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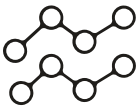
Library QC with the MiSeq™ i100 Series

Assess library quality and optimize library pooling before sequencing on high-throughput systems

Streamline library quality control with same-day results enabled by fast, flexible sequencing



Simplify library rebalancing guided by automated onboard calculations

Increase confidence in results generated on high-throughput systems with high correlation of index representation

M-GL-02871 v1.0

Introduction

To maximize the efficiency of high-throughput sequencing, it is important to know the quality of the starting library. A poor-quality library can undermine the success of large-scale sequencing projects and lead to costly and time-consuming repeat experiments. Historical methods for performing library quality control (QC), such as library quantification/qualification by fluorometry or qPCR, are not functional assays and do not evaluate if the library of interest with the correct indexes has been prepared. The MiSeq i100 Series enables a fast and functional assay of library quality before committing to a full-scale run on the NovaSeq™ 6000 System or NovaSeq X Series, saving time and money while leading to better results.

Using a simple, streamlined workflow, the MiSeq i100 Series generates detailed quality metrics quickly. These metrics can be used to detect sample dropouts, deriving from either failed library prep or index misassignment during run planning, and provide automated calculations for pool rebalancing to ensure balanced index representation across samples. This application note demonstrates a fast, simple, and cost-effective library QC workflow on the MiSeq i100 Series that delivers excellent library representation before sequencing on the NovaSeq X Series or NovaSeq 6000 System (Figure 1).

Library rebalancing with the MiSeq i100 Series

The MiSeq i100 Series can be used as a library QC tool to screen for library dropout and rebalance libraries for a more uniform index representation in a pool. The MiSeq i100 Series features the DRAGEN Library QC app v1.0.13, onboard software that automatically demultiplexes sequencing reads, performs calculations, and provides a report to guide library rebalancing before sequencing on a high-throughput sequencing system (Figure 2). Combining automated rebalancing calculations with index-first sequencing, the MiSeq i100 Series can deliver library demultiplexed information in as little as ~3.2 hours.

Figure 1: Library QC workflow

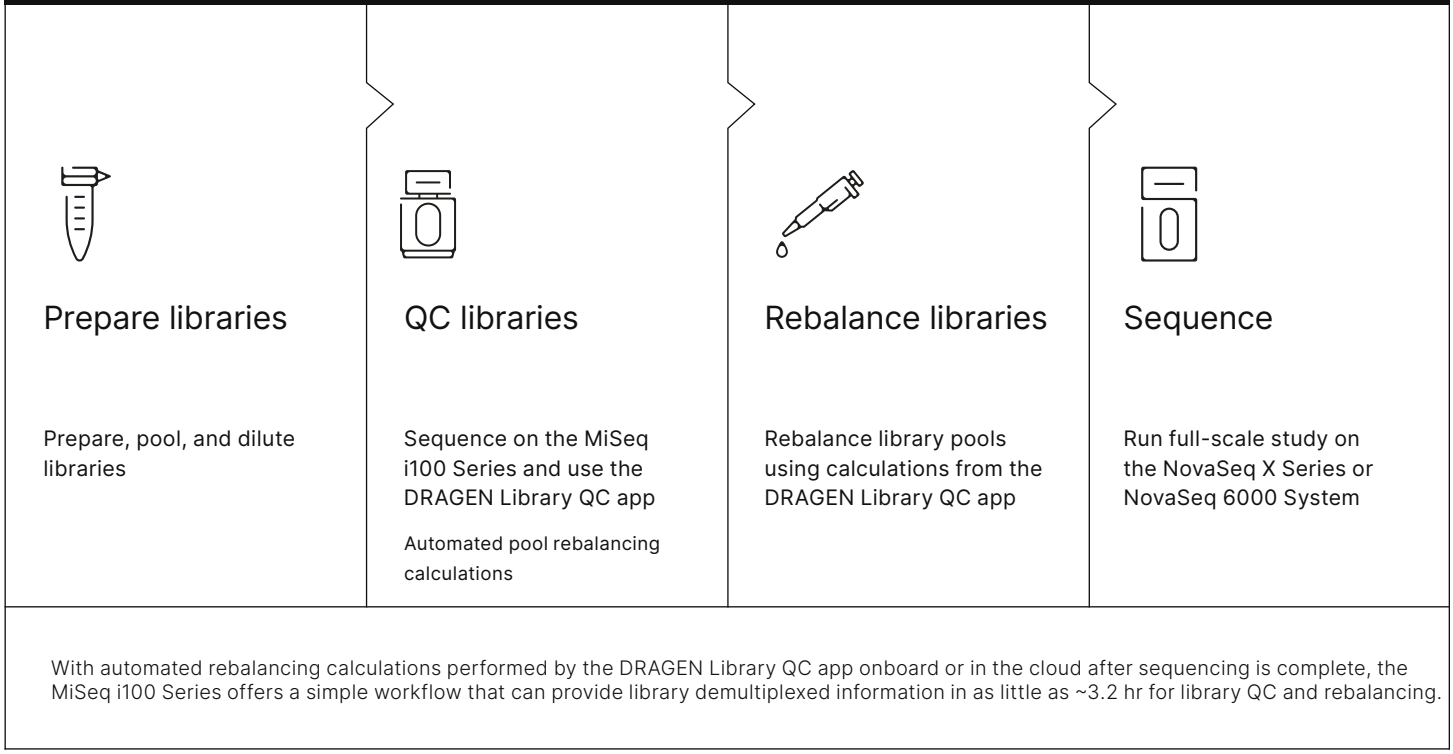
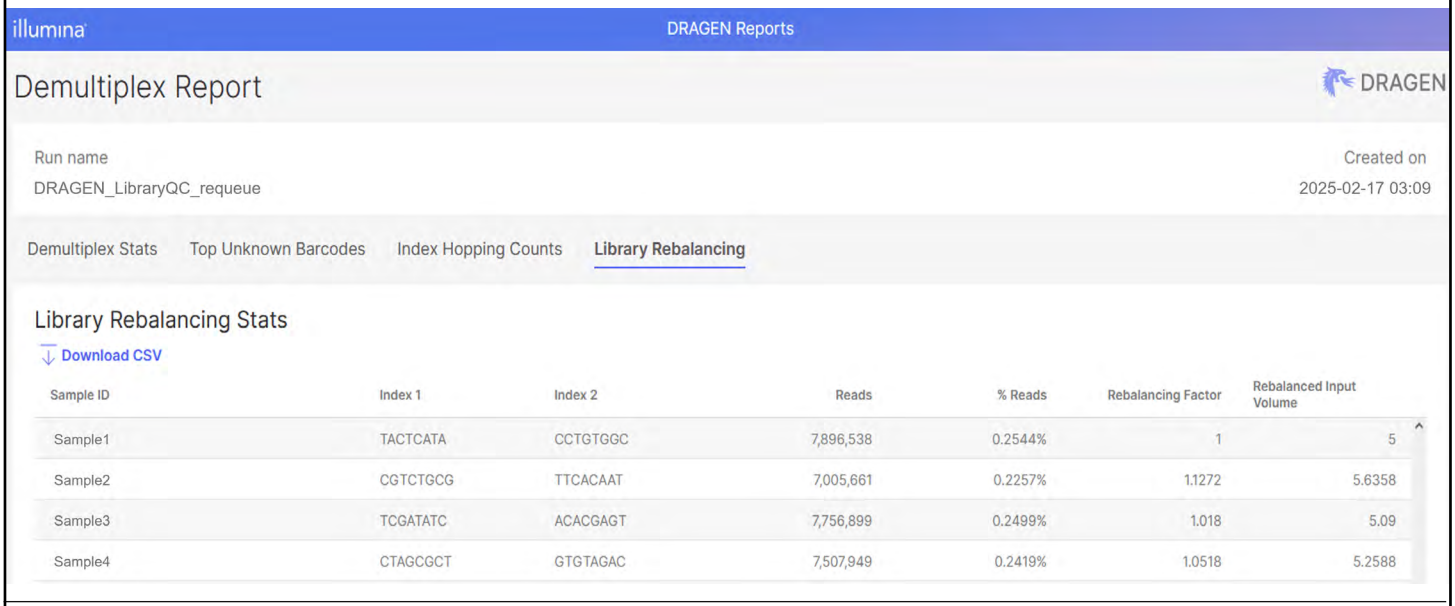


Figure 2: Example report of library rebalancing calculations in the DRAGEN Library QC app



Sample ID	Index 1	Index 2	Reads	% Reads	Rebalancing Factor	Rebalanced Input Volume
Sample1	TACTCATA	CCTGTGGC	7,896,538	0.2544%	1	5
Sample2	CGTCTGCG	TTCACAAT	7,005,661	0.2257%	1.1272	5.6358
Sample3	TCGATATC	ACACGAGT	7,756,899	0.2499%	1.018	5.09
Sample4	CTAGCGCT	GTGTAGAC	7,507,949	0.2419%	1.0518	5.2588

The DRAGEN Library QC app has two modes: FastQC and Full Pipeline (summarized in the MiSeq i100 Series product documentation): FastQC mode runs BCL Convert/demultiplex only and provides rebalancing calculations along with primary metrics.

Methods

Library preparation

Libraries were prepared on the Biomek NGeniuS Next Generation Library Prep System (Beckman Coulter, Catalog no. C62703) from 300 ng input NA12878 genomic DNA (gDNA) (Coriell Institute for Medical Research, Catalog no. NA12878) using Illumina DNA PCR-Free Prep (Illumina, Catalog no. 20041795) with Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples) (Illumina, Catalog no. 20091654) and Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples) (Illumina, Catalog no. 20091656). Libraries were also prepared manually from 100 ng input gDNA using TruSeq™ DNA Nano (Illumina, Catalog, no. 20015965) with IDT for Illumina DNA UD Indexes v2 (96 indexes, 96 samples) (Illumina, Catalog no. 20040870).

Sequencing and rebalancing

Prepared libraries were pooled at equal volumes and sequenced on the MiSeq i100 Plus System with the MiSeq i100 Series 5M Reagent Kit (300 cycles) (Illumina, Catalog no. 20126566) using the 2 × 151 bp run configuration at 24-plex (Table 1). For comparison, the same libraries were sequenced on the iSeq™ 100 System with the iSeq 100 i1 Reagent v2 (300-cycle) kit (Illumina, Catalog no. 20031371).

Sequencing data were analyzed onboard the MiSeq i100 Plus System using the DRAGEN™ Library QC app v1.0.13, which automatically performs calculations for library rebalancing. After library pools were rebalanced, they were sequenced on the NovaSeq X Plus and NovaSeq 6000 Systems using the 2 × 151 bp run configuration to examine the index CV.

Table 1: Library QC on the MiSeq i100 Series

Parameter	Illumina DNA PCR-Free Prep	Illumina DNA PCR-Free Prep	TruSeq DNA Nano
Automation	Beckman Coulter Biomek NGeniuS	Beckman Coulter Biomek NGeniuS	Manual
Genomic DNA	Coriell Human NA12878	Coriell Human NA12878	Coriell Human NA12878
DNA input	300 ng	300 ng	100 ng
Adapters	Illumina Set A UDI 1–24	Illumina Set B UDI 97–120	IDT for Illumina TruSeq UDI 1–24
Loading concentration	120 pM	120 pM	120 pM
% occupancy	91.59%	89.27%	91.43%
% passing filter	80.42%	77.20%	85.02%

Results

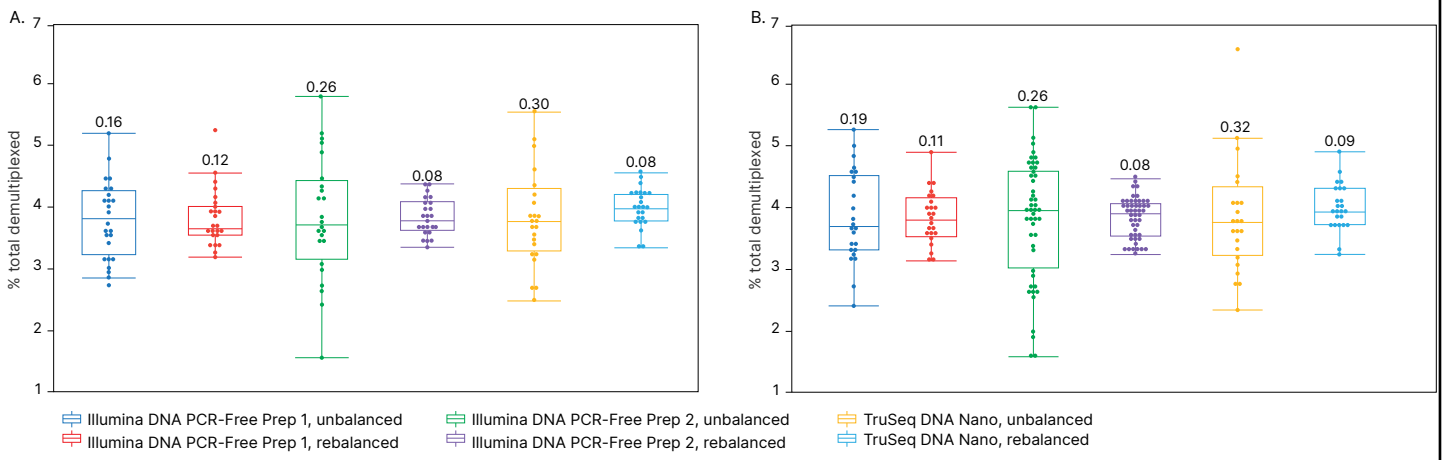
Library rebalancing improves index CV

Libraries prepared with Illumina DNA PCR-Free Prep and TruSeq DNA Nano were sequenced on the MiSeq i100 Series. Library rebalancing factors were obtained with the onboard DRAGEN Library QC app v1.0.13. Rebalanced and unbalanced libraries were sequenced on the NovaSeq X Plus and NovaSeq 6000 Systems. Results show that index CV improvement is observed after rebalancing for all three library pools for both the NovaSeq X Plus (Figure 3A) and NovaSeq 6000 (Figure 3B) Systems.

Correlation of index representation between systems

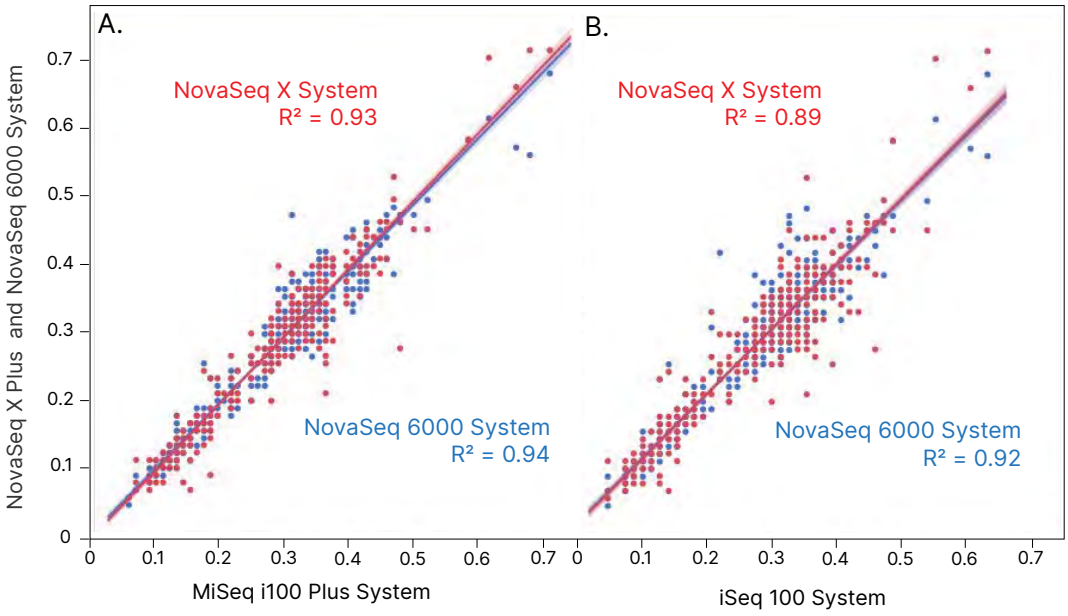
The baseline correlation of index representation was assessed between the MiSeq i100 Plus, iSeq 100, NovaSeq X Plus, and NovaSeq 6000 Systems. A 384-plex Illumina DNA PCR-Free Prep library pool was prepared from Coriell human NA11992 gDNA using the Hamilton STAR automated liquid handler. Pooled libraries were sequenced on the MiSeq i100 Plus System, iSeq 100, NovaSeq X, and NovaSeq 6000 Systems. The demultiplexed information obtained with the MiSeq i100 Plus System shows high correlation with the demultipled information obtained with the NovaSeq X Plus and NovaSeq 6000 Systems with R2 > 0.9 (Figure 4A) and is equivalent to demultiplexed obtained with the iSeq 100 System (Figure 4B).

Figure 3: Library rebalancing with the MiSeq i100 Plus System



Three 24-plex human library pools, Illumina DNA PCR-Free Prep 1, Illumina DNA PCR-Free Prep 2, and TruSeq DNA Nano, were sequenced on the MiSeq i100 Plus System. Library rebalancing factors were obtained with the onboard DRAGEN Library QC app v1.0.13. Rebalanced libraries and unbalanced libraries were then sequenced on the NovaSeq X Plus and NovaSeq 6000 Systems. Index CV (mean value is above each box plot) improvement is observed after rebalancing for all three library pools on both the (A) NovaSeq X Plus and (B) NovaSeq 6000 System.

Figure 4: Correlation of index representation



Index representation on (A) the MiSeq i100 Series shows high correlation with the NovaSeq X Plus and NovaSeq 6000 Systems and is at similar levels to correlation between (B) the iSeq 100 System and the NovaSeq X Plus and NovaSeq 6000 Systems.

Summary

The MiSeq i100 Series provides a fast, simple, and cost-effective workflow for library QC that delivers library demultiplexed information in as little as 3.2 hours. The high correlation of index representation enables prediction of index representation on a high-throughput sequencing system for a given set of index pairs. This library QC function enable users to maximize performance on the NovaSeq X Series and NovaSeq 6000 System.

Learn more

MiSeq i100 Series

NovaSeq X Series

NovaSeq 6000 System

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M-GL-02871 v1.0