

Retrospective Quality Analysis of DNA Samples from the Heidelberg CardioBiobank

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Abstract

This Application Note demonstrates the use of the Agilent 4150 TapeStation system as a quality control (QC) tool to analyze DNA samples stored at the Heidelberg CardioBiobank (HCB). An important quality control step is performed immediately after DNA extraction from blood samples to ensure the storage of only high-quality DNA samples. Subsequently, the QC step can be repeated for previously frozen DNA samples before using them for research purposes. This Application Note focuses on a retrospective analysis of the quality and quantity of stored DNA samples. Analysis of a subset of samples was taken as representative of the DNA quality after nine years of storage at -80°C without any freeze thaw cycles.

Within this timeframe, different DNA extraction methods were used at the HCB, and resulted in varied sample quality levels. Post QC analysis was able to verify the efficiency of the sample extraction and handling processes previously implemented.

Introduction

The HCB is one of the largest biobanks in Germany, with a strong focus on Cardiovascular Diseases. It operates as a hospital-integrated biobank as well as a core biobank for several national and international clinical trials and research projects (Figure 1). The Biobank started with a cluster of standard $-80\text{ }^{\circ}\text{C}$ freezers in which biological samples (predominantly liquid samples) were stored in a study-specific way. Therefore, the collection of the biological samples was performed according to study-specific guidelines. In 2014, the HCB was restructured to establish a large, fully automated $-80\text{ }^{\circ}\text{C}$ -storage system with a capacity of up to 1.2 million samples (custom-built by Liconic AG, Liechtenstein). To ensure that biosamples are “fit for purpose”, we evaluated and optimized the entire workflow for sample collection,

processing, logistics, and storage. It was then possible to develop and implement additional and specific standard operating processes (SOPs) for all pre-analytical processes to achieve the highest quality of samples, which is

mandatory for cutting-edge translational research (Figure 2). To investigate the impact of these processes in biobanks, we analyzed the quality and quantity of DNA samples from a series of randomly selected samples from the

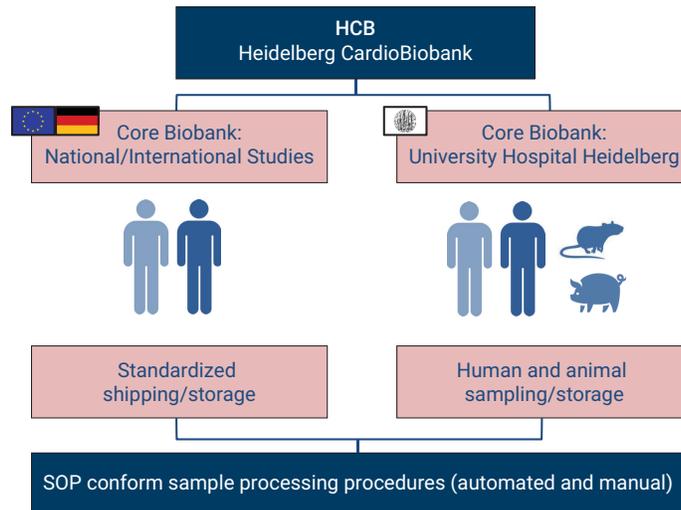


Figure 1. Scheme Heidelberg CardioBiobank (HCB).

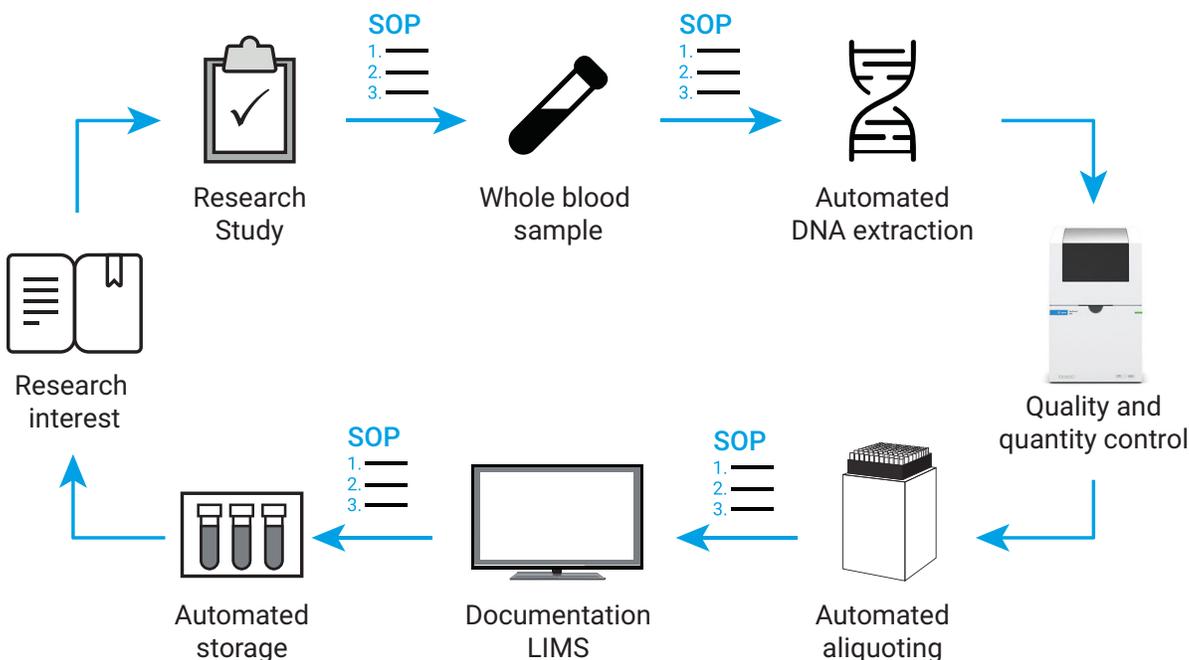


Figure 2. Workflow of whole blood sample processes including DNA extraction. For all pre-analytical processes, SOPs were developed and time standards were set. The SOPs are related to the collection process of biological samples, its transportation, and, if applicable, the automated DNA extraction (Tecan/Promega Freedom EVO HSM workstation). The liquid samples are aliquoted automatically (Tecan Freedom EVO system) and stored in an automated $-80\text{ }^{\circ}\text{C}$ freezer system (customized Liconic STC store). Precise documentation of each processing step is done with the LIMS software CentraXX (Kairos) which records not only the sample data, but also the time stamps of each process.

nine consecutive years between 2010 and 2018 with other established quality management (QM) analysis tools. Additionally, the 4150 TapeStation system was evaluated as a QC tool for quantification and integrity assessment for genomic DNA by automated electrophoresis, and the results were compared.

Experimental

DNA extraction

For the analysis of long-term stored DNA, a random selection of 20 samples per year, from 2010 until 2018, was performed. Before 2015, the blood sampling processes were operated partly under slightly different conditions, for example, in some studies there was no time specification for storage of blood samples at 4 °C before DNA extraction. The following DNA extraction was performed manually with the NucleoSpin Blood XL column kit (Macherey-Nagel, Düren, Germany) using a silica membrane column system for the extraction of DNA from EDTA whole blood (volume 9 mL). As of 2015, the DNA extraction was executed by an automated extraction system (Tecan/Promega Freedom EVO HSM workstation, Tecan, Männedorf, Switzerland) using the ReliaPrep Large Volume HT gDNA Isolation system, which uses a resin bead based extraction technology. The EDTA whole blood samples (volume 9 mL) were stored at 4 °C until sample processing, with a maximum storage time of seven days before starting the automatic extraction of DNA samples. Due to the switch to automated hardware systems, it was also necessary to switch to labware (storage tubes for biological samples) optimized for the automated processes starting in 2014. To exclude possible effects of storage tubes on the quality of the stored samples, we analyzed

two groups of samples from the year 2014. Samples of group 1 from 2014 were stored in tubes from Micronic, and group 2 from 2014 represents samples stored in FluidX tubes. In parallel with the implementation of automated systems, improved SOPs were developed and implemented for all processes related to the handling, processing, and logistics of biological samples.

DNA analysis

The selected DNA samples underwent an identical thawing procedure (18 hours at 4 °C) before an aliquot from each sample was taken for further processing. First, the sample concentration was measured by NanoDrop spectrophotometer (Thermo Fisher, Waltham, USA) to compare the value of the concentration with the measurement taken before freezing in 2010 to 2018. An additional measurement was performed with the Quantus fluorometer (Promega, Madison, USA), which is a fluorescence-based nucleic acid quantification method.

The results from Quantus were used to adjust the final concentration of the DNA samples to the quantitative range of the Agilent Genomic DNA ScreenTape assay of the 4150 TapeStation system (10 to 100 ng/μL). In general, the process was in concordance with the instruction manual. Therefore, all kit components were equilibrated at room temperature for 30 minutes. After dilution of the selected DNA, 10 μL genomic DNA sample buffer and 1 μL genomic DNA sample were carefully aliquoted, vortexed for 1 minute, spun down, and then loaded on the genomic DNA ScreenTape¹. Typically, all available sample positions on 4150 TapeStation controller software were selected. The experiments were performed in triplicates, as displayed with the whole workflow in Figure 3. The Agilent TapeStation analysis software was used to evaluate the DNA sample concentration and the DNA integrity number (DIN).

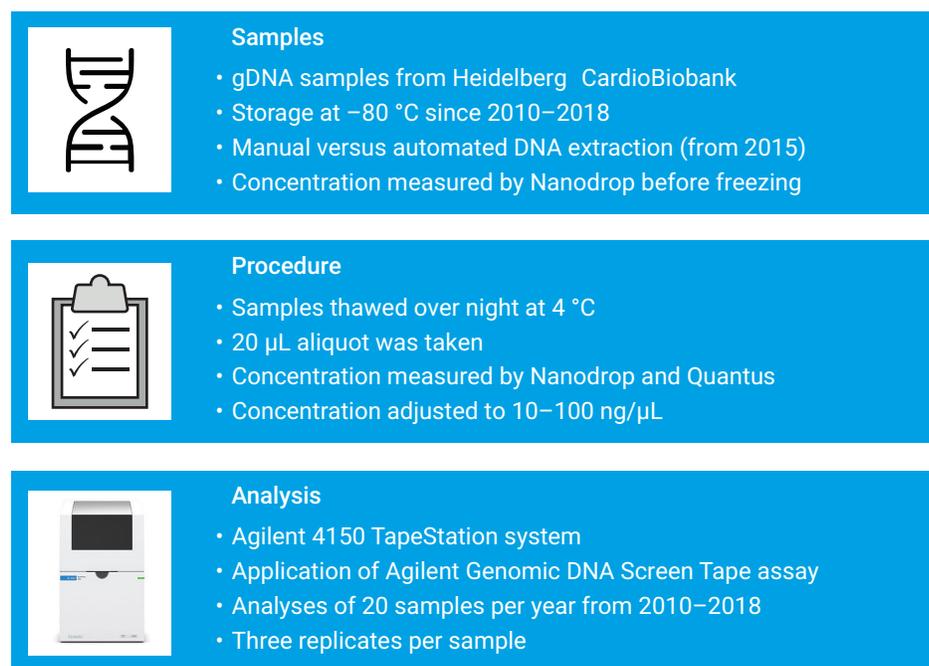


Figure 3. Workflow of DNA quality and quantity analysis.

Results and Discussion

Quality

The DIN algorithm is included in the TapeStation analysis software and provides a quality assessment of the DNA sample by assigning a numerical score from 1 to 10. A high DIN indicates intact gDNA, and a low DIN degraded gDNA². The DNA analysis revealed that the tested DNA samples are of high and consistent quality (DIN >9) from 2014 to the present when automated processes and improved SOPs were in place. By contrast, samples collected before 2014 show higher variation of DNA integrity (Figure 4). Figure 5 shows the comparison of representative samples taken between 2010 and 2018 with the TapeStation gel view and electropherogram overlay. In 2014, the

type of storage tubes was changed due to the implementation of the automated storage and robotics handling systems and their respective requirements. Accordingly, two different sets of

samples (2014/1 and 2014/2) were analyzed. The change from the tube manufacturer Micronic to FluidX had no influence on the quality of the DNA samples.

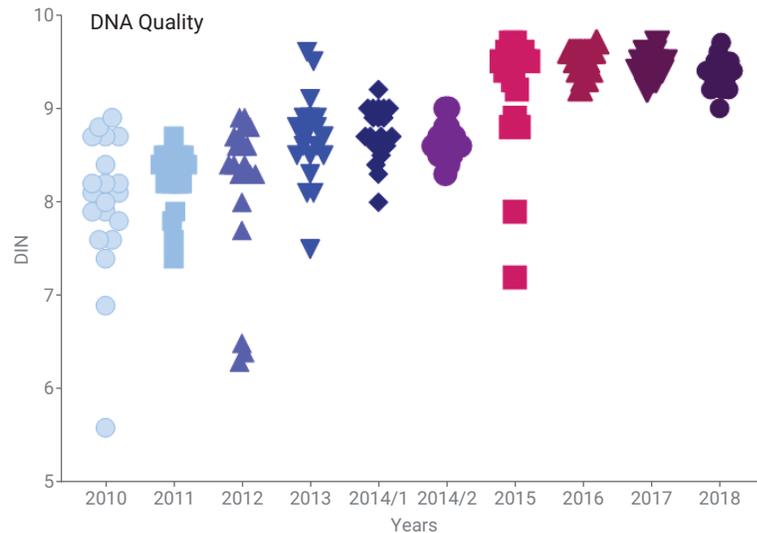


Figure 4. Comparison of sample quality over a period of nine years. For each year, 20 DNA samples were analyzed with the Agilent Genomic DNA ScreenTape assay. In 2014, two groups were analyzed due to the change of labware.

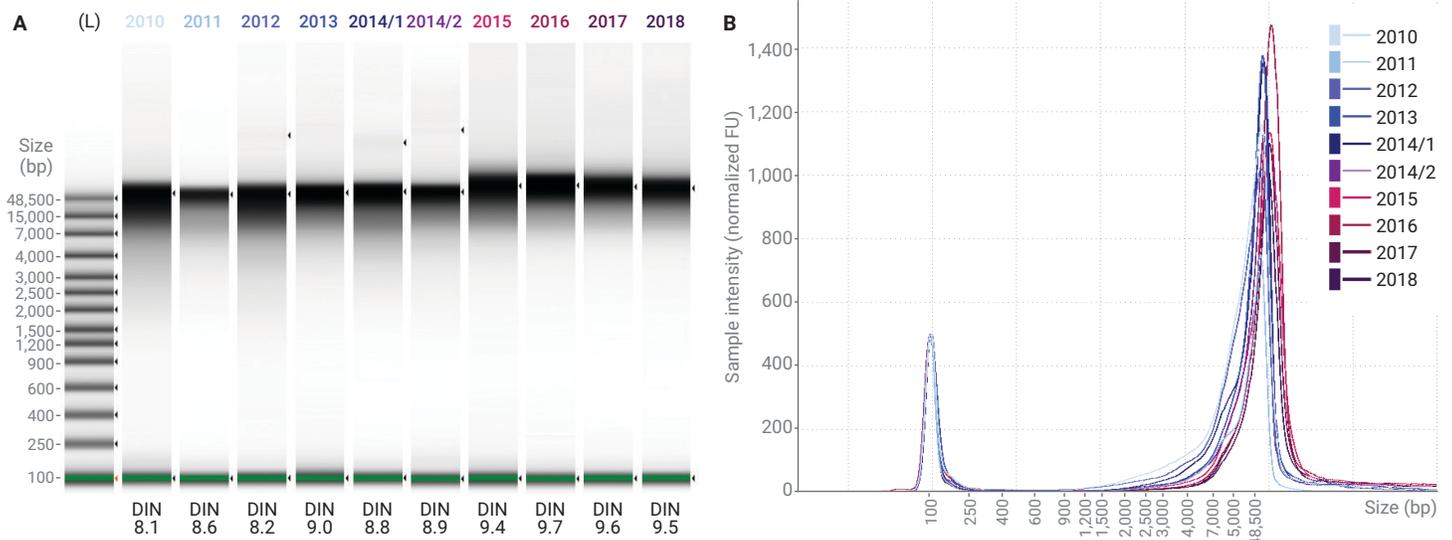


Figure 5. Comparison of representative samples harvested between 2010 and 2018. The DNA sample 2014/1 represent tubes from Micronic and sample 2014/2 tubes from FluidX. The sample integrity increased after 2015, reflected by higher DIN values. A) TapeStation gel view B) Overlay of the electropherograms of 10 samples. Both views enable visual comparison of the sample integrity.

Quantity

The concentration of DNA was routinely measured by a NanoDrop spectrophotometer directly after DNA extraction at the HCB. DNA samples from the years 2010 to 2014 had been extracted manually (see material and methods) and obtained an average DNA concentration of approximately 120 ng/ μ L. The implementation of improved SOPs and the automated DNA extraction method in 2015 resulted in a significant increase of average DNA yield to about 300 ng/ μ L. In this study, after thawing the samples, the DNA quantification by NanoDrop was repeated. The storage at -80°C did not lead to altered sample concentration independent of the storage time (Figure 6). The samples were also measured by Quantus, which fluorometrically evaluates the DNA quantity, as the TapeStation system uses a fluorescence-based approach to determine DNA concentration. All tested samples showed an equivalent DNA concentration on both systems (Figure 7).

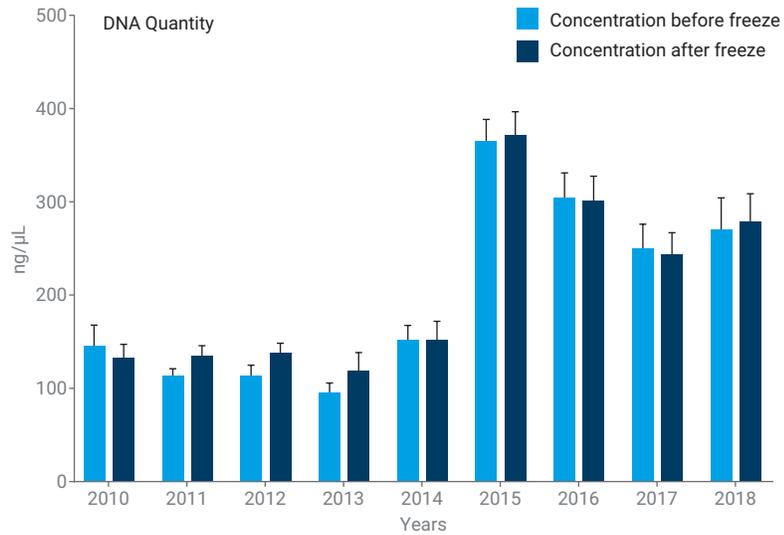


Figure 6. Overview of DNA concentration as measured by NanoDrop spectrophotometer. The increase of DNA concentration was a result of the change from manual to automated DNA extraction methods from 2014 to 2015. All DNA concentrations (conc.) remained stable throughout the years of storage at -80°C .

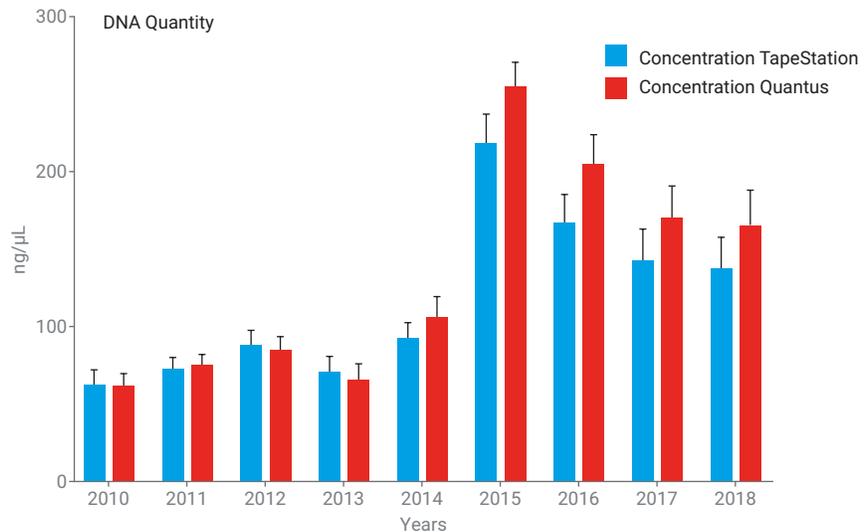


Figure 7. Determination of DNA quantity by Quantus fluorometer and the Agilent 4150 TapeStation system. The increase of the DNA concentration between 2014 and 2015 is a result of the conversion from manual to automated DNA extraction.

Conclusion

From 2010 to 2014, the collection of biological samples at the HCB was performed in some studies under less stringent SOPs compared to 2015 to 2018 (for example, the storage time until processing of blood samples varied from two hours up to several days study dependently). As a result, the quality of the analyzed DNA samples differed from 2010 to 2014.

Because of significant improvements made to processes in 2015, we evaluated workflows such as collection, transportation, and sample preparation procedures. The influence of preprocessing parameters like incubation time and storage modalities on DNA quality was investigated and resulted in the development and implementation of advanced SOPs. In addition to the standardized processes, we implemented automated hardware systems for DNA extraction, which also improved the outcome of the performed

work. Hence, by automating the DNA extraction process, the quantity, quality, and variability of quality of extracted DNA could be significantly increased. The analysis with the Agilent Genomic DNA ScreenTape assay enables comparison of DNA quality by using DIN. The improvements to sample handling and extraction are also reflected in the DNA integrity, leading to DIN values being more consistent at a significantly higher level. Nowadays, the need for personalized and precision medicine is highly apparent, requiring translational research according to best practices ("garbage in equals garbage out"). We showed that the implementation of standardized and almost fully automated sample processing systems leads to the highest quality of biological samples. The use of the Genomic DNA ScreenTape assay with the Agilent 4150 TapeStation system could be smoothly integrated into our workflow as a reliable QC tool in biobanking processes to verify the quality of DNA samples important for downstream applications.

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