E-Supporting Information-1 (ESI-1)

Ionic liquids promote PCR amplification of DNA†

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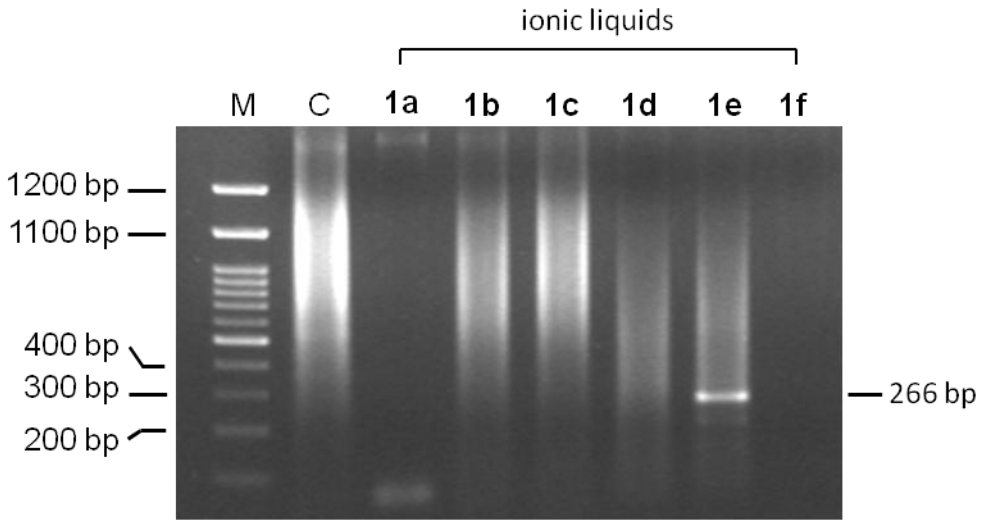

Fig. S1 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids, **1a**-**1f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. The protocol of "Slowdown PCR" described in the section of Materials and Methods was used for gene amplification. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **1a**-**1f**, respectively. Albeit similar in size, the DNA band shown in lane 7 (ionic liquid **1e**) was adduct from nonspecific amplification, after a careful comparison with the correct 266-bp product in gel mobility.

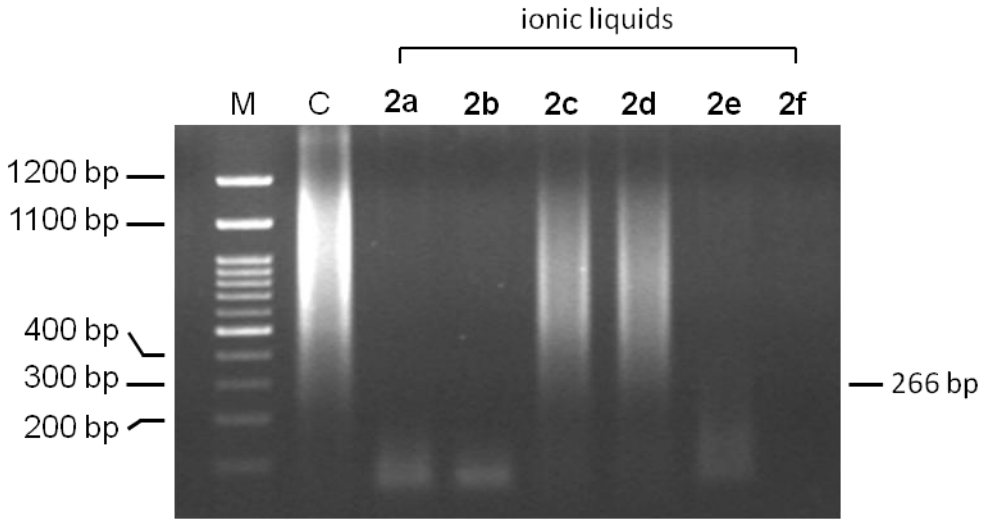

Fig. S2 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids, **2a**-**2f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. The protocol of "Slowdown PCR" described in the section of Materials and Methods was used for gene amplification. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **2a**-**2f**, respectively.

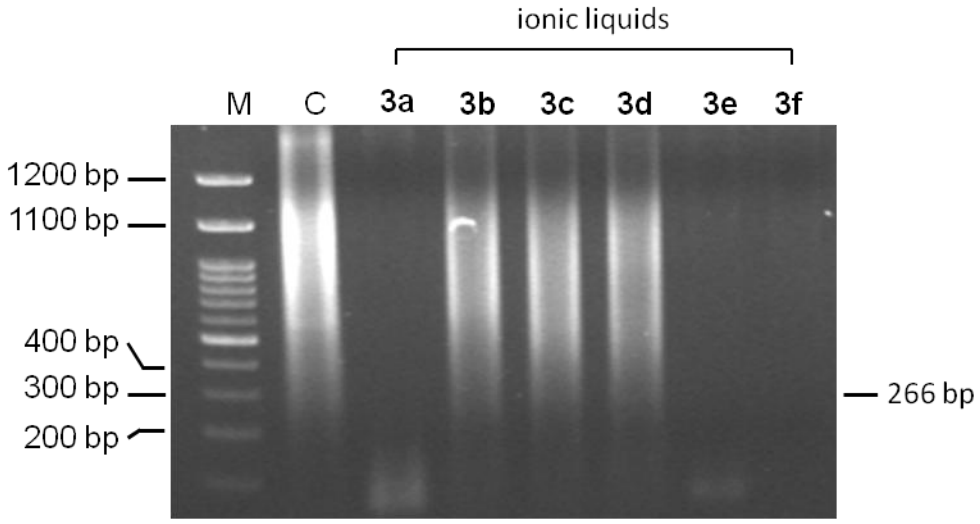

Fig. S3 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids, **3a**-**3f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. The protocol of "Slowdown PCR" described in the section of Materials and Methods was used for gene amplification. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **3a**-**3f**, respectively.

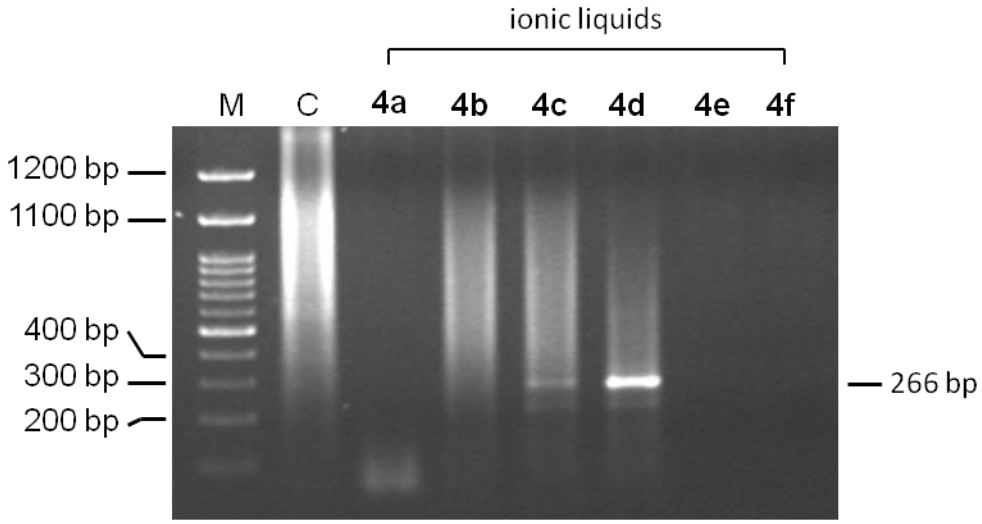

Fig. S4 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids, **4a**-**4f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. The protocol of "Slowdown PCR" described in the section of Materials and Methods was used for gene amplification. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **4a**-**4f**, respectively. DNA sequencing of the adduct isolated from lane 6 (ionic liquid **4d**) confirmed the PCR product to be the high GC content template **A**.

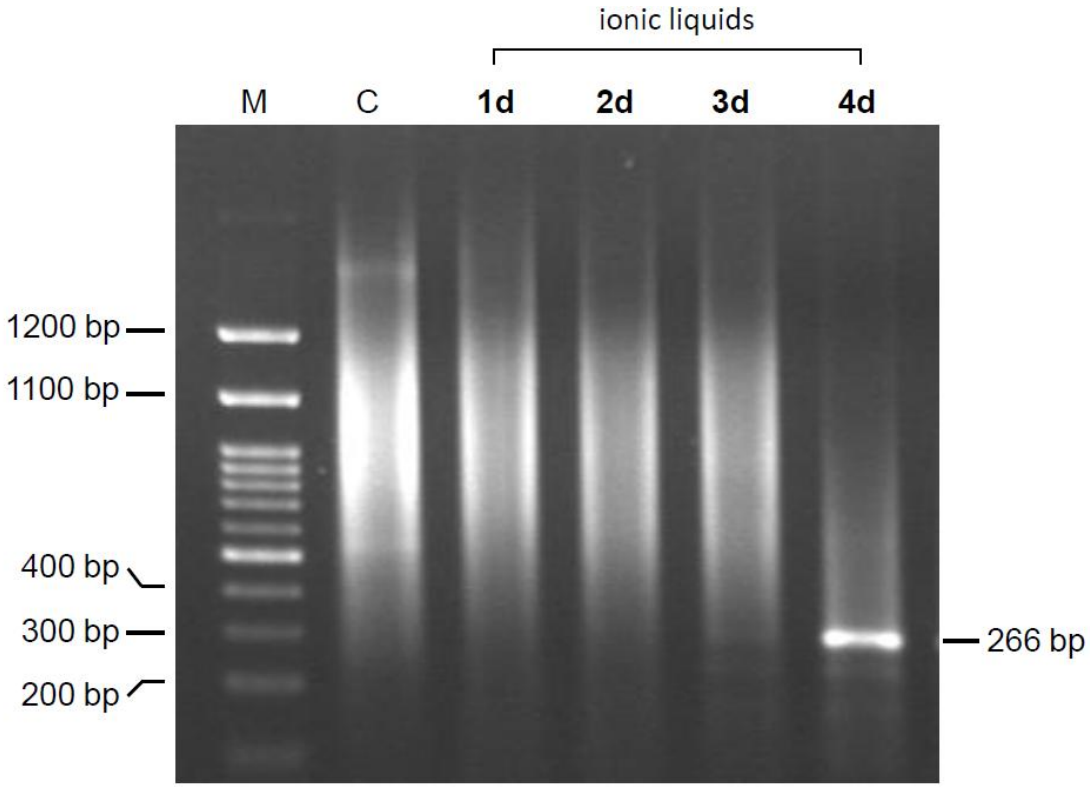

Fig. S5 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids **1d**, **2d**, **3d**, and **4d**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. The protocol of "Slowdown PCR" described in the section of Materials and Methods was used for gene amplification. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-6, ionic liquids **1d**, **2d**, **3d**, and **4d**, respectively.

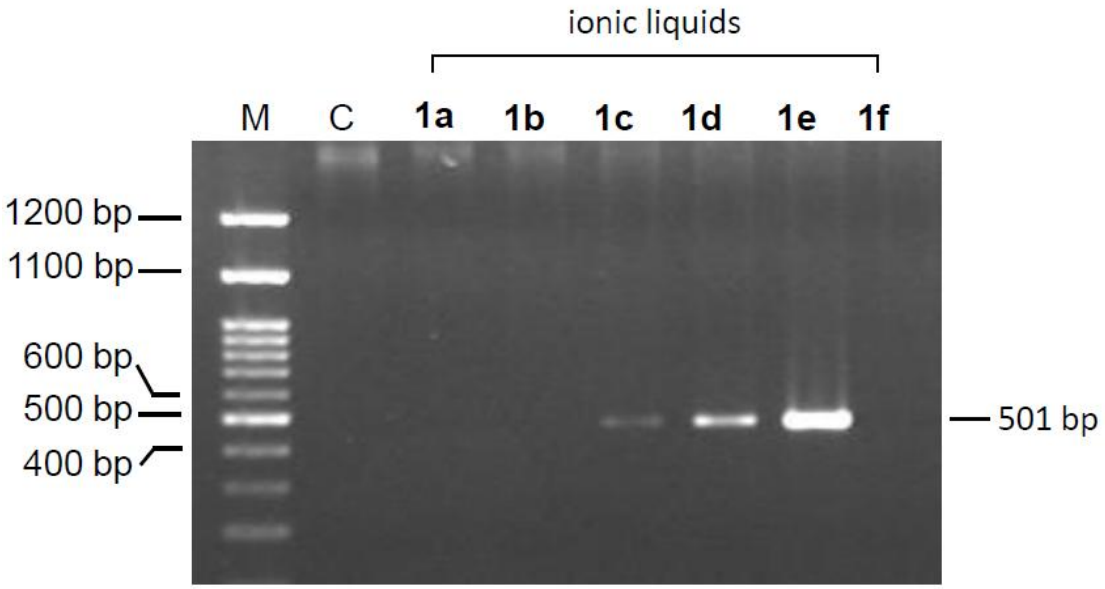

Fig. S6 PCR amplification of normal GC 501-bp DNA template **B** facilitated by ionic liquids, **1a-1f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **1a-1f**, respectively.

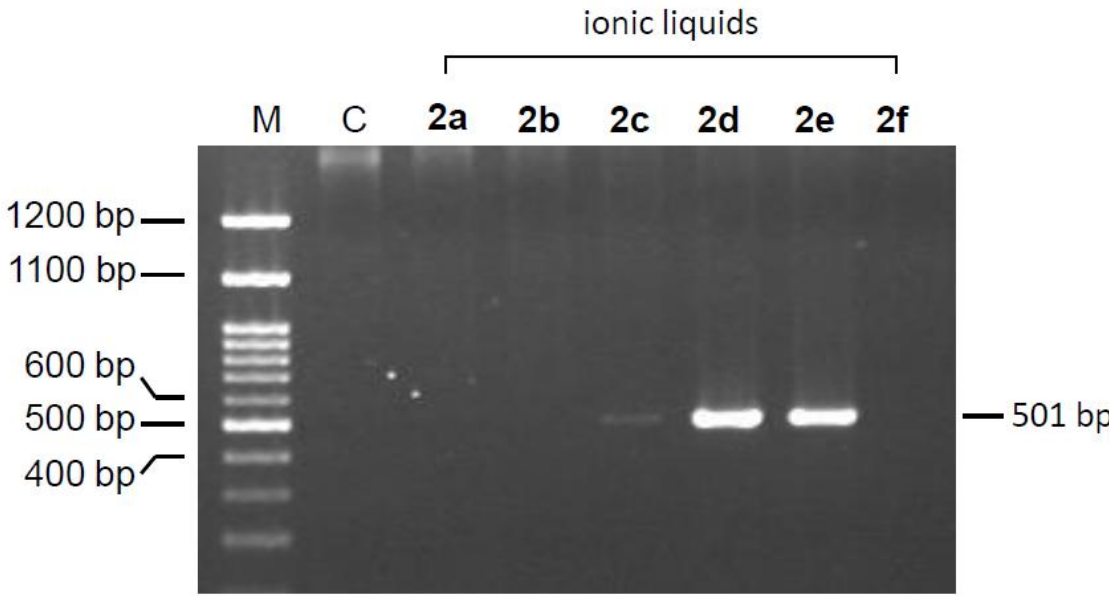

Fig. S7 PCR amplification of normal GC 501-bp DNA template **B** facilitated by ionic liquids, **2a**-**2f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **2a**-**2f**, respectively.

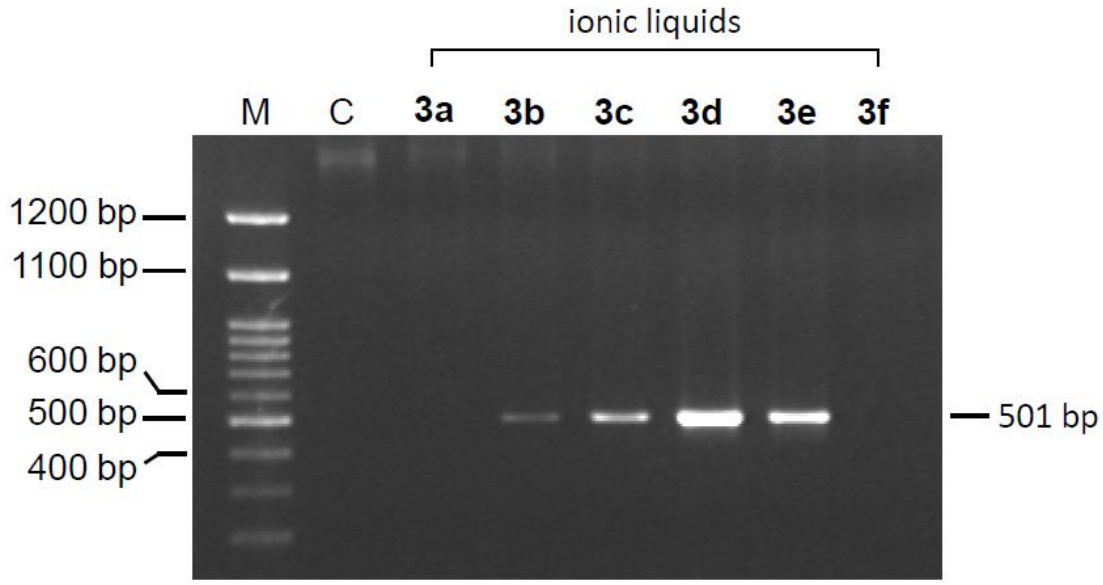

Fig. S8 PCR amplification of normal GC 501-bp DNA template **B** facilitated by ionic liquids, **3a**-**3f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **3a**-**3f**, respectively.

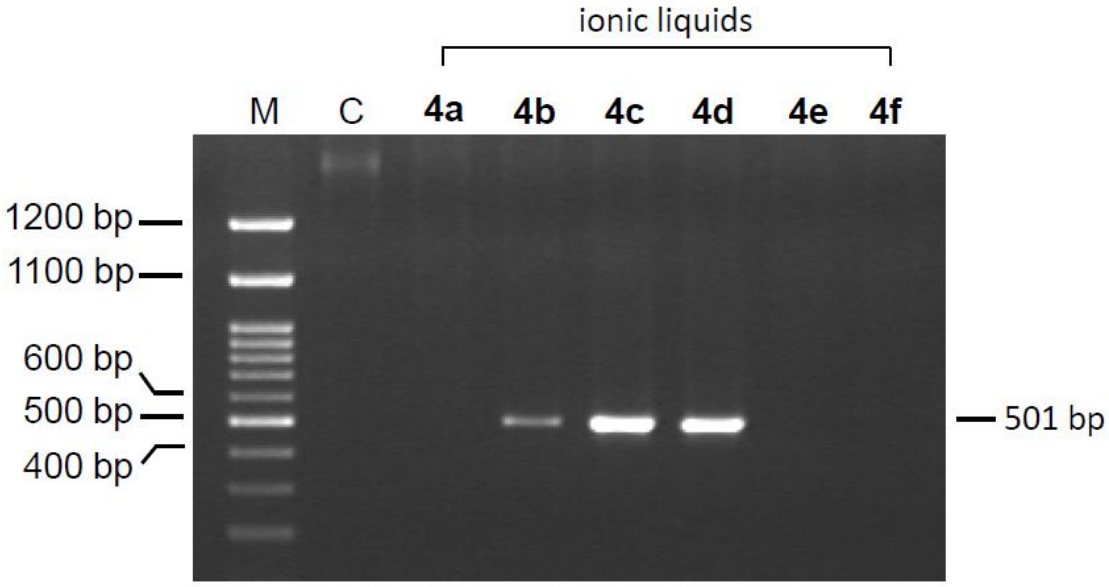

Fig. S9 PCR amplification of normal GC 501-bp DNA template **B** facilitated by ionic liquids, **4a**-**4f**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-8, ionic liquids **4a**-**4f**, respectively. DNA sequencing of the adduct isolated from lane 6 (ionic liquid **4d**) confirmed the PCR product to be the high GC content template **A**.

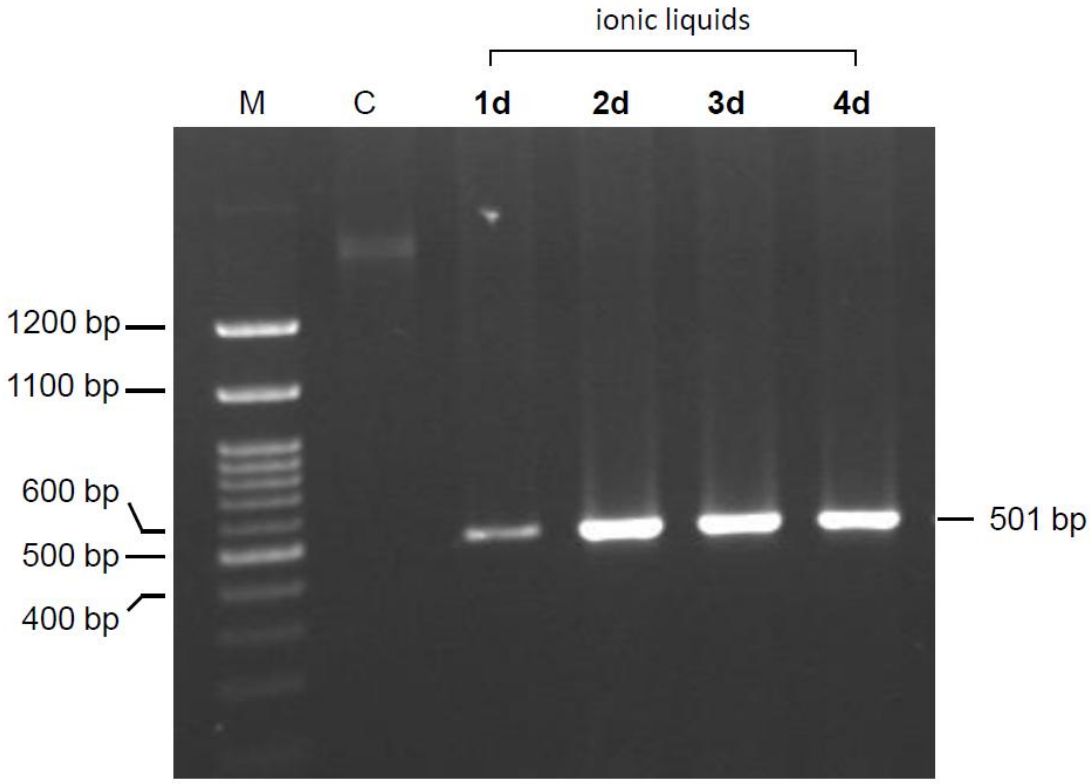

Fig. S10 PCR amplification of GC-rich 266-bp DNA template **A** facilitated by ionic liquids **1d**, **2d**, **3d**, and **4d**. The reaction solution was composed of 2.5 μL 10×PCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa Bio Inc.), 2.5 μL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa Bio Inc.), 0.4 μL primers (25 μM each), and 70 mM ionic liquid. After PCR, a portion of reaction solution (5 μL) was loaded onto a 1% agarose gel. Lane M, 100-bp DNA ladder (GeneDireX); lane C, no ionic liquid as control; lanes 3-6, ionic liquids **1d**, **2d**, **3d**, and **4d**, respectively.

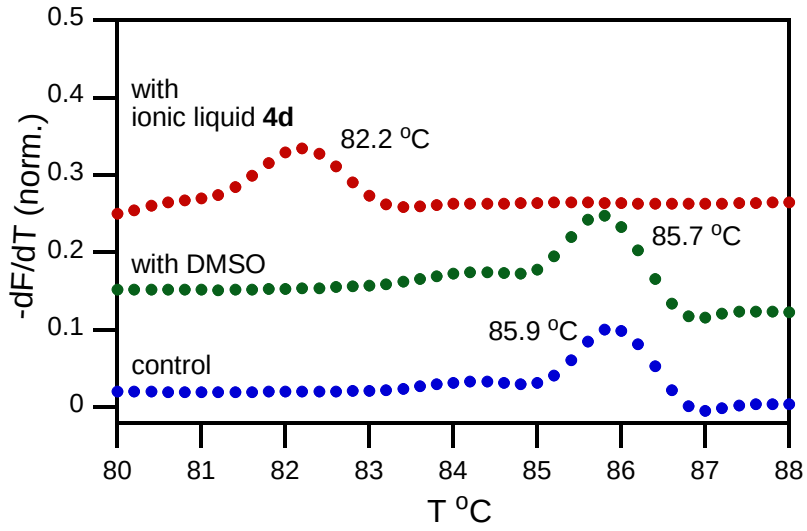

Fig. S11 Fluorescence annealing curves for normal GC DNA duplexes (PCR product **B**, 501 bp) in the presence of ionic liquid **4d** or DMSO (70 mM each). The curves were obtained by differentiating the fluorescence signal from SYBR Green I in the presence of DNA while heating from 60 to 98 °C in increments of 0.2 °C using a realtime PCR instrument (curves were vertically shifted for clarity). The peak positions represent the annealing temperatures T_m, written above each curve. T_m values follow the trend **4d** < DMSO ~ control.

Materials and methods

Primer design

Optimal primers were designed using the Primer premier software 5.0 (PREMIER Biosoft International) with consideration of the following criteria:

(i) Primer length: to achieve effective and specific amplification of template DNA and to prevent formation of secondary structures, it generally requires the optimal length of primers being in the range of 17-24 bp. The search for optimal primers started with primer lengths using the minimum acceptable values. If the primer T_m or the GC% was not within the desired range, nucleotides were added until maximum length was reached.

(ii) T_m: melting temperatures were calculated using the nearest neighbor thermodynamic theory. The range of T_m should provide sufficient thermal window for annealing. Therefore, T_m of all possible primers for the sequencing along the region selected for the search were calculated followed by computing an average T_m. A range is then calculated around this average by using reasonable tight numbers. Optimal GC% was selected to be at least 50% or higher.

(iii) Primer degeneracy, 3' end stability, GC clamps as well as secondary structures such as hairpins, dimers and false priming were all taken into account by designing primers. All primers were purchased from Bio Basic Inc. (Ontario, Canada).

Table 1 Oligonucleotide sequences of primers with calculated T_m and GC content.

Primer	Sequence	T_m (^{o} C)	GC %	PCR product length
GC-rich	5'-AGGACGGGGACTGGTAGC-3'	66.67	60.07	266 bp
	5'-AGGTGTAGGTGCCGAGGAG-3'	63.16	60.27	
Normal GC	5'-GAAGTCGTTACGCCATTCGT-3'	50.00	60.14	501 bp
	5'-AGGTAAATCCGCACACCTTG-3'	50.00	59.99	

PCR conditions

All PCR reactions were performed with 47 ng μl^{-1} genomic DNA of *Streptomyces coelicolor* strain, which was extracted as described by Verhasselt and co-workers [1]. The reactions were carried out in a total volume of 25 µL. PCR contained 2.5 µL 10×PCR buffer supplied by the manufacturer (TaKaRa, 100 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1 µL dNTP (2.5 mM each), 2.5 U Taq Polymerase (TaKaRa), 0.4 µL primers (25 µM each), and 70 mM enhancers or ionic liquids.

Cycling conditions

The S1000 Thermal Cyclers from BIO-RAD (USA) with two different cycling protocols was used; that is, a standard PCR protocol with fixed annealing temperatures, and a 'Slowdown' protocol with a fixed starting annealing temperature followed by various annealing temperatures, prolonged cycles, and small ramp rates.

It was performed as described by Frey and co-workers [2].

Standard PCR condition was: 96 ^{o}C for 6 min to ensure complete first-strand separation followed by 24 cycles of 95 ^{o}C for 20 s, 57 ^{o}C for 30 s, and 72 ^{o}C for 30 s, and final extension at 72 ^{o}C for 10 min.

'Slowdown' PCR condition was used with a generally lowered ramp rate at 2.5 ^{o}C/s and a small cooling rate for reaching annealing temperature at 1.5 ^{o}C/s. PCR was carried out with the following cycling conditions: amplification was proceeded for 48 cycles with 30 s denaturation at 95 ^{o}C, 30 s annealing with a progressively lowered temperature from 70 ^{o}C to 53 ^{o}C at a rate of 1 ^{o}C every third cycle, and a primer extension of 40 s. followed by 15 additional cycles with an annealing temperature of 58 ^{o}C.

Gel electrophoresis

5 μL of PCR products with 1 μL loading buffer were loaded onto 1% agarose gels and visualized under UV-illumination.

Melting curve dissociation analysis

Melting curve analyses were performed with Applied Biosystems StepOne instrument (Carlsbad, CA, USA). Each 20 μL sample contained 2× KAPA SYBR FAST qPCR Master Mix 10 μL (Kapa Biosystems, Woburn, MA, USA), 2 ng μL^{-1} amplified product as template and 70 mM additives (ionic liquid or DMSO). Melting curve analysis was done with temperature range from 60 ^{o}C to 98 ^{o}C at heating ramp of 0.2 ^{o}C. The T_m was directly measured using the software (StepOne version 2.1, Applied

Biosystems) provided by the manufacturer.

DNA sequencing

After PCR amplification, the products **A** and **B** were 266 bp and 501 bp shown in 1% agarose gel, respectively. After purification of the desired DNA product, the amplicon was cloned into a plasmid vector using a TA Cloning Kit (Yeastern Biotech Co., Shijr, Taiwan). Plasmid was isolated from the TA Cloning Kit host using a LiCl-phenol-chloroform extraction method. Clones were verified using Hind III (JENA Bioscience, Jena, Germany), following the manufacturer recommendation. Plasmid insert (PCR amplicon) was sequenced by an external DNA sequencing service (Mission Biotech Co., Taiwan). GenBank Blast search (http://www.ncbi.nlm.nih.gov/BLAST/) was used for amplicon/isolate identification.

The sequence of PCR product **B** with normal GC content (501 bp):

AGGTAAATCCGCACACCTTGTGGCTGAGACCTGATGCCGAGCCGATTGTGGTGAAGTGGATGATCCTATGCTGTCGAGAAAAGCC

TCTAGCGAGTTTCATGGCGGCCCGTACCCTAAACCGACTCAGGTGGTCAGGTAGAGAATACCGAGGCGTTCGGGTGAACTATGGT

TAAGGAACTCGGCAAAATGCCCCCGTAACTTCGGGAGAAGGGGGGCCACACCTGGTGACGAGTTTTGCACTCTGAGCTGGGGGT

GGCCGCAGAGACCAGCGAGAAGCGACCGTTTACTAAAAACACAGGTCCGTGCGAAGCCGTAAGGCGATGTATACGGACTGACGC

CTGCCCGGTGCTGGAACGTTAAGGGGACCGGTTAGTCACATTTCGGTGTGGCGAAGCTGAGAACTTAAGCGCCAGTAAACGGCG

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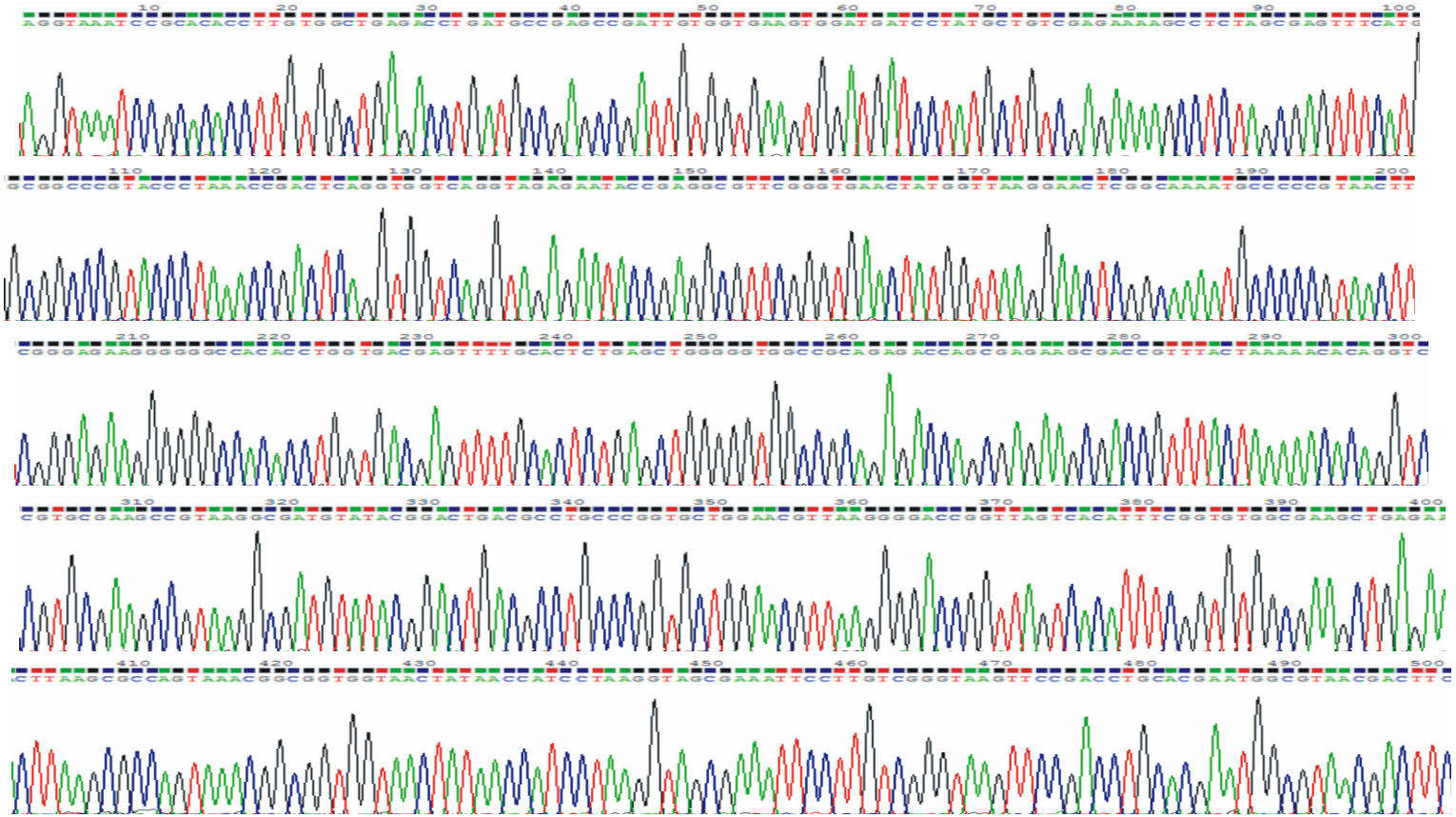

The sequence of PCR product **A** with high GC content (266 bp):

AGGACGGGGACTGGTAGCGGCGCGAGGGGCGGTACGGGTGCGGGAGGACGGCATGGACGGGGCCGACTGGAGCAGGCGGCGA

CTGGTCGGGCTCCTCGCCCGGACCGCCGCCGCGGCGGCGGCACTCCCCGCGGCGGCGTGCGGCGGCCCGGCGGGGACTCAGGC

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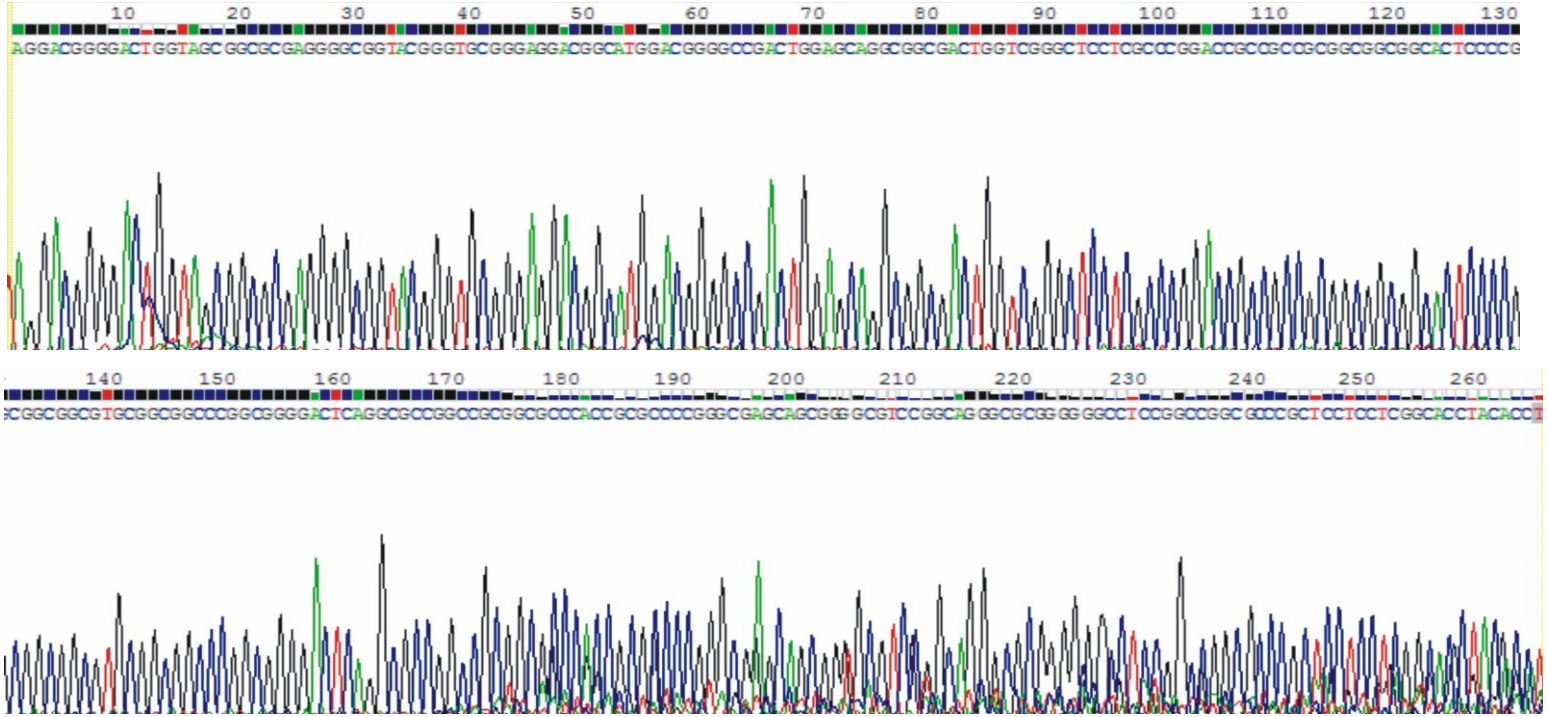

References:

[1] Verhasselt P, Poncelet F, Vits K, Van Gool A, Vanderleyden J. 1989. Cloning and expression of a *Clostridium* acetobutylicum alpha-amylase gene in *Escherichia coli*. FEMS Microbiol. Lett. 59(1-2): 135–140.

[2] Hagen S. Bachmann, Winfried Siffert, Ulrich H. Frey. Successful amplification of extremely GC-rich promoter regions using a novel 'Slowdown PCR' technique. Pharmacogenetics 2003, 13(12): 759-766.