

技术与方法

小麦线粒体DNA的高效提取方法

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摘要 以小麦黄化苗为材料, 通过简单差速离心、DNase I 处理得到无核DNA杂质的线粒体, 用SDS和蛋白酶K裂解线粒体, 经酚/氯仿抽提除去蛋白, 并用RNase A消化而得到单纯线粒体DNA(mtDNA)。对所提取的mtDNA进行紫外吸收光度分析, A_{260}/A_{280} 平均为1.92, A_{260}/A_{230} 平均为2.09, 平均每克黄化苗可提取mtDNA 26.85 mg; 并对mtDNA进行琼脂糖凝胶电泳和RAPD扩增, 均得到清晰的电泳图谱。结果表明: 此提取方法得到的mtDNA, 不但产率高、结构完整, 而且能有效去除核DNA、RNA和蛋白质等杂质, 获得高质量的mtDNA用于PCR反应和各种遗传学分析。研究还发现, 通过调整线粒体裂解温度(先50℃裂解1 h, 再37℃裂解1 h), 亦可大幅度提高mtDNA的产率。

关键词 [小麦](#) [线粒体](#) [mtDNA](#) [提取方法](#) [RAPD](#)

分类号

An efficient method for isolation of mitochondrial DNA in wheat

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Abstract

An efficient method for isolation of mitochondrial DNA (mtDNA) from etiolated tissues of wheat was developed. The protocol consists of mitochondria isolation with differential centrifugation, Dnase I treatment, lysis with SDS and proteinase K, removing protein by TE-saturated phenol/chloroform extraction and a final RNase A treatment for obtaining mtDNA. The mtDNA samples were tested using spectrophotometry and agarose gel electrophoresis. It was proved that the mtDNA isolated by this method not only have the high yield but also structural complete, and contains no impurities, such as nuclear DNA, RNA and protein. The result showed that this high quality mtDNA can be successfully used in PCR and other genetic studies. In addition, it was found that adjusting the lysis temperature has a noticeable effect on the mtDNA yield.

Key words [wheat](#) [mitochondria](#) [mtDNA](#) [isolation method](#) [RAPD](#)

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