9/11/20

Project: VA iGEM 2020 Shared Project
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Nanodrop concentrations for MiniPrep Product

Miniprep Results 9:11.png

GGE114 77 ng/uL
"HIV" 142 ng/uL
pSB1C3-Lux 99 ng/uL

pSB1K3-mRFP 22 ng/uL
pAC-4CL1 25 ng/uL
pMLRT <20 ng/uL * too low; unreliable value

1 uL of a 50 uL prep was used for the analysis

Lab Log

	A	B	C
1	**Procedure**	**Researcher**	**Comments**
2	Glycerol Stock	JB SL	1 mL 50% glycerol, 1 mL culture, 7 plasmids (pUC-STS, pSB1K3, pAC-4CL1, pMLRT, pSB1C3, GGE114, pHIV)
3	Incubation	JB CM	All 7 plasmids, 2 copies of pUC-STS, put in incubator at 8:50 New Colonies were used for each of these 7 cultures, these were not the same colonies used to start the other inoculated cultures. They were derived from the same plates however. -- Did not use heat source
4	QIAprep Spin Miniprep Kit	JB SL	7 plasmids (pUC-STS, pSB1K3, pAC-4CL1, pMLRT, pSB1C3, GGE114, pHIV)
5			

*QIAGEN HiSpeed Plasmid Purification

Introduction

LucidChart Overviews | DNA | Combined Procedures List | Procedure Source

Materials

- QIAGEN HiSpeed Plasmid Maxi Kit **or** QIAGEN HiSpeed Plasmid Mini Kit
- Equitment:
  - Refrigerated Centrifuge
- Total Wait Times:
  - 8 h Incubation at 37°C for Inoculating Culture
  - 15 Min: Centrifuge
  - Pause Point
  - 5 Min Room Temp Incubation
  - 10 Min Room Temp Incubation
  - Pause Point
  - 5 Min Room Temp Incubation

Procedure

Setup (done)

- [x] 1. Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for a final concentration of 100 µg/ml
- [x] 2. Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C
- [x] 3. Pre-chill Buffer P3 at 4°C
- [x] 4. Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see “Using LyseBlue reagent” on page 13

Main Steps (cont. from 9/10 at Step 6)

5. Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm). Use a tube or flask with a volume of at least 4 times the volume of the culture

   Note: If following the Addgene inoculation protocols, the cells will already be in an LB suspension

6. Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids inoculate ▲ 50 ml or ● 150 ml medium. For low-copy plasmids, inoculate ▲ 150 ml or ● 250 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).

   Note: Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter

7. Harvest the bacterial cells by centrifugation at 6000 x g for 15 min at 4°C.

   00:15:00

8. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained

   PAUSE If you wish to stop the protocol and continue later, freeze the cell pellet at –20°C

9. Resuspend the bacterial pellet in ▲ 6 ml or ● 10 ml Buffer P1.

   Note: For efficient lysis, it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1.
   Note: If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

10. Add ▲ 6 ml or ● 10 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min

    Note: Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO2 in the air.
    Note: If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved
    CRITICAL During the incubation prepare the QIAfilter Cartridge: Screw the cap onto the outlet nozzle of the QIAfilter Midi or QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge into a convenient tube or a QIArack

    00:05:00

11. Add ▲ 6 ml or ● 10 ml chilled Buffer P3 to the lysate, and mix immediately and thoroughly by vigorously inverting 4–6 times. Proceed directly to step 12. Do not incubate the lysate on ice

    Note: Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and KDS becomes visible. The buffers must be mixed completely. If the mixture appears still viscous and brownish, more mixing is required to completely neutralize the solution. It is important to transfer the lysate into the QIAfilter Cartridge immediately to prevent later disruption of the precipitate layer.

Note: If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated

12. Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature for 10 min. Do not insert the plunger

    CRITICAL This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging. If, after the 10 min incubation, the precipitate has not floated to the top of the solution, carefully run a sterile pipet tip around the walls of the cartridge to dislodge it

    00:10:00

13. Equilibrate a ▲ HiSpeed Midi or ● HiSpeed Maxi Tip by applying ▲ 4 ml or ● 10 ml Buffer QBT and allow the column to empty by gravity flow.

    Note: Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the HiSpeed Tip to drain completely. HiSpeed Tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column

14. Remove the cap from the QIAfilter outlet nozzle. Gently insert the plunger into the QIAfilter Midi or QIAfilter Maxi Cartridge and filter the cell lysate into the previously equilibrated HiSpeed Tip.

    Note: Filter until all of the lysate has passed through the QIAfilter Cartridge, but do notapply extreme force. Approximately 15 ml or 25 ml of the lysate is generally recovered after filtration.
    Optional: Remove a 300 µl or 120 µl sample of the filtered lysate and save for an analytical gel (sample 1) to determine whether growth and lysis conditions were optimal.

15. Allow the cleared lysate to enter the resin by gravity flow.

    Optional: Remove a 300 µl or 120 µl sample of the flow-through and savefor an analytical gel (sample 2) to determine the efficiency of DNA binding to the QIAGEN resin.

16. Wash the HiSpeed Midi or HiSpeed Maxi Tip with 20 ml or 60 ml Buffer QC.

    Note: Allow Buffer QC to move through the HiSpeed Tip by gravity flow.
    Optional: Remove a 400 µl or 240 µl sample of the wash fraction and savefor an analytical gel (sample 3).

17. Elute DNA with 5 ml or 15 ml Buffer QF.

    Note: Collect the eluate in a tube with a minimum capacity of 10 ml or 30 ml.
    Optional: Remove a 100 µl or 60 µl sample of the eluate and save for an analytical gel (sample 4).
    PAUSE If you wish to stop the protocol and continue later, store the eluate at 4°C.Storage periods longer than overnight are not recommended

18. Precipitate DNA by adding 3.5 ml or 10.5 ml (0.7 volumes) roomtemperatureisopropanol to the eluted DNA. Mix and incubate at room temperature for 5 min.

    Note: All solutions should be at room temperature to minimize salt precipitation.

    00:05:00

19. During the incubation remove the plunger from a 20 ml or 30 ml syringe and attach the QIAprecipitator Midi Module or QIAprecipitator Maxi Module onto the outlet nozzle. Do not use excessive force, bending, or twisting to attach theQIAprecipitator!

    CRITICAL Always remove the QIAprecipitator from the syringe before pulling up the plunger!

20. Place the QIAprecipitator over a waste bottle, transfer the eluate/isopropanolmixture into the 20 ml or 30 ml syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.

    Note: Alternatively, the QIAprecipitator attached to the 20 ml or 30 ml syringe can be placed on a QIAvac 24 Plus or QIAvac 6S manifold. Use of VacConnectors(cat. no. 19407) is recommended for vacuum processing, to raise the QIAprecipitator above the level of adjacent luer extensions. Switch on vacuum to draw the eluate/isopropanol mixture through the QIAprecipitator. Switch off the vacuum once all the liquid has been drawn through.
    CRITICAL Complete the QIAprecipitator procedure (steps 21–26) within 10 min. To prevent detachment of the QIAprecipitator and subsequent loss of DNA andalcohol, do not use excessive force when pushing liquid through theQIAprecipitator.

    00:10:00

21. Remove the QIAprecipitator from the 20 ml or 30 ml syringe and pull out the plunger. Re-attach the QIAprecipitator and add 2 ml 70% ethanol to the syringe. Wash the DNA by inserting the plunger

    Note: Alternatively, if you are using the vacuum procedure, add 2 ml 70% ethanol, andswitch on vacuum to draw the ethanol through the QIAprecipitator. Keep vacuumon for 3 min. Proceed to step 23.

22. Remove the QIAprecipitator from the 20 ml or 30 ml syringe and pull out the plunger. Attach the QIAprecipitator to the 20 ml or 30 ml syringe again, insert the plunger, and dry the membrane by pressing air through the QIAprecipitatorquickly and forcefully. Repeat this step.

23. Dry the outlet nozzle of the QIAprecipitator with absorbent paper to prevent ethanol carryover.

24. Remove the plunger from a new 5 ml syringe and attach the QIAprecipitator ontothe outlet nozzle. Hold the outlet of the QIAprecipitator over a 1.5 ml collectiontube. Add 1 ml of Buffer TE to the 5 ml syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.

    - Ensure that the outlet of the QIAprecipitator is held over the collection tube whenBuffer TE is poured into the syringe, as eluate can drip through the QIAprecipitatorbefore the syringe barrel is inserted.
    - Be careful, as residual elution buffer in the QIAprecipitator tends to foam whenexpelled.
    - Alternatively, if a higher DNA concentration is desired and a reduction in yield ofup to 10% is acceptable, elute with 500 µl Buffer TE. Lower volumes of elutionbuffer are not recommended, since incomplete wetting of the QIAprecipitatormembrane will lead to reduced DNA yields.
    - Water or buffers commonly used to dissolve DNA (e.g., Tris), may also be usedfor elution.
    Note: Buffer TE contains EDTA which may inhibit downstream enzymatic orsequencing reactions.
    Note: Store DNA at –20°C when eluted with water as DNA may degrade in theabsence of buffering and chelating agents.

25. Remove the QIAprecipitator from the 5 ml syringe, pull out the plunger, and reattach the QIAprecipitator to the 5 ml syringe.

26. Transfer the eluate from step 19 to the 5 ml syringe and elute for a second timeinto the same 1.5 ml tube.

Note: This re-elution step ensures that the maximum amount of DNA in theQIAprecipitator is solubilized and recovered.

Note: Be careful, as residual elution buffer in the QIAprecipitator tends to foam when expelled.

*Glycerol Stock

Introduction

LucidChart Overviews | DNA | Combined Procedures List
https://www.addgene.org/protocols/create-glycerol-stock/

Materials

- Overnight Culture
- 2 mL screw-top tubes
- Sterile Loop

Procedure

1. Add 500 µL of overnight culture to 500 µL of 50% glycerol, gently mix

   Note: *Make the 50% glycerol solution by diluting 100% glycerol in dH20.*

2. Freeze at -80 degrees

3. When you would like to get some bacteria, use a sterile loop to scrape some bacteria off the top - DO NOT UN-THAW

4. Grow the bacteria overnight

*QIAprep Spin Miniprep Kit

Introduction

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"This protocol is designed for the purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to Appendix C: Special Applications, page 45."

Materials

› Time:

› Centrifuge for 10 min

Procedure

Protocol

- 1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

  - Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
  - If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

- 2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

  - This protocol is designed for the purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to Appendix C: Special Applications, page 45.
  - If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

- 3. Add 350 µl Buffer N3. Mix immediately and thoroughly by inverting the tube 4–6 times.

  - To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g., ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.
  - If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

- 4. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

  A compact white pellet will form

- 5. Apply 800 µl of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting

6. Centrifuge for 30–60 s. Discard the flow-through.

7. **Recommended:** Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.

   This step is necessary to remove trace nuclease activity when using endA+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains, such as XL-1 Blue and DH5®α, do not require this additional wash step.

8. Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

9. Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.

   CRITICAL Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 min and centrifuge for 1 min

*Culture from Agar Stab (Inoculation)

Introduction

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Materials

- Liquid LB (2xYT)
  - $NaCl_{(S)}$
  - $Tryptone_{(S)}$
  - Yeast Extract
  - dH_2O
- Equitment:
  - Autoclave
  - Shaking Incubator set to 37 °C
- Total Wait Times:
  - Incubation: 12-18 hours

Procedure

Inoculating a Liquid Bacterial Culture - Use heat source to sterilize your workspace

1. Prepare liquid LB. For example, to make 400 mL of LB, weigh out the following into a 500 mL glass bottle:

   4 g NaCl
   4 g Tryptone
   2 g Yeast Extract
   and dH_2O to 400 mL
   Note: *If your lab has pre-mixed LB agar powder, use the suggested amount, instead of the other dry ingredients above.*
   Media without growth (top) and with growth (bottom)
   Loosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) and then loosely cover the entire top of the bottle with aluminum foil. Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at room temperature.

2. When ready to grow your culture, add liquid LB to a tube or flask and add the appropriate antibiotic to the correct concentration (see table below).

Antibiotic Concentrations

	A	B
1	Ampicillin	100 µg/mL
2	Bleocin	5 µg/mL
3	Carbenicillin	100 µg/mL
4	Chloramphenicol	25 µg/mL
5	Coumermycin	25 µg/mL
6	Gentamycin	10 µg/mL
7	Kanamycin	50 µg/mL
8	Spectinomycin	50 µg/mL
9	Tetracycline	10 µg/mL

Note: *If you intend to do a mini-prep you will usually want to start 2 mL in a falcon tube, but for larger preps you might want to use as much as a liter of LB in a 2 L Erlenmeyer flask.*

3. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.

4. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.

5. Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.

6. Incubate bacterial culture at 37°C for 12-18 (12–16 recommended by Qiagen) hr in a shaking incubator.

   Note: *Some plasmids or strains require growth at 30°C. If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.*

7. After incubation, check for growth, which is characterized by a cloudy haze in the media (see linked protocol for info).

   Note: *Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.*
   Note: *A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.*

8. *(Optional)* For long term storage of the bacteria, you can proceed with Creating a Glycerol Stock.

9. You can now isolate your plasmid DNA from the bacterial culture by following Isolating Your Plasmid DNA.

Procedure (Version 2 from Veronica's old lab notebook)

10. Remove the agar plates from the 37° C degree incubator

11. Obtain a 14-mL sterile plastic round-bottom cell culture tubes. Label the tubes. Using sterile technique, pipette 5 mL of the 2xYT media into each tube.

12. Add the appropriate amount of antibiotic (see table above)

Example: Add 5 µL of 100 mg/mL ampicillin stock solution into each tube so that the final concentration of ampicillin in the tube is 100 µg/mL.

13. Light the Bunsen burner

14. Using a sterile loop, pick a colony of cells from the plasmid transformation plate; then, swirl the loop in of the 5 mL media tube.

    Take a metal loop and flame it in the Bunsen burner until it becomes red hot.
    Cool the loop on a side portion of the agar in the petri dish that is free of bacteria so the heat does not kill it
    Scrape up a single large colony of cells.
    Swirl the loop in the media until the cells come off the loop and go into the media.
    Flame the loop again to sterilize the loop.

15. Place the 5 mL culture in a 37° C shaker to be incubated overnight with shaking at about 225-300 rpm.