Keep Me Around: Intron Retention Detection and Analysis

Harold Pimentel\textsuperscript{1}, John G. Conboy\textsuperscript{2}, and Lior Pachter\textsuperscript{*3}

\textsuperscript{1}Department of Computer Science, UC Berkeley.
\textsuperscript{2}Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory.
\textsuperscript{3}Departments of Computer Science, Mathematics and Molecular \& Cell Biology, UC Berkeley.

October 5, 2015

Abstract

Summary: We present a tool, keep me around (kma), a suite of python scripts and an R package that finds retained introns in RNA-Seq experiments and incorporates biological replicates to reduce the number of false positives when detecting retention events. \textit{kma} uses the results of existing quantification tools that probabilistically assign multi-mapping reads, thus interfacing easily with transcript quantification pipelines. The data is represented in a convenient, database style format that allows for easy aggregation across introns, genes, samples, and conditions to allow for further exploratory analysis.

Availability: The source code is available under the GPLv2 license and can be found at: http://github.com/pachterlab/kma

Contact: lpachter@math.berkeley.edu

1 Motivation

Many organisms exhibit intron retention events that can be measured with RNA-Seq [Burgess, 2014, Braunschweig et al., 2014], and recent publications suggest that these events are important constituents of transcriptome regulation. While some existing tools can detect intron retention events [Katz et al., 2010, Anders et al., 2012, Bai et al., 2015], none that we are aware of incorporate biological replicates to reduce the reporting of false-positives. Other tools have been mentioned in the literature, but do not have freely available software [Khodor et al., 2011, Wong et al., 2013, Braunschweig et al., 2014, Boutil et al., 2015]. There is therefore a need for a robust intron retention detection method that is based on rigorous quantification of intron retention followed by assessment of significance using biological replicates.

We present keep me around (kma), a set of tools for detecting intron retention in RNA-Seq experiments that utilizes biological replicates to improve accuracy. \textit{kma} currently uses the transcript quantification method eXpress [Roberts and Pachter, 2013], but is compatible with with any RNA-Seq quantification pipeline.

2 Implementation

\textit{kma} begins by performing a pre-processing step consisting of several python scripts that find “measurable” intronic regions called inclusion regions (regions in which none of the overlapping isoforms contain an exon), together with the corresponding isoforms which could retain the intron called overlap isoforms. \textit{kma} then outputs a table of intron-transcript relationships. This table includes the (1) intron coordinates, (2) intron quantification coordinates (3) transcripts which could potentially retain the intron, and (4) the gene name. The intron quantification coordinates differ from the exact intron coordinates by including a small region of the neighboring exons which is several bases shorter than the read length (Figure 1). This exonic overlap ensures that the reads spanning the intron-exon junctions are included into the intron expression. These

\textsuperscript{*}lpachter@math.berkeley.edu
reads are often valuable information; if they are unique, they give strong evidence for the expression of the intron. kma also outputs a BED track containing intronic quantification coordinates, as well as a FASTA file containing the intronic sequences to quantify against. This pre-processing step only has to be performed once assuming the transcriptome annotation does not change and read size is at least a few bases longer than exon overlap.

kma is designed to leverage existing transcript quantification methods. This allows for the computation of relative abundance of introns as well as transcripts while allowing multi-mapping reads to be processed using well understood models already developed in existing tools [Pachter, 2011; Li et al., 2010]. After the pre-processing step, the intronic sequences are added to the transcriptome and the chosen quantification method is run using the augmented transcriptome. Any method can be used, provided it outputs expression in a unit that is additive, e.g. transcripts per million (TPM).

Once introns and transcripts are quantified from all samples in the experiment, the data can be post-processed and further analyzed in an R package [R Core Team, 2014] that is part of kma. We currently provide functions to read data from eXpress, but it is quite simple to add a new function that reads in other formats; all that is required is the target identifier and corresponding expression estimate. Once data is read in, retention is computed by taking the intron expression (numerator) and summing the expression of the overlapping transcripts plus the intron expression (denominator). This calculation leads to a natural measurement of intron retention, the proportion of the transcript expression containing the intron, also known as the proportion spliced in [Katz et al., 2010].

While we store a special object of class IntronRetention, the majority of the operations depend only on the data stored in database-like data frames with each row being an intron observation from one sample. A common row contains categorical fields intron, sample, condition which serve as a key, along with measurements retention, numerator, denominator, unique reads and various columns for filters. This allows for fast aggregation and manipulation via packages such as dplyr [Wickham and Francois, 2014]. Summaries of retention across subgroups such as specific introns, conditions, or samples can be quickly computed by simple queries. We provide common summaries as functions, but the raw data frame is always available for further analysis. In addition to easy manipulation, this data format is suitable for exploratory analysis in plotting tools such as ggplot2 [Wickham, 2009].

In certain situations, estimates of the retention level can be unreliable due to low coverage in the exonic regions, or high variations in coverage due to biases or repetitive sequences. Coverage filters were implemented based on relative expression or rank, along with a “zero coverage filter”. The zero coverage filter finds the longest spanning region in an intron which has no reads starting in that region. Then, it computes the probability of observing a region of length Z with no reads starting in it, given the intron’s expression. The intron is removed from consideration if the probability is low.

Unlike other publicly available intron retention tools which simply provide an estimate for intron retention per sample, our method provides a resampling hypothesis testing procedure to determine whether the mean is greater than what one would expect due to reshuffling of the given data in those samples. The null distribution is generated from the filtered list by randomly selecting a retention value from each sample per condition B times. For each set of samples, the mean is computed. After the null distribution is generated, the p-value is computed by finding the proportion of null values that the observed mean is greater than.
This allows for a lower false-positive rate when detecting IR events. This procedure also helps shield against samples that have contamination of non-mature mRNA.

3 Discussion

We have developed an R package kma that addresses the issue of finding intron retention events. Since this tool only slightly modifies existing quantification pipelines by introducing an augmented transcriptome, kma can easily be introduced into existing RNA-Seq quantification pipelines. In R, the data is represented in a database-like format that allows for flexible and fast aggregation allowing for exploratory analysis to be carried out relatively easily. We also implemented a hypothesis testing procedure which reduces the likelihood of finding false-positive intron retention events by incorporating biological replicates.

Acknowledgement

HP was supported by the NSF GRFP. JGC and LP were supported in part by NIH R01 DK094699.

References

Simon Anders, Alejandro Reyes, and Wolfgang Huber. Detecting differential usage of exons from RNA-seq data. Genome research, 22(10):2008–2017, 2012.

Yang Bai, Shufan Ji, and Yadong Wang. IRcall and IRclassifier: two methods for flexible detection of intron retention events from RNA-seq data. BMC Genomics, 16(Suppl 2):S9, January 2015. ISSN 1471-2164. doi: 10.1186/1471-2164-16-S2-S9. URL http://www.biomedcentral.com/1471-2164/16/S2/S9.

Paul L Boutz, Arjun Bhutkar, and Phillip A Sharp. Detained introns are a novel, widespread class of post-transcriptionally spliced introns. Genes & development, 29(1):63–80, 2015.

Ulrich Braunschweig, Nuno L. Barbosa-Morais, Qun Pan, Emil N. Nachman, Babak Alipanahi, Thomas Gonatopoulos-Pournatzis, Brendan Frey, Manuel Irimia, and Benjamin J. Blencowe. Widespread intron retention in mammals functionally tunes transcriptomes. Genome Research, page gr.177790.114, September 2014. ISSN 1088-9051, 1549-5469. doi: 10.1101/gr.177790.114. URL http://www.genome.cshlp.org/content/early/2014/09/24/gr.177790.114.

Darren J. Burgess. Alternative splicing: Retaining introns to sculpt gene expression. Nature Reviews Genetics, 15(11):707–707, November 2014. ISSN 1471-0056. doi: 10.1038/nrg3844. URL http://www.nature.com/nrg/journal/v15/n11/full/nrg3844.html.

Yarden Katz, Eric T Wang, Edoardo M Airoldi, and Christopher B Burge. Analysis and design of rna sequencing experiments for identifying isoform regulation. Nature methods, 7(12):1009–1015, 2010.

Yevgenia L. Khodor, Joseph Rodriguez, Katharine C. Abruzzi, Chih-Hang Anthony Tang, Michael T. Marr, and Michael Rosbash. Nascent-seq indicates widespread cotranscriptional pre-mrna splicing in drosophila. Genes & Development, 25(23):2502–2512, 2011. doi: 10.1101/gad.178962.111. URL http://genesdev.cshlp.org/content/25/23/2502.abstract.

Bo Li, Victor Ruotti, Ron M. Stewart, James A. Thomson, and Colin N. Dewey. Rna-seq gene expression estimation with read mapping uncertainty. Bioinformatics, 26(4):493–500, 2010. doi: 10.1093/bioinformatics/btp692. URL http://bioinformatics.oxfordjournals.org/content/26/4/493.abstract.

Lior Pachter. Models for transcript quantification from rna-seq. arXiv, 1104.3889, 2011.

Harold Pimentel, Marilyn Parra, Sherry Gee, Dana Ghanem, Xiuli An, Jie Li, Narla Mohandas, Lior Pachter, and John G. Conboy. A dynamic alternative splicing program regulates gene expression during terminal erythropoiesis. Nucleic Acids Research, 42(6):4031–4042, 2014. doi: 10.1093/nar/gkt1388. URL http://nar.oxfordjournals.org/content/42/6/4031.abstract.

R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2014. URL http://www.R-project.org/.

Adam Roberts and Lior Pachter. Streaming fragment assignment for real-time analysis of sequencing experiments. Nature methods, 10(1):71–73, 2013.

Hadley Wickham. ggplot2: elegant graphics for data analysis. Springer New York, 2009. ISBN 978-0-387-98140-6. URL http://had.co.nz/ggplot2/book

Hadley Wickham and Romain Francois. dplyr: A Grammar of Data Manipulation, 2014. URL http://cran.r-project.org/package=dplyr.

Justin J.-L. Wong, William Ritchie, Olivia A. Ebner, Matthias Selbach, Jason A.W.H. Wong, Yiahou Huang, Dadi Gao, Natalia Pinello, Maria Gonzalez, Kinsha Baidya, Annora Thoeng, Teh-Liane Khoo, Charles G. Bailey, Jeff Holst, and John E.J. Rasko. Orchestrated intron retention regulates normal granulocyte differentiation. Cell, 154(3):583 – 595, 2013. ISSN 0092-8674. doi: http://dx.doi.org/10.1016/j.cell.2013.06.052. URL http://www.sciencedirect.com/science/article/pii/S0092867413008345.