2 × *Taq* Green Master Mix

合生基因 SyngenTech

Size:

Cat.#	Size
BG10109S	40 R(50 μL vol)
BG10109M	200 R(50 μL vol)
BG10109L	1000 R(50 μL vol)

Source: *E.coli* strain with a cloned pol gene from Thermus aquaticus YT1.

Storage temperature: -20℃

Exp.: 12 months(-15℃～-25℃)

Applications:

1. Routine PCR amplification of DNA fragments up to 5 kb.
2. PCR product for TA cloning.
3. Colony PCR.

website: syngen.tech

Description:

2 × *Taq* Green Master Mix contains *Taq* DNA Polymerase, dNTPs, other buffer components and stabilizers. *Taq* DNA Polymerase is a highly thermostable DNA polymerase, that possesses 5' → 3' polymerase activity, 5' → 3' exonuclease activity, but no detectable 3' → 5' exonuclease (proofreading) activity. *Taq* DNA Polymerase is extremely sensitive and able to amplify trace amounts of DNA in the sample reaction.

2 × *Taq* Green Master Mix is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis and ethidium bromide staining. It is not recommended when doing any downstream applications in absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications.

Reagent supplied:

Cat.#	Size	Quantity	Nuclease-Free Water
BG10109S	40 R(50 μL vol)	1 mL	1 mL
BG10109M	200 R(50 μL vol)	5 × 1 mL	5 × 1 mL
BG10109L	1000 R(50 μL vol)	25 × 1 mL	25 x1 mL

Protocol:

To prepare several parallel reactions with minimized pipetting errors, prepare a PCR master mix by mixing Nuclease-Free water, primers and 2 × *Taq* Master Mix. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following component for each 25 μL or 50 μL reaction:

Component	25 μL reaction	50 μL reaction	Final concentration
10 μM forward primer	0.5 μL	1 μL	200 nM
10 μM reverse primer	0.5 μL	1 μL	200 nM
Template DNA	variable	variable	0.1-100 ng
2 × *Taq* Green Master Mix	12.5 μL	25 μL	1 ×
Nuclease-Free water	to 25 μL	to 50 μL	–

3. Gently vortex the samples and spin down.
4. Perform PCR using recommended thermal cycling conditions:

Step	Temperature	Time	Number of cycles
Initial Denaturation	94°C	2-5 min	1
Denaturation	94°C	15-30 s	25-40
Annealing	Tm-5°C	10-30 s	
Extension	68°C	10-60 s/kb	
Final Extension	68°C	5-15 min	1

General guidelines:

1. Template:

High quality, purified DNA templates greatly enhances the success of PCR reaction. Recommended amounts of DNA template for a 50 μL reaction are as follows:

DNA	Amount
Genomic DNA	1 ng-1 μg
Plasmid or Viral DNA	1 pg-1 μg

2. Primers:

Oligonucleotide primers are generally 20-40 nucleotides in length and ideally have a GC content of 40-60%. Software such as Oligo 7 can be used to design or analyze primers. The final concentration of each primer in a PCR reaction may be 0.05-1 μM, typically 0.1-0.5 μM.

3. Denaturation:

An initial denaturation of 2 min at 94°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences or crude samples, a longer denaturation of 2-8 min at 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 2-5 min denaturation at 94°C is recommended.

4. Annealing:

The annealing step is typically 15-60 s. Annealing temperature is based on the Tm of the primer pair and is typically 45-68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting at 5°C below the calculated Tm.

5. Extension:

The recommended extension temperature is 68°C. Extension time are generally 20-30 s/kb for complex, genomic samples, but can be reduced to 10 s/kb for simple templates (plasmid, virus). To determine specific extension time, we recommend 1 min/kb and reduce the extension time until 10 s/kb. 2 min/kb is recommended for crude samples. A final extension of 5 min at 68°C is recommended.

6. Cycle number:

Generally, 25-35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number templates.

7. PCR product:

The PCR products generated by *Taq* DNA Polymerase contain dA overhangs at the 3' end, therefore can be ligated to dT/dU-overhang vectors.

References:

1. Chien A, Edgar D B, Trela J M. Deoxyribonucleic-acid polymerase from extreme thermophile Thermus-aquaticus[J]. *Journal of Bacteriology*, 1976, 127.
2. Kaledin A S, Sliusarenko A G, Gorodetski S I. Isolation and properties of DNA polymerase from extreme thermophylic bacteria Thermus aquaticus YT1-[J]. *Biokhimiia*, 1980, 45(4): 644-651.

2× *Taq* Green Master Mix 使用说明书

【产品规格】

货号	规格
BG10109S	40 R(50 μL vol)
BG10109M	200 R(50 μL vol)
BG10109L	1000 R(50 μL vol)

【产品来源】 重组表达栖热水生菌 DNA 聚合酶基因的大肠杆菌

【储存条件】 -20 ℃

【保质期】 12 个月（-15℃～-25℃）

【应用范围】

1. 常规 PCR 扩增 5 kb 以内的 DNA 片段
2. 生成用于 TA 克隆的 PCR 产物
3. 菌落 PCR

【产品说明】

2 × *Taq* Green Master Mix 是一种加入了 *Taq* DNA 聚合酶，dNTPs，其它一些缓冲液组分和 PCR 稳定剂的预混液。合生基因的 *Taq* DNA 聚合酶对微量的 DNA 样品具有很好的扩增能力。*Taq* DNA 聚合酶具有热稳定性，5' → 3' 端 DNA 聚合酶活性和 5' → 3' 端核酸外切酶活性，无 3' → 5' 端核酸外切酶活性。2 × *Taq* Green Master Mix 推荐在 PCR 产物用于 EB 染色的凝胶电泳实验中使用。但如果下游实验涉及到吸光度和荧光检测，反应体系中的黄色和蓝色染料将会影响这些实验。

【辅酶提供试剂】

货号	规格	数量	无核酸酶水
BG10109S	40 R(50 μL vol)	1 mL	1 mL
BG10109M	200 R(50 μL vol)	5 × 1 mL	5 × 1 mL
BG10109L	1000 R(50 μL vol)	25 x1 mL	25 x1 mL

【操作指南】

如果要同时进行几个平行反应，混合无核酸酶水，反应缓冲液，引物和 2 × *Taq* Green Master Mix 制备 PCR 混合液，为减少加样误差的影响，制备的反应混合液要在需要的反应数量上添加上一个额外反应，分装母液到单个的 PCR 管中，然后加入模板 DNA。

1. 温和震荡后，快速离心瞬态回的所有溶液。
2. 加入 25 μL 或者 50 μL 的组份（见下表）到冰上的薄壁 PCR 管中。

成分	25 μL 反应	50 μL 反应	终浓度
10 μM 正向引物	0.5 μL	1 μL	200 nM
10 μM 反向引物	0.5 μL	1 μL	200 nM
模板 DNA	可变量	可变量	0.1-100 ng
2 × *Taq* Green Master Mix	12.5 μL	25 μL	1 ×
无核酸酶水	至 25 μL	至 50 μL	–

3. 温和震荡并短暂离心。
4. 使用推荐的热循环条件进行 PCR：

步骤	温度	时间	循环数
预变性	94℃	2-5 min	1
变性	94℃	15-30 s	25-40
退火	Tm-5℃	10-30 s	
延伸	68℃	10-60 s/kb	
最后延伸	68℃	5-15 min	1

【使用说明】

1. 模板：高质量、纯化的 DNA 模板会大幅提升 PCR 反应的成功率。对于一个 50 μL 反应体系，推荐量的 DNA 模板如下：

DNA	用量
基因组	1 ng-1 μg
质粒或病毒	1 pg-1 ng

2. 引物：寡核苷酸引物一般长度在 20-40 nt，GC 含量为 40-60%。如 Oligo 7 软件可被用来设计或分析引物。在 PCR 反应中每个引物的终浓度范围是 0.05-1 μM，通常为 0.1-0.5 μM。

3. 变性：94℃ 2 min 预变性即可满足于大部分纯化的 DNA 模板的扩增。对于高 GC 含量序列或者粗样品，推荐使用 2-8 min 的变性时间来完全变性模板。对于菌落 PCR，推荐使用 2-5 min 的预变性时间。

4. 退火：退火时间通常为 15-60 s，退火温度基于引物的 Tm 值，一般在 45℃到 68℃之间。退火温度可以通过低于 Tm-5℃的温度梯度 PCR 进行优化。

5. 延伸：推荐的延伸温度为 68℃。复杂或基因组样品的延伸时间一般为 20-30 s/kb。简单模板（质粒，病毒）可以为 10 s/kb。为确定具体的延伸时间，我们推荐从 1 min/kb 开始降低延伸时间直到 10 s/kb。粗样品推荐使用 2 min/kb。68℃最终延伸时间推荐使用 5 min。

6. 循环数：一般来说，25 到 35 个循环即可以产生足够的 PCR 产物。最高到 45 个循环可用于检测低拷贝模板。

7. PCR 产物：使用 *Taq* DNA 聚合酶产生的 PCR 产物在 3' 端会含有 dA 突出，因此，PCR 产物可以连接到 dT/dU 粘性末端载体上。

【参考文献】

1. Chien A, Edgar D B, Trela J M. Deoxyribonucleic-acid polymerase from extreme thermophile Thermus-aquaticus[J]. *Journal of Bacteriology*, 1976, 127.
2. Kaledin A S, Sliusarenko A G, Gorodetski S I. Isolation and properties of DNA polymerase from extreme thermophylic bacteria Thermus aquaticus YT1-[J]. *Biokhimiia*, 1980, 45(4): 644-651.