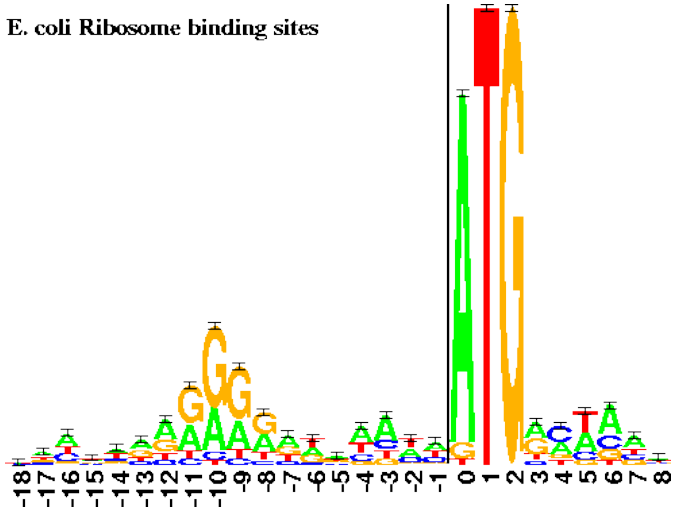
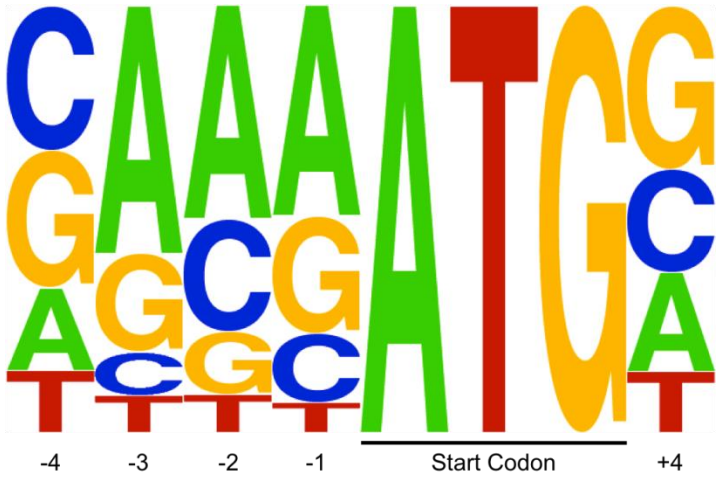
Shine-Dalgarno



Kozak



Orígenes de replicación plásmidos bacterianos

Common Vectors	Copy Number+	ORI	Incompatibility Group	Control
pUC	~500-700	pMB1 (derivative)	A	Relaxed
pBR322	~15-20	pMB1	A	Relaxed
pET	~15-20	pBR322	A	Relaxed
pGEX	~15-20	pBR322	A	Relaxed
pColE1	~15-20	ColE1	A	Relaxed
pR6K	~15-20	R6K*	B	Stringent
pACYC	~10	p15A	B	Relaxed
pSC101	~5	pSC101	C	Stringent
pBluescript	~300-500	ColE1 (derivative) and F1**	A	Relaxed
pGEM	~300-500	pUC and F1**	A	Relaxed

Criterios de elección

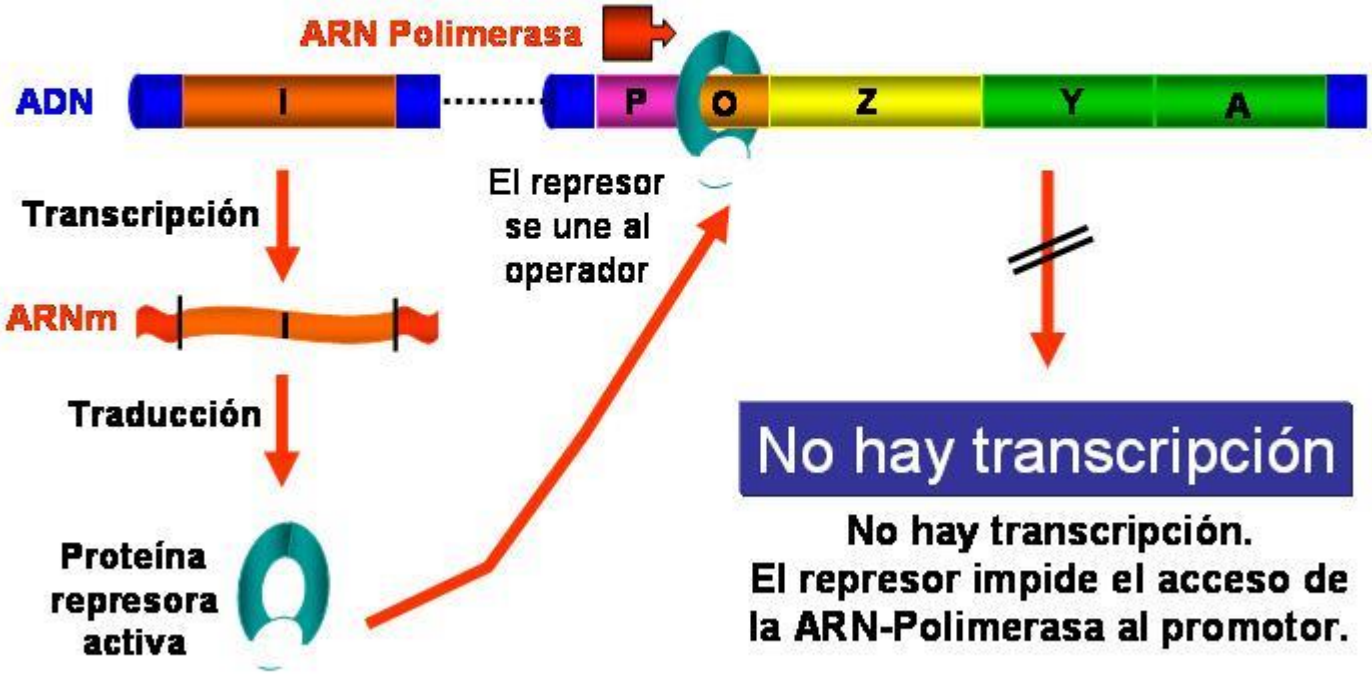
Operón lactosa

- ***El gen z:*** codifica para la ***β-galactosidasa*** que cataliza la hidrolisis de la lactosa en glucosa más galactosa.
- ***El gen y:*** codifica para la ***galactósido permeasa*** que transporta β-galactósidos al interior de la célula bacteriana.
- ***El gen a:*** Este gen no está relacionado con el metabolismo de la lactosa.

El verdadero inductor del sistema es la ***Alolactosa*** y no la lactosa de manera que la ***β-galactosidasa*** transforma la lactosa en ***Alolactosa***. Se utiliza como inductor un análogo sintético de la lactosa que es el ***Isopropil tiogalactósido (IPTG)***. El IPTG no necesita ser transportado por la ***galactósido permeasa*** para entrar en la bacteria.

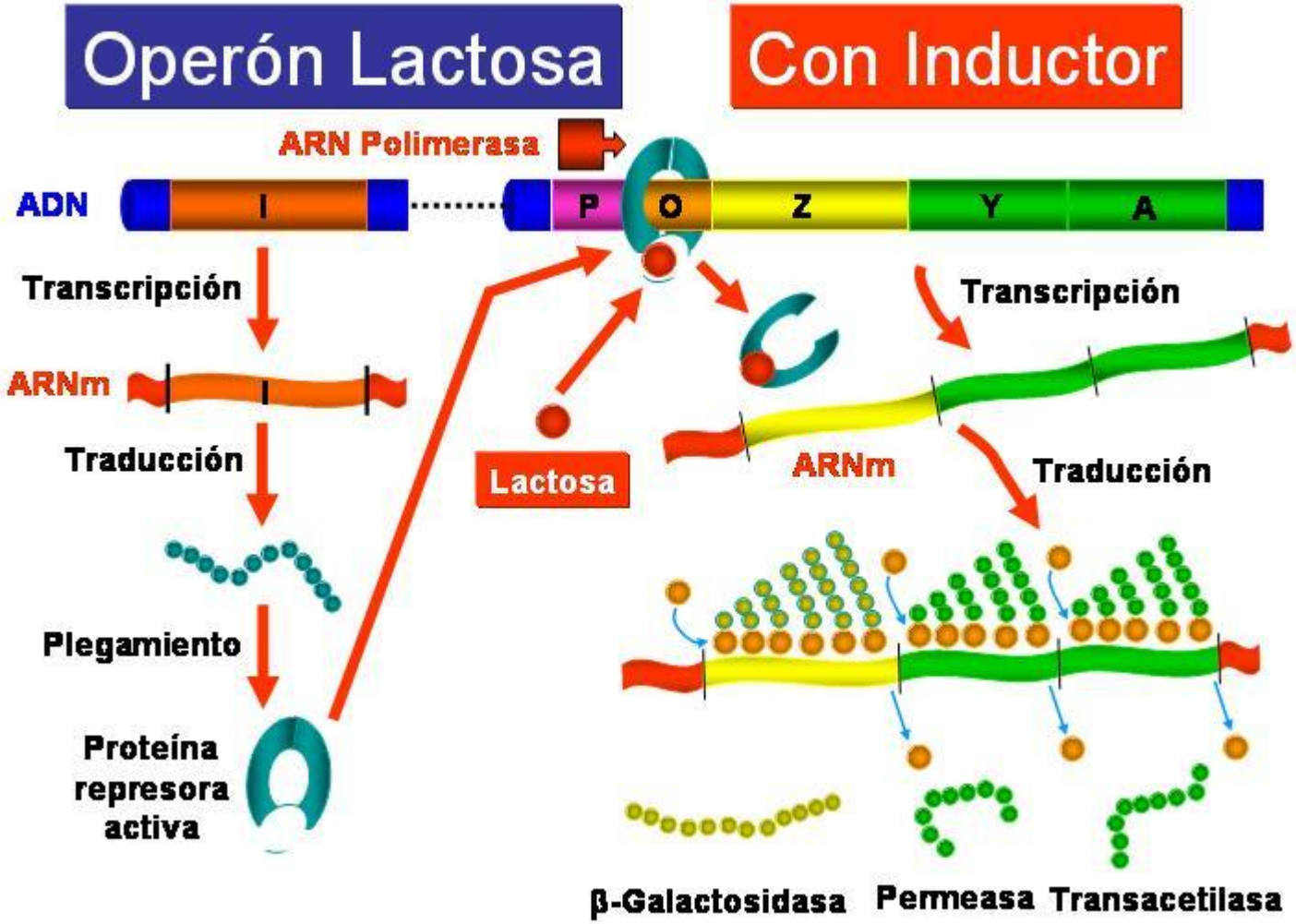
Operón Lactosa

Sin Inductor



No hay transcripción

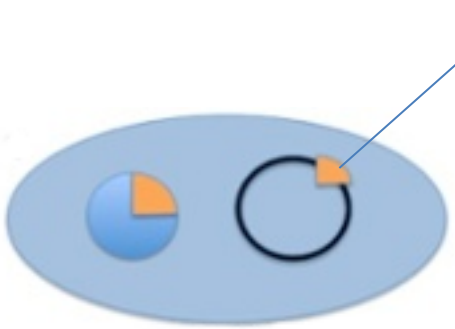
No hay transcripción.
El represor impide el acceso de la ARN-Polimerasa al promotor.


Operón Lactosa
Con Inductor
ARN Polimerasa
ADN
I
P
O
Z
Y
A
Transcripción
ARNm
Traducción
Plegamiento
Proteína represora activa
Lactosa
Transcripción
ARNm
Traducción
β-Galactosidasa
Permeasa
Transacetilasa

α-complementación

LacZ codifica para β-Galactosidasa

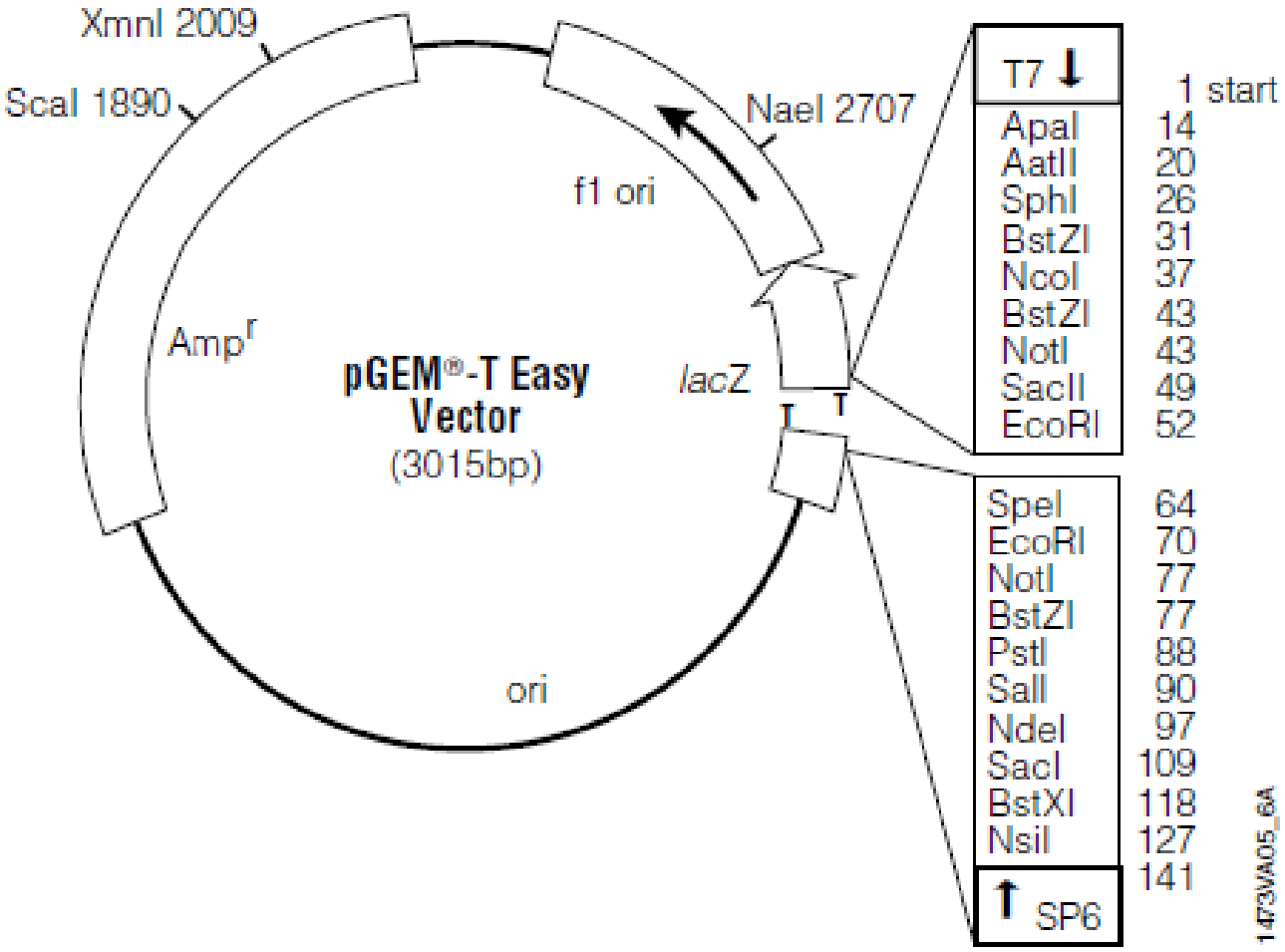
lacZΔM15 β-Galactosidasa inactiva

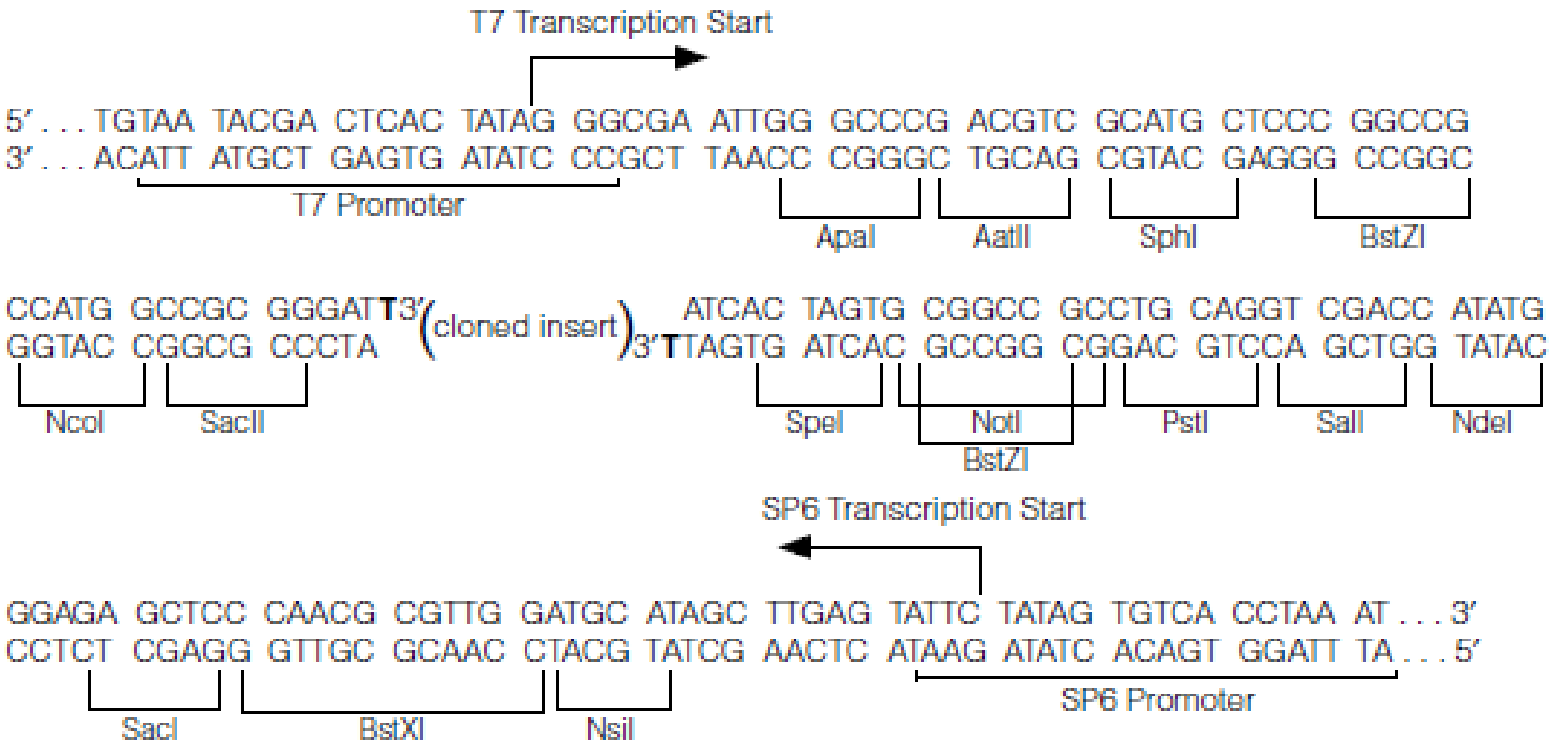


Trans-complementación **Azul**

Blanca

IPTG + x-gal

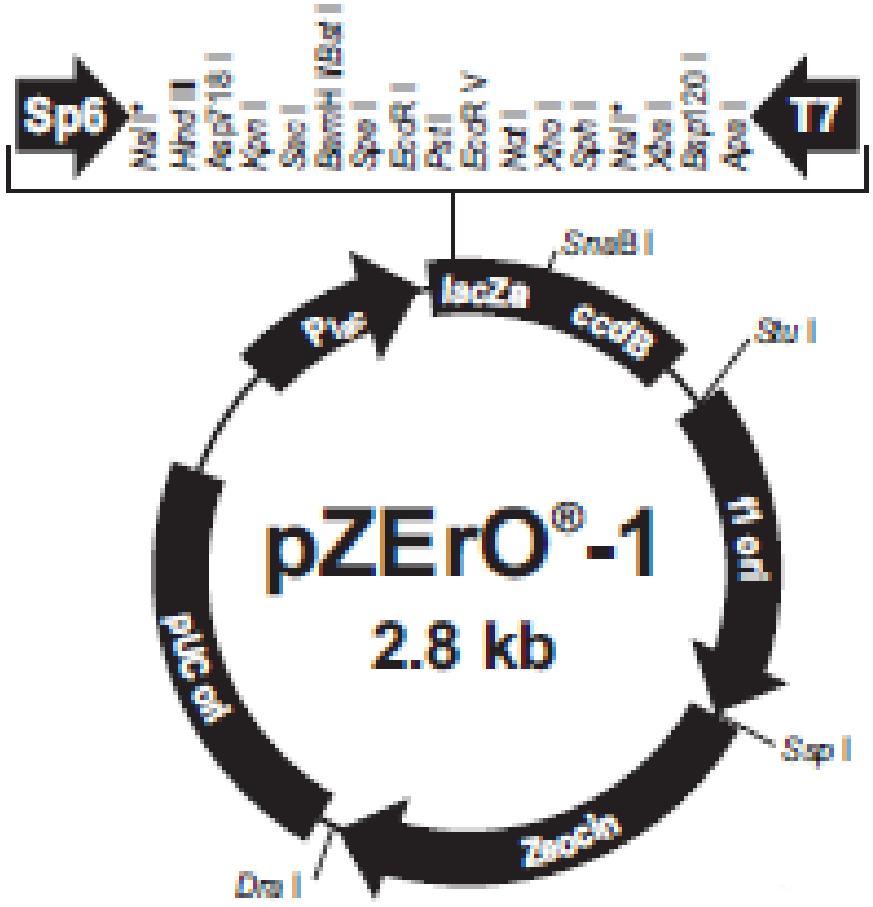

XmnI 2009
ScaI 1890
NaeI 2707
f1 ori
Amp^r
pGEM®-T Easy
Vector
(3015bp)
lacZ
ori
T7
1 start
ApaI 14
AatII 20
SphI 26
BstZI 31
NcoI 37
BstZI 43
NotI 43
SacII 49
EcoRI 52
SpeI 64
EcoRI 70
NotI 77
BstZI 77
PstI 88
SalI 90
NdeI 97
SacI 109
BstXI 118
NsiI 127
141
SP6
1473VA05_6A


T7 Transcription Start
5′ . . . TGTAA TACGA CTCAC TATAG GGCGA ATTGG GCCCG ACGTC GCATG CTCCC GGCCG
3′ . . . ACATT ATGCT GAGTG ATATC CCGCT TAACC CGGGC TGCAG CGTAC GAGGG CCGGC
T7 Promoter
ApaI
AatII
SphI
BstZI
CCATG GCCGC GGGATT3′
GGTAC CGGCG CCCTA
(cloned insert)
3′TTAGTG ATCAC GCCGG CGGAC GTCCA GCTGG TATAC
ATCAC TAGTG CGGCC GCCTG CAGGT CGACC ATATG
NcoI
SacII
SpeI
NotI
BstZI
PstI
SalI
NdeI
SP6 Transcription Start
GGAGA GCTCC CAACG CGTTG GATGC ATAGC TTGAG TATTC TATAG TGTCA CCTAA AT . . . 3′
CCTCT CGAGG GTTGC GCAAC CTACG TATCG AACTC ATAAG ATATC ACAGT GGATT TA . . . 5′
SacI
BstXI
NsiI
SP6 Promoter

ccdB gene

ccd system (control of cell death)

ccdA: antídoto



Genotipos de *E. coli*

TABLE 1 - Common gene mutations found in *E.coli* strains

Gene(s)	Description	Functional Consequence
dam	DNA Adenine methylase mutation (GATC)	Preparing unmethylated DNA, important when trying to cut with certain restriction enzymes (ex: ClaI or XbaI)
dcm	DNA Cytosine methylase mutation (CCWGG)	Preparing unmethylated DNA, important when trying to cut with certain restriction enzymes that are methylation sensitive.
dnaJ	Mutation in a chaperonin gene	Increases the stability of certain expressed proteins
endA, endA1	Endonuclease I (nonspecific cleavage of dsDNA) mutation	Improves plasmid yield
F	Host does (F') or does not (F-) contain the fertility plasmid.	A low copy-number plasmid, encodes proteins needed for bacterial conjugation. Genes listed on F´ are wild-type unless indicated otherwise
fhuA (formerly tonA)	ferric hydroxamate uptake, iron uptake receptor mutation.	T1/T5 Phage resistance
gal	Mutation in galactose metabolism pathway	cells cannot grow on just galactose
gyrA, gyrA96	DNA gyrase mutation	Confers resistance to nalidixic acid
hsdRMS	DNA methylation and degredation system	Used to identify and destroy foreign (unmethylated) DNA
	hsdR(rk-, mk+)	unmethylated DNA not degraded, cell still can methylate DNA
	hsdS(rk-,mk-)	unmethylated DNA not degraded, cell cannot methylate DNA

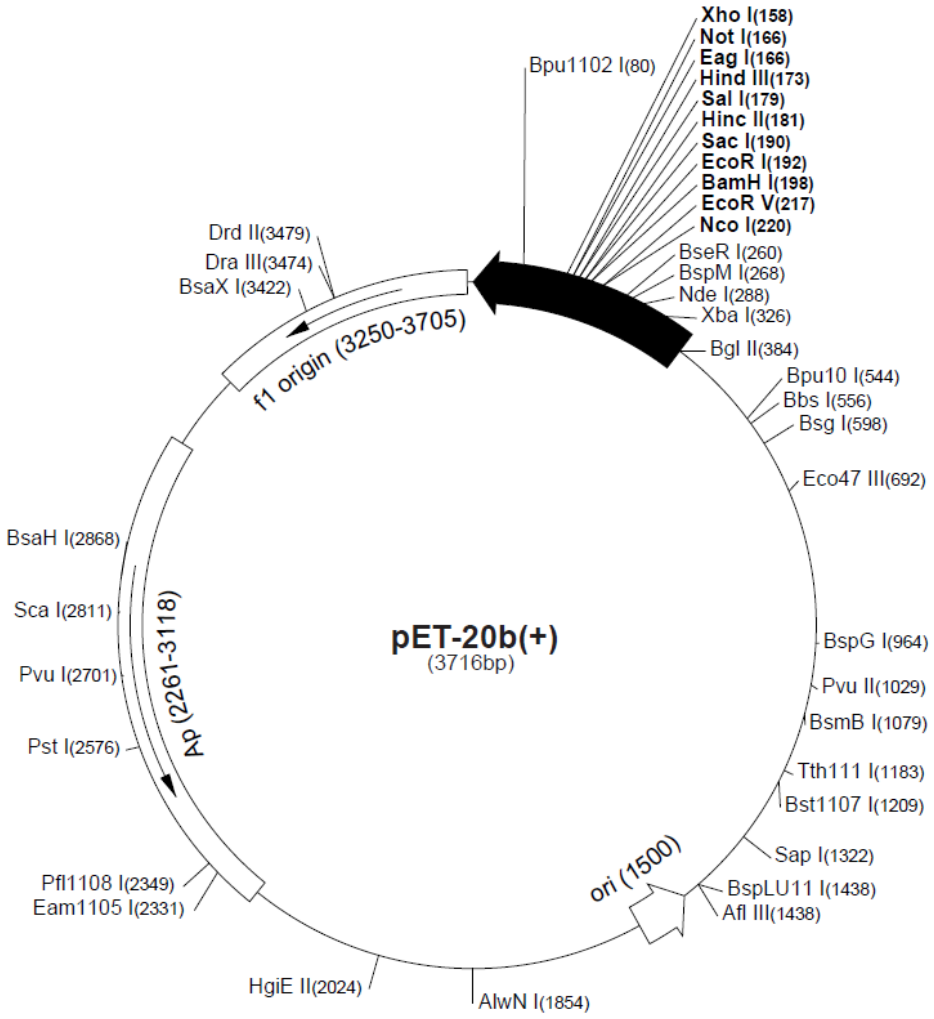
lac	Lac Operon Mutations	blue/white screening of clones
	laclq	lac repressor overproduced, expression from pLac repressed more
	LacZ	β-galactosidase activity abolished
	lacY	Lactose permease inactivated, lactose cannot be taken up by cell
mcrA, mcrBC	Inactivation of pathway that cleaves methylated cytosine DNA	Used to identify and destroy foreign (unmethylated) DNA
mrr, Δ(mcrC-mrr)	Inactivation of pathway that cleaves methylated adenine or cytosine DNA	Used to identify and destroy foreign (unmethylated) DNA
recA, recA1, recA13	Mutation in a DNA-dependent ATPase that is essential for recombination and general DNA repair	Reduces plasmid recombination, increases plasmid stability
recBCD	Exonuclease V activity abolished	Increased plasmid stability, reduced recombination
relA or relA1	Relaxed phenotype, mutation eliminating stringent factor	Allows RNA synthesis in absence of protein synthesis
Ptrc-ccdA		Propagation of ccdB-containing plasmids
Hte		"high transformation efficieny"
deoR	constitutive expression of genes for deoxyribose synthesis	Allows uptake of large plasmids
hee		"high electroporation efficiency"
supE44 (glnV44)		Suppression of the amber (UAG) stop codon by inserting glutamine
supF (tyrT)		Suppression of the amber (UAG) stop codon by inserting tyrosine

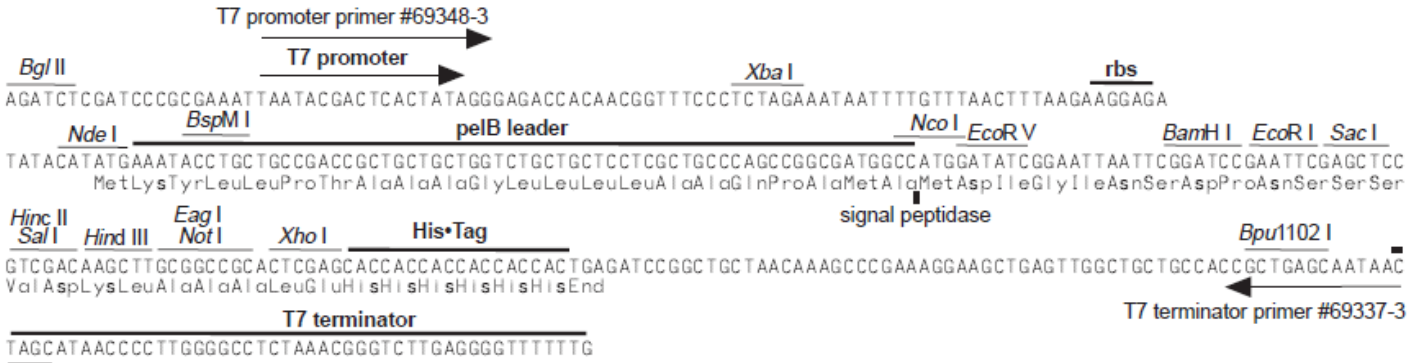
λ-thi-1 or thi1	Mutation in thiamine metabolism	requires exogenous thiamine for growth
ara	disruption of arabinose metabolism pathway	inability to utilize arabinose as a carbon source
leuB	β-isopropyl malate dehydrogenase inactivated	requires exogenous leucine source for growth
proAB	mutation in proline biosynthesis pathway	requires exogenous proline source for growth
rpsL	Mutation in subunit S12 of 30S ribosome	Confers resistance to streptomycin
Tn10		Confers resistance to tetracycline

DH5alpha	General cloning and storage of common plasmids, blue/white screening.	F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ–

Vectores de expresión

pET-20b(+) sequence landmarks	
T7 promoter	353-369
T7 transcription start	352
pelB coding sequence	224-289
Multiple cloning sites (*Nco* I - *Xho* I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
pBR322 origin	1500
bla coding sequence	2261-3118
f1 origin	3250-3705





pET-20b(+) cloning/expression region

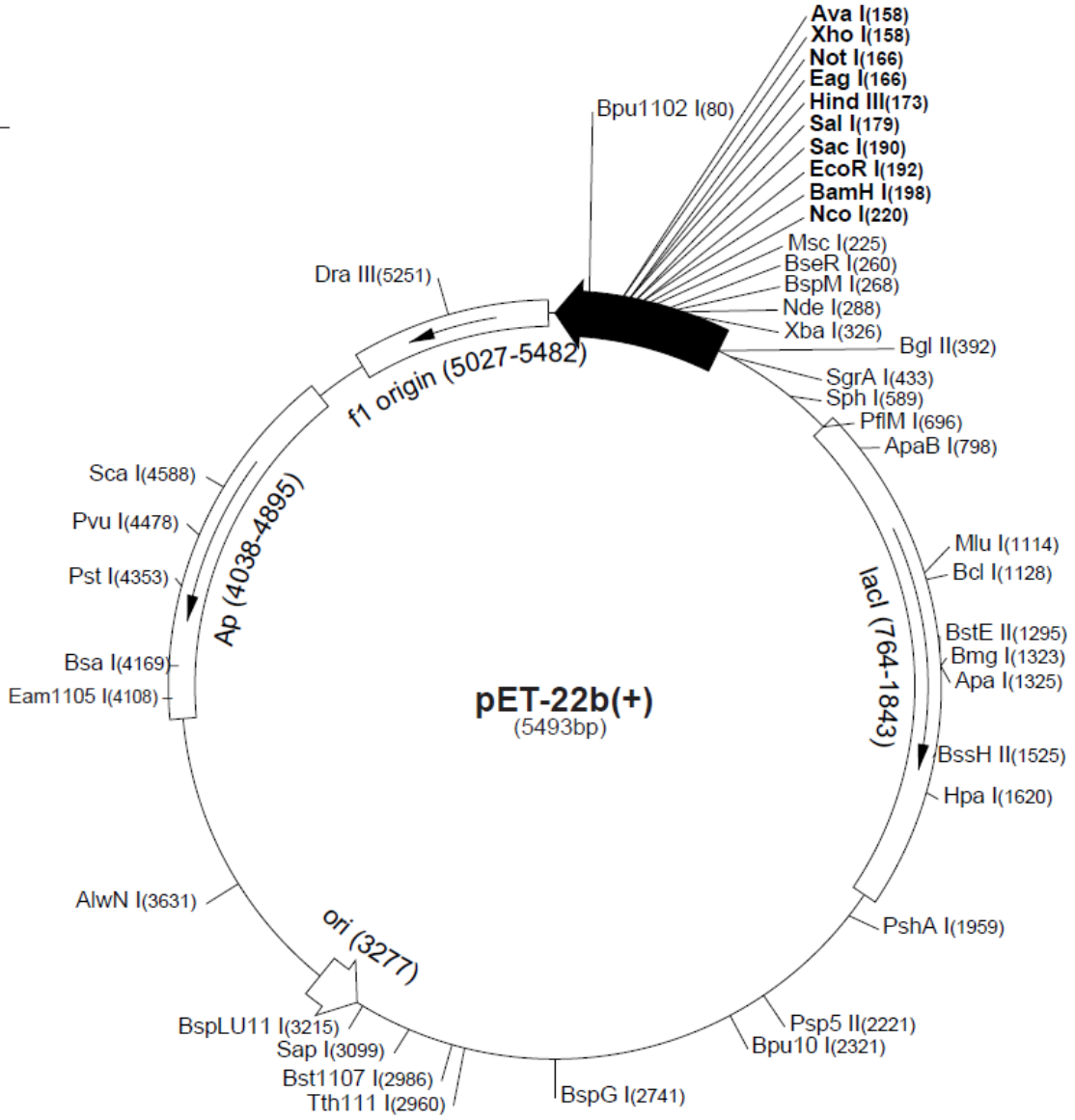
Una cepa para el clonado y otra para expresión.

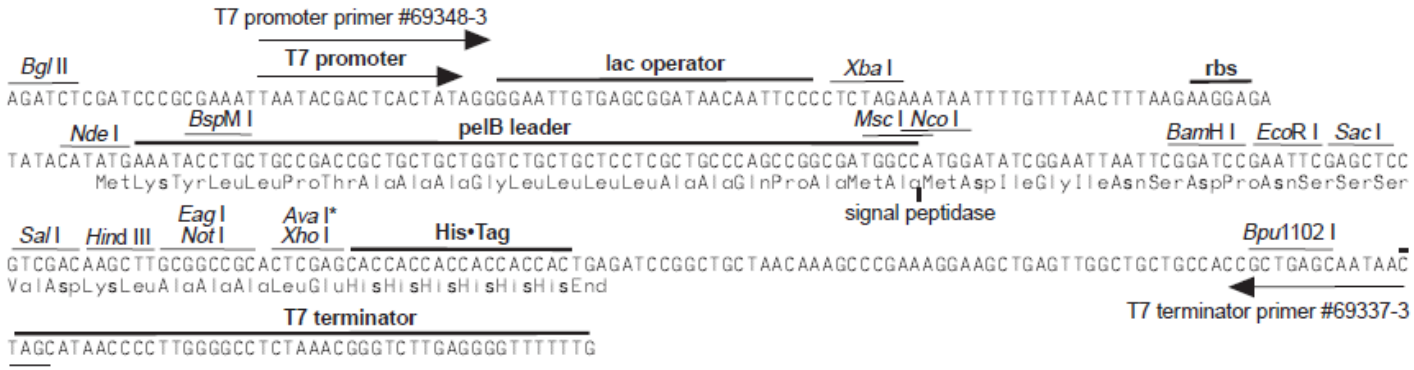
Clonado: una cepa que no codifique T7 RNA polimerasa (JM109 o DH5α)

Expresión: Cepa que exprese T7 RNA polimerasa inducible por IPTG (BL21 (DE3)).

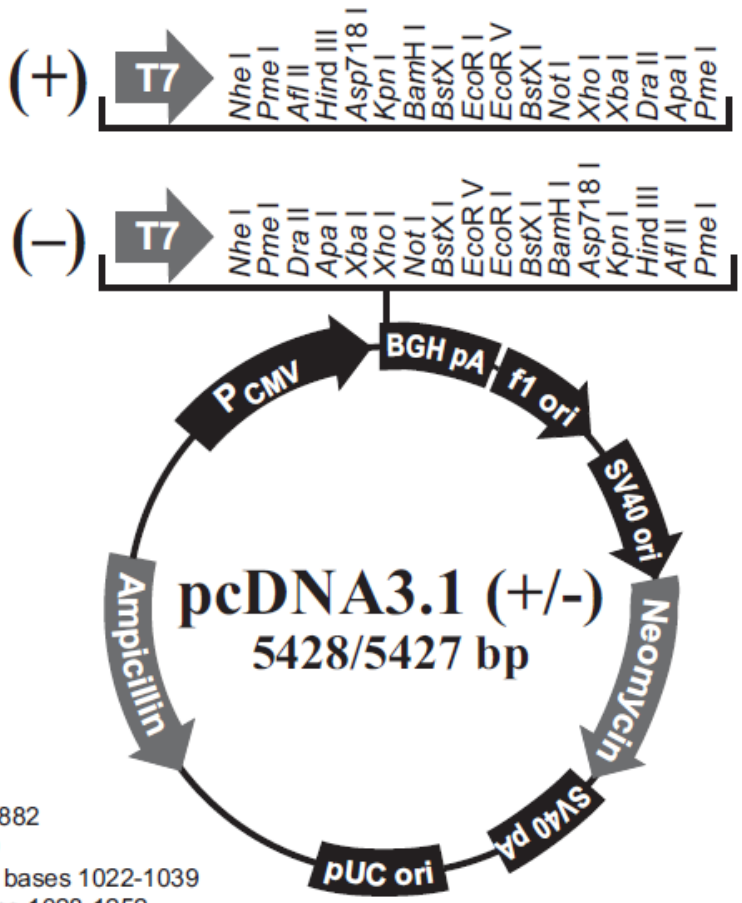
Promotor T7 *lac*

pET-22b(+) sequence landmarks	
T7 promoter	361-377
T7 transcription start	360
pelB coding sequence	224-289
Multiple cloning sites	
(*Nco* I - *Xho* I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	764-1843
pBR322 origin	3277
bla coding sequence	4038-4895
f1 origin	5027-5482



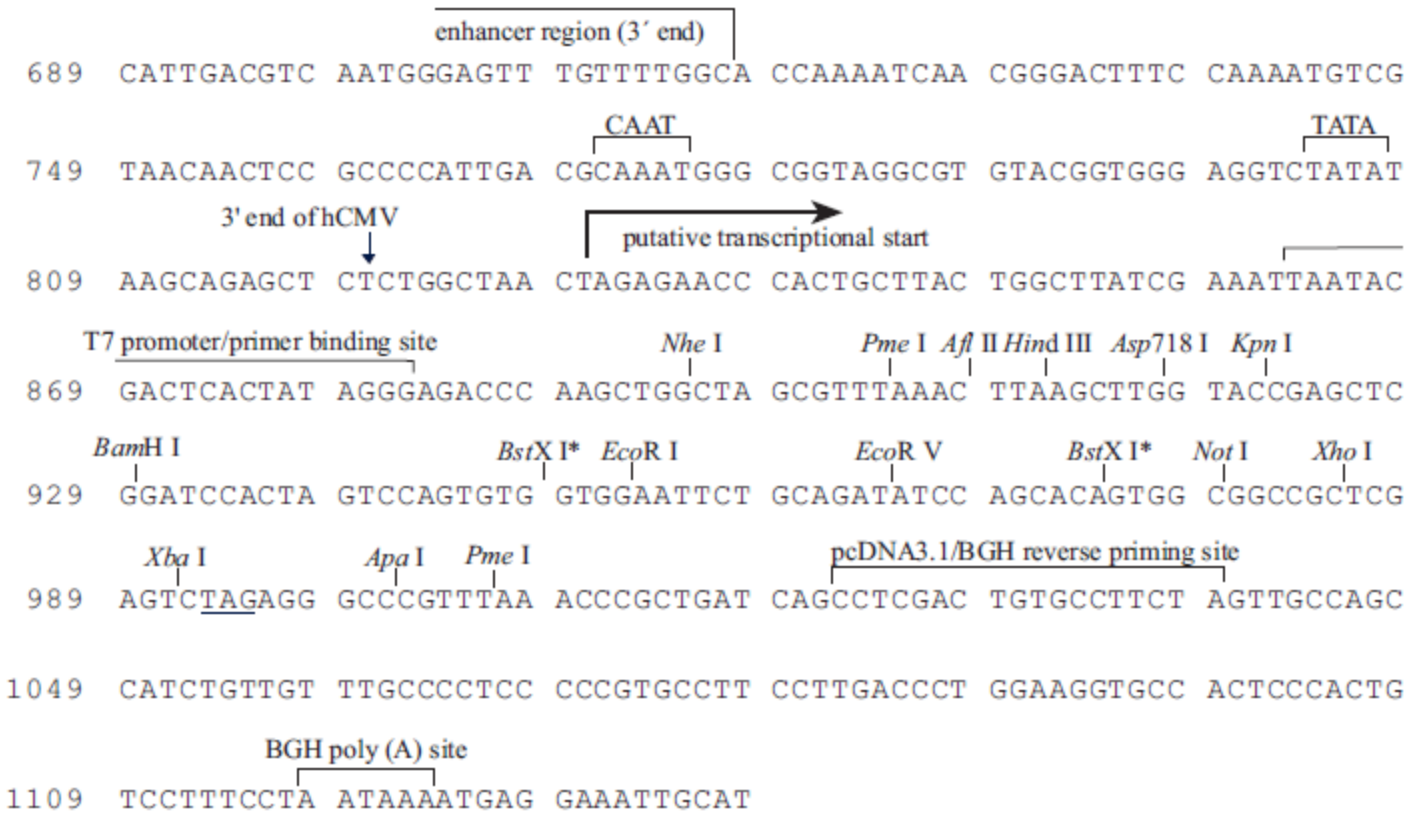


pET-22b(+) cloning/expression region



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)



*Please note that there are two *BstX* I sites in the polylinker.