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Enhancing yields of low and single copy number plasmid DNAs from *Escherichia coli* cells

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ABSTRACT

Many plasmids used for gene cloning and heterologous protein expression in *Escherichia coli* cells are low copy number or single copy number plasmids. The extraction of these types of plasmids from small bacterial cell cultures produces low DNA yields. In this study, we have quantitated yields of low copy and single copy number plasmid DNAs after growth of cells in four widely used broths (SB, SOC, TB, and 2xYT) and compared results to those obtained with LB, the most common *E. coli* cell growth medium. TB (terrific broth) consistently generated the greatest amount of plasmid DNA, in agreement with its ability to produce higher cell titers. The superiority of TB was primarily due to its high levels of yeast extract (24 g/L) and was independent of glycerol, a unique component of this broth. Interestingly, simply preparing LB with similarly high levels of yeast extract (LB24 broth) resulted in plasmid yields that were equivalent to those of TB. By contrast, increasing ampicillin concentration to enhance plasmid retention did not improve plasmid DNA recovery. These experiments demonstrate that yields of low and single copy number plasmid DNAs from minipreps can be strongly enhanced using simple and inexpensive media.

1. Introduction

Molecular biologists routinely use circular plasmids propagated in *Escherichia coli* cells for cloning, expression, sequencing, and mutagenesis of genes. These plasmids are commonly classified into three groups—high copy number (corresponding to hundreds of plasmid DNA molecules per cell), low copy number (typically less than fifty plasmids per cell), and single copy number (approximately one per cell) (Summers, 1998; Feinbaum, 2002; Nordström and Dasgupta, 2006). Most vectors used for simple DNA fragment cloning are high copy number plasmids. The extraction of such plasmids from *E. coli* cells produces high yields, and the DNAs are frequently purified from 1.5 mL of overnight cultures of bacterial cells (referred to as plasmid minipreps). By contrast, many vectors used for expression of proteins are low copy number plasmids. Having reduced copy number (lower gene dosage) can decrease basal, or uninduced, intracellular levels of an expressed protein, which is advantageous because some foreign proteins are toxic even at very low levels and therefore can inhibit growth of the *E. coli* cells. Examples of such plasmids include the widely used pET series of vectors (Novagen), IMPACT system vectors (New England Biolabs), HaloTag vectors (Promega), and several others (Dersch et al., 1994; Mardanov et al., 2007; Cheong et al., 2013; Guan et al., 2013). Yields of these plasmids are greatly reduced compared to high copy number plasmids and therefore larger cell cultures must be inoculated in order to extract sufficient amounts of DNA for detection and manipulation. Plasmids employed for some other procedures such as genomic DNA cloning and sequencing are also low copy or even single copy number plasmids and therefore also produce low DNA yields upon extraction.

The most commonly used liquid medium for growth of *E. coli* cells is LB (Lysogeny Broth), but several other broths have been developed over the years and employed for specific procedures. Examples of these other media include SB (Super Broth), SOC (Super Optimal broth with Catabolite repression), TB (Terrific Broth), and 2xYT (Yeast extract Tryptone) (Tartoff and Hobbs, 1987; Elbing and Brent, 2002; Green and Sambrook, 2012; Lessard, 2013). SOC is added to *E. coli* cells to aid recovery after exposure to high salt concentrations and heat shock in many chemical-based DNA transformation protocols and 2xYT is used in many bacteriophage infection protocols (Elbing and Brent, 2002; Green and Sambrook, 2012). All of these growth media share two components, tryptone and yeast extract, but the amounts of the two nutrients within each broth are variable. Some of the broths contain specific additional chemicals that are beneficial to cell growth such as glycerol, glucose and/or salts.

In the current study, we have tested five well-established *E. coli* broths to determine which one produces the highest yields of low copy and single copy number plasmid DNAs from 1.5 mL minipreps. TB broth consistently produced the greatest DNA yields. The strong

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results with TB were found to be independent of its glycerol content and, instead, were primarily due to its high concentration of yeast extract. Adding increasing amounts of yeast extract to LB broth produced higher DNA yields in a concentration-dependent manner. The results demonstrate that yields of both low and single copy number plasmids isolated using standard alkaline lysis minipreps can be strongly enhanced using simple, inexpensive media containing elevated levels of yeast extract.

2. Materials and methods

2.1. Strains, plasmids and reagents

E. coli Top10 cells containing either the low copy number plasmid pET15b-rKGA (Farrell and Taylor, 2005), the single copy number plasmid pBeloBAC11 (Tillett and Neilan, 1998) (obtained from New England Biolabs), or the high copy number plasmid pRS425 (Christianson et al., 1992) were used for all experiments. Yeast extract (VWR 90004-092) was produced by BD Biosciences and tryptone (VWR 97063-424) was from Amresco. Ampicillin (VWR IB02040) was sold by IBI Scientific. All broths were prepared according to the descriptions published in Current Protocols in Molecular Biology (Elbing and Brent, 2002). Recipes used to prepare 1 L of each broth were the following:

LB: 10 g tryptone, 5 g yeast extract, 5 g NaCl, 1 mL 1 M NaOH.
LB24: 10 g tryptone, 24 g yeast extract, 5 g NaCl, 1 mL 1 M NaOH.
TB: 12 g tryptone, 24 g yeast extract, 4 mL glycerol, 100 mL 0.17 M KH_2PO_4/0.72 M K_2HPO_4.
SB: 32 g tryptone, 20 g yeast extract, 5 g NaCl, 5 mL 1 M NaOH.
2xYT: 16 g tryptone, 10 g yeast extract, 5 g NaCl.
SOC: 20 g tryptone, 5 g yeast extract, 0.58 g NaCl, 3.6 g glucose, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$.

2.2. DNA fluorometry, gel electrophoresis, and cell culture density determination

Cell culture densities were monitored by measuring light scattering at 550 nm using a BioRad Smartspec spectrophotometer after diluting overnight cultures 1:20 into water. DNA fluorometry was performed using a Qubit 2.0 fluorometer (Life Technologies-Invitrogen) in conjunction with the broad range assay protocol. Electrophoretic analysis of purified DNAs employed Horizon 11–14 rigs (Labrepco), 1 × TAE electrophoresis buffer and 0.7–0.8% agarose gels run at approximately 130 V. Gels were stained with ethidium bromide, and photographs were taken using an Alpha Innotech RED instrument.

2.3. Plasmid DNA miniprep protocols

Most plasmid minipreps were performed using a standard alkaline lysis protocol (Elbing and Brent, 2002; Green and Sambrook, 2012) in conjunction with 2–4 mL cell cultures containing 100 μg/mL ampicillin that were shaken overnight (20 — 22h) at ~250 rpm at 37 °C in glass tubes (20 × 150, VWR 47729-584) or plastic snapcap tubes (17 × 100, VWR 60818-725). Briefly, standard alkaline lysis minipreps involved pelleting of 1.5 mL cells in a microcentrifuge for 20 s followed by addition of cold Solution I (50 mM glucose, 25 mM Tris [pH 8.0], 10 mM EDTA), fresh Solution II (0.2 M NaOH, 1% SDS), and cold Solution III (3 M KOAc). After proteins and cell debris were sedimented for 10 min at 21,000 g, supernatants were transferred to a new tube, removing only the liquid that was ~4 mm above the protein pellet to minimize chromosomal DNA contamination. The DNA was precipitated by

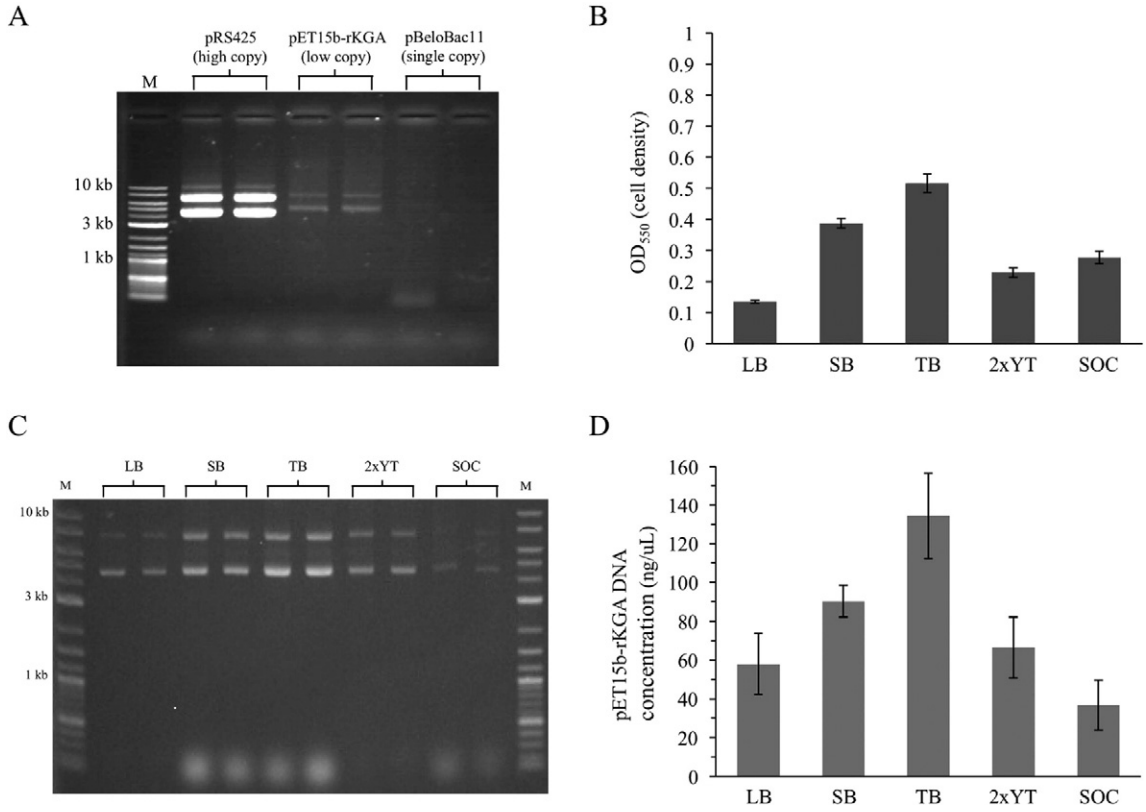

Fig. 1. Assessment of cell culture densities and yields from 1.5 mL plasmid minipreps after propagation in five common broths. (A) Use of the most common broth, LB, produces generous amounts of high copy number DNAs, but not low or single copy number plasmids. Two microliters of each 50 μL prep was loaded onto a 0.7% agarose gel. Results from two independent cultures are shown for each plasmid. (B) TB broth cultures had the highest cell densities after shaking overnight at 37 °C. (C and D) Electrophoresis and DNA fluorometry of plasmid DNAs extracted from each overnight culture by alkaline lysis. Results in B–D are averages of 4 independent cultures. Error bars indicate standard deviations.