Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary information

Novel platform for precise quantification of gene editing products based on microfluidic chip-based digital PCR

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Supplementary Tables

Table S1 The information of Primers and probes used in the study

Species	Primers and Probes	Sequence information（5'-3'）
Oryza sativa	F-primer	TCGCGCTCATTGTCTTCCT
	R-primer	TGGTCTTGAACATTCTCGTTGTG
	FAM-1	CCCCTACTGCCGCCG
	FAM-2	CGTCCCCTACTGCCGCCG
	FAM-3	CCCTACTGCCGCCGCCA
	HEX-1	TGCTGCTCTCGCCGTCCCCT
	HEX-2	CTGCTCTCGCCGTCC
Glycine max	F-primer	GCTGATGCTGGGCATAAACC
	R-primer	CGTAAGAGATTAAATTAGCAGGAAAACC
	HEX	AAGCAAGAGACGTTCTA
	FAM	AGGTGGAAAGGTTTTC
Brassica napus	F1-primer	CGACCTTCCTGGTCCGTACTC
	R1-primer	GCTTGGCAAGAACGGAGAAG
	FAM	TTGCCCATGCTGGCT
	F2-primer	GGCCAGGGCTTCCGTGAT
	R2-primer	CCGTCGTTGTAGAACCATTGG
	HEX	AGTCCTTATGTGCTCCACTTTCTGGTGCA

Table S2 The source of gene-edited plants used in this study

species	Sample number	Genotype		theoretical ratio values	expected ratio values
Oryza sativa	WT	Wild-type		100%	99.17%
	S-1	Heterozygous		50%	49.44%
	S-2	Heterozygous		50%	49.11%
	S-3	Heterozygous		50%	51.06%
	S-4	Homozygous		0%	0%
	S-5	Homozygous		0%	0%
Glycine max	D-1	Heterozygous		10%	10.19%
	D-2	Wild-type		100%	100%
		A-genome	C-genome		
Brassica napus	Y-1	Heterozygous	Homozygous	75%	75%
	Y-2	Heterozygous	WT-type	25%	24.92%

Table S3 The source of gene-edited *Brassica napu*s and *Glycine max* used in this study

species	Sample number	the target genes edited
Brassica napus	WT	(A-genome) GGCCACTTGCCCATGCTGGCTAAGTACGGCCCTGACGT
	WT	(C-genome) GGCCACTTGCCCATGCTGGCTAAGTACGGCCCTGACCT
Brassica napus	Y-1	(A-genome) GGCCACTT**TT**GCCCATGCTGGCTAAGTACGGCCCTGACGT
		(C-genome) GGCCACTT**T**GCCCATGCTGGCTAAGTACGGCCCTGACCT
	Y-2	(A-genome) GGCCACTT**G**GCCCATGCTGGCTAAGTACGGCCCTGACGT
		(C-genome) GGCCACTTGCCCATGCTGGCTAAGTACGGCCCTGACCT
Glycine max	D-1	GAAGCAAGA**A**A**T**GTTCTAGG
	D-2	GAAGCAAGAGACGTTCTAGG

Table S4 The results of cdPCR and ddPCR for detecting diluted samples

Samples (ng/μL)	cdPCR (Copies/μL)					ddPCR (Copies/μL)				
	Parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)	Parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)
10	2083	2077.3	2079	2079.77±2.39	0.11	2093	2111	2124	2109.33±12.71	0.60
5	1082.5	1076.7	1072.2	1077.13±4.22	0.39	1116	1103	1089	1102.67±11.03	1.00
1	188.44	186.14	192.3	188.96±2.54	1.34	216	223	219	219.33±2.87	1.31
0.2	40.42	42.09	41.91	41.47±0.75	1.81	40.2	41.1	40.5	40.6±0.37	0.91
0.04	8.06	8.25	8.11	8.14±0.08	0.98	8.7	9.2	8.4	8.77±0.33	3.76
0.008	8.77	8.52	8.08	8.46±0.29	3.43	6.4	12.9	9.9	9.73±2.66	27.34

Table S5 Results of Mixed Sample Assayed by cdPCR

sample	HEX (Copies/μL)					HEX-FAM (Copies/μL)					mutation frequency
	Parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)	Parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)	
WT	7282.8	7287.7	7347.7	7306.067±29.507	0.40	7267.6	7289.5	7296.7	7284.6±15.156	0.21	WT
50.00%	7260.6	7261.9	7220.3	7247.6±19.311	0.27	3587.1	3606.2	3624	3605.767±18.454	0.51	49.751%
10.00%	7470	7465.9	7498.3	7478.0667±14.404	0.19	776.6	772.4	775.8	774.933±2.230	0.29	10.363%
5.00%	7328.4	7283.6	7247.6	7286.533±33.052	0.45	371.1	369.6	372.8	371.167±1.601	0.43	5.094%
1.00%	7347.7	7282.8	7317.7	7316.067±26.521	0.36	77.7	80.6	84.4	80.9±3.360	4.15	1.109%
0.50%	7296	7253.6	7306.2	7285.267±22.776	0.31	37.1	38.7	36.8	37.533±1.021	2.72	0.515%
0.10%	7282.8	7187.7	7247.7	7239.4±39.266	0.54	7.2	7.7	7.4	7.433±0.252	3.39	0.103%
0.05%	7217.9	7214.3	7258.5	7230.233±20.042	0.28	8.2	4.8	6.6	6.533±1.700	26.04	0.090%

Table S6 Results of Mixed Sample Assayed by ddPCR

sample	HEX (Copies/μL)					HEX-FAM (Copies/μL)					mutation frequency
	parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)	Parallel 1	Parallel 2	Parallel 3	Mean ± error value	RSD (%)	
WT	7300	7320	7290	7303.333±12.472	0.171%	7300	7280	7340	7306.667±24.944	0.75	WT
50.00%	7310	7390	7280	7326.667±46.428	0.634%	3620	3580	3650	3616.667±28.6745	0.79	49.363%
10.00%	7240	7310	7320	7290±35.59	0.488%	760	755	720	745±17.795	2.39	10.219%
5.00%	7190	7260	7230	7226.667±28.674	0.397%	375	380	391	382±6.683	1.75	5.286%
1.00%	7340	7290	7360	7330±29.439	0.402%	80	77	71	76±3.742	4.92	1.037%
0.50%	7270	7250	7330	7283.333±33.9935	0.467%	38	37.4	33.3	36.233±2.089	5.76	0.497%
0.10%	7350	7330	7290	7323.333±24.944	0.341%	7.5	7.25	8.5	7.75±0.540	6.97	0.106%
0.05%	7290	7330	7370	7330±32.6599	0.446%	3	7.8	7.2	6±2.135	35.59	0.082%

Supplementary Figures

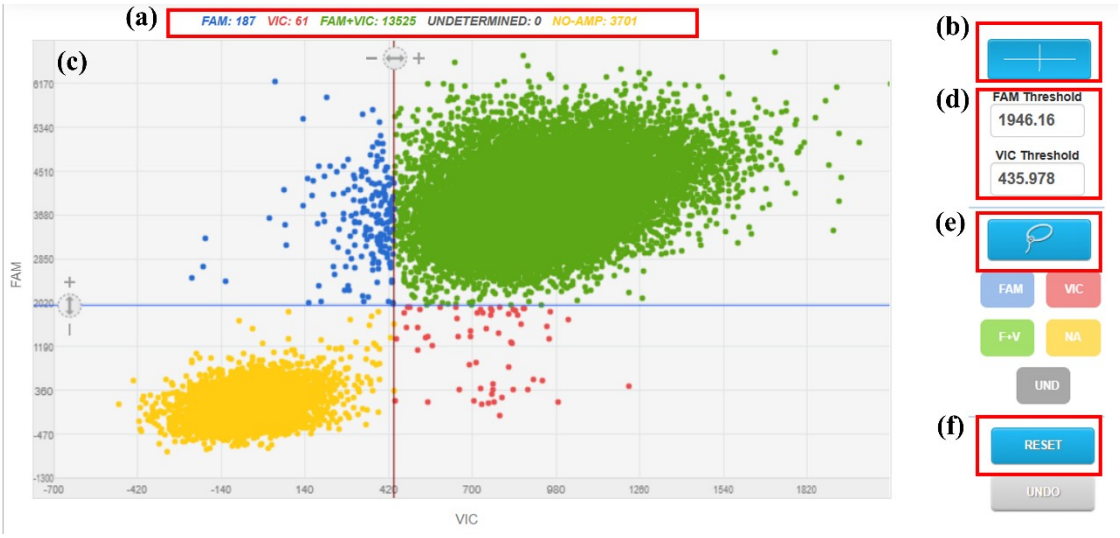


Fig S1 cdPCR operating page features:**(a)** Quality Threshold Requirement; **(b)** Automatic Threshold Segmentation; **(c)** Manual Droplet Illustration with Threshold Setting; **(d)** Box Selection Tool; **(f)** Restore Tool;

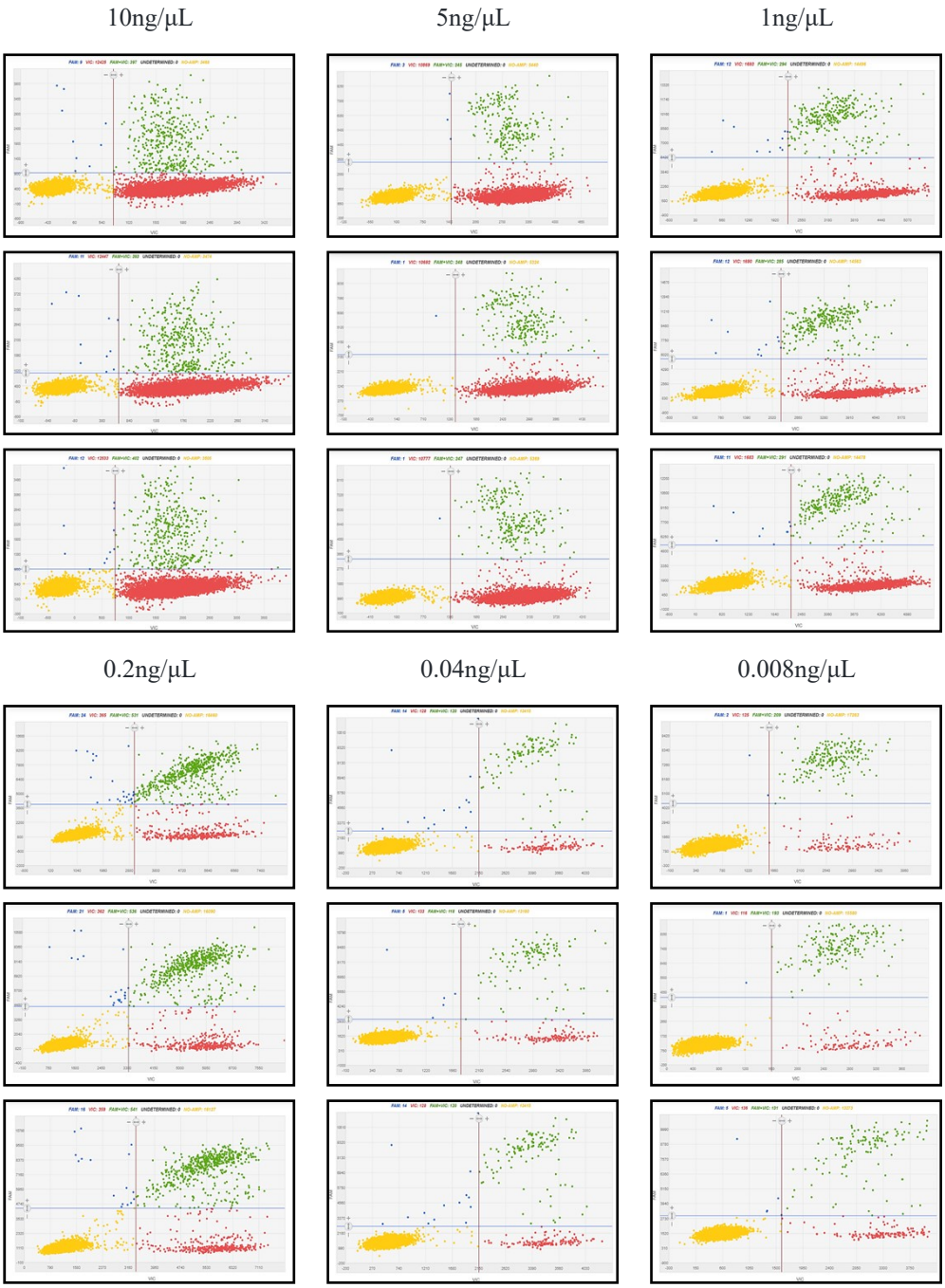
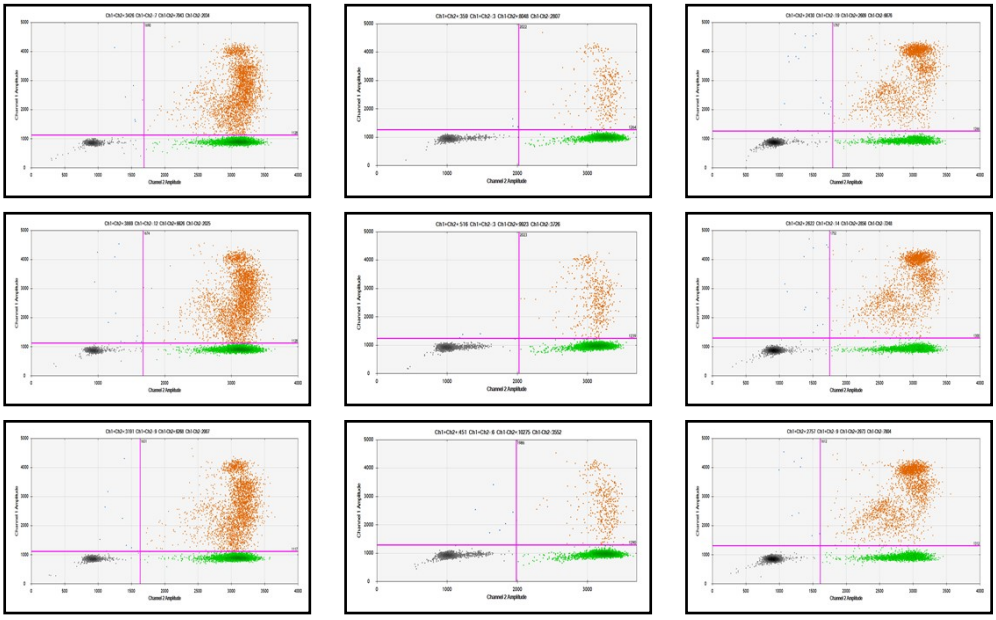


Fig S2 Droplet amplification plot of diluted samples detected by cdPCR. In each experiment, three biological replicates are performed.

10ng/μL 5ng/μL 1ng/μL



0.2ng/μL 0.04ng/μL 0.008ng/μL

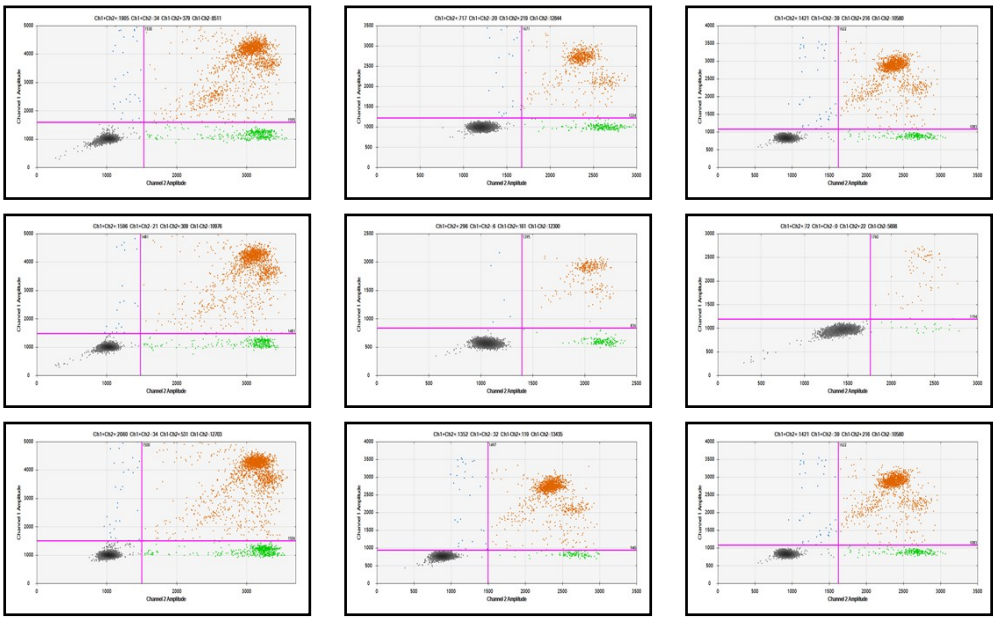


Fig S3 Droplet amplification plot of diluted samples detected by ddPCR. In each experiment, three biological replicates are performed.

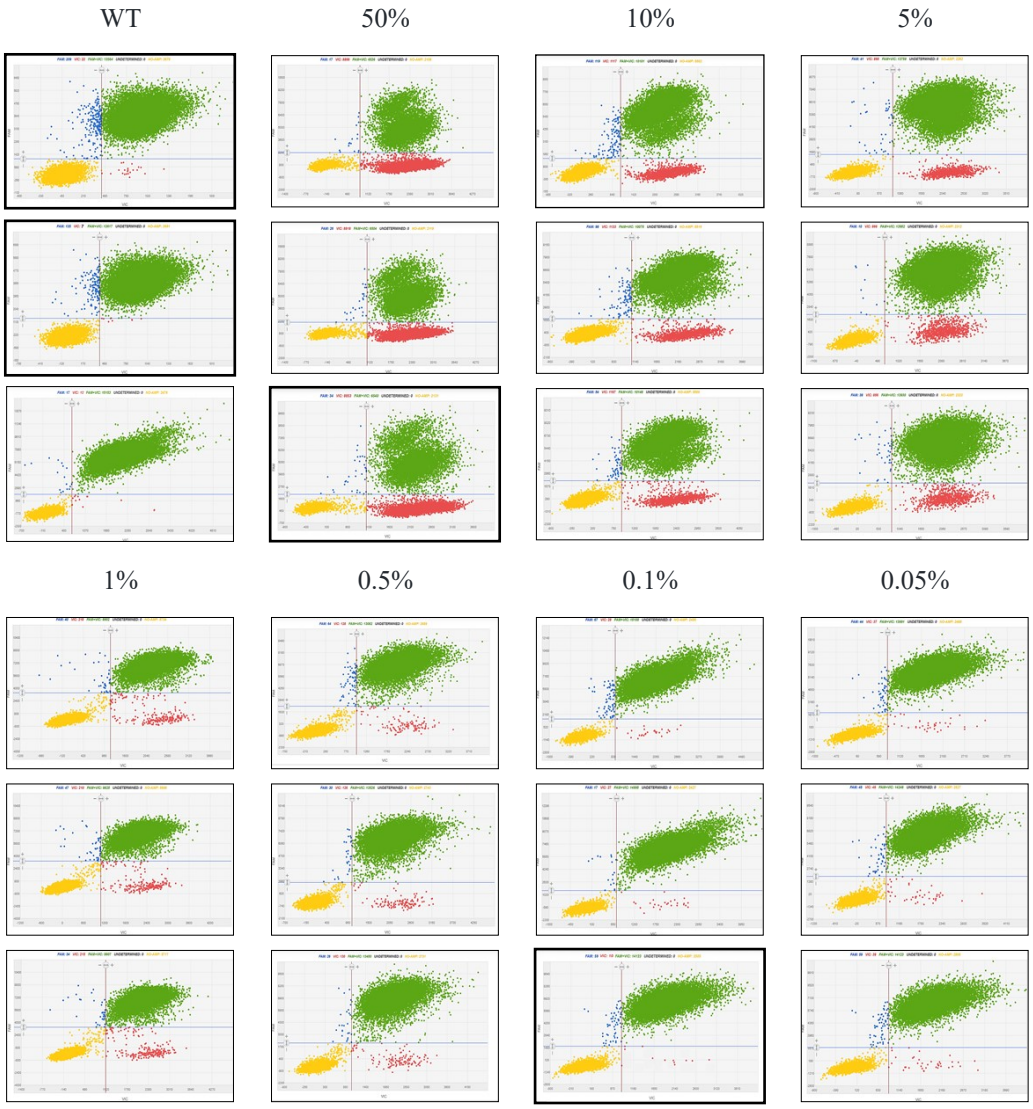


Fig S4 Droplet amplification plots of various mixed samples detected by cdPCR. In each experiment, three biological replicates are performed.

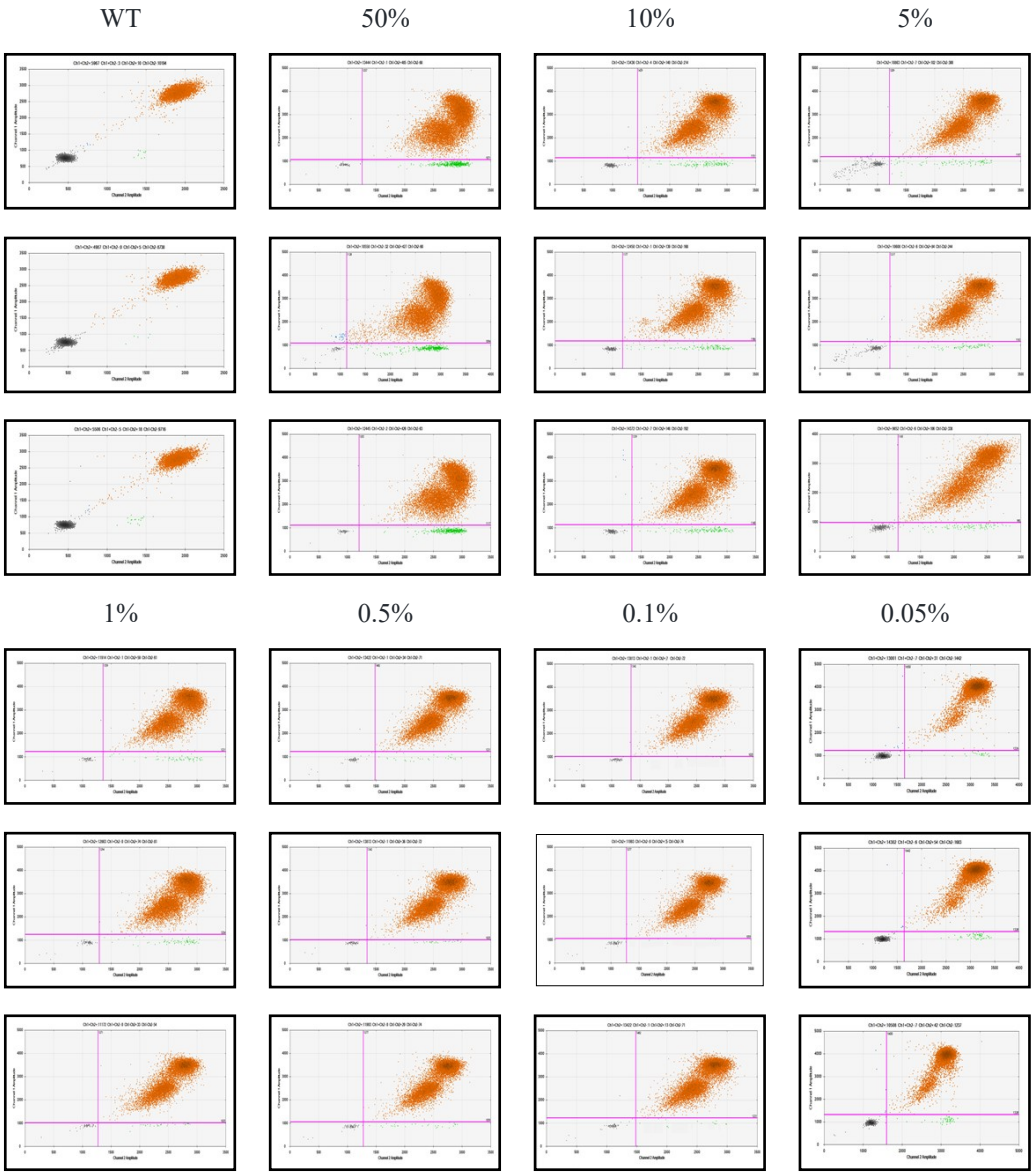


Fig S5 Droplet amplification plots of various mixed samples detected by ddPCR. In each experiment, three biological replicates are performed.

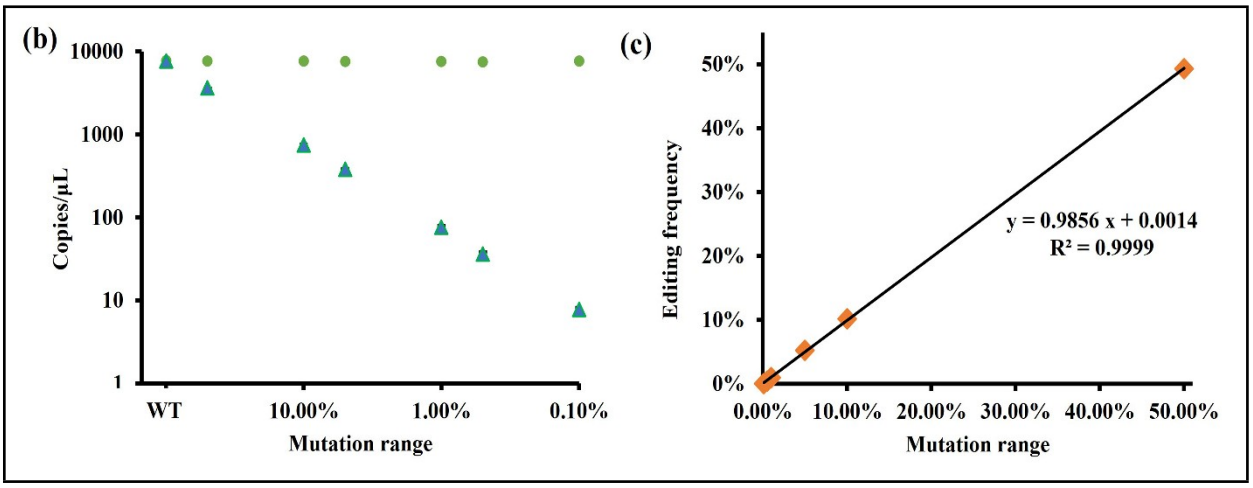


Fig S6(a) Scatter plot showing copy number values and mutation range of different mixed samples detected by ddPCR: green dots and blue triangles represent the concentration of HEX droplets and FAM droplets in each mixed sample, respectively; **(b)** Standard curve showing the editing efficiency determined by ddPCR for different mutation ranges. All data are the average of three replicates ± error value.