SPLICE-q: a Python tool for genome-wide quantification of splicing efficiency

Additional File 1

Supplementary Tables and Figures

Table 1: Summary table of parameters.

Parameter	Description
MinCoverage	Minimum number of reads spanning each splice junction (Default = 10).
MinReadQuality	Mapping quality. By default, only uniquely mapped reads are included (Default = 10).
MinIntronLength	Minimum intron length. Default value is optimal for analysis using human RNA-seq data (Default = 30)
ChromsList	List of chromosome names (Default: chr1-720, I-XVI, 2L, 2R, 3L, 3R, Z, W.)
FilterLevel	(1) keep all introns in the genome regardless of overlaps with other genomic elements. (2) select only introns whose splice junctions do not overlap any exon in different genes (3) select only introns that do not overlap with any exon of the same or different gene (Default).
IERatio	Running mode that additionally outputs the Inverse Intron Expression Ratio (IER). Requires *FilterLevel* 3.
NProcesses	Multiple concurrent processes are used to minimize running times and the number of processes can be adjusted by the user through this parameter.

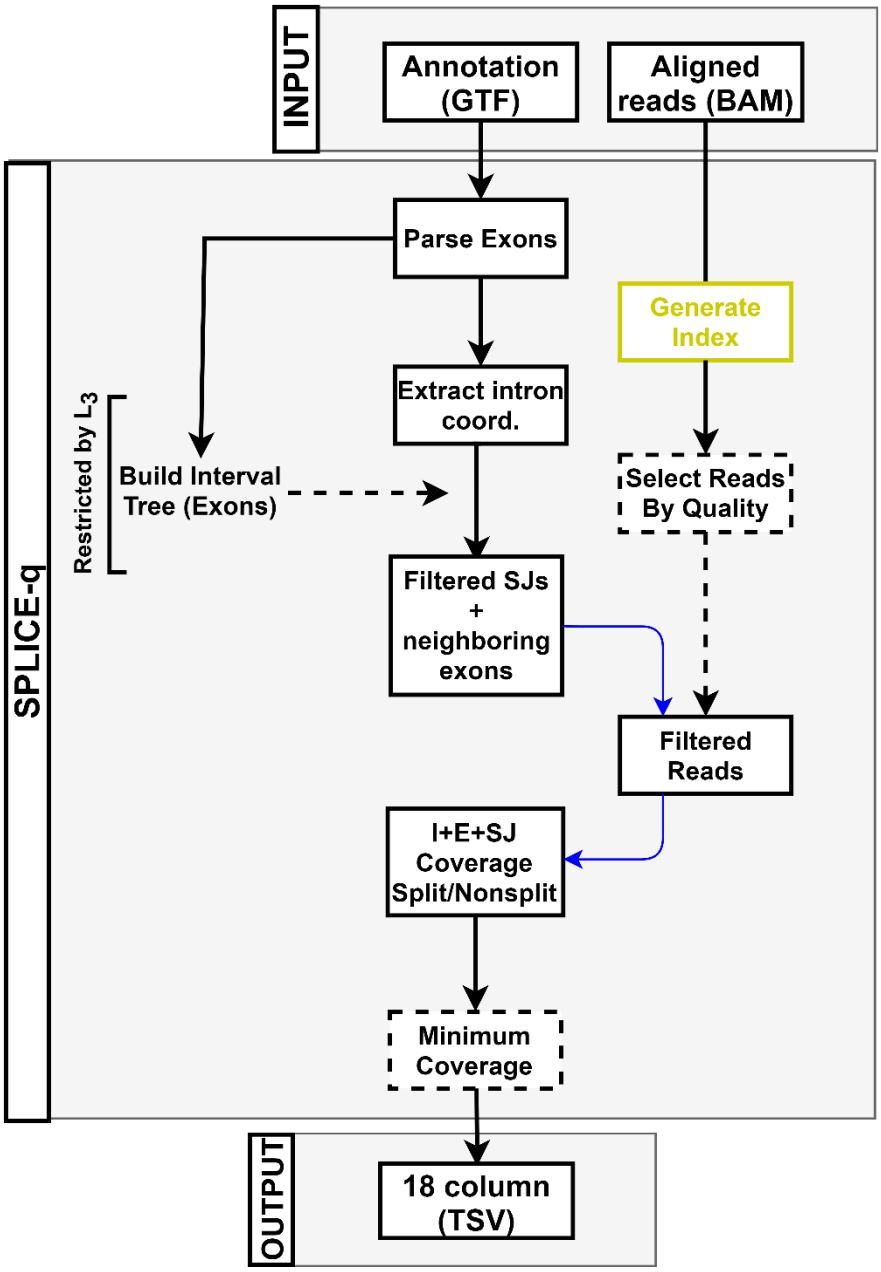
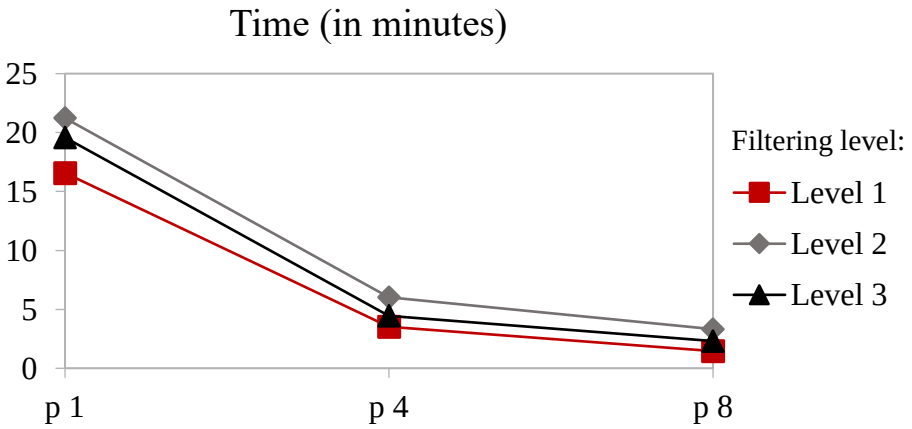


Fig. S1: SPLICE-q's inverse intron expression ratio (IER) workflow. Dashed lines indicated steps which depend on parameter settings. Solid lines represent the mandatory steps of the workflow. Arrows in blue represent a lookup in the data structure they pass through. I = intron; E= exon; SJ = splice junction; TSV = tab-separated values.

a)



b)

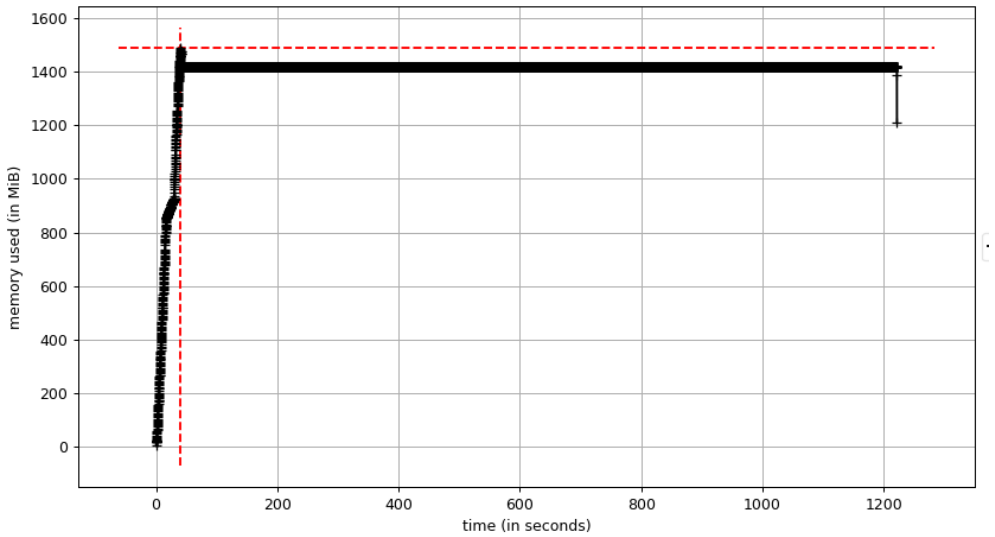


Figure S2: **SPLICE-q's run time and memory usage**. **a)** Run time for approximately 100 million input reads mapped to the human genome (Linux, 64x AMD Opteron 6282 SE, 516GB). **b)** Memory usage for 1.4GB GTF. Time in seconds. p = Number of processes (*NProcesses*).

Materials and Methods

Pulse-chase essay, RNA-seq and read mapping

Human embryonic kidney cells (HEK293) cells were incubated for 15 minutes with 2mM of 5-bromouridine (BrU, pulse). Then, the cells were either collected immediately (0 minutes) or chased for 15, 30 and 60 minutes prior to RNA purification and selection of BrU-labeled RNA as described in [1]. The sequencing library was prepared with the TrueSeq Stranded Total RNA Kit (Illumina). Sequencing was performed in triplicate on the Illumina HiSeq 2500 platform to obtain an average of ~200 million reads per sample. Replicates read coverage are highly correlated with an average $\rho = 0.95$ which satisfies the ENCODE consortium recommendations for biological replicates [2]. The strand-specific reads were mapped to the human reference genome *GRCh38.p10* with STAR v2.7.1a [3] according to recommendations from the STAR manual 2.4.0.1. The genome index for STAR was built on the genome annotation from GENCODE v27[1]. An average of ~85% of the reads in all samples were uniquely mapped. The GEO [4] accession numbers for these sequencing data are GSE92565, GSE83561 and GSE84722.

1 ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release27/gencode.v27.annot ation.gtf.gz

Other datasets

The other datasets processed and analyzed are described below.

Table 1: Datasets used in the study.

Accession/ Reference	Genome/ Annotation	Description
GSE84722 [6]	GRCh38.p10/ gencode v27	Total RNA-seq of HEK293 cells. Sequenced on HiSeq2500.
GSE70378 [7]	Ensembl R64-1-1	*S. cerevisiae* labeled with 4tU labeling for 1.5, 2.5 and 5 minutes. Total RNA-seq also performed. All experiments were performed in triplicate. Sequenced on HiSeq2500.
GSE133626 [8]	GRCh38.p10/ gencode v27	Total RNA from fresh frozen prostate cancer tissue along with a matched normal control sample. Patient 15 of the dataset. Sequenced in duplicate on HiSeq2000.

Statistics and other methods

DeepTools2.0 [5] was used to assess genome-wide similarity of the sequencing replicates. All statistical tests were performed in R 3.6.1 (https://cran.r-project.org/mirrors.html). SPLICE-q's workflow figures were generated with Drawio (https://github.com/jgraph/drawio).

References

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