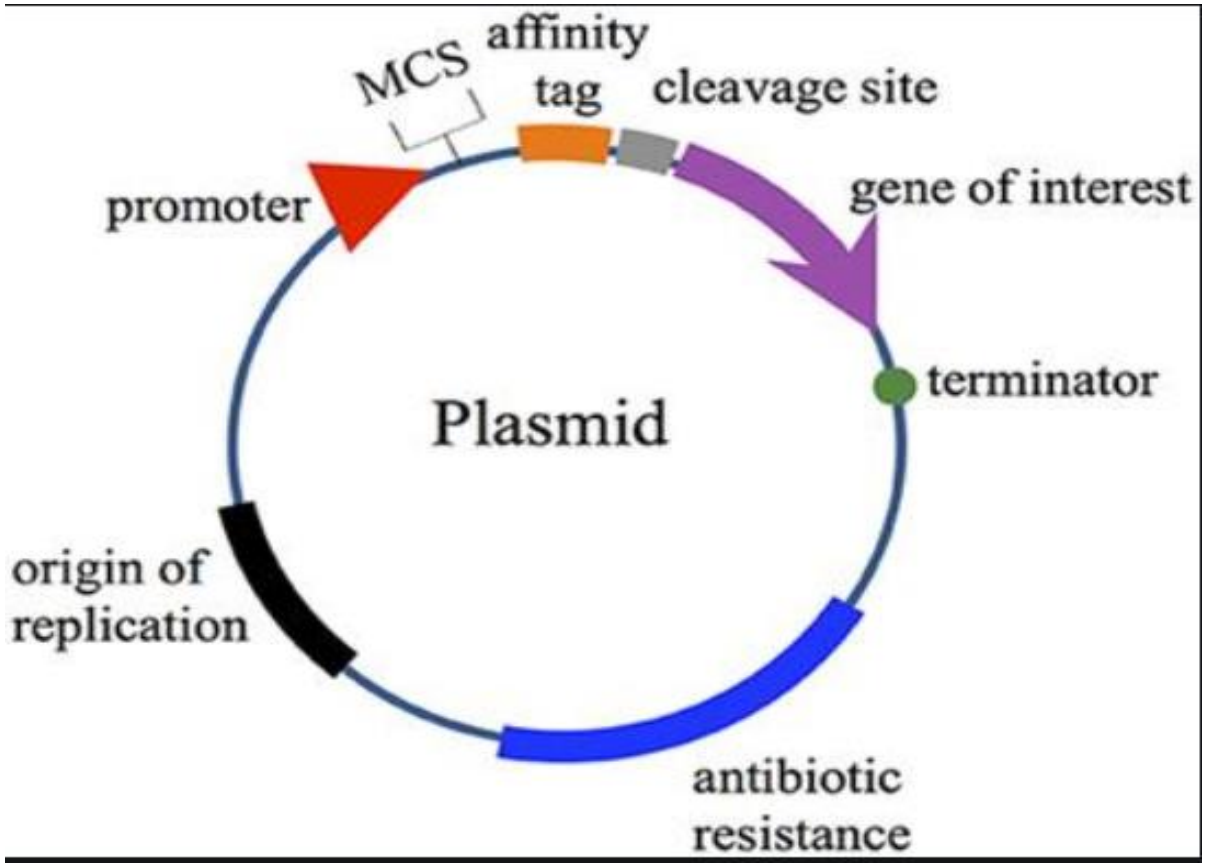
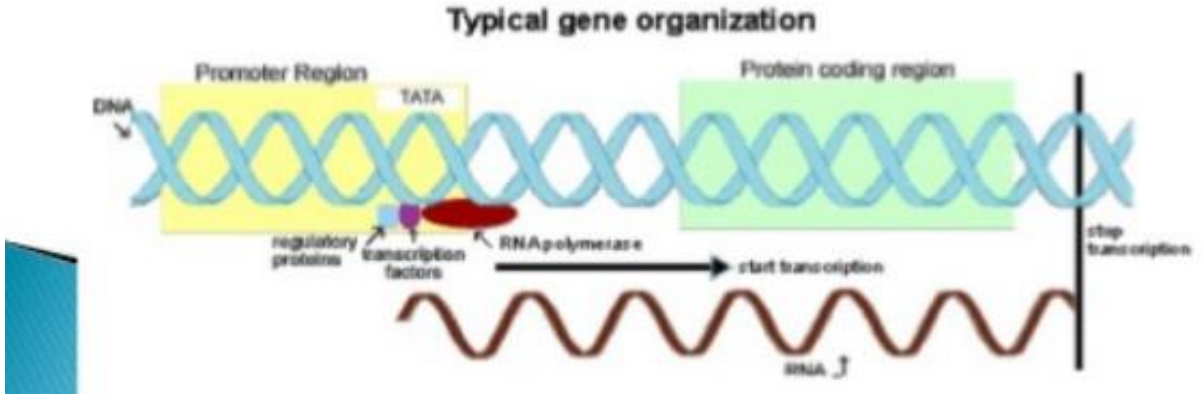
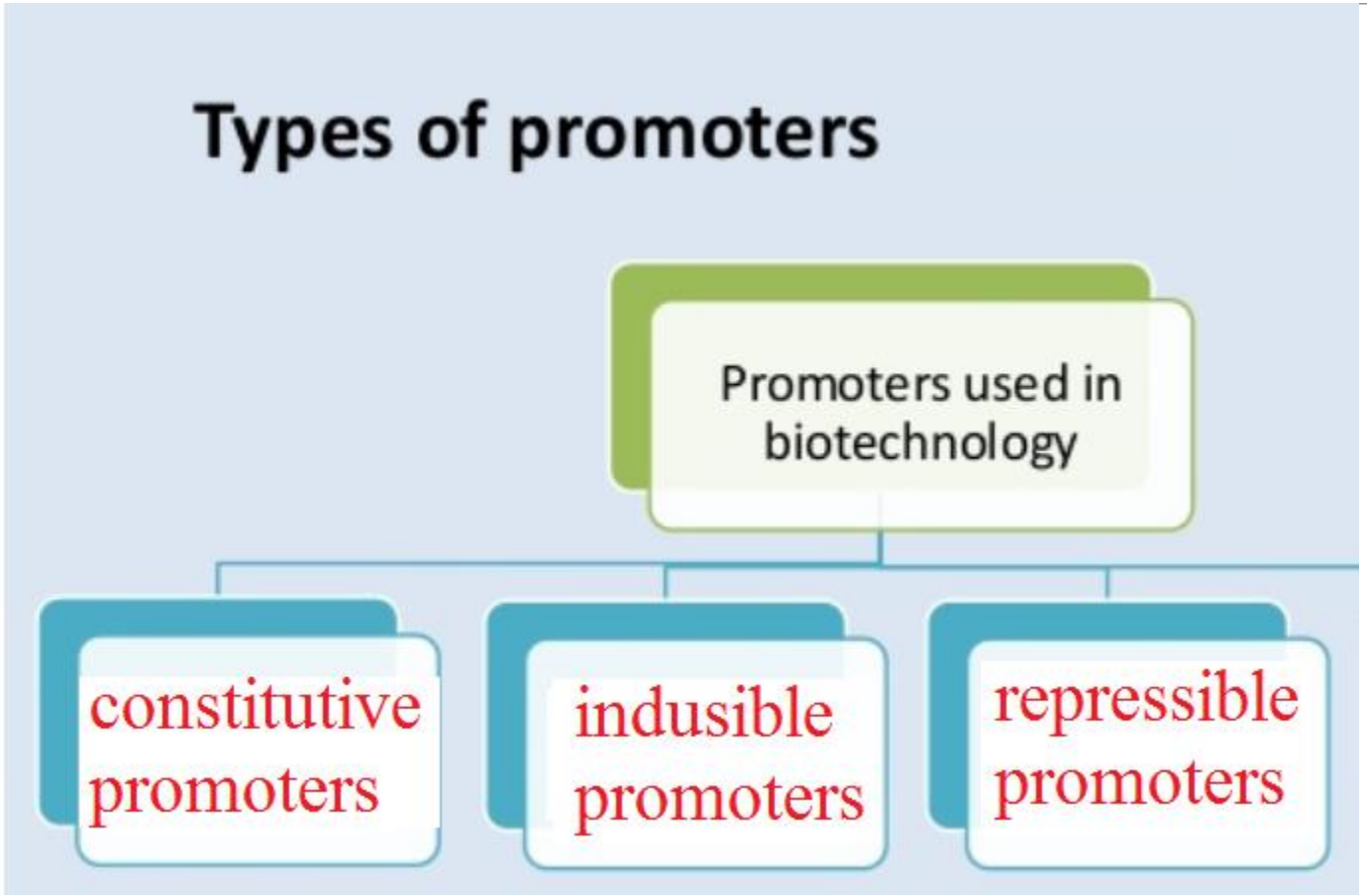

MCS
affinity
tag
cleavage site
promoter
gene of interest
terminator
Plasmid
origin of
replication
antibiotic
resistance

Function of promoter

- RNA polymerase binding site
- Initiation of transcription
- control by regulatory sequences => control the expression of genes




Types of promoters
Promoters used in biotechnology
constitutive promoters
indusible promoters
repressible promoters

Constitutive promoters: level up (low, middle): در همه شرایط بیان شده و تحت تیمار خاصی قرار نمی گیرند.

Inducible: normal (turn/shut off): در حضور ماده تنظیم کننده القاء کننده: gene on

Repressible: normal (turn on): در حضور ماده تنطیمی مهار کننده: gene off

چرا بایستی توالی های تنظیمی (القاء کننده یا مهار کننده شیمیایی) برای کنترل ژن نوترکیب کلون شده استفاده نمود:

۱-اثرات سایتوتوکسیک پروتئین هترولوگوس

۲-بیان هر نوع پروتئین هترولوگوس همراه است با رشد کندتر میزبان و بتدریج حذف خواهد شد.

inducible expression system

s.no.	Host vector system	Inducible expression /promoter
1.	*E .coli*	a.lac Promoter
		b.tac Promoter
		c. *λPL Promoter*
		d.T7 Expression System
2.	**Yeast** *Saccharomyces cerevisiae,* *Pichia pastoris and* *Schizosaccharomyces pombe.*	 *a.GAL System* *b. CUP1 System* a. Alcohol oxidase (*AOX1*) *a.nmt1*

Promoters

- *E.coli* natives
  - *lac, trp, tac, trc, ara*
- Viral, but recognised by *E.coli*
  - λ_L, λ_R, T5
- T7, T7*lac*
  - requires its own RNA polymerase

promoter	-35 region	spacer	-10 region
P_{lac}	TTtACA	18 bp	TATgtT
P_{lacUV5}	TTtACA	18 bp	TATAAT
P_{trp}	TTGACA	17 bp	TtaAcT
P_{tac}	TTGACA	17 bp	TATAAT
λP_L	TTGACA	17 bp	gATAcT
λP_R	TTGACt	17 bp	gATAAT
Consensus	**TTGACA**	**17 bp**	**TATAAT**

lac & *trp*

lac

- Promoter of the *lac* operon
- Repressed by *lacI* gene, which binds downstream of the promoter
- Regulated by galatose or its analogues, in expression work non-hydrolysable IPTG used.

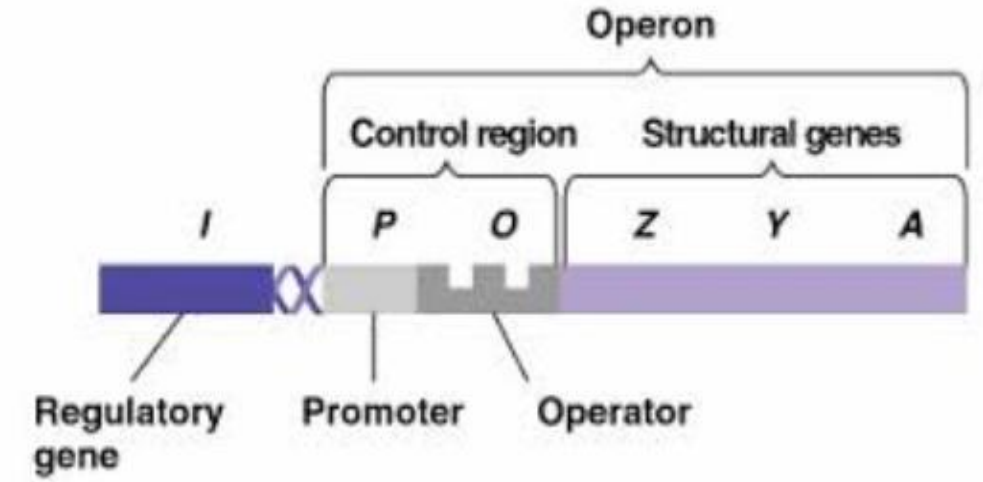
trp

- Promoter of tryptophane biosynthetic enzymes
- Repressed by Trp, so induction done by causing a Trp deficiency with indole-2-acrylic acid

E.coli's own promoters are the first ones ever used to drive overexpression of proteins in bacteria. These are strong promoters, and can be induced with relatively inexpensive chemicals,

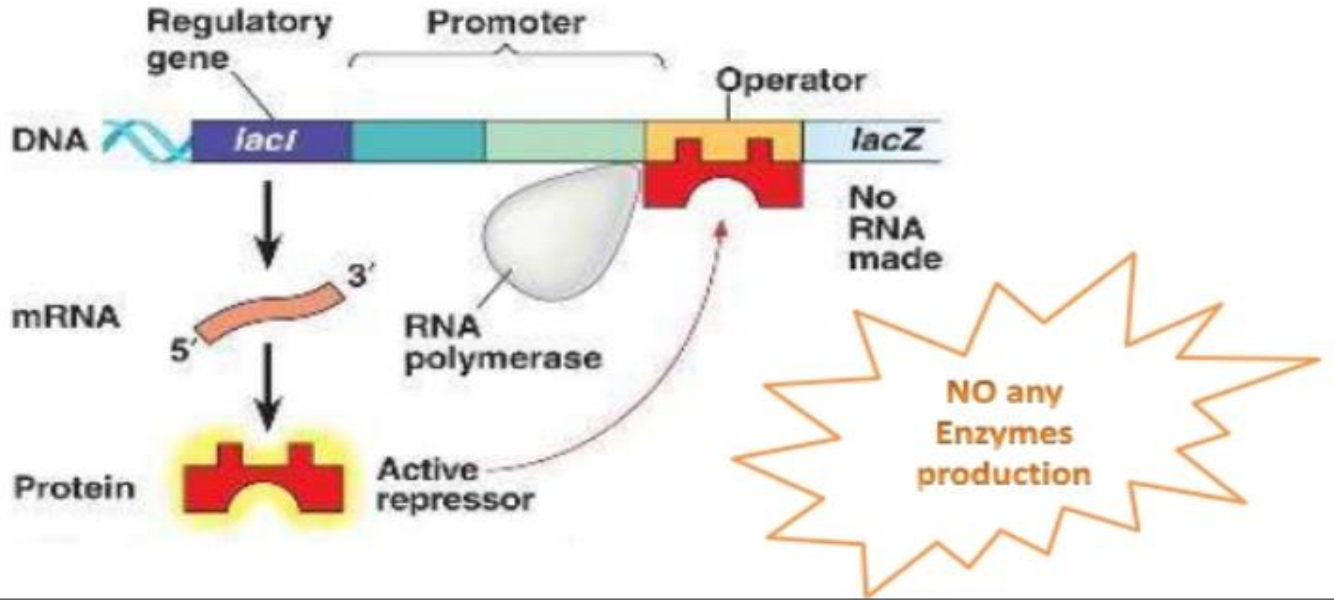
lacI promoter

lacI^{Q} promoter



Lac-operon function

- **when only glucose is present**



Lac Operon In Ecoli

- **Promoter (P)** - aids in RNA polymerase binding
- **Operator (O)** - "on/off" switch - binding site for the repressor protein
- **Repressor (lacI) gene**

Repressor gene (lacI) - produces repressor protein with two binding sites, one for the operator and one for lactose

tac & trc

Synthetic promoters created by fusion of *trp* and *lac* promoters

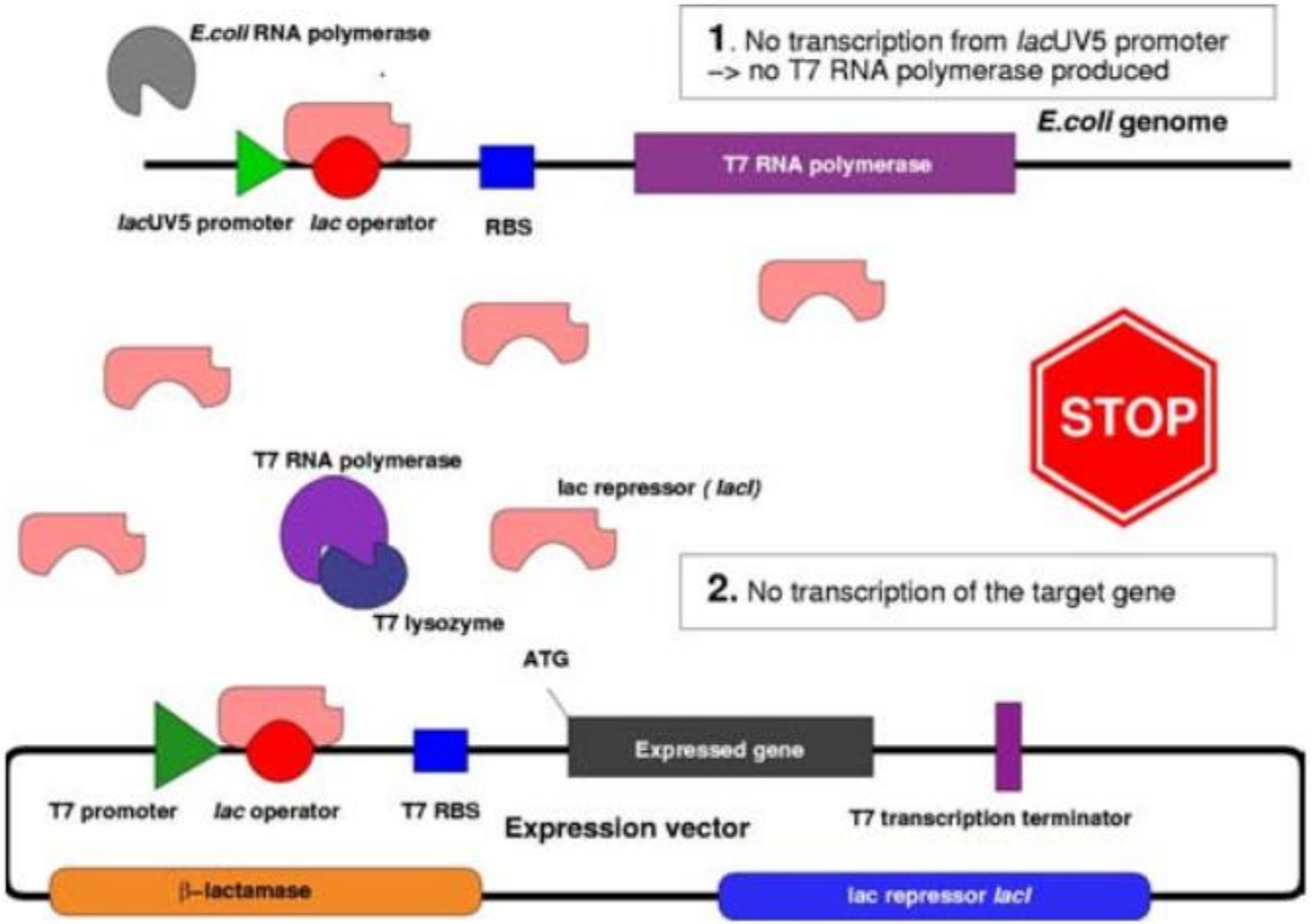
- -35 part from *trp*, -10 from *lac*
- Regulation from *lac* system, *ie.* induced by IPTG
- Originally shown to be much stronger than either of the parent promoters
- Now found in pGEX and pMAL vectors

Although not naturally found in *E.coli* the synthetic tac and trc promoters can be classified as *E.coli* promoters, as they are created by fusing different elements of the *lac* and *trp* promoters making them more powerful than either of the parental promoters alone. Several commercial vector systems still use these, inclusing pMAL and pGEx series, and pTRC series from Invitrogen,

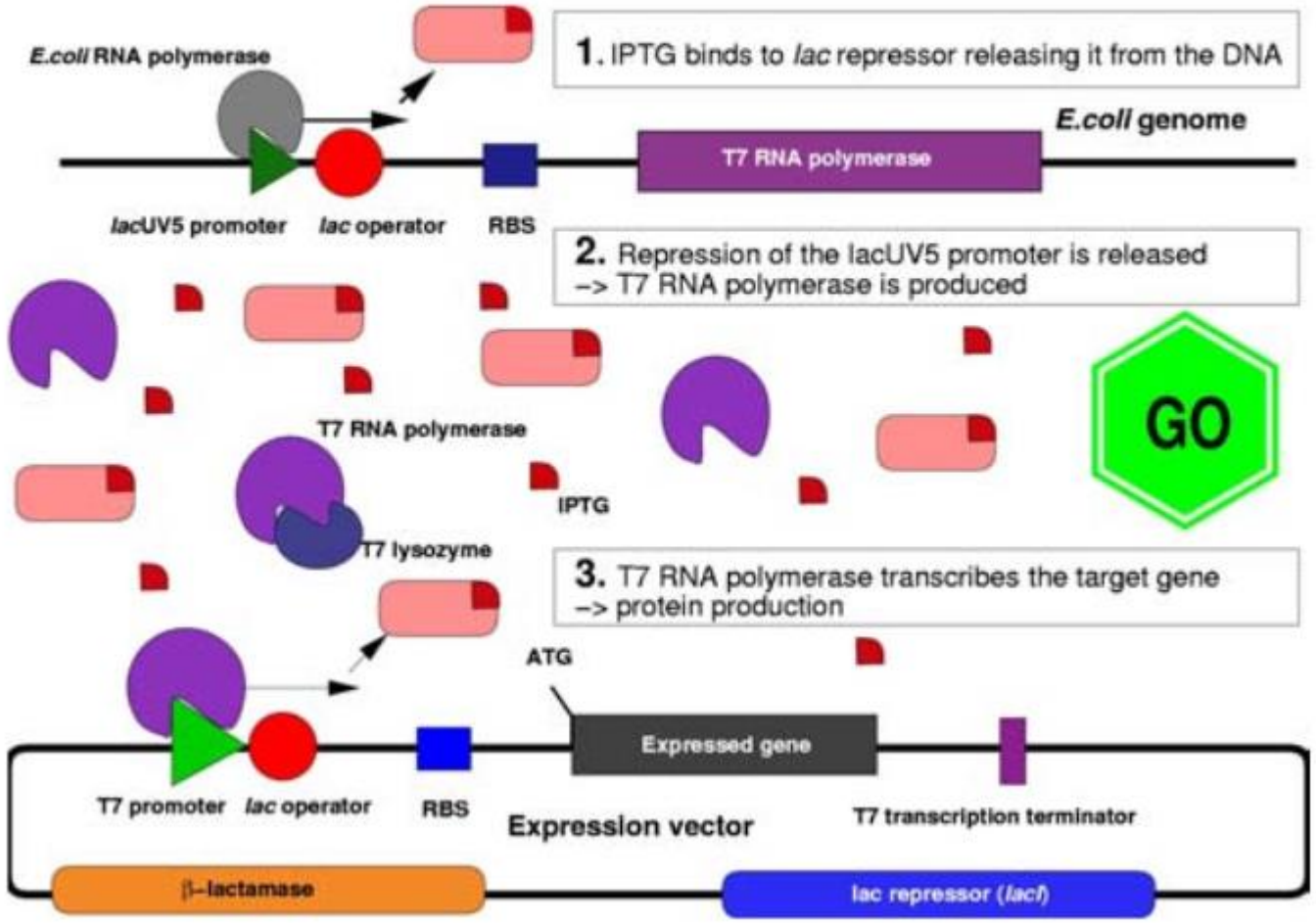
T7 system

- Promoter of the *gene1* of the bacteriophage T7
- Recognised only by the T7 RNA polymerase (T7RP)
  - Faster and more processive enzyme
- Commercialised in the pET series of vectors from Novagen - tens of variants
- T7RP can be inhibited by T7 lysozyme (pLysS/E plasmids)
- Usually combined with *lacO* regulator and *lacI* gene to provide tight regulation of expression (T7*lac*)
- Needs to be combined with a T7 transcription terminator

T7 system before induction

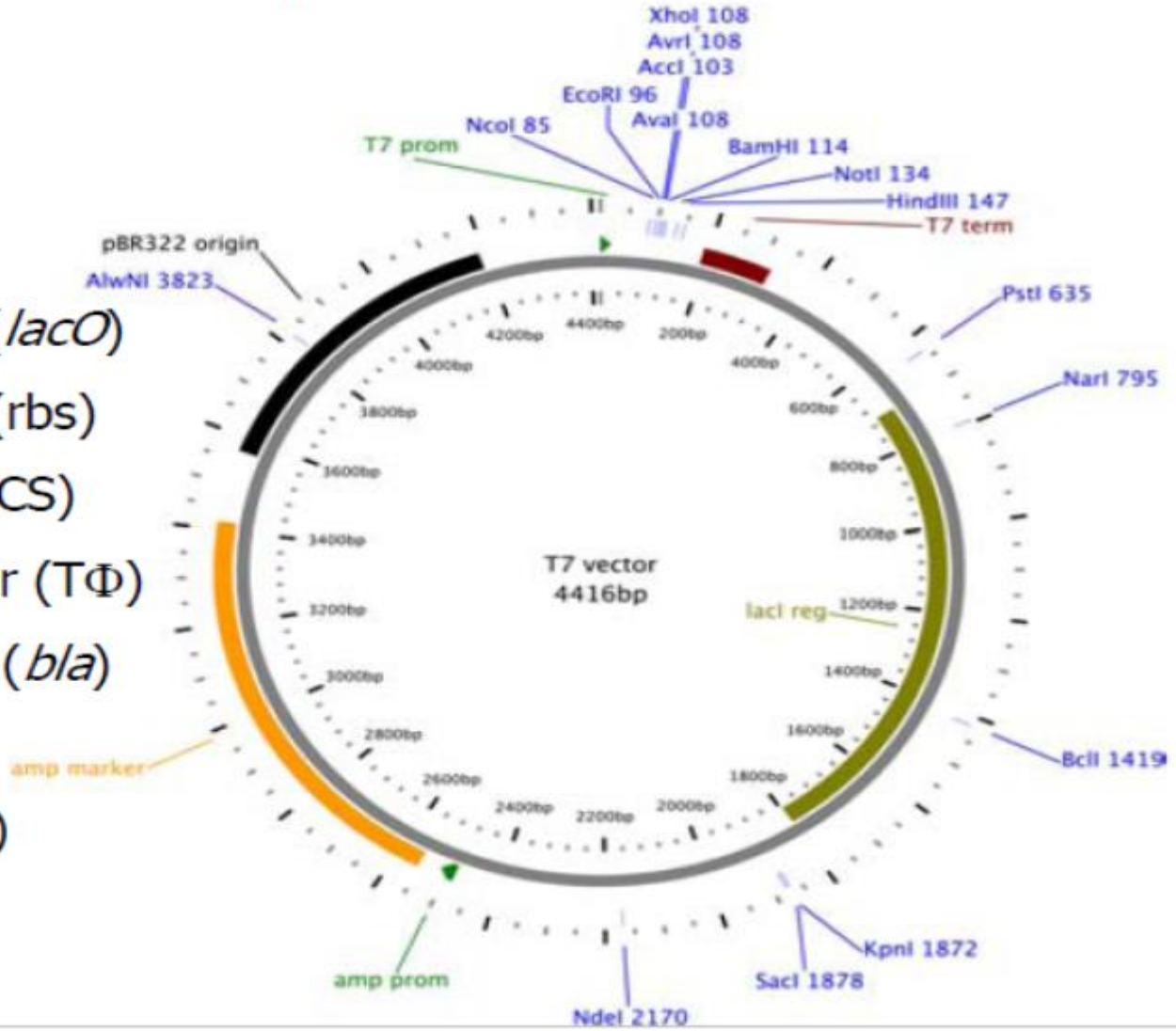


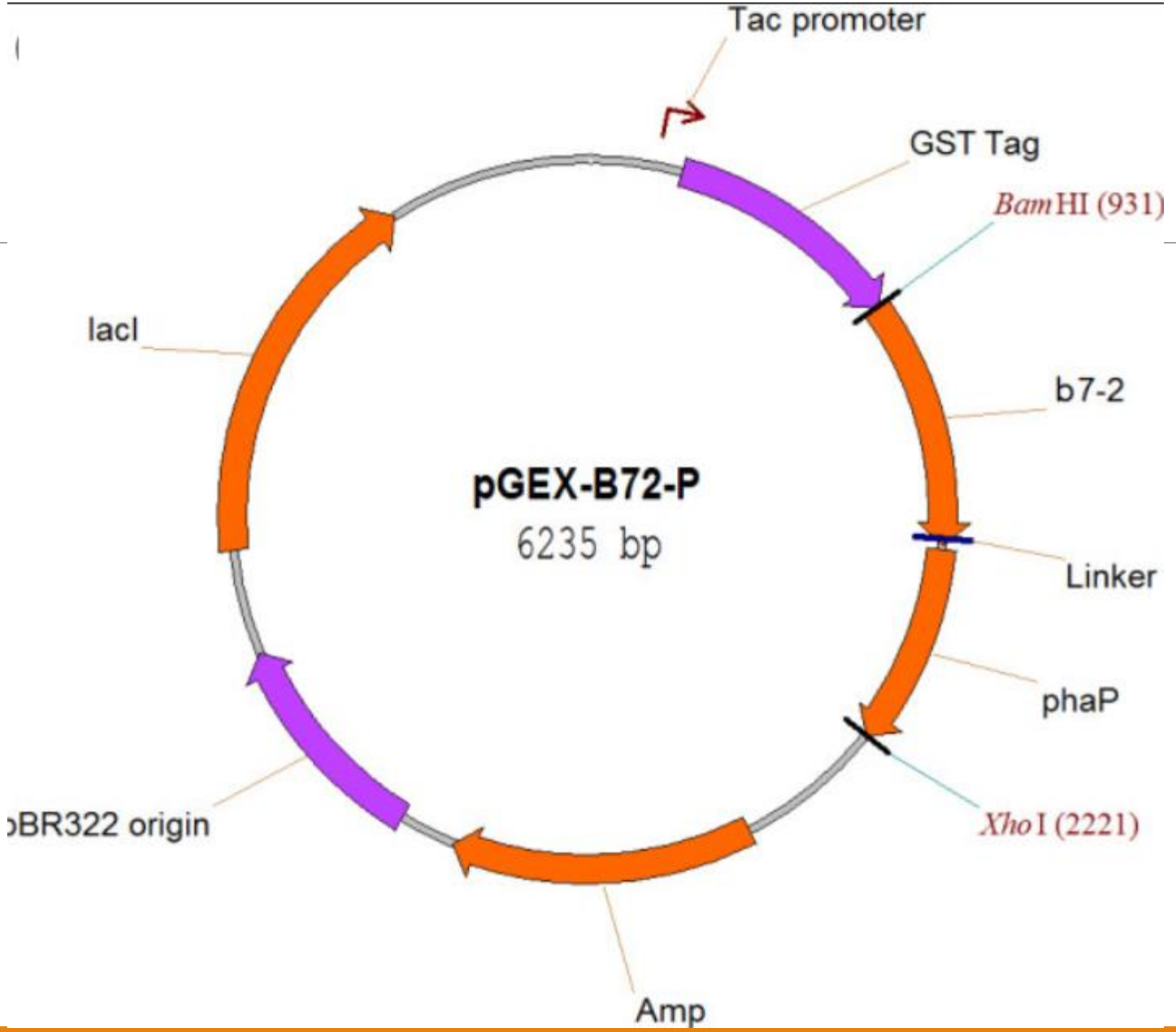
T7 system in action



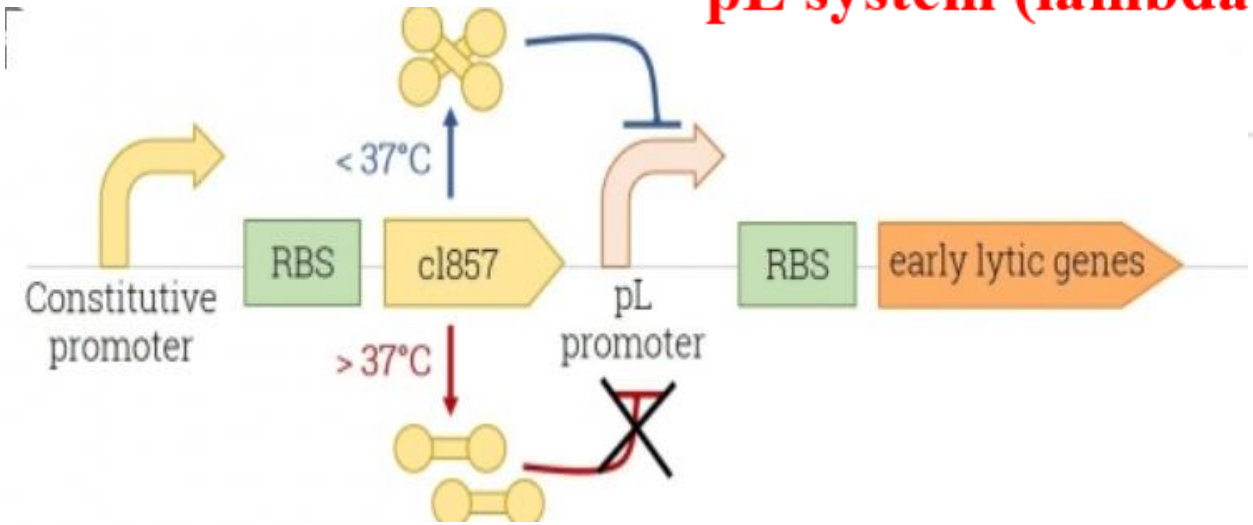
Elements of an expression vector

- Expression cassette
  - Promoter (T7)
  - Regulator binding site (*lacO*)
  - Ribosome binding site (rbs)
  - Multiple cloning site (MCS)
  - Transcription terminator (TΦ)
- Antibiotic resistance gene (*bla*)
- Origin of replication (ori)
- Other control genes (*lacI*)

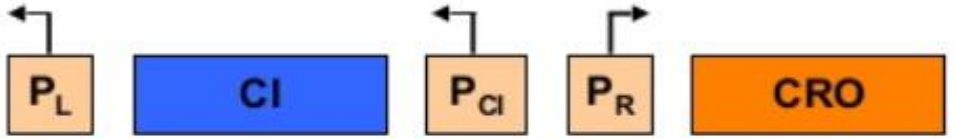



Tac promoter
GST Tag
BamHI (931)
b7-2
Linker
phaP
XhoI (2221)
Amp
pBR322 origin
lacI
pGEX-B72-P
6235 bp

pL system (lambda left promoter=λpL)



Lambda phage (λ) repressor



Three promoters are essential for the transcription of λ phage genome:

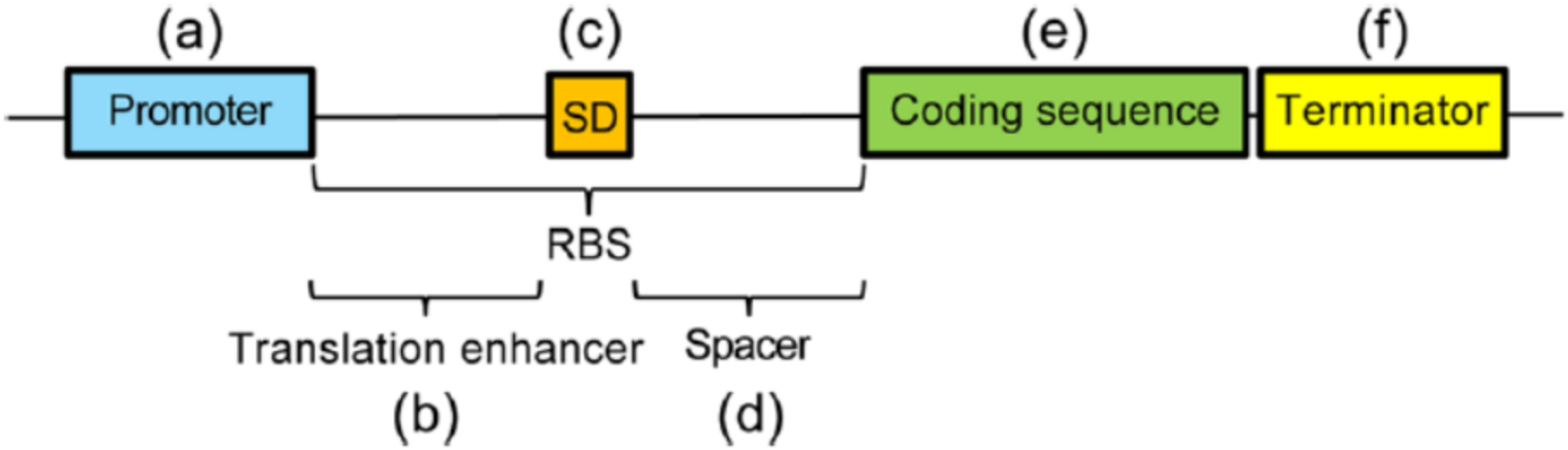
- **P_R:** a promoter for transcribing the rightward genes.
- **P_L:** a promoter to transcribe the leftward genes.
- **P_{CI}:** a promoter to transcribe CI (lambda repressor gene).

Lambda phage (λ) repressor

Lysogenic pathway is achieved when the both P_{CI} is turned ON and P_L and P_R turned OFF.

What is turning these promoters ON and OFF?

Gene cassette



مشکلات مرتبط با تولید پروتئین نوترکیب در اشریشیاکلي و راه حل ؟

1. عدم سیستم Splicing
2. تغییرات پس از ترجمه
3. امکان هضم پروتئین نوترکیب
4. خالص سازی
5. امکان خاتمه پیش از موعد

کاربرد آنزيم هاي مهم در مهندسي ژنتيک

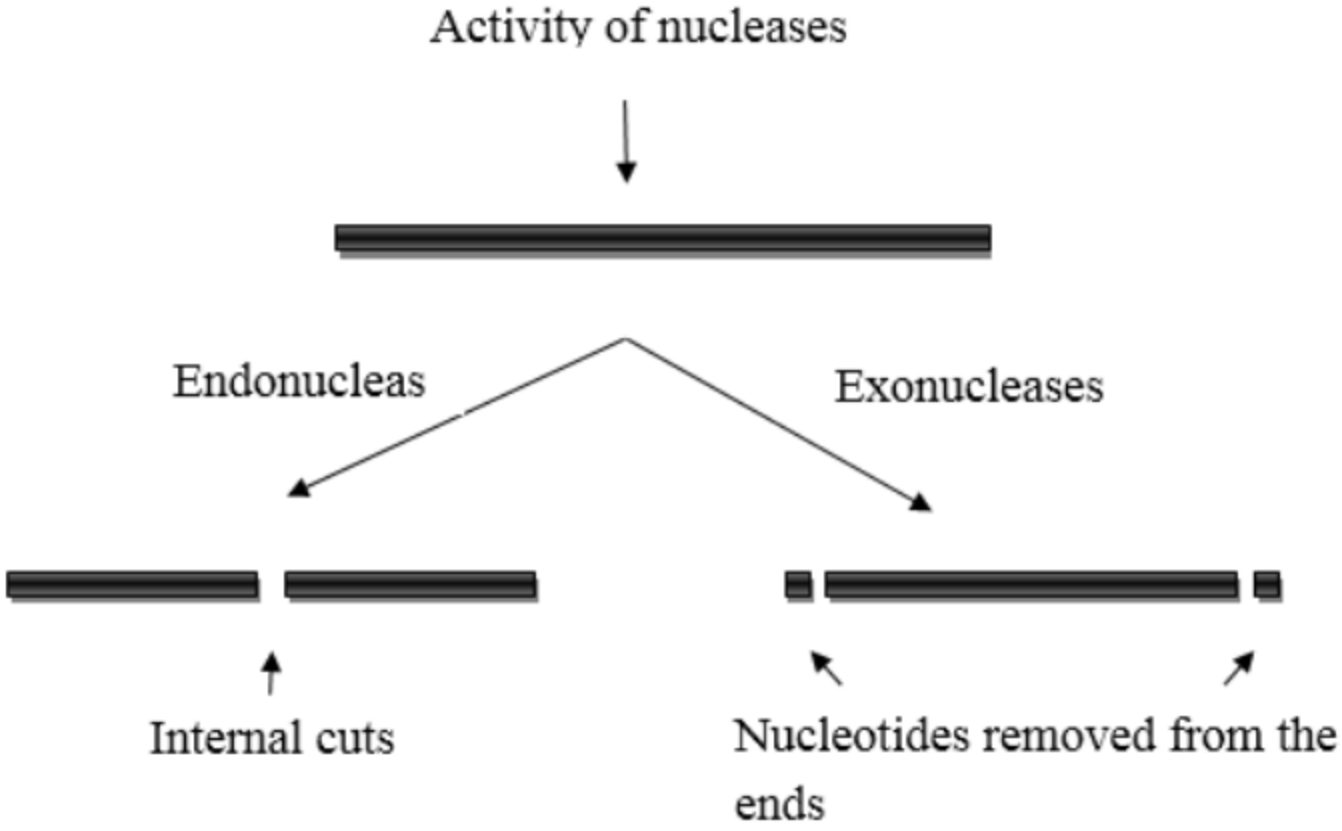
نوکلئازها

ليگازها

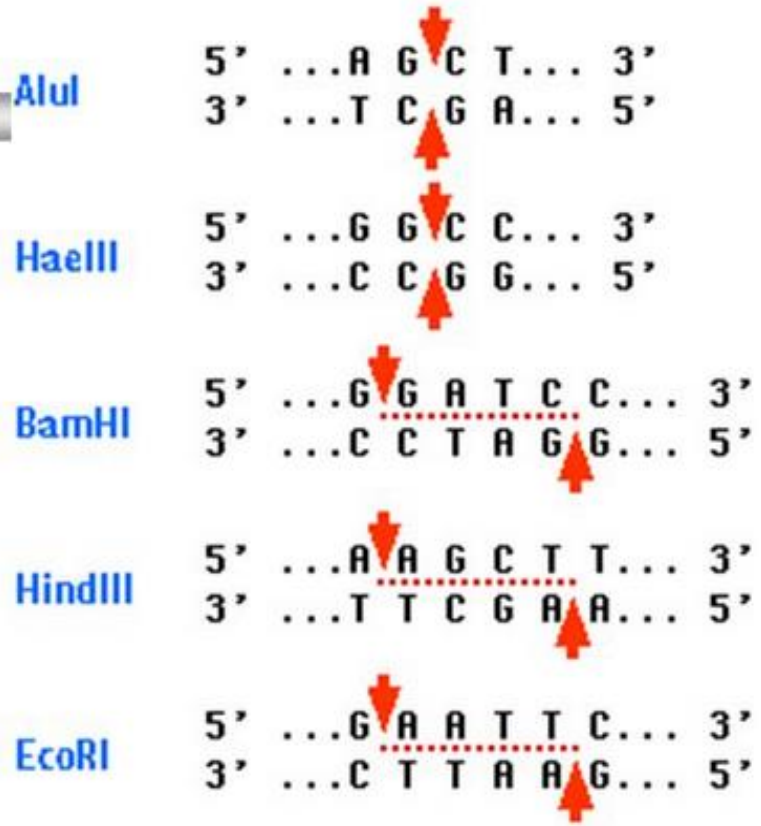
پليمرازها

تغيير دهنده گروههاي شيميايي

توپوازومرازها

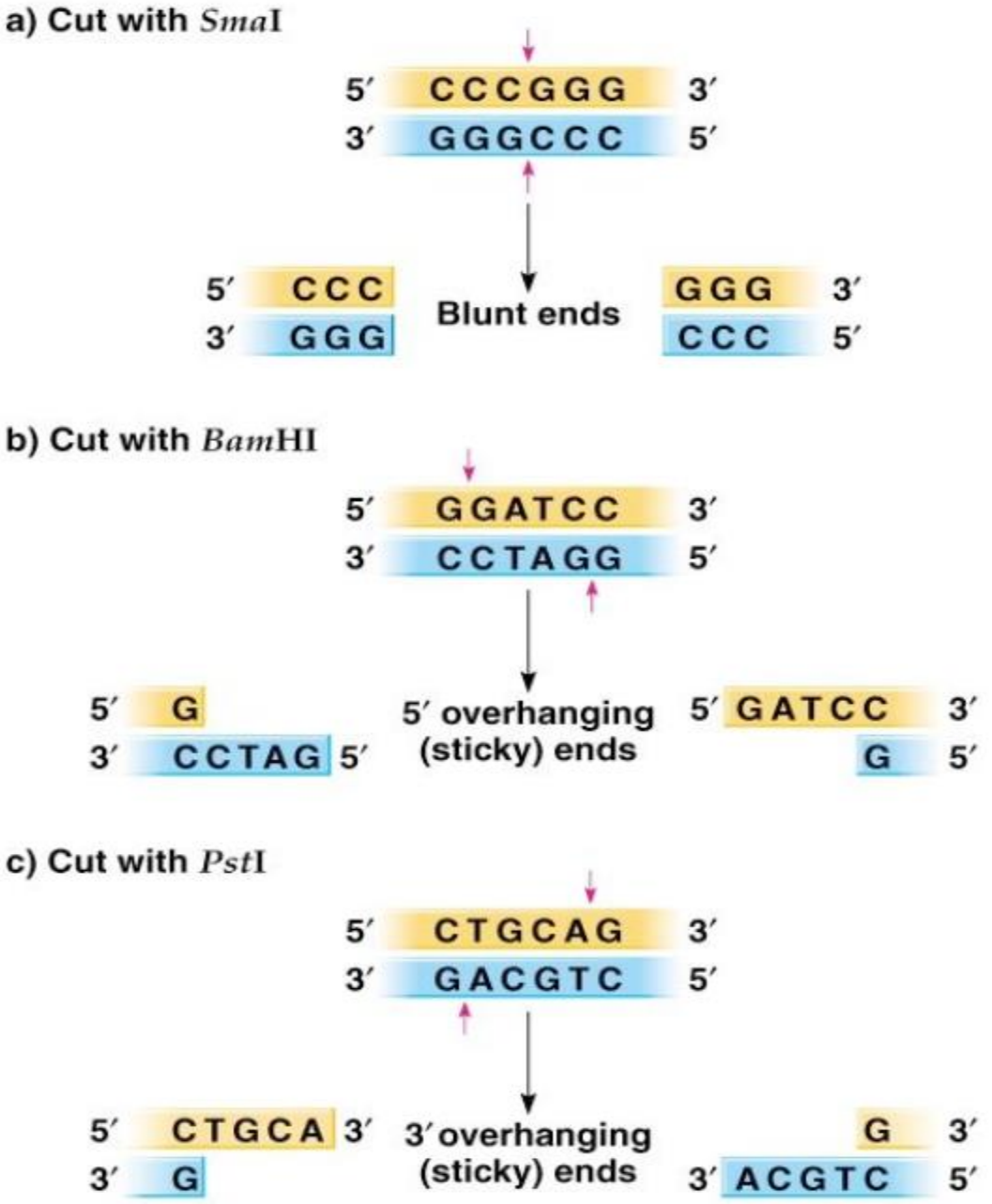

Activity of nucleases
Endonucleas
Exonucleases
Internal cuts
Nucleotides removed from the ends

Blunt & Sticky ends



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends



- Apart from restriction enzymes, there are four useful nucleases that are often used in genetic engineering.
- These are
  - **Bal 31** and
  - **Exonuclease III** (exonucleases), and
  - **Deoxyribonuclease I** (**DNase I**) and
  - **S1-nuclease** (**endonucleases**).

Exonuclease III (*E.coli*)

A double-strand specific, nonprocessive 3´→5´ exodeoxyribonuclease activity; however, 3´-overhangs of ≥4 bases are protected from Exo III activity (1).

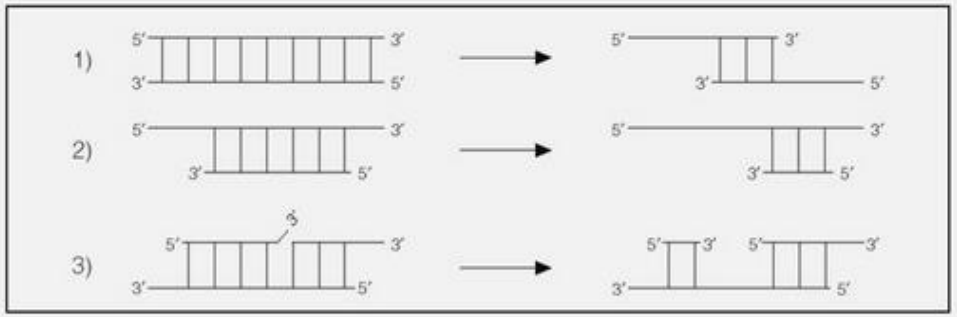


Figure 1. Exonuclease III catalyzes the stepwise removal of mononucleotides starting from a 3´-OH at: 1) blunt ends, 2) recessed ends and 3) nicks. Exonuclease III will also act on 3´-overhangs of less than 4 bases (not shown). Note that the 3´-overhangs shown in 3) are ≥4 bases and therefore not susceptible to Exonuclease III activity.

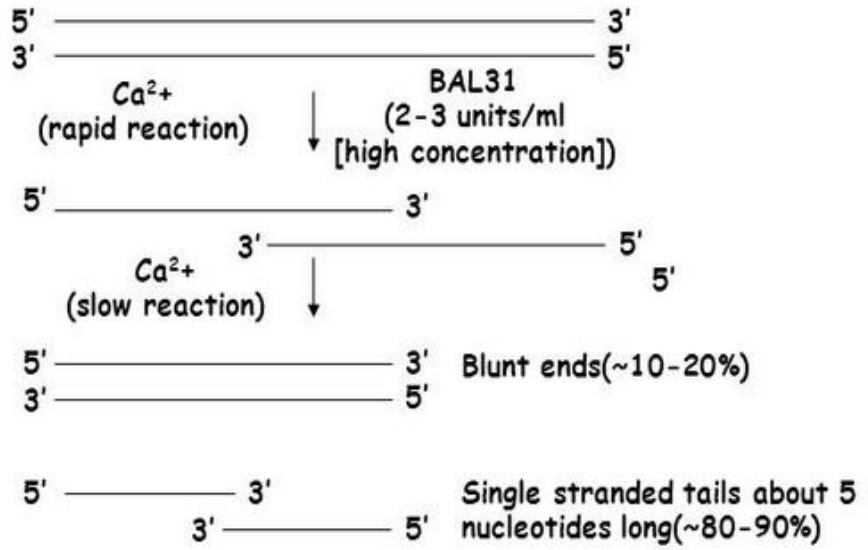
*Bal*31 NUCLEASE

SOURCE

Alteromonas espejiana

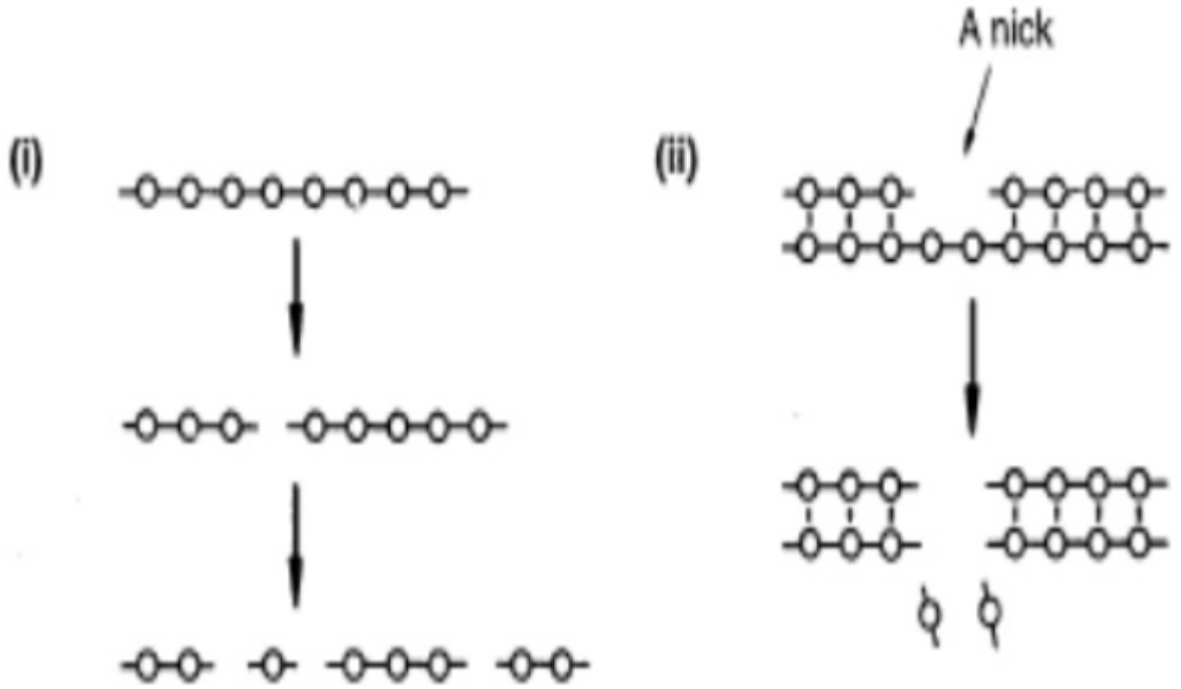
FUNCTION

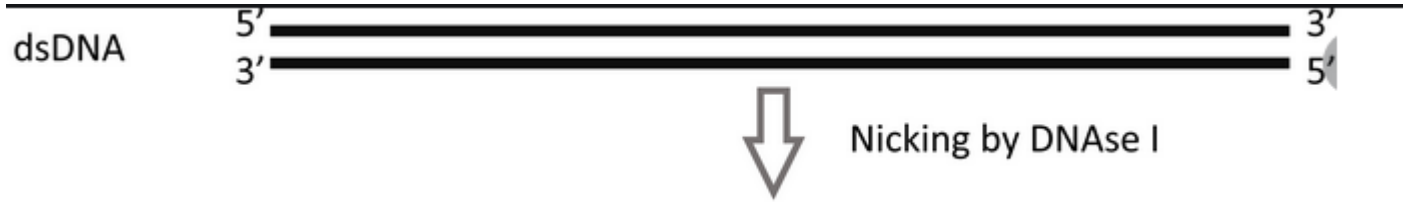
- 3′⟶5′ exonuclease activity that eliminates mononucleotides from dsDNA
- 5′⟶3′ exonuclease activity that works efficiently on ssDNA
- Endonuclease activity that degrades ssDNA slowly and cleaves supercoiled dsDNA as well as mutagenically altered dsDNA



Aspergillus oryzae

S1 nuclease





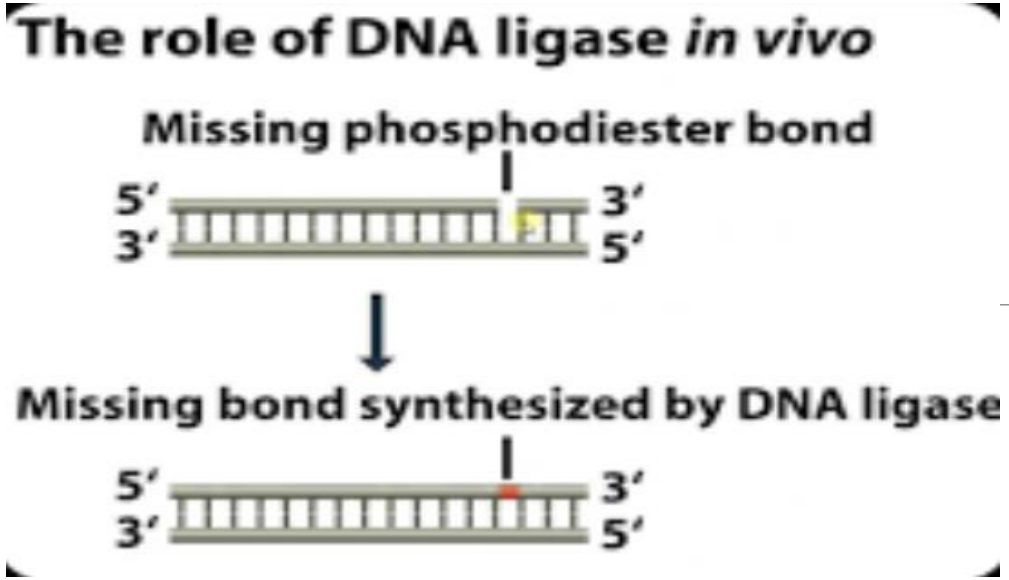
MUNG BEAN ENDONULEASE

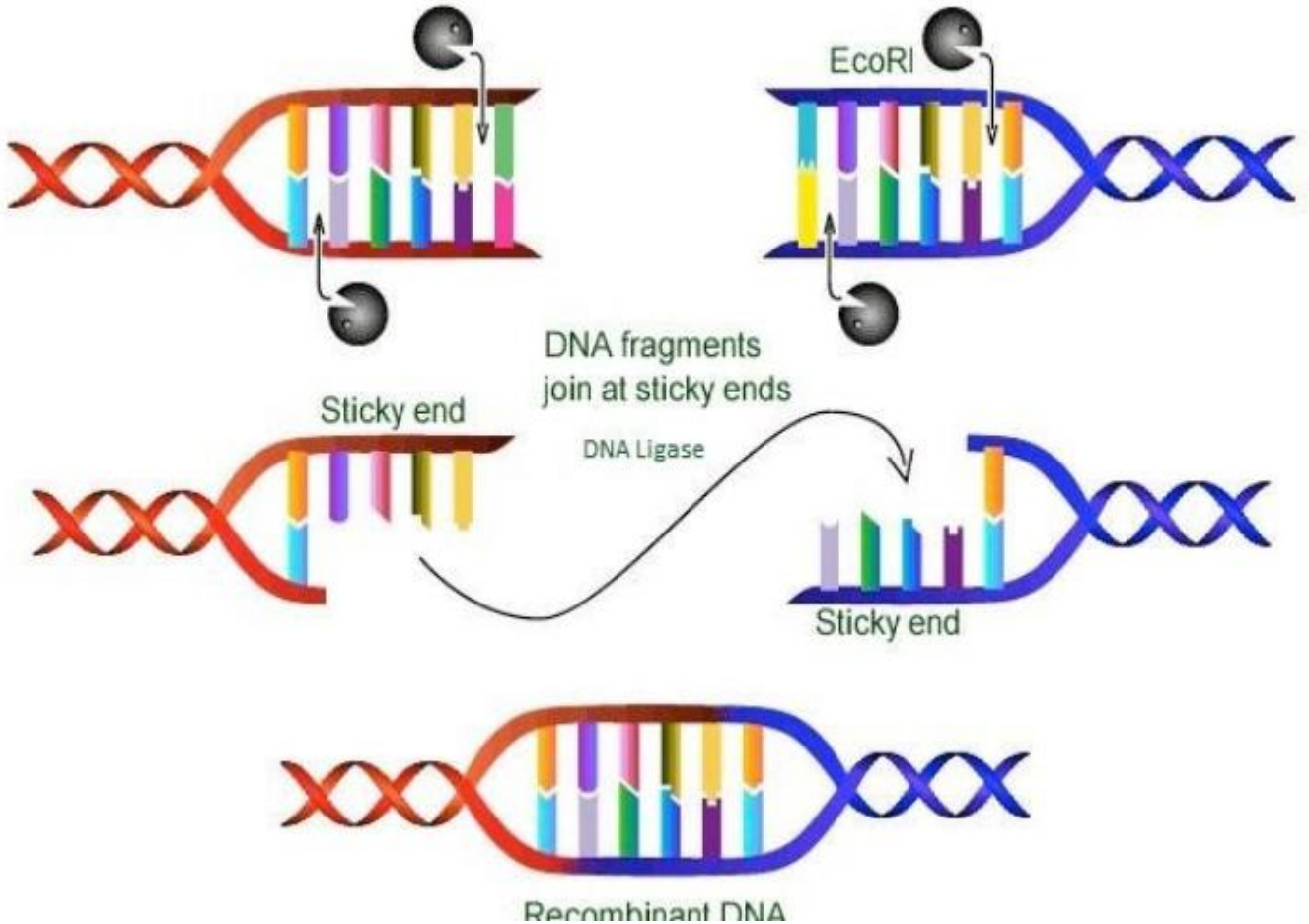
SOURCE

Mung bean sprouts

FUNCTION

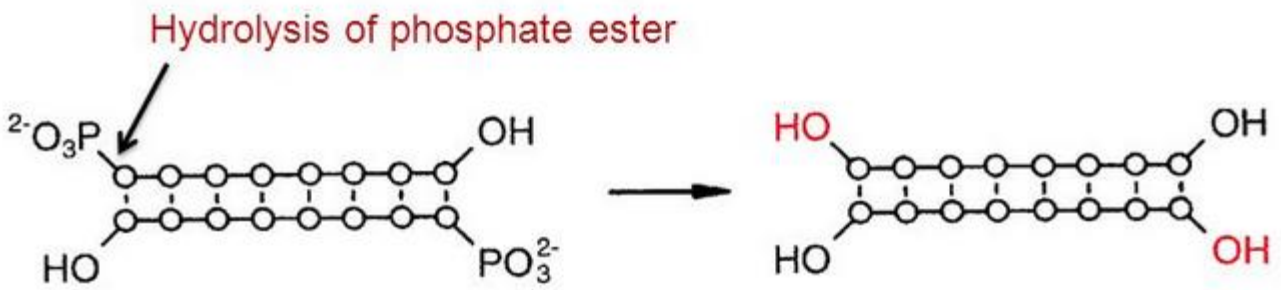
- Single strand specific nuclease that degrades DNA and RNA to 5’-P mononucleotides
- ds DNA, dsRNA and RNA:DNA hybrid are resistant to this enzyme
- Works on nick after it has been enlarged to a gap of many nucleotides


The role of DNA ligase *in vivo*
Missing phosphodiester bond
5′
3′
3′
5′
Missing bond synthesized by DNA ligase
5′
3′
3′
5′


EcoRI
DNA fragments
join at sticky ends
Sticky end
DNA Ligase
Sticky end
Recombinant DNA

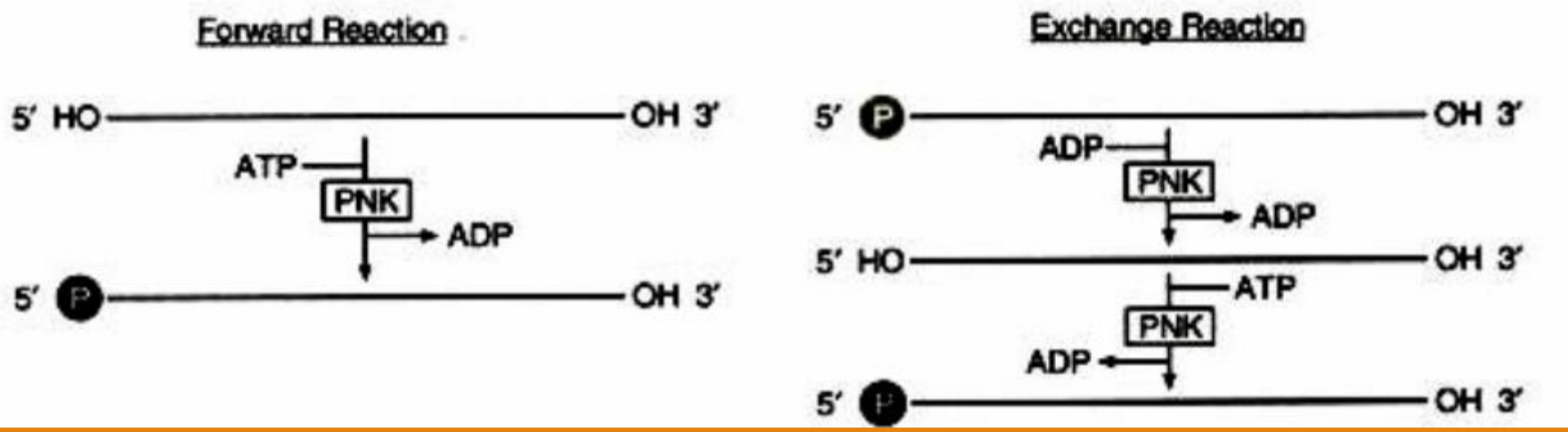
DNA modification enzymes

- **Alkaline phosphatase**:
  - removes the 5' phosphate groups from DNA, normally the vector DNA



Polynucleotide Kinase:

Polynucleotide kinase (PNK) is an enzyme that catalyzes the transfer of a phosphate from ATP to the 5′ end of either DNA or RNA. It is a product of the T4 bacteriophage, and commercial preparations are usually products of the cloned phage gene expressed in E. coli.



Terminal Transferase:

Terminal transferase catalyzes the addition of nucleotides to the 3′ terminus of DNA. Interestingly, it works on single-stranded DNA, including 3′ overhangs of double-stranded DNA, and is thus an example of a DNA polymerase that does not require a primer. It can also add homo-polymers of ribo-nucleotides to the 3′ end of DNA. The much preferred substrate for this enzyme is protruding 3′ ends, but it will also, less efficiently, add nucleotides to blunt and 3′-recessed ends of DNA fragments. Cobalt is a necessary cofactor for