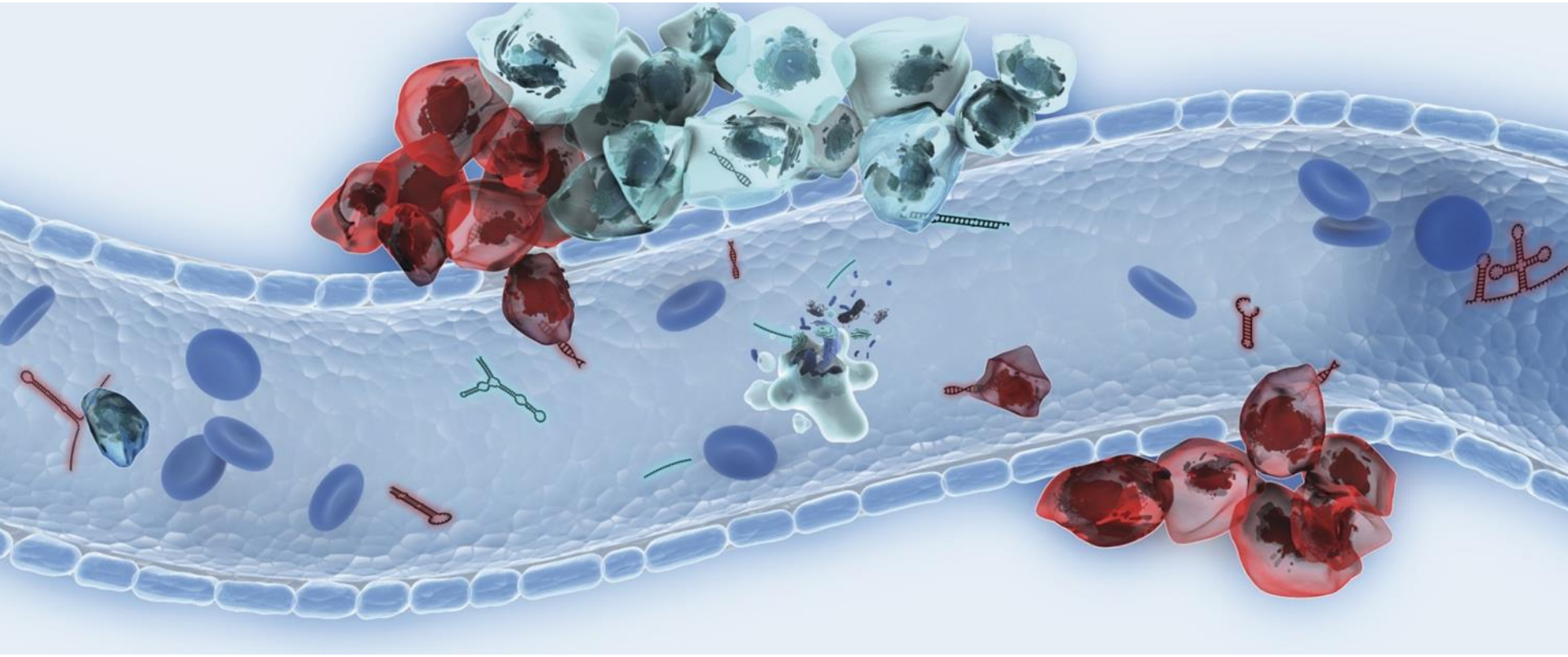

Circulating Cell-Free DNA Pre-analytics: Importance of ccfDNA Stabilization and Extraction for Liquid Biopsy Applications

Martin Schlumpberger, Ass.Director R&D, QIAGEN GmbH

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Call for Workflow Standardization in routine healthcare

- Diagnostic errors cause about 10% of all patient deaths and about 17% of adverse events

  Institute of Medicine (IOM) Report Sept. 2015

- The pre-analytical phase accounts for 46% to 68% of such errors observed during the total testing process

  Medical Laboratory Observer, May 2014

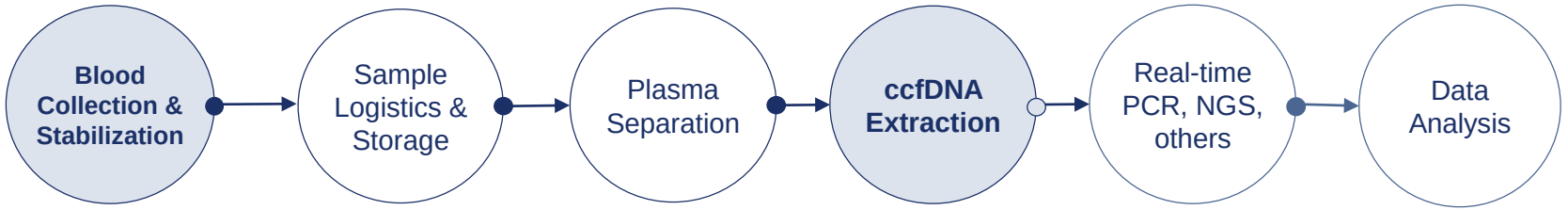
SPIDIA & SPIDIA4P: Standardization and Improvement of Pre-analytical Procedures for *in vitro* Diagnostics *EU FP7-HEALTH (GA. no. 222916) & EU HORIZON 2020 (GA. no. 733112)*

- QIAGEN coordinating both initiatives (16 & 19 Partners)
- 22 CEN Technical Specifications and ISO Standards planned or developed (highly consensus-driven international and European processes)
- E.g., Specifications for pre-examination processes for venous whole blood in Molecular in vitro diagnostic examinations
  - Part 3: Isolated circulating cell-free DNA from plasma CEN/TS 16835-3: 2015

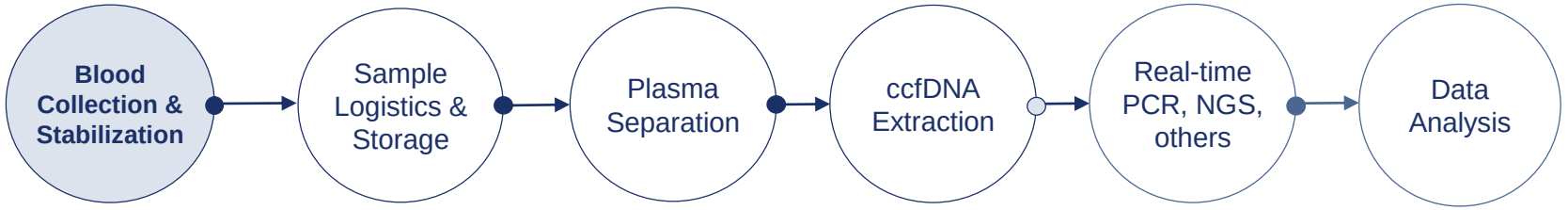
Circulating cell-free DNA: Workflow Challenges

- Usually low concentrations in plasma, serum, urine and other body fluids (1-50 ng DNA/ ml plasma)
- Highly fragmented (<500bp)
- Longer fragments (≥ 500bp) – possible from necrotic processes
- Background DNA can be caused through cell lysis

- **Avoid release of cellular nucleic acids (gDNA)**
- **Enable highly efficient large-volume nucleic acid extraction**
- **Avoid fragment size bias to improve sensitivity**



PAXgene Blood ccfDNA Tube (RUO)* Features



Unique stabilization of extracellular levels of ccfDNA

- Effective stabilization at RT minimizes background gDNA and maximizes ccfDNA yield from plasma
  - White blood cells – helps prevent release of gDNA
  - Red blood cells – helps minimize hemolysis
- Non-crosslinking NA preservation – no DNA modification

BD Vacutainer® plastic tube with BD Hemogard™ safety closure

- Helps minimize risk of tube breakage
- Enhanced safety for healthcare and lab personnel
- Helps minimize contamination between samples
- Provides consistent blood draw volume

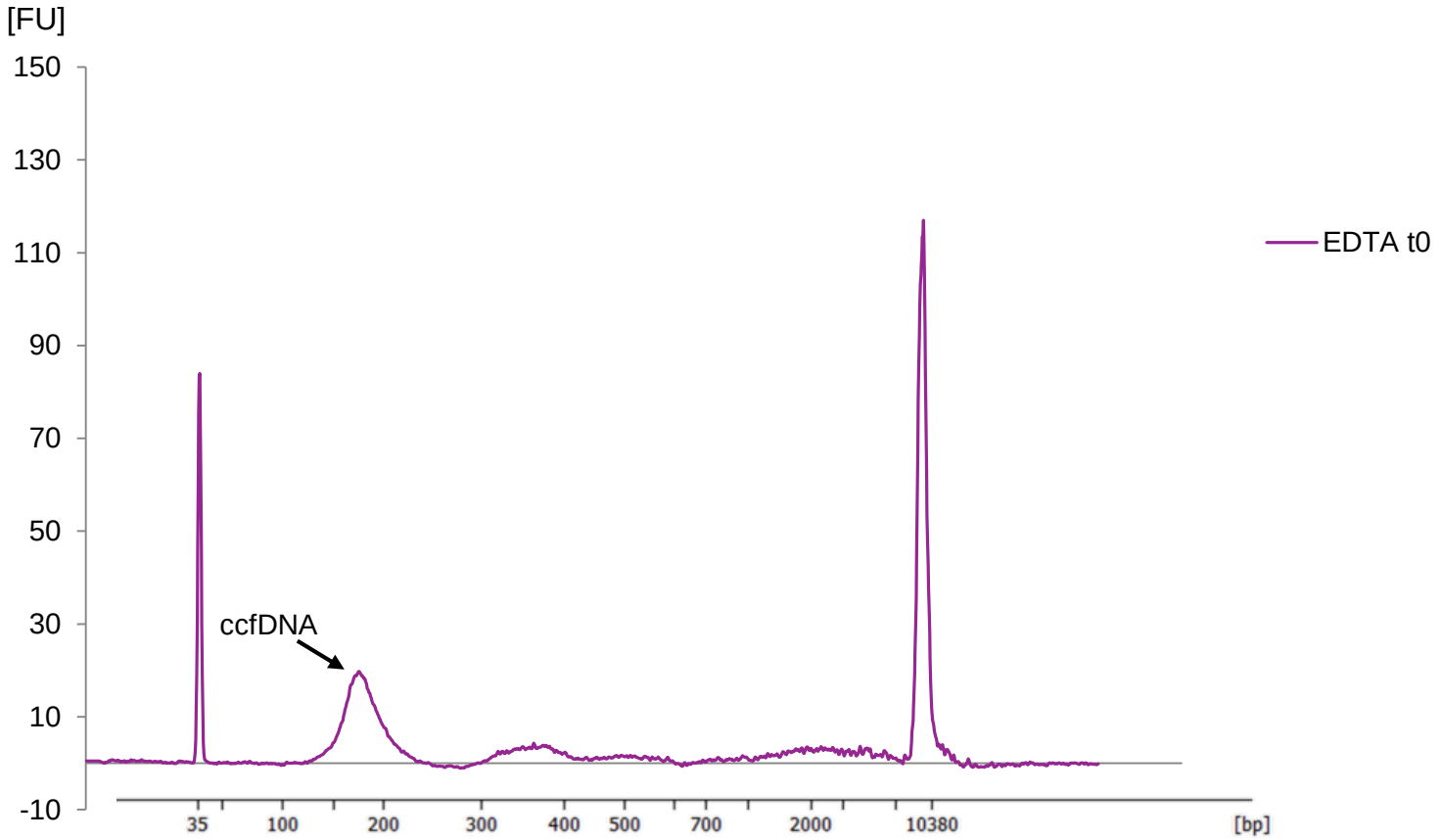
Integrated pre-analytical workflow

- Seamless integration into manual or automated prep with QIAamp® and QIAsymphony® circulating DNA extraction technology

* For Research Use Only. Not for use in diagnostic procedures.

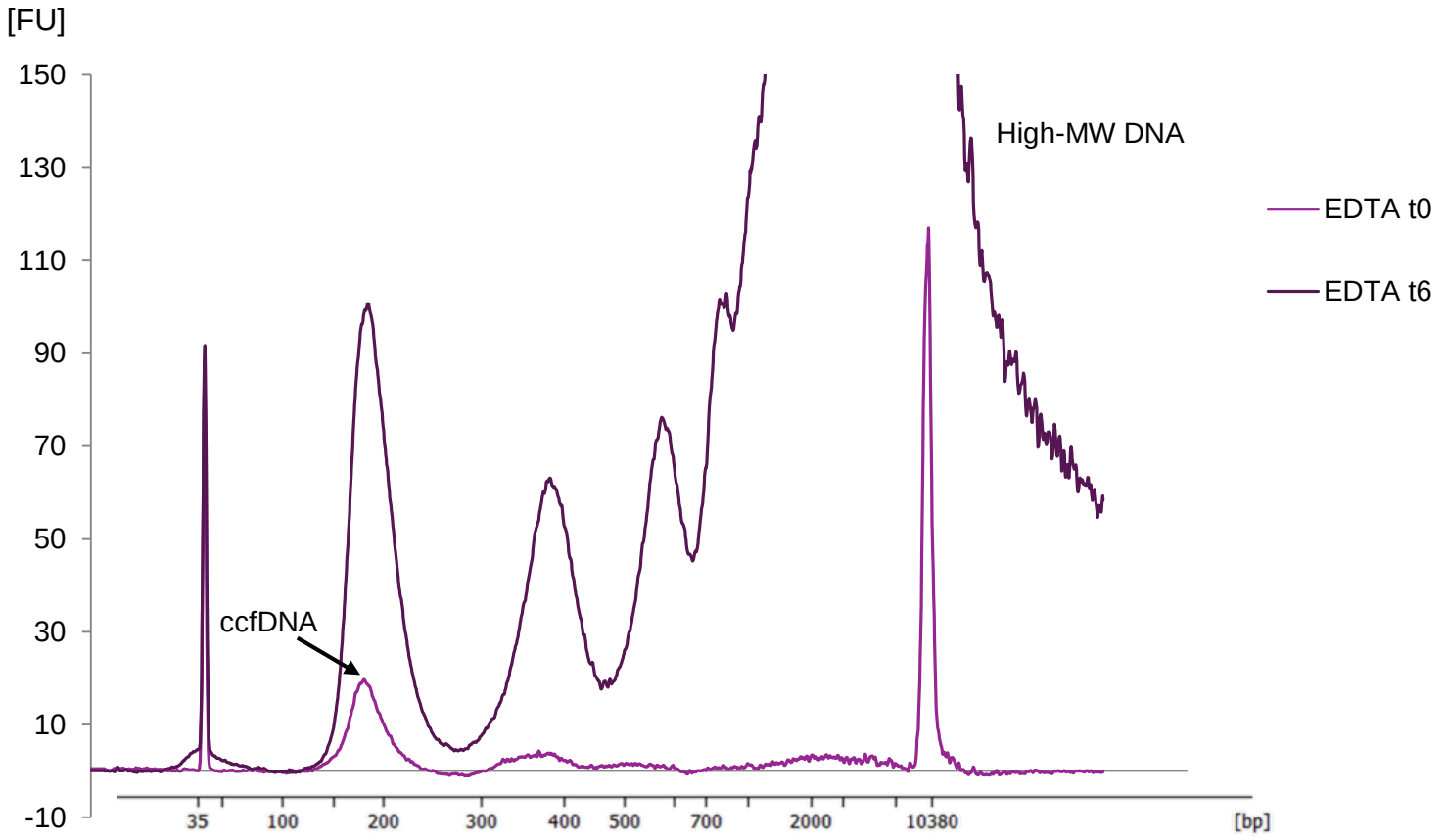
Problem: Need for Stabilization of ccfDNA

- ccfDNA fragment size profile from healthy donor



ccfDNA was extracted from EDTA plasma of 1 subject directly after blood draw (t0). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

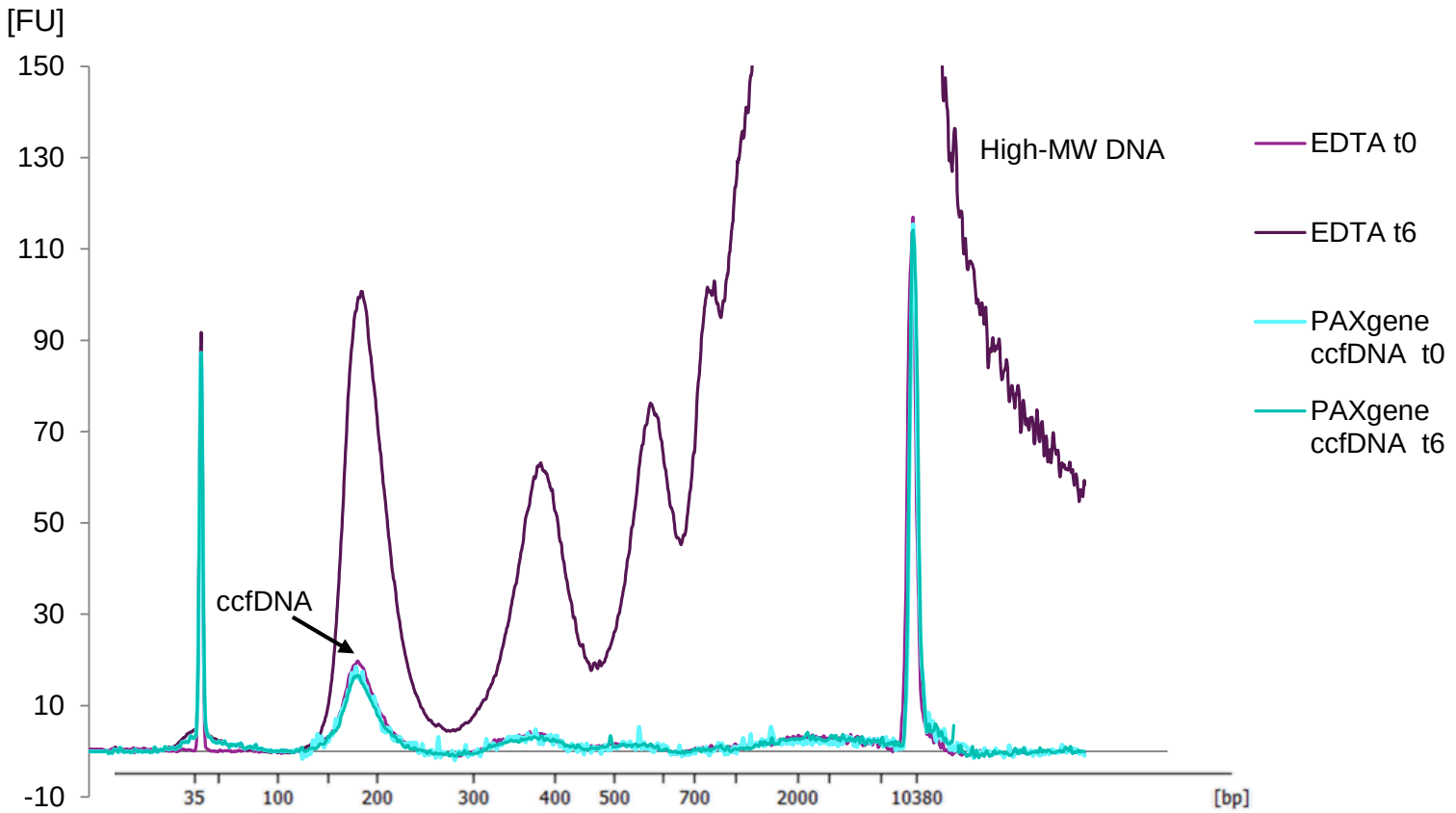
Apoptosis of white blood cells leads to dilution of naturally occuring ccfDNA



ccfDNA was extracted from EDTA plasma of 1 subject directly after blood draw (t0) and after 6 days at room temperature (t6). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

Solution: Prevent Blood Cell Lysis and Release of Genomic DNA

- PAXgene® Blood ccfDNA Tubes (RUO)* help prevent release of gDNA into plasma



ccfDNA was extracted from EDTA and PAXgene plasma of 1 subject directly after blood draw (t0) and after 6 days at room temperature (t6). 1 µl eluate was analyzed using the Agilent® High Sensitivity DNA Kit.

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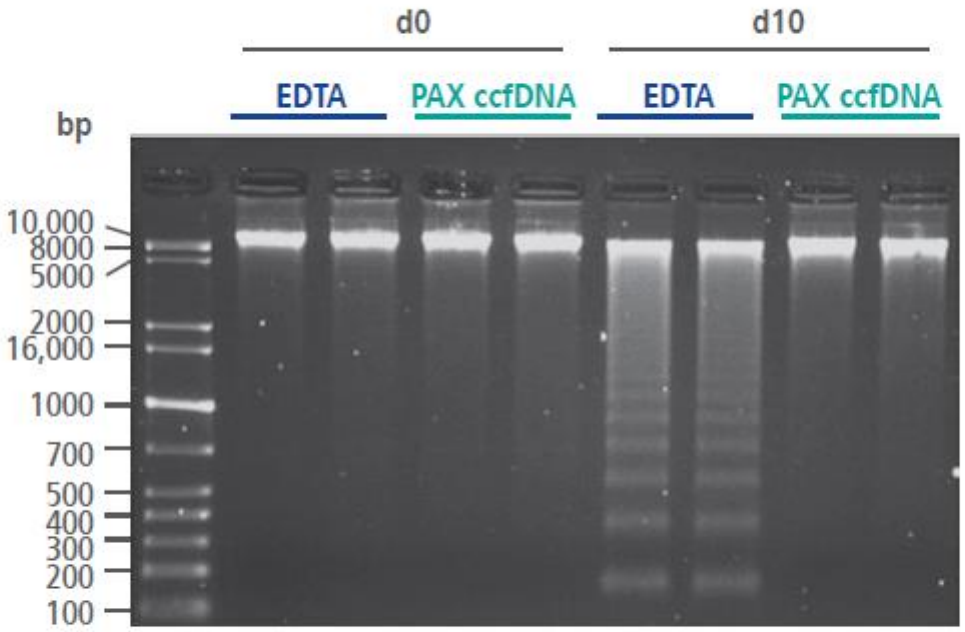
Blood cell stabilization prevents release of fragmented gDNA

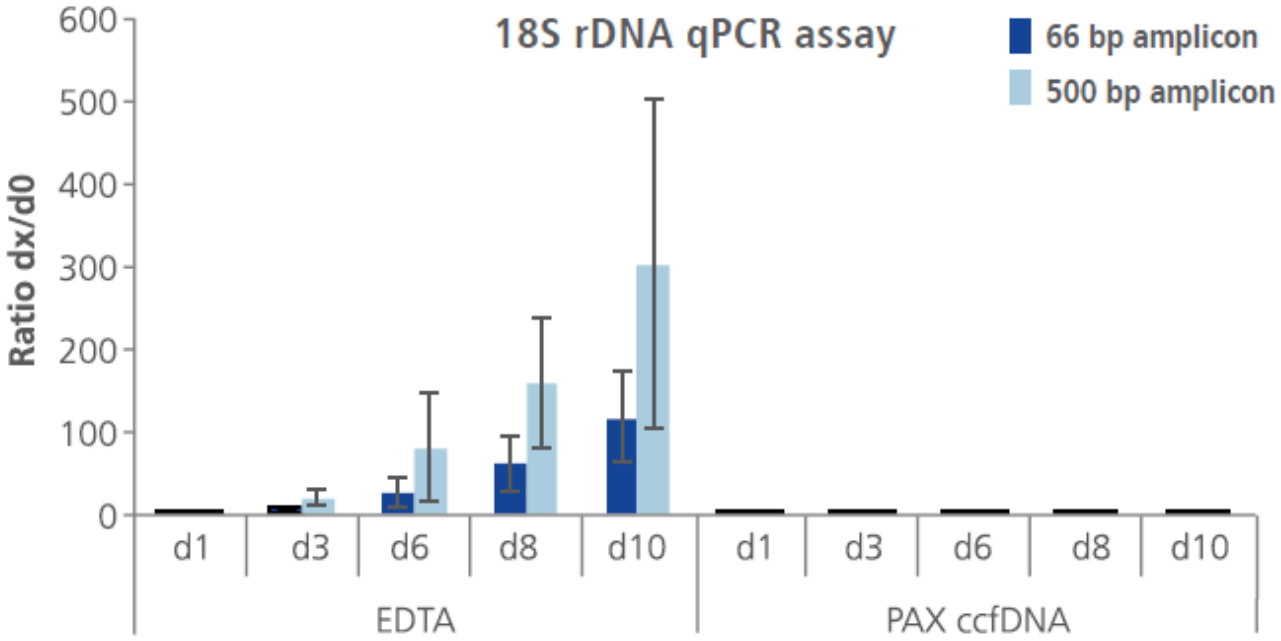
Stabilization of Blood Cells and Prevention of Apoptosis



PAXgene Blood ccfDNA stabilization helps prevent apoptosis of blood cells and fragmentation of genomic DNA. Genomic DNA was extracted from whole blood of 2 subjects using the QIAamp Blood Mini Kit. 400 ng DNA were separated by agarose gel electrophoresis.

Stabilization of whole blood and ccfDNA during transport and storage

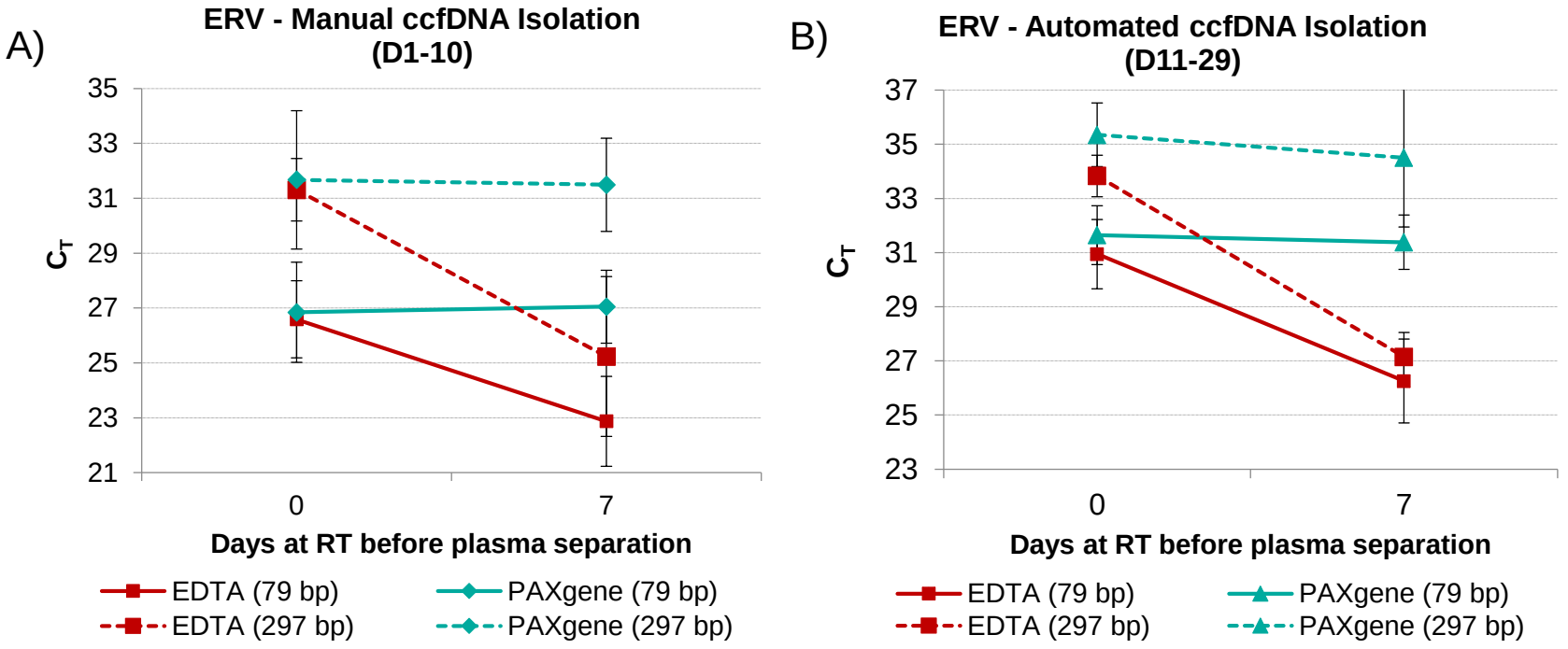
Stabilization of Whole Blood at Room Temperature with No Increase in ccfDNA Levels



PAXgene Blood ccfDNA stabilization helps prevent release of genomic DNA from white blood cells. Plasma was extracted from whole blood of 6 subjects; ccfDNA was isolated and yield was quantified by real-time PCR (18S rDNA gene, 66 bp/500 bp amplicon).

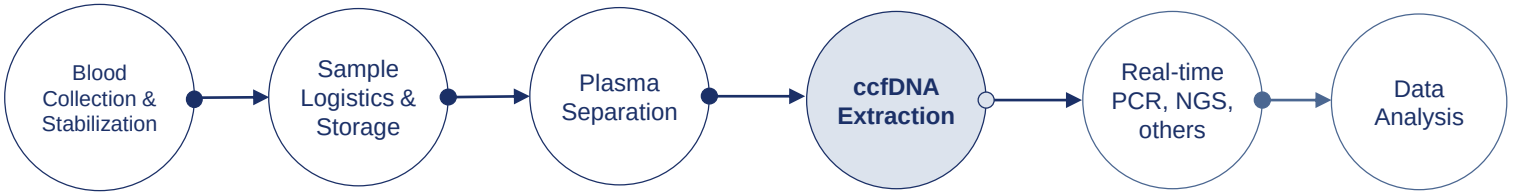
mSHOX2 lung cancer: methylation specific therapy monitoring marker

- Blood from 29 consented lung cancer patients under treatment
- Collected in EDTA and PAXgene Blood ccfDNA Tubes
- Plasma processing immediately or after 7 days storage at RT
- ccfDNA quantified by real-time PCR with ERV (endogenous retrovirus) sequence



Change of target C_T over storage time for ccfDNA from plasma generated from EDTA and PAXgene Blood ccfDNA Tubes. Real-time PCR assays amplifying 2 fragments of the single copy ERV sequence were used to measure DNA content of original eluates after manual (A) and automated (B) ccfDNA isolation. **Data courtesy of Dr. Fleischhacker, UKH Halle/Saale**

Circulating Cell-Free DNA — Preanalytical Workflows



Automated	PAXgene Blood ccfDNA Tubes (RUO)*	1–10 ml Plasma Input	QIAamp MinElute ccfDNA Mini & Midi Kit (MBA)† NEW
Automated	PAXgene Blood ccfDNA Tubes (RUO)	1–10 ml Plasma	EZ1® ccfDNA Mini & Midi Kit (MBA) † NEW
Manual	PAXgene Blood ccfDNA Tubes (RUO)	2.4 or 4.8 ml Plasma	QIAsymphony PAXgene Blood ccfDNA (RUO)*

Integrated Workflow
No protocol modifications or pretreatments. Optimized results.

Complete preanalytical workflow solutions from QIAGEN & PreAnalytiX A QIAGEN / BD Company

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‡ Intended for in vitro diagnostic use.

Optimized extraction: QIAsymphony PAXgene Blood ccfDNA Kit (RUO)*

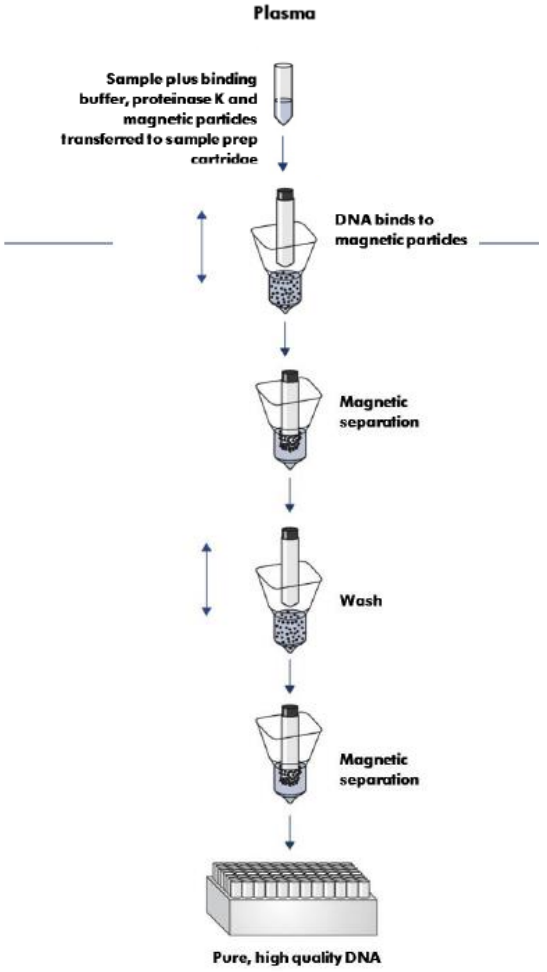
PAXgene Blood ccfDNA Tubes (RUO)	2.4 or 4.8 ml Plasma	QIAsymphony PAXgene Blood ccfDNA (RUO)

Dedicated isolation technology works to streamline and maximize ccfDNA recovery

- **Optimized binding chemistry** for use with PAXgene ccfDNA Tube reagent
- **Optimized input volumes** to accomodate higher volume plasma
- Optional custom protocols for **primary tube handling**

Two protocol lines

- Standard protocol **for small fragment isolation** (≤500 bp)
- Large fragment protocols enable **co-isolation of large fragments** (>500 bp) with flexible elution volume (60, 100, 150 µL)



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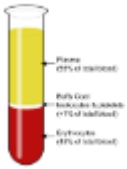
Procedure

10 ml blood drawn into PAXgene ccfDNA tube



Centrifugation to isolate plasma

Option A

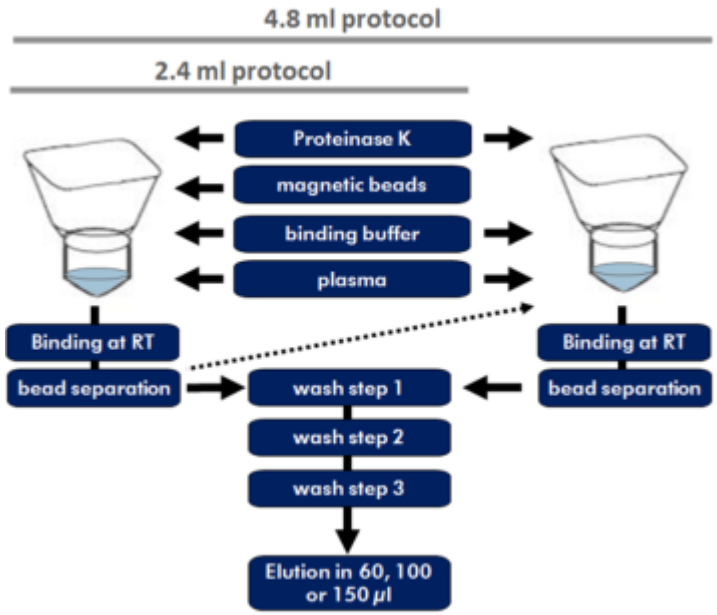
Transfer plasma to new tube

(Optional: repeat centrifugation & plasma transfer to new tube)

Option B (Primary Tube Processing)

Plasma loaded onto QIAsymphony SP to begin automated ccfDNA isolation

ccfDNA for use in NGS, PCR, or other



ccfDNA Extraction: QIAamp MinElute® and EZ1® ccfDNA Kits

Pre- enrichment

Scalable input volume: 1-10 ml

Magnetic bead Technology - no vacuum

Bind ccfDNA

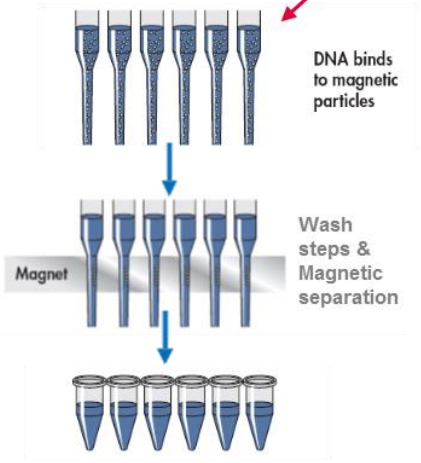
Separate beads on magnet rack

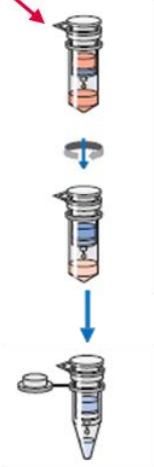
Bead elution in 200 µl

Separate and remove beads

EZ1 ccfDNA Kits on EZ1 Advanced XL or QIAamp MinElute ccfDNA Kits manual/QIAcube

Clean-up





Bind to MinElute column

Wash

Elute ccfDNA

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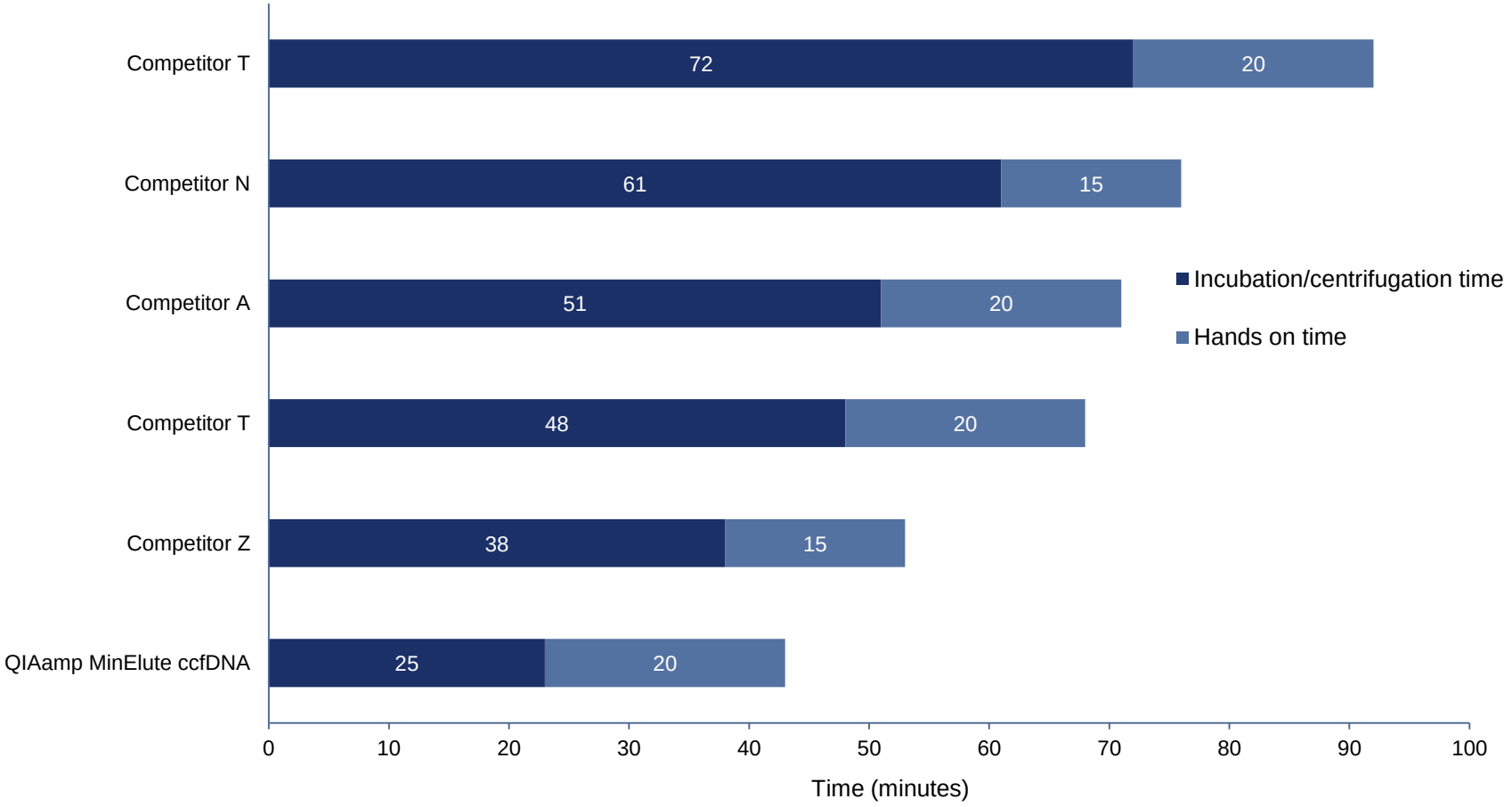
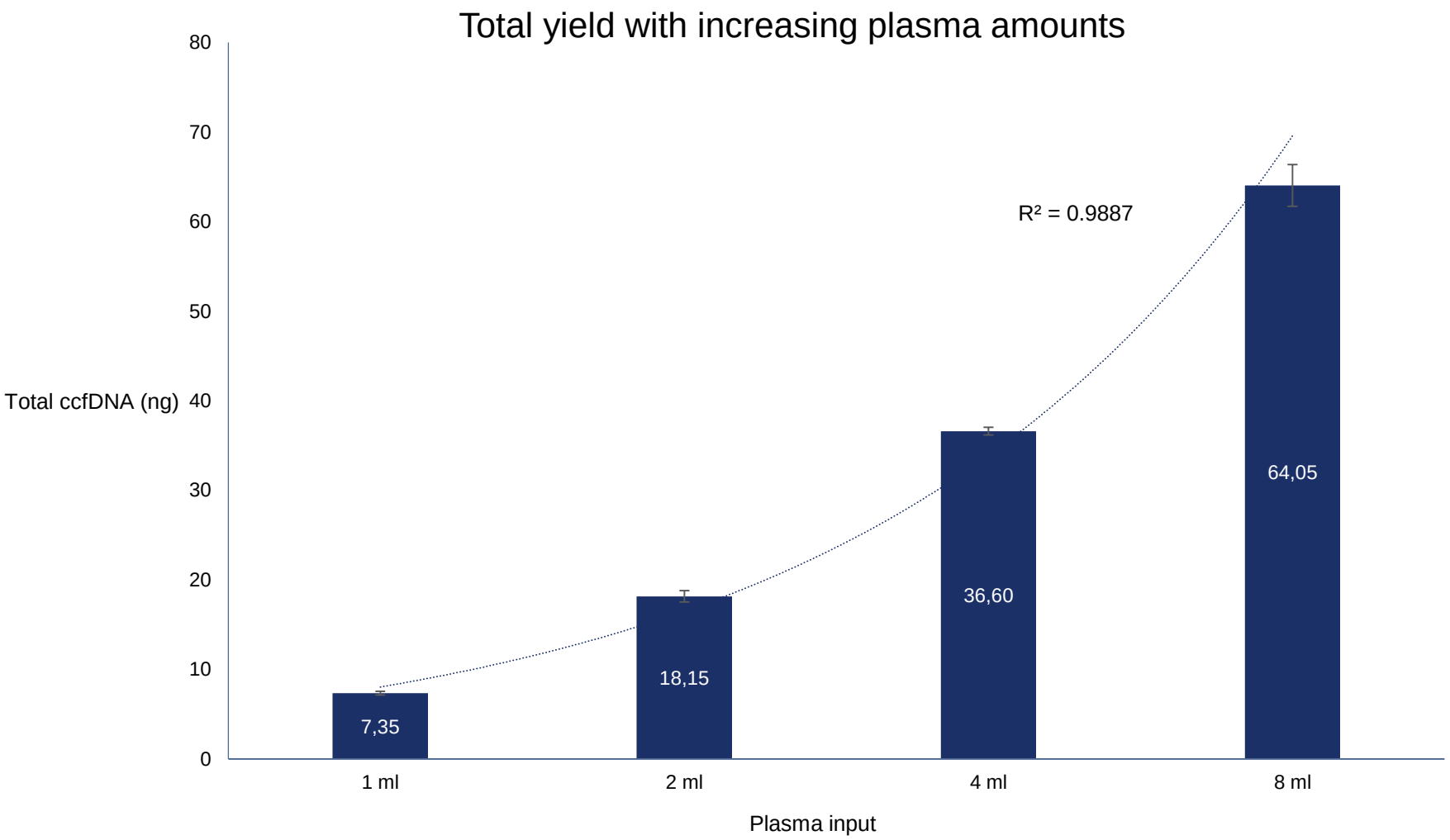
Fast and convenient protocol – Manual solution

Sample processing time – 4 samples (4 ml plasma)



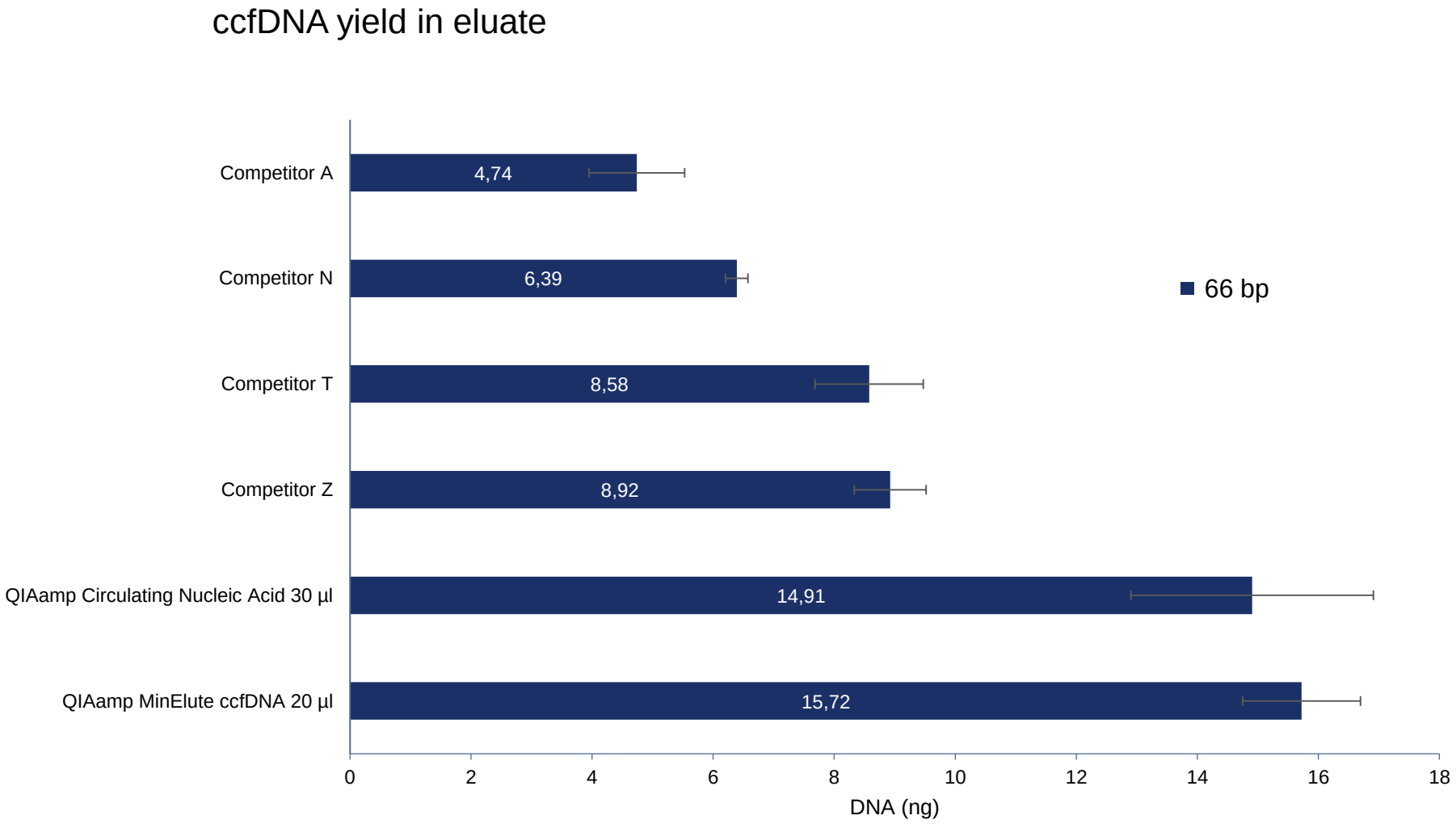
QIAamp MinElute ccfDNA Kit accelerates your ccfDNA extraction.

Scalable sample input volume



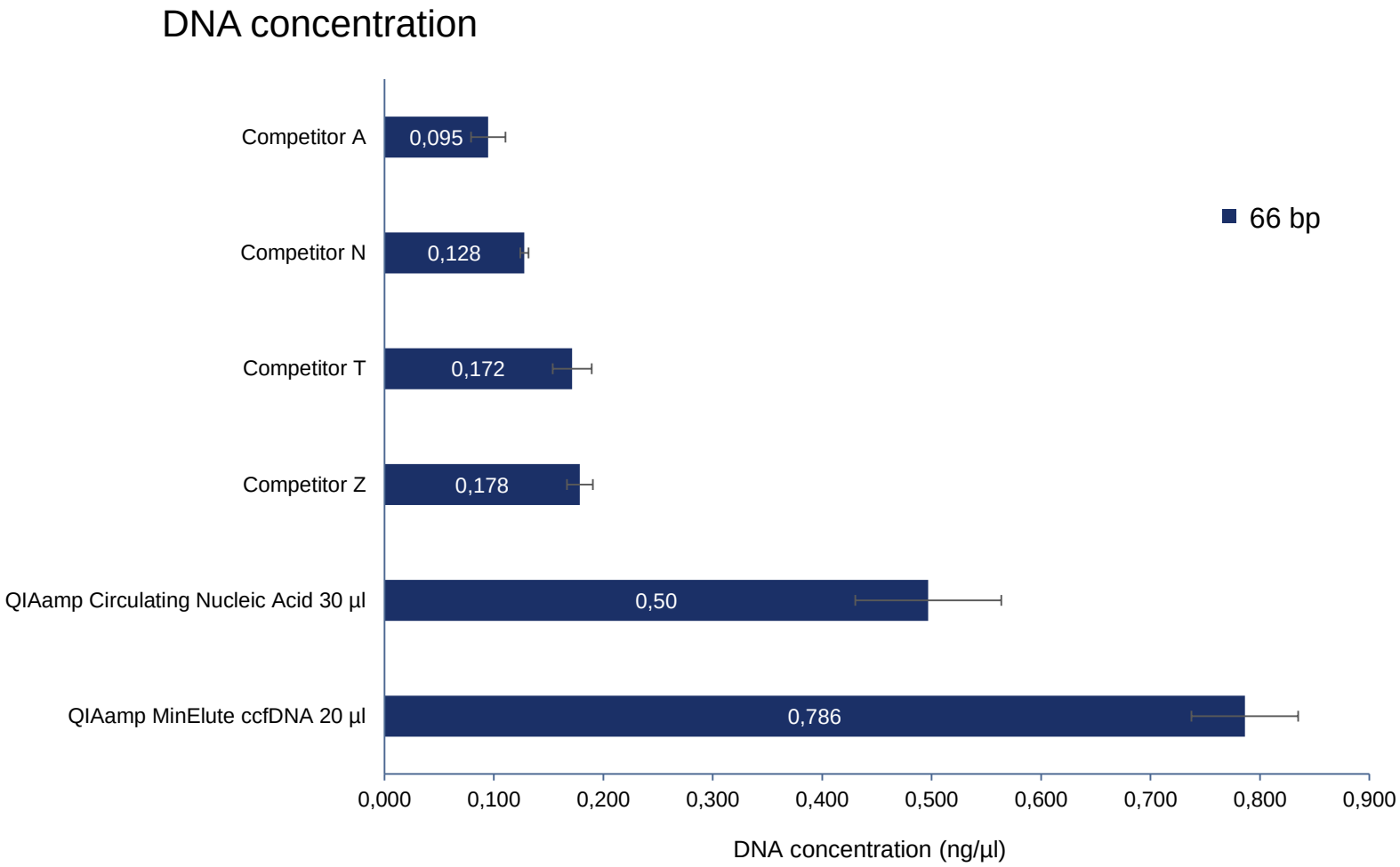
10 ml or higher plasma input is possible.

Efficient recovery of ccfDNA – Highest yields



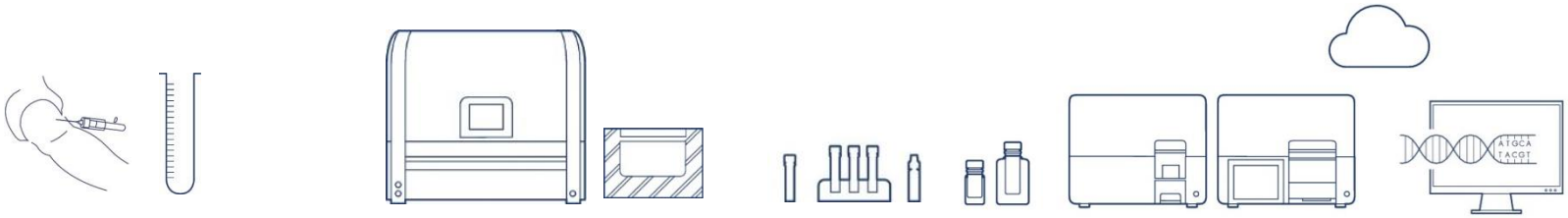
Highest DNA yield ensuring detection of low frequency biomarkers.

Highest concentrations of ccfDNA



Highest DNA concentration ensuring best performance in NGS analysis.

Circulating cell-free DNA NGS workflow



Blood collection and stabilization	ccfDNA extraction	NGS library preparation	Data analysis	Interpretation
• **PAXgene Blood ccfDNA Tubes**	• **QIAamp MinElute ccfDNA Kits** • **EZ1 ccfDNA Kits** • **QIAsymphony** • QIAcube • EZ1 Advanced XL	• QIAseq Targeted DNA Panels • QIAseq Library Preparation Kits	• CLC Genomics Workbench	• Ingenuity Variant Analysis

Thank You!