Pooling RNA-seq and Assembling Models

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Contents

1 Introduction

Pooling RNA-seq and Assembling Models (**PRAM**) is an **R** package that utilizes multiple RNA-seq datasets to predict transcript models. The workflow of PRAM contains four steps. Figure 1 shows each step with function name and associated key parameters. In the later sections of this vignette, we will describe each function in details.

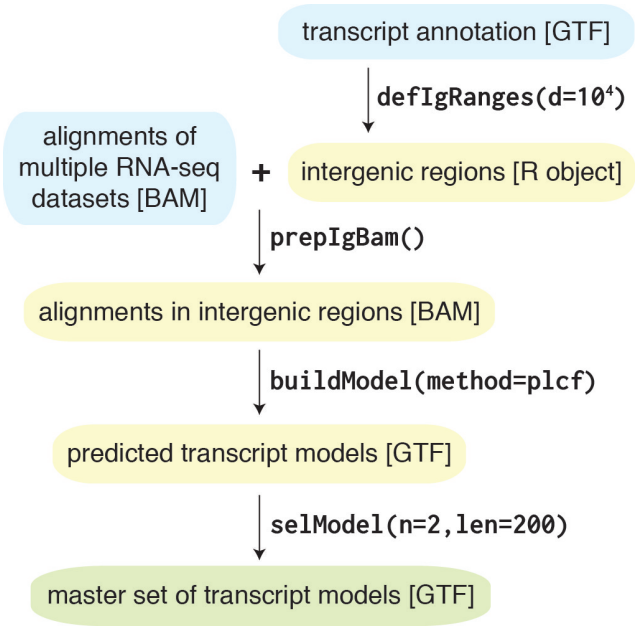

Figure 1: PRAM workflow

2 Installation

Use the following **R** command on **Linux** or **macOS**

```
devtools::install_github('pliu55/pram')
```

3 Quick start

PRAM provides a function `runPRAM()` to let you run through the whole workflow.

For a given gene annotation and RNA-seq alignments, you can predict transcript models in intergenic genomic regions:

```
runPRAM(in_gtf, in_bamv, out_gtf)
```

- `in_gtf`: an input GTF file defining genomic coordinates of existing genes. Required to have an attribute of **gene_id** in the ninth column.
- `in_bamv`: a vector of input BAM file(s) containing RNA-seq alignments. Currently, PRAM only supports strand-specific paired-end RNA-seq with the first mate on the right-most of transcript coordinate, i.e., 'fr-firststrand' by Cufflinks definition.
- `out_gtf`: an output GTF file of predicted transcript models

3.1 Examples

PRAM has included input examples files in its `extdata/demo/` folder. The table below provides a quick summary of all the example files.

Table 1: `runPRAM()`'s input example files

input argument	file name(s)
`in_gtf`	in.gtf
`in_bamv`	SZP.bam, TLC.bam

You can access example files by `system.file()` in **R**, e.g. for the argument `in_gtf`, you can access its example file by

```
system.file('extdata/demo/in.gtf', package='pram')
```

Below shows usage of `runPRAM()` with example input files:

```
in_gtf = system.file('extdata/demo/in.gtf', package='pram')

in_bamv = c( system.file('extdata/demo/SZP.bam', package='pram'),
             system.file('extdata/demo/TLC.bam', package='pram') )

pred_out_gtf = tempfile(fileext='.gtf')

runPRAM(in_gtf, in_bamv, pred_out_gtf)
```

4 Define intergenic genomic ranges: `defIgRanges()`

To predict intergenic transcripts, we must first define intergenic regions by `defIgRanges()`. This function requires a GTF file containing known gene annotation supplied for its `in_gtf` argument. This GTF file should contain an attribue of **gene_id** in its ninth column. We provided an example input GTF file in PRAM package: `extdata/gtf/defIGRanges_in.gtf`.

In addition to gene annotation, `defIgRanges()` also requires user to provide chromosome sizes so that it would know the maximum genomic ranges. You can provide one of the following arguments:

- `chromgrs`: a GRanges object, or
- `genome`: a genome name, currently supported ones are: **hg19**, **hg38**, **mm9**, and **mm10**, or
- `fchromsize`: a UCSC genome browser-style size file, e.g. hg19.

By default, `defIgRanges()` will define intergenic ranges as regions 10 kb away from any known genes. You can change it by the `radius` argument.

4.1 Example

```
defIgRanges( system.file('extdata/gtf/defIgRanges_in.gtf', package='pram'),
             genome = 'hg38' )
```

5 Prepare input RNA-seq alignments: `prepIgBam()`

Once intergenic regions were defined, `prepIgBam()` will extract corresponding RNA-seq alignments from input BAM files. In this way, transcript models predicted at later stage will solely from intergenic regions. Also, with fewer RNA-seq alignments, model prediction will run faster.

Three input arguments are required by `prepIgBam()`:

- `finbam`: an input RNA-seq BAM file sorted by genomic coordinate. Currently, we only support strand-specific paired-end RNA-seq data with the first mate on the right-most of transcript coordinate, i.e. 'fr-firststrand' by Cufflinks's definition.
- `iggrs`: a GRanges object to define intergenic regions.
- `foutbam`: an output BAM file.

5.1 Example

```
finbam = system.file('extdata/bam/CMPRep2.sortedByCoord.raw.bam',
                     package='pram')

iggrs = GenomicRanges::GRanges('chr10:77236000-77247000:+')

foutbam = tempfile(fileext='.bam')

prepIgBam(finbam, iggrs, foutbam)
```

6 Build transcript models: `buildModel()`

`buildModel()` predict transcript models from RNA-seq BAM file(s). This function requires two arguments:

- `in_bamv`: a vector of input BAM file(s)
- `out_gtf`: an output GTF file containing predicted transcript models

6.1 Transcript prediction methods

`buildModel()` has implemented seven transcript prediction methods. You can specify it by the `method` argument with one of the keywords: **plcf**, **plst**, **cfmg**, **cftc**, **stmg**, **cf**, and **st**. The first five denote meta-assembly methods that utilize multiple RNA-seq datasets to predict a single set of transcript models. The last two represent methods that predict transcript models from a single RNA-seq dataset.

The table below compares prediction steps for these seven methods. By default, `buildModel()` uses **plcf** to predict transcript models.

Table 2: Prediction steps of the seven `buildModel()` methods

method	meta-assembly	preparing RNA-seq input	building transcripts	assembling transcripts
plcf	yes	pooling alignments	Cufflinks	no
plst	yes	pooling alignments	StringTie	no

method	meta-assembly	preparing RNA-seq input	building transcripts	assembling transcripts
cfmg	yes	no	Cufflinks	Cuffmerge
cftc	yes	no	Cufflinks	TACO
stmg	yes	no	StringTie	StringTie-merge
cf	no	no	Cufflinks	no
st	no	no	StringTie	no

6.2 Required external software

Depending on your specified prediction method, `buildModel()` requires external software: Cufflinks, StringTie and/or TACO, to build and/or assemble transcript models. You can either specify the software location using the `cufflinks`, `stringtie`, and `taco` arguments in `buildModel()`, or simply leave these three arugments undefined and let PRAM download them for you automatically. The table below summarized software versions `buildModel()` would download when required software was not specified. Please note that, for **macOS**, pre-compiled Cufflinks binary versions 2.2.1 and 2.2.0 appear to have an issue on processing BAM files, therefore we recommend to use version 2.1.1 instead.

Table 3: **`buildModel()`**-required software and recommended version

software	Linux binary	macOS binary	required by
Cufflinks, Cuffmerge	v2.2.1	v2.1.1	**plcf**, **cfmg**, **cftc**, and **cf**
StringTie, StringTie-merge	v1.3.3b	v1.3.3b	**plst**, **stmg**, and **st**
TACO	v0.7.0	v0.7.0	**cftc**

6.3 Example

```
fbams = c( system.file('extdata/bam/CMPRep1.sortedByCoord.clean.bam',
                       package='pram'),
           system.file('extdata/bam/CMPRep2.sortedByCoord.clean.bam',
                       package='pram') )

foutgtf = tempfile(fileext='.gtf')

buildModel(fbams, foutgtf, method='plst')
```

7 Select transcript models: `selModel()`

Once transcript models were built, you may want to select a subset of them by their genomic features. `selModel()` was developed for this purpose. It allows you to select transcript models by their total number of exons and total length of exons and introns.

`selModel()` requires two arguments:

- `fin_gtf`: input GTF file containing to-be-selected transcript models. This file is required to have **transcript_id** attribute in the ninth column.
- `fout_gtf`: output GTF file containing selected transcript models.

By default: `selModel()` will select transcript models with $\geq$ 2 exons and $\geq$ 200 bp total length of exons and introns. You can change the default using the `min_n_exon` and `min_tr_len` arguments.

7.1 Example

```
fin_gtf = system.file('extdata/gtf/selModel_in.gtf', package='pram')

fout_gtf = tempfile(fileext='.gtf')

selModel(fin_gtf, fout_gtf)
```