



## Supporting Information

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Simple and Low-Cost Sampling of Cell-Free Nucleic Acids  
from Blood Plasma for Rapid and Sensitive Detection of  
Circulating Tumor DNA

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## Supplementary

### **Simple and low-cost sampling of cell-free nucleic acids from blood plasma for rapid and sensitive detection of circulating tumor DNA**

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Supplementary Table 1- 3

Supplementary Figure 1-2

**Supplementary Table 1.** Primer sets used for conventional PCR and Sanger sequencing.

| Target gene     |         | Sequence (5' → 3')  |
|-----------------|---------|---|
| <i>Alu</i>      | 247 bp  | F: GTG GCT CAC GCC TGT AAT C<br>R: CAG GCT GGA GTG CAG TGG                    |
|                 | 115 bp  | F: CCT GAG GTC AGG AGT TCG AG<br>R: CCC GAG TAG CTG GGA TTA CA                |
| <i>β-actin</i>  |         | F: GCA CCA CAC CTT CTA CAA TGA<br>R: TGT CAC GCA CGA TTT CCC                  |
| <i>BRAF</i>     | Exon 15 | F: TCA TAA TGC TTG CTC TGA TAG GA<br>R: GGC CAA AAT TTA ATC AGT GGA           |
| <i>KRAS</i>     | G12D    | F: TGT GGT AGT TGG AGC TGG<br>R: TCA TGA AAA TGG TCA GAG AAA CC               |
|                 | G13D    | F: TGT GGT AGT TGG AGC TGG TGA G<br>R: TCA TGA AAA TGG TCA GAG AAA CC         |
|                 | Exon 2  | F: TCA TTA TTT TTA TTA TAA GGC CTG CT<br>R: CAA GAT TTA CCT CTA TTG TTG GAT C |
| <i>18S rRNA</i> |         | F: GCT TAA TTT GAC TCA ACA CGG GA<br>R: AGC TAT CAA TCT GTC AAT CCT CTC       |
| <i>GADPH</i>    |         | F: CCT GGA GAA ACC TGC CAA GTA TG<br>R: AGA GTG GGA GTT GCT TTG AAG TC        |

**Supplementary Table 2.** Comparison of the characteristics of cfDNA isolated using the column-based method and the DTBP platform from 14 colorectal cancer (CRC) samples and 10 healthy control (CTL) samples.

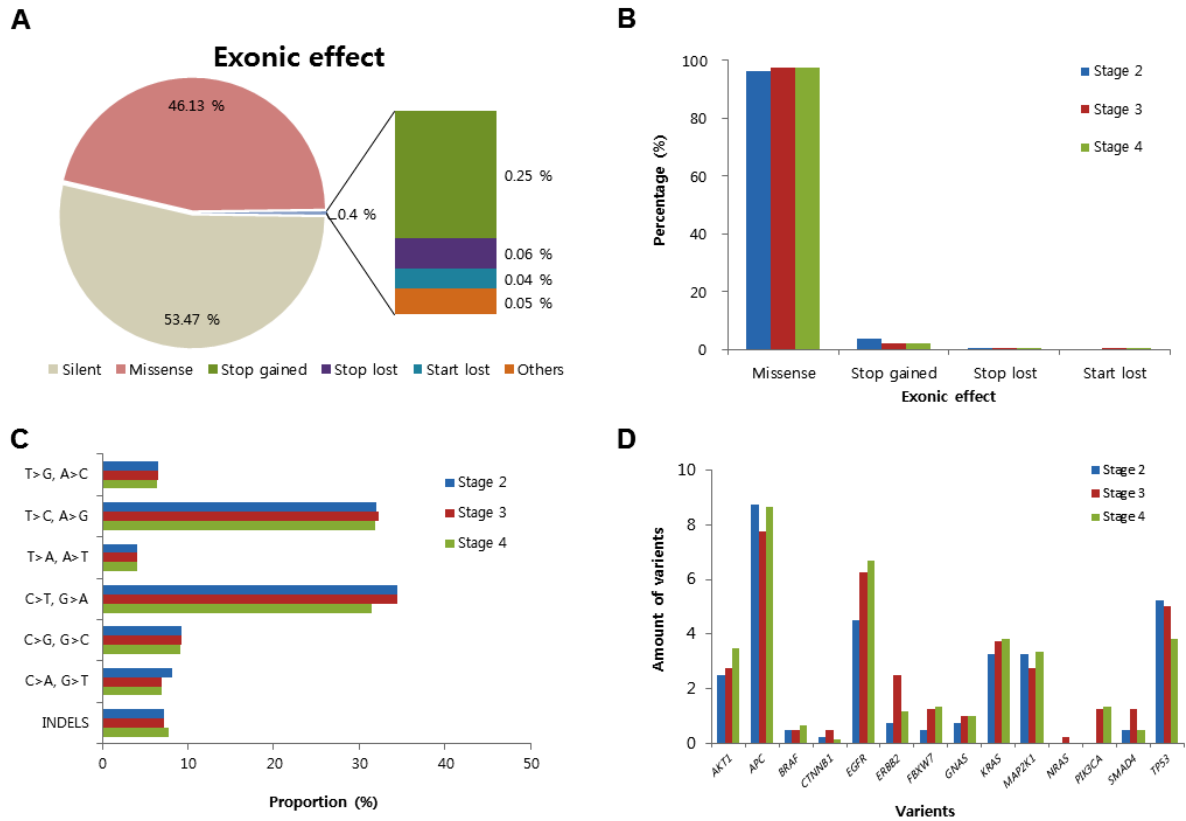
| Nr.               | Concentration<br>(ng/ $\mu\ell$ ) |       | Integrity<br>( <i>Alu</i> 247/115 ratio) |      | $\beta$ -actin $C_T$ |       |       |
|-------------------|-----------------------------------|-------|--|------|----------------------|-------|-------|
|                   | Column                            | DTBP  | Column                                   | DTBP | Column               | DTBP  |       |
| CRC<br>(Patients) | T1                                | 0.574 | 1.12                                     | 0.18 | 0.14                 | 29.95 | 37.98 |
|                   | T2                                | 0.297 | 1.13                                     | 0.26 | 0.06                 | 31.57 | 39.19 |
|                   | T3                                | 1.10  | 1.34                                     | 0.31 | 0.03                 | 30.71 | 37.25 |
|                   | T4                                | 0.626 | 1.08                                     | 0.05 | 0.06                 | 31.93 | 34.96 |
|                   | T5                                | 0.882 | 1.09                                     | 0.12 | 0.12                 | 30.46 | 37.43 |
|                   | T6                                | 0.616 | 0.641                                    | 0.15 | 0.15                 | 30.19 | 33.91 |
|                   | T7                                | 0.667 | 0.786                                    | 0.15 | 0.12                 | 29.7  | 34.98 |
|                   | T8                                | 0.337 | 1.01                                     | 0.08 | 0.03                 | 33.52 | 37.9  |
|                   | T9                                | 0.763 | 1.09                                     | 0.04 | 0.03                 | 31.76 | 38.61 |
|                   | T10                               | 0.298 | 1.46                                     | 0.36 | 0.12                 | 32.11 | 35.18 |
|                   | T11                               | 0.271 | 0.98                                     | 0.26 | 0.08                 | 31.65 | 38.84 |
|                   | T12                               | 0.217 | 0.686                                    | 0.08 | 0.06                 | 33.96 | 38.3  |
|                   | T13                               | 0.237 | 1.39                                     | 0.06 | 0.04                 | 32.77 | 34.93 |
|                   | T14                               | 0.248 | 1.13                                     | 0.35 | 0.1                  | 30.47 | 37.28 |
| CTL<br>(Healthy)  | C1                                | 0.307 | 0.529                                    | 0.38 | 0.29                 | 30.29 | 39.11 |
|                   | C2                                | 0.278 | 1.06                                     | 0.28 | 0.34                 | 30.89 | 38.97 |
|                   | C3                                | 0.427 | 0.403                                    | 0.58 | 0.57                 | 28.18 | 35.84 |
|                   | C4                                | 0.361 | 0.406                                    | 0.77 | 0.38                 | 28.15 | 36.83 |
|                   | C5                                | 0.259 | 0.191                                    | 0.65 | 0.28                 | 30.35 | 36.81 |
|                   | C6                                | 0.509 | 0.523                                    | 0.56 | 0.38                 | 25.96 | 35.09 |
|                   | C7                                | 0.596 | 0.578                                    | 0.98 | 0.29                 | 27.49 | 35.36 |
|                   | C8                                | 0.472 | 0.592                                    | 0.85 | 0.13                 | 28.07 | 36.85 |
|                   | C9                                | 0.404 | 0.937                                    | 0.51 | 0.23                 | 28.58 | 37.94 |
|                   | C10                               | 0.265 | 0.681                                    | 0.33 | 0.3                  | 30.55 | 37.09 |

**Supplementary Table 3.** Results of simple and low-cost ctDNA analyses of samples from 11 colorectal cancer patients using the DTBP platform and Sanger sequencing.

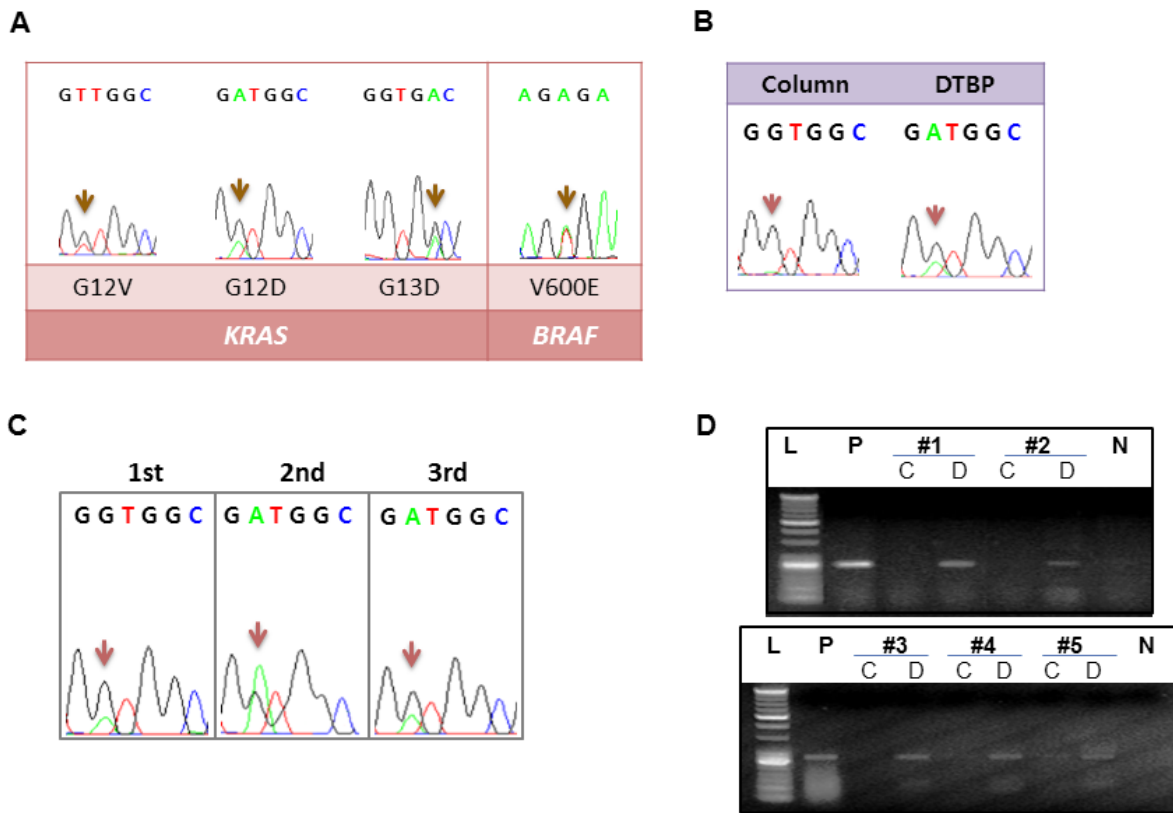
| Nr.        | Tissue       |             | ctDNA             |             | ctDNA        |             |
|------------|--------------|-------------|-------------------|-------------|--------------|-------------|
|            | OncoPanel    |             | Sanger sequencing |             | Biosensor    |             |
|            | <i>BRAF</i>  | <i>KRAS</i> | <i>BRAF</i>       | <i>KRAS</i> | <i>BRAF</i>  | <i>KRAS</i> |
| <b>S1</b>  | WT           | <b>G12D</b> | WT                | <b>G12D</b> | WT           | <b>G12D</b> |
| <b>S2</b>  | WT           | <b>G12D</b> | WT                | <b>G12D</b> | WT           | <b>G12D</b> |
| S3         | WT           | WT          | WT                | WT          | WT           | WT          |
| <b>S4</b>  | WT           | <b>G12D</b> | WT                | WT          | WT           | <b>G12D</b> |
| <b>S5</b>  | WT           | <b>G12V</b> | WT                | <b>G12V</b> | WT           | <b>G12V</b> |
| S6         | WT           | WT          | WT                | WT          | WT           | WT          |
| <b>S7</b>  | <b>V600E</b> | WT          | <b>V600E</b>      | WT          | <b>V600E</b> | WT          |
| S8         | WT           | WT          | WT                | WT          | WT           | WT          |
| <b>S9</b>  | WT           | <b>G12V</b> | WT                | WT          | WT           | WT          |
| <b>S10</b> | WT           | <b>G13D</b> | WT                | <b>G13D</b> | WT           | <b>G13D</b> |
| S11        | WT           | WT          | WT                | WT          | WT           | WT          |

Bold text indicates detected mutations.

WT: wild type



**Supplementary Figure 1.** Genetic characteristics of 14 primary tissue samples from colorectal cancer patients determined using WES. A) Number of exonic effects from 14 tissues. B) Exonic effect dependence on the cancer stage of colorectal cancer (CRC) patients. C) Dependence of the number of nucleotide substitution classes on the cancer stage. D) Dependence on mutation frequencies on the cancer stage in CRC samples.



**Supplementary Figure 2.** Simple and low-cost ctDNA analysis for clinical diagnosis. A) ctDNA was isolated from 11 plasma samples of colorectal cancer patients using the DTBP platform, and the Sanger sequencing was then performed for ctDNA analysis. The mutation sequence identified using the DTBP platform. B) Sequencing results of ctDNA for the detection of the *KRAS* mutation using the column-based method and DTBP platform. C) Reproducibility of the DTBP platform with a different sample tube of the same patient. D) Gel electrophoretic analysis of the PCR products with *KRAS* G12D (left) and *KRAS* G13D (right) mutations extracted from five CRC patients using the column-based method and the DTBP platforms (L: 500bp DNA ladder, P: positive control, #1-2: plasma samples for the G12D mutation, #3-5: plasma samples for the G13D mutation, C: column-based method, D: DTBP platform, and N: negative control).