Molecular Biology: Gene cloning

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LIGATION: THE JOINING OF DNA MOLECULES

The final step in the construction of a recombinant DNA molecule is the joining together of the vector molecule and the foreign (target) DNA to be cloned. This process is referred to as ligation, and the enzyme that catalyses the reaction is called DNA ligase (Brown, 1990).

All living cells produce DNA ligases and within the cell these enzymes carry out the important function of repairing any discontinuities in double-stranded DNA molecules. Such a discontinuity is simply a position where a phosphodiester bond between adjacent nucleotides is missing. Although discontinuities may arise by chance breakage of the cell’s DNA molecules, they are also a natural result of processes such as DNA replication and recombination. Ligases, therefore, play several vital roles in the cell. In a test-tube, purified DNA ligases (e.g. T4 DNA ligase, derived from the T4 bacteriophage) will join together individual DNA molecules or the two ends of the same molecule by catalyzing the formation of a phosphodiester bond between the 5’ phosphate of one strand and the 3’ hydroxyl of the other (Brown, 1990).

Cutting and joining DNA molecules:
(http://www.hort.purdue.edu/hort/courses/HORT250/lecture%2003)

1. Two DNA molecules, one blue and one green, both of which contain the sequence that is recognized by the enzyme EcoRI:

2. The DNA molecules are cut with EcoRI and sticky ends are produced (5’ overhangs):

3. The enzyme DNA ligase is used to join the DNA molecules together. The red lines indicate the formation of covalent bonds:
There are three main methods of joining DNA fragments (www.users.wmin.ac.uk/~redwayk/lectures/):

- **Using sticky (cohesive) ends.** This is the easiest and most commonly used method. Fragment ends must be complementary. Therefore, the same restriction enzyme is used to cut both vector and insert DNA, or two different enzymes are used that produce complementary ends.

- **Using synthetic linkers (adaptors).** Ligases can join blunt ends, but at a lower efficiency. Linkers are chemically synthesized pieces of double-stranded DNA that include a single restriction site for an enzyme that produces sticky ends. DNA ligase will attach linkers to blunt-ended DNA molecules, thus enabling the conversion of blunt ends to sticky ends (after restriction digestion).

- **Using homopolymer tailing.** This technique offers a different approach to the production of sticky ends on a blunt-ended DNA molecule. Long tails of complementary-pairing bases (G and C or T and A) are added to both vector and insert DNA respectively by using the terminal transferase enzyme.