

Supporting Information

Oligonucleotides possessing poly-cytosine tag coupled with magnetic ionic liquids for sequence-specific DNA analysis

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Reagents

Anhydrous diethyl ether (99.0%) was supplied by Avantor Performance Materials Inc. (Center Valley, PA, USA). Cobalt(II) chloride hexahydrate (98%), nickel(II) chloride (98%), hexane (98.5%), bovine serum albumin (BSA), albumin from chicken egg white, DNA sodium salt from salmon testes (stDNA, approximately 20 kbp), Tween 20, Pluronic F-108 (average molecular weight = 14600 g/mol), magnesium chloride hexahydrate and LC-MS grade methanol were all purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol (100%) was purchased from Decon Labs (King of Prussia, PA, USA). Trihexyl(tetradecyl)phosphonium chloride (97.7%) was purchased from Strem Chemicals (Newburyport, MA, USA). Ammonium hydroxide (28-30% solution in water) and 1,1,1,5,5,5-hexafluoroacetylacetone (99%) were purchased from Acros Organics (Morris Plains, NJ, USA). Sso Advanced Universal SYBR Green Supermix was purchased from Bio-Rad Laboratories (Hercules, CA, USA). NEB 5-alpha Competent *Escherichia coli* cells and dimethyl sulfoxide (DMSO) (100%) were obtained from New England Biolabs (Ipswich, MA, U.S.A.). Tris-(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). SYBR Green I (10,000x) was purchased from Life Technologies (Carlsbad, CA, USA). PCR caps and tube strips were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cylinder magnets (0.20 T, 0.66 T, and 0.9 T) were purchased from K&J Magnetics (Pipersville, PA, USA). Deionized water (18.2 M Ω cm) used throughout the study was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All DNA oligonucleotides and primers (primers for qPCR amplification of the 261 bp target sequence were 5'-CAC GCT TAC ATT CAC GCC CT -3' and 5'-CGA GCG TCC CAA AAC CTT CT -3') were purchased from IDT (Coralville, IA, USA). Table S1 shows DNA oligonucleotides used in this study.

Instrumentation

The extraction of ss-DNAs by Co(II)-based MIL was evaluated on an Agilent 1260 HPLC Infinity with a variable wavelength detector (Santa Clara, CA, USA). Real-time quantitative PCR (qPCR) assays were conducted on a CFX96 Touch Real-Time PCR Detection System from Bio-Rad Laboratories. All thermal cycling for hybridization of DNA was performed on a Techne FTgene2D thermal cycler (Burlington, NJ, USA). The concentrations of oligonucleotide standards were detected by a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific). All pH values were measured by an Accumet AB-150 pH meter from Fisher Scientific. The fluorescence intensity of FAM-labeled DNA was monitored by a Synergy H1 Microplate Reader (BioTek Instruments, Inc.). A 0.66 T rod magnet from K&J Magnetics (Plumsteadville, PA, USA) was used for the manipulation of the magnetic extraction phases in all extractions.

HPLC Conditions

The analytical column was a TSK gel DEAE-NPR anion exchange column (35 \times 4.6 mm, 2.5 μ m) with a TSK gel DEAE-NPR guard column (5 mm \times 4.6 mm i.d. \times 5 μ m) from Tosoh Bioscience (King of Prussia, PA). The mobile phase composed of pH 8.0, 20 mM Tris-HCl (A) and pH 8.0, 20 mM Tris-HCl and 1 M NaCl (B). Gradient elution was employed starting at 100% A, rising linearly to 100% B over 9 min, and then returning to the original conditions over 0.01 min and re-equilibrated for 5 min, giving a total cycle

time of 14 min.

Values of extraction efficiency (E) were calculated according to eq 1.

$$E = [1 - C_{aq}/C_{st}] \times 100 \quad (1)$$

Where C_{aq} and C_{st} are the DNA concentration in the aqueous phase following extraction and the concentration of DNA in the standard solution, respectively.

Real-Time Quantitative PCR Assays

A 19 μ L qPCR reaction mix was prepared by mixing 10 μ L of SsoAdvanced Universal SYBR Green Supermix (2x), 2.6 μ L of 50 mM $MgCl_2$, 1 μ L of DMSO, 4.6 μ L of deionized water and 0.8 μ L of 10 μ M forward and reverse primers. For amplification of target DNA, 1 μ L of template target DNA solution was added to the qPCR mixture. The thermal cycling protocol for all qPCR assays was as follows: an initial denaturation step of 5 min at 95.0 $^{\circ}C$ followed by 40 cycles of 10 s at 95.0 $^{\circ}C$ and 30 s at 64.0 $^{\circ}C$.

A four-point calibration curve with a 10-fold dilution series was performed to evaluate the amplification efficiency, which was calculated according to Eq.2, where the slope is the slope of the linear regression in the calibration curve.

$$\text{Amplification efficiency} = [10^{(-1/\text{slope})} - 1] \times 100 \quad (2)$$

As shown in Figure S12, the qPCR amplification efficiency was found to be 97.4% under the conditions employed in this study.

Synthesis of metal-based MIL

The $[P_{66614}^+][Co(hfacac)_3^-]$ or $[P_{66614}^+][Ni(hfacac)_3^-]$ MIL was synthesized according to our previous procedures with a little modification, which was described as followed. 10 mmol of ammonium hydroxide was added to 30 mL of ethanol and the reaction flask was sealed with a rubber septum. 10 mmol of hexafluoroacetylacetone were slowly injected into the reaction mixture by a syringe. After a white vapor disappeared, 3.3 mmol of cobalt (II) chloride hexahydrate or nickel(II) chloride (98%) were added to the mixture. The reaction was allowed to stir at room temperature for 5 hours. The solvent was removed at 40 $^{\circ}C$ under reduced pressure. The crude product was dissolved in diethyl ether and washed with deionized water several times until the aqueous fraction yielded no precipitate during a silver nitrate test. Then diethyl ether was removed in vacuum and the prepared metal salt was dried at 50 $^{\circ}C$ overnight under reduced pressure. A 1 mmol amount of the metal salt was mixed with 1 mmol of trihexyl(tetradecyl)phosphonium chloride in methanol and the reaction was allowed to stir at room temperature for 24 h. The solvent was evaporated at 40 $^{\circ}C$ under reduced pressure. The crude product was dissolved in hexane and the mixture was filtered to remove ammonium chloride. Then, the organic filtrate was washed with deionized water several times and removed at 40 $^{\circ}C$ under vacuum. The prepared $[P_{66614}^+][Co(hfacac)_3^-]$ or $[P_{66614}^+][Ni(hfacac)_3^-]$ MIL was dried at 40 $^{\circ}C$ under vacuum.

The synthetic reproducibility of Co(II)-based MIL was investigated by extraction of a poly-C probe sequence using three batches of MIL. As shown in Fig. S13, the extraction efficiencies of three batches of Co(II)-based MIL were 88.8%, 86.3% and 90.6%, respectively, demonstrating good synthetic reproducibility of the Co(II)-based MIL.

MIL-based extraction of DNA

A 0.44 μM solution of single-stranded or duplex DNA was prepared in 50 μL of deionized H_2O . Then, 1 μL of $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ MIL was added to the aqueous solution and the mixture was shaken at 2000 rpm for 0.5 min to disperse hydrophobic MIL into aqueous solution and extract DNA. The MIL was isolated with a magnet and a 20 μL of aliquot of aqueous phase was subjected to HPLC-UV analysis.

Sequence-selective extraction of target DNA by Poly-C DNA-MIL method

16.9 pmol of poly- C_{20} probe DNA and 1.69 fmol of 261 bp target DNA were hybridized in 50 μL of 25 mM NaCl solution. Then, 1 μL of $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ MIL was added to the aqueous solution and the mixture was shaken at 2000 rpm for 1 min to extract poly- C_{20} probe-target duplex. After decanting the sample solution and washing the MIL with water, the target was released in 25 mM NaCl at 60 $^\circ\text{C}$ for 10 min. Then, a 1 μL aliquot of eluting solution was directly added to qPCR reaction mixture for amplification.

FAM-DNA adsorption and desorption

A 0.1 μM aliquot of FAM-labelled DNA was mixed with 1 μL MIL in 20 μL of 20 mM, pH 8.0 Tris-HCl buffer with 25 mM NaCl for 30 min. Then, an equivalent molar amount of complementary DNA and non-complementary poly-C DNA were added to desorb the FAM-DNA. After 30 min of incubation, the fluorescence intensity was measured using a BioTek Synergy Hybrid H1 microplate reader with an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

Table S1 DNA sequences used and examined in this study. All sequences are listed from the 5' to 3'-end.

Name	Sequences
Oligo 1 (linear, 20% GC content)	TAT TTA GCC GTA TTT TTA TT
Oligo 2 (linear, 20% GC content)	GGG GTA TTT ATA TTT TAT TT
Oligo 3 (linear, 20% GC content)	TAT TTA TAT TTA ATT TCC CC
Oligo 4 (linear, 80% GC content)	TTA TCC CCC CCC CGC CCC CC
Oligo 5 (linear, 80% GC content)	CCC CCC CCC GCC CCC CTT AT
Oligo 6 (linear, 80% GC content)	CTC CCC ACC CAC GCC CTC CC
Oligo 7 (hairpin, 80% GC content)	CGC GGT GGG AGT GGT CCG CG
Oligo 8 (hairpin, 80% GC content)	CGC GGG GGT ATT GGG CCG CG
Oligo 9 (hairpin, 80% GC content)	CGC GTG GGG TTG GGC ACG CG
Poly-C	CCC CCC CCC CCC CCC CCC CC
Poly-A	AAA AAA AAA AAA AAA AAA AA
Poly-T	TTT TTT TTT TTT TTT TTT TT
Poly-GT	GTG TGT GTG TGT GTG TGT GT
FAM-DNA	56-FAM/AAA ACG CCG CAG ACA CAT CC
cDNA	GGA TGT GTC TGC GGC GTT TT
Human telomeric DNA	CCC TAA CCC TAA CCC TAA CCC T
Oligo 4 probe	(TTA TCC CCC CCC CGC CCC CC) (CAC GCT TAC ATT CAC GCC CT)
Oligo 5 probe	(CCC CCC CCC GCC CCC CTT AT) (CAC GCT TAC ATT CAC GCC CT)

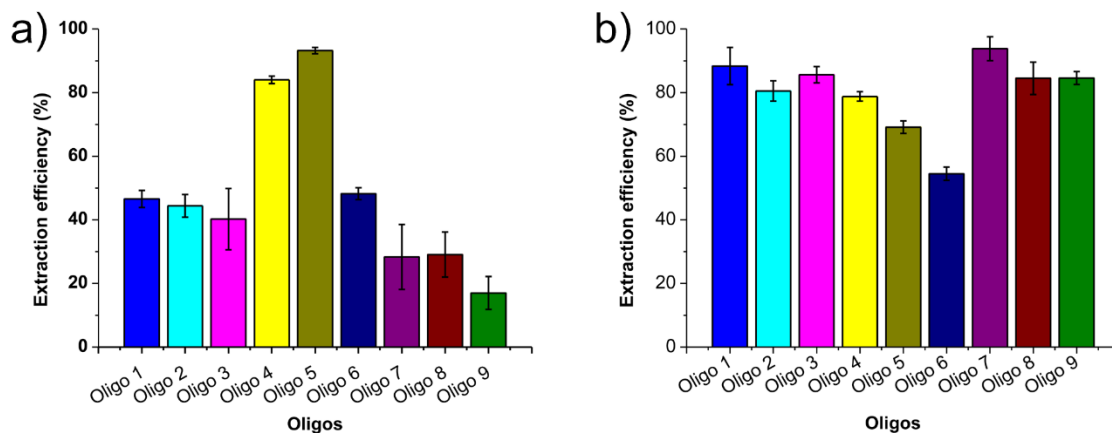


Fig. S1 Extraction efficiencies of 20-mer ss-DNA sequences with different GC content using the Co(II)-based MIL (a) and the Ni(II)-based MIL (b). A solution containing 0.44 μM ss-DNA in 50 μL DI H_2O was extracted using 1 μL of the $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ or $[\text{P}_{66614}^+][\text{Ni}(\text{hfacac})_3^-]$ MIL with a 30 s extraction time and vortex rate of 2000 rpm. The sequences of all oligos are shown in Table S1.

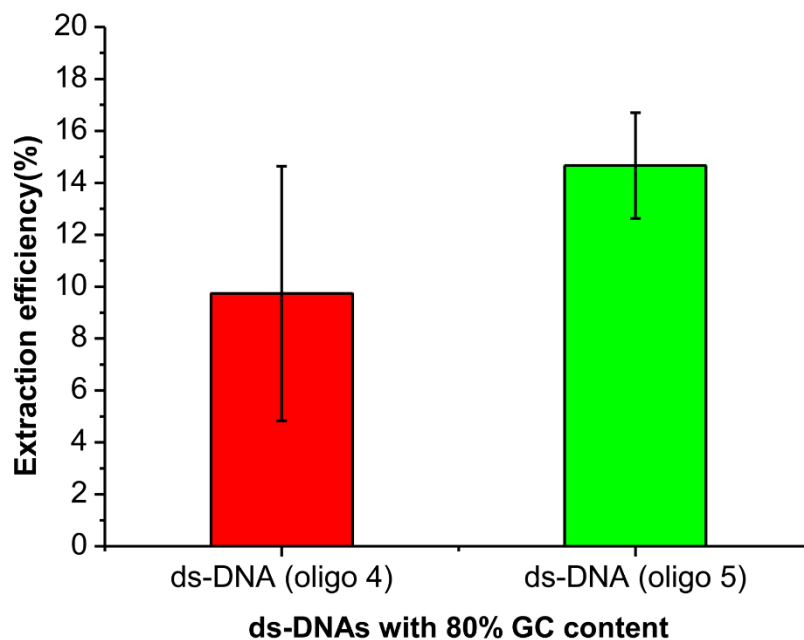


Fig. S2 Extraction efficiencies of 20 bp ds-DNA with 80% GC content using the Co(II)-based MIL. A solution containing 0.44 μM ds-DNA in 50 μL DI H_2O was extracted using 1 μL of the $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ MIL with a 30 s extraction time and a vortex rate of 2000 rpm.

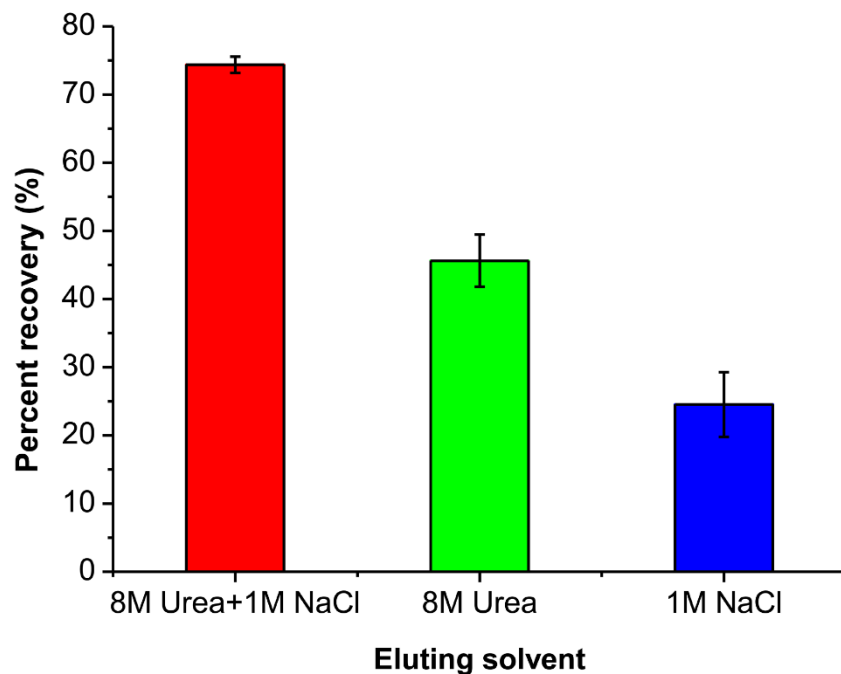


Fig. S3 Recovery of poly-C₂₀ probe DNA using different eluting solvents. A solution containing 0.44 μM poly-C₂₀ probe in 50 μL DI H₂O was extracted using 1 μL of the [P₆₆₆₁₄⁺][Co(hfacac)₃⁻] MIL with a 30 s extraction time and a vortex rate of 2000 rpm. After washing with DI H₂O, the poly-C₂₀ probe DNA was desorbed using 50 μL eluting solvent with a 30 s extraction time and a vortex rate of 2000 rpm.

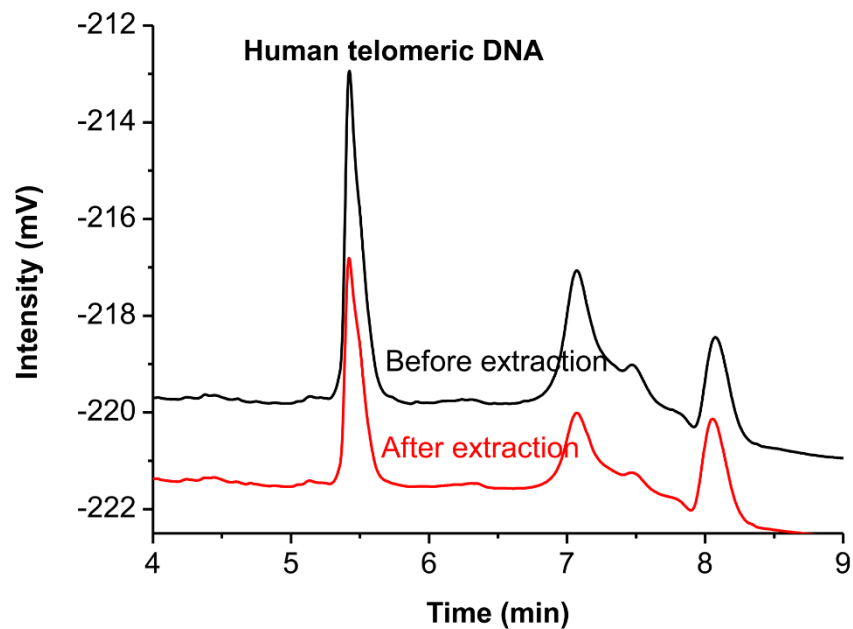


Fig. S4 Chromatograms showing the separation of human telomeric DNA before and after extraction with the Co(II)-based MIL. A solution containing 0.44 μM human telomeric DNA in 50 μL DI H_2O was extracted using 1 μL of the $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ MIL with a 30 s extraction time and a vortex rate of 2000 rpm.

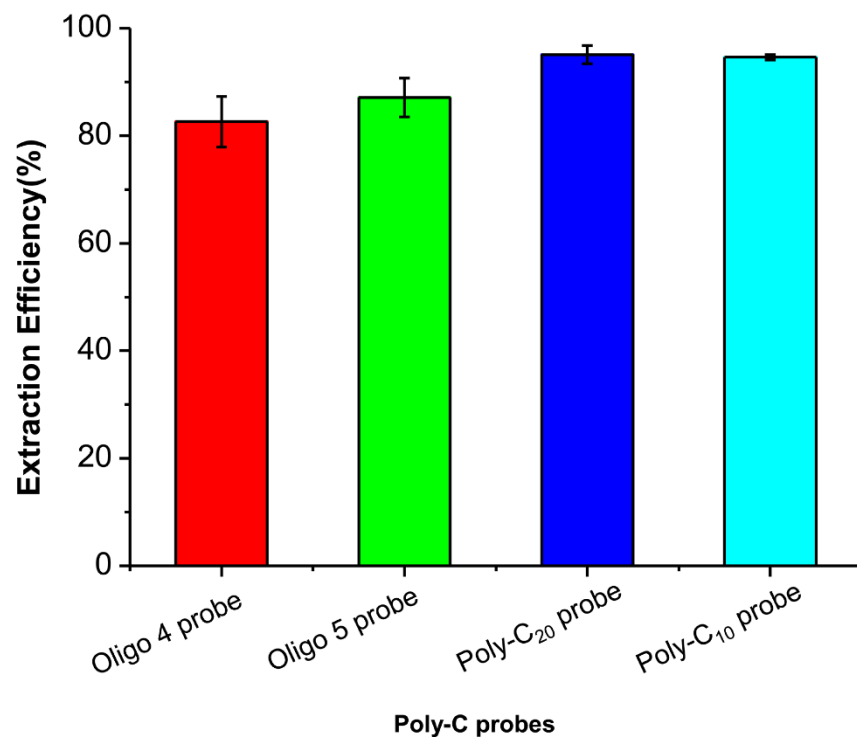


Fig. S5 Extraction efficiencies of poly-C probe sequences by the Co(II)-based MIL. A solution containing 0.44 μM poly-C probe DNA in 50 μL DI H₂O was extracted using 1 μL of the $[\text{P}_{66614}^+][\text{Co}(\text{hfacac})_3^-]$ MIL with a 30 s extraction time and a vortex rate of 2000 rpm.

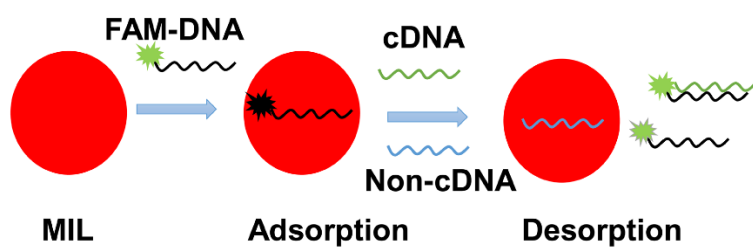


Fig. S6 Scheme depicting adsorption of a FAM-labeled DNA resulting in quenched fluorescence and its subsequent desorption by hybridization to complementary DNA or by displacement with non-cDNA

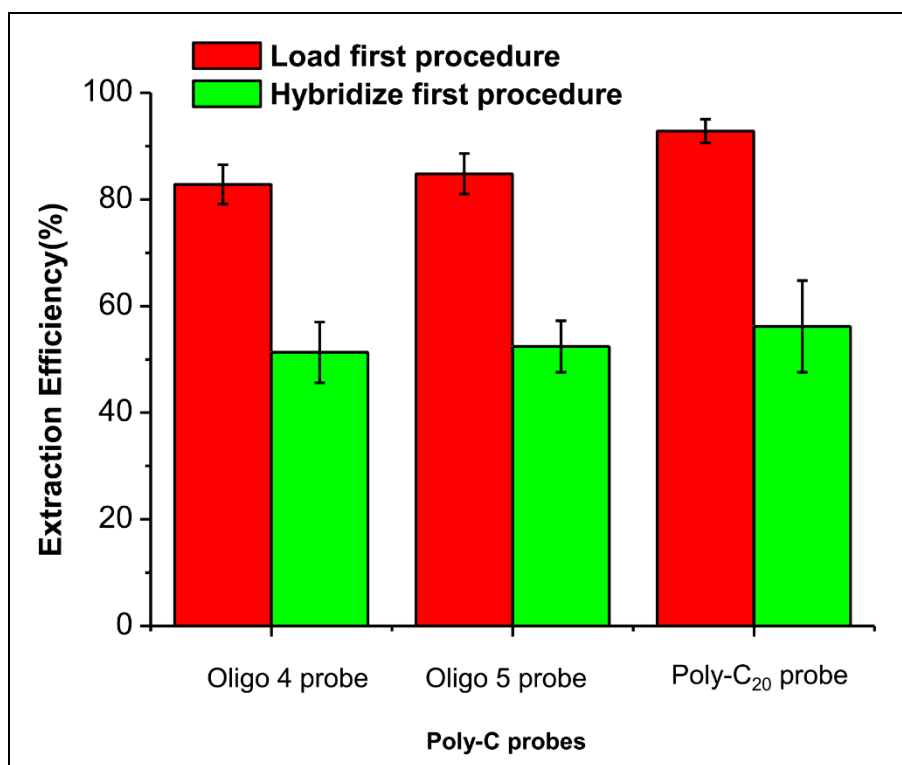


Fig. S7 Extraction efficiencies of 20-mer complementary target DNA (Target 1) using poly-C probes and Co(II)-based MIL by the load first and hybridize first procedures. Extraction conditions: DNA concentration, 0.44 μM ; molar ratio of probe to target, 1:1; total solution volume, 50 μL ; extraction time, 1 min; MIL volume, 1 μL ; rotation rate, 2000 rpm.

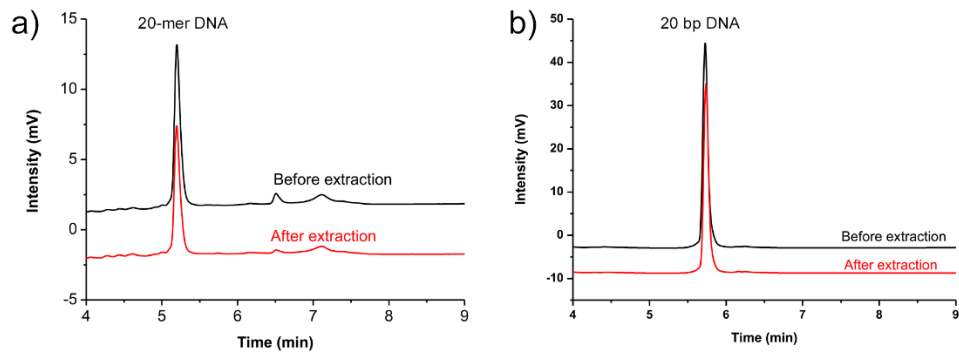


Fig. S8 Representative chromatograms of 20-mer target 1 DNA (a) and its duplex DNA (b) before and after direct extraction with the Co(II)-based MIL. Extraction conditions: DNA concentration, 0.44 μM ; total solution volume, 50 μL ; extraction time, 1 min; MIL volume, 1 μL ; rotation rate, 2000 rpm.

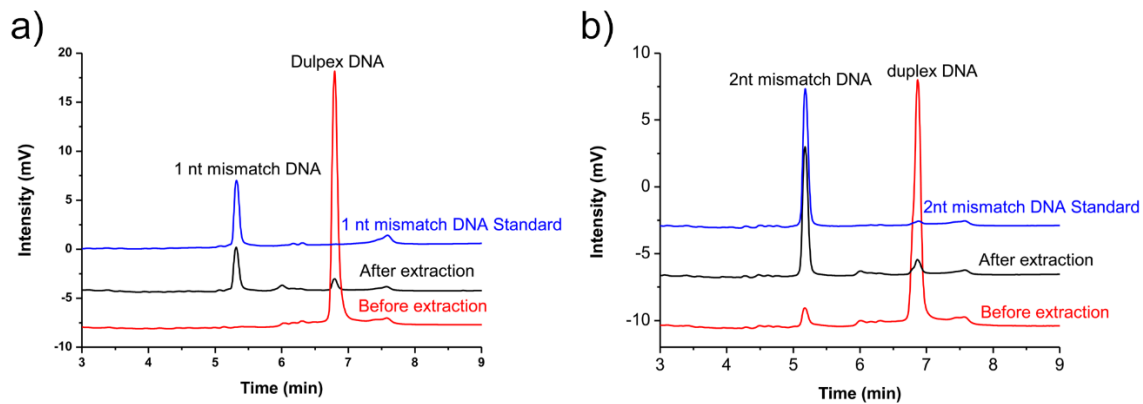


Fig. S9 Chromatograms of 1 nt mismatched DNA (a) and 2 nt mismatched target DNA (b) before and after extraction with the poly-C₂₀ DNA-MIL method. Extraction conditions: DNA concentration, 0.44 μ M; molar ratio of probe to target, 1:1; total solution volume, 50 μ L; extraction time, 1 min; MIL volume, 1 μ L; rotation rate, 2000 rpm.

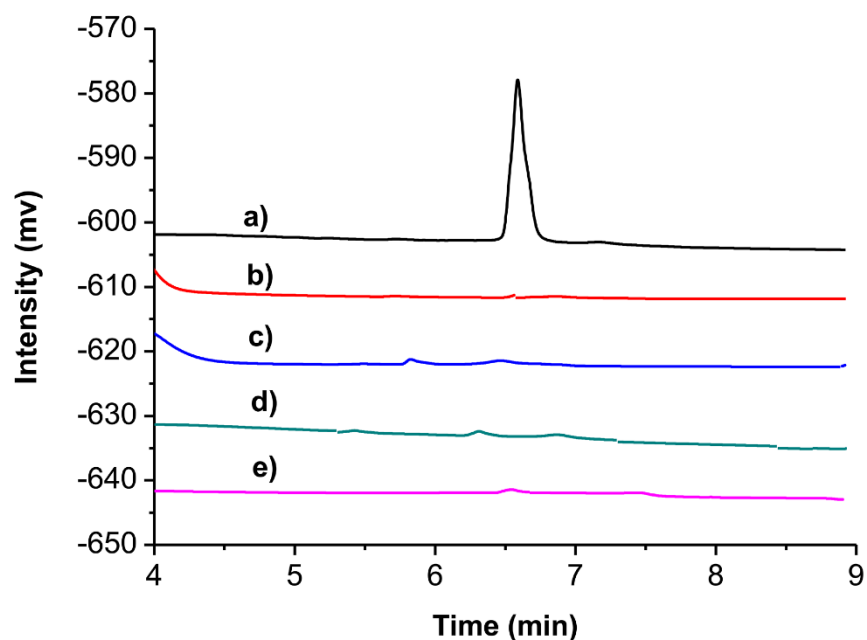


Fig. S10 Chromatograms of a 0.44 μM poly- C_{20} DNA standard solution (a) and 1% solutions of bovine serum albumin (b), albumin from chicken egg white (c), Tween 20 (d), Pluronic F-108 (e) after immersed with poly- C_{20} DNA-MIL for 30 min. 1 μL of Co(II)-based MIL was used to bind 0.44 μM of poly- C_{20} probe DNA in DI H_2O . After washing with DI H_2O , 1% solutions of bovine serum albumin, albumin from chicken egg white, Tween 20, and Pluronic F-108 were respectively added to incubate with poly-C DNA-MIL for 30 min.

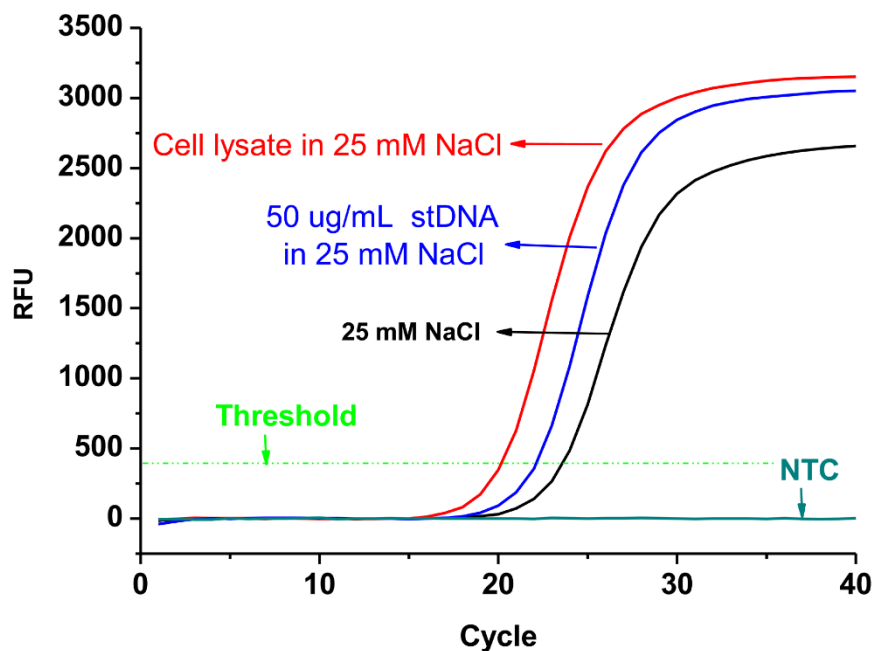


Fig. S11 Representative qPCR amplification plot following poly-C DNA-MIL extraction using poly-C₂₀ probe from different sample matrices: 20 mM NaCl, *E. coli* cell lysate with 20 mM NaCl and 50 µg/mL DNA from salmon testes (stDNA) in 25 mM NaCl. The lysate was prepared from *E. coli* cells using ultrasonication for 10 min. Target DNA: 1.69 fmol; poly-C₂₀ probe: 16.9 pmol; extraction time: 1 min; desorption time: 10 min; desorption method: 25 mM NaCl at 60 °C for 10 min; desorption solvent volume: 50 µL.

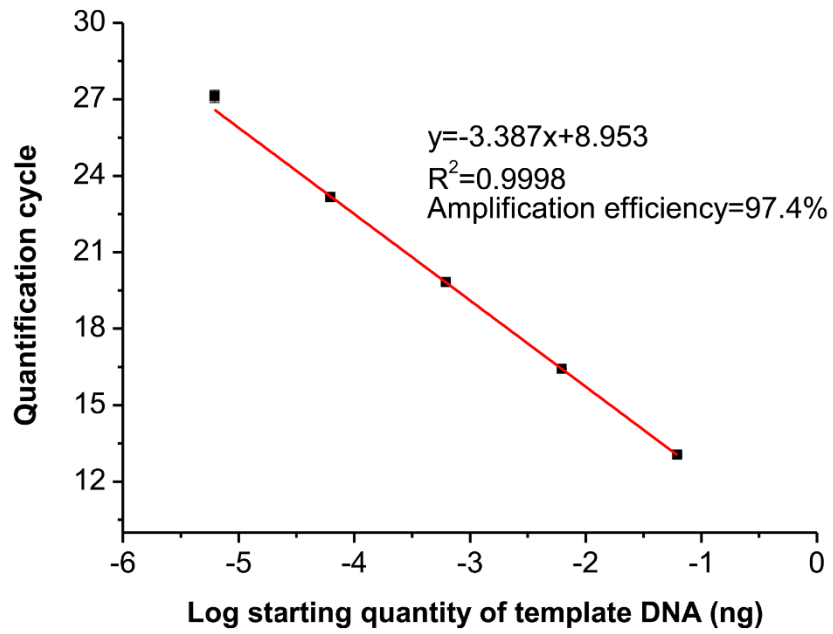


Fig. S12 qPCR calibration curve for a ten-fold dilution series of the 261 bp target sequence.

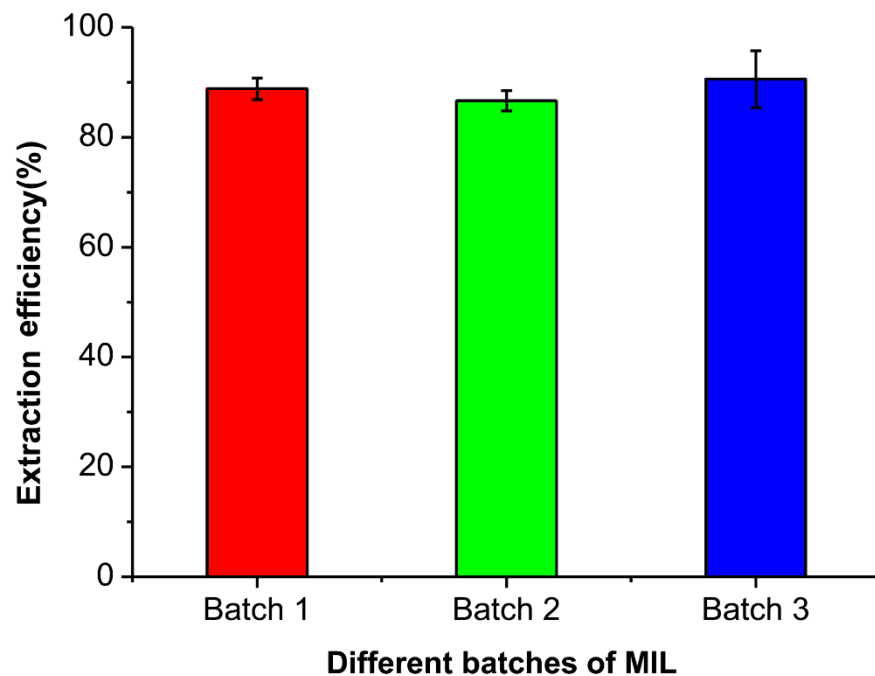


Fig. S13 Extraction efficiencies of poly-C₂₀ probe using three different batches of Co(II)-based MIL. A solution containing 0.44 μM poly-C₂₀ probe DNA in 50 μL DI H₂O was extracted using 1 μL of the [P₆₆₆₁₄⁺][Co(hfacac)₃⁻] MIL with a 30 s extraction time and a vortex rate of 2000 rpm.