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Author(s)

Author One, Author Two and Autor Three

Feroz Khan, MS, PhD,
Editor-in-Chief
feroz.khan@sci-edit.net

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Abstract

A new superior Green Fluorescent Protein (GFP) mutant known asealled superfolder GFP (sfGFP) iswas reported to be more soluble, folds faster folding, and the brightest of brighter than the knownany known GFP mutants. This study aims to create a codon adapted sfGFP tag (TtsfGFP) to that could be used simultaneously forin protein localization andas well as affinity purification studies inof *T. thermophile*. *In vivo* fluorescence spectroscopic analyses of a codon adapted and 6XHis tagged TtsfGFP cassette carrying clones exhibitedshowed that they have ~2-4 fold increase in fluorescence emission as compared towith control groups at 3rd hours. Fluorescencet microscopy results also revealed that TtsfGFP reaches a maximum emission maxima at 100 min, which is much earlier than EGFP and sfGFP controls withat ~240 min. To test the affinity/localization dual tag features, aA *Tetrahymena* ATP dependent DNA ligase domain containing hypothetical gene (H) was cloned into the 32end of 6XHis-TtsfGFP, in order to assess the affinity/localization dual tag feature. Hence, fFluorescencet microscopic analysis of the 6XHis-TtsfGFP-H clone confirmedrevealed its localization into the macronucleus- and micronucleus of vegetative *T. thermophila*. Affinity purifiability purification of TtsfGFP and TtsfGFP-H with Ni-NTA beads was furthermore confirmed using SDS-PAGE and Western blot analysesexperiments. These results indicate that the 6XHis-TtsfGFP tag can be used as a dual tag for protein localization and affinity purification studies inof *T. thermophila*.