Production and Analysis of High Molecular Weight Genomic DNA for NGS Pipelines Using Agilent DNA Extraction Kit (p/n 200600)

Application Note

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Abstract

The Agilent DNA Extraction Kit (p/n 200600) was compared to standard methods such as beadbeating and enzyme treatment for preparation of genomic DNA from the prokaryote *Listeria monocytogenes*. Using this extraction kit, with modifications, to lyse the bacteria and isolate high molecular weight DNA reproducibly yielded high quality DNA suitable for further applications such as polymerase chain reactions to produce amplicons, or for next-generation DNA sequencing. The quality of the high molecular weight DNA, and the comparison of extraction methods, was shown on the Agilent 2200 TapeStation with the Agilent Genomic DNA ScreenTape (p/n 5067-5365) and Agilent Genomic DNA Reagents (p/n 5067-5366).

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Introduction

This application note demonstrates the use of an Agilent DNA extraction kit with microbial samples. The Agilent 2200 TapeStation system, using *Listeria monocytogenes*, was employed for the assessment of genomic DNA (gDNA) samples sizing and quantification. High molecular weight gDNA (HMgDNA) is required for many different assays. DNA for polymerase chain reaction (PCR) was used with an Agilent 2100 Bioanalyzer system to assess PCR amplicons.

Production of HMgDNA is critical to the successful DNA sequencing of microbial genomes. It is possible to sequence the genome of many types of bacteria, but one of the limitations is the ability to lyse the microbe and collect HMgDNA of sufficient quality for use in next generation sequencing (NGS). The 100K Pathogen Genome Project (http://100kgenome.vetmed.ucdavis.edu/) prepares hundreds of DNA samples each day for genome sequencing. As part of that effort, and to use NGS as part of a food-borne outbreak investigation, a protocol that lyses bacterial cells to release HMgDNA ready for sequencing with little to no previous experience is required.

L. monocytogenes, a gram positive, intracellular pathogen, is a major public health concern in the United States due to its high mortality rate and presence in the food supply. In the USA, one in six people becomes ill from eating contaminated food each year. *L. monocytogenes* accounted for 621 documented cases of food-borne illnesses in the USA during 2011, with 22% leading to death [1]. We selected this organism because of the difficulty of lysing gram-positive bacteria and the need to have a fast and robust method that consistently results in HMgDNA.

The Agilent DNA extraction kit (cat #200600) was used, with some modifications, to lyse and extract HMgDNA from *L. monocytogenes*. Following extraction, the DNA quality and quantity was measured with the 2200 TapeStation system to verify the HMgDNA before any NGS work. The extracted gDNA can be used for polymerase chain reaction (PCR), and the Agilent 2100 BioAnalyzer can be used for assessment of PCR amplicons. PCR amplification was done as described by the kit instructions (Black Diamond Genomics, Valrico, FL) using the *Listeria* primer pair to verify the quality of DNA from the isolation method.

Experimental

Bacteria

L. monocytogenes was cultured in Difco BHI Broth (Franklin Lakes, New Jersey) at 37 °C aerobically for 12–16 hours before using it to collect DNA. The amount of cells were normalized to a 600 nm (optical density at 600 nm) = 0.2, which is equal to ~1 x 10^8 cells/mL. For this experiment, 1 mL of the cultured broth was used to collect a cell pellet for genomic DNA extraction after centrifugation at 20,000 xg for 5 minutes.

Genomic DNA extraction

Genomic DNA was extracted from cell pellets and split for use with each of the testing methods. Each method was tested using three replicates.

- **Method 1:** Commercial DNA extraction kit with silica spin column [2,3]
- **Method 2:** Beadbeating with silica spin column [4,5]
- **Method 3:** Lysozyme and mutanolysin lysis and extraction [6,7]
- **Method 4:** Agilent lysing buffer containing SDS with silica spin column [8]
- **Method 5:** Agilent DNA extraction with modified protocol [this study]

The Agilent kit was modified for extraction as follows [9]:

1. Suspend collected cell pellet in 1 mL of Solution 2 and vortex until mixed throughly.
2. Add 1 µL of pronase. Incubate at 60 °C for 1 hour.
3. Place on ice for 10 minutes.
4. Add 500 µL of Solution 3 and invert to mix completely.
5. Place on ice for 5 minutes.
6. Centrifuge at 4,000 xg at 4 °C for 15 minutes and transfer supernatant to a new 1.5-mL microcentrifuge tube.
7. Centrifuge at 20,000 xg at 4 °C for 5 minutes and transfer supernatant to new 1.5-mL microcentrifuge tube.
8. Add 3 µL of RNase to the supernatant and invert to mix completely.
9. Incubate at 37 °C for 15 minutes.
10. Split supernatant into two 1.5-mL microcentrifuge tubes and add an equal volume of isopropanol to each tube.
11. Invert to mix completely.
12. Incubate on ice for 30–60 minutes.
13. Centrifuge DNA at 4,000 xg at 4 °C for 15 minutes.
14. Remove the supernatant (that is, isopropanol).
15. Fill tube containing the DNA pellet to rim with 70% ethanol.
16. Mix completely, centrifuge at 20,000 xg for 5 minutes, and discard ethanol.
17. Air dry and DNA and rehydrate DNA with 100 µL of water and resuspend.
18. Combine both tubes into a single sample. This yields 200 µL DNA.

Genomic DNA analysis

After cell lysis and DNA extraction, the samples were placed in a 96-well plate for the initial quality assessment using the NanoDrop 1000 (NanoDrop Technologies) spectrophotometer. Subsequently, the 2200 TapeStation with the Agilent Genomic DNA ScreenTape (p/n 5067-5365) and Agilent Genomic DNA Reagents (p/n 5067-5366) [10] were also used to evaluate the size of the gDNA, quantification, and quality of the extracted gDNA [11]. Following manufacturer guidelines, the first well of the 96-well plate contained the 3 µL of the Genomic DNA Ladder. Each well following the ladder contained 1 µL of the extracted genomic sample with 10 µL of the Genomic DNA Sample buffer, and the 96-well plate was vortexed on high speed for a few seconds with a brief centrifugation at 500 xg. Then, the plate was placed into the 2200 TapeStation. Each extraction method was analyzed for size and quantity. This protocol was tested with 200 different isolates of *L. monocytogenes* to determine the consistency over individual isolates.

Results and Discussion

The concentration, quality and molecular weight was measured for the gDNA against each lysis and extraction method [12]. The 260 nm/280 nm and 260 nm/230 nm ratios were used to quickly assess the contamination of the gDNA with protein or organics, respectively. The ratio of ≥ 1.8 is acceptable to proceed with additional DNA assessment (Figure 1).

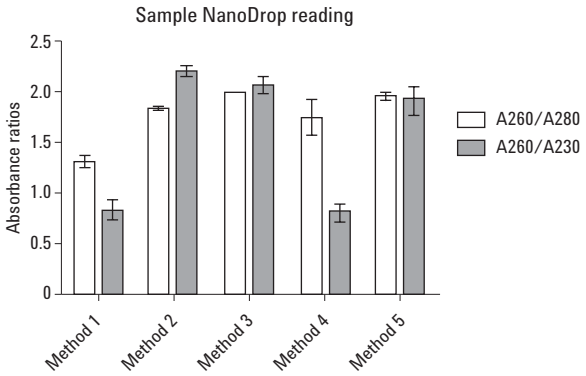

Figure 1. Mean quantification data by NanoDrop including standard error mean (SEM) bars for each extraction method.

Methods 2, 3, and 5 produced gDNA of sufficient quality to proceed with additional assessment of HMgDNA with the 2200 TapeStation. The Genomic DNA ScreenTape used the lower marker of the DNA ladder to quantify the samples in each lane (Figure 2). Methods 2, 3, and 5, produced HMgDNA, which appears as the intense largest band in each lane.

Using the electropheogram mode, each sample was measured to determine the total DNA size and quantity. (Figure 3A–E). HMgDNA size ≥ 50 kb will be acceptable DNA for NGS pipeline.

After demonstrating that the modified Agilent DNA extraction kit lysed and extracted HMgDNA, an additional 200 *L. monocytogenes* samples were processed using the modified protocol.

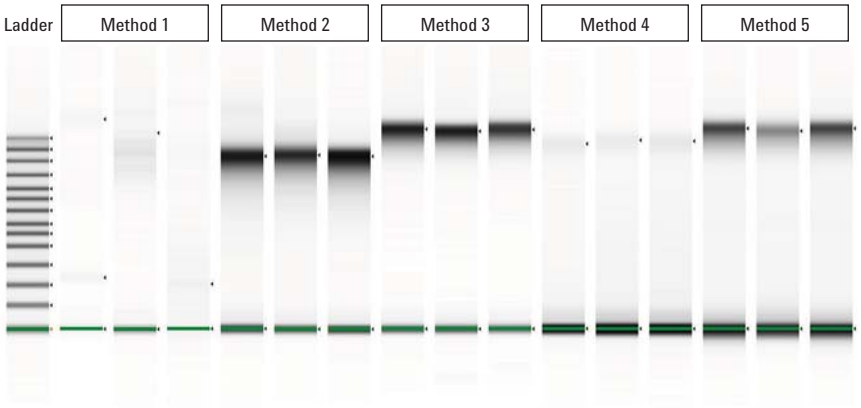

Figure 2. Each replicate of gDNA was examined using the TapeStation to determine degradation and amount of HMgDNA produced from each method.

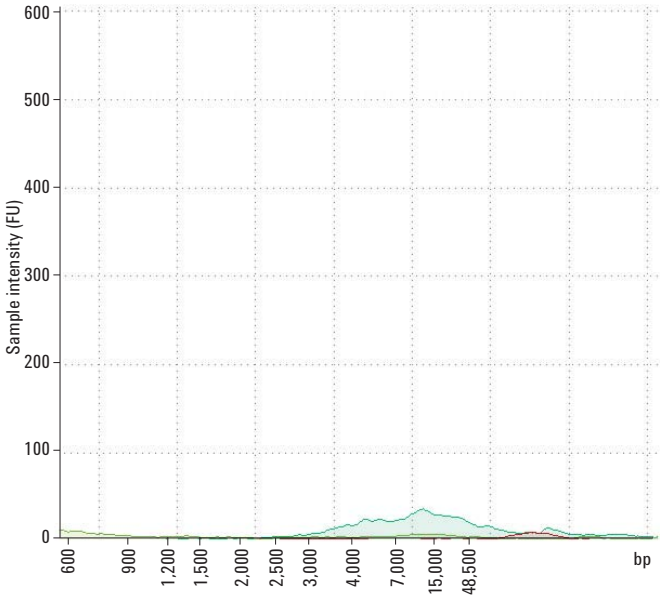

Figure 3A. Electropherogram for extraction Method 1: Commercial DNA extraction kit. In all figures, the internal standard is shown at 100 base pairs.

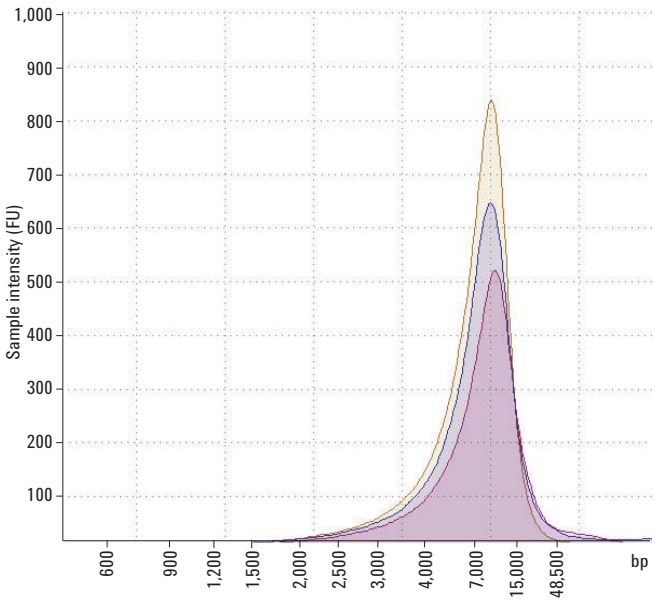

Figure 3B. Electropheogram for extraction Method 2: Beadbeating.

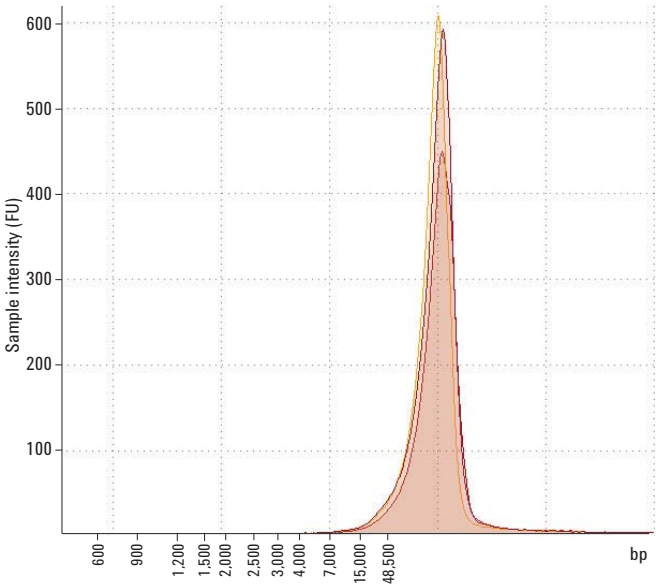

Figure 3C. Electropheogram for extraction Method 3: Lysozyme and mutanolysin.

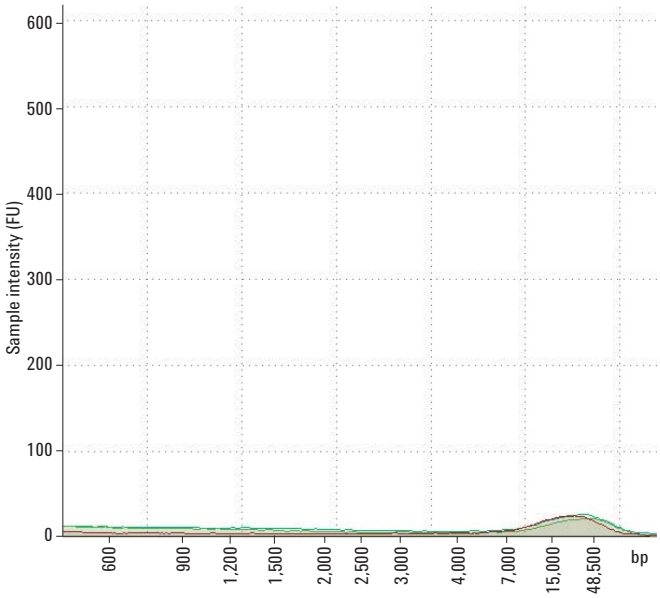

Figure 3D. Electropheogram for extraction Method 4: Lysis buffer containing SDS.

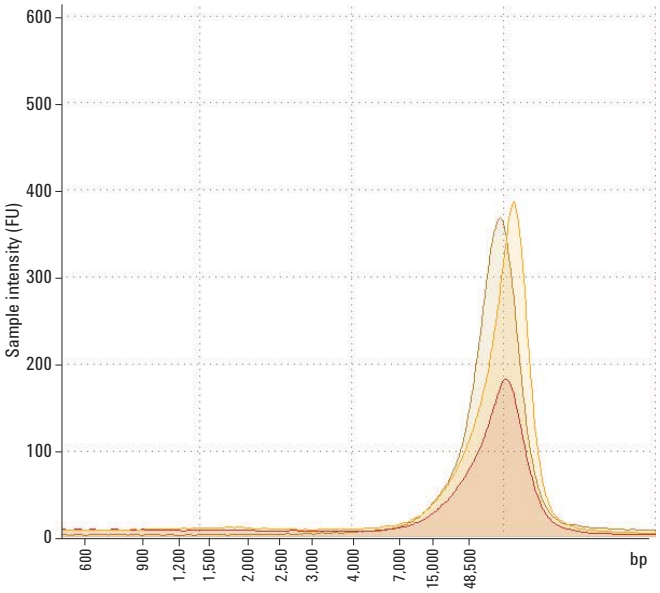

Figure 3E. Electropheogram for extraction Method 5: Agilent DNA Extraction Kit with modified protocol.

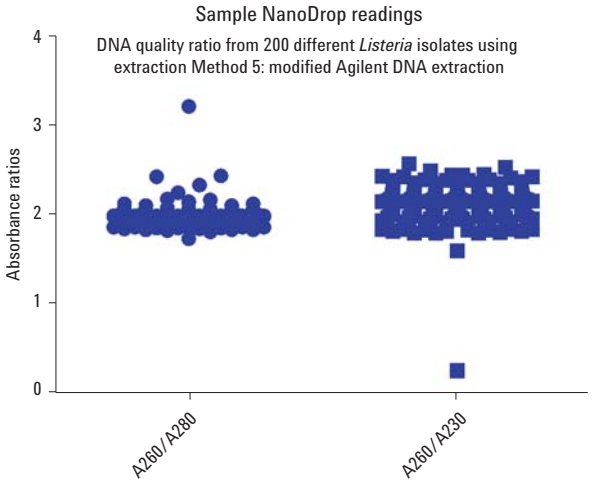

Figure 4. NanoDrop readings of isolates with 99% passed (198/200). The ratio of ≥ 1.8 ratio is acceptable as pure DNA. Average A260/A280 is 1.9 ± 0.08 and average A260/A230 is 2.1 ± 0.16.

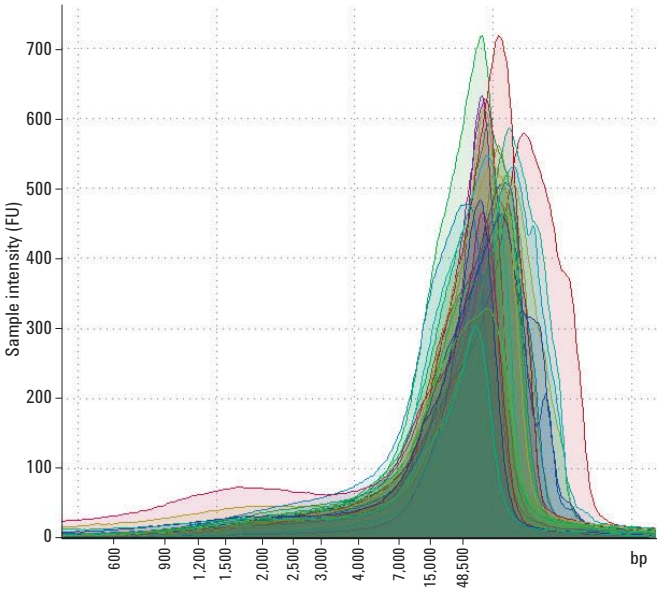

Figure 5. Overlay of electrophoresis showing molecular weight for each Listeria isolate sample. It illustrates the extraction efficiency using Method 5: modified Agilent DNA extraction method.

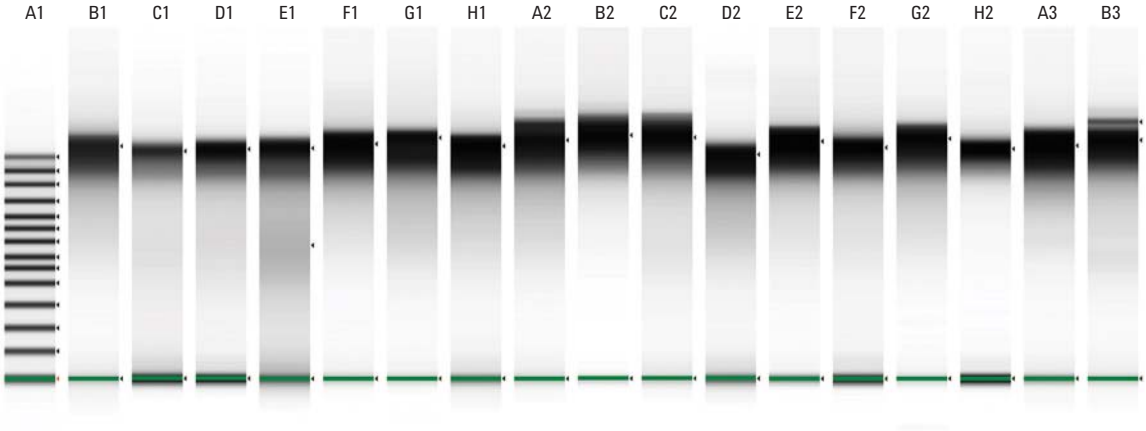

Figure 6. Example image of DNA isolated from Listeria. This is a representative example from 200 different isolates using the TapeStation to determine the gDNA size using extraction Method 5: modified Agilent DNA extraction.

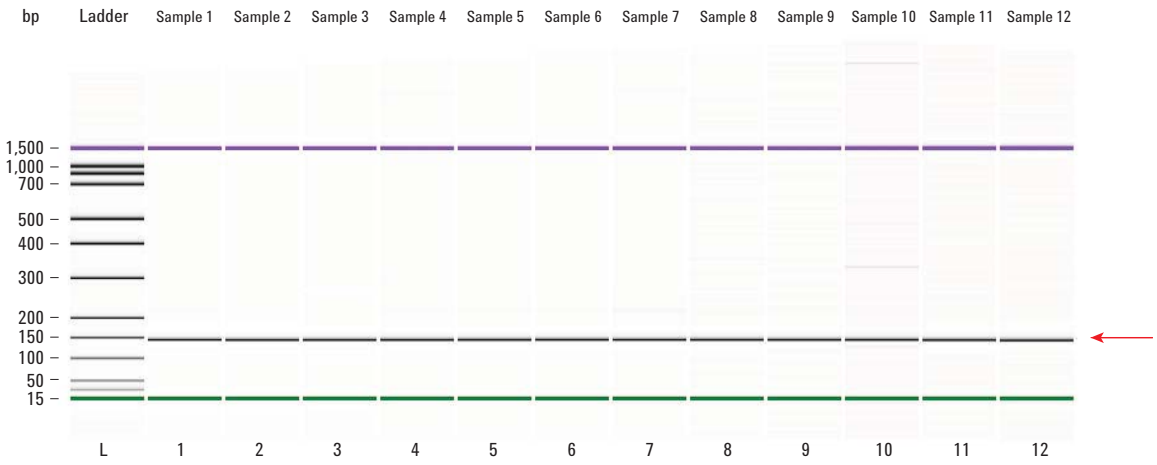

Figure 7. Example image of QC amplicion to verify the gDNA isolated from Listeria using extraction Method 5 (modified Agilent extraction) produced DNA of sufficient quality for PCR. This is a representative example from 200 different isolates using the Agilent 2100 Bioanalyzer with the DNA 1000 chip.

Conclusion

Genomic DNA from *Listeria* using Extraction Method 5 (as shown in Figures 4–6) was also used to demonstrate that the DNA was of sufficient quality to enable PCR and was large enough to use for other applications, such as NGS. Methods 2 and 3 were used as controls, since they are accepted protocols; however, these methods often produce lower molecular weight DNA. The modified protocol with the Agilent DNA extraction kit consistently produced HMgDNA similar to the classical lysing method containing lysozyme and mutanolysin, but without the additional cost of enzymes and in a simplified procedure that is amenable to automation or use by less experienced operators.

Acknowledgment

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