Improved efficacy of whole genome amplification from bacterial cells

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It is often necessary to conduct genetic or genomic analysis with a limiting amount of DNA. Whole genome amplification based on the multiple displacement capacity of DNA polymerases such as φ29 DNA polymerase (multiple displacement amplification; MDA) has been successfully used to amplify eukaryotic genomic DNA with minimum bias (1–3). Although the use of MDA has been limited so far to the genomic analysis of eukaryotic organisms, there may be useful applications of this method to prokaryotic genomes. For bacterial species that can be cultivated in laboratory conditions, pure genomic DNA of a single strain can be feasibly obtained in sufficient amounts necessary for genomic analyses such as genome DNA library construction, molecular typing or genome sequencing. However, more than 99% of microbial species in nature are refractory to cultivation in laboratory growth conditions (4). Consequently, knowledge of the genomic contents of microorganisms has been largely biased toward those that are culturable. In an effort to increase our understanding of the unculturable microorganisms, several strategies have been described (4). MDA is a promising tool to obtain a sufficient amount of genomic DNA from unculturable microbial cells in the environment. For example, the genome sequencing of the unculturable bacterium *Epulopiscium* is currently underway and being made possible in part by the MDA reaction to obtain genomic DNA (5).

For most genomic analyses of unculturable species, it would be necessary to obtain pure genomic DNA from a single strain without contaminating DNA. Such genomic DNA might be obtained by MDA from a single cell or a cluster of a single strain in an environmental niche. For such purposes, using bacterial cells rather than purified genomic DNA as templates in the MDA reaction would be beneficial to maximize the yield of DNA during purification steps. For PCR amplification, template DNA is usually obtained by heating bacterial cells (e.g., 95°C for 2–5 min) to release genomic DNA as well as plasmid DNA. However, it was shown that the heating step could cause depurination of the DNA molecules making them unsuitable as templates for PCR amplification (6). The heating of genomic DNA at 95°C also resulted in a significant decrease in the yield of MDA products in an eukaryotic system (1). In this study, denaturation by heating also resulted in lower locus representation, which is defined as the locus copy number in 1 μg of amplified DNA divided by the locus copy number in 1 μg of genomic DNA control. With denaturation by heating, the locus representation ranged from 2%–80%, while 80%–225% was obtained with nondenatured genomic DNA (1). It was previously demonstrated that the presence of PCR buffer during the heating step prevented depurination in DNA templates, which resulted in better efficacy in PCR amplification (6). In this study, we found that heating bacterial cells in the presence of PCR buffer resulted in DNA templates that increased the efficacy of MDA reactions.

An overnight culture of *Salmonella enteritidis* LK5 (7) grown in Luria Bertani (LB) broth was serially diluted in sterile water. Viable cells were counted by plating the serial dilutions on LB agar plates. The dilutions were used as templates in MDA reactions using the whole genome amplification kit REPLI-g 625S (Molecular Staging, New Haven, CT, USA). A 2-μL aliquot of each dilution containing approximately 600, 60, and 6 colony-forming units (cfu) was mixed with either 0.5 μL double-distilled wa-

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merase were added to each tube. Then the reactions were incubated at 30°C for 2 min and amplified through 30 cycles of 95°C for 30 s, 58°C for 1 min, and 70°C for 1 min. After a final extension at 70°C for 10 min, the reactions were kept at 4°C. As shown in Figure 1, PCR products of 294 bp were amplified from both MDA products obtained from 600 and 60 cfus (Figure 1, lanes 4 and 5, respectively), while the efficiency was much lower with 60 cfus.

In order to determine whether the presence of PCR buffer could improve the efficacy in MDA reactions, the MDA reactions were performed as before, using 2 μL of serial dilutions containing approximately 600, 60, and 6 cfus, except that dilutions were heated for 98°C for 10 min in the presence of 2× cloned Pfu DNA polymerase buffer. There was no gross difference in the yield of MDA products amplified by heating with and without 2× PCR buffer (Figure 2). It was previously reported that MDA reactions initiated with very small inputs of genomic DNA tend to be contaminated with spurious DNA sequences obtained

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>sdf I-F</td>
<td>5'-TGTTTTTATCTGATGCAAGAG-3'</td>
</tr>
<tr>
<td>sdf I-R</td>
<td>5'-CGTCTCGTGGTACCTACAG-3'</td>
</tr>
<tr>
<td>63F</td>
<td>5'-CAGGCTAACACATGCAGAATC-3'</td>
</tr>
<tr>
<td>1393R</td>
<td>5'-ACGGGCGGTGTACAAG-3'</td>
</tr>
<tr>
<td>rpoS-F</td>
<td>5'-AGATCTTCTTCCAGCCAGTGTGT-3'</td>
</tr>
<tr>
<td>rpoS-R</td>
<td>5'-AGATCTCACCGTACGCGTACTACT-3'</td>
</tr>
</tbody>
</table>

All of the oligonucleotides were obtained from Integrated DNA Technologies, Coralville, IA, USA.

**Table 1. PCR Primers and Sequences**

**Figure 1. Multiple displacement amplification (MDA) products of*Salmonella enteritidis* LK5.** Whole genomic DNA was amplified from approximately 600 (lane 2) and 60 (lane 3) colony-forming units (cfus) after releasing genomic DNA by heating at 98°C for 10 min. *S. enteritidis*-specific fragments (294 bp) were amplified from the respective MDA products (lanes 4 and 5). 1-kb DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as a standard marker (M).

**Figure 2. Effect of PCR buffer on multiple displacement amplification (MDA) reaction.** Whole genomic DNA was amplified from approximately 600 (lanes 1 and 2), 60 (lane 3), and 6 (lane 4) colony-forming units (cfus). All samples were heated at 98°C for 10 min in the presence of 2× PCR buffer before the MDA reactions except lane 1, which contained double-distilled water instead. Whole genomic DNA was also amplified using the genomic DNA purified from *Salmonella enteritidis* LK5 as a template (lane 5). 1-kb DNA ladder (Invitrogen) was used as a standard marker (M).
by primer-directed DNA synthesis (2).
To determine whether the presence of the
2× PCR buffer during the heating step
had effects on the quality of the MDA
products, three unlinked genetic loci
were simultaneously amplified in mul-
tiplex PCRs as described above using
those MDA products as templates. The
three sets of primers used in the multi-
plex PCRs (Table 1) were: (i) sdf I-F and
sdf I-R primers for sdf I fragment (8);
(ii) 63F and 1389R for 16S rRNA gene
(9); and (iii) rpoS-F and rpoS-R for the
promoter region and 5′ coding sequence
of rpoS gene. As shown in Figure 3, the
presence of 2× PCR buffer during heat-
ing to release genomic DNA greatly
improved the quality of the resulting
MDA products. From the MDA product
obtained from 600 cfus in the absence of
2× PCR buffer (Figure 3, lane 1), only the
16S rRNA gene sequence was amplified.
In contrast, the MDA products obtained
from 600 and 60 cfus in the presence of
2× PCR buffer allowed amplification of
all three fragments (Figure 3, lanes 2 and
3). The 16S rRNA gene fragment was
amplified even from the MDA product
obtained from 6 cfus in the presence of
2× PCR buffer (Figure 3, lane 4).

In summary, the results of this report
suggests that the addition of PCR buf-
er to 2× final concentration during the
heating step to release genomic DNA
from bacterial cells significantly im-
proved the quality of the MDA products.
We expect that this improvement in the
MDA reaction will help minimize the
number of bacterial cells needed to am-
plify whole genomic DNA. The avail-
ability of genomic DNA of unculturable
bacterial species by the improved MDA
protocol will facilitate characterization
of their genomic contents. The result
also suggests that it may be possible to
amplify whole genomic DNA with min-
imum bias even from a single bacterial
cell after appropriate improvements are
made in the MDA protocol.

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