

BacReady™ Multiplex PCR System

Technical Manual No. 0191

Version 10112010

I	Description	1
II	Applications	2
III	Key Features	2
IV	Shipping and Storage	2
V	Simplified Procedures	2
VI	Detailed Experimental Procedures	2
VII	Examples Using the System	3
VIII	Troubleshooting	5
IX	Order Information	6

I. DESCRIPTION

BacReady™ Multiplex PCR System is a powerful reagent kit for both rapid preparation and multiplex PCR amplification of bacteria genomic DNA and plasmid DNA. Genomic and plasmid DNA are directly released from bacteria (both gram-positive and gram-negative) cells in a single step by adding a proprietary reagent directly to bacteria samples without DNA purification. The genomic and plasmid DNA can then be used immediately in PCR amplification of multiple gene targets (up to >1,000) or stored at 4°C for future use (stable at least 6 months at 4°C).

BacReady™ Multiplex PCR System with enzyme (L00203) contains BR-A buffer and 2X PCR premix. The BR-A buffer is used to lyse cells and to release genomic DNA and plasmid DNA. PCR premix contains PCR buffer (BR-B buffer), dNTP, Mg²⁺ and “HotStart” Script™ DNA polymerase for PCR amplification.

L00203 Components	100 Preps
BR-A buffer	5.00 ml
2X PCR premix	1.00 ml
PCR-grade water	1.00 ml

BacReady™ Multiplex PCR System without enzyme (L00202) is also available from GenScript Corporation. This kit allows our customers to use any other DNA polymerases that they prefer for PCR reaction. The kit contains BR-A buffer and BR-B buffer. The BR-A buffer is used to lyse cells and to release genomic DNA and plasmid DNA. BR-B buffer is an optimized 10X Script™ DNA polymerase buffer (without dNTP). Limited tests at GenScript show that this buffer is compatible with Taq DNA polymerases from other vendors and also increases PCR sensitivity.

L00202 Components	100 Preps
BR-A buffer	5.00 ml
BR-B buffer	0.20 ml
PCR-grade water	1.00 ml

II. APPLICATIONS

This kit is for both genomic DNA and plasmid DNA extraction from gram-positive or gram-negative bacteria.

And for application such as:

- SNP genotyping and mutation detection
- Fast colony screening using PCR
- DNA sequencing and cloning
- Quantitative PCR

III. KEY FEATURES

- ◆ Easy to perform: very simple and rapid procedure to extract both genomic DNA and plasmid DNA in a single step.
- ◆ High specificity: highly specific amplification of DNA using “HotStart” Script™ DNA polymerase (a GenScript proprietary DNA polymerase).
- ◆ Multiplex PCR: up to >1,000 DNA sequences can be amplified using multiplex PCR primers.
Super sensitivity: a single bacteria cell can be used to amplify both genomic DNA and plasmid DNA.

IV. SHIPPING AND STORAGE

This kit is shipped on blue ice. Store the kit at -20°C after receiving.

V. SIMPLIFIED PROCEDURES

1. Thaw BR-A buffer at room temperature and vortex the solution. Add 20 µl of BR-A buffer to 1.0 µl bacteria sample and mix well.
2. Set-up and perform PCR reaction in a PCR cycler.

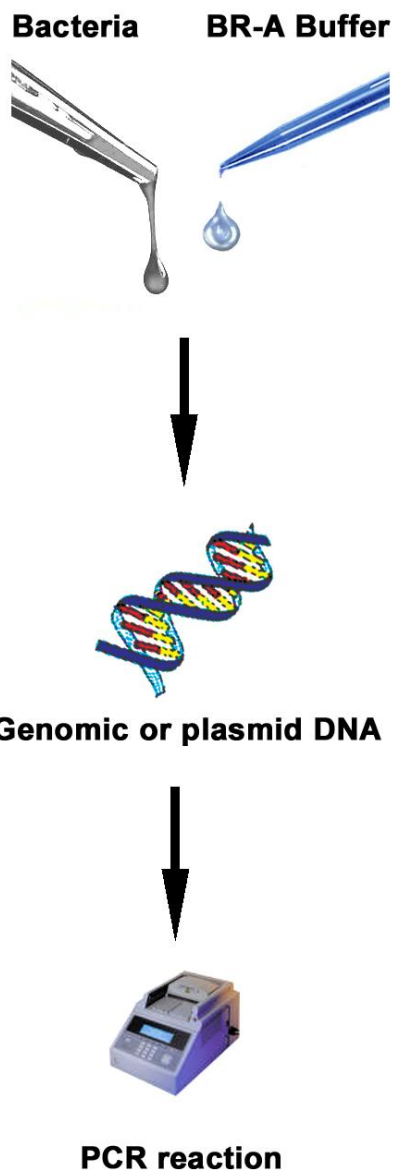
VI. DETAILED EXPERIMENTAL PROCEDURES

A. Genomic and Plasmid DNA Preparation

Thaw BR-A buffer at room temperature and vortex the solution. Add 20 µl of BR-A buffer to 1.0 µl bacteria sample (cell culture or colonies picked from plates) and mix well.

Please note: Genomic DNA is fragile and high molecular weight DNA is sheared easily by mechanical forces. Do not vortex solutions containing genomic DNA.

Overview of Kit Procedure



B. PCR Amplification

One or multiple gene targets can be amplified using a pair of primers or multiple pairs of primers (multiplex PCR) from the genomic or plasmid DNA prepared as described above. PCR reactions can be set up at room temperature since “HotStart” Script™ DNA polymerase is used.

1. Set up 20 µl PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently. The table below is used only as a guide. For multiplex PCR, the primer concentrations and cycling parameters need to be optimized.

Reagent	Volume	Final Concentration
Water, PCR grade	7 µl	
4 µM forward primer	1 µl	200 nM
4 µM reverse primer	1 µl	200 nM
DNA solution	1 µl	
PCR premix	10 µl	
Total	20 µl	

If you are using BacReady™ Multiplex PCR System without enzyme (PCR premix), set up 20 µl PCR reaction following your PCR kit instruction, and use 1 µl of DNA solution prepared. As mentioned before, limited tests at GenScript show that BR-B buffer (without dNTP) is compatible with Taq DNA polymerases from different vendors.

2. The commonly used thermal profiles can be used for PCR amplification. The following two thermal profiles are recommended for the amplification of a single amplicon and multiple amplicons, respectively.

- a. Thermal profiles for amplification of a single amplicon with the primer concentration of 200 nM for each primer.

Activation of Script™ DNA polymerase:	94°C for 15 min
35 PCR cycles of	Denaturation: 94°C for 40 sec
	Annealing: 55-60°C for 1 min
	Extension: 72°C for 30 sec to 2 min (≈1 kb/min)
	Final extension: 72°C for 3 min.

- b. Thermal profiles for amplification of multiple amplicons with the each primer at concentration of 50 nM.

Activation of Script™ DNA polymerase:	94°C for 15 min.
35 PCR cycles of	Denaturation: 94°C for 40 sec
	Annealing: 55-60°C for 2 min
	Extension: ramping from 55°C to 72°C for 5 min
	Final extension: 72°C for 3 min.

VII. EXAMPLES USING THE SYSTEM

A. Gram-negative Bacteria Genomic DNA Preparation and PCR Amplification.

To test genomic DNA preparation from gram-negative bacteria and PCR amplification, a pairs of primers specific for *E. coli* are selected to amplify part of 16S rRNA gene. The expected PCR product is 544 bp. And the sequences of the two primers used in the experiments are:

Forward primer: 5'-GGAAGAAGCTTGCTTCTTTGCTGAC-3'
Reverse primer: 5'-AGCCCGGGGATTTACATCTGACTTA-3'

Genomic DNA's were prepared from four different *E. coli* cultures and amplified using BacReady™ Multiplex PCR System (with enzyme) following the kit instructions. The results were shown in Figure 1.

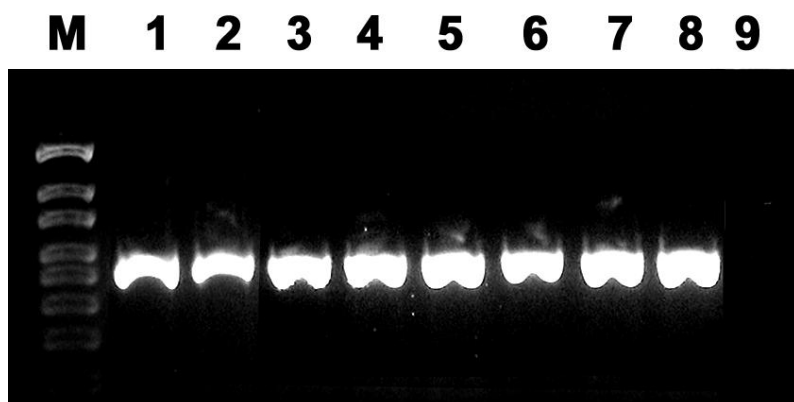


Figure 1. PCR analysis of genomic DNA prepared from bacteria samples. Genomic DNAs were prepared from 4 different *E. coli* cultures and amplified using BacReady™ Multiplex PCR System following the kit instructions. M is 100 bp DNA marker lane. Lane 1 and 2 are culture No. 1; Lane 3 and 4 are culture No. 2, and so on. Lane 9 is a negative control. PCR products are 544 bp.

B. Gram-positive Bacteria Genomic DNA Preparation and PCR Amplification.

To test genomic DNA preparation from gram-positive bacteria and PCR amplification, a pairs of primers specific for gram-positive bacteria are selected to amplify part of rRNA gene. The expected PCR product is 355 bp. And the sequences of the two primers used in the experiments are:

Forward primer: 5'-GCGRCTCTCTGGTCTGTA;
Reverse primer: 5'-GACGACAGCCATGCASCACCTGT.

Genomic DNAs were prepared from five different samples and amplified using BacReady™ Multiplex PCR System (with enzyme) following the kit instructions. The results were shown in Figure 2.

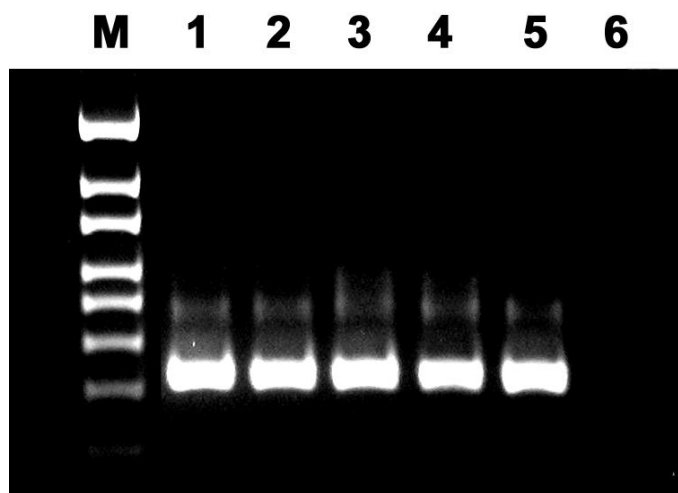


Figure 2. Bacteria genomic DNA was prepared and amplified using BacReady™ Multiplex PCR System (with enzyme). Five different samples were tested. Lane 1 and 2 were yogurt samples; Lane 3, 4 and 5 were bacteria samples picked from slants, they were *Bacillus globigii*, *Bacillus ubtillus* and *Micrococcus leteus*, respectively. Lane 6 is a negative control. PCR products are 355 bp.

Figure 2 shows that all the five DNA samples prepared using the kit produced the expected PCR products. There is little difference between the PCR amplification from 5 samples. This demonstrates the high quality and reproducibility of BacReady™ Multiplex PCR System.

VIII. TROUBLESHOOTING

The table below is guideline for troubleshooting.

Problem	Probable Cause	Solution
No PCR DNA	PCR may be inhibited by components in the samples.	Dilute the DNA 10 fold with BR-A buffer.
	One or more PCR components may be missing.	Always run a positive control side by side with PCR using genomic DNA prepared using the kit.
	PCR conditions are not optimized. The annealing temperature may be too high; More cycles may be needed; The denaturation time may be too short; The extension time may be too short.	Optimize the PCR conditions by decreasing annealing temperature in 2-4°C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments, or increasing the extension time in 1 minute increments. It is recommended to change one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step. Betaine, DMSO and formamide can help amplification of high GC-rich template.
	Genomic DNA is lost especially when a single cell is used.	Do not use pipette tips to mix. Tap the centrifuge tubes gently to mix.
Non-specific DNA products	The primers may not be designed optimally.	Primers may form dimers, or prime at non-specific target sequences. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4°C increments, or decreasing the number of cycles.
High background (with your own Taq polymerase)	Too much Taq DNA polymerase may be used.	Optimize the PCR conditions by decreasing the amount of Taq DNA polymerase in 0.2 unit increments.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

IX. ORDER INFORMATION

BacReady™ Multiplex PCR System without enzyme: Cat. No. L00202
BacReady™ Multiplex PCR System with enzyme: Cat. No. L00203

For Research Use Only.

- The PCR process is covered by U.S. Patent numbers 4683195 and 4683202 issued to Cetus and owned by Hoffman-La Roche Inc. Genscript does not encourage or support the unauthorized use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.
- Patent pending.

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