

Monitoring Long-Term DNA Storage in the Biorepositories at Coriell Institute

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Abstract

Coriell Institute for Medical Research (Coriell) is considered to have the most diverse collection of cell lines, DNA, and other biomaterials, which are available for biomedical research. In 2016, Coriell replaced traditional gel electrophoresis by the TapeStation system with the Agilent Genomic DNA ScreenTape assay. The DNA integrity number (DIN) generated by the TapeStation system was implemented as an objective metric to assess the integrity of genomic DNA. In this application note, we highlight the capabilities of the TapeStation system by a comparison study using genomic DNA (gDNA) samples of varying quality. Further, we describe how Coriell retrieved gDNA samples from long-term storage and qualified these samples with the TapeStation system. By comparing the DIN values of DNA samples extracted between 1990 and 2000, we could confirm the consistent quality and fail-safe storage of DNA at Coriell Institute over 30 years.

Introduction

The Coriell Institute for Medical Research (Coriell) has gained an international reputation in biobanking services by offering collection, processing, cryopreservation, distribution, and information management of biomaterial samples. The Coriell biobank is considered to house one of the most diverse collections of cell lines, DNA, and other biomaterials available for biomedical research. With the development and maintenance of several biorepositories, Coriell is committed to providing the scientific community with well-characterized, high-quality DNA preparations, which are annotated with rich phenotypic data. Key competencies at Coriell are extraction, processing, cryopreservation and fail-safe storage of DNA and other biomaterials. A variety of quality control (QC) assays is conducted on biosamples before long-term storage. The QC process includes quantification using fluorimetry, digestion, identity analysis, purity assessment using spectrophotometry (optical density (OD) ratios), and integrity analysis. Only those samples that pass the entire battery of QC tests are considered for distribution and placed in storage.

Experimental Methods

Instrumentation

An Agilent 4200 TapeStation system (p/n G2991AA) and Agilent Genomic DNA ScreenTape assay (p/n 5067-5365) with Agilent Genomic DNA reagents (p/n 5067-5366) were used for analysis. The integrity of uncut DNA is scored on a scale of 1 to 10, with 10 being highest quality and 1 being poor quality. DNA samples with a DNA score of 7.0 or more are classified as high quality.

The concentration and OD of the DNA samples were determined using a Nanodrop UV-Vis spectrophotometer (Thermo Fischer Scientific, Inc.)

Procedure

1. gDNA samples were extracted between 1990 and 2000 from cell cultures established from human tissue or blood samples.
2. The gDNA samples were extracted using a modified Miller's salting out method. In brief, cultured cells are lysed by addition of anionic detergent. RNA and protein are degraded with the addition of RNase and Proteinase K. After mixing, a salt solution is added, and the insoluble cell debris is removed by centrifugation. Twice the volume of ethanol is added to the supernatant and the resulting DNA precipitate is collected by spooling. The DNA pellet is solubilized in TE buffer (10 mM Tris, pH 8.0/1 mM EDTA).

3. The samples were stored in a -80 °C standard mechanical freezer with temperature monitoring systems.
4. For this study, 50 samples were retrieved using a controlled manual process. To reduce freezer opening time, the pulling process included electronic information about the freezer, section, and box of sample. The samples were collected using a cryocart to enable controlled thawing in the lab.
5. Digestion was performed to verify that DNA could be cut by restriction enzymes. Hydrated DNA (1 µg) was digested with *EcoRI* and *HindIII* restriction enzymes. Thermo Scientific FastDigest EcoRI restriction enzyme (cat. # FD0274) recognizes the G^AAATTC site. Thermo Scientific FastDigest HindIII restriction enzyme (cat. # FD0504) recognizes the A^AAGCTT site. Both enzymes cut at 37 °C in 10 minutes using universal FastDigest Buffer.
6. The cut and uncut DNA samples were diluted to 20 ng/µL. The samples were evaluated using the TapeStation system and traditional gel electrophoresis.

Results and discussion

Implementation of the TapeStation system at Coriell Institute

Before 2016, Coriell qualified gDNA samples by traditional gel electrophoresis with image capturing and visual evaluation. Digestions with *EcoRI* and *HindIII* were run side by side with uncut gDNA to verify that DNA could be digested by restriction enzymes (Figure 1A). Quantification and optical density (OD) were evaluated using a Nanodrop UV-Vis spectrophotometer.

In 2016, the TapeStation system was established in the Coriell Institute to implement the DNA integrity number (DIN) as an objective metric to assess the integrity of extracted gDNA. To highlight the capabilities of the TapeStation system, a comparison study was performed using gDNA samples of varying quality. Figure 1B shows the results from the TapeStation system next to the gel for each sample. The gel view of the TapeStation analysis software provides the familiar approach to compare uncut and digested samples leading to the same conclusions. Further, the DIN value, which is automatically calculated for each sample, offers an objective number for sample integrity. The DIN of the uncut samples correlated with previous quality descriptions assessed by visual evaluation of electrophoresis gels.

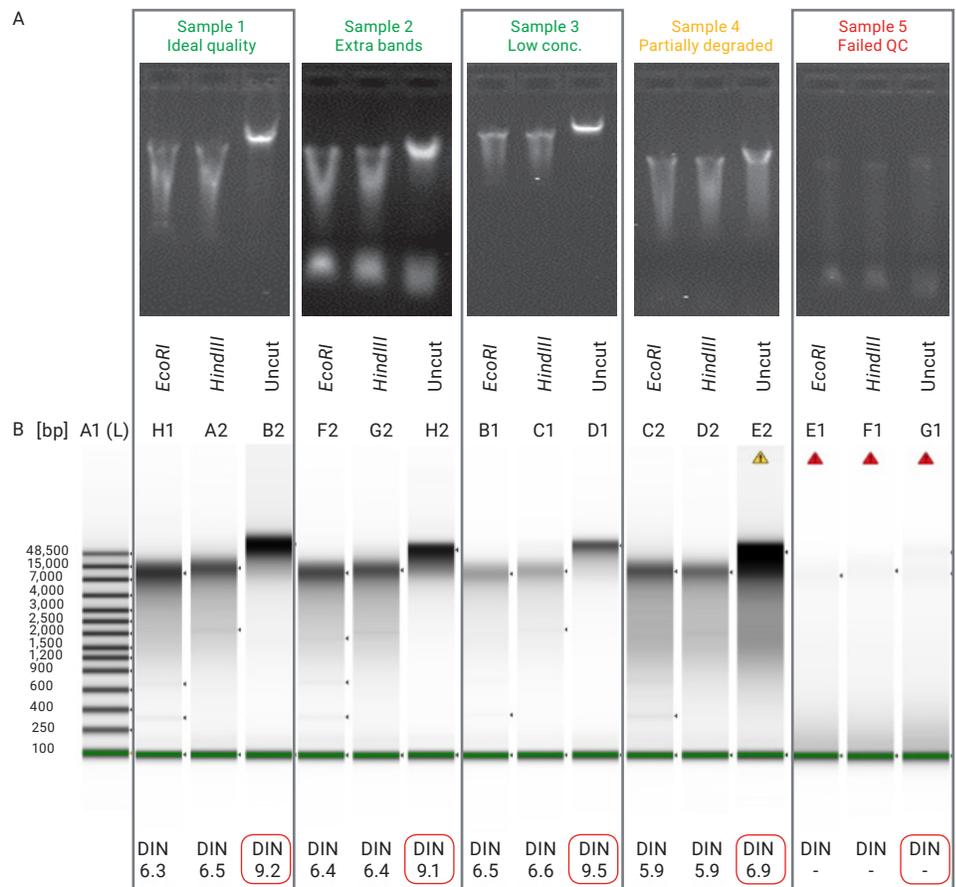


Figure 1. Assessment of five gDNA samples with varying quality. For each sample, uncut gDNA is placed next to *EcoRI* and *HindIII* digestions. A) Image capture of traditional gel electrophoresis B) TapeStation analysis software gel view of the same samples with the DIN highlighted in red for uncut samples. The yellow warning indicates overloading, red warnings indicates sample concentration is outside of the functional range for DIN.

Long-term storage of DNA

Coriell offers long-term storage of biomaterial. Although DNA is viewed as a stable molecule, many conditions can cause loss of DNA bases or strand breakage. A study was conducted to determine DNA stability after long-term storage at -80°C . A batch of 50 samples extracted between 1990 and 2000 were pulled and the concentration and quality

metrics were determined. The concentration was within 10% of the original reading using a spectrophotometric method (Nanodrop) and the 260/280 ratio was approximately 1.75 to 1.80 which is considered as good quality. The DIN score was > 7.0 for all tested samples (Figure 2).

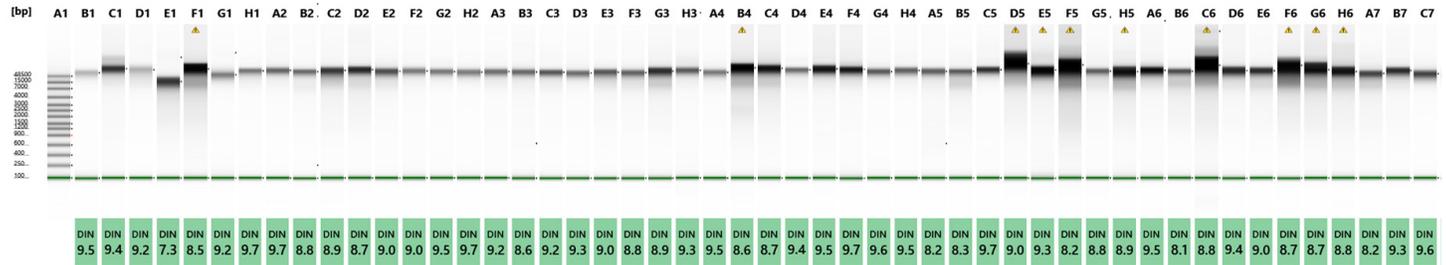


Figure 2. Gel view and DIN of 50 gDNA samples extracted between 1990 and 2000 analyzed with the Genomic DNA ScreenTape assay. Warnings in the lanes show overloading.

The evaluation of the DIN values in relation to the sample's extraction year is shown in Figure 3. A consistent DNA integrity over the decade was observed, revealing consistently reliable DNA extraction. Moreover, the high DIN score for all samples verifies continuously high integrity of DNA samples after 20 to 30 years in long-term storage.

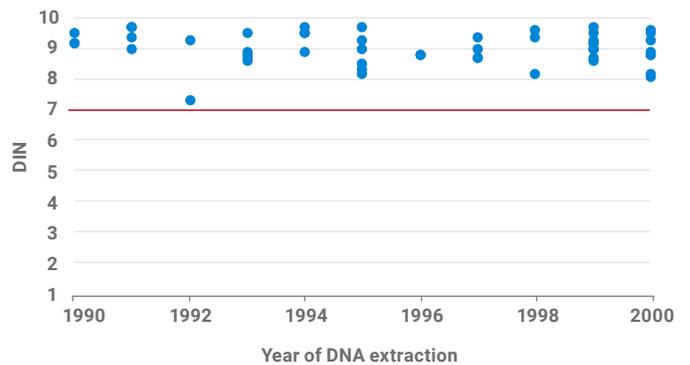


Figure 3. DIN values of 50 gDNA samples extracted between 1990 and 2000 analyzed with the Genomic DNA ScreenTape assay.

Conclusion

The Coriell Institute for Medical Research houses some of the most diverse collections of DNA, RNA, and other biospecimens collected and distributed for use to the international research community. The TapeStation system replaced traditional gel electrophoresis at Coriell in 2016. The TapeStation system offers an automated solution for nucleic acid analysis with the DIN number as an objective measure for DNA integrity independent of the evaluator's experience. The DIN number was used for a retrospective analysis of

the integrity of 50 long-term stored DNA samples extracted between 1990 and 2000. The high DIN value (> 7.0) of all samples verified the high standard of sample handling and fail-safe long-term storage of gDNA samples over decades in the Coriell Institute. With the advent of new QC methods such as the TapeStation system, biobanks can assure that the quality of samples meets the current standard for scientific communities.

www.agilent.com/genomics/tapestation

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