

SCclust T10 Tutorial

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2018-06-06

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1 Introduction

The *SCclust* package implements feature selection based on breakpoints, permutations for FDRs for Fisher test p-values and identification of the clone structure in single cell copy number profiles.

In this tutorial we show how to use *SCclust* package using data, prepared by *sgains* pipeline as described in [Example usage of sGAINS pipeline](#). *SCclust* package is called as the last step in processing data from *sgains* pipeline. In this tutorial we show how *SCclust* package could be used independently from *sgains* pipeline.

We assume that you have an R environment and have installed *SCclust* package as described in the `README.md`.

2 Data

2.1 Data for the T10 case

This tutorial is based on data published in: [Navin N, Kendall J, Troge J, et al. Tumor Evolution Inferred by Single Cell Sequencing. Nature. 2011;472\(7341\):90-94. doi:10.1038/nature09807](#). In particular we will use the data for polygenomic breast tumor T10 case available from SRA. Description of samples for T10 could be found in [Supplementary Table 1 | Summary of 100 Single Cells in the Polygenomic Tumor T10](#)

We are going to run *SCclust* package on prepared by *sgains* pipeline [varbin step](#). You can go through all the step in [sgains T10 tutorial](#) and prepare this data.

For the purposes of this tutorial we recomend you to download already prepared [varbin data](#) from [example data](#). Apart from `varbin` T10 data you will need the binning scheme used in the analysis, that could be found [here](#). And also we will need `cytoBand.txt` for HG19 that you can download it from UCSC Genome Browser.

2.2 Collect the Necessary Data

Let us create a directory, where to store all the data used in this tutorial:

```
mkdir T10data
cd T10data
```

and let us download and extract T10 varbin data:

```
wget -c \
  https://github.com/KrasnitzLab/SCclust/releases/download/v1.0.0RC3/navin_t10_varbin_data.tar.gz
tar zxvf navin_t10_varbin_data.tar.gz
rm navin_t10_varbin_data.tar.gz
```

Let us also download and extract the binning scheme used in preparation of varbin data:

```
wget -c \
  https://github.com/KrasnitzLab/SCclust/releases/download/v1.0.0RC3/hg19_R50_B20k_bins_boundaries.txt.gz
gunzip hg19_R50_B20k_bins_boundaries.txt.gz
```

And finally let us download the cytoBand.txt for Human reference genome *hg19*:

```
wget -c \
  http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/cytoBand.txt.gz
gunzip cytoBand.txt.gz
```

Our data directory should have following structure:

```
.
|-- T10data
|   |-- cytoBand.txt
|   |-- hg19_R50_B20k_bins_boundaries.txt
|   |-- varbin
|       |-- SRR052047.varbin.20k.txt
|       |-- SRR052148.varbin.20k.txt
|       |-- SRR053437.varbin.20k.txt
|       ...
```

2.3 Explore the Downloaded Data

We are going to use *SCclust* package so let us load it:

```
library("SCclust")
```

2.3.1 Binning schema

```
gc_df <- read.csv("T10data/hg19_R50_B20k_bins_boundaries.txt", header = T, sep='\t')
knitr::kable(head(gc_df))
```

Describe the data.

bin.chrom	bin.start	bin.start.abspos	bin.end	bin.length	mappable.positions	gc.content
chr1	0	0	859077	859077	131390	0.4357746
chr1	859077	859077	999002	139925	131390	0.6280936
chr1	999002	999002	1141973	142971	131391	0.6026537
chr1	1141973	1141973	1280121	138148	131390	0.6284347
chr1	1280121	1280121	1435418	155297	131390	0.5757548

bin.chrom	bin.start	bin.start.abspos	bin.end	bin.length	mappable.positions	gc.content
chr1	1435418	1435418	1603686	168268	131391	0.5690862

2.3.2 Cytobands and Centromeres for HG19

```
cytobands <- read.csv("T10data/cytoBand.txt", header = F, sep='\t')
knitr::kable(head(cytobands))
```

Describe the data.

V1	V2	V3	V4	V5
chr1	0	2300000	p36.33	gneg
chr1	2300000	5400000	p36.32	gpos25
chr1	5400000	7200000	p36.31	gneg
chr1	7200000	9200000	p36.23	gpos25
chr1	9200000	12700000	p36.22	gneg
chr1	12700000	16200000	p36.21	gpos50

The main reason we need `cytoBand.txt` is to get the location of centromeres. Since centromere areas contain a lot of repetitive sequences they are excluded from analysis when segmenting and clustering samples.

To find regions where centromeres are located we are using `calc_centroareas` function:

```
centroareas <- calc_centroareas(cytobands)
knitr::kable(head(centroareas, 5))
```

	chrom	from	to
33	1	120600000	128900000
393	2	83300000	102700000
508	3	87200000	98300000
556	4	48200000	52700000
604	5	46100000	58900000

So, in `centroareas` for each chromosome we have the region where the centromere is located.

2.3.3 Varbin Samples Data

For each varbin sample

```
sample_df <- read.csv("T10data/varbin/SRR052047.varbin.20k.txt", header=T, sep='\t')
knitr::kable(head(sample_df))
```

Describe the data.

chrom	chrompos	abspos	bincount	ratio
chr1	0	0	51	0.3327000
chr1	859077	859077	57	0.3718412
chr1	999002	999002	89	0.5805941
chr1	1141973	1141973	53	0.3457471

chrom	chrompos	abspos	bincount	ratio
chr1	1280121	1280121	99	0.6458294
chr1	1435418	1435418	63	0.4109824

```
sample_df <- read.csv("T10data/varbin/SRR052148.varbin.20k.txt", header=T, sep='\t')
knitr::kable(head(sample_df))
```

chrom	chrompos	abspos	bincount	ratio
chr1	0	0	125	0.5530061
chr1	859077	859077	69	0.3052594
chr1	999002	999002	90	0.3981644
chr1	1141973	1141973	57	0.2521708
chr1	1280121	1280121	98	0.4335568
chr1	1435418	1435418	84	0.3716201

3 Auto Generated

Vignettes are long form documentation commonly included in packages. Because they are part of the distribution of the package, they need to be as compact as possible. The `html_vignette` output type provides a custom style sheet (and tweaks some options) to ensure that the resulting html is as small as possible. The `html_vignette` format:

- Never uses retina figures
- Has a smaller default figure size
- Uses a custom CSS stylesheet instead of the default Twitter Bootstrap style

3.1 Vignette Info

Note the various macros within the `vignette` section of the metadata block above. These are required in order to instruct R how to build the vignette. Note that you should change the `title` field and the `\VignetteIndexEntry` to match the title of your vignette.

3.2 Styles

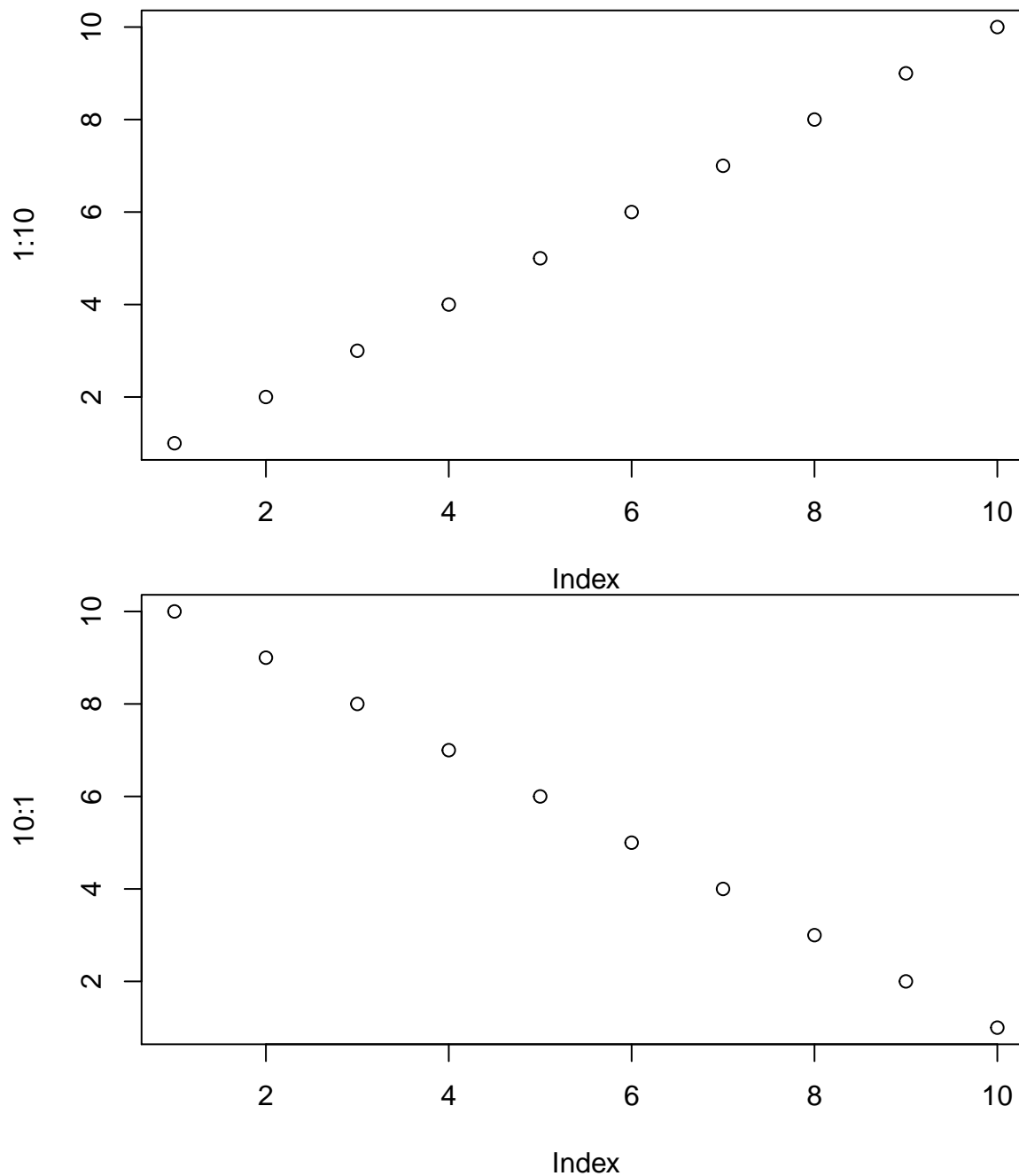
The `html_vignette` template includes a basic CSS theme. To override this theme you can specify your own CSS in the document metadata as follows:

```
output:
  rmarkdown::html_vignette:
    css: mystyles.css
```

3.3 Figures

The figure sizes have been customised so that you can easily put two images side-by-side.

```
plot(1:10)
plot(10:1)
```



You can enable figure captions by `fig_caption: yes` in YAML:

```
output:
  rmarkdown::html_vignette:
    fig_caption: yes
```

Then you can use the chunk option `fig.cap = "Your figure caption."` in **knitr**.

3.4 More Examples

You can write math expressions, e.g. $Y = X\beta + \epsilon$, footnotes¹, and tables, e.g. using `knitr::kable()`.

¹A footnote here.

	mpg	cyl	disp	hp	drat	wt	qsec	vs	am	gear	carb
Mazda RX4	21.0	6	160.0	110	3.90	2.620	16.46	0	1	4	4
Mazda RX4 Wag	21.0	6	160.0	110	3.90	2.875	17.02	0	1	4	4
Datsun 710	22.8	4	108.0	93	3.85	2.320	18.61	1	1	4	1
Hornet 4 Drive	21.4	6	258.0	110	3.08	3.215	19.44	1	0	3	1
Hornet Sportabout	18.7	8	360.0	175	3.15	3.440	17.02	0	0	3	2
Valiant	18.1	6	225.0	105	2.76	3.460	20.22	1	0	3	1
Duster 360	14.3	8	360.0	245	3.21	3.570	15.84	0	0	3	4
Merc 240D	24.4	4	146.7	62	3.69	3.190	20.00	1	0	4	2
Merc 230	22.8	4	140.8	95	3.92	3.150	22.90	1	0	4	2
Merc 280	19.2	6	167.6	123	3.92	3.440	18.30	1	0	4	4

Also a quote using >:

“He who gives up [code] safety for [code] speed deserves neither.” ([via](#))