Rapid DNA Extraction and Amplification Kit

Project number: D669986

Storage conditions: -20° C.

Products

individual parts making up a compound	50T
Buffer SA	15ml
2×PCR MasterMix	1ml
Proteinase K	12.5mg
Proteinase K Storage Buffer	1.25mL

Products

This kit adopts a unique buffer system containing all the reagents for rapid preparation of genomic DNA and PCR amplification, and is suitable for one-step extraction of genomic DNA from various plant and animal tissues and bacteria and for PCR amplification. The whole extraction process does not require liquid nitrogen grinding, organic solvent extraction, anhydrous ethanol precipitation, and the quality of extracted DNA is stable. The 2×PCR MasterMix provided in this kit is a highly compatible PCR reagent that can amplify DNA samples efficiently and specifically, which includes DNA polymerase, dNTPs, MgCl2, reaction buffer, PCR reaction enhancer and so on. It is characterized by fast and easy, high sensitivity, high specificity, good stability, etc. It is especially suitable for high throughput screening.

Pre-experiment Preparation and Important Notes

1. Add the specified amount of Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity.
2. Repeated freezing and thawing of the samples should be avoided, as this will result in smaller DNA fragments and a decrease in the amount of extracted DNA.
3. Before use, please check Buffer SA for crystallization or precipitation. If crystallization or precipitation occurs, please re-dissolve Buffer SA in a 56℃ water bath.
4. The PCR MasterMix provided with this product is 2×, when using it, you need to add template and primer, and add RNase-Free Water to make up the volume, so that its concentration is 1× to carry out the reaction.

procedure

1. Fetch:

Plant material: take about 10 mg of sample in a centrifuge tube (provided);

Animal material: take about 10 mg of sample in a centrifuge tube (provided);

Bacteria: Take 200-800 μL of bacteria in good growth condition in a centrifuge tube (self-provided) and collect the bacteria.

2. Add 200 μL of Buffer SA and vortex to mix.

Note: In the case of plant leaves and animal tissues, they should be ground with a pestle and mortar as much as possible: in the case of plant seeds, they should be crushed and finely ground beforehand; bacterial and 1-3 mm rat-tail samples can be directly vortex lysed.

3. Add 10μL of Proteinase K, mix well, incubate at 56℃ for 10 minutes, and treat at 95℃ for 5 minutes.

Note: 1) In the case of animal tissue samples, the incubation time at 56° C may be extended to 30 minutes as appropriate; if there is any incompletely digested tissue, it should be removed as thoroughly as possible after centrifugation in the next step.

2) Be careful not to exceed 5 minutes when treating at 95° C.

4. 13,000 rpm (~17,900 x g), centrifugation for 5 minutes.

5. Transfer the supernatant to a new centrifuge tube (self-prepared) and use it directly for PCR amplification, or store the solution at 4℃ or -20℃.

6. PCR amplification:

1) PCR reaction system:

The following examples are conventional PCR reaction systems and reaction conditions, which should be improved and optimized according to the template, primer structure and target fragment size in actual operation.

reagents	20 μL system	final concentration
2×PCR MasterMix	10 μL	1×
Forward Primer, 10 μM	1 μL	0.4 μM
Reverse Primer, 10 μM	1 μL	0.4 μM
Template DNA	1-2 μL	
RNase-free Water	up to 20 μL	

Note: Please use the final concentration of 0.2-0.6μM as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the concentration of primer can be increased; if a non-specific reaction occurs, the concentration of primer can be decreased, thus optimizing the reaction system.

2) PCR reaction conditions:

move	temp	timing
premutability	94° C	2min
denaturation	94° C	30s
annealing (metallurgy)	55-65° C	30s 30-40 cycles
reach	72° C	60s
ultimate extension	72° C	5min

Note: 1) In general, the annealing temperature is 5℃ lower than the melting temperature of the amplification primer Tm, and the annealing time is generally 30-60 seconds. When the desired amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; when a non-specific reaction occurs, the annealing temperature should be raised, thus optimizing the reaction conditions.
(2) The extension time is set according to the size of the fragment to be amplified, and the amplification efficiency of Taq DNA Polymerase included in this product is 1kb/30s .
3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, the amplification is insufficient; if the number of cycles is high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.
(3) Result detection: 5 μL of reaction product was taken at the end of the reaction and directly detected by agarose gel electrophoresis.