Resource Summary Report

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Ligation Protocol with T4 DNA Ligase

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Proper Citation

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.7khhkt6

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Group: iGEM Wageningen 2019

Summary: The final step in the construction of a recombinant plasmid is connecting the insert DNA (gene or fragment of interest) into a compatibly digested vector backbone. This is accomplished by covalently connecting the sugar backbone of the two DNA fragments. This reaction, called ligation, is performed by the T4 DNA ligase enzyme. The DNA ligase catalyzes the formation of covalent phosphodiester linkages, which permanently join the nucleotides together. After ligation, the insert DNA is physically attached to the backbone and the complete plasmid can be transformed into bacterial cells for propagation.The majority of ligation reactions involve DNA fragments that have been generated by restriction enzyme ligation (See Protocols). Most restriction enzymes digest DNA asymmetrically across their recognition sequence, which results in a single stranded overhang on the digested end of the DNA fragment. The overhangs, called "sticky ends", are what allow the vector and insert to bind to each other. When the sticky ends are compatible, meaning that the overhanging base pairs on the vector and insert are complementary, the two pieces of DNA connect and ultimately are fused by the ligation reaction.

Affiliations: Wageningen University

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