Feb 13, 2026

Plant DNA extraction with Qiagen Genomic Tip

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqpxoxvk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.36wgqpxoxvk5/v1

Protocol Citation: CHIARA NATALI, Claudio Ciofi 2026. Plant DNA extraction with Qiagen Genomic Tip . **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqpxoxvk5/v1

Protocol status: Working
We use this protocol and it's working

Created: December 03, 2025

Last Modified: February 13, 2026

Protocol Integer ID: 234118

Keywords: HMW-DNA, BGE, plant hmw dna extraction, genomic tip, plant dna, plant dna extraction, dna extraction, Biodiversity Genomics Europe, plant dna extraction with qiagen genomic tip, plant dna extraction, qiagen genomic tip, use with qiagen genomic, qiagen genomic, isolation of genomic dna, genomic dna, dna, plant leaf, extraction

Funders Acknowledgements:
Biodiversity Genomics Europe receives funding from the European Union's Horizon Europe Research and Innovation Action
Grant ID: 101059492

Disclaimer

The duration times presented are estimations only.

Abstract

This protocol is designed for isolation of genomic DNA from 1–2 g of starting material (plant leaf) with a QIAGEN Genomic-tip 500/G. However, it can be scaled down for use with QIAGEN Genomic-tip 100/G.

Attachments

DOCX

Qiagen Genomic Tip.d...

3.5MB

Guidelines

THE QIAGEN PRINCIPLE
Each disposable QIAGEN Genomic-tip packed with QIAGEN resin is designed to operate by gravity flow. QIAGEN protocols are optimized for use with fixed sample volumes corresponding to the capacity of the QIAGEN Genomic-tip used (see table below).

	QIAGEN Genomic-tip		
	20/G	100/G	500/G
DNA	1–20 µg	10–100 µg	80–500 µg
Blood	0.1_1 ml	1_5 ml	5_20 ml
Cells	5×10^6	2×10^7	1×10^8
Tissue	20 mg	100 mg	400 mg
Yeast	1.5×10^9	7.0×10^9	3.5×10^{10}
Bacteria	4.5×10^9	2.2×10^{10}	1.0×10^{11}

Specifications for different QIAGEN Genomic-tips

QIAGEN Genomic-tips are available in a variety of sizes for preparation of as little as 20 µg or as much as 500 µg of DNA. The maximum DNA-binding capacities of QIAGEN Genomic-tips 20/G, 100/G, and 500/G are 20 µg, 100 µg, and 500 µg, respectively. Actual yields will depend on the tissue, body fluid, or cell type used. Overloading tips with an excessive amount of sample will lead to reduced flow rates, extend the time required for loading, washing, and elution, and may affect the purity and yield of the eluted DNA.

QIAGEN Genomic-tips contain a unique, patented anion-exchange resin. The resin consists of defined silica beads with a particle size of 100 µm, a large pore size, and a hydrophilic surface coating. DNA purification on QIAGEN resin is based on the interaction between negatively charged phosphates of the DNA backbone and positively charged DEAE (Dietilamminoetil) groups on the surface of the resin. Impurities such as RNA, protein, carbohydrates, and small metabolites are washed from QIAGEN resin with medium-salt buffers, while DNA remains bound until eluted with a high-salt buffer.

The following guidelines refer respectively to the use of Genomic-tip 20/G, Genomic-tip 100/G or Genomic-tip 500/G and to the use of the QBT, QC, QF solutions thus composed:

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution. Adjust the volume to 1 liter with distilled water.

QC: Dissolve 58.44 g NaCl, 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml distilled water. Adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

Materials

- QIAGEN Genomic-Tip 500/G (or 100/G, if scaled down)
- Materials for grinding plant leaf material in liquid nitrogen (details not covered in this protocol)
- Bottles for 1 liter of buffers
- NaCl
- MOPS (free acid)
- NaOH
- Distilled water
- Isopropanol
- Triton X-100
- Tris base
- HCl
- 50 ml screw cap tubes
- Pipettes + standard and wide-bore pipette tips
- Tris-Cl
- CTAB
- PEG 6000 or 8000
- EDTA
- ß-mercaptoethanol
- RNase A
- Vortexer
- Shaking water bath, if available
- Chloroform/isoamyl alcohol (24:1)
- Cooling centrifuge (>5000 x g)
- A tip holder or QIArack
- 70% ethanol
- buffer, e.g., TE buffer, pH 8.0, or 10 mM Tris-Cl, pH 8.5
- Oven or similar for pre-warming of some buffers
- shaker
- vacuum centrifuge
- Nanodrop or similar UV/Vis spectrophotometer
- Qubit fluorometer and 1X dsDNA HS Assay kit
- Pippin Pulse Gel Electrophoresis using 50 - 100 ng of DNA (gDNA) and Quick-Load 1 kb Extend DNA Ladder (size range: 0.5 kb to 48.5 kb) - or some other method to determine DNA size distribution with

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and work in a fume hood to perform the laboratory procedures in this protocol. In addition, chloroform-resistant gloves must be worn, and chloroform waste bottles should be stored in the fume hood.

Waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

Liquid waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

See best practices, thawing and handling instructions, and safety instructions for all the kit reagents, as well as instructions and safety data sheets for other reagents used in this protocol **before starting**.

If the tissue sample is ground in cryogenic conditions, take care to follow appropriate safety measures when performing this.

Before start

Prepare buffers mentioned in Guidelines, and Carlson lysis buffer needed in step 3.

Preparation of the lysate

1h 40m

1 Grind 1–2 g of leaf material in liquid nitrogen with a mortar and pestle. 1h

Safety information

Users should have appropriate training for handling of liquid nitrogen in order to perform this step in a safe manner.

2 Transfer tissue powder into a 50 ml screw-cap tube.

3 Add 10 ml of Carlson lysis buffer pre-warmed to 74°C. 2m

	Carlson lysis buffer
	100 mM Tris·Cl, pH 9.5
	2 % CTAB
	1.4 M NaCl
	1 % PEG 6000 or 8000
	20 mM EDTA

Recipe for Carlson lysis buffer

4 Add 25 µl β-mercaptoethanol and 100 µl of RNase A (20 mg/ml).
Vortex at full speed for 5–10 s. 1m

Safety information

β-mercaptoethanol is toxic; dispense in a fume hood and wear appropriate protective clothing.

5 Incubate at 74°C for 00:20:00 in a shaking water bath. 20m

Note

If a shaking water bath is not available, gently shake the samples every 5 min during incubation.

6 Cool the samples to room temperature, add 1 volume of chloroform/isoamyl alcohol (24:1), and vortex at full speed for 5–10 seconds. 4m

7 Centrifuge at 5,000 x g for 00:10:00 at 4°C. 10m

8 Transfer aqueous upper phase to a fresh 50 ml screw-cap tube. 1m

9 Add 1 volume of distilled water and adjust the pH to 7.0 using HCl. 2m

Note

Generally 100–200 µl of 25% HCl is required.

10 Proceed to next section of the protocol.

Setup of QIAGEN Genomic-tips 15m

11 Equilibrate a QIAGEN Genomic-tip with 10 ml of Buffer QBT, and allow the QIAGEN Genomic-tip to empty by gravity flow.

1. Place a QIAGEN Genomic-tip over a tube using a tip holder or into a QIArack over the waste tray.
2. Equilibrate the QIAGEN Genomic-tip with 10 ml of Buffer QBT described above. Flow begins automatically by reduction in surface tension due to the presence of detergent (0.15% Triton X-100) in the equilibration buffer. Allow the QIAGEN Genomic-tip to drain completely. The flow of buffer will stop when the meniscus reaches the upper frit. The frit prevents the QIAGEN Genomic-tip from running dry, allowing it to be left unattended. Do not force out the remaining buffer, as this will necessitate restarting the flow with a syringe and adapter.

Note

The binding, washing, and elution conditions for QIAGEN resin are strongly influenced by pH.

DNA binding on QIAGEN Genomic-tips

1h 30m

12 The cleared lysate is loaded onto a pre-equilibrated QIAGEN Genomic-tip by gravity flow. The salt and pH conditions of the lysate and the superior selectivity of the QIAGEN resin ensure that only DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained and appear in the flow-through fraction.

Note

Vortexing the genomic DNA has a minimal effect on the size of the DNA, and it accelerates the QIAGEN procedure by eliminating poor flow rates associated with clogging.

- Vortex the sample (from step 9) for 10 seconds at maximum speed and apply it to the equilibrated QIAGEN Genomic-tip. Allow it to enter the resin by gravity flow.

The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip. Once the QIAGEN Genomic-tip is loaded with the clear and particle-free sample, flow will begin unassisted. Allow gravity to determine the flow rate. Particularly concentrated genomic DNA lysates may exhibit diminished flow rates due to increased viscosity. It may also be helpful to dilute the lysate with an equal volume of Buffer QBT prior to loading.

DNA washing on Qiagen Genomic Tips

1h 30m

13 The QIAGEN Genomic-tip is then washed with a medium-salt buffer (Buffer QC). This buffer completely removes any remaining contaminants, such as traces of RNA and protein (e.g., RNase A), without affecting the binding of the DNA. Buffer QC also disrupts nonspecific interactions and allows removal of nucleic acid-binding proteins without the use of phenol. The low concentration of ethanol in the wash buffer eliminates nonspecific hydrophobic interactions, further enhancing the purity of the

bound
DNA.

1. Wash the QIAGEN Genomic-tip with 2 × 15 ml of Buffer QC.
2. Allow Buffer QC to move through the QIAGEN Genomic-tip by gravity flow. Two washes are sufficient to remove all contaminants in the majority of DNA preparations.

It is particularly important not to force out residual Buffer QC. Traces of Buffer QC will not affect the elution step.

DNA elution and precipitation

14 The DNA is then efficiently eluted from the QIAGEN Genomic-tip with a high-salt buffer (Buffer QF).
The eluted DNA is desalted and concentrated by isopropanol precipitation. Precipitation is carried out at room temperature (15-25°C) to minimize coprecipitation of salt. After centrifugation, the DNA pellet is washed with 70% ethanol to remove residual salt and to replace the isopropanol with ethanol, which is more volatile and easily removed.

Note

Use of Buffer QF pre-warmed to 50°C will increase yields.

15 Place the QIAGEN Genomic-tip over a clean 30 ml collection tube.

Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

16 Elute with 1 × 15 ml of Buffer QF, and collect the eluate. Flow begins automatically. Allow the QIAGEN Genomic-tip to drain by gravity flow.

17 Precipitate the DNA by adding 10.5 ml (0.7 volumes) room-temperature (15–25°C) isopropanol to the eluted DNA. Recover the precipitated DNA as described in next step.

18 Mix and centrifuge immediately at >5000 x g for at least 00:15:00 at 4°C. Carefully remove the supernatant.

Note

All solutions should be at room temperature to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 5000 x g is the minimal force required for efficient precipitation. Higher g-force is recommended where possible.

Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

19 Wash the centrifuged DNA pellet with 4 ml of **cold** 70% ethanol.

Note

The 70% ethanol removes precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

A second wash with cold 70% ethanol may improve results in more sensitive applications.

20 Vortex briefly and centrifuge at >5000 x g for 00:10:00 at 4°C.

21 Carefully remove the supernatant without disturbing the pellet.

22 After careful and complete removal of the supernatant with a pipet, the pellet should be briefly dried by vacuum centrifuge before resuspending in a small volume of suitable buffer. Overdrying the pellet will make the DNA difficult to redissolve.

23 Resuspend the DNA in 0.1-2 ml of a suitable buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5. DNA dissolves best under slightly alkaline conditions (pH 8.0 8.5) and does not dissolve easily in acidic buffers).

24 Dissolve the DNA overnight on a shaker at room temperature. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided.

Determination of yield, purity and length on the DNA

8h 30m

25 DNA yield is usually determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Purity is determined by calculating the ratio of

30m

absorbance at 260 nm to absorbance at 280 nm and ratio of absorbance at 260 nm to absorbance at 230 nm. Pure DNA has an A260/A280 ratio of 1.7 - 1.9 and an A260/A230 ratio of 2.0 - 2.2.

Concentration [ng/µl]	
A260/280	
A260/230	

26 Fluorimetric measurements are more accurate and should be used if precise concentrations are needed. Take a 1 µl aliquot from each sample and measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit.

Required Concentration [ng/µl]	
Yield [ng]	

27 Resolve DNA size distribution with Pippin Pulse Gel Electrophoresis using 50 - 100 ng of DNA (gDNA) and Quick-Load 1 kb Extend DNA Ladder (size range: 0.5 kb to 48.5 kb).

Note

Also other suitable methods can be used to resolve size distribution.

Protocol references

QIAGEN Isolation of genomic DNA from plants (QG07.doc Sep-01)
QIAGEN Genomic DNA Handbook - June 2015