Important Workflow Update

Updated library loading concentration of the NovaSeq 6000 and NextSeq 500/550 and pre-amplification annealing temperature

Dear Valued Researcher,

10x Genomics is committed to providing customers the best experience to support their research needs. To ensure optimal assay performance, we occasionally refine our workflow recommendations. We have three updates on the Single Cell Gene Expression Flex assay workflow as described below:

We have updated our recommended loading concentrations on the NextSeq 500/550 and NovaSeq 6000. For NextSeq 500/550, we consistently observed lower sequencer output when loading Flex libraries at the previously recommended concentration. To improve sequencer output, we've adjusted our loading concentration so users can maximize their sequencing runs. In some cases, for NovaSeq 6000, we have observed loading concentration outside of our recommended range can lead to poor run performance, lower Q30 scores, possible introduction of sequencing artifacts, and, counterintuitively, lower total data output (i.e., fewer usable reads) as compared to optimally loaded runs.

To improve the robustness of sequencing runs, we are updating our loading recommendations for the NextSeq 500/550 and NovaSeq 6000 as follows:

- NextSeq 500/550: 2.5 pM
- NovaSeq 6000 Standard Workflow: 100 pM – 150 pM
- NovaSeq 6000 XP Workflow: 150 pM – 200 pM

For other Illumina sequencers (e.g., NextSeq 1000/2000), the recommended loading concentrations remain unchanged. For loading other library types onto NextSeq 500/550 or NovaSeq 6000, continue to follow the recommendations in the respective User Guide. The most up-to-date User Guides can be found on our Support Site. For additional details on sequencing best practices, please review the following Q&A articles:

- How do alternative quantification methods other than qPCR impact library loading?
- How do I pool 10x libraries for Illumina sequencing?

Additionally, we have updated the pre-amplification annealing temperature (adjusted from 67°C to 63°C) across all Fixed RNA Profiling protocols to accommodate Barcode Oligo Capture compatibility (Feature Barcode technology for Protein using TotalSeq™-C). This has been applied to all User Guides to simplify the user experience. For additional details regarding this update, please review the following Q&A article:

- Why is the annealing temperature during Pre-Amplification updated from 67°C to 63°C across all Fixed RNA Profiling protocols?

Please forward this notification to end-user personnel within your organization. If you have any questions regarding this notification, please contact your Field Application Scientist directly, or email our Support Team at support@10xgenomics.com.

Contact us
10xgenomics.com | info@10xgenomics.com

CG000716 - Rev A - Flyer - Important Workflow Update