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Genome analysis

Palimpsest: an R package for studying mutational and structural variant signatures along clonal evolution in cancer

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Abstract

Summary: Cancer genomes are altered by various mutational processes and, like palimpsests, bear the signatures of these different processes. The *Palimpsest* R package provides a complete workflow for the characterization and visualization of mutational signatures and their evolution along tumor development. The package covers a wide range of functions for extracting both base substitution and structural variant signatures, inferring the clonality of each alteration and analyzing the evolution of mutational processes between early clonal and late subclonal events. *Palimpsest* also estimates the probability of each mutation being due to each process to predict the mechanisms at the origin of driver events. *Palimpsest* is an easy-to-use toolset for reconstructing the natural history of a tumor using whole exome or whole genome sequencing data.

Availability and implementation: *Palimpsest* is freely available at www.github.com/FunGEST/Palimpsest.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Mutational signature analysis is a powerful approach to understand the origin of somatic mutations in cancer (Alexandrov *et al.*, 2013). This approach can be extended to any kind of genomic alteration including structural variants (Helleday *et al.*, 2014). Mutational processes may evolve over time, giving different mutational signatures in early clonal and late subclonal mutations (Nik-Zainal *et al.*, 2012; Rubanova *et al.*, 2018). Next-generation sequencing data also allow to reconstruct the timing of copy-number alterations (CNAs)

by distinguishing clonal/subclonal events and using the proportion of duplicated mutations to time chromosome duplications (Greenman *et al.*, 2012). Finally, different mutational processes have different propensities to target specific loci depending on the local chromatin state (Polak *et al.*, 2015; Sabarinathan *et al.*, 2016) and sequence context (Letouzé *et al.*, 2017). Understanding how mutational processes evolve during tumorigenesis and alter specific driver genes is critical to better understand the natural history of cancers and eventually predict and influence their clinical behavior.
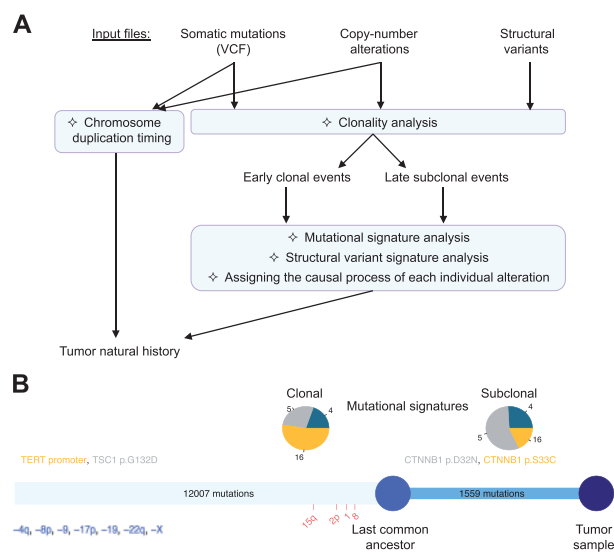
Fig. 1. (A) Workflow of a typical analysis with *Palimpsest*. **(B)** Oncogenic timeline of a tumor representing the number of clonal/subclonal mutations, the contribution of mutational processes to each step and the timing of driver mutations (colored according to their most likely causal mutational process) and CNAs

To the best of our knowledge, no comprehensive workflow integrates mutational signature and clonality analyses to reconstruct the natural history of a tumor. In addition, no automated tools for extracting structural variant signatures, timing chromosome duplications and estimating the process at the origin of each mutation are currently available. The *Palimpsest* R package provides an extensive toolset to explore and visualize the diverse processes generating somatic alterations in a tumor, their evolution along tumorigenesis and their interaction with driver genes.

2 Features of *palimpsest*

Palimpsest takes as input somatic mutations and structural variants (optional), copy-number data and a minimal sample annotation file indicating gender and tumor purity. Figure 1A and Supplementary Figure S1 illustrate a typical Palimpsest analysis, as described step by step below.

Mutational signature analysis. Palimpsest allows both *de novo* extraction of novel mutational signatures or quantification of previously described signatures using non-negative matrix factorization (NMF), as implemented in the *NMF* R package (Gaujoux and Seoighe, 2010). Utilities are provided to visualize signatures, transcriptional strand biases and inter-mutation distance, and to compare signatures using cosine similarity.

Structural variant signature analysis. Palimpsest implements an adaptation of the mutational signature analysis framework for structural variants (SVs). SVs are first classified into 38 categories according to the type (deletion, tandem duplication, inversion and interchromosomal translocation) and size of rearrangements (Nik-Zainal *et al.*, 2016). NMF then allows extracting new or known structural variant signatures and their contribution to each tumor genome.

Predicting the process at the origin of each individual mutation. Once the mutation catalogue of a tumor has been deconvoluted as the addition of several mutational processes, *Palimpsest* estimates the probability of each individual mutation being due to each process using simple Bayesian statistics (Letouzé *et al.*, 2017). This key feature, available for both point mutations and structural variants, allows predicting the processes most likely at the origin of driver events in each tumor.

Clonality analysis and evolution of mutational signatures. Palimpsest integrates variant allele fraction, tumor purity and local copy number to estimate the proportion of tumor cells harboring each mutation and classify them as clonal or subclonal. Then, the package extracts and compares mutational signatures operative in clonal and subclonal mutations to explore the evolution of mutational processes along tumorigenesis.

Timing CNAs. Several tools have been described to differentiate clonal from subclonal CNAs, which can be directly specified in the CNA input file. In addition, *Palimpsest* will estimate the molecular timing of duplications using the proportion of duplicated/non-duplicated somatic mutations.

Oncogenic timelines. Finally, *Palimpsest* integrates the results of clonality and mutational signature analyses to generate a comprehensive representation of the natural history of the tumor (Fig. 1B).

3 Conclusions

Unraveling what mutational processes generate driver mutations and fuel the initiation and progression of tumor clones is crucial to better understand the natural history of cancers and optimize personalized patient care. *Palimpsest* integrates a broad range of functions within a single convenient workflow to provide a complete picture of tumorigenesis. The minimum basic input data make the package easy-to-use in any cancer genomic study downstream classical variant calling and copy number analyses.

References

Alexandrov,L.B. *et al.* (2013) Signatures of mutational processes in human cancer. *Nature*, **500**, 415–421.

Gaujoux,R. and Seoighe,C. (2010) A flexible R package for nonnegative matrix factorization. *BMC Bioinformatics*, **11**, 367.

Greenman,C.D. *et al.* (2012) Estimation of rearrangement phylogeny for cancer genomes. *Genome Res.*, **22**, 346–361.

Helleday,T. *et al.* (2014) Mechanisms underlying mutational signatures in human cancers. *Nat. Rev. Genet.*, **15**, 585–598.

Letouzé,E. *et al.* (2017) Mutational signatures reveal the dynamic interplay of risk factors and cellular processes during liver tumorigenesis. *Nat. Commun.*, **8**, 1315.

Nik-Zainal,S. *et al.* (2012) The life history of 21 breast cancers. *Cell*, **149**, 994–1007.

Nik-Zainal,S. *et al.* (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*, **534**, 47–54.

Polak,P. *et al.* (2015) Cell-of-origin chromatin organization shapes the mutational landscape of cancer. *Nature*, **518**, 360–364.

Rubanova,Y. *et al.* (2018) TrackSig: reconstructing evolutionary trajectories of mutation signature exposure. *bioRxiv*, 260471.

Sabarinathan,R. *et al.* (2016) Nucleotide excision repair is impaired by binding of transcription factors to DNA. *Nature*, **532**, 264–267.