

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 2.0 and 2.1 and Sequencing Kit 2.1. 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)
Diffusion	<250	Unsupported	Unsupported
	>250 (fixed insert sizes)	2 - 8	Unsupported
	>15K Size Selected Libraries	2 - 8*	2 - 8*
	Non-Size Selected Libraries	2 - 8*	Unsupported
MagBead	<15K	Unsupported	Unsupported
	>15K	2 - 16	
	Iso-Seq	40 - 50	

*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.

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 by this formula:

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

Some guidelines, generalized by mean insert size, are shown in the table below. It is important to note that these are guidelines and that experiments are recommended to determine the most accurate times for specific applications.

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

Revision History (Description)	Version	Date
Initial release.	01	February 2018

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