

Dual-molecular barcode sequencing detects rare variants in tumor and cell free DNA in plasma

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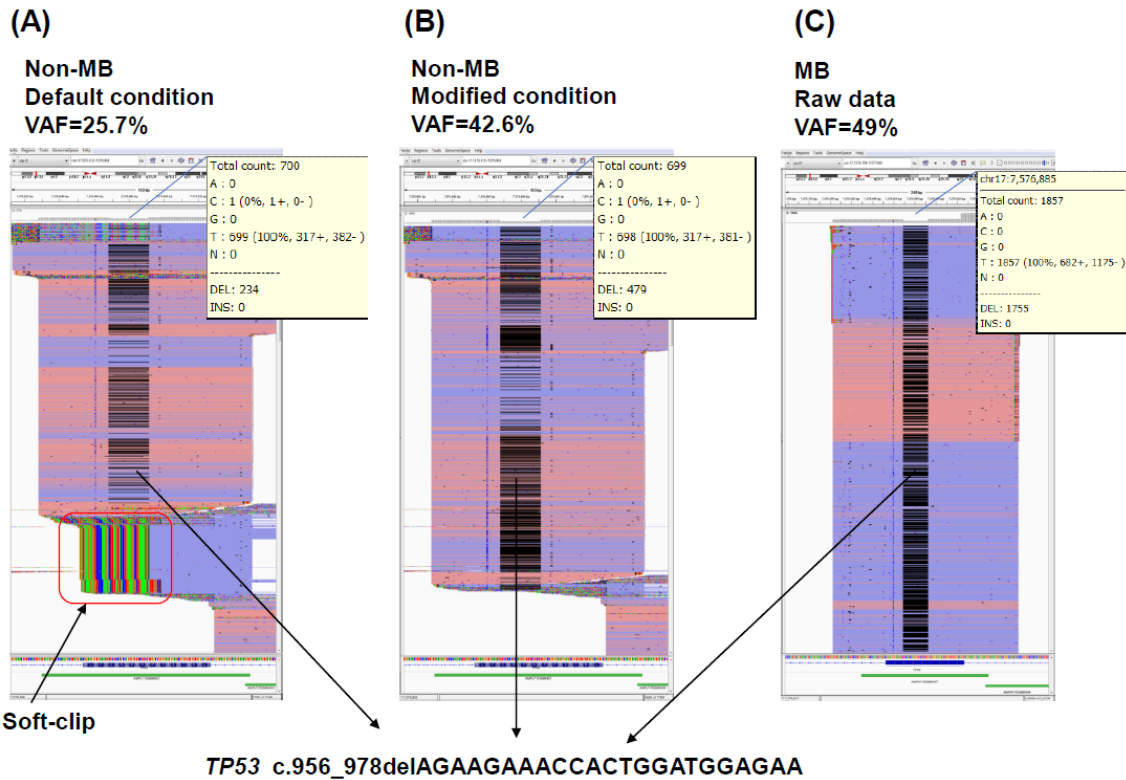
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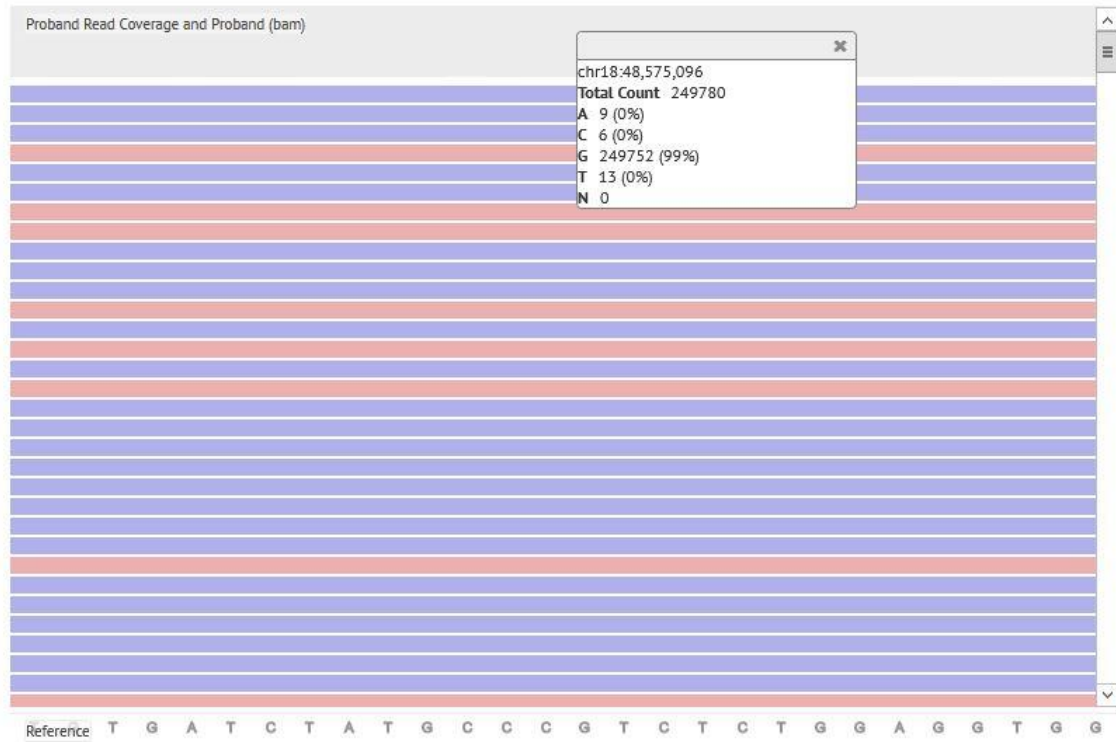
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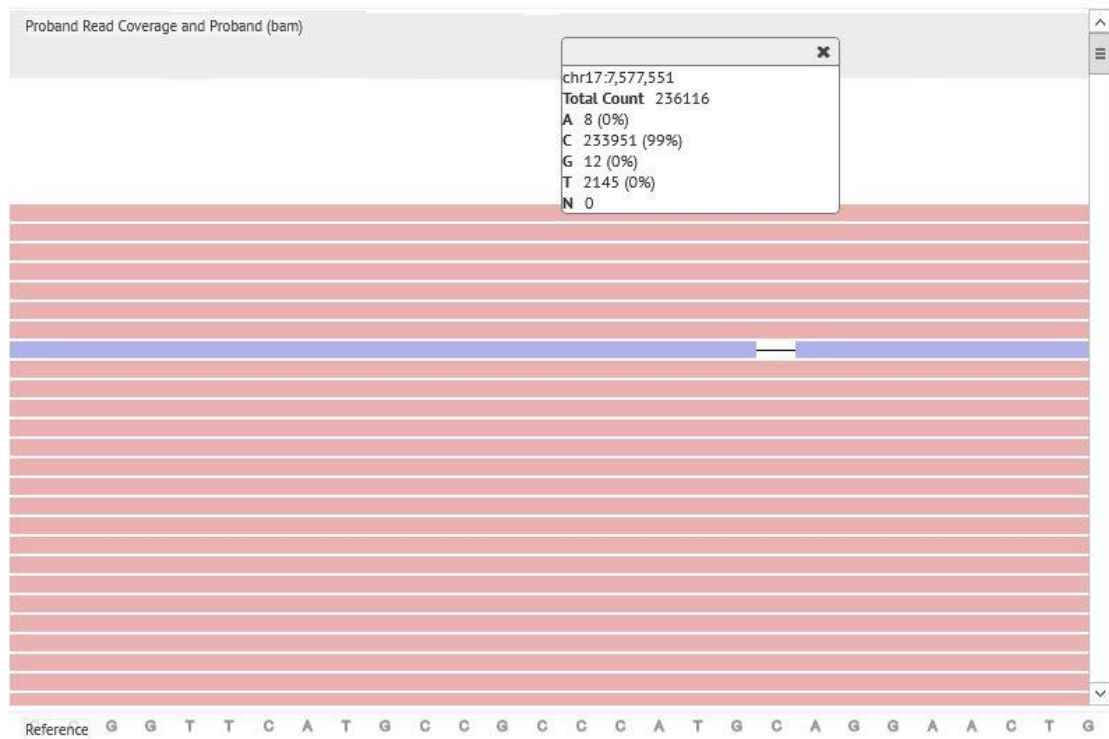
Supplemental Figure 1

Modified mapping condition improves the sequencing data. **(A-C)** BAM files of non-molecular barcode (Non-MB) and molecular barcode (MB) sequencing were visualized by IGV. Individual forward and reverse sequencing reads were represented by red and purple horizontal lines, respectively. Black line represents the 23 bp deletion mutation at *TP53* (c.956_978delIAGAAGAAACCACTGGATGGAGAA) **(A)** Sequence reads are aligned with default condition. Soft clipped bases appear as multi-colour in the red line. **(B)** After applied modified condition, full length reads are mapped on the reference. **(C)** Raw data of MB sequence was visualized. VAF, variant allele fraction.



Supplemental Figure 2

Amplicon ultra-deep sequencing data is visualized by Ion Reporter Genome Viewer. The variant allele fraction is 0.0036% at the location (chr18:48,575,096: *SMAD4*, c.290G>A, p.R97H).



Supplemental Figure 3

Amplicon ultra-deep sequencing data is visualized by Ion Reporter Genome Viewer.

The variant allele fraction is 0.91% at the location (chr17:7,577,551: *TP53*, c.730G>A p.G244S).

Supplemental Table 1. Patient characteristics and sample information

| Case number | Tumor type | Tumor size (mm) | Stage | Sample description | The fraction of tumour cells | Timing of plasma sampling |
|--------------------|----------------------------|----------------------------|--------------|----------------------------------|---|----------------------------------|
| #1 | Cholangiocarcinoma | 15 | IV | Primary tumor, biopsy | 70% | Before treatment |
| #2 | Gallbladder cancer | 31 | IV | Primary tumor, biopsy | 90% | Before treatment |
| #3 | Cholangiocarcinoma | 14 | II | Primary tumor, surgical specimen | 30% | Before treatment |
| #4 | Pancreatic cancer | 28 | IV | Primary tumor, cytology | 70% | Before treatment |
| #5 | Non-small cell lung cancer | 20 | IIIA | Primary tumor, surgical specimen | 80% | Before treatment |
| #6 | Non-small cell lung cancer | 140 | IIIA | Primary tumor, surgical specimen | 90% | Before treatment |
| #7 | Non-small cell lung cancer | 31 | IIIA | Primary tumor, surgical specimen | 70% | Before treatment |
| #8 | Non-small cell lung cancer | 35 | IIIB | Primary tumor, surgical specimen | 90% | Before treatment |
| #9 | Non-small cell lung cancer | 35 | IIB | Primary tumor, surgical specimen | 70% | Before treatment |
| #10 | Non-small cell lung cancer | 25 | IA | Primary tumor, surgical specimen | 80% | Before treatment |
| #11 | Non-small cell lung cancer | 30 | IIIA | Primary tumor, surgical specimen | 90% | Before treatment |
| #12 | Non-small cell lung cancer | 35 | IB | Primary tumor, surgical specimen | 90% | Before treatment |

Supplemental Table 2. Sequencing and coverage data

| Case number | Tumor type | Sample | Mapped reads | On target | Mean depth | Uniformity | Assay |
|-------------|-----------------------------|--------|--------------|-----------|------------|------------|-------|
| #1 | Cholangiocellular carcinoma | Plasma | 3,754,464 | 97.2% | 58,575 | 100.0% | MB |
| #2 | Gallbladder cancer | Plasma | 2,514,168 | 97.1% | 39,538 | 99.0% | MB |
| #3 | Cholangiocellular carcinoma | Plasma | 3,472,708 | 96.9% | 53,377 | 98.3% | MB |
| #4 | Pancreatic cancer | Plasma | 6,094,694 | 96.2% | 91,855 | 98.3% | MB |
| #5 | Non-small cell lung cancer | Plasma | 2,957,896 | 97.7% | 54,551 | 96.9% | MB |
| #6 | Non-small cell lung cancer | Plasma | 1,586,321 | 98.7% | 29,145 | 94.9% | MB |
| #7 | Non-small cell lung cancer | Plasma | 2,625,945 | 96.7% | 47,506 | 97.1% | MB |
| #8 | Non-small cell lung cancer | Plasma | 2,476,376 | 96.2% | 44,623 | 96.9% | MB |
| #9 | Non-small cell lung cancer | Plasma | 2,458,344 | 96.1% | 43,489 | 93.2% | MB |
| #10 | Non-small cell lung cancer | Plasma | 867,018 | 97.4% | 15,853 | 96.9% | MB |
| #11 | Non-small cell lung cancer | Plasma | 3,549,516 | 97.2% | 64,744 | 96.9% | MB |
| #12 | Non-small cell lung cancer | Plasma | 2,313,442 | 98.8% | 42,864 | 94.1% | MB |
| #1 | Cholangiocellular carcinoma | Tumor | 8,322,428 | 96.5% | 128,044 | 98.3% | MB |
| #2 | Gallbladder cancer | Tumor | 3,735,040 | 98.1% | 62,707 | 92.1% | MB |
| #3 | Cholangiocellular carcinoma | Tumor | 4,236,568 | 98.4% | 69,233 | 96.9% | MB |
| #4 | Pancreatic cancer | Tumor | 5,983,945 | 98.7% | 95,415 | 99.2% | MB |
| #5 | Non-small cell lung cancer | Tumor | 355,964 | 98.7% | 6,762 | 96.9% | MB |
| #6 | Non-small cell lung cancer | Tumor | 470,015 | 98.0% | 8,760 | 96.9% | MB |
| #7 | Non-small cell lung cancer | Tumor | 499,853 | 98.7% | 9,401 | 94.9% | MB |
| #8 | Non-small cell lung cancer | Tumor | 374,209 | 99.1% | 7,067 | 96.9% | MB |

| | | | | | | | |
|-----|-----------------------------|--------|------------|-------|-------|-------|--------|
| #9 | Non-small cell lung cancer | Tumor | 412,872 | 98.7% | 7,806 | 96.9% | MB |
| #10 | Non-small cell lung cancer | Tumor | 401,632 | 97.9% | 7,539 | 94.9% | MB |
| #11 | Non-small cell lung cancer | Tumor | 529,937 | 98.6% | 9,896 | 96.9% | MB |
| #12 | Non-small cell lung cancer | Tumor | 448,788 | 97.9% | 8,375 | 96.9% | MB |
| #1 | Cholangiocellular carcinoma | Buffy | 4,457,587 | 96.4% | 1,712 | 93.5% | Non-MB |
| #2 | Gallbladder cancer | Buffy | 3,284,762 | 96.6% | 1,265 | 88.6% | Non-MB |
| #3 | Cholangiocellular carcinoma | Buffy | 2,967,476 | 96.8% | 1,161 | 87.9% | Non-MB |
| #4 | Pancreatic cancer | Buffy | 6,872,346 | 96.0% | 2,598 | 95.1% | Non-MB |
| #5 | Non-small cell lung cancer | Buffy | 7,005,018 | 96.6% | 2,598 | 94.1% | Non-MB |
| #6 | Non-small cell lung cancer | Buffy | 2,072,413 | 97.0% | 731 | 86.6% | Non-MB |
| #7 | Non-small cell lung cancer | Buffy | 5,400,620 | 96.5% | 2,010 | 96.0% | Non-MB |
| #8 | Non-small cell lung cancer | Buffy | 10,970,074 | 97.1% | 4,068 | 96.1% | Non-MB |
| #9 | Non-small cell lung cancer | Buffy | 4,226,844 | 97.4% | 1,570 | 96.3% | Non-MB |
| #10 | Non-small cell lung cancer | Buffy | 2,265,267 | 96.9% | 797 | 88.5% | Non-MB |
| #11 | Non-small cell lung cancer | Buffy | 5,197,102 | 97.6% | 1,959 | 85.2% | Non-MB |
| #12 | Non-small cell lung cancer | Buffy | 2,124,972 | 96.5% | 744 | 88.6% | Non-MB |
| #1 | Cholangiocellular carcinoma | Plasma | 4,186,864 | 87.5% | 1,322 | 64.6% | Non-MB |
| #2 | Gallbladder cancer | Plasma | 2,308,019 | 80.2% | 661 | 76.6% | Non-MB |
| #3 | Cholangiocellular carcinoma | Plasma | 2,208,811 | 89.5% | 735 | 92.3% | Non-MB |
| #4 | Pancreatic cancer | Plasma | 2,051,196 | 89.2% | 690 | 88.3% | Non-MB |
| #5 | Non-small cell lung cancer | Plasma | 1,564,526 | 91.3% | 539 | 93.8% | Non-MB |
| #6 | Non-small cell lung cancer | Plasma | 2,211,838 | 62.0% | 302 | 87.8% | Non-MB |

| | | | | | | | |
|-----|-----------------------------|--------|------------|-------|-------|-------|--------|
| #7 | Non-small cell lung cancer | Plasma | 1,226,665 | 90.6% | 417 | 91.7% | Non-MB |
| #8 | Non-small cell lung cancer | Plasma | 3,187,923 | 87.3% | 1,026 | 96.6% | Non-MB |
| #9 | Non-small cell lung cancer | Plasma | 2,140,283 | 90.1% | 704 | 91.7% | Non-MB |
| #10 | Non-small cell lung cancer | Plasma | 1,825,259 | 67.0% | 336 | 89.0% | Non-MB |
| #11 | Non-small cell lung cancer | Plasma | 1,649,419 | 90.4% | 560 | 92.6% | Non-MB |
| #12 | Non-small cell lung cancer | Plasma | 1,448,017 | 67.0% | 257 | 88.0% | Non-MB |
| #1 | Cholangiocellular carcinoma | Tumor | 3,216,705 | 96.6% | 1,147 | 83.0% | Non-MB |
| #2 | Gallbladder cancer | Tumor | 12,303,197 | 97.7% | 4,634 | 82.4% | Non-MB |
| #3 | Cholangiocellular carcinoma | Tumor | 6,452,140 | 96.9% | 2,454 | 89.7% | Non-MB |
| #4 | Pancreatic cancer | Tumor | 5,447,544 | 97.0% | 2,086 | 89.0% | Non-MB |
| #5 | Non-small cell lung cancer | Tumor | 11,350,163 | 97.5% | 4,251 | 81.3% | Non-MB |
| #6 | Non-small cell lung cancer | Tumor | 1,485,554 | 68.0% | 184 | 75.0% | Non-MB |
| #7 | Non-small cell lung cancer | Tumor | 6,747,121 | 94.9% | 2,440 | 79.6% | Non-MB |
| #8 | Non-small cell lung cancer | Tumor | 5,060,613 | 97.4% | 1,856 | 95.2% | Non-MB |
| #9 | Non-small cell lung cancer | Tumor | 4,497,132 | 97.5% | 1,655 | 95.7% | Non-MB |
| #10 | Non-small cell lung cancer | Tumor | 2,910,896 | 96.7% | 980 | 75.3% | Non-MB |
| #11 | Non-small cell lung cancer | Tumor | 6,685,444 | 97.5% | 2,488 | 81.0% | Non-MB |
| #12 | Non-small cell lung cancer | Tumor | 2,487,433 | 96.3% | 842 | 77.5% | Non-MB |

MB, molecular barcode; Non-MB, non-molecular barcode