Detection of somatic variants and *EGFR* mutations in cell-free DNA from non-small cell lung cancer patients by ultra-deep sequencing using the ion ampliseq cancer hotspot panel and droplet digital polymerase chain reaction

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Mutation detection accuracy of ICP and ddPCR using reference cfDNA

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	ICP			ddPCR				
erence cfDNA	0	5%	1%	0.1%	0	5%	1%	0.1%
p.E746_A750del	0	7.82	1.54	0.21	0	7.51	1.23	0.14
p.T790M	5/10043 (0.05)	5.52	0.71	0.25	0	4.40	0.96	0.17
p.L858R	2/17844 (0.01)	4.10	0.28	0.08	0	5.26	1.02	0.10
G12D	3/13805 (0.02)	5.99	0.88	0.07	0	7.84	4.07	0.33
Q61K	6/33744 (0.02)	5.63	0.69	0.11				
E545K	4/18620 (0.02)	6.81	1.46	0.19				
	p.E746_A750del p.T790M p.L858R G12D Q61K	p.E746_A750del 0 p.T790M 5/10043 (0.05) p.L858R 2/17844 (0.01) G12D 3/13805 (0.02) Q61K 6/33744 (0.02)	p.E746_A750del 0 7.82 p.T790M 5/10043 (0.05) 5.52 p.L858R 2/17844 (0.01) 4.10 G12D 3/13805 (0.02) 5.99 Q61K 6/33744 (0.02) 5.63	p.E746_A750del 0 7.82 1.54 p.T790M 5/10043 (0.05) 5.52 0.71 p.L858R 2/17844 (0.01) 4.10 0.28 G12D 3/13805 (0.02) 5.99 0.88 Q61K 6/33744 (0.02) 5.63 0.69	ICP erence cfDNA 0 5% 1% 0.1% p.E746_A750del 0 7.82 1.54 0.21 p.T790M 5/10043 (0.05) 5.52 0.71 0.25 p.L858R 2/17844 (0.01) 4.10 0.28 0.08 G12D 3/13805 (0.02) 5.99 0.88 0.07 Q61K 6/33744 (0.02) 5.63 0.69 0.11	ICP erence cfDNA 0 5% 1% 0.1% 0 p.E746_A750del 0 7.82 1.54 0.21 0 p.T790M 5/10043 (0.05) 5.52 0.71 0.25 0 p.L858R 2/17844 (0.01) 4.10 0.28 0.08 0 G12D 3/13805 (0.02) 5.99 0.88 0.07 0 Q61K 6/33744 (0.02) 5.63 0.69 0.11	ICP erence cfDNA 0 5% 1% 0.1% 0 5% p.E746_A750del 0 7.82 1.54 0.21 0 7.51 p.T790M 5/10043 (0.05) 5.52 0.71 0.25 0 4.40 p.L858R 2/17844 (0.01) 4.10 0.28 0.08 0 5.26 G12D 3/13805 (0.02) 5.99 0.88 0.07 0 7.84 Q61K 6/33744 (0.02) 5.63 0.69 0.11 0	ICP ddPCR erence cfDNA 0 5% 1% 0.1% 0 5% 1% p.E746_A750del 0 7.82 1.54 0.21 0 7.51 1.23 p.T790M 5/10043 (0.05) 5.52 0.71 0.25 0 4.40 0.96 p.L858R 2/17844 (0.01) 4.10 0.28 0.08 0 5.26 1.02 G12D 3/13805 (0.02) 5.99 0.88 0.07 0 7.84 4.07 Q61K 6/33744 (0.02) 5.63 0.69 0.11 0.00 0.

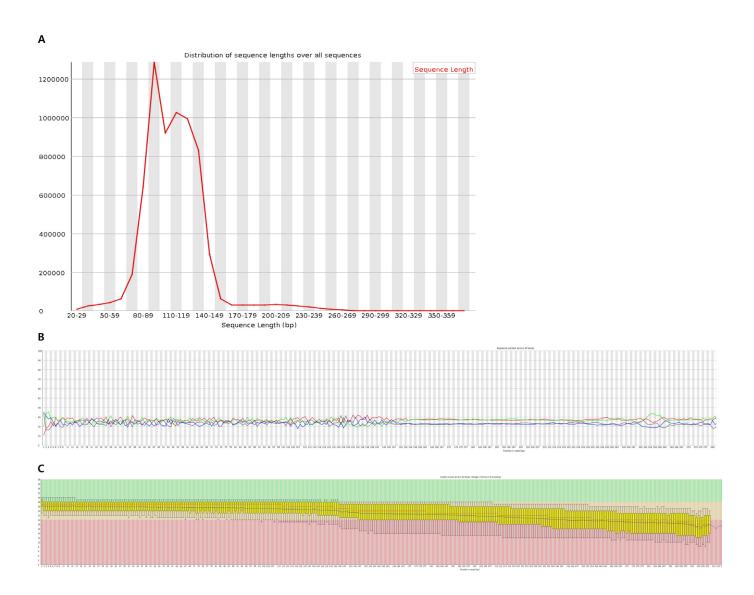
Supplementary Table 2A: Details of variants detected from 123 cfDNA using ultra-deep sequencing ICP. See Supplementary Table 2A

Supplementary Table 2B: Number of variants according to the cell type

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Variants (n)	Overall (123)	Adc (93)	Sqc (20)	Others (10)				
0	12	7	4	1				
1	15	8	6	1				
2~10	81	66	9	6				
11~20	10	9	0	1				
21~40	3	2	1	0				
100~	2	1	0	1				
Average	6.93	6.83	3.85	14.1				
Median	4	4	2	3				

Supplementary Table 3: EGFR mutational screening using the Ion ampliSeq cancer	hotspot panel
(ICP) in cfDNA samples from 123 patients with lung cancer. See Supplementary_Tab	le_3

Supplementary Table 4: *EGFR* mutations (including low-frequency mutations) in cfDNA based on ICP in 56 patients who were treated with an EGFR TKI. See Supplementary_Table_4



Supplementary Figure 1: Sequencing coverage. (A) Distribution of sequence lengths over all sequences of cfDNA. The most sequence lengths are between 60 to 170 bp. (B) GC content across all bases of cfDNA. (C) Quality scores across all bases of cfDNA.