Protocol for Monarch Genomic DNA Extraction (NEB #T3010) Part 1: Sample Lysis - Gram-positive Bacteria using NEBExpress T4 Lysozyme (NEB #P8115)

Overview

This protocol enables the genomic DNA extraction from up to 2 x 10^9 gram-positive bacteria. This protocol can successfully extract genomic DNA from *B.subtilis* and *B.thuringiensis*, but needs to be tested for other gram-positive bacteria on case-by-case basis.If the Gram positive bacterial species of interest cannot be lysed with NEBExpress® T4 lysozyme, consider using other lysing enzymes such as lysostaphin, labiase, mutanolysin or achromopeptidase.

Materials needed:

- 50 mM Tris-HCl, 0.5 mM EDTA pH 7.5 is required (not supplied). Alternatively, TE Buffer can be used.
- NEBExpress T4 Lysozyme (NEB #P8115)

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.
- 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 Lysis

PART 2: Genomic DNA Binding and Elution

Part 1: Sample Lysis

1. **Harvest a maximum of up to 2 x 10^9 gram-positive bacteria by centrifugation for 1 minute at > 12,000 x g. Discard supernatant.**

2. **Add 100 µl of 50 mM Tris-Cl, 0.5 mM EDTA pH 7.5 (or TE Buffer) and resuspend bacterial pellet by pipetting or vortexing.**

3. **Add 10 µl T4 Lysozyme (NEB #P8115) and mix by pipetting or vortexing briefly. Incubate at room temperature for 5 minutes.** Lysates may remain turbid to some degree.

4. **Add 10 µl Proteinase K and 100 µl Tissue Lysis Buffer and vortex briefly. Incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed (1400 rpm or 2000 rpm if available).** If lysates were not clear after T4 Lysozyme incubation, they will soon become clear after adding the Proteinase K and Tissue Lysis Buffer if T4 Lysozyme works for the respective bacteria species. If a thermal mixer is not available, vortex samples several times during incubation and continuously for 1 minute at the end of the incubation.

5. **Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (1400 rpm or 2000 rpm if available).**

6. **Proceed to Part 2: Genomic DNA Binding and Elution.**