Cloning of a His-tagged Firefly Luciferase gene into a pET expression vector.

Luciferase is an enzyme that catalyzes the ATP dependent-oxidation of luciferin resulting in the generation of light. We are interested in affinity purification of the Firefly luciferase using a bacterial overexpression system. We used the cloning vector pGEM-luc. The goal of the project is to generate a recombinant DNA construct of a His-tagged luciferase gene in a pET expression vector that can be overexpressed in E.coli and the luciferase protein purified using Affinity purification. The pET 30a vector an expression vector with an N-terminal His-tag was chosen as the expression vector of choice. To clone the luciferase gene into the pET vector in the proper orientation, PCR was used to obtain the luciferase gene fragment with restriction sites for Bam H1 and Sac1 flanking the gene. The gene was then ligated into the pET vector.