Ultra-High Efficient Colony PCR for High Throughput Screening of Bacterial Genes

Mohammad Abu Hena Mostofa Jamal^{1,4} · Satya Priya Sharma¹ · Hea-Jong Chung^{1,5} · Hyeon-Jin Kim² · Seong-Tshool Hong¹ · Seungkoo Lee³

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Abstract Current colony PCR methods are not suitable for screening genes encoded in genomic DNA and are limited to *E. coli* host strains. Here, we describe an ultra-high efficient colony PCR method for high throughput screening of bacterial genes embedded in the genomic DNA of any bacterial species. This new technique expands colony PCR method to several hosts as well as offers a rapid, less expensive and reliable bacterial genomic DNA extraction.

Keywords Colony PCR · DNA extraction · High throughput screening · Sonication · Sequencing

Colony PCR is one of the most commonly used methods to quickly screen for the presence of desired plasmids in *E. coli* colonies without need for culturing individual colonies and preparing plasmid DNA before analysis [1, 2]. Current colony PCR methods are basically limited to

E. coli bacterial species and only screen for plasmid DNA present at greater than several dozen copy numbers per cell [3, 4]. Accordingly, it is not possible to directly screen genes embedded in genomic DNA by current colony PCR techniques. Moreover, host strains of bacterial species for colony PCR are typically limited to *E. coli*, [5] which means that PCR of genomic DNA directly from bacterial colonies is generally not feasible. At the same time, PCR of genomic DNA directly from bacterial colonies is a daily necessity in many molecular biology labs for high throughput screening of genes, as in the case of PCR amplification of small-subunit ribosomal DNA sequences of bacterial species [6]. Our new method has focused on high throughput screening of bacterial gene embedded in genomic DNA and to spread the advantages of quick screening to all bacterial species.

Here, we present a new colony PCR approach that is rapid and non-toxic, uses less equipment, and requires minimum labor and/or time to extract quality genomic DNA from bacteria that are as pure and concentrated as that prepared using other extraction techniques. We performed this colony PCR for high throughput screening of bacterial gene embedded in genomic DNA by means of mechanical disruption using sonicator. We developed this alternative method of colony PCR and genomic DNA extraction from bacteria while working with a guanidine-detergent-based cell wall lysing method [7, 8] for genomic DNA isolation (Table 1). The use of the guanidine-detergent made the DNA extraction procedure easy, rapid, reliable and efficient, but the presence of guanidine isothiocyanate in the mixture made the process hazardous for users [9]. In the current procedure, we eliminated the use of the toxic guanidine detergent and established a less harmful method for bacterial genomic DNA isolation (Table 1).

Mohammad Abu Hena Mostofa Jamal and Satya Priya Sharma have contributed equally to this work.

✉ Seungkoo Lee
jsklee@kangwon.ac.kr

¹ Department of Biomedical Sciences and Institute for Medical Science, Chonbuk National University Medical School, Chonbuk National University, Jeonju, South Korea

² JINIS BDRD Institute, JINIS Biopharmaceuticals Co., Waju, Chonbuk, Korea

³ Department of Anatomic Pathology, School of Medicine, Kangwon National University Hospital, Kangwon National University, Chuncheon, Gangwon 24341, South Korea

⁴ Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh

⁵ Department of Microbiology, Seonam University Medical School, Namwon, Chonbuk 55321, South Korea

Table 1 A detailed stepwise bacterial DNA extraction method through DNAzol and new ultra-high efficient colony PCR methods

Steps	DNAzol method	New method
Washing	1. Take a single colony in 500 µL filter sterilized autoclaved distilled water or 500 µL bacterial culture (OD value 0.5) in 1.5 mL Eppendorf tube 2. Centrifuge for 1 min at 11,000 rpm 3. Discard the supernatant 4. Add 500 µL autoclaved distilled water, vortex and centrifuge for 1 min at 1300 rpm. And discard the supernatant	1. Take a single colony in 500 µL filter sterilized autoclaved distilled water or 500 µL bacterial culture (OD value 0.5) in 1.5 mL Eppendorf tube 2. Centrifuge for 1 min at 11,000 rpm 3. Discard the supernatant 4. Add 500 µL autoclaved distilled water, vortex and centrifuge for 1 min at 1300 rpm. And discard the supernatant
Cell wall lysis	5. Add 100 µL DNAzol reagents 6. Sonicate at 4 °C for 10 min. After every 2.5 min keep the tube on ice for 1 min	5. Add 100 µL filtered sterilized autoclaved distilled water 6. Sonicate at 4 °C for 10 min. After every 2.5 min keep the tube on ice for 1 min
Separation	7. After sonication keep the sample on ice for 30 min according to the DNAzol kit's instruction 8. Centrifuge for 10 min at 11,000 rpm 9. Transfer the fresh supernatant to a fresh 1.5 Eppendorf tube 10. Add 50 µL 100% ethanol, invert 6 times and keep stand for 3 min 11. Centrifuge for 2 min at 11,000 rpm 12. Remove the supernatant	7. After sonication keep the sample on ice for 10 min to settle down the cell debris in the bottom N/R N/R N/R N/R N/R
Alcohol washing	13. Add 500 µL 75% ethanol for washing. Mix and keep stand for 3 min and centrifuge for 2 min at 11,000 rpm and remove the supernatant. Repeat the steps for 2 times	N/R
Extraction	14. Dry the tube keeping open the tube to the air for 15 to 20 min 15. Add 60 µL filtered autoclaved distilled water and 30 µL 8 mM NaOH and mix to dissolve the DNA	8. Transfer 60 µL fresh supernatant to a fresh tube and use the supernatant as DNA sample 9. Though the fresh supernatant can be used as template, we can add 30 µL 8 mM NaOH to 60 µL fresh supernatant to preserve the DNA for subsequent using

N/R not required

Table 2 A comparative assessment of DNAzol method for bacterial genomic DNA isolation and new ultra-high efficient colony PCR method

Bacterial species	DNAzol method			New method		
	DNA concentration (ng/µL)	260/280 OD ratio	Sequence similarity	DNA concentration (ng/µL)	260/280 OD ratio	Sequence similarity
<i>E. coli</i> KCTC 2571	137.04	1.88	97.3%	127.8	1.93	99%
<i>Klebsiella pneumoniae</i> KCTC 12385	163.03	2.12	99%	208.53	1.94	99%
<i>Pseudomonas aeruginosa</i> KCTC 1750	94.04	1.93	99.9%	172.04	1.98	99.6%
<i>Lactobacillus acidophilus</i> KCTC 3179	178.06	1.99	99.8%	212.30	1.93	99.9%
<i>Bacillus cereus</i> KCTC 1014	212.32	2.13	99.3%	384.91	1.77	99.9%
<i>Staphylococcus aureus</i> KCTC 1916	133.53	2.11	98.8%	734.01	2.74	99%
Other parameters						
Time	Almost 1 h 30 min			30 min		
Toxicity	Toxic			Non-toxic		
Chemical	DNAzol kit, 100% Alcohol, Autoclaved filter sterilized distilled water, 8 mM NaOH			Autoclaved filter sterilized distilled water, 8 mM NaOH		
Cost	0.75 USD ~ 1 USD/sample			Almost free of cost		
Equipment requirement	Centrifuge, sonicator, autoclave			Sonicator, autoclave, centrifuge (optional)		

We used 16S-rDNA sequence-based bacterial species identification to check the quality, reliability, and specificity of extracted genomic DNA from the guanidine-

detergent-based method and our new method. We compared the quality and quantity of the extracted DNA, as well as extraction time, extraction cost, human effort, and

Fig. 1 Downstream application and effectiveness of new colony PCR method. **a** PCR result using universal primer 27 F and 1492 R of genomic DNA isolated using DNAzol method and new method. After 10 min sonication gram negative *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and gram positive *Bacillus cereus*, *Lactobacillus acidophilus* showed band having similar intensity of PCR band from genomic DNA isolated by DNAzol method whereas band

intensity of *Staphylococcus aureus* was lower at this stage. **b** Genomic DNA from *Staphylococcus aureus* colony also showed band after increasing sonication time to 20 min. **c** Representative figure of 16S rDNA sequencing shows electrogram and sequence similarity result of genomic DNA isolated from new method comparing with DNAzol method

hazards between the two methods. DNAzol reagent (Invitrogen catalog Number 10503-027) was used as the guanidine-detergent for bacterial genomic DNA isolation. Six different bacterial species, three gram-negative (*E. coli* KCTC 2571, *Klebsiella pneumoniae* KCTC 12385, *Pseudomonas aeruginosa* KCTC 1750), and three gram-positive (*Staphylococcus aureus* KCTC 1916, *Bacillus cereus* KCTC 1014, *Lactobacillus acidophilus* KCTC 3179), were aerobically grown on tryptic soya agar medium for comparative analysis. A single colony of the six selected bacterial species was used for further parallel subsequent procedures (Table 1). We can also use 500 μ L of bacterial suspension maintained at an OD 0.5 at 600 instead of single colony. In the next step, bacterial suspensions were washed twice with ddH₂O for the removal of residual agar and resuspended in 100 μ L of ddH₂O or DNAzol, respectively,

for sonication. Sonication was performed with a water-based ultra-sonicator (Saehan SH-1025D/1050D, Saehan Ultrasonic Co., Korea) at a 40 kHz oscillation frequency for 10 min. Importantly, the sample temperature was maintained below 4 °C throughout the sonication procedure. If in the sonication machine there is no facility to maintain the temperature at 4 °C, the sonicator should be filled with ice. And samples were kept on the ice for a 1 min rest after each two and half minute cycle of sonication. Thereafter, samples were placed on ice for 10 min to allow settling of cell debris, and supernatants were carefully transferred into new tubes for DNA quality checks. The purity and yield of extracted bacterial genomic DNA from both methods were measured using a BioSpec-nano (Shimadzu Biotech, Japan) [10]. Lastly, all extracted bacterial DNA from both methods were used as templates

in nested PCR under conditions suggested for EF-Taq DNA polymerase (Sol Gent, South Korea) for 16S-rDNA gene amplification using universal 27F/1492R primers [11, 12] and amplified DNAs, followed by subsequent Sanger sequencing for bacterial species identification [13–15].

Genomic DNA quality analysis revealed that the DNA extracted via the new method was pure and sometimes higher concentrated compared to that extracted with DNAzol (Table 2). Without using RNase we get good PCR product and subsequent result of sequencing. Furthermore, if we need to remove RNA impurities, we can use RNase as optional steps (1:10 of volume ratio of RNase and ddH₂O) like other commercially available kit (NucleoSpin Tissue, REF, 740952.250, GenElute™ Bacterial Genomic DNA Kit, sigma). The mechanical breakup of the bacterial cell wall using sonication at a very low temperature (4 °C) is the underlying principal of this procedure. The controlled sonication process tears the bacterial outer covering and releases the genomic DNA into the mixture. The constant low temperature (4 °C) retains the stability of genomic DNA by restricting the enzymatic lysis of DNA by temperature-sensitive DNase enzymes [16]. Ten minutes of

sonication was sufficient for most bacterial strains to obtain the desired PCR amplicons, with the exception of *S. aureus*, possibly due to the shape, size, cell wall composition or culture quality of the bacteria (Fig. 1a). Twenty minutes of sonication produced better results of *S. aureus* (Fig. 1b). So, in mechanical disruption, these phenomenon are crucial. Thus, Gram-negative bacteria appear to be preferable for this method. Sometimes less than 10 min sonication is enough for cell wall disruption but 10 min sonication is appropriate for broad range of bacteria screening. This mechanical disruption may degrade physically fragile whole genomic DNA but it gives very efficient and rapid colony PCR to identify bacteria or screen genes embedded in genomic DNA. All bacterial identification was performed using NCBI BLAST searches [17–19] and results were obtained if more than 99% similarity existed with a bacterial species (Table 2; Fig. 1), confirming the authenticity of our new genomic DNA extraction method for colony PCR.

Our new, non-toxic, ultra-high efficient colony PCR method is significantly faster, less expensive, less laborious, and most importantly, as reliable and precise as other currently used procedures to extract bacterial genomic

Table 3 Comparison between previously described colony PCR methods with our method

Factors	References					
	[20]	[21]	[22]	[23]	[24]	Our method
Bacterial screening spectrum	Limited to <i>E. coli</i>	Not mentioned	Several bacterial fish pathogens	Limited to <i>E. coli</i> and <i>S. cerevisiae</i>	Limited to <i>Staphylococcus</i> spp.	Broad range of including both Gram's (+) and (–) bacterial strains
Culture condition	Solid culture and liquid culture both	Not mentioned	Solid culture	Solid culture	Solid culture	Solid culture and liquid culture both
DNA extraction						
Bacterial cell lysis	Lysed at 100 °C	Not lysed	Not lysed	Not lysed	Not lysed	Lysed by ultrasonication at 4 °C
DNA release in matrix	After lysis at 100 °C	During PCR	During PCR	During PCR	During PCR	During sonication at 4 °C
Processing time	<4 h	Not mentioned	Overnight	Not mentioned	Not mentioned	≈ 30 min
DNA quality	Not measured	Not measured	Not measured	Not measured	Not measured	Good quality of DNA
Key motive of the experiment	Screening of recombinant clones	High throughput screening including single bacterial cell	To reduce the cost and time in fish pathogenic bacteria diagnosis	Direct PCR of DNA without prior DNA purification	To validate novel RT-PCR methods for the detection of <i>mecA</i> in <i>Staphylococcus</i> spp.	High throughput screening of bacterial genes embedded in the genomic DNA of any bacterial species
Special equipment	No special equipment	Droplet microfluidic device	No special equipment	No special equipment	Light Cycler 2.0 instrument	Ultrasonication (40 kHz)
Cost	Low cost	Not mentioned	Low cost	Not mentioned	Low cost	Low cost

DNA (Table 2). However, our new method is fundamentally similar to the previously described colony PCR techniques [20–24]. Despite the fundamental similarity, this new innovative colony PCR technique has several notable methodological advantages compared to already published producers, summarized in Table 3. Accordingly, our technique is much easier to performed, covering broader spectrum of bacterial species and extracted DNA can be used for the any kind of gene identification or multiple downstream processes independently to the colony PCR due its purity and high concentration. Thus, Incorporation of this procedure for high throughput screening can reduce workloads, particularly in molecular-based microbiology labs, by minimizing the time and effort needed for microbial genomic DNA isolation and identification of various genes embedded in genomic DNA.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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