MINIPREP KIT

- Reagents:
  - Miniprep kit
  - LB medium
  - Agar medium LB
  - eppendorf tubes 1.5 mL
- Material:
  - Micropipettes 1000 uL, 100 uL, 10 uL
  - Pipette tips 1000 uL, 100 uL, 10 uL

- Equipment:
  - Centrifugation

3.A. Preparation of E. coli

1. Use a single, well isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) to inoculate 1–10ml of LB medium (containing the same antibiotic). We recommend LB culture medium. Rich media, such as Terrifi c Broth, produce high cell densities that may overload the DNA purifi cation system.

2. Incubate overnight (12–16 hours) at 37°C in a shaking incubator. Incubation time can be optimized to increase the plasmid DNA yield for a given culture volume. However, it has been observed that as a culture ages the DNA yield may begin to decrease due to cell death and lysis within the culture.

Note: An A600 reading of 2–4 ensures that cells have reached the proper growth density for harvesting and plasmid DNA isolation.

For high-copy-number plasmids, do not process more than 5ml of bacterial culture. If more than 5ml of culture is processed, the capacity of the Wizard® SV Minicolumn will be exceeded and no increase in plasmid yield will be obtained.

For low-copy-number plasmids, it may be necessary to process larger volumes of bacterial culture (up to 10ml) for recovery of suffi cient DNA. Processing greater than 10ml of culture will lead to insuffi cient clearing of the bacterial lysate and thus increased contaminants in the plasmid DNA.

3.B. Production of a Cleared Lysate

Note: Throughout the remainder of this document, the supplied Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA), Neutralization Solution (NSB) and Column Wash Solution (CWA) are referred to as Cell Resuspension

Solution, Cell Lysis Solution, Neutralization Solution and Column Wash Solution, respectively.

1. Harvest 1–5ml (high-copy-number plasmid) or 10ml (low-copy-number plasmid) of bacterial culture by centrifugation for 5 minutes at 10,000 x g in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.

2. Add 250µl of Cell Resuspension Solution and completely resuspend the cell pellet by vortexing or pipetting. It is essential to thoroughly resuspend the cells. If they are not already in a microcentrifuge tube, transfer the resuspended cells to a sterile 1.5ml microcentrifuge tube(s).

Note: To prevent shearing of chromosomal DNA, do not vortex after Step 2. Mix only by inverting the tubes.

3. Add 250µl of Cell Lysis Solution and mix by inverting the tube 4 times (do not vortex). Incubate until the cell suspension clears (approximately 1–5 minutes).

Note: It is important to observe partial clearing of the lysate before proceeding to addition of the Alkaline Protease Solution (Step 4); however, do not incubate for longer than 5 minutes.

4. Add 10µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for 5 minutes at room temperature.

Alkaline protease inactivates endonucleases and other proteins released during the lysis of the bacterial cells that can adversely aff ect the quality of the isolated DNA. **Do not exceed 5 minutes of incubation with Alkaline Protease** Solution at Step 4, as nicking of the plasmid DNA may occur.

5. Add 350µl of Neutralization Solution and immediately mix by inverting the tube 4 times (do not vortex).

6. Centrifuge the bacterial lysate at maximum speed (around 14,000 × g) in a microcentrifuge for 10 minutes at room temperature.

Centrifugation Protocol

Prepare plasmid DNA purifi cation units by inserting one Spin Column into one 2ml Collection Tube for each sample.

1. Transfer the cleared lysate (approximately 850µl, Section 3.B, Step 6) to the prepared Spin Column by decanting. Avoid disturbing or transferring any of the white precipitate with the supernatant.

Note: If the white precipitate is accidentally transferred to the Spin Column, pour the Spin Column contents back into a sterile 1.5ml microcentrifuge tube and centrifuge for another 5–10 minutes at maximum speed. Transfer the resulting supernatant into the same Spin Column that was used initially for this sample. The Spin Column can be reused but only for this sample.

2. Centrifuge the supernatant at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the fl owthrough from the Collection Tube. Reinsert the Spin Column into the Collection Tube.

3. Add 750µl of Column Wash Solution, previously diluted with 95% ethanol, to the Spin Column.
4. Centrifuge at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the fl owthrough. Reinsert the Spin Column into the Collection Tube.
5. Repeat the wash procedure using 250µl of Column Wash Solution
6. Centrifuge at maximum speed in a microcentrifuge for 2 minutes at room temperature.
7. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at maximum speed. 8. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube.
9. Elute the plasmid DNA by adding 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at maximum speed for 1 minute at room temperature in a microcentrifuge.
10. After eluting the DNA, remove the assembly from the 1.5ml microcentrifuge tube and discard the Spin Column.
11. DNA is stable in water without addition of a buff er if stored at –20°C or below. DNA is stable at 4°C in TE buff er. To store the DNA in TE buff er, add 11µl of 10X TE buff er to the 100µl of eluted DNA. Do not add TE buff er if the DNA is to be used for automated fl uorescent sequencing.
12. Cap the microcentrifuge tube and store the purifi ed plasmid DNA at –20°C or below.