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雷蒙德氏棉叶绿体基因组Fosmid文库构建

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Construction of a Fosmid Library of Chloroplast Genome in *Gossypium raimondii*

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摘要 采用高盐、低pH值法提取雷蒙德氏棉叶绿体DNA；通过物理剪切法获得随机断裂的DNA片段；剪切片段末端、补平修饰后与pCC1FOS载体连接；用噬菌体包装蛋白包装重组DNA，侵染大肠杆菌EPI300，构建了雷蒙德氏棉叶绿体基因组文库。对于叶绿体DNA剪切，以1 mL注射器中等速度吸打18次为最佳参数。叶绿体基因组Fosmid文库滴度为1×10^4 cfu · mL^{-1}，插入片段大小平均为38 kb，最终筛选出39个克隆用于后续研究，覆盖叶绿体基因组9.2倍。以叶绿体特异标记筛选出能够覆盖雷蒙德氏棉叶绿体全基因组的6个克隆：F66，F46，F28，F8，F55和F3，为基因组结构和功能基因分析提供了良好的基础。

关键词： 雷蒙德氏棉 叶绿体DNA Fosmid文库

Abstract： In this paper, a Fosmid library of *G. raimondii* chloroplast genome was constructed. The chloroplast DNA was isolated by high ionic strength and low pH buffer method. The DNA was randomly sheared and cloned into pCC1FOS vector. Recombinant DNA was packaged with the Lambda Packaging Extracts, then transfected into *E. coli* strain EPI300. The best sheared parameter employed in the study was 18 times with middle speed using a 1 mL injector. The library of chloroplast genome (titer: 1×10^4 cfu · mL^{-1}) was obtained in which the average inserted DNA fragment was 38 kb. Thirty-nine clones covering 9.2 fold the chloroplast genome were selected by selection marker to be further analyzed. Six clones, F66, F46, F28, F8, F55, and F3, which could span *G. raimondii* complete genome, were screened out by cotton chloroplast markers. The library would be a valuable resource for study on genome structure and functional genes investigation in cotton.

Keywords： Gossypium raimondii cpDNA Fosmid library

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