



This is a simple and standard quick prep to isolate plasmid DNA. If using high copy DNA 1-2 ml culture will suffice. Use no more than 5 - 10 ml culture for trouble plasmids or low copy plasmids.

A key to success in protocols like this is to know *where the desired component of the reaction is located at all times*: the **supernatant** or the **pellet**. (In this case, plasmid DNA is the desired component.)

### Cell Culture - PREPARE 1-5 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.
- 2) **Culture overnight WITH SHAKING** at an angle at 37°C. This is best done after noon 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.

#### BEFORE STARTING:

- Add RNaseA and LyseBlue to P1 (see buffer instructions)
- Check Buffer P2 for SDS precipitation due to low storage temperatures (in winter, the room's temp will cause some ppt). If necessary, dissolve the SDS by warming to 37°C.
- Pre-Chill Buffer P3 at 4°C.

#### Bacterial Culture Information:

- From a freshly grown bacterial colony, select an isolated colony and inoculate 5.0 ml of LB media with the appropriate antibiotic in a loosely capped (use the plastic tube caps or cotton plug. *See the bacterial culture handout for antibiotic and media preparation information.*)
- Incubate the culture in a rolling mixer for 12 – 14 hours. Much longer and the cells will be too dense to work well and cells may have begun to lyse and die. Less time will result in poor or no DNA yield.

#### Alkaline Lysis Protocol (5 ml culture preparation)

1. **Resuspend pellet. Add 200 µl Buffer P1. Resuspend and transfer to a microcentrifuge tube.**
  - Ensure that RNaseA has been added to the buffer.
  - No clumps should be visible
  - If using Lyse Blue, ensure all particles are completely dissolved by vortexing buffer P1 PRIOR to use.
  - The bacteria should be resuspended completely by vortexing or pipetting up and down.
2. **Lyse cells. Add 200 µl Buffer P2 and mix by inverting the tube 4-6 times.**
  - Incubate at room temp for 5 min.
  - DO NOT VORTEX or vigorously invert– will shear genomic DNA causing contaminant in your elution.
  - DO NOT allow lysis reaction to go longer than 5 min.
  - Solution should turn blue. 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. **Precipitate protein and SDS with KOAc. Add 300 µl Buffer P3. Mix immediately and thoroughly by inverting 4-6 times.**
  - 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. 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. **Clarify Lysate:** Centrifuge at 10 min at 13,000 rpm (~17,900 x g) in a table-top centrifuge.

- Remember to leave the hinge of the tube on the outside edge of the centrifuge
  - A tight white pellet should form on the outside/side bottom of the tube. This is the cell debris, SDS-potassium ions and trapped genomic DNA. Plasmid DNA is in solution (supernate) of this centrifugation.
- 5. Precipitate Plasmid DNA: Precipitation of DNA with isopropanol should be carried out at room temperature in order to minimize salt precipitation.**
- Transfer supernatant to fresh-labeled tube. Supernate should be close to 700  $\mu$ l. Discard pellet.
  - Add 0.7 volume (490  $\mu$ l) of room temperature isopropanol and mix by inversion.
  - Centrifuge at max speed in microfuge for 20 min at room temp. Plasmid DNA will ppt.
  - Remove supernatant solution and use kimwipe to wick off remaining solution.
  - Carefully add 1 ml of ice cold 70% ethanol and resuspend the pellet by a short vortex.
  - Centrifuge to ppt the plasmid DNA at max rpm ( $\sim$ 17,900 x g) for 30 min at 4°C.
  - The pellet will be difficult to see (some describe it as glass) and carefully remove ethanol. Close the cap and invert or "flick" the tube with your finger. Then recentrifuge as before. The 70% ethanol serves to remove precipitated salt, as well as to replace isopropanol with the more volatile ethanol, making the DNA easier to redissolve. You may need to tip the tube upside down on Kimwipes to remove residual ethanol.
  - Marking the outside of the tube before centrifugation allows the pellet to be easily located. *Watch the tube hinge placement for identification of the pellet.*

**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 CO<sub>2</sub> 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.

### **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<sub>2</sub>EDTA.2H<sub>2</sub>O   3.72 g  
HCl                            to pH 8  
H<sub>2</sub>O                            to 1000 ml

*Dissolve the Tris and EDTA in 800 ml H<sub>2</sub>O and adjust the pH to 8. Make the volume up to 1000 ml with H<sub>2</sub>O.*

**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<sub>2</sub>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<sub>2</sub>O                            500 ml  
Potassium Acetate        294.5 g  
Glacial Acetic Acid        110 ml  
H<sub>2</sub>O                            to 1000 ml

*Add the potassium acetate to 500 ml of H<sub>2</sub>O 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 H<sub>2</sub>O.*

### **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.