FAQ ABOUT ccfDNA AND CTCs

What is the difference between ccfDNA and CTCs?
Circulating cell-free DNA (ccfDNA), isolated from the plasma portion of a blood draw, can originate from apoptotic and necrotic tumor cells, whereas circulating tumor cells (CTCs) are living cells that have shed from the primary tumor and circulate in the bloodstream. The incidence of CTCs is very rare (1 in 1,000,000), but the tumor component of the ccfDNA can be as high as 10%.

What recent advances have enabled use of CTCs for guiding cancer treatment?
New methods for CTC enrichment enable interrogation of these rare cells. Molecular analysis can provide an invaluable tool for early stage detection of cancer, neoplastic progression, and recurrence monitoring.

Why are CTCs of interest in clinical research?
CTCs can lead to subsequent growth of tumors in distant organs. Recent studies have demonstrated that CTCs reflect the molecular features of cells within tumor masses, and could be considered a “liquid biopsy,” providing real-time monitoring of a patient’s disease status. In contrast to ccfDNA, CTCs can be cultured and serve as an unlimited source for clinical research studies.

What studies involving ccfDNA and CTCs have been performed on the MassARRAY System?
Following is a synopsis of key publications in chronological order.

STUDIES ON THE MASSARRAY SYSTEM

Multi-purpose utility of circulating plasma DNA testing in patients with advanced cancers
A study used Agena Bioscience’s OncoCarta™ Panel, which has a sensitivity of ≤ 10% mutation frequency, to analyze the concordance of critical hot-spot mutations in matched circulating plasma DNA and archival tumor tissue from patients with late stage advanced solid tumors from the Royal Marsden NHS Foundation Trust. The overall median circulating plasma DNA concentration was 17 ng/ml (range: 0.5-1600). Disease states included colorectal cancer, melanoma, breast cancer, and ovarian cancer. The overall concordance between paired FFPE tumors and circulating plasma was 60%, suggesting the utility of this approach.
Perkins G, de Bono J et al. PLOS ONE, 11, 2012;7(11); e47020.

Detection of EGFR T790M mutation in plasma DNA from patients refractory to EGFR tyrosine kinase inhibitor
A secondary epidermal growth factor receptor (EGFR) mutation, T790M, leads to acquired resistance to EGFR-tyrosine kinase inhibitors. Plasma samples from 75 non-small cell lung cancer patients were obtained after discontinuation of gefitinib or erlotinib. The assay sensitivity using the single base extension chemistry (SABER, a precursor to UltraSEEK™) on the MassARRAY System was 0.3% with mixed oligonucleotides. The mutation was detected in 21 of the 75 plasma samples (28%); 14 of 21 samples were confirmed by subcloning and subsequent sequencing. This study introduces a feasible method for determining plasma T790M mutation status for monitoring EGFR-TKI therapy.

Clinical validation of an ultra high-throughput spiral microfluidics for the detection and enrichment of viable circulating tumor cells
A novel method based on spiral microfluidics enabled isolation of CTCs from 7.5 ml blood volumes in less than 5 minutes. Retrieved cells are unlabeled and viable for downstream molecular analysis including targeted mutation...
The ultra-sensitive MassARRAY System was used to detect the presence of an \textit{EGFR}-activating mutation in both isolated CTCs and plasma cell-free DNA, and demonstrated concordance with the original tumor biopsy samples. Sensitivity was tested to 1.5\% in one of the CTC samples.

Khoo BL and Lim CT et al. \textit{PLOS ONE}, 07,2014;9(7), e99409.

\textbf{Ultrasensitive MALDI-TOF-based mutation panel is more sensitive than ARMS method in ccfDNA obtained from plasma in melanoma patients}

A method capable of detecting low frequency mutant alleles in tumor and ccfDNA could provide invaluable information for treatment options. Using the UltraSEEK Oncogene Panel, comprised of 26 hotspot mutations, we evaluated paired tumor and ccfDNA from 122 patients diagnosed with melanoma. A key driver mutation, \textit{BRAF} V600E, was successfully identified in every positive admixture sample down to 0.1\% frequency with our panel, while an alternate PCR-based approach (ARMs) could detect to 1.0\%. The study demonstrates that the sensitivity achieved with the MassARRAY approach is superior and provides an added benefit of multiplexed analysis where needed, saving precious clinical material.

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\section*{PRODUCTS AND SERVICES}

Agena Bioscience offers the UltraSEEK method for low abundance mutation detection at ≤ 1\% frequency. You may purchase predesigned panels or order custom assays specific to your research needs.

The UltraSEEK method consists of standard PCR amplification of both wild type and mutant DNA followed by single base extension (Fig. 1). The extension reaction utilizes a single mutation-specific chain terminator labeled with biotin-streptavidin for solid phase capture. After capture, wash and elution, the resultant products are spotted onto a SpectroCHIP\textsuperscript{®} Array for MALDI-TOF analysis on the MassARRAY System.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig1.png}
\caption{UltraSEEK mutation detection process with the MassARRAY System}
\end{figure}

The UltraSEEK Oncogene Panel offers pre-designed, targeted assays for 26 hot spot mutations in 12 oncogenes. The panel has been validated against Horizon Diagnostic DNA standards to as low as 0.2\% analytical sensitivity. \textit{EGFR} T790M is one of the key mutations included to aid in clinical cancer research.

Agena Bioscience can adapt the UltraSEEK method for any set of assays where ultrasensitive mutation detection is desired. Please inquire with your local representative regarding Assays by Agena custom services.