

QIAGEN Supplementary Protocol:

Isolation of genomic DNA from tissue using the QIAGEN-tip 2500

This protocol is designed for the rapid, easy, and non-toxic preparation of up to 2 mg genomic DNA from not more than 2 g of tissue using QIAGEN-tip 2500. QIAGEN® Genomic-tips 20/G, 100/G, and 500/G can also be used with this protocol by reducing the amount of starting material according to the table on page 2. The purified genomic DNA ranges in size from 50–150 kb.

Storage of tissue samples

It is well known that DNA yield will decrease when tissue samples are stored at either 2–8°C or –20°C without previous treatment. To avoid this, we recommend adding the tissue sample to 20% glycerol or 20% DMSO and storing in liquid nitrogen. Frozen tissue need not be thawed before beginning the procedure.

Sample amounts

QIAGEN protocols are optimized for use with fixed cell densities, to match the capacity of the QIAGEN-tip used. Overloading tips with DNA from an excessive number of cells (too much tissue) will reduce the performance of the system. Yield of DNA and flow characteristics of the QIAGEN-tips depend on the number of cells in the sample and on the size of the genome.

Tissue samples may vary in the number of cells they contain, depending on the organ, the age of the donor, and the organism the sample came from. There is a good correlation between cell number and weight in different organs and amongst different mammalian species. Weighing the sample before starting the preparation is recommended.

Liver and spleen are transcriptionally very active organs. Therefore tissue samples derived from these organs have a very high protein and RNA content. For this reason, when preparing genomic DNA from liver or spleen, only 75–85% of the standard amount of starting material should be used. The table on the following page gives you maximum sample amounts to start with, which may be helpful.

Important notes before starting

- To obtain maximum purity and optimum flow rates it is very important not to overload the QIAGEN-tip 2500.
- All buffers must be equilibrated to room temperature before use.
- If using QIAGEN Protease in step 4, follow the instructions given on the label or product sheet for reconstitution before use.

Recommended maximum amounts of starting tissue

QIAGEN-tip	20/G	100/G	500/G	2500
maximum DNA yield	15–20 μ g	70–90 μ g	350–450 μ g	~2 mg
Heart	20 mg	100 mg	400 mg	2.00 g
Lung	20 mg	100 mg	400 mg	2.00 g
Muscle	20 mg	100 mg	400 mg	2.00 g
Brain	20 mg	100 mg	400 mg	2.00 g
Mouse tail	20 mg	100 mg	400 mg	2.00 g
Liver	15 mg	80 mg	350 mg	1.75 g
Spleen	15 mg	80 mg	350 mg	1.75 g

Procedure

This is the standard procedure for isolation of genomic DNA from different tissues (e.g., lung, liver, spleen, tumors, and mouse tails) with QIAGEN-tip 2500. When using QIAGEN Genomic-tips 20/G, 100/G, and 500/G, please follow the Tissue Protocol in the *QIAGEN Genomic DNA Handbook*.

1. Each preparation requires the following reagent amounts:

Reagent	Amount
Buffer G2	45 ml
Buffer QBT	35 ml
Buffer QC	100 ml
Buffer QF	35 ml
RNase A (100 mg/ml solution)	90 μ l
QIAGEN Protease or Proteinase K	2.5 ml

2. For each prep, add 90 μ l RNase A stock solution (100 mg/ml) to 45 ml Buffer G2 before use.

RNase A should be added to Buffer G2 to a final concentration of 200 μ g/ml and will be stable for 6 months when stored at 2–8°C.

- 3. Do not use more than 2 g of tissue (1.75 g of liver or spleen). Thoroughly homogenize the tissue mechanically in 45 ml of Buffer G2 (with RNase A) using a homogenizer (e.g., Ultra Turrax[®], Polytron[®]). Alternatively, homogenize tissue to a fine powder with liquid nitrogen in a precooled mortar and pestle before adding Buffer G2 (in step 4).**

For frozen tissue samples stored in 20% glycerol or 20% DMSO, pellet the sample by centrifugation, and discard the supernatant before homogenization.

Add the RNase A to Buffer G2 according to step 2 before adding the buffer to the tissue sample.

Take care to homogenize the tissue as thoroughly as possible. This step is critical for lysis and for a good flow rate through the QIAGEN-tip 2500.

- 4. Transfer homogenate or powdered tissue from Step 3 to a 50 ml screw-cap tube. Add 2.5 ml of QIAGEN Protease or Proteinase K solution to the homogenate, or 45 ml Buffer G2 and 2.5 ml of QIAGEN Protease or Proteinase K solution to the powdered tissue. Mix well by vortexing.**

Add the RNase A to Buffer G2 according to step 2 before adding the buffer to the ground tissue.

- 5. Incubate at 50°C for 2 h.**

Incubation time depends on how well the tissue sample was homogenized in Step 3. Lysates should be clear after incubation. If particulate matter can still be observed after 2 hours, extend the incubation time until the lysate is clear to avoid clogging the QIAGEN-tip 2500. Cellular debris can also be pelleted by centrifuging at 5000 x g, 4°C, for 10 min.

Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and potentially infectious viral proteins. The excess of proteolytic enzyme digests the denatured proteins into smaller fragments. Buffer G2 and proteolytic enzymes, in combination, strip the genomic DNA of all bound proteins, facilitating more efficient removal during purification.

RNase A eliminates RNA from the genomic DNA preparation.

Take a 250 µl aliquot and save it for an analytical gel (aliquot 1).

- 6. Equilibrate a QIAGEN-tip 2500 with 35 ml of Buffer QBT, and allow the QIAGEN-tip 2500 to empty by gravity flow.**

Place a QIAGEN-tip 2500 over a tube using a QIArack placed in a waste tray. Equilibrate the QIAGEN-tip 2500 with the volume of Buffer QBT described above. Flow begins immediately, due to the presence of detergent (0.15% Triton[®] X-100) in the equilibration buffer. Allow the QIAGEN-tip 2500 to drain completely. The flow of buffer will stop when the meniscus reaches the upper frit. The frit prevents the QIAGEN-tip 2500 from running dry, and allows it to be left unattended. Do not force out the remaining buffer, as this will necessitate restarting the flow with a syringe and adapter.

7. Vortex the sample from Step 5 for 5 s (at maximum speed) and apply onto the equilibrated QIAGEN-tip 2500. Allow it to enter the resin by gravity flow.

Vortexing genomic DNA has very little effect on its size, and accelerates the QIAGEN procedure by eliminating poor flow rates associated with clogging. DNA samples prepared with up to 20 seconds of vortexing show only a slight reduction in size from 50–150 kb (majority 80–100 kb) to 50–130 kb (majority 75–95 kb).

Once the QIAGEN-tip 2500 is loaded with the clear and particle-free sample, flow will begin unassisted. Allow gravity to determine the flow rate. The flow rate will depend on the number of cells from which the DNA was prepared and on the size distribution of the DNA.

Particularly concentrated genomic DNA lysates may flow more slowly due to increased viscosity. Flow can be assisted by the application of gentle positive pressure using a disposable syringe and the appropriate adapter. Also, it may be helpful to dilute the lysate with an equal volume of dilution buffer (e.g., Buffer QBT) prior to loading.

When using positive pressure, do not allow the flow rate to exceed 40–60 drops/min for QIAGEN-tip 2500.

Take a 250 µl aliquot and save it for an analytical gel (aliquot 2).

8. Wash the QIAGEN-tip 2500 with 2 x 50 ml of Buffer QC.

Allow Buffer QC to move through the QIAGEN-tip 2500 by gravity flow. Two washes are sufficient to remove all contaminants in the majority of DNA preparations. If necessary, a third wash may be performed.

Flow can be assisted by the application of gentle positive pressure using a disposable syringe and the appropriate adapter. When using positive pressure, do not allow the flow rate to exceed 40–60 drops/min for QIAGEN-tip 2500. It is particularly important not to force out residual wash Buffer QC. Traces of Buffer QC will not affect the elution step.

Take a 300 µl aliquot from the first 50 ml wash and save it for an analytical gel (aliquot 3).

9. Elute the genomic DNA with 35 ml of Buffer QF.

Place the QIAGEN-tip 2500 over a clean collection tube. Flow will begin immediately after the addition of Buffer QF. Drain the QIAGEN-tip 2500 by allowing it to empty by gravity flow. It is not necessary to force the remaining elution buffer from the QIAGEN-tip 2500.

Flow can be assisted by the application of gentle positive pressure using a disposable syringe and the appropriate adapter. When using positive pressure, do not allow the flow rate to exceed 40–60 drops/min for QIAGEN-tip 2500.

Take a 200 µl aliquot and save it for an analytical gel (aliquot 4).

10. Add 0.7 volumes of isopropanol (24.5 ml) previously equilibrated to room temperature. Spool out the DNA using a glass rod or precipitate the DNA by inverting the tube 10–20 times. Centrifuge at >5000 x g at 4°C for at least 15 min and carefully remove the supernatant.

Precipitation of DNA with isopropanol should be carried out with all solutions equilibrated to room temperature, to minimize salt precipitation.

5000 x g is the minimum g-force required for efficient precipitation. Higher g-force is recommended where possible. The sample is centrifuged at 4°C so that it does not overheat. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt containing pellets that result from ethanol precipitation. It is a good idea to mark the expected location of the pellet on the tube before centrifugation.

- 11. Immediately transfer spooled DNA to a microfuge tube containing 2–5 ml of the buffer of choice for resuspension. Alternatively, wash centrifuged DNA with 10 ml of cold 70% ethanol, air dry for 10 min, and resuspend in a suitable volume (2–5 ml) of the buffer of choice (e.g., TE, pH 8.0). Dissolve DNA overnight on a shaker or at 55°C for 1–2 hours.**

The DNA pellet should be vortexed briefly in 70% ethanol, and then re-pelleted by centrifugation. 70% ethanol serves to remove any residual salt, as well as to replace the isopropanol with the more volatile ethanol, making the DNA easier to redissolve. After careful and complete removal of the ethanol supernatant with a pipette, the pellet should be air-dried briefly (for approximately 10 min) before redissolving in a small volume of the buffer of choice (TE, pH 8.0). Overdrying the pellet will make the DNA very difficult to redissolve. Pipetting the DNA up and down to promote redissolving may cause shearing, and should be avoided. If glass tubes have been used for the precipitation, take care to wash down the walls of the tube with buffer, to recover all the DNA. If the DNA is difficult to dissolve, the solution may be too acidic. DNA dissolves best in slightly alkaline conditions (pH 8.0–8.5).

- 12. To determine the concentration and yield of DNA, take an aliquot of the sample and dilute it 1:5 in storage buffer. Calibrate the spectrophotometer using the same buffer and measure A_{260} and A_{280} , or make a scan from 220–320 nm.**

For pure DNA, the A_{260}/A_{280} ratio should be 1.7–1.9.

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Abbreviated procedure

1. If using QIAGEN Protease in step 3 below, follow the instructions given on the label or product sheet for reconstitution before use. Add 90 μ l RNase A to 45 ml Buffer G2, for each prep.
2. Do not use more than 2 g of tissue (1.75 g liver or spleen). Homogenize with a homogenizer in 45 ml of Buffer G2 (with RNase A), or grind to fine powder in liquid nitrogen with mortar and pestle.
3. Transfer homogenate or powdered tissue to a screw-cap tube. To the homogenate add 2.5 ml of QIAGEN Protease or Proteinase K. To the powdered tissue add 45 ml of Buffer G2 (with RNase A) and 2.5 ml of QIAGEN Protease or Proteinase K.
4. Incubate at 50°C for 2 h.
5. Equilibrate QIAGEN-tip 2500 with 35 ml of Buffer QBT.
6. Vortex sample for 3–5 s at maximum speed and apply onto the QIAGEN-tip 2500.
7. Wash with 2 x 50 ml of Buffer QC.
8. Elute with 35 ml of Buffer QF.
9. Add 24.5 ml of isopropanol. Spool DNA or centrifuge at >5000 x g for at least 15 min.
10. Wash, dry, and redissolve the DNA.

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