## Sequence: NewSequence

```
(Sense primer)       5' CACCATGAATTCACTCAGTGAAGCC 3'
                          ||||||||||||||||||||||
                3' (1) NNNGTACTTAAGTGAGTCACTTCGG (25) 5'

(Anti-sense primer)  5' GCAATCAGTTTACCAGAACATCTGCAG 3'
                       |||||||||||||||||||||||||||
             3' (1242) CGTTAGTCAAATGGTCTTGTAGACGTC (1216) 5'
```

**Properties:**

| | Rating | Seq No | Length | Tm [° C] | GC% | ΔG [kcals/mol] | Activity [μg/OD] | Degeneracy | Ta Opt [° C] |
|---|---|---|---|---|---|---|---|---|---|
| **Sense** | **56** | **1** | **25** | **64.8** | **48.0** | **-44.9** | **32.0** | **1** | **--** |
| **Anti-sense** | **58** | **1242** | **27** | **65.7** | **44.4** | **-47.9** | **31.4** | **1** | **--** |
| **Product** | **51** | **--** | **1242** | **86.3** | **42.0** | **--** | **--** | **--** | **54.9** |

**Secondary structures of sense primer:**

1. **Most stable hairpin:**

**ΔG =-2.6 [kcals/mol] (3' Hairpin)**

```
┌TCACTTAAGTACCAC 5'
C |||||  ¦
└AGTGAAGCC 3'
```

1. **Most stable dimer:**

**ΔG =-12.4 [kcals/mol]**

```
        5' CACCATGAATTCACTCAGTGAAGCC 3'
           ¦    ||||||||    ¦
 3' CCGAAGTGACTCACTTAAGTACCAC 5'
```

2. **ΔG =-8.4 [kcals/mol]**

```
 5' CACCATGAATTCACTCAGTGAAGCC 3'
          ¦  |||||| ¦¦¦¦¦¦  ¦
       3' CCGAAGTGACTCACTTAAGTACCAC 5'
```

3. **ΔG =-5.4 [kcals/mol]**

```
                5' CACCATGAATTCACTCAGTGAAGCC 3'
                      ||||
 3' CCGAAGTGACTCACTTAAGTACCAC 5'
```

1. **Most stable false priming site:**

**ΔG =-12.4 [kcals/mol]; Product = 0**

```
         3' CCGAAGTGACTCACTTAAGTACCAC 5'
                   ¦¦¦¦ ||||||||
 5'  (262)  AAAGCTTCTGACTGAATTCAACAAA (286) 3'
```

2. **ΔG =-12.4 [kcals/mol]; Product = 972**

```
         5' CACCATGAATTCACTCAGTGAAGCC 3'
            ¦    ||||||||   ¦ ¦  ¦
 3'  (269)  GACTGACTTAAGTTGTTTAGGTGAC (293) 5'
```

**Secondary structures of anti-sense primer:**

1. **Most stable hairpin:**

**ΔG =-0.9 [kcals/mol] (3' Hairpin)**

```
┌AAGACCATTTGACTAACG 5'
C ||||
└ATCTGCAG 3'
```

2. **ΔG =0.6 [kcals/mol] (3' Hairpin)**

```
┌CCATTTGACTAACG 5'
A    ¦ |||
└GAACATCTGCAG 3'
```

3. **ΔG =0.8 [kcals/mol] (3' Hairpin)**

```
┌ATTTGACTAACG 5'
C ¦ ¦     |||
└CAGAACATCTGCAG 3'
```

1. **Most stable dimer:**

**ΔG =-11.2 [kcals/mol] (3' Dimer)**

```
 5' GCAATCAGTTTACCAGAACATCTGCAG 3'
                         ||||||
                      3' GACGTCTACAAGACCATTTGACTAACG 5'
```

2. **ΔG =-6.1 [kcals/mol] (3' Dimer)**

```
   5' GCAATCAGTTTACCAGAACATCTGCAG 3'
      |||     ¦ ¦   ¦ ¦     ¦¦¦
 3' GACGTCTACAAGACCATTTGACTAACG 5'
```

**3.** **ΔG =-5.1  [kcals/mol]**

```
5' GCAATCAGTTTACCAGAACATCTGCAG 3'
                ||||   ||||
                ||||   ||||
          3' GACGTCTACAAGACCATTTGACTAACG 5'
```

**4.** **ΔG =-4.6  [kcals/mol] (3' Dimer)**

```
5' GCAATCAGTTTACCAGAACATCTGCAG 3'
        ||| |   | |   | |||
        ||| |   | |   | |||
  3' GACGTCTACAAGACCATTTGACTAACG 5'
```

**1.** **Most stable false priming site:**

**ΔG =-14.4  [kcals/mol]; Product = 0**

```
        5' GCAATCAGTTTACCAGAACATCTGCAG 3'
             |   | ||||||||| ||
             |   | ||||||||| ||
3'  (373)  GTTCTTTAAAATGGTCTGGTCACACCT (399) 5'
```

**2.** **ΔG =-11.2  [kcals/mol] (3' False priming site); Product = 0**

```
         5' GCAATCAGTTTACCAGAACATCTGCAG 3'
              |   ||        |  | ||||||
              |   ||        |  | ||||||
3'  (1011)  AATGTCTCCTCCCTCGTCTTCGACGTC (1037) 5'
```

**3.** **ΔG =-11.2  [kcals/mol] (3' False priming site); Product = 1058**

```
         3' GACGTCTACAAGACCATTTGACTAACG 5'
            |||||| ||     |       |
            |||||| ||     |       |
5'  (1032)  CTGCAGCTGCCACCGCTGTAGTAGGAT (1058) 3'
```

**4.** **ΔG =-11.2  [kcals/mol] (3' False priming site); Product = 0**

```
         5' GCAATCAGTTTACCAGAACATCTGCAG 3'
                       |   |  || ||||||
                       |   |  || ||||||
3'  (1195)  GAGGTAAACCTTTTACAAGTGGACGTC (1221) 5'
```

**Secondary structures of primer pair:**

**1.** **Most stable cross dimer:**

**ΔG =-4.6  [kcals/mol] (3' Cross dimer)**

```
5' CACCATGAATTCACTCAGTGAAGCC 3'
                  |||     |
                  |||     |
            3' GACGTCTACAAGACCATTTGACTAACG 5'
```