

# 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 proximal 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 syntenic k-mers that they share, or alignment.

## 2 Package Structure

---

Currently SynExtend contains only one set of functions, but will be expanded in the future.

### 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 archaea genomes: *Nitrosopumilus adriaticus*, *Nitrosopumilus piranensis*, and a *Candidatus Nitrosopumilus*.

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

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```
##      clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##      clusterExport, clusterMap, parApply, parCapply, parLapply,
##      parLapplyLB, parRapply, parSapply, parSapplyLB
## The following objects are masked from 'package:stats':
##
##      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

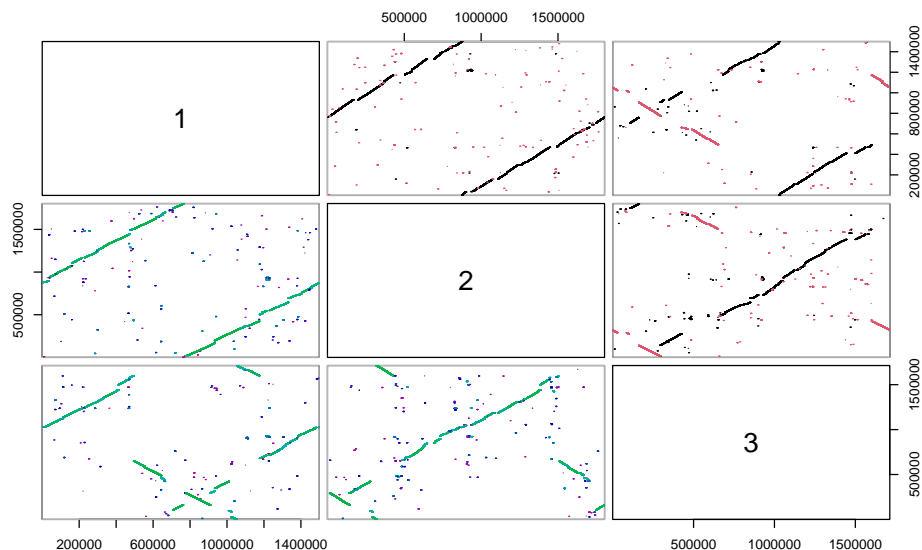
DBPATH <- system.file("extdata",
                      "VignetteSeqs.sqlite",
                      package = "SynExtend")

Syn <- FindSynteny(dbFile = DBPATH)
## =====
##
## Time difference of 8.07 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 clear visual representations of the data inside. The genomes used in this example are all from the same genus, and should be expected to be somewhat closely related.

```
Syn
##           1           2           3
## 1         1 seq   43% hits 58% hits
## 2 177 blocks         1 seq 39% hits
## 3 133 blocks 207 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    18 932191 197117      0      0
## [2,]      1      1      0    14 932212 197138      0      0
## [3,]      1      1      0    45 932268 197194      3      1
## [4,]      1      1      0    17 932350 197276      0      0
## [5,]      1      1      0    20 932377 197303      0      0
## [6,]      1      1      0    24 932416 197342      1      2
print(head(Syn[[2, 1]]))
##      index1 index2 strand score start1 start2 end1 end2 first_hit
## [1,]      1      1      0 40740 932191 197117 1091978 359925      1
## [2,]      1      1      0 34439 495594 1507344 623248 1649448    1975
## [3,]      1      1      0 31996 771868 13735 918407 154427    3561
## [4,]      1      1      0 16601 328609 1298395 463732 1437247    5212
## [5,]      1      1      0 14806 705863 1746189 760642 1799647    6849
## [6,]      1      1      0 14003 40177 943557 155993 1070853    7482
##      last_hit
## [1,]      1974
## [2,]     3560
## [3,]     5211
## [4,]     6848
## [5,]     7481
## [6,]     8694
```

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

To take advantage of these syntenic 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]]`.

```
GeneCalls <- vector(mode = "list",
                    length = ncol(Syn))

GeneCalls[[1L]] <- gffToDataFrame(GFF = system.file("extdata",
                                                    "GCA_006740685.1_ASM674068v1_genomic.gff.gz",
                                                    package = "SynExtend"),
                                Verbose = TRUE)

## =====
## Time difference of 28.90044 secs
GeneCalls[[2L]] <- gffToDataFrame(GFF = system.file("extdata",
                                                    "GCA_000956175.1_ASM95617v1_genomic.gff.gz",
                                                    package = "SynExtend"),
                                Verbose = TRUE)

## =====
## Time difference of 39.7893 secs
GeneCalls[[3L]] <- gffToDataFrame(GFF = system.file("extdata",
                                                    "GCA_000875775.1_ASM87577v1_genomic.gff.gz",
                                                    package = "SynExtend"),
                                Verbose = TRUE)

## =====
## Time difference of 39.33171 secs

names(GeneCalls) <- seq(length(GeneCalls))
```

SynExtend's `gffToDataFrame` function will directly import gff files into a useable 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        1     307    621   gene gene-Nisw-00010
## 2         1        1     673   1182   gene gene-Nisw-00015
## 3         1        0    1271   1621   gene gene-Nisw-00020
## 4         1        1    1603   1914   gene gene-Nisw-00025
## 5         1        0    2013   2225   gene gene-Nisw-00030
## 6         1        1    2222   3313   gene gene-Nisw-00035
##      Range      Product  Coding  Translation_Table  Contig
##   <IRangesList> <character> <logical> <character> <character>
## 1      307-621  DNA-binding protein    TRUE          11  CP035425.1
## 2      673-1182 DNA-directed RNA pol..    TRUE          11  CP035425.1
## 3     1271-1621 hypothetical protein    TRUE          11  CP035425.1
## 4     1603-1914 MarR family transcri..    TRUE          11  CP035425.1
## 5     2013-2225 hypothetical protein    TRUE          11  CP035425.1
## 6     2222-3313 deoxyhypusine synthase    TRUE          11  CP035425.1
```

## Using SynExtend

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(SytenyObject = Syn,
                           GeneCalls = GeneCalls,
                           LimitIndex = FALSE,
                           Verbose = TRUE)

##
## Reconciling genecalls.
## =====
## Finding connected features.
## =====
## Time difference of 3.181271 secs
```

The `Links` object generated by `NucleotideOverlap` is a raw representation of positions on the syteny map where shared k-mers link genes between paired genomes. As such, it is analagous in shape to objects of class `Syteny`. 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 1740 Pairs 1807 Pairs
## 2 14814 Kmers    1 Contig 1840 Pairs
## 3 16722 Kmers 15127 Kmers    1 Contig
```

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

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

##
## Preparing overhead data.
## Overhead complete.
## Collecting pairs.
## =====
## Time difference of 37.23505 secs
```

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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 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_1080	1	0.9931940	1	1	315
## 2	1_1_1	2_1_1081	178	0.9878461	6	57	315
## 3	1_1_2	2_1_1082	237	0.9734190	8	60	510
## 4	1_1_3	2_1_1083	252	0.9612734	8	69	351
## 5	1_1_4	2_1_1083	9	0.9651535	1	9	312
## 6	1_1_4	2_1_1084	134	1.0000000	6	30	312

##	p2FeatureLength	Adjacent	TetDist	PIDType	PredictedPID
## 1	417	1	0.07524152	AA	0.4152832
## 2	309	1	0.06476061	AA	0.7832442
## 3	543	2	0.04352695	AA	0.8178053
## 4	387	2	0.05609274	AA	0.8442963
## 5	387	2	0.09458148	AA	0.4622422
## 6	312	2	0.07030926	AA	0.7950427

`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 0.06195211 secs  
##  
## Assigning final root:  
##
```

```
=====  
## Time difference of 0.07099795 secs  
##  
## Assigning single linkage clusters.  
## =====  
## Time difference of 0.710489 secs
```

```
SeqsByCluster <- ExtractBy(x = LinkedPairs1,  
                           y = SingleLinkageClusters,  
                           Method = "clusters",  
                           DBPATH = DBPATH,
```

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```
Verbose = TRUE)

##
## Preparing overhead data.
## Overhead complete.
## =====
## Time difference of 3.53257 mins

head(SeqsByCluster)
## [[1]]
## AStringSet object of length 6:
##      width seq                                     names
## [1]   105 MLMDETREPHGQEQTKKSDETIA...DTDAVAGIGRMTSTIEIVLVKI* 1_1_1
## [2]   139 MLLPAEIESKTLIPALRAILAKK...NIDEQVCKECENMLLKPGGSVY* 1_1_1794
## [3]   139 MLLPAEIESKTLIPALRAILAKK...NIDEQVCKECENMLLKPGGSVY* 2_1_1080
## [4]   103 MEETTEPYGQEQTKESEGTHI...DTAEAPGIGSMTSTIEIILNKI* 2_1_1081
## [5]   139 MLLPAEIESKTLIPALRAILAKK...DIDEQVCKECENMLLKPGGSVY* 3_1_1268
## [6]   103 MEETSEPYGQEQTKKSDIAIHI...DTENAAGIGRMTSTIEIILIKN* 3_1_1269
##
## [[2]]
## AStringSet object of length 3:
##      width seq                                     names
## [1]   170 MSDANNTVEVVEAEDEIPATEEI...ITIRRVLPNGDYQNIPIDYFEK* 1_1_2
## [2]   181 MIRLYNVLRKFCYLSDVKEAPLV...ITIRRVLPNGDFQNIPIIDYFEK* 2_1_1082
## [3]   183 MIRLYNVLRKNRYLSANKTKVV...ITIRRVLPNGDYQNIPIDYFEK* 3_1_1270
##
## [[3]]
## AStringSet object of length 6:
##      width seq                                     names
## [1]   117 MEIRGKAKICCDLKRHLSPRTVG...DNIDALKDVKSGDVLCIYEETA* 1_1_3
## [2]   104 MGGAKKPTVAKKDTSSGSKESKK...EKGIVKRVGGYSGHLYQAVSS* 1_1_4
## [3]   129 MSTSSVSRKQLILEIRGKAKISC...GDIDALKNVKSGDVLCIYEETA* 2_1_1083
## [4]   104 MGGAKKPTAANKDKSAGSKDTKK...IKGTVKRVGGYSGHLYQAVSS* 2_1_1084
## [5]   129 MSTASVSRKQLILEIKGKAKISC...ENIDALKDVKSGDVLCIYEETA* 3_1_1271
## [6]   104 MGGAKKPTAAKDTSSNTKDSKK...EKGIVKKVGGYSGHLYQAVSS* 3_1_1272
##
## [[4]]
## AStringSet object of length 6:
##      width seq                                     names
## [1]    71 MGVSXGAKCNVDGCDNDGARSL...YKEYKESKDDRDLERARFDKF* 1_1_5
## [2]   364 VDPHFHFGKDIPHIKLDPKMTIE...YEKLSNDYFKNPVNKKRSKKKN* 1_1_6
## [3]    71 MGIVSKGAKCNVDGCDQDGARSL...YKEWKESKDDRDLERARFDKF* 2_1_1085
## [4]   354 VDPHEFHFGKDIPHIKLDPNMTIE...KRLYKLDKLYEKLREDYSKNP* 2_1_1086
## [5]    71 MGVSXGAKCNVDGCDKDGARSL...YKEYKESKDDRELERARYDRF* 3_1_1273
## [6]   366 MVVDPHKFHFGKDIPHIKLDPKMT...YEKLSKDYFKNPVKKRVKKKN* 3_1_1274
##
## [[5]]
## AStringSet object of length 3:
##      width seq                                     names
## [1]   112 MDIIDLHDPQRVNRSPDAVEILL...TSNLGISGLILRSVISLRGKLE* 1_1_7
## [2]   112 MKIIDLHDPQRVDKSPDDVEILM...ISNLGISALILRSIICLREKLD* 2_1_1087
## [3]   113 MMDIVDLHDPQRVNRIPDKTKVL...TSNLGISGLILRSVISLRGKTS* 3_1_1275
```

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```
##
## [[6]]
## AStringSet object of length 3:
##      width seq                                     names
## [1]  623 MRILQLHCDSIEYTPKKEIKSA...SGKPFTGLNQSSHLSKRPQLMV* 1_1_12
## [2]  621 MRILQLHCDSIEYTPKKEIKSA...KEKPFTGLNLPKYLSKRPQLMV* 2_1_1094
## [3]  622 MRILQLHCDSIEYTPKKEIKSA...SGKPFTGLNQAMHLSKRPQLMV* 3_1_1276
```

### Session Info:

```
sessionInfo()
## R version 4.1.0 (2021-05-18)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Mojave 10.14.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/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] stats4      parallel  stats      graphics  grDevices  utils      datasets
## [8] methods     base
##
## other attached packages:
## [1] SynExtend_1.4.1      DECIPHER_2.20.0      RSQLite_2.2.7
## [4] Biostrings_2.60.0    GenomeInfoDb_1.28.0  XVector_0.32.0
## [7] IRanges_2.26.0       S4Vectors_0.30.0     BiocGenerics_0.38.0
## [10] BiocStyle_2.20.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.6           knitr_1.33           magrittr_2.0.1
## [4] zlibbioc_1.38.0      bit_4.0.4            rlang_0.4.11
## [7] fastmap_1.1.0        blob_1.2.1           stringr_1.4.0
## [10] tools_4.1.0          xfun_0.23            DBI_1.1.1
## [13] htmltools_0.5.1.1    bit64_4.0.5          yaml_2.2.1
## [16] digest_0.6.27        crayon_1.4.1         bookdown_0.22
## [19] GenomeInfoDbData_1.2.6 BiocManager_1.30.15  vctrs_0.3.8
## [22] bitops_1.0-7         RCurl_1.98-1.3       cachem_1.0.5
## [25] memoise_2.0.0        evaluate_0.14        rmarkdown_2.8
## [28] stringi_1.6.2        compiler_4.1.0       pkgconfig_2.0.3
```