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Plasmid Cloning - PureLink Kit

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Ty Flanagan[1]

[1]University of Minnesota

Ty Flanagan
University of Minnesota

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is for plasmid cloning using *E. coli* and the PureLink Quick Plasmid Miniprep Kit. See the Plasmid Ext - PureLink Quick Plasmid Miniprep Kit Protocol.pdf on the Goolge Drive.

Troubleshooting

Grow modified *E. coli* overnight

12m

1 Prepare working space for bacterial work. 5m

- 70% ethanol spray down.
- Bunsen burner.
- Gloves.
- Follow aseptic technique.

2 Prepare 2 tubes with 2.5 mL LB liquid broth with 50 ug/mL of ampicillin. 3m

Note

Use a non-sealing tube, or at the very end, before incubation, keep the tube cap slightly unscrewed.

The 5 mL non-sealing tubes are recommended.

3 Inocculate the media with one "tip-worth" of frozen bacterial stock. Use a 1000 uL pipette tip to physically scrape the frozen stock to collect the bacteria in the pipette tip's tip. Mix the media with the pipette tip. 2m

4 Incubate the culture at 37 °C in the shaking incubator overnight. 2m

Note

Keep volumes low relative to tube size as to prevent spilling. Also, make sure that the tube is supported as to not move much.
Do not overtighten screw-on caps.

Follow PureLink Quick Plasmid Miniprep Kit Protocol

58m

5 Begin heating 200 µL of TE Buffer in a 1.5 mL tube at 70 °C using a dry bath. 5m

6 Centrifuge the cultures and decant the media. 5m

12000 x g, 00:03:00

7 Add 250 µL of Resuspension Buffer (R3) and completely resuspend the bacteria pellet using the pipette. 5m

Note

If using a newer kit, make sure that RNase A has been added to the R3 solution.

8 Add 250 µL of Lysis Buffer (L7). Gently mix by inversion 3 times. Incubate at room temp for 00:05:00 . 7m

Note

Do not vortex.

9 Add 350 uL of Precipitation Buffer (N4). Quickly after addition, mix by inversion or vigorous shaking. 4m

Note

Do not vortex.

10 Centrifuge. 10m

15000 x g, 00:10:00

11 Transfer the supernatant into a spin column with a 2 mL wash tube. Centrifuge and discard the flowthrough. Reuse the same wash tube until noted. 5m

12000 x g, 00:01:00

12 Wash the spin column with 500 µL of Wash Buffer (W10). Incubate at room temp for 00:01:00 . Centrifuge and discard flowthrough. 6m

12000 x g, 00:01:00

Note

If using a newer kit, make sure that ethanol has been added to the W10 solution.

13 Add 700 µL of Wash Buffer (W9). Centrifuge and discard flowthrough. 6m

12000 x g, 00:01:00

Centrifuge and place spin column into new 1.5 mL tube.

12000 x g, 00:01:00

Note

If using a newer kit, make sure that ethanol has been added to the W9 solution.

14 Add 75 µL of the preheated TE Buffer (TE). Incubate at room temp for 00:01:00 2m

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15 Centrifuge and discard the column. 3m

12000 x g, 00:02:00

Note

Store DNA at 4 °C for short term and -20 °C for long term. Freeze-thaw cycles damage DNA products, but frozen is better for long term storage. ~4 weeks or 30 days is general guidance on shelf life or refrigerator DNA.