Short Technical Reports

Nucleic acid contamination of glycogen used in nucleic acid precipitation and assessment of linear polyacrylamide as an alternative co-precipitant

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BioTechniques 47:1019-1022 (December 2009) doi 10.2144/000113276
Keywords: glycogen; linear polyacrylamide; co-precipitant; contamination; nucleic acid; carrier; stable-isotope probing; genomics

Molecular-grade glycogen is widely used to recover nanogram or picogram quantities of DNA and RNA across molecular biology applications in the life sciences. As a result, its purity is critical to obtain reliable results. Using agarose gel electrophoresis, we detected pg/µL (DNA) to ng/µL (RNA) concentrations of nucleic acid in two of the nine glycogen samples obtained from commercial suppliers. Denaturing gradient gel electrophoresis of 16S rRNA gene PCR-amplified products indicated that an additional two samples contained detectable contamination. We also tested a synthetic polymer co-precipitant, linear polyacrylamide (LPA); none of the four samples tested with LPA were detectably contaminated. The partial 16S rRNA gene sequence associated with the contaminated samples of the shellfish-derived glycogen was nearly identical to the sequence of *Actinobacteria lwoffii*, which has been isolated from mussels previously. By testing the recovery of low-nanogram amounts of DNA with multiple precipitants and simulated experimental conditions, we demonstrated that LPA was a preferable co-precipitant for sensitive protocols.

Introduction

Glycogen is routinely used to aid in the precipitation and recovery of nucleic acids in many molecular biology applications. These include gene expression analysis (1–3), RNA precipitation following tissue microdissection (4), DNA methylation studies (5), DNA-protein interactions (6), cancer research (7,8), as well as the detection of other human diseases, such as HIV provirus in host DNA (9). Glycogen is also applied extensively for microbial community analyses, including the analysis of nucleic acids from the human microbiome (10), microbial communities in low-biomass environmental samples coupled with whole-community genome amplification (11), and stable-isotope probing (SIP) protocols focusing on the detection of functional groups within a microbial community (12,13). A recently published procedure for environmental genomic DNA sequencing with next-generation sequencing technologies (e.g., 454 pyrosequencing) includes glycogen as a carrier for precipitation (www.454.com/downloads/protocols/NatureMethods_metagenomics.pdf). Because of the small quantities of nucleic acid being manipulated (e.g., nanogram or picogram quantities) and the sensitive nature of the downstream analyses in many studies, it is imperative that co-precipitants (other than yeast-derived tRNA, for example) be free from nucleic acid.

Despite the critical importance of co-precipitant reagent purity for the manipulation of small amounts of nucleic acid, commercially available supplies of molecular-grade glycogen have never been rigorously assessed for nucleic acid contamination. Because the biological sources of glycogen for commercial distribution are typically mussels and oysters, the synthetic nature of alternative co-precipitants [e.g., linear polyacrylamide (LPA)] would suggest higher purity. LPA has been demonstrated as a suitable co-precipitant for ethanol precipitations recovering DNA fragments greater than 20 base pairs (14). Here, we used PCR and denaturing gradient gel electrophoresis (DGGE) to test the hypothesis that co-precipitants from biological origin are associated with nucleic acid contamination, and test both glycogen and LPA for the ability to recover small amounts of DNA in isopropanol and polyethylene glycol (PEG) precipitations, with and without the addition of cesium chloride (CsCl) to the precipitation. The latter condition simulates precipitations commonly associated with RNA-SIP and DNA-SIP protocols (12,13).

Materials and methods

Co-precipitant samples

We obtained a total of nine glycogen samples and four LPA samples (Table 1) in 2008 and 2009 to assess possible nucleic acid contamination. We assigned a letter designation randomly to each sample to maintain anonymity (A-I for glycogen samples; J-M for LPA samples). For one sample (sample A), we obtained multiple production lots from the same supplier (A1 and A2).

PCR and denaturing gradient gel electrophoresis (DGGE)

We amplified a ~500-bp fragment of bacterial 16S rRNA (63f-GC and 518r; Invitrogen, Carlsbad, CA, USA) to detect DNA contamination of co-precipitants. PCR was conducted in 50-µL reactions, each containing 5 µL 10× PCR buffer (New England BioLabs, Ipswich, MA, USA), 200 µM dNTPs (New England BioLabs), 25 pmol each primer (Invitrogen), 5 µg BSA (Sigma-Aldrich, St. Louis, MO, USA) and 1.25 U *Taq* DNA polymerase (New England BioLabs). A total of 20 µg each glycogen sample (samples A-I) and approximately 30 µg LPA (samples J-M) were used as template. PCR was carried out in a DNA Engine thermocycler (Bio-Rad, Hercules, CA, USA) and the protocol consisted of 95°C for 4 min; 30 cycles of 95°C for 60 s, 55°C for 60 s, and 72°C for 60 s; followed by a final extension at 72°C for 10 min. Aliquots (4 µL) of each PCR product were

run on a 1% agarose gel, which was subsequently stained with a 1 µg/mL ethidium bromide solution. All 14 PCR products were also run on a 6% DGGE polyacrylamide gel consisting of a denaturing gradient of 40% to 70% on a CBS DGGE system (CBS Scientific, Solana Beach, CA, USA) at 85V for 14 h at 60°C, as described previously (15). Following electrophoresis, gels were stained with SYBR green I nucleic acid stain (Invitrogen) and imaged on a Typhoon 9400 imager (Amersham, Pittburgh, PA, USA).

Direct agarose gel electrophoresis of co-precipitant

Glycogen samples A1/A2 and D (200 µg each) were run on a 1% agarose gel containing 1:10,000 concentration of Gel Red nucleic acid stain (Biotium, Hayward, CA, USA), and visualized on an AlphaDigiDoc RT (Alpha Innotech, San Leandro, CA, USA).

Quantification of nucleic acid in glycogen samples

DNA and RNA present in contaminated glycogen samples (A1/A2 and D) were quantified by comparison with known amounts of bacterial genomic DNA (*Methylococcus capsulatus* str. Bath) or soil-extracted RNA run on an agarose gel stained with Gel Red (Biotium) and SYBR Green II (Invitrogen) to estimate DNA and RNA concentrations, respectively.

Sequencing

PCR products (~500 bases) for samples A2 and D were sequenced directly using the reverse primer (518r, Invitrogen) on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by the Molecular Biology Facility at the University of Waterloo. Both sequences were identical and combined. The sequence was compared with GenBank (16) using the megaBLAST program (17) and submitted to GenBank with accession no. GQ139361.

DNA precipitation with polyethylene glycol and isopropanol

Each precipitation was done in triplicate with 50 ng or 5 ng *M. capsulatus* str. Bath genomic DNA, 1 µL (20 µg) uncontaminated glycogen (sample B) or 4 µL (20 µg) of LPA (sample J). Polyethylene glycol (PEG) precipitations were performed by adding DNA to either 100 µL TE (10 mM Tris, 1 mM EDTA) or 100 µL of a 7.163 M cesium chloride (CsCl) solution. The CsCl precipitation was done to simulate SIP gradient fraction recovery of nucleic acids (12,13). Following this, the co-precipitant and two volumes of PEG solution (30% PEG 6000, 1.6 M NaCl) were added to each precipitation and incubated at room temperature for

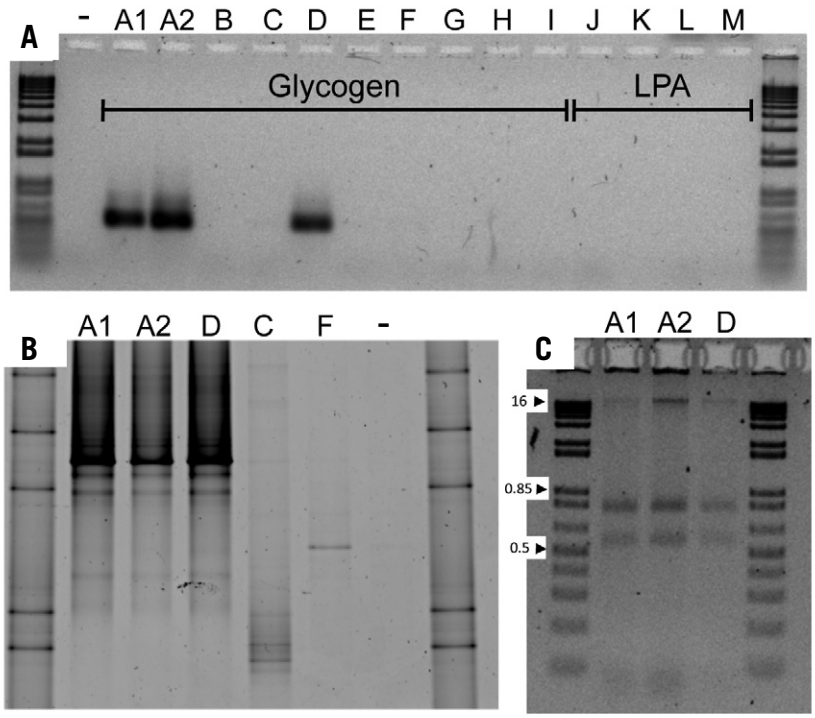

Figure 1. Assessing nucleic acid contamination. (A) Contamination of glycogen (samples A-I) and LPA (samples J-M) obtained from commercial suppliers was assessed by electrophoresis of 16S rRNA gene PCR products (~500 bp total amplicon size) on a 1% agarose gel ('-' refers to the negative control). (B) Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene PCR products from contaminated glycogen samples on a polyacrylamide gel ('-' refers to the negative control). In order to run comparable PCR product amounts in each lane, the PCR product volumes loaded were 1.5 µL (A2), 3 µL (A1, D), and 18 µL (C, F, negative control). (C) Electrophoresis of glycogen aliquots on an agarose gel. For (A) and (C), a 1-kb ladder was loaded at either end of the gels at 100 ng and 5 ng, respectively. For (B), outside lanes contain DGGE markers. For (C), ladder band sizes (kb) are shown for reference.

Table 1. Glycogen and LPA samples used in this study

Catalog number	Company	Location
	Glycogen samples	
AM9510	Ambion	Austin, TX, USA
AM9515	Ambion	Austin, TX, USA
A2168	AppliChem	Boca Raton, FL, USA
361507	Calbiochem	Gibbstown, NJ, USA
R0561	Fermentas	Burlington, ON, CA
10814010	Invitrogen	Carlsbad, CA, USA
10901393001	Roche	Branford, CT, USA
G1767	Sigma-Aldrich	St. Louis, MO, USA
2301440	5PRIME	Gaithersburg, MD, USA
	LPA samples	
A6587	AppliChem	Boca Raton, FL, USA
Bio-37075	Bioline	Taunton, MA, USA
69049	Novagen	Madison, WI, USA
70748	Novagen	Madison, WI, USA

2 h. Isopropanol precipitations were carried out using 250 µL ammonium acetate (7.5 M), 750 µL isopropanol, and either 500 µL TE or 100 µL CsCl solution combined with 400 µL TE (for SIP simulation). Each DNA precipitation was incubated at -20°C for 2 h. For both PEG and isopropanol precipitations, DNA was recovered by a 30-min centrifugation at 13,000× *g*, followed by washing with 70% ethanol and a 10-min centrifugation. Pellets were air-dried for 10 min, then suspended in 10 µL TE buffer. Two-nanogram equivalents from the triplicate precipitations (0.4 µL of the 50-ng and 4 µL of the 5-ng precipitations) were run on a 1% agarose gel stained with Gel Red and analyzed with Alpha View software (Alpha Innotech) to assess recovery efficiency.

Results and discussion

In order to assess the purity of commercially available co-precipitants, we analyzed 13 samples from 9 suppliers, including 9 glycogen samples and 4 samples of LPA. Primers targeting bacterial 16S ribosomal RNA genes were used in PCR amplifications, using aliquots of all samples as template (A1, A2, B–M). We found that 2 of the 9 glycogen samples (A1/A2 and D) amplified bacterial 16S rRNA genes that were detectable on an agarose gel; none of the four LPA samples tested positive (Figure 1A). Of the positive glycogen samples, both lots from sample A (A1 and A2) were found to be positive for bacterial 16S rRNA genes, indicating that this was not an isolated occurrence.

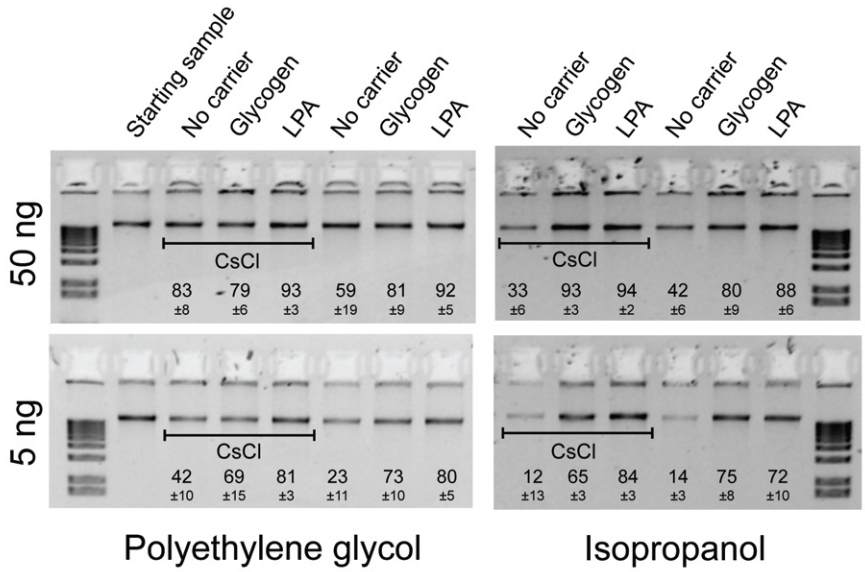

Figure 2. Comparison of glycogen (sample B) and linear polyacrylamide (LPA; sample J) nucleic acid precipitations efficiency. Precipitations of *M. capsulatus* str. Bath genomic DNA (50 ng or 5 ng) were carried out with co-precipitants with either polyethylene glycol solution (PEG) or isopropanol. Comparisons are included for precipitation in the presence or absence of cesium chloride (CsCl). Average recovery (%) is reported on the gel with SEM based on triplicate precipitations. Single representative gel images are included here.

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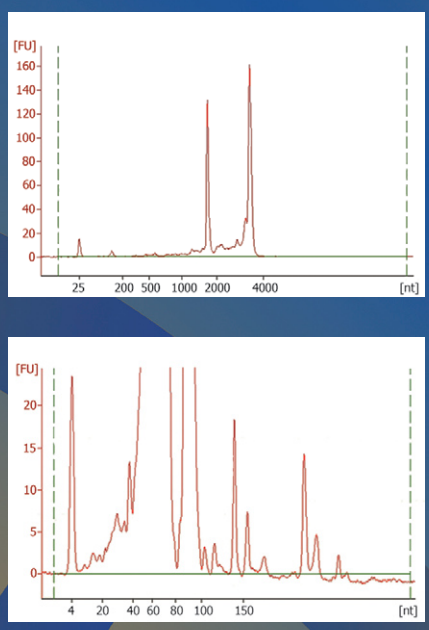

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Amplified 16S rRNA gene PCR products were separated by DGGE to generate high-sensitivity fingerprint patterns for all glycogen and LPA samples obtained in this study. Single predominant bands were clearly visible in samples A (A1 and A2) and D (Figure 1B), indicating that contamination was a result of nucleic acid from a single bacterial organism. Weaker bands also present in the gel may be due to multiple ribosomal RNA operons; similar 'patterns' have been detected with other pure cultures that we have tested (data not shown). Although not apparent on the agarose gel, unique DGGE banding patterns were also associated with samples C and F (Figure 1B), demonstrating distinct contaminant DNA associated with those sources of glycogen as well. None of the other glycogen or LPA samples generated banding patterns on DGGE. Altogether, four (A1/A2, D, C, F) of the nine tested glycogen samples were contaminated to varying degrees, as detected by these PCR-based approaches.

In order to test the quantity and quality of nucleic acid contamination, aliquots of glycogen samples A1, A2, and D were electrophoresed on an agarose gel (Figure 1C). In these three cases, genomic DNA and ribosomal RNA bands were clearly visible with UV transillumination. The identity of the genomic DNA and ribosomal RNA bands was confirmed by treatment of glycogen aliquots with nucleases. Nucleic acid concentrations present in glycogen samples A1, A2, and D were quantified at 12 pg/µL, 77 pg/µL, and 17pg/µL for DNA, and 3 ng/µL, 4 ng/µL, and 2 ng/µL for RNA, respectively. The relative migration of glycogen-associated DNA and ribosomal RNA bands in agarose gels was comparable to the migration of nucleic acids from other bacterial pure culture extracts (data not shown). Furthermore, exposure of glycogen aliquots to 30 min of UV light in tubes placed directly on a transilluminator prevented subsequent PCR amplification, but did not affect the ability of glycogen to act as an effective co-precipitant (data not shown).

Purified PCR products from contaminated samples A2 and D were sequenced directly. Identical sequence data were obtained and combined. A 385-base sequence was used in a BLAST analysis. The closest cultured hit was an *Acinetobacter lwoffii* 16S rRNA gene sequence with 99% similarity. *A. lwoffii* has been reported to be an inhabitant of mussels (18), a commercially available source of molecular-grade glycogen.

We assessed LPA as an alternative co-precipitant to glycogen. Using non-contaminated glycogen sample B and LPA sample J for comparison, we assessed the efficiency of these two co-precipitants at recovering two different quantities of genomic DNA (50 ng and 5 ng; Figure 2). We found no significant difference in DNA recovery efficiency between glycogen and LPA, regardless of the starting amount of DNA or the particular precipitant used (PEG solution or isopropanol). Both co-precipitants were effective in the presence of CsCl, simulating conditions for which nucleic acid is recovered from gradient fractions during SIP experiments (12,13).

High-quality reagents for specialized manipulations of nucleic acid are an essential requirement for high-throughput sequencing and genomics, gene-expression analysis, characterization of low-biomass microbial communities, SIP experiments, flow-sorting of single cells, and microbiome-related research. We have demonstrated that glycogen is comparable to LPA. However, due to purity issues, if glycogen is employed, pre-treatment with UV light is recommended to destroy contaminating nucleic acids prior to use of this co-precipitant in life science research applications.

Due to the increased purity afforded by the chemical synthesis of LPA, comparable performance, and similar cost as a co-precipitant, we have established that LPA is preferable to glycogen for routine molecular applications.

Acknowledgments

This work was supported by a Discovery Grant to J.D.N. from the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank colleagues and collaborators who have shared research results affected by contaminated glycogen. Hataichanok Sae Yang, Taybah Bhatti, and Brendan J. McConkey are thanked for helpful discussions. We appreciate the contribution of several companies for providing glycogen and LPA samples free of charge.

Competing interests

The authors declare no competing interests.

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Received 27 May 2009; accepted 23 September 2009.

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