**MetaMutationalSigs: Comparison of mutational signature analyzers made easy**

**Abstract:**

**Summary:**

Mutations that result in cancers are caused by several mutational processes; mutational signature analysis can identify the contribution of these processes to observed mutational patterns. Recently, several packages have been developed for mutational signature analysis, with each using different methodology and yielding significantly different results. Because of the nontrivial differences in tools’ results, researchers may desire to survey and compare the available tools, in order to objectively evaluate their results for their specific research question, such as which mutational signatures are prevalent in different cancer types. There is a need for a software that can aggregate results from different packages and present them in a user-friendly way to facilitate effective comparison.

**Availability and implementation:**

MetaMutationalSigs is implemented using R and python and is available for installation using Docker and available at: [https://github.com/PalashPandey/MetaMutationalSigs](https://github.com/PalashPandey/MetaMutationalSigs" \t "_blank)

**Introduction:**

Cancerous tumors acquire several types of mutations in the form of single nucleotide variants, insertions and deletions, copy number changes and chromosomal aberrations. These mutations are hypothesized to be caused by multiple mutational processes operative in cancer leaving behind specific footprints in the DNA that can by captured by tumor mutation signature analysis. It is becoming increasingly evident that these tumor mutation signatures are not only important for understanding cancer evolution but also may have therapeutic implications, thus this an a very active and important area of research [1,2,3].

The basic idea behind mutational signatures is that mutational processes create specific patterns of mutations. Thus, it follows that if one can identify these patterns in a given sample then they can essentially detect the corresponding mutational processes. The possible mutations are grouped into 6 mutation types based on the base where the mutation was observed. These 6 mutation types are C>A, C>G, C>T, T>A, T>C, and T>G. Now, these 6 types of mutations are further divided based on their location, i.e. other bases that are in their immediate proximity giving us the 96 mutation types that are termed the single based substitution context. Alexandrov et al. first developed and applied this idea to The Cancer Genome Atlas (TCGA) [7] data to identify the first iteration of 30 mutational signatures termed COSMIC signatures [5], which came be used as the de facto reference for signature refitting. They then expanded their analysis to more data from Pan-Cancer Analysis of Whole Genomes (PCAWG) [7] resulting in multiple signatures using different contexts, such as double base substitutions (DBS) and insertions/deletions (indels), that are in addition to the reference single base substitution (SBS) signatures [8].

The mutational signature analysis workflow involves multiple steps that require different amounts of time and processing power. Briefly, the workflow starts from BAM files that are aligned to a reference genome and then proceeds to the variant calling step which outputs the VCF files. These steps are usually very resource-intensive and thus do not allow for much experimentation on personal computers (the downstream steps of variant filtering and annotation are much faster). The final step, the mutational signature analysis, is the least resource-intensive and, therefore, is easier for users to compare multiple methods on their desktop. Therefore, to facilitate comprehensive mutational signature analysis, we developed the package, MetaMutationalSigs. We create a wrapper for multiple packages [10,11,12,13] typically used for mutation signature analysis, and provide a standard format for their outputs so that they can be effectively compared. We have also standardized the input formats accepted by various packages to ease interoperability. With our previous experience of visualizations for genomic data [Lan et al.], we also implement standard visualizations for the results of all mutational signature analysis packages to ensure easy analysis. MetaMutationalSigs software is easy to install and use through Docker.

**Approach:**

The two major methods typically used for mutational signature analysis are signature refitting and de-novo signature extraction. Signature refitting methods try to reconstruct the observed mutational pattern in the sample (the frequencies of 96 types of mutations) using linear combinations of known signatures (COSMIC 30, SBS, ID, etc.), these methods work quite well on small sample sizes and single samples and are widely used with such data [6]. Signature extraction methods try to infer signatures from a given dataset of samples, then the extracted signatures are compared with known reference signatures. Each extracted signature is assigned to a known signature if their cosine similarity exceeds a set threshold [4]. There are a few important caveats to signature extraction as recently discussed in [9]: 1) a novel signature can be very similar to several reference signatures and the assignment is not always perfect and 2) the threshold for assignment plays a crucial role but is not widely agreed upon, using a different threshold can change the assignment. [9]

We chose signature refitting as our primary task and implemented high performing packages as identified in [9] that were implemented in R. We implement DeconstructSigs [10], MutationalPatterns [11], Sigfit [12], Sigminer [13], these tools build up on other tools such as [14, 15]. Our package outputs several data files in CSV format ready for further analysis and visualization using external packages along with visualizations of the signature exposures as described in Table 1.

We use root mean squared error (RMSE) between the reconstructed and actual signals (a metric commonly used in signal processing [Rosen] )as our performance metric for comparison of these packages.

Table 1.

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| --- | --- | --- |
| File Name | Format | Description |
| Heatmap\_exposures\_all\_sigs\_legacy.pdf | pdf | Exposures for all COSMIC 30 signatures. |
| Heatmap\_exposures\_all\_sigs\_SBS.pdf | pdf | Exposures for all COSMIC SBS signatures. |
| Heatmap\_legacy.pdf | pdf | Heatmap for difference between the predicted exposures by different tools. One for each sample. |
| Heatmap\_SBS.pdf | pdf | Heatmap for difference between the predicted exposures by different tools. One for each sample. |
| legacy\_pie\_charts.html | html | Interactive pie charts of 30 legacy signature exposures, per sample and for each tool. |
| sbs\_pie\_charts.html | html | Interactive pie charts of SBS signature exposures, per sample and for each tool. |
| legacy\_rmse\_bar\_plot.png | png | Reconstruction error using 30 legacy COSMIC signatures for each tool. |
| sbs\_rmse\_bar\_plot.png | png | Reconstruction error using COSMIC SBS signatures for each tool. |
| toolname\_results\legacy\_sample\_error.csv | csv | Data used to create the bar plot. |
| toolname\_results\legacy\_sample\_exposure.csv | csv | data used to create heatmap and pie chart. |
| toolname\_results\sbs\_sample\_error.csv | csv | Data used to create the bar plot. |
| toolname\_results\sbs\_sample\_exposure.csv | csv | Data used to create heatmap and pie chart. |

Figure 1

A) Workflow for mutational signature analysis. Our tool MetaMutationalSigs is at the final level of analysis. B) Heatmap of Euclidean distance between the predicted exposures of COSMIC legacy signatures by different tools for a sample. C) Heatmap of Euclidean distance between the predicted exposures of COSMIC SBS signatures by different tools for a sample. D) RMSE of the reconstruction error using for each tool and reference signatures, lower is better. Prevalence of SBS:ID:DBS::100:10:1. E) Heatmap of COSMIC legacy signature exposures, one row per sample. F) Heatmap of COSMIC SBS signature exposures, one row per sample.

**Discussion:**

The massive increase in the number of software packages has made managing dependencies quite burdensome, coupled with incompatible data formats for signature matrices can make mutational signature analysis difficult and hard to reproduce. Our package provides an easy way of performing these setup related tasks so one can focus more on the analysis. Investigators should keep in mind that refitting approaches need a priori knowledge about the samples for effective interpretation [16] and the results should not be used as is without a sanity check.

Future work for this project would focus on expanding the tool to work with more packages and keep the reference signatures updated as new versions are released. Due to the open-source nature of the project, we also welcome additional feature requests using the project link on GitHub <https://github.com/PalashPandey/MetaMutationalSigs>

**References:**

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