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线粒体转录复合物相关蛋白原核表达与纯化

刘光,杨瑞锋,石秉炀,刘德培*

中国医学科学院 北京协和医学院 基础医学研究所医学分子生物学国家重点实验室,北京 100005

Prokayotic Expression and Purification of Mitochondrial Transcription Complex Proteins

LIU Guang, YANG Rui-feng, SHI Bing-yang, LIU De-pei*

National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences,CAMS and PUMC, Beijing 100005, China

摘要 **目的**获取人线粒体转录因子A（TFAM）、线粒体转录因子B1（TFB1M）、线粒体转录因子B2（TFB2M）基因片段，高效表达和纯化带有谷胱甘肽转移酶（GST）标签的 GST-TFAM、GST-TFB1M、GST-TFB2M融合蛋白。 **方法** 设计引物扩增得到TFAM、TFB1M、TFB2M的cDNA片段，通过引入的酶切位点克隆至表达载体pET42a，构建重组表达载体，并导入*E.coli* BL21宿主菌中，异丙基硫代半乳糖苷(IPTG)诱导表达重组的GST融合蛋白，谷胱甘肽琼脂糖珠纯化表达产物，十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）进行分析鉴定。 **结果** 获得pET42a-TFAM、pET42a-TFB1M、pET42a-TFB2M表达质粒，测序结果与GenBank的基因序列一致。SDS-PAGE分析结果显示，重组GST-TFAM、GST-TFB1M、GST-TFB2M融合蛋白分别在相对分子质量56 000、67 000、 69-000 处出现特异性蛋白条带，经GST亲和层析纯化后，得到高纯度的融合蛋白。 **结论** 成功构建了基因重组体pET42a-TFAM、pET42a- TFB1M、pET42a-TFB2M，制备了GST-TFAM、GST-TFB1M、GST-TFB2M融合蛋白。

关键词： 线粒体转录因子A 线粒体转录因子B1 线粒体转录因子B2 融合蛋白 纯化

Abstract： Objective To obtain human mitochondrial transcription factor A (TFAM), mitochondrial transcription factor B1 (TFB1M), and mitochondrial transcription factor B2 (TFB2M) that were expressed efficiently in *E.coli* BE21 and to purify the target proteins. Methods TFAM, TFB1M, and TFB2M segments were designed and synthesized. After having been sequenced, the reconstructed expression vectors were constructed by enzyme digestion and by cloning into an expression vector pET42a. Then the reconstructed vectors were transformed into *E.coli* BL21. Recombinant glutathione S transferase (GST) fusion proteins were expressed via the induction of IsoPropyl β-D-ThioGalactoside (IPTG) and purified by glutathione Sepharose 4B. Results The expression plasmids of pET42a-TFAM, pET42a-TFB1M, and pET42a-TFB1M were successfully constructed. The sequences of the cloned gene segments were identical with GenBank reported. The protein bands with relative molecular masses of 56.000, 67.000, and 69.000 appeared on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after the expressed GST-TFAM, GST-TFB1M, and GST-TFB2M fusion proteins were separated by SDS-PAGE. The expressed fusion proteins were purified to high purity. Conclusion The recombinant plasmids pET42a-TFAM,pET42a-TFB1M, and pET42a-TFB2M were successfully costructed, and the GST-fused target proteins were prepared.

Keywords： mitochondrial transcription factor A mitochondrial transcription factor B1 mitochondrial transcription factor B2 fusion protein purification

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Corresponding Authors: 刘德培 Email: liudp@pumc.edu.cn

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