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A Set of Genetic Constructs for Binase and Barstar Overproduction

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Abstract

© 2016, Springer Science+Business Media New York.Low molecular weight guanyl-preferring ribonuclease (RNase) secreted by Bacillus pumilus is referred to as binase and regarded as a potential agent to target oncogenic diseases and viral infections. Depending on research purposes, native binase or tagged and mutated forms are required for application. Specific inhibition of binase activity is achieved by binding to RNase inhibitor barstar. Native and mutant forms of binase are routinely obtained by ion-exchange chromatography, while tagged versions of binase, which can be purified faster and less tedious than non-tagged, are currently unavailable. Here, recombinant DNA techniques were employed to improve binase overexpression in bacterial host cells, its purification, and subsequent detection in downstream assays using affinity tag. Here, recombinant DNA techniques were employed to improve binase overexpression in bacterial host cells. Binase-barstar genetic cassette was modified to obtain strong two-gene operon with the possibility of rapid exchange of binase and/or barstar genes with homologous or mutated ones as well as the generation of C-terminally His-tagged binase and/or barstar. The binase-barstar operon was introduced into pET26b vector as well as into pET26b-pET15b hybrid vector that was created in this study. As a result, plasmids allowed the induced production of extracellular non-tagged, N- or C-terminal His-tagged binase and synchronous intracellular production of non-tagged or C-His-tagged barstar. The constructs can be further sub-cloned in gram-positive bacterial hosts to ensure native production conditions.

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Keywords

Barstar, Binase, His-tag, Overexpression, RNase

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