

Supplementary

Table S1 Sequence information of plasmids

Plasmid	Gene	COSMIC ID	Amino acid change	Nucleotide change	Mutation site	Sequence information
Plasmid-1	<i>PIK3CA</i>	763	p.E545K	c.1633G>A	>3:178936091	TCCAGAGGGGAAAAATATGACAAAGAAAGCTATATAAGATATTATTTATTTTACAGAGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAAATGACAAAGAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTA*AGCAGGAGAAAGATTTCTATGGAGTCCACAGGTAAGTGCTAAAATGGAGATTCTCTGTTTCTTTTCTTTATTACAGAAAAATAACTGAATTTGGCTGATCTCAGCATGTTTACCATACCTATTGGAATAAATAAAGCAGAATTTAC
Plasmid-2	<i>EGFR</i>	6224	p.L858R	c.2573T>G	>7:55259515	CTTCCCATGATGATCTGTCCCTCACAGCAGGGTCTTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGTGCACCGCGACCTGGCAGCCAGGAACGTAAGTGGTGAACACCGCAGCATGTCAAGATCACAGATTTTGGGCG*GGCCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAAGAGGCCAAAGTAAGGAGGTGGCTTTAGGTTCAGCCAGCATTTTCTGACACCAGGGAACAGGCTGCCCTCCACTAGCTGTATTGTTAACACATGCAGGGGAGGATG
Plasmid-3	<i>EGFR</i>	6225	p.E746_A750delELREA	c.2236_2250del15GGAATTAAGAGAAGC	>7:55242466	TCGGGGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGGCACCATCTCACAATTGCCAGTTAACGTTCTTCTCTCTCTGTGCATAGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATCCCGTCGCTATCAAGG*AATTAAGAGAAGCAACATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTCTGCTTTGCTGTGTGGGGTCCATGGCTCTGAACCTCAGGCCACCCTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC
Plasmid-4	<i>EGFR</i>	6240	p.T790M	c.2369C>T	>7:55249071	GTCCATGTGCCCTCCTTCTGGCCACCATGCGAAGCCACACTGACGTGCCTCTCCCTCCCTCCAGGAAGCCTACGTGATGGCCAGCGTGGACAACCCCCACGTGTGCCGCTGCTGGGCATCTGCCTCACCTCCACCGTGCAGCTCATCAT*GCAGCTCATGCCCTTCGGCTGCCTCCTGGACTATGTCGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAACTGGTGTGTGCAGATCGCAAGGTAATCAGGGAAGGAGATACGGGGAGGGGAGATAAGGAGCCAGGATCCT
Plasmid-5	<i>KRAS</i>	521	p.G12D	c.35G>A	>12:25398284	ATGGTCAGAGAAACCTTTATCTGTATCAAAGAATGGTCTGCACCAGTAATATGCATATTAACAAGATTACCTCTATTGTTGGATCATATTCGTCACAAAATGATTCTGAATTAGCTGTATCGTCAAGGCACCTTTGCCACCCAT*GAGCTCCAACACCACAAGTTTATATTCAGTCATTTTCAGCAGGCCCTATAATAAAAATAATGAAAATGTGACTATATTAGAACATGTCACACATAAGGTTAATACTATCAAACTCCACCGTACCTTTAATAACAACTCACCTT
Plasmid-6	<i>NRAS</i>	580	p.Q61K	c.181C>A	>1:115256530	CACAAAGATCATCCTTTTCAGAGAAAATAATGCTCCTAGTACCTGTAGAGGTTAATATCCGCAAATGACTTGCTATTATTGATGGCAAATACACAGAGGAAGCCTTCGCTGTCTCATGATTGGTCTCTCATGGCACTGTACTCTTT*TCAGCTGTATCCAGTATGTCCAACAAACAGGTTTACCATCTATAACCCTTGTCTTCTGTAAGAATCCTGGGGTGTGGAGGTAAGGGGGCAGGGAGGGAGGGAAGTTCAATTTTATTAACACAGGGAATGCAATGCTATTG
Plasmid-7	<i>KRAS</i>	549	p.Q61K	c.181C>A	>12:25380277	TACTCCTAATGTCAGCTTATTATATTCAATTTAAACCCACCTATAATGGTGAATATCTCAAATGATTTAGTATTATTTATGGCAAATACACAAAGAAAGCCCTCCCAGTCCCTCATGACTGGTCCCTCATTGCACTGTACTCCTTT*ACCTGCTGTGTCGAGAATATCCAAGAGACAGGTTTCTCCATCAATTACTACTTGCTTCTGTAGGAATCCTGAGAAGGGAGAAACACAGTCTGGATTATTACAGTGCACCTTTACTTCAAAAAAGGTGTATATACAACCAACAACAA
Plasmid-8	<i>BRAF</i>	475	p.V600E	c.1799_1800TG>AA	>7:140453135	TATAGTTGAGACCTTCAATGACTTTCTAGTAACCTCAGCAGCATCTCAGGGCCAAAAATTAATCAGTGGAAAAATAGCCTCAATTTACCATCCACAAAATGGATCCAGACAACCTGTTCAAACCTGATGGGACCCACTCCATCGAGATTTT*CTGTAGCTAGACCAAAATCACCTATTTTACTGTGAGGTCTTCATGAAGAAATATCTGAGGTGTAGTAAGTAAAGGAAAACAGTAGATCTCATTTTCTATCAGAGCAAGCATTATGAAGAGTTTAGGTAAGAGATCTAATTTCTAT
Plasmid-9	<i>AKT1</i>	33765	p.E17K	c.49G>A	>14:105246551	CCGAGAGGCCAAGGGGATACTTACGCGCCACAGAGAAGTTGTTGAGGGGAGCCTCACGTTGGTCCACATCCTGCGGCCGCTCCTTGTAGCCAATGAAGGTGCCATCATTCTTGGAGGAGGAAGTAGCGTGGCCGCCAGGTCTTGTGACTT*CCCTACAGACGTGCGGGTGGTGGAGAGCCACGCACACTCTACCCGTCAGACCCCTGCCAGGCAGCCAGGCAGGAAGTGGGTGTGCCAGGACAGATGTGCTGGGATGCCTGAGTCCAGGGGGCAGGCGCGGTACGGGAGCTGTTTCTTAG
Plasmid-10	<i>KIT</i>	1314	p.D816V	c.2447A>T	>4:55599321	TAGTTTTCACTCTTTACAAGTTAAAATGAATTTAAATGGTTTTCTTTCTCCTCCAACCTAATAGTGTATTACAGAGACTTGGCAGCCAGAAATATCCTCCTTACTCATGGTCCGATCACAAAGATTTGTGATTTTGGTCTAGCCAGAGT*CATCAAGAATGATTTAATATGTGGTTAAAGGAAACGTGAGTACCATTCTGCTTGACAGTCCGCAAAGGATTTTATGTTTCAACTTTTCGATAAAAATTTGTTTCTGTGATTTTCATAATGAAATCCTGTCTAGGGATATCACAC

The letters before * represent the mutation site. COSMIC, the Catalogue of Somatic Mutations in Cancer.

Table S2 Information of patients

Sample ID	Vaf (L-library) (%)	Input (ng)	Cancer type	Vaf (detected by ds-library) (%)
Sample 1	0.27	10	Lung cancer	0.50
Sample 2	0.75	10	Lung cancer	0.50
Sample 3	1.36	10	Lung cancer	0.52
Sample 4	0.70	10	Lung cancer	0.57
Sample 5	0.21	10	Lung cancer	0.57
Sample 6	1.38	10	Lung cancer	0.76
Sample 7	0.66	10	Lung cancer	0.87
Sample 8	1.36	10	Lung cancer	0.99
Sample 9	0.44	10	Lung cancer	1.16
Sample 10	0.88	10	Lung cancer	1.17
Sample 11	0.68	10	Lung cancer	1.20
Sample 12	2.15	10	Lung cancer	1.43
Sample 13	0.62	5	Colorectal cancer	1.70
Sample 14	2.18	5	Lung cancer	1.73
Sample 15	4.40	5	Lung cancer	2.82
Sample 16	6.11	2	Lung cancer	4.19
Sample 17	4.97	5	Lung cancer	2.22
Sample 18	4.35	5	Lung cancer	3.93
Sample 19	1.40	2.55	Lung cancer	2.74
	4.51	2.55	Lung cancer	4.89
Sample 20	2.19	5	Lung cancer	3.25
Sample 21	6.62	2	Lung cancer	4.19
Sample 22	3.31	5	Esophageal squamous cell carcinomas	1.96
Sample 23	3.36	5	Lung cancer	2.48
Sample 24	1.89	10	Lung cancer	1.41
Sample 25	2.97	5	Cholangiocarcinoma	3.21
Sample 26	1.38	10	Liver cancer	1.19

L-library, library using a large proportion of magnetic beads.

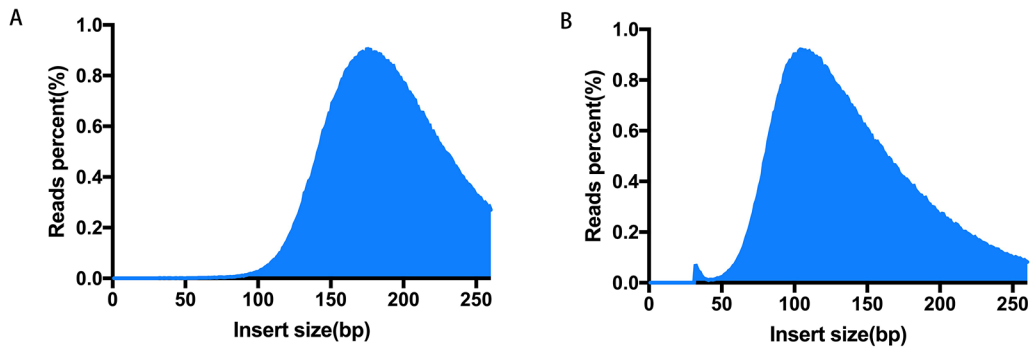


Figure S1 Fragment sizes of the L- and R-libraries in the Horizon cfDNA standard. (A) Insert size distribution of the R-library preparation methods. (B) Insert size distribution of the L-library preparation methods with L-library showing significant enrichment of the <math>< 150</math> bp fragments. L-library, library using a large proportion of magnetic beads; R-library, library using a regular proportion of magnetic beads. cfDNA, cell-free DNA.

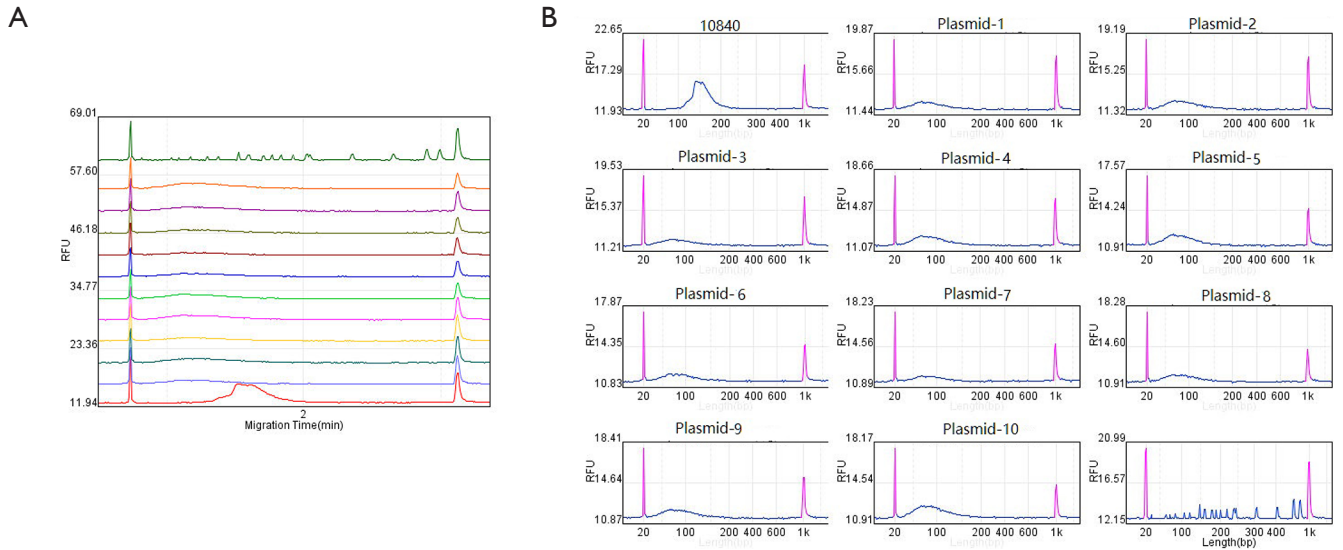


Figure S2 The sizes of fragmented plasmid and cell line DNA.

Table S3 Variant allele frequency of 10 mutations selected in plasmid-simulated cfDNA samples with the L- and R-library

DNA input	Mutation	10^{-5}		5×10^{-7}		5×10^{-8}	
		L-library (%)	R-library (%)	L-library (%)	R-library (%)	L-library (%)	R-library (%)
20 ng	PIK3CA-E545K	46.59	32.89	2.82	1.30	NA	NA
	EGFR-L858R	69.65	58.29	8.39	4.89	1.03	NA
	EGFR-19DEL	41.02	31.33	2.36	1.97	0.43	NA
	EGFR-T790M	73.10	56.97	10.71	4.51	1.49	NA
	KRAS-G12D	56.18	39.44	5.91	2.61	1.27	0.62
	NRAS-Q61K	66.44	43.76	7.39	2.16	0.71	0.68
	KRAS-Q61K	39.07	22.02	2.28	0.64	NA	NA
	BRAF-V600E	40	28.79	2.09	0.81	0.33	NA
	AKT1-E17K	70.91	55.34	9.98	3.86	0.91	NA
	KIT-D816V	60.33	47.29	6.14	2.65	0.31	NA
5 ng	PIK3CA-E545K	44.79	34.73	2.63	1.32	NA	NA
	EGFR-L858R	70.38	59.62	8.57	4.53	NA	NA
	EGFR-19DEL	41.23	35.46	3.96	2.01	NA	NA
	EGFR-T790M	72.28	59.36	12.77	5.34	1.15	0.89
	KRAS-G12D	55.81	38.55	4.53	3.53	0.94	NA
	NRAS-Q61K	66.25	45.14	6.09	2.33	1.04	NA
	KRAS-Q61K	39.89	18.27	1.90	1.47	NA	NA
	BRAF-V600E	41.49	25.36	1.60	1.78	NA	NA
	AKT1-E17K	73.34	54.36	8.44	3	0.61	NA
	KIT-D816V	62.79	46.84	5.15	2.34	0.78	NA

“NA” indicates that the mutation was not detected. L-library, library using a large proportion of magnetic beads; R-library, library using a regular proportion of magnetic beads. cfDNA, cell-free DNA.

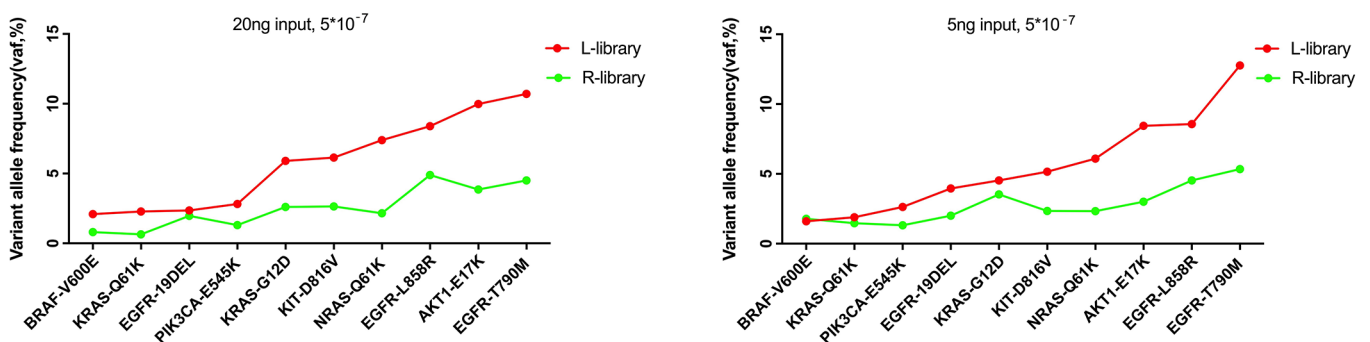


Figure S3 Variant allele frequency of 10 mutations in the 5×10^{-7} plasmid-simulated cfDNA samples with the L- and R-library. L-library, library using a large proportion of magnetic beads; R-library, library using a regular proportion of magnetic beads. cfDNA, cell-free DNA.

Table S4 Variant allele frequency of selected 10 mutations in the plasmid-simulated cfDNA samples with the L- and R-library using different read lengths

Sample, DNA input	Mutation	30–90 bp		91–150 bp		>150 bp		All	
		L-library (%)	R-library (%)	L-library (%)	R-library (%)	L-library (%)	R-library (%)	L-library (%)	R-library (%)
10 ⁻⁵ , 20 ng	PIK3CA-E545K	90.23	NA	54.80	46.65	39.37	31.45	46.59	32.89
	EGFR-L858R	95.02	95.58	79.08	74.89	63.57	56.54	69.65	58.29
	EGFR-19DEL	27.53	NA	54.21	40.35	41.98	31.43	41.02	31.33
	EGFR-T790M	97.33	97.70	83.57	78.17	64.87	54.67	73.10	56.97
	KRAS-G12D	91.61	93.78	63.06	54.61	45.78	36.71	56.18	39.44
	NRAS-Q61K	94.85	94.57	71.14	56.97	53.63	40.31	66.44	43.76
	KRAS-Q61K	90.18	NA	46.68	37.34	29.08	20.20	39.07	22.02
	BRAF-V600E	86.66	86.27	50.54	44.92	33.37	27.31	40.00	28.79
	AKT1-E17K	96.97	97.61	78.53	73.75	60.96	52.89	70.91	55.34
	KIT-D816V	94.89	92.83	72.05	65.44	53.80	45.39	60.33	47.29
5×10 ⁻⁷ , 20 ng	PIK3CA-E545K	29.50	NA	2.71	2.56	1.53	1.23	2.82	1.30
	EGFR-L858R	54.11	NA	11.98	10.10	5.97	4.38	8.39	4.89
	EGFR-19DEL	NA	NA	4.27	4.80	2.27	1.98	2.36	1.97
	EGFR-T790M	56.41	NA	15.58	10.17	6.72	3.86	10.71	4.51
	KRAS-G12D	39.91	NA	6.88	4.99	3.61	2.36	5.91	2.61
	NRAS-Q61K	38.69	NA	9.02	3.89	4.68	1.60	7.39	2.16
	KRAS-Q61K	27.19	NA	2.45	1.38	1.12	0.64	2.28	0.64
	BRAF-V600E	13.71	NA	3.10	1.80	1.67	0.75	2.09	0.81
	AKT1-E17K	61.94	NA	13.43	8.66	6.49	3.50	9.98	3.86
	KIT-D816V	48.08	NA	9.19	5.45	4.86	2.54	6.14	2.65
5×10 ⁻⁸ , 20 ng	PIK3CA-E545K	NA	NA	NA	NA	NA	NA	NA	NA
	EGFR-L858R	NA	NA	1.62	NA	0.78	NA	1.03	NA
	EGFR-19DEL	NA	NA	1.29	NA	NA	NA	0.43	NA
	EGFR-T790M	NA	NA	2.93	NA	1.10	NA	1.49	NA
	KRAS-G12D	11.89	NA	1.27	NA	0.67	NA	1.27	0.62
	NRAS-Q61K	NA	NA	0.77	1.14	NA	0.70	0.71	0.68
	KRAS-Q61K	NA	NA	NA	NA	NA	NA	NA	NA
	BRAF-V600E	NA	NA	0.66	NA	NA	NA	0.33	NA
	AKT1-E17K	NA	NA	1.43	NA	0.65	NA	0.91	NA
	KIT-D816V	NA	NA	NA	0.56	NA	NA	0.31	NA
10 ⁻⁵ , 5 ng	PIK3CA-E545K	87.77	NA	51.69	48.07	36.79	33.51	44.79	34.73
	EGFR-L858R	95.22	NA	78.86	76.01	61.94	58.25	70.38	59.62
	EGFR-19DEL	29.50	NA	53.50	47.68	42.15	35.49	41.23	35.46
	EGFR-T790M	96.55	NA	80.53	79.38	61.44	56.74	72.28	59.36
	KRAS-G12D	92.48	NA	62.22	55.33	45.08	36.15	55.81	38.55
	NRAS-Q61K	94.55	NA	66.22	59.11	50.67	42.07	66.25	45.14
	KRAS-Q61K	96.72	NA	46.99	31.64	29.86	17.31	39.89	18.27
	BRAF-V600E	89.23	NA	50.95	39.33	32.74	22.83	41.49	25.36
	AKT1-E17K	97.42	NA	79.31	70.29	61.21	50.93	73.34	54.36
	KIT-D816V	97.34	NA	72.06	64.22	54.49	44.50	62.79	46.84
5×10 ⁻⁷ , 5 ng	PIK3CA-E545K	NA	NA	2.80	2.65	1.70	1.34	2.63	1.32
	EGFR-L858R	NA	NA	11.99	10.73	5.25	4.43	8.57	4.53
	EGFR-19DEL	NA	NA	7.67	3.40	4.08	1.89	3.96	2.01
	EGFR-T790M	NA	NA	18.22	11.79	7.77	5.04	12.77	5.34
	KRAS-G12D	NA	NA	6.44	5.11	3.12	3.11	4.53	3.53
	NRAS-Q61K	NA	NA	6.59	4.52	3.35	2.37	6.09	2.33
	KRAS-Q61K	NA	NA	1.92	NA	0.71	0.89	1.90	1.47
	BRAF-V600E	NA	NA	1.57	3.34	0.83	1.58	1.60	1.78
	AKT1-E17K	NA	NA	10.51	6.39	5.02	2.36	8.44	3.00
	KIT-D816V	NA	NA	6.96	5.23	3.51	2.14	5.15	2.34
5×10 ⁻⁸ , 5 ng	PIK3CA-E545K	NA	NA	NA	NA	NA	NA	NA	NA
	EGFR-L858R	NA	NA	NA	NA	NA	NA	NA	NA
	EGFR-19DEL	NA	NA	NA	NA	NA	NA	NA	NA
	EGFR-T790M	NA	NA	NA	NA	1.06	NA	1.15	0.89
	KRAS-G12D	NA	NA	NA	NA	NA	NA	0.94	NA
	NRAS-Q61K	NA	NA	NA	NA	NA	NA	1.04	NA
	KRAS-Q61K	NA	NA	NA	NA	NA	NA	NA	NA
	BRAF-V600E	NA	NA	NA	NA	NA	NA	NA	NA
	AKT1-E17K	NA	NA	NA	NA	NA	NA	0.61	NA
	KIT-D816V	NA	NA	1.52	NA	0.71	NA	0.78	NA

“NA” indicates that the mutation was not detected. L-library, library using a large proportion of magnetic beads; R-library, library using a regular proportion of magnetic beads. cfDNA, cell-free DNA.