Extraction and analysis of circulating cell-free DNA from plasma samples for lung cancer research

Abstract

We report a method for the extraction of circulating cell-free DNA (cfDNA) from plasma samples using the Applied Biosystems™ MagMAX™ Cell-Free DNA Isolation Kit followed by digital PCR and next-generation sequencing analysis. Extraction and analysis of cfDNA was successfully performed from the plasma of 3 donors in good health and 11 donors with lung cancer. The results show that the MagMAX Cell-Free DNA Isolation Kit offers efficient recovery of high-quality cfDNA for downstream research applications and enables time savings compared to traditional methods.

Introduction

Development of drugs targeting driver genes involved in lung cancer progression has become essential for the advancement of treatment options. In addition, the tolerance acquisition mechanisms of cells to these targeted therapies has been clarified, leading to the development of second- and third-generation drugs to allow post-tolerance therapy strategies. Companion diagnostics to help confirm tolerance acquisition mechanisms after primary treatment is indispensable for these new generations of drugs. Since it is often difficult to conduct an invasive second biopsy during or after treatment, there is considerable interest in developing less-invasive genetic tests that utilize peripheral blood. It is well known that the peripheral blood of cancer patients contains tissue-derived cfDNA [1]. As genetic abnormalities have been analyzed using cfDNA for many cancer types such as lung cancer, breast cancer, pancreatic cancer, colon cancer, and prostate cancer, the interest in developing minimally invasive genetic tests has increased.

Lung cancer is a leading cause of death worldwide, with the majority of cases attributed to non-small cell lung carcinoma [2]. Mutation of the epidermal growth factor receptor gene (*EGFR*) is frequently observed in non-small cell lung carcinoma. For lung cancers positive in this mutation, first-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs) are applied, which often have a remarkable effect, but eventually develop tolerance [3]. The T790M mutation of *EGFR* is recognized in approximately half of drug-resistant tumors, and third-generation EGFR-TKIs are effective against lung cancers with this mutation. Before treatment with targeted therapies, it is essential to confirm the T790M mutation using companion diagnostics. As previously described, it is often difficult to perform a second biopsy of the tumor tissue, so genetic testing using cfDNA from peripheral blood is of interest to many researchers studying practical methods to replace a tissue biopsy.

Genetic testing using cfDNA has significant challenges that are not typically associated with traditional testing using tumor tissues. First, the test must be compatible with a very small amount of nucleic acid, since cfDNA can be collected from only a few milliliters of peripheral blood. Second, the test requires a highly sensitive method that can detect a tumor-derived cfDNA mutation existing at low frequency, since peripheral blood also contains circulating DNA derived from normal cells. These challenges can be overcome with highly sensitive detection technologies such as digital PCR as well as simple methods to efficiently extract cfDNA from peripheral blood. Here we report the results of an evaluation of the MagMAX Cell-Free DNA Isolation Kit by Prof. Kazuto Nishio and Dr. Kazuko Sakai, Department of Genome Biology, KINDAI University, Faculty of Medicine, Osaka, Japan.

Materials and methods

After donor consent and approval by the ethics committee of the university, peripheral venous blood from 3 donors in good health and 11 donors with lung cancer were collected using EDTA blood collection tubes, from which the plasma was separated by centrifugation.

Extraction of cfDNA from approximately 1 mL plasma was performed as described in the MagMAX Cell-Free DNA Isolation Kit user guide. A Bioanalyzer™ instrument (Agilent) was used to confirm the size of extracted DNA. The yield of cfDNA was calculated by quantifying the number of copies of the RNaseP gene using Applied Biosystems™ TaqMan™ Copy Number Assays.

Results

Using plasma samples from healthy donors, an average of 581 copies (maximum 716 copies, minimum 404 copies) of cfDNA per 1 mL of plasma was extracted (Table 1). Bioanalyzer instrument results showed that only DNA near 170 bases was present after extraction, which is normally considered to be derived from cfDNA, and high molecular weight DNA was not found (Figure 1). Since macromolecular DNA is primarily derived from the mononuclear cells mixed in the plasma, it becomes a hindrance in subsequent experiments and analysis. Using plasma samples from donors with lung cancer, an average of 4,127 copies (maximum 10,477 copies, minimum 974 copies) of cfDNA was extracted from 0.5 mL to 1.9 mL of plasma (Table 2). Extracted cfDNA was also examined for the *EGFR* T790M mutation using digital PCR and amplicon sequencing using the Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2. High-quality somatic mutation and sequencing analysis results were obtained (data not shown).

Table 1. cfDNA yield from plasma samples of healthy donors.

Sample	Plasma amount (mL)	cfDNA yield (No. of copies)
1	1.0	624
2	1.0	716
3	1.0	404

Table 2. cfDNA yield from plasma samples of donors with lung cancer.

Sample	Plasma amount (mL)	cfDNA yield (No. of copies)
1	1.2	6,050
2	0.9	10,477
3	1.2	6,403
4	0.8	4,677
5	0.5	974
6	0.7	1,187
7	1.7	1,160
8	2.0	2,251
9	1.9	3,676
10	1.7	3,384
11	1.8	5,154

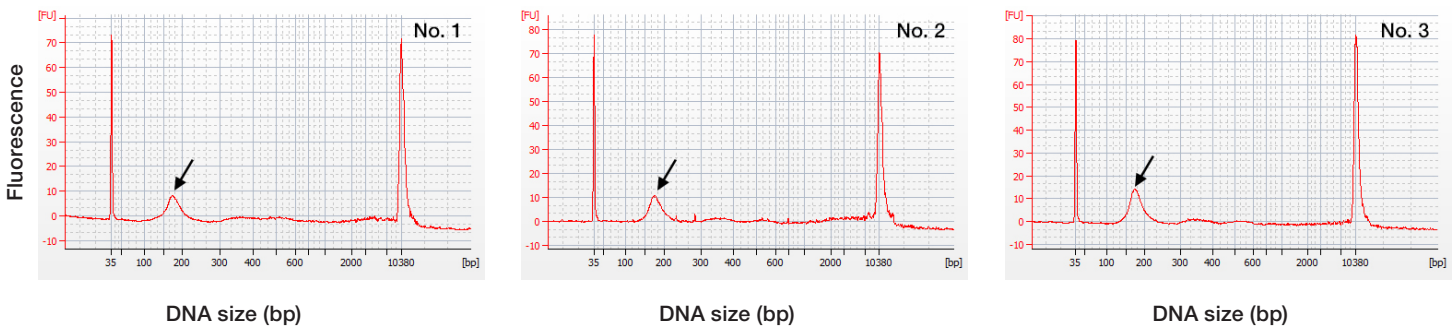

Figure 1. Bioanalyzer instrument results for cfDNA extracted from the plasma samples of healthy donors.

Discussion

In this study, cfDNA was extracted from the plasma samples of multiple donors using the MagMAX Cell-Free DNA Isolation Kit. The results indicate that the cfDNA yield per mL of plasma from donors with lung cancer is approximately 6 times higher compared to the cfDNA yield per mL of plasma from healthy donors. The yield of cfDNA obtained is comparable to traditional extraction methods, and is sufficient to analyze by digital PCR, targeted next-generation sequencing, and other genetic analysis methods.

It is important to use cfDNA of consistent quality before proceeding with genetic analysis. Results from the Bioanalyzer instrument showed that only DNA around 170 bp was found, and high molecular weight DNA was not detected. Digital PCR and amplicon sequencing were also readily performed with the extracted cfDNA. Together, these results suggest that high-quality cfDNA was obtained from the kit.

As the development of high-throughput technology has progressed, advancements in the ease of use and sample-processing capability of nucleic acid extraction methods are in greater demand. The MagMAX Cell-Free DNA Isolation Kit uses a magnetic bead–based DNA extraction method, which is adaptable to automation. Proteinase and heat treatment are unnecessary with this kit, enabling all processes to be conducted at room temperature.* Compared to traditional cfDNA extraction methods, there is also a significant reduction in processing and hands-on time (Figure 2). The genome biology class at the KINDAI University School of Medicine extracts cfDNA from as many as 1,000 samples annually. For large numbers of samples, utilizing the MagMAX Cell-Free DNA Isolation Kit instead of traditional methods can help increase workflow efficiencies and speed time to results.

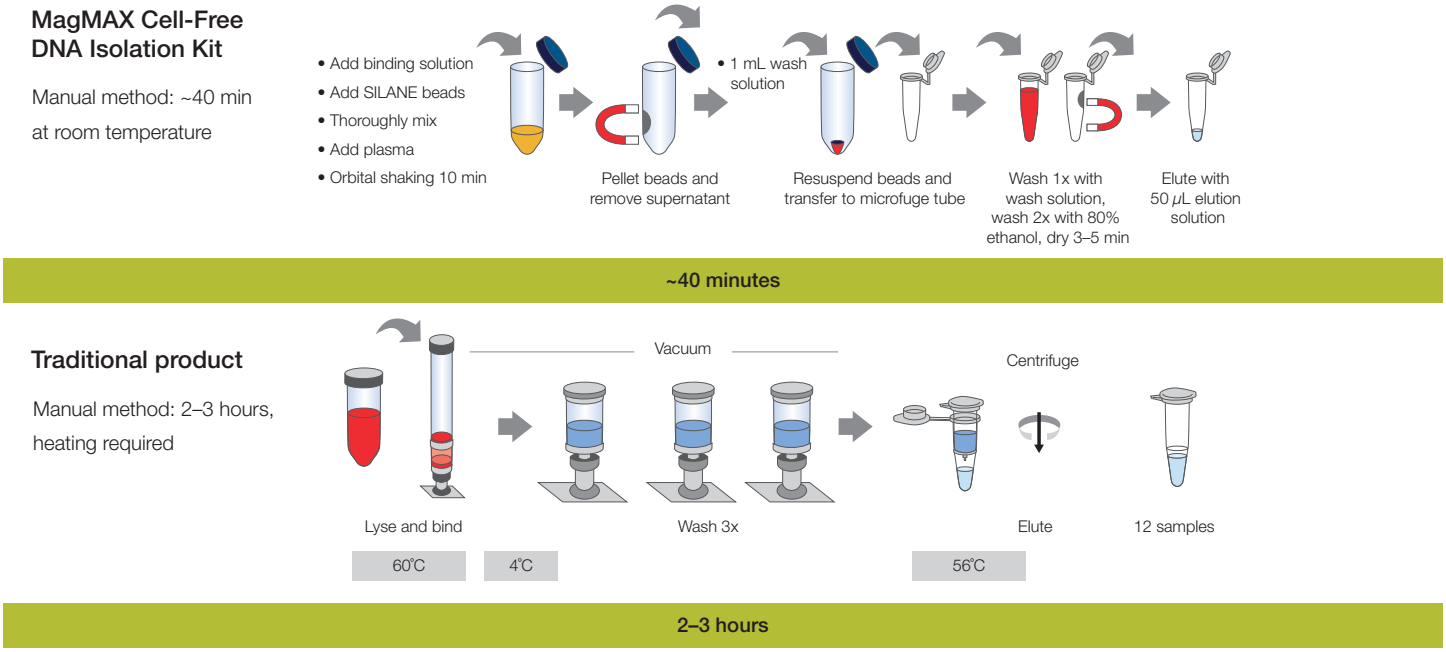

Figure 2. MagMAX Cell-Free DNA Isolation Kit protocol compared to a traditional column-based protocol.

* Applicable when EDTA tubes are used. When Streck tubes are used, a proteinase digestion is recommended. See the product manual for details.

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References

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