Public Database의 이용(1) - SignalP (version 4.1)

2015. 8.

KIST 이철주

Secretion pathway prediction

ProteinCenter (Proxeon Bioinformatics, Odense, Denmark; http://www.cbs.dtu.dk/services)

SignalP (version 4.1)

(http://www.cbs.dtu.dk/services/SignalP)

Classical secretion pathway 예상

hidden Markov model algorithms 이용[i]

SignalP uses amino acid sequences to predict the existence and location of signal peptide cleavage sites. The hidden Markov model algorithms calculate the probability of whether the submitted sequence contains a signal peptide or not. A protein is considered classically secreted if it receives a default cutoff values Y (0.5 > D-cutoff for TM networks, 0.45 > D-cutoff for no-TM model networks). [ii]

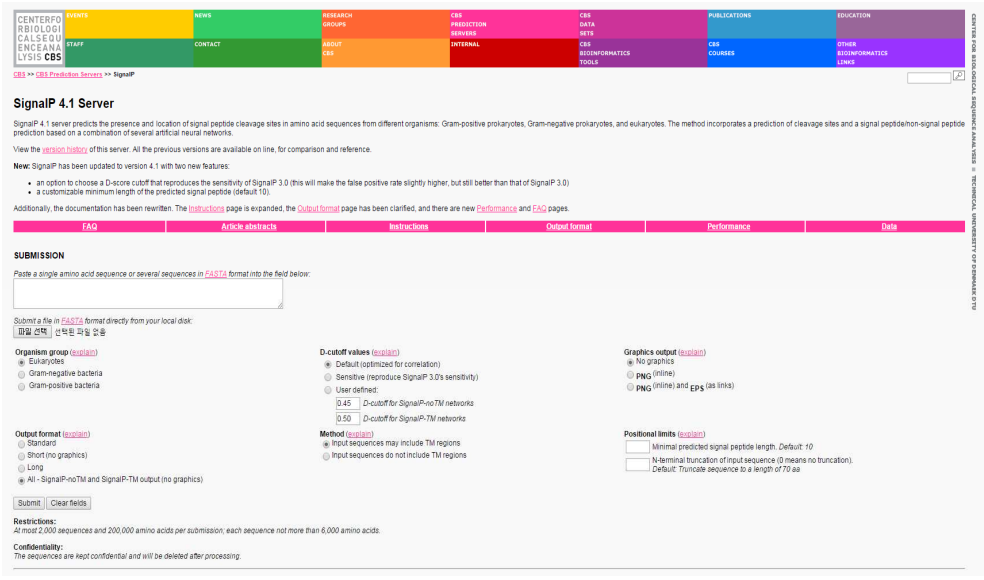


Fig 1. SignalP homepage

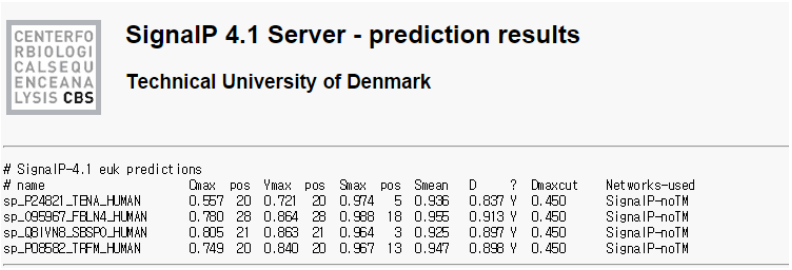
■ Parameter

1. Fasta 형식 또는 파일 준비

- 한 번의 submission에 2,000 entries, 총 200,000 amino acids가능
- 하나의 entry는 6,000 amino acids를 넘지 않아야 함 (넘을 경우 N-terminal에서부터 6000번째까지만 사용)

2. Organism group 선택

3. Output format: data 정리 및 분석 작업을 위해서는 "short" 선택



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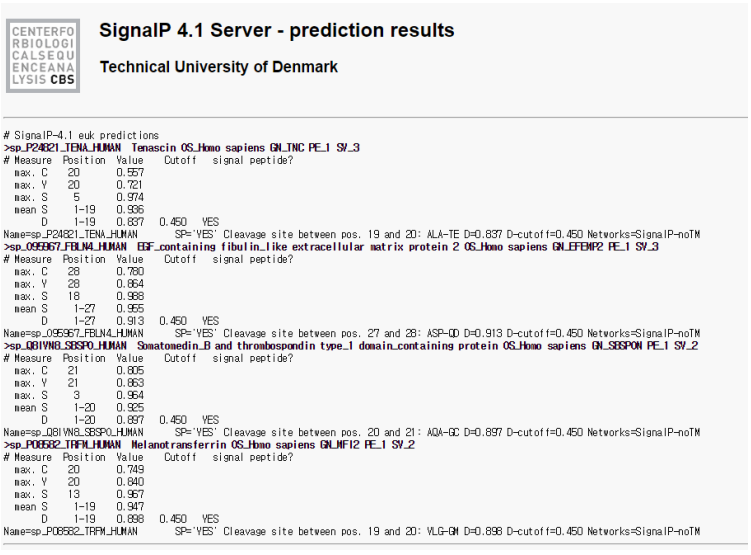
SignaIP 4.1 Server - prediction results

Technical University of Denmark

```
# SignaIP-4.1 euk predictions
# name                    Cmax  pos  Ymax  pos  Smax  pos  Smean   D     ?  Dmaxcut    Networks-used
sp_P24821_TENA_HUMAN      0.557  20  0.721  20  0.974   5  0.936   0.837 Y  0.450      SignaIP-noTM
sp_O95967_FBLN4_HUMAN     0.780  28  0.864  28  0.988  18  0.955   0.913 Y  0.450      SignaIP-noTM
sp_Q81VN8_SBSPO_HUMAN     0.805  21  0.863  21  0.964   3  0.925   0.897 Y  0.450      SignaIP-noTM
sp_P08582_TRFM_HUMAN      0.749  20  0.840  20  0.967  13  0.947   0.898 Y  0.450      SignaIP-noTM
```

Fig 2. Output format "short"



CENTERFO RBIOLOGI CALSEQU ENCEANA LYSIS CBS

SignaIP 4.1 Server - prediction results

Technical University of Denmark

```
# SignaIP-4.1 euk predictions
>sp_P24821_TENA_HUMAN  Tenascin OS=Homo sapiens GN=TNC PE=1 SV=3
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    20       0.557
  max. Y    20       0.721
  max. S    5        0.974
  mean S    1-19     0.936
       D    1-19     0.837   0.450   YES
Name=sp_P24821_TENA_HUMAN      SP='YES' Cleavage site between pos. 19 and 20: ALA-TE D=0.837 D-cutoff=0.450 Networks=SignaIP-noTM
>sp_O95967_FBLN4_HUMAN  EGF_containing fibulin_like extracellular matrix protein 2 OS=Homo sapiens GN=EFEMP2 PE=1 SV=3
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    28       0.780
  max. Y    28       0.864
  max. S    18       0.988
  mean S    1-27     0.955
       D    1-27     0.913   0.450   YES
Name=sp_O95967_FBLN4_HUMAN     SP='YES' Cleavage site between pos. 27 and 28: ASP-QD D=0.913 D-cutoff=0.450 Networks=SignaIP-noTM
>sp_Q81VN8_SBSPO_HUMAN  Somatomedin_B and thrombospondin type_1 domain_containing protein OS=Homo sapiens GN=SBSPON PE=1 SV=2
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    21       0.805
  max. Y    21       0.863
  max. S    3        0.964
  mean S    1-20     0.925
       D    1-20     0.897   0.450   YES
Name=sp_Q81VN8_SBSPO_HUMAN     SP='YES' Cleavage site between pos. 20 and 21: AQA-GC D=0.897 D-cutoff=0.450 Networks=SignaIP-noTM
>sp_P08582_TRFM_HUMAN  Melanotransferrin OS=Homo sapiens GN=MFI2 PE=1 SV=2
# Measure  Position  Value    Cutoff   signal peptide?
  max. C    20       0.749
  max. Y    20       0.840
  max. S    13       0.967
  mean S    1-19     0.947
       D    1-19     0.898   0.450   YES
Name=sp_P08582_TRFM_HUMAN      SP='YES' Cleavage site between pos. 19 and 20: YLG-GN D=0.898 D-cutoff=0.450 Networks=SignaIP-noTM
```

Fig3. Output format "standard"

4. D-cutoff value: default 권장 (0.45이상 SignalP-noTM networks, 0.5이상 SignalP-TM networks)

   *TM: transmembrane state (sequence의 hydrophobicity에 따라 integral transmembrane sequence 예상, 4개 이상 존재하면 TM으로 인지)

5. Method: default 권장 (input sequences may include TM regions)

6. Graphics output: 다수의 proteome 분석 시 default 권장 (no graphic)

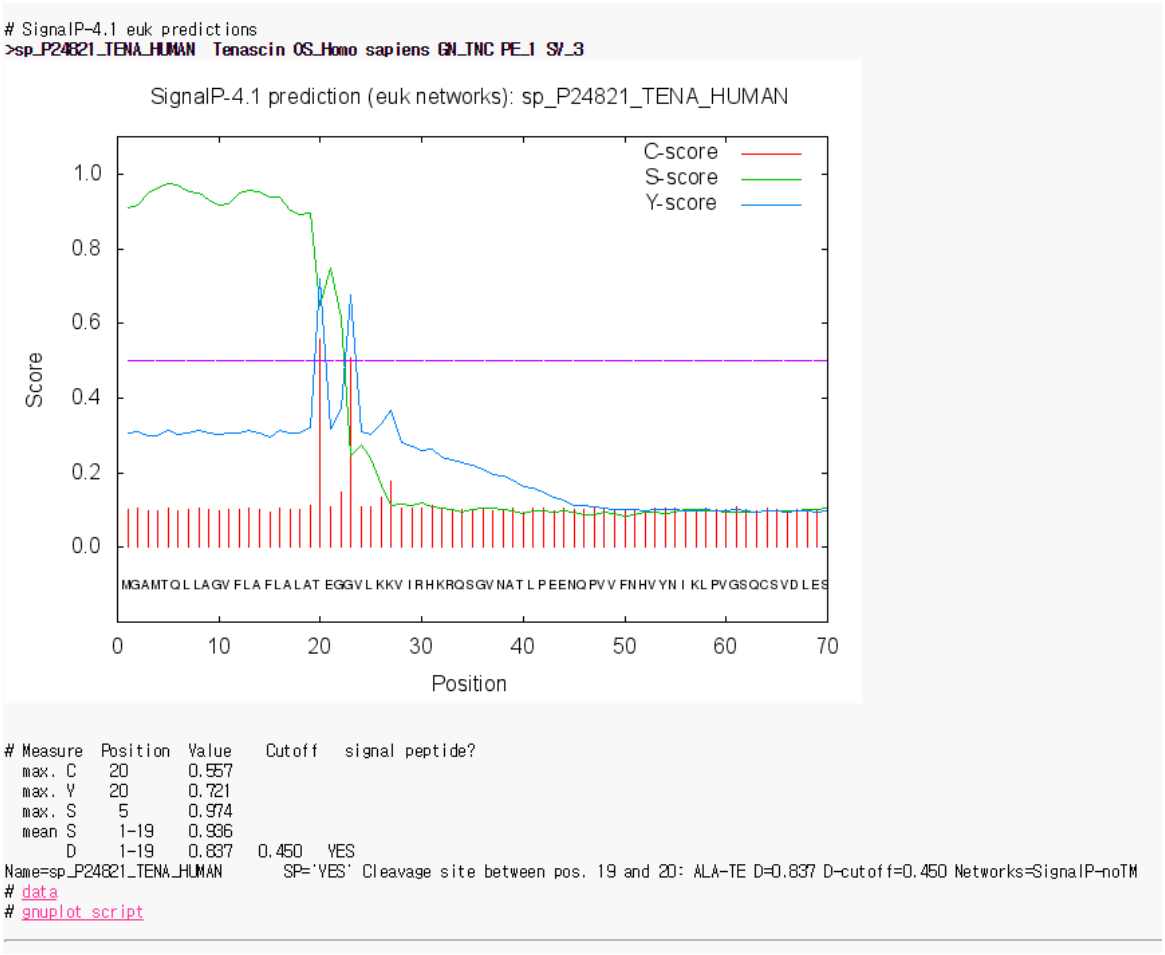


Fig 4. Graphics output "PNG (inline)" 선택 시, output

■ 결과 해석

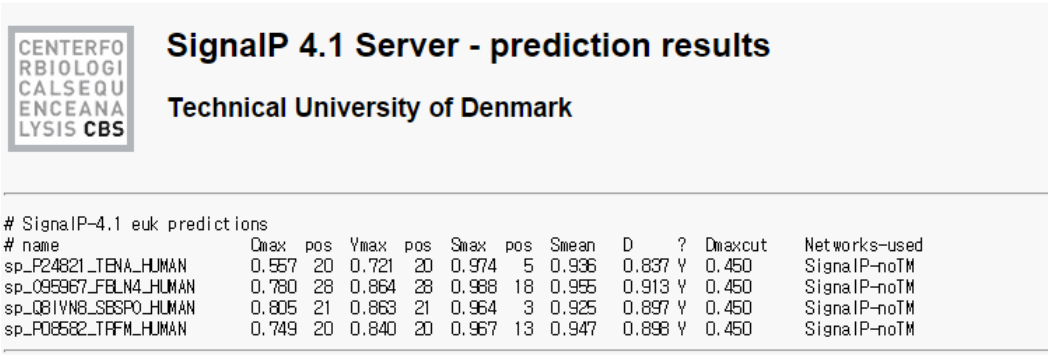


Fig 5. SignalP의 prediction results

D (D-cutoff)

? (Y: D-cutoff 0.45 ≥ SignalP-noTM, 0.5 ≥ SignalP-TM, N: 0.45< SignalP-noTM, 0.5 < SignalP-TM)

Public Database의 이용(2) - SecretomeP (version 2.0)

2015. 8.

KIST 이철주

SecretomeP (version 2.0)

- (http://www.cbs.dtu.dk/services/SecretomeP)
- nonclassical secretion pathway 예상
- neural network 이용

SecretomeP uses a neural network that combines six protein characteristics to determine if a protein is nonclassically secreted. These characteristics include the number of atoms, number of positively charged residues, presence of transmembrane helices, presence of low-complexity regions, presence of pro-peptides and subcellular localization. A protein is considered nonclassically secreted if it receives an NN-score ≥ 0.5.[iii]

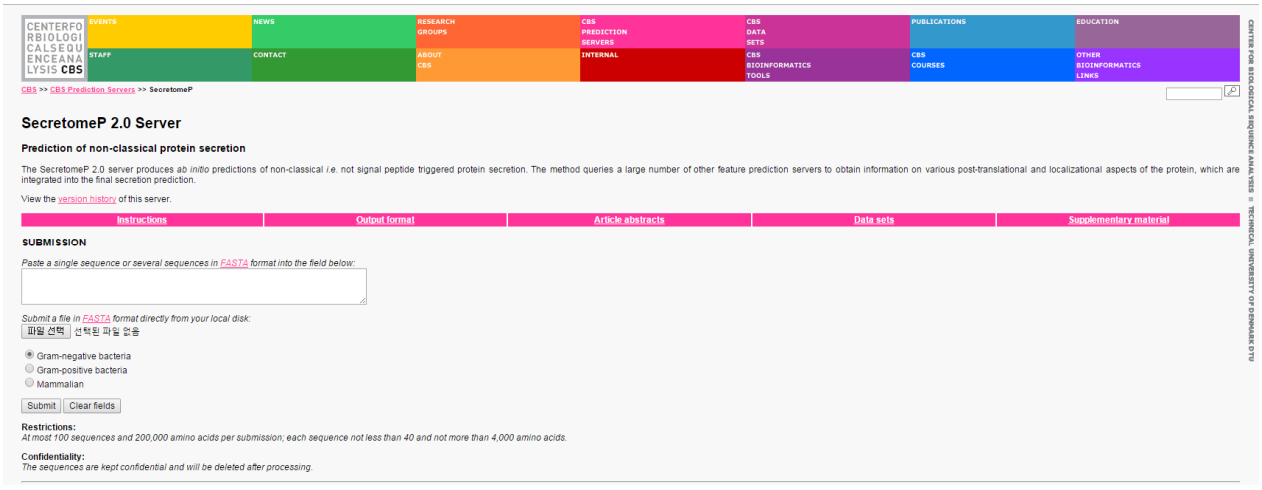


Fig 6. SecretomeP homepage

■ **Parameter**

1. Fasta 형식 또는 파일 준비
   - 한 번의 submission에 100 entries, 총 200,000 amino acids가능
   - 하나의 entry는 40~4,000 amino acids여야 함. (넘을 경우 N-terminal에서부터 4,000번째까지만 사용)

2. Organism group 선택

■ **결과 해석**

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Secretome 2.0 Server - prediction results

Technical University of Denmark

Non-classically secreted proteins should obtain an NN-score exceeding the normal

```
# SecretomeP 1.0f predictions

# Name        NN-score  Odds   Weighted   Warning
#                         by prior
# ================================================================
ADA17_HUMAN     0.190   0.372     0.001    signal peptide predicted by SignalP
ARVC_HUMAN      0.532   1.103     0.002    -
ASNS_HUMAN      0.427   0.730     0.001    -
ATRN_HUMAN      0.298   0.560     0.001    -
CATF_HUMAN      0.625   1.717     0.003    signal peptide predicted by SignalP
CBPE_HUMAN      0.464   0.933     0.002    signal peptide predicted by SignalP
CLCA_HUMAN      0.864   4.778     0.010    -
CLH2_HUMAN      0.443   0.868     0.002    -
CO5A1_HUMAN     0.053   0.129     0.000    signal peptide predicted by SignalP
CRDL1_HUMAN     0.683   1.959     0.004    signal peptide predicted by SignalP
CRIM1_HUMAN     0.521   1.140     0.002    signal peptide predicted by SignalP
EGFL7_HUMAN     0.590   1.465     0.003    signal peptide predicted by SignalP
FGFR4_HUMAN     0.611   1.514     0.003    signal peptide predicted by SignalP
FINC_HUMAN      0.371   0.686     0.001    signal peptide predicted by SignalP
FPRP_HUMAN      0.711   2.390     0.005    signal peptide predicted by SignalP
GDF15_HUMAN     0.892   5.521     0.011    signal peptide predicted by SignalP
GRN_HUMAN       0.590   1.342     0.003    signal peptide predicted by SignalP
H2A1H_HUMAN     0.563   1.969     0.004    -
IBP3_HUMAN      0.819   3.877     0.008    signal peptide predicted by SignalP
```

Fig 7. SecretomeP의 prediction results

SignalP 에 의해 signal peptide가 예상된 된 단백질을 제외하고 NN-score가 0.5이상인 경우, nonclassical secretion pathway를 통해 secretion 되었다고 예상

Public Database의 이용(3) - TMHMM (version 2.0)

2015. 8.

KIST 이철주

TMHMM (version 2.0)

- (http://www.cbs.dtu.dk/services/TMHMM)
- transmembrane helices in integral membrane proteins 예상
- hidden Markov model 이용

The TMHMM program is based on a hidden Markov model approach. The hidden Markov model can incorporate hydrophobicity, charge bias, helix lengths and grammatical constraints into one model for which algorithms for parameter estimate and prediction already exist.[iv]

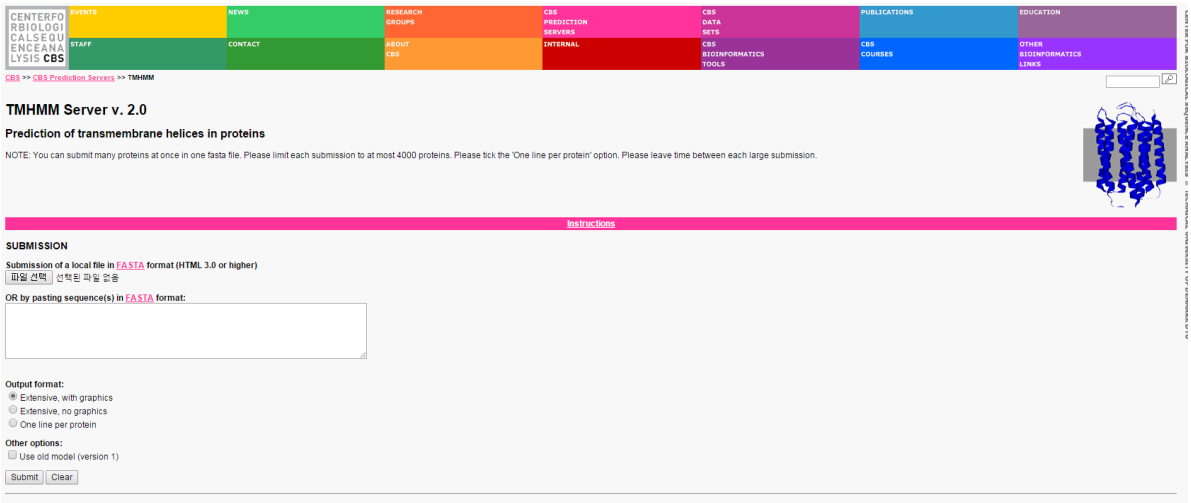


Fig 8. TMHMM homepage

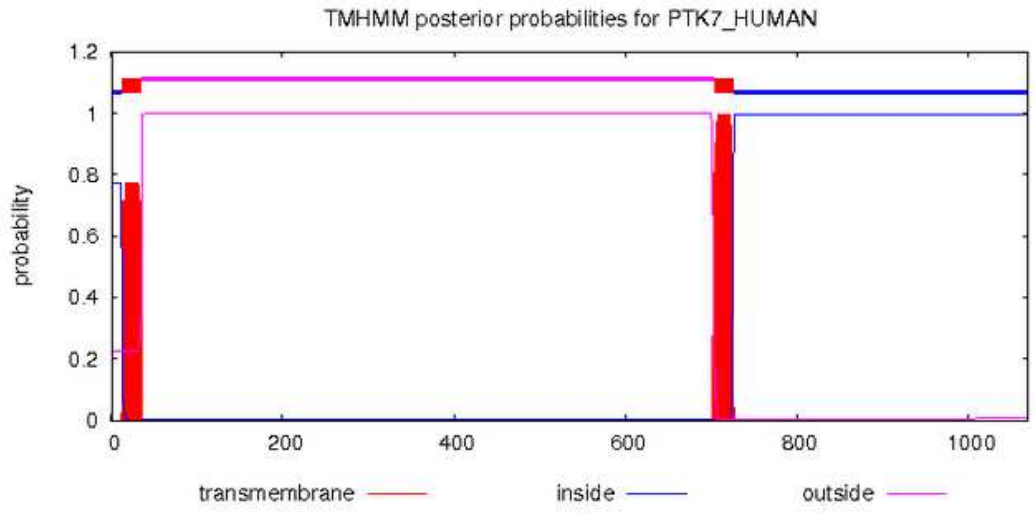
■ Parameter

1. Fasta 형식 또는 파일 준비
   - 한 번의 submission에 4,000 entries 가능

2. Output format 선택 : one line per protein 추천

■ 결과해석

```
# PTK7_HUMAN Length: 1070
# PTK7_HUMAN Number of predicted TMHs:  2
# PTK7_HUMAN Exp number of AAs in TMHs: 39.8346499999999999
# PTK7_HUMAN Exp number, first 60 AAs:  17.43834
# PTK7_HUMAN Total prob of N-in:        0.77345
# PTK7_HUMAN POSSIBLE N-term signal sequence
PTK7_HUMAN      TMHMM2.0        inside      1    12
PTK7_HUMAN      TMHMM2.0        TMhelix     13   35
PTK7_HUMAN      TMHMM2.0        outside     36   703
PTK7_HUMAN      TMHMM2.0        TMhelix     704  726
PTK7_HUMAN      TMHMM2.0        inside      727  1070
```



plot in postscript, script for making the plot in gnuplot, data for plot

Fig 9. TMHMM의 prediction results (Extensive, with graphics)

TMHMM result

HELP with output formats

```
GDF15_HUMAN     len=308 ExpAA=3.03      First60=3.03    PredHel=0       Topology=o
LOXL3_HUMAN     len=753 ExpAA=0.48      First60=0.47    PredHel=0       Topology=o
CRDL1_HUMAN     len=450 ExpAA=0.01      First60=0.01    PredHel=0       Topology=o
MMP10_HUMAN     len=476 ExpAA=0.51      First60=0.46    PredHel=0       Topology=o
TGFB1_HUMAN     len=390 ExpAA=7.17      First60=7.16    PredHel=0       Topology=o
LAMC2_HUMAN     len=1193        ExpAA=0.26      First60=0.26    PredHel=0       Topology=o
QPCT_HUMAN      len=361 ExpAA=4.36      First60=4.29    PredHel=0       Topology=o
LTBP1_HUMAN     len=1721        ExpAA=2.46      First60=2.46    PredHel=0       Topology=o
GRN_HUMAN       len=593 ExpAA=0.03      First60=0.03    PredHel=0       Topology=o
CO5A1_HUMAN     len=1838        ExpAA=5.38      First60=5.37    PredHel=0       Topology=o
IBP3_HUMAN      len=291 ExpAA=4.22      First60=4.22    PredHel=0       Topology=o
LAMB3_HUMAN     len=1172        ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
STC1_HUMAN      len=247 ExpAA=0.01      First60=0.01    PredHel=0       Topology=o
EGFL7_HUMAN     len=273 ExpAA=5.36      First60=5.36    PredHel=0       Topology=o
RNT2_HUMAN      len=256 ExpAA=8.19      First60=8.19    PredHel=0       Topology=o
INHBA_HUMAN     len=426 ExpAA=0.01      First60=0.01    PredHel=0       Topology=o
MIS_HUMAN       len=560 ExpAA=0.57      First60=0.56    PredHel=0       Topology=o
FINC_HUMAN      len=2386        ExpAA=0.17      First60=0.17    PredHel=0       Topology=o
CBPE_HUMAN      len=476 ExpAA=10.01     First60=10.00   PredHel=0       Topology=o
H2A1H_HUMAN     len=128 ExpAA=1.63      First60=1.13    PredHel=0       Topology=o
MFGM_HUMAN      len=387 ExpAA=2.94      First60=2.94    PredHel=0       Topology=o
RS12_HUMAN      len=132 ExpAA=0.01      First60=0.01    PredHel=0       Topology=o
PTK7_HUMAN      len=1070        ExpAA=39.83     First60=17.44   PredHel=2       Topology=i13-35o704-726i
ATRN_HUMAN      len=1429        ExpAA=26.26     First60=0.05    PredHel=1       Topology=o1279-1301i
TPIS_HUMAN      len=249 ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
MET_HUMAN       len=1390        ExpAA=25.70     First60=0.61    PredHel=1       Topology=o933-955i
RPESP_HUMAN     len=264 ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
MSLN_HUMAN      len=630 ExpAA=19.57     First60=13.00   PredHel=1       Topology=i9-31o
FPRP_HUMAN      len=879 ExpAA=22.24     First60=0.06    PredHel=1       Topology=o831-853i
ASNS_HUMAN      len=561 ExpAA=0.02      First60=0.01    PredHel=0       Topology=o
NOTUM_HUMAN     len=496 ExpAA=0.04      First60=0.02    PredHel=0       Topology=o
CRIM1_HUMAN     len=1036        ExpAA=32.05     First60=10.15   PredHel=1       Topology=o939-961i
TRFM_HUMAN      len=738 ExpAA=0.16      First60=0.06    PredHel=0       Topology=o
UB2D4_HUMAN     len=147 ExpAA=0.01      First60=0.01    PredHel=0       Topology=o
ADA17_HUMAN     len=824 ExpAA=21.54     First60=0.01    PredHel=1       Topology=o672-694i
SF3B4_HUMAN     len=424 ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
ARVC_HUMAN      len=962 ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
CATF_HUMAN      len=484 ExpAA=0.45      First60=0.44    PredHel=0       Topology=o
LTBP4_HUMAN     len=1624        ExpAA=7.83      First60=7.83    PredHel=0       Topology=o
FGFR4_HUMAN     len=802 ExpAA=6.96      First60=0.09    PredHel=0       Topology=o
CLCA_HUMAN      len=248 ExpAA=0.00      First60=0.00    PredHel=0       Topology=o
CLH2_HUMAN      len=1640        ExpAA=1.26      First60=0.00    PredHel=0       Topology=o
NDRG3_HUMAN     len=375 ExpAA=1.16      First60=0.00    PredHel=0       Topology=o
```

Fig 10. TMHMM의 prediction results (one line per protein)

- Length (“len=”): the length of the protein sequence.
- Number of predicted TMHs: The number of predicted transmembrane helices.
- Exp number of AAs in TMHs (“ExpAA=”): The expected number of amino acids intransmembrane helices. If this number is larger than 18 it is very likely to be a transmembrane protein (OR have a signal peptide).
- Exp number, first 60 AAs (“First60=”): The expected number of amino acids in transmembrane helices in the first 60 amino acids of the protein. If this number more than a few, you should be warned that a predicted transmembrane helix in the N-term could be a signal peptide.
- Total prob of N-in: The total probability that the N-term is on the cytoplasmic side of the membrane.
- POSSIBLE N-term signal sequence: a warning that is produced when "Exp number, first 60 AAs" is larger than 10.
- "PredHel=": The number of predicted transmembrane helices by N-best.
- "Topology=": The topology predicted by N-best. ('i' if the loop is on the inside or 'o' if it is on the outside)

[i] Nielsen H, Krogh A. Prediction of signal peptides and signal anchors by a hidden Markov model. Proc Int Conf Intell Syst Mol Biol. 1998;6:122-30.

[ii] Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature methods. 2011;8:785-6.

[iii] Bendtsen JD, Jensen LJ, Blom N, Von Heijne G, Brunak S. Feature-based prediction of non-classical and leaderless protein secretion. Protein Eng Des Sel. 2004;17:349-56.

[iv] Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol. 2001;305:567-80.