PCR (models: ExtraGene 3200 and 9700) performance analysis

Project Number: SMI-2011

Project period: Jul. 1, 2012 to Dec. 31, 2012

Executive Unit: Department of Bioresources, Da-Yeh University, Taiwan

Executive Chair: Chorng-Horng Lin

Project Contact Person: Chorng-Horng Lin

Implementation units

Department of Bioresources

Name of contact person: Chorng-Horng Lin

Contact Tel :04 -8511-888 ext 6214

Fax Number :04 -8511-326

Mail: clin@mail.dyu.edu.tw

Authorized by

ExtraGene Inc.

Name of contact person: Peter Huang

Contact Tel :04 -24930999

Fax Number :04 -2422777

Mail: service@extragene-web.com

Abstracts

The polymerase chain reaction (PCR) is an easy, economic, convenient and biochemical technology in molecular biology for the amplification of thousands to millions of copies of a particular DNA sequence. The method consists of repetitive cycles of three steps: denaturation, hybridization, and polymerase extension, that raising temperature to break hydrogen bonds for formation of the single strand DNAs, then lowering temperature to allow the primers to anneal to the template DNAs, at last increasing temperature to the optimal condition for the Taq DNA polymerase to perform the polymerization. Because that the appropriate temperature and reaction time will be modulated based on the length and the specificity of the primers and the templates, the progresses of the PCR machine evolve the PCR reaction. Presently PCR technology is widely used in DNA cloning for sequencing, DNA-based phylogeny, functional analysis of genes, the diagnosis of hereditary diseases, the identification of genetic fingerprints, and the detection and diagnosis of infectious diseases. This report analyzes and compares two PCR machines: ExtraGene 3200 and 9700, with other brands of PCR machines on the performance of the PCR reaction, such as semiquantitative PCR with serial dilution of the templates or different number of cycles, the touchdown PCR, and the gradient PCR. The last two experiments only apply to the 9700 model. The results show that the 3200 model takes less time than other PCR machine in the completion time. The 9700 model performs as well as other PCR machines in touchdown PCR and gradient PCR, and since the model can set simultaneously in the temperature gradient of 12, to enhance its usefulness.

Keywords:. Polymerase Chain Reaction (PCR), ExtraGene 3200, ExtraGene 9700

1. Introduction

PCR (Polymerase chain reaction) is a simple convenient, economic method to amply a large number of DNAs, published in 1985 on the use of DNA polymerase to copy the Beta-globin gene for the detection of sickle cell anemia [1]. According to the founder, Dr. Mullis's own description, the concept of this method is originated in a Friday night April 1983, while he was carrying a female colleague in Northern California Bungalow road. At that time he was responsible for the synthesis of short DNA sequences in the company. Based on the principle of dideoxyribonucleotide sequencing method, the use of pairs of primers, high temperature to separate the double-stranded DNA into single strand, primers annealing, DNA polymerase to perform polymerization and the cycle repeats of these steps could achieve the purpose of a large number of DNAs amplification [2, 3]. By PCR method, a small amount of DNA could be amplified million times in a short time, applied in medical diagnoses, the DNA sequence of ancient creatures could be effectively reproduced etc., Dr. Mullis won the Nobel Prize in Chemistry in 1993 [4].

With the advances in biotechnology, the PCR methods and their applications have progressed extremely broad. For example, DNA with point mutation could be synthesized and constructed by PCR amplification simply with specific mutated primers [5]. PCR has been applied to the analysis of gene expression levels. Because as the number of cycle increases, the PCR product eventually would reach the saturation, the Investigator might reduce the number of cycle or the PCR reaction was conducted with different number of cycles to make the PCR product in the linear relationship for quantitative analysis. Another method was based on different known concentrations of the standard and the unknown concentration of the experimental group subjected to PCR analysis and quantitative comparison [6]. Such method also known as semi-quantitative PCR method, has been applied to the analysis of mouse sperm mother cell development and performance of the X chromosome gene, using beta-actin gene as the standard [7], applied to the heat shock protein gene expression analysis[8], analysis of cytomegalovirus DNA in serum and its incidence relevance in liver transplant patient [9], and using the same sample cells for the quantitative analysis of different genes expression [10]. Unlike semi-quantitative PCR, real-time quantitative PCR (Real-time PCR) method takes advantage of the PCR machine combined with a fluorescence detection system that the PCR cycle can be recorded with the amounts of

fluorescence, and the fluorescence can be calibrated in the conjugated primer or the competitive primers to achieve quantitative purpose [11, 12]. Addition to the simple PCR machine, i.e. to amply DNA with a fixed temperature and cycles, in order to efficiently increase the success of the PCR reactions, some PCR machines with changeable PCR reaction conditions have been designed and on the market, such as touchdown PCR that after the end of a cycle the annealing temperatures could drop to reduce the interference from non-specific products, especially for the complex genome analysis[13], for example the replication of soil bacterial 16S ribosomal DNA for the denaturing gradient gel electrophoresis analysis (DGGE) [14], applied to the diagnosis of the filamentous fungi [15], for the construction of the cDNA library [16]. Temperature gradient PCR is to perform PCR reaction with setting of different annealing temperatures, thus the optimal PCR condition could be achieved in one reaction time, such as the correct PCR products could be obtained for the unknown template DNA with the temperature gradient PCR method [17], using the difference in the primer to template bonding, the appropriate annealing temperature to distinguish between wild-type and mutant could be modulated by the temperature gradient PCR [18], to identify the different annealing temperature with multiple sets of primers for the detection of infected cells by *Pseudomonas aeruginosa* strains, [19]. Because PCR technology is extensively used, the above description is only applied for this report.

ExtraGene Inc. commissioned our laboratory the performance analysis of two PCR models, ExtraGene 3200 and 9700. ExtraGene 3200 is a simple model, with the capacity of 24 PCR reactions, and access to the 100 group setup, without touchdown and temperature gradient PCR. ExtraGene 9700 model can accommodate two specifications of 96 and 256 PCR reactions and can be set for touchdown PCR and temperature gradient PCR test. In order to compare the PCR machines of ExtraGene3200 and ExtraGene9700 with other brands, such as the iXXX Thermal Cycler (Bxx-xxx , USA), MXXX Thermal Cycler (Bxx-xxx), A- PC-xxx (Axxx, Japan) and 2xxx Thermal Cycler(AXX, USA), the different PCR conditions, such as the serial dilutions of template PCR, different number of cycles PCR, touchdown PCR and temperature gradient PCR were carried out and the performance was analyzed.

2. Materials and Methods

2.1 DNA material

Template DNAs used in this experiment were the soil bacteria 16s ribosomal DNA chromosomal DNA cloned into yT & A vector (Yeastern, Taipei, Taiwan) and soil bacteria genomic DNAs [20].

2.2 PCR, Polymerase Chain Reaction

The PCR reaction volume was 20 μL, containing primer 500 pmole of (Table 1) [14, 21], dNTP 200 μmoles, 10X PCR buffer (200 mM Tris-HCl, pH 8.8, 20 mM $MgSO_4$ ($7H_2O$), 100 mM KCl , 1% Triton X-100, 100 mM $(NH_4)_2SO_4$, 1 mg / ml BSA) and 1.5U HiFi DNA polymerase (Yeastern). PCR machines used in this study were ExtraGene 3200,9700 (ExtraGene Inc., Taiwan), iXXX Thermal cycler (Bxx-xxx), Mxxx Thermal cycler (Bxx-xxx), A- PC-xxx (Japan), and 2xxx Thermal Cycler (AXX).

2.2.1 PCR with varied template concentration

Serial dilutions of plasmid DNA 1/5, 1/50, 1/500, were used for PCR amplification. The PCR amplification was carried out with the following temperature profile: Initial denaturation at 95 ℃ for 5 min followed by 30 cycles of denaturation at 95 ℃ for 15 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 45 s, with a final extension at 72 ^{o}C for 10 min. The PCR products were resolved on 1.0% agarose gel.

2.2.2 PCR with varied number of cycles

Diluted 1/5 of plasmid DNAs were used as a template and the PCR condition was the same as the 2. 2.1, but with varied 20, 24, 28 and 30 cycles. The PCR products were resolved on 1.0% agarose gel.

2.2.3 Touchdown PCR

The template DNA was extracted from the chromosomal DNA of soil bacteria. The variable V3 region of 16s rDNA gene sequences from 341 to 534 of *Escherichia coli* (*E. coli*) was amplified by PCR described by Muyzer et al. [14]. PCR was performed by

using the following protocol: 1 min at 94℃, 1 min at 65℃, and 3 min at 72℃ with 0.5℃ touchdown every cycle during annealing for 20 cycles, followed by 10 cycles with an annealing temperature of 55℃ and a final cycle consisting of 10 min at 72℃.The amplified products were analyzed by electrophoresis in 2.0% (w/vol) agarose gels and stained with ethidium bromide.

2.2.4 The temperature gradient PCR

The template DNA was extracted from the chromosomal DNA of soil bacteria. The full-length sequence of the bacterial 16S ribosomal DNA was amplified. The PCR amplification was carried out with the following temperature profile: Initial denaturation at 95 ℃ for 5 min followed by 30 cycles of denaturation at 95 ℃ for 15 s, annealing at 63 ℃ to 47 ℃ for 30 s and extension at 72 ℃ for 2 min, with a final extension at 72 ^{o}C for 10 min. The 1500bp of PCR products were resolved on 1.0% agarose gel.

2.3 Image J analysis of PCR products

PCR products were analyzed by gel electrophoresis, photography, and Image J software analysis [22] to obtain quantitative data of the integration area.

Results and Discussion

The PCR test was first carried out with serial dilutions of the templates, 16S rDNA gene cloning plasmid DNAs (Fig. 1) With the decline of the template concentration, the PCR product also showed decremented. According to the Image J software analysis, the area amount of PCR product of the 1/500 dilution of plastid DNA with ExtraGene 3200 models was used as a reference value for the contrast correction, and the relative area values were 1, 0.09, 0, 0.6, for the PCR products with ExtraGene 3200 (1/500), ExtraGene 9700 (1/500), iXXX Thermal cycler (1/500), and Mxxx Thermal cycler (1/500) respectively. Comparison with the remaining 1/5 and 1/50 dilution of plasmids PCR reactions also showed the same results that the ExtraGene 3200 showed the best performance and the models 9700 showed the second.

For the PCR test with a different number of cycles, the area-based scores of the PCR products from models ExtraGene 3200 and 9700 were compared with other models and the results showed the pros and cons (Fig. 2 and 3). But on the record of the completion time for 24 cycles, there were 58,60,66,75 minutes for models ExtraGene 3200, 9700, iXXX Thermal Cycler, and A- PC-xxx (Japan) respectively (Figure 2), and 51, 56, 66, 75 minutes for the models ExtraGene 3200, 2720 Thermal Cycler, iXXX Thermal Cycler, A- PC-xxx resepctively (Figure 3). The results showed that models ExtraGene 3200 and 9700 can perform the PCR reaction well in relative short time.

The performance of touchdown PCR for model ExtraGene 9700 was showed in Figure 4. The touchdown PCR method was referred to Muyzer et al. [14], applied to the amplification of the variable V3 region of the 16S ribosomal DNA of soil bacteria for DGGE analysis. Initially the annealing temperature was set at 65 ^{o}C, with each cycle the annealing temperature with a decrement of 0.5 ^{o}C, annealing temperatures would drop to 55 ^{o}C after 20 cycles. Thus the specificity and yield of the PCR product can be increased, suitable for microbial phase specific genes analyzes. The Image J software analysis results showed the production yield performance of model ExtraGene 9700 ranked No. 1, sequentially followed by A- PC-xxx and iXXX Thermal cycler models (Figure 4).

Finally, the temperature gradient PCR tests were compared between models ExtraGene 9700 and Bxx-xxx the iXXX Thermal Cycler that the entire length of 16S ribosomal DNA was amplified from a mixture of soil bacteria DNA (Figure 5). The results showed that if the annealing temperature is high, the PCR reaction would be preferred, and the PCR product would decrease in accordance with the annealing temperature drop. The PCR performance of ExtraGene 9700 and iXXX Thermal cycler models were similar, but because the temperature gradient of ExtraGene 9700 model can be set to 12, compared to iXXX Thermal cycler, only 8 can be setting, the ExtraGene 9700 model showed more selective.

The PCR reaction was very sensitive, because the small amount of contaminant or error from the operator usually results in the non-specific product formation or production deviation. Additionally, whether the machines are old or new might deviate the PCR performance in the comparison of several PCR models? ExtraGene3200 and 9700 models were able to perform the PCR reaction with the expected results, consistent with other models,

especially in the outstanding yield performance.

Conclusions

1. The PCR reaction of ExtraGene3200 and 9700 models meet the expected results, and the yield is excellent, compared to the other models tested.

2. The PCR reaction time of ExtraGene3200 and 9700 model is shorter than the time of other models, showed the better time efficiency.

3. The ExtraGene 9700 can be set to a 12 temperature gradient, showed the selective advantage on the experimental design.

References

1. Saiki, R.K., et al., *Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia.* Science, 1985. **230**(4732): p. 1350-4.

2. Mullis, K.B., *The unusual origin of the polymerase chain reaction.* Sci Am, 1990. **262**(4): p. 56-61, 64-5.

3. Mullis, K., et al., *Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction.* Cold Spring Harb Symp Quant Biol, 1986. **51 Pt 1**: p. 263-73.

4. Nobel Prize Press Release, 1993; Available from: http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1993/press.html.

5. Ho, S.N., et al., *Site-directed mutagenesis by overlap extension using the polymerase chain reaction.* Gene, 1989. **77**(1): p. 51-9.

6. Gause, W.C. and J. Adamovicz, *The use of the PCR to quantitate gene expression.* PCR Methods Appl, 1994. **3**(6): p. S123-35.

7. McCarrey, J.R., D.D. Dilworth, and R.M. Sharp, *Semiquantitative analysis of X-linked gene expression during spermatogenesis in the mouse: ethidium-bromide staining of RT-PCR products.* Genet Anal Tech Appl, 1992. **9**(4): p. 117-23.

8. Abe, K., K. Kogure, and Y. Itoyama, *Rapid and semiquantitative analysis of HSP72 and HSC73 heat shock mRNAs by mimic RT-PCR.* Brain Res, 1995. **683**(2): p. 251-3.

9. Evans, P.C., et al., *Qualitative and semiquantitative polymerase chain reaction testing for cytomegalovirus DNA in serum allows prediction of CMV related disease in liver transplant recipients.* J Clin Pathol, 1998. **51**(12): p. 914-21.

10. Marone, M., et al., *Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample.* Biol Proced Online, 2001. **3**: p. 19-25.

11. VanGuilder, H.D., K.E. Vrana, and W.M. Freeman, *Twenty-five years of quantitative PCR for gene expression analysis.* Biotechniques, 2008. **44**(5): p. 619-26.

12. Dhanasekaran, S., T.M. Doherty, and J. Kenneth, *Comparison of different standards for real-time PCR-based absolute quantification.* J Immunol Methods, 2010. **354**(1-2): p. 34-9.

13. Don, R.H., et al., *'Touchdown' PCR to circumvent spurious priming during gene amplification.* Nucleic Acids Res, 1991. **19**(14): p. 4008.

14. Muyzer, G., E.C. de Waal, and A.G. Uitterlinden, *Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.* Appl Environ Microbiol, 1993. **59**(3): p. 695-700.

15. Brandt, M.E., et al., *Utility of random amplified polymorphic DNA PCR and TaqMan automated detection in molecular identification of Aspergillus fumigatus.* J Clin Microbiol, 1998. **36**(7): p. 2057-62.

16. Fietto, J.L., R. DeMarco, and S. Verjovski-Almeida, *Use of degenerate primers and touchdown PCR for construction of cDNA libraries.* Biotechniques, 2002. **32**(6): p. 1404-8, 1410-1.

17. Rose, T.M., et al., *Analysis of 4.3 kilobases of divergent locus B of macaque retroperitoneal fibromatosis-associated herpesvirus reveals a close similarity in gene sequence and genome organization to Kaposi's sarcoma-associated herpesvirus.* J Virol, 2003. **77**(9): p. 5084-97.

18. Lee, K.P., et al., *TIGRINA d, required for regulating the biosynthesis of tetrapyrroles in barley, is an ortholog of the FLU gene of Arabidopsis thaliana.* FEBS Lett, 2003. **553**(1-2): p. 119-24.

19. Fothergill, J.L., et al., *Diagnostic multiplex PCR assay for the identification of the Liverpool, Midlands 1 and Manchester CF epidemic strains of Pseudomonas aeruginosa.* J Cyst Fibros, 2008. **7**(3): p. 258-61.

20. 王彥文, *巴克素對土壤菌相影響之研究*, in *分子生物科技學系碩士班* 2009, 大葉大學.

21. Edwards, U., et al., *Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA.* Nucleic Acids Res, 1989. **17**(19): p. 7843-53.

22. Rasband, W.; Available from: http://rsb.info.nih.gov/ij/.

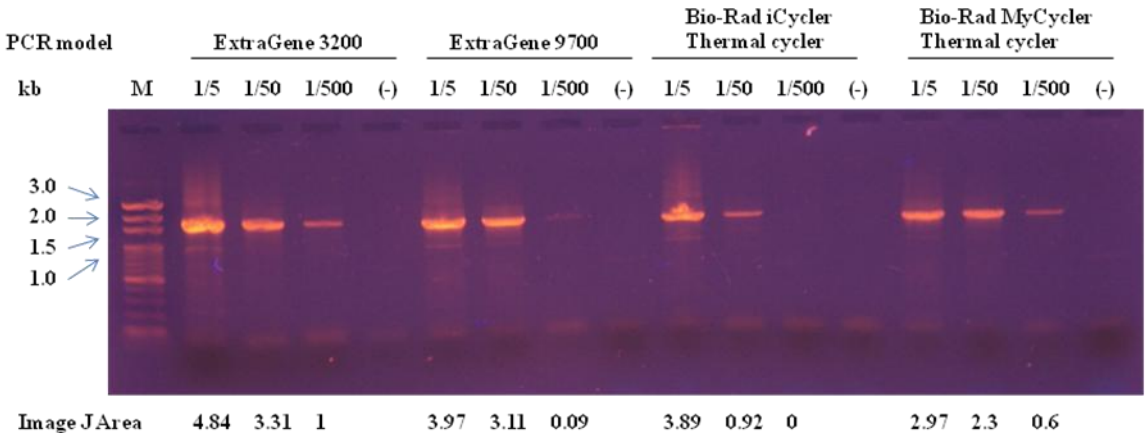

Figure 1. PCR with the serial diluted template DNA. The plasmid DNA of soil bacteria 16s rDNA gene was serial diluted of 1/5, 1/50 and 1/500 for PCR reaction. 5 L of the PCR solution was applied for gel electrophoresis, photography, and analysis with Image J program. PCR amplification was carried out with the following temperature profile: initial denaturation at 95 ℃ for 5 min followed by 30 cycles of denaturation at 95 ℃ for 15 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 45 s, with a final extension at 72 ℃ for 10 min. M represents the 100bp DNA marker. (-) represents the negative control of water.

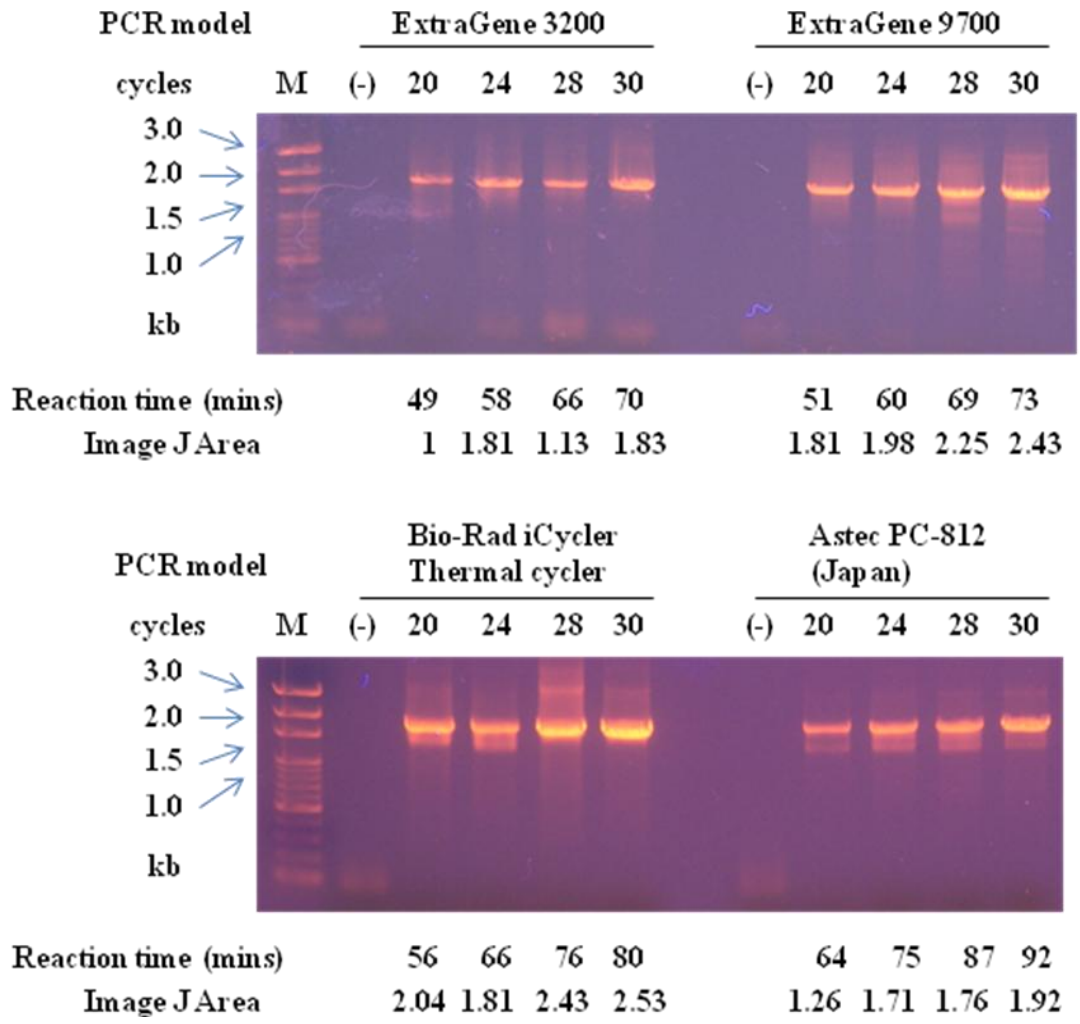

Figure 2. The serial cycles of PCR reaction. PCR amplification was carried out similar to the condition of Figure 1, with the cycles of 20, 24, 28 and 30 and 1/5 dilution of the plasmid DNA as the template. M represents the 100bp DNA marker. (-) represents the negative control of water.

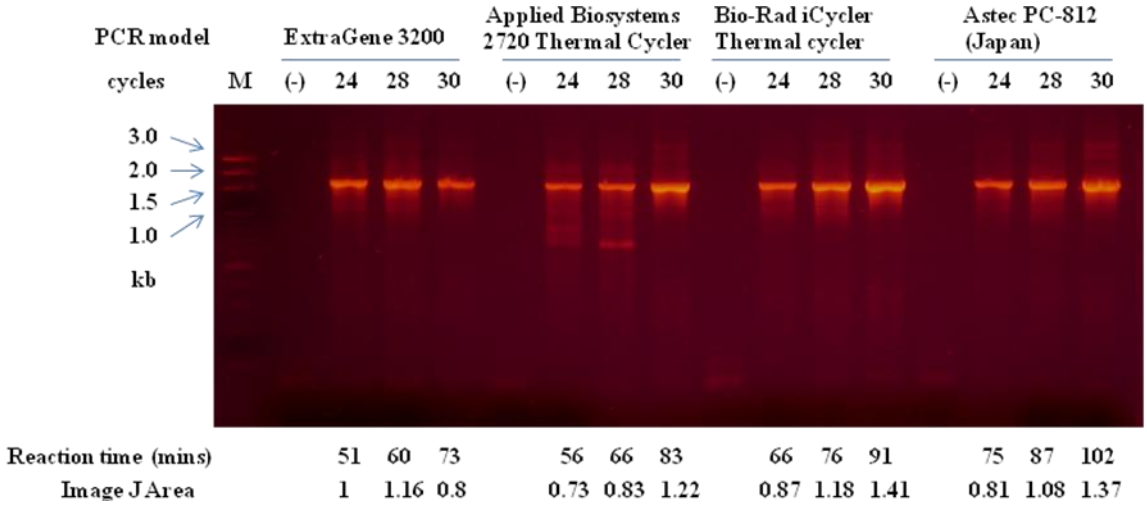

Figure 3. The serial cycles of PCR reaction. PCR amplification was carried out similar to the condition of Figure 1, with the cycles of 24, 28 and 30 and 1/5 dilution of the plasmid DNA as the template. M represents the 100bp DNA marker. (-) represents the negative control of water.

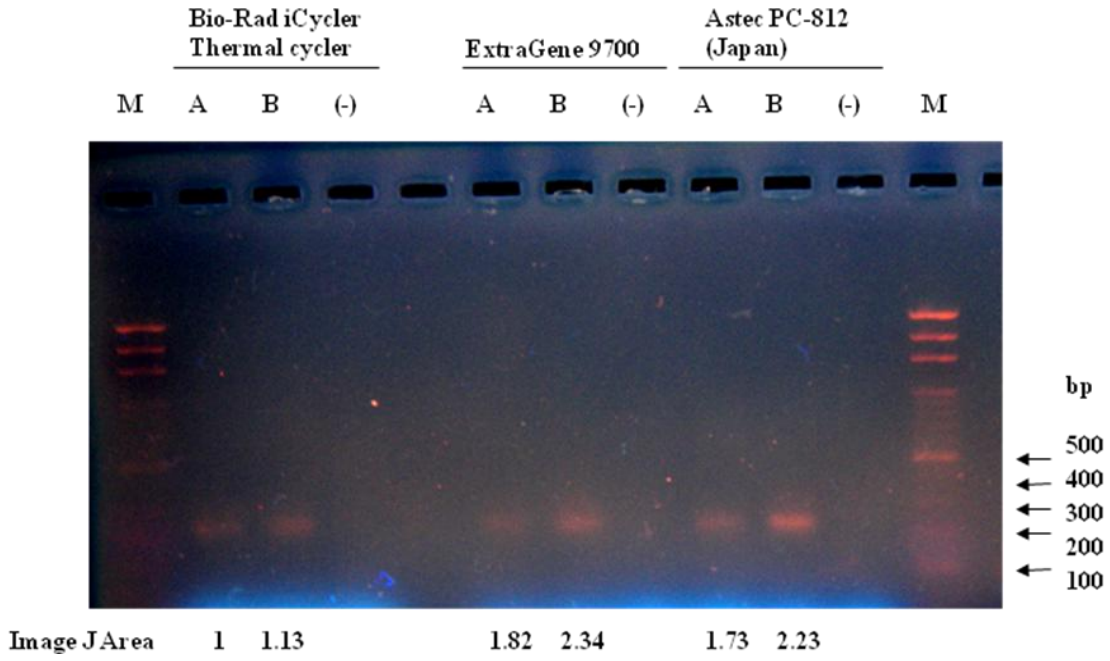

Figure 4. Touchdown PCR. The variable V3 region of 16S rDNA gene sequences from 341 to 534 nucleotides of *Escherichia coli* was amplified by PCR, following Muyzer et al. (1993). The amplification was done with the following temperature profile: Initial denaturation at 95℃ for 5 min, 20 cycles of denaturation at 95℃ for 15 sec, annealing at 65℃ (with 0.5℃ reduction after every cycle) for 30 sec, extension at 72℃ for 30 sec, followed by 10 cycles of denaturation at 95℃ for 15 sec, annealing at 55℃ for 30 sec, extension at 72℃ for 30 sec and a final extension at,72℃ for 10 min. A and B represent the bacterial genomic DNAs from different soils. M represents the 100bp DNA marker. (-) represents the water as the negative control.

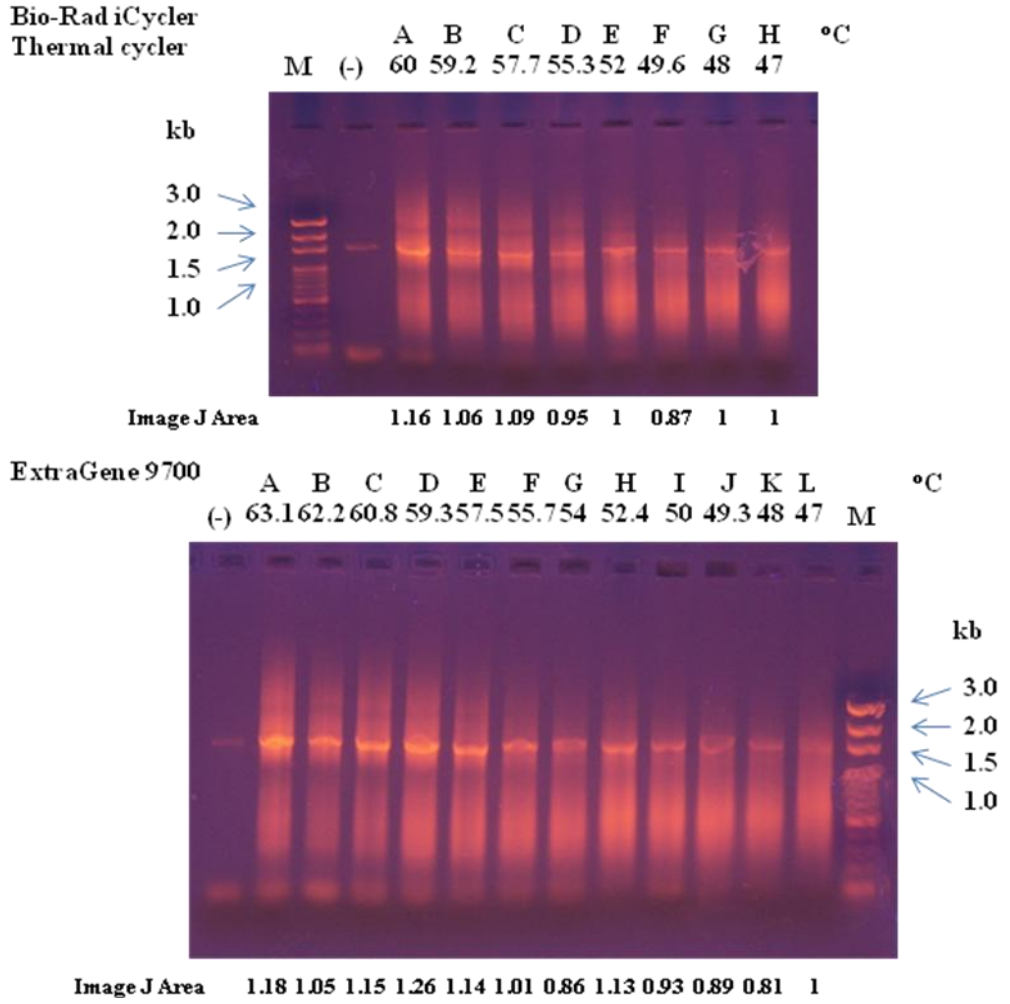

Figure 5. Gradient PCR. The soil bacterial 16S rDNA gene was amplified by PCR. The amplification was done with the following temperature profile: Initial denaturation at 95℃ for 5 min, 30 cycles of denaturation at 94℃ for 1 min, 30 cycles of annealing at range 63℃ to 47℃ for 1 min, extension at 72℃ for 2 min and a final extension at,72℃ for 10 min. Letters A to L represent the different annealing temperature. M represents the 100bp DNA marker. (-) represents the water as the negative control.

Table 1. The PCR reaction primers

引子	序列(5’ – 3’)
M13 Forward[a]	GGTTTTCCCAGTCACGAC
M13 Reverse[a]	GGAAACAGCTATGACCATGC
341F[b]	CCTACGGGAGGCAGCAG
534R[b]	ATTACCGCGGCTGCTGG[*]
pA8[c]	AGAGTTTGATCCTGGCTCAG
pH1541[c]	AAGGAGGTGATCCAGCCGCA

a. Primers used in different template concentrations PCR is extremely different number of cycles PCR.

b. Decreasing PCR primers used in.

c. Primers used in the temperature gradient PCR.

[*]GC Extended sequence:
CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG