Market-Ready Cell Line-derived cfDNA Reference Materials for Liquid Biopsy Quality Assurance

Kirsten Simon[1], Carsten Uhlig[5], Martin Jähne[2], Steffen Uhlig[4], Stefan Holdenrieder[5], Abel Bronkhorst[3]
1 - *QD Labs LP (Delaware, USA)*, 2 - *new diagnostics GmbH (Dresden, Germany)*, 3 - *CEBIO GmbH-Center of Evaluation of Biomarker (Munich, Germany)*,
4 - *QuoData GmbH (Berlin, Germany)*, 5 - *DHM German Heart Center Munich (Munich, Germany)*

Overview

Purpose:
Authentic cfDNA reference materials from various cancer and non-cancer cell lines with biologically relevant characteristics for internal and external quality control to ensure reliable data and clinical decision-making

Methods:
Mammalian cell cultivation techniques, DNA extraction, cfDNA quantification, sizing and characterization, dPCR, NGS, quality control

Results:
Establishment of processes and workflows compliant to ISO 17034:2016 [1] to produce mono-nucleosomal wild-type cfDNA from HMEC-1 cells

Introduction

The expanding liquid biopsy market demands reliable reference materials for cfDNA analysis. Current commercial solutions face limitations in authenticity and supply consistency. An ISO 17034:2016 compliant workflow generates characterized cfDNA reference materials through optimized cell line processing which can be used for interlaboratory comparisons, for method validation and for assessing laboratory proficiency within the scope of liquid biopsies. Quality assessment includes DNA quantification and size distribution analysis in the first stage. This strategic collaboration between QuoData's validation expertise and clinical diagnostics experience delivers a robust solution for liquid biopsy quality assurance, addressing critical market needs.

Methods

- Production of first batches of mono-nucleosomal (167 bp) wild-type cfDNA using HMEC-1 cells.
- cfDNA quantification using Invitrogen Qubit 4 Fluorometer. Characterization of cfDNA with Agilent Bioanalyzer 2100 and Agilent TapeStation.
- Assessment of homogeneity and stability for the individual cfDNA batches following the experimental design from ISO 13528:2022 [2].
- Internal and external calibration and testing procedures for equipment and devices for compliance with the relevant requirements of ISO 17025:2017 [3] to ensure accuracy of results.

Workflows

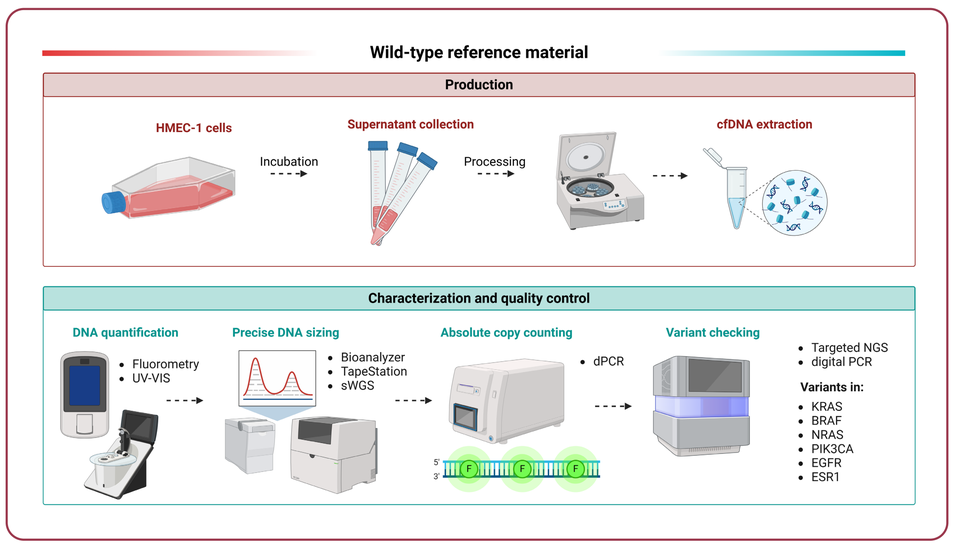

***Figure 1:** Workflow to produce wild-type cfDNA, mono-nucleosomal (~167 bp) from HMEC-1 cells including characterization and quality control.*

- Establishment of an exemplary production workflow for the extraction of cfDNA from HMEC-1 cells with subsequent characterization.
- Determination of cfDNA concentration using fluorometry and size cfDNA using Bioanalyzer Agilent 2100 and TapeStation.
- Application of further quality control measures with dPCR and targeted NGS for cancer cell lines carrying various SNVs.

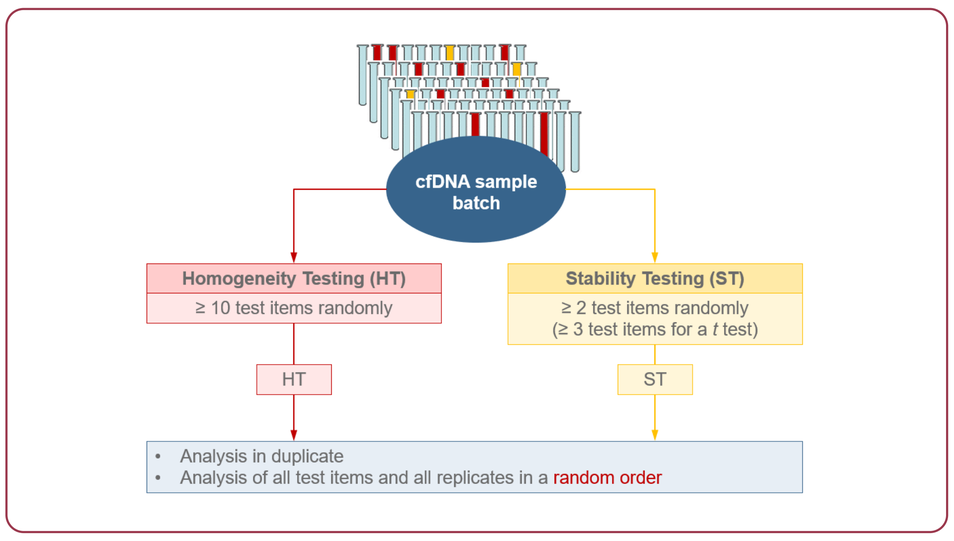

***Figure 2:** Experimental design for homogeneity and stability testing for cfDNA sample batches.*

- H&S testing of cfDNA concentration and size is accomplished following the experimental design from ISO 13528:2022 for each sample batch.
- Additional stability testing includes various shipment and storage conditions.
- Assessment of homogeneity and stability using QuoData's proven software PROLab.

Conclusions

1. First establishment of cell line cultivation and analytical workflows to produce wild-type cfDNA reference material with authentic biological signatures within a quality-controlled environment.
2. Processes compliant to ISO 17034:2016 make the reference material ready for the use for e.g., in proficiency testing, in-house method validation and routine quality assurance.
3. Established processes and quality control measures can be transferred to the production of cfDNA from various cancer and non-cancer cell lines.

Literature

[1] International Standardization Organisation [ISO], 2016, (ISO 17034:2016)
[2] International Standardization Organisation [ISO], 2022, (ISO 13528:2022)
[3] International Standardization Organisation [ISO], 2017, (ISO 17025:2017)

www.cebio-med.de
www.new-diagnostics.com
www.quodata.de
www.deutsches-herzzentrum-muenchen.de
liquid-biopsy.qd-labs.com