



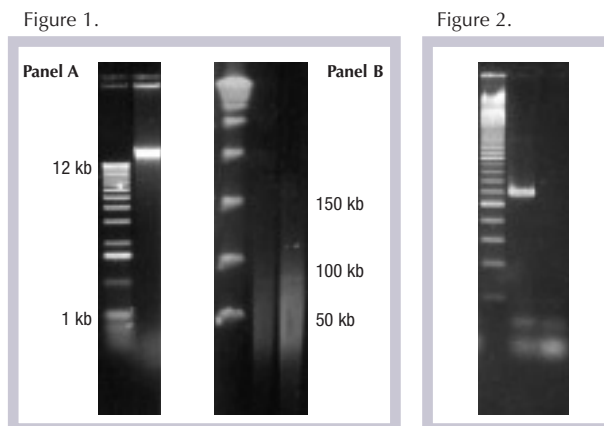
## Introducing MasterPure™ Gram Positive DNA Purification Kit

Bruce W. Jarvis and Les M. Hoffman, EPICENTRE

The MasterPure™ Gram Positive DNA Purification Kit contains all of the reagents needed to purify high-yields of PCR-ready DNA specifically from gram-positive bacteria. The kit's Ready-Lyse™ Lysozyme and Gram Positive Cell Lysis Solutions effectively lyse the sturdy cell walls of these bacteria. Ready-Lyse Lysozyme is provided as a stable solution of a non-mammalian, non-avian recombinant lysozyme, eliminating the need to prepare an enzyme solution. In contrast to other commonly-used lysozymes, this enzyme has high specific activity and has no net charge at neutral pH, so it does not bind to and, thus, decrease DNA yields.<sup>1</sup>

The complete, enzymatic lysis of the bacteria with the MasterPure Gram Positive DNA Purification Kit results in high yields of high molecular weight, PCR-ready DNA. From 1-ml aliquots of an overnight *Bacillus subtilis* culture, yields averaged 9 to 12 µg of DNA. Figure 1A shows a large DNA band above the 12-kb marker band when 300 ng of *B. subtilis* DNA, purified with the kit, was subjected to electrophoresis. The purified *B. subtilis* DNA was further characterized by pulsed-field gel electrophoresis (Figure 1B) and found to range in size from 30 to 80 kb. This size range is convenient for making fosmid libraries. Another important indication of the quality of the DNA is the ability to use it directly in PCR without further clean-up steps. For Figure 2, 0.5 ng of *B. subtilis* DNA was amplified by 30 cycles of PCR<sup>2</sup> and the expected 600-bp product was resolved on an agarose gel.

The MasterPure Gram Positive DNA Purification Kit has been successfully used to purify DNA from a variety of dif-

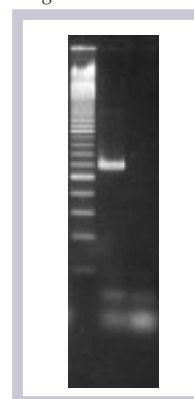


**Figure 1. Electrophoretic Analysis of DNA purified using the MasterPure™ Gram Positive DNA Purification Kit.** Panel A. DNA from *B. subtilis* (ATCC 6051) was separated on a 1% agarose gel and stained with SYBR® Gold. Lane 1, kilobase ladder. Lane 2, 300 ng of *B. subtilis* DNA. Panel B. Pulsed-field gel electrophoresis of *B. subtilis* (ATCC 6051) DNA, Lane 1, Phage lambda ladder. Lane 2, 300 ng of DNA. Lane 3, 600 ng of DNA.

ferent microbes. The necessary incubation time with Ready-Lyse Lysozyme varies with the species. Incubation times and yields for some common gram-positive bacteria are given in Table 1.

Two other commercially available kits for purifying gram-positive DNA were tested and compared to the MasterPure Kit. For one supplier, DNA purification from gram-positive bacteria is an adaptation of the kit's general protocol, so no lytic enzyme is provided. This kit uses a spin-column format and recommends that the user determine the number of cells and potential DNA yield in the samples before beginning the purification. The kit produced 30% as much DNA as the MasterPure Kit. The second supplier's kit, surprisingly, produced only 1% of the

Figure 2.



**Figure 2. PCR Product from DNA purified using the MasterPure™ Gram Positive DNA Purification Kit.** *B. subtilis* DNA (1 µl, 0.5 ng) was amplified by PCR using forward primer 5'-AGGGTCATTG-GAAACTGGG and reverse primer 5'-CGTGTGTAGCCAGGTCATA. The cycling conditions were 95°C for 2 minutes and then 30 cycles of 95°C (45 seconds); 55°C (45 seconds); 72°C (45 seconds), followed by 72°C for 2 minutes. Lane 1, 100-bp ladder. Lane 2, PCR product from 0.5 ng of *B. subtilis* DNA. Lane 3, no template PCR.

DNA yield as the MasterPure Kit. We believe this low yield is due to incomplete cell lysis by the kit's lytic enzyme, which cleaves the polysaccharide bonds found in yeast cell walls, but not found in bacterial cell walls. In summary, the MasterPure Gram Positive DNA Purification Kit purifies high yields of intact, PCR-ready DNA from a variety of gram-positive bacteria. All necessary reagents, including an enzyme that effectively lyses these bacteria, are provided and the method is readily scalable to larger volumes.

### Acknowledgment

We thank Dilara Begum for the pulsed-field gel data.

### References

- Hoffman, L.M. and Jarvis, B.W. (2003) *EPICENTRE Forum*. **10**(3), 3.
- Kuske, C.R. et al. (1998) *J. Bacteriol.* **64**(7), 2463.
- Hoffman, L. and Moan, E. (1998) *EPICENTRE Forum* **5**(1), 1.

[www.epicentre.com/masterpure\\_gpdna.asp](http://www.epicentre.com/masterpure_gpdna.asp)

### MasterPure™ Gram Positive DNA Purification Kit

MGP04020	20 reactions
MGP04100	100 reactions

#### Contents:

Gram Positive Cell Lysis Solution  
MPC Protein Precipitation Reagent  
Ready-Lyse™ Lysozyme  
Proteinase K  
TE Buffer  
RNase A

**Table 1. Incubation times needed for DNA recovery from gram positive bacteria.**

Species	Culture Medium	Ready-Lyse™ Incubation Time	DNA Yield* from 1-ml culture (µg)
<i>Bacillus subtilis</i>	Brain-Heart Infusion (BHI)	30 minutes	9.0
<i>Listeria monocytogenes</i>	BHI	Overnight	3.3
<i>Staphylococcus aureus</i>	BHI	Does not require Ready-Lyse	8.0
<i>Streptococcus mutans</i>	Todd-Hewitt	Overnight	3.0
<i>Lactococcus lactis</i>	M17	30 minutes	1.1

\*Yield was determined by fluorescence with Hoechst dye 33258.<sup>3</sup>