新定量PCR数据处理方法的理论探讨　A New Method of Processing Quantitative PCR Data

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摘要　日新月异的生命科学技术的发展及临床医学科学研究的需求，一般的PCR技术已远远不能满足工作的需要。PE公司在进行了大量的PCR动力学研究的基础上，发现了利用荧光标记探针在PCR循环过程中积累的荧光强度达到仪器捡出阈值时，系统的初始模板数量与循环次数之间有线性关系,据此建立了目前的PE 7700 、PE 5700仪器的定量PCR技术,开创了PCR技术的新局面。但是由于这一技术的误差较大，尚不能满足生命科学及临床医学科学研究的需求，因此需要继续研究新的定量PCR技术。PCR动力学数学模型是根据PCR 技术的原理提出的，能够准确描述PCR反应产物分子数量积累规律的动力学方程，给出了PCR产物数量或者荧光强度与初始模板数量及其他反应条件间的函数关系。利用这一关系，根据PCR反应已积累的产物数量，可以实现准确的定量PCR分析，得到初始模板数量达到定量PCR的目的。使用动力学数学模型做定量PCR分析，其结果的误差仅与使用的荧光强度数值的精确度相关。使用精确到6位数的荧光强度数据，模板数自100~1 000 000区间定量结果的准确性可达99%以上。本文根据模拟实验数据进行了初步的定量PCR分析，结果提示，目前的定量PCR仪器使用PCR动力学模型理论处理分析数据，定量分析的结果会比目前的CT值方法在准确性方面提高几十倍以上，可以满足各方面研究工作误差水平的需要。

Abstract:Today standard PCR can't satisfy the need of biotechnique development and clinical research any more.After numerous dynamic research,PE company found there is a linear relation between initial template number and cycling time when the accumulating fluorescent product is detectable.Therefore,they developed a quantitative PCR technique to be used in PE7700 and PE5700.But the error of this technique is too great to satisfy the need of biotechnique development and clinical research.A better quantitative PCR technique is needed.The mathematical model submitted here is combined with the achievement of relative science,and based on the PCR principle and careful analysis of molecular relationship of main members in PCR reaction system.This model describes the function relation between product quantity or fluorescence intensity and initial template number and other reaction conditions,and can reflect the accumulating rule of PCR product molecule accurately.Accurate quantitative PCR analysis can be made use this function relation.Accumulated PCR product quantity can be obtained from initial template number.Using this model to do quantitative PCR analysis,result error is only related to the accuracy of fluorescence intensity or the instrument used.For an example,when the fluorescence intensity is accurate to 6 digits and the template size is between 100 to 1 000 000,the quantitative result accuracy will be more than 99%.The difference of result error is distinct using same condition,same instrument but different analysis method.Moreover,if the PCR quantitative analysis system is used to process data,it will get result 80 times of accuracy than using CT method.

关键词　定量PCR　PCR动力学　动力学数学模型　荧光定量PCR Key words　quantitative PCR　PCR dynamics dynamic mathematic model　fluorescent quantitative PCR

分类号

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