The Twinkle Factory

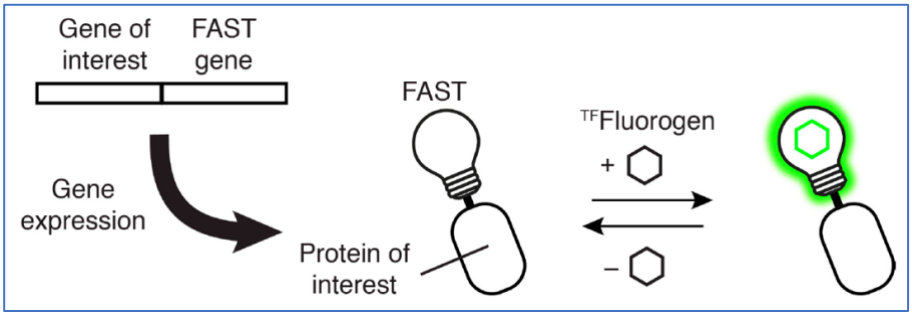
Stain different, tag FAST.

Expedite metabolic engineering of anaerobes with FAST of The Twinkle Factory

Abstract – Luc Lenglet – CLOSTRIDIUM XVI – Toulouse, September 14-17th, 2022

Fluorescence labeling of proteins has been a long-lasting obstacle for the community of researchers in the anaerobic world. Hence an irresistible challenge for biophysicists! And since CLOSTRIDIUM XV in München, a new technology has indeed emerged, FAST of The Twinkle Factory.

FAST is a fluorescent labeling technology introduced by researchers of ENS-PSL (Paris) in 2016. FAST enables the specific fluorescent labeling of any protein of interest (POI). The protocol is based on the instantaneous and reversible formation of a fluorescent molecular assembly between the small (14 kDa), genetically encoded, proteic tag FAST and various fluorogenic ligands (^{TF}Fluorogens). ^{TF}Fluorogens are dark in water and strongly fluoresce only when bound to FAST. They hence enable to detect and image FAST-tagged proteins with high contrast without the need of washing the excess of fluorogenic ligands. Furthermore, while labeling of FAST-tagged proteins with a ^{TF}Fluorogen is non-covalent, it can easily be reversed by washing when necessary. Since initial disclosure, FAST has been through a number of evolutions, *e.g.*, splitFAST for protein-protein interaction tracking, frFAST for far-red labeling, greenFAST and redFAST for orthogonal multicolor labeling, making it a versatile tool for cell biology.



One further feature is of utmost relevance in the field of solvent- and acid-forming Clostridia: FAST does not require oxygen for being fluorescent, unlike fluorescent proteins. It can hence fluorescence-label proteins in weakly oxygenated or strictly anaerobic environments. In 2019, Prof. Papoutsakis first reported the potential of FAST for studies involving cell sorting, sporulation dynamics, and population characterization in pure as well as mixed cultures such as those in various native or synthetic microbiomes and syntrophic cultures. Since then, a number of teams worldwide have implemented FAST in *Clostridium acetobutylicum, C. ljungdahlii, Acetobacterium woodii, Eubacterium limosum*, etc. hence demonstrating its versatility and its potential to be the Swiss Army knife for metabolic engineering of Clostridia.

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