Protocol for Expression and Purification of Nef

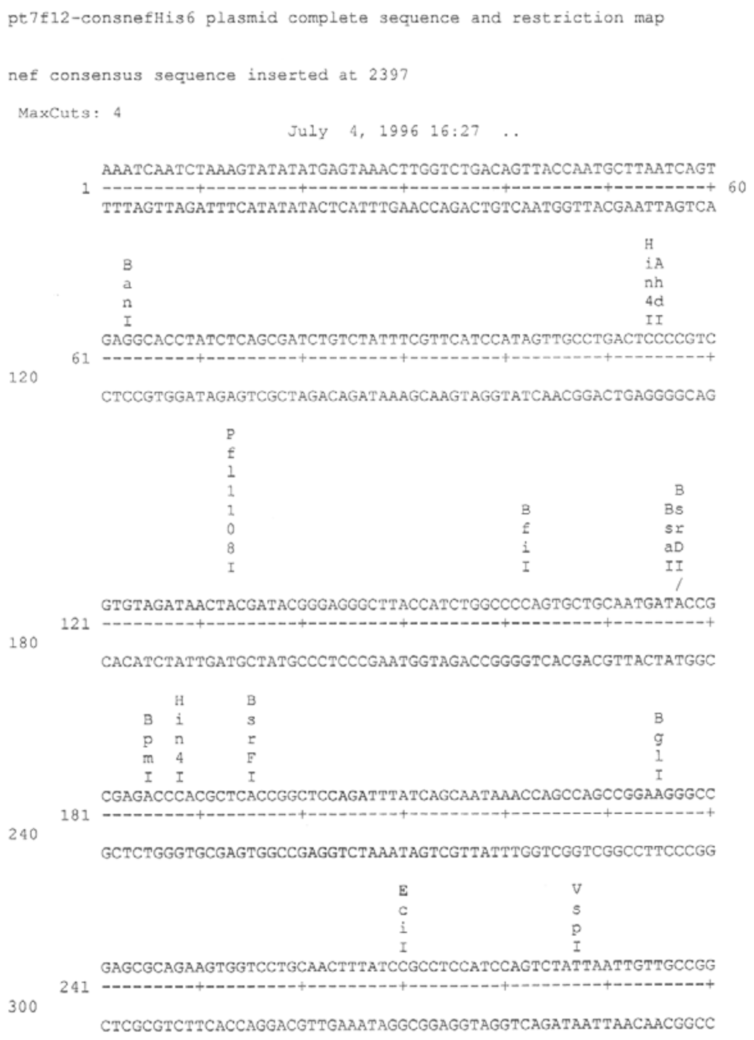
1. transform BL21 DE3 cells with pT7f12consnefHis6
2. plate on 2xYT, carbenicillin (100µg/ml),glucose (0.4%)
3. 37°C o/n
4. pick one colony into 50ml 2xYT carb, glucose (conc. as above)
5. grow to OD600 of 0.5
6. inoculate 1L 2xYT carb cultures with 10ml of starter culture
7. grow to OD600 of 0.5 to 1
8. induce with 1ml 1M IPTG/ L (1 mM IPTG final)
9. grow for 2 hrs 37°C, 300 rpm
10. spin GS-3 5,5000 rpm 4°C 5' (5000xg)
11. decant sup
12. wash 1X with ice cold PBS
13. spin GS-3 5,5000 rpm 4°C 5' (5000xg)
14. decant sup (can freeze pellet at -20°C)
15. resuspend pellet in 100 ml sonication buffer with lysozyme:
    300mM NaCl (33.3 ml 5M NaCl/ 500ml)
    50mM NaPO4 (6.7g dibasic sodium phosphate/ 500ml)
    4mM BME (140µl stock/ 500ml)
    pH to 7.5 w./ HCl
    add 0.5g lysozyme to 100ml of above

16. incubate on ice 30'
17. sonicate on ice (Braun sonicator):
    1cm tip (pre chilled on ice)
    30 pulses at 30" each with 30 seconds between pulses
    power on low
    pulse on continual
    output all the way up
18. spin 19Krpm SS-34 30' 4°C (43000xg)

****Note -- all column steps were done at 4°C

19. load sup at 0.5ml/min onto nickel column (5ml resin from Qiagen) pre-equilibrated with at least 5 column volumes of sonication buffer with BME pH 7.5 (see above) (The 6 histidines on the C terminal end of the protein allow the protein to bind to the column)
20. wash column at 0.5ml/min with 250ml sonication buffer with BME pH 7.5 until OD280<0.05
21. wash column at 0.5ml/min with 150ml sonication buffer 10% glycerol (no BME) pH 6.0 until OD280<0.05
22. elute Nef with 30ml gradient of sonication buffer with glycerol (no BME) from pH 6.0 to pH 2.0 -- collect 1ml fractions into 1ml sonication buffer with 10% glycerol pH 8.0 (no BME)
23. analyze fractions by SDS-PAGE on 15% gel (Coomassie stain)

I get a yield of about 500µg to 1mg of Nef per liter of culture. An internal initiation product of about 21 KD was copurified with the full length (~31 KD) nef and comprised about 20% of the purified product.



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pt7f12-consnefHis6 plasmid complete sequence and restriction map

nef consensus sequence inserted at 2397

 MaxCuts: 4
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         AAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGT
       1 ---------+---------+---------+---------+---------+---------+ 60
         TTTAGTTAGATTTCATATATACTCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCA

                                                              H
           B                                                  iA
           a                                                  nh
           n                                                  4d
           I                                                  II
         GAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC
      61 ---------+---------+---------+---------+---------+---------+
120
         CTCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAG

                     P
                     f
                     l
                     1                                           B
                     1                            B             Bs
                     0                            f             sr
                     8                            i             aD
                     I                            I             II
                                                                 /
         GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCG
     121 ---------+---------+---------+---------+---------+---------+
180
         CACATCTATTGATGCTATGCCCTCCCGAATGGTAGACCGGGGTCACGACGTTACTATGGC

                H      B
             B  i      s                                       B
             p  n      r                                       g
             m  4      F                                       l
             I  I      I                                       I
         CGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCC
     181 ---------+---------+---------+---------+---------+---------+
240
         GCTCTGGGTGCGAGTGGCCGAGGTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGG

                                      E                V
                                      c                s
                                      i                p
                                      I                I
         GAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
     241 ---------+---------+---------+---------+---------+---------+
300
         CTCGCGTCTTCACCAGGACGTTGAAATAGGCGGAGGTAGGTCAGATAATTAACAACGGCC
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                                                 P
                                                 s
                                                 p
                                                 1        B
                                             F   4        s
                                             s   0        r
                                             p   6        D
                                             I   I        I
         GAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACA
     301 ---------+---------+---------+---------+---------+---------+
360
         CTTCGATCTCATTCATCAAGCGGTCAATTATCAAACGCGTTGCAACAACGGTAACGATGT

                                                      B
               M                                      s
               s                                      a
               l                                      W
               I                                      I
         GGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
     361 ---------+---------+---------+---------+---------+---------+
420
         CCGTAGCACCACAGTGCGAGCAGCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCT

         TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT
     421 ---------+---------+---------+---------+---------+---------+
480
         AGTTCCGCTCAATGTACTAGGGGGTACAACACGTTTTTTCGCCAATCGAGGAAGCCAGGA

             B                  G
             sP               Ed                     M
             iv               ai                     s
             Eu               eI                     l
             II               II                     I
              /                 /
         CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTG
     481 ---------+---------+---------+---------+---------+---------+
540
         GGCTAGCAACAGTCTTCATTCAACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGAC

                                                               ST
                                                               ca
                                                               at
                                                               II
                                                                /
         CATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA
     541 ---------+---------+---------+---------+---------+---------+
600
         GTATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAGACACTGACCACTCATGAGT
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                                                                H
                                          B                 B   i
                            B             s                 s B n B
                            c             i                 a c c s
                            g             E                 H g I b
                            I             I                 I I I I
         ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACA
     601 ---------+---------+---------+---------+---------+---------+
660
         TGGTTCAGTAAGACTCTTATCACATACGCCGCTGGCTCAACGAGAACGGGCCGCAGTTGT

                                                              P
                                                 B            s
                                                 s            p     B
                                                 i            1     c
                                        D        H            4X    e
                                        r        K            0m    8
                                        a        A            6n    3
                                        I        I            II    I
                                                               /
         CGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCT
     661 ---------+---------+---------+---------+---------+---------+
720
         GCCCTATTATGGCGCGGTGTATCGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGA

                                      M
                                      s                             B
                                      p                             s
                                      A                             s
                                      1                             S
                                      I                             I
         TCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACT
     721 ---------+---------+---------+---------+---------+---------+
780
         AGCCCCGCTTTTGAGAGTTCCTAGAATGGCGACAACTCTAGGTCAAGCTACATTGGGTGA

              B
           E  s
          Ac  i              T                T
          po  H              a                a
          a5  K              q                q
          L7  A              I                I
          II  I              I                I
           /
         CGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAA
     781 ---------+---------+---------+---------+---------+---------+
840
         GCACGTGGGTTGACTAGAAGTCGTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTT

                                                     M
                                                     s
                                                     l
                                                     I
         ACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTC
     841 ---------+---------+---------+---------+---------+---------+
900
         TGTCCTTCCGTTTTACGGCGTTTTTTCCCTTATTCCCGCTGTGCCTTTACAACTTATGAG
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                                                                 B
                  E        S                              R     s
                  a        s                              c     r
                  r        p                              a     B
                  I        I                              I     I
         ATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGA
     901 ---------+---------+---------+---------+---------+---------+
960
         TATGAGAAGGAAAAAGTTATAATAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCT

                                                                   B
                                                                   s
                                                                   p
                                                                   2
                                                                   4
                                                                   I
         TACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGA
     961 ---------+---------+---------+---------+---------+---------+
1020
         ATGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCT

                      B  A
                      s  a                 R
                      a  t                 c
                      H  I                 a
                      I  I                 I
         AAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGG
    1021 ---------+---------+---------+---------+---------+---------+
1080
         TTTCACGGTGGACTGCAGATTCTTTGGTAATAATAGTACTGTAATTGGATATTTTTATCC

                   E
                   c
                   o
              B    O
              s    1 B
              s    0 b
              S    9 s
              I    I I
         CGTATCACGAGGCCCTTTCGTCTTCAAGAATTAAAAGGATCTAGGTGAAGATCCTTTTTG
    1081 ---------+---------+---------+---------+---------+---------+
1140
         GCATAGTGCTCCGGGAAAGCAGAAGTTCTTAATTTTCCTAGATCCACTTCTAGGAAAAAC

                                         B
                                         s
               R                         p
               c                         2
               a                         4
               I                         I
         ATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCG
    1141 ---------+---------+---------+---------+---------+---------+
1200
         TATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAGCAAGGTGACTCGCAGTCTGGGGC
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                                                   B
                                                   c
                                                   e
                                                   8
                                                   3
                                                   I
         TAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGC
    1201 ---------+---------+---------+---------+---------+---------+
1260
         ATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACG

                                MH
                                sg
                                pi
                                AE
                                1I
                                II
         AAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTC
    1261 ---------+---------+---------+---------+---------+---------+
1320
         TTTGTTTTTTTGGTGGCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAG

             E                        B
             c                        s
             o                        p
             5                        2
             7                        4
             I                        I
         TTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGT
    1321 ---------+---------+---------+---------+---------+---------+
1380
         AAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACA

         AGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGC
    1381 ---------+---------+---------+---------+---------+---------+
1440
         TCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACG

                                                    B
                          A                         c
                          l               M         e
                          w               m         8
                          N               e         3
                          I               I         I
         TAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT
    1441 ---------+---------+---------+---------+---------+---------+
1500
         ATTAGGACAATGGTCACCGACGACGGTCACCGCTATTCAGCACAGAATGGCCCAACCTGA
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                                                                  B
                                      M                           s
                       B              s   B                   A   i
                       s              p   s                   p   H
                       a              A   i                   a   K
                       W              1   E                   L   A
                       I              I   I                   I   I
          CAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACAC
     1501 ---------+---------+---------+---------+---------+---------+
 1560
          GTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTG

          AGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAG
     1561 ---------+---------+---------+---------+---------+---------+
 1620
          TCGGGTCGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGTAACTC

                H                                 B
                a                       E      M  s
                e                       c      m  a
                I                       i      e  W
                I                       I      I  I
          AAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCG
     1621 ---------+---------+---------+---------+---------+---------+
 1680
          TTTCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCCAGC

                       B
                       s
                       s
                       S
                       I
          GAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTG
     1681 ---------+---------+---------+---------+---------+---------+
 1740
          CTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGAC

                                                   B
                                                   c
                                  D                e              E
                                  r                8              c
                                  d                3              i
                                  I                I              I
          TCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA
     1741 ---------+---------+---------+---------+---------+---------+
 1800
          AGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAACACTACGAGCAGTCCCCCCGCCT

          GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTT
     1801 ---------+---------+---------+---------+---------+---------+
 1860
          CGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAA
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                B
                s
               Ap
               fL
               lU  N
               Il  s
               Il  p
               II  I
                /
         TTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCT
    1861 ---------+---------+---------+---------+---------+---------+
1920
         AACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGA

                          B                  B
                          s                  s
                          r                  i
                          B                  E
                          I                  I
         TTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCG
    1921 ---------+---------+---------+---------+---------+---------+
1980
         AACTCACTCGACTATGGCGAGCGGCGTCGGCTTGCTGGCTCGCGTCGCTCAGTCACTCGC

                         H
            ES           a
            aa           e
            rp           I
            II           I
             /
         AGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCAC
    1981 ---------+---------+---------+---------+---------+---------+
2040
         TCCTTCGCCTTCTCGCGGACTACGCCATAAAAGAGGAATGCGTAGACACGCCATAAAGTG

                             B
                             s
                 B       A   i
                 g       p   H       T
                 l       a   K       a
                 I       L   A       t
                 I       I   I       I
         ACCGCATAAGATCTTGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCC
    2041 ---------+---------+---------+---------+---------+---------+
2100
         TGGCGTATTCTAGAACCACGTGAGAGTCATGTTAGACGAGACTACGGCGTATCAATTCGG
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                                         T
                                         t
                                  B      h
                                  s     B1
                                  a     f1
                                  A     i1
                                  I     II
                                         /
          AGTATATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCA
     2101 ---------+---------+---------+---------+---------+---------+
 2160
          TCATATATGTGAGGCGATAGCGATGCACTGACCCAGTACCGACGCGGGGCTGTGGGCGGT

                M
                s
          B     p                  D
          s     A                  r
          b     1                  d
          I     I                  I
          ACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCT
     2161 ---------+---------+---------+---------+---------+---------+
 2220
          TGTGGGCGACTGCGCGGGACTGCCCGAACAGACGAGGGCCGTAGGCGAATGTCTGTTCGA

                     B
                     s           N
                     m           s
                     B           p
                     I           I
          GTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCG
     2221 ---------+---------+---------+---------+---------+---------+
 2280
          CACTGGCAGAGGCCCTCGACGTACACAGTCTCCAAAAGTGGCAGTAGTGGCTTTGCGCGC

                      N                 N     V             B
                      s                 s     s             s
                      p                 p     p             a
                      V                 V     I             I
          AGGCCCAGCGATTCGAACTTCTGATAGACTTCGAAATTAATACGACTCACTATAGGGAGA
     2281 ---------+---------+---------+---------+---------+---------+
 2340
          TCCGGGTCGCTAAGCTTGAAGACTATCTGAAGCTTTAATTATGCTGAGTGATATCCCTCT

                                                          T
                         X                                a      N  M
                         b                                q      d  s
                         a                                I      e  l
                         I                                I      I  I
          CCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATG
     2341 ---------+---------+---------+---------+---------+---------+
 2400
          GGTGTTGCCAAAGGGAGATCTTTATTAAAACAAATTGAAATTCTTCCTCTATATGTATAC

          GGTGGCAAGTGGTCAAAACGTAGTGTGAGTGGATGGCCTGCTGTAAGAGAAAGAATGAGG
     2401 ---------+---------+---------+---------+---------+---------+
 2460
          CCACCGTTCACCAGTTTTGCATCACACTCACCTACCGGACGACATTCTCTTTCTTACTCC
```

```
             B
             p
             u
             1
             1                             B  AX
             0                             s  vh
             2                             a  ao
             I                             I  II
                                               /
         CGAGCTGAGCCAGCAGCAGAGGGAGTGGGAGCAGTATCTCGAGACCTGGAAAAACATGGA
    2461 ---------+---------+---------+---------+---------+---------+
2520
         GCTCGACTCGGTCGTCGTCTCCCTCACCCTCGTCATAGAGCTCTGGACCTTTTTGTACCT

         GCAATTACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAA
    2521 ---------+---------+---------+---------+---------+---------+
2580
         CGTTAATGTTCATCGTTATGTCGTCGATGGTTACGACGAACACGGACCGATCTTCGTGTT

                                        B
                        B               s
                        s               u   B   K
                        e               3   a   p
                        R               6   n   n
                        I               I   I   I
         GAGGAGGAAGAAGTGGGTTTTCCAGTCAGACCTCAGGTACCTTTAAGACCAATGACTTAC
    2581 ---------+---------+---------+---------+---------+---------+
2640
         CTCCTCCTTCTTCACCCAAAAGGTCAGTCTGGAGTCCATGGAAATTCTGGTTACTGAATG

                    B
                    g                D
                    l                r
                    I                a
                    I                I
         AAGGCAGCAGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGTTAATT
    2641 ---------+---------+---------+---------+---------+---------+
2700
         TTCCGTCGTCATCTAGAATCGGTGAAAAATTTTCTTTTCCCCCCTGACCTTCCCAATTAA

                          H  ER
                          i  cl               A
                          n  oe               c
                          4  RA               c
                          I  VI               I
         TACTCCCAAAAAAGACAAGATATCCTTGATCTGTGGGTCTACCACACACAAGGCTACTTC
    2701 ---------+---------+---------+---------+---------+---------+
2760
         ATGAGGGTTTTTTCTGTTCTATAGGAACTAGACACCCAGATGGTGTGTGTTCCGATGAAG
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                                                  E   T
                                                  c   a
                                                  o   q
                                                  R   I
                                                  V   I
           CCTGATTGGCAGAACTACACACCAGGGCCAGGAATCAGATATCCACTGACCTTTGGGTGG
      2761 ---------+---------+---------+---------+---------+---------+
2820
           GGACTAACCGTCTTGATGTGTGGTCCCGGTCCTTAGTCTATAGGTGACTGGAAACCCACC

                   S                      E
                   p                      a
                   e                      r
                   I                      I
           TGCTTCAAACTAGTACCAGTTGAGCCAGAGAAGGTAGAAGAGGCCAATGAAGGAGAGAAC
      2821 ---------+---------+---------+---------+---------+---------+
2880
           ACGAAGTTTGATCATGGTCAACTCGGTCTCTTCCATCTTCTCCGGTTACTTCCTCTCTTG

           AACTGCTTGCTACACCCTATGAGCCAGCATGGGATGGATGACCCAGAGAAAGAAGTATTA
      2881 ---------+---------+---------+---------+---------+---------+
2940
           TTGACGAACGATGTGGGATACTCGGTCGTACCCTACCTACTGGGTCTCTTTCTTCATAAT

                                                                BB
                                                   A            ss
                                                   v            ap
                                                   a            WE
                                                   I            II
                                                                 /
           GTGTGGAAGTTTGACAGCAAACTAGCATTTCATCACATGGCCCGAGAGCTGCATCCGGAG
      2941 ---------+---------+---------+---------+---------+---------+
3000
           CACACCTTCAAACTGTCGTTTGATCGTAAAGTAGTGTACCGGGCTCTCGACGTAGGCCTC

                                                                 P
                                                        H        s
                                                        i        p
                                                        n        1
           ST                                      P    d     D  4
           ca                                      s    I     r  0
           at                                      t    I     a  6
           II                                      I    I     I  I
            /
           TACTACAAAGACTGCCACCATCACCATCACCATTGACTGCAGCCCAAGCTTTAAACGTTA
      3001 ---------+---------+---------+---------+---------+---------+
3060
           ATGATGTTTCTGACGGTGGTAGTGGTAGTGGTAACTGACGTCGGGTTCGAAATTTGCAAT

            S         A          A
            s         p          p
            p         o          o
            I         I          I
           ATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGG
      3061 ---------+---------+---------+---------+---------+---------+
3120
           TATAAAACAATTTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCC
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                                                                 B
                                                                 s
                                                                 b
                                                                 I
         CCGAAATCGGAAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTG
    3121 ---------+---------+---------+---------+---------+---------+
3180
         GGCTTTAGCCTTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAAC

                                             UB
                                  B          bs
                                  p          aa
                                  m          DX
                                  I          II
         TTCCAGTTTGGAACAAGAGTCCACTATTGAGGAACGTGAACTCCAGCGTCAAAGGGCGAA
    3181 ---------+---------+---------+---------+---------+---------+
3240
         AAGGTCAAACCTTGTTCTCAGGTGATAACTCCTTGCACTTGAGGTCGCAGTTTCCCGCTT

                                       D
                                      Br   D
                                      sa   r
                                      aI   d
                                      AI   I
                                      II   I
                                       /
         AAACCGTCTATCGGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGG
    3241 ---------+---------+---------+---------+---------+---------+
3300
         TTTGGCAGATAGCCCCGCTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACC

                                                     B
               B                                     a
               a                                     n
               n                                     I
               I                                     I
         GGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTT
    3301 ---------+---------+---------+---------+---------+---------+
3360
         CCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAA

                    N
                   Bg                                         B
                   so                                         s
                   rA                                         r
                   FI                                         B
                   IV                                         I
                    /
         GACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCG
    3361 ---------+---------+---------+---------+---------+---------+
3420
         CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGC
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```
         H       H
         a       a
         e       e
         I       I
         I       I
         CTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTA
    3421 ---------+---------+---------+---------+---------+---------+
3480
         GATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAAT

                              H
                              i
                              n               H
                              d      C        i
                              I      l        n
                              I      a        4
                              I      I        I
         ATGCGCCGCTACAGGGCGCGTAAGCTTATCGATGATAAGCTGTCAAACATGAGAATT
    3481 ---------+---------+---------+---------+---------+------- 3537
         TACGCGGCGATGTCCCGCGCATTCGAATAGCTACTATTCGACAGTTTGTACTCTTAA

Enzymes that do cut and were not excluded:

    AatII     AccI   AflIII     AhdI    AlwNI    ApaLI     ApoI     AvaI
     BanI    BanII     BbsI   Bce83I     BcgI     BfiI     BglI    BglII
     BpmI Bpu1102I     BsaI    BsaAI    BsaHI    BsaWI    BsaXI     BsbI
    BseRI    BsiEI  BsiHKAI    BsmBI   Bsp24I    BspEI BspLU11I    BsrBI
    BsrDI    BsrFI    BssSI   Bsu36I     ClaI     DraI   DraIII     DrdI
    DrdII     EaeI     EarI     EciI   Eco57I EcoO109I    EcoRV     FspI
    GdiII    HaeII   HgiEII    Hin4I   HincII  HindIII     KpnI     MmeI
     MslI   MspA1I     NdeI   NgoAIV     NspI     NspV Pfl1108I Psp1406I
     PstI     PvuI     RcaI    RleAI     SapI     ScaI     SpeI     SspI
    TaqII     TatI  Tth111I    UbaDI     VspI     XbaI     XhoI     XmnI

Enzymes that do not cut:

    AflII     ApaI    ApaBI     AscI    AvrII     BaeI    BamHI     BclI
     BmgI     BplI   Bpu10I    BsaBI     BsgI     BsmI    BspGI    BspMI
    BsrGI   BssHII Bst1107I   BstEII    BstXI     DsaI     EagI Eco47III
    EcoNI    EcoRI     FseI     HpaI     MluI     MscI     MunI     NarI
     NcoI     NheI     NotI     NruI     NsiI     PacI    PflMI    PinAI
     PmeI     PmlI    PpuMI    PshAI    PvuII    RsrII    SacII     SalI
    SanDI    SexAI     SfiI     SgfI    SgrAI     SmaI    SnaBI     SphI
     SrfI Sse8387I Sse8647I     SstI     StuI     StyI     SunI     SwaI
     XcmI

Enzymes excluded; MinCuts: 1  MaxCuts: 4

  AceIII    Bsp24I Bsp1286I    BstYI     HaeI     SfcI    TaqII Tth111II
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