Whole genome scaffolding using SaaRclust

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Package

SaaRclust 0.99

Contents

1 Introduction

Strand-seq is a single-cell sequencing technique able to preserve contiguity of individual parental homologues in single-cell (Falconer et al. 2012). Each parental homolog undergoes independent random segregation during cell division, leading to a unique strand state profile in Strand-seq data. Strand-seq distinguishes three possible template strand states for each chromosome of a diploid genome. The Watson-Watson (WW) strand state is characteristic of two Watson (reads aligned to minus strand) templates inherited from both parental homologues. The Crick-Crick (CC) strand state is characteristic of two Crick (reads aligned to plus strand) templates inherited from both parental homologues. Lastly, the Watson-Crick (WC) strand state is characteristic of a Watson and Crick template being inherited from either parental homologue (Sanders et al. 2017). Such Strand-seq signal can be used to assign contigs or long sequencing reads to a chromosome of origin. This feature has been shown to be valuable for scaffolding early build genome assemblies as well finding chimeric or misoriented contigs (Hills et al. 2013). We do so using SaaRclust, an R based package that implements a novel latent variable model and a corresponding Expectation Maximization (EM) algorithm in order to reliably cluster contigs or long sequencing reads by chromosome. SaaRclust was previously introduced for this in silico separation of long sequencing reads by chromosome and direction (Ghareghani et al. 2018). Here we have extended its functionalities to be able to scaffold contig stage assemblies and to detect and correct assembly error such as chimeric or misoriented contigs. SaaRclust employs an Expectation-Maximization (EM) soft clustering algorithm to handle the uncertainty arising from the sparse Strand-seq data. The main idea underlying our clustering algorithm is that contigs originating from the same chromosome share the same directionality pattern of aligned Strand-seq reads across multiple single cells, that differs from contigs originating from a different chromosome. The EM algorithm is based on iterating between assigning strand states for each Strand-seq library and chromosome and assigning chromosomes to each contig, which are both hidden information at the beginning. EM converges to a local optimum solution of the maximum likelihood problem, e.g., maximizing the likelihood of observed data (number of directional aligned Strand-seq reads to long reads), given the model parameters (strand states), and we have shown SaaRclust to be able to assign even individual long sequencing reads to chromosomes of origin.

2 Minimal parameters

Here are the minimal parameters required to successfully run genome scaffolding using SaaRclust.

bamfolder: A folder name where minimap file(s) is stored.
outputfolder: A folder containing BAM files with Strand-seq reads aligned to a denovo assembly.
pairedEndReads: Make sure to set to TRUE if paired-end reads are being used.
assembly.fasta: A denovo assembly FASTA file, if one want to export scaffolded denovo assembly in FASTA format.

For more details on available parameters please run.

```
library(SaaRclust)
?scaffoldDenovoAssembly
```

3 Quick Start

Run SaaRclust using pre-computed binned counts of Strand-seq reads aligned to the GRCh38. To speed up this process we sent bin size to 5 Mbp and included sequences of 10 Mbp and longer. Because Strand-seq data have been aligned to GRCh38 with chromosomes 1-22, X and Y we have set 'desired.num.clusters' parameter to 24.

```
bamfolder <- system.file("extdata", package = "SaaRclust")
scaffoldDenovoAssembly(bamfolder = bamfolder,
                       outputfolder = bamfolder,
                       store.data.obj = TRUE,
                       reuse.data.obj = TRUE,
                       pairedEndReads = TRUE,
                       bin.size = 5000000,
                       step.size = 5000000,
                       bin.method = 'dynamic',
                       prob.th = 0.25,
                       ord.method = 'greedy',
                       min.contig.size = 10000000,
                       concat.fasta = FALSE,
                       num.clusters = 100,
                       desired.num.clusters = 24,
                       min.region.to.order = 1000000,
                       remove.always.WC = TRUE,
                       mask.regions = FALSE)
```

4 Parameter settings recomendations

min.contig.size: This parameter should be set at least 2x the N50 read length used for the assembly

4.1 Soft Clustering

If RData object containing hard clustering results is already available you can run only soft clustering.

```
# Setting some variables

HC.input='SaaRclust_results/Clusters/hardClusteringResults.RData'
minimap.file='SaaRclust_exampleData/NA12878_WashU_PBreads_chunk9126.maf.gz'
```

```
# If theta.param & pi.param are set to NULL SaaRclust will try to load them from HC.input.

SaaRclust(minimap.file=minimap.file, outputfolder='SaaRclust_results', num.clusters=47,
EM.iter=100, alpha=0.1, minLib=10, upperQ=0.95, theta.param=NULL, pi.param=NULL, logL.th=1,
theta.constrain=FALSE, store.counts=FALSE, HC.input=HC.input)
```

4.2 Recomendations

In order to run both, hard and soft clustering in a single command.

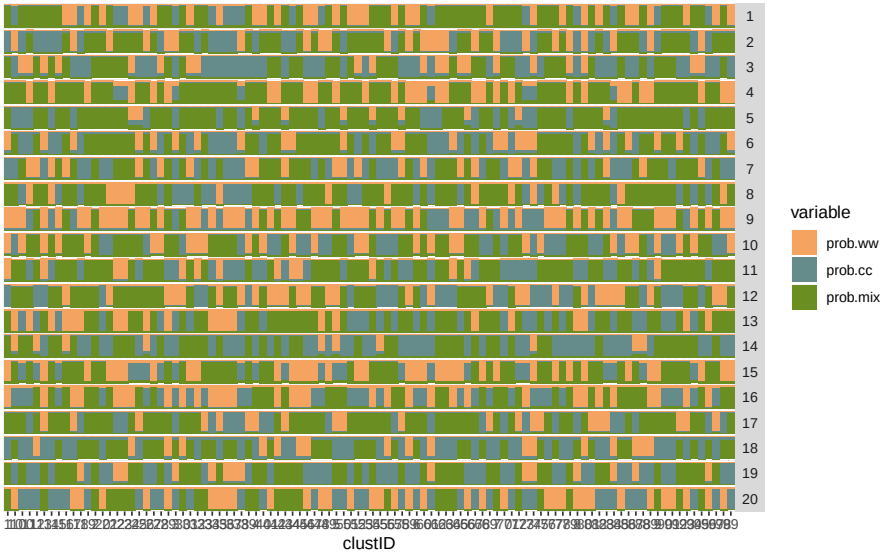
```
# Hard clustering
# Remember to set HC.only=FALSE

runSaaRclust(inputfolder=inputfolder, outputfolder="SaaRclust_results", num.clusters=54,
EM.iter=100,alpha=0.01, minLib=10, upperQ=0.95, logL.th=1, theta.constrain=FALSE,
store.counts=FALSE, store.bestAlign=TRUE, numAlignments=3000, HC.only=FALSE, verbose=TRUE)
```

4.3 Plot clustering accuracy plots

NOTE: Working only for data from the original publication.
Rscript below plots clustering accuracy measures presented in the orignal paper (Fig4 b,d,c)
Before running the script make sure that biovizBase and ggplot2 packages are installed on your machine.

```
## Load the package
library(SaaRclust)
## Get example files
example.data <- system.file("extdata", "softClust_100K_5e+06bp_dynamic.RData",
                            package = "SaaRclust")
EM.obj <- get(load(example.data))
## Plot theta parameter
plotThetaEstimates(theta.param = EM.obj$theta.param[1:20])
```

Whole genome scaffolding using SaaRclust



5 Session Info

```
devtools::session_info()
## - Session info -------------------------------------------------------------
##  setting  value
##  version  R version 3.5.3 (2019-03-11)
##  os       Ubuntu 18.04.2 LTS
##  system   x86_64, linux-gnu
##  ui       X11
##  language (EN)
##  collate  en_US.UTF-8
##  ctype    en_US.UTF-8
##  tz       America/Los_Angeles
##  date     2020-03-27
##
## - Packages -----------------------------------------------------------------
##  package              * version   date       lib source
##  assertthat             0.2.1     2019-03-21 [1] CRAN (R 3.5.3)
##  backports              1.1.4     2019-04-10 [1] CRAN (R 3.5.3)
##  bamsignals             1.14.0    2018-10-30 [1] Bioconductor
##  BH                   * 1.69.0-1  2019-01-07 [1] CRAN (R 3.5.3)
##  Biobase                2.42.0    2018-10-30 [1] Bioconductor
##  BiocGenerics         * 0.28.0    2018-10-30 [1] Bioconductor
##  BiocManager            1.30.4    2018-11-13 [1] CRAN (R 3.5.3)
##  BiocParallel           1.16.6    2019-02-10 [1] Bioconductor
##  BiocStyle            * 2.10.0    2018-10-30 [1] Bioconductor
##  Biostrings             2.50.2    2019-01-03 [1] Bioconductor
##  bitops                 1.0-6     2013-08-17 [1] CRAN (R 3.5.3)
##  bookdown               0.12      2019-07-11 [1] CRAN (R 3.5.3)
##  callr                  3.3.2     2019-09-22 [1] CRAN (R 3.5.3)
##  cli                    1.1.0     2019-03-19 [1] CRAN (R 3.5.3)
##  cluster                2.0.7-1   2018-04-09 [4] CRAN (R 3.5.0)
##  codetools              0.2-16    2018-12-24 [4] CRAN (R 3.5.2)
##  colorspace             1.4-1     2019-03-18 [1] CRAN (R 3.5.3)
##  cowplot              * 1.0.0     2019-07-11 [1] CRAN (R 3.5.3)
##  crayon                 1.3.4     2017-09-16 [1] CRAN (R 3.5.3)
##  data.table             1.12.8    2019-12-09 [1] CRAN (R 3.5.3)
##  DelayedArray           0.8.0     2018-10-30 [1] Bioconductor
##  desc                   1.2.0     2018-05-01 [1] CRAN (R 3.5.3)
##  devtools               2.1.0     2019-07-06 [1] CRAN (R 3.5.3)
##  digest                 0.6.21    2019-09-20 [1] CRAN (R 3.5.3)
##  dplyr                  0.8.3     2019-07-04 [1] CRAN (R 3.5.3)
##  evaluate               0.14      2019-05-28 [1] CRAN (R 3.5.3)
##  foreach                1.4.4     2017-12-12 [1] CRAN (R 3.5.3)
##  fs                     1.3.1     2019-05-06 [1] CRAN (R 3.5.3)
##  GenomeInfoDb         * 1.18.2    2019-02-12 [1] Bioconductor
##  GenomeInfoDbData       1.2.0     2019-04-10 [1] Bioconductor
##  GenomicAlignments      1.18.1    2019-01-04 [1] Bioconductor
##  GenomicRanges        * 1.34.0    2018-10-30 [1] Bioconductor
##  ggplot2              * 3.2.1     2019-08-10 [1] CRAN (R 3.5.3)
```

```
##  glue                  1.3.1     2019-03-12 [1] CRAN (R 3.5.3)
##  gtable                0.3.0     2019-03-25 [1] CRAN (R 3.5.3)
##  htmltools             0.4.0     2019-10-04 [1] CRAN (R 3.5.3)
##  igraph                1.2.4.1   2019-04-22 [1] CRAN (R 3.5.3)
##  IRanges             * 2.16.0    2018-10-30 [1] Bioconductor
##  iterators             1.0.10    2018-07-13 [1] CRAN (R 3.5.3)
##  knitr                 1.25      2019-09-18 [1] CRAN (R 3.5.3)
##  labeling              0.3       2014-08-23 [1] CRAN (R 3.5.3)
##  lattice               0.20-38   2018-11-04 [4] CRAN (R 3.5.1)
##  lazyeval              0.2.2     2019-03-15 [1] CRAN (R 3.5.3)
##  lifecycle             0.1.0     2019-08-01 [1] CRAN (R 3.5.3)
##  lpSolve               5.6.13.3  2019-08-19 [1] CRAN (R 3.5.3)
##  magrittr              1.5       2014-11-22 [1] CRAN (R 3.5.3)
##  Matrix                1.2-16    2019-03-08 [4] CRAN (R 3.5.2)
##  matrixStats           0.54.0    2018-07-23 [1] CRAN (R 3.5.3)
##  memoise               1.1.0     2017-04-21 [1] CRAN (R 3.5.3)
##  munsell               0.5.0     2018-06-12 [1] CRAN (R 3.5.3)
##  pillar                1.4.2     2019-06-29 [1] CRAN (R 3.5.3)
##  pkgbuild              1.0.3     2019-03-20 [1] CRAN (R 3.5.3)
##  pkgconfig             2.0.3     2019-09-22 [1] CRAN (R 3.5.3)
##  pkgload               1.0.2     2018-10-29 [1] CRAN (R 3.5.3)
##  plyr                  1.8.4     2016-06-08 [1] CRAN (R 3.5.3)
##  prettyunits           1.0.2     2015-07-13 [1] CRAN (R 3.5.3)
##  processx              3.4.1     2019-07-18 [1] CRAN (R 3.5.3)
##  ps                    1.3.0     2018-12-21 [1] CRAN (R 3.5.3)
##  purrr                 0.3.2     2019-03-15 [1] CRAN (R 3.5.3)
##  R6                    2.4.0     2019-02-14 [1] CRAN (R 3.5.3)
##  RColorBrewer        * 1.1-2     2014-12-07 [1] CRAN (R 3.5.3)
##  Rcpp                  1.0.2     2019-07-25 [1] CRAN (R 3.5.3)
##  RCurl                 1.95-4.12 2019-03-04 [1] CRAN (R 3.5.3)
##  remotes               2.1.0     2019-06-24 [1] CRAN (R 3.5.3)
##  reshape2              1.4.3     2017-12-11 [1] CRAN (R 3.5.3)
##  rlang                 0.4.0     2019-06-25 [1] CRAN (R 3.5.3)
##  rmarkdown             1.16      2019-10-01 [1] CRAN (R 3.5.3)
##  rprojroot             1.3-2     2018-01-03 [1] CRAN (R 3.5.3)
##  Rsamtools             1.34.1    2019-01-31 [1] Bioconductor
##  S4Vectors           * 0.20.1    2018-11-09 [1] Bioconductor
##  SaaRclust           * 0.99      2020-03-25 [1] local
##  scales              * 1.0.0     2018-08-09 [1] CRAN (R 3.5.3)
##  sessioninfo           1.1.1     2018-11-05 [1] CRAN (R 3.5.3)
##  stringi               1.4.3     2019-03-12 [1] CRAN (R 3.5.3)
##  stringr               1.4.0     2019-02-10 [1] CRAN (R 3.5.3)
##  SummarizedExperiment  1.12.0    2018-10-30 [1] Bioconductor
##  testthat              2.1.1     2019-04-23 [1] CRAN (R 3.5.3)
##  tibble                2.1.3     2019-06-06 [1] CRAN (R 3.5.3)
##  tidyr                 1.0.0     2019-09-11 [1] CRAN (R 3.5.3)
##  tidyselect            0.2.5     2018-10-11 [1] CRAN (R 3.5.3)
##  TSP                   1.1-7     2019-05-22 [1] CRAN (R 3.5.3)
##  usethis               1.5.1     2019-07-04 [1] CRAN (R 3.5.3)
##  vctrs                 0.2.0     2019-07-05 [1] CRAN (R 3.5.3)
##  withr                 2.1.2     2018-03-15 [1] CRAN (R 3.5.3)
```

```
##  xfun                  0.10      2019-10-01 [1] CRAN (R 3.5.3)
##  XVector               0.22.0    2018-10-30 [1] Bioconductor
##  yaml                  2.2.0     2018-07-25 [1] CRAN (R 3.5.3)
##  zeallot               0.1.0     2018-01-28 [1] CRAN (R 3.5.3)
##  zlibbioc              1.28.0    2018-10-30 [1] Bioconductor
##
## [1] /home/porubsky/R/x86_64-pc-linux-gnu-library/3.5
## [2] /usr/local/lib/R/site-library
## [3] /usr/lib/R/site-library
## [4] /usr/lib/R/library
```

Report any issues here:

References

Falconer, Ester, Mark Hills, Ulrike Naumann, Steven S S Poon, Elizabeth A Chavez, Ashley D Sanders, Yongjun Zhao, Martin Hirst, and Peter M Lansdorp. 2012. "DNA Template Strand Sequencing of Single-Cells Maps Genomic Rearrangements at High Resolution." *Nat. Methods* 9 (11): 1107–12.

Ghareghani, Maryam, David Porubsky, Ashley D Sanders, Sascha Meiers, Evan E Eichler, Jan O Korbel, and Tobias Marschall. 2018. "Strand-Seq Enables Reliable Separation of Long Reads by Chromosome via Expectation Maximization." *Bioinformatics* 34 (13): i115–i123.

Hills, Mark, Kieran O'Neill, Ester Falconer, Ryan Brinkman, and Peter M Lansdorp. 2013. "BAIT: Organizing Genomes and Mapping Rearrangements in Single Cells." *Genome Med.* 5 (9): 82.

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