

Spatial genomics enables multi-modal study of clonal heterogeneity in tissues

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Tongtong Zhao^{1,2,7}, Zachary D. Chiang^{1,2,3,7}, Julia W. Morriss^{1,2}, Lindsay M. LaFave^{2,4,5}, Evan M. Murray^{1,2}, Isabella Del Priore^{4,5}, Kevin Meli^{4,5}, Caleb A. Lareau^{1,2}, Naeem M. Nadaf¹, Jilong Li¹, Andrew S. Earl^{1,2,3}, Evan Z. Macosko^{1,6}, Tyler Jacks^{1,4,5}, Jason D. Buenrostro^{1,2,3,8} & Fei Chen^{1,2,3,8}✉

The state and behaviour of a cell can be influenced by both genetic and environmental factors. In particular, tumour progression is determined by underlying genetic aberrations^{1–4} as well as the makeup of the tumour microenvironment^{5,6}. Quantifying the contributions of these factors requires new technologies that can accurately measure the spatial location of genomic sequence together with phenotypic readouts. Here we developed slide-DNA-seq, a method for capturing spatially resolved DNA sequences from intact tissue sections. We demonstrate that this method accurately preserves local tumour architecture and enables the de novo discovery of distinct tumour clones and their copy number alterations. We then apply slide-DNA-seq to a mouse model of metastasis and a primary human cancer, revealing that clonal populations are confined to distinct spatial regions. Moreover, through integration with spatial transcriptomics, we uncover distinct sets of genes that are associated with clone-specific genetic aberrations, the local tumour microenvironment, or both. Together, this multi-modal spatial genomics approach provides a versatile platform for quantifying how cell-intrinsic and cell-extrinsic factors contribute to gene expression, protein abundance and other cellular phenotypes.

Tissue function requires precise spatial organization of cell types, whose states are influenced by cell-intrinsic genetic factors and extrinsic environmental cues. In cancer, clonal populations of tumour cells evolve a diverse repertoire of DNA mutations, copy number alterations (CNAs), and large chromosomal rearrangements^{1,2}, resulting in intra-tumour genetic heterogeneity, which is associated with an increased risk of drug resistance, metastasis and relapse^{3,4}. Concomitantly, surrounding normal cells that make up the tumour microenvironment communicate to form spatial neighbourhoods with distinct biochemical and biomechanical properties^{5,6} that influence cell migration and invasion^{7,8}, as well as drug permeability⁹. Decoupling and quantifying these genetic aberrations and environmental cues within a tumour is critical to understanding cancer progression and improving treatments.

Current methods for delineating intratumour genetic heterogeneity include deep sequencing to quantify mutant allele frequencies^{10,11} and single-cell whole-genome sequencing^{12–14}. These methods leverage genetic alterations that occur during the evolution of the tumour to reconstruct phylogenetic cell lineages^{10–14}, but do not measure spatial organization. By contrast, multi-region sequencing methods^{15–17} such as laser-capture microdissection (LCM) preserve spatial context, but are mostly limited to clearly observable late-stage cancers and require manual selection of cells, constraining throughput and

de novo discovery. The recently developed in situ genome sequencing¹⁸ enables untargeted spatial measurements of DNA, but focuses on high-resolution imaging of chromosome structure, precluding analysis of tissue sections. It therefore remains poorly understood how tumour clones are organized within a tissue, and to what extent cancer progression is driven by clone-specific genetic aberrations or environmental cues, highlighting a need for new methods that can integrate genomic, transcriptomic and spatial measurements at scale.

Spatially resolved DNA sequencing

Slide-seq^{19,20} (hereafter referred to as slide-RNA-seq) is a scalable technology that uses barcoded bead arrays to capture spatially resolved genome-wide expression. Here we developed slide-DNA-seq, a method that enables spatially resolved DNA sequencing from intact tissues.

We first generate a spatially indexed array of 3-mm beads, as developed for slide-RNA-seq^{19,20}. Each 10- μ m polystyrene bead contains a unique DNA barcode that corresponds to a spatial location and is read out using sequencing by ligation chemistry^{19,20}. We then cryosection tissues and transfer a single 10- μ m-thick fresh-frozen section onto the sequenced bead array (Fig. 1a). To enable unbiased capture of DNA, the tissue section is treated with HCl to remove histones and transposed with Tn5 to create genomic fragments flanked by custom adapter

¹Broad Institute of MIT and Harvard, Cambridge, MA, USA. ²Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA. ³Gene Regulation Observatory, Broad Institute of MIT and Harvard, Cambridge, MA, USA. ⁴Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁵David H. Koch Institute, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁶Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA. ⁷These authors contributed equally: Tongtong Zhao, Zachary D. Chiang.

⁸These authors jointly supervised this work: Jason D. Buenrostro, Fei Chen. ✉e-mail: jason_buenrostro@harvard.edu; chenf@broadinstitute.org

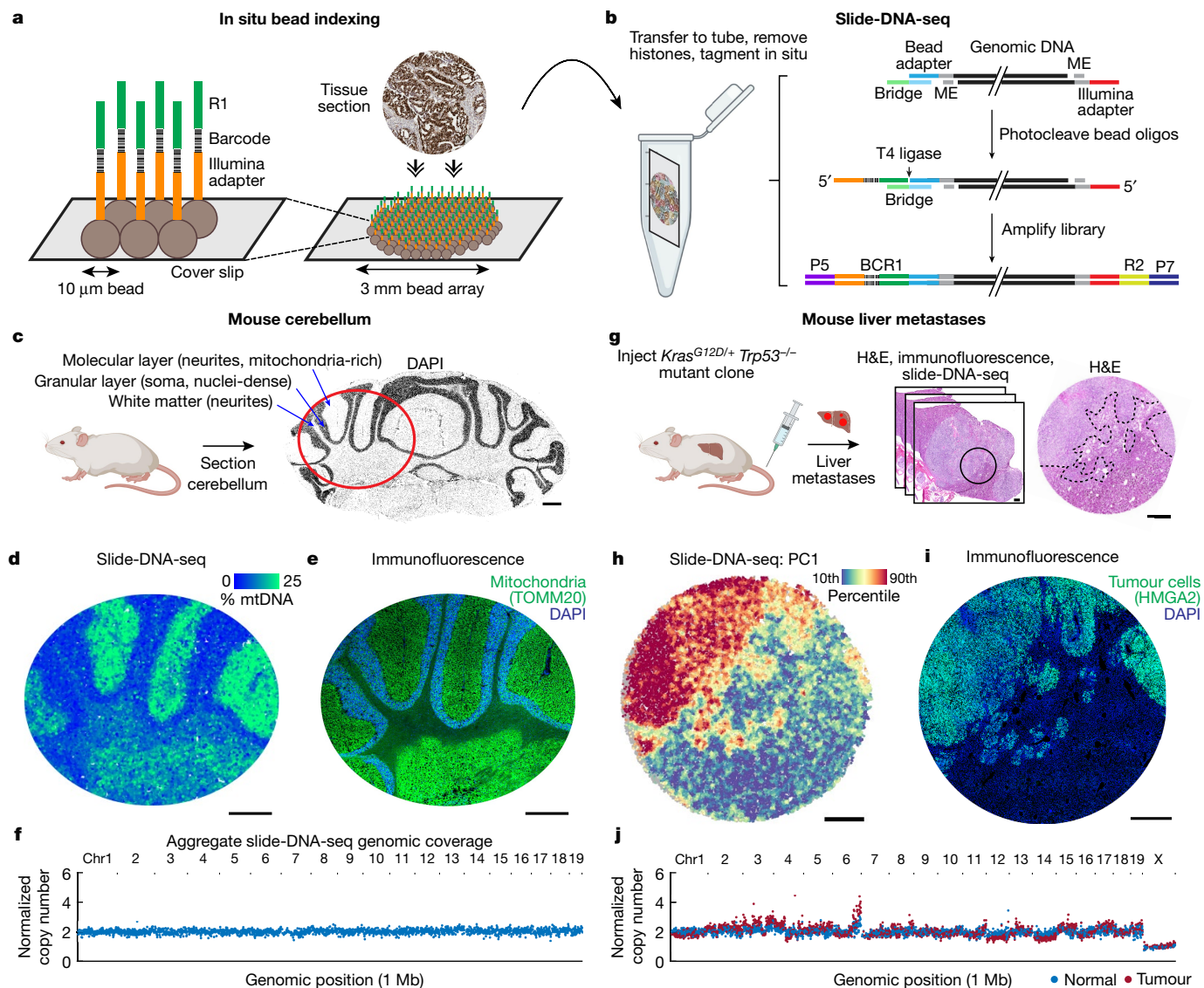


Fig. 1 | Slide-DNA-seq enables spatially resolved DNA sequencing.

a, Schematic of in situ bead indexing. An array of randomly deposited beads is spatially indexed by in situ sequencing of DNA barcodes. Fresh-frozen tissue is cryosectioned onto the array. **b**, Schematic of slide-DNA-seq library construction. Genomic DNA is transposed with Tn5. Hybridization of a bridge oligonucleotide enables ligation of photocleaved, spatially indexed bead oligonucleotides to genomic fragments. BC, barcode; ME, mosaic ends; P5/P7, Illumina adaptor; R1, Illumina read 1; R2, Illumina read 2. **c**, A DAPI-stained cryosection of a mouse cerebellum. The red circle indicates the approximate region shown in **d**, **e**. **d**, Slide-DNA-seq of a cerebellar section with beads coloured by percentage of fragments aligned to mitochondrial genome. mtDNA, mitochondrial DNA. **e**, Adjacent section to the one in **d**, stained with

DAPI and antibody against the mitochondrial protein TOMM20. **f**, Normalized copy number per 1-Mb genomic bin for aggregated beads from **d**, **g**. Serial sections from *Kras^{G12D/+}; Trp53^{-/-}* liver metastases were processed for H&E staining. Centre, circle indicates the region in **h**, **i**. Right, dotted lines indicate tumour boundary. **h**, Slide-DNA-seq of mouse liver section with beads coloured by principal component 1 scores (PC1). For visualization, scores for each bead are smoothed by 50 PC neighbours and 10 spatial neighbours (36 μm diameter). **i**, Adjacent section to the one in **h**, stained with DAPI and antibody against the tumour marker HMGA2. **j**, Normalized copy number per 1-Mb genomic bin for aggregated normal and tumour beads from the liver section in **h**. Scale bars, 500 μm. Grey beads are shown for spatial context but are excluded from the analysis.

sequences^{21,22} (Supplementary Table 1). We then photocleave spatial barcodes from the beads, ligate them to proximal genomic fragments, and PCR amplify the resulting DNA sequencing library (Fig. 1b). Following library construction, we perform high-throughput paired-end sequencing and use DNA barcodes to associate each genomic fragment with a spatial location on the bead array. These associations enable us to reconstruct the spatial organization of DNA in a tissue without imaging the sample under a microscope. We developed optimizations for tissue fixation, histone removal and bridge oligonucleotide hybridization that collectively maximize library size, make chromatin uniformly accessible to Tn5 (Extended Data Fig. 1), and preserve tissue architecture (Extended Data Fig. 2). Following our initial optimizations, each

array contains 20,000 to 40,000 beads with a median 165 to 421 fragments per bead (tumour tissues; Extended Data Fig. 3). Furthermore, we developed a proof-of-concept protocol variant that uses repeated Tn5 tagmentation to improve yield, resulting in a tenfold increase in genomic fragments (Extended Data Fig. 3, Supplementary Methods). Detailed metrics for all tissues analysed in this study are listed in Supplementary Table 2.

To determine the spatial and genomic resolution of this approach, we first applied slide-DNA-seq to the mouse cerebellum, which contains distinct nuclei-dense (soma) and mitochondria-rich (neurites) regions (Fig. 1c). We reasoned that these patterns should be reflected in the spatial distribution of nuclear versus mitochondrial DNA fragments.

Indeed, striations of nuclear versus mitochondrial DNA content were apparent from slide-DNA-seq data (Fig. 1d, Extended Data Fig. 3). We then used these patterns to measure our spatial resolution by performing immunofluorescence and DAPI staining on serial tissue sections of the same cerebellum, resulting in a lateral diffusion estimate of approximately 25 μm (Fig. 1e, Extended Data Fig. 4, Supplementary Methods). To measure genomic resolution, we corrected the data for sequence biases and normalized coverage using bulk sequencing of the same tissue (Extended Data Fig. 5, Supplementary Methods, Supplementary Discussion). Using this approach, 99.78% of non-overlapping 1-Mb genomic bins had a normalized copy number between 1.5 and 2.5 (Fig. 1f, Extended Data Fig. 6). Together, these data show that slide-DNA-seq can spatially localize genomic information within normal tissues.

Detecting spatial distribution of CNAs

We next applied slide-DNA-seq to measure the spatial distribution of copy number alterations (CNAs) in a tumour section using genetically engineered mouse models of lung adenocarcinoma that are known to harbour chromosomal amplifications and deletions²³. First, we isolated and expanded a single tumour clone from a *Kras*^{G12D/+}/*Trp53*^{-/-} (KP) mouse lung tumour^{24,25} and injected this clone into the tail vein of a mouse, giving rise to large metastases in the liver (Fig. 1g). We then collected multiple serial sections of liver metastases to perform slide-DNA-seq, along with haematoxylin and eosin (H&E) staining and immunofluorescence for HMGA2, a late-stage tumour marker. To characterize tumour heterogeneity within the tissue, we developed a slide-DNA-seq analysis workflow comprising two main tasks: (1) de novo identification and spatial localization of clonal populations, and (2) characterization of genomic CNAs for each clone.

First, to detect and localize tumour clones within a tissue, we smoothed bead data on the basis of spatial proximity ($k = 50$ nearest beads, approximately 110 μm diameter; Extended Data Fig. 3; median $18,587 \pm 5,300$ fragments) and performed principal component analysis (PCA) to find co-associated genomic regions with variable coverage across the tissue. We then used these regions to assign a clonal identity to each bead on the slide-DNA-seq array by k -means clustering (Extended Data Fig. 7, Supplementary Methods). When we applied this approach to the slide-DNA-seq array from the liver metastases, principal component 1 (PC1, 2.89% variance explained) showed spatial patterning (Fig. 1h) that was visually concordant with immunofluorescence on a serial section against the late stage tumour marker HMGA2^{26–28} (Fig. 1i). To validate whether this approach could be used to identify genetically distinct tumour clones, we performed downsampling on bulk sequencing of 4 tumour cell lines and found robust accuracy (99.38%) with as few as 1,000 fragments per sample (Extended Data Fig. 8, Supplementary Methods), suggesting that this strategy is sufficient for slide-DNA-seq data.

The second task in the analysis workflow is to characterize the CNAs present in each tumour clone. To do this, we aggregated data from hundreds to thousands of raw beads on the basis of the cluster assignments from the first task and visualized the genomic coverage of each cluster at 1-Mb resolution. When applied to the liver metastases array, the tumour-associated cluster displayed significant CNAs, including the amplification of chromosome (chr)6 that is characteristic for *Kras*-induced lung tumours²³, whereas the normal cluster showed comparatively uniform coverage (Fig. 1j). Further comparisons to a biological replicate performed on a serial section revealed visually concordant tissue architecture, as well as high correlation between tumour copy number profiles (Pearson's $r = 0.986$; Extended Data Fig. 9). To quantify the accuracy of the copy number analysis, we used the diploid mouse cerebellum data to systematically evaluate coverage at a range of bin sizes and spatial resolutions (Extended Data Fig. 10, Supplementary Methods). Together, these results demonstrate that our slide-DNA-seq analysis workflow enables de novo discovery and

localization of tumour regions at approximately 1-Mb genomic resolution (Supplementary Discussion).

Spatial genomics of metastatic clones

To demonstrate that our experimental and computational approach can distinguish between clones within a tissue, we injected multiple clones originating from two independently derived metastatic KP tumours into the tail vein of a mouse, which gave rise to large metastases in the liver. We then performed H&E staining and identified a region of the tissue that appeared to have two spatially distinct metastases (Fig. 2a). Immunohistochemistry on the same region of a serial section revealed that the two varied in protein levels of tumour marker HMGA2²⁶, suggesting that they may originate from different metastatic clones (Extended Data Fig. 11a).

We then applied slide-DNA-seq to a third serial section of the same liver tissue. Using the PCA approach described above, we found that both PC1 and PC2 explained substantial variance (4.21% and 2.50%, respectively), allowing the beads to be assigned to 3 distinct clusters on the basis of their genomic profiles (Fig. 2b). One of these clusters was visually concordant with H&E staining of normal tissue, whereas the other two appeared to correspond to the different metastases. We developed a permutation test to spatially localize statistically significant CNA gains or losses present in one or both of the metastases, and detected differential regions on chr6, chr15 and chr19 (Fig. 2c, Supplementary Methods). We then tested the aggregate genomic coverage in selected regions for statistical significance (two-sided Wilcoxon rank sum test; P -values in Fig. 2d), providing further evidence that they were seeded by different clones. Additionally, we observed that one clone was probably triploid, which we confirmed independently using flow cytometry (Extended Data Fig. 12).

To test whether genetic differences between the two clones were reflected in cell state, we performed slide-RNA-seqV2²⁰ on a fourth serial section and collected paired single-nucleus RNA sequencing (snRNA-seq). Unsupervised clustering of the snRNA-seq data and spatial projection²⁹ onto slide-RNA-seq beads (Methods, Supplementary Table 3) revealed that the two metastases were transcriptionally distinct (Fig. 2e, Extended Data Fig. 11b, c); there were 3,732 genes differentially expressed between the two clones (Fig. 2f, Supplementary Table 4; two-sided z -test, false discovery rate < 0.01 , $\log_2(\text{fold change}) > 1$, minimum of 100 transcripts). Clone A had higher expression of late-stage tumour markers, including *Hmga2* (lung metastases), *Tm4sf1* (JAK-STAT signalling) and *Vim* (cell motility), whereas the top hits for clone B included *Aqp5* (loss of lineage identity) and epithelial-to-mesenchymal transition markers *SIOOa4* and *Vcan*²⁵ (Fig. 2f, g). Although both clones exhibited epithelial-to-mesenchymal transition and metastasis expression signatures, these differentially expressed genes may reflect divergent paths of tumour evolution. Furthermore, we found differential monocyte localization ($P = 0.0002$; permutation test) into clone B, reflecting a higher degree of immune infiltration (Extended Data Fig. 11e, f, Supplementary Methods). Altogether, these data demonstrate that paired slide-DNA-seq and slide-RNA-seq enable spatial characterization of genetically distinct metastatic tumour clones and their associated cell states.

Subclone detection in human colon cancer

We then sought to determine whether slide-DNA-seq could detect clonal heterogeneity de novo in a primary human tumour. We selected a sample from a stage IIIB colorectal tumour, because colorectal cancer is one of the most common causes of cancer-related deaths worldwide and 84% of such tumours display chromosome instability^{30,31}. As before, we performed H&E staining, multiplexed immunohistochemistry and slide-DNA-seq on serial sections (Fig. 3a). First, we examined H&E staining, observing many localized aggregates of tumour cells about

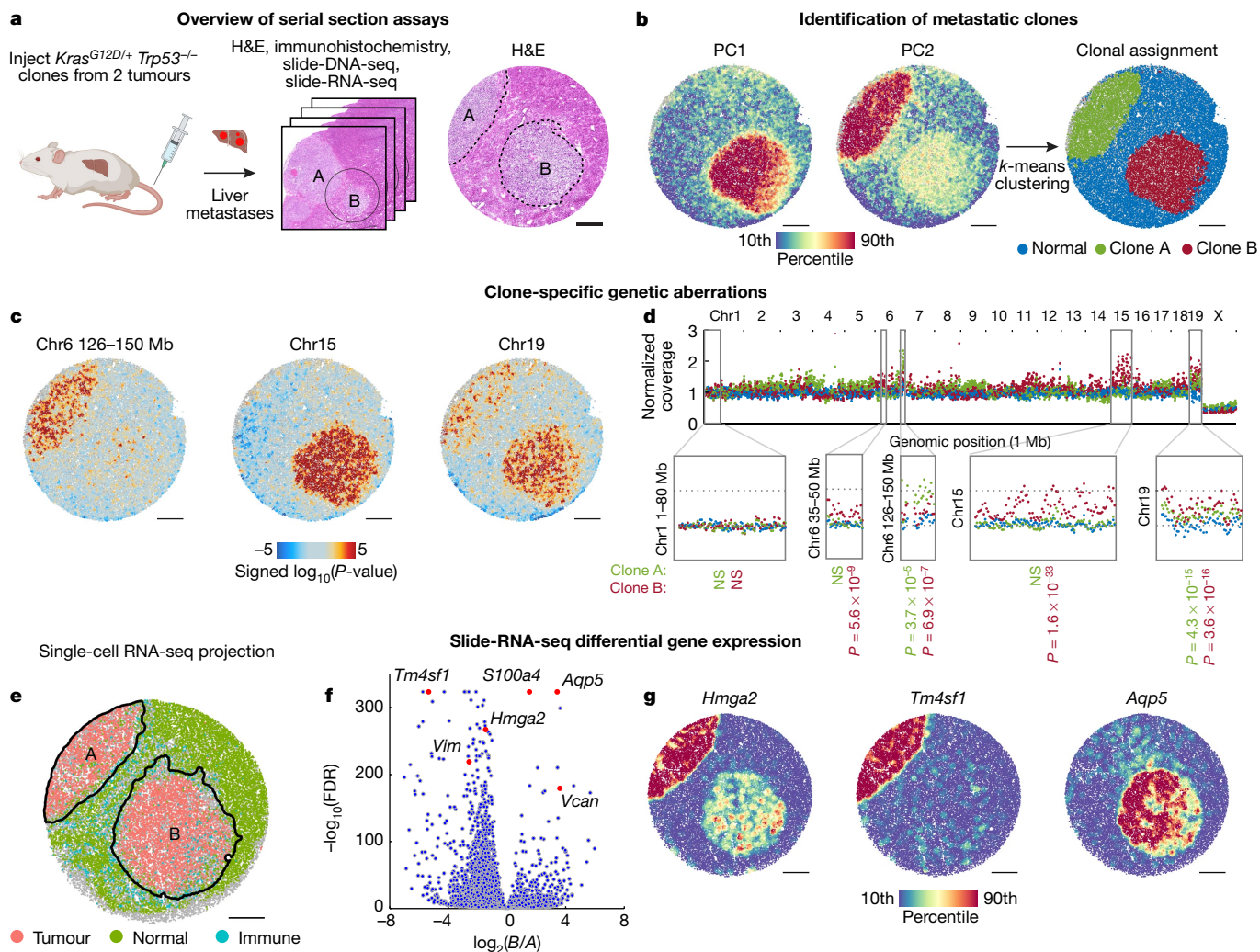


Fig. 2 | Paired slide-DNA-seq and slide-RNA-seq characterize the genetics and transcriptomes of distinct metastatic clones. a–g, Serial sections from *Kras^{G12D/+}Trp53^{-/-}* liver metastases were processed for H&E staining (centre, circle indicates analysed region; right, dotted lines indicate tumour boundaries), immunohistochemistry (Extended Data Fig. 5), slide-DNA-seq (**b–d**) and slide-RNA-seq (**e–g**). **b**, PC1 (left) and PC2 (middle) of slide-DNA-seq genomic coverage. Beads clustered using *k*-means (*k* = 3) and annotated as normal, clone A or clone B (right). **c**, Genomic region enrichment signed *P*-values for chromosomes 6 (126–150 Mb), 15 and 19 (two-sided permutation test, not adjusted for multiple comparisons). Amplifications, red; deletions, blue. **d**, Genomic coverage profiles of aggregate normal (blue), clone A (green),

and clone B (red) beads from **b**. Genomic coverage normalized to 1 to compare profiles of different ploidy (Extended Data Fig. 12). *P*-values calculated using two-sided Wilcoxon rank sum test to compare clone and normal coverage. NS, not significant. **e**, Slide-RNA-seq of mouse liver serial section coloured by tumour, normal and immune cell classes, as assigned from single-cell projection. Detailed cell-type labels are shown in Extended Data Fig. 13. **f**, Differentially expressed genes between clones A and B. Genes shown in **g** or referred to in text are labelled and indicated by red dots. **g**, Normalized expression of selected genes from **f**. Scale bars, 500 μ m. Grey beads are shown for spatial context but are excluded from the analysis.

100–500 μ m in diameter. We hypothesized that each of these aggregates could arise from a single clonal lineage, suggesting constraints on migration or, alternatively, that each aggregate could contain a mixture of cells from different lineages, indicating cell intermixing.

To distinguish between these two possibilities, we performed PCA and unsupervised clustering on the slide-DNA-seq data as described above, which resulted in three distinct clusters of genomic profiles (Fig. 3b). One of these clusters had a spatial distribution that was visually concordant with normal tissue in the H&E staining (Fig. 3b right, blue), but also included regions of moderate PC1 scores, suggesting a low abundance of cancer cells harbouring CNAs. By contrast, the other two clusters displayed high PC1 scores and were spatially restricted to distinct tumour aggregates, supporting the hypothesis that each aggregate originates from a single lineage. This finding is consistent with reports suggesting that individual colorectal tumour cells seed a glandular organization in which neighbouring cells share a recent

common ancestor^{32,33}. We validated the tumour architecture detected by slide-DNA-seq through co-registration of the slide-DNA-seq array, H&E staining and immunohistochemistry for the tumour marker MKI67 and the immune marker CD45 (Fig. 3c).

We then set out to characterize the genetic aberrations of the identified subclones. We found several genetic aberrations, including chr8q amplification and loss of chr15 and chr18, that were shared across all tumour regions (Fig. 3d, e, Supplementary Methods), indicating that they arose early in tumour evolution and may have had an important role in tumour initiation. The chr8q amplification contains genes known to promote tumour progression, including the proto-oncogenes *MYC* and *MYBL1*³⁴, whereas deletion of chr15 results in loss of multiple genes required for genome stability, including *TP53BP1*³⁵, *RAD51*³⁶ and *FAN1*³⁷. Supporting these observations, chr8q gain and chr18 loss were identified as typical early events in an evolutionary history of 60 colorectal tumours¹⁰. In contrast to these shared aberrations, we observed

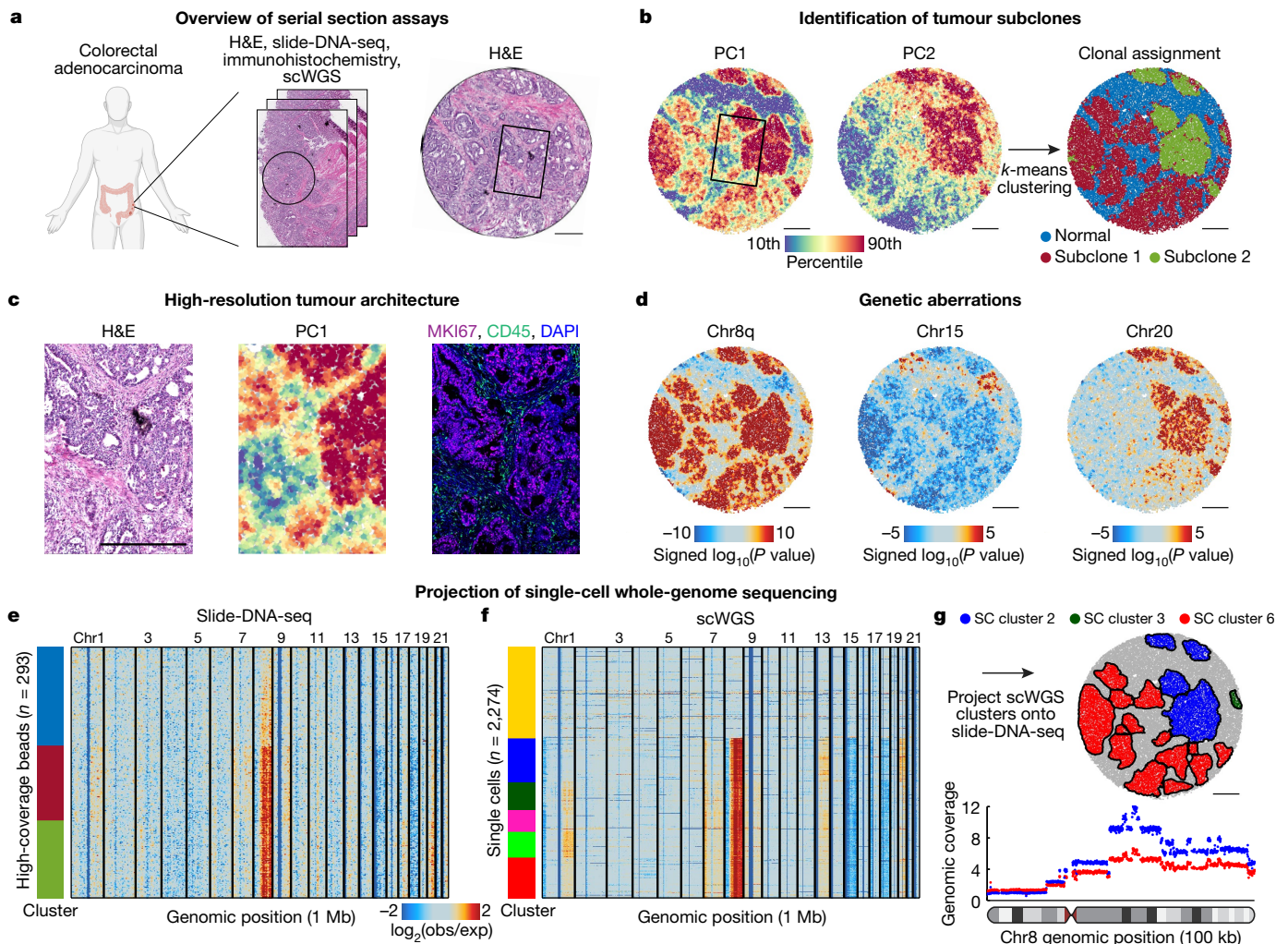


Fig. 3 | De novo identification of spatial tumour clones in primary human colorectal cancer. **a–g**, Serial sections of primary human colorectal tumour were processed for H&E staining (right, **c**), slide-DNA-seq (**b–e**), and multiplexed immunohistochemistry (**c**)—scWGS was performed on the same sample (**f, g**). **b**, PC1 (left) and PC2 (middle) of slide-DNA-seq genomic coverage. Beads clustered using *k*-means (*k* = 3) and annotated as normal, subclone 1 or subclone 2 (right). **c**, Magnified view of boxed regions in **a, b**. Right, serial section stained with antibodies against MKI67, CD45 and DAPI. **d**, Genomic region enrichment signed *P*-values for chromosomes 8q, 15 and 20 (two-sided

permutation test, not adjusted for multiple comparisons). **e**, Copy number profiles for 293 high-coverage slide-DNA-seq beads. **f**, Copy number profiles for 2,274 single cells profiled via scWGS. Profiles within each cluster are ordered by the PC1 score in **e, f, g**. Matched single-cell clusters projected onto slide-DNA-seq array (top). Genomic coverage of chromosome 8 at 100 kb resolution for single-cell clusters 2 and 6 (bottom). Scale bars, 500 μ m. Amplifications, red; deletions, blue (**d–f**). Grey beads are shown for spatial context but are excluded from the analysis.

subclonal amplifications of chr1q, chr7, and chr20, which presumably occurred at a later stage of evolution (Fig. 3d, e). Notably, previous analyses of colorectal cancers classified chr7p amplification as a typically clonal (rather than subclonal) event, whereas both loss and gain of chr20p were identified as frequent subclonal aberrations^{10,38}. The detection and temporal classification of these events demonstrate the utility of slide-DNA-seq for studying the evolution of clonal heterogeneity.

To validate these genetic aberrations, we performed single-cell whole-genome sequencing (scWGS) on the same colorectal tumour. This approach sampled cells from the entirety of the tumour (100-fold more material than the slide-DNA-seq tissue section), so we expected to potentially identify additional subclones. In line with this expectation, analysis of 2,274 high-coverage single-cell CNA profiles resulted in one normal cluster and five tumour clusters, some of which resembled the slide-DNA-seq CNA profiles (Fig. 3f). We then sought to project the high-coverage sequencing onto the slide-DNA-seq array to identify CNAs at enhanced resolution (Supplementary Methods). The spatial regions predominantly matched two separate scWGS clusters,

supporting the analysis using slide-DNA-seq only, but we also found a small region with distinct genetic aberrations that was revealed only with the higher coverage of the scWGS data (Fig. 3g, top, Extended Data Fig. 13). Having demonstrated improved spatial resolution, we then re-analysed the matched scWGS clusters at 100 kb genomic resolution, revealing a complex CNA landscape in chromosome 8 (Fig. 3g, bottom). Together, these analyses validate that slide-DNA-seq alone is sufficient for de novo discovery and localization of distinct tumour clones within a tissue and show that CNA characterization can be enhanced through integration with scWGS.

Multi-modal analysis of clonal heterogeneity

Finally, to demonstrate the unique capabilities of a multi-modal spatial sequencing approach, we sought to quantify how tumour transcriptional programs are controlled by both genetics and environmental cues. We first performed H&E staining, slide-DNA-seq and slide-RNA-seqV2 on serial sections from a nearby region of the

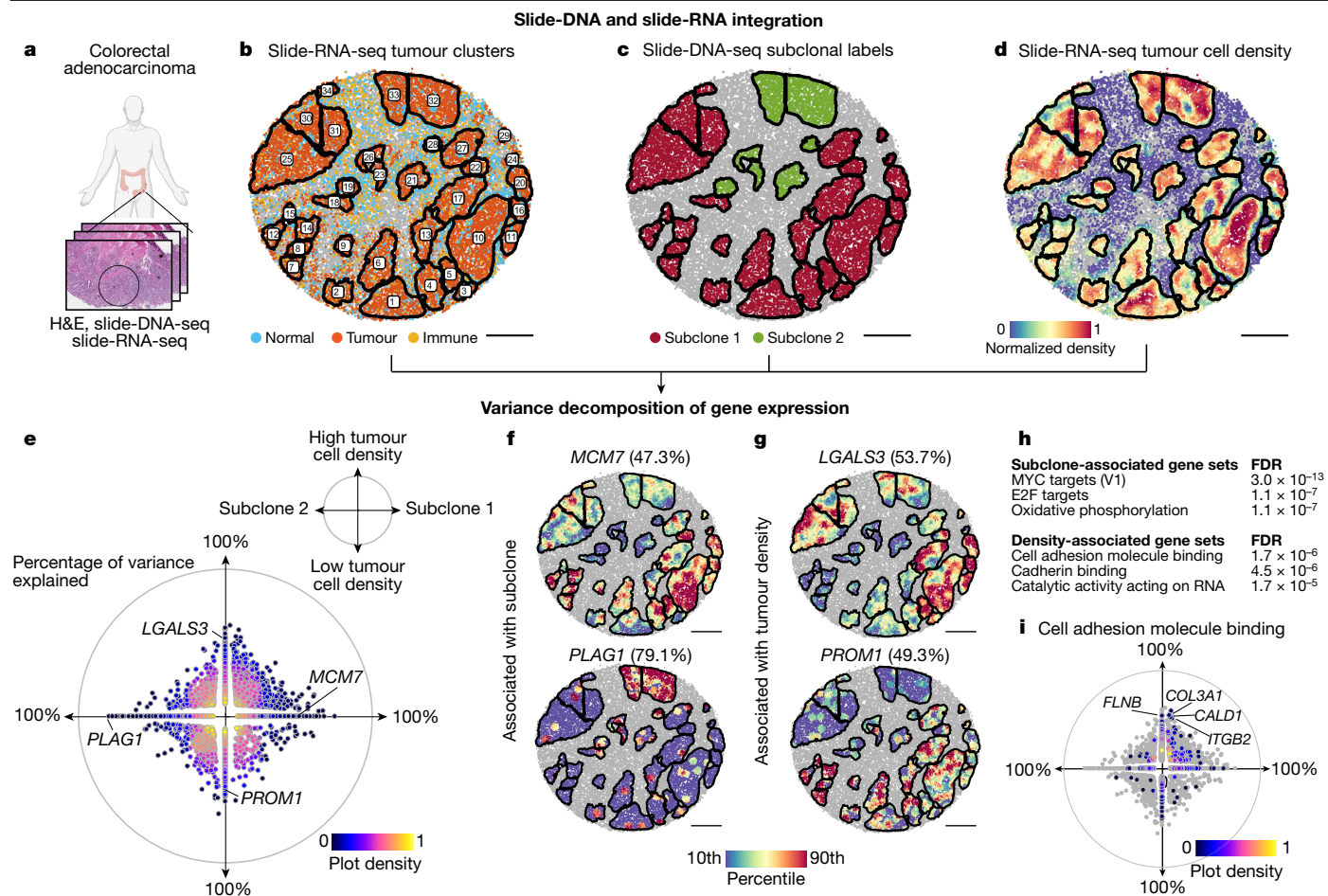


Fig. 4 | Decomposition of transcriptional programs driven by genetic aberrations and tumour density. **a–i**, Serial sections from nearby region of human colorectal tumour from Fig. 3 were processed for H&E staining, slide-DNA-seq (Extended Data Fig. 14) and slide-RNA-seq of a colon tumour section with beads coloured by assignment to normal, tumour or immune clusters. Black lines denote boundaries of spatially distinct tumour regions. **c**, Subclone labels for spatial tumour regions (defined via co-registration with slide-DNA-seq serial section) plotted on slide-RNA-seq array from **b**. **d**, Tumour density plotted on the slide-RNA-seq array from **b**. **e**, Genes plotted by percentage of variance explained by subclone (x-axis)

and/or tumour density (y-axis), coloured by plot density ($n = 2,148$; stepwise regression, $P < 0.05$). **f**, Top subclone-associated genes, with expression plotted for spatial tumour regions. **g**, Same as **f** but for top tumour density-associated genes. **h**, Selected gene sets significantly associated with either subclone or tumour density. **i**, Cell adhesion molecule-binding genes ($n = 544$) plotted by percent variance explained by subclone (x-axis) and tumour density (y-axis), coloured by plot density. All other genes from **e** are shown in grey. Scale bars, 500 μm . Grey beads shown for spatial context but excluded from analysis.

colorectal tumour (Fig. 4a) and co-registered the arrays to integrate pathological, genomic and transcriptomic information. We then identified spatially distinct regions of tumour cells (Fig. 4b, Supplementary Methods) and proceeded to assign each one with a subclonal identity (Fig. 4c) and quantify the local tumour density (Fig. 4d, Supplementary Methods). Comparison with the H&E staining validated the spatial architecture of the subclones identified by slide-DNA-seq as well as the tumour density quantified by slide-RNA-seq (Extended Data Fig. 14).

Given both subclonal identity (cell-intrinsic) and tumour density (cell-extrinsic) measurements, we set out to deconvolve how these factors contribute to the transcriptional programs of the colorectal tumour. To this end, we used a variance-decomposition approach that, for each gene, calculates the percentage of gene expression variance explained by subclonal identity, tumour density and unexplained variance (Supplementary Methods). Of the 25,074 genes detected by slide-RNA-seq 412 genes were significantly associated with subclonal identity, 638 genes were associated with tumour density, and 1,098 genes were associated with a combination of both ($P < 0.05$, variance explained $> 30\%$, Fig. 4e, Supplementary Table 5). Genes associated with subclonal identity included known cancer genes located in amplified

regions, such as *PLAG1*, an oncogene on chr8q³⁹, and *MCM7*, a MYC target gene on chr7q that is involved in DNA replication initiation⁴⁰ (Fig. 4f). Notable tumour density-associated genes included *LGALS3*⁴¹ (also known as galectin-3), which contributes to immunosuppression in the tumour microenvironment, and *PROM1* (also known as CD133), which is important for intestinal homeostasis, regeneration and tumour initiation⁴² (Fig. 4g).

As well as characterizing individual genes, we also performed gene set enrichment analysis to determine which molecular pathways were associated with subclonal identity or tumour density (Fig. 4h, Supplementary Methods). This analysis showed that subclonal identity primarily altered the expression of genes involved in cell growth and proliferation, with MYC- and E2F-target genes representing the top hallmark gene sets for subclone 1 (Extended Data Fig. 15). By contrast, genes associated with high tumour density were most enriched for cell adhesion molecule- and cadherin-binding properties (Fig. 4i, Extended Data Fig. 15), including the extracellular matrix component gene *COL3A1*, actin modulator genes *FLNB* and *CALD1*, and the mechanotransduction regulator gene *ITGB2* (also known as CD18). Notably, extracellular matrix stiffness and remodelling are thought to promote

cell proliferation and tumour progression⁴³, which may contribute to high tumour cell density. Overall, these analyses demonstrate the utility of this multi-modal approach for decoupling and quantifying contributions of genetic and environmental factors to gene expression.

Discussion

This study demonstrates that slide-DNA-seq can detect clonal heterogeneity, characterize the copy number alterations of each clone and analyse their spatial distribution within a tissue. These capabilities, in combination with processing of serial sections for histopathology and slide-RNA-seq enable high-resolution multi-omic characterization of intratumoural heterogeneity⁴⁴. Additionally, integration with single-cell whole-genome sequencing may enable spatial characterization of complex subclonal events, such as loss of heterozygosity or extrachromosomal DNA amplifications⁴⁵. Going forward, we anticipate that slide-DNA-seq will be particularly useful for large-scale efforts to create atlases of tumour evolution¹⁰, adding spatial information to studies of clonal heterogeneity. It may also drive new frontiers in clinical diagnoses as a complement to standard pathology assays such as H&E staining, karyotyping and DNA fluorescence in situ hybridization.

Spatially resolved DNA sequencing may also enable advances in many fields beyond cancer genomics, including spatially resolved metagenomics⁴⁶, evaluation of gene therapy delivery⁴⁷, synthetic DNA data storage⁴⁸ and lineage tracing in healthy tissues⁴⁹. The core of this technology—that is, fragmenting and barcoding DNA in situ to preserve spatial information for next-generation sequencing—is compatible with other sequencing-based assays. For example, direct tagmentation of the DNA without HCl treatment, or converting methylated cytosines to dihydrouracil before amplification, would enable spatially resolved measurements of chromatin accessibility and DNA methylation, respectively^{22,50}. In summary, slide-DNA-seq enables new opportunities to chart the spatial organization of cell states in human development, homeostasis and disease.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-021-04217-4>.

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Article

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Raw sequencing data are available from the Sequence Read Archive under accession PRJNA768453. Spatial barcode locations and counts matrices are available from the Broad Institute Single Cell Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP1278). GC-content tracks for hg19 and mm10 were downloaded from the UC Santa Cruz Genome Browser. k36 mappability tracks for both genomes were downloaded from <https://bismap.hoffmanlab.org/>. Replication timing data were downloaded from Gene Expression Omnibus accession GSM923451 for hg19 and GSE137764 for mm10. Tn5 insertion bias tracks for both genomes were generated using the bias command from pyatac (<https://nucleoatc.readthedocs.io/en/latest/pyatac/>). Gene sets were downloaded from the Molecular Signatures Database Collections (<http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp>).

Code availability

Code for the in situ bead indexing is available from <https://github.com/broadchenf/SlideSeq>. Code for all analyses is available from https://github.com/buenrostrolab/slide_dna_seq_analysis and archived at <https://doi.org/10.5281/zenodo.5553305>.

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Author contributions T.Z. and J.W.M. developed the protocol and performed experiments. Z.D.C. developed the computational processing pipeline. T.Z., Z.D.C., J.D.B., and F.C. performed analyses. L.M.L., I.D.P. and K.M. assisted with the mouse experiments under the supervision of T.J. E.M.M. performed the in situ bead indexing and slide-RNA-seq experiments under the supervision of E.Z.M. and F.C. J.L. wrote the in situ bead-indexing pipeline. N.M.N. assisted with the 10x experiment. C.A.L. and A.S.E. assisted with the computational processing pipeline. T.Z., Z.D.C., J.D.B. and F.C. wrote the manuscript with input from all authors. E.Z.M., T.J., J.D.B. and F.C. supervised this work.

Competing interests E.Z.M. and F.C. are listed as inventors on a patent application related to slide-seq. T.J. is a member of the board of directors of Amgen and Thermo Fisher Scientific. He is also a co-founder of Dragonfly Therapeutics and T2 Biosystems. T.J. serves on the scientific advisory board of Dragonfly Therapeutics, SQZ Biotech and Skyhawk Therapeutics, and is the president of Break Through Cancer. J.D.B. holds patents related to ATAC-seq and is on the scientific advisory board for Camp4, Seqwell and Celsee. F.C. is a paid consultant for Celsius Therapeutics and Atlas Bio. E.Z.M. is a paid consultant for Atlas Bio. T.Z., E.Z.M., J.D.B. and F.C. have filed a patent application based on this work.

Additional information

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Correspondence and requests for materials should be addressed to Jason D. Buenrostro or Fei Chen.

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