Genome Conformation Profiling from as few as 1000 Cells

Application Note

1. Introduction

The three-dimensional (3D) genome organization plays a key role in gene regulation. Chromatin conformation capture (3C) technology, including Hi-C, has been instrumental in enabling researchers to unravel the spatial organization of chromosomes. This chemistry enables the genome-wide mapping of chromatin contacts allowing features such as A/B compartments and topologically associated domains (TADs) to be identified at an unprecedented scale[1].

Traditionally, Hi-C methods have required a minimum of 1 million cells to obtain high resolution genome topology which has restricted the use of certain sample types (e.g. FACS, fine needle aspirates etc.) or the use of rare samples. Recently, Dovetail Genomics released the Omni-C™ Kit, a modification of the Dovetail™ Hi-C approach that replaces restriction enzymes with a sequence-independent endonuclease. A low input protocol enabling as few as 100,000 cells to be interrogated with minimal changes to the workflow is available. However, this is a not a strict threshold, as the input can be reduced significantly further.

To expand the use of sample types previously limited by Hi-C, this work demonstrates the use of the Omni-C Kit with ultra-low input cell counts down to 1,000 cells. Omni-C™ libraries generated from ultra-low input cell counts are of comparable quality to those made with the standard input of 1 million cells with minimal changes to the workflow.

2. Materials and Methods

The two-day ultra-low input Omni-C™ workflow[1] was initiated by crosslinking GM12878 cells to form a stable 3D nucleosome complex. Next, the ultra-low input samples were processed in the same manner as with the standard protocol with the exception of the *in-situ* digestion conditions. Here, the enzyme concentration was lowered to the appropriate amount necessary to achieve the correct digestion profile for each cell count.

To assess library quality, an initial round of low depth (1-2 M reads) sequencing was performed and mapped to the hg38 reference genome. Quality criteria were calculated using the Omni-C™ QC Pipeline, which evaluates each library based on four metrics: 1) the inter-chromosomal *trans* reads, 2) the short range (< 1 kbp) intra-chromosomal *cis* reads, 3) the long range (>1 kbp) intra-chromosomal *cis* reads, and 4) the predicted library complexity at 300 M reads. Next, the libraries were deeply sequenced (360-790 M reads) and A/B compartments were identified with Juicer.

3. Results and Discussion

The Omni-C™ protocol reliably generates ample proximity ligated DNA suitable for library generation across a wide range of input amounts **(Figure 1)**. As expected, the amount of DNA recovered after proximity ligation decreases with a reduction in input material. However, libraries can still be generated with the amount recovered from 1 K cells. Importantly, the quality of long-range information is preserved across the range of sample inputs tested **(Figure 2**).

Figure 1 – Abundant amount of proximity ligated DNA retrieved.

Amount of proximity ligated DNA (ng) retrieved after crosslink reversal for each ultra-low input library (n = 2 or 3).

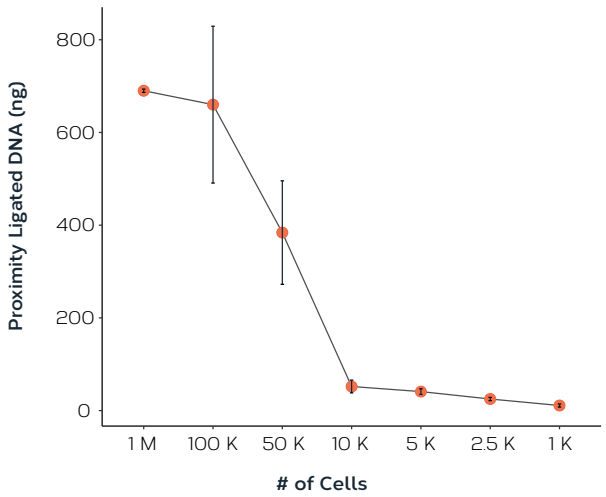
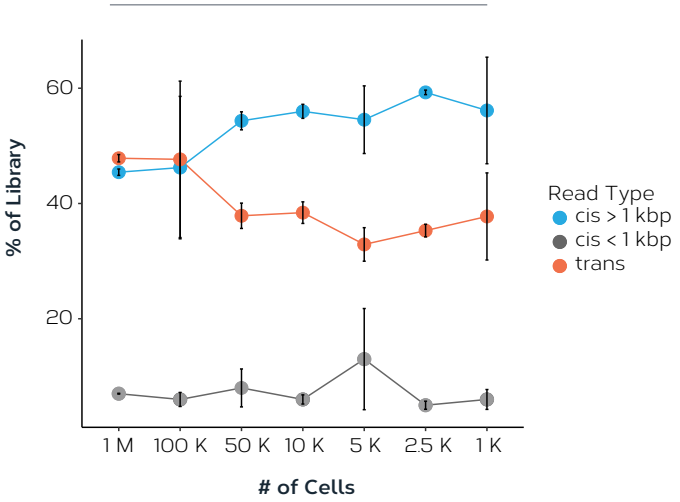


Figure 2– High complexity libraries generated from ultra-low input cell counts.

This graph shows the library quality for each sample. The grey circles represent the short-range intra-chromosomal cis interactions (< 1 Kb), the blue circles represent the long-range intra-chromosomal cis interactions (> 1 Kb), and the orange circles represent the inter-chromosomal trans reads (n = 2 or 3).



The libraries generated were observed to be of high quality based on the low amounts of short-range *cis* interactions (< 1 kbp) and enrichment of long-range *cis* interactions (> 1 kbp) with high complexity. Library complexity is reduced in libraries generated from 5 K - 1 K inputs, which aligns with the expectation that decreases in DNA used for library generation reduces the complexity of the library **(Figure 3)**. Despite the change in complexity, the long-range interactions remained comparable across the cell inputs tested as demonstrated. For applications where complexity is not such a critical component, libraries generated from an input of less than 10 K cells are still viable and enable A/B compartment calling down to 1 K cells, as noted in the principal coordinate correlation being >0.9 between 1 M and 1 K cell inputs.

In addition to assessing the quality of ultra-low input libraries, A/B compartments were analyzed for a range of ultra-low input cell counts. To gain a better understanding of the active and inactive compartments, libraries generated from ultra-low input cell counts with the Omni-C™ Kit identified A/B compartments with as few as 1 K cells **(Figure 4)**. The principle component 1 (PC1) between the standard input of 1 M cells and an ultra-low input of 1 K cells display very similar profiles and align well with the corresponding domains. This is further evidenced by the principal coordinate correlation (PCC) analysis, which indicates a good correlation between the 1 M cell input and the 1 K cell input, with the PCC being >0.9 **(Figure 5)**.

Additionally, the Omni-C™ workflow captures multi-kilobase topological features at reduced cell counts, as low as 10 K cells **(Figure 6)**. The reduction of input material does not alter the genome conformation analyses. When comparing the contact matrices between 10 K cells and 1 M cells, at similar library qualities, the domains present in the 1 M cells are reflected in the 10 K cell contact matrix. This suggests that topological studies can be conducted with high fidelity by th Omni-C workflow with a significantly reduced cell input.

4. Conclusions

The genome topology of samples can now be interrogated using the Omni-C™ Kit with ultra-low input cell counts with minimal change to the Omni-C workflow. As demonstrated here, these low input libraries contain sufficient Hi-C contact information to enable the identification of topology features such as A/B compartments and domains.

Figure 3 – High quality libraries generated from ultra-low input cell counts.

The bar graph represents the predicted library complexity at a 300 M reads for each library (n = 2 or 3).

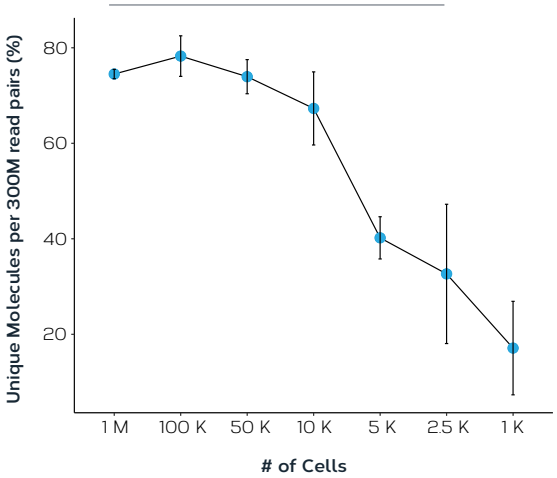


Figure 4 – A/B compartments are comparable with the ultra-low input of 1 K cells and the standard input of 1 M cells.

A contact matrix was generated from 1 K cells (chr11:15-25 Mb at a resolution of 32 kb). PC1 was obtain from contact maps at 100 kb resolution. PC1 designates the A/B compartments which are comparable between the standard input of 1 M cells and ultra-low input of 1 K cells. A/B compartments from both sample inputs align with domains shown in the contact matrix.

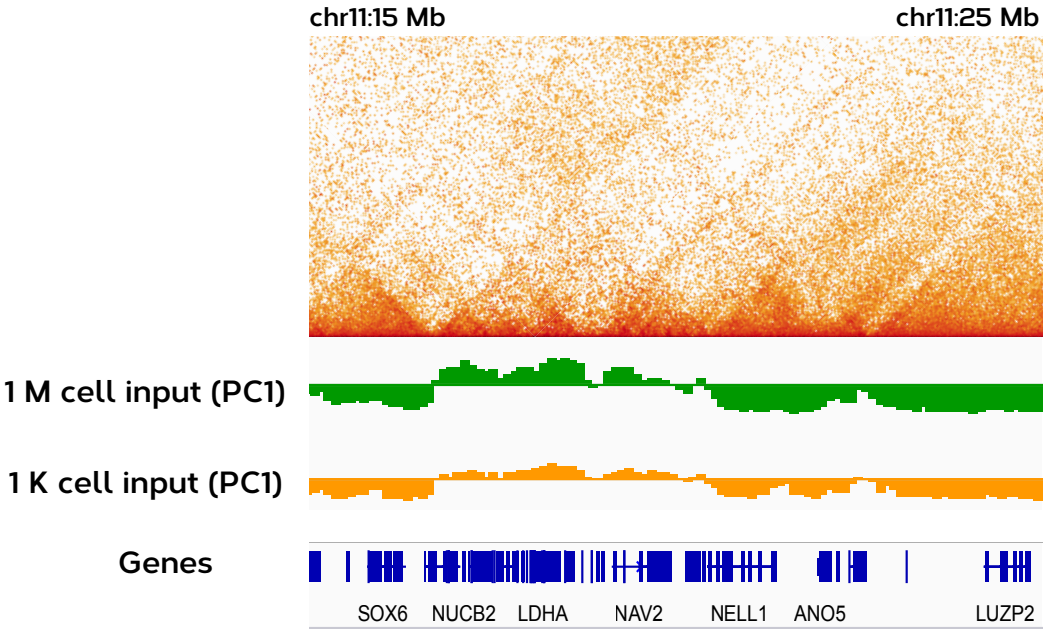


Figure 5 – PCC analysis indicates that ultra-low 1 K cell input captures A/B compartments similarly to the standard 1 M cell input.

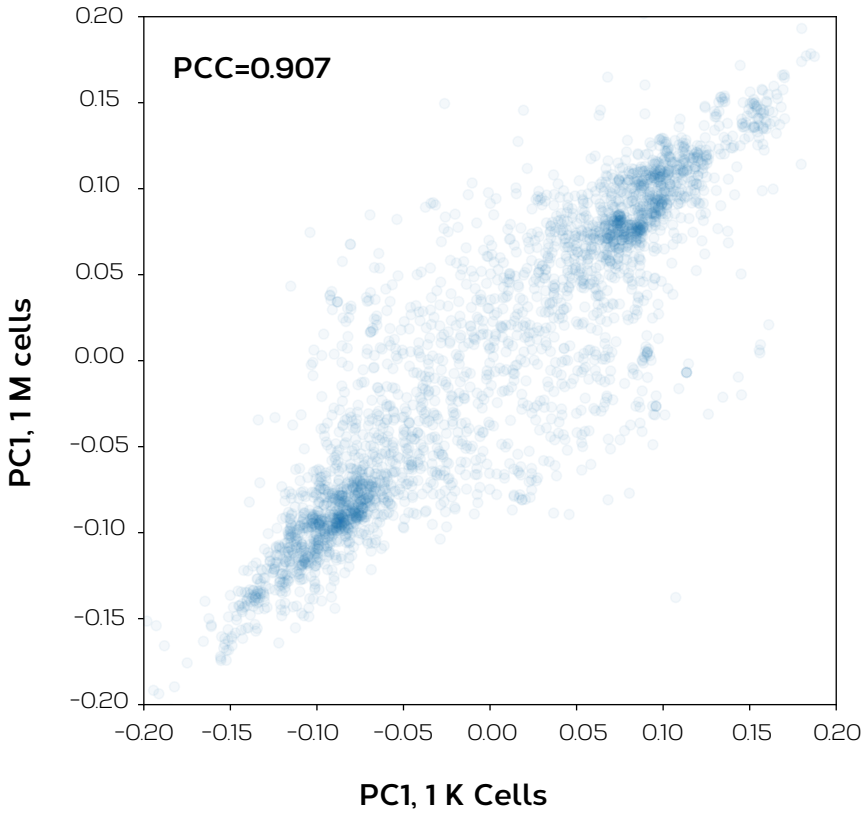
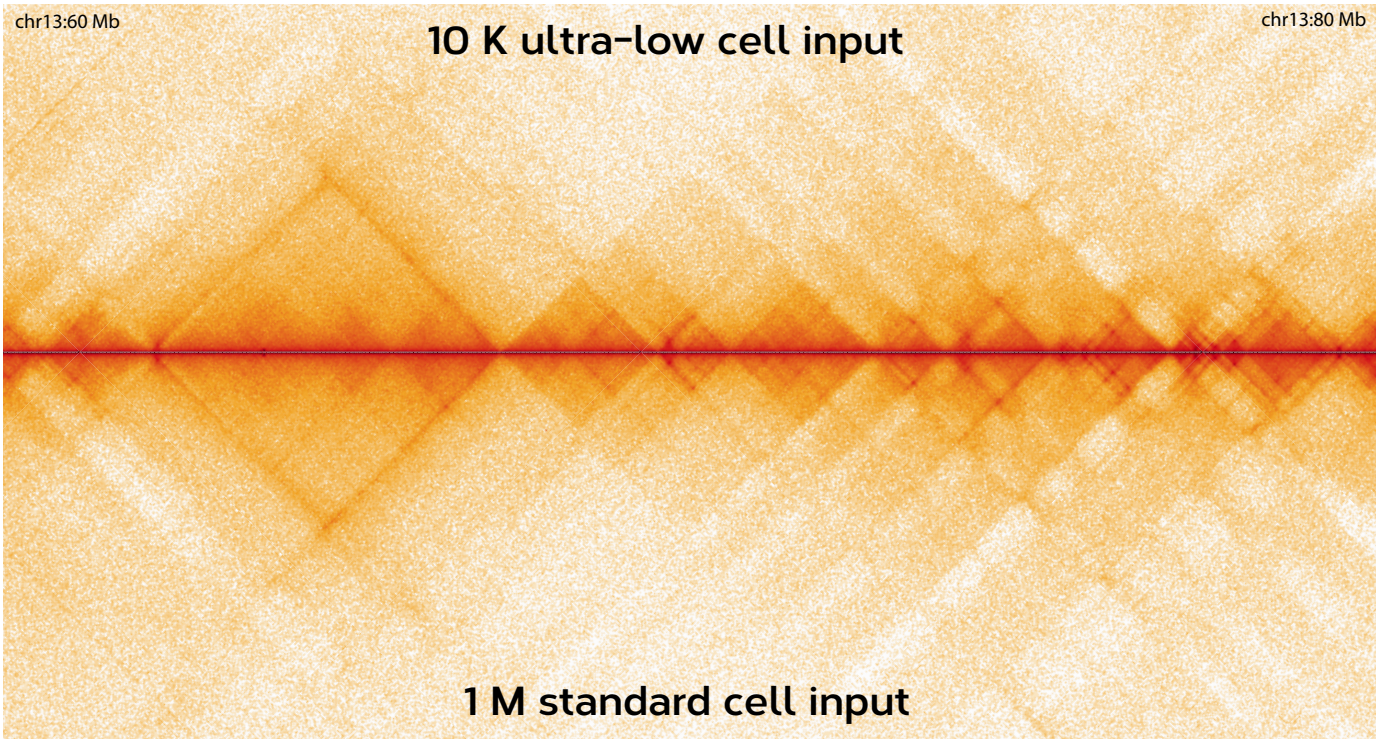


Figure 6 – Identification of topological features is comparable between ultra-low input of 10 K cells and a standard input of 1 M cells.

Top contact map shows topological domains at 10 K cells, bottom contact map shows topological domains at 1 M cells. Deep sequencing performed using 2 x 150 bp at chr13:60-80 Mb and binned at 32 kb resolution.



References

[1]Dovetail Genomics Omni-C™ Tech Note [dovetailgenomics.com/omni-c-kit]

To place an order or for more information:
visit us at www.dovetailgenomics.com or send an email to info@dovetail-genomics.com