# What is Molecular Cloning?

Worksheet

Molecular cloning is the process of isolating a DNA fragment, inserting it into a vector such as a plasmid, and introducing that recombinant molecule into a host cell so it is copied many times.

## Questions

1. Which enzyme joins DNA fragments together during cloning?

A) Restriction enzyme
B) DNA ligase
C) DNA polymerase
D) Reverse transcriptase

2. What is a common vector used to carry a DNA insert into bacteria?

A) Ribosome
B) Plasmid
C) Lysosome
D) tRNA

3. In blue-white screening, a white colony indicates:

A) No insert present
B) An insert was successfully cloned
C) A failed transformation
D) Vector self-ligation

4. Transformation efficiency TE = 510 cfu/g; you transform 4 ng of DNA and plate 25% of the reaction. Expected colonies?

A) 500
B) 5,000
C) 50,000
D) 500,000

5. A competent E. coli strain has a transformation efficiency of 110 cfu/g. You transform 2 ng of ligated plasmid and plate 20% of the reaction. How many colonies do you expect?

6. A plasmid vector (3 kb) is cut with EcoRI and ligated to an insert (1.2 kb) also cut with EcoRI. What is the size of the resulting recombinant plasmid?

7. After transformation, colonies are grown on X-gal/IPTG plates for blue-white screening. What does a white colony indicate?

8. Define: What is molecular cloning?

9. Define: What is a plasmid vector?

10. Define: What two enzymes are essential for cloning?

# Answer Key

1. B) DNA ligase - DNA ligase seals the phosphodiester backbone, joining the insert and vector.

2. B) Plasmid - Plasmids are small circular DNA molecules commonly used as cloning vectors.

3. B) An insert was successfully cloned - White colonies have a disrupted lacZ gene, meaning the insert interrupted it.

4. C) 50,000 - N = (510) 0.004 0.25 = 50,000 colonies.

5. TE = 110 cfu/g, mass = 2 ng = 0.002 g, fraction plated = 0.2 N = TE m f N = (110) 0.002 0.2 = 40,000 colonies

6. Vector = 3 kb, insert = 1.2 kb Ligation joins them into one circular recombinant molecule Recombinant plasmid = 3 + 1.2 = 4.2 kb

7. The vector's lacZ gene is disrupted by the multiple cloning site A white colony has no functional -galactosidase, meaning the insert is present A blue colony has an intact lacZ gene, meaning the vector self-ligated without an insert

8. Making many identical copies of a DNA fragment by inserting it into a vector and propagating it in a host cell.

9. A small circular DNA molecule used to carry and replicate a DNA insert inside a host cell.

10. Restriction enzymes (which cut DNA at specific sites) and DNA ligase (which joins DNA fragments together).