Final Exam, BS99A

Final Exam, BS99B

- There will be assigned seating (charts posted at PSLH doors at 10:00 am).
- Bring your student ID.
- Bring a pencil.
- No calculators, no pagers, no hats, etc...
- Multiple choice AND essay questions will cover lectures since the mid-term.
- After 12:15pm, please don't get up until all exams have been collected.

- Questions regarding grading should be directed ***in writing*** and with a ***detailed explanation*** to the TA.

Please fill out ***teaching evaluations*** (for my section only) and turn them in after today's lecture.

Last Lecture: Expression Systems for Recombinant Proteins

Please report typos, errors etc. by EMAIL (mention the title of this page).

Lecture 9

Expression Systems for Recombinant Proteins

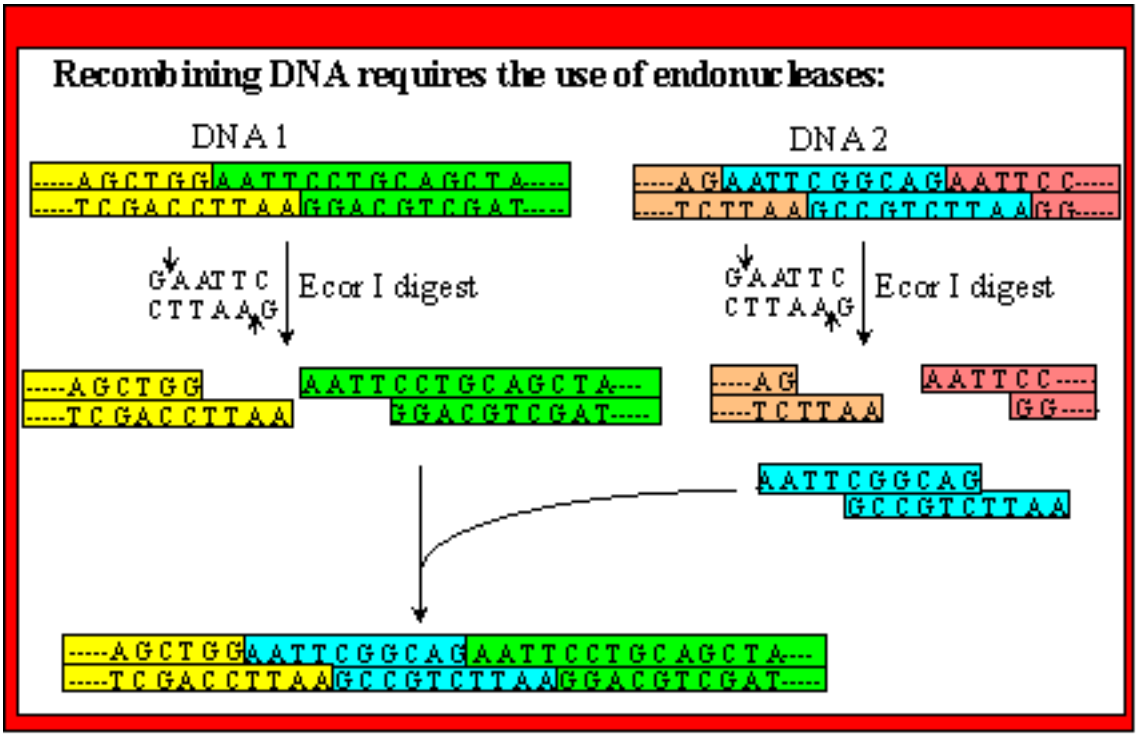
Recombinant protein expression is the foundation of today's biomolecular research and the thriving Biotech industry.

Goal: Overproduction of proteins for structural & functional studies and for medical & industrial applications.

As a 199 research student chances are high that ***you*** will engage in some aspect of ***recombinant protein expression.***

These techniques rely on:

- the ***universality*** of the Genetic Code
- ***knowing*** the Genetic Code
- the relative similarity of the translational machinery (ribosome)
- the rapid progress in molecular biology/genetic engineering over the past few decades:
    - sequence-specific ***nucleic acid hybridization*** (1961)
    - sequence-specific ***cleavage*** of DNA (1962)
    - DNA cloning/amplification (early 70s)
    - DNA sequencing (mid 70s)
    - Cutting and pasting pieces of DNA from one source into another:
        - excise with sequence-specific ***restriction endonuclease*** (more than 100 known, normally employed for defense by bacteria)
        - ***hybridize*** sticky ends
        - reform covalent phosphodiester bonds with ***DNA ligase*** (1967)



Examples of Recombinant Protein Products

- Hormones
  - Insulin: Diabetes
  - Human thyroid stimulating hormone
- Blood clotting factors
  - Coagulation factor VIII : hemophilia A.
  - Coagulation factor IX: hemophilia B.
- Interferons
  - interferon-(alpha)-2a: chronic hepatitis C.
  - gamma interferon: hepatitis B, C, herpes and viral enteritis.

- Immunization agents
  - Hepatitis B vaccine: a non-infectious vaccine derived from Hepatitis B surface antigen (HBSA) produced in yeast cells.

- Research enzymes
  - Restriction endonucleases
  - Endoglycosidases: PNGase F

Next: DNA Cloning

Please report typos, errors etc. by EMAIL (mention the title of this page).

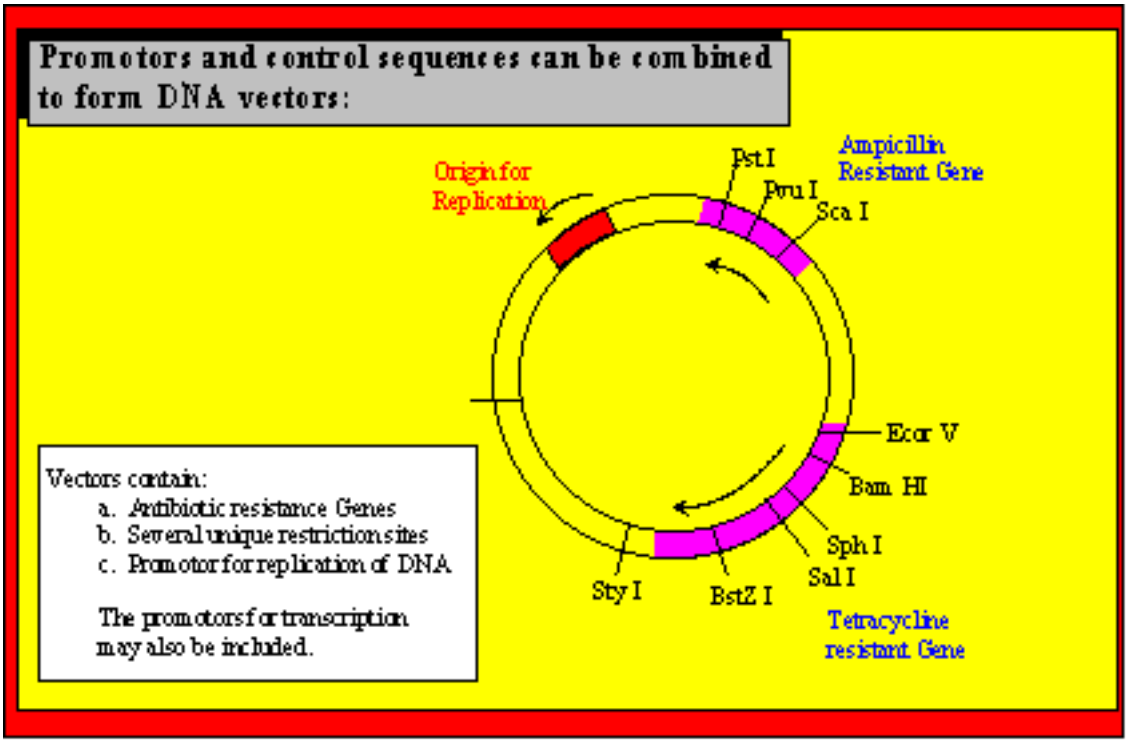
DNA Cloning

Cloning:

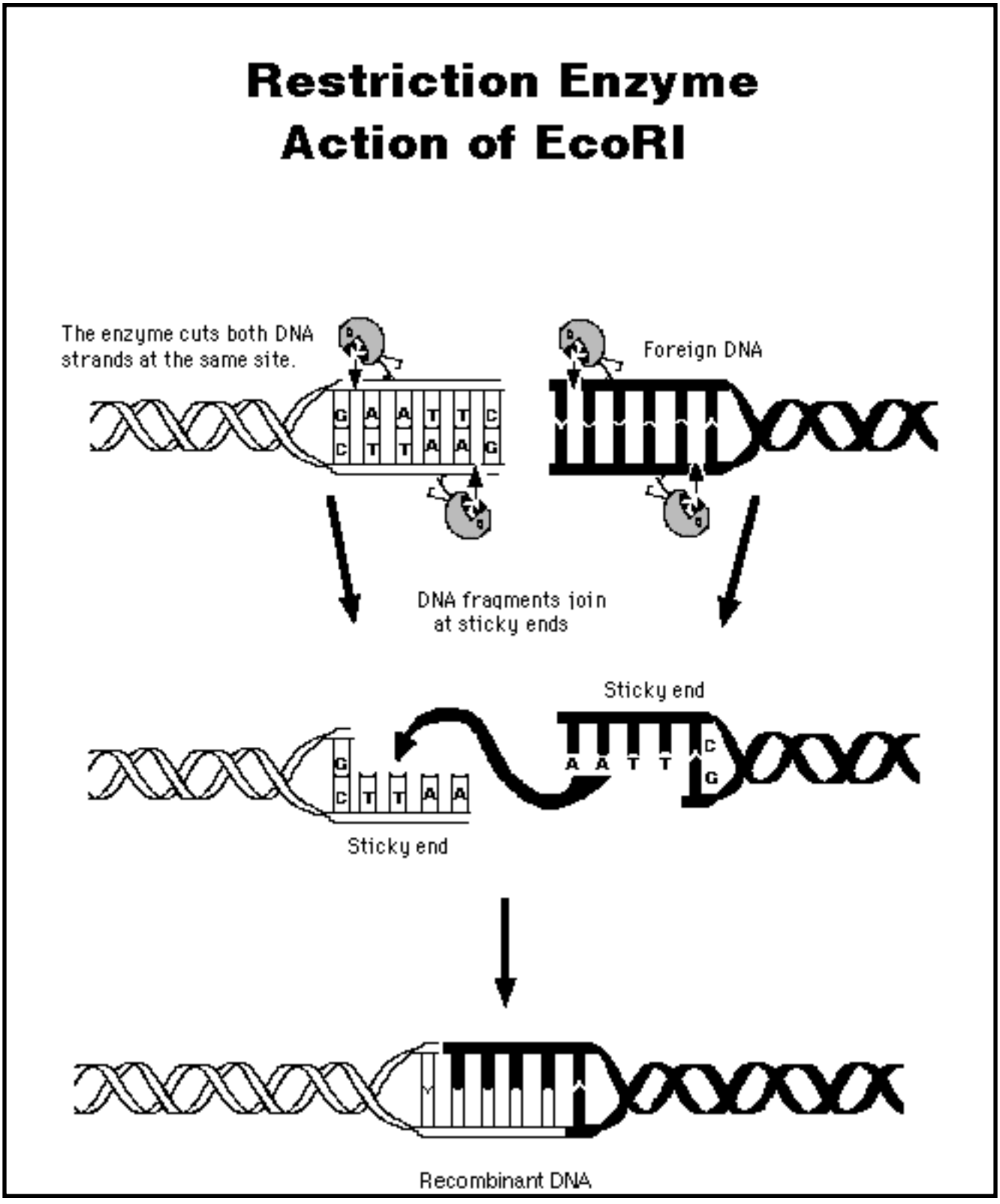
1. Cell fractionation by conventional chromatographic methods to yield about a microgram of protein.
2. The protein is analyzed to yield the identity of the first 30 amino acids - its N-terminal amino acid sequence.
3. The Genetic Code is used to ***predict nucleotide sequences*** corresponding to this amino acid sequence.
4. ***DNA fragments*** complementary to these sequences (oligomers of 15-20 bases) are chemically synthesized.
5. The DNA fragments are then ***hybridized*** (base-paired) ***with total cellular mRNA***.
6. Long cDNA segments are produced from mRNAs complementary to the ***DNA fragments*** using the enzyme ***reverse transcriptase***.
7. Large amounts of this cDNA is obtained by cloning into plasmids (***amplification***).
8. ***Selection*** of the right clone (several steps).
9. Finally, the cloned cDNA is incorporated into an ***expression vector*** or ***plasmid*** and transferred into bacterial or yeast cells.
10. This is the starting point for scaled-up production of large amounts of the protein.

Expression vectors or *plasmids* must contain:

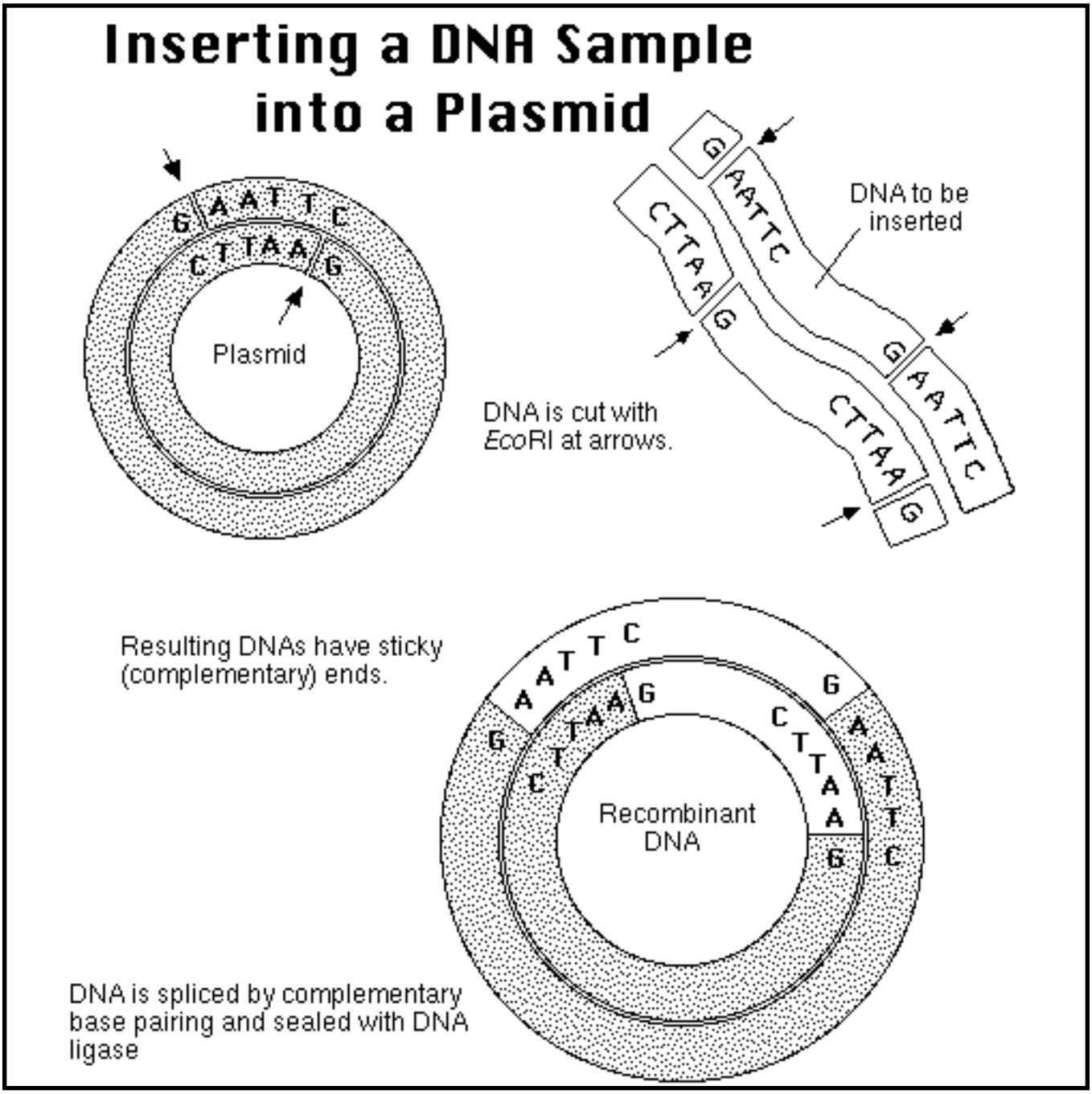
- origin of replication: DNA polymerase
- selectable marker(s): antibiotic resistance
- promoter: recognized by RNA polymerase
- multiple clonig sites (restriction enzyme sites): cutting/pasting of DNA fragments



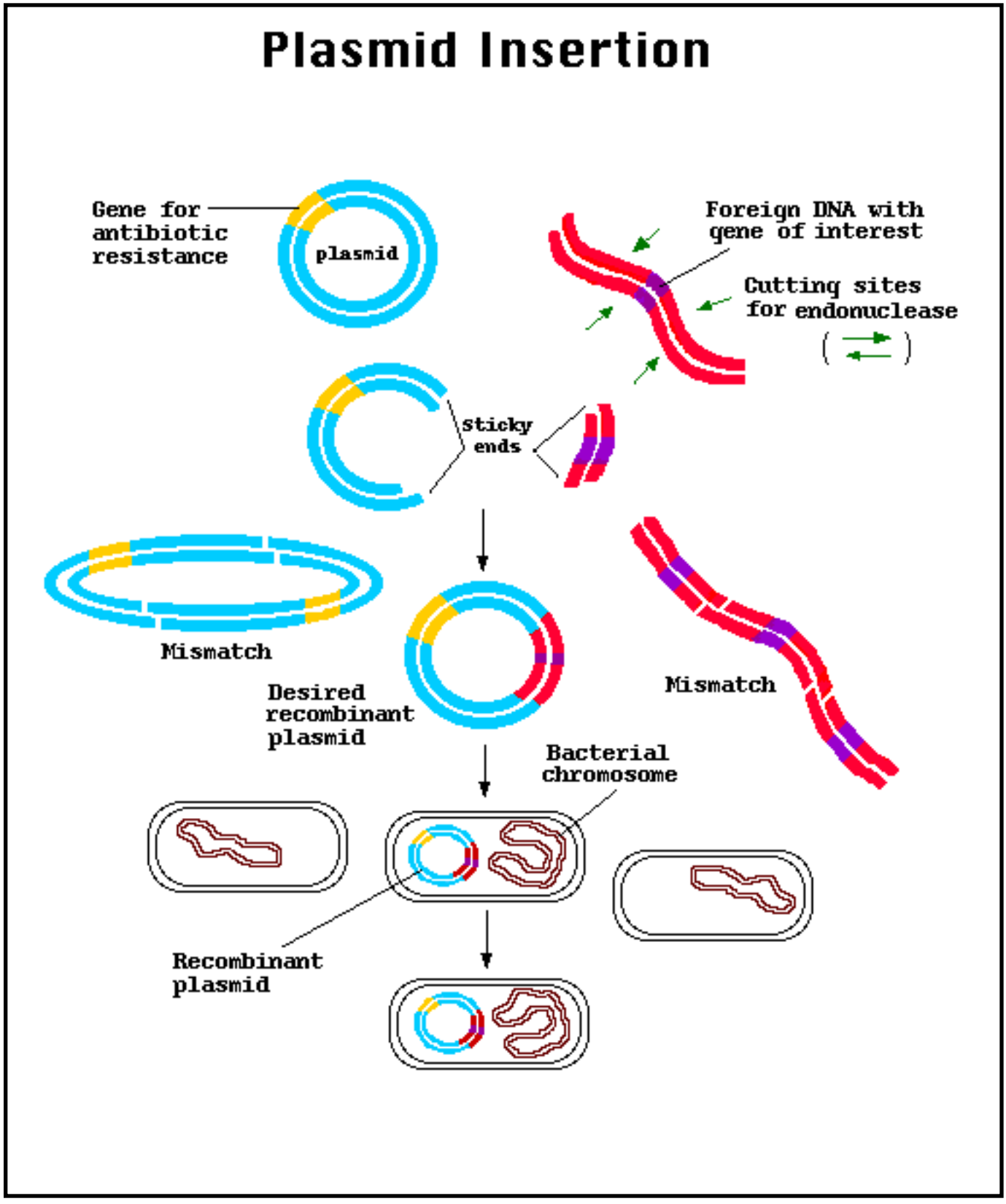
Plasmids are small circular molecules of extrachromosomal, double-stranded DNA. They occur naturally in both bacteria and yeast where they replicate as independent units. Unlike chromosomal DNA, plasmids usually occur as multiple copies.



Restriction endonuclease EcoRI cuts double-stranded DNA and generates sticky ends. Sticky ends hybridize (base-pair) to each other and **DNA ligase** reforms covalent phosphodiester backbone.



A piece of DNA can be inserted into a plasmid if both the circular plasmid and the source of DNA have recognition sites for the same restriction endonuclease.



General scheme for insertion of gene of interest into an expression plasmid.

Next: Expression Systems

Please report typos, errors etc. by EMAIL (mention the title of this page).

Expression Systems

The DNA on the plasmid is transcribed to mRNA which in turn is translated to protein.

Types of expression systems:

- Bacterial: plasmids, phages
- Yeast: expression vectors: plasmids, yeast artifical chromosomes (YACs)
- Insect cells: baculovirus, plasmids
- Frog oocytes: injected mRNA
- Mammalian:
  - viral expression vectors (gene therapy):
    - SV40
    - vaccinia virus
    - adenovirus
    - retrovirus
  - Stable cell lines (CHO, HEK293)

Bacterial expression systems:

- Usually ***E. coli***
- A specific gene on a plasmid can produce 1-30% of the total protein.
- For ***native protein***:
  - Ligate gene to bacterial promoter on plasmid.
  - Watch for frame shift!
- For ***fusion protein***:
  - DNA for gene of interest is inserted after the 3' or before the 5' terminus of "carrier" gene (GST, GFP).
  - Watch for frame shift!
  - Advantages over native protein expression:
    - synthesized at high levels like a normal bacterial gene
    - often results in more stable product than native protein
    - fusion protein is generally larger than most ***E. coli*** proteins: easy identification & purification
    - exploit functional features of carrier protein in purification
    - **Caveat:** often low yields when cleaving carrier from target protein with protease
- For ***His-tagged protein***:
  - By mutation introduce multiple histidine codons (6 or more) at the N-terminus or C-terminus
  - Purification over Nickel column: only proteins with poly-His tag will bind tightly

Common problems with bacterial expression systems:

- Low expression levels:
  - change promoter
  - change plasmid
  - change cell type
  - add rare tRNAs for rare codons on second plasmid

- Severe protein degradation:
  - use proteasome inhibitors and other protease inhibitors
  - try induction at lower temperature

- Missing post-translational modification: co-express with kinases etc.

- Glycosylation will not be carried out:
  - use yeast or mammalian expression system

- Misfolded protein (inclusion bodies):
  - co-express with GroEL, a chaperone
  - try refolding buffers

Next: Summary

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Summary

- Recombinant protein expression relies on the ***universality*** of the Genetic Code
- It employs a host of sophisticated techniques to analyze, manipulate and copy DNA (***genetic engineering***)
- The gene of interest (DNA) is cloned into an ***expression plasmid*** or ***vector***
- The DNA on the plasmid is transcribed to mRNA which in turn is translated to protein

Next: Final Exam

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