

Loading and Pre-Extension Recommendations for the Sequel® System

Quick Reference Card

Please refer to the tables below for loading and pre-extension recommendations for all insert sizes.

Loading Method	Insert Size (bp)	SMRTbell Template Prep Kit v1.0 (pM, on-plate concentration)	SMRTbell Express Template Prep Kit (pM, on-plate concentration)	Target % P1*
Diffusion	<250	Not Supported	Not Supported	Not Supported
	>250 (Fixed insert sizes)	2 - 8	Not Supported	~70%
	>15K Size-Selected Libraries	2 - 8 **	2 - 8 **	>50%
	Microbial Multiplexing (Size Selected and Non-Size-Selected)	6 - 8 **	Not Supported	50 - 65%
	Non-Size-Selected Libraries	2 - 8 **	Not Supported	>50%
	Iso-Seq Libraries	2 - 8 **	Not Supported	<70%
MagBead	<15K	Not Supported	Not Supported	Not Supported
	>15K	2 - 16		>50%
	Iso-Seq Libraries	40 - 50		<70%

* Recommended for optimal total bases per SMRT Cell. 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 loading higher than classic Poisson distribution.

Pre-Extension Time Recommendations

Pre-extension is a Software feature that allows SMRTbell molecules to reach rolling circle replication (when the polymerase is most stable) before movie collection is initiated.

Pre-extension is highly recommended for all insert sizes except size-selected large insert libraries.

Generalized pre-extension guidelines by mean insert size and applications are summarized in the table below. Further optimization of pre-extension time is recommended for specific applications to maximize read length and yield. Pre-extension is not recommended for samples prepared with the Express Template Prep Kit v1.

Mean Insert Size / Applications	Minimum Pre-Extension Time (minutes)
≤1 kb	30
2 kb	45
4 kb	90
6 kb	135
10 kb	220
Microbial Multiplex	120
Iso-Seq Libraries	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
Updated loading and pre-extension recommendations for all SMRTbell insert sizes as a result of SMRT Link v6.0.0 release. New recommendations for loading Iso-Seq Libraries. Added "Minimum" to table header for "Pre-Extension Time".	04	October 2018

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