

## Estimation of pseudo counts

The equation used to estimate frequencies in a weight matrix is

$$p_a = \frac{\alpha \cdot f_a + \beta \cdot g_a}{\alpha + \beta}$$

where  $\alpha$  is the number of sequence in the multiple alignment (minus 1),  $\beta$  is the weight on prior (or weight on pseudo counts),  $f_a$  is the observed frequency for amino acid a and  $g_a$  is the pseudo frequency for amino acid a.

The pseudo frequency is estimated using the relation

$$g_a = \sum_b f_b \cdot q(a|b)$$

where  $f_b$  is the observed frequency for amino acid b, and  $q(a|b)$  is the Blosum substitution frequency for the amino acid a, conditional on the observation of amino acid b.

Once you have estimated the frequency  $p_a$ , the weight matrix values are calculated using the relation

$$W_a = 2 * \frac{\log(\frac{p_a}{q_a})}{\log 2}$$

where  $p_a$  is the frequencies of amino acid a at position i in the motif, and  $q_a$  is the background frequency of amino acid a (see last page).

The Blosum62 substitution matrix and a table of the 20 background frequencies are given on the last page.

Say, you have the following 6 sequences

EDRYK  
EHYLK  
QGHLP  
EHL YR  
EHQEA  
EHYLR

Estimate the observed frequencies ( $f_a$ ), the pseudo frequencies ( $g_a$ ), and the combined frequencies  $p_a$  at P1 for the 20 amino acids (fill out the table below). Use  $\beta=5$  and no sequence weighting.

Say you want to calculate the values for A. Then

$$f(A) = 0.0$$

$$g(A) = f(E) \cdot q(A|E) + f(Q) \cdot q(A|Q) = 5/6 \cdot 0.06 + 1/6 \cdot 0.06 = 0.06$$

$$p(A) = (5 \cdot 0.0 + 5 \cdot 0.06) / 10 = 0.03$$

$$w(A) = 2 \cdot \log(0.03 / 0.074) / \log(2) = -2.61$$

since the other 18 amino acids have frequency values of 0.

	$f_a$	$g_a$	$p_a$	$w_a$
A	0	0.06	0.03	-2.61
R	0	0.053	0.027	-1.93
N	0	0.04	0.02	-2.33
D	0	0.083	0.042	-0.75
C	0	0.01	0.005	-4.64
Q	0.167	0.085	0.126	3.78
E	0.833	0.267	0.550	6.70
G	0	0.04	0.02	-3.78
H	0	0.03	0.015	-1.59
I	0	0.022	0.011	-5.30
L	0	0.042	0.021	-4.50
K	0	0.082	0.041	-1.01
M	0	0.012	0.006	-4.19
F	0	0.018	0.009	-4.72
P	0	0.028	0.014	-2.92
S	0	0.06	0.03	-1.85
T	0	0.04	0.02	-2.70
W	0	0.01	0.005	-2.76
Y	0	0.02	0.01	-3.36
V	0	0.032	0.016	-4.41

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	0.29	0.03	0.03	0.03	0.02	0.03	0.04	0.08	0.01	0.04	0.06	0.04	0.02	0.02	0.03	0.09	0.05	0.01	0.02	0.07
R	0.04	0.34	0.04	0.03	0.01	0.05	0.05	0.03	0.02	0.02	0.05	0.12	0.02	0.02	0.02	0.04	0.03	0.01	0.02	0.03
N	0.04	0.04	0.32	0.08	0.01	0.03	0.05	0.07	0.03	0.02	0.03	0.05	0.01	0.02	0.02	0.07	0.05	0.00	0.02	0.03
D	0.04	0.03	0.07	0.40	0.01	0.03	0.09	0.05	0.02	0.02	0.03	0.04	0.01	0.01	0.02	0.05	0.04	0.00	0.01	0.02
C	0.07	0.02	0.02	0.02	0.48	0.01	0.02	0.03	0.01	0.04	0.07	0.02	0.02	0.02	0.02	0.04	0.04	0.00	0.01	0.06
Q	0.06	0.07	0.04	0.05	0.01	0.21	0.10	0.04	0.03	0.03	0.05	0.09	0.02	0.01	0.02	0.06	0.04	0.01	0.02	0.04
E	0.06	0.05	0.04	0.09	0.01	0.06	0.30	0.04	0.03	0.02	0.04	0.08	0.01	0.02	0.03	0.06	0.04	0.01	0.02	0.03
G	0.08	0.02	0.04	0.03	0.01	0.02	0.03	0.51	0.01	0.02	0.03	0.03	0.01	0.02	0.02	0.05	0.03	0.01	0.01	0.02
H	0.04	0.05	0.05	0.04	0.01	0.04	0.05	0.04	0.35	0.02	0.04	0.05	0.02	0.03	0.02	0.04	0.03	0.01	0.06	0.02
I	0.05	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.27	0.17	0.02	0.04	0.04	0.01	0.03	0.04	0.01	0.02	0.18
L	0.04	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.12	0.38	0.03	0.05	0.05	0.01	0.02	0.03	0.01	0.02	0.10
K	0.06	0.11	0.04	0.04	0.01	0.05	0.07	0.04	0.02	0.03	0.04	0.28	0.02	0.02	0.03	0.05	0.04	0.01	0.02	0.03
M	0.05	0.03	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.10	0.20	0.04	0.16	0.05	0.02	0.04	0.04	0.01	0.02	0.09
F	0.03	0.02	0.02	0.02	0.01	0.01	0.02	0.03	0.02	0.06	0.11	0.02	0.03	0.39	0.01	0.03	0.03	0.02	0.09	0.06
P	0.06	0.03	0.02	0.03	0.01	0.02	0.04	0.04	0.01	0.03	0.04	0.04	0.01	0.01	0.49	0.04	0.04	0.00	0.01	0.03
S	0.11	0.04	0.05	0.05	0.02	0.03	0.05	0.07	0.02	0.03	0.04	0.05	0.02	0.02	0.03	0.22	0.08	0.01	0.02	0.04
T	0.07	0.04	0.04	0.04	0.02	0.03	0.04	0.04	0.01	0.05	0.07	0.05	0.02	0.02	0.03	0.09	0.25	0.01	0.02	0.07
W	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.03	0.05	0.02	0.02	0.06	0.01	0.02	0.02	0.49	0.07	0.03
Y	0.04	0.03	0.02	0.02	0.01	0.02	0.03	0.02	0.05	0.04	0.07	0.03	0.02	0.13	0.02	0.03	0.03	0.03	0.32	0.05
V	0.07	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.16	0.13	0.03	0.03	0.04	0.02	0.03	0.05	0.01	0.02	0.27

# Background frequencies

A 0.07400  
R 0.05200  
N 0.04500  
D 0.05400  
C 0.02500  
Q 0.03400  
E 0.05400  
G 0.07400  
H 0.02600  
I 0.06800  
L 0.09900  
K 0.05800  
M 0.02500  
F 0.04700  
P 0.03900  
S 0.05700  
T 0.05100  
W 0.01300  
Y 0.03200  
V 0.07300