SunLong Biotech Co.,LTD
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2×Taq plus PCR Master Mix

****Product Composition****

Cat. No.	SLPCR243	
2×Taq plus PCR Master Mix	1 ml	1 ml×5
ddH2O	1 ml	1 ml×5
Manual	1	1

****Storage and Shelf Life****

- Store at -20° C for a shelf life of over two years; store at 2~8° C for a shelf life of 6 months. Repeated freeze-thaw cycles (up to 16 times) do not affect performance.

****Product Description****

2×Taq plus PCR Master Mix is an optimized, double-concentration PCR premix. It is suitable for PCR requiring high fidelity and long amplification fragments, capable of amplifying fragments up to 10 kb from complex genomic DNA. PCR enhancers and protein stabilizers synergistically improve PCR efficiency and sensitivity, making it ideal for low-copy template amplification. The product is easy to use: simply take 0.5 times the PCR reaction volume of 2×Taq plus PCR Master Mix, add primers and template, and adjust the volume with ddH_2O.

2 × Taq plus PCR Master Mix contains Taq enzyme and a certain proportion of pfu DNA Polymerase. Most amplified products have an additional A base at the 3' end, allowing direct cloning into T-Vectors. The product contains both red and yellow electrophoresis indicator dyes, which do not inhibit PCR or affect EB staining. The relative migration distances of the dyes are shown in Table 1:

****Table 1: Agarose Gel Concentration and Dye Migration Distance****

Gel Concentration	Red Dye Migration	Yellow Dye Migration
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

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****PCR Reaction Components****

1. **Template DNA Purity**: Many residual nucleic acid extraction reagents can affect PCR, including proteases, protein denaturants (e.g., SDS, guanidine salts), high concentrations of salts (e.g., KAc, NaAc, sodium octanoate), and high concentrations of EDTA. For low-purity templates (e.g., templates obtained by boiling), do not exceed 1/10 of the PCR reaction volume (e.g., for a 50 µl reaction, do not add more than 5 µl of template). If the template DNA purity is poor, use the PCR Clean-Up Kit to purify and concentrate the template DNA. After purification, the template can be used up to 1/2 of the PCR reaction volume.

2. ****Template DNA Amount**:** Even trace amounts of DNA can be used as a PCR template, but for reaction stability, it is recommended to use at least 10^4 copies of the target sequence in a 50 µl reaction. Recommended template DNA amounts:

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

If using PCR products as templates for re-amplification, dilute the products at least 1,000 to 10,000 times before use to avoid smeared or non-specific bands.

3. ****Primer Concentration**:** Typically, primers are prepared at a concentration of 10 µM (50×), with a working concentration of 0.2 µM. Excessive primers may cause non-specific amplification, while insufficient primers may reduce amplification efficiency.

****PCR Parameters****

1. ****Initial Denaturation**:** Typically 94° C for 1~5 minutes. Excessive temperature or time may reduce Taq enzyme activity.
2. ****Annealing**:** Annealing temperature is critical for PCR. Too high a temperature may reduce yield, while too low a temperature may cause primer dimers or non-specific amplification. For initial PCR attempts, try an annealing temperature 5° C below the Tm (if the two primers have different Tms, use the lower Tm). Primer synthesis companies usually provide the Tm, or it can be estimated using the formula: Tm = 2° C × (A+T) + 4° C × (G+C). The optimal annealing temperature should be determined by gradient PCR.
3. ****Extension**:** The extension temperature is typically 72° C, and the extension time depends on the length of the target DNA fragment, calculated at 500 bp per minute. Excessive extension time may increase non-specific products. After the final cycle, extend for an additional 5~10 minutes to ensure complete double-stranded products.
4. ****Cycle Number**:** Typically 25~35 cycles. For low-copy templates, the number of cycles can be increased. Excessive cycles may increase non-specific amplification and reduce specific products.

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Procedure

1. Thaw 2×Taq plus PCR Master Mix, ddH_2O, template DNA, and primers at room temperature, then place on ice.
2. Mix the thawed components by inverting, and prepare the PCR reaction as follows:

Component	Volume
2×Taq plus PCR Master Mix	25 µl
Primer 1 （10 µM）	1 µl
Primer 2 （10 µM）	1 µl
Template	n µl
ddH_2O	(23-n) µl
Total	50 µl

Note: The above example is for a 50 µl reaction. Adjust the volumes proportionally for other reaction sizes.

3. Gently flick the PCR tube to mix, then centrifuge briefly to settle the solution.
4. Example PCR cycling conditions:

94℃ 3 min

94℃ 30 sec
※55℃ 30 sec
§ 72℃ 1 min
} 30 Cycles

72℃ 5 min

※Use the actual optimal annealing temperature.
§ Calculate based on 500 bp per minute.

5. **Result Detection**: Take 5~10 µl of the PCR product for agarose gel electrophoresis.

Agarose Concentration	Optimal Linear DNA Resolution Range
0.5%	1,000~30,000 bp
0.7%	800~12,000 bp
1.0%	500~10,000 bp
1.2%	400~7,000 bp
1.5%	200~3,000 bp
2.0%	50~2,000 bp