**Primer list (with T7 tag):**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene Name | No. | 　 | Primer Plus Tag |
| A-region1 | 1 | F | aggaagagagGTTATAGTATTGTTGGGAGGGAGG |
| R | cagtaatacgactcactatagggagaaggctAACCCCTTAACCTTTTCAACC |
| 2 | F | aggaagagagTTATAGTATTGTTGGGAGGGAGG |
| R | cagtaatacgactcactatagggagaaggctTCAAATCCAACCAACTAAACAAA |
| 3 | F | aggaagagagGGTTGAAAAGGTTAAGGGGTT |
| R | cagtaatacgactcactatagggagaaggctACTCAAATCCAACCAACTAAACAA |
| A-region2 | 4 | F | aggaagagagAGGGTTTGAAGTTTTGGAGAGTGT |
| R | cagtaatacgactcactatagggagaaggctACAAAACCCTCAATCCCTATAAA |
| 12 | F | aggaagagagGTAGATTGTGGATAGTTTAGA |
| R | cagtaatacgactcactatagggagaaggctAAAAAAACCTAACCCTAAAAAA |

**Methylation Design Report**

**A-Region\_1**
<http://www.epidesigner.com/epidesigner/9395070768/results2.html>



**Comment:** You can choose primer #2; or choose #1 and #3 for the bisulfite product-PCR- experiment.

Primer 1 Tm Sequence

Left primer 60.34 GTTATAGTATTGTTGGGAGGGAGG

Right primer 59.65 AACCCCTTAACCTTTTCAACC

PRODUCT  Size: 194,   No of CpG's : 7, Coverage : 5

**Primer 2** Tm Sequence

Left primer 59.66 TTATAGTATTGTTGGGAGGGAGG

Right primer 59.8 TCAAATCCAACCAACTAAACAAA

PRODUCT  Size: 288,   No of CpG's : 15, Coverage : 12

Primer 3 Tm Sequence

Left primer 59.65 GGTTGAAAAGGTTAAGGGGTT

Right primer 60.18 ACTCAAATCCAACCAACTAAACAA

PRODUCT  Size: 119,   No of CpG's : 8, Coverage : 7

**RNase cleavage prediction:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| #1 |  |  |  |  | **#2** |  |  |  |
| **CpG Unit** | **CpG Site** | **Comment** |  | **CpG Unit** | **CpG Site** | **Comment** |
| CpGUnit\_1 | CpG\_1 | 　 | 　 |  | CpGUnit\_1 | CpG\_1 | 　 | 　 |
| CpGUnit\_2 | CpG\_2 | L\_mass | SN2SM4 |  | CpGUnit\_2 | CpG\_2 | L\_mass | SN2SM4 |
| CpGUnit\_3 | CpG\_3 | L\_mass | SN2SM4 |  | CpGUnit\_3 | CpG\_3 | L\_mass | SN2SM4 |
| CpGUnit\_4 | CpG\_4 | L\_mass | 　 |  | CpGUnit\_4 | CpG\_4 | L\_mass | SN3SM2 |
| CpGUnit\_5 | CpG\_5 | L\_mass | SN2SM4 |  | CpGUnit\_5 | CpG\_5 | L\_mass | SN2SM4 |
| CpGUnit\_6 | CpG\_6 | L\_mass | 　 |  | CpGUnit\_6 | CpG\_6 | L\_mass | 　 |
| CpGUnit\_7 | CpG\_7 | 　 | 　 |  | CpGUnit\_7 | CpG\_7 | 　 | 　 |
| #3 |  |  |  |  | CpGUnit\_8 | CpG\_8 | L\_mass | SN3SM2 |
| **CpG Unit** | **CpG Site** | **Comment** |  | CpGUnit\_9 | CpG\_9.10.11 | 　 | 　 |
| CpGUnit\_1 | CpG\_1 | L\_mass | 　 |  | CpGUnit\_10 | CpG\_12.13.14 | 　 | 　 |
| CpGUnit\_2 | CpG\_2.3.4 | 　 | 　 |  | CpGUnit\_11 | CpG\_15 | 　 | 　 |
| CpGUnit\_3 | CpG\_5.6.7 | 　 | 　 |  |  |  |  |  |
| CpGUnit\_4 | CpG\_8 | 　 | 　 |  |  |  |  |  |

**Sequence:**

GACGAGCGGGAAGGGGACGCAG**CCACAGCATTGTCTGGGAGGGAGGGGAGAAGCTCCGCCCCTAGAGGGGAGGGGAGAAGAGGAGGGGGCAGGAGACAAGGGGAGGAAAGGCGAAGGCAAGGCGAAGGGGTGGAGAGTGATATGAAGAGCGAGAGAAAAGAGAGGACAGCCGAGCAGATCCGGTAGGGGCTAAAGGCCAAGGGGCTGGAGGGAGGGAGAGGAAGGAGGAGGGGAGCGAGGAGGGCGGGGCGCCCGGTCCCCAGTCGCGCGGCCACCGCCTGCCCAGCCTGGCTGGATTTGA**GCCCGGCCCGCCGATCGCTGGCCGAGGCGCAGCTGAAGCAGCCCCCCAGCCGCGGCGCCCCCACTTTCCAACTCCACGGTTCCTGCGGCGCGGGCGCGATGCGGGCGCGGGGCGCGG

**A-Region\_2**

<http://www.epidesigner.com/epidesigner/9689718930/results2.html>



**Comment:** You can choose primer #4; or choose #12 for the bisulfite product-PCR- experiment.

Primer 4 Tm Sequence

Left primer 60.64 AGGGTTTGAAGTTTTGGAGAGTGT

Right primer 60.43 ACAAAACCCTCAATCCCTATAAA

PRODUCT  Size: 368,   No of CpG's : 31, Coverage : 20

**Primer 12** Tm Sequence

Left primer 55.9 GTAGATTGTGGATAGTTTAGA

Right primer 57.95 AAAAAAACCTAACCCTAAAAAA

PRODUCT  Size: 418,   No of CpG's : 34, Coverage : 29

**RNase cleavage prediction:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| #4 |  |  |  |  | **#12** |  |  |  |
| **CpG Unit** | **CpG Site** | **Comment** |  | **CpG Unit** | **CpG Site** | **Comment** |
| CpGUnit\_1 | CpG\_1 | 　 | 　 |  | CpGUnit\_1 | CpG\_1.2.3 | 　 | 　 |
| CpGUnit\_2 | CpG\_2.3.4 | 　 | 　 |  | CpGUnit\_10 | CpG\_16 | 　 | 　 |
| CpGUnit\_3 | CpG\_5 | 　 | 　 |  | CpGUnit\_11 | CpG\_17 | 　 | 　 |
| CpGUnit\_4 | CpG\_6.7.8 | 　 | 　 |  | CpGUnit\_12 | CpG\_18 | 　 | SN2SM2 |
| CpGUnit\_5 | CpG\_9.10.11 | 　 | 　 |  | CpGUnit\_13 | CpG\_19 | 　 | 　 |
| CpGUnit\_6 | CpG\_12 | 　 | 　 |  | CpGUnit\_14 | CpG\_20.21.22 | 　 | 　 |
| CpGUnit\_7 | CpG\_13.14.15.16 | H\_mass | 　 |  | CpGUnit\_15 | CpG\_23 | 　 | SN1SM2 |
| CpGUnit\_8 | CpG\_17.18.19 | H\_mass | 　 |  | CpGUnit\_16 | CpG\_24.25.26.27.28.29 | H\_mass | 　 |
| CpGUnit\_9 | CpG\_20 | 　 | 　 |  | CpGUnit\_17 | CpG\_30 | 　 | 　 |
| CpGUnit\_10 | CpG\_21.22.23 | H\_mass | 　 |  | CpGUnit\_18 | CpG\_31.32.33 | 　 | 　 |
| CpGUnit\_11 | CpG\_24 | L\_mass | 　 |  | CpGUnit\_19 | CpG\_34 | 　 | 　 |
| CpGUnit\_12 | CpG\_25.26.27 | H\_mass | 　 |  | CpGUnit\_2 | CpG\_4 | 　 | SN1SM2 |
| CpGUnit\_13 | CpG\_28 | 　 | 　 |  | CpGUnit\_3 | CpG\_5 | 　 | 　 |
| CpGUnit\_14 | CpG\_29 | 　 | 　 |  | CpGUnit\_4 | CpG\_6.7 | 　 | 　 |
| CpGUnit\_15 | CpG\_30 | 　 | 　 |  | CpGUnit\_5 | CpG\_8.9 | 　 | 　 |
| CpGUnit\_16 | CpG\_31 | L\_mass | 　 |  | CpGUnit\_6 | CpG\_10 | L\_mass | 　 |
|  |  |  |  |  | CpGUnit\_7 | CpG\_11.12.13 | 　 | 　 |
|  |  |  |  |  | CpGUnit\_8 | CpG\_14 | 　 | SN2SM2 |
|  |  |  |  |  | CpGUnit\_9 | CpG\_15 | L\_mass | 　 |

**Sequence:**

GTCCGAAGAAAGTTGTGGCTTGGAAAATGGGCGTCCTTCCTTCTTTTTGAGCCCGCGATGGGAAGGCGCTCCCACCCCATCCCCGGGGTCTCTGAGACTTCCTGGCGAGGTGGGGCGGGGCCCGCGGCAGGTGCAGGGCTG**GGGAGGGCCTGACAGCCCTGGAGAGTGCCCGACCCCAGTCCGCCGCCGTCTTCTAGGCGGATTCCCCAGAGGCAGGTCCCAGTCCCCGGCGCGGGGAAATTTAGGCGGCGCGCTTCAGAGGGGCGCAGGGTGCGCGGGGGCCGTTGGCCCTCCGCTCTCGCCCCGAGCGAGGCAGGTGCCGTGGGCGGCCCCGGCTACTTCTGCATTTCGAGCGCTTACTTTGCATTCAGCTCTAAGTCGGAGAGCTCTGGGGACAGCCGAGAGTGCCAGTGGCCTCATAGGGAGACTGAGGGTTCTGTTTCACCTCGGGCGCCGTGTGGGTCTGGGGCTGTCCACAGCACCTCTGC**GCCTCAGT