# Part II: Genomic-tip Protocol

## Protocol for Isolation of Genomic DNA from Blood, Cultured Cells, Tissue, Yeast, or Bacteria

**All samples must first be prepared according to the relevant Sample Preparation and Lysis Protocol (pages 17–43).**

Black denotes mini-prep (Genomic-tip 20/G) volumes, blue denotes midi-prep (Genomic-tip 100/G) volumes, red denotes maxi-prep (Genomic-tip 500/G) volumes.

1. **Equilibrate a QIAGEN Genomic-tip 20/G, Genomic-tip 100/G, or Genomic-tip 500/G with 1 ml, 4 ml, or 10 ml of Buffer QBT, and allow the QIAGEN Genomic-tip to empty by gravity flow.**

   Place a QIAGEN Genomic-tip over a tube using a tip holder or into a QIArack over the waste tray. Equilibrate the QIAGEN Genomic-tip with the volume of buffer described in the protocol. 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.

2. **Vortex the sample (from the last step of the specific sample preparation and lysis protocol) for 10 s at maximum speed and apply it to the equilibrated QIAGEN Genomic-tip. Allow it to enter the resin by gravity flow.**

   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. The average size of genomic DNA is reduced by only 10 kb when vortexed for up to 20 s (see Figure 3, page 12).

   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. The flow rate will depend on the sample source, the number of cells from which the DNA sample was prepared, and on genome size.

   Particularly concentrated genomic DNA lysates may exhibit diminished flow rates due to increased viscosity. Flow can be assisted by the application of gentle positive pressure using a disposable syringe and the appropriate adapter. It might also be helpful to dilute the lysate with an equal volume of Buffer QBT prior to loading.

When using positive pressure, do not allow the flow rate to exceed 4–10 drops/min, 10–20 drops/min, or 20–40 drops/min.

☞ *Take a 300 µl, 300 µl, or 150 µl aliquot and save it for an analytical gel (aliquot 2).*

3. **Wash the QIAGEN Genomic-tip with 3 x 1 ml, 2 x 7.5 ml, or 2 x 15 ml of Buffer QC.**

   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. An additional wash is occasionally necessary if large culture volumes or bacterial strains containing large amounts of carbohydrate are used.

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

   ☞ *Take a 1200 µl, 600 µl, or 300 µl aliquot and save it for an analytical gel (aliquot 3).*

4. **Elute the genomic DNA with 2 x 1 ml, 1 x 5 ml, or 1 x 15 ml of Buffer QF.**

   Place the QIAGEN Genomic-tip over a clean 10 ml, 10 ml, or 30 ml collection tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps. Elute with the appropriate volume of Buffer QF, and collect the eluate. Flow begins automatically. Allow the QIAGEN Genomic-tip to drain by gravity flow.

   Use of Buffer QF prewarmed to 50°C will increase yields.

   ☞ *Take a 600 µl, 300 µl, or 120 µl aliquot and save it for an analytical gel (aliquot 4).*

5. **Precipitate the DNA by adding 1.4 ml, 3.5 ml, or 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Recover the precipitated DNA as described in step 5A or step 5B.**

5A. **Precipitate the DNA by inverting the tube 10 to 20 times, and spool the DNA using a glass rod. Proceed with step 6A.**

5B. **Alternatively, mix and centrifuge immediately at >5000 x *g* for at least 15 min at 4°C. Carefully remove the supernatant. Proceed with step 6B.**

   All solutions should be at room temperature in order 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. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

**6A. Immediately transfer the spooled DNA to a microcentrifuge tube containing 0.1–2 ml of a suitable buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5). Dissolve the DNA overnight on a shaker or at 55°C for 1–2 h.**

**6B. Wash the centrifuged DNA pellet with 1 ml, 2 ml, or 4 ml of cold 70% ethanol. Vortex briefly and centrifuge at >5000 x *g* for 10 min at 4°C. Carefully remove the supernatant without disturbing the pellet. Air-dry for 5–10 min, and resuspend the DNA in 0.1–2 ml of a suitable buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5). Dissolve the DNA overnight on a shaker or at 55°C for 1–2 h.**

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.

After careful and complete removal of the ethanol supernatant with a pipet, the pellet should be air-dried briefly before resuspending in a small volume of suitable buffer. Overdrying the pellet will make the DNA difficult to redissolve. Resuspend the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. DNA dissolves best under slightly alkaline conditions (pH 8.0–8.5) and does not dissolve easily in acidic buffers.

### Determination of yield, purity, and length of the DNA

Yields of genomic DNA will depend on the number of cells and the capacity of the QIAGEN Genomic-tip used. DNA yield is usually determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: for example, an eluate containing 25–50 ng of DNA/µl ($A_{260}$ = 0.5–1) should not be diluted with more than four volumes of buffer. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 nm and 280 nm, or scan absorbance from 220–320 nm. (A scan will show whether there are other factors affecting absorbance at 260 nm.) Readings on a spectrophotometer are not always accurate, particularly if a single wavelength measurement is taken rather than a scan, and should always be verified by visual examination of the DNA on an agarose gel. Fluorimetric measurements are more accurate and should be used if precise concentrations are needed.

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an $A_{260}/A_{280}$ ratio of 1.7–1.9.

The precise length of genomic DNA is determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. Standard PFGE conditions are as follows: 1.5% agarose gel in 0.5x TBE electrophoresis buffer; switch intervals, 1–10 s; run time, 16 h; voltage, 170 V.

## Analytical gel

To analyze the purification procedure or to find where a problem may have occurred if yields are low, take a proportional aliquot of each of the samples marked in the specific protocol. Precipitate each of the aliquots 1–4 with 0.7 volumes of isopropanol. Rinse the pellets with 70% ethanol, drain well, and resuspend in 20 µl of TE, pH 8.0. Add the appropriate loading buffer, and use 10 µl of the samples for analysis on a 0.5% agarose gel. Run the gel until the bromophenol blue is near the bottom, and stain it briefly in an ethidium bromide solution. An example of an analytical gel can be found in Figure 6 below.

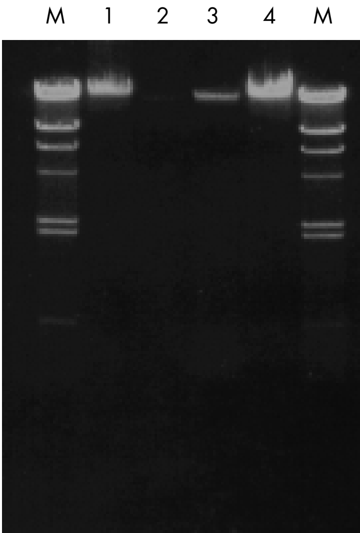


***Figure 6.*** *Agarose gel analysis of the genomic DNA purification procedure.* ***1:*** *nuclear lysate;* ***2:*** *flow-through fraction;* ***3:*** *wash fraction;* ***4:*** *eluate containing pure genomic DNA.* ***M:*** *markers.*