

GOSJ: An R package for adjusting bias from gene structure on identifying differentially expressed genes in RNA-Seq data analysis

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May 13, 2016

Motivation:

Differential exons or splicing junctions usage analysis is very helpful for detecting alternative splicing events. There are several studies that researchers attempt to understand alternative splicing events by performing differential exons or/and splicing junctions usage analysis. In these analysis, the differentially expressed gene are derived based on differential exon or/and splicing junction usage analysis, then subsequent gene set or/and pathway analysis are performed based on differentially expressed gene list. However, it is observed that the derived differentially expressed gene list from differential exon or/and splicing junction analysis is biased by the number of exons or/ and splicing junctions within gene, and cause further bias in subsequent gene set and pathway analysis.

Results:

We demonstrate this bias using published data from a study of differential splicing junction usage in Myelodysplastic syndromes(MDS) and a data set that we obtained in a study of differential exons and splicing junctions usage in blood cancer. We show that the identified differentially expressed genes through using differential usage of gene features(exons and/or splicing junctions) are biased by the number of features within gene, and subsequently lead to the bias in following Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) enrichment analysis. We develop an approach to adjust this bias. A R package(GOSJ) based on this approach is also implemented to make this method be available for other researchers. This GOSJ package is accessible on <https://github.com/aiminy/GOSJ.git>.

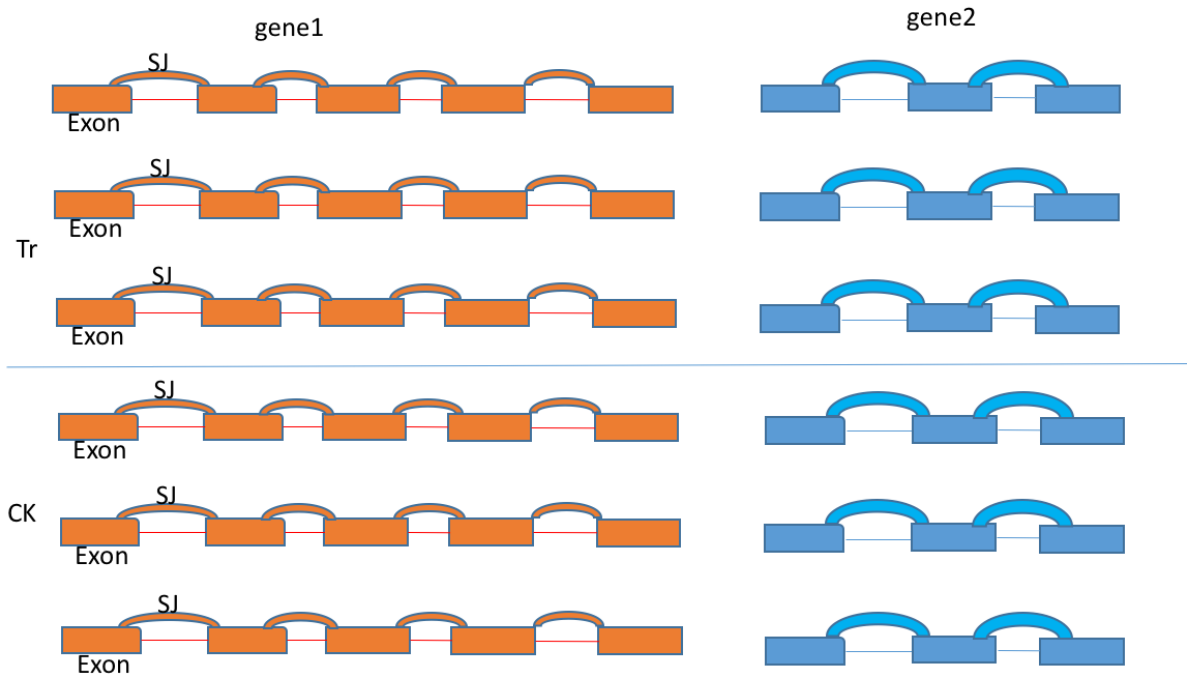
Introduction

RNA-Seq data has been extensively used to measure expression of transcripts, and to investigate alternative splicing, allele specific expression and RNA editing. Several methods have been developed to study alternative splicing. One of these methods is to focus on studying differential feature(exons or/and splicing junction) usage to understand alternative splicing. In these studies, the counts of features are obtained using the annotated gene model(see the following Figure), and are used to calculate usage of these features through the following method:

$$Usage\ of\ feature\ i = \frac{counts\ of\ feature\ i}{counts\ of\ other\ features\ excluding\ feature\ i}$$

Based on the usage of exons and splicing junctions, differential usage between two conditions are identified. The differentially expressed genes are derived from the differential usage of exons and splicing junctions within genes, subsequent gene sets or/and pathway analysis are performed using this derived differentially expressed genes. It is observed that identifying differentially expressed genes using this approach is biased by the number of exons and splicing junctions.

There are several previous studies that showed using RNA-Seq data to study differentially expressed genes and subsequent gene sets or pathways analysis could be biased by read counts of transcripts and length



The probability to declare that gene is differentially expressed(p value in DE) is related to gene structure(how many exons or/and how many SJ) if using gene sub-feature based counts

Figure 1: Bias from gene structure

of transcripts. Several methods are also developed to correct these biases either in the step of identifying differentially genes or in the step of performing gene sets enrichment analysis(Gao et al. 2011). A well known R package for correcting bias in gene set enrichment analysis, GoSeq, is available(Young et al. 2010). A logistic regression based approach is also developed for adjusting length bias of transcript in Gene Ontology enrichment analysis(Mi et al. 2012).

Similar situations are also observed in analyzing genome-wide methylation data. Several studies found that number of methylation within genes could cause a bias to identify diffenertially methylated genes. An R package is also available for correcting this bias(Phipson, Maksimovic, and Oshlack 2016).

However, bias on identifying differntilly expressed genes due to different gene structure and its corrections have not been addressed in detail. In this paper, we explore to identify the bias factor on RNA-Seq data related to gene structure, we demonstrate that by identifying right bais factors could lead to the better adjustment on DE analysis and the following gene GO term and pathway enrichment analysis. A R package based on this analysis procedure is also developed.

RESULTS AND DISCUSSIONS

Analysis on several real data sets

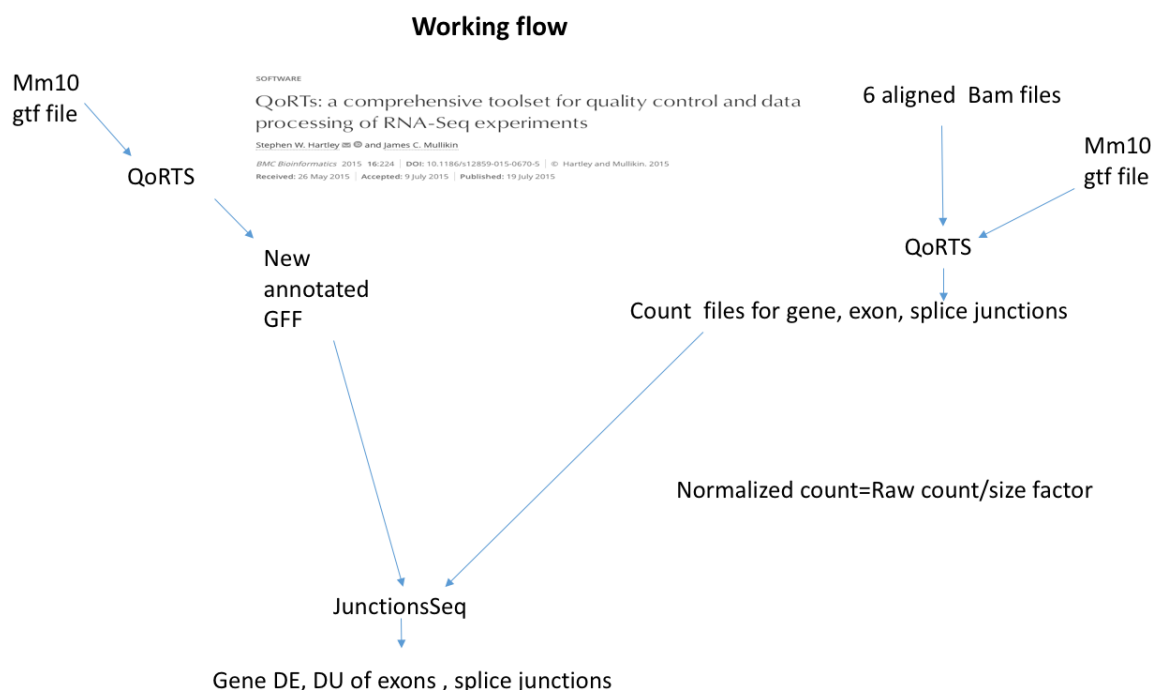


Figure 2: working flow

1. Reanalysis for a data set in Myelodysplastic syndromes(MDS) study
2. Analysis for a data aset we obtained in blood cancer study

A data set including 6 samples is used for developing this approach. This 6 samples belong to two conditions, and there are 3 samples in each condition. The reads in each sample is aligned to the mouse mm10 reference

genome using Tophat. Gene models is based on UCSC mm10 refSeq. We performed analysis based on 2 scenarios:

In the first scenario, we use gene-based counts to identify differentially expressed genes, then using this differentially expressed gene list to identify the relationship between the proportion of DE and the possible bias factors such as gene length, number of exons and number of splicing junctions.

In the second scenario, Ensemble-annotated exons and junction-spanning read counts are calculated, and a negative binomial model is applied to these counts to identify differential exon and junction usage while the overall gene expression changes are controlled. In this step, the p value of differential usage of each subfeature of each gene are determined, and used to calculate the genewise p value based on the following method described in DEXSeq R package (Anders, Reyes, and Huber 2012):

let p_{il} be the p value for l subfeature (exon or splicing junction) of gene i , then genewise p value is calculated using the following method:

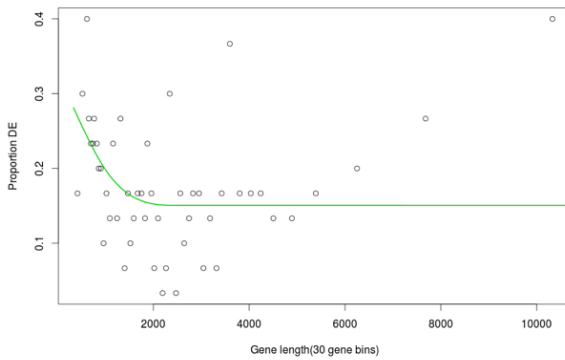
$$P(\text{at least 1 type I error among } m \text{ tests}) = \frac{\sum_{i=1}^M 1 - (1 - \theta)^{n_i}}{|i : \exists p_{il} < \theta|}$$

Based on these genewise p values, we set 0.05 as threshold to define differentially expressed gene list. We firstly applied logistic regression to identify the relationship between the differentially expressed gene list and possible bias factors such as the number splicing junction and exons.

Secondly, we use these differentially expressed gene list to identify the relationship between the proportion of DE in gene sets and the possible bias factors such as gene length, number of exons and number splicing junctions.

Differential expression as a function of gene length or number of splicing junctions

Gene length



Number of splicing junctions

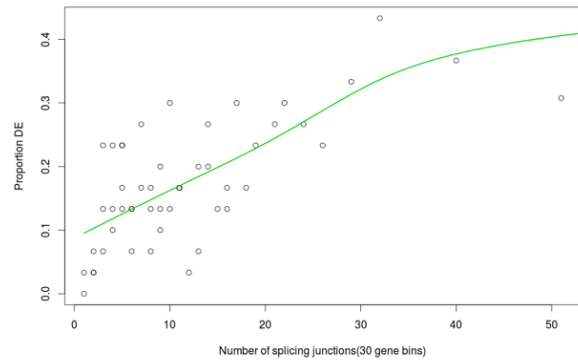
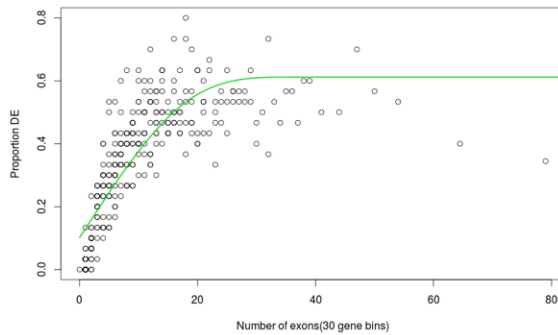


Figure 3: Bias from sj and GL

- Correction for GO term enrichment analysis
- Correction for KEGG pathway analysis.
- Correction for hallmark gene sets

Differential expression as a function of #exons or #exons plus #SJ(Splicing Junctions)

With exons



With exons plus SJs

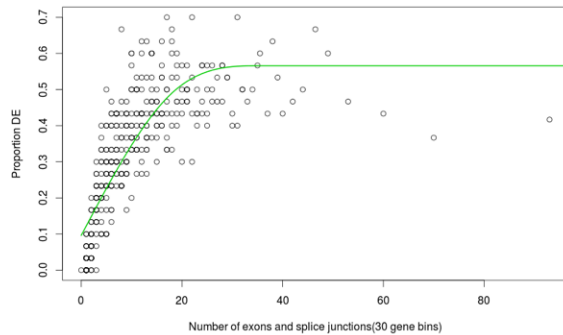


Figure 4: Bias from sj and exons

Generate permuted data set based on the data set from blood cancer.

Using the idea in (Geeleher et al. 2013), we explore to fix gene structure in the data set in blood cancer, and use permutation to generate more data sets to demonstrate the bias from gene structure. We have about 13000 genes, each gene has certain number of subfeatures, in this permutation setting, we fix the number of subfeatures for each gene as the real data, and perform the following permutations:

- Permutating sample label
- Permutating mapping counts

By this way, we explore to identify the same enriched gene sets and pathways of GO and KEGG. If there are high consistencies between the results from permuted data sets and that of real data sets, this indicates that the number of subfeatures of genes biases gene set or/and pathway analysis.

Conclusion

In summary, our work identified the potential bias in RNA-Seq data analysis when using differential exons or/and splicing junction usage to represent differentially expressed gene, and using these differentially expressed genes to perform subsequent gene set and pathway analysis. We further suggest a bias correction approach that could provide a more accurate gene set and pathway analysis. We implemented this method into an R package, and we believe that this R package could help other researchers when they perform similar type of RNA-Seq data analysis.

Reference

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