High-Throughput Analysis of Foodborne Bacterial Genomic DNA Using Agilent 2200 TapeStation and Genomic DNA ScreenTape System

Application Note

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Abstract

The initial step in Next Generation Sequencing is to construct a library from genomic DNA. To gain the optimum result, extracted DNA must be of high molecular weight with limited degradation. High-throughput sequencing projects, such as the 100K Pathogen Genome Project, require methods to rapidly assess the quantity and quality of genomic DNA extracts. In this study, assessment of the applicability of the Agilent 2200 TapeStation was done using genomic DNA from nine foodborne pathogens using several accepted high-throughput methods. The Agilent 2200 TapeStation System with Genomic DNA ScreenTape and Genomic DNA Reagents was easy to use with minimal manual intervention. An important advantage of the 2200 TapeStation over other high-throughput methods was that high molecular weight genomic DNA quality and quantity can be quantified apart from lower molecular weight size ranges, providing a distinct advantage in the library construction pipeline and over other methods available for this important step in the Next Generation Sequencing process.

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Introduction

Large scale sequencing projects, such as the 100K Foodborne Pathogen Genome Project (http://100kgenome.vetmed.ucdavis.edu/), require high-throughput procedures from DNA extraction to library construction and sequencing. This effort to sequence the genomes of 100,000 microbes important to food security represents a consortium of government, academic, and industrial partners in a global effort to make these sequences public. In the preparation pipeline for sequencing such a large number of microbial genomes, DNA quantification (and size qualification) is extremely important in many steps in the pipeline (Figure 1).

DNA quantification is routinely achieved by different methods. The commonly used methods are based on UV absorbance of nucleic acids or are dye-based. However, each commonly used method has shortcomings. UV absorbance spectroscopy quantifies all types of nucleic acids (A_{260} measures single- and double-stranded, short and large, RNA as well as DNA). Dye-based measures such as Qubit, PicoGreen, or Quant-iT quantify the concentration of dsDNA using a fluorescent intercalating dye, which requires the bases to be stacked in a double helix to bind and fluoresce strongly. However, none of these methods determine the size (base pair, bp) of the microbial genomic DNA (gDNA) quantified and routinely overestimate the gDNA concentration because they measure degraded fragments in addition to high molecular weight (HMW) gDNA. DNA extracts are often analyzed on agarose sieving gels, but this approach is not suitable in a high-throughput workflow because estimation of sizes against a ladder coupled with densitometry to determine concentration offers low resolution and cannot be automated. Even the Agilent 2100 Bioanalyzer can only size fragments up to 12,000 bp, much smaller than gDNA, and is also not high-throughput. Obtaining HMW gDNA with limited degradation is essential to gain optimum result in library construction and sequencing. Recently, an Agilent DNA extraction kit was used to generate HMW gDNA from a wide range of isolates of the foodborne pathogen *Listeria monocytogenes* [9]. Evaluation of the size of the gDNA extract is crucial, since the next step in library preparation for automated sequencing is shearing the gDNA to an optimal size range depending on the sequencer to be used. Having HMW gDNA is essential as a starting point before using any shearing procedure (nebulization, sonication, enzymatic) [1].

A method that could quantify and qualify gDNA extracts directly in a 96-well plate format would increase dramatically the throughput required for such an ambitious project as the 100K Foodborne Pathogen Genome Project. Agilent Technologies recently introduced the 2200 TapeStation system for DNA quantification and sizing with the capability for 96-well plate sample format, along with the fast analysis time and constant cost per sample. This system offers the characteristics required by a workflow of microbial library preparation for a project of this magnitude.

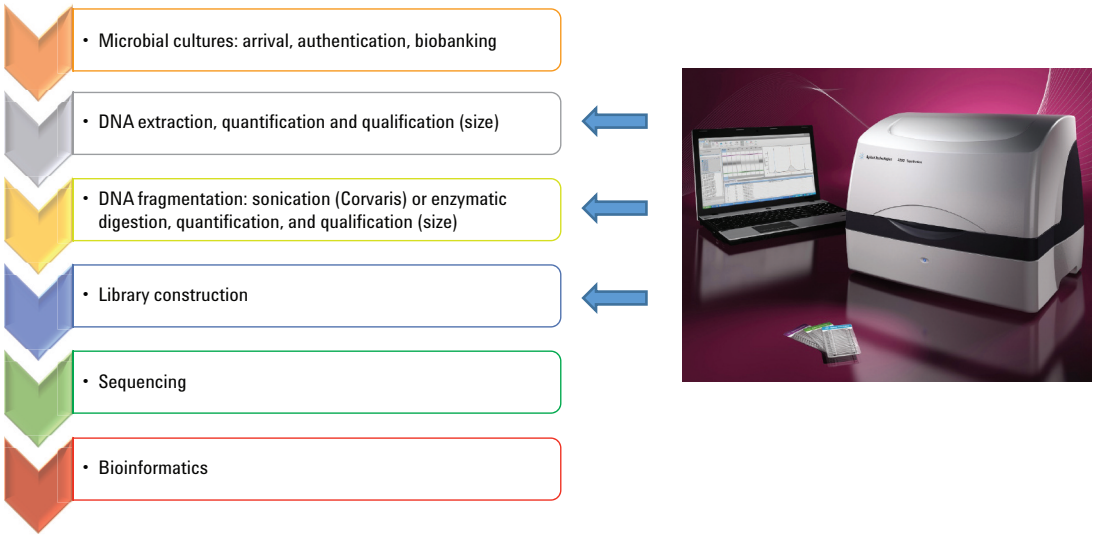

Figure 1. 100K Foodborne Pathogen Genome Project Workflow. The arrows show at which steps the TapeStation could be used in the library construction/sequencing workflow.

DNA analyses on the 2200 TapeStation system are performed using the ready-to-use Genomic DNA ScreenTape and Genomic DNA Reagents [2]. The 2200 TapeStation instrument automatically loads the prepared samples from the 96-well plate onto the Genomic DNA ScreenTape. Electrophoresis and imaging of an electropherogram or a gel image, as well as analysis, are all automated within the instrument.
This application note presents a comparative study between the quantification of microbial gDNA obtained with the 2200 TapeStation using Genomic DNA ScreenTape System to other commonly employed methods.

Experimental

Bacterial culture and DNA extraction

The bacteria used in this application note are listed in Table 1. Bacteria were cultured in their respective media on agar plates at 37 °C aerobically for 12–16 hours before sampling for DNA extraction. The equivalent of a 2-mm^2 square surface of a colony was collected for gDNA extraction.

Table 1. Microbes Used in This Study and Their Culture Media

ID#	Microbe	Culture medium[1]
1	*Lactococcus* sp.	BHI
2	*Acinetobacter* sp.	BHI
3	*Staphylococcus aureus*	BHI
4	*Escherichia coli*	TSA
5	*Listeria* sp.	TSA
6	*Salmonella enterica* serovar Muenster	LB
7	*Salmonella enterica* serovar Saint Paul	LB
8	*Salmonella enterica* serovar Javiana	LB
9	*Salmonella enterica* serovar Newport	LB

[1] Brain Heart Infusion (BHI; Difco, Franklin Lakes, New Jersey); Tryptic Soy Agar (TSA; Difco, Franklin Lakes, New Jersey); Luria Broth (LB; Difco, Franklin Lakes, New Jersey).

DNA extraction

Lactococcus sp.*, Acinetobacter* sp.*, Staphylococcus aureus,* and *Listeria* sp. cells were extracted using a method based on bead beating with purification using a silica spin column [3,4]. *Escherichia coli* and *Salmonella enterica* serovars were extracted with a commercial DNA extraction kit followed by purification using a silica spin column [5,6].

Instrumentation and reagent kits for microbial genomic DNA QA/QC

gDNA extracts were analyzed in triplicate (analytical replicates) with all the methods listed below, except for the microplate-based method measuring absorbance at 260 nm. This latter method required a larger volume (≥ 80 µL per well) than those available for multiple replicates; only one replicate was then analyzed.

The gDNA extracts were quantified using the following methods: UV-Vis absorbance at 260 nm using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts) or using a DTX880 Multimode Detector (Beckman Coulter, Pasadena, California) with UV transparent 96-well plates (CLS3635-50EA; Sigma-Aldrich, St. Louis, Missouri); using a Quant-iT High Sensitivity dsDNA Assay Kit (Q33120; Invitrogen, Carlsbad, California).

Quantification, sizing, and integrity analysis using the Agilent Genomic DNA ScreenTape Assay

Agilent Technologies provided the 2200 TapeStation System (G2964A), Genomic DNA ScreenTape (5067-5365), and Genomic DNA Reagents (5067-5366) (Waldbronn, Germany), and these were used in accordance with manufacturer instructions [7,8].

The samples for the Genomic DNA ScreenTape assay were prepared by mixing 1 µL of gDNA sample with 10 µL of Genomic DNA sample buffer. A 3 µL amount of Genomic DNA ladder was placed in the first well of a 96-well plate, followed by the samples. The prepared plate was vortexed on high speed, centrifuged, then placed in the 2200 TapeStation instrument [8].

2200 TapeStation Analysis Software A.01.04 was used to analyze the data. Electropherograms from analysis of bacterial gDNA were aligned and scaled to MW range. The "Region" view function in the software was used to integrate specific regions of the electropherograms: 35 to 450 bp was used for the lower marker (100 bp at 8.5 ng/µL) and the area from 450 to > 60,000 bp was used for quantification of gDNA in each sample.

Results and Discussion

Genomic DNA from nine representative foodborne bacteria was extracted and analyzed with the 2200 TapeStation. This application note demonstrates that TapeStation is the instrument of choice to analyze, in a high-throughput workflow, bacterial gDNA for quantification and size distribution determination. It provides a rapid assessment of the quantity and quality (size) of the DNA in the gel/electropherogram view modes. It precisely quantifies gDNA from a specific size range, an essential step prior to DNA fragmentation and library construction.

Bacterial gDNA quality assessment: quantity and quality (size)

Visualization of the bacterial gDNA extracts using the gel and electropherogram view modes (Figures 2 and 3) allows a rapid assessment of the gDNA quantity and size that matches a high-throughput workflow. Figures 2 and 3 show a representative subset of the nine gDNA extracts analyzed. A rapid analysis showed that the concentration of gDNA in some of these extracts was low (3, 6 to 9) compared to bacterial extracts 1, 2, 4, and 5. However, in all bacterial DNA extracts, the sizes (bp) of the highest point of the gDNA peak in electropherograms were above the required length (> 10 kb), thus, qualifying them to be used as inputs in the library construction pipeline (Figures 2 and 3, Table 2).

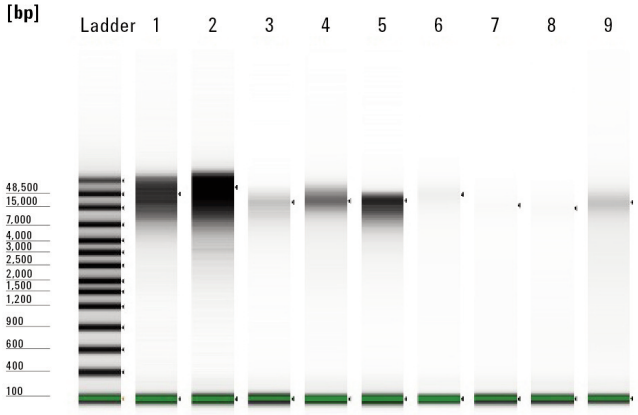

Figure 2. Representative gel images of the bacterial gDNA extracts analyzed with Agilent 2200 TapeStation instrument and Genomic DNA ScreenTape system. Bacteria IDs are defined in Table 1.

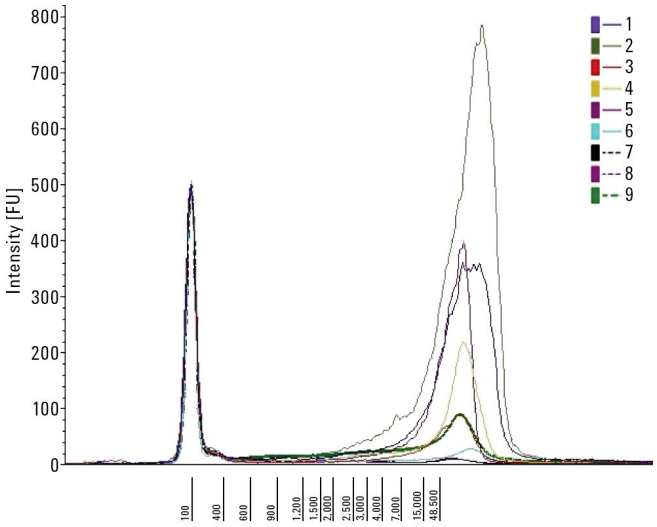

Figure 3. Overlaid representative electrophoregrams of bacterial gDNA extracts with Agilent 2200 TapeStation instrument and Genomic DNA ScreenTape system. Bacteria IDs are defined in Table 1.

Table 2. Sizes of Highest Point of gDNA Peaks in Bacterial Extracts Electropherograms

ID#	Bacteria	Size (bp)[1]	n
1	*Lactococcus* sp.	56,365 ± 1,870	3
2	*Acinetobacter* sp.	60,000	3
3	*Staphylococcus aureus*	17,624 ± 1,757	3
4	*Escherichia coli*	17,976 ± 2,192	3
5	*Listeria* sp.	23,977 ± 3,330	3
6	*Salmonella enterica* serovar Muenster	39,392 ± 11,127	3
7	*Salmonella enterica* serovar Saint Paul	16,187 ± 1,717	2
8	*Salmonella enterica* serovar Javiana	14,828 ± 726	3
9	*Salmonella enterica* serovar Newport	17,624 ± 1,442	3

[1] Average ± Standard error of the mean. When the peak size was > 60,000 bp, "60,000" was used in the calculations.

Quantification of bacterial gDNA using UV-based methods

We included a first-pass step to quantify and qualify the DNA after extraction to obtain the ratios to calculate the contamination of organic (A_{260}/A_{230}) and proteins (A_{260}/A_{280}) in addition to the concentration. A NanoDrop 2000 UV-Vis spectrophotometer was previously used, but, due to its low throughput, we adopted a UV-based method in a 96-well plate format. This assay could be performed with any microplate reader. The concentrations estimated by NanoDrop ranged from 28 to 208 ng/µL, and from 30 to 180 ng/µL for the UV-based method in microplate format (Figure 4). Both methods gave comparable DNA concentrations but Nanodrop measurements were overestimated compared to the ones obtained by the UV-based microplate method. The latter method is not destructive and requires the use of a larger volume of extract, thus giving a better estimate on a more representative sample. We then adopted the UV-based method in 96-well plate format as our first-pass step after DNA extraction for quantification and quality control.

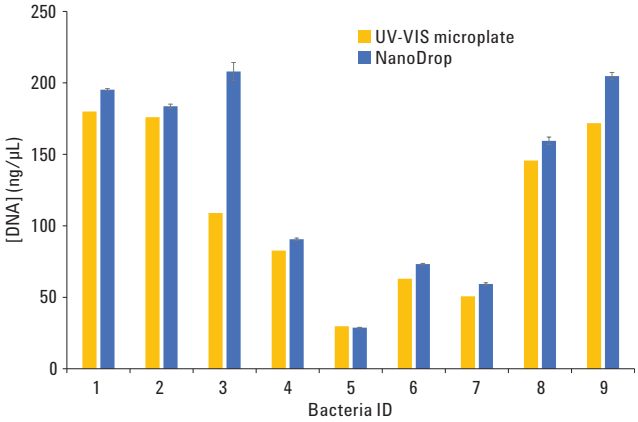

Figure 4. Quantification of gDNA in bacterial extracts determined by UV-Vis absorbance at 260 nm measured with a NanoDrop 2000 UV-Vis spectrophotometer (n = 3 per extract) and with a DTX880 Multimode Detector (microplate reader) (n = 1 per extract). Bacteria IDs are defined in Table 1.

Quantification of bacterial gDNA using TapeStation and a fluorescence-based method

It is well known that UV-based methods overestimate the DNA concentration. Nucleobases, nucleosides, nucleotides, and DNA single-(ss) and double-stranded (ds), of any size, will contribute to UV-absorbance at 260 nm. However, for DNA fragmentation and library construction steps, input of specific quantities of HMW double-stranded DNA are required. Fluorescence-based methods using an intercalated dye are commonly used to quantify dsDNA. One of the methods, Qubit, is the method of choice in a low-throughput workflow. In a high-throughput workflow, the equivalent microplate version, Quant-iT, could be used in a situation in which a large number of libraries are to be prepared, for example, with a robotic platform such as the Agilent Bravo Automated Liquid Handling Platform. Both methods are well accepted as reference methods in quantifying dsDNA prior to DNA fragmentation and library construction. To demonstrate the equivalence of Quant-iT and TapeStation methods, we quantified gDNA from a diverse set of foodborne bacterial pathogens (Table 1). Figure 5 shows the results of this comparison: gDNA extracts value from 3.2 to 63.0 ng/µL with the Quant-iT method, and from 1.6 to 59.7 ng/µL with TapeStation. The two methods proved to be equivalent in quantifying the gDNA. Moreover, TapeStation permits quantification of various sizes of dsDNA. Quant-iT quantifies any double-stranded nucleic acid of any length, and will also measure the short, degraded fragments in gDNA extractions.

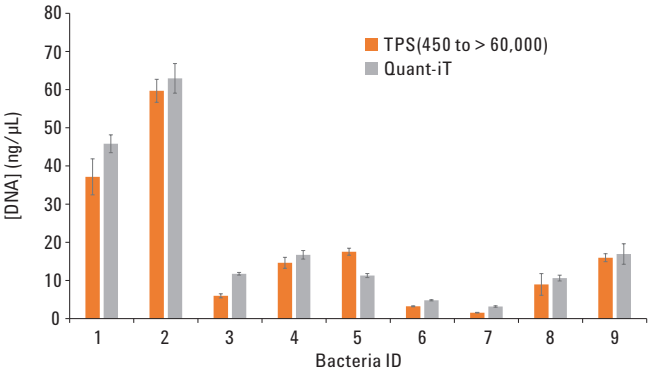

Figure 5. High-throughput gDNA quantification in bacterial extracts using the Quant-iT High Sensitivity dsDNA assay (microplate fluorescence-based) and 2200 TapeStation System with Genomic DNA ScreenTape and Genomic DNA Reagents. gDNA extracts were analyzed in triplicate (analytical replicates). Bacteria IDs are defined in Table 1.

Figure 6 shows an example of an electropherogram in which, as a wider range of molecular weights of gDNA are included in the integration, the resulting gDNA concentrations tend to be close to Quant-iT estimate. The advantage of TapeStation is the unique capacity to quantify double-stranded gDNA across either the whole range of size or for a specific size range. This advantage could introduce a new paradigm in DNA fragmentation and library construction in which the input of double-stranded gDNA could be quantified precisely in the size range of interest. For example, in the library construction workflow for long read sequencing technologies, gDNA of 30 kb and more need to be used as input. Only TapeStation offers the capability to quantify precisely the DNA input of selected size range in high-throughput.

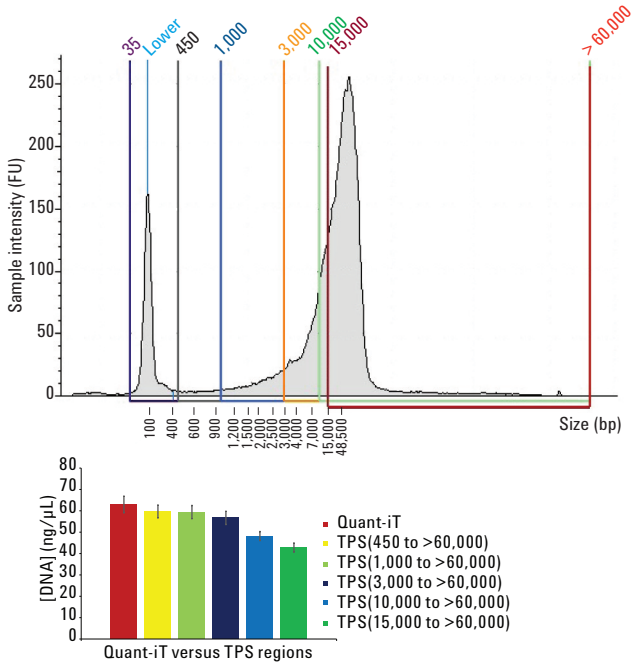

Figure 6. Representative electropherogram from gDNA extract of bacterium ID #2 (Acinetobacter sp.) with regions of different size ranges selected and integrated. TapeStation quantifies dsDNA of various sizes. Quant-iT quantifies double-stranded nucleic acid of any length. As a broader region of the electropherogram is integrated, the corresponding quantity of dsDNA reaches the value obtained with the Quant-iT method.

Direct quantification and qualification of bacterial gDNA by Agilent 2200 TapeStation

TapeStation could be directly used to quantify and qualify DNA in extracts without any other prior step or analysis by an alternative means (such as a UV- or fluorescence-based method). As an example, for our initial effort in the 100K Foodborne Pathogen Genome Project, our team extracted DNA from more than 1,600 cultures of *Escherichia*, *Listeria*, *Salmonella*, and *Vibrio* spp. Quantification of the DNA was performed using a NanoDrop 2000 UV-Vis spectrophotometer. The average concentration was 365 ± 4 ng/µL (average ± standard error of the mean, n = 1,623) with a minimum concentration of 22 and a maximum of 1,228 ng/µL (only four extracts had concentrations above 1,000 ng/µL). Thus, using a 1/10 dilution of any extract, their DNA concentration could have been estimated directly using TapeStation thus improving the throughput. The linear concentration range of the instrument was up to 100 ng/µL [8]. Moreover, TapeStation analysis also allows estimation of the size range of the DNA molecules extracted. Measuring absorbance at 260 nm after DNA extraction using UV-based methods could become optional.

Conclusion

The Agilent 2200 TapeStation system offers an easy-to-use system appropriate for part of a 96-well plate high-throughput workflow to prepare libraries for genomic sequencing on a large scale. This system does quantification and sizing of the gDNA samples with minimum manual intervention. The Genomic DNA ScreenTape and the Genomic DNA Reagents are already prepared, and only require the extracted DNA samples to be put into 96-well plates. As an important advantage, DNA QC using the 2200 TapeStation permits quantification of DNA of required size range for further steps in the library construction pipeline. Combined with the added advantages of high-throughput compatibility, ease-of-use, minimal manual intervention, and constant cost per sample regardless of throughput, the 2200 TapeStation system is ideal for the assessment of gDNA quantity and size distribution.

References

1. S. Wilkening, M.M Tekkedil, G. Lin, E.S. Fritsch, W. Wei, J. Gagneur, D.W. Lazinski, A. Camilli, L.M. Steinmetz "Genotyping 1000 yeast strains by next-generation sequencing" *BMC Genomics* 14:90, 2013.
2. A. Padmanaban, D. McDade Walker "Analysis of high molecular weight genomic DNA using the Agilent 2200 TapeStation and Genomic DNA ScreenTape" Agilent Technologies (p/n 5990-1797EN), 2013.
3. OPS diagnostics laboratory products for sample preparation: http://www.opsdiagnostics.com/applications/applicationtable/microorganism/bacteria.htm.
4. K. Rantakokko-Jalava, J. Jalava "Optimal DNA isolation method for detection of bacteria in clinical specimens by broad-range PCR" *Journal of Clinical Microbiology* 40:4211–4217, 2002.
5. Qiagen QIAamp DNA Mini Kit: http://www.qiagen.com/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/qiaamp-dna-mini-kit.
6. S.A. Greenspoon, M.A. Scarpetta, M.L. Drayton, S.A. Turek "QIAamp spin columns as a method of DNA isolation for forensic casework" *Journal of Forensic Sciences* 43:1024–1030, 1998.
7. Agilent 2200 TapeStation User Manual, Agilent Technologies (p/n G2964-90001).
8. Agilent Genomic DNA ScreenTape System Quick Guide, Agilent Technologies (p/n G2964-90040 rev.B).
9. N. Kong, W. Ng, V. Lee, B.C. Weimer, L. Kelly "Production and Analysis of High Molecular Weight Genomic DNA for NGS Pipelines Using Agilent DNA Extraction Kit (p/n 200600)" Agilent Technologies (p/n 5991-3722EN).

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