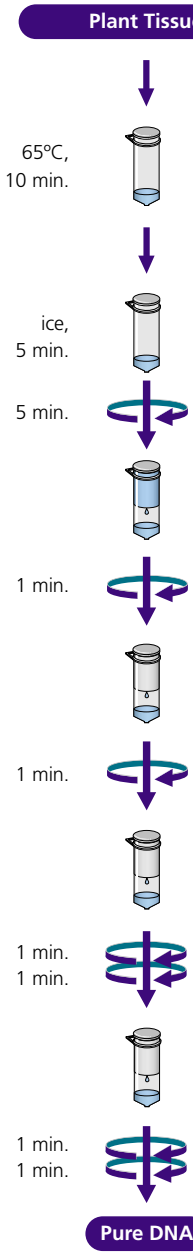
GENELUTE PLANT GENOMIC DNA KIT



All spins at ≥ 12,000 x g

1 Release DNA from tissue

- ❑ Grind plant tissue in liquid nitrogen.
- ❑ Lyse up to 100 mg ground plant tissue with 350 µl of lysis solution (Part A) + 50 µl of lysis solution (Part B) Vortex & invert to mix thoroughly.
- ❑ *Incubate at 65 °C for 10 min.*

2 Remove debris

- ❑ Add 130 µl precipitation solution. Invert to mix. *Incubate on ice 5 min.*
- ❑ Pellet debris 5 min.
- ❑ Transfer supernatant to blue filtration column. *Spin 1 min.*

3 Bind DNA to column

- ❑ Add 700 µl binding solution to filtrate. Mix thoroughly by inversion.
- ❑ Transfer 700 µl of mixture to binding column. *Spin 1 min.* Discard flow through.
- ❑ Repeat with remainder of mixture. Transfer column to new collection tube.

4 Wash to remove contaminants

- ❑ Add 500 µl wash solution to column. *Spin 1 min.* Transfer column to new collection tube.
  Note: Ethanol must be added to wash solution concentrate before first use.
- ❑ Add second 500 µl wash solution to column. *Spin 1 min.*

5 Elute purified DNA

- ❑ Transfer column to new collection tube.
- ❑ Add 100 µl elution solution (pre-warmed to 65 °C) to column. *Spin 1 min.*
- ❑ Repeat elution.

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Problem	Reason	Solution
Binding column clogged	Sample size was too large	For future preparations, use less plant tissue. To salvage the current preparation, increase g-force and/or spin longer until lysate passes through the binding column. Yield of DNA may be reduced.
	Tissue disruption was insufficient	Thoroughly disrupt the starting material according to step 1 of the protocol. If using alternative disruption methods, make sure you are effectively disrupting the tissue.
Low Yield	State of starting plant tissue	Yields will vary between different types of plant tissues and plant species. If possible, use the youngest leaves or tissues. If samples are being stored for future use, flash freezing in liquid nitrogen is recommended.
	Tissue disruption was insufficient	See above.
	Residual ethanol in eluate	Ethanol from the final wash must be eliminated before eluting the DNA. A longer or additional spin is needed to dry the membrane. If eluate containing ethanol contacts the column, repeat the centrifugation step before eluting DNA.
	Wash Solution Concentrate was not diluted before use	Confirm the Wash Solution concentrate was properly diluted with ethanol before use.
	Elution is incomplete	Confirm that DNA was eluted in 200 µl Elution Solution. A 5 minute incubation at room temperature after Elution Solution has been added to the binding column will improve yields with most types of material. You may also perform a second and third elution on the binding column using 200 µl Elution Solution for each elution.
	Water was used for elution instead of Elution Solution	Elution Solution is recommended for optimal yields and storage of end product. If water is used to elute DNA, confirm the pH is at least 7.0, to avoid acidic conditions, which would subject the DNA to acid hydrolysis when stored for long periods of time.
Purity of DNA lower than expected: A_{260}/A_{280} ratio is too low	Purification was incomplete	Use less tissue for future preparations.
	Background reading is high due to silica fines	Spin DNA sample at maximum speed for 1 minute; use supernatant to repeat absorbance readings.
Purity of DNA lower than expected: A_{260}/A_{280} ratio is too high	RNA contamination	Include RNase A treatment step in future isolations.
Sheared DNA	Manipulation of sample was excessive	All pipeting steps should be accomplished as gently as possible. Wide orifice pipet tips are recommended to help eliminate potential shearing. If minimally sheared genomic DNA is desired in downstream applications (e.g., long amplification PCR), mix with gentle pipeting or inversion until homogeneous instead of vortexing.
	Old sample or sample has undergone repeated freeze/thaw cycles	Old starting material may yield degraded DNA in the eluate. Fresh cell and tissue preparations should be used immediately or frozen in liquid nitrogen and stored at -70 °C until needed.
Inhibition of downstream applications	Residual ethanol in eluate	After the final wash of the binding column do not allow the flow-through liquid to contact the column. Re-spin the column, if necessary, by emptying the flow-through liquid from the collection tube and centrifuging the binding column for an additional 1 minute at maximum speed (12,000-16,000 x g).
	Excess salt in eluate	Make sure that binding column is transferred to a new collection tube before adding Wash Solution.