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Quick Protocol for Extraction and Purification of Genomic DNA

New England Biolabs[1]

[1]New England Biolabs

2 *Works for me* This protocol is published without a DOI.

New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

Isabel Gautreau
New England Biolabs

ABSTRACT

Genomic DNA Purification Consists of Two Stages:

- Part 1: Sample Lysis
- Part 2: Genomic DNA Binding and Elution

EXTERNAL LINK

https://neb.com/protocols/2019/04/30/quick-protocol-for-extraction-and-purification-of-genomic-dna-using-the-monarch-genomic-dna-purification-kit-neb-t3010

PROTOCOL CITATION

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OWNERSHIP HISTORY

Sep 27, 2019	Anita Broellochs	protocols.io
Jun 20, 2020	New England Biolabs Tech Support	New England Biolabs
Jun 23, 2020	Isabel Gautreau	New England Biolabs

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GUIDELINES

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.

MATERIALS

NAME	CATALOG #	VENDOR
Monarch® Genomic DNA Purification Kit	T3010	New England Biolabs

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

- 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).

PART 1: SAMPLE LYSIS

1 Please select the sample material that you want to use:
- Cultured Cells
- Mammalian Whole Blood (non-nucleated)
- Nucleated Whole Blood (birds, reptiles)
- Animal Tissue

Step 1 includes a Step case.

Cultured Cells

Mammalian Whole Blood (non-nucleated)

Nucleated Whole Blood (birds, reptiles)

Animal Tissue

step case

Cultured Cells

2 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 **00:01:00** 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.

3 

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.

4 

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

5

Incubate for **00:05:00** 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.

PART 2: GENOMIC DNA BINDING AND ELUTION

6

Add **400 µl** gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for **00:00:05** – **00:00:10** .

Thorough mixing is essential for optimal results.

7 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 8.

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.

8

Close the cap and centrifuge: first for **00:03:00** at **1000 x g** to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for **00:01:00** at maximum speed (> **12000 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.

9

Transfer column to a new collection tube and add **500 µl** gDNA Wash Buffer.

10

Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge immediately for **00:01:00** at maximum speed (**12000 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.

11

Reinsert the column into the collection tube. Add **500 µl** gDNA Wash Buffer and close the cap. Centrifuge immediately for **00:01:00** at maximum speed (> **12000 x g**), then discard the collection tube and flow through.

12 Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included).

13

Add **35 µl** – **100 µl** preheated (**60 °C**) gDNA Elution Buffer, close the cap and incubate at **Room temperature** for **00:01:00** .

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.

14

Centrifuge for **00:01:00** at maximum speed (> **12000 x g**) to elute the gDNA.