

Assessing Quality of DNA

CTRNet Standard Operating Procedure			
Assessing Quality of DNA			
SOP Number:	05.004	Version:	e1.1
Supersedes:	05.004 e1.0	Category:	Quality Assurance

Approved By:	CTRNet College of Advisors	
	Per: Peter Watson and Anne-Marie Mes-Masson	

1.0 PURPOSE

Quality assurance is fundamental to the successful operation of a biobank offering tissue specimens and derivatives for research purposes. A high level of molecular integrity is essential for avoiding inconsistencies and variables in research studies. Nucleic acid quality is critically important for many techniques utilized in genomic analysis, for the meaningful interpretation of results and for the facilitation in the comparison of results across independent laboratories. CTRNet-registered biobanks should be confident that they are providing adequate samples for the specified research purpose. Ideally, testing procedures should be in place to monitor and assess the quality of the samples in the collection.

2.0 SCOPE

This Standard Operating Procedure (SOP) outlines minimum assessment and testing that should be in place to evaluate the quality of deoxyribonucleic acid (DNA) extracted in the biobank in order to provide investigators with a product that is consistent with their needs.

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

Note: When adopting this SOP for local use please reference CTRNet.

- 3.1 CTRNet Policy: POL 5 Records and Documentation
- 3.2 CTRNet Policy: POL 7 Material and Information Handling
- 3.3 CTRNet Standard Operating Procedure: SOP 5.001 Assessing Quality of Tissue Specimens
- 3.4 CTRNet Standard Operating Procedure: SOP 5.002 Assessing Quality of Nucleic Acids
- 3.5 CTRNet Standard Operating Procedure: SOP 08.02.004 Blood Derivatives: Extraction of DNA
- 3.6 CTRNet Standard Operating Procedure: SOP 08.03.008 Tissue Derivatives - Extraction of DNA

*3.7 CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material
Waste Management*

4.0 ROLES AND RESPONSIBILITIES

This SOP applies to all biobank personnel involved in writing, revising, reviewing, approving and maintaining SOPs.

Biobank Personnel	Responsibility/Role
Lab technician	Conducts and assists with quality assurance procedures. Records and documents outcomes.

Assessing Quality of DNA

5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	Materials and Equipment (Site Specific)
4200 or 4150 TapeStation System	
Loading tips (5067-5598, 1pk or 5067-5599, 10pk)	
Optical Tube 8x Strip (401428) and Optical Cap 8x Strip (401425)	
Rack for Streck tubes	
Vortex mixer IKA MS3 with 96-well sample plate adapter	
96-well sample plates (5042-8502) and 96-well Plate Foil Seal (5067-5154) (4200 TapeStation system only)	
Volumetric micropipettes for handling volumes from 1 to 15 µL	
Centrifuges for tube strips and 96-well sample plates	
5067-5365 Genomic DNA ScreenTape (includes 7 ScreenTape devices)	
5067-5366 Genomic DNA Reagents (includes Genomic DNA Ladder & Genomic DNA Sample Buffer)	
5067-5630 Cell-free DNA ScreenTape (includes 7 ScreenTape devices)	
5067-5631 Cell-free DNA Reagents (includes Cell-free DNA Ladder and Cell-free DNA Sample Buffer)	

6.0 DEFINITIONS

See the CTRNet Program Glossary: <https://biobanking.org/webs/glossary>

7.0 PROCEDURES

7.1 Quality Assessment – genomic DNA by using the Agilent 4200 or 4150 TapeStation System

The following procedure is based on the use of the Agilent 4150 (G2992AA) and 4200 (G2991AA) TapeStation systems with Genomic DNA ScreenTapes and Reagents to determine the concentration and integrity of genomic DNA samples. It provides a read-out for sample quantity and quality and has the added advantage of requiring small amounts of the sample. A quality score, the DNA Integrity number (DIN), is automatically calculated and can be used to establish thresholds.

7.1.1 Prepare run

- a. Allow Genomic DNA Reagents (5067-5366) to equilibrate at room temperature for 30 minutes.
- b. Launch the Agilent TapeStation Controller software.
- c. Flick the Genomic DNA ScreenTape device (5067-5365) and insert it into the ScreenTape nest of the TapeStation instrument.
- d. Select required sample positions in the TapeStation Controller software.
- e. The required consumables (tips, further ScreenTape devices) are displayed in the TapeStation Controller software. Make sure that all materials and reagents are prepared for next steps
- f. Vortex reagents and samples. Spin down before use.

7.1.2 Prepare ladder in one well of a complete tube strip

- a. For 1 or 2 ScreenTape devices: pipette 10 μ L Genomic DNA Sample Buffer and 1 μ L Genomic DNA Ladder at position A1 in a tube strip or the 96 well plate.
- b. For more than 2 ScreenTape devices: pipette 20 μ L Genomic DNA Sample Buffer and 2 μ L Genomic DNA Ladder at position A1 in a tube strip or the 96 well plate.

7.1.3 Prepare samples

- a. For each sample, pipette 10 μ L Genomic DNA Sample Buffer and 1 μ L DNA sample in a tube strip or 96-well sample plate.
- b. Apply caps to tube strips and/or foil seals to 96-well sample plates.
- c. Mix liquids using the IKA MS3 vortexer at 2000 rpm for 1 min.
- d. Spin down samples and ladder for 1 min.

7.4.4 Sample Analysis

- a. Load samples into the TapeStation instrument.
- b. Place ladder in position A1 on tube strip holder.
- c. Carefully remove caps of tube strips. Visually confirm that liquid is positioned at the bottom.
- d. Click Start.
- e. The TapeStation Analysis software opens automatically after the run and displays results.

For more information about using the 4200 & 4150 TapeStation systems to assess the quality of genomic DNA refer to Section 8.1.

Assessing Quality of DNA

For the use with the Agilent 2200 TapeStation system a specific Quick Guide is available online.

7.2 Quality Assessment – cell-free DNA by using the Agilent 4200 or 4150 TapeStation System

The following procedure is used by the quality control centre of CTRNet and is based on the use of the Agilent 4150 (G2992AA) and 4200 (G2991AA) TapeStation systems with Cell-free DNA ScreenTapes and Reagents to determine the concentration and integrity of cell-free DNA samples. It provides a read-out for sample quantity and quality and has the added advantage of requiring small amounts of the sample. A quality score, the %cfDNA, is automatically calculated and can be used to establish thresholds.

7.1.1 Prepare run

- a. Allow Cell-free DNA Reagents to equilibrate at room temperature for 30 minutes.
- b. Launch the Agilent TapeStation Controller software.
- c. Flick the Cell-free DNA ScreenTape device and insert it into the ScreenTape nest of the TapeStation instrument.
- d. Select required sample positions in the TapeStation Controller software.
- e. The required consumables (tips, further ScreenTape devices) are displayed in the TapeStation Controller software. Make sure that the materials and reagents are prepared for the next steps.
- f. Vortex reagents and samples. Spin down before use.

7.1.2 Prepare ladder in one well of a complete tube strip

- a. For 1 ScreenTape device: pipette 2 μ L Cell-free DNA Sample Buffer and 2 μ L Cell-free DNA Ladder at position A1 in a tube strip.
- b. For 2 ScreenTape devices: pipette 4 μ L Cell-free DNA Sample Buffer and 4 μ L Cell-free DNA Ladder at position A1 in a tube strip.
- c. For more than 2 ScreenTape devices: pipette 15 μ L Cell-free DNA Sample Buffer and 15 μ L Cell-free DNA Ladder at position A1 in a tube strip.

7.1.3 Prepare samples

- a. For each sample, pipette 2 μ L Cell-free DNA Sample Buffer and 2 μ L DNA sample in a tube strip or 96-well sample plate.
- b. Apply caps to tube strips and/or foil seals to 96-well sample plates.
- c. Mix liquids using the IKA MS3 vortexer at 2000 rpm for 1 min.
- d. Spin down samples and ladder for 1 min.

7.4.4 Sample Analysis

- a. Load samples into the TapeStation instrument. Place ladder in position A1 on tube strip holder.
- b. Carefully remove caps of tube strips. Visually confirm that liquid is positioned at the bottom.
- c. Close the lid of the machine.
- d. Click Start on the software screen and follow the instructions.
- e. The TapeStation Analysis software opens automatically after the run and displays results.

Assessing Quality of DNA

For more information about using the 4200 & 4150 TapeStation systems to assess the quality of cell-free DNA refer to Section 8.2.

The Agilent Cell-free DNA ScreenTape assay is exclusively available for use with the Agilent 4150 or 4200 TapeStation systems (not for 2200 TapeStation system).

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Quality Assessment – genomic DNA by using the Agilent 4200 or 4150 TapeStation System

- Agilent Genomic DNA ScreenTape Quick Guide for TapeStation Systems:
https://www.agilent.com/cs/library/usermanuals/public/gDNA_QuickGuide.pdf
- Agilent Genomic DNA ScreenTape System Quick Guide (specific for 2200 TapeStation system):
https://www.agilent.com/cs/library/usermanuals/public/ScreenTape_gDNA_QG.pdf
- Performance Characteristics of the Genomic DNA ScreenTape Assay for the 4150 TapeStation System:
<https://www.agilent.com/cs/library/technicaloverviews/public/technicaloverview-gdna-4150-tapestation-5994-0497en-agilent.pdf>
- High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation System:
<https://www.agilent.com/cs/library/technicaloverviews/public/5991-6629EN.pdf>
- Monitoring Long-Term DNA Storage in the Biorepositories at Coriell Institute:
<https://www.agilent.com/cs/library/applications/application-coriell-biorepositories-din-tapestation-5994-1362en-agilent.pdf>
- Retrospective Quality Analysis of DNA Samples from the Heidelberg CardioBiobank:
<https://www.agilent.com/cs/library/applications/application-dna-quality-heidelberg-cardiobiobank-4150-tapestation-5994-0811en-agilent.pdf>
- Standardized DNA and RNA Sample Quality Control:
https://www.agilent.com/cs/library/posters/public/Copy%20of%20Standardized_DNA_RNA_sample_quality_control_PR7000-1612_poster_Agilent.pdf

8.2 Quality Assessment – cell-free DNA by using the Agilent 4200 or 4150 TapeStation System

- Agilent Cell-free DNA ScreenTape Quick Guide for TapeStation Systems:
https://www.agilent.com/cs/library/usermanuals/public/cfDNA_QuickGuide.pdf
- Performance Characteristics of the Cell-free DNA ScreenTape Assay:
<https://www.agilent.com/cs/library/technicaloverviews/public/technicaloverview-cfdna-assay-tapestation-5994-1390EN-agilent.pdf>

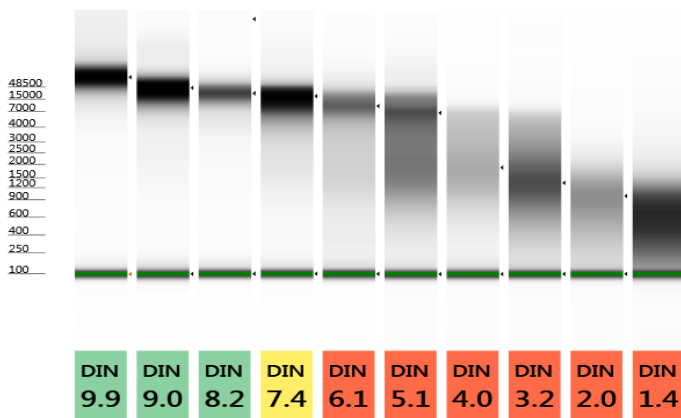
Assessing Quality of DNA

- Cell-free DNA Quality and Quantity Assessment – A Method Comparison:
https://www.prealanalytix.com/storage/download/PAXgene_Blood_ccfDNA_Tube_RUO/Posters/PROM-15083-001_POS_PX_ccfDNA_RUO_QualQuantAssess_CNAPS_0919_ROW.pdf
- Sample QC with the Cell-free DNA ScreenTape assay:
<https://www.agilent.com/cs/library/posters/public/poster-cfdna-assay-tapestation-5994-0337en-agilent.pdf>
- Monitoring the Impact of Pre-analytical Parameters on cfDNA Quality:
<https://www.agilent.com/cs/library/posters/public/PR7000-7173%20cfDNA%20quality%20parameters.pdf>

9.0 APPENDICES

9.1 Appendix A – Interpreting genomic DNA samples analyzed on a TapeStation system with the DIN

The DNA Integrity number DIN ranges from 1 for degraded to 10 intact genomic DNA. The DIN is automatically calculated and displayed below the gel image, based on defined thresholds a color coding can be applied.

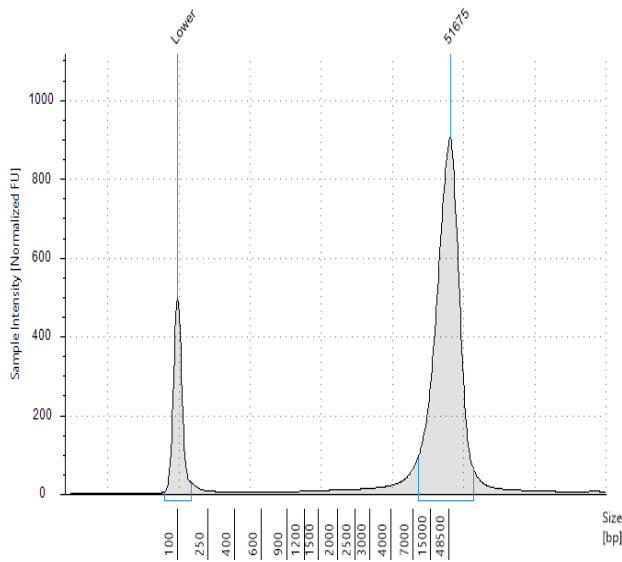


Below are diagrams displaying High Quality DNA, and Highly degraded DNA.

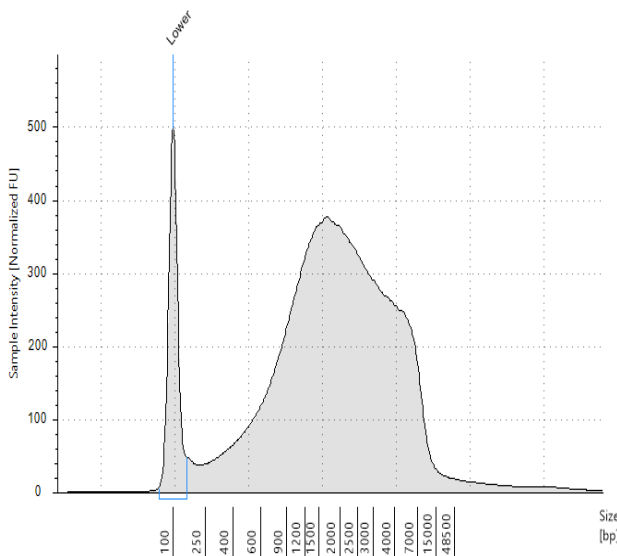
A. Electropherogram showing intact DNA with a DIN of 9.1

Intact DNA is characterized by clear high molecular weight DNA peak above 60.000 bp and low amount of smaller DNA fragments.

Assessing Quality of DNA



B. Electropherogram showing degraded DNA with a DIN of 3.9
The high molecular weight DNA above 60.000 bp is completely degraded into smaller DNA fragments.

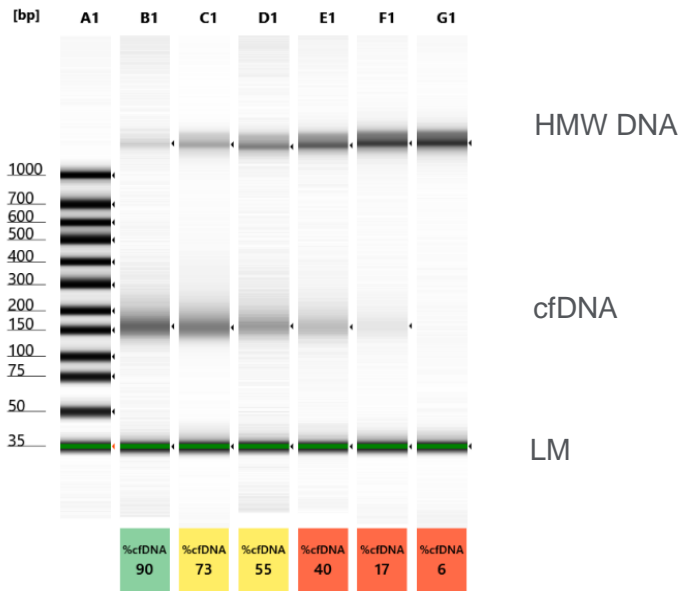


9.2 Appendix B – Interpreting cell-free DNA samples analyzed on a TapeStation system with the %cfDNA

The %cfDNA ranges from 0 for no cell-free DNA to 100% pure cell-free DNA without high molecular weight DNA contamination. The %cfDNA is automatically calculated the amount of the

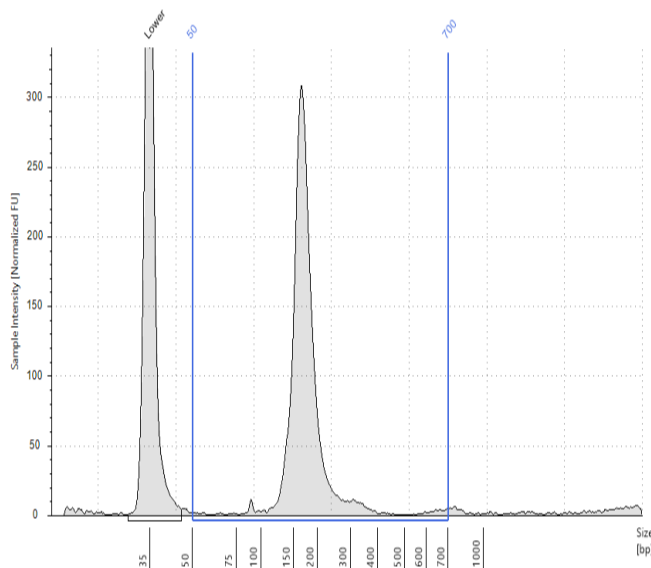
Assessing Quality of DNA

sample between 50 and 700bp and is displayed below the gel image, based on defined thresholds a color coding can be applied.



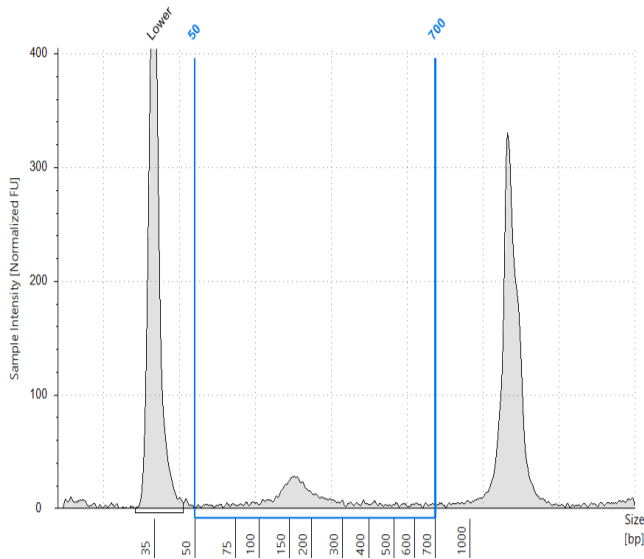
Below are diagrams displaying pure cell-free DNA, and cell-free DNA with an increased amount of high molecular weight DNA.

A. Electropherogram showing pure cell-free DNA with a %cfDNA of 90
 Pure cell-free DNA is characterized by a fragment around 170 bp representing the DNA around one histone (mono nucleosomal DNA). In some cases, additional peaks around 370 and 510 bp can be present representing the DNA around 2 or 3 histones (multi nucleosomal DNA).



Assessing Quality of DNA

B. Electropherogram showing cell-free DNA with a huge amount of high molecular weight DNA contamination and an %cfDNA of 17.
The high molecular weight DNA originating from cells going through apoptosis can contaminate cell-free DNA samples. The high molecular weight DNA is above 800 bp.



10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions