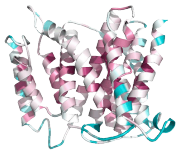
Plasmid Purification
DMAE Protocol



INTRODUCTION

DMAE is an ion exchanger (pH values of the functional groups of the resin is 1.5 lower than DEAE) which is well suited for binding acidic biopolymers like DNA. Thus the negatively charged DNA will bind to the resin in buffers lower pH (6.4). We use MOPS for low pH buffers as pH of Tris can drift upwards and may cause elution or inhibit binding.

500 ml preps will require 8 ml columns while 16 ml columns should be used for 1 liter preps. The following protocol is for 1 liter cultures. The binding capacity is 1.5 – 2.0 mg per cc of resin.

PREPARATION

- 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 37oC.
- Pre-Chill Buffer P3 at 4oC.
- Use FRESH cultures from a newly inoculated plate. The plate should be no more than one week old.
- Prepare a water bath at 42oC if starting with comp cells.

PROTOCOL

Culture (1 liter preparation)

1. **Transform (Day One - start at 2 pm finish at 8 am)** :Transform using Top 10 Chemically Competent E. coli (cat C4040-10)cells from invitrogen. These are DH10B strain geneotype cells and good for plasmid purification. 5 ul of cells per 2 ul of DNA.
   - Thaw vial of comp cells on ice. DO NOT LET WARM. Transfer unused cells to CHILLED microfuge tube (10 ul aliquots) and replace in -80oC freezer.
   - Pipet 2 ul plasmid DNA to 5 ul comp cells. Mix by tapping gently. Do not mix by pipetting.
   - Incubate the vial on ice for 30 min.
   - Heat shock at 42oC. Do not mix or shake. Place on ice following heat shock.
   - Add 50 ul of pre-warmed sterile SOC.
   - Place vial in microfuge rack on its side and secure with tape to avoid loss of the vial. Shake the vial at 37oC for exactly one hour at 225 rpm in a shaking incubator.
   - Spread the entire transformation on a labeled LB agar plate with the appropriate antibiotic. Invert plates and incubate 6 hrs to overnight.

2. **Culture (Day Two – Start at 8 am 4-6 hours for starter followed with overnight incubation):** From a fresh colony, start two, 10ml LB cultures containing antibiotic. Use a 50 ml falcon tube with the cap loose. Place in a tube rack on shaker.
   - OPTIONAL: If time, at the end of the 4 hr incubation, spin down 1 ml of culture and perform a Qiagen mini prep spin purification. Use the results of an agarose electrophoresis (10 ul of eluted sample) to determine the correct size plasmid with highest yield. This is not necessary for most preparations. However, when getting low yields (less than 1 mg) this step should be conducted.
   - Around 3 pm, expand 2 ml of starter culture to 1 liter of LB media in 2 liter flask. Only need 500 ml if using MY media.
   - Incubate for 16 hours (should be finished around 7-8 am). Grow at 37oC. Shake at ~300 RPM.
   - Centrifuge at 4000 rpm in 250 ml centrifuge bottles for 15 min. Combine pellets into one bottle. Remove all traces of supernatent. Record mass of pellet. A 1 liter preparation should yield 6-7 g of wet weight. Freeze at this point or continue to lysis and purification.

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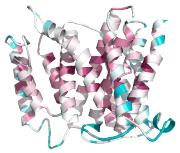

Lysis (1 liter preparation)

1. **Resuspend pellet**. Add 15 ml of P1 per gram of pellet. Use a 500 ml centrifuge bottle.
   - The bacteria should be resuspended completely by vortexing or pipetting up and down or place in the shaker incubator until NO cell clumps remain.

2. **Lyse cells**. Add 15 ml of P2 per gram of pellet. Mix by inverting the tube 4-6 times, and incubate at room temp for 5 min.
   - DO NOT VORTEX or vigorously invert– will shear genomic DNA which will be found as a 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 15 ml of P3 per gram of pellet. Mix immediately and thoroughly by vigorously 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 PPT.
   - Filter the flocculent material with cheesecloth.
   - Divide sample between two 250 ml conical bottles.
   - Centrifuge at 5000 RPM for 15 min (4500g) to remove most of the ppt.
   - Filter clarified lysate through a Whatman GF/A filter (cat 1600-820) using a vacuum bottle.
   - *SAVE* 50 ul of lysate for gel analysis.

5. **Endotoxin Removal Buffer**: Add 1/10th volume of Buffer ER to the cleared lysate and incubate on ice for 30 min. After addition of Buffer ER< the lysate may appear turbid, but will become clear again during the incubation on ice.

Chromatography (1 liter preparation)

1. **Prepare Column:**
   - To pack a column, obtain two polyethylene frits and one 50 ml column.
   - Place one frit in the bottom of a closed column. Flow 100 ml of storage buffer through the empty column. Soak the top frit in storage solution for 10 min prior to use. This will aid in improving flow rate.
   - Fill with 16 ml of water. Indicate the level on the column with a marker.
   - Add 32 ml of 50% DMAE slurry to the column, allow the resin to settle for a few minutes and open the column. Add EQB buffer if needed to avoid the column from going dry.
   - Add or remove resin to reach the 16 ml mark and close column.
   - Add frit to top of resin. Do not compress the resin bed, your flow rate will slow down considerably.
   - Equilibrate the column by washing 150 ml of EQB through the column. Do not let go dry.
   - Store with several ml of EQB buffer for no more than two days.

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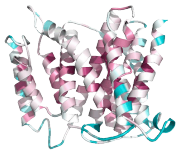

2. **Purification**: Be careful to avoid running the column dry.
   - Apply the clarified lysate to the column. Save 50 ul if you wish to analyze flow through.
   - Wash the column with 150 ml of EQB.
   - Elute with 100 ml of Elution Buffer (45°C).

3. **DNA Precipitation**:
   - Add an equal volume of isopropyl alcohol (IPA) to the eluted DNA fraction. Mix by inversion.
   - Store at -20oC for one hour.
   - Centrifuge at 5000 RPM in sterile 50 ml falcon tubes for 30 min.
   - Decant supernatent.
   - Swirl 10 ml of 70% ethanol into each 50 ml falcon tube.
   - Combine into one tube.
   - Centrifuge at 5000 RPM in swinging bucket rotor for 30 min.
   - IN STERILE HOOD!!!!! Decant supernatent and tip upside down to drain.
   - Dry using filtered N2 gas IN HOOD.
   - Add 1 – 2 ml of Endofree water or TE. Swirl (light vortexing) to dislodge plasmid pellet.
   - Allow to sit at room temp overnight to solubilize plasmid pellet.
   - Determine DNA concentration – if needed dilute DNA to 1 mg/ml using sterile water or sterile TE.

4. **Regeneration**: Wash the column with the following solutions
   - 100 ml Base Sans Solution
   - 200 ml water
   - 100 ml Acid Sans Solution
   - 200 ml water
   - 150 ml Storage Buffer

BUFFERS
Lysis

P1		P2		P3	
50 mM Tris base	6.06 g	200 mM NaOH	8 g	H_2O	500 ml
10 mM $Na_2EDTA.2H_2O$	3.72 g	1% SDS	10 g	Potassium Acetate	294.5 g
HCl	*to pH 8*	H_2O	*to 1000 ml*	Glacial Acetic Acid	110 ml
H_2O	*to 1000 ml*			H_2O	*to 1000 ml*
Dissolve the Tris and EDTA in 800 ml H_2O and adjust the pH to 8. Make the volume up to 1000 ml with H_2O.		*Dissolve NaOH in 800 ml of water, then add SDS while stirring with stir bead. Q.S to 1000ml with water.*		*Add the potassium acetate to 500 ml of H_2O and dissolve by agitation. 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_2O.*	
Just prior to use, add RNAseA 50 ug/ml AND LyseBlue to the amount of P1 needed for that day.					

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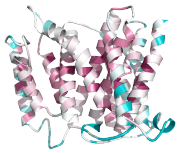

Purification

EQB 50 mM MOPS, pH 6.40 630 mM Sodium Chloride 15 % IPA	**ELUTION** 50 mM Tris-HCL, pH 8.7 1.5 M NaCl	**ER** 50 mM MOPS pH 6.40 700 mM Sodium Chloride 2% Triton X-114 15% IPA
Acid Sans Soln 0.5N HCL 2M NaCl	**Base Sans Soln** 0.5 M NaOH 2M NaCl	**STORAGE** 150 mM Sodium Chloride 20% EtOH

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.