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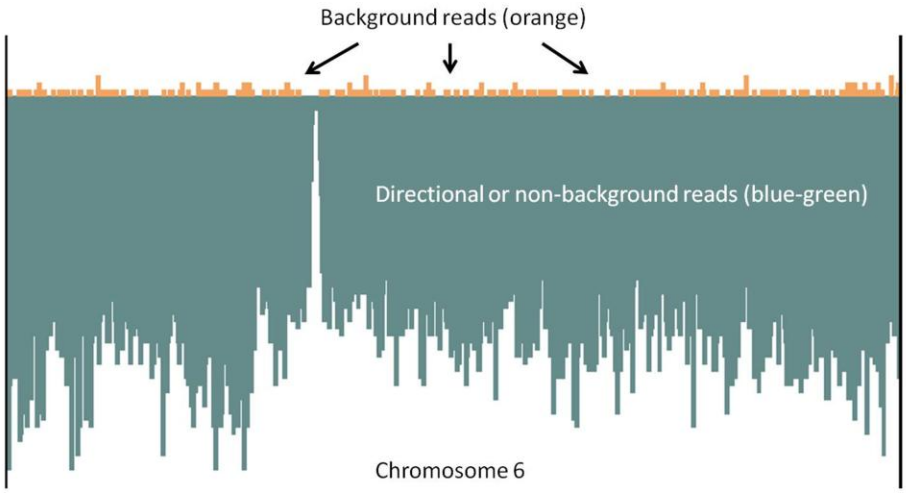
Supplemental information

Construction of Strand-seq libraries in open nanoliter arrays

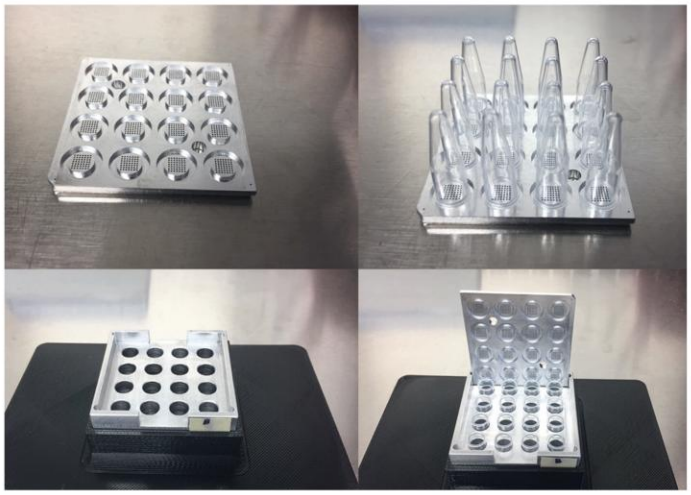
Vincent C.T. Hanlon, Daniel D. Chan, Zeid Hamadeh, Yanni Wang, Carl-Adam Mattsson, Diana C.J. Spierings, Robin J.N. Coope, and Peter M. Lansdorp

Supplemental Figures

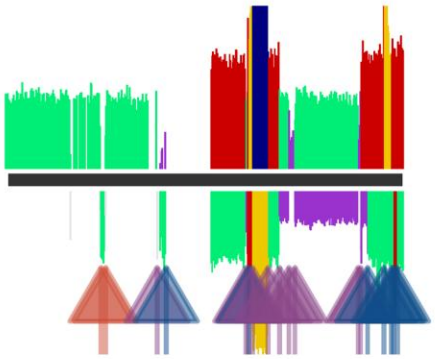
a.



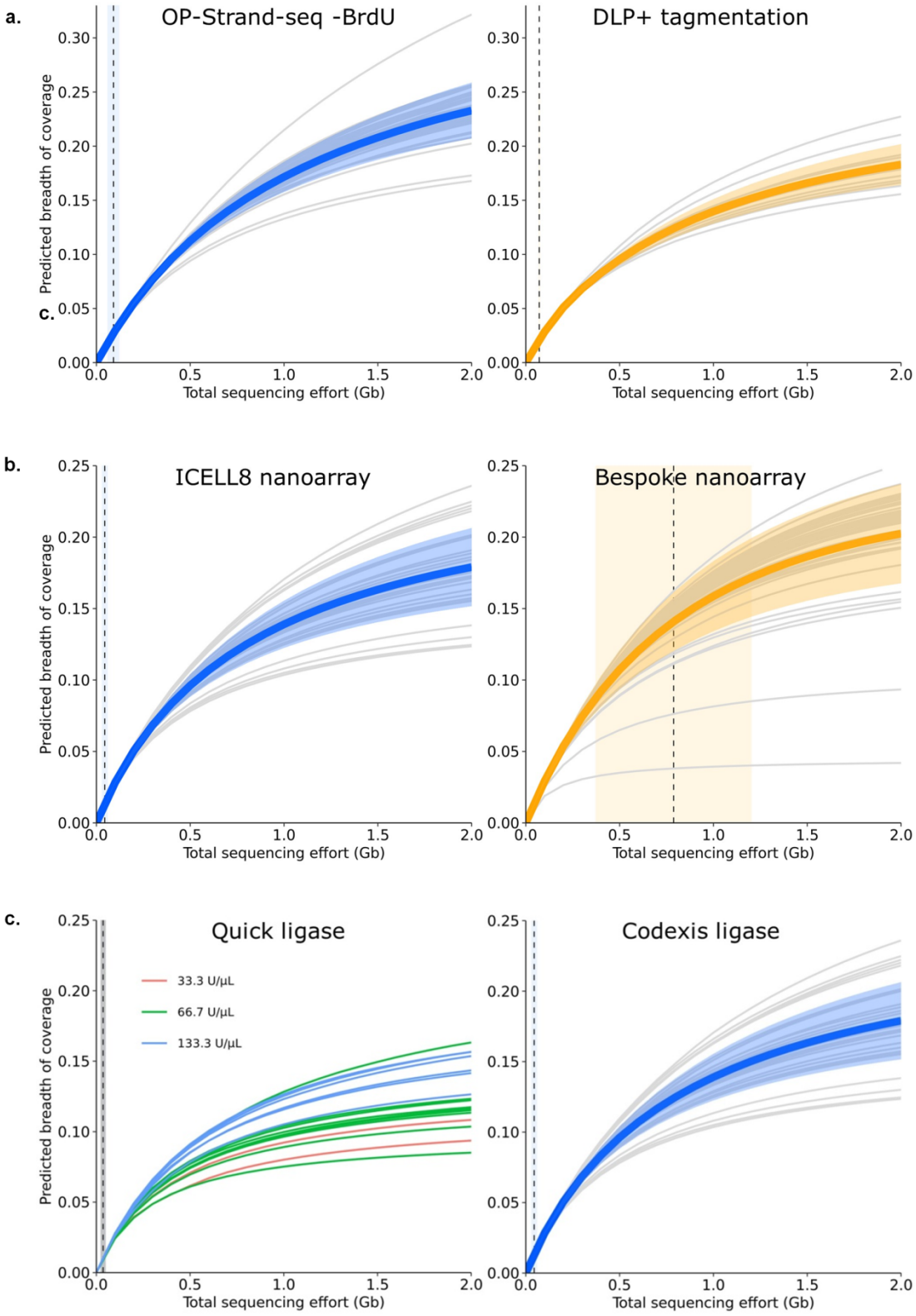
b.



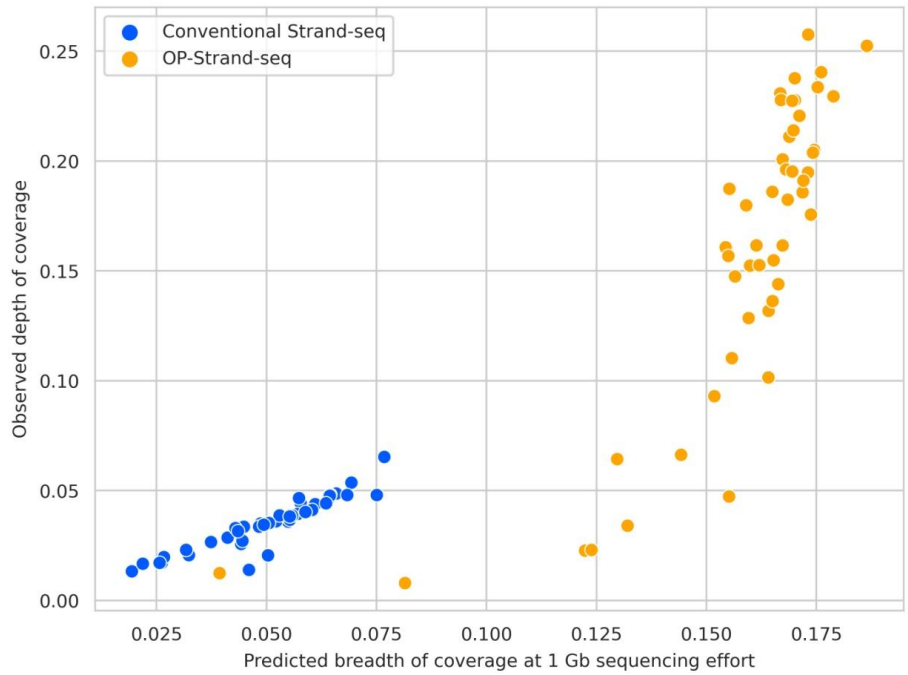
c.



Supplemental Fig. S1. Illustrations of OP-Strand-seq techniques and analysis. Related to the STAR Methods and Figs. 2-3. **a,** Mapped reads colored by orientation for a single Strand-seq library illustrate the term "background", which refers to non-directional reads. Background reads come from library fragments for which either the nascent strand did not incorporate BrdU or the UV treatment did not create nicks. Such fragments result in reads that map to the reference genome in both orientations rather than just one. When two homologous chromosomes yield directional reads with the same orientation (as shown here; called Watson-Watson or Crick-Crick), reads with the opposite orientation are background reads. We quantify background as the fraction of mapped reads that have this opposite orientation relative to the fraction of directional reads. Background reads are also present in Watson-Crick regions, where two homologous chromosomes yield directional reads in opposite orientations, but if that is the case the background reads are invisible in the sense that they cannot be distinguished from directional reads based on their orientation alone. **b,** Bespoke aluminum nanoarrays for construction of OP-Strand-seq libraries. Each of the 16 clusters with up to 49 individual libraries can be harvested by centrifugation into separate tubes (with the lids cut off). The multiple cluster design allows for separation of libraries made from different sample types for analysis and flexibility in sequencing. In addition, the design allows optimization of library construction by looking at library yield rather than sequencing results (required using arrays without clusters). At bottom, a centrifuging adapter for harvesting libraries is shown. **c,** The four consecutive CNV breakpoints on chromosome 16 identified using Aneufinder (Bakker et al., 2016) that we selected for further analysis. Purple indicates monosomy, green is disomy, red is trisomy, yellow is tetrasomy, and navy blue is pentasomy. The arrows at bottom indicate where AneuFinder detected breakpoints. Bins of forward reads are displayed on top of the black line, and bins of reverse reads are displayed below it.


a.
OP-Strand-seq -BrdU
DLP+ tagmentation
b.
ICELL8 nanoarray
Bespoke nanoarray
c.
Quick ligase
33.3 U/µL
66.7 U/µL
133.3 U/µL
Codexis ligase
Predicted breadth of coverage
Total sequencing effort (Gb)

Supplemental Fig. S2. Complexity comparisons of nanoliter-volume, single-cell libraries. Related to the Results and Discussion and the STAR Methods. The sequencing effort per library is shown as vertical dashed lines (means) and bands (standard deviations; too narrow to be visible for the DLP+ libraries). **a,** OP-Strand-seq libraries prepared in an ICELL8 nanoarray (left) and a bespoke nanoarray (right; from the 78 libraries referred to in the Results and Discussion). We used overnight ligation for libraries in the ICELL8 nanoarray, but not for those in the bespoke nanoarray (see STAR Methods). The ICELL8 libraries were sequenced at very low read density. **b,** DLP+ libraries prepared by Laks et al. (library ID A90553A, sample GM18507; Laks et al., 2019) in comparison with OP-Strand-seq (overnight Codexis ligation in an ICELL8 chip). For the OP-Strand-seq libraries, BrdU incorporation, Hoechst 33258 addition and UV treatment, which reduce complexity, were omitted for a fairer comparison with DLP+ (i.e., so that both protocols made scWGS libraries). Note that Preseq (Daley and Smith, 2014) produced complexity estimates for only a small subset of the 612 DLP+ libraries. **c,** Complexity comparison of libraries made with Quick ligase and Codexis ligase in an ICELL8 chip, after overnight ligation. We show the final concentration at ligation of three Quick ligase treatments, where 66.7 U/µL is equivalent by volume to the Codexis ligase treatment. For Quick ligase, we recommend using 133.3 U/µL.



Supplemental Fig. S3. Depth of coverage is plotted against a point estimate of library complexity, i.e., the predicted breadth of coverage obtained with 1 Gb of sequencing. Of the 78 OP-Strand-seq libraries and the 78 conventional Strand-seq libraries, we show only those for which complexity curves are available. Related to the STAR Methods.