SUPPLEMENTARY FILE

Table S1 Primers used for the amplification of mutant fragments

Primers	Direction	Sequences
19del	Forward	5’-TGGGCAGCATGTGGCACCATCT-3’
	Reverse	5’- CATGGACCCCCACA-3’
*L858R-1	Forward	5’-GTCCCTCACAGCAGGGTCTTC-3’
	Reverse	5’-CTTTGCCTCCTTCTGCATGGTAT-3’
*L858R-2	Forward	5’-GGCAGCCAGGAACGTACTGGTG-3’
	Reverse	5’-TGTCAGGAAAATGCTGGCTGACCT-3’
T790M	Forward	5’-CCTCCTTCTGGCCACCATGC-3’
	Reverse	5’-CCAATATTGTCTTTGTGTTCCCGG-3’

*L858R-1 and L858R-2 fragments detect the same exon 21 L858R mutation site. The only difference was the length of nucleotides next to the mutation site.

Table S2 The information of the NGS-based cancer IVDs of the eight participants

#	ctDNA extraction	FFPE extraction	DNA fragmentation	Isolation technique	Quality control	Sequencing platform	Minimum ctDNA quantity (ng)	Minimum FFPE quantity (ng)	Read length (bp)	Sequencing kit
1	QIAamp Circulating Nucleic Acid Kit (Qiagen)	QIAamp DNA FFPE Tissue Kit (Qiagen)	NEBNext dsDNA Fragmentase (NEB)	Hybrid capture	Qubit 2.0 and ABI7500 Real-Time PCR system (ThermoFisher)	NextSeq CN500 (Berry Genomics)	10	120	2x150	NextSeq 500 Mid Output Kit v2 (300 cycles) (Illumina)
2	QIAseq cfDNA All-in-One Kit (Qiagen)	TIANamp FFPE DNA kit (Tiangen)	LE220 High Performance Ultra-sonicator (Covaris)	Hybrid capture	LabChip GX Touch HT Capillary Electrophoresis (PerkinElmer) and ABI7500 Real-Time PCR system (ThermoFisher)	NextSeq 500 (Illumina)	30	500	2x150	NextSeq 500 Mid Output Kit v2 (150 cycles) (Illumina)
3	MagMAX Cell-Free DNA Isolation Kit (ThermoFisher)	blackPREP FFPE DNA Kit (Analytik Jena)	Covaris M220 (Covaris)	Hybrid capture	Agilent Bioanalyzer 2100 (Agilent Tech), Qubit 3.0, and ABI7500 Real-Time PCR system (ThermoFisher)	HiSeq X Ten (Illumina)	10	N/A	2x150	HiSeq Ten Reagent Kit v2.5 (Illumina)
4	QIAamp Circulating Nucleic Acid Kit (Qiagen)	GeneRead DNA FFPE kit (Qiagen)	QIAseq FX DNA Library Kit (Qiagen)	Hybrid capture	Agilent 4200 TapeStation Instrument (Agilent Tech)	NextSeq 500 (Illumina)	40	300	2x150	NextSeq 500/550 High Output Kit v2 (300 cycles) (Illumina)
5	GenoPrep	QIAamp	N/A	Multiplex	Qubit 2.0	Ion Proton	10	10	200	Ion AmpliSeq

	DNA extraction kit (Genosaber)	DNA FFPE Tissue Kit (Qiagen)		PCR	(ThermoFisher) and Agilent 4200 TapeStation Instrument (Agilent Tech)	(ThermoFisher)				(Thermo Fisher)
6	MagMAX Cell-Free DNA Isolation Kit (ThermoFisher)	QIAamp DNA FFPE Tissue Kit (Qiagen)	Bioruptor sonication system (Diagenode)	Hybrid capture	Agilent Bioanalyzer 2100 (Agilent Tech), and LabChip GX Touch HT Capillary Electrophoresis (PerkinElmer)	NextSeq 550AR (Annoroad)	10	500	*2x150 2x75	NextSeq 550 High Output Kit (Illumina)
7	QIAamp Circulating Nucleic Acid Kit (Qiagen)	QIAamp DNA FFPE Tissue Kit (Qiagen)	Bioruptor sonication system (Diagenode)	Hybrid capture	Agilent 4200 TapeStation Instrument (Agilent Tech), and ABI7500 Real-Time PCR system (Thermo Fisher)	NextSeq 500 (Illumina)	15	50	2x75	NextSeq 500 High Output Kit (Illumina)
8	QIAamp Circulating Nucleic Acid Kit (Qiagen)	QIAamp DNA FFPE Tissue Kit (Qiagen)	NEBNext dsDNA Fragmentase (NEB)	Hybrid capture	Agilent Bioanalyzer 2100 (Agilent Tech), and Qubit 2.0 (ThermoFisher)	BGISEQ-500 (BGI)	10	100	2x50	BGISEQ-500RS High-throughput sequencing kit (PE50) (BGI)

*The read length of participant #6 was 2? 50bp for plasma and 2? 5bp for FFPE samples.

Abbreviations: ctDNA, circulating tumor deoxyribonucleic acid; FFPE, formalin-fixed, paraffin-embedded; bp, base pair; N/A, not applicable.

Table S3 Testing parameters of the eight participants

	Average depth of coverage					**Q20 (%)**					**On-target rate (%)**				
	Plasma			FFPE		Plasma			FFPE		Plasma			FFPE	
#	Q0	Q1	Q2	Q1	Q2	Q0	Q1	Q2	Q1	Q2	Q0	Q1	Q2	Q1	Q2
1	2173	338	697	554	875	*99.96/ 99.63	*99.96/ 99.60	*99.96/ 99.62	*99.96/ 99.56	*99.97/ 99.61	93.88	93.54	93.89	93.18	93.22
2	4200	4100	4200	4800	6100	NE	NE	NE	NE	NE	20.00	19.00	20.00	21.00	22.00
3	13152	13160	7756	NE	NE	52.38	49.14	49.08	48.99	49.09	86.78	86.73	86.54	NE	NE
4	2467	2850	3014	2913	1732	NE	NE	NE	NE	NE	34.91	26.16	26.84	60.26	61.72
5	376000	351000	408000	446000	421000	80.04	80.39	78.38	79.32	73.02	97.36	97.08	95.93	99.84	99.83
6	2580	2351	2367	2343	2356	90.02	89.53	88.92	94.88	94.51	15.42	15.23	15.37	17.50	17.22
7	4863	5260	4981	941	1004	NE	NE	NE	NE	NE	43.04	43.20	40.73	18.89	25.28
8	3252	3156	3531	862	841	NE	NE	NE	NE	NE	26.15	25.85	25.68	21.25	21.52

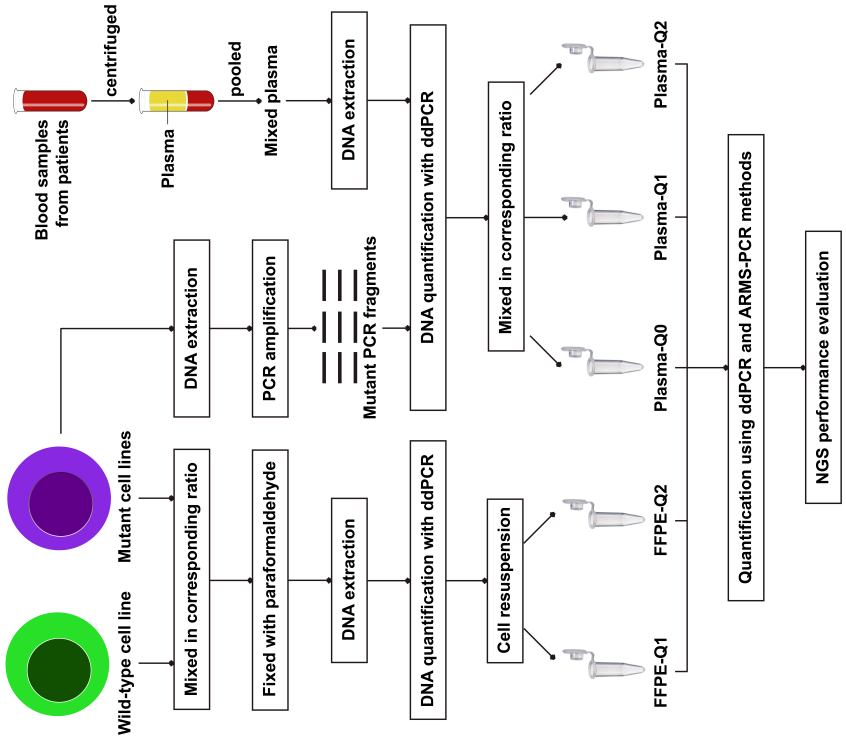
*Two values of Q20 were presented in participant #1 as bi-directional sequencing was used.
Abbreviations: FFPE, formalin-fixed, paraffin-embedded; NE, not evaluated.

Table S4 Number of reads of each target mutation of the eight participants

	19del reads (wild-type/mutant)					**L858R reads (wild-type/mutant)**					**T790M reads (wild-type/mutant)**				
	Plasma			FFPE		Plasma			FFPE		Plasma			FFPE	
#	Q0	Q1	Q2	Q1	Q2	Q0	Q1	Q2	Q1	Q2	Q0	Q1	Q2	Q1	Q2
1	2083/1	N/A	453/7	516/7	452/22	2224/23	N/A	817/29	657/16	605/55	1451/12	N/A	811/36	462/9	262/22
2	N/A	4417/6	4369/9	4961/38	6415/ 204	4752/47	4514/46	4721/60	4627/76	6108/ 421	4436/9	4214/4	4183/11	4544/80	6239/ 472
3	16373/ 127	17025/ 107	9973/ 147	N/A	N/A	16022/ 92	16270/ 252	9956/ 336	N/A	N/A	14903/ 108	15412/ 141	8238/ 229	N/A	N/A
4	4357/8	3061/17	4053/70	4224/53	2222/84	2682/49	2532/81	3453/88	3394/64	1978/ 164	2126/5	2344/38	3161/ 160	2325/52	1490/ 113
5	149700/ 300	87422/ 404	134927/ 1047	109528/ 993	12747/ 348	320394/ 1296	255327/ 2635	354268/ 7611	265395/ 4334	237598/ 16258	52295/ 121	36609/ 183	41254/ 553	26319/ 396	31321/ 1726
6	4082/45	3439/36	3653/18	N/A	N/A	3240/28	2885/48	2899/ 188	2087/32	2142/ 121	2186/3	2055/51	2338/ 168	2426/48	2501/ 197
7	5284/0	5924/9	5518/24	1057/6	1108/22	5385/70	6115/ 134	6126/ 162	955/15	1086/89	6051/31	6474/69	6785/ 123	954/20	1134/89
8	3891/0	3701/0	4105/0	861/9	813/64	3026/8	3024/22	3288/32	558/11	480/38	6335/14	5602/19	6156/60	798/10	747/57

Abbreviations: del, deletion; T, threonine; M, methionine; L, leucine; R, arginine; FFPE, formalin-fixed, paraffin-embedded; NE, not evaluated.

Figure S1 The workflow of the preparation of reference materials



For the preparation of FFPE reference materials, wildtype cells were spiked with mutant cells and the mixtures were then fixed with paraformaldehyde. For the preparation of plasma reference materials, nucleic acid fragments containing 19del, L858R or T790M mutations were amplified from the gDNA of H1650 and H1975 cell lines using corresponding primers and were spiked into pooled plasma.