Agilent

Trusted Answers

Automated Nucleic Acid Sample QC in NGS Workflows

Agilent TapeStation systems

Contents

Importance of Nucleic Acid Quality Control in NGS

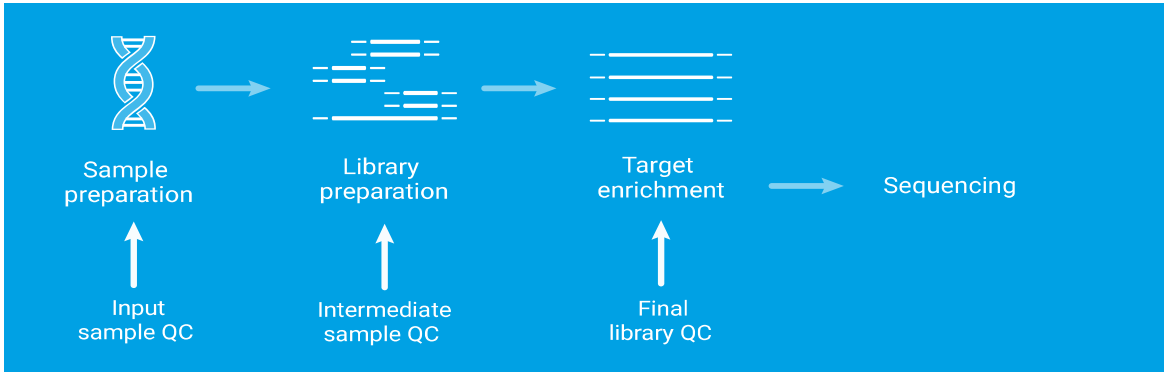
High-quality DNA and RNA samples support strong next-generation sequencing (NGS) results. To help prevent suboptimal sequencing data and avoid wasting time and resources, quality control (QC) steps are recommended at various points in the NGS workflow, from initial sample assessment to final library qualification for NGS library preparation protocols.

This eBook explains how the benefits of the Agilent TapeStation systems and ScreenTape technology offer idal and easy-to-use sample QC solutions for NGS workflows. It also provides scientific application examples and links to additional reading material.

Quality control steps during NGS library preparation

Streamline Your NGS Library Preparation with These Sample QC Steps

- **Input sample QC:** simplify decision-making by tailoring library preparation protocols to your sample concentration and quality
- **Intermediate sample QC:** sample integrity can impact library yield and complexity, so check your sample after fragmentation and adaptor ligation steps
- **Final library QC:** avoid overamplification due to excessive PCR cycles and/or yield and accurately determine library concentration for optimal flow cell loading



Benefits of the TapeStation systems

Ease of use
Fully automated sample processing with ready-to-use ScreenTape consumables for easy sample QC, no matter your sample type

Save time
Get quick answers in as little as one to two minutes per sample will allow you to get to your results faster

Flexibility
Easily switch between DNA and RNA assays with scalable sample throughput (1 - 96 samples) tailored to the needs of your lab

Optimize efficiency
Save money, resources, and optimize your lab's efficiency with constant cost per-sample, minimal intervention, and user-independent results

The Role of Sample Quality Metrics in NGS Library Workflows Preparation

The process of taking a starting nucleic acid sample to a sequencing-ready library can involve multiple purification, ligation, amplification, and/or sizing steps. Building sample QC checkpoints into your NGS workflow helps you ensure each of these steps are optimized and allow you to identify potential paint points with objective quality metrics.

The Agilent TapeStation systems generate these metrics by assigning numerical values for profile variations related to sample quality and integrity. Using these instruments, you can obtain valuable DNA and RNA sample from not just routine, high-quality sample types but also from more challenging specimens, including cell-free DNA and formalin-fixed paraffin-embedded (FFPE) tissue.

The Agilent TapeStation systems offer several sample quality metrics tailored to your specific nucleic acid sample type, including:

DIN

DIN – DNA integrity number

A DNA quality score assigned to gDNA samples on a scale of 1 (strongly degraded) to 10 (highly intact) DNA

%cfDNA

%cfDNA – % Cell-free DNA

The percentage of cfDNA subcomponents, compared to total sample DNA, that is present in the region between 50 and 700 bp. Lower %cfDNA corresponds to greater high-molecular-weight (HMW) DNA contamination

RIN^e

RIN^e – RNA Integrity Number (RIN) equivalent

An RNA quality score calculated on a scale of 1 (strongly degraded) to 10 (highly intact) RNA, equivalent to the RIN used by the 2100 Agilent Bioanalyzer

DV_{200}

DV_{200} – Distribution Value 200

A quality metric for degraded RNA isolated from FFPE tissue, the DV_{200} identifies the percentage of RNA fragments over 200 nucleotides in size. This helps determine the minimal input required for successful library preparation and reproducible results

Application note

Optimization of an Agilent NGS Workflow for the Characterization of NSCLC DNA Samples in a Molecular Diagnostic and Anatomic Pathology Laboratory

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Introduction

The Anatomical Pathology laboratory of the SS Annunziata Hospital in Chieti, Italy, is a reference center for lung cancer. This lab uses NGS methods to characterize formalin-fixed paraffin-embedded (FFPE) and plasma samples with the aim of identifying gene aberrations that are the targets of new drugs. Thirty-nine previously characterized samples from 37 patients with advanced non-small cell lung cancer (NSCLC) were employed in this study, consisting of 2 plasma and 37 FFPE samples (three of which belonged to the same patient). The samples were characterized using a complete Agilent workflow, which included the Agilent 4200 TapeStation system for sample quality control of gDNA, cfDNA, and NGS libraries prior to sequencing. The data obtained from their work demonstrated the feasibility and advantages of introducing this Agilent workflow into their routine practice.

Results

- Identified a range of DNA integrity in FFPE-extracted NSCLC gDNA (DIN = 1.7 to 8) and plasma-derived cfDNA samples (%cfDNA = 70 to 72%) analyzed on the 4200 TapeStation system
- Showed that NGS library recovery did not necessarily correlate with starting input sample mass (likely due to variable sample integrity)
- Found a positive correlation between DIN value of a sample and the number of on-target reads
- Demonstrated the reliability and accuracy of an NGS workflow, powered by the Agilent MagnisDx NGS Prep system and Agilent SureSelect Cancer All-In-One Lung panel, which features a three-day turnaround time for characterizing difficult samples

The Benefits of Sample QC

cfDNA quantification and qualification enabled the Anatomical Pathology laboratory to optimize their workflow for the detection of somatic variants in lung tumor samples, including several clinically relevant variants that were difficult to detect.

Application note

Quality Control of Cell-Free DNA Samples Analyzed with Next-Generation Sequencing

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Introduction

Next-generation sequencing (NGS) is an essential tool for analyzing biosamples in clinical research. Cell-free DNA (cfDNA) has become an important sample type for cancer research and, therefore, it is important that robust NGS results can be obtained from cfDNA. However, cfDNA analyses can be challenging due to its low concentration and possible contamination from high-molecular weight (HMW) DNA, both necessitating reliable quality control. The Agilent 4200 TapeStation system is a vital quality control (QC) tool in NGS workflows. The TapeStation systems and Agilent Cell-free DNA ScreenTape assay provide a %cfDNA quality metric for determining the quality of input cfDNA for downstream processes. The Sample Processing Lab (SPL) collaborated with the Genomics and Proteomics Core Facility (GPCF), both part of the German Cancer Research Center (Deutsches Krebsforschungzentrum, DKFZ) to track 13 cfDNA samples with the TapeStation system from initial QC with the %cfDNA metric, through the NGS workflow, to the final sequencing results. All 13 samples reported a %cfDNA greater than 83% with successful library preparation and sequencing metrics.

Results

- The cfDNA ScreenTape assay confirmed consistent sizing of the extracted cfDNA corresponding to mono-, di, and tri-nucleosome peaks
- Researchers determined relatively high cfDNA purity (%cfDNA ranging from 83 to 97% of total extracted cfDNA) with minimal HMW contamination from their current extraction protocol
- cfDNA quantification helped researchers optimize PCR cycles during library construction based on total input amount
- Researchers were able to confirm size distribution of final NGS libraries with all samples falling within the 200 to 400 bp size QC threshold

The Benefits of Sample QC

The SPL is currently optimizing cfDNA extraction protocols from liquid biopsies. By demonstrating consistent sizing, cfDNA concentration, and minimal HMW contamination, sample QC helps ensure optimal extraction procedures that increase reliability and success in downstream sequencing applications.

Application note

Quality Control of Magnis SureSelect XT HS Workflow with Agilent Automated Electrophoresis Solutions

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Introduction

The Agilent 4150 TapeStation system performs reliable sample quality control (QC) through various steps of the Agilent SureSelect XT HS target enrichment and next-generation sequencing (NGS) library preparation workflow on the Agilent Magnis NGS prep system. The workflow includes QC steps for the incoming samples, intermediate products during library preparation, and final libraries. The performance and applicability of the 4150 TapeStation system in qualification and quantification of libraries at different QC steps utilizing the Agilent TapeStation DNA ScreenTape assays is reviewed. The TapeStation DNA ScreenTape assays provided comparable results to the equivalent assays of the Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems. Final library QC metrics were correlated to the outcome of QC metrics from sequencing data.

Results

- Sample QC at input, precapture library, and postcapture library steps demonstrates high sample reproducibility of many sample types on the Magnis NGS Prep system
- Direct comparison of the 4150 TapeStation system to the 2100 Bioanalyzer and 5200 Fragment Analyzer systems shows comparable performance between all three automated electrophoresis instruments
- Integration of the TapeStation system with the Magnis NGS Prep system allows monitoring and optimization at every step of the sample QC process

The Benefits of Sample QC

Using the 4150 TapeStation system, researchers demonstrated that the Magnis NGS Prep system provided reproducibility on par with manual preps, offering a reliable means for automated library preparation.

TapeStation Interactive Lab for Sample Quality Control

If you would like to learn more about the TapeStation systems, ScreenTape technology, and other applications, please visit the TapeStation interactive lab with exclusive videos, literature, and much more information.