## **Application of Chi-Square test in WEGO 2.0**

Suppose we have 3 valid input files from samples s1, s2 and s3. To test if there is a sample difference in gene enrichments involved in biological process, WEGO constructed a contingency table of 3×2 (see Table 1) separating the genes by samples or annotated as GO terms of biological process.

If  $O_{ij}$  is the observed number and  $E_{ij}$  is the expected number of genes corresponding to the j<sup>th</sup> sample and the i<sup>th</sup> condition (whether annotated as GO terms), then chi-square is:

$$\chi^{2} = \sum_{i} \sum_{j} \frac{(O_{ij} - E_{ij})^{2}}{E_{ij}}$$
(1)

where  $E_{ij}$  is obtained as  $E_{ij} = \frac{R_i \times C_j}{TotalSum}$  (2).

	s1	s2	d3	Total
In biological process	g11	g12	g13	R1=g11+g12+g13
Not in biological process	g21	g22	g23	R2=g21+g22+g23
Total	C1=g11+g21	C2=g12+g22	C3=g13+g23	Total Sum= g11+g12+g13+ g21+g22+g23

Table 1 Example of contingency table of 3 input samples

The P-value is obtained when projecting the value of chi-square to the chi-square distribution with 2 degrees of freedom.