An Introduction to Liquid Biopsies: ctDNA Sequencing Technical Topics

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Advancing Diagnosis and Discovery

Disclosure

Invitae (ArcherDx): Shareholder

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Outline

cfDNA

ctDNA detection methods

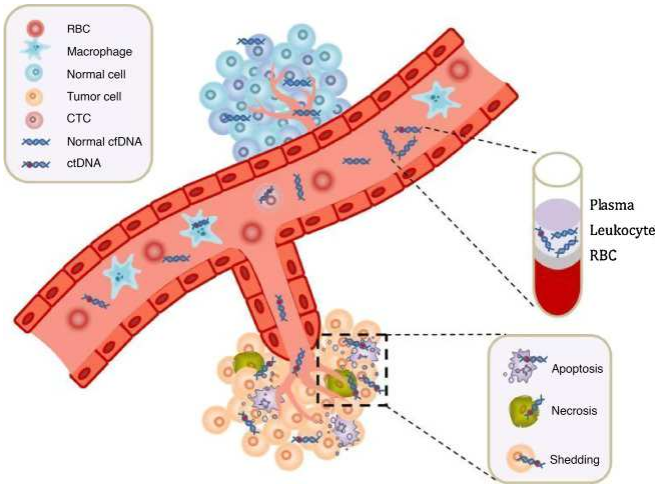
Sequencing coverage depth

cfDNA collection and preparation

Unique molecular identifiers (UMIs)

Caveats when using UMIs

Cell-Free DNA in Plasma



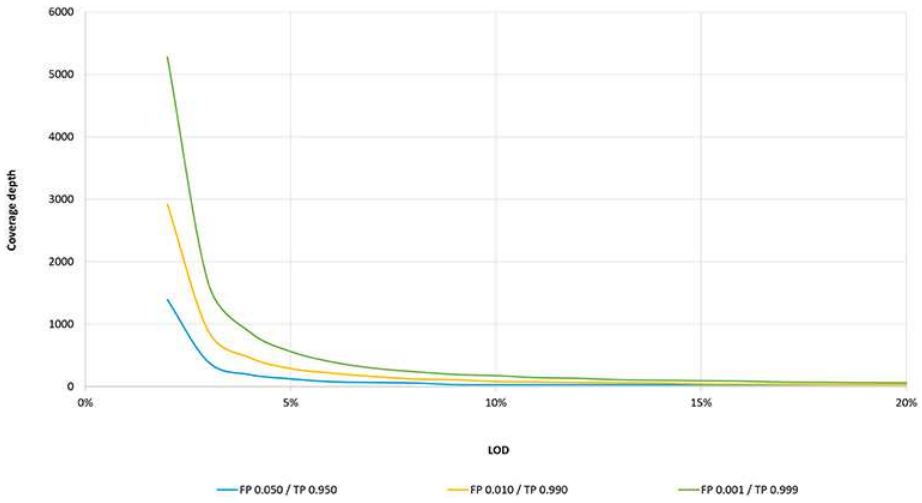
Hahn et al *Kidney Cancer 3* 2019

ctDNA Detection Methods

Analysis Type	Sensitivity	Targets	Applications	Advantages	Limitations	Cost
qPCR	0.01-0.1%	Hotspots	Detection, monitoring, targeted therapy	High specificity/sensitivity, rapid, simple	No multiplexing, known mutations	$
Digital PCR	0.01-0.1%	Hotspots, gene fusions, CNV	Detection, monitoring, targeted therapy	Up to 5 targets, high specificity/sensitivity, quantification, rapid, simple	Limited multiplexing, known mutations	$
Targeted NGS Panel	0.0001%-2%	Known & unknown mutations, indels, CNV, chromosomal rearrangements	Detection, monitoring, classification, targeted therapy	High specificity/sensitivity, error correction, better multiplexing	Complex workflow, sequencing instrument required, bioinformatics	$$
Whole exome sequencing	5%	Coding, non-coding, promoter, UTR regions	Detection, monitoring, targeted therapy, classification	Discovery, signatures, CNV, fusion, rearrangements, neoantigens, TMB	Low sensitivity, complex workflow, sequencing instrument required, bioinformatics	$$$
Whole genome sequencing	5-10%	Structural variants, fragmentation pattern, genome wide CNV, methylation, TMB	Classification/origin, early detection	Shallow sequencing cancer profiling/signatures	Variable/low sensitivity/specificity, lots of sequencing, bioinformatics	$$$$

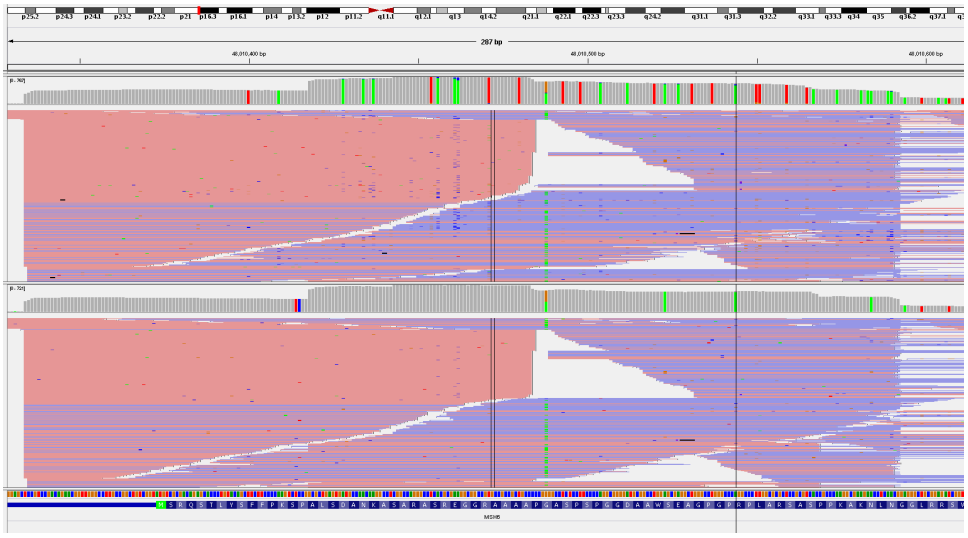
Adapted from Table 1, Bohers et al *Pharmaceuticals* 2021

Sequencing Coverage Depth Relative to Allelic Fraction



Petrackova et al *Frontiers in Oncology* 2019

NGS Sequencing Errors



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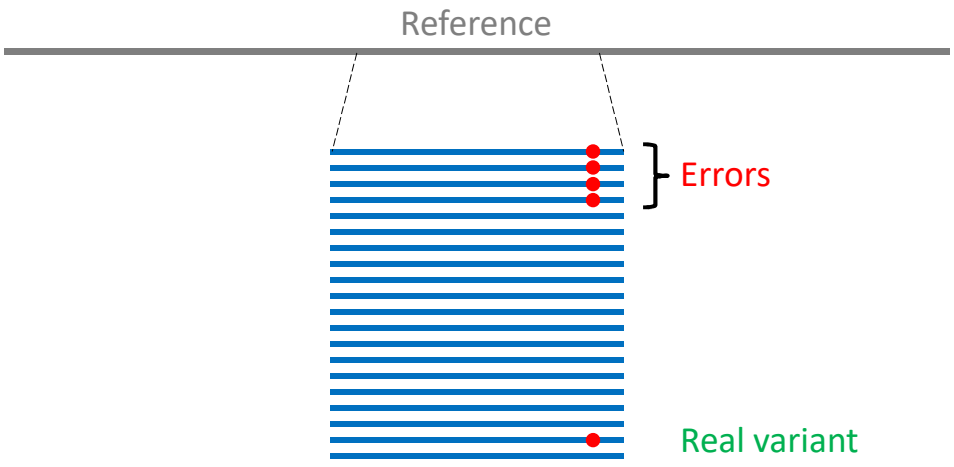
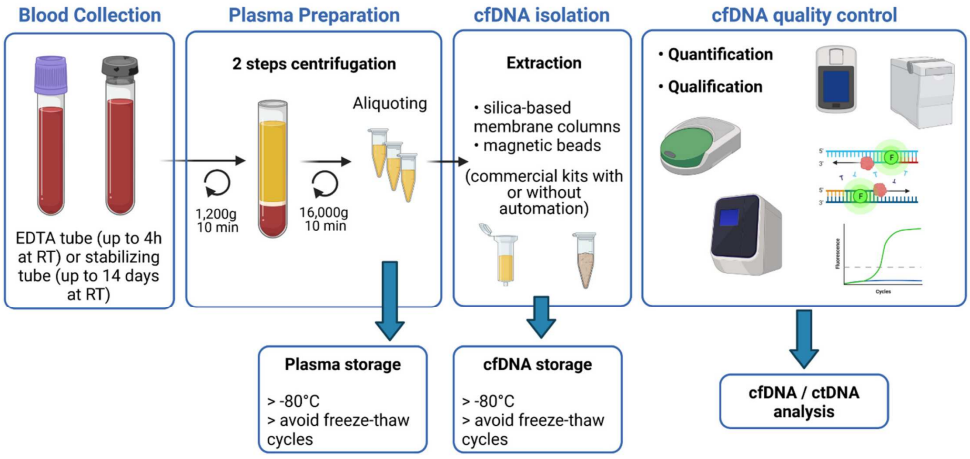
Distinguishing Real Variants from Sequencing Errors

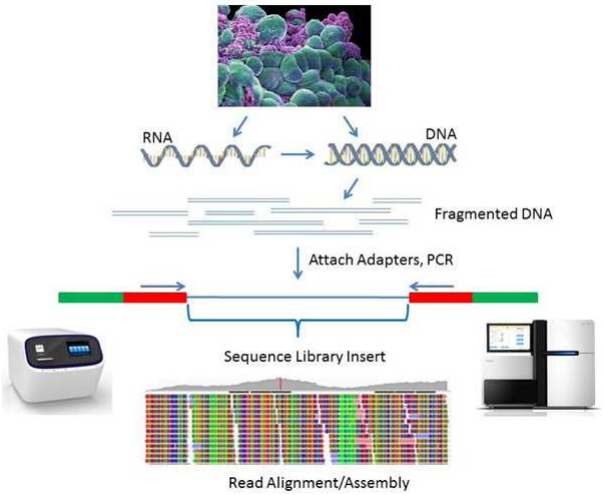


Pre-Analytical Collection and Preparation of cfDNA



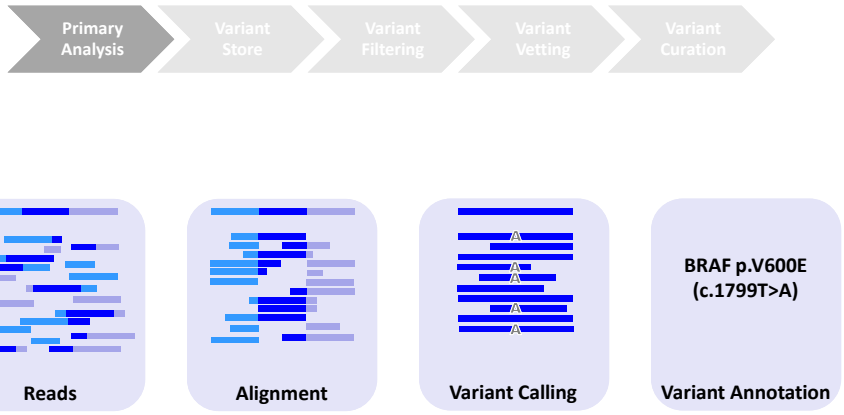
Bohers et al *Pharmaceuticals* 2021

NGS Library Construction



Head et al *Biotechniques* 2018

Clinical Genomics Workflow: Primary Analysis



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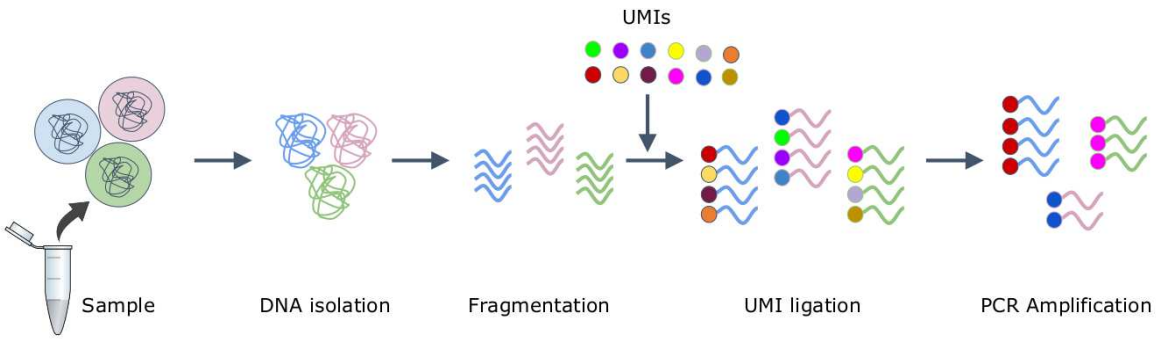
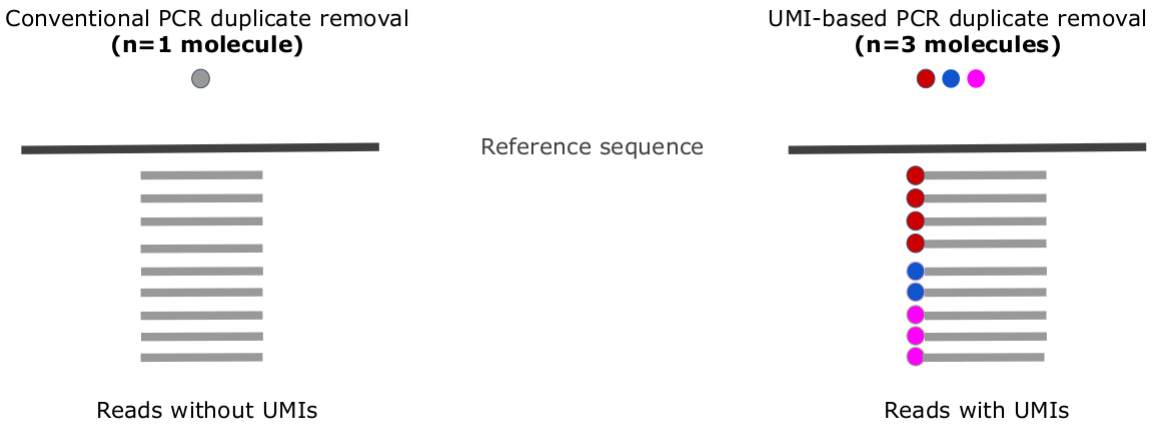
Unique Molecular Identifiers/Molecular Barcode (UMI, UMT, RMT)



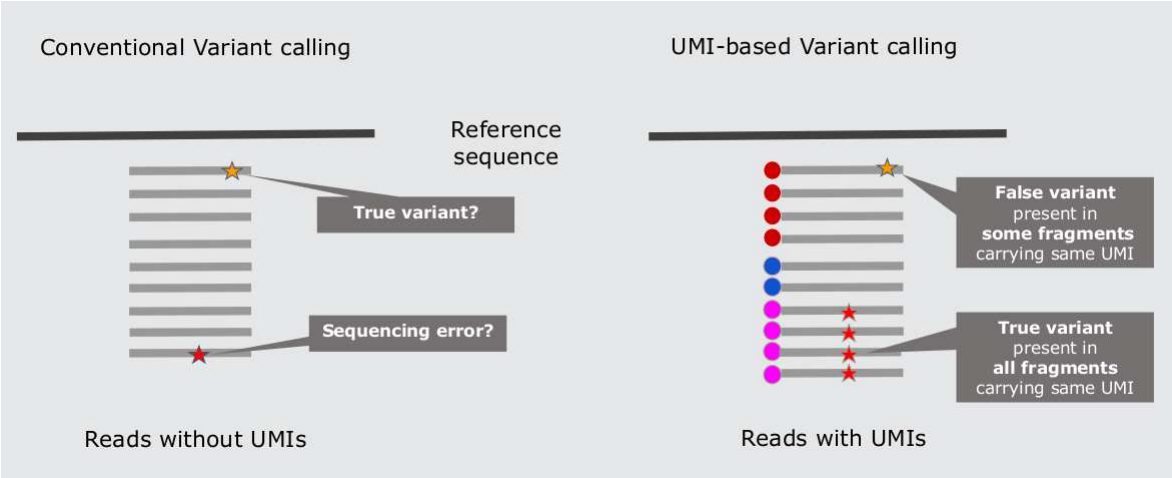
http://blog.avadis-ngs.com (01/2018)

UMI-Based PCR Duplicate Removal/De-Duplication



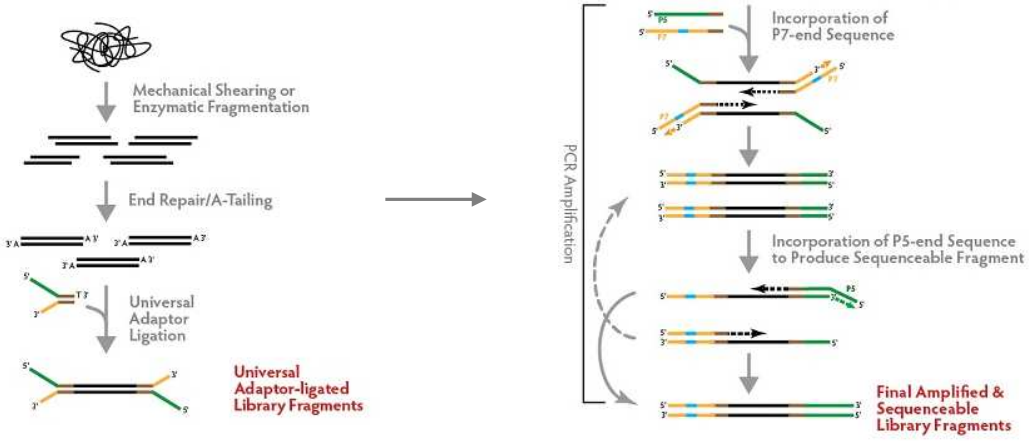
http://blog.avadis-ngs.com (01/2018)

UMI-Based Variant Calling Error Correction



http://blog.avadis-ngs.com (01/2018)

UMI Caveat: Adapter Carryover

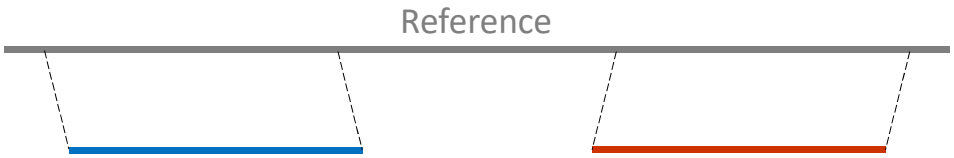


https://biocat.com

UMI Caveat: Barcode Sequencing Error

ATCGCGTA → ATCGCCTA

UMI Caveat: Barcode Uniqueness Across the Library



UMI = ATCGCGTA

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Maximum barcode diversity with 8 bases → 4^8 = 65536

*UMI uniqueness across a library
should consider genomic alignment position

Summary

- Ultra sensitive detection of tumor variants from ctDNA requires very high sequencing coverage
- Thorough sampling of DNA molecules/library fragments/sequencing reads is critical for analyzing cell-free DNA by NGS
- Unique molecular identifiers (UMIs) are required for sequencing error correction to enable confident variant calling for cell-free DNA applications
- A complete solution including special library construction techniques and bioinformatic tools are required to successfully leverage UMIs for cell-free DNA testing