ClusteredMutations: Looking for a (Mutation) Shower.

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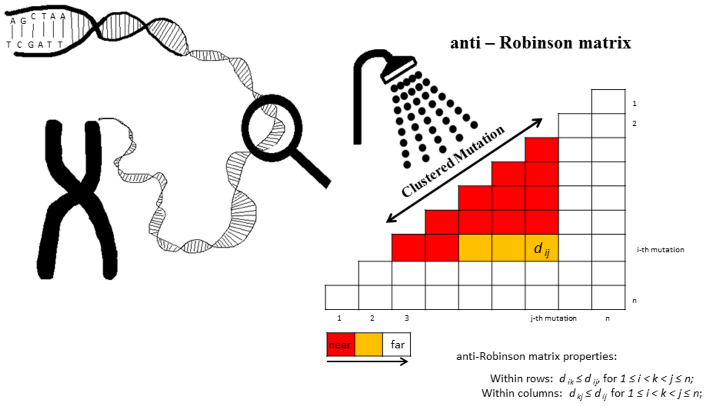
Abstract

This vignette shows the steps to identify the hyper-mutated zones, i.e., groups of closely spaced mutations, with a data set of somatic substitution mutations from a primary breast cancer whole genome with a germline mutation in BRCA1 using *ClusteredMutations*.

keywords: *cancer genome, somatic mutation, mutation showers, clustered mutations, kataegis, anti-Robinson matrix*


A G C T A A
T C G A T T
anti – Robinson matrix
Clustered Mutation
d_{ij}
1
2
i-th mutation
n
1
2
3
j-th mutation
n
near
far
anti-Robinson matrix properties:
Within rows: $d_{ik} \le d_{ij}$, for $1 \le i < k < j \le n$;
Within columns: $d_{kj} \le d_{ij}$ for $1 \le i < k < j \le n$;

1 Example and applications.

In the following example, a data set (PD4107a) of somatic substitution mutations from a primary breast cancer whole genome with a germline mutation in BRCA1 [1, 4] is used to locate the hyper-mutated zones using *ClusteredMutations*.
First, *ClusteredMutations* package and PD4107a data set are loaded.

```
> library(ClusteredMutations)
> data(PD4107a)
```

showers() is called with a change in the default setting to identify the complex mutations. Complex mutations are those regions with two or more mutations with each mutation separated by less than 10 bp from their nearest neighbor; therefore, min=2 and max=10 are used. Because complex mutations likely originate from trans-lesion synthesis (TLS) past a single DNA lesion[3], Roberts et al.[6, 5] proposed treating them as a single event. In this example, all somatic substitution mutations are used, including complex mutations.

```
> data.showers<-showers(data=PD4107a, chr=Chr, position=Position, min=2, max=10)
> head(data.showers, n=10)
```

```
   chr      pend    pstart nend nstart distance number
1    1  18331461  18331460   29     28        1      2
2    1  49584638  49584628  116    115       10      2
3    1  84702722  84702721  244    243        1      2
4    1  96832689  96832688  272    271        1      2
5    1 112246806 112246804  345    344        2      2
6    1 164753620 164753619  436    435        1      2
7    2  39902729  39902728  118    117        1      2
8    2  51867763  51867761  155    154        2      2
9    2  69402832  69402831  206    205        1      2
10   2  89459416  89459415  260    259        1      2
```

The classic graph (Figure 1) to localize the regional clustering of mutations is the rainfall plot[4]. *imd()* permits the generation of a data set with the inter-mutational distance (IMD), the distance between each somatic substitution and the substitution immediately prior[4], and extra information, for example: base substitutions.

```
> extra <- factor(c(),levels=c("T>C","T>G","T>A","C>T","C>G","C>A"))
> extra[PD4107a$Ref_base=="A" & PD4107a$Mutant_base=="G"]<-"T>C"
> extra[PD4107a$Ref_base=="T" & PD4107a$Mutant_base=="C"]<-"T>C"
> extra[PD4107a$Ref_base=="A" & PD4107a$Mutant_base=="C"]<-"T>G"
> extra[PD4107a$Ref_base=="T" & PD4107a$Mutant_base=="G"]<-"T>G"
> extra[PD4107a$Ref_base=="A" & PD4107a$Mutant_base=="T"]<-"T>A"
> extra[PD4107a$Ref_base=="T" & PD4107a$Mutant_base=="A"]<-"T>A"
> extra[PD4107a$Ref_base=="G" & PD4107a$Mutant_base=="A"]<-"C>T"
> extra[PD4107a$Ref_base=="C" & PD4107a$Mutant_base=="T"]<-"C>T"
> extra[PD4107a$Ref_base=="G" & PD4107a$Mutant_base=="C"]<-"C>G"
> extra[PD4107a$Ref_base=="C" & PD4107a$Mutant_base=="G"]<-"C>G"
> extra[PD4107a$Ref_base=="G" & PD4107a$Mutant_base=="T"]<-"C>A"
> extra[PD4107a$Ref_base=="C" & PD4107a$Mutant_base=="A"]<-"C>A"
> PD4107a$extra<-extra
> rainfall<-imd(data=PD4107a,chr=Chr,position=Position,extra=extra)
> plot(rainfall$number, rainfall$log10distance, col=c("yellow", "green",
+       "pink", "red", "black", "blue")[rainfall$extra], pch=20,
+       ylab="Intermutation distance (bp)", xlab="PD4107a", yaxt="n")
> axis(2, at=c(0, 1, 2, 3, 4, 6), labels=c("1", "10", "100", "1000",
+     "10000", "1000000"), las=2, cex.axis=0.6)
> legend("topleft", legend = levels(rainfall$extra), col=c("yellow",
+       "green", "pink", "red", "black", "blue"), pch=20, horiz=TRUE,
+       text.font=4, bg='lightblue')
```

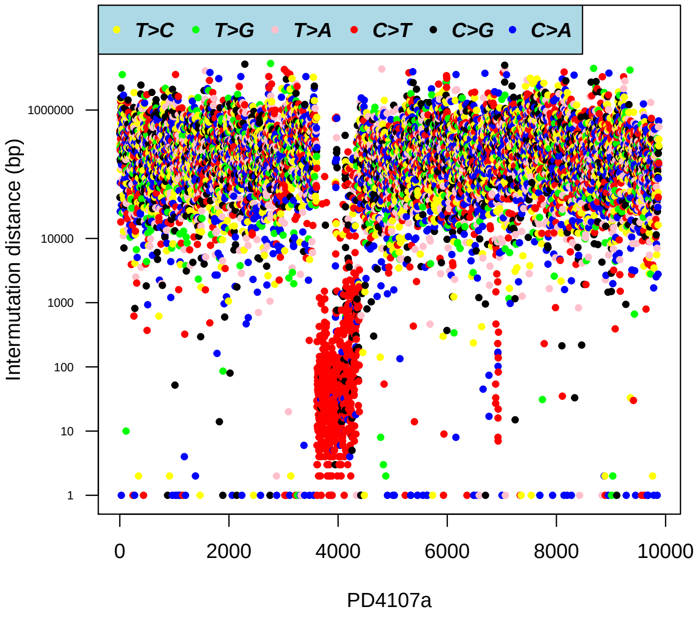


Figure 1: Rainfall plot of somatic substitution mutations from a patient with breast cancer (PD4107a).

Figure 1 is the rainfall plot of PD4107a. The horizontal axis presents the number of consecutive mutations, the vertical axis indicates the distance between each somatic substitution and the substitution immediately prior (inter-mutational distance). Four features were observed in the rainfall plot of PD4107a (Figure 2):
1) Many of the somatic mutations have IMD greater than 10000 bp; thus, two consecutive mutations are sufficiently remote to be candidates to belong to the regional clustering of substitution mutations.
2) There were mutations with distances less than 10 bp, i.e., complex mutations[6, 5].
3) There were mutation zones with IMD less than 1000 bp on chromosomes 6 and 12. These zones can be hyper-mutated regions.
4) The preponderance of C>T and C>G substitutions is present in the candidate zones.
The rainfall plot identifies candidate hyper-mutated zones. Visual assessment can be erroneous (Figure 2). *showers()* is called with the default setting. There are no clustered mutations.

```
> set.seed(42)
> position<-c( c((runif(1001,min=1,max=10000001))),
+ c(c(10110001,10110011,10110021,10110031,10110041,10120000,
+ 10120001,10120011,10120021,10120031,10130000,
+ 10130001,10130011,10130021,10130031,10140000,
+ 10140001,10140011,10140021,10140031,10150000,
+ 10150001,10150011,10150021,10150031,10160000,
+ 10160001,10160011,10160021,10160031,10170000,
+ 10170001,10170011,10170021,10170031,10180000,
+ 10180001,10180011,10180021,10180031,10190000,
+ 10210001,10210011,10210021,10210031,10220000,
+ 10220001,10220011,10220021,10220031,10230000,
+ 10230001,10230011,10230021,10230031,10240000,
+ 10240001,10240011,10240021,10240031,10250000,
+ 10250001,10250011,10250021,10250031,10260000,
+ 10260001,10260011,10260021,10260031,10270000,
+ 10270001,10270011,10270021,10270031,10280000,
+ 10280001,10280011,10280021,10280031,10290000) +
+ round(runif(81,min=0,max=500))),
+ c(round(runif(991,min=10296000,max=20200000))))
> rainfall<-imd(position=position)
> #Rainfall plot for PD4107a cancer sample;
> plot(rainfall$number, rainfall$log10distance, pch=20,
+       ylab="Intermutation distance (bp)", xlab="Example", yaxt="n")
> axis(2, at=c(0, 1, 2, 3, 4, 6), labels=c("1", "10", "100", "1000",
+      "10000", "1000000"), las=2, cex.axis=0.6)
> theta <- seq(0, 2 * pi, length = 200)
> lines(x = 100 * cos(theta) + 1050, y = sin(theta) + 1.5, col="red")
```

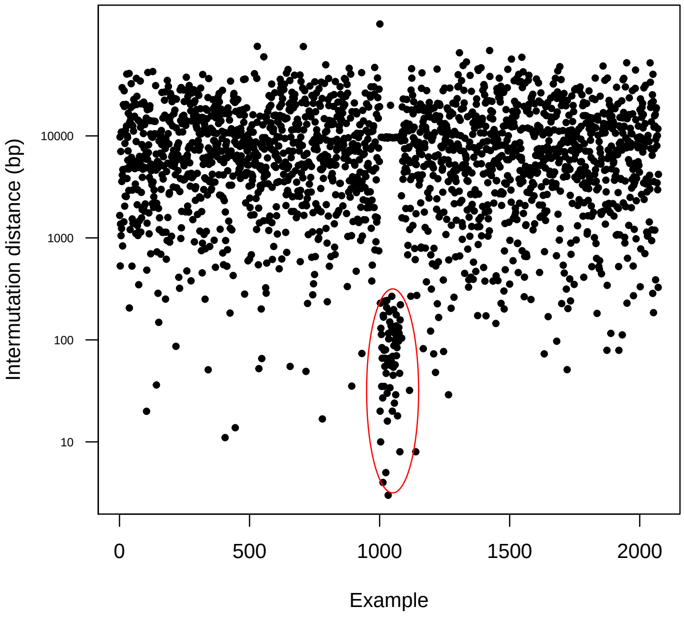


Figure 2: Rainfall plot of an example data set of somatic substitution mutations.

showers() function in the example data set finds 0 hyper-mutated zones.

```
> showers(position=position)
```

```
[1] chr      pend     pstart   nend     nstart   distance number
<0 rows> (or 0-length row.names)
```

showers() function in the PD4107a data set finds 21 hyper-mutated zones with 674 mutations, *features()* shows the mutation positions in the chromosome with additional information.

```
> showers(data=PD4107a,chr=Chr,position=Position)
```

```
   chr      pend    pstart nend nstart distance number
1    6 126239586 126233148  466    370     6438     97
2    6 126279808 126274096  512    467     5712     46
3    6 126376071 126371860  525    513     4211     13
4    6 126394625 126392175  545    526     2450     20
5    6 126437625 126430855  708    546     6770    163
6    6 130423519 130419337  797    740     4182     58
7    6 130438324 130433693  849    798     4631     52
8    6 130489124 130483574  887    851     5550     37
9    6 131796572 131788326  915    904     8246     12
10   6 131818990 131810251  939    916     8739     24
11   6 132401366 132396811  960    948     4555     13
12   6 132552483 132544956  978    968     7527     11
13   6 132603528 132599455 1025    979     4073     47
14   6 133554257 133550552 1043   1038     3705      6
15   6 133716520 133707397 1066   1049     9123     18
16   6 134025191 134015015 1088   1075    10176     14
17   6 134118849 134115823 1096   1089     3026      8
18   6 135262041 135256447 1112   1104     5594      9
19   6 137982971 137979704 1133   1127     3267      7
20   6 138015423 138010978 1140   1134     4445      7
21  12  10508274  10505228   54     43     3046     12
```

showers() function uses the anti-Robinson properties. For example, for a sample of DNA sequence of cancer cell with 14 somatic substitution mutations (Figure 3) and if the hyper-mutated zone contains those segments with $>=$ 5 consecutive mutations with a distance of $<=$ 100 bp, then:
1) the distance between the mutations 3 and 7 is

$$d_{37} = 106$$

2) The distance is increased when moving away from the main diagonal, by row

$$d_{37} = 106 <= d_{38} = 116$$

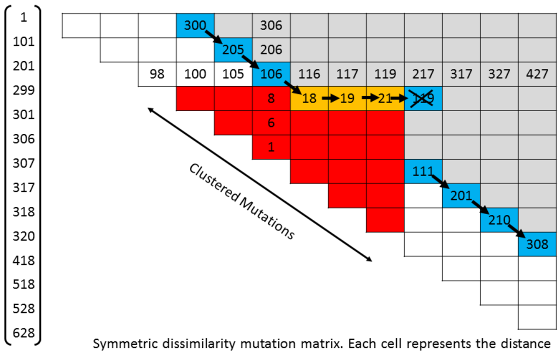
and by column

$$d_{37} = 106 <= d_{27} = 206$$

3) The length of the hyper-mutated zone is determined by row or by column based on anti-Robinson properties: j - i + 1 = 10 - 4 + 1 = 7.
4) The calculated distance to the identified hyper-mutated zones is equal to: n - min + (number of the hyper-mutated zones in the sample) + 1 = 14 - 5 + 1 + 1 = 11.

```
> example1<-c(1,101,201,299,301,306,307,317,318,320,418,518,528,628)
> 10**(dissmutmatrix(position=example1,upper=TRUE))
```

```
      1    2    3    4    5    6    7    8    9   10   11   12   13   14
1         100  200  298  300  305  306  316  317  319  417  517  527  627
2   100       100  198  200  205  206  216  217  219  317  417  427  527
3   200  100         98  100  105  106  116  117  119  217  317  327  427
4   298  198   98          2    7    8   18   19   21  119  219  229  329
5   300  200  100    2          5    6   16   17   19  117  217  227  327
6   305  205  105    7    5          1   11   12   14  112  212  222  322
7   306  206  106    8    6    1        10   11   13  111  211  221  321
8   316  216  116   18   16   11   10         1    3  101  201  211  311
9   317  217  117   19   17   12   11    1         2  100  200  210  310
10  319  219  119   21   19   14   13    3    2        98  198  208  308
11  417  317  217  119  117  112  111  101  100   98       100  110  210
12  517  417  317  219  217  212  211  201  200  198  100        10  110
13  527  427  327  229  227  222  221  211  210  208  110   10       100
14  627  527  427  329  327  322  321  311  310  308  210  110  100
```



The position of somatic substitution mutations in the DNA of cancer cells.

Symmetric dissimilarity mutation matrix. Each cell represents the distance in base pairs between the chromosomal position of somatic mutations. For this example, hyper-mutated region: min>=5, max=<100.

Figure 3: Symmetric dissimilarity mutation matrix for a sample of DNA sequence of cancer cell with 14 somatic substitution mutations.

dissmutmatrix() obtains the symmetric dissimilarity mutations matrix. This function computes and returns the distance matrix computed using the Euclidean distance measure to compute the distances between all pairs of positions of somatic mutations. This matrix can be plotted using the *dissplot()* function of the *seriation* R package[2]. Plotting the distance matrix helps to visualize and identify mutation clusters in addition to locating the micro-clustered mutated regions within the macro-clustered mutated zones that occur during the oncogenic process. The plot is applied to chromosome 6 of the PD4107 data set (Figure 4). The distances, in logarithm of base 10, are colored according to the existing color palette. Observed clusters of mutations, or candidates for hyper-mutated zones (less than 5000 bp between distant mutations), are shown by orange and red squares (20 regions).

```
> mut.matrix <- dissmutmatrix(data=PD4107a, chr=Chr,
+ position=Position, subset=6)
> dissplot(mut.matrix, method=NA, options=list( col = c("black",
+ "navy", "blue", "cyan", "green", "yellow", "orange", "red",
+ "darkred", "darkred", "white")))
```

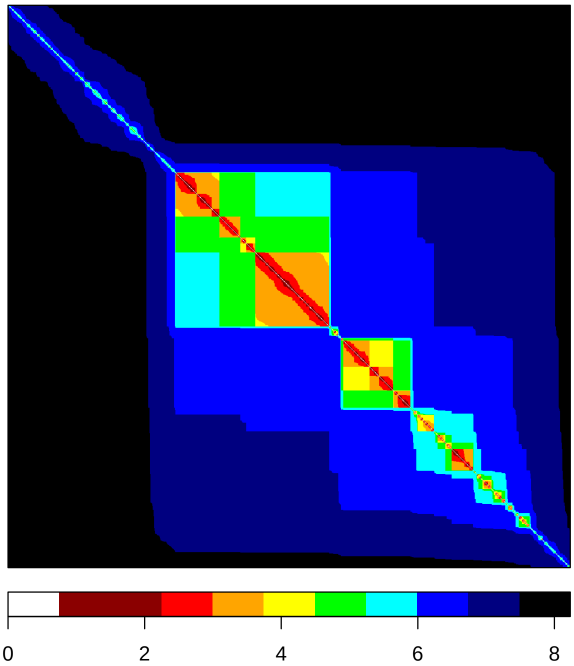


Figure 4: Graphical representation of the anti-Robinson matrix, i.e, the dissimilarity plot, generated from the mutation positions on chromosome 6.

2 Conclusion.

The anti-Robinson matrix properties can be used to identify and view all small highly mutated zones within the oncogenic process. The properties of this matrix are implemented in an R package: *ClusteredMutations*.

References

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