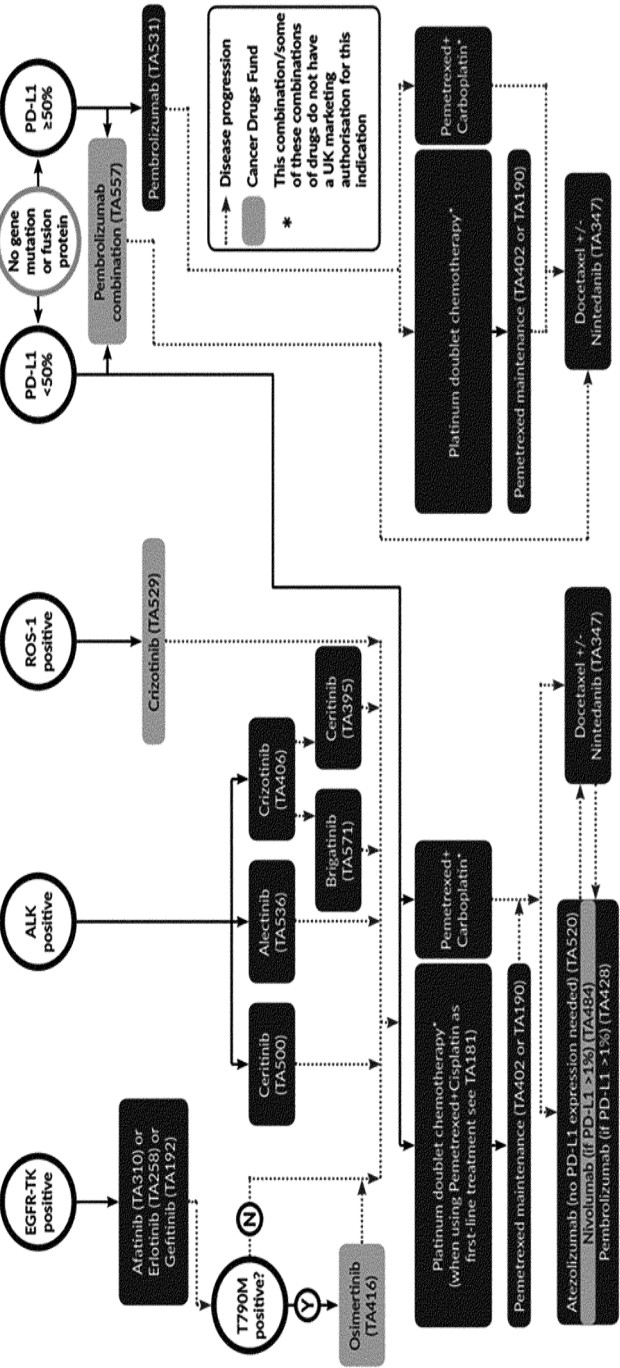
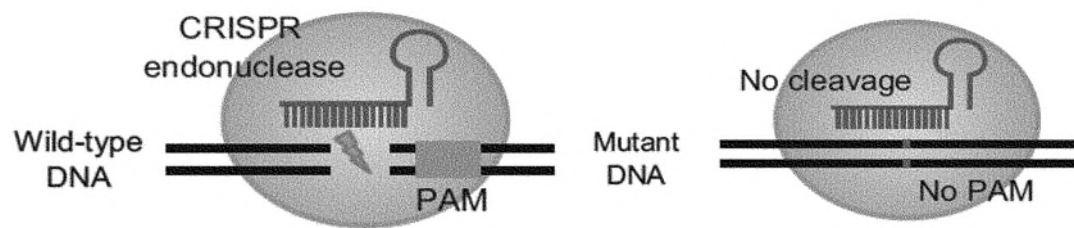


【FIG. 1】



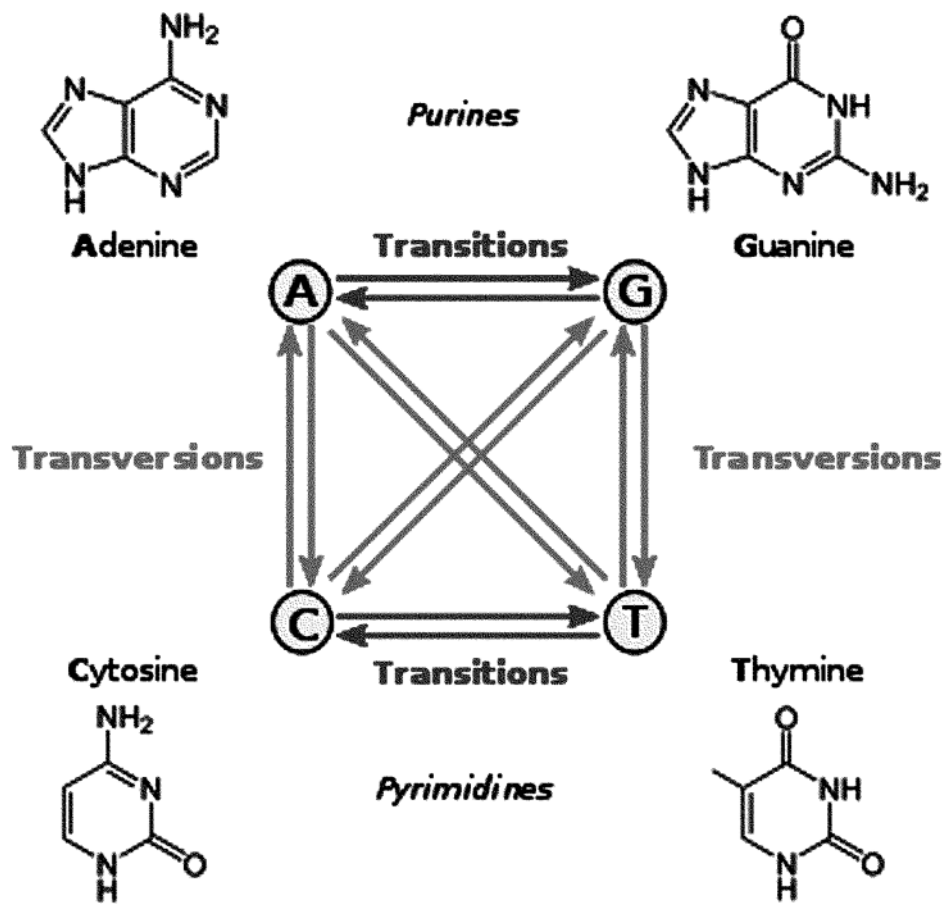
【FIG. 2】



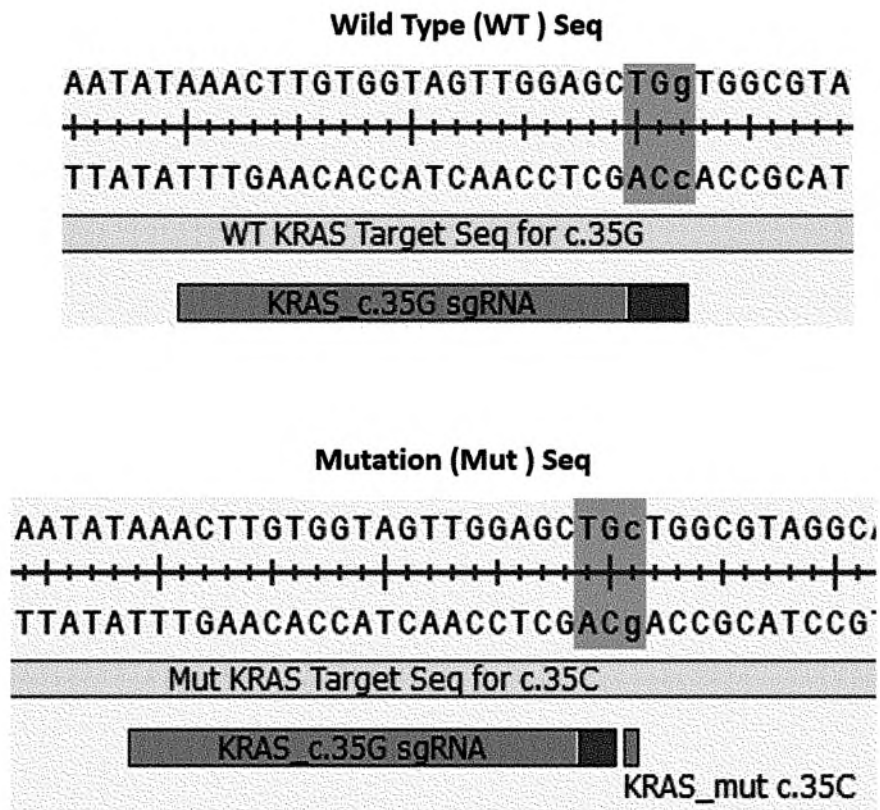
【FIG. 3a】



【FIG. 3b】

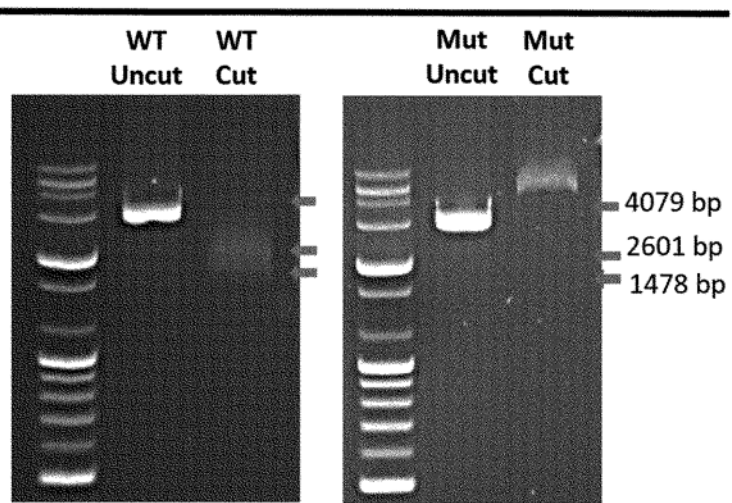


【FIG. 4a】

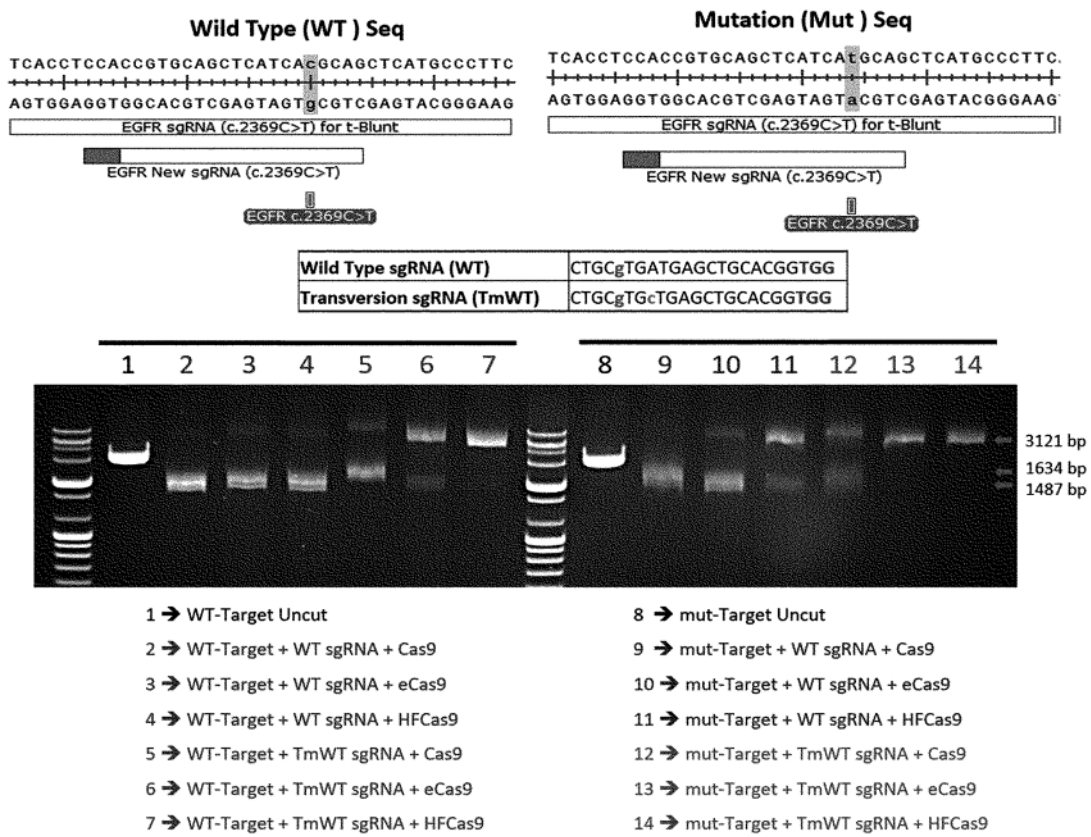


【FIG. 4b】

Cas9 Protein + KRAS IVT sgRNA + Linearized Plasmids (WT & mut G>C)



【FIG. 5】

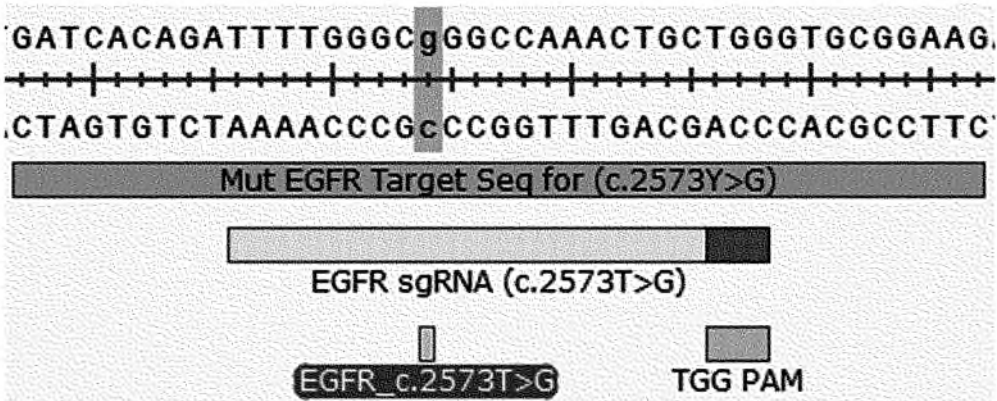


【FIG. 6a】

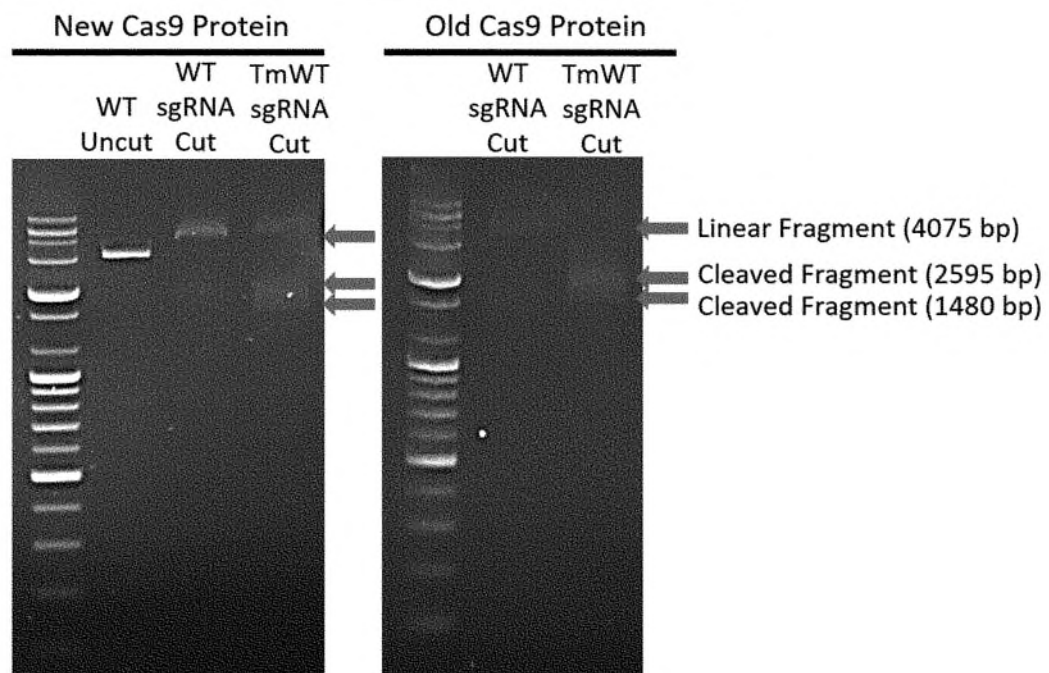
Linearized Plasmids Contains Wild Type (WT) Seq



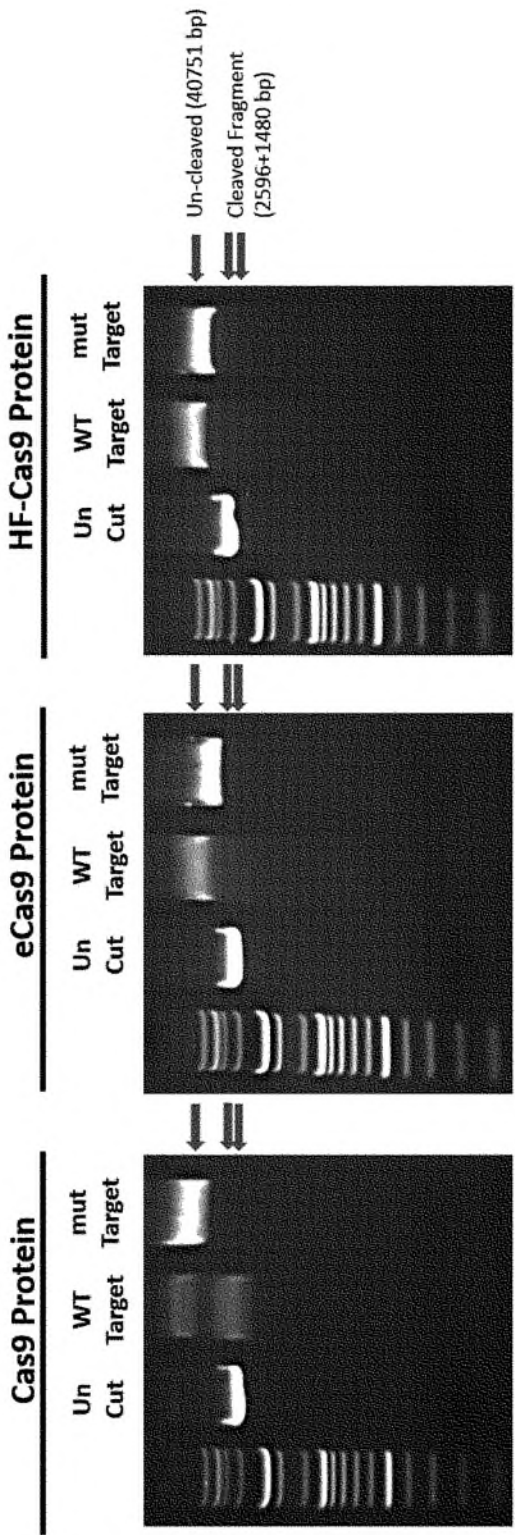
Linearized Plasmids Contains Mutation (Mut) Seq



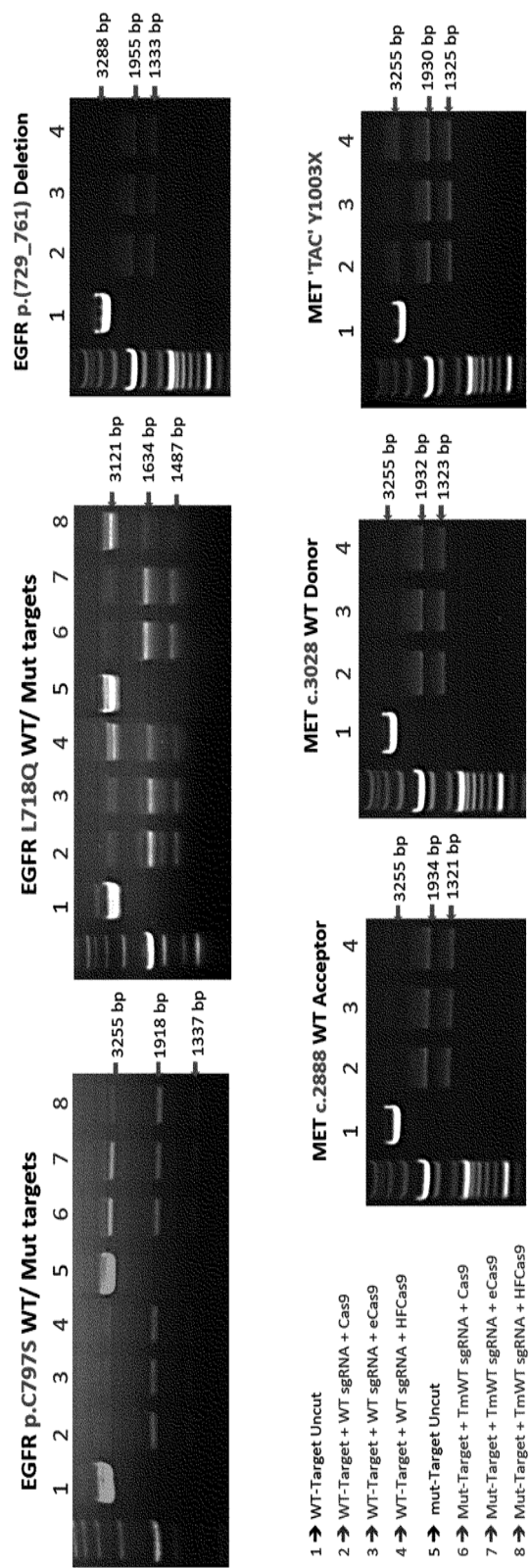
【FIG. 6b】

Cas9 Protein + EGFR IVT sgRNA + Linearized Wild Type Plasmid

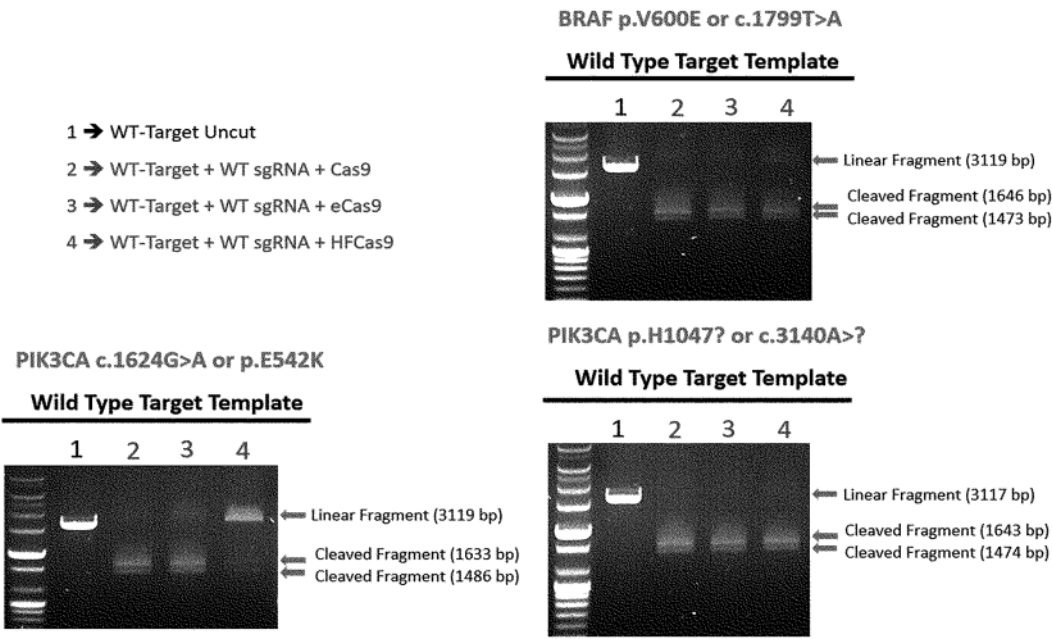
【FIG. 7】



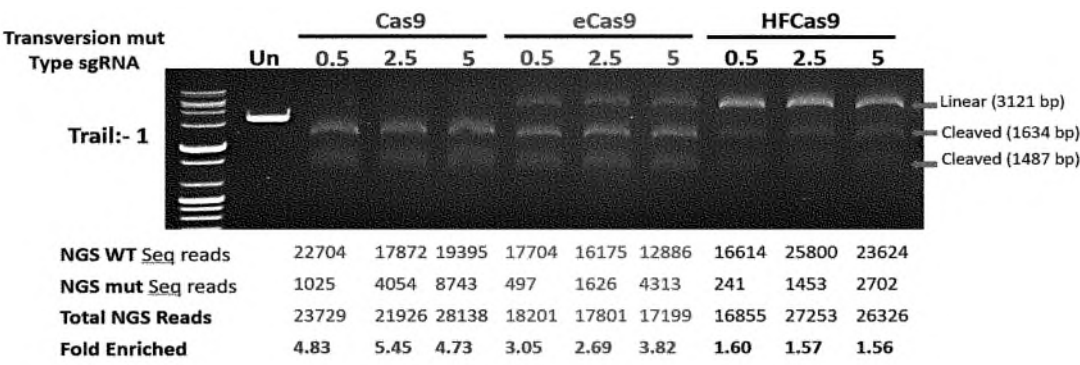
【FIG. 8】



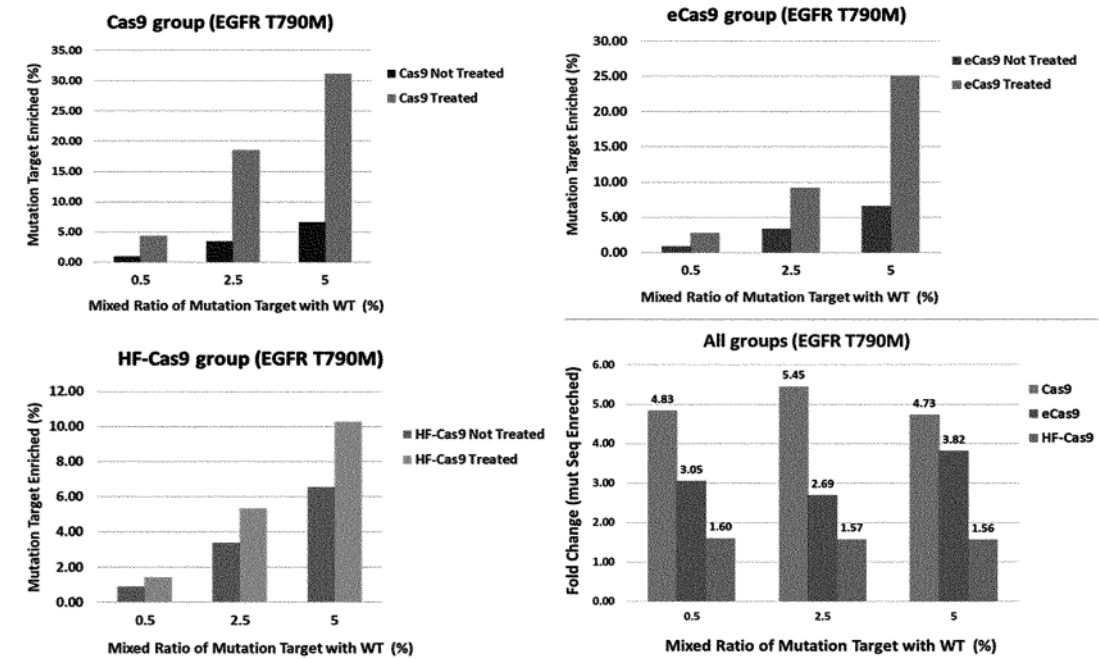
【FIG. 9】



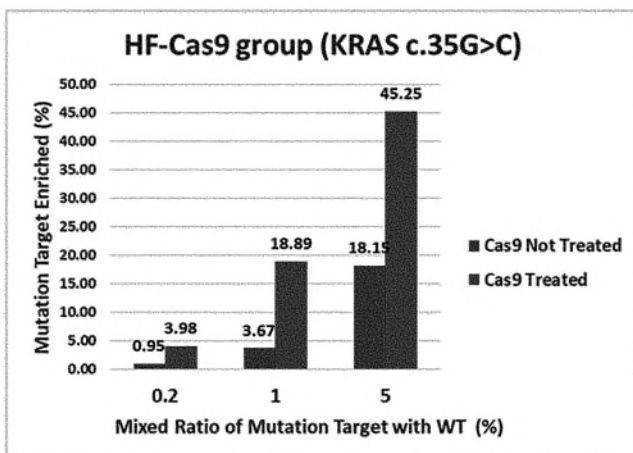
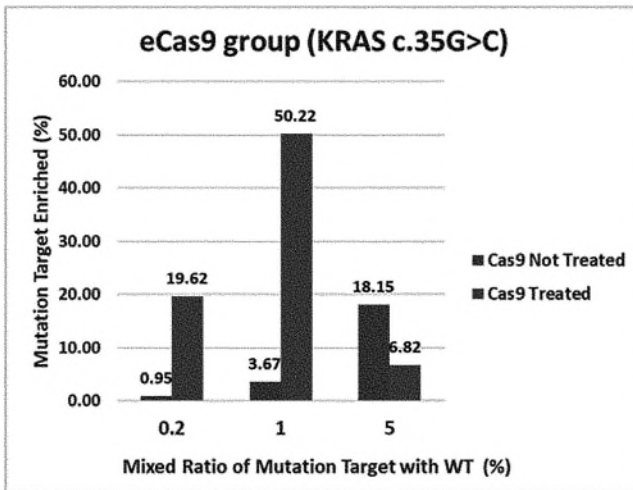
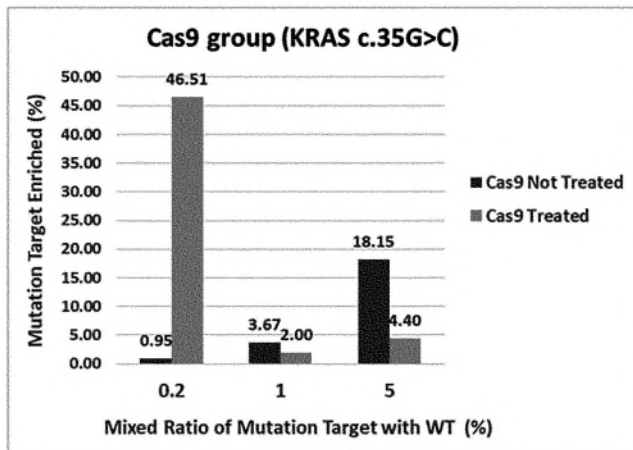
【FIG. 10】



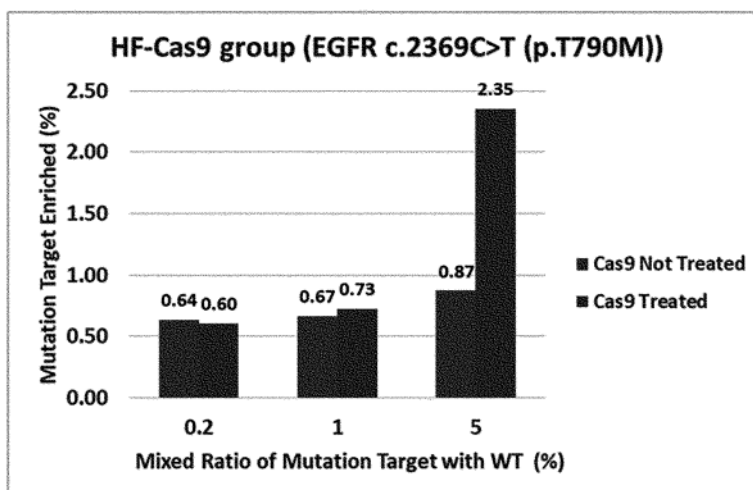
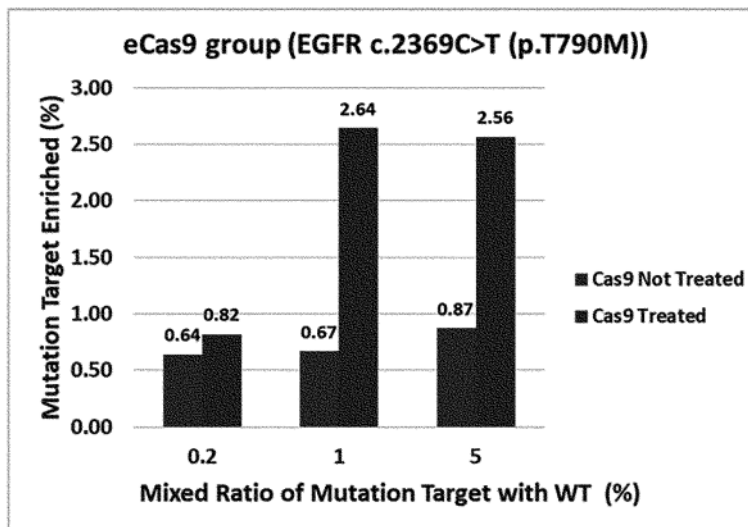
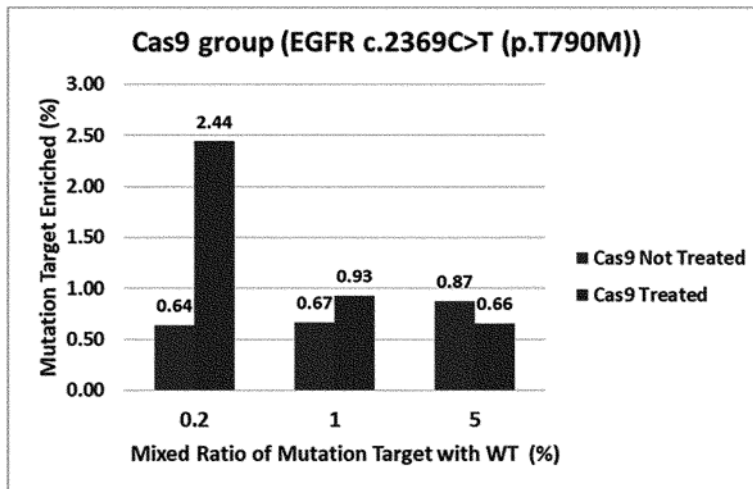
【FIG. 11】



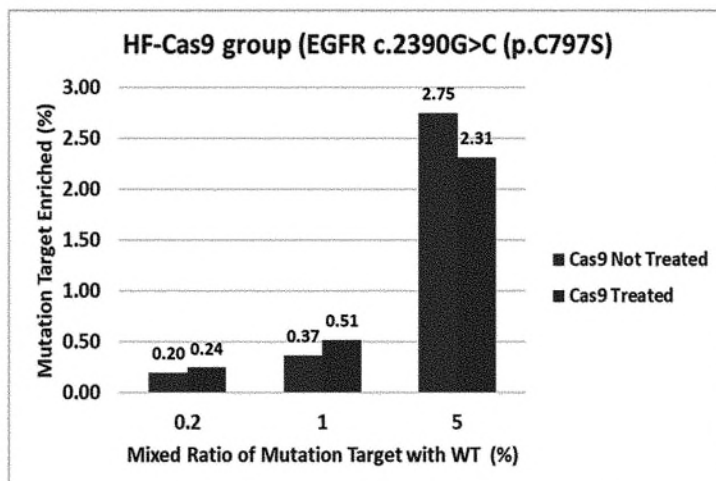
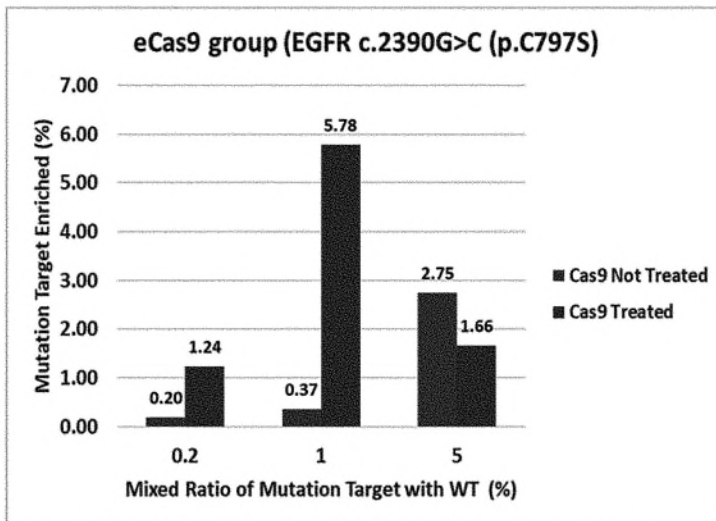
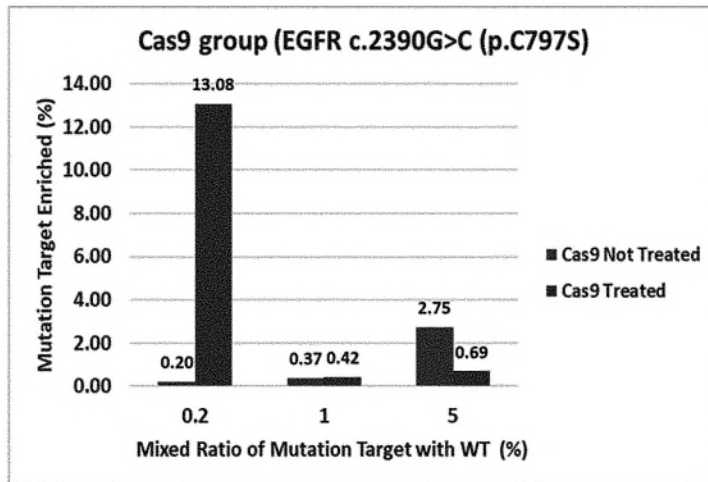
【FIG. 12】



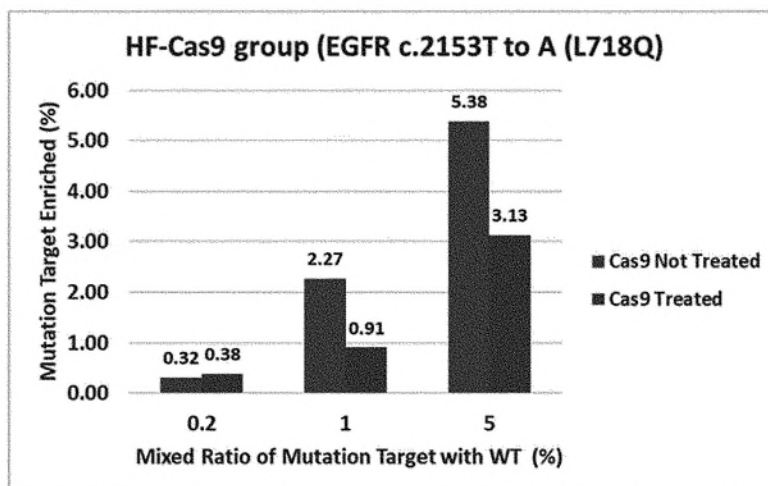
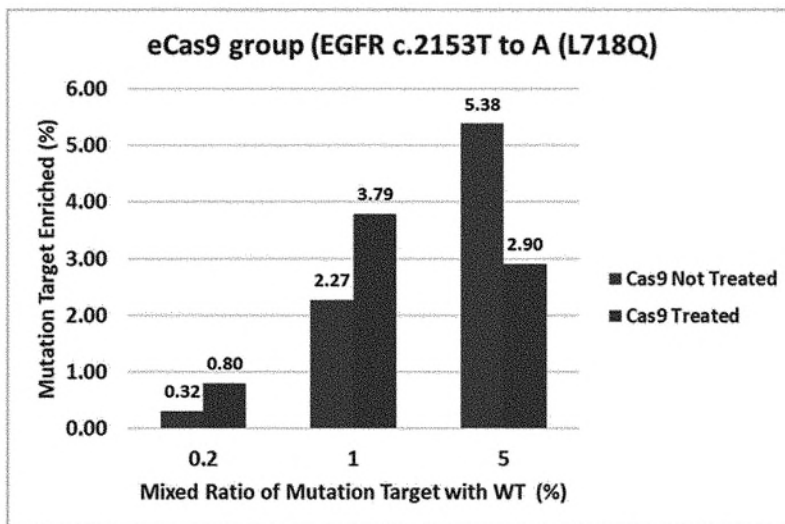
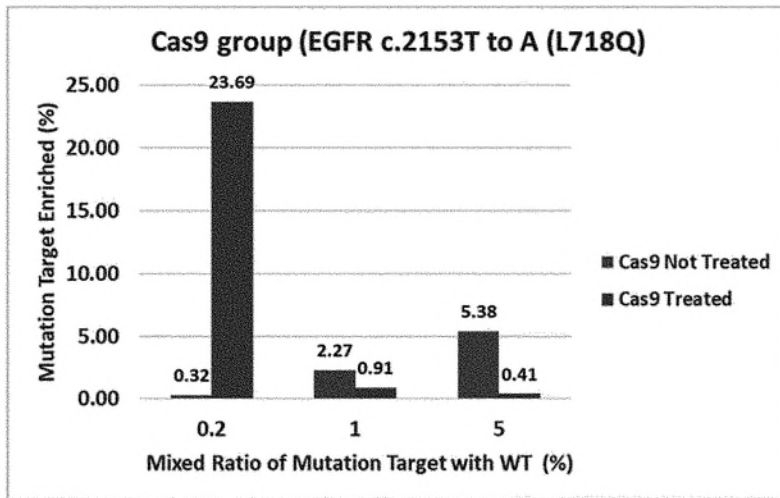
【FIG. 13】



【FIG. 14】



【FIG. 15】



sgRNA activity
confirmed

EGFR p.T790M,

EGFR 15bp Deletion →

KRAS p.G12? →

Table 1: 5% Multiplex I cfDNA Reference Standard (Part No.: HD777)

Gene	Variant	Amplicon size (bp)	Expected Af (%)	WT Conc. (copies/μl)	Mutant Conc. (copies/μl)	Total Conc. (copies/μl)
EGFR	T790M	117.0	5.0	2120	109	2229
EGFR	delE746-A750	110/95	5.0	2388	146	2534
EGFR	L858R	78.0	5.0	4092	203	4295
EGFR	V769-D770insASV	122/131	5.0	2108	97	2205
NRAS	Q61K	83.0	6.3	3316	224	3540
NRAS	A59T	66.0	6.3	3628	262	3890
PIK3CA	E545K	81.0	6.3	3340	210	3550
KRAS	G12D	80.0	6.3	3152	197	3349

Table 3: 0.1% Multiplex I cfDNA Reference Standard (Part No.: HD779)

Gene	Variant	Amplicon size (bp)	Expected Af (%)	WT Conc. (copies/μl)	Mutant Conc. (copies/μl)	Total Conc. (copies/μl)
EGFR	T790M	117.0	0.10	2228	3	2231
EGFR	delE746-A750	110/95	0.10	2428	2	2430
EGFR	L858R	78.0	0.10	4188	4	4192
EGFR	V769-D770insASV	122/131	0.10	2224	2	2226
NRAS	Q61K	83.0	0.13	3536	5	3541
NRAS	A59T	66.0	0.13	4012	5	4017
PIK3CA	E545K	81.0	0.13	3528	5	3533
KRAS	G12D	80.0	0.13	3268	5	3273

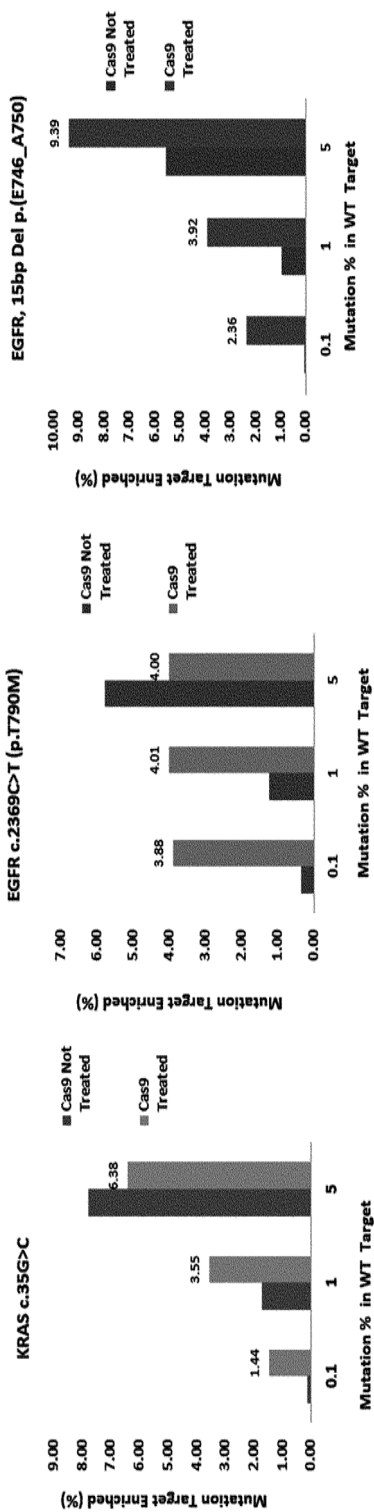
Table 2: 1% Multiplex I cfDNA Reference Standard (Part No.: HD778)

Gene	Variant	Amplicon size (bp)	Expected Af (%)	WT Conc. (copies/μl)	Mutant Conc. (copies/μl)	Total Conc. (copies/μl)
EGFR	T790M	117.0	1.0	2256	24	2280
EGFR	delE746-A750	110/95	1.0	2468	30	2498
EGFR	L858R	78.0	1.0	4384	40	4424
EGFR	V769-D770insASV	122/131	1.0	2204	19	2223
NRAS	Q61K	83.0	1.3	3568	46	3614
NRAS	A59T	66.0	1.3	4360	56	4416
PIK3CA	E545K	81.0	1.3	3576	44	3620
KRAS	G12D	80.0	1.3	3308	36	3344

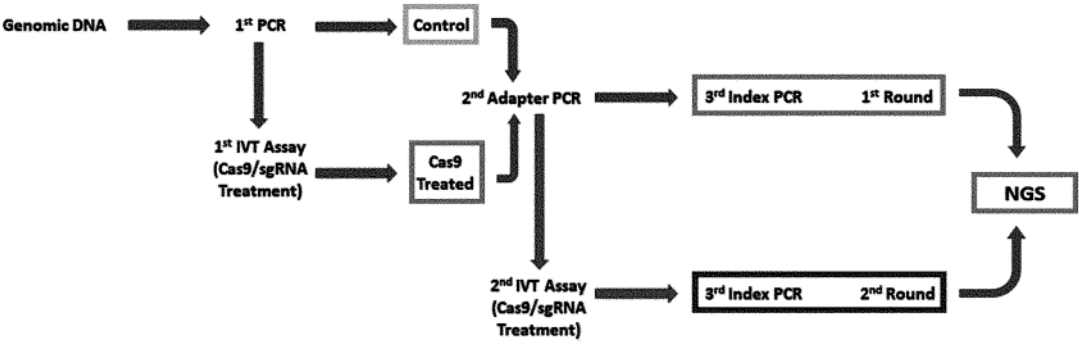
Table 4: 100% Multiplex I wt cfDNA Reference Standard (Part No.: HD776)

Gene	Variant	Amplicon size (bp)	Expected Af (%)	WT Conc. (copies/μl)	Mutant Conc. (copies/μl)	Total Conc. (copies/μl)
EGFR	T790M	117.0	0.00	2248	0.7	2249
EGFR	delE746-A750	110/95	0.00	2572	0.0	2572
EGFR	L858R	78.0	0.00	4408	0.1	4408
EGFR	V769-D770insASV	122/131	0.00	2216	0.0	2216
NRAS	Q61K	83.0	0.00	3492	0.1	3492
NRAS	A59T	66.0	0.00	4184	1.0	4185
PIK3CA	E545K	81.0	0.00	3628	0.6	3629
KRAS	G12D	80.0	0.00	3380	1.1	3381

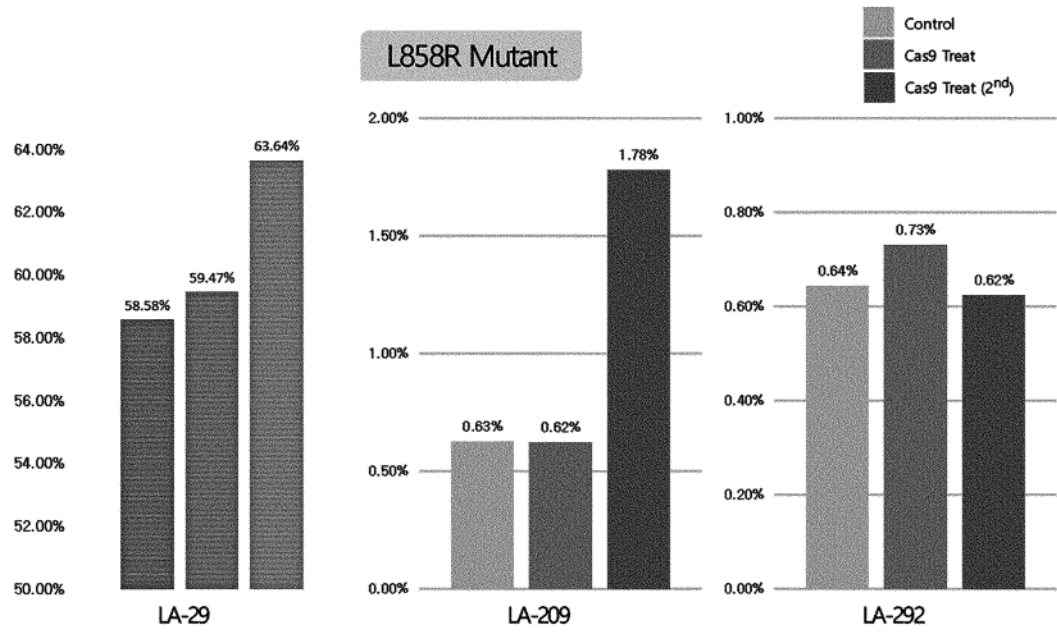
【FIG. 17】



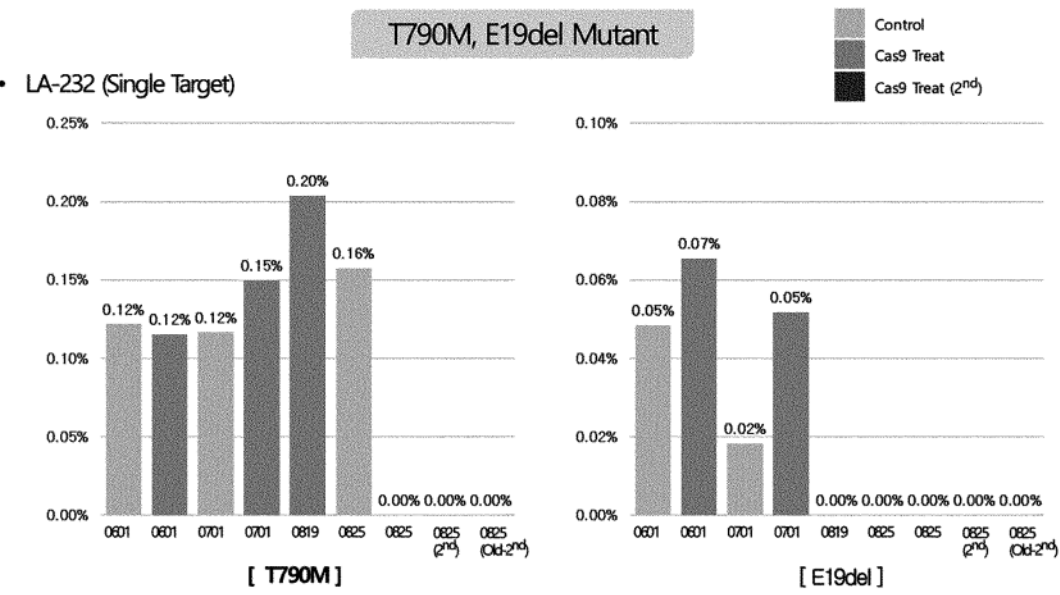
【FIG. 18】



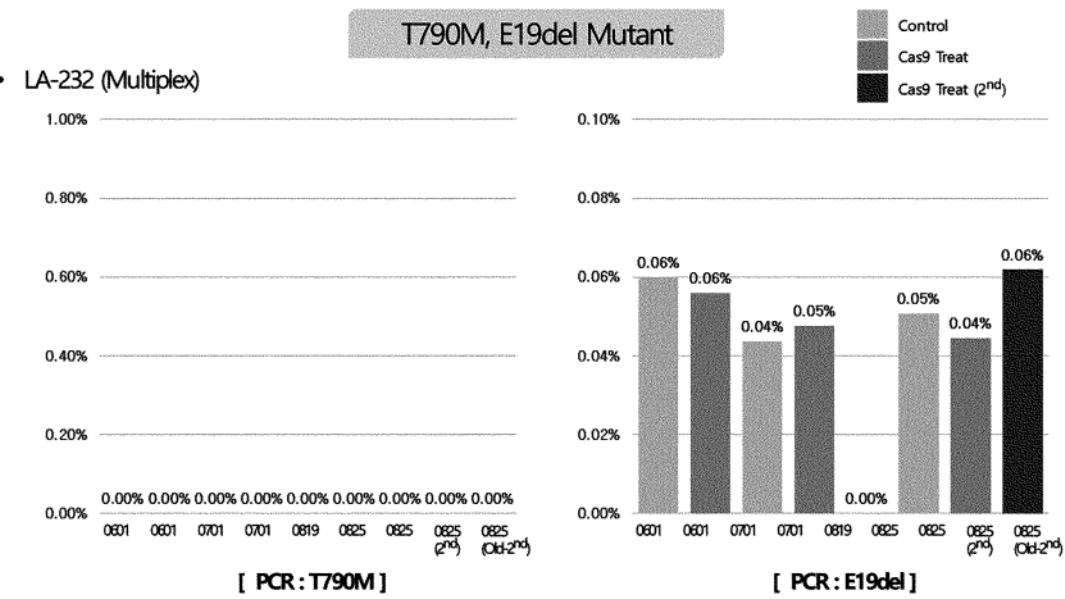
【FIG. 19】



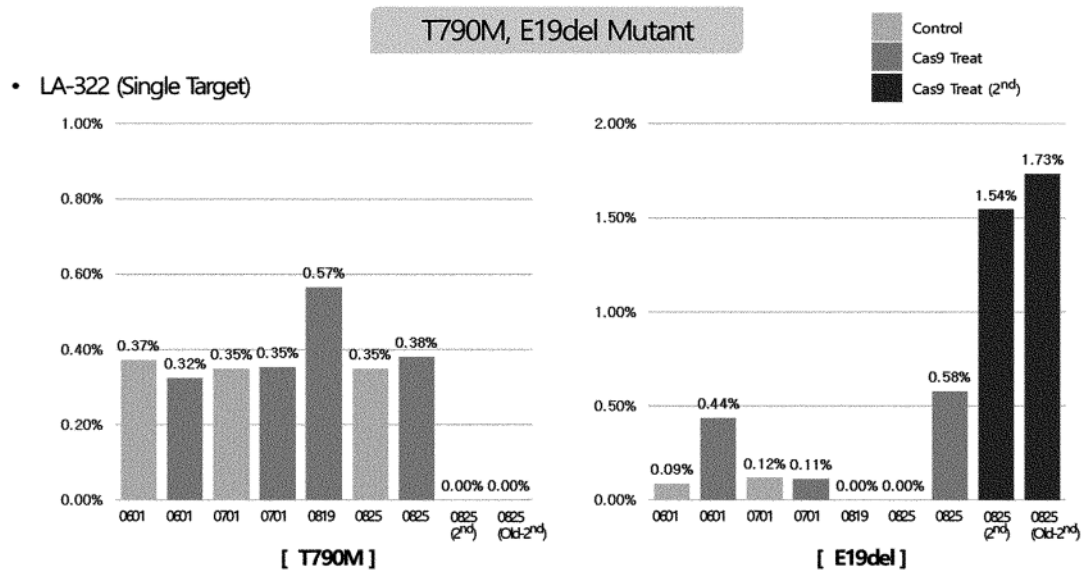
【FIG. 20】



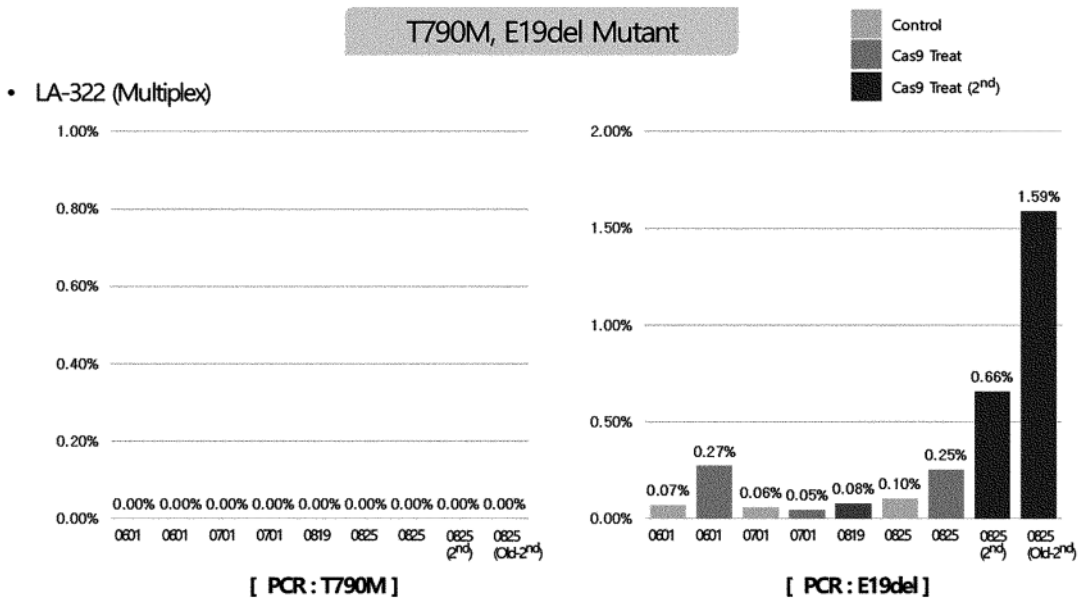
【FIG. 21】



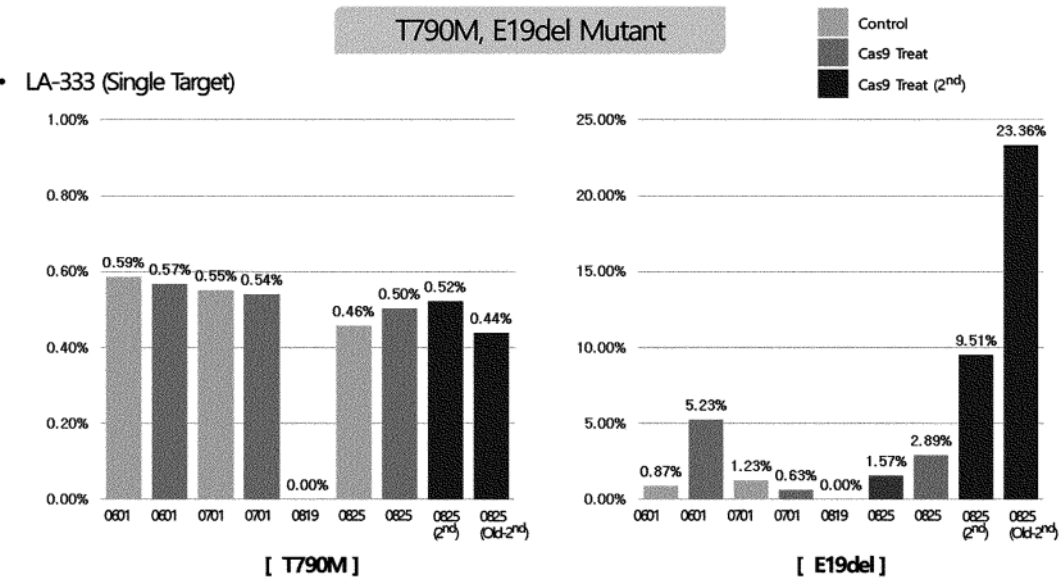
【FIG. 22】



【FIG. 23】



【FIG. 24】



【FIG. 25】

