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一个改进的PCR过程中聚合酶复制精确性测定系统的建立

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摘要 在大肠杆菌中建立了一套用于测定 DNA聚合酶在PCR过程中复制精确性的系统, 并测定了耐热FD DNA聚合酶在PCR扩增过程中的复制精确性。这一系统主要包括以质粒pUC118和pUC119为出发质粒所构建的一套共6个突变质粒, 分别为pFDFP118和pFDFP119(+1移码突变)、pFDFM118和pFDFM119(-1移码突变)、pFDFU118和pFDFU119(碱基置换突变)。这些突变质粒均不能进行lacZ-α互补反应, 因此在含有X-Gal和IPTG的培养基上菌落呈白色。同时还构建了PCR产物克隆载体pFDFL118和pFDFL119。以上述突变质粒为模板进行PCR反应, 取反应产物和pFDFL118或pFDFL119连接后转化到大肠杆菌中。若在PCR过程中发生回复突变, 则转化子在含有X-Gal和IPTG的培养基上呈蓝色。由转化子中蓝白菌落个数即可计算出DNA聚合酶的复制差错率。用这一系统测得FD DNA耐热聚合酶的复制差错率为10 E-5-10 E-6。

关键词 PCR DNA聚合酶 复制精确性 碱基置换突变 移码突变

分类号

Constuction of an improved System for the Determination of Fidelity of Polymerase in PCR

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Abstract

A system used for the determination of fidelity of DNA polymerase in PCR was developen in E.coli and was used to determine the fidelity of FD DNA polymerase in PCR amplication.Frame shift and base substitution mutations were created in vitro in the lacZ gene in pUC118 and pUC119. As a result, a set of six derived plasmids namely pFDFM118 and pFDFM119 (-1 frame shift), pFDFP118 and pFDFP119 (+1 frame shift), pFDFU118 and pFDFU119 (base substitution) were obtained.All of them failed to carry out lacZ a-complementation in E.coli MV1184 and the colonies appeared white on medium with X-Gal and IPTG consequently.PCR reaction was carried out using these derived plasmids as templates and the PCR products were ligated to specially constructed cloning vectors pFDFL118 or pFDFL119,and the ligated products were used to trans form MV1184.If any back mutation happens to occur during PCR,the transformants would appear blue on medoum with X-Gal and IPTG. By scoring the number of blue and white colonies,the fidelity of DNA polymerase can be calculated.With this system the error of replication of the FD DNA polymerase was found to be 10⁻⁵~10⁻⁶.

Key words PCR DNA polymerase Fidelity of replication Base substitution mutation Frame shift mutation

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