Studying the expression of genes at mRNA level is a basic step for many biological studies. The fast advancing next generation sequencing technology has opened a new page for measuring RNA abundances by sequencing or RNA-Seq. RNA-Seq has revealed many new observations on the characteristics of mRNA expression especially on the wide existence of alternative splicing. The estimation of gene expression values and identification of differentially expressed genes become a challenging task in this context. We have developed a series of methods on the estimation of expression of alternative isoforms and of the whole gene, and comparing differential expression and differential splicing of genes between samples or groups of samples. One basic challenge is the modeling RNA-Seq data that have non-uniform distribution of reads on the transcripts. We developed a non-parametric method to model and correct for the non-uniform distribution of reads. Experiments on both simulated and real data showed that the method can estimate the expression of isoforms and of the whole genes more accurately, and can identify major isoforms with higher fidelity.