

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

### Quick Reference Card

Refer to the table below for loading recommendations for the Sequel System. Note that the Sequel Sequencing Plate 3.0 should be used for all applications.

Applications	Data Type	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
De Novo Assembly – Continuous Long Reads (>15 kb)	CLR	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	2 - 8
De Novo Assembly - Microbial Multiplexing (10 – 15 kb)	CLR	Express Prep 2.0	Binding Kit 3.0	v4	4	1.2X AMPure PB Beads	6 - 12
De Novo Assembly - Low DNA Input or 15 kb)	CCS	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	2 - 8
De Novo Assembly – HiFi Reads or Variant Detection – HiFi Reads (15 – 25 kb)	CCS	Express Prep 2.0	Binding Kit 3.0	v2	4	1.2X AMPure PB Beads	5 - 7
Structural Variation Detection (>15 kb)	CLR	Express Prep 2.0	Binding Kit 3.0	v2	4	1.2X AMPure PB Beads	2 - 8
Shotgun Metagenomics (10 kb)	CCS	Express Prep 2.0	Binding Kit 3.0	v2	4	1.2X AMPure PB Beads	5 - 7
Amplicon Sequencing (including 16S Amplicons)	CCS	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	2 - 8
Iso-Seq Method (short, standard, long) or Single-Cell Iso-Seq Method	CCS	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X ProNex Beads	2 - 8

Target % P1 loading is 50% to 70% for all listed applications. This P1 loading range is recommended for optimal yield per SMRT Cell (defined as maximized raw yield for long insert reads, and unique molecular yield for HiFi 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 100 pM.

## Pre-Extension and Movie 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.

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 data yield.

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
De Novo Assembly – Continuous Long Reads (>15 kb)	0	10
De Novo Assembly - Microbial Multiplexing (10 – 15 kb)	2	10
De Novo Assembly - Low DNA Input (15 kb)	2	20
De Novo Assembly – HiFi Reads or Variant Detection – HiFi Reads (15 – 25 kb)	8	20
Structural Variation Detection (>15 kb)	0	10
Shotgun Metagenomics (10 kb, AMPure PB bead size-selection)	8	20
Amplicons (≥3 kb)	Use default values in Run Design	6 - 20
Amplicons (<3 kb)	Use default values in Run Design	8 - 10
16S (1.6 - 2.5 kb)	1.3	10
Iso-Seq Method (short, standard, long)	4	20

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
Updated to include SMRTbell Express Template Prep Kit 2.0.	05	February 2019
Removed SMRTbell Express Template Prep Kit column. Changed "Not Supported" values for Diffusion >250 bp and Iso-Seq libraries.	06	May 2019
Updated Diffusion Loading recommendations for SMRTbell Express Template Prep Kit 2.0 >250 bp and Iso-Seq libraries.	07	June 2019
Updated QRC for Sequel. New Table 1 contains more detailed information for clarity.	08	September 2019
Corrected Large Insert and Microbial Multiplexing Movie Collection time from 15 to 10 hours.	09	October 2019
Removed reference to Sequel "II" in introductory sentence.	10	October 2019
Updated to include parameters for several applications.	11	November 2020

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