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#### AUTHOR CONTRIBUTIONS

S.B. and J.P. designed this project. J.P. and L.C. constructed the web version of the Digenome-seq program. D.K., G.-H.H., S.K. and S.-T.K. gave critical comments on the web panel. J.-S.K. and S.B. supervised the research.

#### COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the [online version of the paper](#).

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## E-scape: interactive visualization of single-cell phylogenetics and cancer evolution

**To the Editor:** Populations of cancer cells emerge through branched evolutionary processes characterized by variable cell fitness and differential growth. Cell population dynamics fundamentally impact biological properties and clinical endpoints, motivating key questions asked by cancer-focused biomedical investigators. Recently, it has become possible to analyze how mutations are distributed across time<sup>1,2</sup> and space<sup>3,4</sup> in cell populations, with implications for our understanding of treatment resistance, cancer progression<sup>5</sup> and metastasis<sup>4</sup>.

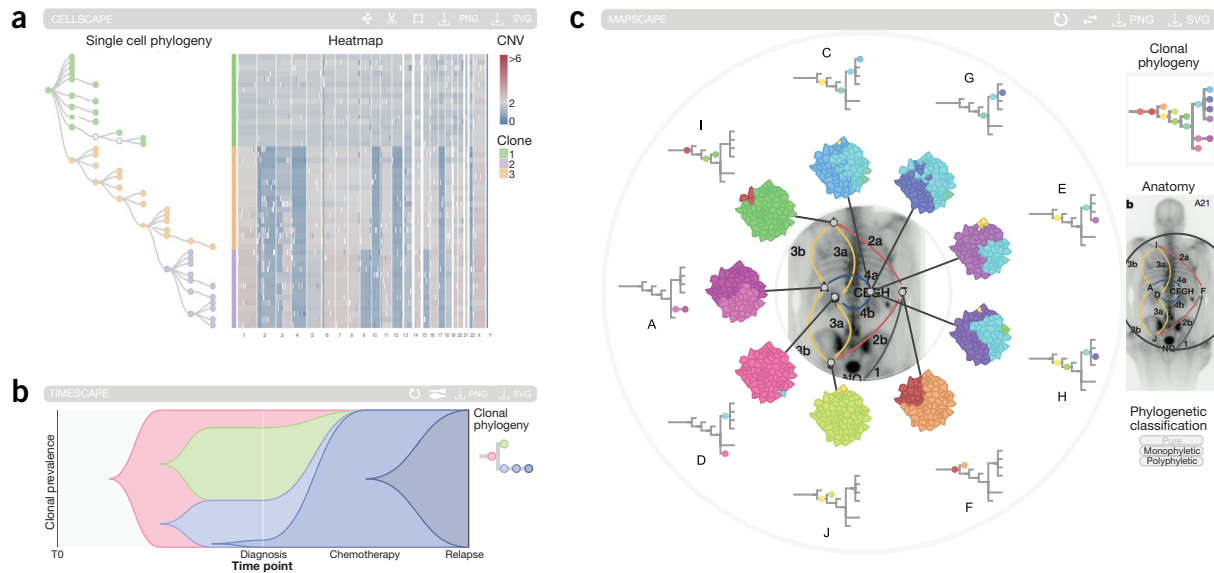
Interactive visualization of clonal dynamics may greatly facilitate clinical and biological interpretation. We have developed E-scape (evolutionary landscapes) as an open-source, browser-based visualization suite (**Supplementary Software**) to render complex relationships between cancer evolution data in an intuitive, interactive framework for biomedical investigators (**Fig. 1**). E-scape consists of three visualization tools deployed as R html widgets (**Supplementary Fig. 1** and **Supplementary Methods**): (i) TimeScope for time series analysis (<https://bioconductor.org/packages/timescape/>); (ii) MapScope for spatial distribution analysis (<https://bioconductor.org/packages/mapscope/>); and (iii) CellScope for single-cell analysis, including analysis of the phylogenetic and population dynamics of cancer (<https://bioconductor.org/packages/cellscape/>). We illustrate E-scape using both single-cell and bulk whole-genome sequencing data from diverse studies of clonal evolution in cancer<sup>1–6</sup> (**Supplementary Tables 1–7**). Links to interactive versions of all figures are available in **Supplementary Note 1**.

CellScope facilitates the interactive browsing of single-cell clonal evolution data sets (**Fig. 1a**). The tool requires two main inputs (**Supplementary Fig. 1** and **Supplementary Table 8**): (i) single-cell mutation content in the form of either copy number segments or targeted point mutations and (ii) a single-cell phylogeny with optional branch lengths. The output of CellScope is an interactive interface displaying two complementary and dynamically linked views: a single-cell phylogeny that expresses the evolutionary progression of clonal lineages and a heatmap representing the mutation status in each cell for each genomic locus. The CellScope toolbar is equipped with several features that enable toggling between tree layouts, incorporating optional edge distances, and selecting genomic profiles of interest to highlight corresponding cells in the phylogeny (**Supplementary Methods**).

TimeScope is a tool for navigating clonal dynamics over time (**Fig. 1b**). TimeScope requires two inputs (**Supplementary Fig. 1** and **Supplementary Table 9**): (i) the clonal phylogeny and (ii) clonal prevalences. It can also accept a table of targeted mutations observed in each clone and their allele prevalences over time. The resulting view displays clonal prevalence vertically and time horizontally, and mouse-over of a clone (or branch) in the phylogeny highlights the clone in the temporal view and vice versa. The mutation table is linked through click events (**Supplementary Fig. 2**), and additional interactive features are available in the toolbar (**Supplementary Methods**). TimeScope enables automatic generation of temporal views for large numbers of samples, and it reveals patterns of clonal expansions<sup>5</sup> (**Supplementary Fig. 3**). CellScope can optionally integrate a TimeScope view in cases where single cells can be assigned to timepoints (e.g., **Supplementary Fig. 4**); this enables interactive exploration of clonal evolution at single-cell resolution.

MapScope integrates clonal prevalence, clonal hierarchy, anatomic, and mutational information to provide interactive visualization of spatial mappings of clones (**Fig. 1c**). MapScope inputs (**Supplementary Fig. 1** and **Supplementary Table 10**) (i) a clonal phylogeny, (ii) clonal prevalences, (iii) an image reference (which may be a medical image or drawing; see **Supplementary Methods**), and (iv) pixel locations for each sample on the referenced image. MapScope can also accept a table of mutations for each clone and their variant allele frequencies in each sample. For each sample, the resulting view contains a cropped anatomical image surrounded by representations of clonal prevalences and a phylogeny of clones present in the sample. MapScope provides two visual encodings of clonal prevalences (**Supplementary Fig. 5**), a cellular aggregate depiction inspired by Eirew *et al.*<sup>2</sup>, where colored areas are proportional to clonal prevalence, and a donut chart of prevalences. The layout of clones within the aggregate (**Supplementary Methods**) is schematic; it does not represent *in situ* collocation of clones. The prevalence and tree representations are interactively linked (**Supplementary Fig. 6** and **Supplementary Methods**). The use of MapScope alongside TimeScope enables exploration of spatially distinct samples over multiple timepoints; for example, **Supplementary Figure 7** reveals how primary clones in a right uterus tumor likely led to a brain relapse and a subsequent right pelvic mass in the peritoneal cavity<sup>4</sup>.

Collectively, the E-scape suite helps researchers engage effectively with cancer evolution data sets by combining all components needed to study the dynamics of disease progression. We anticipate the suite will facilitate improved biological



**Figure 1** | The E-scape tool suite. **(a)** A CellScape visualization of triple-negative breast cancer single-cell data from Wang *et al.*<sup>6</sup>. The heatmap depicts genome-wide copy number variations (CNVs) (columns) across single cells (rows). Copy number gains and losses are encoded by red and blue color gradients, respectively. The tree illustrates the evolutionary relationships between cells. Colored annotations indicate clonal membership of cells. **(b)** A TimeScape visualization of acute myeloid leukemia patient 933124 data from Ding *et al.*<sup>1</sup>. Clonal prevalences (vertical axis) are plotted across timepoints (gray lines, horizontal axis) for each clone (colors), and the evolutionary relationships between clones are captured by the phylogenetic tree and TimeScape layout. **(c)** A MapScape visualization of metastatic prostate cancer data from Gundem *et al.*<sup>3</sup>. Spatially distinct tumor samples are indicated on an anatomical image. Colors represent distinct clones, phylogenetic trees depict evolutionary relationships between clones, and clonal prevalences per site are proportional to the corresponding colored area of the cellular aggregate representation. Insets (right) depict global clonal phylogeny over all samples, anatomical context and phylogenetic classification filters.

understanding of clonal evolution in cancer towards translation into the clinical domain.

**Data availability statement.** All data presented were previously published and are provided in **Supplementary Methods** and **Supplementary Tables 1–7**.

*Note: Any Supplementary Information and Source Data files are available in the online version of the paper.*

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