

Loading and Pre-Extension Recommendations for Sequel® II/IIe Systems

Quick Reference Card

Refer to the table below for loading recommendations for the Sequel II and Sequel IIe Systems. Note that the sample quality, size, and binding efficiency may affect loading concentrations. This may result in optimum loading concentrations as low as 20 pM or as high as 150 pM. Use Sequel II Sequencing Plate 2.0 for all application types.

Note: If your SMRT® Link software shows default settings for Sample Setup and Run Design that differ from this Quick Reference Card, follow the guidance provided in this QRC. For questions regarding SMRT Link Sample Setup or Run Design recommendations for specific applications, please contact PacBio [Technical Support](#).

Applications	Data Type	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
De Novo Assembly – Microbial Multiplexing (10 kb – 15 kb)	CLR	Express Prep 2.0	Binding Kit 2.2*	v5*	1	1.2X AMPure® PB Beads	70 – 100
De Novo Assembly – Low DNA Input (15 kb)	HiFi	Express Prep 2.0	Binding Kit 2.2*	v5*	1	1.2X AMPure PB Beads	30 – 70
De Novo Assembly – Ultra-Low DNA Input or Variant Detection – Ultra-Low DNA Input (10 kb – 12 kb)	HiFi	Express Prep 2.0	Binding Kit 2.2*	v5*	1	1.2X ProNex Beads	50 - 70
De Novo Assembly - HiFi Reads or Variant Detection – HiFi Reads (15 kb – 18 kb)	HiFi	Express Prep 2.0	Binding Kit 2.2	v5	1	1.2X AMPure PB Beads	30 - 70
Shotgun Metagenomics (10 kb)	HiFi	Express Prep 2.0	Binding Kit 2.2*	v5*	1	1.2X AMPure PB Beads	30 – 70
Amplicons (≥3 kb)	HiFi	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Amplicons (< 3 kb)	HiFi	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 150
16S Amplicons (1.6 kb - 2.5 kb)	HiFi	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 100
Iso-Seq / Single-Cell Iso-Seq Method (standard samples)	HiFi	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X ProNex Beads	40 - 80
Iso-Seq / Single-Cell Iso-Seq Method (focus on long transcripts)	HiFi	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X ProNex Beads	50 - 100

Target % P1 is 50 to 70. Recommended for optimal yield per SMRT Cell (defined as maximized raw yield for long insert CLR 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.

* Also compatible with Sequel II Binding Kit 2.0. If using Binding Kit 2.0, we recommend using Sequencing Primer v4, except for Shotgun Metagenomics, where we recommend Sequencing Primer v2. If your SMRT Link Sample Setup software version does not specify “Sequel II Binding Kit 2.2”/“Sequencing Primer v5” as default values, you must manually select “Sequel II Binding Kit 2.2”/“Sequencing Primer v5” in order to use these reagents with these applications. After selecting “Sequel II Binding Kit 2.2”/“Sequencing Primer v5” in SMRT Link Sample Setup, we recommend using a 1-hr Polymerase Binding Time.

Pre-Extension and Movie Time Recommendations

Pre-extension is a Run Design feature that allows SMRTbell template 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 yield.

Applications	Pre-Extension Time (hr)	Adaptive Loading Target (P1 + P2)	Movie Collection Time (hr)
De Novo Assembly - Microbial Multiplexing (10 kb – 15 kb)	2*	0.85 (Binding Kit 2.2)*	15
De Novo Assembly – Low DNA Input (15 kb)	2*	0.85 (Binding Kit 2.2)*	30
De Novo Assembly – Ultra-Low DNA Input or Variant Detection – Ultra-Low DNA Input (10 kb – 12 kb)	2*	0.85 (Binding Kit 2.2)*	30
De Novo Assembly – HiFi Reads or Variant & SV Detection – HiFi Reads (15 kb – 18 kb)	2*	0.85 (Binding Kit 2.2)*	30
Shotgun Metagenomics (10 kb)	2*	0.85 (Binding Kit 2.2)*	30
Amplicons (≥3 kb)	Use default values in Run Design	N/A	10 - 30
Amplicons (<3 kb)	Use default values in Run Design	N/A	10
16S Amplicons (1.6 kb - 2.5 kb)	0.5	N/A	10
Iso-Seq / Single-Cell Iso-Seq Method (standard samples)	2	N/A	24
Iso-Seq / Single-Cell Iso-Seq Method (focus on long transcripts)	2	N/A	24

* If using Sequel II Binding Kit 2.2 for these applications, use a 2-hr Pre-Extension Time and enable Adaptive Loading (set Adaptive Loading Target (P1 + P2) = 0.85). If using Binding Kit 2.0 for these applications, use a 2-hr Pre-Extension Time and do not enable Adaptive Loading.

Revision History (Description)	Version	Date
Initial release.	01	April 2019
Added loading recommendations for Iso-Seq and 16S applications.	02	June 2019
Updated recommendations for the new Binding Kit and Sequencing plate	03	September 2019
Updated to add multiplex options for various applications.	04	November 2019
Updated to add Ultra-Low DNA and several other parameter changes.	05	November 2020
Updated to add Sequel II Polymerase 2.2	06	April 2021
Added "Also compatible with Binding Kit 2.2 and Sequencing Primer v5" in footer of first table. Updated De Novo Assembly – HiFi Reads or Variant & SV Detection – HiFi Reads insert sizes to 15 kb to 18 kb).	07	May 2021
Updated Binding Kit, Sequencing Primer, Pre-Extension Time and Adaptive Loading for Microbial Multiplexing, Low-DNA and Ultra-Low DNA for De Novo Assembly and Shotgun Metagenomics applications.	08	July 2021

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