Green Fluorescent Protein Purification as a Didactic Tool During Practical Classes For Undergraduates Students of UFAM

Faria, J.A.Q.A\textsuperscript{1}; Bezerra I.C.\textsuperscript{2}; Wöhlke, J.L\textsuperscript{2}; Filho, S.A\textsuperscript{2}

\textsuperscript{1} Departamento de Ciências Fisiológicas, ICB, UFAM, AM; \textsuperscript{2}Centro de Apoio Multidisciplinar – ICB, UFAM, AM Brazil

INTRODUCTION: The Green Fluorescent Protein (GFP), originated from the jellyfish \textit{Aequorea victoria} has broadly applicability for cellular and molecular biology research. Its spectral characteristics make it practical to be detect by UV-A (black light) lamp during the purification procedure. Moreover, this approach implementation during a practical class allows the exploring of fluorescence features. OBJECTIVES: the purpose of this investigation was to teach the concepts and principles of protein purification during a practical class using recombinant GFP protein. MATERIAL E METHODS: Transformed E. coli JM110 expressing GFP were resuspended in buffer solution (Tris-HCl 20 mM pH 8.0, 150 mM NaCl, 5 mM EDTA, 20% (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}) following the sonication step. The lysate was submitted to the purification through hydrophobic interaction chromatography column (HIC). After analysis of chromatogram, some collected fractions were quantified by Bradford assay and evaluated by SDS-PAGE. Besides that, the GFP presences were measured at an excitation wavelength of 488 nm on a spectrofluorimeter. RESULTS AND DISCUSSION: Before the experiments, the students were encouraged to explore the biochemistry characteristics of GFP, assessing protein data banks and published articles. These guided questions conducted to discussion of the purification strategy choosen. The GFP purification enabled the visual observation of chromatography principles necessary for the theory assimilation. During the chromatography running, we used a UV-A lamp which allowed a greatly exploration of concepts beyond this technique such as the sample injection, the GFP column retention, and the elution step. The chromatogram obtained were analysed and correlated to the collected fractions. Our next step was the efficiency analysis generated by the GFP measurement, total protein quantification and the analytical method SDS-PAGE. CONCLUSION: Collectively, we observed in this class the clear development of chromatography concepts by adopting the GFP protein as a target for purification protocol.

Keywords: Biochemistry Practical Class, GFP protein purification, Purification Protocol