# Monarch® Plasmid Miniprep Kit from NEB

- Pellet 1 – 5 mL bacterial culture by centrifugation for 30 seconds. Discard supernatant.
- Resuspend pellet in 200 µL **Plasmid Resuspension Buffer (B1)**. Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.
- Add 200 µL **Plasmid Lysis Buffer (B2)**, gently invert tube 5 – 6 times, and incubate at room temperature for 1 minute. Color should change to dark pink, and solution will become transparent and viscous. Do not vortex.
- Add 400 µL of **Plasmid Neutralization Buffer (B3)**, gently invert tube until neutralized, and incubate at room temperature for 2 minutes. Sample is neutralized when color is uniformly yellow and precipitate forms. Do not vortex.
- Centrifuge lysate for 2 – 5 minutes. For culture volumes > 1 mL, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Pellet should be compact; spin longer if needed.
- Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow through.
- Re-insert column in the collection tube and add 200 µl of **Plasmid Wash Buffer 1**. Centrifuge for 1 minute. Discarding the flow through is optional.
- Add 400 µl of **Plasmid Wash Buffer 2** and centrifuge for 1 minute.
- Transfer column to a clean 1.5 mL microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow through. If there is any doubt, re-spin the column for 1 minute.

- Add ≥ 30 µL **DNA Elution Buffer** to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA, (≥ 10 kb), heating the elution buffer to 50 °C prior to use can improve yield.

From: New England BioLabs Inc.