

BloodReady™ Multiplex PCR System

Technical Manual No. 0174

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	4
VIII	Troubleshooting	6
IX	Order Information	7

I. DESCRIPTION

BloodReady™ Multiplex PCR System is a powerful reagent kit for both rapid genomic DNA preparation and multiplex PCR amplification. Genomic DNA is directly released from blood cells in a single step by adding a proprietary reagent directly to blood samples without DNA purification. The genomic 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).

BloodReady™ Multiplex PCR System with enzyme (GenScript, L00197) contains BR-A buffer and PCR premix. The BR-A buffer is used to lyse cells and to release genomic DNA. PCR premix contains PCR buffer (BR-B buffer), dNTP, Mg²⁺ and “HotStart” Script™ DNA polymerase for PCR amplification.

L00197 Components	100 Preps
BR-A buffer	5.00 ml
2X PCR premix	1.00 ml
PCR-grade water	1.00 ml

BloodReady™ PCR System without enzyme (GenScript, L00196) 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. 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.

L00196 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 the genomic DNA extraction from blood.

And for application such as:

- SNP genotyping and mutation detection
- Target detection in transgenic mice
- DNA sequencing and cloning
- Quantitative PCR

III. KEY FEATURES

- ◆ Easy to perform: Very simple and rapid procedure to extract genomic DNA in a single step
- ◆ High specificity: Highly specific amplification of genomic 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: Genomic DNA from a single blood cell has been successfully used in multiplex PCR amplification of more than 1000 amplicons and subsequent DNA genotyping assays.

IV. SHIPPING AND STORAGE

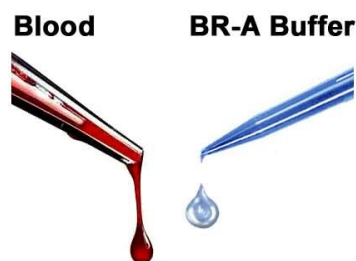
This kit is shipped on blue ice. Store the kit at -20°C after receiving.

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 blood sample and mix well.
2. Set-up and perform PCR reaction in a PCR cycler.

VI. DETAILED EXPERIMENTAL PROCEDURES

Overview of Kit Procedure



Genomic DNA



PCR reaction

A. Genomic DNA preparation

Thaw BR-A buffer at room temperature and vortex the solution. Add 20 μ l of BR-A buffer to 1.0 μ l blood sample (BR-A buffer should be at least 20 volumes of blood sample) and mix well.

Please note: (1) The genomic DNA solution is red from blood cells but does not affect PCR.
 (2) Genomic DNA is fragile and high molecular weight DNA is sheared easily by mechanical forces. Do not vortex solutions containing genomic DNA.

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 DNA prepared as described above. PCR reactions can be set up at room temperature since “HotStart” Script™ DNA polymerase is used.

Set up 20 μ l 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. Please only use 1 μ l of genomic DNA for 20 μ l of PCR reaction.

Reagent	Volume	Final Concentration
water, PCR grade	7 μ l	
4 μ M forward primer	1 μ l	200 nM
4 μ M reverse primer	1 μ l	200 nM
genomic DNA	1 μ l	
PCR premix	10 μ l	
total	20 μ l	

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

1. 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
 40 PCR cycles: Denaturation: 94°C for 40 sec
 Annealing: 55 - 60°C for 1 min
 Extension: 72°C for 30 sec to 2 min (\approx 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.
40 PCR cycles: 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. Blood cell genomic DNA preparation and PCR amplification.

Genomic DNAs were prepared from nine different blood samples and amplified using BloodReady™ Multiplex PCR System (with enzyme) following the kit instructions. The results were shown in Figure 1.

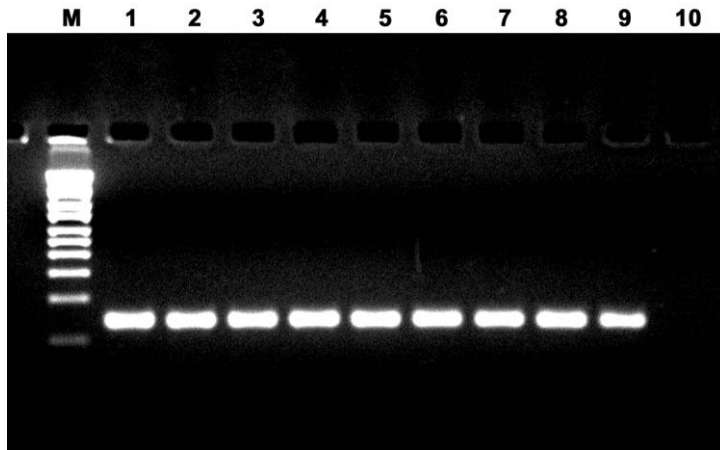


Figure 1. PCR analysis of genomic DNA prepared from blood samples. Genomic DNAs were prepared from 9 different blood samples and amplified using BloodReady™ Multiplex PCR System following the kit instructions. M is 100 bp DNA marker lane. Lane 1 to 9 are 9 blood samples. Last lane is a negative control. PCR products are 142 bp.

The sequences of the two primers used in the experiments are:

Forward primer: 5'-TCCAGCTGTGCAGTTCTCCAAAACA-3'

Reverse primer: 5'- ATTCCAGAGGGGTGACTACACATT-3'

Figure 1 shows that the target PCR product of 142 bp fragment is seen from all 9 genomic DNA samples. There is little difference between genomic DNA samples extracted from different blood samples. This demonstrates the high quality and reproducibility of BloodReady™ Multiplex PCR System.

B. Multiplex PCR amplification.

Human genomic DNAs prepared using Blood Ready™ Multiplex PCR System (with enzyme) were also amplified in a multiplex PCR following the kit instructions. 12 pairs of primers were designed to amplify 12 different human genes with the sequences shown below:

No.	DNA size (bp)	Forward Primer sequence	Reverse Primer sequence
1	142	ATTGTAGGGAAATGTCTGTCTGAT	ACACCAATCTCTACATCATAAGGAG
2	133	AGTGATCATGCTGTTTTCTC	GATTTTTATCCTGTTTGTGCC
3	126	TCAAATAATTGTTCCAAAGTAGCA	AAAAATGACCTTTGCAAGTACATT
4	119	TGATTATTGGGAAAAGATCTGAGAC	ACAAACCCACTTTTCATCACA
5	112	AAGCATACTGTGAGAGTGCACA	AGGCCAATGGGTAATGGTAAATCCC
6	105	CACCTCTGACTTCTCAGGTGT	GCCTCTAACATTCTGTTTAGGAGA
7	100	GTAAAGAATTCAATGAGTATGCCA	CTTGTTTGCAGGGTGATGCCATTT
8	96	TGTCCCTCTGAATAATTGTAGAA	ATGTCTGAGTTAAATACCACACAG
9	90	TAAGACAGTTTTCTTGGAGAGTAAACATTG	TTTTTCAAAGTCTTCAGATATGGT
10	85	CTCCAACACACAGAACAGGAGGGAGGAAT	TAATGGAAGGAGTAGCCCAACT11
11	80	TCATATTAAGCAACTAATATTTGTGCCATC	CATCTGGTGCCCATGTGTGTC
12	76	TCCCGTCACCTGAAACTGCTGTCACC	GCATATTTGGTGGAAAAGTCTACAG

The results were shown in Figure 2.

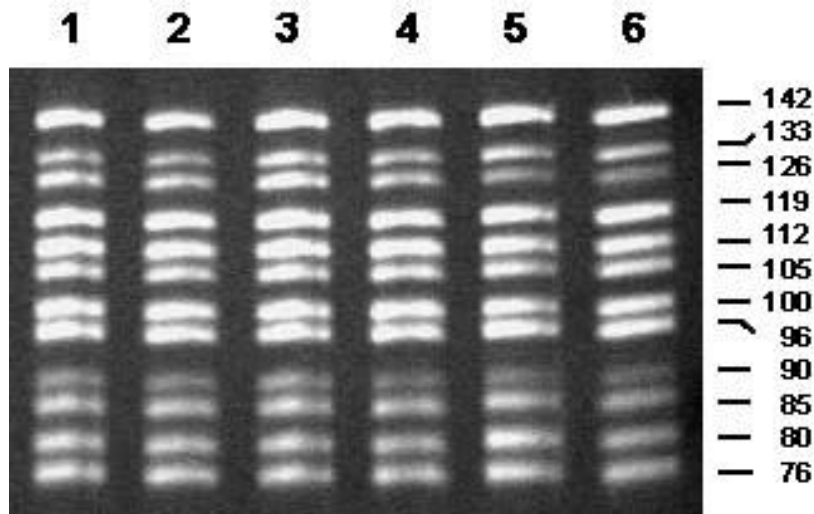


Figure 2. Human genomic DNA was prepared and amplified using BloodReady™ Multiplex PCR System (with enzyme). PCR DNA sizes are shown on the right.

Lane 1 and 2 using 100 blood cells.
Lane 3 and 4 using 25 blood cells.
Lane 5 and 6 using 5 blood cells.

Figure 2 shows that all the 12 target PCR products of different sizes are amplified in a multiplex PCR from as few as 5 blood cells. There is little difference between multiplex PCR using 100 blood cells from that using 5 blood cells. Again, this demonstrates the high quality and reproducibility of BloodReady™ 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 blood.	Dilute the genomic DNA 10 fold with PCR-grade water.
	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 0.5 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

BloodReady™ Multiplex PCR System without enzyme: Cat. No. L00196
 BloodReady™ Multiplex PCR System with enzyme: Cat. No. L00197

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.

GenScript USA Inc.
 860 Centennial Ave., Piscataway, NJ 08854
 Tel: 732-885-9188, 732-885-9688
 Fax: 732-210-0262, 732-885-5878
 Email: info@genscript.com
 Web: <http://www.genscript.com>