Electroforesis en gel de DNA

Método analítico de visualización del DNA basado en la migración del DNA a través de un gel

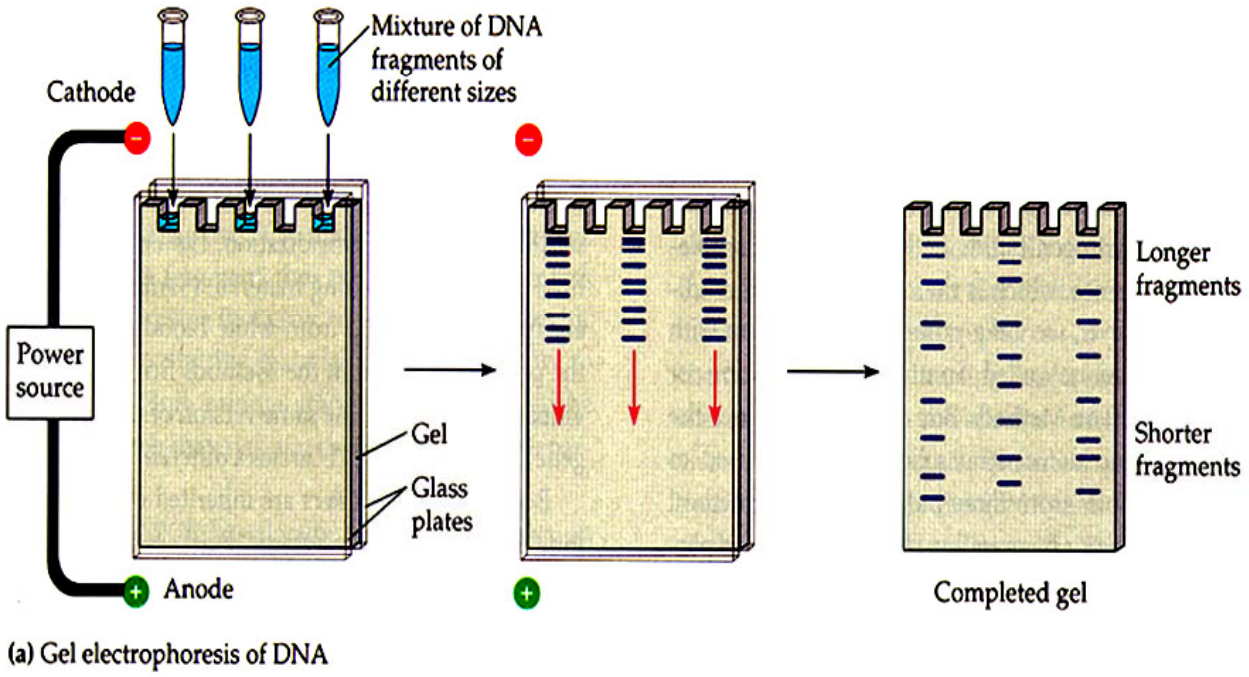
Gel Polímero de agarosa

Polímero de acrilamida o poliacrilamida

$$V = q E / f$$

V= velocidad de migración
E= gradiente de potencial eléctrico (Volts/cm)
q= carga eléctrica efectiva de la partícula
f= coeficiente de fricción (tamaño y forma)

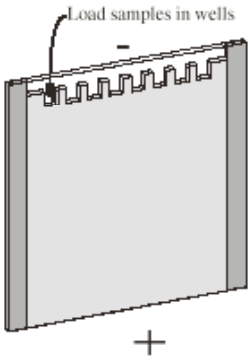
Gel electrophoresis of DNA



(a) Gel electrophoresis of DNA

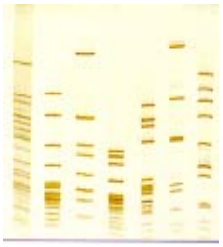
Electroforesis en gel de DNA

Gel de poliacrilamida

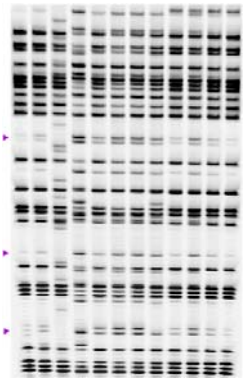


Electroforesis vertical

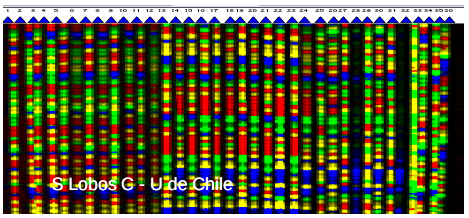
Tinción con nitrato de plata



Marcaje radiactivo



Marcaje fluorescente

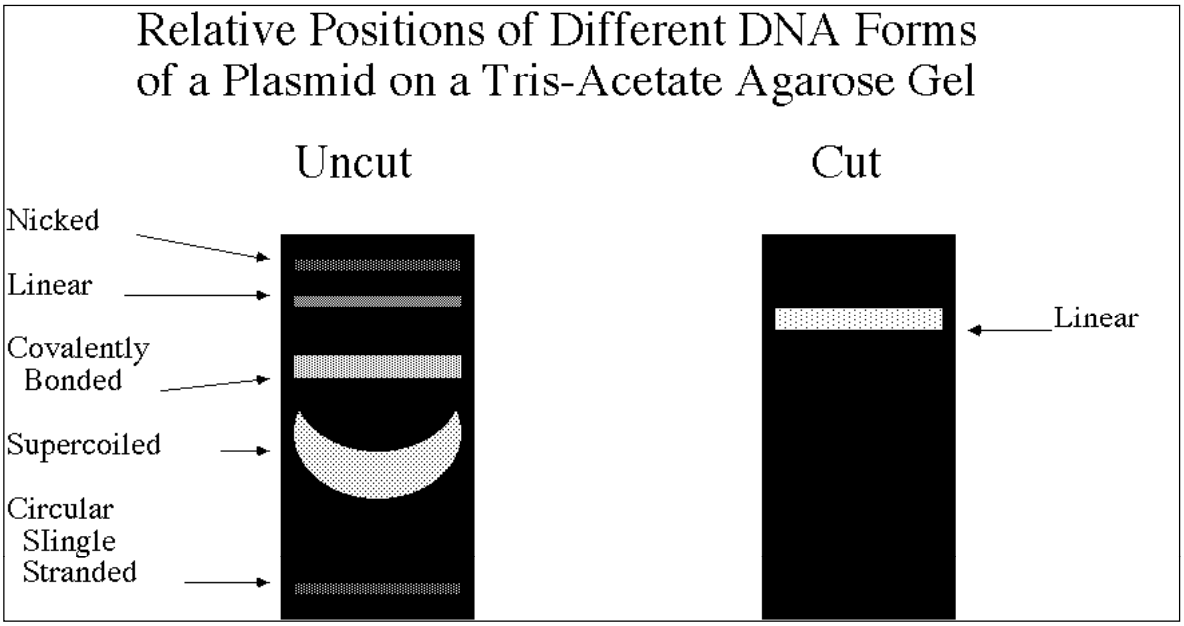


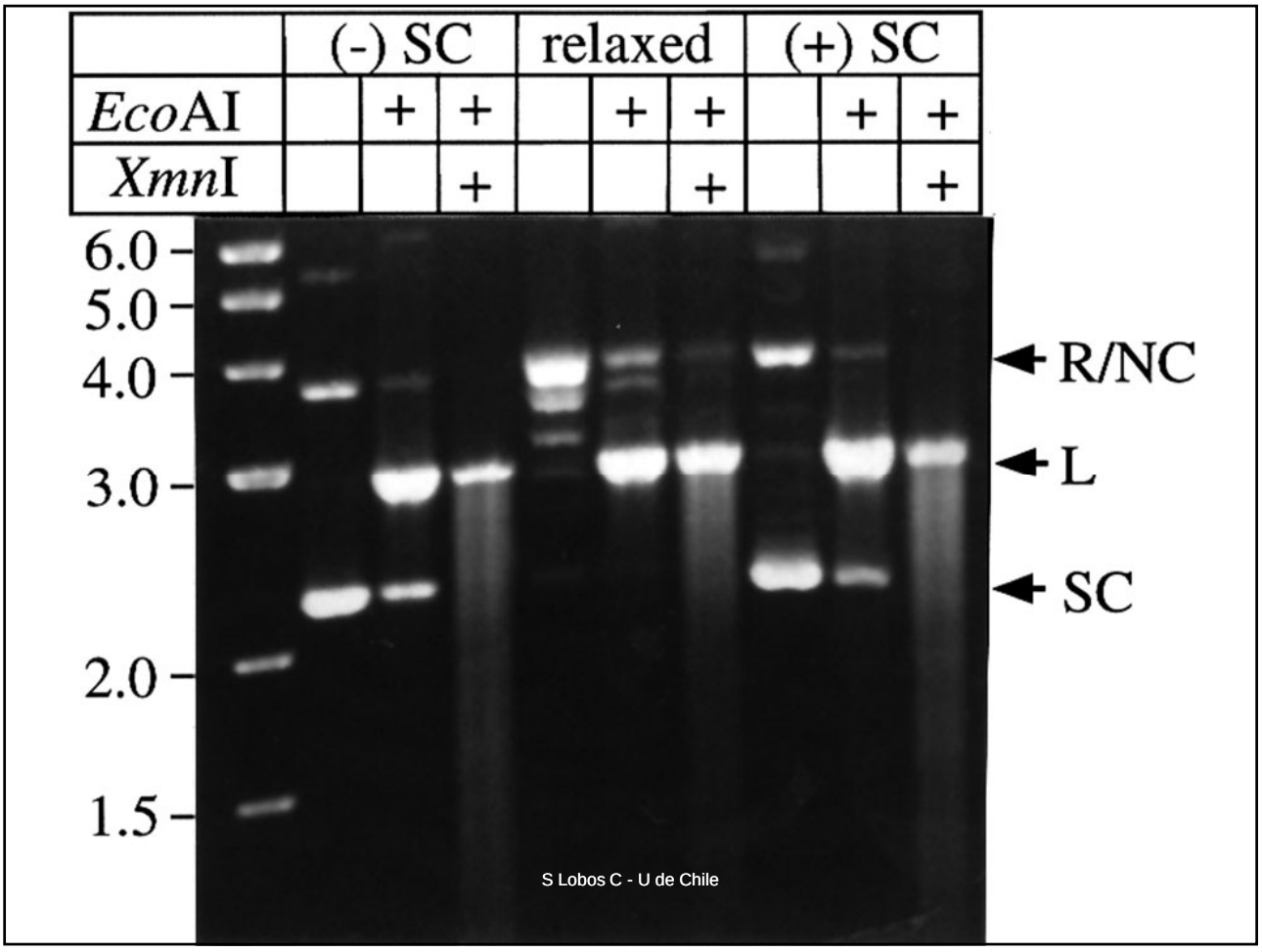
Aspectos que afectan la migración del ADN

- Tamaño del DNA
- Tamaño del poro (concentración del gel)
- Voltaje aplicado
- Conformación del DNA

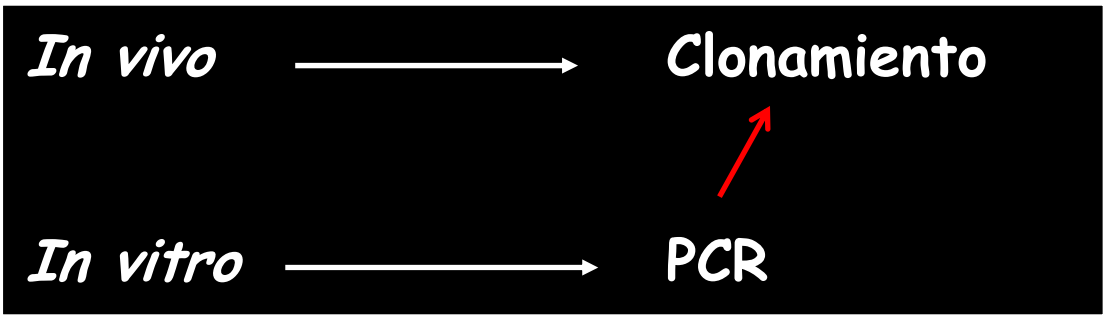
$$V = q E / f$$

Aspectos que afectan la migración del ADN




(-) SC
relaxed
(+) SC
EcoAI
XmnI
+
6.0
5.0
4.0
3.0
2.0
1.5
R/NC
L
SC
S Lobos C - U de Chile

Amplificación de DNA



Clonamiento

Amplificación *IN VIVO* de secuencias determinadas de DNA basada en el uso de:

- -Vectores
- -Ligasa
- -Células vivas
- -etc

Tipos de vectores de clonamiento

- **Plasmidio** - Molécula de DNA circular extracromosómico que se replica de modo autónomo dentro de una célula bacteriana
Límite de clonación: 100 a 10,000 pb ó 0.1-10 kb.

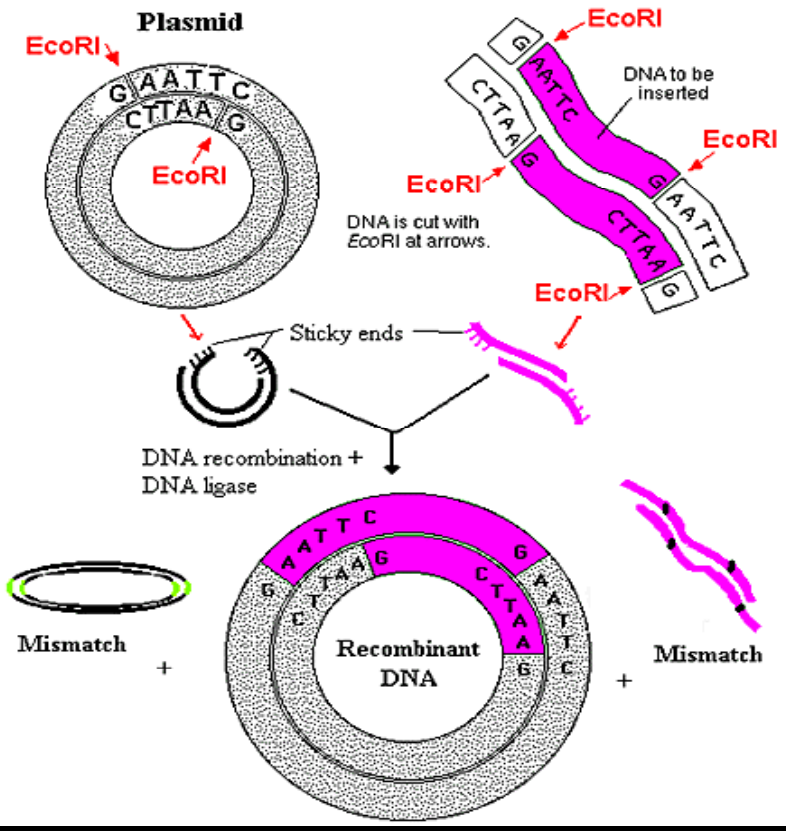
- **Fago** - Derivado del bacteriófago lambda; moléculas de DNA lineal, su DNA puede ser reemplazado por DNA extraño sin alterar el ciclo celular . Límite de clonación: 8-20 kb.

- **Cosmidio** - Molécula de DNA circular extracromosómica que combina aspectos de plasmidios y fagos; Límite de clonación: 35-50 kb.

Tipos de vectores de clonamiento

- **Bacterial Artificial Chromosomes (BAC)** - Basados en plasmidios mini -F bacterianos. Límite de clonación: 75-300 kb.

- **Yeast Artificial Chromosomes (YAC)** - Cromosoma artificial que contiene telómeros, origen de replicación, un centrómero de levadura y un marcador para su identificación en células de levadura. Límite de clonación: 100-1000 kb.



Introducción de un fragmento de DNA en un plasmidio

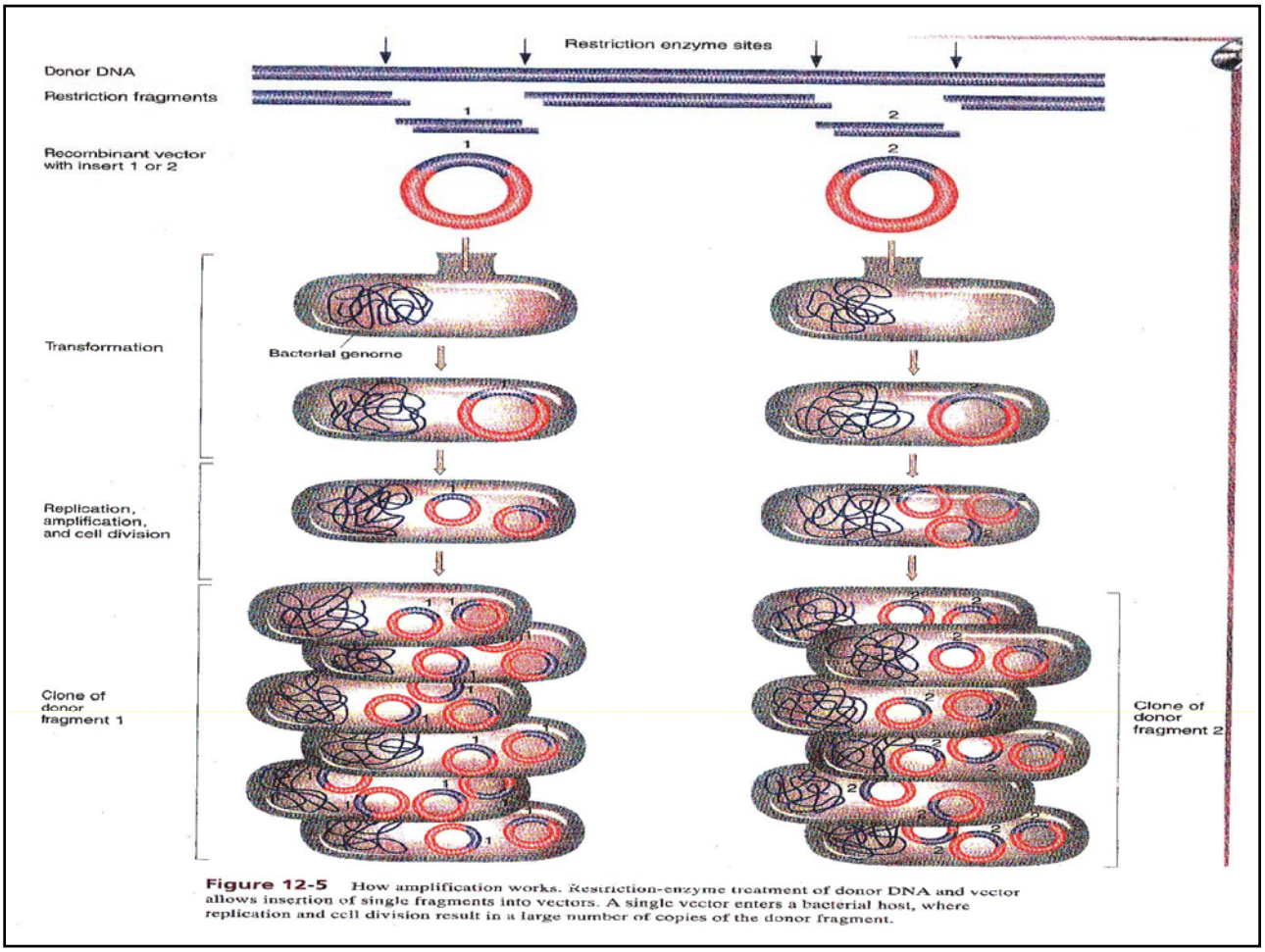


Figure 12-5 How amplification works. Restriction-enzyme treatment of donor DNA and vector allows insertion of single fragments into vectors. A single vector enters a bacterial host, where replication and cell division result in a large number of copies of the donor fragment.

TABLE 14-15
Plasmids[a]

Plasmid	Mass, Mdal	No. copies/ chromosome	Self-transmissible	Other phenotypic features[b]
Colicinogenic factors				
ColE1	4.2	10–15	no	colicin E1 (membrane changes)
ColE2 (*Shigella*)	5.0	10–15	no	colicin E2 (DNase)
ColE3	5.0	10–15	no	colicin E3 (ribosomal RNase)
ColV2	94	1–2	yes	colicin V2; F pilus
ColIb (*Salmonella*)	62	1–2	yes	colicin Ib; I pilus
Fertility factors				
F	62	1–2	yes	F pilus
Flac	95	1–2	yes	F pilus; *lac* operon
ColV2 (see above)				
Resistance factors				
R100	70	1–2	yes	Cm^R, Sm^R, Su^R, Tc^R
R64	78	(limited)	yes	Tc^R, Sm^R
R6K	25	12	yes	Ap^R, Sm^R
R-pSC101	5.8	1–2	no	Tc^R
Defective phages				
λdv	4.2	~50	no	none
λdv gal	6.1	(50)	no	*gal* operon
Recombinants				
pDM500	9.8	~20	no	*Drosophila melanogaster* histone genes
pSY211	10.7	1–2	no	*oriC*, Ap^R
pJS5	2.6	~20	no	*oriC*, *asn* gene product
pBR322[c]	2.9	~20	no	Ap^R, Tc^R, high copy number
pBR345	0.7	~20	no	ColE1-type replication
Unknown				
15T⁻ minicircles	1.4	~20	no	none

[a] *DNA Insertion Elements, Plasmids and Episomes* (Bukhari, A. I., Shapiro, J. A., and Adhya, S. L., eds.) *CSHL*, 1977.
[b] Ap, ampicillin; Cm, chloramphenicol; R, resistance; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; *oriC*, origin of *E. coli* chromosome; *asn*, asparagine.
[c] Useful plasmid for genetic engineering. See Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heymeker, H. L., and Boyer, H. W. (1977) *Gene* 2, 95; Sutcliffe, J. G. (1978) *N.A. Res.* 5, 2721.

Los vectores plasmidiales: Un poco de historia

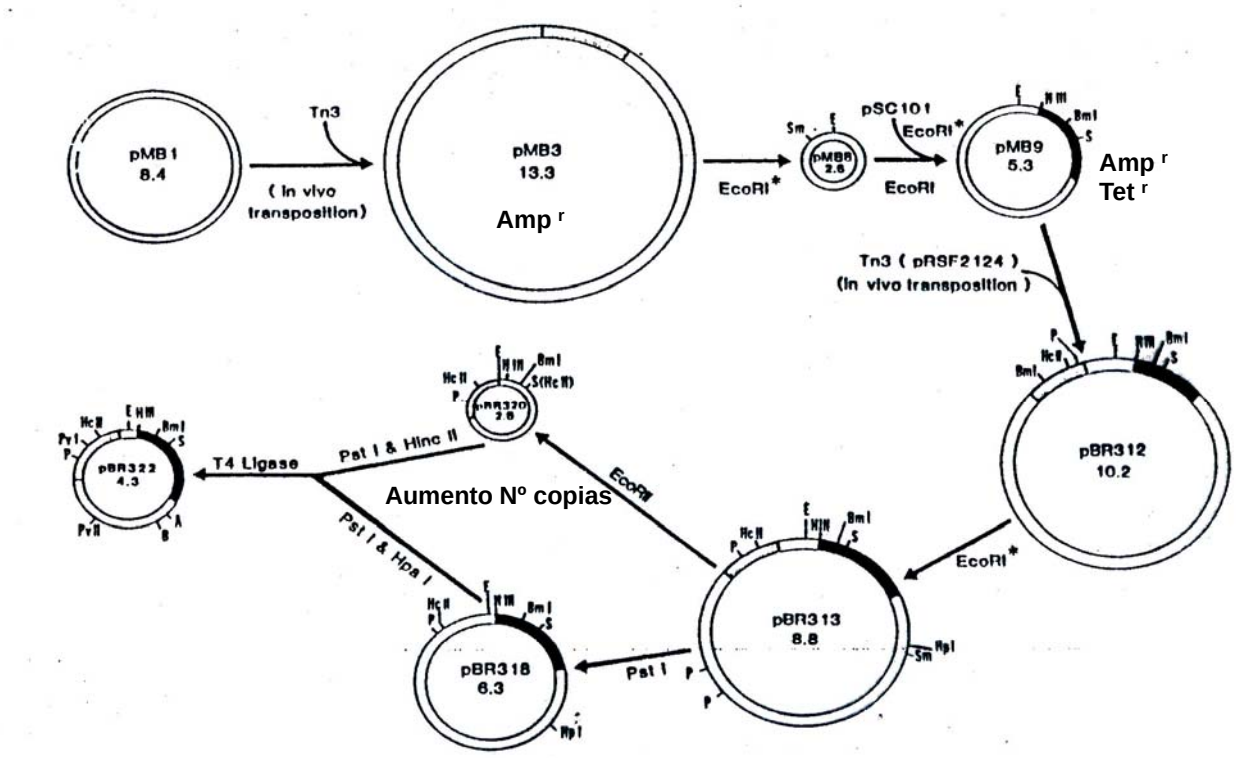


Figure 1.4a
Schematic representation showing the steps in the construction of pBR322 from pMB1, pSC101 and pRSF2124. A, *Aval*; B, *Ball*; Bml, *Bam*HI; E, *Eco*RI; HIII, *Hind*III; HcII, *Hinc*II; HpI, *Hpa*I; P, *Pst*I; PvI, PvuI; PvII, PvuII; S, *Sal*I; Sm, *Sma*I. (Adapted from *Life Sciences* vol. 25, F. Bolivar, "Molecular Cloning Vectors," 1979, Pergamon Press, Ltd.)

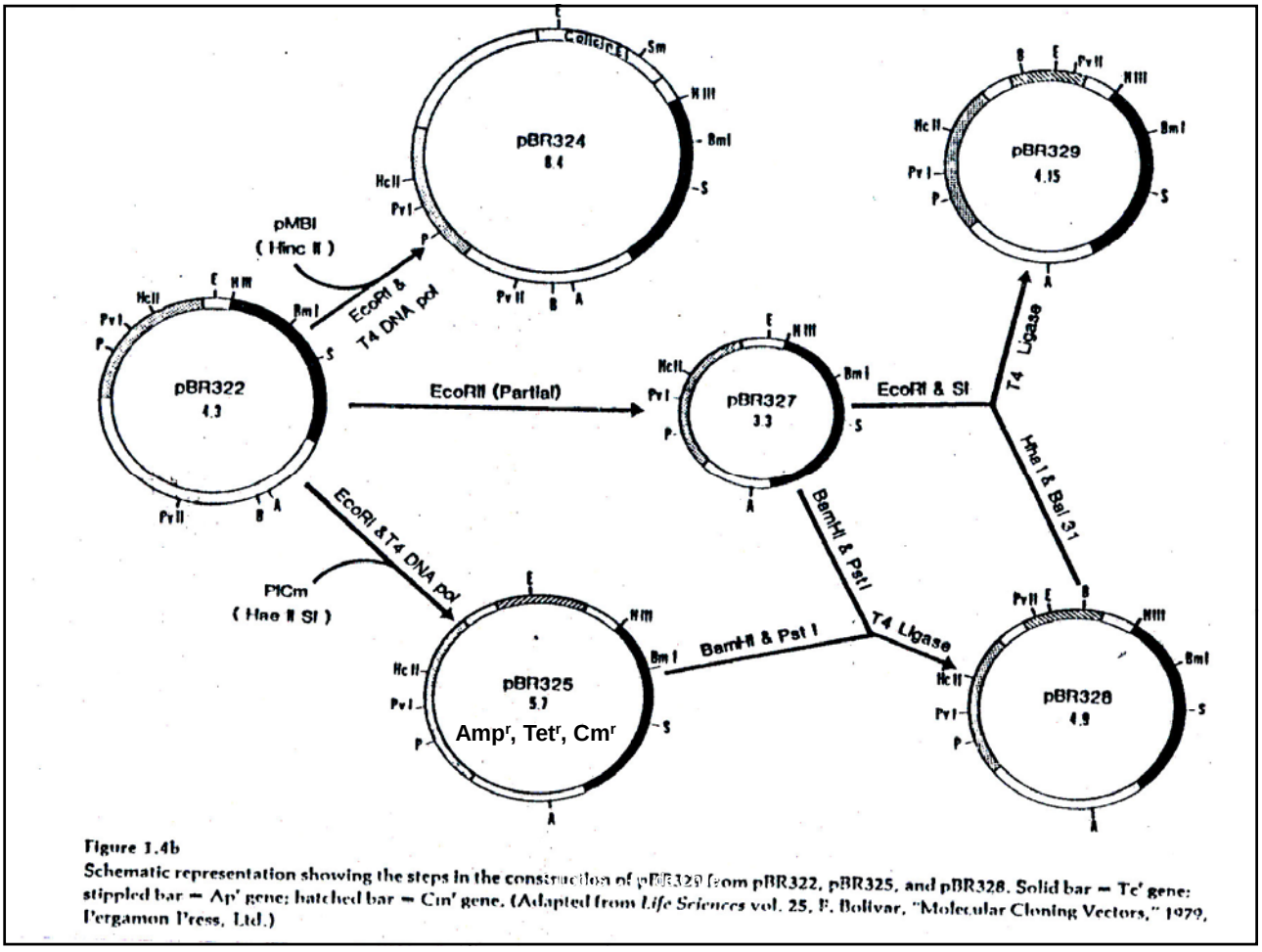
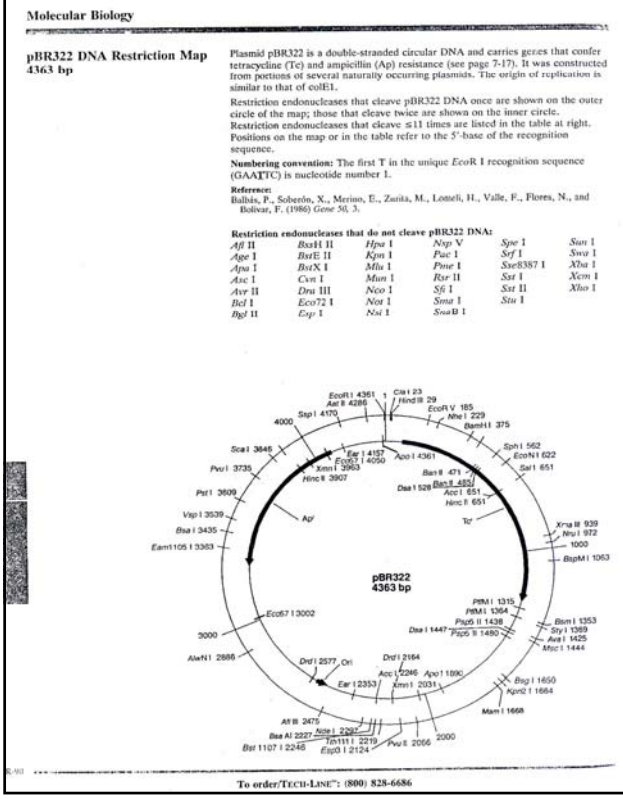


Figure 1.4b
Schematic representation showing the steps in the construction of pBR329 from pBR322, pBR325, and pBR328. Solid bar = Tc' gene; stippled bar = Ap' gene; hatched bar = Cm' gene. (Adapted from *Life Sciences* vol. 25, F. Bolivar, "Molecular Cloning Vectors," 1979, Pergamon Press, Ltd.)

Selección de recombinantes
Inactivación por inserción versus α-complementación



Molecular Biology

pBR322 DNA Restriction Map
4363 bp

Plasmid pBR322 is a double-stranded circular DNA and carries genes that confer tetracycline (Tc) and ampicillin (Ap) resistance (see page 7-17). It was constructed from portions of several naturally occurring plasmids. The origin of replication is similar to that of colE1.

Restriction endonucleases that cleave pBR322 DNA once are shown on the outer circle of the map; those that cleave twice are shown on the inner circle. Restriction endonucleases that cleave ≤11 times are listed in the table at right. Positions on the map or in the table refer to the 5'-base of the recognition sequence.

Numbering convention: The first T in the unique *Eco*R I recognition sequence (GAATTC) is nucleotide number 1.

Reference:
Balbás, P., Soberón, X., Merino, E., Zurita, M., Lomeli, H., Valle, F., Flores, N., and Bolivar, F. (1986) *Gene* 50, 3.

Restriction endonucleases that do not cleave pBR322 DNA:

Afl II	*Bsa*H I	*Hpa* I	*Nsp* V	*Spe* I	*Sun* I
Age I	*Bst*E II	*Kpn* I	*Pac* I	*Srf* I	*Swa* I
Apa I	*Bst*X I	*Mlu* I	*Pme* I	*Sse*8387 I	*Xba* I
Asc I	*Cvn* I	*Mun* I	*Rsr* II	*Sst* I	*Xcm* I
Avr II	*Dra* III	*Nco* I	*Sfi* I	*Sst* II	*Xho* I
Bcl I	*Eco*72 I	*Not* I	*Sma* I	*Stu* I	
Bgl II	*Esp* I	*Nsi* I	*Sna*B I		

pUC18 and
pUC19 DNA Restriction Maps
2686 bp

Plasmids pUC18 and pUC19 are *E. coli* cloning vectors. They contain the *Pvu* II/*Eco*R I fragment of pBR322 which carries the β-lactamase gene (ampicillin resistance, Ap^r) and the origin of replication. A *Hae* III fragment (position 239–681) containing the α-peptide of the *lacZ* (β-galactosidase) gene and a multiple cloning site of one of the M13mp sequencing vectors was combined with the pBR322 fragment to form the original vector from which pUC18 and pUC19 are derived. Thus, as with the M13mp vectors (p. 289), insertion of DNA at the multiple cloning site results in interruption of the α-peptide, producing colorless, rather than blue, colonies on medium containing ampicillin and X-gal or Bluo-gal. Plasmids pUC18 and pUC19 differ only in the orientation of the multiple cloning site: in pUC18 the *Hind* III site is closest to the *lac* promoter; in pUC19 the *Eco*R I site is closest. This permits DNA fragments with two different restriction ends to be "forced" into the multiple cloning site with a specified end adjacent to the promoter.

Restriction endonucleases that cleave pUC18 or pUC19 DNA are shown on the outer circle of the map, and those that cleave twice are shown on the inner circle. All restriction endonucleases cleaving pUC19 DNA ≤20 times are listed in the table at right. The nucleotide positions refer to the 5'- base of the recognition sequence.

Numbering convention: The first T in the sequence TCGCGCGTTTC is nucleotide number 1. This base corresponds to position 2024 in pBR322.

Reference:
Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* 33, 103.

Restriction endonucleases which do not cleave pUC18 or pUC19 DNA:

Afl II	*Bsm* I	*Dra* III	*Nco* I	*Rsr* II	*Sty* I
Apa I	*Bsp*M II	*Eco*47 III	*Nhe* I	*Sfi* I	*Tth*111 I
Asu II	*Bst*I II	*Eco*R V	*Not* I	*Sma*B I	*Xho* I
Acc II	*Bst*E II	*Esp* I	*Nru* I	*Sna* I	*Xma* III
Bcl I	*Bst*X I	*Hpa* I	*Nsi* I	*Spe* I	
Bcl I	*Cla* I	*Mlu* I	*Pfl*M I	*Sst* II	
Bgl II	*Cvn* I	*Nae* I	*Ppu*M I	*Stu* I	

pUC18 multiple cloning site and primer binding region: 371–480

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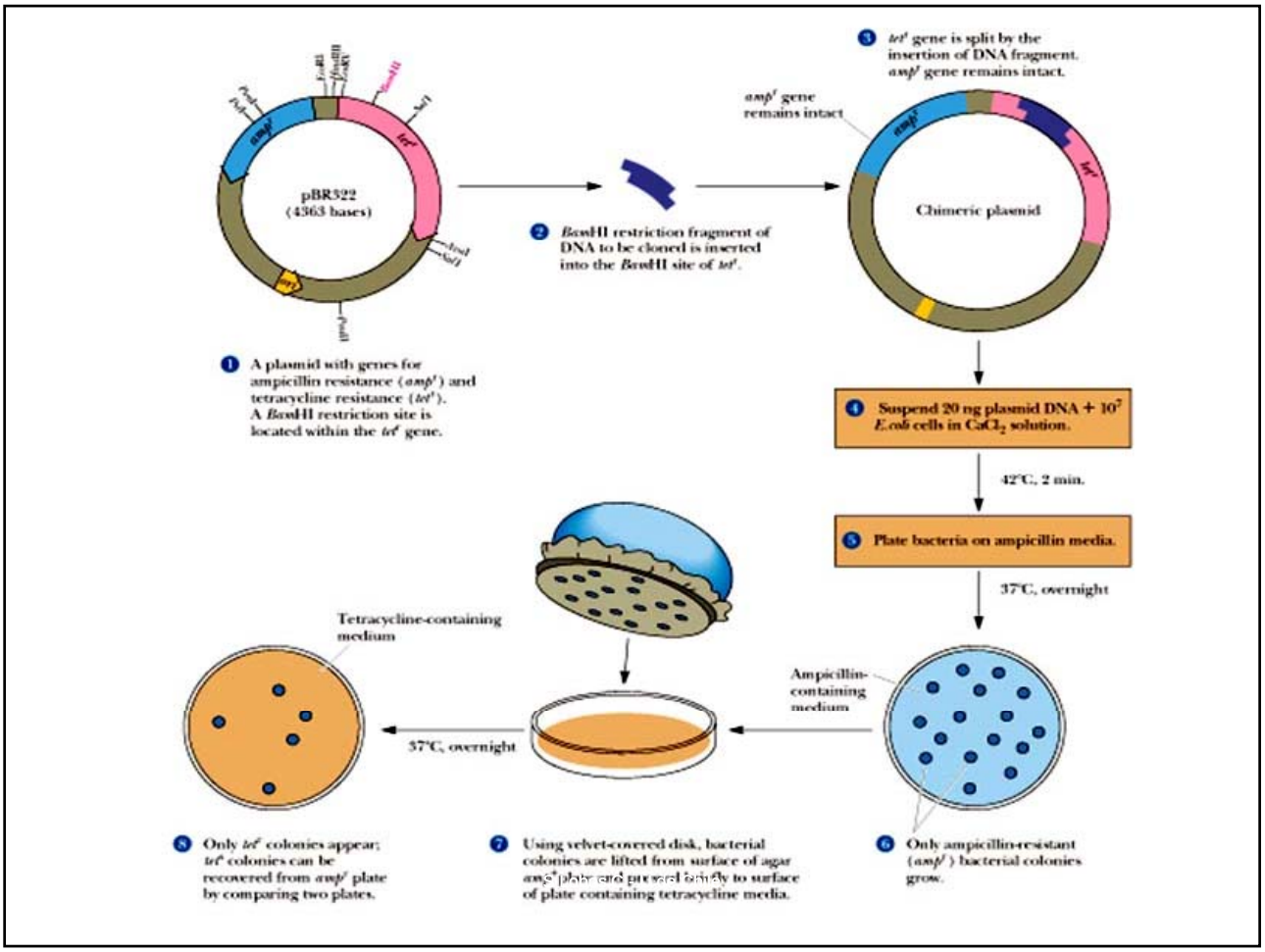

pBR322
(4363 bases)
1 A plasmid with genes for ampicillin resistance (amp^r) and tetracycline resistance (tet^r). A BamHI restriction site is located within the tet^r gene.
2 BamHI restriction fragment of DNA to be cloned is inserted into the BamHI site of tet^r.
3 tet^r gene is split by the insertion of DNA fragment. amp^r gene remains intact.
amp^r gene remains intact
Chimeric plasmid
4 Suspend 20 ng plasmid DNA + 10^7 E.coli cells in CaCl2 solution.
42°C, 2 min.
5 Plate bacteria on ampicillin media.
37°C, overnight
Ampicillin-containing medium
6 Only ampicillin-resistant (amp^r) bacterial colonies grow.
7 Using velvet-covered disk, bacterial colonies are lifted from surface of agar amp^r plate and pressed briefly to surface of plate containing tetracycline media.
37°C, overnight
Tetracycline-containing medium
8 Only tet^r colonies appear; tet^s colonies can be recovered from amp^r plate by comparing two plates.

pUC18 and pUC19 DNA Restriction Maps 2686 bp

Plasmids pUC18 and pUC19 are *E. coli* cloning vectors. They contain the *Pvu* II/*Eco*R I fragment of pBR322 which carries the β-lactamase gene (ampicillin resistance, Ap^r) and the origin of replication. A *Hae* II fragment (position 239-681) containing the α-peptide of the *lacZ* (β-galactosidase) gene and a multiple cloning site of one of the M13mp sequencing vectors was combined with the pBR322 fragment to form the original vector from which pUC18 and pUC19 are derived. Thus, as with the M13mp vectors (p. 219), insertion of DNA at the multiple cloning site results in interruption of the α-peptide, producing colorless, rather than blue, colonies on medium containing ampicillin and X-gal or Bluo-gal. Plasmids pUC18 and pUC19 differ only in the orientation of the multiple cloning site: in pUC18 the *Hind* III site is closest to the *lac* promoter; in pUC19 the *Eco*R I site is closest. This permits DNA fragments with two different restriction ends to be "forced" into the multiple cloning site with a specified end adjacent to the promoter.

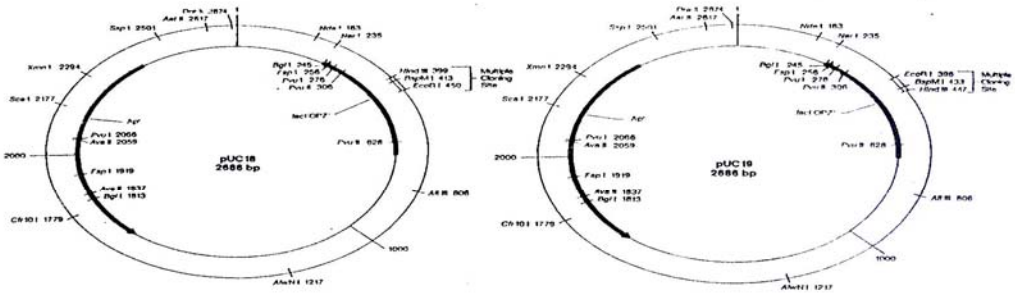
Restriction endonucleases that cleave pUC18 or pUC19 DNA are shown on the outer circle of the map, and those that cleave twice are shown on the inner circle. All restriction endonucleases cleaving pUC19 DNA ≤20 times are listed in the table at right. The nucleotide positions refer to the 5′- base of the recognition sequence.

Numbering convention: The first T in the sequence TCGCGCGTTTC is nucleotide number 1. This base corresponds to position 2074 in pBR322.

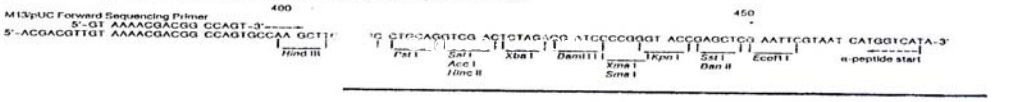
Reference:
Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene 33*, 103.

Restriction endonucleases which do not cleave pUC18 or pUC19 DNA:

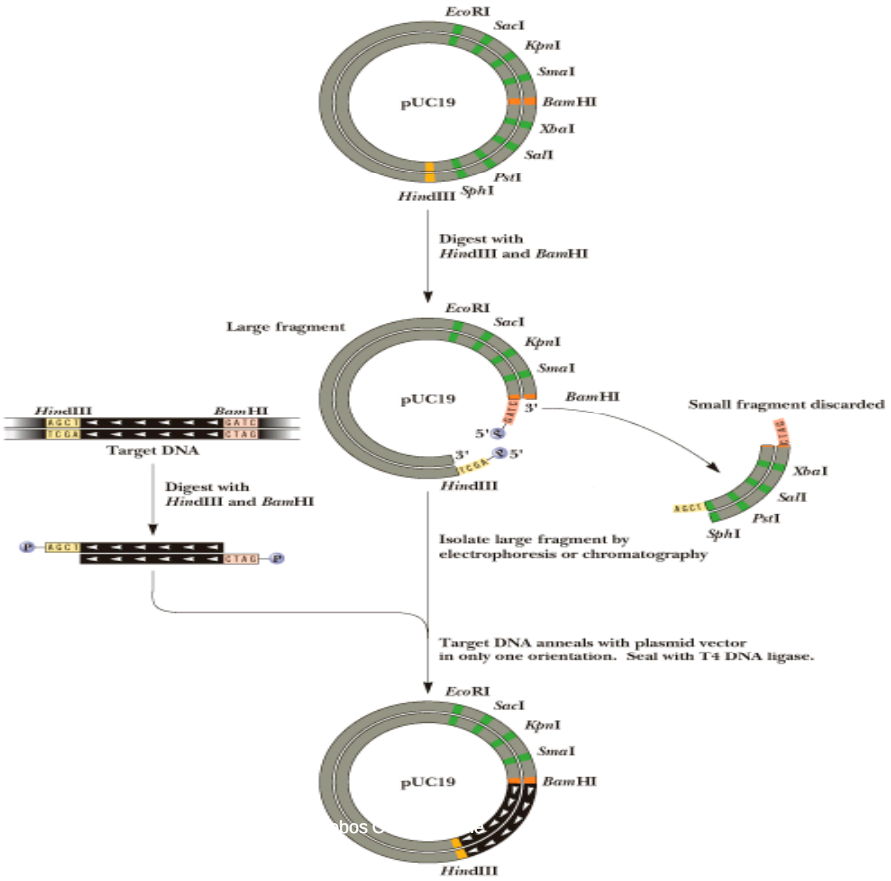
Aff II	*Bsm* I	*Dra* III	*Nco* I	*Rsr* II	*Sty* I
Apa I	*Bsp*M II	*Eco*47 III	*Nhe* I	*Sfi* I	*Tth*111 I
Asu II	*Bss*H II	*Eco*R V	*Not* I	*Sna*B I	*Xho* I
Avr II	*Bst*E II	*Esp* I	*Nru* I	*Spe* I	*Xma* III
Bal I	*Bst*X I	*Hpa* I	*Nsi* I	*Spl* I	
Bcl I	*Cla* I	*Mlu* I	*Pfl*M I	*Sst* II	
Bgl II	*Con* I	*Nae* I	*Ppu*M I	*Stu* I	



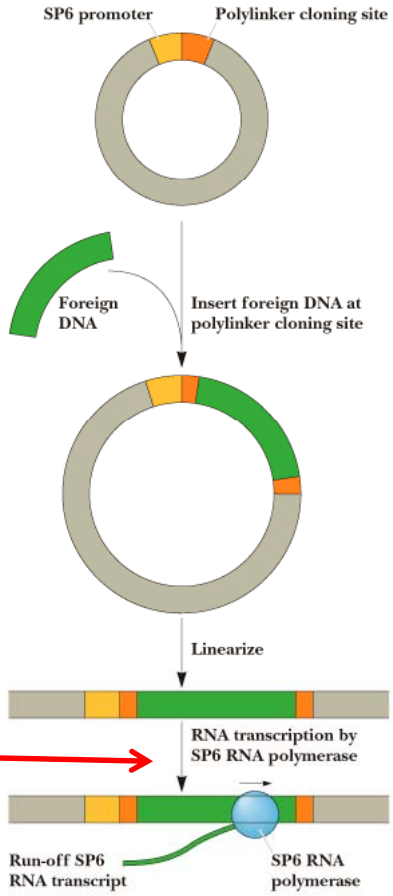
pUC18 multiple cloning site and primer binding region: 371-480



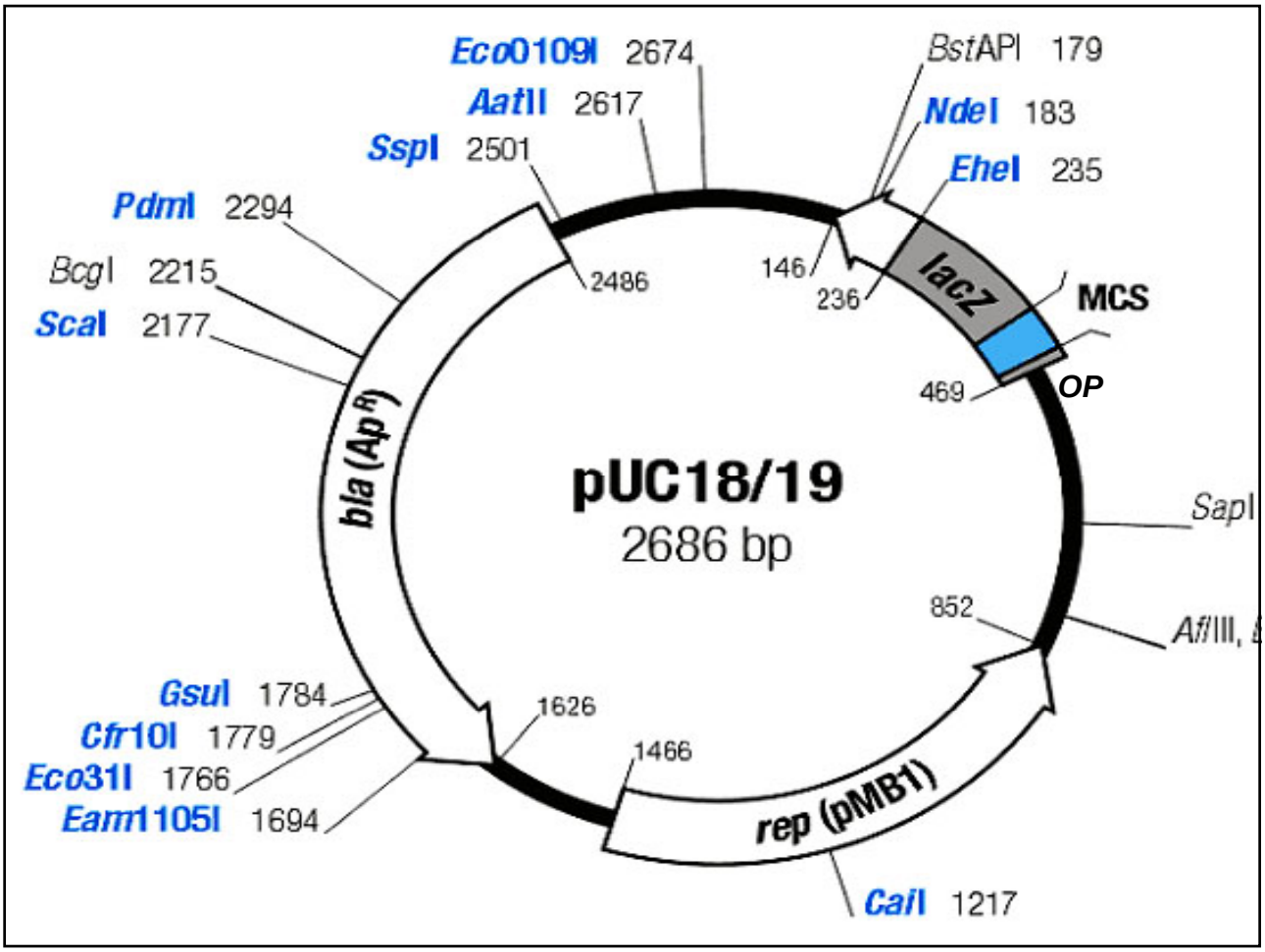
Clonamiento por inserción direccional



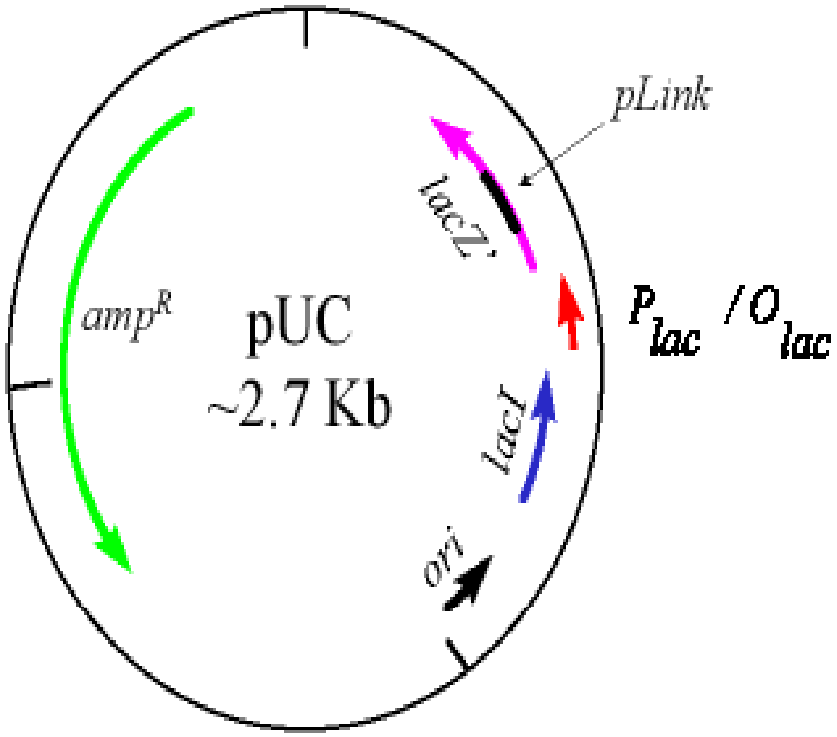
Transcripción "*in vitro*"



Salmonella typhimurium SP6 phage


Eco0109I 2674
AatII 2617
SspI 2501
PdmI 2294
BcgI 2215
ScaI 2177
BstAPI 179
NdeI 183
EheI 235
2486
146
236
lacZ
MCS
469
OP
bla (Ap^R)
pUC18/19
2686 bp
SapI
852
AflIII,
GsuI 1784
Cfr10I 1779
Eco31I 1766
Eam1105I 1694
1626
1466
rep (pMB1)
CaiI 1217

Serie de vectores plasmidiales pUC. Selección de recombinantes por α-complementación

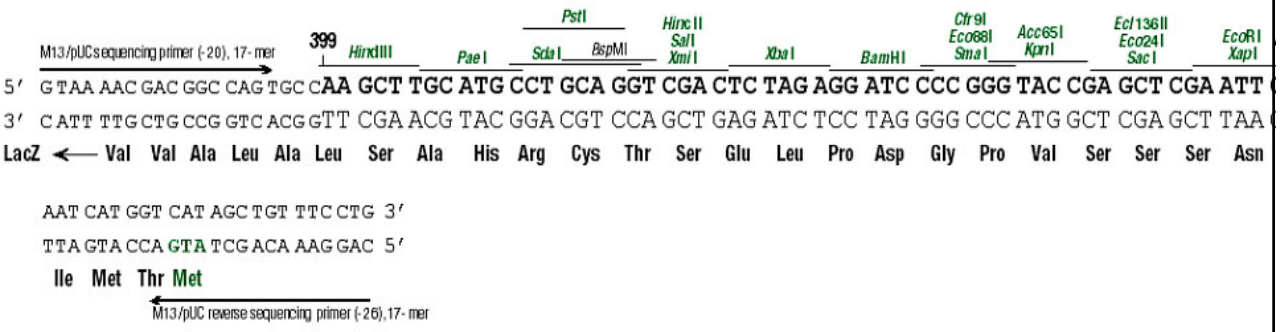


Carece del gen *rop y tiene una mutación* t^s *en el RNA II. A 37ºC puede llegar a 70 copias por célula y a 42ºC más de 200 copias por célula.*

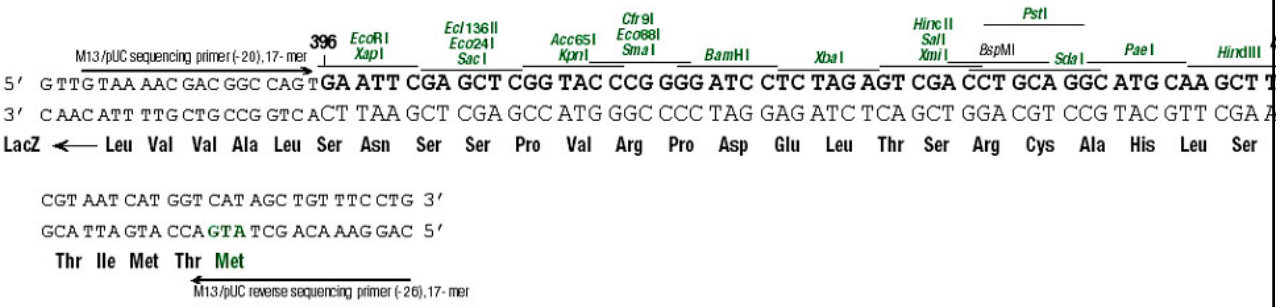
Posee los primeros 58 aa de la β-galactosidasa (péptido alfa o α).

Dentro del péptido α posee un sitio de múltiple clonamiento (MCS) en perfecto marco de lectura con el péptido α.

Polylinker o Multiple Cloning Site (MCS) – pUC18

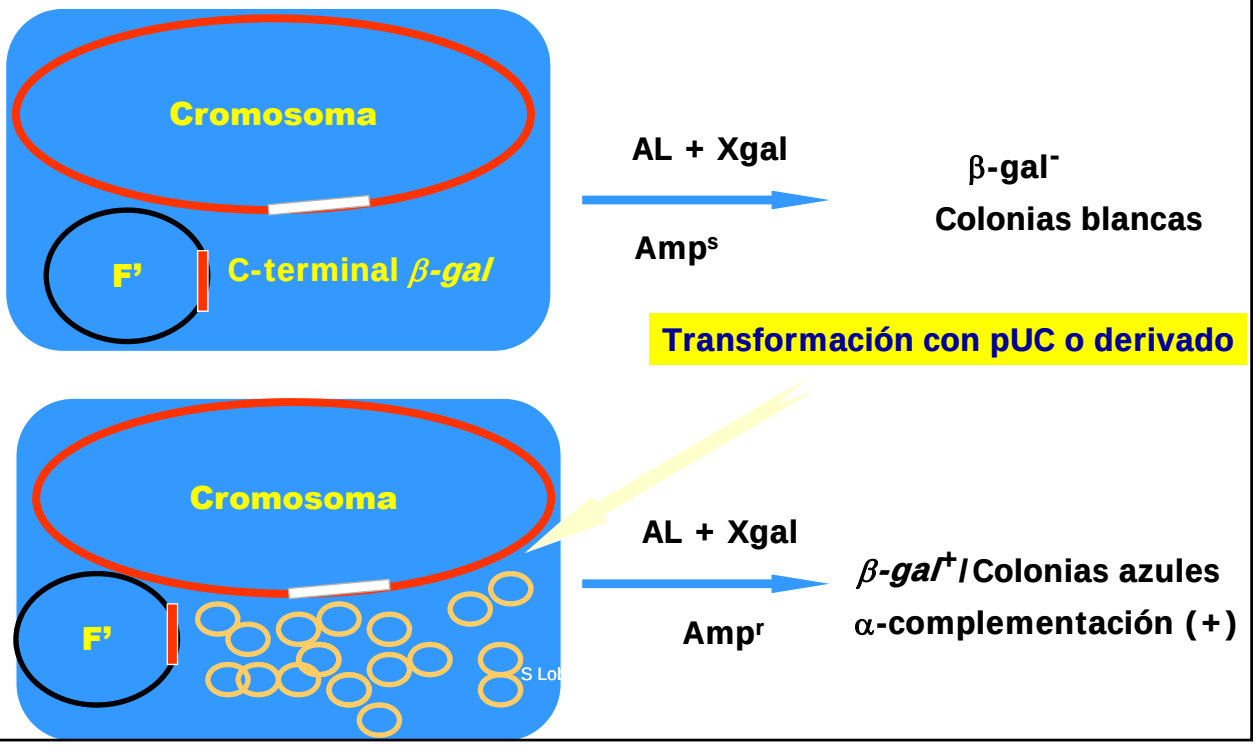


Polylinker o Multiple Cloning Site (MCS) – pUC19



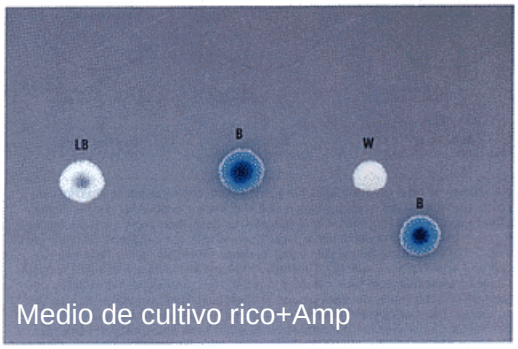
α - complementación es una complementación Intra-alélica

E. coli **JM109 (F'traD36 proA+B+ lacI^q ▲(lacZM15/▲(lac-proAB)glnV44 e14 gyrA96 recA1 endA1 thi hsdR17**



Principal ventaja: Screening Azul/Blanco

- **Cuando se inserta un fragmento de DNA en el polylinker el péptido α no se produce.**

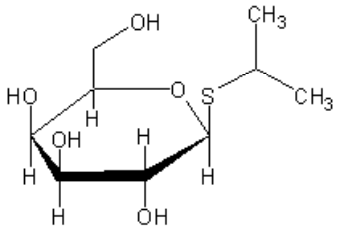


Medio de cultivo rico+Amp

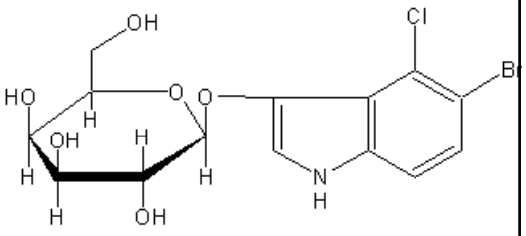
- Por lo tanto colonias bacterianas que poseen el plasmidio con un inserto de DNA son blancas.

Principal ventaja: Screening Azul/Blanco

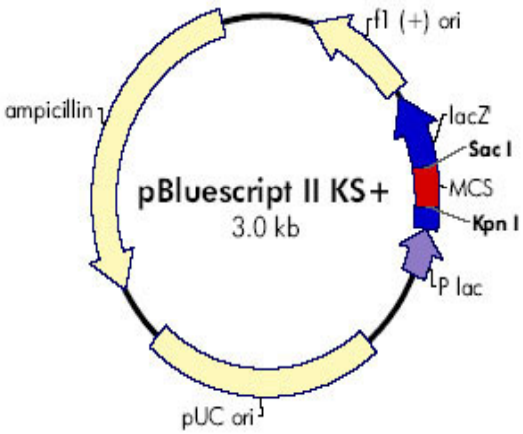
- **Inductor: ISOPROPYL-ß-D-THIOGALACTOPIRANOSIDO (IPTG)**



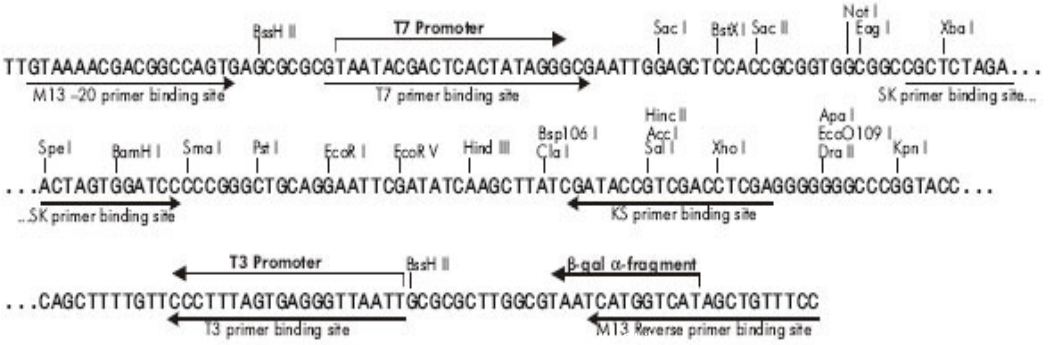
- **Indicador colorimétrico: 5-bromo-4-chloro-3-indoxyl-beta-D-galactopiranosido (X-gal), colonies turn blue**



f1 (+) origin 135–441
β-galactosidase α-fragment 460–816
multiple cloning site 653–760
***lac* promoter** 817–938
pUC origin 1158–1825
ampicillin resistance (*bla*) ORF 1976–2833



pBluescript II KS (+/–) Multiple Cloning Site Region
(sequence shown 598–826)



pSP18 and pSP19 DNA Restriction Maps 3004/3012 bp

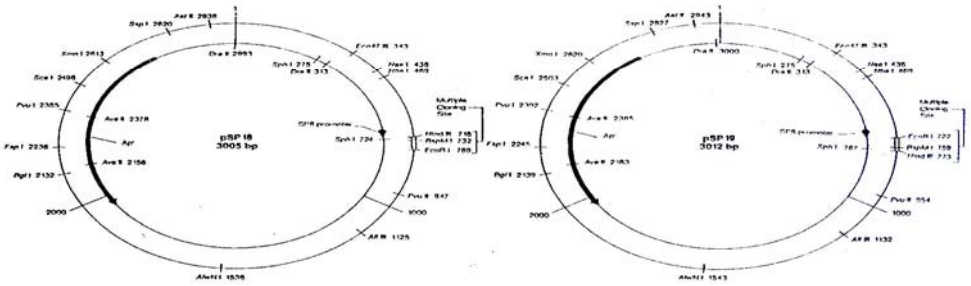
The pSP plasmids are designed for use with SP6 RNA polymerase. They were constructed at Bethesda Research Laboratories from a pUC derivative containing an SP6 promoter and a DNA fragment containing the M13mp18 or mp19 multiple cloning site. DNA inserts in these vectors can be transcribed by SP6 RNA polymerase to produce multiple RNA copies of one strand of the insert. The sequences of the multiple cloning sites with the SP6 promoter are shown below. Listings of the complete sequences of these plasmids are available from BRL Technical Services.

Restriction endonucleases that cleave pSP18 or pSP19 DNA are shown on the outer circle of the map, and those that cleave twice are shown on the inner circle. The nucleotide positions refer to the 5'- base of the recognition sequence.

Numbering convention: The first T in the sequence TCGCGCGTTTC is nucleotide number 1. This base corresponds to position 2074 in pBR322.

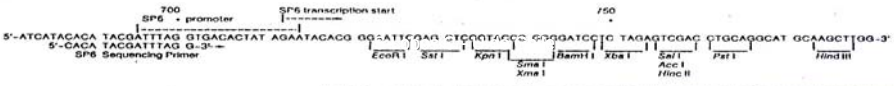
Restriction Endonucleases which do not cleave pSP18 and pSP19 DNA:

Afl II	*Bsm* I	*Dra* III	*Not* I	*Sna*B I	*Xho* I
Apa I	*Bsp*M II	*Eco*R V	*Nru* I	*Spe* I	*Xma* III
Asu II	*Bss*H II	*Esp* I	*Nsi* I	*Spl* I	
Avr II	*Bst*E II	*Hpa* I	*Pfl*M I	*Sst* II	
Bal I	*Bst*X I	*Mlu* I	*Ppu*M I	*Stu* I	
Bcl I	*Cla* I	*Nco* I	*Rsr* II	*Sty* I	
Bgl II	*Cvn* I	*Nde* I	*Sfi* I	*Tth*111 I	

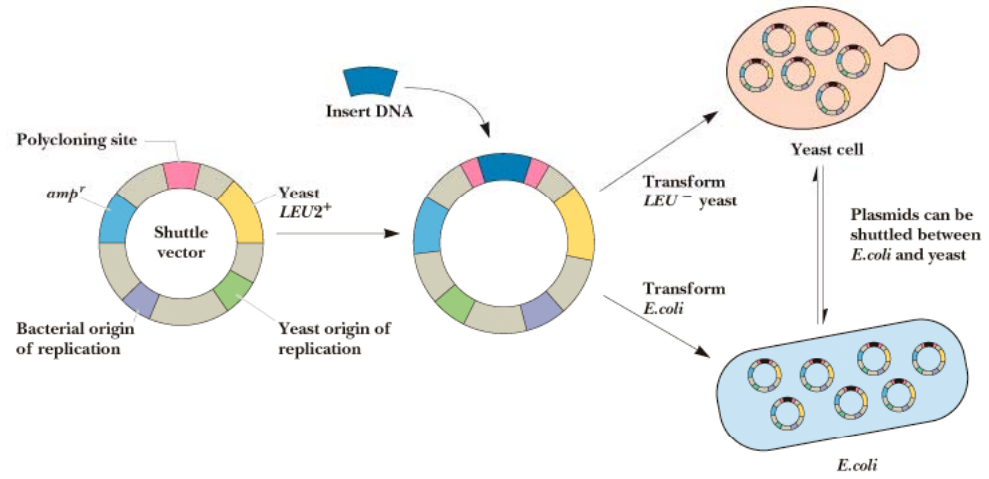


pSP18 promoter, multiple cloning site and primer binding region: 681-780

pSP19 promoter, multiple cloning site and primer binding region: 681-780



Garrett & Grisham: Biochemistry, 2/e
Figure 13.10



Saunders College Publishing

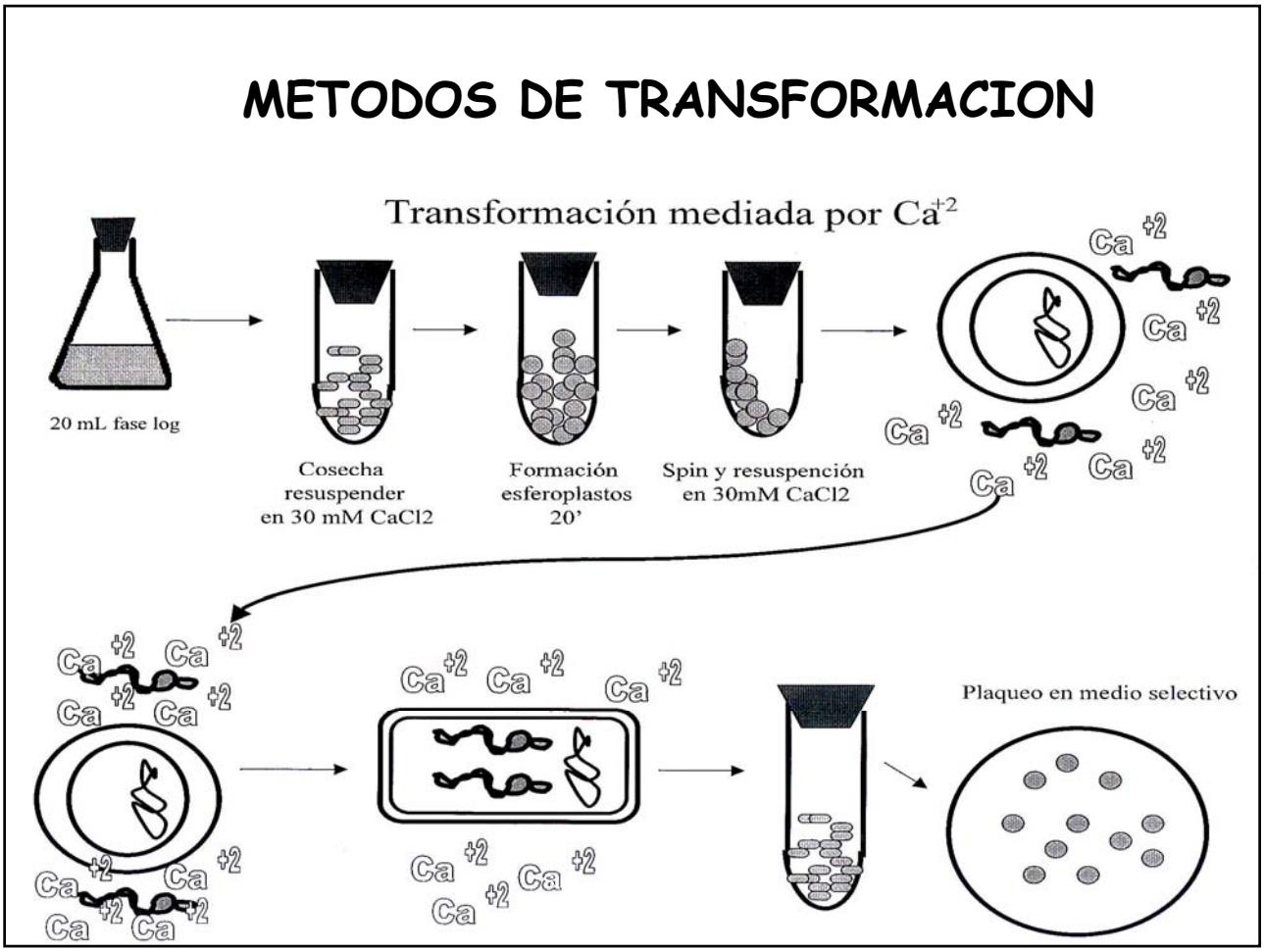
Células bacterianas hospederas

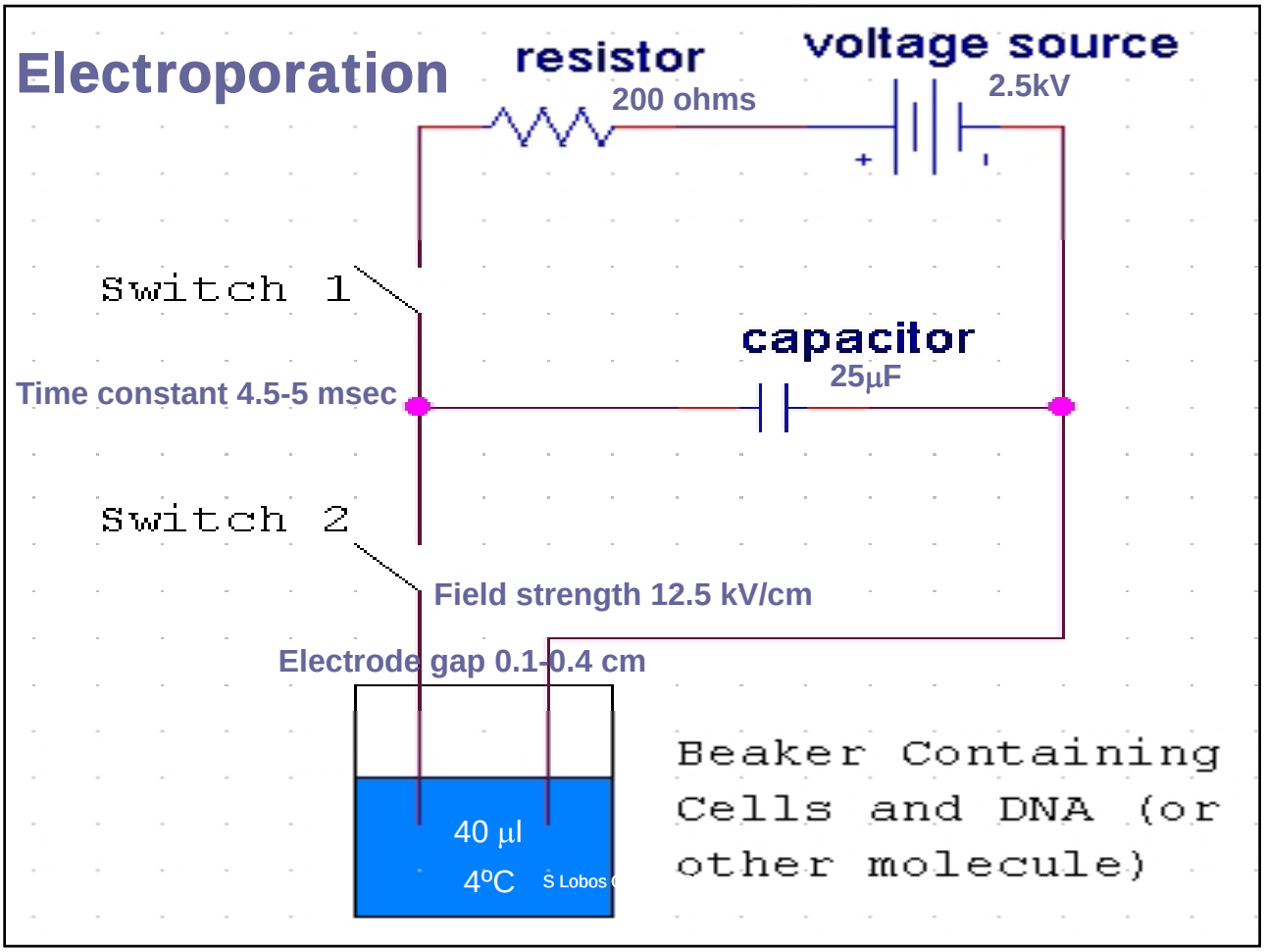
deben cumplir algunos requisitos

System	Description	Effect on cloning experiments
EcoKI, EcoBI	Type I restriction- modification systems are encoded by host *hsdR*, *hsdM* and *hsdS* genes (1). Products of all three genes form a multisubunit enzyme possessing both endonucleolytic and methylation activities (1). The enzyme acts as a methylase when the recognition sequence is hemimethylated, methylating the unmodified strand. When the recognition site is completely unmodified, the enzyme acts as an endonuclease and hydrolyzes DNA. In addition, products of *hsdM* and *hsdS* form a complex capable of modifying specific DNA targets. EcoKI recognizes the sequence 5'...AAC(N)$_6$GTGC...3' (2), *Eco*BI recognizes the sequence 5'...TGA(N)$_8$TGCT...3' (3).	The majority of laboratory strains of *E.coli* are derived from wild-type strains K-12 and B, which possess the EcoKI and EcoBI restriction-modification systems, respectively. *hsdS* mutations abolish both DNA modification and restriction; *hsdR* mutants are modification-proficient but restriction-deficient. If plasmid DNA which contains an EcoKI or EcoBI recognition sequence is isolated from a strain lacking the EcoKI or EcoBI methylation function (i.e. *hsdM* or *hsdS* mutants) and is introduced into an *hsdR*$^+$*M*$^+$*S*$^+$ recipient strain, transformation efficiency may be markedly reduced. Modification of EcoKI or EcoBI DNA targets can result in reduced cleavage by restriction endonucleases whose targets overlap the recognition sites.
Dam	DNA adenine methylase is encoded by the *dam* gene of *E.coli*. It modifies the adenine residue at the N^6-position on both strands within the sequence 5'...GATC (4, 5).	• Dam methylase-modified plasmids with either the *E.coli* chromosomal replication origin or the pMB1 plasmid origin transform *E.coli dam* mutants poorly (6, 7). This effect is suppressed completely if transforming DNA is not methylated (8). Methylated or unmethylated plasmids transform *dam*$^+$ strains efficiently. • DNA isolated from *E.coli dam*$^+$ strain can be difficult to hydrolyze by restriction endonucleases whose targets overlap (partially or completely) the Dam

Dcm	DNA cytosine methylase is encoded by the *dcm* gene of *E.coli*. It modifies the internal cytosine residue at the C^5-position on both strands within the sequence 5'...CC(A/T)GG-3' (9, 10).	DNA isolated from *E.coli dcm+* strain can be difficult to hydrolyze by restriction endonucleases whose targets overlap (partially or completely) the Dcm recognition site, *see*.
McrA	The *E.coli* chromosome-encoded restriction system named McrA (for **M**odified **C**ytosine **R**estriction) is directed against DNA methylated at the sequence 5'...Cm^5CGG, which is its only known target (11).	The $McrA^+$ phenotype of the host can interfere with the cloning of appropriately modified DNA (or genes coding for DNA modification enzymes of either CCGG or overlapping specificities). McrA phenotypes of some laboratory *E.coli* strains are presented in reference 12.
McrBC	McrBC is the other chromosome-encoded *E.coli* restriction system, which, like McrA, is specific for modified cytosine. It is encoded by two neighbor genes, *mcrB* and *mcrC* (13). McrBC requires the presence of two (G/A)mC recognition elements (where mC is 5-hydroxymethylcytosine, N^4-methylcytosine or 5-methylcytosine (13)) appropriately spaced in the substrate DNA (14). DNA cleavage occurs in region between two recognition elements (14). The optimal separation between elements is 55-103 base pairs (14).	The $McrBC^+$ phenotype of the host can interfere with the cloning of appropriately modified DNA or genes coding for DNA modification enzymes of overlapping specificity. McrBC phenotypes of some laboratory *E.coli* strains are presented in reference 12.
Mrr	The *E.coli* chromosome-encoded restriction system named Mrr (for **M**ethylated adenine **r**ecognition and **r**estriction) acts against DNA containing both N^6-methyladenine and 5-methylcytosine (15-17). No simple consensus sequence causing Mrr-associated restriction has been determined.	The precise specificity of Mrr is uncertain, but it requires the presence of either modified adenine of cytosine. Thus, Mrr^+ phenotype of the host can interfere with the cloning of appropriately modified DNA or genes coding for DNA modification enzymes of overlapping specificity.

METODOS DE TRANSFORMACION




Electroporation
resistor
200 ohms
voltage source
2.5kV
+
-
Switch 1
capacitor
25μF
Time constant 4.5-5 msec
Switch 2
Field strength 12.5 kV/cm
Electrode gap 0.1-0.4 cm
40 μl
4ºC
S Lobos
Beaker Containing Cells and DNA (or other molecule)


S Lobos C - U de Chile

Biobalística (bombardeo con partículas)

Gen Gun