

Using SynExtend

Nicholas Cooley

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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, 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 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

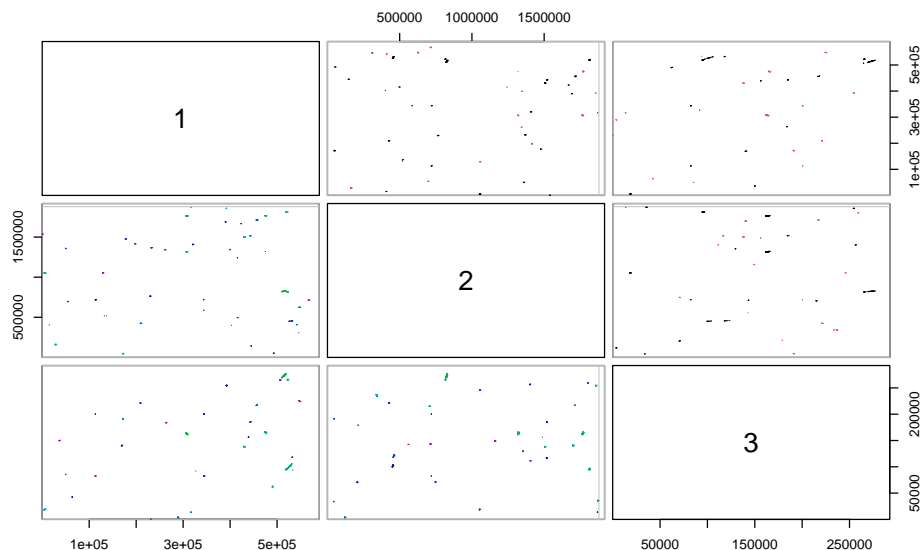
DBPATH <- system.file("extdata",
                      "Endosymbionts.sqlite",
                      package = "SynExtend")

Syn <- FindSynteny(dbFile = DBPATH)
## =====
##
## Time difference of 6.16 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
## 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)
```

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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
```

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.

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

## =====
## Time difference of 14.75235 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
```

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

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```
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 0.5610831 secs
```

The `Links` object generated by `NucleotideOverlap` is a raw representation of positions on the synteny 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 3.500978 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 to `Model = "Global"`, is simply a prediction of whether the involved nucleotides are likely

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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 0.006542921 secs  
##  
## Assigning final root:  
##
```

```
=====  
## Time difference of 0.007014036 secs  
##  
## Assigning single linkage clusters.  
## Assignments complete.  
##  
## Time difference of 0.01918697 secs
```

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

```
##  
## Extracting Sequences:
```

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```
## =====
##
## Arranging Sequences:
## =====
##
## Time difference of 0.31353 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 ATGGATTGAGACGATATTAAGAA...ATGTGAGAAATTTAGATGTATAA 1_1_4
## [2] 2484 ATGACTACAATGACCAAAGAAGA...CGGTTAAGAATTTGGATATTAA 2_1_1146
## [3] 2385 ATGATTAATTATGATTCTTCGAA...CAAATTTGACTTAGATTTTAA 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 ATGGCTCAACAAAATGATGCAGG...TAAAAGAAGAGGCCTTGTTTAA 2_1_156
##
## [[5]]
## DNASTringSet object of length 3:
##      width seq                      names
## [1] 2385 ATGTGCGCAAGAGACTTGATGG...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...GTATGCGTGGAGAAATAACTGA 1_1_43
## [2] 1347 ATGCGTAAACAACAAGTAAAAAC...GTCTTAAGAGTTTtagccattaa 2_1_769
```

Session Info:

```
sessionInfo()
## R version 4.2.0 RC (2022-04-19 r82224)
## Platform: x86_64-apple-darwin17.0 (64-bit)
```


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```
## Running under: macOS Mojave 10.14.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] parallel stats4      stats      graphics  grDevices utils      datasets
## [8] methods   base
##
## other attached packages:
## [1] SynExtend_1.8.0      DECIPHER_2.24.0      RSQLite_2.2.12
## [4] Biostrings_2.64.0    GenomeInfoDb_1.32.0  XVector_0.36.0
## [7] IRanges_2.30.0       S4Vectors_0.34.0     BiocGenerics_0.42.0
## [10] BiocStyle_2.24.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.8.3          compiler_4.2.0        BiocManager_1.30.17
## [4] bitops_1.0-7          tools_4.2.0           zlibbioc_1.42.0
## [7] digest_0.6.29         bit_4.0.4             evaluate_0.15
## [10] memoise_2.0.1         pkgconfig_2.0.3       rlang_1.0.2
## [13] cli_3.3.0             DBI_1.1.2             yaml_2.3.5
## [16] xfun_0.30             fastmap_1.1.0         GenomeInfoDbData_1.2.8
## [19] stringr_1.4.0         knitr_1.38            vctrs_0.4.1
## [22] bit64_4.0.5           rmarkdown_2.14        bookdown_0.26
## [25] blob_1.2.3            magrittr_2.0.3        htmltools_0.5.2
## [28] stringi_1.7.6         RCurl_1.98-1.6        cachem_1.0.6
## [31] crayon_1.5.1
```