**Supporting Information**

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| **Date of Publication** | **Tool Name** | **Specificity** | **Level of analysis** | **Statistical Summary** | **Statistic Type** |
| 2010 | MISO [(55)](https://www.zotero.org/google-docs/?ZGHI3L) | Splicing | Event | Uses a probabilistic Bayesian framework to model and detect splicing events. Percentage spliced-in (PSI) values were calculated for splice sites or alternative exons. Hypothesis testing uses posterior probabilities and Bayes factors. | Probabilistic |
| 2010 | DESeq2 / DESeq [(21)](https://www.zotero.org/google-docs/?QsQBS0) | General | Transcript/Gene | Uses a generalized linear model (GLM) fit to the negative binomial (NB) distribution. Addresses gene-specific dispersion variation. Uses a variance stabilizing transformation (VST) to address Poisson-like distribution. Hypothesis testing through the Wald test is used between conditions to test for differential expression. False discovery rate (FDR) control using the Benjamini-Hochberg procedure. | Parametric |
| 2012 | DEXSeq [(47)](https://www.zotero.org/google-docs/?5GhEk7) | Splicing | Exon/Transcript | Adapted from DESeq2 and edgeR's generalized linear model (GLM). Models read counts per exon using a GLM fitted to the negative binomial (NB) distribution. Addresses dispersion variation through shrinkage. Uses a variance stabilizing transformation (VST) to address Poisson-like distribution. Hypothesis testing through likelihood-ratio test (LRT) to test for differential usage. False discovery rate (FDR) control using the Benjamini-Hochberg (BH) procedure. | Parametric |
| 2012 | Cuffdiff2 [(73)](https://www.zotero.org/google-docs/?OPws1S) | General | Transcript | Multivariate mixed model fit to a beta negative binomial (NB) distribution with multi-read correction. Trimmed mean of m-values (TMM) normalization applied. Hypothesis testing using the likelihood ratio test (LRT) to test for differential expression. False discovery rate control using the Benjamini-Hochberg (BH) procedure. | Parametric |
| 2013 | Splicing  Compass [(74)](https://www.zotero.org/google-docs/?RQ4aAH) | Splicing | Event | Employs geometric angles to increase sensitivity on exon read counts. Designed for the detection of more complex splicing events. Hypothesis testing using a one-sided t-test and Benjamini Hochberg (BH) correction to control false discovery rate (FDR). | Parametric |
| 2013 | DSGseq [(29)](https://www.zotero.org/google-docs/?lw2R7Z) | Splicing | Exon | Uses a negative binomial (NB) model on exon counts to detect differential spliced genes. Hypothesis testing using NB statistics without classical P value significance. | Parametric |
| 2013 | GLiMMPs [(44)](https://www.zotero.org/google-docs/?hkEOhp) | Splicing | Exon | Uses a generalized linear mixed model (GLMM) to fit the negative binomial (NB) distribution to account for phylogenetic relationships as a source of random effects. hypothesis testing using a likelihood ratio test (LRT). detects splicing quantitative trait loci (QTLs). | Parametric |
| 2013 | riff-parametric [(30)](https://www.zotero.org/google-docs/?2M5mID) | Splicing | Exon (Regions) | Uses a negative binomial (NB) model on smaller exonic regions that are indicative of relative isoform abundance. Hypothesis testing was performed using diff.parametric equation per exonic region with Bonferroni corrections. Has an option for rDiff-nonparametric if there is no gene annotation. | Mixed |
| 2013 | EBSeq [(48)](https://www.zotero.org/google-docs/?49pSNa) | Splicing | Transcript/Gene | Uses hierarchical modelling under an empirical Bayesian framework under a negative binomial distribution (NB). Hypothesis testing using posterior probabilities to evaluate probabilities per gene. | Probabilistic |
| 2014 | rMATs [(40)](https://www.zotero.org/google-docs/?onEkDF) | Splicing | Event | Detects splicing events using splice site junctions. Uses a joint probability model to compare splicing events between conditions using both the normal and negative binomial distribution. Employs a hierarchical Bayesian framework to estimate percent-spliced-in (PSI) values for each splicing event. Hypothesis testing is conducted using a likelihood ratio test (LRT). False discovery rate (FDR) control is applied using benjamini-hochberg (BH) correction. | Non-parametric |
| 2014 | SeqGSEA [(32)](https://www.zotero.org/google-docs/?Ri15ZB) | Splicing | Transcript | Adapted from DESeq2 and DSGSeq's generalized linear model (GLM) on negative binomial (NB) distributions. Incorporates a ranked-based strategy for overrepresented gene sets. Employs gene set enrichment analysis (GSEA). | Parametric |
| 2015 | SUPPA [(39)](https://www.zotero.org/google-docs/?l3GTKK) | Splicing | Event | Detects splicing events using event annotations. Uses a linear mixed model (LMM) under the beta distribution. Percent spliced-in (PSI) values are calculated per splicing event annotated. Hypothesis testing uses the Wilcoxin-Mann-Whitney U test to compare PSI distributions. False discovery rate (FDR) control is applied using Benjamini-Hochberg (BH) correction. Applies the Pareto principle. | Non-parametric |
| 2015 | dSpliceType [(75)](https://www.zotero.org/google-docs/?PblPKA) | Splicing | Event | Utilises a multivariate statistical model for splicing events. Change-point analysis followed by a parametric statistical test using the Schwarz information criterion (SIC). | Parametric |
| 2015 | JunctionSeq [(31)](https://www.zotero.org/google-docs/?VX45dy) | Splicing | Exon/Splice Junctions | Adapted from DEXseq's generalized linear model (GLM) fitted to the negative binomial (NB) distribution. Read counts are instead mapped to splice/exon-exon junctions. Uses Bayesian estimation to compute posterior probabilities for splicing changes. False discovery rate (FDR) control using the Benjamini-Hochberg (BH) procedure. | Parametric |
| 2015 | NOISeq [(43)](https://www.zotero.org/google-docs/?OQ1PBX) | Splicing | Transcript/Gene | Specifically designed for small or irregular sample sizes. Uses a non-parametric model under a Bayesian approach. Hypothesis testing using posterior probabilities to evaluate probabilities per gene. | Non-  parametric |
| 2016 | MAJIQ [(26)](https://www.zotero.org/google-docs/?9619Nf) | Splicing | Event | Detects local splicing variations (LSVs) encompassing event complexity. Uses beta distribution modelling for inclusion levels under a Bayesian framework. Utilises posterior distributions for multiple hypothesis testing. Percentage spliced index (PSI) calculated using likelihood estimates. | Non-  parametric |
| 2016 | DRIMSeq [(34)](https://www.zotero.org/google-docs/?tAJS2G) | Splicing | Transcript | Uses a Dirichlet-multinomial model (Bayesian framework) fitted to a negative binomial distribution. Constructs a "splicing index," which represents the relative abundance of alternative splicing isoforms. An exact test is used to test for differential expression. False discovery rate (FDR) control using the Benjamini-Hochberg procedure. | Parametric |
| 2017 | LeafCutter [(57)](https://www.zotero.org/google-docs/?HGRscA) | Splicing | Event | Detects splicing quantitative trait loci (SQTL) using splice junctions with percentage spliced in (PSI) values. Differential intron excision. Dirichlet-multinomial generalized linear model. Hypothesis testing using permutation testing under a Bayesian framework. | Parametric |
| 2018 | WHIPPET[(41)](https://www.zotero.org/google-docs/?JzzIqZ) | Splicing | Event | Uses entropy based statistics. Employs a Bayesian framework to estimate the posterior probability of different splicing outcomes based on observed RNA-Seq read counts, allowing for the identification and quantification of splicing variations. | Non-parametric |
| 2019 | DARTS [(46)](https://www.zotero.org/google-docs/?ABy3pk) | Splicing | Event | Uses a trained deep neural network (DNN) model accompanied by empirical Bayesian statistics for hypothesis testing. | Non-  parametric |
| 2019 | IsoformSwitchAnalyzeR [(36)](https://www.zotero.org/google-docs/?vUr4LC) | Splicing | Transcript | Modular package. Primarily utilises DEXSeq model to test for isoform switches but includes Cuffdiff or Saturn options. Facilitates further annotation of features such as open reading frames (ORF), coding potential and nonsense-mediated decay sensitivity of transcripts. | Parametric |
| 2021 | DTUrtle [(35)](https://www.zotero.org/google-docs/?b5nd1V) | Splicing | Transcript | Adapted directly from DRIMSeq's Dirichlet-multinomial model (DMM). Hypothesis testing uses a likelihood ratio test (LRT). Two-stage statistical procedures were applied using stage's false discovery rate (FDR) corrections through the Benjamini-Hochberg (BH) procedure. | Parametric |

**Supplementary Table 1:** **Statistical summary of current differential splicing tools with categorisation into parametric, non-parametric or probabilistic based on the testing statistic employed.** The summary captures the model and distribution type, hypothesis testing methods, and corrective procedures. Categorised are the three types of splicing analysis by output: exon-based, transcript-based and event-based.