

Biotechnology uses recombinant DNA technology to study many different biological topics. The details we have learned about molecular biology within the cell (DNA replication, RNA transcription, protein expression) come to play here, in understanding how to manipulate DNA outside the cell.

Look at the attached map of a circular DNA molecule called pET28b (pET28b is a plasmid) and answer the following questions to demonstrate your mastery of the details about molecular biology.

- 1) There is a gene encoded in this plasmid that allows bacteria carrying this plasmid to grow in the presence of kanamycin (an antibiotic). What are the numbers of the DNA bases that make up that gene?

- 2) Where is the Origin of Replication? What happens at the ORI?

- 3) What happens at the T7 promoter site?

- 4) What happens at the RBS?

- 5) Write the palindromic sequences for the following restriction endonucleases:

BamHI

NcoI

NdeI

HindIII

EcoRI

- 6) Why is NcoI a good restriction endonuclease to use at the 5' end of a gene when cloning in a protein-coding gene? Hint – to which end of the protein does the 5' end of the gene correspond?