Branchpointer: prediction of human splicing branchpoint sites

Beth Signal

2016-12-19

Branchpointer: prediction of human splicing branchpoint sites

Introduction

Preparations

Download genome annotations

Branchpointer requires a genome annotation derived from a GTF file and the fasta sequence for this genome annotation. We will be using the GENCODE annotation (http://www.gencodegenes.org/releases/current.html) as an example, although others and custom annotations can be used.

Create or move to a working directory where these files can be stored. Note that these can be large files (>1GB) when uncompressed

```
wget ftp://ftp.sanger.ac.uk/pub/gencode/Gencode_human/release_24/gencode.v24.annotation.gtf.gz
gunzip gencode.v24.annotation.gtf.gz

wget ftp://ftp.sanger.ac.uk/pub/gencode/Gencode_human/release_24/GRCh38.p5.genome.fa.gz
gunzip GRCh38.p5.genome.fa.gz
```

1. Read in exon annotations

readExonAnnotation will generate an exon annotation table from a gtf, and save it in the same location

```
exons <- readExonAnnotation("gencode.v24.annotation.gtf")
```

After this step has been performed once, the exon file can be specified instead to save reformatting the gtf file again.

```
exons <- readExonAnnotation("gencode.v24.annotation.exons.txt")
```

We will load in a small formatted exon annotation file from the package data

```
small_exons <- system.file("extdata","gencode.v24.annotation.exons.small.txt",
                           package = "branchpointer")
exons <- readExonAnnotation(small_exons)
```

Branchpoint predictions

2. Read query and calculate location attributes

2.1. Intronic window queries

Query regions must contain a branchpoint window - that is the region located at -18 to -44 from the 3' splice site. Each region given will be treated as only one query, and associated with the closest 3' exon. To cover multiple 3'exons, please provide branchpointer with seperate region queries. For known regions, queries can be supplied as a table:

```r
query_intron <- system.file("extdata","intron_example.txt",
                            package = "branchpointer")
query_intron <- readQueryFile(query_intron,query_type = "region")

pander::pander(query_intron, row.names=FALSE, split.table=130, style="rmarkdown")
```

id	chromosome	chrom_start	chrom_end	strand
BRCA1_intron	chr17	43106534	43106634	-
BRCA2_intron	chr13	32376570	32376669	+

Then location information can be retrieved using

```r
query_intron <- getQueryLoc(query_intron,query_type="region",exons = exons)

pander::pander(query_intron, row.names=FALSE, split.table=130, style="rmarkdown")
```

Table 2: Table continues below

	id	chromosome	chrom_start	chrom_end	strand	to_3prime	to_5prime	same_gene	exon_3prime	exon_5prime
BRCA1_intron	BRCA1_intron	chr17	43106551	43106577	-	18	3914	TRUE	ENSE00003541068.1	ENSE00001888888.1
BRCA2_intron	BRCA2_intron	chr13	32376626	32376652	+	18	1246	TRUE	ENSE00003461148.1	ENSE00002167182.1

For large numbers of queries (>500), it is recomended to use parallelisation to speed up computation. This can be done by setting `use_parallel=TRUE` and supplying a `cores` number greater than 1 to functions with this argument. Note that if the number of specified cores is greater than the number available, the maximum number available will be utilised

```r
query_intron <- getQueryLoc(query_intron,query_type="region",
                            exons = exons, use_parallel=TRUE, cores=4)
```

Alternatively, to generate branchpoint window region queries by exon annotations, the exon annotation file can be used: Note that when searching for genes, transcripts, or exons, the ids used must be in the same format as in the annotation file (i.e ENSG00000XXXXXX, ENST00000XXXXXX, ENSE00000XXXXXX). If you are unsure of a id, aliases can typically be found through ensembl (ensembl.org), or through a biomaRt query.

```r
query_intron_make <- makeRegions("ENSE00003541068.1", "exon_id", exons)

pander::pander(query_intron_make, row.names=FALSE, split.table=130, style="rmarkdown")
```

Table 4: Table continues below

id	chromosome	chrom_start	chrom_end	strand	to_3prime	to_5prime	same_gene
ENSE00003541068.1	chr17	43106551	43106577	-	18	3914	TRUE

exon_3prime	exon_5prime
ENSE00003541068.1	ENSE00001888888.1

```r
#or for multiple ids
query_intron_make <- lapply(c("ENSE00003541068.1", "ENSE00003461148.1"),
                           makeRegions, "exon_id", exons)

query_intron_make <- do.call("rbind", query_intron_make)
```

2.2. SNP queries

Query SNPs should be located nearby a branchpoint window to have any potential effects on branchpoint architecture SNP queries can be supplied as a table formatted as follows:

```r
query_snp <- system.file("extdata","SNP_example.txt", package = "branchpointer")
query_snp <- readQueryFile(query_snp,query_type = "SNP")
```

Alternatively, appropriate attributes can be pulled from biomart when a list of refsnp ids is provided:

```r
mart <- useMart("ENSEMBL_MART_SNP", dataset="hsapiens_snp",host="www.ensembl.org")

query_snp <- snpToQuery(c("rs17000647","rs5031002","rs998731"), mart_snp = mart)
```

By default, all SNPs retrieved will be unstranded, and hence further processing will be done on both strands

Location information can be retrieved using

```r
query_snp <- getQueryLoc(query_snp,query_type="SNP",exons = exons, filter = FALSE)
```

Each SNP will be associated with the closest 3' exon. If SNPs are distal from branchpoint windows, the max_dist argument will remove any greater than the specified distance. Filtering prior to exon associations can speed up processing in instances where it is unknown if the majority of SNPs fall nearby branchpoint windows.

Queries can be provided as stranded or unstranded. In the case of unstranded queries, any value except `"+"` or `"-"` will cause branchpointer to run on both strands.

3. Get sequence attributes for query regions

Sequences covering each site +/- 250 nt are retrieved using bedtools. The absolute location of the bedtools binary must be provided for calls from within R. To find the location of your installed bedtools binary, using the command line type:

```
which bedtools
```

If chromosome names in the .fasta genome file do not match those in the query (i.e chr1 in query, 1 in .fasta), the argument `rm_chr` should be set to `FALSE`.

This will generate a data.frame with a row for each site (of 27) in branchpoint window regions. If a SNP query type is provided, this will also perform an in silico mutation of the sequence.

All features required for the model to predict branchpoint probability are contained within this data.frame.

When performing this step for multiple cases simultaneously, a unique_id can be provided to prevent incorrect .fasta files being read in during the sequence retreival step.

```
#for query regions
query_attributes_intron <- getBranchpointSequence(query_intron,
                                        query_type = "region",
                                        genome = "GRCh38.p5.genome.fa",
                                        bedtools_location="/Applications/apps/bedtools2/bin/bedtools")


#for query SNPs
query_attributes_snp <- getBranchpointSequence(query_snp,
                                        query_type = "SNP",
                                        genome = "GRCh38.p5.genome.fa",
                                        bedtools_location="/Applications/apps/bedtools2/bin/bedtools")
```

Alternatively, a BSgenome object can be used instead of specifying a genome .fa file and using bedtools Available BSgenomes are listed on the Bioconductor AnnotationData packages page

```
#install the correct genome from Bioconductor
source("https://bioconductor.org/biocLite.R")
biocLite("BSgenome.Hsapiens.UCSC.hg38")
```

```
#load in BSgenome
suppressMessages(library(BSgenome.Hsapiens.UCSC.hg38))
genome <- BSgenome.Hsapiens.UCSC.hg38

#for query regions
query_attributes_intron <- getBranchpointSequence(query_intron,
                                        query_type = "region",
                                        useBSgenome = TRUE,
                                        BSgenome = genome)


#for query SNPs
query_attributes_snp <- getBranchpointSequence(query_snp,
                                        query_type = "SNP",
                                        useBSgenome = TRUE,
                                        BSgenome = genome)
```

4. Predict branchpoint probabilities

Probabilities for each site within the window are then evaluated using the branchpointer model. We recommend use of the cutoff probability 0.5 to distinguish branchpoints and non-branchpoint sites. U2 binding energy can be used as a measurement of branchpoint strength when the probability score is above the cutoff.

```
branchpoint_predictions_intron <- predictBranchpoints(query_attributes_intron)

keep_bps <- which(branchpoint_predictions_intron$branchpoint_prob > 0.5)

branchpoints_intron <- branchpoint_predictions_intron[keep_bps,]

pander::pander(head(arrange(branchpoints_intron, plyr::desc(branchpoint_prob))),
               row.names=FALSE, split.table=130, style="rmarkdown")
```

Table 6: Table continues below

id	branchpoint_prob	nucleotide	distance	allele_status	chromosome	strand	end	exon_3prime	exon_5prime	U2_binding_energy
BRCA2_intron	0.8107	A	21	REF	chr13	+	32376649	ENSE00003461148.1	ENSE00002167182.1	2.5
BRCA2_intron	0.5898	A	22	REF	chr13	+	32376648	ENSE00003461148.1	ENSE00002167182.1	1.8
BRCA2_intron	0.5672	A	30	REF	chr13	+	32376640	ENSE00003461148.1	ENSE00002167182.1	2.2
BRCA2_intron	0.5324	A	26	REF	chr13	+	32376644	ENSE00003461148.1	ENSE00002167182.1	1.6
BRCA1_intron	0.5093	A	30	REF	chr17	-	43106563	ENSE00003541068.1	ENSE00001888888.1	0.1

```
branchpoint_predictions_snp <- predictBranchpoints(query_attributes_snp)

snp_stats <- predictionsToStats(branchpoint_predictions_snp, query_snp)

pander::pander(snp_stats, row.names=FALSE, split.table=130, style="rmarkdown")
```

Table 8: Table continues below

Table 9: Table continues below

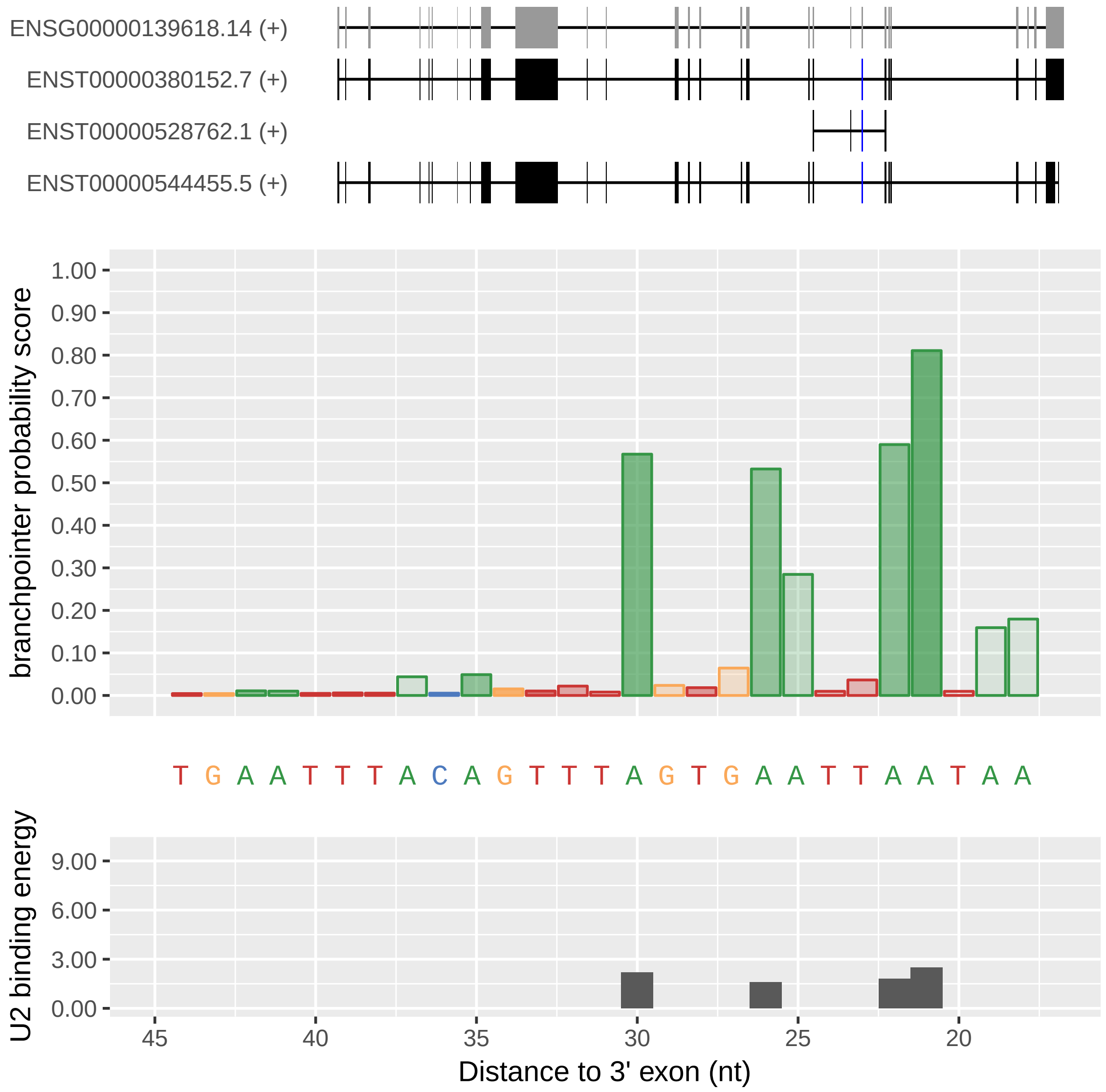
	id	chromosome	chrom_start	strand	ref_allele	alt_allele	BP_num_REF	BP_num_ALT	deleted_n	created_n	dist_to_exon	dist_to_BP_REF	dist_to_BP_ALT	max_prob_REF	max_prob_ALT	max_U2_REF	max_U2_ALT
1	rs17000647_pos	chr4	75556520	+	C	A	4	3	1	0	21	-1	-1	0.9711	0.8713	6.5	3
2	rs5031002_pos	chrX	67722783	+	G	A	0	1	0	1	44	NA	0	0.4563	0.6564	NA	0.5
6	rs998731_neg	chr8	80183160	-	C	T	1	2	0	1	22	-4	0	0.6077	0.7966	0.5	1.6

The window scores can be plotted using plotBranchpointWindow(), with optional plots for gene and isoform structure. The main panel displays the probability scores of each site within the branchpoint window. The opacity of the bars is representative of relative U2 binding energy (darker = stronger), and the lower panel shows U2 binding energy for all sites above the provided probability cutoff.

Branchpoint predictions in an intronic window

BRCA2 intron (ENSE00002167182.1 - ENSE00003461148.1).

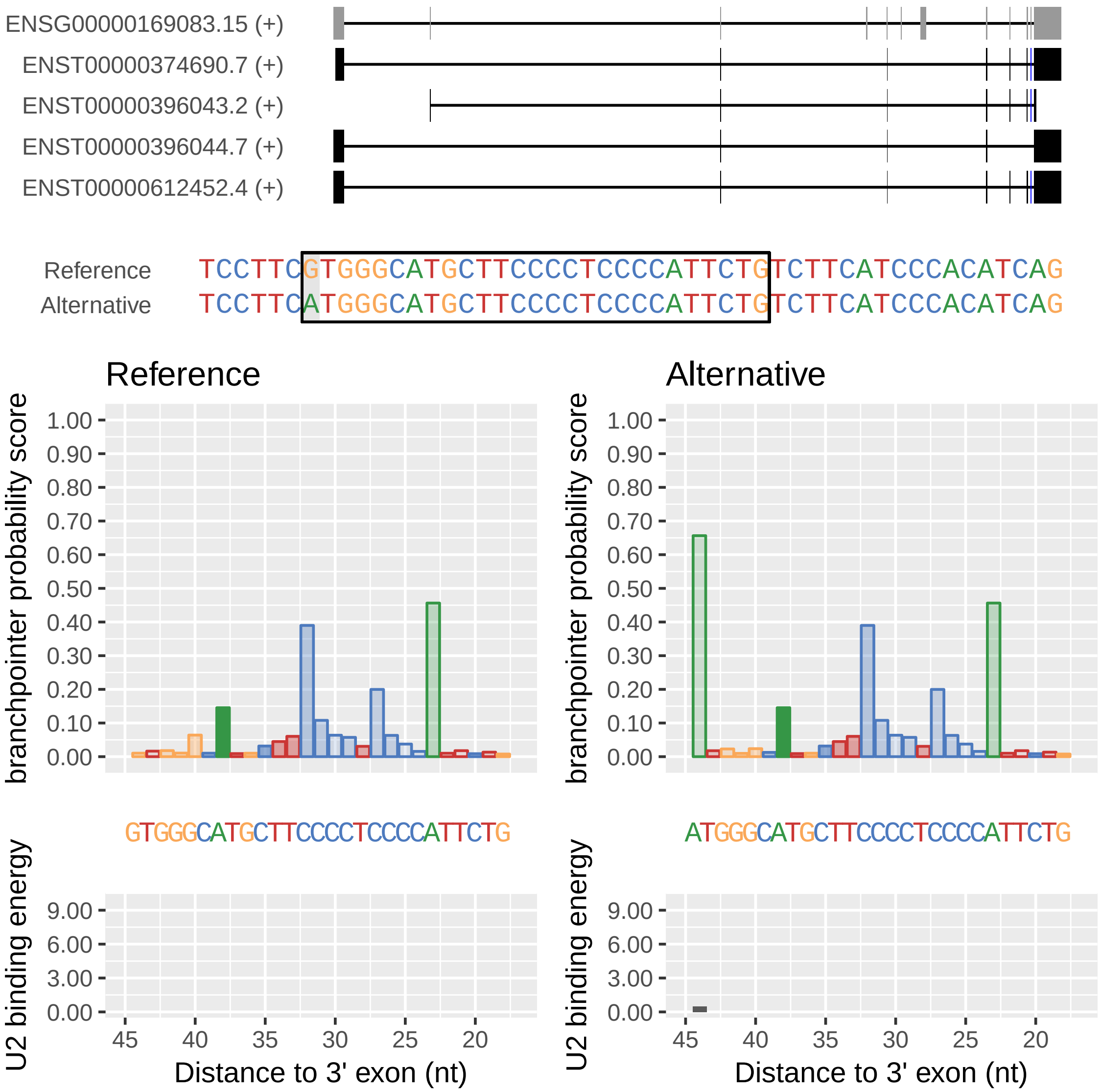
```
plotBranchpointWindow(query_intron$id[2], branchpoint_predictions_intron,
                      query_attributes_intron,
                      probability_cutoff = 0.5,plot_mutated = FALSE,
                      plot_structure = TRUE,exons = exons)
```



Branchpoint predictions in reference and alternative sequence

rs17000647 in C4orf26 intron 1.

```
plotBranchpointWindow(query_snp$id[2], branchpoint_predictions_snp,query_attributes_snp,
                      probability_cutoff = 0.5,plot_mutated = TRUE,
                      plot_structure = TRUE,exons = exons)
```



Output table column descriptions

Several data.frames are created and used to predict branchpoints using branchpointer.

getQueryLoc

column name	description
id	identifier for query
chromosome	chromosome name (i.e. chr1)
chrom_start	chromosome location of the start of the window (for region), or location of the SNP (for SNP)
chrom_end	chromosome location of the end of the window (for region). Not included for SNP queries.
strand	chromosome strand
to_3prime	distance (in nucleotides) to the closest annotated 3' exon

column name	description
to_5prime	distance (in nucleotides) to the closest annotated 5' exon
same_gene	(boolean) are the closest 3' and 5' exons from the same parent gene?
exon_3prime	exon_id of the closest annotated 3' exon
exon_5prime	exon_id of the closest annotated 5' exon

getBranchpointSequence

column name	description
id	identifier for site-specific query (id+to_3prime+REF/ALT)
chromosome	chromosome name (i.e. chr1)
end	chromosome location of the site specific query
strand	chromosome strand
seq	RNA sequence covering the 501 nucleotides surrounding the site
exon_3prime	exon_id of the closest annotated 3' exon
exon_5prime	exon_id of the closest annotated 5' exon
to_3prime	distance (in nucleotides) to the closest annotated 3' exon
to_5prime	distance (in nucleotides) to the closest annotated 5' exon
ppt_start	distance (in nucleotides) to the polypyrimidine tract
ppt_run_length	length of the polypyrimidine tract
canon_hit1	distance (in nucleotides) to the 1st AG dinucleotide
canon_hit2	distance (in nucleotides) to the 2nd AG dinucleotide
canon_hit3	distance (in nucleotides) to the 3rd AG dinucleotide
canon_hit4	distance (in nucleotides) to the 4th AG dinucleotide
canon_hit5	distance (in nucleotides) to the 5th AG dinucleotide
seq_neg5	sequence identity of the nucleotide -5nt from the tested site
seq_neg4	sequence identity of the nucleotide -4nt from the tested site
seq_neg3	sequence identity of the nucleotide -3nt from the tested site
seq_neg2	sequence identity of the nucleotide -2nt from the tested site
seq_neg1	sequence identity of the nucleotide -1nt from the tested site
seq_pos0	sequence identity of the tested site
seq_pos1	sequence identity of the nucleotide +1nt from the tested site
seq_pos2	sequence identity of the nucleotide +2nt from the tested site
seq_pos3	sequence identity of the nucleotide +3nt from the tested site
seq_pos4	sequence identity of the nucleotide +4nt from the tested site
seq_pos5	sequence identity of the nucleotide +5nt from the tested site

predictBranchpoints

column name	description
id	identifier for query
branchpoint_prob	branchpoint probability score
nucleotide	nuceotide at tested site
distance	distance (in nucleotides) to the closest annotated 3' exon
allele_status	REF (reference sequence) or ALT (alternative sequence)
chromosome	chromosome name (i.e. chr1)
strand	chromosome strand
end	chromosome location of the site specific query
exon_3prime	exon_id of the closest annotated 3' exon

column name	description
exon_5prime	exon_id of the closest annotated 5’ exon
U2_binding_energy	binding energy of the sequence surrounding the testing site to the U2 snRNA

predictionsToStats

column name	description
id	identifier for query
chromosome	chromosome name (i.e. chr1)
chrom_start	chromosome location of the SNP
strand	chromosome strand
ref_allele	nucleotide identity of the reference allele
alt_allele	nucleotide identity of the alternative allele
BP_num_REF	number of branchpoints in the tested window with the reference sequence
BP_num_ALT	number of branchpoints in the tested window with the alternative sequence
deleted_n	number of branchpoints that become deleted in the alternative sequence
created_n	number of branchpoints that become created in the alternative sequence
dist_to_exon	distance (in nucleotides) to the closest annotated 3’ exon
dist_to_BP_REF	distance (in nucleotides) to the closest branchpoint in the reference sequence
dist_to_BP_ALT	distance (in nucleotides) to the closest branchpoint in the alternative sequence
max_prob_REF	maximum branchpoint probability score in the reference sequence
max_prob_ALT	maximum branchpoint probability score in the alternative sequence
max_U2_REF	maximum U2 binding energy of predicted branchpoints in the reference sequence (NA if no branchpoints)
max_U2_ALT	maximum U2 binding energy of predicted branchpoints in the alternative sequence (NA if no branchpoints)