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Fungi Genomic DNA Extraction Kit

Cat No.: EXD0220

Package: 50T/100T

Storage: At room temperature(15℃-25℃) in dry place for 1 year.

Kit Contents

Component	50T	100T
RNase A	1 ml	1 ml × 2
Proteinase K	1 ml	1 ml × 2
Beads	6 g	11 g
Solution A	10 ml	20 ml
Solution B	10 ml	20 ml
Washing Buffer	15 ml	15 ml × 2
Elution Buffer	10 ml	20 ml
Adsorption Column	50 Units	100 Units
Collection Tube	50 Units	100 Units
Instruction	1 Piece	1 Piece

Product Description

Fungi are Heterotroph with nucleus and cell wall, there are many species and genera, more than 10,000 genera and more than 100,000 species have been reported. Fungi are generally divided into three kinds, namely yeast, mould and mushroom. Yeast and mould can be treated with Beads, mushroom are directly ground with liquid nitrogen. After pretreatment, bacteria solution can be adsorbed with siliceous membrane to obtain fungi genomic DNA with high purity. The extracted DNA is large yield and good integrity, it can be directly used for a variety of routine operations, including PCR/Real Time PCR, sequencing, southern blot, mutant analysis, SNP, etc.

Protocol

Add fresh opened absolute ethanol in Washing Buffer before use, volume is based on the label of bottle as a reference. Put cap back on bottle and shake well. All centrifuge steps are performed at room temperature(15℃-25℃).

1. Sample treatment:

1) Yeast: Take 1-2ml cultured bacteria solution, centrifuge at 12,000rpm for 1min, discard supernatant. Add 200μl Solution A, 20μl RNase A and 100mg Beads, place on high-speed vortex mixer for 5-10min.

2) Mould(spore can also be treated in the same way): Take 50-100mg hyphae, add 200μl Solution A, grind with glass mortar. Add 20μl RNase A and 100mg Beads, place on high-speed vortex mixer for 30min.

3) Mushroom: Take 50-100mg sample, add sample into mortar, pour proper amount of liquid nitrogen, grind immediately, repeat three times. If there isn't liquid nitrogen in the laboratory, add 200μl Solution A, grind with glass mortar. Add 200μl Solution A, 20μl RNase A and 100mg Beads, place on high-speed vortex mixer for 5min.

2. Add 20μl Proteinase K(10mg/ml), mix thoroughly, digest at 55℃ water bath for 30min, invert tube several times in the meanwhile. Centrifuge at 12,000rpm for 2min. Transfer supernatant into a new centrifuge tube. If there is precipitation, centrifuge at 12,000 rpm for 2min again.

3. Add 200μl Solution B in supernatant, mix thoroughly. White precipitate may form, redissolve at 55℃ water bath for 5min, precipitate will disappear, it does not affect DNA extraction. If solution does not become clear, prolong digestion time please. Because it means that sample is not completely digested, which may lead to less and impure extracted DNA, and it may also cause column blocking after loading.

4. Add 200μl absolute ethanol, mix thoroughly. White precipitate may form, it won't affect DNA extraction. Add mixture into Adsorption Column, place for 2min.

5. Centrifuge at 12,000rpm for 1min, discard waste liquid, put Adsorption Column back into Collection Tube.

6. Add 600μl Wash Buffer in Adsorption Column(added absolute ethanol), centrifuge at 12,000rpm for 1min, discard waste liquid, put Adsorption Column into Collection Tube.

7. Repeat wash step 6 with another 600μl Washing Buffer.

8. Centrifuge at 12,000rpm for 2min, place Adsorption Column at room temperature(15℃-25℃) or 50℃ warm box for a few minutes to remove residual Washing Buffer in Adsorption Column, otherwise ethanol in Washing Buffer will affect the follow-up experiments such as enzyme digestion and PCR.

9. Put Adsorption Column into a new clean centrifuge tube, drop 50-200μl Elution Buffer preheated at 65℃ water bath in center of adsorption membrane(tip don't touch membrane), place at room temperature(15℃-25℃) for 5min. Centrifuge at 12,000rpm for 1 min.

10. Add Elution Buffer got from step 9 centrifuge tube to Adsorption Column, place at room temperature(15℃-25℃) for 2min, centrifuge at 12,000rpm for 2min, obtain high quality fungi genomic DNA .

Notes

1. Due to various of fungi, some fungi are particularly hard to treat, they can be ground in liquid nitrogen, shake with Beads, digest with Proteinase K. Generally, it will obtain Fungi Genomic DNA. If extracted DNA can't be detected by electrophoresis, the reason is that extracted DNA concentration is low, but it can be used for PCR and get a good result.

2. If precipitation occurs in Solution A or Solution B, redissolve at 55℃ water bath for a moment, precipitation will disappear, it does not affect DNA extraction.

3. If extracted DNA concentration is low, please extend time of Beads treatment. If DNA bands are short and dispersion, reduce time of Beads treatment.

4. Volume of Elution Buffer shouldn't be less than 50μl, if volume is too small, it will affect recovery efficiency. pH value of the Elution buffer may also affect elution efficiency, if using water as Elution buffer, please ensure pH8.0(it can use NaOH to adjust pH value), elution efficiency will be reduced if pH value is lower than 7.0. DNA product should be stored at -20℃ to prevent DNA degradation.
5. DNA concentration and purity detection(higher concentration): size of obtained genomic DNA fragment is related with storage time, shear force and other factors. The concentration and purity of obtained DNA fragment can be detected by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak at OD_{260}. When OD_{260}=1, it is equal to 50μg/ml of double-stranded DNA and 40μg/ml single-stranded DNA. OD_{260} / OD_{280} ratio should be 1.7 to 1.9, if deionized water is used instead of Elution buffer during elution operation, ratio will be lower, because pH value and ion will affect absorbance, but it does not mean low purity.