Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

2× GC-Rich PCR Mix instructions

Composition

Cat. No.	7006100	7006500
2×GC-Rich PCR Mix	1 ml	1 ml×5
GC-Rich Buffer	0.5 ml	0.5 ml×5
ddH_2O	1 ml	1 ml×5
Instructions	1	1

Storage

The storage period is more than 2 years at -20°C, and the validity period is 6 months when stored at 2~8°C. Repeated freeze-thaw 16 times will not affect the use effect.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

2×GC-Rich PCR Mix is an optimized two-fold concentration PCR master mix. It is suitable for PCR amplification with high fidelity requirements, long amplification fragments and high GC content. The specially formulated GC-Rich Buffer amplifies DNA fragments up to 81% GC and up to 10 kb in length. The product is easy to use, only need to take 2×GC-Rich PCR Mix 0.5 times of the volume of the PCR system and appropriate GC-Rich Buffer, add primers and template, and make up the volume with ddH_2O.

2× GC-Rich PCR Mix contains Taq enzyme and a certain proportion of Pfu DNA Polymerase, and most of the amplified target products have an A base attached to the 3′ end, which can be directly cloned in T-Vector. The product contains two types of indicator dyes for electrophoresis, red and yellow. They do not inhibit PCR or affect EB color development, and their relative electrophoresis migration distances are shown in the following table.

Gel Concentration	Red Dye	Yellow Dye
0.8%	2000 bp	~80 bp
1.0%	1500 bp	~40 bp
1.5%	1000 bp	~20 bp
2.0%	500 bp	<10 bp
2.5%	350 bp	<10 bp
3.0%	200 bp	<10 bp

Components of the PCR System

1. Purity of template DNA: Many residual nucleic acid extraction reagents can affect the PCR reaction, including proteases, protein denaturants (e.g., SDS, guanidine salts), high concentrations of salts (KAc, NaAc, sodium caprylate, etc.), and high concentrations of EDTA. Do not use more than 1/10 of the PCR system for less pure templates (e.g., 5 µl of the sample for 50 µl PCR system). If the template DNA purity is too poor, the Simgen DNA Purification Kit (Cat. No.2101050) is recommended to purify and concentrate the template DNA, and the amount of template used after purification can be as much as 1/2 of the volume of the PCR system.
2. Template DNA dosage: A very small amount of DNA can also be used as a PCR template, but to ensure the stability of the reaction, it is recommended to use more than 10^4 copies of the target sequence as a template for the 50 µl system. Recommended dosage of template DNA:

   Human Genomic DNA: 0.05 µg~0.5 µg/50 µl PCR system
   E. coli genomic DNA: 10 ng~100 ng/50 µl PCR system
   λ DNA: 0.5 ng~5 ng/50 µl PCR system
   Plasmid DNA: 0.1 ng ~ 10 ng/50 µl PCR system

   If the amplification product is to be used as a template for reamplification, the amplification product should be diluted at least 1,000 to 10,000-fold before using it as a template, otherwise smeared bands or non-specific bands may occur.
3. Primer concentration: Typically, each primer is prepared at a concentration of 10 µM (50×) and a working concentration of 0.2 µM. Too much primer may result in non-specific amplification, and too little primer may reduce amplification efficiency.

PCR parameter settings

1. Initial denaturation: The general initial denaturation is 94°C, 1~5 min. Denaturation temperatures that are too high or too long can result in loss of Taq enzyme activity.
2. Annealing: Annealing temperature is key for PCR, as too high a temperature may reduce yield, and too low a temperature may result in primer-dimers or non-specific amplification. For the first attempt at PCR amplification, it is recommended to try a lower than 5 °C Tm (if the two primer Tm are different, refer to the lower Tm) as the annealing temperature. Generally, primer synthesis companies will provide the Tm of the synthesized primers, and the primer Tm can also be estimated according to this formula: Tm = 2°C×(A+T) + 4°C× (G+C). The optimal annealing temperature needs to be determined by gradient PCR.
3. Extension: The extension temperature is typically 72°C, and the length of extension time depends on the length of the DNA fragment of interest, and the desired extension time is calculated at 1 kb/min, which may result in a non-specific increase. After the end of the cycle, continue to extend for 5~10 min to obtain the complete double-stranded product.
4. Number of cycles: Generally, 25~35 cycles are used, and the number of cycles can be increased appropriately with a low copy template. Excessive number of cycles may increase non-specific amplification and decrease specific products.
5. Usage of GC-Rich Buffer: When the ratio of 2× GC-Rich PCR Mix to GC-Rich Buffer is 2:1, DNA fragments with 81% GC content can be amplified, but the amplification yield is not the highest. Users can reduce the amount of GC-Rich Buffer according to the GC content of the amplified fragment to obtain a higher amount of amplification product.

Protocol

1. Thaw 2×GC-Rich PCR Mix, GC-Rich Buffer, ddH_2O, template DNA, and primers at room temperature and place on ice.
2. After thawing, the components were inverted up and down to mix evenly, and the PCR system was prepared according to the following compositions:

2×GC-Rich PCR Mix	20 µl
GC-Rich Buffer	10 µl
Primer 1（10 µM）	1 µl
Primer 2（10 µM）	1 µl
template	n µl
ddH_2O	(8-n) µl
Total	40 µl

* The above example is the maximum usage of GC-Rich Buffer, and users can reduce the usage of GC-Rich Buffer according to the actual situation.
* The above example is for the addition of components for a 40 µl reaction, if additional volumes of the reaction are required, please increase or decrease the components proportionally.

3. Flick the PCR reaction tube to mix well, and spin down the solution to the bottom of the tube.
4. Example of PCR reaction cycle setup

Step	Temperature(℃)	Time	Number Of Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	30
Annealing	※55°C	30 sec	
Extension	§ 72°C	1 min	
Final Extension	72°C	5 min	1

※The actual optimal annealing temperature shall prevail.
§ Calculated at 1 kb/min.

5. Results: 5-10 µl of the amplified product was directly detected by agarose electrophoresis.
Relationship between agarose gel concentration and optimal resolution range for linear DNA:

Agarose	Optimal linear DNA resolution range
0.5%	1,000~30,000
0.7%	800~12,000
1.0%	500~10,000
1.2%	400~7,000
1.5%	200~3,000
2.0%	50~2,000