Plasmid Miniprep

Overview

This protocol covers the isolation of plasmid DNA from *E. coli* via miniprep (alkaline lysis) using Qiagen reagents. This protocol also covers the creation of a glycerol stock for preservation of plasmid containing bacteria

Materials

- *E. coli* culture grown overnight (14-16 hours) containing plasmid to be isolated.
    - Culture should be in late exponential growth phase (turbid but not overly so). Overlong growth of culture will lead to lower yields and lower quality plasmid. Shortened growth of culture leads to lower yield
    - The maximum volume for a single miniprep column is 5mL of culture. Note that higher culture volumes are associated with higher yield.[i]
    - Standard culture volume is 4mL (3mL for miniprep, .5mL for glycerol stock)
- Temporary 1.7mL centrifuge tubes (1 per sample)
    - Each tube should be labeled with a key (typically numerical) that corresponds to a specific culture tube. This key should be logged on your benchling.
- Econospin Mini Spin Column (1 per sample)
    - Make sure you use the correct column (30-40µg binding capacity). Do not use the "min-elute columns". **Correct columns are blue and stored at room temperature.**
    - Label the column with the same numerical keys as your temporary 1.7mL centrifuge tubes. Note, label the column (top part), not the collection tube which will be disposed of.
- Final 1.7mL centrifuge tubes (2 per sample)
    - 1 tube is need for final miniprep tube and another for the glycerol stock
    - Each tube should be labeled with the construct name, plasmid backbone, the miniprep/glycerol stock number (abbreviate mp/gs. [e.g. mp#1]), the date and initials.
    - Initials and date only need to be located on the sides of tubes, not the top.
    - Glycerol stocks and miniprep numbers should correspond (i.e. mp#1 should come from the same culture as gs#1).
- 50% Glycerol
    - 50:50 volume to volume solution of Sterile H_2O and Glycerol
    - Used to help cryopreservation of bacteria
- Buffer P1 (Resuspension Buffer)
    - Buffer stored at 4C (fridge)
    - Comprised of: 50mM Tris-HCl pH 8.0, 10mM EDTA, 100µg RnaseA
    - Used to resuspend bacteria in buffered solution. RnaseA breaks down RNA after cells are lysed.[ii]
- Buffer P2 (Lysis buffer)
    - Comprised of: 200mM NaOH, 1% SDS

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[i] One would expect the plasmid gained per unit volume of culture to be negative

[ii] While for our work we would consider RNA a fairly benign/minor contaminant, it represents a major contaminant for researchers working with eukaryotic transfections, or more commonly with in vitro transcriptions.

    - Used to break down cell walls/membranes and denature proteins
- Buffer N3
    - Comprised of: 4.2 M Gu-HCl, 0.9 M Potassium acetate, pH 4.8
    - Neutralizes the base from the lysis buffer.
- Buffer PE (Wash buffer)
    - Comprised of:[iii] 10 mM Tris-HCl pH 7.5, 80% ethanol. Used to wash away proteins, salts, and various other contaminants.
- Buffer EB (Elution Buffer)
    - Comprised of: 10 mM Tric-cl pH 8.5
    - Used to elute DNA from column
- Buffer EB (Elution Buffer)
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Procedure

1. Transfer 1.5mL of each culture to the appropriate **temporary tube** (P1000 set to 750µl recommended).
    - Vortex or pipette up and down culture before transfer, otherwise bacteria will clump on the bottom.
2. Pellet cells by centrifugation in microcentrifuge at 6000x RPM.
3. Discard (pour out) liquid.
    - Do not bang or shake tube vigorously as this can lead to loss of bacterial pellet.
    - Try to remove as much liquid as can be done in a reasonably quickly manner. You will repeat this step so it does not matter if every last drop is gone.
4. Repeat steps 1-3 until desired volume of culture is spun down.
5. Remove excess liquid by blotting the tube on a paper towel. Be careful to not dislodge the pellet.
    - Note that the paper towel should not go inside the tube/touch the pellet.
    - Get as much as can be reasonably removed. Excess liquid will reduce yield/purity, but there is a limit to what can reasonably be removed without pipetting.
6. **Resuspend** pellet in **250µl of cold buffer P1**. (To resuspend either vortex tube or pipette up and down on pellet until no solids remain). Pipetting is recommended.

[iii] Qiagen has not revealed the exact recipe, this is a close approximation.
Source: https://openwetware.org/wiki/Qiagen_Buffers

7. Add **250µl of buffer P2**. **Gently invert at least 6 times** to mix. **Do not shake, do not vortex.**
8. Incubate for **1-4 minutes**.
    - Note: Incubation should not be allowed to continue for longer than 4 minutes (including time taken to neutralize). The true upper limit of incubation time is 5 minutes. **Do not allow incubation to proceed longer than this as it will damage DNA**.
9. Neutralize lysis buffer by adding **350µl of N3** to tube and **gently inverting at least 6 times to mix**. A white precipitate should form. **Do not shake, do not vortex.**
10. Pellet cell debris by centrifugation for 5-10 minutes at 13,300 rpm.
    - Make sure to face hinges of tubes outwards so debris collects on the same area of the tube
11. Aspirate liquid (being sure to avoid collection of precipitate) and apply to appropriate spin column. Spin for 1 minute at 6,000 rpm. Throw away temporary tube.
    - Maximum volume that can be used at once is 800µl. In the event there is too much liquid simply spin multiple times.
    - Adding precipitate to columns will cause reduced purity/protein contamination. Avoiding dispensing pipette in tube. It is expected that there will be a small volume that is impossible to aspirate without precipitate. Leave that volume behind.
12. Discard flow through (dump out fluid from lower collection tube, then replace the upper column).
    - Note: be careful to not bring the bottom of the column in contact with the liquid.
13. Add 800µl of buffer PE. Allow columns to stand for 1 minute. Spin for 1 minute at 13,300 rpm. Discard flow through.
14. Dry column by spinning empty column for 1 minute at 13,300 rpm.
15. Remove upper column from collection tube and transfer to the appropriate final miniprep tube. Throw away collection tube.
16. For **high copy** plasmids (e.g. 1C3), apply **25µl** of Buffer EB[iv] to the column membrane. For **low copy** plasmids add **15µL** of **Buffer EB** to the column membrane. Do not pierce membrane
    - Note: The volume of elution buffer can be adjusted up or down. Higher elution volumes lead to higher total yields (ng of DNA) but lower concentrations (ng/µL). Lower volumes yield lower total yields but higher

[iv] Pre-warming elution buffer to 50C is supposed to increase yield, especially of large plasmids.

concentrations. The minimum total volume (i.e. elution buffer used in steps 16-18) is 30µl.

17. Let incubate 1 minute. Spin at 13,300 RPM.
    - For improved yield, let sit for 4 minutes instead.
18. Repeat steps 17 and 18.[v]
19. Throw away column. Measure concentration and purity via nanodrop. Record purities in log.
20. Store minipreps in the awaiting confirmation box at -20 and record their names, locations, concentrations and purities on the awaiting confirmation spreadsheet on google drive.
21. Create a glycerol stock by adding 500µl of culture to 500µl of **50% glycerol** in your final glycerol stock tube. Invert tubes to mix.
22. Store in the awaiting confirmation box at -80C and record initials, dates and locations on the awaiting confirmation spreadsheet on google drive.
23. It is recommended to clean your pipette by wiping with 70% ethanol.

[v] Alternatively spin through the full volume and the elute again using the EB buffer just spun through (as per what is done in PCR purification). This method might lead to slightly higher yields, but is somewhat more labor intensive (in my opinion). Either works fine.