BioCore
Exploring Scientific Community

International Journal of Biotechnology and Bioengineering

Research Article
ISSN 2475-3432

The Real Time Polymerase Chain Reaction: An Excellent Model Technology in Gene Expression and Analysis in Different Scientific Researches

Muhammad Aslam[1], Muhammad Sulaman Saeed[*1], Shahid Sattar[1], Shoukat Sajad[2], Muhammad Sajjad[2], Muhammad Rehan[3]

[1]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*

[2]*Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan*

[3]*Department of Agronomy, University of Agriculture, Faisalabad, Pakistan*

Abstract

Kary Banks Mullis in 1984 was the scientist who invented the technique of polymerase chain reaction (PCR), this technique further modified into real-time PCR. The specific role of Real-Time PCR in observing and expressing genes in real time has altered the scientific era of 21st century in especially life sciences. Its excellent role in quantitative traits measurement, genetic potential variability in intra and inter organisms, in the early hours diagnosis of disorders, forensic fields and many others has revolutionized the scientific research. Here, we will briefly discuss various aspects of real-time PCR, including its technique, applications and challenges in all scientific fields like medicine, environment, animals and plants

Keywords: Real-Time PCR, Applications, Disorders, Genetic potential, Challenges

Corresponding author: **Muhammad Sulaman Saeed**

Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Tel: +92331-7300437

E-mail: sulaman_saeed@yahoo.com

Citation: Muhammad Sulaman Saeed et al. (2019), The Real Time Polymerase Chain Reaction: An Excellent Model Technology in Gene Expression and Analysis in Different Scientific Researches. Int J Biotech & Bioeng. 5:4, 54-58

Received: September 10, 2019
Accepted: September 13, 2019
Published: September 25 , 2019

Introduction

Kary Banks Mullis was the American biochemist who invented the technique of Polymerase Chain Reaction (PCR) in 1984; it was a great step towards revolutionizing the science. After this, Real-Time PCR abbreviated as RT-PCR has become a useful and common technique for detection and quantification of expression of elected genes [1]. This tool to identify PCR products in real time during the chain reaction has been remained available for the last nine years but there was a great increase during last 2 years. There has been great work in the field of genomics, medicine, cell biochemistry, biophysiology and cytogenetics underlying the phenotypic and genotypic expression of cell regulation [2]. The recent advancements in biological sciences during the past decades assist us in deep understanding of knowledge about network of various genetic systems that perform integrated intracellular functions in separated manner, i.e., the gene regulation of phenotypic expression of under observation genotype. But here the one major point is notable that major portion of the genome is going to be unknown to researchers and interrelationships between enzymes, signaling moving substances and many small molecules is still little bit known. To have complete information about metabolic pathways, gene regulation, gene expression, transcription factors, medicine and many other small molecules is still needed [3].

Gene expression and regulation have been utilized mainly to understand the interrelationships between ecological pressurized or weakened phenotypes and the cellular expression systems. The specific primers detection based identification system is useful to inform us about more knowledge of the aetiology, ecology and epidemiology of living organism's pathogenic microorganisms like in plants and animals [4].

In simply, we can say that laboratory techniques along with nested PCR for identification on presence or absence of microbial DNA in the extracts from many plant matrices. In order to provide different products of PCR to consumers, there are also many other methods of PCR technology [5]. Many are dependent on the utilization of internal transcription spacer regions within the nuclear ribosomal genes clusters because they are specific loci for the designed framework of PCR-based detection tools. So, these specific clusters can be easily available by utilizing universal primers and primarily are present in higher copy number of the cell but technically many inter-specific sequences modification for the framework of species specific modified primers [6]. This RT-PCR gives us quantitative genotyping management and detection system of single nucleotide polymorphism and allelic discrimination when a small portion of the measured sample containing the mutations. This RT-PCR is best suited for many genes observation dependent on the flourochomes and analyzing of melted curves of the amplified products. As, RT-PCR is a best tool for absolute comparisons of the all transcripts involving in the under observation tissues but here is a great problem with PCR products parameters counting [7]. Due to less efficient RNAs extraction technologies, there is little bit less application of RT-PCR in the field of diagnostics of plants and animals cells but PCR inhibiting factors like plant polysaccharides and polyphenolics [8]. The major error in the PCR technology programming comes due to improper ways of handling PCR. The extraction, purification and mixing of nucleic acids into different compounds used in PCR technology is a really serious step. Recently, methods used for gene expression investigations start with a template synthesis step in which nucleic acids are totally freed of sticky proteins and then they are purified. The step by step processes of PCR are very tough because every time we have to shift materials from tube to tube. So, there is loss of material during shifting which creates problems [9-12]. The extraction of DNA and RNA from any living source is very time consuming and very complex. The isolation of RNA is especially very problematic because the RNA molecule is highly sensitive to enhanced temperatures and is degenerated by RNAses, which have to be promptly inactivated leading cell lysis. There are many commercial available molecular kits for PCR. The RT-PCR machines give precise calibration of kinetics of the PCR taking place inside the tube and promptly, show the visual representation of the PCR products following each series [13]. The data taken through RT-PCR is very accurate as compared to general PCR machine. There are commercially many companies which have the websites with complete protocols and output information investigated [14-16].

In this review paper, we discuss the common criteria and technical components of PCR technology, for fast functional genomics. Examples are also provided to show the efficacy of results of plant pathology research work and verification of targets for mammalian investigations.

Applications in Different Fields

Medicine

The newly developed techniques of nucleic acid amplifications have developed the medical diagnostics. Recent technologies that permit the diagnostics of different diseases through nucleic acid amplification are becoming very standardized [17]. The applications of these new molecular techniques in the field of diagnostics of diseases are becoming much expanded. In the medicinal field first of all, we look at the disease of Cancer [18]. Cancer comes from the addition of genetic polymorphism and mutation and/or sporadic somatic polymorphism in DNA repair, cell cycle and growth signaling genes [19]. We have now modern techniques of diagnostics of cancer but with surgical and medical treatment of cancer, still it remains a great cause of mortality during every year. At the earlier stage of cancer, the detection of cancer and its symptoms is very difficult to observe due to its complex multidimensional nature and heterogeneity [20-22]. However, with the invention of RT-PCR the earlier diagnostics of cancer is possible with small quantity of nucleic acids of different samples. Many of the cancerous parts of the body are detected in detection system by using different probes or by using marker genes expression. The accuracy of single marker gene is not great enough for clinical implications [23]. By adopting a genetic expression panel, the detection of Breast Cancer, Carcinoma of bladder, colorectal cancer and other types of cancers is feasible upto a good limit which was impossible during the past few decades. Now, six out of ten applications are made for the blood cancer detection. So, we can say that RT-PCR has revolutionized the medical world [24].

Field of Bacteriology

Earlier antibiotic therapy was dependent on the identification of Gram stain classification method. The identification of the presence of bacterial pathogens was earlier observed by the conventional PCR but later it was boosted up by using the RT-PCR. Fluorescence hybridization probes permitted a fast observation of low amounts of bacterial DNA and a right positive and negative gram stain classification technique [25]. RT-PCR made easier to detect the bacteria by hybridization technique without any hindrances of culturing bacteria into culture media which was a tough job before the invention of PCR. A fast detection of the pathogen will make easier to detect the pathogen and to choose the right antibiotic at the right stage which will kill the bacteria. The detection and observation of the mycobacterial infections on the earlier stages is very difficult because of lower specificity and sensitivity but on the later stages it may become sensitive and specific to be detected.

Field of Virology

Many of the protocols of RT-PCR have been made to detect and to quantify the viruses from viral infected human samples [26]. Various techniques are developed to detect the viruses especially relevant to human diseases. The identification of HSV1 and HSV2 was made possible by using TaqMan probes and it was, we can say, alternative to the conventional PCR. Recently, an observation, differentiation and quantification between HSV1 and HSV2 genotypes were attained by using different primers and probes which are called Light cycler induced targeting HSV DNA polymerase genes [27]. A sexually transmitted disease which is genital herpes is transmitted through virus but it is accurately detected by the RT-PCR and is documented well. Moreover, the RT-PCR has also aided to investigate the interactions between virus and host; this all is studied in the RT-PCR detection system with great efficiency [26]. RT-PCR has a great role in detecting the chronic conditions. Normally, the patients with less immunity tend to be more infected by viruses. RT-PCR multifactorial complex tools have been developed for virus specimens' identification [28].

Detection of Protozoa

As RT-PCR invention has revolutionized the molecular biology, so it has also good access to detect the parasitic Protozoa of medical field interest. RT-PCR has different protocols to detect the infections of Protozoa in humans but a disease caused by Protozoa, called Malaria remains still limited in identification by PCR due to its expensive molecular protocols [29]. RT-PCR have newly developed techniques for identification of chagas disease, amoebic dysentery, visceral leishmaniasis, giardiasis causing long lasting gastroenteritis and toxoplasmosis in the amniotic fluid of pregnant women and in the immune compromised patients. Protozoa cause the epidemic diseases around the world which are very problematic [30].

Detection of Fungi

Many diseases in humans are caused by the Aspergillus species [31]. There are many conventional methods which were used earlier to detect the fungal infections; these include cell culturing, histopathology, biochemical microscopy, PCR, probe detection, CFU quantification and identification, broth dilution and staining following by microscopic investigations [32]. The efficiency of these methods remain slower at some moments due to less efficient tool used. Quantitative and qualitative RT-PCR tools have also been made for other fungi like Coccidioides sp., and many others [33].

Food Science

Mycotoxins have a great role as food contaminants and they are now become a big source of bad effects on food items. To cope with such a situation, a fast and less expensive diagnostic system of food borne pathogen around the world for food pathogen detection have been developed for industry and human health [34]. Well-organized PCR-based methods for the detection and identification of the food-borne pathogens have been developed with analytical and diagnostic efficacy. In Food science, RT-PCR has strong role to detect the pathogens as Salmonella sp, which is one the most alarming pathogen of the food products [35].

Food borne viral contaminations are major worldwide leading disorders in humans. There is now innovation by the developing of RT-PCR by which we can successfully investigate the presence of viruses from blood and serum [36]. This methodology is very useful for Hepatitis B virus detection [37-40]. Other food item borne viruses are rotaviruses and gastrointestinal viruses. In shortly, we can say that the detection of these viruses directly from these food items is very difficult task.

Forensic Sciences

There are now advanced technologies developed for the detection of DNAs in Forensic Science. Through short tandem repeats, we can detect the DNAs of different organisms. One of the most common technologies used is PCR that permits efficient genotype knowledge and information from the specimens [41]. Forensic science mainly depends upon the slot blot technique which is much time consuming and laborious. RT-PCR has got a high standard among the tools used in Forensic science [42]. The DNA samples used in the investigations are checked by the fluorogenic probes and same situation was implemented on the discriminate sex. In this method, different samples of DNA are taken and they are passed through the PCR and are labeled with respective probe and then probe identifies each band of DNA [43-44]. Then, there is a computational analysis of DNA by using tannic acid which compares the amplifications efficacies of unknown DNA specimens with neat and clean standards. Moreover, DNA degradation is also investigated through forensic sciences.

Plant Sciences

Justification of Microarray Consequences

RT-PCR has specific role to study the gene expression designs. This study relates to the activation of genes, signal transfer, biosynthesis machinery and metabolic pathways. Gene expression investigations made easier identification of directions of stress signaling conditions of the abiotic and biotic environments. Such conditions of house-keeping genes have been studied in Potato. The efl alpha was the most stable gene among seven tested genes in biotic and abiotic conditions. Normally, the microarray confirmation through RT-PCR is highly well comparable as compared to other methods which are usually a best assay. So, here the main point is notable that justification of microarray results through RT-PCR is better than other methods [45].

Plant-Microorganism Interrelations

There is a vital association between plant and the associated microbe that forms a special relationship where the microbe is a dependent element. Early diagnosis of the attack of parasite can maintain plant health but only through RT-PCR [46]. As the severe attack of parasite can harm the plant population and can cause epidemic situations to the population of plants. In PCR, the methodology is that we take different samples of infected plant parts and then they are mixed with the PCR elements [47]. These parasites are further identified by PCR products computation by using different probes. Several reports are reported about the diagnosis of diseases related to plant-microbe parasitic relation. In past, there came many epidemics due to parasitic diseases like Rust and Smut etc [48].

Species Differentiation

In plants, there are several species within a genus which correlates with each other and there is just a small difference between these species. The difference is just at a specific site when we take the cytogenetic data of these species; there comes just a small difference among chromosomes of the different species [49-52]. So, the expression analyses of different plants are checked through gene expression in which sample is taken from the plant and is utilized in PCR. So, the PCR identifies the different products through probe hybridization and all computational arrangements are made by checking the gene expression of these products. The southern blot, northern blot and western blot techniques are used to detect different species. In all these techniques, the major data comes from the RT-PCR which detects and specifies the different species of plant [50].

Conclusion

RT-PCR has become best tool for the detection and quantification of expressing profiles of under investigation genes. This review itself shows that RT-PCR technique is a best technique to express the gene expression. This tool allows the quantitative and qualitative genotyping and shows detection of the single nucleotide polymorphism as well as Genetic variation. The RT-PCR has a great role in Medical field, Forensic science, Botany, Zoology, Mycology, Bacteriology, Virology and many other fields. In short, RT-PCR is a best tool in Molecular Biology [53].

References

1. Schaad, N.W. and Fredrick, R.D. Real-time PCR and its application for rapid plant disease diagnostics. Can. J. Plant Pathol. 2002,24: 250-258.

2. Monis, P.T. and Giglio, S. Nucleic acid amplification-based techniques for pathogen detection and identification. Infect. Genet. Evo. 2006, 6: 2-12.

3.Kalow, W. Pharmacogenetics and personalised medicine. Fundament. Clin. Pharmacol. 2002, 16: 337-342.

4. Severino, G. and Del Zompo, M. Adverse drug reactions: role of pharmacogenomics. Pharmacol. Res. 2004, 49: 363-373.

5.Kalow, W. Pharmacogenetics and pharmacogenomics: origin, status, and the hope for personalized medicine. Pharmacogenomics J. 2006, 6: 162-165.

6. Kirk, B.W., Feinsod, M., Favis, R., Kliman, R.M. and Barany, F. Single nucleotide polymorphism seeking long term association with complex disease. Nucleic Acids Res. 2002, 30: 3295-3311.

7. Alaoui-Jamali, M.A. and Xu, Y.J. Proteomic technology for bio-marker profiling in cancer: an update. J. Zhejiang Univ. Sci. B 2006, 7: 411-420.

8. Liefers, G.J. and Tollenaar, R.A.E.M. Cancer genetics and their application to individualized medicine. Eur. J. Cancer 2002, 38: 872-879.

9. Stahlberg, A., Zoric, N., Aman, P. and Kubista, M. Quantitative real-time PCR for cancer detection: the lymphoma case. Expert. Rev. Mol. Diagn. 2005, 5: 221-230.

10. Khanna, C. and Helman, L.J. Molecular approaches in pediatric oncology. Annu. Rev. Med. 2006, 57: 83-97.

11. Stahlberg, A., Zoric, N., Aman, P. and Kubista, M. Quantitative real-time PCR for cancer detection: the lymphoma case. Expert. Rev. Mol.

Diagn. 2005, 5: 221-230.
12. Mackay, I.M. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004, 10: 190-212.
13. Hoebeeck, J.V.D.L., Poppe, R., De Smet, B., Yigit, E., Claes, N., Zewald, N., de Jong, R., De Paepe, G.J., Speleman, A. and Van-desompele, F. Rapid detection of VHL exon deletions using real-time quantitative PCR. Lab. Investig. 2005, 85: 24-33.
14.Epsy, M.J., Uhl, J.R., Sloan, L.M., Buckwalter, S.P., Jones, M.F., Vetter, E.A., Yao, J.D.C., Wengenack, N.L., Rosenblatt, J.E., Cockerill, F.R. and Smith, T.F. Real-time PCR in clinical microbi-ology: Applications for routine laboratory testing. Clin. Microbiol. Rev. 2006, 19: 165-256.
15. Kaltenboeck, B. and Wang, C. Advances in real-time PCR: appli-cation to clinical laboratory diagnostics. Adv. Clin. Chem. 2005, 40: 219-259.
16. Weidmann, M., Meyer-König U. and Hufert, F.T. Rapid detection of herpes simplex virus and varicella-zoster virus infections by real-time PCR. J. Clin. Microbiol. 2003, 41: 1565-1568.
17. Legoff, J., Bouhlal, H., Gresenguet, G., Weiss, H., Khonde, N., Hocini, H., Desire, N., Si Mohamed, A., Longo, J.D., Chemin, C., Frost, E., Pepin, J., Malkin, J.E., Mayaud, P. and Belec, L. Real-time PCR quantification of genital shedding of herpes simplex vi-rus (HSV) and human immunodeficiency virus (HIV) in women coinfected with HSV and HIV. J. Clin. Microbiol. 2006, 44: 423-432.
18. Fleming, D.T., McQuillan, G.M., Johnson, R.E., Nahmias, A.J., Aral, S.O. and Lee, F.K. St Louis, M.E. Herpes simplex virus type 2 in the United States, 1976 to 1994. N. Engl. J. Med. 1997, 337: 1105-1111.
19. Ryncarz, A.J., Goddard, J., Wald, A., Huang, M.L., Roizman, B. and Corey, L. Development of a high-throughput puantative assay for detecting herpes simplex virus DNA in clinical samples. J. Clin. Microbiol. 1999, 37: 1941-1947.
20. Espy, M.J., Ross, T.K., Teo, R., Svien, K.A., Wold, A.D., Uhl, J.R. and Smith, T.F. Evaluation of LightCycler PCR for implementation of laboratory diagnosis of herpes simplex virus infections. J. Clin. Microbiol. 2000a, 38: 3116-3118.
21. Espy, M.J., Teo, R., Ross, T.K., Svien, K.A., Wold, A.D., Uhl, J.R. and Smith, T.F. Diagnosis of varicella-zoster virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. 2000b, 38: 3187-3189.
22. Koenig, M., Reynolds, K.S., Aldous, W. and Hickman, M. Com-parison of Light-Cycler PCR, enzyme immunoassay, and tissue culture for detection of herpes simplex virus. Diagn. Microbiol. In-fect. Dis. 2001, 40: 107-110.
23. Burrows, J., Nitsche, A., Bayly, B., Walker, E., Higgins, G. and Kok, T. Detection and subtyping of Herpes simplex virus in clinical samples by LightCycler PCR, enzyme immunoassay and cell cul-ture. BMC Microbiol. 2002, 2: 12.
24. Aldea, C., Alvarez, C.P., Folgueira, L., Delgado, R. and Otero, J.R. Rapid detection of herpes simplex virus DNA in genital ulcers by real-time PCR using SYBR green I dye as the detection signal. J. Clin. Microbiol. 2002, 40: 1060-1062.
25. Wald, A., Huang, M.L., Carrell, D., Selke, S. and Corey, L. Polym-erase chain reaction for detection of herpes simplex virus (HSV) DNA on mucosal surfaces: comparison with HSV isolation in cell culture. J. Infect. Dis. 2003, 188: 1345-1351.
26. van Doornum, G.J., Guldemeester, J., Osterhaus, A.D. and Ni-esters, H.G. Diagnosing herpesvirus infections by real-time ampli-fication and rapid culture. J. Clin. Microbiol. 2003, 41: 576-580.
27. Schmutzhard, J., Riedel, H.M., Wirgart, B.Z. and Grillner, L. De-tection of herpes simplex virus type 1, herpes simplex virus type 2 and varicella-zoster virus in skin lesions. Comparison of real-time PCR, nested PCR and virus isolation. J. Clin. Virol. 2004, 29: 120-126.
28. Kimura, H., Morita, M., Yabuta, Y., Kuzushima, K., Kato, K., Kojima, S., Matsuyama, T. and Morishima, T. Quantative analysis of Epstein-Barr virus load by using a real-time PCR assay. J. Clin. Microbiol. 1999, 37: 132-136.
29. MacKenzie, J., Gallagher, A., Clyton, R.A., Perry, J., Eden, O.B., Ford, A.M. Greaves, M.G. and Jarrett, R.F. Screening for herpesvi-rus genomes in common acute lymphoblastic leukemia. Leukemia 2001, 15: 415-421.
30. Templeton, K.E., Scheltinga, S.A., Beersma, M.F., Kroes, A.C. and Claas, E.C. Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B vi-ruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4. J. Clin. Microbiol. 2004, 42: 1564-1569.
31. Klaschik, S., Lehmann, L.E., Raadts, A., Book, M., Hoeft, A. and Stuber, F. Real-time PCR for detection and differentiation of gram-positive and gram-negative bacteria. J. Clin. Microbiol. 2002, 40: 4304-4307.
32. Kraus, G., Cleary, T., Miller, N., Seivright, R., Young, A.K., Spruill, G. and Hnatyszyn, H.J. Rapid and specific detection of the mycobacterium tuberculosis complex using fluorogenic probes and real time PCR. Mol. Cell. Probes 2001, 15: 375-383.
33. Lachnik, J., Ackermann, B., Bohrssen, A., Maass, S., Diephaus, C., Puncken, A., Stermann, M. and Bange, F.C. Rapid-cycle PCR and fluorimetry for detection of mycobacteria. J. Clin. Microbiol. 2002, 40: 3364-3373.
34. Coppenraet, E.S.B.V., Lindeboom, J.A., Prins, J.M., Peeters, M.F., Claas, E.C.J. and Kuijper, E.J. Real-time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of Mycobacterial lymphadenitis in children. J. Clin. Microbiol. 2004, 42: 2644-2650.
35. Sedlacek, L., Rifai, M., Feldmann, K. and Bange, F.C. LightCy-clerbased differentiation of Mycobacterium abscessus and Myco-bacterium chelonae. J. Clin. Microbiol. 2004, 42: 3284-3287.
36. Stermann, M., Bohrssen, A., Diephaus, C., Maass, S. and Bange, F.C. Polymorphic nucleotide within the promoter of nitrate reduc-tase (NarGHJI) is specific for Mycobacterium tuberculosis. J. Clin. Microbiol. 2003, 41: 3252-3259.
37. Broccolo, F., Scarpellini, P., Locatelli, G., Zingale, A., Brambilla, A.M., Cichero, P., Sechi, A., Lazzarin, A., Lusso, P. and Malnati, M.S. Rapid diagnosis of mycobacterial infections and quantitation of Mycobacterium tuberculosis load by two real-time calibrated PCR assays. J. Clin. Microbiol. 2003, 41: 4565-4572.
38. Desjardin, L.E., Chen, Y., Perkins, M.D., Teixeira, L., Cave, M.D. and Eisenach, K.D. Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. J. Clin. Microbiol. 1998, 36: 1964-1968.
39. Rhee, J.T., Piatek, A.S., Small, P.M., Harris, L.M., Chaparro, S.V., Kramer, F.R. and Alland, D. Molecular epidemiologic evaluation of transmissibility and virulence of Mycobacterium tuberculosis. (1999) J. Clin. Microbiol. 1999, 37: 1764-1770.
40. Rondini, S., Mensah-Quainoo, E., Troll, H., Bodmer, T. and Pluschke, G. Development and application of real-time PCR assay for quantification of Mycobacterium ulcerans DNA. J. Clin. Mi-crobiol. 2003, 41: 4231-4237.
41. Viedma, D.G.D., del Sol Diaz Infantes, M., Lasala, F., Chaves, F., Alcala, L. and Bouza, E. New real-time PCR able to detect in a single tube multiple rifampin resistance mutations and high-level isoniazid resistance mutations in Mycobacterium tuberculosis. J. Clin. Microbiol. 2002, 40: 988-995.
42. Marı´n, M., de Viedma, D.G., Ruı´z-Serrano, M.J. and Bouza, E. Rapid direct detection of multiple rifampin and isoniazid resis-tance mutations in Mycobacterium tuberculosis in respiratory sam-ples by

real-time PCR. Antimicrob. Agents Chemother. 2004, 48: 4293-4300.
43. Torres, M.J., Criado, A., Palomares, J.C. and Aznar, J. Use of real-time PCR and fluorimetry for rapid detection of rifampin and isoni-azid resistance-associated mutations in Mycobacterium tuberculo-sis. J. Clin. Microbiol. 2000, 38: 3194-3199.
44. Piatek, A.S., Telenti, A., Murray, M.R., El-Hajj, H., Jacobs, W.R., Kramer, F.R. and Alland, D. Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing. Antimicrob. Agents Chemother. 2000, 44: 103-110.
45. Wada, T., Maeda, S., Tamaru, A., Imai, S., Hase, A. and Kobaya-shi, K. Dual-probe assay for rapid detection of drug-resistant My-cobacterium tuberculosis by real-time PCR. J. Clin. Microbiol. 2004, 42: 5277-5285.
46. Bell, C.A., Uhl, J.R., Hadfield, T.L., David, J.C., Meyer, R.F., Smith, T.F. and Cockerill, F.R. Detection of Bacillus anthracis DNA by LightCycler PCR. J. Clin. Microbiol. 2002, 40: 2897-2902.
47. Moser, M.J., Christensen, D.R., Norwood, D. and Prudent, J.R. Multiplexed detection of anthrax-related toxin genes. J. Mol. Di-agn. 2006, 8: 89-96.
48. Pham, A.S., Tarrand, J.J., May, G.S., Lee, M.S., Kontoyiannis, D.P. and Han, X.Y. Diagnosis of invasive mold infection by real-time quantitative PCR. Am. J. Clin. Pathol. 2003, 119: 38-44.
49. Sanguinetti, M., Posteraro, B., Pagano, L., Pagliari, G., Fianchi, L., Mele, L., La Sorda, M., Franco, A. and Fadda, G. Comparison of real-time PCR, conventional PCR, and galactomannan antigen de-tection by enzymelinked immunosorbent assay using bronchoal-veolar lavage fluid samples from hematology patients for diagnosis of invasive pulmonary aspergillosis. J. Clin. Microbiol. 2003, 41: 3922-3925.
50. Bowman, J.C., Abruzzo, G.K., Anderson, J.W., Flattery, A.M., Gill, C.J., Pikounis, V.B., Schmatz, D.M., Liberator, P.A. and Douglas, C.M. Quantitative PCR assay to measure Aspergillus fu-migatus burden in a murine model of disseminated aspergillosis: demonstration of efficacy of caspofungin acetate. Antimicrob. Agents Chemother. 2001, 45: 3474-3481.
51. Costa, C., Vidaud, D., Olivi, M., Bart-Delabesse, E., Vidaud, M. and Bretagne, S. Development of two real-time quantitative TaqMan PCR assays to detect circulating Aspergillus fumigatus DNA in serum. J. Microbiol. Methods 2001a, 44: 263-269.
52. Maaroufi, Y., De Bruyne, J.M., Duchateau, V., Georgala, A. and Crokaert, F. Early detection and identification of commonly en-countered Candida species from simulated blood cultures by using a real-time PCR based assay. J. Mol. Diagn. 2004, 6: 108-114.
53. White, P.L., Williams, D.W., Kuriyama, T., Samad, S.A., Lewis, M.A. and Barnes, R.A. Detection of Candida in concentrated oral rinse cultures by real-time PCR. J. Clin. Microbiol. 2004, 42: 2101-2107.