

Supporting Information

Magnetic Ionic Liquids as PCR-compatible Solvents for DNA Extraction from Biological Samples

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Reagents. Trioctylamine and trihexyl(tetradecyl)phosphonium chloride were purchased from Acros Organics (NJ, USA) and Strem Chemicals (Newburyport, MA, USA), respectively. Benzyl bromide, iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), sodium dodecyl sulfate (SDS), albumin from chicken egg white, deferoxamine mesylate salt, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). T4 gp32 was obtained from Affymetrix (Santa Clara, CA, USA). Sodium hydroxide and potassium acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Synthetic oligonucleotide primers were purchased from IDT (Coralville, IA, USA), while the modified pET-32 plasmid was obtained from EMD Millipore (Billerica, MA, USA). NEB 5-alpha Competent *E. coli* cells and Phusion High-Fidelity DNA Polymerase were purchased from New England Biolabs (Ipswich, MA, USA). Agarose and tris(hydroxymethyl)aminomethane (Tris) were obtained from P212121 (Ypsilanti, MI, USA). SYBR Safe DNA gel stain was purchased from Life Technologies (Carlsbad, CA, USA) and a 1 Kb Plus DNA Ladder (250–25,000 bp) was purchased from Gold Biotechnology, Inc. (St. Louis, MO, USA). Bromophenol blue was purchased from Santa Cruz Biotech (Dallas, TX, USA). QIAquick Gel Extraction and QIAamp DNA Mini Kits were purchased from QIAGEN (Valencia, CA, USA). Deionized water (18.2 M Ω cm) obtained from a Milli-Q water purification system was used for all buffers and solutions (Millipore, Bedford, MA, USA).

Synthesis of MILs. Synthesis of the $[\text{P}_{6,6,14}]^+[\text{FeCl}_4]^-$ and $[(\text{C}_8)_3\text{BnN}]^+[\text{FeCl}_3\text{Br}]^-$ MILs were carried out using previously reported procedures.^{1,2}

PCR Amplification and Gel Analysis. All PCR experiments were carried out with a total reaction volume of 50 μL . For standard reactions, a 25.9 ng quantity of pDNA containing the 879 bp MTAP gene was used as the template. Forward and reverse primers with sequences of 5'-TGC TGT TCC AGG GAC CT-3' and 5'-GAA TTC GGA TCC GGA CGC-3' were added to the mixture at 0.2 μM . Each reaction was prepared with 10 μL of 5X Phusion HF buffer, 1 μL of 10 mM dNTPs, and 1 unit of high fidelity Phusion DNA polymerase. The reaction mixture was diluted to 50 μL with deionized water. Temperature settings for PCR included: initial denaturation at 95 °C for 5 minutes followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 45 s, and extension at 72 °C for 45 s. A final extension step at 72 °C for 5 min was employed, after which the samples were cooled to 4 °C.

Cell Cultures. Competent *E. coli* cells were transformed with a modified pET-32 plasmid (pDNA) encoding human 5'-methylthioadenosine phosphorylase (MTAP). Approximately 25 ng of purified pDNA was added to a microcentrifuge tube containing 10 μL of NEB 5-alpha Competent *E. coli* cells and gently mixed. The solution was placed on ice for 30 min. The microcentrifuge tube was set in a water bath at 42 °C for 42 s, removed from heat, and immediately chilled on ice for 5 min. Then, 950 μL of Luria Bertani (LB) media was added to the solution. The mixture was incubated at 37 °C for 1 h and subsequently transferred into 120 mL of LB media with 100 $\mu\text{g mL}^{-1}$ carbenicillin at 37 °C for 24 h.

MIL-based Extraction of pDNA from Crude Bacterial Cell Lysate. A 2 mL aliquot of *E. coli* cell culture was centrifuged at 1,380g for 8 minutes and resuspended in 300 μ L of 20 mM Tris, 0.1 mM EDTA buffer (pH 8). A 120 μ L volume of 0.2 N NaOH, 1% (w/v) SDS was then added to the sample. After gently mixing the solution, 80 μ L of 3 M potassium acetate (pH 4.8) were added and the solution diluted to a final volume of 2 mL. The crude cell lysate was then extracted with 20 μ L of either the $[P_{6,6,6,14}^+][FeCl_4^-]$ MIL or the $[(C_8)_3BnN^+][FeCl_3Br^-]$ MIL using a dispersive droplet extraction approach.³ The MIL was retrieved from the crude lysate using a 0.66 T rod magnet and rinsed with deionized water. A 0.5 μ L volume of the pDNA-enriched MIL was then transferred into a PCR mixture with 80 mM Tris (pH 8), 1 mM EDTA, 1 mM $MgCl_2$, and 400 ng/ μ L ovalbumin as additives.

DNA Sequencing. Following PCR amplification and gel electrophoresis, the MTAP amplicon was excised from the agarose gel and extracted using a QIAquick Gel Extraction kit. For each sequencing reaction, 40 ng of template and 10 pmol of each primer were submitted to an external DNA sequencing service (Eurofins Genomics, Huntsville, AL).

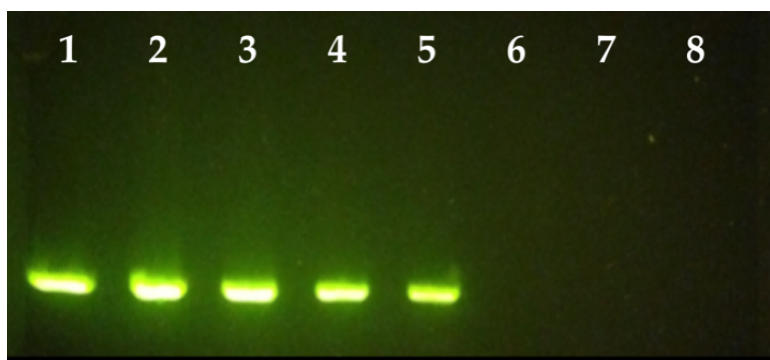
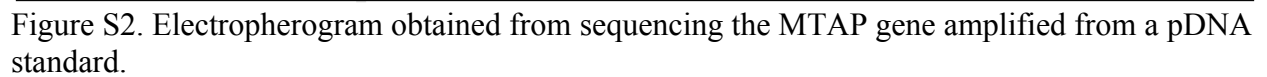
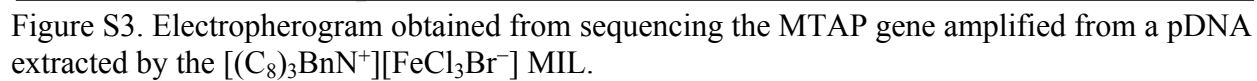
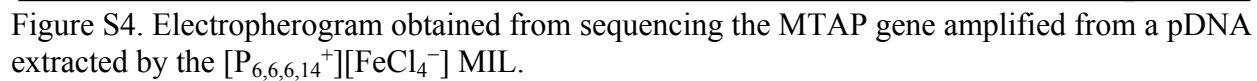


Figure S1. Effect of FeCl₃ on the PCR amplification of an 879 bp gene from pDNA. Lane 1) 25.9 ng of pDNA in a standard PCR mix, 2) 0.1 μ M FeCl₃, 3) 1 μ M FeCl₃, 4) 5 μ M FeCl₃, 5) 10 μ M FeCl₃, 6) 20 μ M FeCl₃, 7) 30 μ M FeCl₃, 8) 40 μ M FeCl₃.







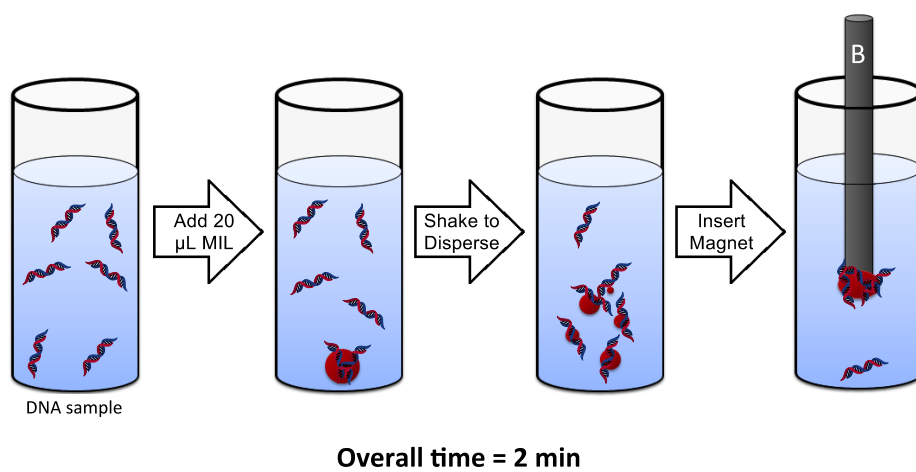


Figure S5. Schematic of MIL-based dispersive droplet extraction of DNA using the $[(\text{C}_8)_3\text{BnN}^+][\text{FeCl}_3\text{Br}^-]$ MIL.

References

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