

Protocol for Monarch Genomic DNA Extraction (NEB #T3010)

Part 1: Sample Lysis - Buccal Swabs

Overview

This protocol facilitates the gDNA extraction from various types of buccal swabs.

Before You Begin:

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS (not supplied) is required.
- If available, a microcentrifuge pre-chilled to 4°C is recommended.
- Set a thermal mixer (e.g. ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- 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 Preparation

PART 2: Genomic DNA Binding and Elution

Protocol

1. Add 1 ml cold PBS to 1.5 ml reaction tube.
2. Scrape inside of cheek with commercial collection device (according to manufacturer's recommendations).
3. Cut off / break off buccal swab cell collection piece and place in the tube with the PBS.
4. Vortex for 10 seconds to release cell material, then remove swab from the PBS.
5. Pellet cellular material by centrifugation for 30 seconds (2000 x g) at 4°C if possible.
6. With a pipette, remove most of the PBS, leaving ~100 µl of PBS behind with the cell pellet.

7. Resuspend the pellet by vortexing.
8. Add 10 μ l Proteinase K and 3 μ l RNase A, and vortex briefly.
9. Add 100 μ l Cell Lysis Buffer, vortex briefly, then incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed (2000 rpm, or maximum speed available).
10. [Proceed to Part 2: Genomic DNA Binding and Elution.](#)

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](#)