

Using SynExtend

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1 Introduction

`SynExtend` is a package of tools for working with objects of class `Synteny` built from the package `DECIPHER`'s `FindSynteny()` function.

Synteny maps provide a powerful tool for quantifying and visualizing where pairs of genomes share order. Typically these maps are built from predictions of orthologous pairs, where groups of pairs that provide contiguous and sequential blocks in their respective genomes are deemed a 'syntenic block'. That designation of synteny can then be used to further interrogate the predicted orthologs themselves, or query topics like genomic rearrangements or ancestor genome reconstruction.

`FindSynteny` takes a different approach, finding exactly matched shared k-mers and determining where shared k-mers, or blocks of proximate shared k-mers are significant. Combining the information generated by `FindSynteny` with locations of genomic features allows us to simply mark where features are linked by syntenic k-mers. These linked features represent potential orthologous pairs, and can be easily evaluated on the basis of the k-mers that they share, or alignment.

2 Package Structure

Currently `SynExtend` contains one set of functions for performing orthology predictions, as well as a rearrangement estimation function that is currently under construction.

2.1 Installation

1. Install the latest version of R using [CRAN](#).
2. Install `SynExtend` in R by running the following commands:

```
if (!requireNamespace("BiocManager",  
                      quietly = TRUE)) {  
  install.packages("BiocManager")  
}  
BiocManager::install("SynExtend")
```

3 Usage

Using the `FindSynteny` function in `DECIPHER` build an object of class `Synteny`. In this tutorial, a prebuilt `DECIPHER` database is used. For database construction see `?Seqs2DB` in `DECIPHER`. This example starts with a database containing three endosymbiont genomes that were chosen to keep package data to a minimum.

```
library(SynExtend)  
## Loading required package: DECIPHER  
## Loading required package: Biostrings  
## Loading required package: BiocGenerics  
##  
## Attaching package: 'BiocGenerics'  
## The following objects are masked from 'package:stats':  
##
```

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```
##      IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##      Filter, Find, Map, Position, Reduce, anyDuplicated, aperm, append,
##      as.data.frame, basename, cbind, colnames, dirname, do.call,
##      duplicated, eval, evalq, get, grep, grepl, intersect, is.unsorted,
##      lapply, mapply, match, mget, order, paste, pmax, pmax.int, pmin,
##      pmin.int, rank, rbind, rownames, sapply, setdiff, sort, table,
##      tapply, union, unique, unsplit, which.max, which.min
## Loading required package: S4Vectors
## Loading required package: stats4
##
## Attaching package: 'S4Vectors'
## The following object is masked from 'package:utils':
##
##      findMatches
## The following objects are masked from 'package:base':
##
##      I, expand.grid, unname
## Loading required package: IRanges
## Loading required package: XVector
## Loading required package: GenomeInfoDb
##
## Attaching package: 'Biostrings'
## The following object is masked from 'package:base':
##
##      strsplit
## Loading required package: RSQLite
## Loading required package: parallel
##
## Attaching package: 'SynExtend'
## The following object is masked from 'package:stats':
##
##      dendrapply

DBPATH <- system.file("extdata",
                      "Endosymbionts.sqlite",
                      package = "SynExtend")

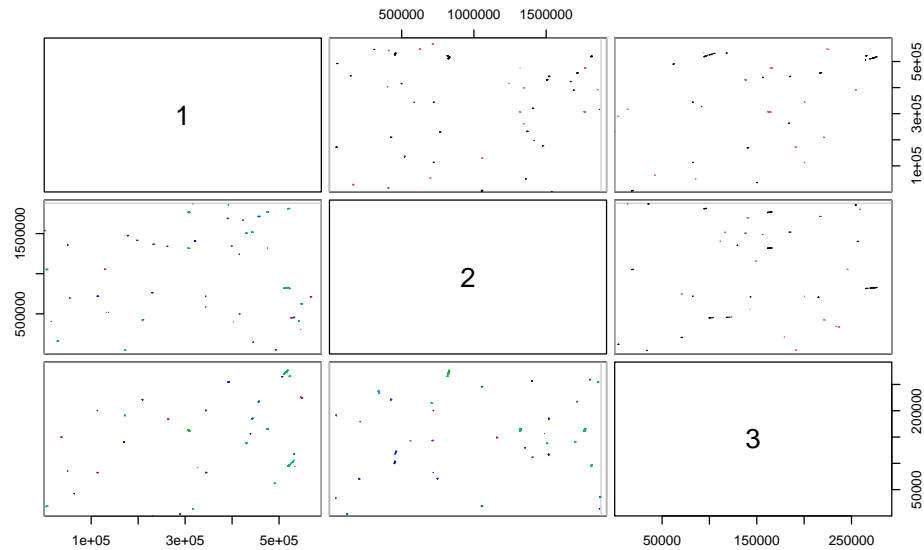
Syn <- FindSynteny(dbFile = DBPATH)
## =====
##
## Time difference of 7.04 secs
```

Synteny maps represent where genomes share order. Simply printing a synteny object to the console displays a gross level view of the data inside. Objects of class `Synteny` can also be plotted to provide clear visual representations of the data inside. The genomes used in this example are distantly related and fairly dissimilar.

```
Syn
##      1      2      3
```

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```
## 1      1 seq 1.4% hits  3% hits
## 2 46 blocks    2 seqs 3.7% hits
## 3 31 blocks 36 blocks    1 seq
pairs(Syn)
```



Data present inside objects of class `Synteny` can also be accessed relatively easily. The object itself is functionally a matrix of lists, with data describing exactly matched k-mers present in the upper triangle, and data describing blocks of chained k-mers in the lower triangle. For more information see `?FindSynteny` in the package DECIPHER.

```
print(head(Syn[[1, 2]]))
##      index1 index2 strand width start1 start2 frame1 frame2
## [1,]      1      1      0    62 510107 820531      1      3
## [2,]      1      1      0    27 510220 820644      1      3
## [3,]      1      1      0    27 511315 821517      1      3
## [4,]      1      1      0    33 511477 821676      1      3
## [5,]      1      1      0    30 511837 822042      1      3
## [6,]      1      1      0    27 512044 822252      1      3
print(head(Syn[[2, 1]]))
##      index1 index2 strand score start1 start2 end1 end2 first_hit
## [1,]      1      1      0   323 510107 820531 518022 827923      1
## [2,]      1      1      0   237 521728 815602 522899 816821     24
## [3,]      1      1      0   147 429274 1502877 430720 1504314     35
## [4,]      1      1      0   135 518406 1810735 521309 1813715     49
## [5,]      1      1      0   135   4142 1051916   6221 1054103     59
## [6,]      1      1      0   117 171167   46819 173077   49020     71
##      last_hit
## [1,]        23
## [2,]        34
## [3,]        48
## [4,]        58
## [5,]        70
## [6,]        78
```

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The above printed objects show the data for the comparison between the first and second genome in our database.

To take advantage of these synteny maps, we can then overlay the gene calls for each genome present on top of our map.

Next, GFF annotations for the associated genomes are parsed to provide gene calls in a use-able format. GFFs are not the only possible source of appropriate gene calls, but they are the source that was used during package construction and testing. Parsed GFFs can be constructed with `gffToDataFrame`, for full functionality, or GFFs can be imported via `rtracklater::import()` for limited functionality. GeneCalls for both the `PairSummaries` and `NucleotideOverlap` functions must be named list, and those names must match `dimnames(Syn)[[1]]`.

```
# generating genecalls with local data:
GC <- gffToDataFrame(GFF = system.file("extdata",
                                       "GCF_021065005.1_ASM2106500v1_genomic.gff.gz",
                                       package = "SynExtend"),
                    Verbose = TRUE)

## =====
## Time difference of 11.17983 secs

# in an effort to be space conscious, not all original gffs are kept within this package
GeneCalls <- get(data("Endosymbionts_GeneCalls", package = "SynExtend"))
```

SynExtend's `gffToDataFrame` function will directly import gff files into a usable format, and includes other extracted information.

```
print(head(GeneCalls[[1]]))
## DataFrame with 6 rows and 11 columns
##      Index Strand Start Stop Type ID
##      <integer> <integer> <integer> <integer> <character> <character>
## 1      1      0      1 1362 gene gene-Gromo_RS00005
## 2      1      0     1652 2740 gene gene-Gromo_RS00010
## 3      1      0     2745 3905 gene gene-Gromo_RS00015
## 4      1      0     4016 6400 gene gene-Gromo_RS00020
## 5      1      0     6496 8598 gene gene-Gromo_RS00025
## 6      1      0     8722 9822 gene gene-Gromo_RS00030
##      Range Product Coding Translation_Table
##      <IRangesList> <character> <logical> <character>
## 1      1-1362 chromosomal replicat.. TRUE 11
## 2     1652-2740 DNA polymerase III s.. TRUE 11
## 3     2745-3905 AAA family ATPase TRUE 11
## 4     4016-6400 DNA topoisomerase (A.. TRUE 11
## 5     6496-8598 hypothetical protein TRUE 11
## 6     8722-9822 MFS transporter TRUE 11
##      Contig
##      <character>
## 1 NZ_CP069247.1
## 2 NZ_CP069247.1
## 3 NZ_CP069247.1
## 4 NZ_CP069247.1
## 5 NZ_CP069247.1
## 6 NZ_CP069247.1
```

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Raw GFF imports are also acceptable, but prevent alignments in amino acid space with `PairSummaries()`.

```
X01 <- rtracklayer::import(system.file("extdata",
                                       "GCA_000875775.1_ASM87577v1_genomic.gff.gz",
                                       package = "SynExtend"))

class(X01)
print(X01)
```

`SynExtend`'s primary functions provide a way to identify where pairs of genes are explicitly linked by syntenic hits, and then summarize those links. The first step is just identifying those links.

```
Links <- NucleotideOverlap(SyntenyObject = Syn,
                           GeneCalls = GeneCalls,
                           Verbose = TRUE)

##
## Reconciling genecalls.
## =====
## Finding connected features.
## =====
## Time difference of 0.405941 secs
```

The `Links` object generated by `NucleotideOverlap` is a raw representation of positions on the syntenic map where shared k-mers link genes between paired genomes. As such, it is analogous in shape to objects of class `Synteny`. This raw object is unlikely to be useful to most users, but has been left exposed to ensure that this data remains accessible should a user desire to have access to it.

```
class(Links)
## [1] "LinkedPairs"
print(Links)
##           1           2           3
## 1  1 Contig  55 Pairs  49 Pairs
## 2 238 Kmers  2 Contigs 57 Pairs
## 3 296 Kmers 337 Kmers  1 Contig
```

This raw data can be processed to provide a straightforward summary of predicted pairs.

```
LinkedPairs1 <- PairSummaries(SyntenyLinks = Links,
                              DBPATH = DBPATH,
                              PIDs = FALSE,
                              Verbose = TRUE)

##
## Preparing overhead data.
## Overhead complete.
## Collecting pairs.
## =====
## Time difference of 3.3424 secs
```

The object `LinkedPairs1` is a `data.frame` where each row is populated by information about a predicted orthologous pair. By default `PairSummaries` uses a simple model to determine whether the k-mers that link a pair of genes are likely to provide an erroneous link. When set

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to `Model = "Global"`, is simply a prediction of whether the involved nucleotides are likely to describe a pair of genomic features whose alignment would result in a PID that falls within a random distribution. This model is effective if somewhat permissive, but is significantly faster than performing many pairwise alignments.

```
print(head(LinkedPairs1))
##          p1      p2 ExactMatch Consensus TotalKmers MaxKmer p1FeatureLength
## 1 1_1_1 2_1_1682      63 0.9867718         2      33         1362
## 2 1_1_4 2_1_1146     397 0.9918857        12      60         2385
## 3 1_1_13 2_1_426      29 0.9957898         1      29          82
## 4 1_1_23 2_1_156     330 0.9975095        10      45         1068
## 5 1_1_40 2_1_1492     131 0.9908460         4      41         2385
## 6 1_1_43 2_1_769      87 0.9881375         3      32         1275
##      p2FeatureLength Adjacent      TetDist PIDType PredictedPID
## 1              1344         0 0.04894316      AA      0.4081521
## 2              2484         0 0.03564127      AA      0.5693577
## 3               84         0 0.11731328      NT      0.5568290
## 4             1053         0 0.05201223      AA      0.6978996
## 5             2448         0 0.04585398      AA      0.3463909
## 6             1347         0 0.04718792      AA      0.4450279
```

`PairSummaries` includes arguments that allow for aligning all pairs that are predicted, via `PIDs = TRUE`, while `IgnoreDefaultStringSet = FALSE` indicates that alignments should be performed in nucleotide or amino acid space as is appropriate for the linked sequences. Setting `IgnoreDefaultStringSet = TRUE` will force all alignments into nucleotide space.

As of `SynExtend` v 1.3.13, the functions `ExtractBy` and `DisjointSet` have been added to provide users with direct tools to work with `PairSummaries` objects.

```
SingleLinkageClusters <- DisjointSet(Pairs = LinkedPairs1,
                                     Verbose = TRUE)
```

```
##
## Assigning initial root:
## =====
## Time difference of 0.006132126 secs
##
## Assigning final root:
##
```

```
## =====
## Time difference of 0.002350092 secs
##
## Assigning single linkage clusters.
## Assignments complete.
##
## Time difference of 0.01349401 secs
```

```
# extract the first 10 clusters
Sets <- ExtractBy(x = LinkedPairs1,
                 y = DBPATH,
                 z = SingleLinkageClusters[1:10],
                 Verbose = TRUE)
##
```

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```
## Extracting Sequences:
## =====
##
## Arranging Sequences:
## =====
##
## Time difference of 0.385998 secs

head(Sets)
## [[1]]
## DNASTringSet object of length 2:
##      width seq                                     names
## [1] 1362 ATGAGTAGTGAAATTTCTAATGT...TTAAAGCAAAATTGATGAAATAA 1_1_1
## [2] 1344 ATGTTGACAAAACCCGCAAGA...CTAGACGCAATTTAGAAGCTTAA 2_1_1682
##
## [[2]]
## DNASTringSet object of length 3:
##      width seq                                     names
## [1] 2385 ATGGATTGACACGATATTAAGAA...ATGTGAGAAATTTAGATGTATAA 1_1_4
## [2] 2484 ATGACTACAATGACCAAAGAAGA...CGGTTAAGAATTTGGATATTTAA 2_1_1146
## [3] 2385 ATGATTAATTATGATTCTTCGAA...CAAATTTTGACTTAGATTTTAA 3_1_28
##
## [[3]]
## DNASTringSet object of length 2:
##      width seq                                     names
## [1] 82 GCCCAGGTGGCGGAATGGTAGAC...AGGTTCAAGTCCTCTTCTGGGTA 1_1_13
## [2] 84 GCGGTCATGGCGGAATGGTAGA...ATGTTGAGTCATCTTGACCGCA 2_1_426
##
## [[4]]
## DNASTringSet object of length 2:
##      width seq                                     names
## [1] 1068 TTGAATAAGAATCAAAATGATGA...AAGATGTACTTGAAGATGTATAA 1_1_23
## [2] 1053 ATGGCTCAACAAATGATGCAGG...TAAAAGAAGAGGCCTTGGTTTAA 2_1_156
##
## [[5]]
## DNASTringSet object of length 3:
##      width seq                                     names
## [1] 2385 ATGTGCGCAAGAGACTTGGATGG...GCTTTAAATTGGAGAAGATATAA 1_1_40
## [2] 2448 TTGGCCAAGAATTTAAAAATAAA...CCTATTTAGACCAAGAGCTATAA 2_1_1492
## [3] 1500 ATGTCGTATAAAGAAAAAAAAT...TTAAATCATATATTCAAGAATGA 3_1_100
##
## [[6]]
## DNASTringSet object of length 2:
##      width seq                                     names
## [1] 1275 ATGTCCGATGTATTGCTATCAAT...GTATGCGTGGAGAAATAACTTGA 1_1_43
## [2] 1347 ATGCGTAAACAACAAGTAAAAAC...GTCTTAAGAGTTTGTAGCCATTAA 2_1_769
```

Session Info:

```
sessionInfo()
## R version 4.3.0 RC (2023-04-13 r84266)
```


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```
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS Monterey 12.6.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib; LAPACK version
##
## locale:
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] parallel stats4      stats      graphics  grDevices  utils      datasets
## [8] methods  base
##
## other attached packages:
## [1] SynExtend_1.12.0  DECIPHER_2.28.0  RSQLite_2.3.1
## [4] Biostrings_2.68.0 GenomeInfoDb_1.36.0 XVector_0.40.0
## [7] IRanges_2.34.0    S4Vectors_0.38.1 BiocGenerics_0.46.0
## [10] BiocStyle_2.28.0
##
## loaded via a namespace (and not attached):
## [1] vctrs_0.6.1      crayon_1.5.2      cli_3.6.1
## [4] knitr_1.42       rlang_1.1.0       xfun_0.38
## [7] DBI_1.1.3        bit_4.0.5         RCurl_1.98-1.12
## [10] htmltools_0.5.5  rmarkdown_2.21    evaluate_0.20
## [13] bitops_1.0-7     fastmap_1.1.1     yaml_2.3.7
## [16] memoise_2.0.1    bookdown_0.33     BiocManager_1.30.20
## [19] compiler_4.3.0   blob_1.2.4        pkgconfig_2.0.3
## [22] digest_0.6.31    GenomeInfoDbData_1.2.10 bit64_4.0.5
## [25] tools_4.3.0      zlibbioc_1.46.0   cachem_1.0.7
```