中国寄生虫学与寄生虫病杂志 2007 25 (6): 451-456 ISSN: 1000-7423 CN: 31-1248/R

论著

多重PCR技术检测恶性疟原虫抗药性相关分子标志的方法研究

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收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的 建立恶性疟原虫5个主要抗药性相关基因的单管多重PCR扩增方法，用于恶性疟原虫抗药性分子标志检测。 方法 依据各基因参考序列，运用Primer Premier 5.0和Oligo 6.0软件，设计5对特异性引物，采用Hot Start Taq DNA聚合酶，设置递增延伸温度，对恶性疟原虫标准株（3D7、Dd2和HB3）、分离株（FCC1/HN、 CMH/YN）、现场标本（来源于海南、 云南和缅甸）、 近缘虫种对照（间日疟原虫、 伯氏疟原虫、 食蟹猴疟原虫、 杜氏利什曼原虫和牛源隐孢子虫）和空白对照（以H2O为模板）进行5个抗药性相关基因（包括恶性疟原虫氯喹抗性转运蛋白基因*Pfcrt*、多药抗性基因*Pfmdr1*、二氢喋酸合成酶基因*Pfdhps*、二氢叶酸还原酶基因*Pfdhfr*和三磷酸腺苷酶第6亚基基因*PfATPase6*）的单管多重PCR扩增，2%琼脂糖凝胶电泳鉴定扩增结果，测定扩增产物序列，并与参考序列（3D7株）比对。 结果 经电泳，恶性疟原虫标准株、分离株和现场标本的多重PCR扩增产物均可见5条目标条带。测序结果与参考序列比对，高度同源，最低同源性为98.5%。模板DNA量达0.1 ng即满足扩增要求，近缘虫种对照和空白对照未见扩增产物。 结论 多重PCR技术实现了单管1次反应完成5个抗药性相关基因的扩增，该方法灵敏，特异性好，有助于提高恶性疟原虫抗药性分子标志的检测效率。

关键词 恶性疟原虫 药物抗性 单核苷酸多态性 多重PCR

分类号

Multiplex PCR for Analysis of the *Plasmodium falciparum* Drug Resistance Molecular Markers

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Abstract

Objective To develop a multiplex PCR protocol for amplification of five Plasmodium falciparum drug resistance related genes， thereby facilitate the rapid and high throughput analysis of the drug resistance molecular markers. Methods Five pairs of primers were designed according to the reference sequences by using Primer Premier 5.0 and Oligo 6.0 software. Drug resistance related genes， including *P. falciparum* chloroquine resistance transporter （*Pfcrt*）， multi-drug resistance 1 （*Pfmdr1*）， dihydropteroate synthetase （*Pfdhps*）， dihydrofolate reductase （*Pfdhfr*） and sarco/endo-plasmic reticulum Ca^{2+}-ATPase（*PfATPase6*）， were amplified by single-tube multiplex PCR using Hot Start Taq DNA Poly-merase among negative controls （*P. vivax*， *P. berghei*， *P. cynomolgi*， *Leishmania donovani*， *Cryptosporidium andersoni*）， blank control （using H_2O as template）， as well as *P. falciparum* laboratory isolates （3D7， Dd2， HB3， FCC1/HN and CMH/YN） and field samples （collected from Yunnan， Hainan of China and Myanmar）. After amplification， the PCR products were analyzed by agarose gel electrophoresis. The sequencing results were aligned to the reference sequence using BLAST. Results Five expected bands at 315， 437， 514， 594 and 770 bp were obtained with no additional or nonspecific products in *P. falciparum* laboratory isolates and field samples. The sequencing results were identical with the reference sequence except the polymorphism sites， and exhibited more than 98.5% homology. The multiplex amplification was performed successfully starting from 0.1 ng of DNA template. No band was observed in negative controls and blank control. Conclusion The present study establishes a method to amplify five *Plasmodium falciparum* drug resistance related genes harboring 21 SNPs by one-tube reaction. The multiplex PCR protocol showing high specificity and sensitivity is more convenient and efficient in analyzing the *P. falciparum* drug resistance molecular markers as compared with traditional nested PCR.

Key words

Plasmodium falciparum Drug resistance Single nucleotide polymorphism Multiplex polymerase chain reaction

DOI:

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