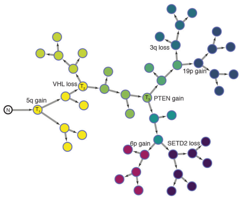
May 12, 2020

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



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.bfhpjj5n

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Fang Wang

DOI: **dx.doi.org/10.17504/protocols.io.bfhpjj5n**

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

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

Protocol status: Working

Created: April 23, 2020

Last Modified: May 12, 2020

Protocol Integer ID: 36111

Keywords: single cell technology, tumor evolution, copy number alteration,

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.

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

Software

MEDALT	NAME
Fang Wang and Qihan Wang	DEVELOPER

Extract input dataset

Dataset

Single cell copy number profile generated by single cell DNA seq	NAME
https://github.com/KChen-lab/MEDALT/blob/master/example/scDNA.CNV.txt	LINK

Dataset

Single cell copy number profile inferred from single cell RNA se	NAME
https://github.com/KChen-lab/MEDALT/blob/master/example/scRNA.CNV.txt	LINK

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

Command

```
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

TXT scDNA.CNV.txt

Command

```
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.
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

    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

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!
```

Note

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

Expected result

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

TXT CNV.tree.txt

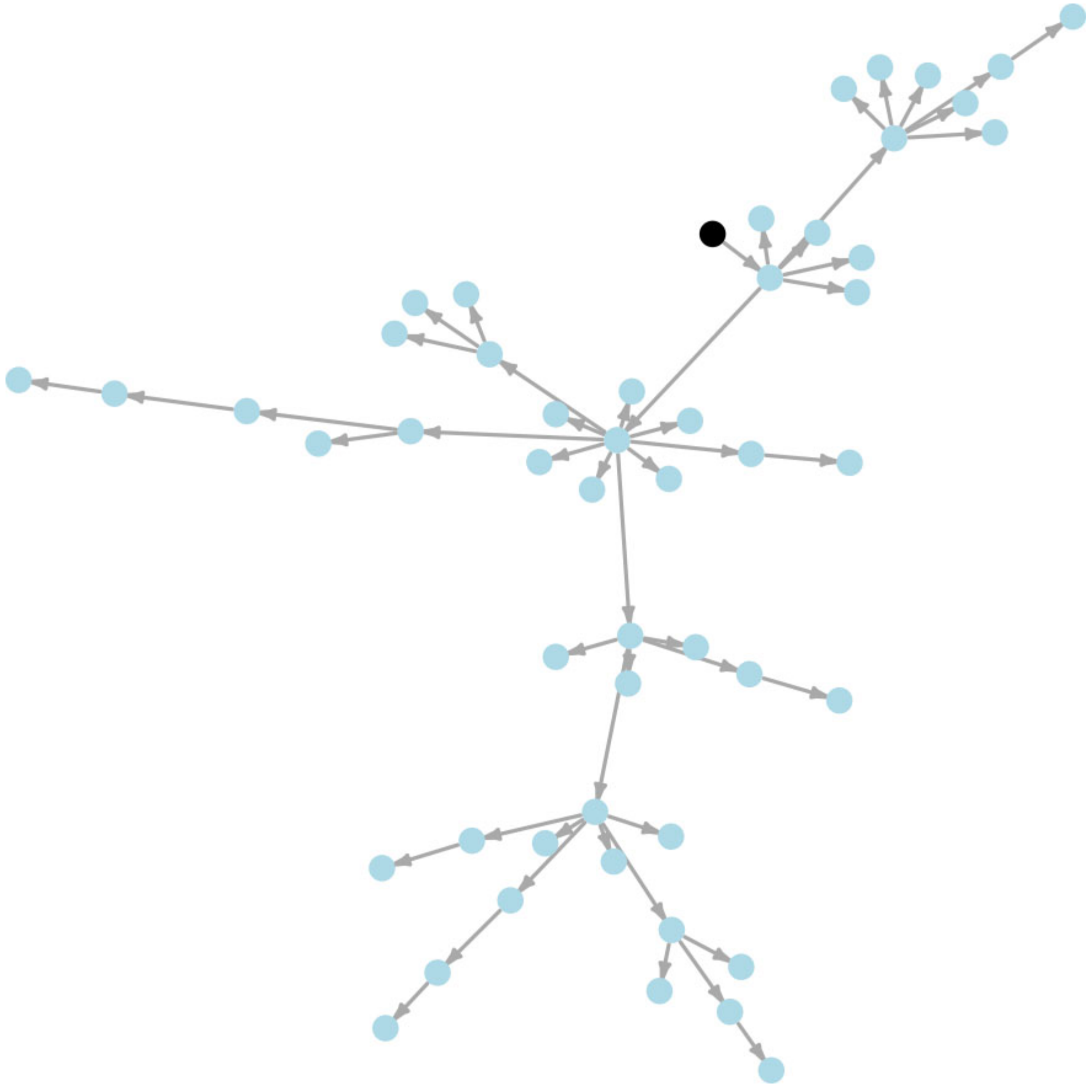
(2) segmental.LSA.txt which includes broad CNAs significantly associated with lineage expansion.

TXT segmental.LSA.txt

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

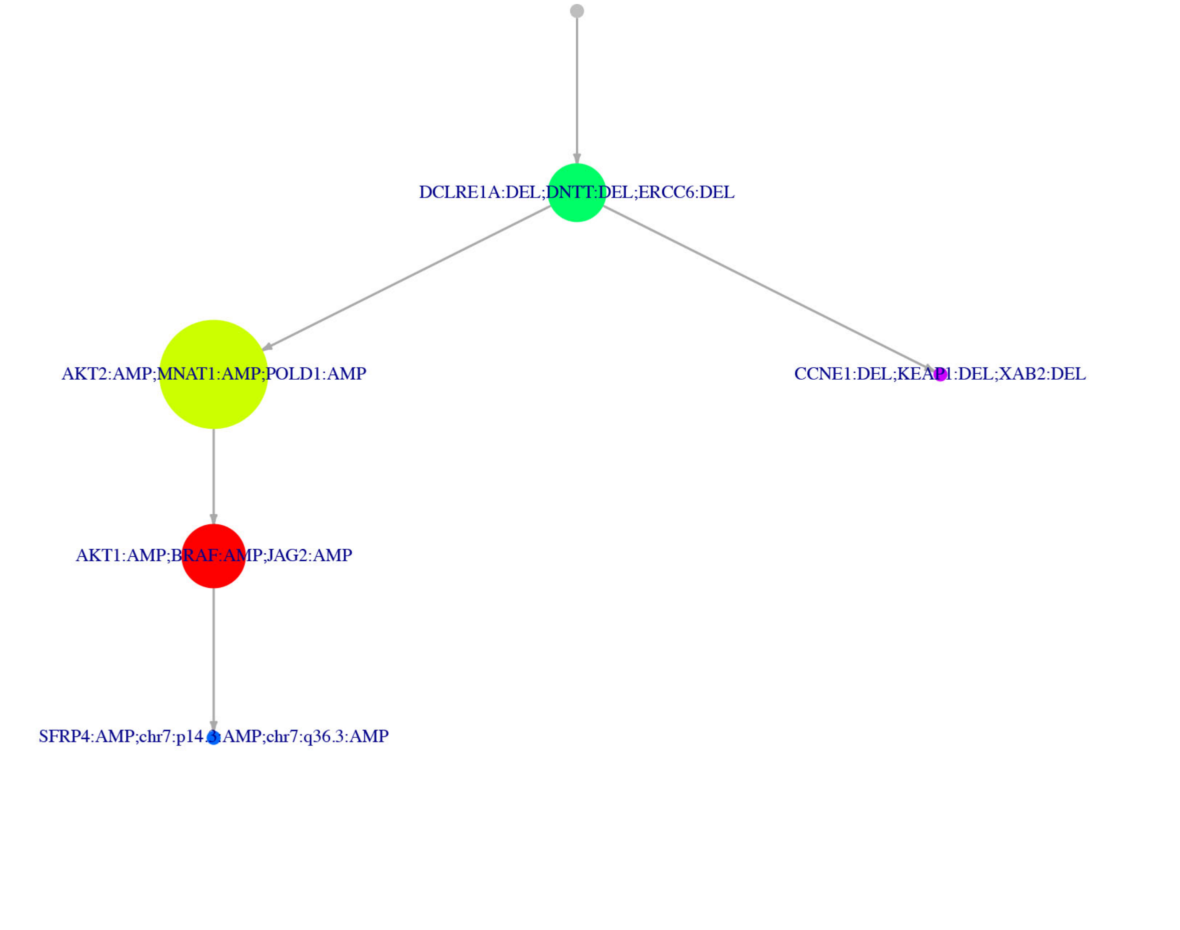
TXT gene.LSA.txt

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.


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

Note

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

TXT scRNA.CNV.txt

Command

```
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,
    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
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Loading required package: matrixStats

Attaching package: ‘matrixStats’

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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’:

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```

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

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Loading required package: Rsamtools

Attaching package: ‘VariantAnnotation’

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

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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

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’:
```

```
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!
```

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CNV.tree.txt

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segmental.LSA.txt

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gene.LSA.txt

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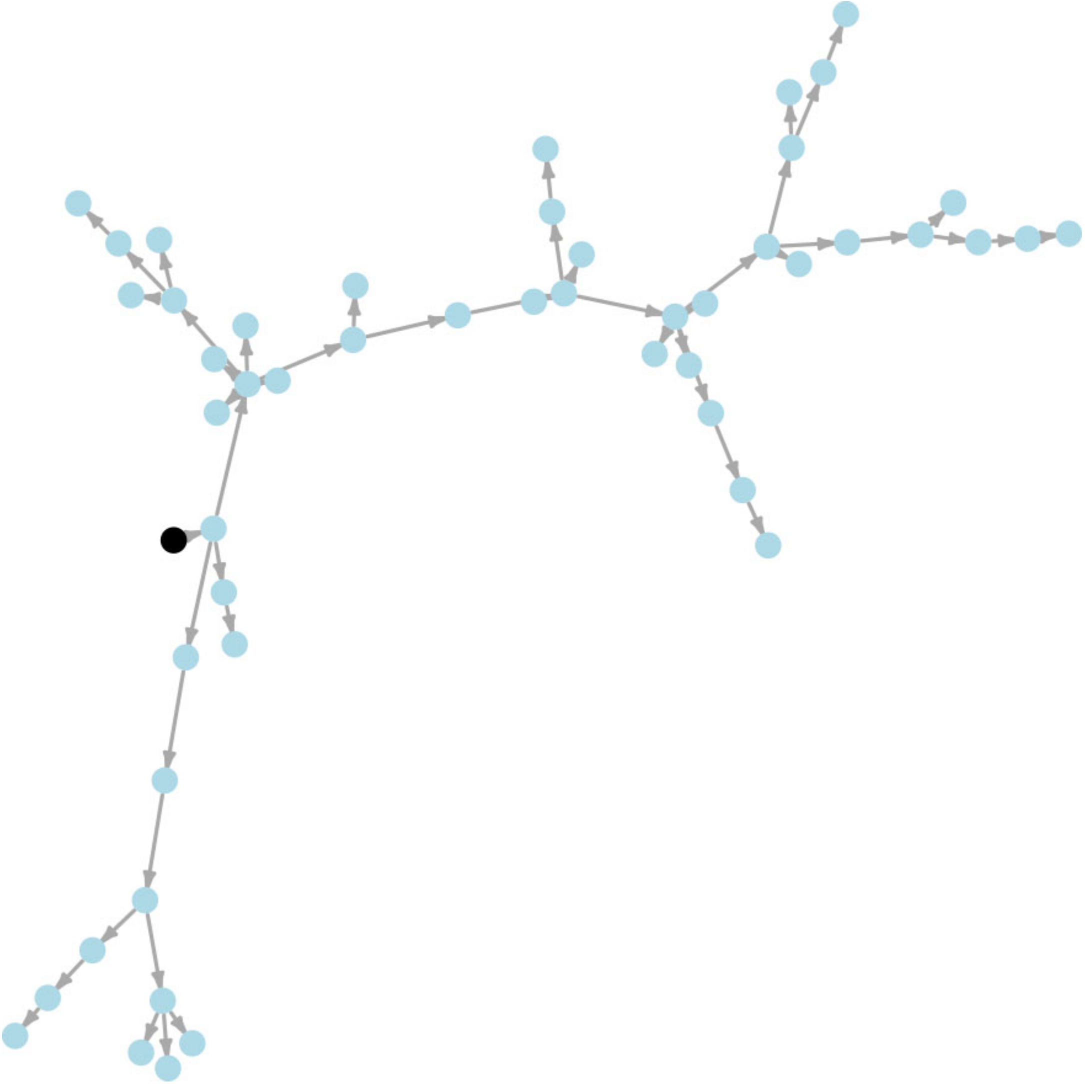
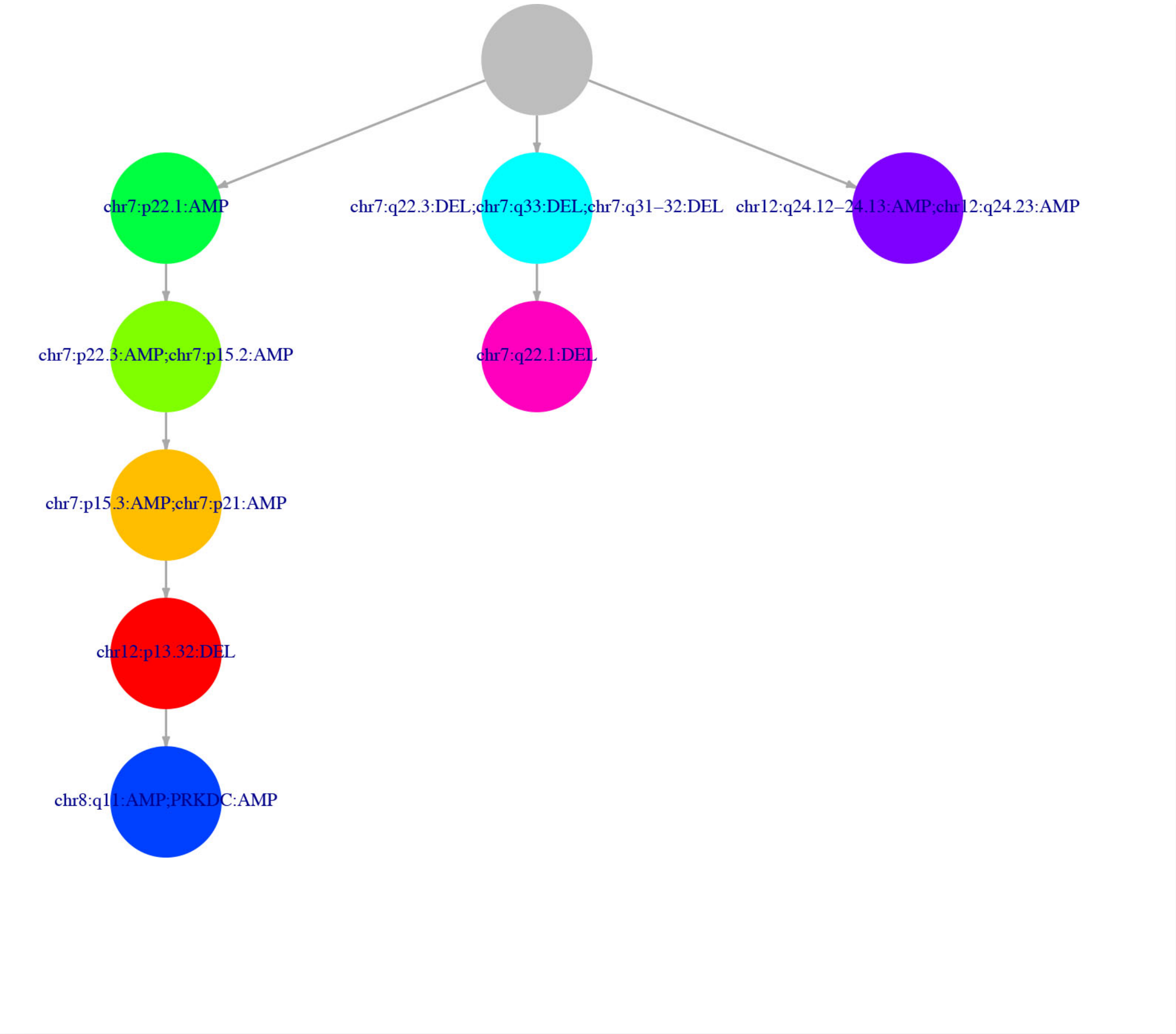


(2) LSA.tree.pdf which is a visualization of identified CNAs by igraph.


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