10x GENOMICS®

TECHNICAL NOTE

10x Barcode Continuity during Library Sequencing

INTRODUCTION

The schematic of the 10x Chromium™ Genome Library fragment places the 16 base pair barcode as the first 16 base pairs of the library insert (Fig. 1), which will be sequenced as the first 16 bases of Read 1.

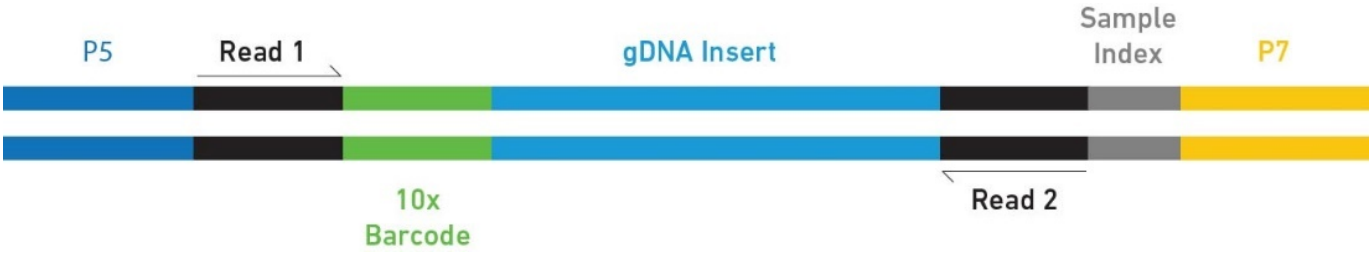

Fig. 1. Schematic overview of fragment of a final 10x Chromium™ Genome library.

DISCUSSION

The operator of an Illumina® HiSeq® 4000 or HiSeq® X Series sequencer will sometimes abort a run after imaging of the first cycle is completed to check the flow cell surface for uneven intensities, bubbles, scratches and general cluster distribution. Then, a new run will be started to resume sequencing of Read 1. However, the data from the previous cycle is lost and, if precautions are not taken, the dataset will now start with the nucleotide in the second position, identifying it as the first nucleotide in the sequence data. The result is a one-base frameshift in the numerical assignment of each base pair in the sequence (Fig. 2).

The frameshift has a significant impact on the sequencing of 10x Chromium™ Genome libraries. The first 16 cycles of Read 1 contain the 10x Barcode information. Therefore, in this scenario only the last 15 base pairs of the 10x Barcode will be read while the information for the first base pair is lost during the initial cycle when the run is aborted. If the complete 10x Barcode sequence is unavailable, the fragment cannot be assigned to the correct partition and the library will need to be resequenced. A significantly reduced fraction of reads with barcodes matching the whitelist may be evidence of this effect.

Note that this effect is unlikely to occur on MiSeq® and NextSeq® Series sequencers as the option to abort a sequencing run and then resume is not provided. In contrast, HiSeq® Series instruments are susceptible to this issue, e.g., HiSeq® 4000 and HiSeq® X Series sequencers are able to abort and then resume a run, resulting in the loss of the first cycle data.

If a run is aborted after the first cycle and before resuming sequencing, a complete re-hybridization step must be performed to prevent the loss of 10x Barcode information for 10x Chromium™ Genome libraries sequenced on the HiSeq® 4000 or HiSeq® X Series sequencers. In this way the synthesized first base will be denatured and washed away with the sequencing primer, allowing the system to start again with a fresh hybridization of the sequencing primer to its appropriate first position on the 10x Barcode. This will successfully capture all 16 bases of the 10x Barcode, all of which are required to determine the fragments' original partitions, and enable downstream analysis of the data. However, if the sequencing run has been completed, the library will need to be resequenced in order to retrieve the accurate 10x Barcode sequence information. Then, the fragments can be assigned to the correct partitions and the library can undergo a complete analysis.

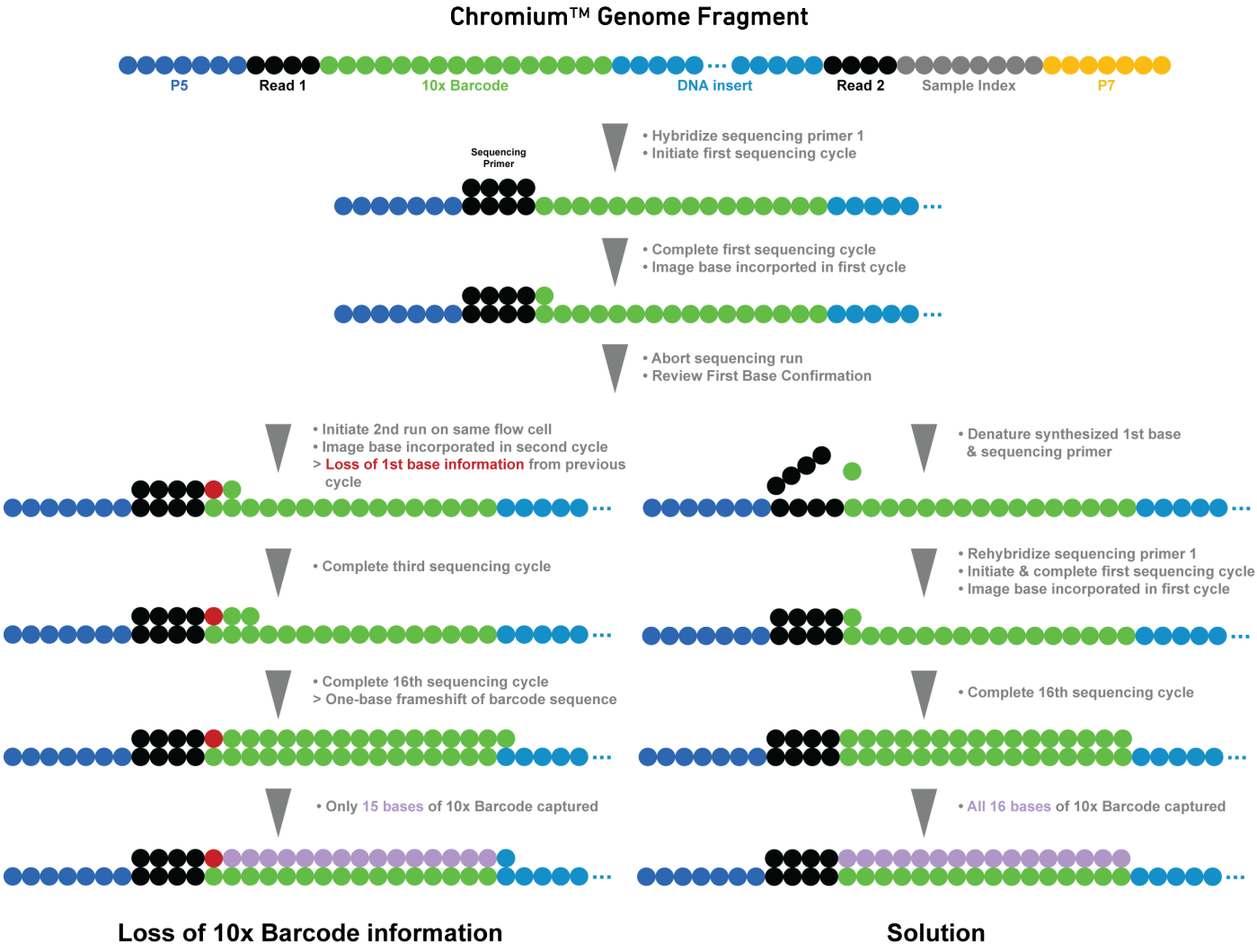

Fig. 2. Illustration on how to prevent the loss of 10x Barcode sequencing information.

Notices

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Legal Notices

Customer Information and Feedback

For technical information or advice, please contact our Customer Technical Support Division online at any time.

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