

Introduction

Today you will ligate your linearized plasmid backbone with your PCR product by mixing the two in the presence of ATP and an enzyme, T4 DNA ligase. During the ligation reactions, hydrogen bonds will form between the overhangs on the fragments, and then the ligase will repair the phosphate backbone, creating a stable circular plasmid.

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DNA ligation

Hopefully, your ligation reactions will generate your desired construct, the pCX-NXX backbone carrying the EGFP gene lacking the first 32 amino acids. You will transform the ligation reactions into bacteria. During “transformation,” a single plasmid from the ligation mixture enters a single bacterium and, once inside, replicates and expresses the genes it encodes. One of the genes on the pCX-NNX plasmid leads to ampicillin-resistance. Thus, a transformed bacterium will grow on agar medium containing ampicillin. Untransformed cells will die before they can form a colony on the agar surface.

Most bacteria do not usually exist in a “transformation ready” state, but the bacteria can be made permeable to the plasmid DNA, and cells that are capable of transformation are referred to as “competent.” Competent cells are extremely fragile and should be handled gently, specifically kept cold and not vortexed. The transformation procedure is efficient enough for most lab purposes, with efficiencies as high as 10^9 transformed cells per microgram of DNA, but it is important to realize that even with high efficiency cells only 1 DNA molecule in about 10,000 is successfully transformed.

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Bacterial transformation