Plasmid preparation

Plasmid preparation was performed using the Zyppy™ Plasmid Miniprep Kit from Zymo Research.

1. Centrifuge 1.5 mL of bacterial culture for 30 seconds at maximum speed.
2. Discard the supernatant and repeat step 1.
3. Add 600 µL of TE or water to the bacterial cell pellet and resuspend completely.
4. Add 100 µL of 7x Lysis Buffer, mix by inverting the tube 4 to 6 times, and incubate for 1-2 minutes. Do not vortex.
5. Add 350 µL of cold Neutralization Buffer and mix thoroughly. Do not vortex.
6. Centrifuge for 2 to 4 minutes at 11,000x g.
7. Place a Zymo-Spin™ IIN column in a Collection Tube and transfer the supernatant from step 6 into the Zymo-Spin™ IIN column.
8. Centrifuge the Zymo-Spin™ IIN for 15 seconds at 11,000x g.
9. Discard the flow-through and return the Zymo-Spin™ IIN column to the same Collection Tube.
10. Add 200 µL of Endo-Wash Buffer to the column and centrifuge for 30 seconds at 11,000x g.
11. Add 400 µL of Zyppy™ Wash Buffer to the column and centrifuge for 1 minute at 11,000x g.
12. Transfer the column into a clean 1.5 mL microcentrifuge tube and add 30 µL of Zyppy™ Elution Buffer directly to the column matrix and incubate for 1 minute at room temperature.
13. Centrifuge for 30 seconds at 11,000x g to elute the plasmid DNA.