HMMER

Laboratory of Bioinformatics I
Module 2

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http://biofold.org/

Biomolecules
Folding and
Disease

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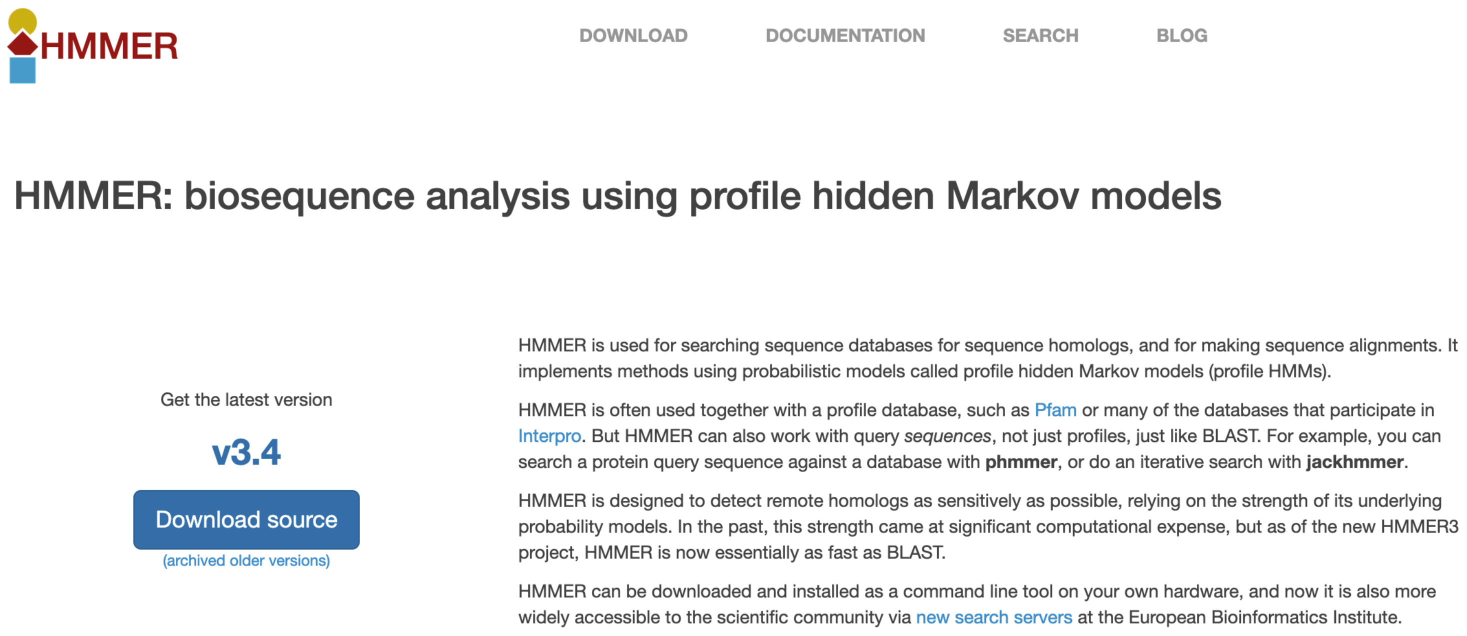
HMMER



HMMER: biosequence analysis using profile hidden Markov models

HMMER is used for searching sequence databases for sequence homologs, and for making sequence alignments. It implements methods using probabilistic models called profile hidden Markov models (profile HMMs).

HMMER is often used together with a profile database, such as Pfam or many of the databases that participate in Interpro. But HMMER can also work with query *sequences*, not just profiles, just like BLAST. For example, you can search a protein query sequence against a database with **phmmer**, or do an iterative search with **jackhmmer**.

HMMER is designed to detect remote homologs as sensitively as possible, relying on the strength of its underlying probability models. In the past, this strength came at significant computational expense, but as of the new HMMER3 project, HMMER is now essentially as fast as BLAST.

HMMER can be downloaded and installed as a command line tool on your own hardware, and now it is also more widely accessible to the scientific community via new search servers at the European Bioinformatics Institute.

Eddy SR (1998) *Profile hidden Markov models*. **Bioinformatics** 14:755-763
Eddy SR (2008) *A Probabilistic Model of Local Sequence Alignment That Simplifies Statistical Significance Estimation.* **PLoS Comp. Biol**. 4: e1000069
Eddy SR (2011) *Accelerated profile HMM searches.* **PLoS Comp. Biol**. 7:e1002195

http://hmmer.org

Why HMMER?

HMMER is an Hidden Markov Model based tool used for

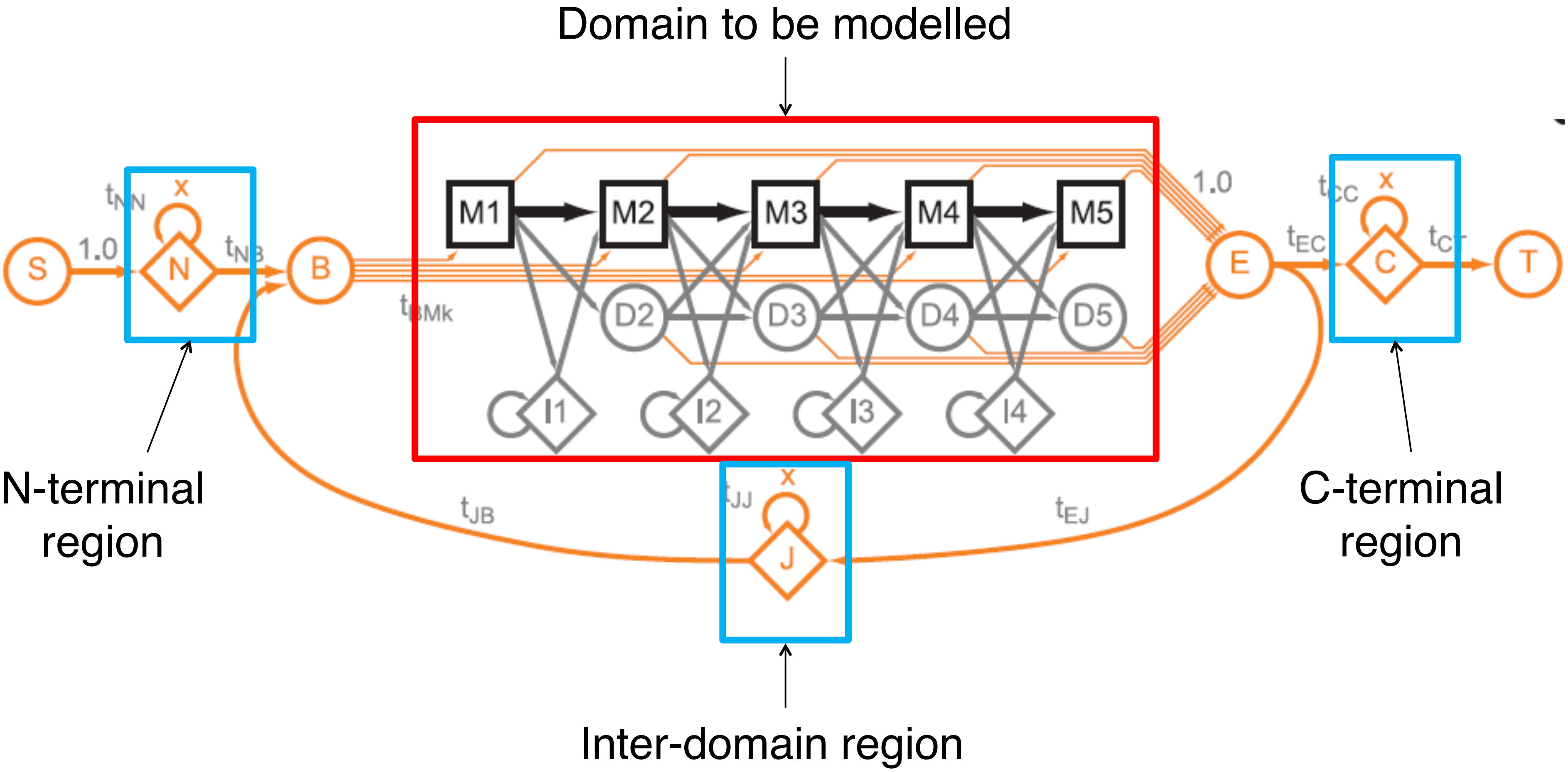
- searching sequence databases for sequence homologs
- make sequence alignments

HMMER is designed to detect remote homologs relying on the strength of its underlying probability models.

The new version of HMMER (HMMER3) is as fast as BLAST.

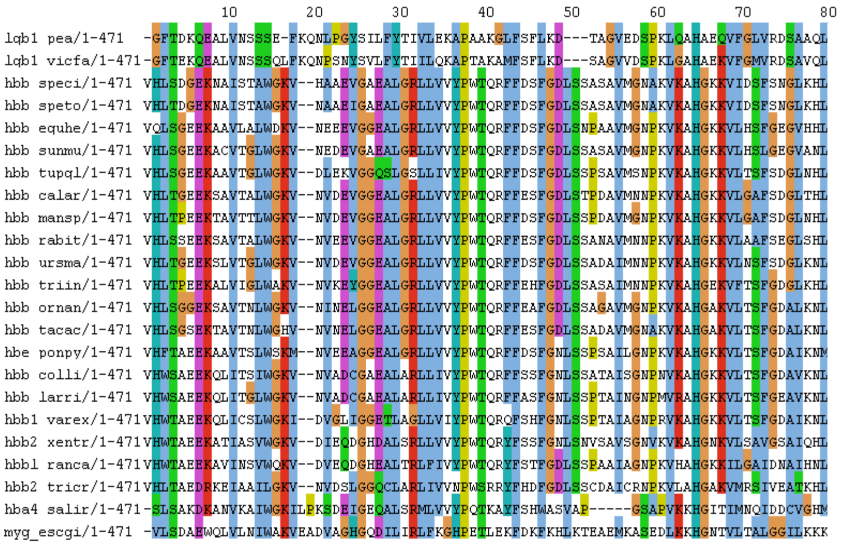
HMMER: General Model

The domain model has multiple hits and for each hit insertion and deletion

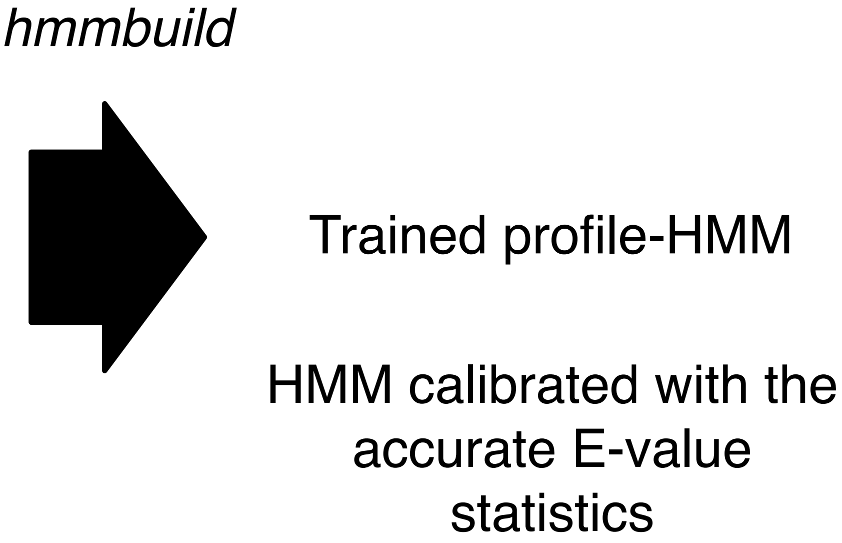


Aligning a protein family

Takes the aligned sequences, checks for redundancy and sets the emission and the transitions probabilities of a HMM, setting the parameters for the Extreme value distribution



```
                           10        20        30        40        50        60        70        80
lqb1 pea/1-471   -GFTDKQEALVNSSSE-FKQNLPGYSILFYTIVLEKAPAAKGLFSFLKD---TAGVEDSPKLQAHAEQVFGLVRDSAAQL
lqb1 vicfa/1-471-GFTEKQEALVNSSSQLFKQNPSNYSVLFYTIILQKAPTAKAMFSFLKD---SAGVVDSPKLGAHAEKVFGMVRDSAVQL
hbb speci/1-471 VHLSDGEKNAISTAWGKV--HAAEVGAEALGRLLVVYPWTQRFFDSFGDLSSASAVMGNAKVKAHGKKVIDSFSNGLKHL
hbb speto/1-471 VHLTDGEKNAISTAWGKV--NAAEIGAEALGRLLVVYPWTQRFFDSFGDLSSASAVMGNAKVKAHGKKVIDSFSNGLKHL
hbb equhe/1-471 VQLSGEEKAAVLALWDKV--NEEEVGGEALGRLLVVYPWTQRFFDSFGDLSNPAAVMGNPKVKAHGKKVLHSFGEGVHHL
hbb sunmu/1-471 VHLSGEEKACVTGLWGKV--NEDEVGAEALGRLLVVYPWTQRFFDSFGDLSSASAVMGNPKVKAHGKKVLHSLGEGVANL
hbb tupql/1-471 VHLSGEEKAAVTGLWGKV--DLEKVGGQSLGSLLIVYPWTQRFFDSFGDLSSPSAVMSNPKVKAHGKKVLTSFSDGLNHL
hbb calar/1-471 VHLTGEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMNNPKVKAHGKKVLGAFSDGLTHL
hbb mansp/1-471 VHLTPEEKTAVTTLWGKV--NVDEVGGEALGRLLVVYPWTQRFFDSFGDLSSPDAVMGNPKVKAHGKKVLGAFSDGLNHL
hbb rabit/1-471 VHLSSEEKSAVTALWGKV--NVEEVGGEALGRLLVVYPWTQRFFESFGDLSSANAVMNNPKVKAHGKKVLAAFSEGLSHL
hbb ursma/1-471 VHLTGEEKSLVTGLWGKV--NVDEVGGEALGRLLVVYPWTQRFFDSFGDLSSADAIMNNPKVKAHGKKVLNSFSDGLKNL
hbb triin/1-471 VHLTPEEKALVIGLWAKV--NVKEYGGEALGRLLVVYPWTQRFFEHFGDLSSASAIMNNPKVKAHGEKVFTSFGDGLKHL
hbb ornan/1-471 VHLSGGEKSAVTNLWGKV--NINELGGEALGRLLVVYPWTQRFFEAFGDLSSAGAVMGNPKVKAHGAKVLTSFGDALKNL
hbb tacac/1-471 VHLSGSEKTAVTNLWGHV--NVNELGGEALGRLLVVYPWTQRFFESFGDLSSADAVMGNAKVKAHGAKVLTSFGDALKNL
hbe ponpy/1-471 VHFTAEEKAAVTSLWSKM--NVEEAGGEALGRLLVVYPWTQRFFDSFGNLSSPSAILGNPKVKAHGKKVLTSFGDAIKNM
hbb colli/1-471 VHWSAEEKQLITSIWGKV--NVADCGAEALARLLIVYPWTQRFFSSFGNLSSATAISGNPNVKAHGKKVLTSFGDAVKNL
hbb larri/1-471 VHWSAEEKQLITGLWGKV--NVADCGAEALARLLIVYPWTQRFFASFGNLSSPTAINGNPMVRAHGKKVLTSFGEAVKNL
hbb1 varex/1-471VHWTAEEKQLICSLWGKI--DVGLIGGETLAGLLVIYPWTQRQFSHFGNLSSPTAIAGNPRVKAHGKKVLTSFGDAIKNL
hbb2 xentr/1-471VHWTAEEKATIASVWGKV--DIEQDGHDALSRLLVVYPWTQRYFSSFGNLSNVSAVSGNVKVKAHGNKVLSAVGSAIQHL
hbbl ranca/1-471VHWTAEEKAVINSVWQKV--DVEQDGHEALTRLFIVYPWTQRYFSTFGDLSSPAAIAGNPKVHAHGKKILGAIDNAIHNL
hbb2 tricr/1-471VHLTAEDRKEIAAILGKV--NVDSLGGQCLARLIVVNPWSRRYFHDFGDLSSCDAICRNPKVLAHGAKVMRSIVEATKHL
hba4 salir/1-471-SLSAKDKANVKAIWGKILPKSDEIGEQALSRMLVVYPQTKAYFSHWASVAP-----GSAPVKKHGITIMNQIDDCVGHM
myg_escgi/1-471 -VLSDAEWQLVLNIWAKVEADVAGHGQDILIRLFKGHPETLEKFDKFKHLKTEAEMKASEDLKKHGNTVLTALGGILKKK
```



hmmbuild

Trained profile-HMM

HMM calibrated with the accurate E-value statistics

Scoring the Sequence

After the training, the model M associates to a sequence s the probability $P(s \mid M)$

This probability answers the question:

What is the probability for a model M (e.g. describing the Globins) to generate the sequence s?

BUT the question we want to answer to is:

Given a sequence s, does it belong to the class described by the model M? (e.g. is it a Globin?)

We need to compute $P(M \mid s)$!!

A Priori Probabilities

$$P(M \mid s) = \frac{P(s \mid M)\,P(M)}{P(s)}$$

A priori probabilities

$P(M)$ is the probability of the model (i.e. of the class described by the model) BEFORE we know the sequence:

can be estimated as the abundance of the class

$P(s)$ is the probability of the sequence in the sequence space.

Cannot be reliably estimated!!

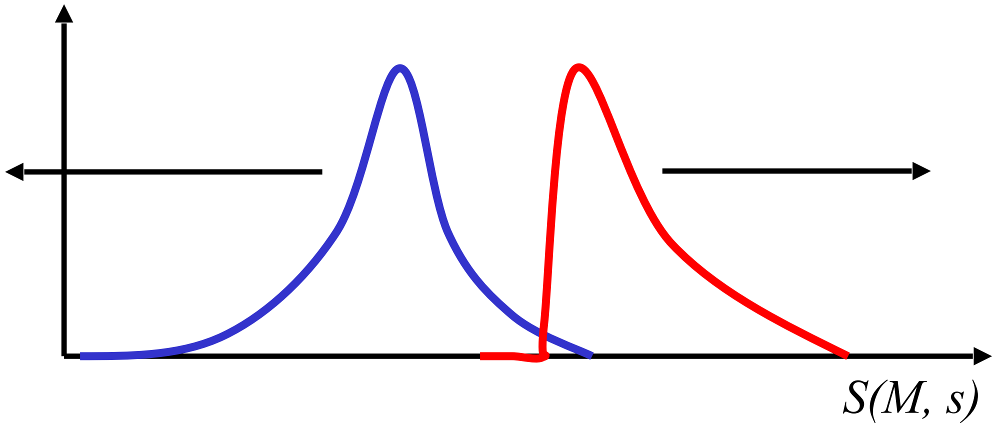
The Null Model

Null Model: a model that generates ALL the possible sequences with probabilities depending ONLY on letter (e.g. residue) statistical abundance (in HMMER3, by default, mean residue frequencies in Swiss-Prot 50.8 (October 2006)

Log odd score (in bits)

$$S(M, s) = \log_2 \frac{P(s \mid M)}{P(s \mid N)}$$

Sequences NOT belonging to model M

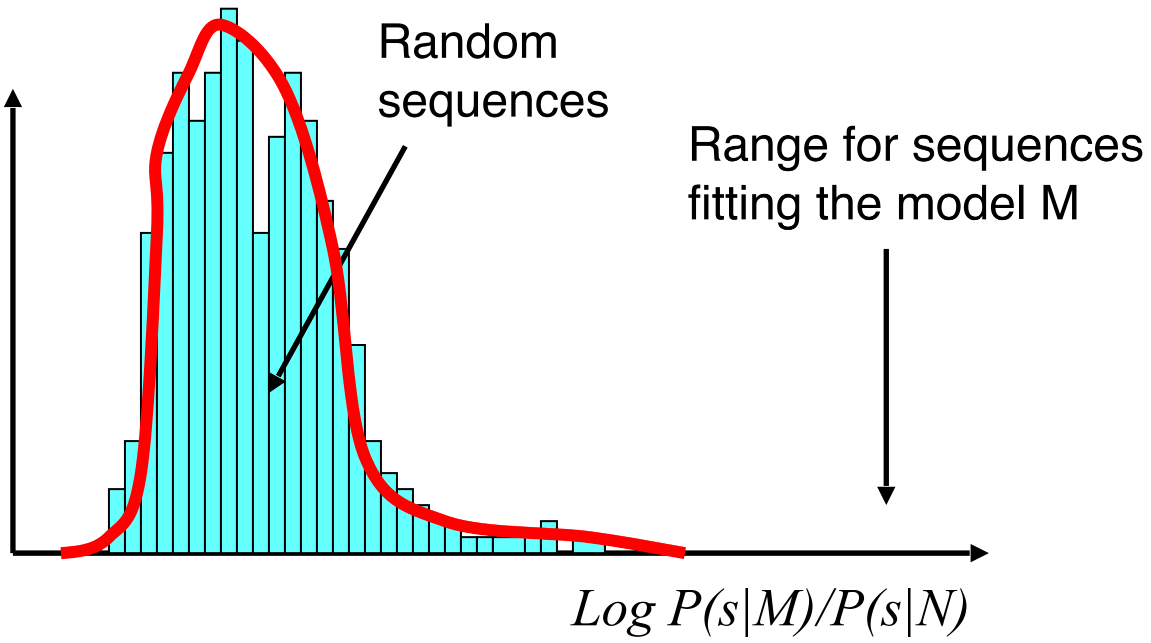


Sequences belonging to model M

In this case we need a threshold and a statistic for evaluating the significance (E-value, P-value)

Extreme Value Distribution

Given a trained model M, a number of N (default 200 in HMMER3) random sequences are generated and scored with the model.



Random sequences

Range for sequences fitting the model M

The random distribution is fitted with a Gumbel distribution, by estimating λ and μ

$$P(S \geq t) = 1 - \exp\left[-e^{-\lambda(t-\mu)}\right]$$

The E-value

After setting λ and μ

$$P(S \geq t) = 1 - \exp\left[-e^{-\lambda(t-\mu)}\right]$$

gives the probability of finding random matches with score > t:
This is by definition the P-value corresponding to the score t

The E-value(t), namely expected number of random sequences with a score > t, is obtained with the relation

$$P = 1 - e^{-E}$$

E-value vs P-value

If E is the expected (average) number of occurrences of a rare event, we can adopt the Poisson's statistics to estimate the probability of observing a of such events.

$$p(a) = e^{-E}\frac{E^a}{a!}$$

P-value (P) is the probability of observing at least one rare event, that is

$$P = 1 - p(0)$$

$$P = 1 - e^{-E}$$

HMMER Installation

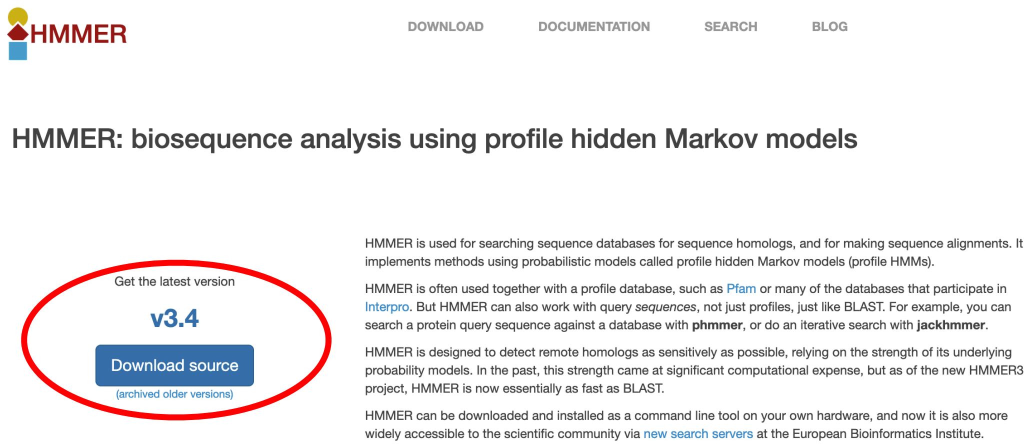
For Debian/Ubuntu Linux distributions

```
apt-get install hmmer
apt-get install hmmer-doc
```

Root privileges are needed
User guide http://eddylab.org/software/hmmer/Userguide.pdf

Otherwise:

Download the Linux version →



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http://hmmer.org

Globin Alignment

The multiple sequence alignment is provided in Stockholm format

```
# STOCKHOLM 1.0

HBB_HUMAN   ........VHLTPEEKSAVTALWGKV....NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVL
HBA_HUMAN   .........VLSPADKTNVKAAWGKVGA..HAGEYGAEALERMFLSFPTTKTYFPHF.DLS.....HGSAQVKGHGKKVA
MYG_PHYCA   .........VLSEGEWQLVLHVWAKVEA..DVAGHGQDILIRLFKSHPETLEKFDRFKHLKTEAEMKASEDLKKHGVTVL
GLB5_PETMA  PIVDTGSVAPLSAAEKTKIRSAWAPVYS..TYETSGVDILVKFFTSTPAAQEFFPKFKGLTTADQLKKSADVRWHAERII

HBB_HUMAN   GAFSDGLAHL...D..NLKGTFATLSELHCDKL..HVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANAL
HBA_HUMAN   DALTNAVAHV...D..DMPNALSALSDLHAHKL..RVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVL
MYG_PHYCA   TALGAILKK....K.GHHEAELKPLAQSHATKH..KIPIKYLEFISEAIIHVLHSRHPGDFGADAQGAMNKALELFRKDI
GLB5_PETMA  NAVNDAVASM..DDTEKMSMKLRDLSGKHAKSF..QVDPQYFKVLAAVIADTVAAG.........DAGFEKLMSMICILL

HBB_HUMAN   AHKYH......
HBA_HUMAN   TSKYR......
MYG_PHYCA   AAKYKELGYQG
GLB5_PETMA  RSAY.......
//
```

HEADER:	`# STOCKHOLM 1.0`
END:	`//`
GAP:	`.`

Stockholm Format

More information can be added. They can be used to guide the HMM training.

```
#=GC SS_cons    Secondary structure (consensus)

#=GC RF         Reference annotation
```

Often the consensus RNA or protein sequence is used as a reference
Any non-gap character (e.g. x's) can indicate consensus/conserved/match columns
. or - indicate insert columns
~ indicate unaligned insertions
Upper and lower case can be used to discriminate strong and weakly conserved residues respectively

```
#=GC MM         Model Mask
```

Indicates which columns in an alignment should be masked, such that the emission probabilities for match states corresponding to those columns will be the background distribution. Masked positions are marked with “m”

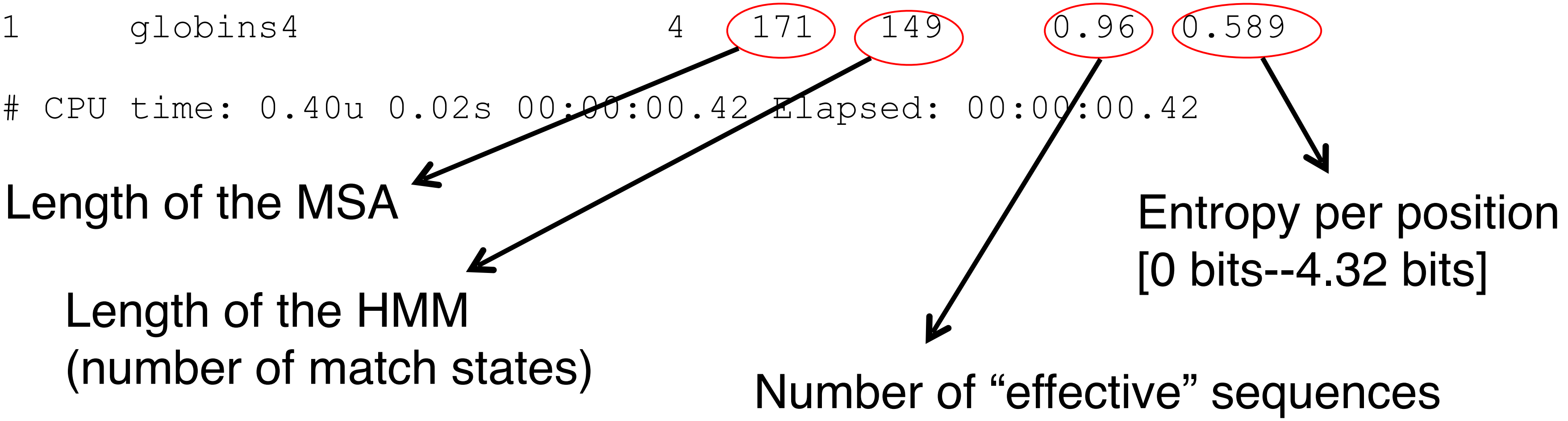
Building the HMM model

SINTAX:
hmmbuild <hmm_file> <msa_file>

```
# hmmbuild :: profile HMM construction from multiple sequence alignments
# HMMER 3.1b2 (February 2015); http://hmmer.org/
# Copyright (C) 2015 Howard Hughes Medical Institute.
# Freely distributed under the GNU General Public License (GPLv3).
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
# input alignment file:             globins4.sto
# output HMM file:                  globins4.hmm
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

# idx name                  nseq  alen  mlen eff_nseq re/pos description
#---- -------------------- ----- ----- ----- -------- ------ -----------
1     globins4                 4   171   149     0.96  0.589 

# CPU time: 0.40u 0.02s 00:00:00.42 Elapsed: 00:00:00.42
```



Length of the MSA

Length of the HMM
(number of match states)

Number of “effective” sequences

Entropy per position
[0 bits--4.32 bits]

HMM Model Output

HEADER: General info

Use information from RF and MM lines in Stockholm

Build a consensus sequence

Use information from SS_cons line in Stockholm

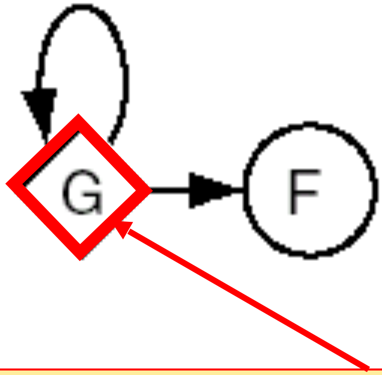
Map with respect to the alignment file

```
HMMER3/f [3.1b2 | February 2015]
NAME  globins4
LENG  149
ALPH  amino
RF    no
MM    no
CONS  yes
CS    no
MAP   yes
DATE  Sat Mar  8 23:36:44 2014
NSEQ  4
EFFN  0.964844
CKSUM 2027839109
STATS LOCAL MSV       -9.9014  0.70957
STATS LOCAL VITERBI  -10.7224  0.70957
STATS LOCAL FORWARD   -4.1637  0.70957
HMM          A        C        D        E        F        G        H       …
            m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  … 10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

Statistical parameters needed for E-value calculations (μ, λ)

NULL Model

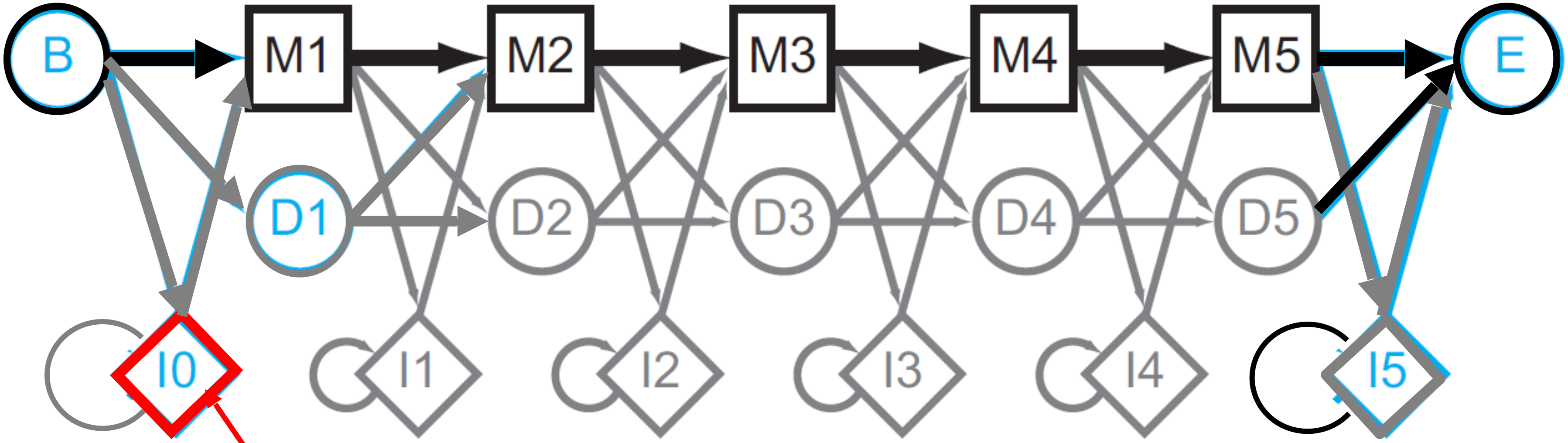
Score = -ln (p) or ‘*’ if p=0
Where p = is function of the natural abundance of residues
Swiss-Prot 50.8 (October 2006)



```
HMM         A        C        D        E        F        G        H      …
           m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  … 10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

0-states (emissions)

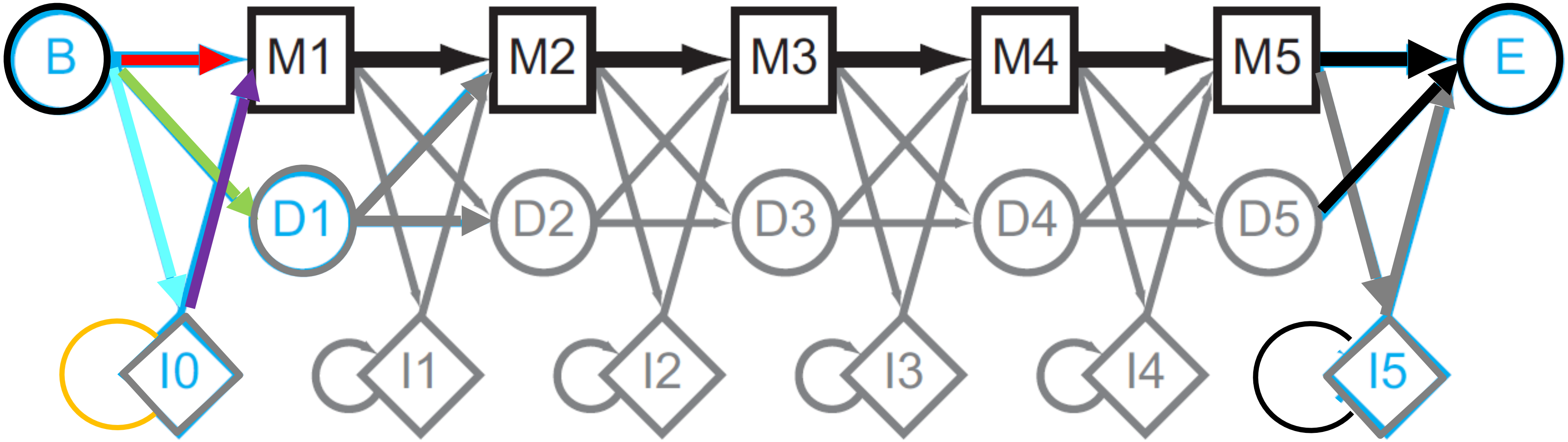
Score = -ln (p) or '*' if p=0



```
HMM          A        C        D        E        F        G        H      …
           m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  …  10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

0-states (transitions)

Score = -ln (p) or ‘*’ if p=0



```
HMM          A        C        D        E        F        G        H       …
           m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
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      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  …  10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```
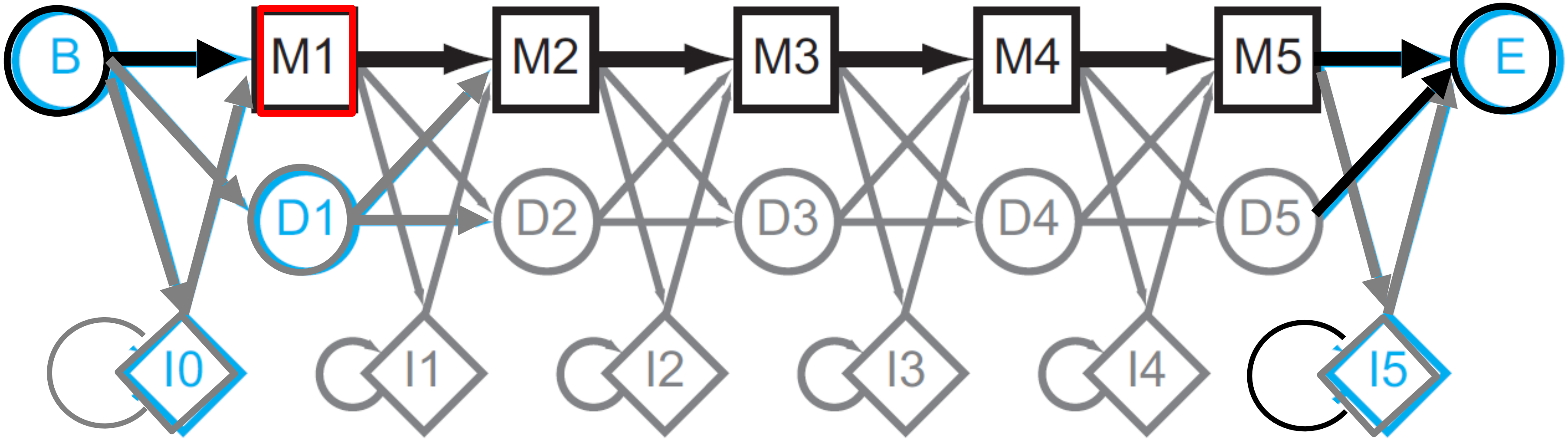

1-states (emissions)

Score = -ln (p) or ‘*’ if p=0



Corresponding position in initial MSA

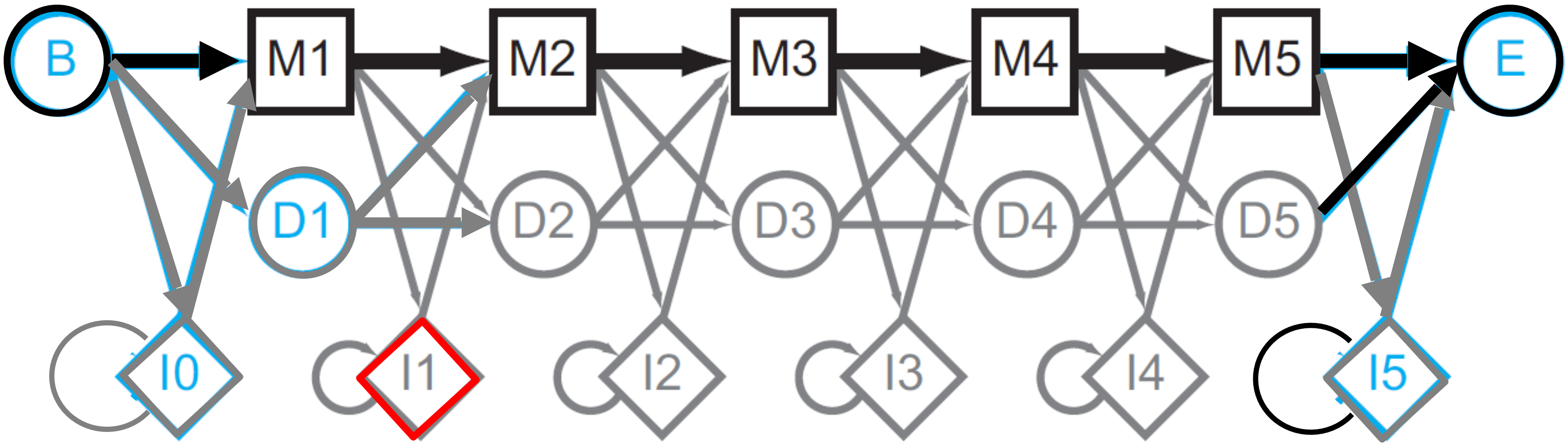
Consensus residue (lowest score)

```
HMM          A        C        D        E        F        G        H     ...
            m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  ...
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  ...
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  ...  9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  ...
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  ... 10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  ...
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

Column annotation for RF, MM, SS_cons (if present)

States of layer 1 (emissions)

Score = -ln (p) or ‘*’ if p=0



```
HMM          A        C        D        E        F        G        H       …
           m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069   …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516   …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
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          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494   …
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382   … 10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494   …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```
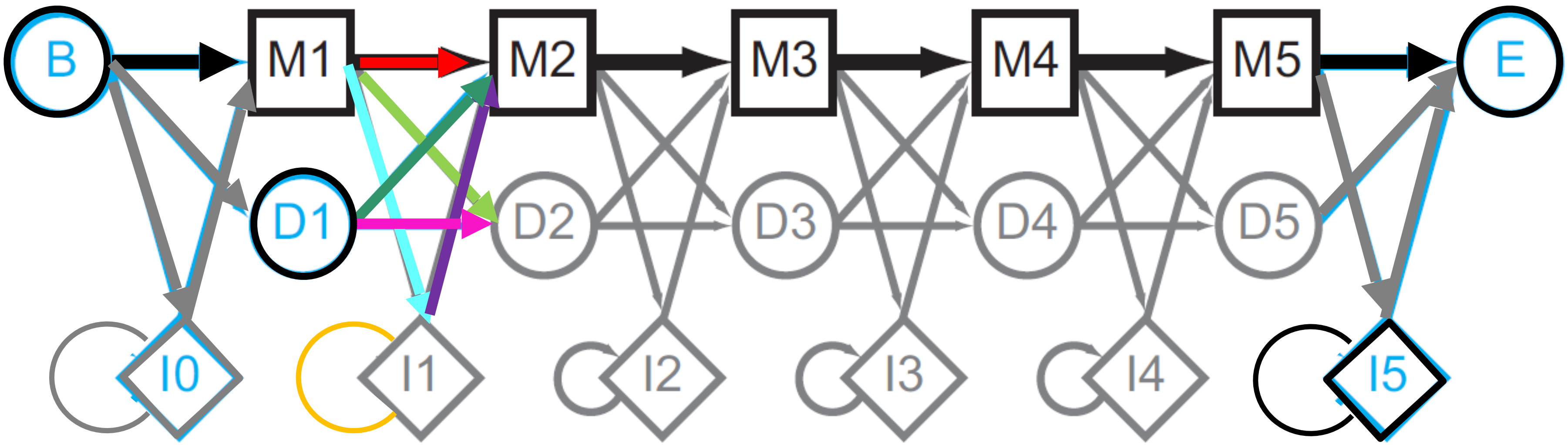

States of layer 1 (transitions)

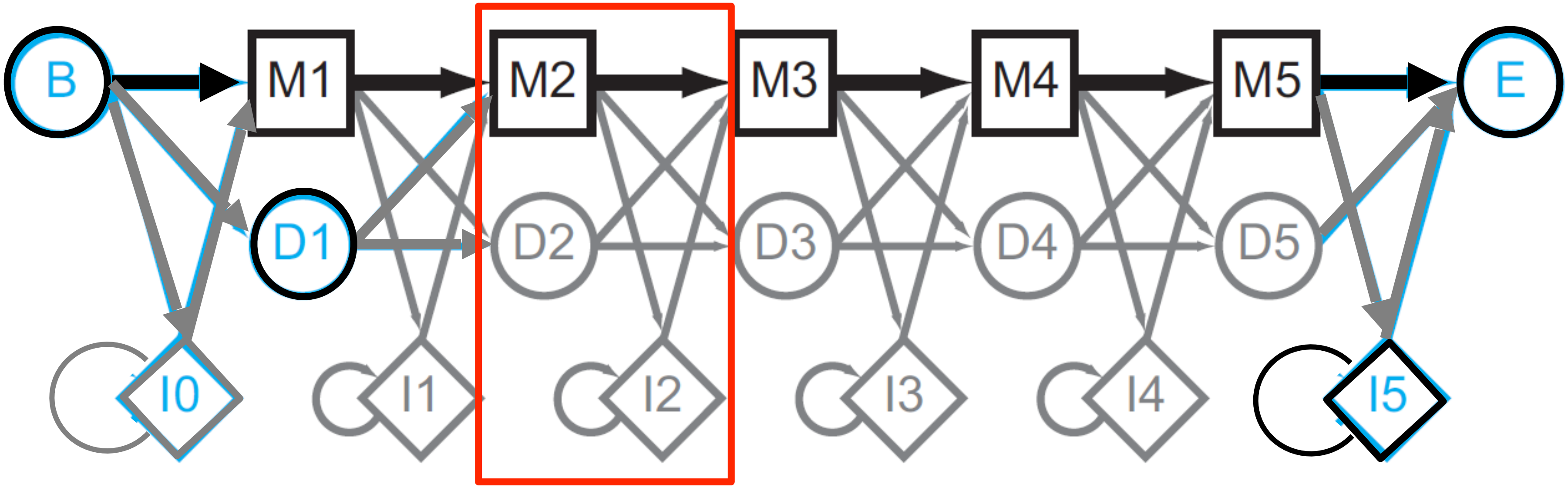
Score = -ln (p) or ‘*’ if p=0



```
HMM          A        C        D        E        F        G        H        …
           m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO   2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
          0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
      1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
      2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  …  10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

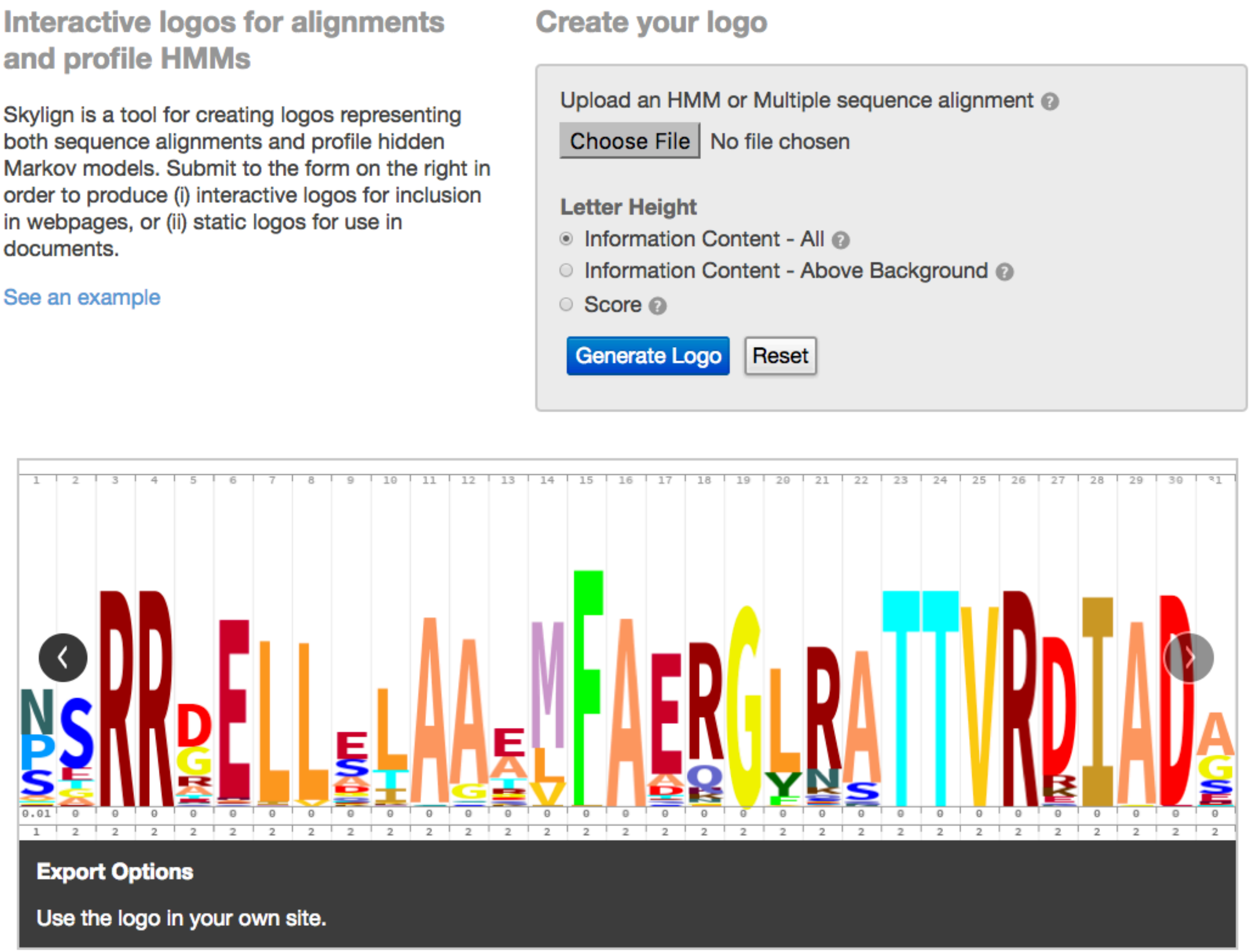
States of Layer 2

Score = -ln (p) or ‘*’ if p=0



```
HMM          A        C        D        E        F        G        H       …
            m->m     m->i     m->d     i->m     i->i     d->m     d->d
  COMPO    2.36553  4.52577  2.96709  2.70473  3.20818  3.02239  3.41069  …
           2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  …
           0.57544  1.78073  1.31293  1.75577  0.18968  0.00000        *
       1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  …   9 v - - -
           2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
           0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
       2   2.62748  4.47174  3.31917  2.82619  3.63815  3.49607  2.75382  …  10 v - - -
           2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  …
           0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
```

Skylign



Interactive logos for alignments and profile HMMs

Skylign is a tool for creating logos representing both sequence alignments and profile hidden Markov models. Submit to the form on the right in order to produce (i) interactive logos for inclusion in webpages, or (ii) static logos for use in documents.

See an example

Create your logo

Upload an HMM or Multiple sequence alignment

No file chosen

Letter Height

● Information Content - All

○ Information Content - Above Background

○ Score

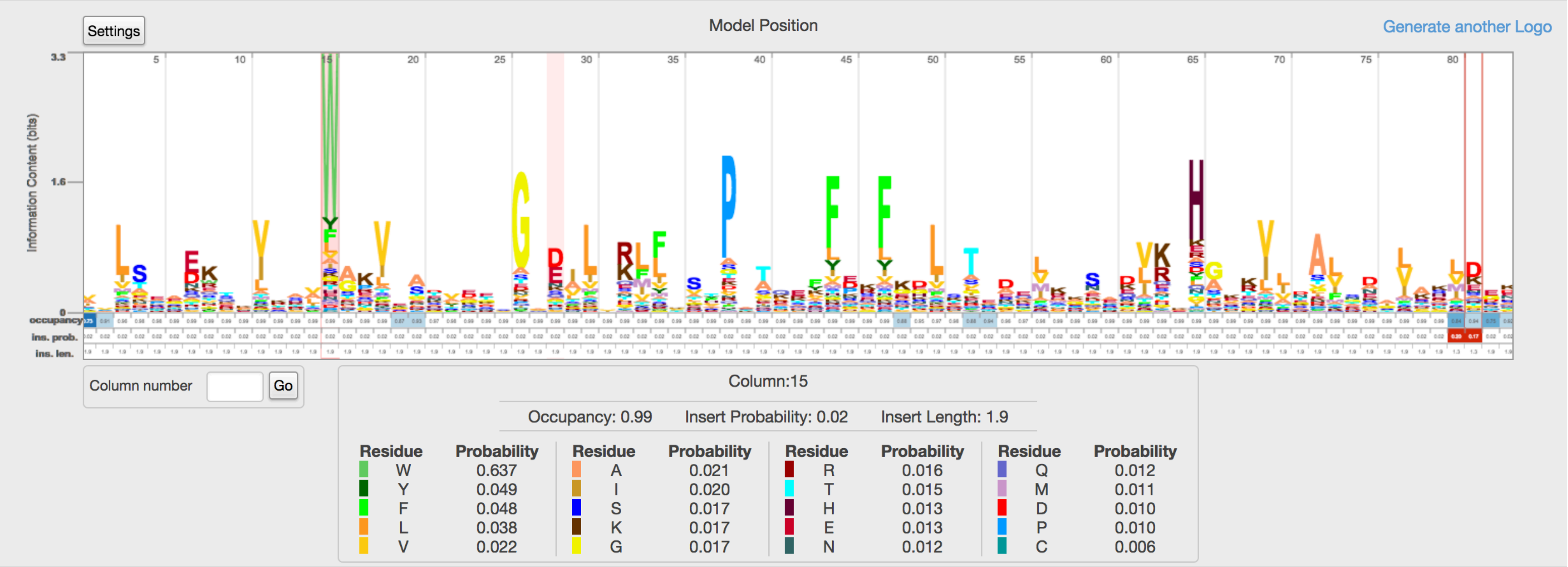
Export Options

Use the logo in your own site.

http://www.skylign.org

Skylign Output

Can be computed with the hmmlogo command



Column:15

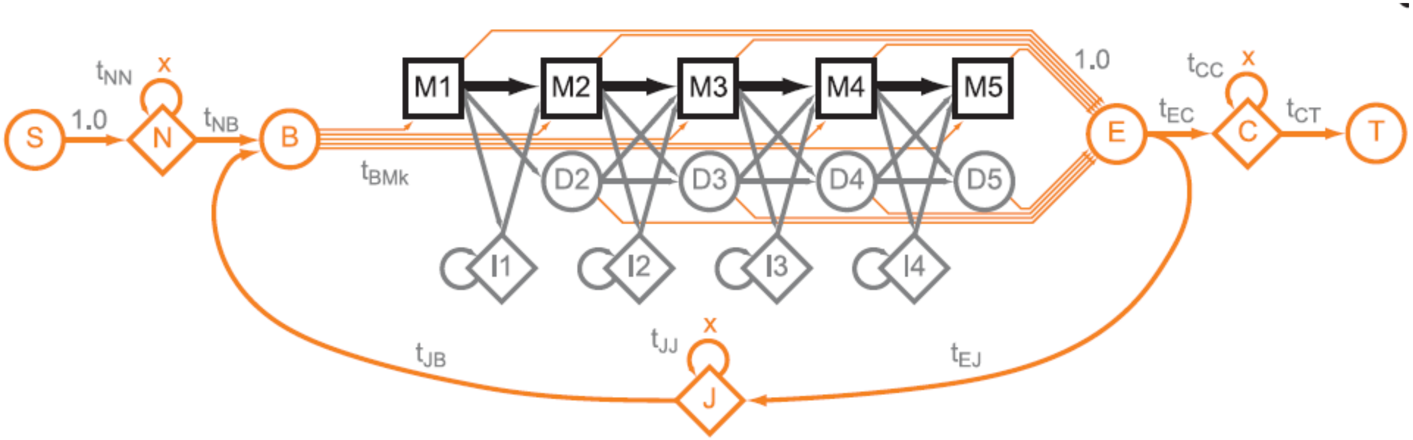
Occupancy: 0.99 Insert Probability: 0.02 Insert Length: 1.9

Residue	Probability	Residue	Probability	Residue	Probability	Residue	Probability
■ W	0.637	■ A	0.021	■ R	0.016	■ Q	0.012
■ Y	0.049	■ I	0.020	■ T	0.015	■ M	0.011
■ F	0.048	■ S	0.017	■ H	0.013	■ D	0.010
■ L	0.038	■ K	0.017	■ E	0.013	■ P	0.010
■ V	0.022	■ G	0.017	■ N	0.012	■ C	0.006

http://www.skylign.org

Generate sample sequence

Trained HMM



hmmemit

Generate (sample) sequences
from a profile HMM

Generate Sequences

hmmemit can be used to generate sequence using the Null model

```
SINTAX:
hmmemit [-N <num>] <hmm_file>
```

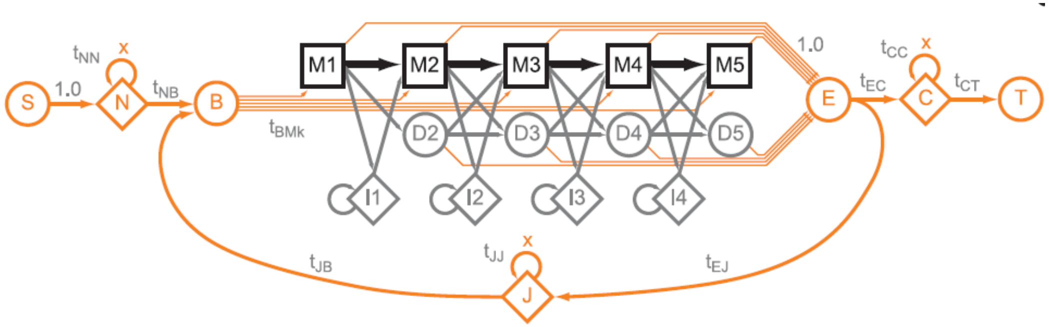
```
hmmemit –N 2 globins4.hmm
```

```
>globins4-sample1
EIPLMDLTEMESIWSGVNAAYKQVGKEEIVMMLQSLPTTVETFEKFHGNVSLDTEYKYRE
EYTKHAKTLLGAMLAASLSLKQHTENLDHLSKQLAAKVSIGPRPPRLCQRAAVTVLKAKF
PKNYTKHAMASSKKAMSDQEDLLDGKYK
>globins4-sample2
SHIEINPLEAVADLYTTLVIESQYDTPRIQSLHSLEWKKPAACYYRRNFDSFSDVTTTNM
MRVSASLRKMTMRVINAFITISATRDNHVQRIIPNAEDHSHKKSNAIDFKAIGVLPEISL
KMVPCRHPQDMGNEIHSIEEGLKEGGESADVRY
```

Search Matching Sequences

Set of sequences

Trained HMM



hmmsearch

List of sequences that match
the HMM (sorted by E-value)

Search Sequence

SINTAX:

`hmmsearch <hmmfile> <seqdb>`

Reads a set of sequences and finds occurrences of the modelled domain. Accepted formats include fasta, embl, genbank, ddbj, uniprot, stockholm, pfam, a2m, and afa.

```
# hmmsearch :: search profile(s) against a sequence database
# HMMER 3.1b1 (May 2013); http://hmmer.org/
# Copyright (C) 2013 Howard Hughes Medical Institute.
# Freely distributed under the GNU General Public License (GPLv3).
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
# query HMM file:                  globins4.hmm
# target sequence database:        tutorial/globins45.fa
# output directed to file:         search1.txt
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Query:       globins4  [M=149]
Scores for complete sequences (score includes all domains):
   --- full sequence ---   --- best 1 domain ---    -#dom-
    E-value  score  bias    E-value  score  bias    exp  N  Sequence
    ------- ------ -----    ------- ------ -----   ---- --  --------
    8.7e-67  215.6   2.9    9.7e-67  215.4   2.9    1.0  1  MYG_ESCGI
    1.1e-65  211.9   0.1    1.3e-65  211.8   0.1    1.0  1  HBB_MANSP
```

Model Line

```
Domain annotation for each sequence (and alignments):
>> MYG_ESCGI
#   score  bias c-Evalue i-Evalue hmmfrom hmm to alifrom ali to envfrom env to acc
--- ------ ---- -------- -------- ------- ------ ------- ------ ------- ------ ---
1 !  215.4 2.9   9.7e-67  9.7e-67      2   149 .]      1   147 [.      1   147 [. .99

  Alignments for each domain:
  == domain 1  score: 215.4 bits;  conditional E-value: 9.7e-67
globins4  2 vLseaektkvkavWakveadveesGadiLvrlfkstPatqefFekFkdLstedelkksadvkk
            vLs+ae++ v+++Wakveadv+++G+diL+rlfk +P+t+e+F+kFk+L+te+e+k+s+d+kk
MYG_ESCGI 1 VLSDAEWQLVLNIWAKVEADVAGHGQDILIRLFKGHPETLEKFDKFKHLKTEAEMKASEDLKK
            69*************************************************************

globins4  102 dpkyfkllsevlvdvlaarlpkeftadvqaaleKllalvakllaskYk 149
              ++ky++++s+++++vl++r+p++f+ad+qaa++K+l+l++k++a+kYk
MYG_ESCGI 100 PIKYLEFISDAIIHVLHSRHPGDFGADAQAAMNKALELFRKDIAAKYK 147
              ***********************************************7 PP
```

IN MODEL LINE:Capital letters represent the most conserved (high information content) positions. Dots (.) in this line indicate insertions in the target sequence with respect to the model.

MIDLINE indicates matches between the query model and target sequence. + indicates positive score(“conservative substitution”, with respect to what the model expects at that position).

BOTTOM LINE represents the posterior probability of each aligned residue. 0: 0-5%, 1: 5-15%, .. 9: 85-95%, *: 95-100% posterior probability. You can use these posterior probabilities to decide which parts of the alignment are well determined or not.

Searching Globin Sequence

`hmmsearch -o search1.txt  globins4.hmm  tutorial/globins45.fa`

```
# hmmsearch :: search profile(s) against a sequence database
# HMMER 3.1b1 (May 2013); http://hmmer.org/
# Copyright (C) 2013 Howard Hughes Medical Institute.
# Freely distributed under the GNU General Public License (GPLv3).
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
# query HMM file:                  globins4.hmm
# target sequence database:        tutorial/globins45.fa
# output directed to file:         search1.txt
# - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Query:       globins4  [M=149]
Scores for complete sequences (score includes all domains):
   --- full sequence ---   --- best 1 domain ---    -#dom-
    E-value  score  bias    E-value  score  bias    exp  N  Sequence
    ------- ------ -----    ------- ------ -----   ---- --  --------
    8.7e-67  215.6   2.9    9.7e-67  215.4   2.9    1.0  1  MYG_ESCGI
    1.1e-65  211.9   0.1    1.3e-65  211.8   0.1    1.0  1  HBB_MANSP
```

Score is in bits.
Bias is a correction: pay attention when it is on the same order of magnitude of the score (biased compositions/repetitive seq)

E-values

- if both E-values are significant (<< 1), the sequence is likely to be homologous to your query.

- if the full sequence E-value is significant but the single best domain E-value is not, the target sequence is probably a multidomain remote homolog: it contains multiple weakly-scoring domains, even if no single domain is solidly significant on its own; but we need to check if it’s just a repetitive sequence.

The Alignment

```
Domain annotation for each sequence (and alignments):
>> MYG_ESCGI
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  215.4   2.9   9.7e-67   9.7e-67       2     149 .]       1     147 [.       1     147 [. 0.99

  Alignments for each domain:
  == domain 1  score: 215.4 bits;  conditional E-value: 9.7e-67
   globins4   2 vLseaektkvkavWakveadveesGadiLvrlfkstPatqefFekFkdLstedelkksadvkkHgkkvldAlsdalakldekleaklkdLselHakklkv 101
                vLs+ae++ v+++Wakveadv+++G+diL+rlfk +P+t+e+F+kFk+L+te+e+k+s+d+kkHg++vl+Al+ +l+k ++++ea+lk+L+++Ha+k+k+
  MYG_ESCGI   1 VLSDAEWQLVLNIWAKVEADVAGHGQDILIRLFKGHPETLEKFDKFKHLKTEAEMKASEDLKKHGNTVLTALGGILKK-KGHHEAELKPLAQSHATKHKI 99
                69****************************************************************************.99******************* PP

   globins4 102 dpkyfkllsevlvdvlaarlpkeftadvqaaleKllalvakllaskYk 149
                ++ky++++s+++++vl++r+p++f+ad+qaa++K+l+l++k++a+kYk
  MYG_ESCGI 100 PIKYLEFISDAIIHVLHSRHPGDFGADAQAAMNKALELFRKDIAAKYK 147
                ***********************************************7 PP
```

In a match column, residues are upper case, and a ’-’ character means a deletion relative to the consensus.
In an insert column, residues are lower case, and a ’.’ is padding.

Insertions in a profile HMM are unaligned

BOTTOM LINE represents the posterior probability of each aligned residue. 0: 0-5%, 1: 5-15%, .. 9: 85-95%, *: 95-100% posterior probability.

Match Scores

```
Domain annotation for each sequence (and alignments):
>> MYG_ESCGI
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  215.4   2.9   9.7e-67   9.7e-67       2     149 .]       1     147 [.       1     147 [. 0.99
```

!: pass both per-domain and per-sequence E-value thresholds (0.001).
?: pass only one E-value threshold

c-Evalue: conditional E-value: statistical significance of the domain given that we know that the sequence is a true homolog

i-Evalue: independent E-value: statistical significance of the best domain identified in the sequence.

Then the portions of the aligned HMM and the sequence are provided

Envelope is the best aligned sequence portion

Acc: mean per residue alignment a–posteriori probability

Statistics Summary

```
Internal pipeline statistics summary:
-------------------------------------
Query model(s):                              1  (149 nodes)
Target sequences:                           45  (6519 residues searched)
Passed MSV filter:                        45  (1); expected 0.9 (0.02)
Passed bias filter:                       45  (1); expected 0.9 (0.02)
Passed Vit filter:                        45  (1); expected 0.0 (0.001)
Passed Fwd filter:                        45  (1); expected 0.0 (1e-05)
Initial search space (Z):                 45  [actual number of targets]
Domain search space  (domZ):              45  [number of targets
                                             reported over threshold]
# CPU time: 0.02u 0.01s 00:00:00.03 Elapsed: 00:00:00.03
# Mc/sec: 32.38
//
```

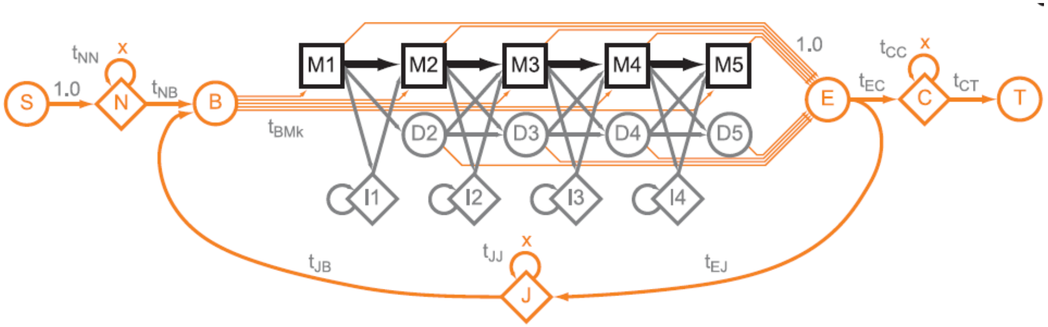
MSV: Multi-Segment Viterbi filter: sort of «local» BLAST-like alignments (heuristic)

Expected counts must be much lower than real counts.
Reported thresholds (in parenthesis) are in terms of P-values

MSA with HMM

Set of sequences

Trained HMM



hmmalign

Alignment of all sequences to the model

HMMALIGN

SINTAX:

hmmalign [-options] <hmmfile> <seqdb>

Reads a set of sequences and builds a MSA based on the model. Accepted formats include FASTA, EMBL, GenBank, DDBJ, UniProt, Stockholm, and SELEX.

```
# STOCKHOLM 1.0

MYG_ESCGI          .-VLSDAEWQLVLNIWAKVEADVAGHGQDILIRLFKGHPETLEKFDKFKHLKTEAEMKASEDLKK
#=GR MYG_ESCGI  PP ..69*************************************************************
MYG_HORSE          g--LSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKK
#=GR MYG_HORSE  PP 8..89************************************************************
MYG_PROGU          g--LSDGEWQLVLNVWGKVEGDLSGHGQEVLIRLFKGHPETLEKFDKFKHLKAEDEMRASEELKK
#=GR MYG_PROGU  PP 8..89************************************************************
MYG_SAISC          g--LSDGEWQLVLNIWGKVEADIPSHGQEVLISLFKGHPETLEKFDKFKHLKSEDEMKASEELKK
#=GR MYG_SAISC  PP 8..89************************************************************
```

In a match column, residues are upper case, and a '-' character means a deletion relative to the consensus.
In an insert column, residues are lower case, and a '.' is padding.

Insertions in a profile HMM are unaligned

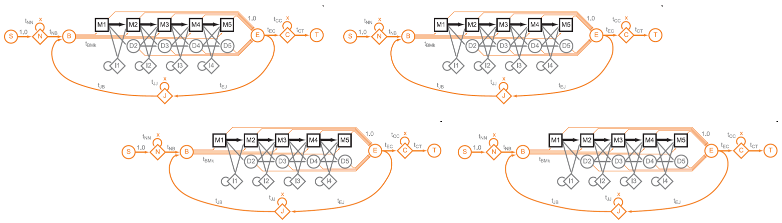
BOTTOM LINE represents the posterior probability of each aligned residue. 0: 0-5%, 1: 5-15%, .. 9: 85-95%, *: 95-100% posterior probability.

Scan HMM Library

Protein Sequence

```
>protein_id
VQLTVETITELAKNSYVAWGLSAAPISQNK
GKNGLHKFYFKMDNSEDFFEKLQELAGKDE
TYKGANIRWLGENVFDANSTIVSQDQEHHS
AEVMDSLSRELHAKVARYDMAYVEYLSMCI
APGFFANNEPIGAVECVSGIAHKMLKLIAA
LLSAKY
```

HMM Library



hmmscan

List of HMMs that best match the sequence

HMMPRESS & HMMSCAN

```
SINTAX:
hmmpress [-options] <hmmfile>

hmmscan  [-options] <hmmdb> <seqfile>
```

1. Generate a unique file putting together with “cat” all the previously generate hmm models. Use press to generate an hmm library.

2. Scan the new sequence against the hmm library

```
Query:       7LESS_DROME  [L=2554]
Accession:   P13368
Description: RecName: Full=Protein sevenless;          EC=2.7.10.1;
Scores for complete sequence (score includes all domains):
   --- full sequence ---   --- best 1 domain ---    -#dom-
    E-value  score  bias    E-value  score  bias    exp  N  Model    Description
    ------- ------ -----    ------- ------ -----   ----  --  --------- -----------
    5.6e-57  178.0   0.4    3.5e-16   47.2   0.9    9.4  9  fn3       Fibronectin
    1.1e-43  137.2   0.0    1.7e-43  136.5   0.0    1.3  1  Pkinase   Protein kinas
```

Exercise

In the tutorial directory (https://goo.gl/DsE2im) two protein MSAs are present: Pkinase.sto (protein kinase) and fn3.sto (fibronectin 3).

- Build two HMMs for the two alignment
- Check if the sequence 7LESS_DROME contains protein kinase or fibronectin3 domains. If yes, how many?