pAAVD-Grin1(1300)-CAG-Crimson-PolyA(rbG)-Grin1(1200)

2024-07-29

4.6k

ITR- Grin1in9(stuffer,1327bp)-CAG-crimson-polyA(rbGlob)- Grin1in9(stuffer,1184bp)-ITR



2024-09-12 NP测序结果：

CAG部分（多个连续G碱基）若很重要建议sanger测序  
466：Grin1in9(stuffer,1327bp)部分polyT若很重要建议sanger测序

1038：del c

1045：a-g

其上重复序列部分需要sanger测序核实

StartOfSeq

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCacgcgt TCTAGGGAGAGAGGGGGTCTGAAGGCTGTAGTCAAACATCGATAACCAGAAGCATCCATCACGATGCCAATGGCTGTTAGGAATGTTTCTGGCCTCACTGGTTTGTTCAATTTGATTTTATATGTATGAGTGTTTTGCCTACCTGTATATGTAGATACCATGTGCTCTCCTGGTGCCCACAGAATTCAGGATATTGGATGGCCTGTAACTGGAATGGATGGTTGTAAGCCACCGTGTGGGCGGTAAGAACAGAAACCGGGTCTTCTGTAAGACCTTCGGGTGCTCATAACTGCTCACCCACTTGTCTAACCCCATCACTAATTTTTTTTTTTTTGACAGCGCTAGCAAAAACACATAATAGAAAAATATGATCCTGCTTCCTTCTCTCTTCCTTCTCAAGACTCCCAGTGAGGGCACCTCCTCCAGTGCCTGTCACCATGCCTTGCCTCACTGTCCACTCCTGCCTGGGCTACCTACCCCTCTGTTTTTTGCATCCGATATAGACCCTCAGAAACCAACTGTGTTCCACTCTGGCATCCCCCAGACTTCTACCTGTCCCTCAACTATGAAACTGTAGACCCTAGAGAATAGGGAGGGAGAATGTCAAAGCTGAAGAGGCAGCCTTGAAAGAGAAGCATTAACTAGGCATGGTGGAGCATGCCTTTAAACCCAGCACTTTGGAAGTAGATGAACAAGAATCAGAAGTTCAAGGTCCTCCTCAGCTAAAAGCCAGCAGGGACAGCCTTGACTCTATGAAGGCTCTTTCTTTTTTGTGGTGGGACATCAAGAAGAGTTCATTTGGCTTAGACTTTCATATTGTGTTCCATCATTGAAGGAAGTCAGGACAGGAACTTAAACCGGGCAGAAGCCTGGAAGCAGGAGCTGATGCAGAGGCCTGGAgGGGTGCTGTTGATGCAGAGGCCCTGGAGGGGTGCTGCTGATGCAAAGGCCCTGGAGGGGTGCTGCTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGATGGGTGCTGCTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGAGGGGTGCTGCTCACTGGCTTGCTCCTTATGGCTTGCTTGGCTTGCTTTCTTATAAAACCCGGAACCACCCGCCCAGGGTGGCTTCACCTACCATGGGTTTGCCCCTCTCTCCTTCAATGGCTATTTAAGAAAATGCTTTACAGGCTGGCCT

agctagcCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTGGATCCgcgatcgcc

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GTTGGTCACAAAGCTGGCTGACTAACCAATGCAGCACAGTTACTGTGCTCCAGGCTTAGAAGAAAACTTGTCAAAAATAATATTAGTGATAATGATAACAATAATAAAAAACAGTGGGACTGGAGAGATGGCTCAGTGGTTTAGAGCCCTGGCTATTCTCCCATAGGACCCGGGTTCAAGTCCCAGTACCCACACAGCAGCTCACAACCATCTGTATCTATAGTTCCAGGGGATCTAATGTTCTTTCTGACCTCTGTGGGTTCTGTATGCACCTGGAGTACAAACGTGTATGCTGGCAAAACACCCATACACTTAAAATAGAGAAGATATTCTCTCAGAGGTTAAGAGAAATTGTTGCTCTTCCAAAAAAAAAAAAAAAAAAAAAAAAAACTGGGCTCAGTTCCCAGCACCCACATGGTAGTTACAACTATGTGTAGCTTCAGTTCCAGGGGATTTGACGCCTCCTTCTGGTTTCTGTGGACCCCTGCATGTACGTGATACACATACATACACTGAGGCACACACACACACATAAGATAAAAACAAAAGATAAATATATAAGTGATAAAAGTGATAGAGAAAGACATGGGGCATTATCCTCCATCCTCTGTGTACTTGCTCATATACATGAATACACAGGAACACATACATACATATAAACATATACATACATGTAAACATGTATACATATATACATACATATAAAACTACCTAAAAGGTAGTTTTTTAAAAGATGAGGATCAAAGACTGAAGGCTACATGCCCATGGAGGCCAGGGTAGCTTTGGAAAGGACACAGCCTTTCTGCTCCCTGATTTGTCCGAGACAGTGCCTGGTGCACACTTCCTGCACAGTAGCACTATGATAGAATAATGTATAAAGGGGCTGGAGAGATGGCTCAGTGGTTGAGAGCACTGACTGCTCTTCAGAAGGTCCTGAGTTCAATTCCCGGCAACCACATGATGGCTCACAACCATCCGTAATGAGATCTGGCACCCTCTTCTGGAGTGTTTGAAGACAGCTACAGTGTACTTACATATAATAAATAAATAAATCTTTTTTTAAAAAATCTGAAAAATAAAAGAAGAATGTATAAGCTTAAGCTGCAGCCTGAGAGAAAGGGGCCACAGGAGAGGTCGCAGTACTGCAGGATGCATTTTTTGATGAGTCAGGGCAGGTGGCTATA

CacgtgCGGACCGAGCGGCCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

GGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCtTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACtCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGtCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTtCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT

EndOfSeq

Backbone: Cut pAAV-CMV-GFP-PolyA P1628

图示

描述已自动生成

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Ends** | **Coordinates** | **Length (bp)** |
| 1 | PmlI-MluI | 2622-139 | 2896 (BB for AAV) |
| 2 | EcoRI-PmlI | 1316-2621 | 1306 |
| 3 | MluI-EcoRI | 140-1315 | 1176 |

共4个insert

酶切+PCR法

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **insert** | **PCR template** | **F-Primer** | **R-Primer** | Length |
| 1 (Grin1in9-1) | Mus gDNA | T6181  T6183 | T6182  T6184 | 2.7k  1.3k |
| 2 (CAG- Crimson) | P2163 | NheI-PmeI | 酶切 | 1.6k |
| 3 (polyA-s rbGlob) | P1629 | T6185 | T6186 | 126bp |
| 4 (Grin1in9-2) | Mus gDNA | T6187  T6189 | T6188  T6191 | 1.4k  1.2k |

pAAV-U1a-SpCas9-polyA P1629

图示, 示意图

描述已自动生成

T6181-Grin-2.7k-F CTGTTTGCAGTTCAGGCACC Tm=60C

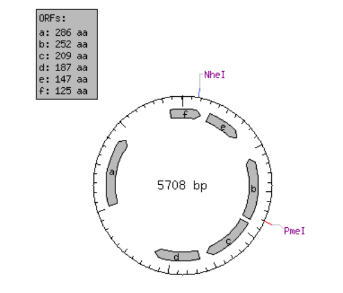
T6182-Grin-2.7k-R TGGCTGGTGTGGAACTTGTT Tm=60C

T6183-Grin-1.3k-F ATCACTAGGGGTTCCTGCGGCCGCACGCGTTCTAGGGAGAGAGGGGGTCTGA Tm=79C

T6184-Grin-1.3k-R GTCATTATTGACGTCAATGGgctagctAGGCCAGCCTGTAAAGC Tm=69C

pAAV-CAG-Crimson-Wpre-polyA P2163

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Ends** | **Coordinates** | **Length (bp)** |
| 1 | PmeI-NheI | 1807-166 | 4068 |
| 2 | NheI-PmeI | 167-1806 | 1640（CAG-Crimson） |



T6185-Crim-PArb-F CGCCACCACCTGTTCCAGTAGgtttaaacgAtctttttccctctgccaaaaattatggg Tm=71C

T6186-PArb-R CTTTGTGACCAACcgagtgagagacacaaaaaattcc Tm=65C

T6187-Grin-1.4k-F TGAATGTGGATCCCAGCACC Tm=60C

T6188-Grin-1.4k-R AGCCTAAAGCCTCCCTCTGA Tm=60C

T6189-Grin-1.2k-F gtctctcactcgGTTGGTCACAAAGCTGGCT Tm=68C

T6191-Grin-1.2k-R GTTCCTGCGGCCGCTCGGTCCGCACGTGTATAGCCACCTGCCCTGACTCAT

0 Tm=79C

附Gin1 intron9序列信息（Stuffer）

>mm39\_dna range=chr2:25188709-25192910 5'pad=0 3'pad=0 strand=- repeatMasking=none

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ACTTGTCCTAATCCAGAGATGTCTTTGACGTAGCTTCGGATGCAGTGGCTGTGTTGTCCATCTGCTGCTGGCAGGCAGGCACCATGGCCTCCAGTCTGGTTGAGGAGTTACCATCTGCTAATCTTTCCTTTCTTGAGACTAGGGCAGAGGAATGAGAGCTTGGGCCACTTGGAAGCCAGTAAGAACAGGTGTTCTTGATAAGTTTAAGCTATCCTCTCTTCCCAGGGTGCTGATGTCTTCCAAGTATGCAGATGGAGTGACTGGCCGTGTGGAATTCAATGAGGATGGGGACCGGAAGTTTGCCAACTATAGTATCATGAACCTGCAGAACCGCAAGCTGGTGCAAGTGGGCATCTACAATGGTACCCATGTAGGTGGGGGTCATGAGGGGGTGGGGGCTGGGGCCTTAGGGTCCTGGGGCCAAGACCCCTGCGTGGCCACCCTCCATCTCATACTCCCACCCCCAGGTCATCCCAAATGACAGGAAGATCATCTGGCCAGGAGGAGAGACAGAGAAGCCTCGAGGATACCAGATGTCCACCAGACTAAAGGTGGGGACCCCACACTCCCCAATATTACCATCTGTGATGTTTCATCTATTCCTTCAGGCCAGGGGGAAAGGGGATGAAGTCACAGAACACAGATAGAGGAGGTGGAGGCTGTGCCAGCACCTGGCTCTGGCGCACATGGCAGAGGAAGCAGGTGTGGAGGTGGGGGAAGGAGGAGCAGCAGTTTCTGCCCAACAGGGTCTTTGCAGTACTCTATCCTGGCCCAGCTCTAGTCCAAGGAAGAACTGGCAGAGGCAGAGGGACAGTGGGTGGATGCTTTGGGTCCCAGTGCAGAGTTTAGCTTTAAAAAGTGTCCTATTTCTCCTTGGGCACCCAGGAACCCCTTCTTAAGCTGTCTTCTTCCTGTATAGTCTCTTTTCCTCGGCCTGATTTACACTTTCCTTGGGTTCATCCATGGACCCCTCCCTCTGCCTAAGGGCTATTCTGAGAATTCCTCTTTACGATGAGCCACAGTGTTCCCAGCTCTGATACTGTAGATCACCAAAGGCAGCCTCTGCAGTGCCAAGTGCCTAGCCAACCTTCTCTTAGTGCCTTCATCAAATGTCTCAGCCCTACTAACTCTCTGAGCTAACCCCTGCAGTGGGGGCTCATTCTCCAGTGAGTACCTGCCTGCAGTTCTCACAGGACAGTGAGAGTGTCGCCTTGCAAGGCCTGTGTGATAACTGTTTGCAGTTCAGGCACCTAGTGTTGAAAAGTGTGAGAGTGCAGGGAGGTGGTGGCATGTCCCTTTAATCCCAGAGGCAGCGGCACACAGATCTCTATGGGTTTGAGGCCAGCTTGGTCTACACAGCTTATTCTAGGACAGCCAGGGCTACACAAAGAAACCCTGTCTTGAAAAGCCAAGGGGGAGGGCTGGAGAGATGGCTCAGCCCTTAGTAGCATTGGCTGCTTTTCCAGAGGTCATGAGTTCAATTCCCAGCAACCACATGGTGGCTCACAACCATCTGTAATGGGATCCAACGCCCTTTTCTTGTGTCAGCGACAGCATGTTCATATACATAAAATAAATAAGACTTAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGGAAGAAAGAAAGAAAAGGAAGAAAAGAAAAGTCTAGGGAGAGAGGGGGTCTGAAGGCTGTAGTCAAACATCGATAACCAGAAGCATCCATCACGATGCCAATGGCTGTTAGGAATGTTTCTGGCCTCACTGGTTTGTTCAATTTGATTTTATATGTATGAGTGTTTTGCCTACCTGTATATGTAGATACCATGTGCTCTCCTGGTGCCCACAGAATTCAGGATATTGGATGGCCTGTAACTGGAATGGATGGTTGTAAGCCACCGTGTGGGCGGTAAGAACAGAAACCGGGTCTTCTGTAAGACCTTCGGGTGCTCATAACTGCTCACCCACTTGTCTAACCCCATCACTAATTTTTTTTTTTTTGACAGCGCTAGCAAAAACACATAATAGAAAAATATGATCCTGCTTCCTTCTCTCTTCCTTCTCAAGACTCCCAGTGAGGGCACCTCCTCCAGTGCCTGTCACCATGCCTTGCCTCACTGTCCACTCCTGCCTGGGCTACCTACCCCTCTGTTTTTTGCATCCGATATAGACCCTCAGAAACCAACTGTGTTCCACTCTGGCATCCCCCAGACTTCTACCTGTCCCTCAACTATGAAACTGTAGACCCTAGAGAATAGGGAGGGAGAATGTCAAAGCTGAAGAGGCAGCCTTGAAAGAGAAGCATTAACTAGGCATGGTGGAGCATGCCTTTAAACCCAGCACTTTGGAAGTAGATGAACAAGAATCAGAAGTTCAAGGTCCTCCTCAGCTAAAAGCCAGCAGGGACAGCCTTGACTCTATGAAGGCTCTTTCTTTTTTGTGGTGGGACATCAAGAAGAGTTCATTTGGCTTAGACTTTCATATTGTGTTCCATCATTGAAGGAAGTCAGGACAGGAACTTAAACCGGGCAGAAGCCTGGAAGCAGGAGCTGATGCAGAGGCCCTGGAAGGGTGCTGTTGATGCAGAGGCCCTGGAGGGGTGCTGCTGATGCAAAGGCCCTGGAGGGGTGCTGCTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGATGGGTGCTGCTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCCTGGAGGGGTGCTGTTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGAGGGGTGCTGCTGATGCAGAGGCCTGGAGGGGTGCTGCTCACTGGCTTGCTCCTTATGGCTTGCTTGGCTTGCTTTCTTATAAAACCCGGAACCACCCGCCCAGGGTGGCTTCACCTACCATGGGTTTGCCCCTCTCTCCTTCAATGGCTATTTAAGAAAATGCTTTACAGGCTGGCCTATAGCCTAATCTTATGGAGGAATTTTTTCTATTTTTTAAAATTAATTTATTCCTCTCTAGTGTATTACATCCCAACTGTACTCTCCCTTTCATCCCTGTCTTAAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGGAAGGAAGGAAGGAAGGAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAAGAAAAGCATGCCAGGCAATGGTGACACACACTTTAATTCTGTCACTCAGGAGGCAGAGGCAGGAACAGATAAATCTCTGTGAGTTCAAGGACAGCCTGGTCTACAGAGCAAATTTCAGGATAGCCAGGGCTACACAGAGAAACCTTGTCTTGGAAAAAACAAACAAACAAACACAAAACAAAAAACAACAAACAGGAGCTGTAGAGATGGTTCAGTGGTTAAGAGCACTGGCTGCTCTTCAGAGGTCCTGAGTTCAATTCCCAGCAACCACATGGTGGCTCACAACCATCTACAATGAGATCTGATGCCCTCTTCTGGTGTGTCTGAAGACAGCCACAGTGTATTCACATACATAAAATAAATAGGTCTCAAGCCGGGCGTGGTGGCACACGCCTTTAATCCCAGCACTCGGGAGGCAGAGGCAGGCGGATTTCTGAGTTCGAGGCCAGCCTGGTCTACAAAGTGAGCTCCAGGACAGCCAGAGCTATAAAGAGAAACCCTGTCTCGAAAAACCAAAAAAAAAAAAAAAAATCTCTCTAAAAAACAAACAATGCTGAGGTGGTAGGAAATACCTTTAATCCTTGTATTCAGTAGGAAGAGTGGGGTGGAGCTCTGTCAGTTCAAGGCCAGCATGGTCTACAAAACAAGTTCCACACCAGCCAGAGCTACAAGGCAAAGATACAAGACCCTGACTAAAAAAAAAAAAAAAAAAACAGGGCTCAGTGGGTAAGAGCACCCAACTGCTCTTCCGAAGGTCTAGAGTTCAAATCCCAGAAACCACATGGTGGCTCACAACACAACCATCTGTAATGAGATCTGATGCCCTCTTCTGGAGTGTCTGAATACAGCTACAGTGTACTTGTATATAATAAATAAATCTAAAAAAACAAAAAATAAAAAAGTAACTAGCCCAGTGGGTCACATGAATGTGGATCCCAGCACCCATGTAAAAGTCTGGACATGGCTGCACACTGTGCCCATCATAGCCCTTGGTAGAAGGAGTGGGCAGAGGCAGGTTGGTCACAAAGCTGGCTGACTAACCAATGCAGCACAGTTACTGTGCTCCAGGCTTAGAAGAAAACTTGTCAAAAATAATATTAGTGATAATGATAACAATAATAAAAAACAGTGGGACTGGAGAGATGGCTCAGTGGTTTAGAGCCCTGGCTATTCTCCCATAGGACCCGGGTTCAAGTCCCAGTACCCACACAGCAGCTCACAACCATCTGTATCTATAGTTCCAGGGGATCTAATGTTCTTTCTGACCTCTGTGGGTTCTGTATGCACCTGGAGTACAAACGTGTATGCTGGCAAAACACCCATACACTTAAAATAGAGAAGATATTCTCTCAGAGGTTAAGAGAAATTGTTGCTCTTCCAAAAAAAAAAAAAAAAAAAAAAAAAACTGGGCTCAGTTCCCAGCACCCACATGGTAGTTACAACTATGTGTAGCTTCAGTTCCAGGGGATTTGACGCCTCCTTCTGGTTTCTGTGGACCCCTGCATGTACGTGATACACATACATACACTGAGGCACACACACACACATAAGATAAAAACAAAAGATAAATATATAAGTGATAAAAGTGATAGAGAAAGACATGGGGCATTATCCTCCATCCTCTGTGTACTTGCTCATATACATGAATACACAGGAACACATACATACATATAAACATATACATACATGTAAACATGTATACATA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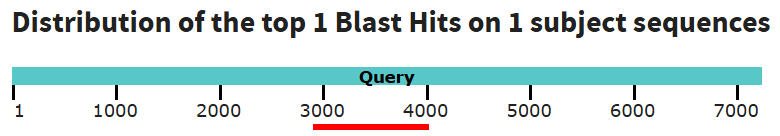
P2389

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| --- | --- | --- | --- |
| **#** | **Ends** | **Coordinates** | **Length (bp)** |
| 1 | PvuI-PvuI | 2428-5926 | 3499 |
| 2 | EcoRI-PvuI | 327-2427 | 2101 |
| 3 | PvuI-EcoRI | 5927-326 | 1588 |

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| --- | --- | --- | --- |
| **#** | **Ends** | **Coordinates** | **Length (bp)** |
| 1 | PstI-PstI | 7-2772 | 2766 |
| 2 | PstI-PstI | 4590-6 | 2605 |
| 3 | PstI-PstI | 2773-4350 | 1578 |
| 4 | PstI-PstI | 4392-4589 | 198 |
| 5 | PstI-PstI | 4351-4391 | 41 |

SEQ T2273-Crimson-seq-F CCCTCCACTGAGCGCAACTA

P2389A T2273-Crimson-seq-F CCCTCCACTGAGCGCAACTA



Query 2897 GTGCTGAAGGGCGAGAACCACATGGCGCTGAAGCTGAAGGGCGGCGGCCACTACCTGTGT 2956

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Sbjct 1 GTGCTG-A-GGCGAGAA-CACATGGCGCTGAAGCTGAAGGGCGGCGGCCACTACCTGTGT 57

Query 2957 GAGTTCAAGTCCATCTACATGGCCAAGAAGCCCGTGAAGCTGCCCGGCTACCACTACGTG 3016

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Sbjct 58 GAGTTCAAGTCCATCTACATGGCCAAGAAGCCCGTGAAGCTGCCCGGCTACCACTACGTG 117

Query 3017 GACTACAAGCTCGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAGCAGTACGAG 3076

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Sbjct 118 GACTACAAGCTCGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAGCAGTACGAG 177

Query 3077 CGCGCCGAGGCCCGCCACCACCTGTTCCAGTAGGTTTAAACGATCTTTTTCCCTCTGCCA 3136

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Sbjct 178 CGCGCCGAGGCCCGCCACCACCTGTTCCAGTAGGTTTAAACGATCTTTTTCCCTCTGCCA 237

Query 3137 AAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAAT 3196

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 238 AAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAAT 297

Query 3197 TTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGTTGGTCACAAA 3256

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Sbjct 298 TTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGTTGGTCACAAA 357

Query 3257 GCTGGCTGACTAACCAATGCAGCACAGTTACTGTGCTCCAGGCTTAGAAGAAAACTTGTC 3316

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Sbjct 358 GCTGGCTGACTAACCAATGCAGCACAGTTACTGTGCTCCAGGCTTAGAAGAAAACTTGTC 417

Query 3317 AAAAATAATATTAGTGATAATGATAACAATAATAAAAAACAGTGGGACTGGAGAGATGGC 3376

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Sbjct 418 AAAAATAATATTAGTGATAATGATAACAATAATAAAAAACAGTGGGACTGGAGAGATGGC 477

Query 3377 TCAGTGGTTTAGAGCCCTGGCTATTCTCCCATAGGACCCGGGTTCAAGTCCCAGTACCCA 3436

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Sbjct 478 TCAGTGGTTTAGAGCCCTGGCTATTCTCCCATAGGACCCGGGTTCAAGTCCCAGTACCCA 537

Query 3437 CACAGCAGCTCACAACCATCTGTATCTATAGTTCCAGGGGATCTAATGTTCTTTCTGACC 3496

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Sbjct 538 CACAGCAGCTCACAACCATCTGTATCTATAGTTCCAGGGGATCTAATGTTCTTTCTGACC 597

Query 3497 TCTGTGGGTTCTGTATGCACCTGGAGTACAAACGTGTATGCTGGCAAAACACCCATACAC 3556

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Sbjct 598 TCTGTGGGTTCTGTATGCACCTGGAGTACAAACGTGTATGCTGGCAAAACACCCATACAC 657

Query 3557 TTAAAATAGAGAAGATATTCTCTCAGAGGTTAAGAGAAATTGTTGCTCTTCCaaaaaaaa 3616

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Sbjct 658 TTAAAATAGAGAAGATATTCTCTCAGAGGTTAAGAGAAATTGTTGCTCTTCC---AAAAA 714

Query 3617 aaaaaaaaaaaaaaaaaaCTGGGCTCAGTTCCCAGCACCCACATGGTAGTTACAACTATG 3676

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Sbjct 715 AAAAAAAAAAAAAAAAAACTGGGCTCAGTTCCCAGCACCCACATGGTAGTTACAACTATG 774

Query 3677 TGTAGCTTCAGTTCCAGGGGATTTGACGCCTCCTTCTGGTTTCTGTGGACCCCTGCATGT 3736

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Sbjct 775 TGTAGCTTCAGTTCCAGGGGATTTGACGCCTCCTTCTGGTTTCTGTGGACCCCTGCATGT 834

Query 3737 ACGTGATACACATACATACACTGAGGcacacacacacacataagataaaaacaaaagata 3796

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Sbjct 835 ACGTGATACACATACATACACTGAGGCACACACACACACATAAGATAAAAACAAAAGATA 894

Query 3797 aatatataagtgataaaagtgatagagaaagaCATGGGGCATTATCCTCCATCCTCTGTG 3856

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Sbjct 895 AATATATAAGTGATAAAAGTGATAGAGAAAGACATGGGGCATTATCCTCCATCCTCTGTG 954

Query 3857 TACTTGCTcatatacatgaatacacaggaacacatacatacatataaacatatacataca 3916

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Sbjct 955 TACTTGCTCATATACATGAATACACAGGAACACATACTTACATATAAACATATACATACA 1014

Query 3917 tgtaaacatgtatacatatatacatacatataAAACTACCTAAAAGGTAGTTTTTTAAAA 3976

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Sbjct 1015 TGTAAACATGTATACATATATACCTACATATAAAACTACCTAAAAGGTAGTTTTTTAAAA 1074

Query 3977 GATGAGGATCAAAGACTGAAGGCTAC 4002

||||||||||||||||||||||||||

Sbjct 1075 GATGAGGATCAAAGACTGAAGGCTAC 1100

crimson-polyA(rbGlob)- Grin1in9(stuffer,1184bp)

CTACAAGCTCGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAGCAGTACGAGCGCGCCGAGGCCCGCCACCACCTGTTCCAGTAGgtttaaacgAtctttttccctctgccaaaaattatggggacatcatgaagccccttgagcatctgacttctggctaataaaggaaatttattttcattgcaatagtgtgttggaattttttgtgtctctcactcgGTTGGTCACAAAGCTGGCTGACTAACCAATGCAGCACAGTTACTGTGCTCCAGGCTTAGAAGAAAACTTGTCAAAAATAATATTAGTGATAATGATAACAATAATAAAAAACAGT

