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).

Required Materials:

- LB Growth Media
- BL21 DE3 *E. coli*
- 14 mL bacterial culture tubes
- LB+antibiotic plates
- 0.1 M IPTG
- 1 % Triton-X
- 50 uL of 200 uM substrate (either CMU-C or CMU-X2)
- 96 well plate

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. Measure OD and normalize to 0.1 OD.
4. Add 1 uL of 0.01 OD bacteria to 49 uL of growth media (LB+antibiotic) to well of 96 well plate. Incubate shaking for 17 hours.
5. At 17 hours, add 0.1 uL of 0.1 M IPTG for 1 hour.
6. After 1 hour, add 1% Triton-X with 50 uL of 200 uM enzyme substrate (CMU-C or CMU-X2). Incubate for another 18n hours, shaking.
7. After 18 hours, use a plate reader to measure excitation at 365 nm and emission at 450 nm for a glycosidase of interest well, empty vector-containing bacteria well, and a well containing just LB+antibiotic.
8. Subtract background Relative Light Units (RLU) from both the glycosidase reading and negative control reading and divide the glycosidase reading by the negative control reading.