Feb 16, 2025

Bacterial Transformation Maxi Prep & Mini Prep

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvm95q6l3p/v1

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Protocol Citation: Yujie Fan, Chelsie Steele 2025. Bacterial Transformation Maxi Prep & Mini Prep . **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm95q6l3p/v1

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

Created: January 07, 2025

Last Modified: February 16, 2025

Protocol Integer ID: 117860

Keywords: bacterial transformation maxi prep, mini prep, transformation

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020495

Abstract

Bacterial Transformation Maxi Prep & Mini Prep

Materials

E-coli
Plasmid Plus
ZymoPURE Plasmid Maxiprep Kit
ZYPPY Plasmid Miniprep Kit

High Efficiency Transformation for NEB Stable Medium Competent E. Coli

17h 35m 30s

1 Thaw a tube of NEB Stable Competent *E. coli (C3040H)* cells on ice for 10 minutes. In the meantime, pre-warm selection plates with the appropriate antibiotitic resistance in the 30°C incubator. *Note: For best clone stability, incubate plates and liquid cultures at 30°C and prepare plasmid DNA from fresh transformants (from plates not greater than 3 days old). Store unstable clones as plasmid DNA, rather than cell-based glycerol stocks.*

30m

2 Add 1 -2 µl containing 100 pg – 100 ng of plasmid DNA to the cell mixture (that is, the E. Coli cells). Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex and do not touch the part of the tube that has the cells to avoid warming the cells.

30s

3 Place the mixture on ice for 30 minutes. In the meantime, set mixer HC to 42°C in preparation for next step.

5m

4 Heat shock at exactly 42°C for exactly 30 seconds (no shaking, speed 0). Do not mix. Place on ice for 5 minutes. Do not mix. In the meantime, set mixer HC down to 30°C to prepare for the next step.

5 Pipette 950 µl of room temperature NEB10-beta/Stable Outgrowth Medium (Cat No B9035S, Bio Labs) into the E.Coli/DNA mixture and place at 30°C for 60 minutes. Shake the tube horizontally at 250 rpm or rotate.

6 Mix the cells thoroughly by flicking the tube and inverting. Then spread 50-100 µl of cells or diluted cells onto a selection plate. That is, pipette 100uL in the middle and use a spreader. Incubate plates 24 hrs at 30°C or overnight at 37°C. We are incubating at 30°C.

Pick Colonies to Sequence

7 Disinfect the working surface and wipe down the excess alcohol. Set up bacteria sterile test tubes and Plasmid Plus medium (Cat No 446300, HTS labs). Add the appropriate antibiotic resistance gene. For us, we added CRB in a 1:1000 dilution to the medium.

8 Fill each bacteria tube with 5mL of the plasmid medium.

9 Collect selection plate from the incubator. Flip the plate to be right-side up. Find a region that is sparse so that you can pick a single colony. Use a p10 pipette to put a single colony on the end of the pipette tip. Then, eject the entire pipette into the sterile tube with medium and cap the tube.

10 Place the tubes in the bacteria shaker overnight. The next day, use the QIAprep Spin Miniprep Kit for Miniprep.

Miniprep to isolate plasmid DNA

11 Follow the centrifuge method for the QIAprep Spin Miniprep Kit.

12 Pellet 2mL of the bacterial overnight culture from the shaker by centriguation at 10,000g for 3 minutes at room temperature. Use a 2mL microcentrifuge tube.

13 Resuspend the pelleted cells in 250uL Buffer P1 and transfer to a new microcentrifuge tube.

14 Add 250uL Buffer P2 to the tube and mix thouroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than five minutes.

15 Add 350uL Buffer N3 to the tube and mix immediately and thoroughly by inverting the tube 4-6 times.

16 Centrifuge the tube for 10 minutes at 17,900g.

17 Transfer 800uL of the supernatant to the QIAprep 2.0 spin column. Centrifuge at same conditions for 1 minute and discard the flow-through.

18 Wash the QIAprep 2.0 spin column by adding 500uL Buffer PB. Centrifuge for one minute and discard the flow through.

19 Wash the QIAPrep 2.0 spin column by adding 750uL Buffer PE. Centrifuge for one minute and discard the flow throw.

20 Centrifuge again for an addition one minute to remove residual wash buffer.

21 Place the QIAprep 2.0 column in a clean and labeled 1.5mL microcentrifuge tube. To elute the DNA, add 50uL Buffer EB to the center of the QIAprep 2.0 spin column. Let stand for one minute and then centrifuge at same conditions for another minute. Nanodrop concentration and keep on ice.

22 Send out the samples for sequencing.

23 To prepare for maxi-prep, obtain recyclable 500mL plastic flasks as well as previously stored sterile tubes from the 4°C fridge that contain bacteria cells from the Miniprep (you should have 3mL left if you used around 2mL for miniprep).

24 To each flask, add 1mL from the sterile tube from Miniprep, and then add an additional 50mL of the plasmid medium that contains the appropriate antibiotic. Cover the ring of the flask and place in a bacteria to grow the culture overnight.

Maxi Prep

16h

25 In order to purify the bacteria, the protocol ZymoPURE Plasmid Maxiprep Kit was followed. (Cat No D4202 & D4203, Zymo Research)

26 Transfer the bacteria culture from the flask into a 50mL canonical tube. Centrifuge in the bacteria centrifuge for 10 minutes at around 7000g. Discard the supernatant and follow the protocol for plasmid DNA purification.

27 Add 14mL of ZymoPURE P1 to the bacterial cell pellet and re-suspend completely by vortexing.

28 Add 14mL of ZymoPURE P2 and immdeiatey mix by gently inverting the tube 6 times. Do not vortex. Let Sit at room temperature for 2-3 minutes. Cells are completely lysed when the solution apperas clear, purple, and viscous.

29 Add 14mL of ZymoPURE P3 and mix gently but thoroughly by inversion. Do not vortex. Invert the tube an additional 5 times after the sample turns completely yellow. A yellowish precipitate will form, in my experience, takes a while.

30 Before syringing, centrifuge the tube at 7000g for 5 minutes.

31 Grab and label with ZymoPURE Syringe Filter-X tubes, ensure the plug is attached to the Luer Lock at the bottom of the tubes. Then, place the syringe filter upright in a new 50mL. tube and load the lysate in the syring filter. Remove the Luer Lock plug from the bottom of the syring and place the plunger in the syringe to push the solution through the ZymoPURE Syringe Filter-X in one continuous motion.

32 To determine how much ZymoPURE Binding Buffer to the tube, multiply the amount of lysate you collect by 0.4 and then add that much of the buffer to the tube. Mix thoroughly by inverting the tube 8 times.

33 Connect labeled Zymo-Spin V-PX columns onto the vaccuum manifold. With the vacuum off, add the entire lysate mixture into the Zymo-Spin V-PX column and then turn on the vacuum until all of the liquid passes through the column completely.

34 Remove and discard the 50mL Reservoir from the top of the Zymo-Spin V-PX column. With the vacuum off, add 10mL of ZymoPURE Wash 1 to the column. Turn on the vacuum until all of the liquid has passed completely through the column.

35 With the vacuum off, add 10mL of ZymoPURE Wash 2 to the column. Turn on the vaccuum until all of the liquid has passed completely through the column. Repeat this wash with the Wash 2 buffer, but then keep the vacuum on for an additional two minutes after the liquid has passed completely through the column.

36 Remove and discard the 15mL Reservoir-X and place the Zymo-Spin V-PX column in a 2mL collection tube. Centrifuge at 16,000g for 1 minute to remove residual wash buffer.

37 Transfer the column in a new and long-term labeled microcentrifuge tube and add 400uL of ZymoPURE Elution Buffer directly to the center of the column matrix. Wait 2 minutes, and then centrifuge at 16,000g for 1 minute in a microcentrifuge.

38 Nanodrop the concentration and store in -20°C.