Genomic DNA Extraction from plant including fungi, spore, mycelium and green algae using Exgene™ Plant SV mini

Here are additional GeneAll *Specialist's tips* for higher extraction efficiency.

1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 100 mg (wet) or 25 mg (dried) of ground tissue into a 1.5 ml or 2.0 ml microcentrifuge tube.

   Quick and complete disruption of tissue is essential for good result in preparation.
   Grinding under liquid nitrogen is the best method for good result, however other methods such as bead-beater or rotor-stator homogenizer can be a good alternative.
   Lyophilized tissue can be ground at room temperature.

   The purified DNA is of the highest quality and is fully compatible with downstream PCR, real-time PCR, Southern blotting, microarray technology applications.

   GA Tips

   - The remaining ground tissue powders can be stored at -70°C in a 50 ml tube for DNA extraction for more than a year.
   - For larger amounts of plant tissue, it is recommended to use a Midi (Cat. No. 117-226) or MAXI kit (Cat. No. 117-310).

2. Add 400 µl of Buffer PL and 4 µl of RNase A solution (100 mg/ml, provided). Vortex vigorously.

   Any clumps should not be visible.
   Mix the lysate by pipetting or vortexing to remove any tissue clumps.

   GA Tips

   - It is recommended to warm the Buffer PL at 65℃ before use.
   - Vortex the mixture vigorously for 1 minute to ensure thorough mixing without clumps.
     If the mixture is not mixed well or becomes sticky when the tube is tilted, add an additional 400 µl of Buffer PL and 4 µl of RNase A, and vortex again.
     If the sample still is not mixed well, repeat the addition of Buffer PL and RNase A until the mixture is thoroughly mixed.
     If clumps persist even after adding sufficient Buffer PL, the clumps can be loosened using the tip of a 1 ml pipette tip.
   - For improved yield and extraction of high-molecular-weight genomic DNA, Proteinase K (not provided) can be added to the sample at a concentration of 1/40 of the total volume after Buffer PL and RNase A treatment.
   - After Proteinase K treatment, incubate at 55℃ for 1 hour, followed by 15 to 30 minutes at 65~70℃ (Step 3).

3. Incubate for 10~15 min at 65℃. Mix 2~3 times during incubation by inverting or vortexing.

   Occasional mixing will accelerate the lysis.

   GA Tips

   - Invert every 5 minutes (~30 times) during the incubation.

4. Add 140 µl of Buffer PD to the lysate. Vortex to mix, and incubate for 5 min on ice.
   (Optional :) Centrifuge for 5 min at full speed (>10,000 x g or 14,000 rpm).

   For some plants, the lysate becomes very viscous or sticky after addition of Buffer PD, and this leads to shearing of DNA or clogging of EzSep™ Filter.
   In this case, removal of precipitates by optional centrifugation will be helpful before proceeding to next step.

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5. Apply the lysate to the EzSep™ Filter (blue) and centrifuge for 2 min at full speed.

   It may be requisite to use wide-bore tip or to cut the end off the pipet tip to apply the viscous lysate to the EzSep™ Filter.
   Small pellet can be formed in the collection tube after centrifugation.
   Be careful not to disturb this pellet in next step 6.

6. Transfer the pass-through to a new 1.5 ml microcentrifuge tube by pipetting or decanting carefully not to disturb the cell debris pellet.

   About 450 µl of lysate is recovered typically.
   Recovered volume of lysate can be varied depending on the plant tissue used.
   Check the correct volume of lysate for optimal binding condition in next step.

GA Tips

- If the volume of pass-through exceeds 800 µl, transfer it to a 15 ml tube.

7. Add 1.5 volumes of Buffer BD to the lysate and mix immediately by pipetting or inverting.

   Adjust the volume of Buffer BD on the basis of correct volume of lysate.
   For 450 µl lysate, add 675 µl Buffer BD.
   Immediate mixing is important for optimal binding conditions.
   A precipitate can be formed after addition of Buffer BD but this will not affect the preparation.

GA Tips

- Invert 30~50 times.

8. Apply 700 µl of the mixture from step 7 to the SV column (green) sitting in collection tube. Centrifuge for 30 sec, and discard the pass-through. Reuse the collection tube.

   Any precipitate which may have formed in mixture should be included in transfer.

GA Tips

- Possible to load 700 µl of solution three times for a total of 2,100 µl per column.

9. Repeat step 8 with remaining sample.

10. Apply 700 µl Buffer CW to the SV column, centrifuge for 30 sec and discard the pass-through, and re-insert the SV column to the collection tube.

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11. Add 300 µl of Buffer CW to the SV column. Centrifuge for 2 min.
Transfer carefully the SV column to a new 1.5 ml microcentrifuge tube (not provided).

Care must be taken at the removal of SV column from the collection tube so the column does not come into contact with the pass-through fraction, as this will result in carryover of ethanol.
Residual ethanol in eluate may interfere with the subsequent reactions.
If carryover of Buffer CW occurs, centrifuge again for 1 min before proceeding to next step.

GA Tips

- Centrifuge for 1 minute with the column tube facing opposite to that in step 11 to remove any remaining Buffer CW from the column.

12. Add 100 µl of Buffer AE directly onto the center of SV column membrane. Incubate for 5 min at room temperature and centrifuge for 1 min.

Elution volume can be decreased to 50 µl for high concentration of DNA, but this will slightly decrease in overall DNA yield.
If maximum recovery of DNA is preferred or the starting materials contain large amount of DNA, elution can be done in 200 µl of Buffer AE.

GA Tips

- Incubate for 5 at ~65°C instead of RT to increase yield.

13. Repeat step 12.

More 20~40% DNA can be obtained by repeat of eluting.
A new 1.5 ml microcentrifuge tube can be used to prevent dilution of the first eluate.