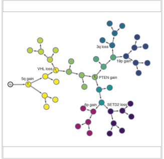

May 12, 2020

Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile

Fang Wang[1], Qihan Wang[2], Vakul Mohanty[1], Shaoheng Liang[1], Jinzhuang Dou[1], Jincheng Han[1], Darlan Conterno Minussi[1], Ruli Gao[3], Li Ding[4], Nicholas Navin[1], Ken Chen[5]

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1 Works for me dx.doi.org/10.17504/protocols.io.bfhpjj5n

Fang Wang

ABSTRACT

This protocol describes two innovative algorithms:

1) A minimal event distance aneuploidy lineage tree (MEDALT) inference algorithm allows implementing genetically meaningful distances and is scalable to current single-cell datasets containing thousands of cells, and

2) A statistical routine, Lineage Speciation Analysis (LSA), enables prioritization of CNAs and genes that are non-randomly associated with the observed lineage expansion and thereby are potentially functionally important.

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2020.04.12.038281v1.full

1 Install Python 2.7 and R 3.5
Download MEDALT tool from https://github.com/KChen-lab/MEDALT.git

MEDALT 1.0
by Fang Wang and Qihan Wang

Extract input dataset

Single cell copy number profile generated by single cell DNA seq

Single cell copy number profile inferred from single cell RNA se

2 Decompress gzipped files (MEDALT-1.0.tar.gz)

```
tar -zxvf MEDALT-1.0.tar.gz
cd MEDALT-1.0

#help document
python scTree.py -h

Usage: python scTree.py <-P path> <-I input> <-D datatype>

Input integer copy number profile. Columns correspond to chromosomal position.
Rows correspond to cells.

Options:
  --version             show program's version number and exit
  -h, --help            Show this help message and exit.
  -P PATH, --Path=PATH  Path to script
  -I INPUT, --Input=INPUT
                        Input file
  -G GENOME, --Genome=GENOME
                        Genome version hg19 or hg38
  -O OUTPUT, --Output=OUTPUT
                        Output path
  -D DATATYPE, --Datatype=DATATYPE
                        The type of input data. Either D (DNA-seq)
                        or R (RNA-seq).
  -W WINDOWS, --Windows=WINDOWS
                        the number of genes you want to merge when
                        you input copy number profile inferred from
                        scRNA-seq. Default 30.
  -R PERMUTATION, --Permutation=PERMUTATION
                        Whether reconstructed permuted tree (T) or
                        not (F). If not, permuted copy number
                        profile will be used to perform LSA. Default
                        value is F due to time cost.
```

3 Run the example data generated based on single cell DNA sequencing technology

scDNA.CNV.txt

```
python scTree.py -P ./ -I ./example/scDNA.CNV.txt -D D -G hg19 -O
./example/outputDNA


Transfer data to segmental level
Inferring MEDALT.
MEDALT inferrence finish
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
MEDALT inference finish.
Performing LSA.
Loading required package: BiocGenerics
Loading required package: parallel

Attaching package: ‘BiocGenerics’

The following objects are masked from ‘package:parallel’:

    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’:

    anyDuplicated, append, as.data.frame, basename, cbind, colMeans,
    colnames, colSums, dirname, do.call, duplicated, eval, evalq,
    Filter, Find, get, grep, grepl, intersect, is.unsorted, lapply,
    lengths, Map, mapply, match, mget, order, paste, pmax, pmax.int,
    pmin, pmin.int, Position, rank, rbind, Reduce, rowMeans, rownames,
    rowSums, sapply, setdiff, sort, table, tapply, union, unique,
    unsplit, which, which.max, which.min

Loading required package: S4Vectors
Loading required package: stats4

Attaching package: ‘S4Vectors’

The following object is masked from ‘package:base’:

    expand.grid

Loading required package: IRanges
Loading required package: GenomicRanges
Loading required package: GenomeInfoDb
Loading required package: Biostrings
Loading required package: XVector

Attaching package: ‘Biostrings’

The following object is masked from ‘package:base’:

    strsplit

Loading required package: BSgenome
Loading required package: rtracklayer
Loading required package: GenomicFeatures
Loading required package: AnnotationDbi
Loading required package: Biobase
Welcome to Bioconductor
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.

Loading required package: VariantAnnotation
Loading required package: SummarizedExperiment
Loading required package: DelayedArray
Loading required package: matrixStats

Attaching package: ‘matrixStats’

The following objects are masked from ‘package:Biobase’:

    anyMissing, rowMedians

Loading required package: BiocParallel

Attaching package: ‘DelayedArray’

The following objects are masked from ‘package:matrixStats’:

    colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges

The following object is masked from ‘package:Biostrings’:

    type

The following objects are masked from ‘package:base’:

    aperm, apply

Loading required package: Rsamtools

Attaching package: ‘VariantAnnotation’

The following object is masked from ‘package:base’:

    tabulate

Loading required package: GenomicAlignments
There were 20 warnings (use warnings() to see them)

Attaching package: ‘igraph’

The following objects are masked from ‘package:DelayedArray’:

    path, simplify

The following objects are masked from ‘package:rtracklayer’:

    blocks, path
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
The following object is masked from ‘package:Biostrings’:

    union

The following object is masked from ‘package:GenomicRanges’:

    union

The following object is masked from ‘package:IRanges’:

    union

The following object is masked from ‘package:S4Vectors’:

    union

The following objects are masked from ‘package:BiocGenerics’:

    normalize, path, union

The following objects are masked from ‘package:stats’:

    decompose, spectrum

The following object is masked from ‘package:base’:

    union

Warning message:
package ‘igraph’ was built under R version 3.5.2

Attaching package: ‘DescTools’

The following object is masked from ‘package:igraph’:

    %c%

Warning message:
package ‘DescTools’ was built under R version 3.5.2
[1] Visualization MEDALT!
null device
          1
[1] LSA segmentation!
[1] Calculating CFL
[1] Calculating permutation CFL
[1] Estimate emperical p value
[1] Estimate parallel evolution
null device
          1
Done!
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

R packages (igraph, HelloRanges and DescTools) are loaded.

Three text files are expected:
(1) CNV.tree.txt which is an rooted directed tree including three columns: parent node, child node and distance.

CNV.tree.txt

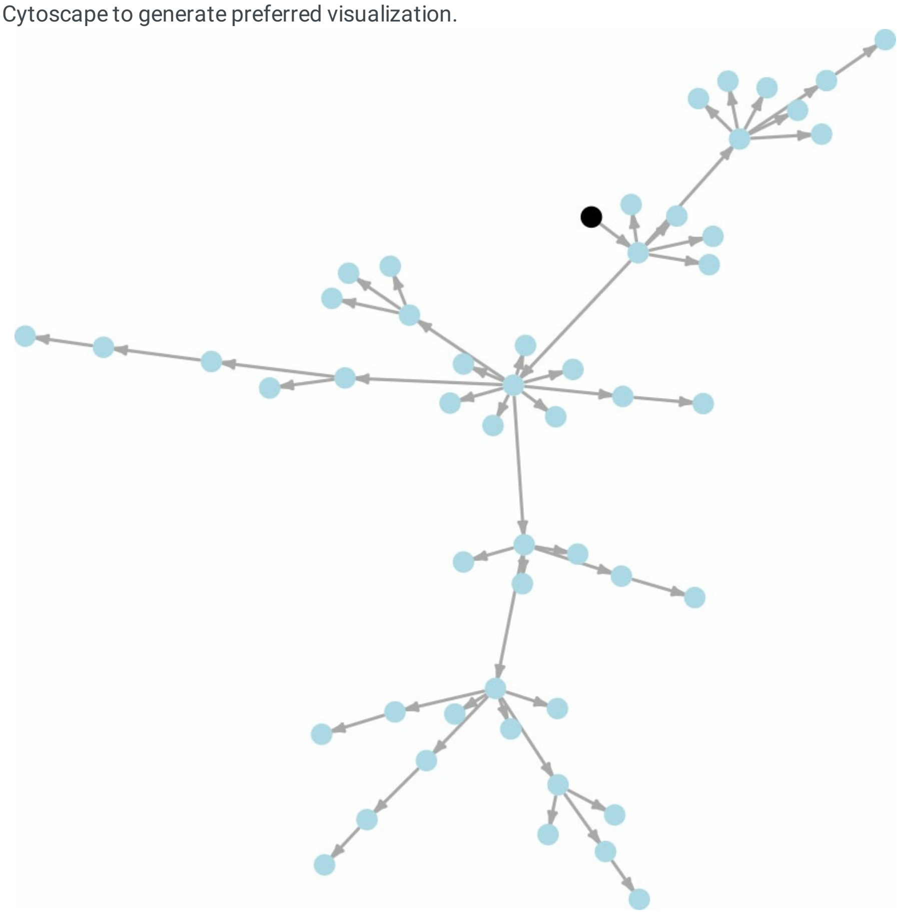
(2) segmental.LSA.txt which includes broad CNAs significantly associated with lineage expansion.

segmental.LSA.txt

(3) gene.LSA.txt which includes focal (gene) CNAs significantly associated with lineage expansion.

gene.LSA.txt

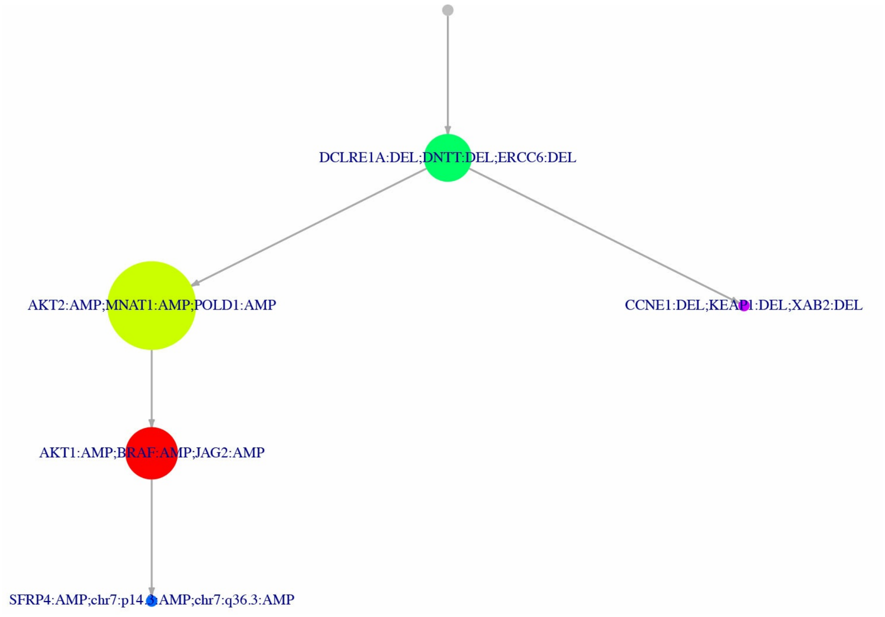
Two figures are also expected:
(1) singlecell.tree.pdf which is a visualization of MEDALT by igraph. You also can input CNV.tree.txt into Cytoscape to generate preferred visualization.



Each node represents a cell, each edge represents a kinship between two cells, arrows point towards younger cells, and the root represents a normal diploid cell.

(2) LSA.tree.pdf which is a visualization of identified CNAs by igraph.

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n


DCLRE1A:DEL;DNTT:DEL;ERCC6:DEL
AKT2:AMP;MNAT1:AMP;POLD1:AMP
CCNE1:DEL;KEAP1:DEL;XAB2:DEL
AKT1:AMP;BRAF:AMP;JAG2:AMP
SFRP4:AMP;chr7:p14.3:AMP;chr7:q36.3:AMP

We run the example data only through permuting copy number profile instead of reconstructing tree based on permuted copy number profile. The seting can be changed via -R T.

4 Run the example data inferred using inferCNV based on single cell RNA sequencing technology

scRNA.CNV.txt

```
python scTree.py -P ./ -I ./example/scRNA.CNV.txt -D R -G hg19 -O
./example/outputRNA

Transfer data to segmental level
The number of genes which are merger into the bin is default value 30. If you
want change it please specify the value through -W
Inferring MEDALT.
MEDALT inferrence finish.
Performing LSA.
Loading required package: BiocGenerics
Loading required package: parallel

Attaching package: ‘BiocGenerics’

The following objects are masked from ‘package:parallel’:

    clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
    clusterExport, clusterMap, parApply, parCapply, parLapply,
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
    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’:

    anyDuplicated, append, as.data.frame, basename, cbind, colMeans,
    colnames, colSums, dirname, do.call, duplicated, eval, evalq,
    Filter, Find, get, grep, grepl, intersect, is.unsorted, lapply,
    lengths, Map, mapply, match, mget, order, paste, pmax, pmax.int,
    pmin, pmin.int, Position, rank, rbind, Reduce, rowMeans, rownames,
    rowSums, sapply, setdiff, sort, table, tapply, union, unique,
    unsplit, which, which.max, which.min

Loading required package: S4Vectors
Loading required package: stats4

Attaching package: ‘S4Vectors’

The following object is masked from ‘package:base’:

    expand.grid

Loading required package: IRanges
Loading required package: GenomicRanges
Loading required package: GenomeInfoDb
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Loading required package: XVector

Attaching package: ‘Biostrings’

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Loading required package: rtracklayer
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Loading required package: AnnotationDbi
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Welcome to Bioconductor

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    'browseVignettes()'. To cite Bioconductor, see
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Loading required package: VariantAnnotation
Loading required package: SummarizedExperiment
Loading required package: DelayedArray
Loading required package: matrixStats
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
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The following objects are masked from ‘package:DelayedArray’:

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The following objects are masked from ‘package:rtracklayer’:

    blocks, path

The following object is masked from ‘package:Biostrings’:

    union

The following object is masked from ‘package:GenomicRanges’:

    union

The following object is masked from ‘package:IRanges’:
```

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

```
    union

The following object is masked from ‘package:S4Vectors’:

    union

The following objects are masked from ‘package:BiocGenerics’:

    normalize, path, union

The following objects are masked from ‘package:stats’:

    decompose, spectrum

The following object is masked from ‘package:base’:

    union

Warning message:
package ‘igraph’ was built under R version 3.5.2

Attaching package: ‘DescTools’

The following object is masked from ‘package:igraph’:

    %c%

Warning message:
package ‘DescTools’ was built under R version 3.5.2
[1] Visualization MEDALT!
null device
          1
[1] LSA segmentation!
[1] Calculating CFL
[1] Calculating permutation CFL
[1] Estimate emperical p value
[1] Estimate parallel evolution
null device
          1
Done!
```

Three text files are expected:
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CNV.tree.txt

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segmental.LSA.txt

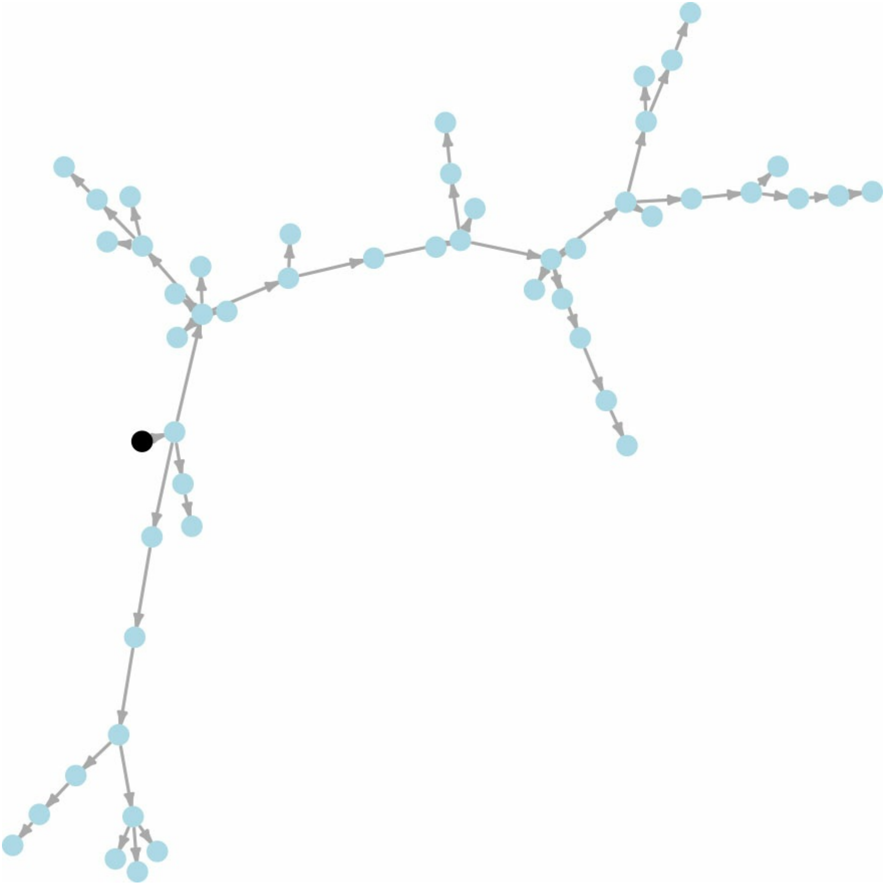
Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

(3) gene.LSA.txt which includes focal (gene) CNAs significantly associated with lineage expansion.

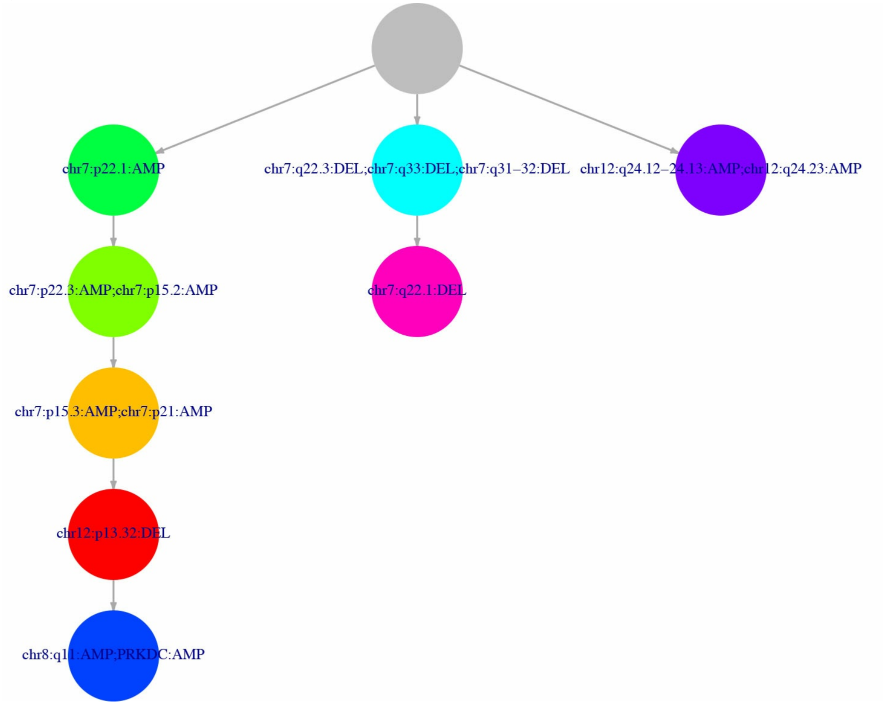
gene.LSA.txt

Two figures are also expected:

(1) singlecell.tree.pdf which is a visualization of MEDALT by igraph.



(2) LSA.tree.pdf which is a visualization of identified CNAs by igraph.


chr7:p22.1:AMP
chr7:q22.3:DEL;chr7:q33:DEL;chr7:q31–32:DEL
chr12:q24.12–24.13:AMP;chr12:q24.23:AMP
chr7:p22.3:AMP;chr7:p15.2:AMP
chr7:q22.1:DEL
chr7:p15.3:AMP;chr7:p21:AMP
chr12:p13.32:DEL
chr8:q11:AMP;PRKDC:AMP

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n

Citation: Fang Wang, Qihan Wang, Vakul Mohanty, Shaoheng Liang, Jinzhuang Dou, Jincheng Han, Darlan Conterno Minussi, Ruli Gao, Li Ding, Nicholas Navin, Ken Chen (05/12/2020). Minimal Event Distance Aneuploidy Lineage Tree (MEDALT) inference based on single cell copy number profile. https://dx.doi.org/10.17504/protocols.io.bfhpjj5n