

## Cloning and expression of a recombinant form of the Cov-2 N Protein in *E.Coli*

### 1- RT-PCR with N-specific primers

#### Used primers

NF1 5' cagGGATCCGatgtctgataatggaccccaaa 3' **BamHI**, for periplasmic expression

NR1 5' ctcGTCGACggcttgagttgagtcagcactgc 3' **SalI** site

#### Digestion of the vector pET26 with BamHI Xho1

#### Digestion of the Insert with BamHI SalI

#### Ligation (Xho1 and SalI are compatible)

```
ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCAGCCGGCGATGGCC  
ATGGATATCGGAATTAATTCGGATCCGatgtctgataatggaccccaaaatcagcga  
28321 aatgcacccccgcattacgtttgggtggaccctcagattcaactggcagtaaccagaatgga  
28381 gaacgcagtgggggcgcgatcaaaaacaacgtcggccccaaggtttaccaataatactgcg  
28441 tcttggttcaccgctctcactcaacatggcaaggaagaccttaaattccctcgaggacaa  
28501 ggcgttccaattaacaccaatagcagtcocagatgacaaaattggctactaccgaagagct  
28561 accagacgaattcgtgggtggtgacggtaaaatgaaagatctcagtccaagatggtatctc  
28621 tactacctaggaactggggccagaagctggacttccctatgggtgctaacaaagacggcatc  
28681 atatggggttgcaactgagggagccttgaatacaccaaaaagatcacattggcaccgcgaat  
28741 cctgctaacaatgctgcaatcgtgctacaacttctcaaggaacaacattgccaaaaggc  
28801 ttctacgcagaagggagcagagggcggcagtcgaagcctcttctcgttctcatcacgtagt  
28861 cgcaacagttcaagaaattcaactocaggcagcagtaggggaacttctcctgctagaatg  
28921 gctggcaaatggcgggtgatgctgctcttggcttctgctgctgcttgacagattgaaccagctt  
28981 gagagcaaaaatgtctggttaaaggccaacaacaagcgaactgtcactaagaaatct  
29041 gctgctgaggcttctaagaagcctcggcaaaaacgtactgccactaaagcatacaatgta  
29101 acacaagctttcggcagacgtggtocagaacaaaaccaaggaaaatttgggggaccaggaa  
29161 ctaatacagacaaggaactgattacaaacattggccgcaaatgcacaatttgccccagc  
29221 gcttcagcgttcttcgggaatgtcgcgcattggcatggaagtcacaccttcgggaacgtgg  
29281 ttgacctacacaggtgccatcaaatgggatgacaaagatccaaatttcaaagatcaagtc  
29341 atthttgctgaataagcatattgacgcatacaaaaacattcccaccaacagagcctaaaaag  
29401 gacaaaaagaagaaggctgatgaaactcaagccttaccgcagagacagaagaaacagcaa  
29461 actgtgactcttctcctgctgagatttgatgatttctccaacaattgcaacaatcc  
29521 atgagcagtgctgactcaactcaagccGTCGAGCACCACCACCACCACCACTGA
```

Translate

#### Peptide signal PelB

```
MKYLPTAAAGLLLLAAQPAMAMDIGINSDPMSDNQPQNQRNAPRITFGGSDSTGSNQNNGERSGARSKQRRP  
QGLPNNASWFTALTQHGEDLKFPRGQGVPIINTNSSPDDQIGYRRARRRIRGGDGKMKDLSRWYFYFLGTGP  
EAGLPYGANKDGIWVATEGALNTPKDHIGTRNPANNAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSRSRNSS  
RNSTPGSSRGTS PARMAGNGGDAALALLLLDRLNQLKESKMSGKQQQQQQT VTKSAEASKPRQKRTATKAY  
NVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDK  
DPNFKDQVILLNKHIDAYKTFPTEPKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQOSMSSADSTQ  
AVEHHHHHH
```

## N protein as expressed in periplasmic area

**Theoretical pI/Mw (average) for the user-entered sequence:**

10 20 30 40 50 60  
MDIGINSDPM SDNGPQNRN APRITFGGPS DSTGSNQNGE RSGARSKQRR PQGLEPNNTAS

70 80 90 100 110 120  
WFTALTQHGK EDLKFPRGQG VPINTNSSPD DQIGYRRAT RRIRGGDGKM KDLSRWYFY

130 140 150 160 170 180  
YLGTGPEAGL PYGANKDGII WVATEGALNT PKDHIGTRNP ANNAIVLQL PQGTTLPKGF

190 200 210 220 230 240  
YAEGSRGGSQ ASSRSSRSR NSSRNSTPGS SRGTSPARMA GNGGDAALAL LLLDRLNQL

250 260 270 280 290 300  
SKMSGKGQQQ QGQTVTKKSA AEASKKPRQK RTATKAYNVT QAFGRRGPEQ TQGNFGDQEL

310 320 330 340 350 360  
IRQGTDYKHW PQIAQFAPSA SAFFGMSRIG MEVTPSGTWL TYTGAIKLDD KDPNFKDQVI

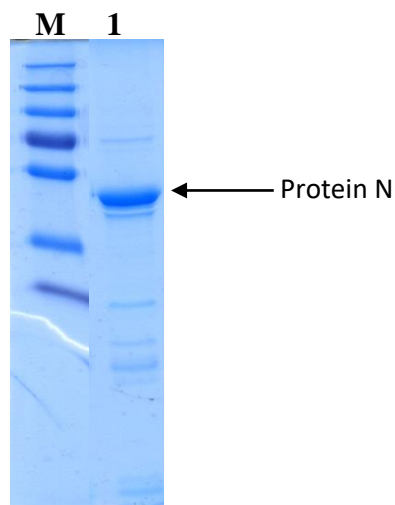
370 380 390 400 410 420  
LLNKHIDAYK TFPPTPEPKD KKKKADETQA LPQRQKKQQT VTLLPAADLD DFSKQLQQSM

430  
SSADSTQAVE HHHHHH

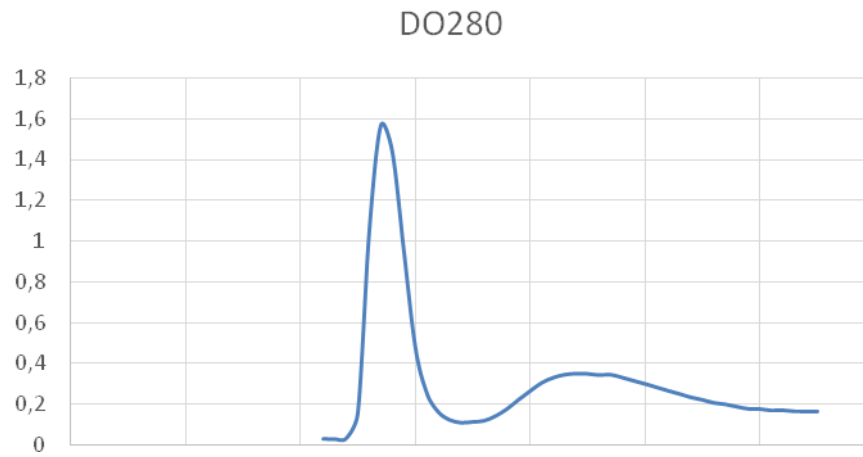
**Theoretical Mw: 47619.83 Da**

**Theoretical pI: 9.95**

**Purification of the recombinant N protein from the periplasmic space of BL21 on a Nickel Sepharose column**



## Purification of recombinant N on a Sepharose G50 column



## Analysis of the protein on SDS-PAGE gel

