



The Specificity of Restriction Enzymes and the Insertion of a Target Gene into a Vector

1. Cut the double stranded DNA helices out of the paper (page 2) and tape them together in the order described.
2. Take the transparency restriction enzymes (molecular scissors) and determine where your specific enzyme will cut the double helix. Using colored pencils, mark the “cut” line.
3. If your restriction enzyme will cut the strand in two places, how many pieces of DNA will you have after the restriction digest?
4. If your restriction enzyme will cut the strand in one place, how many pieces of DNA will you have after the restriction digest?
5. The blue box represents an Origin of Replication while the purple box represents the Ampicillin resistance gene. Discuss or describe why these specific sequences are necessary in a vector.
6. Mark on the target gene exactly where the BgIII and EcoRI restriction enzymes would cut.
7. Using scissors, cut the target DNA and the vector DNA in the exact locations that the RE would cut.
8. Using glue as the ligase enzyme, anneal the cut ends of the target DNA and vector DNA.

C G
A T
T A
A T
A T
G C
G C
C G
C G
A T
A T
G C
C G
A T
T A
A T
A T
G C
C G
T A
C G
G C
A T
G C
C G
A T
T A
A T
A T
G C
G C
A T
One

G C
C G
G C
T A
T A
A T
G C
C G
C G
T A
C G
G C
A T
G C
G C
T A
T A
T A
T A
A T
T A
C G
C G
T A
G C
G C
A T
Two

A T
G C
T A
G C
G C
C G
A T
G C
T A
C G
T A
C G
A T
G C
T A
G C
C G
T A
T A
T A
C G
G C
A T
G C
A T
G C
G C
C G
Three

T A
T A
A T
C G
C G
G C
T A
T A
A T
C G
C G
G C
T A
G C
C G
G C
C G
G C
C G
C G
T A
T A
A T
A T
G C
G C
C G
G C
Four

T A
A T
G C
C G
C G
T A
T A
C G
G C
A T
A T
G C
G C
C G
C G
C G
T A
A T
G C
G C
C G
T A
Five

G C
G C
C G
C G

HpaII

G C
G C
G C
C G
C G
C G

XmaI

C G
T A
C G
G C
A T
G C

SacI

C G
T A
T A
A T
A T
G C

Eco RI

T A
C G
T A
A T
G C
A T

BglII

C G
C G
T A
A T
G C
G C

Bam HI

C G
C G
T A
G C
G C

AvaII

T A
T A
C G
G C
A T
A T

HindIII

Page 4 (Target Gene—Run on Colored Paper)

C G
A T
G C
G C
C G
A T
G C
T A
C G
T A
A T
G C
A T
T A
G C
G C
C G
T A
T A
C G
T A
T A
A T
A T
G C
C G
G C