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Expression and Purification of a Redox-Sensitive Green Fluorescent Protein Fused with Anthrax Toxin Lethal Factor

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Pathogenicity of Anthracis Bacillus is induced by its exotoxin composed of protective antigen (PA) lethal factor (LF) and edema factor (EF) that together target mammalian cells. As previous studies have suggested, it is important to study the structure of the targeted receptor (ANTXR2) as there has been evidence showing that the disruption of the disulfide bonds present in the receptor ectodomain inhibits the PA-mediated LF translocation across the membranes of mammalian cells, suggesting that cellular redox potential plays an important role in the anthrax toxin action. Here, we have constructed a fusion-gene containing the Nterminal Lethal Factor and a redox-sensitive green fluorescent protein (roGFP) which will allow for tracking of the redox potential while anthrax toxin travels within the host cell. The LFN-roGFP gene was constructed by using over-lap PCR allowing for linkage of the genes with the incorporation of BamHI/NdeI restriction sites that will allow for subsequent insertion into a pet-15b vector.