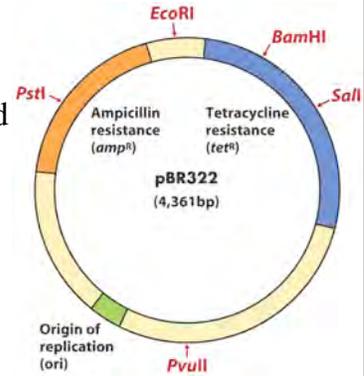


Chem 431C Lecture 10c

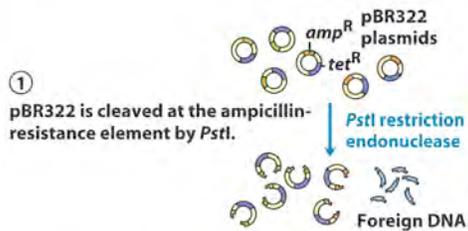
Today:

- Quiz 9:50 - 10:00
- Specific topics in Chapt 9 10:00-10:30
- Review for Final; 10:30-10:40

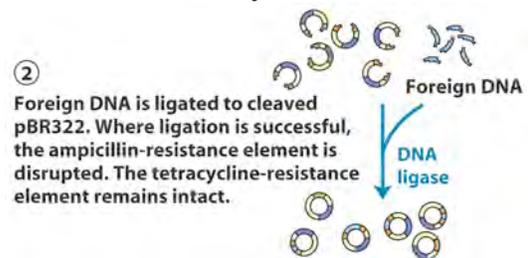
Constructed E.coli plasmid pBR322



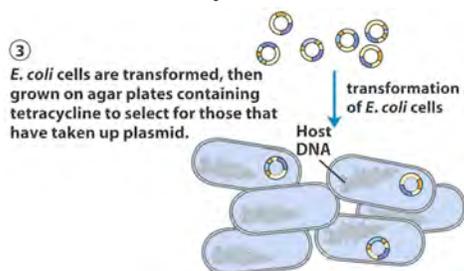
Use of pBR322 to clone foreign DNA & identify transformed cells



Use of pBR322 to clone foreign DNA & identify transformed cells

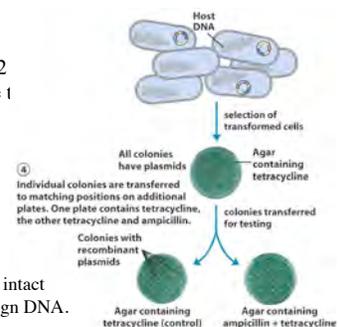


Use of pBR322 to clone foreign DNA & identify transformed cells



First, select transformed cells containing pBR322 using its resistance to tetracycline. Note that *amp* and *tet* genes serve as "markers".

Missing "spots" lack intact *amp* => contain foreign DNA.



Cloning vectors and amplification

4 cloning vectors used in E.coli:

- 1) Plasmids
- 2) Bacteriophages
- 3) Bacterial artificial chromosomes (BACs)
- 4) Yeast artificial chromosomes (YACs)

Bacteriophages

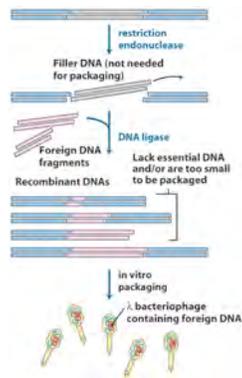
Bacteriophages

-efficient for delivering 45 kbp of DNA into bacterium

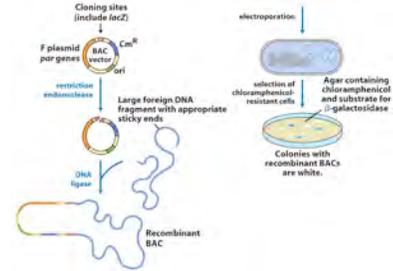
-2 key features of importance:

- a) 1/3 λ genome nonessential & replaceable with foreign DNA
- b) DNA packaged as infectious phage only if 40 k bp - 53 kbp long (constraint ensures packaging of recombinant DNA only).

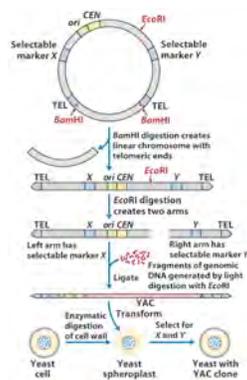
Bacteriophages



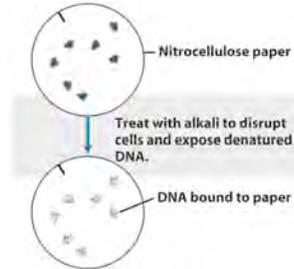
Bacterial Artificial Chromosomes (BACs)



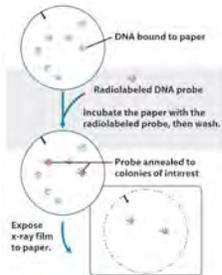
Yeast Artificial Chromosomes (YACs)



Detection of specific DNA sequence by hybridization



Detection of specific DNA sequence by hybridization



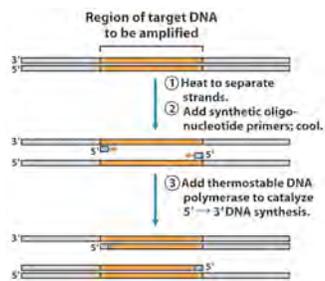
PCR = polymerase chain reaction

PCR amplifies specific gene sequences

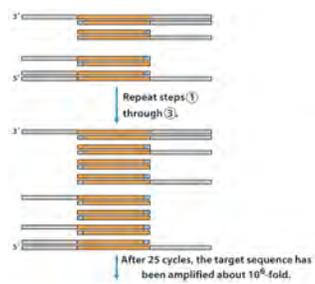
3 steps :

- DNA separated by heating
 - annealed to an excess of short synthetic DNA primers
 - new DNA synthesized by polymerization
- these steps are repeated 25-30 cycles
DNA pol TaqI (from thermophilic bacteria) used

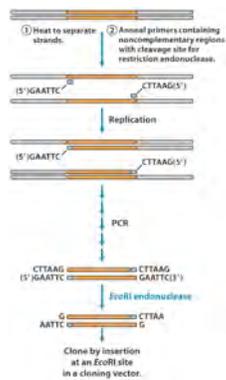
PCR continuation...



PCR = continuation...



DNA amplified by PCR can be cloned



The End

The Final Exam

Guidelines will be posted in chem 431c website.
Please note that they represent goals - as far as the % distribution of the points.

Emphasis will be on depth and breadth of knowledge not on memorization.

Concepts will be tested. Comparisons. Creative thinking - what happens if...?

The exam...

Wednesday, Dec 10, 2009 at 8 am - 10:30 am

150 points

Chapters 8, 22, 24-28, 9

Roughly 50% of exam is on post Test 2 material

Emphasis on Replication, Transcription, Translation

No multiple choice. Short answer and Essay type questions

Be prepared to "draw" cell components to illustrate mechanisms.

The exam...

Detailed very short answer type of questions like...

1. Write down the balanced equation catalyzed by nitrogenase.
2. What key ribozyme is involved in translation?
3. _____ is the central enzyme in the entry of reduced nitrogen in most organism. It is regulated by _____ (type of regulation).
4. The enzyme that catalyzes the formation of deoxyribonucleotides from ribonucleotides is _____ and it has two different regulatory sites: the _____ and the _____ sites.

The exam...

Some brief answer questions might be like:

- 1) State the purpose of PCR. In a sentence, describe notable features in the technique.
- 2) The main difference between type I and type II restriction endonucleases is...
- 3) 5 different processes (and thus potential points of regulation) which affect the steady state concentration of a protein are:
- 4) In the regulation of an operon, what differentiates positive from negative regulation is...

The exam...

Medium size questions (some are comparisons)

- 1) Does the double helix model of Watson and Crick logically imply the following?
 - a) Chargaff's Rules of DNA base composition
 - b) Meselson and Stahl's experimental results
- 2) Compare and contrast telomerase, TaqI DNA pol I and RNA replicase

The exam...

Longer questions:(some are open ended)

- 1) Illustrate the replication fork. Would it be more efficient if a cell had a hypothetical DNA polymerase that could synthesize in the 3' -> 5' direction?
- 2) What happens when replication and transcription "collide"? What are the implications for the directionality of transcription in relation to replication?
- 3) Describe the status of the lac operon when neither glucose of lactose are present? Is repression of lac operon absolute? Comment on the advantage of your answer to the cell.
- 4) What is a necessary discovery before you can propose a "Protein (not RNA) World"? Explain.

Have a good review, a successful
Final Exam and good holiday
break!