Gene expression

PLRS: a flexible tool for the joint analysis of DNA copy number and mRNA expression data

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ABSTRACT
Summary: DNA copy number and mRNA expression are commonly used data types in cancer studies. Available software for integrative analysis arbitrarily fixes the parametric form of the association between the two molecular levels and hence offers no opportunities for modelling it. We present a new tool for flexible modelling of this association. PLRS uses a wide class of interpretable models including popular ones and incorporates prior biological knowledge. It is capable to identify the gene-specific type of relationship between gene copy number and mRNA expression. Moreover, it tests the strength of the association and provides confidence intervals. We illustrate PLRS using glioblastoma data from The Cancer Genome Atlas.

Availability and implementation: PLRS is implemented as an R package and available from Bioconductor (as of version 2.12; http://bioconductor.org). Additional code for parallel computations is available as Supplementary Material.

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1 INTRODUCTION
DNA copy number aberrations are characteristics of the cancer cell. These aberrations are gains and losses of chromosomal DNA, which may alter expression levels of mRNA transcripts. The identification of genes for which an abnormal copy number affects gene expression is important in cancer studies, as these genes are likely to be relevant for tumorigenesis. Here, we present a new tool for exploratory and confirmatory analysis of such effects.

For a given gene, copy number and mRNA expression are generally believed to be concordant. The exact form of the association is usually not established. In fact, the shape is likely to differ between genes because of the presence of different (post-)transcriptional regulatory mechanisms. Tools that investigate the interaction between the two molecular levels assist in better understanding of regulatory mechanisms.

Numerous software packages have been proposed for joint analysis of copy number and gene expression data (Chari et al., 2008; Lê Cao et al., 2009; Lee and Kim, 2009; Louhimo and Hautaniemi, 2011; Salari et al., 2010; van Wieringen et al., 2006). However, most of these fix the association between DNA and RNA a priori, typically a linear or piecewise constant line. Hence, these approaches do not permit investigation or identification of the shape of the association. Recently, the need for more subtle models has been highlighted (Leday et al., 2013; Nemes et al., 2012; Solvang et al., 2011) to reflect the biological mechanisms between the two molecular levels. Here, we describe the R package PLRS that implements the framework recently proposed by Leday et al. (2013). PLRS uses piecewise linear regression splines, which allow multiple linear lines, and are a wide class of interpretable models including the linear and piecewise constant ones. It enforces concordance by restricting relevant model parameters. In addition, PLRS tests the strength of the overall association, identifies its functional shape and provides confidence intervals for the estimated curve. We illustrate PLRS using a dataset from 160 glioblastoma samples obtained from The Cancer Genome Atlas (TCGA).

2 MODEL
PLRS models cis-relationships between copy number and mRNA expression by piecewise linear regression splines (Leday et al., 2013). The relevance of this class of models is multifold. Unlike other methods, PLRS combines copy number data from various steps of the preprocessing, namely, the segmented and called data (van de Wiel et al., 2011). Segmented data are continuous (log2-values) and provide the (relative) amount of DNA copies (gene dosage), whereas called data represent discrete states associated with the various types of copy number aberration; the biological literature commonly distinguishes four of these: ‘loss’ (less than two copies of genomic DNA), ‘gain’ (two to four copies), ‘reference’ (two copies), and ‘amplification’ (more than four copies). Second, PLRS allows the effect of DNA on mRNA to differ across types of aberrations. This is biologically plausible: the efficacy of mechanisms that compensate for genomic aberrations may differ between losses, gains and amplifications. Third, good interpretability is ensured by the piecewise linearity of the model and a set of restrictions on the parameters. For example, copy number is concordant with gene expression and ‘normal’ copy number cannot severely alter gene expression.

In this context, the R package PLRS implements various statistical procedures to detect which and how gene copy number abnormalities alter the gene expression level. Identification of the functional form of the association is achieved by model selection, which automatically merges copy number states when their
association with mRNA expression can be captured with one regression line. Simultaneous confidence intervals on the selected curve are provided for more detailed description. Finally, a statistical test evaluates the significance of the overall association by testing the null hypothesis: copy number does not affect mRNA expression, leading to a single horizontal line.

3 RESULTS

We applied PLRS to a dataset of 160 glioblastoma tumour samples obtained from TCGA (http://cancergenome.nih.gov; Verhaak et al., 2010) for which copy number (Agilent CGH Microarray 244A) and mRNA expression (Agilent 244K platform) were available. We found that for many known cancer genes, the expression level is strongly associated with DNA aberrations (cf. Supplementary Material). Figure 1 depicts the DNA–mRNA association for four genes, including known aberrations (MET, ERCC2, and AGAP2). Clearly, relationships are different and demonstrate that the flexibility of the PLRS model allows new insights in the association. For gene MET, we observe that the effect of amplifications extends that of gains more than proportionally. For ERCC2, the expression level of samples with loss and normal copy number differs in average and expression increases linearly with dosage. Amplifications of gene AGAP2 have a strong effect on mRNA expression, whereas gains have none. The effect as defined by PLRS is broad and expressed by both an intercept and a slope for each copy number aberration state. The variety of models resulting from PLRS contrasts with most other methods, which impose a unique parametric form to all genes. Our method lets the data decide what is most appropriate. As a consequence, PLRS has more power than other standard methods for detecting relatively large effects occurring in small subgroups of samples (Leday et al., 2013). Note that other non-linear techniques, e.g. based on mutual information, can be competitive but less interpretable.

4 CONCLUSION

PLRS is a tool for flexible modelling of the association between DNA copy number and mRNA expression. We demonstrated its potential to reveal interesting relationships. It is particularly useful for (i) a detailed understanding of the relationship between DNA copy number and mRNA expression and (ii) powerful detection of copy number-induced sample subgroup-specific effects, thereby acknowledging heterogeneity of many cancers. The software can also be used for studying the effect of DNA copy number on microRNA expression.

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REFERENCES