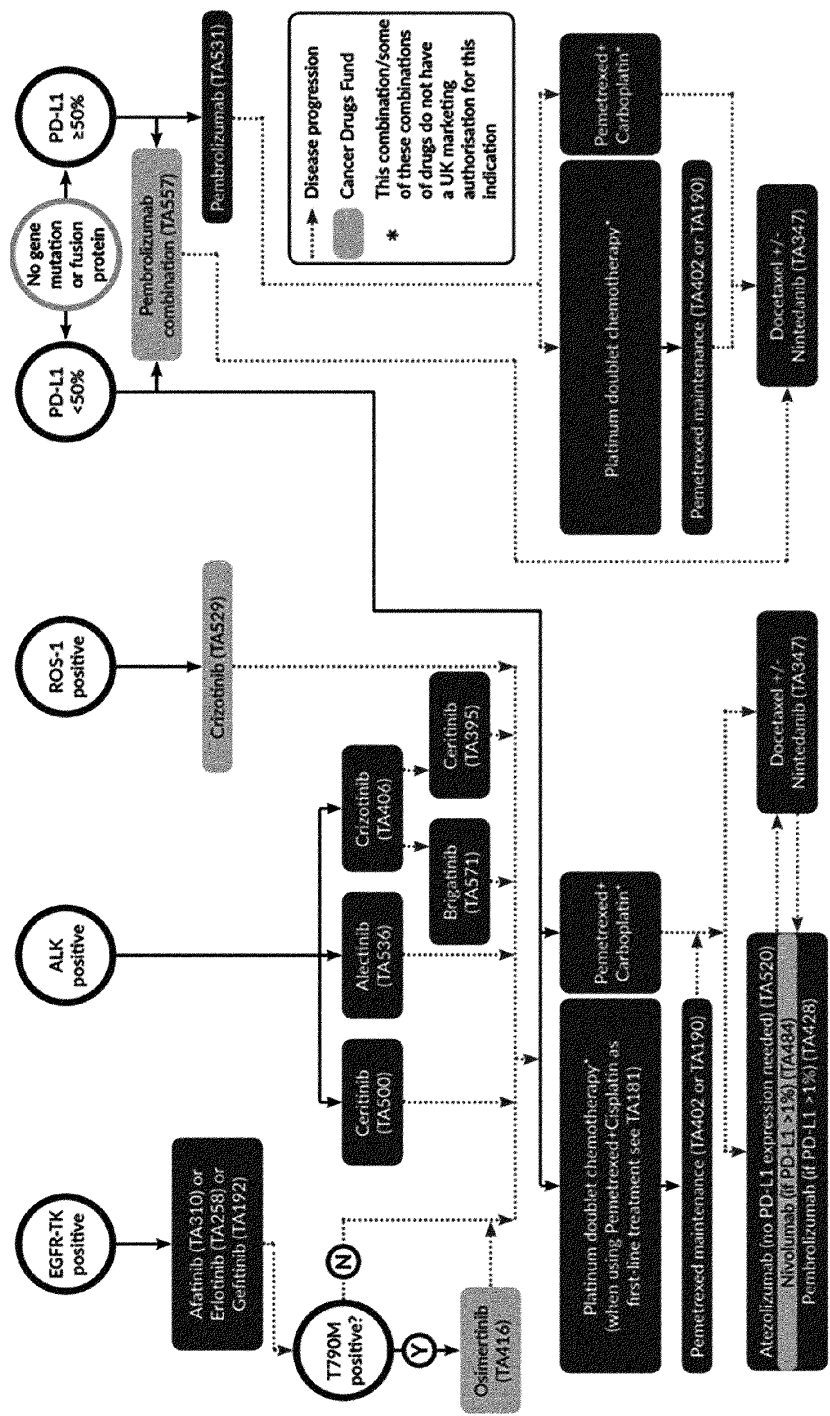
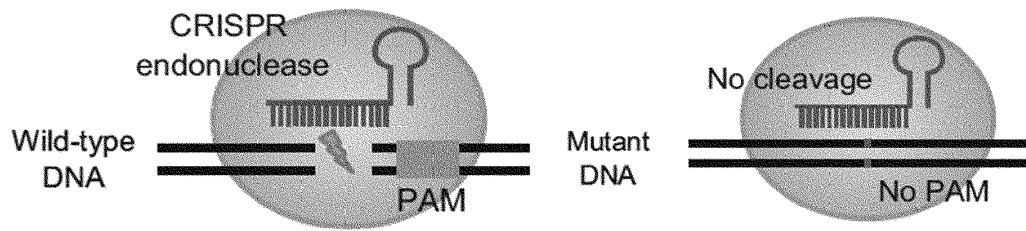


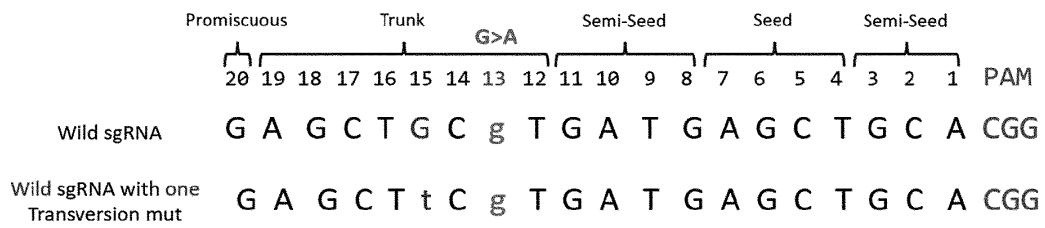
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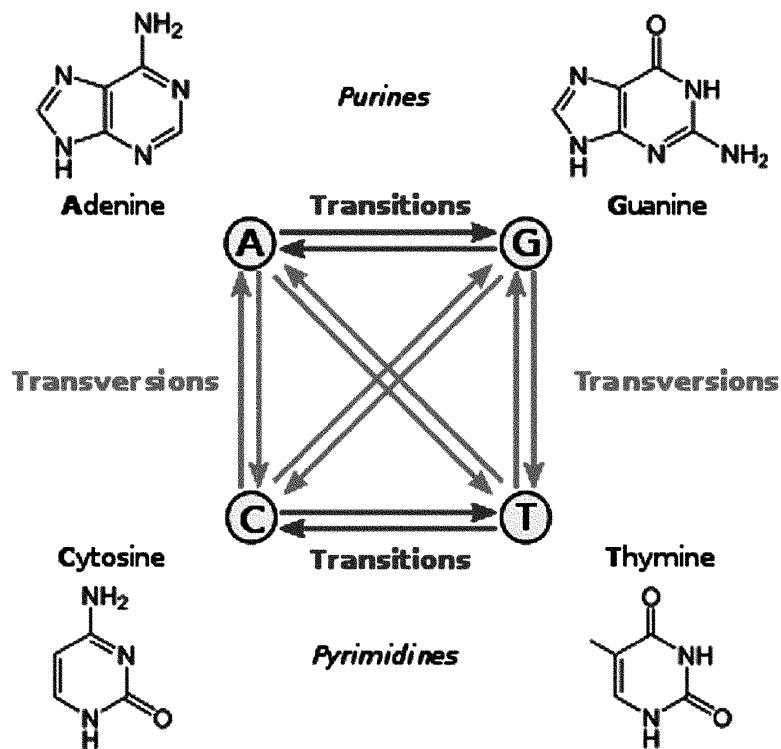
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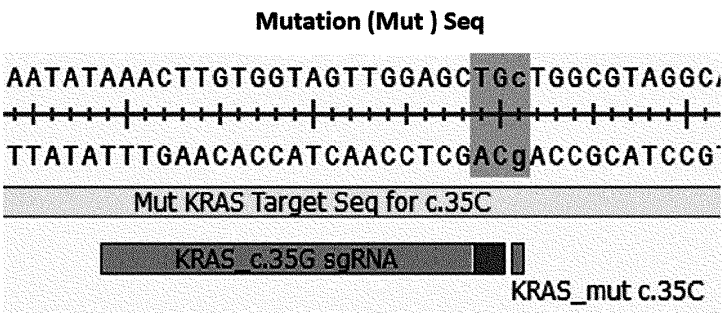
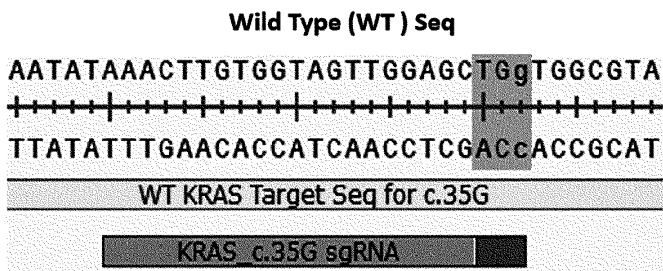
[도3a]



[도3b]

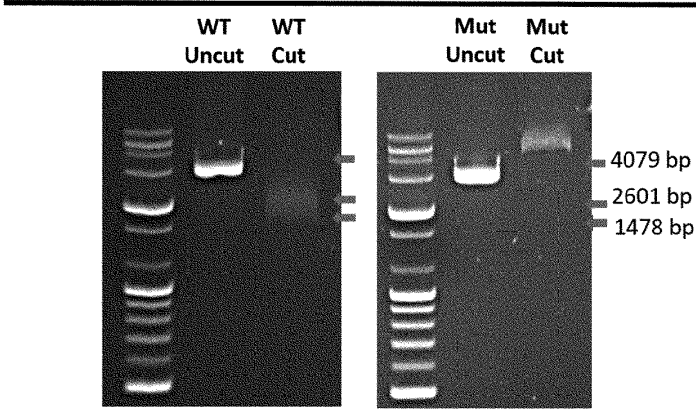


[Figure 4a]

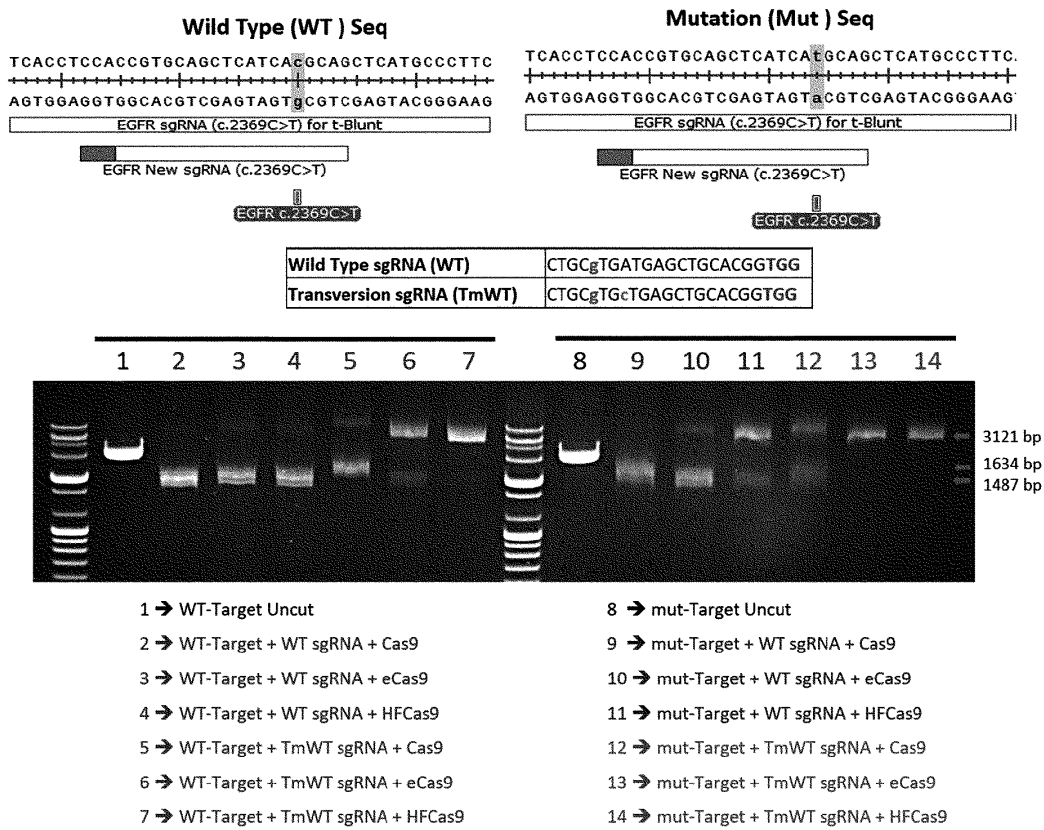


[Figure 4b]

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



[圖5]



[Figure 6a]

Linearized Plasmids Contains Wild Type (WT ) Seq

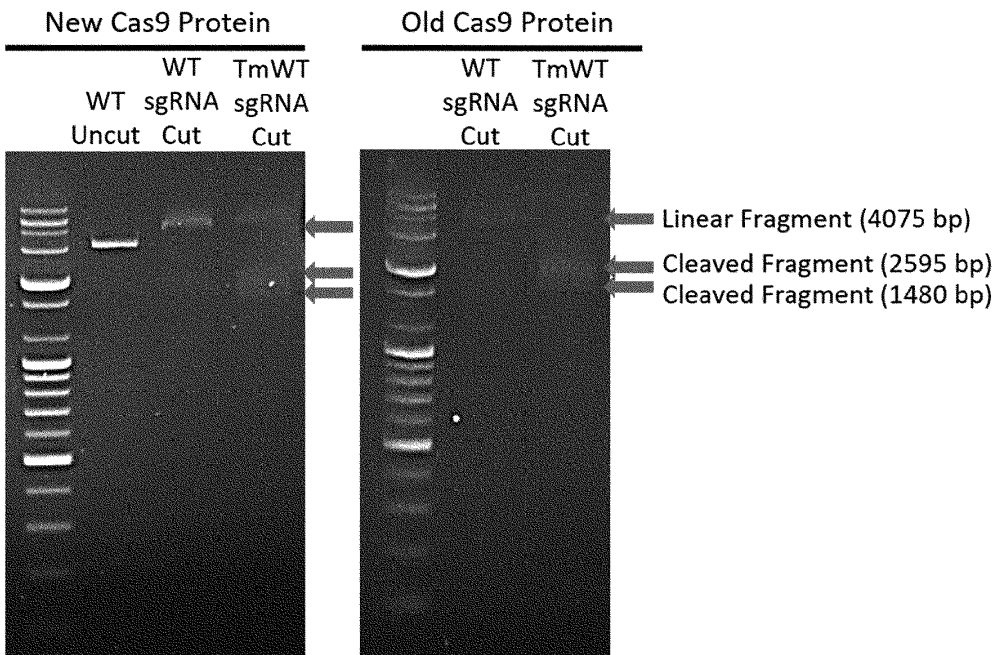


Linearized Plasmids Contains Mutation (Mut ) Seq

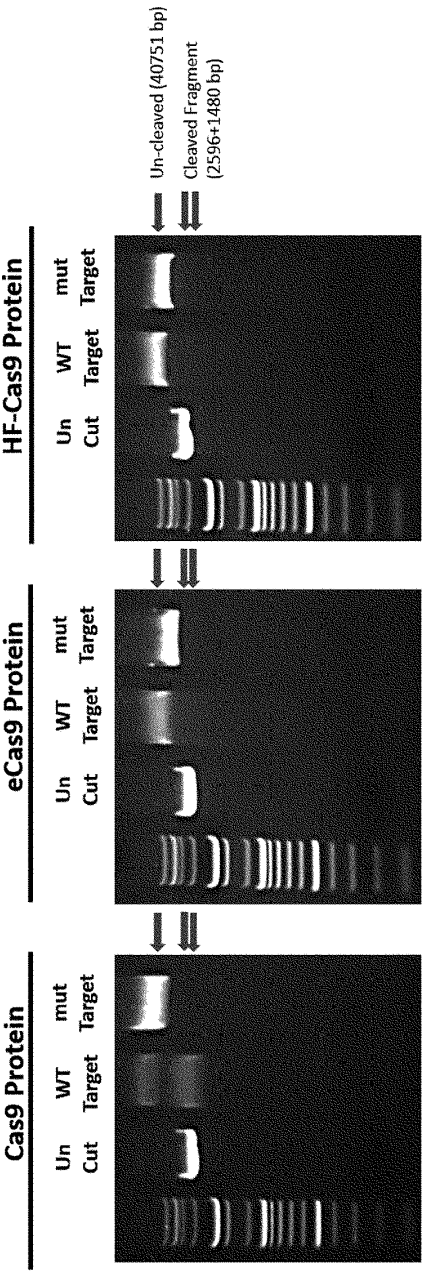


[Figure 6b]

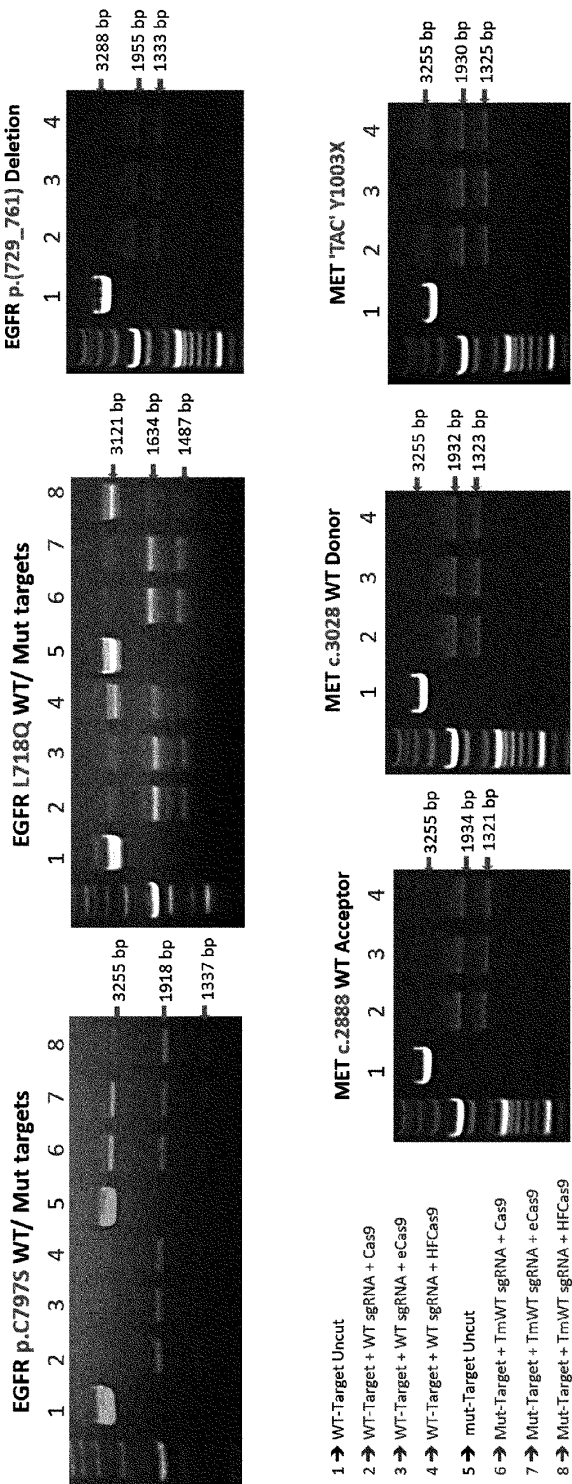
Cas9 Protein + EGFR IVT sgRNA + Linearized Wild Type Plasmid



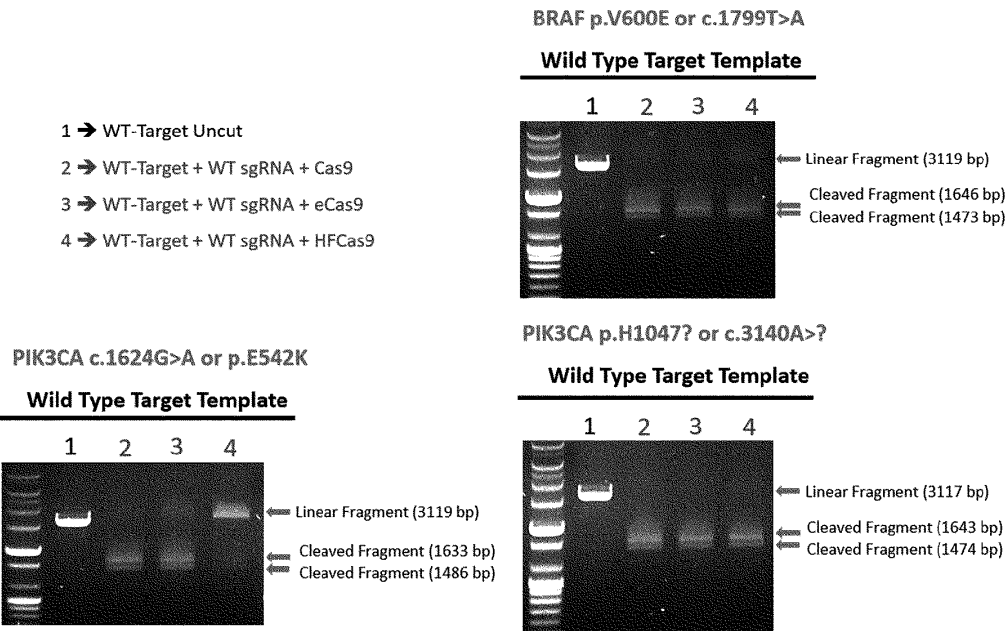
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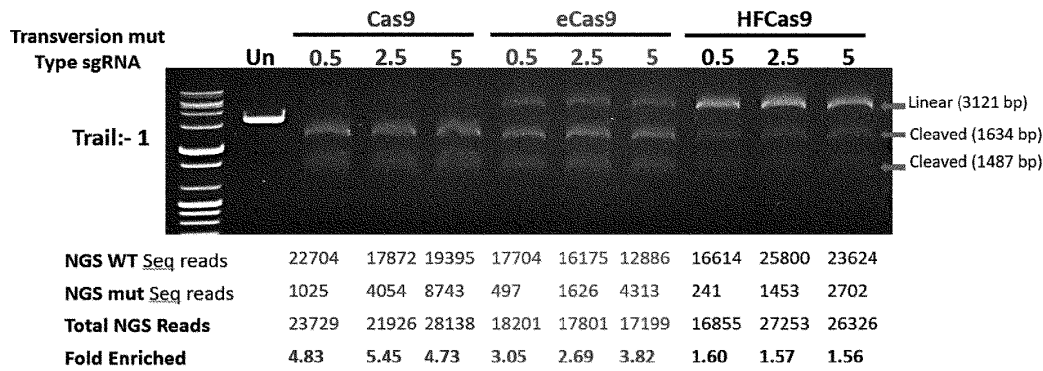
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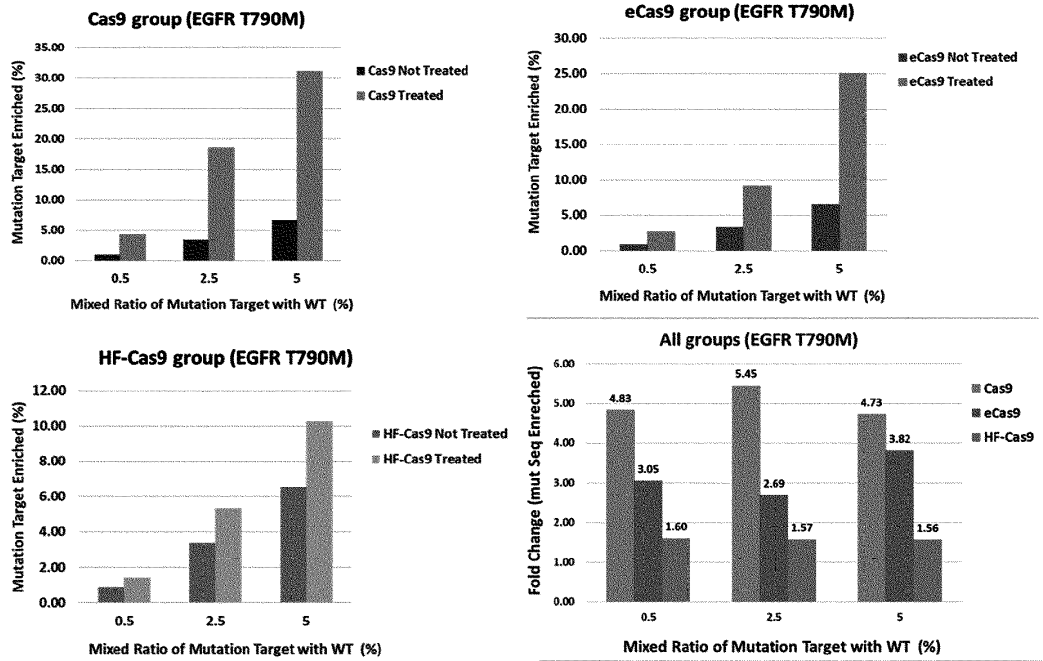
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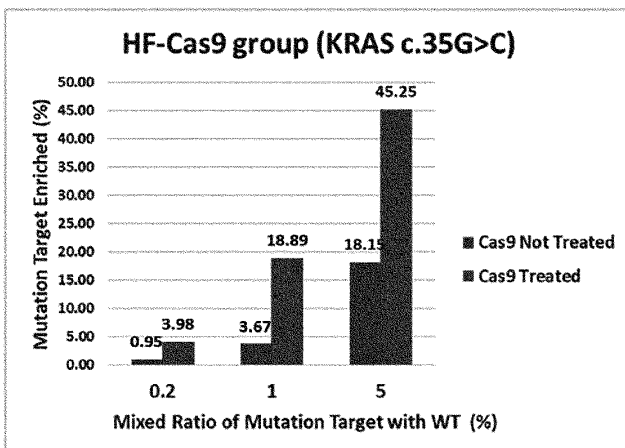
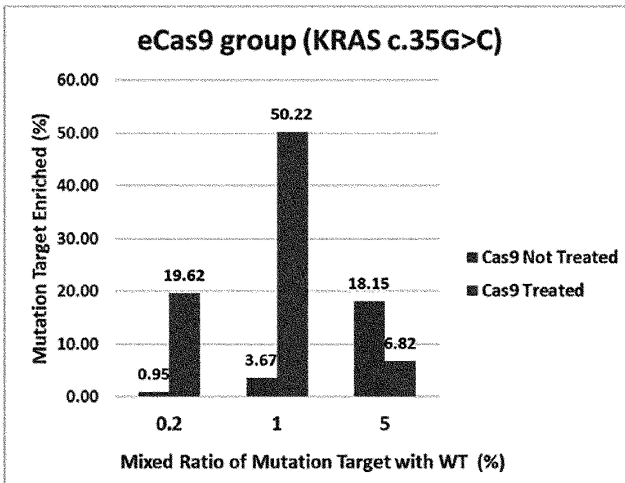
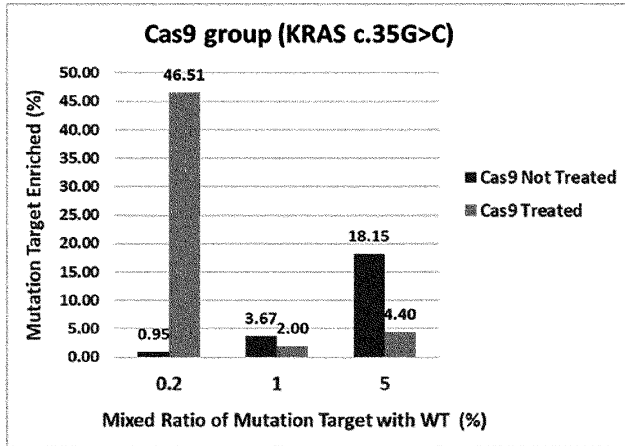
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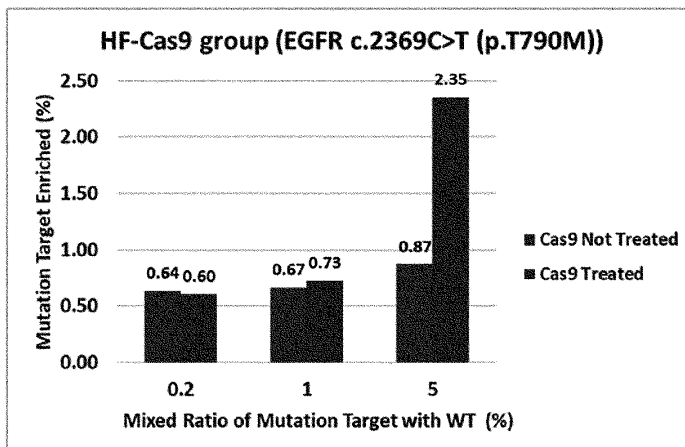
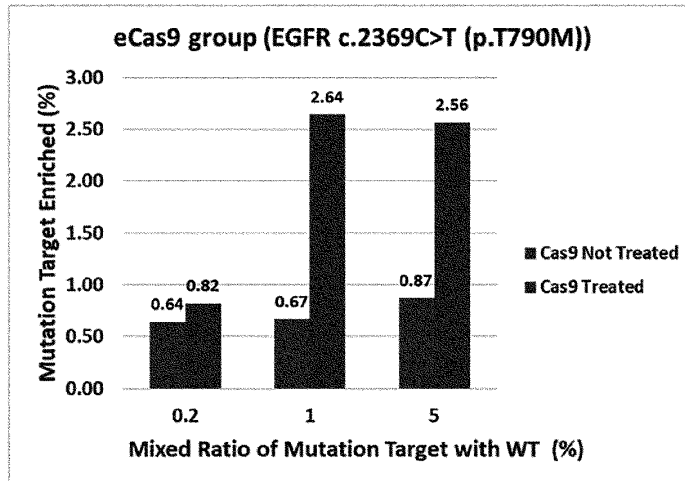
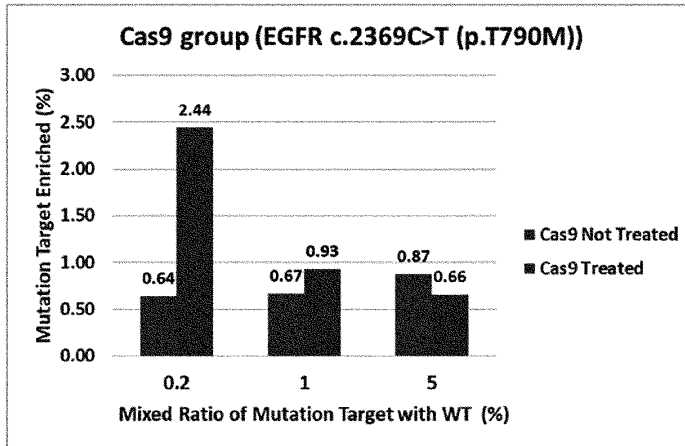
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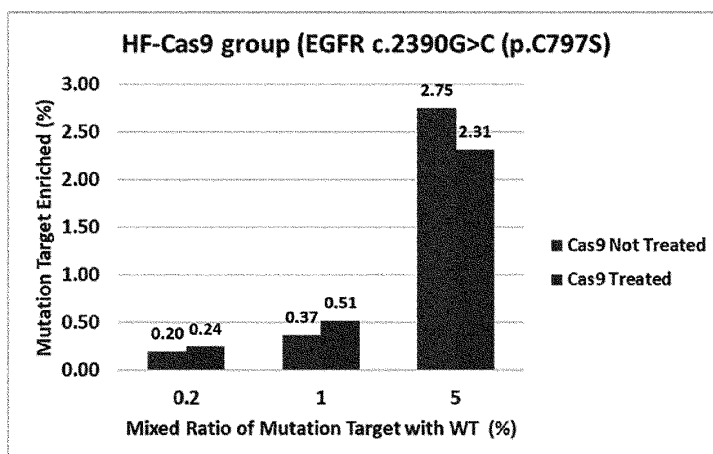
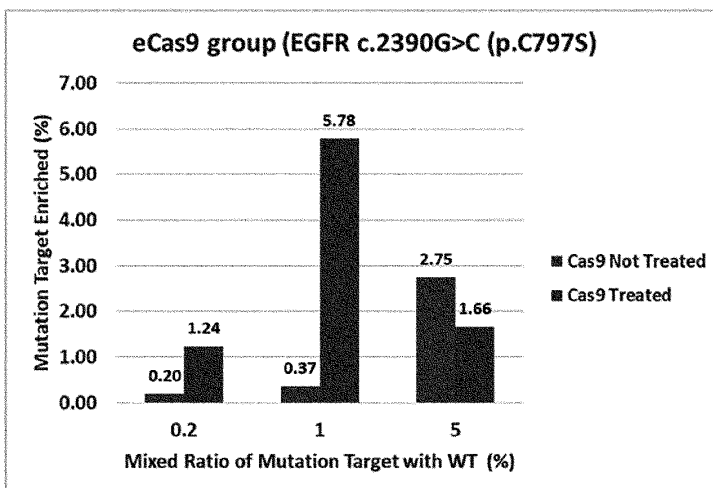
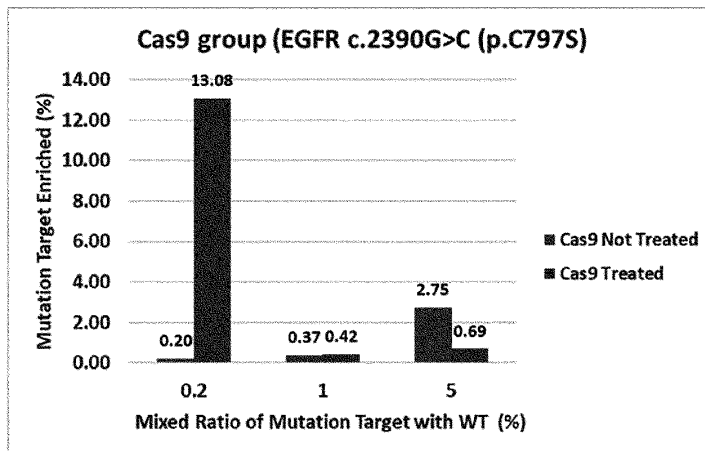
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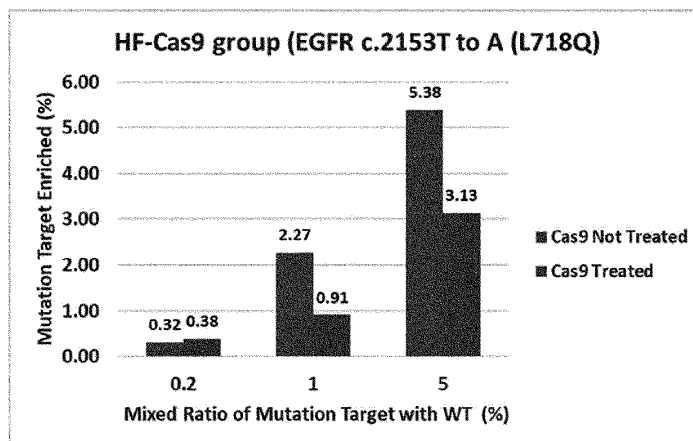
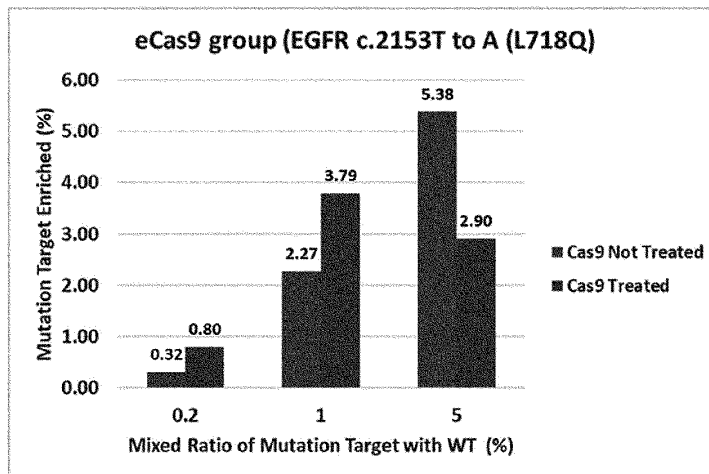
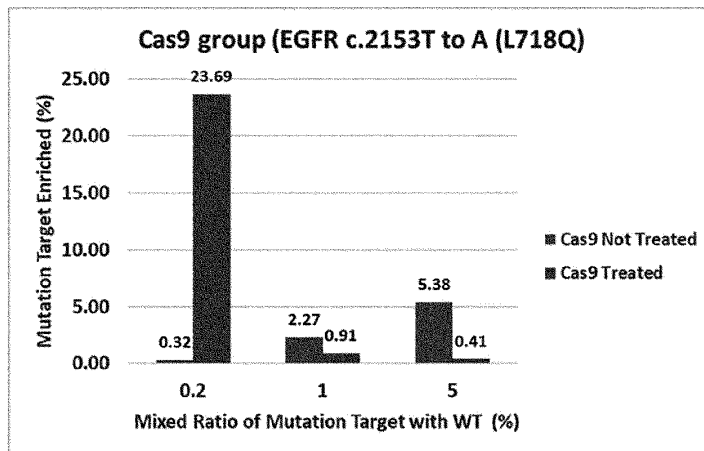
[도13]



[도14]



[도15]



[516]

sgRNA activity  
confirmed

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

| Gene   | Variant         | Amplicon size (bp) | Expected AE (%) | WT Conc (copies/ $\mu$ l) | Mutant Conc (copies/ $\mu$ l) | Total Conc (copies/ $\mu$ l) |
|--------|-----------------|--------------------|-----------------|---------------------------|-------------------------------|------------------------------|
| 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                         |

KRAS p.G12?

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

| Gene   | Variant         | Amplicon size (bp) | Expected AE (%) | WT Conc (copies/ $\mu$ l) | Mutant Conc (copies/ $\mu$ l) | Total Conc (copies/ $\mu$ l) |
|--------|-----------------|--------------------|-----------------|---------------------------|-------------------------------|------------------------------|
| 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                         |

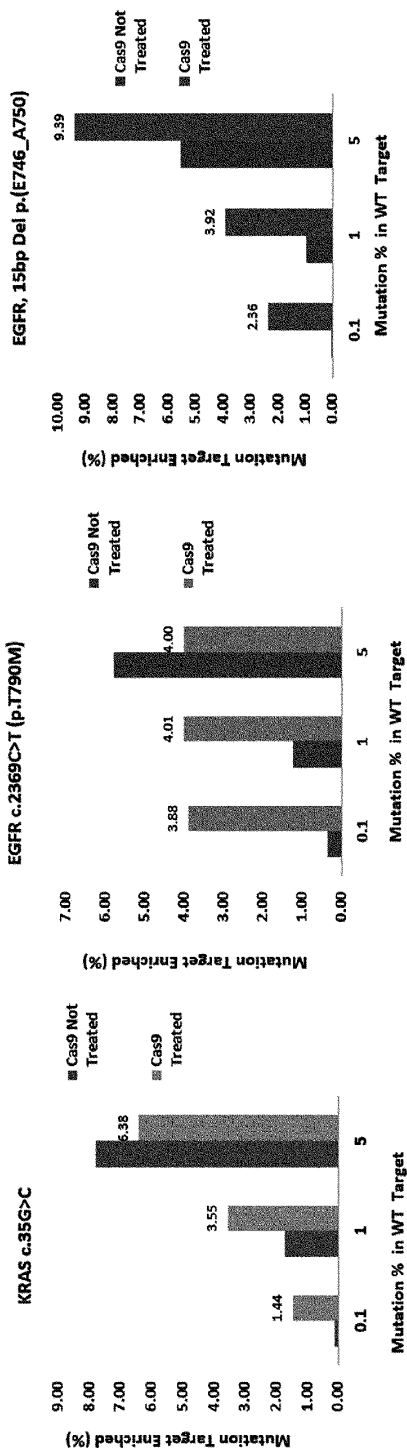
Table 3: 0.1% Multiplex l cfDNA Reference Standard (Part No.: HD779)

| Gene   | Variant         | Amplicon size (bp) | Expected AE (%) | WT Conc (copies/ $\mu$ l) | Mutant Conc (copies/ $\mu$ l) | Total Conc (copies/ $\mu$ l) |
|--------|-----------------|--------------------|-----------------|---------------------------|-------------------------------|------------------------------|
| 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                         |

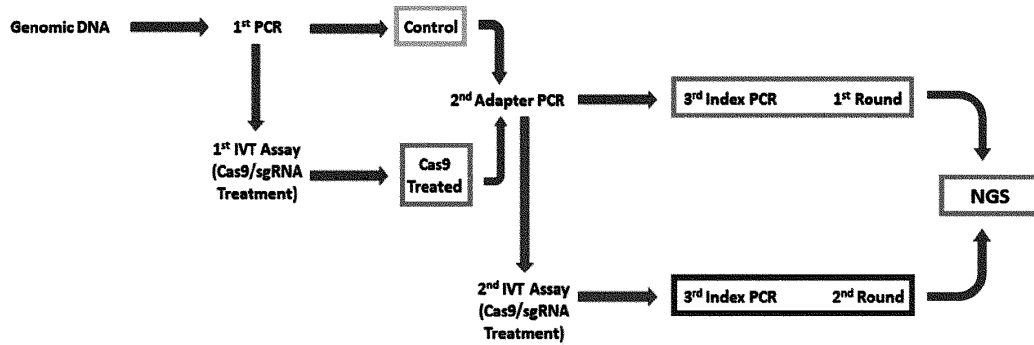
Table 4: 100% Multiplex l wt cfDNA Reference Standard (Part No.: HD776)

| Gene   | Variant         | Amplicon size (bp) | Expected AE (%) | WT Conc (copies/ $\mu$ l) | Mutant Conc (copies/ $\mu$ l) | Total Conc (copies/ $\mu$ l) |
|--------|-----------------|--------------------|-----------------|---------------------------|-------------------------------|------------------------------|
| 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                         |

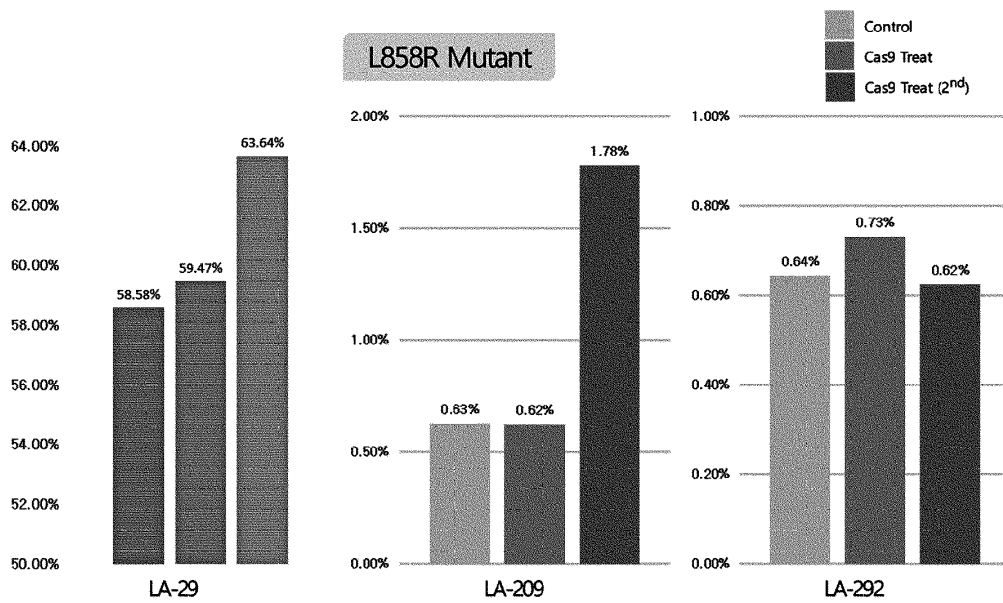
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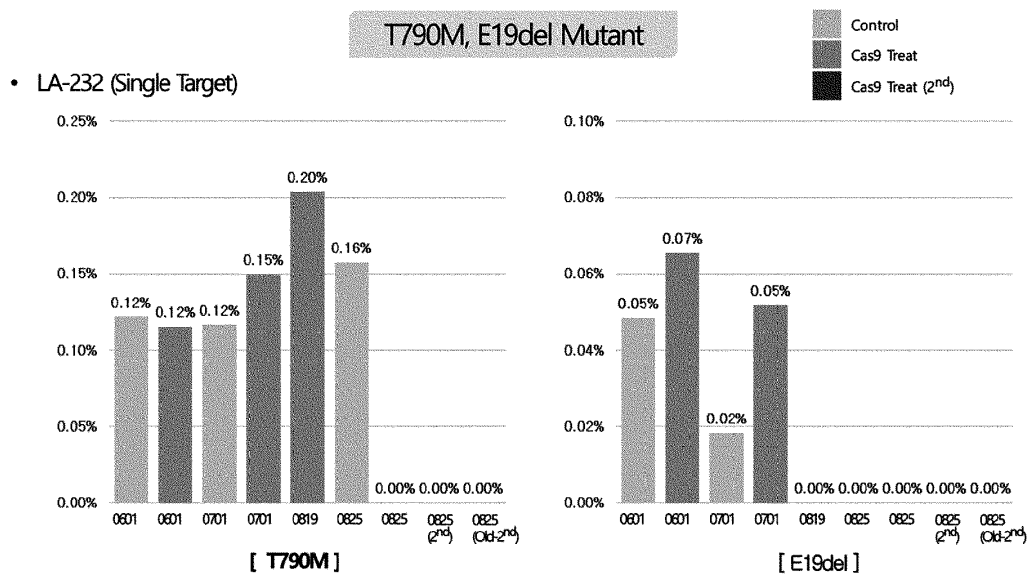
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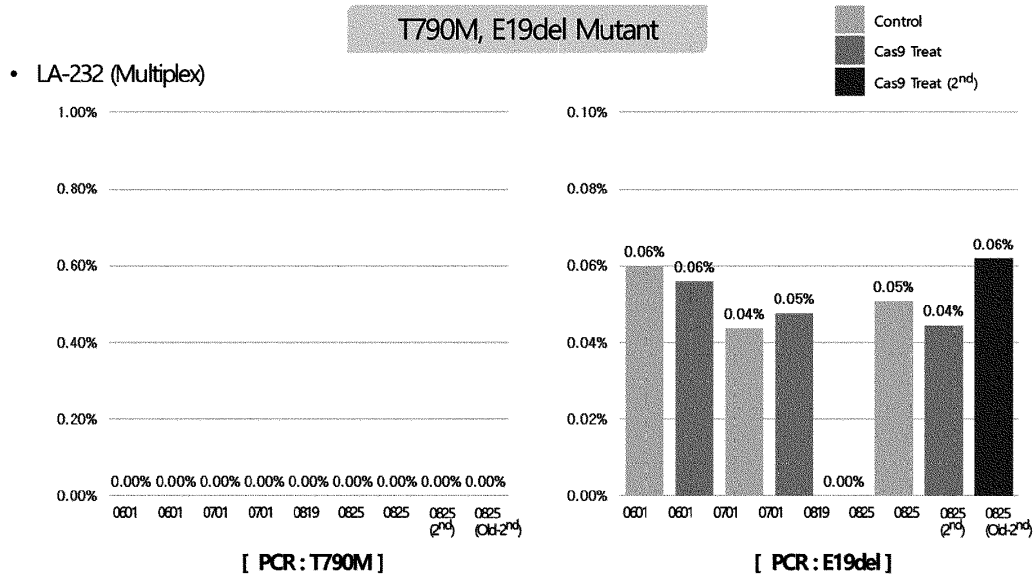
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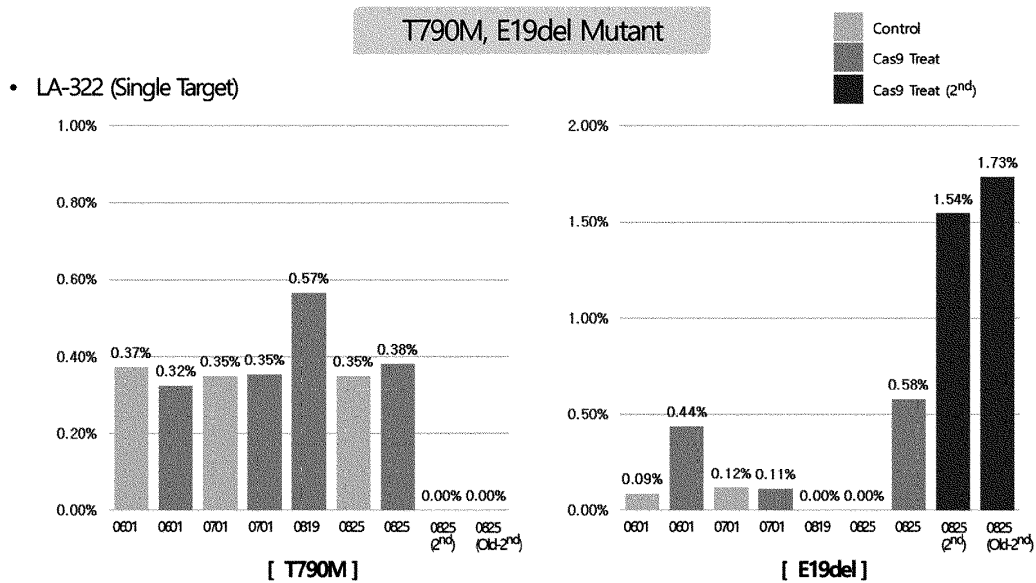
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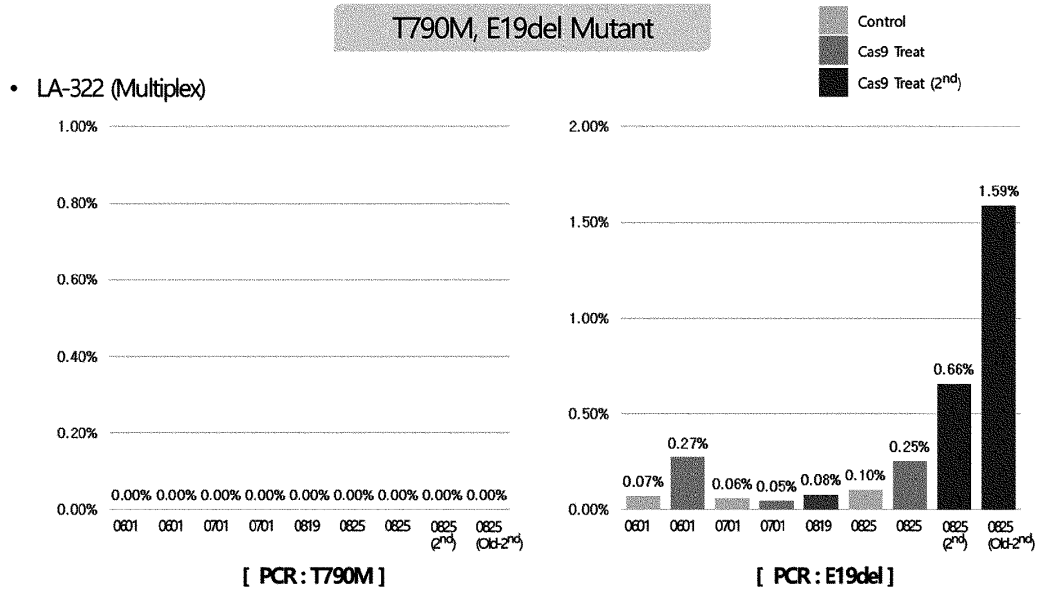
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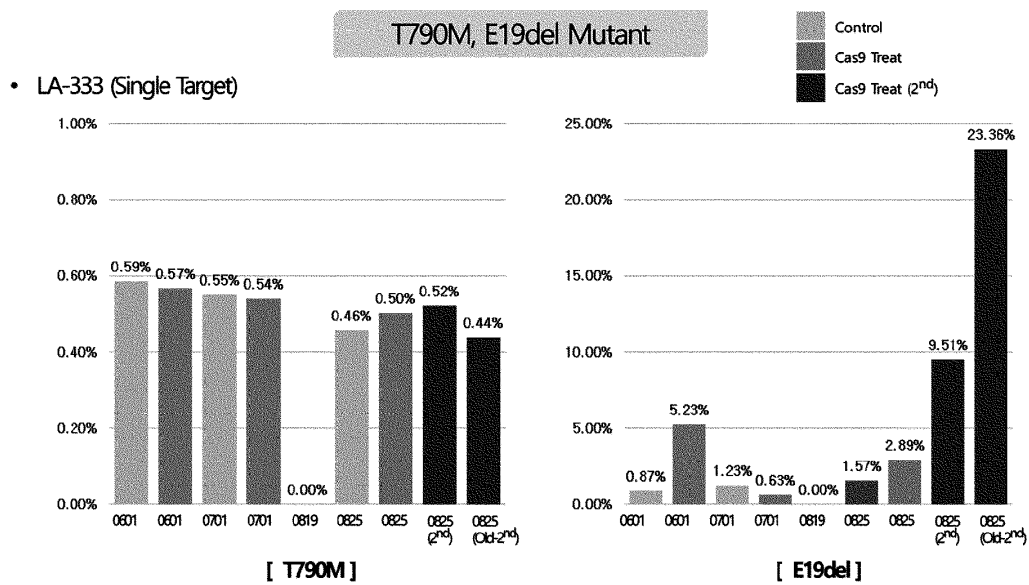
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[도 23]



[도 24]



[도 25]

