Sequence analysis

Snaptron: querying splicing patterns across tens of thousands of RNA-seq samples

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Associate Editor: Alfonso Valencia

Received on February 16, 2017; revised on August 3, 2017; editorial decision on August 30, 2017; accepted on August 31, 2017

Abstract

Motivation: As more and larger genomics studies appear, there is a growing need for comprehensive and queryable cross-study summaries. These enable researchers to leverage vast datasets that would otherwise be difficult to obtain.

Results: Snaptron is a search engine for summarized RNA sequencing data with a query planner that leverages R-tree, B-tree and inverted indexing strategies to rapidly execute queries over 146 million exon-exon splice junctions from over 70,000 human RNA-seq samples. Queries can be tailored by constraining which junctions and samples to consider. Snaptron can score junctions according to tissue specificity or other criteria, and can score samples according to the relative frequency of different splicing patterns. We describe the software and outline biological questions that can be explored with Snaptron queries.

Availability and implementation: Documentation is at http://snaptron.cs.jhu.edu. Source code is at https://github.com/ChristopherWilks/snaptron and https://github.com/ChristopherWilks/snaptron-experiments with a CC BY-NC 4.0 license.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The Sequence Read Archive (SRA) is a repository of sequencing data containing over 12 petabases (Leinonen et al., 2011). Archives like the SRA allow researchers to reproduce past studies, combine data in new ways, and leverage data that would otherwise be too expensive or difficult to generate. But there is no convenient way to pose scientific questions against the archives without first downloading and re-analyzing data.

Snaptron is a search engine for querying splicing patterns in large, pre-analyzed collections of human RNA sequencing (RNA-seq) samples. Snaptron lends valuable context and support to hypotheses related to splicing patterns in human. Snaptron’s query planner combines the strengths of different indexing strategies—R-trees, B-trees and term-document inverted indices—to rapidly answer queries (Supplementary Fig. S1). While past efforts have sought to enable querying of sequencing and expression data (Kolesnikov et al., 2014; Petryszak et al., 2016; Solomon and Kingsford, 2016), Snaptron is unique both in the breadth of splicing data it can query and in its ability to rapidly answer sophisticated questions.

2 Materials and methods

We first used Rail-RNA (Nellore et al., 2015) to analyze archived human RNA-seq samples, as described previously (Collado-Torres et al., 2017; Nellore et al., 2016a,b). Rail-RNA outputs is a table summarizing evidence for exon-exon splice junctions across all samples. We also created tables detailing metadata for each sample. This is the source material for Snaptron as well as for the
Snaptron also annotates each junction with: (i) gene annotation status (Supplementary Table S1), (ii) count of samples with one or more reads covering the junction and (iii) junction coverage statistics, such as sum and mean, summarized over all samples with evidence for the junction.

Snaptron user may query any of these four compilations of human RNA-seq samples: SRAv1 contains 43M junctions called from 21,504 public samples from the SRA. SRAv2 contains 81M junctions called from 44,427 public samples from the SRA. GTEx contains 29M junctions called from 9,622 samples from the v6 GTEx data freeze. TCGA contains 37M junctions called from 11,284 samples from TCGA.

3 Results

Supplementary Note 4 points to all software used for these results.

3.1 Assessing putative novel junctions

Snaptron’s junction calls were made without use of gene annotation, so it can assess prevalence of annotated or unannotated events without bias. We demonstrate this by partly recreating the Goldstein et al. (2016) study, which searched for unannotated cassette exons in Illumina RNA-seq data from 16 tissues. A cassette exon was called novel if neither extreme coincided with an annotated junction, but the exon was in an annotated gene. Goldstein et al. (2016) found 249 novel exons and validated 216 in a separate cohort.

To study these 249 exons using Snaptron, we posed shared-sample-count (SSC) queries that gathered evidence for the exons in the SRAv2 and GTEx compilations and scored exons according to the number of samples with evidence for the exon (details in Supplementary Note S5). Of the 249 putative exons, 236 (94.8%) occurred in both the SRAv2 and GTEx compilations. We found 204 of the 236 were validated by Goldstein et al. (2016), while the remaining 32 failed validation. The validated exons had significantly higher SSC than the others (Supplementary Fig. S4), indicating the SSC query is a rapid, in-silico method for measuring prevalence and reliability of a putative novel event.

Also, though the original study considered the 236 exons to be unannotated, Snaptron results showed that 132 were annotated, most by the more inclusive SIBgenes (https://genome.ucsc.edu/cgi-bin/hgTrackUi? db=hg38&g=sibGene) and ACEview (Thierry-Mieg and Thierry-Mieg, 2006) tracks. Thus, Snaptron makes it easy to understand the annotation status of splicing events with respect to a wide range of annotations.

3.2 Assessing tissue specificity

In a repetitive element locus (REL) exonizaton event, part of the interspersed repeat is spliced into a surrounding gene as an exon. Darby et al. (2016) report numerous such events in human, including some specific to brain or blood. We used Snaptron to assess tissue specificity of five events where the spliced-in exon was not annotated. We used an SSC query to confirm the five events occur in both the SRAv2 and GTEx compilations (more than 39 samples in both cases). We then used a tissue specificity (TS) query to measure specificity of the five REL exons with respect to the more comprehensive GTEx compilation (details in Supplementary Note S6).

Results showed all five exonization events were tissue-specific (Kruskal-Wallis $P<1\times10^{-5}$). In this way, Snaptron can measure a splicing pattern’s tissue specificity, a proxy for biological function.

3.3 Ranking samples according to splicing pattern

We performed an experiment modeled on Nellore et al. (2016a)”s analysis of the anaplastic lymphoma kinase (ALK) gene’s ALK$^{ATI}$ variant isoform. ALK is mutated or aberrantly expressed in some cancers, notably in the form of the ALK$^{ATI}$ variant, characterized by an alternative transcription initiation (ATI) site (Wiesner et al., 2015). We used Snaptron to show the ALK$^{ATI}$ variant and related EML4-ALK fusion can be found in non-cancer samples.

We used a junction inclusion ratio (JIR) query to rank samples according to how often the excised junctions (missing in ALK$^{ATI}$) occurred relative to the junctions present in both spliceforms (details
in Supplementary Note 7). The top 10 samples ranked by JIR match those reported by Nellore et al. (2016a), including unexpected melanocyte and macrophage samples. This shows how Snaptron can rank samples according to the relative prevalence of a splicing pattern, such as a splicing signature for a disease phenotype. Snaptron also supports a percent spliced in (PSI) query that adapts JIR to the common case of an alternatively spliced cassette exon.

3.4 Graphical user interface
We built a graphical user interface (GUI) to demonstrate how Snaptron queries can enable exploration and visualization of splice junctions across tens of thousands of samples (Fig. 1). A GUI user can (i) select a gene or region of interest, (ii) filter and color-code junctions according to summaries like shared sample count or average coverage and (iii) distinguish annotated from unannotated junctions. Supplementary Note 8 provides GUI links.

4 Discussion
Snaptron combines multiple indexing and database systems in a way that allows rapid queries, which can constrain flexible combinations of both structured interval and numeric data, and less structured textual metadata. This enables convenient new ways to explore and visualize splicing patterns over tens of thousands of individuals, measure the prevalence and reliability of putative novel splicing events, measure tissue specificity of possibly functional splicing patterns, and find samples with characteristic splicing patterns.

Acknowledgements
We thank Princy Parsana, Leonardo Collado-Torres, Jonathan Ling and Leonard Goldstein for advice and assistance. We thank Sarven Sabunciyan for providing REL exonization events. We thank Joyce Wilks, Elisabeth Wilks and Anand Malpani for help with naming.

Funding
CW, AN and BL were supported by NIH/NIGMS grant 1R01GM118568 to BL.

Conflict of Interest: none declared.

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