

## 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 Blosom 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 Blosom62 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.

	$f_a$	$g_a$	$p_a$	$w_a$
A				
R				
N				
D				
C				
Q				
E				
G				
H				
I				
L				
K				
M				
F				
P				
S				
T				
W				
Y				
V				

Blosum substitution frequencies. Each row gives the frequency of  $q(J|I)$ . That is the first row gives the frequencies of observing the 20 amino acids given you have observed the amino acid I.

	P(J I)																			
	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 ( $q_a$ )

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