Quick Protocol for Extraction and Purification of Genomic DNA

Overview

We recommend that first-time users of this kit review the product manual before starting; it provides additional information to consider at various steps. This quick protocol is meant for experienced users. The manual also contains protocols for reaction cleanup and extraction of gDNA from additional sample types.

Before You Begin:

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the gDNA Wash Buffer concentrate as indicated on the bottle label.
- Set a thermal mixer (e.g. ThermoMixer®) or, if not available, a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35-100 µl per sample).
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Genomic DNA Extraction Consists of Two Stages:

PART 1: Sample Lysis

PART 2: Genomic DNA Binding and Elution

PART 1: SAMPLE LYSIS

Cultured Cells:

1. Start with a cell pellet containing 1 x 10^4 – 5 x 10^6 cells (typical starting amount is 1 x 10^6 cells).

   Frozen cells: thaw cell pellet slowly on ice and loosen by flicking the tube several times. Resuspend in 100 µl cold PBS by pipetting up and down.

   Fresh cells: pellet by centrifugation at 1000 x g for 1 minute and resuspend in 100 µl cold PBS by pipetting up and down. Ensure pellet is resuspended completely. If using lower cell inputs, the use of carrier RNA may be beneficial; see product manual for details.

2. Add 1 µl Proteinase K and 3 µl RNase A to the resuspended pellet and mix by vortexing briefly to ensure the enzymes are efficiently dispersed. Do not add the enzymes and the Cell Lysis Buffer simultaneously, as the high viscosity of the lysate will prevent equal distribution of the enzymes. Addition of RNase A can be omitted if a low percentage of co-purified RNA will not affect downstream applications.

3. Add 100 µl Cell Lysis Buffer and vortex immediately and thoroughly. The solution will rapidly become viscous.

4. Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm). Incubation for longer than 5 minutes is not necessary, but will not negatively affect the quality of the purified gDNA. If a thermal mixer is not available, use a heating block and vortex occasionally.

5. Proceed to step 1 of Part 2: Genomic DNA Binding and Elution.

Mammalian Whole Blood (non-nucleated)

1. Transfer 100 µl of whole blood to a 1.5 ml microfuge tube. If processing less than 100 µl of blood, add cold PBS to bring the total volume to 100 µl. For pre-aliquoted frozen samples, do not thaw; add Proteinase K, RNase A and Blood Lysis Buffer to the frozen sample in step 2.

2. Add 10 µl Proteinase K, 3 µl RNase A and 100 µl of Blood Lysis Buffer. Mix immediately by vortexing. For frozen samples, do not thaw; add reagents and lysis buffer to frozen sample and immediately proceed to step 3. When working with multiple samples, prepare a master mix of the three reagents to save pipetting steps. Addition of RNase A can be omitted if a low percentage of co-purified RNA will not affect downstream applications.

3. Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm). Incubation for longer than 5 minutes is not necessary, but will not negatively affect the quality of the purified gDNA from most species. If a thermal mixer is not available, use a heating block and vortex occasionally.

4. Proceed to step 1 of Part 2: Genomic DNA Binding and Elution.

Nucleated Whole Blood (birds, reptiles)

1. Transfer 10 µl whole blood to a 1.5 ml microfuge tube.

2. Add 90 µl cold PBS and mix by vortexing.

3. Add 10 µl Proteinase K and 3 µl RNase A, and mix again by vortexing. Do not add the enzymes and the Blood Lysis Buffer simultaneously, as the high viscosity of the lysate will prevent equal distribution of the enzymes. Addition of RNase A can be omitted if a low percentage of co-purified RNA will not affect downstream applications.

4. Add 100 µl Blood Lysis Buffer and vortex thoroughly. The solution will rapidly become viscous.

5. Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm). Incubation for longer than 5 minutes is not necessary, but will not negatively affect the quality of the purified gDNA.

6. Proceed to step 1 of Part 2: Genomic DNA Binding and Elution.

Animal Tissue

1. Cut tissue into small pieces to ensure rapid lysis and high yields. Weigh the appropriate tissue amount and place in a 1.5 ml microfuge tube (see table below or Choosing Input Amounts for recommended input amounts)

STARTING MATERIAL	RECOMMENDED INPUT AMOUNT
Rodent tail	up to 25 mg
Brain	up to 12 mg
Fibrous tissue (muscle, heart)	up to 25 mg
Ear clips, skin	up to 10 mg
Liver, lung	up to 15 mg
Spleen, kidney	up to 10 mg

2. Add Proteinase K (according to the table below) and 200 µl of Tissue Lysis Buffer to each sample. Mix immediately by vortexing. Ensure tissue particles are able to move freely in the lysis mix and do not remain stuck on the bottom of the tube. When working with multiple samples, prepare a master mix of Tissue Lysis Buffer and Proteinase K to save pipetting steps.

TISSUE TYPE	PROTEINASE K AMOUNT
Brain, Kidney, Skin, Ear Clips	3 µl
All other tissues	10 µl

3. Incubate at 56°C in a thermal mixer with agitation at full speed (1400 rpm) until tissue pieces have completely dissolved (typically 30-60 minutes). If time is not limiting, additional incubation up to 3 hours can further improve yields and decrease residual RNA. If an incubator with agitation is not available, use a tube rotator placed within an incubator, shaking water bath or a heating block (vortex samples every 5-15 minutes to speed up lysis).

4. [Note: The following step can be omitted when working with fresh or frozen tissue amounts <15 mg. Centrifuge for 3 minutes at maximum speed (12,000 x *g*) to pellet debris. Transfer the supernatant to a fresh microfuge tube. This prevents residual debris from clogging the membrane binding sites and helps to reach maximal yield and purity. It is especially important to perform this step if sample appears turbid, contains residual particles, when working with stabilized tissue, or when working with brain or fibrous tissues.]

5. Add 3 µl of RNase A to the lysate, vortex thoroughly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed. This step can be skipped if a low percentage of co-purified RNA will not affect downstream applications.

6. Proceed to step 1 of Part 2: Genomic DNA Binding and Elution.

PART 2: GENOMIC DNA BINDING AND ELUTION

1. **Add 400 µl gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for 5-10 seconds.** Thorough mixing is essential for optimal results.

2. **Transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area. Proceed immediately to step 3.** Do not reload the same column with more sample; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge. Avoid touching the upper column area with lysate/binding mix and avoid transferring foam that may have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate.

3. **Close the cap and centrifuge: first for 3 minutes at 1,000 x *g* to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for 1 minute at maximum speed (> 12,000 x *g*) to clear the membrane. Discard the flow-through and the collection tube.** For optimal results, ensure that the spin column is placed in the centrifuge in the same orientation at each spin step (for example, always with the hinge pointing to the outside of the centrifuge); ensuring the liquid follows the same path through the membrane for binding and elution can slightly improve yield and consistency.

4. **Transfer column to a new collection tube and add 500 µl gDNA Wash Buffer. Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge immediately for 1 minute at maximum speed (12,000 x *g*), and discard the flow through.** The collection tube can be tapped on a paper towel to remove any residual buffer before reusing it in the next step. Inverting the spin column containing wash buffer prevents salt contamination in the eluate.

5. **Reinsert the column into the collection tube. Add 500 µl gDNA Wash Buffer and close the cap. Centrifuge immediately for 1 minute at maximum speed (>12,000 x *g*), then discard the collection tube and flow through.**

6. **Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included). Add 35-100 µl preheated (60°C) gDNA Elution Buffer, close the cap and incubate at room temperature for 1 minute.** Elution in 100 µl is

recommended, but smaller volumes can be used and will result in more concentrated DNA but a reduced yield (20–25% reduction when using 35 µl). Eluting with preheated elution buffer will increase yields by ~20–40% and eliminates the need for a second elution. For applications in which a high DNA concentration is required, using a small elution volume and then eluting again with the eluate may increase yield (~10%). The elution buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA) offers strong protection against enzymatic degradation and is optimal for long term storage of DNA. However, other low-salt buffers or nuclease-free water can be used if preferred. For more details on optimizing elution, please refer to "Considerations for Elution & Storage" in the product manual.

7. **Centrifuge for 1 minute at maximum speed (> 12,000 x *g*) to elute the gDNA.**

Additional Resources you may find helpful:

- Monarch Spin gDNA Extraction Kit Product Manual
- Choosing Input Amounts for the Monarch Spin gDNA Extraction Kit
- Troubleshooting Guide for Genomic DNA Extraction & Purification
- Factors Affecting DNA Quality when Purifying gDNA from Blood and Tissues with the Monarch Spin gDNA Extraction Kit
- Guidelines for Handling Tissue Samples when using the Monarch Spin gDNA Extraction Kit

Have any questions?

Our tech support scientists would be happy to help. Email us at info@neb.com.