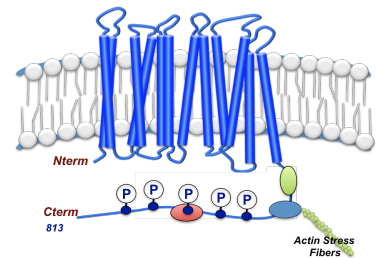


DNA Mini Prep Protocol

Qiagen Spin Protocol



Plasmid Prep: Follow the Qiagen protocol if there is confusion or a discrepancy.

Cell Culture - PREPARE TWO 5 ml CULTURES: LABEL EACH TUBE WITH THE NAME OF PLASMID AND YOUR NAME.

- 1) **Select a single well isolated colony** from your freshly transformed cell culture. Alternatively a streak from a glycerol stock can be used if re-streaked before using. Use a toothpick or pipette tip to transform the colony into a 5 ml LB media with the appropriate antibiotic in loosely capped tube. Leave tip in the liquid. Use the 5 ml culture tubes with loose caps. Not falcon tubes or flasks.
- 2) **Culture overnight WITH SHAKING** at an angle at 37°C. This is best started after 12pm but before 5pm.
- 3) **Centrifuge the Cells.** Harvest the cells by centrifugation at 3,000 x g for 5 minutes at 4°C. Remove all traces of supernatant by inverting the centrifuge tube until all media has been drained. The pellet can then be frozen at -20°C for later use. Use the swinging bucket rotor in the 5 ml culture tube

Qiagen QIAprep Mini prep DNA Purification Method

Plasmid isolation Procedure (taken directly from the Qiagen Handbook with additional notation for clarity)

1) Resuspend the bacterial pellet in 250 µl of Buffer P1.

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 of Buffer P2 and mix the sample by inversion 4-6 times. Incubate at room temp for 5 min.

Mix Gently by inverting the tube. 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 suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Vortexing will result in shearing of the genomic DNA, leaving free chromosomal fragments to contaminate the plasmid DNA. This will result in a co-purification of both DNA types and will ruin any further experiments. The lysed cells be very thick, almost like snot. The solution should become slightly more clear if the cells have lysed. This solution contains NaOH and will alkaline lyse the cells.

Incubate for exactly 5 minutes. Longer incubations will lead to inappropriate degradation of genomic DNA. SDS solubilizes the phospholipid and protein components. NaOH denatures the DNA as well as proteins. Optimal lysis time allows maximal release of plasmid DNA from the cells without release of genomic DNA. Long exposure to alkaline conditions may cause the plasmid DNA to become irreversibly denatured. This DNA will run faster on a gel and is resistant to restriction enzyme digestion.

3) Add 350 µl of Buffer N3/P3 and mix immediately and thoroughly by inverting the tube 4-6 times..

After addition of this buffer the solution becomes cloudy and very viscous. The lysate is neutralized by the addition of acidic potassium acetate in the neutralization buffer. The high salt (K⁺) concentration causes the SDS to become insoluble and precipitate. Plasmid DNA is too small to be trapped in the precipitate while genomic DNA and protein is. Any remaining SDS in solution will inhibit DNA binding to the column later so make certain that the solution is well mixed.

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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 be come cloudy.

If LyseBlue 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

Remove and save 10 µl of the supernatant for an analytical gel. Save as cell lysis.

5) Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

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

7) Recommended: Wash the QIAprep 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 alpha do not require this additional wash step

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

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

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

Important: SAVE THIS ELUTION - this contains your plasmid.

12) Determine the concentration of DNA in the sample.

- Determine the absorbance of your sample at 260 and 280 nm.
- The concentration of DNA will be 10 times the absorbance at 260 nm. The units will be mg/ml.
- The actual purity of the sample can be determined by taking the ratio of absorbance at 260 to 280 nm. *If the ratio is greater than 1.8 the absorption is due to nucleic acids. A high quality prep should have a ratio of 2.0 to 1.8. If the ratio is below 1.6 there may be proteins or other organic contaminants and the DNA can be extracted by chloroform phenol extraction for a final clean up.*

Typical loads for DNA gel. 10-20 µl of eluted DNA mixed with 5 µl of 6X DNA Sample Buffer. Load the entire sample. A 1 Kb ladder will work for most plasmid preps. 0.8 -1.2 % agarose gels will serve most simple restriction digests and tests of the DNA gel.

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Preparation Notes:

- To ensure high yields of pure DNA, use no more than 3 ml LB culture for high-copy number plasmids (e.g., pUC, pBluescript®). For low-copy-number plasmids (e.g., pBR322), use no more than 10 ml LB culture and refer to the recommendations on page 13. We do not recommend the use of rich media such as TB or 2xYT for culture. When low-copy-number plasmids containing the ColE1 replication origin are prepared, the yield can be improved by amplification in the presence of chloramphenicol (34 mg/ml). They should then be treated as high-copy-number plasmids.
- Add the provided RNase A solution to Buffer P1 before use (spin down RNase A briefly before use). Buffer P1 should then be stored at 2–8°C and is stable for 6 months.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- After use, the bottle containing Buffer P2 should be closed immediately to avoid any reaction between the NaOH and CO₂ in the air. If the buffer is left open for any length of time, it should be prepared fresh from stock solutions.
- Chill Buffer P3 at 4°C.
- Optional: To confirm purification or to identify a problem, samples may be taken at specific steps for analysis on an agarose gel. Appropriate samples and volumes are indicated in the protocol below.

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25 x TEA Buffer

1M Tris, 15mM EDTA, 125 mM Na acetate (pH 7.8) To 750 ml of distilled water add 121 g of Tris base, 10.2g of sodium acetate, 18.6 g of EDTA. Adjust the pH to 7.8 with glacial acetic acid and QS to 1 liter. Store at 4°C

Lysis

P1

50 mM Tris base	6.06 g
10 mM Na ₂ EDTA.2H ₂ O	3.72 g
HCl	to pH 8
H ₂ O	to 1000 ml

Dissolve the Tris and EDTA in 800 ml H₂O and adjust the pH to 8. Make the volume up to 1000 ml with H₂O.

Just prior to use, add RNaseA 50 µg/ml AND LyseBlue to the amount of P1 needed for that day.

P2

200 mM NaOH	8 g
1% SDS	10 g
H ₂ O	to 1000 ml

Dissolve NaOH in 800 ml of water, then add SDS while stirring with stir bead. Q.S to 1000ml with water.

P3

H ₂ O	500 ml
Potassium Acetate	294.5 g
Glacial Acetic Acid	110 ml
H ₂ O	to 1000 ml

Add the potassium acetate to 500 ml of H₂O and dissolve by agitation. Will be 3.0 M K Acetate buffer when finished. Add the glacial acetic acid slowly with gentle swirling and test the pH (it should be between 5.4 and 5.6). If not, pH using acetic acid. Make the volume up to 1000 ml with H₂O.

Using LyseBlue reagent (1000X = 43 mg/ml thymolphthalein in ethanol)

LyseBlue is a color indicator that provides visual identification of optimum buffer mixing. LyseBlue can be added to the resuspension buffer (Buffer P1) bottle before use. Alternatively, smaller amounts of LyseBlue can be added to aliquots of Buffer P1, enabling single plasmid preparations incorporating visual lysis control to be performed.

LyseBlue reagent should be added to Buffer P1 at a ratio of 1:1000 to achieve the required working concentration (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue precipitates after addition into Buffer P1. This precipitate will completely dissolve after addition of Buffer P2. Shake Buffer P1 before use to resuspend LyseBlue particles.

The plasmid preparation procedure is performed as usual. After addition of Buffer P2 to Buffer P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved. Upon addition of neutralization buffer (Buffer S3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.