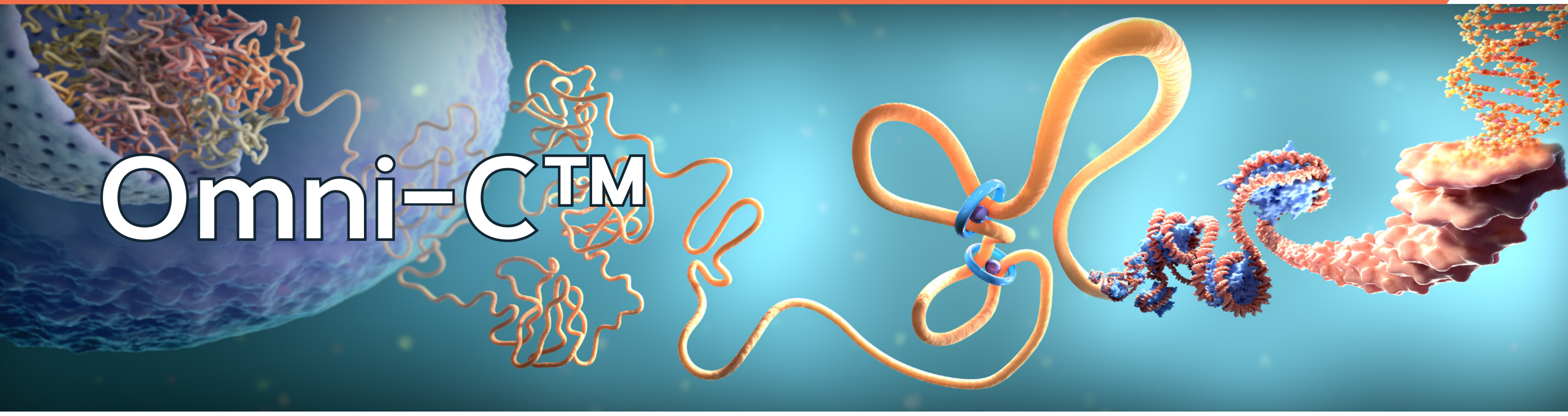


Omni-C™

Introduction

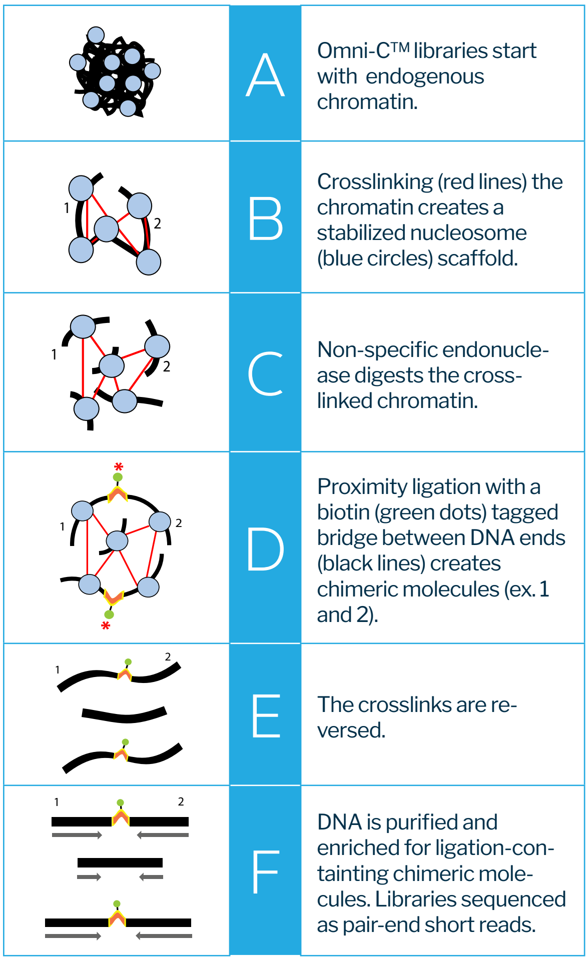
The discovery and adoption of chromatin capture methods have greatly accelerated the study of genome topology and enhanced the quality of genome assemblies. Genome conformation mapping technologies, including Dovetail Genomics™ Hi-C, have facilitated an unprecedented view of the three-dimensional (3-D) organization of the genome through sequencing technology. Methods like Hi-C have enabled the study of 3-D features such as topologically associated domains (TADs) and chromatin looping that are important in gene regulation and epigenetics. Moreover, the 3-D genomic structure can be leveraged for genome assembly by informing the scaffolding of contigs at chromosome scale. While Hi-C has enabled researchers in these areas, there are still notable drawbacks to this method due to the use of restriction enzymes (RE) to digest chromatin. Most notably, approximately 20% of the mappable human genome is blind to Hi-C due to low RE site density and analyses of Hi-C data are dependent on capturing these RE sites.

Here we introduce Omni-C™ Technology, a sequence-independent endonuclease-based Dovetail Genomics™ proximity-ligation protocol, which aims to address the limitations of RE-based Hi-C approaches **(Figure 1)**. By employing an endonuclease, Omni-C™ increases the genomic coverage of a proximity-ligation assay, therefore expanding the efficiency of each sequencing run by covering more of the genome and reducing biases imposed by RE site density. As such, Omni-C™ libraries importantly offer greater and more even genome coverage increasing the discovery potential for genome features from SNPs to large scale conformational changes.

Product

Omni-C™ is an 8-reaction kit to generate sequence-independent endonuclease-based Hi-C libraries that provides more uniform coverage across the genome. The Omni-C™ assay is a 2-day workflow: Day 1 - Sample Prep & Proximity Ligation; Day 2 - Library Generation resulting in an Illumina ready sequencing library (Figure 2). To ensure reaction efficiency, Omni-C offers quality

Figure 1 – Endonuclease-based molecular biology diagram.



A — Omni-C™ libraries start with endogenous chromatin.

B — Crosslinking (red lines) the chromatin creates a stabilized nucleosome (blue circles) scaffold.

C — Non-specific endonuclease digests the cross-linked chromatin.

D — Proximity ligation with a biotin (green dots) tagged bridge between DNA ends (black lines) creates chimeric molecules (ex. 1 and 2).

E — The crosslinks are reversed.

F — DNA is purified and enriched for ligation-containting chimeric molecules. Libraries sequenced as pair-end short reads.

The Omni-C™ process starts with endogenous chromatin, which is fixed in place (cross-linking) with formaldehyde. After cross-linking, an in-situ chromatin digestion is achieved with an endonuclease. Cells are lysed to release digested chromatin for proximity ligation, cross-link reversal and library assembly. The result is an Illumina ready The Omni-C™ library control (QC) checks that are predictive of final library quality enabling protocol success to be easily assessed at three points in the process using standard molecular biology approaches (Figure 3). The built-in QC check points occur at strategic stages throughout the assay and are designed to be clear indicators of a successful reaction before progressing on to subsequent stages in the protocol. Omni-C is currently validated on mammalian cells and tissues, with work in progress to confirm a broad range of other sample types **(Figure 4)**. Accompanying the Omni-C™ assay is an open-source informatic QC tool to assess the library quality. Omni-C sequencing data is fully compatible with the vast array of freely available open-source NGS sequence analysis tools.

Figure 2 - Omni-C™ 2-day workflow

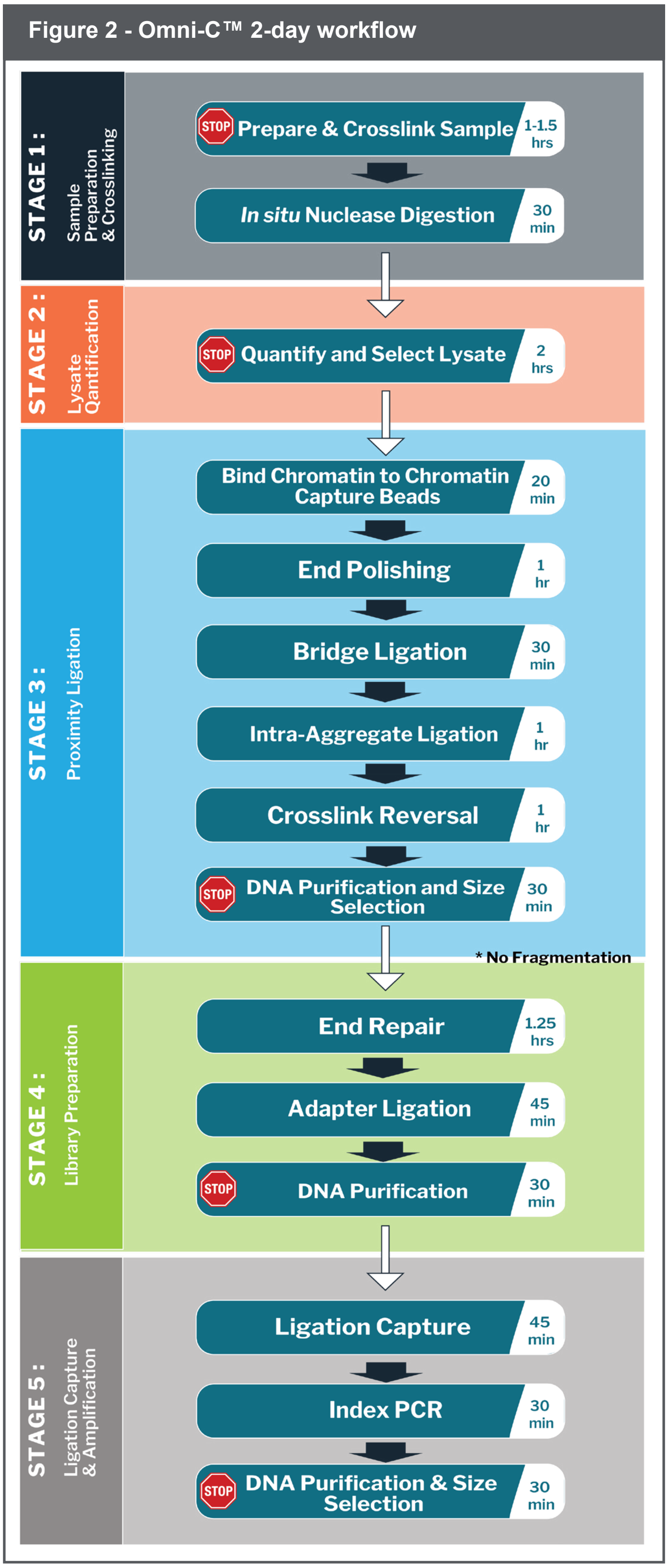
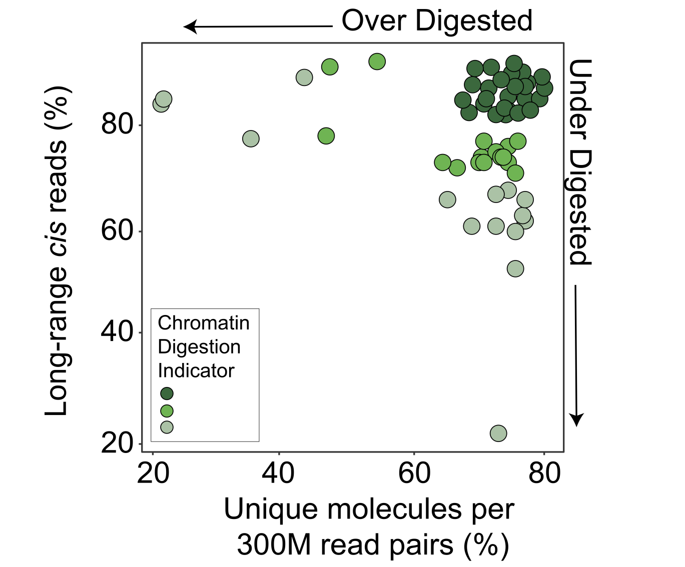

STAGE 1 :
Sample Preparation & Crosslinking
STOP Prepare & Crosslink Sample 1-1.5 hrs
In situ Nuclease Digestion 30 min
STAGE 2 :
Lysate Qantification
STOP Quantify and Select Lysate 2 hrs
STAGE 3 :
Proximity Ligation
Bind Chromatin to Chromatin Capture Beads 20 min
End Polishing 1 hr
Bridge Ligation 30 min
Intra-Aggregate Ligation 1 hr
Crosslink Reversal 1 hr
STOP DNA Purification and Size Selection 30 min
* No Fragmentation
STAGE 4 :
Library Preparation
End Repair 1.25 hrs
Adapter Ligation 45 min
STOP DNA Purification 30 min
STAGE 5 :
Ligation Capture & Amplification
Ligation Capture 45 min
Index PCR 30 min
STOP DNA Purification & Size Selection 30 min

Figure 3 – Chromatin Digestion Index is predictive of library quality



Omni-C™ libraries were generated across various sample inputs (n=51). The CDI is informative about both library complexity, plotted as the percent of unique molecules projected per 300 million read pairs, and proportion of long-range cis reads (number of cis reads >1Kbp as a percent of total cis reads). Samples that are over digested during sample prep produce libraries that are of low complexity while maintaining a high proportion of long-range information, while samples that are under digested are still complex but are deficient in long-range information.

Data Highlights

Quality

The two-day Omni-C™ workflow consistently produces endonuclease-based Hi-C libraries that exhibit high complexity and enrichment of long-range *cis* reads. The built-in QC steps enables users to determine library quality before sequencing. The Chromatin Digestion Index (CDI) quantitatively predicts the complexity and the expected proportion of long-range cis reads in for each reaction **(Figure 3)**. Regardless of sample type, Omni-C™ generated libraries with the high complexity and long-range information **(Figure 4)**.

Figure 4 – Validation of sample input types

Cells and tissues from human and mice were used as inputs to validate Omni-C™. All libraries were sequenced between 20-40 million 2x150bp read pairs and processed through the Dovetail Genomics Omni-C™ QC pipeline.

***A)** Long-range cis read pairs are plotted as a percent of total cis reads in the library and **B)** complexity is plotted as percent of unique molecules projected per 300 million read pairs.*

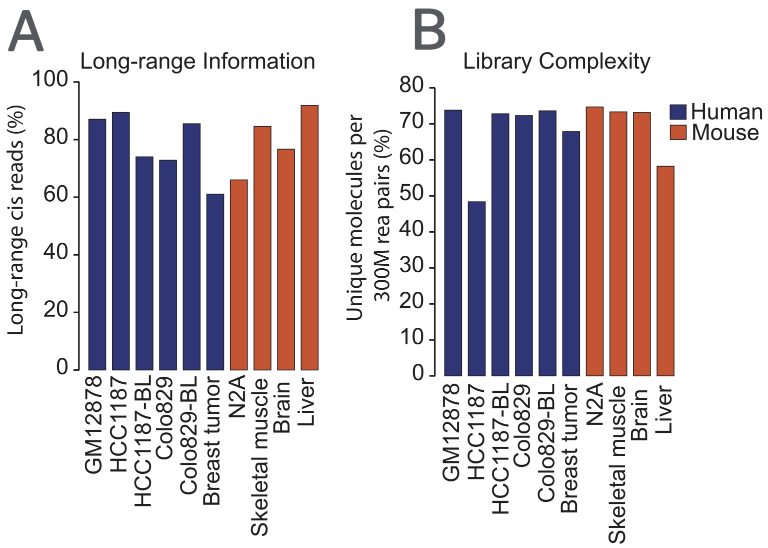


Table 1 – The Omni-C Assay accommodates a broad range in input material

Omni-C™ libraries were generated from cell and tissue inputs from both human and mice. The resulting libraries were sequenced to 20-40 million read pairs (2x150 bp) and assessed on the Omni-C™ QC pipeline.

Sample	Type	Species	Amount	% Long-range *cis*	% Unique molecules per 300M read pairs
GM12878	Cell	Human	1M	87.1%	82.0%
GM12878	Cell	Human	500K	84.6%	80.7%
GM12878	Cell	Human	250K	84.8%	78.7%
GM12878	Cell	Human	100K	87.1%	81.3%
Breast Tumor	Tissue	Human	10mg	76.7%	73.0%
Skeletal Muscle	Tissue	Mouse	10mg	91.8%	58.3%
Liver	Tissue	Mouse	10mg	82.5%	76.0%
Brain	Tissue	Mouse	10mg	71.8%	69.3%
Brain	Tissue	Mouse	5mg	71.0%	77.3%

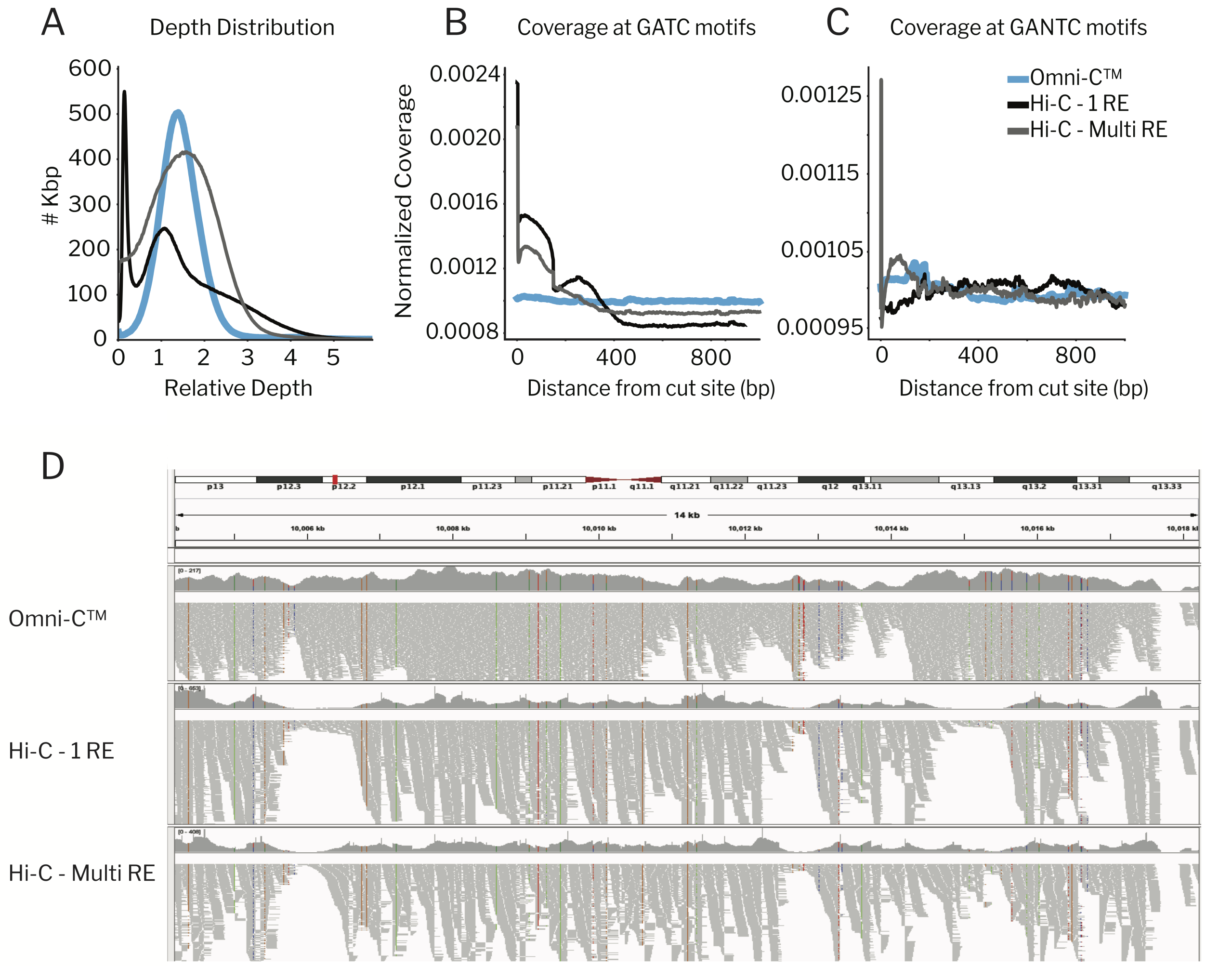
Flexibility

Omni-C™ is also designed to be a flexible assay. The Omni-C Assay accommodates a broad range in input material. The normal workflow calls for 1 million cells or 50 mg of tissue, the low input protocol uses starting material as low as 100K cells and 5 mg of tissue. Omni-C™ still yields high complexity libraries at lower starting inputs **(Table 1)**. Omni-C™ is also compatible with targeted enrichment procedures such hybrid capture, thereby reducing sequence burden and increasing resolution around sites of interest.

Coverage

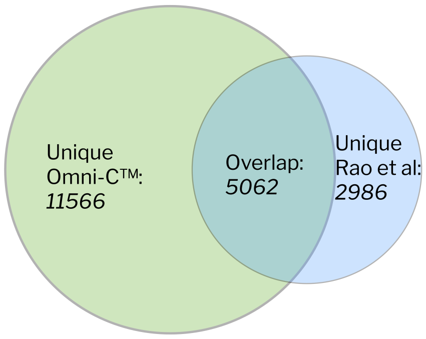
The endonuclease-based Omni-C™ provides superior coverage across the genome (Table 2). The Omni-C™ Assay data exhibit a narrow per base coverage histogram when compared to other RE-based Hi-C approaches demonstrating a more even sequence distribution across the entire genome. Typical Hi-C approaches miss a significant portion of the genome, leading to wider histograms and significant portions of the genome with no coverage at all **(Figure 5a)**. This uneven coverage is also reflected in the strong bias of reads at RE sites, whereas Omni-C™ libraries show no such bias **(Figure 5b)**. As such, Omni-C™ data capture single-nucleotide information in a manner that is independent of RE site proximity. When viewing Omni-C™ data in the Integrated Genome Viewer (IGV), it is clear where RE-based Hi-C falls short in coverage and misses SNPs **(Figure 5c)**. The improved coverage that is inherent to Omni-C™ enables a more exhaustive view of the genome in down-stream analyses, which opens up the potential for SNP calling and phasing.

Figure 5 – Coverage analysis



Deeply sequenced Omni-C™ (1 billion read-pairs) libraries were compared to RE-based Hi-C libraries for coverage. ***A)*** *Per base coverage, in Kbps. Coverage at RE sites,* ***B)*** *GATC (DpnII, MboI, Sau3AI) and* ***C)*** *GANTC (HinFI) are plotted as the average of the absolute value both upstream and downstream of RE sites.* ***D)*** *IGV view of coverage across a 14 Kbp window. Colored vertical lines indicate single nucleotide polymorphisms.*

Figure 6 – Loop detection



Omni-C™ libraries from cell line GM12878 was sequenced to 1.77 billion read pairs and loops were called using HiCCUPs. The resulting loops were then compared to loops found in Rao et al., 2014 (4.9 Billion read pairs).

Table 2 – Contact map resolution at a fixed sequencing depth

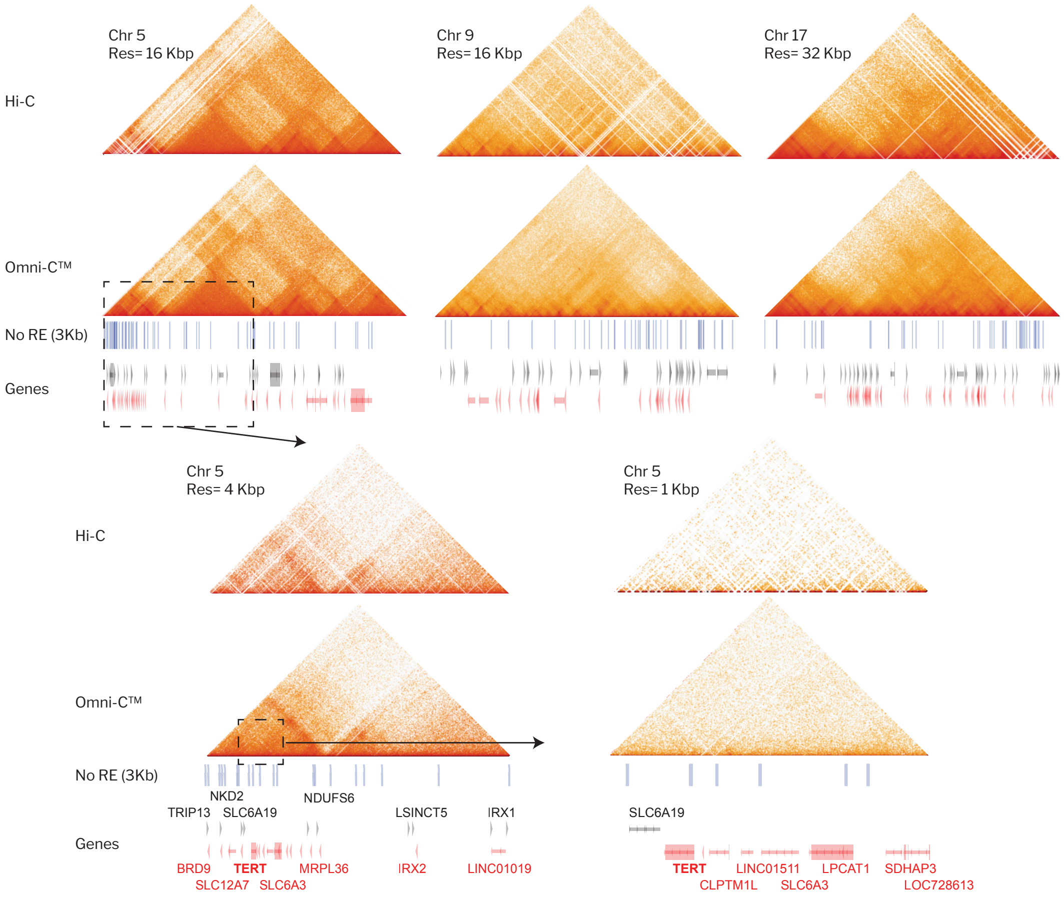
Assay	Resolution	Sequencing Depth (Read Pairs)	Genome Covered (%)
Omni-C™	5 kbp	1 Billion	93.23%
Hi-C	5 kbp	1 Billion	86.30%

Topology

In addition to uniform coverage, Omni-C™ delivers on conformation. Loop calling with Omni-C™ data from GM12878 via HiCCUPS detected 16,628 chromatin loops with 5,062 overlapping with Rao et al., 2014, with 3-fold less than was used in the Rao et al. 2014 study (Figure 6). Overlapping loop calls between Omni-C™ and Rao et al., are similar in number to other such comparisons.

The contact matrices generated from Omni-C™ libraries present a more complete view of genome conformation. During contact matrix balancing, areas with low coverage are essentially normalized with zero value in the denominator resulting in contact maps with blank vectors at these low coverage sites. Here we highlight three regions where Omni-C™ reveals topological features not captured in RE-based Hi-C (Figure 7). TERT, a gene that is vital in telomere maintenance and is often overexpressed in many lung cancers, presents a challenge for RE-based Hi-C contact matrices. The contact matrix generated by Omni-C™ captures a region of chromosome 5, which is known to be a cancer susceptibility locus. The second example (7b) demonstrates a ~5Mbp section on chromosome 9 that lacks sufficient Hi-C coverage to produce an uninterrupted contact map. Omni-C™'s uniformity generates a much more complete contact matrix at this site, which contains genes associated with apoptosis signaling. The last example is chromosome 17. Again, Omni-C™ produces a more comprehensive contact matrix where RE-based Hi-C falls short. Here topology around a potential oncogene, FASN, can now be seen in the Omni-C™ data. FASN is often overexpressed in breast cancers and understanding the looping and conformation impacting FASN, could

Figure 7 – Contact matrices from Omni-C™ libraries generate more complete contact matrices



Blank bands in the contact matrix occur during contact matrix balancing in regions were coverage is too low. Low coverage regions cause the contacts to be normalized with a zero value in the denominator which results in blank vectors across these low-coverage regions. The examples are Chr5, a cancer susceptibility locus, which is often over expressed in lung cancer. The second is Chr9 which displays a contact matrix with ~5Mbp region of poor mapping that encompasses the TRAF2 gene that plays a key role apoptotic signaling. The third example is Chr17, containing a suspected oncogene, FASN, which is often over expressed in breast cancer. Blow each comparison are blue boxes denoting 3 Kbp regions that are devoid of RE sites, and gene tracks in black and red arrows. Under these comparisons are zoomed in sections of chr5 that encompasses TERT at 4 Kbp and 1 Kbp resolutions.

lead to a better understanding of why this gene is overexpressed in breast cancer.

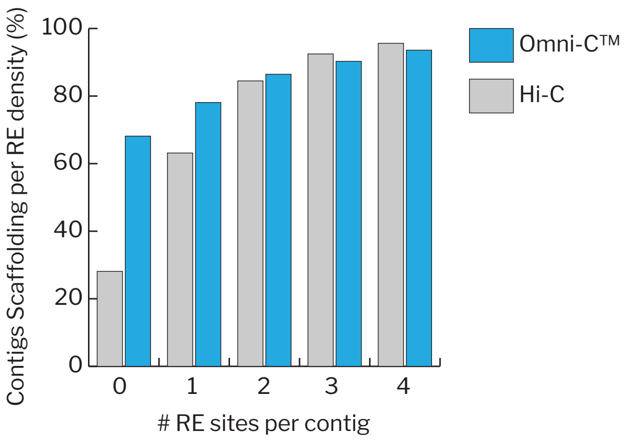
Scaffolding

A staple application of proximity ligation data is scaffolding contigs for genome assembly. The ability of Hi-C data to scaffold correctly depends on the RE site density captured within each assembled contig. The analysis on scaffolding a human genome shows RE-dependent Hi-C scaffolding misses contigs that Omni-C™ can include (Figure 8). As Omni-C™ is RE agnostic, it can scaffold contigs more efficiently than Hi-C data, where RE frequency per contig is low. In regions of the genome where RE frequencies increase, the quality of the scaffold based on Hi-C and Omni-C data respectively are similar indicating that it is the lack of RE density that results in the quality difference observed in RE sparse regions.

Summary

Here we presented data that showcases Omni-C™, an endonuclease-based Hi-C kit, from Dovetail Genomics. The robust protocol provides quality control steps that are predictive of library performance before sequencing. The workflow of Omni-C™ does not drastically alter the typical RE-based Hi-C workflow and can incorporate low input samples. Omni-C™ offers uniform coverage across the genome without over representing RE sites. This demonstrated uniformity of coverage provides a more complete view of the genome through proximity ligation.

Figure 8 – Omni-C™ is more efficient at scaffolding contigs with low RE site density



The human genome, HG38, was cut into contigs of random size. Libraries were made from GM12878 on both Omni-C™ and RE-based Hi-C. The contigs were then scaffolded using HiRise. The scaffolded contigs were binned into groups by the number of RE sites per contig. Scaffolding efficiency was determined by normalizing the number of contigs scaffolded by the total number of contigs in each RE site group.