ClusteredMutations: Looking for a (Mutation) Shower.

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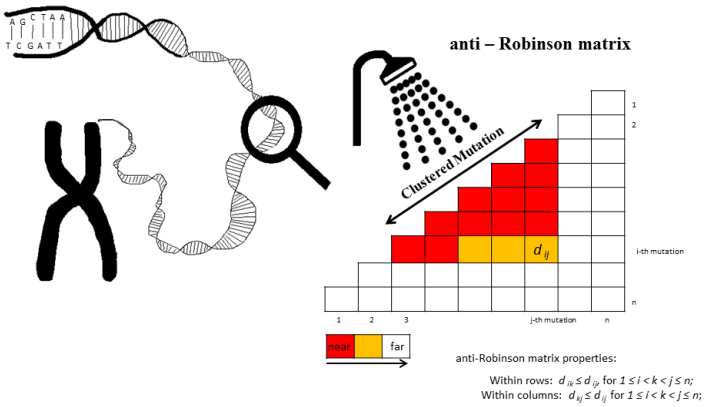
Abstract

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*



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')
```

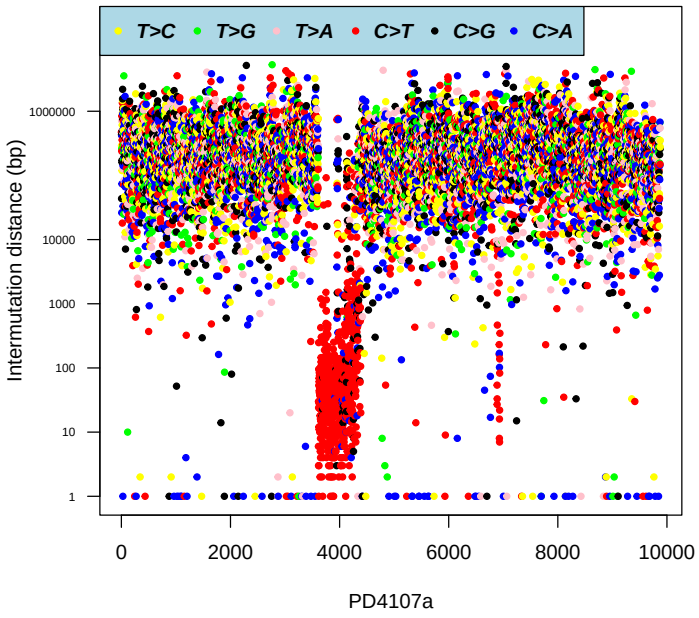


Figure 1: Rainfall plot of somatic substitution mutations from a patient with breast cancer (PD4107a).

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")
```

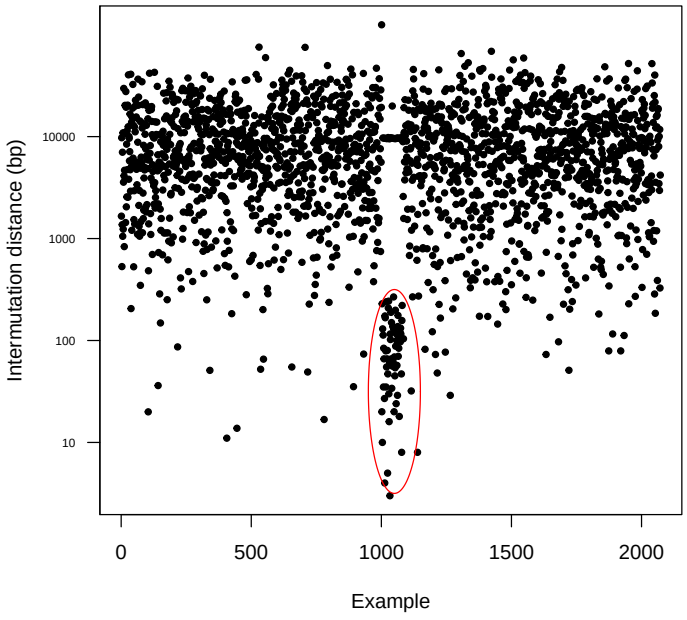


Figure 2: Rainfall plot of an example data set of somatic substitution mutations.

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> (o 0- extensión 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	

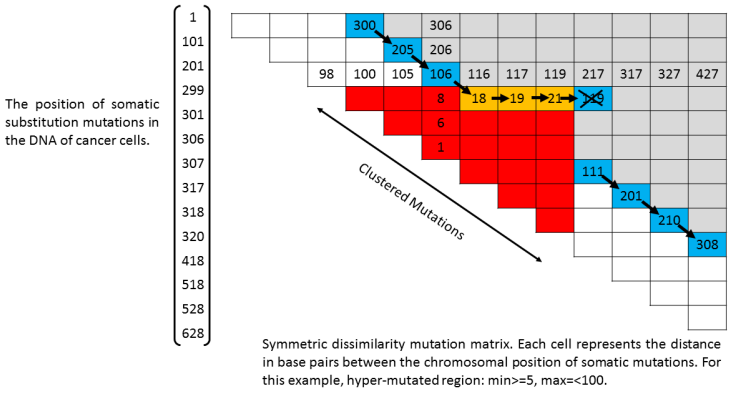


Figure 3: Symmetric dissimilarity mutation matrix for a sample of DNA sequence of cancer cell with 14 somatic substitution mutations.

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 hypermutated 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")))
```

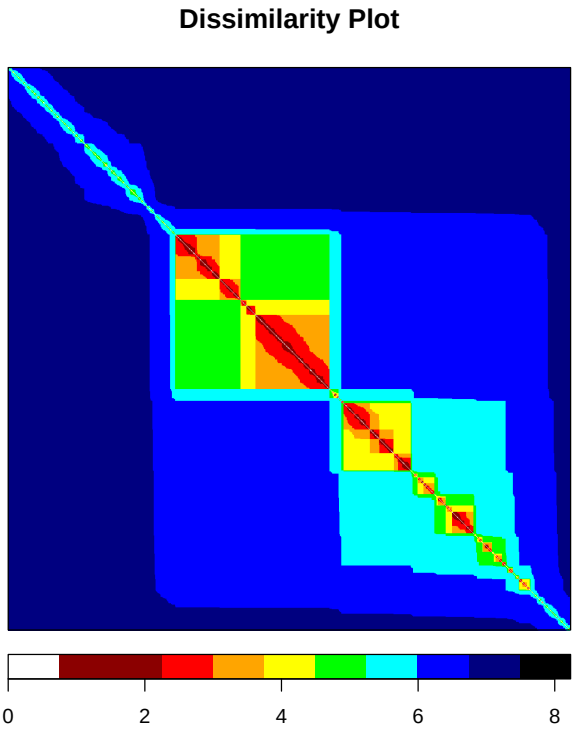


Figure 4: Graphical representation of the anti-Robinson matrix, i.e, the dissimilarity plot, generated from the mutation positions on chromosome 6.

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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