The DNA Integrity Number (DIN): A novel Approach for Objective Integrity Classification of Genomic DNA Samples

Marcus Gassmann[1], Eva Schmidt[1], Adam Inche[2], Isabel Pechtl[1] and Barry McHoull[2]
1) Agilent Technologies, Waldbronn, Germany; 2) Agilent Technologies, Edinburgh, UK

Agilent Technologies

Introduction

Genomic DNA (gDNA) is used as starting material in the experimental workflow of many applications in molecular biology. The integrity of the DNA critically affects the success of many downstream experiments like array CGH or next generation sequencing. Purification techniques for gDNA are fundamental to ensure best results in downstream procedures, but optimized quality control of the purified gDNA is equally important. Therefore electrophoretic analysis of the gDNA sample is highly recommended as the respective downstream applications can be expensive and time consuming. The 2200 TapeStation system in conjunction with the Genomic DNA ScreenTape assay provides an excellent solution for assessing the quantity, integrity and overall quality of gDNA starting material. The ScreenTape device is a pre-packaged microfluidic consumable designed for performing electrophoretic applications in a microscale format. It is used in combination with the 2200 TapeStation instrument.

Degradation of gDNA is typically a gradual process in which high-molecular weight DNA is fragmented into smaller species. It can occur either enzymatically, chemically or mechanically. Judging the integrity of DNA by visual evaluation of the electropherogram trace is subjective and can be error-prone. In order to standardize this assessment, a novel algorithm was developed to score gDNA samples on the 2200 TapeStation system. The DNA integrity number (DIN) is calculated from several features obtained from the electrophoretic trace and provides a numerical value from 1 (degraded) to 10 (intact). The DIN is independent from instrument, reagent batch and sample concentration and can be used as objective measure for determining the integrity of gDNA.

Material and Methods

Material and samples

Commercially available human gDNA (Promega) was fragmented using ultrasonication and needle shearing or a combination of both to generate a set of different samples representing a wide degradation/integrity range.

Enzymatic fragmentation was performed using dsDNA Fragmentase® (NEB) treatment for a range of time periods. FFPE-tissue-derived samples were kindly provided by customers.

The 2200 TapeStation system (Agilent Technologies, PN G2965AA) was operated using the TapeStation Analysis Software (Revision A01.05) in combination with Genomic DNA ScreenTape consumables (PN 5067-5365) and Genomic DNA reagents (PN 5067-5366)

Genomic DNA analysis with the 2200 TapeStation system

DNA analysis was performed according to the Genomic DNA ScreenTape system Quick Guide. In brief, gDNA samples were mixed with Genomic DNA Sample Buffer. The Genomic DNA ScreenTape, loading tips and samples were then placed in the 2200 TapeStation instrument. The 2200 TapeStation system loaded, electrophoresed, imaged and presented digitally analyzed results in less than 2 minutes per sample. The DIN is reported from TapeStation Analysis Software version A.01.05 onwards. DIN functionality is backward compatible with data files generated with previous software versions.

Figure 1: The Agilent 2200 TapeStation system

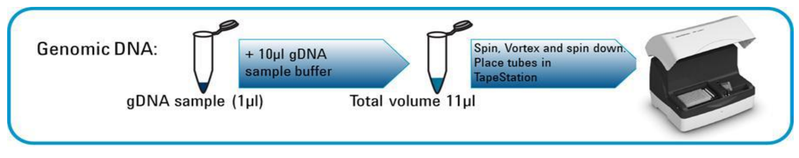

Figure 2: Genomic DNA sample preparation protocol

Results

Analysis of artificially degraded gDNA samples in different concentrations

A set of 15 gDNA samples with a varying degree of degradation were analyzed using the 2200 TapeStation system and the Genomic DNA ScreenTape assay. The samples were tested at four different concentrations covering the quantitative range of the Genomic DNA Assay (10, 30, 60 and 100 ng/µl). The TapeStation Analysis Software provides a gel view (Fig. 3) and an electropherogram view (Fig. 4). The DIN is calculated for each sample.

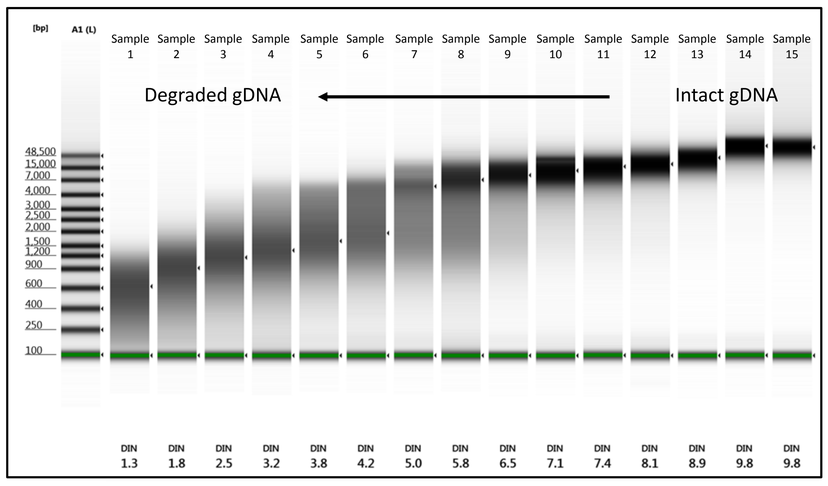

Figure 3: Gel image of gDNA samples showing a different degree of degradation

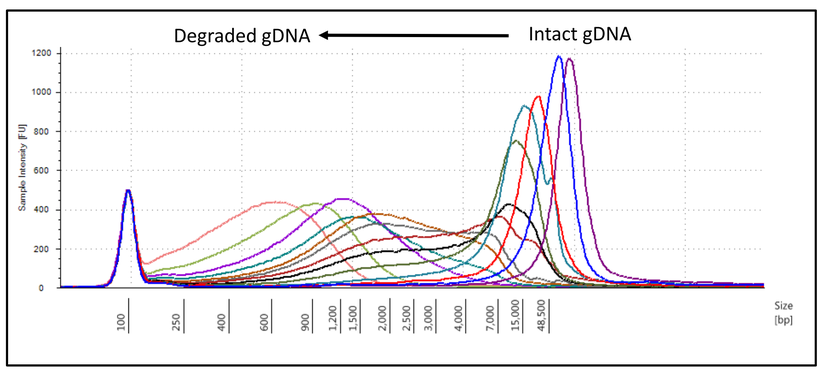

Figure 4: Electropherogram Overlay of a gDNA degradation series

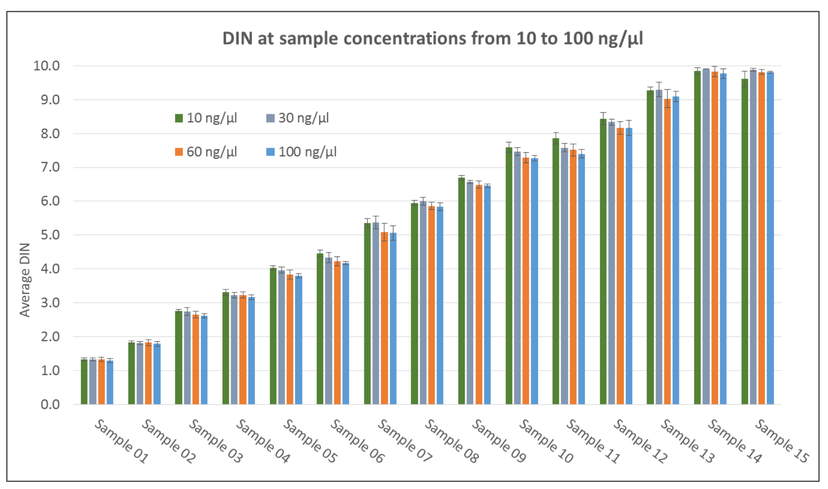

Figure 5: Average DIN for the 15 gDNA samples at concentrations from 10 ng/µl to 100 ng/µl. Error bars represent one standard deviation (n=10 for samples at 10, 30 and 100 ng/µl; n=44 for samples at 60 ng/µl).

DIN robustness using various reagent and ScreenTape batches

In order to show that the DIN algorithm is reagent and ScreenTape batch independent, a subset of the samples were analyzed using either three different reagent batches (Fig 6), or five different ScreenTape batches (Fig 7).

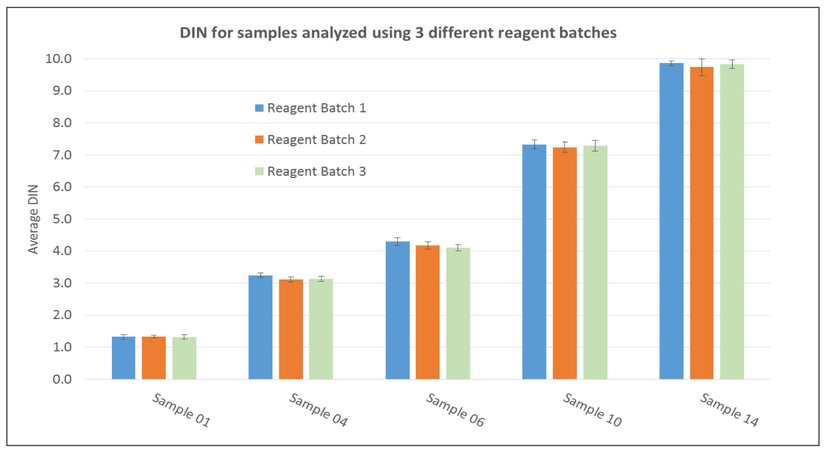

Figure 6: Average DIN for 5 gDNA samples (60 ng/µl). Samples were analysed using 3 different ScreenTape reagent batches. Error bars represent one standard deviation (n=10).

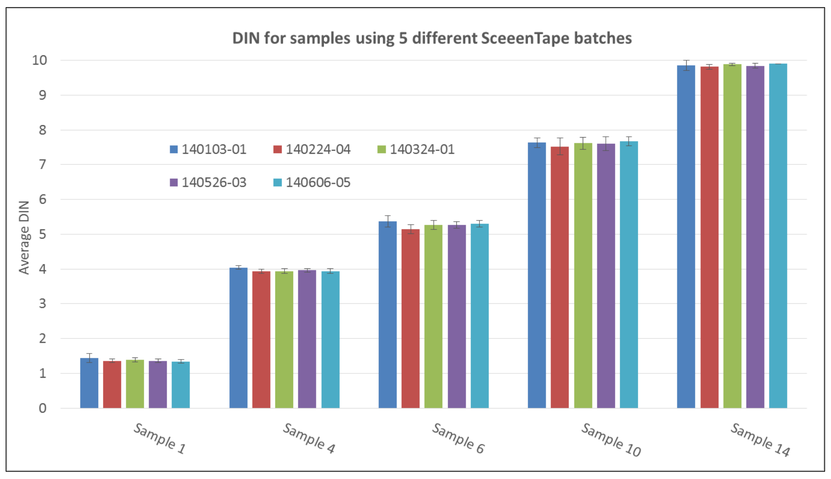

Figure 7: Average DIN for 5 gDNA samples (25 ng/µl). Samples were analysed using 5 different ScreenTape batches. Error bars represent one standard deviation (n=12).

Analysis of FFPE tissue -derived samples

Different samples isolated from FFPE tissue sections were analyzed on the Genomic DNA ScreenTape assay (Fig 8). The observed DIN range was 1.1 to 6.6.

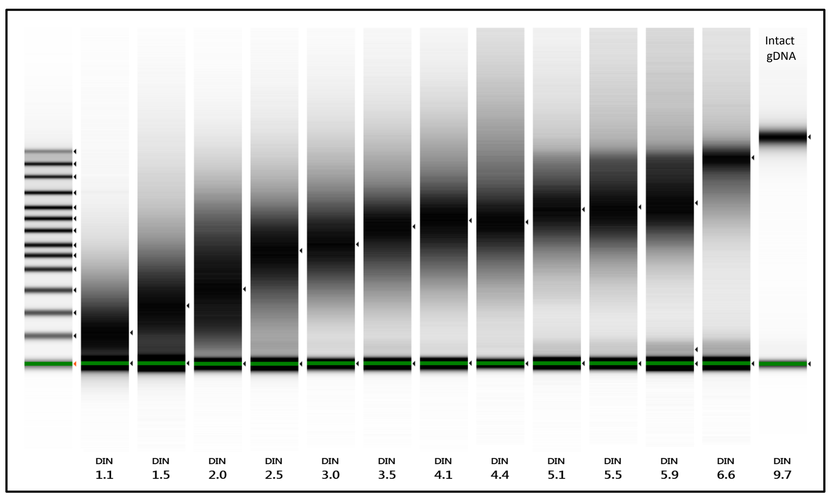

Figure 8: Gel image different samples purified from FFPE tissue sections

Enzymatic fragmentation of Genomic DNA

Human gDNA was digested using NEBNext® dsDNA Fragmentase®. Treatment was performed for a range of time periods (Fig 9).

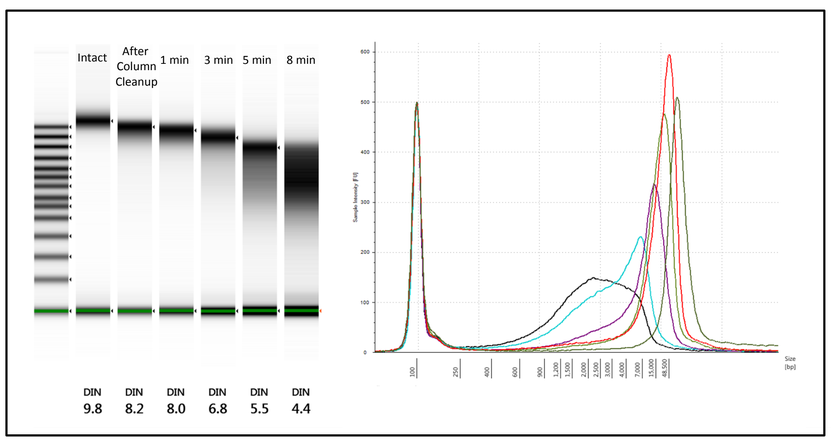

Figure 9: Human gDNA was treated with dsDNA Fragmentase® from 1 to 8 min. The progress of fragmentation correlates to a decrease of the DIN from 9.8 to 4.4

Conclusions

- **The DNA Integrity Number (DIN) provides a numerical value from 1 (degraded) to 10 (intact) for genomic DNA samples analyzed on the 2200 TapeStation system**
- **Scoring is dependent on the degradation state and can be used as an objective measure for determining gDNA sample integrity**
- **The DIN is very robust and reproducible and is independent of instrument, reagent batch and sample concentration**
- **The DIN algorithm can assess standard and FFPE-tissue derived gDNA samples**
- **DIN calculation is available in the TapeStation Analysis Software version A.01.05**