Miniprep-Promega

Introduction

As of 7/20/17
Revised protocol for higher yield.
Make sure overnight culture was 5mL total
Underlined areas are changed by Chelsea
Waste:
1. Dump the liquid into the plastic Miniprep waste bin
2. Toss solids into biohazard

Materials

- Autoclaved 2mL centrifuge tubes
- Autoclaved 1.5 mL centrifuge tubes
- (630 uL) x (# of samples) of clean Nuclease-free water

Procedure

Prepare Lysate

1. Centrifuge 1.5 mL of bacterial culture for 1 minute at maximum speed (14000 rpm) in a microcentrifuge. Discard the supernatant.
2. Add an additional 1.5 mL of bacterial culture to the same tube and repeat Step 1.
3. Add 600 uL of nuclease free water to the cell pellet, and resuspend completely by pipetting up and down
4. Add 100 uL of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.

   Wait 2 minutes after adding Cell Lysis Buffer, and make sure the liquid is not viscous
5. Add 350 uL of cold (4-8C) Neutralization Solution, and mix thoroughly by inverting ~ 15 times.

   The solution will become orangish-yellow and have lots of debris
6. Centrifuge at maximum speed in a microcentrifuge for 10 minutes (14000 rpm)
7. Transfer the supernatant (~900 uL) to a PureYield Minicolumn without disturbing the cell debris pellet
8. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 1 minute
9. Discard the flowthrough (into MINIPREP WASTE), and place the minicolumn into the same Collection Tube.

Wash

10. Add 200 uL of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifube at maximum speed in a microcentrifuge for 1 minute.

11. Add 400 uL of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 1 minute.

12. Repeat step 11 for full wash

Elute

13. Transfer the minicolumn to a 1.5 mL microcentrifuge tube, then add 30 uL of nuclease free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.

14. Centrifuge for 30 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at -20 C

    Use the nanodrop to determine the concentration
    Put 1 uL of sample onto the nanodrop for each measurement
    Make sure to wipe down the nanodrop with a kimwipe between every measurement