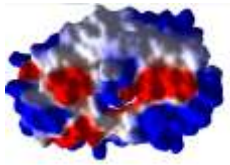
1. 多线程搜结构模板
2. 迭代片段组装
3. 在蛋白质功能数据库中重穿线3D模型

PyMOL

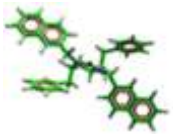

The PyMOL Molecular Graphics System
File Edit Build Movie Display Setting Scene Mouse Wizard Plugin Help
COMPND 2 COMPLEXED WITH XK263 OF DUPONT MERCK 1HVR 4
ObjectMolecule: Read secondary structure assignments.
ObjectMolecule: Read crystal symmetry information.
Symmetry: Found 6 symmetry operators.
CmdLoad: "C:\Users\xuxiaoshu\Desktop\1HVR.pdb" loaded as "1HVR".
You clicked /1HVR/1HVR/A/ILE`62/CA
Selector: selection "sele" defined with 9 atoms.
Error: you must first pick an atom to replace.
Error: you must first pick an atom to replace.
Error: you must first pick an atom to replace.
PyMOL>
Reset Zoom Orient Draw Ray
Unpick Deselect Rock Get View
|< < Stop Play > >| MClear
Command Builder
Rebuild Abort
PyMOL Viewer
all
1HVR
(sele)
Mouse Mode 3-Button Viewing
Selecting Residues
PyMOL>

蛋白质与配体分子对接分析

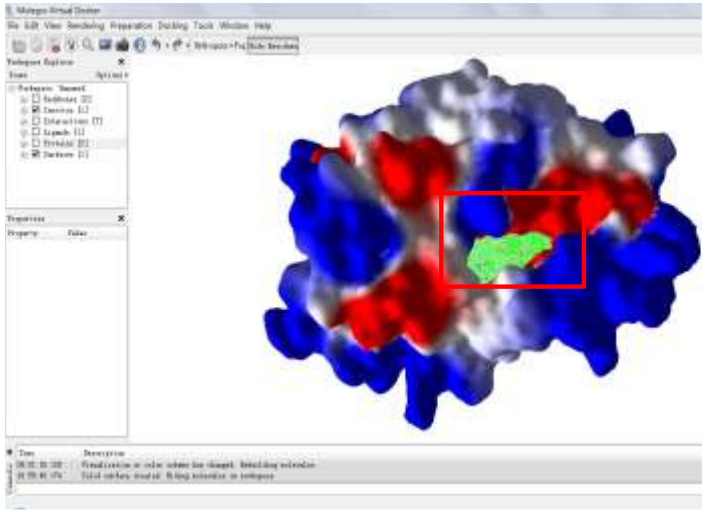
HIV-1 protease



PDB ID: 1HVR

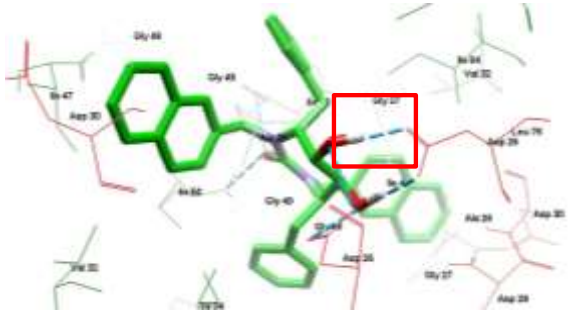


XK263



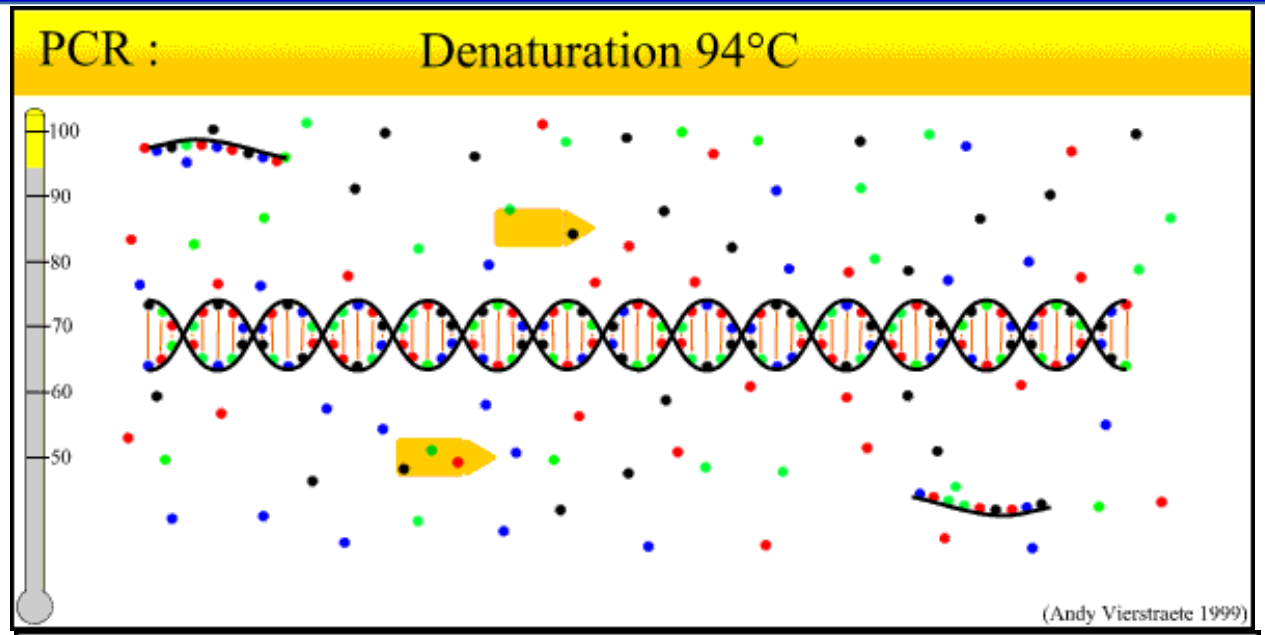
预测活性中心

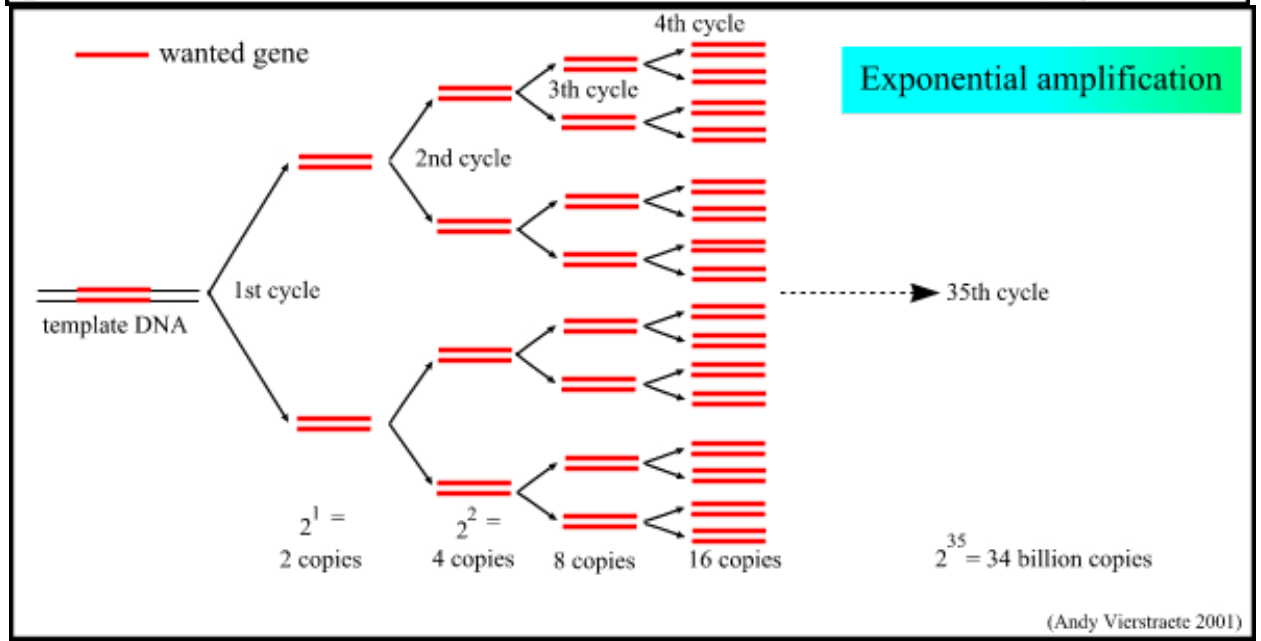
Docking



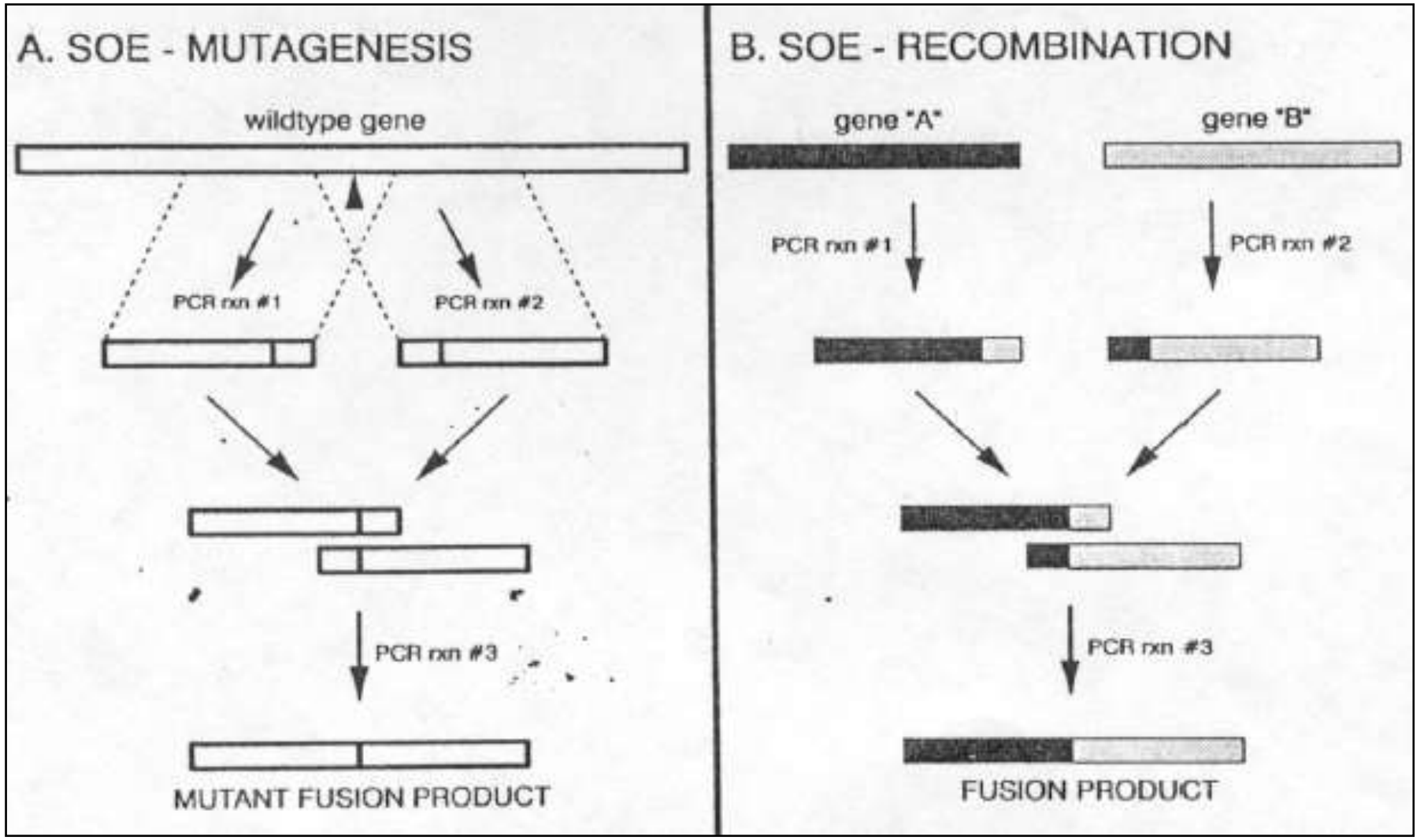
配体与蛋白质间氢键

PCR原理

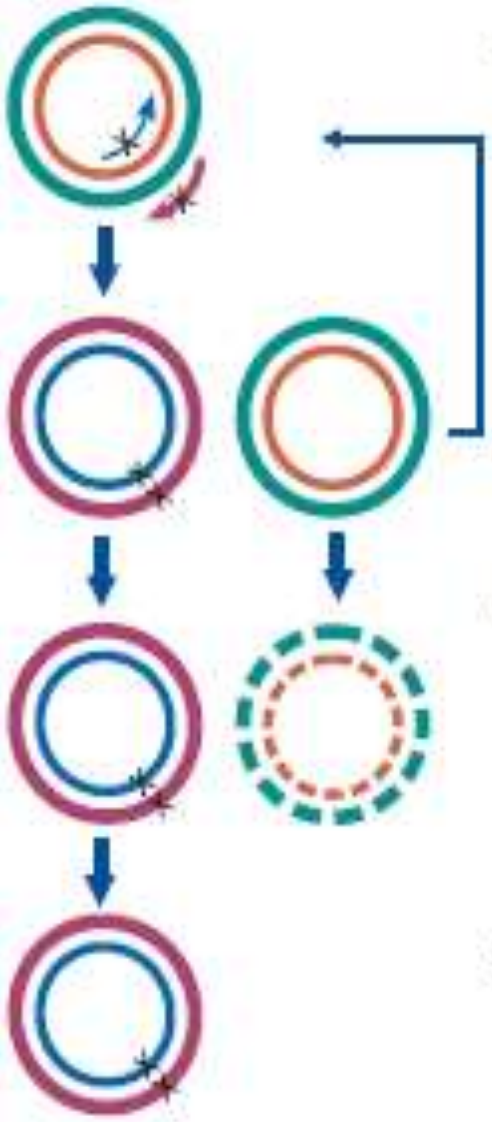

PCR :
Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
Exponential amplification
35th cycle
2^1 = 2 copies
2^2 = 4 copies
8 copies
16 copies
2^{35} = 34 billion copies
(Andy Vierstraete 2001)

定向突变 - PCR


A. SOE - MUTAGENESIS
wildtype gene
PCR rxn #1
PCR rxn #2
PCR rxn #3
MUTANT FUSION PRODUCT
B. SOE - RECOMBINATION
gene "A"
gene "B"
PCR rxn #1
PCR rxn #2
PCR rxn #3
FUSION PRODUCT

Stratagene Mutagenesis Kit Selection Guide



1. Mutant Strand Synthesis
Perform thermal cycling to:
- Denature DNA template
- Anneal mutagenic primers containing desired mutation
- Extend and incorporate primers with *PfuUltra*™ DNA polymerase

2. *Dpn* I Digestion of Template
Digest parental methylated and hemimethylated DNA with Dpn I

3. Transformation
Transform mutated molecule into competent cells for nick repair

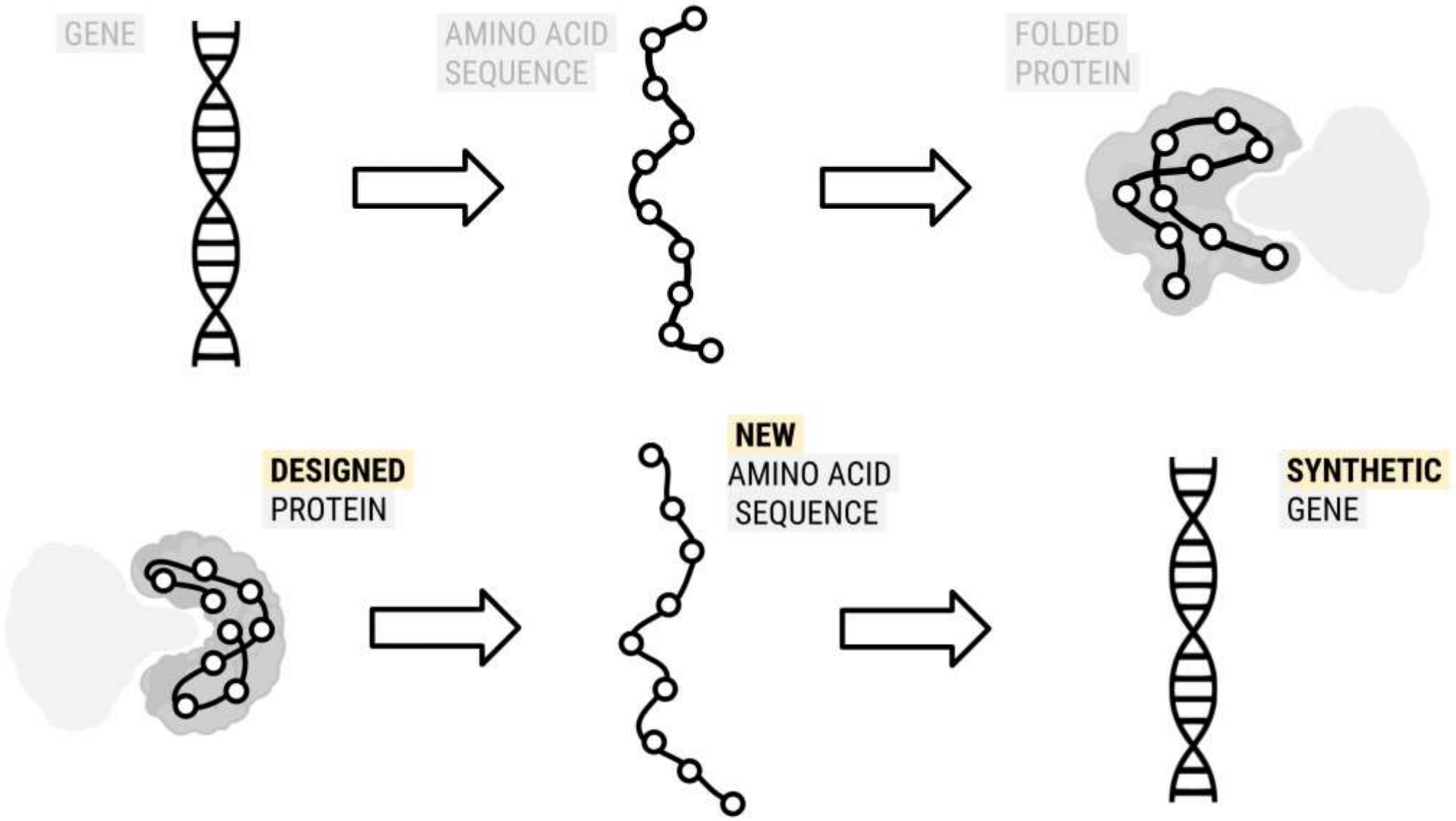
APPLICATION	PRODUCT	ADVANTAGES
Random mutagenesis for large fragments	GeneMorph® II Kit	+ Balanced mutational spectrum + Easily adjust mutation frequency + Robust amplification up to 6 kb
Targeted random mutagenesis on entire protein or small protein domains coupled with an easy cloning method	GeneMorph® II EZ Clone Kit	+ Balanced mutational spectrum + Robust amplification and randomization of sequences up to 3.5 kb + Eliminates difficult restriction cloning + Easily adjust mutation frequency
Highest fidelity site-directed mutagenesis	QuikChange® II Kit	+ Greater than 80% efficiency + Non-PCR strategy minimizes errors + Single day protocol saves time + Eliminates difficult restriction cloning
For large plasmids; highest fidelity and efficient site-directed mutagenesis	QuikChange® II XL Kit	+ Optimized reagents for large constructs > 8kb + Non-PCR method minimizes errors + Eliminates difficult restriction cloning + Single day protocol saves time
High efficiency, complex multi-site mutagenesis	QuikChange® Multi Kit	+ Save time introducing multiple mutations + Perform saturation mutagenesis + Eliminates difficult restriction cloning + Single day protocol saves time

有理设计小结

- **工具**
  - 酶数据库　　Brenda
  - 结构预测　　I-Tasser
  - 分子图像　　PyMOL
  - 定点突变，序列重组　　PCR

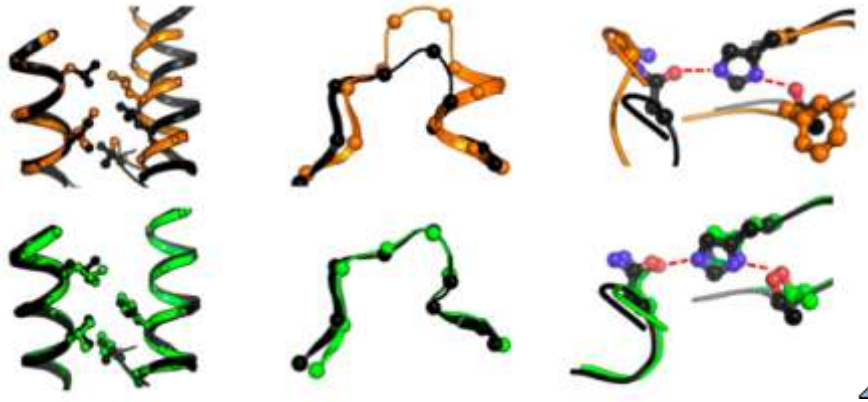
- **目标**
  - 热稳定性，pH曲线　　表面氨基酸替换
  - 催化效率　　需了解催化机理
  - 底物特异性　　需了解催化机理

从头设计

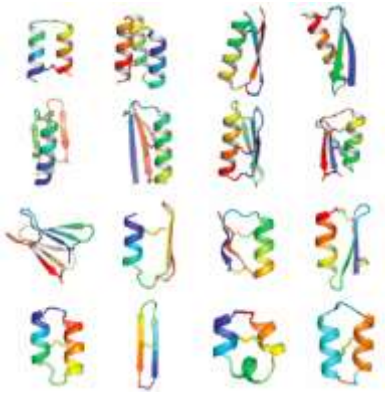


https://www.ipd.uw.edu/research/basic-areas/

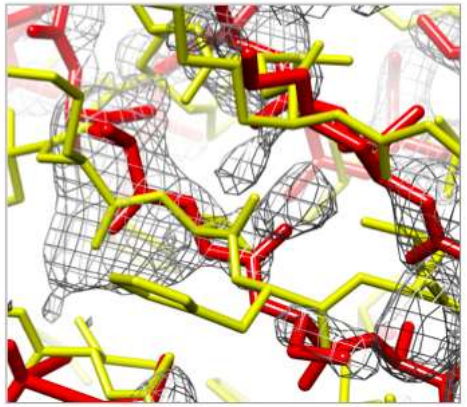
从头设计流程



1.力场开发与抽样算法



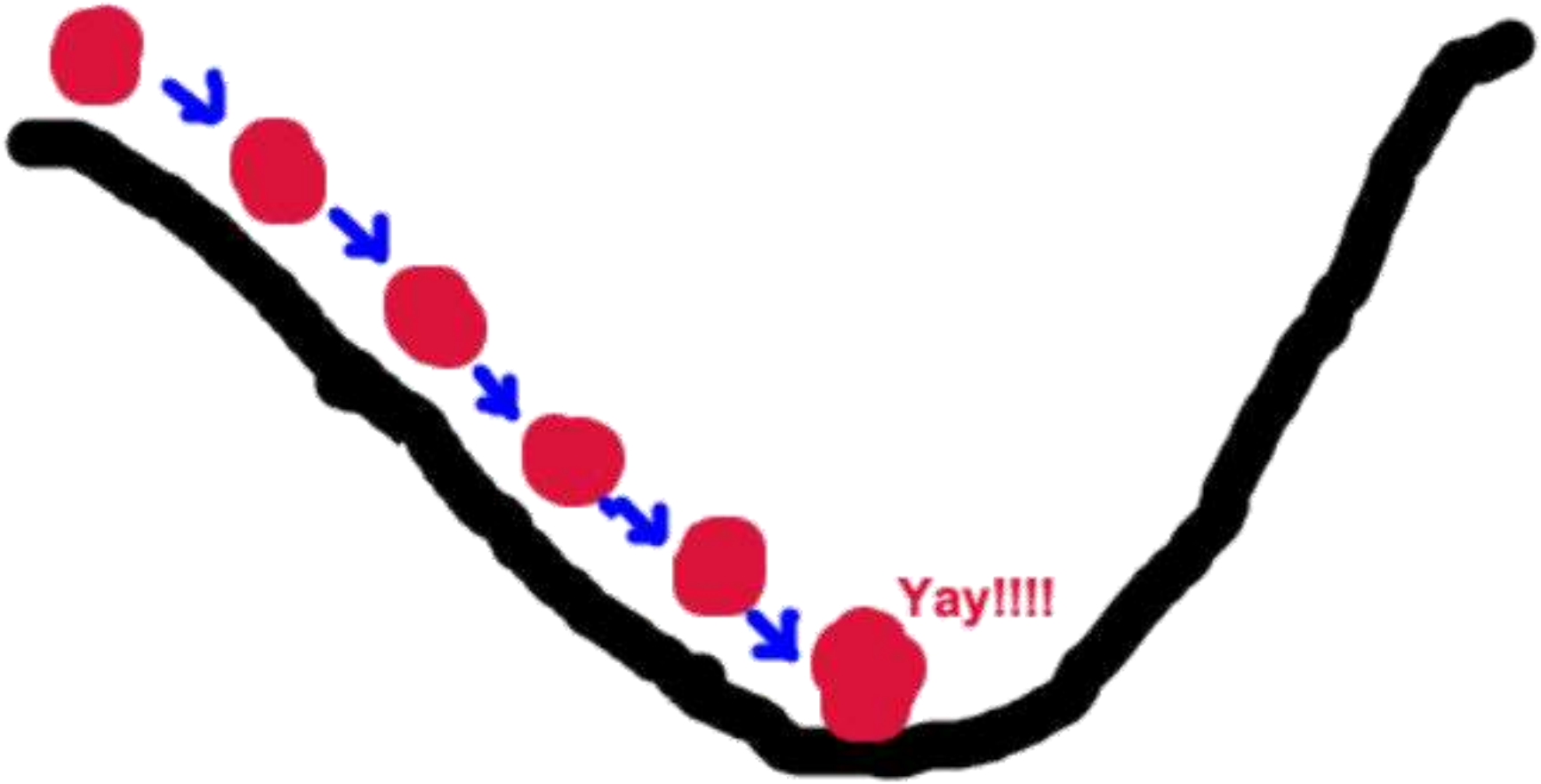
2.高通量测试



3.结构鉴定

https://www.ipd.uw.edu/research/basic-areas/

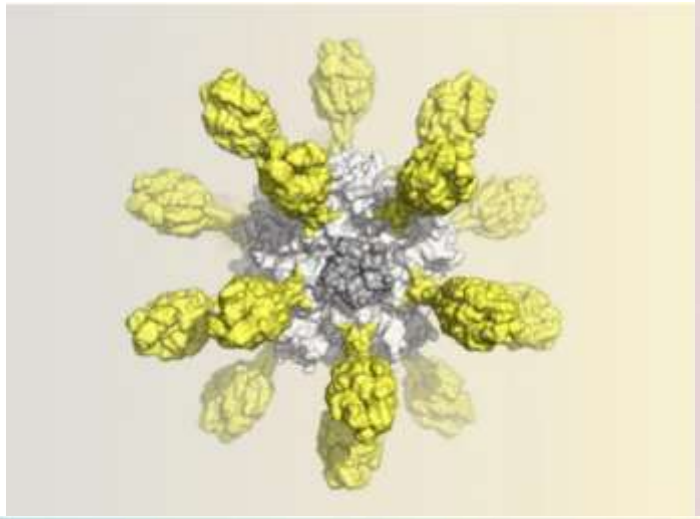
从头设计梯度下降法



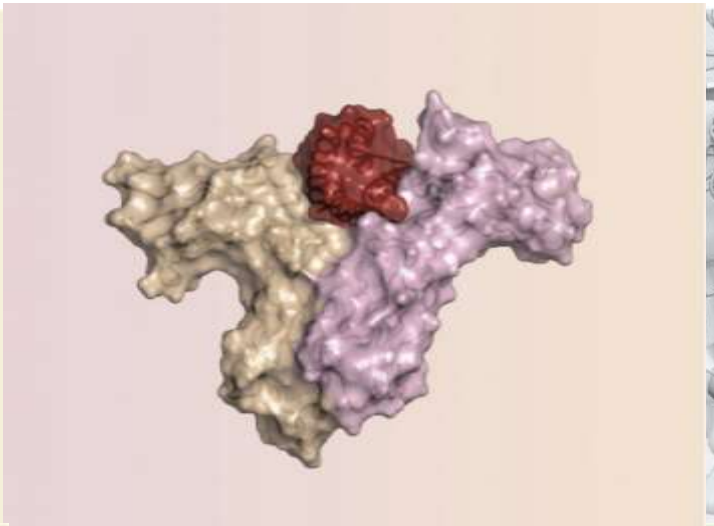
改变形状→计算能量→ 直到不能更低

从头设计

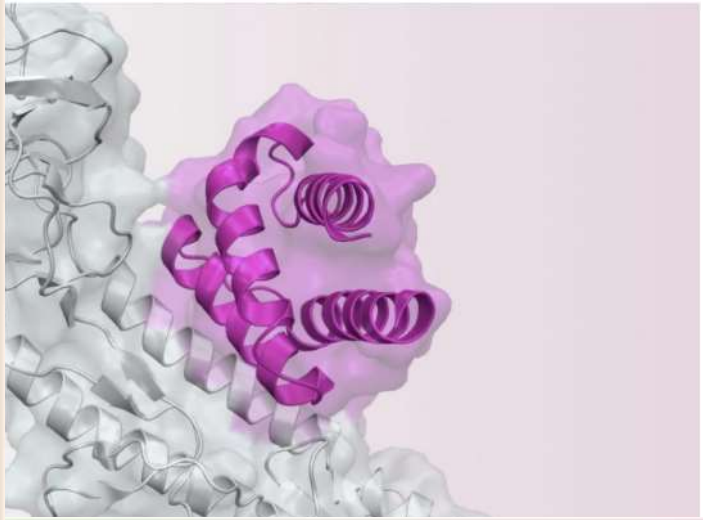
疫苗与抗原表位

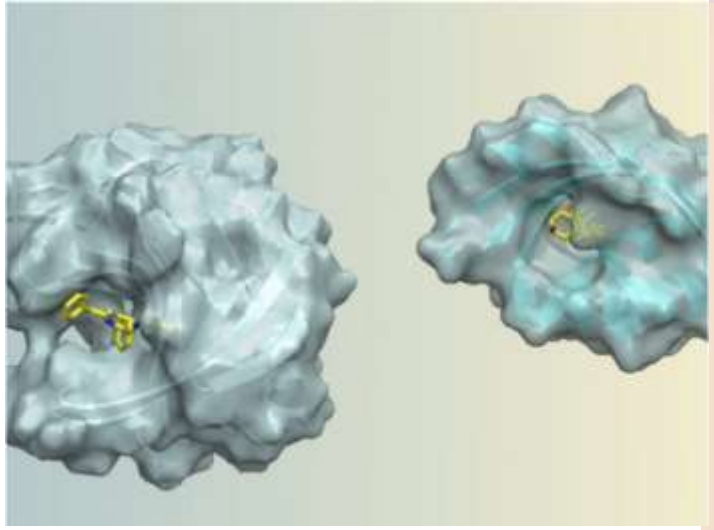


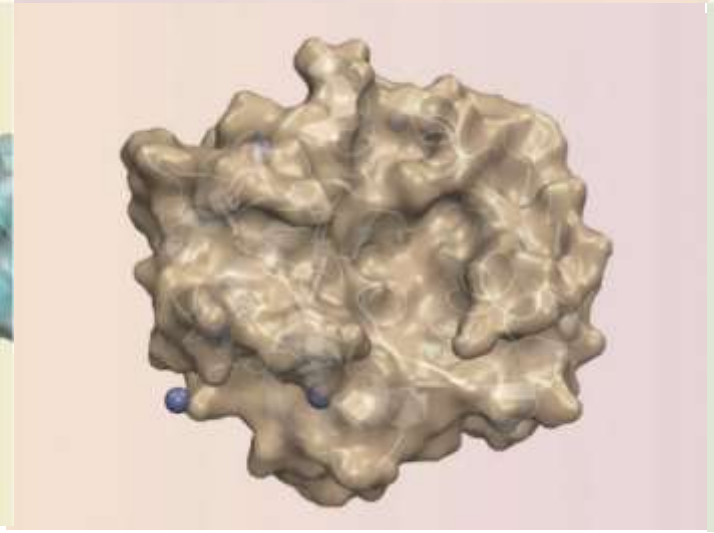
癌症免疫疗法

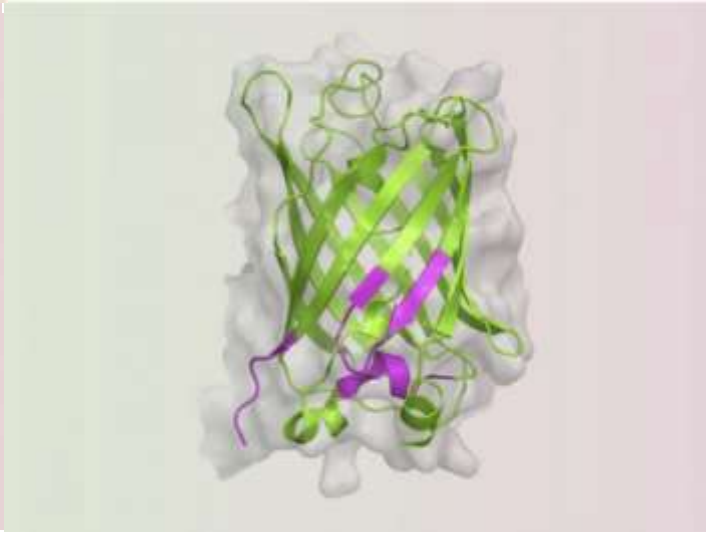


流感







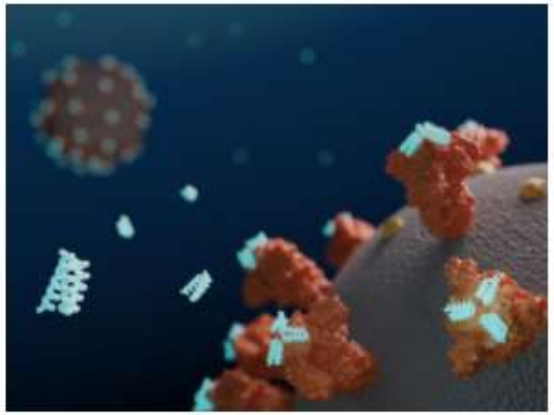


生物传感器

麸质过敏

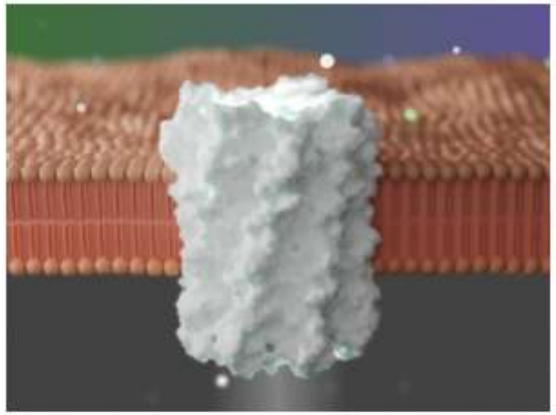
免疫沉默

从头设计



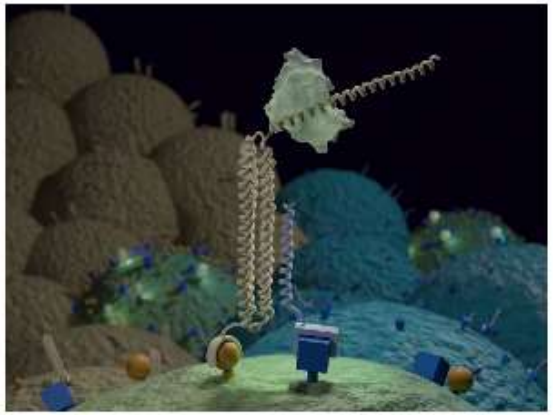
De novo minibinders target SARS-CoV-2 Spike protein

SEPTEMBER 9, 2020



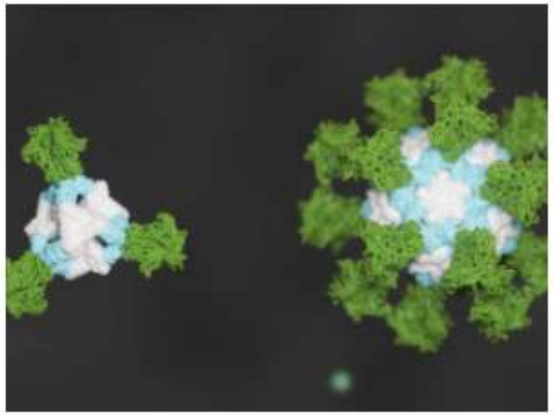
Selective ion channels designed from scratch

AUGUST 31, 2020



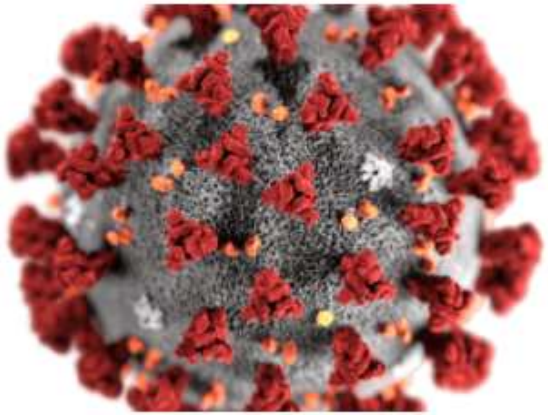
Introducing Co-LOCKR: designed protein logic for cell targeting

AUGUST 20, 2020



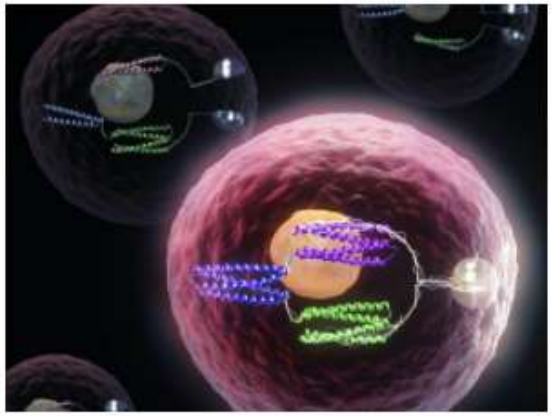
De novo nanoparticles as vaccine scaffolds

AUGUST 5, 2020



Rosetta's role in fighting coronavirus

APRIL 18, 2020



De novo design of protein logic gates

APRIL 2, 2020

从头设计

1.新骨架

2.结合小分子

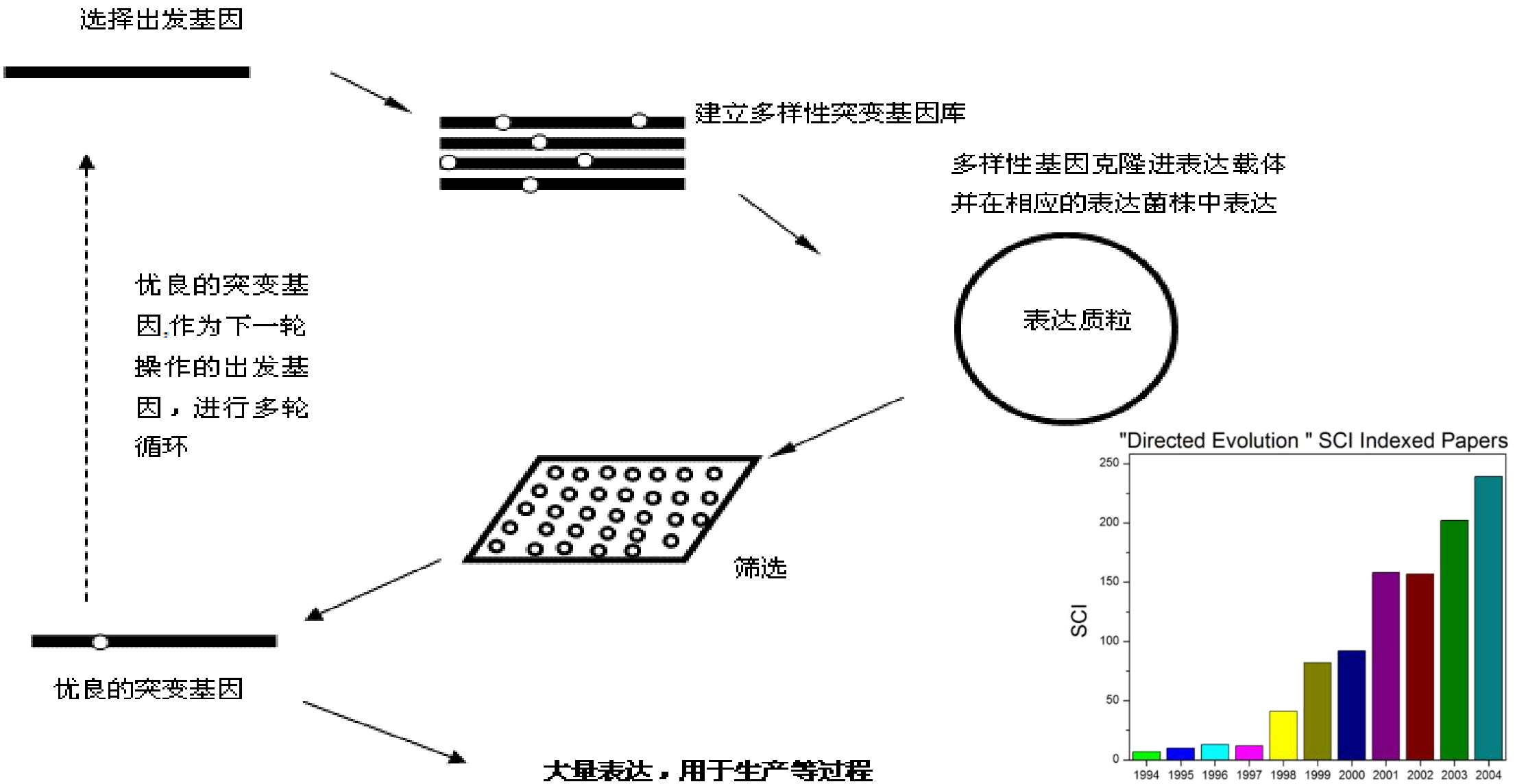
3.自组装纳米材料和疫苗

4.设计新酶催化反应类型

https://www.ipd.uw.edu/research/basic-areas/

休息10分钟

蛋白质的定向进化


选择出发基因
建立多样性突变基因库
多样性基因克隆进表达载体
并在相应的表达菌株中表达
表达质粒
筛选
优良的突变基因
优良的突变基因,作为下一轮操作的出发基因，进行多轮循环
大量表达，用于生产等过程

"Directed Evolution " SCI Indexed Papers
SCI
Year
0
50
100
150
200
250
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004

高频随机突变导入方法

DNA聚合酶	碱基错配率/每轮复制	
大肠杆菌体内	1	X 10^{-10}-10^{-8}
Replicase	1.03	X 10^{-4}
Vent(exo$^-$)	1.9	X 10^{-4}
Taq	2.0-21	X 10^{-5}
KlenTaq	5.1	X 10^{-5}
Vent	2.4-5.7	X 10^{-5}
Pfu, PfuTurbo	1.6	X 10^{-6}

易错PCR error-prone PCR (= PCR with many errors/mutations)

- 无校对DNA聚合酶　e.g. Taq, Mutazyme
- 非优化条件　e.g. Mn2+ 代替 Mg2+, 调整4种dNTP相对浓度

体内高频随机突变

以大肠杆菌XL1-Red作为基因复制宿主

XL1-Blue strain: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI*^{q} *ZΔM15 Tn10* (Tet^{r})]

正常菌株

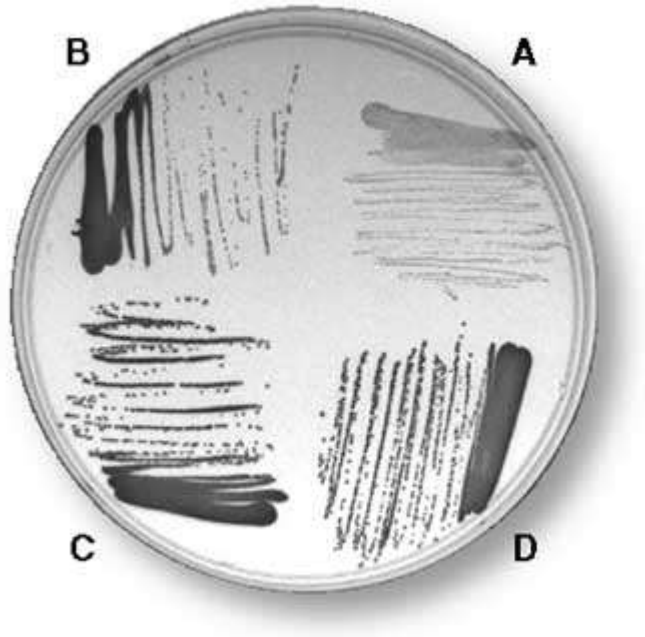
XL1-Red strain: *endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10* (Tet^{r})

DNA修复缺陷

Sequence of a 1-kb Segment from pGC10

CLONE #	CHANGE(S) OBSERVED
1	1-bp insertion at base 182
2	G- A transition at base 148
3	none
4	none
5	T - A transversion at base 42
6	none
7	T - C transition at base 243
8	G - A transition at base 103
9	none
10	none

Plasmid pGC10, a pBluescript phagemid derivative, was propagated for approximately 30 generations in XL1-Red. Its DNA was then isolated and retransformed into XL1-Blue. Ten clones were selected at random, and a 1-kb segment was sequenced.

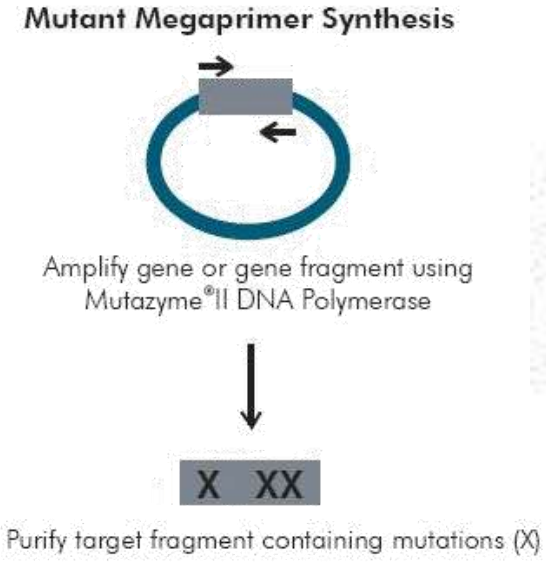


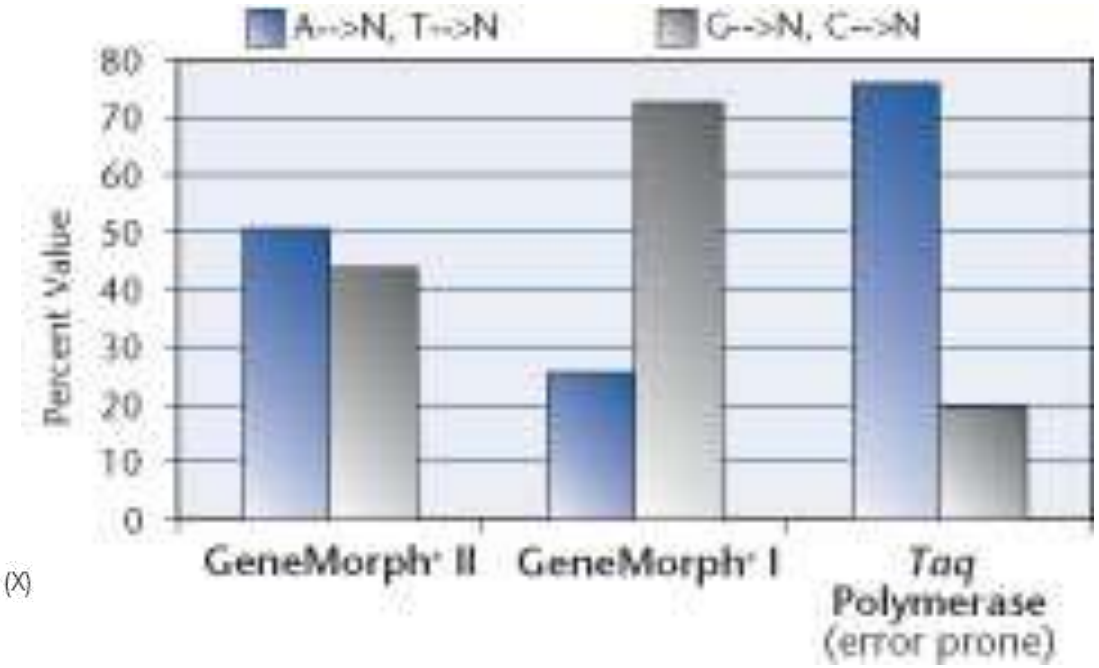
Mutants of the *P. furiousus* Alkaline Phosphatase Gene: Morphology of clones on LB plates containing BCIP indictor substrate.
A: Parent Pfu alkaline phosphatase clone.
B: Pfu 5. C: Pfu 5-1. D: Pfu 5-2.

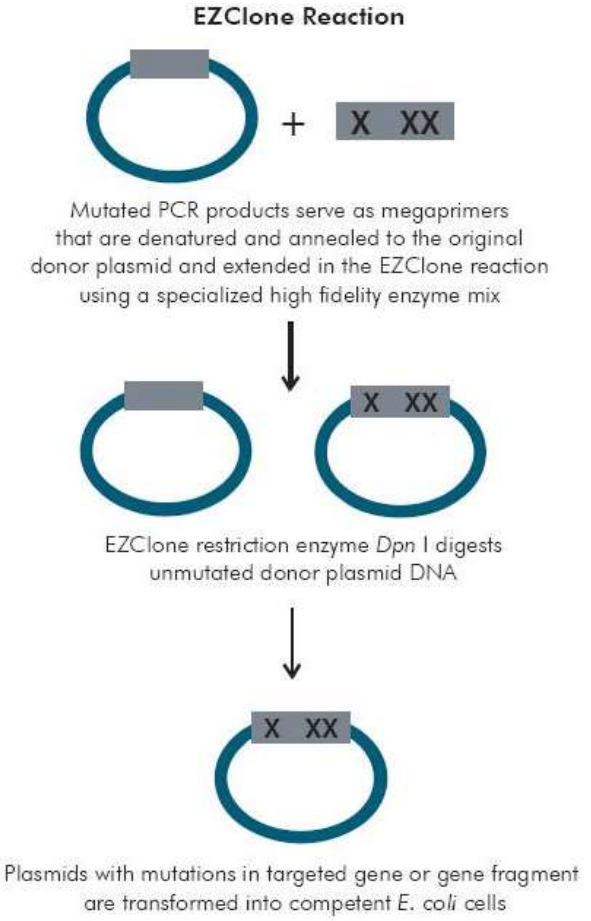
培养到平台期 平均2Kb发生1个碱基突变

Greener, A. and Callahan, M. (1994), *Strategies*. 7: 32-34

Random Mutagenesis kit

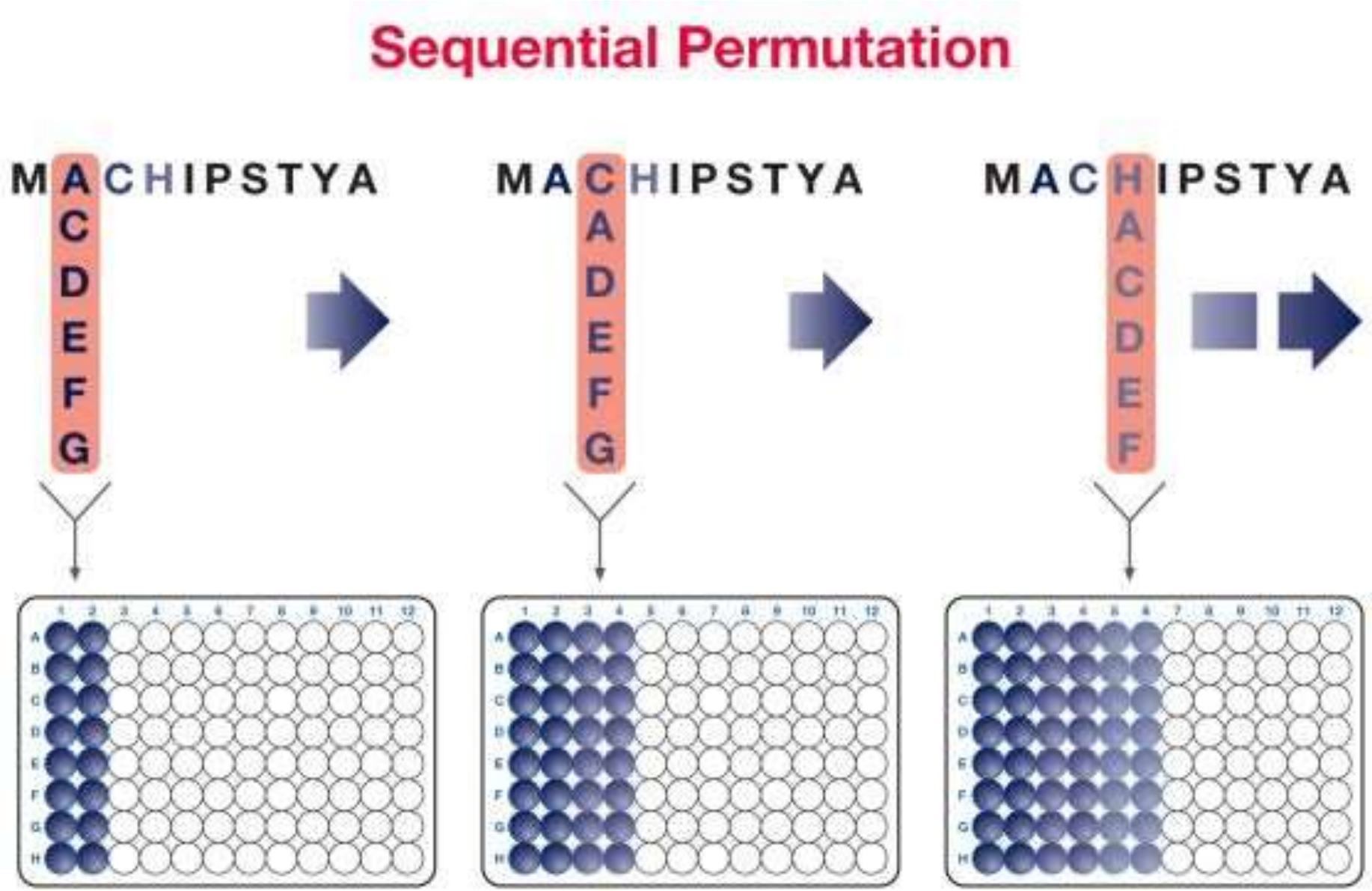






突变率 0.1 - 1.6% /PCR, 相当于1 - 16个碱基突变/基因

逐点饱和突变技术


Sequential Permutation
MACHIPSTYA
ACDEFG
MACHIPSTYA
CADEFG
MACHIPSTYA
HACDEF

逐点饱和突变技术

GeneArt® Site-Saturation Mutagenesis Custom Service Requirements Form

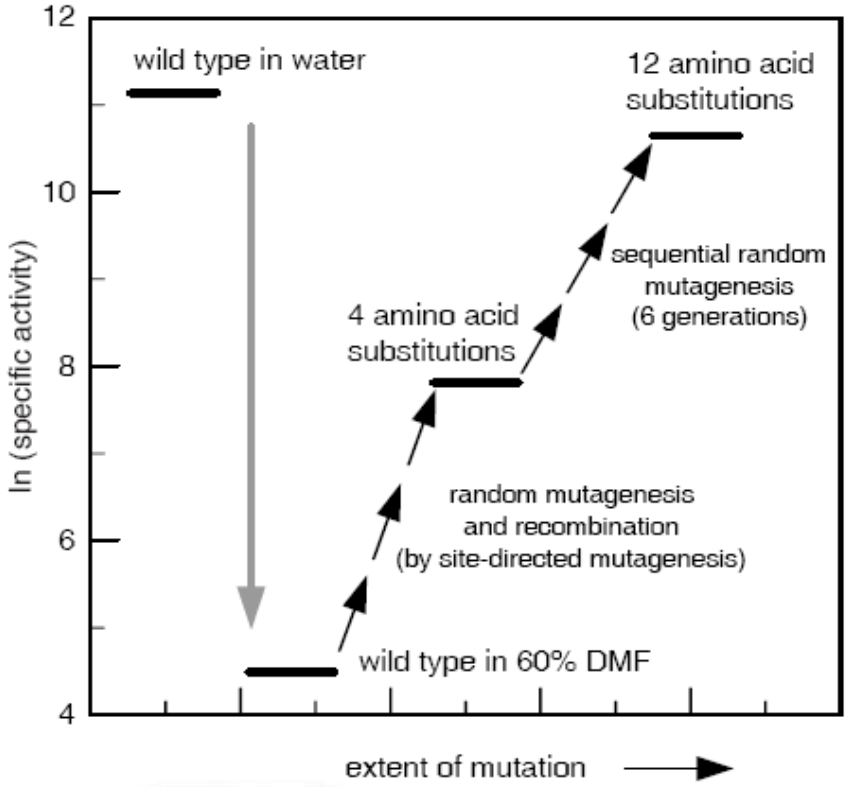
life technologies™

Please send the completed form as well as any questions to GeneArtSupport@lifetech.com

Once we have received the necessary project information, we will follow up with a quote of price and turnaround time.

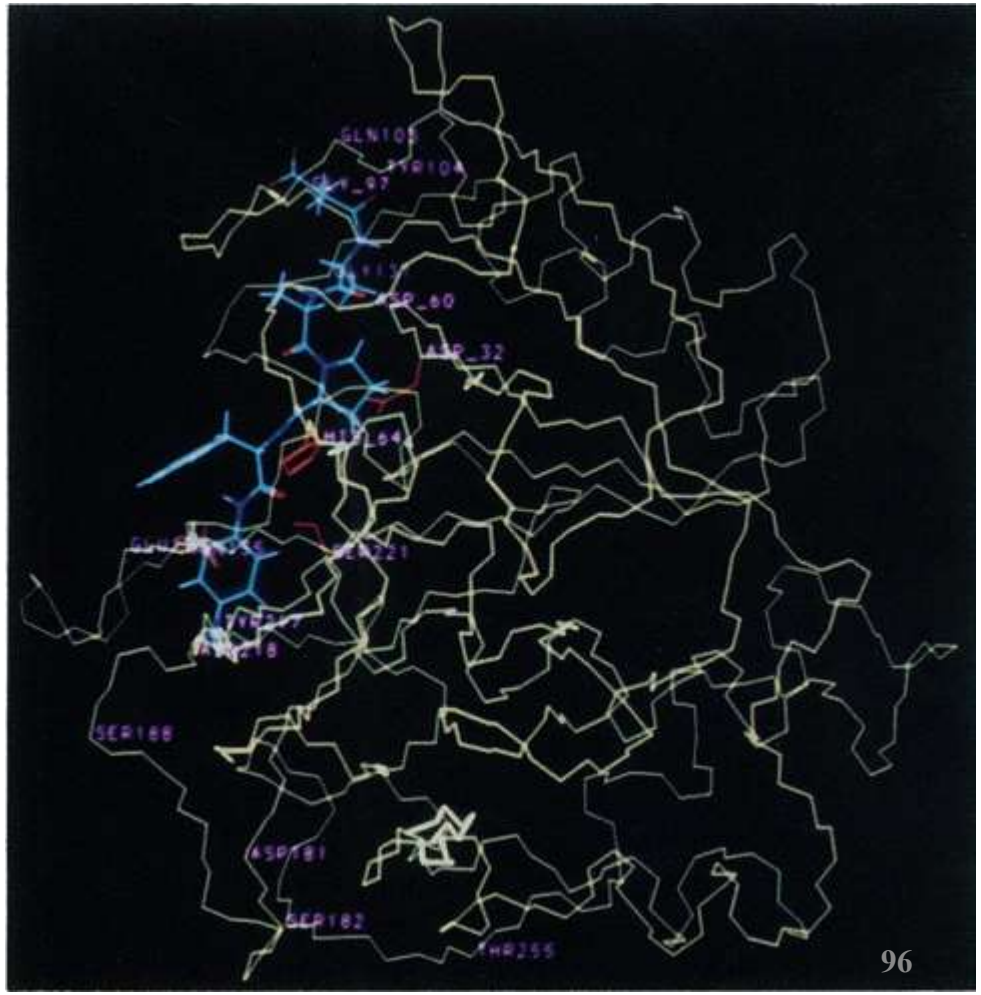
Customer Information			
Name			
Institution			
Address			
1. Permutations and strain			
1.1 Name of the sequence			
1.2 ORF length			
1.3 SSM type (A16, M16; 19; Pool of one position, Pool of all positions)			
1.4 Complete sequence: (Please paste in the complete sequence of the mastergene and mark the codon (3 bp) where counting of permutated positions should start).			
1.5 Number of deg. Positions (per project)			
1.6.1 Organism			
1.6.2 Strain			
1.7 Mastergene (template)	customer provided	no	
	Synthesised *de novo* *(designed by GeneArt® for this project)*	yes	*Gene No* / *Codon usage table*

定向进化提高枯草芽孢杆菌蛋白酶E在DMF水溶液的活力



Chen and Arnold. *PNAS* 1993
You and Arnold. *Protein Eng* 1996

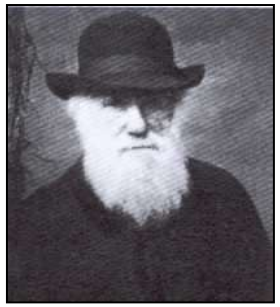
Frances H. Arnold
2018 诺贝尔化学奖



自然进化

进化

逐步过程

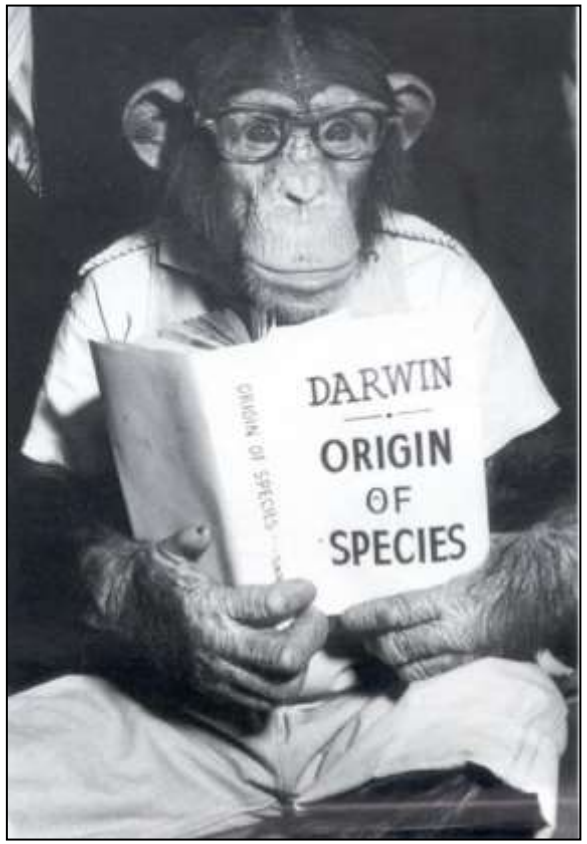


自然进化

- 自发突变
- 重组
- 自然选择

达尔文进化理论

生命的复杂性是**突变**，**重组**和**自然选择**算法的结果



农业进化

加速进化

快速改变

农业进化

- 自发突变
- 育种
- 筛选

人类的作物，花卉和家禽家畜育种实践有几千年历史

实验室进化

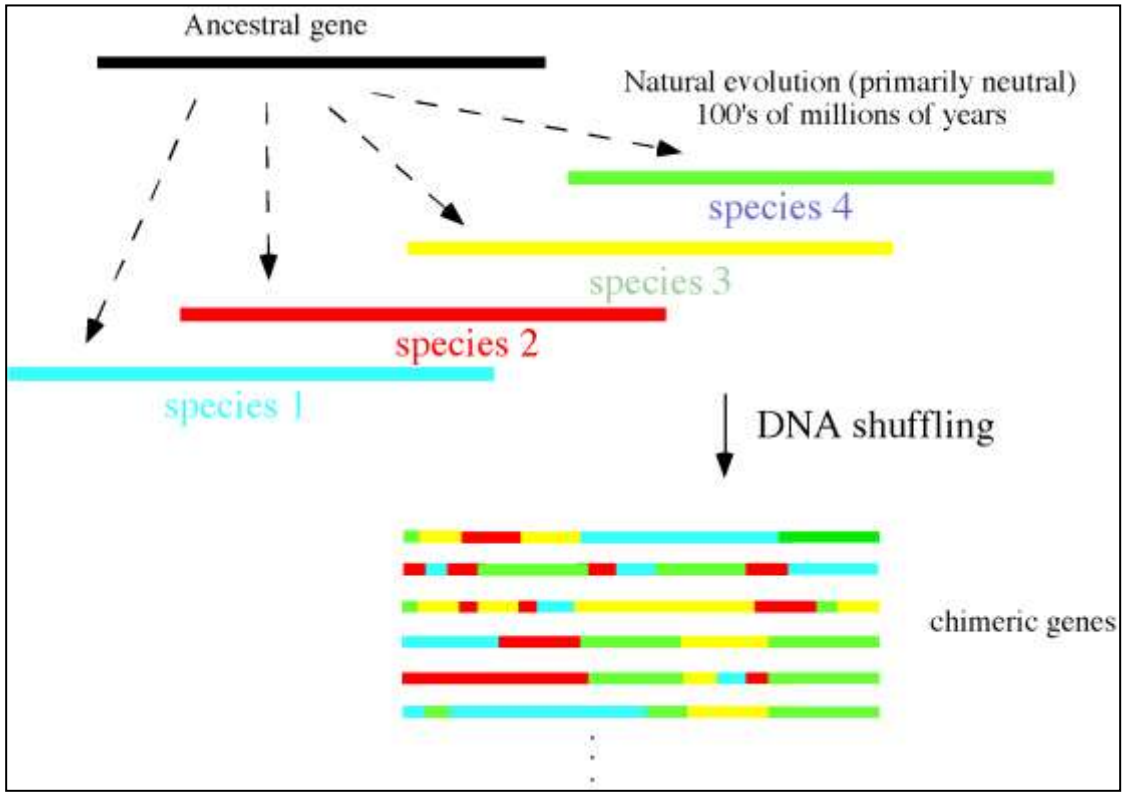
革命

极快改变

实验室进化

- 加快突变速度
- 分子育种
- 筛选

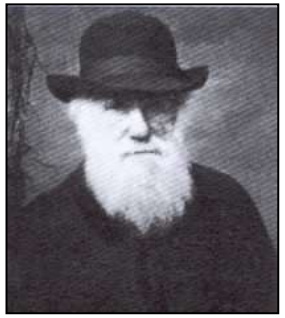
实验室进化不同于自然进化在于其明确的进化目标. 在此意义上它是 “定向” 并且更像育种。此外，它非常快。



进化比较

进化

逐步过程



自然进化

- 自发突变
- 重组
- 自然选择

加速进化

快速改变

农业进化

- 自发突变
- 育种
- 筛选

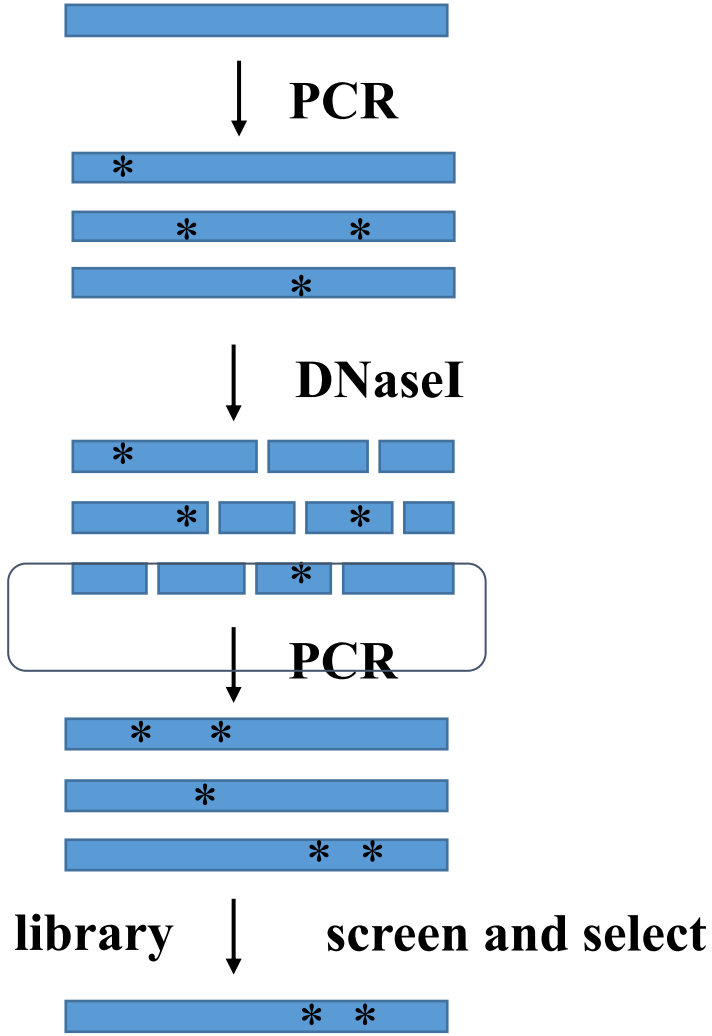
革命

极快改变

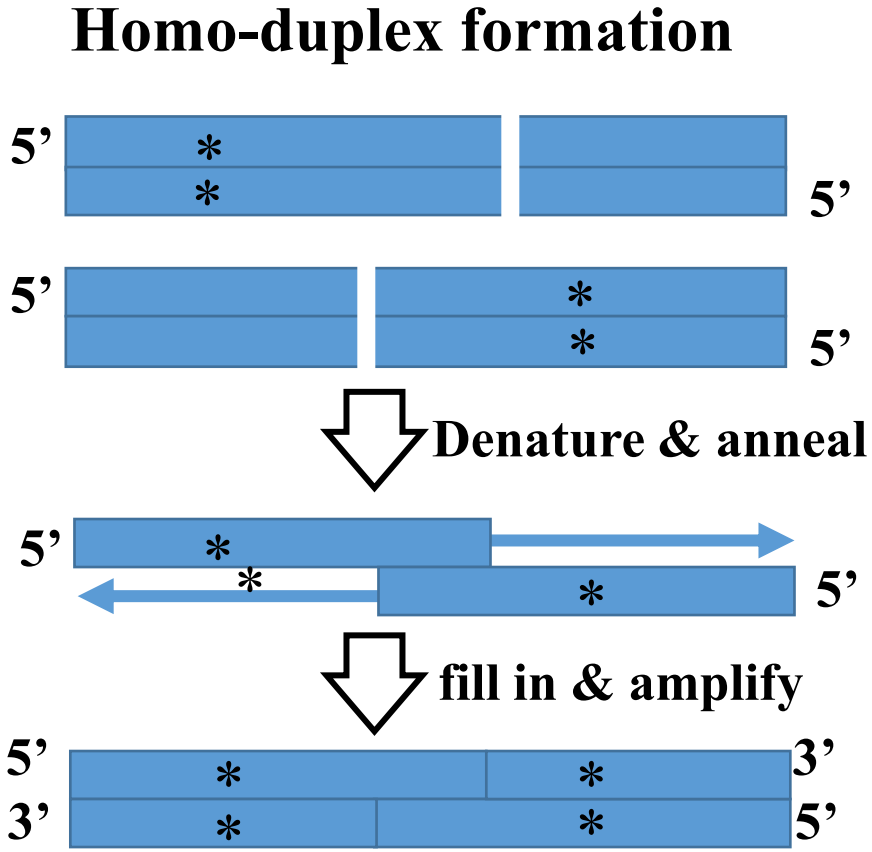
实验室进化

- 加快突变速度
- 分子育种
- 筛选

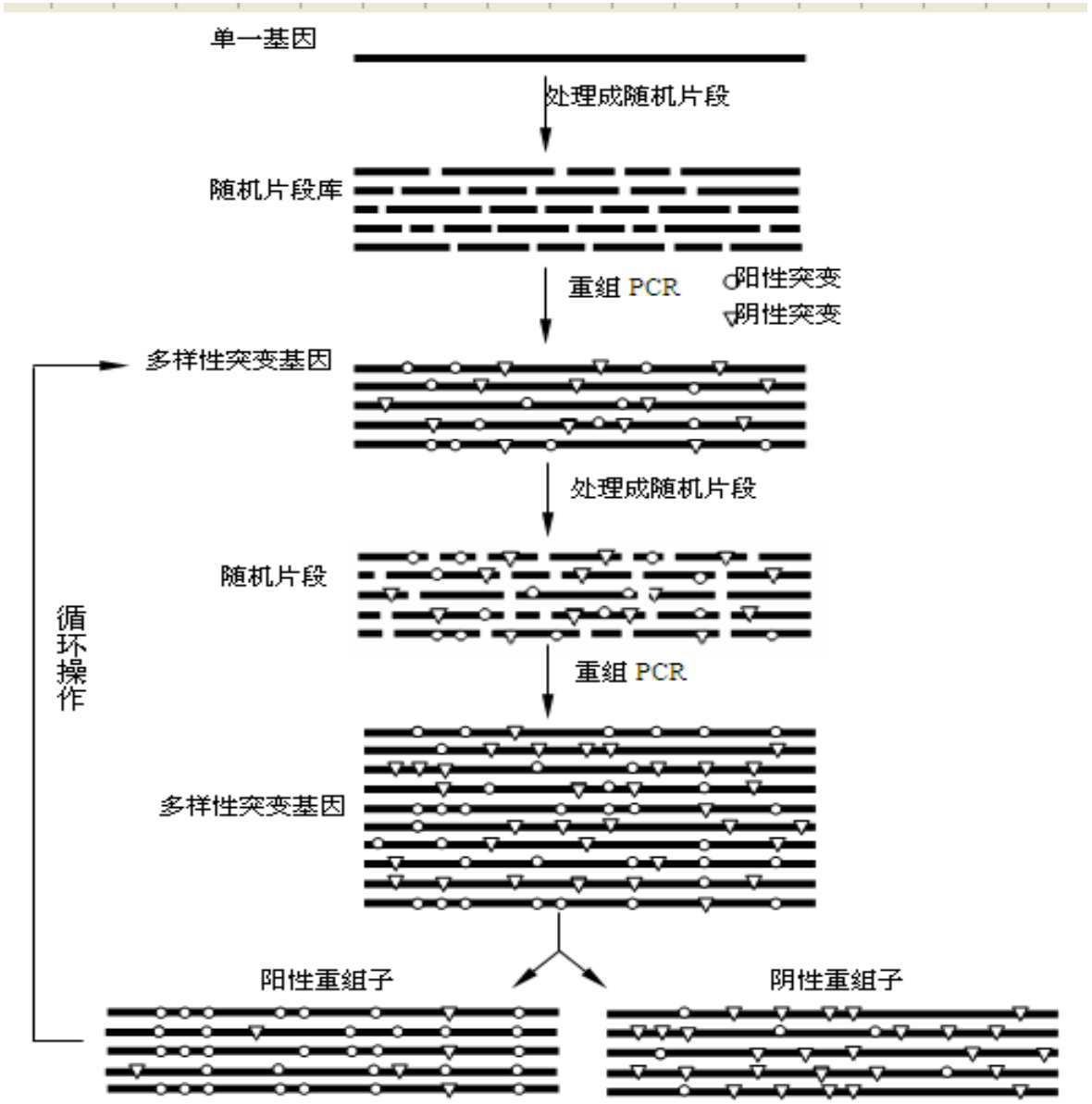
Error-prone PCR



DNA shuffling

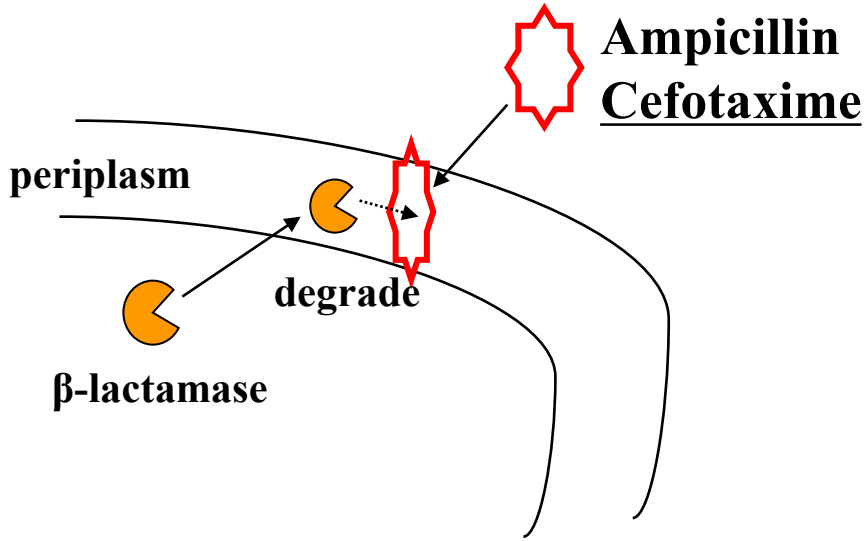


DNA Shuffling



DNA Shuffling – β-lactamase case

β-Lactamase
: hydrolysis of beta-lactam antibiotics



TEM-1 W.T.

↓ **MIC = 0.02 ug/ml**

shuffling - 3 cycle

↓ **X 16,000**

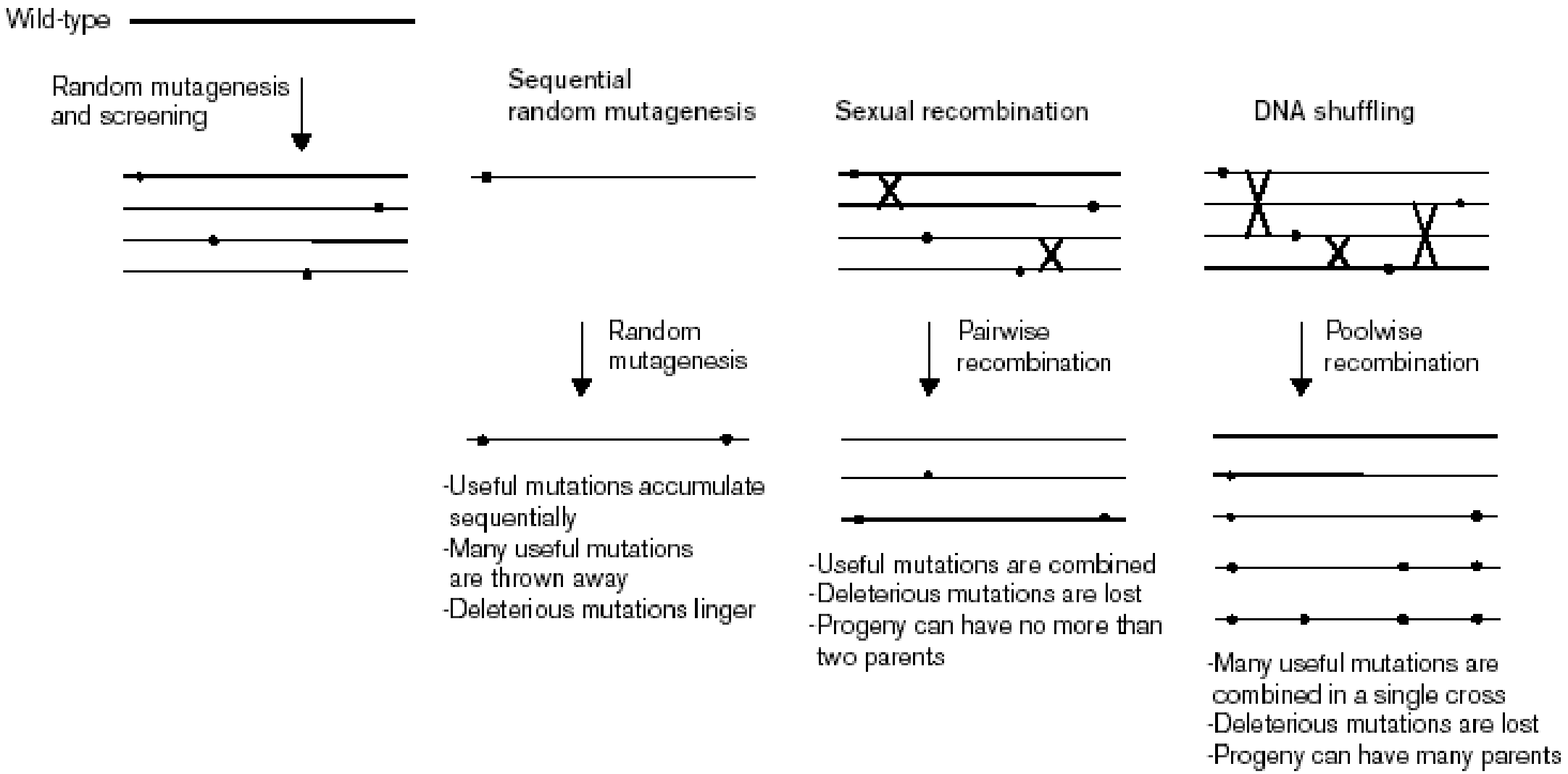
Back cross over - 2 cycle

↓ **X 32,000**

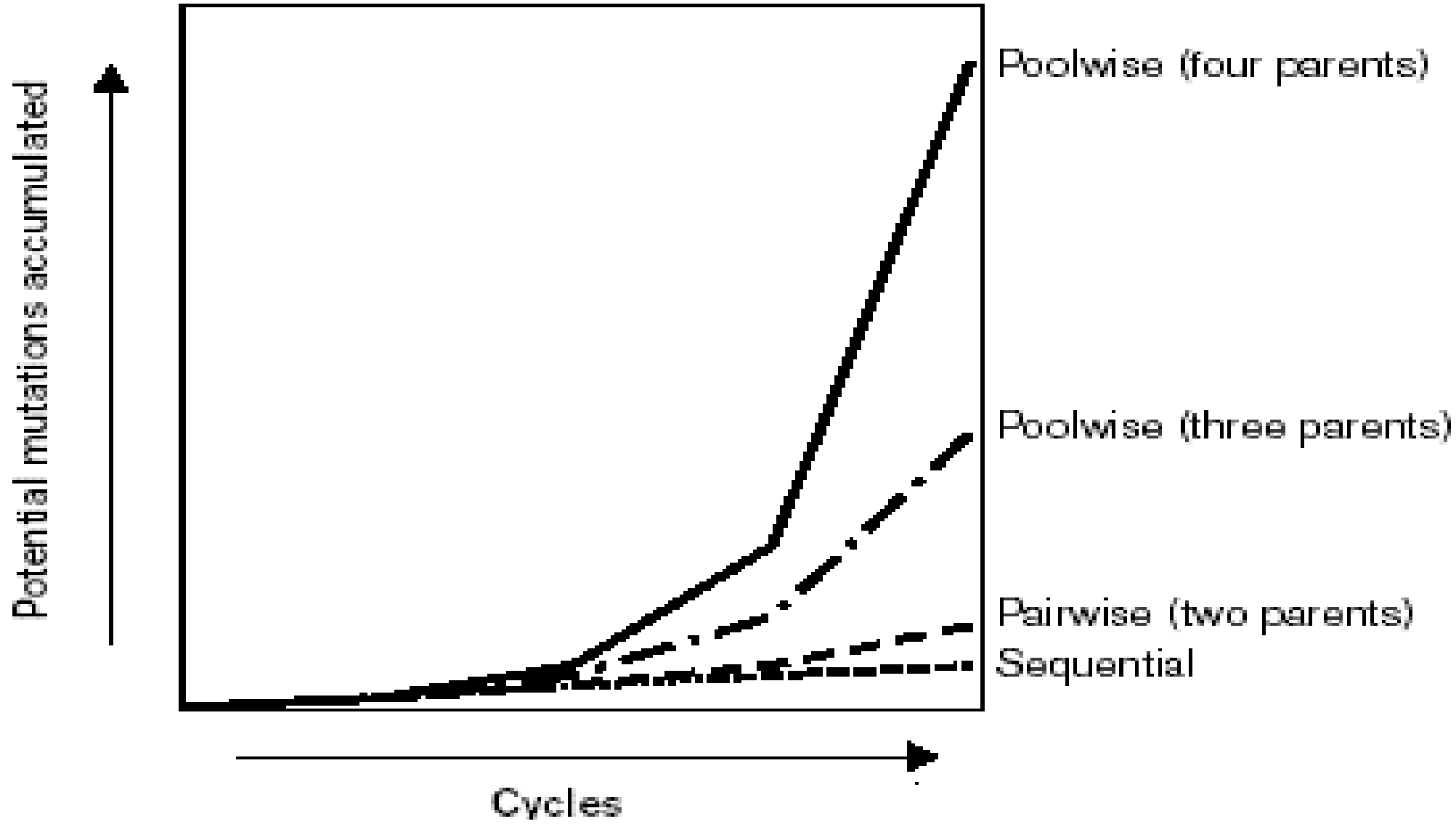
MIC = 640 ug/ml

(Stemmer, W.P.C. *Nature* 1994 370:389-391)

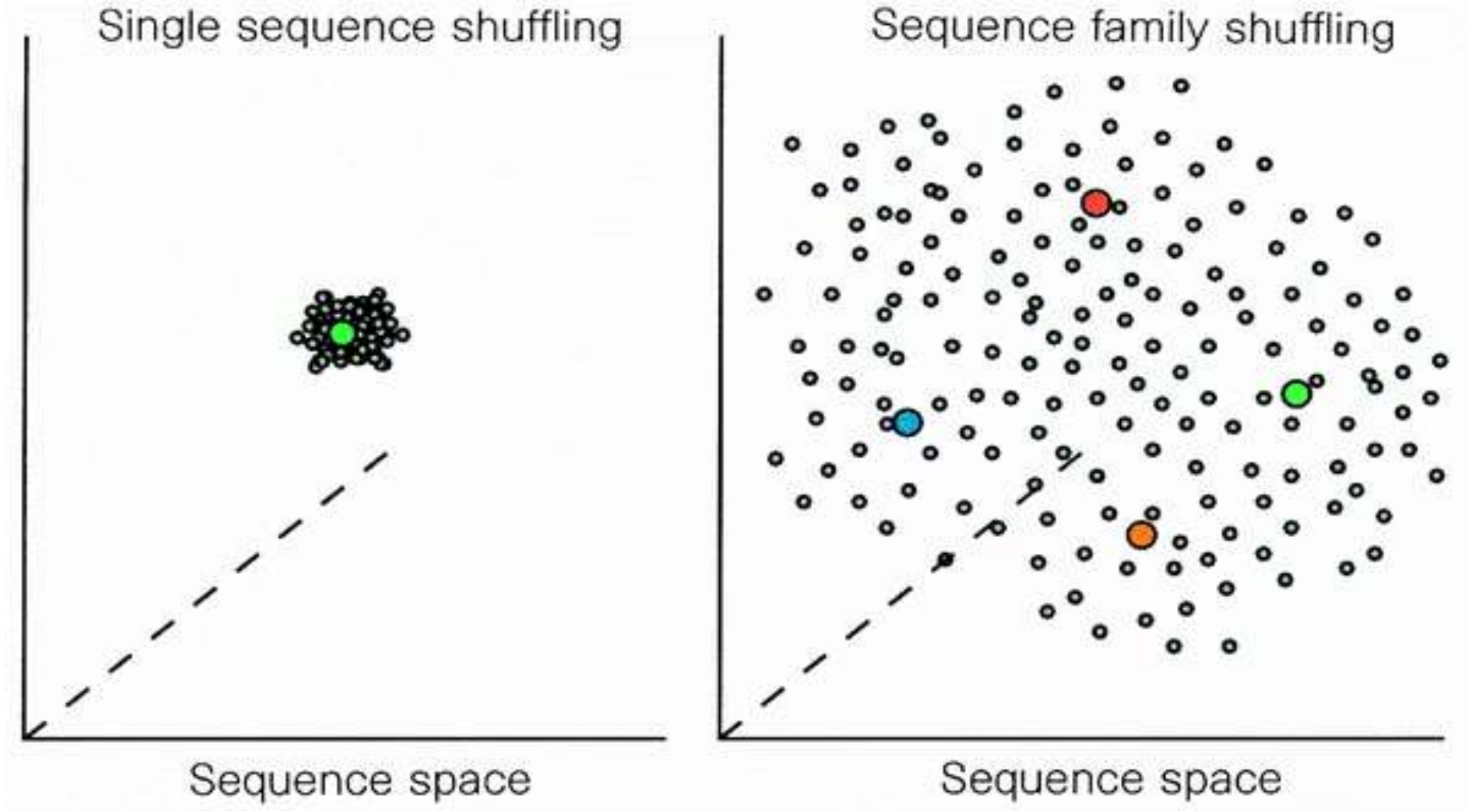
定向进化方法比较


Wild-type
Random mutagenesis and screening
Sequential random mutagenesis
Sexual recombination
DNA shuffling
Random mutagenesis
Pairwise recombination
Poolwise recombination
-Useful mutations accumulate sequentially
-Many useful mutations are thrown away
-Deleterious mutations linger
-Useful mutations are combined
-Deleterious mutations are lost
-Progeny can have no more than two parents
-Many useful mutations are combined in a single cross
-Deleterious mutations are lost
-Progeny can have many parents

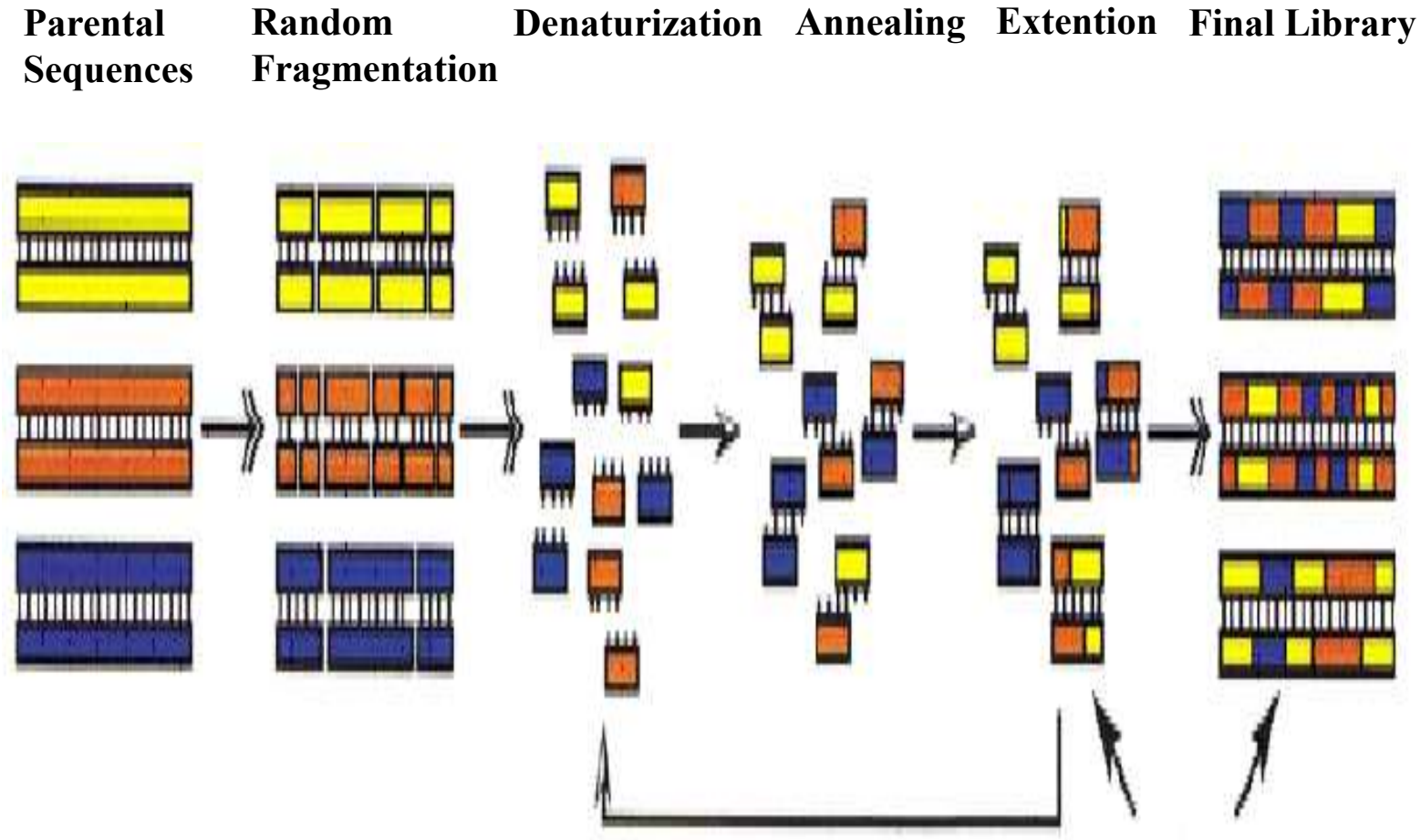
多亲本重组提升突变积累速度



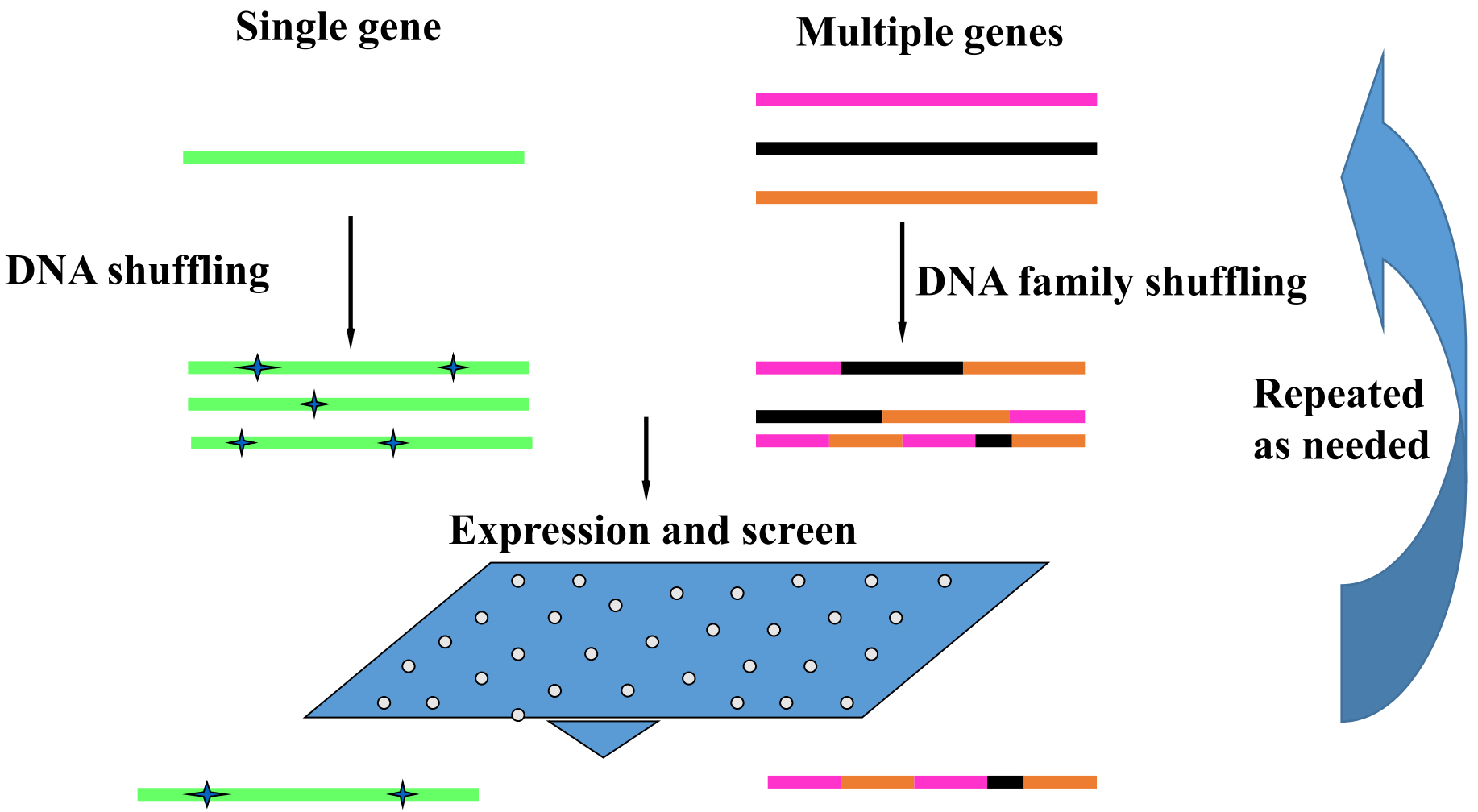
单基因Shuffling和多基因Shuffling获得的突变库



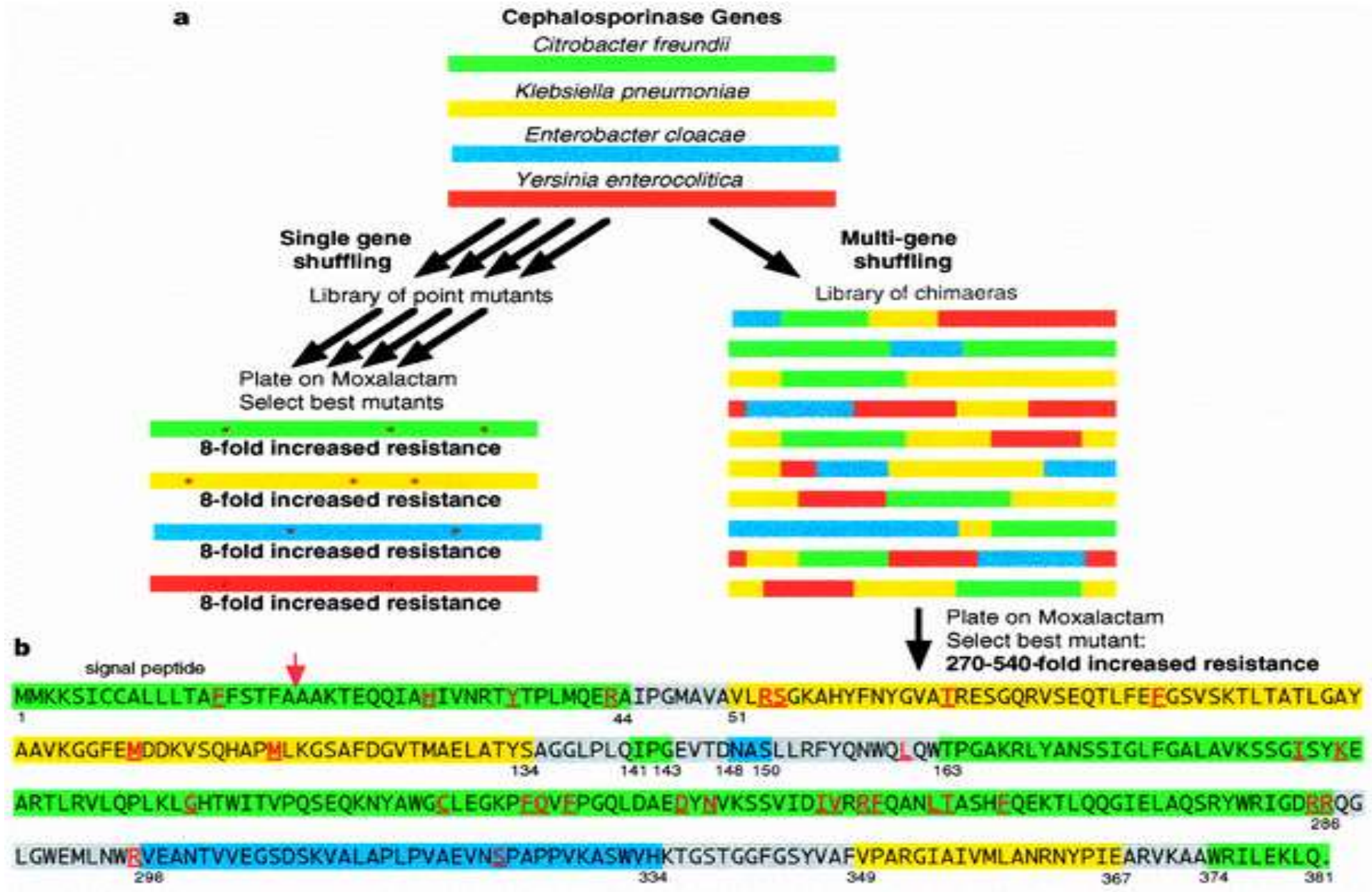
DNA Family shuffling



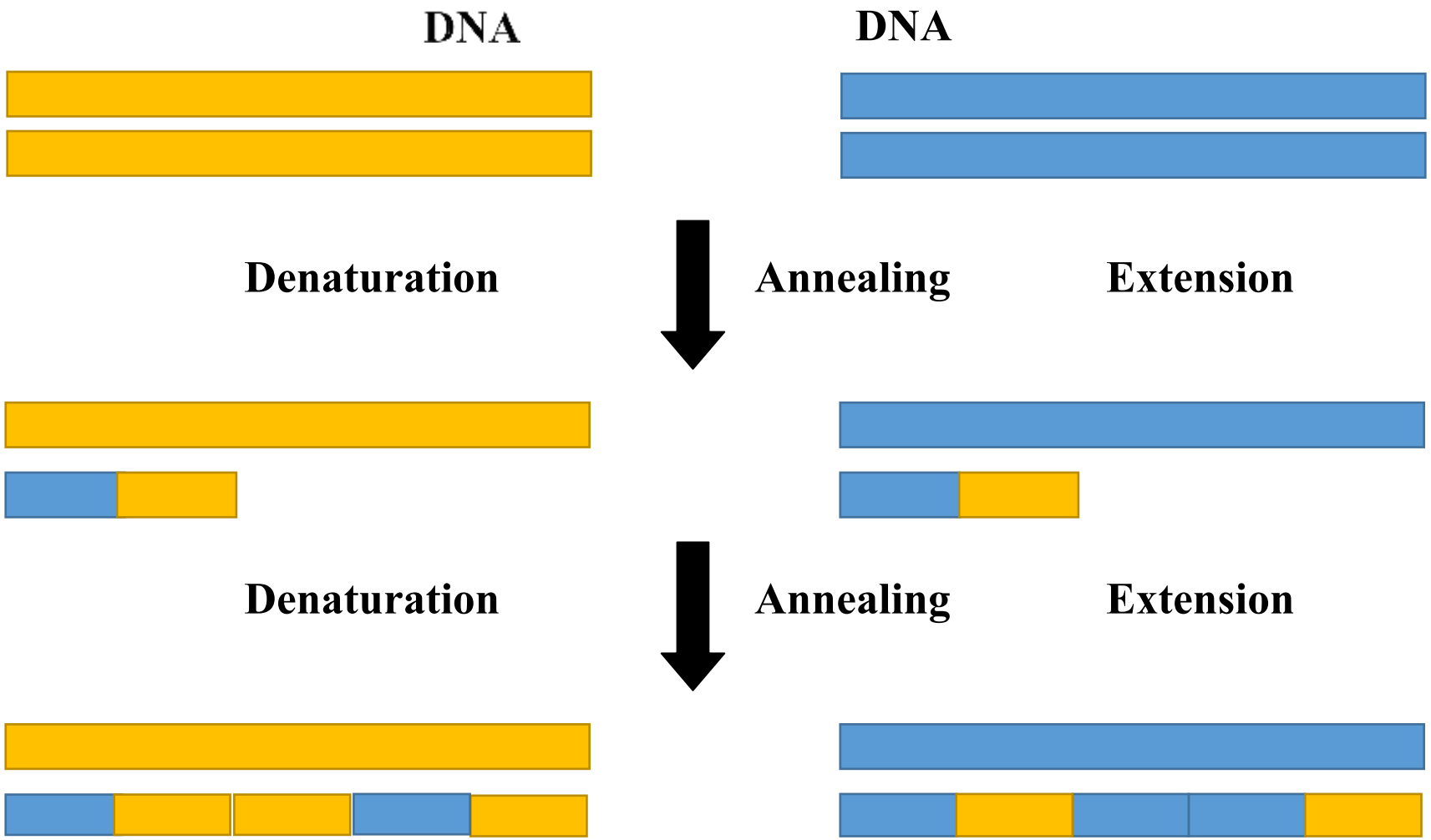
定向进化的基本过程


Single gene
Multiple genes
DNA shuffling
DNA family shuffling
Repeated
as needed
Expression and screen

Comparison of single sequence shuffling versus sequence family shuffling

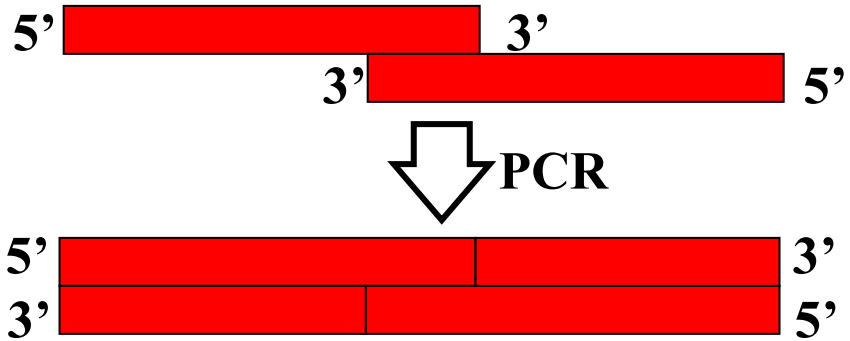


Staggered Extension Process(StEP)



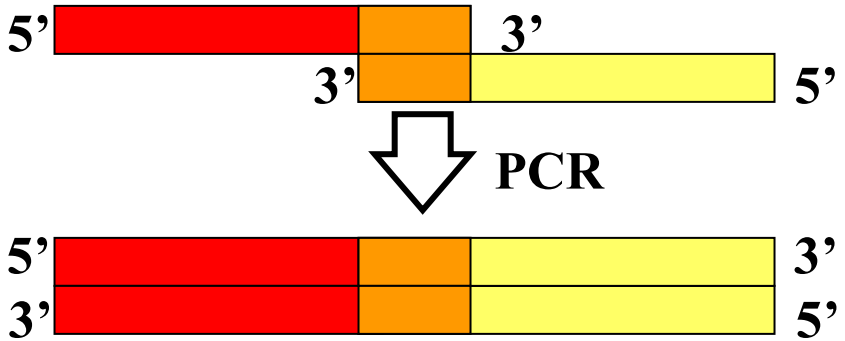
DNA shuffling - efficiency depends on homology

Homo-duplex formation



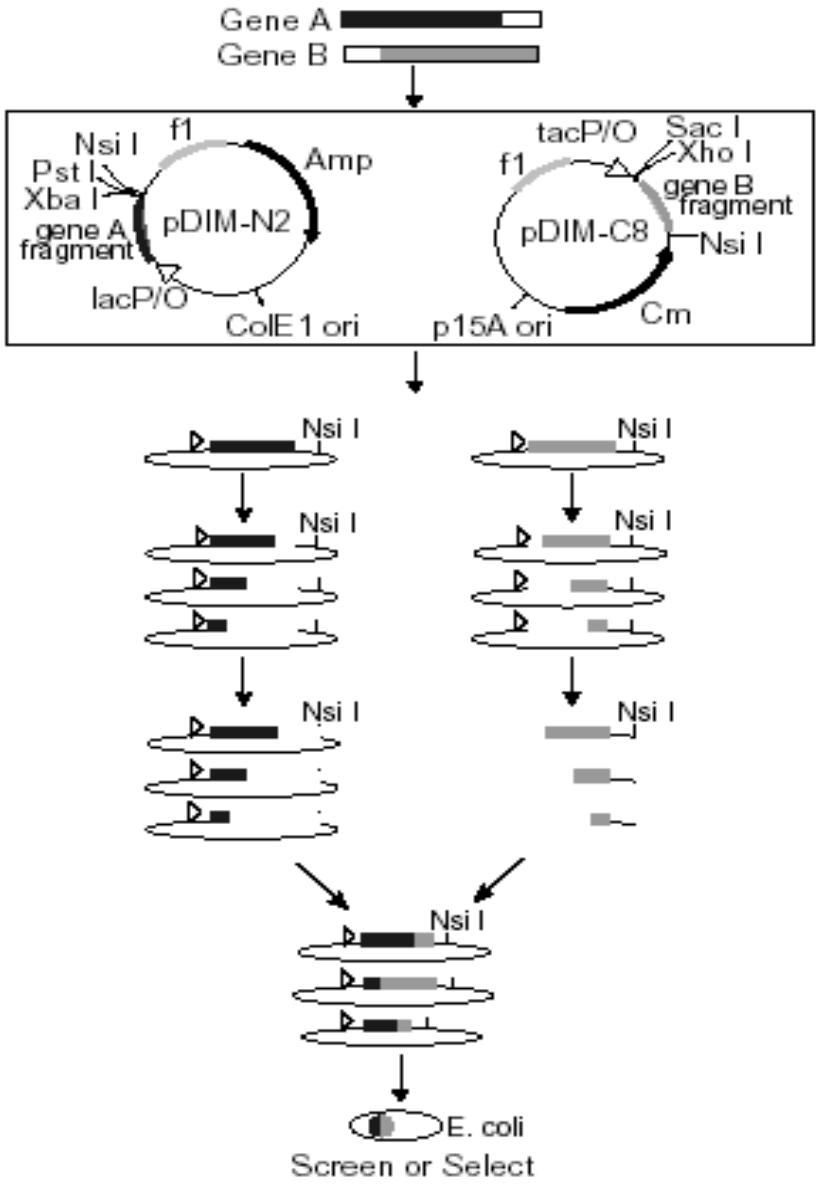
Resembles synthesis of synthetic gene (Directed Mutagenesis)

Hetero-duplex formation



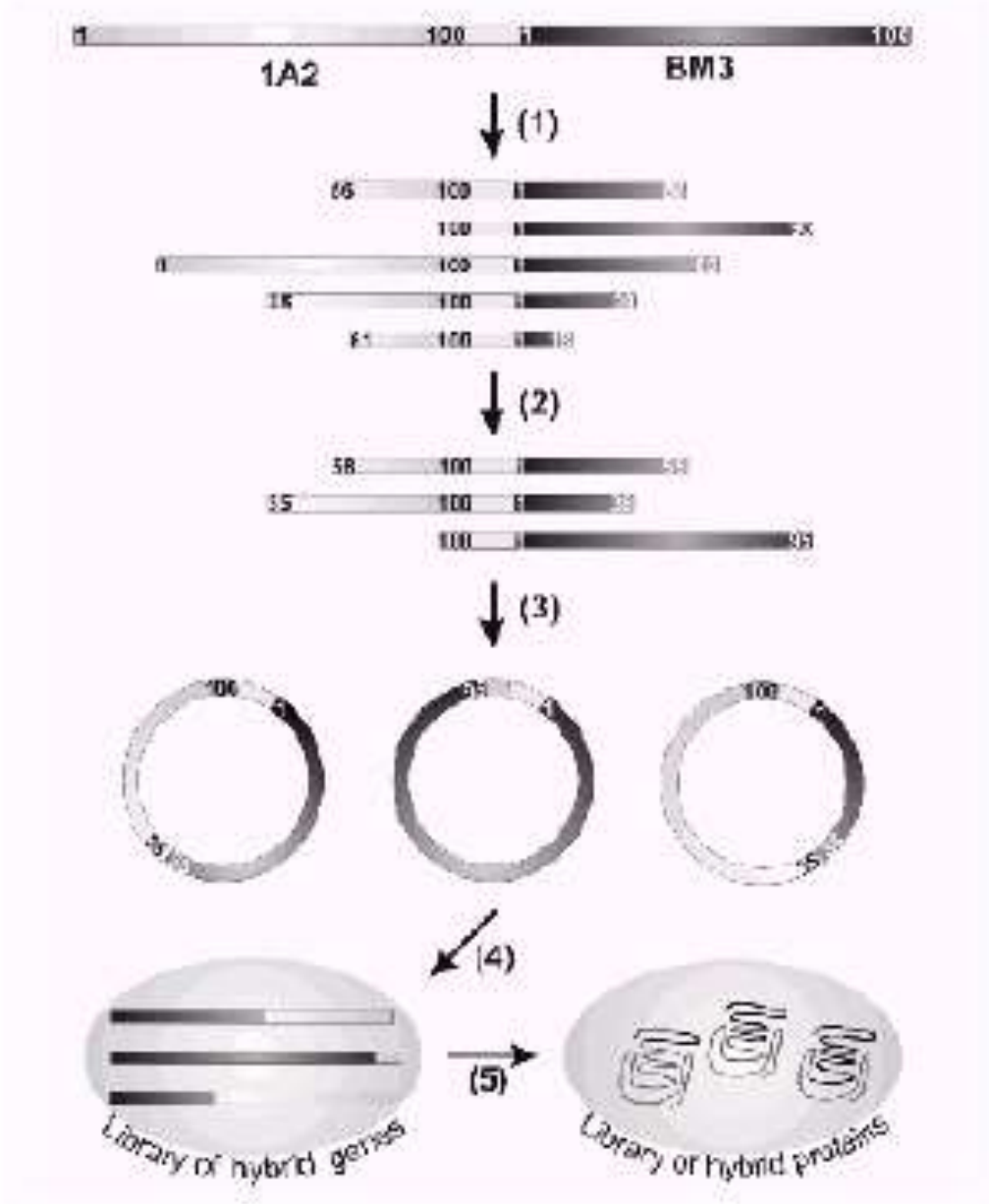
Resembles synthesis of gene fusions (Directed Mutagenesis)

Incremental Truncation for the Creation of HYbrid enzymes (ITCHY)



对随机挑选的混组酶进行测序证明ITCHY比普通混组方法在两个基因的非同源区发生了更多的交叉。而且通过ITCHY发现的嵌合酶比普通DNA混组方法获得杂合酶的活性相同或更高。

Sequence Homology-Independent Protein Recombination (SHIPREC)



应用于膜结合人细胞色素P450和可溶性细菌P450亚铁血红素结构域的混组，将混组基因库和氯霉素乙酰基转移酶基因融合，筛选到2个在细菌中可溶性比人P450高的P450杂合酶。

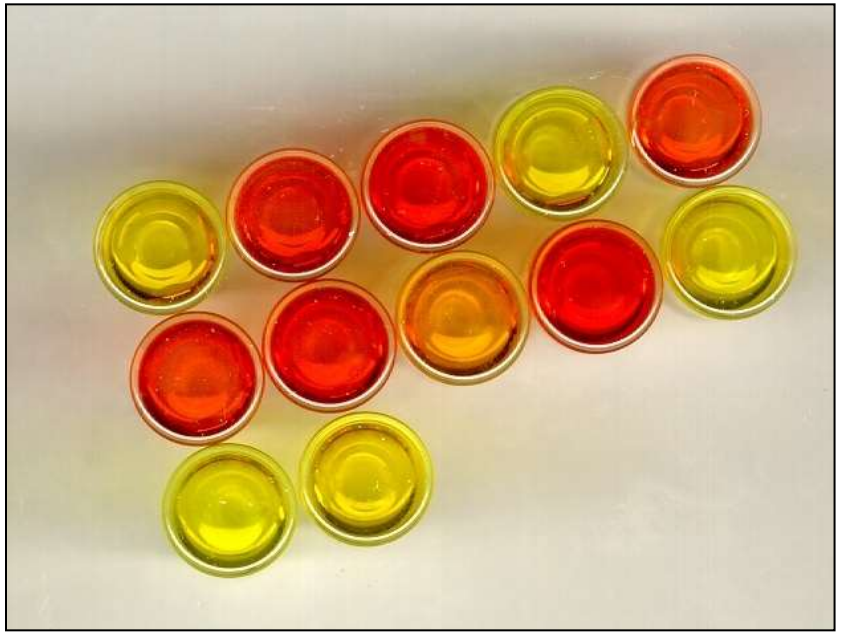
实验室进化 - 进化方法比较

- **error-prone PCR**
  2-6 nucleotide mutations / DNA molecule
  1-3 amino acid substitutions / protein molecule

- **DNA shuffling**
  high homology at DNA level (> 60% identity)
  - fragmentation by DNase (multiple recombination)
  - restriction enzymes (multiple recombination)

- **low homology at DNA level**
  - domain swapping (single-site recombination; ITCHY)
  - shuffling of "synthetic genes" (multiple recombination)

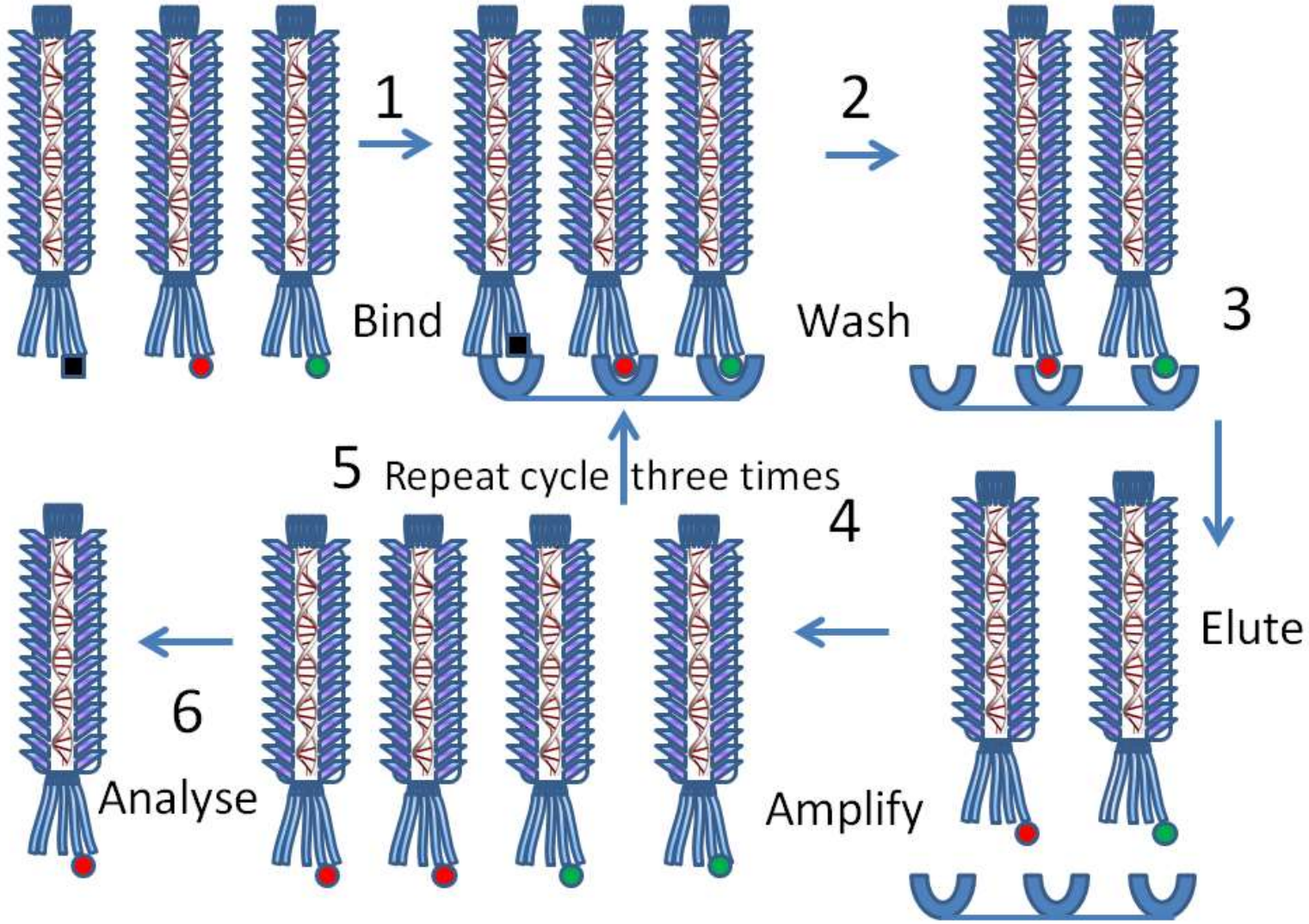
高通量筛选 - - 无理设计的有理部分



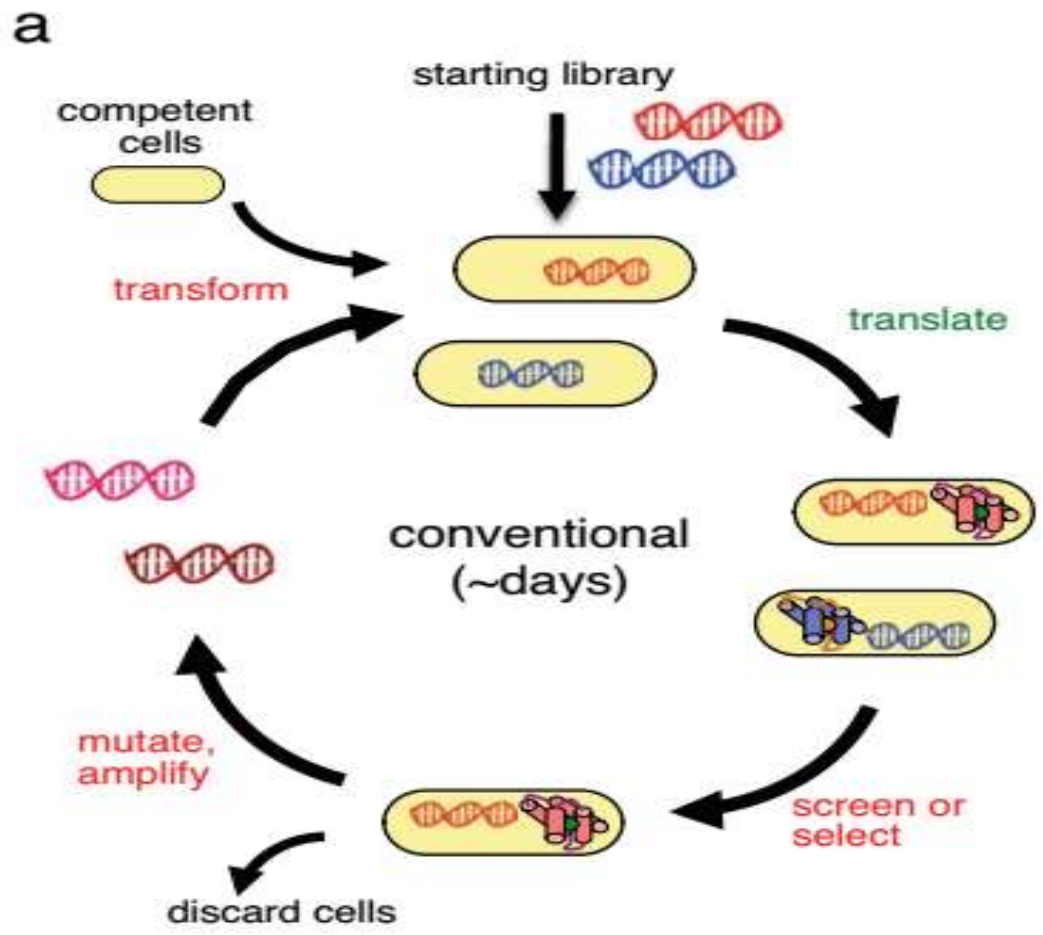
screening筛选

selection选择

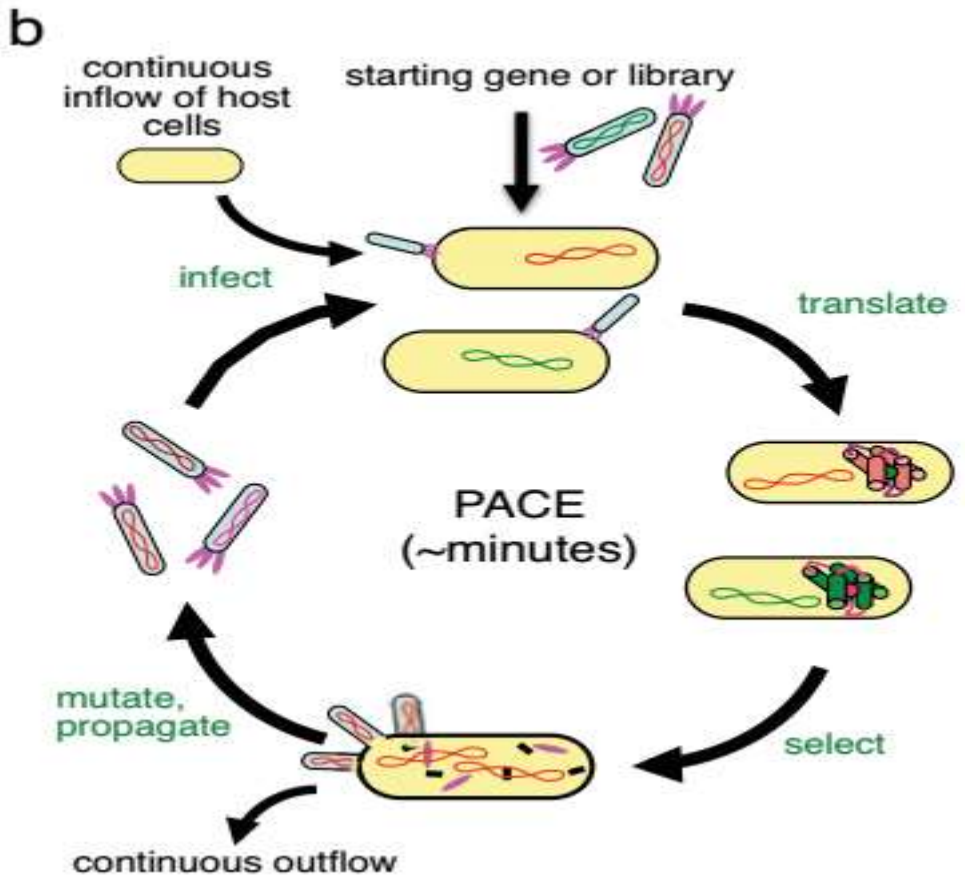
噬菌体展示技术（2018诺贝尔奖）



如何实现酶的连续进化?



红色字体表示需要研究人员参与，绿色表示不需要



噬菌体辅助的连续进化

Kevin M. Esvelt ,et al.2011

体内高频随机突变

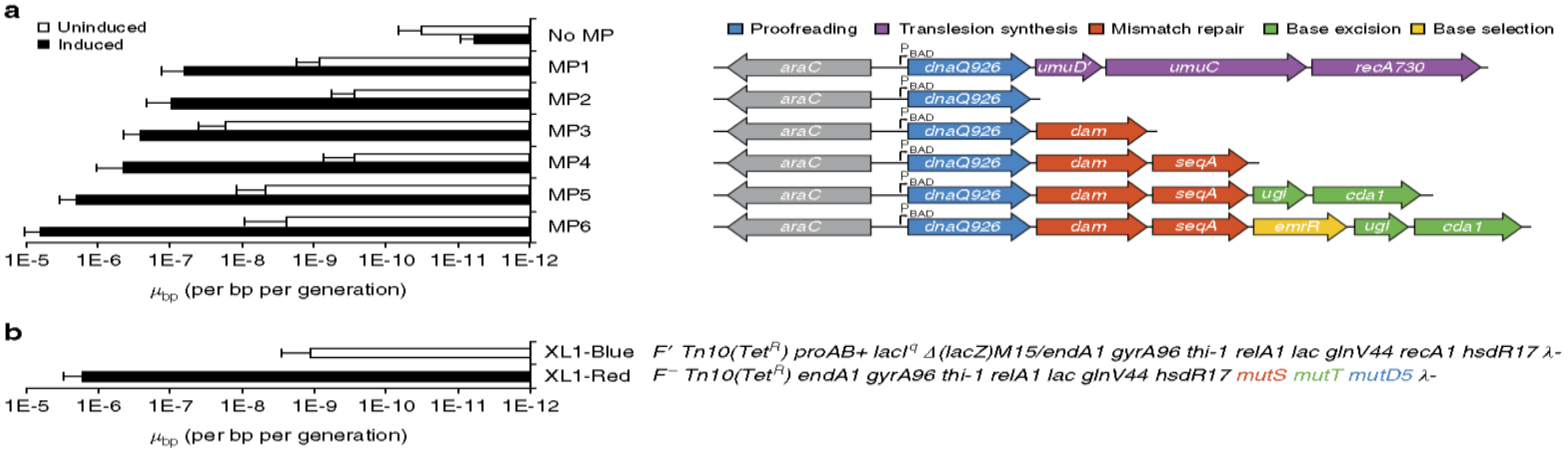
XL1-Blue strain: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI^q ZΔM15 Tn10* (Tet^r)]

正常菌株

XL1-Red strain: *endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10* (Tet^r)

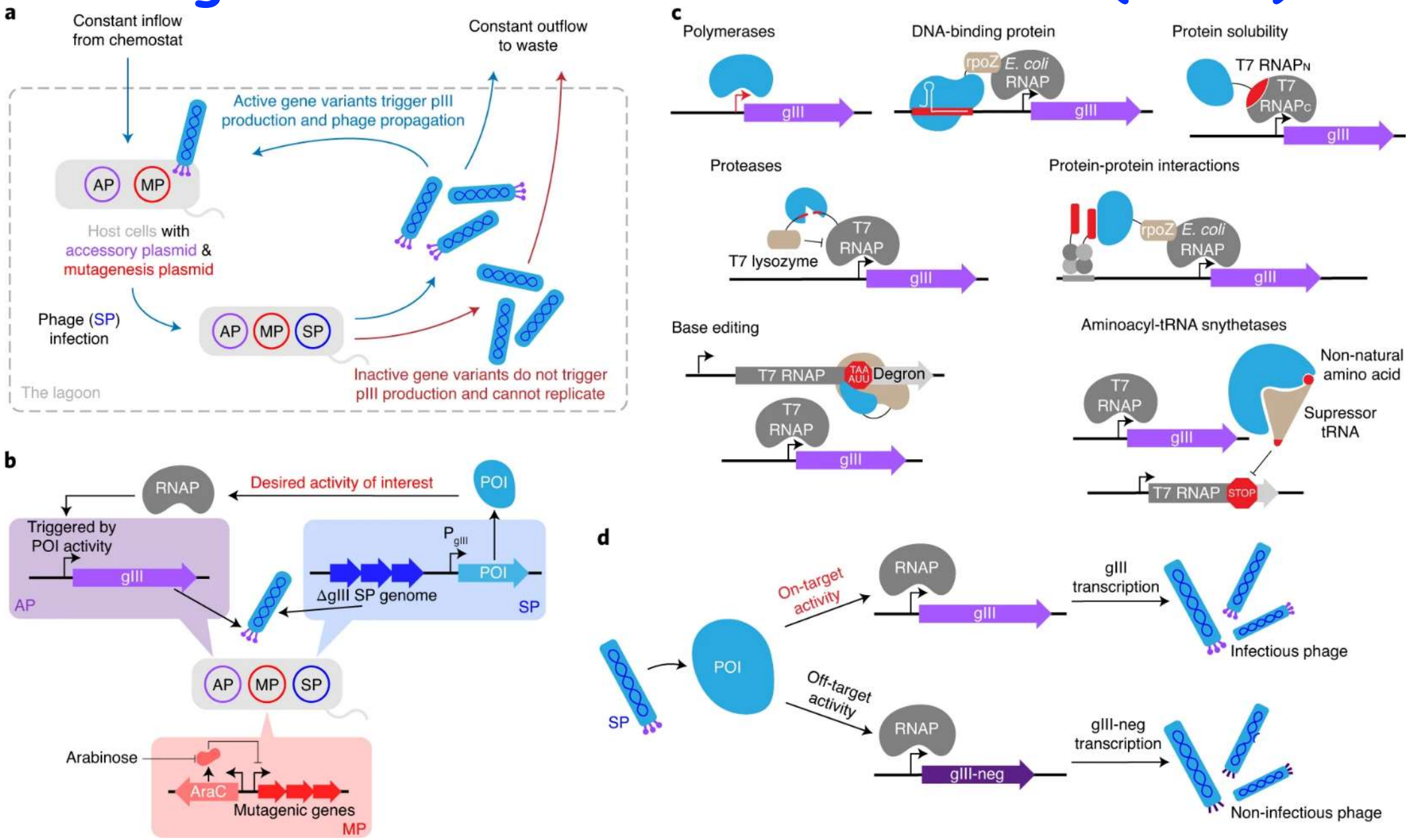
DNA修复缺陷

Greener, A. and Callahan, M. (1994), *Strategies.* 7: 32-34



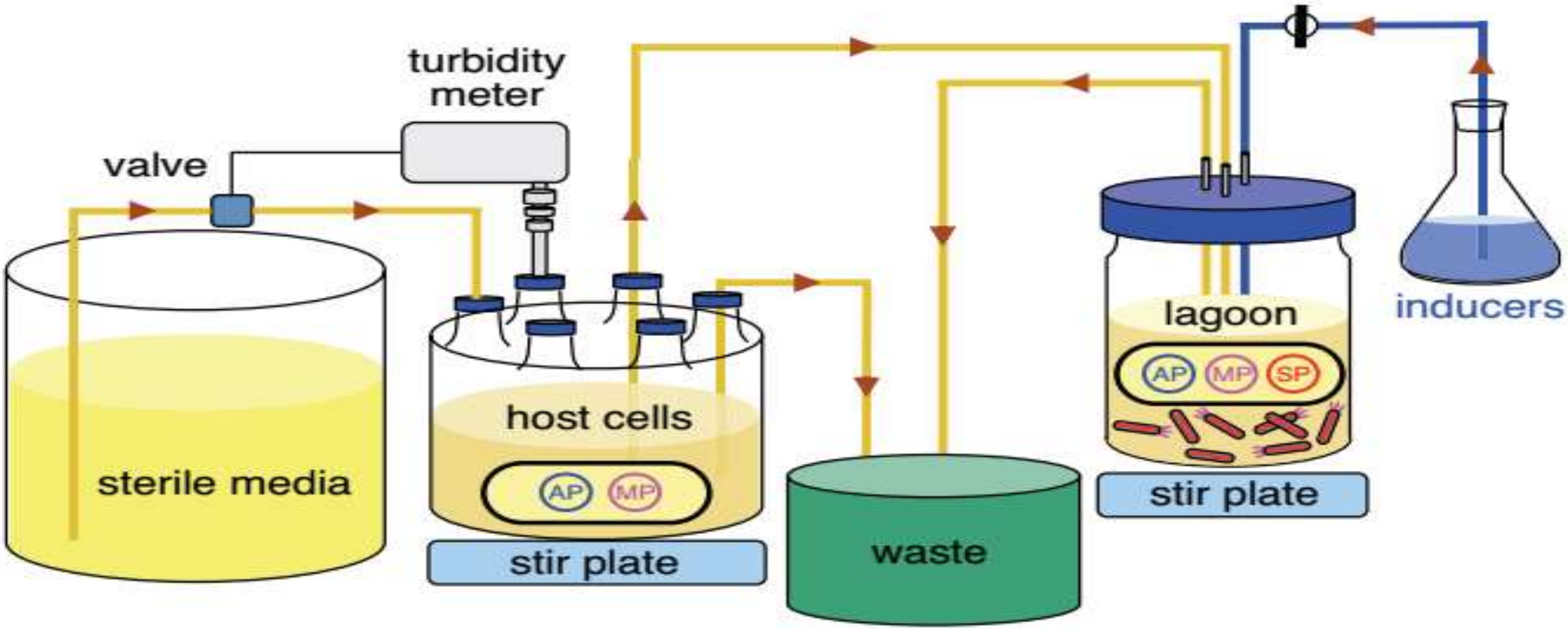
Liu D* et al. Nature Commun 2015

Phage-Assisted continuous evolution (PACE)



Liu D* et al. *Nature Protocols* 2020

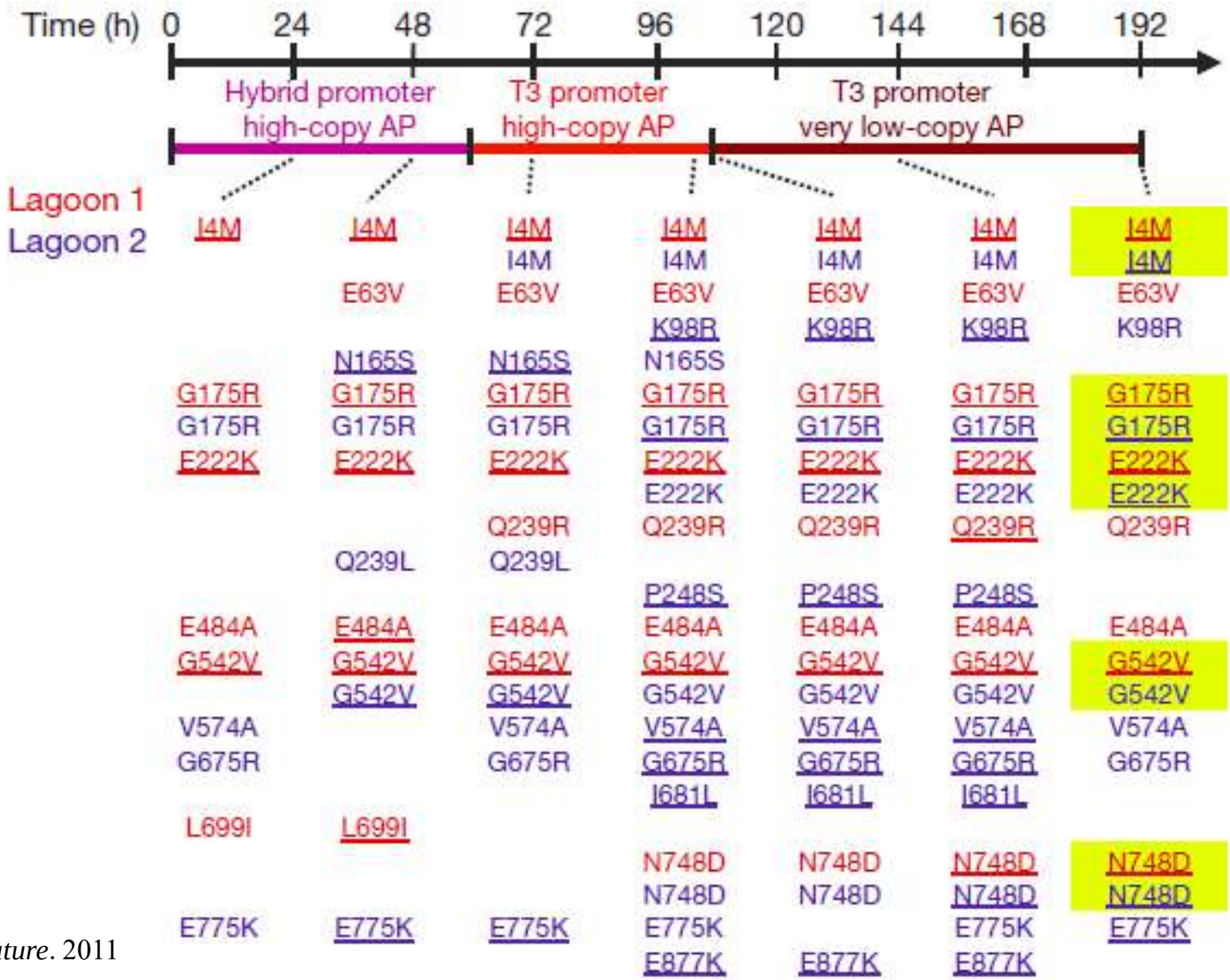
噬菌体辅助进化



MP: Mutagenesis Plasmid
AP: Accessory Plasmid
SP: Selection Plasmid

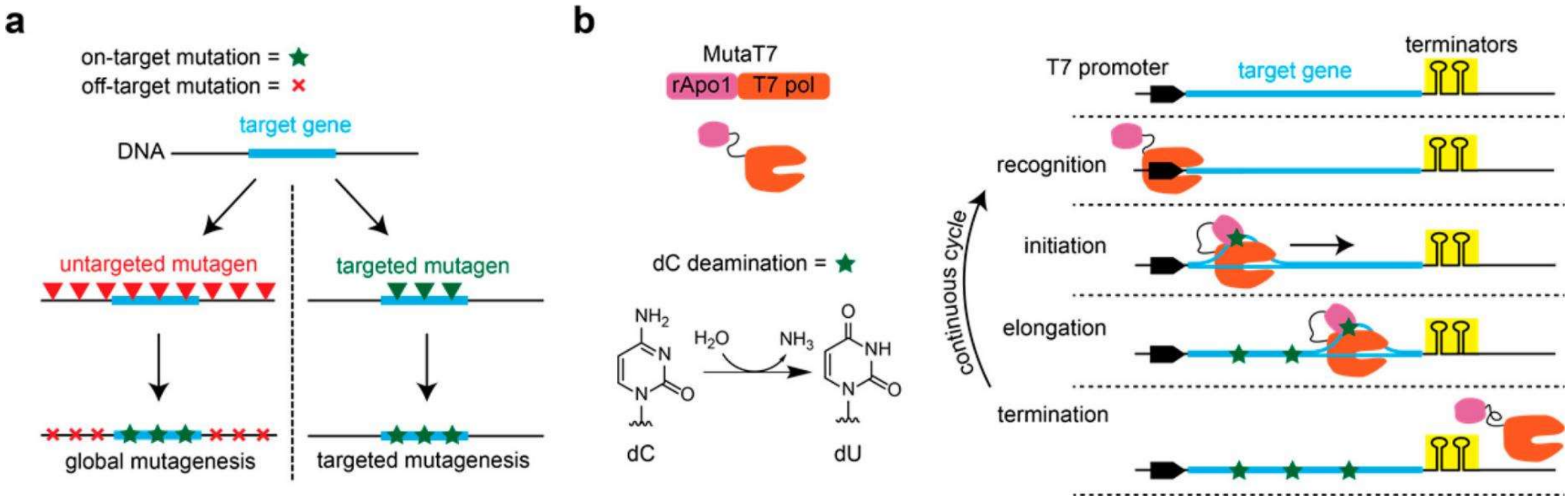
Kevin M. Esvelt ,et al.2011

PACE识别T3启动子的T7 RNA聚合酶



Kevin M. Esvelt., et al. *Nature*. 2011

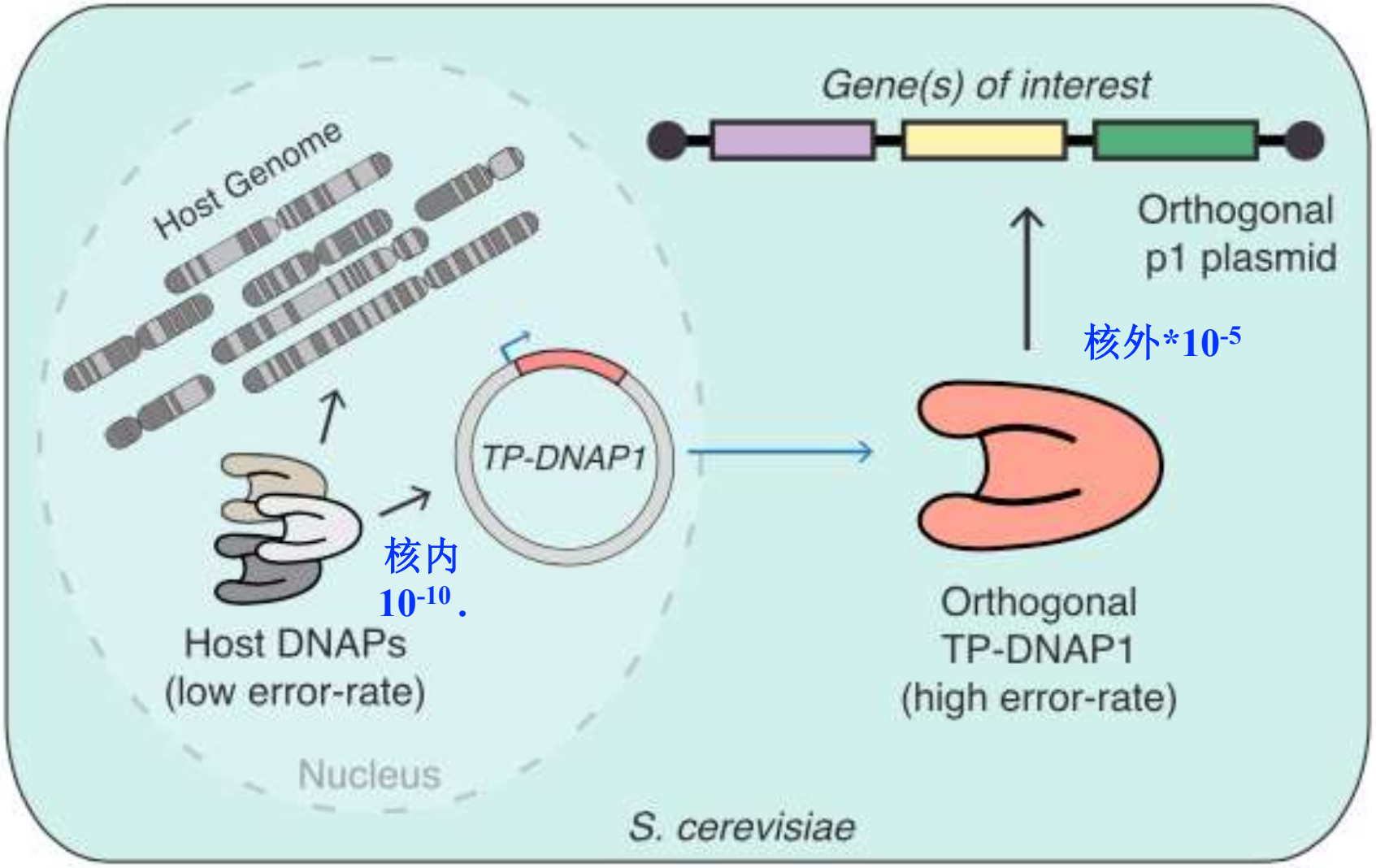
体内特定序列突变系统—T7RNA聚合酶+胞嘧啶脱氨酶



(a) Schematic illustrating global versus targeted mutagenesis. (b) MutaT7 construct and the targeted mutagenesis cycle.

DOI: (10.1021/jacs.8b04001)

Orthogonal DNA replication system (OrthoRep)

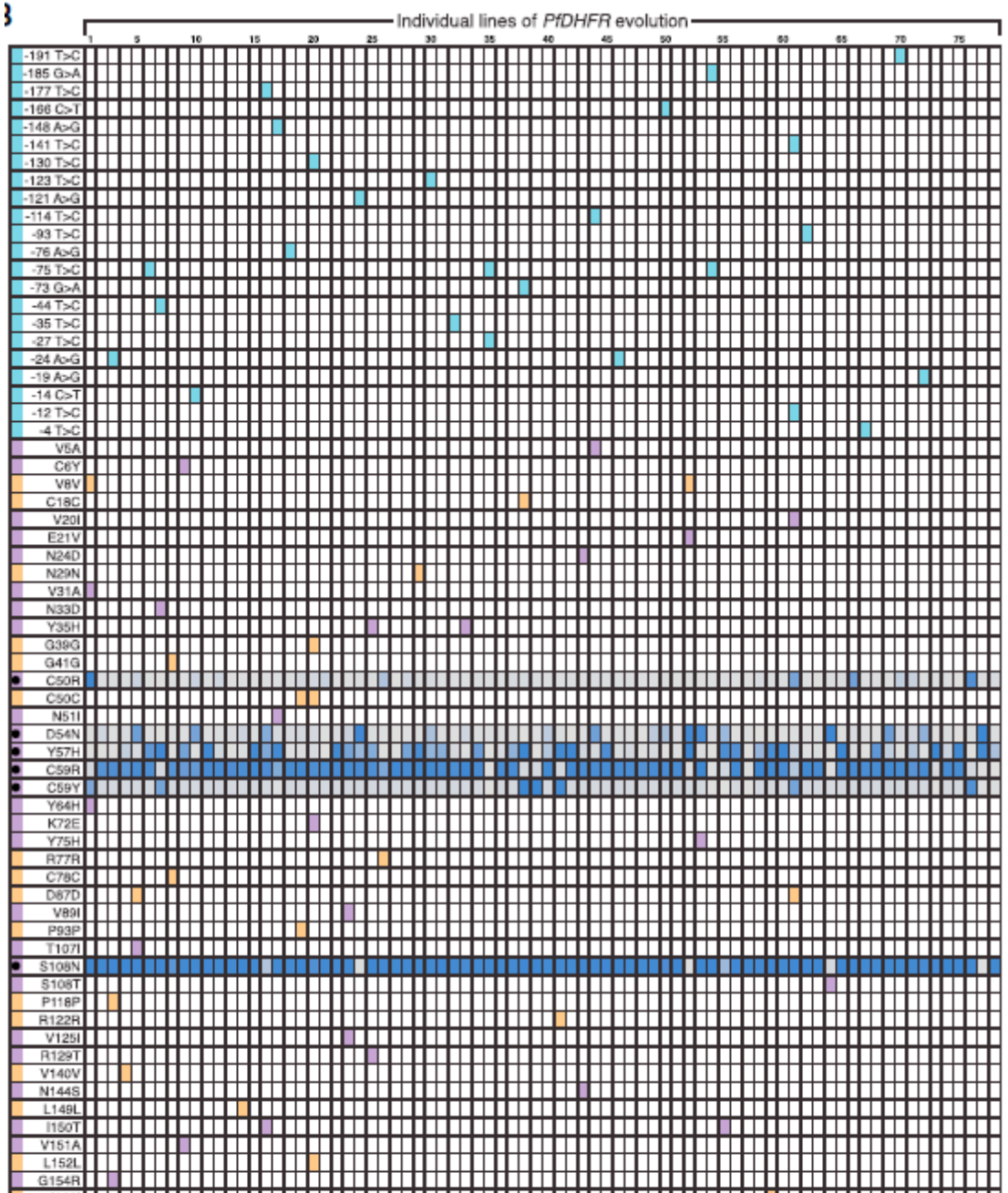


DNAPs: DNA polymerases

TP: Terminal Protein

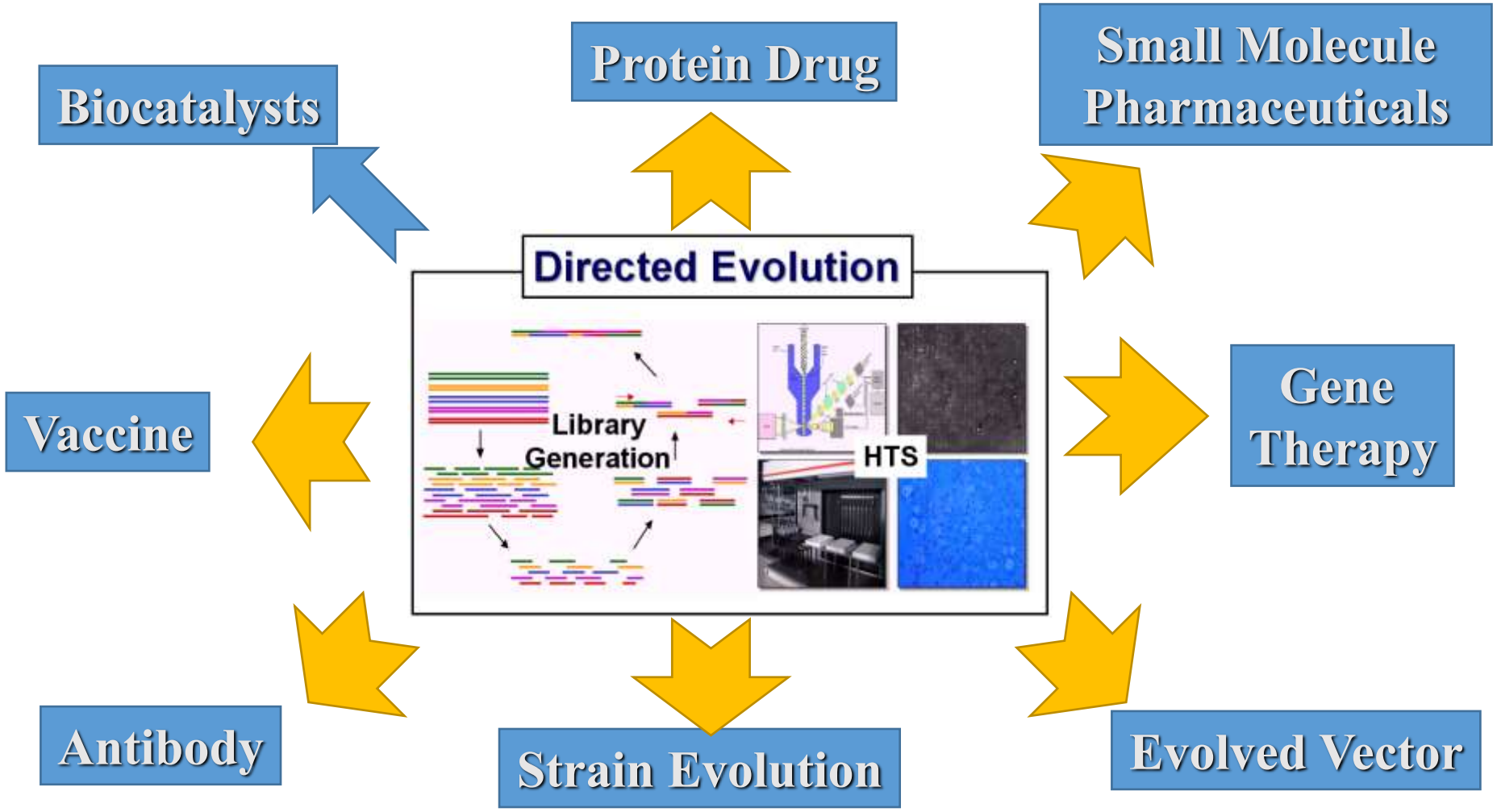
Arjun Ravikumar., et al. *Cell*. 2018

OrthoRep进化高活性二氢叶酸还原酶



Arjun Ravikumar., et al. *Cell*. 2018

定向进化的应用


Protein Drug
Small Molecule Pharmaceuticals
Biocatalysts
Directed Evolution
Library Generation
HTS
Vaccine
Gene Therapy
Antibody
Strain Evolution
Evolved Vector

定向进化的局限性

筛选包含所有可能突变体的库不现实：

含100氨基酸的酶：有20^{100}种可能组合

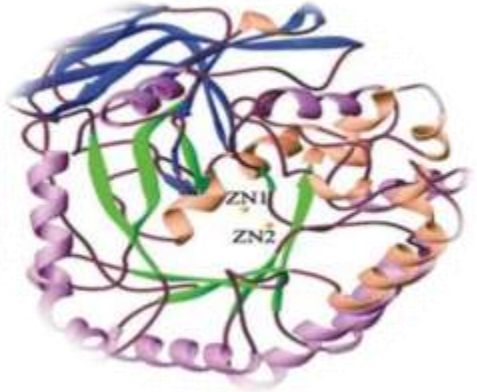
- 找到减少所需库容量的方法
  选择亲本基因
- 发展高效快速的筛选方法
  发展智能测定

定向进化小结

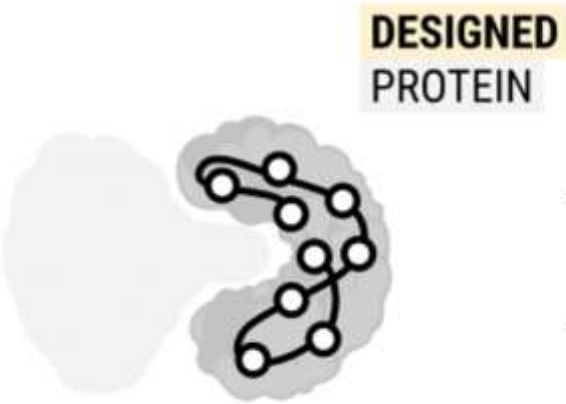
- 定向进化是更有效的蛋白质改造方法
- 突变/重组方法成熟
- 筛选能力是成败关键

各种设计与改造方法的结合

有理



天然



从头

无理

小结 蛋白质工程实验策略

选择起始基因建立测活体系和/或筛选方法

如果知道结构功能关系，先采用有理设计方法

随机突变微调并高通量筛选

没有合适起始基因才从头设计

课程要点总结

- 大肠杆菌表达优化流程
- 蛋白质分析工具

大肠杆菌表达实验策略（学术版）

✓首选pET24或pET28（如果需要纯化）与BL21(DE3)

✓TB培养基37°生长到1-1.5OD，18 ℃生长1小时到3OD ，0.5mM IPTG诱导19小时至OD达10

- **高表达低可溶的解救措施→**

1. 降温低至15℃
2. 换培养基为2xYT或ZYP5052(自诱导)，换表达宿主
3. 截短N端和/或C端2-10个氨基酸残基
4. 与MBP等高可溶性蛋白融合表达
5. 化学诱导分子伴侣、共表达分子伴侣/作用蛋白或提供配体

Graslund et al 2008 Nature Methods

酶改造工具

- 酶数据库 Brenda
- 结构预测 Swiss-Model/I-TASSER
- 分子图像 PyMOL

微信公众号

课后请多提宝贵意见
助我进步，再次谢谢大家！

2017年《实验生物学》期末考试

题目：《详述重组表达你所感兴趣的某个蛋白质的技术路线》

总分16.5（及格分9.9分）

得分段分布：

8~9.5分：不合格分段：5份（1份8分，1个9分，3个9.5分）

对题意理解不明，答题毫无逻辑；三大块主要内容（序列获取、表达系统选择和表达优化）最多提到一块内容。

10~11.5分：及格，低分段：27份

有基本逻辑，答题中能艰难看出三大块主要内容，或者只提到其中两块内容并有基本描述。

11.5~13.5：中等分段：32份

提到三块主体内容，并给出基本描述。

14~15.5：高分段：31份

逻辑清楚，三块主要内容都能答到，但有明显缺漏或部分描述有误。

16分：5份

三块主要内容明确，且得分项按条罗列明确，表述基本无误。

评分标准

1、 序列（目的基因）获取（共5分，提到就给3分。下列具体方法两条各1分；对于具体案例，只要提到获取相应基因，3-5分酌情给分）

1.1 序列已知：文库中亚克隆，直接(RT)PCR获得，合成

1.2 序列未知：联配同类酶的已发表序列，根据保守氨基酸序列设计简并引物扩增基因；纯化酶，测定部分氨基酸序列，设计简并引物扩增基因；以方法1，2设计DNA探针进行southern杂交和/或菌落杂交；纯化酶，制备抗体，用免疫印迹的方法筛选阳性克隆；基因组测序

2、 表达系统选择（共4.5分，提到就给2分；提到大肠杆菌/pET系统加1分，描述出优势加0.5分。 提到优先根据文献中条件，次选大肠系统-酵母系统-其他系统，并阐述优略及选择标准，打3.5分及以上）

3、 表达优化（共7分，提到就给3分。提到需要提高表达量&可溶性加1分，下列具体策略三条各1分。）

A 高表达低可溶：降温低至18°C；LB换TB培养基；与MBP融合表达；共表达分子伴侣改善可溶性；蛋白质工程改造

B 高可溶低表达：换Rosetta宿主；密码子优化

C 低表达低可溶：优化可溶性——优化表达