# HMM for Alignments

**Laboratory of Bioinformatics I**
**Module 2**

26 March, 2019

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http://biofold.org/

Biomolecules
Folding and
Disease

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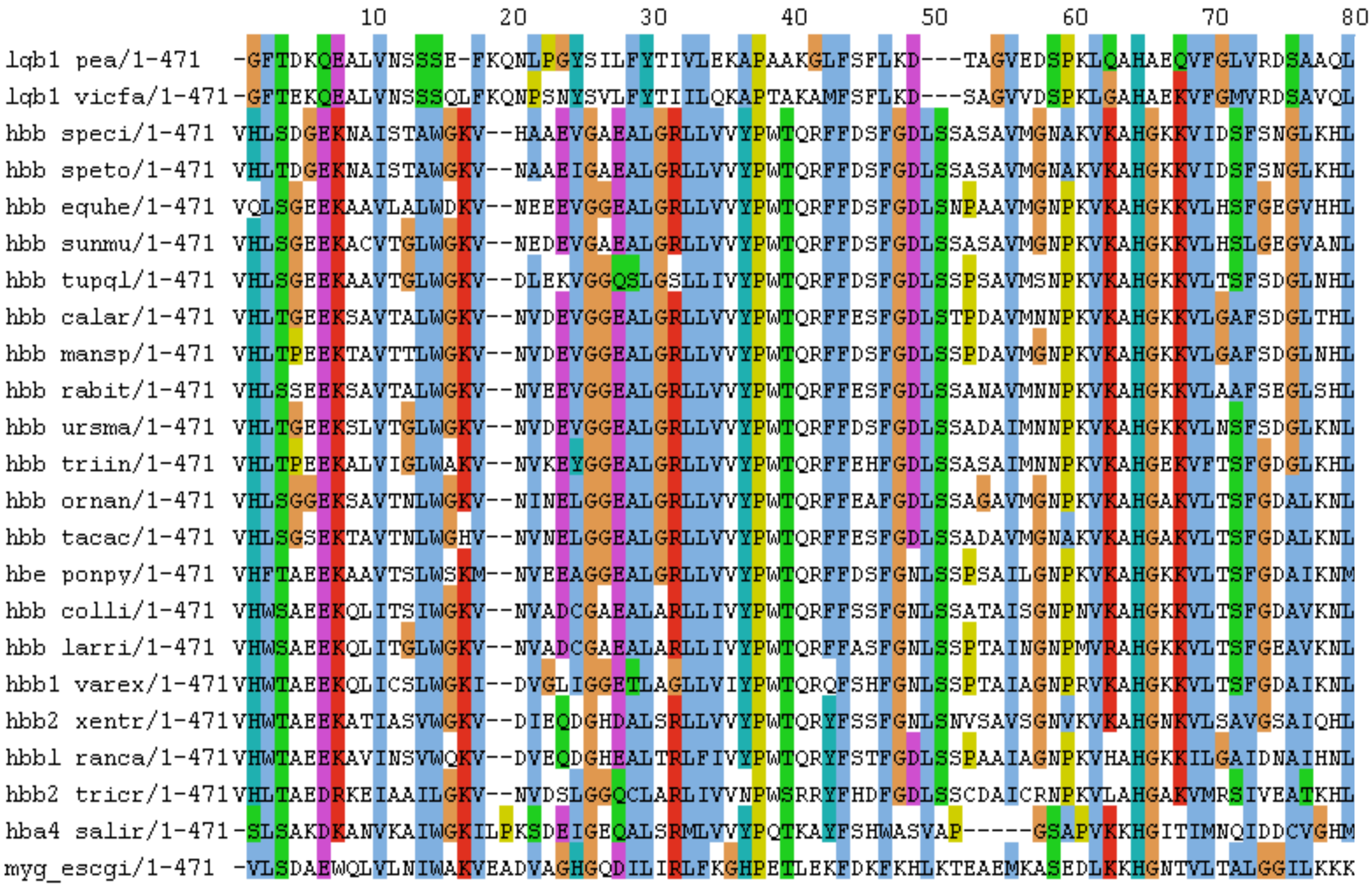
# Alignment of globins

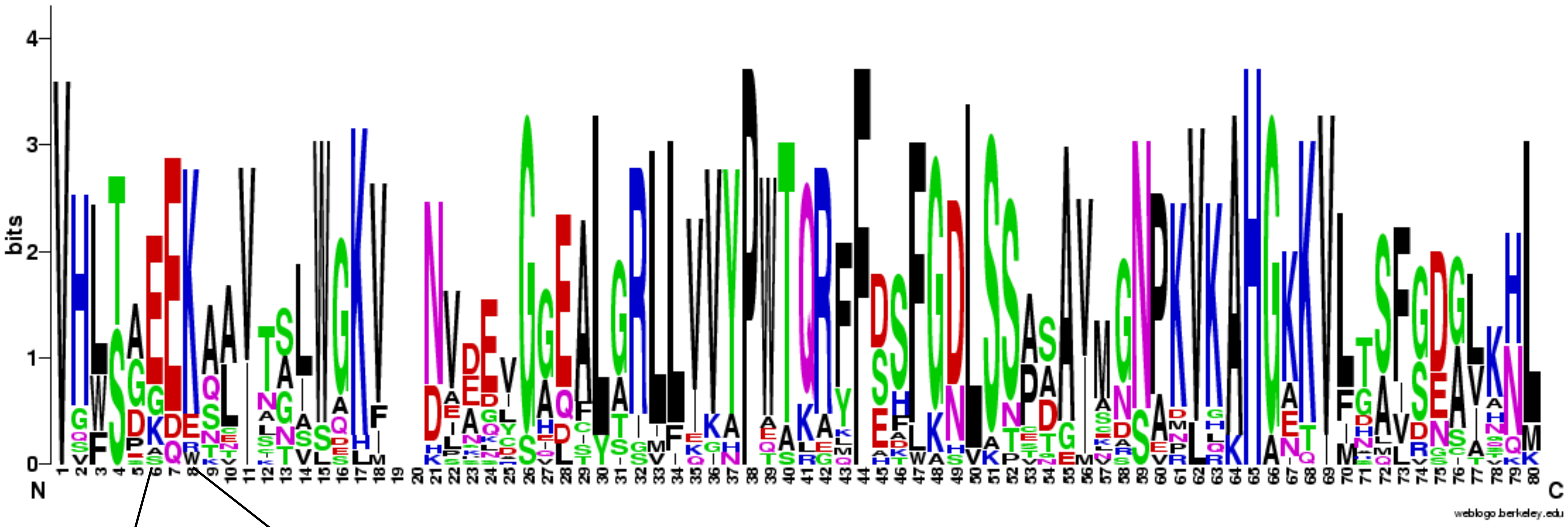
Different positions are not equivalent

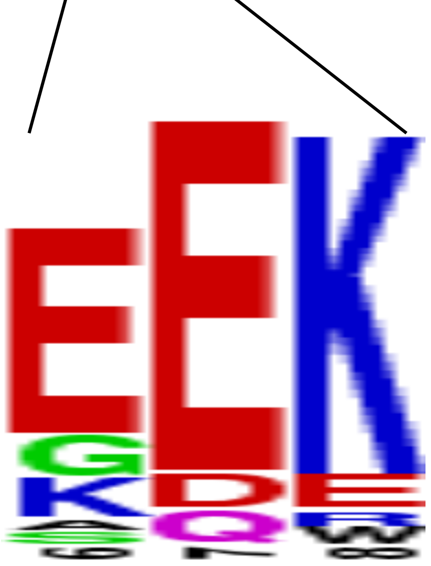


```
                            10        20        30        40        50        60        70        80
lqb1 pea/1-471   -GFTDKQEALVNSSSE-FKQNLPGYSILFYTIVLEKAPAAKGLFSFLKD---TAGVEDSPKLQAHAEQVFGLVRDSAAQL
lqb1 vicfa/1-471 -GFTEKQEALVNSSSQLFKQNPSNYSVLFYTIILQKAPTAKAMFSFLKD---SAGVVDSPKLGAHAEKVFGMVRDSAVQL
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/1-471 VHWTAEEKQLICSLWGKI--DVGLIGGETLAGLLVIYPWTQRQFSHFGNLSSPTAIAGNPRVKAHGKKVLTSFGDAIKNL
hbb2 xentr/1-471 VHWTAEEKATIASVWGKV--DIEQDGHDALSRLLVVYPWTQRYFSSFGNLSNVSAVSGNVKVKAHGNKVLSAVGSAIQHL
hbbl ranca/1-471 VHWTAEEKAVINSVWQKV--DVEQDGHEALTRLFIVYPWTQRYFSTFGDLSSPAAIAGNPKVHAHGKKILGAIDNAIHNL
hbb2 tricr/1-471 VHLTAEDRKEIAAILGKV--NVDSLGGQCLARLIVVNPWSRRYFHDFGDLSSCDAICRNPKVLAHGAKVMRSIVEATKHL
hba4 salir/1-471 -SLSAKDKANVKAIWGKILPKSDEIGEQALSRMLVVYPQTKAYFSHWASVAP-----GSAPVKKHGITIMNQIDDCVGHM
myg_escgi/1-471  -VLSDAEWQLVLNIWAKVEADVAGHGQDILIRLFKGHPETLEKFDKFKHLKTEAEMKASEDLKKHGNTVLTALGGILKKK
```

# Sequence logo

A more flexible alignment score is needed to align protein families



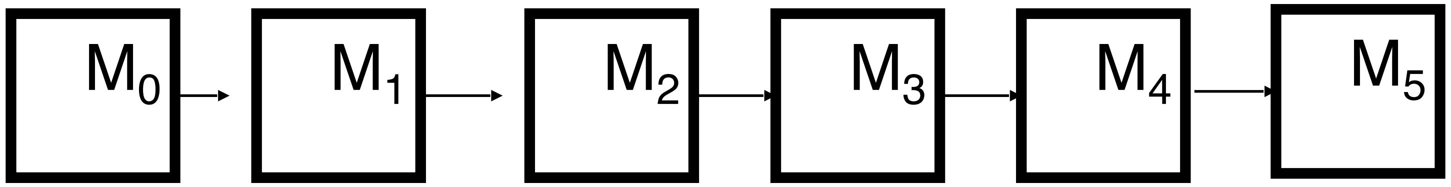


The substitution score may depend on the position.

http://weblogo.berkeley.edu/

# How to Align?

Each state represent a position in the alignment.



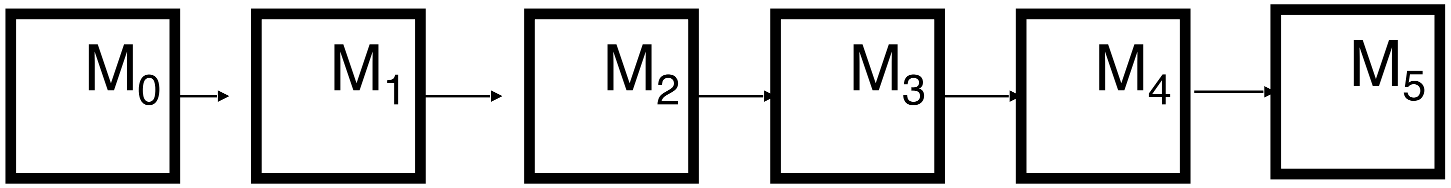
|  |  |  |  |  |  |
|---|---|---|---|---|---|
| A | C | G | G | T | A |
| $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |
| A | C | G | A | T | C |
| $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |
| A | T | G | T | T | C |
| $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |

Each position has a peculiar composition

# From Sequences to Model

Given a set of sequences we can train a model by estimating the emission probability

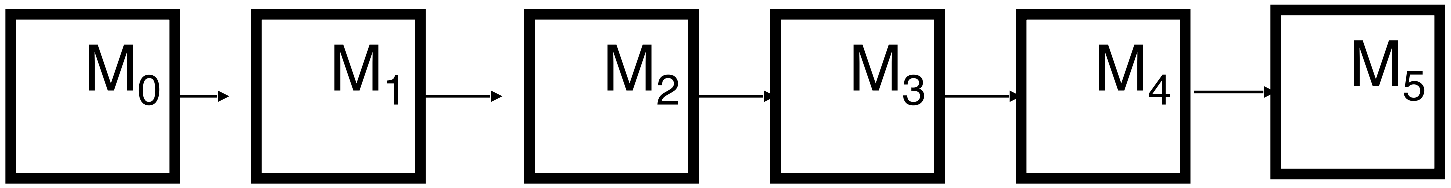
| | | | | | |
|---|---|---|---|---|---|
| A | C | G | G | T | A |
| A | C | G | A | T | C |
| A | T | G | T | T | C |



| | | | | | | |
|---|---|---|---|---|---|---|
| A | 1 | 0 | 0 | 0.33 | 0 | 0.33 |
| C | 0 | 0.66 | 0 | 0 | 0 | 0.66 |
| G | 0 | 0 | 1 | 0.33 | 0 | 0 |
| T | 0 | 0.33 | 0 | 0.33 | 1 | 0 |

# Scoring a Sequence

Given the model we can calculate the probability of the a new aligned sequence

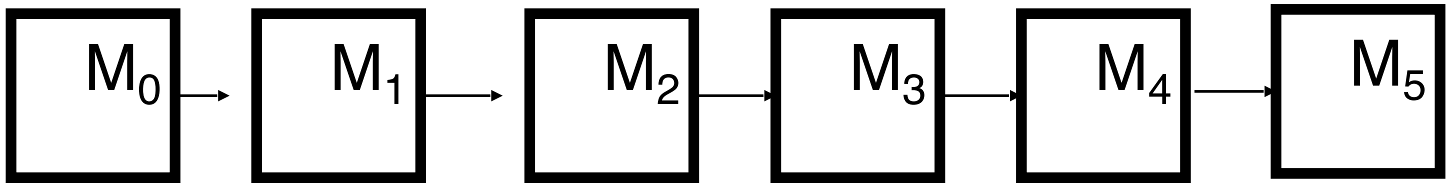


| | | | | | | |
|---|---|---|---|---|---|---|
| A | 1 | 0 | 0 | 0.33 | 0 | 0.33 |
| C | 0 | 0.66 | 0 | 0 | 0 | 0.66 |
| G | 0 | 0 | 1 | 0.33 | 0 | 0 |
| T | 0 | 0.33 | 0 | 0.33 | 1 | 0 |
| | A | C | G | A | T | C |

$$P(s|M) = 1 \times 0.66 \times 1 \times 0.33 \times 1 \times 0.66$$

# Alignments with Gaps

A strategy to introduce gaps is needed

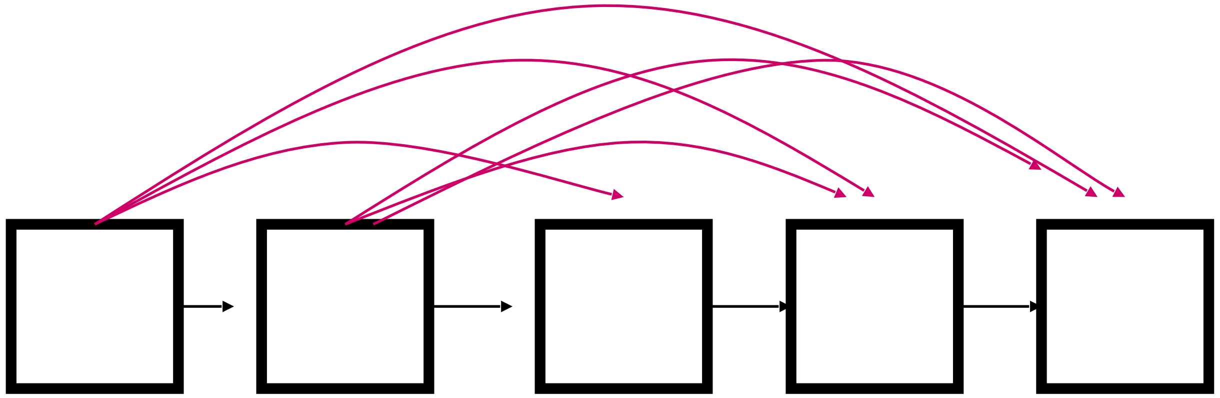


| | | | | | | |
|---|---|---|---|---|---|---|
| A | 1 | 0 | 0 | 0.33 | 0 | 0.33 |
| C | 0 | 0.66 | 0 | 0 | 0 | 0.66 |
| G | 0 | 0 | 1 | 0.33 | 0 | 0 |
| T | 0 | 0.33 | 0 | 0.33 | 1 | 0 |

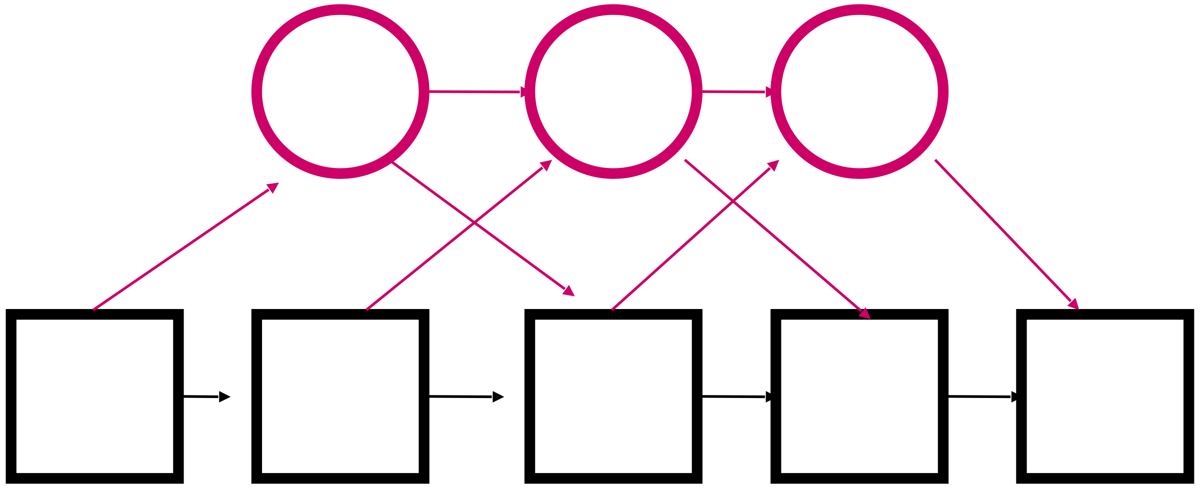
| | | | | | |
|---|---|---|---|---|---|
| A | | G | A | T | C |
| $M_0$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $M_5$ |

# Silent States

Different topology to model gaps

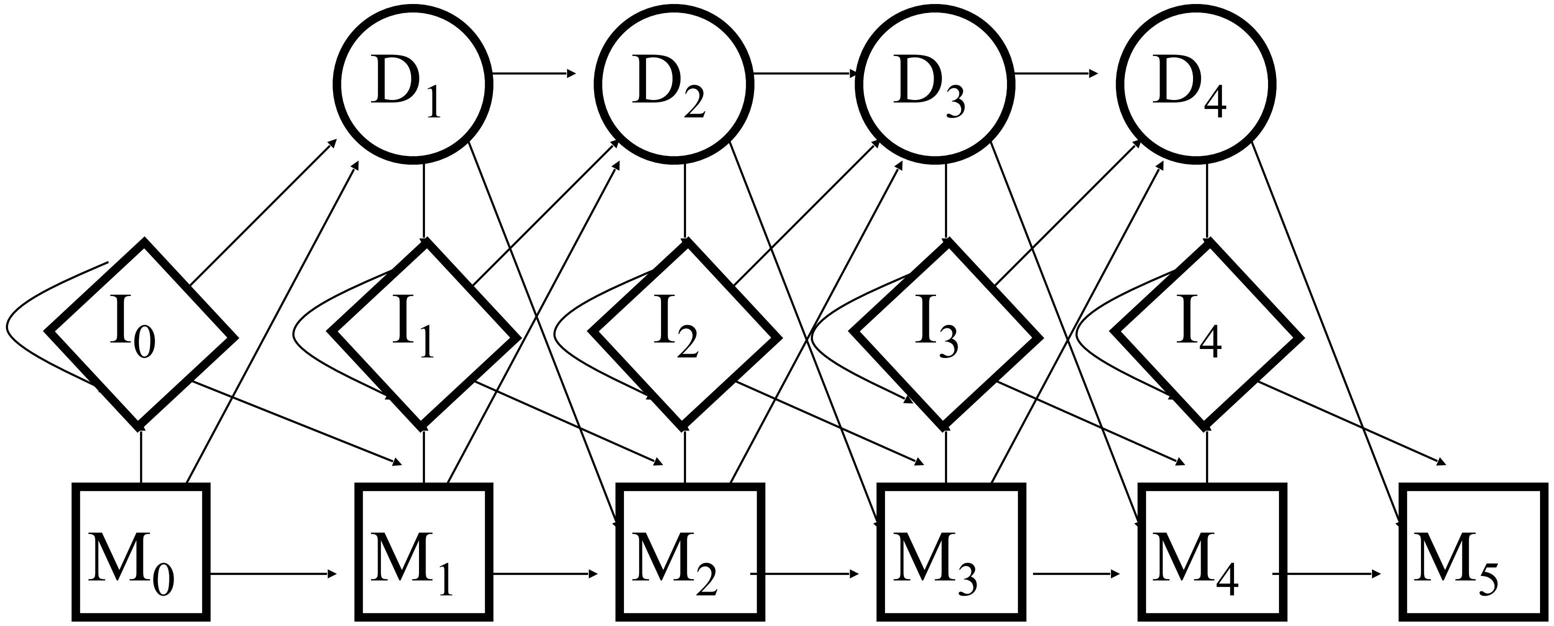


N(N-1)/2  transitions



To reduce the number of parameters we can use states that doesn’t emit any character
4N-8  transitions

# Profile HMM



Delete states

Insert states

Match states

A C G G T A

$M_0$ $M_1$ $M_2$ $M_3$ $M_4$ $M_5$

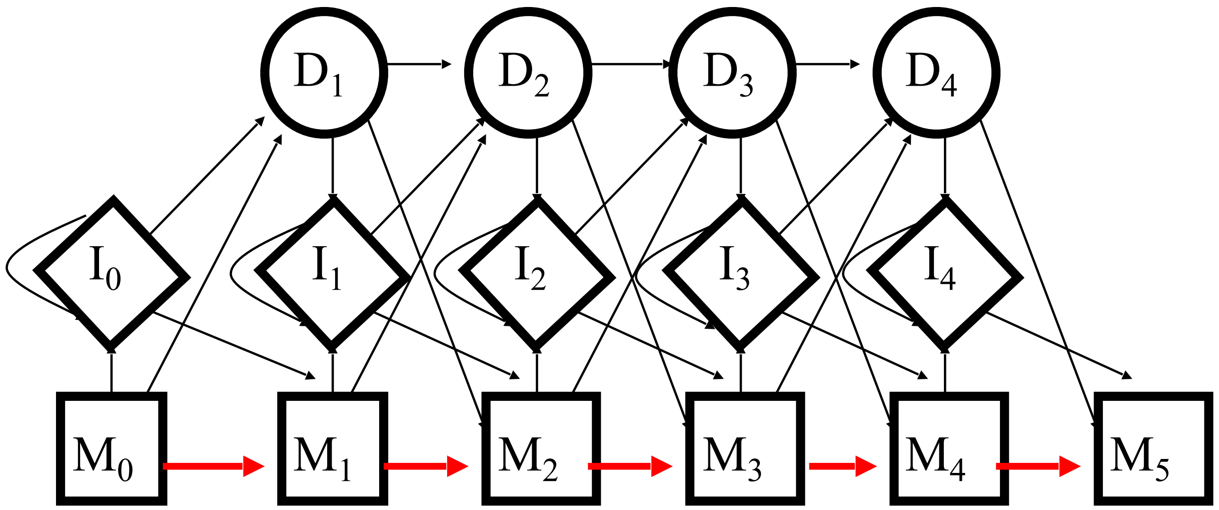
A C G C A G T C

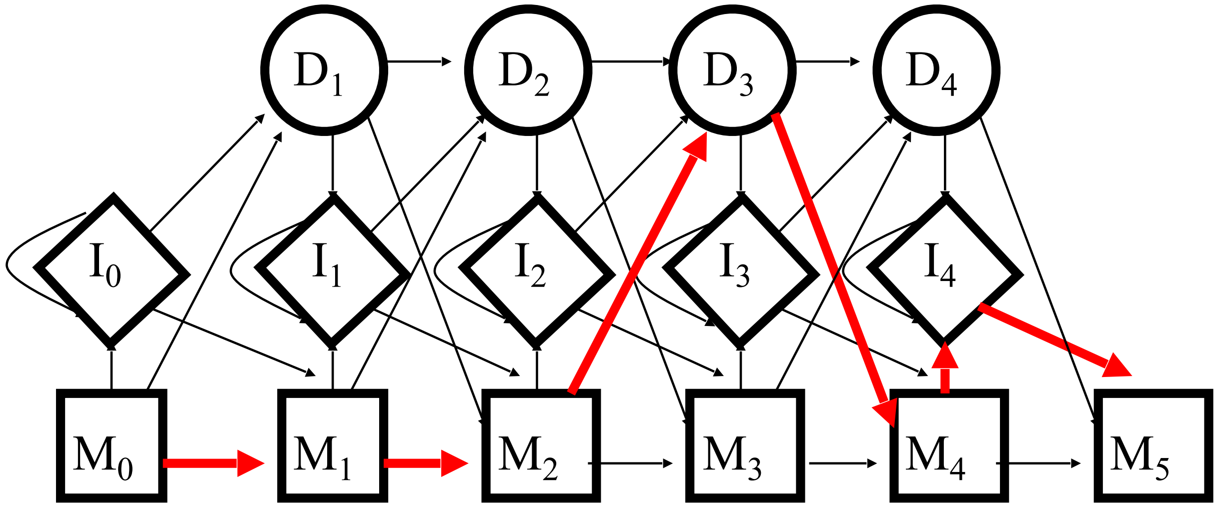
$M_0$ $I_0$ $I_0$ $M_1$ $M_2$ $M_3$ $M_4$ $M_5$

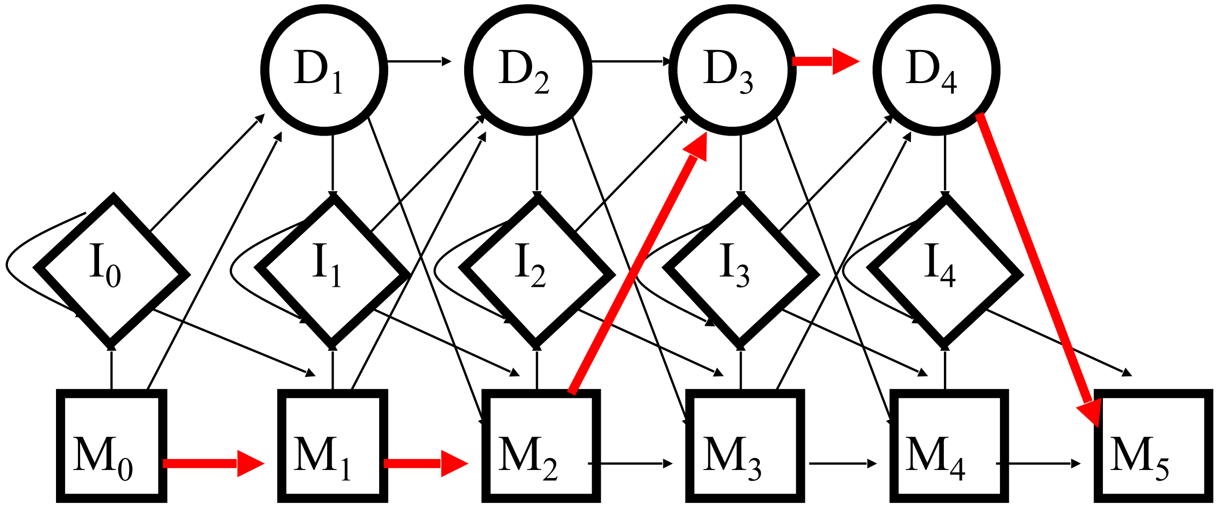
A G A T C

$M_0$ $D_1$ $M_2$ $M_3$ $M_4$ $M_5$

# Example of Alignment


$D_1$ $D_2$ $D_3$ $D_4$
$I_0$ $I_1$ $I_2$ $I_3$ $I_4$
$M_0$ $M_1$ $M_2$ $M_3$ $M_4$ $M_5$


$D_1$ $D_2$ $D_3$ $D_4$
$I_0$ $I_1$ $I_2$ $I_3$ $I_4$
$M_0$ $M_1$ $M_2$ $M_3$ $M_4$ $M_5$


$D_1$ $D_2$ $D_3$ $D_4$
$I_0$ $I_1$ $I_2$ $I_3$ $I_4$
$M_0$ $M_1$ $M_2$ $M_3$ $M_4$ $M_5$

***Sequence 1***

A S T R A L

*Viterbi path*

| | | | | | |
|---|---|---|---|---|---|
| $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |
| A | S | T | R | A | L |

***Sequence 2***

A S T A I L

*Viterbi path*

| | | | | | | |
|---|---|---|---|---|---|---|
| $M_0$ | $M_1$ | $M_2$ | $D_3$ | $M_4$ | $I_4$ | $M_5$ |
| A | S | T | | A | I | L |

***Sequence 3***

A R T I

*Viterbi path*

| | | | | | |
|---|---|---|---|---|---|
| $M_0$ | $M_1$ | $M_2$ | $D_3$ | $D_4$ | $M_5$ |
| A | R | T | | | I |

# Alignment Calculation

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | | $M_5$ | *Sequence 1* |
| A | S | T | R | A | | L | |
| $M_0$ | $M_1$ | $M_2$ | $D_3$ | $M_4$ | $I_4$ | $M_5$ | *Sequence 2* |
| A | S | T | | A | I | L | |
| $M_0$ | $M_1$ | $M_2$ | $D_3$ | $D_4$ | | $M_5$ | *Sequence 3* |
| A | R | T | | | | I | |

Grouping by vertical layers

| | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| $s_1$ | A | S | T | R | A | L |
| $s_2$ | A | S | T | | AI | L |
| $s_3$ | A | R | T | | | I |

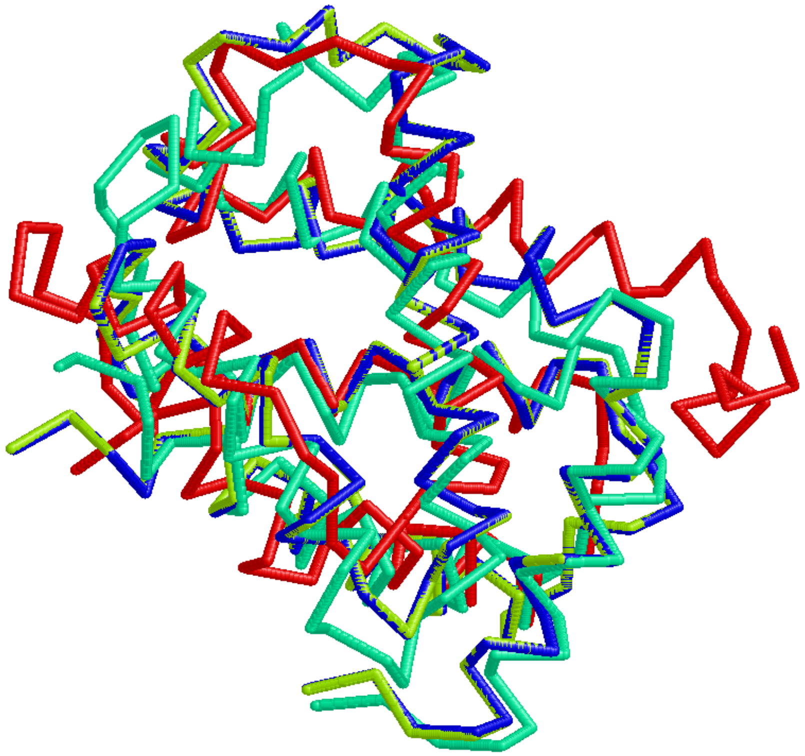
Alignment

```
ASTRA-L
AST-AIL
ART---I
```

-Log P(s I M)      Is an alignment score

# Alignment of Globins

```
           AAAAAAAAAAAAAAAA   BBBBBBBBBBBBBBBBCCCCCCCCCCC
                                                     DDDD
---------VLSPADKTNVKAAWGKVGA--HAGEYGAEALERMFLSFPTTKTYFPHF-DL
--------VHLTPEEKSAVTALWGKV----NVDEVGGEALGRLLVVYPWTQRFFESFGDL
---------VLSEGEWQLVLHVWAKVEA--DIAGHGQDILIRLFKHHPETLEKFDRFKHL
----------LSADQISTVQASFDKVKG------DPVGILYAVFKADPSIMAKFTQFAG-
PIVDTGSVAPLSAAEKTKIRSAWAPVYS--TYETSGVDILVKFFTSTPAAQEFFPKFKGL
--------GALTESQAALVKSSWEEFNA--NIPKHTHRFFILVLEIAPAAKDLFS-FLK-
---------GLSAAQRQVIAATWKDIAGADNGAGVGKDCLIKFLSAHPQMAAVFG-FSG-


 DDDDDDDEEEEEEEEEEEEEEEEEEEEE            FFFFFFFFFFFF  FFGGG
                            F                      GG  GG
S-----HGSAQVKGHGKKVADALTNAVAHV---D--DMPNALSALSDLHAHKL--RVDPV
STPDAVMGNPKVKAHGKKVLGAFSDGLAHL---D--NLKGTFATLSELHCDKL--HVDPE
KSEAEMKASEDLKKHGVTVLTALGAILKK----K-GHHEAELKPLAQSHATKH--KIPIK
KDLESIKGTAPFETHANRIVGFFSKIIGEL--P---NIEADVNTFVASHKPRG---VTHD
TTADQLKKSADVRWHAERIINAVNDAVASM--DDTEKMSMKLRDLSGKHAKSF--QVDPQ
GTSEVPQNNPELQAHAGKVFKLVYEAAIQLQVTGVVVTDATLKNLGSVHVSKG---VADA
---AS---DPGVAALGAKVLAQIGVAVSHL--GDEGKMVAQMKAVGVRHKGYGNKHIKAQ


GGGGGGGGGGGGGGGG     HHHHHHHHHHHHHHHHHHHHHHHHHH

NFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR------
NFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH------
YLEFISEAIIHVLHSRHPADFGADAQGAMSKALELFRKDIAAKYKELGYQG
QLNNFRAGFVSYMKAHT--DFA-GAEAAWGATLDTFFGMIFSKM-------
YFKVLAAVIADTVAAG---------DAGFEKLMSMICILLRSAY-------
HFPVVKEAILKTIKEVVGAKWSEELNSAWTIAYDELAIVIKKEMNDAA---
YFEPLGASLLSAMEHRIGGKMNAAAKDAWAAAYADISGALISGLQS-----
```



*Bashford et al.* J Mol Biol 1987

# Globins HMM

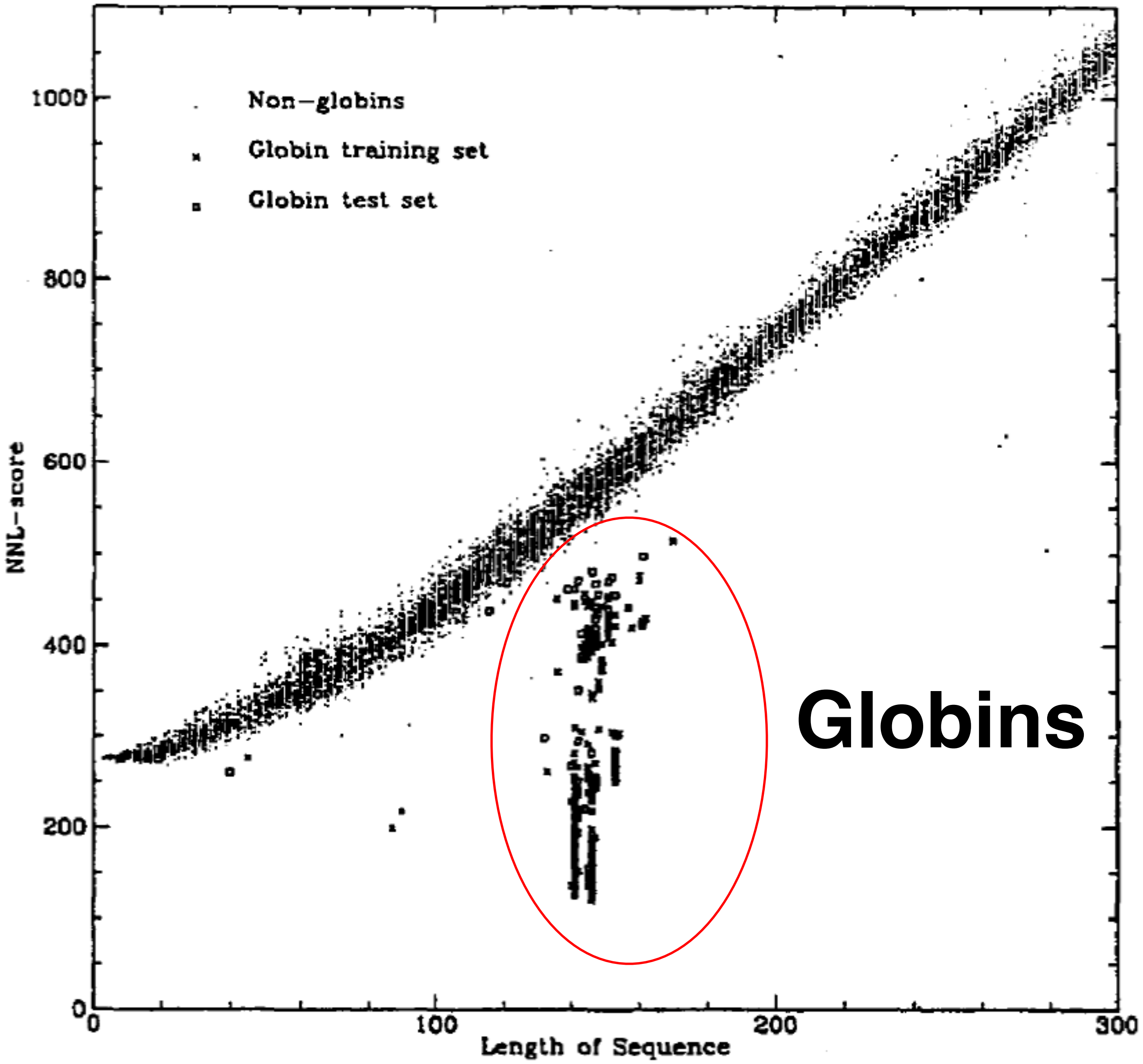
HMM are calculate from a training set of 400 unaligned sequences. After the HMM is built, it is used to obtain a multiple alignment of all the training sequences. This is the alignment of the 7 globins as aligned with the trained model.

```
           AAAAAAAAAAAAAAAA   BBBBBBBBBBBBBBBCCCCCCCCCCCC
                                                     DDDD
           ***************    ************************
V.........LSPADKTNVKAAWGKVGA..HAGEYGAEALERMFLSFPTTKTYFPHFD-L
Vh........LTPEEKSAVTALWGKV--..NVDEVGGEALGRLLVVYPWTQRFFESFGDL
V.........LSEGEWQLVLHVWAKVEA..DVAGHGQDILIRLFKSHPETLEKFDRFKHL
-.........LSADQISTVQASFDKV--..KGDPVGI--LYAVFKADPSIMAKFTQFAGK
PivdtgsvapLSAAEKTKIRSAWAPVYS..TYETSGVDILVKFFTSTPAAQEFFPKFKGL
Ga........LTESQAALVKSSWEEFNA..NIPKHTHRFFILVLEIAPAAKDLFSFLK-G
G.........LSAAQRQVIAATWKDIAGadNGAGVGKDCLIKFLSAHPQMA---AVFG-F

 DDDDDDDEE EEEEEEEEEEEEEEEEEEE            FFFFFFFFFF   FFFFG
                             F                         GGGG
                   ***********            *********       **
SHGSAQVKGH-GKK.----VADALTNAVAHVDD.....MPNALSALSDLHA...HKLRVD
STPDAVMGNPKVKA.HGKKVLGAFSDGLAHLDN.....LKGTFATLSELHC...DKLHVD
KTEA-EMKASEDLKkHGVTVLTALGAILKKKGH.....HEAELKPLAQSHA...TKHKIP
DLES-IKGTAPFET.HANRIVGFFSKIIGELPN.....IEADVNTFVASHK...PR-GVT
TTADQLKKSADVRW.HAERIINAVNDAVASMDDtek..MSMKLRDLSGKHA...KSFQVD
TSEVPQ-NNPELQA.HAGKVFKLVYEAAIQLQVtgvvvTDATLKNLGSVHV...SK-GVA
SGAS----DPGVAA.LGAKVLAQIGVAVSHLGDegk..MVAQMKAVGVRHKgygNK-HIK

GGGGGGGGGGGGGGGGGG     HHHHHHHHHHHHHHHHHHHHHHHHHH
******************    ************************
PVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKY......R
PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKY......H
IKYLEFISEAIIHVLHSRHPGDFGADAQGAMNKALELFRKDIAAKYkelgyqG
HDQLNNFRAGFVSYMKAH--TDF-AGAEAAWGATLDTFFGMIFSKM......-
PQYFKVLAAVIADTVAA---GD------AGFEKLMSMICILLRSAY......-
DAHFPVVKEAILKTIKEVVGAKWSEELNSAWTIAYDELAIVIKKEMnda...A
AQYFEPLGASLLSAMEHRIGGKMNAAAKDAWAAAYADISGALISGLq.....S
```

*Krogh et al.* J Mol Biol. 1994

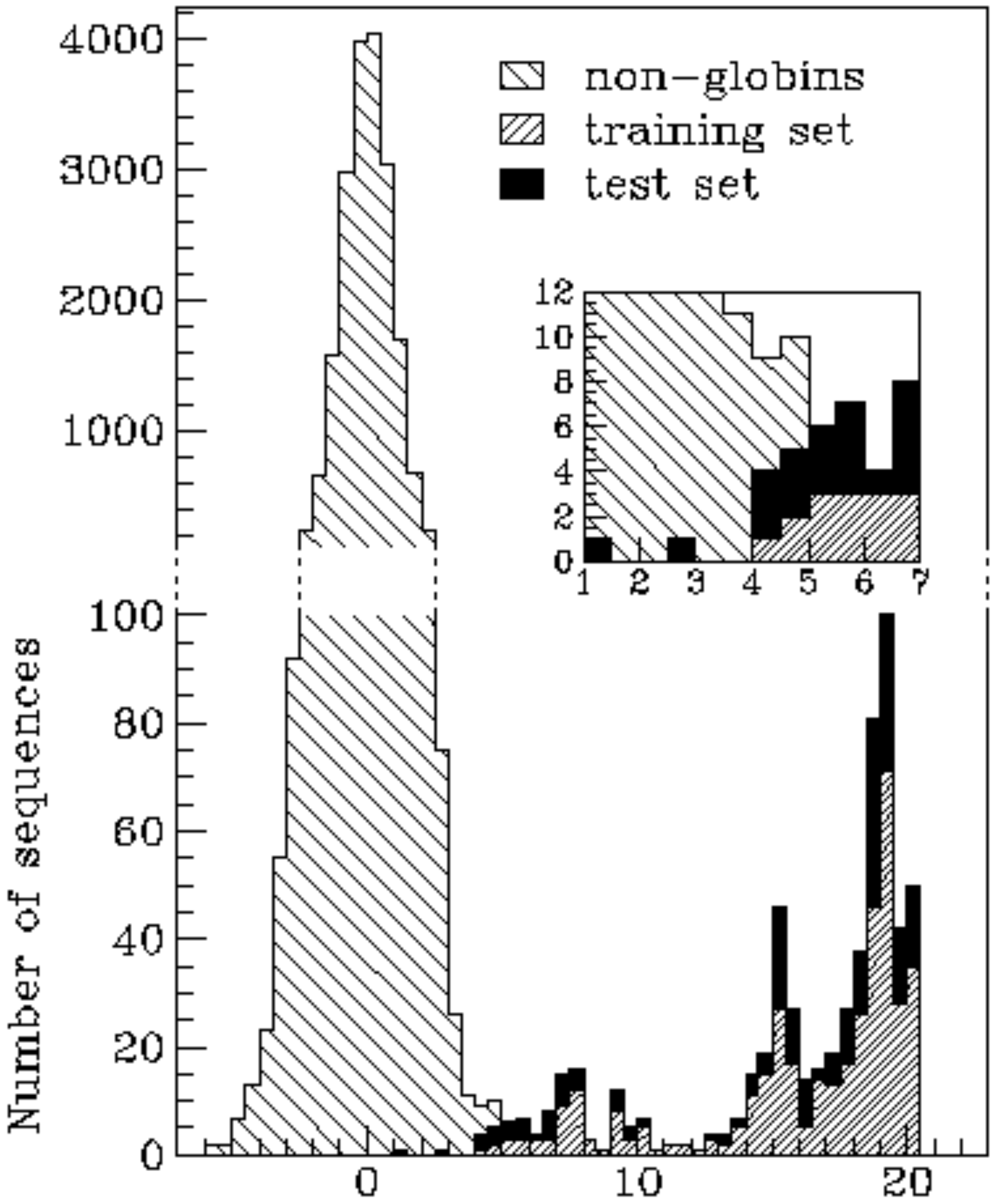
# Globin Classification

The NLL-score is calculated to discriminate between Globin and non-Globin protein sequences



NLLscore = —log P(s|M)

# Score distribution

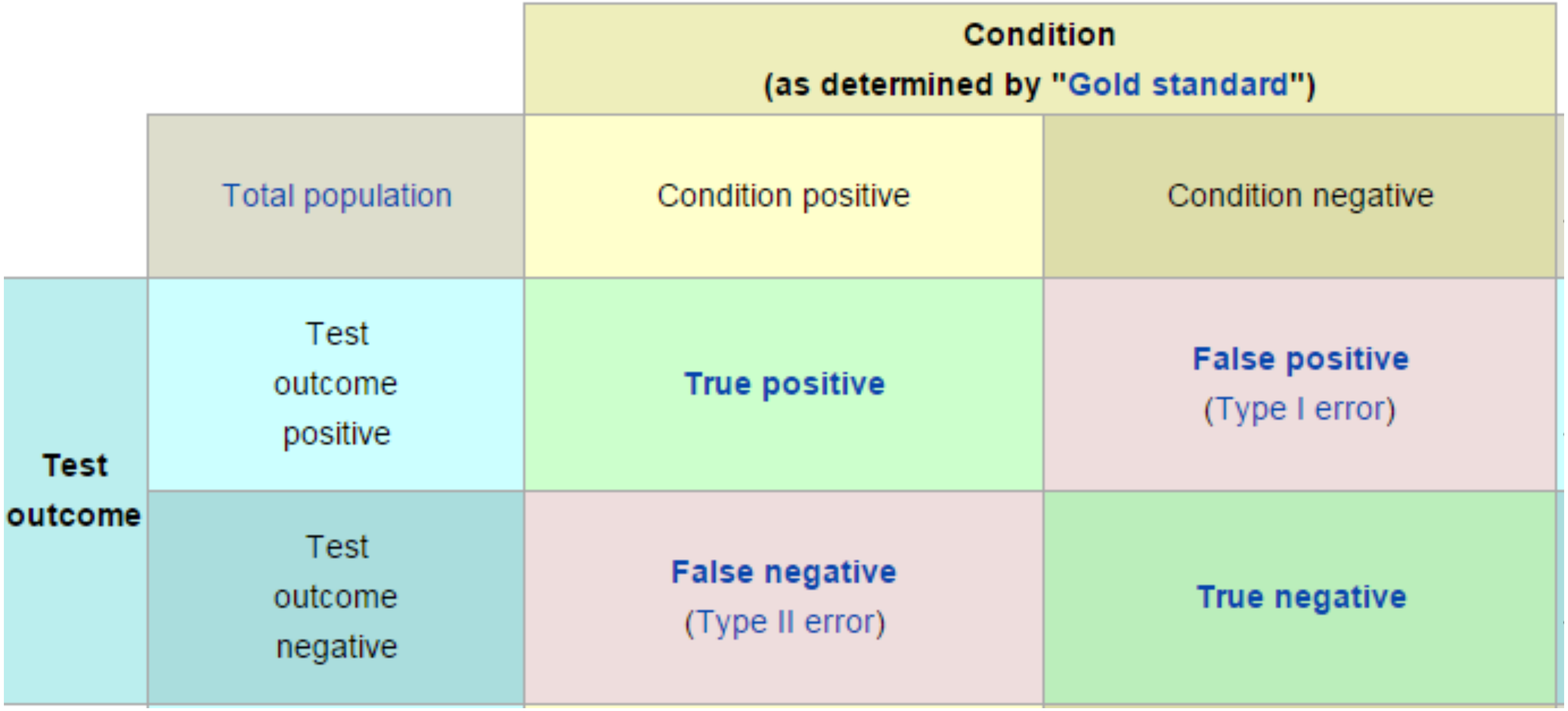


$$\text{Z-score} = \frac{\text{NLL (s) - <NLL>}}{\sigma \ \text{(NLL)}}$$

With mean and standard deviation computed on sets of sequences with similar length

# Confusion Matrix

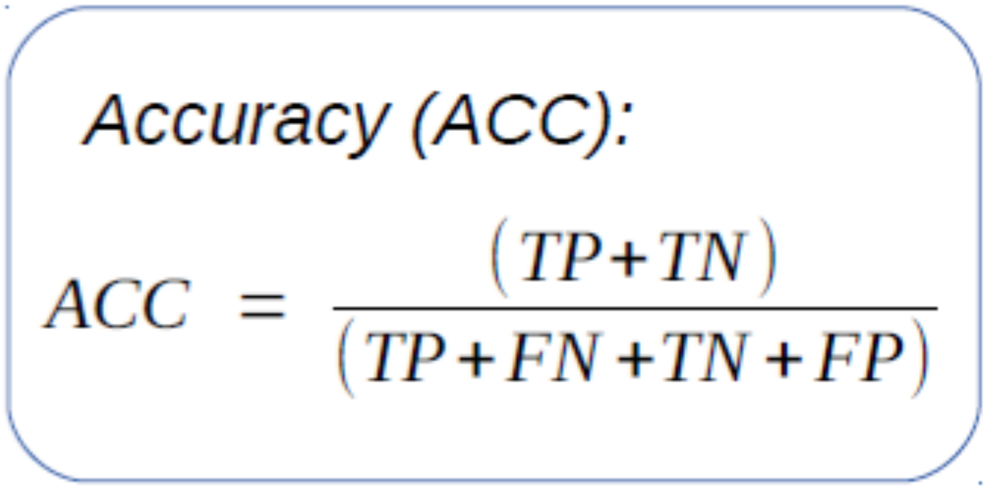
A 2x2 matrix for calculating the performance of prediction methods



| | | Condition<br>(as determined by "Gold standard") | |
|---|---|---|---|
| | Total population | Condition positive | Condition negative |
| Test outcome | Test outcome positive | **True positive** | **False positive**<br>(Type I error) |
| | Test outcome negative | **False negative**<br>(Type II error) | **True negative** |

# Overall Accuracy

How many predictions are correct on the overall?



*Accuracy (ACC):*

$$ACC = \frac{(TP+TN)}{(TP+FN+TN+FP)}$$

Is it an informative enough score?

# Dataset Unbalance

Accuracy can be strongly biased because of class unbalance. It is not very informative

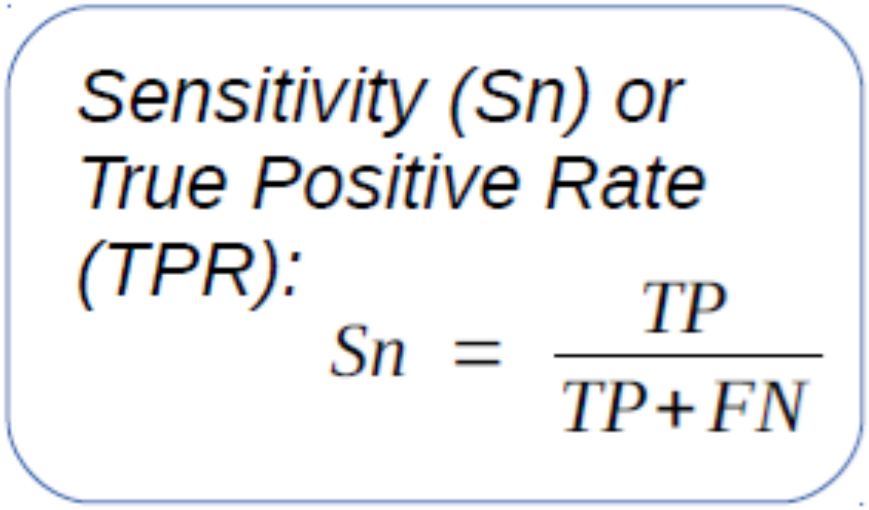
| | Class 1 | Class -1 |
|---|---|---|
| Prediction 1 | 90 | 10 |
| Prediction -1 | 0 | 0 |

Acc = 0.9
ALL the examples are predicted in the class 1:
Very bad predictions

| | Class 1 | Class -1 |
|---|---|---|
| Prediction 1 | 81 | 1 |
| Prediction -1 | 9 | 9 |

Acc = 0.9
It seems a much more reasonable prediction

# Class Specific Measures

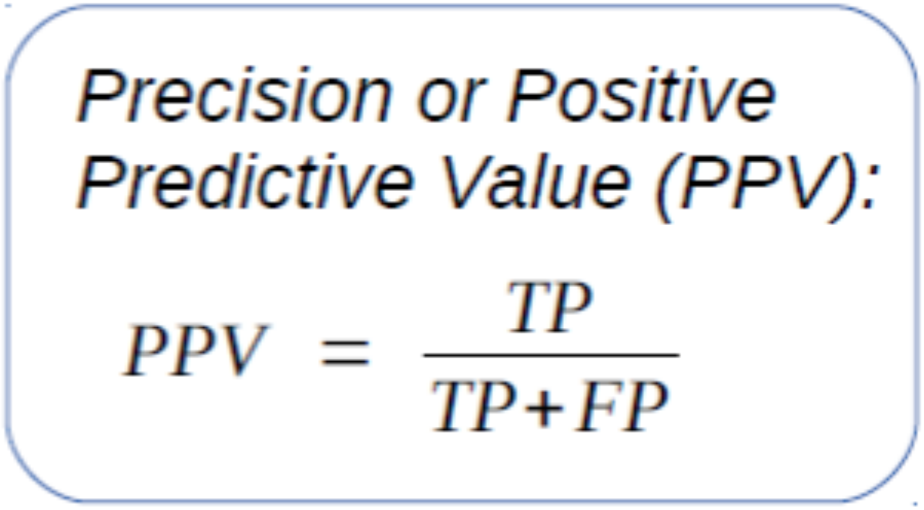


*Sensitivity (Sn) or True Positive Rate (TPR):*

$$Sn = \frac{TP}{TP+FN}$$

It answer to the question:

How many of the real positive examples are correctly predicted?



*Precision or Positive Predictive Value (PPV):*

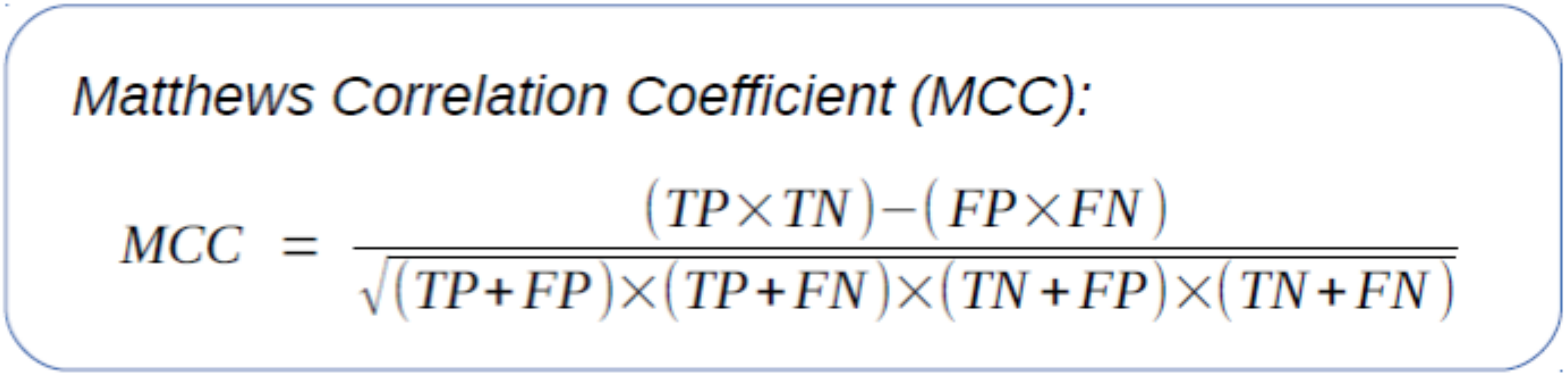
$$PPV = \frac{TP}{TP+FP}$$

It answer to the question:

How many of the positive predictions are correct?

It is sometimes referred as Specificity

# Matthews Correlation



*Matthews Correlation Coefficient (MCC):*

$$MCC = \frac{(TP \times TN) - (FP \times FN)}{\sqrt{(TP+FP) \times (TP+FN) \times (TN+FP) \times (TN+FN)}}$$

It answer to the question:

Is the prediction really correlated with the real classes?

It is 0 in case of random prediction
It is 1 only in case of perfect prediction
It is -1 only in case of completely wrong prediction

It is the Pearson’s correlation coefficient for categorical classes

# MCC and Unbalance

MCC is not affected by dataset unbalance

| | Class 1 | Class -1 |
|---|---|---|
| Prediction 1 | 90 | 10 |
| Prediction -1 | 0 | 0 |

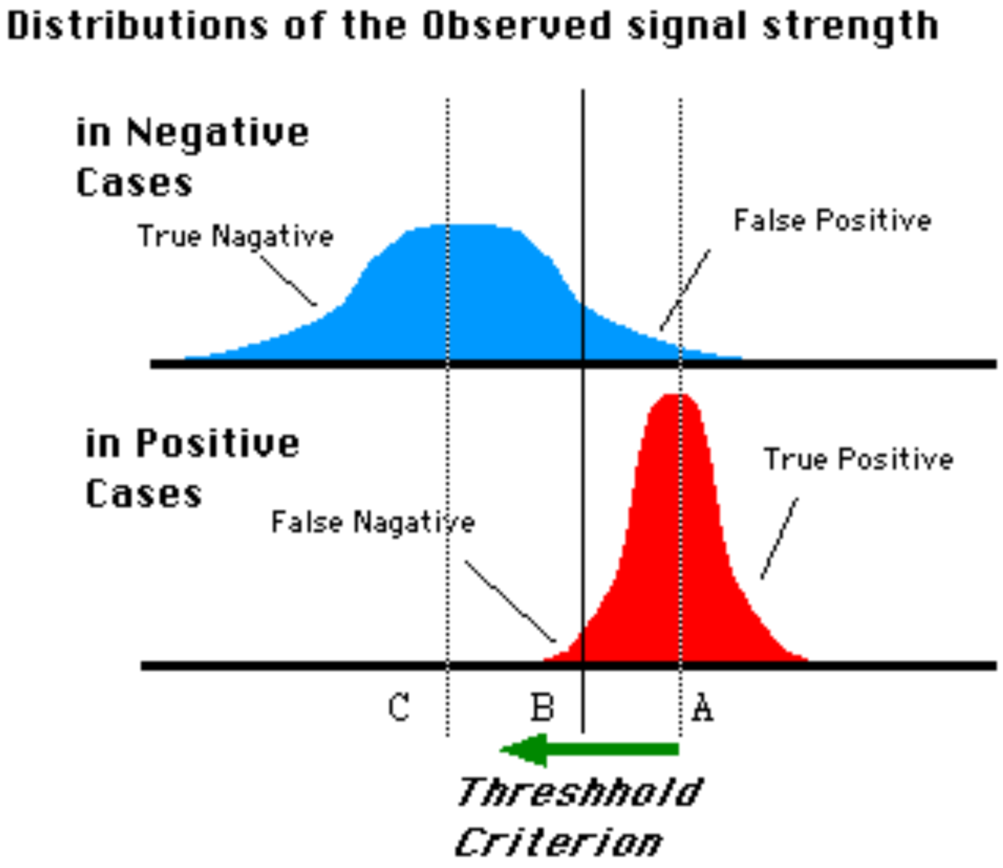
Acc = 0.9
All the examples are predicted in the class 1:
MCC = 0.0
Very bad predictions

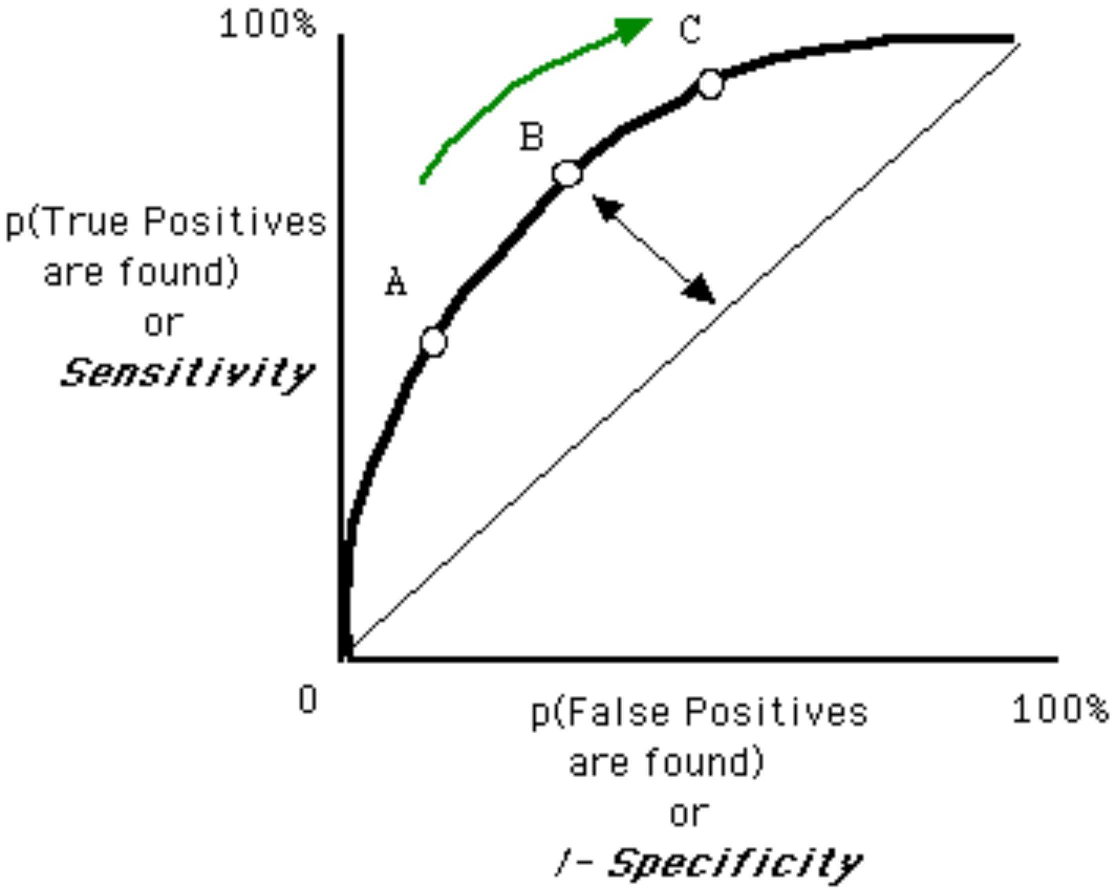
| | Class 1 | Class -1 |
|---|---|---|
| Prediction 1 | 81 | 1 |
| Prediction -1 | 9 | 9 |

Acc = 0.9
MCC = 0.62
Predictions are good

# ROC Curve

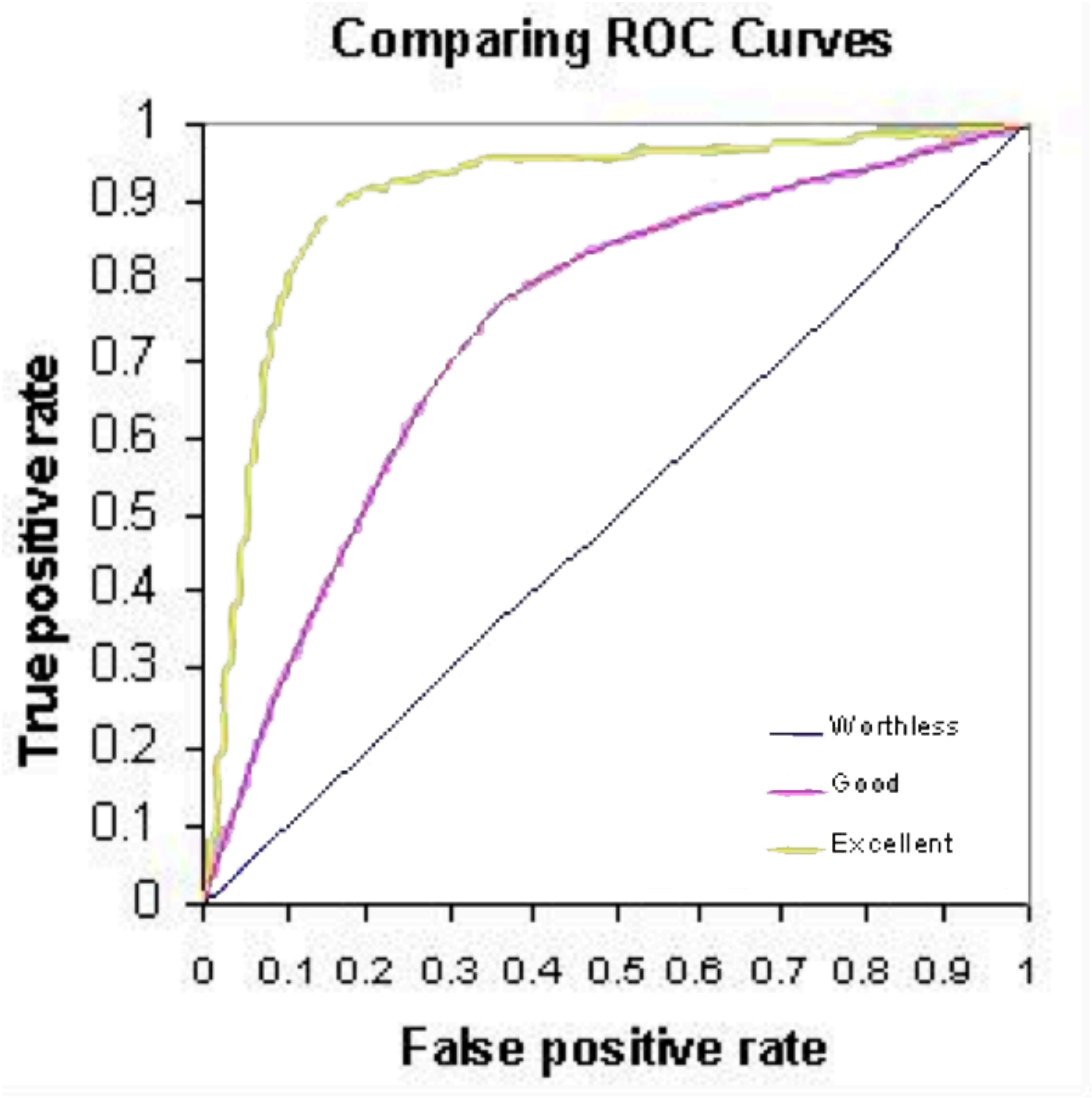
The Receiver Operating Characteristics depends on a parameter, TPR and FPR can be plotted at varying values of the parameter





# Area Under Curve

The Area Under the ROC Curve (AUC) is used to measure the perforce of a predictor



AUC=0.5 → Random prediction

AUC=1 → Perfect prediction