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Are Enzymes Required for the Extraction and Isolation of DNA in Conjunction with Mechanical Homogenization?

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Introduction

Enzymes are biological catalysts that play a role in the isolation of nucleic acids from tissue and microbial sample types. In 1973, Maria Gross-Bellard was one of the first researchers to use Proteinase K in the isolation of DNA from mammalian cells[1]. Today, enzymes are commonly provided in DNA isolation kits to enable cellular and organelle disruption and for the removal of contaminating proteins. DNA kit enzymes vary based on the target sample. While Proteinase K is commonly used in the isolation of DNA from mammalian cells and tissues, lyticase and lysozyme areenzymes used to degrade the cell walls of yeast and bacteria and are frequently included in microbial DNA isolation kits. While effective, enzymes represent a significant percentage of DNA kit cost and require special storage and handling conditions. While standard lysis buffers coupled with enzymes can be used to disrupt soft tissues and cells, mechanical homogenizers are routinely employed for disruption of tougher tissues and cells with robust cell walls. In these cases, are enzymes necessary to obtain high concentrations of DNA?

In this study, DNA extractions were performed using commercially available DNA extraction kits from three sample types: bacteria, yeast and tissues. DNA was extracted per the kit manufacturer's instructions and in conjunction with mechanical homogenization via bead milling. The analysis was then repeated without the use of enzymes DNA concentration and quality was evaluated by spectrophotometry and electrophoresis respectively for each sample type and processing method.

Methods

Frozen *Rattus norvegicus* brain tissue (Bioreclеamation) was cut into five 30 mg sections. *Escherichia coli (E. coli)* was grown overnight in LB broth at 37°C. *E. coli* cells were isolated in 3 mL of media. *Saccharomyces cerevisiae (S. cerevisiae)* was grown in Sabouraud Dextrose (SBD) broth for one week at 30°C. *S. cerevisiae* cells were isolated in 3 mL of media.

DNA EXTRACTION

DNA extraction was performed using three Omni Bead Mill DNA Purification kits specific for tissues, bacteria cells and yeast cells (Cat # 26-007, 26-008, and 26-009). Sample dissociation for each kit is performed through bead mill homogenization and enzyme treatment as described in table 1. To determine the effect of combining enzyme treatment with mechanical homogenization, DNA was extracted using only mechanical homogenization and mechanical homogenization combined with a series of different enzyme treatments as described in table 2.

DNA for all samples was eluted in 100 µL of EB buffer and concentration was determined on the Nanodrop 2000 (Thermo Fisher Scientific) as seen in table 2.

AGAROSE GEL ELECTROPHORESIS

For all samples, corresponding DNA and TBE/Urea loading buffer (Table 2) was added to a 1% agarose gel and electrophoresed at 140 V for 45 minutes. Gels were stained with Ethidium Bromide (EtBr) in DI water for 20 minutes and visualized on a Gel-Doc EZ system (Biorad) as seen in Figures 1 - 2.

PCR AMPLIFICATION

Bacteria and yeast DNA samples that were digested enzymatically with mechanical homogenization were diluted to 1 pg/µL.

Conserved regions of the 16S and 18S rDNA gene were detected via PCR using Molzym's universal 16S and 18S rDNA primers respectively. To one tube for bacterial gene amplification, 1 µL bacterial genomic DNA, 10 µL 2.5X Mastermix 16S, 0.8 µL MolTaq, and 14 µL DI water. To one tube for yeast gene amplification, 1 µL yeast genomic DNA, 10 µL 2.5X Mastermix 18S, 0.8 µL MolTaq, and 14 µL DI water. Samples were placed in a T-100™ Thermo cycler (Biorad) and ran according to Table 3. 9 µL of each sample with 1 µL of DNA staining solution and 2 µL of gel loading dye along with 5 µL of 1Kb prestained ladder were loaded to a 2% agarose gel and electrophoresed at 140 V for 45 minutes. Gels were stained with Ethidium Bromide (EtBr) in DI water for 20 minutes and visualized on a Gel-Doc EZ system (Figure 3). The amplified regions (approximately 450 bp and 310 bp for bacteria and yeast respectively) were confirmed.

Table 1: Sample DNA Extraction Methods

Sample ID	Method	Settings (Bead Ruptor)	Enzyme	Duration
Rat Small Intestine	Enzymatic only	N/A	Proteinase K	2 hrs
Rat Small Intestine	Mechanical + Enzymatic	5 m/s x 10 sec	Proteinase K	1 hrs
Rat Small Intestine	Mechanical + Enzymatic	5 m/s x 10 sec	Proteinase K	30 min
Rat Small Intestine	Mechanical only	5 m/s x 10 sec	N/A	N/A
E. coli	Enzymatic only	N/A	Lysozyme + Proteinase K	10 min, 1 hr
E. coli	Mechanical + Enzymatic	4.35 m/s x 45 sec	Lysozyme + Proteinase K	10 min, 1 hr
E. coli	Mechanical + Enzymatic	4.35 m/s x 45 sec	Lysozyme + Proteinase K	5 min, 30 min
E. coli	Mechanical + Enzymatic	4.35 m/s x 45 sec	Lysozyme	10 min
E. coli	Mechanical + Enzymatic	4.35 m/s x 45 sec	Proteinase K	1 hr
E. coli	Mechanical only	4.35 m/s x 45 sec	N/A	N/A
S. cerevisiae	Enzymatic only	N/A	Lyticase + Proteinase K	30 min, 1 hr
S. cerevisiae	Mechanical + Enzymatic	5 m/s for 2 x 30 sec, 30s dwell	Lyticase + Proteinase K	30 min, 1 hr
S. cerevisiae	Mechanical + Enzymatic	5 m/s for 2 x 30 sec, 30s dwell	Lyticase + Proteinase K	15 min, 30 min
S. cerevisiae	Mechanical + Enzymatic	5 m/s for 2 x 30 sec, 30s dwell	Lyticase	30 min
S. cerevisiae	Mechanical + Enzymatic	5 m/s for 2 x 30 sec, 30s dwell	Proteinase K	1 hr
S. cerevisiae	Mechanical only	5 m/s for 2 x 30 sec, 30s dwell	N/A	N/A

Methods (cont)

Table 2: DNA Yields vs. Extraction Process

Sample	Extraction Method	Avg. Concentration (ng/µL)	Avg. 260/280
S. cerevisiae	Mechanical + Lyticase +Proteinase K	17	1.84
S. cerevisiae	Mechanical + Lyticase	9.8	1.69
S. cerevisiae	Mechanical + Proteinase K	6.8	1.55
S. cerevisiae	Mechanical + lyticase (1/2) + Proteinase K (1/2	12	1.70
S. cerevisiae	Mechanical only	6.0	1.65
Rat small intestine	Mechanical +Proteinase K	1186	1.88
Rat small intestine	Mechanical +Proteinase K (1/2)	971.2	1.87
Rat small intestine	Mechanical only	810.6	1.88
E. coli	Mechanical + Lysozyme +Proteinase K	48.5	1.85
E. coli	Mechanical + Lysozyme	30.1	1.90
E. coli	Mechanical + Proteinase K	20.3	1.93
E. coli	MechanicL + Lysozyme (1/2) + Proteinase K (1/2)	50.1	1.81
E. coli	Mechanical only	39.1	1.92

Table 3: Thermo cycler Directions

Temperature	Time	Cycles
95°C	1 min	
95°C	5 sec	40
55°C	5 sec	
72°C	25 sec	
4°C	Hold	

Results

Herein, we evaluated the requirement of enzymes in the extraction of DNA in conjunction with mechanical homogenization using the Omni Bead Mill Nucleic Acid Purification Kits. Three different sample types were evaluated E. col , S. cerevisiae and rat intestine. In all cases, DNA was extracted from the samples per the manufacturer's recommendations with and without the use of enzymes in conjunction with mechanical homogenization via bead milling (Table 1). DNA concentrations were observed to decrease as enzymes digestions steps were removed from the extraction process. With the exception of E. coli, mechanical homogenization alone (without the addition of enzymes) produced the lowest yields. For S. cerevisiae and rat intestine the addition of the secondary enzyme incubation (Proteinase K and Lyticase) increase DNA yield by 45%. The addition of a secondary digestion step in the E. coli and S. cerevisiae increased DNA by 2.2% and 91% respectively when compared to mechanical dissociation alone. Reducing the enzyme incubation time for ½ (total time savings 1 hr) reduced DNA yield by 18% and 29% for rat intestine and S. cerevisiae samples respectively. Reducing the incubation time for the E. coli samples did not reduce DNA yields (Table 2).

To evaluate genomic DNA integrity all samples were separated and visualized on a 1% argarose gel (Figures 1-2). For the E. coli preparation, samples extracted with the use of mechanical homogenization and lysozyme exhibited the least amount of DNA shearing relative to the control (no mechanical dissociation). S. cerevisiae samples subjected to mechanical disruption showed the most amount of DNA shearing relative to the control.

To evaluate if the DNA was suitable for PCR, E. coli and S. cerevisiae samples were subjected to PCR amplification of a conserved 16S and 18S gene. Figure 3 shows the resulting 2% agarose gel that was stained with EtBr showing tight bands of the 16S and 18S rDNA genes at 450 and 310 bp for the E. coli and S. cerevisiae samples in lanes 3 and 4.

Conclusions

- Suitable DNA for PCR can be obtained from samples without long enzymatic incubation steps
- Mechanically disrupting samples without the use of enzymes decreases overall extraction time

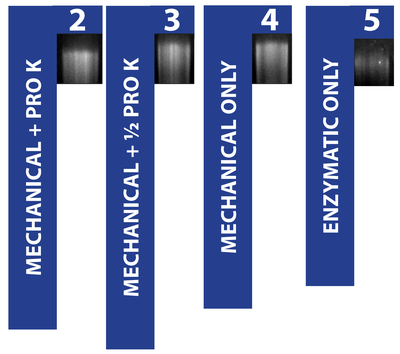

Figure 1: DNA extraction of Rat small intestine samples

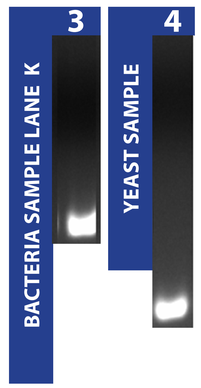

Figure 3: PCR amplified 16S and 18S rDNA genes of bacteria and yeast samples

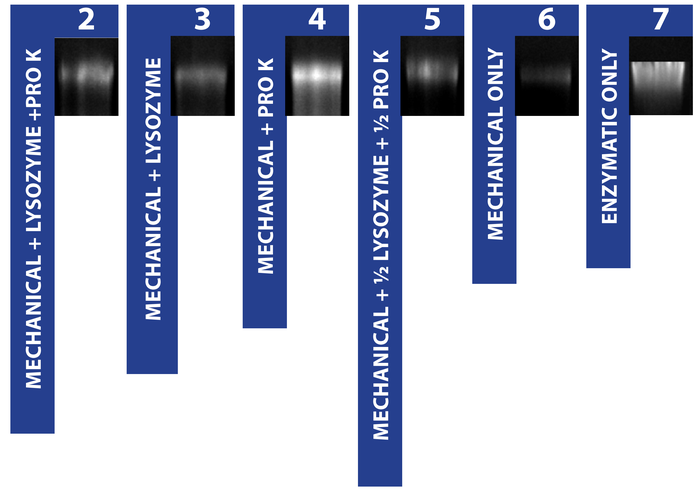

Figure 2: DNA extraction of bacteria samples

References

1. *Gross-Bellard, Maria, Pierre Oudet, and Pierre Chambon. "Isolation of High-Molecular-Weight DNA from Mammalian Cells." European Journal of Biochemistry 36.1 (1973): 32-38.*