Development of a New Method for Rapid Purification and Analysis of Circular Plasmid DNAs from Yeast Cells

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Abstract

It is common practice to purify circular plasmid DNAs from small cultures of *E. coli* bacterial cells for subsequent analysis by gel electrophoresis. By contrast, extraction and electrophoretic analysis of circular DNAs from small cultures of yeast and other eukaryotic cells is difficult because of the small numbers of these molecules inside cells. Existing methods employed in budding yeast (*S. cerevisiae*) require extraction from a large volume of cultured cells; the partially purified plasmids are then typically transformed into *E. coli* cells and extracted via plasmid DNA minipreps for gel analysis. In the current study, we have developed a new method for extracting plasmids from small yeast cell cultures that permits visualization of the circular DNAs by electrophoresis. The new approach was shown to be superior to two other common yeast DNA extraction methods, as it produced more plasmid DNA and less contaminating chromosomal DNA and RNA. The method was tested by cloning a *RAD52* gene-containing fragment into an expression vector inside yeast cells. DNAs from the transformants were then analyzed directly by gel electrophoresis after performing yeast minipreps. The new method allows yeast plasmids to be analyzed quickly, eliminating the requirement for subsequent transformation into *E. coli* cells.

Introduction

The haploid *S. cerevisiae* cell nucleus comprises ~14,000 kilobases (kb) of DNA, distributed among 16 linear chromosomal DNAs ranging from 250 to 2000 kb (1). Additionally, some strains harbor 50 to 60 copies of a 6.3-kb plasmid, known as the 2-µm plasmid (1). Extracting chromosomal DNA from yeast cells involves various methods to disrupt yeast cells and release DNA, followed by protein removal and precipitation of DNA using ethanol or isopropanol (2). Subsequent treatment with RNase enzymes eliminates any remaining small RNA molecules (3). Unlike bacterial protocols, purifying plasmid DNAs from small yeast cultures presents challenges due to low levels of the plasmids and high levels of chromosomal DNA and RNA contaminants. In this study, we developed a method for extracting plasmids from small yeast cultures (minipreps) that allows immediate visualization of circular DNAs via gel electrophoresis. This method increased plasmid DNA yield and reduced contamination by chromosomal DNA and RNA.

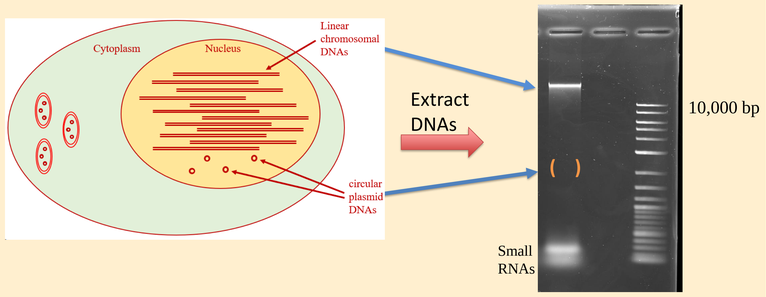

Figure 1. Eukaryotic cells contain large chromosomal DNAs and small circular DNAs. The circular DNAs are difficult to detect on a standard agarose gel because their mass is much lower than that of chromosomal DNA.

Goals of Project

- The primary goal of this project was to develop and test a new rapid method for isolating plasmids from small yeast cell cultures that allow immediate visualization of circular plasmid DNAs by gel electrophoresis.
- Several variables were tested, and a new method was developed to lyse cells with lyticase and the detergent SDS, remove proteins with potassium acetate, and concentrate DNA with isopropanol. Samples were treated with RNase twice.

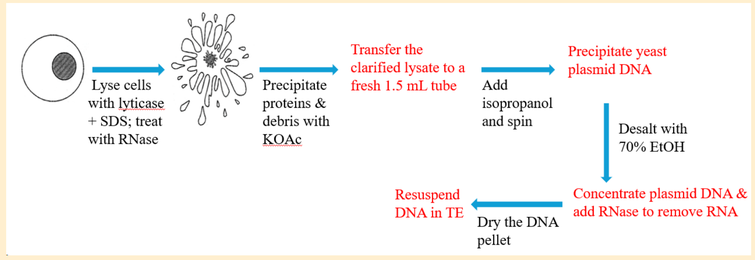

Figure 2. Steps of the new method. Yeast cells were lysed with lyticase and SDS detergent, treated with RNase, and then incubated with heat. Proteins were precipitated with potassium acetate and isopropanol was used to precipitate DNA. Plasmid DNA was concentrated by desalting and removing RNAs. Finally, DNA was resuspended in TE buffer & RNased again.

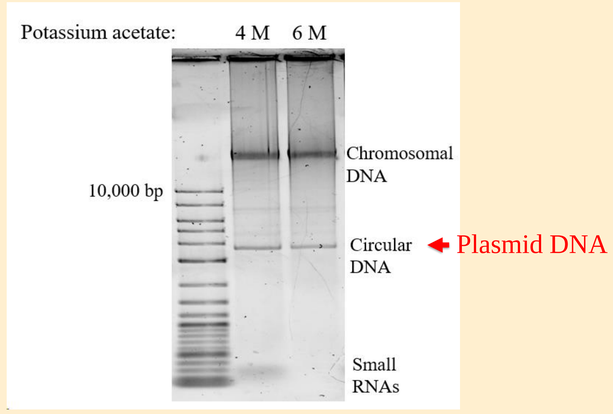

Figure 3. Example of a test to determine how much Potassium Acetate was optimum. Removal of proteins with 6 M KOAc removed more RNA.

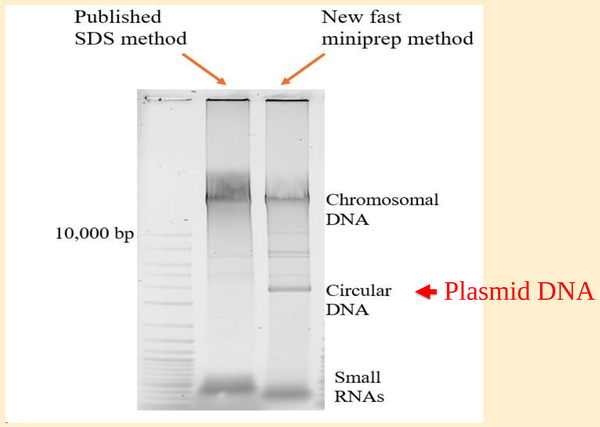

Figure 4. Our new fast miniprep method yields more plasmid DNA and less chromosomal DNA than a previously published SDS (SET) method (4).

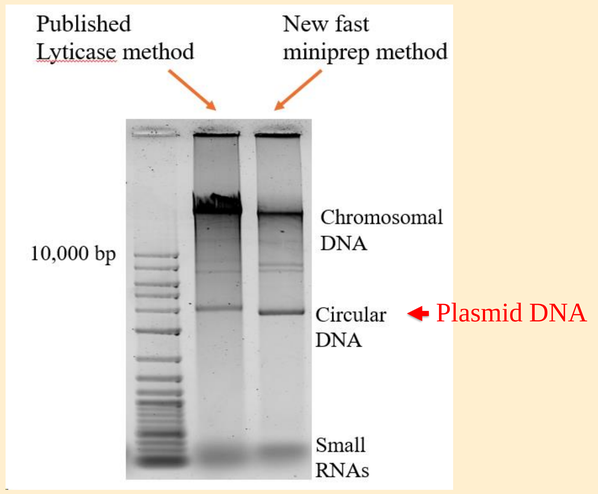

Figure 5. The new fast miniprep method also yields more circular plasmid DNA and less chromosomal DNA than a previously published method that also used Lyticase (5). The new miniprep method was much faster.

Conclusions

- The new fast miniprep method surpasses existing methods in isolating circular plasmids from small yeast cell cultures for analysis by gel electrophoresis
- 6 M potassium acetate was better for eliminating RNAs than 4 M, did not improve yields of circular DNA
- The new method was much better at producing detectable circular plasmid DNA than a previously published SDS method (the SET method)
- The new fast method generated more plasmid DNA with less contaminating chromosomal DNA and RNA and took much less time than a well-established Lyticase method

References

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