2009

PLANT SV MINI
PLANT SV MIDI
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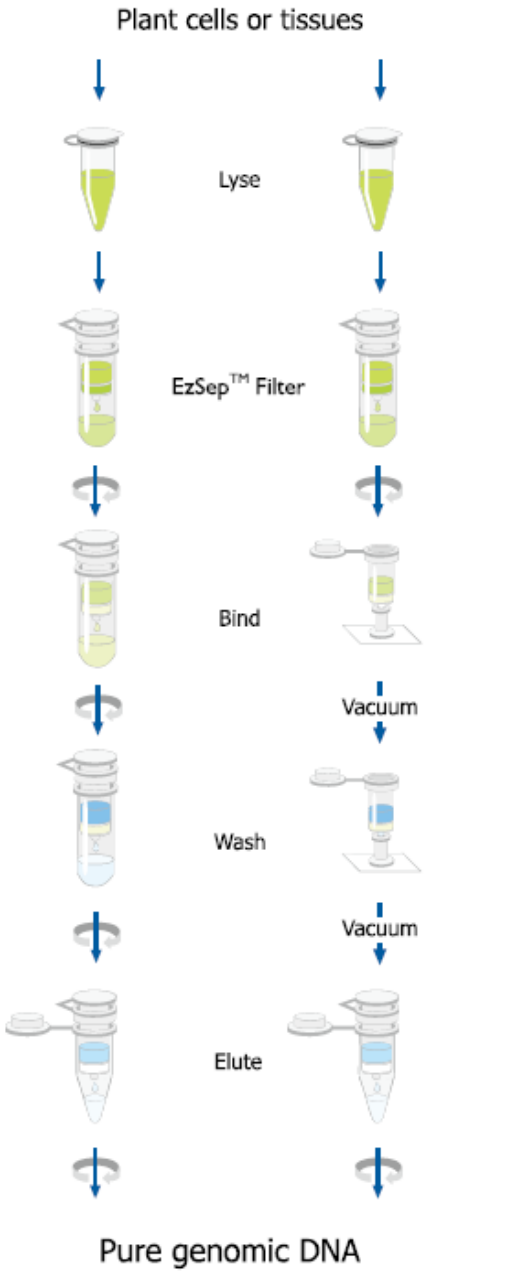
DNA PURIFICATION HANDBOOK

GeneAll

Plant SV Kit Procedures

in microcentrifuges on vacuum manifolds



GeneAll® Exgene™ Plant SV mini

Before experiment

Unless there is an other indication, all centrifugation steps should be performed at full speed (>10,000 xg or 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.

Buffer PL may precipitate upon storage at cold ambient temperature. If so, dissolve it in 65°C water bath.

■ *Prepare the below;*

- » *65°C water bath or heating block*
- » *1.5 mL and 2 mL Micro centrifuge tubes*
- » *Microcentrifuge*

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 ml 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.

2. Add 400 ul of Buffer PL and 4 ul 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.

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

Occasional mixing will accelerate the lysis.

4. Add 140 ul 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 xg 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.

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 tube by pipetting or decanting carefully not to disturb the cell debris pellet.

About 450 ul of lysate is recovered typically. Recoverd 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.

7. Add 1.5 vol 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 ul lysate, add 675 ul 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.

8. **Apply 700 ul of the mixture from step 7 to the GeneAll® 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.

9. **Repeat step 8 with remaining sample.**

10. **Apply 700 ul 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.**

11. **Add 300 ul of Buffer CW to the SV Column. Centrifuge for 2 min. Transfer carefully the SV Column to a new 1.5 ml tube (not provided).**
   Care must be taken at the removal of GeneAll® 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.

12. **Add 100 ul 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 ul 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 ul of buffer AE.

13. **Repeat step 12.**
   More 20 ~ 40 % DNA can be obtained by repeat of eluting.
   A new 1.5 ml tube can be used to prevent dilution of the first eluate.