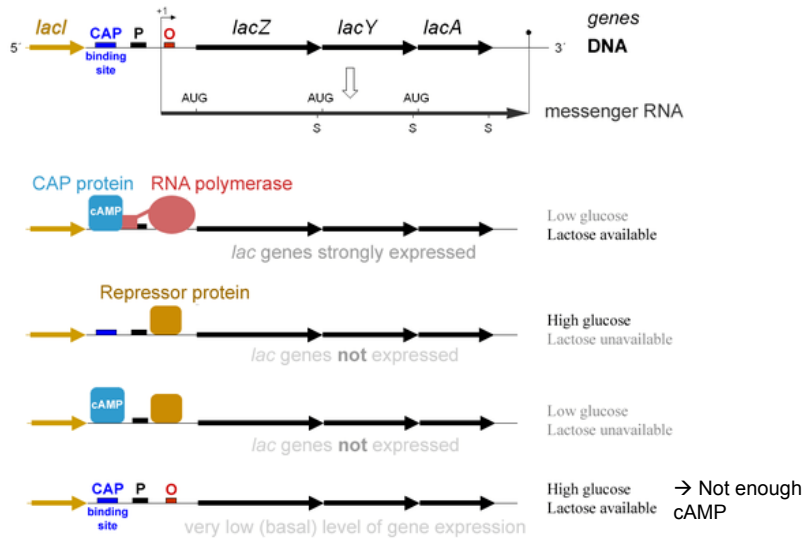
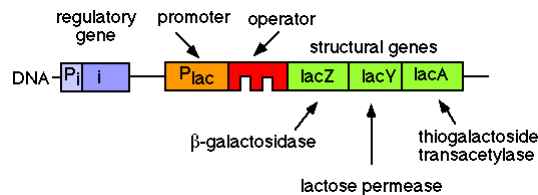


Role of the promoter: Inducible Expression

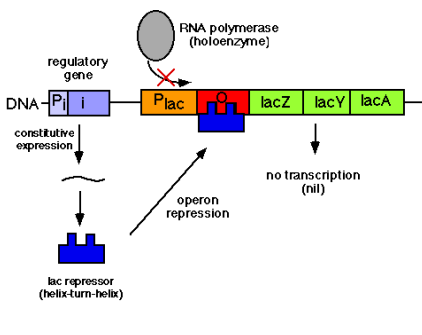
The *lac* Operon and its Control Elements



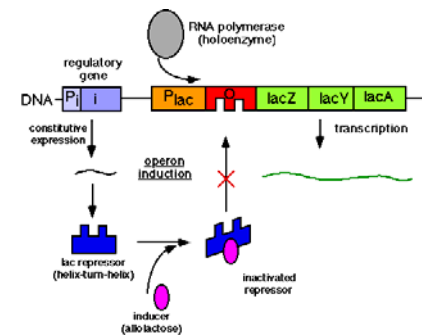
Role of the promoter: Inducible Expression



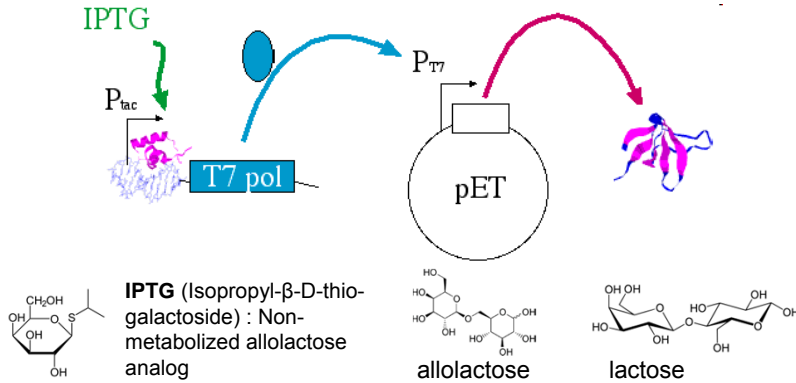
Uninduced (No IPTG)



Induced (added IPTG)

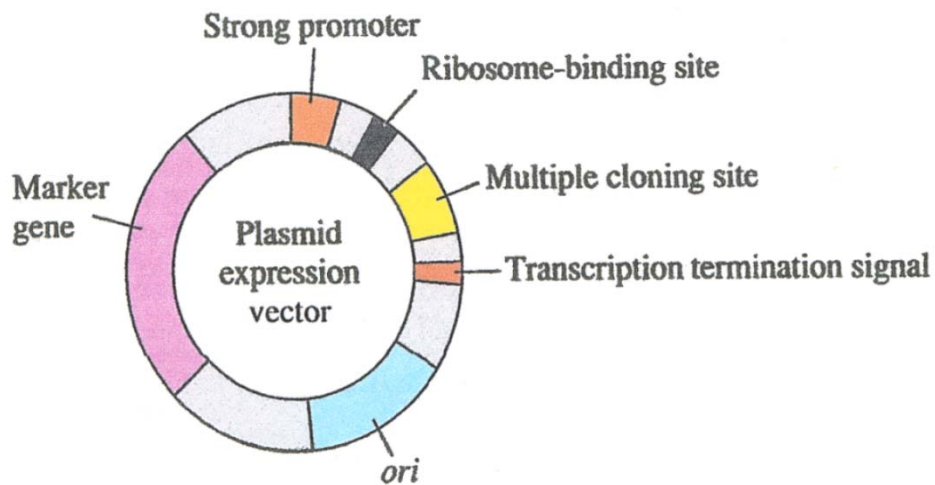


Role of the promoter: Inducible Expression

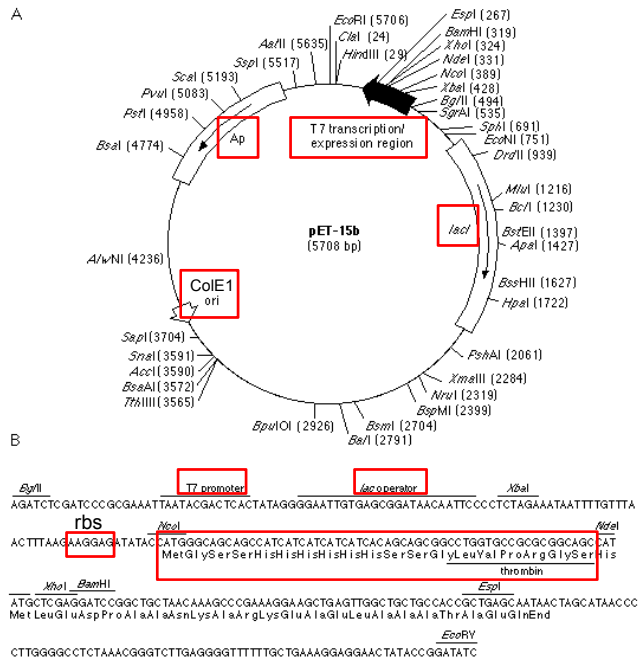


- **T7 promoter:** Promoter to work specifically with T7 RNA polymerase (T7: a bacteriophage = bacterial virus). Present also w/ lac operator
 * To express protein under T7 promoter (e.g., pET vectors), you need an *E. coli* strain with **DE3** in its chromosome (a λ prophage) carrying the **T7 RNA polymerase gene** and lacI to encode lac repressor.
- **Tac promoter:** Trp/lac hybrid promoter. Transcribed by *E. coli* RNA polymerase. E.g., pGEX vectors

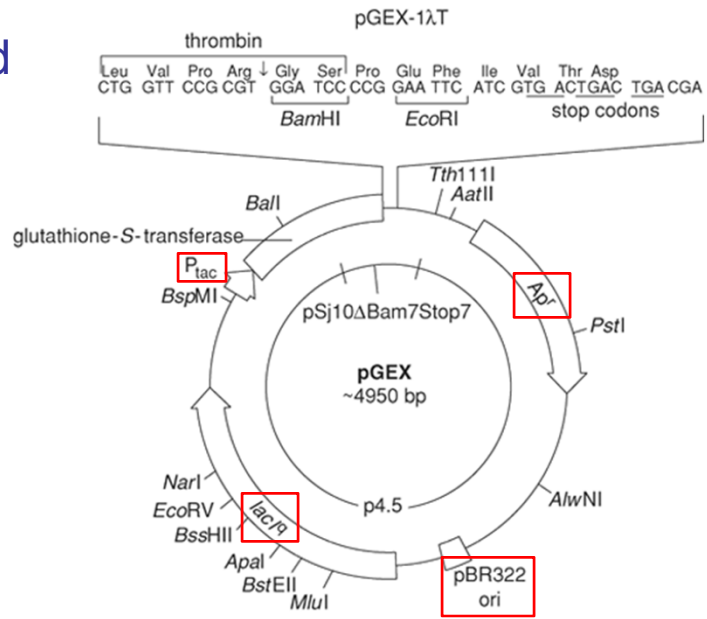
Other Elements



Plasmid map



Plasmid map



Overview of Cloning → Expression

Vector

: Miniprep

*source: transformed bacteria

→ Restriction digest

→ Purify (Agarose gel)

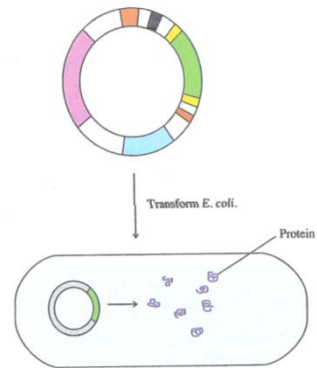
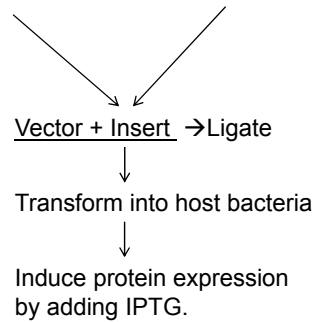
Insert (including gene of interest)

: PCR

→ Purify

→ Restriction digest

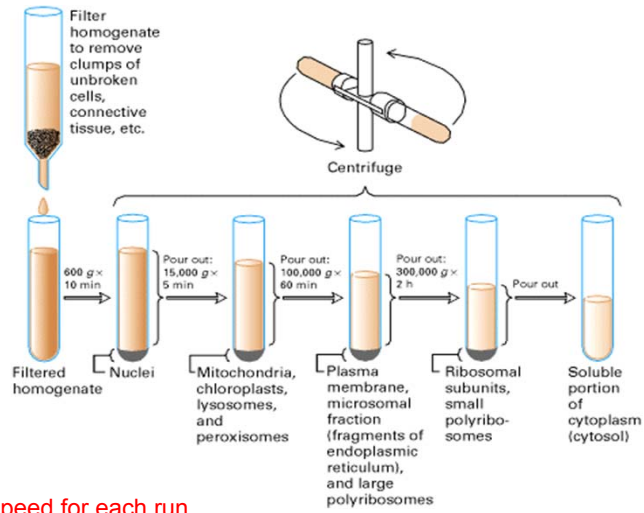
→ Purify



Separation/Purification

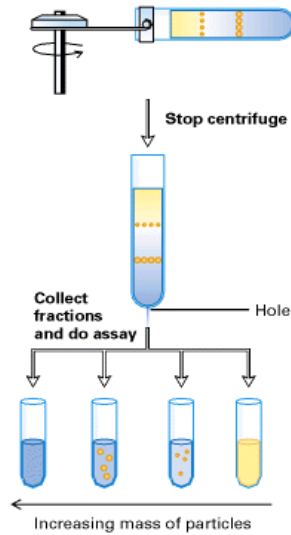
- Centrifugation
- Salting Out
- Chromatography*

Differential centrifugation



*Increase speed for each run

Gradient centrifugation



*Fixed speed at a ultra-high speed, but give a density gradient in the medium using sucrose or CsCl

Fractional Precipitation (Salting out)

- Surface residues interact w/ H_2O (hydration).
- Increasing $[(NH_4)_2SO_4]$ competes with protein residues for available H_2O .
- Protein-protein interaction is promoted through enhanced **hydrophobic interactions** under such high salt \rightarrow Proteins precipitate.
- Not precise, but good way to concentrate.
- Every protein has its own solubility limit in $(NH_4)_2SO_4$.

