Guidelines for Updated Loading Recommendations and Required Use of Sequel® Internal Control

User Bulletin

This User Bulletin describes PacBio's best practices for loading recommendations and a reminder on the required use of the Sequel® Internal Control. It is highly recommended that you read this before loading your sample on the Sequel System.

New General Loading Guidance

For best results using Sequel System Software Suite (v5.1.0), we recommend that you load higher than classic Poisson distribution (e.g. 37%). Please refer to PacBio's Quick Reference Card, Diffusion Loading and Pre-Extension Time Recommendations for the Sequel System, for more information including starting loading concentrations.

1. We recommend that for most applications and sample types, set target P1 value at >50%. Poisson statistics still apply, and we want to target only 1 active polymerase per ZMW. Pre-extension can help eliminate some >1 sequencing polymerase/ZMW to allow the target loading to increase from P1 ~37 to >50%.
2. As P1's increase, there may be some decrease in read length and this should be monitored.
3. We recommend monitoring the P0 value for sample overloading. We recommend that you set target P0 values at ~20%. **Note**: If the P0 values are <10%, then the SMRT® Cell is overloaded.
4. For application-based loading, we recommend the following:
   - Iso-Seq® libraries, and amplicons with pre-extension, will benefit if you target P1 at ~70% and keep P2 <20%.
   - For *de novo* libraries generated from the SMRTbell® Express Template Prep Kit, we recommend targeting P1 ~50%.
   - For microbial multiplex samples, we recommend targeting P1 ~50-65%.

PacBio terminology:

- P0: The Percentage of ZMWs that are Empty.
- P1: The Percentage of ZMWs that are Productive.
- P2: The Percentage of ZMWs that are not P1 or P0.

Required Use of Sequel Internal Control

As a reminder, PacBio requires the use of the Sequel Internal Control for consumables to be eligible for reimbursement.

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