Content

Plant DNA Extraction Kit

Composition

Plant DNA Extraction Kit Cat. No.	5 Preps 3201005	50 Preps 3201050	250 Preps 3201250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Buffer PL	3 ml	30 ml	150 ml
Buffer K	2 ml	20 ml	100 ml
Buffer WA (concentrate)	1.9 ml	12 ml	60 ml
Buffer WB (concentrate)	1.5 ml	10 ml	50 ml
Buffer TE	1.2 ml	12 ml	60 ml
Instructions	1	1	1

Storage

1. All the reagents and components can be stored for up to two years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2-8℃.
2. The product stored at 2~8℃ should be restored to room temperature before use.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, liquid nitrogen and mortar may be required.
2. 1.5 ml centrifuge tubes, pipettes, and tips.
3. Protective equipment such as latex gloves, disposable masks, and paper towels.
4. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Vortexer, water bath or dry thermostat.
6. Buffer TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, Simgen Cat. No. 9006500) may be required.
7. RNase A (Simgen Cat. No. 8001001) may be required.
8. Proteinase K (Simgen Cat. No. 8000224) may be required.
9. Grinding Rods (Simgen Cat. No. D-050) may be required.
10. Lysozyme (Simgen Cat. No. 8009500) may be required.

Quality Assurance

Hangzhou Simgen Biotechnology Co., Ltd. guarantees that the products provided are qualified products that have passed the quality inspection. If user finds that the product cannot meet the experimental requirements, please stop using the product immediately and contact Simgen technical support for help. E-mail: technical@simgen.cn, Tel: 400-0099-857.

According to our past experience, the difference in samples used by users is the most important reason for not meeting the requirements, if you need to isolate and purify DNA from some rare samples, please be sure to communicate with our technical support before proceeding with the experiment.

Precautions

Buffer PL, Buffer K and Buffer WA contain irritating compounds, please wear latex gloves and protective glasses when handling to avoid contamination of skin, eyes, and inhalation of the mouth and nose. If it gets contaminated with skin or eyes, rinse immediately with plenty of water or saline, and seek medical help if necessary.

Introduction

This product is suitable for extracting DNA from plants, fungi, stool, bacteria, arthropods, etc., and can also be used to extract DNA from Blood and animal tissues. The specially designed buffer solution and Spin Column efficiently adsorb only large fragments of genomic DNA, so RNA can be removed without additional RNase digestion in most cases. The operation steps are very simple and fast, only need to lyse sample and then precipitate the insoluble matter of the sample. The supernatant containing DNA will add to the Spin Column for DNA adsorption, and then wash the DNA with Buffer WA and Buffer WB. The high-purity genomic DNA elute with Buffer TE is suitable for various molecular biology experiments.

Analysis And Description of The Operation Steps

1. The starting sample dosage and the reference yield of DNA

Samples	Amount	DNA Yield (μg)
Plant tissues, filamentous fungi, mushrooms	50~150 mg fresh tissue or 10~30 mg dried tissue	3~25
Senescent leaves, seeds, plant tuber (with a lot of starch).	100~200 mg	1~15
Yeast	5~10 ml yeast culture	1~5
Stool	30~50 mg	5~15
Arthropod	50~100 mg	1~10
Skeleton	50~100 mg	5~50
Animal tissues	50~100 mg	10~30
Bacteria	3~5 ml bacteria culture	10~30
Mammalian anticoagulant Blood, saliva, bone marrow	300 μl	3~25
Blood with nucleated erythrocytes such as birds, amphibians, fish, etc	5~10 μl	15~25
Cultured cells	$5\sim10\times10^6$ cells	5~25

2. Sample lysis

Samples with strong cell walls, such as plant tissue, fungi, or yeast, need to be grind into a homogenate with a mortar. If the fiber content of plant tissues is high, liquid nitrogen must also be added to grind to make the sample fully broken. The bacterial cell wall can be lysed with lysozyme. Samples with high protein content should also be supplemented with proteinase K digesting protein, the purpose of which is to promote the complete separation of DNA and protein in the sample.

3. Selective precipitation of proteins and cell debris

Buffer K will precipitate most of the proteins in the solution, and in order to maintain the solubilized state of the genomic DNA in the solution and prevent the protein precipitation from taking away the genomic DNA, this step requires vigorous shaking (vortex shaking for 30 sec). The precipitated protein is centrifuged at high speed and aggregated at the bottom of the 1.5 ml tube, where it is separated from the genomic DNA.

4. Column purification techniques

1) DNA binding

The DNA containing the supernatant obtained by centrifugation is poured into the Spin Column for a short centrifugation for a few secs, and the DNA is adsorbed to the silica membrane of the Spin Column, and PCR inhibitors or impurities that inhibit downstream molecular biological reactions are filtered out. The specially designed buffer system and preferably silica membrane only efficiently adsorb of genomic DNA, and RNA can be removed from most samples without additional RNase digestion, except for samples with very high RNA content (yeast, insects, etc.).

2) Washing

Buffer WA effectively washes away a small amount of protein that often remains on the Spin Column.

Buffer WB washes away any remaining Buffer WA on the membrane, ensuring that the pure DNA adsorbs on the Spin Column.

In the process of DNA binding and washing, only the solution needs to be filtered through the Spin Column, so there are no strict requirements for centrifugation speed or centrifugation time, and the "short run" mode in the centrifuge can be selected to save operation time.

3) Spin dries by centrifugation

Place the Spin Column back into the 2 ml Collection Tube and centrifuge at 14,000 rpm for 1 min (if the centrifuge does not reach 14,000 rpm, we recommend centrifugation at least 12,000-13,000 rpm for 2 min.) The role of:

A. Fully discard the Buffer WB by centrifugation.
B. Buffer WB filtrate can also be centrifuged if it accidentally contaminates the bottom of the Spin Column during discarding.

4) Elute DNA

A. We recommend using Buffer TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) provided in the kit to elute DNA for long-term stable storage. DNA can also be eluted with deionized water, but the pH of the deionized water should be greater than 7, otherwise the elution efficiency of the DNA will be affected.
B. Pre-warmed Buffer TE or deionized water can improve the elution efficiency of DNA.
C. Adding Buffer TE or deionized water to the Spin Column and incubate for longer periods of time (up to 3-5 min) can improve the elution efficiency of DNA.
D. After centrifugation and spin-drying, the Spin Column can be directly added to elution of DNA without opening the lid of the Spin Column to volatilize residual ethanol, and over-drying of the Spin Column will be detrimental to the elution of DNA.
E. The eluted genomic DNA fragments ranged from 200 bp to 50 kb, with the main DNA fragments concentrated around 30 kb.
F. For the sake of product safety, if the centrifuge does not have a leak-proof lid, we recommend changing the centrifugation conditions to 8000 rpm for 1 min while eluting DNA to avoid the 1.5 ml tube lid from falling off.

5. Efficiency and purity of DNA recovery

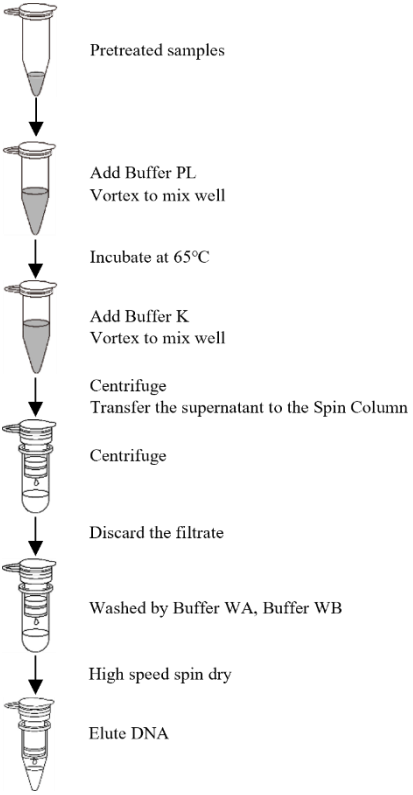
The DNA obtained from samples of the same quality varies greatly between different species of plants due to their large differences in water content. DNA should be extracted from the same tissue sample of the same plant. If the amount of DNA in the sample is low, consider increasing

the amount of sample used to increase the DNA yield, but it should be noted that the maximum amount of sample used does not exceed twice the recommended amount.
The silica gel membrane of the Spin Column equipped with this product has a large pore size, and the adsorption efficiency for RNA or small fragments of DNA is low, if the DNA in the sample is degraded (such as FFPE tissue, cadavers, etc.), this kit should not be selected.
The mass of DNA is typically estimated by measuring the absorbance value at 260 nm, which is converted to an optical density value of 1 OD equivalent to a concentration of 50 μg/μl double-stranded DNA. DNA purity is usually estimated using A260/A280, and the A260/A280 ratio for pure DNA should be between 1.7–1.9. The salt residue of DNA is usually estimated using A260/A230, and the A260/A230 ratio for pure DNA should be between 1.8–2.5.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 65℃ and incubate Buffer PL and Buffer TE to 65℃.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the bottle and tick the box on the label to mark "Ethanol Added".

Diagram of the operation flow



Protocol For Plant Tissues, Filamentous Fungi, And Mushrooms

1. **Weigh 300~500 mg (100~200 mg dry tissue) sample in a mortar, grind the sample to powder form with liquid nitrogen, and then weigh 50~100 mg (10~20 mg of dried tissue) of the tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen.**

* In order to grind the tissue into powder form, and to avoid the difficulty of weighing the grind tissue powder due to thawing, the sample can be ground by repeated grinding with liquid nitrogen multiple times. Plant tissues must be ground to powder form **(as fine as flour)** in order to adequately destroy the cell walls of plant cells (especially filamentous fungi), otherwise the recovery efficiency of the final DNA will be severely affected.
* For plant tissues with low fiber content, weigh 100 mg sample into a mortar, add 100 µl Buffer PL incubated to 65℃ and grind to a homogenate at room temperature, then add 400 µl Buffer PL incubated to 65℃ to grind several times, and transfer all the homogenate (including the resulting foam) to a clean 1.5 ml centrifuge tube and proceed directly to the 65℃ incubations in step 2 for 10 min.

2. **Add 500 µl Buffer PL incubated to 65℃, vortex for 30 sec to mix, and incubate at 65℃ for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**
3. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
4. **Transfer the supernatant from step 3 into a Spin Column (The Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.
* Pigments from some plants may remain on the filter of the Spin Column, and an additional ethanol wash step can be added (add 700 µl absolute ethanol and centrifuge at 12,000 rpm for 30 sec).

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified DNA may be mixed with ethanol.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65℃ to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

9. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**

Protocol For Seeds or Tubers

1. **Weigh 100~200 mg of sample into a mortar and add 100 µl Buffer TE (Simgen Cat. No. 9006500, not provided), grind to homogenization at room temperature.**

* If the sample is very dry, increase the Buffer TE appropriately.
* If the size of the seed or tuber is large, cut the part containing the germ or spores to extract the DNA.

2. **Add 500 µl 65°C pre-warmed Buffer PL to dilute the grind samples, all transfer to a self-supplied 1.5 ml centrifuge tube, and incubated at 65°C for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**
3. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
4. **Transfer the supernatant from step 3 into a Spin Column (The Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.
* Pigments from some plants may remain on the filter of the Spin Column, and an additional ethanol wash step can be added (add 700 µl absolute ethanol and centrifuge at 12,000 rpm for 30 sec).

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65°C to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

9. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**

Protocol For Yeast

1. **Collect 5~10 ml yeast cultured overnight and discard the supernatant.**

* If yeast is collected with a 1.5 ml centrifuge tube, the enrichment cells can be centrifuged multiple times at 12,000 rpm for 30 sec.

2. **Add 200 µl Buffer TE (Simgen Cat. No. 9006500, not provided), directly pipette several times with the tip to suspend the yeast pellet, transfer it into a mortar. Add liquid nitrogen to soak the yeast solution (the solution will immediately coagulate into a lump when it touches the liquid nitrogen) and grind vigorously until the yeast to powder form.**
3. **When the powdered yeast begins to thaw, transfer the homogenate to a 1.5 ml centrifuge tube.**

* If the pellet is not ground into powder, it should be supplemented with liquid nitrogen to continue grinding, otherwise the recovery efficiency of the final DNA will be seriously affected.
* In the absence of liquid nitrogen, the cell wall can be fully destroyed by direct grinding for about 15 min. If the liquid is evaporated during this process, add an appropriate amount of Buffer TE to continue grinding, then transfer the homogenate into a 1.5 ml centrifuge tube, and follow step 4.

4. **Add 2 µl RNase A (Simgen Cat. No. 8001001, not provided), incubate at 65℃ for 5 min.**
5. **Add 300 µl Buffer PL and 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided), close the lid, vortex shaking for 30 sec to mix, incubate at 65℃ for 20 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**
6. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
7. **Transfer the supernatant from step 6 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

9. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

10. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

11. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 60~100 µl Buffer TE incubated to 65℃ to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

12. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**

Protocol For Stool

1. **Collect 30~50 mg stool with a 1.5 ml centrifuge tube and add 250 µl Buffer TE (Simgen Cat. No. 9006500, not provided) vortex and shake until all stool pellets are suspended.**

* If the stool is in liquid form, transfer 50 µl stool directly. If DNA is extracted from mouse stool, it can be used with a grinding rod (Simgen Cat. No. D-050, not provided) grinding stool pellets.

2. **Add 250 µl Buffer PL, shake vigorously to mix well, incubate at 95°C for 5 min.**

* If only the DNA of intestinal cells or the DNA of gram-negative bacteria in the stool needs to be tested, incubate at 70°C for 5 min is only required.

3. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
4. **Transfer the supernatant from step 3 into a Spin Column (The Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65°C to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

9. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**

Protocol For Blood or Animal Cells

For non-nucleated red blood cell anticoagulated blood, saliva or bone marrow samples, follow step 1a.
For nucleated red blood cell anticoagulated blood samples, follow step 1b.
For animal culture cell samples, follow step 1c.

1a. Add 20 µl Proteinase K (Simgen Cat. 8000224, not provided) to the bottom of a 1.5 ml centrifuge tube, and add 300 µl blood, saliva, or bone marrow. Proceed to Step 2.

* If the volume of anticoagulated blood is less than 300 µl, add PBS solution to adjust the final blood volume to 300 µl.

1b. Add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided) to the bottom of a 1.5 ml centrifuge tube and add 290 µl Buffer TE (Simgen Cat. No. 9006500, not provided), and then add 5~10 µl blood. Proceed to Step 2.

1c. Collect 5~10×10^6 cells with a 1.5 ml centrifuge tube and add 290 µl Buffer TE (Simgen Cat. No. 9006500, not provided) and add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided). Proceed to Step 2.

2. **Add 200 µl Buffer PL incubated to 65℃, vortex for 30 sec to mix, and incubate at 65℃ for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**
3. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
4. **Transfer the supernatant from step 3 into a Spin Column (The Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65℃ to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

9. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**

Protocol For Bacteria

For gram-negative bacteria, follow step 1a.
For gram-positive bacteria, follow step 1b.

1a. Collect 3~5 ml gram-negative bacteria culture with a 1.5 ml centrifuge tube and add 200 µl Buffer TE (Simgen Cat. No. 9006500, not provided), vortex shaking to fully suspend bacteria. Proceed to Step 2.

1b. Collect 3~5 ml gram-positive bacterial culture with a 1.5 ml centrifuge tube and add 100 µl Buffer TE (Simgen Cat. No. 9006500, not provided), vortex shaking to fully suspend bacteria. Add 100 µl lysozyme (Simgen Cat. No. 8009500, not provided), vortex shaking for about 15 sec to mix, incubate at 37°C for 30 min. Proceed to Step 2.

* Some divalent cations can inhibit the activity of lysozyme, if the bacterial medium contains divalent cations (such as MRS medium, etc.), a washing step should be added after centrifugation to collect the bacteria: add 1 ml distilled water, centrifuge at 12000 rpm for 30 sec after vortexing to suspend bacteria, discard the distilled water, add 200 µl Buffer TE, vortex and shake to fully suspend the bacteria.
* Preparation method of lysozyme solution: 100 mg/ml lysozyme solution is prepared according to the ratio of adding 100 mg lysozyme per 1 ml deionized pure water.
* Lysozyme solution will seriously reduce the lysic efficiency after freezing and thawing, please try to use freshly prepared or freeze-thaw lysozyme solution no more than once.

2. Add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided) and 250 µl Buffer PL incubated to 65°C, vortex with shaking for 30 sec to mix, and incubate at 65°C for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.

* If DNA is extracted from freshly grown bacteria, some of the RNA from the bacteria may be extracted together, but the presence of RNA does not affect PCR-related experiments. If you want to remove RNA completely, follow these steps: add 250 µl Buffer PL incubated to 65°C and 2 µl RNase A (Simgen Cat. No. 8001001, not provided), vortex for 30 sec to mix, and incubate at 65°C for 5 min. Add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided), vortex for 30 sec to mix well, incubate at 65°C for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help release the DNA.

3. Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.

4. Transfer the supernatant from step 3 into a Spin Column (The Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.

5. Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

6. Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.

* Ensure that absolute ethanol has been added to Buffer WB.

7. Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65℃ to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

9. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**

Protocol For Animal Tissues

1. **Cut 50~100 mg human or animal tissue (one section of rat tail 0.4-0.6 cm or two sections of mouse tail 0.4-0.6 cm) with a scalpel, and then chop the tissue into a homogeneous with the tip of the knife and transfer the tissue to a 1.5 ml centrifuge tube.**

* The tissue particles must be chopped into a homogenous to shorten the digestion time of the tissue.
* For samples containing a lot of dense connective tissue, such as skin, tendons, rat tails, etc., use the tip of a scalpel to cut the sample into as few pieces as possible to shorten the dissolution time.

2. **Add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided), and then add 200 µl Buffer TE (Simgen Cat. No. 9006500, not provided) incubated to 65℃, vortex shaking for a few secs to disperse the tissue homogenate.**
3. **Add 300 µl Buffer PL incubated to 65℃, vortex for 30 sec to mix, and incubate at 65℃ for 30 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**

* For samples with a large amount of dense connective tissue, incubate at 65℃ overnight can be used to fully lyse the tissue and increase the amount of DNA recovered.

4. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
5. **Transfer the supernatant from step 4 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65℃ to the Spin**

Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

10. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**

Protocol For Arthropods (Insects, Etc.), Bone Tissues

1. **Weigh 300~500 mg (arthropods including exoskeletons) sample in a mortar, grind the sample into powder form with liquid nitrogen, and then weigh 50~100 mg of the tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen.**
2. **Add 200 µl Buffer TE (Simgen Cat. No. 9006500, not provided) and vortex incubated to 65℃ and shake for a few secs to disperse the tissue homogenate.**

* If DNA is extracted from live arthropods, an additional 2 µl RNase A (Simgen Cat. No. 8001001, not provided), close the lid, shake vigorously to mix, and incubate at 65℃ for 5 min before entering step 3.

3. **Add 20 µl Proteinase K (Simgen Cat. No. 8000224, not provided) and 300 µl Buffer PL incubated to 65℃, vortex and shake for 30 sec to mix, and incubate at 65℃ for 10 min. During the incubation, shake the centrifuge tube vigorously several times every 2~3 min to help the release of DNA.**
4. **Add 350 µl Buffer K, close the lid, shake vigorously for 15 sec, vortex for 30 sec. Centrifuge at 13,000 rpm for 5 min.**
5. **Transfer the supernatant from step 4 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* If there are lipids floating on top of the supernatant, carefully transfer the supernatant to a Spin Column with a pipette without bringing in the floating.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, close the lid, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the purified nucleic acid may be mixed with ethanol.

9. **Discard the 2 ml Collection Tube, place the Spin Column in a new clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 65℃ to the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the tube lid coming off and damaging the centrifuge.

10. Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.

Analysis of Common Problems

1. DNA cannot be recovered or the recovery efficiency of DNA is low

Possible causes:

1） The sample contained low levels of DNA and high levels of water or polysaccharide derivatives. Try doubling the amount of sample used. (Note that for each additional 50 mg of sample, decrease the amount of Buffer PL by 50 µl).

2） The content of phenols or mucopolysaccharides in the sample is too high, and the precipitate takes away the DNA. Select the Simgen Plant/Fungal DNA Extraction Kit (Simgen Cat. No. 3200050) to extract the DNA.

3） Senescent plant leaves were selected. The amount of DNA in senescent leaves will be severely reduced, if conditions allow, try to choose freshly grown plant leaves to extract DNA.

4） Improper storage, resulting in degradation of DNA in the sample. For tissue samples with high water content, they should be stored at -20℃, and -70℃ is recommended.

5） No absolute ethanol was added to Buffer WA or Buffer WB. Absolute ethanol should be added in proportion, if other reagents are added by mistake, please ask Simgen technical department for help.

6） The elution efficiency of DNA is poor. Refer to point 4 "Elute DNA" in Column Purification Techniques on page 3 to optimize the elution protocol for DNA.

2. A260/A280 ratio is too high

The purified DNA contains too much RNA. If DNA is being extracted from a live sample, 2 µl of RNase A (Simgen Cat. No. 8001001, not provided) can be added at the same time as Buffer PL to reduce RNA carryover.

3. A260/A230 value is abnormal

1） The A260/A230 ratio for pure DNA should be between 1.8-2.5, and if the A260/A230 deviates significantly from this range, be careful to observe the silica membrane on the Spin Column for residual plant pigments. If the pigment residue is severe, add 1~2 additional absolute ethanol washes after washing the Spin Column with Buffer WB (add 600 µl absolute ethanol to the Spin Column and centrifuge at 12,000 rpm for 30 sec).

2） The wash sequence of Buffer WA and Buffer WB was incorrectly used. Make sure to wash the Spin Column in the correct order.

4. Follow-up experiments with DNA did not work well

1） Excessive salt residue. Note the wash sequence of Buffer WA and Buffer WB, making

sure to wash the Spin Column in the correct order.

2） Excess ethanol residue. Note that the high-speed spin dry step should not be omitted, and the empty Spin Column should be carefully removed to avoid inversion, so as not to allow the residual filtrate at the bottom of the 2 ml Collection Tube to come into contact with the Spin Column.

3） Too much DNA was used as a PCR template. In general, 100-500 ng of DNA is appropriate to add to a 50 μl PCR reaction as a template (single copy gene).

5. DNA is sheared into small fragments, and severe tailing occurs during electrophoresis

1） Samples were repeatedly frozen and thawed multiple times. Try to avoid repeated freezing and thawing of samples, or splitting samples into small pieces before cryopreservation.

2） The sample is too old. Stale samples contain only fragments of broken DNA.