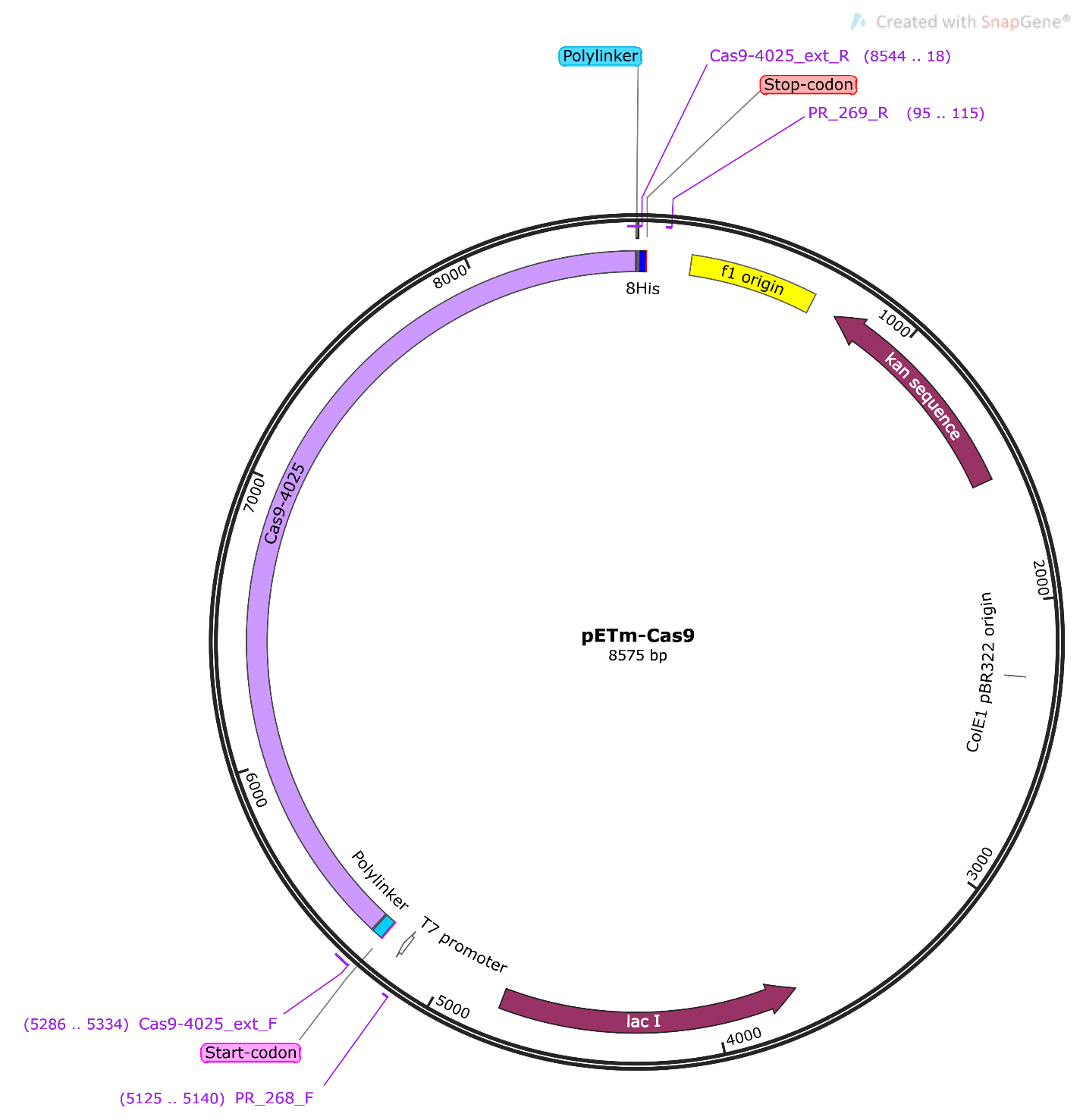
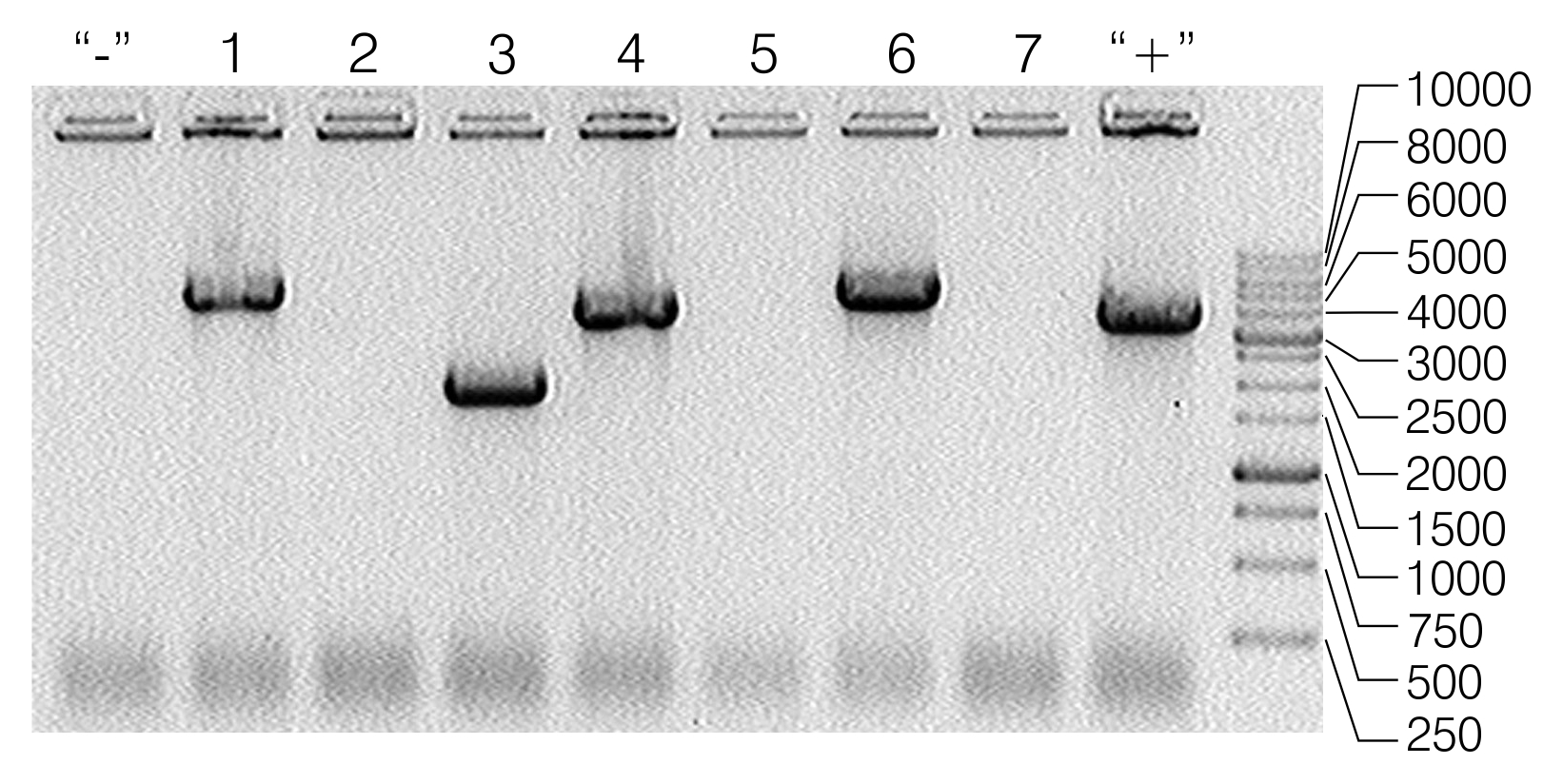
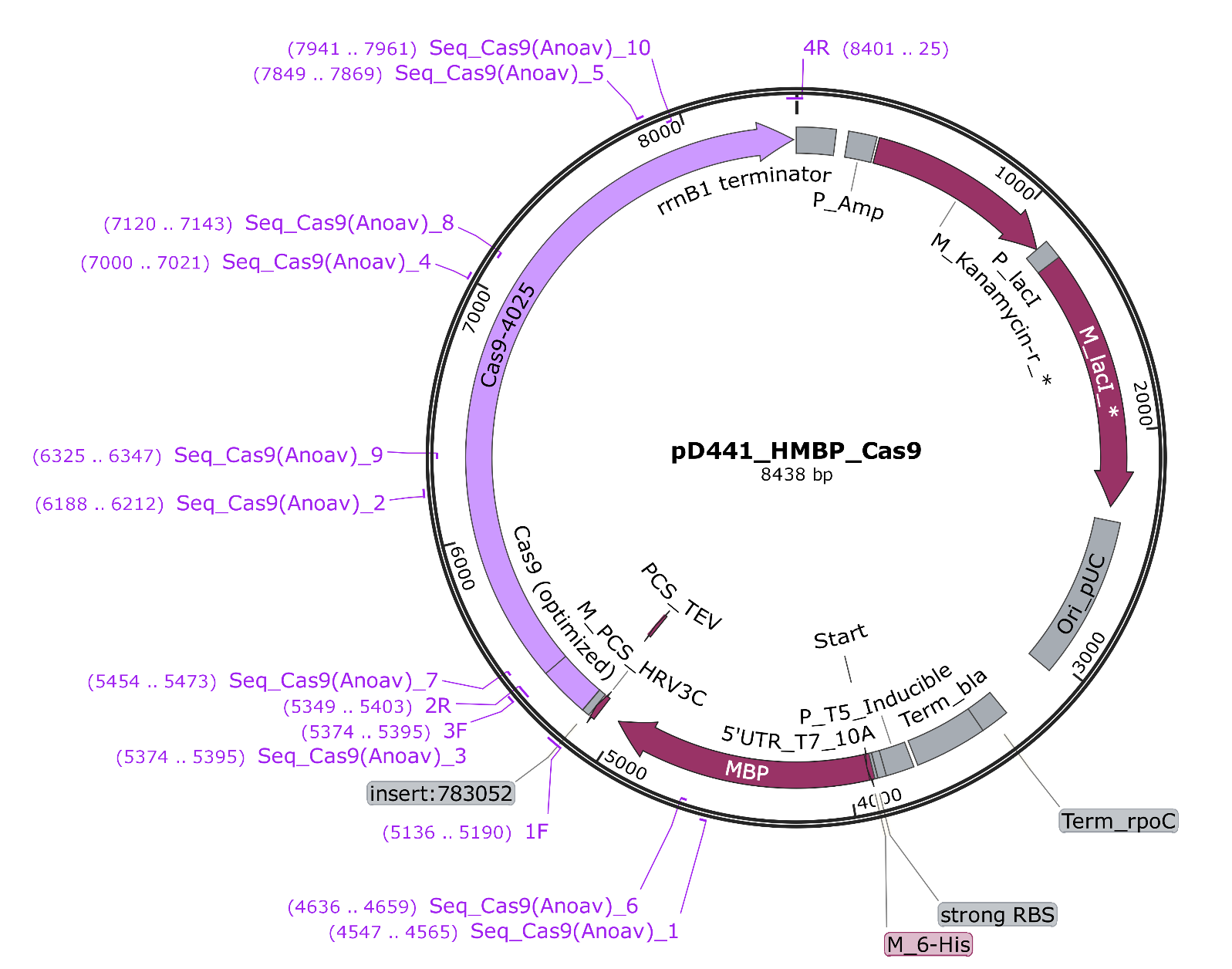
**Supplementary Materials:**

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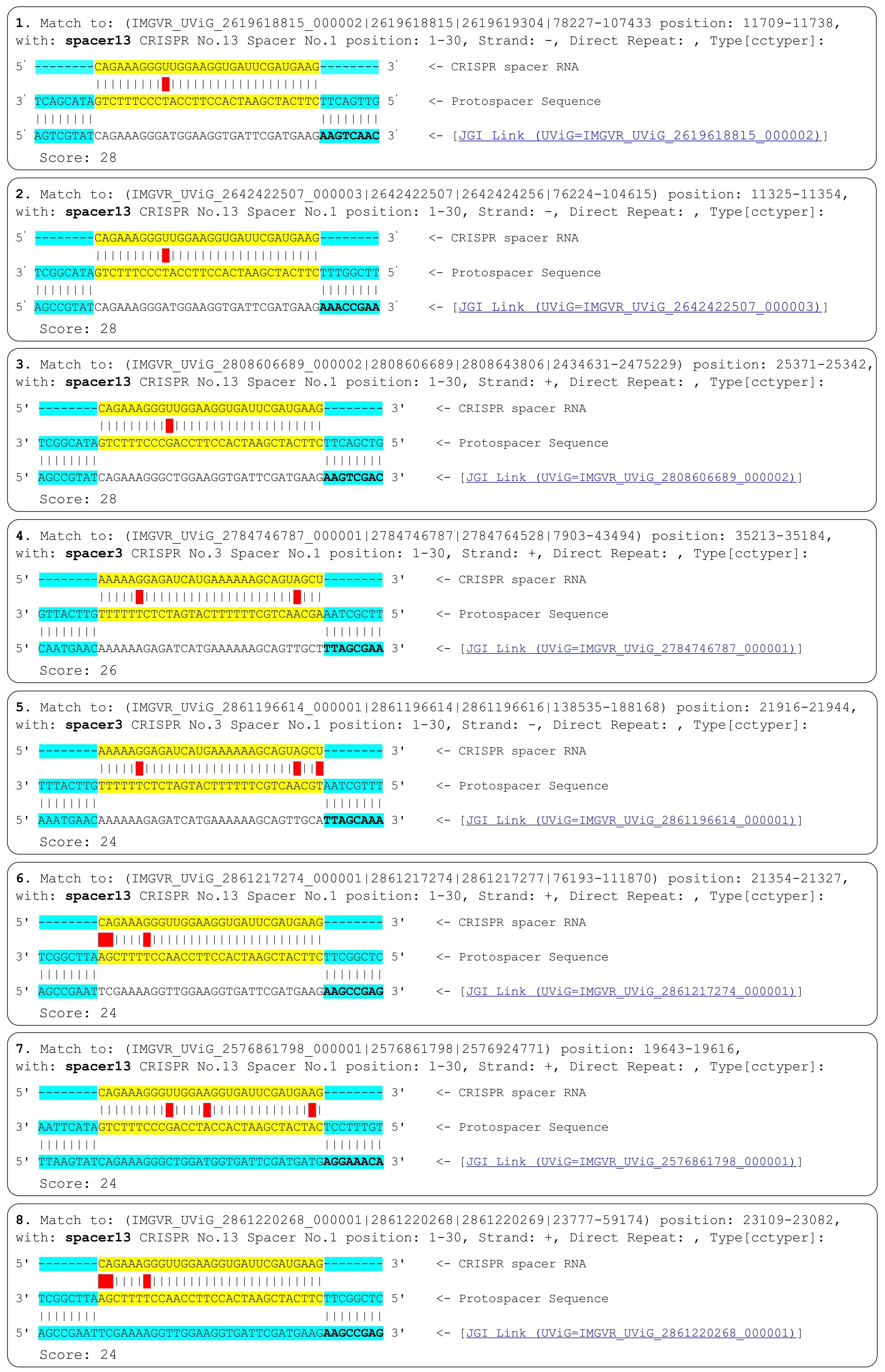
**Figure S1.** pETm-AnoCas9 map.



**Figure S2.** Analysis of PCR products after electrophoresis in 1% agarose gel. The expected product length was 3,580 bp in the case of the *anoCas9* insertion.Lane “-“ – negative control, lane “+“ – positive control (ligation mixture was used as a template).Lanes 1-7 refer to the colonies 1-7, respectively.

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**Figure S3.** pD441-HMBP-AnoCas9 map.

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**Figure S4.** CRISPRTarget spacer analysis. First 8 targets are presented that were used to predict and create the PAM sequence logo.

**Table S1.** Amplification primers used for the generation of FAM-labeled dsDNA PAM-templates.

|  |  |
| --- | --- |
| **Name** | **Structure (5’-3’)** |
| Uni-Spacer-F | caagaaggagatgtctggggatgtcagggatgcatttgtggccat |
| FAM-Uni-R | ttctcatctgtgccagcacccttcatggatttgtaaagtttgtcggcaaagaagaga |
| Spacer-PAM-R\_1 | ttgtcggcaaagaagagaggcttgttcttgacactttgaacaatggccacaaatgcatc |
| Spacer-PAM-R\_2 | ttgtcggcaaagaagagaggcttgttcttgacacttggaacaatggccacaaatgcatc |
| Spacer-PAM-R\_3 | ttgtcggcaaagaagagaggcttgttcttgacacttcgaacaatggccacaaatgcatc |
| Spacer-PAM-R\_4 | ttgtcggcaaagaagagaggcttgttcttgacacttagaacaatggccacaaatgcatc |
| Spacer-PAM-R\_5 | ttgtcggcaaagaagagaggcttgttcttgacactttgatcaatggccacaaatgcatc |
| Spacer-PAM-R\_6 | ttgtcggcaaagaagagaggcttgttcttgacacttggatcaatggccacaaatgcatc |
| Spacer-PAM-R\_7 | ttgtcggcaaagaagagaggcttgttcttgacacttcgatcaatggccacaaatgcatc |
| Spacer-PAM-R\_8 | ttgtcggcaaagaagagaggcttgttcttgacacttagatcaatggccacaaatgcatc |
| Spacer-PAM-R\_9 | ttgtcggcaaagaagagaggcttgttcttgacactttgagcaatggccacaaatgcatc |
| Spacer-PAM-R\_10 | ttgtcggcaaagaagagaggcttgttcttgacacttggagcaatggccacaaatgcatc |
| Spacer-PAM-R\_11 | ttgtcggcaaagaagagaggcttgttcttgacacttcgagcaatggccacaaatgcatc |
| Spacer-PAM-R\_12 | ttgtcggcaaagaagagaggcttgttcttgacacttagagcaatggccacaaatgcatc |
| Spacer-PAM-R\_13 | ttgtcggcaaagaagagaggcttgttcttgacactttgaccaatggccacaaatgcatc |
| Spacer-PAM-R\_14 | ttgtcggcaaagaagagaggcttgttcttgacacttggaccaatggccacaaatgcatc |
| Spacer-PAM-R\_15 | ttgtcggcaaagaagagaggcttgttcttgacacttcgaccaatggccacaaatgcatc |
| Spacer-PAM-R\_16 | ttgtcggcaaagaagagaggcttgttcttgacacttagaccaatggccacaaatgcatc |
| Spacer-PAM-R\_18 | ttgtcggcaaagaagagaggcttgttcttgacacgtcgaccaatggccacaaatgcatc |
| Spacer-PAM-R\_19 | ttgtcggcaaagaagagaggcttgttcttgacacctcgaccaatggccacaaatgcatc |
| Spacer-PAM-R\_20 | ttgtcggcaaagaagagaggcttgttcttgacacatcgaccaatggccacaaatgcatc |
| Spacer-PAM-R\_21 | ttgtcggcaaagaagagaggcttgttcttgacacttcgacctatggccacaaatgcatc |
| Spacer-PAM-R\_22 | ttgtcggcaaagaagagaggcttgttcttgacacgtcgacctatggccacaaatgcatc |
| Spacer-PAM-R\_23 | ttgtcggcaaagaagagaggcttgttcttgacacctcgacctatggccacaaatgcatc |
| Spacer-PAM-R\_24 | ttgtcggcaaagaagagaggcttgttcttgacacatcgacctatggccacaaatgcatc |
| Spacer-PAM-R\_25 | ttgtcggcaaagaagagaggcttgttcttgacacttcgaccgatggccacaaatgcatc |
| Spacer-PAM-R\_26 | ttgtcggcaaagaagagaggcttgttcttgacacgtcgaccgatggccacaaatgcatc |
| Spacer-PAM-R\_27 | ttgtcggcaaagaagagaggcttgttcttgacacctcgaccgatggccacaaatgcatc |
| Spacer-PAM-R\_28 | ttgtcggcaaagaagagaggcttgttcttgacacatcgaccgatggccacaaatgcatc |
| Spacer-PAM-R\_29 | ttgtcggcaaagaagagaggcttgttcttgacacttcgacccatggccacaaatgcatc |
| Spacer-PAM-R\_30 | ttgtcggcaaagaagagaggcttgttcttgacacgtcgacccatggccacaaatgcatc |
| Spacer-PAM-R\_31 | ttgtcggcaaagaagagaggcttgttcttgacacctcgacccatggccacaaatgcatc |
| Spacer-PAM-R\_32 | ttgtcggcaaagaagagaggcttgttcttgacacatcgacccatggccacaaatgcatc |
| Spacer-PAM-R\_33 | ttgtcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc |
| Spacer-PAM-R\_34 | ttgtcggcaaagaagagaggcttgttcttgacacttcgtcgtatggccacaaatgcatc |
| Spacer-PAM-R\_35 | ttgtcggcaaagaagagaggcttgttcttgacacttcgtcctatggccacaaatgcatc |
| Spacer-PAM-R\_36 | ttgtcggcaaagaagagaggcttgttcttgacacttcgtcatatggccacaaatgcatc |
| Spacer-PAM-R\_37 | ttgtcggcaaagaagagaggcttgttcttgacacttcggcttatggccacaaatgcatc |
| Spacer-PAM-R\_38 | ttgtcggcaaagaagagaggcttgttcttgacacttcggcgtatggccacaaatgcatc |
| Spacer-PAM-R\_39 | ttgtcggcaaagaagagaggcttgttcttgacacttcggcctatggccacaaatgcatc |
| Spacer-PAM-R\_40 | ttgtcggcaaagaagagaggcttgttcttgacacttcggcatatggccacaaatgcatc |
| Spacer-PAM-R\_41 | ttgtcggcaaagaagagaggcttgttcttgacacttcgccttatggccacaaatgcatc |
| Spacer-PAM-R\_42 | ttgtcggcaaagaagagaggcttgttcttgacacttcgccgtatggccacaaatgcatc |
| Spacer-PAM-R\_43 | ttgtcggcaaagaagagaggcttgttcttgacacttcgccctatggccacaaatgcatc |
| Spacer-PAM-R\_44 | ttgtcggcaaagaagagaggcttgttcttgacacttcgccatatggccacaaatgcatc |
| Spacer-PAM-R\_45 | ttgtcggcaaagaagagaggcttgttcttgacacttcgacttatggccacaaatgcatc |
| Spacer-PAM-R\_46 | ttgtcggcaaagaagagaggcttgttcttgacacttcgacgtatggccacaaatgcatc |
| Spacer-PAM-R\_48 | ttgtcggcaaagaagagaggcttgttcttgacacttcgacatatggccacaaatgcatc |
| Spacer-PAM-R\_33 | ttgtcggcaaagaagagaggcttgttcttgacacttcgtcttatggccacaaatgcatc |

**Table S2.** Sequences of CRISPR array elements derived from the *Anoxybacillus flavithermus* genome.

|  |  |
| --- | --- |
| **Name** | **Structure (5’-3’)** |
| CRISPR repeat | gtaatagttcccctgaggttattgctgtgttatgat |
| CRISPR spacer 1 | aaaatgacacacttatttgataaaaagaaa |
| CRISPR spacer 2 | ccgaaagttttagactcactataaatcctc |
| CRISPR spacer 3 | aaaaaggagatcatgaaaaaagcagtagct |
| CRISPR spacer 4 | cgtgggtcaattcggtaaatggaagttttt |
| CRISPR spacer 5 | tgctacacttttgcaccgaaacggagcgga |
| CRISPR spacer 6 | agaccagtccaactagaaattaattttaga |
| CRISPR spacer 7 | tgaagaaaaagaaaactgtagagaaacaac |
| CRISPR spacer 8 | tcatcagcaaaaatgtatagcgagaacggc |
| CRISPR spacer 9 | tttctgtctagcagaacaactatttctgaa |
| CRISPR spacer 10 | tttgctactatgatagaaagaacattagtg |
| CRISPR spacer 11 | tttctgtctagcagaacaactatttccgaa |
| CRISPR spacer 12 | tttctgtctagcagaacaactatttcggcg |
| CRISPR spacer 13 | cagaaagggttggaaggtgattcgatgaag |
| CRISPR spacer 14 | ccaaattcatgttttaagaacccgctgata |
| CRISPR spacer 15 | atcatttaacgtcttactgatccgcgtatt |
| CRISPR spacer 16 | tggtggtgatatgcctaataacgaattaaa |

**Table S3.** Structure of dsDNA templates used as a library to determine AnoCas9 PAM specificity. The PAM motif is highlighted in green.

|  |  |
| --- | --- |
| **Name** | **Structure (5’-3’)** |
| Spacer-PAM \_1 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGTTCAAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_2 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGTTCCAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_3 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGTTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_4 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGTTCTAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_5 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGATCAAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_6 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGATCCAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_7 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGATCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_8 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGATCTAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_9 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGCTCAAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_10 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGCTCCAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_11 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGCTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_12 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGCTCTAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_13 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCAAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_14 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCCAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_15 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_16 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCTAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_18 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCGACGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_19 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCGAGGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_20 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATTGGTCGATGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_21 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_22 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGTCGACGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_23 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGTCGAGGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_24 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGTCGATGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_25 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATCGGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_26 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATCGGTCGACGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_27 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATCGGTCGAGGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_28 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATCGGTCGATGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_29 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATGGGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_30 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATGGGTCGACGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_31 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATGGGTCGAGGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM \_32 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATGGGTCGATGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_33 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAAGACGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_34 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATACGACGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_35 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGACGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_36 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATATGACGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_37 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAAGCCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_38 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATACGCCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_39 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGCCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_40 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATATGCCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_41 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAAGGCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_42 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATACGGCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_43 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAGGGCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_44 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATATGGCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_45 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATAAGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_46 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATACGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |
| Spacer-PAM-R\_48 | CAAGAAGGAGATGTCTGGGGATGTCAGGGATGCATTTGTGGCCATATGTCGAAGTGTCAAGAACAAGCCTCTCTTCTTTGCCGACAAACTTTACAAATCCATGAAGGGTGCTGGCACAGATGAGAA |