

iPAC: identification of Protein Amino acid Mutations

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Abstract

The **iPAC** package provides a novel tool to identify somatic mutation clustering of amino acids while taking into account their three dimensional structure. Currently, **iPAC** maps the protein's amino acids into a one dimensional space while preserving, as best as possible, the three dimensional local neighbor relationships. Mutation clusters are then found by considering if pairwise mutations are closer together than expected by chance alone via the the *Nonrandom Mutation Clustering* (NMC) algorithm [Ye et al., 2010]. Finally, the clustering results are mapped back onto the original protein and reported back to the user. A paper detailing this methodology and results is currently in preparation. *Additional methodologies based on different algorithms will be added in the future.*

1 Introduction

Recently, there have been significant pharmacological advances in treating oncogenic driver mutations [Croce, 2008]. Several methods that rely on amino acid mutational clusters have been developed in order to identify these mutations. One of the most recent methods was presented by Ye et al. [2010]. Their algorithm identifies mutation clusters by calculating whether pairwise mutations are closer on the the line than expected by chance alone when assuming that each amino acid has an equal probability of mutation. As their algorithm relies on considering the protein in linear form, it can potentially exclude clusters that are close together in 3D space but far apart in 1D space. This package is specifically designed to overcome this limitation.

Currently, this package has two methods that deal with the 3D structure of the protein: 1) linear and 2) MDS [Borg and Groenen, 1997]. The user should primarily use MDS as it is more statistically rigorous. We include the linear method as an example that the general package is itself flexible. Should the user want to map the protein to 1D space using their own algorithm, they can thus do so.

	cluster_size	start	end	number	p_value
V12	2	12	13	131	1.979447e-235
V12	1	12	12	100	6.486735e-188
V12	11	12	22	132	3.220145e-145
V12	12	12	23	133	6.524053e-142
V12	50	12	61	138	4.338908e-65
V13	1	13	13	31	2.732914e-39
V12	106	12	117	139	2.341227e-23
V12	135	12	146	149	1.356584e-20
V13	10	13	22	32	4.487362e-12
V13	11	13	23	33	1.279256e-11
V146	1	146	146	10	1.918440e-08

The results from *Code Example 1* show all the statistically significant clusters found, including the size of the cluster, the start and end positions and the number of mutations in that cluster.

3 Remapping Algorithm

3.1 Matching the Mutation and Position Information

Before we can run the 3D clustering algorithm while, we first need to come up with an alignment between the mutational information provided by a source such as COSMIC [Forbes et al., 2008] and the positional information provided by a source such as the PDB [Berman et al., 2000]. Such an alignment is necessary because mutational information is typically provided on the "canonical" amino acid numbering which often differs from the numbering used in the PDB database. Thus amino acid #i from the PDB database might not be amino acid #i from the mutational database.

To solve this problem, we consider the mutational database to contain the "canonical" ordering of the protein. We then attempt to map the structural information to the canonical ordering and create a new matrix of residues, their canonical counts, and their positions in 3D space. If successful, we then have a relational structure between the two databases allowing us to refer to amino acid #i where i represents the same amino acid in both databases.

We have created two methods that allow one to construct such a matrix: 1)*get.Positions* and 2)*get.AlignedPositions*.

The first method, *get.Positions* attempts to create the position matrix directly from the CIF file in the PDB database. It returns a list of several items, the first of which is \$Positions, which must later be passed to the *ClusterFind* method. Due to the complexity of CIF files, *get.Positions* currently works on approximately 70% of the structures in the PDB database.

The second method, *get.AlignedPositions* performs a pairwise alignment algorithm to align the canonical protein ordering with the XYZ positions in the PDB. Since *get.AlignedPositions* runs an alignment algorithm, the ordering might not be perfect and we recommend the user to verify the results. However,

from our testing, the alignment procedure works quite well. Furthermore, since *get.AlignedPositions* does not have to consider as many aspects of the CIF file, it is more robust and often works when *get.Positions* fails.

Let us first consider the *get.Positions* function. We will consider three examples, one for KRAS protein and two for the PIK3CA protein. For each example, we need to input the location of the CIF file (this holds the structural information), the location of the FASTA file (this holds the canonical protein sequence) and the sidechain that we want to use from the CIF file.

As the entire position sequence is too long to print, we first save the result and then print the first 10 rows of the position matrix. The remaining elements of the result are printed in full.

Code Example 2: Extracting positions using the *get.Positions* function

```
> library(iPAC)
> CIF<-"https://files.rcsb.org/view/3GFT.cif"
> Fasta<-"https://www.uniprot.org/uniprot/P01116-2.fasta"
> KRAS.Positions<-get.Positions(CIF, Fasta, "A")
> names(KRAS.Positions)

[1] "Positions"          "External.Mismatch" "PDB.Mismatch"
[4] "Result"

> KRAS.Positions$Positions[1:10,]

  Residue Can.Count SideChain XCoord  YCoord ZCoord
1     MET          1         A 62.935  97.579 30.223
2     THR          2         A 63.155  95.525 27.079
3     GLU          3         A 65.289  96.895 24.308
4     TYR          4         A 64.899  96.220 20.615
5     LYS          5         A 67.593  96.715 18.023
6     LEU          6         A 65.898  97.863 14.816
7     VAL          7         A 67.664  98.557 11.533
8     VAL          8         A 66.263 100.550  8.617
9     VAL          9         A 67.484  99.500  5.194
10    GLY         10         A 66.575 100.328  1.605

> KRAS.Positions$External.Mismatch

  PDB.Residue Canonical.Residue Canonical.Num
1           H                Q                61

> KRAS.Positions$PDB.Mismatch

  PDB.Residue Canonical.Residue Canonical.Num      Remark
19           H                Q                61 SEE REMARK 999

> KRAS.Positions$Result
```

```
[1] "OK"
```

Observe that the final element in *Code Example 2* is “OK”. That is because the only mismatched residue (at position 61), was documented in the CIF file as well. Thus it is considered a “reconciled” mismatch. It is up to the user to decide if they want to include it in the position sequence that is passed on to the *ClusterFind* method or to remove it.

Code Example 3: Final example of the `get.Positions` function

```
> CIF <- "https://files.rcsb.org/view/2RD0.cif"
> Fasta <- "https://www.uniprot.org/uniprot/P42336.fasta"
> PIK3CAV2.Positions <- get.Positions(CIF, Fasta, "A")
> names(PIK3CAV2.Positions)

[1] "Positions"          "External.Mismatch" "PDB.Mismatch"
[4] "Result"

> PIK3CAV2.Positions$Positions[1:10,]

  Residue Can.Count SideChain XCoord YCoord ZCoord
1     GLY         8         A 88.344 61.306 112.918
2     GLU         9         A 90.119 58.543 111.029
3     LEU        10         A 92.954 56.400 109.709
4     TRP        11         A 93.105 53.251 107.542
5     GLY        12         A 91.616 50.221 109.372
6     ILE        13         A 90.825 52.285 112.474
7     HIS        14         A 87.540 54.192 112.953
8     LEU        15         A 88.806 56.633 115.544
9     MET        16         A 92.435 57.520 116.178
10    PRO        17         A 93.481 57.378 119.852

> PIK3CAV2.Positions$External.Mismatch

NULL

> PIK3CAV2.Positions$PDB.Mismatch

NULL

> PIK3CAV2.Positions$Result

[1] "OK"
```

Observe that the final result in *Code Example 3* is “OK”. Here we use a different file location for the canonical sequence – the UNIPROT database. Here, the canonical sequence is slightly different and matches up exactly to the extracted positions. As there is only 1 isoform listed on UNIPROT for PIK3CA we suggest using the same source for both the mutational and canonical position

information. For example, if your mutation data was obtained from COSMIC, you should use COSMIC to get the canonical protein sequence.

Let us now consider the *get.AlignedPositions* function. This function automatically drops positions that do not match up.

Code Example 4: Extracting positions using the *get.AlignedPositions* function

```
> CIF<- "https://files.rcsb.org/view/2RD0.cif"
> Fasta <- "https://www.uniprot.org/uniprot/P42336.fasta"
> PIK3CAV3.Positions<-get.AlignedPositions(CIF,Fasta , "A")
> names(PIK3CAV3.Positions)

[1] "Positions"          "Diff.Count"          "Diff.Positions"     "Alignment.Result"
[5] "Result"

> PIK3CAV3.Positions$Positions[1:10,]

  Residue Can.Count SideChain XCoord YCoord ZCoord
14  GLY           8         A 88.344 61.306 112.918
15  GLU           9         A 90.119 58.543 111.029
16  LEU          10         A 92.954 56.400 109.709
17  TRP          11         A 93.105 53.251 107.542
18  GLY          12         A 91.616 50.221 109.372
19  ILE          13         A 90.825 52.285 112.474
20  HIS          14         A 87.540 54.192 112.953
21  LEU          15         A 88.806 56.633 115.544
22  MET          16         A 92.435 57.520 116.178
23  PRO          17         A 93.481 57.378 119.852

> PIK3CAV3.Positions$External.Mismatch

NULL

> PIK3CAV3.Positions$PDB.Mismatch

NULL

> PIK3CAV3.Positions$Result

[1] "OK"
```

Both *get.AlignedPositions* and *get.Positions* are still in beta and are provided to the user for convenience only. Changes by the PDB or COSMIC to their file structure might result in errors and it is up to the user to ensure the correct data is supplied to the *ClusterFind* function.

3.2 Finding Clusters in 3D Space

Now that we have the positional data, we can find the mutational clusters while taking into account the 3D protein structure. We begin by selecting a method to map the protein down to a 1D space.

The first method, "linear", fixes a specified point (x_0, y_0, z_0) and then calculates the distance from each amino acid to that point. The amino acids are then rearranged in order from the shortest distance to the longest distance. The second method, "MDS", uses Multidimensional Scaling [Borg and Groenen, 1997] to map the protein to a 1D space. We strongly encourage the user to employ the MDS method as it is more statistically rigorous. The linear method is provided as an example to show how other mapping paradigms might be implemented.

A diagram of either the MDS or linear mapping can be displayed when the *ClusterFind* method is run. As the mapping algorithms are different, the mapping images created are different as well. The linear method will generate an image of the distances from (x_0, y_0, z_0) to each amino acid. These distance will be drawn as dotted green lines from each amino acid to the fixed point. Conversely, the MDS methodology will create lines from each amino acid to the x-axis which will mark where on the line the amino acid is positioned.

We begin by first running the algorithm on KRAS using the MDS method followed by the linear method. For a full list of all the possible parameters, simply type '?ClusterFind' after loading the **iPAC** library.

Code Example 5: Running the ClusterFind method with a MDS mapping

```
> #Extract the data from a CIF file and match it up with the canonical protein sequence.
> #Here we use the 3GFT structure from the PDB, which corresponds to the KRAS protein.
> CIF<-"https://files.rcsb.org/view/3GFT.cif"
> Fasta<-"https://www.uniprot.org/uniprot/P01116-2.fasta"
> KRAS.Positions<-get.Positions(CIF,Fasta, "A")
> #Load the mutational data for KRAS. Here the mutational data was obtained from the
> #COSMIC database (version 58).
> data(KRAS.Mutations)
> #Identify and report the clusters.
> ClusterFind(mutation.data=KRAS.Mutations,
+             position.data=KRAS.Positions$Positions,
+             create.map = "Y", Show.Graph = "Y",
+             Graph.Title = "MDS Mapping",
+             method = "MDS")

[1] "Running Remapped"
[1] "Running Full"
[1] "Running Culled"
$Remapped
  cluster_size start end number      p_value
```

V136	1	12	12	100	8.932390e-183
V136	2	12	13	131	3.908116e-165
V121	49	13	61	38	3.097954e-124
V124	134	13	146	49	1.017093e-122
V136	106	12	117	139	1.362730e-119
V121	57	61	117	6	3.016259e-106
V124	30	117	146	11	1.666182e-102
V124	135	12	146	149	3.813215e-90
V121	50	12	61	138	2.682445e-87
V93	10	13	22	32	3.434148e-73
V93	96	22	117	8	1.140497e-65
V73	11	13	23	33	1.429105e-53
V73	95	23	117	7	4.204875e-48
V93	11	12	22	132	3.398568e-39
V142	1	13	13	31	8.571798e-38
V73	12	12	23	133	1.963564e-23
V142	105	13	117	39	4.563510e-12
V124	1	146	146	10	3.897757e-08
V121	86	61	146	16	6.115605e-07

\$OriginalCulled

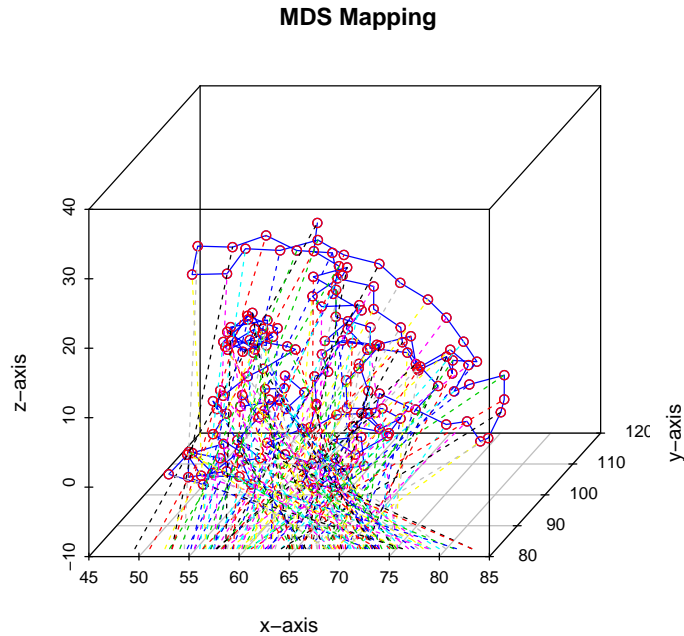
	cluster_size	start	end	number	p_value
V12	2	12	13	131	9.453887e-229
V12	1	12	12	100	7.630495e-183
V12	11	12	22	132	1.554973e-138
V12	12	12	23	133	3.526333e-135
V12	50	12	61	138	2.824800e-58
V13	1	13	13	31	8.857871e-38
V12	106	12	117	139	4.538089e-17
V12	135	12	146	149	3.853241e-13
V13	10	13	22	32	8.603544e-11
V13	11	13	23	33	2.553752e-10
V146	1	146	146	10	5.331155e-08

\$Original

	cluster_size	start	end	number	p_value
V12	2	12	13	131	1.979447e-235
V12	1	12	12	100	6.486735e-188
V12	11	12	22	132	3.220145e-145
V12	12	12	23	133	6.524053e-142
V12	50	12	61	138	4.338908e-65
V13	1	13	13	31	2.732914e-39
V12	106	12	117	139	2.341227e-23
V12	135	12	146	149	1.356584e-20
V13	10	13	22	32	4.487362e-12
V13	11	13	23	33	1.279256e-11


```
V146          1  146 146    10  1.918440e-08
```

```
$MissingPositions  
  LHS RHS  
[1,] 168 188
```



Code Example 6: Running the ClusterFind method with a Linear mapping

```
> #Extract the data from a CIF file and match it up with the canonical protein sequence.  
> #Here we use the 3GFT structure from the PDB, which corresponds to the KRAS protein.  
> CIF<-"https://files.rcsb.org/view/3GFT.cif"  
> Fasta<-"https://www.uniprot.org/uniprot/P01116-2.fasta"  
> KRAS.Positions<-get.Positions(CIF,Fasta, "A")  
> #Load the mutational data for KRAS. Here the mutational data was obtained from the  
> #COSMIC database (version 58).  
> data(KRAS.Mutations)  
> #Identify and report the clusters.  
> ClusterFind(mutation.data=KRAS.Mutations,  
+             position.data=KRAS.Positions$Positions,  
+             create.map = "Y", Show.Graph = "Y",  
+             Graph.Title = "Linear Mapping",  
+             method = "Linear")
```

[1] "Running Remapped"

[1] "Running Full"

[1] "Running Culled"

\$Remapped

	cluster_size	start	end	number	p_value
V90	1	12	12	100	8.862875e-183
V90	2	12	13	131	2.234056e-175
V90	135	12	146	149	2.965106e-158
V90	50	12	61	138	1.092881e-149
V90	11	12	22	132	9.127863e-90
V66	57	61	117	6	3.393497e-89
V90	12	12	23	133	3.393497e-89
V66	30	117	146	11	2.804316e-86
V66	105	13	117	39	5.838579e-79
V66	96	22	117	8	8.125462e-61
V66	95	23	117	7	2.427091e-60
V66	106	12	117	139	2.047918e-48
V95	1	13	13	31	8.857871e-38
V95	134	13	146	49	1.438417e-26
V95	49	13	61	38	9.252899e-22
V100	1	146	146	10	3.897757e-08
V100	86	61	146	16	1.607373e-05
V95	11	13	23	33	8.412719e-04
V95	10	13	22	32	8.663956e-04

\$OriginalCulled

	cluster_size	start	end	number	p_value
V12	2	12	13	131	9.453887e-229
V12	1	12	12	100	7.630495e-183
V12	11	12	22	132	1.554973e-138
V12	12	12	23	133	3.526333e-135
V12	50	12	61	138	2.824800e-58
V13	1	13	13	31	8.857871e-38
V12	106	12	117	139	4.538089e-17
V12	135	12	146	149	3.853241e-13
V13	10	13	22	32	8.603544e-11
V13	11	13	23	33	2.553752e-10
V146	1	146	146	10	5.331155e-08

\$Original

	cluster_size	start	end	number	p_value
V12	2	12	13	131	1.979447e-235
V12	1	12	12	100	6.486735e-188
V12	11	12	22	132	3.220145e-145
V12	12	12	23	133	6.524053e-142
V12	50	12	61	138	4.338908e-65

```

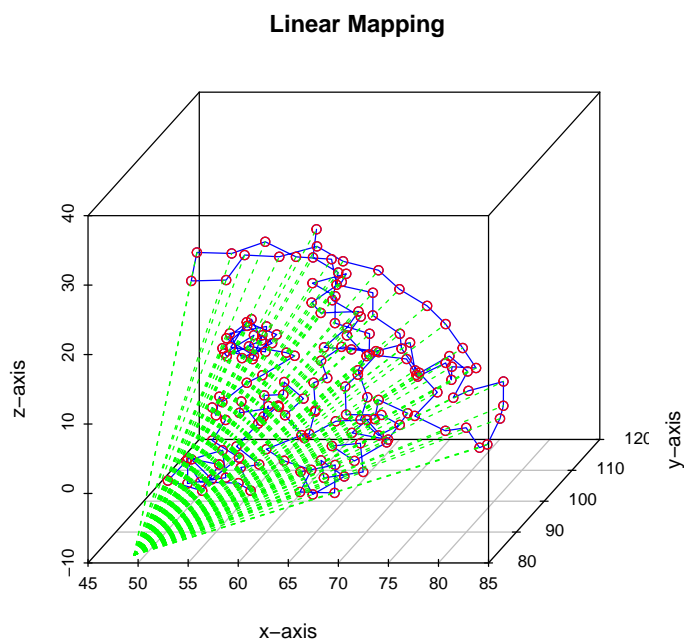
V13          1    13  13    31  2.732914e-39
V12         106   12 117   139  2.341227e-23
V12         135   12 146   149  1.356584e-20
V13          10    13  22    32  4.487362e-12
V13          11    13  23    33  1.279256e-11
V146         1   146 146    10  1.918440e-08

```

```

$MissingPositions
  LHS RHS
[1,] 168 188

```



As can be seen from *Code Examples 5* and *6* above, the *ClusterFind* method returns a list of four elements. The first element, `$Remapped`, displays all the clusters found while taking into account the 3D structure of the protein by utilizing either the linear or MDS methodology. The next element, `$OriginalCulled`, displays all the clusters found using the original NMC algorithm after removing all the amino acids for which we do not have (x, y, z) positions. The `$Original` element displays all the clusters found using the NMC algorithm without removing the amino acids for which we do not have 3D positional information.

If the user wants to compare the results generated when taking protein structure into account versus those when protein structure is ignored, it is recommended that the user compare the matrices in `$Remapped` versus `$OriginalCulled`. In this way, the user is considering the differences that arise strictly

from the protein structure as the amino acids with missing 3D positions have been removed prior to the analysis.

Finally, the `$MissingPositions` element displays a matrix of all the amino acids for which we had mutational data but for which we did not have positional data. For instance, in *Code Example 5*, the mutation data matrix had 188 columns while we were able to extract positional information only for amino acids 1-167. Furthermore, amino acid 61 was excluded from the final position matrix when the `get.AlignedPositions` function was run as the amino acid listed in the CIF file did not match the canonical sequence in the FASTA file. As such, the `$MissingPositions` element has a matrix of 2 rows as follows:

```
$MissingPositions
      LHS RHS
[1,]  61  61
[2,] 168 188
```

References

- H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, and P.E. Bourne. The protein data bank. *Nucleic Acids Research*, 28(1):235–242, January 2000. ISSN 13624962. doi: 10.1093/nar/28.1.235. URL www.pdb.org.
- Ingwer Borg and Patrick J. F Groenen. *Modern multidimensional scaling : theory and applications*. Springer, New York, 1997. ISBN 0387948457 9780387948454.
- Carlo M Croce. Oncogenes and cancer. *The New England Journal of Medicine*, 358(5):502–511, January 2008. ISSN 1533-4406. doi: 10.1056/NEJMra072367. URL <http://www.ncbi.nlm.nih.gov/pubmed/18234754>. PMID: 18234754.
- S A Forbes, G Bhamra, S Bamford, E Dawson, C Kok, J Clements, A Menzies, J W Teague, P A Futreal, and M R Stratton. The catalogue of somatic mutations in cancer (COSMIC). *Current Protocols in Human Genetics / Editorial Board, Jonathan L. Haines ... [et Al.]*, Chapter 10:Unit 10.11, April 2008. ISSN 1934-8258. doi: 10.1002/0471142905.hg1011s57. URL <http://www.ncbi.nlm.nih.gov/pubmed/18428421>. PMID: 18428421.
- Jingjing Ye, Adam Pavlicek, Elizabeth A Lunney, Paul A Rejto, and Chi-Hse Teng. Statistical method on nonrandom clustering with application to somatic mutations in cancer. 11(1):11, 2010. ISSN 1471-2105. doi: 10.1186/1471-2105-11-11. URL <http://www.biomedcentral.com/1471-2105/11/11>.