默克集团公司简介

世界上历史最悠久的医药化工企业，其历史可追溯到1668年，总部位于德国达姆斯达特

在64个国家拥有分支机构，员工近40000人

2010年收购 Millipore- Merck Millipore

2011年收购清大天一

默克密理博

生命科学部——基础研究，药物研发上游

医药化学解决方案部——生物药物生产中试至生产

生物制药工艺部——生物药物生产中试至生产

实验室纯水部——科研及工业高品质水机及系统

实验室基础部——各类化学实验室

医药化学解决方案业务部

上游过程

细胞培养基

- 化学限定细胞培养基
- 个性化细胞培养基
- 单一添加成分

重组或来源于动物的细胞培养添加物

- 澳洲胎牛血清

生物反应器 10L-4000L

下游过程

过程中所需添加的物质

- Benzonase® 核酸内切酶
- 酶及其他特别添加物

过程中所需的化学物质
EMPROVE® bio

- 矿物质
- 缓冲液
- 各种辅助化学物质

制剂

生物制剂过程中所需的各种物质

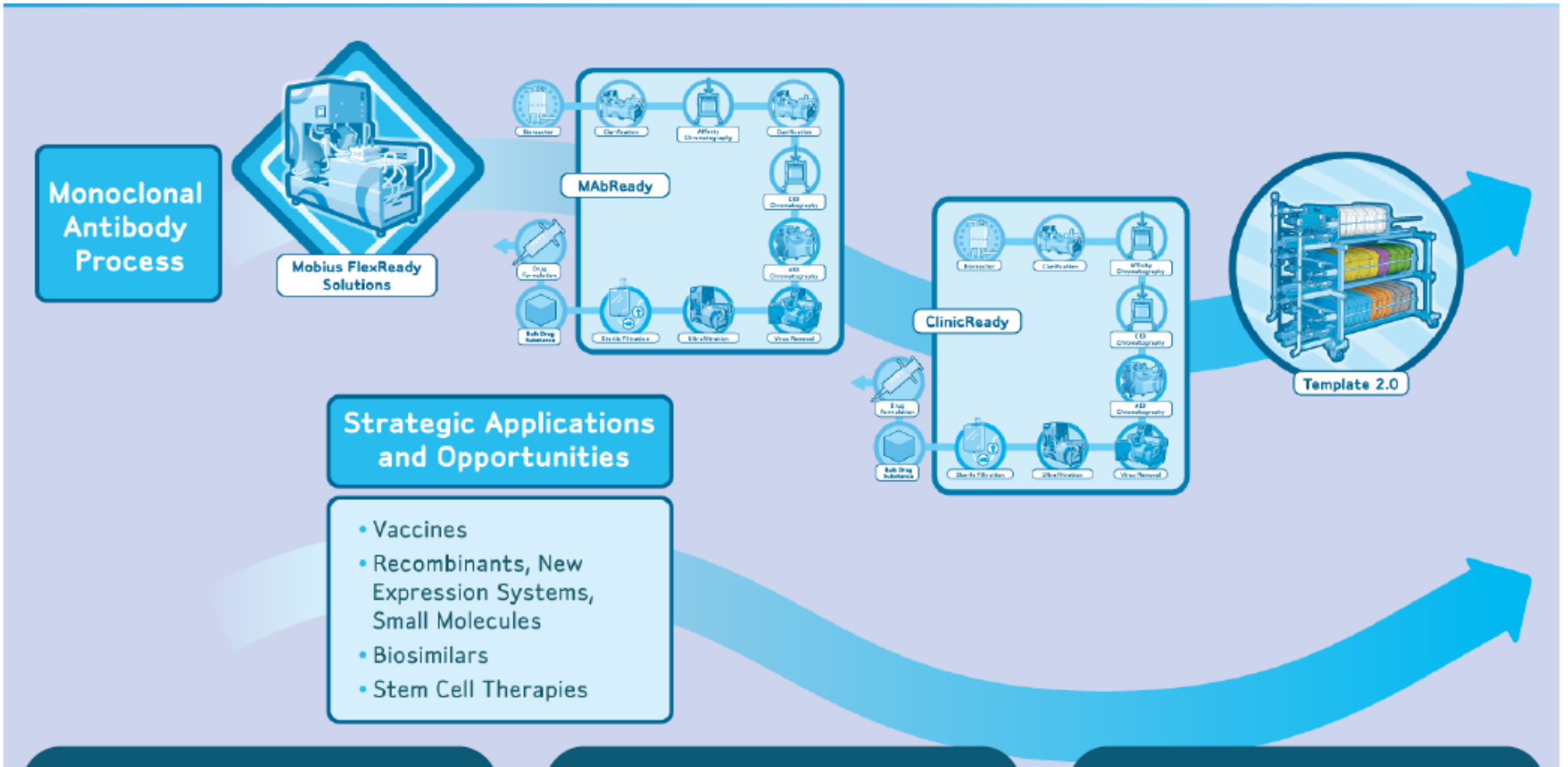
药物输送物质

- 聚乙二醇化修饰技术
- 微脂粒药物代谢技术

在线清洗液

氢氧化钠, 异丙醇
客户定制化清洗液（高纯水配置，符合欧盟、美国药典要求的清洗剂）

默克密理博—生物制药工艺部



专业知识

- 生物制药行业超过30年的工艺开发和生产专业知识
- 帮助符合法规的要求及咨询服务

领先技术

- 澄清、除病毒及除菌过滤
- 超滤及层析
- 一次性技术
- 无菌取样

卓越能力

- 为细菌及哺乳动物细胞系统进行工艺开发和优化
- 工艺放大及缩小
- 临床药物准备

提高活性蛋白表达水平，事半功倍

——默克密理博Novagen重组表达平台介绍与经验分享

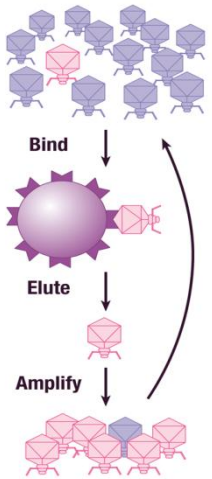
何煜 (Allen He)　June 2013

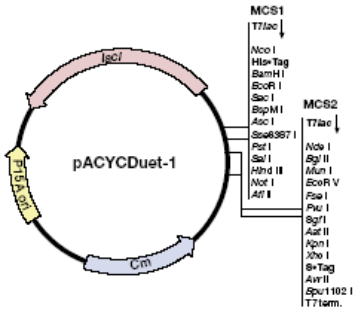
400-889-1988-2 欢迎垂询

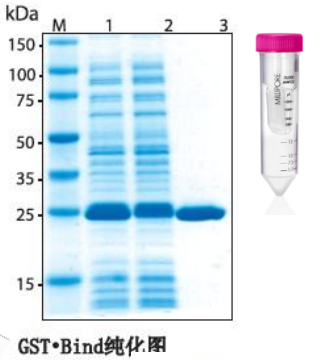
默克密理博支持生物制药公司研发部门

各种规格的优质生化试剂：抗生素，缓冲液，表面活性剂……

基因克隆	原核蛋白表达	真核细胞培养与表达	产物纯化	质量控制
快速优化目的片段克隆	高产表达抗原或活性蛋白产物	制备抗体和活性蛋白	高效抗体或活性蛋白纯化	验证产物质量的多种平台







Western Blot
ELISA
流式细胞
多因子检测
信号通路抑制剂
药物吸收/扩散
细胞毒理
抗体
……
……

T7Select噬菌体展示系统

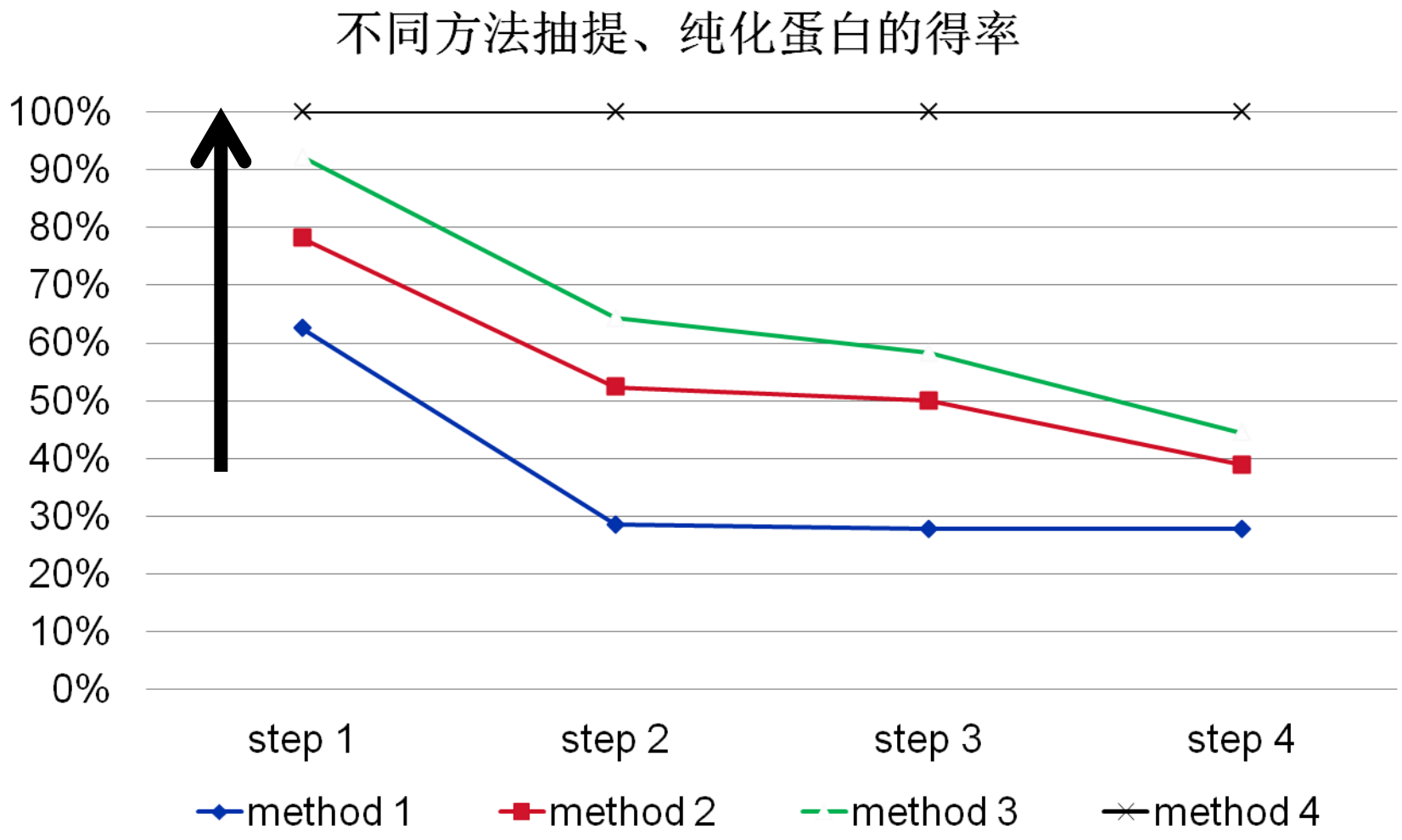
PCR克隆

pET载体及表达宿主菌
LIC 1天快速表达克隆
蛋白复性

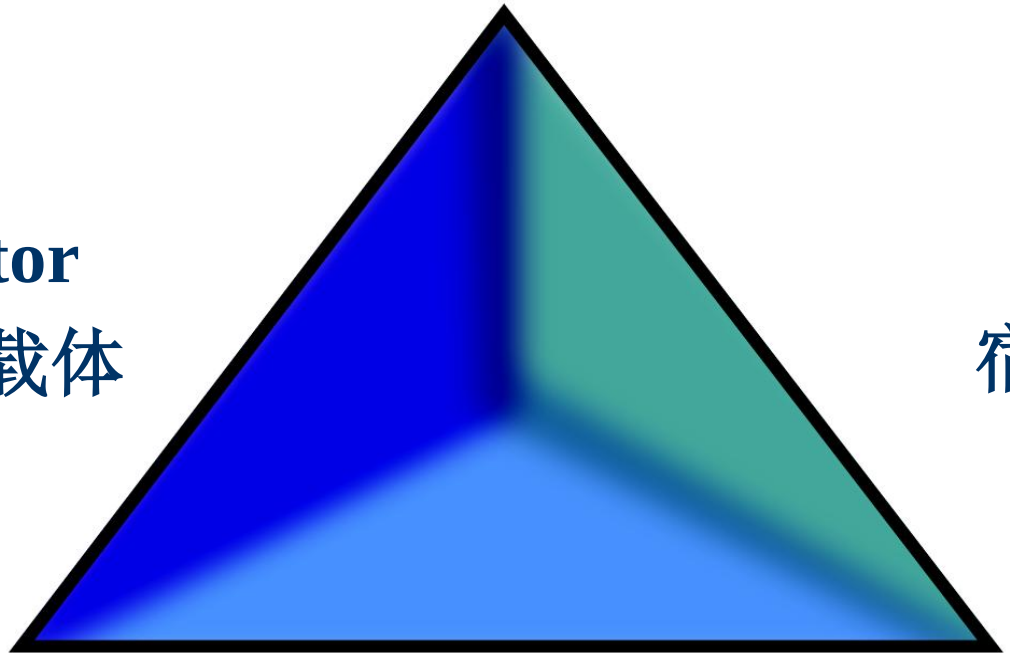
昆虫细胞表达快速评估与高产平台
无菌过滤与细胞培养
快速准确细胞计数器

His/GST亲和纯化
高通量蛋白抽提纯化
抗体纯化
蛋白透析与超滤

如果能大幅提高蛋白产量……



重要原则：选择合适的表达平台（宿主）



Vector
表达载体

Host
宿主细胞

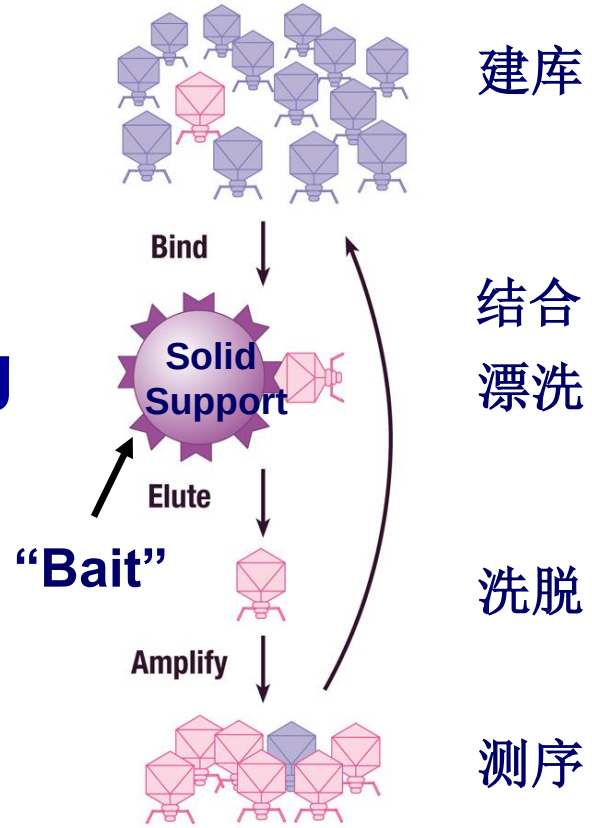
Growth conditions
培养条件

表达什么？本身就是个问题……

T7Select® Phagedisplay噬菌体展示技术

- 快速克隆新基因
- 快速优化基因

Biopanning
生物淘筛



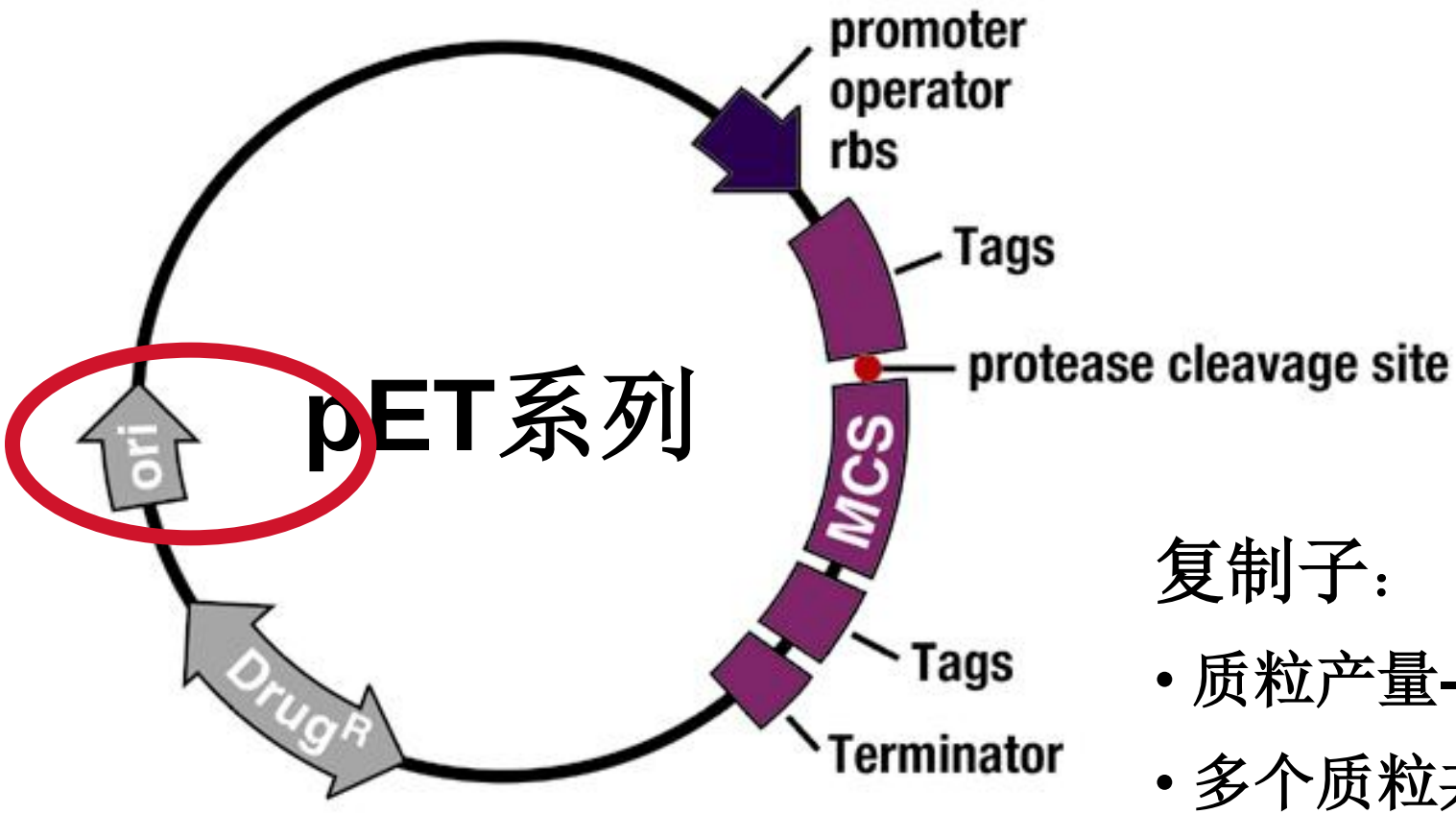
经美国食品和药物管理局（FDA）批准的重组抗体

目前有400多项关于抗体治疗的临床研究正在进行，其中42项已经进入III期实验或正由II期转入III期；174项正在进行I期或由I期转入II期实验（Stenfan Dubel于**2005年5月**报道）

Avastin（阿瓦斯丁）：抗血管内皮生长因子（VEGF）抗体，抑制肿瘤血管生成；用于治疗肠癌
Herceptin（赫赛汀）：抗酪氨酸激酶受体（抗HER2/neu）抗体，能显著抑制HER2/neu过度表达的乳腺癌细胞的增殖
Humira：风湿性关节炎
Leukosite：B细胞白血病
Mylotarg：急性髓性白血病
Rapitva：2003年获批用于中度、重症斑状牛皮癣、银屑病
Synagis：呼吸道合胞体病毒感染（1998年获批，最早的人源化抗体药物）
Tysabri：多发性硬化
Xolair：过敏性哮喘
Zenapax（以及Simulect舒莱）：抗CD肾移植后的排斥反应；Zenapax是由于哮喘及其他呼吸道系统疾病的人源化抗体
Cotara：多种癌症
Erbitux（爱必妥）：默克雪兰诺；抗表皮生长因子受体（EGFR抗体）；2004年获批用于治疗直结肠癌
Remicade：抗肿瘤坏死因子（TNF-alpha）抗体，治疗风湿性关节炎，克罗恩病，节段性回肠炎
Reopro（阿昔单抗）：抗血凝
Rituximab（利妥昔）和Alemtuzimab：抗CD20；先用于非霍奇金淋巴瘤，后尝试用于免疫缺陷相关淋巴瘤和中枢神经淋巴瘤。Rituxan 2006年获批用于成人
Simulect：肾移植后的排斥反应
Zevalin：淋巴瘤，风湿性关节炎
h-R3：治疗头颈部、胃、肺、乳腺等的上皮源性肿瘤

超过30%的都采用了噬菌体展示技术

重要原则：选择合适的载体

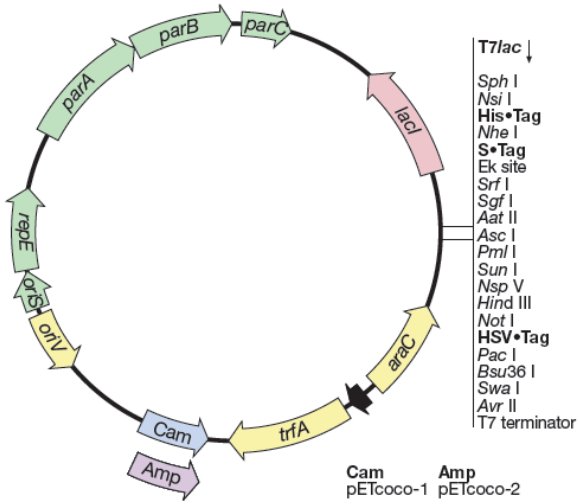


复制子：

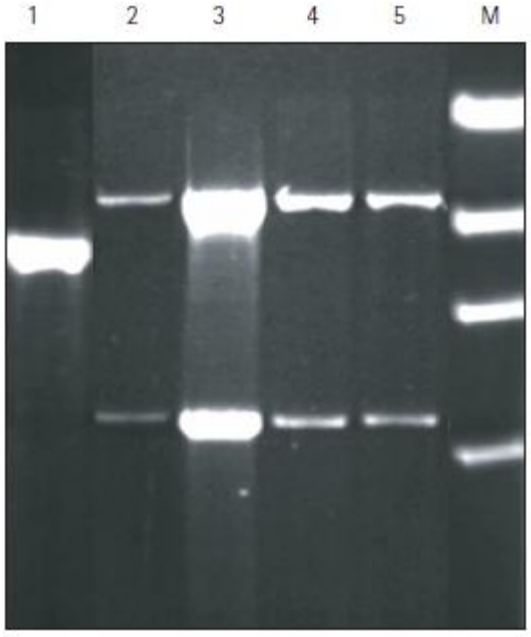
- 质粒产量-蛋白产量
- 多个质粒共表达

T7-driven Expression Vector Families

Vector Family	Replicon (source)	Copy Number
pET	ColE1 (pBR322)	~ 40
pETDuet™		
pETcoco™	Mini-F/RK2 (pBeloBAC11, RK2)	1, amplifiable to ~ 40
pETBlue™	ColE1 (pUC)	> 500
pTriEx™		
pBiEx™		
pACYCDuet™	P15A (pACYC184)	10–12
pRSF	RSF1030	> 100
pCDF	CloDF13	20–40
pCOLADuet™	ColA	20-40

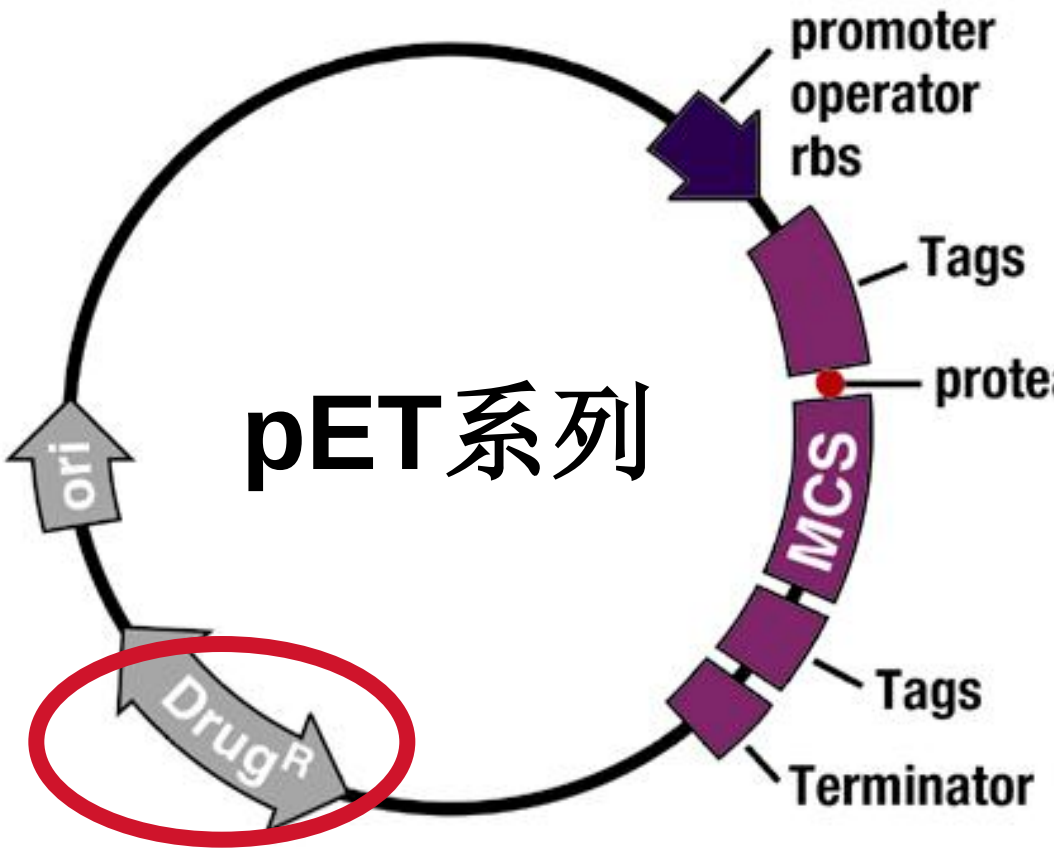


pETcoco-1 and pETcoco-2 vectors



Lane	Sample
1	pET-24.lacZ, uninduced
2	pETcoco-1.lacZ, uninduced
3	pETcoco-1.lacZ, induced (arabinose)
4	pETcoco-1.lacZ, induced (arabinose, then IPTG)
5	pETcoco-1.lacZ, induced (IPTG)
M	λ HindIII markers

重要原则：选择合适的载体



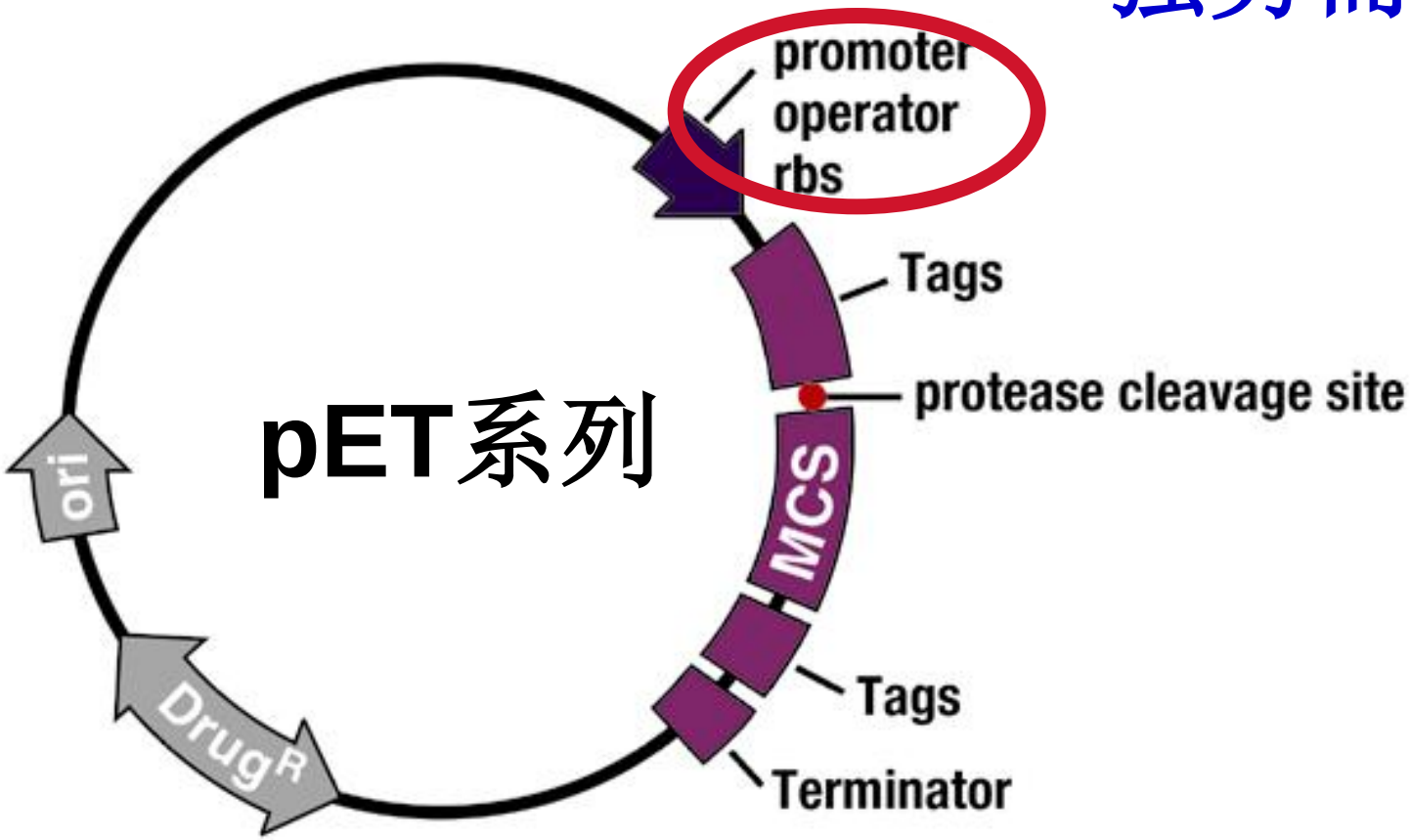
筛选标记：

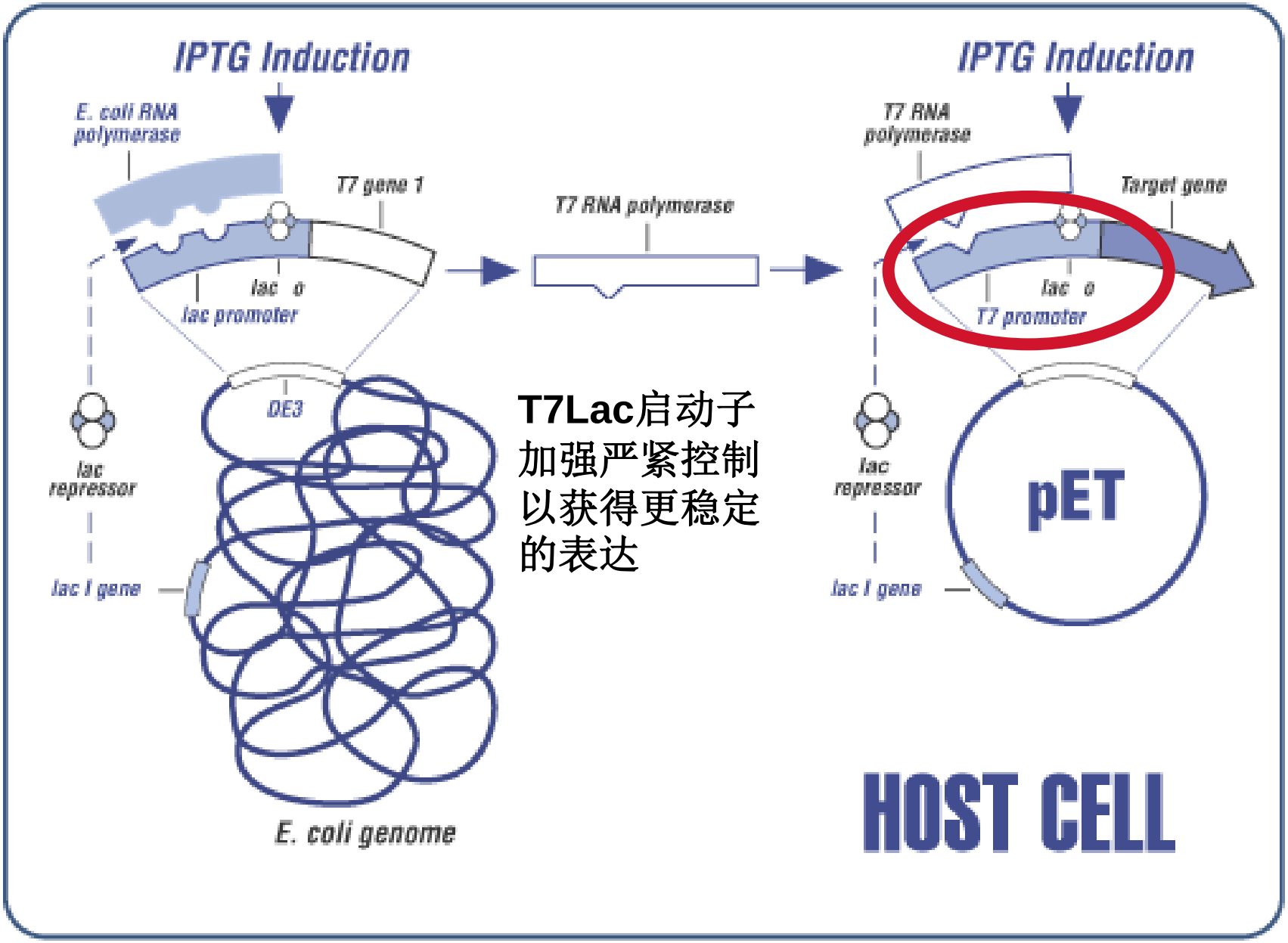
- 常用青霉素/卡那霉素
- 链霉素/四环素等

重要原则：选择合适的载体

启动子：带有**T7 Lac**启动子的**pET**系列表达载体成为金标

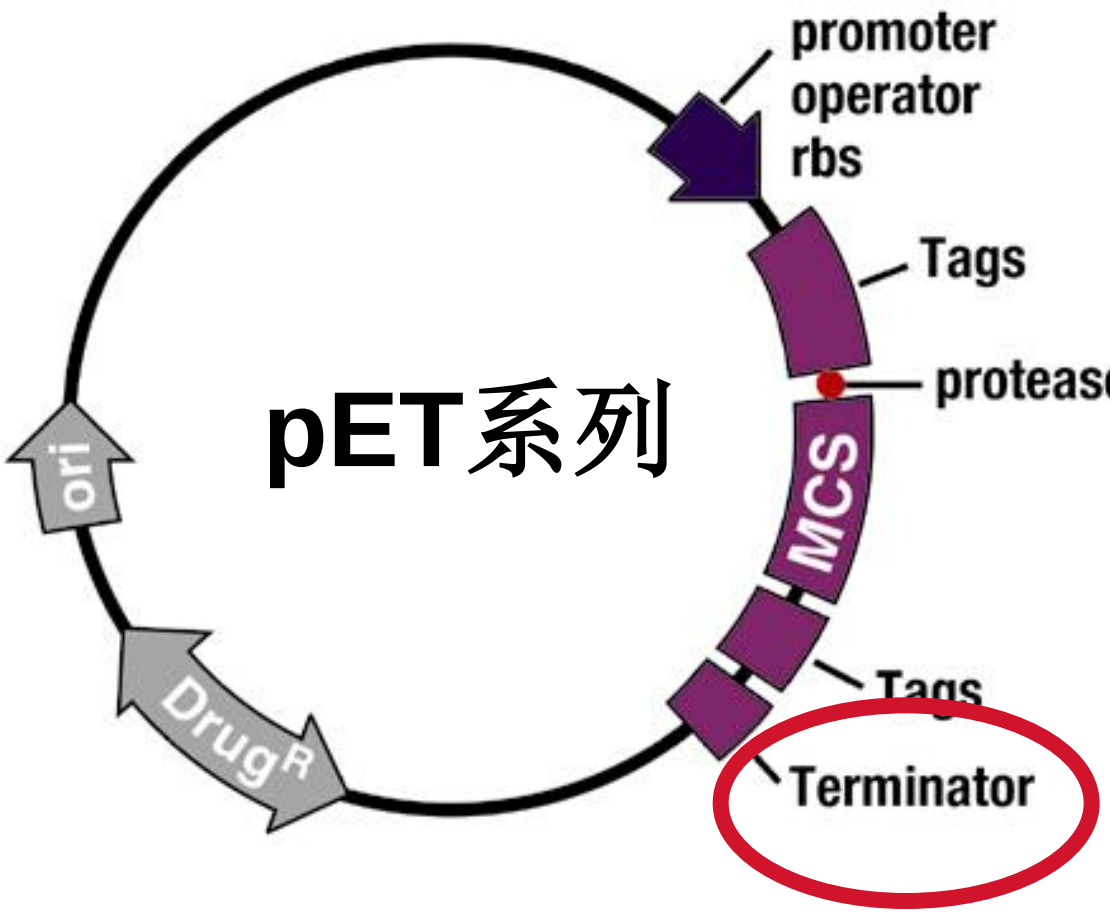
强势而可控





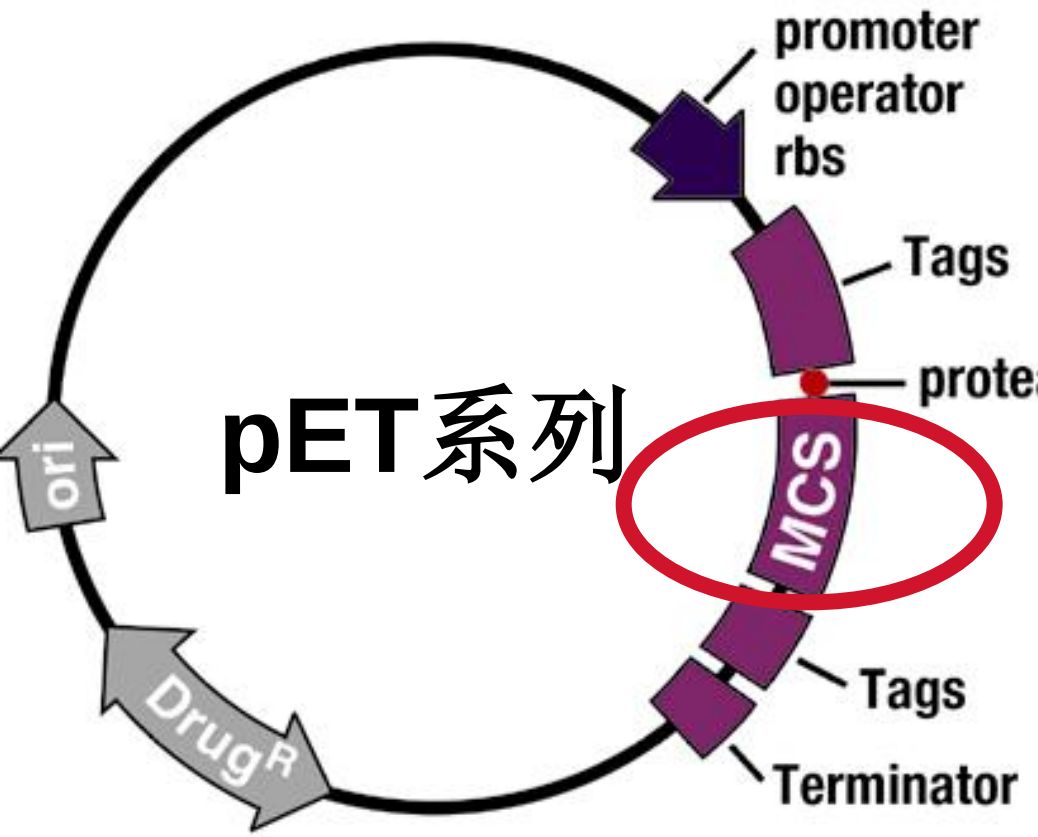
T7Lac启动子加强严紧控制以获得更稳定的表达

重要原则：选择合适的载体



终止子：转录水平的终止，也可考虑翻译水平的终止

重要原则：选择合适的载体

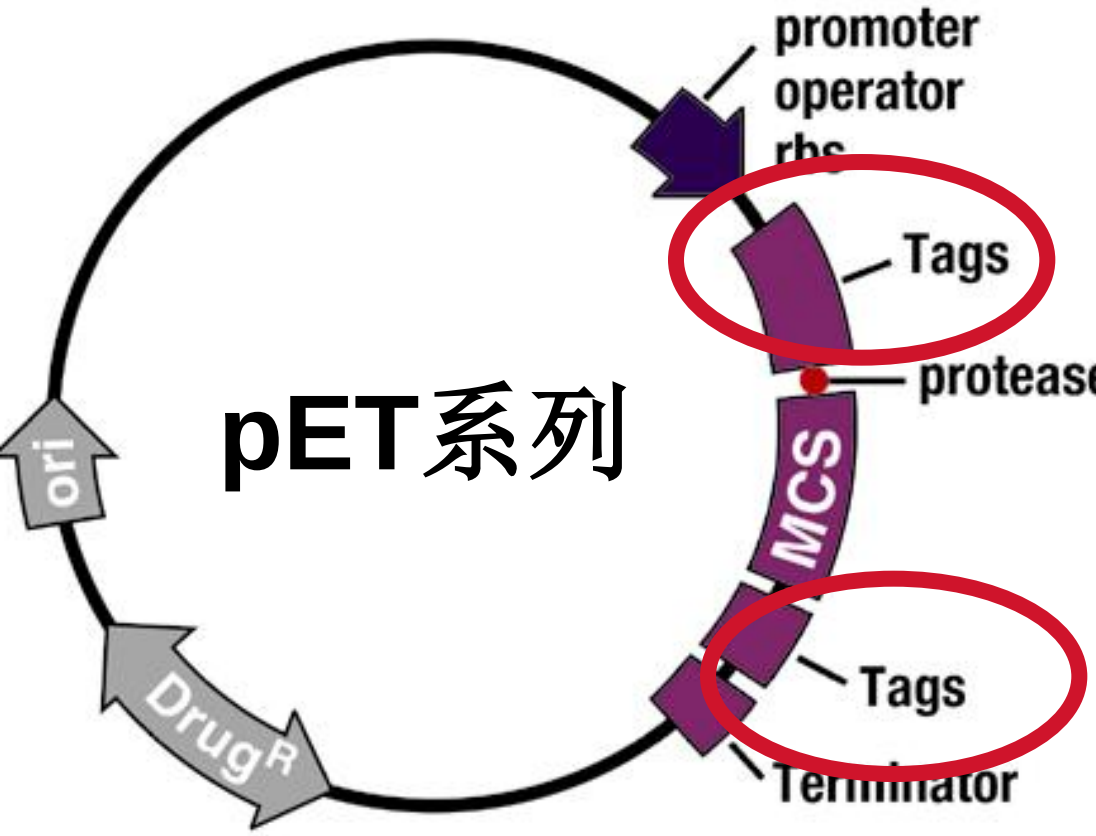


多克隆位点：

- pET系列共同的设计便于亚克隆
- 决定了载体来源序列在蛋白产物上的位置和应用

重要原则：选择合适的载体

- 提高产量和稳定性
- 蛋白定位与分泌
- 可溶性
- 特异性亲和纯化
- 检测**



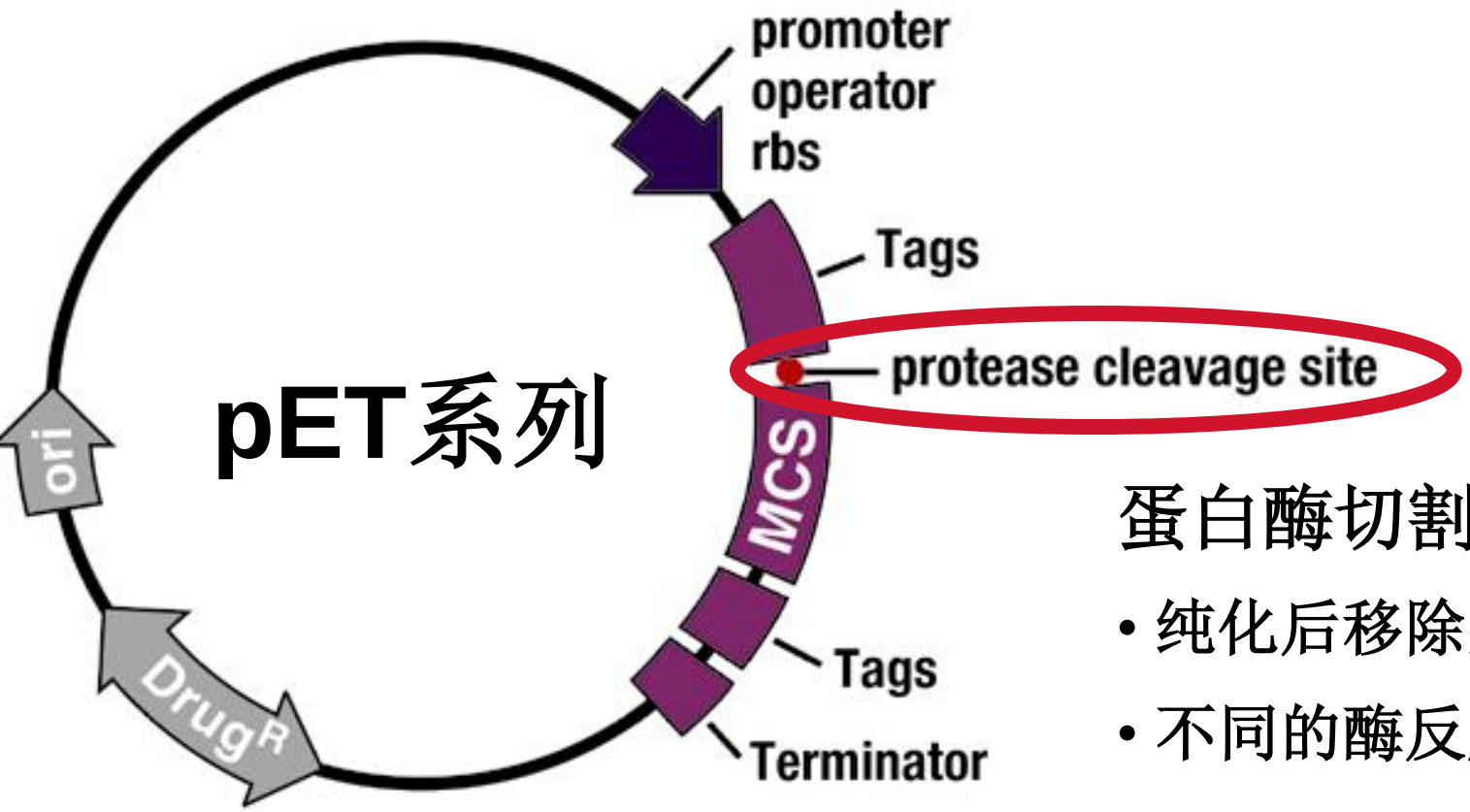
融合标签：

- 不同大小的C-或N-端标签的不同功能与对后续操作的影响
- 是否需要或容忍标签的存在

Fusion Tags Available for pET Constructs

Tag	N/C Terminal or Internal (I)	Size (aa)	Basis for Detection and/or Purification	Applications	pET Vector Series
Dsb•Tag	N	208 (DsbA) 236 (DsbC)	potential periplasmic localization	soluble protein, periplasmic disulfide bond formation, isomerization	39, 40
GST•Tag	N	220	glutathione affinity, mAb, enzymatic activity	purification, Western blot, quantitative assay	41, 42, 49, 60-DEST
His•Tag	N, C, I	6, 8, or 10	mAb, metal chelation chromatography (native or denaturing)	His•Bind® resin purification, Western blot	14–16, 19–52, (53-62)-DEST
HSV•Tag	C	11	mAb	Western blot, immunofluorescence	25, 27, 43.1, 44
KSI	N	125	highly expressed hydrophobic domain	small protein/peptide production/purification, insoluble protein	31
Nus•Tag	N	495	mAb	soluble protein, cytoplasmic disulfide bond formation in *trxB*⁻ hosts, Western blot	43.1, 44, 50, (57, 58)-DEST
pelB	N	20	potential periplasmic localization	protein export/folding	20, 22, 25, 26, 27
S•Tag	N, I	15	mAb, S-protein (104 aa) affinity	Western blot, quantitative assay, purification	29, 30, 32, 39, 40, 41, 42, 43.1, 47, 48, 49, 50, (54, 56, 58)-DEST
Strep•Tag II	N	8	mAb, Strep•Tactin affinity	purification, Western blot	51, 52, (53, 55, 57, 59, 61, 62)-DEST
T7•Tag	N, I	11	mAb	Western blot, immunoprecipitation, purification	3, 9, 11, 17, 21, 23, 24, 28
Trx•Tag	N	109	mAb, thioredoxin	soluble protein, cytoplasmic disulfide bond formation in *trxB*⁻ and *gor*⁻ hosts, Western blot	32, 48, 59-DEST

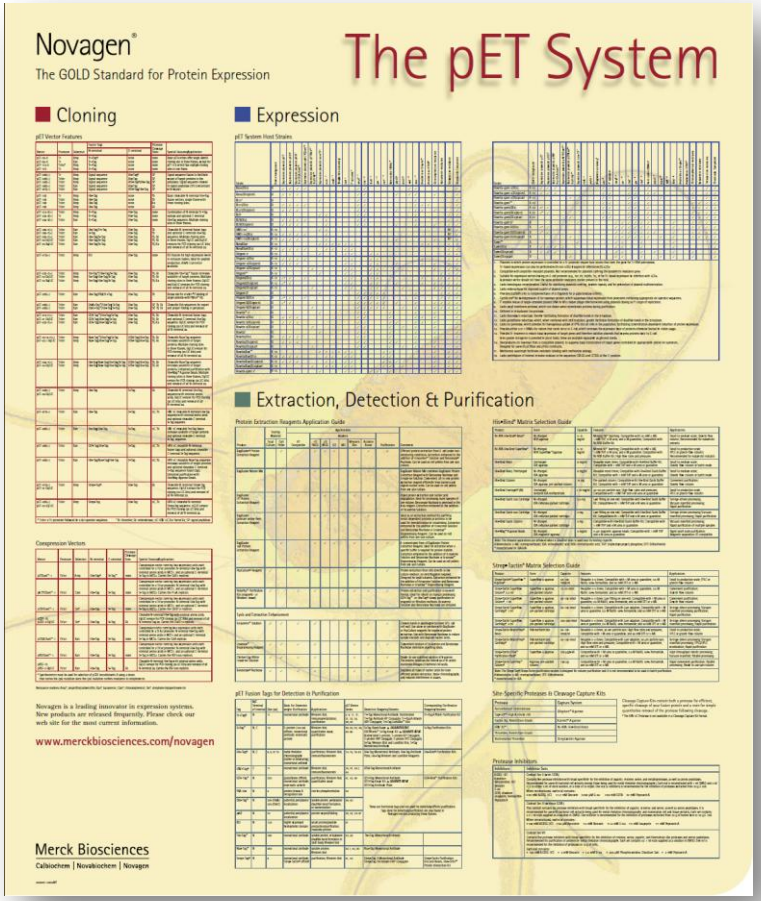
重要原则：选择合适的载体

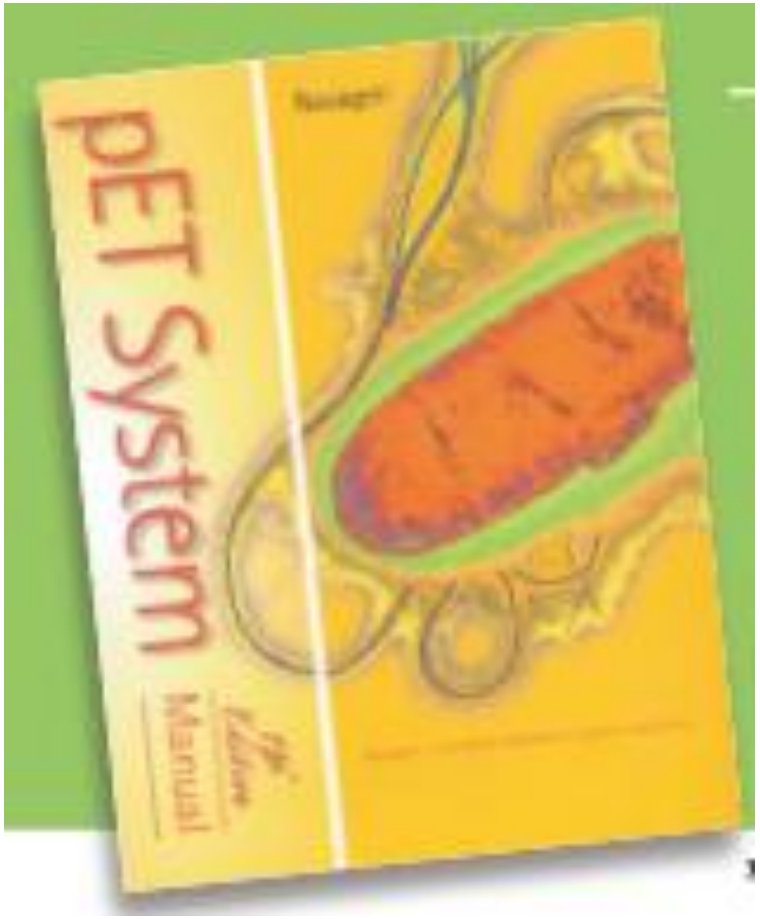


蛋白酶切割序列：

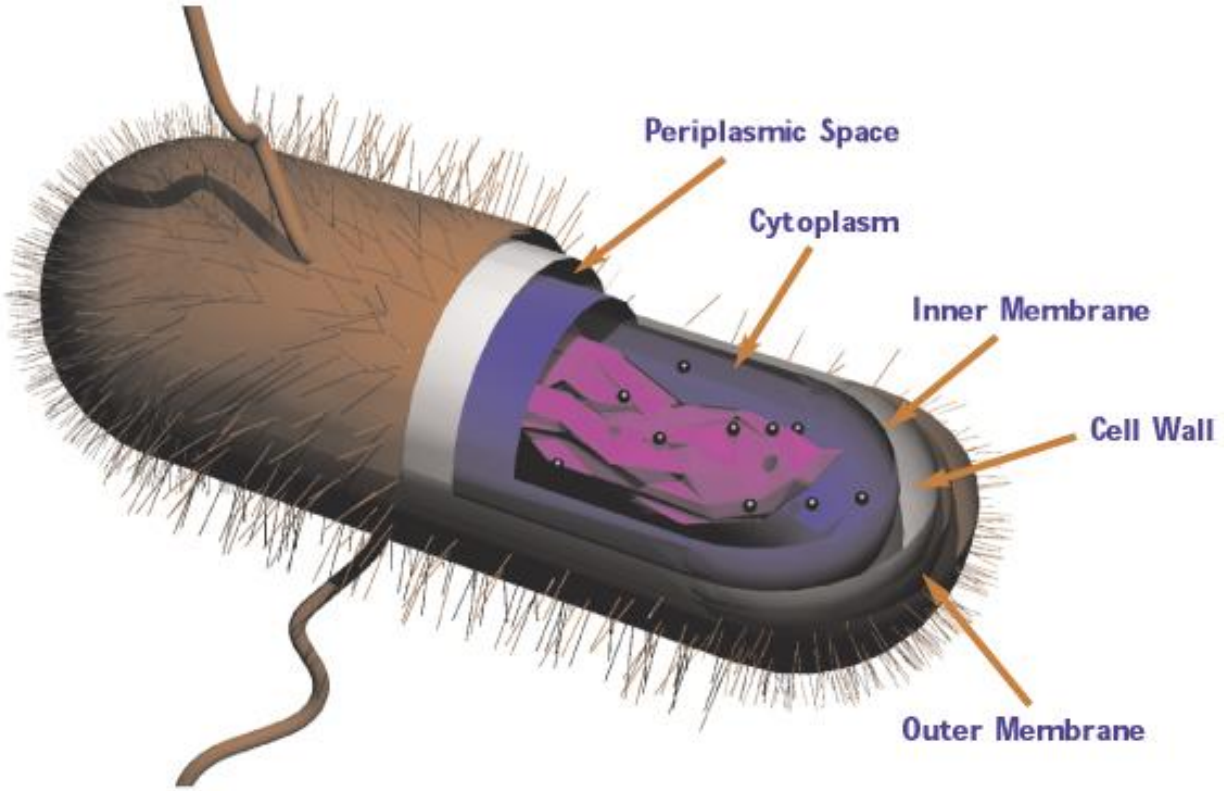
- 纯化后移除外源序列
- 不同的酶反应条件不同

pET系统手册：原核表达教科书





增加蛋白活性/可溶性的策略：载体



融合标签

分泌信号

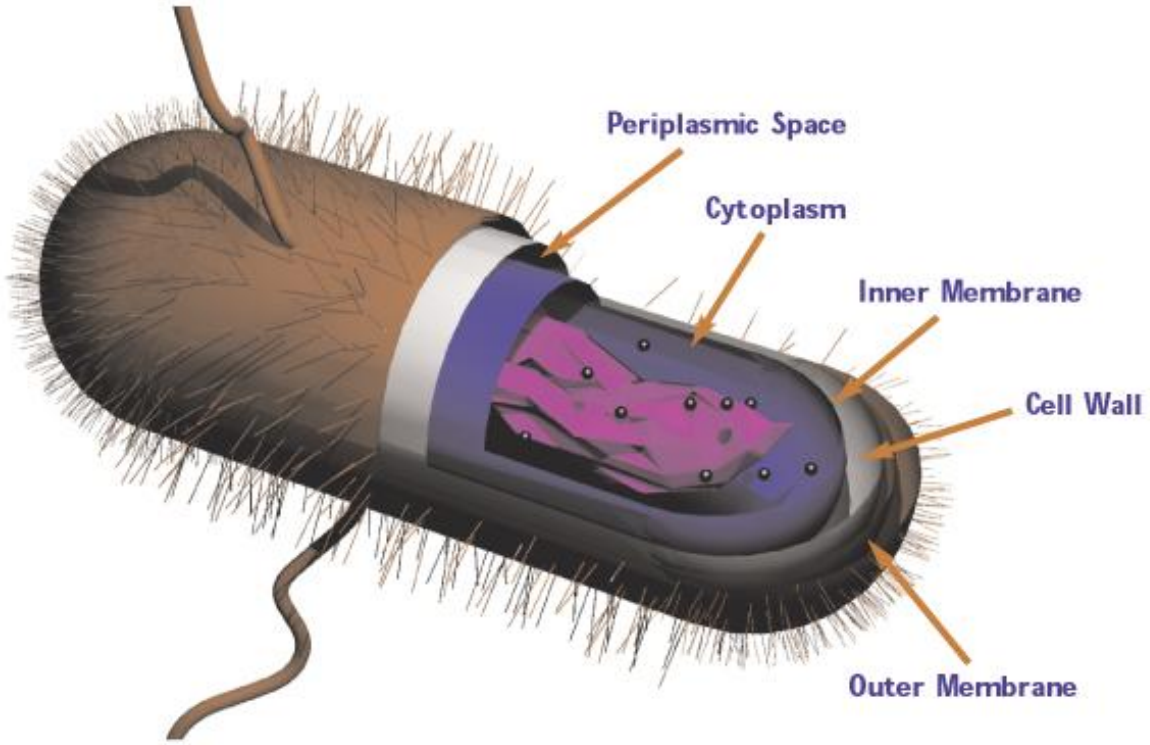
- ***pelB* leader (22 aa)**
- ***ompT* leader (20 aa)**

增加蛋白活性/可溶性的策略：载体

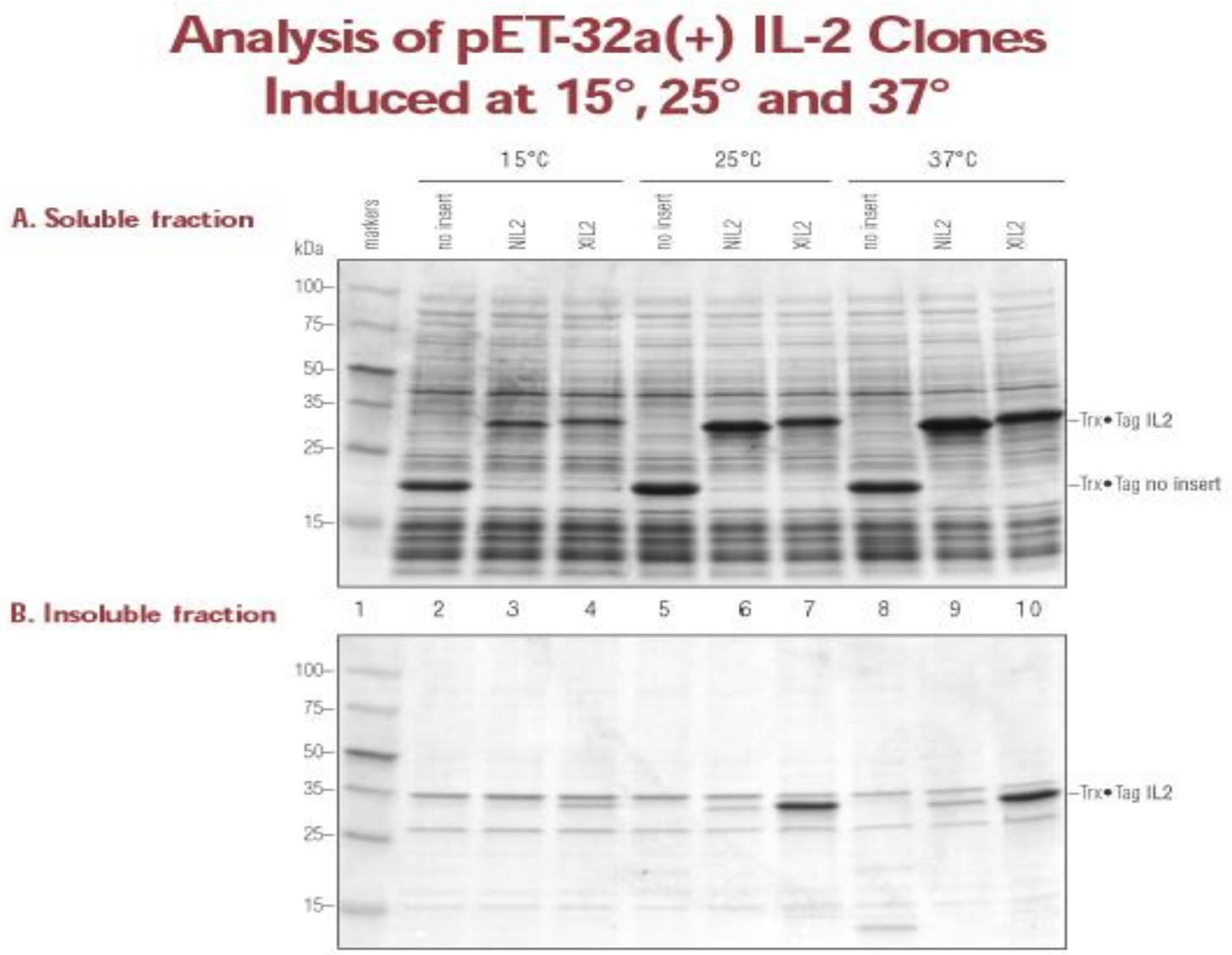
融合标签

融合表达的氧化酶

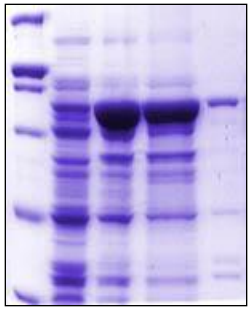
- 氧化还原酶 (208 aa, *DsbA*的基因产物)
- 二硫键异构酶 (236 aa, *DsbC*的基因产物)
- 硫氧还蛋白 (109 aa，trx)



经典案例：IL-2的工业化生产



www.biotech.ou.edu——预测蛋白可溶性



University of Oklahoma
School of Chemical Engineering and Materials Science
Recombinant Protein Solubility Prediction

Submit Query

Reset

Type (or cut and paste) your protein sequence below, click on the "Submit" button, and the solubility probability of your protein will be calculated. The statistical model predicts protein solubility **assuming the protein is being overexpressed in *Escherichia coli***. If there are numbers, spaces, or other characters in your sequence, don't worry, they won't affect the calculation. For more information on the solubility model used here, see the references below.

References:

- **R.G. Harrison.** 2000. Expression of soluble heterologous proteins via fusion with NusA protein. *inNovations*. **11:**4-7. PDF file
- **Davis, G.D., Elisee, C., Newham, D.M. and R.G. Harrison**. 1999. New fusion protein systems designed to give soluble expression in *Escherichia coli*. *Biotechnol. Bioeng.* **65(4):**382-8. PubMed Abstract
- **Wilkinson, D.L. and R.G. Harrison.** 1991. Predicting the solubility of recombinant proteins in Escherichia coli. *Bio/Technology*. **9:** 443-448. PubMed Abstract

增加蛋白活性/可溶性的策略：载体

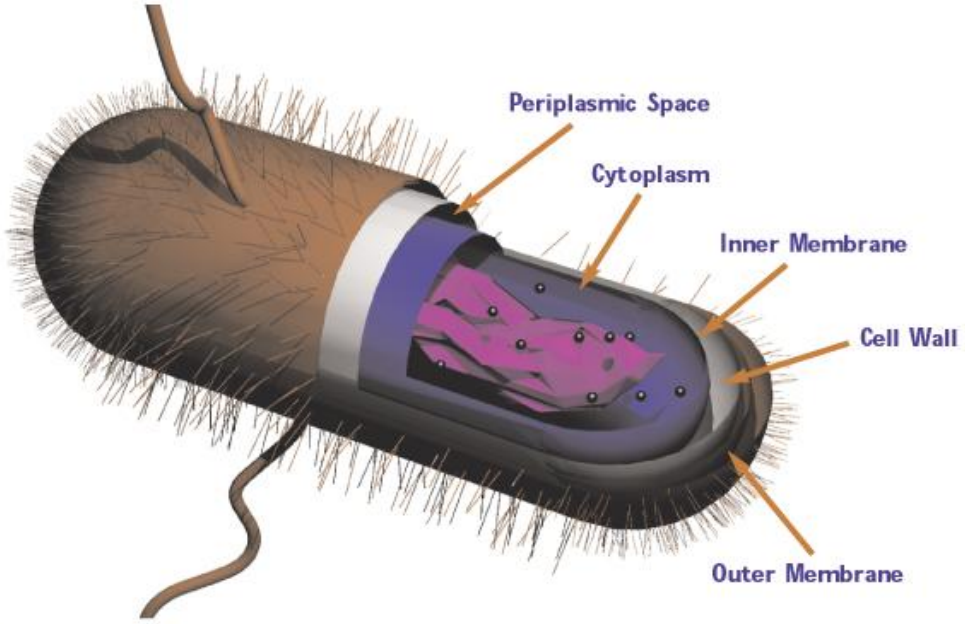
融合标签

分泌信号

- ***pelB* leader (22 aa)**
- ***ompT* leader (20 aa)**

融合表达的氧化酶

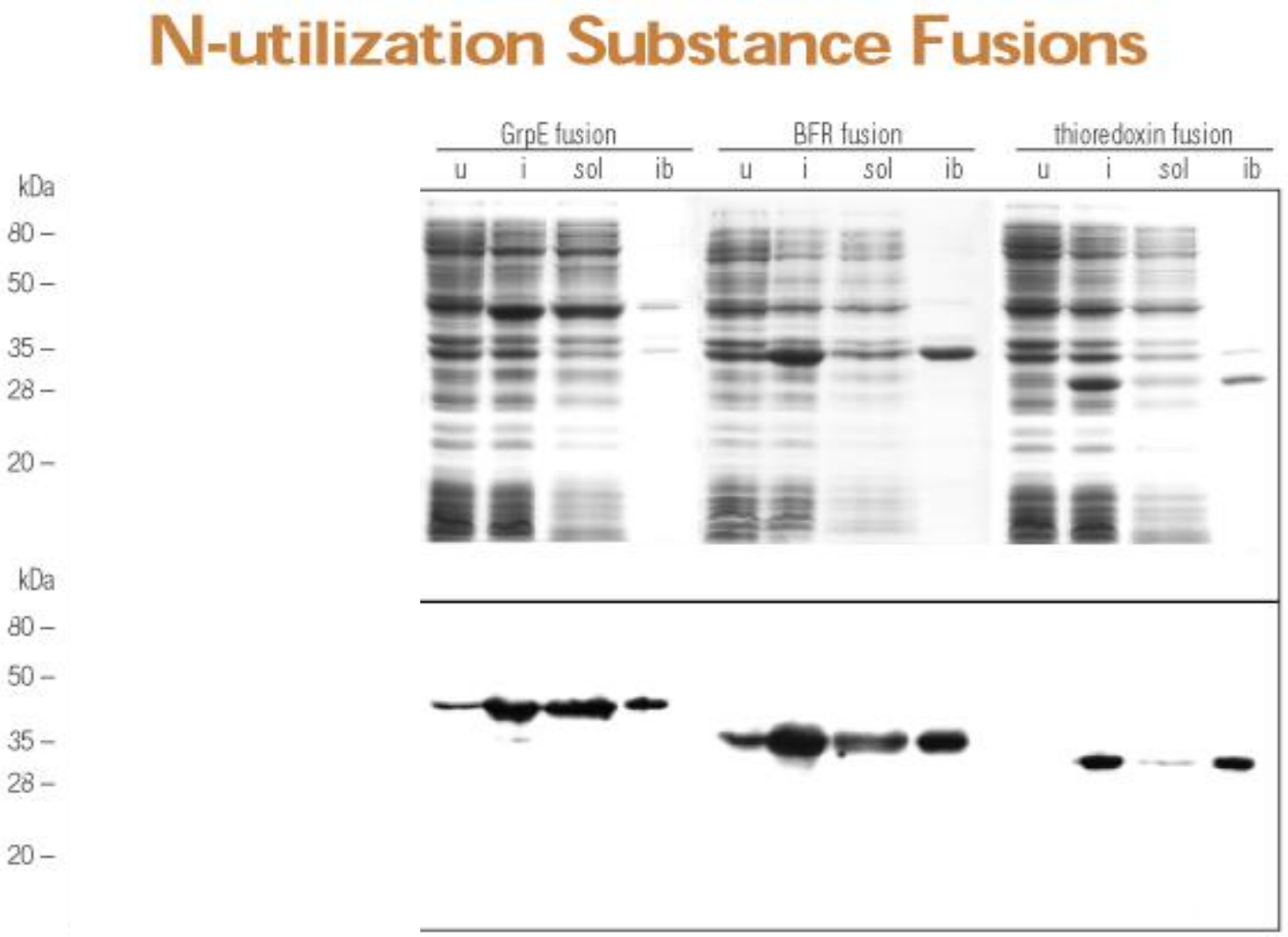
- **氧化还原酶 (208 aa, *DsbA*的基因产物)**
- **二硫键异构酶 (236 aa, *DsbC*的基因产物)**
- **硫氧还蛋白 (109 aa，trx)**



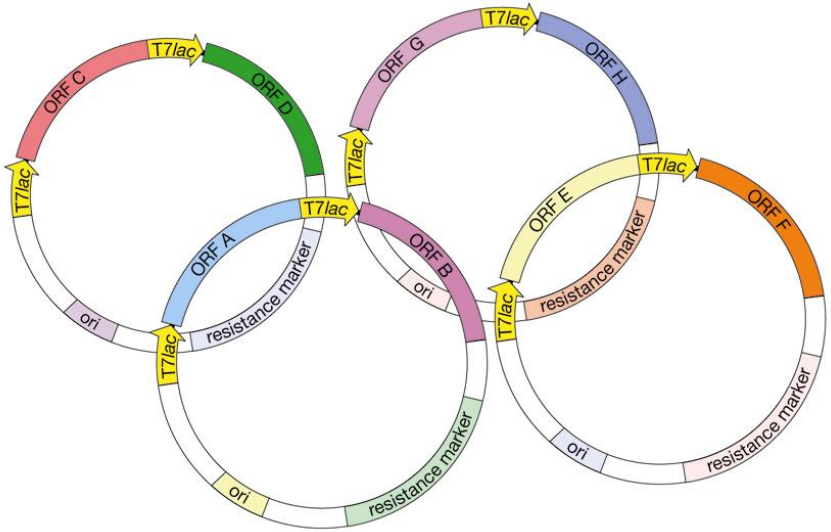
高可溶性蛋白

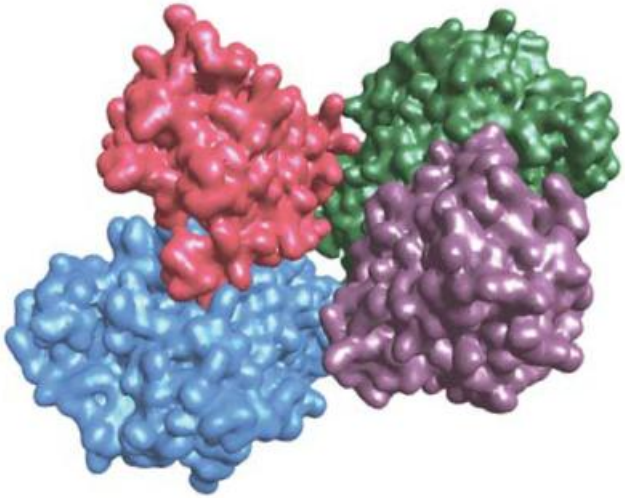
- **谷胱甘肽还原酶GST (220 aa)**
- ***NusA* (495 aa)**

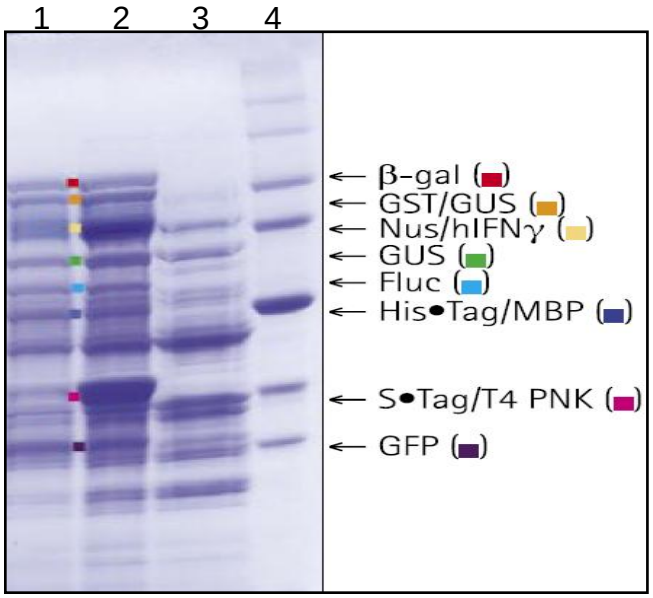
经典案例：IL-3可溶性改善



在一个表达系统中实现1—8个蛋白共同表达



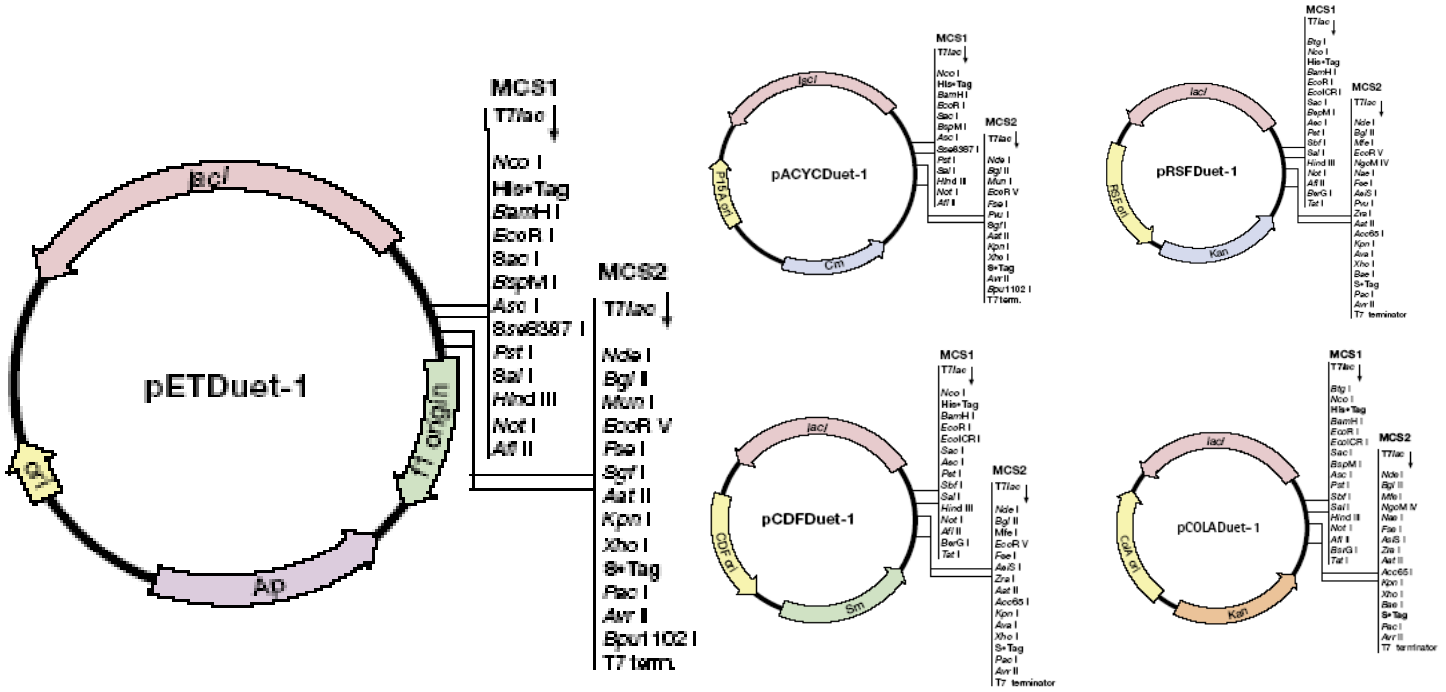




Lanes:

1 pRSFDuet-1, pACYCDuet-1, pETDuet-1, pCDFDuet-1 combination
2 pCOLADuet-1, pACYCDuet-1, pETDuet-1, pCDFDuet-1 combination
3 Uninduced control
4 Perfect Protein™ Markers

多亚基蛋白表达、目的蛋白-辅因子共表达变得灵活、简便可控



Plasmid	Replicon (source)	Copy Number	Resistance
pETDuet-1	ColE1 (pBR322)	~40*	Ampicillin (carbenicillin)
pACYCDuet-1	P15A (pACYC184)	10–12*	Chloramphenicol
pCDFDuet-1	CloDF13	~20–40*	Streptomycin, spectinomycin
pRSFDuet-1	RSF1030	> 100*	Kanamycin
pCOLADuet-1	ColA	~20–40	Kanamycin

* Copy number estimates are based on agarose gel analysis (3).

pET系列载体：满足各种表达要求

- Strep•Tag ® II：pET-51,-52；提高纯度，>95%
- HRV 3C： pET-47,-48,-49,-50；低温切割保护蛋白
- NusA： pET-43.1, -44, -50b；提高可溶性
- GST： pET-41,-42；提高可溶性，纯化，活性检测
- Trx： pET-32；提高可溶性
- site-specific ^{32}P-labeling： pET-33；目的蛋白标记
- Peptide Expression： pET-31，小肽表达
- pET-28, 29, 30：常用带HisTag的载体
- Duet Coexpression Vectors：双阅读框载体，蛋白复合物表达
- LIC：提高克隆效率的载体设计

稳定高产表达

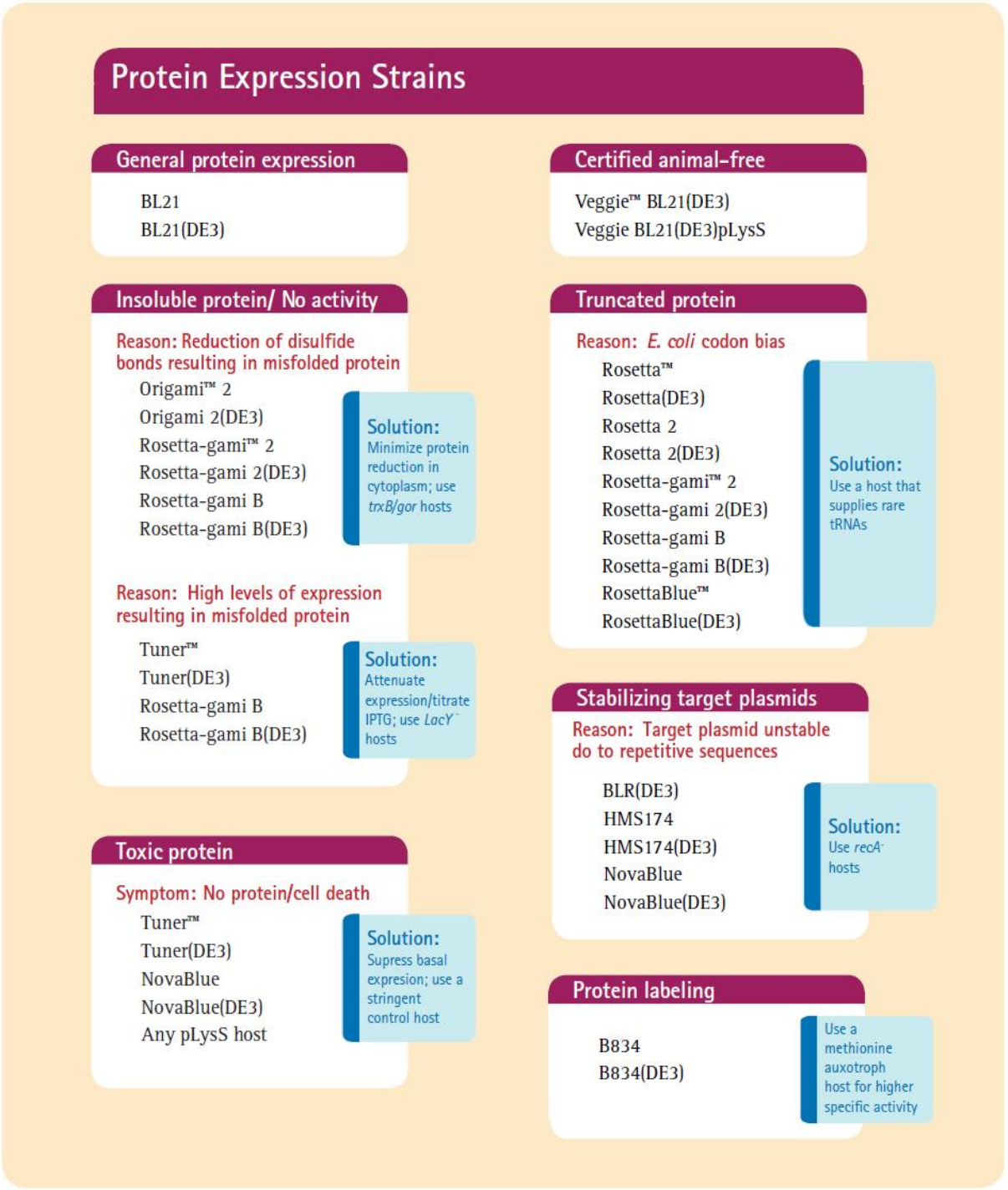
提高蛋白可溶性与活性

多基因共表达

提供纯化便利

不带外源序列的蛋白产物

宿主细胞


Protein Expression Strains
General protein expression
BL21
BL21(DE3)
Certified animal-free
Veggie™ BL21(DE3)
Veggie BL21(DE3)pLysS
Insoluble protein/ No activity
Reason: Reduction of disulfide bonds resulting in misfolded protein
Origami™ 2
Origami 2(DE3)
Rosetta-gami™ 2
Rosetta-gami 2(DE3)
Rosetta-gami B
Rosetta-gami B(DE3)
Solution: Minimize protein reduction in cytoplasm; use trxB/gor hosts
Reason: High levels of expression resulting in misfolded protein
Tuner™
Tuner(DE3)
Rosetta-gami B
Rosetta-gami B(DE3)
Solution: Attenuate expression/titrate IPTG; use LacY- hosts
Truncated protein
Reason: E. coli codon bias
Rosetta™
Rosetta(DE3)
Rosetta 2
Rosetta 2(DE3)
Rosetta-gami™ 2
Rosetta-gami 2(DE3)
Rosetta-gami B
Rosetta-gami B(DE3)
RosettaBlue™
RosettaBlue(DE3)
Solution: Use a host that supplies rare tRNAs
Stabilizing target plasmids
Reason: Target plasmid unstable do to repetitive sequences
BLR(DE3)
HMS174
HMS174(DE3)
NovaBlue
NovaBlue(DE3)
Solution: Use recA- hosts
Toxic protein
Symptom: No protein/cell death
Tuner™
Tuner(DE3)
NovaBlue
NovaBlue(DE3)
Any pLysS host
Solution: Supress basal expresion; use a stringent control host
Protein labeling
B834
B834(DE3)
Use a methionine auxotroph host for higher specific activity

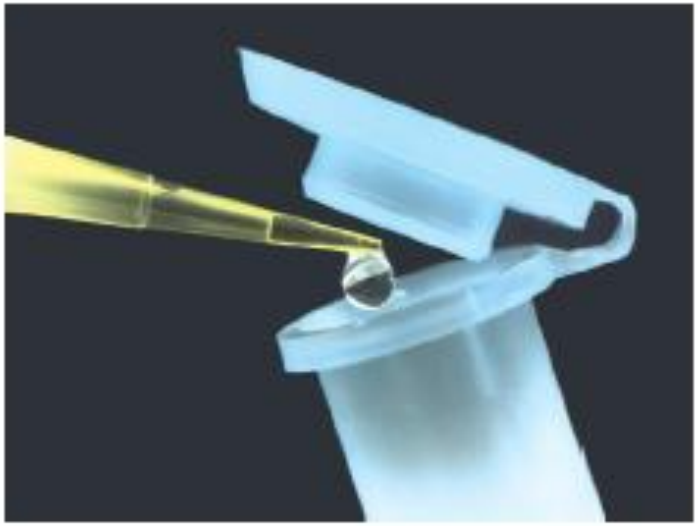
重要原则：宿主菌的种类与品质

区别：

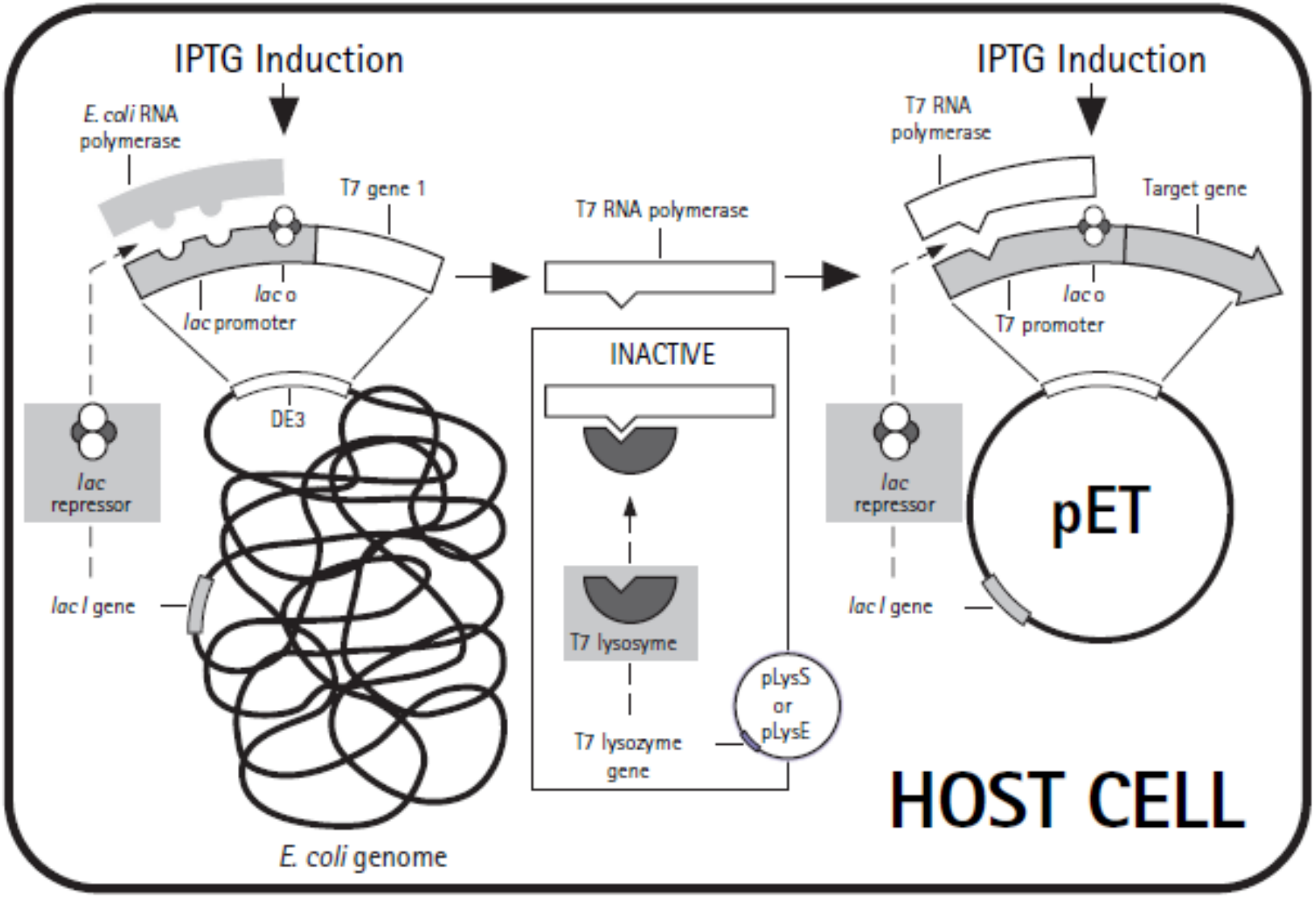
BL21

BL21（DE3）

BL21（DE3）*pLySs*

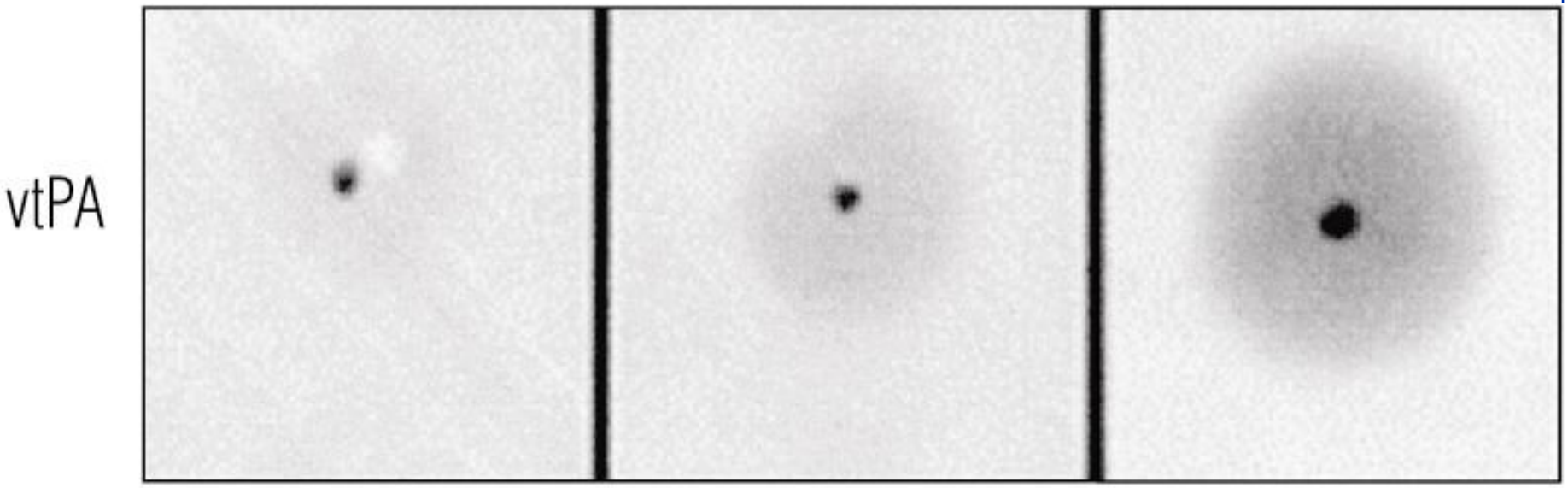


pLysS系列：提高毒性蛋白表达产量与稳定性



增加蛋白活性/可溶性的策略：Origami

二硫键的正确形成与折叠



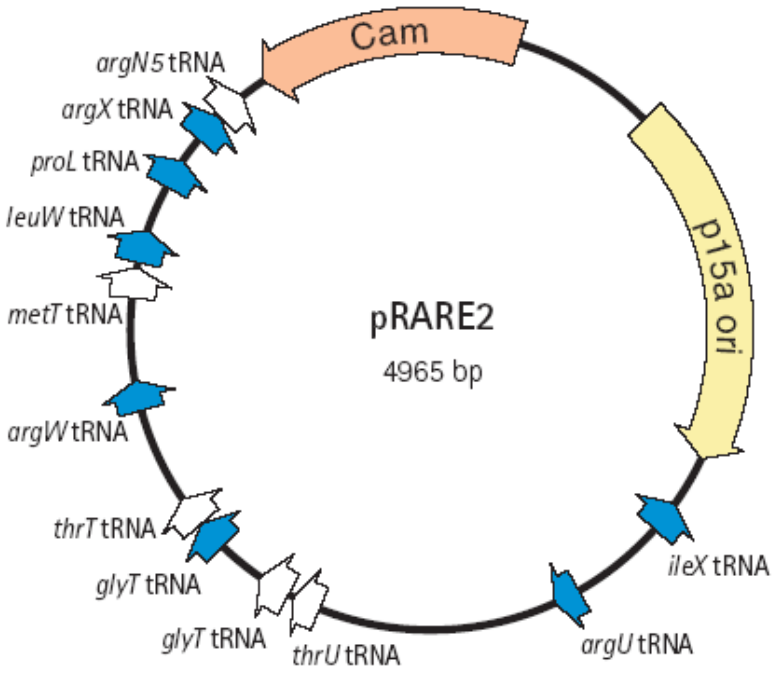
Tissue plasminogen activator (9 disulfide bonds) was expressed in three different hosts. 10 μg of soluble protein was spotted onto fibrin agarose plates and incubated for 24 h at 37° C. Zone of clearing measures biological activity of tPA.

获得全长蛋白，提高产量：Rosetta

Table 1. Arg, Gly, Ile, Leu and Pro codon usage in *E. coli*

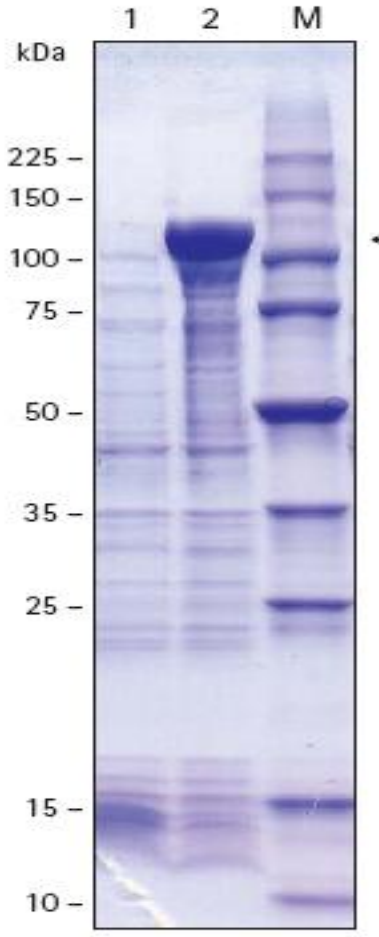
amino acid	codon	fraction in all genes	fraction in Class II
Arg	AGG	0.022	0.003
Arg	AGA	0.039	0.006
Arg	CGG	0.098	0.008
Arg	CGA	0.065	0.011
Arg	CGU	0.378	0.643
Arg	CGC	0.398	0.330
Gly	GGG	0.151	0.044
Gly	GGA	0.109	0.020
Gly	GGU	0.337	0.508
Gly	GGC	0.403	0.428
Ile	AUA	0.073	0.006
Ile	AUU	0.507	0.335
Ile	AUC	0.420	0.659
Leu	UUG	0.129	0.034
Leu	UUA	0.131	0.055
Leu	CUG	0.496	0.767
Leu	CUA	0.037	0.008
Leu	CUU	0.104	0.056
Leu	CUC	0.104	0.080
Pro	CCG	0.525	0.719
Pro	CCA	0.191	0.153
Pro	CCU	0.159	0.112
Pro	CCC	0.124	0.016

Codon usage is expressed as the fraction of all possible codons for a given amino acid. "All genes" is the fraction represented in all 4,290 coding sequences in the *E. coli* genome (6). "Class II" is the fraction represented in 195 genes highly and continuously expressed during exponential growth (7).



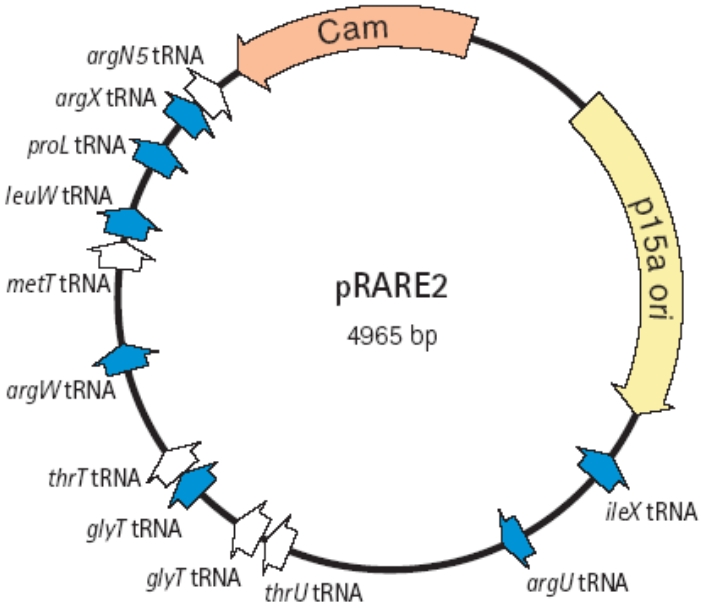
获得全长蛋白，提高产量：Rosetta

克服稀有密码子导致的低产与不完整问题



← β-gal (5 × CGG mutant)

Lane	Sample
1	Rosetta(DE3) total cell extract (induced)
2	Rosetta 2(DE3)total cell extract (induced)
M	Perfect Protein™ Markers, 10–225 kDa



Effect of consecutive CGG rare codons on target protein expression
A pET-15b recombinant plasmid containing five consecutive CGG codons near the 5'end of the β-gal coding region was transformed into Rosetta™(DE3) amd Rosetta 2(DE3) cells. The cells were induced with IPTG for 3 hr and harvested by centrifugation.

几种有特色的细胞株

Strain	DrugR	Genotype	Description
Rosetta™2	cam	F- ompT hsdSB(rB-mB-) gal dcm lacY1 pRARE	protease deficient, lac permease deficient, rare tRNAs
RosettaBlue™	cam, tet	endA1 hsdR17 (rK12-mK12 +) supE44 thi-1 recA1 gyrA96 relA1 lac F'[proA+B+ lacIqZDM15::Tn10 pRARE	K12, rare tRNAs
Origami™2	tet, Str, kan	Dara-leu7697 DlacX74 DphoAPvu II phoR araD139 ahpC galE galK rpsL F'[lac+(lacIq)pro] gor522::Tn10 trxB::Str,Tet	K12, oxidizing cytoplasm
Origami B	tet, kan	F- ompT hsdSB(rB-mB-) gal dcm lacY1 ahpC gor522::Tn10 trxB::kan	protease deficient, lac permease deficient, oxidizing cytoplasm
Rosetta-gami™	tet, kan, cam	Δ*ara-leu7697* Δ*lacX74* Δ*phoAPvu* II *phoR araD139 ahpC galE galK rpsL* F′[*lac*$^{+}$(*lacI*^{q})*pro*] *gor522*::Tn*10 trxB*::kan pRARE	K12, rare tRNAs, oxidizing cytoplasm
Rosetta-gami B	tet, kan, cam	F^{-} *omp*T hsdS$_{B}$($r_{B}^{-}m_{B}^{-}$) *gal dcm lacY1 ahpC gor522::*Tn*10 trxB::*kan pRARE	protease deficient, *lac* permease deficient, rare tRNAs, oxidizing cytoplasm

无动物来源产品满足特殊需要

Veggie Peptone

Veggie Yeast Extract

Veggie™ Singles™ Competent Cells

Veggie NovaBlue Competent

Veggie BL21(DE3) and Veggie BL21(DE3)pLysS

rLysozyme Solution, Veggie™ Grade

Animal-Free Protease Inhibitor Cocktails

Benzonase® 核酸酶减少样品粘度，提高蛋白产率

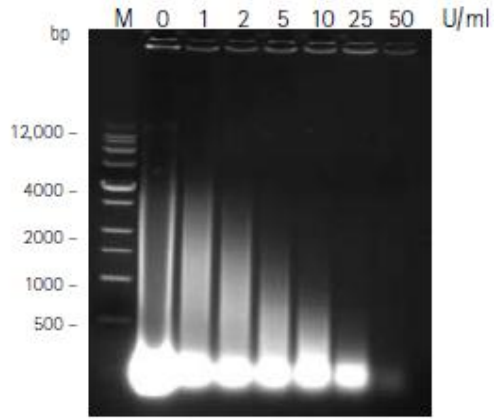
生物工程生产，来自***Serratia Marcescens***

是**2个30 kDa亚基的二聚体**

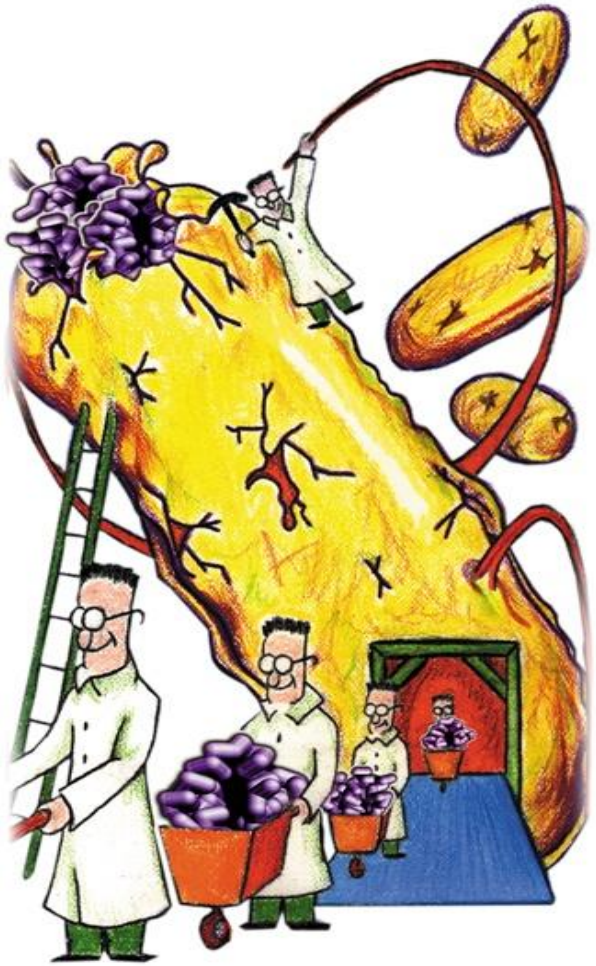
Viscosity reduction by Benzonase

Benzonase高效去除蛋白产物中的核酸干扰

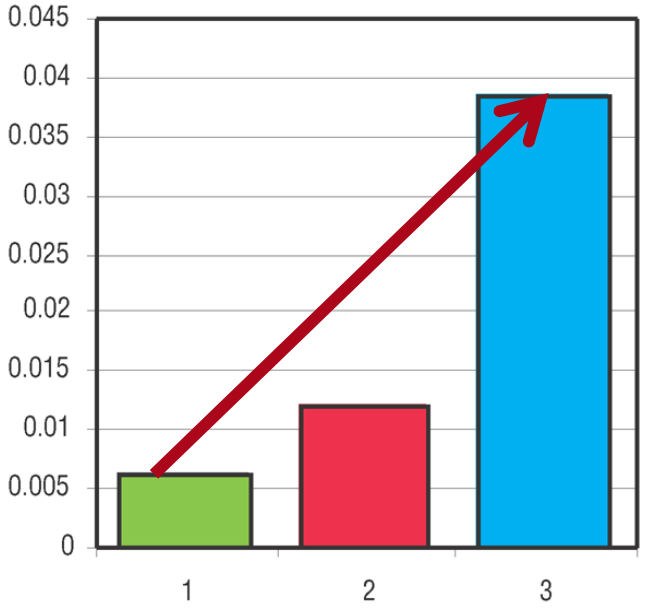
- 迅速、安全地降低样品粘度
- 减少色谱柱堵塞、耗损
- 减少样品分离中各种物质的相互干扰
- 提高纯化速度和效率



替代超声波的抽提试剂大幅提高活性蛋白产量



抽提分离条件会带来活性蛋白产量成倍的差异



pET41a（+）活性GST重组蛋白含量检测：

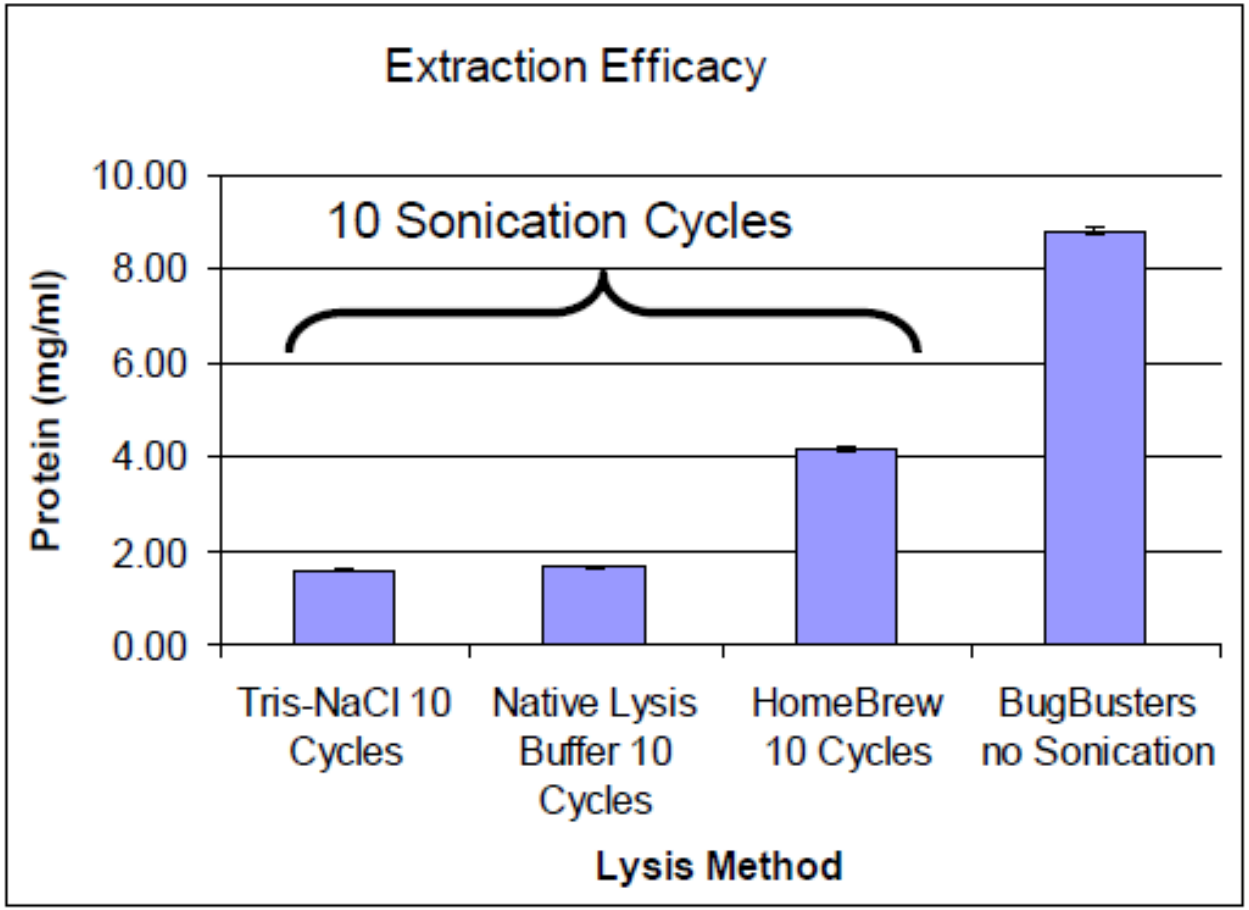
（1）超声波处理

（2）实验室常用裂解液

（3）BugBuster温和抽提试剂

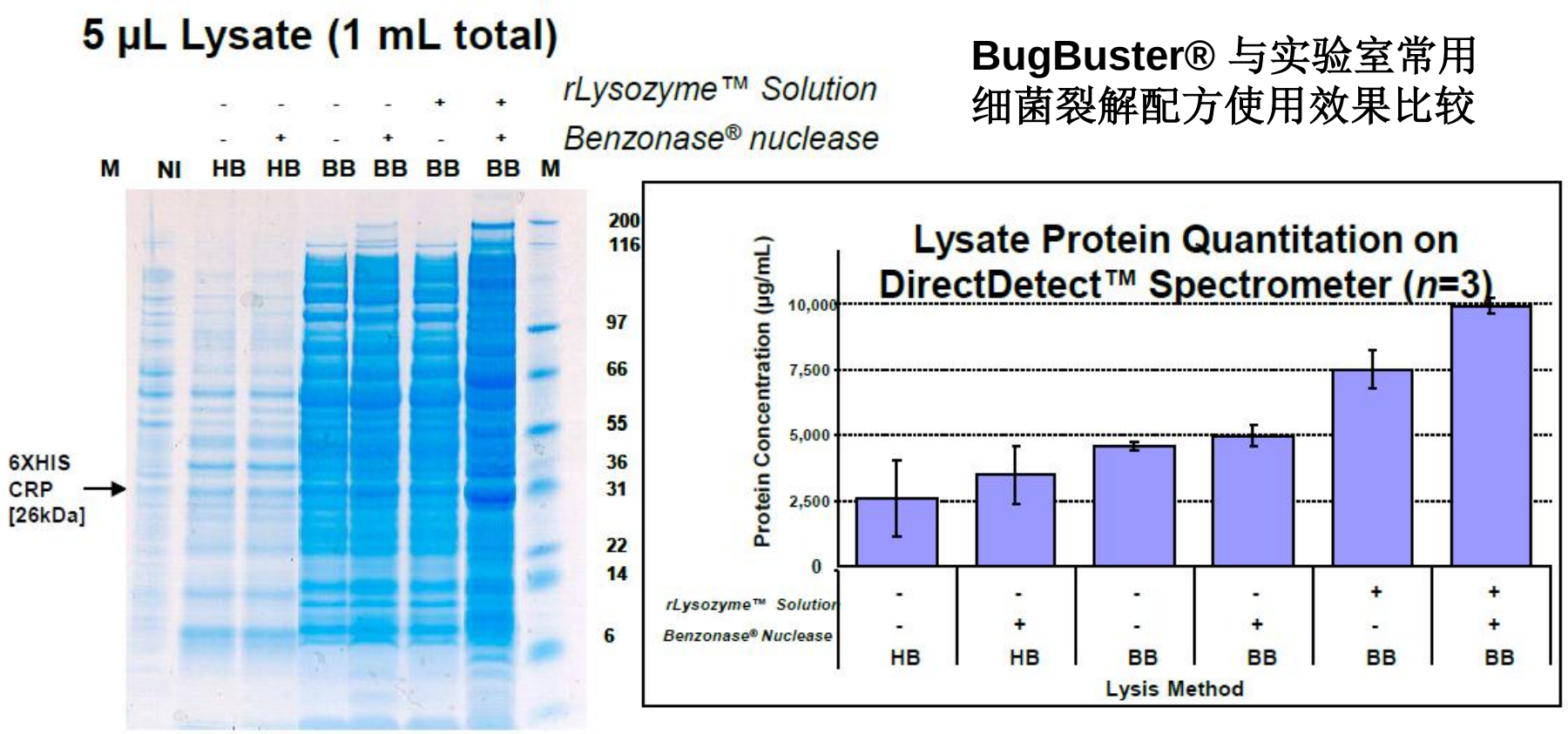
BugBuster对于活性要求高的样品、多样本平行处理、小体积样品、线性缩放处理等有着特别的价值

替代超声波的抽提试剂大幅提高活性蛋白产量



实验室常用溶菌配方往往还是离不开超声波处理，然而释放蛋白的效果，以及超声对于蛋白的损害可能来自剪切力、气泡张力、热效应等等

替代超声波的抽提试剂大幅提高活性蛋白产量

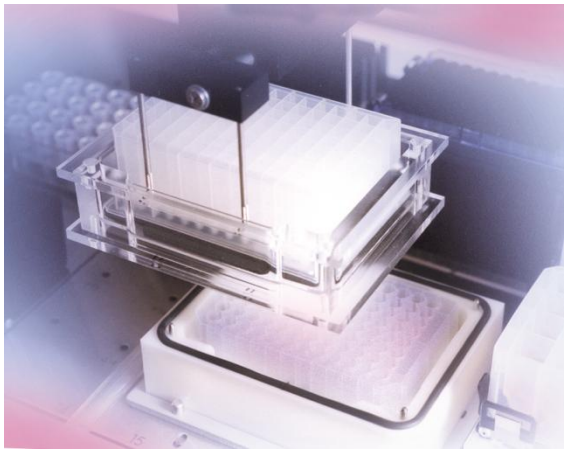


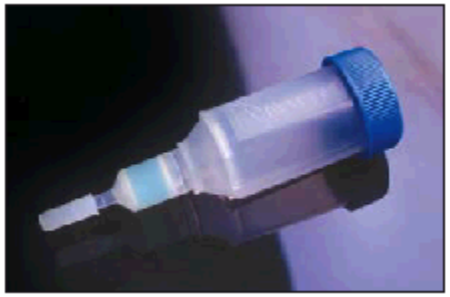
BugBuster® 与实验室常用细菌裂解配方使用效果比较

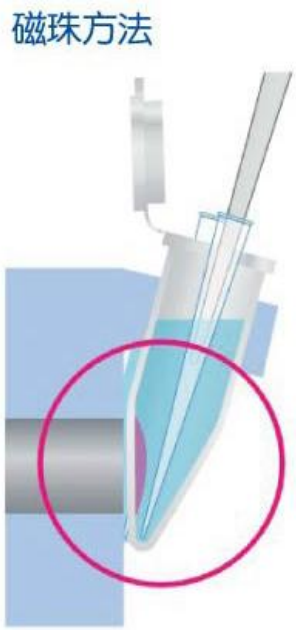
- **BugBuster® Master Mix蛋白产量显著提高**
- **Benzonase®核酸酶和rLysozyme™高效溶菌酶与BugBuster一起使用综合效果好**
- **没有Benzonase®核酸酶处理时常常遇到样品粘稠问题**

亲和纯化

- His•Tag® sequence
- GST•Tag™ sequence
- S•Tag™ sequence
- HSV•Tag® sequence
- T7•Tag® sequence
- Strep •Tag sequence



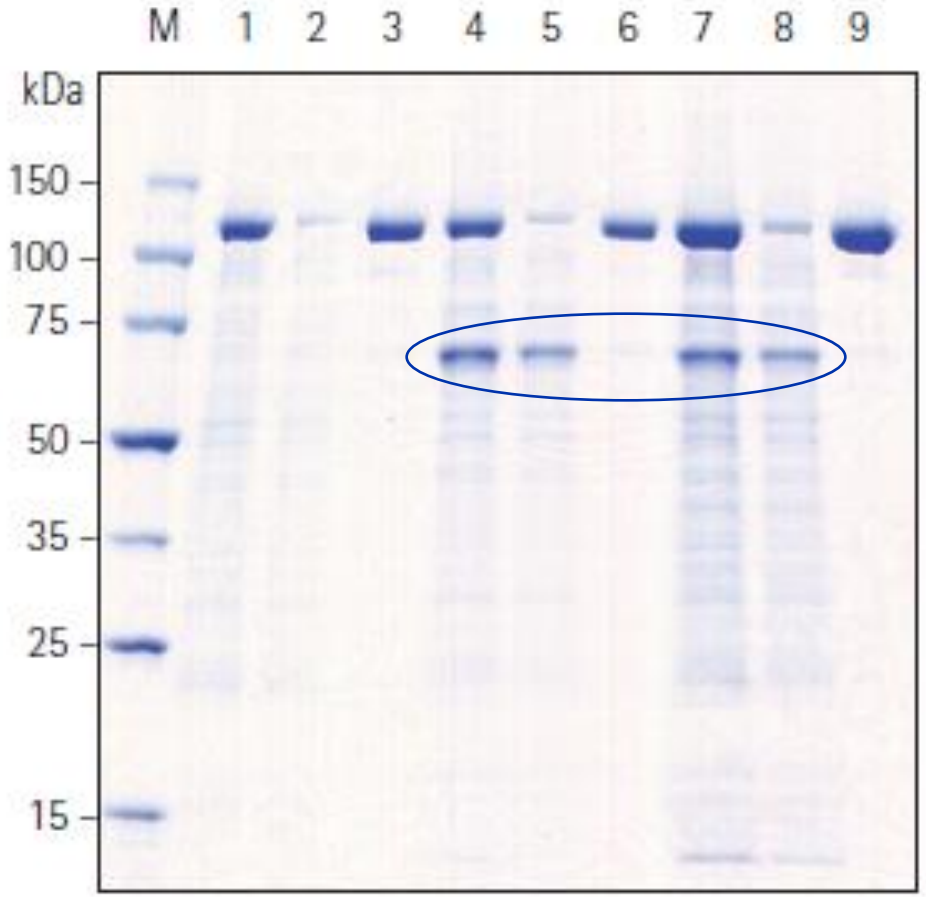




新型磁珠法提高了操作通量

获得高产完整一致的目的蛋白

- 表达全长蛋白
- 减少蛋白降解或被剪切
- 减少核酸干扰
- 特异性好的亲和纯化方法
- 合适的树脂用量



亲和纯化-浓缩-除盐/换液： Amicon Pro

大量手工处理时间

全程时间太长，样品易降

样品经多次转移，损失不小

得率很低

纯化完了要换种装置进行透析

透析完了还需进行浓缩操作

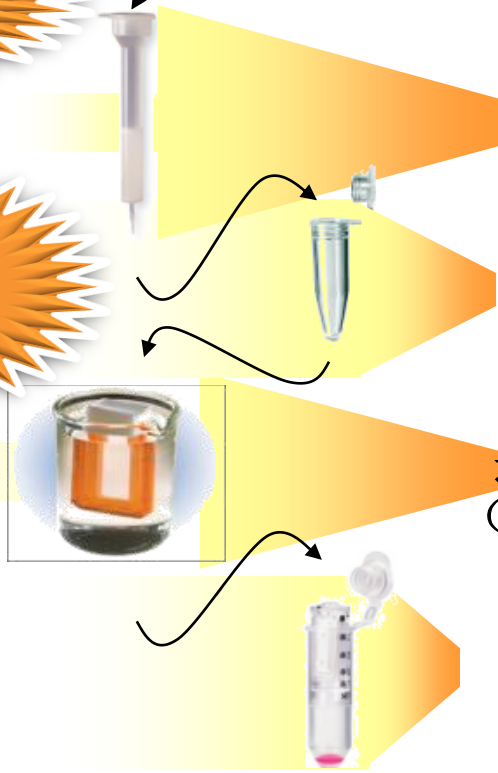
样品

纯化

收集

透析/ 摸渗滤（换液，除盐）

浓缩



新

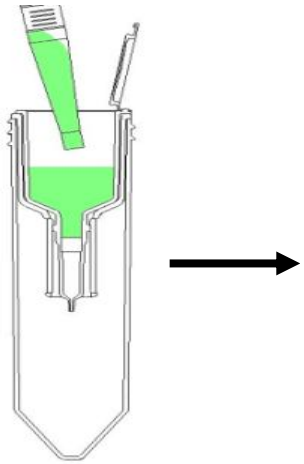
Amicon Ultra 0.5

多步操作
多种装置
多次样品转移

全新一体化设计，将所有处理放在一个装置中进行，不需要样品转移

Amicon® Pro System

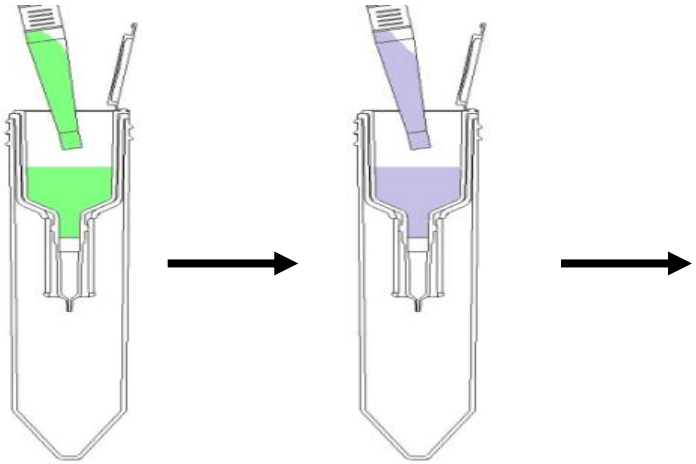
漂洗树脂



Amicon® Pro System

漂洗树脂

加入粗样
与树脂孵育

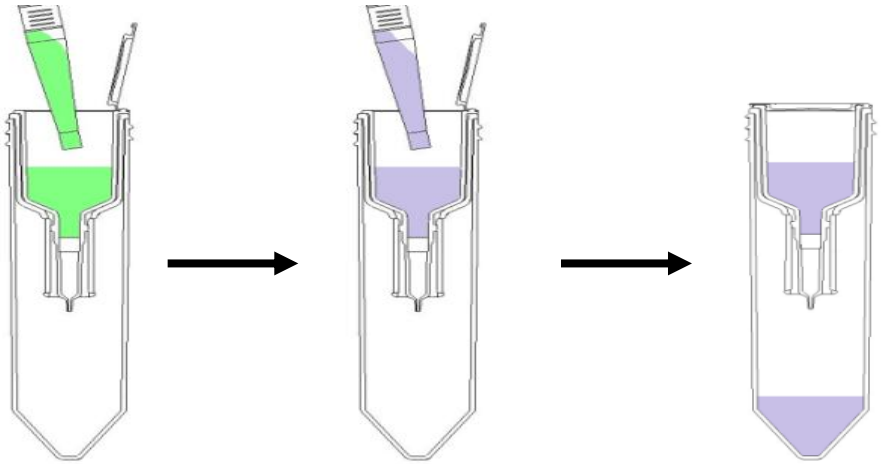


Amicon® Pro System

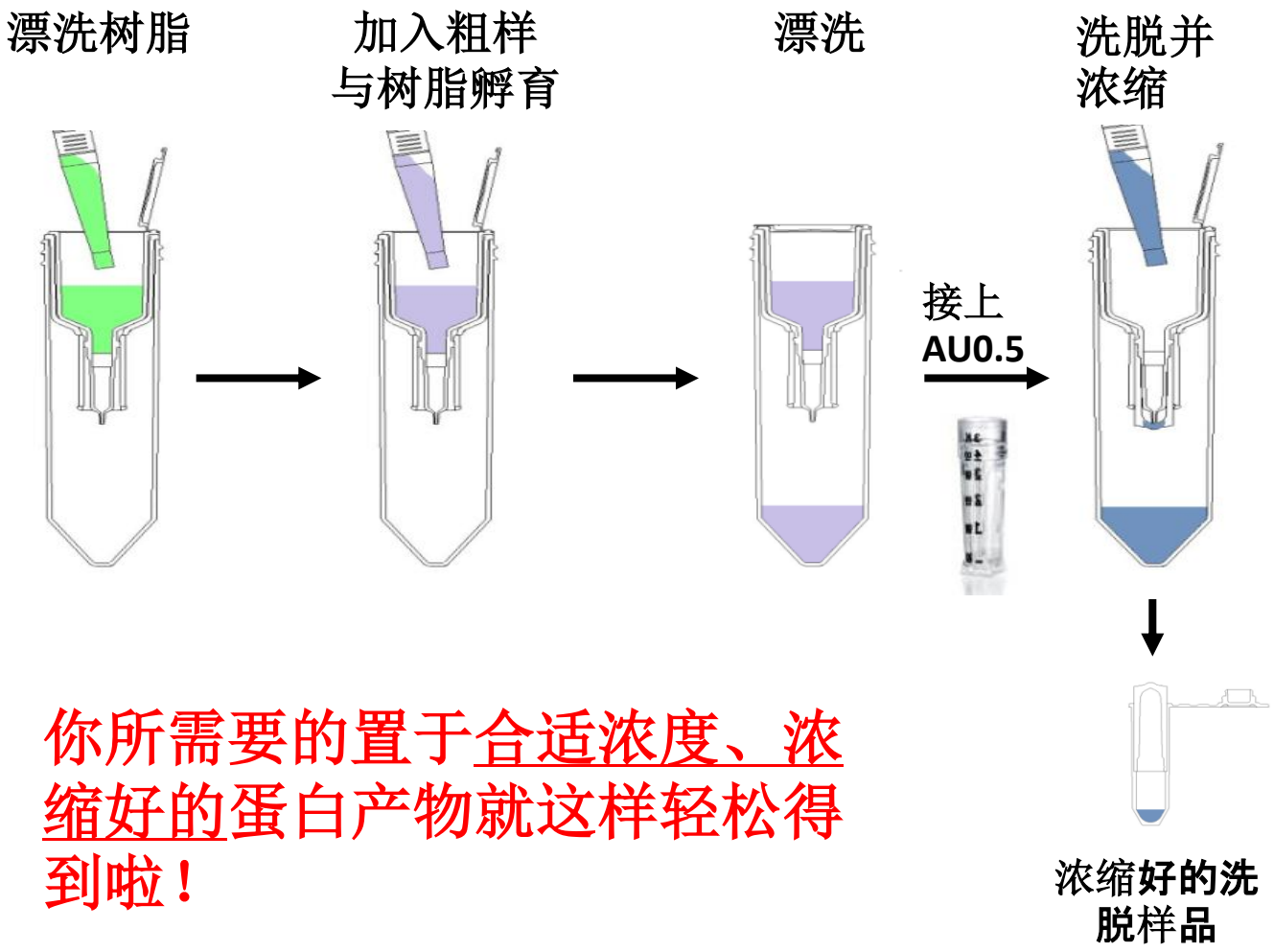
漂洗树脂

加入粗样
与树脂孵育

漂洗

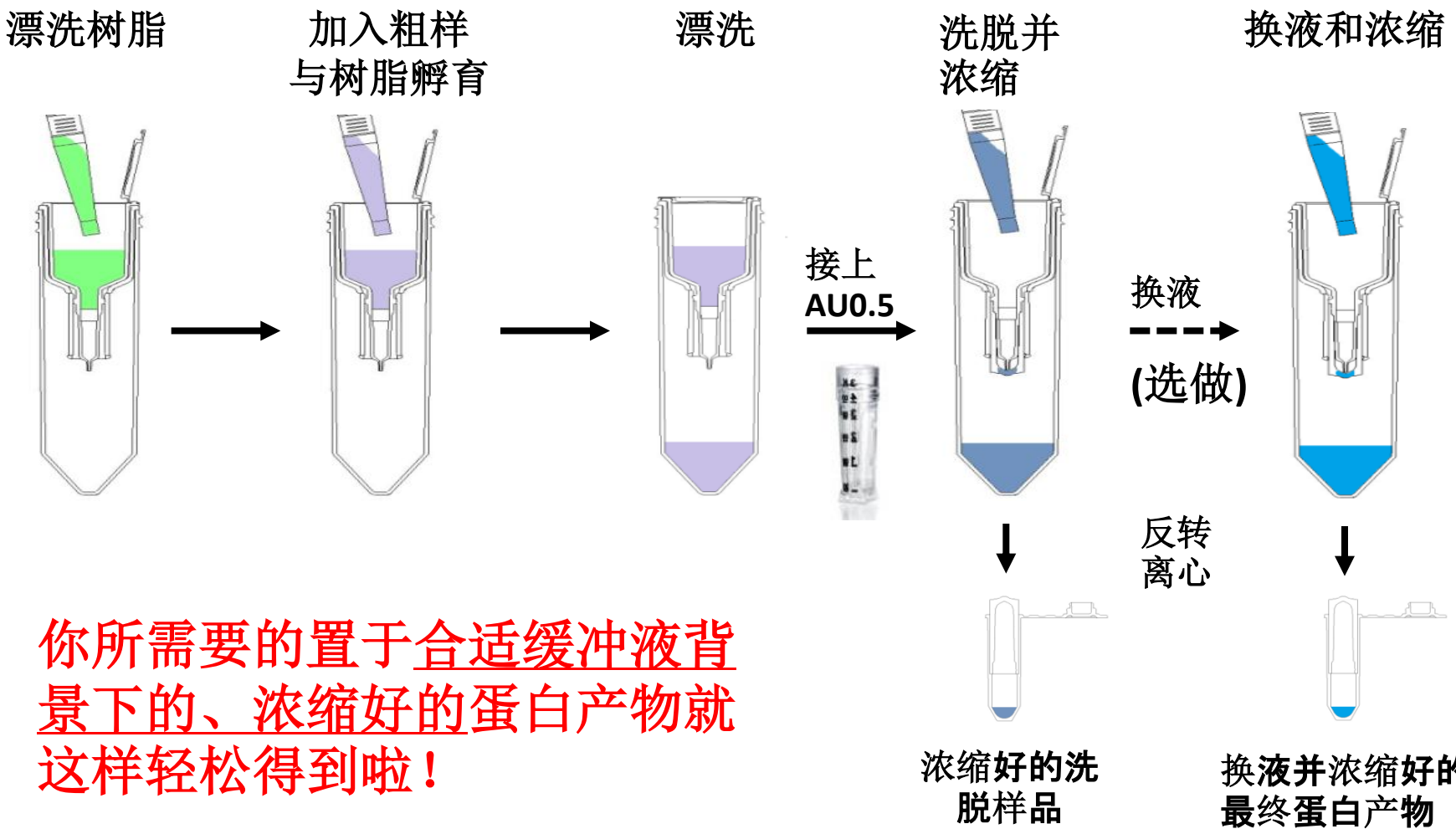


Amicon® Pro System



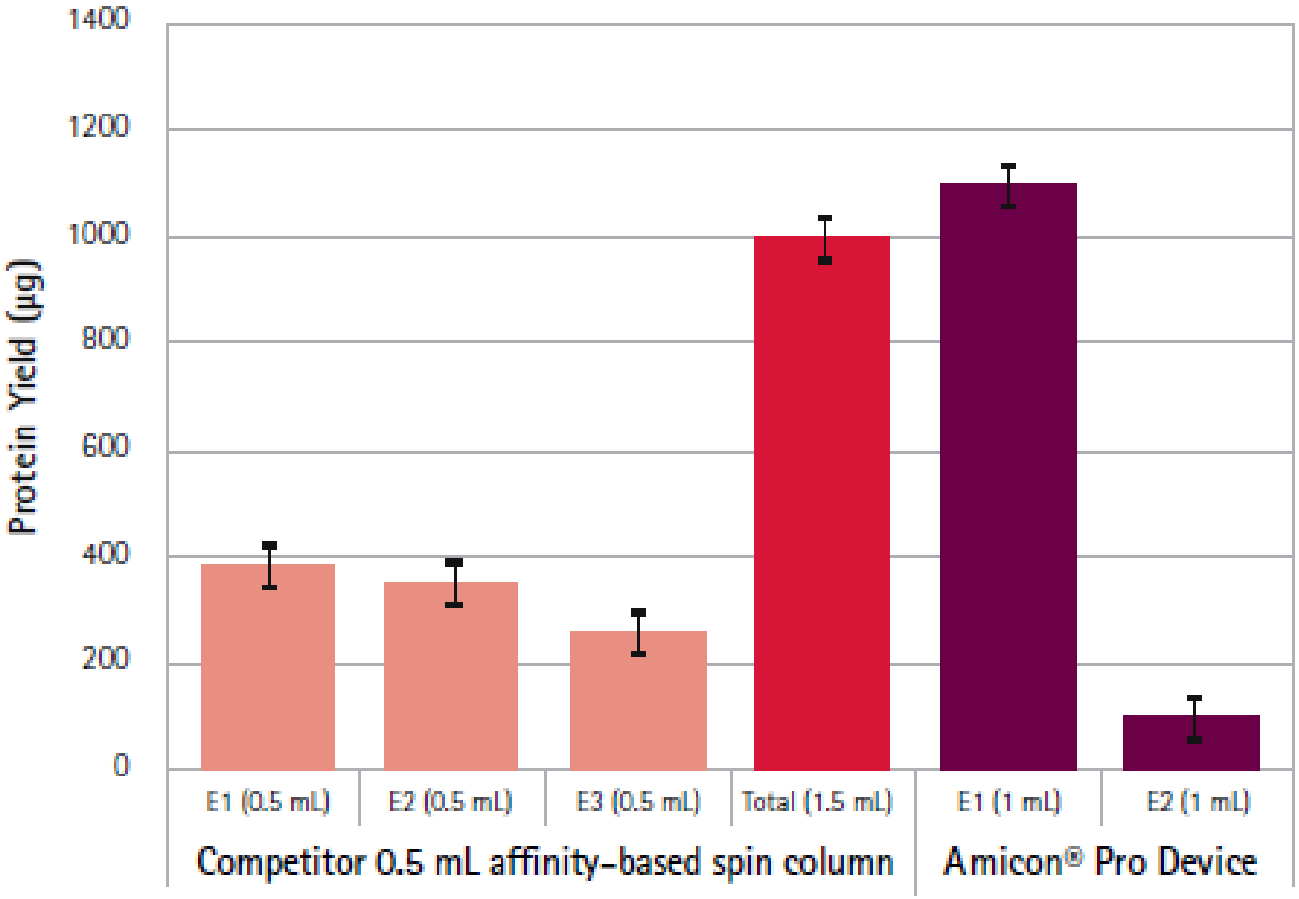
你所需要的置于合适浓度、浓缩好的蛋白产物就这样轻松得到啦！

Amicon® Pro System



你所需要的置于合适缓冲液背景下的、浓缩好的蛋白产物就这样轻松得到啦！

一步洗脱，高蛋白回收率


Protein Yield (µg)
1400
1200
1000
800
600
400
200
0
E1 (0.5 mL)
E2 (0.5 mL)
E3 (0.5 mL)
Total (1.5 mL)
E1 (1 mL)
E2 (1 mL)
Competitor 0.5 mL affinity-based spin column
Amicon® Pro Device

应用举例(1)：亲和纯化+换液+/-浓缩

GE His Trap Columns

Qiagen Protein Spin Columns

Pierce spin columns

Amicon Pro与传统亲和纯化方法操作比较		
纯化+浓缩+换液	0.5ml甩柱+超滤管	**Amicon Pro**
操作时间*	67min	**29min**
离心次数	12	**6**
样品转移次数	1	**0**

*两种方法中都有的60min孵育时间没有计算在内

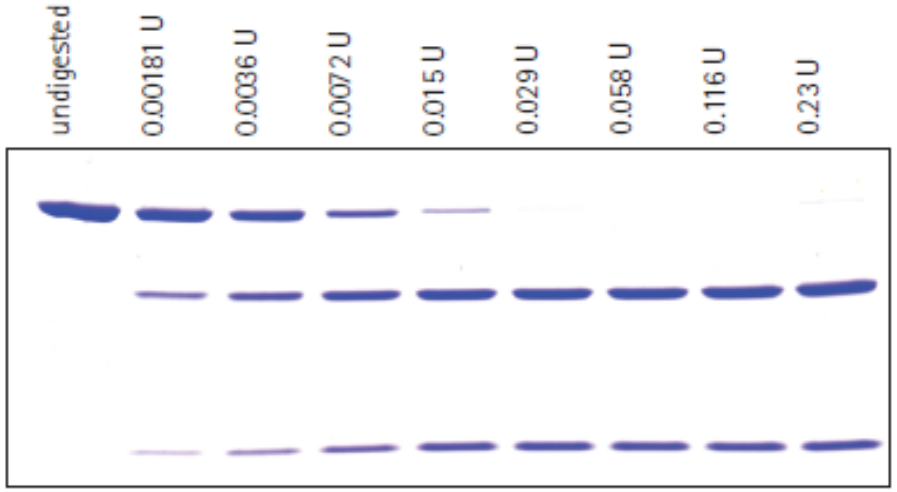
应用举例(2)：透析换液——以抗体标记为例

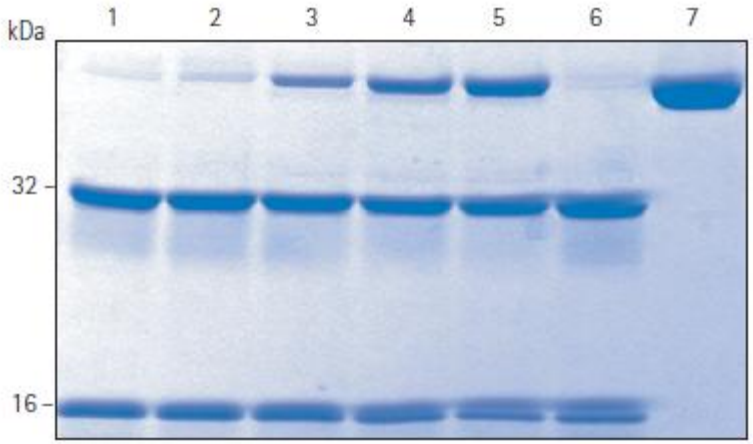
操作步骤	透析法换液	Amicon Pro
缓冲液置换	过夜	15min
FITC标记	3h	30min
移除未标记的FITC并换液	过夜	15min
总用时	3天	1h
抗体得率	39%	72%

Amicon Pro与传统抗体标记方法操作比较		
抗体标记*	标记前后用透析法换液	Amicon Pro
操作时间	3天	60min
操作步骤	11	5
样品转移次数	3	0
起始抗体需要量	1mg	50ug

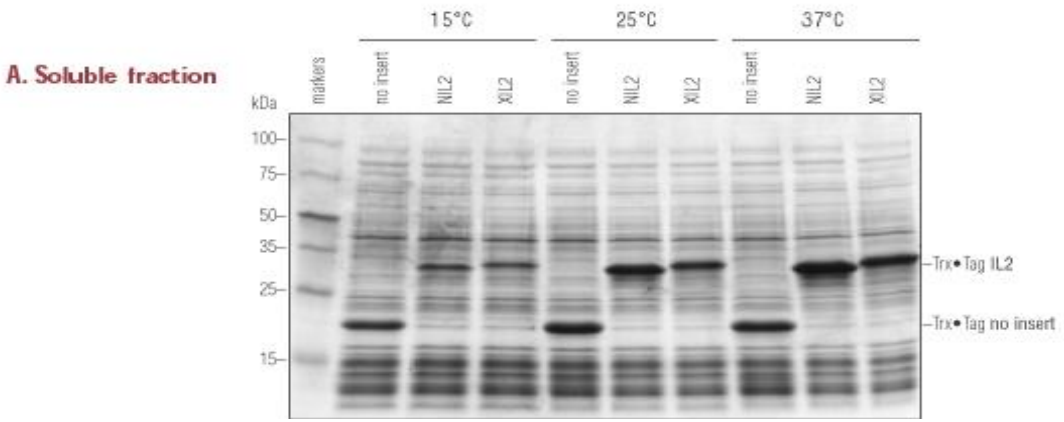
去除目的蛋白上的多余序列

Thrombin	Leu Val Pro Arg Gly Ser
Factor Xa	Ile Glu Gly Arg
Enterokinase	Asp Asp Asp Asp Lys
HRV 3C	Leu Glu Val Leu Phe Gln Gly Pro

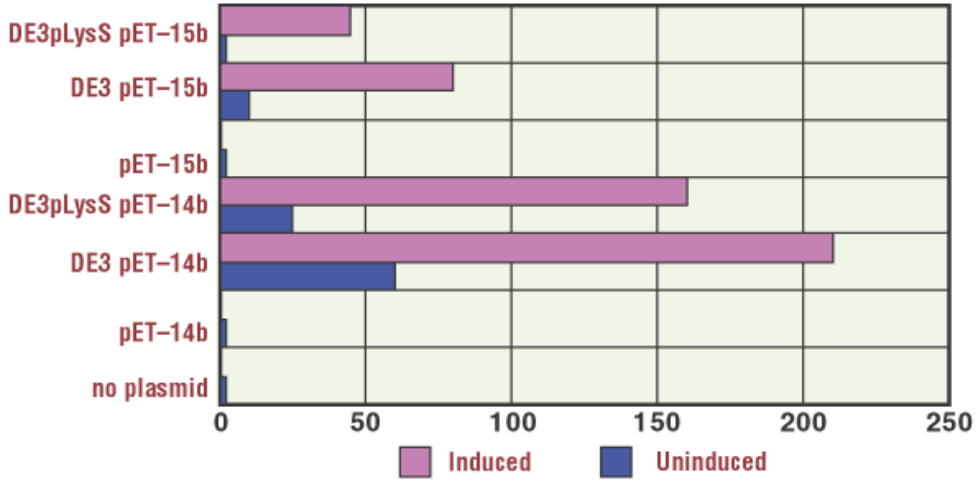




Analysis of pET-32a(+) IL-2 Clones Induced at 15°, 25° and 37°



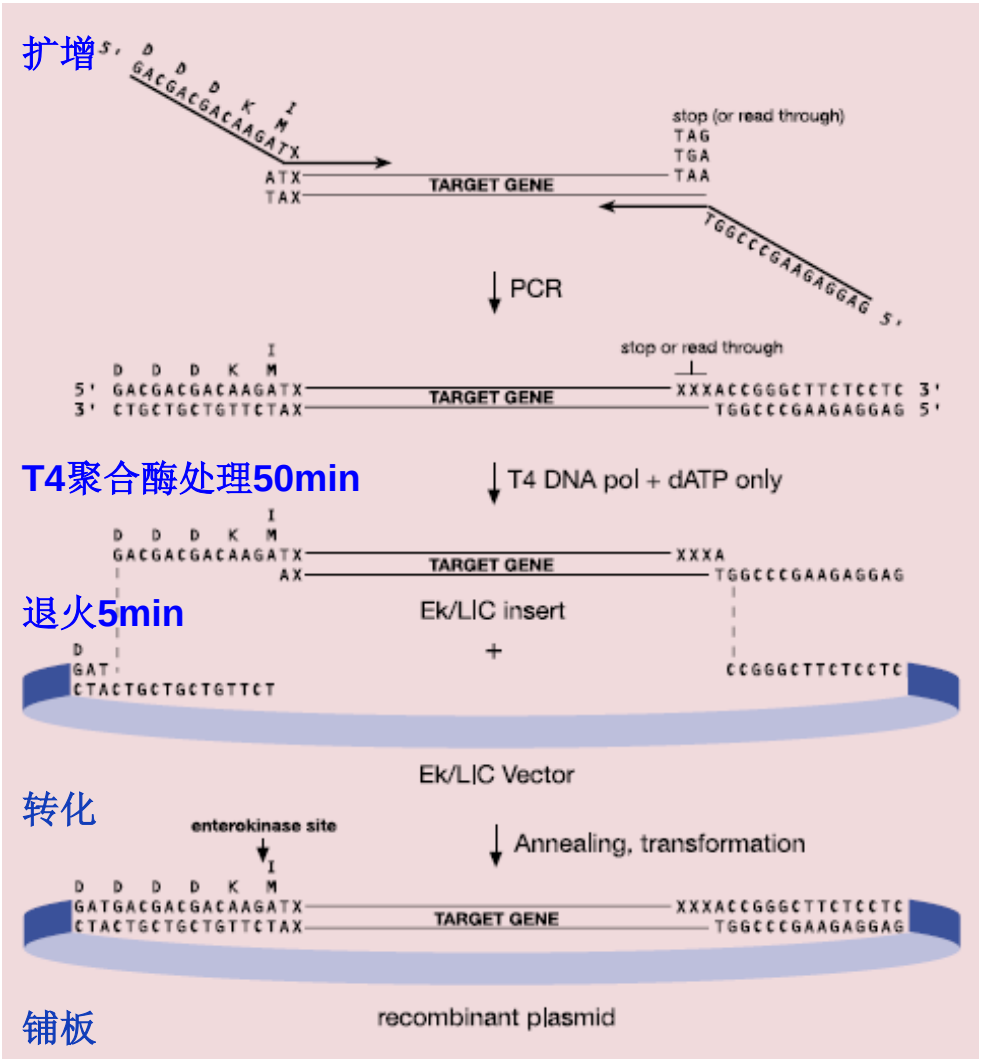
Effect of Host/Vector Combination on Expression Level

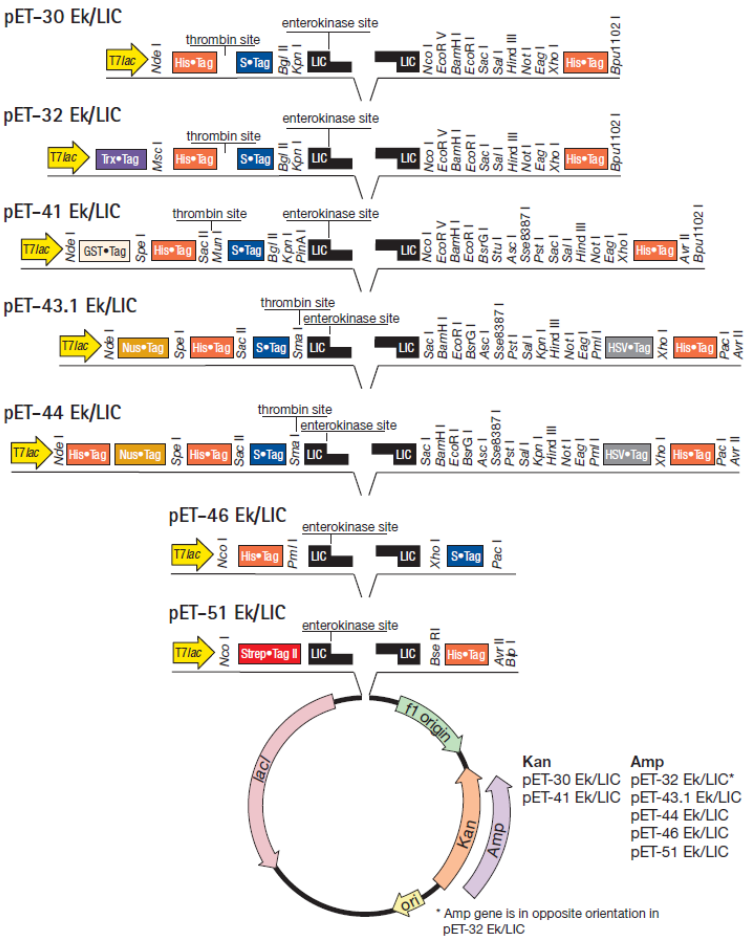


载体-宿主-发酵条件组合多因素互动带来不同结果，数据平行比较是个问题！

高通量克隆表达
抽提纯化与分析

LIC高效克隆方法：1天获得表达克隆





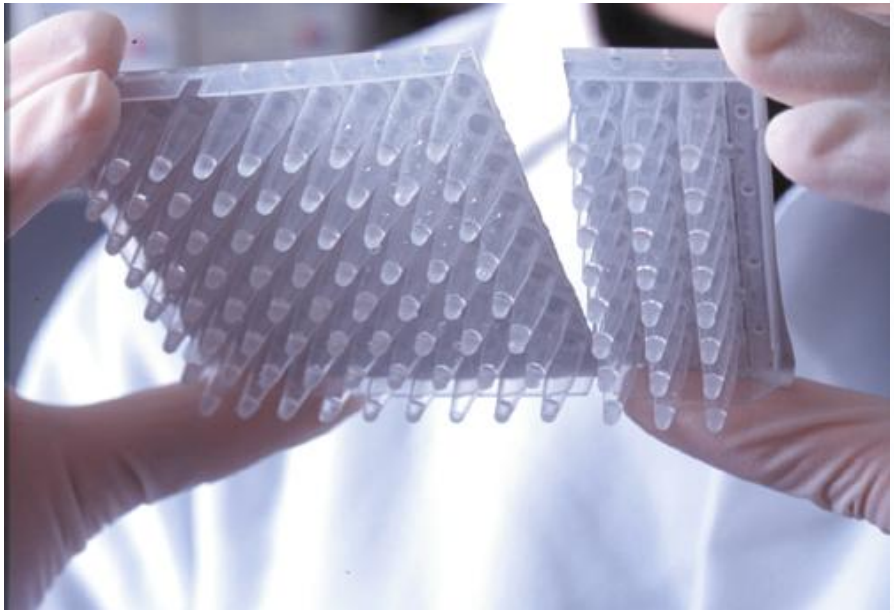
高通量克隆表达

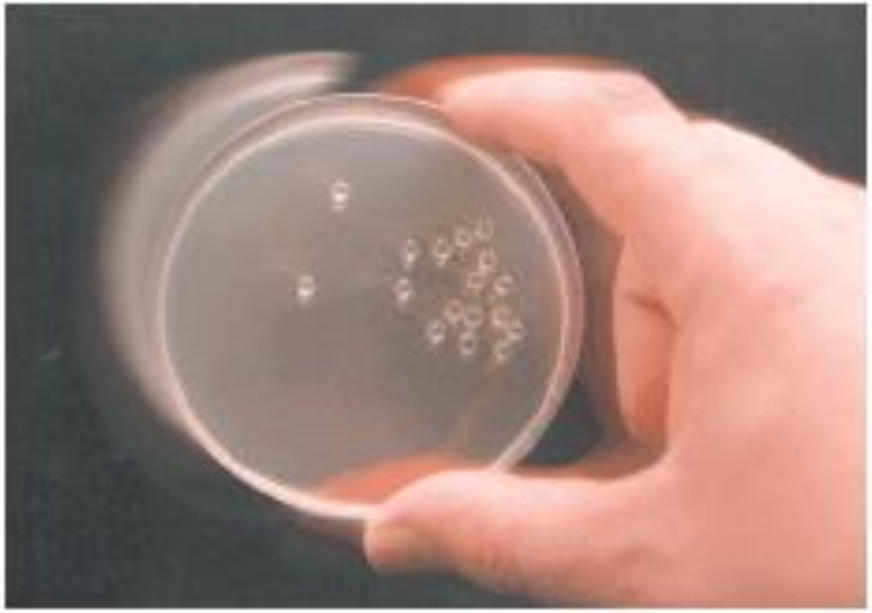
直接使用的预制感受态细胞：

- 克隆型
- 表达型

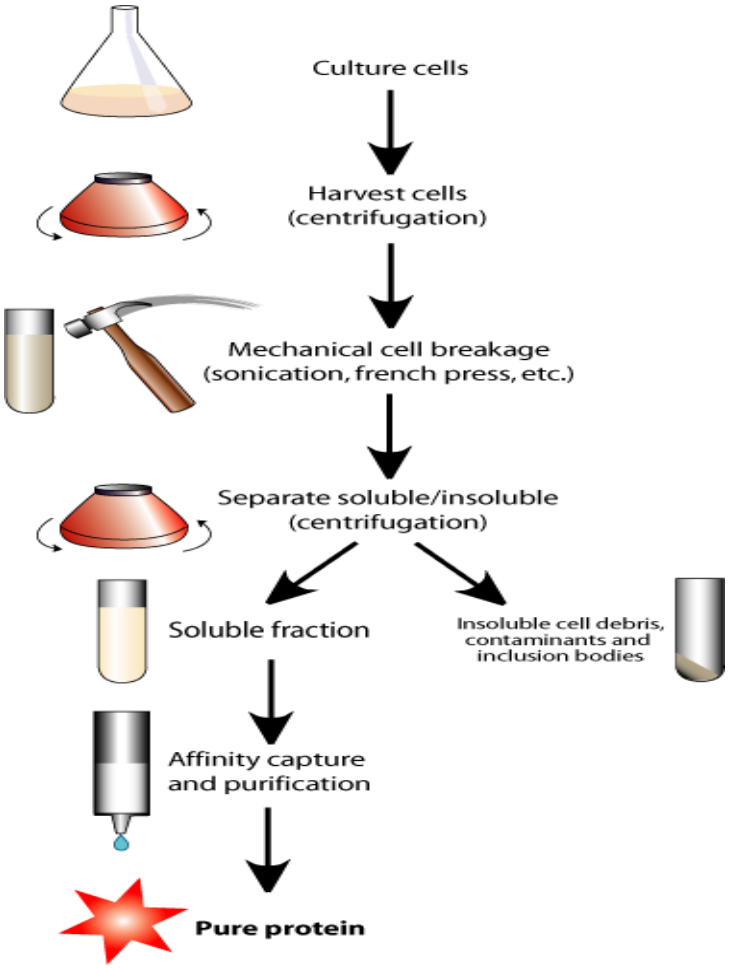
方便又环保的

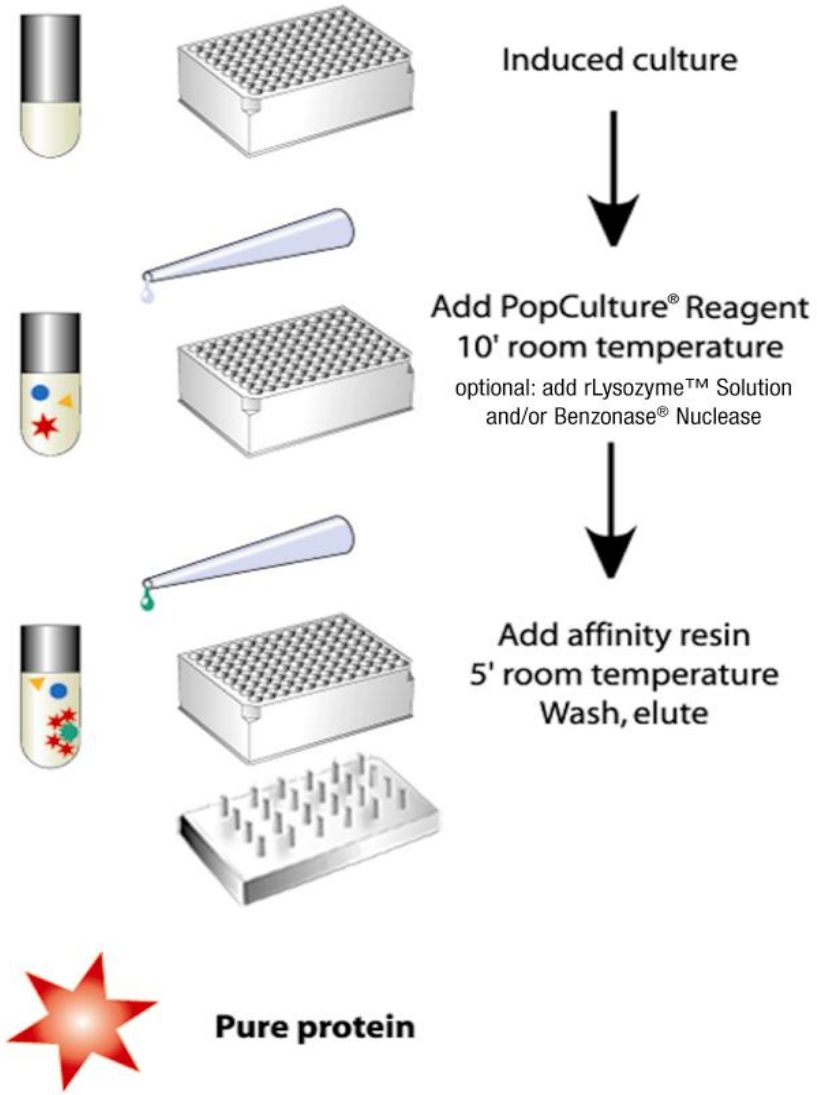
ColiRoller 铺板珠





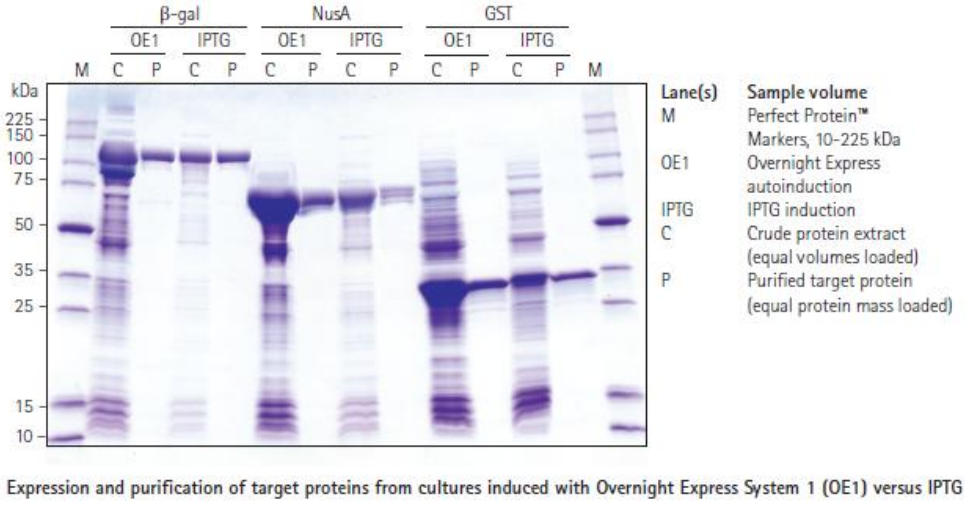
高通量诱导表达、抽提、纯化策略





Overnight Express™ 自动诱导表达培养基

- 应用于**pET**等乳糖操纵子诱导表达系统
- 完全不需要进行**OD**值监控、多次放大和添加**IPTG**等步骤
- 更高的细胞浓度、更高活性蛋白产量

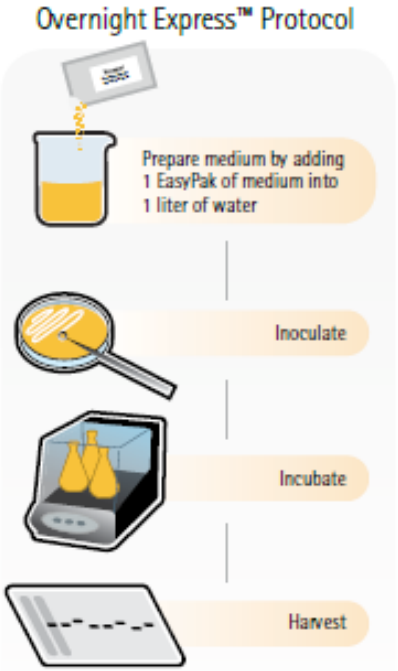


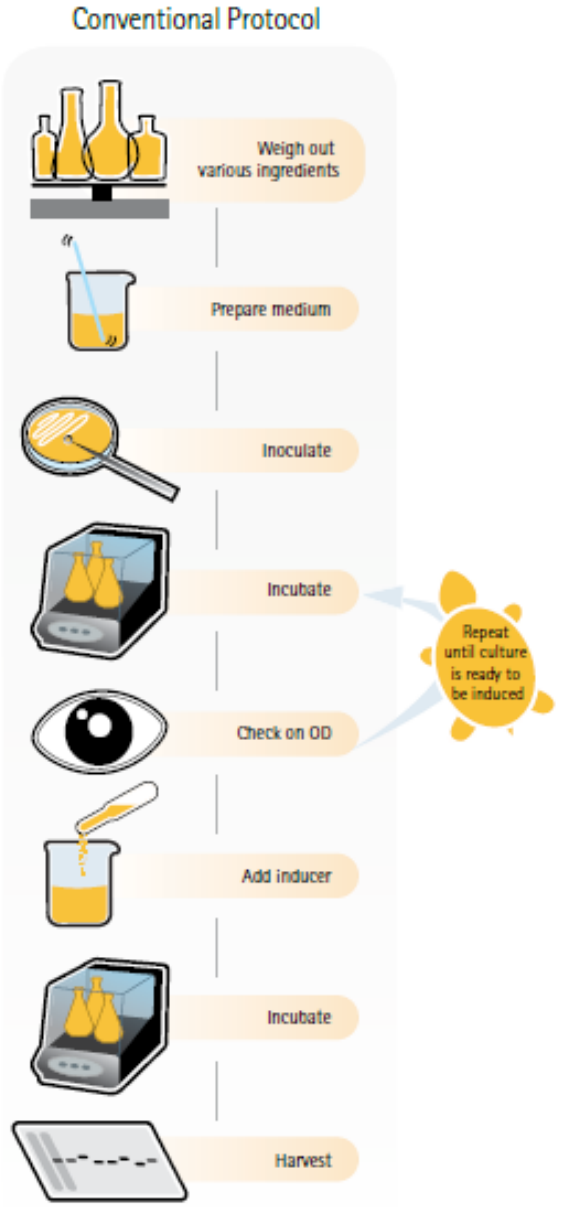
Expression and purification of target proteins from cultures induced with Overnight Express System 1 (OE1) versus IPTG

Overnight Express™ 培养基提高蛋白产量、活性和操作通量

Medium	Protein	Cell Harvest OD_{600} OE1[2]	Cell Harvest OD_{600} IPTG[3]	Pure Protein Yield[1] OE1	Pure Protein Yield[1] IPTG
TB	β-gal	20.7	9.7	675	225
TB	NusA	20.0	8.5	690	240
TB	GST	20.2	15.2	750	255

Overnight Express™ 自动诱导表达培养基





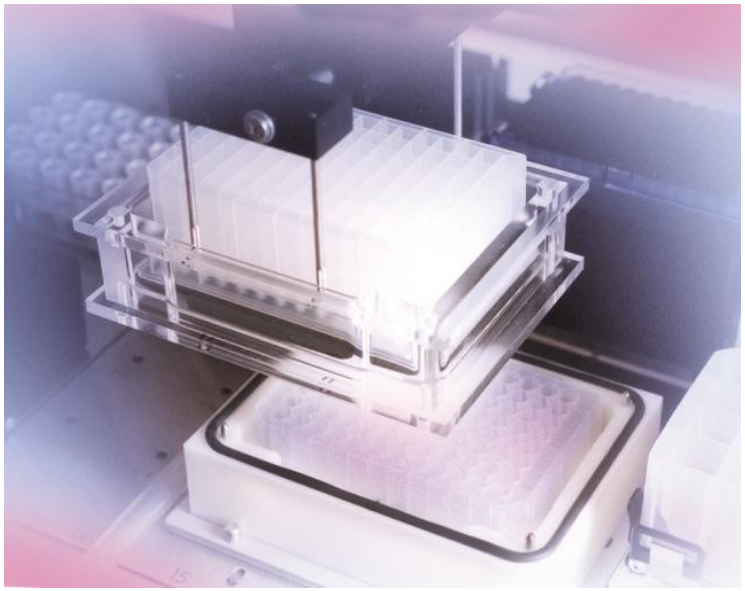
Overnight Express™ systems... for a good night's sleep

中通量/高通量亲和纯化

His•Tag®

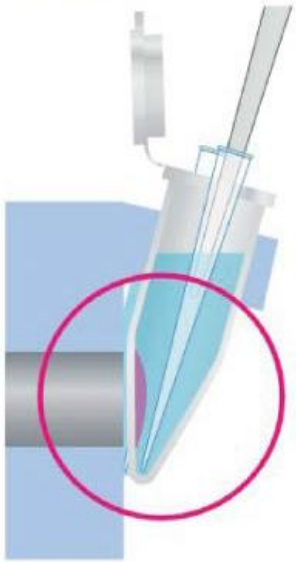
GST•Tag™

Protein A or G



ROBOT FRIENDLY

磁珠方法



PureProteome——新型磁珠法提高了操作通量

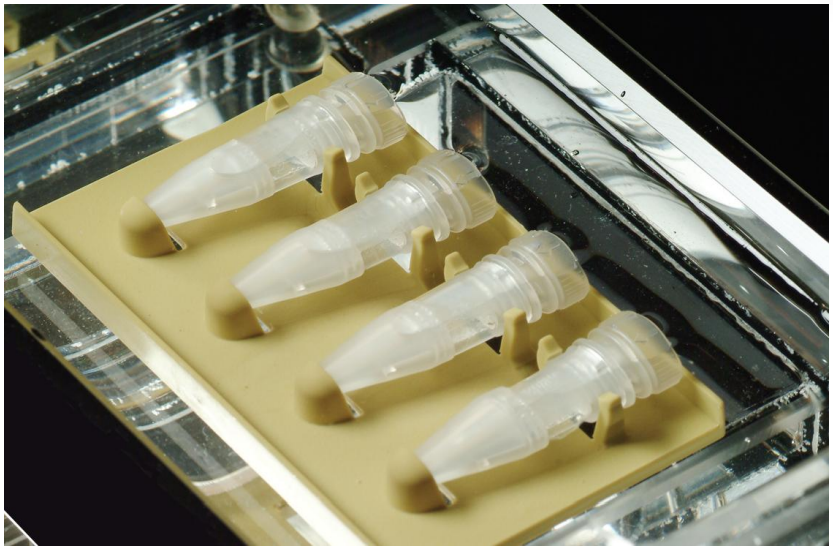
Amicon Pro——纯化、浓缩/、换液一体化设计

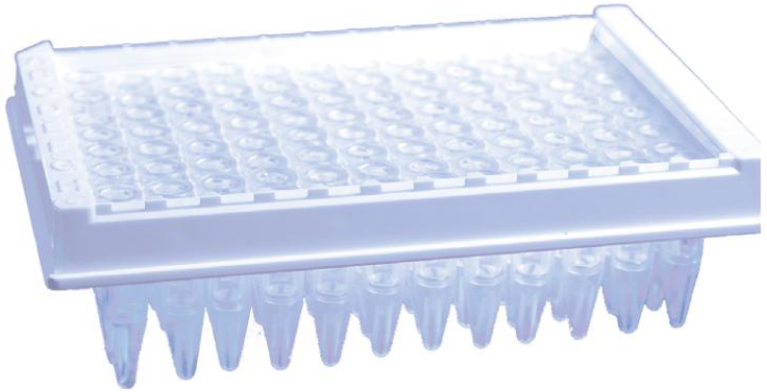
原核表达系统

包涵体/无活性是显著的难题

D-Tube™ 透析管——复性好帮手

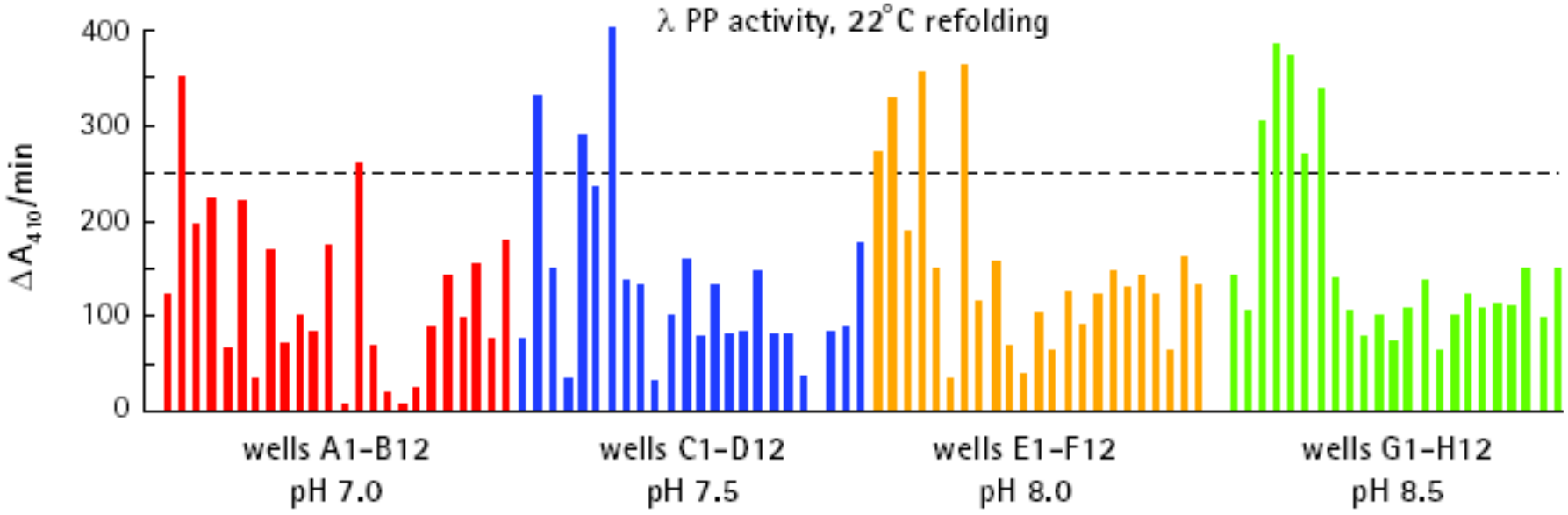
- 旋盖式透析管设计
- 没有样品渗漏或丢失
- 采用移液器操作，简便易行
- 核酸和蛋白电洗脱
- 样品回收率高



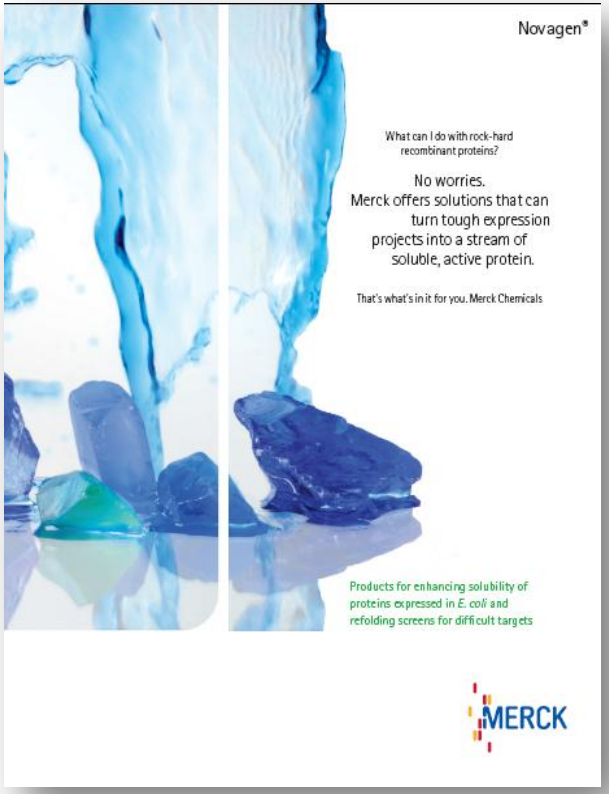


iFold 1/2/3 蛋白复性系统

快速摸索200多个优化复性条件，提高蛋白复性成功率



原核表达平台的蛋白可溶性优化策略

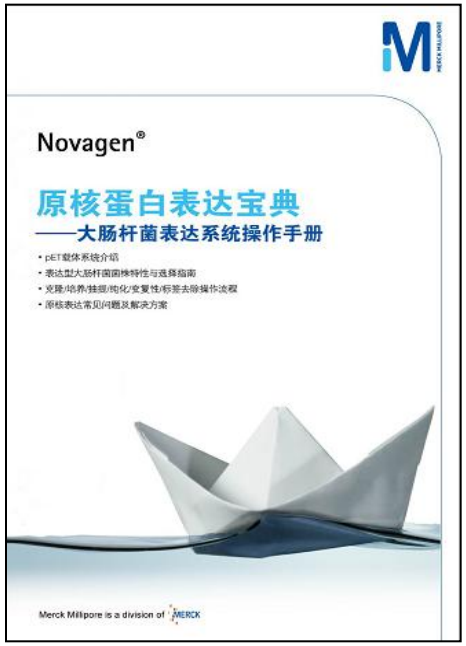


Novagen原核表达系统

从载体、共表达策略、衍生菌株、体外复性……

提供获取更高产的活性可溶蛋白解决方案

您还可以登记索取的资料.....



- 蛋白研究工具产品手册（第三版修订版）
- **pET系统手册英文版或中文版**
- 可溶性策略手册
- 昆虫表达系统手册
- **Strep标签表达、纯化、分析手册**

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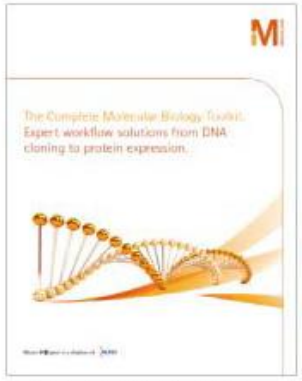
- ✓ 蛋白互作与功能研究
- ✓ Western Blot技术与产品

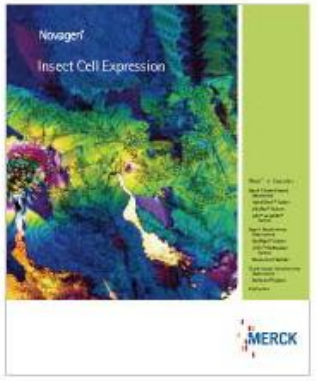


从克隆到表达产品手册

pET系统挂图
（载体–宿主菌–蛋白抽提–纯化–去除标签）

原核蛋白表达宝典

增强原核表达蛋白可溶性技术手册



昆虫细胞蛋白表达技术手册