

# Loading and Pre-Extension Recommendations for the Sequel® II System

## Quick Reference Card

Please refer to the tables below for loading and pre-extension recommendations for large insert and High Fidelity libraries.

Loading Method	Insert Size	SMRTbell Template Prep Kit 1.0 (pM, on-plate concentration)	SMRTbell Express Template Prep Kit 2.0 (pM, on-plate concentration)	Target % P1*
Diffusion	> 15 kb	Not Supported	15 - 50	50 - 70
	> 30 kb	Not Supported	15 - 50	50 - 70
	11-13 kb High Fidelity Libraries	25 – 75	Not Supported	50 - 70

\* Recommended for optimal yield per SMRT Cell (defined as maximized raw yield for long insert reads, and unique molecular yield for High Fidelity Reads.) 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 optimum loading concentrations as low as 10 pM or as high as 50 pM.

### 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 recommended for High-Fidelity libraries only.

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.

Mean Insert Size / Applications	Minimum Pre-Extension Time (minutes)
> 15 kb	No pre-extension
> 30 kb	No pre-extension
High Fidelity Libraries (11 kb – 13 kb)	120

Revision History (Description)	Version	Date
For validation	Validation	April 2019

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