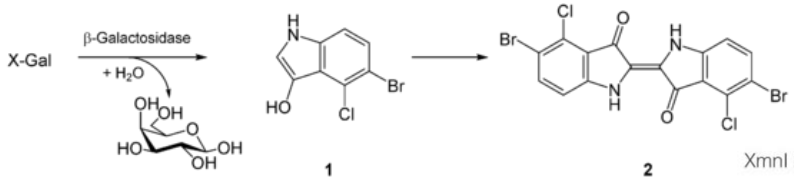
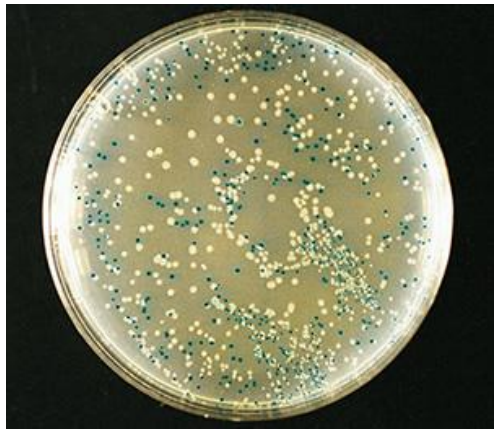
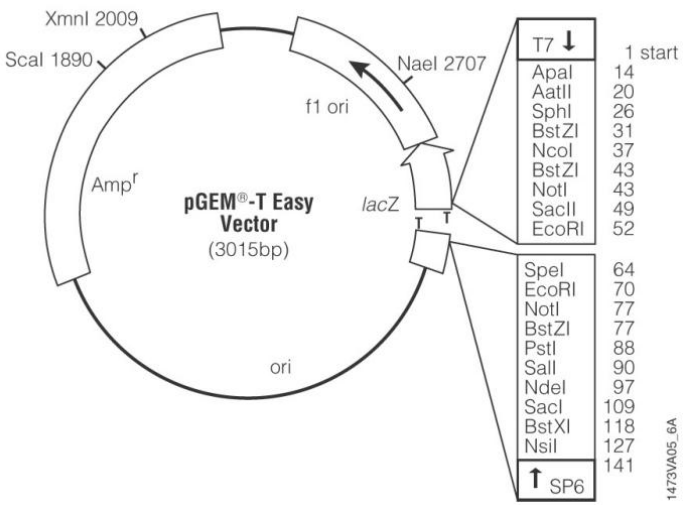
Cloning strategies

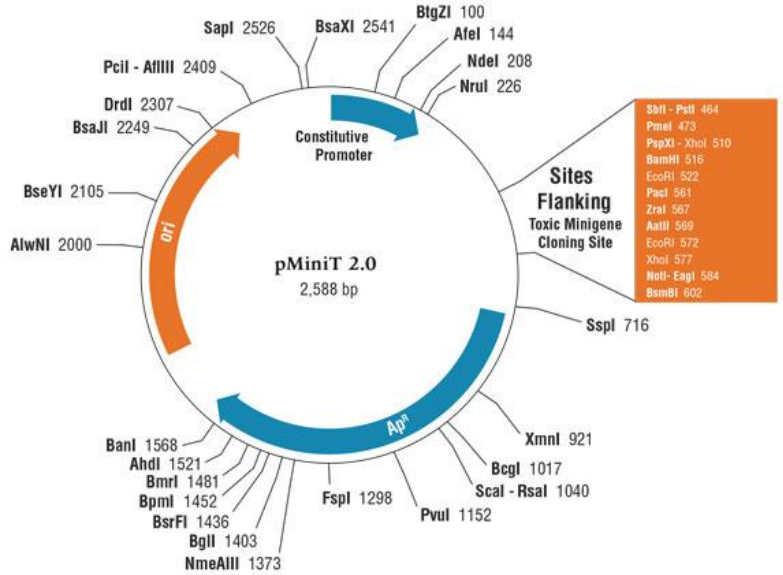
– TA cloning

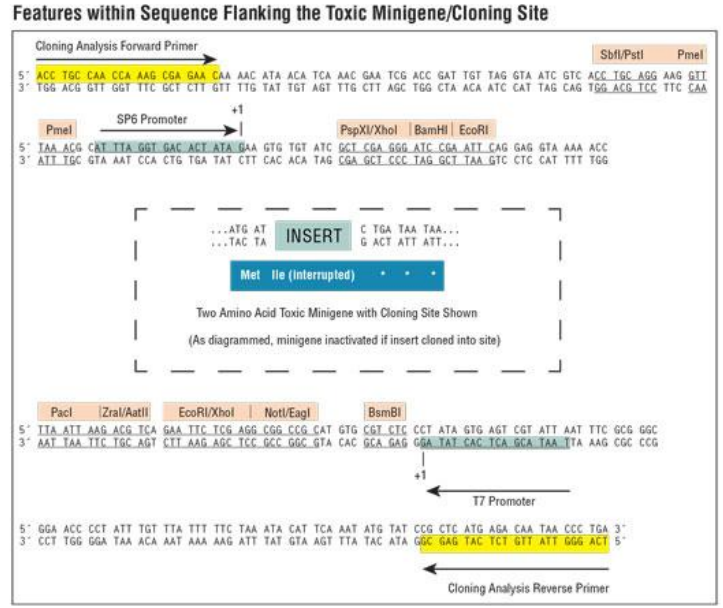
- using the property of Taq DNA polymerase to add A to the 3' end
- **pMiniT 2.0** (toxic mini-genes) (NEB)
- **pGEM-Teasy** (blue-white selection) (Promega)







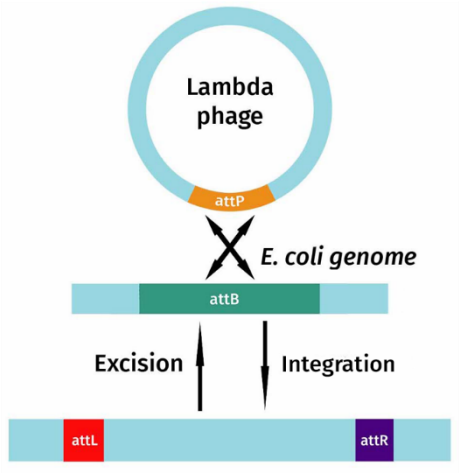


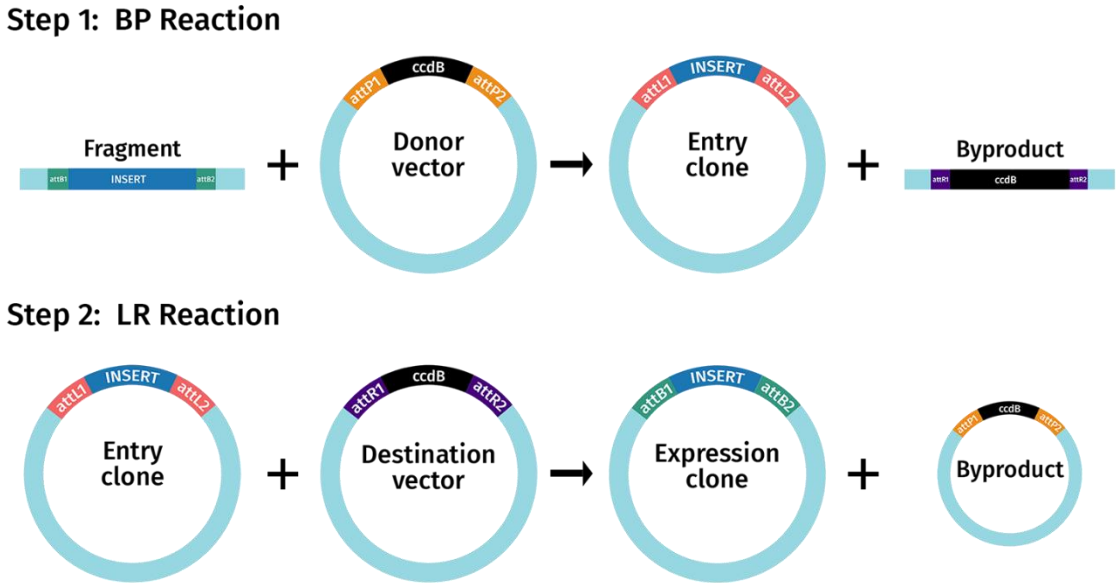


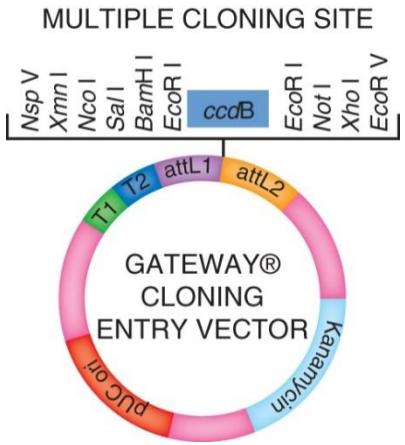
LABORATORY OF MOLECULAR PATHOLOGY

Cloning strategies

- **GATEWAY** cloning vectors (Invitrogen-Thermo)
  - use of phage lambda integrase and excisionase enzymes
  - use of **ENTRY** and **DESTINATION** vectors
  - the BP reaction removes the gene of interest from attR sites and inserts it into attL sites.
  - the LR reaction removes the gene of interest from attL sites and inserts it into attR sites







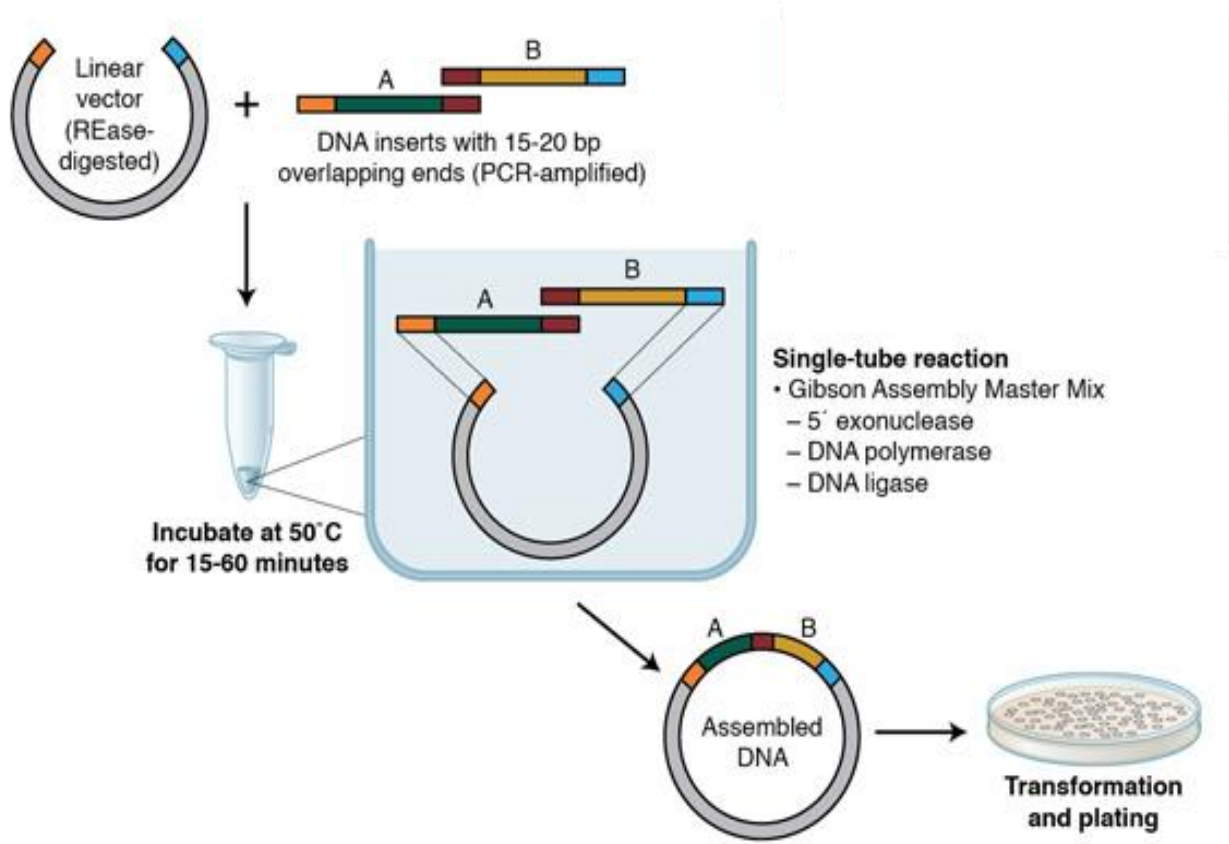
Clark and Pazdernik, 2016

https://www.youtube.com/watch?v=e_ShyrRrufM

LABORATORY OF MOLECULAR PATHOLOGY

Cloning strategies

- In 2009 Dr. Daniel Gibson and colleagues at the J. Craig Venter Institute developed a new method to easily assemble multiple linear DNA fragments
- **Advantages**
  I. There is no need for specific restriction sites.
  II. Join any fragments regardless of order.
  III. The reaction takes place in one tube.
- **Gibson's Mix consists of three different enzymes**
  I. T5 Exonuclease
  II. Phusion DNA Polymerase
  III. Taq DNA ligase

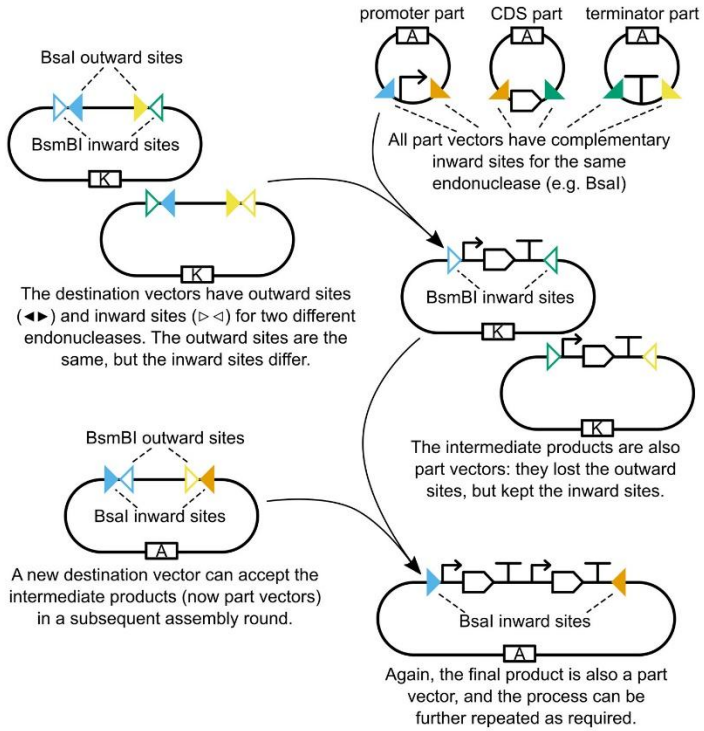


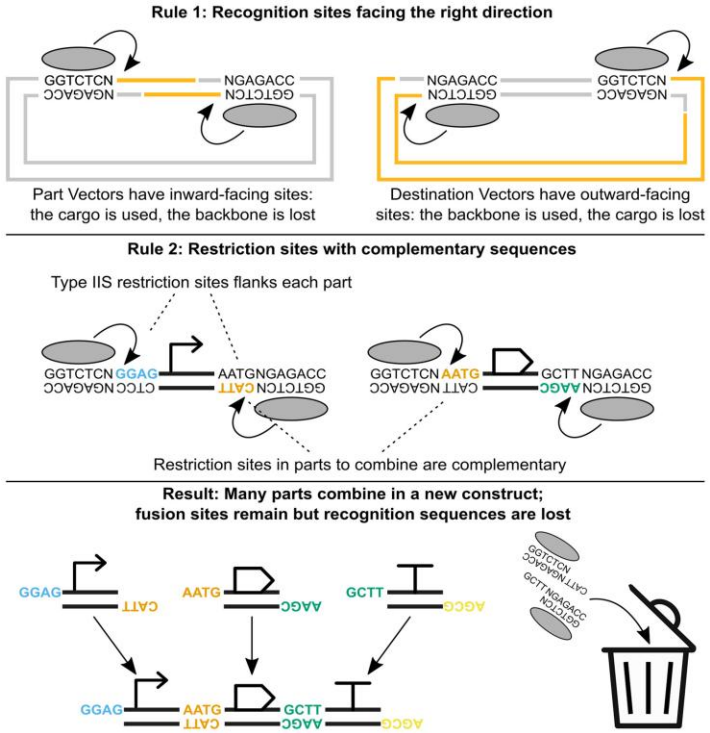
https://www.youtube.com/watch?v=tlVbf5fXhp4

LABORATORY OF MOLECULAR PATHOLOGY

Cloning strategies

- Golden Gate assembly achieves hierarchical assembly of DNA parts by utilizing Type IIS restriction enzymes to produce user-specified sticky ends on cut DNA fragments.



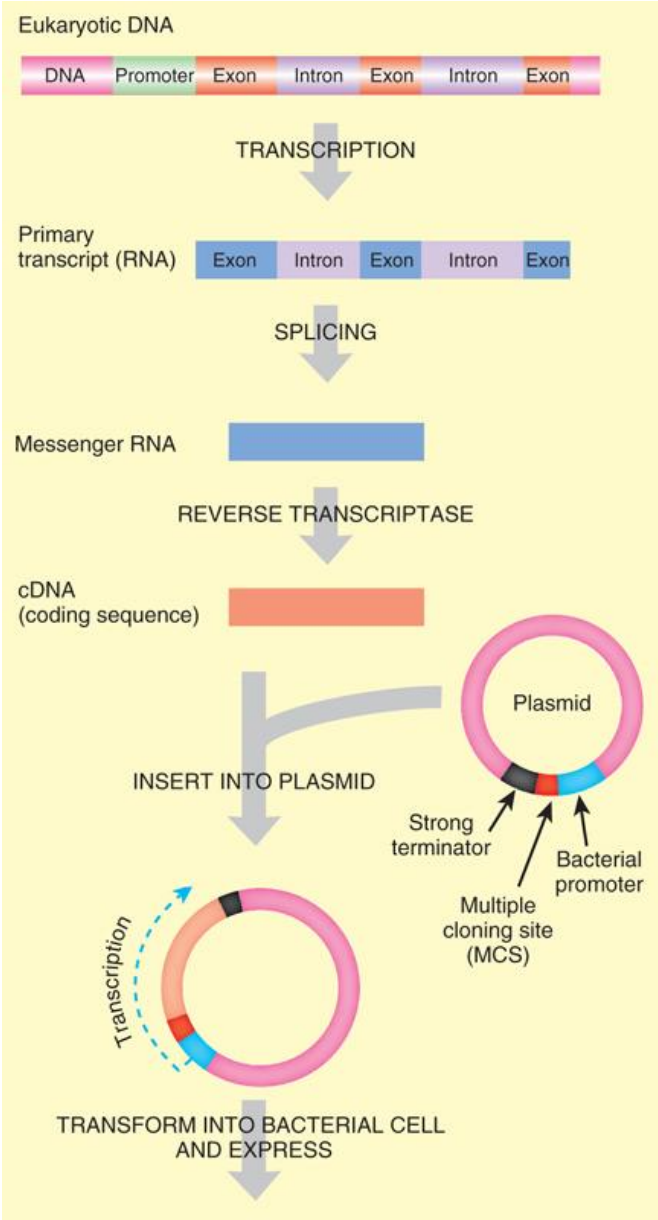


https://www.neb.com/en/tools-and-resources/video-library/golden-gate-assembly-workflow?autoplay=1

LABORATORY OF MOLECULAR PATHOLOGY

Bacterial Expression Vectors

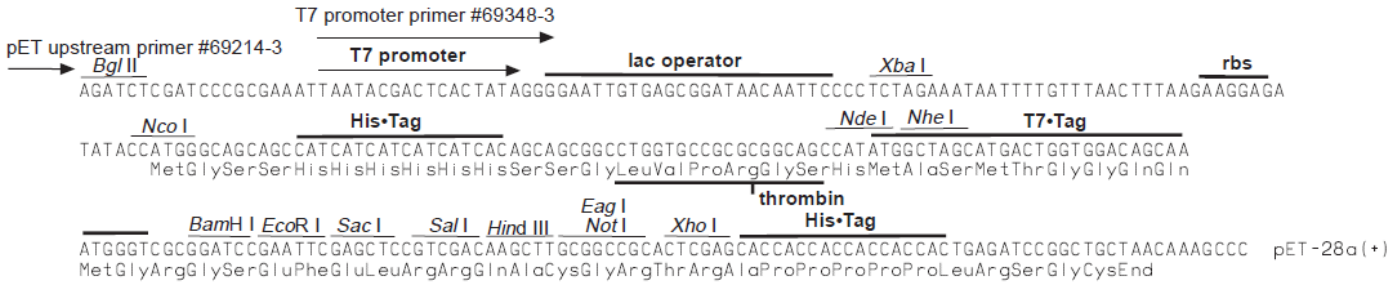
- Special plasmids (expression vectors) are used to increase proteins expression
  - strong promoter, adequate *ori* site, selection marker for antibiotic
- Expression of eukaryotic proteins is more problematic
  - promoter modification, absence of splicing, low rate of translation
  - weak interaction of the ribosome with the RBS site, mRNA instability, limited amount of tRNA
- The necessity of using specially modified vectors

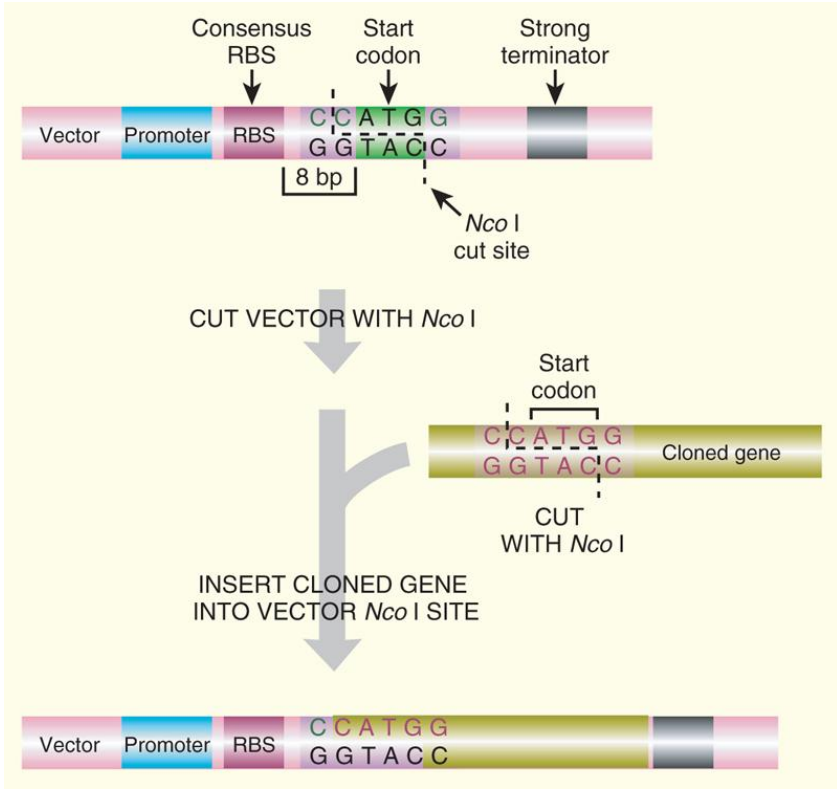


Clark and Pazdernik, 2016

Translational Expression Vectors

- Designed for protein expression
  - maximum translation initialization
  - consensus RBS site
  - ATG codon at an optimal distance of 8 bases from the RBS
  - cloning site directly in the ATG codon (*Nco* I)
- The possibility of further complications in protein folding



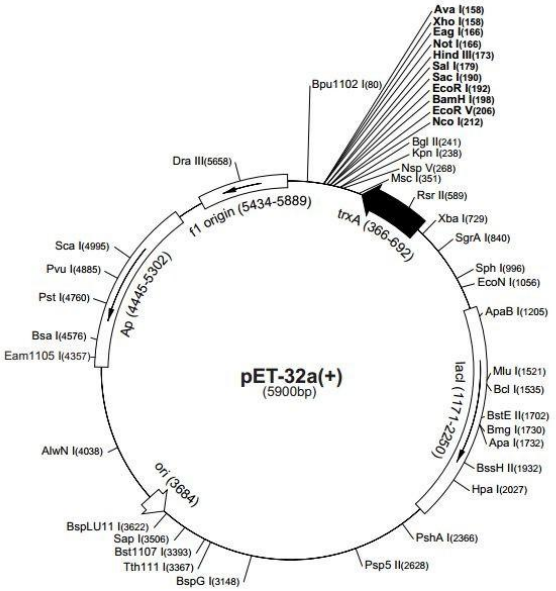


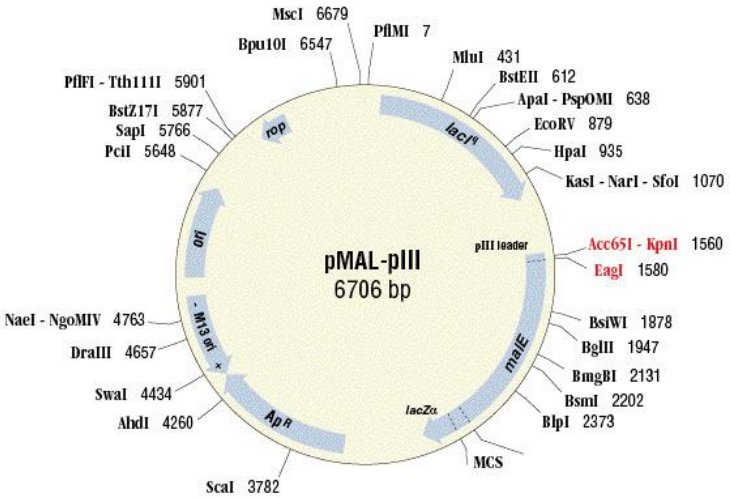
Clark and Pazdernik, 2016

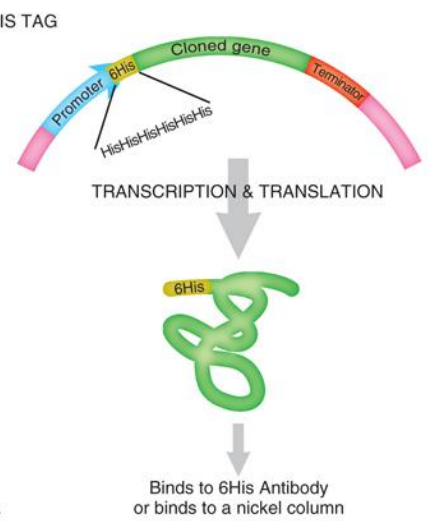
LABORATORY OF MOLECULAR PATHOLOGY

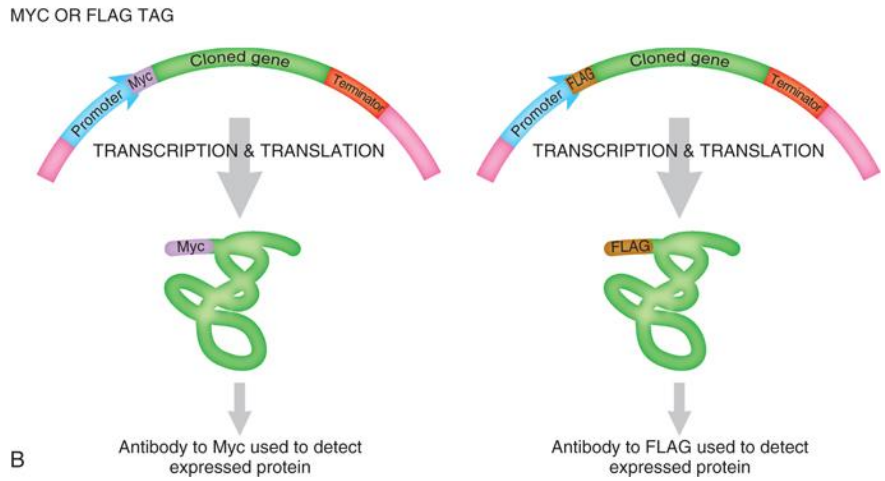
Translational Expression Vectors

- pET, prSET *E. coli* T7 expression vectors
  - expression in BL21(DE3)pLysS cells
- pMAL expression vectors
  - carry maltose-binding protein (MBP)





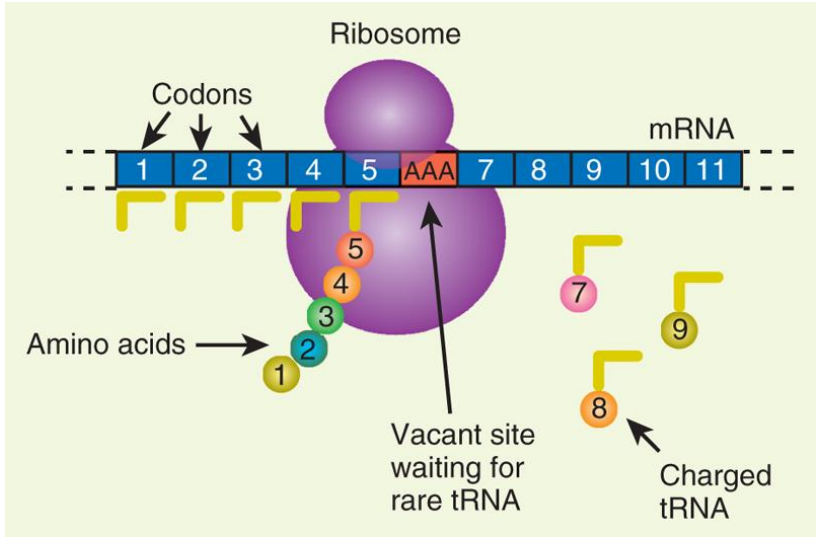




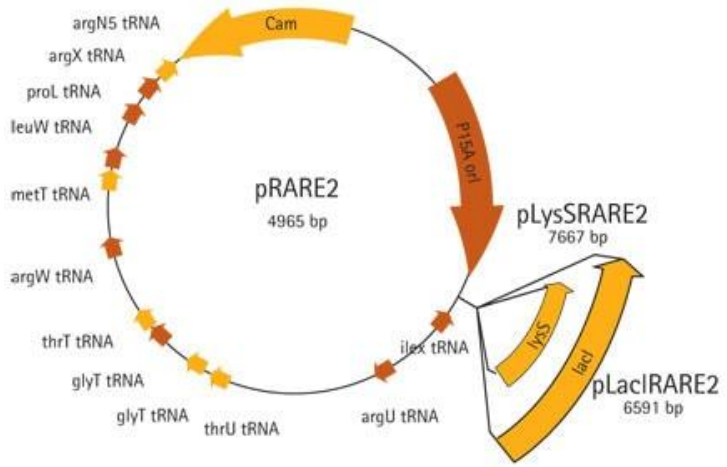
LABORATORY OF MOLECULAR PATHOLOGY

Codons Effect

- Protein expression in other organisms (eukaryotic in bacteria)
- Different organisms prefer different codons for a given AA
  - optimization of the codons used in gene synthesis
  - up to a 10-fold increase in production
  - delivery of tRNA carrying rare codons to the organism
  - ***E. coli* ROSETTA** – seven tRNAs for rare codons (AGA, AGG, AUA, CUA, GGA, CCC, and CGG)



Clark and Pazdernik, 2016



LABORATORY OF MOLECULAR PATHOLOGY