Accel-NGS® 2S PCR-Free Library Quantification for Achieving Optimal Cluster Densities

Library Quantification

Bioanalyzer traces of Accel-NGS 2S PCR-free libraries reflect the presence of fully-adapted library molecules; however, the partially single-stranded adapters of PCR-free libraries retard migration of library molecules on the Bioanalyzer and overestimate the size of these libraries. Consult the Expected Results section of the Accel-NGS 2S PCR-free DNA Library Kit Instruction Manual for more information.

Without PCR enrichment for fully adapted molecules with these PCR-free libraries to generate fully double-stranded DNA (dsDNA) molecules, Bioanalyzer electropherograms (or dsDNA Qubit® readings) are unreliable for library quantification. Therefore, we recommend using a qPCR-based library quantification kit to quantify your libraries and accurately load the sequencing instrument. There are many commercially-available qPCR kits available for library quantification.

MiSeq® Cluster Density Data

MiSeq cluster densities in the following table were obtained from 10 pM loading concentration from PCR-free and +PCR-amplified Accel-NGS 2S libraries. All sequencing was performed on the same instrument.

Library Preparation Kit	PCR Status	Average Cluster Density (K/mm^2) (n=7)
Accel-NGS 2S PCR-free	PCR-free	930
Accel-NGS 2S Plus	+PCR	878

HiSeq® 2500 Cluster Density Data

HiSeq Rapid Run cluster densities in the table below were obtained from various loading concentrations from PCR-free and +PCR-amplified Accel-NGS 2S libraries. Sequencing was performed on multiple instruments (n=2 lanes).

Instrument	Library Preparation Kit	PCR Status	Loading Concentration (pM)	Cluster Density (K/mm^2)
HiSeq #1	Accel-NGS 2S PCR-free	PCR-free	6.5	719
HiSeq #1	Accel-NGS 2S Plus	+PCR	6.0	815
HiSeq #2	Accel-NGS 2S PCR-free	PCR-free	8.5	1115
HiSeq #3	Accel-NGS 2S Plus	+PCR	8.0	875

Conclusion

Observed cluster densities are not dependent on library amplification for the MiSeq or HiSeq 2500. Therefore, no special adjustments are required for loading PCR-free libraries vs. PCR-amplified libraries on these instruments.

However, if you use Swift's PCR-free libraries on the patterned flow cells, including the HiSeq 3000/4000 and HiSeq X Ten, you will need to follow Illumina's® loading recommendations for the TruSeq® Nano kit as opposed to the TruSeq PCR-free kit. This requires you to load 2-3 nM onto the flow cell as opposed to the recommended 1-2 nM for the TruSeq PCR-free kit in order to obtain proper clustering. Please consult Illumina's technical support and the cBot™ 2 System Guide (pg. 19-26) for loading PCR-free vs. PCR-amplified libraries on the HiSeq 3000/4000 and HiSeq X Ten instruments.

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