Additional file 2

Additional text – **Relationship between** $R_{iw}(b,l)$ and $R_{sequence}(l)$:

The individual information content of each individual splice site was calculated using the following equation [29, 30]:

$$R_{sequence}(l) = 2 + \sum_{b \in A.C.G.T} f(b,l) \log_2 f(b,l)$$
 (2)

where, f(b,l) is the probability of nucleotide b at position l. To calculate the information content $(R_i,bits)$ of each splice site, at first, an individual information weight matrix is generated from the frequencies of each nucleotide at each position. The individual information weight matrix $R_{i,w}(b,l)$ can be calculated from the following equation [29, 30]:

$$R_{i,w}(b,l) = 2 + \log_2 f(b,l)$$
 (3)

The information content of each splice site was calculated by summing up $R_{i,w}(b,l)$ at each position of the splice site sequences. The relationship between $R_{i,w}(b,l)$ and $R_{sequence}(l)$ is discussed below.

In a set of aligned sequences the jth sequence is represented by a matrix s(b,l,j), where b is the base at position l. The individual information of sequence j is the dot product between the sequence and the weight matrix $R_{i,w}(b,l)$ as given below:

$$R_{i}(j) = \sum_{l} \sum_{b=1}^{T} s(b, l, j) R_{iw}(b, l)$$

$$\tag{4}$$

The frequency matrix f(b,l) is created by aligning n individual sequences and given by:

$$f(b,l) = \frac{1}{n} \sum_{i=1}^{n} s(b,l,j)$$
(5)

As the frequencies also sum to one, we can write:

$$\sum_{b=A}^{T} f\left(b, l\right) = 1 \tag{6}$$

The mean information content of n sequences used to create the frequency matrix f(b,l) is given by:

$$E(R_i) = \frac{1}{n} \sum_{i=1}^{n} R_i(j) \tag{7}$$

From equation (3) and (4) we get:

$$R_{i}(j) = \sum_{l} \sum_{b=A}^{T} s(b, l, j) [2 + \log_{2} f(b, l)]$$
 (8)

From equation (7) and (8) we get:

$$E(R_i) = \frac{1}{n} \sum_{i=1}^{n} \sum_{l} \sum_{b=A}^{T} s(b, l, j) [2 + \log_2 f(b, l)]$$
 (9)

From equation (5) and (9) we get:

$$E(R_i) = \sum_{l} \sum_{b=A}^{T} f(b, l) [2 + \log_2 f(b, l)]$$
 (10)

$$= \sum_{l} \sum_{b=A}^{T} 2f(b,l) + \sum_{l} \sum_{b=A}^{T} f(b,l) \log_{2} f(b,l)$$

From equation (6) and (10) we get:

$$E(R_i) = \sum_{l} 2 + \sum_{l} \sum_{b=A}^{T} f(b, l) \log_2 f(b, l)$$
(11)

The right hand side of equation (11) is similar to that of $R_{sequence}(l)$ in equation (2). Hence, it can be deduced that the average of individual information content is the average information content of the sites.