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 MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 10xPCR buffer (100 mM tris–HCl, pH 8.3, 500 mM KCl, 15 mM MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 MgCl₂; 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 $4d < DMSO \sim$ 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.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>$T_m$ (°C)</th>
<th>GC %</th>
<th>PCR product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-rich</td>
<td>5’-AGGACGGGGACTGGTAGC-3’</td>
<td>66.67</td>
<td>60.07</td>
<td>266 bp</td>
</tr>
<tr>
<td></td>
<td>5’-AGGTGTAGGGCTCGGAGG-3’</td>
<td>63.16</td>
<td>60.27</td>
<td></td>
</tr>
<tr>
<td>Normal GC</td>
<td>5’-GAAGTCGGTTACGCACCTGT-3’</td>
<td>50.00</td>
<td>60.14</td>
<td>501 bp</td>
</tr>
<tr>
<td></td>
<td>5’-AGGTAATCCGCACACCTTG-3’</td>
<td>50.00</td>
<td>59.99</td>
<td></td>
</tr>
</tbody>
</table>

**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 °C for 6 min to ensure complete first-strand separation followed by 24 cycles of 95 °C for 20 s, 57 °C for 30 s, and 72 °C for 30 s, and final extension at 72 °C for 10 min.

‘**Slowdown’ PCR** condition was used with a generally lowered ramp rate at 2.5 °C/s and a small cooling rate for reaching annealing temperature at 1.5 °C/s. PCR was carried out with the following cycling conditions: amplification was proceeded for 48 cycles with 30 s denaturation at 95 °C, 30 s annealing with a progressively lowered temperature from 70 °C to 53 °C at a rate of 1 °C every third cycle, and a primer extension of 40 s. followed by 15 additional cycles with an annealing temperature of 58 °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 °C to 98 °C at heating ramp of 0.2 °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):

AGGTAAATCCGCACACCTTGTGGCTGAGACCTGATGCGCCAGCAGATTGTGGGTGAATGGATGATCCTATGCTGTCGAGAAAAGCC
TCTAGCGAGTTTCATGGCGGCCCGTACCCTAAACCACAGCTCAAGCTAGTAGAAATACCGAGGGTGTCGGGTGAACTATGGT
TAAGGAAACTCGGGCAAAATGCCCCCGTAACCTTGCGGAGAAAGGGGGGCCACACCTGTGAGACGGAGTTTTTGACTCTGACGCTGGG
GGCCGCAGAGCAGCAGAGCGACCGCCGTTTACTAAACACAGGTCCGTGCGAAAGCCGTAAAGCAGATGTATACGGACTGACGC
CTGCCCGGTGCTGGAACGTTAAGGGGACCGGTTCACATTTTCTGCTTGGTCAGAGCTGAAGCTAAAGCCCGAGTAAACCGCG
GTGGTAAGTCTATAACCATCCTAAAGGTAGCGAAATTCCTTTGTCCCGGTAAGTTCCGACCTGCAACGAAATGGCGTAACGACTTC
The sequence of PCR product A with high GC content (266 bp):

AGGACGGGGACTGTAGCGGCGGAGGGGGCGGTACGGTGCTCGGAGGACGCGCCTGGACCTGACACTGGAGCAGGGCGGCGA
CTGGTCGGGACTCTGCTGCCCCGACCGCCGCGGCCGCGCGGCGACTCCCCGCCGCGCGGTGCCGGCGGCCCCGCGGGAGACTCAGGC
GCCGCGCGGCGGCCACCCGCCGCCCCCCGGCGACACCGGGGCTCCCAGGCGGCGGGGGCCCTCGCCGGCGGCCCGCTCC
TCCTCGGCCACCTACACCT
References:
