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DNA Ligation Reactions

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Abstract

In molecular biology, ligation refers to the joining of two DNA fragments through the formation of a phosphodiester bond. An enzyme known as a ligase catalyzes the ligation reaction. In the cell, ligases repair single and double strand breaks that occur during DNA replication. In the laboratory, DNA ligase is used during molecular cloning to join DNA fragments of inserts with vectors – carrier DNA molecules that will replicate target fragments in host organisms.

This video provides an introduction to DNA ligation. The basic principle of ligation is described as well as a step-by-step procedure for setting up a generalized ligation reaction. Critical aspects of ligation reactions are discussed, such as how the length of a sticky end overhang affects the reaction temperature and how the ratio of DNA insert to vector should be tailored to prevent self-ligation. Molecular tools that assist with ligations like the Klenow Fragment and shrimp alkaline phosphatase (SAP) are mentioned, and applications , such as proximity ligations and the addition of linkers to fragments for sequencing are also presented.

Transcript

Ligation can be defined as the act of joining, and in biology the term refers to an enzymatic reaction that joins two biomolecules with a covalent bond. This video describes the application of DNA ligation in molecular biology research.

In the cell, DNA ligases are enzymes that identify and seal breaks in DNA by catalyzing the formation of phosphodiester bonds between the 3'-hydroxyl and 5'-phosphate groups of the DNA backbone. Ligation occurs as part of normal cellular processes, such as DNA replication, to repair single and double strand DNA breaks.

In the laboratory, DNA ligases is routinely used in molecular cloning - a process that joins endonuclease-digested DNA fragments, or inserts, with an endonuclease-digested vector, such as a plasmid, so that the fragment can be introduced into host cells and then replicated.

Endonuclease digestions involve the use of restriction endonucleases, or restriction enzymes, which create nicks at specific stretches of DNA.

These nicks can resemble single strand breaks producing 3' and 5' overhangs, called sticky ends or double strand breaks with no overhangs, called blunt-ends. Ligating sticky ends is advantageous, because the complimentary overhanging base pairs stabilize the reaction. Because blunt end ligations don't have any complimentary base pairing, the ligation is less efficient and more difficult for the enzyme to join the ends. Sticky and blunt ends cannot, under normal circumstances, be ligated together.

However, the Klenow fragment, the product of DNA polymerase 1, digested with subtilisin can convert sticky ends to blunt ends. Klenow possesses 3' to 5' exonuclease activity that chews up 3' overhangs and polymerase activity that blunts 5'overhangs by extending the 3' end of the complementary strand.

When the goal is to insert a gene into a plasmid, resealing of vector DNA, called self-ligation, is a common undesirable outcome for a ligation reaction. Alkaline phosphatase treatment of vector DNA post-digestion removes 5'phosphates on both ends and prevents this undesirable outcome.

As we mentioned previously, vector and insert DNAs are digested with endonucleases prior to beginning a ligation. Following gel-purification of digested vector and insert, DNA concentrations are measured a spectrophotometer to determine the concentration of the purified vector and insert.

From this concentration, the number of molecules of insert or vector in 1 µl can be determined based on the average molecular weight for a DNA base pair and the number of base pairs in each fragment. Based on the calculated molecular concentration of vector and insert, a 3 to 1 ratio of insert to vector is calculated, to determine the volume of vector and insert used in the reaction. This 3 to 1 ratio of DNA insert to vector is desirable, because it ups the probability of the insert being ligated into vector versus vector ligating itself.

Now that we have determined the amount of vector and insert DNA to use in the reaction, we proceed to set up the ligation reaction on ice. The order of adding in which reaction components should be added to your microfuge tube is as follows: sterile water enough to a make a 10 µl final volume, in our case we'll use 4 µl, 1 µl 10X of ligation buffer, 1 µl 10mM of ATP, 1 µl of vector and 3 µl insert DNA, as calculated, and finally 1 µl DNA Ligase. The reaction is mixed thoroughly, centrifuged and incubated at the appropriate temperature.

Whether you are doing a sticky or blunt end ligation impacts the temperature and duration of the ligation reaction. For example, a sticky end ligation with a six base pair overhang can be carried out near room temperature for about 1 hr, because the complementary ends stabilize the joining of fragments. Short overhangs or blunt end ligations should be carried out between 14-20˚C overnight.

Now that we learned how to set up a ligation reaction, let's have a look at some of the applications of this procedure.

Ligations can be used to directly insert PCR-amplified fragments into linearized plasmids. Here you see a researcher taking a sample of frozen mouse brain, isolating genomic DNA from it, and then subjecting it to bisulfite PCR, which is a PCR-based method to detect methylated DNA. PCR products are then directly ligated into the plasmid to create a library of genes that are methylated in that particular brain region.

Ligations can be used to attach oligonucleotide linkers, which contain binding sites for PCR primers, to purify DNA fragments. When working with tumor samples, scientists can use this approach to sequence tumor genomic DNA, with the hope of identifying tumor-causing mutations.

In this video, ligation is performed on DNA isolated from formaldehyde fixed cells and subsequently treated with a restriction enzyme and klenow in presence of biotin, which is then used to pull down ligated DNA. This DNA is then amplified using PCR and the products sequenced to identify chromatin interactions at various scales as shown.

You have now learned about DNA ligase, various principles involved in setting up ligation in the laboratory, potential problems and fixes and various applications of ligation in molecular biology research. Thanks for watching.