# Sensitive Detection of 5% Somatic ctDNA Variants Using MagPurix® cfDNA Extraction in a Liquid Biopsy NGS workflow

## Executive Summary

This study evaluated a complete liquid biopsy pipeline for detecting somatic variants at 5% variant allele frequency (VAF) in circulating tumor DNA (ctDNA) using plasma cfDNA. A key component of the workflow was automated extraction with the **MagPurix® 12 System** and **MagPurix® CFC DNA Extraction Kit (ZP02017)**, starting from plasma collected in Streck Cell-Free DNA BCT tubes.

MagPurix® provided sufficient cfDNA yield and the expected fragment size profile (120–220 bp peak) to support an optimized NGS protocol (Twist cfDNA library prep + Illumina MiSeq) and a dedicated bioinformatics pipeline. Using 0% and 5% reference standards, the combined NGS + confirmatory SNaPshot workflow achieved 94.12% sensitivity and 99% specificity for detecting clinically relevant somatic variants at 5% VAF, demonstrating that MagPurix®-extracted cfDNA is highly suitable for sensitive liquid biopsy applications.

## Key Findings

- **cfDNA yield compatible with demanding NGS workflows**
  - Duplicate MagPurix® extractions from patient samples yielded, for example, ~46–56 ng cfDNA in 45 µL for one case (RX84.2023) and ~10–12 ng for a lower-yield case (RX87.2023), both adequate for Twist cfDNA library preparation (≥10–30 ng input).

- **High-quality cfDNA fragment profile for ctDNA detection**
  - TapeStation analysis of MagPurix®-extracted cfDNA showed the expected cfDNA fragment pattern with a main peak between 120–220 bp and an additional peak around 300 bp, matching the theoretical cfDNA distribution.

- **Enabling sensitive detection of 5% somatic variants**
  - Using MagPurix®-extracted cfDNA, the optimized NGS pipeline (Twist library prep with UMIs, 300× target coverage, and custom “Unbalance” variant-calling) successfully detected somatic variants present at 5% VAF in a 5% cfDNA reference standard.
  - When two independent somatic runs were combined, the method showed only 3 false negatives out of 51 variants, corresponding to 94.12% sensitivity.

- **High diagnostic reliability when coupled with orthogonal confirmation**
  - Initial NGS analysis displayed good but imperfect specificity; however, by confirming clinically relevant variants with a targeted SNaPshot assay, the authors report an overall specificity of 99% for the workflow.

## Conclusion

Within this validated liquid biopsy workflow, the MagPurix® 12 System and MagPurix® CFC DNA Extraction Kit provided high-quality cfDNA from plasma, with yields and fragment profiles fully compatible with advanced NGS and ctDNA variant detection. The complete process, built

on MagPurix® extraction, enabled detection of 5% VAF somatic variants with 94.12% sensitivity and 99% specificity after orthogonal confirmation.

For clinical and research laboratories developing liquid biopsy assays, MagPurix® offers a reliable, automated, and sample-efficient extraction platform that supports sensitive detection of low-frequency tumor variants and helps ensure confidence in downstream molecular findings.

## Reference

Mareso, C., Crosta, L., De Vita, M. G., Cristofoli, F., Tanzi, B., Benedetti, S., et al. (2024). Assessing the efficacy of an innovative diagnostic method for identifying 5% variants in somatic ctDNA. *Gene*, *928*, 148771. https://doi.org/10.1016/j.gene.2024.148771