

CMSC499A: Mutation Signature Explorer

Mark Keller *

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Abstract

Identification and analysis of patterns in data can be difficult without visualization tools. Datasets of somatic mutations in cancer are no exception. Recent whole-genome sequencing projects have produced large amounts of mutation data to be explored. Of great importance is the classification of different mutational processes and the mutational signatures² they leave behind. As new mutational signatures continue to be discovered, observation of the levels of signature activity, called signature exposure, helps show how the underlying mutational processes differ across cancer types, time, and environmental variables. Visualizations of mutational signatures and mutation datasets in the context of these variables can help researchers to quickly observe trends and relationships in this data, verifying current knowledge or prompting further analysis. This paper describes a mutation visualization tool developed this semester that allows simple somatic mutation datasets and mutation signatures to be explored interactively and provides users with publication-ready graphics.

1 Introduction

Mutation signatures are distinctive patterns of somatic mutations generated by different mutational processes over the course of a lifetime². These patterns have been extracted from mutation datasets using computational and mathematical techniques. The concept of mutational signatures has been validated by in vitro experiments that perform knockouts of DNA repair genes and then observe that mutational signatures result¹⁸.

Observation of mutational signatures and knowledge of the mutational processes that cause them can improve cancer diagnosis and treatment. Mutational signatures have been used to predict BRCA1/BRCA2 deficiency in breast cancers, allowing therapeutic sensitivity to be distinguished as early as the first biopsy⁶. In colorectal cancers, mutational signatures have enabled identification of base excision repair (BER) genomic instability that can prompt genetic counselling and immunotherapy measures that take into consideration the high mutation load¹⁴.

There are many open questions about mutational signatures. Currently, mutational signatures are represented as probability distributions over 96 categories of single-nucleotide variant (SNV) mutations. These 96 categories include the reference base, the variant base, and one flanking base pair in each direction. It is not known how significant these flanking base pairs are, how many to consider, or to what extent these depend on one another¹⁵. The mutational processes (or levels of noise) that cause some of the currently published mutational signatures remain unknown. Methods to consider other types of mutations (indels and rearrangements) are only being formulated now¹⁸.

*advised by Professor Max Leiserson

Mutation Signature Explorer is a web-based (<https://mutation-signature-explorer.lrggr.io>) mutation data browser that enables exploration of mutational signatures, SNV mutations, and mutation density. This tool allows users to choose sequencing project datasets by cancer type, as well as mutational signature combinations (of which can be selected based on cancer-type-specific presets) before plotting this data. Web-based interactive visualizations allow for exploration across datasets and features in a way that is accessible and fast. Data exploration through these visualizations can be used to make initial inferences that lead to further statistical analysis, or, alternatively, can be used to verify and communicate relationships found through prior analysis.

Each type of visualization produced by the Mutation Signature Explorer aims to enable further examination of a trend or relationship which has been hypothesized or presented in existing literature. The following are types of visualizations that can be generated and their motivations:

1. It is well known that tobacco and alcohol usage increases risk of many types of cancers^{7,13}. A question of interest is how mutational signatures relate to smoking status and other clinical variables. Alexandrov *et al.* 2016 finds that smokers exhibit increases in mutations attributed to COSMIC signatures 2, 4, 5, 13, and 16¹. Kim *et al.* 2016 also notes an association between signature 5 and smoking¹⁰.

To illustrate estimated contributions of a selected combination of mutational signatures, a stacked bar plot can be generated, showing estimated signature exposures for a sample, along with tobacco and alcohol usage indicators. Exposure values can be normalized to sum to one for each sample, or kept relative to the total number of mutations in each sample. This plot type was inspired by Figure 4 in Kim *et al.* 2016 that displays estimated contributions of 4 different signatures and tobacco usage over cohorts of urothelial cancer tumor samples¹⁰.

2. Localized hypermutation, known as kataegis, was first discussed by Taylor *et al.* 2013¹⁶, in which whole-genome sequencing of breast cancers showed regions with mutation “rainfalls” - greater than 5 mutations with significantly short intermutational distances. Mutations in regions of kataegis have been shown to occur frequently at C base pairs preceded by a 5-prime T base pair, due to APOBEC activity during double-strand break repair¹⁶. Since this introduction in 2013, kataegis has been shown to occur in additional tumor types². COSMIC signatures 2 and 13 have been associated with APOBEC activity, and by extension, kataegis². There is recent interest in rigorous statistical identification and verification of kataegis¹⁷.

To examine mutation clusters, specifically those occurring in regions of kataegis, a rainfall plot can be generated for each sample. Mutations are plotted horizontally based on their genome location, and vertically by the distance (in bp) to the previous mutation. Mutations are colored according to one of 96 mutation categories (5' flanking base pair, single-nucleotide variant, 3' flanking base pair). Rainfall plots are commonly used to examine regions of hypermutation, as they allow these regions to be easily identified by tight vertical clusters of mutations. Similar rainfall plots can be seen in Figure 4 of Nik-Zainal *et al.* 2012¹² and in Figure 6 of Alexandrov *et al.* 2013².

To identify samples containing instances of localized hypermutation, kataegic events can be highlighted along the genome in a second type of plot. Along the vertical axis are samples, and along the horizontal axis is the genome. Users can zoom and pan along each chromosome, and easily pinpoint kataegis events by the dark bars located on mutations in kataegis regions. Samples are grouped by sequencing project and cancer type. This plot acts as a rainfall plot selector, as each sample bar can be clicked to generate a corresponding rainfall plot. To our knowledge, this style of plot has not been used before to visualize kataegis.

3. A question that prompted the development of this visualization tool is whether certain mutational signatures are associated with mutations in specific genomic locations or regions. One way to examine this is to look at mutation signature activity across the genome with a “Manhattan plot”. The Manhattan plot gets its name from the resemblance of a skyline along the genome, and is typically used to visualize the output of statistical significance tests used in genome-wide association studies⁸. To generate this plot, signatures are assigned to individual mutations (see Methods section for details) and then grouped into bins by chromosome location and signature. The horizontal axis represents the genome location, and the vertical axis represents the number of mutations within a bin. To our knowledge, this is a new method for exploring mutational signatures along the genome.

The rest of this paper is organized into the following sections: Methods, Case Studies, and Conclusions. Included in the Methods section are discussions of open-source libraries and frameworks used to create the browser application, the steps taken to process raw mutation and clinical datasets and the sources of these datasets, and the algorithms used to compute signature exposures. The Case Studies section presents two use cases for this tool: exploration of the relationship between alcohol usage and mutational signatures, and verification of the mutation categories typically present in regions of hypermutation. Future directions for development of the Mutation Signature Explorer are outlined in the Conclusions section.

2 Methods

Using the Vue JavaScript framework, the application is made up of reusable components that encapsulate templates, functions, and variables. This encapsulation promotes modularity, and therefore ease of maintainability. Using the data-driven documents JavaScript library (D3.js)⁴, each type of plot is tailored to suit the data types and data sets presented. Showing donor clinical variables, such as smoking and alcohol usage, and their relationship to mutation signatures, requires this fine control that D3 provides. In addition, D3 contains APIs for easy implementation of custom interactive features, such as highlighting, panning, and zooming. Interactivity extends beyond single plots, linking plots together based on variables such as chromosome region and donor.

Data processing for plots occurs in two stages. Simple somatic mutation datasets and donor clinical datasets from sequencing projects are downloaded and processed into a uniform format. This conversion must be specified for each sequencing project, as each provides datasets in a different format. Currently, processing has been completed for simple somatic mutation and clinical datasets from the Pan-Cancer Analysis of Whole Genomes (PCAWG) study. Once into a uniform format, these datasets are stored in a bucket in the UMIACS object store, and can be processed dynamically by a web server as specific requests are made for visualizations. Usage of the object store allows future developers to quickly get the application up and running without needing to perform this initial time-consuming data processing step. Dynamic processing is performed by a web application written in Python with the Flask framework. The Pandas and NumPy packages are used for data manipulation in both of these processing stages.

Signature exposures are estimated using a quadratic programming approach detailed by Huang *et al.* 2017⁹. To generate the aforementioned Manhattan Plot showing signatures across the genome, signatures are assigned to mutation categories for each sample by first computing estimated signature exposures, then taking the maximum of the product of each signature’s probability for the mutation category and the sample’s exposure to the same signature. Signatures present in

each cancer type, or signature combination “presets”, are based on those specified in publications of mutational signatures. For example, the presences of COSMIC signatures can be found in Figure 3 of Alexandrov *et al.* 2013².

Code for the Mutation Signature Explorer is open-source and available through the following repositories: <https://github.com/lrgr/mutation-signature-explorer>, <https://github.com/lrgr/mutation-signature-explorer-server>. While this software can be run locally, accessing a hosted version may be easier for some users. Currently, an instance of the Flask-powered web server is deployed to Heroku. An instance of the front end (Vue/D3) component is deployed through GitHub pages, and uses Travis CI for continuous integration and deployment. This makes access and usage as simple as connecting via a web browser.

3 Case Studies

3.1 Alcohol Usage and Mutational Signatures

One use case for this tool is the analysis of relationships between signature exposures and clinical variables. Interactive sorting and dynamic calculations of signature exposures allow trends to be observed quickly. Although the relationship between liver cancer and alcohol consumption has long been documented³, the relationship between mutational signatures and alcohol consumption has not been extensively examined. Recently, Li *et al.* 2018 identified a mutational signature associated with alcohol usage in esophageal squamous cell carcinoma that resembles COSMIC signatures 1, 2, 13 and 16¹¹. To look for a relationship in liver cancer with the Mutation Signature Explorer, we can select the PCAWG Liver Cancer - NCC, JP cohort, along with the COSMIC LICA signatures preset for liver cancer. Then, to examine the alcohol usage clinical variable, we can select the Signature Exposures with Clinical Data plot. Using the sorting functionality, we first sort by the alcohol usage clinical variable. With no trends immediately apparent, we can now sort by signature values. Sorting by COSMIC signature 12 and normalizing the signature exposures, we can see that only two alcohol-free donors are in the top 14 of 28 donors (Figure 1). Hovering over the samples and looking at the y-axis, we can see that the contribution of signature

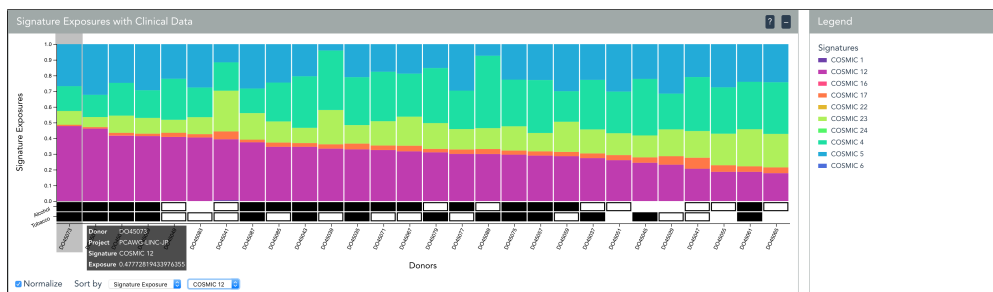


Figure 1: Screenshot of Signature Exposures with Clinical Data plot with PCAWG-LINC-JP data, normalized and sorted by COSMIC signature 12

12 ranges from over 10% to just under 50% for all samples. Using this information, one could perform further statistical analysis on this data or seek out additional liver cancer mutation data to analyze.

Interestingly, when sorting by COSMIC signatures 1 and 16 - signatures both present in liver cancer and that resemble the Li *et al.* 2018 signature, a similar trend in alcohol usage arises (only

two alcohol-free donors in the top half of donors). However, the contributions from these two signatures are too low to be visible for any sample in the plot.

3.2 Hypermutation and Mutation Category

Another use case is the analysis of the relationship between hypermutation and mutation category. For SNVs, the mutation category is the 5' flanking base pair, the mutation reference base pair, the variant base pair, and the 3' flanking base pair. Mutations are assigned colors based on mutation categories on the rainfall plots to allow this type of relationship (or lack thereof) to be quickly spotted. The prevalence of mutations with reference base C and 5' T in regions of kataegis is well documented¹⁶.

To verify this relationship with the Mutation Signature Explorer, we can choose the PCAWG UK Breast Triple Negative/Lobular Cancer cohort and add a Kataegis Plot. Next, Rainfall Plots can be generated by choosing donors with kataegis events on chromosome 1. By hovering over mutations in kataegis regions on these plots and looking at the categories on the legend, we see an abundance of orange-colored C[C>T]X mutations and some blue T[C>T]X mutations in these regions. Figure 2 shows these rainfall plots and the mutation category legend. These plots could be used to discover further trends occurring in regions of hypermutation, such as significant rates of mutations that fall into categories with patterns other than T[C>T]X and C[C>T]X in specific cancer types. Such discoveries can be communicated to others easily, as Mutation Signature Explorer URLs include information about current plot states.

4 Conclusions

Future development of the Mutation Signature Explorer should include the expansion of functionality through implementation of more plot types. Specifically, visualization of mutational signatures themselves - as distributions over mutation categories - would be a simple but informative feature to add. This would give users that are unfamiliar with mutational signatures a better sense of how the signatures are used in the other plot types. These could look like interactive versions of the plots presented in Figure 2 of Alexandrov *et al.* 2013².

Visualization of mutation rates, in the form of interactive mutational prevalence plots, should be used to show relationships between mutation rates and variables such as cancer type, clinical data, mutational signatures, and kataegis rates. A mutation prevalence plot by cancer type is presented in Figure 1 of Alexandrov *et al.* 2013².

Another Manhattan plot should be implemented, showing mutation rates along the genome by mutation category.

A plot showing the location of genes should be linked to existing plots that present data along the genome. This type of plot showing gene locations can be found in Figure 1 of Chelaru *et al.* 2014⁵.

Existing plots should be modified to allow analysis of mutation types other than SNVs. For example, since kataegis has been hypothesized to be associated with rearrangements, the rainfall and kataegis plots should be modified to show structural somatic mutations. The kataegis plot should also be modified to show survival status, which has also been hypothesized to be associated with kataegis.

Mutation datasets from additional sources should be processed to allow for stronger conclusions to be made using this tool. When additional mutational signatures are discovered, they should

be added to the database to ensure that users have access to the most relevant and accurate visualizations possible. Local dataset visualization should be further enabled, to extend functionality beyond the public datasets we have processed.

References

- [1] Ludmil B Alexandrov, Young Seok Ju, Kerstin Haase, Peter Van Loo, Iñigo Martincorena, Serena Nik-Zainal, Yasushi Totoki, Akihiro Fujimoto, Hidewaki Nakagawa, Tatsuhiro Shibata, et al. Mutational signatures associated with tobacco smoking in human cancer. *Science*, 354(6312):618–622, 2016.
- [2] Ludmil B Alexandrov, Serena Nik-Zainal, David C Wedge, Samuel AJR Aparicio, Sam Behjati, Andrew V Biankin, Graham R Bignell, Niccolo Bolli, Ake Borg, Anne-Lise Børresen-Dale, et al. Signatures of mutational processes in human cancer. *Nature*, 500(7463):415, 2013.
- [3] F Xavier Bosch, Josepa Ribes, Mireia Diaz, and Ramon Cleries. Primary liver cancer: world-wide incidence and trends. *Gastroenterology*, 127(5):S5–S16, 2004.
- [4] Michael Bostock, Vadim Ogievetsky, and Jeffrey Heer. D³ data-driven documents. *IEEE transactions on visualization and computer graphics*, 17(12):2301–2309, 2011.
- [5] Florin Chelaru, Llewellyn Smith, Naomi Goldstein, and Héctor Corrada Bravo. Epiviz: interactive visual analytics for functional genomics data. *Nature methods*, 11(9):938, 2014.
- [6] Helen Davies, Dominik Glodzik, Sandro Morganella, Lucy R Yates, Johan Staaf, Xueqing Zou, Manasa Ramakrishna, Sancha Martin, Sandrine Boyault, Anieta M Sieuwerts, et al. Hrdetect is a predictor of brca1 and brca2 deficiency based on mutational signatures. *Nature medicine*, 23(4):517, 2017.
- [7] Richard Doll and A Bradford Hill. Lung cancer and other causes of death in relation to smoking. *British medical journal*, 2(5001):1071, 1956.
- [8] Greg Gibson. Hints of hidden heritability in GWAS. *Nature genetics*, 42(7):558, 2010.
- [9] Xiaoqing Huang, Damian Wojtowicz, and Teresa M Przytycka. Detecting presence of mutational signatures in cancer with confidence. *Bioinformatics*, 34(2):330–337, 2017.
- [10] Jaegil Kim, Kent W Mouw, Paz Polak, Lior Z Braunstein, Atanas Kamburov, Grace Tiao, David J Kwiatkowski, Jonathan E Rosenberg, Eliezer M Van Allen, Alan D D’Andrea, et al. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. *Nature genetics*, 48(6):600, 2016.
- [11] XC Li, MY Wang, M Yang, HJ Dai, BF Zhang, W Wang, XL Chu, X Wang, H Zheng, RF Niu, et al. A mutational signature associated with alcohol consumption and prognostically significantly mutated driver genes in esophageal squamous cell carcinoma. *Annals of Oncology*, 2018.
- [12] Serena Nik-Zainal, Ludmil B Alexandrov, David C Wedge, Peter Van Loo, Christopher D Greenman, Keiran Raine, David Jones, Jonathan Hinton, John Marshall, Lucy A Stebbings, et al. Mutational processes molding the genomes of 21 breast cancers. *Cell*, 149(5):979–993, 2012.

- [13] Collaborative Group on Hormonal Factors in Breast Cancer et al. Alcohol, tobacco and breast cancer—collaborative reanalysis of individual data from 53 epidemiological studies, including 58 515 women with breast cancer and 95 067 women without the disease. *British journal of cancer*, 87(11):1234, 2002.
- [14] Camilla Pilati, Jayendra Shinde, Ludmil B Alexandrov, Guillaume Assié, Thierry André, Zofia Hélias-Rodzewicz, Romain Ducoudray, Delphine Le Corre, Jessica Zucman-Rossi, Jean-François Emile, et al. Mutational signature analysis identifies mutyh deficiency in colorectal cancers and adrenocortical carcinomas. *The Journal of pathology*, 242(1):10–15, 2017.
- [15] Yuichi Shiraishi, Georg Tremmel, Satoru Miyano, and Matthew Stephens. A simple model-based approach to inferring and visualizing cancer mutation signatures. *PLoS genetics*, 11(12):e1005657, 2015.
- [16] Benjamin JM Taylor, Serena Nik-Zainal, Yee Ling Wu, Lucy A Stebbings, Keiran Raine, Peter J Campbell, Cristina Rada, Michael R Stratton, and Michael S Neuberger. DNA deaminases induce break-associated mutation showers with implication of APOBEC3B and 3A in breast cancer kataegis. *Elife*, 2, 2013.
- [17] Fouad Yousif, Stephenie Prokopec, Ren X Sun, Fan Fan, Christopher M Lalansingh, David H Park, Lesia Szyca, PCAWG Network, and Paul C Boutros. The origins and consequences of localized and global somatic hypermutation. *bioRxiv*, page 287839, 2018.
- [18] Xueqing Zou, Michel Owusu, Rebecca Harris, Stephen P Jackson, Joanna I Loizou, and Serena Nik-Zainal. Validating the concept of mutational signatures with isogenic cell models. *Nature communications*, 9(1):1744, 2018.

A Examples of Publication-Ready Figures

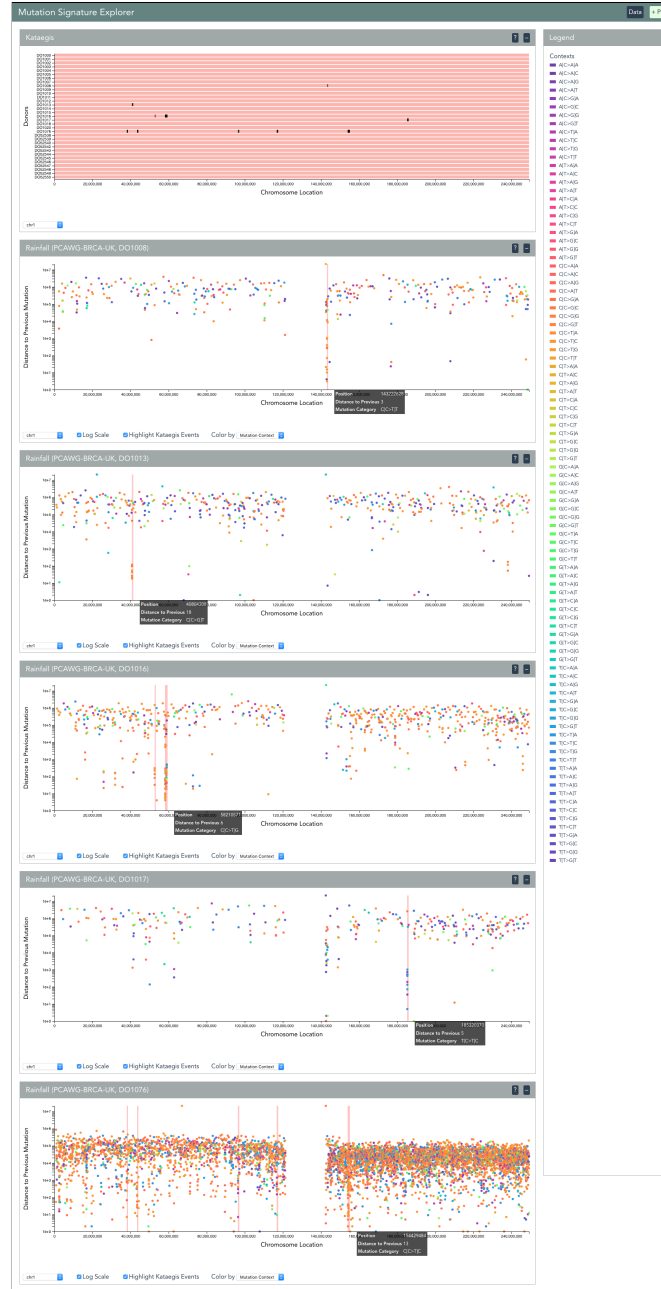


Figure 2: Screenshot of Rainfall Plots with PCAWG-BRCA-UK data for Chromosome 1

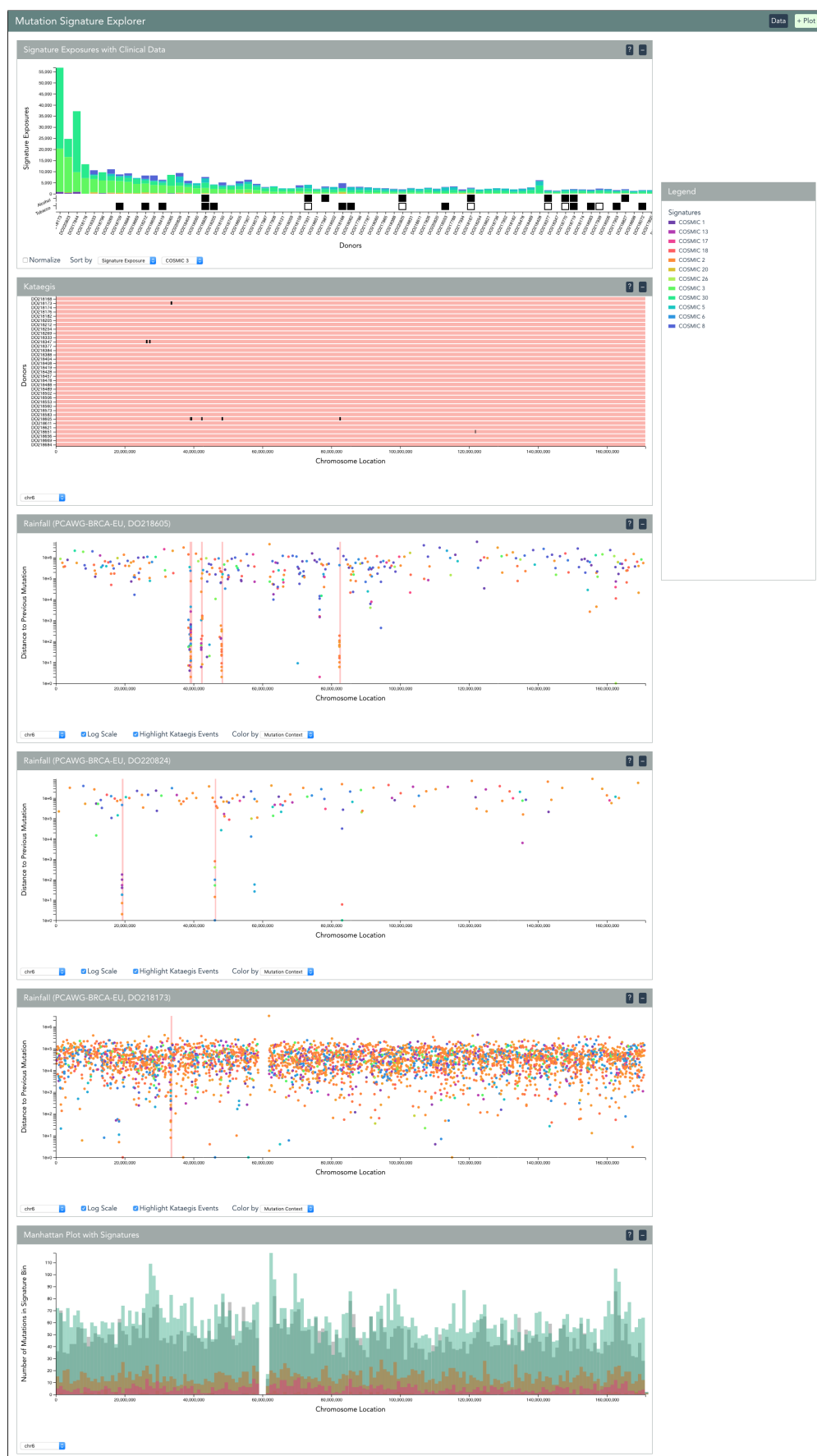
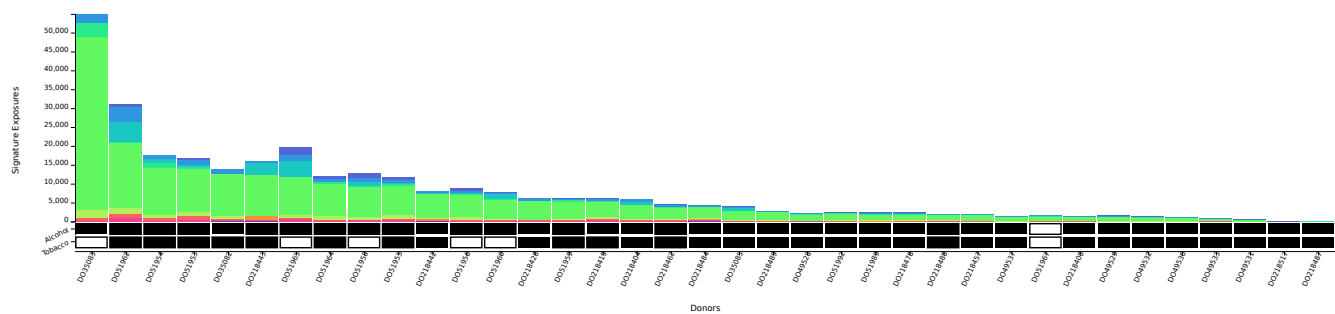
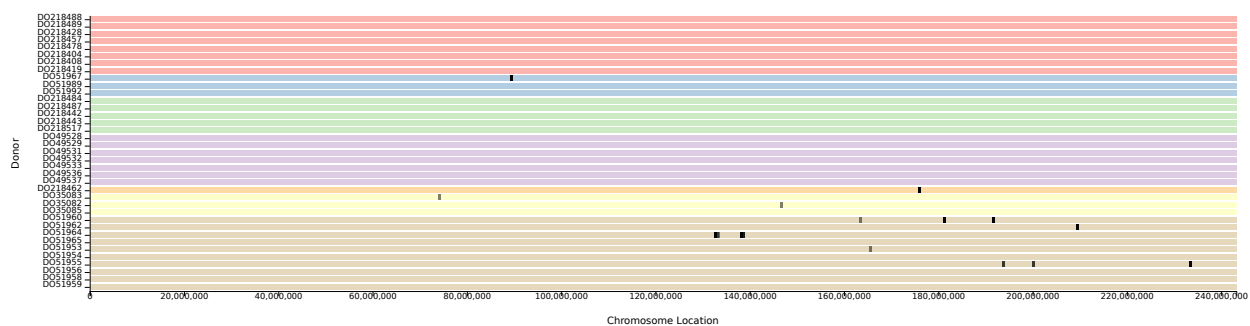


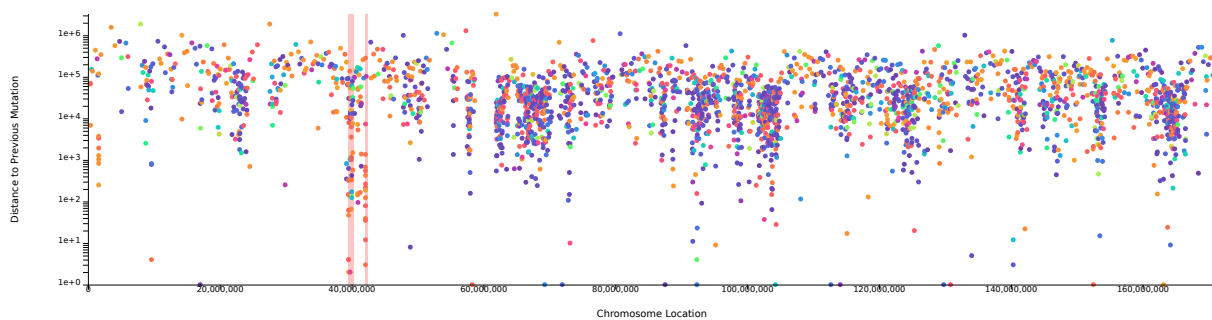
Figure 3: Screenshot



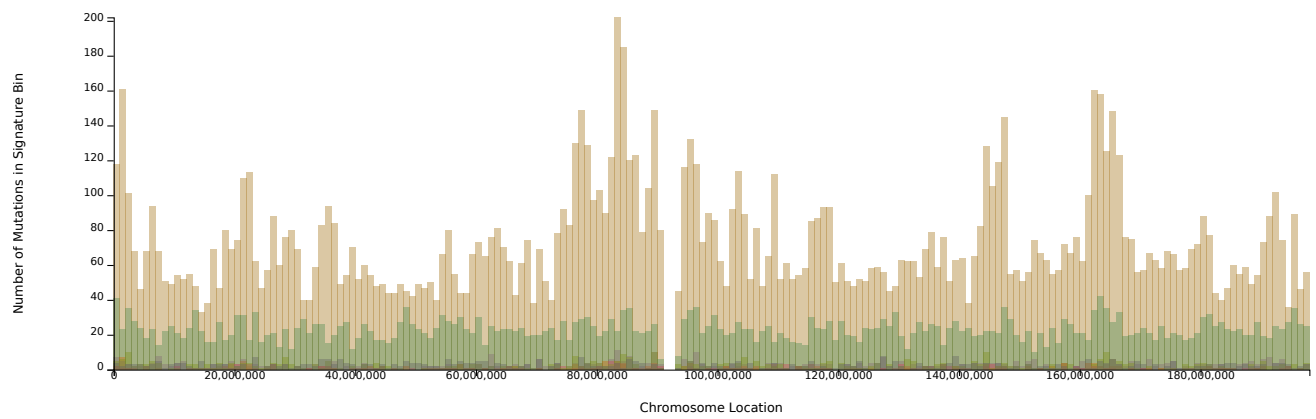
Example Figure 1: Signature Exposures with Clinical Variables



Example Figure 2: Kataegis Plot (Chromosome 2)



Example Figure 3: Rainfall Plot (Chromosome 6)



Example Figure 4: Manhattan Plot (Chromosome 2)