Monitoring Genomic DNA Fragmentation upon Apoptosis Induction Using the Genomic DNA ScreenTape Assay with the Agilent 2200 TapeStation System

Application Note

Nucleic Acid Analysis

Author

Arunkumar Padmanaban
Agilent Technologies, Inc.
Bangalore, India

Abstract

The Agilent Genomic DNA ScreenTape assay in conjunction with the Agilent 2200 TapeStation system automates the analysis of gDNA from cells that are undergoing apoptosis. With flexible sample throughput, the 2200 TapeStation system and Genomic DNA ScreenTape can be successfully used in cell cycle studies to analyze gDNA.

The Genomic DNA ladder-like fragmentation pattern upon electrophoretic analysis is a key biochemical assay to confirm cell death. In this Application Note, human leukemia cell line U937 was treated with camptothecin for different time points, and the cell death was confirmed by analyzing the genomic DNA integrity using the Genomic DNA ScreenTape assay.

Agilent Technologies

Introduction

Apoptosis, or programmed cell death (PCD), is a critical process for the elimination of cells to maintain balance in cell population. Apoptosis is a normal cellular mechanism that occurs during developmental stage, aging, and in response to some immune reactions[1]. Abnormalities in apoptosis, however, can lead to imbalances in cellular regulation. Excessive cell death can cause autoimmune diseases, while little or no apoptosis can result in tumor growth. Understanding the molecular mechanism, signaling pathways, and cellular machinery involved in apoptosis has increased with more research aiming to elucidate the process. Because of this knowledge of apoptosis, it is possible to identify important clinical candidates to treat several associated diseases.

PCD is characterized by several cellular processes such as cell shrinkage, nuclear condensation, and the formation of apoptotic bodies. In other processes, chromosomal DNA fragmentation by endonucleases is considered to be the biochemical hallmark of apoptosis[2]. Chromosomal DNA is cleaved at nucleosome sites resulting in nucleosomal oligomers in multiples of 180–200 bp in length[3]. Various biochemical techniques have been developed to analyze cells that are undergoing DNA fragmentation. Agarose gel electrophoresis analysis of the extracted genomic DNA (gDNA) from apoptotic cells gives a visual indication of the DNA fragmentation.

Methods

Materials

The Agilent 2200 TapeStation system (G2964AA) with Agilent TapeStation Analysis Software [revision A01.05 (SR1)], Genomic DNA ScreenTape (5067-5365) and Genomic DNA Reagents (5067-5366) were obtained from Agilent Technologies, Inc. The NanoDrop 1000 spectrophotometer and Qubit 1.0 Fluorometer with Qubit dsDNA BR assay kit were purchased from Thermo Scientific and Life Technologies respectively. Camptothecin and human myelomonocytic leukemia cell line (U937), RPMI-1640 medium, L-glutamine, and fetal bovine serum were purchased from Sigma-Aldrich. The DNeasy Blood and Tissue kit was purchased from Qiagen. The Apoptotic DNA Ladder kit was purchased from Roche, and supplied apoptosis-induced lyophilized control U937 cells were used as a positive control for analysis.

Cell culture

U937 cells were grown in suspension culture in complete media (RPMI-1640, 10 % FBS and 2 mM L-glutamine) at 37 °C in a 5 % CO_2 atmosphere until they reached 90 % confluency. Cells were maintained in exponential phase by media replacement every 2 days, and fresh media was provided before the day of chemical induction.

Apoptosis induction

Apoptosis induction was carried out as described earlier[4]. Briefly, camptothecin (CPT) was reconstituted in 0.5 M NaOH to 50 mg/mL (150 mM), CPT stock was diluted in blank media (RPMI-1640 and 10 % FBS) before use, and cells were treated with a final concentration of 5 µg/mL. An equal volume of 0.5 M NaOH in blank media was added to the control cell culture flask. The cells were incubated for time points T = 0, 3, 8, 24, and 48 hours. The positive control from the Apoptotic DNA Ladder kit contains 2×10^6 lyophilized U937 cells that were treated with 4 µg/mL camptothecin for 3 hours.

The pretreated and lyophilized cells were resuspended in PBS, and the gDNA extraction was processed similar to samples.

Genomic DNA analysis

At each incubation time point, the cells were collected by centrifugation, and cell pellets containing 2×10^6 cells were stored at –80 °C until further use. Genomic DNA was extracted from cell pellets and the lyophilized U937 cells from Roche kit using the Qiagen DNeasy Blood and Tissue kit following the manufacture guidelines. The extracted gDNA was quantified using NanoDrop, Qubit, and was also analyzed using the 2200 TapeStation system. For analysis, 1 µL of sample was mixed with 10 µL of sample buffer, vortexed, and centrifuged to collect the samples. The samples were then placed in the 2200 TapeStation system with the Genomic DNA ladder to gather quantitative and qualitative data. Samples from each time point were analyzed in triplicate for all three platforms.

Results and Discussion

The Genomic DNA ScreenTape assay was used to monitor the fragmentation of gDNA upon apoptosis induction by camptothecin. The progression of the cell death due to sequence-specific fragmentation of the gDNA was quantitatively analyzed, comparing the integrity of gDNA and the appearance of oligomers.

Evaluation of apoptosis

Camptothecin, a known apoptosis inducer, was used to induce apoptosis in U937 cell lines. To evaluate the progression of cell death, the DNA fragmentation pattern was monitored using the Genomic DNA ScreenTape assay. The gDNA extracted from cells after different time points of camptothecin treatment were analyzed using the Genomic DNA ScreenTape assay with a positive control.

The analyzed gDNA samples show decreasing peak integrity and the appearance of several distinct peaks over time, as shown in the gel image in Figure 1.

The distinct peaks that appear after CPT treatment are oligomers with an average size difference of 180 bp. The experimental controls T = 0 and 48 hours confirm that the fragmentation is triggered by CPT treatment.

The laddering pattern of the samples is comparable to that of the positive control, showing a similar fragmentation of the genomic DNA. The difference between samples versus the commercially available positive control in laddering pattern might be due to tissue culture conditions, for example cell density or cell harvest.

The average size of the large gDNA peak as determined by the TapeStation Analysis Software can also be used as an indicator to assess DNA integrity, as presented in Figure 2. The integrity of the gDNA samples as expressed in kilobases (kb) shows increasing fragmentation on higher time point treatments.

The Genomic DNA ScreenTape assay also quantifies the samples in a single run. The samples from all time points were quantified and compared with the NanoDrop and Qubit assays, and presented in Figure 3. The data show a comparable quantification between the 2200 TapeStation system and Qubit, which uses a dsDNA specific dye, and is not impacted by other contaminations. NanoDrop values were observed to be higher, as the UV measurements were affected by the digested oligonucleotides, ssDNA, and other buffer components.

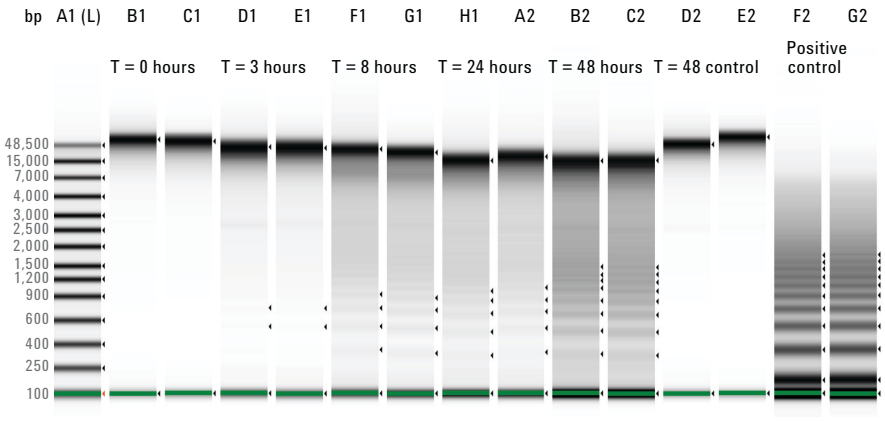

Figure 1. Gel image showing quality of extracted gDNA from U937 cells at different time points of CPT treatment with controls.

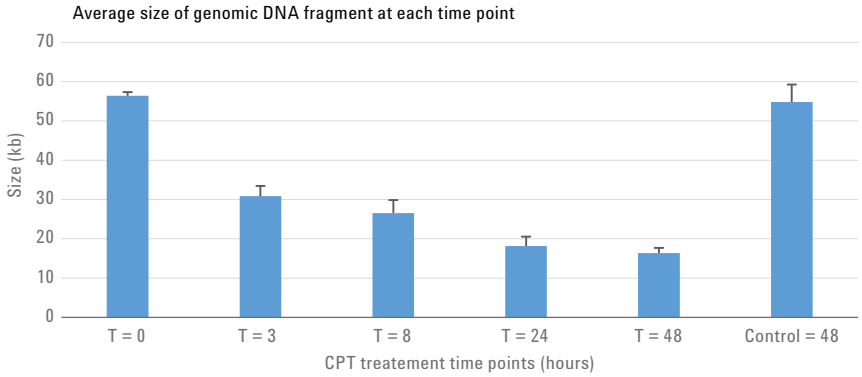

Figure 2. Comparison of the average peak size of the extracted genomic DNA samples compared to the control. (n = 3 per time point).

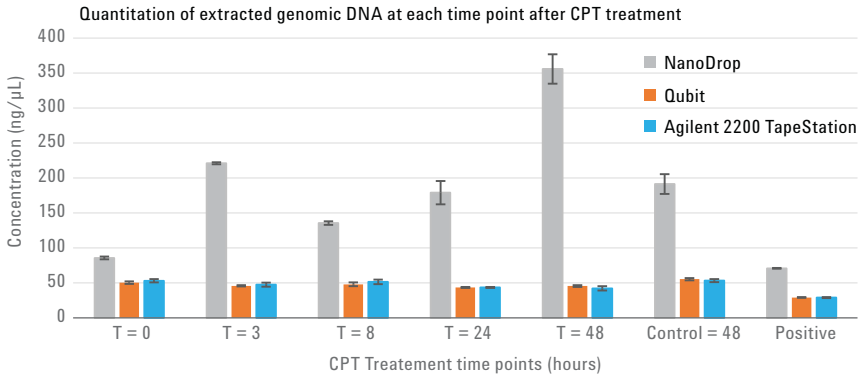

Figure 3. Sample quantities determined by three platforms: NanoDrop, Qubit, and Agilent 2200 TapeStation system. Error bars indicate standard deviation. (n = 3 per time point/platform).

DIN Metrics

The Genomic DNA ScreenTape assay also provides a quantitative measure of sample integrity, as the DNA Integrity Number (DIN), ranging from 1 to 10, where 1 indicates highly degraded and 10 indicates highly intact DNA[5]. Although the DIN has been developed for the smear analysis of intact gDNA and any degradation products, it can also be used to some extent to quantitatively measure the intactness of genomic DNA samples within the apoptotic pathway, as shown in Figure 4. This gives an indicator to measure the progression of apoptosis after drug treatment.

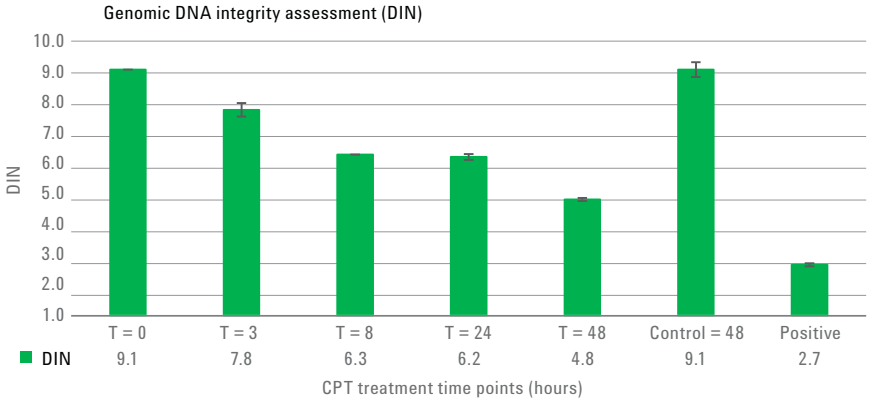

Figure 4. Comparison of DIN numbers of extracted gDNA samples from different time points. (n = 3 per time point).

Conclusion

This Application Note shows that the Agilent Genomic DNA ScreenTape assay can be successfully used to monitor changes in the integrity of gDNA upon induction of apoptosis by providing a gel image that shows the distinctive laddering pattern typical of PCD. Moreover, the Genomic DNA ScreenTape assay can be used for quantitative and qualitative analysis of the gDNA samples using a sample volume as small as 1 µL, and returning results within 2 minutes per sample.

References

1. Elmore, S. Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology* **2007**.
2. Zhang, J. H; Xu, M. DNA fragmentation in apoptosis. *Cell Research* **2000**.
3. Bortner, C. D; Oldenburgh, N; Cidlowski, J. The role of DNA fragmentation in apoptosis. *Trends in Cell Biology* **1995**.
4. Khare, S; *et al.* Early changes in metabolism of leukemic cell lines upon induction of apoptosis by cytotoxic drugs. *European Journal of Pharmacology* **2003**.
5. Gassmann, M; McHoull, B. DNA Integrity Number (DIN) with the Agilent 2200 TapeStation System and the Agilent Genomic DNA ScreenTape Assay. *Agilent Technologies*, publication number 5991-5258EN, **2014**.

www.agilent.com/genomics/Tapestation

Published in the USA, December 1, 2015
5991-5888EN

Agilent Technologies