Plasmid Isolation

Introduction

Once transformation has been successful it is common to create a liquid culture for further research. Follow this protocol for creating plasmid isolation from your liquid culture.
Protocol for GeneJET Plasmid Miniprep Kit.
Source: https://www.protocols.io/view/plasmid-isolation-miniprep-protocol-for-genejet-pl-7j4hkqw

Materials

- Bunsen burner and matches
- Sterile LB-media
- Liquid culture
- Eppendorf tube
- GeneJET Plasmid Miniprep Kit: K0502: Thermo Fisher

Procedure

1. Centrifuge overnight culture at 4700rpm for 5 minutes and discard the supernatant. (2ml tubes per culture)
2. Add 250 µL Resuspension solution and resuspend the cells in this. Transfer the mix to an Eppendorf tube.
3. Add 250 µL Lysis solution and invert the tube 4-6 times.
4. Add 350 µL Neutralization solution and invert the tube 4-6 times.
5. Centrifuge for 5 minutes and transfer the supernatant to a GeneJET spin column tube.
6. Centrifuge for 1 minute. Empty the flowthrough and place the column back in the collection tube.
7. Add 500 µL Wash buffer and centrifuge for 1 minute. Discard the flowthrough and place the column back.
8. Repeat step 7.
9. Centrifuge for an additional minute to remove all residual ethanol. Place the column in a fresh Eppendorf.
10. Add 20 µL of either an Elution buffer or MilliQ to the column and incubate at room temperature for 1 minute.
11. Centrifuge the column for 1 minute.
12. Discard the column and store the purified DNA in -20ºC.