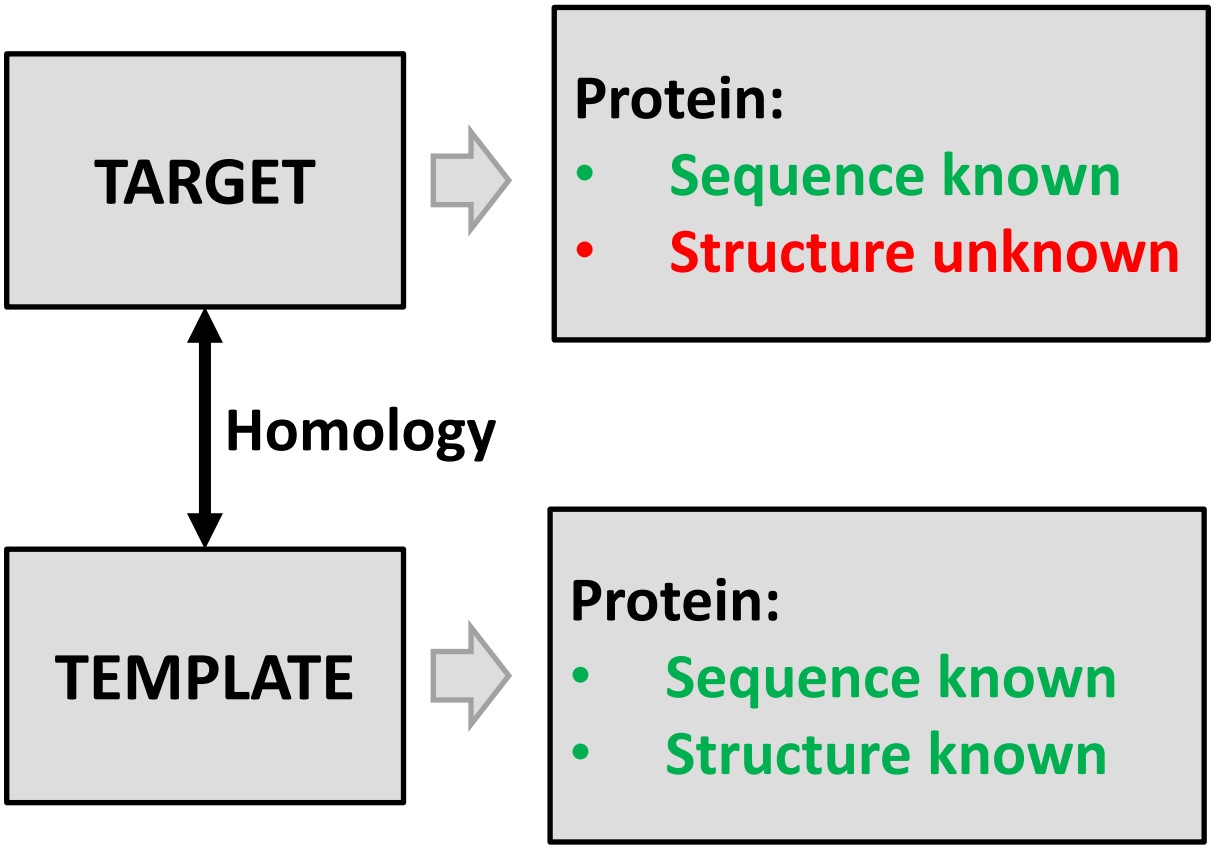
Structural biology

Practice 2: Hidden Markov Models and HMMer

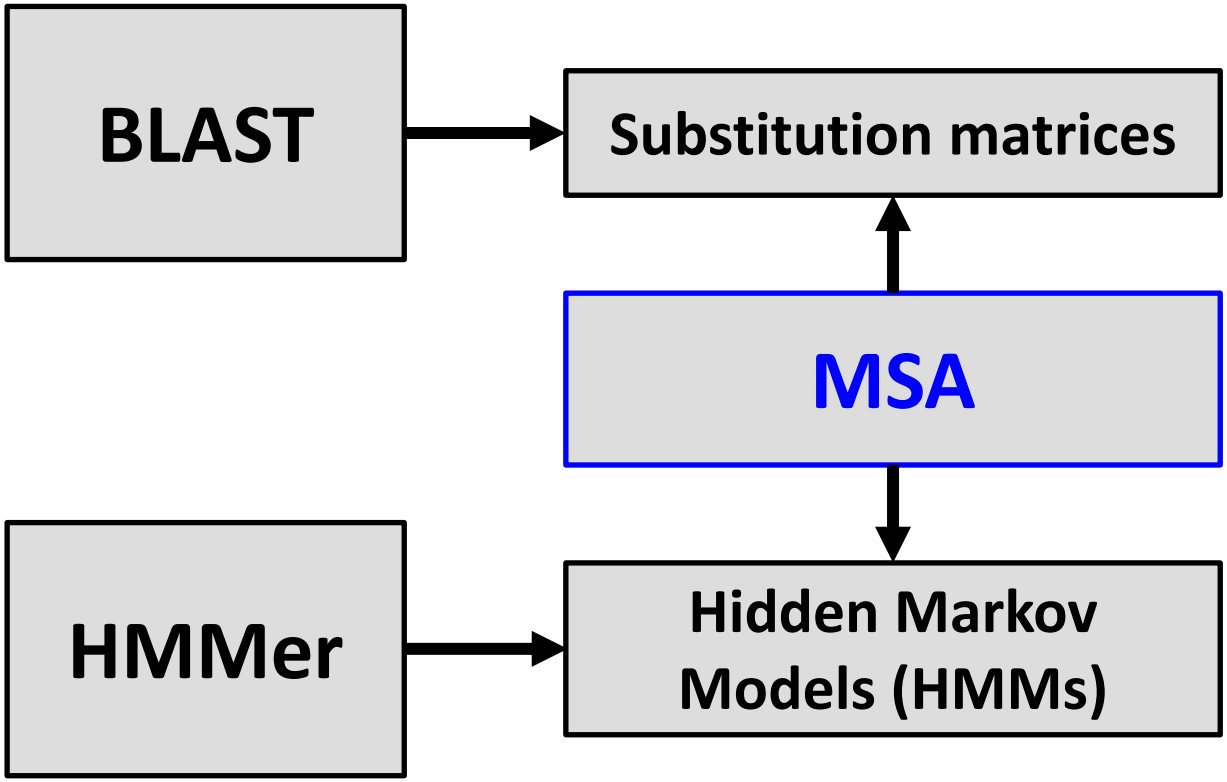
Course 2022-2023

Target and template



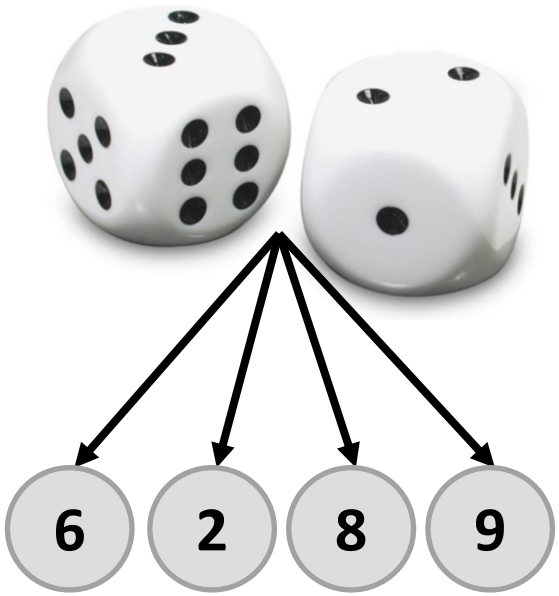
Hidden Markov Models and substitution matrices

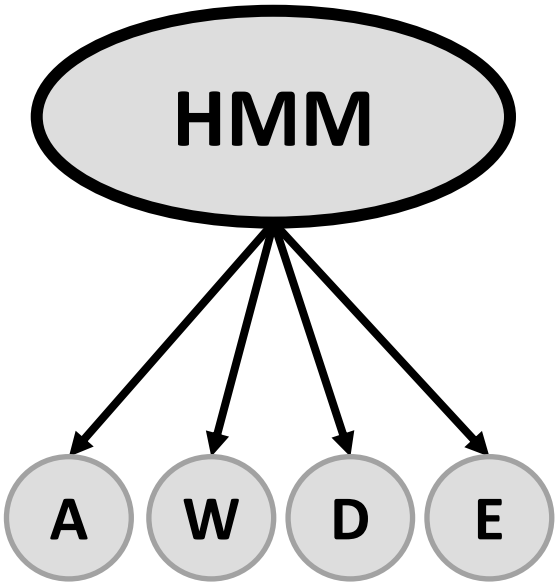
Hidden Markov Models (HMMs) are equivalent to substitution matrices



What is a HMM?

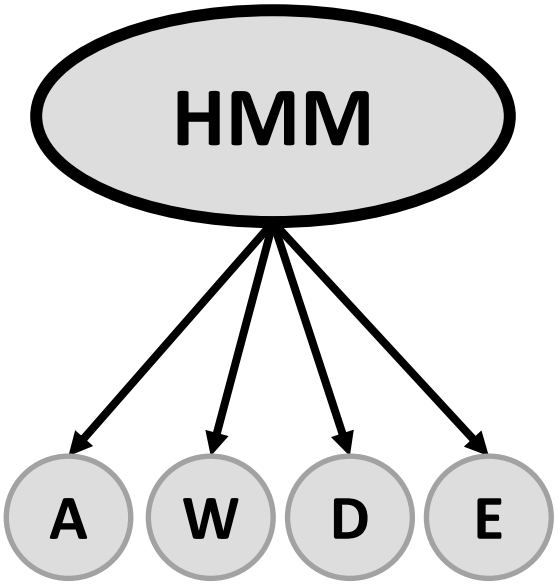
The same way that dice generate numbers, HMM generate amino acids





What is a HMM?

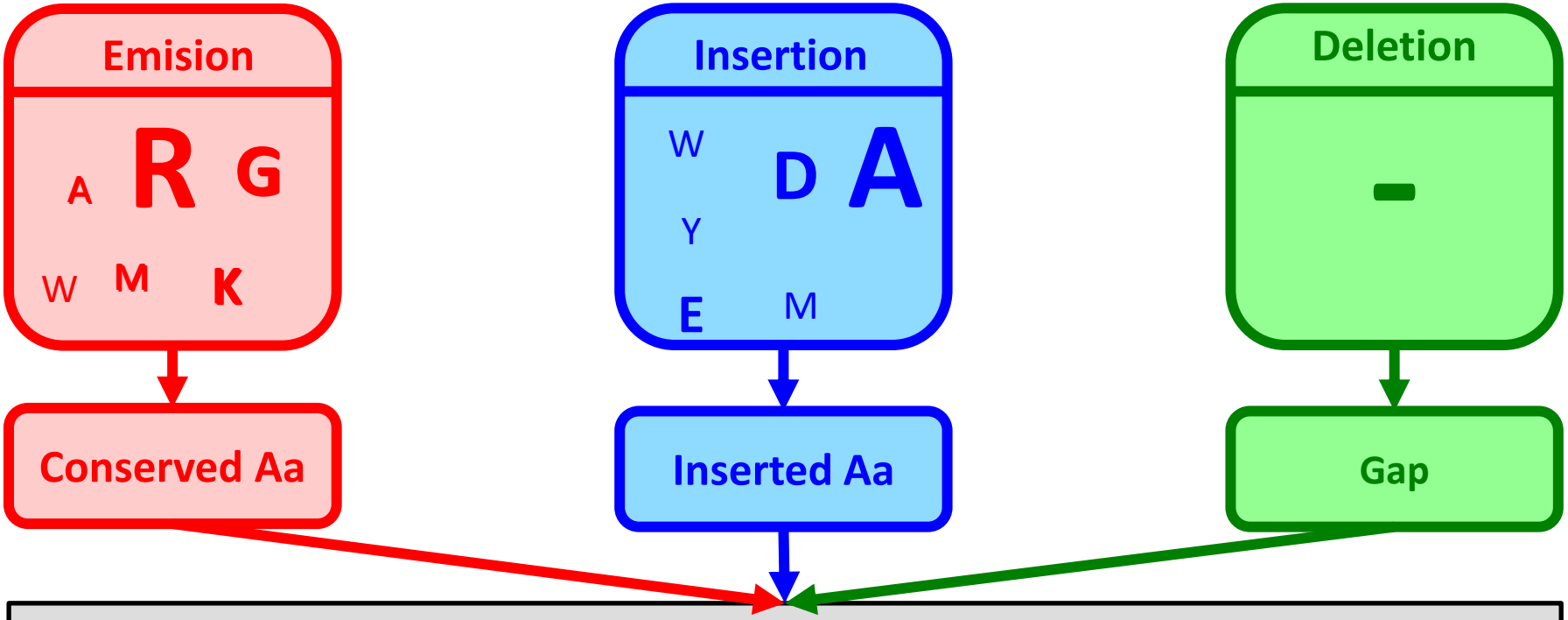
Each amino acid is produced with a specific probability contained inside the HMM



P(prot) = P(A) x P(W) x P(D) x P(E)

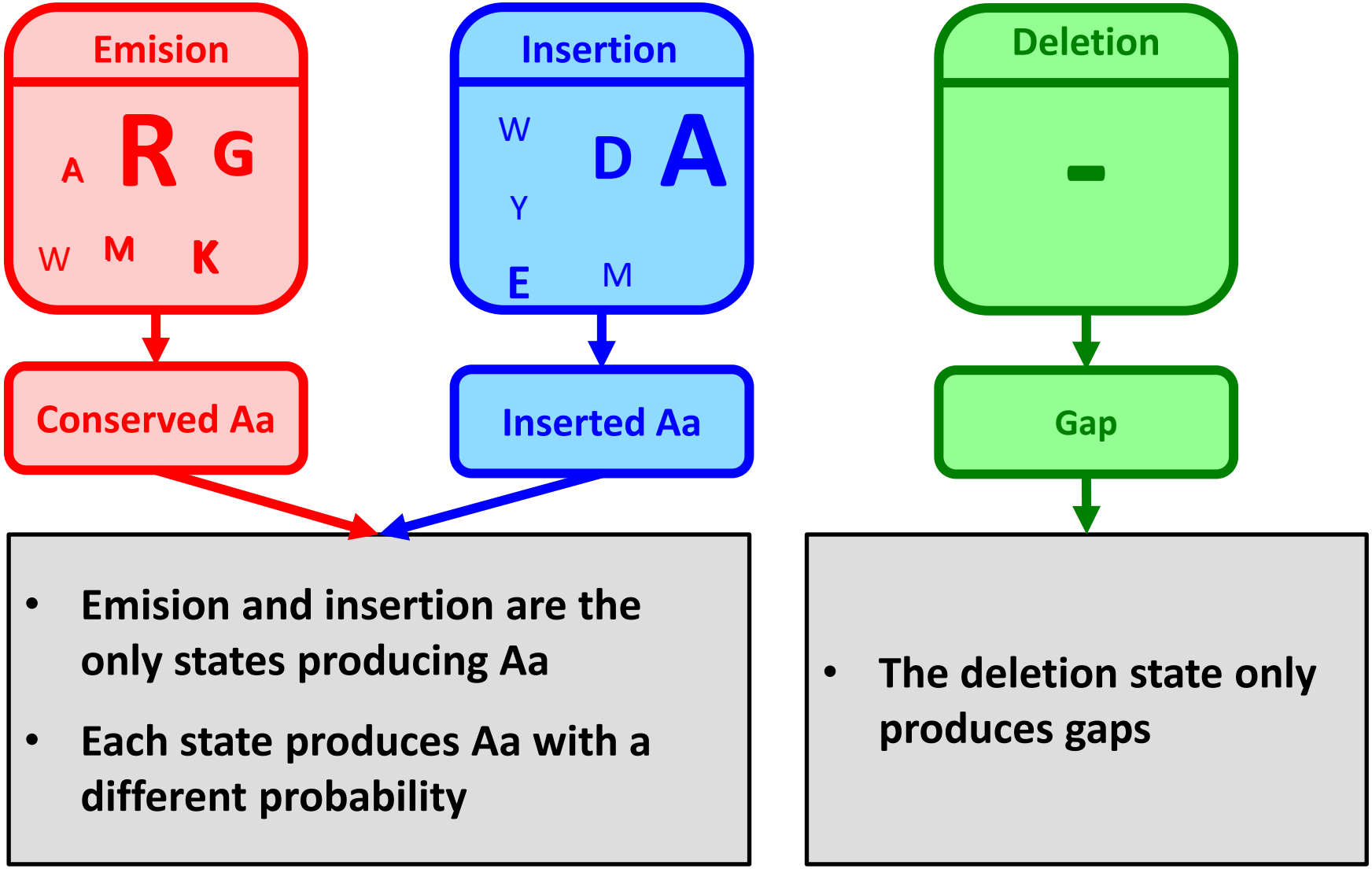
What is a HMM?

HMMs have states, each state has its own probabilities for producing amino acids



In reference with the sequences contained in the MSA used to create the HMM

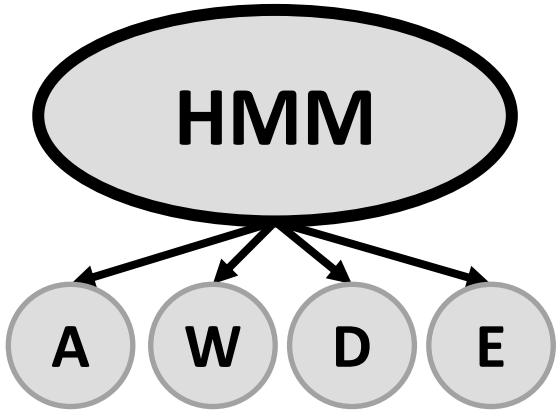
What is a HMM?



- Emision and insertion are the only states producing Aa
- Each state produces Aa with a different probability

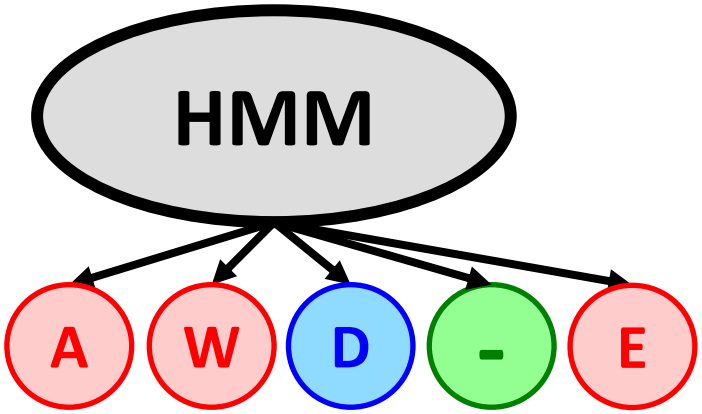
- The deletion state only produces gaps

What is a HMM?



$$P(prot) = P(A) \times P(W) \times P(D) \times P(E)$$

What is a HMM?

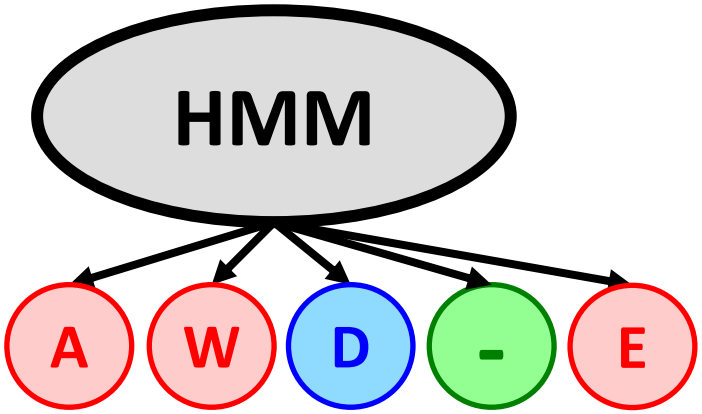


$$P(prot) = P_e(A) \times P_e(W) \times P_i(D) \times P_e(E)$$

What is a HMM?

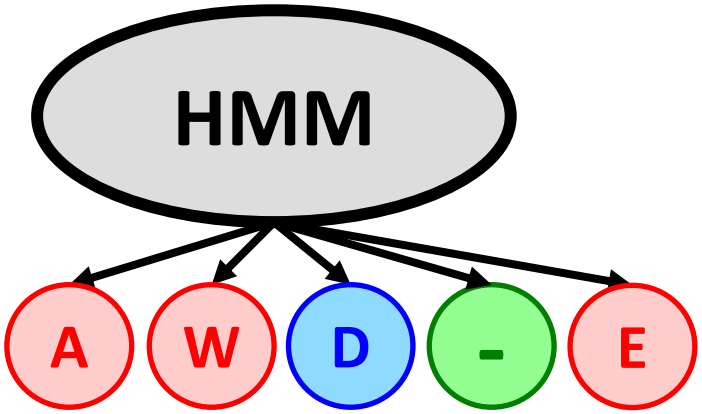
Transitions from state to state also depend on probabilities contained in the HMM

What is a HMM?



$$P(prot) = Pt(ee) \times P_e(A) \times Pt(ee) \times P_e(W) \times Pt(ei) \times P_i(D) \times Pt(id) \times Pt(de) \times P_e(E)$$

What is a HMM?



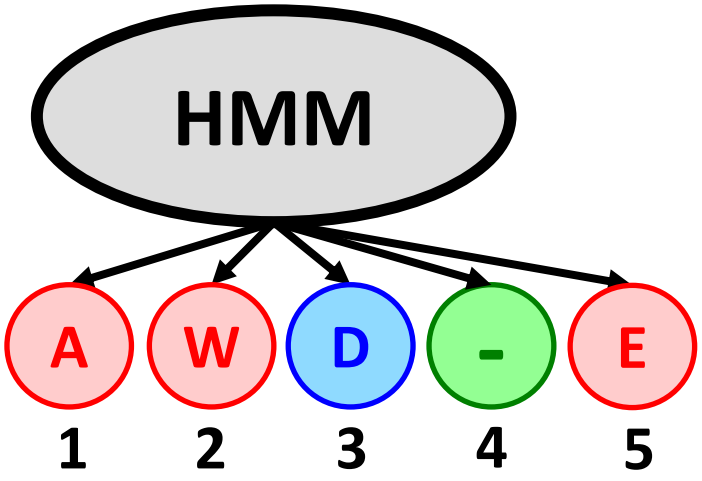
$$P(prot) = Pt(ee) \times P_e(A) \times Pt(ee) \times P_e(W) \times Pt(ei) \times P_i(D) \times Pt(id) \times Pt(de) \times P_e(E)$$

When the HMM introduces gaps in the sequence only considers the probability of moving inside the deletion state

What is a HMM?

All probabilities in a HMM are specific for each one of the positions in the HMM

What is a HMM?



$$P(prot) = P_{t1}(ee) \times P_{e1}(A) \times$$

$$P_{t2}(ee) \times P_{e2}(W) \times P_{t3}(ei) \times P_{i3}(D) \times$$

$$P_{t4}(id) \times P_{t5}(de) \times P_{e5}(E)$$

What is a HMM?

A HMM can create the same sequence using different state paths

Path 1: A W D - E

Path 2: A W - D E

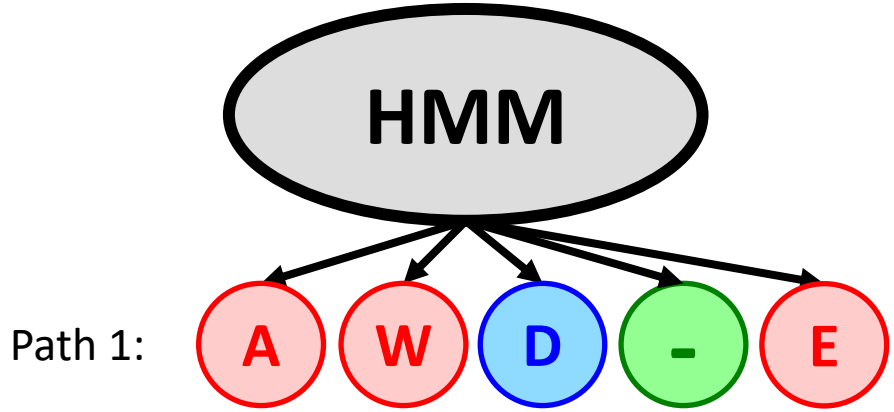
Path 3: A W - D E

Path 4: A W D E -

Etc…

What is a HMM?

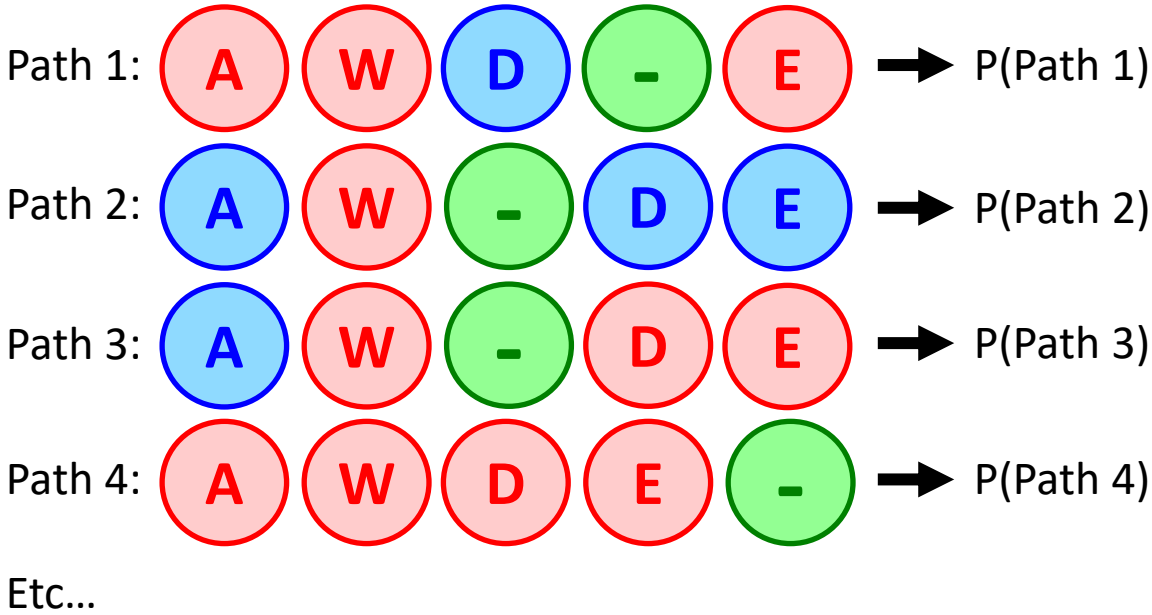
The probability of a HMM making one protein sequence through one specific path is the product of all the probabilities involved



P(path1) = P_{t1}(ee) x P_{e1}(A) x P_{t2}(ee) x P_{e2}(W) x P_{t3}(ei) x P_{i3}(D) x P_{t4}(id) x P_{t5}(de) x P_{e5}(E)

What is a HMM?

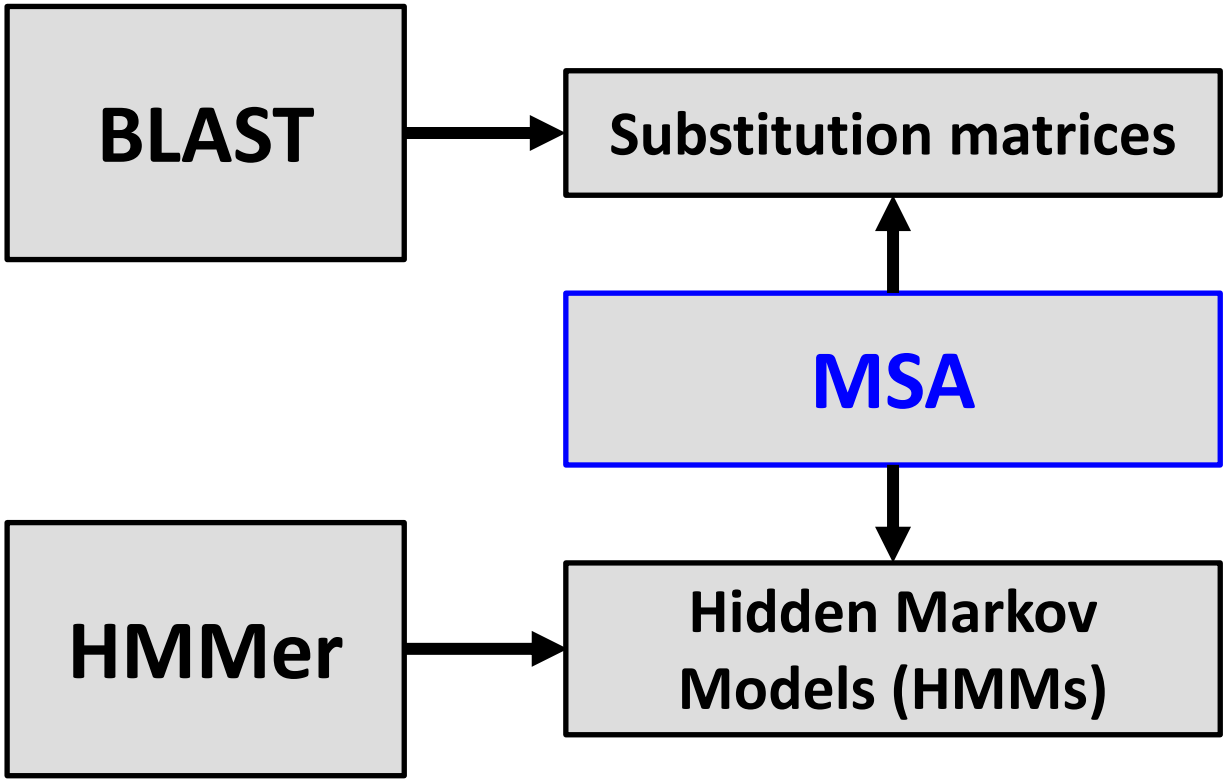
The probability of a HMM making one protein sequence through any path is the addition of the probabilities for each path



P(total) = P(path1) + P(path2) + P(path3) + P(path4) + Etc…

What is a HMM?

Hidden Markov Models (HMMs) are equivalent to substitution matrices



Create a HMM with hmmbuild

Create a HMM from a MSA using hmmbuild

Step 1: Creating a HMM using hmmbuild

To generate a HMM of a particular family of sequences we need a previous alignment of these sequences. This MSA, named seed, will be turn into a HMM by using the program hmmbuild. Here is an example of HMM usage:

- **hmmbuild [model_HMM] [alignment]**

The alignment has to be in STOCKHOLM format, like **globins4.sto**. You will find the required files in the folder HMMER within the directory of exercise_2. We run this as an example:

```
hmmbuild  globins4.hmm  globins4.sto
```

Create a HMM with hmmbuild

How does a HMM look from the inside?

```
HMMER3/f [3.1b2 | February 2015]
NAME  globins4
LENG  149
ALPH  amino
RF    no
MM    no
CONS  yes
CS    no
MAP   yes
DATE  Tue Jan  5 18:22:24 2021
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        I        K        L        M        N        P        Q
     R        S        T        V        W        Y
            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  2.90041  2.55332  2.35210  3.67329  3.19812  3.45595  3.16091
  3.07934  2.66722  2.85475  2.56965  4.55393  3.62921
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  3.29302  2.67763  2.69377  4.24712  2.90369  2.73719  3.18168
  2.89823  2.37879  2.77497  2.98431  4.58499  3.61525
          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  2.40482  3.29230  2.54324  3.63799  3.55099  3.93183  3.61602
  3.56580  2.71897  2.84104  1.67328  5.32720  4.10031      9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
          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  3.03401  2.75280  2.74783  3.65114  3.24714  2.62341  3.12082
  3.11124  2.79244  2.89355  1.88003  5.06315  3.77128     10 v - - -
```

Create a HMM with hmmbuild

How does a HMM look from the inside?

```
HMMER3/f [3.1b2 | February 2015]
NAME  globins4
LENG  149
ALPH  amino
RF    no
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DATE  Tue Jan  5 18:22:24 2021
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
```

General information

Probabilities

```
HMM          A        C        D        E        F        G        H        I        K        L        M        N        P        Q
  R        S        T        V        W        Y
           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  2.90041  2.55332  2.35210  3.67329  3.19812  3.45595  3.16091
  3.07934  2.66722  2.85475  2.56965  4.55393  3.62921
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  3.29302  2.67763  2.69377  4.24712  2.90369  2.73719  3.18168
  2.89823  2.37879  2.77497  2.98431  4.58499  3.61525
          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  2.40482  3.29230  2.54324  3.63799  3.55099  3.93183  3.61602
  3.56580  2.71897  2.84104  1.67328  5.32720  4.10031      9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
          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  3.03401  2.75280  2.74783  3.65114  3.24714  2.62341  3.12082
  3.11124  2.79244  2.89355  1.88003  5.06315  3.77128     10 v - - -
```

Create a HMM with hmmbuild

How does a HMM look from the inside?

```
HMM          A        C        D        E        F        G        H        I        K        L        M        N        P        Q
    R        S        T        V        W        Y
            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  2.90041  2.55332  2.35210  3.67329  3.19812  3.45595  3.16091
  3.07934  2.66722  2.85475  2.56965  4.55393  3.62921
          2.68640  4.42247  2.77497  2.73145  3.46376  2.40504  3.72516  3.29302  2.67763  2.69377  4.24712  2.90369  2.73719  3.18168
  2.89823  2.37879  2.77497  2.98431  4.58499  3.61525
          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  2.40482  3.29230  2.54324  3.63799  3.55099  3.93183  3.61602
  3.56580  2.71897  2.84104  1.67328  5.32720  4.10031      9 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
          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  3.03401  2.75280  2.74783  3.65114  3.24714  2.62341  3.12082
  3.11124  2.79244  2.89355  1.88003  5.06315  3.77128     10 v - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
      3   3.50771  4.88753  4.66754  4.31907  3.27776  4.35743  4.88268  2.50779  4.08449  0.57907  3.22569  4.56607  4.74802  4.37991
  4.20749  3.97946  3.79191  2.62059  5.25407  4.04279     11 L - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
          0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
      4   2.34080  4.28719  3.51550  3.22063  4.37406  3.06195  4.29366  3.74891  3.24370  3.47337  4.31943  3.39310  3.80273  3.56072
  3.55390  1.08280  2.00280  3.23325  5.72380  4.49519     12 s - - -
          2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
```

Create a HMM with hmmbuild

How does a HMM look from the inside?

Legend

```
HMM          A        C        D        E        F        G        H        I        K        L        M        N        P        Q
   R         S        T        V        W        Y
            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  2.90041  2.55332  2.35210  3.67329  3.19812  3.45595  3.16091
 3.07934  2.66722  2.85475  2.56965  4.55393  3.62921
                                    145  3.46376  2.40504  3.72516  3.29302  2.67763
                                   8499  3.61525
                                    577  0.18968  0.00000        *
     1   1.70038  4.17733  3.76164  3.36686  3.72281  3.29583  4.27570  2.40482  3.29230  2.54324  3.63799  3.55099  3.93183  3.61602
 3.56580  2.71897  2.84104  1.67328  5.32720  4.10031      9 v - - -
         2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
 2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
         0.03156  3.86736  4.58970  0.61958  0.77255  0.34406  1.23405
     2                                815  3.49607  2.75382  3.03401  2.75286
 3.11124                              7128     10 v - - -
                                      354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
 2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
         0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
     3   3.50771  4.88753  4.66754  4.31907  3.27776  4.35743  4.88268  2.50779  4.08449  0.57907  3.22569  4.56607  4.74802  4.37991
 4.20749  3.97946  3.79191  2.62059  5.25407  4.04279     11 L - - -
         2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
 2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
         0.02321  4.17053  4.89288  0.61958  0.77255  0.48576  0.95510
     4   2.34080  4.28719  3.51550  3.22063  4.37406  3.06195  4.29366  3.74891  3.24370  3.47337  4.31943  3.39310  3.80273  3.56072
 3.55390  1.08280  2.00280  3.23325  5.72380  4.49519     12 s - - -
         2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146
 2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
```

Position inside the HMM

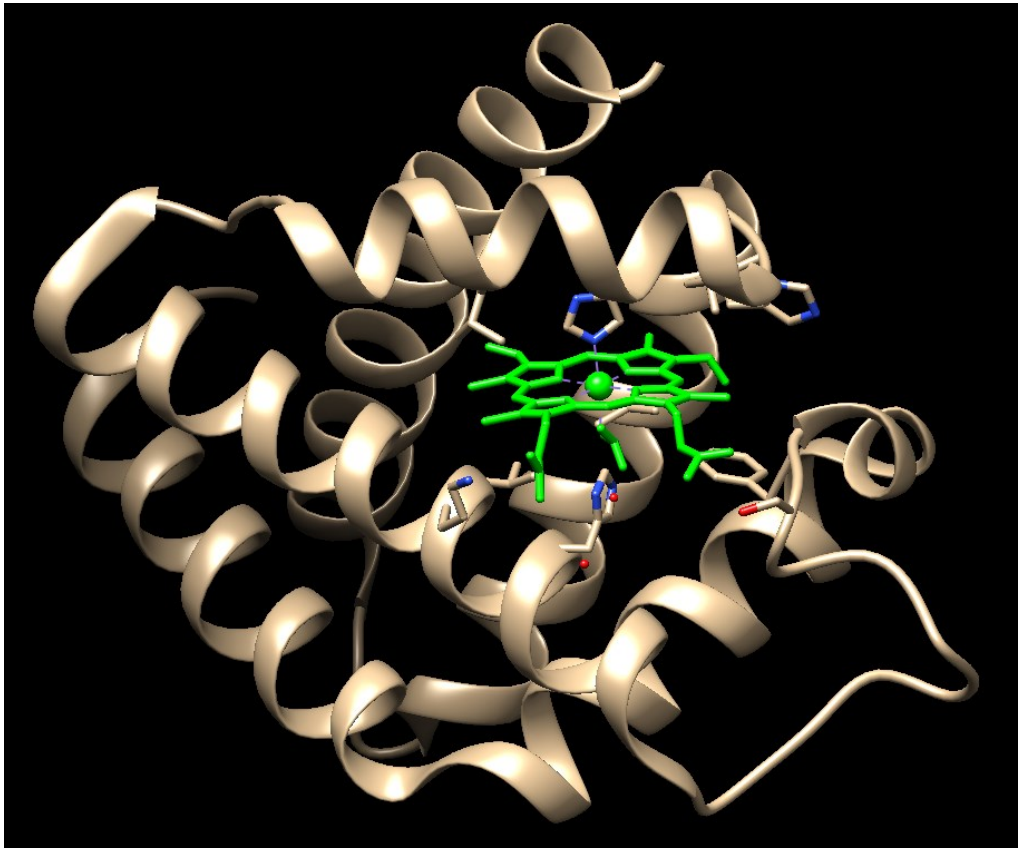
Probabilities of emission

Probabilities of transition

Probabilities of insertion

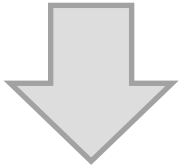
Create a HMM with hmmbuild

We created a HMM that is informative for the globin domain



Create a HMM with hmmbuild

It is common to use HMMs that are informative for specific protein domains



We can call them profiles

Find sequences using HMMs with hmmsearch

Search for templates using hmmsearch

```
hmmsearch  globins4.hmm  /mnt/NFS_UPF/soft/databases/blastdat/pdb_seq
> globins_pdb.out
```

hmmsearch finds proteins in a database that match a HMM

↓

Finds sequences that are likely to be produced by the input HMM

Find sequences using HMMs with hmmsearch

Take a look to the hmmsearch output

```
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 Description
    ------- ------ -----    ------- ------ -----   ---- --  -------- -----------
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2  1abw_A    mol:protein length:283  HEMOGLOBIN-BASED BLOOD SUBS
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2  1aby_A    mol:protein length:283  HEMOGLOBIN
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2  1c7c_A    mol:protein length:283  PROTEIN (DEOXYHEMOGLOBIN (A
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2  1o1p_A    mol:protein length:283  Hemoglobin Alpha chain
     5e-119  396.4   8.1    8.1e-59  201.0   0.9    2.0  2  1c7d_A    mol:protein length:284  PROTEIN (DEOXYHEMOGLOBIN (A
   8.2e-117  389.2   8.0    1.1e-57  197.3   0.9    2.0  2  1o1n_A    mol:protein length:285  Hemoglobin Alpha chain
   1.6e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2  1o1j_A    mol:protein length:283  Hemoglobin Alpha chain
   1.7e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2  1o1m_A    mol:protein length:285  Hemoglobin Alpha chain
   5.7e-114  379.9   7.0    3.4e-56  192.4   0.7    2.0  2  1o1l_A    mol:protein length:283  Hemoglobin Alpha chain
    1.4e-65  222.9   3.3    1.6e-65  222.7   3.3    1.0  1  1cp5_A    mol:protein length:154  PROTEIN (MYOGLOBIN)
```

Find sequences using HMMs with hmmsearch

Take a look to the hmmsearch output

```
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 Description
    ------- ------ -----    ------- ------ -----   ---- --   -------- -----------
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1abw_A    mol:protein length:283  HEMOGLOBIN-BASED BLOOD SUBS
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1aby_A    mol:protein length:283  HEMOGLOBIN
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1c7c_A    mol:protein length:283  PROTEIN (DEOXYHEMOGLOBIN (A
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1o1p_A    mol:protein length:283  Hemoglobin Alpha chain
     5e-119  396.4   8.1    8.1e-59  201.0   0.9    2.0  2   1c7d_A    mol:protein length:284  PROTEIN (DEOXYHEMOGLOBIN (A
   8.2e-117  389.2   8.0    1.1e-57  197.3   0.9    2.0  2   1o1n_A    mol:protein length:285  Hemoglobin Alpha chain
   1.6e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2   1o1j_A    mol:protein length:283  Hemoglobin Alpha chain
   1.7e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2   1o1m_A    mol:protein length:285  Hemoglobin Alpha chain
   5.7e-114  379.9   7.0    3.4e-56  192.4   0.7    2.0  2   1o1l_A    mol:protein length:283  Hemoglobin Alpha chain
    1.4e-65  222.9   3.3    1.6e-65  222.7   3.3    1.0  1   1cp5_A    mol:protein length:154  PROTEIN (MYOGLOBIN)
```

Why do we have different results for the full sequence and for the best domain?

Find sequences using HMMs with hmmsearch

Take a look to the hmmsearch output

```
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 Description
    ------- ------ -----    ------- ------ -----   ---- --   -------- -----------
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1abw_A    mol:protein length:283  HEMOGLOBIN-BASED BLOOD SUBS
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1aby_A    mol:protein length:283  HEMOGLOBIN
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1c7c_A    mol:protein length:283  PROTEIN (DEOXYHEMOGLOBIN (A
   4.9e-119  396.4   8.1      8e-59  201.0   0.9    2.0  2   1o1p_A    mol:protein length:283  Hemoglobin Alpha chain
     5e-119  396.4   8.1    8.1e-59  201.0   0.9    2.0  2   1c7d_A    mol:protein length:284  PROTEIN (DEOXYHEMOGLOBIN (A
   8.2e-117  389.2   8.0    1.1e-57  197.3   0.9    2.0  2   1o1n_A    mol:protein length:285  Hemoglobin Alpha chain
   1.6e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2   1o1j_A    mol:protein length:283  Hemoglobin Alpha chain
   1.7e-114  381.7   7.3    1.7e-56  193.4   0.7    2.0  2   1o1m_A    mol:protein length:285  Hemoglobin Alpha chain
   5.7e-114  379.9   7.0    3.4e-56  192.4   0.7    2.0  2   1o1l_A    mol:protein length:283  Hemoglobin Alpha chain
    1.4e-65  222.9   3.3    1.6e-65  222.7   3.3    1.0  1   1cp5_A    mol:protein length:154  PROTEIN (MYOGLOBIN)
```

Why do we have different results for the full sequence and for the best domain?

→

Proteins can have more than one domain

Find domains using HMMs with hmmsearch

Search for fibronectin type-3 domains in a protein sequence using hmmsearch

```
hmmbuild  fn3.hmm  fn3.sto
```

```
hmmsearch  fn3.hmm  7LESS_DROME.fa  >  fn3.out
```

hmmsearch finds regions in protein sequences that match a HMM

↓

Finds regions in the sequence that are likely to be produced by the input HMM

Find domains using HMMs with hmmsearch

Take a look to the hmmsearch output

```
Query:       fn3  [M=86]
Accession:   PF00041.13
Description: Fibronectin type III domain
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   Description
    ------- ------ -----    ------- ------ -----   ---- --  --------   -----------
    1.9e-57  178.0   0.4    1.2e-16   47.2   0.9    9.4  9  7LES_DROME  SEVENLESS PROTEIN (EC 2.7.1.112).

Domain annotation for each sequence (and alignments):
>> 7LES_DROME  SEVENLESS PROTEIN (EC 2.7.1.112).
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 ?   -1.3   0.0      0.17      0.17      61      74 ..     396     409 ..     395     411 .. 0.85
   2 !   40.7   0.0   1.3e-14   1.3e-14       2      84 ..     439     520 ..     437     521 .. 0.95
   3 !   14.4   0.0     2e-06     2e-06      13      85 ..     836     913 ..     826     914 .. 0.73
   4 !    5.1   0.0    0.0016    0.0016      10      36 ..    1209    1235 ..    1203    1259 .. 0.82
   5 !   24.3   0.0   1.7e-09   1.7e-09      14      80 ..    1313    1380 ..    1304    1386 .. 0.82
   6 ?    0.0   0.0     0.063     0.063      58      72 ..    1754    1768 ..    1739    1769 .. 0.89
   7 !   47.2   0.9   1.2e-16   1.2e-16       1      85 [.    1799    1890 ..    1799    1891 .. 0.91
   8 !   17.8   0.0   1.8e-07   1.8e-07       6      74 ..    1904    1966 ..    1901    1976 .. 0.90
   9 !   12.8   0.0   6.6e-06   6.6e-06       1      86 []    1993    2107 ..    1993    2107 .. 0.89
```

Find domains using HMMs with hmmsearch

Take a look to the hmmsearch output

List of hits

```
Query:       fn3  [M=86]
Accession:   PF00041.13
Description: Fibronectin type III domain
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   Description
    ------- ------ -----    ------- ------ -----   ---- --  --------   -----------
    1.9e-57  178.0   0.4    1.2e-16   47.2   0.9    9.4  9  7LES_DROME  SEVENLESS PROTEIN (EC 2.7.1.112).
```

Results per domain

```
Domain annotation for each sequence (and alignments):
>> 7LES_DROME  SEVENLESS PROTEIN (EC 2.7.1.112).
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 ?   -1.3   0.0      0.17      0.17      61      74 ..     396     409 ..     395     411 .. 0.85
   2 !   40.7   0.0   1.3e-14   1.3e-14       2      84 ..     439     520 ..     437     521 .. 0.95
   3 !   14.4   0.0     2e-06     2e-06      13      85 ..     836     913 ..     826     914 .. 0.73
   4 !    5.1   0.0    0.0016    0.0016      10      36 ..    1209    1235 ..    1203    1259 .. 0.82
   5 !   24.3   0.0   1.7e-09   1.7e-09      14      80 ..    1313    1380 ..    1304    1386 .. 0.82
   6 ?    0.0   0.0     0.063     0.063      58      72 ..    1754    1768 ..    1739    1769 .. 0.89
   7 !   47.2   0.9   1.2e-16   1.2e-16       1      85 [.    1799    1890 ..    1799    1891 .. 0.91
   8 !   17.8   0.0   1.8e-07   1.8e-07       6      74 ..    1904    1966 ..    1901    1976 .. 0.90
   9 !   12.8   0.0   6.6e-06   6.6e-06       1      86 []    1993    2107 ..    1993    2107 .. 0.89
```

Find domains using HMMs with hmmsearch

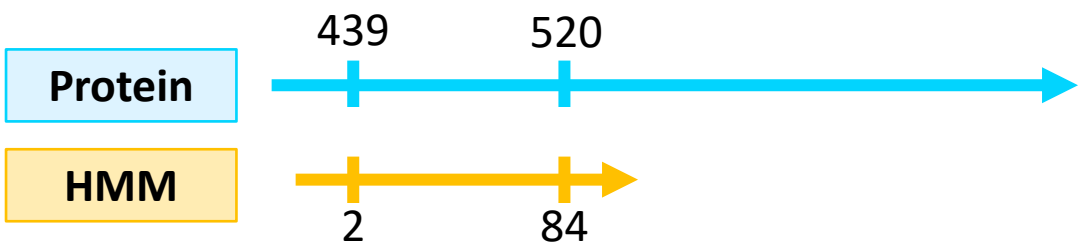
Take a look to the hmmsearch output
(Results per domain section)

```
Domain annotation for each sequence (and alignments):
>> 7LES_DROME  SEVENLESS PROTEIN (EC 2.7.1.112).
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 ?   -1.3   0.0      0.17      0.17      61      74 ..     396     409 ..     395     411 .. 0.85
   2 !   40.7   0.0   1.3e-14   1.3e-14       2      84 ..     439     520 ..     437     521 .. 0.95
   3 !   14.4   0.0     2e-06     2e-06      13      85 ..     836     913 ..     826     914 .. 0.73
   4 !    5.1   0.0    0.0016    0.0016      10      36 ..    1209    1235 ..    1203    1259 .. 0.82
   5 !   24.3   0.0   1.7e-09   1.7e-09      14      80 ..    1313    1380 ..    1304    1386 .. 0.82
   6 ?    0.0   0.0     0.063     0.063      58      72 ..    1754    1768 ..    1739    1769 .. 0.89
   7 !   47.2   0.9   1.2e-16   1.2e-16       1      85 [.    1799    1890 ..    1799    1891 .. 0.91
   8 !   17.8   0.0   1.8e-07   1.8e-07       6      74 ..    1904    1966 ..    1901    1976 .. 0.90
   9 !   12.8   0.0   6.6e-06   6.6e-06       1      86 []    1993    2107 ..    1993    2107 .. 0.89
```

Find domains using HMMs with hmmsearch

We can align HMMs with a protein sequence

hmmfrom	hmm to		alifrom	ali to
61	74	..	396	409
2	84	..	439	520
13	85	..	836	913
10	36	..	1209	1235
14	80	..	1313	1380
58	72	..	1754	1768
1	85	[.	1799	1890
6	74	..	1904	1966
1	86	[]	1993	2107



alifrom and ali to tell us where are the protein domains in our sequence

Find domains using HMMs with hmmsearch

hmmsearch shows the alignment between the HMM and each of the domains

We can align HMMs with a protein sequence

```
== domain 2  score: 40.7 bits;  conditional E-value: 1.3e-14
                 ---CEEEEEEECTTEEEEEEE--S--SS--SEEEEEEEETTTCCGCEEEEEETTTSEEEEES--TT-EEEEEEEEETTEE-E CS
       fn3    2 saPenlsvsevtstsltlsWsppkdgggpitgYeveyqekgegeewqevtvprtttsvtltgLepgteYefrVqavngagegp 84
                 saP   ++ +  ++ l ++W p +  +gpi+gY++++++++++  + e+ vp+   s+ +++L++gt+Y++ +  +n++gegp
7LES_DROME 439 SAPVIEHLMGLDDSHLAVHWHPGRFTNGPIEGYRLRLSSSEGNA-TSEQLVPAGRGSYIFSQLQAGTNYTLALSMINKQGEGP 520
                 78999999999*****************************9998.*********************************9997 PP
```

Find domains using HMMs with hmmsearch

We can align HMMs with a protein sequence

HMM

```
== domain 2  score: 40.7 bits;  conditional E-value: 1.3e-14
                 ---CEEEEEEECTTEEEEEEE--S--SS--SEEEEEEEETTTCCGCEEEEEETTTSEEEEES--TT-EEEEEEEEETTEE-E CS
      fn3    2 saPenlsvsevtstsltlsWsppkdgggpitgYeveyqekgegeewqevtvprtttsvtltgLepgteYefrVqavngagegp 84
                 saP   ++ +  ++ l ++W p +  +gpi+gY++++++++++  + e+ vp+   s+ +++L++gt+Y++ +  +n++gegp
7LES_DROME 439 SAPVIEHLMGLDDSHLAVHWHPGRFTNGPIEGYRLRLSSSEGNA-TSEQLVPAGRGSYIFSQLQAGTNYTLALSMINKQGEGP 520
               789999999999*****************************9998.*********************************9997 PP
```

Protein sequence

Alignment score

HMMs contain probabilities of producing Aa on each position:

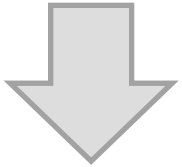
1. HMM position 1 aligns with Aa 1 in the protein sequence ➡ $score_1$
2. HMM position 2 aligns with Aa 2 in the protein sequence ➡ $score_2$

Etc…

Find HMMs that fit a sequence with hmmscan

There are several HMM databases on the internet

How can I know what HMM from a database fits my target sequence?



Using hmmscan

Find HMMs that fit a sequence with hmmscan

hmmscan finds what HMMs from a database match a protein sequence

- **What domains has my target sequence?**
- **Where are these domains in the sequence?**

Find HMMs that fit a sequence with hmmscan

Create a database of HMMs using hmmpress

```
hmmbuild  Pkinase.hmm  Pkinase.sto
```

Then, concatenate all the generated HMMs in one file:

```
cat  globins4.hmm  fn3.hmm  Pkinase.hmm  >  minifam
```

In order to check sequences and profiles very fast, we compress and index the database file using **hmmpress**. Here is a usage example:

- **hmmpress [database]**

We run then:

```
hmmpress  minifam
```

Find HMMs that fit a sequence with hmmscan

Execute hmmscan using this new database and the 7LES_DROME sequence

Now we can search what is the best profile for a given target sequence using the command hmmscan. Here is a usage example:

- **hmmscan (options) [Database_HMM] [sequence] > [output]**

For example we can use the sequence of 7LES_DROME to search for the best profile in the database previously generated. Run:

```
hmmscan  minifam  7LESS_DROME.fa  >  7LESS_DROME_minifam.out
```

Find HMMs that fit a sequence with hmmscan

Take a look to the hmmscan output

```
Query:       7LES_DROME  [L=2554]
Accession:   P13368
Description: SEVENLESS PROTEIN (EC 2.7.1.112).
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 type III domain
      3e-44  139.0   0.0    4.7e-44  138.3   0.0    1.3  1  Pkinase   Protein kinase domain
```

List of HMM matching our sequence

Find HMMs that fit a sequence with hmmscan

Take a look to the hmmscan output

Results per domain

```
Domain annotation for each model (and alignments):
>> fn3  Fibronectin type III domain
   #    score  bias  c-Value  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 ?   -1.3   0.0      0.33       0.5      61      74 ..     396     409 ..     395     411 .. 0.85
   2 !   40.7   0.0   2.6e-14   3.8e-14       2      84 ..     439     520 ..     437     521 .. 0.95
   3 !   14.4   0.0   4.1e-06   6.1e-06      13      85 ..     836     913 ..     826     914 .. 0.73
   4 !    5.1   0.0    0.0032    0.0048      10      36 ..    1209    1235 ..    1203    1259 .. 0.82
   5 !   24.3   0.0   3.4e-09     5e-09      14      80 ..    1313    1380 ..    1304    1386 .. 0.82
   6 ?    0.0   0.0      0.13      0.19      58      72 ..    1754    1768 ..    1739    1769 .. 0.89
   7 !   47.2   0.9   2.3e-16   3.5e-16       1      85 [.    1799    1890 ..    1799    1891 .. 0.91
   8 !   17.8   0.0   3.7e-07   5.5e-07       6      74 ..    1904    1966 ..    1901    1976 .. 0.90
   9 !   12.8   0.0   1.3e-05     2e-05       1      86 []    1993    2107 ..    1993    2107 .. 0.89
```

We already saw the results for the fibronectin type 3 domain

Find HMMs that fit a sequence with hmmscan

Take a look to the hmmscan output

Results per domain

```
>> Pkinase  Protein kinase domain
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  138.3   0.0   3.1e-44   4.7e-44       2     256 ..    2210    2479 ..    2209    2482 .. 0.85
```

Find HMMs that fit a sequence with hmmscan

Take a look to the hmmscan output

Alignment between HMM and domains

```
Alignments for each domain:
== domain 1  score: 138.3 bits;  conditional E-value: 3.1e-44
                 EEEEEEEEETTEEEEEEEETTTTE....EEEEEEEEHHHCCCCCCHHHHHHHHHHHHHSSSSB--EEEEEEETTEEEEEEE--TS-BHHHHHH.... CS
   Pkinase     2 elleklGsGsfGkVykakkkktgk....kvAvKilkkeeekskkektavrElkilkklsHpnivkllevfetkdelylvleyveggdlfdllk.... 90
                 +ll+ lGsG+fG+Vy+++ k++ +    +vA+K l+k  ++  +     +E++++ +++H+niv+l++++ + +++ l++e++e+gdl ++l+
7LES_DROME 2210 KLLRFLGSGAFGEVYEGQLKTEDSeepqRVAIKSLRKGASEFAELL---QEAQLMSNFKHENIVRLVGICFDTESISLIMEHMEAGDLLSYLRaara 2303
                 67899********88776655444444*******9998887764...4*****************************************9988 PP

                 .......HHHST-HHHHHHHHHHHHHHHHHHHHTTEE-S--SGGGEEEETTTEE.......EE--GTT.E..EECSS-C-S--S..-GGGS-HHHHC CS
   Pkinase    91 .......kegklseeeikkialqilegleylHsngiiHrDLKpeNiLldkkgev.......kiaDFGlakklekssekltlvg..treYmAPEvll 171
                            ls  e+  ++  +++g +yl +++++HrDL   N+L++++          ki DFGla+ ++ks+   ++  g    ++m+PE l
7LES_DROME 2304 tstqepqPTAGLSLSELLAMCIDVANGCSYLEDMHFVHRDLACRNCLVTESTGStdrrrtvKIGDFGLARDIYKSDYYRKEGEGllPVRWMSPESLV 2400
                 887766555666*************************************9554445999************988887777766622679******** PP

                 CS-CTHHHHHHHHHHHHHHHHH.SS-TTSSSHHCCTHHHHSSHHH......TTS.....HHHHHHHHHHT-SSGGGSTTHHHHHT CS
   Pkinase   172 kakeytkkvDvWslGvilyellt.gklpfsgeseedqleliekilkkkleedepkssskseelkdlikkllekdpakRltaeeilk 256
                  +   t+++DvW++Gv+++e+lt g+ p+  +   ++ e++++++++   ++ p  ++  e+l +l+  ++++dp +R++++++++
7LES_DROME 2401 -DGLFTTQSDVWAFGVLCWEILTlGQQPYAAR---NNFEVLAHVKEGGRLQQ-PPMCT--EKLYSLLLLCWRTDPWERPSFRRCYN 2479
                 .9999***************999899999999...55666655555443333.33344..89*******************99887 PP
```

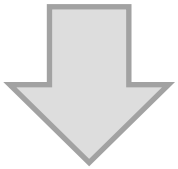
Comparing hmmsearch and hmmscan

The outputs of hmmsearch and hmmscan have the same organization

	hmmsearch	hmmscan
List of hits	Hits are protein sequences that fit the input HMM	Hits are HMMs that fit the input protein sequence
Results per domain	No difference	No difference
Alignments between HMMs and domains	No difference	No difference

Make MSAs with hmmalign

HMMs are more versatile tan substitution matrices



We can use HMMs to make MSAs

Make MSAs with hmmalign

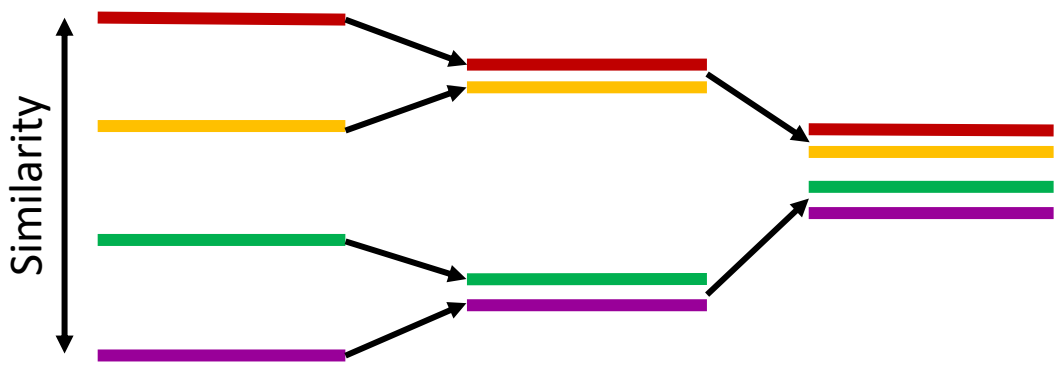
HMMs are better than agglomerative methods to make MSAs like clustalw

- **The alignment is made according with the specific information of the HMM**
- **The alignment is made faster**

Make MSAs with hmmalign

HMMs make alignments faster than clustalw

Clustalw

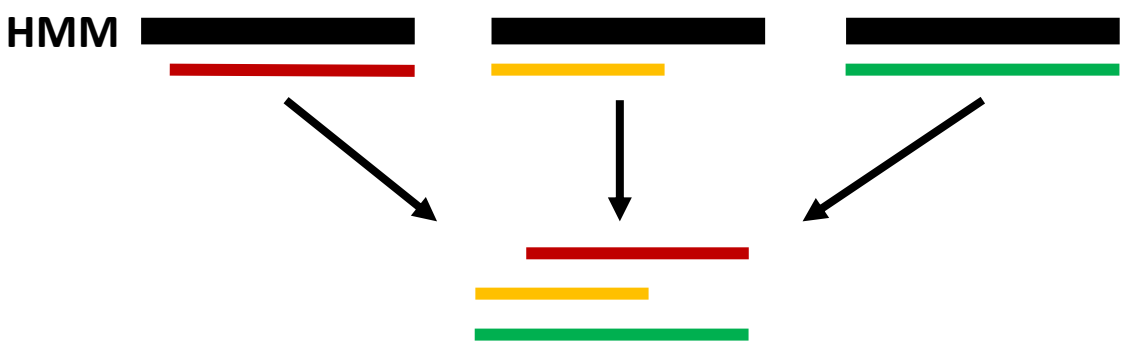


This takes a lot of time for a large number of sequences

Make MSAs with hmmalign

HMMs make alignments faster than clustalw

HMMs



Only one alignment per sequence

Make MSAs with hmmalign

Use hmmalign to make a MSA with globin sequences

- **hmmalign [model_HMM] [file_with_sequences] > [output]**

We can show this with the file globins45.fa. Run the following commands and test the speed of both approaches, hmmalign and clustalw:

```
hmmalign  globins4.hmm  globins45.fa  >  globins45_hmm.sto
```

```
clustalw  globins45.fa
```

Make MSAs with hmmalign

Change the format of the output MSA

```
perl /mnt/NFS_UPF/soft/perl-lib/aconvertMod2.pl -in h -out c
<globins45_hmm.sto>globins45_hmm.clu
```

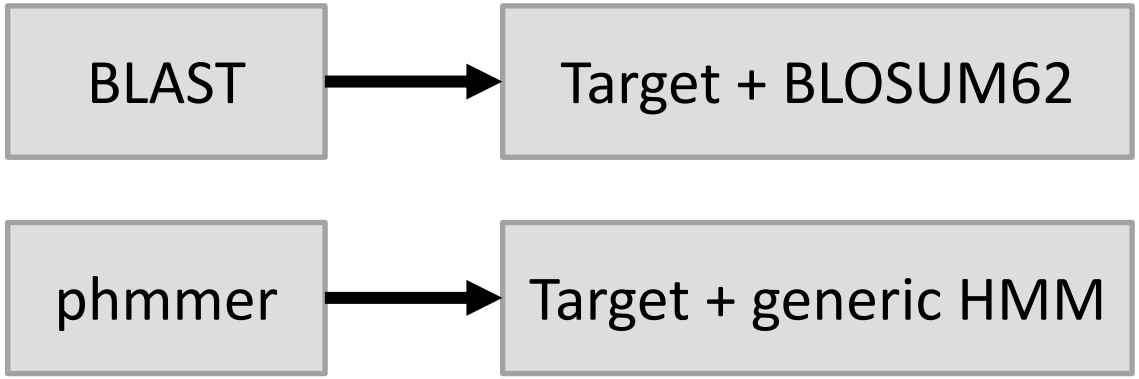
Find homologous proteins with phmmer and jackhmmer

How can I find templates for my target using HMMs?

- **Using a HMM from the domain of my target**
- **Using phmmer or jackhmmer**

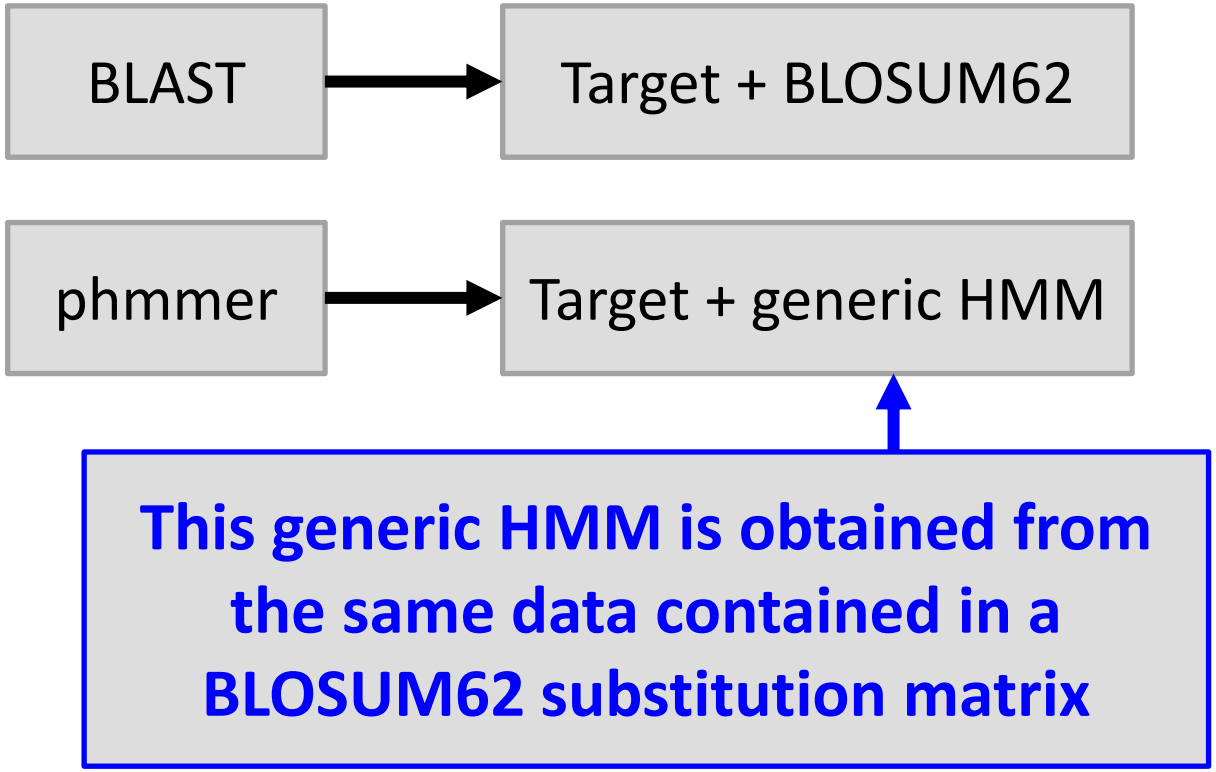
Find homologous proteins with phmmer and jackhmmer

phmmer is similar to BLAST



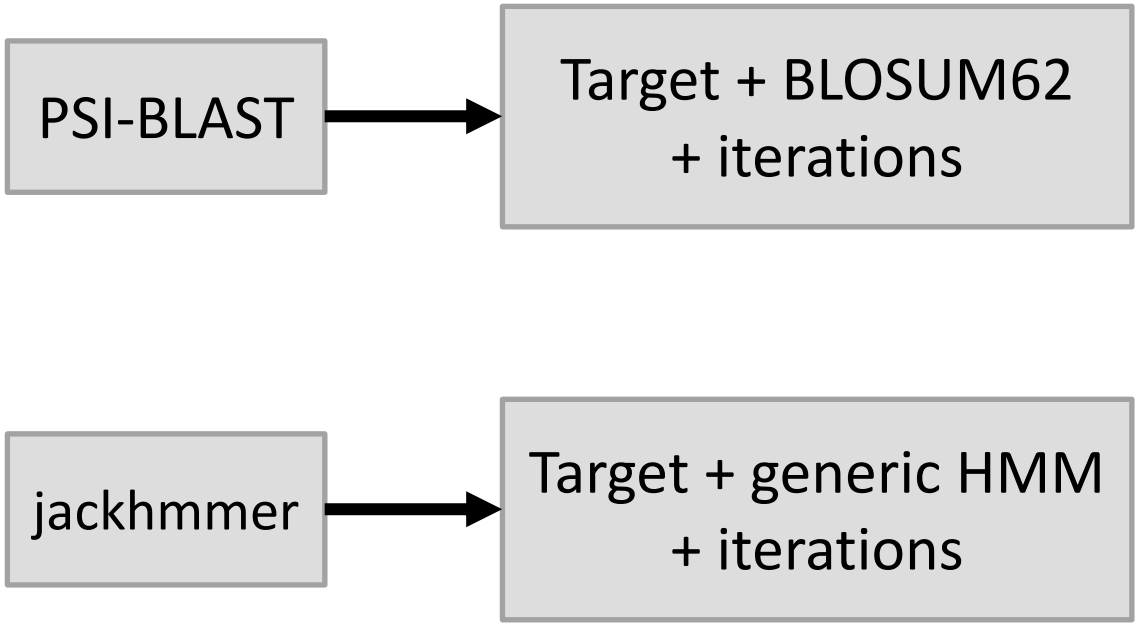
Find homologous proteins with phmmer and jackhmmer

phmmer is similar to BLAST



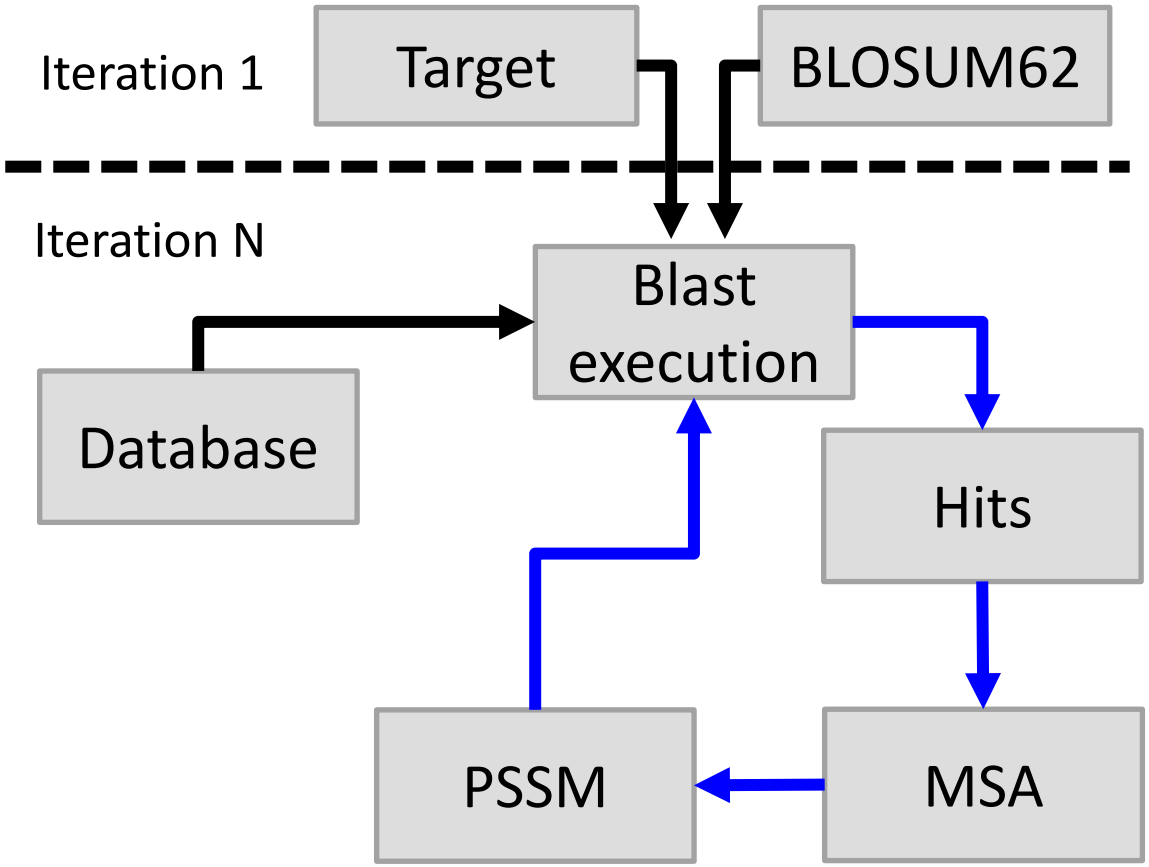
Find homologous proteins with phmmer and jackhmmer

jackhmmer is similar to PSI-BLAST



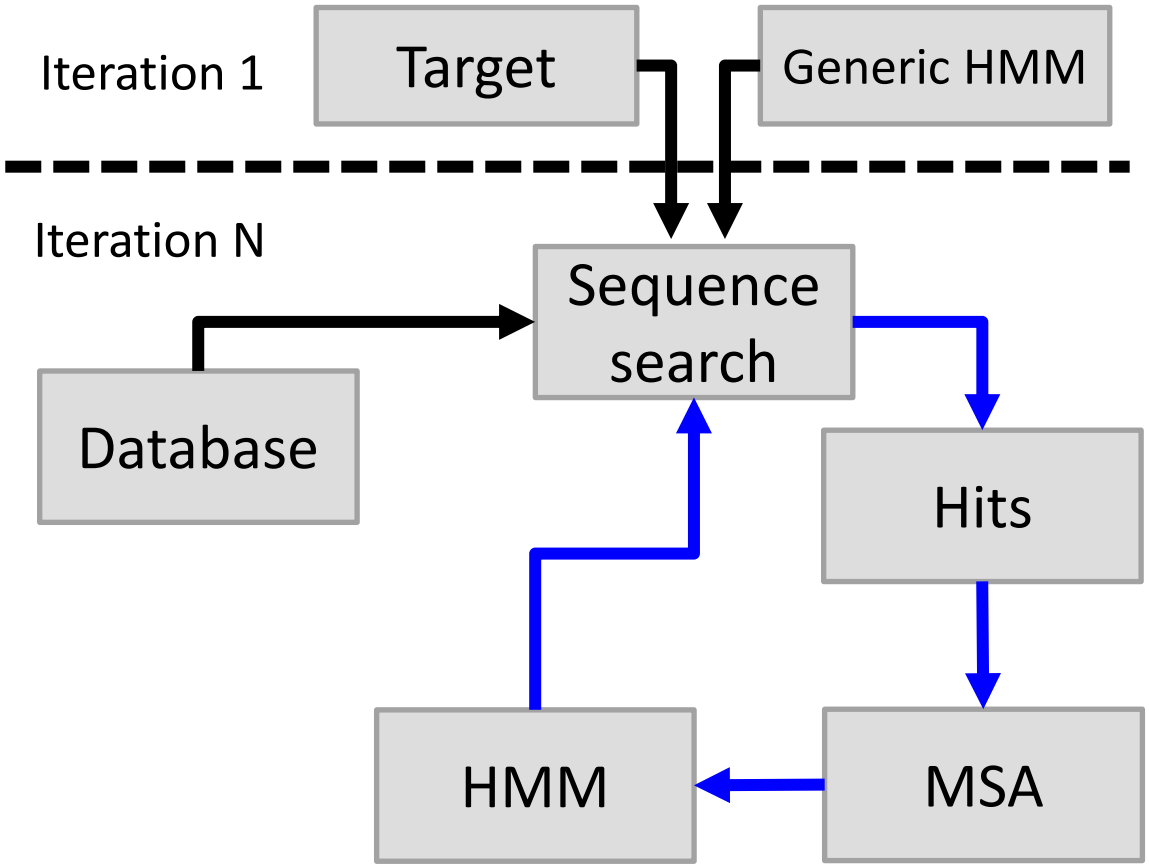
Find homologous proteins with phmmer and jackhmmer

PSI-BLAST creates a new PSSM at each iteration



Find homologous proteins with phmmer and jackhmmer

Jackhmmer creates a new HMM at each iteration



Find homologous proteins with phmmer and jackhmmer

Execute phmmer and jackhmmer and compare the results

```
jackhmmer  hbb_human globins45.fa  >  globins_jackhmmer.out
```

```
phmmer  hbb_human  globins45.fa  >  globins_phmmer.out
```

Using PFAM

PFAM is an extense and reliable database of HMMs

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Pfam 33.1 (May 2020, 18259 entries)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. More...

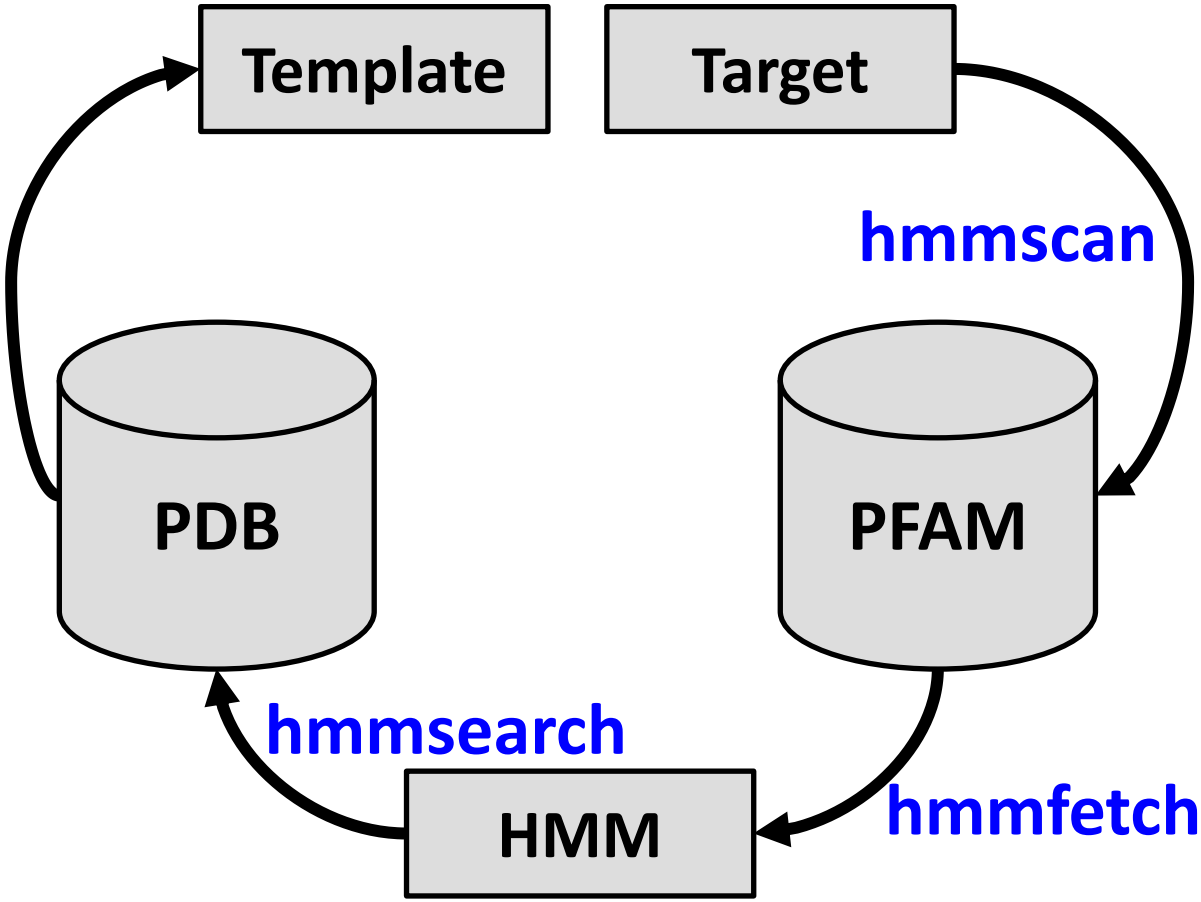
QUICK LINKS	YOU CAN FIND DATA IN PFAM IN VARIOUS WAYS...
SEQUENCE SEARCH	Analyze your protein sequence for Pfam matches
VIEW A PFAM ENTRY	View Pfam annotation and alignments
VIEW A CLAN	See groups of related entries
VIEW A SEQUENCE	Look at the domain organisation of a protein sequence
VIEW A STRUCTURE	Find the domains on a PDB structure
KEYWORD SEARCH	Query Pfam by keywords
JUMP TO	enter any accession or ID [Go] [Example]

Enter any type of accession or ID to jump to the page for a Pfam entry or clan, UniProt sequence, PDB structure, etc.

Or view the help pages for more information

Using PFAM

How to use PFAM to find templates for our target?



Using PFAM

How to use PFAM to find templates for our target?

3 programs involved:

- **hmmscan:** finds what HMMs from a database match the input sequence
- **hmmfetch:** extracts a HMM from a database
- **hmmsearch:** finds what sequences from a database match the input HMM

Using PFAM

Execute hmmscan on the pfam database

```
hmmscan  /mnt/NFS_UPF/soft/databases/pfam-3/Pfam-A.hmm  hbb_human.fa
> hb_human_db.out
```

Using PFAM

Take a look to the hmmscan output

```
Query:       HBB_HUMAN  [L=146]
Description: Human beta hemoglobin.
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
    ------- ------ -----    ------- ------ -----   ---- --  --------     -----------
    6.1e-30  103.6   0.0    4.5e-29  100.8   0.0    1.9  2  Globin        Globin
  ------ inclusion threshold ------
       0.12   11.9   0.7       0.35   10.4   0.0    2.1  2  BCA_ABC_TP_C  Branched-chain amino acid ATP-binding cassette


Domain annotation for each model (and alignments):
>> Globin  Globin
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  100.8   0.0   6.6e-33   4.5e-29       1     108 []       7     111 ..       7     111 .. 0.98
   2 ?    0.7   0.1     0.085   5.8e+02      52      72 ..     123     143 ..     116     145 .. 0.81
```

Using PFAM

Execute hmmfetch to extract the HMM we want from the PFAM database

- **hmmfetch [database_HMM] [name_HMM] > [file_HMM]**

Therefore, in our example, assuming we have found a domain_target:

Step 6.2) extract the profile(s) from PFAM that correspond to the domains of the target sequence which are found in the column indicated as "model" (see example in step 3 of this tutorial). Let's assume the name of the model we have found for hbb_human is "domain_hbb", then we execute the command:

```
hmmfetch  /mnt/NFS_UPF/soft/databases/pfam-3/Pfam-A.hmm  "domain_hbb"
> domain_hbb.hmm
```

Is this the name of the HMM that we want to get?

Using PFAM

Take a look to the hmmscan output

```
Query:       HBB_HUMAN  [L=146]
Description: Human beta hemoglobin.
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
    ------- ------ -----    ------- ------ -----   ---- -- --------      -----------
    6.1e-30  103.6   0.0    4.5e-29  100.8   0.0    1.9  2  Globin        Globin
  ------ inclusion threshold ------
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 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  100.8   0.0   6.6e-33   4.5e-29       1     108 []       7     111 ..       7     111 .. 0.98
   2 ?    0.7   0.1     0.085   5.8e+02      52      72 ..     123     143 ..     116     145 .. 0.81
```

Using PFAM

Execute hmmfetch to extract the HMM we want from the PFAM database

- **hmmfetch [database_HMM] [name_HMM] > [file_HMM]**

Therefore, in our example, assuming we have found a domain_target:

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```
hmmfetch  /mnt/NFS_UPF/soft/databases/pfam-3/Pfam-A.hmm
> domain_hbb.hmm
```

Globin

Using PFAM

Is this globins HMM different to the one we used at the beggining of the practice?

Using PFAM

Is this globins HMM different to the one we used at the beggining of the practice?

YES:

- **HMMs from the PFAM database are manually curated and very reliable**
- **The two HMMs are made with a different number of sequences**

Using PFAM

Execute hmmsearch to search for proteins containing the globin domain in the PDB

Step 6.3) Search for sequences with known structure that contain the same domain as our target using **hmmsearch**:

```
hmmsearch  domain_hbb.hmm  /mnt/NFS_UPF/soft/databases/blastdat/pdb_seq
> hbb_pdb_by_HMM.out
```

Programs from practical 1

BLAST	Find homologous sequences to a target
PSI-BLAST	Find homologous sequences to a target using iterations
clustalw	Make MSAs

Programs from practical 2

hmmbuild	Create HMMs from MSAs
hmmsearch	Find matches of a HMM in a database of sequences
hmmscan	Find matches of a sequence in a database of HMMs
hmmpress	Build a database of HMMs
hmmalign	Make MSAs using a HMM
phmmer	Find homologous sequences to a target
jackhmmer	Find homologous sequences to a target using iterations
hmmfetch	Extract a HMM from a database
aconvertMod2.pl	Change the format of MSAs

Databases from practicals 1 and 2

PDB

- Proteins with available structure
- Biased
- Redundant

SCOP

- Classification of protein structures (from the PDB) into domains

PFAM

- HMMs for protein domains

Uniprot (AKA SwissProt)

- Proteins with available sequence
- Non-biased
- Non-redundant

Exercises

You can try the exercises before the syncronic class

QUESTIONS FROM THE TUTORIAL

1) Compare the results of phmmer, jackhmmer with the results of hmmsearch using "domain_hbb.hmm" (see hbb_pdb_by_HMM.out) when searching homologs in pdb_seq for hbb_human.

2) If a protein sequence has more than one domain in PFAM, do you think the result of using hmmsearch and jackhmmer will be the same? Why? Test the example with 7LES_DROME in SwissProt.

3) In practice 2.1 we used PSI-BLAST to fish sequences in the database uniprot_sprot.fasta and generate a PSSM profile which was used for searching homologs in PDB. Check the manual of HMMER3.0 and create your own protocol in which you use the program jackhmmer in a similar approach: use SwissProt database to generate the HMM profile and perform the search in pdb_seq.

4) Use hmmscan to search the best model(s) for 7LES_DROME in PFAM and search the homologs in PDB with this/these model(s). Compare the results of this search with the results of your protocol search in question 3. What are the differences? Why?

5) Use your protocol described in question 3 to search homologs of 7LES_DROME in PDB and compare with the results of the protocol described in practice 2.1 when using PSI-BLAST.

6) Use the sequence target.fa from practice 2.1. Apply phmmer, jackhmmer and the protocols of questions 3 and 4 to find homologs in PDB. What's the fold of this sequence? Compare the result with the homologs found in practice 2.1

7) Use hmmalign and FetchFasta.pl to align the sequence of target.fa and its homologs of PDB

8) If you have to align the sequence 7LES_DROME and its homologs of PDB what's the best model to use? Produce the alignment with the models from question 4 and your protocol in question 3 to show your answer.

9) What are the folds of the following sequences?

   a. *problem1/serc_myctu.fa*

   b. *problem2/p72_mycmy.fa*

   c. *problem3/lip_staau.fa*

   d. *problem4/orc1_human.fa*

10) Find what are all the domains in the sequence 7LES_DROME. If you wanted to find templates for its Pkinase domain, what HMM would you choose and why?

Exercises

Exercise 10

```
Query:       7LES_DROME  [L=2554]
Accession:   P13368
Description: SEVENLESS PROTEIN (EC 2.7.1.112).
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
    ------- ------ -----    ------- ------ -----   ---- --  --------       -----------
    9.5e-92  306.6   0.0    1.6e-91  305.9   0.0    1.4  1  Pkinase_Tyr     Protein tyrosine kinase
    8.1e-52  173.1   0.6    3.9e-12   46.0   0.8    9.5  9  fn3             Fibronectin type III domain
    1.2e-40  139.2   0.0    1.8e-40  138.6   0.0    1.3  1  Pkinase         Protein kinase domain
     0.0047   16.8   0.0       0.17   11.7   0.0    3.5  4  Interfer-bind   Interferon-alpha/beta receptor, fibronectin
  ------ inclusion threshold ------
      0.054   13.4   0.1       0.24   11.3   0.1    2.1  2  CarboxypepD_reg Carboxypeptidase regulatory-like domain
```

Exercises

Exercise 10

```
Query:       7LES_DROME  [L=2554]
Accession:   P13368
Description: SEVENLESS PROTEIN (EC 2.7.1.112).
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
    ------- ------ -----    ------- ------ -----   ---- --  --------       -----------
    9.5e-92  306.6   0.0    1.6e-91  305.9   0.0    1.4  1  Pkinase_Tyr     Protein tyrosine kinase
    8.1e-52  173.1   0.6    3.9e-12   46.0   0.8    9.5  9  fn3             Fibronectin type III domain
    1.2e-40  139.2   0.0    1.8e-40  138.6   0.0    1.3  1  Pkinase         Protein kinase domain
     0.0047   16.8   0.0       0.17   11.7   0.0    3.5  4  Interfer-bind   Interferon-alpha/beta receptor, fibronectin
  ------ inclusion threshold ------
      0.054   13.4   0.1       0.24   11.3   0.1    2.1  2  CarboxypepD_reg Carboxypeptidase regulatory-like domain
```

Exercises

Exercise 10

```
>> fn3  Fibronectin type III domain
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 ?   -1.0   0.0      0.64   1.8e+03      60      73 ..     396     409 ..     395     411 .. 0.85
   2 !   40.9   0.0   5.5e-14   1.5e-10       1      83 [.     439     520 ..     439     521 .. 0.95
   3 !   14.4   0.0   9.8e-06     0.027      14      83 ..     838     912 ..     827     914 .. 0.72
   4 !    4.9   0.0    0.0094        26      10      35 ..    1210    1235 ..    1203    1259 .. 0.81
   5 !   23.4   0.0   1.5e-08   4.2e-05      13      79 ..    1313    1380 ..    1306    1385 .. 0.81
   6 ?    0.3   0.0      0.26   7.2e+02      57      72 ..    1754    1769 ..    1736    1769 .. 0.89
   7 !   46.0   0.8   1.4e-15   3.9e-12       1      84 [.    1800    1890 ..    1800    1891 .. 0.91
   8 !   18.0   0.0   7.4e-07     0.002       5      73 ..    1904    1966 ..    1901    1976 .. 0.90
   9 !    9.8   0.0   0.00027      0.73       1      85 []    1994    2107 ..    1994    2107 .. 0.87
```

Exercises

Exercise 10

```
>> Pkinase_Tyr  Protein tyrosine kinase
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  305.9   0.0   5.8e-95   1.6e-91       1     259 []    2209    2481 ..    2209    2481 .. 0.97
```

```
>> Pkinase  Protein kinase domain
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  138.6   0.0   6.7e-44   1.8e-40       2     256 ..    2210    2479 ..    2209    2482 .. 0.85
```

Why do we have two matches with HMMs that are informative for the Pkinase domain?

Exercises

Exercise 10

```
>> Pkinase_Tyr  Protein tyrosine kinase
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  305.9   0.0   5.8e-95   1.6e-91       1     259 []    2209    2481 ..    2209    2481 .. 0.97
```

```
>> Pkinase  Protein kinase domain
   #    score  bias  c-Evalue  i-Evalue hmmfrom  hmm to    alifrom  ali to    envfrom  env to     acc
 ---   ------ ----- --------- --------- ------- -------    ------- -------    ------- -------    ----
   1 !  138.6   0.0   6.7e-44   1.8e-40       2     256 ..    2210    2479 ..    2209    2482 .. 0.85
```

The two HMMs are recognizing the same domain: we select the HMM that recognizes this domain with best E-values