

Lab 2

Restriction Digestion of Plasmid DNA

(pARA and pKAN-R)



Pre Lab Readiness

- Familiarity and proper use of micropipettes
 - Remember the 1st and 2nd stops
- Understand enzymes
 - Not consumed in reaction
 - Affected by time, pH, temp
 - Biological catalyst made of protein
- Principles of Restriction Enzymes
 - BamH1
 - HindIII



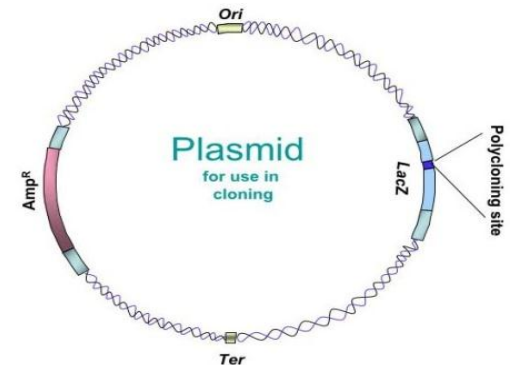
Why are we doing this?

To Understand how Recombinant DNA is made!

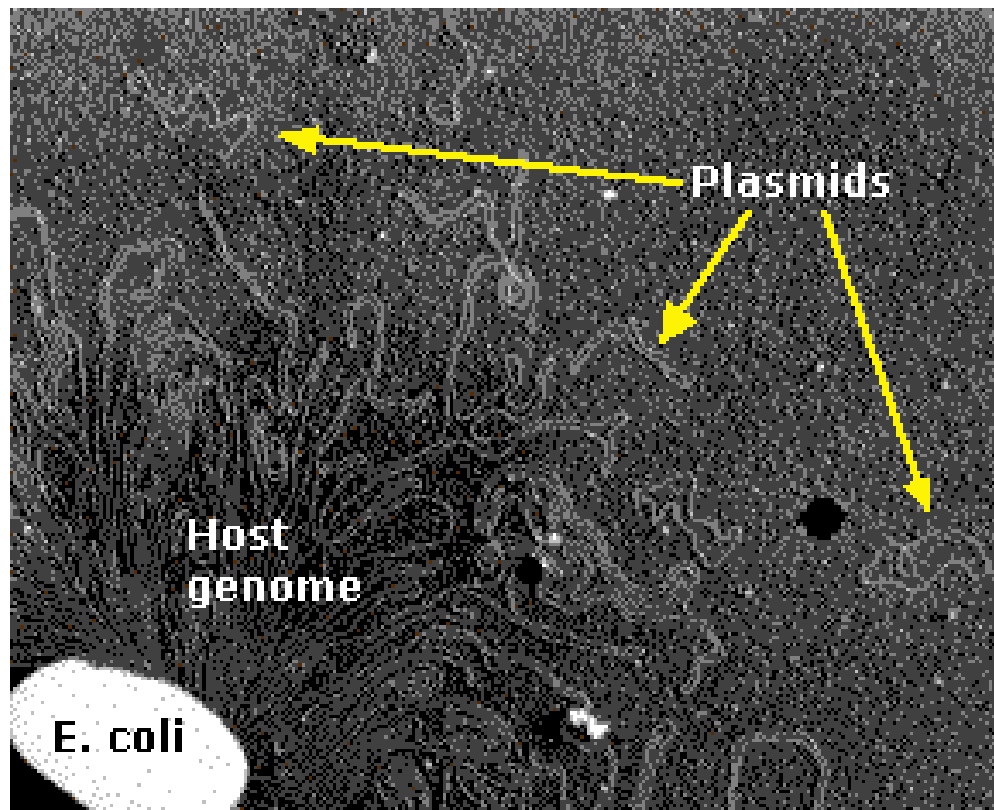
Lab 2 Terms

What is a Plasmid?

- Small circular DNA molecule
- Capable of self replication
- May contain an antibiotic resistant gene(s) and/or other gene(s)



Cool Picture of Plasmids!



Electron micrograph of an *E. coli* cell ruptured to release its DNA.

The tangle is a portion of a single DNA molecule containing over 4.6 million base pairs encoding approximately 4,300 genes.

The small circlets are plasmids.

(Courtesy of Huntington Potter and David Dressler, Harvard Medical School.)

Lab 2 terms

- **Plasmid**- circular molecule of DNA found in bacterial cells
 - **pARA and pKAN-R are the two plasmid used in lab 2**
- **DNA**- the double-stranded molecule that encodes genetic information composed of nucleotides (A,T,C and G)
- **Gene** - a section of DNA that codes for a protein
- **Gene expression** - the process by which the DNA information is converted to mRNA and then to protein
- **Antibiotic** - a molecule that inhibits growth or destroys bacteria
 - **Ampicillin and kanamycin are two examples used in experiment 2**
- **Selectable marker**- allows for the selection of bacteria that harbor the plasmid and those that do not

Lab 2 terms

- **Restriction enzyme** - an enzyme that cuts DNA at a location dictated by a particular sequence of base pairs
 - **BamHI and Hind III are the enzymes used in Lab 2**
- **Restriction site** - the recognition site on the DNA molecule that has the correct sequence of base pairs for an enzyme to recognize and cut
- **Red fluorescent protein (rfp)** - a protein derived from a sea anemone that fluoresces when exposed to UV light
- **Recombinant DNA** - DNA that has been joined together, which now carries genes from two or more organisms.
 - **In the case of Lab 2: from a eukaryotic sea anemone and a prokaryotic bacteria, *E. coli*.**

Lab 2 terms

Sticky ends - are unpaired base pairs that are produced from the digestion of a DNA molecule by restriction enzymes

DNA molecule with *Bam*H I and *Hind* III restriction sites (underlined). The arrows indicate sites where enzymes will cut the sugar-phosphate backbone of the DNA molecule.

The lower DNA molecule indicates the location of the “sticky ends” (bold).



Standards Evaluation

- Biology; Cell Biology Standard 1b
- Students know enzymes are proteins that catalyze biochemical reactions without altering the reaction's equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings