

Diffusion Loading and Pre-Extension Time Recommendations for the Sequel® System

Quick Reference Card

Diffusion Loading

PacBio recommends Diffusion Loading when using the Sequel Binding Kit v2.0 and v2.1 and Sequencing Kit v2.1. Note that the SMRTbell® Express Template Prep Kit is **only** compatible with Diffusion Loading. The loading recommendations are shown in the table below and are for the Sequel System **only**.

PacBio still recommends MagBead Loading for the PacBio RS II System and Iso-Seq® applications.

Loading Method	Insert Size (bp)	SMRTbell Template Prep Kit v1.0 (pmol on-plate concentration)	SMRTbell Express Template Prep Kit (pmol on-plate concentration)	Target Fraction of Productive Loading* (% P1)
Diffusion	<250	Unsupported	Unsupported	Unsupported
	>250 (Fixed insert sizes)	2 - 8	Unsupported	~70%
	>15K Size-Selected Libraries	2 - 8 **	2 - 8 **	>50%
	Microbial Multiplex (Non-Size-Selected Libraries)	3 - 6 **	Unsupported	50 - 65%
	Microbial Multiplex (Size-Selected Libraries)	6 - 8 **	Unsupported	50 - 65%
	Non-Size-Selected Libraries	2 - 8 **	Unsupported	>50%
MagBead	<15K	Unsupported	Unsupported	Unsupported
	>15K	2 - 16		>50%
	Iso-Seq Method	40 - 50		~70%

* Targeted fraction of productive loading (P1) are recommended as optimal for sequencing yield. Indications for overloaded libraries can be gauged by P0 values. Note: If P0 values are <10% then the SMRT Cell is overloaded.

** Sample quality, size, and binding efficiency may affect loading concentrations, which may result in some loading as low as 1 pM or as high as 20 pM.

For best results, we recommend that you load higher than classic Poisson distribution. For more information, please refer to the PacBio document "User Bulletin - Guidelines for Updated Loading Recommendations and Required Use of Sequel Internal Control".

Pre-Extension Time Recommendations

Pre-extension is a feature associated with versions of the Sequel Sequencing Kits v2.0 (and greater). **Pre-extension is highly recommended for all runs except for inserts <1 kb and size-selected long insert libraries.**

PacBio does not recommend pre-extension for small insert libraries because the polymerase will have made one complete circle before data acquisition starts. In general, the pre-extension time is calculated using this formula:

$$\text{Pre-extension time (minutes)} = \text{Insert Size (bp)} \times 2 / 1.5 \text{ (bases/second)} / 60 \text{ (seconds)}$$

Generalized guidelines by mean insert size and applications are shown in the table below. Further optimization of pre-extension time is recommended for specific applications not listed to maximize sequencing yield, and barcode recovery if multiplexing.

Mean Insert Size / Applications	Pre-Extension Time (minutes)
<1 kb	N/A
1 kb	>30
2 kb	45
4 kb	90
6 kb	135
10 kb	220
Microbial Multiplex	120
Iso-Seq Method	240
Size Selected Libraries	N/A

Revision History (Description)	Version	Date
Initial Release	01	March 2018
Updated to include Microbial Multiplexing information (internal release only)	02 (Internal Only)	April 2018
Updated to include Microbial Multiplexing information	03	May 2018

For Research Use Only. Not for use in diagnostic procedures. © Copyright 2018, Pacific Biosciences of California, Inc. All rights reserved. Information in this document is subject to change without notice. Pacific Biosciences assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of Pacific Biosciences products and/or third party products. Please refer to the applicable Pacific Biosciences Terms and Conditions of Sale and to the applicable license terms at <https://www.pacb.com/legal-and-trademarks/terms-and-conditions-of-sale/>. Pacific Biosciences, the Pacific Biosciences logo, PacBio, SMRT, SMRTbell, Iso-Seq and Sequel are trademarks of Pacific Biosciences. BluePippin and SageELF are trademarks of Sage Science, Inc. NGS-go and NGSengine are trademarks of GenDx. FEMTO Pulse and Fragment Analyzer are trademarks of Advanced Analytical Technologies. All other trademarks are the sole property of their respective owners.