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大肠杆菌表达的*Taq* DNA聚合酶的纯化

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摘要 *Taq* DNA聚合酶是分子生物学研究中最常用的热稳定DNA聚合酶之一, 与其他热稳定DNA聚合酶具有相似的特征, 其纯化策略不但有潜在的应用前景, 也对同类聚合酶的分离具有指导意义。已报道的适宜大量制备*Taq*酶的方案所需成本较高, 而文章介绍了一种利用国产阳离子交换树脂廉价制备*Taq*酶的方案。在本方案中, 采用热变性、$(NH_4)SO_4$沉淀与724离子交换层析分离大肠杆菌表达的*Taq*酶, 约18 g Na型树脂干粉一次可回收比活约8 131.98 U/mg、总酶活2.2×10^5 U、近27.07 mg *Taq*酶。纯化的产率可达48.92%, 纯化倍数约59.35。所制酶SDS-PAGE电泳只检测到94 kDa单一蛋白条带, 未检测到DNA核酸酶污染, 与商品酶的PCR扩增能力无区别。此纯化方法成本低, 适合实验室一般性的制备和生产应用。

关键词: *Taq* DNA 聚合酶 724树脂 阳离子交换层析

Abstract: *Taq* DNA polymerase is one of the most commonly thermostable DNA polymerases in molecular biological researches, which shares its basic characters with others of the family, thereby its purifying strategy could be used not only in itself production but also in the extraction of the others as a reference. At present, the protocols reported for large scale preparation of *Taq* DNA are high cost, so a cheaper method was described here. In this protocol, by heat de-naturation, ammonium sulfate precipitation and cation exchange chromatography of 724 resin, about 18 g powder of Na form resin could recover about 27.07 mg of *Taq* enzyme. The total activity and specific activity were approximately 2.2×10^5 U and 8131.98 U/mg. The total yield was about 48.92% with 59.35 of purification folds. Analysis of quality of purified enzyme indicated that only one protein 94 kDa was identified against SDS-PAGE and the remnant of DNA nuclease was not detected. For PCR reaction, The amplification ability of purified *Taq* polymerase was not different from that of the commercially available ones. This method reported in the present study is effective and low cost, making it suitable for general purification in laboratories or business production.

Keywords: *Taq* DNA polymerase, 724 resin, cation-exchange chromatography

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