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Project: VA iGEM 2020 Shared Project
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Lab Log

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*Culture from Agar Stab (Inoculation)

Introduction

LucidChart Overviews | DNA | Combined
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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

1. Check all control and DNA strain plates. Our XL1-Blue control dish grew contaminated colonies so we threw all 5 petri dishes using the XL1-Blue Cell Line away. Continued with solely the pduABJKNUT in JM109 cell line.

- [x] 2. 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.

NOTE: this was already made for us (see 9/10/20 for details on media). Used Amp for our 2 plasmids

3. 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.*

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

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

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

7. 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.*

8. 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.*

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

10. 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)

11. Remove the agar plates from the 37° C degree incubator
12. 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.
13. 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.
14. Light the Bunsen burner
15. 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.
16. Place the 5 mL culture in a 37° C shaker to be incubated overnight with shaking at about 225-300 rpm.

*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."
Note: Process was used to create 2 pduABJKNUT (JM109 Successful culture) DNA deposits

Materials

› Time:

› Centrifuge for 10 min

Procedure

Protocol

- ✔ 1. Collect 3 total ml of culture supernatant and centrifuge for 2 min at 13,000 rpm.
- ✔ 2. Use pressure valve to remove supernatant.
- ✔ 3. 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.
- ✔ 4. 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.
- ✔ 5. 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.

- [x] 6. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

  A compact white pellet will form

- [x] 7. Apply 800 µl of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting

- [x] 8. Centrifuge for 30–60 s. Discard the flow-through.

- [ ] 9. **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.

- [x] 10. Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

- [x] 11. 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.

- [x] 12. 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