Recitation Section 17 Answer Key
April 11-12, 2005

Recombinant DNA and Cloning

A. The Tools

Recombinant DNA is a set of tools that allows scientists to move between genetics, biochemistry and molecular biology – allowing us to determine how the parts of a cell or organism work.

1. What does it mean to clone a gene?
Cloning DNA means to isolate a gene or fragment of DNA away from the other DNA of an organism and be able to propagate this piece.

2. What is a vector?
Vectors (or plasmids) are naturally occurring small pieces of circular DNA that can replicate in various organisms. In this course we concentrate on plasmids, which are designed to propagate in microorganisms.
3. What do the restriction enzymes do? How are they physically able to perform this function?
Restriction enzymes (naturally occurring in bacteria) make it possible to cut DNA at predetermined sequences without damaging the DNA sequence.
All interactions in biology are based on shape and chemical identities of the interacting surfaces. Restriction enzymes recognize particular sequences in DNA by making physical contact with the portions of the bases exposed in the grooves of the DNA molecule. Once properly positioned over the recognition site, the enzyme is able to perform a symmetric cut.

4. What is a library?
A library is a set of vectors containing DNA inserts that collectively represent all of the DNA in a genome or all of RNAs expressed in a cell. Each vector has only one piece of DNA or cDNA, but collectively the vectors contain all of the information.

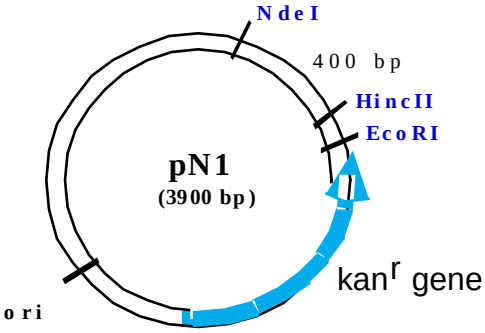
5. What three features must vectors or plasmids have? Why?
Plasmids and vectors have origins of replication that allows for replication in organisms.
A plasmid or a vector has a restriction site recognized by a restriction enzyme, where a piece of DNA can be inserted.
A selectable marker to allow us to select or screen the library for the presence of an organism containing the nucleic acid fragment of interest.

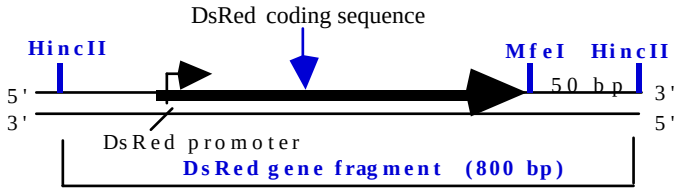
6. Where did the techniques and reagents used in recombinant DNA applications come from?
Many of the techniques and reagents used in recombinant DNA technology are adaptations of processes that exist in nature.

7. What is the purpose of the recombinant DNA technology?
Recombinant DNA technology allows scientists to manipulate the genetic composition of a cell. This allows us to create organisms of interests and study their properties. For example, the technology allows us to find the nucleic acid fragment encoding the gene of interest or to express large quantities of the protein of interest in a new species or under a different set of conditions.

B. Application

You have been given a purified DNA preparation of pN1, a 3900bp circular plasmid, which contains a bacterial origin of replication (ori) and the gene for kanamycin resistance (kan^r). In addition, you also have a preparation of an 800bp linear DNA fragment (isolated from a HincII restriction digest of DNA from another source) that contains the entire gene for DsRed (a red fluorescent protein). These two DNA molecules with their known restriction enzyme sites are shown below:





The restriction enzymes listed below cut the following sequences:

HincII cuts*: 5'-GTPy|PuAC-3' / 3'-CAPu|PyTG-5'

NdeI cuts: 5'-CA|TATG-3' / 3'-GTAT|AC-5'

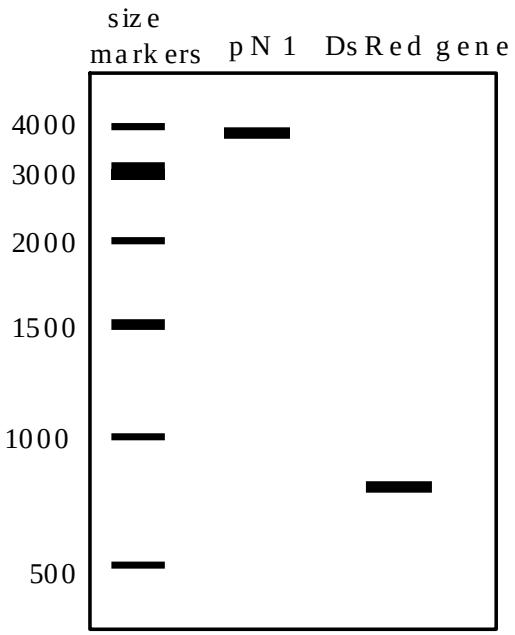
MfeI cuts: 5'-C|AATTG-3' / 3'-GTTAA|C-5'

EcoRI cuts: 5'-G|AATTC-3' / 3'-CTTAA|G-5'

* Py = pyrimidine, either T or C
Pu = purine, either A or G

You want to produce a new plasmid, which will be called pDsRedN1. It will contain genes that confer both kanamycin resistance and red fluorescence. To accomplish this task, you have available the four restriction enzymes, HincII, NdeI, MfeI, and EcoRI. The recognition sequences where the enzymes cut the DNA are shown above.

1. To begin the cloning process, you digest both pN1 and DsRed DNA fragment with HincII and separate the DNA samples by size on an agarose gel. Draw the pattern you would predict on the diagram below.



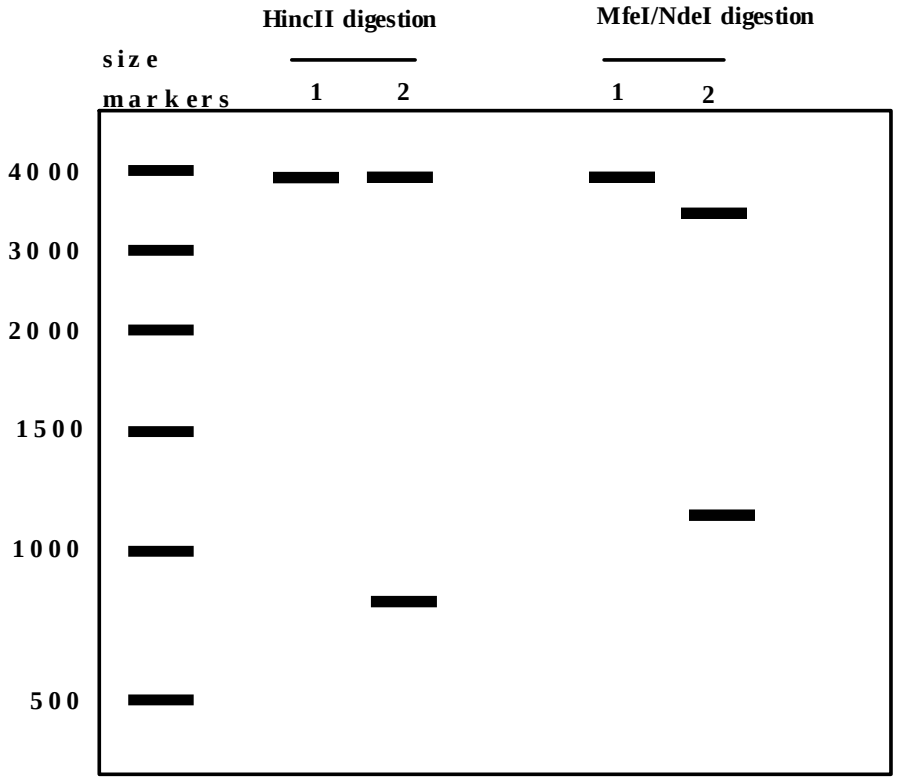
Under special conditions, plasmid DNA can enter *E. coli* cells. The plasmid DNA functions as normal DNA, *i.e.*, genes on the plasmid can be transcribed and translated.
E. coli cells that have incorporated a plasmid are said to be transformed.

2. Where have we encountered a transformation before?
In the Griffith and Avery experiments, live but not virulent bacteria mixed with dead virulent bacteria gave rise to the live virulent bacteria. The process was termed transformation, and the non virulent bacteria were said to be transformed with the genetic material of the dead virulent bacteria. Avery determined that the genetic material was DNA.

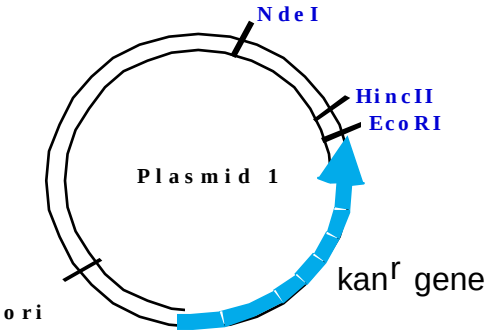
You isolate the DNAs from the gel, mix them together in a tube and ligate them with the enzyme DNA ligase. You take your ligation mix and add it to *E. coli* cells which are then spread on kanamycin-containing plates (solid media in petri dishes) and grown overnight to isolate bacterial colonies.

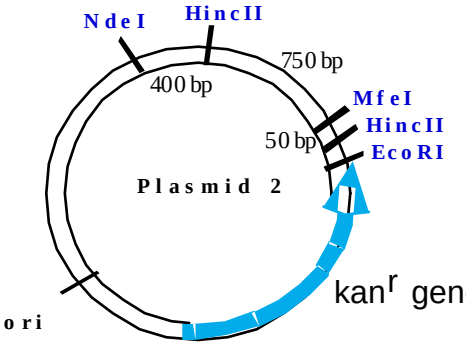
3. Why is it necessary to grow the cells on plates containing kanamycin?
When you mix the DNA and bacteria in the transformation protocol, most of the bacteria in the transformation reaction do not take up a plasmid. The presence of kanamycin in the media prevents all these other bacteria from growing and allows you to select only those containing the kanamycin resistance gene.

Each bacterial cell that received a plasmid should grow up into a bacterial colony on a petri dish containing kanamycin media. When the plasmid DNA from two of these colonies: plasmids 1 and 2, are analyzed by restriction enzyme analysis with a) HincII alone and b)MfeI and NdeI together, a distinct pattern is observed for each of the plasmids. The patterns seen after electrophoretic separation of the DNA fragments on a size separation gel are shown in the figure below.

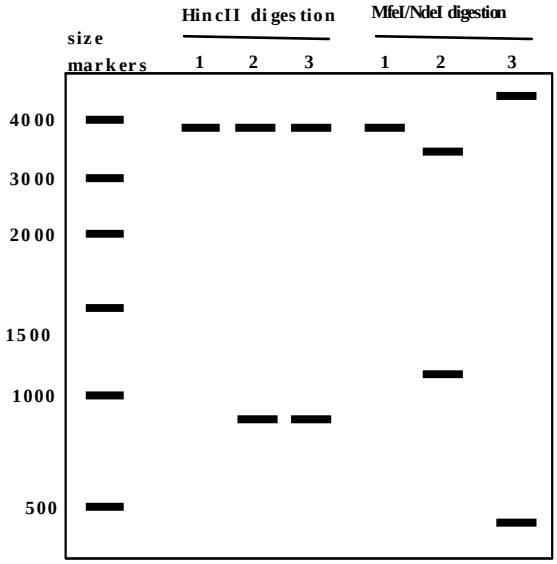


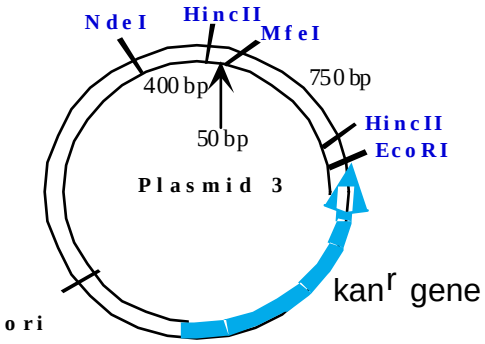
4. Make a diagram for each of the plasmid molecules, 1 and 2 based on the restriction patterns shown in the gel above.





5. Make a diagram of what the plasmid would look like if the DsRed gene fragment had been inserted in the opposite orientation. Also, make a diagram of what the gel would look like if the HincII and MfeI/NdeI digests of this plasmid were separated by electrophoresis.





6. You would like to generate a single product pDsRedN1 with the DsRed gene fragment in a unique orientation. Use any of the restriction enzymes provided to design such a procedure.

Use HincII and EcoRI to cut the pN1 plasmid and use HincII and MfeI to cut the DsRed gene fragment. This procedure generates two different sets of compatible "sticky" ends on the vector and insert. The HincII blunt ends will be ligated on one end and the compatible EcoRI and MfeI overhangs will hybridize on the other which will require the DsRed gene to insert in only one orientation.