Clonado de genes
y
Expresión de proteínas

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CONICET
U N C

FCQ

Subcloning

↓

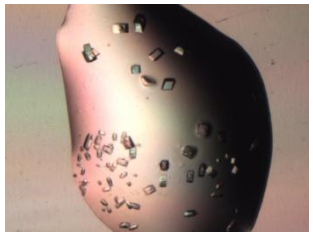
Expression

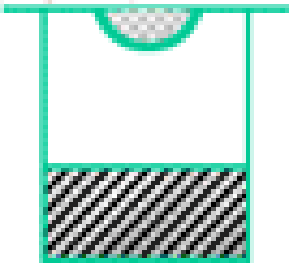
↓

Purification → Biochemical characterization

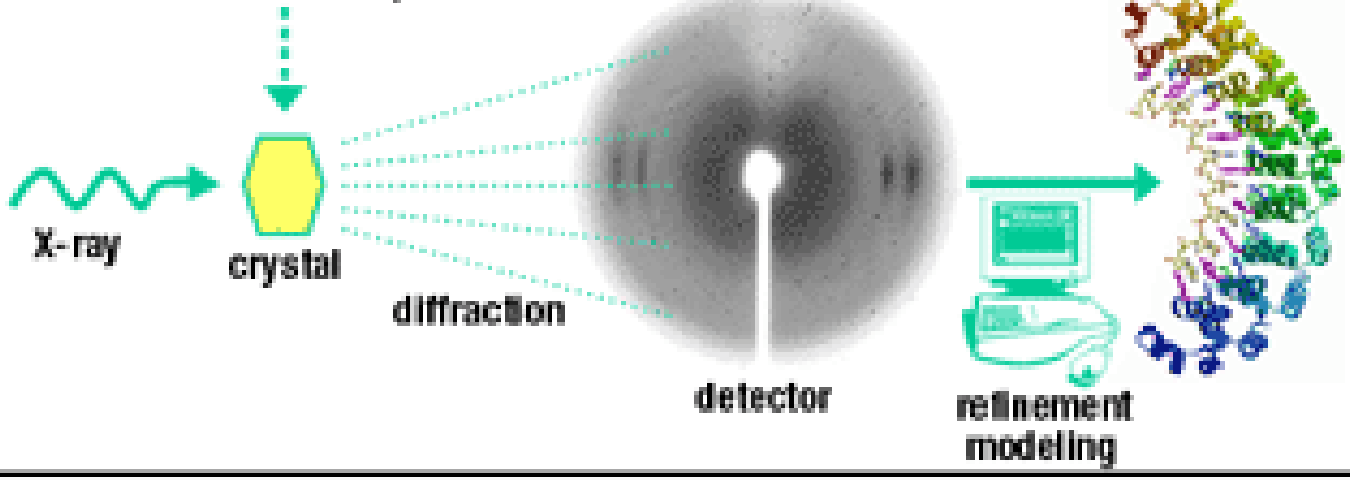
↓

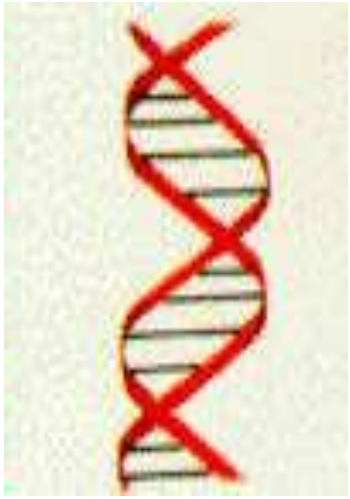
Crystallization: Hanging Drop with Protein



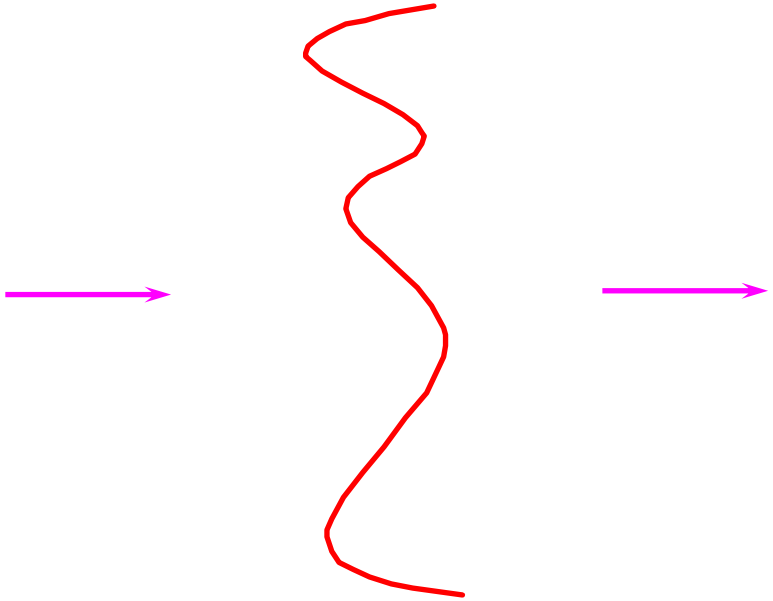


Reservoir with Precipitant

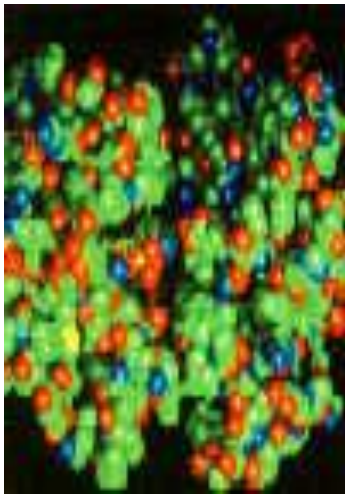




Gen (DNA)

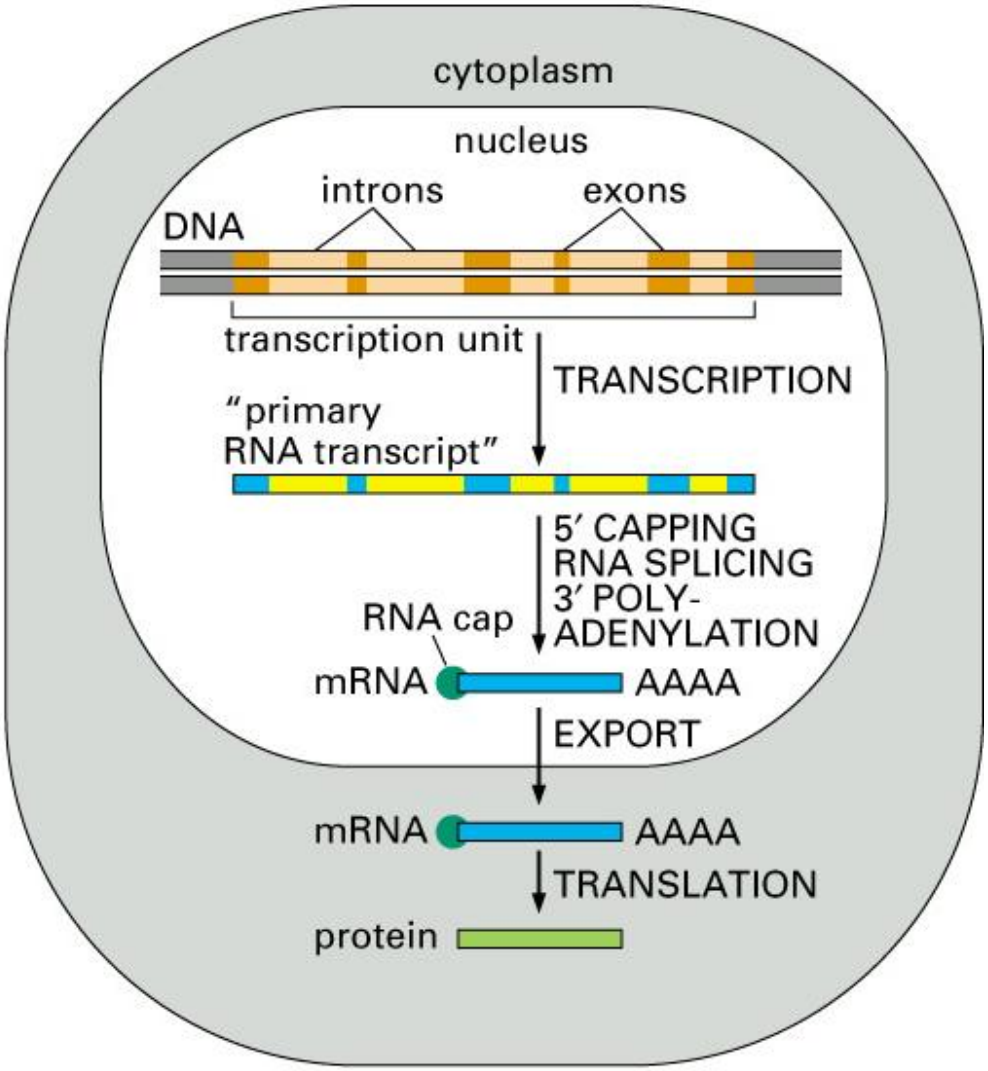


mRNA

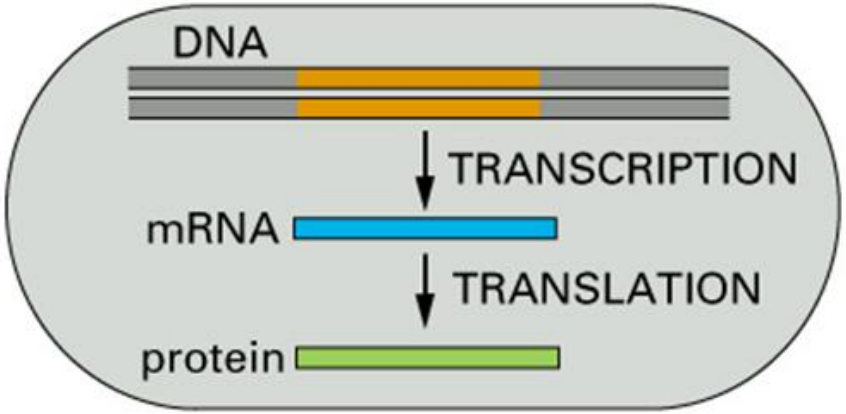


Proteína

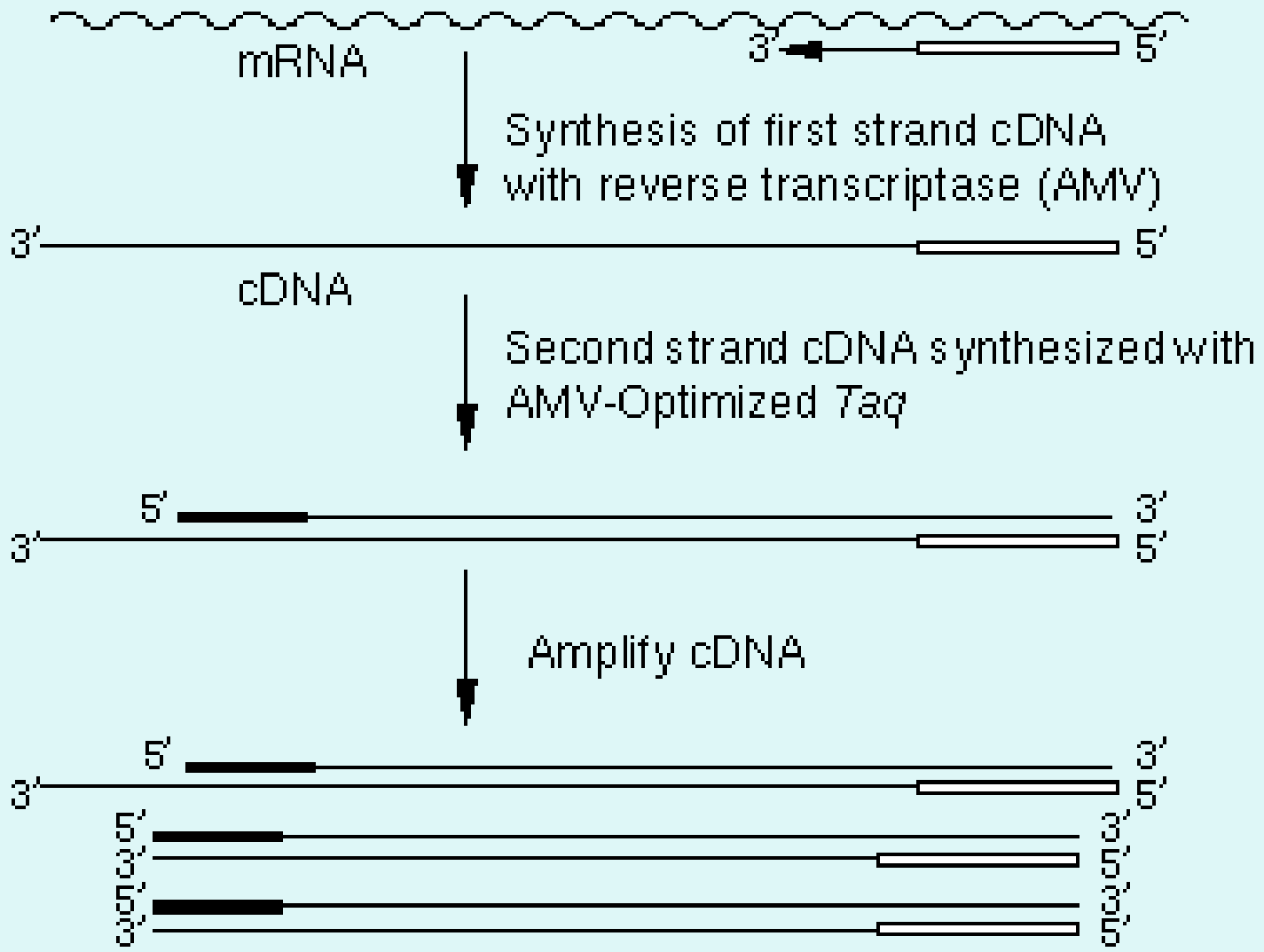
EUCARYOTES



PROCARYOTES



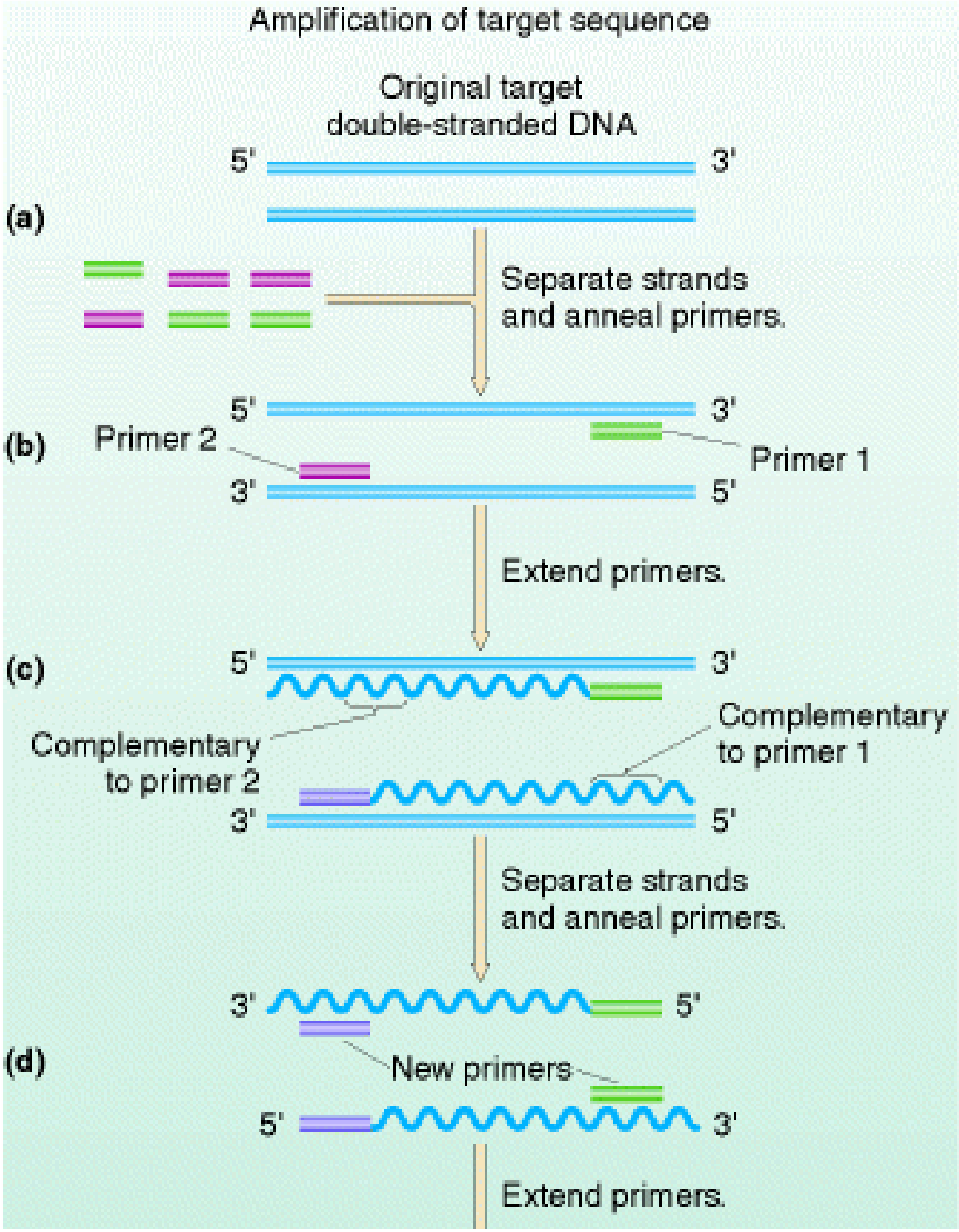
Reverse transcription

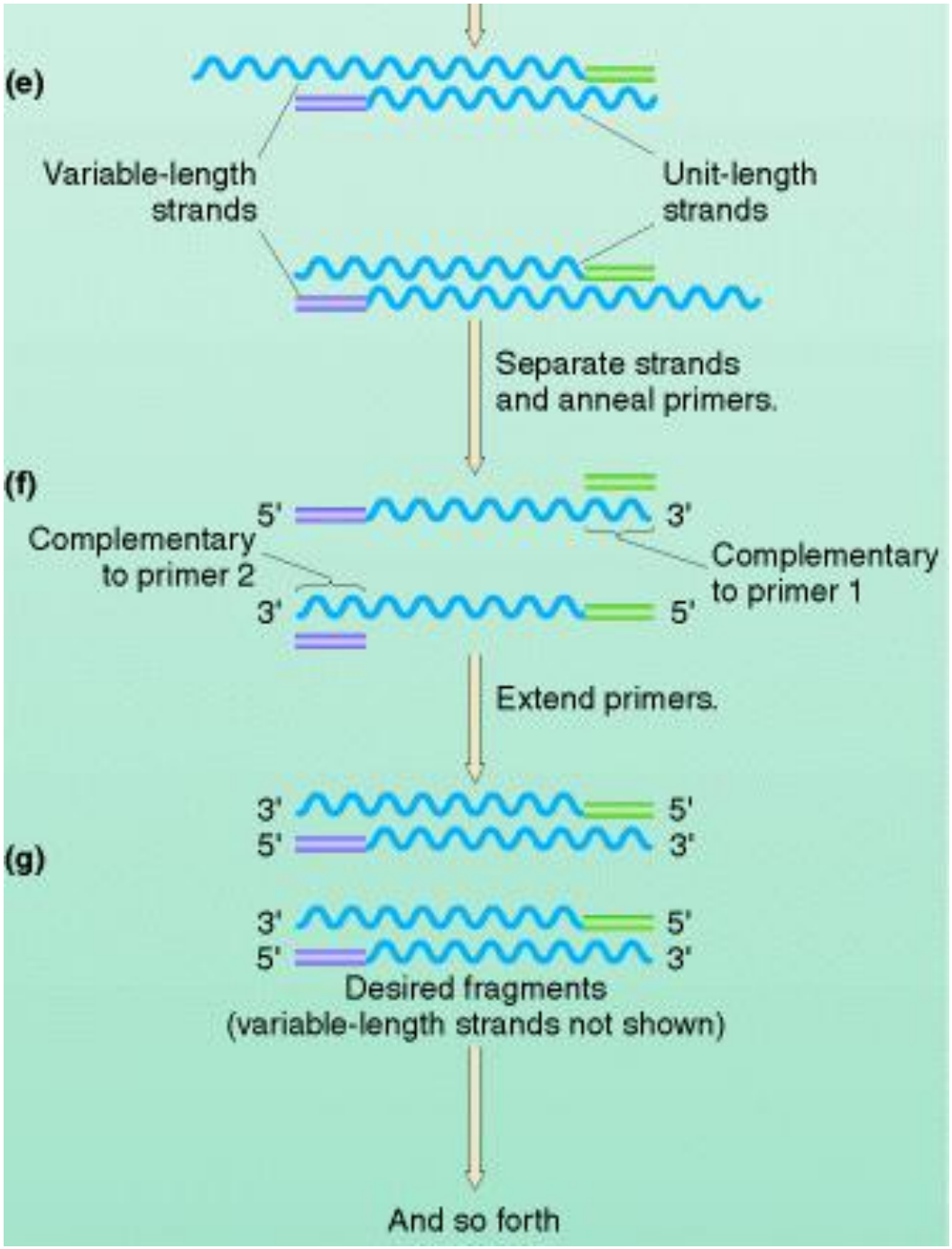


PCR

NdeI

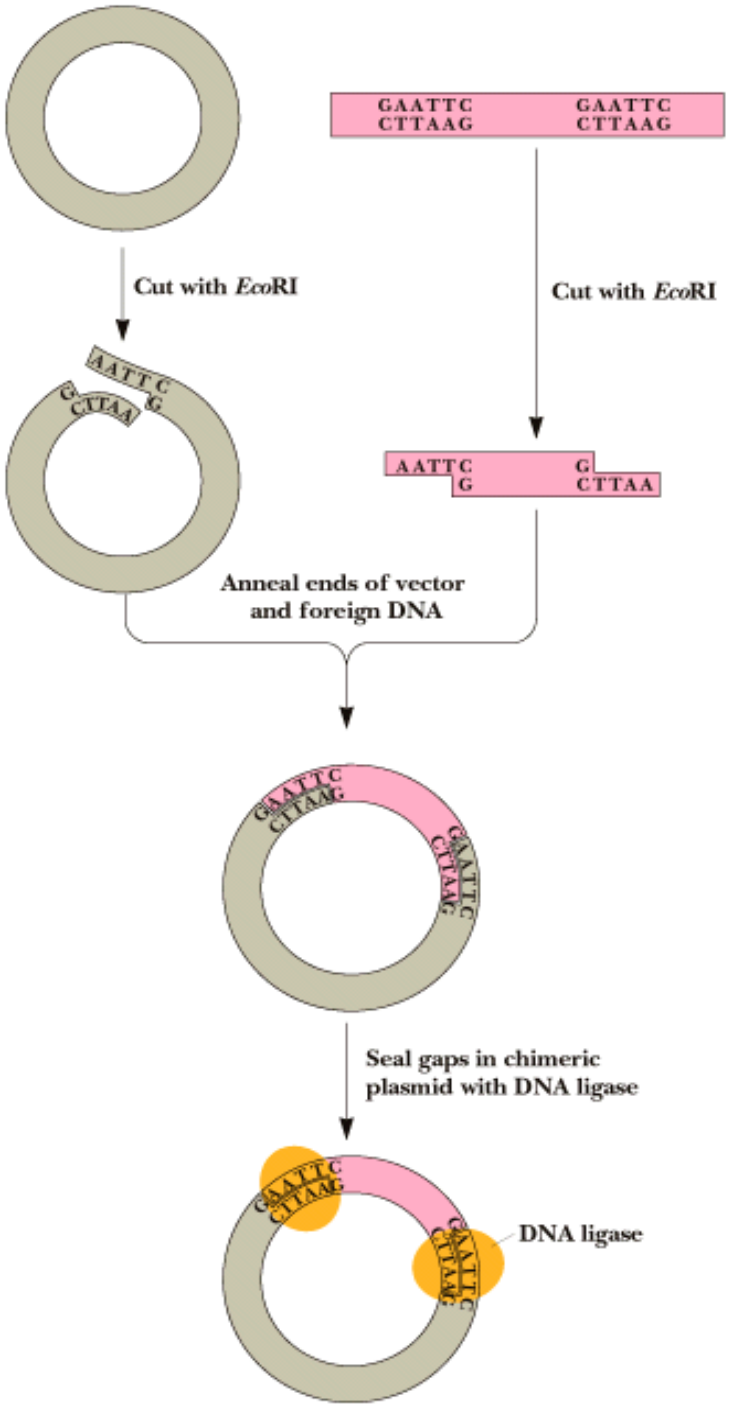
NNCATATGNNNNNNNNNN




(e)
Variable-length strands
Unit-length strands
Separate strands and anneal primers.
(f)
5'
3'
Complementary to primer 2
Complementary to primer 1
3'
5'
Extend primers.
(g)
3'
5'
5'
3'
3'
5'
5'
3'
Desired fragments
(variable-length strands not shown)
And so forth

- Enzimas de restricción

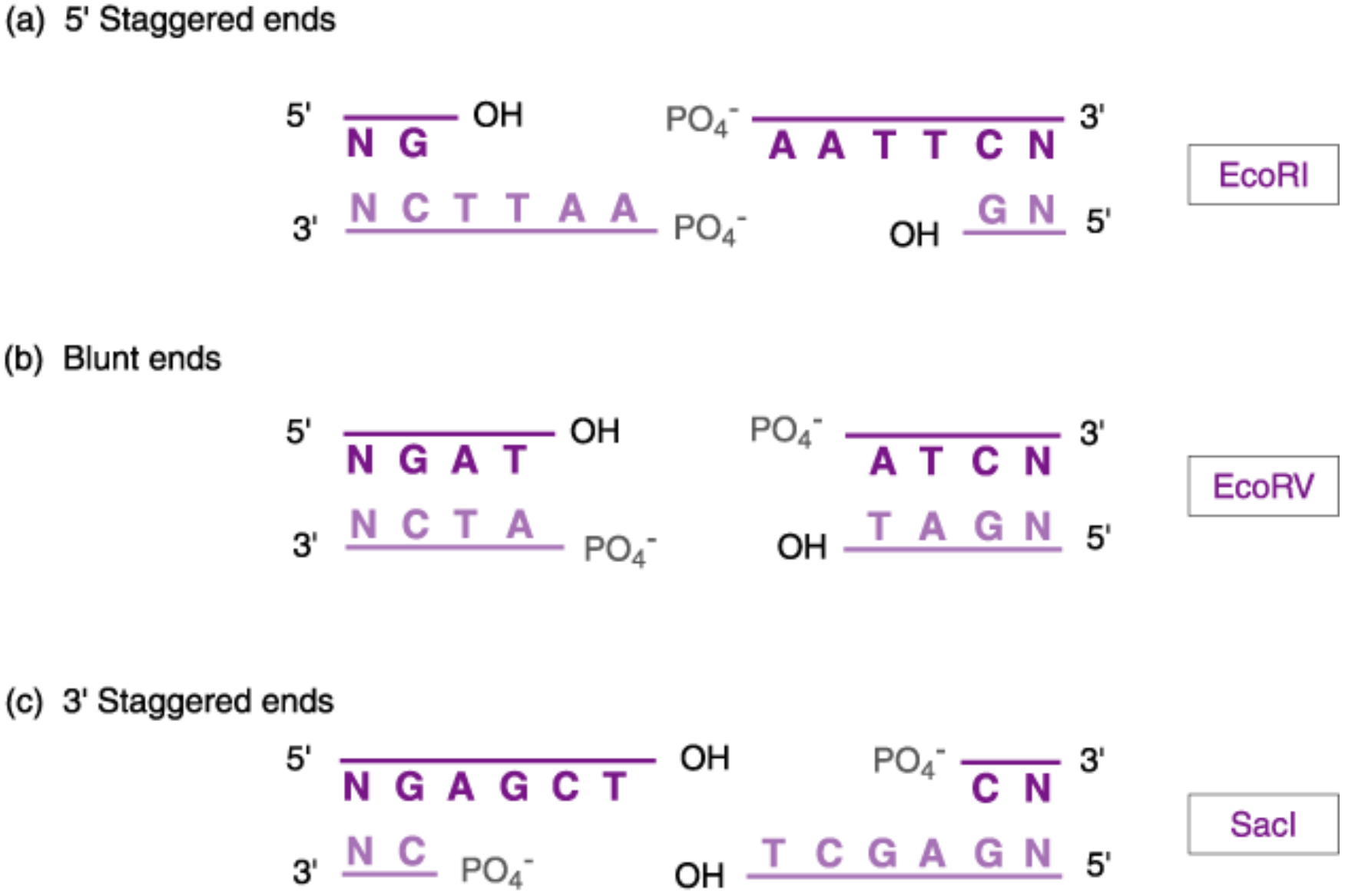
- Ligasa



- Enzimas de restricción

- Ligasa

Enzimas de restricción



Sensibilidad de las ER a la metilación del ADN

Dam(G^{m}ATC), Dcm(C^{m}CWGG) and CpG(^{m}CG) Methylation

Legend

●	Not Sensitive
■	Blocked
□ ol	Blocked by Overlapping
□ sol	Blocked by Some Combinations of Overlapping
◆	Impaired
◇ ol	Impaired by Overlapping
◇ sol	Impaired by Some Combinations of Overlapping

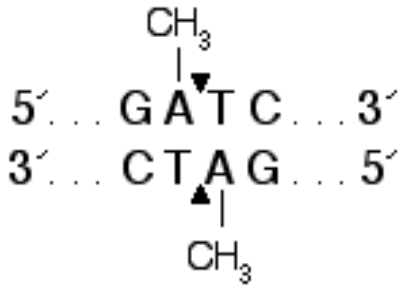
Enzyme	Sequence	Dam	Dcm	CpG
BamH I	GGATCC	●	●	●
Cla I	ATCGAT	□ ol	●	■
Kas I	GGCGCC	●	●	■
Kpn I	GGTACC	●	●	●
Mbo I	GATC	■	●	◇ ol
Sau3A I	GATC	●	●	□ ol
Dpn II	GATC	■	●	●

Single Letter Code

B = C or G or T
D = A or G or T
H = A or C or T
K = G or T
M = A or C
N = A or C or G or T
R = A or G
S = C or G
V = A or C or G
W = A or T
Y = C or T

DpnI

Recognition Site:



MboI

Recognition Site:

Methylation Sensitivity:
dam methylation: Blocked
dcm methylation: Not sensitive
CpG methylation: Impaired by overlapping

DpnII

Recognition Site:

Methylation Sensitivity:
dam methylation: Blocked
dcm methylation: Not sensitive
CpG methylation: Blocked by overlapping

Sau3AI

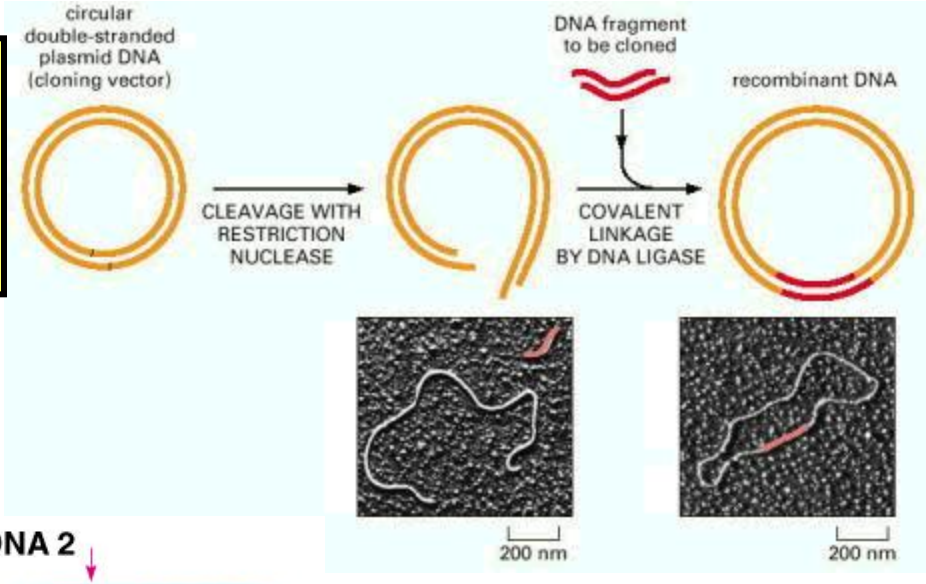
Recognition Site:

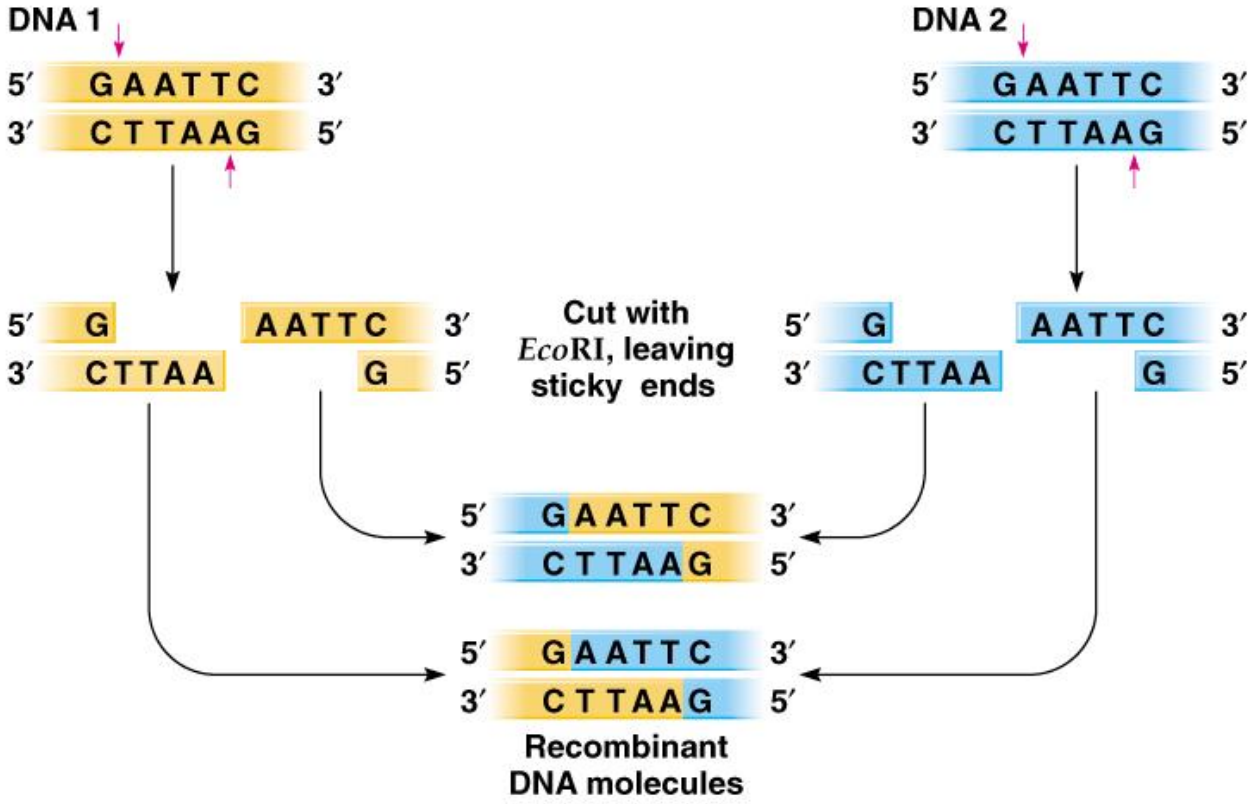
Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Blocked by overlapping

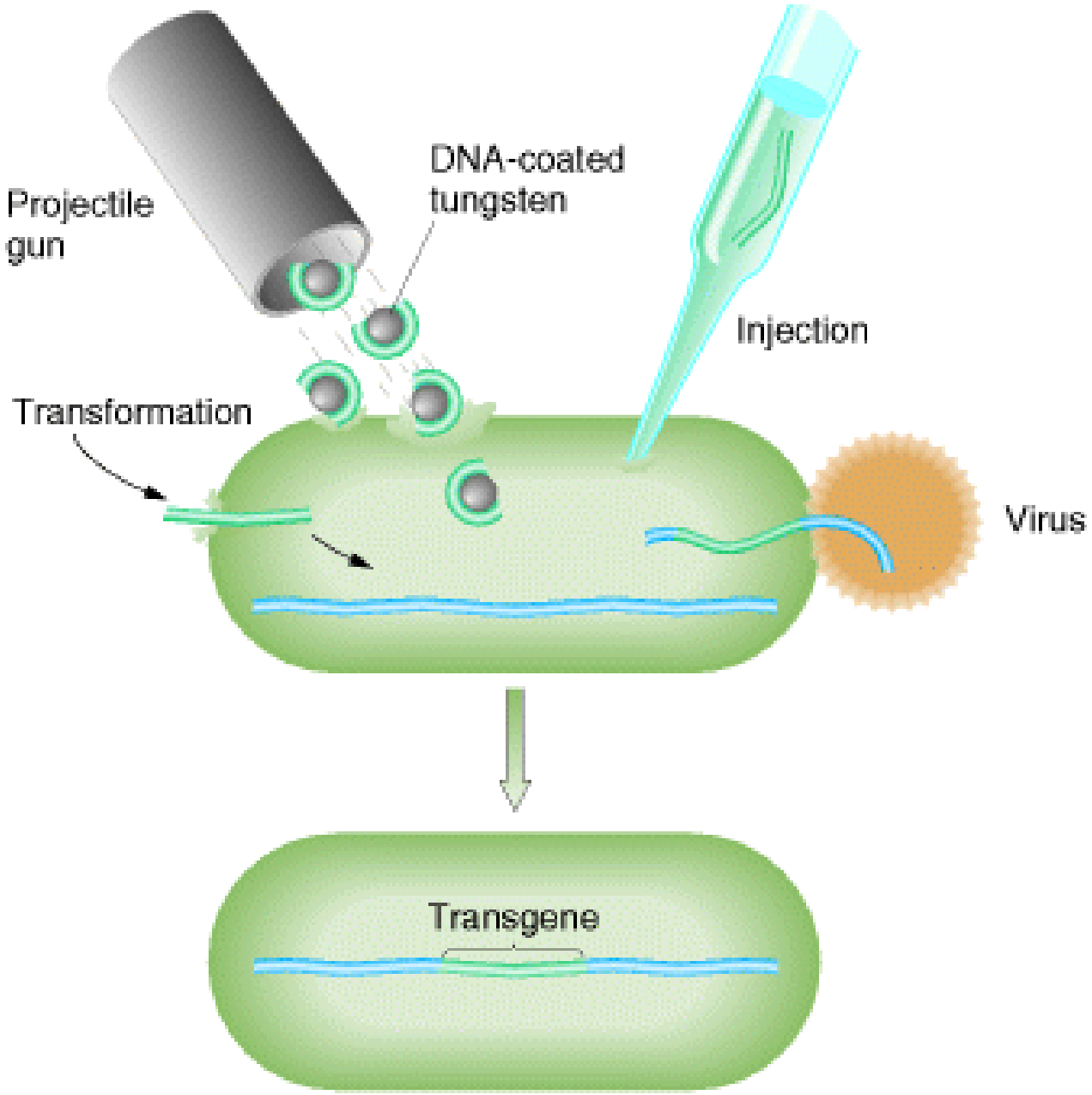
Extremos cohesivos compatibles

BamH I (G/GATCC)	Bcl I, Dpn II	Alw I, Dpn II
	Bgl II, BstY I (R/GATCY)	Alw I, BstY I, Dpn II
	BstY I (G/GATCC)	Alw I, BamH I, BstY I, Dpn II, Nla IV
Ban I		
(G/GTACC)	Acc65 I	Acc65 I, Ban I, Kpn I, Nla IV, Rsa I
(G/GCGCC)	Kas I	Ban I, BsaH I, Hae II, Hha I, Kas I, Nar I, Nla IV
(G/GTACC)	BsiW I, BsrG I	Rsa I
Ban II		
(GGGCC/C)	Apa I, Bsp1286 I (GGGCC/C)	Apa I, Ban II, Bsp1286 I, Hae III, Nla IV, Sau96 I
(GAGCT/C)	Bsp1286 I (GAGCT/C), Sac I	Alu I, Ban II, BsiHKA I, Bsp1286 I, Sac I
Bcl I (T/GATCA)	BamH I, BstY I (R/GATCY)	Alw I, Dpn II
	Bgl II, Mbo II	Dpn II
Bfa I (C/TAG)	Ase I, Csp6 I, Mse I, Nde I	--
Bgl II (A/GATCT)	BamH I, BstY I (R/GATCY)	Alw I, BstY I, Dpn II
	Bcl I, Dpn II	Dpn II

Ligado de fragmentos de ADN






Projectile gun
DNA-coated tungsten
Injection
Transformation
Virus
Transgene

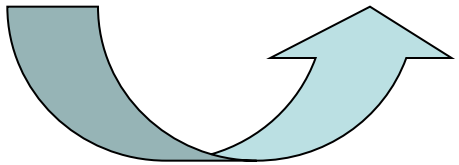
- **Electroporación**

→ 1 célula/10^2-10^3 células

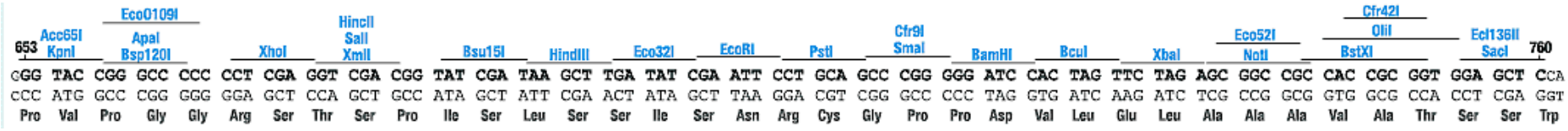
- Tratamiento con **$CaCl_2$** mas shock térmico

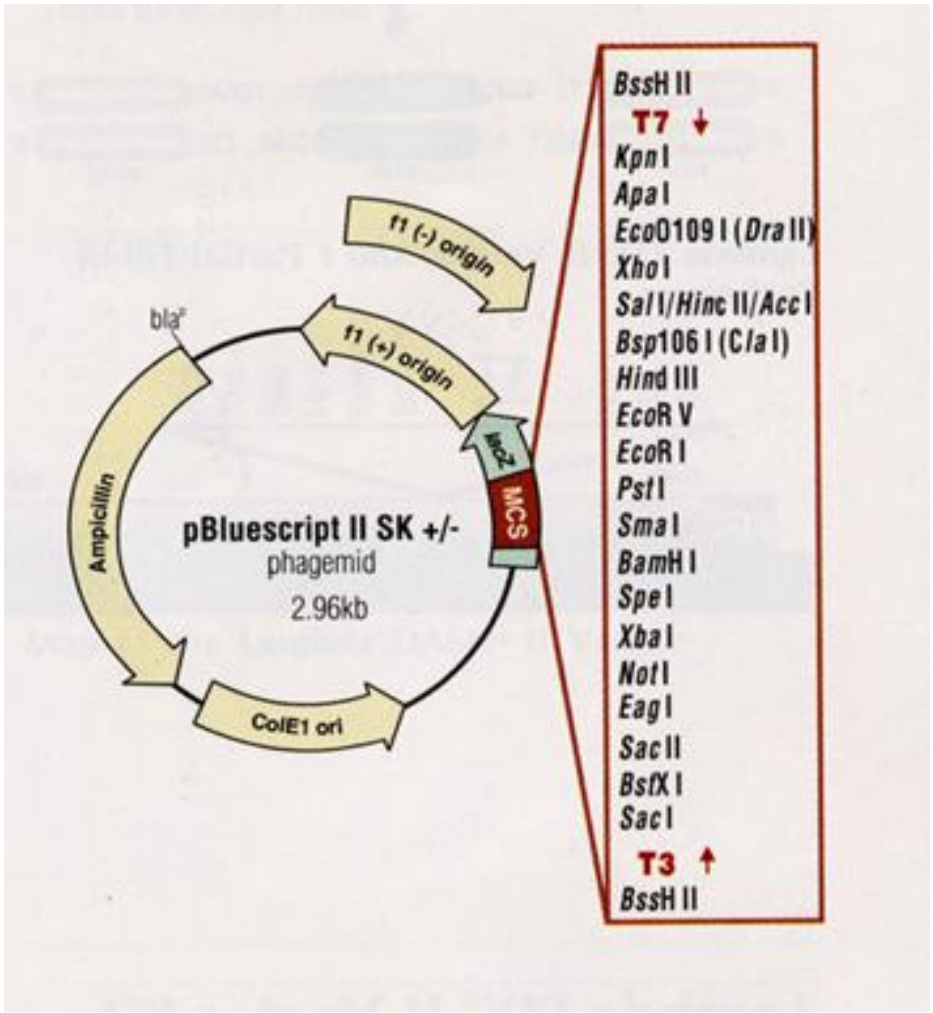
→ 1 célula/10^5-10^6 células

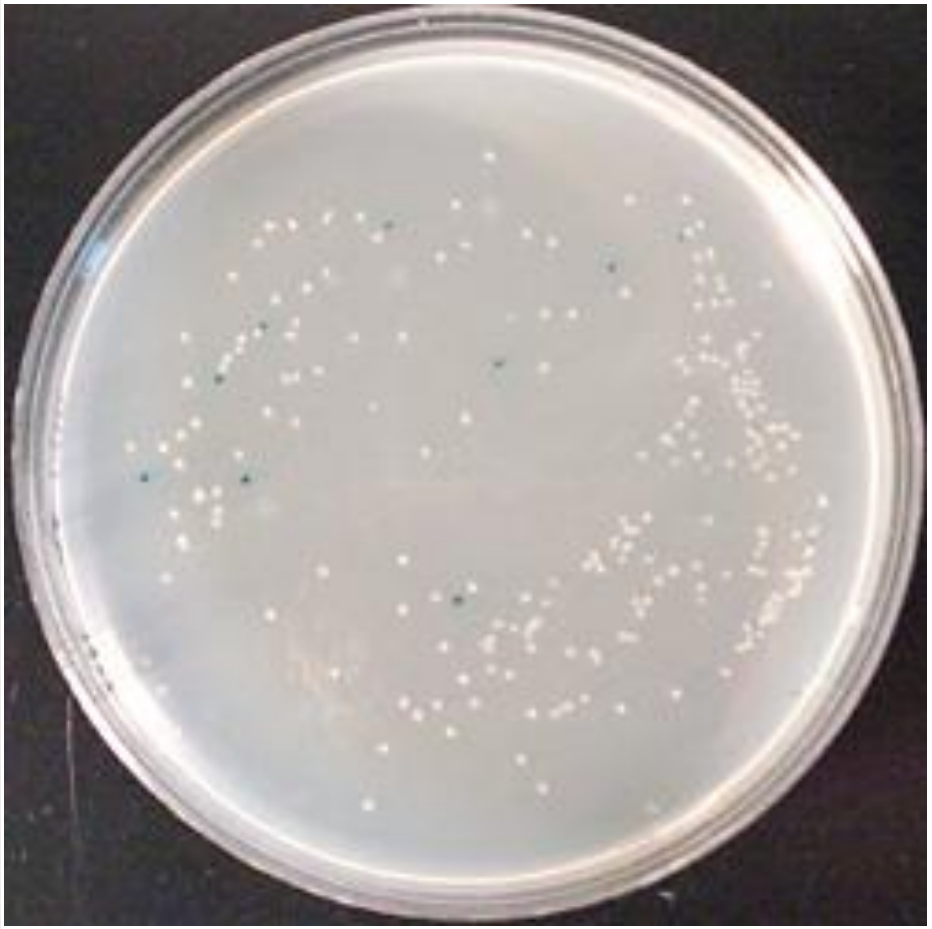
Amplificación del gen clonado y purificación del vector de clonado - expresión

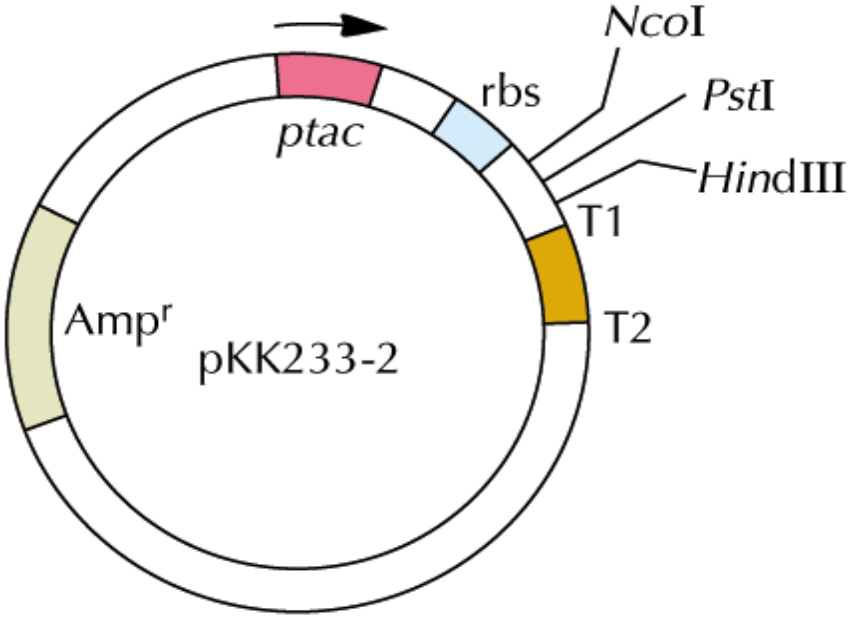


Plásmidos


653
Acc65I KpnI
EcoO109I ApaI Bsp120I
XhoI
HincII SalI XmiI
Bsu15I
HindIII
Eco32I
EcoRI
PstI
Cfr9I SmaI
BamHI
BcuI
XbaI
Eco52I NotI
Cfr42I OliI BstXI
EcI136II SacI
760
CGG TAC CGG GCC CCC CCT CGA GGT CGA CGG TAT CGA TAA GCT TGA TAT CGA ATT CCT GCA GCC CGG GGG ATC CAC TAG TTC TAG AGC GGC CGC CAC CGC GGT GGA GCT CCA
CCC ATG GCC CGG GGG GGA GCT CCA GCT GCC ATA GCT ATT CGA ACT ATA GCT TAA GGA CGT CGG GCC CCC TAG GTG ATC AAG ATC TCG CCG GCG GTG GCG CCA CCT CGA GGT
Pro Val Pro Gly Gly Arg Ser Thr Ser Pro Ile Ser Leu Ser Ser Ile Ser Asn Arg Cys Gly Pro Pro Asp Val Leu Glu Leu Ala Ala Ala Val Ala Thr Ser Ser Trp

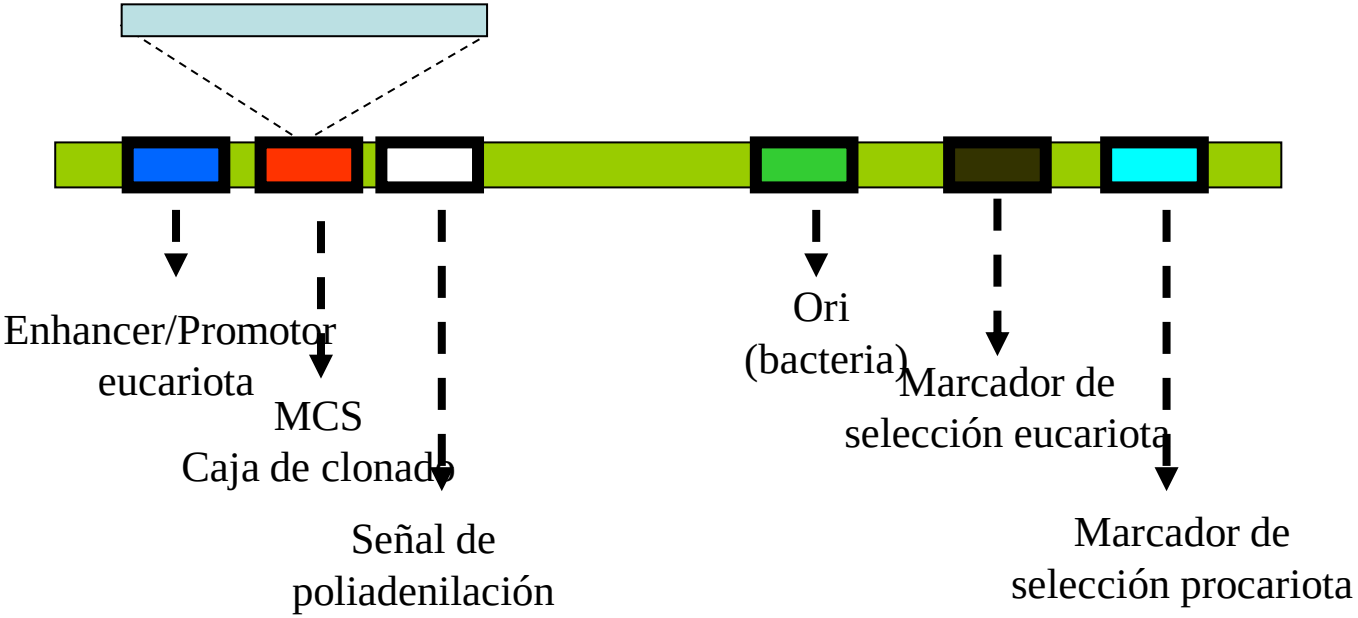

BssH II
T7
Kpn I
Apa I
EcoO109 I (Dra II)
Xho I
Sal I/Hinc II/Acc I
Bsp106 I (Cla I)
Hind III
EcoR V
EcoR I
Pst I
Sma I
BamH I
Spe I
Xba I
Not I
Eag I
Sac II
BstX I
Sac I
T3
BssH II
f1 (-) origin
f1 (+) origin
bla
lacZ
MCS
Ampicillin
pBluescript II SK +/-
phagemid
2.96kb
ColE1 ori



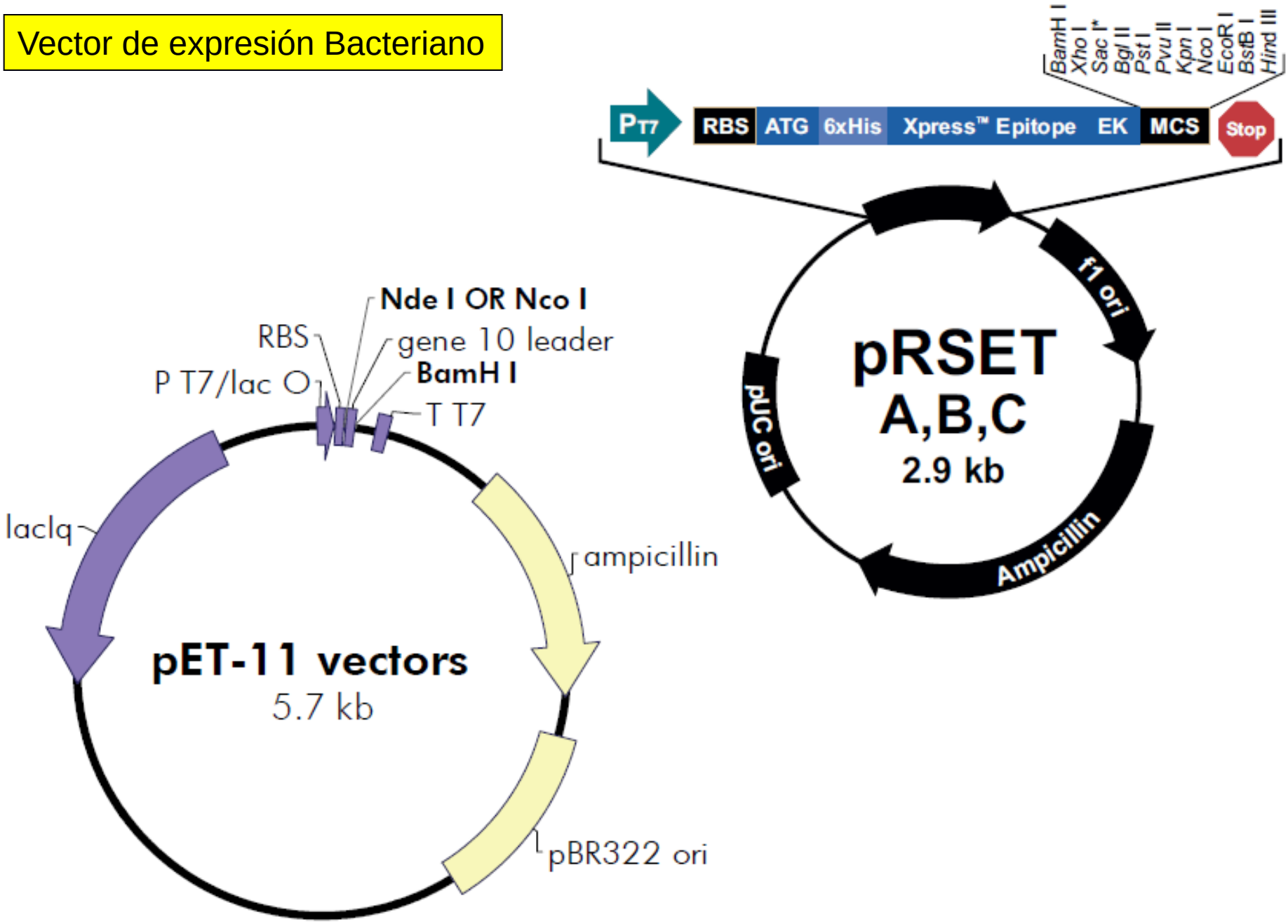


Vector de expresión Bacteriano

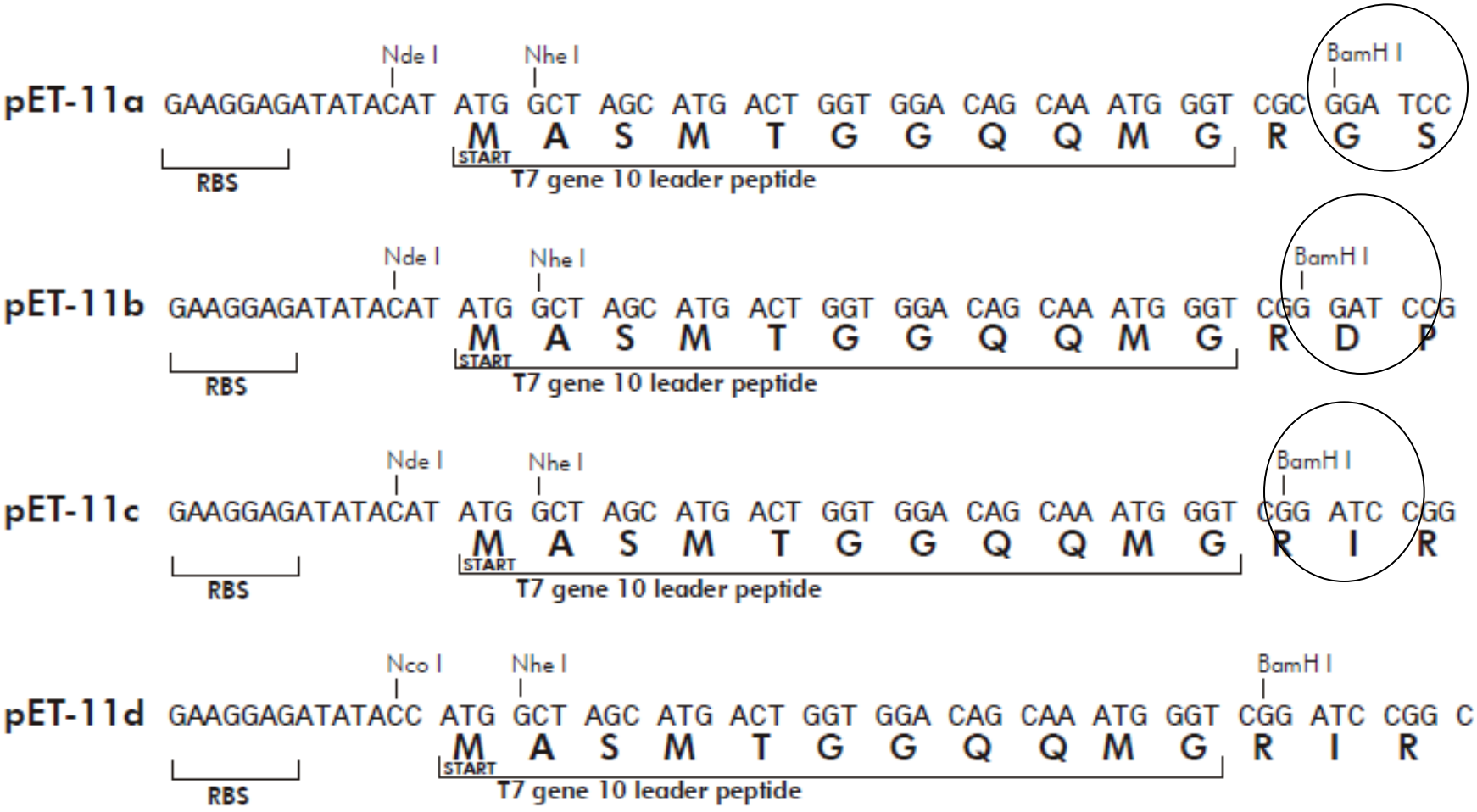
Vector de expresión Eucariota

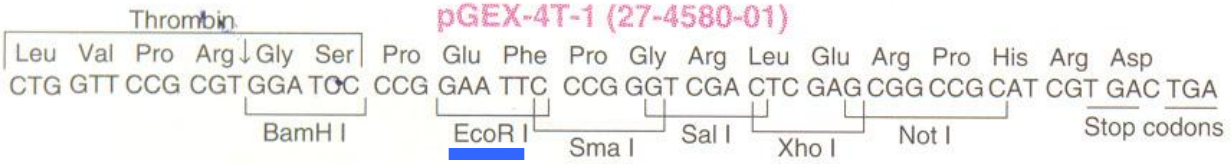


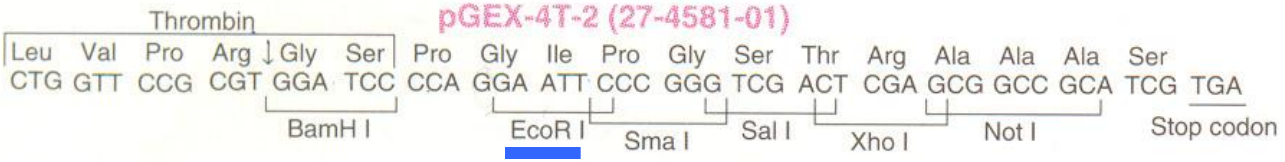
Vector de expresión Bacteriano

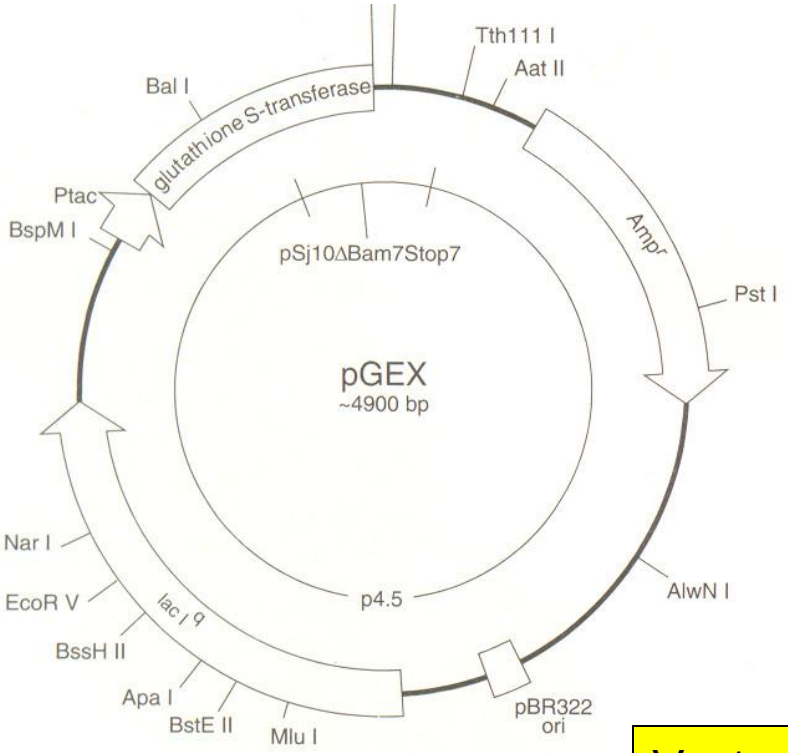


Vector de expresión Bacteriano





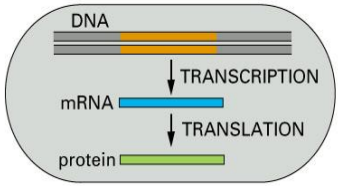




Vector de expresión Bacteriano

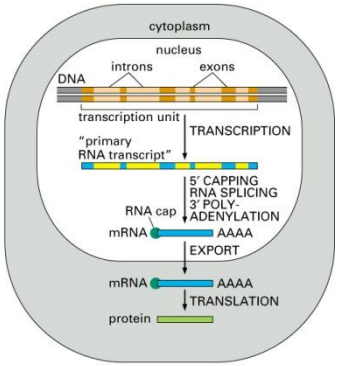
PROTEINAS NATIVAS: Se pueden purificar…… PERO, existen proteínas solo presentes en tejidos de difícil acceso (humanos) o presentes en cantidades extremadamente pequeñas.
PROTEINAS MODIFICADAS O NUEVAS (no existentes en la naturaleza)

Obtención de proteínas recombinantes



Células procariotas (cultivo)

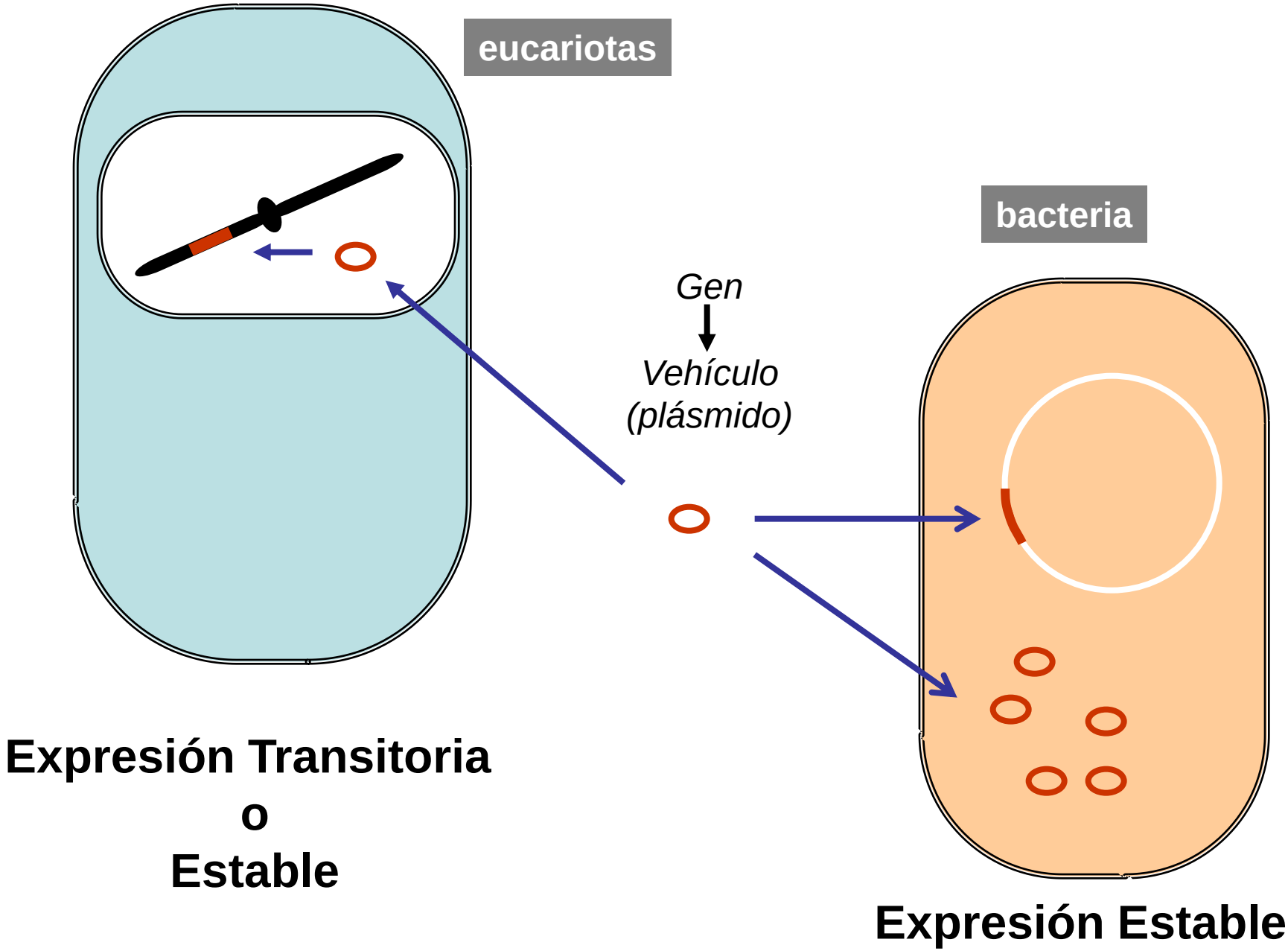
GENES AISLADOS

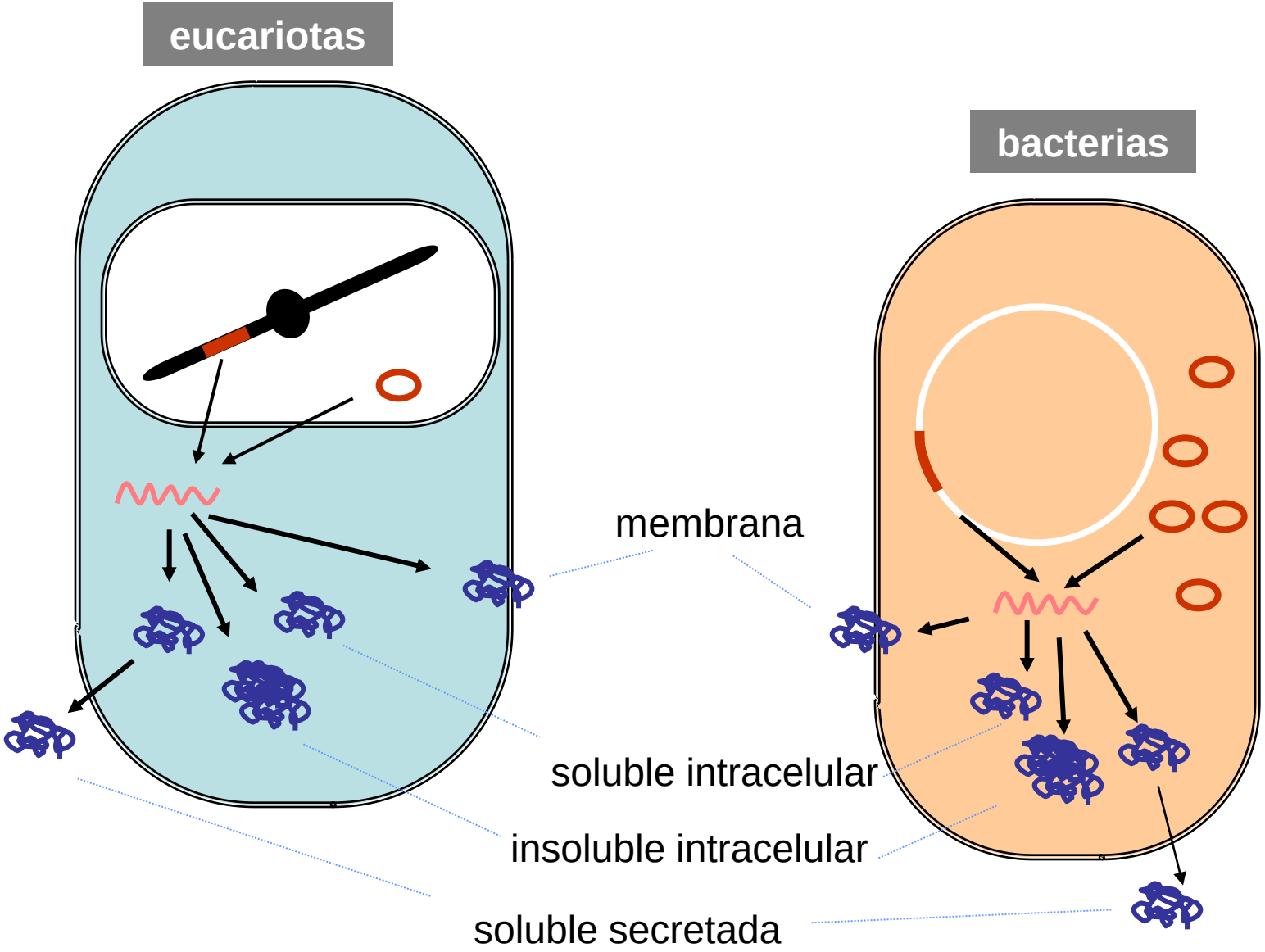


Células eucariotas (cultivo)

Animales

Vegetales


eucariotas
bacteria
Gen
Vehículo
(plásmido)
Expresión Transitoria
o
Estable
Expresión Estable


eucariotas
bacterias
membrana
soluble intracelular
insoluble intracelular
soluble secretada

EXPRESION DE PROTEINAS EN SISTEMAS PROCARIOTAS Y EUCARIOTAS

PROCARIOTAS

Ventajas

- Facil manipulación
- Altos niveles de expresión (1-30%)
- Bajos costos y simplicidad en la producción a gran escala

Desventajas

- Plegamiento incorrecto
- Ausencia de modificaciones postraducción
- Bajos niveles de secreción

EUCARIOTAS

Ventajas

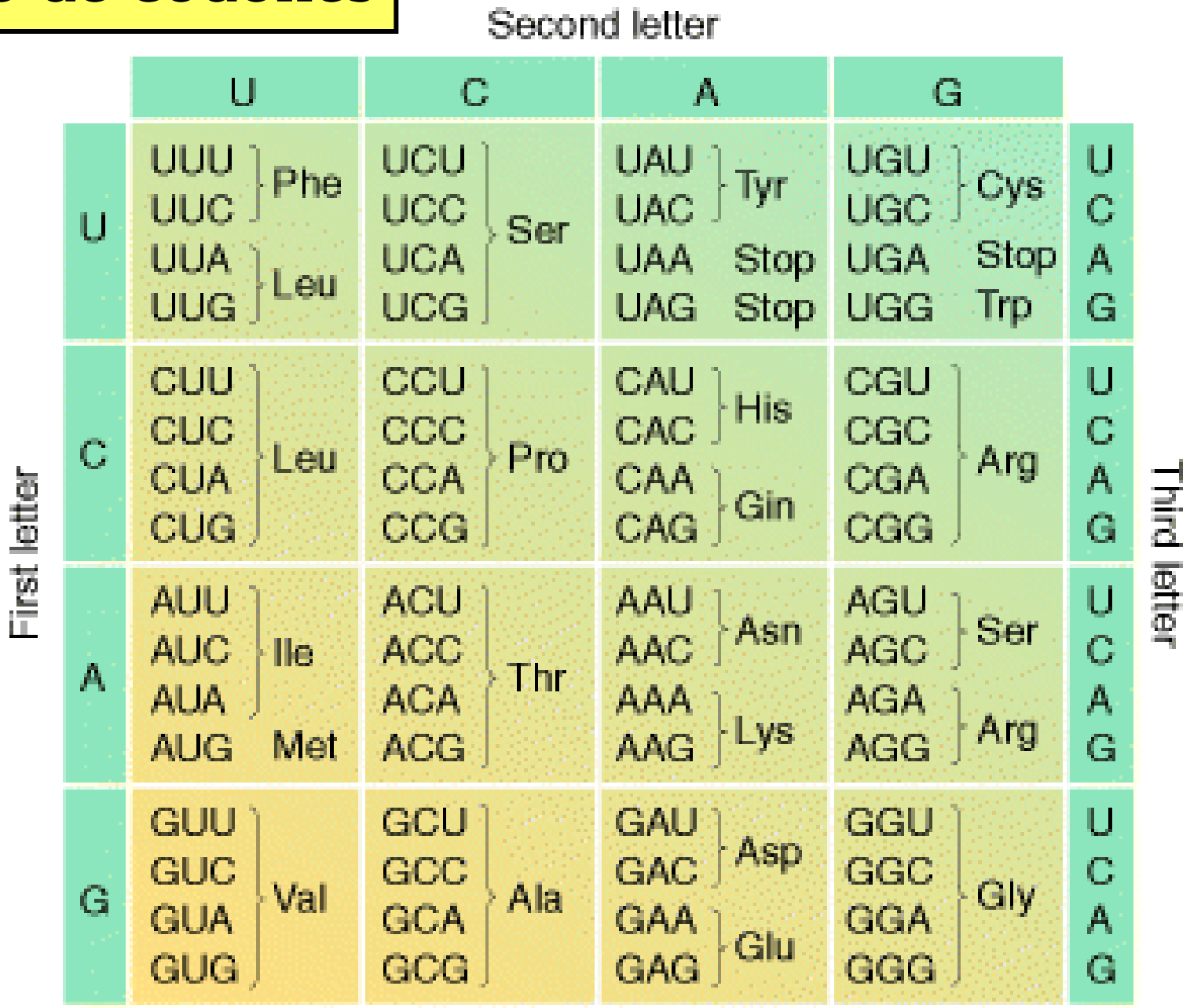
- Plegamiento correcto, actividad biológica
- Modificaciones postraducción
- Correcta localización subcelular

Desventajas

- Manipulación compleja
- Costos elevados

Los genes de bacterias, hongos, plantas y animales pueden ser manipulados en bacterias y ser reintroducidos en las células procariotas o eucariotas en donde permanecerán como material extracromosomico o se integraran al ADN dependiendo del tipo celular y el vector utilizado.

Uso de codones



USO DE CODONES EN E. COLI & HUMANOS

Table 3.2 The genetic code and codon usage in *E. coli* and humans

Codon	Amino acid	Frequency of use in:	
		E. coli	Humans
GAG	Glutamic acid	0.30	0.59
GAA	Glutamic acid	0.70	0.41
CGG	Arginine	0.08	0.19
CGA	Arginine	0.05	0.10
CGU	Arginine	0.42	0.09
CGC	Arginine	0.37	0.19
AGG	Arginine	0.03	0.22
AGA	Arginine	0.04	0.21
CCG	Proline	0.55	0.11
CCA	Proline	0.20	0.27
CCU	Proline	0.16	0.29
CCC	Proline	0.10	0.33
UGA	Stop	0.30	0.61
UAG	Stop	0.09	0.17
UAA	Stop	0.62	0.22

W126–W131 Nucleic Acids Research, 2007, Vol. 35, Web Server issue
doi:10.1093/nar/gkm219

OPTIMIZER: a web server for optimizing the codon usage of DNA sequences

Pere Puigbò[1], Eduard Guzmán[1,2], Antoni Romeu[1] and Santiago Garcia-Vallvé[1,*]

[1]Evolutionary Genomics Group, Biochemistry and Biotechnology Department, Faculty of Chemistry, Rovira i Virgili University (URV), c/Marcel·li Domingo, s/n. Campus Sescelades, 43007 Tarragona, Spain and [2]Institut Català de la Salut, Àrea Bàsica de Salut, Tarragona 2, Spain

Received January 29, 2007; Revised March 22, 2007; Accepted March 28, 2007

http://genomes.urv.es/OPTIMIZER/

Leading Edge
Minireview

Cell

How the Sequence of a Gene Can Tune Its Translation

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[1]Department of Microbiology, Ohio State Biochemistry Program, and Center for RNA Biology, The Ohio State University, Columbus, OH 43210, USA
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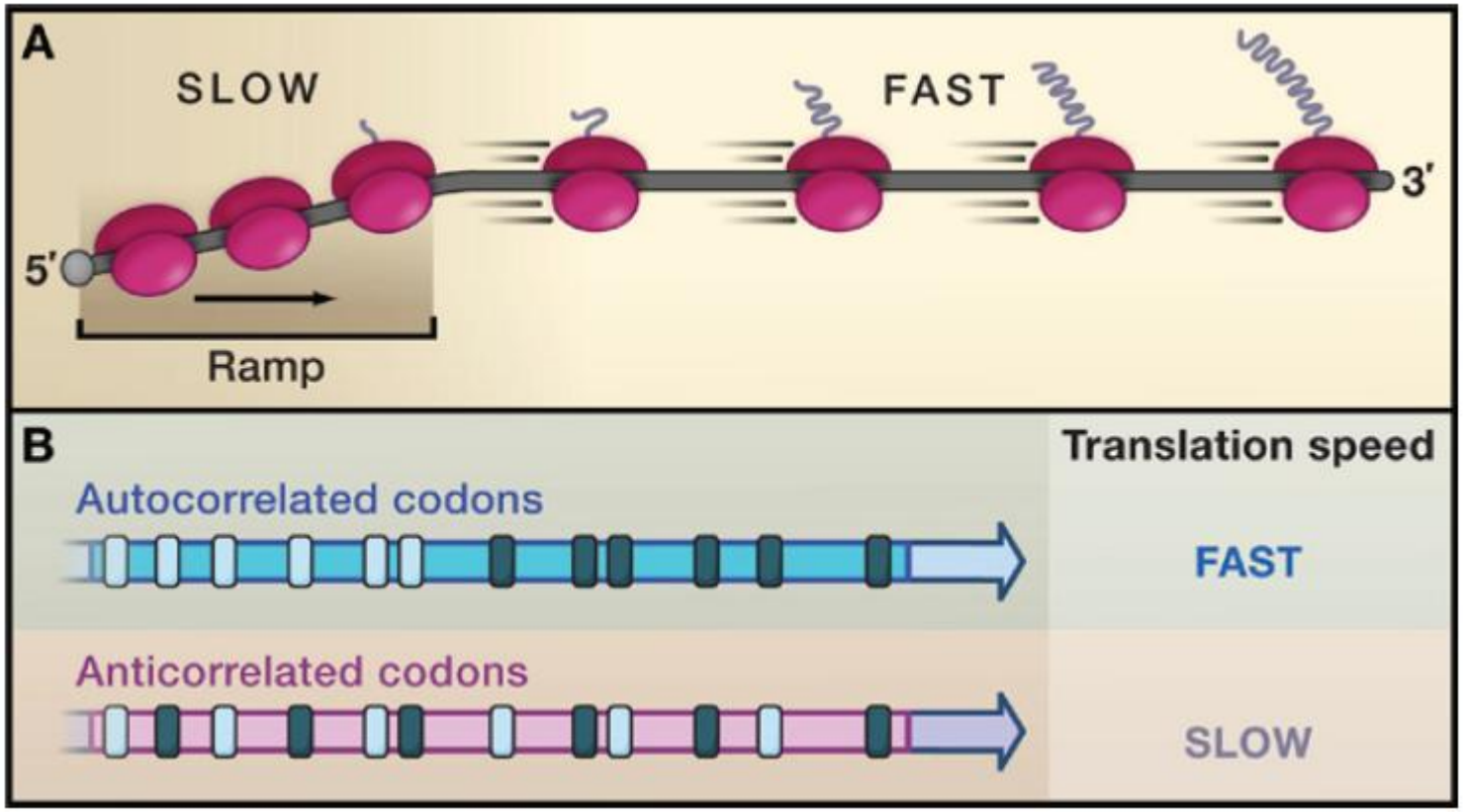
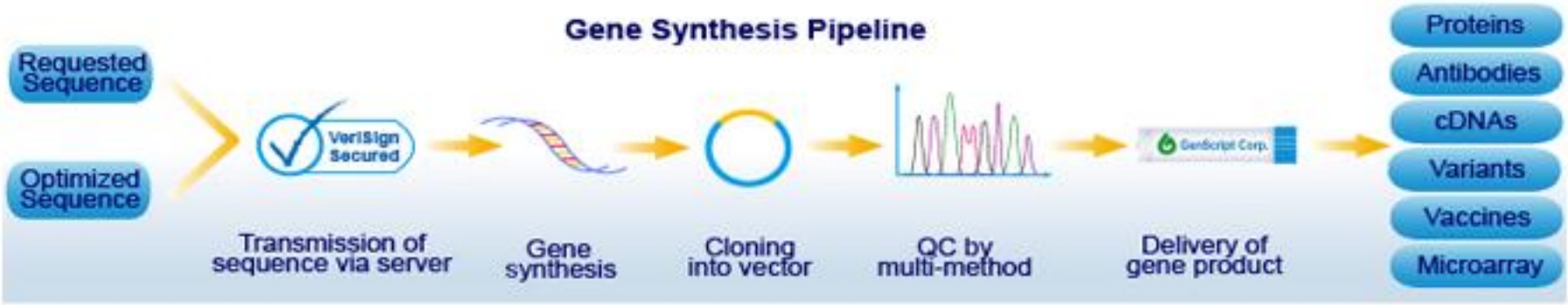
DOI 10.1016/j.cell.2010.03.033

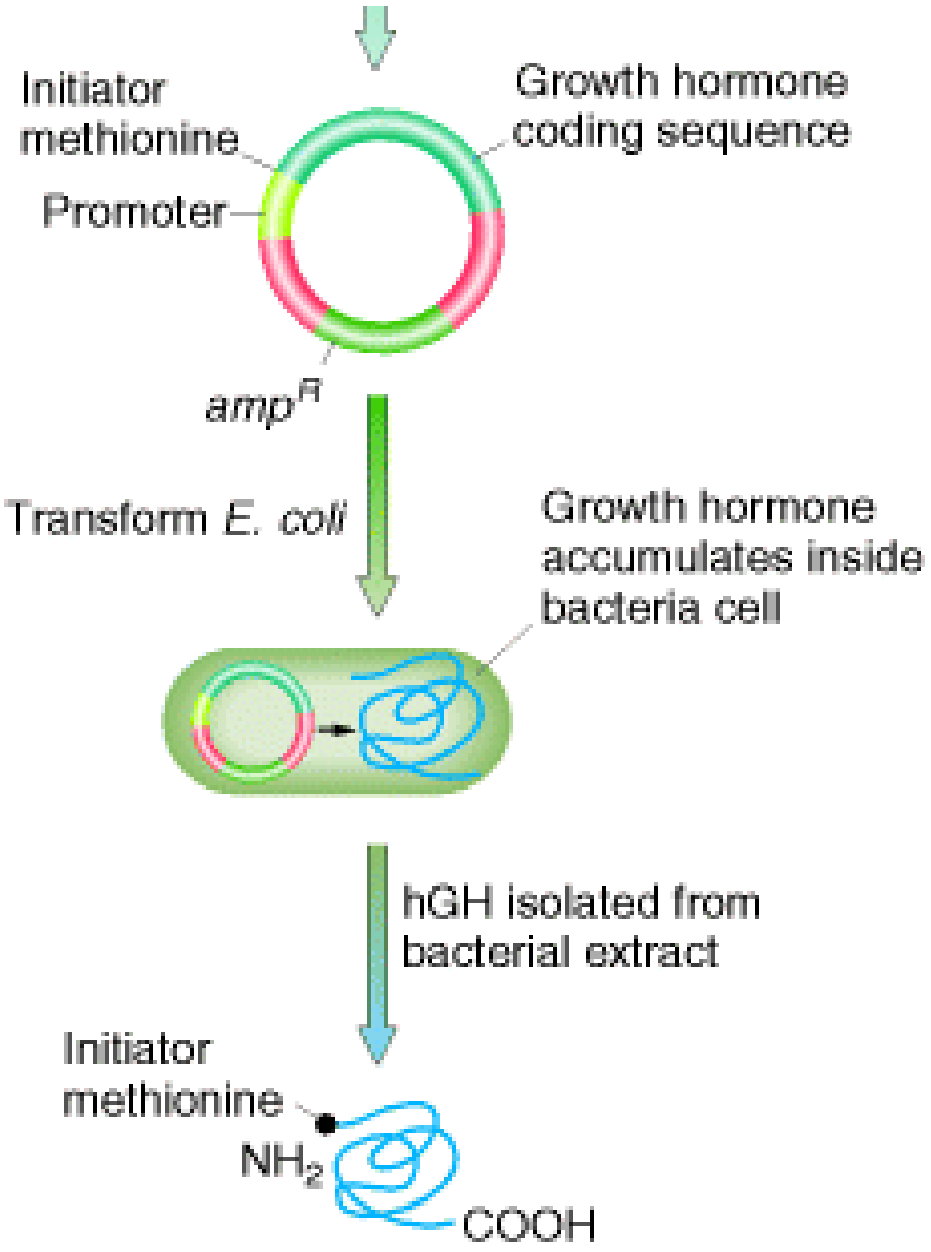
Sixty-one codons specify 20 amino acids, offering cells many options for encoding a polypeptide sequence. Two new studies (Cannarrozzi et al., 2010; Tuller et al., 2010) now foster the idea that patterns of codon usage can control ribosome speed, fine-tuning translation to increase the efficiency of protein synthesis.

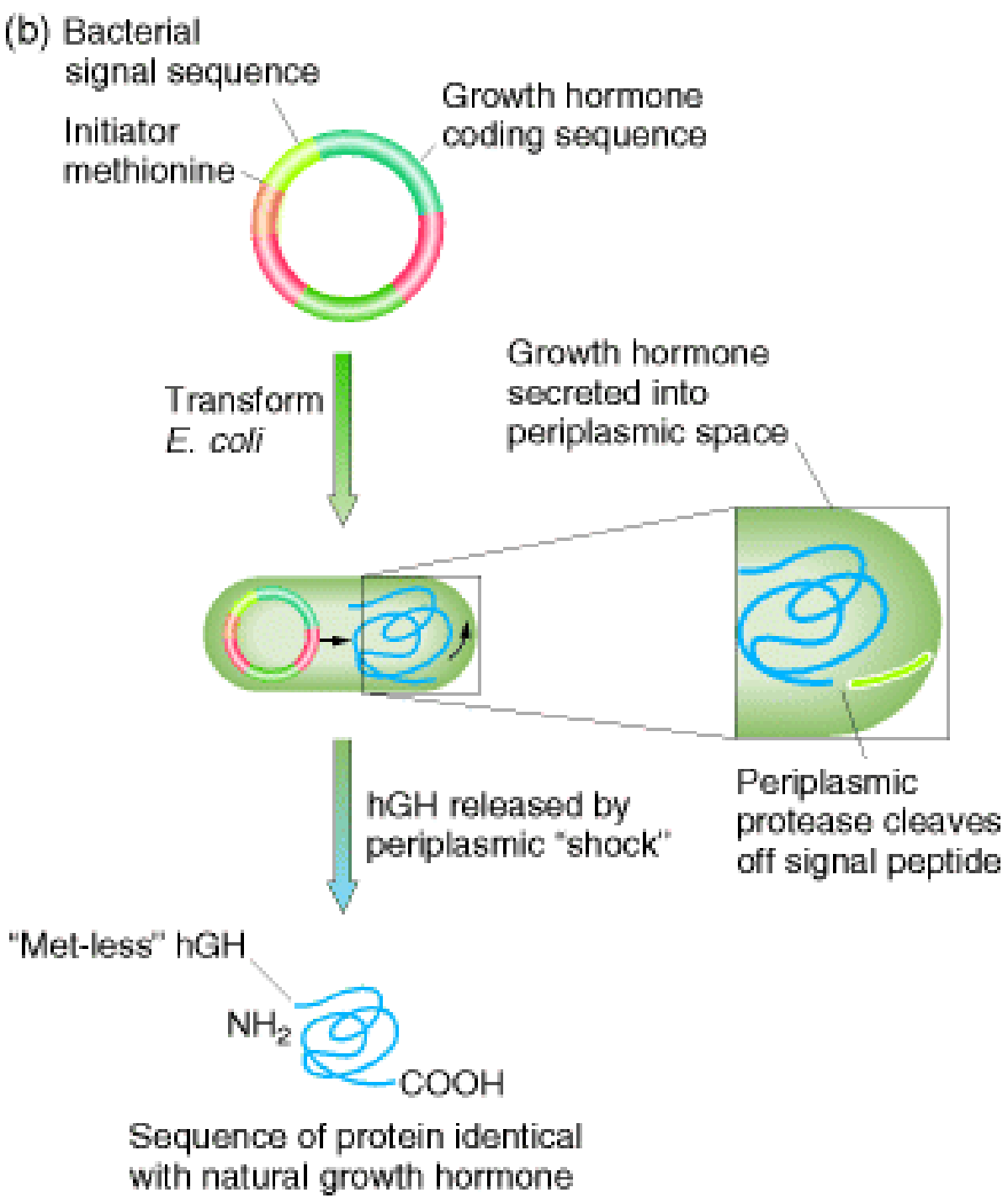

A
SLOW
FAST
5′
3′
Ramp
B
Translation speed
Autocorrelated codons
FAST
Anticorrelated codons
SLOW

1 bp	0,39
10 bp	3,90
100 bp	39,00
1000 bp	390,00

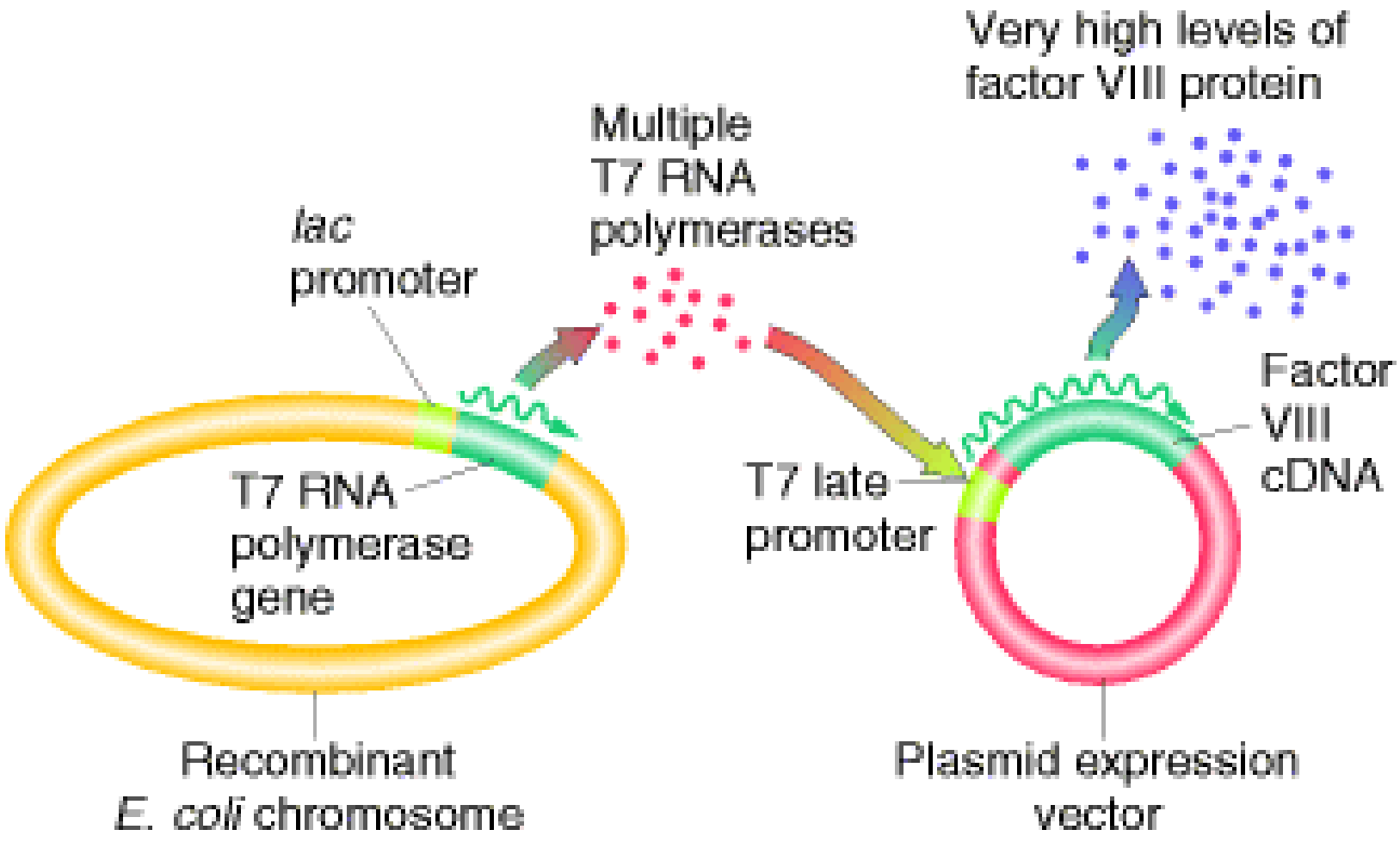
http://www.genscript.com/


Gene Synthesis Pipeline
Requested Sequence
Optimized Sequence
VeriSign Secured
GenScript Corp.
Transmission of sequence via server
Gene synthesis
Cloning into vector
QC by multi-method
Delivery of gene product
Proteins
Antibodies
cDNAs
Variants
Vaccines
Microarray

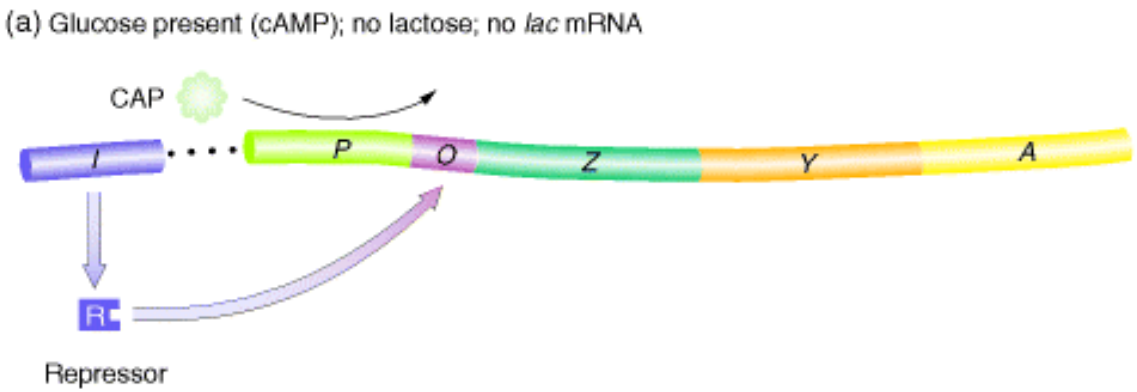

Initiator methionine
Promoter
Growth hormone coding sequence
amp^R
Transform *E. coli*
Growth hormone accumulates inside bacteria cell
hGH isolated from bacterial extract
Initiator methionine
NH_2
COOH

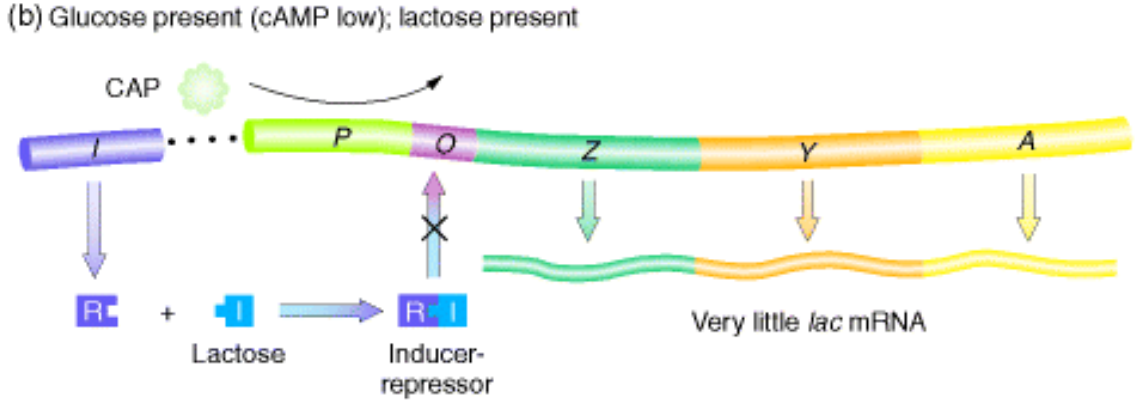

(b) Bacterial signal sequence
Initiator methionine
Growth hormone coding sequence
Transform E. coli
Growth hormone secreted into periplasmic space
hGH released by periplasmic "shock"
Periplasmic protease cleaves off signal peptide
"Met-less" hGH
NH2
COOH
Sequence of protein identical with natural growth hormone

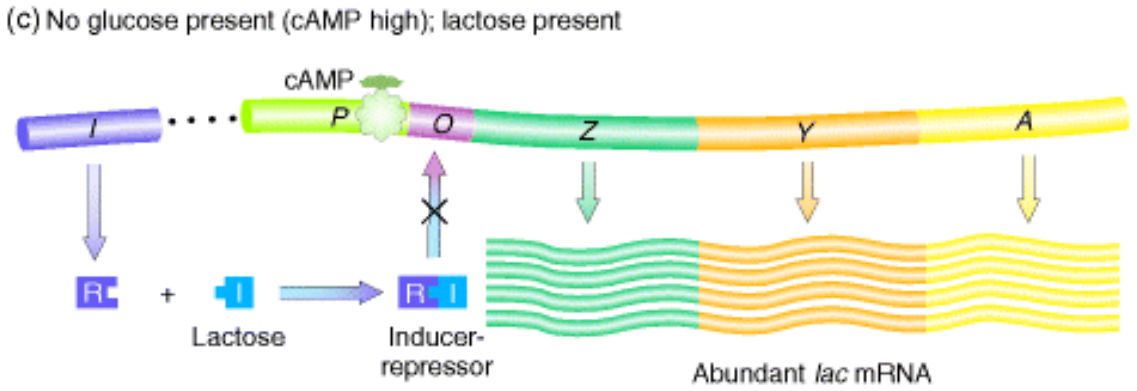
Expresión de genes en bacterias

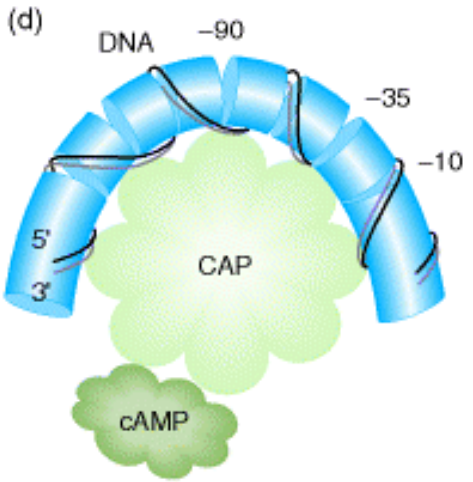


Control negativo y positivo del operón Lac

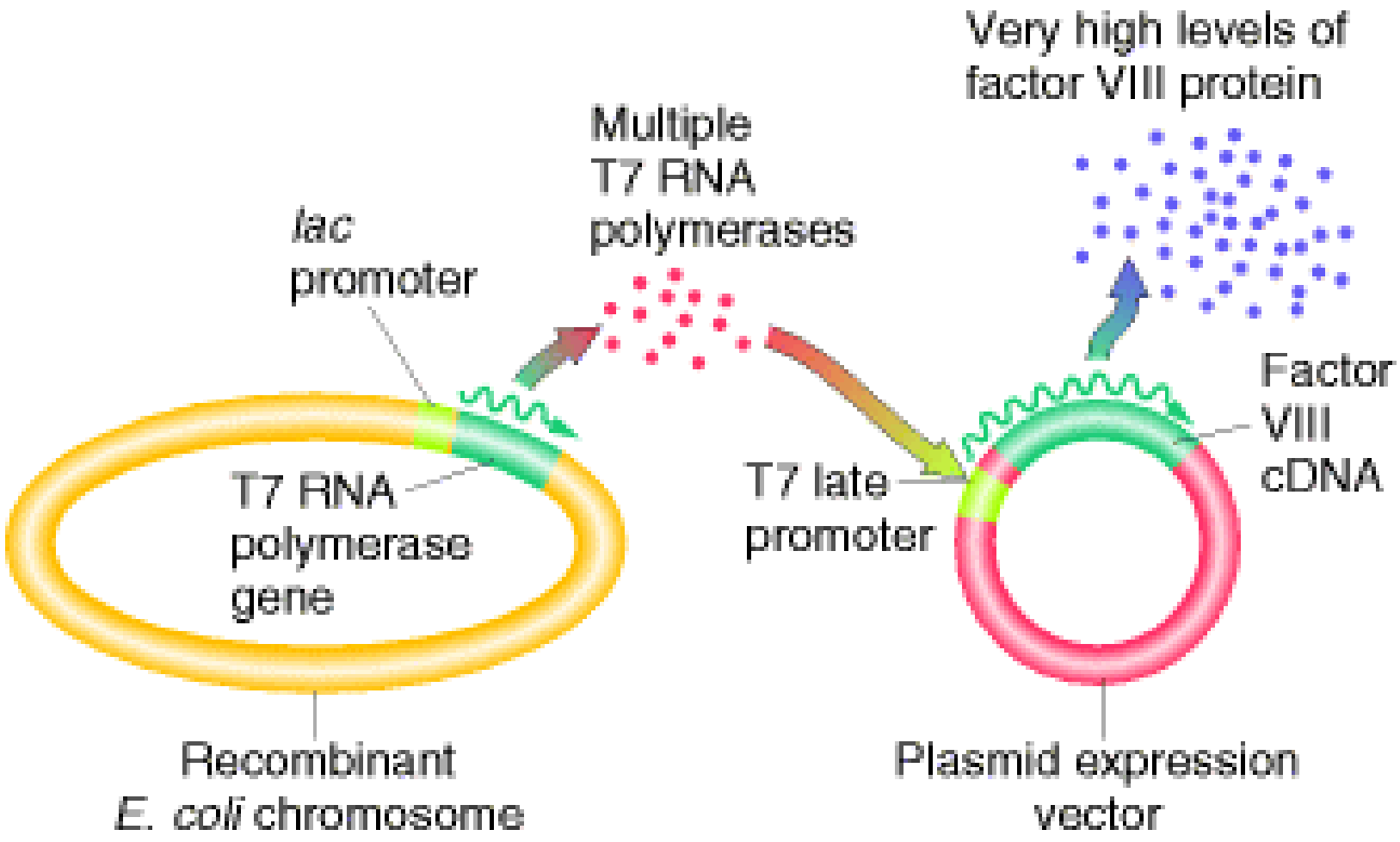








Expresión de genes en bacterias



Recent Patents on Biotechnology **2010**, *4*, 23-29

Secretory Production of Recombinant Proteins in *Escherichia coli*

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Abstract: Extracellular production of heterologous proteins using the *Escherichia coli* cell factory offers several advantages over intracellular production and mammalian culture. Properly folded proteins can be rapidly accumulated in the culture media, and downstream processes for isolation and purification can be much simplified. Efforts to enhance the secretory production of target proteins can be largely classified as selection and modification of the signal peptide, coexpression of proteins to assist translocation and folding, improvement of periplasmic release, and protection of target proteins from degradation and contamination. Here, we review recent patents on the secretory production of recombinant proteins in *E. coli*.

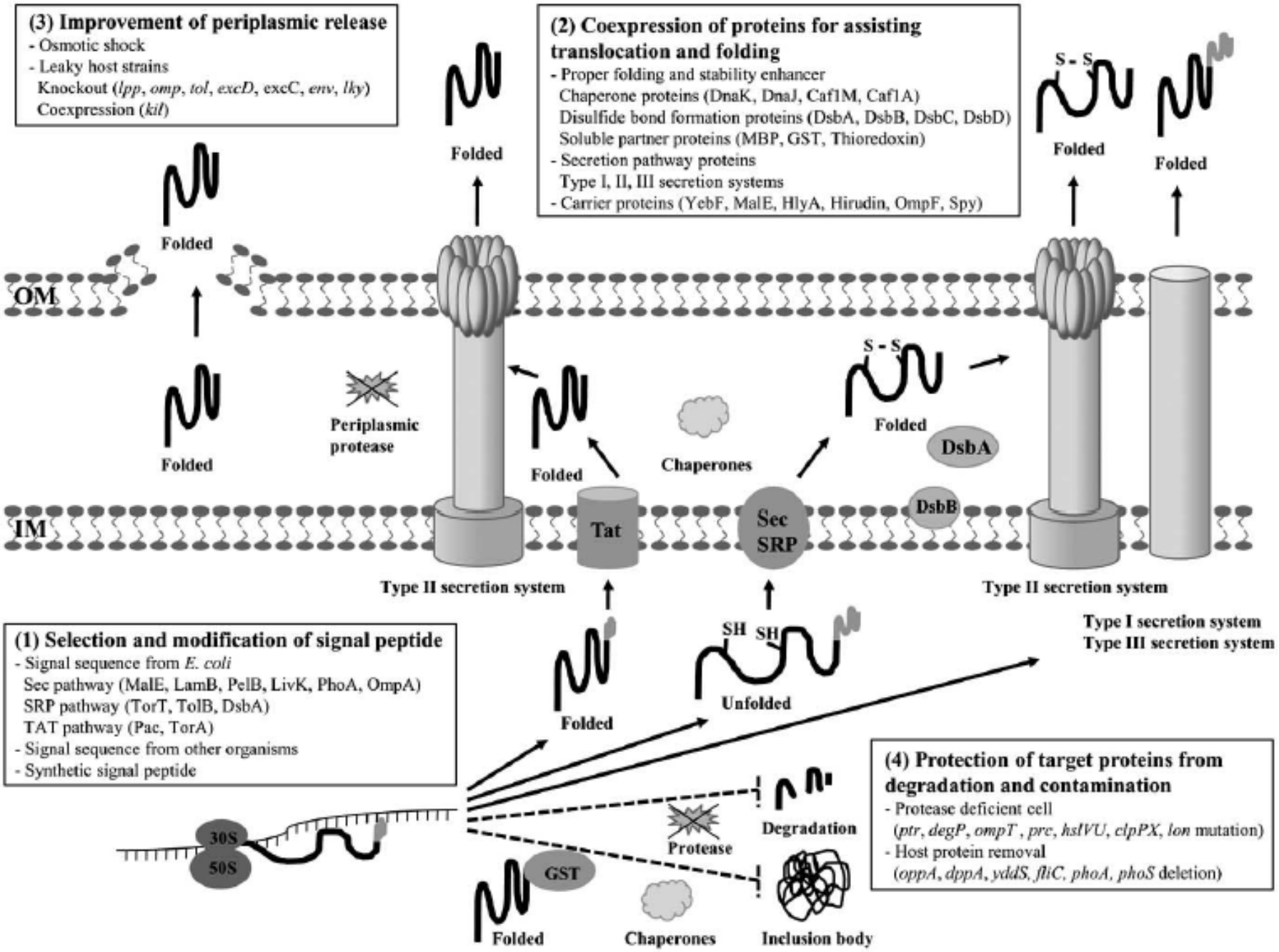

(3) Improvement of periplasmic release
- Osmotic shock
- Leaky host strains
Knockout (lpp, omp, tol, excD, excC, env, lky)
Coexpression (kil)
(2) Coexpression of proteins for assisting translocation and folding
- Proper folding and stability enhancer
Chaperone proteins (DnaK, DnaJ, Caf1M, Caf1A)
Disulfide bond formation proteins (DsbA, DsbB, DsbC, DsbD)
Soluble partner proteins (MBP, GST, Thioredoxin)
- Secretion pathway proteins
Type I, II, III secretion systems
- Carrier proteins (YebF, MalE, HlyA, Hirudin, OmpF, Spy)
Folded
S - S
OM
IM
Periplasmic protease
Chaperones
DsbA
DsbB
Tat
Sec SRP
Type II secretion system
Type I secretion system
Type III secretion system
SH SH
Unfolded
(1) Selection and modification of signal peptide
- Signal sequence from E. coli
Sec pathway (MalE, LamB, PelB, LivK, PhoA, OmpA)
SRP pathway (TorT, TolB, DsbA)
TAT pathway (Pac, TorA)
- Signal sequence from other organisms
- Synthetic signal peptide
30S
50S
Protease
Degradation
GST
(4) Protection of target proteins from degradation and contamination
- Protease deficient cell
(ptr, degP, ompT , prc, hslVU, clpPX, lon mutation)
- Host protein removal
(oppA, dppA, yddS, fliC, phoA, phoS deletion)
Inclusion body

Table 1. Examples of Commercialized Protein Secretion Systems

Feature	Company	Product
Sec, SRP, TAT pathway signal sequence	AthenaES®	The ACES™ Signal Sequence Kit
Sec pathway signal sequence (PelB)	Progen	pOPE 101 vector
Sec pathway signal sequence (PelB, OmpT)	Novagen®	pET-12, 20, 22, 25, 26, 27 vectors
Sec pathway signal sequence (OmpA)	IBA	pASK-IBA2, 4, 6, 12, 14, 16, 32, 44 vectors
Sec pathway signal sequence (OmpA)	SIGMA	pFLAG-ATS™, pFLAG-CTS™ vector
Leader peptide from the bacteriophage fd gene III protein (gIII)	Invitrogen	pBAD/gIII vector
Coexpression of chaperone proteins	TAKARA BIO INC.	Chaperone Plasmid Set
Enhanced cytoplasmic disulfide formation (*trxB*, *gor* mutant strain)	Novagen®	Orgami™, Orgami B series
Enhanced cytoplasmic disulfide formation (*trxB*, *gor*, *ahpC* mutant and DsbC expression strain)	NEW ENGLAND BioLabs® INC.	SHuffle™ strains
Enhanced disulfide formation fusion (DsbA, DsbC)	Novagen®	pET-39, 40 vectors
Soluble protein fusion (MBP)	NEW ENGLAND BioLabs® INC.	pMAL™ Protein Fusion and Purification System
Soluble protein fusion (Trx, GST, NusA)	Novagen®	pET-32, 41, 42, 43.1 vectors
Soluble protein fusion (GST)	GE Healthcare	pGEX vectors
Carrier protein fusion (YebF)	AthenaES®	The ACES™ YebF Protein Export Kit
Carrier protein fusion (MBP fused with M13 pIII leader sequence)	NEW ENGLAND BioLabs® INC.	pMal-pIII vector
Enhanced secretion (Modified outer membrane strain)	Wacker Chemie AG	The WACKER secretion system

SISTEMAS DE PURIFICACIÓN PARA PROTEÍNAS DE FUSIÓN

Table 6.3 Some fusion systems used to facilitate the purification of foreign proteins produced in *E. coli*

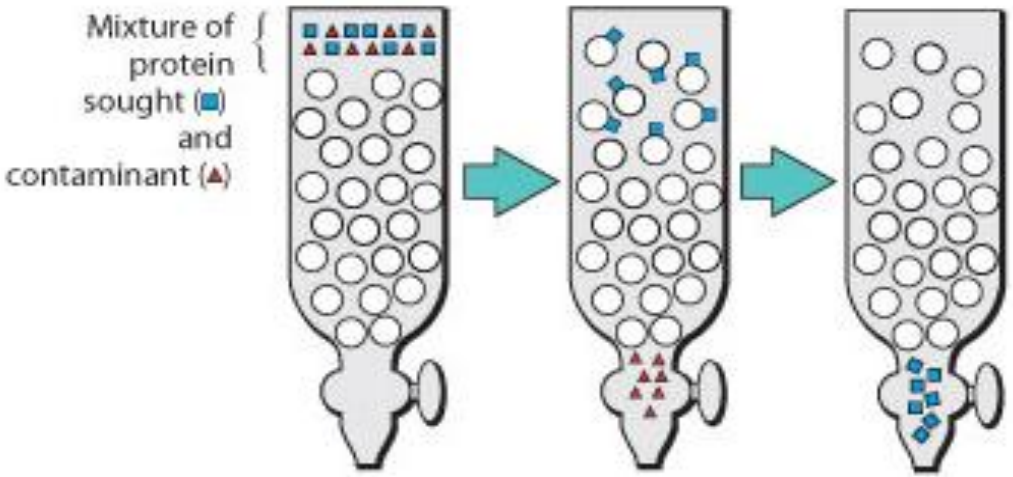
Fusion partner	Size	Ligand	Elution condition
ZZ	14 kDa	IgG	Low pH
His tail	6–10 aa	Ni^{2+}	Imidazole
Strep-tag	10 aa	Streptavidin	Iminobiotin
PinPoint	13 kDa	Streptavidin	Biotin
MBP	40 kDa	Amylose	Maltose
β-Lactamase	27 kDa	Phenyl-boronate	Borate
GST	25 kDa	Glutathione	Reducing agent
Flag	8 aa	Specific MAb	Low calcium

Adapted from Nygren et al., 1994, *Trends Biotechnol.* **12:**184–188.

Abbreviations: aa, amino acids; kDa, kilodaltons; ZZ, a fragment of *Staphylococcus aureus* protein A; His, histidine; Strep-tag, a peptide with affinity for streptavidin; PinPoint, a protein fragment which is biotinylated in vivo in *E. coli*; MBP, maltose binding protein; GST, glutathione *S*-transferase; Flag, a peptide recognized by enterokinase.

Proteína a purificar

Ligando



Centricon

Amicon

Microcon

pET ™ Protein Fusion and Purification System

(Expression and Purification of Proteins and Cloned Genes)

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
DsbA•Tag™ DsbC•Tag™	N	208 (DsbA) 236 (DsbC)	potential periplasmic localization	DB, DI, PE, SP	39, 40
GST•Tag™	N	220	monoclonal antibody, enzymatic activity, glutathione affinity	AP, IF, IP, QA, WB	41, 42, 49
His•Tag®	N, C, I	6, 8, or 10	monoclonal antibody, metal chelation chromatography (native or denaturing)	AP, IF, WB	14–16, 19–52
HSV•Tag®	C	11	monoclonal antibody	IF, WB	25, 27, 43.1, 44
KSI	N	125	highly expressed hydrophobic domain	PP	31
Nus•Tag™	N, I	495	promotes cytoplasmic solubility, monoclonal antibody	SP, WB	43.1, 44, 50
pelB	N	20	potential periplasmic localization	PE	20, 22, 25, 26, 27
PKA site	I	5	protein kinase A recognition site	PS	33
S•Tag™	N, I	15	S-protein (104 aa) affinity, monoclonal antibody	AP, IF, IP, QA, WB	29, 30, 32, 34–37, 39–50
Strep•Tag® II	N	8	monoclonal antibody, engineered streptavidin affinity	AP, WB	51, 52
T7•Tag®	N, I	11	monoclonal antibody	AP, IF, IP, WB	3, 9, 11, 17, 21, 23, 24, 28, 33
Trx•Tag™	N	109	monoclonal antibody, promotes disulfide bond formation	DB, SP	32, 48

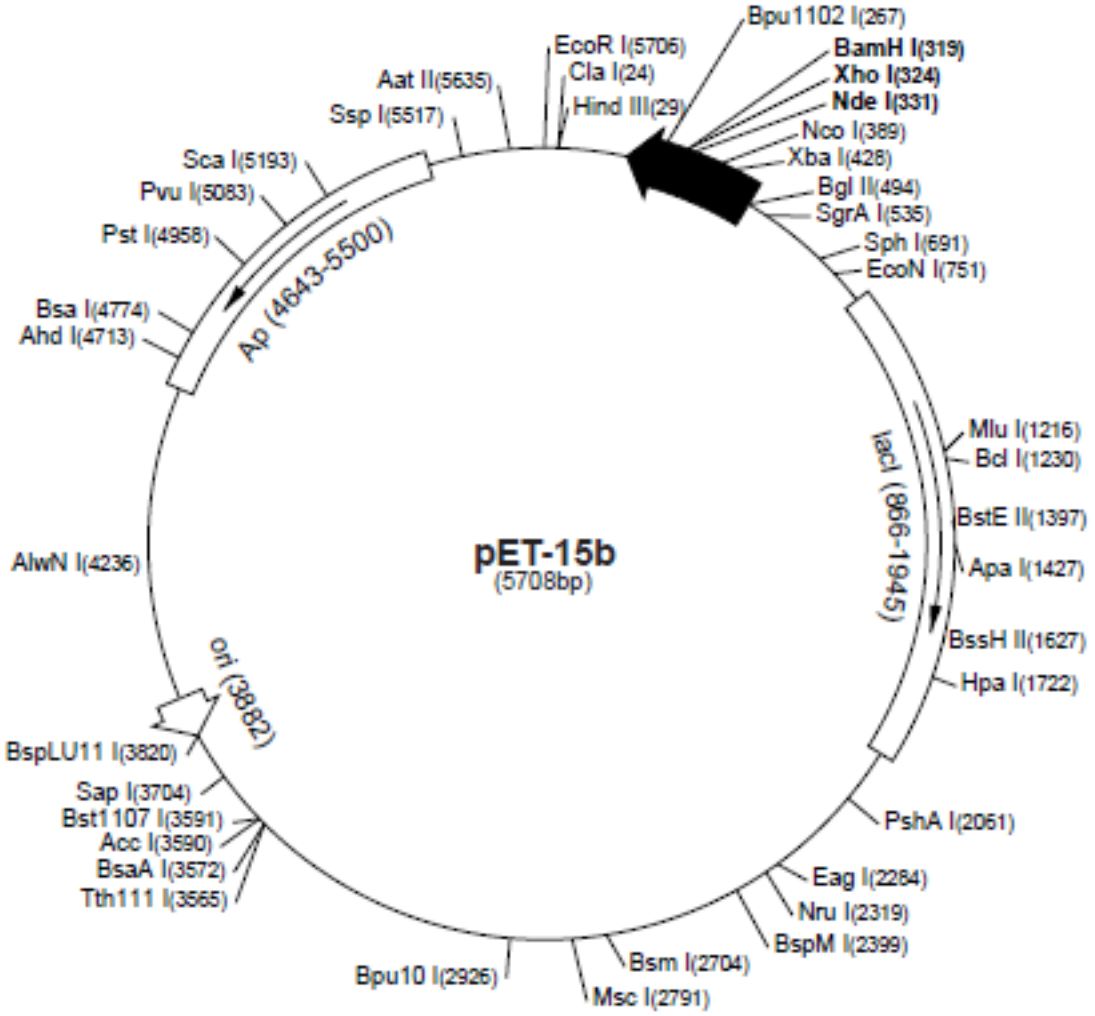
AP: affinity purification
DB: disulfide bond formation
DI: disulfide bond isomerization
WB: Western blotting
IP: immunoprecipitation
PE: protein export
PP: small protein/peptide production
IF: immunofluorescence
QA: quantitative assay
SP: soluble protein
PS: *in vitro* phosphorylation

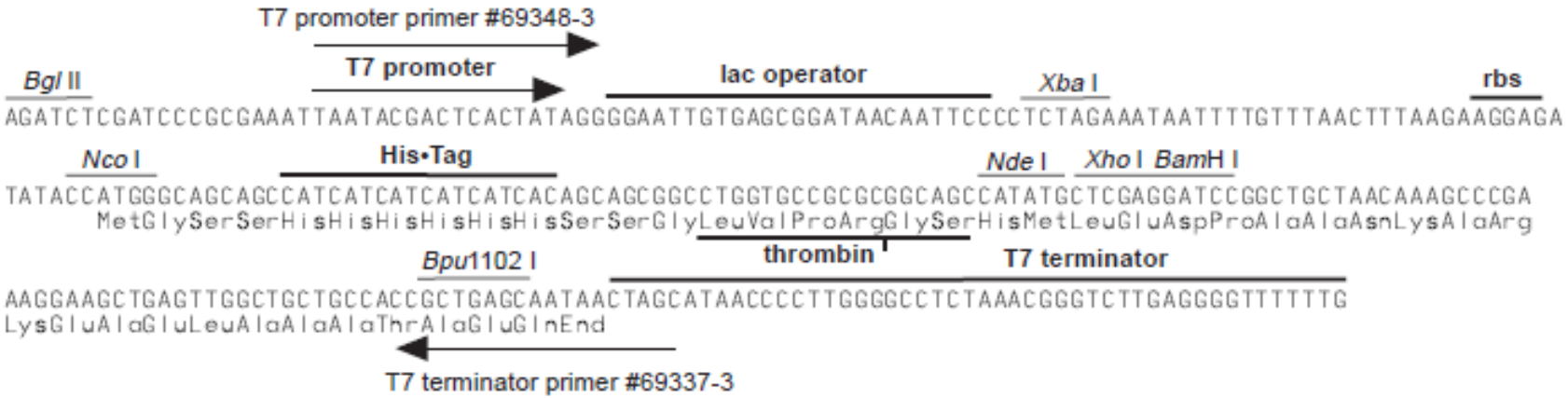
Vector	amp®	kan®	T7	T7/lac	His•Tag®	T7•Tag®	S•Tag™	Trx•Tag™	KSI	HSV•Tag®	PKA	Dsb•Tag™	GST•Tag™	Nus•Tag™	Strep•Tag® II	protease	signal seq.
pET-3a–d	●		●			N											
pET-9a–d		●	●			N											
pET-11a–d	●			●		N											
pET-14b	●		●		N											T	
pET-15b	●			●	N											T	
pET-16b	●			●	N											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(+)	●			●	L,C		I	N								T, E	
pET-32 Ek/LIC	●			●	L,C		I	N								T, E	
pET-32 Xa/LIC	●			●	L,C		I	N								T, X	
pET-33b(+)		●		●	N,C	I					I					T	
pET-39b(+)		●		●	L,C		I					N				T, E	●
pET-40b(+)		●		●	L,C		I					N				T, E	●
pET-41a–c(+)		●		●	L,C		I						N			T, E	
pET-41 Ek/LIC		●		●	L,C		I						N			T, E	
pET-42a–c(+)		●		●	L,C		I						N			T, X	
pET-43.1a–c(+)	●			●	L,C		I			C				N		T, E	
pET-43.1 Ek/LIC	●			●	L,C		I			C				N		T, E	
pET-44a–c(+)	●			●	N,L,C		I			C				I		T, E	
pET-45b(+)	●			●	N		C									E	

Vector amp® kan® 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-15b sequence landmarks

T7 promoter	453-469
T7 transcription start	452
His•Tag coding sequence	362-380
Multiple cloning sites	
(*Nde* I - *Bam*H I)	319-335
T7 terminator	213-259
lacI coding sequence	(866-1945)
pBR322 origin	3882
bla coding sequence	4643-5500




T7 promoter primer #69348-3
T7 promoter
lac operator
Bgl II
Xba I
rbs
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Nco I
His•Tag
Nde I
Xho I
BamH I
TATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCTCGAGGATCCGGCTGCTAACAAAGCCCGA
MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetLeuGluAspProAlaAlaAsnLysAlaArg
thrombin
Bpu1102 I
T7 terminator
AAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
LysGluAlaGluLeuAlaAlaAlaThrAlaGluGlnEnd
T7 terminator primer #69337-3

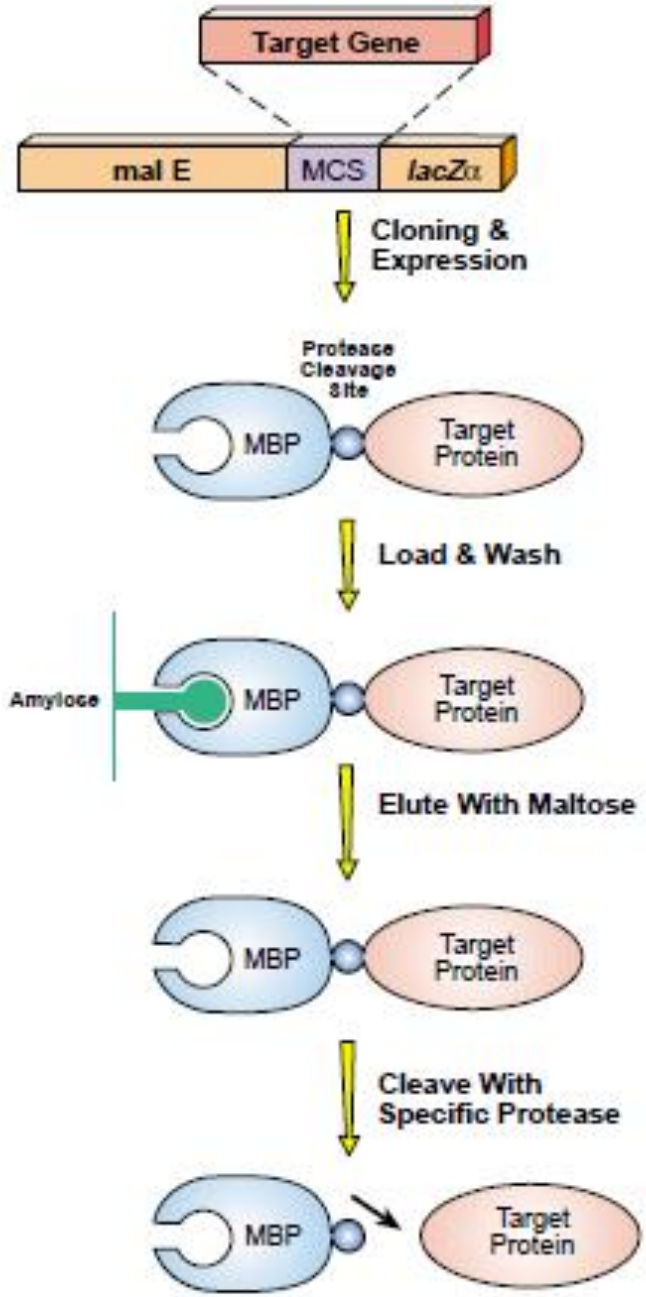
Strain	Deriv	Genotype	Description/Application	Antibiotic Resistance[1]
B834	B	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm met*	*met* auxotroph, parent of BL21, control non-expression[2] host	none
B834(DE3)[3]	B	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm met* (DE3)	*met* auxotroph, parent of BL21, general expression[3] host, ^{35}S-met labeling	none
B834(DE3)pLysS[3]	B	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm met* (DE3) pLysS (Cam^R)	*met* auxotroph, parent of BL21, high-stringency expression [3,4] host, ^{35}S-met labeling	Chloramphenicol (34 µg/ml)
BL21	B834	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm*	control non-expression[2] host	none
BL21(DE3)[3]	B834	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm* (DE3)	general purpose expression[3] host	none
BL21(DE3)pLysS[3]	B834	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm* (DE3)pLysS (Cam^R)	high-stringency expression [3,4]	Chloramphenicol (34 µg/ml)
BLR	BL21	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm Δ(srl-recA)306*::Tn *10* (Tet^R)	*recA*⁻ control non-expression[2] host recommended for use with tandem repeats	Tetracycline (12.5 µg/ml)
BLR(DE3)[3]	BL21	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm* (DE3) Δ*(srl-recA)306*::Tn *10* (Tet^R)	*recA*⁻ expression[3] host recommended for use with tandem repeats	Tetracycline (12.5 µg/ml)
BLR(DE3)pLysS[3]	BL21	F⁻ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm* (DE3) Δ*(srl-recA)306*::Tn *10* pLysS (Cam^R, Tet^R)	*recA*⁻ high-stringency expression [3,4] host recommended for use with tandem repeats	Chloramphenicol (34 µg/ml) Tetracycline (12.5 µg/ml)
HMS174	K-12	F⁻ *recA1 hsdR(r_{K12}^- m_{K12}^+)* (Rif^R)	control non-expression[2] host	Rifampicin (200 µg/ml)
HMS174(DE3)[3]	K-12	F⁻ *recA1 hsdR(r_{K12}^- m_{K12}^+)* (DE3) (Rif^R)	*recA*⁻ K-12 expression[3] host	Rifampicin (200 µg/ml)
HMS174(DE3)pLysS[3]	K-12	F⁻ *recA1 hsdR(r_{K12}^- m_{K12}^+)* (DE3) pLysS (Cam^R, Rif^R)	*recA*⁻ K-12 high-stringency expression[3,4] host	Chloramphenicol (34 µg/ml) Rifampicin (200 µg/ml)
NovaBlue	K-12	*endA1 hsdR17(r_{K12}^- m_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac* F′ *[proA⁺ B⁺ lacI^q ZΔM15*::Tn *10]* (Tet^R)	non-expression[2] host, general purpose cloning host, plasmid preps	Tetracycline (12.5 µg/ml)
NovaBlue(DE3)	K-12	*endA1 hsdR17(r_{K12}^- m_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac* (DE3) F′ *[proA⁺ B⁺ lacI^{q}ΔZ M15*::Tn *10]* (Tet^R)	*recA*⁻ *endA*⁻ K-12 *lacI^q* expression[3] host recommended for use with NovaTope® System	Tetracycline (12.5 µg/ml)
NovaBlue T1^R	K-12	*endA1 hsdR17(r_{K12}^- m_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac tonA* F′ *[proA⁺ B⁺ lacI^{q}Δ Z M15*::Tn *10]* (Tet^R)	non-expression[2] host, general purpose cloning, plasmid preps, T1 and T5 phage resistant	Tetracycline (12.5 µg/ml)

Strain	Deriv	Genotype	Description/Application	Antibiotic Resistance[1]
Origami(DE3)pLysS[5]	K-12	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F′ *[lac⁺ lacI^q pro]* (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Kan^R, Str^R, Tet^R)	high-stringency[3,4] expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm	Chloramphenicol (34 µg/ml) Kanamycin (15 µg/ml) Streptomycin (50 µg/ml)[6] Tetracycline (12.5 µg/ml)
Origami 2[5]	K-12	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F′ *[lac⁺ lacI^q pro] gor522*::Tn*10 trxB* (Str^R, Tet^R)	control non-expression[2] host; kanamycin sensitive	Streptomycin (50 µg/ml)[6] Tetracycline (12.5 µg/ml)
Origami 2(DE3)[5]	K-12	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F′ *[lac⁺ lacI^q pro]* (DE3) *gor522*::Tn*10 trxB* (Str^R, Tet^R)	general expression 3 host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in E. coli cytoplasm; kanamycin sensitive	Streptomycin (50 µg/ml)[6] Tetracycline (12.5 µg/ml)
Origami 2(DE3)pLysS[5]	K-12	*Δara–leu7697Δ lacX74Δ phoA PvuII phoR araD139 ahpC galE galK rpsL* F′ *[lac⁺ lacI^q pro]* (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Str^R, Tet^R)	high-stringency[3,4] expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm; kanamycin sensitive	Chloramphenicol (34 µg/ml) Streptomycin (50 µg/ml)[6] Tetracycline (12.5 µg/ml)
Origami™ B[5]	Tuner™ (B strain)	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm lacY1 ahpC gor522*::Tn*10 trxB* (Kan^R, Tet^R)	control non-expression[2] host	Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Origami B(DE3)[5]	Tuner (B strain)	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm lacY1 ahpC* (DE3) *gor522*::Tn*10 trxB* (Kan^R, Tet^R)	general expression[3] host; contains Tuner *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation	Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Origami B(DE3)pLysS[5]	Tuner (B strain)	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm lacY1 ahpC* (DE3) *gor522*::Tn*10 trxB* pLysS (Cam^R, Kan^R, Tet^R)	high-stringency[3,4] expression host; contains Tuner *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation	Chloramphenicol (34 µg/ml) Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Rosetta™	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* pRARE[6] (Cam^R)	control non-expression[2] host	Chloramphenicol (34 µg/ml)
Rosetta(DE3)[3]	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* (DE3) pRARE[6] (Cam^R)	general expression[3] host; provides six rare codon tRNAs	Chloramphenicol (34 µg/ml)
Rosetta(DE3)pLysS[3]	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* (DE3) pLysSRARE[6] (Cam^R)	high-stringency[3,4] expression host; provides six rare codon tRNAs	Chloramphenicol (34 µg/ml)
Rosetta 2	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* pRARE2[7] (Cam^R)	control non-expression[2] host	Chloramphenicol (34 µg/ml)
Rosetta 2(DE3)[3]	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* (DE3) pRARE2[7] (Cam^R)	general expression[3] host; provides seven rare codon tRNAs	Chloramphenicol (34 µg/ml)
Rosetta 2(DE3)pLysS[3]	BL21	F⁻ *ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm* (DE3) pLysSRARE2[6] (Cam^R)	high-stringency[3,4] expression host; provides seven rare codon tRNAs	Chloramphenicol (34 µg/ml)
RosettaBlue™	K-12	*endA1 hsdR17(r$_{K12}^-$ m$_{K12}^+$) supE44 thi-1 recA1 gyrA96 relA1 lac* F′ *[proA⁺B⁺ lacI^qΔZM15*::Tn*10]* pRARE[6] (Cam^R, Tet^R)	control non-expression[2] host	Chloramphenicol (34 µg/ml) Tetracycline (12.5 µg/ml)
RosettaBlue(DE3)	K-12	*endA1 hsdR17(r$_{K12}^-$ m$_{K12}^+$) supE44 thi-1 recA1 gyr96 relA1 lac* (DE3) F′ *[proA⁺B⁺ lacI^qΔZM15*::Tn*10]* pRARE[6] (Cam^R, Tet^R)	*recA⁻ endA⁻* K-12 *lacI^q* general expression[3] host; provides six rare codon tRNAs	Chloramphenicol (34 µg/ml) Tetracycline (12.5 µg/ml)

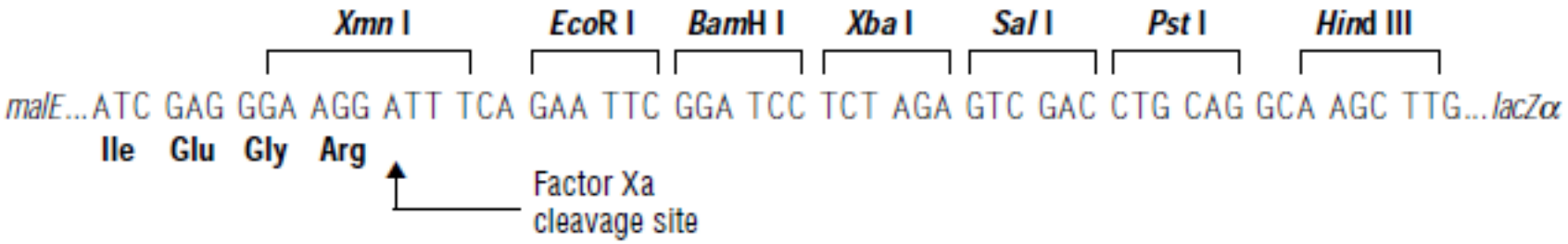
Strain	Deriv	Genotype	Description/Application	Antibiotic Resistance[1]
Rosetta-gami™ 2[5]	Origami (K-12)	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* F′ *[lac⁺ lacI^q pro] gor522*::Tn *10 trxB* pRARE2[7] (Cam^R, Str^R, Tet^R)	control non-expression[2] host	Chloramphenicol (34 µg/ml) Streptomycin (50 µg/ml)[8] Tetracycline (12.5 µg/ml)
Rosetta-gami 2(DE3)[5]	Origami (K-12)	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F′ *[lac⁺ lacI^q pro] gor522*::Tn *10 trxB* pRARE2[7] (Cam^R, Str^R, Tet^R)	general expression[3] host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm, provides seven rare codon tRNAs, kanamycin sensitive	Chloramphenicol (34 µg/ml) Streptomycin (50 µg/ml)[8] Tetracycline (12.5 µg/ml)
Rosetta-gami 2(DE3)pLysS[5]	Origami (K-12)	*Δara–leu7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F′ *[lac⁺ lacI^q pro] gor522*::Tn *10 trxB* pLysSRARE2[7] (Cam^R, Str^R, Tet^R)	high-stringency[4, 4] expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm, provides seven rare codon tRNAs; kanamycin sensitive	Chloramphenicol (34 µg/ml) Streptomycin (50 µg/ml)[8] Tetracycline (12.5 µg/ml)
Rosetta-gami B[5]	Tuner™ (B strain)	F$^-$ *ompT hsdSB(r_B^- m_B^-) gal dcm lacY1 ahpC gor522*::Tn *10 trxB* pRARE (Cam^R, Kan^R, Tet^R)	control non-expression[2] host	Chloramphenicol (34 µg/ml) Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Rosetta-gami B(DE3)[5]	Tuner (B strain)	F$^-$ *ompT hsdSB(r_B^- m_B^-) gal dcm lacY1 ahpC* (DE3) *gor522*::Tn *10 trxB* pRARE (Cam^R, Kan^R, Tet^R)	general expression[3] host; contains Tuner *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation, provides six rare codon tRNAs	Chloramphenicol (34 µg/ml) Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Rosetta-gami B(DE3) pLysS[5]	Tuner (B strain)	F$^-$ *ompT hsdSB(r_B^- m_B^-) gal dcm lacY1 ahpC* (DE3) *gor522*::Tn *10 trxB* pLysSRARE (Cam^R, Kan^R, Tet^R)	high-stringency[4, 4] expression host; contains Tuner *lac* permease mutation and *trxB/gor* mutations for cytoplasmic disulfide bond formation, provides six rare tRNAs	Chloramphenicol (34 µg/ml) Kanamycin (15 µg/ml) Tetracycline (12.5 µg/ml)
Tuner™	BL21	F$^-$ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1*	control non-expression[2] host	none
Tuner(DE3)	BL21	F$^-$ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1* (DE3)	general expression[3] host; *lac* permease mutation allows control of expression level	none
Tuner(DE3)pLysS	BL21	F$^-$ *ompT hsdS$_B$(r_B^- m_B^-) gal dcm lacY1* pLysS (DE3) (Cam^R)	high-stringency[4, 4] expression host; *lac* permease mutation allows control of expression level	Chloramphenicol (34 µg/ml)

pMAL™ Protein Fusion and Purification System

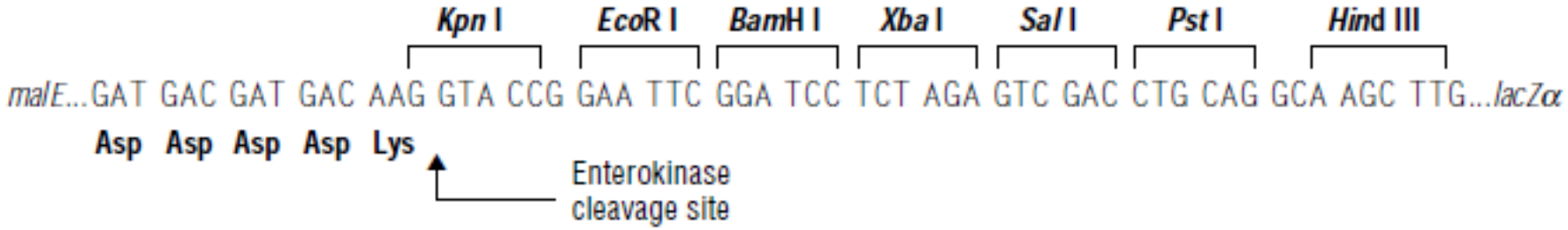
(Expression and Purification of Proteins and Cloned Genes)


Target Gene
mal E
MCS
lacZα
Cloning &
Expression
Protease
Cleavage
Site
MBP
Target
Protein
Load & Wash
Amylose
MBP
Target
Protein
Elute With Maltose
MBP
Target
Protein
Cleave With
Specific Protease
MBP
Target
Protein

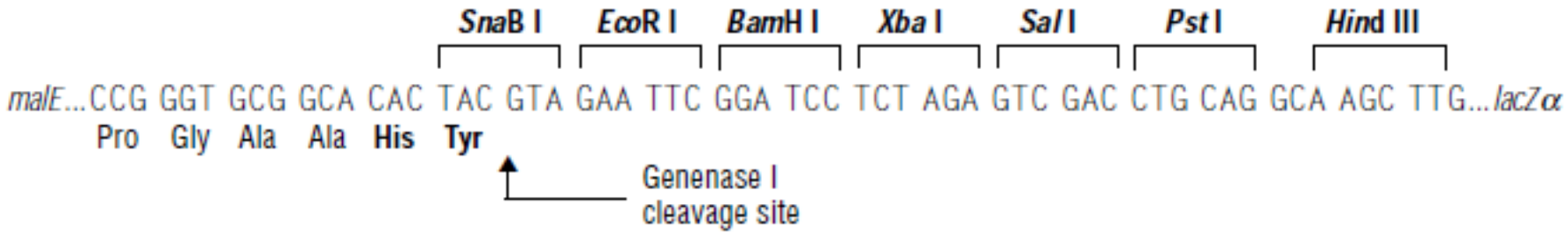
pMAL™-c2X, -p2X polylinker:

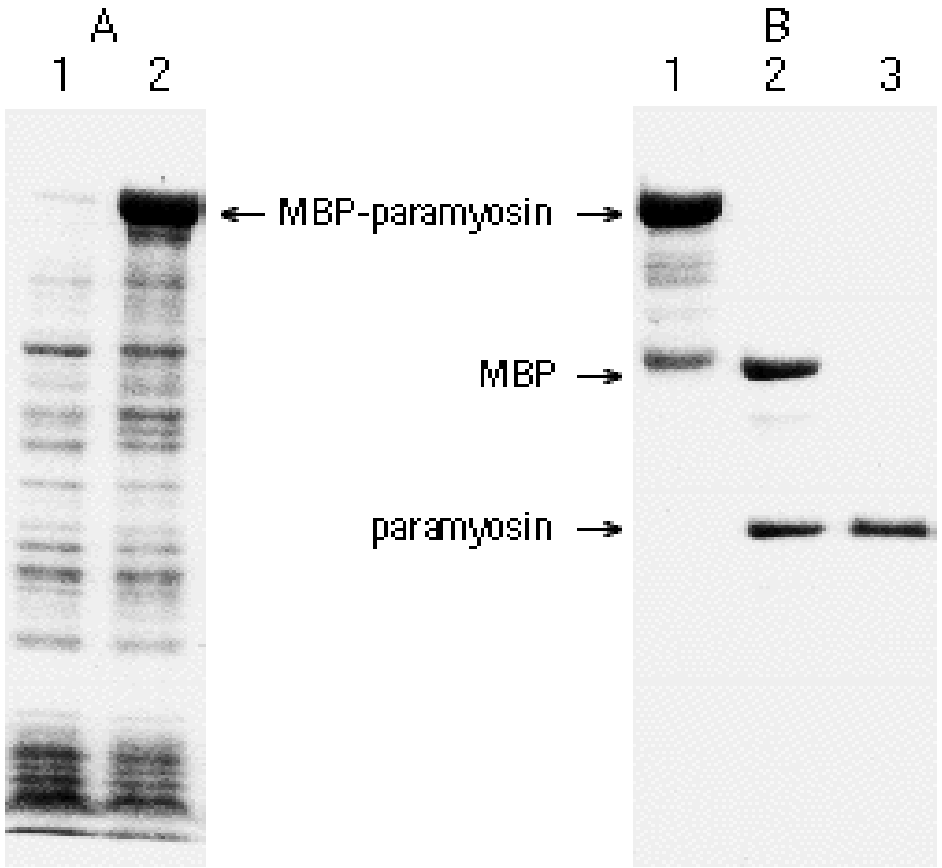


pMAL-c2E, pMAL-p2E Polylinker



pMAL-c2G, pMAL-p2G Polylinker



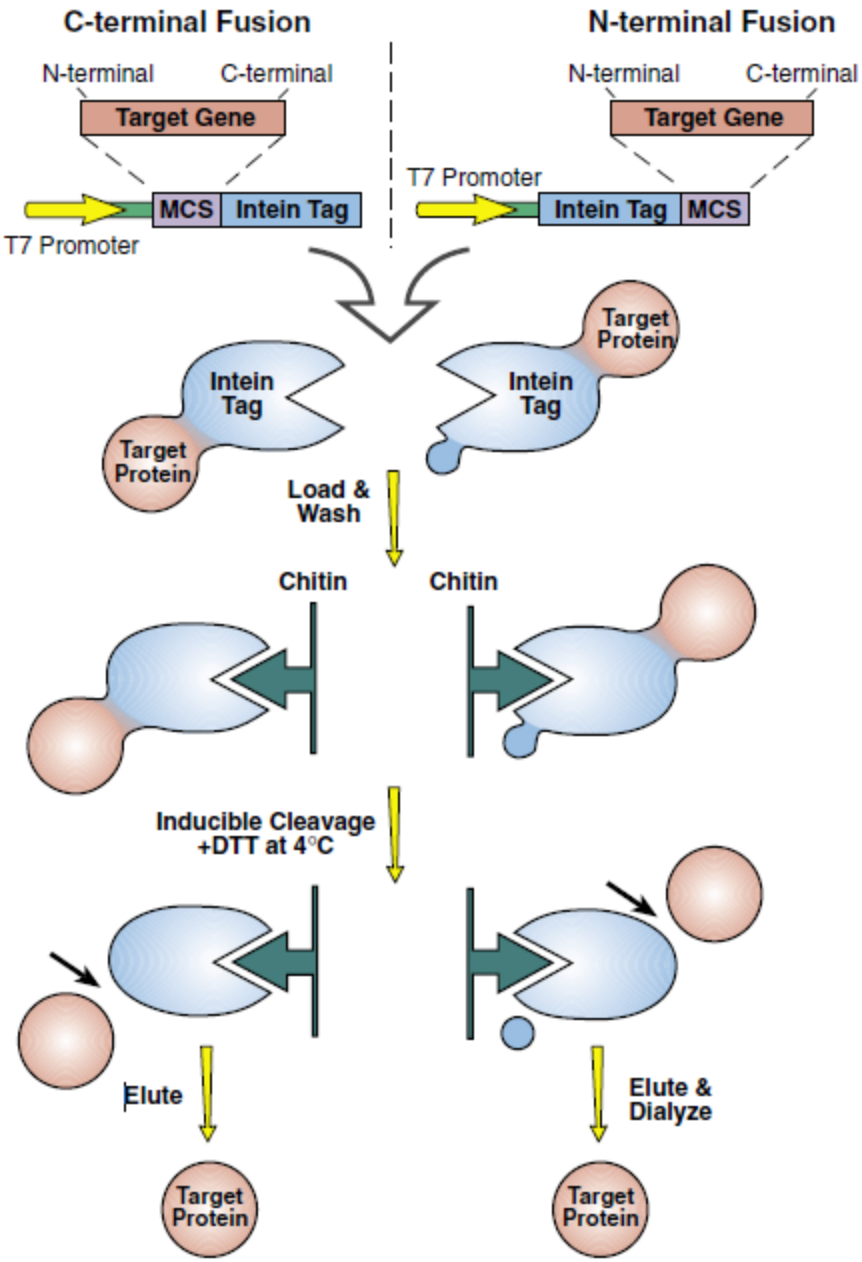


SDS-polyacrylamide gel electrophoresis of fractions from the purification of MBP-paramyosin-ΔSal.
A: Lane 1: uninduced cells. Lane 2: induced cells.
B: Lane 1: purified protein eluted from amylose column with maltose. Lane 2: purified protein after Factor Xa cleavage. Lane 3: paramyosin fragment eluted from second amylose column.

IMPACT™-CN

Protein Purification System Now Featuring Fusion to C- or N- Terminus of the Target Protein

For additional information, including vector sequences and frequently asked questions, see the NEB website: www.neb.com


C-terminal Fusion
N-terminal
C-terminal
Target Gene
MCS
Intein Tag
T7 Promoter
N-terminal Fusion
N-terminal
C-terminal
Target Gene
T7 Promoter
Intein Tag
MCS
Intein Tag
Target Protein
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Target Protein
Load & Wash
Chitin
Chitin
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Elute
Elute & Dialyze
Target Protein
Target Protein

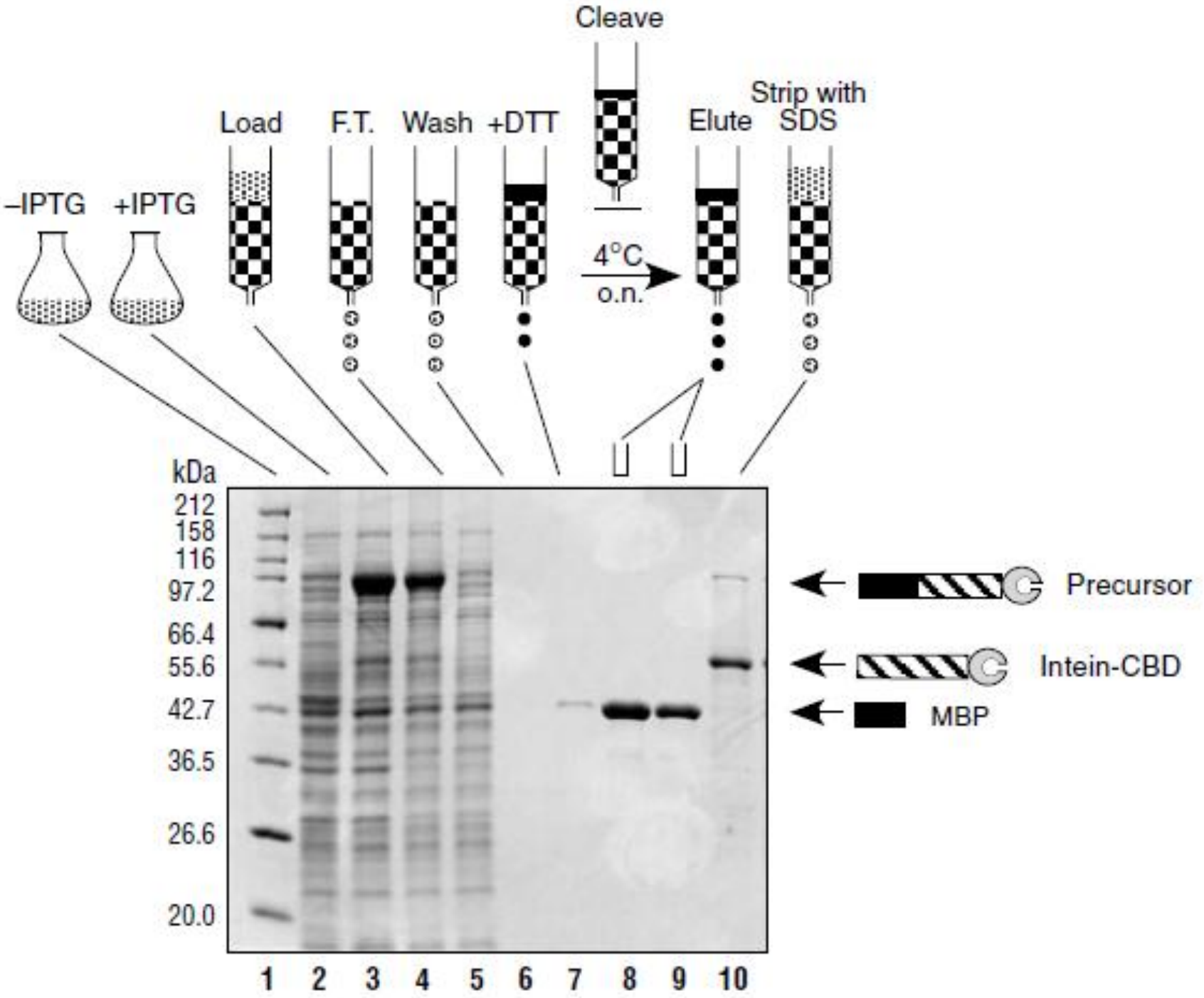
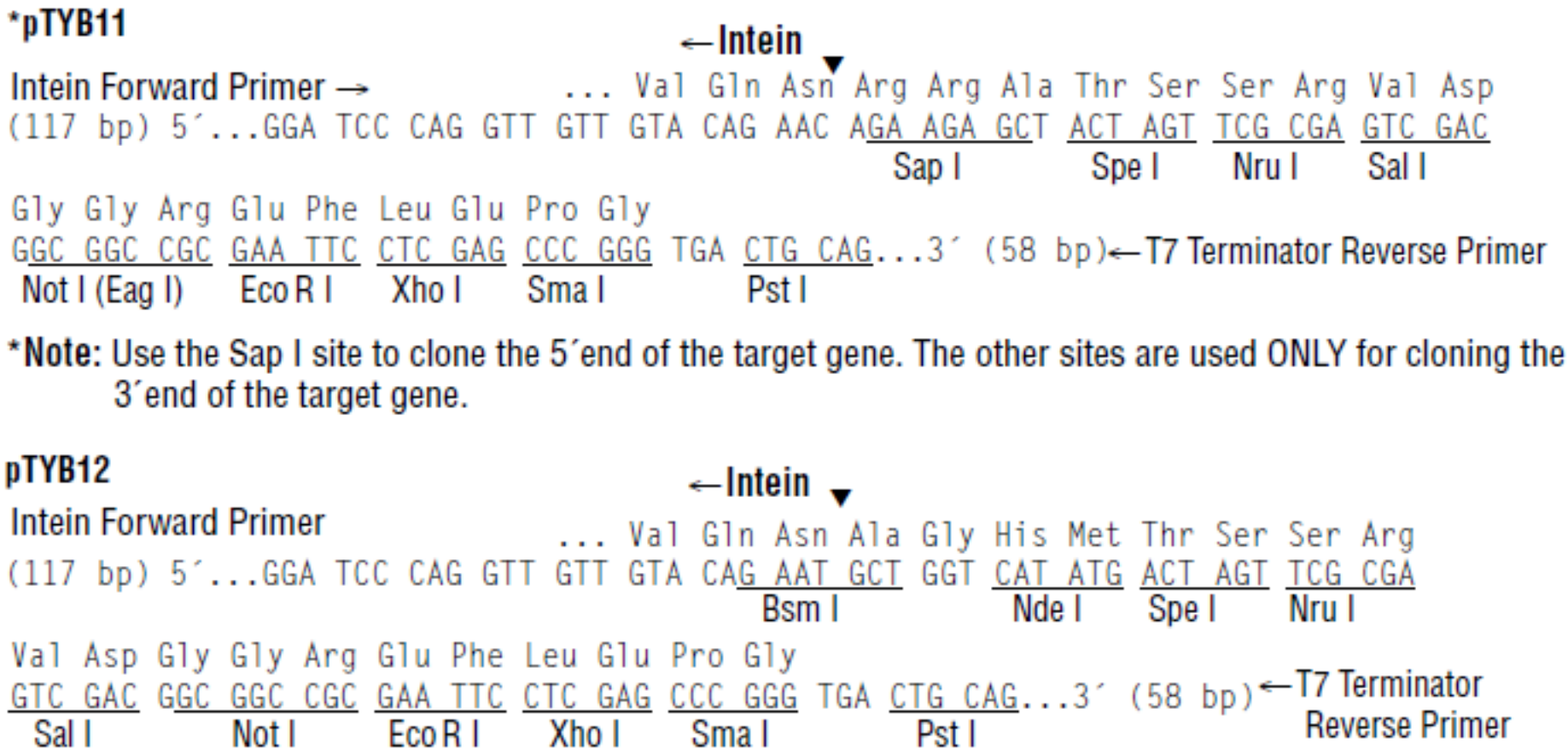

Cleave
Load
F.T.
Wash
+DTT
Elute
Strip with SDS
–IPTG
+IPTG
4°C
o.n.
kDa
212
158
116
97.2
66.4
55.6
42.7
36.5
26.6
20.0
1 2 3 4 5 6 7 8 9 10
Precursor
Intein-CBD
MBP

Table 2A: *Effect of the C-terminal residue of a target protein on DTT-induced cleavage of the intein tag when pTYB1 is used as the cloning vector.*

C-terminal Residue of the Target Protein	*In vivo* Cleavage	*In vitro* Cleavage with DTT (40 mM) 4°C	16°C
Gly	–	+++	+++
Ala	–	+++	+++
Ile*	–	+	+
Leu*	–	+	+++
Met*	–	+++	+++
Phe*	–	+++	+++
Val*	–	+	++
Gln*	–	+++	+++
Ser	–	++	+++
Trp*	–	+++	+++
Tyr*	–	+++	+++
Lys*	–	+++	+++
Thr*	25%	++	+++
Glu*	50%	++	+++
His*	50%	++	++
Arg*	75%	not determined	not determined
Asp	100%	not determined	not determined
Asn	–	–	–
Cys	–	–	–
Pro	–	–	–

Table 2B: *Effect of the first N-terminal residue of a target protein on the DTT-induced Cleavage of the intein tag when pTYB11 is used as the cloning vector.*

	% Cleavage After 16 Hours*			% Cleavage After 40 Hours*		
N-Terminal Residue of the Target Protein	**4°C**	**16°C**	**23°C**	**4°C**	**16°C**	**23°C**
Met Ala Gln	40–60	> 80	> 95	60–90	> 90	> 95
Gly Leu Asn Trp Phe Tyr	10–40	50–80	75–95	40–60	> 90	> 90
Val Ile Asp Glu Lys Arg His	< 10	30–50	50–80	10–20	70–90	70–95
Pro	< 10	< 10	< 10	< 10	< 10	< 10
Thr	7	40	80	20	80	> 90
Ser	not determined	not determined	not determined	not determined	not determined	not determined
Cys	not determined	not determined	not determined	not determined	not determined	not determined



Nucleótidos

GTGCCGCGGATTTTATTTCGAGTTACAAGAATTGAAGGAAGACGATTATTATG
GGATTACTTTATCTGATGATTCTGATCATCAGTTTTTGCTTGGATCCCAGGTTG
TTGTACAGAAT

(115 nucleotidos)

invitrogen™

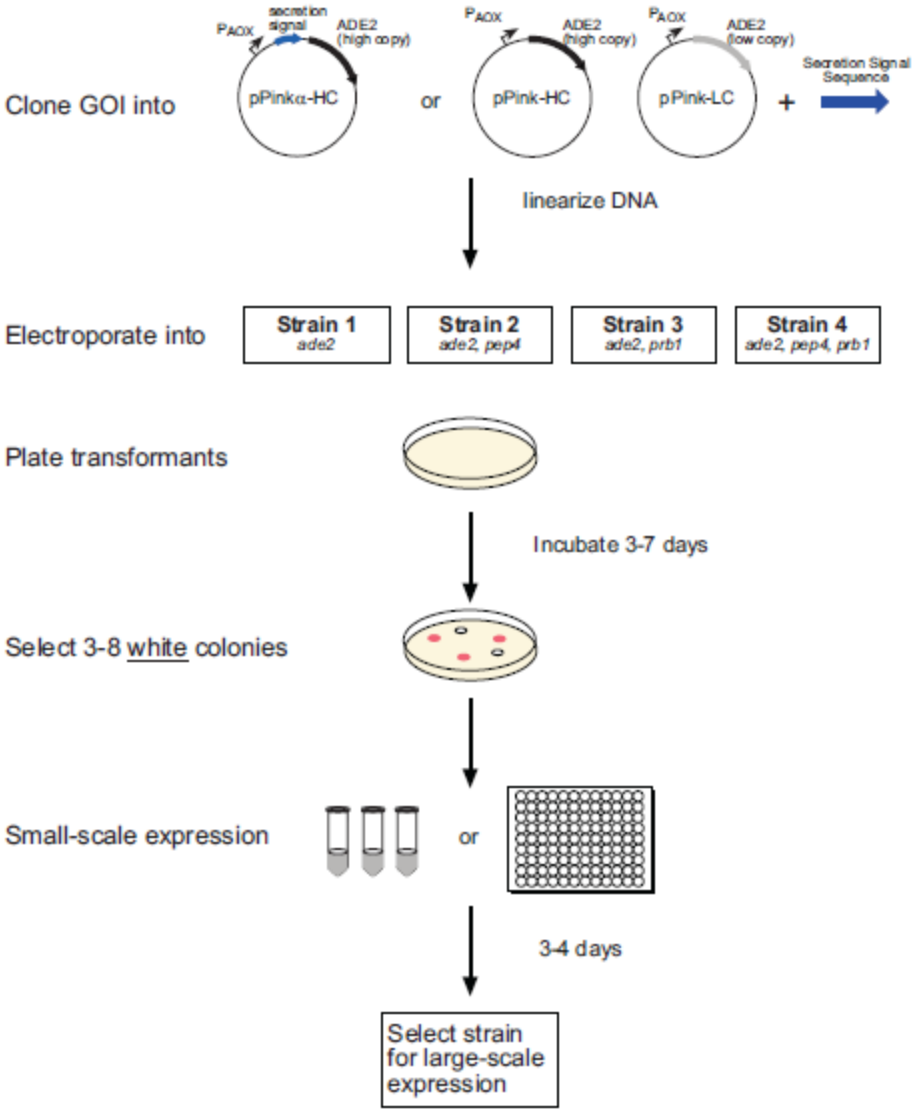
PichiaPink™ Expression System

For High-level and Large-scale Expression and Secretion of Bioactive Recombinant Proteins in *Pichia pastoris*

Catalog nos. A11150, A11151, A11152, A11153, and A11154

Experimental Process

The overall experimental process is presented below. More information about recombination and integration in *Pichia* is provided in a review by Higgins and Cregg (Higgins & Cregg, 1998).



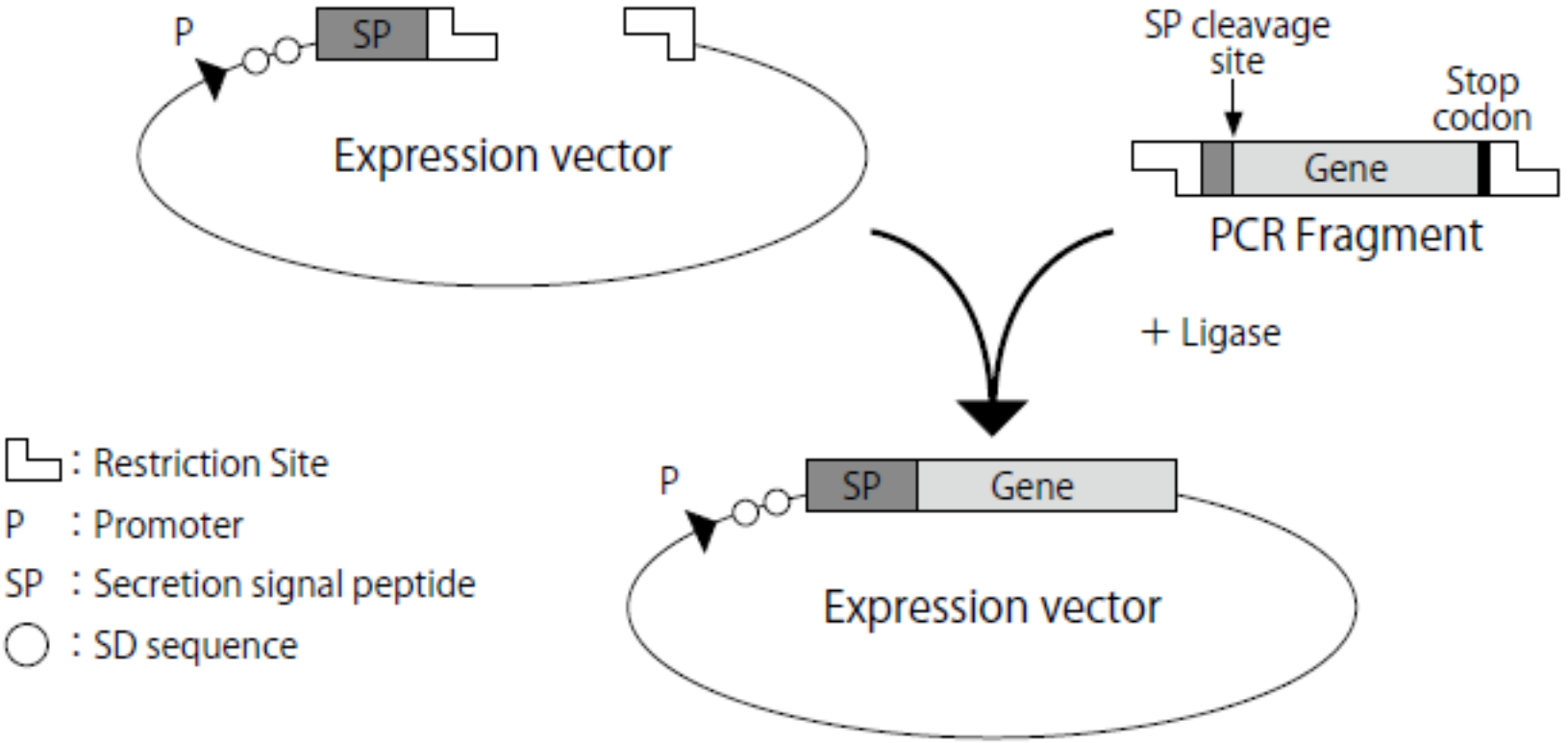
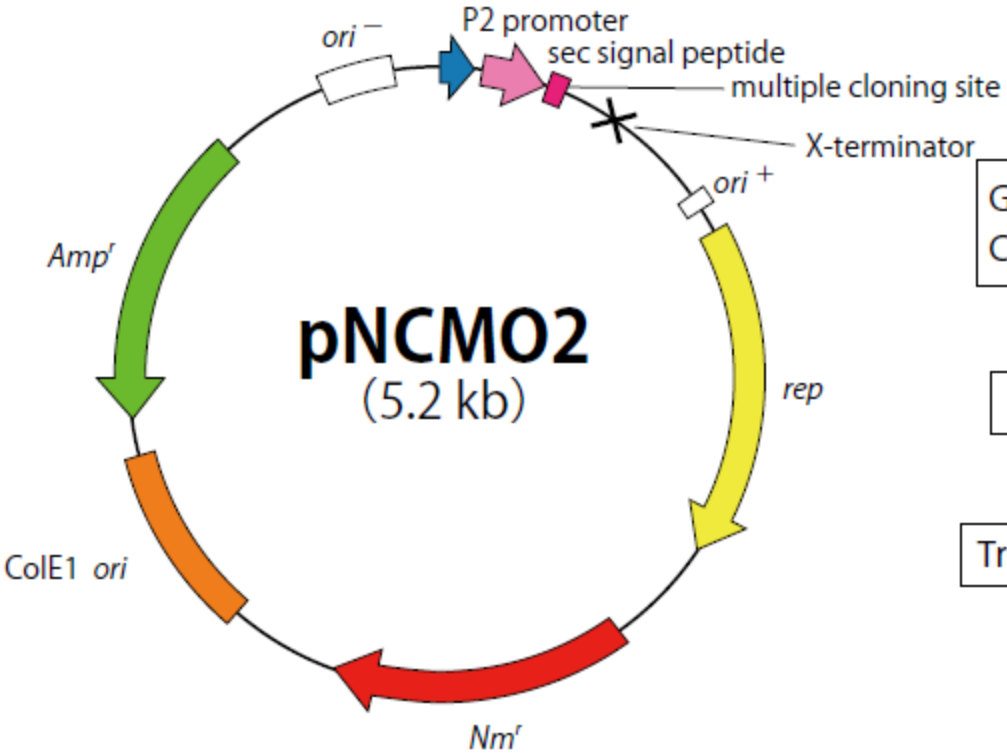


Fig. 3. Construction of *B. choshinensis* expression plasmid



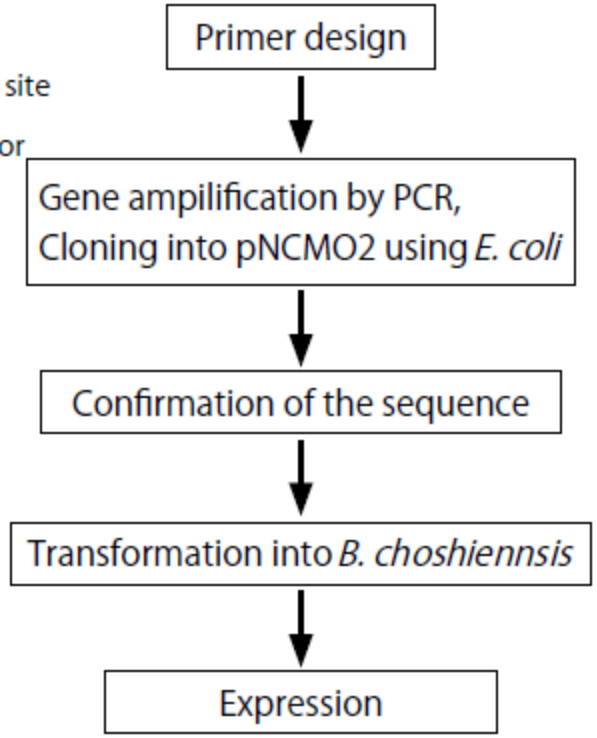


Fig 1. Map of pNCMO2

Because of the strong promoter activity of P2 promoter, it shows detrimental effect on the growth of the transformants. In that case, the use of pNY326 is recommended.

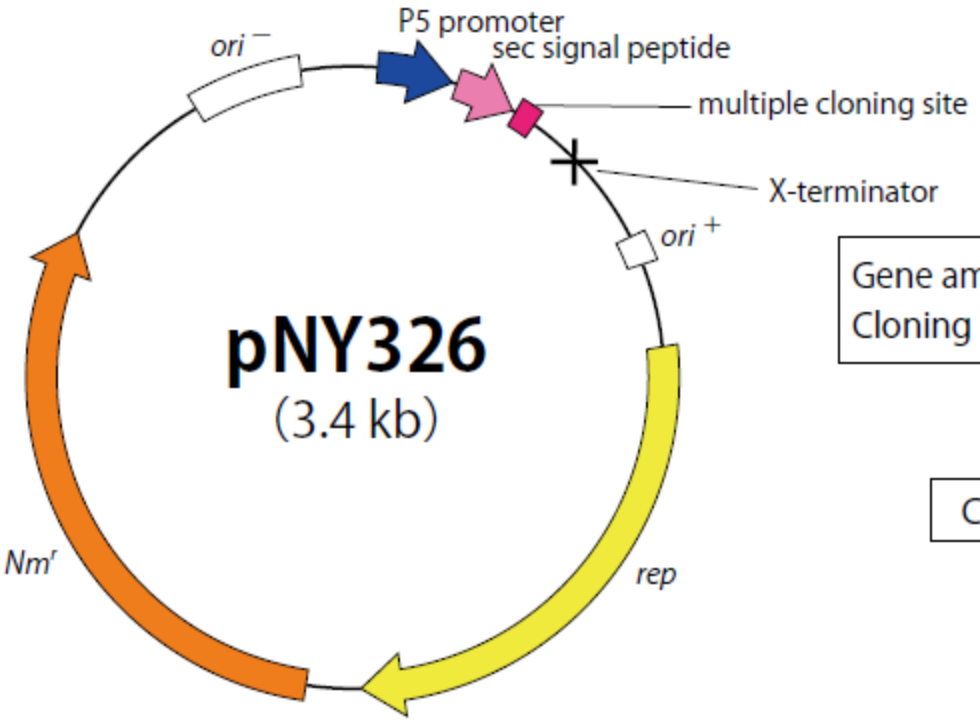


Fig 2. Map of pNY326

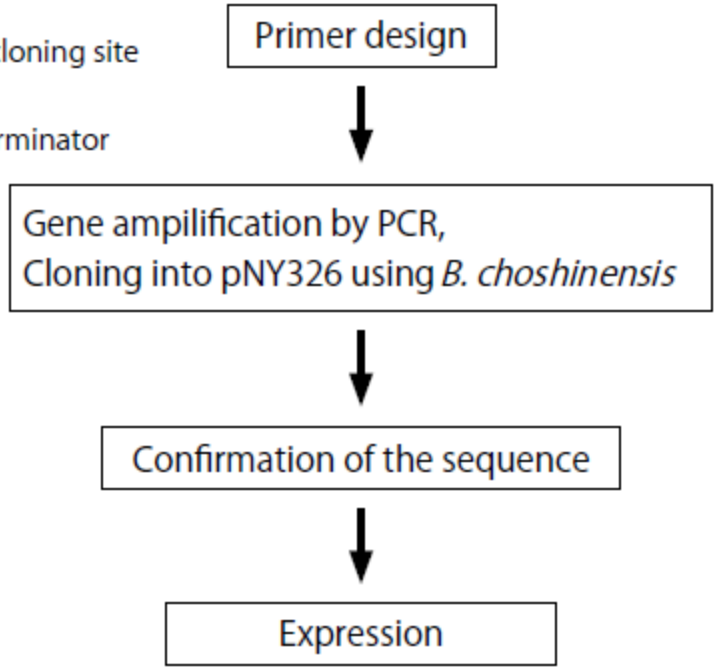


Table 1. Some examples of successful production of heterologous proteins using *B. choshinensis* host-vector system.

Proteins	Origins	Production (g/L)	References
Enzymes			
α-amylase	*B. licheniformis*	3.7	
Sphingomyelinase	*B. cereus*	3.0	
Xylanase	*B. halodurans*	0.2	
CGTase	*B. macerans*	1.5	2)
Chitosanase	*B. circulans*	1.4	
Hyper thermo-stable protease	*A. pernix*	0.1	
Hyper thermo-stable nuclease	*P. horikoshii*	0.7	
PDI	human	1.0	3)
Antigens			
Surface antigen	*E. rhusiopathiae*	0.9	
Surface antigen	*T. pallidum*	0.8	
Cytokines			
EGF	human	1.5	4)
IL-2	human	0.6	5)
NGF	mouse	0.2	
IFN-γ	chicken	0.5	6)
TNF-α	bovine	0.4	
GM-CSF	bovine	0.2	
GH	flounder	0.2	

Trends Biotechnol. 2010 Jan;28(1):37-45. Epub 2009 Nov 10.

Review

Elastin-like polypeptides revolutionize recombinant protein expression and their biomedical application

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[2] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

312 Biophysical Journal Volume 97 July 2009 312–320

Influence of the Amino-Acid Sequence on the Inverse Temperature Transition of Elastin-Like Polymers

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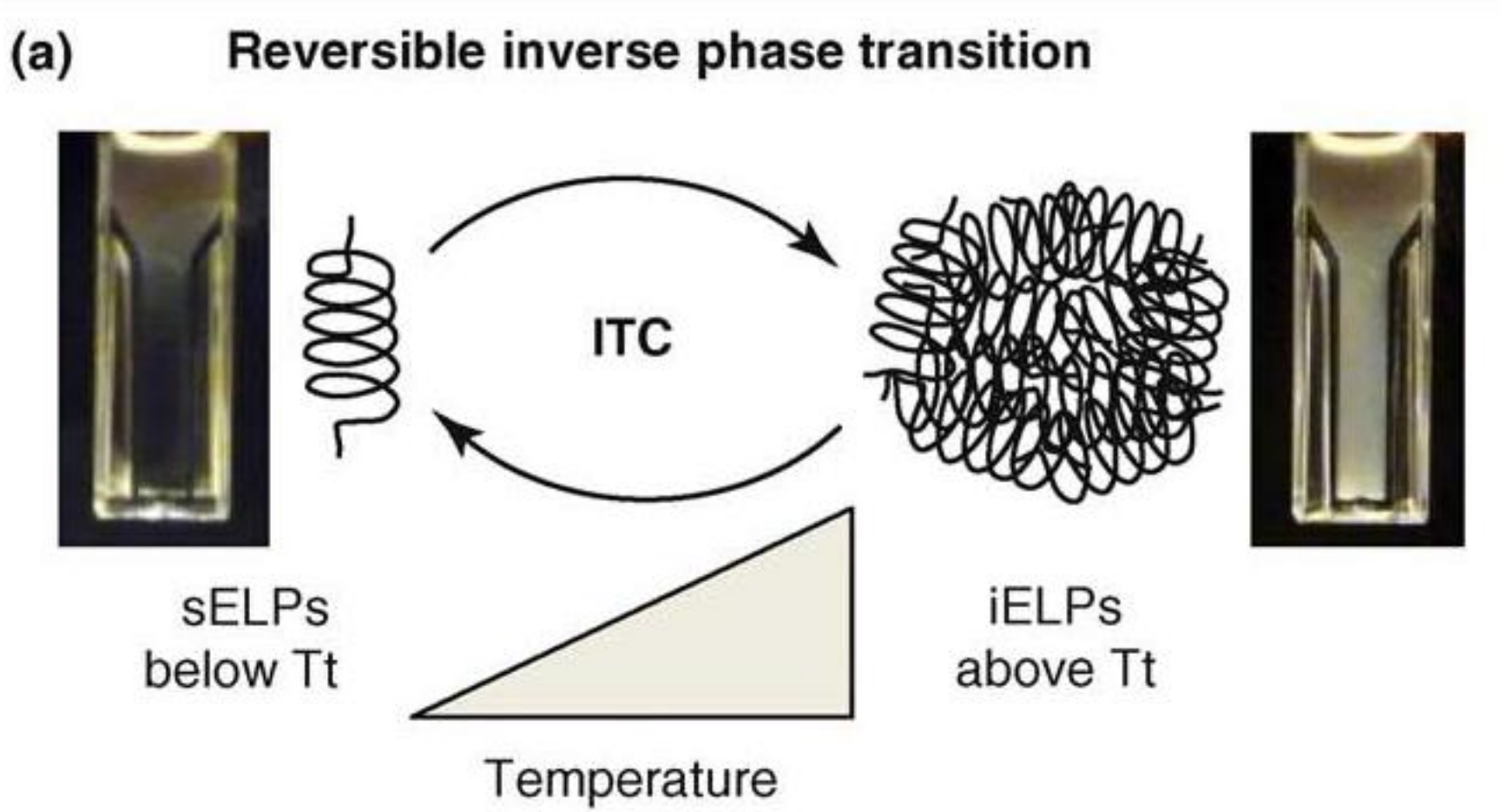
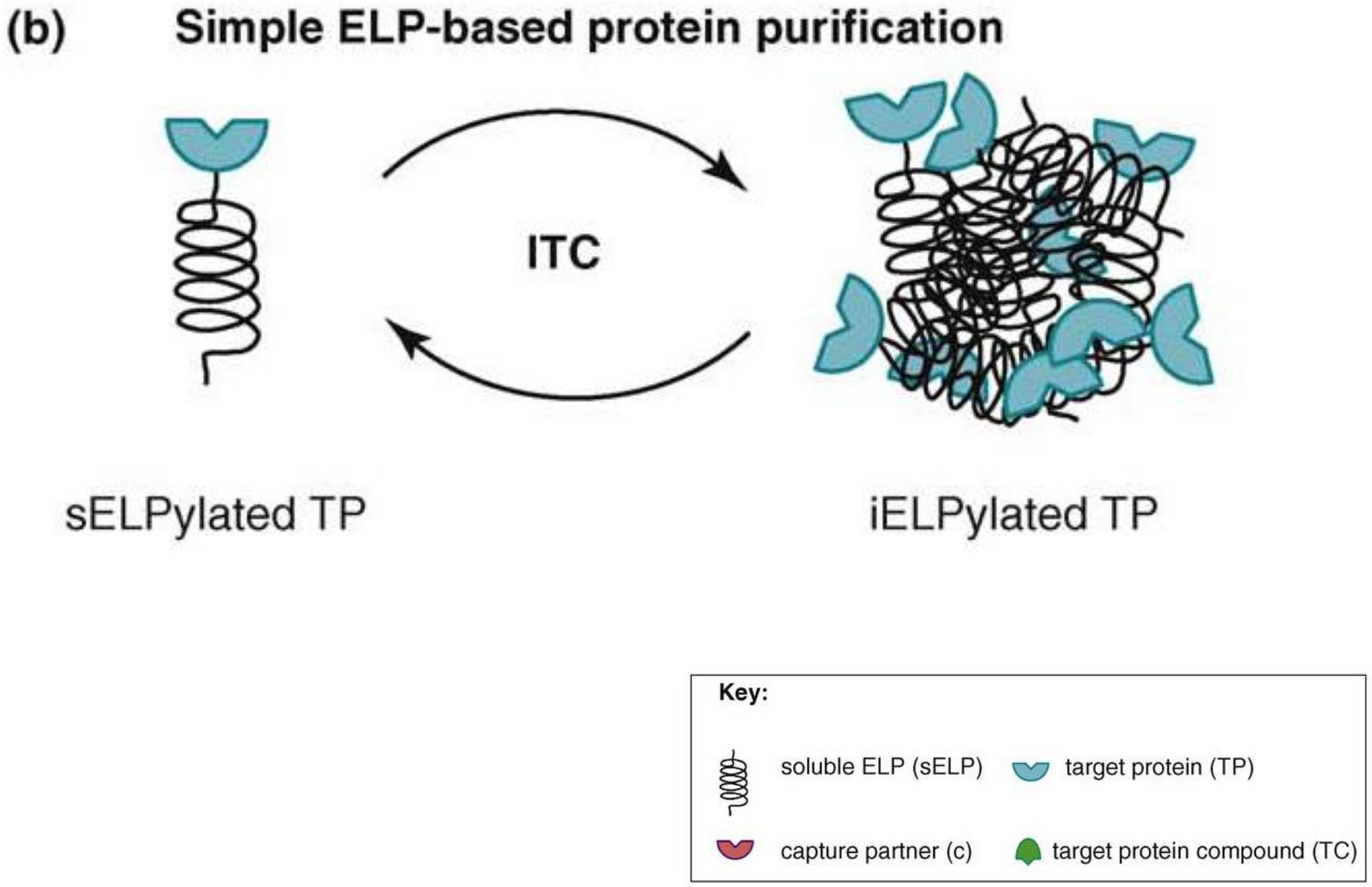
G.I.R. Bioforge, Universidad de Valladolid, Centro de I+D, and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, Valladolid, Spain

ABSTRACT This work explores the dependence of the inverse temperature transition of elastin-like polymers (ELPs) on the amino-acid sequence, i.e., the amino-acid arrangement along the macromolecule and the resulting linear distribution of the physical properties (mainly polarity) derived from it. The hypothesis of this work is that, in addition to mean polarity and molecular mass, the given amino-acid sequence, or its equivalent—the way in which polarity is arranged along the molecule—is also relevant for determining the transition temperature and the latent heat of that transition. To test this hypothesis, a set of linear and di- and triblock ELP copolymers were designed and produced as recombinant proteins. The absolute sequence control provided by recombinant technologies allows the effect of the amino-acid arrangement to be isolated while keeping the molecular mass or mean polarity under strict control. The selected block copolymers were made of two different ELPs: one exhibiting temperature and pH responsiveness, and one exhibiting temperature responsiveness only. By changing the arrangement and length of the blocks while keeping other parameters, such as the molecular mass or mean polarity, constant, we were able to show that the sequence plays a key role in the smart behavior of ELPs.

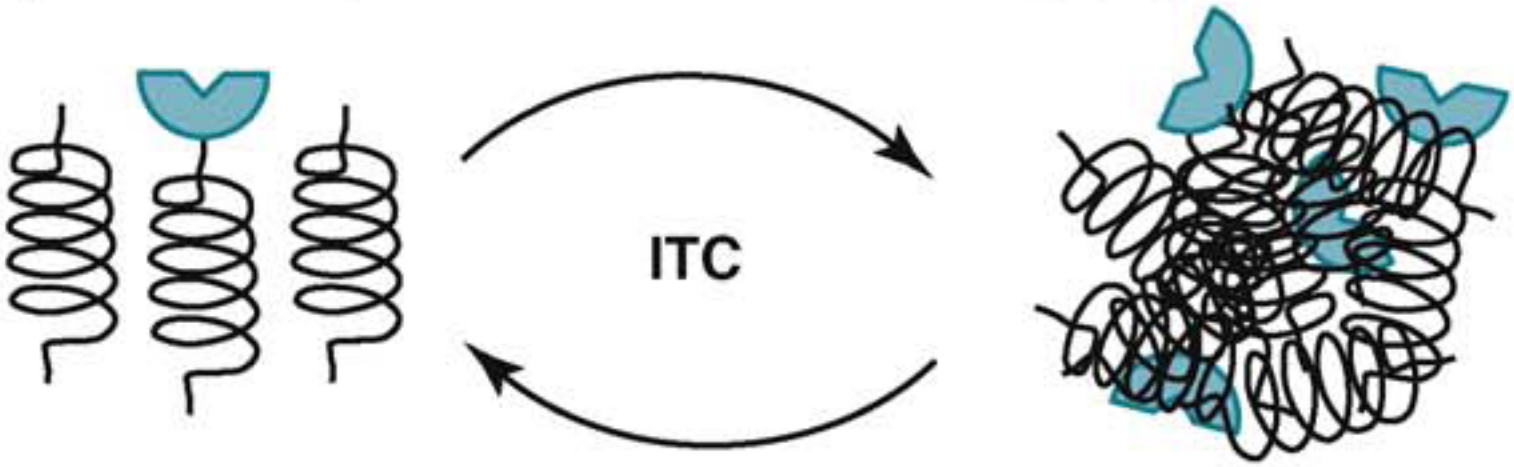
TABLE 1 Amino-acid sequence of the elastin-like block copolymers investigated (abbreviation indicates the kind of block and the number of pentapeptides in each block)

	Sequence	Molecular mass/kDa	Abbreviation
i	$(VPAVG)_{62}$	26.3	A62
ii	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{15}$	31.9	E75
iii	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{20}$	30.5	E50A20
iv	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{40}$	38.5	E50A40
v	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{60}$	47.0	E50A60
vi	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{20}$-$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$	51.9	E50A20E50
vii	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{40}$- $[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$	59.5	E50A40E50
viii	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{60}$- $[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$	67.9	E50A60E50
ix	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{20}$-$(VPAVG)_{40}$	59.5	E100A40
x	$[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$LG_{10}L$ -$(VPAVG)_{60}$	47.8	E50-G_L-A60
xi	$[[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{20}]$ - $[[(VPGVG)_2\ (VPGEG)\ (VPGVG)_2]_{10}$-$(VPAVG)_{20}]$	59.5	E50A20E50A20

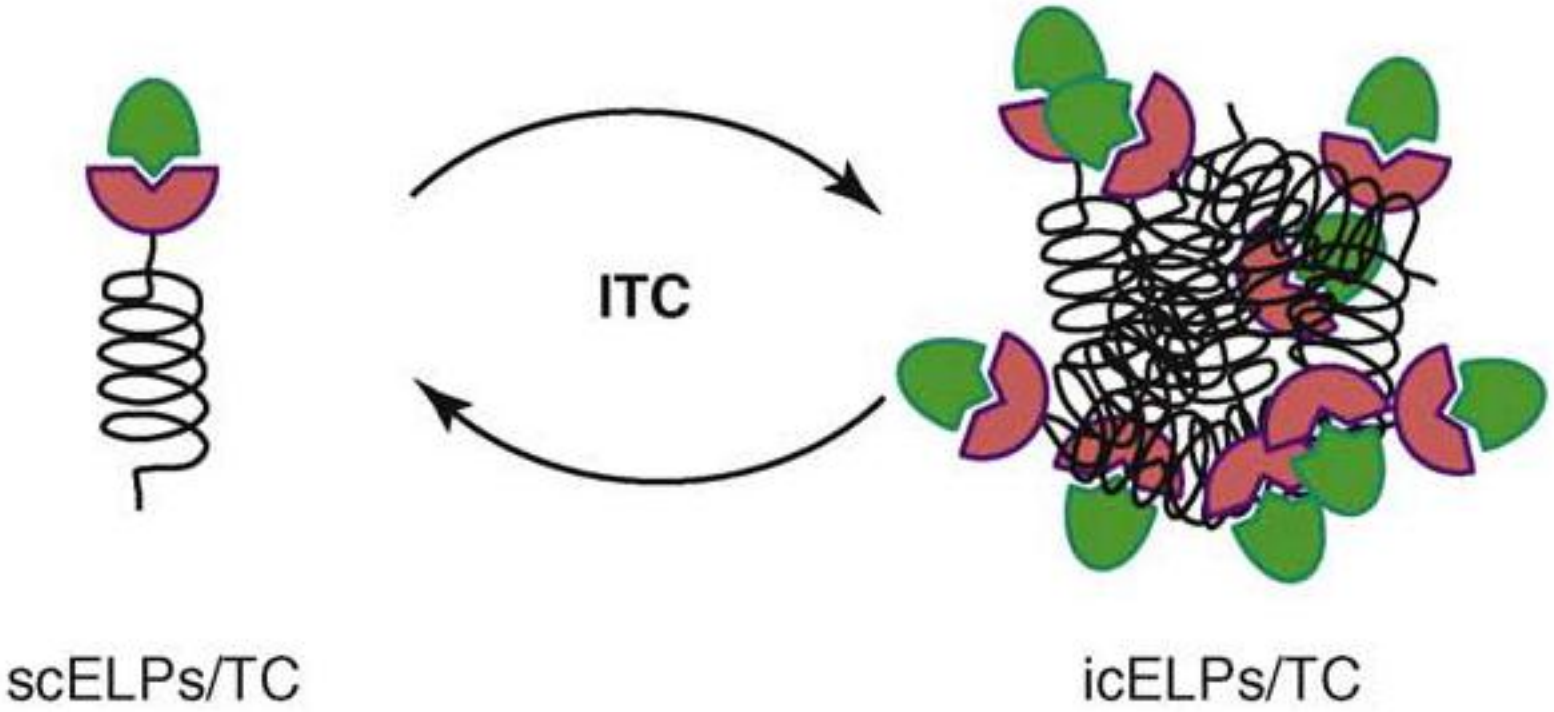

(a) Reversible inverse phase transition
ITC
sELPs
below Tt
iELPs
above Tt
Temperature
Key:
soluble ELP (sELP)
target protein (TP)
capture partner (c)
target protein compound (TC)


(b) Simple ELP-based protein purification
ITC
sELPylated TP
iELPylated TP
Key:
soluble ELP (sELP)
target protein (TP)
capture partner (c)
target protein compound (TC)

(c) Protein purification via ELP-coaggregation



(d) Purification via ELP-mediated affinity capture



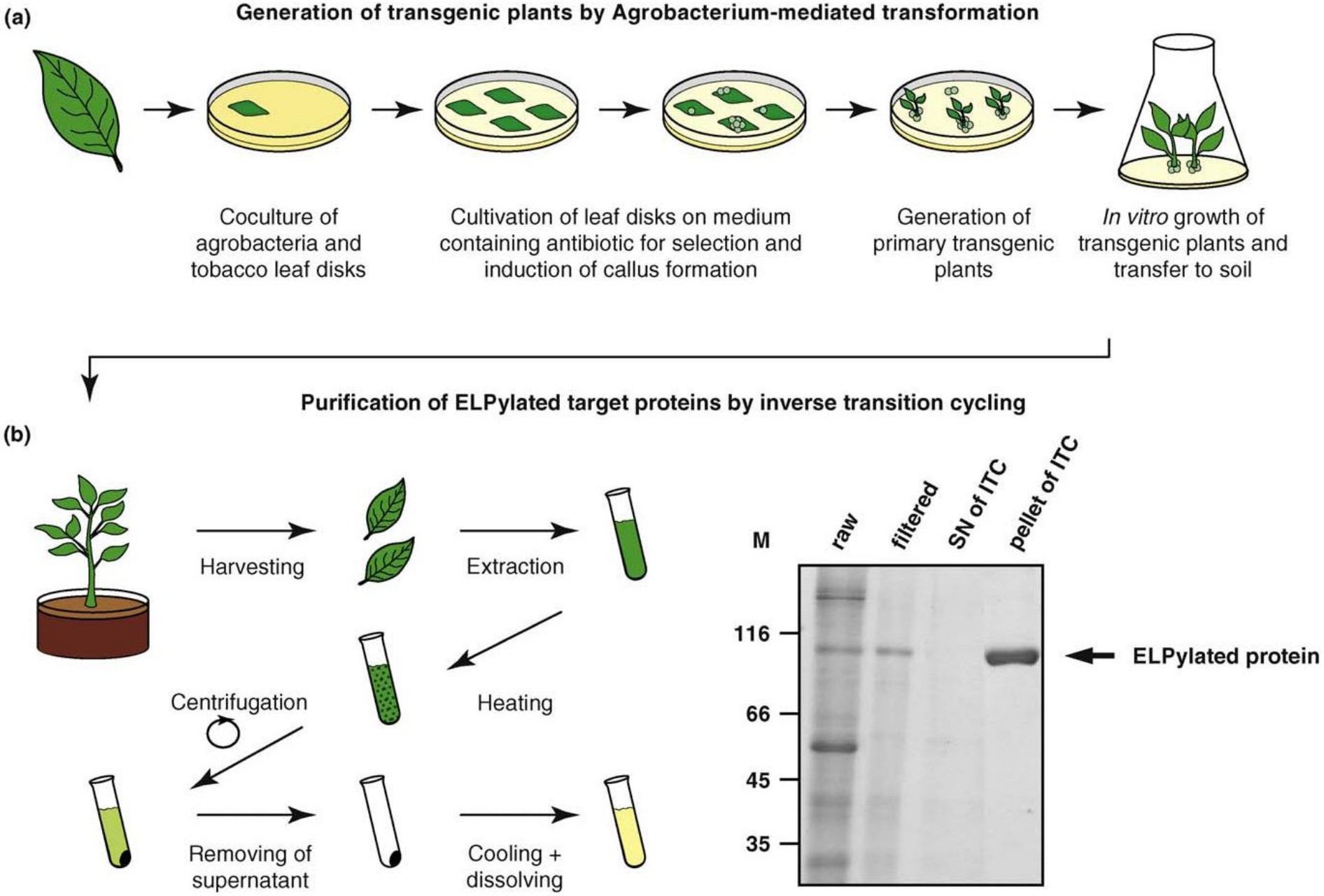
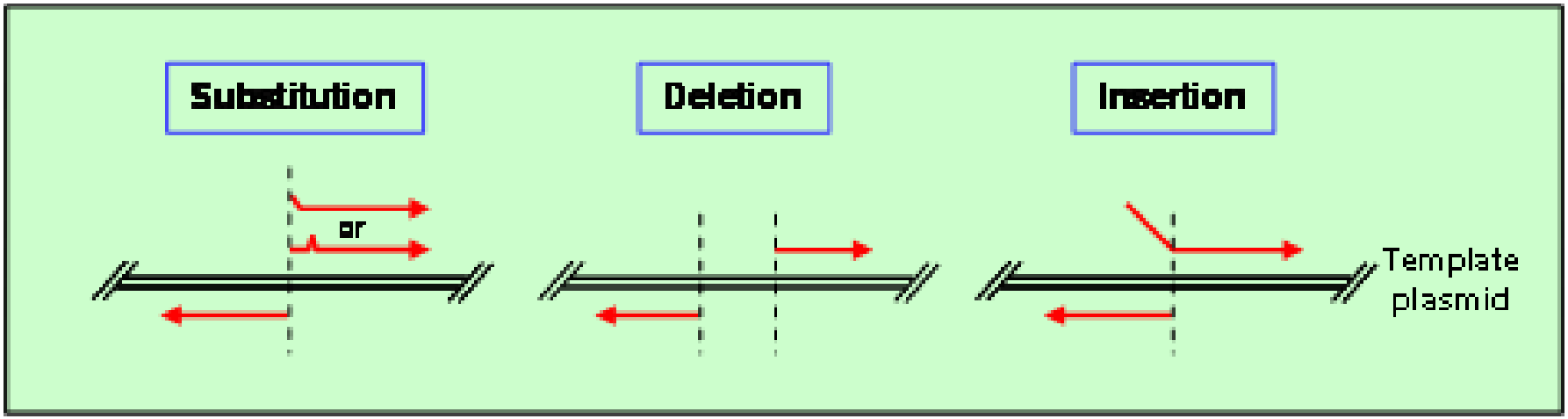
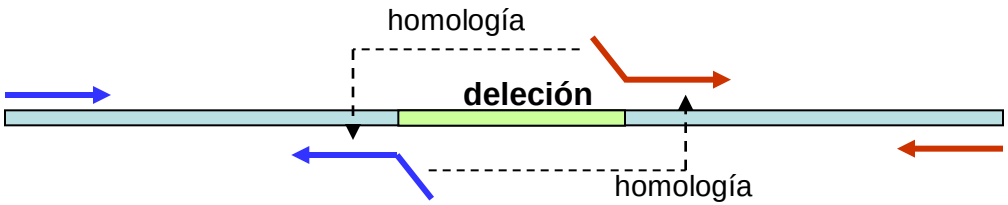

(a)
Generation of transgenic plants by Agrobacterium-mediated transformation
Coculture of agrobacteria and tobacco leaf disks
Cultivation of leaf disks on medium containing antibiotic for selection and induction of callus formation
Generation of primary transgenic plants
In vitro growth of transgenic plants and transfer to soil
Purification of ELPylated target proteins by inverse transition cycling
(b)
Harvesting
Extraction
Heating
Centrifugation
Removing of supernatant
Cooling + dissolving
M
raw
filtered
SN of ITC
pellet of ITC
116
66
45
35
ELPylated protein

Table 1. ELPs

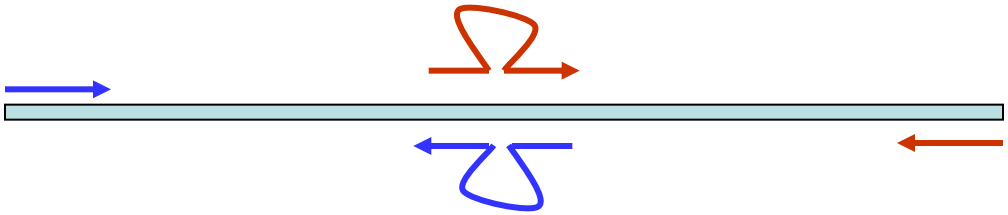
Recombinant protein	Plant expression system	Accumulation level	Purification	Biological functionality	References
(Gly-Val-Gly-Val-Pro)$_{121}$	Tobacco suspension cultures	N. D.	Not done	N. D.	[68]
(Gly-Val-Gly-Val-Pro)$_{121}$	Tobacco leaves	0.01–0.05% TSP	ITC	N. D.	[69]
(Gly-Val-Gly-Val-Pro)$_{121}$	Tobacco chloroplasts	N. D.	Not done	N. D.	[71]
SO1-(Val-Pro-Gly-Xaa-Gly)$_{100}$	Tobacco leaves	0.5–4% TSP	ITC (80 mg/kg FW)	Growth of anchorage-dependent mammalian cells	[72]
scFv-(Val-Pro-Gly-Xaa-Gly)$_{100}$	Tobacco seeds	25% TSP	Not done	Comparable binding affinities to unfused scFv	[82]
Mini-sgp130-(Val-Pro-Gly-Xaa-Gly)$_{100}$	Tobacco leaves	N. D.	ITC (141 μg/g FW)	Inhibition of sIL-6R-mediated trans-signaling	[83]
MaSp2-(Val-Pro-Gly-Val-Gly)$_{27}$	Tobacco leaves	0.25% TSP; 0.75% TSP	Not done	N. D.	[76]
mIL4-(Val-Pro-Gly-Val-Gly)$_{27}$	Tobacco leaves	0.75% TSP; 0.086% TSP	Not done	N. D.	[76]
hIL10-(Val-Pro-Gly-Val-Gly)$_{27}$	Tobacco leaves	0.55% TSP (transient); 0.27% TSP (stable)	Not done	N. D.	[76]
mAb 2F5-ELP	Tobacco leaves	0.2-0.6% TSP	ITC	Comparable binding parameters to CHO-derived non-fused 2F5	[78,79]
scFv-(Val-Pro-Gly-Val-Gly)$_{28}$	Tobacco leaves	0.08-0.1% TSP; 0.8% TSP	ITC (1.5 mg from 660 g FW)	Binding to corresponding antigen	[81]
GFP-(Val-Pro-Gly-Val-Gly)$_n$	Tobacco leaves	0.2-0.5%TSP, 21% TSP (n=10 and p19)	ITC	-	[70,77]
IL10-(Val-Pro-Gly-Val-Gly)$_n$	Tobacco leaves	0.2-0.5% TSP, 4.5% TSP (n=5 and p19)	ITC	N. D.	[77]
EPO-(Val-Pro-Gly-Val-Gly)$_n$	Tobacco leaves	<0.2% TSP	ITC	Binding to human EPO receptor	[70,77]
mAb 2G12-ELP	Tobacco leaves and seeds	0.1-1% TSP	ITC	HIV-1 neutralization	[80]

Proteínas modificadas?

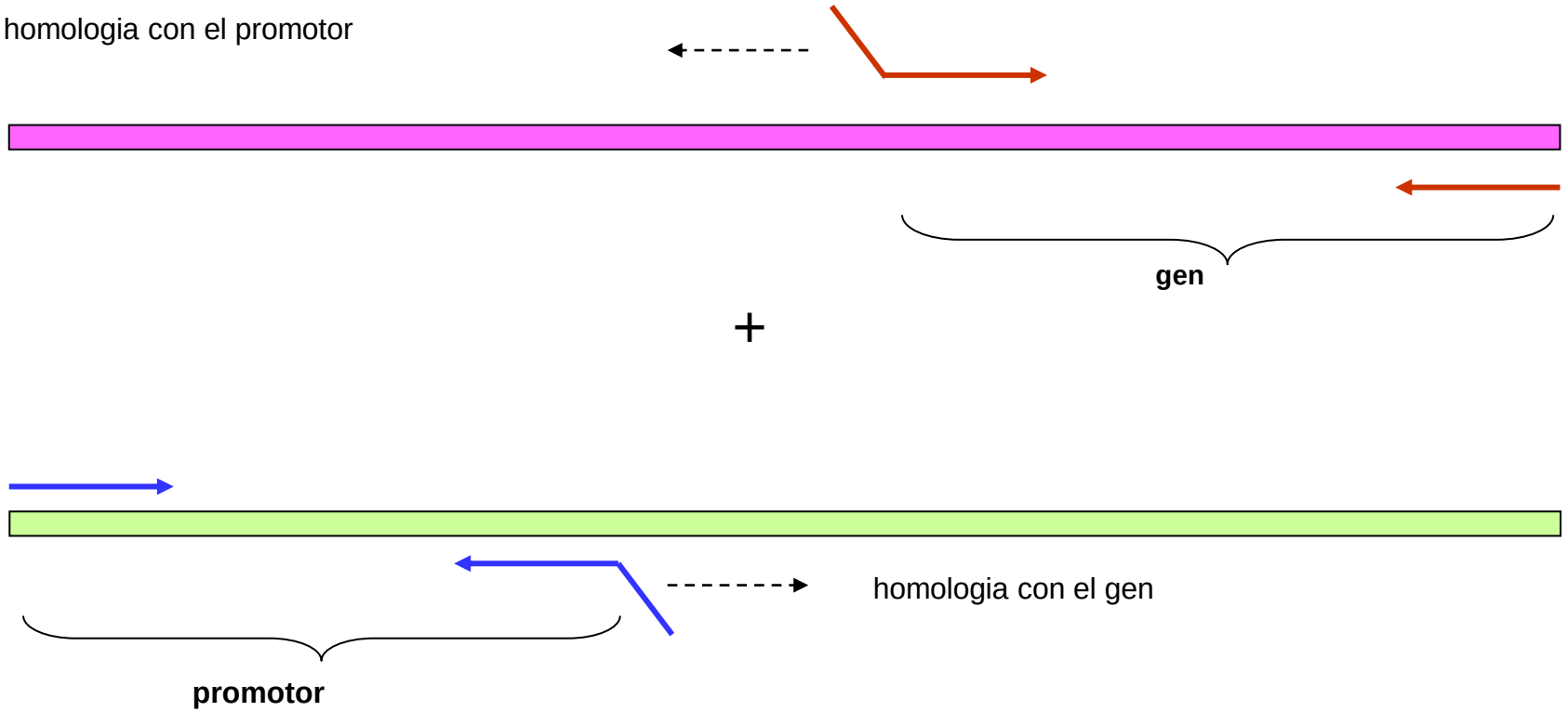




DELECIÓN



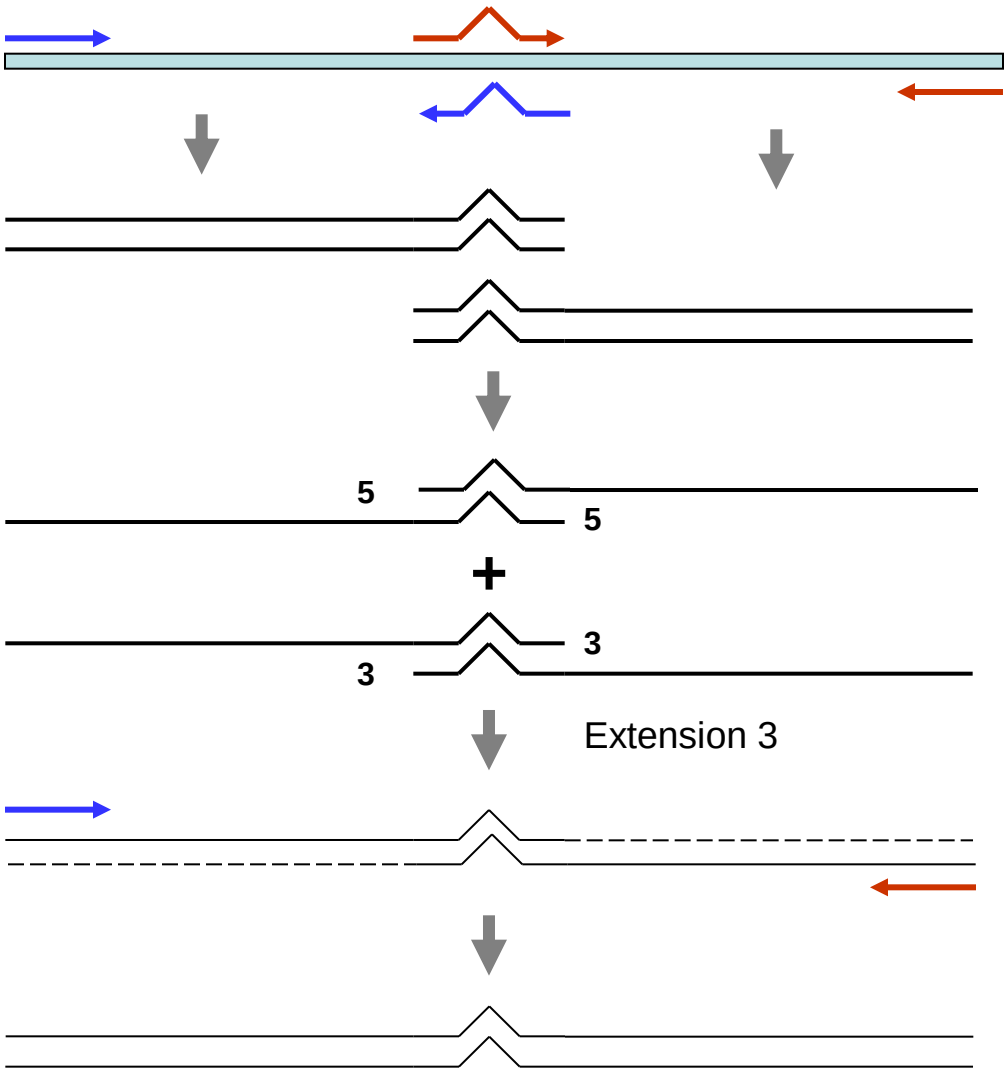
INSERCIÓN



Quimeras

PCR recombinante

MUTAGENESIS CON PCR



PCR con primers mutados
(dos reacciones)

Eliminar los primers,
desnaturalizar/renaturalizar

PCR con primers externos

http://watcut.uwaterloo.ca/watcut/watcut/template.php?act=silent_new

WatCut: An on-line tool for restriction analysis, silent mutation scanning, and SNP-RFLP analysis

Restriction analysis | Silent mutation analysis | SNP-RFLP analysis | Select enzymes | Preferences | Help

Search your primer for mutations that create novel restriction sites without changing the encoded protein sequence.

Type or paste your oligo sequence here:

Name of your oligo:

Clear Form | Submit Sequence

Select from previous projects: ldsfkjhaks (51 bp) Show

Oligo name: **dslkfjI** Bases: **30** GC content: **70** T_m: **71** **°C**

Sequence: CTGGTGCTGGTGGACATGCACGCGGCCCAC

Select the correct reading frame:

Coding Strand	Reading frame	Encoded protein sequence
Forward	(•) 1	CTG GTG CTG GTG GAC ATG CAC GCG GCC CAC Leu Val Leu Val Asp Met His Ala Ala His
	( ) 2	C TGG TGC TGG TGG ACA TGC ACG CGG CCC AC - Trp Cys Trp Trp Thr Cys Thr Arg Pro --
	( ) 3	CT GGT GCT GGT GGA CAT GCA CGC GGC CCA C -- Gly Ala Gly Gly His Ala Arg Gly Pro -
Reverse	( ) -1	CTG GTG CTG GTG GAC ATG CAC GCG GCC CAC Gln His Gln His Val His Val Arg Gly Val
	( ) -2	C TGG TGC TGG TGG ACA TGC ACG CGG CCC AC - Pro Ala Pro Pro Cys Ala Arg Pro Gly --
	( ) -3	CT GGT GCT GGT GGA CAT GCA CGC GGC CCA C -- Thr Ser Thr Ser Met Cys Ala Ala Trp -

Analyze

WatCut: An on-line tool for restriction analysis, silent mutation scanning, and SNP-RFLP analysis

Results of silent mutation scan **Oligo: dslkfjI** **Enzymes: Filtered**

Bases: 30 **GC content: 70%** **T_m: 71 °C**

DNA / protein sequence:

```
CTG GTG CTG GTG GAC ATG CAC GCG GCC CAC
Leu Val Leu Val Asp Met His Ala Ala His
```

Show mutations: From 0 to >3 Sort by enzyme Update page Print version

Save checked enzymes as new set: Save Check all Uncheck all

Enzyme	Site / mutations:	Base changes	$T_{m\,template}$, °C	$T_{m\,self}$, °C
HincII	CTGGTGCTGGTTGACATGCACGCGGCCCAC	1	66	70
NsiI	CTGGTGCTGGTGGACATGCATGCGGCCCAC	1	66	70
NspI	CTGGTGCTGGTGGACATGCACGCGGCCCAC	0	71	71
NspI	CTGGTGCTGGTGGACATGCATGCGGCCCAC	1	66	70
SalI	CTGGTGCTGGTCGACATGCACGCGGCCCAC	1	68	71
ScaI	CTAGTACTGGTGGACATGCACGCGGCCCAC	2	62	68
SpeI	CTGGTACTAGTGGACATGCACGCGGCCCAC	2	62	68
SphI	CTGGTGCTGGTGGACATGCATGCGGCCCAC	1	66	70
TatI	CTAGTACTGGTGGACATGCACGCGGCCCAC	2	62	68

Lps	CATATGAGTGAAGCACCGCGTATCC	NdeI	-
Stopa	GAATTCGGTACCTCAGCGTCCGCGCAGGAAGAGCTTGTCCAG	KpnI-EcoRI	-
Sals	GTCGACATGCACGCGGCCCACGAGC	SalI	-
Sala	GTCGACCAGCACCAGGCCGTGGGCGTTC	SalI	-
M468As	GTCGACGCGCACGCGGCCCACGAGCGGATCAC	SalI	M468A
H469As	GTCGACATGGCCGCGGCCCACGAGCGGATCAC	SalI	H469A
H472As	GTCGACATGCACGCGGCCGCCGAGCGGATCACCTACGAGC	SalI	H472A
E473As	GTCGACATGCACGCGGCCCACGCGCGGATCACCTACGAGCGCCT	SalI	E473A
R474As	GTCGACATGCACGCGGCCCACGAGGCGATCACCTACGAGCGCCTCAAG	SalI	R474A
Y477As	GTCGACATGCACGCGGCCCACGAGCGGATCACCGCCGAGCGCCTCAAGGTGGCGATGG	SalI	Y477A
E478As	GTCGACATGCACGCGGCCCACGAGCGGATCACCTACGCGCGCCTCAAGGTGGCGATGGCCAGC	SalI	E478A

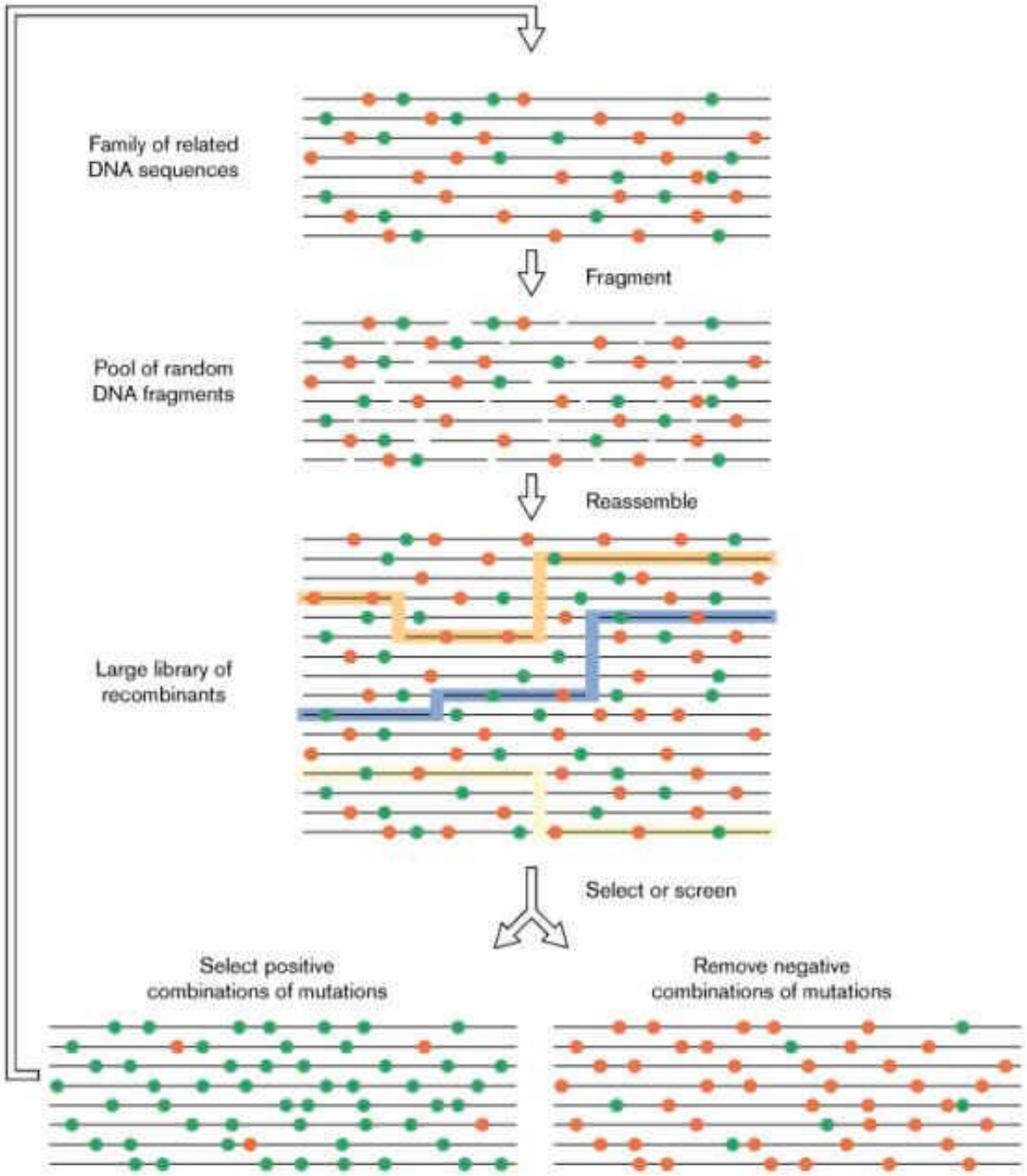
Rapid evolution of a protein in vitro by DNA shuffling.
Stemmer WP.
Nature. 1994 Aug 4;370(6488):389-91.

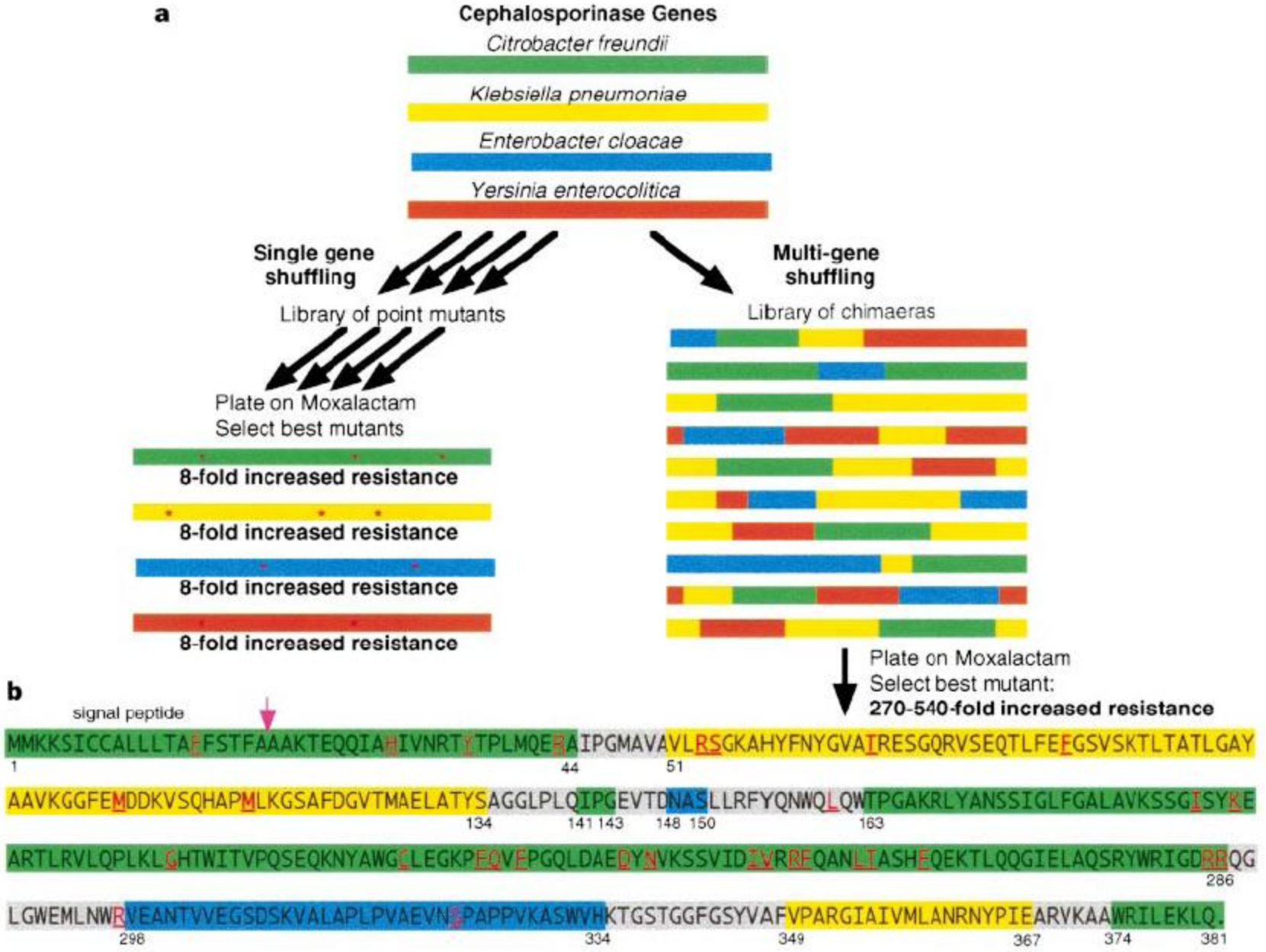
DNA shuffling of a family of genes from diverse species accelerates directed evolution.
Crameri A, Raillard SA, Bermudez E, Stemmer WP.
Nature. 1998 Jan 15;391(6664):288-91.

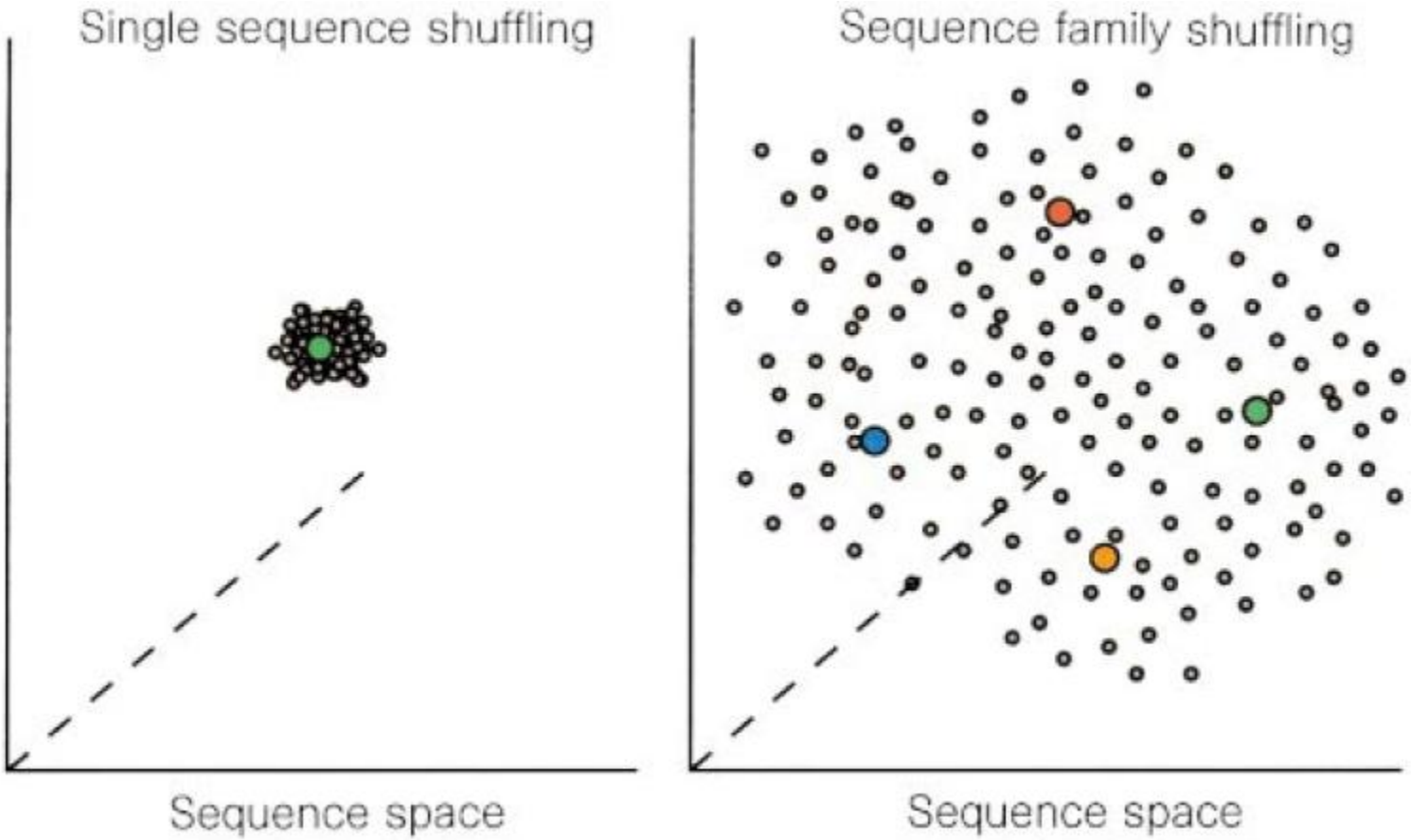
Genome shuffling leads to rapid phenotypic improvement in bacteria.
Zhang YX, Perry K, Vinci VA, Powell K, Stemmer WP, del Cardayré SB.
Nature. 2002 Feb 7;415(6872):644-6.

Genome shuffling of Lactobacillus for improved acid tolerance.
Patnaik R, Louie S, Gavrilovic V, Perry K, Stemmer WP, Ryan CM, del Cardayré S.
Nat Biotechnol. 2002 Jul;20(7):707-12.

DNA shuffling Methodology




a
Cephalosporinase Genes
Citrobacter freundii
Klebsiella pneumoniae
Enterobacter cloacae
Yersinia enterocolitica
Single gene shuffling
Multi-gene shuffling
Library of point mutants
Library of chimaeras
Plate on Moxalactam
Select best mutants
8-fold increased resistance
8-fold increased resistance
8-fold increased resistance
8-fold increased resistance
Plate on Moxalactam
Select best mutant:
270-540-fold increased resistance
b
signal peptide
MMKKSICCALLLTAFFSTFAAAKTEQQIAHIVNRTYTPLMQERAIPGMAVAVLRSGKAHYFNYGVAIRESGQRVSEQTLFEFGSVSKTLTATLGAY
1
44
51
AAVKGGFEMDDKVSQHAPMLKGSAFDGVTMAELATYSAGGLPLQIPGEVTDNASLLRFYQNWQLQWTPGAKRLYANSSIGLFGALAVKSSGISYKE
134
141 143
148 150
163
ARTLRVLQPLKLGHTWITVPQSEQKNYAWGCLEGKPFQVFPGQLDAEDYNVKSSVIDIVRRFQANLTASHFQEKTLQQGIELAQSRYWRIGDRRQG
286
LGWEMLNWRVEANTVVEGSDSKVALAPLPVAEVNSPAPPVKASWVHKTGSTGGFGSYVAFVPARGIAIVMLANRNYPIEARVKAAWRILEKLQ.
298
334
349
367
374
381


Single sequence shuffling
Sequence space
Sequence family shuffling
Sequence space

GenScript
The Biology CRO

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- Baculovirus/insect cell production up to grams
- Mammalian cell production up to grams

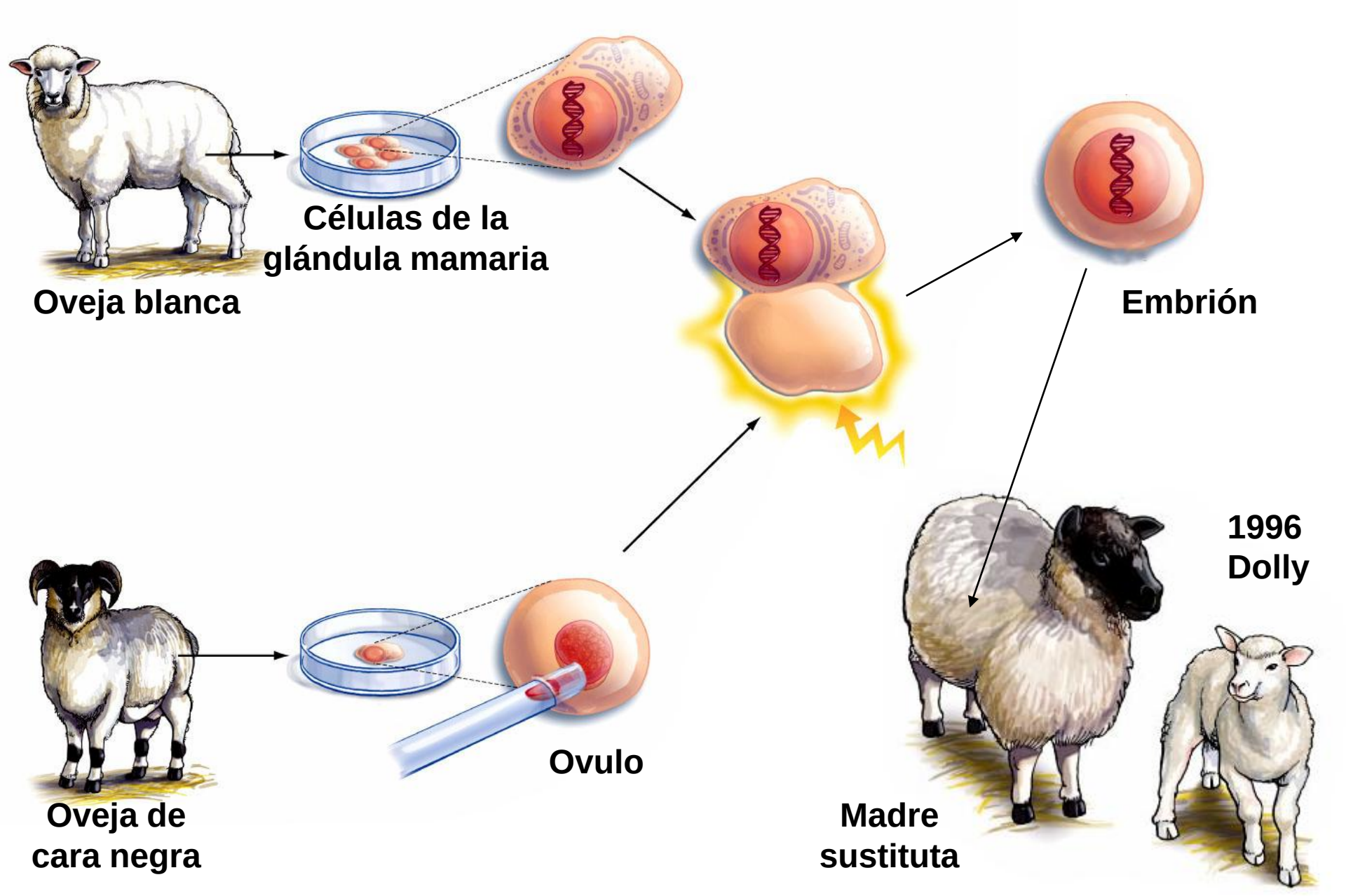
Bioprocessing Services

- Mammalian protein expression service
- Stable cell line development & protein production

OptimumGene™ Codon Optimization

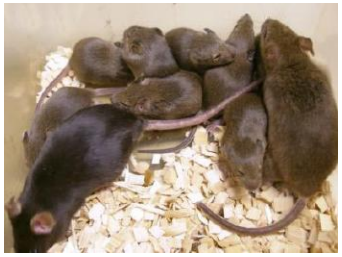
—— Significant increase in protein expression

Clonado reproductivo



Clonado reproductivo

2008
"Production of healthy cloned mice from bodies frozen at 20°C for 16 years"



2008
"Cloning endangered gray wolves (*Canis lupus*) from somatic cells collected postmortem"

2009
"Resurrection of a bull by cloning from organs frozen without cryoprotectant in a -80°C freezer for a decade"

2009
"First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning"

SCIENCE VOL 319 29 FEBRUARY 2008

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison III, Hamilton O. Smith*

We have synthesized a 582,970–base pair *Mycoplasma genitalium* genome. This synthetic genome, named *M. genitalium* JCVI-1.0, contains all the genes of wild-type *M. genitalium* G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and to allow for selection. To identify the genome as synthetic, we inserted "watermarks" at intergenic sites known to tolerate transposon insertions. Overlapping "cassettes" of 5 to 7 kilobases (kb), assembled from chemically synthesized oligonucleotides, were joined by in vitro recombination to produce intermediate assemblies of approximately 24 kb, 72 kb ("1/8 genome"), and 144 kb ("1/4 genome"), which were all cloned as bacterial artificial chromosomes in *Escherichia coli*. Most of these intermediate clones were sequenced, and clones of all four 1/4 genomes with the correct sequence were identified. The complete synthetic genome was assembled by transformation-associated recombination cloning in the yeast *Saccharomyces cerevisiae*, then isolated and sequenced. A clone with the correct sequence was identified. The methods described here will be generally useful for constructing large DNA molecules from chemically synthesized pieces and also from combinations of natural and synthetic DNA segments.

3 AUGUST 2007 VOL 317 SCIENCE

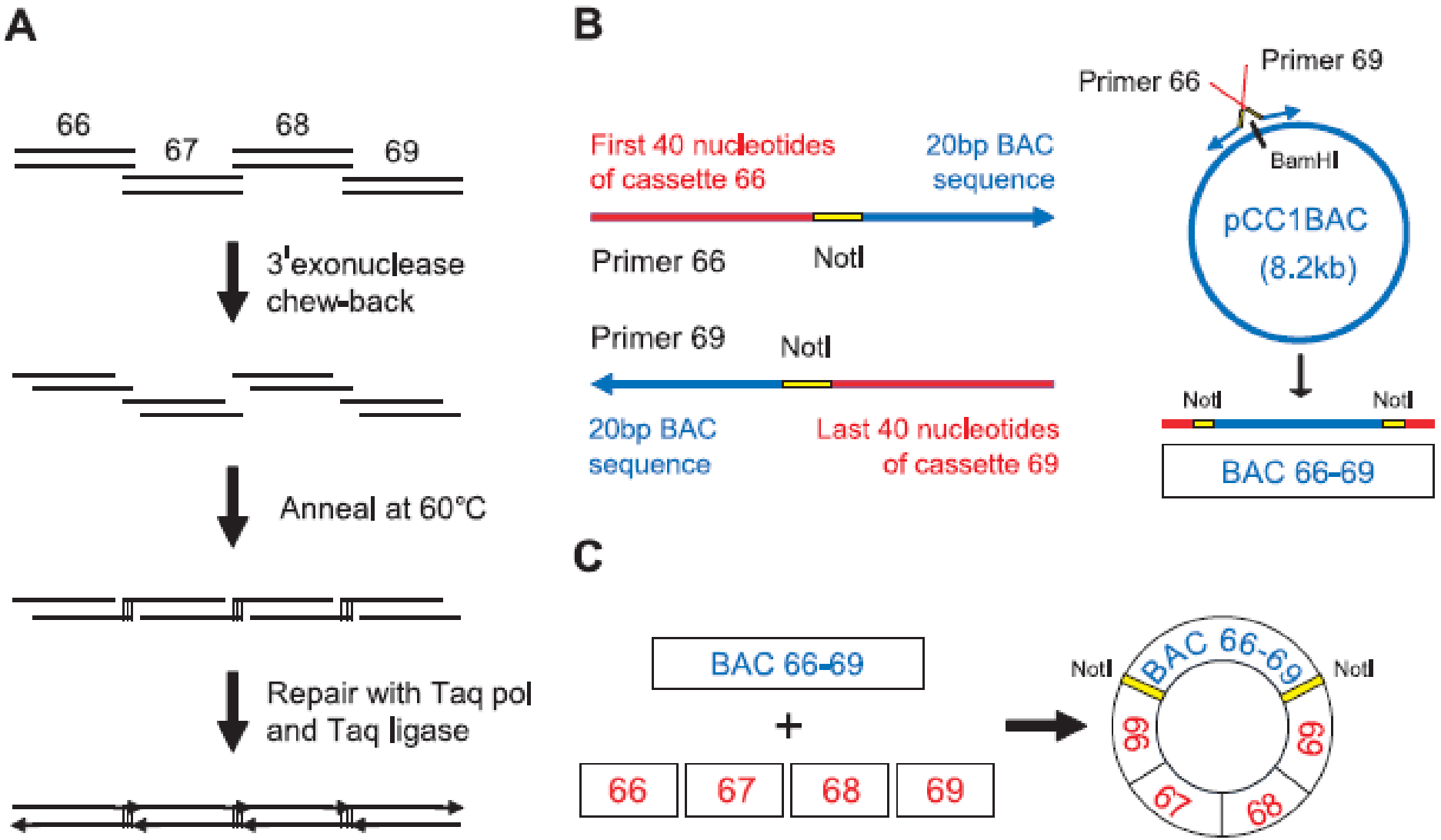
Genome Transplantation in Bacteria: Changing One Species to Another

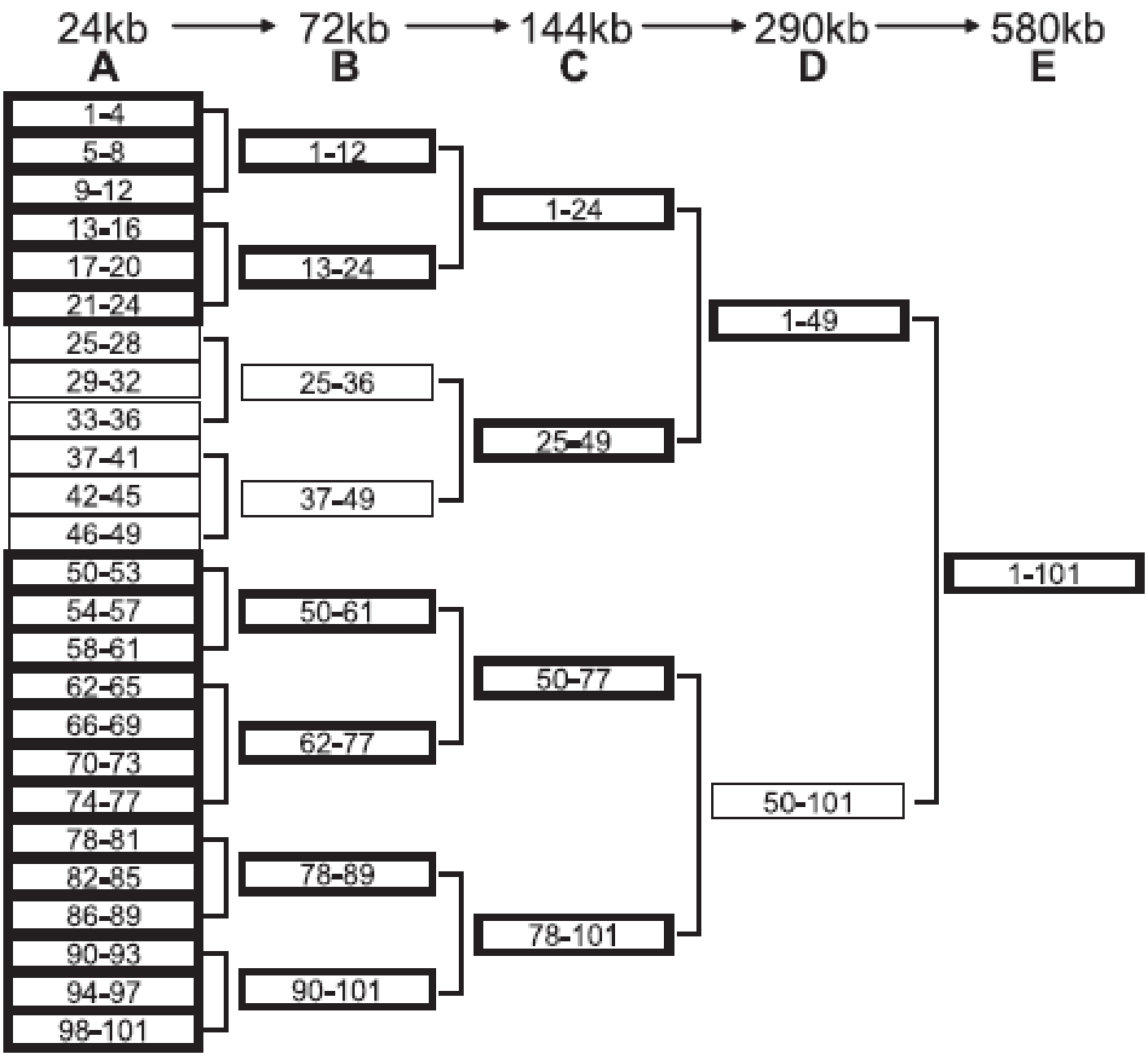
Carole Lartigue, John I. Glass,* Nina Alperovich, Rembert Pieper, Prashanth P. Parmar, Clyde A. Hutchison III, Hamilton O. Smith, J. Craig Venter

As a step toward propagation of synthetic genomes, we completely replaced the genome of a bacterial cell with one from another species by transplanting a whole genome as naked DNA. Intact genomic DNA from *Mycoplasma mycoides* large colony (LC), virtually free of protein, was transplanted into *Mycoplasma capricolum* cells by polyethylene glycol–mediated transformation. Cells selected for tetracycline resistance, carried by the *M. mycoides* LC chromosome, contain the complete donor genome and are free of detectable recipient genomic sequences. These cells that result from genome transplantation are phenotypically identical to the *M. mycoides* LC donor strain as judged by several criteria.

Transformación, Transducción, Conjugación

Synthetic genomics


A
66
67
68
69
3'exonuclease chew-back
Anneal at 60°C
Repair with Taq pol and Taq ligase
B
First 40 nucleotides of cassette 66
20bp BAC sequence
Primer 66
NotI
Primer 69
NotI
20bp BAC sequence
Last 40 nucleotides of cassette 69
Primer 66
Primer 69
BamHI
pCC1BAC (8.2kb)
NotI
NotI
BAC 66-69
C
BAC 66-69
+
66
67
68
69
NotI
BAC 66-69
NotI
66
67
68
69


24kb
72kb
144kb
290kb
580kb
A
B
C
D
E
1-4
5-8
9-12
13-16
17-20
21-24
25-28
29-32
33-36
37-41
42-45
46-49
50-53
54-57
58-61
62-65
66-69
70-73
74-77
78-81
82-85
86-89
90-93
94-97
98-101
1-12
13-24
25-36
37-49
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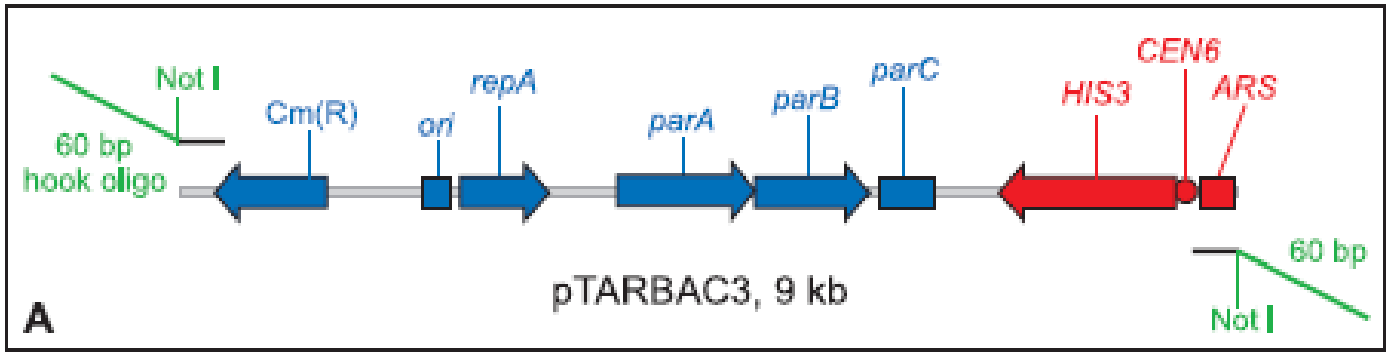
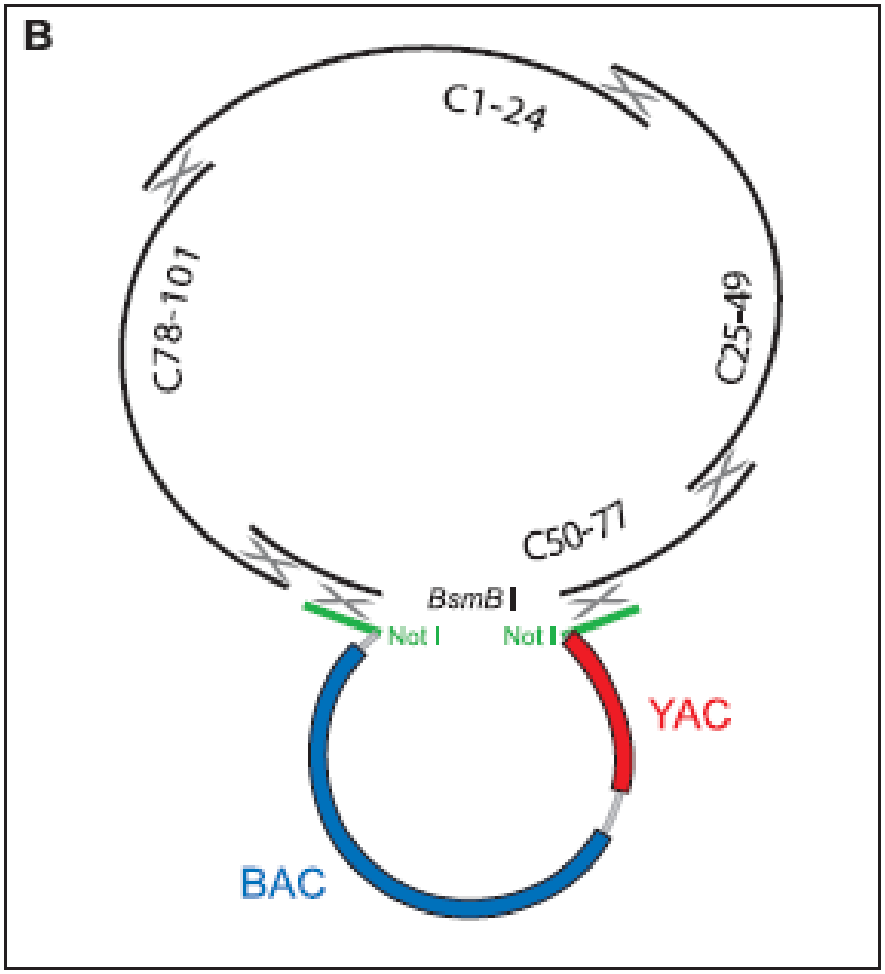
1-24
25-49
50-77
78-101
1-49
50-101
1-101


Not I
60 bp
hook oligo
Cm(R)
ori
repA
parA
parB
parC
HIS3
CEN6
ARS
60 bp
Not I
pTARBAC3, 9 kb
A


B
C1-24
C25-49
C50-T1
C78-101
BsmB I
Not I
Not I
YAC
BAC

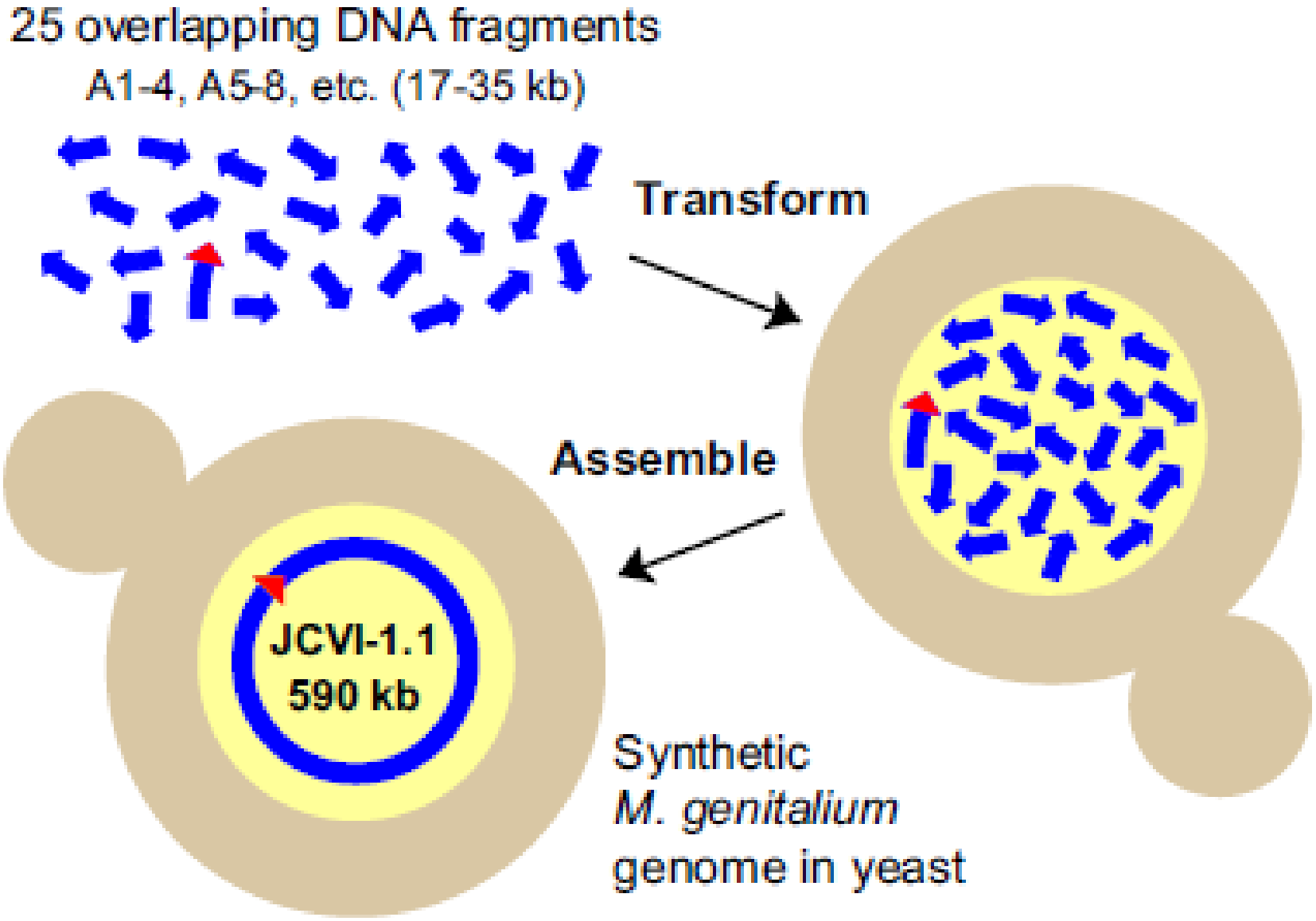
20404–20409 | PNAS | December 23, 2008 | vol. 105 | no. 51

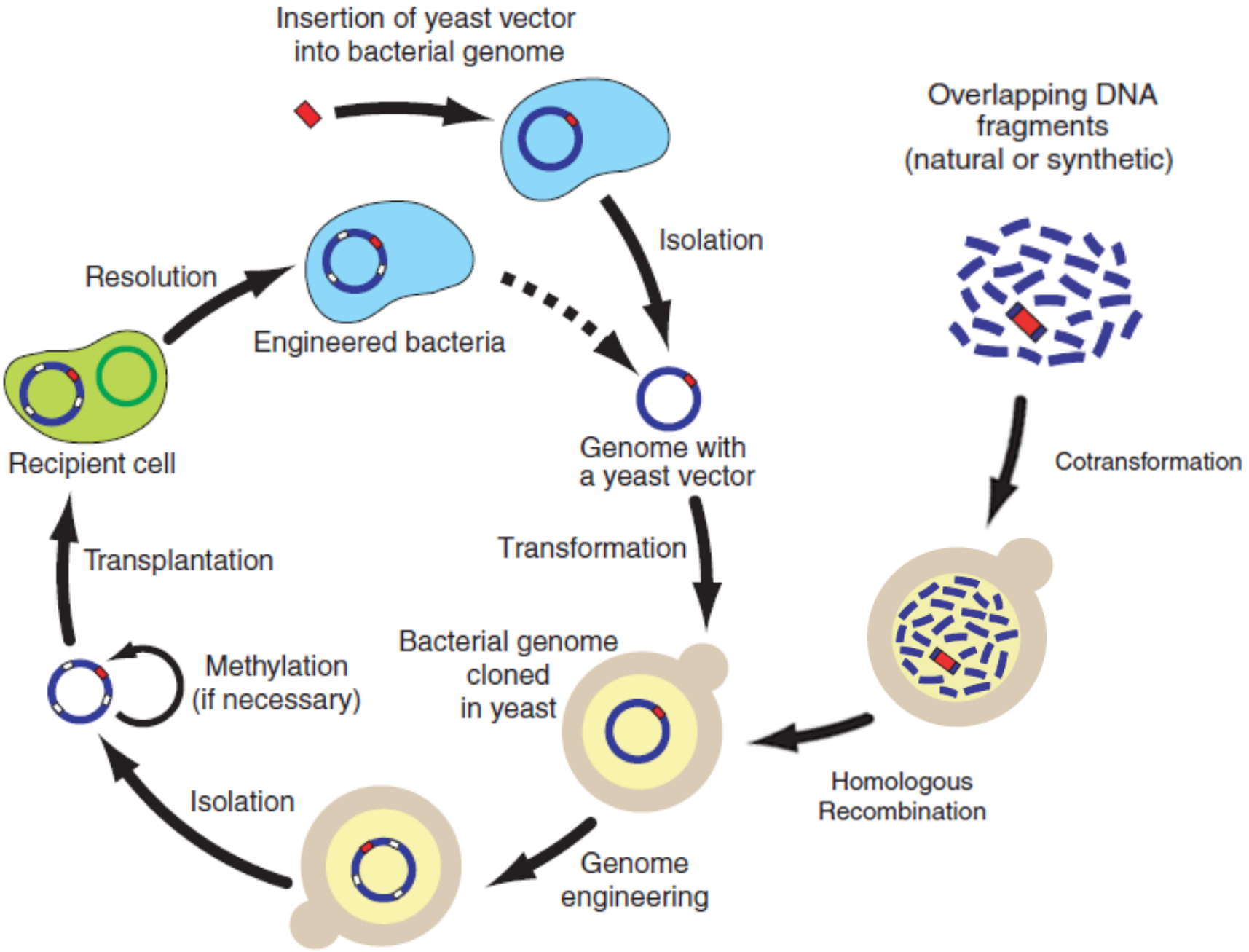
One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome

Daniel G. Gibson[a,1], Gwynedd A. Benders[b], Kevin C. Axelrod[a], Jayshree Zaveri[a], Mikkel A. Algire[a], Monzia Moodie[a], Michael G. Montague[a], J. Craig Venter[a], Hamilton O. Smith[b], and Clyde A. Hutchison III[b,1]

[a]The J. Craig Venter Institute, Synthetic Biology Group, Rockville, MD 20850 and [b]The J. Craig Venter Institute, Synthetic Biology Group, San Diego, CA 92121

We previously reported assembly and cloning of the synthetic *Mycoplasma genitalium* JCVI-1.0 genome in the yeast *Saccharomyces cerevisiae* by recombination of six overlapping DNA fragments to produce a 592-kb circle. Here we extend this approach by demonstrating assembly of the synthetic genome from 25 overlapping fragments in a single step. The use of yeast recombination greatly simplifies the assembly of large DNA molecules from both synthetic and natural fragments.


25 overlapping DNA fragments
A1-4, A5-8, etc. (17-35 kb)
Transform
Assemble
JCVI-1.1
590 kb
Synthetic
M. genitalium
genome in yeast


Insertion of yeast vector
into bacterial genome
Overlapping DNA
fragments
(natural or synthetic)
Isolation
Resolution
Engineered bacteria
Recipient cell
Genome with
a yeast vector
Cotransformation
Transplantation
Transformation
Methylation
(if necessary)
Bacterial genome
cloned
in yeast
Homologous
Recombination
Isolation
Genome
engineering