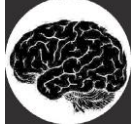
	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
DNA Miniprep Module Hours: 3.0	Effective Date: 1/16/2025 PRQs: <ul style="list-style-type: none"> - Autoclave LB/Agar Sterilization 	Revision 1.0 Author: Z. Hazlett-Klein Checked by Editor: M. Stowell

Background

Proper DNA cloning is a stepwise procedure. As discussed in the bacterial transformation SOP, target gene sequences can be cloned into plasmid vectors and transformed into competent bacterial strains for amplification (cloning) of the plasmid DNA. Following plasmid transformation and bacterial growth, one must isolate and purify the target plasmid away from the host bacterial cell contents. When done at the small scale (purifying up to 20 µg plasmid DNA), this DNA purification technique is known as a *DNA miniprep*. If scaled up, i.e. purifying larger amounts of DNA from larger growth cultures, this technique can be referred to as a DNA midiprep (50-100 µg DNA) or even DNA maxiprep (up to 1 mg DNA).¹ Whichever scale of method chosen, each use the same sequence of steps to lyse the host cells and purify plasmid DNA.


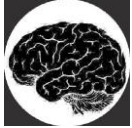
Purification of plasmid DNA via miniprep from bacterial host cells is accomplished in three main steps. First, bacterial cells are lysed using an alkaline buffer and the lysate cleared via centrifugation. Next the soluble fraction is passed through a microcentrifuge spin filter (Fig 1) containing a silica membrane for adsorption of DNA and elution of the remaining components. After multiple buffer washes, the purified plasmid DNA is eluted from the spin column and stored for future use at -20°C.²



Figure 1. Microcentrifuge spin filters. The filter (small well with silica membrane) is placed inside a microcentrifuge tube. Centrifugation passes solutions through the filter and the eluant collects at the bottom of the microcentrifuge tube.

DNA minipreps are available as boxed kits that contain all the reagents, equipment, and instructions required to purify plasmid DNA. In the Skills Center, we will be using kits prepared by either Qiagen or ThermoFisher.

1. Purpose

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The purpose of this SOP is to instruct students on the background and procedures necessary for plasmid DNA purification using miniprep kits.

2. Scope

This procedure applies to qualified skills center users.

3. Responsibility



- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
- 3.3. It is the responsibility of the user to become trained on the procedure.

4. Definitions

- 4.1 DNA Mini/midi/maxi-prep: The process of purifying DNA from a host cell at small, medium, and large scale amounts of cells/desired DNA, respectively.
- 4.2 Microcentrifuge spin filter (Fig 1): A filtration device that can be used to pass cell lysate through, collect DNA, and wash away unbound cellular components.
- 4.3 Lysate: The soluble fraction of lysed cells.
- 4.4 Eluate: The portion of the lysate that passes through the silica membrane filter without binding. The eluate is often discarded in this process.

5. Materials/Equipment

- 5.1 DNA cloning bacterial colonies (either as plated colonies or frozen glycerol stock)
- 5.2 LB media
- 5.3 Antibiotic specific to the resistance gene within the plasmid DNA of interest
- 5.4 Round bottomed culture tubes (polypropylene or glass)
- 5.5 DNA Minprep kit (We use Quintara Bio or Thermofisher)
- 5.6 37°C shaking incubator

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5.7 Ice in insulated ice bucket

5.8 4°C centrifuge

5.9 UV Vis Spectrophotometer and 50 – 2000 µl UVette (cuvette)

6. Procedures

6.1 Retrieve source of DNA cloning bacterial colonies containing cloned plasmid DNA.

6.1.1 Can either use your LB agar plate with bacterial colonies containing cloned plasmid DNA (end product of bacterial transformation protocol), or obtain a glycerol stock of the same cloned bacterial cells.

6.2 Inoculate 5 ml of LB + 1X plasmid selection antibiotic with plasmid cloning bacterial cells in round bottomed culture tubes.

6.2.1 Using a sterile toothpick or pipette tip, either pick colony from plate or scrape frozen surface of bacteria stored glycerol stock and immediately place in culture tube with LB + antibiotic.

6.3 Shake small batch of cloning cells at 37°C overnight.

6.4 Collect DNA miniprep kit (Qiagen or Thermofisher)



6.4.1 Find instruction card inside kit and collect all reagents and materials needed for DNA miniprep.

NOTE: One or more reagents might be kept at 4°C for storage. Collect and store on ice till needed.

6.4 Pellet 5 ml cells in 4°C centrifuge for 10 minutes at 5000 rpm. Keep on ice.

6.5 Remove LB media by aspiration or pipetting. Be careful not to aspirate cells.

6.6 Follow miniprep kit instruction card to purify plasmid DNA from bacterial cloning cells. Protocol for QIAprep Spin Miniprep Kit provided below⁴:

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<p style="text-align: center;">DNA Miniprep</p> <p style="text-align: center;">Module Hours: 3.0</p>	<p>Effective Date: 1/16/2025</p> <p>PRQs:</p> <ul style="list-style-type: none"> - Autoclave LB/Agar Sterilization 	<p>Revision 1.0</p> <p>Author: Z. Hazlett-Klein</p> <p>Checked by</p> <p>Editor: M. Stowell</p>

Quick-Start Protocol
February 2015

QIAprep[®] Spin Miniprep Kit

The QIAprep Spin Miniprep Kit (cat. nos. 27104 and 27106) can be stored at room temperature (15–25°C) for up to 12 months.


For more information, please refer to the most recent version of the *QIAprep Miniprep Handbook*, which can be found at: www.qiagen.com/handbooks.



For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at: www.qiagen.com/contact.

Notes before starting

- Optional: Add LyseBlue reagent to Buffer P1 at a ratio of 1 to 1000.
- Add the provided RNase A solution to Buffer P1, mix and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 13,000 rpm (~17,900 *x g*) in a conventional table-top microcentrifuge.



1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 *x g*) for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.

Sample to Insight


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4. Add 350 μ l Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
 5. Centrifuge for 10 min at 13,000 rpm (~17,900 \times g) in a table-top microcentrifuge.
 6. Apply 800 μ l supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. For centrifuge processing, follow the instructions marked with a triangle (\blacktriangle). For vacuum manifold processing, follow the instructions marked with a circle (\bullet). \blacktriangle Centrifuge for 30–60 s and discard the flow-through, or \bullet apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
 7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. \blacktriangle Centrifuge for 30–60 s and discard the flow-through, or \bullet apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
- Note:** This step is only required when using endA+ strains or other bacteria strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. \blacktriangle Centrifuge for 30–60 s and discard the flow-through, or \bullet apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source. Transfer the QIAprep 2.0 spin column to the collection tube.
 9. Centrifuge for 1 min to remove residual wash buffer.
 10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 μ l Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.
 11. If the extracted DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, QIAprep® (QIAGEN Group). 1091289 02/2015 HB-1921-001 © 2015 QIAGEN, all rights reserved.

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6.7 Finish with eluting the purified plasmid DNA from spin column.

6.8 Determine the concentration of the purified plasmid DNA using UV Vis Spectrophotometry.

6.8.1 Use the 50 µl-2000 µl cuvette. There will not be a very large volume of eluted Plasmid DNA to begin with. Therefore, dilute the sample 1:10 in elution buffer to a final volume of 50 µl and pipette into cuvette carefully without introducing bubbles.

6.8.2 Measure the absorbance peak of the double stranded plasmid DNA at 260 nm wavelength. Consider the direction the cuvette is placed into the spectrometer. Depending on the path of light through the cuvette, the path length (which will be used to calculate concentration) will be different (Reference: <https://online-shop.eppendorf.us/US-en/Laboratory-Consumables-44512/Cuvettes-59968/Eppendorf-UVette-PF-9817.html>).

6.8.3 Measure the concentration of the eluted DNA, using Beers-Law, using the extinction factor for dsDNA: $0.020 (\mu\text{g/ml})^{-1} \text{ cm}^{-1}$ at 260nm

6.8.4 Make sure to multiply this concentration by 10 to address the 1:10 dilution of the sample.

6.9 Prepare DNA sample to be sent off for sequencing using available DNA sequencing services. For Quintarabio sequencing instructions and order form see https://www.quintarabio.com/service/dna_sequencing.



6.9.1 Follow sequencing outsource instructions to ensure that 1) your plasmid DNA is diluted to the proper concentration, 2) the proper primers are either requested or added to the DNA sample to be shipped, and 3) all info is filled out in order form and the form printed and packaged with the sample to be shipped.

6.10 Once sequencing results are returned, determine if the purified DNA contains the desired gene inserts. This may take up to a week for results to be delivered.

7. Troubleshooting

7.1 There is no absorbance when reading the DNA sample

Check to make certain the UV/VIS is set to the correct wavelength

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9. Module Mastery Task

9.1 Obtain bacterial cloning cells containing transformed plasmid: Either grown on agar plate with selective growth media or by glycerol stock.

9.2 Grow a 5 ml culture of these cells in LB + selection antibiotic.

9.3 Follow steps in plasmid DNA miniprep kit to purify the plasmid DNA from their bacterial host cells.

9.3.1 Which miniprep kit was used?

9.3.2 Indicate each step in the miniprep that corresponds with the three main steps of plasmid DNA purification (see Background).

9.4 When plasmid DNA is purified, indicate the concentration of your purified DNA.

9.5 Prepare and send off this sample for sequencing.

9.5.1 What are your 5' and 3' sequencing primers?

9.5.2 What gene do you expect to obtain the sequence for?

9.6 Provide evidence from sequencing results that you successfully purified the plasmid DNA of interest.