

# Part I: Sample Preparation and Lysis Protocols

## Blood

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.

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg, 90 µg, or 400 µg of genomic DNA from up to 1 ml, 5 ml, or 20 ml of whole blood or  $5 \times 10^5$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  lymphocytes or buffy-coat cells (leukocyte-rich blood concentrate). The purified genomic DNA ranges from 20 to 150 kb in size.

### Storage of blood samples

Storage of blood samples without previous treatment leads to reduced yields of DNA. For best results, use one of the following procedures.

- For short-term storage (up to 3 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 4°C.

Two alternatives are available for long-term storage:

- Perform steps 1–5 of the protocol beginning on page 20, and then freeze the nuclear pellet at –20°C. When ready to complete the purification, continue with step 6.
- Collect blood in tubes containing a standard anticoagulant and store tubes at –70°C. Frozen blood should be thawed in a 37°C water bath with mild agitation before beginning the procedure.

### Recommended cell densities

Blood is a complex mixture of cells, proteins, metabolites, and many other substances. About 56% of human blood volume is comprised of cells, more than 99% of which are erythrocytes. Human erythrocytes and thrombocytes (platelets, 0.5% of blood components) do not contain nuclei and are therefore unsuitable for preparation of genomic DNA. The only blood cells that contain nuclei are leukocytes (0.3% of cellular blood components).

Blood samples may vary widely in the number of leukocytes they contain, depending on the health of the donor. Healthy blood, for example, contains fewer than  $10^7$  leukocytes per ml, while blood from an infected donor may have a tenfold higher leukocyte concentration. Although a QIAGEN Genomic-tip 100/G (capacity = 100 µg genomic DNA) can handle up to 5 ml of healthy whole blood, it would be overloaded if more than 0.5 ml of blood from an unhealthy donor were used.

**A. Isolation of genomic DNA from whole blood of “healthy” donors (leukocyte concentrations from  $5 \times 10^6$ /ml to  $1 \times 10^7$ /ml)**

Ensure that there are no more than  $1 \times 10^7$  leukocytes per ml of blood, and use no more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  total leukocytes in your sample. Place sample in a 10 ml or 50 ml screw-cap tube or in two 50 ml screw-cap tubes. Proceed with protocol, beginning on page 20.

**B. Isolation of genomic DNA from whole blood of “unhealthy” donors**

**1. Leukocyte concentrations higher than  $1 \times 10^7$ /ml**

Ensure that there are no more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  leukocytes in your sample. Using PBS, adjust the volume to 1 ml, 5 ml, or  $2 \times 10$  ml in a 10 ml or 50 ml screw-cap tube or in two 50 ml screw-cap tubes. Proceed with protocol, beginning on page 20.

**2. Leukocyte concentrations lower than  $5 \times 10^6$ /ml**

Ensure that the blood sample volume contains at least  $1 \times 10^6$ ,  $5 \times 10^6$ , or  $2.5 \times 10^7$  leukocytes in total. Pellet the cells by centrifugation (15 min,  $1000 \times g$ ). Resuspend the sample in 0.1 ml, 1 ml, or 5 ml of PBS in a 10 ml, 10 ml, or 50 ml screw-cap tube. Proceed with protocol, beginning on page 20.

**C. Isolation of genomic DNA from buffy-coat preparations (do not use more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  buffy-coat cells)**

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and will yield approximately 5–10 times more DNA than an equivalent volume of blood.

Prepare buffy coat by centrifuging whole blood for 10 min at  $3300 \times g$  at room temperature. After centrifugation, three different fractions are obtained: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

Do not use more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  buffy-coat cells.

Using PBS, adjust the volume to 1 ml, 5 ml, or  $2 \times 10$  ml in a 10 ml or 50 ml screw-cap tube or in two 50 ml screw-cap tubes. Mix. Proceed with protocol, beginning on page 20.

**D. Isolation of genomic DNA from lymphocyte preparations (do not use more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  lymphocytes)**

**1. Ficoll gradient preparation of lymphocytes**

Use a common Ficoll® gradient product (lymphocyte-separation medium). Warm the lymphocyte-separation medium to room temperature before use.

For details of the procedure, consult the package insert provided with the lymphocyte separation medium.

Use no more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  total lymphocytes in your sample. Resuspend the sample in 1 ml, 5 ml, or  $2 \times 10$  ml of PBS in a 10 ml or 50 ml screw-cap tube or in two 50 ml screw-cap tubes. Proceed with protocol, beginning on page 20.

**2. Dextran sedimentation**

To 1 ml, 5 ml, or 20 ml of whole blood add 0.35 ml, 1.7 ml, or 6.6 ml of an aqueous solution of 6% Dextran (MW: 250,000; Sigma; not included in the kit). Mix well.


Put the tube in a rack and allow sedimentation to take place for 45 min. (Keeping the tube tilted will accelerate the sedimentation). Carefully remove the lymphocyte-rich supernatant to avoid contaminating it with the erythrocyte-rich top layer or the sediment. Use an aliquot with no more than  $5 \times 10^6$ ,  $2 \times 10^7$ , or  $1 \times 10^8$  total lymphocytes. Using PBS, adjust the volume to 1 ml, 5 ml, or  $2 \times 10$  ml in a 10 ml or 50 ml screw-cap tube or in two 50 ml screw-cap tubes. Proceed with protocol, beginning on page 20.

## Sample Preparation and Lysis Protocol for Blood

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.

This is the standard sample preparation and lysis protocol for whole blood, buffy-coat cells, and lymphocytes, prior to genomic DNA isolation. Part II, the Genomic-tip protocol, begins on page 44

### Important notes before starting

- Please read subsection A, B, C, or D of this procedure on pages 18–19 to determine how to prepare the sample and the appropriate amount to use.
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Please refer to the section on cell densities (pages 17–19).
- Equilibrate Buffer C1 and distilled water to 4°C. Buffer C1 should always be stored at 2–8°C.
- Equilibrate all other buffers to room temperature (15–25°C). These buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, a solution of QIAGEN Protease should be carefully prepared. See step 2 for details of preparation. Alternatively, QIAGEN Proteinase K solution may be used directly.
- Steps 3–7 of the protocol are carried out in standard 10 ml, 50 ml, or 50 ml screw-cap tubes.
- If using frozen blood or blood which has been stored at 2–8°C, please refer to the section on storage of blood samples (page 17).
- Optional: remove aliquots at the steps indicated with the symbol  in order to monitor the procedure on an analytical gel (page 47).

## Reagents required per prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
C1 (2–8°C)	1.25 ml	6 ml	22 ml
G2	1 ml	5 ml	10 ml
QBT	2 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
Distilled water (2–8°C)	3.75 ml	18 ml	66 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
QIAGEN Protease or Proteinase K stock solution	25 µl	95 µl	200 µl

1. **Prepare Buffers C1, G2, QBT, QC, and QF according to the instructions on pages 56–57. Alternatively, the Genomic DNA Buffer Set or the QIAGEN Blood & Cell Culture DNA Kit can be used (for ordering information see page 61).**
2. **Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See pages 57–58 for further details and page 63 for ordering information.

Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 6.

3. **Use 0.1–1 ml, 1–5 ml, or 5–20 ml whole blood or 1 ml, 5 ml, or 20 ml of buffy-coat or lymphocyte suspension (pages 18–19). Add 1 volume (0.1–1 ml, 1–5 ml, or 5–20 ml) of ice-cold Buffer C1 and 3 volumes of ice-cold distilled water (0.3–3 ml, 3–15 ml, or 15–60 ml). Mix by inverting the tube several times until the suspension becomes translucent. Incubate for 10 min on ice.**

Buffer C1 and distilled water must be cold. Keep on ice during use.

Buffer C1 lyses the cells but stabilizes and preserves the nuclei. Erythrocytes lyse first, releasing the hemoglobin and making the suspension translucent. Frozen blood samples do not visibly change upon lysis.

**4. Centrifuge the lysed blood at 4°C for 15 min at 1300 x g. Discard the supernatant.**

After centrifugation, the small, nuclear pellet is still slightly red due to residual hemoglobin, which is removed in the next step. Centrifugation in a swing-out rotor will make the pellet easier to see.

**5. Add 0.25 ml, 1 ml, or 2 ml of ice-cold Buffer C1 and 0.75 ml, 3 ml, or 6 ml of ice-cold distilled water. Resuspend the pelleted nuclei by vortexing. Centrifuge again at 4°C for 15 min at 1300 x g. Discard the supernatant.**

This wash step removes all residual cell debris and hemoglobin from the nuclear pellet. If the pellet is not white, repeat the wash.

At this point the pellet may be frozen and stored at -20°C if desired. When ready to complete the purification procedure, continue with step 6 of the protocol. The yield of DNA will be the same as for fresh blood samples.

**6. Add 1 ml, 5 ml, or 10 ml of Buffer G2, and completely resuspend the nuclei by vortexing for 10–30 s at maximum speed.**

Resuspend the nuclei as thoroughly as possible by vortexing. This step is critical for a good flow rate on the QIAGEN Genomic-tip.

**7. Add 25 µl, 95 µl, or 200 µl of QIAGEN Protease or Proteinase K stock solution, and incubate at 50°C for 30–60 min.**

See step 2 for preparation of stock solutions.

The length of incubation depends on how well the nuclei were resuspended in step 6. If the suspension is not homogeneous after vortexing, a full 60 min incubation is recommended to avoid clogging the QIAGEN Genomic-tip.

Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and viral particles. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification. It is important that the lysate becomes clear at this stage. If necessary, extend the incubation time, or pellet the particulate matter by centrifugation for 10 min at 5000 x g, 4°C.

☞ Take a 300 µl, 300 µl, or 150 µl aliquot and save it for an analytical gel (aliquot 1).

**8. Proceed with Part II, the Genomic-tip protocol, on page 44.**

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.