

Recombinant DNA and Cloning

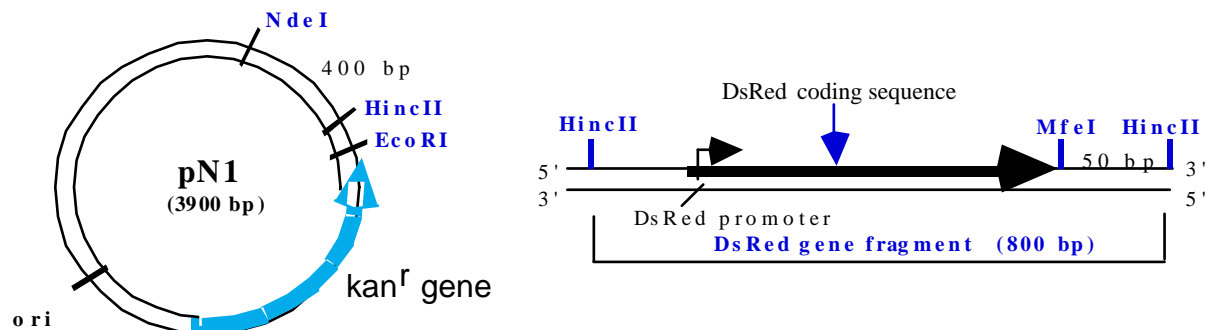
A. The Tools

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?
2. What is a vector?
3. What do the restriction enzymes do? How are they physically able to perform this function?
4. What is a library?
5. What three features must vectors or plasmids have? Why?
6. Where did the techniques and reagents used in recombinant DNA applications come from?
7. What is the purpose of the recombinant DNA technology?

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

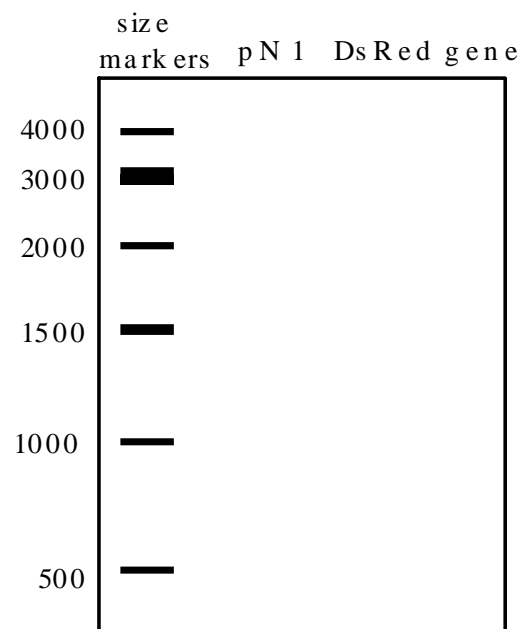
MfeI cuts: 5' -C | AATTG-3' EcoRI cuts: 5' -G | AATTC-3'
 3' -GTTAA | C-5' 3' -CTTAA | G-5'

* 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.

- 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.



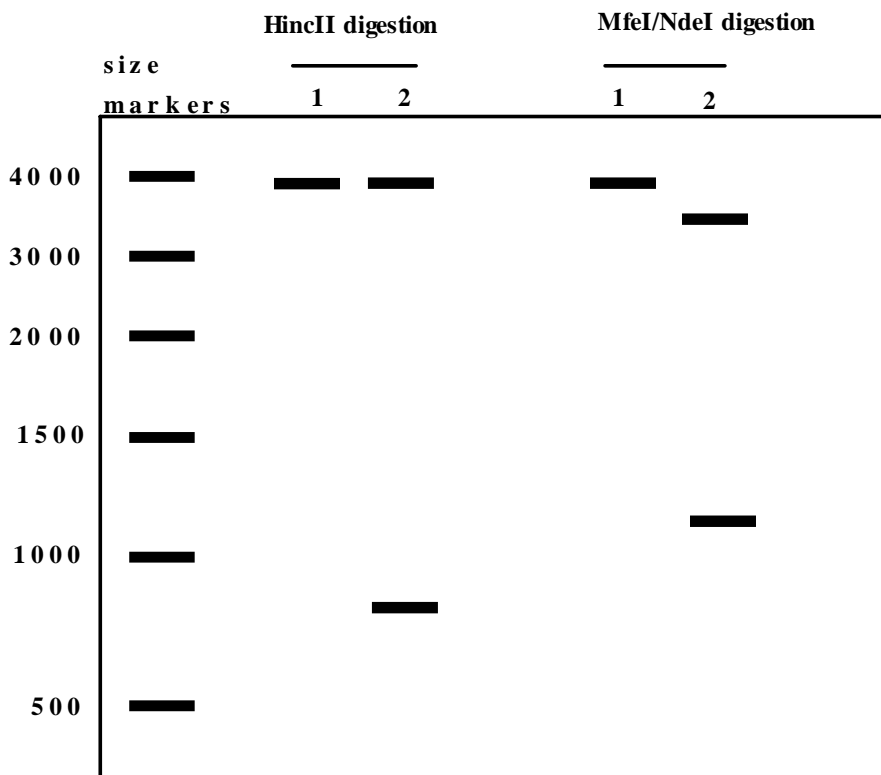
Under special conditions, plasmid DNA can enter *E. coli* cells. The plasmid DNA functions as normal DNA, *i.e.*, genes on the plasmid can be transcribed and translated. *E. coli* cells that have incorporated a plasmid are said to be transformed.

2. Where have we encountered a transformation before?

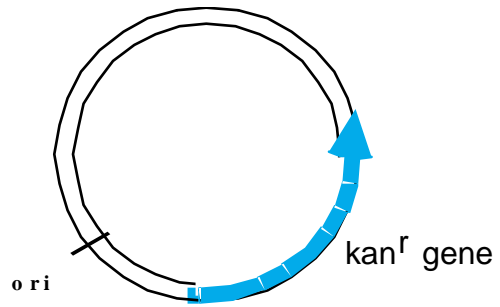
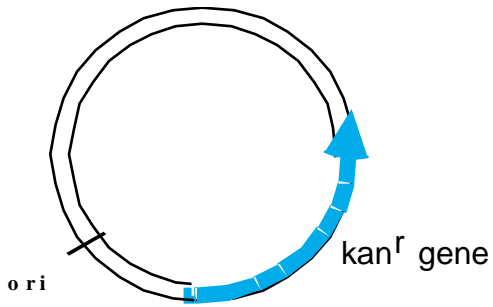
You isolate the DNAs from the gel, mix them together in a tube and ligate them with the enzyme DNA ligase. You take your ligation mix and add it to *E. coli* cells which are then spread on kanamycin-containing plates (solid media in petri dishes) and grown overnight to isolate bacterial colonies.

3. Why is it necessary to grow the cells on plates containing kanamycin?

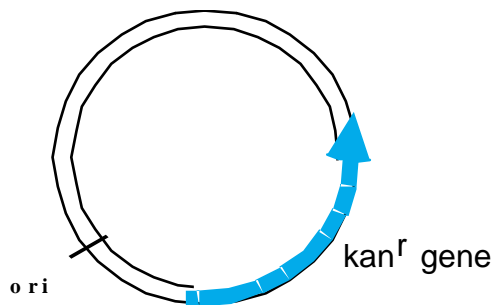
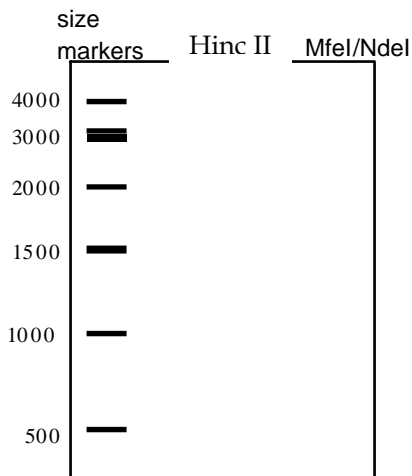
Each bacterial cell that received a plasmid should grow up into a bacterial colony on a petri dish containing kanamycin media. When the plasmid DNA from two of these colonies: plasmids 1 and 2, are analyzed by restriction enzyme analysis with a) *HincII* alone and b) *MfeI* and *NdeI* together, a distinct 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.