Fluorophore Assessment of Glycosidase Activity

2017 Protocols

Adapted from Chen, H. M., Armstrong, Z., Hallam, S. J., & Withers, S. G. (2016). Synthesis and evaluation of a series of 6-chloro-4-methylumbelliferyl glycosides as fluorogenic reagents for screening metagenomic libraries for glycosidase activity. Carbohydrate research, 421, 33-39.

Purpose:

Purpose of this protocol is to assess the enzymatic activity of glycosidases using fluorophores conjugated to substrates of interest (such as cellobiose or xylobiose). Once the enzyme of interest cleaves substrate, the fluorophore is released enabling quantification of enzymatic activity.

Required Materials:

- LB Growth Media
- BL21 DE3 *E. coli*
- 14 mL bacterial culture tubes
- LB+antibiotic plates
- 0.1 M IPTG
- 1% Triton-X in 50 mM Potassium Acetate Buffer pH 7.0
- 200 uM substrate (either CMU-C or CMU-X2) in DMSO
- DeepWell 96 well microplate
- 96 well microplate (clear bottom)

Procedure:

1. Transform your glycosidase of interest in an expression vector under control of the lac promoter into BL21 DE3 *E. coli* (refer to transformation protocol for more detail). Grow overnight at 37°C.
2. Grow up single colony in 5 mL LB media+antibiotic batch culture at 37°C overnight, shaking.
3. Re-seed overnight culture into new media+antibiotic and grow at 37°C until OD reaches 0.5.
4. When OD reaches 0.5, induce with 1:1000 0.1M IPTG for 4 hours.
5. After 4 hours, transfer 50 uL solution to clear bottom 96 well microplate and add 50 uL 200 μM enzyme substrate (CMU-C or CMU-X2) in 1% Triton-X/Potassium Acetate Buffer. Incubate for 18h hours at 37°C, shaking.
6. After 18 hours, use a plate reader to measure excitation at 365 nm and emission at 450 nm for a glycosidase of interest,
7. Controls for this experiment are empty vector (EV) and LB alone. LB alone serves as a background control, allowing you to subtract all relative light units from background. You then normalize your enzyme of interest to EV, giving you a fold increase of fluorophore release which is directly correlated to enzymatic activity.