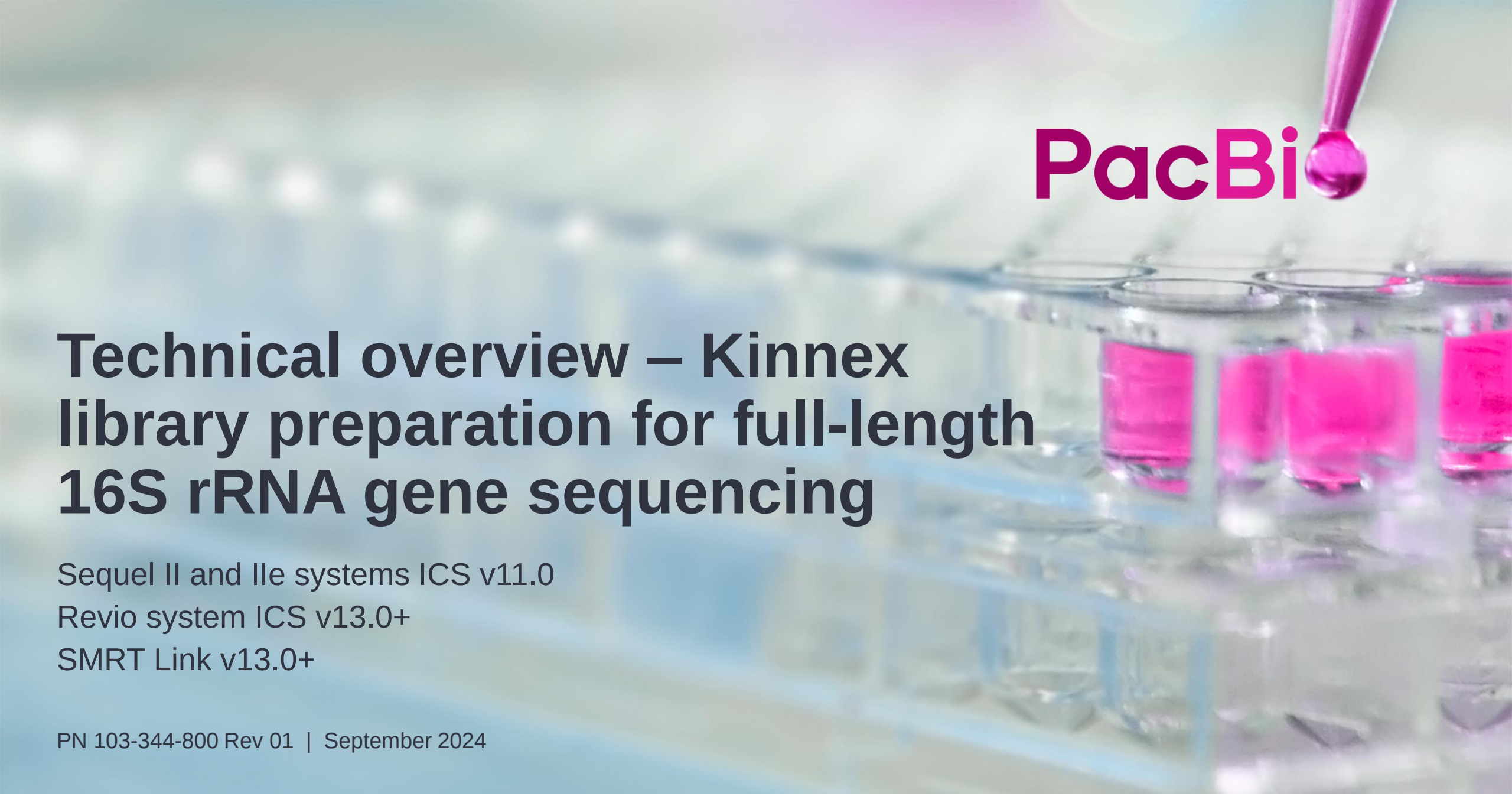


Technical overview – Kinnex library preparation for full-length 16S rRNA gene sequencing

Sequel II and IIe systems ICS v11.0
Revio system ICS v13.0+
SMRT Link v13.0+

PN 103-344-800 Rev 01 | September 2024

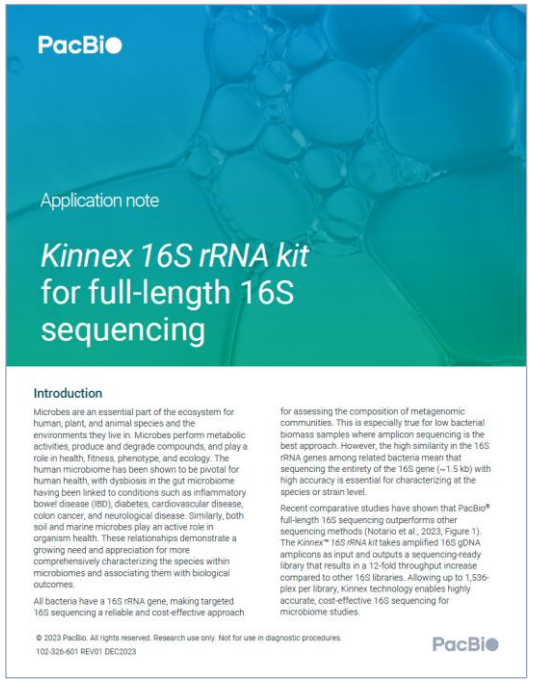
Technical overview – Kinnex library preparation for full-length 16S rRNA gene sequencing

Technical Overview

1. Kinnex 16S rRNA method overview
2. Kinnex 16S rRNA library preparation workflow details
3. Kinnex 16S rRNA sequencing preparation workflow details
4. Kinnex 16S rRNA example sequencing performance data
5. Kinnex 16S rRNA data analysis workflow overview
6. Technical documentation & applications support resources

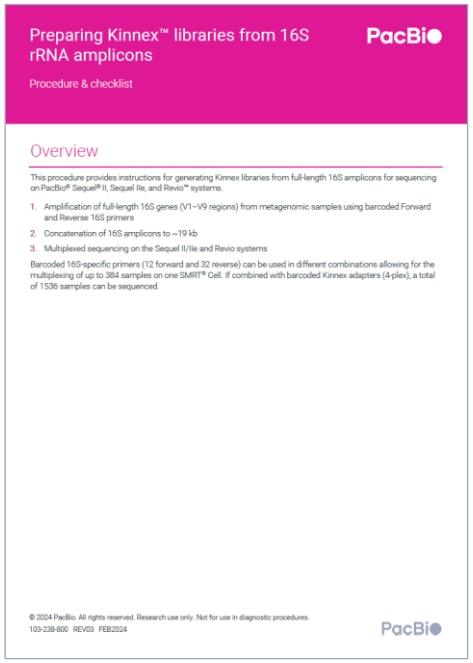
Kinnex library preparation for full-length 16S rRNA gene sequencing: Getting started

Application-specific literature → **Application-specific protocol** → **Application-specific technical overview** → **Library preparation, sequencing & analysis**



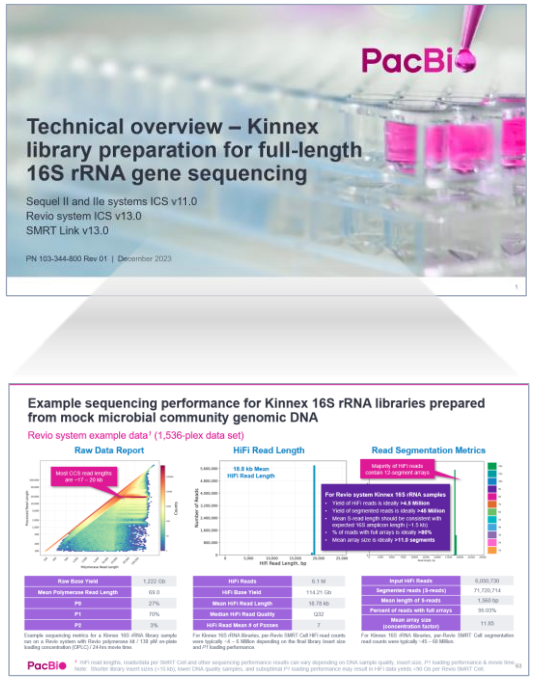
Application note – Kinnex 16S rRNA kit for full-length 16S sequencing (102-326-601)

Summary overview of application-specific library preparation and data analysis workflow recommendations.



Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800)

Technical documentation containing application-specific library preparation protocol details.



Technical overview – Kinnex library preparation for full-length 16S rRNA gene sequencing (103-344-800)

Technical overview presentations describe sample preparation details for constructing Kinnex HiFi libraries for specific applications. Example sequencing performance data for a given application are also summarized.

16S gene amplification
(Third-party PCR reagents)

1 – 2 ng input gDNA amount per sample

Use barcoded forward & reverse 16S primers

Kinnex library preparation
(Kinnex PCR 12-fold kit + Kinnex concatenation kit)

Use barcoded 16S amplicons to generate Kinnex library containing 12-segment array

Sample multiplexing up to 1,536-plex

SMRT sequencing
(Sequel II/IIe & Revio systems)

Perform ABC* and sequence Kinnex libraries on PacBio long-read systems

Data analysis
(SMRT Link and GitHub tools)

Use SMRT Link Read Segmentation data utility to split arrayed 16S HiFi reads

Perform tertiary analysis using GitHub tools or custom pipeline

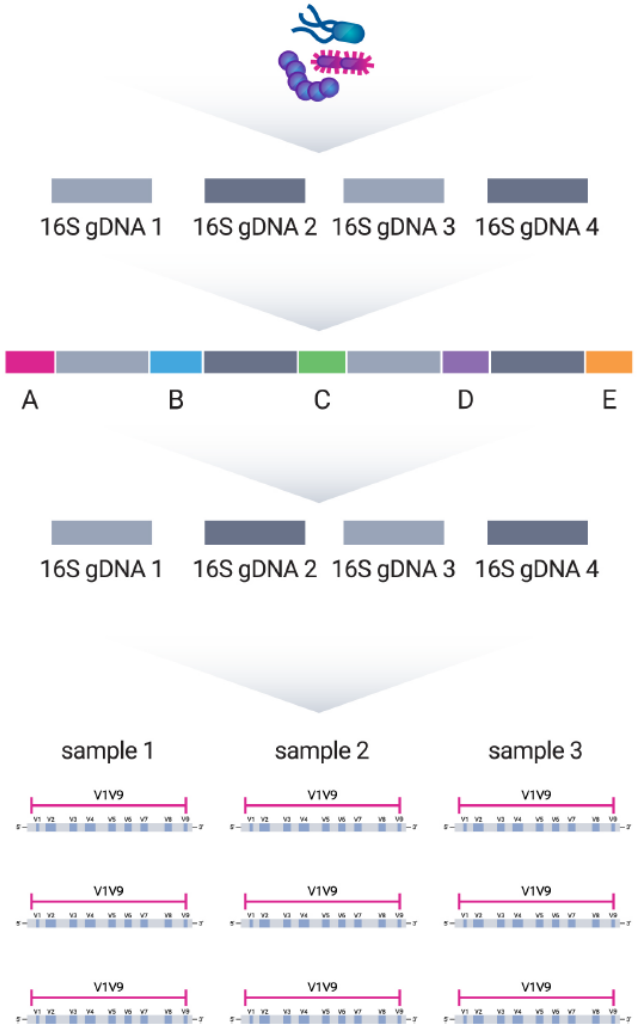
PacBio

* ABC = Anneal primer / Bind polymerase / Clean up bound complex

Kinnex 16S rRNA method overview

Kinnex 16S rRNA method overview

Use Kinnex full-length RNA kit to perform high-accuracy, full-length isoform sequencing with PacBio long-read systems



16S amplicon generation

Kinnex library preparation

PacBio sequencing

Read segmentation + amplicon demultiplexing

- Official protocol to generate barcoded 16S amplicons compatible with Kinnex 16S rRNA kit
- Protocol supports up to 384-plex multiplexing
- 2-day Kinnex library preparation using Kinnex 16S rRNA kit (103-072-100)
- SMRT Link Run Design support for ‘Kinnex 16S rRNA’ application type option with auto-analysis (read segmentation only)[1]
- Demultiplex 16S amplicon barcodes in SMRT Link to generate per-sample read BAM files
- Analyze per-sample BAM files using GitHub tools or other custom 16S analysis pipeline

[1] Kinnex 16S rRNA kit requires SMRT Link v13.0 or higher.

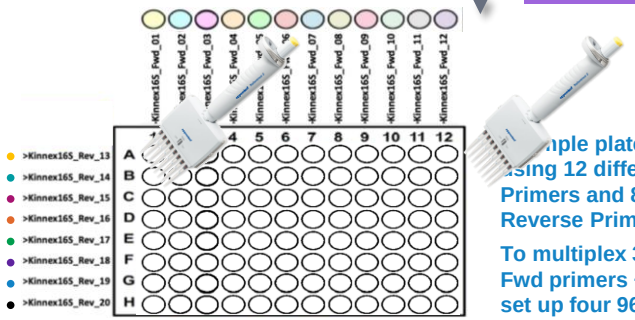
Kinnex 16S rRNA method overview (cont.)

Full-length 16S gene amplification

Bacterial gDNA fragments isolated from metagenomic samples

Recommended gDNA input for full-length 16S PCR amplification = 1-2 ng

PCR amplification of full-length 16S genes[1]



Sample plate layout for 96-plex PCR design using 12 different 16S Barcoded Forward Primers and 8 different 16S Barcoded Reverse Primers.

To multiplex 384 samples, use 12 barcoded Fwd primers + 32 barcoded Rev primers and set up four 96-well PCR reaction plates.

Can barcode up to 384 samples during 16S gene amplification step using PacBio barcoded Fwd and Rev primers[1]

Forward (F) PCR primer (Kinnex16S_Fwd_ XX[1])

Reverse (R) PCR primer (Kinnex16S_Rev_ XX[1])

Dual-barcoded full-length 16S amplicon product

Contains Kinnex PCR handle 1

Equal-volume pooling & cleanup

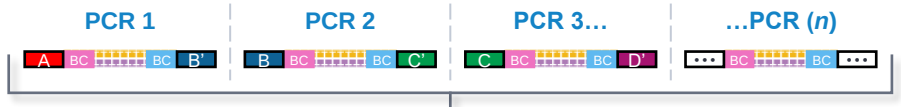
Contains Kinnex PCR handle 2

Pooled barcoded full-length 16S amplicons for input into Kinnex library construction

Kinnex library prep, sequencing & analysis

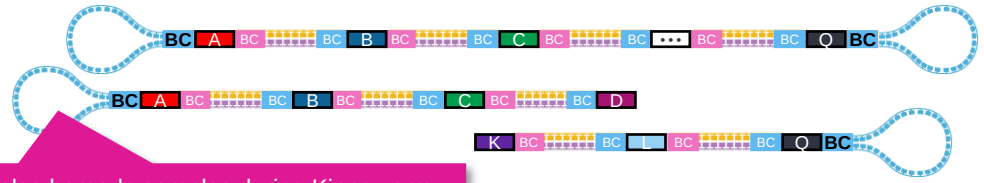
Amplified 16S products containing a different PacBio barcode at each end (Fwd BC ≠ Rev BC)

Kinnex PCR (12 parallel PCR Rx to append Kinnex segmentation adapters)



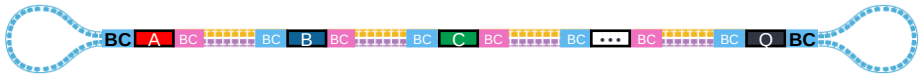
Pooling & Kinnex array formation

Barcoded Kinnex terminal adapter (bcM0001-bcM0004)[2]



Can also barcode samples during Kinnex array formation using up to 4 different barcoded Kinnex terminal SMRTbell adapters[2]

DNA damage repair & nuclease Tx



Anneal Kinnex sequencing primer / Bind polymerase / Cleanup (ABC)

Purified polymerase-bound 12-segment Kinnex library

Long-read sequencing & data analysis

Sequel II, Sequel IIe or Revio system

Third-party metagenomic secondary analysis tools

[1] 12 different 16S barcoded Forward PCR primers + 32 different 16S barcoded Reverse PCR primers are available for 16S gene amplification step to multiplex up to 384 samples.

[2] Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

Kinnex 16S rRNA library preparation procedure description

Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800) describes the workflow for generating Kinnex libraries from full-length 16S amplicons for sequencing on PacBio Sequel II, Sequel IIe, and Revio systems

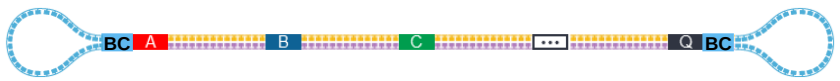
Overview

This procedure provides instructions for generating Kinnex libraries from full-length 16S amplicons for sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

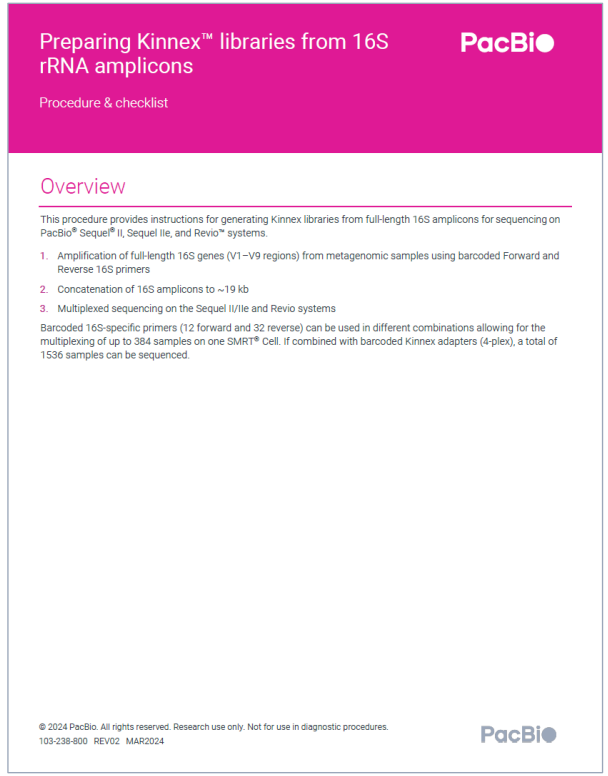
1. Amplification of full-length 16S genes (V1–V9 regions) from metagenomic samples using barcoded Forward and Reverse 16S primers
2. Concatenation of 16S amplicons to ~19 kb
3. Multiplexed sequencing on the Sequel II/IIe and Revio systems

Barcoded 16S-specific primers (12 forward and 32 reverse) can be used in different combinations allowing for the multiplexing of up to 384 samples on one SMRT® Cell. If combined with barcoded Kinnex adapters (4-plex), a total of 1536 samples can be sequenced.

Kinnex 16S rRNA kit
103-072-100 (12 rxn)



Kinnex 16S rRNA library template (~18 kb)
Contains 12 concatenated full-length 16S amplicon segments


Preparing Kinnex™ libraries from 16S rRNA amplicons

PacBio

Procedure & checklist

Overview

This procedure provides instructions for generating Kinnex libraries from full-length 16S amplicons for sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

1. Amplification of full-length 16S genes (V1–V9 regions) from metagenomic samples using barcoded Forward and Reverse 16S primers
2. Concatenation of 16S amplicons to ~19 kb
3. Multiplexed sequencing on the Sequel II/IIe and Revio systems

Barcoded 16S-specific primers (12 forward and 32 reverse) can be used in different combinations allowing for the multiplexing of up to 384 samples on one SMRT® Cell. If combined with barcoded Kinnex adapters (4-plex), a total of 1536 samples can be sequenced.

103-238-800 REV02 MAR2024

PacBio

PacBio Documentation (103-238-800)

- Kinnex 16S rRNA library prep protocol uses **Kinnex 16S rRNA kit**
  → **Do not use** SMRTbell prep kit 3.0 with this protocol

Kinnex 16S rRNA kit components

Kinnex 16S rRNA kit provides full support for Kinnex library prep workflow starting with 16S amplicon DNA as input

Kinnex 16S rRNA kit bundle (103-072-100)

Includes Kinnex PCR kit, Kinnex concatenation and ancillary DNA cleanup reagents needed for incorporation of Kinnex segmentation adapters and Kinnex array formation for generating Kinnex 16S rRNA libraries.

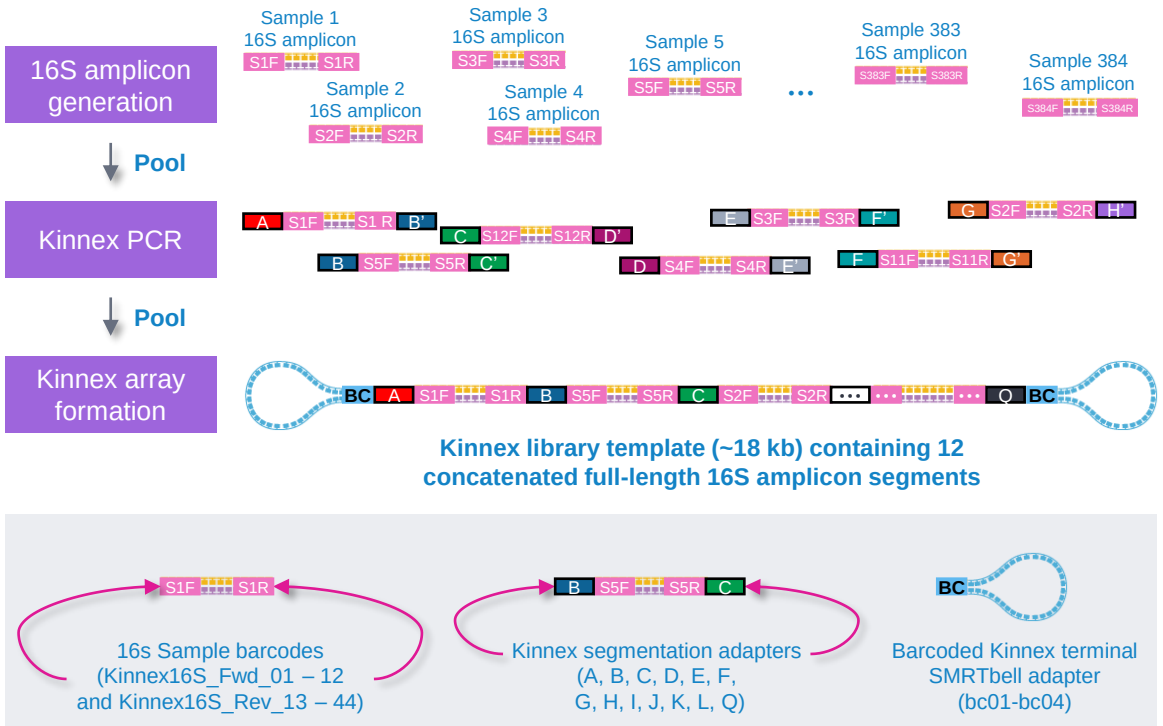
Kinnex 16S rRNA kit components		
Component		**Description**
1		**Kinnex PCR 12-fold kit (12 rxn)** • Contains reagents for Kinnex PCR to incorporate segmentation adapters
2		**Kinnex concatenation kit (12 rxn)** • Contains reagents for Kinnex array formation and SMRTbell template construction • Includes barcoded Kinnex adapter mixes (bcM0001 – bcM0004)
3		**SMRTbell cleanup beads** • For DNA cleanup
4		**Elution buffer** • For DNA cleanup

Kinnex 16S rRNA library barcoding options for sample multiplexing

Kinnex 16S rRNA library preparation procedure supports up to 1,536-plex sample multiplexing

Kinnex 16S rRNA library preparation procedure supports **up to 1,536-plex** sample multiplexing through combined use of:

- → 12 different 16S barcoded Forward PCR primers[1] (Kinnex16S_Fwd_01 – Kinnex16S_Fwd_12)
- → 32 different 16S barcoded Reverse PCR primers[1] (Kinnex16S_Rev_13 – Kinnex16S_Rev_44)
- → 4 different barcoded Kinnex terminal SMRTbell adapters (bc01 – bc04)



[1] Refer to ***Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons*** (103-238-800) for forward and reverse barcoded 16S PCR primer sequences.

Kinnex 16S rRNA experimental design considerations

Kinnex 16S rRNA application use case recommendations for PacBio systems

	Sequel II and IIe systems		Revio system	
Experimental goal	Determine the microbial diversity (phylogeny and taxonomy) of bacteria in a metagenomic sample			
Sample multiplexing[1]	Up to 384 samples per SMRT Cell 8M (384-plex)		Up to 1,536 samples per Revio SMRT Cell (1536-plex)	
Expected coverage per sample[2]	**96-plex**	**260 K**	**96-plex**	**625 K**
	192-plex	**130 K**	**192-plex**	**313 K**
	384-plex	**65 K**	**384-plex**	**156 K**
	768-plex	33 K	**768-plex**	**78 K**
	1,536-plex	16 K	**1,536-plex**	**39 K**
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800)			
Metagenomic DNA input amount input into 16S gene amplification	1-2 ng of input gDNA per metagenomic sample			
16S amplicon DNA input into Kinnex library prep workflow	35 ng of purified pooled 16S amplicon DNA			
SMRT Link data analysis workflows	Read Segmentation			
Community data analysis tools	pb-16S-nf			

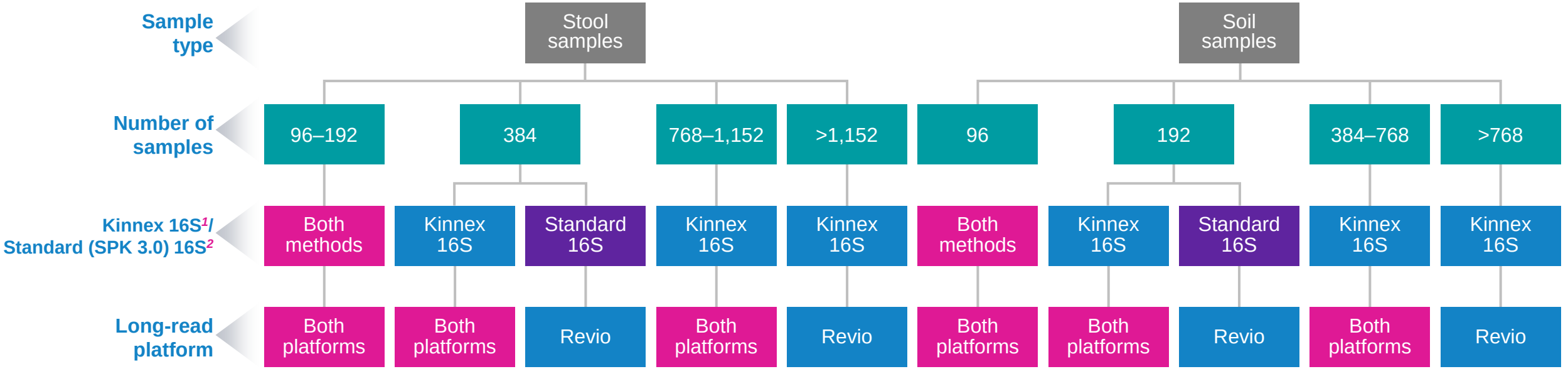
[1] Kinnex concatenation kit (103-071-800) can support up to 1,536-plex sample multiplexing through the combined use of 12 different 16S barcoded Forward PCR primers + 32 different 16S barcoded Reverse PCR primers and 4 different barcoded Kinnex terminal SMRTbell adapters during Kinnex 16s rRNA library construction.

[2] With proper full array formation and adequate sequencing, one SMRT Cell on the Sequel II, IIe, and Revio systems are expected to achieve 20–25 million and 50–60 million 16S sequences, respectively. For most 16S analysis applications, typically aim for ~30-50 K reads/sample.

PacBio

Kinnex 16S rRNA experimental design considerations (cont.)

PacBio full-length 16S protocol and PacBio long-read sequencing platform recommendations for different sample types



Stool samples typically need 10–20k average reads/sample; soil samples need 30–50k

[1] Refer to ***Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons*** (103-238-800).

[2] Refer to ***Procedure & checklist – Amplification of bacterial full-length 16S rRNA gene with barcoded primers*** (101-599-700) and ***Procedure & checklist – Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0*** (102-359-000).

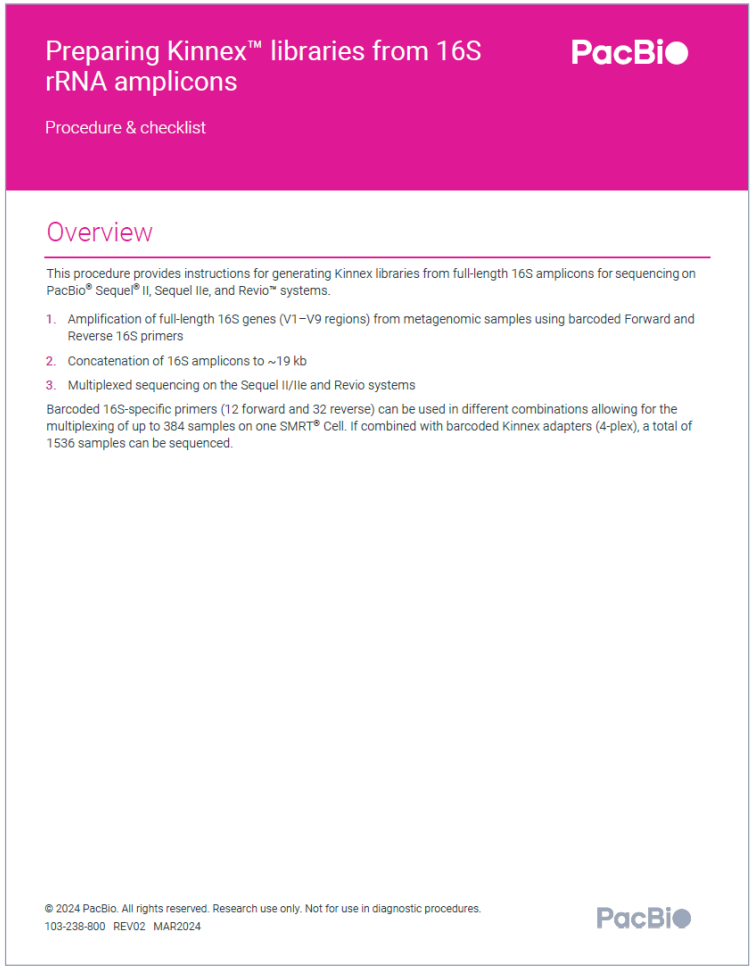
Kinnex 16S rRNA library preparation workflow details

Procedure & checklist – Preparing Kinnex libraries from 16S amplicons (103-238-800)

Procedure & checklist 103-238-800 describes the workflow for generating Kinnex libraries from full-length 16S amplicons for sequencing on PacBio Sequel II, Sequel IIe, and Revio systems[1]

Procedure & checklist contents

1. Barcoded 16S gene-specific forward and reverse primer oligo synthesis and storage recommendations.
2. Bacterial input genomic DNA QC recommendations and general best practices for reagent & sample handling.
3. Enzymatic workflow steps for PCR amplification of 16S gene with barcoded primers.
4. Enzymatic workflow steps for construction of 12-segment Kinnex arrays from amplified 16S DNA products.
5. Enzymatic workflow steps for DNA damage repair & nuclease treatment of Kinnex libraries.
6. Workflow steps for final cleanup of Kinnex SMRTbell libraries using SMRTbell cleanup beads.


Preparing Kinnex™ libraries from 16S rRNA amplicons

PacBio

Procedure & checklist

Overview

This procedure provides instructions for generating Kinnex libraries from full-length 16S amplicons for sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

1. Amplification of full-length 16S genes (V1–V9 regions) from metagenomic samples using barcoded Forward and Reverse 16S primers
2. Concatenation of 16S amplicons to ~19 kb
3. Multiplexed sequencing on the Sequel II/IIe and Revio systems

Barcoded 16S-specific primers (12 forward and 32 reverse) can be used in different combinations allowing for the multiplexing of up to 384 samples on one SMRT® Cell. If combined with barcoded Kinnex adapters (4-plex), a total of 1536 samples can be sequenced.

103-238-800 REV02 MAR2024

PacBio

PacBio Documentation (103-238-800)

[1] Refer to **SMRT Link Sample Setup** for recommended sample setup conditions for sequencing Kinnex full-length RNA library samples on PacBio Sequel II/IIe & Revio systems.

Kinnex 16S rRNA library construction workflow overview

Procedure & checklist – Preparing Kinnex libraries using the Kinnex 16S rRNA kit (103-238-800)

	Cleanup	DNA QC	Walk-away time*	
1 16S full-length sequence capture	N/A	N/A	1.5 hrs	**Day 1 (~4 hrs*)**
Safe stop				
2 Pooling & SMRTbell cleanup	1.1X SMRTbell cleanup beads	Qubit dsDNA HS assay	0.5 hrs	
Safe stop				
3 Kinnex PCR & SMRTbell cleanup	1.1X SMRTbell cleanup beads	Qubit dsDNA HS assay	1.5 - 2.0 hrs	
Safe stop				
4 Kinnex array formation & DNA repair	N/A	N/A	1.5 hrs	**Day 2 (~2.5 hrs*)**
5 SMRTbell cleanup	1.0X SMRTbell cleanup beads	N/A	20 min	
Safe stop				
6 Nuclease treatment	N/A	N/A	15 min	
7 Final cleanup with SMRTbell cleanup beads	1.0X SMRTbell cleanup beads	Qubit dsDNA HS assay Femto Pulse gDNA 165 kb kit	20 min	

* **Excludes** hands-on time for setting up enzymatic reaction steps and additional time required to perform DNA sizing QC and DNA concentration QC.

General best practices recommendations for preparing Kinnex 16S rRNA libraries

Preparation of barcoded 16S gene-specific forward and reverse primers

- We recommend resuspending stock oligos with a target concentration of 100 µM in 10 mM Tris-HCl pH 8.0–8.5 (elution buffer) or low TE (10 mM Tris-HCl with 0.1 mM EDTA)
- To prepare oligo working solutions, diilute each primer individually to 2.5 µM in 10 mM
- Tris-HCl pH 8.0–8.5 (elution buffer) or low TE. For example, add 5 µL of 100 µM primer stock to 195 µL of 10 mM Tris-HCl pH 8.0–8.5 buffer. This volume of diluted oligo is sufficient for running more than 50 PCR reactions
- Always mix primer stocks well before preparing dilutions. Prior to use, verify that the concentration of each diluted oligo solution is 2.5 µM by directly measuring the OD260 value using a Nanodrop system
- Aliquot the diluted oligos in 96-well plates in the format shown in the plate maps below

Plate map for preparing barcoded 16S gene-specific forward primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	Fwd_01	Fwd_02	Fwd_03	Fwd_04	Fwd_05	Fwd_06	Fwd_07	Fwd_08	Fwd_09	Fwd_10	Fwd_11	Fwd_12
B												
C	Fwd_01	Fwd_02	Fwd_03	Fwd_04	Fwd_05	Fwd_06	Fwd_07	Fwd_08	Fwd_09	Fwd_10	Fwd_11	Fwd_12
D												
E	Fwd_01	Fwd_02	Fwd_03	Fwd_04	Fwd_05	Fwd_06	Fwd_07	Fwd_08	Fwd_09	Fwd_10	Fwd_11	Fwd_12
F												
G	Fwd_01	Fwd_02	Fwd_03	Fwd_04	Fwd_05	Fwd_06	Fwd_07	Fwd_08	Fwd_09	Fwd_10	Fwd_11	Fwd_12
H												

Plate map for preparing barcoded 16S gene-specific reverse primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	Rev_13		Rev_21		Rev_29		Rev_37					
B	Rev_14		Rev_22		Rev_30		Rev_38					
C	Rev_15		Rev_23		Rev_31		Rev_39					
D	Rev_16		Rev_24		Rev_32		Rev_40					
E	Rev_17		Rev_25		Rev_33		Rev_41					
F	Rev_18		Rev_26		Rev_34		Rev_42					
G	Rev_19		Rev_27		Rev_35		Rev_43					
H	Rev_20		Rev_28		Rev_36		Rev_44					

* **Room temperature** (RT) is defined as any temperature in the range of 18 – 23°C for this protocol.

General best practices recommendations for preparing Kinnex 16S rRNA libraries (cont.)

DNA input and reagent & sample handling

Required DNA input amount

- Recommended input bacterial genomic DNA amount per sample is 1–2 ng
- Expected 16S amplicon size is approximately 1,500 bases and typical 16S PCR product yields are 50–300 ng per sample

DNA QC & handling recommendations for bacterial gDNA isolated from metagenomic samples

- For best results, characterize bacterial gDNA samples thoroughly and normalize gDNA concentrations before use
- Bring gDNA samples to room temperature and mix well by pipetting to ensure sample homogeneity, then measure gDNA concentration using Qubit dsDNA HS assay reagents
- Assess sample purity using a Nanodrop system → OD260/280 should be between 1.8 and 2.0 for purified double-stranded DNA
- To ensure pipetting accuracy, plan to deliver 1–2 ng of gDNA to each individual PCR reaction in a constant 5 µL volume
  - Normalize sample gDNA concentration to 0.2–0.4 ng/µL in 10 mM Tris-HCl pH 8.0–8.5 (elution buffer) prior to setting up PCR reactions.
  - Note: Nuclease-free water and Elution buffer (EB) from PacBio can be used in place of 10mM Tris-HCl pH 8.0–8.5 for gDNA normalization.
- Based on prior PacBio experience, QIAgen Powerfecal Pro kit extracts DNA of sufficient quality for this workflow.

Reagent and sample handling

- Thaw PCR Ready Mix on ice and mix well before use
- Note: All PCR reactions described in this procedure must be set up and kept on ice until read to load onto thermal cycler instrument
  - High proofreading activity of the enzyme in the PCR Ready Mix will rapidly degrade primers at room temperature

* **Room temperature** (RT) is defined as any temperature in the range of 18 – 23°C for this protocol.

16S full-length sequence capture

Perform PCR amplification of 16S gene with barcoded forward and reverse primers

1 16S full-length sequence capture
↓ Safe stop
2 Pooling & SMRTbell cleanup
↓ Safe stop
3 Kinnex PCR & SMRTbell cleanup
↓ Safe stop
4 Kinnex array formation & DNA repair
↓
5 SMRTbell cleanup
↓ Safe stop
6 Nuclease treatment
↓
7 Final cleanup with SMRTbell cleanup beads

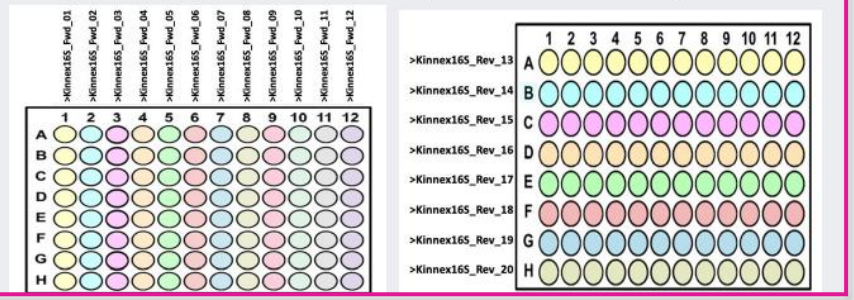
1. PCR amplification of 16S gene with barcoded primers

✔ Step	Instructions
	Thaw the PCR Ready Mix, briefly vortex to mix, and place on ice. Note that all PCR reactions must be set up on ice; the high proofreading activity of the enzyme will result in rapid primer degradation at room temperature.
	Thaw plates containing the diluted forward and reverse primers. Briefly spin the plate to ensure that the entire volume is at the bottom of each well.
1.1	

16S PCR Master Mix 1 components	1 sample	N	For 96-plex	For 192-plex	For 384-plex
PCR-grade Water	1.5 µL	1.5 x N x 1.1	158 µL	317 µL	634 µL
2X KAPA HiFi HotStart ReadyMix	12.5 µL	12.5 x N x 1.1	1320 µL	2640 µL	5280 µL
Total	14 µL	14 x N x 1.1	1478 µL	2957 µL	5914 µL

1.2 Transfer 14 µL of the prepared 16S PCR Master Mix 1 into a 96-well PCR plate for each 96-plex. For a 96-plex experiment design, use one 96-well plate. For a 384-plex experiment design, use four 96-well plates. Add 5 µL (1ng) of each diluted gDNA sample to each well containing 16S PCR Master Mix 1 on ice.

1.3 The figures below illustrate an example plate layout for setting up a 96-plex PCR design using twelve different 16S Barcoded Forward Primers and eight different 16S Barcoded Reverse Primers. Please refer to Appendix - 384 barcodes layout for all of the plate designs.



- Prepare PCR Master Mix[1] on ice for processing up to 384 metagenomic DNA samples (include 10% overage)
- Ensure all reagents are thawed and mixed prior to usec

- **IMPORTANT:** All PCR reactions described in this procedure must be set up and kept on ice until read to load onto thermal cycler instrument
  - High proofreading activity of the enzyme in the PCR Ready Mix will rapidly degrade primers at room temperature

[1] Kapa PCR mix (HiFi HotStart ReadyMix) and 16S primer oligos are user-supplied and must be purchased from a third-party vendor.

16S full-length sequence capture (cont.)

Perform PCR amplification of 16S gene with barcoded forward and reverse primers

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

1. PCR amplification of 16S gene with barcoded primers

1.4 On ice, add 3 µL of the **Barcoded Forward Primers (2.5 µM)** to wells containing 19 µL of gDNA and 16S PCR Master Mix followed by 3 µL of the **Barcoded Reverse Primers (2.5 µM).** The final concentration of the barcoded forward and reverse primers in each well is 0.3 µM. The final reaction volume in each well is 25 µL. Mix well by pipetting. Seal the plates to prevent evaporation during PCR. Briefly spin the plate in a refrigerated centrifuge (4°C) to ensure that the entire sample volume is at the bottom of each well.

1.5 Set up a thermal cycler with the program shown below. **Set the lid temperature to 105°C and pre-heat the thermal cycler until the lid temperature reaches 105°C and before adding the 96-well PCR plate. Keep the 96-well PCR plates on ice until the lid is pre-heated.**

The duration of PCR is around 1 hour.

Step	Temperature	Duration	Cycle
Initial Denaturation	95 °C	3 min	1
Denaturation	98 °C	20 s	20
Annealing	57 °C	30 s	
Extension	72 °C	75 s	
Final Extension	72 °C	5 min	1
Hold	4 °C	Hold	

1.6 **Spot-check amplification results by directly loading 1 µL of one or more PCR products onto an Agilent Bioanalyzer Chip.**

The expected target amplicon size is ~1500 bp, and the amount of amplicon material generated from each sample should be comparable as assessed by analyzing the relative intensity of the ~1500 bp PCR product. (Figure 1)

1.7 Proceed to pooling and SMRTbell cleanup in the next step.

SAFE STOPPING POINT -- Store at 4°C or -20°C for long-term storage

- **IMPORTANT:** Pre-heat thermal cycler until lid temperature reaches 105˚C before loading the 96-well PCR plate.
  - Keep the 96-well PCR plates on ice until the lid is pre-heated.

- Expected 16S amplicon size is approximately 1,500 bases and typical 16S PCR product yields are 50–300 ng per sample

16S PCR amplicon pooling & SMRTbell cleanup

Pool barcoded 16S PCR amplicons and perform cleanup using 1.1X SMRTbell cleanup beads

1 16S full-length sequence capture

Safe stop

2 Pooling & SMRTbell cleanup

Safe stop

3 Kinnex PCR & SMRTbell cleanup

Safe stop

4 Kinnex array formation & DNA repair

5 SMRTbell cleanup

Safe stop

6 Nuclease treatment

7 Final cleanup with SMRTbell cleanup beads

- Minimum amount of pooled 16S amplicon DNA needed to proceed with Kinnex PCR = **35 ng**

2.1 Pooling of barcoded 16S PCR amplicons

✔	Step	Instructions
	2.1.1	If PCR products are of the expected size and comparable quantity, pool equal volumes of each PCR reaction in a clean DNA LoBind microcentrifuge tube according to the recommendations below: • For a 96-plex experiment design, we recommend pooling **10 µL** from each PCR reaction. • For a 192-plex or higher-plex experiment design, we recommend pooling **5 µL** from each PCR reaction. Typical total yield from each 25 µL PCR reaction is ~50-300 ng. If doing less than 96-plex, pool **20uL from each PCR reaction into subsequent steps, but sure there is at least 35ng into the Kinnex PCR step.** Store unused PCR reactions at -20ºC for future use if desired.
	2.1.2	Proceed to SMRTbell cleanup in the next step.

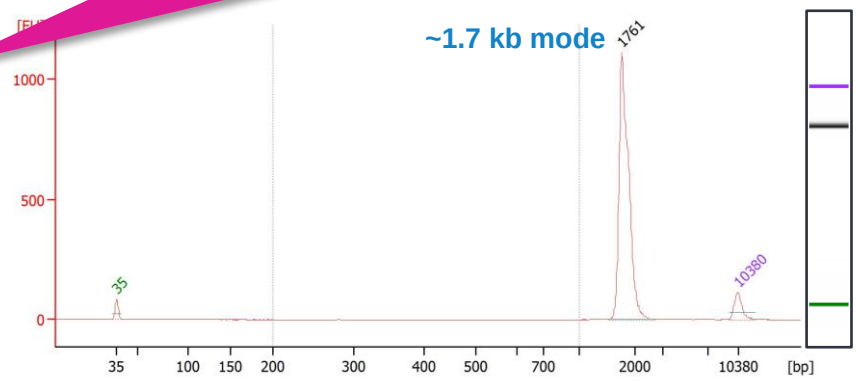
- **For 96-plex:** Pool 10 µL from each PCR reaction
- **For 192-plex or higher:** Pool 5 µL from each PCR reaction
- **For <96-plex:** Pool 20 µL from each PCR reaction into subsequent steps
- Typical total yield from each 25 µL PCR reaction is **~50-300 ng**
- Ensure there is **at least 35 ng** of pooled amplicon DNA to proceed to the Kinnex PCR step

2.2 Cleanup of pooled 16S PCR amplicon using 1.1X SMRTbell cleanup beads

✔	Step	Instructions
	2.2.1	Add **1.1X** v/v (volume over volume) of resuspended, room-temperature SMRTbell cleanup beads to the tube of pooled 16S amplicon. Note: Please use a 5 mL LoBind tube if the volume is more than 2mL.
	2.2.2	Pipette-mix the beads until evenly distributed.
	2.2.3	Quick-spin the tube in a microcentrifuge to collect liquid.
	2.2.14	Slowly pipette off the cleared supernatant without disturbing the beads. Transfer the supernatant to a **single new 1.5mL LoBind tube or tube strip**. Discard the old tube with beads.
	2.2.15	Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit using **1 µL** aliquot from the LoBind tube. Typical total yield from each 25 µL PCR reaction is ~50–300 ng.

SAFE STOPPING POINT -- Store at 4°C or -20°C for long-term storage

- Perform 1.1X SMRTbell bead cleanup at room temp.



Example Bioanalyzer DNA sizing QC analysis results for pooled 16S amplicon DNA samples generated from mock microbial community genomic DNA (ATCC MSA-1003 20 Strain Staggered Mix).

Kinnex PCR

In this step, incorporate programmable Kinnex segmentation adapter sequences into amplified DNA products

1 16S full-length sequence capture

Safe stop

2 Pooling & SMRTbell cleanup

Safe stop

3 Kinnex PCR & SMRTbell cleanup

Safe stop

4 Kinnex array formation & DNA repair

5 SMRTbell cleanup

Safe stop

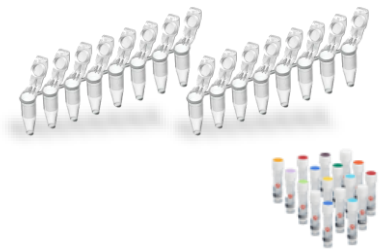
6 Nuclease treatment

7 Final cleanup with SMRTbell cleanup beads

Set up 12 parallel Kinnex PCR reactions per 16S sample with premixed Kinnex primers to generate amplified DNA products containing programmable sequences at both ends

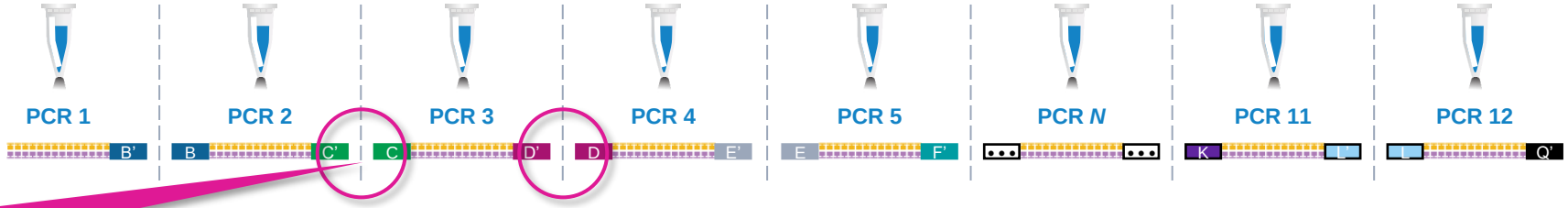
Pooled barcoded 16S amplicon DNA sample after 1.1X SMRTbell bead cleanup

Distribute barcoded DNA sample + Kinnex PCR reaction mix into 12 PCR tubes or 12 wells in a 96-well plate



OR

Perform Kinnex PCR by adding premixed Kinnex primer pair solutions



Example: Amplified DNA products from Kinnex PCR 3 contain flanking segmentation adapter sequences that are **complementary** to the ends of DNA products from Kinnex PCR 2 & Kinnex PCR 4

Pool 12 Kinnex PCR products together and perform 1.1X SMRTbell bead cleanup

Kinnex PCR-amplified DNA library insert sample ready for Kinnex array formation step

PacBio

Kinnex PCR (cont.)

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

3.1 Prepare Kinnex primers premix

✔	Step	Instructions
	3.1.1	Thaw the following components. The entire volume of primers can be transferred to an 8-strip tube for ease of use with a multi-channel pipette.

12X concatenation		Tube color	P/N
1	Kinnex primer mix A	Orange	103-107-800
2	Kinnex primer mix B	Orange	103-107-900
3	Kinnex primer mix C	Orange	103-108-000
4	Kinnex primer mix D	Orange	103-108-100
5	Kinnex primer mix E	Orange	103-108-200
6	Kinnex primer mix F	Orange	103-108-300
7	Kinnex primer mix G	Orange	103-108-400
8	Kinnex primer mix H	Orange	103-153-000
9	Kinnex primer mix I	Orange	103-153-100
10	Kinnex primer mix J	Orange	103-153-200
11	Kinnex primer mix K	Orange	103-153-300
12	Kinnex primer mix LQ	Orange	103-144-000

3.1.2	Briefly vortex to mix, and quick-spin to collect liquid. Place the primer mixes on ice and proceed to the preparation of the Kinnex PCR master mix.

- Can transfer entire volume of primers to PCR tubes for ease of use with multi-channel pipettes (12 primer mix tubes)

Kinnex PCR (cont.)

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

3.2 Kinnex PCR

✔	Step	Instructions
		Thaw the following components on ice, briefly vortex to mix, and quick-spin to collect liquid. Place reagents on ice. Add the below components to a LoBind tube, pulse vortex to mix and quick-spin. Place master mix on ice.

Master mix components	Volume for 12X concatenation*
PCR-grade water	132-X µL
Kinnex PCR Mix (103-107-700)	165 µL
35 ng of purified amplicons from Step 2.2.14*	X µL
Total volume	297 µL

X= 35 (ng)/ purified pooled DNA concentration from step 2.2.14

*10% overage included.

	3.2.1	
	3.2.2	Distribute **22.5 µL** of Master Mix 2 into each 12 PCR tubes (for 12X concatenation) on ice.
	3.2.3	Add **2.5 µL** of Kinnex primers premix into each of 12 PCR tubes of Step 3.2.2 on ice.
	3.2.4	Set up the thermal cycler as shown below with lid temperature set to 105°C. Keep samples on ice and do not add samples to thermal cycler until the lid has reached 105°C. The duration of PCR is approximately 35 minutes.

Step	Temperature	Duration	Cycle
Initial Denaturation	98°C	3 min	1
Denaturation	98°C	20 s	
Annealing	68°C	30 s	9
Extension	72°C	90 s	
Final Extension	72°C	5min	1
Hold	4°C	Hold	

SAFE STOPPING POINT -- Store at 4°C or -20°C for long-term storage

- Set up Kinnex PCR reactions **ON ICE**
- PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.

- **Critical step! Correct setup of all 12 Kinnex PCR reactions is required** – any missing/incorrect MAS primer pairs will result in no/low SMRTbell yield

- Set up on ice and add PCR reaction to thermal cycler after lid has preheated to 105°C to avoid digestion of primers by polymerase exonuclease activity

Kinnex PCR (cont.)

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

3.3 Pooling of 12 Kinnex PCR products and 1.1x SMRTbell cleanup

✔	Step	Instructions
	3.3.1	Add 23 µL from each of the 12 PCR reactions into a 1.5 mL tube for a total volume of 276 µL.
	3.3.2	Add **1.1X** v/v (volume over volume, 304 µL) of resuspended, room-temperature SMRTbell cleanup beads to the tube of pooled Kinnex PCR amplicon.
	3.3.3	Pipette-mix the beads or invert the tube until evenly distributed.
	3.3.4	Quick-spin the tube in a microcentrifuge to collect liquid.
	3.3.5	Incubate at **room temperature** for **10 minutes** to allow DNA to bind the beads.
	3.3.6	Place the tube in a magnetic separation rack until the beads separate fully from the solution.
	3.3.7	Slowly pipette off the cleared supernatant without disturbing the beads. Discard the supernatant.
	3.3.8	Slowly dispense **1000 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into the tube. After **30 seconds**, remove the 80% ethanol and discard.
	3.3.9	Repeat the previous step.
	3.3.10	Remove residual 80% ethanol: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in a magnetic separation rack until the beads separate fully from the solution. • Pipette off residual 80% ethanol and discard.
	3.3.11	Remove the tube from the magnetic rack. **Immediately** add **40 µL** of **Elution buffer** to the tube and resuspend the beads by pipetting 10 times or until evenly distributed.
	3.3.12	Quick-spin the tube in a microcentrifuge to collect liquid.
	3.3.13	Incubate at **room temperature** for **5 minutes** to elute DNA.
	3.3.14	Place the tube in a magnetic separation rack until the beads separate fully from the solution.
	3.3.15	Slowly pipette off the cleared supernatant without disturbing the beads. Transfer the supernatant to a PCR **tube strip**. Discard the old tube with beads.
	3.3.16	Make a 1:10 dilution of the sample and measure the DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Typical yield is 5–9 µg.

- Pool exactly 23 µL from each Kinnex PCR reaction in a clean 1.5 mL DNA LoBind tube[1] for a total combined volume of 184 µL

- Add exactly 304 µL of SMRTbell cleanup beads (1.1X)
- Kinnex PCR mix significantly increases stringency of SMRTbell clean up beads, so accurate pipetting is critical

- Perform DNA concentration QC using Qubit dsDNA HS kit
- Typical yield of Kinnex PCR products is 5–9 µg
- Verify there is sufficient yield of Kinnex PCR products (min. 4 µg) to proceed to Kinnex array formation step

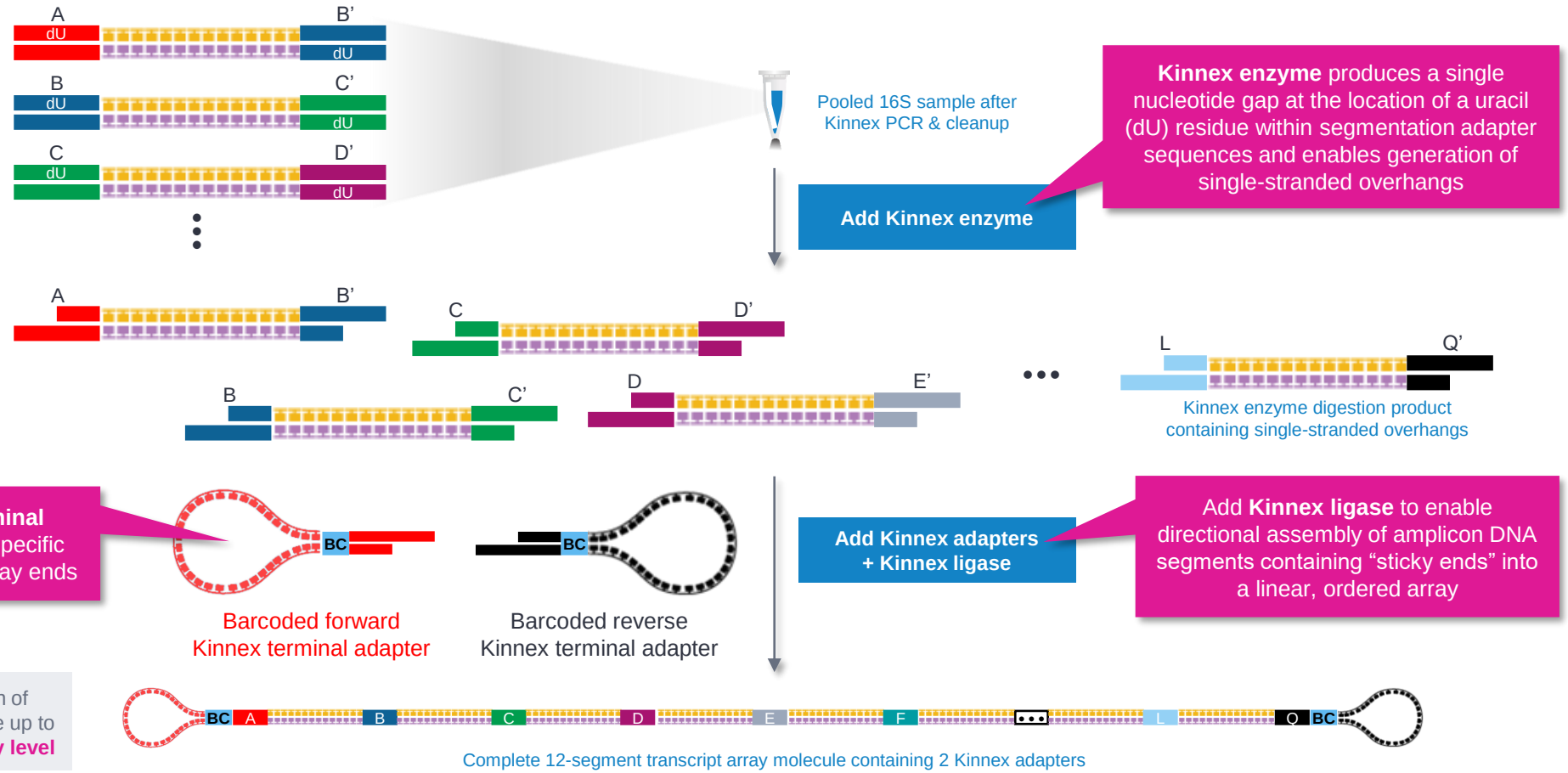
PacBio

Kinnex array formation

In this step, assemble 16S amplicons (“segments”) containing programmable ends into a linear array

1 16S full-length sequence capture
↓ Safe stop
2 Pooling & SMRTbell cleanup
↓ Safe stop
3 Kinnex PCR & SMRTbell cleanup
↓ Safe stop
4 Kinnex array formation & DNA repair
↓
5 SMRTbell cleanup
↓ Safe stop
6 Nuclea[illegible]
7 Fina[illegible] with SMRTbell cleanup beads

Treat pooled Kinnex PCR products with Kinnex enzyme to create single-stranded overhangs to enable subsequent directional assembly of 16S amplicons into a linear, ordered array



Kinnex library prep workflow supports incorporation of PacBio barcodes at the array formation step to enable up to **4-plex sample multiplexing at the SMRTbell library level**

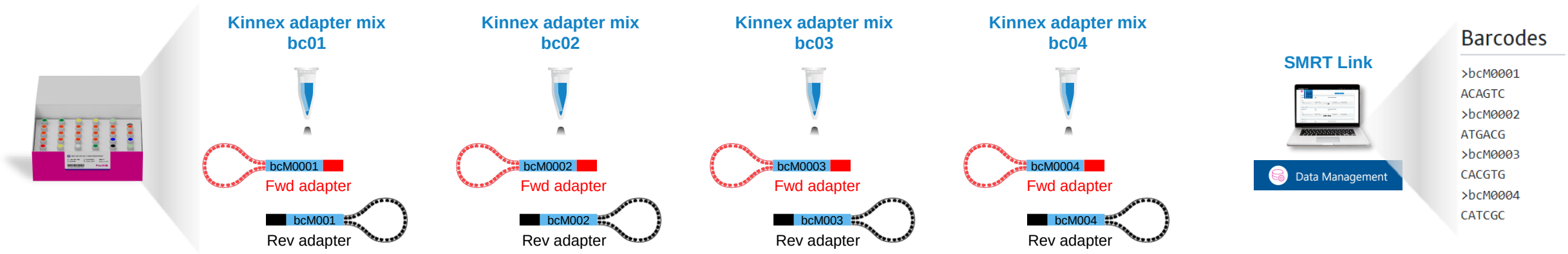
PacBio

* **Note:** Four barcoded terminal Kinnex adapters (Kinnex adapter bc01-04) are available for Kinnex array formation step. **Note:** Kinnex concatenation workflow is not compatible with standard SMRTbell adapters from SMRTbell prep kit 3.0 and is also not compatible with SMRTbell barcoded adapter plate 3.0.

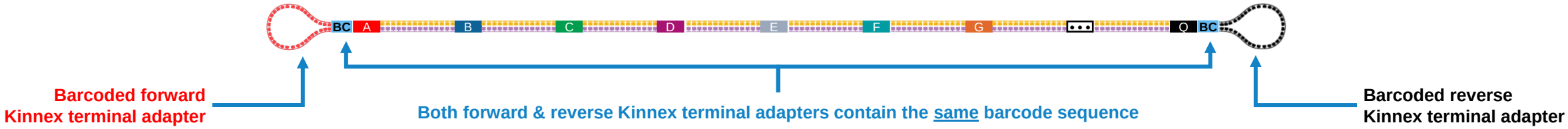
Kinnex array formation (cont.)

Kinnex terminal adapters incorporate barcode sequences to enable up to 4-plex sample multiplexing at the library level

- Kinnex adapters contain barcode sequences[1] to enable (optional) sample multiplexing at the SMRTbell library level (up to 4-plex)
  - Forward and reverse Kinnex adapter pairs are pre-mixed in Kinnex concatenation kits
  - Kinnex concatenation kits contain a total of 4 barcoded Kinnex adapter mixes (bc01-bc04) to enable multiplexing of up to 4 samples per SMRT Cell



Example complete Kinnex library molecule containing barcoded Kinnex terminal adapters at both ends



[1] Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

Kinnex array formation (cont.)

Kinnex terminal adapters use a new design that enables improved SMRT sequencing performance

- Kinnex adapters enable:
  - Longer polymerase read length → Improved HiFi conversion rate (HiFi reads/Total *P1* reads)
  - Improved *P1* loading efficiency

Forward and reverse Kinnex terminal adapters have **different structures**

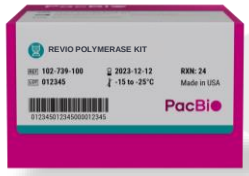
Annealed & bound Kinnex library molecule

- New Kinnex adapter design requires a different sequencing primer (Kinnex sequencing primer 103-179-000)

Sequel II binding kit 3.2 and cleanup beads (102-333-300)

Sequencing primer 3.2

Kinnex sequencing primer (103-179-000)



Revio polymerase kit[2] (102-817-600)

Standard sequencing primer

Kinnex sequencing primer (103-179-000)

[1] If needed, Kinnex sequencing primer may be provided free-of-charge to any customers that are still using older Sequel II binding kits or older Revio polymerase kits that do not already contain the primer.

[2] Revio polymerase kit includes SMRTbell cleanup beads.

Kinnex array formation & DNA damage repair (cont.)

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

7 Final cleanup with SMRTbell cleanup beads

4. Kinnex array formation

Step	Instructions
4.1.1	In a 0.2 mL PCR tube, add 5 µg of sample from Step 3.3.15, in 39 µL of volume (128 ng/µL). Pipette with Elution buffer going into this step if the sample is too concentrated.
	Add 2 µL of Kinnex adapter barcode 01–04 mix (select a single barcode per sample). Note: if not barcoding, select any Kinnex adapter barcode for use.

Tube color	Component	Volume
Blue	Kinnex adapter	2.0 µL

4.1.2 Add the following components in the listed order.

If processing multiple samples, make a master mix with 10% overage. Pipette to mix.

Tube color	Component	Volume
White	Kinnex array and repair buffer (103-110-300)	7.0 µL
Red	Kinnex enzyme (103-110-400)	4.0 µL
Yellow	Kinnex ligase (103-110-500)	6.0 µL
	Total RM1 volume	17 µL

4.1.3 Add 17 µL of master mix to the PCR tube containing sample and Kinnex barcode adapter. Pipette-mix and run the Kinnex primer digestion/ligation program with the lid set to 55°C.

Step	Temperature	Duration
1	45°C	60 min
2	4°C	Hold

4.1.4 After running the Kinnex primer digestion/ligation program, add 2 µL of DNA repair mix directly to the Kinnex primer digestion/ligation sample.

Tube color	Component	Volume
Green	DNA repair mix (103-110-000)	2 µL

4.1.5 Thoroughly mix by pipetting up and down 10 times and then quick-spin to collect all liquid.

4.1.6 Run the DNA Damage Repair Program with the lid set to >55°C.

Step	Temperature	Duration
1	45°C	30 min
2	4°C	Hold

- Recommended input amount to proceed with Kinnex array formation is **5 µg** of Kinnex PCR amplicons (from Step 3)
  - Proceeding with <3 µg is **not recommended** since lower input amounts may lead to insufficient final library yields to enable optimal sequencing results

- **IMPORTANT:** If combining multiple barcoded Kinnex libraries for sequencing, make sure each library uses one of the 4 different Kinnex barcoded adapters

- Perform **DNA Damage Repair** step to repair nicked / damaged DNA sites within newly formed Kinnex array products

PacBio

SMRTbell bead cleanup

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

5. 1X SMRTbell bead cleanup

✔	Step	Instructions
	5.1	Add 60 µL (1X v/v) of resuspended, room temperature SMRTbell cleanup beads to each sample.
	5.2	Pipette-mix the beads until evenly distributed and quick-spin in a microcentrifuge to collect liquid.
	5.3	Incubate at room temperature for 10 minutes to allow the DNA to bind the beads.
	5.4	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	5.5	Slowly pipette off the cleared supernatant without disturbing the beads. Discard the supernatant.
	5.6	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
	5.7	Repeat the previous step.
	5.8	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until the beads separate fully from the solution. • Pipette off residual 80% ethanol and discard.
	5.9	Remove the tube strip from the magnetic rack. Immediately add 40 µL of Elution buffer to each tube and resuspend the beads by pipetting 10 times or until evenly distributed. Quick-spin the tube strip in a microcentrifuge to collect liquid.
	5.10	Leave at room temperature for 5 minutes to elute DNA.
	5.11	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
		SAFE STOPPING POINT -- Store at 4°C or -20°C for long-term storage

- Perform **1.0X** SMRTbell bead cleanup at room temp.

Nuclease treatment & final SMRTbell bead cleanup

Perform nuclease treatment and final SMRTbell bead cleanup to remove incomplete SMRTbell template arrays

1 16S full-length sequence capture

Safe stop

2 Pooling & SMRTbell cleanup

Safe stop

3 Kinnex PCR & SMRTbell cleanup

Safe stop

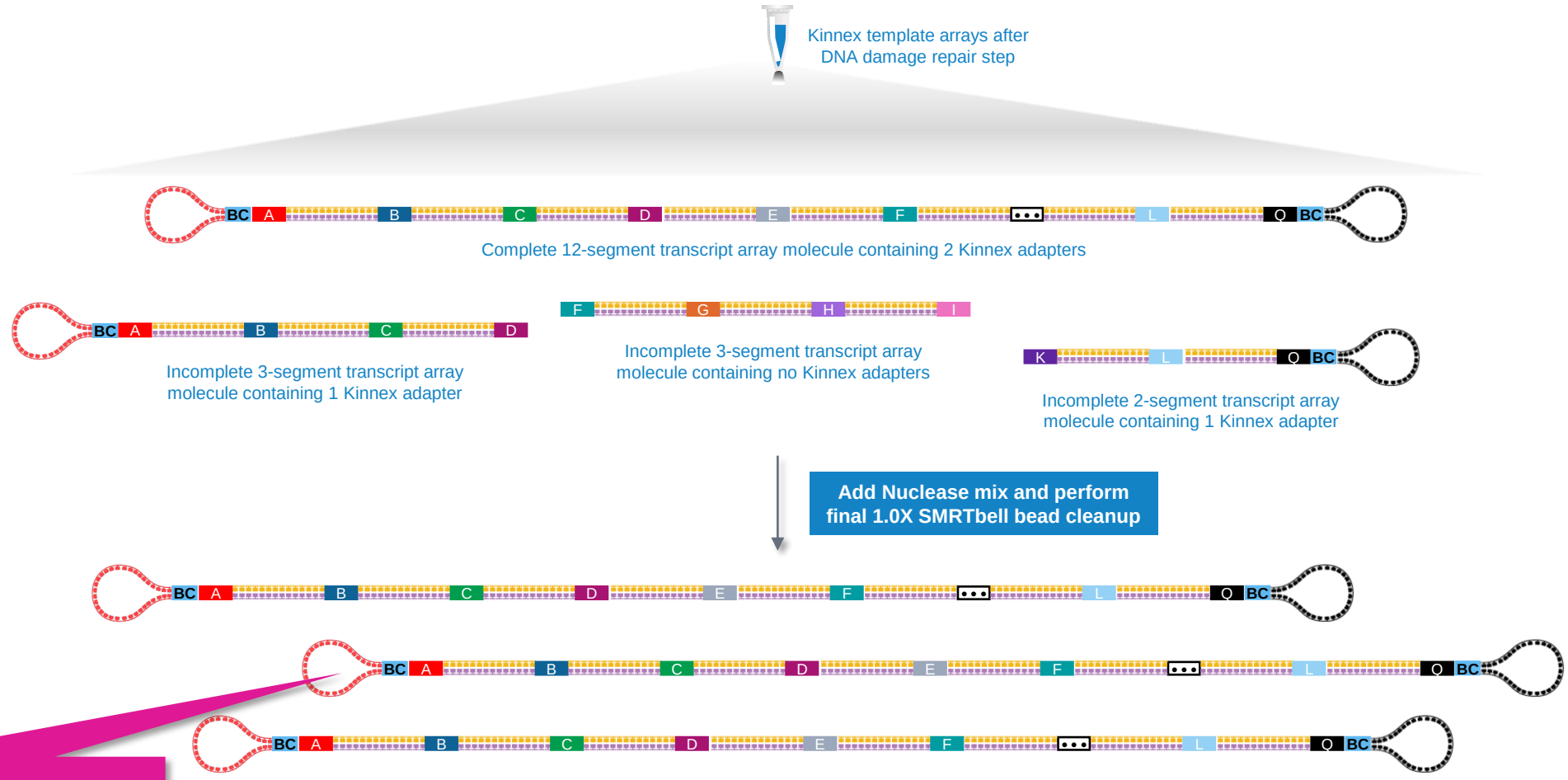
4 Kinnex array formation & DNA repair

5 SMRTbell cleanup

Safe stop

6 Nuclease treatment

7 Final cleanup with SMRTbell cleanup beads



- After nuclease treatment, most remaining SMRTbell templates are complete (full-length) 12-segment transcript array molecules capped with Kinnex adapters

PacBio

Nuclease treatment

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

Final Kinnex library yield is typically sufficient to load **≥2 SMRT Cells**

6. Nuclease treatment

✔	Step	Instructions
	6.1	Add the following components to a new microcentrifuge tube. Adjust the component volumes for the number of samples being prepared, plus 10% overage. Pipette-mix the master mix. For individual preps, add components directly to each sample from the previous step in the order and volume listed below. **Nuclease master mix** Tube: Light purple — Component: Nuclease buffer (103-110-200) — Volume: 5 µL Tube: Light green — Component: Nuclease mix (103-110-100) — Volume: 5 µL Total volume: 10 µL
	6.2	Add 10 µL of Nuclease Master mix to each sample. The total volume should be 50 µL.
	6.3	Thoroughly mix by pipetting up and down 10 times and then quick-spin to collect all liquid.
	6.4	Run the nuclease treatment program with the lid set to >47°C. Step 1: 37°C — 15 min Step 2: 4°C — Hold

- Perform nuclease treatment for **15 min**

Final cleanup with SMRTbell cleanup beads

Procedural notes

1 16S full-length sequence capture

↓ Safe stop

2 Pooling & SMRTbell cleanup

↓ Safe stop

3 Kinnex PCR & SMRTbell cleanup

↓ Safe stop

4 Kinnex array formation & DNA repair

↓

5 SMRTbell cleanup

↓ Safe stop

6 Nuclease treatment

↓

7 Final cleanup with SMRTbell cleanup beads

Final Kinnex library yield is typically sufficient to load **≥2 SMRT Cells**

7. Final cleanup with 1.0X SMRTbell cleanup beads

✔	Step	Instructions
	7.1	Add 50 µL (1X v/v) of resuspended, room temperature SMRTbell cleanup beads to each sample from the previous step. Pipette-mix the beads until evenly distributed.
	7.2	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
	7.3	Incubate at room temperature for 10 minutes to allow DNA to the bind beads.
	7.4	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	7.5	Slowly pipette off the cleared supernatant without disturbing the beads.
	7.6	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
	7.7	Repeat the previous step.
	7.13	Slowly pipette off the cleared supernatant without disturbing the beads. Transfer the supernatant to a new 0.5 mL LoBind tube or a PCR tube strip. Discard the old tube strip with beads.
	7.14	Take a 1 µL aliquot from each tube. Make a 1:5 dilution of the sample in Elution buffer and measure the DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Calculate the total mass. Expect 10–20% recovery of the starting Kinnex-PCR product.
		Recommended: Further dilute each aliquot to 250 pg/µL with EB. Measure the final SMRTbell library size distribution with a Femto Pulse system (see Figure 1).
	7.15	Proceed to the SMRT® Link Sample Setup to prepare the SMRTbell library for sequencing. **DNA concentration must be less than 60 ng/µL to proceed to ABC.** Using a concentration >60 ng/µL will result in low loading on the sequencer.
	7.16	Store SMRTbell libraries at 4°C if sequencing within the week. Long-term storage should be at -20°C. Minimize freeze-thaw cycles when handling SMRTbell libraries.

Note: Due to diverse sources of bacterial genomic DNA, there might be contaminants that affect the sequencing performance. An additional clean-up of final SMRTbell library using 3.1X diluted Ampure PB (35% v/v, part number 100-265-900) or Monarch Genomic DNA Purification Kit (#T3010S) has been shown to remove contaminants effectively.

PROTOCOL COMPLETE

- Perform **DNA concentration QC** on final purified Kinnex RNA library using a Qubit dsDNA HS assay
  - Typical final SMRTbell library yield from 5 µg of input DNA into Kinnex array formation is **~10 – 20%** – a much higher observed yield might suggest incomplete digestion of partial SMRTbell templates
  - **Troubleshooting tip:** If SMRTbell library yield is higher than expected and *P1* loading is lower than expected, consider repeating the nuclease treatment step

- Perform **DNA sizing QC** on final purified Kinnex 16S rRNA library using a Femto Pulse system (expected final library insert size is **~17 – 18 kb**)

- Kinnex 16S rRNA final SMRTbell library concentration must be **≤60 ng/ µL** to proceed with SMRT Link sample setup (ABC[1])
  - → Using a concentration above 60 ng/µL will result in lower loading during sequencing

Kinnex 16S rRNA library prep inputs & expected step yields

Final Kinnex library yield is typically sufficient to load ≥2 SMRT Cells

Kinnex 16S rRNA

Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800)

Library insert generation

PCR amplification of F-L 16S genes
IN: 1-2 ng of bacterial gDNA per sample

SMRTbell bead cleanup
OUT: 100-300 ng of PCR products per sample

Pool barcoded 16S PCR amplicons

SMRTbell bead cleanup

Kinnex PCR

Kinnex PCR
IN: 35 ng of pooled 16S amplicons

SMRTbell bead cleanup
OUT: 5-9 µg of amplified products after pooling

Kinnex array formation

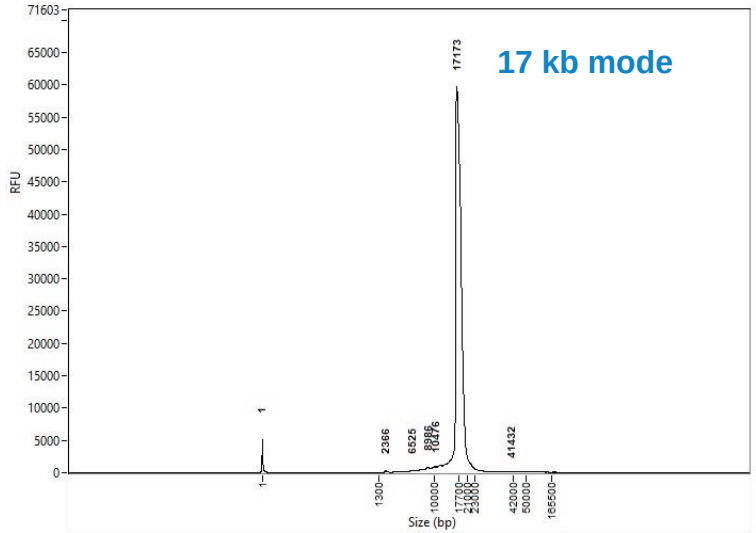
Kinnex digestion / Ligation
IN: 5 µg of pooled Kinnex PCR products

DNA damage repair

SMRTbell cleanup
OUT: 80% recovery from Kinnex digestion Rx input

Nuclease treatment
IN: 40 µL of DDR reaction products

SMRTbell bead cleanup
OUT: 10-20% recovery from Kinnex digestion Rx input



Example Femto Pulse DNA sizing QC analysis results for final Kinnex 16S rRNA library prepared with pooled 16S amplicon DNA samples generated from mock microbial community genomic DNA (ATCC MSA-1003 20 Strain Staggered Mix).

Example Kinnex 16S rRNA library prep yields

gDNA input for 16S PCR	1.1 ng
16S amplicon DNA input for Kinnex array formation	6000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1080 ng (18%)

[1] **Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~20% when using 16S amplicon DNA samples generated from mock microbial community genomic DNA (ATCC MSA-1003 20 Strain Staggered Mix).**

Final Kinnex library yield is typically sufficient to load **≥2 SMRT Cells**

Kinnex 16S rRNA sequencing preparation workflow details

Sample Setup & Run Design recommendations for Kinnex 16S rRNA libraries

SMRT Link supports Kinnex 16S rRNA sequencing preparation & analysis workflow for PacBio systems[1]

Select desired **application type** in SMRT Link

SMRT Link v13.0+

- Sample Setup
- Runs

Recommended target *P1* loading range for Revio system is **~50 – 70%**

Application type

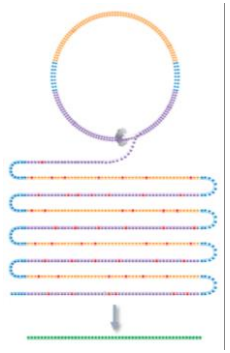
Kinnex 16S rRNA[1]

Whole genome sequencing
- Human WGS
- Microbial assembly
- Other WGS

RNA sequencing
- Iso-Seq method
- MAS-Seq single cell
- Kinnex single-cell RNA
- Kinnex full-length RNA

Viral sequencing
- Adeno-associated virus

Metagenomics
- Kinnex 16S rRNA



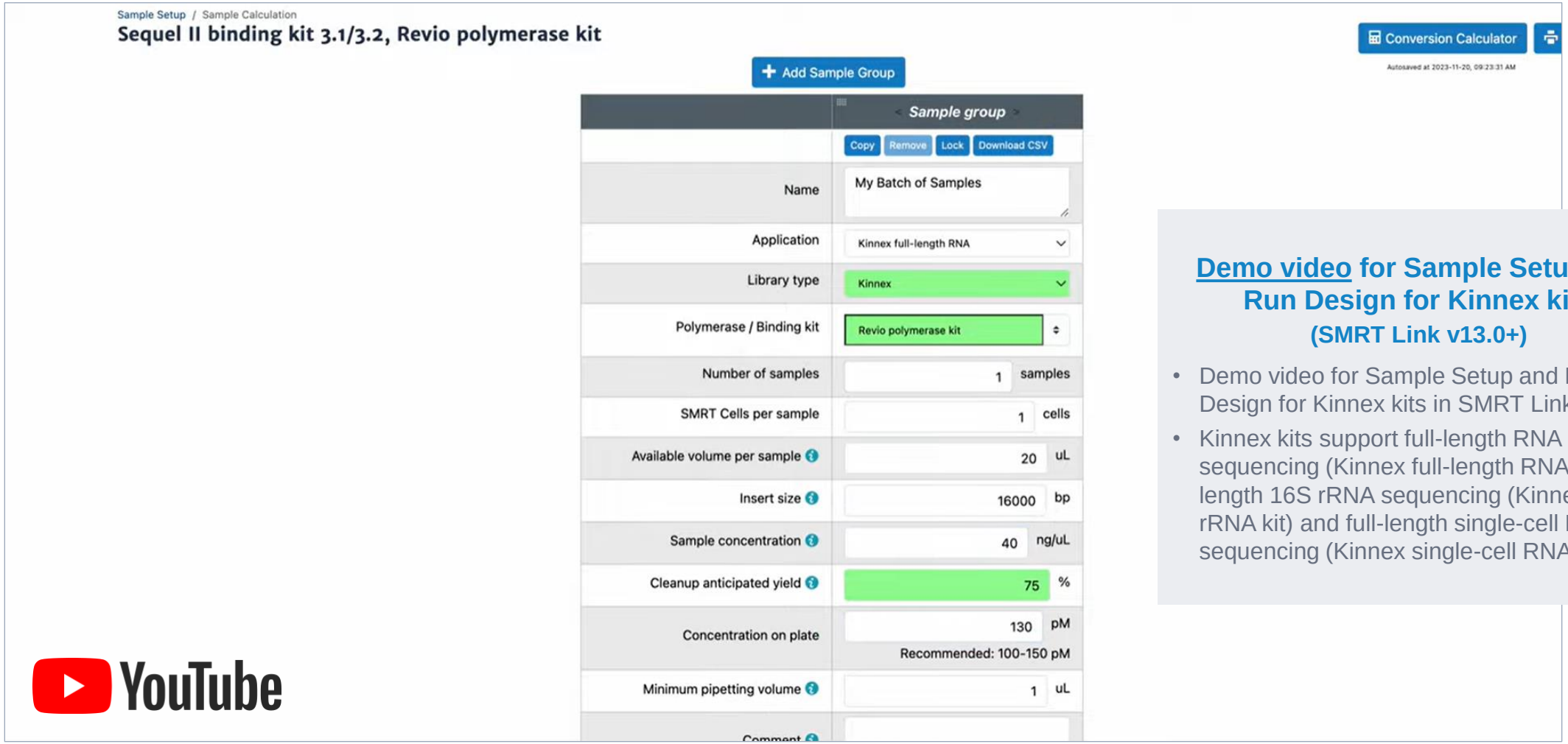
HiFi read

SMRT Link module	Key setup parameters For Kinnex libraries	Sequel II/IIe system recommended settings for Kinnex libraries	Revio system recommended settings for Kinnex libraries
Sample setup	**Library type**	Kinnex	
	Primer	Kinnex sequencing primer	
	Binding/Polymerase kit[1]	Sequel II binding kit 3.2 (includes Kinnex sequencing primer)	Revio polymerase kit (includes Kinnex sequencing primer)
	Concentration on plate	40 – 60 pM	100 – 150 pM
Runs → Run design	**Adapter / Library type**	SMRTbell Adapter Design = SMRTbell Kinnex Prep Kit	Library type = Kinnex
	Movie collection time	30 hrs	24 hrs
	Use adaptive loading	YES	
	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = NO CCS Analysis Output - Include Kinetics Information = NO	Consensus Mode = MOLECULE

[1] Kinnex 16S rRNA kit requires SMRT Link v13.0 or higher.

SMRT Link Sample Setup and Run Design for Kinnex kits video demonstration

Video demonstration of SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits supporting full-length RNA sequencing, single-cell RNA sequencing and full-length 16S rRNA sequencing



Demo video for Sample Setup and Run Design for Kinnex kits
(SMRT Link v13.0+)

- Demo video for Sample Setup and Run Design for Kinnex kits in SMRT Link v13.0+
- Kinnex kits support full-length RNA sequencing (Kinnex full-length RNA kit), full-length 16S rRNA sequencing (Kinnex 16S rRNA kit) and full-length single-cell RNA sequencing (Kinnex single-cell RNA kit)

SMRT Link Sample Setup procedure for Kinnex 16S rRNA libraries

Revio system

Sequel II and IIe systems

	Sample group
	Copy / Remove / Lock / Download CSV
Name	Kinnex full-length 16S rRNA library demo
Application	Kinnex 16S rRNA
Library type	Kinnex
Polymerase / Binding kit	Revio polymerase kit
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	20 uL
Insert size	18000 bp
Sample concentration	40 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	130 pM Recommended: 100-150 pM
Minimum pipetting volume	1 uL
Comment	Kinnex library containing array of 12 FL 16S amplicon segments

	Sample group
	Copy / Remove / Lock / Download CSV
Name	Kinnex full-length 16S rRNA library demo
Application	Kinnex full-length RNA
Library type	Kinnex
Polymerase / Binding kit	Sequel® II Binding Kit 3.2
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	20 uL
Insert size	18000 bp
Sample concentration	40 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	50 pM Recommended: 40-60 pM
Minimum pipetting volume	1 uL
Comment	Kinnex library containing array of 12 FL 16S amplicon segments

- Select **application type** to autofill fields in green

IMPORTANT: Specify **Library type = Kinnex**

- Library type field determines sequencing primer type to use for annealing step
  - → Kinnex libraries require use of **Kinnex sequencing primer**[1]

- Select **Revio polymerase kit** for Revio system and **Sequel II Binding Kit 3.2** for Sequel II/IIe systems

- Recommended Kinnex 16S rRNA library input concentration for sample setup is **20 – 60 ng/µL**

- Recommended OPLC range is **100 – 150 pM** for Revio system and **40 – 60 pM** for Sequel II/IIe systems

- **Recommended target *P1* loading range**
- Revio system: **~50 – 70%**
- Sequel II and IIe systems: **~60 – 80%**

[1] Sample Setup **Library type** field specifies structure of SMRTbell library and determines sequencing primer type to use for annealing step. For Kinnex libraries, the forward and reverse Kinnex terminal SMRTbell adapters have different structures and require use of Kinnex sequencing primer for primer annealing step.

SMRT Link Run Design procedure for Revio system

Sample and run information

Select desired **application type** to autofill Library Type, Polymerase Kit & Movie Acquisition Time recommended settings

Kinnex 16S rRNA

Plate 1, Well A01: Kinnex 16S rRNA library demo

Application (Required)	Kinnex 16S rRNA
Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	Kinnex 16s rRNA library demo
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	18000
Polymerase Kit (Required)	Revio polymerase kit
Movie Acquisition Time (hours)	24

Specify **Kinnex** library type (instead of Standard or AAV)[1]

Specify **Revio polymerase kit**

Specify **Use Adaptive Loading = YES**

Use Adaptive Loading: YES / NO

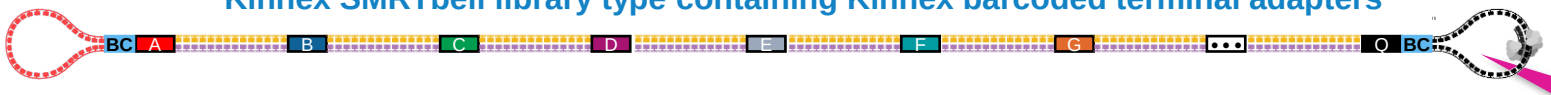
Specify **Insert Size**

Recommend **24 hrs** movie collection for Revio Kinnex samples

Standard SMRTbell library type containing standard SPK3 barcoded terminal adapters

Forward and reverse standard terminal adapters have the **same structure**

Kinnex SMRTbell library type containing Kinnex barcoded terminal adapters



Forward and reverse Kinnex terminal adapters have **different structures**

Library Type field determines which adapter finding algorithm is used during post-primary analysis[1]

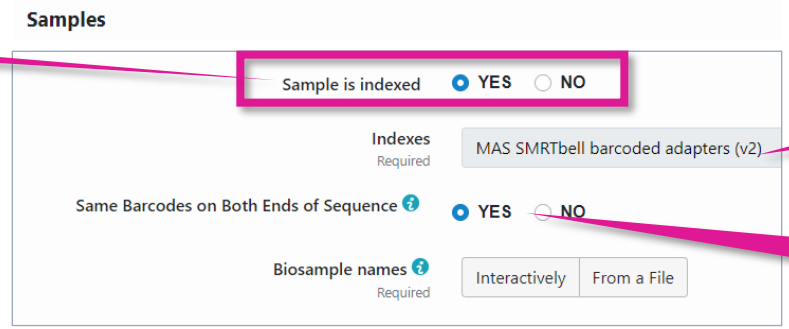
[1] **Note:** When sequencing a Kinnex library sample, if 'Standard' library type is mistakenly selected instead of 'Kinnex' then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.

SMRT Link Run Design procedure for Revio system (cont.)

Sample indexing (barcoding) information

Kinnex 16S rRNA

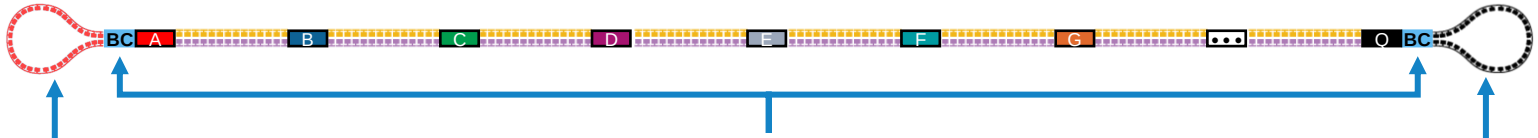
Default = **YES** for **Sample is indexed**



Specify **Indexes** FASTA
= **MAS SMRTbell barcoded adapters (v2)**

Specify **YES** for **Same barcodes on both ends of sequences**

Example complete Kinnex library molecule containing barcoded Kinnex terminal MAS adapters[1] at both ends



Barcoded forward Kinnex terminal adapter

Both forward & reverse Kinnex terminal adapters contain the same barcode sequence

Barcoded reverse Kinnex terminal adapter

Example interactive biosample name specification for a multiplexed Kinnex library sample

SMRT Link

Data Management

MAS SMRTbell barcoded adapter indexes

```
>bcM0001
ACAGTC
>bcM0002
ATGACG
>bcM0003
CACGTG
>bcM0004
CATCGC
```

[1] Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-bcM0004) are available for Kinnex array formation step. Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

SMRT Link Run Design procedure for Revio system (cont.)

Run options and data options

Kinnex 16S rRNA

Run Options

Library Concentration (pM) Required: 130

On-plate loading concentration is **required** for Revio samples

Data Options

Include Base Kinetics: YES / NO

Default = **NO** for **Include Base Kinetics**

Consensus Mode: MOLECULE / STRAND

Assign Data To Project: General Project

Default **Consensus Mode = MOLECULE**[1]

Can leave Include Base Kinetics and Consensus Mode fields at their default settings for Kinnex library samples

[1] **Molecule Consensus Mode** → generates a single circular consensus sequencing read for a given library insert molecule. **Strand Consensus Mode** → generates a consensus sequence for each strand (forward & reverse) of a given library insert molecule.

SMRT Link Run Design procedure for Sequel II/IIe systems

Sample information and run information

Kinnex 16S rRNA

- Select desired Kinnex application from the **Application** field drop-down menu
- The following fields are auto-populated with default recommended values and high-lighted in green:
  - **SMRTbell Adapter Design**
    - → SMRTbell Kinnex Prep Kit
  - **Binding Kit**
    - → Sequel II Binding Kit 3.2
  - **Sequencing Kit**
    - → Sequel II Sequencing Plate 2.0 (4 rxn or 1 rxn)
  - **DNA Control Complex**
    - → Sequel II DNA Internal Control Complex 3.2
  - **Movie Time per SMRT Cell**
    - → 30 hrs
  - **Pre-Extension Time**
    - → 2 hrs

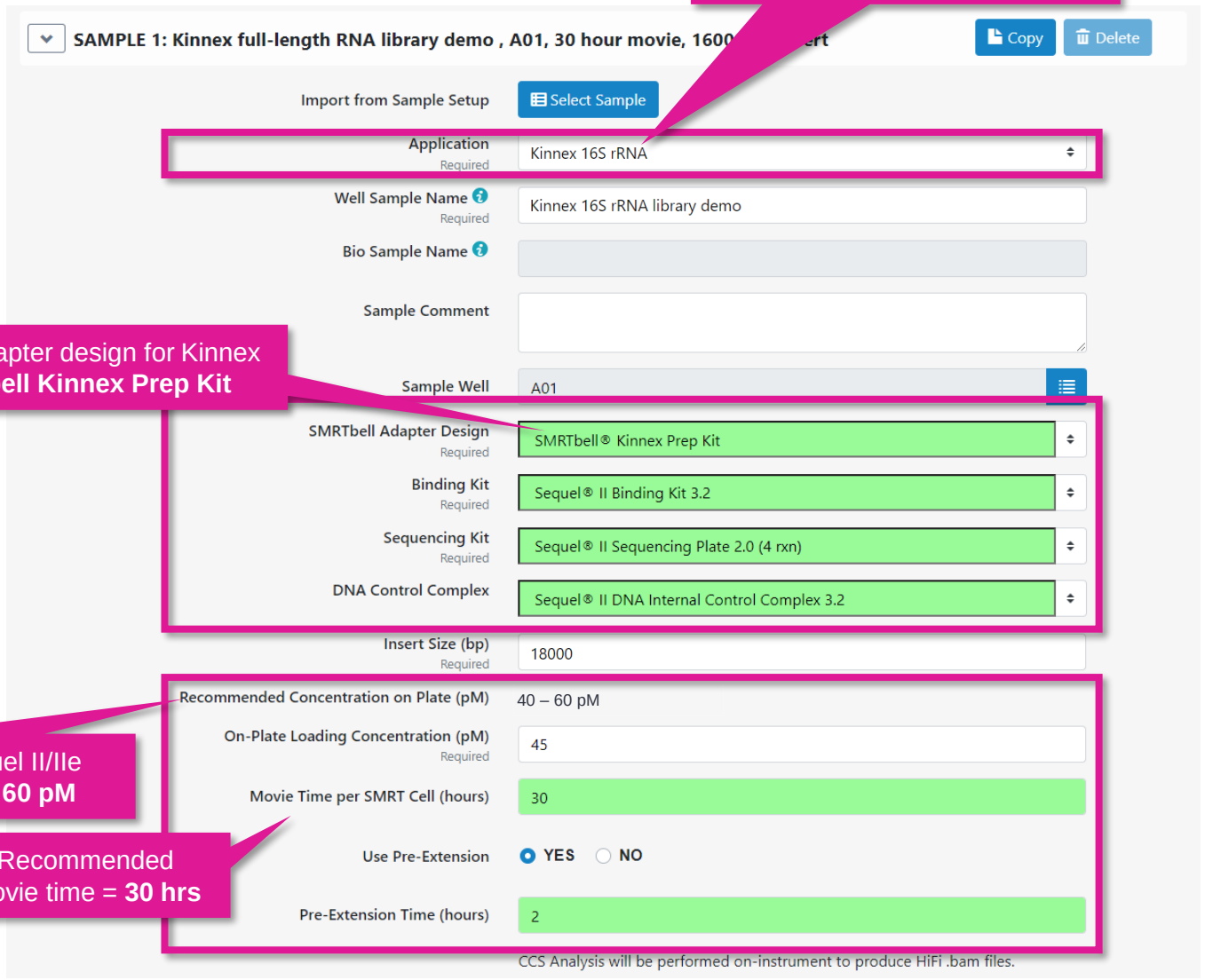
SMRTbell Adapter Design field determines which adapter finding algorithm is used during post-primary analysis[1]

Select desired Kinnex application type from drop-down menu

Default SMRTbell adapter design for Kinnex samples is **SMRTbell Kinnex Prep Kit**

Recommended OPLC for Sequel II/IIe Kinnex library samples is **40 – 60 pM**

Recommended movie time = **30 hrs**



Example sample information entered into a Sequel IIe system run design worksheet for a Kinnex 16S rRNA library sample.

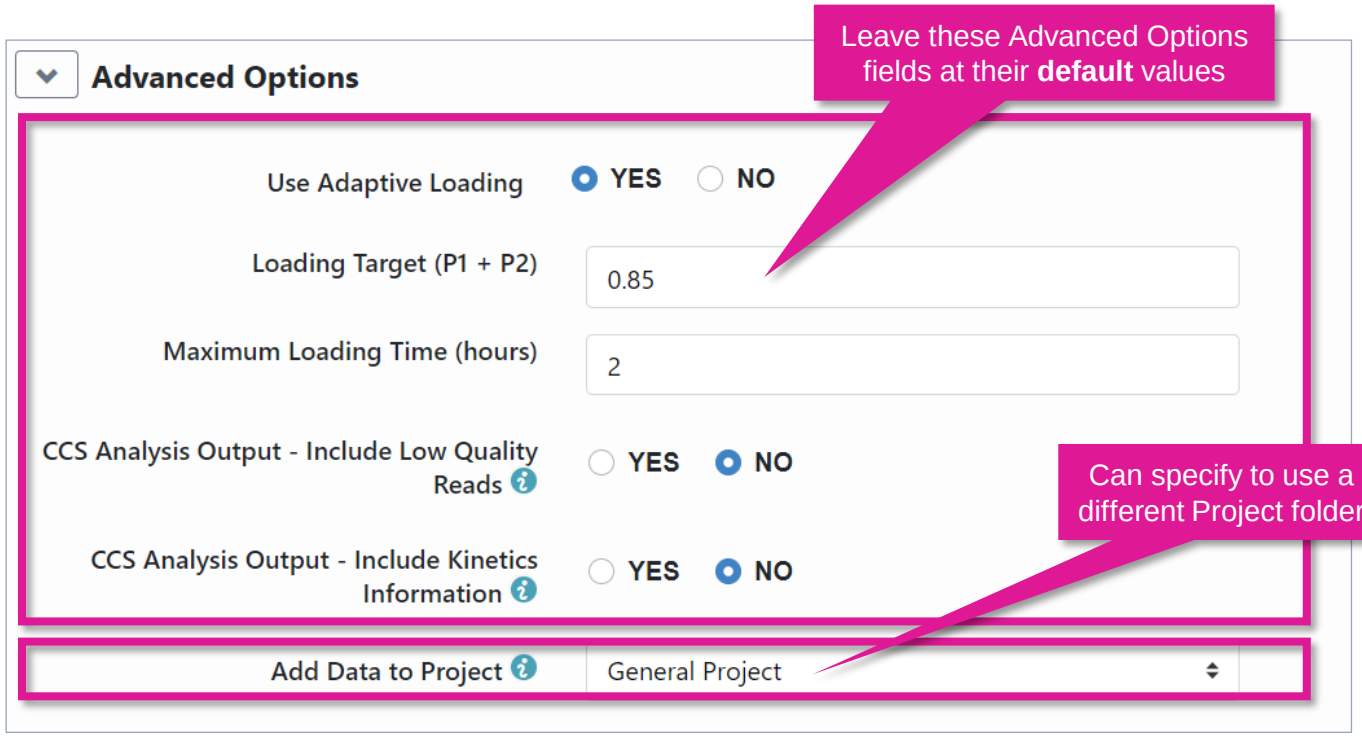
[1] **Note:** When sequencing a Kinnex library sample, if 'Overhand-SMRTbell Prep Kit 3.0' is mistakenly selected instead of 'SMRTbell Kinnex Prep kit' in the SMRTbell Adapter Design field, then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.

SMRT Link Run Design procedure for Sequel II/IIe systems (cont.)

Advanced options

Kinnex 16S rRNA

- For all Kinnex library samples, leave the following **Advanced Options** fields at their **default settings**
  - ❑ **Use Adaptive Loading**
    - → YES
  - ❑ **Loading Target (P1 + P2)**
    - → 0.85
  - ❑ **Maximum Loading Time**
    - → 2 hours
  - ❑ **CCS Analysis Output - Include Low Quality Reads**
    - → NO
  - ❑ **CCS Analysis Output - Include Kinetics Information**
    - → NO
  - ❑ **Pre-Extension Time**
    - → 2 hrs
- If desired, specify to use an alternative project folder for the **Add Data to Project** field



Example default Advanced Options settings entered into a Sequel IIe system run design worksheet for a Kinnex 16S rRNA library sample.

SMRT Link Run Design procedure for Sequel II/IIe systems (cont.)

Barcoded sample options

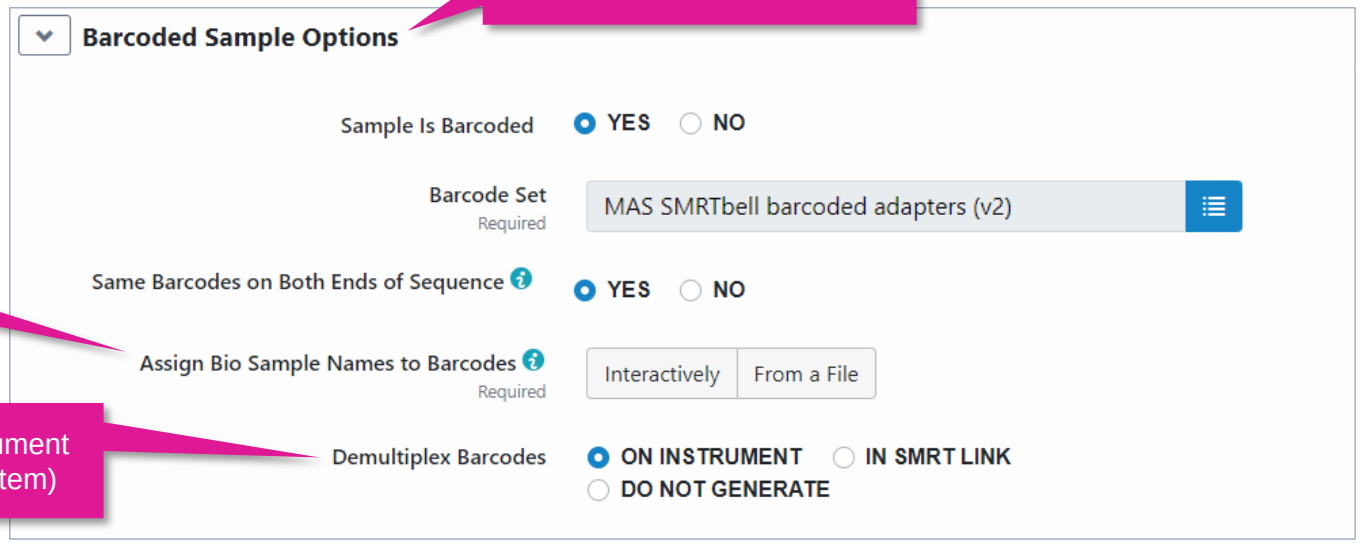
Kinnex 16S rRNA

- For Kinnex library samples, can leave most **Barcoded Sample Options** fields at their **default settings**

Specify Bio Sample Names, either interactively or by downloading a CSV file (**Interactively** or **From a file**)

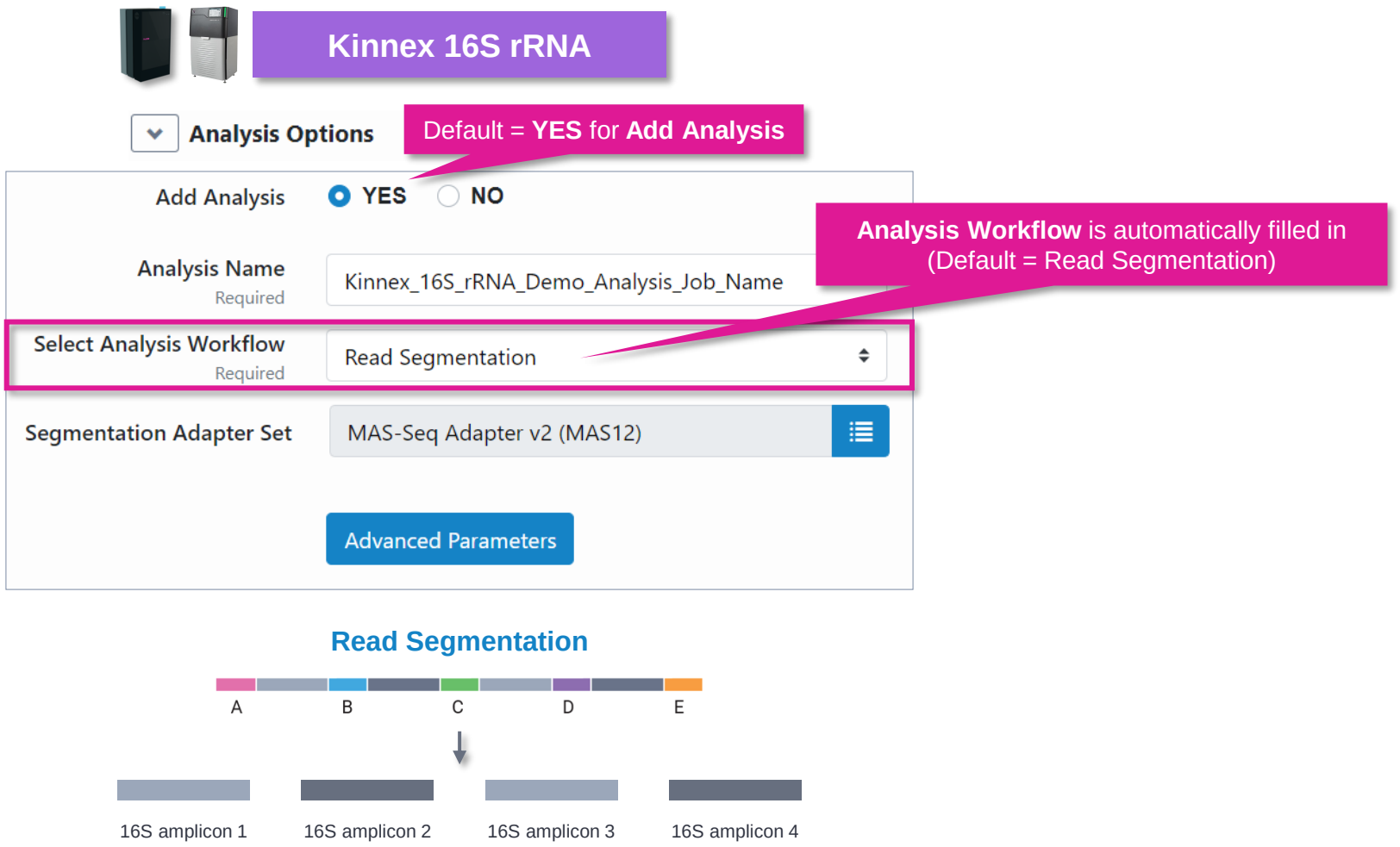
If desired, specify to perform barcode demultiplexing on-instrument or in SMRT Link (default = **On-instrument** for Sequel IIe system)

Can leave most of these fields at their default **values**



Example default Barcoded Sample Options settings entered into a Sequel IIe system run design worksheet for a Kinnex full-length RNA library sample.

SMRT Link Run Design analysis options for Revio system and Sequel II/IIe systems



Read Segmentation data utility splits arrayed HiFi reads at segmentation adapter positions, generating segmented reads (S-reads) which are the comprising fragments.

SMRT Link Run Design analysis options for Revio system and Sequel II/IIe systems (cont.)

Kinnex 16S rRNA

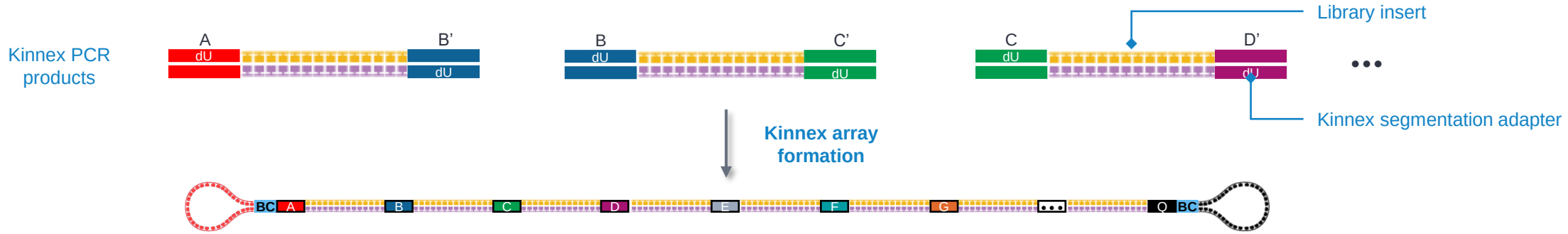
Analysis Options

Add Analysis	YES / NO
Analysis Name (Required)	Kinnex_16S_rRNA_Demo_Analysis_Job_Name
Select Analysis Workflow (Required)	Read Segmentation
Segmentation Adapter Set	MAS-Seq Adapter v2 (MAS12)

Advanced Parameters

Specify **Segmentation Adapter Set** that corresponds to the Kinnex library concatenation method used

→ For Kinnex 16S rRNA samples, specify **MAS-Seq Adapter v2 (MAS12)**



Complete array molecule containing concatenated library insert segments

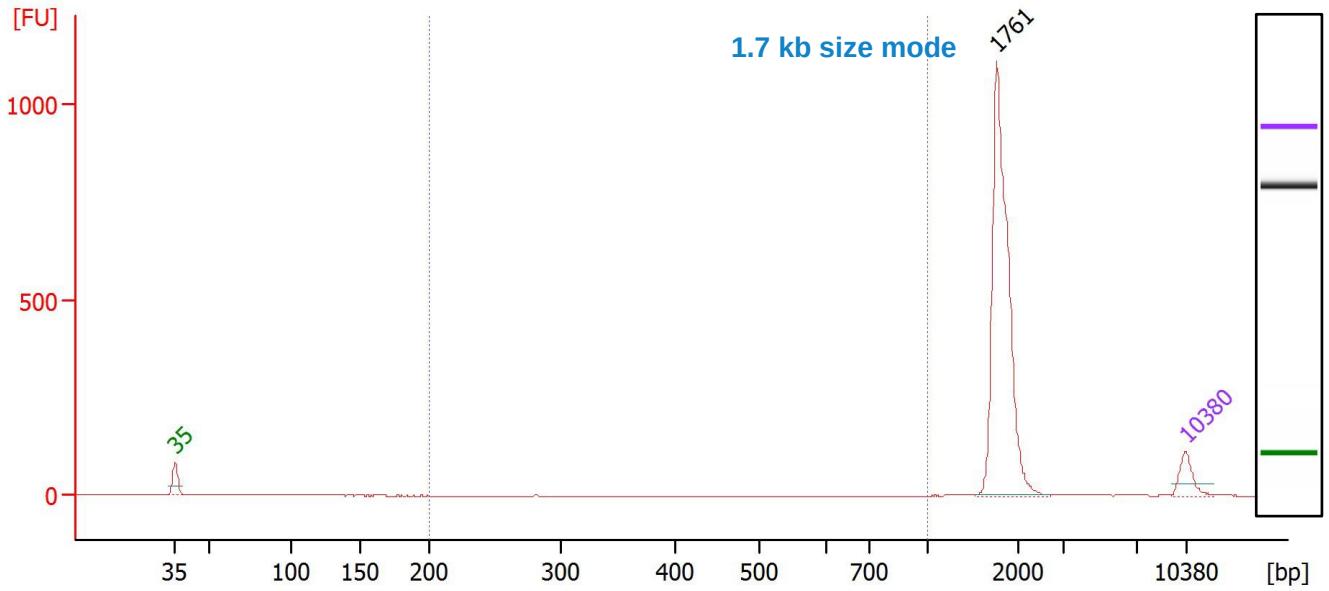
Kinnex 16S rRNA example sequencing performance data

Example Kinnex 16S rRNA library preparation QC results

Final Kinnex library yield is typically sufficient to load **≥2 SMRT Cells**

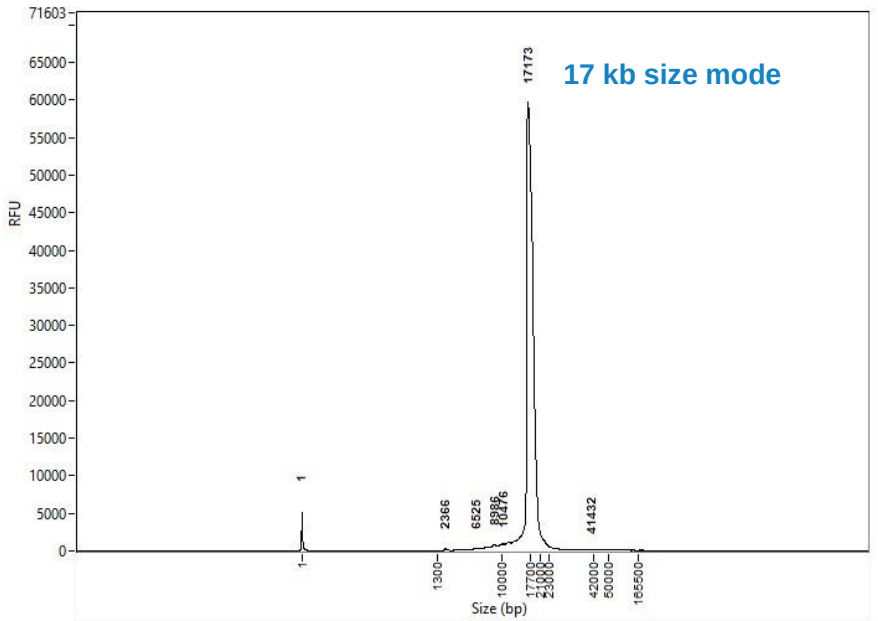
Kinnex full-length 16S RNA library prepared from mock microbial community genomic DNA

16S amplicon DNA QC



Example Bioanalyzer DNA sizing QC analysis results for pooled 16S amplicon DNA samples generated from mock microbial community genomic DNA (ATCC MSA-1003 20 Strain Staggered Mix).

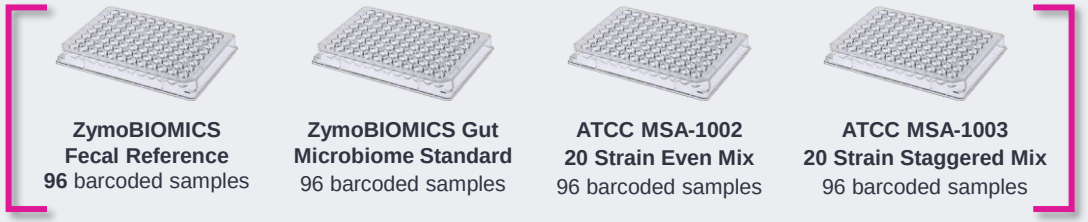
Final Kinnex 16S rRNA library QC



Example Femto Pulse DNA sizing QC analysis results for final Kinnex 16S rRNA library.

Sample multiplexing design for Kinnex 16S rRNA library

384-plex 16S PCR x 4-plex Kinnex adapter barcoding → Total sample multiplex level = **1,536-plex**



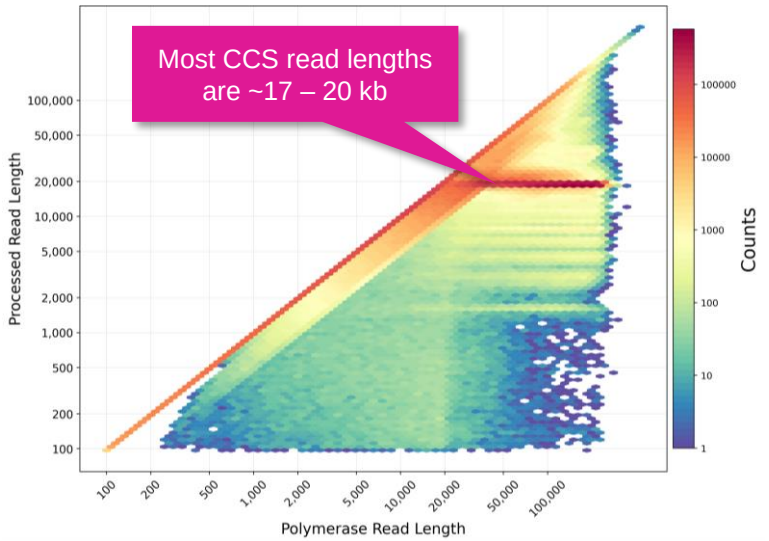
gDNA input for 16S PCR	1.1 ng
16S amplicon DNA input Kinnex PCR products for Kinnex array formation	6000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1080 ng (18%)

[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~20% when using mock microbial community genomic DNA for Kinnex full-length 16S rRNA library construction.

Example sequencing performance for Kinnex 16S rRNA libraries prepared from mock microbial community genomic DNA

Revio system example data[1] (1,536-plex data set)

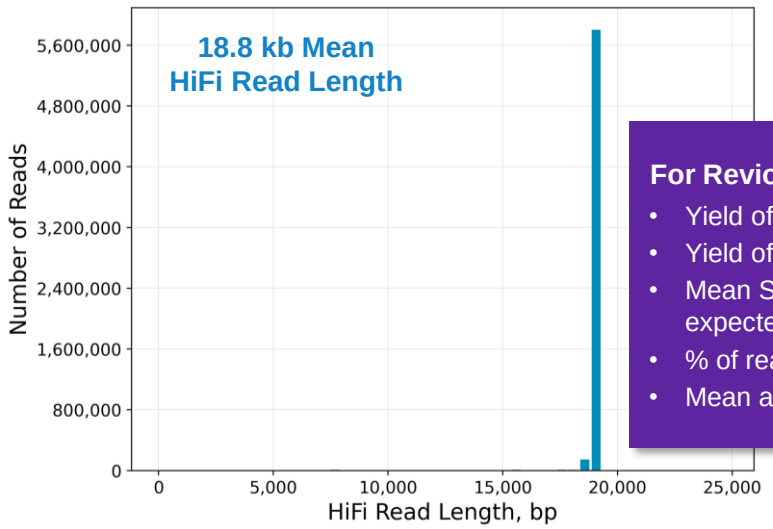
Raw Data Report



Raw Base Yield	1,222 Gb
Mean Polymerase Read Length	69.0
P0	27%
P1	70%
P2	3%

Example sequencing metrics for a Kinnex 16S rRNA library sample run on a Revio system with Revio polymerase kit / 130 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

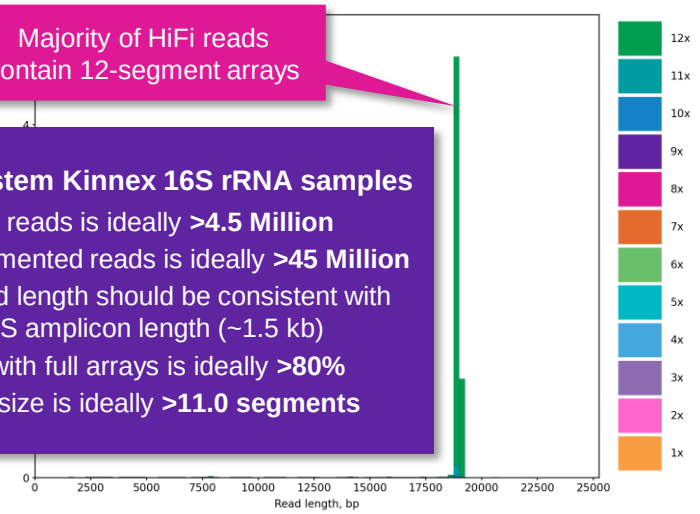
HiFi Read Length



HiFi Reads	6.1 M
HiFi Base Yield	114.21 Gb
Mean HiFi Read Length	18.78 kb
Median HiFi Read Quality	Q32
HiFi Read Mean # of Passes	7

For Kinnex 16S rRNA libraries, per-Revio SMRT Cell HiFi read counts were typically ~4 – 6 Million depending on the final library insert size and *P1* loading performance.

Read Segmentation Metrics



For Revio system Kinnex 16S rRNA samples

- Yield of HiFi reads is ideally **>4.5 Million**
- Yield of segmented reads is ideally **>45 Million**
- Mean S-read length should be consistent with expected 16S amplicon length (~1.5 kb)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **>11.0 segments**

Input HiFi Reads	6,050,730
Segmented reads (S-reads)	71,720,714
Mean length of S-reads	1,560 bp
Percent of reads with full arrays	95.03%
Mean array size (concentration factor)	11.85

For Kinnex 16S rRNA libraries, per-Revio SMRT Cell segmentation read counts were typically ~45 – 60 Million.

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal *P1* loading performance may result in HiFi data yields <90 Gb per Revio SMRT Cell.

Improving sequencing performance of “difficult” 16S samples

Performing AMPure PB bead size-selection on Kinnex full-length 16S rRNA libraries can help improve *P1* loading of challenging metagenomic samples

Sample Name	P1 %	Gb Yield	Mean Length	Mean QV
16S_collaborator_SOP	26	46	18,813 bp	Q29
16S_collaborator_3.1X AMPure	**80**	**87**	**18,851 bp**	**Q28**

Some bacterial 16S samples may have carry-over contaminants present leading to low *P1* loading on Revio and Sequel II/IIe systems

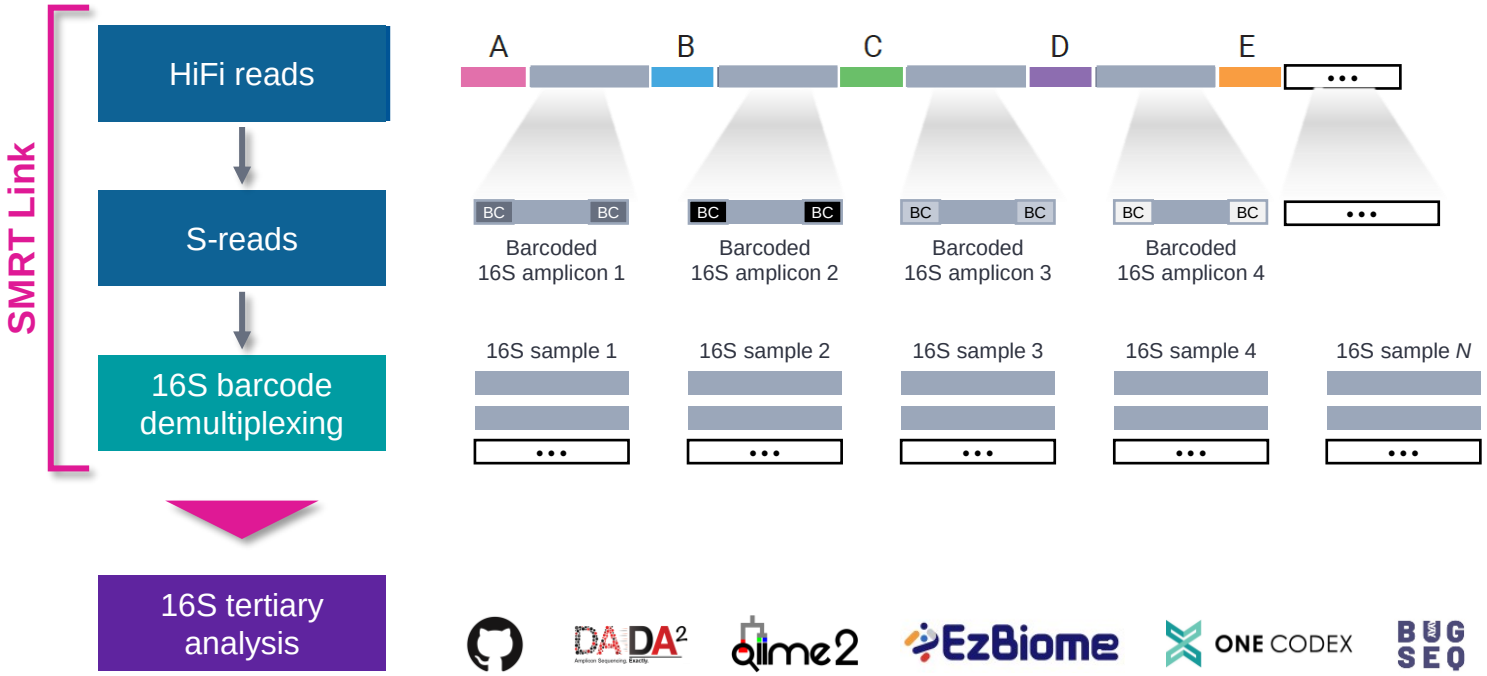
→ Using AMPure size-selection (3.1X 35% AMPure PB beads) can help mitigate this issue

Kinnex 16S rRNA data analysis workflow overview

Kinnex 16S rRNA bioinformatics workflow overview

SMRT Link Read Segmentation processes HiFi reads generated from Kinnex 16S rRNA libraries to produce individual segmented reads (S-reads) that are compatible with tertiary analysis tools

SMRT Link read segmentation and barcode demultiplexing workflow[1]



Example Kinnex 16S rRNA data set

- ZymoBIOMICS Fecal Reference with TruMatrix Technology (human) [Link]
- Mixture: ZymoBIOMICS Gut Microbiome Standard, ZymoBIOMICS Fecal Reference with TruMatrix™ Technology, ATCC 20 Strain Even Mix Genomic Material, ATCC 20 Strain Staggered Mix Genomic Material [Link]

Read segmentation

- Use SMRT Link Read Segmentation data utility to split arrayed Kinnex library HiFi reads into individual segmented reads (S-reads) that represent the original barcoded 16S amplicon sequences

Demultiplex barcodes

- Use SMRT Link Demultiplex barcodes data utility to separate sequence reads by barcode

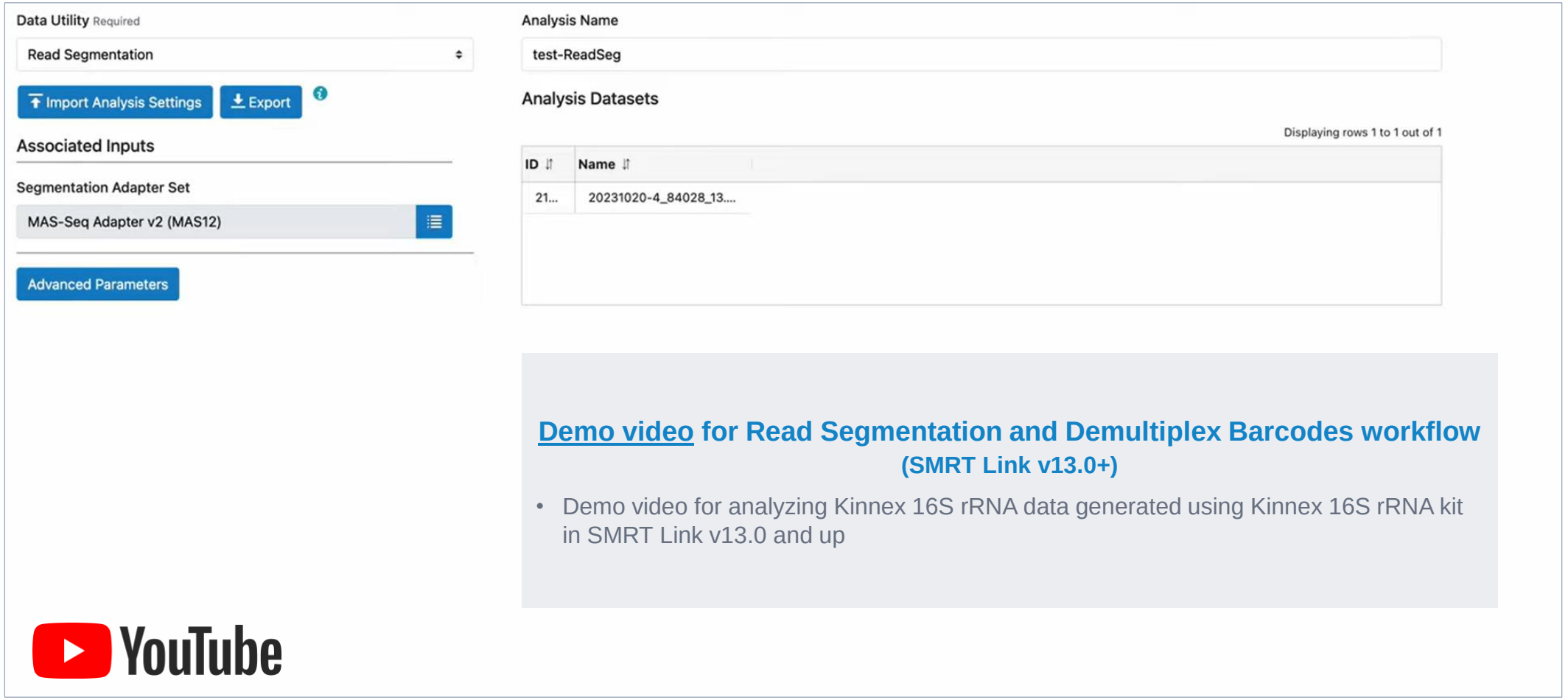
Tertiary analysis

- Use Github or other third-party tools (e.g., PacBio GitHub, *DADA2*, *QIIME2*, *microbiomehelper*, *One Codex*, *EZBiome*, *BugSeq*)

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for Read Segmentation and Demultiplex Barcodes data utilities. (A video tutorial is also available for viewing.)

SMRT Link Read Segmentation and Demultiplex Barcodes video demonstration

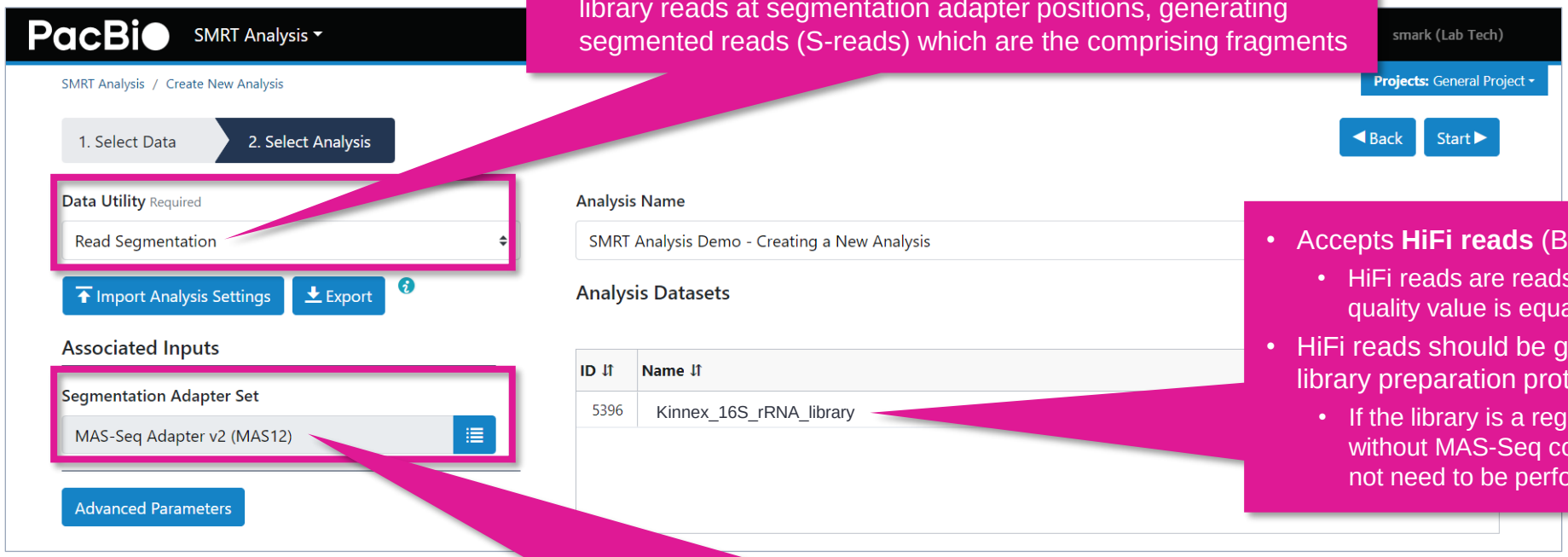
Video demonstration of SMRT Link Read Segmentation and Demultiplex Barcodes workflow for analysis of Kinnex 16S rRNA samples



SMRT Link Read Segmentation setup

Specify **Read Segmentation** data utility in SMRT Link[1]

- **Read Segmentation** data utility splits arrayed Kinnex HiFi library reads at segmentation adapter positions, generating segmented reads (S-reads) which are the comprising fragments



- Accepts **HiFi reads** (BAM format) as input
  - HiFi reads are reads generated with CCS analysis whose quality value is equal to or greater than 20
- HiFi reads should be generated using the Kinnex 16S rRNA library preparation protocol (103-238-800)
  - If the library is a regular (non-Kinnex) 16S monomer library without MAS-Seq concatenation, Read Segmentation does not need to be performed.

- For Kinnex 16S rRNA libraries, specify Segmentation Adapter Set = **MAS-Seq Adapter v2 (MAS12)**
- Specify a FASTA file, provided by PacBio, containing segmentation adapters. If you need a custom segmentation adapter set, click Advanced Parameters and use a custom FASTA file formatted as described in the SMRT Link User Guide

[1] See ***SMRT Link User Guide*** (103-008-800) for detailed descriptions of parameter settings for Read Segmentation data utility.

Example SMRT Link Read Segmentation data utility processing results[1] for Kinnex 16S rRNA library prepared from mock microbial community gDNA

SMRT Link Read Segmentation data utility job report – Summary Metrics and Segmentation Statistics

Summary Metrics

Value	Analysis Metric
6,050,730	Reads
71,720,714	Segmented reads (S-reads)
1,560	Mean length of S-reads
95.03 %	Percent of reads with full arrays
11.85	Mean array size (concatenation factor)

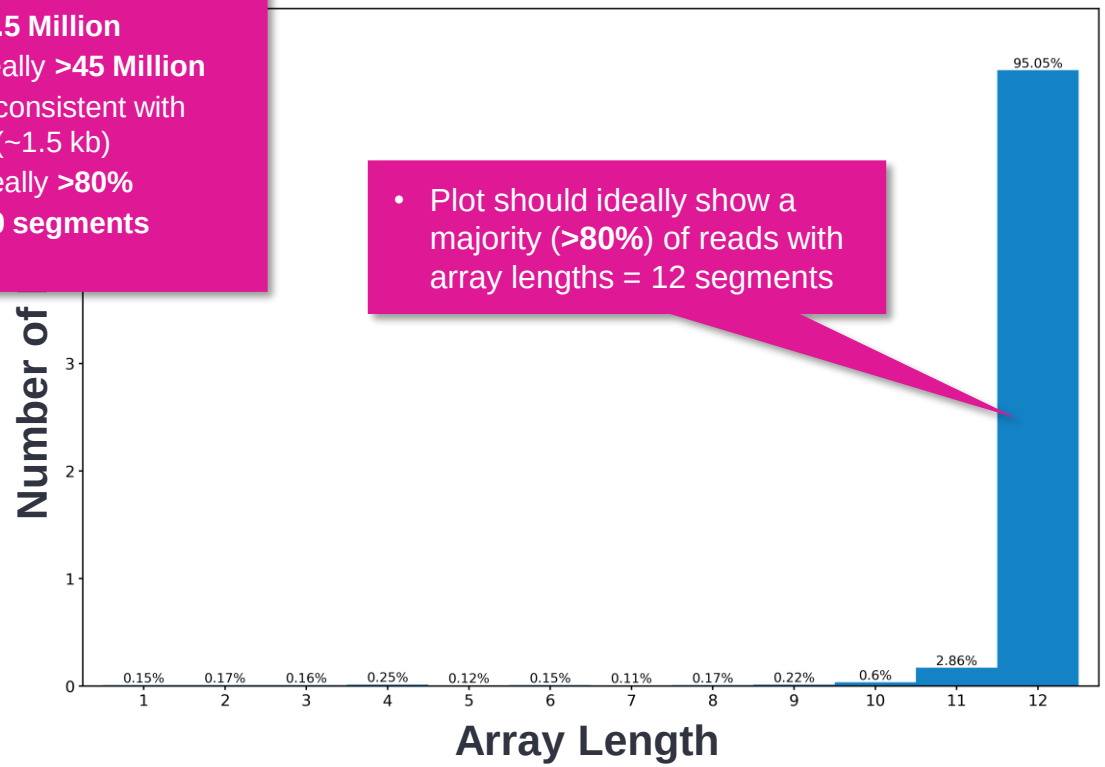
Example Revio system data shown.

For Revio system Kinnex 16S rRNA samples

- Yield of HiFi reads is ideally **>4.5 Million**
- Yield of segmented reads is ideally **>45 Million**
- Mean S-read length should be consistent with expected 16S amplicon length (~1.5 kb)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **>11.0 segments**

- **Reads:** Number of input arrayed HiFi reads
- **Segmented reads (S-reads):** Number of generated S-reads
- **Mean length of S-reads:** Mean read length of generated S-reads
- **Percent of reads with full arrays:** Percentage of input HiFi reads containing all adapter sequences in the order listed in the segmentation adapter FASTA file
- **Mean array size:** Mean number of fragments (or S-reads) found in input reads

Segmentation Statistics



- Plot should ideally show a majority (**>80%**) of reads with array lengths = 12 segments

Histogram distribution of the number of S-reads per HiFi read. (Example Revio system data shown.)

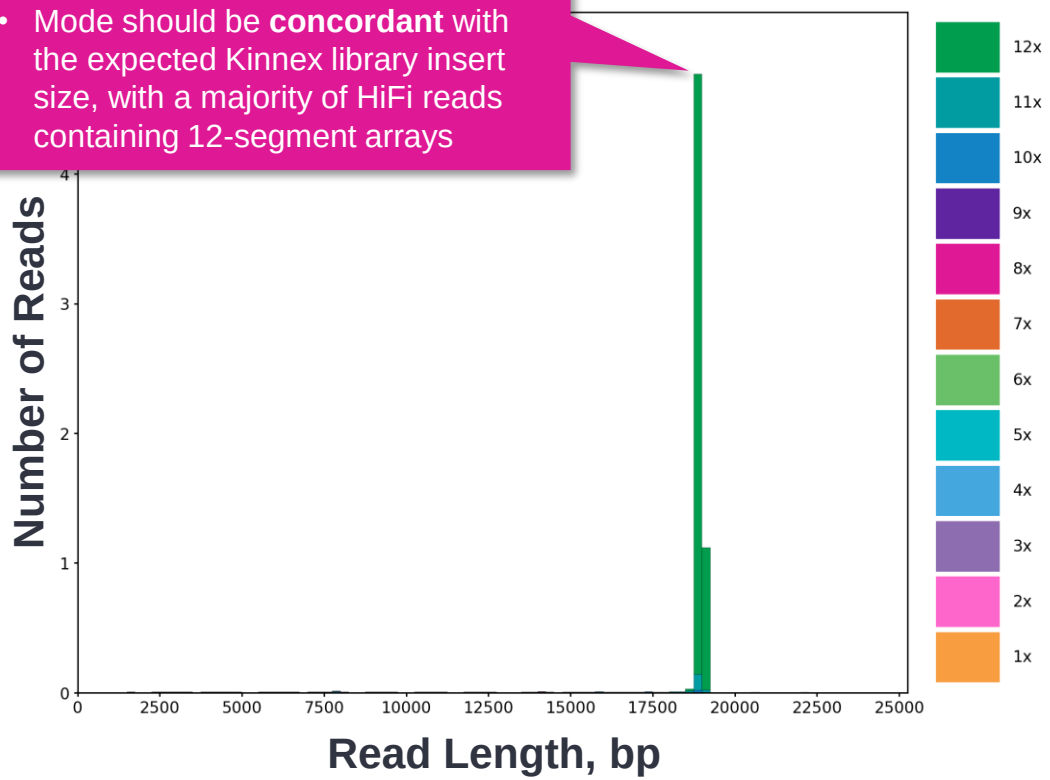
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. For Sequel IIe systems, we recommend aiming for ~60 – 80% *P1* loading. For Revio system, we recommend aiming for ~50 – 70% *P1* loading.

Example SMRT Link Read Segmentation data utility processing results[1] for Kinnex 16S rRNA library prepared from mock microbial community gDNA (cont.)

SMRT Link Read Segmentation data utility job report – Length of Reads and S-read Length Distribution

Length of Reads

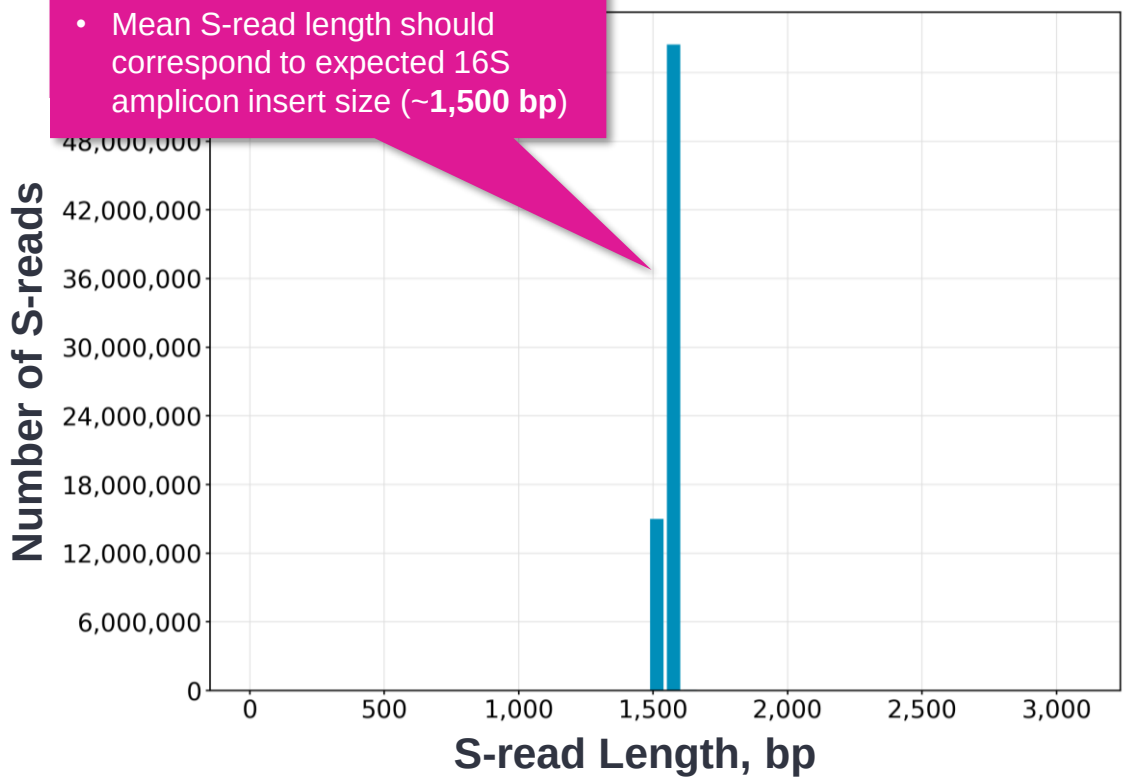
- Mode should be **concordant** with the expected Kinnex library insert size, with a majority of HiFi reads containing 12-segment arrays



Histogram distribution of the number of HiFi reads by read length, in base pairs. (Example Revio system data shown.)

S-read Length Distribution

- Mean S-read length should correspond to expected 16S amplicon insert size (~**1,500 bp**)



Histogram distribution of the number of S-reads by HiFi read length, in base pairs. (Example Revio system data shown.)

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. For Sequel IIe systems, we recommend aiming for ~60 – 80% *P1* loading. For Revio system, we recommend aiming for ~50 – 70% *P1* loading.

Technical documentation & applications support resources

Technical resources for Kinnex full-length 16S rRNA library preparation, sequencing & data analysis

Metagenomic DNA sample preparation resources

- Based on prior PacBio experience, the PowerFecal Pro DNA Kit (QIAGEN) extracts DNA of sufficient quality for the Kinnex full-length 16S rRNA library preparation workflow.

Kinnex 16S rRNA library preparation literature & other resources

- Application note – Kinnex 16S rRNA kit for full-length 16S sequencing (102-326-601)
- Brochure – Scalable, cost-effective RNA sequencing with PacBio Kinnex kits (102-326-597)
- Procedure & checklist – Preparing Kinnex libraries using Kinnex 16S rRNA kit (103-238-700)
- Technical overview – Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing (103-343-700)
- Technical overview – Kinnex library preparation using Kinnex 16S rRNA kit (103-344-800)
- Video tutorial – SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits [Link]

Data analysis resources

- SMRT Link v12.0 MAS-Seq troubleshooting guide (102-994-400)
- SMRT Link v13.1 Kinnex single-cell troubleshooting guide (103-516-100)
- SMRT Link software installation guide [Link]
- SMRT Link user guide [Link]
- SMRT Tools reference guide [Link]
- Video tutorial – Analyzing Kinnex 16S rRNA data in SMRT Link [Link]

Technical resources for Kinnex full-length 16S rRNA library preparation, sequencing & data analysis

Posters & publications

- ASM Microbe Poster (2023) – Increasing throughput of full-length 16S sequencing using concatenation [Link]

Webinars

- PacBio webinar (2023) – Creating a reliable microbiome testing service for companion animal health using PacBio sequencing [Link]

Example PacBio data sets

Application	Dataset	Data type	PacBio system
Kinnex 16S rRNA sequencing	ZymoBIOMICS Fecal Reference with TruMatrix Technology (human) [Link]	HiFi long read	Sequel II & Revio systems
	Mixture: ZymoBIOMICS Gut Microbiome Standard, ZymoBIOMICS Fecal Reference with TruMatrix™ Technology, ATCC 20 Strain Even Mix Genomic Material, ATCC 20 Strain Staggered Mix Genomic Material [Link]	HiFi long read	Sequel II & Revio systems

www.pacb.com