High-throughput analysis of DNA samples using the D1K ScreenTape assay and the Agilent 2200 TapeStation system

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Introduction

Recent advances in genomics demands to look at a wealth of genetic information in a short period of time. DNA analysis using slab gel electrophoresis and capillary electrophoresis are widely being used as a QC step in next generation sequencing and microarray studies. However, often these techniques lack the speed and involve more manual steps to perform the assay. The Agilent 2200 TapeStation system meets this demand by providing a simple to use automated electrophoresis platform with variable throughput capabilities from one or two samples up to a 96-well plate. The Agilent 2200 TapeStation system along with the D1K ScreenTape assay can analyze a full 96-well plate in less than 100 minutes with minimal manual intervention.

Figure 1: The Agilent 2200 TapeStation System

The D1K ScreenTape device has 16 individual, pre-packaged separation channels and can size DNA fragments between 35 and 1000bp. The 2200 TapeStation instrument offers flexibility to utilize 8-way strip tubes as well as 96-well plates for low and high-throughput requirements. Here in this study, we demonstrate the capability of the Agilent 2200 TapeStation system in accurate and reproducible sizing of DNA samples from a 96-well sample plate ensuring success in downstream applications.

Materials & Methods

The DNA samples comprising D1K Ladder, Fermentas NoLimits™ fragments of sizes 800, 400 and 200bp, Fermentas GeneRuler™ Low Range DNA ladder were used for assessment of the D1K ScreenTape assay. PCR amplifications were carried out using the Agilent SureCycler 8800 for five housekeeping genes using the PCR program wizard and Herculase II enzyme. The schematic diagram of the sample preparation workflow and analysis is illustrated in Figure 1 showing the layout of sample positions and dilutions.

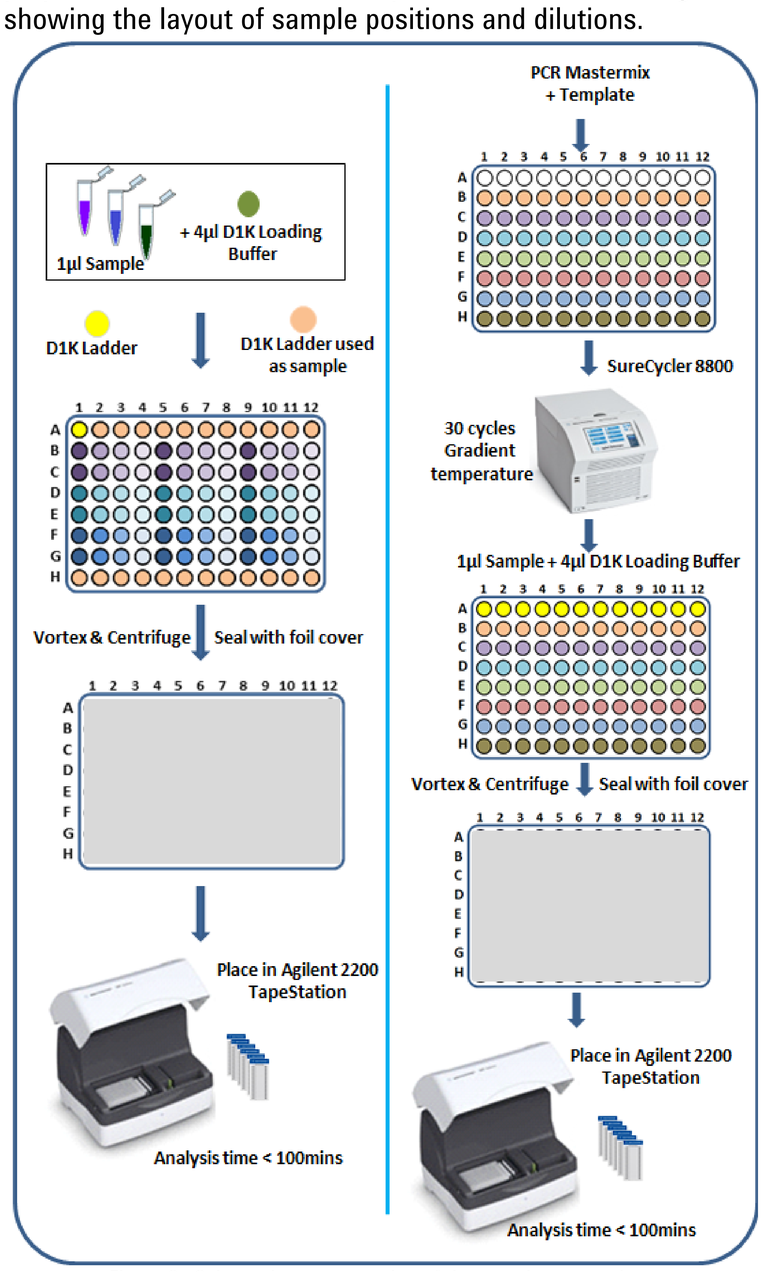


Figure 2: Schematic representation of the sample preparation

The D1K ScreenTape assay was also assessed for its integration with the Agilent SureCycler 8800 for applications such as high-throughput PCR analysis. The robustness of the D1K ScreenTape assay was assessed by extending the analysis time to 4.5 hours.

Results & Discussion

Electrophoretic separation and sizing of DNA samples by the D1K ScreenTape assay was assessed. The sizing accuracy and precision of the D1K ScreenTape assay was studied by analyzing triplicates of 96-well sample plate. The sizing correlation of run ladder and software ladder was also studied. The sizing robustness of the D1K ScreenTape assay was demonstrated by extending the analysis time to 4.5 hours instead of 100 minutes. The integration of Agilent 2200 TapeStation system for high-throughput PCR applications is also assessed.

Figure 3a and 3b showing separation of different DNA samples and PCR products analyzed on the D1K ScreenTape assay.

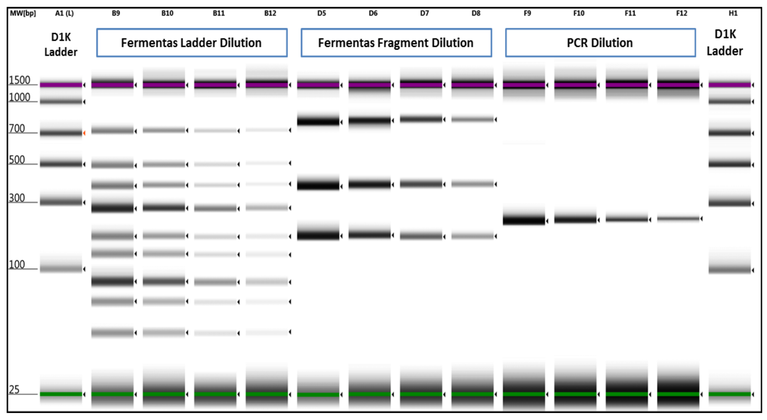


Figure 3a: A typical DNA analysis using the D1K ScreenTape assay.

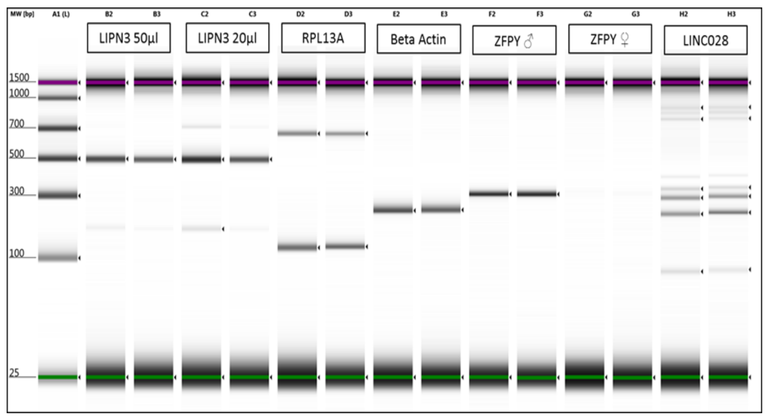


Figure 3b: Analysis of PCR products using the D1K ScreenTape assay.

The reported sizes of DNA samples were collated from all plate replicates and then plotted against the actual fragment size. The study shows that there is very high positive correlation between reported sizes and actual sizes demonstrating the high sizing accuracy of the D1K ScreenTape assay with R2 greater than 0.99.

Agilent 2200 TapeStation system also offers a software ladder which can be inserted after completion of the run through the TapeStation Analysis Software. The Software ladder replaces the requirement to run a ladder for each analysis saving valuable sample space. A correlation graph (Figure 4) of sizing accuracy using a run ladder and the software ladder shows that there is a high positive relation with a R2 of greater than 0.99.

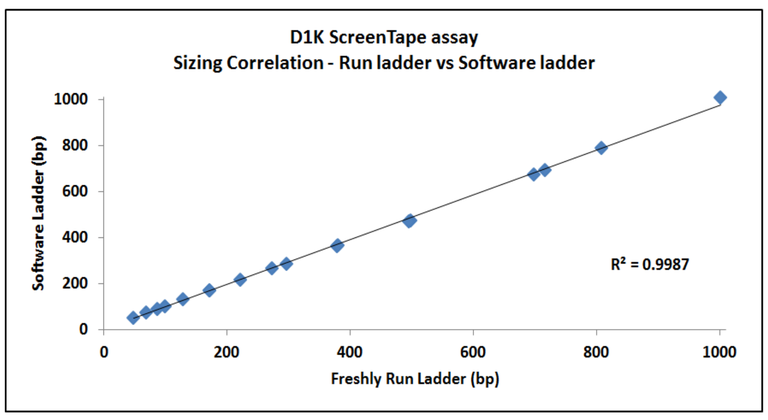


Figure 4: Sizing correlation between run ladder and software ladder

The inter-assay and the intra-assay sizing reproducibility of the D1K ScreenTape assay for run ladder and software ladder is presented in the table 1 below. The sizing accuracy of the D1K ScreenTape assay was demonstrated to be well within the stated specification of ±10% with sizing precision (CV) of less than 2%.

D1K ScreenTape Sizing	Run ladder		Software Ladder	
	Inter-assay	Intra-assay	Inter-assay	Intra-assay
Mean Accuracy %	94.9	94.9	92.8	92.8
Mean CV%	1.5	1.4	1.4	1.5

The sizing robustness of the Agilent D1K ScreenTape assay in separation and sizing of DNA samples was assessed by analyzing a 96-well sample plate over an extended time period of 4.5 hours. The study demonstrates that the D1K ScreenTape assay is capable of sizing DNA sample reproducibly even on an extended analysis period.

The sizing of each fragments of the Fermentas ladder was collated for each time point and for all the plate replicates. The graph plotted between the reported sizes and the actual sizes from each time point shows the excellent reproducibility of Agilent 2200 TapeStation system in analyzing DNA samples even on extended analysis time. The precision of sizing for each fragment between each analysis over the time period is shown in Figure 6.

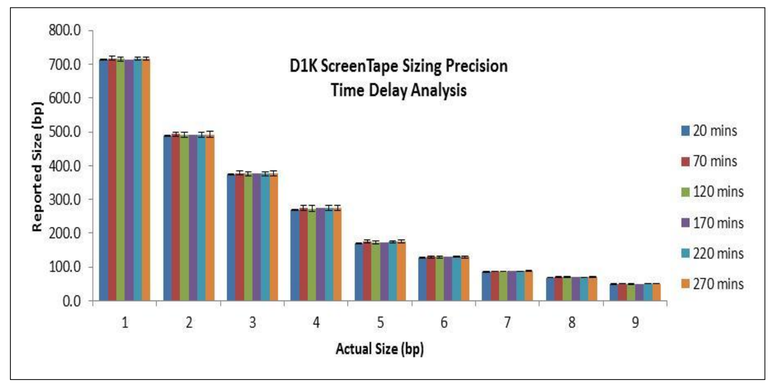
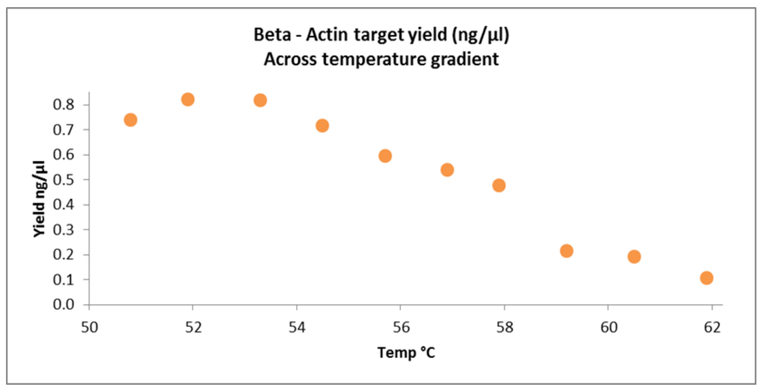


Figure 4: Sizing correlation between run ladder and software ladder

The fast, automated electrophoresis platform with flexible throughput offers an excellent tool for the analysis of PCR reactions for various parameters. The individual lanes of the D1K ScreenTape device allows analysis of PCR products amplified at different temperatures without cross contamination. This feature combined with 12 temperature gradient zones of the Agilent SureCycler 8800 enables study and optimization of PCR conditions for different primers in a single reaction. Figure 7 shows the differences in amplicon yield of beta-actin gene across the temperature gradient zone. The amplification with 12 temperature zone of the Agilent SureCycler 8800 followed by analysis on the Agilent 2200 TapeStation system helps in optimizing the annealing temperature for several primer pairs in a single run.



The D1K ScreenTape assay can be also used to optimize the annealing temperature for eliminating amplification of non-target sequence. A primer was designed to amplify a segment of TBP (TATA Box binding Protein) gene and PCR was carried out with a temperature gradient. Analysis using the D1K ScreenTape assay showed non-specific amplification of complimentary sequences based on the temperature (Figure 9). With this data, selection of a specific cycle profile which minimizes non-specific amplification is made very easy.

Conclusions

- Agilent 2200 TapeStation instrument and the D1K ScreenTape assay can be used as reliable system for high-throughput analysis of DNA samples.
- The sizing accuracy of 95% and precision CV of 2% are well within the stated specification of the D1K ScreenTape assay.
- Software ladder and run ladder shows a very high positive correlation in sizing DNA samples with R^2 greater than 0.99.
- The system also shows high reproducibility in sizing and quantification of DNA samples even on extended time period of 4.5 hours.
- Agilent SureCycler 8800 along with the Agilent 2200 TapeStation system provides an excellent workflow solution for high-throughput analysis of PCR products.