Abstract

Biobanks and biorepositories play a crucial role in medical research by offering robust, readily available, and even rare, biospecimen samples for clinical, biomedical, and research studies. As biobanks continue to collect samples from a growing number of sources and may store them for both short- and long-term tenures, the need for implementing general guidelines for consistent, optimal operations is necessary. These requirements include quality control (QC) measures to be taken during sample collection, prior to sample accession, and upon use of the sample, in order to ensure biological material and data collections of highest possible quality. Various international institutions and standard-setting bodies, including International Organization for Standardization (ISO) and International Society for Biological and Environmental Repositories (ISBER), have thus put forth recommendations for biobank best practices. ISBER Recommendations for Repositories has identified the Agilent quality metrics as a way to assess the integrity and fragmentation of DNA and RNA. The Agilent automated electrophoresis instruments including the TapeStation, Femto Pulse, Bioanalyzer, and Fragment Analyzer systems provide quality metrics that are essential analysis tools for determining the quality of nucleic acids. The various quality metrics include the DNA integrity number (DIN) and genomic DNA quality number (GQN) for DNA, %cfDNA for cell-free DNA, and RNA integrity number (RIN), RIN equivalent (RIN\^e), and RNA quality number (RQN) for RNA assessment. Here, we demonstrate how the automated electrophoresis instruments are used within biobanks, and how their use can aid biobanks in determining best practices for handling, extraction, storage, and shipping of nucleic acids.
Introduction

Biobanks emerged due to many researchers collecting large numbers of specimens for their studies, which ultimately led to the necessity of a single location for storage and collection of samples for future research. Through the years, with new scientific insights continually emerging, these same samples collected for one research study, over time, became useful for other researchers experiments or multicenter cohorts. Biobanks have become a reliable source for samples that are not always readily available to researchers. Thus, it is important to store samples of high quality with complete documentation so they can be used for future studies. Best practices for biobanks were established to harmonize the biobanking community through common procedures, standards, and quality control.

Over the last years, organizations such as the International Organization for Standardization (ISO) and the International Society for Biological and Environmental Repositories (ISBER) have developed standards and recommendations for biobanks. ISO is an international standard-setting body composed of representatives from various national standards organizations promoting worldwide proprietary, industrial, and commercial standards. ISBER is a global biobanking organization that provides a forum for the dissemination of state-of-the-art policies, processes, and research findings, in addition to creating educational and training opportunities. Mounting evidence indicates that pre-analytical procedures including collection, processing, storage, and shipping of biospecimens can affect the detection of molecular analytes in downstream analyses. Biospecimen quality has undoubtedly become important to the success of biomedical research, compelling many organizations to recommend quality standards. These standards will promote biospecimen consistency and quality and optimize access to biospecimens and their associated data.

ISO and ISBER biospecimen fit for purpose guidelines

Many standards and recommendations have been established for biobanks and repositories by ISO¹ and ISBER² in order to identify, document, and make available samples fit for purpose for biomedical research. Specific for biobanks, ISO 20387:2018 states in section 7.8.1.1 that "critical activities having an impact on the quality of the biological material and associated data shall be identified by the biobank, provider, recipient, or user. The biobank shall establish, document, and implement quality control (QC) procedures related to such activities." In addition, section 7.8.1.2 adds that "the biobank shall provide biological material and associated data fit for purpose. The biobank shall define a minimum set of QC procedures to be performed on the biological material and associated data or a subset of it. Exceptions can be justified for rare or legacy biological material elimination." Section 7.8.2.9 adds "that the biobank shall use approaches to provide objective evidence to demonstrate the comparability of biological material quality (the processing or testing output), where such approaches are available and appropriate."

Similarly, ISBER guidelines for quality control in section E2 note that across all repositories and specimen types, QC processes include the four "pillars" of collection quality:

1. Authenticity—correctly assigned identity;
2. Purity—freedom from contamination;
3. Stability—capability of a sample material to retain the initial value of a measured quantity for a defined period of time within specific limits when stored under defined conditions;

The pillars of purity and stability specifically address the integrity of nucleic acid specimens. The ISBER Best Practices states in E2.4 quality control considerations for nucleic acid specimens that “DNA and RNA can be assessed for integrity and fragmentation (e.g., molecular weight, DNA Integrity Number, RNA Integrity Number), quantity/concentration, and purity”. 
Quality control considerations for nucleic acids

High-quality nucleic acids are necessary for successful analysis in a variety of applications and quality control can help determine which samples are suitable for downstream analysis. DNA and RNA are obtained by extraction from many different types of tissue samples. Not all tissue types are equally stable and some sample extraction methods can promote degradation, thus the integrity of the sample can vary. In addition, pre-analytical procedures including collection, processing, storage, and shipping of biospecimens can affect the integrity of nucleic acids. Nucleic acid samples such as formalin-fixed paraffin embedded (FFPE), ancient samples, and RNA are easily degraded due to chemical fixation, time, temperature, enzyme digestion, and improper handling. Intact, high-quality genomic DNA (gDNA) is displayed as a smear of very large sized fragments, while total RNA will have very distinct ribosomal peaks. If a sample is significantly degraded with only small fragments existing, it will result in poor sequencing results, loss of coding areas of interest, and gaps in the full-length RNA and gDNA. Knowing input nucleic acid quality helps provide guidance as to which samples are fit for purpose for a particular research study or workflow. It also aids in directing changes needed to optimize a workflow, such as: input concentration, fragmentation conditions, the amount of library used in enrichment, and the number of PCR cycles to be used in amplification steps.

Agilent instruments

The Agilent automated electrophoresis portfolio offers several instruments including the TapeStation, Femto Pulse, Bioanalyzer, and Fragment Analyzer systems that provide various quality metrics to analyze nucleic acid samples. In addition, all the instruments provide sizing, quantification, and quality analysis of DNA and RNA, while specializing in different throughput capabilities, resolution, and application requirements for individual lab demands.

Quality metrics

The quality metrics referred to in ISBER Best Practices such as the DIN, GQN, RIN, RIN\* and RQN provide the user with an objective and reliable assessment of the integrity of a sample (Table 1). This allows the biobank to easily identify high-quality samples, provides a reference tool that aids in sample selection for research studies, and helps establish or identify issues with quality procedures related to handling, extraction methods, shipping, and storage. In addition, researchers can establish quality metric standards in their workflows, for example by examining the RIN\* in RNA degradation studies or the DIN in FFPE samples. This saves time and money by reducing human error and variation between user assessments, while easily identifying unfit starting materials that would lead to poor results. Quality metrics help to establish quality procedures for handling, extraction methods, shipping, and storage. Side-by-side comparisons of different handling techniques and extraction methods help determine best practices, while before-and-after measurements verify reliability of shipping and long-term storage protocols.

Table 1. Overview of quality metrics for the Agilent automated electrophoresis portfolio.
Genomic DNA and FFPE – DIN, GQN

Genomic DNA is easily sheared with everyday handling, mixing, and multiple freeze-thaw events. DNA from fresh, frozen, or FFPE tissue can be assessed with the TapeStation, Fragment Analyzer, and Femto Pulse systems. FFPE and gDNA integrity can be assessed by the quality metric scores DIN and GQN. The DIN algorithm from the TapeStation software provides a numerical assessment of the DNA quality by assigning each sample a score from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN suggests a strongly degraded gDNA sample (Figure 1). The GQN quality metric generated by the Femto Pulse and Fragment Analyzer systems is designed to allow for easy analysis of gDNA and sheared DNA. The user defines a size threshold they deem appropriate for their specific application. The GQN value is then calculated based on the fraction of the total measured concentration of the sample that lies above the specified size threshold. The GQN scores samples on a scale of 0 to 10, where 0 indicates that none of the sample exceeds the threshold and 10 indicates 100% of the sample lies above the threshold value (Figure 2).

![Figure 1](image1.png)  
*Figure 1.* Mouse gDNA samples with different DNA integrity were analyzed by the Agilent 4200 TapeStation system with the Agilent Genomic DNA ScreenTape assay. The quality metric DIN decreases with increased degradation. This figure has been reproduced from Agilent publication number 5991-6629EN.

![Figure 2](image2.png)  
*Figure 2.* Sheared gDNA from PacBio separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. A) Separations on the electropherogram; B) Average smear size and quality metric GQN at 30 kb. LM = lower marker. This figure has been reproduced from Agilent publication number 5994-0520EN.

<table>
<thead>
<tr>
<th>Average smear size (bp)</th>
<th>GQN Set at 30 kb</th>
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</thead>
<tbody>
<tr>
<td>12,147</td>
<td>0</td>
</tr>
<tr>
<td>23,339</td>
<td>1.5</td>
</tr>
<tr>
<td>45,304</td>
<td>6.4</td>
</tr>
<tr>
<td>57,789</td>
<td>7.1</td>
</tr>
<tr>
<td>73,267</td>
<td>7.8</td>
</tr>
<tr>
<td>94,045</td>
<td>7.8</td>
</tr>
<tr>
<td>109,968</td>
<td>8.2</td>
</tr>
<tr>
<td>164,292</td>
<td>8.8</td>
</tr>
</tbody>
</table>
RNA – RIN, RIN®, RQN

RNA from fresh or frozen tissue can be assessed with the RIN (Bioanalyzer), RIN® (TapeStation), or RQN (Femto Pulse and Fragment Analyzer). All three RNA quality metrics consider the electrophoretic separation of the RNA sample, including the ribosomal bands and the presence or absence of degradation products in front of the 18S peak. They are calculated using a scale from 1 to 10. A high RIN, RIN®, or RQN indicates highly intact RNA, and a low number suggests a strongly degraded RNA sample7,8 (Figure 3). Several studies have been performed demonstrating the equivalences of the RIN to the RIN®9, and the RIN to the RQN10.

![Figure 3. Varying degrees of degraded total RNA was analyzed on A) the Agilent Bioanalyzer system with the Eukaryote Total RNA Nano assay; B) the Agilent Fragment Analyzer system with the HS RNA kit (15 nt). The quality metrics RIN and RQN decrease with increasing degradation. These figures have been reproduced from Agilent publication numbers 5989-1165EN7 and 5994-0521EN8.](image)
FFPE RNA – DV\textsubscript{200}

FFPE RNA samples are difficult to use, as degradation due to fixation and storage conditions is often quite extensive. It is important to evaluate the quality of each FFPE RNA sample before proceeding with library preparation to eliminate highly degraded samples containing RNA fragments smaller than the optimal sequencing size range. Although RIN, RIN\textsuperscript{e}, and RQN values are reliable metrics for evaluating the quality of RNA isolated from fresh and frozen tissue or cell culture, they are not a definitive measure of RNA quality from FFPE samples. To solve this problem, a quality metric developed by Illumina, the DV\textsubscript{200}, calculates the percentage of RNA fragments greater than 200 nucleotides in size. The DV\textsubscript{200} metric is then used to determine the minimal RNA input required for successful library preparation and reproducible results. Given the strong correlation between DV\textsubscript{200} values and library yield, the DV\textsubscript{200} metric is ideal for assessing FFPE RNA quality before library construction with the Bioanalyzer\textsuperscript{11}, TapeStation\textsuperscript{12}, Femto Pulse, and Fragment Analyzer systems\textsuperscript{13}.

Cell-free DNA – %cfDNA

As minimally invasive sample collection methods, or liquid biopsy, has gained relevance in clinical research, cell-free DNA (cfDNA) has become an important input material for NGS, creating the need for a reliable quality metric. High-molecular-weight (HMW) DNA in the cfDNA sample can interfere with library yield and sequencing quality. The TapeStation software and Cell-free DNA ScreenTape assay features a new quality metric, %cfDNA (Figure 4). This reflects the percentage of cfDNA subcomponents that are present in the preset region between 50 and 700 bp in relation to the total sample DNA. The %cfDNA metric allows the user to evaluate sample quality and identify whether a sample contains a sufficient percentage of cfDNA for downstream processes\textsuperscript{14}.

**Figure 4.** Overlay of cfDNA samples with differing quality. The quality metric %cfDNA decreases with increasing high molecular weight genomic DNA over 700 bp. This figure has been reproduced from Agilent publication number 5994-1390EN\textsuperscript{14}.
There are many analytical variables that need to be considered to maintain high nucleic acid quality. Every step starting with method of collection and labware; to turnaround time, handling, extraction methods, and temperature; to transportation conditions and final storage of the sample will influence nucleic acid quality (Figure 5). Comparing the quality metric of the sample after each analytical variable allows a user to determine which method, at each step, minimizes the impact on the quality of the nucleic acid. An increasing number of biobanks have been using quality metrics to determine best practices for each step along the nucleic acid life cycle.

**Figure 5.** Pre-analytical variables that can affect nucleic acid quality.
Genomic DNA extraction methods

A variety of methods are available for isolation of gDNA from eukaryotic and prokaryotic sources that are tailored towards the specific downstream application. Consequently, the size and quality of the isolated gDNA is affected by the different extraction methods, driving a need for reliable quality assessment of gDNA samples to determine which extraction method to use. Sizing and quality of gDNA was compared between five different extraction methods on the Femto Pulse system with the Genomic DNA 165 kb kit15 (Figure 6). The yeast gDNA smear size and integrity varied depending on the extraction method. Method A, a phenol-chloroform extraction, and method B, another liquid extraction method, resulted in the largest intact gDNA smear. Method C, a spin-column extraction method, and method D, a membrane extraction method, displayed similar sizing in the middle of the five different extraction methods. Method E, another spin-column extraction method, resulted in the smallest size. The genomic quality number, GQN, decreased with the decreasing size of the gDNA samples. The Femto Pulse and GQN demonstrated which extraction method maintained the integrity and size of gDNA.

Automation of genomic DNA extraction

Automation of nucleic acid extractions has become increasingly popular to improve efficiency. It is important when changing extraction methods that the nucleic acid quality and yield does not decrease. This is especially important with FFPE samples, where the nucleic acid quality is already compromised due to chemical fixation. It is essential to compare each manual or automated method and not to lump all automated processes together, as each are unique and will have varying results. Mathieson et al. compared the quality of extracted gDNA with a manual extraction kit and two different automated methods with the TapeStation and the DIN quality metric16. They found the manual method resulted in a statistically higher average DIN value of 5.95 (p< 0.003) compared to 5.7 for both automated processes (Figure 7). Comparing the resulting DIN quality metric for the manual and automated extraction process provided clear cut data for the decision process when determining which extraction process to implement.

#### Table 1: Genomic DNA Smear Size and GQN

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Average Smear Size (bp)</th>
<th>Average GQN&lt;sub&gt;50 kb&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>163,670</td>
<td>8.2</td>
</tr>
<tr>
<td>B</td>
<td>103,111</td>
<td>7.4</td>
</tr>
<tr>
<td>C</td>
<td>76,778</td>
<td>6.1</td>
</tr>
<tr>
<td>D</td>
<td>62,165</td>
<td>3.0</td>
</tr>
<tr>
<td>E</td>
<td>27,388</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Figure 6. Electropherogram traces from the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. Yeast *Saccharomyces cerevisiae* gDNA was extracted by five different protocols: A) phenol-chloroform extraction; B) liquid extraction; C and E) spin-column extraction; and D) membrane extraction. LM = lower marker. Average smear size and GQN<sub>50 kb</sub> from corresponding extraction methods. This figure has been reproduced from Agilent publication number 5994-0754EN15.

Figure 7. Comparison of DIN from 30 FFPE tissue blocks where gDNA was extracted using manual, QiaCube, ExScale DNA only, and ExScale simultaneous DNA/RNA methods. DIN values range from 1 to 10, with higher numbers denoting less-degraded DNA. The boxes are the 25th to 75th percentiles, intersected by the median. The whiskers show the 10th and 90th percentiles, and each spot represents one remaining outlier. The difference in DIN between any two extraction methods is statistically significant (p<0.05), with the exception of the QiaCube/ExScale DNA only and the ExScale DNA/ExScale simultaneous comparisons. DIN, DNA Integrity Number. This figure has been modified and reproduced from Mathieson et al.16. The publisher for this copyrighted material is Mary Ann Liebert, Inc. publishers.
The Heidelberg Biobank in Germany performed a retrospective quality analysis of gDNA\(^\text{17}\) after many changes were made to the biobank processes. Samples before 2014 were manually extracted and handled with study-specific guidelines. In 2015, the biobank was restructured and implemented automated extraction methods and optimized workflows with additional standard operating procedures for sample processing, logistics, and storages. The quality of gDNA between 2010 to 2014 and after 2015 was assessed by the TapeStation system and the DIN quality metric. The DNA quality analysis revealed a higher and more consistent DIN > 9 after implementation of automated processes and improved SOPs in 2015. In contrast, samples analyzed prior to any improvements showed a higher variation in gDNA quality (Figure 8). The TapeStation and DIN quality metric demonstrated that the implemented process changes that were made to improve workflows did indeed result in less variation and higher-quality gDNA.

**Genomic DNA extraction over time**

Coriell Institute for Medical Research (Coriell) is considered to have the most diverse collection of cell lines, DNA, and other biomaterials available for biomedical research. In 2016, Coriell replaced traditional gel electrophoresis with the TapeStation system and the Genomic DNA ScreenTape assay\(^\text{18}\). The DIN was implemented to confirm gDNA stability and consistent quality after long-term storage of gDNA at –80 °C over a 30-year period. The DIN revealed consistent and reliable gDNA extraction between 1990 and 2000 (Figure 9). A high-quality DIN of > 7 was noted for all samples. The TapeStation and DIN confirmed that gDNA integrity was indeed maintained after long-term storage at –80 °C and corroborated that best practices for long-term storage were implemented.

![Figure 8](image8.png)

**Figure 8.** Comparison of sample quality over a period of 9 years. For each year, 20 gDNA samples were analyzed with the 4150 TapeStation system and the Genomic DNA ScreenTape assay. In 2014, two groups were analyzed due to the change of labware. This figure has been reproduced from Agilent publication number 5994-0811EN\(^\text{17}\).

![Figure 9](image9.png)

**Figure 9.** DIN values of 50 gDNA samples extracted between 1990 and 2000 were analyzed on the Agilent 4200 TapeStation with the Agilent Genomic DNA ScreenTape assay. This figure has been reproduced from Agilent publication number 5994-1362EN\(^\text{18}\).
Genomic DNA mixing

Handling methods can greatly affect the size and quality of gDNA. It is easily sheared with everyday handling, mixing, and multiple freeze-thaw events, so proper handling of gDNA is important to help maintain the integrity of the sample. If a sample is handled too roughly, it can lead to degradation of the sample. The Femto Pulse system is ideal for sizing and quality analysis of large gDNA, with a pulsed method that replaces overnight pulsed-field gel electrophoresis (PFGE).

Promega gDNA was mixed by several different methods: wide-bore pipette tip, narrow-bore pipette tip, microplate shaker, and two different vortexers and analyzed on the Femto Pulse system with the Genomic DNA 165 kb kit\(^{19}\). The rough shaking from the two vortexers greatly degraded and decreased the size of the gDNA sample compared to the gentler hand mixing with the pipette tips (Figure 10). The Femto Pulse aided in determining which mixing method should be implemented to maintain the integrity and size of gDNA.

Shipping and handling

Biospecimens are often shipped multiple times before reaching the end user. They travel from collection facilities, to laboratories responsible for processing and extraction, to biobanks for storage, and then finally to the end user. The Biobanque de Picardie used the DIN as a vital QC tool to monitor the integrity of gDNA when shipping from laboratory to laboratory (Figure 11)\(^ {20}\).

![Figure 10](image1.png)

Figure 10. The impact of mixing on the size of gDNA smears was analyzed on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. Commercially available Promega gDNA samples were mixed with diluent marker using a variety of different mixing techniques, including the recommended wide-bore pipet (black), a narrow-bore pipet (blue), a touch mixer Vortex 1 (red), and two plate shakers, Vortex 2 (orange), and Vortex 3 (green). This figure has been reproduced from Agilent publication number 5994-2723EN\(^ {19}\).

![Figure 11](image2.png)

Figure 11. Schematic representation of project logistics for the Chronic Kidney Disease - Renal Epidemiology and Information Network (CKD-REIN) study. This figure has been reproduced from Agilent publication number 5994-2921EN\(^ {20}\).
This additional QC step was necessary to verify the fulfillment of the substantial quality requirements in relation to initial sample handling, storage organization, and subsequent transfer. A second laboratory, Centre National de Recherche en Génomique Humaine (CNRGH), reanalyzed 394 samples for gDNA integrity on the 4200 TapeStation system with the Genomic DNA ScreenTape assay (Figure 12). A total of 32 (8.1%) reported identical DIN values, 94 samples (24%) had a difference between 0 and 0.2 (within the standard deviation of the TapeStation21), and for 197 samples (50%), the DIN difference ranged between 0.2 and 0.6. Only 10 samples out of 394 (2.5%) gave a DIN difference exceeding 1. The low average of DIN differences (0.2) indicated that shipping and handling of samples between the laboratories was performed successfully in a way that did not affect gDNA integrity.

Quality confirmation after power outage

The Center for Biospecimen Research and Development (CBRD) runs New York University Langone’s centralized biobank and was one of many laboratories to be affected by the power outage from Hurricane Sandy in October of 201222. The majority of samples were frozen and kept in −80 °C freezers. With no power, they were unable to track the rising temperatures in the freezers and determine if samples were exposed to ambient temperatures that can cause degradation. To determine the effect the power outage and temperature change had on the quality of these frozen tissue samples, nucleic acid samples collected before and after the storm were analyzed with the Fragment Analyzer system and the GQN quality metric. The DNA samples reported a GQN of 8.0 for samples before and after the power outage (Figure 13). RNA was assessed with the RQN, ranging from 8.1–8.3, and the 28Ss/18S ratio of rRNA at 1.83–1.87 for samples before and after the hurricane, respectively. A GQN or RQN > 7 was considered of sufficient quality DNA and RNA for downstream applications. The Fragment Analyzer system confirmed that emergency liquid nitrogen and dry ice was able to maintain nucleic acid sample quality during the power outage.

Figure 12. Comparison of DIN values before and after shipping between the Biobanque de Picardie and CNRGH. This figure has been reproduced from Agilent publication number 5994-2921EN20.

Figure 13. (a) Results of capillary electrophoresis of extracted DNA from select 2012 and 2013 specimens compared with a ladder of known nucleotide lengths and two control DNA samples with known high fidelity. (b) Results of capillary electrophoresis of extracted RNA from select 2012 and 2013 specimens compared against a ladder of known nucleotide lengths. The bands at ~4 and ~2 kb mark the 28S and 18S subunits of ribosomal RNA, respectively. This figure has been modified and reproduced from Mendoza et al.22. The publisher for this copyrighted material is Mary Ann Liebert, Inc. publishers.
Storage temperatures of paraffin-embedded tissue and fixation preservatives

Many samples stored at biobanks are paraffin-embedded tissues (PET) that are important for biomedical research and biomarker development. Fixation of these tissues with formalin has become the standard method for preservation since the late 19th century. While formalin fixation provides excellent preservation of tissue morphology, it causes cross-linking and chemical modification of nucleic acids and proteins. These chemical modifications affect the outcomes of cDNA synthesis from RNA, RT-qPCR, and microarray gene expression analysis. Consequently, more tissue-friendly fixation preservatives are continually being investigated. Long-term storage of these tissues can also affect the RNA and DNA extracted from these tissues. A comparative study was performed to evaluate the effect of long-term storage of formalin-fixed paraffin-embedded (FFPE) and PAXgene Tissue-fixed (a formalin replacement), paraffin-embedded (PFPE) tissue at different temperatures on nucleic acid stability and usability in PCR. Integrity of DNA isolated from rat FFPE and PFPE tissues stored at 22 °C room temperature, 4 °C, and –20 °C for 9 years was assessed on the TapeStation and the Genomic DNA ScreenTape assay (Figure 14). DNA from tissue stored at 22 °C was highly degraded as seen from the smear over the smaller size range of 0.3 to 1.5 kb. DNA quality improved at 4 °C and –20 °C with increased size and less fragmentation. Overall, tissue preserved with PFPE and stored at –20 °C resulted in the largest, least-fragmented DNA. A storage temperature effect was observed for qPCR performance for DNA isolated from both FFPE and PFPE tissue. Overall amplification efficiency was higher from DNA stored at lower temperatures compared to room temperature. Due to the negative impact of chemical modification from formalin, DNA from PFPE performed better than FFPE DNA.

![Figure 14. Storage of PET blocks for 9 years at lower temperatures prevents DNA from degradation. Analysis of DNA integrity from PFPE (A) and formalin-fixed paraffin-embedded (FFPE) (B) tissues of rat liver, kidney, spleen, lung and intestine on Agilent 4200 TapeStation system with genomic DNA Analysis ScreenTape assay. PET blocks were stored prior to DNA extraction for 108 months at 22°C, 4°C, and -20°C. DNA was extracted from 3x 10µm sections in triplicate. This figure has been modified and reproduced from Groelz et al. This is an open access article distributed under the terms of the Creative Commons Attribution License.](image-url)
RNA was also extracted from FFPE and PFPE tissue stored at 22 °C (room temperature), 4 °C, −20 °C, and −80 °C and analyzed with the Bioanalyzer system and the RIN quality metric\(^\text{23}\) (Figure 15). Storage of tissue at room temperature led to RNA degradation directly after storage began, with RIN scores between 2 and 3. RNA degradation slowed down with a RIN above 6, when tissue blocks were stored at 4 °C for up to 24 months. Freezing both FFPE and PFPE tissue blocks prevented RNA from degradation and maintaining a RIN above 7 for 108 months. Downstream results from RT-qPCR were improved with storage at 4 °C and below and with PFPE tissue. The TapeStation and Bioanalyzer systems helped biobanks to determine the best storage temperature for paraffin-fixed tissue in order to provide quality samples for future research studies.

**Figure 15.** RNA integrity from rat PET blocks decrease depending on storage temperature. For each of five different tissue types (liver, kidney, spleen, lung and intestine) and four different temperatures (22°C, 4°C, −20°C, and −80°C) 3 blocks of FFPE and PFPE rat tissue were stored. RNA was extracted from block 1 after 3, 6 and 12 months (m), from block 2 after 24, 36, 48 and 72 m and from block 3 after 108 m of storage. Mean RNA integrity number (RIN) values with standard deviation from FFPE (A) and PFPE tissue (B) are shown for triplicate extractions from five different tissue types for each fixation method, storage time point and temperature (total RIN values \( n = 942 \)). Dotted line indicates block change. This figure has been modified and reproduced from Groelz et al\(^\text{23}\). This is an open access article distributed under the terms of the Creative Commons Attribution License.
Cryogenic long-term storage

The Ontario Tumour Bank (OTB) has been collecting samples for cancer research since 2014. As part of its ongoing commitment to quality, OTB participates in the Integrated Biobank of Luxembourgh Biospecimen Proficiency Testing Program. To ensure high-quality samples stored in cryogenic conditions over time, they assessed a random selection of frozen samples each year for RNA and DNA integrity\(^2\). RNA was extracted from cryogenic frozen samples, analyzed on the Bioanalyzer with the RNA 6000 Nano kit, and assigned an RIN (Figure 16). The authors stated that "The RIN value generated by Agilent’s Bioanalyzer is commonly accepted as a standardized indicator of total RNA quality and integrity.” The abundance of endogenous and exogenous RNAses renders RNA more susceptible to degradation than DNA. As RNA is less stable than DNA, RNA quality is a generally recognized criterion by which tissue “quality” can be measured. The majority of samples had a RIN > 6.5 and were of acceptable quality. The Bioanalyzer and quality metric RIN aided in determining that extended long-term cryogenic storage of tissue did not affect the quality of RNA and remains a viable option for long-term storage of tissue samples.

Conclusion

Biobanks continue to grow, storing samples from multiple sources. ISO and ISBER have put forth recommendations on how to standardize processes for biobanks and repositories. In this application note, we have shown examples of how quality metrics provided by the TapeStation, Femto Pulse, Bioanalyzer, and Fragment Analyzer systems help determine if a sample is fit-for-purpose and if best practices have been followed. Quality metrics provide a highly useful, objective measurement for determining the integrity of samples during nucleic acid extraction, handling, and shipping. Quality control allows a consistent and reliable way to measure the integrity of any given sample, providing confidence to both the biobank and their customers in the quality of the stored samples.
Appendix

Equipment maintenance, repair and replacement

According to ISBER Best Practices C12. Equipment maintenance, repair and replacement, "a system for preventative maintenance and repair of storage equipment, supporting systems, and facilities should be in place. System maintenance should be performed at regular, established intervals per manufacturer’s recommendation and as determined as fit for purpose aligned with the repository’s practices." Performing routine assessments and modifications to the equipment per the manufacturer’s specifications keeps the instruments used in the repository in top shape. In addition, in C12.2 Verification of Equipment Functionality, "the proper performance of all equipment and related software should be verified or qualified prior to use or following repairs that affect the instrument’s operating capabilities. Documentation of the testing should be maintained and made available for audits." ISO section 6.5 also specifies guidelines for equipment verification upon installation, maintenance, documentation, support, and replacement.

Agilent IQ/OQ services and maintenance

The Agilent compliance solution provides an integrated and reliable tool for quality control of nucleic acids. The compliance solution is comprised of the 4150 and 4200 TapeStation, Femto Pulse, Fragment Analyzer, and the 2100 Bioanalyzer instruments, software, services, as well as application-specific consumables. A certified engineer ensures the instrument and software are installed correctly, documenting the completeness of shipping, the operating environment, and the components of the system. Audit-ready documentation is supplied for both the instrument and software. OQ is performed to verify and document the system's ability to meet specified performance criteria ensuring basic accuracy after it is installed in the selected environment. OQ should be performed to verify the functionality of the hardware and software, thereby qualifying the system.

Information management systems

ISBER Best Practices section I covers the necessity of repository information management systems. "Effective tracking systems should be in place to ensure that specimens can be tracked accurately from the site at which they are collected through their entire life cycle at the repository. Critical components of these systems include the use of unique specimen identifiers, appropriate specimen labels, electronic data inventory systems for specimen tracking, consent form and/or permit tracking." ISO section 7.10 also specifies that biobanks have a procedure for implementation, modification, and use of a computer system software, hardware, and database.

The software packages for the TapeStation, Femto Pulse Fragment Analyzer, and Bioanalyzer systems allow integration into a laboratory information management system (LIMS). Agilent offers an information management system called SLIMS, which combines a LIMS and electronic laboratory notebook (ELN) in a single system, enabling comprehensive workflow management. SLIMS facilitates data collection and record keeping, improves compliance, and streamlines processes. The system is designed to support the requirements of ISO17025, 21 CFR Part 11, HIPAA, and CLIA. SLIMS elevates sample repository management with integration with freezer systems, instruments, and EMR systems. SLIMS does more than managing sample location, as it facilitates reliable, compliant material traceability by assisting users to find out if a particular sample was correctly handled prior to storage, to review who manipulated it, and see freezer temperature changes over time.
References

4. Use of the Agilent 4200 TapeStation for Quality Control in the Whole Exome Sequencing Workflow at the German Cancer Research Center (DKFZ). Agilent Technologies application note, publication number 5991-7615EN, 2016.
5. High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation. Agilent Technologies technical overview, publication number 5991-6629EN, 2016.


