High-Quality Nucleic Acid Extraction from Challenging Oncology Samples

Chi Hoang

Application Specialist

Agenda

Overview: Cancer and Cancer Genomics

FFPE: Challenges and Solutions

Liquid Biopsy: Challenges and Solutions

Publications

Agenda

Overview: Cancer and Cancer Genomics

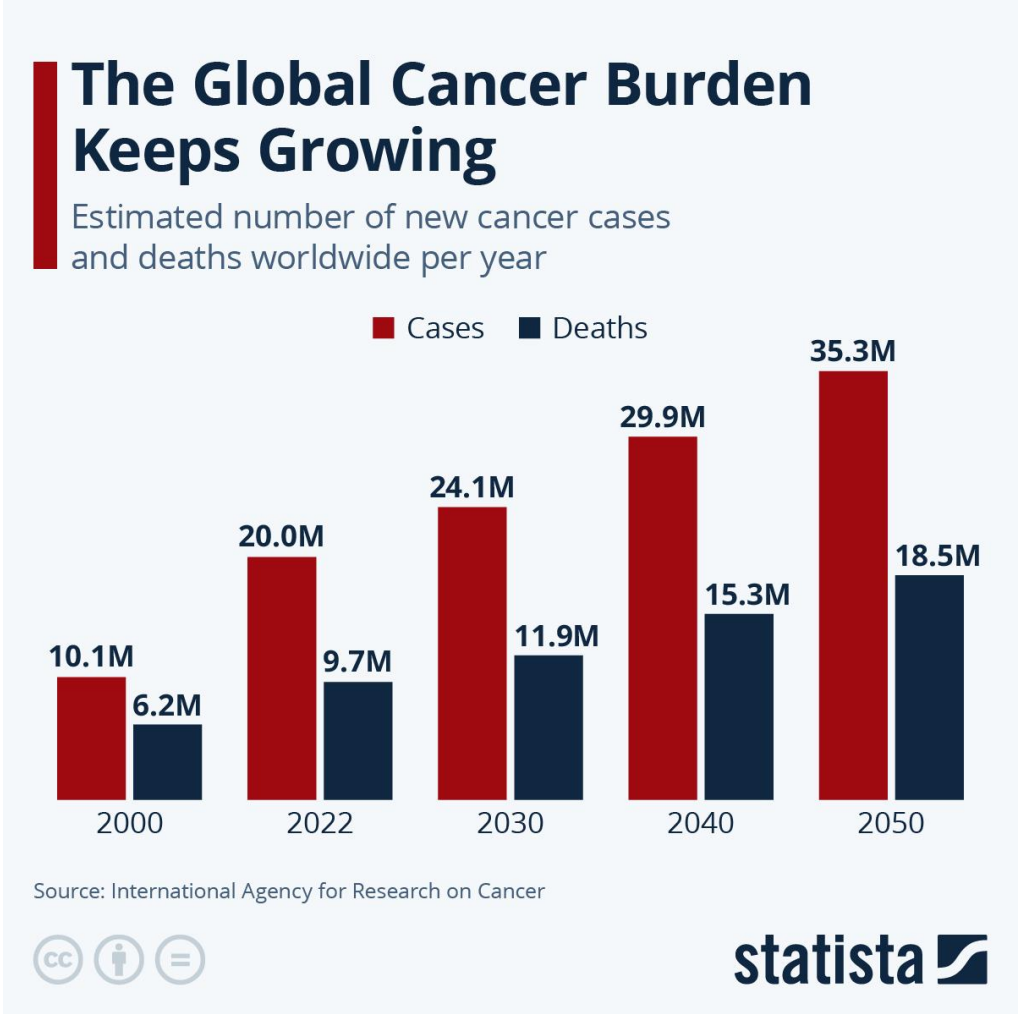
FFPE: Challenges and Solutions

Liquid Biopsy: Challenges and Solutions

Publications

What is Cancer ?

- Cancer is a disease caused by uncontrolled cell growth and division
- It can originate in almost any tissue or organ and may spread (metastasize) to other parts of the body.
- 5-10% are caused by Inherited genomic variants (germline mutations), and most of them are caused by acquired genomic variants (somatic mutations)



Cancer Genomics

- Cancer genomics is the study of the totality of DNA sequence and gene expression differences between tumour cells and normal host cells

- Helping clinicians:

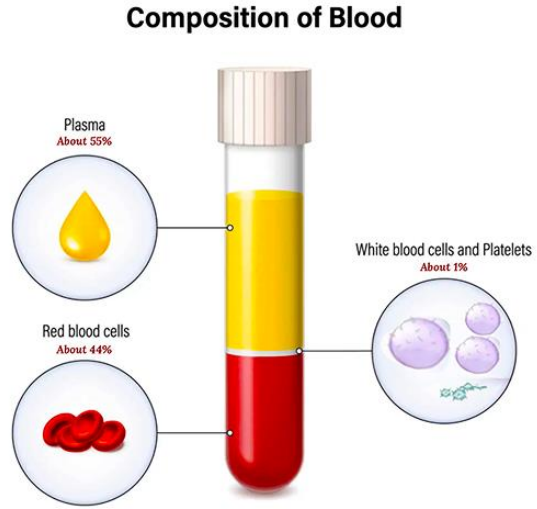
Cancer Screening for Early Detection	Diagnosis / Prognosis	Targeted Treatment	Monitoring treatment response and recurrence (MRD)
RB1 for Retinoblastoma	***BCR/ABL*** *for CML*	**c-KIT, PDGFRA, BRAF for** Gastrointestinal Stromal Tumor	**PML/RARA t(15;17)** for APL

Cancer Genomics

- In recent years, the combination of next-generation sequencing (NGS) and advanced computational data analysis approaches has revolutionised our understanding of the genomic underpinnings of cancer development and progression.

Typical Oncology Sample Types

- **FFPE tissues:** Common in Vietnam hospitals for biopsy archiving, often degraded
- Fresh frozen tissues: Used in research and specialized centers
- **Liquid Biopsy (plasma, serum, urine):** Gaining traction for non-invasive liquid biopsies
- Bone marrow aspirates: Frequently used in leukemia diagnostics
- Fine-needle aspirates: Common in field-based or resource-limited cancer screenings



Agenda

Overview: Cancer and Cancer Genomics

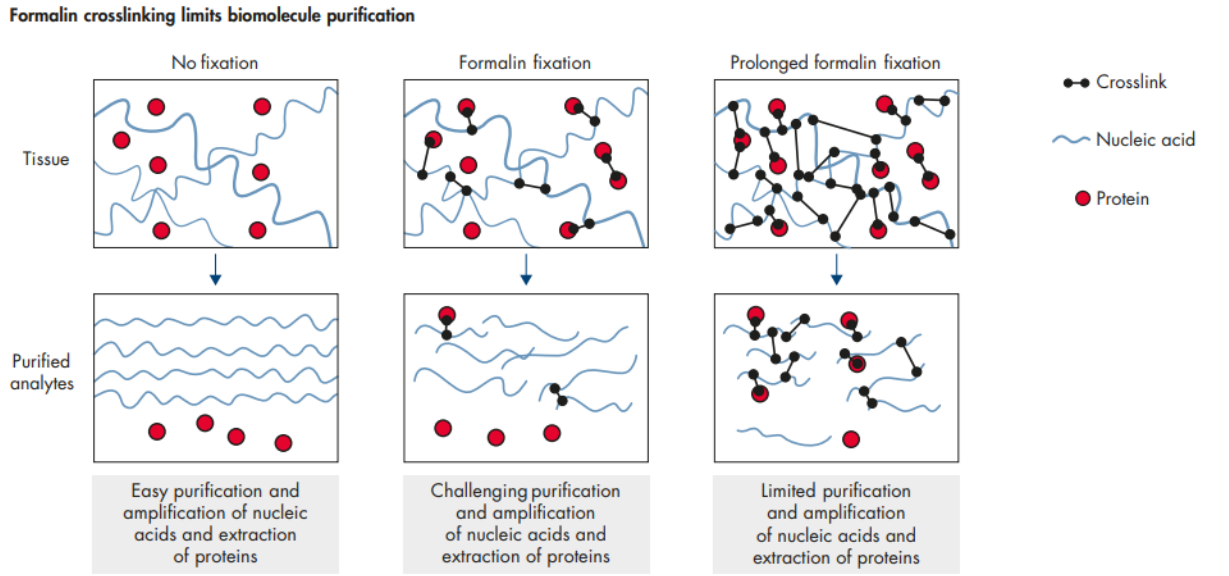
FFPE: Challenges and Solutions

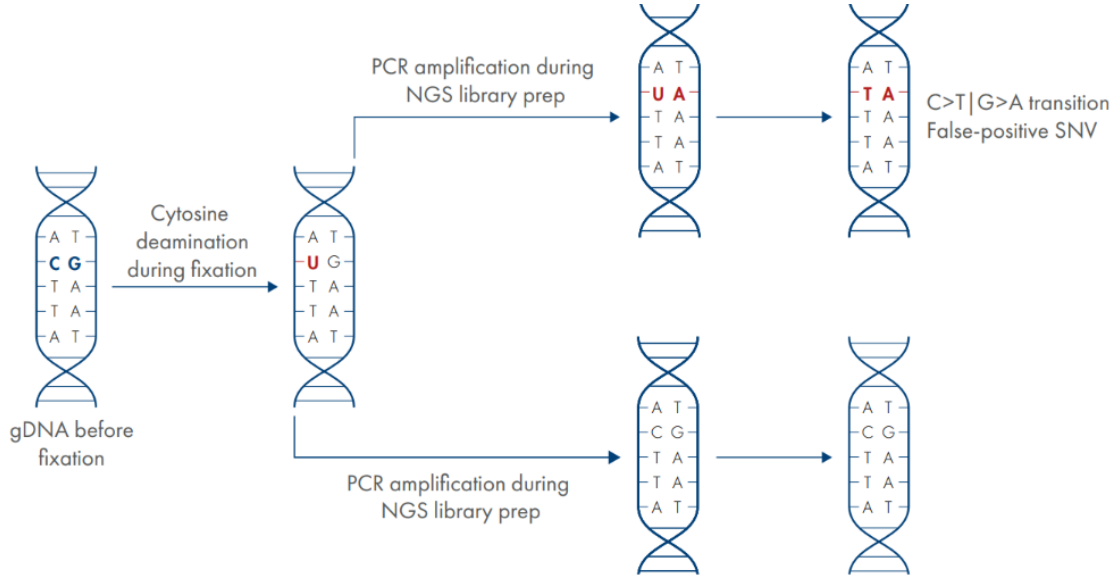
Liquid Biopsy: Challenges and Solutions

Publications

FFPE - Challenges

1. Precious, **irretrievable samples** you cannot afford to waste
2. **Formalin crosslinking** interfering with downstream analysis
3. **Highly fragmented** nucleic acids
4. **Low** DNA, RNA, and protein **yields**
5. Potentially **inaccurate genomic**, transcriptomic, and proteomic data





FFPE: Solutions – QIAGEN Sample Collection

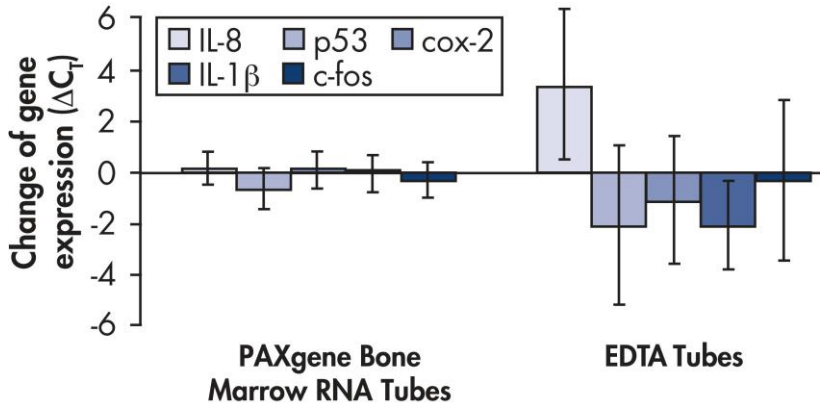
Tumor tissue

PAXgene Tissue System‡

- Formalin-free preservation of morphology and biomolecules
- Improved molecular results from fixed tissues
- Tissue storage and archiving for later processing
- RNA, miRNA, DNA and/or proteins from one sample

PAXgene Bone Marrow RNA System‡

- Immediate stabilization of intracellular RNA
- RNA remains stable for days at room temperature or when kept refrigerated
- Long-term sample storage at –20°C or –70°C



FFPE: Solutions – QIAGEN Sample Extraction

Precious, irretrievable samples	• Simultaneous purification of DNA & RNA • Allow reliable comparison of genomic and transcriptomic data
Formalin crosslinking	• QIAGEN® Deparaffinization Solution • Optimized procedure: input volume, temperature, duration, special lysis
Fragmented nucleic acids	• Optimized Procedure: how to treat FFPE
Low yields	• High recovery efficiency • Eliminate inhibitors
Inaccurate genomic data	• UNG treatment

QIAamp DNA FFPE Kits

- High recovery of amplifiable DNA
- Paraffin removal for full decrosslinking without xylene
- UNG treatment to remove artificial cytosine deamination
- Automatable kits for EZ2 Connect available

AllPrep DNA/RNA FFPE Kit

- Simultaneous purification of DNA and RNA from the same sample
- Effectively separation of RNA and DNA
- Automatable kits for EZ2 Connect available

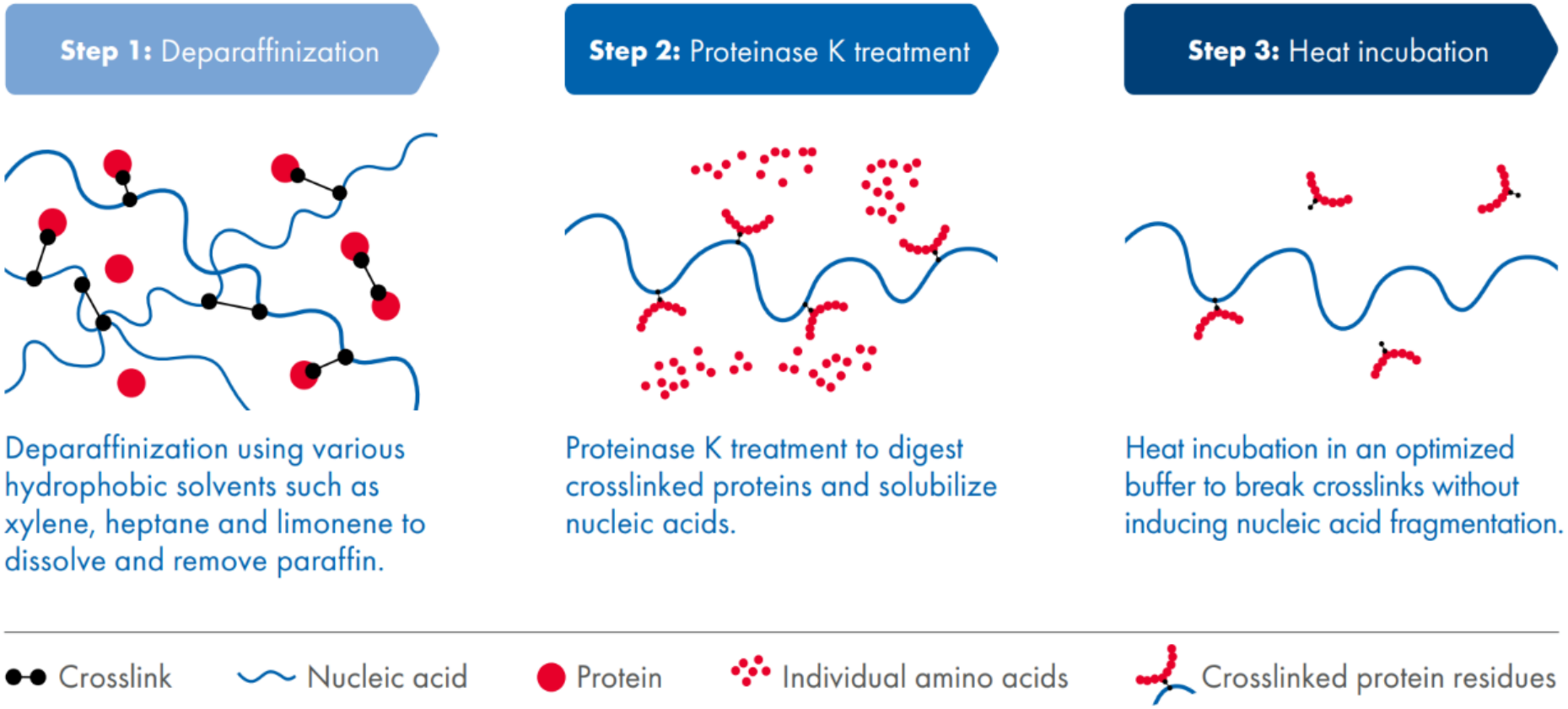
RNeasy FFPE Kits

- Purification of total RNA
- Novel method to overcome formalin crosslinking
- Automatable kits for EZ2 Connect available

miRNeasy FFPE Kits

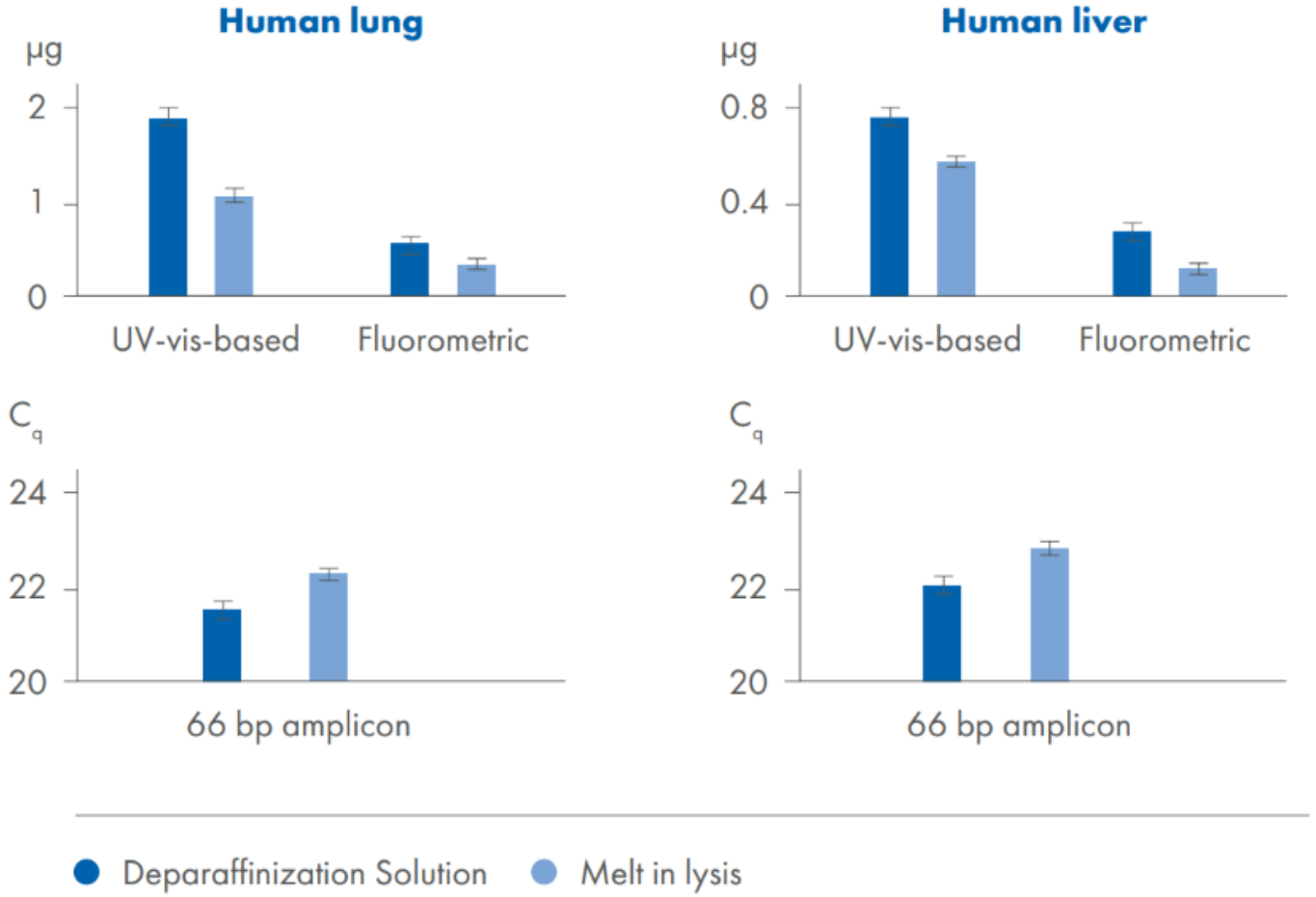
- Purification of total RNA, including small RNAs
- Novel method to overcome formalin crosslinking
- Automation possible on QIAcube Connect

FFPE: Solutions – QIAGEN Sample Extraction – Crosslinking & Yields



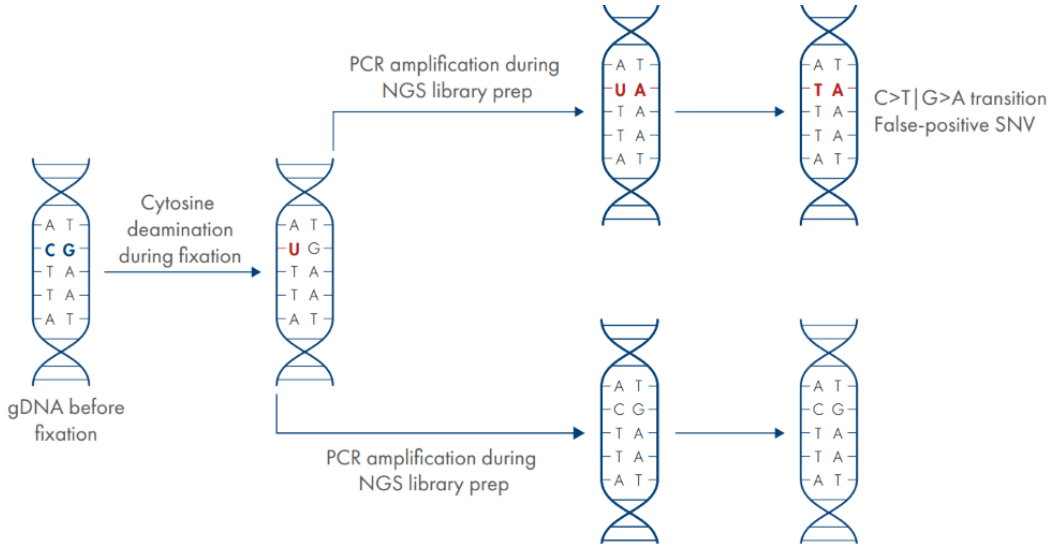
Key steps in nucleic acid preparation from FFPE samples.

FFPE: Solutions – QIAGEN Sample Extraction – Crosslinking & Yields



Efficient deparaffinization. Deparaffinization of 2 x 10 µm FFPE tissue sections from human lung and human liver

FFPE: Solutions – QIAGEN Sample Extraction – inaccurate genomic data



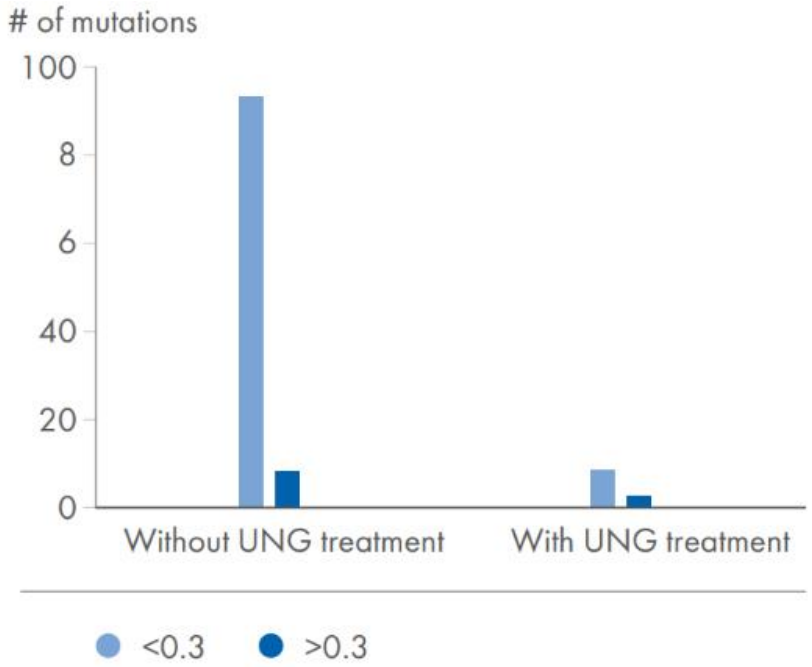


Figure 4. Dramatic reduction in artifactual C→T | G→A transitions. DNA was extracted from liver cancer samples, either with the incorporated UNG treatment step – a feature of the QIAamp® DNA FFPE Advanced UNG Kit – or without UNG treatment. The results show that UNG treatment removed over 90% of low-frequency novel mutations that are most likely to be artifactual.

Agenda

Overview: Cancer and Cancer Genomics

FFPE: Challenges and Solutions

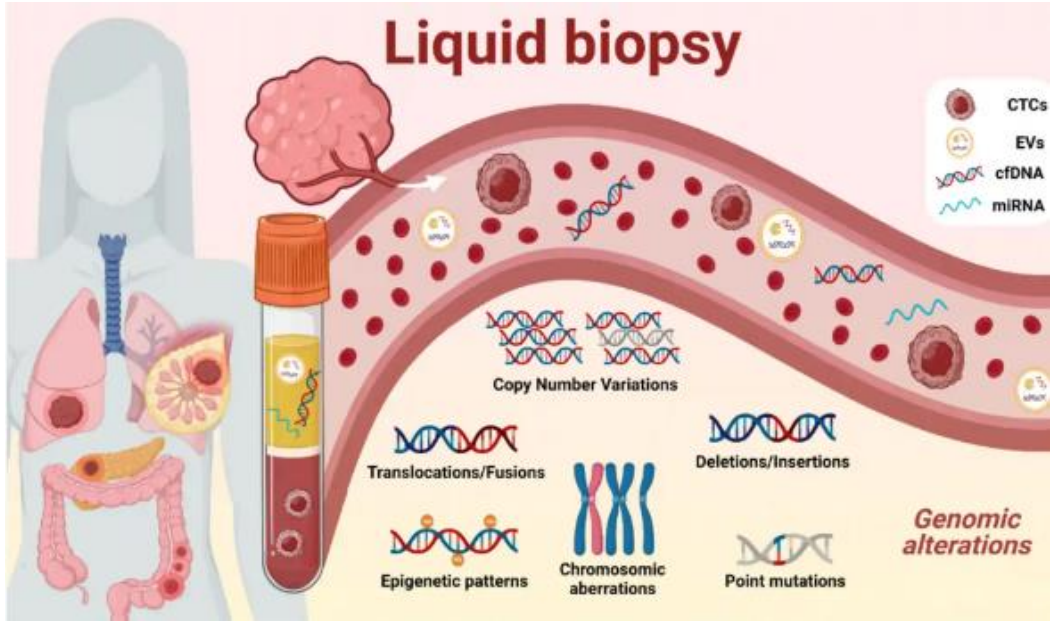
Liquid Biopsy: Challenges and Solutions

Publications

Liquid Biopsy

Liquid biopsy is a minimally or non-invasive method for the detection and analysis of biomarkers from body fluids such as:

- Blood
- Serum or plasma
- Urine
- Saliva
- Cerebrospinal fluid



In cancer research, liquid biopsy complements tissue biopsy and often overcomes its limitations such as:

- Not enough availability of tumor tissue
- A hard-to-reach tumor
- Need for regular monitoring
- Need for more molecular insights from heterogeneous tumors

The most common liquid biopsy analytes are:

- Cell-free circulating DNA (cfDNA)
- Cell-free circulating miRNA (cfRNA)
- Extracellular vesicles (EVs) and related content
- Circulating tumor cells (CTCs) and related content
- Proteins

Liquid Biopsy - Challenges

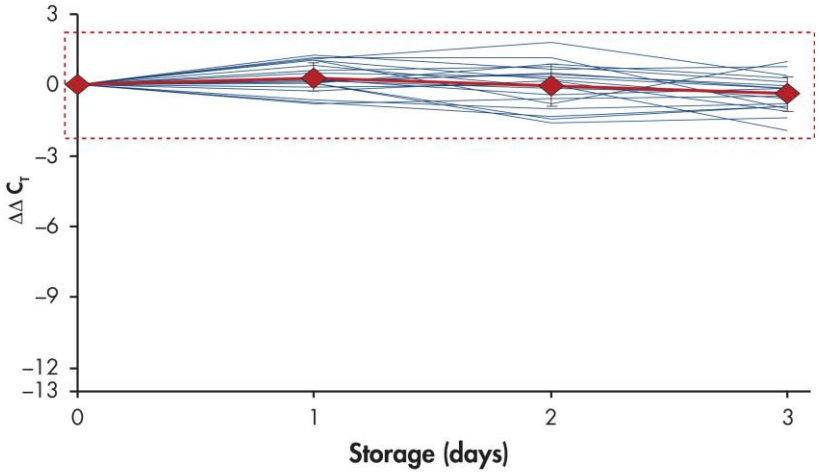
1. Low and highly variable concentrations, ranging from 1–50 ng/mL in healthy individuals
2. The potential for experimental bias through contamination with cellular DNA
3. The half-life of cfDNA in blood ranges from 15 minutes to 2.5 hours
4. PCR inhibitors

It requires a workflow that:

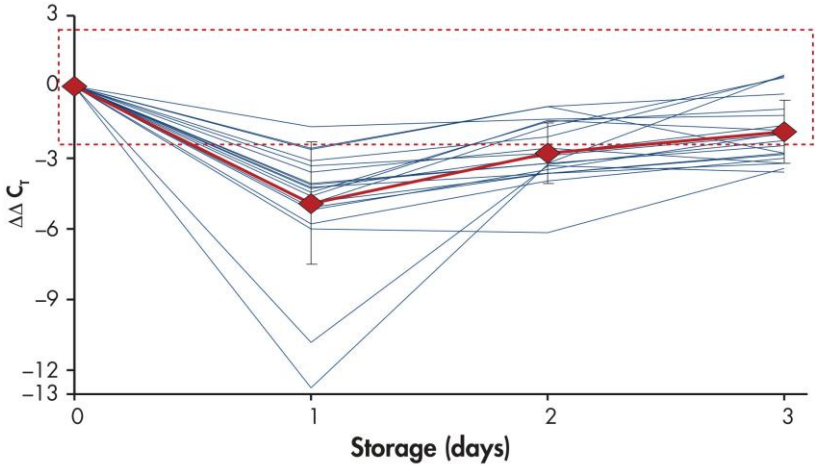
1. **Avoids release of cellular nucleic acids (gDNA)**
2. **Enables highly efficient largevolume nucleic acid extraction**
3. **Avoids fragment size bias to improve sensitivity**

Liquid Biopsy –Solutions – QIAGEN Sample Collection

A PAXgene Blood RNA Tubes



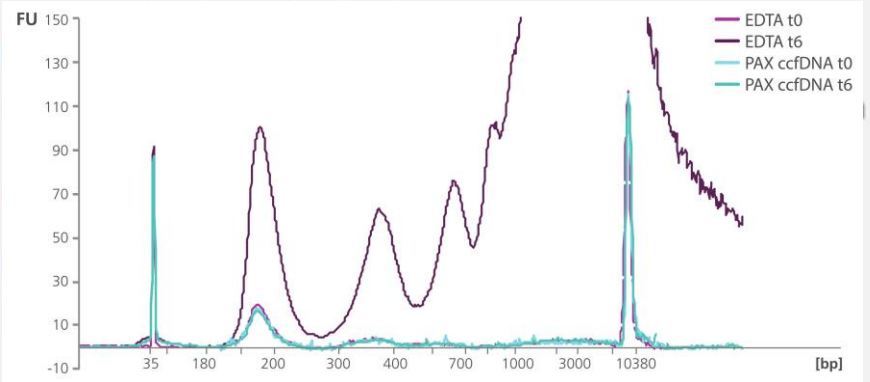
B EDTA



Liquid biopsy

PAXgene Blood ccfDNA Tube †

- Unique formaldehyde-free, non-crosslinking stabilizing chemistry
- Primary tube handling protocols for cfDNA-extraction on QIAsymphony SP available
- Liquid biopsy multianalyte workflow: Detection of cfDNA, gDNA, and CTC RNA and circulating RNAs from the same blood draw‡

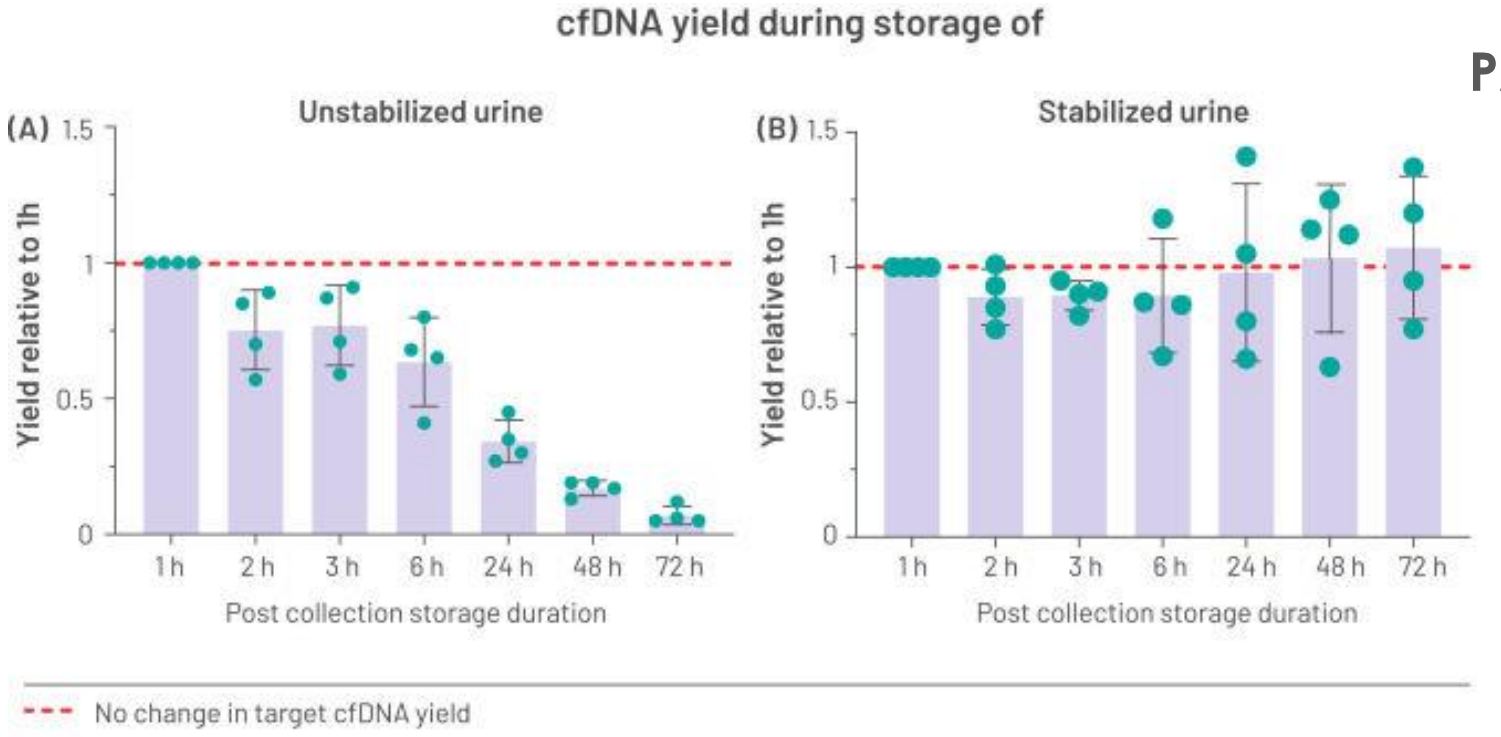


PAXgene Blood RNA Tube (IVD)*

- Single tube for collection, storage and transport of intracellular RNA
- Manual and automated RNA purification kits of total RNA and including miRNA available‡

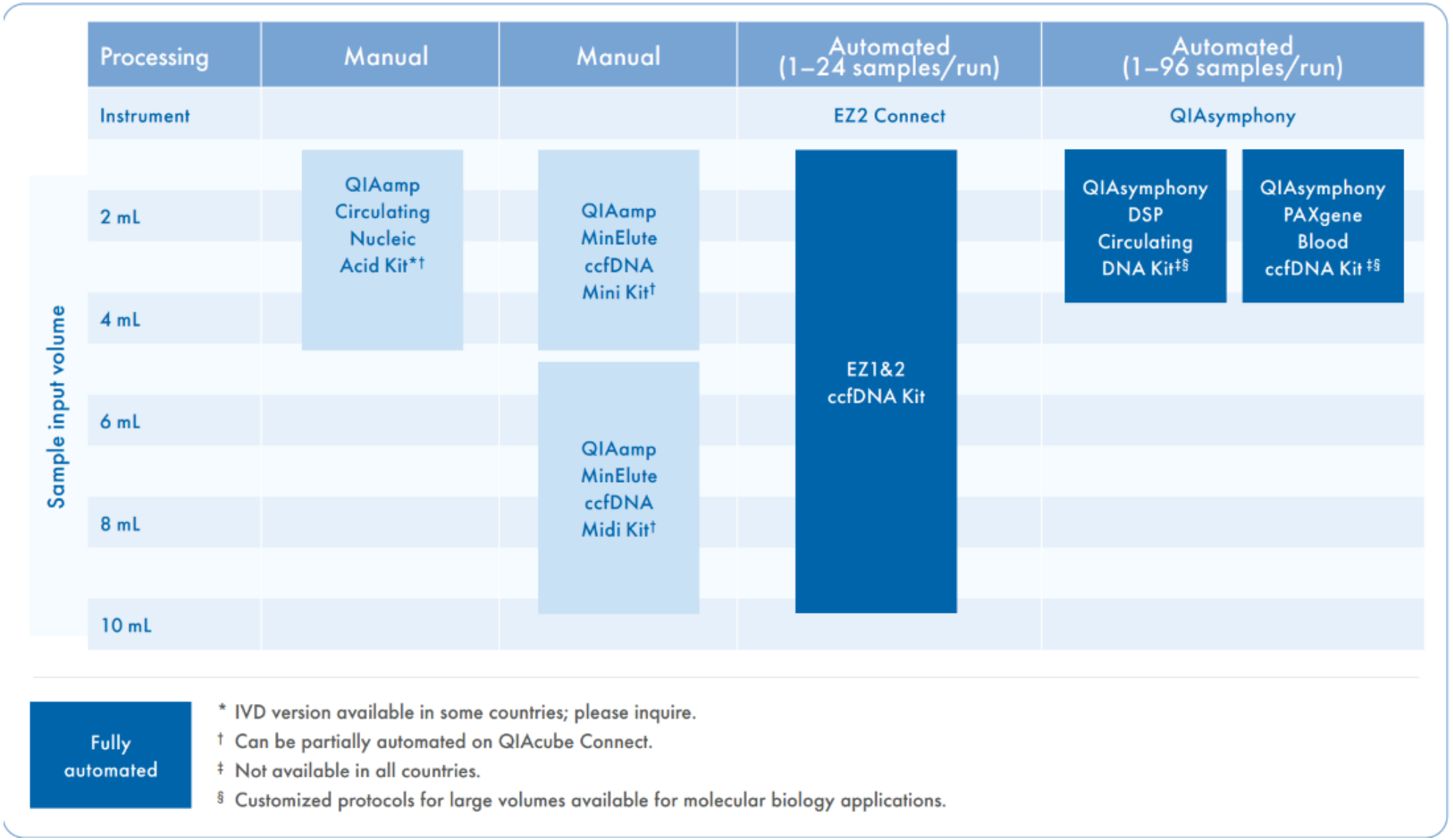
Liquid Biopsy –Solution – QIAGEN Sample Collection

- cfDNA is stabilized by minimization of cfDNA degradation, by minimization of gDNA release and by minimization of bacterial growth over urine storage
- The additive in the tube is non-crosslinking and does not modify biomolecules like cfDNA



PAXgene Urine Liquid Biopsy Set

Liquid Biopsy –Solutions – QIAGEN Sample Extraction



Automated purification

Instrument	Sample volume	Samples/run	Suitable for	Features
EZ2 Connect with QIAsphere 	Up to 8 mL	Up to 24	• CTC mRNA • cfDNA • FFPE DNA & RNA • Cells • DNA or RNA from fresh or frozen tissue	• Boosts reproducibility and convenience with prefilled reagent cartridges • Minimize manual steps with onboard pipetting, heating and automated piercing of prefilled cartridges
QIAcube Connect with QIAsphere 	Up to 10 mL	Up to 12	• cfDNA • FFPE DNA & RNA • Cells • DNA and/or RNA from fresh or frozen tissue	• Automates manual workflows; no change in chemistry required • More than 70 QIAGEN kits with over 180 standard protocols for DNA, RNA and protein sample processing • Optional protocol customization expands the use to over 3000 protocols
QIAsymphony SP 	Up to 4 mL; Customized protocols up to 10 mL for RUO	1–96 samples in 4 batches	• cfDNA from plasma • gDNA from blood • DNA from FFPE • DNA or RNA from fresh or frozen tissue	• Flexible loading of new samples during a run • Protocols customizable (protocol steps, labware, etc.) on demand • Traceability and LIMS compatibility

Agenda

Overview: Cancer and Cancer Genomics

FFPE: Challenges and Solutions

Liquid Biopsy: Challenges and Solutions

Publications

Publications

Evaluation of commercial kits for purification of circulating free DNA

Russell J. Diefenbach [a c], Lee Jenny H. [a c], Richard F. Kefford [b c d], Helen Rizos [a c]

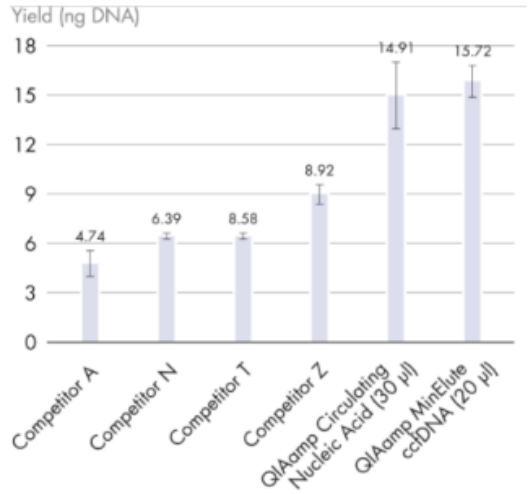
Show more

+ Add to Mendeley Share Cite

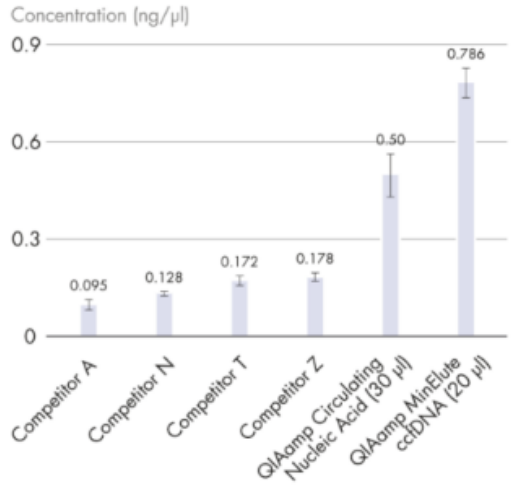
https://doi.org/10.1016/j.cancergen.2018.08.005 ↗ Get rights and content ↗

Highlights

- Spin column-based kits outperform magnetic bead-based commercial cfDNA kits.
- The Qiagen spin column-based cfDNA kit remains the gold standard.
- The Qiagen magnetic bead-based cfDNA kit is an alternative when factoring in price.



- Total yield of a 66 bp cfDNA fragment (4 mL plasma) is shown in comparison to 4 other commercially available kits.
- The QIAamp MinElute ccfDNA Kit provided the highest DNA yield.



- cfDNA concentrations of the 66 bp fragment were compared to the 4 competitor kits.
- The QIAamp MinElute ccfDNA Kit provided the highest DNA concentration in the final eluate.

Publications

Reliability and performance of commercial RNA and DNA extraction kits for FFPE tissue cores

Palak G. Patel[1,2☯], Shamini Selvarajah[1☯¤], Karl-Philippe Guérard[3], John M. S. Bartlett[4], Jacques Lapointe[3], David M. Berman[1,2], John B. A. Okello[1,2], Paul C. Park[1]*

1 Department of Pathology & Molecular Medicine, Queen's University, Kingston, Ontario, Canada, **2** Division of Cancer Biology & Genetics, Queen's Cancer Research Institute, Queen's University, Kingston, Ontario, Canada, **3** Department of Surgery, Division of Urology, McGill University and the Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada, **4** Diagnostic Development Program, Ontario Institute for Cancer Research (OICR), Toronto, Ontario, Canada

☯ These authors contributed equally to this work.
¤ Current address: Trillium Health Partners–Credit Valley Site, Mississauga, Ontario, Canada
* pcp1@queensu.ca, pcpark@gmail.com

Abstract

Cancer biomarker studies often require nucleic acid extraction from limited amounts of formalin-fixed, paraffin-embedded (FFPE) tissues, such as histologic sections or needle cores. A major challenge is low quantity and quality of extracted nucleic acids, which can limit our ability to perform genetic analyses, and have a significant influence on overall study design. This study was aimed at identifying the most reliable and reproducible method of obtaining sufficient high-quality nucleic acids from FFPE tissues. We compared the yield and quality of nucleic acids from 0.6-mm FFPE prostate tissue cores across 16 DNA and RNA extraction protocols, using 14 commercially available kits. Nucleic acid yield was determined by fluorometry, and quality was determined by spectrophotometry. All protocols yielded nucleic acids in quantities that are compatible with downstream molecular applications. However, the protocols varied widely in the quality of the extracted RNA and DNA. Four RNA and five DNA extraction protocols, including protocols from two kits for dual-extraction of RNA and DNA from the same tissue source, were prioritized for further quality assessment based on the yield and purity of their products. Specifically, their compatibility with downstream reactions was assessed using both NanoString nCounter gene expression assays and reverse-transcriptase real-time PCR for RNA, and methylation-specific PCR assays for DNA. The kit deemed most suitable for FFPE tissue was the AllPrep kit by Qiagen because of its yield, quality, and ability to purify both RNA and DNA from the same sample, which would be advantageous in biomarker studies.

Publications

Targeted Sequencing of Plasma-Derived vs. Urinary cfDNA from Patients with Triple-Negative Breast Cancer

Henrike Herzog [1], Senol Dogan [2], Bahriye Aktas [1] and Ivonne Nel [1,*]

[1] Department of Gynecology, Medical Center, University of Leipzig, 04103 Leipzig, Germany
[2] Soft Matter Physics Division, Peter-Debye-Institute, University of Leipzig, 04103 Leipzig, Germany
* Correspondence: ivonne.nel@medizin.uni-leipzig.de; Tel.: +49-3419720083

2.3. Isolation and Quantification of Cell-Free DNA (cfDNA)

Aliquots of plasma and urine samples were thawed immediately and only once prior to cfDNA extraction to minimize freeze–thaw effects [22]. Isolation and quantification of cfDNA was performed as described elsewhere [23]. Subsequently, cfDNA was isolated from 4 mL of plasma and 10 mL of matching urine supernatant for each patient using the QIAamp MinElute ccfDNA Midi Kit (QIAGEN, Hilden, Germany) according to the manufacturers' instructions. To obtain a maximum yield of nucleic acids, cfDNA from plasma was eluted in 50 µL and cfDNA from urine in 20 µL ultraclean water, respectively. Concentrations of the isolated cfDNA were quantified fluorometrically using a Qubit 2.0 fluorometer and the Qubit dsDNA HS Assay kit (Cat. No. Q32851, Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturers' instructions, and samples were stored at −20 °C prior to targeted sequencing.

Publications

Detection of Oncogene Hotspot Mutations in Female NSCLC Tumor DNA and Cell-Free DNA

by **Ieva Drejeriene** [1,2,*], **Saulius Cicenas** [3], **Diana Stanciute** [3], **Arnoldas Krasauskas** [1,3] and **Jurate Gruode** [2,4]

[1] Faculty of Medicine, Vilnius University, 03101 Vilnius, Lithuania
[2] Klaipeda University Hospital, 92288 Klaipeda, Lithuania
[3] National Cancer Institute, 08406 Vilnius, Lithuania
[4] Faculty of Health Science, Klaipeda University, 92294 Klaipeda, Lithuania
[*] Author to whom correspondence should be addressed.

Cancers **2024**, *16*(9), 1770; **https://doi.org/10.3390/cancers16091770**

Submission received: 7 March 2024 / Revised: 16 April 2024 / Accepted: 21 April 2024 / Published: 3 May 2024

(This article belongs to the Section **Cancer Biomarkers**)

2.2. DNA Preparation

Two types of samples were collected from each patient: tumor tissue (fresh or formalin-fixed paraffin embedded (FFPE)) and EDTA plasma. DNA was extracted from fresh tissue using the QIAsymphony DSP Virus/Pathogen Midi Kit (Qiagen, Hilden, Germany), from FFPE using the QIAamp DNA FFPE Tissure Kit (Qiagen, Hilden, Germany), and cfDNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) following the manufacturers' instructions. DNA was quantified with a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, California, US), and cfDNA was quantified with Therascreen EGFR Plasma RGQ PCR Kit (Qiagen, Hilden, Germany).

Thank you for your attention. Questions?

Liquid Biopsy –Solutions – QIAGEN Sample Extraction

Kit	Technology	Starting volume	Processing time	Automation	Samples/ run	Eluate volumes	Good to know
QIAamp MinElute ccfDNA Mini Kit (50)	Magnetic bead and spin column	1–4 mL	45 min	Manual		20 µL	Obtain highly concentrated cfDNA from plasma or serum due to low elution volumes
QIAamp MinElute ccfDNA Midi Kit (50)		5–10 mL	70 min	Partially on QIAcube Connect	Up to 12	30 µL	
QIAamp Circulating Nucleic Acid Kit (50)	Silica membrane	Up to 5 mL	<2 h	Manual using vacuum with QIAvac 24 Plus		20–150 µL	Described in more than 81,000** publications – referred as the gold-standard kit for cfDNA extraction
		Up to 4 mL urine					
EZ1&2 ccfDNA Kit (48)	Magnetic bead	1–2 mL	35 min	EZ2 Connect	Up to 24	70 µL	Your kit of choice for flexible cfDNA automated preparation
		3–4 mL	46 min				
		5–8 mL	70 min				
QIAsymphony PAXgene Blood ccfDNA Kit (192*,†)	Magnetic bead	2.4 mL	1 h 5 min	QIAsymphony SP	24	60, 100 or 150 µL	Optimized isolation chemistry for plasma in PAXgene Blood ccfDNA Tubes; separate protocol lines for small and large cfDNA fragments
			4 h 20 min		96		
		4 mL or 4.8 mL	1 h 25 min		24		
			5 h 40 min		96		
QIAamp ccfDNA/RNA Kit (50)	Silica membrane	1–4 mL	45 min	Manual, partially on QIAcube Connect	Up to 12	14–20 µL	Purification of cfDNA and cfRNA into one eluate; vacuum-free protocol using spin column technology