Protocol A: Preparation of endotoxin-free carrier DNA for transfection

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Abstract

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Protocol

Grow an overnight culture of DH5alpha E. coli transformed with pUC19 in LB

Step 1.

• Transform plasmid according to standard protocols
• Luria-Bertani broth (Miller Formula) with 50 µg/ml carbenecillin or 100 µg/ml ampicillin
• Grow at 37°C

Grow 1 l cultures of transformed E. coli in superbroth from small overnight culture

Step 2.

• 1L Superbroth: 15 mM MgCl2, 32 g Peptone, 20 g Yeast Extract, 5 g NaCl
• Innoculate 2 ml of overnight culture per liter of superbroth
• Grow in 2.8-liter, baffled Fernbach flasks at 37°C and 200 rpm for 16 hours

Harvest cells by spinning at >3500xg for 15 min and 4°C

Step 3.

• Discard supernatant
• Use immediately for DNA purification or store long term by flash freezing pelletin liquid nitrogen and storing at -80°C

Purify DNA using Qiagen or Zymo Gigaprep Kit

Step 4.

• Qiagen Gigaprep Kit Cat No. 12191
• Zymo Gigaprep Kit Cat No. D4204
• Purify according to manufacturers instructions

Concentrate DNA with and ethanol precipitation

Step 5.

• Add one-tenth the volume of 3 M Sodium Acetate, pH 5.2 to one volume of DNA
• Add 2.5 volumes of ethanol to the solution of DNA and sodium acetate
• Mix well
• Pellet DNA by spinning for 30 min at 20,000xg and 4°C

• Discard supernatant
• Resuspend pellet in one-tenth the original volume with 10 mM Tris-Acetate, pH 8.0
• If some DNA is still not soluble, spin the sample for 10 min at 20,000xg and 4°C and keep the supernatant

Remove residual endotoxin (lipopolysaccharide) with a PEG precipitation of DNA

Step 6.
• To 100 µl restriction of DNA, add 750 µl of 5 M NaCl and 750 µl of 40% (w/v) PEG 8000 and mix well.
• Incubate at -20°C for ≥1 h
• Pellet DNA by spinning for 30 min at 20,000xg and 4°C.
• Remove supernatant and resuspend pellet in 100 µl of 10 mM Tris-Acetate, pH 8.0
• Repeat PEG precipitation.
• After the second precipitation, resuspend the plasmid in 200 µl of 300 mM Sodium Acetate, pH 5.2
• Add 500 µl of 200 proof ethanol
• Incubate at -20°C for 30 min
• Pellet DNA by spinning for 30 min at 20,000xg and 4°C
• Discard superntant and resuspend the pellet in a minimal volume of 10 mM Tris-acetate, pH 8.0 that has been filtered through a 0.22 µm filter

Quantify DNA and resuspend to a final concentration of 20 µg/µl

Step 7.
• Prepare a small aliquot of twenty-fold dilution of DNA for spectrophotometry
• Quantify the DNA by UV-Vis spectrophotometry using the approximation of 1 OD at 260 nm = 50 ng/µl of DNA
• Adjust the volume of DNA for final conentration of 20 µg/µl.

Store DNA at -20°C in 200 µl aliquots

Step 8.

HIGHLY RECOMMENDED: Purchase carrier DNA from a company

Step 9.
It is far more cost and time effective to purchase DNA from a manufacturer according to your specifications. We have previously purchased DNA from Nature Technology (http://www.natx.com). Please note that *S. rosetta* is very senstive to EDTA. Make sure to have your DNA resuspended in a buffer without EDTA