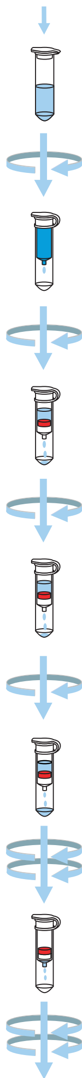
G2N 350 | G2N 70 | G2N 10

GENELUTE PLANT GENOMIC DNA KIT



All spins at ≥ 12,000 x g

1 Prepare plant tissue

- ❑ Grind plant tissue in liquid nitrogen.

2 Release DNA from tissue

- ❑ 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.*

3 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.*

4 Prepare binding column

- ❑ Add 500 µl Column Preparation Solution to binding column.
- ❑ *Spin 1 min.* Discard flow-through.

5 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.

6 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 3 min.*

7 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.

Order/Customer Service *Phone* 1-800-325-3010 *Fax* 1-800-325-5052 *Technical Service* 1-800-325-5832
World Headquarters 3050 Spruce St. St. Louis MO 63103 (314)771-5750 www.sigma-aldrich.com

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Problem	Reason	Solution
Binding column clogged	Sample is 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. The DNA yield may be reduced.
	Tissue is insufficiently disrupted	Thoroughly disrupt the starting material according to step 1 of the protocol. If using alternative methods, make sure that you are effectively disrupting the tissue.
DNA yield is low	Sample may be old or degraded	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-freeze in liquid nitrogen and store at –70 °C.
	Tissue is insufficiently disrupted	See above.
	The eluate contains residual ethanol from wash	Ethanol from the final wash must be eliminated before eluting the DNA. A longer or additional spin is required to dry the membrane. If the flow-through liquid containing ethanol contacts the binding column, repeat the centrifugation step before eluting the DNA.
	Wash Solution concentrate was not diluted before use	Confirm that the Wash Solution concentrate was properly diluted with ethanol before use.
	DNA elution is incomplete	Confirm that the DNA was eluted in 100 µl Elution Solution. A second and third elution using 100 µl of Elution Solution may be performed. 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.
	Water was used for elution instead of Elution Solution	Elution Solution is recommended for optimal yields and storage of the purified DNA. If water is used to elute the DNA, confirm that the pH is at least 7.0, to avoid acidic conditions which may subject the DNA to acid hydrolysis when stored for long periods of time.
Purity of the DNA is lower than expected: A_{260}/A_{280} ratio is too low	Purification is incomplete	Reduce the initial volume of the sample.
	Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purity of the DNA is lower than expected: A_{260}/A_{280} ratio is too high	Genomic DNA is contaminated with RNA	Include an RNase A treatment in future isolations or treat the final product with RNase A Solution and repurify.
DNA is sheared	DNA sample was excessively manipulated	All pipetting steps should be accomplished as gently as possible. Wide-bore pipette tips are recommended to help eliminate potential shearing. Do not vortex.
	Sample is old, degraded, or has undergone repeated freeze/thaw cycles	Old starting material may yield degraded DNA in the eluate. Fresh preparations should be used immediately or be frozen in liquid nitrogen and stored at –70 ˚C until needed.
Downstream applications are inhibited	Ethanol is carried over into the final genomic DNA preparation	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, after emptying the collection tube, for an additional 1 minute at maximum speed (12,000-16,000 *x g*).
	Salt is carried over into the final genomic DNA preparation	Make sure that binding column is transferred to a new collection tube before adding the Wash Solution. Wash twice with 500 µl of Wash Solution.