Product introduction

The kit uses ion exchange silica packed media to bind DNA at the appropriate salt ion concentration using gravity flow, and then elute the HMW high molecular weight DNA after changing the salt ion and pH conditions. It is also compatible with the extraction of large fungal samples. The extracted genomes are of good integrity, high purity, stable and reliable quality, and can meet the downstream applications in triple sequencing.

Product features

Wide application: Suitable for a wide range of sample types including polysaccharide polyphenolic plants and macrofungi.

Completely non-toxic: Toxic organic reagents such as phenol/chloroform are not required.

High purity: Polysaccharide co-sedimentation agent and polyphenol adsorption are combined to completely remove secondary metabolites.

High molecular weight: Approximately 48 kb of genomic DNA can be extracted.

Precautions: Please be sure to read this precaution before using the kit.

1. Repeated freeze-thawing of the samples should be avoided, as this may result in smaller DNA fragments and lower extraction volumes.
2. If the buffer HMW1 precipitates, it can be dissolved in a 37°C water bath and used after shaking well.

Operating steps

Column equilibration:

Add 5 ml of equilibrium binding solution HMW3 to the HMW gDNA adsorption column, and let the equilibrium binding solution HMW3 flow through the adsorption column by gravity.

Note: The columns treated with equilibrium binding solution are best used immediately, leaving them for too long will affect the use effect.

Sample pre-treatment:

Take 0.5 g-2 g of plant tissue and add liquid nitrogen to grind it thoroughly and transfer to a 50 ml collection tube.

Note: 1)Try to use young and tender leaf tissue for extraction.

2) For samples with high polysaccharide polyphenol content, the sample loading volume can be moderately reduced.

3) For large fungal samples, refer to the pre-treatment method for plant samples.

Genomic DNA adsorption and purification:

1. Add 8 ml of buffer HMW1, 100 μl of nucleic acid protectant ST, 200 μl of Proteinase K and one polyphenol adsorbent tablet (NKY tablets) to the above treated sample, shake rapidly and mix well, then place the centrifuge tube in a 50C water bath for 30 min.
2. Add 200 μl of RNaseA (10 mg/ml), mix well, and let stand at room temperature for 5 min.
3. Add 2 ml of polysaccharide co-sedimentation agent HMW2, mix well, and let the tube stand on ice for 5 min.
4. Centrifuge at 6,000 rpm for 10 min at 4°C, and transfer the supernatant to a centrifuge tube containing a HMW filter column (HMW Filter).
5. Centrifuge at 2,000 rpm for 30 s at 4°C, discard the HMW filter column, add twice the volume of the filtrate to the equilibrium binding solution HMW3 (approximately 11-13 ml volume), and mix upside down for 30 s.
6. Carefully pour the mixture from step 5 into an equilibrated HMW gDNA Tip column until all solutions flow through the column and discard the effluent.

7. Add 10 ml of rinse solution HMW4 to the HMW gDNA adsorption column (HMW gDNATip) until all the solution flows through the column and discard the effluent.

Note: For particularly viscous or impurity-rich samples, an additional 5 ml of rinse solution HMW4 can be added.

8. Take a clean 50 ml centrifuge tube to collect the eluate and add 7.5 ml of eluate HMW5 to the HMW gDNA Tip until all the solution flows through the column.

9. Discard the HMW gDNA column (HMW gDNA Tip), add 5 ml of isopropanol to the solution from step 8 and mix well; centrifuge at 8,000 rpm for 10 min at 4°C and carefully pour off the supernatant.

Note: The position of the precipitate can be marked on the centrifuge tube to avoid precipitate loss when pouring off the supernatant.

10. Rinse the precipitate by adding 2 ml of 70% ethanol to the tube containing the precipitate and centrifuge at 8,000 rpm for 5 min at 4°C. Carefully discard half of the supernatant.

Note: Rinsing of the precipitate should not be done by vigorous vortexing, but by gently shaking the centrifuge tube until the precipitate is resuspended.

11. Transfer the remaining supernatant and precipitate to a 1.5 ml centrifuge tube with a cut blue tip and centrifuge at 8,000 rpm for 5 min at 4°C.

12. The residual liquid at the bottom of the tube was discarded with a gun tip and dried at room temperature for 5-10 min.

13. Add 100-300 μl of elution buffer TB to dissolve genomic DNA and store in appropriate conditions.

Note: The eluate can be heated at 50 °C for 10 min to fully solubilize the DNA.

14. (Optional step) For individual extremely complex samples, if the purity of the extracted product is not satisfied, it is recommended to purify it once using 0.8x volume of Beckman XP or Tiangen NG316 magnetic beads according to the operating instructions.

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HMW Plant Genomic DNA Kit 1

Cat. No.: **NG411-T1**

Product composition

Product composition	NG411-T1 (10 preps)
HMW1(Buffer HMW1)	90 ml
HMW2 (Buffer HMW2)	25 ml
NKY tablets	10 slices
HMW3 (Buffer HMW3)	250 ml
HMW4 (Buffer HMW4)	150 ml
HMW5 (Buffer HMW5)	90 ml
TB (Buffer TB)	15 ml
ST (Buffer ST)	1 ml
RNase A (10 mg/ml)	2 ×1 ml
Proteinase K	2 ×1 ml
HMW Filter	10 Pcs
HMW gDNA Tip	10 Pcs
Collection Tubes (50 ml))	10 Pcs

Storage conditions

The kit can be stored for 12 months under dry conditions at room temperature (15-30℃). If the solution produces precipitation, the solution in the kit should be left at room temperature for a period of time before use, and if necessary, it can be preheated in a 37℃ water bath for 10min to dissolve the precipitation.