# CSE 549: Computational Biology

Substitution Matrices

Stony Brook University

# How should we score alignments

So far, we've looked at "arbitrary" schemes for scoring mutations. How can we assign scores in a more meaningful way?

### Are these scores

| | A | C | G | T |
|---|---|---|---|---|
| A | 5 | -5 | -3 | -5 |
| C | -5 | 5 | -5 | -3 |
| G | -3 | -5 | 5 | -5 |
| T | -5 | -3 | -5 | 5 |

### better than these scores?

| | A | C | G | T |
|---|---|---|---|---|
| A | 4 | -1 | -1 | -1 |
| C | -1 | 4 | -1 | -1 |
| G | -1 | -1 | 4 | -1 |
| T | -1 | -1 | -1 | 4 |

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Are these scores          better than these scores?

| | A | C | G | T |
|---|---|---|---|---|
| A | 5 | -5 | -3 | -5 |
| | | | 5 | |
| G | | -5 | 5 | -5 |
| T | -5 | -3 | -5 | 5 |

| | A | C | G | T |
|---|---|---|---|---|
| A | 4 | -1 | -1 | -1 |
| | | | | |
| G | -1 | -1 | 4 | -1 |
| T | -1 | -1 | -1 | 4 |

**One option — "learn" the substitution / mutation rates from real data**

# How should we score alignments

**Main Idea: Assume** we can obtain (through a potentially burdensome process) a collection of high quality, high confidence sequence alignments.

We have a collection of sequences which, presumably, originated from the same ancestor — differences are mutations due to divergence.

**Learn** the frequency of different mutations from these alignments, and use the frequencies to derive our scoring function.

# BLOSUM62 matrix

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | -2 | -2 | -2 | 0 | 0 | 0 | 0 | -2 | -2 | -3 | -2 | -1 | -2 | 0 | 0 | 0 | -2 | -3 | 0 | A |
| | | 5 | -2 | -3 | -3 | 0 | -1 | -2 | 0 | -3 | -4 | 1 | -3 | -3 | -2 | -2 | 0 | 0 | -3 | -4 | R |
| | | | 5 | 0 | 0 | 0 | -2 | 0 | 0 | -4 | -5 | -2 | -3 | -3 | -2 | 0 | 0 | -2 | -2 | -5 | N |
| | | | | 5 | -4 | 0 | 1 | -1 | 0 | -5 | -6 | -3 | -4 | -4 | 0 | -2 | -2 | -2 | -2 | -5 | D |
| | | | | | 8 | -2 | -3 | -1 | -1 | 0 | -2 | -3 | 0 | -1 | -1 | 1 | 0 | 0 | -2 | 0 | C |
| | | | | | | 5 | 2 | 0 | 0 | -2 | -4 | 0 | -2 | -3 | 0 | 0 | 0 | 0 | -2 | -3 | Q |
| | | | | | | | 5 | 0 | 0 | -3 | -4 | 0 | -3 | -3 | 0 | 0 | 0 | -2 | -3 | -3 | E |
| | | | | | | | | 6 | 0 | -4 | -5 | -2 | -3 | -2 | -2 | 0 | 0 | 0 | -2 | -3 | G |
| | | | | | | | | | 6 | -3 | -4 | 0 | -2 | 0 | 0 | 0 | 0 | 0 | 2 | -2 | H |
| | | | | | | | | | | 4 | 0 | -3 | 2 | 0 | -2 | -3 | 0 | 0 | -3 | 2 | I |
| | | | | | | | | | | | 4 | -4 | 0 | 0 | -3 | -4 | -3 | 0 | -4 | 0 | L |
| | | | | | | | | | | | | 4 | -2 | -4 | -1 | -2 | 0 | 0 | -3 | -4 | K |
| | | | | | | | | | | | | | 6 | 0 | -3 | -3 | -2 | 0 | -3 | 2 | M |
| | | | | | | | | | | | | | | 6 | -3 | -2 | -2 | 2 | 2 | 0 | F |
| | | | | | | | | | | | | | | | 7 | 0 | 0 | -2 | -3 | 0 | P |
| | | | | | | | | | | | | | | | | 4 | 2 | -2 | -2 | -3 | S |
| | | | | | | | | | | | | | | | | | 5 | -1 | -3 | 0 | T |
| | | | | | | | | | | | | | | | | | | 9 | 2 | -1 | W |
| | | | | | | | | | | | | | | | | | | | 7 | -3 | Y |
| | | | | | | | | | | | | | | | | | | | | 4 | V |

Brick, Kevin, and Elisabetta Pizzi. "A novel series of compositionally biased substitution matrices for comparing Plasmodium proteins." BMC bioinformatics 9.1 (2008): 236.
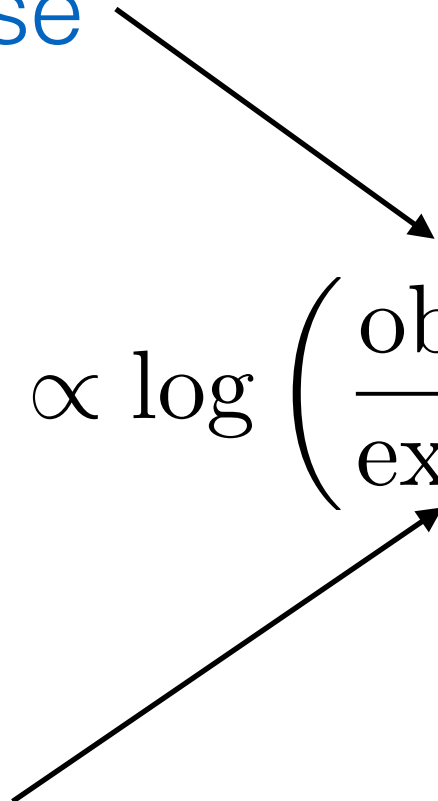
# Probabilities to Scores

Assuming we have a reasonable process by which to compute frequencies, how can we use this to obtain a score?

# Probabilities to Scores

Assuming we have a reasonable process by which to compute frequencies, how can we use this to obtain a score?

Hypothesis we wish to test; two amino acids are correlated because they are homologous.

$$\text{score} = \log \text{ odds ratio} = s_{AB} \propto \log \left( \frac{\text{observed}}{\text{expected}} \right)$$

Null hypothesis; two amino acids occur independently (and are uncorrelated and unrelated).

Eddy, Sean R. "Where did the BLOSUM62 alignment score matrix come from?." Nature biotechnology 22.8 (2004): 1035-1036.

# Probabilities to Scores

$$\text{score} = \log \text{ odds ratio} = s_{AB} \propto \log\left(\frac{\text{observed}}{\text{expected}}\right)$$
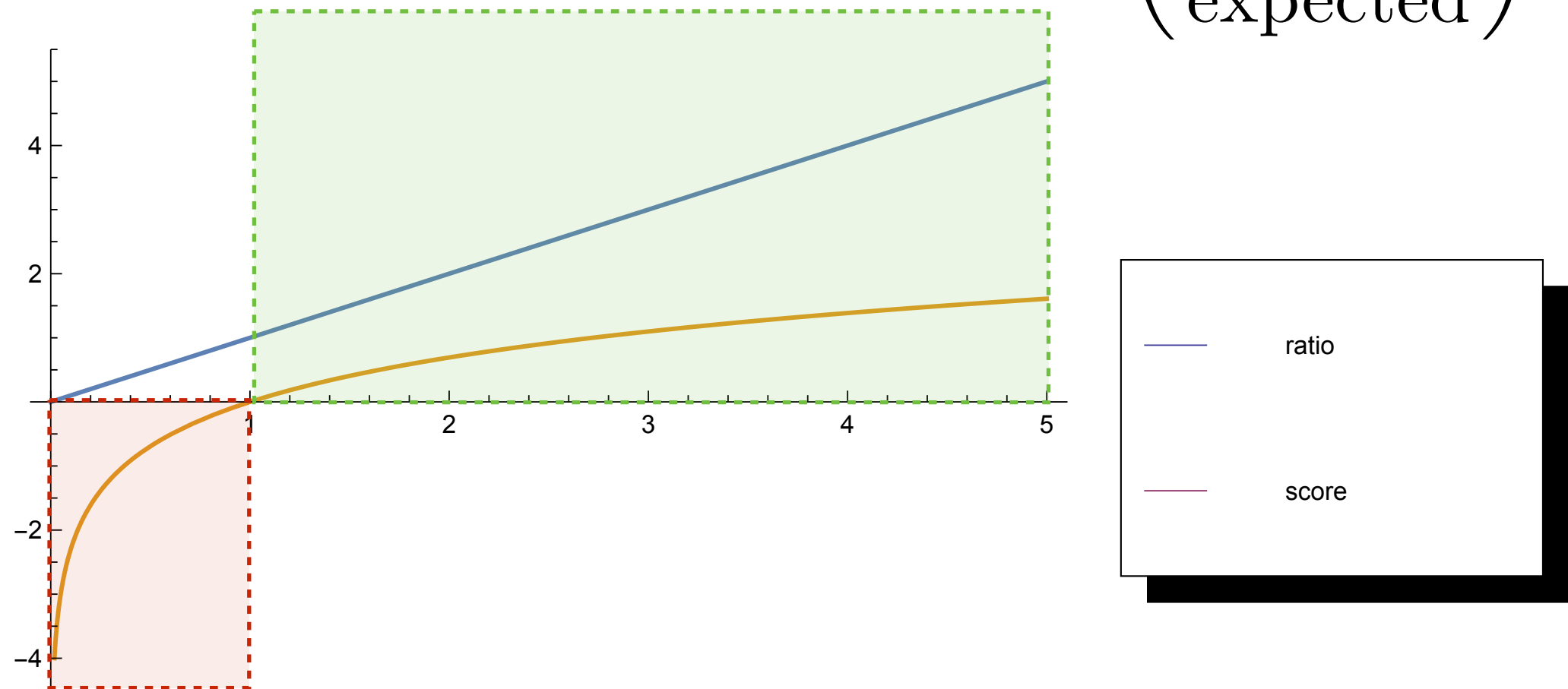


Positive scores mean we find "conservative substitutions"

Negative scores mean we find "nonconservative substitutions"

Eddy, Sean R. "Where did the BLOSUM62 alignment score matrix come from?." Nature biotechnology 22.8 (2004): 1035-1036.
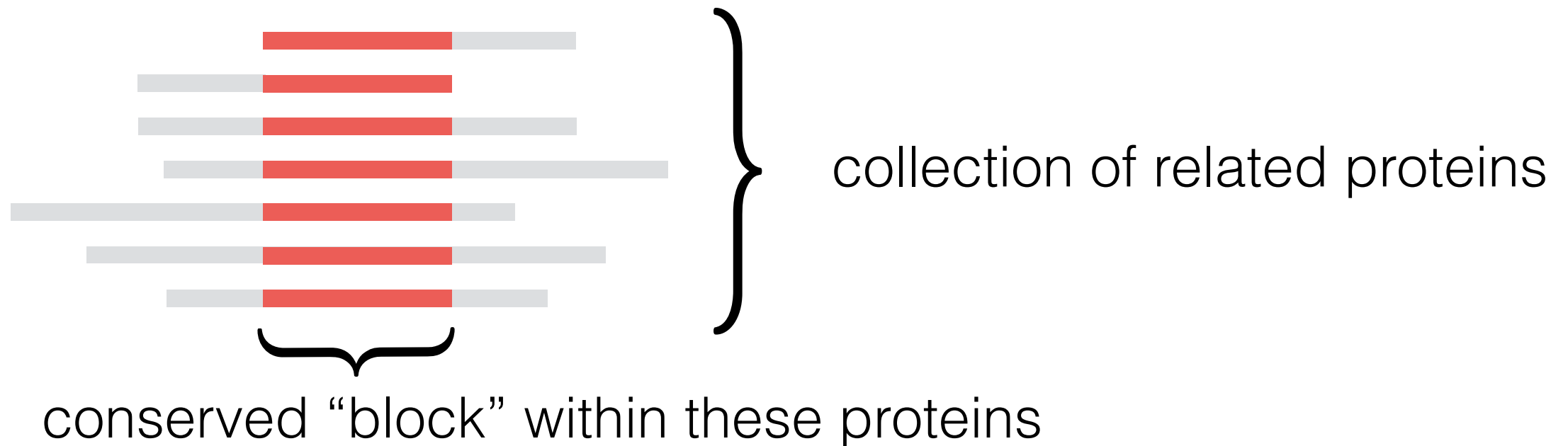
# BLOSUM matrix

Introduced by Henikoff & Henikoff in 1992

Start with the BLOCKS database (H&H '91)

1. Look for conserved (gapless, <=62% identical) regions in alignments.

2. Count all pairs of amino acids in each column of the alignments.

3. Use amino acid pair frequencies to derive "score" for a mutation/replacement

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix

Start with the BLOCKS database (H&H '91)

1. Look for conserved (gapless) regions in alignments.



collection of related proteins

conserved "block" within these proteins

sequences too similar are "clustered" & represented by either a single sequence, or a weighted combination of the cluster members

BLOSUM r: the matrix built from blocks with no more than r% of similarity – e.g., BLOSUM62 is the matrix built using sequences with no more than 62% similarity.*

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix

Start with the BLOCKS database (H&H '91)

2. Count all pairs of amino acids in each column of the alignments.
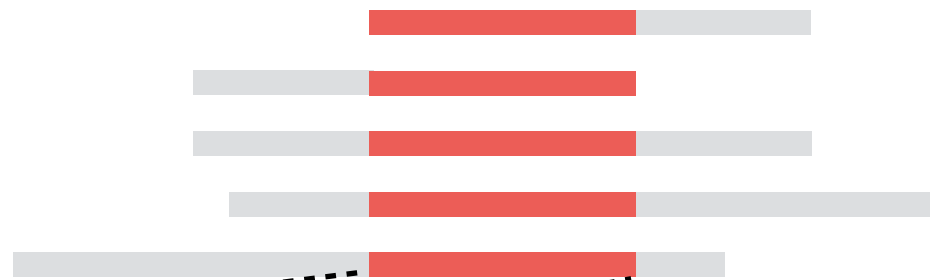


FPTADAGGRS

FVTADALGRS

FPTPDAGLRN

FVTAEAGIRQ

FPTAEAGGRS

$$c_{AB}^{(i)} = \begin{cases} \binom{c_A^{(i)}}{2} & \text{if } A = B \\ c_A^{(i)} \times c_B^{(i)} & \text{otherwise} \end{cases}$$
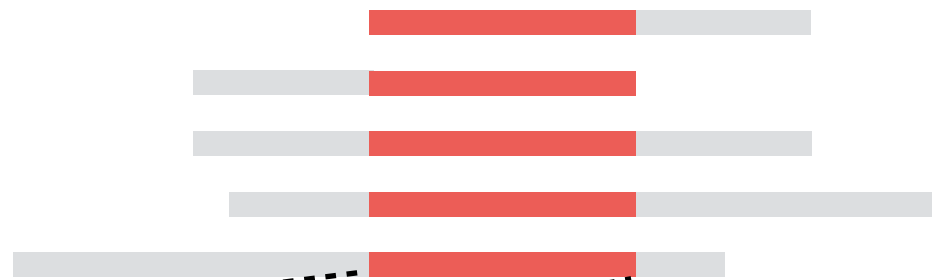
$c_A^{(i)} = $ num. of occurrences of $A$ in column $i$

What is the intuition behind this expression?

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix

Start with the BLOCKS database (H&H '91)

2. Count all pairs of amino acids in each column of the alignments.



FPTADAG**G**RS
FVTADAL**G**RS
FPTPDAG**L**RN
FVTAEAG**L**RQ
FPTAEAG**G**RS

Example:

$$c_{GG}^{(i)} = \binom{3}{2} = 3$$

$$c_{GL}^{(i)} = 3 \times 2$$

$$c_{LL}^{(i)} = \binom{2}{2} = 1$$

In this column, there are 3 ways to pair G with G, 6 potential ways to pair G with L and 1 potential way to pair L with L.

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# Computing Scores

3. Use amino acid pair frequencies to derive "score" for a mutation/replacement

Total # of potential align. between A & B:
$$c_{AB} = \sum_i c_{AB}^{(i)}$$

Total number of pairwise char. alignments:
$$T = \sum_{A \geq B} c_{AB}$$

Normalized frequency of aligning A & B:
$$q_{AB} = \frac{c_{AB}}{T}$$

# BLOSUM matrix



FPTADAGGRS

FVTADALGRS

FPTPDAGLRN

FVTAEAGLRQ

FPTAEAGGRS

In our example, we get

$$q_{GL} = \frac{0+0+0+0+0+0+4+6+0+0}{10\frac{(5)(4)}{2}} = \frac{10}{100}$$

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix



FPTADAGGRS

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In our example, we get

$$q_{GL} = \frac{0+0+0+0+0+0+4+6+0+0}{10\frac{(5)(4)}{2}} = \frac{10}{100}$$

why does this denominator work?

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix



FPTADAGGRS

FVTADALGRS

FPTPDAGLRN

FVTAEAGLRQ

FPTAEAGGRS

$c_{VP} = 2*3 = 6$

$c_{PP} = 3 \text{ choose } 2 = 3$

$c_{VV} = 2 \text{ choose } 2 = 1$

So $c_{VP} + c_{PP} + c_{VV} = 10 = 5 \text{ choose } 2$

In our example, we get

$$q_{GL} = \frac{0+0+0+0+0+0+4+6+0+0}{10\frac{(5)(4)}{2}} = \frac{10}{100}$$

why does this denominator work?

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# BLOSUM matrix



FPTADAGGRS

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FPTAEAGGRS

In our example, we get

$$q_{GL} = \frac{0+0+0+0+0+0+4+6+0+0}{10\frac{(5)(4)}{2}} = \frac{10}{100}$$

total column sum is always # rows choose 2

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# Computing Scores

3. Use amino acid pair frequencies to derive "score" for a mutation/replacement

Probability of occurrence of amino acid A in any {A,B} pair:

$$p_A = q_{AA} + \sum_{A \neq B} q_{AB}$$

Expected likelihood of each {A,B} pair, assuming independence:

$$e_{AB} = \begin{cases} (p_A)(p_B) = (p_A)^2 & \text{if } A = B \\ (p_A)(p_B) + (p_B)(p_A) = 2(p_A)(p_B) & \text{otherwise} \end{cases}$$

# Computing Scores

Recall the original idea (likelihood → scores)

$$\text{score} = \log \text{ odds ratio} = s_{AB} \propto \log\left(\frac{\text{observed}}{\text{expected}}\right)$$

$$\text{score} = \log \text{ odds ratio} = s_{AB} = \text{Round}\left(\left(\frac{1}{\lambda}\right)\log_2\left(\frac{q_{AB}}{e_{AB}}\right)\right)$$

Scaling factor used to produce scores that can be rounded to integers; set to 0.5 in H&H '92.

Henikoff, S.; Henikoff, J.G. (1992). "Amino Acid Substitution Matrices from Protein Blocks". PNAS 89 (22): 10915–10919.

# Scores are data-dependent

## distribution of amino acids across columns matters

| GG | GW |
|----|----|
| GA | GA |
| WG | GW |
| WA | GA |
| NG | GN |
| GA | GA |
| GA | GA |

$p_G = 0.5$

$e_{GG} = 0.25$

$q_{GG} = 0.214$

$s_{GG} = \text{Round}[(2)\log_2(0.214 / 0.25)]$
$= \text{Round}[(2)(-0.22)] = 0$

$p_G = 0.5$

$e_{GG} = 0.25$

$q_{GG} = 0.5$

$s_{GG} = \text{Round}[(2)\log_2(0.5 / 0.25)]$
$= \text{Round}[(2)(1)] = 2$

# Scores are data-dependent

| {G,W} observed a lot | {G,W} observed rarely |
|:---:|:---:|
| GG | GW |
| GA | GA |
| WG | GW |
| AW | GA |
| NG | GN |
| GA | GA |
| GA | AG |

$p_G = 0.5 \qquad p_W = 0.143$

$e_{GW} = 0.143$

$q_{GW} = 0.167$

$s_{GW} = \text{Round}[(2)\log_2(0.167 / 0.143)]$
$= \text{Round}[(2)(0.224)] = 0$

$p_G = 0.5 \qquad p_W = 0.143$

$e_{GW} = 0.143$

$q_{GW} = 0.048$

$s_{GW} = \text{Round}[(2)\log_2(0.048 / 0.143)]$
$= \text{Round}[(2)(-1.575)] = -3$

adopted from: http://www.bioinfo.rpi.edu/bystrc/courses/biol4540/lecture5.pdf

# Example

FPTADAGGRS

FVTADALGRS

FPTPDAGLRN

FVTAEAGLRQ

FPTAEAGGRS

$$c_{AB} = \sum_i c_{AB}^{(i)} \longrightarrow$$

## Matrix of $c_{AB}$ values

|   | A | D | E | F | G | L | N | P | Q | R | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 16 |   |   |   |   |   |   |   |   |   |   |   |   |
| **D** |   | 3 |   |   |   |   |   |   |   |   |   |   |   |
| **E** |   | 6 | 1 |   |   |   |   |   |   |   |   |   |   |
| **F** |   |   |   | 10 |   |   |   |   |   |   |   |   |   |
| **G** |   |   |   |   | 9 |   |   |   |   |   |   |   |   |
| **L** |   |   |   |   | 10 | 1 |   |   |   |   |   |   |   |
| **N** |   |   |   |   |   |   | 0 |   |   |   |   |   |   |
| **P** | 4 |   |   |   |   |   |   | 3 |   |   |   |   |   |
| **Q** |   |   |   |   |   |   | 1 |   | 0 |   |   |   |   |
| **R** |   |   |   |   |   |   |   |   |   | 10 |   |   |   |
| **S** |   |   |   |   |   |   | 3 |   | 3 |   | 3 |   |   |
| **T** |   |   |   |   |   |   |   |   |   |   |   | 10 |   |
| **V** |   |   |   |   |   |   |   | 6 |   |   |   |   | 1 |

# Example

## Matrix of q$_{AB}$ values

**C$_{AB}$**

$$q_{AB} = \frac{c_{AB}}{T}$$

$$p_A = q_{AA} + \sum_{A \neq B} \frac{q_{AB}}{2}$$
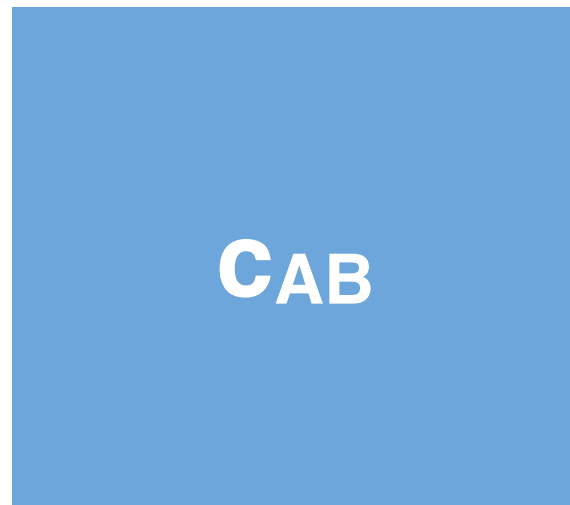
|   | A | D | E | F | G | L | N | P | Q | R | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.16 | | | | | | | | | | | | |
| D | | 0.03 | | | | | | | | | | | |
| E | | 0.06 | 0.01 | | | | | | | | | | |
| F | | | | 0.1 | | | | | | | | | |
| G | | | | | 0.09 | | | | | | | | |
| L | | | | | 0.1 | 0.01 | | | | | | | |
| N | | | | | | | 0 | | | | | | |
| P | 0.04 | | | | | | | 0.03 | | | | | |
| Q | | | | | | | 0.01 | | 0 | | | | |
| R | | | | | | | | | | 0.1 | | | |
| S | | | | | | | 0.03 | | 0.03 | | 0.03 | | |
| T | | | | | | | | | | | | 0.1 | |
| V | | | | | | | | 0.06 | | | | | 0.01 |

| P$_A$ | P$_D$ | P$_E$ | P$_F$ | P$_G$ | P$_L$ | P$_N$ | P$_P$ | P$_Q$ | P$_R$ | P$_S$ | P$_T$ | P$_V$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.18 | 0.06 | 0.04 | 0.1 | 0.14 | 0.06 | 0.02 | 0.08 | 0.02 | 0.1 | 0.06 | 0.1 | 0.04 |
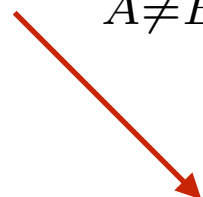
# Example

## Matrix of $e_{AB}$ values

|   | A | D | E | F | G | L | N | P | Q | R | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0.0324 | | | | | | | | | | | | |
| **D** | 0.0216 | 0.0036 | | | | | | | | | | | |
| **E** | 0.0144 | 0.0048 | 0.0016 | | | | | | | | | | |
| **F** | 0.0360 | 0.0120 | 0.0080 | 0.0100 | | | | | | | | | |
| **G** | 0.0504 | 0.0168 | 0.0112 | 0.0280 | 0.0196 | | | | | | | | |
| **L** | 0.0216 | 0.0072 | 0.0048 | 0.0120 | 0.0168 | 0.0036 | | | | | | | |
| **N** | 0.0072 | 0.0024 | 0.0016 | 0.0040 | 0.0056 | 0.0024 | 0.0004 | | | | | | |
| **P** | 0.0288 | 0.0096 | 0.0064 | 0.0160 | 0.0224 | 0.0096 | 0.0032 | 0.0064 | | | | | |
| **Q** | 0.0072 | 0.0024 | 0.0016 | 0.0040 | 0.0056 | 0.0024 | 0.0008 | 0.0032 | 0.0004 | | | | |
| **R** | 0.0360 | 0.0120 | 0.0080 | 0.0200 | 0.0280 | 0.0120 | 0.0040 | 0.0160 | 0.0040 | 0.0100 | | | |
| **S** | 0.0216 | 0.0072 | 0.0048 | 0.0120 | 0.0168 | 0.0072 | 0.0024 | 0.0096 | 0.0024 | 0.0120 | 0.0036 | | |
| **T** | 0.0360 | 0.0120 | 0.0080 | 0.0200 | 0.0280 | 0.0120 | 0.0040 | 0.0160 | 0.0040 | 0.0200 | 0.0120 | 0.0100 | |
| **V** | 0.0144 | 0.0048 | 0.0032 | 0.0080 | 0.0112 | 0.0048 | 0.0016 | 0.0064 | 0.0016 | 0.0080 | 0.0048 | 0.0080 | 0.0016 |

$p_A$

$q_{AB}$
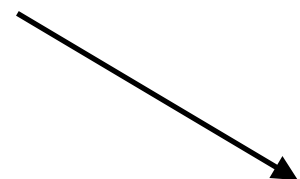
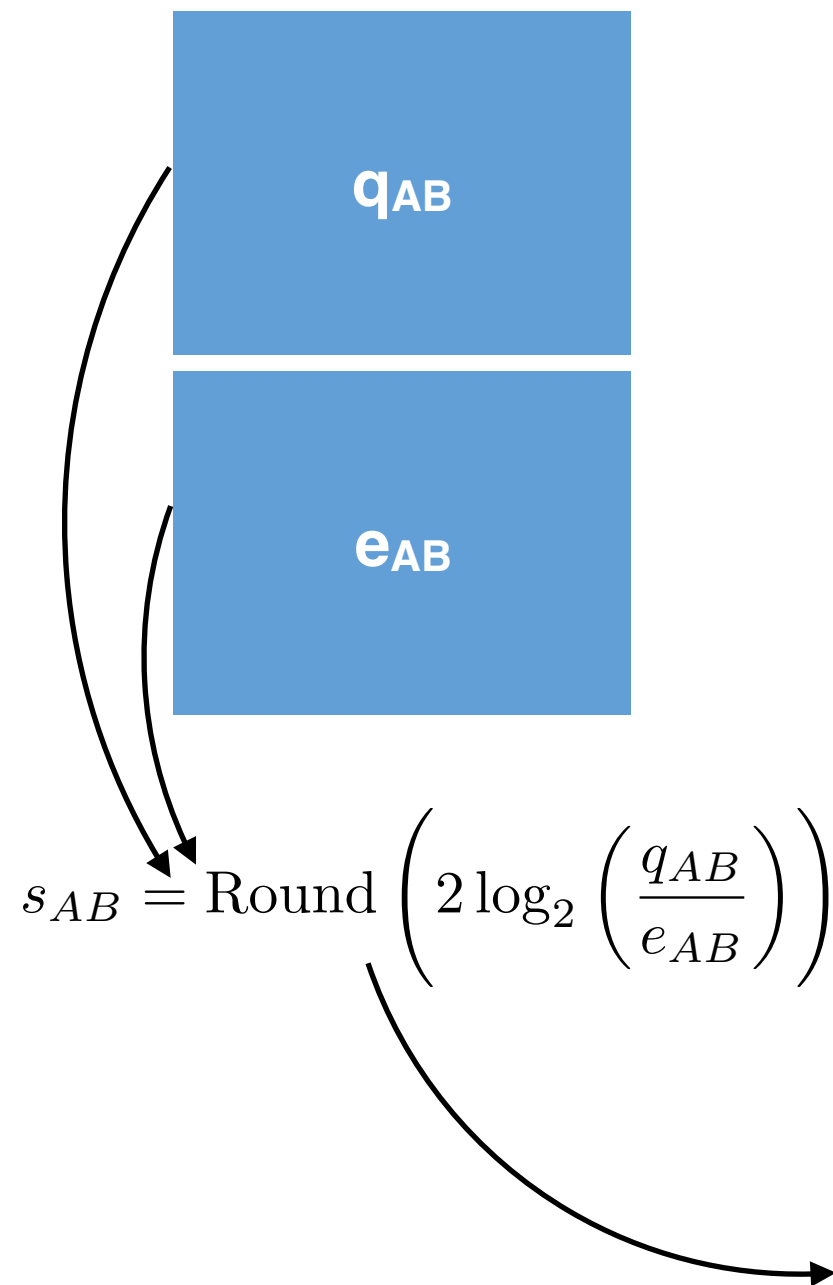$$e_{AB} = \begin{cases} (p_A)(p_B) = (p_A)^2 & \text{if } A = B \\ (p_A)(p_B) + (p_B)(p_A) = 2(p_A)(p_B) & \text{otherwise} \end{cases}$$

# Example

## Matrix of scores

|   | A | D | E | F | G | L | N | P | Q | R | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 |   |   |   |   |   |   |   |   |   |   |   |   |
| **D** |   | 6 |   |   |   |   |   |   |   |   |   |   |   |
| **E** |   | 7 | 5 |   |   |   |   |   |   |   |   |   |   |
| **F** |   |   |   | 7 |   |   |   |   |   |   |   |   |   |
| **G** |   |   |   |   | 4 |   |   |   |   |   |   |   |   |
| **L** |   |   |   |   | 5 | 3 |   |   |   |   |   |   |   |
| **N** |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **P** | 1 |   |   |   |   |   |   | 4 |   |   |   |   |   |
| **Q** |   |   |   |   |   |   | 7 |   |   |   |   |   |   |
| **R** |   |   |   |   |   |   |   |   |   | 7 |   |   |   |
| **S** |   |   |   |   |   |   | 7 |   | 7 |   | 6 |   |   |
| **T** |   |   |   |   |   |   |   |   |   |   |   | 7 |   |
| **V** |   |   |   |   |   |   |   |   | 6 |   |   |   | 5 |

$$s_{AB} = \text{Round}\left(2\log_2\left(\frac{q_{AB}}{e_{AB}}\right)\right)$$

$q_{AB}$

$e_{AB}$

Blank cells are "missing data" (i.e. no observed values); wouldn't happen with sufficient training data.

# Dealing with sequence redundancy

E.g., for BLOSUM-80, group sequences that are >80% similar

```
TCMN_STRGA ( 331)   IADLGGGDGWFLAQILRRHPHATGLLMDLPRVA   74
TCMO_STRGA ( 173)   FVDLGGARGNLAAHLHRAHPHLRATCFDLPEME   81
ZRP4_MAIZE ( 204)   LVDVGGGIGAAAQAISKAFPHVKCSVLDLAHVV   68

COMT_EUCGU ( 205)   VVDVGGGTGAVLSMIVAKYPSMKGINFDLPHVI   42  ⎤
CHMT_POPTM ( 204)   LVDVGGGTGAVVNTIVSKYPSIKGINFDLPHVI   41  ⎬  1 sequence (1/3 for each)
COMT_MEDSA ( 204)   LVDVGGGTGAVINTIVSKYPTIKGINFDLPHVI   47  ⎦

CRTF_RHOSH ( 205)   LMDVGGGTGAFLAAVGRAYPLMELMLFDLPVVA   59
OMTA_ASPPA ( 250)   VVDVGGGRGHLSRRVSQKHPHLRFIVQDLPAVI   47
```

- Sequences are not independent because they are closely related, in this case COMT_EUCGU, CHMT_POPTM, and COMT_MEDSA are all >80 identical, and the others are more different

- BLOSUM approach accounts for this by treating the group of 3 as a count of 1

- One then gets a Weighted (BLOSUM 80) count of transitions for column 1:

$$c_{FF} = 0 \quad c_{FI} = 1 \quad c_{FL} = 2.67 \quad c_{FV} = 1.33$$
$$c_{II} = 0 \quad c_{IL} = 2.67 \quad c_{IV} = 1.33$$
$$c_{LL} = 2.33 \quad c_{LV} = 3.33$$
$$c_{VV} = 0.33$$

(slide from Michael Gribskov)

# Other Scoring Matrices

## PAM vs. BLOSUM

| PAM | BLOSUM |
|---|---|
| To compare the closely related sequences, PAM matrices with lower numbers are created. | To compare the closely related sequences, BLOSUM matrices with higher numbers are created. |
| To compare the distantly related proteins, PAM matrices with high numbers are created. | To compare the distantly related proteins, BLOSUM matrices with low numbers are created. |

| PAM | BLOSUM |
|---|---|
| PAM100 | BLOSUM90 |
| PAM120 | BLOSUM80 |
| PAM160 | BLOSUM60 |
| PAM200 | BLOSUM52 |
| PAM250 | BLOSUM45 |

# Other Scoring Matrices

## PAM vs. BLOSUM

| PAM | BLOSUM |
|---|---|
| Based on global alignments of closely related proteins. | Based on local alignments of protein segments. |
| PAM1 is the matrix calculated from comparisons of sequences with no more than 1% divergent | BLOSUM 62 is calculated from comparisons of sequences no less than 62% identical |
| Other PAM matrices are extrapolated from PAM1 | Other BLOSUM matrices are not extrapolated, but computed based on observed alignments at different identity percentage |
| Larger numbers in name denote larger evolutionary distance | Larger numbers in name denote higher sequence similarity (& therefore smaller evolutionary distance) |
| Based on explicit, Markovian, model of evolution | Not based on any explicit model of evolution, but learned empirically from alignments |

from: http://en.wikipedia.org/wiki/BLOSUM

# What about gap penalties?

Despite some work[+], the setting of gap penalties is still much more arbitrary than the selection of a substitution matrix.

[*]Gap penalty values are designed to reduce the score when an alignment has been disturbed by indels. The value should be small enough to allow a previously accumulated alignment to continue with an insertion of one of the sequences, but should not be so large that this previous alignment score is removed completely.

Changing the gap function can have significant effects on reported alignments. People often resort to "defaults" to avoid having to justify a choice.

[+]Reese, J. T., and William R. Pearson. "Empirical determination of effective gap penalties for sequence comparison." Bioinformatics 18.11 (2002): 1500-1507.

[*] http://en.wikipedia.org/wiki/Gap_penalty