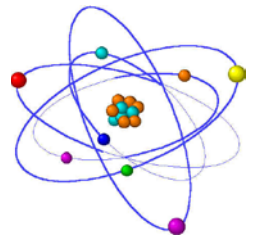
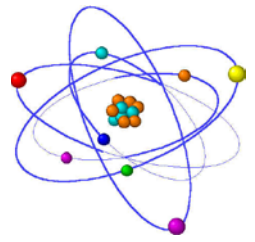


Biochemistry Lab



Controlled Protein Expression

Reading the Strains



Two general types of strains biochemists are interested in:

- DNA factories – have characteristics that promote plasmid DNA replication

Example:

DH5 α -

*fhuA2 Δ (argF-lacZ)U169 phoA glnV44 Φ 80 Δ (lacZ)M15 gyrA96 recA1
relA1 endA1 thi-1 hsdR17*

- Protein factories

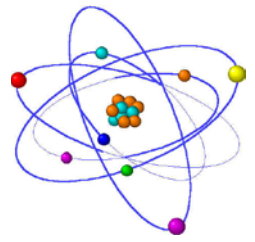
Example:

BL21(DE3)

*F⁻ ompT gal dcm lon hsdS_B(r_B⁻ m_B⁻) λ (DE3 [lacI lacUV5-T7 gene 1 ind1
sam7 nin5])*

A reasonably thorough summary of these strains can be found [here](#)

Reading the Strains

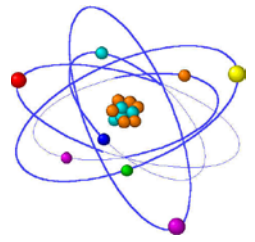


Cloning Strains (DH5 α)

General ideas –

1. Get rid of or mutate genes that promote recombination with genomic DNA (*recA1*, *gyrA96*)
2. Get rid of nucleases (*endA1*)
3. Optimize transformation of unmethylated DNA (*hsdR17*)
4. Promote RNA synthesis in the absence of protein synthesis (*relA1*)
5. T1 Phage resistant (*fhuA2*)
6. Alter function of some genes ($\Delta(\text{lacZYA-argF})\text{U169 } \phi 80\text{lacZ}\Delta\text{M15}$)
7. Suppress UAG stop codon (*glnV44*)

Reading the Strains

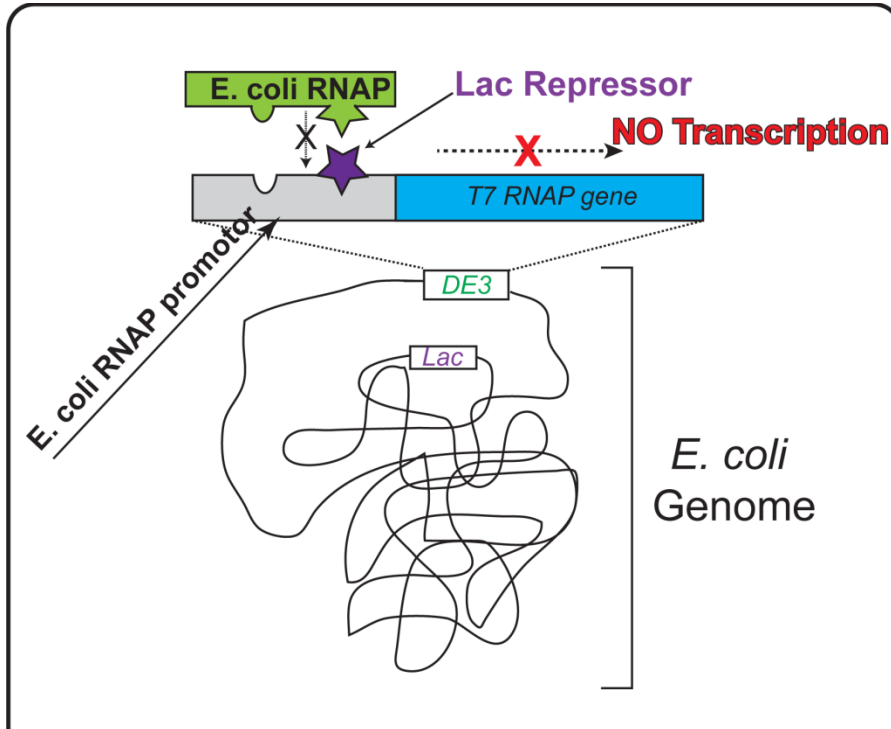
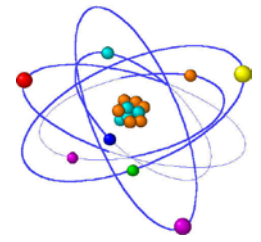


Expression Strains (BL21 (DE3))

General ideas –

1. Get rid of or mutate protease genes (*ompT*, *lon*)
2. T7 RNA polymerase included under control of the optimized Lac operator (λ (DE3 [*lacI lacUV5-T7 gene 1 ind1 sam7 nin5*]))
3. Certain methylated sequences cannot be made by this strain and will be degraded if incorporated (*hds_B*)
4. (*r_B⁻ m_B⁻*) tell us that this strain lacks the recombinase and methylase systems

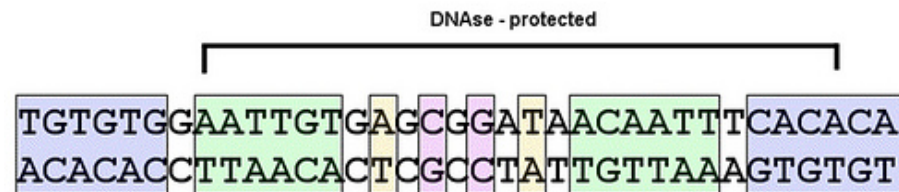
DE3 Lysogen



The T7 DE3 lysogen is directly incorporated into the *E. coli* genome.

The lac operator sequence is incorporated just upstream of the T7 RNA polymerase gene.

Lac Operator



Allows biochemists to control the expression of the T7 RNA Polymerase

Lac Repressor

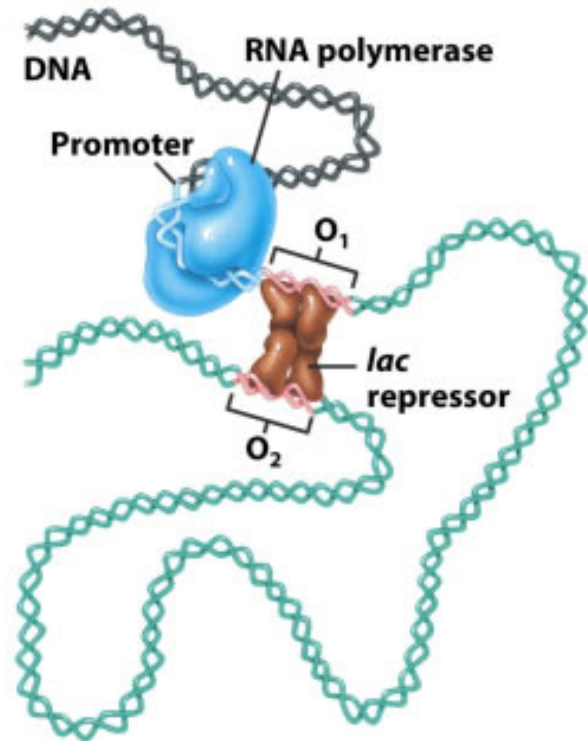
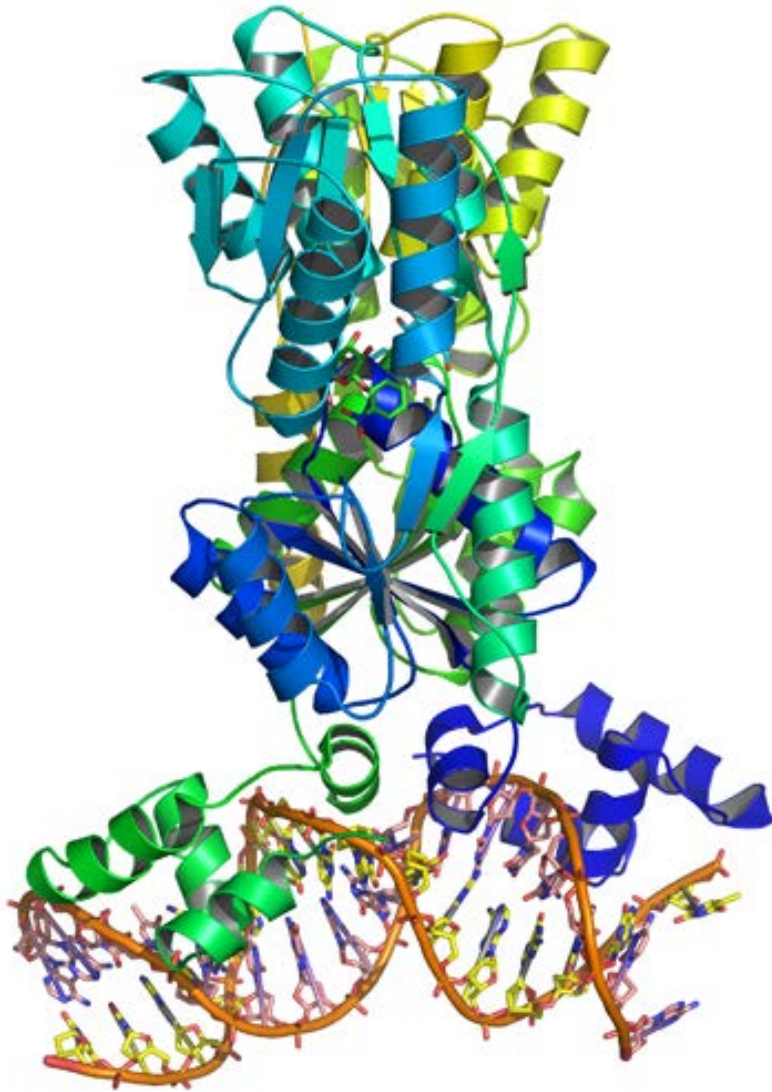
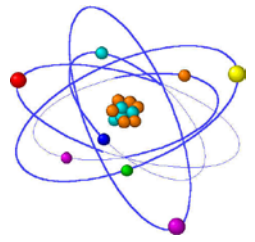
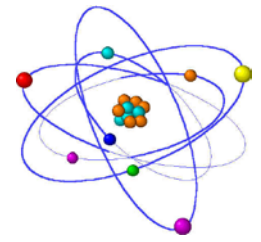


Figure 21-17 Principles of Biochemistry, 4/e
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DE3 Lysogen – Lac operon

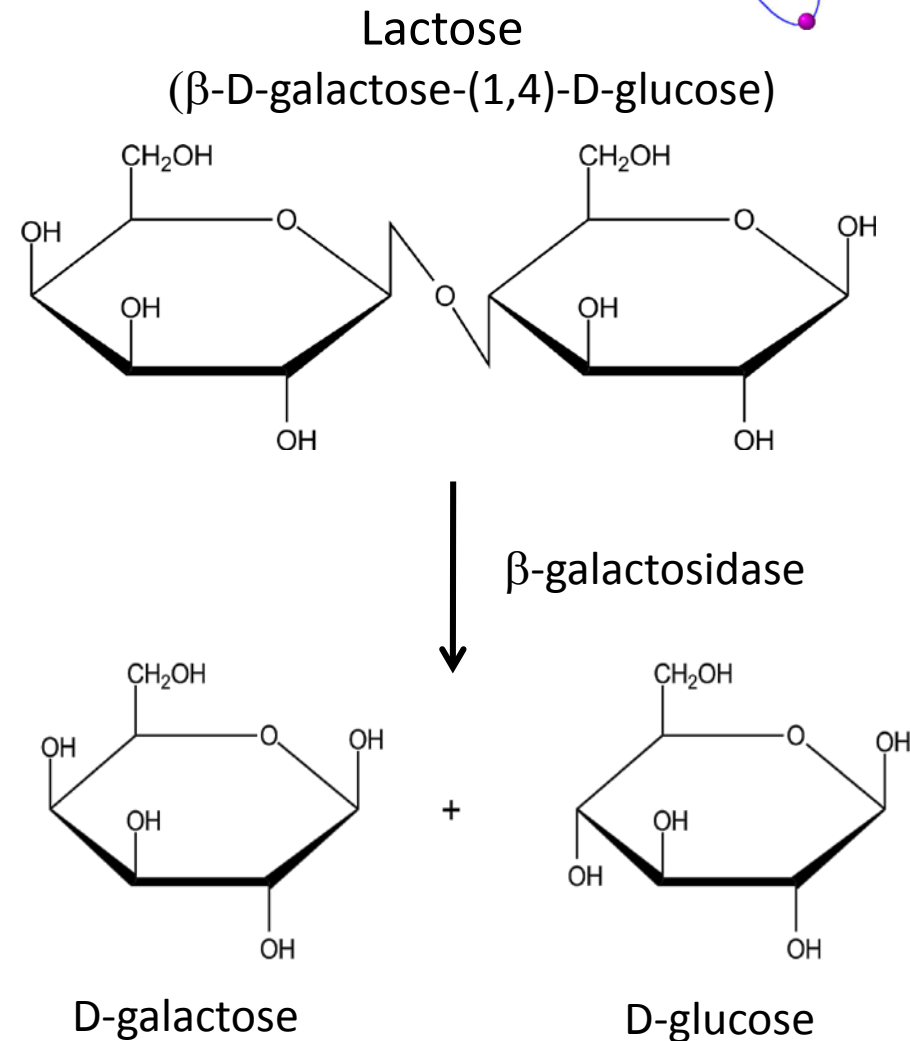


The natural function of the Lac Repressor is to sense and respond to intracellular lactose concentrations.

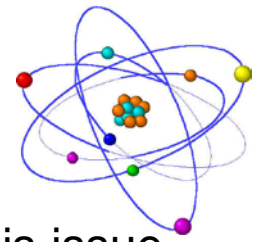
β -galactosidase \rightarrow galactoside hydrolase

E. coli can not make lactose, so only lactose acquired from the media can trigger derepression.

Why might this be problematic?



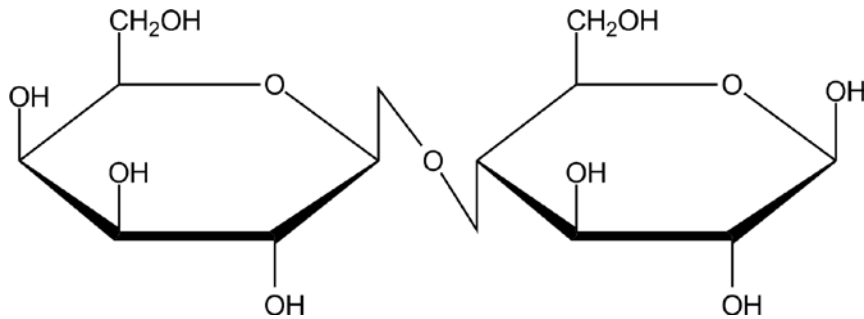
DE3 Lysogen – Lac operon



Non-hydrolyzable lactose mimic, IPTG, is used to avoid the hydrolysis issue.

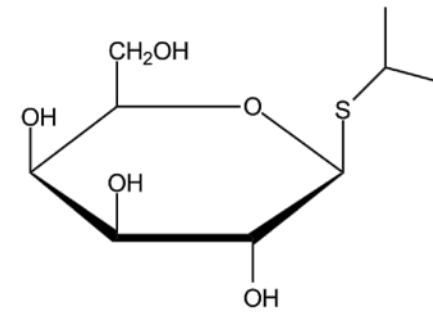
Lactose

(β -D-galactose-(1,4)-D-glucose)

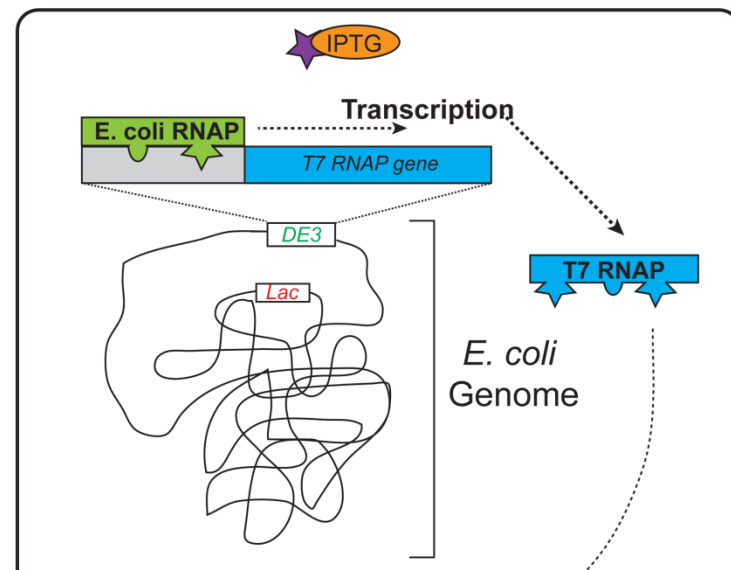
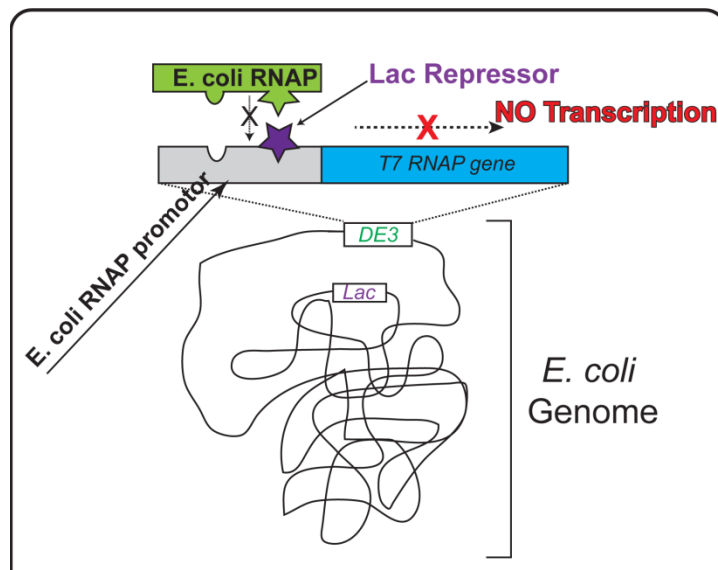


No IPTG

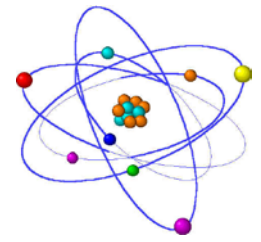
Isopropyl- β -D₁-thiogalactoside



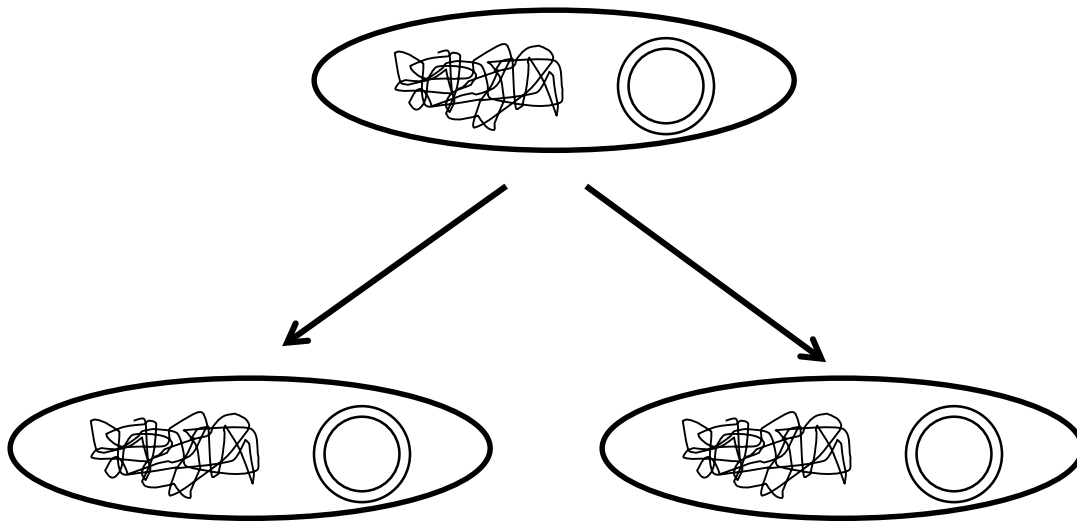
IPTG added



Plasmid DNA

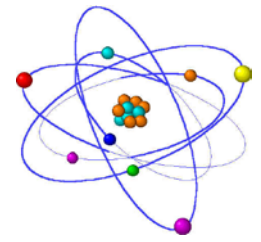


The T7 RNA Polymerase is NOT a natural protein in *E. coli*, so producing it will have no effect unless exogenous DNA is introduced into the cytosol.



A plasmid is a double stranded DNA construct, commonly circular, that is stable in the cytosol of bacteria and can replicate independently of the genomic DNA.

Plasmid DNA

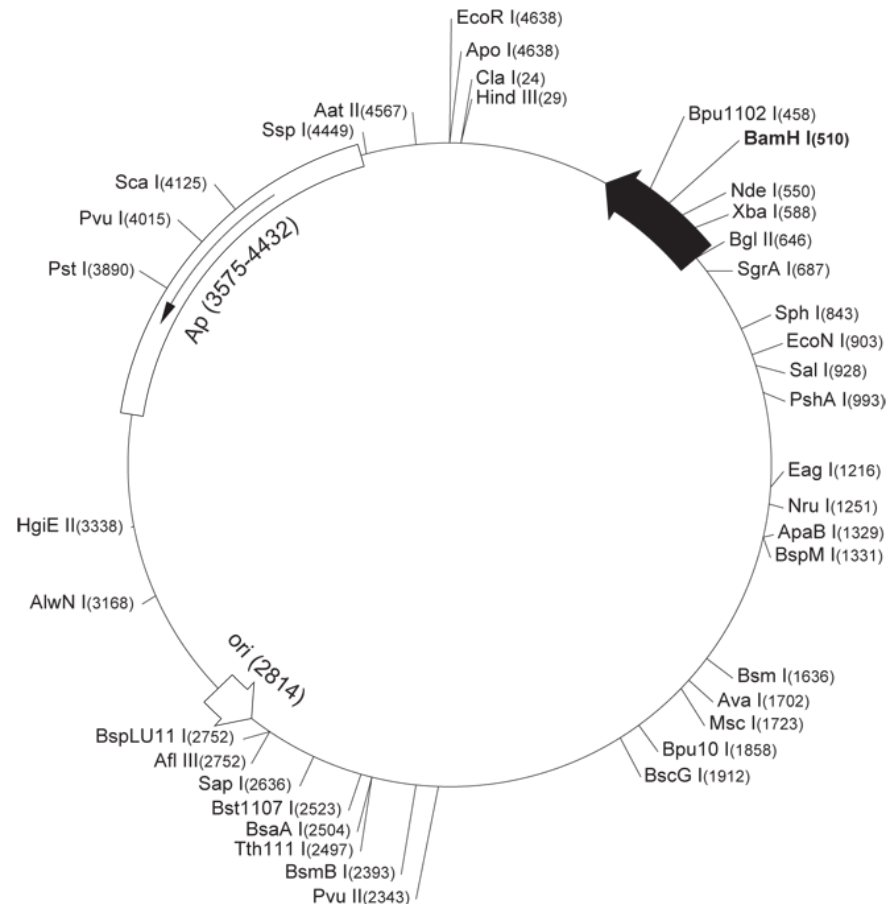


Useful features of plasmids for protein expression

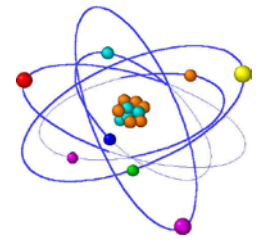
Origin of replication - allows the *E. coli* machinery to recognize and replicate the plasmid.

Antibiotic resistance – allows selection of only bacteria carrying the plasmid

Gene of interest - codes for the protein of interest



Plasmid DNA



Useful features of plasmids for protein expression

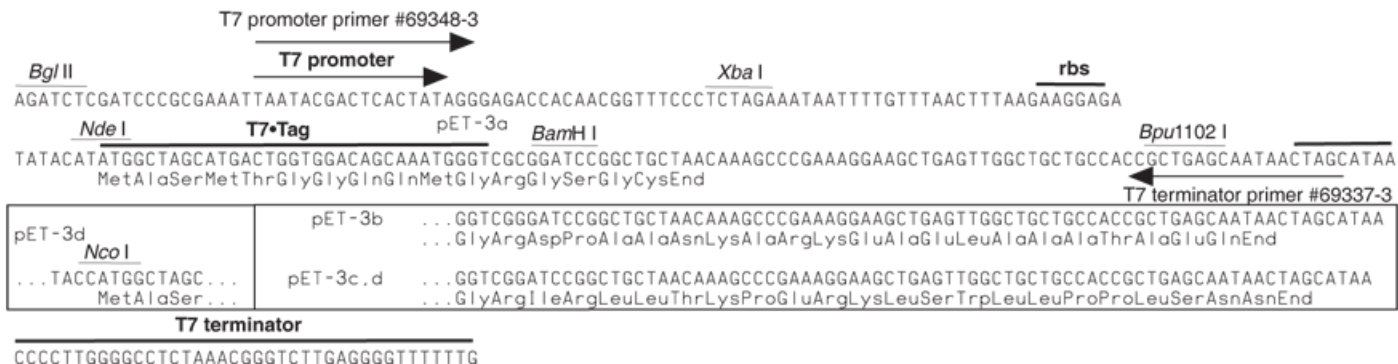
Gene of interest - gets inserted into the multiple cloning site

T7 promoter – Binding site for T7 polymerase

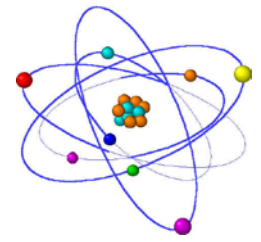
T7 terminator – T7 polymerase will fall off here

RBS – Ribosome binding site

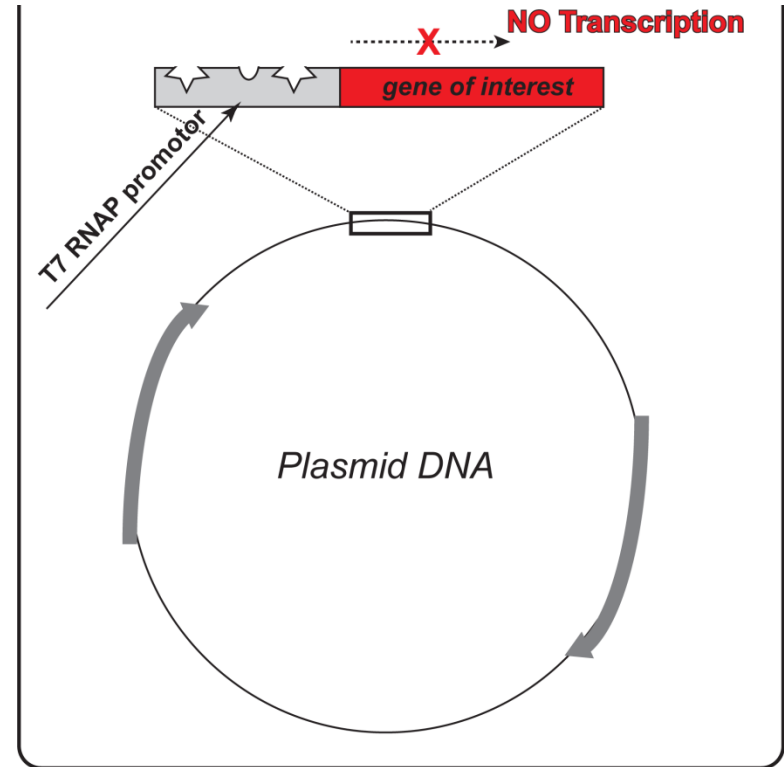
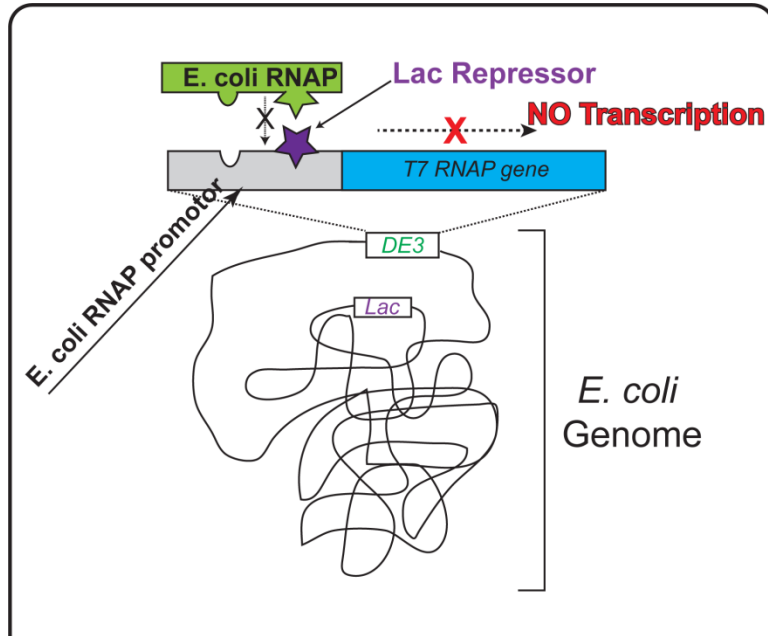
Restriction Sites – Allows the gene to be inserted at the desired location



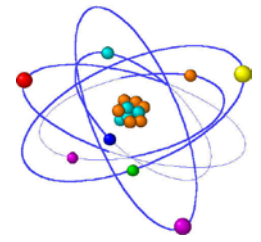
The Big Picture



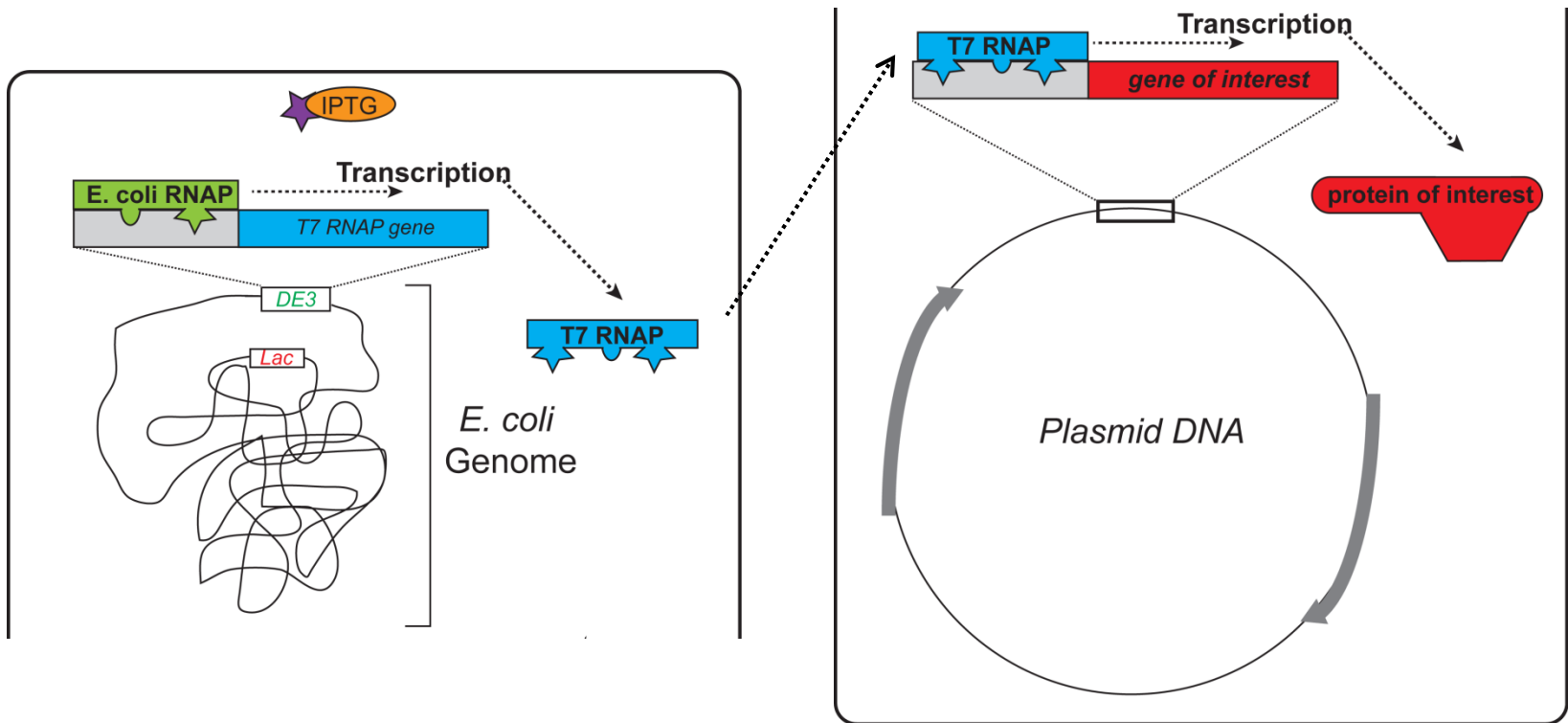
No IPTG



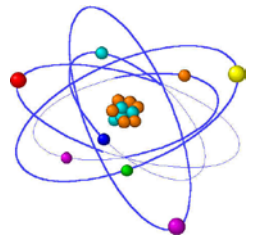
The Big Picture



IPTG Added



This week's experiment:



Do you remember how to make a buffer?