Supporting Information

Preservation of DNA in Nuclease-rich Samples using Magnetic Ionic Liquids

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Synthesis of MILs. The synthesis of the \([\text{P}^{66614+}]\text{[FeCl}_4^-\text{]}\), \([\text{N}^{888Bn+}\text{][FeCl}_3\text{Br}^-\text{]}\), and \([\text{P}^{66614+}]_2\text{[MnCl}_4^{2-}\text{]}\) MILs were adapted from previously reported procedures.\(^1,2\) For the \([\text{P}^{66614+}]\text{[FeCl}_4^-\text{]}\) and \([\text{P}^{66614+}]_2\text{[MnCl}_4^{2-}\text{]}\) MILs, equimolar quantities of the \([\text{P}^{66614+}][\text{Cl}^-\text{]}\) and iron(III) chloride hexahydrate (FeCl\(_3\cdot6\text{H}_2\text{O}\)) or manganese(II) chloride tetrahydrate (MnCl\(_2\cdot4\text{H}_2\text{O}\)), respectively, were stirred in methanol for 24 h. The methanol was then evaporated \textit{in vacuo} and the crude product washed with 5 × 40 mL of deionized water. Residual solvent was removed \textit{in vacuo}. For preparation of the \([\text{N}^{888Bn+}\text{][FeCl}_3\text{Br}^-\text{]}\) MIL, 8 mmol of trioctylamine and 10 mmol of benzyl bromide were dissolved in chloroform under reflux conditions for 72 h. Chloroform was removed by rotovap and excess starting material removed by washing the crude product with 5 × 40 mL of hexane to yield \([\text{N}^{888Bn+}\text{][Br}^-\text{]}\). After drying in a vacuum oven, the bromide salt was reacted with 12 mmol of FeCl\(_3\cdot6\text{H}_2\text{O}\) in methanol for 24 h. The methanol was then removed \textit{in vacuo} and the product washed with 5 × 40 mL of deionized water. The product was finally dried \textit{in vacuo}.

Recovery of sDNA. Recovery of sDNA from the MIL phase was undertaken using a previously reported solid phase extraction (SPE) procedure.\(^3\) The MIL sample containing DNase I and sDNA was dissolved in 1 mL of 3 M potassium acetate and loaded onto a silica column. sDNA was eluted from the column using 10 mM Tris, 0.1 mM EDTA (pH 8.5) and precipitated with absolute ethanol. Purified sDNA was reconstituted in 20 µL of 10 mM Tris (pH 8.5) and analyzed by agarose gel electrophoresis.

PCR Conditions. The total reaction volume for all PCR experiments was 50 µL. 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 final concentrations of 0.2 µM. A custom PCR buffer\(^4\) consisting of 80 mM Tris, 1 mM EDTA, 400 ng µL\(^{-1}\) ovalbumin, 1X Phusion HF buffer, an additional 1 mM MgCl\(_2\), 5 mM dNTPs, and 1 U of high fidelity Phusion DNA polymerase. The reaction mixture was diluted to 50 µL with deionized water. Temperature settings for PCR were as follows: 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. Final extension was employed at 72 °C for 5 min and the samples then cooled to 4 °C.

Transformations and Cell Cultures. A 0.5 µL aliquot of sample containing pDNA, DNase I, and the \([\text{P}^{66614+}]_2\text{[MnCl}_4^{2-}\text{]}\) MIL was transferred to a microcentrifuge tube containing 20 µL of competent \textit{E. coli} cells and placed on ice for 30 min. The sample was then subjected to heat shock for 30 s at 42 °C, removed from the heat source, and immediately placed on ice for 5 min. The cells were suspended in 950 µL of SOC media and incubated for 1 h at 37 °C. A 250 µL aliquot of the transformed cells was then spread on Luria Bertani agar (100 µg mL\(^{-1}\) carbenicillin). The plate was incubated overnight at 37 °C.
Figure S1. $^1$H NMR for $[\text{N}_{888\text{Bn}}^+][\text{Br}^-]$. 
Figure S2. $^{13}$C NMR of $[N_{888Bn}^+] [Br^-]$. 
Figure S3. Absorption spectrum for the \([N_{888Bn}^{-}][FeCl_{2}\text{Br}^{-}]\) MIL in methanol.
Table S1. Phase transition behavior of the three studied MILs.

<table>
<thead>
<tr>
<th>MIL</th>
<th>( T_g ) /°C</th>
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<tbody>
<tr>
<td>([\text{P}_{66614}^+])[\text{FeCl}_4^-]</td>
<td>(-71^b)</td>
</tr>
<tr>
<td>([\text{N}_{88888\text{Bn}}^+])[\text{FeCl}_3\text{Br}^-]</td>
<td>(-53^c)</td>
</tr>
<tr>
<td>([\text{P}_{66614}^+])_2[\text{MnCl}_4^{2-}]</td>
<td>(-69^b)</td>
</tr>
</tbody>
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\(^a\)Glass transition temperatures \( (T_g) \) as determined by differential scanning calorimetry (DSC). \(^b\)From reference 1. \(^c\)From reference 2.
Figure S4. Schematic representation of SDE experiments for the determination of DNase I partitioning behavior between aqueous solution and the hydrophobic MIL phase. Extraction conditions: MIL volume: 20 µL; sample volume: 1.25 mL; buffer composition: 10 mM Tris (pH 8.5); DNase I concentration: 1,000 µg mL\(^{-1}\); stir rate: 85 rpm.
Figure S5. DNA sequencing results of the MTAP gene amplified from pDNA template. Prior to amplification, pDNA was stored in the [P$_{66614}^+$][FeCl$_4^-$] MIL with 20 U of DNase I for 72 h at room temperature.
Figure S6. DNA sequencing results of the MTAP gene amplified from pDNA template. Prior to amplification, pDNA was stored in the $\left[\text{N}_{888\text{Br}}^+\right]\left[\text{FeCl}_3\text{Br}^-\right]$ MIL with 20 U of DNase I for 72 h at room temperature.
Figure S7. DNA sequencing results from the MTAP gene amplified from a pDNA standard.
Figure S8. PCR amplification of the 879 bp MTAP gene from pDNA stored within MIL for 72 h and room temperature. Initially, 5 µg of pDNA were spiked into 20 µL of MIL and incubated for 1 h at room temperature prior to the addition of 20 U of DNase I.
Figure S9. PCR amplification of the 879 bp MTAP gene from pDNA template. Lane 1 shows a standard and lane 2 shows the amplicon obtained from a PCR mixture spiked with 1 U of DNase I immediately prior to thermal cycling.
Figure S10. Colonies on a carbenicillin selection plate after transformation of competent *E. coli* cells with pDNA treated with DNase I within the $[\text{P}_{6664}^\text{+}]_2[\text{MnCl}_4^-]$ MIL. The mixture was incubated for 1 day at room temperature prior to heat shock transformation.
References


