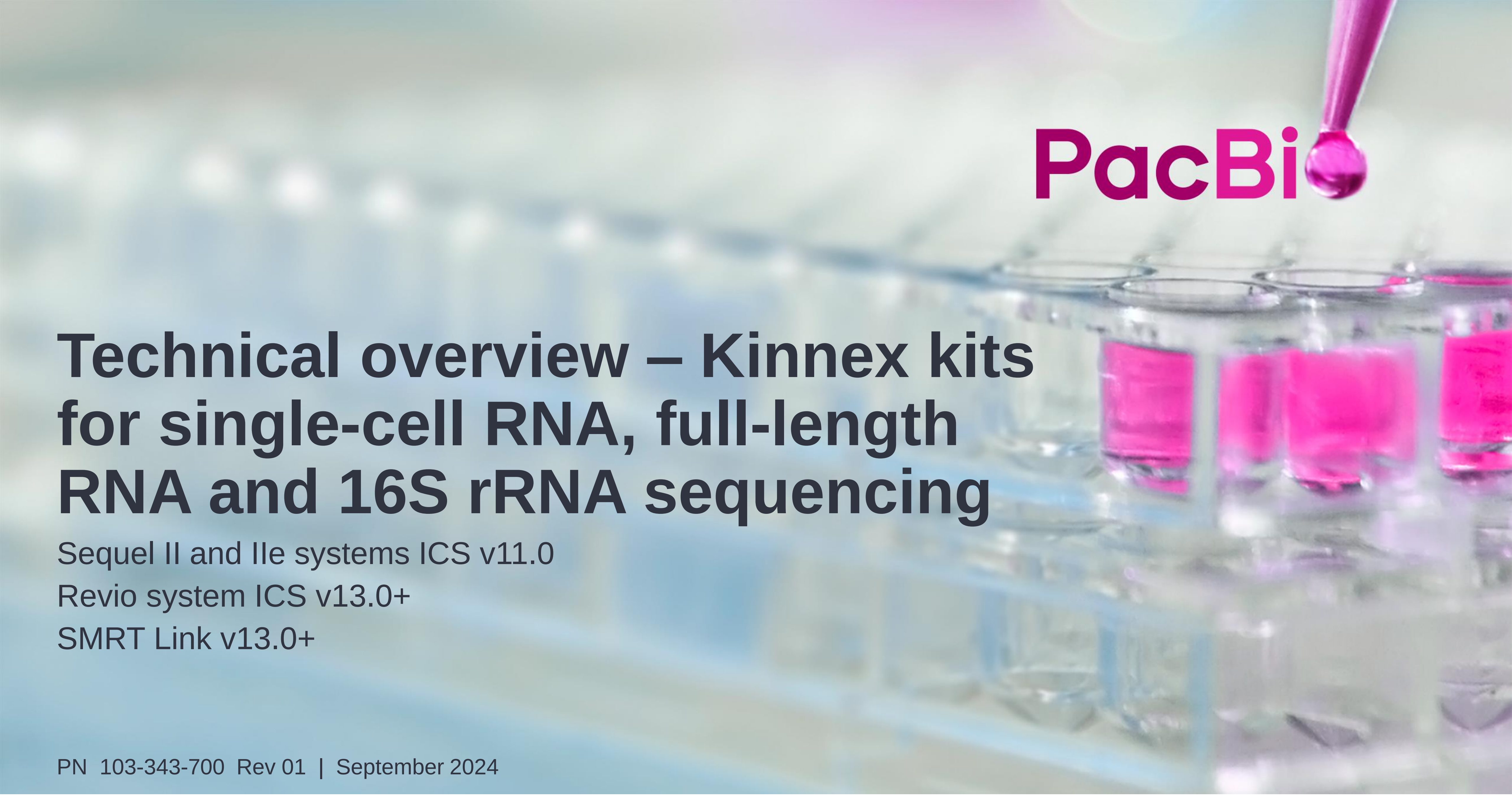


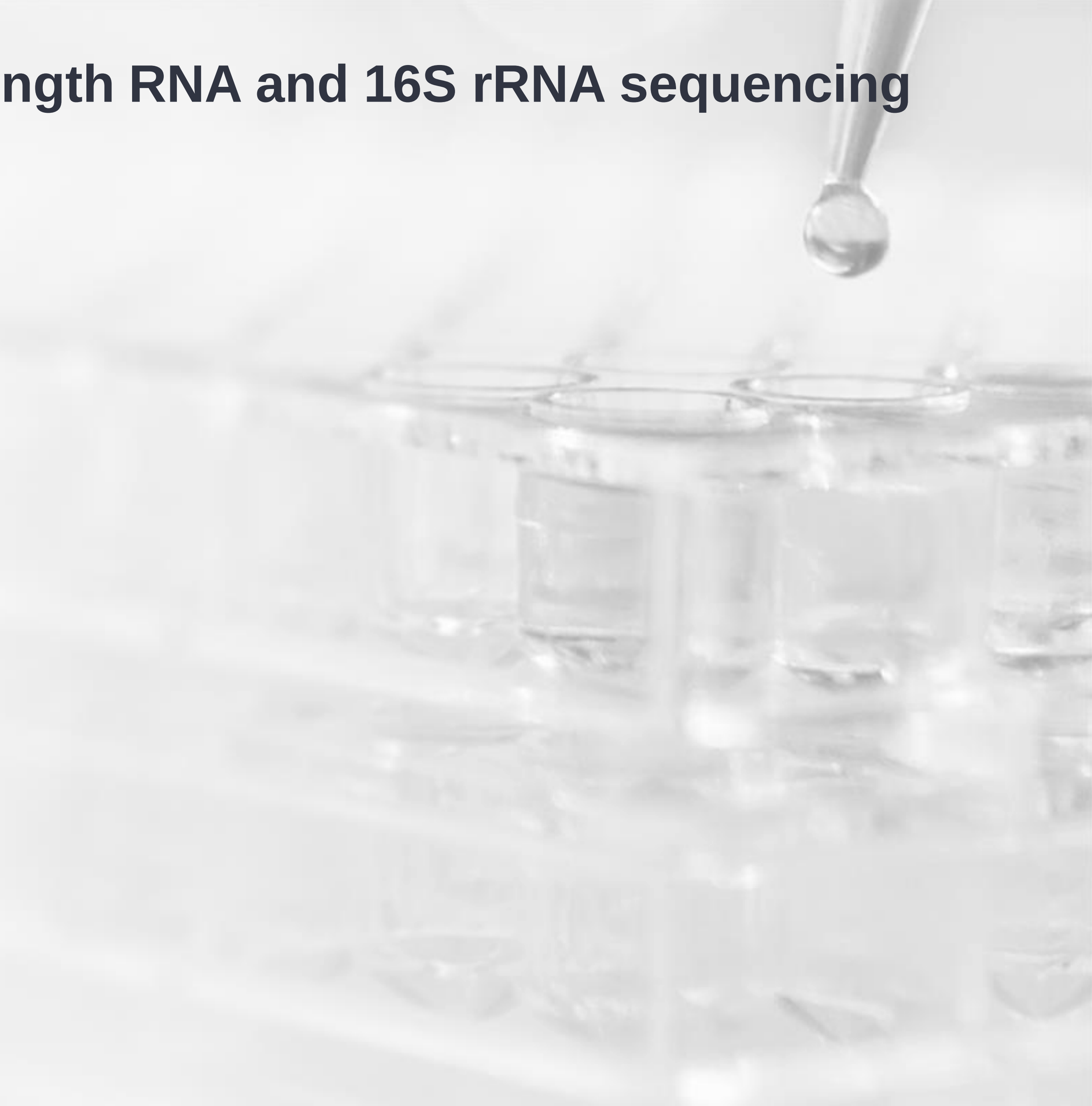
PacBio

Technical overview – Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing

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

PN 103-343-700 Rev 01 | September 2024

Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing



Technical overview

1. Kinnex product introduction
2. Kinnex product configuration overview
3. Kinnex library preparation & sequencing workflow overview
4. Kinnex single-cell RNA workflow key highlights
5. Kinnex full-length RNA workflow key highlights
6. Kinnex 16S rRNA workflow key highlights
7. Technical documentation & applications support resources

Kinnex product introduction

Kinnex kits for single-cell RNA, full-length RNA, and 16S rRNA sequencing

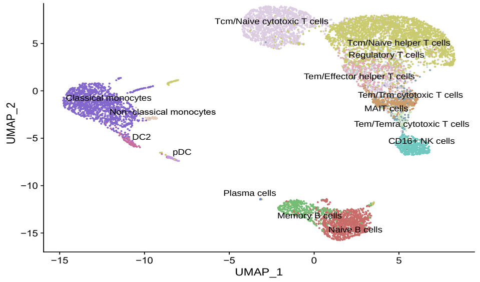
Kinnex kits offer scalable, cost-effective RNA sequencing solutions

Kinnex single-cell RNA kit[1]

Single-cell RNA sequencing
[(10x Chromium Single Cell 3’ or 5’ cDNA input)]

Up to 4-plex Kinnex library
[16-fold concatenation]

80M reads (Revio SMRT Cell)
[40M reads (Sequel II/IIe SMRT Cell 8M)]



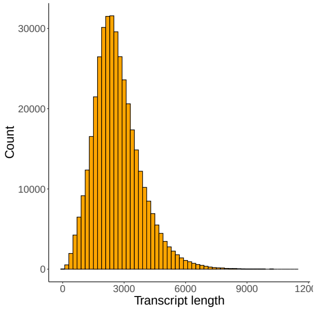
Identify cell type-specific isoform expression

Kinnex full-length RNA kit[2]

Full-length
RNA sequencing

Up to 48-plex Kinnex library
[8-fold concatenation]

40M reads (Revio SMRT Cell)
[15M reads (Sequel II/IIe SMRT Cell)]



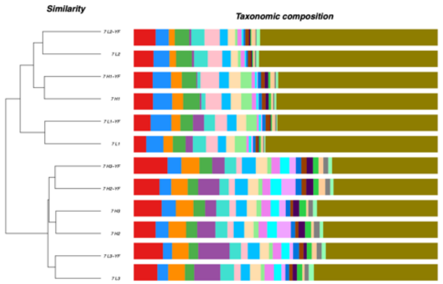
Full-length transcripts from 1–10 kb for bulk RNA samples

Kinnex 16S rRNA kit[2]

Full-length 16S rRNA
for species identification

Up to 1,536-plex Kinnex library
[12-fold concatenation]

60M (Revio SMRT Cell)
[25M reads (Sequel II/IIe SMRT Cell)]



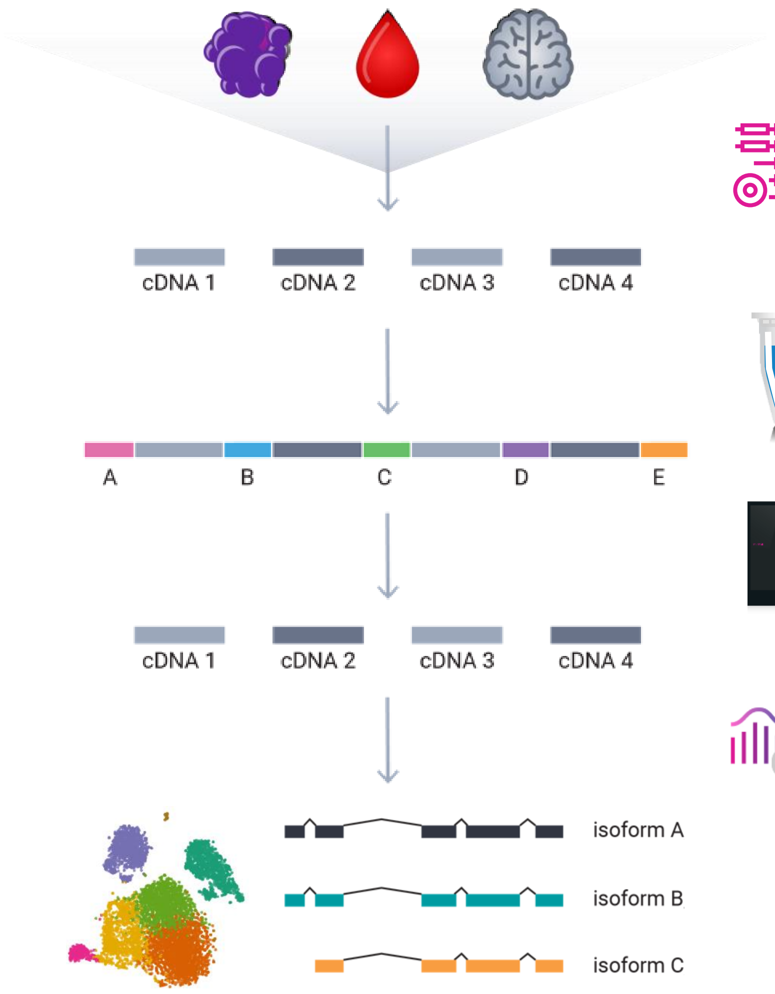
Taxonomic profiling for human, animal, and environmental samples

---

[1] **Note:** Kinnex single-cell RNA kit requires SMRT Link v13.1 or higher.

[2] Kinnex full-length RNA kit and Kinnex 16S rRNA kit requires SMRT Link v13.0 or higher.

Kinnex single-cell RNA kit for single-cell isoform sequencing



Single-cell cDNA generation

Kinnex library preparation

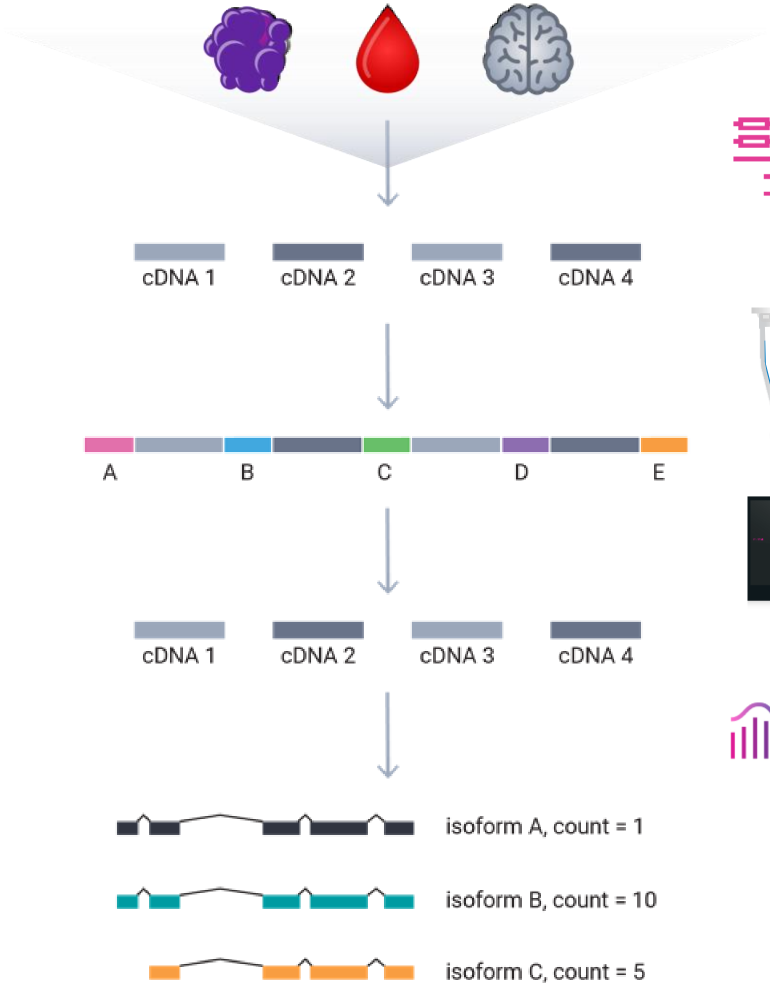
PacBio sequencing

Read segmentation + single-cell Isoform analysis

- 10x Chromium Single Cell 3’ kit (v3.1) and 5’ kit (v2)
- 15–75 ng cDNA input
- 3,000 to 10,000 target cell recovery
- 2-day Kinnex library preparation using Kinnex single-cell RNA kit
- Barcoded Kinnex adapters support up to 4-plex multiplexing
- SMRT Link Run Design support for ‘Kinnex single-cell RNA’ application type option with auto-analysis (read segmentation + single-cell isoform analysis)[1]
- SMRT Link single-cell Iso-Seq isoform-classification software to identify novel genes and isoforms
- Output compatible with tertiary single-cell analysis tools (e.g., *Seurat*, *Scanpy*, *Kana*)

[1] **Note:** Kinnex single-cell RNA kit requires SMRT Link v13.1 or higher.

Kinnex full-length RNA kit for high-accuracy, full-length isoform sequencing



cDNA generation

Kinnex library preparation

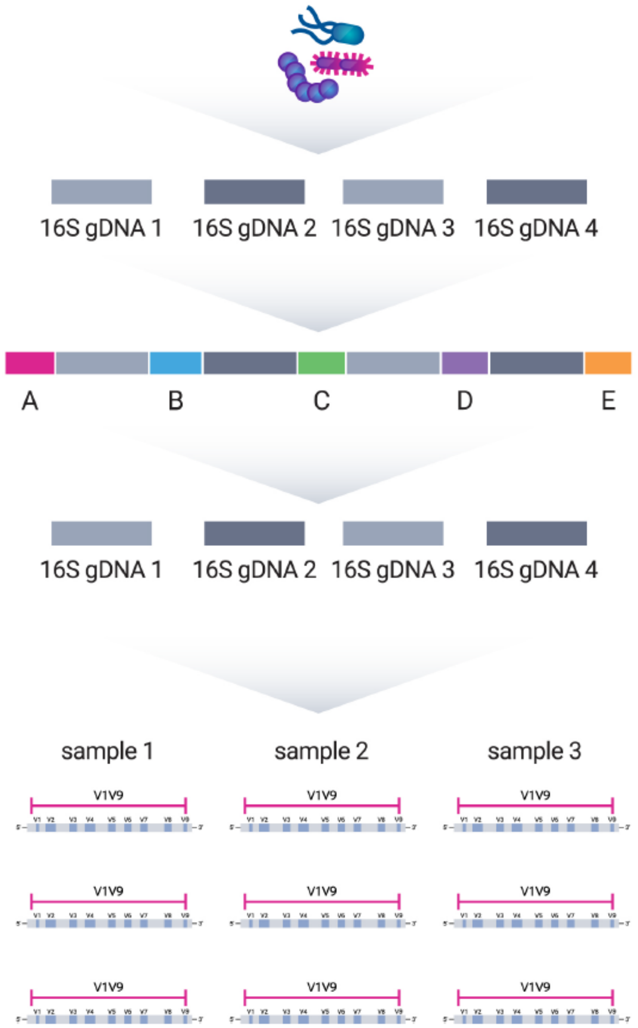
PacBio sequencing

Read segmentation + Isoform analysis

- Input 300 ng total RNA, RIN ≥7
- Generate up to 12-plex barcoded cDNA using Iso-Seq express 2.0 kit (103-071-500)
- 2-day Kinnex library preparation using Kinnex full-length RNA kit (103-072-000)
- SMRT Link Run Design support for ‘Kinnex full-length RNA’ application type with auto-analysis (read segmentation + isoform analysis)[1]
- SMRT Link Iso-Seq isoform-classification software to identify novel genes and isoforms with abundance information

[1] Kinnex full-length RNA kit and Kinnex 16S rRNA kit requires SMRT Link v13.0 or higher.

Kinnex 16S rRNA kit for full-length 16S sequencing



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 full-length RNA kit and Kinnex 16S rRNA kit requires SMRT Link v13.0 or higher.

Kinnex product configuration overview

Kinnex product design overview and supported applications

Kinnex kits utilize the MAS-Seq concatenation method to increase throughput on Sequel II/IIe & Revio systems

Step

Library insert generation

Kinnex PCR

Kinnex array formation

NEW! Support for 10x 5’ single-cell kit

Kinnex single-cell

3’ or 5’ single-cell cDNA generation kit

Kinnex capture primers*

Kinnex capture beads kit

Kinnex single-cell concatenation kit + ancillary cleanup reagents

10x GENOMICS

Remove TSO artifacts from single-cell cDNA

*Incl. with Kinnex single-cell concatenation kit

103-076-000

103-242-000

102-158-300
SMRTbell cleanup beads

101-633-500
Elution buffer

Kinnex FL RNA

Iso-Seq express 2.0 Kit

NEW! Includes 1st-strand cDNA synthesis reagents + barcoded cDNA primers

Kinnex PCR 8-fold kit

103-071-500

Generate and amplify cDNA from starting total bulk RNA input

103-071-600

Kinnex concatenation kit + ancillary cleanup reagents

103-071-800

102-158-300
SMRTbell cleanup beads

101-633-500
Elution buffer

Kinnex 16S

Bacterial 16S PCR protocol

Users to order barcoded 16S PCR primers from 3rd-party vendor

Kinnex PCR 12-fold kit

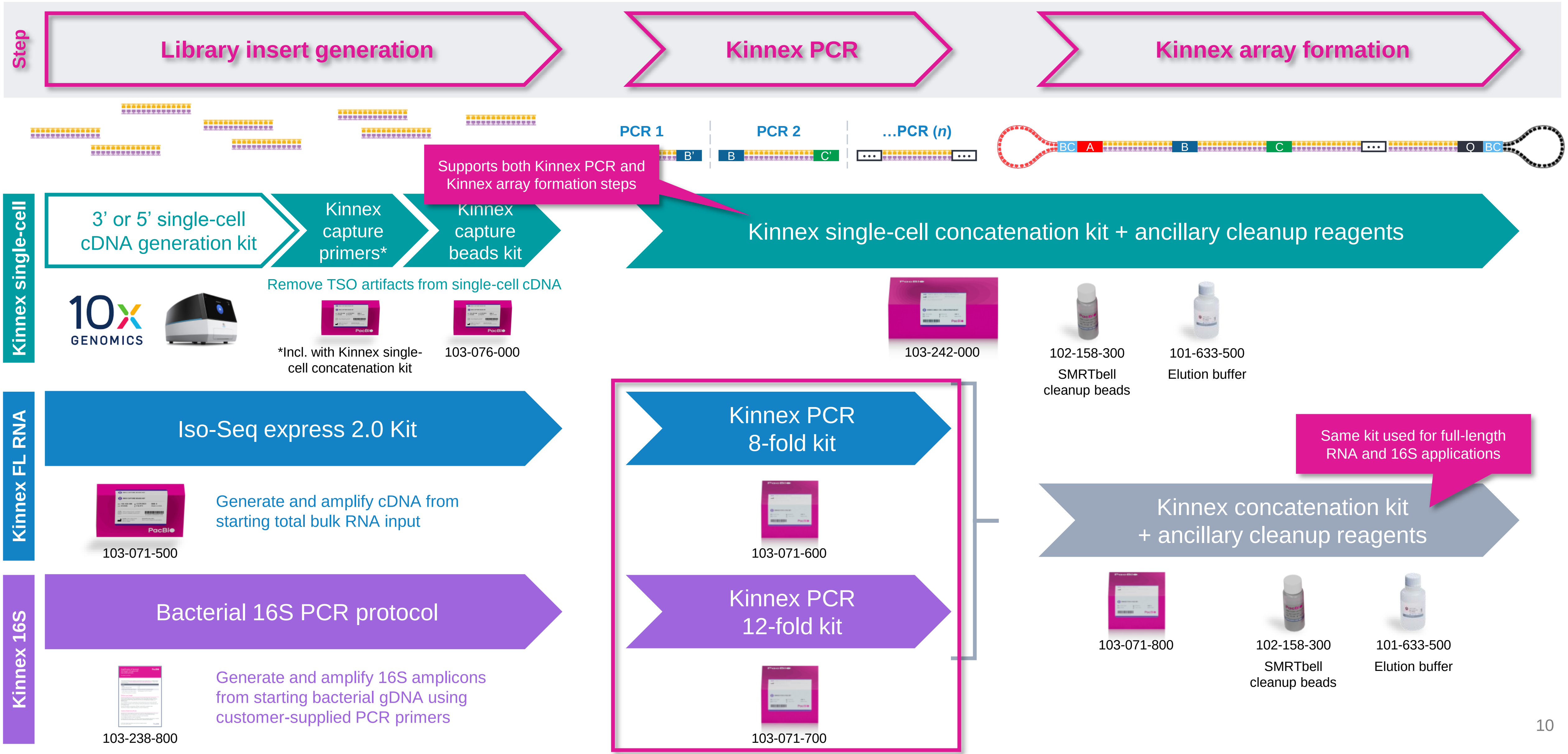
103-238-800

Generate and amplify 16S amplicons from starting bacterial gDNA using customer-supplied PCR primers

103-071-700

Kinnex product design overview and supported applications

Kinnex kits utilize the MAS-Seq concatenation method to increase throughput on Sequel II/IIe & Revio systems



Step

Library insert generation

Kinnex PCR

Kinnex array formation

Kinnex single-cell

3' or 5' single-cell cDNA generation kit

Kinnex capture primers*

Kinnex capture beads kit

Supports both Kinnex PCR and Kinnex array formation steps

Kinnex single-cell concatenation kit + ancillary cleanup reagents

Remove TSO artifacts from single-cell cDNA

*Incl. with Kinnex single-cell concatenation kit

103-076-000

103-242-000

102-158-300
SMRTbell cleanup beads

101-633-500
Elution buffer

Kinnex FL RNA

Iso-Seq express 2.0 Kit

Generate and amplify cDNA from starting total bulk RNA input

103-071-500

Kinnex PCR 8-fold kit

103-071-600

Same kit used for full-length RNA and 16S applications

Kinnex concatenation kit + ancillary cleanup reagents

103-071-800

102-158-300
SMRTbell cleanup beads

101-633-500
Elution buffer

Kinnex 16S

Bacterial 16S PCR protocol

Generate and amplify 16S amplicons from starting bacterial gDNA using customer-supplied PCR primers

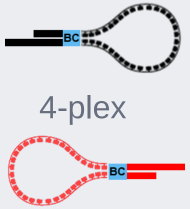
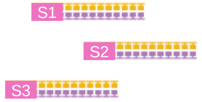
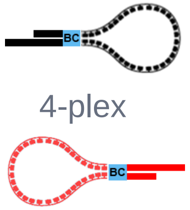
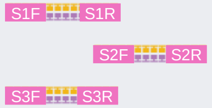
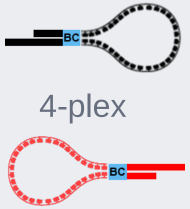
103-238-800

Kinnex PCR 12-fold kit

103-071-700

Kinnex bundle kit product features

All three Kinnex bundle kit products are compatible with Sequel II/IIe and Revio systems

	Kinnex protocol	Kinnex bundle kit product	Concatenation factor	Sample multiplexing capacity			Throughput per SMRT Cell	SMRT Link support
				Amplicon multiplexing	Library multiplexing	Total multiplexing capacity		
Kinnex single-cell	Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) 	**Kinnex single-cell RNA kit** **(103-072-200)** **12 rxn** 	**16-fold**	None	 4-plex	**4-plex**	**~30-40 M** (Sequel II/IIe) **~80-100 M** (Revio)	**Full** Demux BC Read seg. Sc-Iso-Seq
Kinnex FL RNA	Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700) 	**Kinnex full-length RNA kit** **(103-072-000)** **12 rxn** 	**8-fold**	12-plex (Using Iso-Seq express 2.0 kit[1]) 	 4-plex	**48-plex**	**~15 M** (Sequel II/IIe) **~40 M** (Revio)	**Full** Demux BC Read seg. Iso-Seq
Kinnex 16S rRNA	Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800) 	**Kinnex 16S rRNA kit** **(103-072-100)** **12 rxn** 	**12-fold**	384-plex (Using customer-supplied 16S PCR primers) 	 4-plex	**1,536-plex**	**~25 M** (Sequel II/IIe) **~60 M** (Revio)	**Partial** Demux BC Read seg.

---

[1] **Note:** Iso-Seq express 2.0 kit (103-071-500) is **not included** in Kinnex full-length RNA bundle kit and must be purchased separately from PacBio.

Kinnex bundle kit product components

Kinnex full-length RNA and 16S rRNA kits share a common workflow; Kinnex single-cell RNA kit uses existing MAS-Seq single-cell workflow with additional 10x 5’ single-cell cDNA and sample multiplexing support

	Kinnex protocol	Kinnex bundle kit product	Kinnex bundle kit included components				Not included in Kinnex bundle kit but required for application-specific library prep workflow
Kinnex single-cell	Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) 	**Kinnex single-cell RNA kit** **(103-072-200)** **12 rxn** 	 Kinnex capture beads kit 103-076-000 (12 rxn)	 Kinnex single-cell concatenation kit 103-242-000 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300	**10x Genomics Chromium Single Cell 3’ or 5’ reagent kits**
Kinnex FL RNA	Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700) 	**Kinnex full-length RNA kit** **(103-072-000)** **12 rxn** 	 Kinnex PCR 8-fold kit 103-071-600 (12 rxn)	 Kinnex concatenation kit 103-071-800 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300	 **Iso-Seq express 2.0 kit**[1] 103-071-500 (24 rxn)
Kinnex 16S rRNA	Preparing Kinnex libraries from 16S rRNA amplicons (103-238-800) 	**Kinnex 16S rRNA kit** **(103-072-100)** **12 rxn** 	 Kinnex PCR 12-fold kit 103-071-700 (12 rxn)	 Kinnex concatenation kit 103-071-800 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300	**16S gene amplification PCR primers (customer-supplied)**[2]

---

[1] **Note:** Iso-Seq express 2.0 kit (103-071-500) is **not included** in Kinnex full-length RNA bundle kit and must be purchased separately from PacBio.

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

Kinnex library preparation & sequencing workflow overview

Kinnex library preparation workflow overview

Kinnex single-cell RNA (2 days)[1]

PacBio [103-254-300](103-254-300)

Preparing Kinnex libraries using Kinnex single-cell RNA kit

Library insert generation

1 Input 3’ or 5’ sc-cDNA quality control

2 TSO PCR + cleanup

○ Safe stop

3 TSO artifact removal + cleanup

○ Safe stop

Kinnex PCR

4 Kinnex PCR + cleanup

○ Safe stop

Day 1 (~6 hrs)

Kinnex array formation & cleanup

5 Kinnex array formation + cleanup

○ Safe stop

6 DNA damage repair + cleanup

○ Safe stop

7 Nuclease treatment

8 Final SMRTbell bead cleanup

Day 2 (~3 hrs)

Kinnex full-length RNA (2 days)[1]

PacBio 103-238-700

Preparing Kinnex libraries using the Kinnex full-length RNA kit

Library insert generation

1 Input total RNA quality control

2 cDNA synthesis + cleanup

○ Safe stop

3 cDNA amplification + cleanup

Pool barcoded samples if multiplexing

○ Safe stop

Kinnex PCR

4 Kinnex PCR + cleanup

○ Safe stop

Day 1 (~5 hrs)

Kinnex array formation & cleanup

5 Kinnex array formation

6 DNA damage repair + cleanup

○ Safe stop

7 Nuclease treatment

8 Final SMRTbell bead cleanup

Day 2 (~3 hrs)

Kinnex 16S rRNA (2 days)[1]

PacBio 103-238-800

Preparing Kinnex libraries from 16S rRNA amplicons

Library insert generation

1 Input metagenomic gDNA quality control

2 PCR amplify full-length 16S genes

○ Safe stop

3 16S amplicon pooling + cleanup

Pool barcoded samples if multiplexing

○ Safe stop

Kinnex PCR

4 Kinnex PCR + cleanup

○ Safe stop

Day 1 (~3 hrs)

Kinnex array formation & cleanup

5 Kinnex array formation

6 DNA damage repair + cleanup

○ Safe stop

7 Nuclease treatment

8 Final SMRTbell bead cleanup

Day 2 (~3 hrs)

---

[1] Workflow times shown are for processing up to 12 Kinnex single-cell RNA samples, up to 24 Kinnex full-length RNA samples, and up to 1,536 Kinnex 16S rRNA samples.

Kinnex library insert generation key workflow steps

Single-cell cDNA sample preparation

Single-cell cDNA synthesis, amplification & cleanup

10x GENOMICS

Full-length single-cell 3' cDNA product (CBC = 10x cell barcode)

Min. cDNA input for TSO artifact removal step = 15 ng

OR

Full-length single-cell 5' cDNA product (CBC = 10x cell barcode)

Min. cDNA input for TSO artifact removal step = 15 ng

TSO artifact removal[1]

PacBio

103-254-300

3' single-cell cDNA with TSO artifacts removed

5' single-cell cDNA with TSO artifacts removed

Cleanup

Single-cell cDNA sample ready for Kinnex PCR step

Full-length RNA sample preparation

Total RNA isolation & QC

Example: QIAGEN RNeasy Plus kit

Minimum total RNA input for cDNA synthesis = 300 ng

cDNA synthesis, amplification & cleanup

Can barcode up to 12 samples during cDNA amplification step using PacBio barcoded Fwd Iso-Seq primers

Barcoded full-length cDNA product

Pooling of barcoded cDNA samples & cleanup

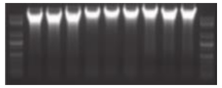
If multiplexing, perform equal-mass pooling of barcoded cDNA samples

Pooled, full-length cDNA sample ready for Kinnex PCR step

16S RNA sample preparation

Bacterial metagenomic DNA isolation & QC

Example: QIAGEN Powerfecal Pro kit



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

PCR amplification of full-length 16S genes

Can barcode up to 384 samples during 16S gene amplification step using combinatorial dual-indexing with barcoded Fwd + Rev 16S gene-specific primers

Dual-barcoded 16S amplicon product

Pooling of barcoded 16S PCR amplicons & cleanup

Perform equal-volume pooling of barcoded 16S samples

Pooled, full-length 16S amplicon sample ready for Kinnex PCR step

---

[1] Video tutorial is available: See *TSO artifact removal demo for PacBio MAS-Seq for 10x Single Cell 3' kit* [Click to view the online video: Link]

[2] Can use 12 different barcoded Iso-Seq Forward PCR primers to multiplex up to 12 samples per library prep Rx

[3] Can use 12 different 16S barcoded Forward PCR primers + 32 different 16S barcoded Reverse PCR primers to multiplex up to 384 samples per library prep Rx

Application-specific Kinnex library insert generation workflow comparison

MAS-Seq for 10x single-cell 3'

Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit (102-678-600)

MAS-Seq for 10x 3' concatenation kit (102-407-900)

MAS capture beads kit (102-428-400)

TSO PCR → SMRTbell bead cleanup → TSO artifact removal → SMRTbell bead cleanup

1.0 hr | 20 min | 1.0 hr | 20 min

~3 hrs

Kinnex single-cell

Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

Kinnex single-cell concatenation kit (103-242-000)

Kinnex capture beads kit (103-076-000)

TSO PCR → SMRTbell bead cleanup → TSO artifact removal → SMRTbell bead cleanup

1.0 hr | 20 min | 1.0 hr | 20 min

~3 hrs

Kinnex FL RNA

Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

Iso-Seq express 2.0 kit (103-071-500)

cDNA synthesis → SMRTbell bead cleanup → cDNA amplification → SMRTbell bead cleanup

1.3 hrs | 20 min | 1.0 hr | 20 min

~3 hrs

Kinnex 16S rRNA

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

Full-length 16S gene amplification protocol (103-238-800) using customer-supplied PCR primers

PCR amplification of F-L 16S genes → Pooling of barcoded 16S PCR amplicons → SMRTbell bead cleanup

1.3 hrs | 10 min | 20 min

~2 hrs

PacBio

Kinnex PCR key workflow steps

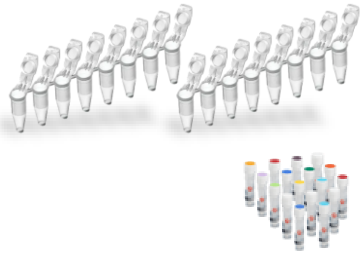
Kinnex PCR step incorporates programmable segmentation adapter sequences into library insert samples

Application	Concatenation factor
Kinnex full-length RNA	8-fold
Kinnex 16S rRNA	12-fold
Kinnex single-cell RNA	16-fold

Set up 8/12/16 parallel PCR reactions per sample to generate amplified library inserts containing programmable sequences at both ends

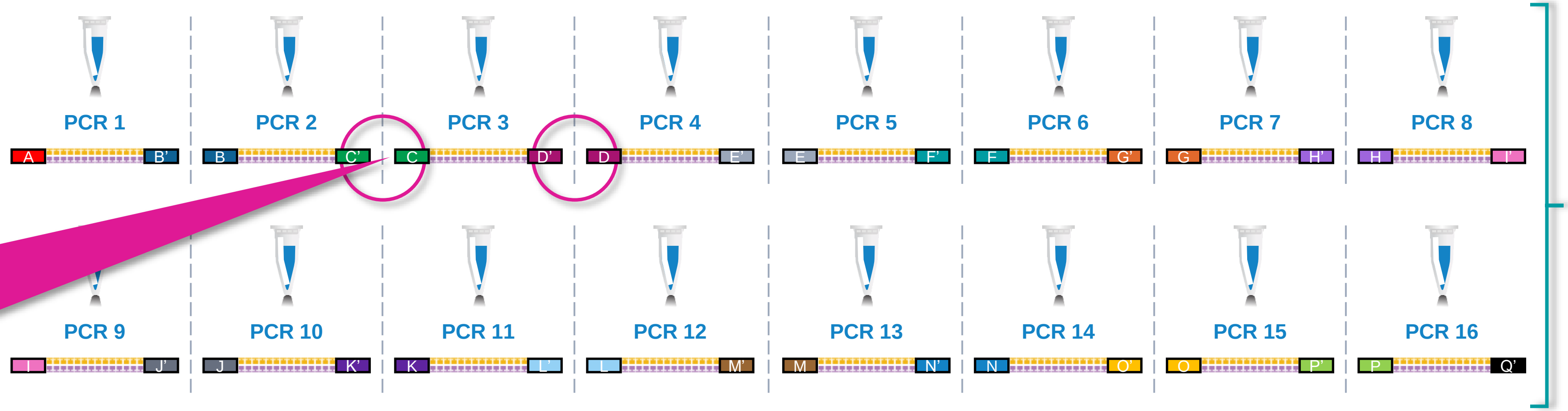
Library insert sample (single-cell cDNA *OR* full-length cDNA *OR* 16S amplicon DNA)

Distribute library insert sample + Kinnex PCR reaction mix into 8/12/16 PCR tubes or 8/12/16 wells in a 96-well plate



OR

Perform PCR by adding premixed Kinnex primer pair solutions



Example Kinnex PCR setup for Kinnex single-cell RNA (16-fold concatenation factor)

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 8/12/16 Kinnex PCR products together and perform SMRTbell bead cleanup

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

PacBio

Application-specific Kinnex PCR workflow comparison

Kinnex PCR thermal cycler program

MAS-Seq for 10x single-cell 3'

Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit (102-678-600)

MAS-Seq for 10x 3' concatenation kit (102-407-900)

Kinnex PCR (16-mer) → SMRTbell bead cleanup

1.5 hrs | 20 min

~2 hrs

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

Kinnex single-cell

Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

Kinnex single-cell concatenation kit (103-242-000)

Kinnex PCR (16-mer) → SMRTbell bead cleanup

1.5 hrs | 20 min

~2 hrs

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

Kinnex FL RNA

Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

Kinnex PCR 8-fold kit (103-071-600)

Kinnex PCR (8-mer) → SMRTbell bead cleanup

1.5 hrs | 20 min

~2 hrs

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

Kinnex 16S rRNA

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

Kinnex PCR 12-fold kit (103-071-700)

Kinnex PCR (12-mer) → SMRTbell bead cleanup

1.0 hr | 20 min

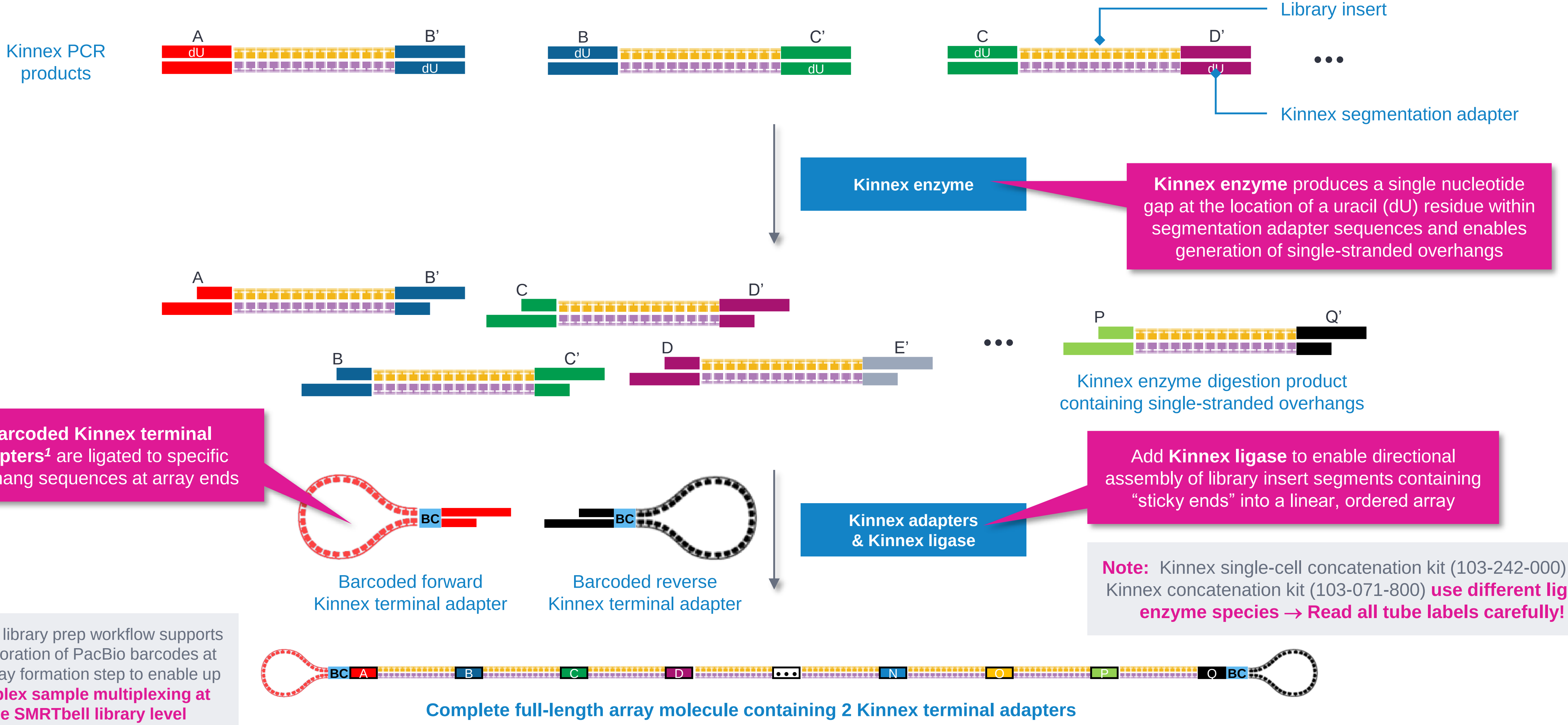
~1.5 hrs

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

PacBio

Kinnex array formation key workflow steps

Kinnex array formation step involves assembly of Kinnex PCR products (“segments”) containing a library insert + programmable “sticky ends” into a linear array



Kinnex PCR products

Library insert

Kinnex segmentation adapter

Kinnex enzyme

Kinnex enzyme produces a single nucleotide gap at the location of a uracil (dU) residue within segmentation adapter sequences and enables generation of single-stranded overhangs

Kinnex enzyme digestion product containing single-stranded overhangs

Barcoded Kinnex terminal adapters[1] are ligated to specific overhang sequences at array ends

Barcoded forward Kinnex terminal adapter

Barcoded reverse Kinnex terminal adapter

Kinnex adapters & Kinnex ligase

Add **Kinnex ligase** to enable directional assembly of library insert segments containing “sticky ends” into a linear, ordered array

Note: Kinnex single-cell concatenation kit (103-242-000) and Kinnex concatenation kit (103-071-800) **use different ligase enzyme species → Read all tube labels carefully!**

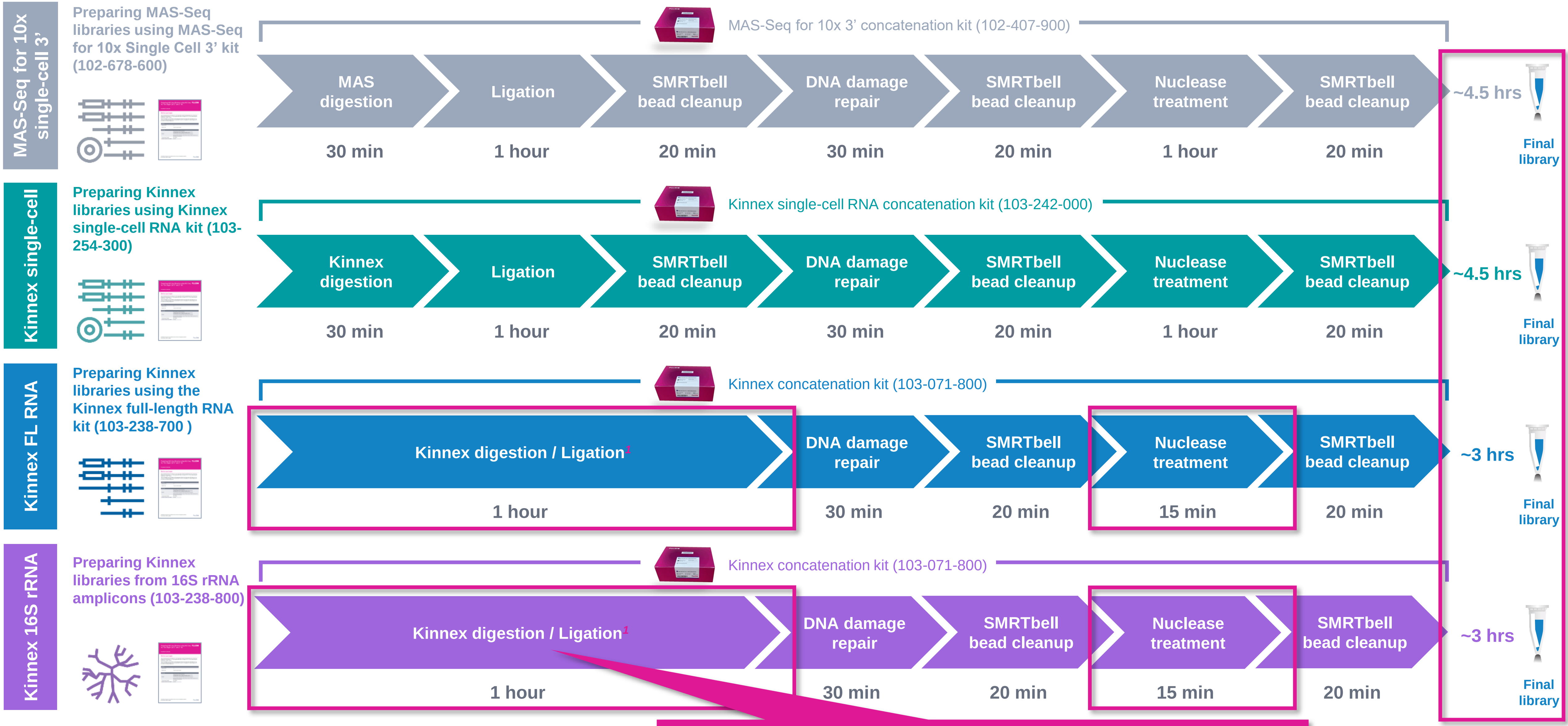
Kinnex library prep workflow supports incorporation of PacBio barcodes at the array formation step to enable up to **4-plex sample multiplexing at the SMRTbell library level**

Complete full-length array molecule containing 2 Kinnex terminal adapters

---

[1] Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-bcM0004) 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.

Application-specific Kinnex array formation workflow comparison



MAS-Seq for 10x single-cell 3'

Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit (102-678-600)

MAS-Seq for 10x 3' concatenation kit (102-407-900)

MAS digestion	Ligation	SMRTbell bead cleanup	DNA damage repair	SMRTbell bead cleanup	Nuclease treatment	SMRTbell bead cleanup	~4.5 hrs
30 min	1 hour	20 min	30 min	20 min	1 hour	20 min	Final library

Kinnex single-cell

Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

Kinnex single-cell RNA concatenation kit (103-242-000)

Kinnex digestion	Ligation	SMRTbell bead cleanup	DNA damage repair	SMRTbell bead cleanup	Nuclease treatment	SMRTbell bead cleanup	~4.5 hrs
30 min	1 hour	20 min	30 min	20 min	1 hour	20 min	Final library

Kinnex FL RNA

Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

Kinnex concatenation kit (103-071-800)

Kinnex digestion / Ligation[1]	DNA damage repair	SMRTbell bead cleanup	Nuclease treatment	SMRTbell bead cleanup	~3 hrs
1 hour	30 min	20 min	15 min	20 min	Final library

Kinnex 16S rRNA

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

Kinnex concatenation kit (103-071-800)

Kinnex digestion / Ligation[1]	DNA damage repair	SMRTbell bead cleanup	Nuclease treatment	SMRTbell bead cleanup	~3 hrs
1 hour	30 min	20 min	15 min	20 min	Final library

Kinnex digestion + ligation performed as a single reaction instead of two separate sequential reactions and SMRTbell bead cleanup is not required afterwards
→ Kinnex full-length RNA & 16S rRNA protocols are more **streamlined**

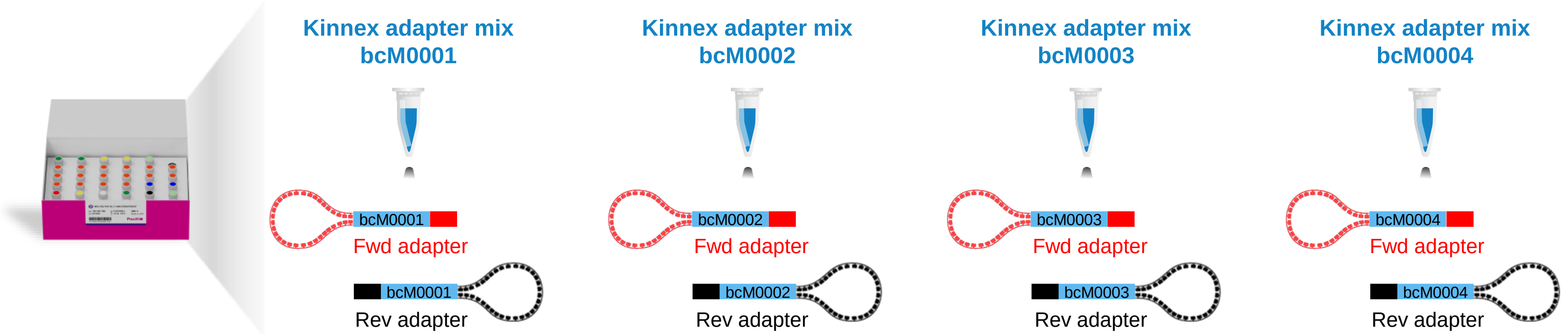
PacBio

[1] Kinnex digestion / ligation reaction does not require addition of ligatio

New Kinnex SMRTbell adapter design

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

- New 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 (bcM0001-bcM0004) to enable multiplexing of up to 4 samples per SMRT Cell

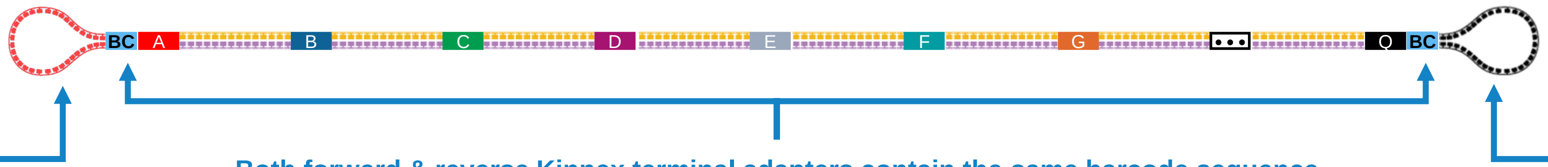


SMRT Link

Barcodes

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

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



Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

---

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

New Kinnex SMRTbell adapter design (cont.)

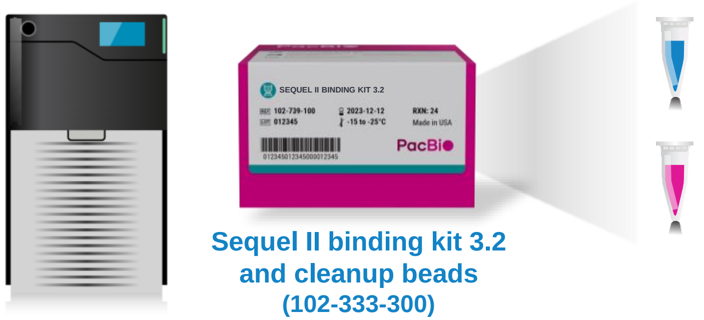
Kinnex terminal adapters use a new design that enables improved SMRT sequencing performance

- 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)



Sequencing primer 3.2

Kinnex sequencing primer (103-179-000)

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

Standard sequencing primer

Kinnex sequencing primer (103-179-000)

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

---

[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.

Application-specific Kinnex library prep input & expected step yield comparison

MAS-Seq for 10x single-cell 3'

Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit (102-678-600)

Library insert generation

- TSO PCR — **IN:** 15 ng, 60-75 ng or 75 ng of 10x 3' or 5' cDNA
- SMRTbell bead cleanup — **OUT: ~200 ng – 1 µg of amplified products**
- TSO Removal — **IN:** 150 ng – 1 µg of TSO PCR products
- SMRTbell bead cleanup — **OUT: ≥50 ng of TSC artifact-depleted cD**

Kinnex PCR

- Kinnex PCR
- SMRTbell bead cleanup — **mplified oling**

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

Kinnex array forma

- Kinnex digestion — **IN:** 10 µg of pooled MAS PCR products
- Ligation
- SMRTbell cleanup — **OUT: ≥5 µg of MAS array products**
- DNA damage repair — **IN:** 5 µg of MAS array products
- SMRTbell cleanup
- uclease ment
- SMRTbell bead cleanup — **OUT: 10-25% recovery from DDR Rx input**

Kinnex single-cell

Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

Library insert generation

- TSO PCR — **IN:** 15 ng, 60-75 ng or 75 ng of 10x 3' or 5' cDNA
- SMRTbell bead cleanup — **OUT: ~200 ng – 1 µg of amplified products**
- TSO removal — **IN:** 150 ng – 1 µg of TSO PCR products
- SMRTbell bead cleanup — **OUT: ≥50 ng of TSO artifact-depleted cDNA**

Kinnex PCR

- Kinnex PCR — **Min. IN:** 50 ng* of TSO artifact-depleted cDNA
- SMRTbell bead cleanup — **OUT: ≥10 µg of amplified products after pooling**

Kinnex array formation

- Kinnex digestion — **IN:** 5 µg of pooled Kinnex PCR products
- Ligation
- SMRTbell cleanup
- 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: 10-25% recovery from Kinnex digestion Rx input**

Kinnex FL RNA

Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

Library insert generation

- cDNA synthesis — **IN:** 300 ng of total RNA per sample
- SMRTbell bead cleanup
- cDNA amplification
- SMRTbell bead cleanup — **OUT: ≥100 ng of amplified cDNA per sample**

Kinnex PCR

- Kinnex PCR — **IN:** 55 ng of amplified cDNA (single or multiplex)
- SMRTbell bead cleanup — **OUT: 6-12 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: 10-25% recovery from Kinnex digestion Rx input**

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

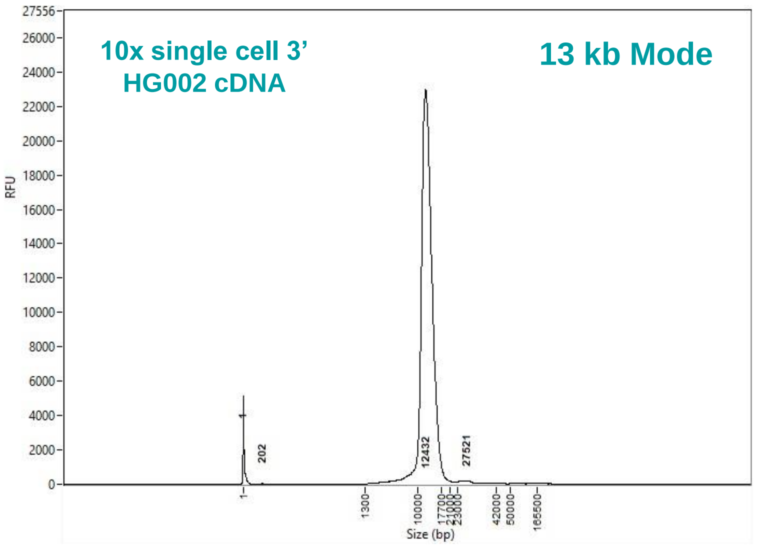
- Kinnex PCR — **IN:** 35 ng of pooled 16S amplicons
- SMRTbell bead cleanup — **OUT: 5-9 µg of amplified products after pooling**

Kinnex array formation

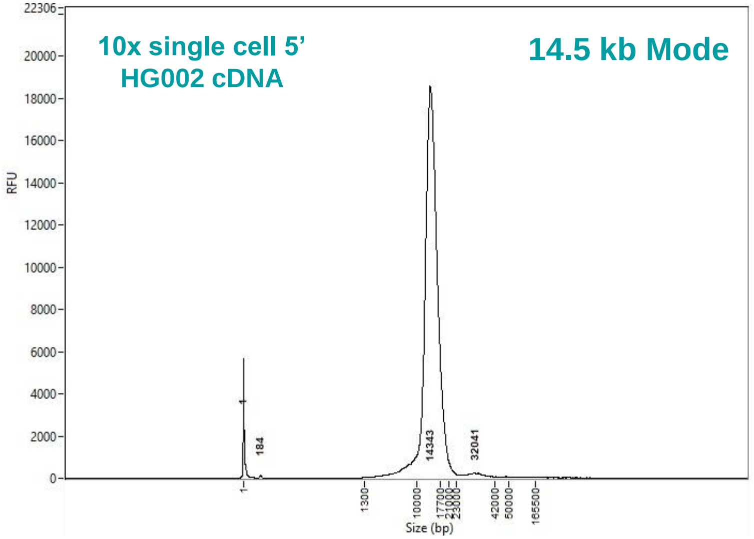
- Kinnex digestion / Ligation — **IN:** 5 µg of pooled Kinnex PCR products
- DNA damage repair
- SMRTbell cleanup — **OUT: 80% recovery from Kinnex digestion Rx input**
- Nuclease treatment — **IN:** 40 µL of DDR reaction products
- SMRTbell bead cleanup — **OUT: 10-20% recovery from Kinnex digestion Rx input**

Example Femto Pulse DNA sizing QC results for final Kinnex SMRTbell libraries

Kinnex single-cell RNA
(16-fold target concatenation)

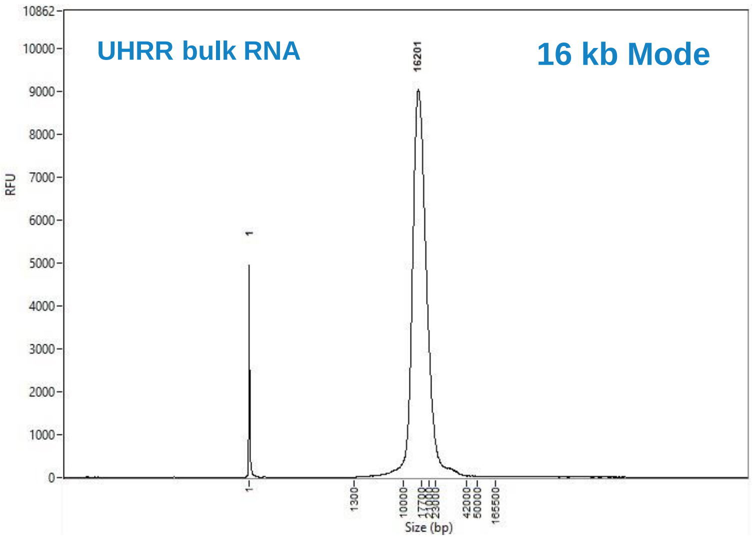

10x single cell 3’
HG002 cDNA

13 kb Mode


10x single cell 5’
HG002 cDNA

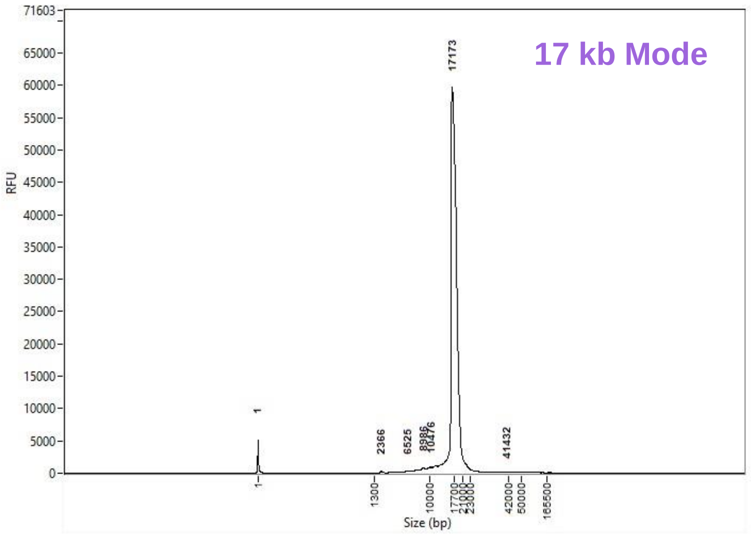
14.5 kb Mode

Kinnex full-length RNA
(8-fold target concatenation)


UHRR bulk RNA

16 kb Mode

Kinnex 16S rRNA
(12-fold target concatenation)


17 kb Mode

Kinnex library type	Kinnex library size range
Kinnex single-cell RNA	~12 – 16 kb
Kinnex full-length RNA	~12 – 16 kb
Kinnex 16S rRNA	~17 – 18 kb

Kinnex sequencing preparation workflow overview

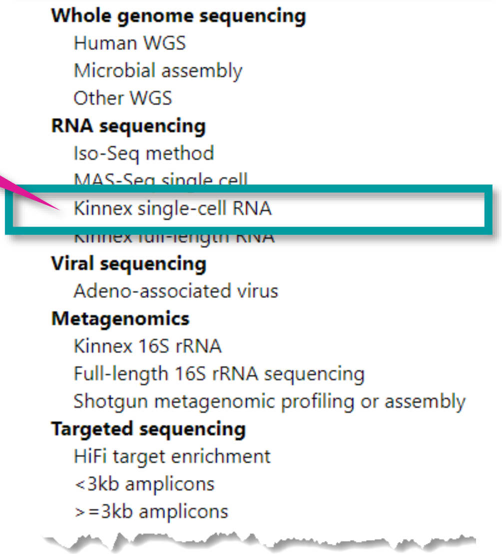
Sample Setup & Run Design recommendations for Kinnex libraries

Follow SMRT Link instructions to prepare Kinnex libraries for sequencing[1,2]

Select **desired Kinnex application type** in SMRT Link Sample Setup & Runs modules

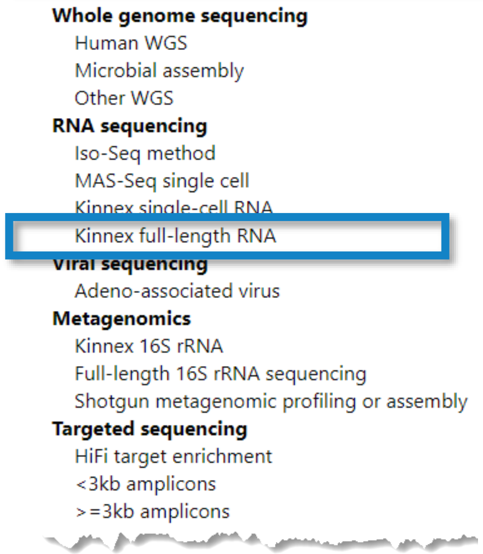
After specifying your application type, **SMRT Link** **auto-fills** selected Sample Setup & Run Design parameter fields with default recommended values

Kinnex single-cell RNA[1]



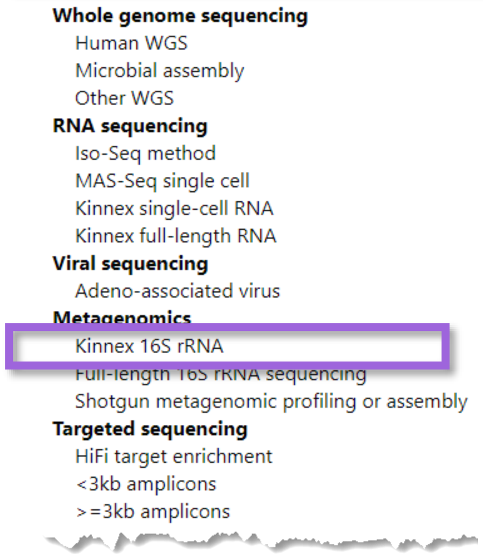
Whole genome sequencing
Human WGS
Microbial assembly
Other WGS
RNA sequencing
Iso-Seq method
MAS-Seq single cell
Kinnex single-cell RNA
Kinnex full-length RNA
Viral sequencing
Adeno-associated virus
Metagenomics
Kinnex 16S rRNA
Full-length 16S rRNA sequencing
Shotgun metagenomic profiling or assembly
Targeted sequencing
HiFi target enrichment
<3kb amplicons
>=3kb amplicons

Kinnex full-length RNA[2]



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
Full-length 16S rRNA sequencing
Shotgun metagenomic profiling or assembly
Targeted sequencing
HiFi target enrichment
<3kb amplicons
>=3kb amplicons

Kinnex 16S rRNA[2]



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
Full-length 16S rRNA sequencing
Shotgun metagenomic profiling or assembly
Targeted sequencing
HiFi target enrichment
<3kb amplicons
>=3kb amplicons

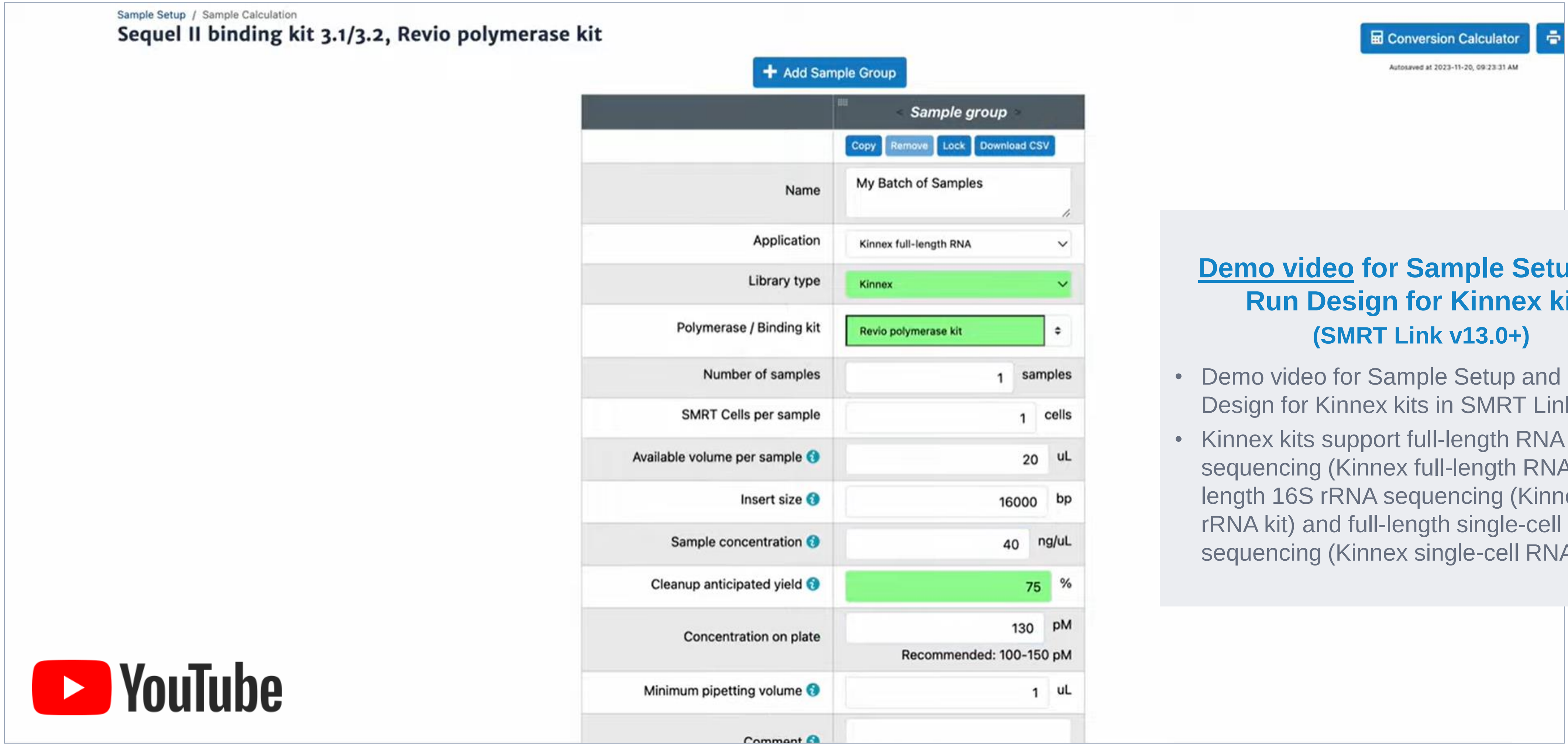
SMRT Link module	Key setup parameters	Sequel II/IIe system recommended settings	Revio system recommended settings
Sample setup	**Library type**	Kinnex	
	Primer	Kinnex sequencing primer	
	Binding/Polymerase kit[1]	Sequel II binding kit 3.2 (includes Kinnex sequencing primer)	Revio polymerase kit (includes Kinnex sequencing primer)
	Concentration on plate	40 – 60 pM	100 – 150 pM
Runs → Run design	**Library type**	Kinnex	
	Movie collection time	30 hrs	24 hrs
	Use adaptive loading	YES	

[1] **Note:** Kinnex single-cell RNA kit requires SMRT Link v13.1 or higher.

[2] Kinnex full-length RNA kit and 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



Sequel II binding kit 3.1/3.2, Revio polymerase kit

Autosaved at 2023-11-20, 09:23:31 AM

Name	My Batch of Samples
Application	Kinnex full-length RNA
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	16000 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	

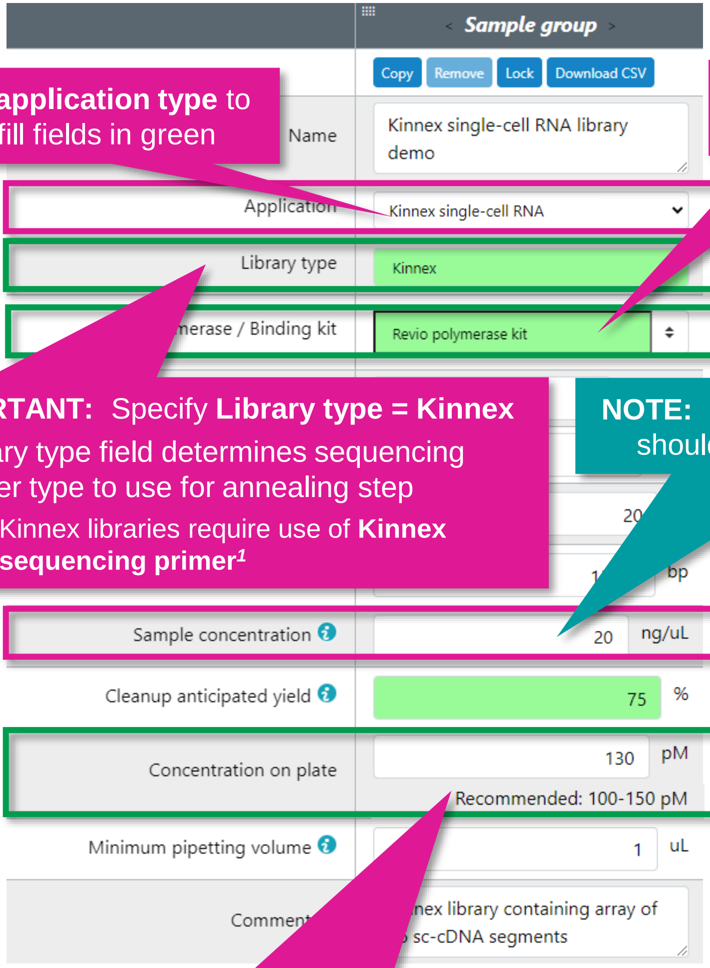
Demo video for Sample Setup and Run Design for Kinnex kits
(SMRT Link v13.0+)

- 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)

View Video tutorial – ***SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits*** at: YouTube Link

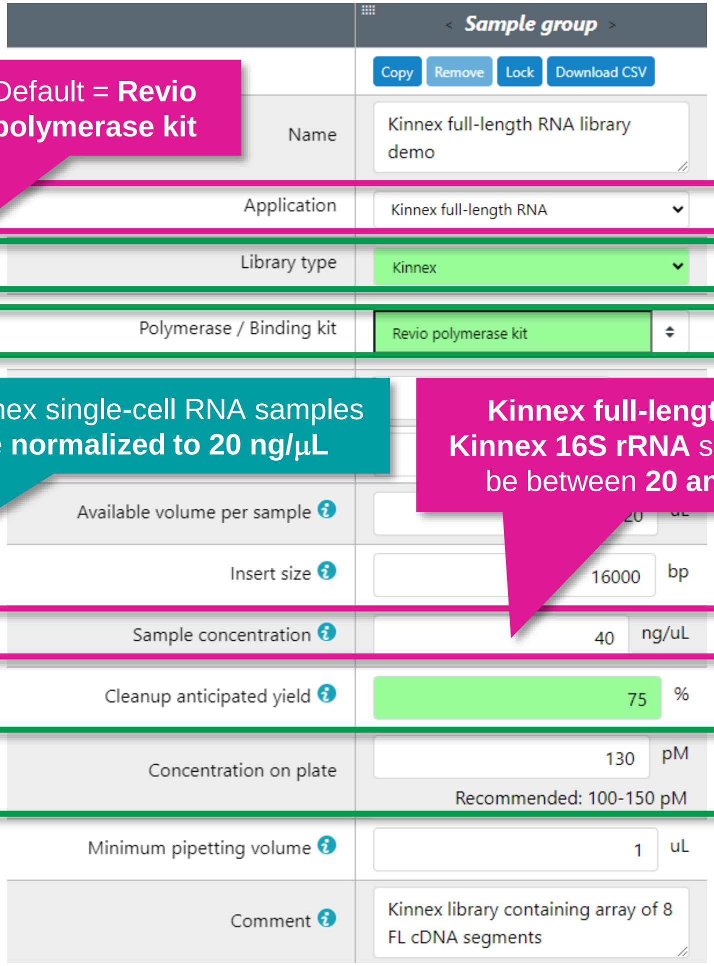
SMRT Link Sample Setup procedure for Revio system

Kinnex single-cell RNA



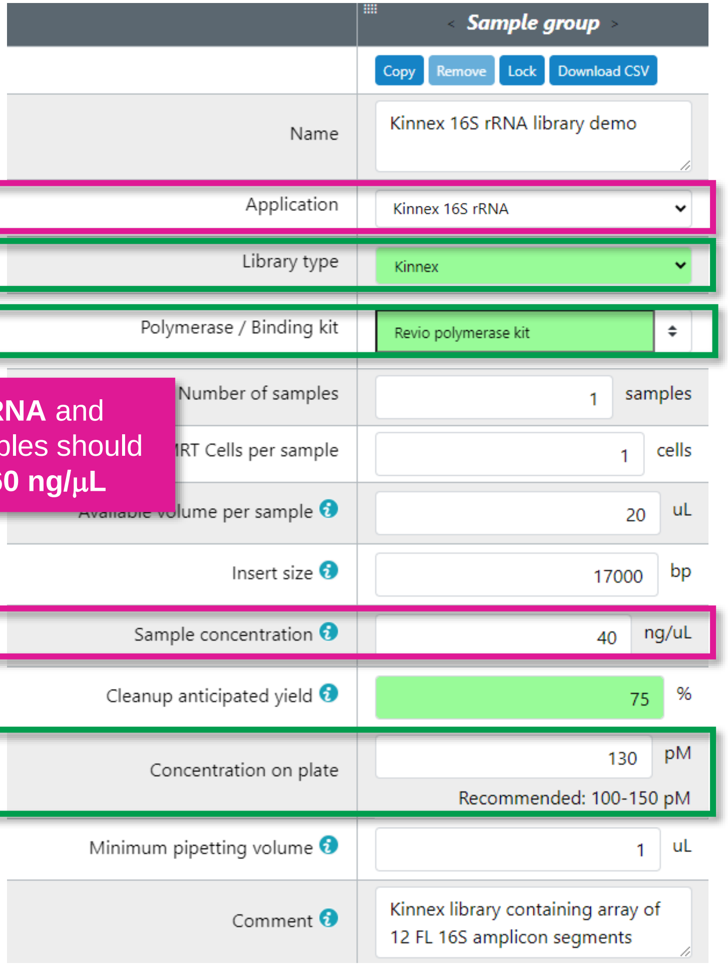
Name	Kinnex single-cell RNA library demo
Application	Kinnex single-cell RNA
Library type	Kinnex
…merase / Binding kit	Revio polymerase kit
	20…
	1… bp
Sample concentration	20 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	130 pM Recommended: 100-150 pM
Minimum pipetting volume	1 uL
Comment…	…nex library containing array of …sc-cDNA segments

Kinnex full-length RNA



Name	Kinnex full-length RNA library demo
Application	Kinnex full-length RNA
Library type	Kinnex
Polymerase / Binding kit	Revio polymerase kit
Available volume per sample	20 uL
Insert size	16000 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 8 FL cDNA segments

Kinnex 16S rRNA



Name	Kinnex 16S rRNA library demo
Application	Kinnex 16S rRNA
Library type	Kinnex
Polymerase / Binding kit	Revio polymerase kit
Number of samples	1 samples
…RT Cells per sample	1 cells
…vailable volume per sample	20 uL
Insert size	17000 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

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

Default = **Revio polymerase kit**

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]

NOTE: Kinnex single-cell RNA samples should be **normalized to 20 ng/μL**

Kinnex full-length RNA and **Kinnex 16S rRNA** samples should be between **20 and 60 ng/μL**

Recommended starting OPLC range is **100 – 150 pM** for Revio Kinnex samples

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

---

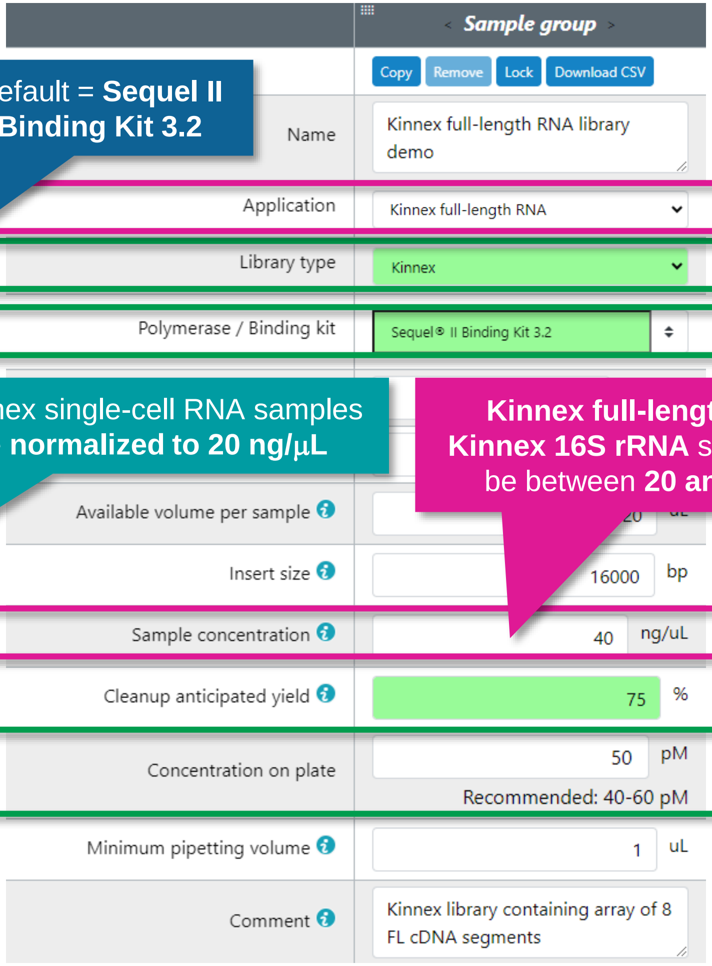
[1] Sample Setup **Library type** field specifies structure of SMRTbell library and determines sequencing primer type to use for annealing step. For Kinnex libraries, the forward and reverse Kinnex terminal SMRTbell adapters have different structures and require use of Kinnex sequencing primer for primer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

SMRT Link Sample Setup procedure for Sequel II/IIe systems

Kinnex single-cell RNA

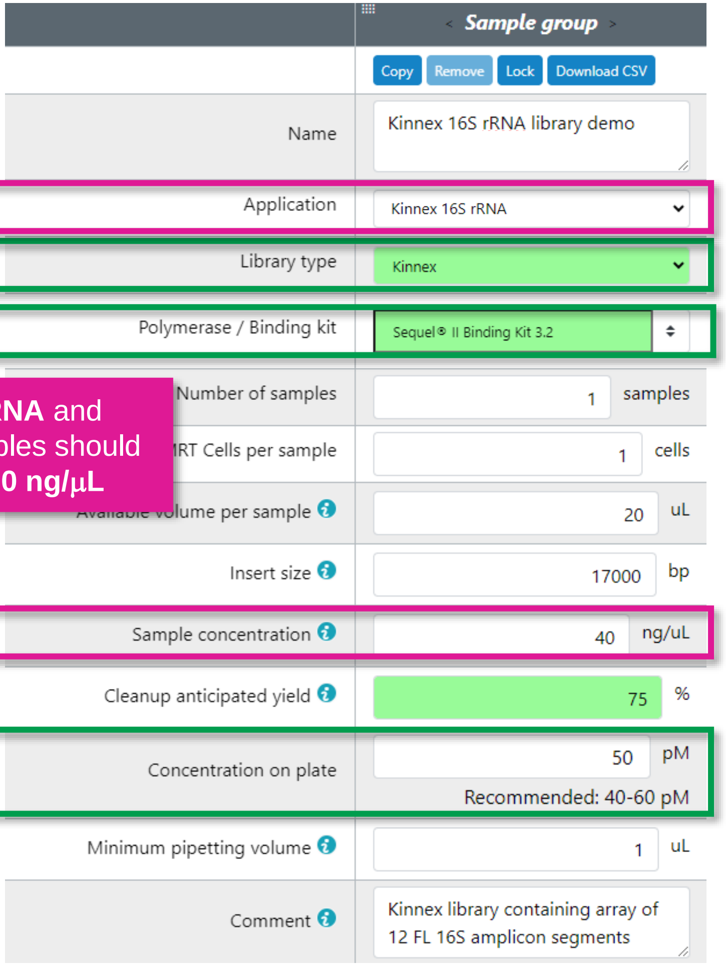
Name	Kinnex single-cell RNA library demo
Application	Kinnex single-cell RNA
Library type	Kinnex
...erase / Binding kit	Sequel® II Binding Kit 3.2
	20
	1... bp
Sample concentration	20 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	50 pM Recommended: 40-60 pM
Minimum pipetting volume	1 uL
Comment	...ex library containing array of ...sc-cDNA segments

Kinnex full-length RNA



Name	Kinnex full-length RNA library demo
Application	Kinnex full-length RNA
Library type	Kinnex
Polymerase / Binding kit	Sequel® II Binding Kit 3.2
Available volume per sample	...20 uL
Insert size	16000 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 8 FL cDNA segments

Kinnex 16S rRNA



Name	Kinnex 16S rRNA library demo
Application	Kinnex 16S rRNA
Library type	Kinnex
Polymerase / Binding kit	Sequel® II Binding Kit 3.2
Number of samples	1 samples
...RT Cells per sample	1 cells
Available volume per sample	20 uL
Insert size	17000 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

Default = **Sequel II Binding Kit 3.2**

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]

NOTE: Kinnex single-cell RNA samples should be **normalized to 20 ng/µL**

Kinnex full-length RNA and **Kinnex 16S rRNA samples** should be between **20 and 60 ng/µL**

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

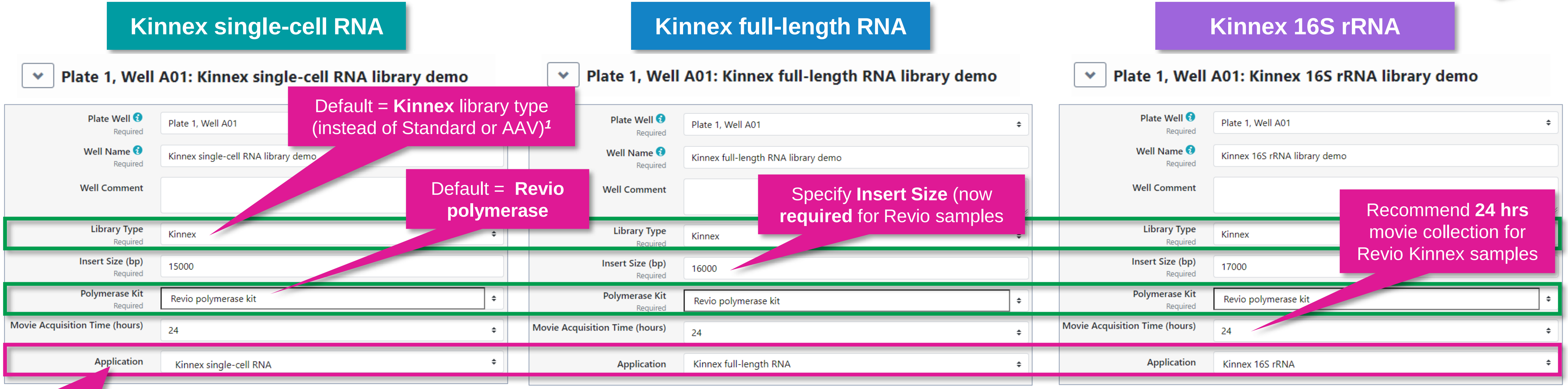
Recommended target *P1* loading range for Sequel II/IIe system is ~60 – 80%

---

[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



Kinnex single-cell RNA

Plate 1, Well A01: Kinnex single-cell RNA library demo

Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	Kinnex single-cell RNA library demo
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	15000
Polymerase Kit (Required)	Revio polymerase kit
Movie Acquisition Time (hours)	24
Application	Kinnex single-cell RNA

Kinnex full-length RNA

Plate 1, Well A01: Kinnex full-length RNA library demo

Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	Kinnex full-length RNA library demo
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	16000
Polymerase Kit (Required)	Revio polymerase kit
Movie Acquisition Time (hours)	24
Application	Kinnex full-length RNA

Kinnex 16S rRNA

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

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)	17000
Polymerase Kit (Required)	Revio polymerase kit
Movie Acquisition Time (hours)	24
Application	Kinnex 16S rRNA

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

Default = **Revio polymerase**

Specify **Insert Size** (now **required** for Revio samples

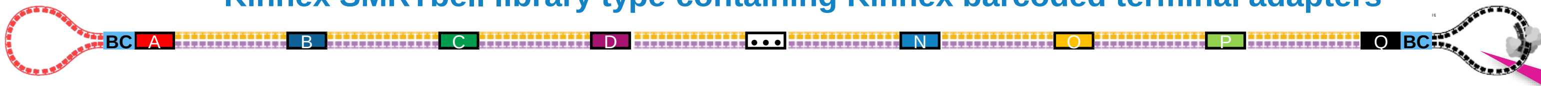
Recommend **24 hrs** movie collection for Revio Kinnex samples

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

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]

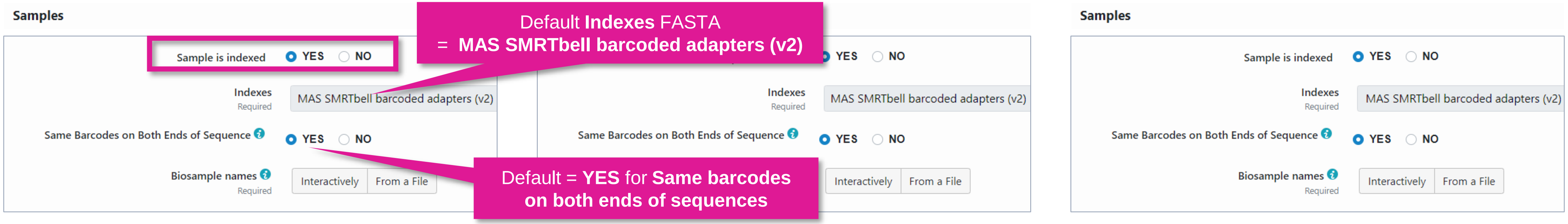
---

[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 single-cell RNA | **Kinnex full-length RNA** | **Kinnex 16S rRNA**



Samples

Sample is indexed	● YES ○ NO
Indexes Required	MAS SMRTbell barcoded adapters (v2)
Same Barcodes on Both Ends of Sequence	● YES ○ NO
Biosample names Required	Interactively | From a File

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

Default = **YES** for **Same barcodes on both ends of sequences**

	● YES ○ NO
Indexes Required	MAS SMRTbell barcoded adapters (v2)
Same Barcodes on Both Ends of Sequence	● YES ○ NO
	Interactively | From a File

Samples

Sample is indexed	● YES ○ NO
Indexes Required	MAS SMRTbell barcoded adapters (v2)
Same Barcodes on Both Ends of Sequence	● YES ○ NO
Biosample names Required	Interactively | From a File

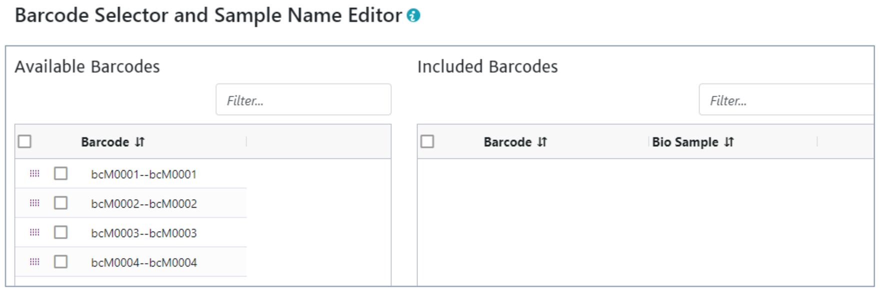
Example complete Kinnex library molecule containing barcoded Kinnex terminal MAS adapters[1] at both ends

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



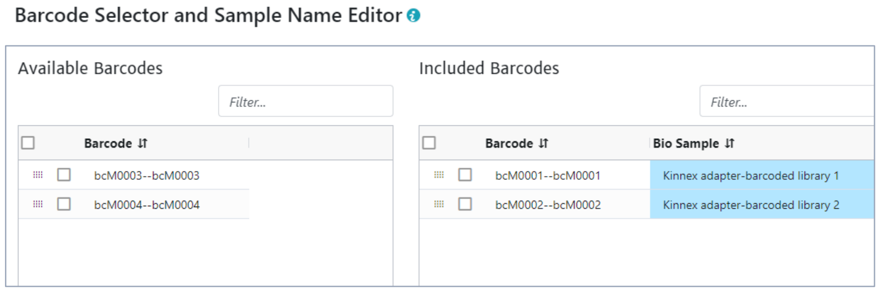
Barcode Selector and Sample Name Editor

Available Barcodes

☐	Barcode
☐	bcM0001--bcM0001
☐	bcM0002--bcM0002
☐	bcM0003--bcM0003
☐	bcM0004--bcM0004

Included Barcodes

☐	Barcode	Bio Sample



Barcode Selector and Sample Name Editor

Available Barcodes

☐	Barcode
☐	bcM0003--bcM0003
☐	bcM0004--bcM0004

Included Barcodes

☐	Barcode	Bio Sample
☐	bcM0001--bcM0001	Kinnex adapter-barcoded library 1
☐	bcM0002--bcM0002	Kinnex adapter-barcoded library 2

SMRT Link

Data Management

MAS SMRTbell barcoded adapter indexes

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

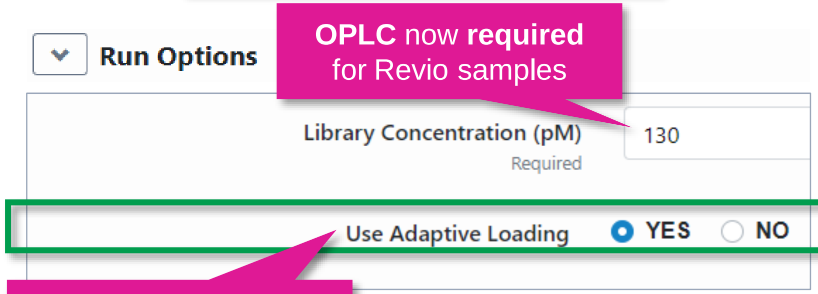
---

[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 single-cell RNA



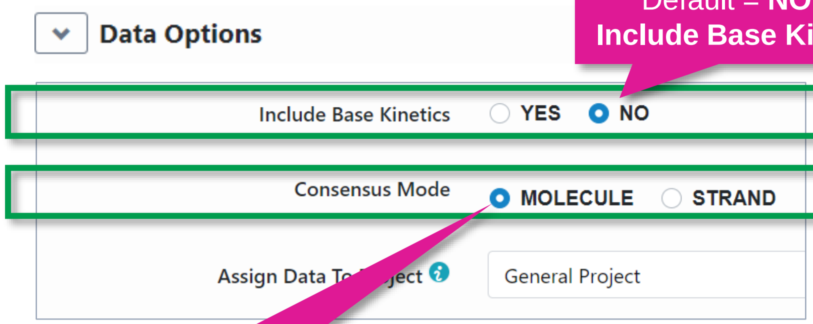
Run Options

Library Concentration (pM) Required: 130

Use Adaptive Loading ● YES ○ NO

OPLC now required for Revio samples

Default = **YES** for **Use Adaptive Loading**



Data Options

Include Base Kinetics ○ YES ● NO

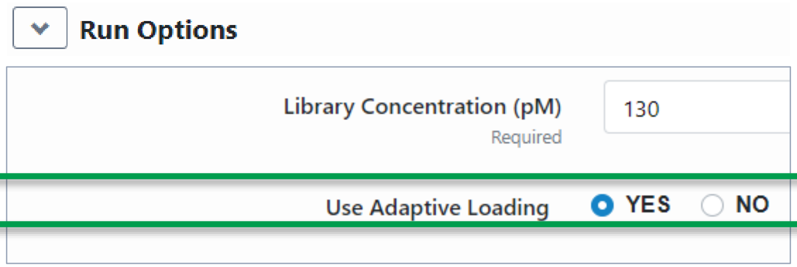
Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

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

Default Consensus Mode = MOLECULE[1]

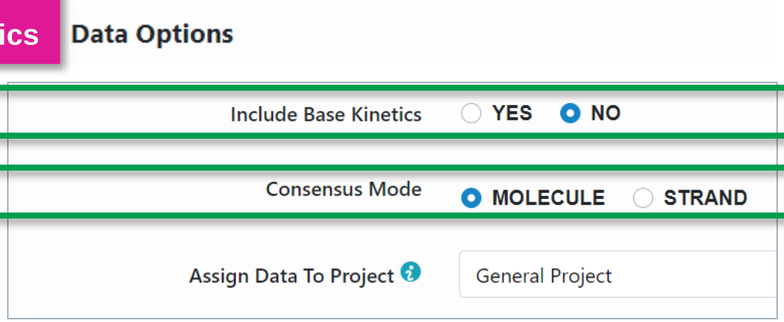
Kinnex full-length RNA



Run Options

Library Concentration (pM) Required: 130

Use Adaptive Loading ● YES ○ NO



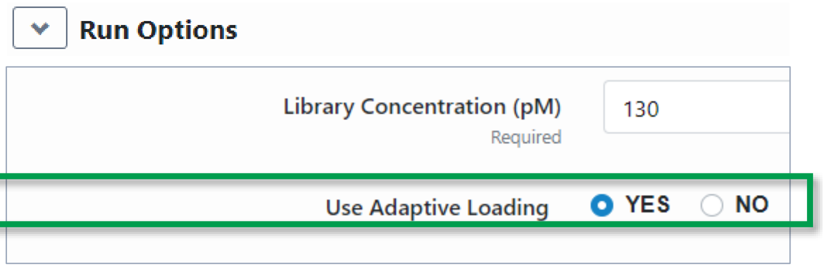
Data Options

Include Base Kinetics ○ YES ● NO

Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

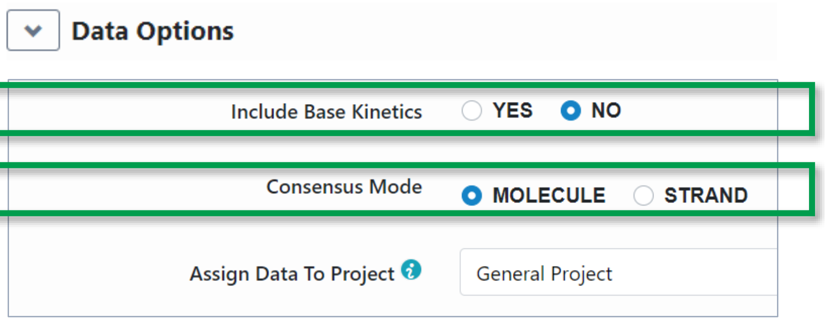
Kinnex 16S rRNA



Run Options

Library Concentration (pM) Required: 130

Use Adaptive Loading ● YES ○ NO



Data Options

Include Base Kinetics ○ YES ● NO

Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

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

---

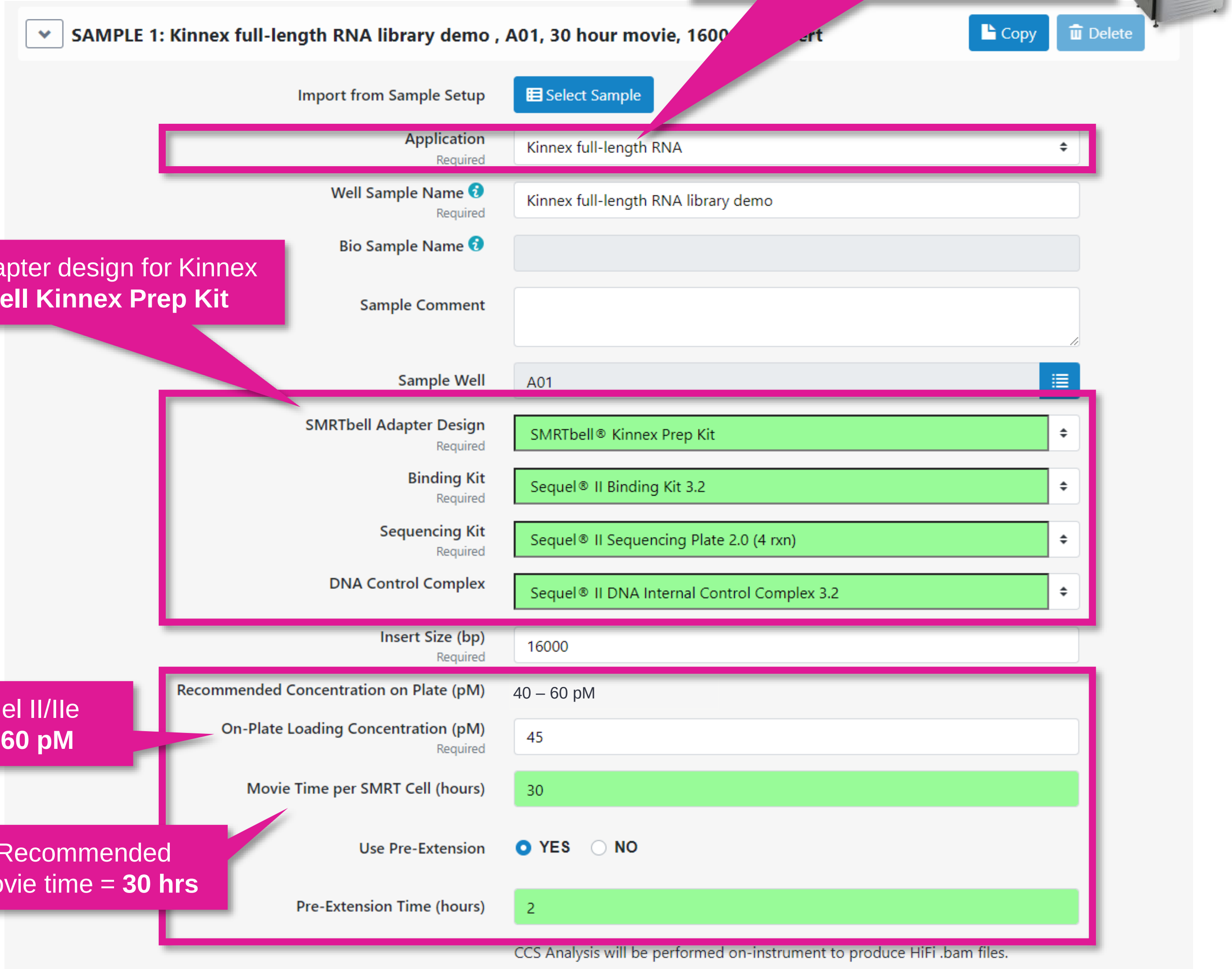
[1] Molecule Consensus Mode → generates a single circular consensus sequencing read for a given library insert molecule. Strand Consensus Mode → generates a consensus sequence for each strand (forward & reverse) of a given library insert molecule.

SMRT Link Run Design procedure for Sequel II/IIe systems

Sample information and run information

- 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]



SAMPLE 1: Kinnex full-length RNA library demo , A01, 30 hour movie, 1600 ert	
Import from Sample Setup	Select Sample
Application Required	Kinnex full-length RNA
Well Sample Name Required	Kinnex full-length RNA library demo
Bio Sample Name	
Sample Comment	
Sample Well	A01
SMRTbell Adapter Design Required	🟩 SMRTbell® Kinnex Prep Kit
Binding Kit Required	🟩 Sequel® II Binding Kit 3.2
Sequencing Kit Required	🟩 Sequel® II Sequencing Plate 2.0 (4 rxn)
DNA Control Complex	🟩 Sequel® II DNA Internal Control Complex 3.2
Insert Size (bp) Required	16000
Recommended Concentration on Plate (pM)	40 – 60 pM
On-Plate Loading Concentration (pM) Required	45
Movie Time per SMRT Cell (hours)	🟩 30
Use Pre-Extension	● YES ○ NO
Pre-Extension Time (hours)	🟩 2

CCS Analysis will be performed on-instrument to produce HiFi .bam files.

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 full-length RNA library sample.

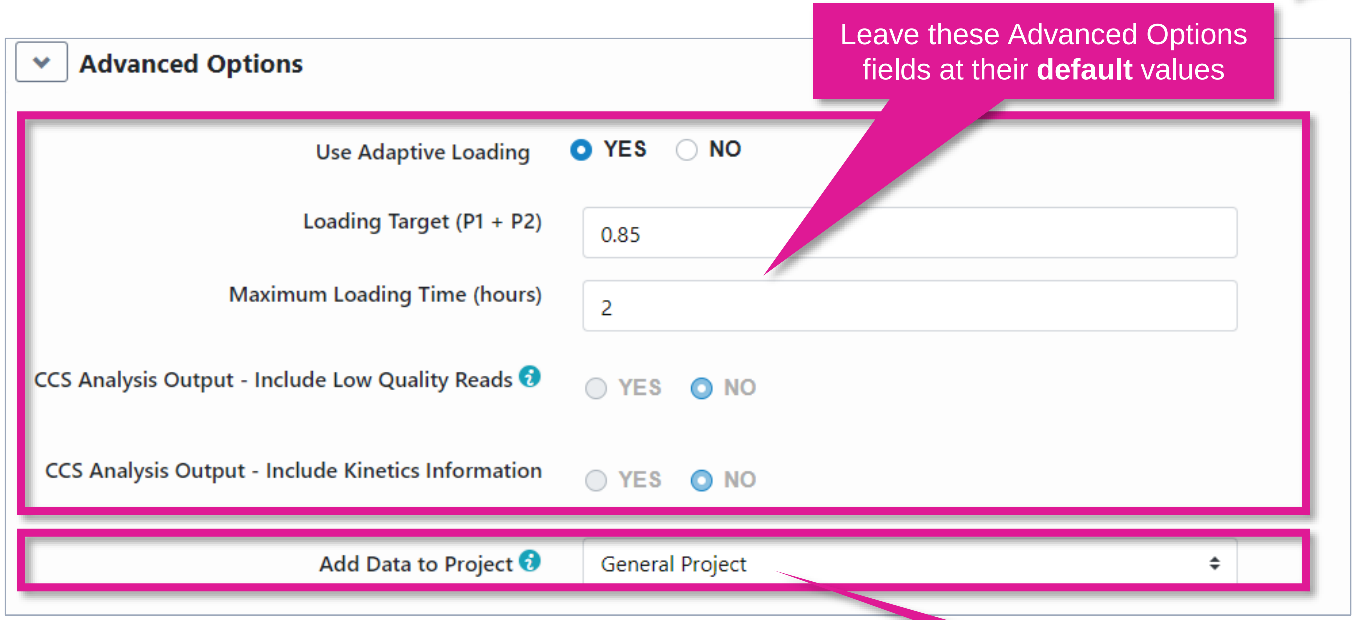
---

[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

- 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



Advanced Options

Use Adaptive Loading ● YES ○ NO

Loading Target (P1 + P2) 0.85

Maximum Loading Time (hours) 2

CCS Analysis Output - Include Low Quality Reads ○ YES ● NO

CCS Analysis Output - Include Kinetics Information ○ YES ● NO

Add Data to Project General Project

Leave these Advanced Options fields at their **default** values

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

Can specify to use a different Project folder

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

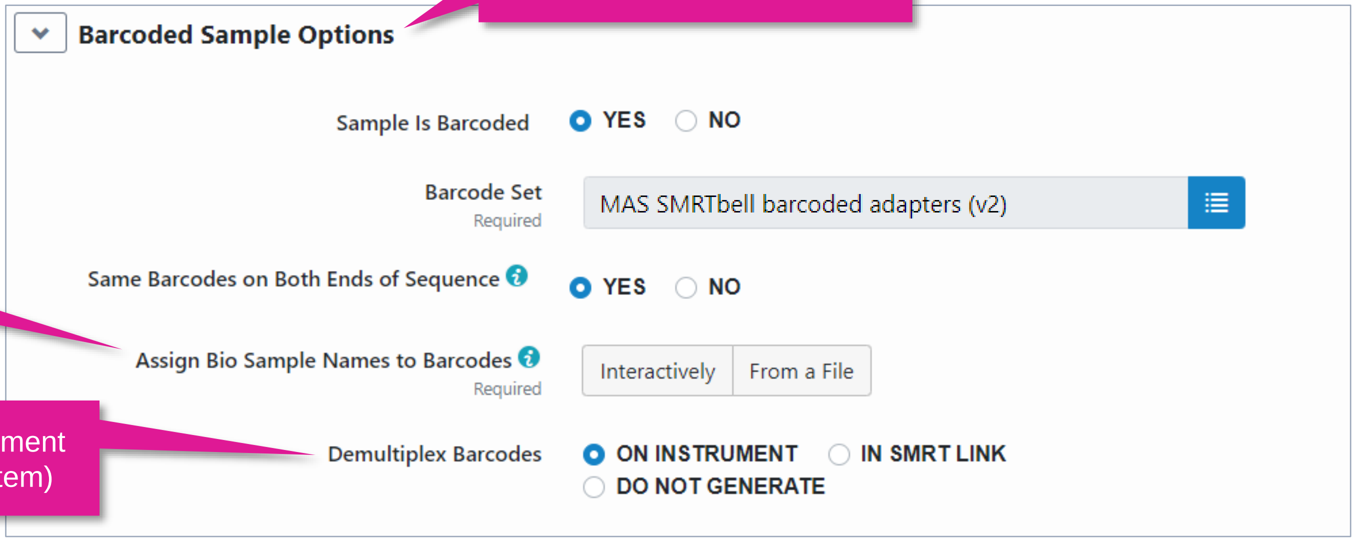
Barcoded sample options

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

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

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)



Barcoded Sample Options

Sample Is Barcoded: ● YES ○ NO

Barcode Set (Required): MAS SMRTbell barcoded adapters (v2)

Same Barcodes on Both Ends of Sequence: ● YES ○ NO

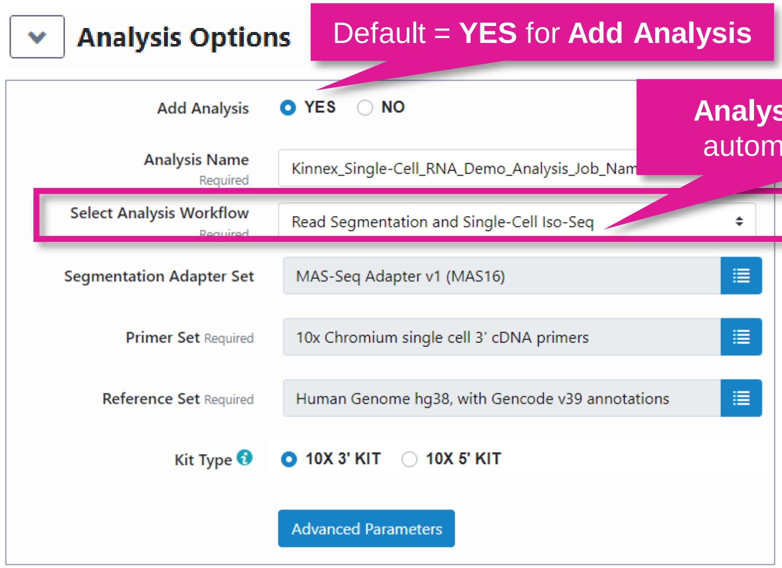
Assign Bio Sample Names to Barcodes (Required): Interactively | From a File

Demultiplex Barcodes: ● ON INSTRUMENT ○ IN SMRT LINK ○ DO NOT GENERATE

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 & Sequel II/IIe systems

Kinnex single-cell RNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Nam

Select Analysis Workflow Required: Read Segmentation and Single-Cell Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

Primer Set Required: 10x Chromium single cell 3' cDNA primers

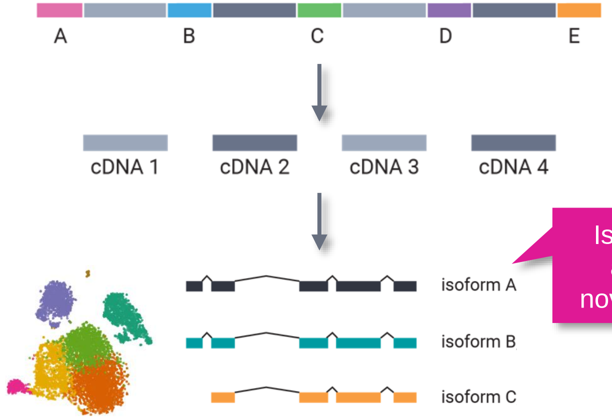
Reference Set Required: Human Genome hg38, with Gencode v39 annotations

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

Default = **YES** for **Add Analysis**

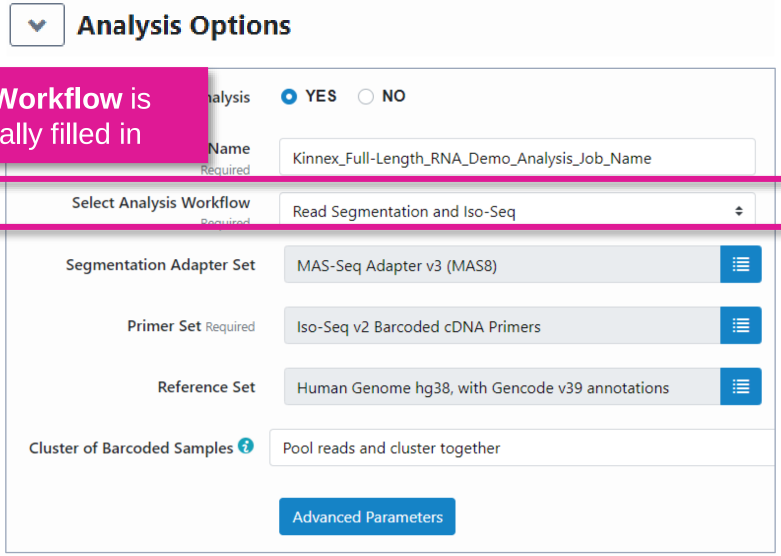
Analysis Workflow is automatically filled in

Read Segmentation and Single-Cell Iso-Seq



Isoform-classification analysis to identify novel genes & isoforms

Kinnex full-length RNA



Analysis Options

alysis ● YES ○ NO

Name Required: Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation and Iso-Seq

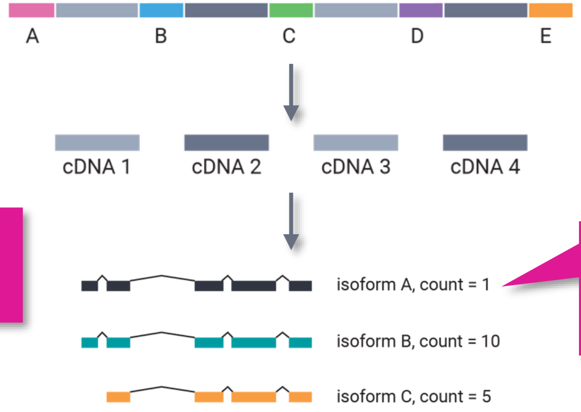
Segmentation Adapter Set: MAS-Seq Adapter v3 (MAS8)

Primer Set Required: Iso-Seq v2 Barcoded cDNA Primers

Reference Set: Human Genome hg38, with Gencode v39 annotations

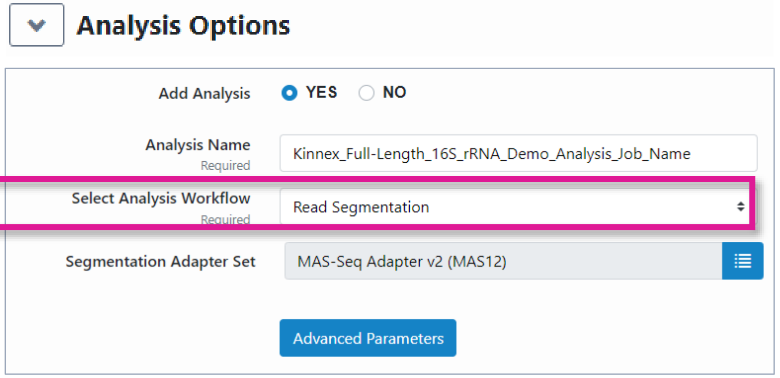
Cluster of Barcoded Samples: Pool reads and cluster together

Read Segmentation and Iso-Seq



Isoform-classification analysis to identify novel genes & isoforms with abundance information (raw counts & normalized counts per million)

Kinnex 16S rRNA



Analysis Options

Add Analysis ● YES ○ NO

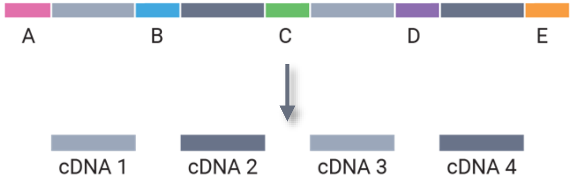
Analysis Name Required: Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation

Segmentation Adapter Set: MAS-Seq Adapter v2 (MAS12)

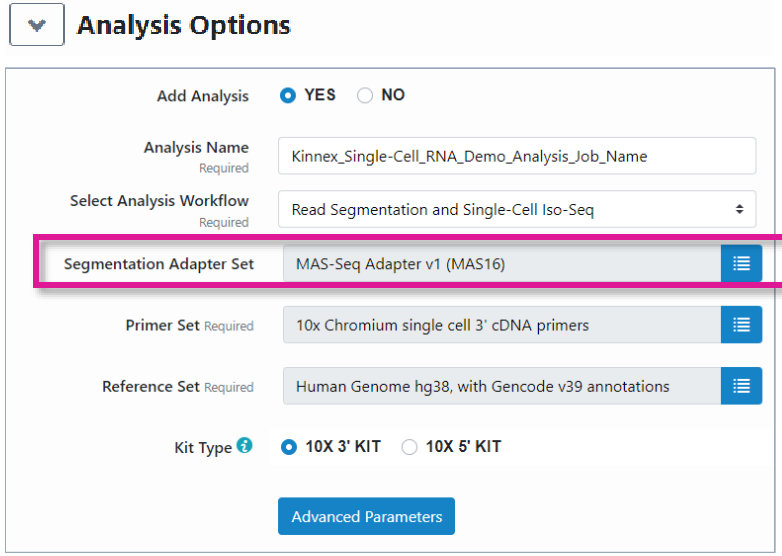
Split arrayed HiFi reads at adapter positions, generating segmented reads (S-reads)

Read Segmentation



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

Kinnex single-cell RNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation and Single-Cell Iso-Seq

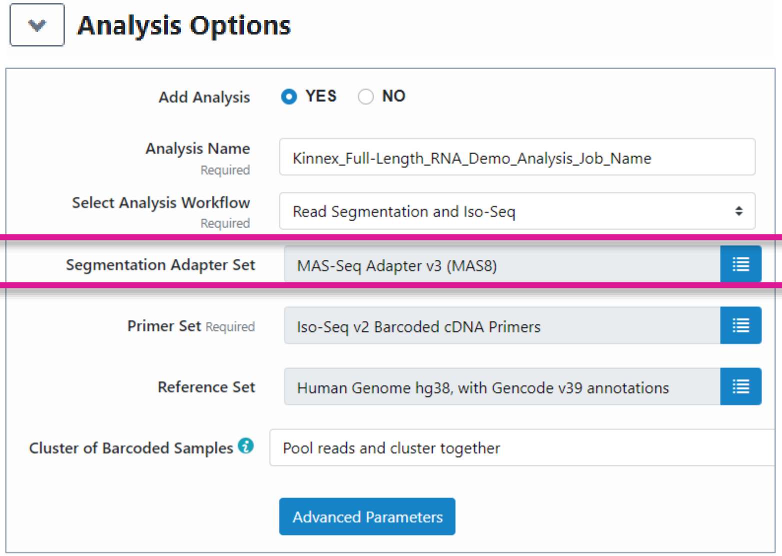
Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

Primer Set Required: 10x Chromium single cell 3' cDNA primers

Reference Set Required: Human Genome hg38, with Gencode v39 annotations

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

Kinnex full-length RNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation and Iso-Seq

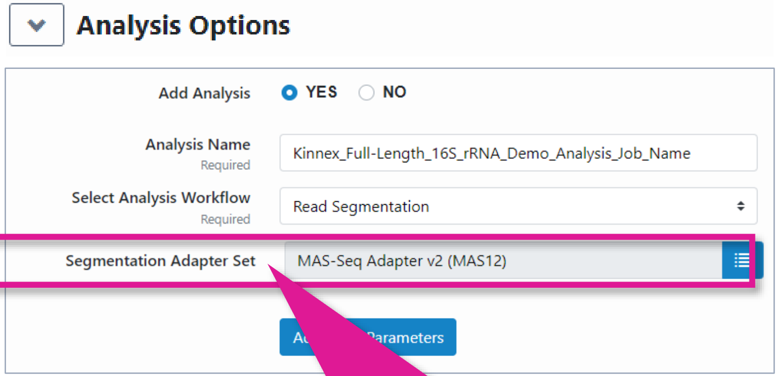
Segmentation Adapter Set: MAS-Seq Adapter v3 (MAS8)

Primer Set Required: Iso-Seq v2 Barcoded cDNA Primers

Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Kinnex 16S rRNA



Analysis Options

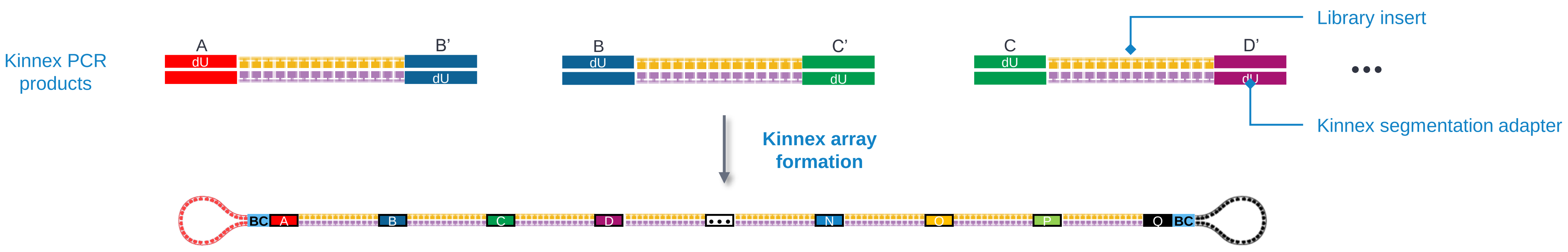
Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation

Segmentation Adapter Set: MAS-Seq Adapter v2 (MAS12)

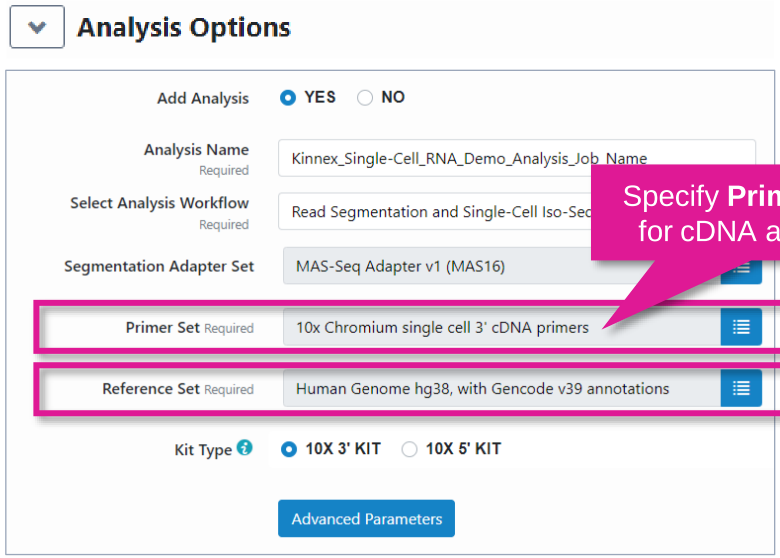
Specify **Segmentation Adapter Set** that corresponds to the Kinnex library concatenation method used



Complete array molecule containing concatenated library insert segments

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

Kinnex single-cell RNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name (Required): Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow (Required): Read Segmentation and Single-Cell Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

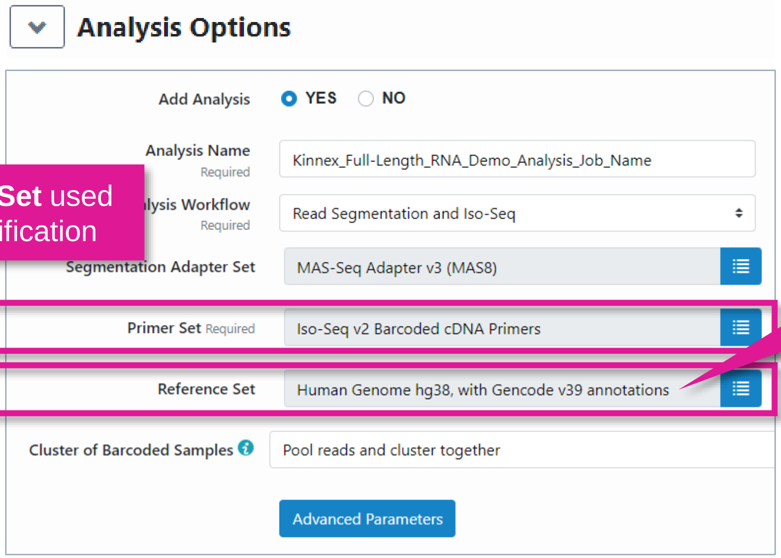
Primer Set (Required): 10x Chromium single cell 3' cDNA primers

Reference Set (Required): Human Genome hg38, with Gencode v39 annotations

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

Specify **Primer Set** used for cDNA amplification

Kinnex full-length RNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name (Required): Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow (Required): Read Segmentation and Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v3 (MAS8)

Primer Set (Required): Iso-Seq v2 Barcoded cDNA Primers

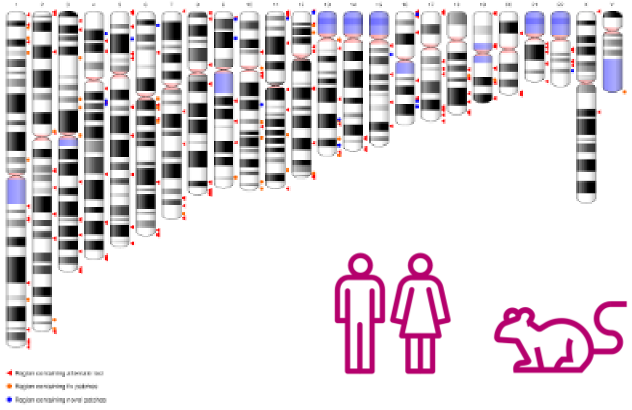
Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Specify **Reference** Set. Default sets are:

- Human Genome hg38, with Gencode v39 annotations
- Mouse Genome mm39, with Gencode vM28 annotations

Specify reference genome & annotation sets to align high quality isoforms to, and to collapse isoforms mapped to the same genomic loci.



For Kinnex single-cell 3' RNA analysis, select '10x Chromium single cell 3' cDNA primers'

OR

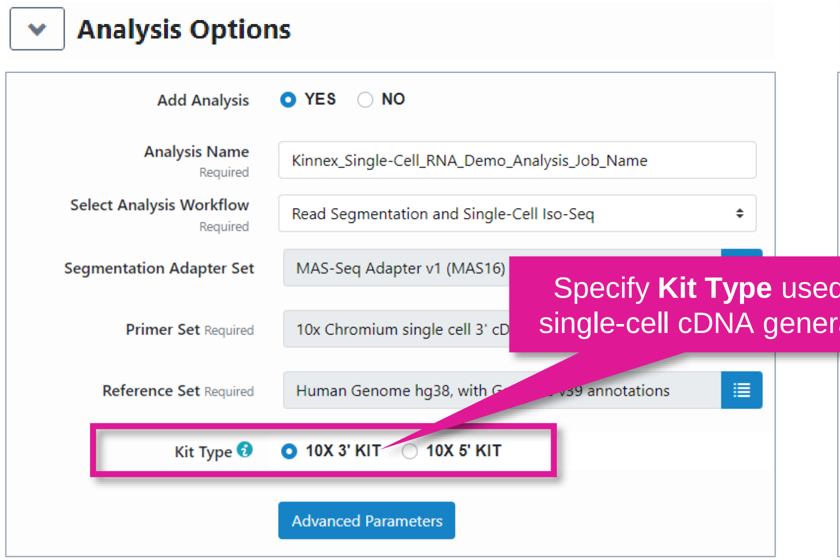
For Kinnex single-cell 5' RNA analysis, select '10x Chromium single cell 5' cDNA primers'

For Kinnex full-length RNA analysis, select 'Iso-Seq v2 Barcoded cDNA Primers'

Specify primer sequence file in FASTA format to **identify cDNA primers for removal** (include the 5' and 3' cDNA primers)

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

Kinnex single-cell RNA


Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation and Single-Cell Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

Primer Set Required: 10x Chromium single cell 3' cD

Reference Set Required: Human Genome hg38, with G v39 annotations

Kit Type ● 10X 3' KIT ○ 10X 5' KIT

Specify **Kit Type** used for single-cell cDNA generation

Specification of Kit Type (10x 3’ Kit or 10x 5’ Kit) **determines which set of 10x barcode sequences to use**, and also affects **UMI and single-cell barcode design settings**[1]

5’ [CBC][UMI]TTTTTTTTTT LIBRARY INSERT CCC TSO 3’
3’ [CBC][UMI]AAAAAAAAAA LIBRARY INSERT GGG TSO 5’

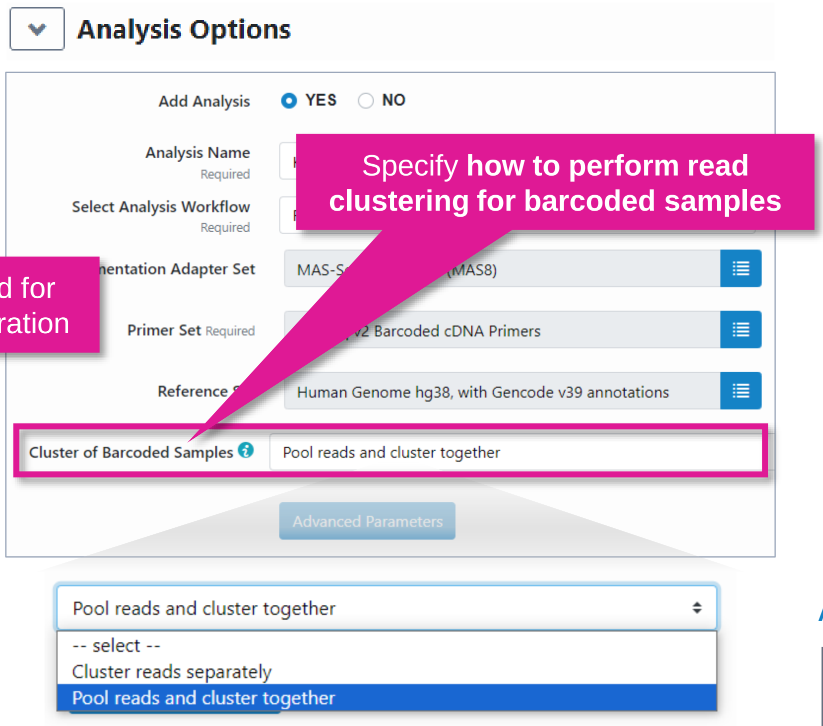
For Kinnex single-cell 3’ RNA analysis, select ‘10x 3’ Kit’

OR

5’ [CBC][UMI][TSO]GGG LIBRARY INSERT AAAAAAAAAA 3’
3’ [CBC][UMI][TSO]CCC LIBRARY INSERT TTTTTTTTTT 5’

For Kinnex single-cell 5’ RNA analysis, select ‘10x 5’ Kit’

Kinnex full-length RNA


Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set: MAS-S (MAS8)

Primer Set Required: v2 Barcoded cDNA Primers

Reference: Human Genome hg38, with Gencode v39 annotations

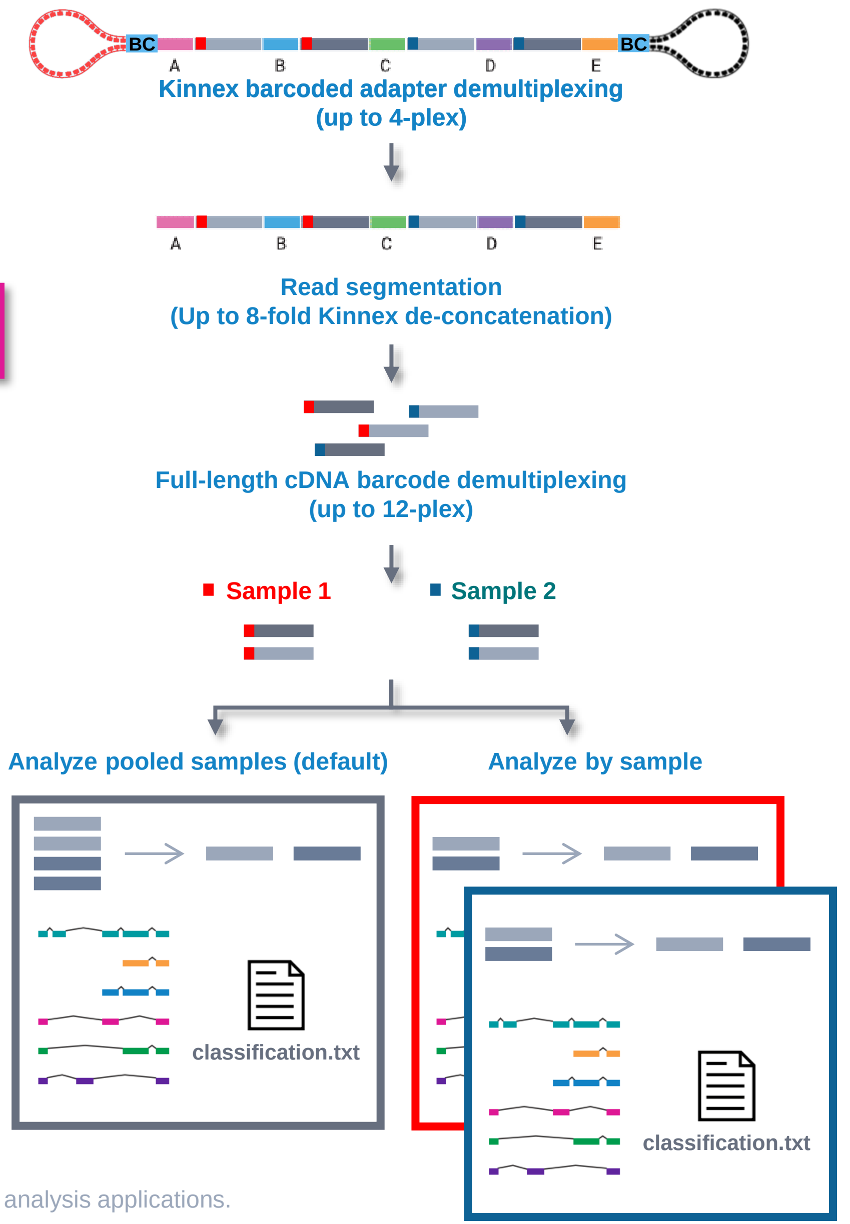
Cluster of Barcoded Samples: Pool reads and cluster together

Pool reads and cluster together
- -- select --
- Cluster reads separately
- Pool reads and cluster together

Specify **how to perform read clustering for barcoded samples**

Specification of **Cluster of Barcoded Samples** setting **determines whether all FLNC reads will be pooled for clustering** (Does not apply to non-barcoded samples.)

Clustering options for Kinnex full-length RNA samples

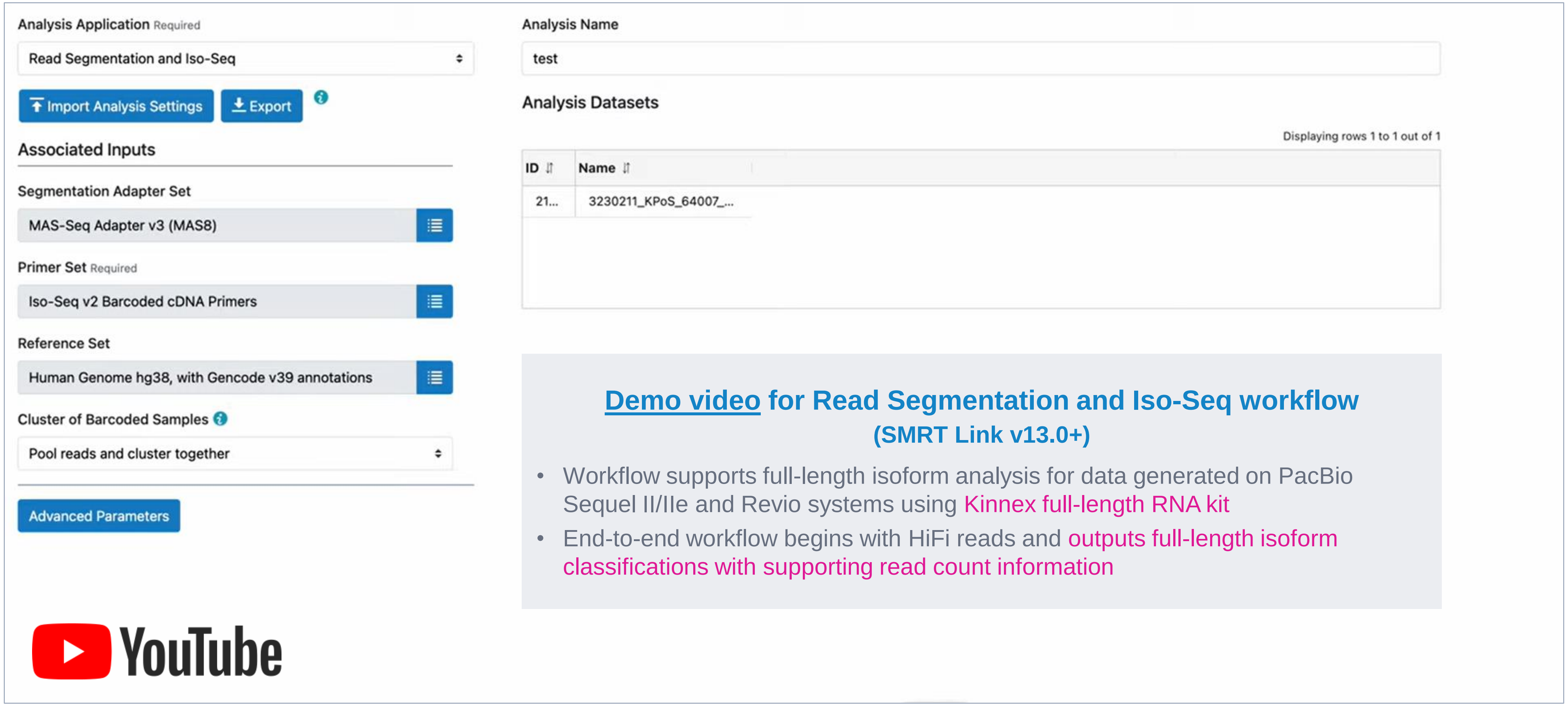


---

[1] See *SMRT Link User Guide* documentation for detailed descriptions of advanced parameters for Iso-Seq / Single-Cell Iso-Seq analysis applications.

SMRT Link Read Segmentation and Iso-Seq analysis video demonstration

Video demonstration of SMRT Link Read Segmentation and Iso-Seq application workflow for analysis of Kinnex full-length RNA samples



Analysis Application Required: Read Segmentation and Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v3 (MAS8)

Primer Set Required: Iso-Seq v2 Barcoded cDNA Primers

Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Analysis Name: test

ID	Name
21...	3230211_KPoS_64007_...

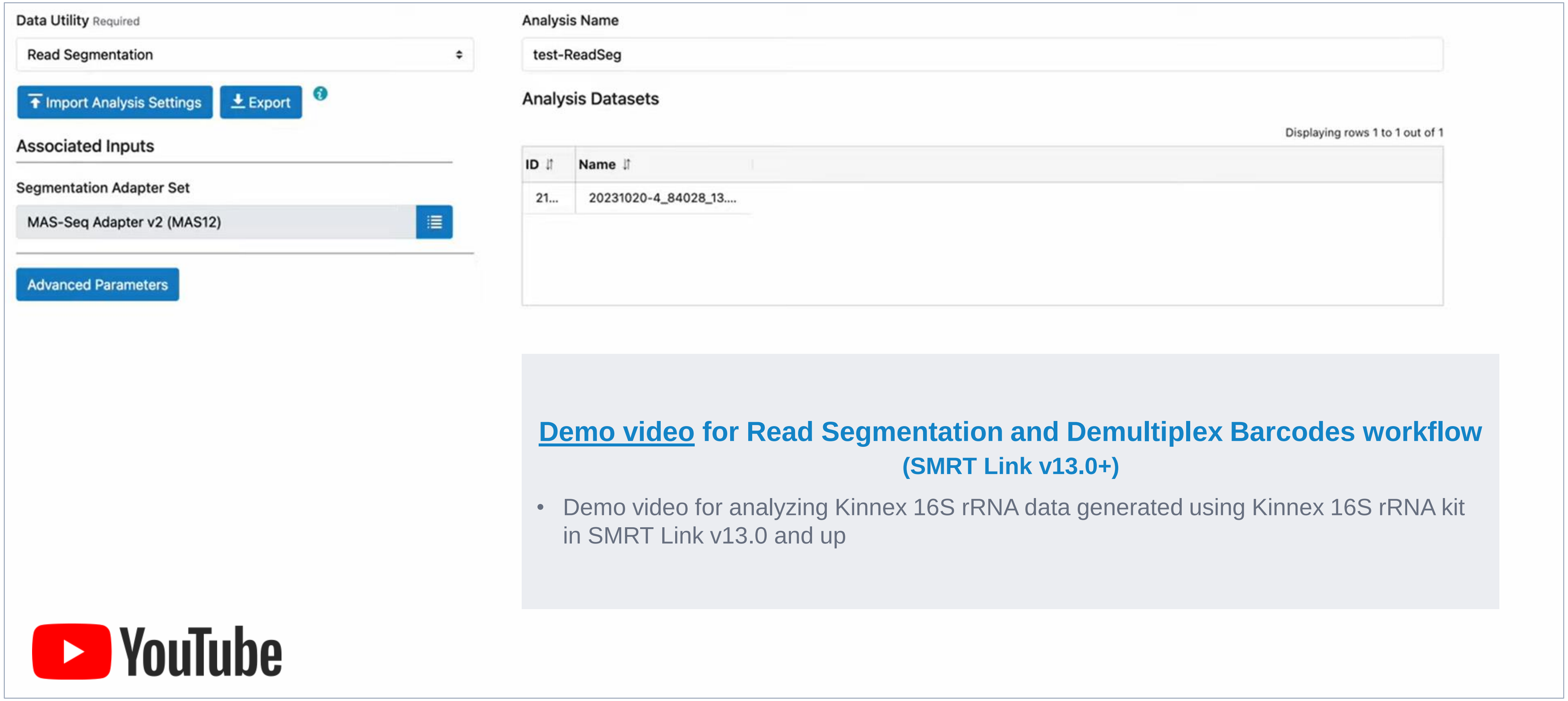
Demo video for Read Segmentation and Iso-Seq workflow
(SMRT Link v13.0+)

- Workflow supports full-length isoform analysis for data generated on PacBio Sequel II/IIe and Revio systems using Kinnex full-length RNA kit
- End-to-end workflow begins with HiFi reads and outputs full-length isoform classifications with supporting read count information

View Video tutorial – ***Read Segmentation and Iso-Seq workflow in SMRT Link*** at: YouTube Link

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



Data Utility Required: Read Segmentation

Analysis Name: test-ReadSeg

Associated Inputs

Segmentation Adapter Set: MAS-Seq Adapter v2 (MAS12)

Analysis Datasets

Displaying rows 1 to 1 out of 1

ID	Name
21...	20231020-4_84028_13....

[Demo video](#) for Read Segmentation and Demultiplex Barcodes workflow (SMRT Link v13.0+)

- Demo video for analyzing Kinnex 16S rRNA data generated using Kinnex 16S rRNA kit in SMRT Link v13.0 and up

View Video tutorial – ***Analyzing Kinnex 16S rRNA data in SMRT Link*** at: [YouTube Link](#)

Kinnex single-cell RNA library preparation & sequencing workflow key highlights

Kinnex single-cell RNA library preparation procedure description

Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3’ or 5’ single cell cDNA using the **Kinnex single-cell RNA kit**[1] for library preparation and sequencing on PacBio Sequel II, Sequel IIe & Revio systems.

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples

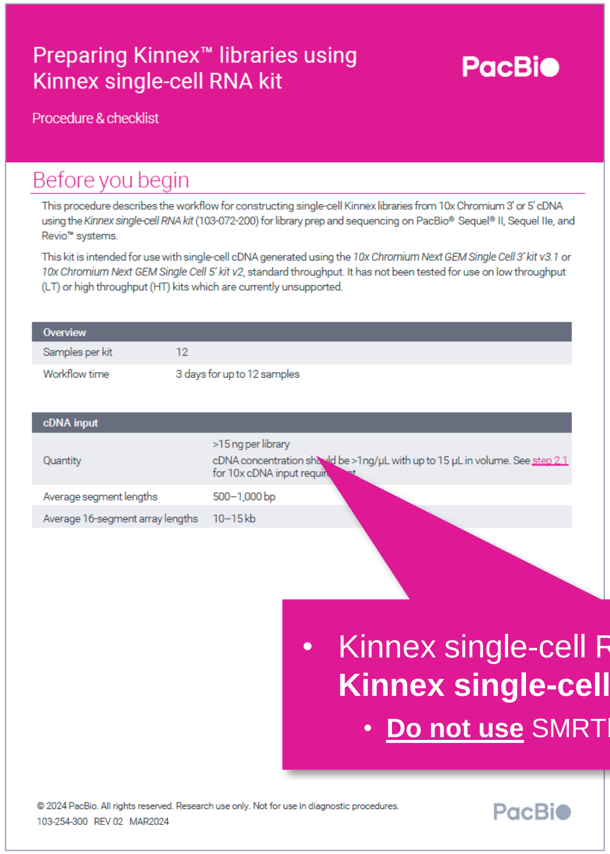
cDNA input	
Quantity	>15 ng per library cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,000 bp
Average 16-segment array lengths	10–15 kb

Kinnex single-cell RNA kit
(103-072-200)

Concatenation factor

16-fold

Kinnex library template (~10 – 15 kb) containing 16 concatenated single-cell cDNA segments



Preparing Kinnex™ libraries using
Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3’ or 5’ cDNA using the *Kinnex single-cell RNA kit* (103-072-200) for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3’ kit v3.1* or *10x Chromium Next GEM Single Cell 5’ kit v2*, standard throughput. It has not been tested for use on low throughput (LT) or high throughput (HT) kits which are currently unsupported.

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples

cDNA input	
Quantity	>15 ng per library cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,000 bp
Average 16-segment array lengths	10–15 kb

103-254-300 REV 02 MAR2024

- Kinnex single-cell RNA library prep protocol uses **Kinnex single-cell RNA kit**
  - **Do not use** SMRTbell prep kit 3.0 with this protocol

PacBio Documentation (103-254-300)

---

[1] Please contact PacBio Technical Support for questions about compatibility of the Kinnex library preparation workflow with other 10x Chromium kits.

Comparison of *Kinnex single-cell RNA kit* vs. *MAS-Seq for 10x Single Cell 3’ kit*

	Kinnex single-cell RNA kit (103-072-200)	**MAS-Seq for 10x Single Cell 3’ kit (102-659-600)**
# reactions	**12**	8
Concatenation	16-fold	
Compatibility	10x Chromium Single Cell 3’ kit (v3.1) **10x Chromium Single Cell 5’ kit (v2)**	10x Chromium Single Cell 3’ kit (v3.1)
Sample multiplexing support	**4-plex using Kinnex barcoded adapters**	None
Workflow time	2 days	
SMRT Link support	Yes (since SMRT Link v13.1)	Yes (since SMRT Link v11.1)
Sequencing primer	**Kinnex sequencing primer**	Standard sequencing primer
Sequencing OPLC	**50 pM (Sequel II/IIe)** **130 pM (Revio)**	85 pM (Sequel II/IIe) 225 pM (Revio)
Run time	Sequel II/IIe: 30hr movie with adaptive loading Revio: 24hr movie with adaptive loading	
S-read yield	~30-40 M reads (Sequel II/IIe) ~80-100 M reads (Revio)	

Kinnex single-cell RNA experimental design considerations

Kinnex single-cell RNA application use case recommendations for PacBio systems

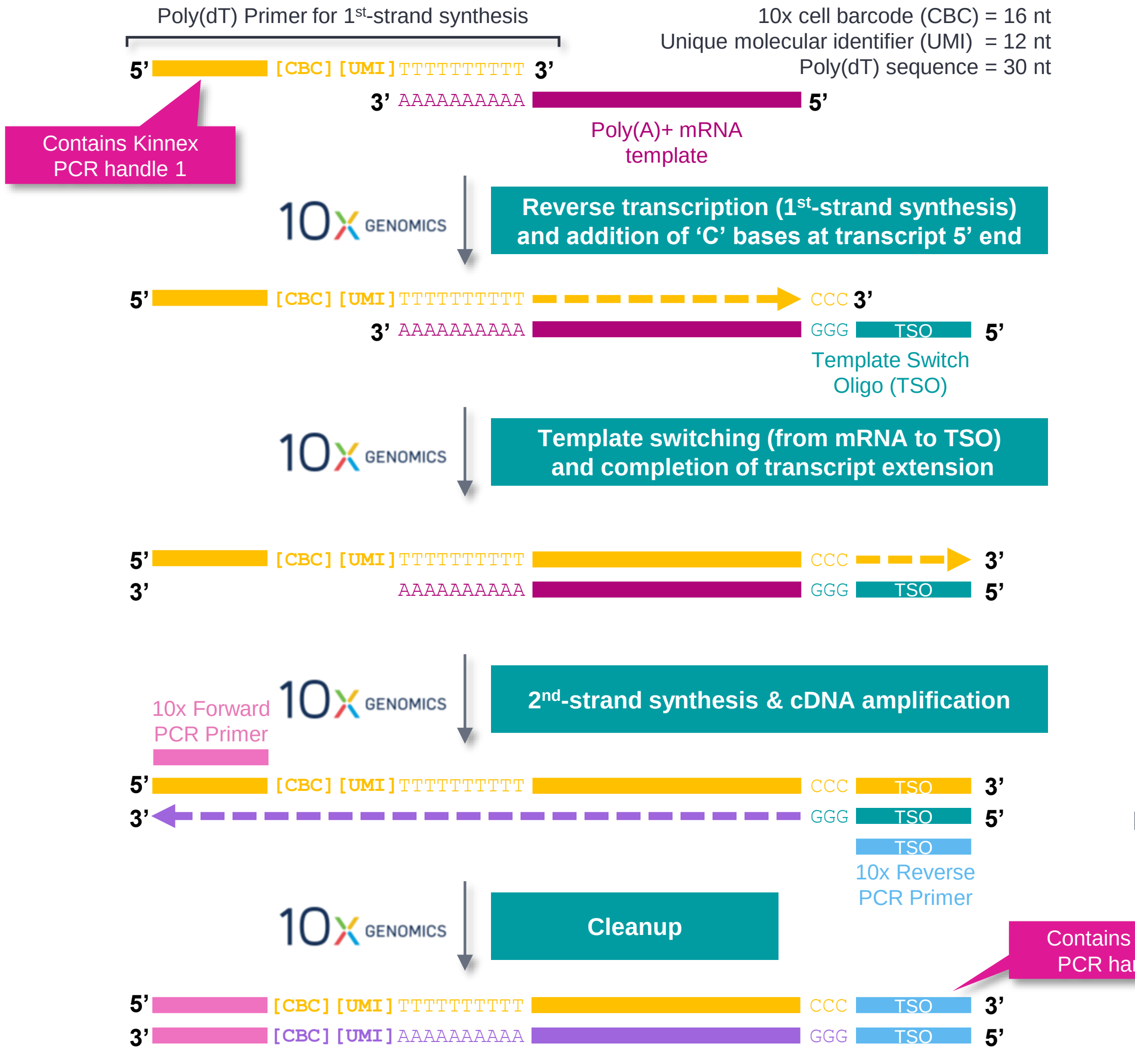
	Sequel II and IIe systems	Revio system
Experimental goal	Characterize alternative splicing in single cells / cell types	
Sample multiplexing[1]	Not recommended	Up to 2 samples per Revio SMRT Cell (2-plex)
Cell input into 10x Chromium single cell 3' or 5' cDNA generation workflow	3,000 – 10,000 cells for running a single (non-multiplexed) sample on one Sequel II SMRT Cell 8M)	3,000 – 6,000 cells per sample if multiplexing 2 samples per Revio SMRT Cell (2-plex) 8,000 – 10,000 cells per sample if running a single (non-multiplexed) sample on one Revio SMRT Cell
Expected coverage	Obtain ≥3,000 – 10,000 unique reads/single cell	Obtain up to ~10,000 unique reads/single cell
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)	
Single-cell cDNA input into Kinnex library prep workflow	15-75 ng of 10x Chromium 3' or 5' single cell cDNA	
SMRT Link data analysis workflows	Read Segmentation and Single-cell Iso-Seq Analysis	
Community data analysis tools	Annotation & quantification: SQANTI3 Differential analysis: TappAS Fusion calling: pbfusion Visualization: SWAN	

---

[1] Kinnex single-cell concatenation kit (103-242-000) can support up to 4-plex sample multiplexing per SMRT Cell through the use of four different barcoded Kinnex terminal SMRTbell adapters during Kinnex single-cell RNA library construction.

Kinnex single-cell RNA method overview

Single-cell cDNA sample preparation*



Poly(dT) Primer for 1st-strand synthesis

10x cell barcode (CBC) = 16 nt
Unique molecular identifier (UMI) = 12 nt
Poly(dT) sequence = 30 nt

Contains Kinnex PCR handle 1

Poly(A)+ mRNA template

Reverse transcription (1st-strand synthesis) and addition of 'C' bases at transcript 5' end

Template Switch Oligo (TSO)

Template switching (from mRNA to TSO) and completion of transcript extension

10x Forward PCR Primer

2nd-strand synthesis & cDNA amplification

10x Reverse PCR Primer

Cleanup

Contains Kinnex PCR handle 2

10x barcoded single-cell cDNA for input into PacBio Kinnex library construction

Min. cDNA input for TSO artifact removal step = 15 ng

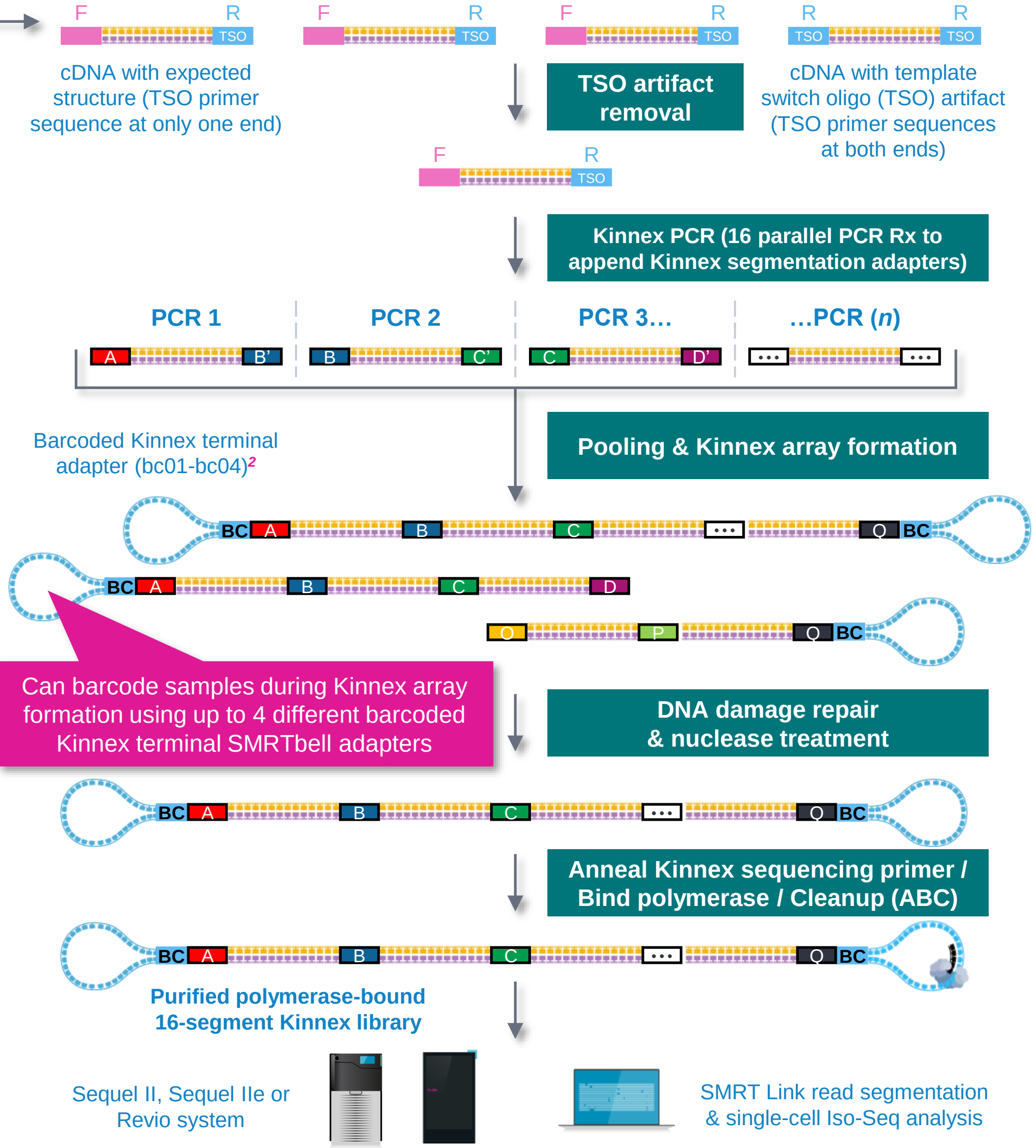
Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit

Procedure & checklist

Before you begin

Procedure & checklist (103-254-300)

Kinnex library prep, sequencing & analysis



cDNA with expected structure (TSO primer sequence at only one end)

TSO artifact removal

cDNA with template switch oligo (TSO) artifact (TSO primer sequences at both ends)

Kinnex PCR (16 parallel PCR Rx to append Kinnex segmentation adapters)

Barcoded Kinnex terminal adapter (bc01-bc04)[2]

Pooling & Kinnex array formation

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

DNA damage repair & nuclease treatment

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

Purified polymerase-bound 16-segment Kinnex library

Sequel II, Sequel IIe or Revio system

SMRT Link read segmentation & single-cell Iso-Seq analysis

---

* Refer to 10x Genomics Support website to download 10x Chromium single cell 3'/5' reagent kit user guides.

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

Kinnex single-cell RNA library preparation procedural notes

Library insert generation

TSO PCR
SMRTbell bead cleanup
TSO removal
SMRTbell bead cleanup

TSO PCR

- Set up TSO PCR reactions **ON ICE** using the **CORRECT** TSO PCR primer set (3' 10x cDNA → 3' TSO PCR primer; 5' 10x cDNA → 5' TSO PCR primer)
  - PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.

TSO removal [Video demonstration]

- **IMPORTANT:** For bead capture steps, allow enough time for beads to magnetize as binding buffer is highly viscous; pipette mix with care and **avoid generating bubbles**
  - Wide-bore pipette tips are recommended to help minimize foaming (specifically when resuspending Kinnex capture beads)
- **Fully resuspend beads** during all wash steps to remove artifact cDNA effectively
- **Fully resuspend beads** before DNA quantification using Qubit dsDNA HS assay

SMRTbell bead cleanup

- Prior to Kinnex array formation, perform a **1.5X** SMRTbell bead cleanup
  - If the cDNA contains smaller fragments <200 bp, it is recommended to increase the SMRTbell cleanup bead ratio to 1.8 – 2.0X

Kinnex PCR

Kinnex PCR (16-mer)
SMRTbell bead cleanup

Kinnex PCR

- Set up Kinnex PCR reactions **ON ICE**
  - PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.
- Perform Kinnex PCR using **25 ng** of cDNA after TSO removal step

Kinnex array formation

Kinnex digestion
Ligation
SMRTbell bead cleanup
DNA damage repair
SMRTbell bead cleanup
Nuclease treatment
SMRTbell bead cleanup

Kinnex digestion

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

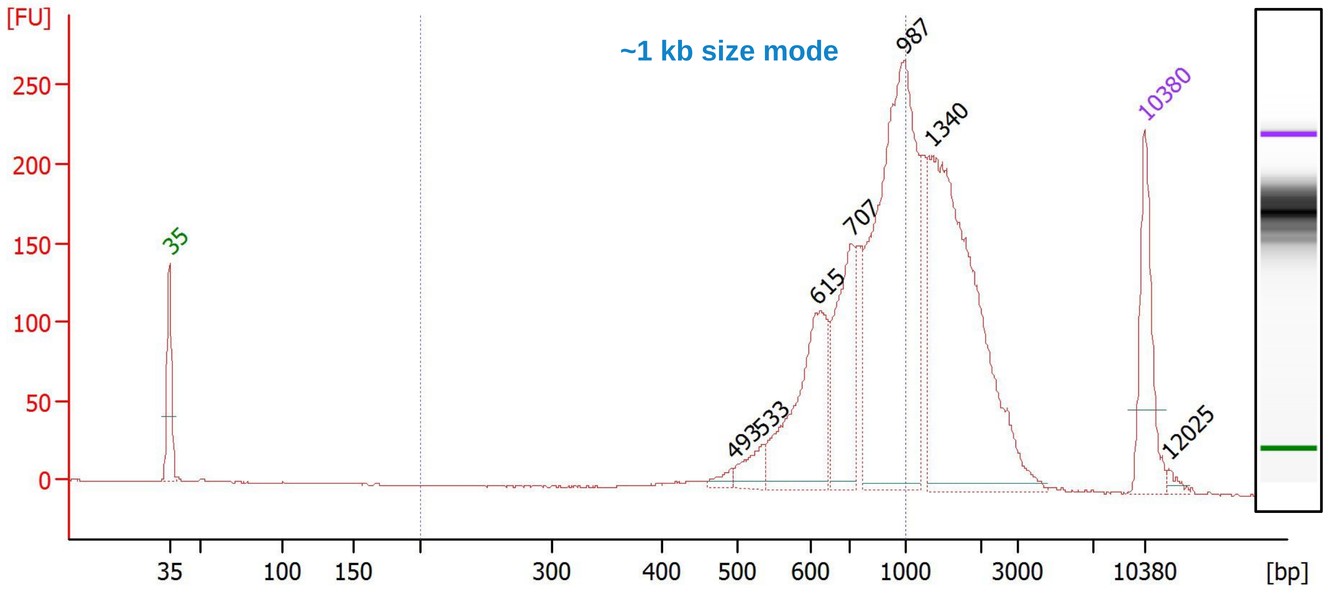
Final SMRTbell bead cleanup

- Perform **1.0X** SMRTbell bead cleanup on final library

Example Kinnex single-cell RNA library preparation QC results

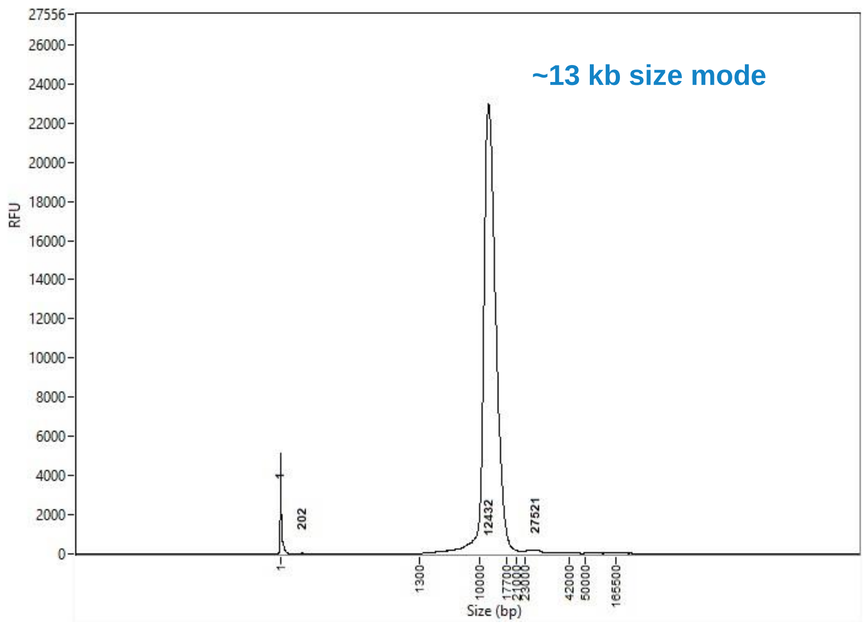
Kinnex single-cell 3’ RNA library prepared with human cDNA

Kinnex PCR DNA sizing QC (Single-cell 3’ cDNA)



Example Bioanalyzer DNA sizing QC analysis results for Kinnex PCR products generated for a 10x Chromium single-cell 3’ cDNA samples prepared from a human cell line (HG002).

Final Kinnex single-cell RNA library QC



Example Femto Pulse DNA sizing QC analysis results for final Kinnex single-cell RNA library.

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

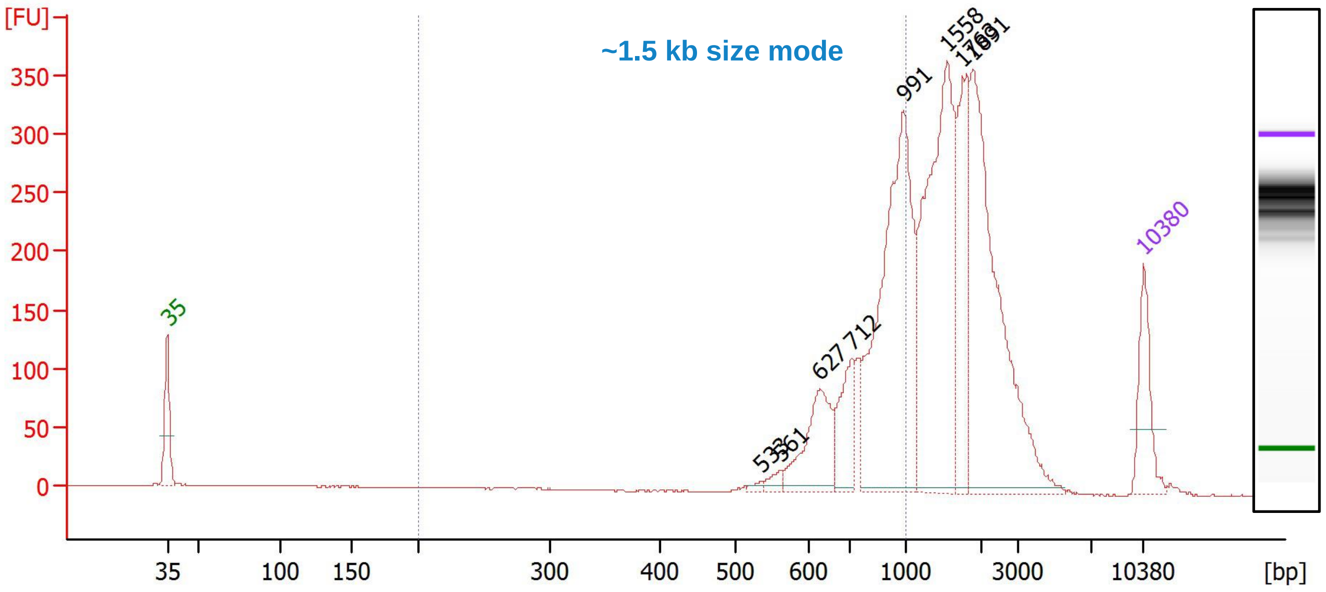
10x single cell 3’ cDNA input	15 ng
cDNA input for Kinnex array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1100 ng (22.0%)

[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~25% when using single-cell 3’ cDNA samples for Kinnex single-cell RNA library construction.

Example Kinnex single-cell RNA library preparation QC results (cont.)

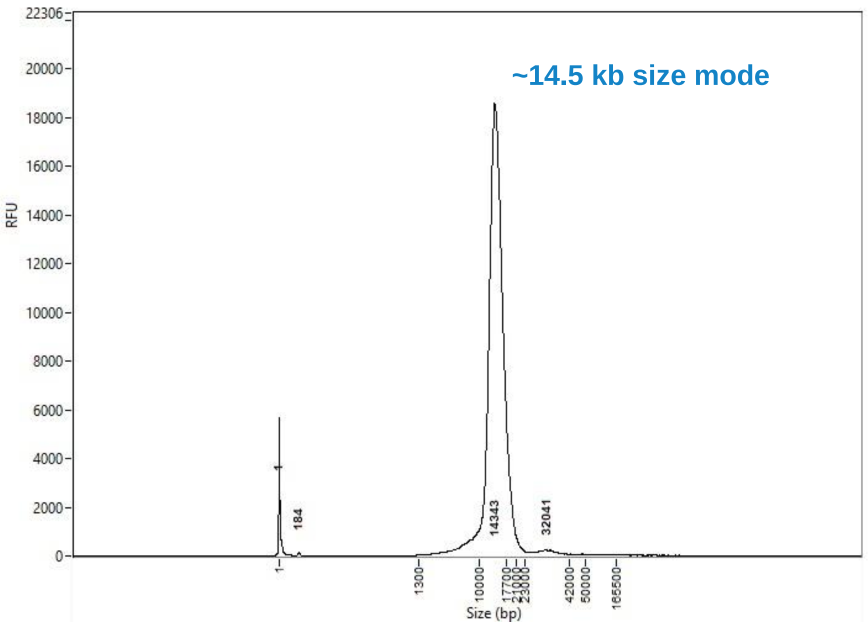
Kinnex single-cell 5’ RNA library prepared with human cDNA

Kinnex PCR DNA sizing QC (Single-cell 5’ cDNA)



Example Bioanalyzer DNA sizing QC analysis results for Kinnex PCR products generated for a 10x Chromium single-cell 5’ cDNA samples prepared from a human cell line (HG002).

Final Kinnex single-cell RNA library QC



Example Femto Pulse DNA sizing QC analysis results for final Kinnex single-cell RNA library.

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

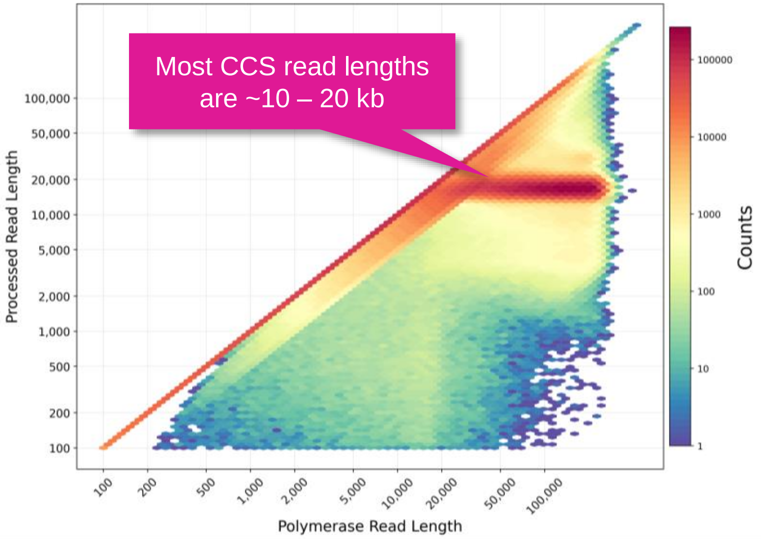
10x single cell 5’ cDNA input	15 ng
cDNA input for Kinnex array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1008 ng (20.2%)

[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~25% when using single-cell 5’ cDNA samples for Kinnex single-cell RNA library construction.

Example sequencing performance for Kinnex single-cell RNA libraries prepared with human cDNA

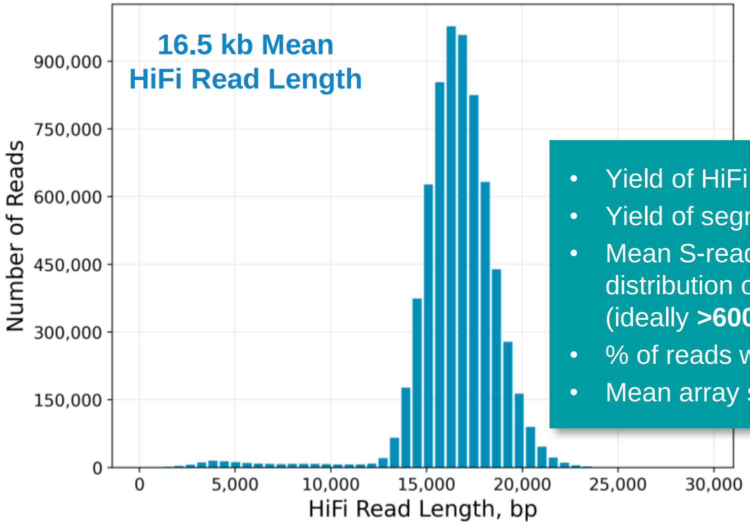
Revio system example data[1] – Kinnex single-cell RNA 3’ library sample

Raw Data Report



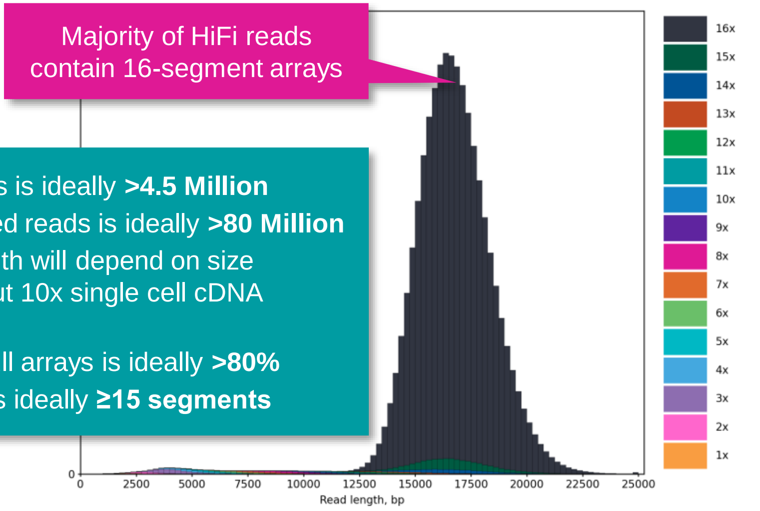
Most CCS read lengths are ~10 – 20 kb

HiFi Read Length



16.5 kb Mean HiFi Read Length

Read Segmentation Metrics



Majority of HiFi reads contain 16-segment arrays

- Yield of HiFi reads is ideally **>4.5 Million**
- Yield of segmented reads is ideally **>80 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

Raw Base Yield	1,289 Gb
Mean Polymerase Read Length	73.16 kb
P0	27%
P1	70%
P2	3%

Example sequencing metrics for a human Kinnex single-cell RNA 3’ library sample run on a Revio system with Revio polymerase kit / 130 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

HiFi Reads	6.7 M
HiFi Base Yield	111.24 Gb
Mean HiFi Read Length	16.55 kb
Median HiFi Read Quality	Q28
HiFi Read Mean # of Passes	8

For human Kinnex single-cell RNA libraries, per-Revio SMRT Cell HiFi read counts were typically ~4 – 7 Million depending on the final library insert size and *P1* loading performance.

Input HiFi Reads	6,673,602
Segmented reads (S-reads)	104,869,257
Mean length of S-reads	1,031 bp
Percent of reads with full arrays	93.89%
Mean array size (concentration factor)	15.71

For Kinnex single-cell RNA libraries, per-Revio SMRT Cell segmentation read counts were typically >80 Million.

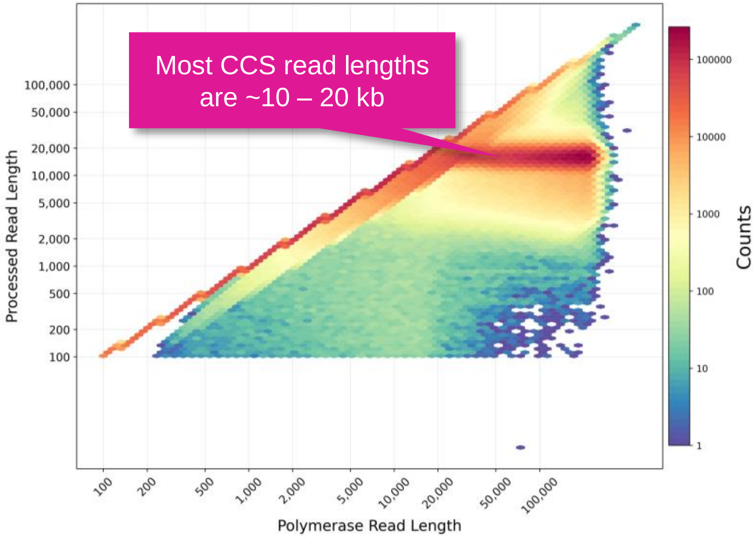
---

[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.

Example sequencing performance for Kinnex single-cell RNA libraries prepared with human cDNA

Revio system example data[1] – Kinnex single-cell RNA 5’ library sample

Raw Data Report

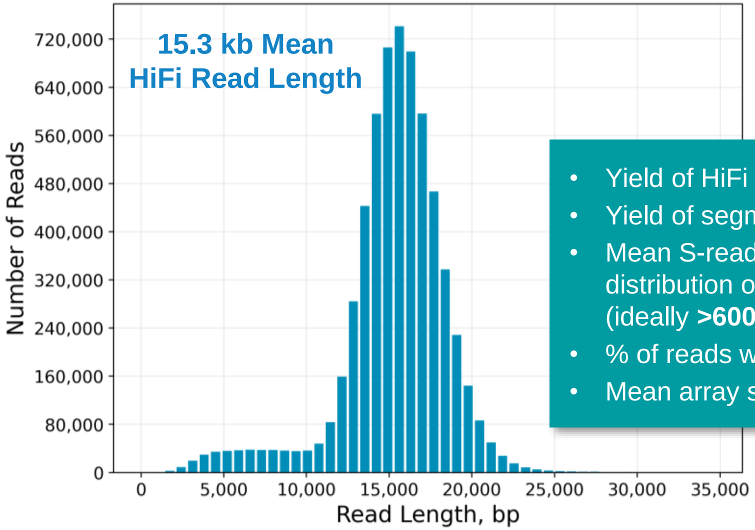


Most CCS read lengths are ~10 – 20 kb

Raw Base Yield	1,116 Gb
Mean Polymerase Read Length	74,7 kb
P0	40%
P1	59%
P2	1%

Example sequencing metrics for a human Kinnex single-cell RNA 5’ 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

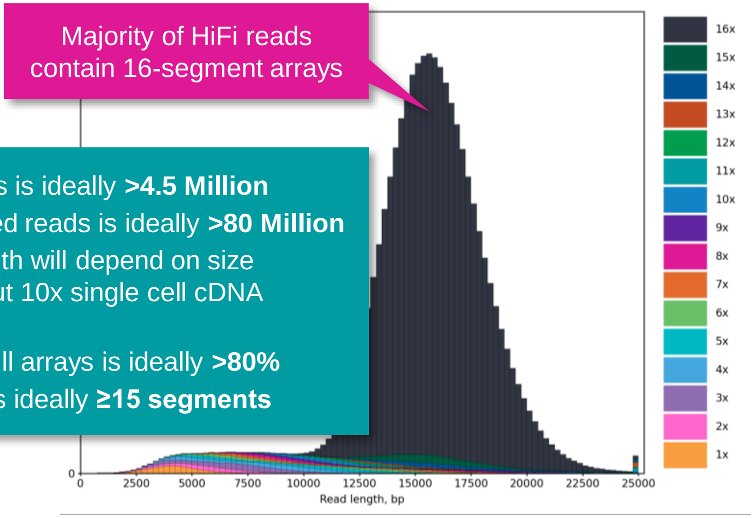


15.3 kb Mean HiFi Read Length

HiFi Reads	6.1 M
HiFi Base Yield	93.7 Gb
Mean HiFi Read Length	15.3 kb
Median HiFi Read Quality	Q30
HiFi Read Mean # of Passes	9

For human Kinnex single-cell RNA libraries, per-Revio SMRT Cell HiFi read counts were typically ~4 – 7 Million depending on the final library insert size and *P1* loading performance.

Read Segmentation Metrics



Majority of HiFi reads contain 16-segment arrays

- Yield of HiFi reads is ideally **>4.5 Million**
- Yield of segmented reads is ideally **>80 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

Input HiFi Reads	6,104,086
Segmented reads (S-reads)	91,323,803
Mean length of S-reads	980 bp
Percent of reads with full arrays	87.46%
Mean array size (concentration factor)	14.96

For Kinnex single-cell RNA libraries, per-Revio SMRT Cell segmentation read counts were typically >80 Million.

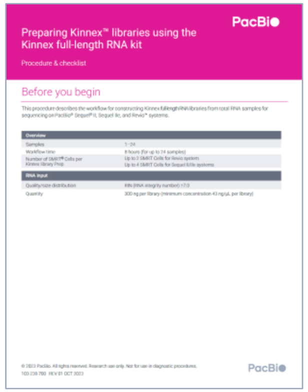
---

[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.

Kinnex full-length RNA library preparation & sequencing workflow key highlights

Kinnex full-length RNA library preparation procedure description

Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700) describes the workflow for constructing Kinnex libraries from total RNA samples using the **Kinnex full-length RNA kit** for sequencing on PacBio Sequel II, Sequel IIe & Revio systems.



PacBio

Preparing Kinnex™ libraries using the Kinnex full-length RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing Kinnex full-length RNA libraries from total RNA samples for sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

Overview	
Samples	1–24
Workflow time	8 hours (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	Up to 2 SMRT Cells for Revio system Up to 4 SMRT Cells for Sequel II/IIe systems
RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/µL per library)

© 2023 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.
103-238-700 REV 01 OCT 2023

PacBio

PacBio Documentation
(103-238-700)

Kinnex full-length RNA kit
(103-072-000)

Iso-Seq express 2.0 kit[1]
103-071-500 (24 rxn)

Concatenation factor

8-fold

Kinnex PCR 8-fold kit
103-071-600 (12 rxn)

Kinnex concatenation kit
103-071-800 (12 rxn)

SMRTbell cleanup beads
102-158-300

Elution buffer
101-633-500

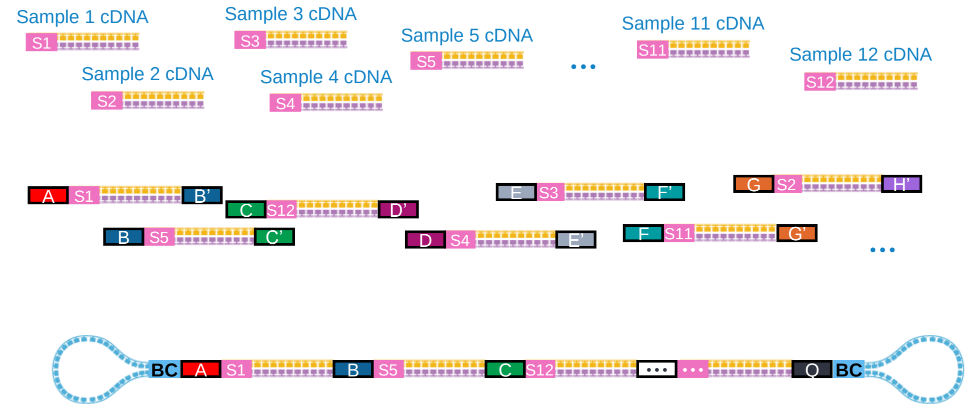
Overview	
Samples	1–24
Workflow time	8 hours (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	Up to 2 SMRT Cells for Revio system Up to 4 SMRT Cells for Sequel II/IIe systems
RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/µL per library)

- Kinnex full-length RNA library prep protocol uses **Kinnex full-length RNA kit** and **Iso-Seq express 2.0 kit[1]**
  - **Do not use** SMRTbell prep kit 3.0 with this protocol

Kinnex full-length RNA library barcoding options

Kinnex full-length RNA library preparation procedure supports **up to 48-plex** sample multiplexing through combined use of:

→ **12 different barcoded cDNA amplification PCR primers**
→ **4 different barcoded Kinnex terminal SMRTbell adapters**



cDNA synthesis

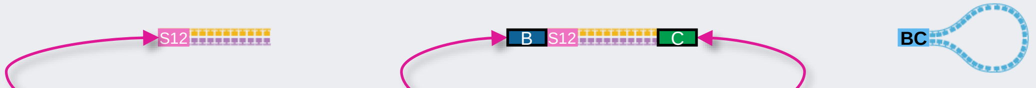
Pool

Kinnex PCR

Pool

Array formation

Kinnex library template (~18 kb) containing 8 concatenated full-length cDNA segments



Kinnex cDNA Sample barcode
(bc01-bc12)

Kinnex segmentation adapters
(A, B, C, D, E, F, G, H, Q)

Barcoded Kinnex terminal SMRTbell adapter
(bc01-bc04)

PacBio

*[1] **Note:** Iso-Seq express 2.0 kit (103-071-500) is **not included** in Kinnex full-length RNA bundle kit and must be purchased separately from PacBio.

Comparison of *Iso-Seq express 2.0 kit* vs. *Iso-Seq express oligo kit*

	Iso-Seq express 2.0 kit (103-071-500)	**Iso-Seq express oligo kit (101-737-500)**
Description	Includes Iso-Seq Express template switching oligo, barcoded cDNA PCR Primers, and other reagents needed for performing 1st-strand cDNA synthesis and PCR amplification of cDNA products	Includes Iso-Seq express template switching oligo and non-barcoded cDNA PCR Primer to be used in conjunction with third-party reagents for performing 1st-strand cDNA synthesis and PCR amplification of cDNA products
# reactions	24	24
Storage	Refer to product insert for storage instructions	-70°C to -80°C
Compatible SMRTbell library types	Standard (non-concatenated) library Kinnex (concatenated) library	Standard (non-concatenated) library
Sample multiplexing support	Includes 12 barcoded Iso-Seq PCR primers (bc01 – bc12) for up to 12-plex sample multiplexing	Requires additional purchase of barcoded PCR primers for cDNA amplification from a third-party vendor
Kit contents	Iso-Seq RT buffer Iso-Seq RT primer mix Iso-Seq RT enzyme mix Iso-Seq cDNA PCR mix Iso-Seq template switch oligo Iso-Seq cDNA amplification primer Iso-Seq primers (bc01 – bc12)	Iso-Seq Express template switching oligo Iso-Seq Express cDNA PCR Primer
SMRT Link support for barcoded cDNA primer Iso-Seq libraries	YES (select 'Iso-Seq v2 Barcoded cDNA Primers' for primer set to use for Iso-Seq analysis application)	YES (select 'Iso-Seq 12 Barcoded cDNA Primers' or 'Custom cDNA Primers' for primer set to use for Iso-Seq analysis application)

Kinnex full-length RNA experimental design considerations

Kinnex full-length RNA application use case recommendations for PacBio systems

Example application	Human genetics disease studies	Biopharma for identifying highly expressed targets	Plant & animal whole genome annotation
Experimental goal	Isoform discovery and quantification of moderate-to-rare transcripts	Isoform discovery of high expressed transcripts	Comprehensive transcript annotation in a species
Example study design	Disease vs. normal tissues with multiple replicates	Disease cohort with >20+ samples	Plant or animal with multiple tissue types
Target depth of coverage per sample	10 M reads per sample	5 M reads per sample	≤5 M reads per sample
Sample multiplexing[1]	**Sequel II/IIe system:** Up to 2 samples per SMRT Cell 8M (2-plex)	**Sequel II/IIe system:** Up to 3 samples per SMRT Cell 8M (3-plex)	**Sequel II/IIe system:** Up to 3 tissue types per SMRT Cell 8M (3-plex)
	Revio system: Up to 4 samples per Revio SMRT Cell (4-plex)	**Revio system:** Up to 8 samples per Revio SMRT Cell (8-plex)	**Revio system:** Up to 8 tissue types per Revio SMRT Cell (8-plex)[2]
Expected data throughput (per SMRT Cell)	**Sequel II/IIe system:** 15 M reads per SMRT Cell 8M divided by *N* samples		
	Revio system: 40 M reads divided by *N* samples		
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)		
Total RNA input into Kinnex library prep workflow	300 ng total RNA (RIN ≥7) for 1st-strand cDNA synthesis		
SMRT Link data analysis workflows	Read Segmentation and Iso-Seq analysis application with option to “pool reads and cluster together” to get a master isoform classification file with per-sample full-length read counts		
Community data analysis tools	Annotation & quantification: PIGEON, SQANTI3, Differential analysis: TappAS, Fusion calling: pbfusion, Visualization: SWAN		

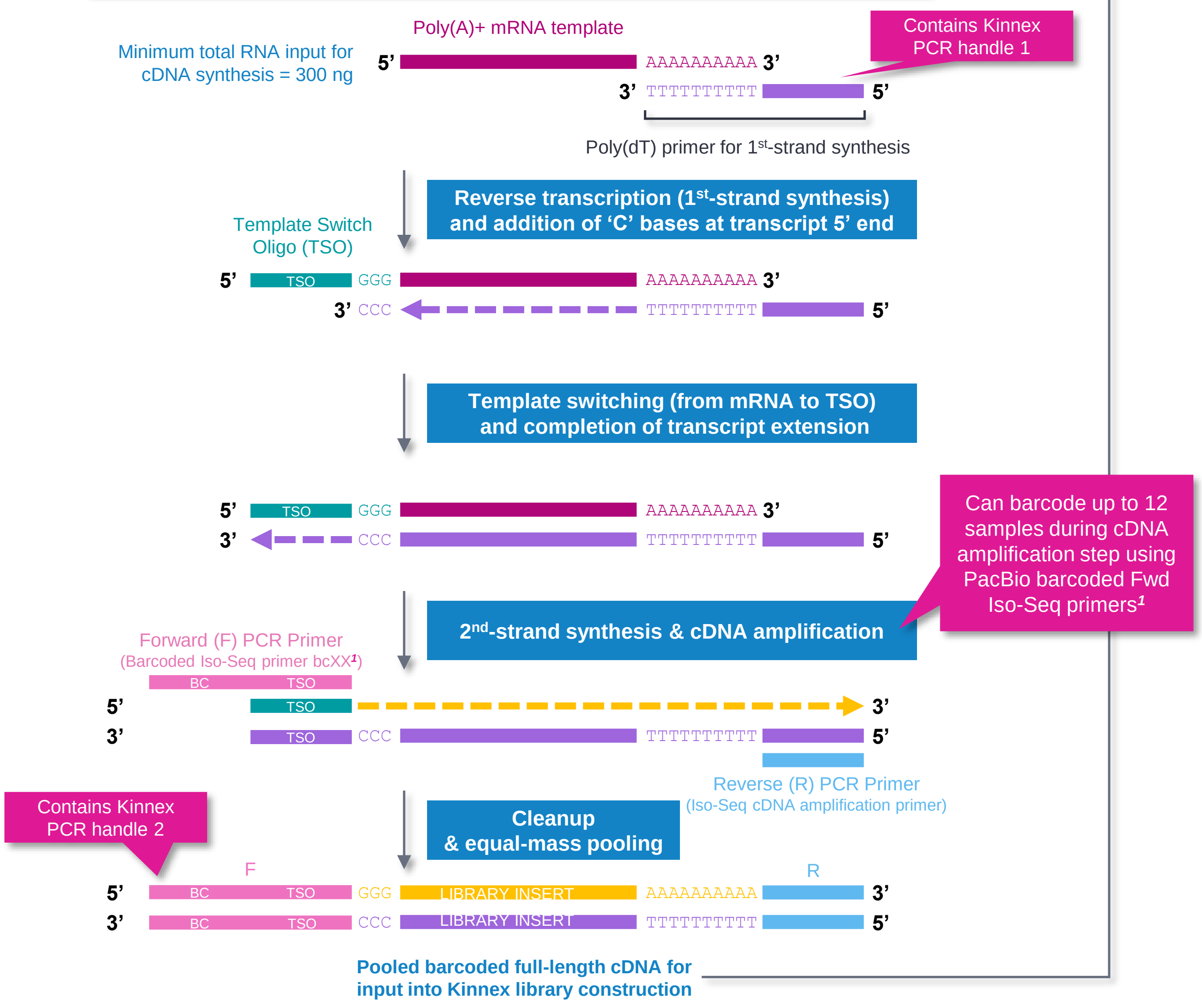
---

[1] Kinnex concatenation kit (103-071-800) can support up to 48-plex sample multiplexing through the combined use of 12 different barcoded cDNA amplification primers and 4 different barcoded Kinnex terminal SMRTbell adapters during Kinnex full-length RNA library construction.

[2] If targeting <5 M transcripts reads per sample → can multiplex up to 12 tissues types per Revio SMRT Cell.

Kinnex full-length RNA method overview

Full-length cDNA synthesis & amplification



Minimum total RNA input for cDNA synthesis = 300 ng

Poly(A)+ mRNA template

Contains Kinnex PCR handle 1

Poly(dT) primer for 1st-strand synthesis

Template Switch Oligo (TSO)

Reverse transcription (1st-strand synthesis) and addition of 'C' bases at transcript 5' end

Template switching (from mRNA to TSO) and completion of transcript extension

Can barcode up to 12 samples during cDNA amplification step using PacBio barcoded Fwd Iso-Seq primers[1]

2nd-strand synthesis & cDNA amplification

Forward (F) PCR Primer
(Barcoded Iso-Seq primer bcXX[1])

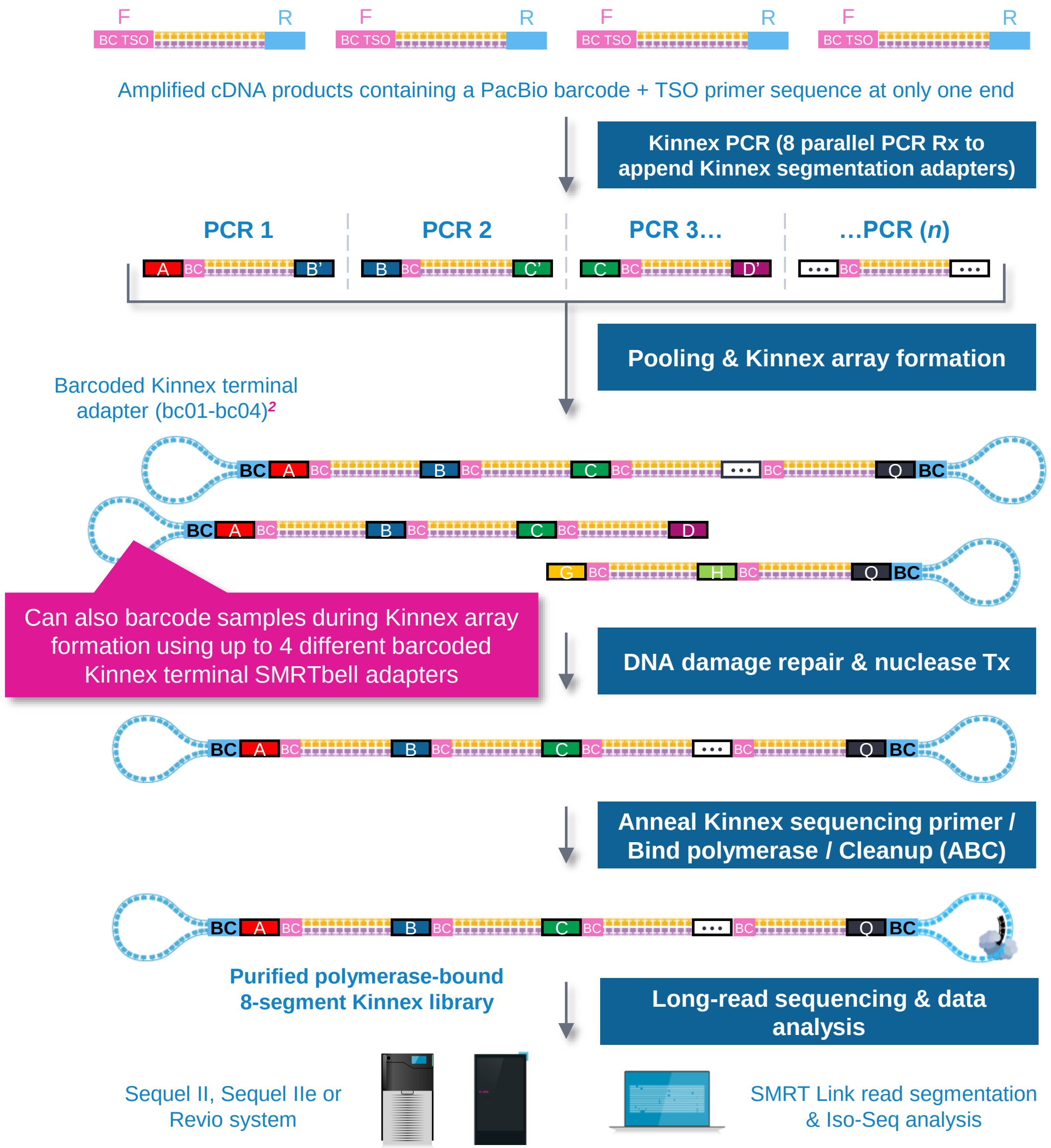
Reverse (R) PCR Primer
(Iso-Seq cDNA amplification primer)

Contains Kinnex PCR handle 2

Cleanup & equal-mass pooling

Pooled barcoded full-length cDNA for input into Kinnex library construction

Kinnex library prep, sequencing & analysis



Amplified cDNA products containing a PacBio barcode + TSO primer sequence at only one end

Kinnex PCR (8 parallel PCR Rx to append Kinnex segmentation adapters)

Pooling & Kinnex array formation

Barcoded Kinnex terminal adapter (bc01-bc04)[2]

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

DNA damage repair & nuclease Tx

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

Purified polymerase-bound 8-segment Kinnex library

Long-read sequencing & data analysis

Sequel II, Sequel IIe or Revio system

SMRT Link read segmentation & Iso-Seq analysis

---

[1] Twelve barcoded Iso-Seq primers (Iso-Seq primer bc01–12) are available for cDNA amplification step.

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

Kinnex full-length RNA library preparation procedural notes

Library insert generation

cDNA synthesis
SMRTbell bead cleanup
cDNA amplification
SMRTbell bead cleanup

cDNA amplification

- **Set up on ice** and add PCR reaction to thermal cycler **after the lid has preheated to 105°C** to avoid digestion of primers by polymerase exonuclease activity
- Barcoded primers are used during cDNA amplification
  - 12 barcoded forward primers (bc01-bc12) available for use in combination with Iso-Seq cDNA amplification primer

SMRTbell bead cleanup

- Previous non-Kinnex full-length RNA (bulk monomer) Iso-Seq protocol (*Procedure & checklist – Preparing Iso-Seq libraries using SMRTbell prep kit 3.0* [102-396-000]) offered 3 options for post-cDNA amplification SMRTbell bead cleanup: 0.82X, 0.86X, and 0.95X → For simplification, Kinnex full-length RNA protocol now only specifies to use **0.9X**

Kinnex PCR

Kinnex PCR (8-mer)
Pool + SMRTbell bead cleanup

Kinnex PCR

- Can transfer entire volume of primers to PCR tubes for ease of use with multi-channel pipettes (8 primer mix tubes)
- **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

Pooling of 8 Kinnex PCR products + SMRTbell bead cleanup

- **Pool exactly 23 µL** from each Kinnex PCR reaction for a total combined volume of **184 µL**
- Add exactly **193 µL** of SMRTbell cleanup beads (**1.05X**)
- Kinnex PCR mix significantly increases stringency of SMRTbell clean up beads, so **accurate pipetting is critical**

Kinnex array formation

Kinnex digestion / Ligation
DNA damage repair
SMRTbell bead cleanup
Nuclease treatment
SMRTbell bead cleanup

Kinnex digestion

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

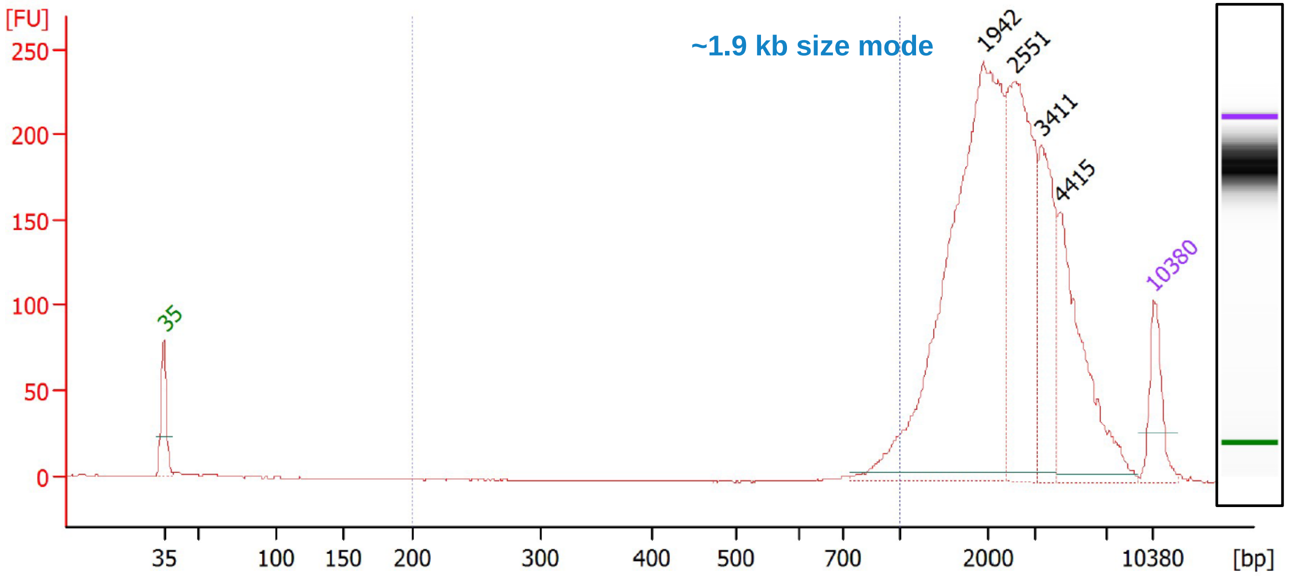
Final SMRTbell bead cleanup

- Perform **1.0X** SMRTbell bead cleanup on final library

Example Kinnex full-length RNA library preparation QC results

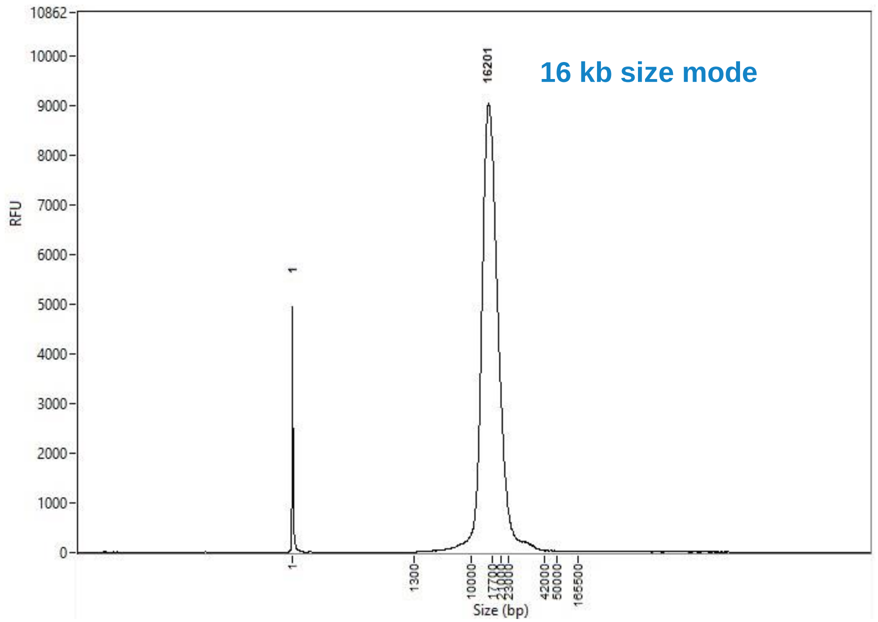
Kinnex full-length RNA library prepared with human UHRR total RNA

Amplified full-length cDNA QC



Example Bioanalyzer DNA sizing QC analysis results for amplified full-length cDNA generated from a universal human RNA reference (UHRR) total RNA sample.

Final Kinnex full-length RNA library QC



Example Femto Pulse DNA sizing QC analysis results for final Kinnex full-length RNA library.

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

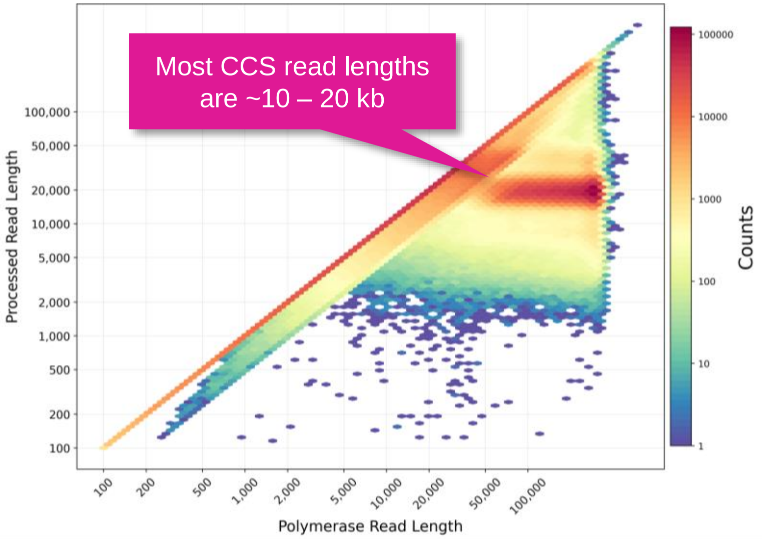
Total RNA input for cDNA synthesis	300 ng
cDNA input for Kinnex array formation	5900 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1460 ng (24.7%)

[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~25% when using UHRR total RNA samples for Kinnex full-length RNA library construction.

Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA

Sequel IIe system example data[1]

Raw Data Report

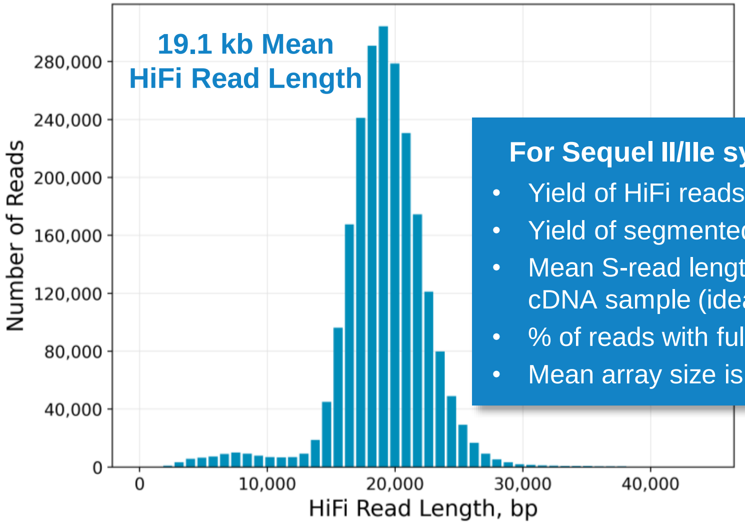


Most CCS read lengths are ~10 – 20 kb

Raw Base Yield	652 Gb
Mean Polymerase Read Length	115.36 kb
P0	28%
P1	71%
P2	1%

Example sequencing metrics for a Universal Human Reference RNA (UHRR) Kinnex full-length RNA library sample run on a Sequel IIe system with Binding Kit 3.2 (Polymerase 2.2) / 80 pM on-plate loading concentration (OPLC) / 30-hrs movie time / 2-hrs pre-extension time.

HiFi Read Length

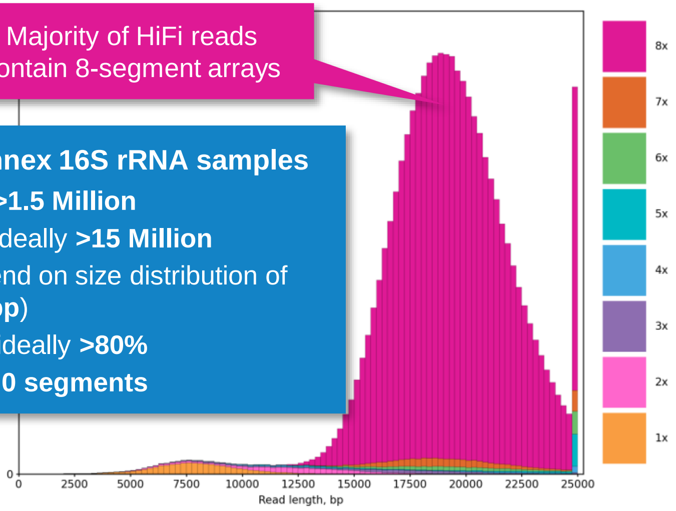


19.1 kb Mean HiFi Read Length

HiFi Reads	2.3 M
HiFi Base Yield	43.2 Gb
Mean HiFi Read Length	19.1 kb
Median HiFi Read Quality	Q32
HiFi Read Mean # of Passes	10

For UHRR Kinnex full-length RNA libraries, per-SMRT Cell 8M HiFi read counts typically ranged from ~2 – 3 Million depending on the final library insert size.

Read Segmentation Metrics



Majority of HiFi reads contain 8-segment arrays

For Sequel II/IIe system Kinnex 16S rRNA samples

- Yield of HiFi reads is ideally **>1.5 Million**
- Yield of segmented reads is ideally **>15 Million**
- Mean S-read length will depend on size distribution of cDNA sample (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **>7.0 segments**

Reads	2,260,039
Segmented reads (S-reads)	17,213,165
Mean length of S-reads	2,420 bp
Percent of reads with full arrays	91.07%
Mean array size (concentration factor)	7.62

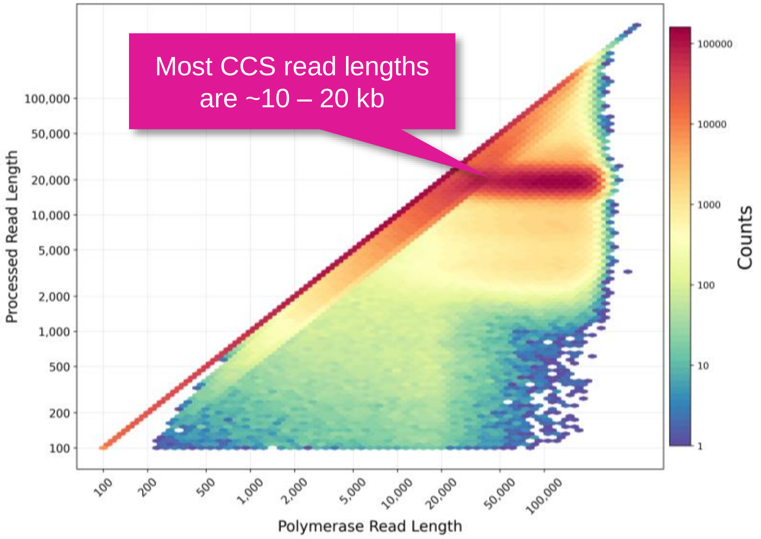
For UHRR Kinnex libraries, per-SMRT Cell 8M segmentation read counts were typically ~15 – 20 Million.

[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 <30 Gb per Sequel II SMRT Cell 8M.

Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA

Revio system example data[1]

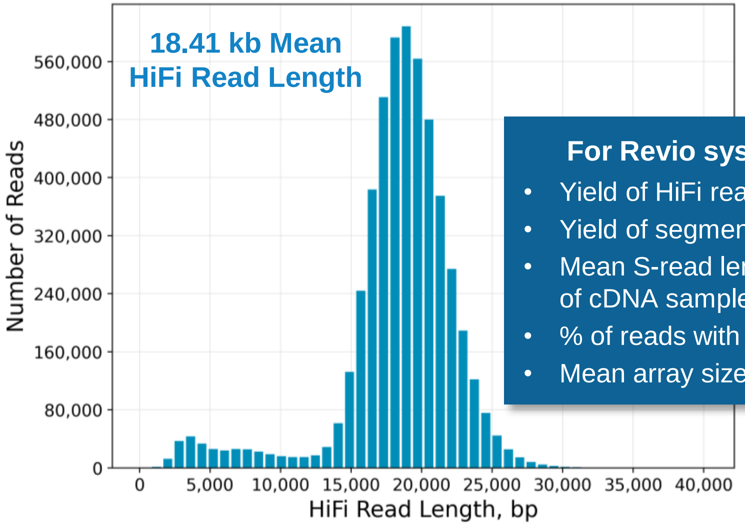
Raw Data Report


Most CCS read lengths are ~10 – 20 kb

Raw Base Yield	1,168 Gb
Mean Polymerase Read Length	58.6 kb
P0	16%
P1	79%
P2	5%

Example sequencing metrics for a Universal Human Reference RNA (UHRR) Kinnex full-length RNA library sample run on a Revio system with Revio polymerase kit / 130 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

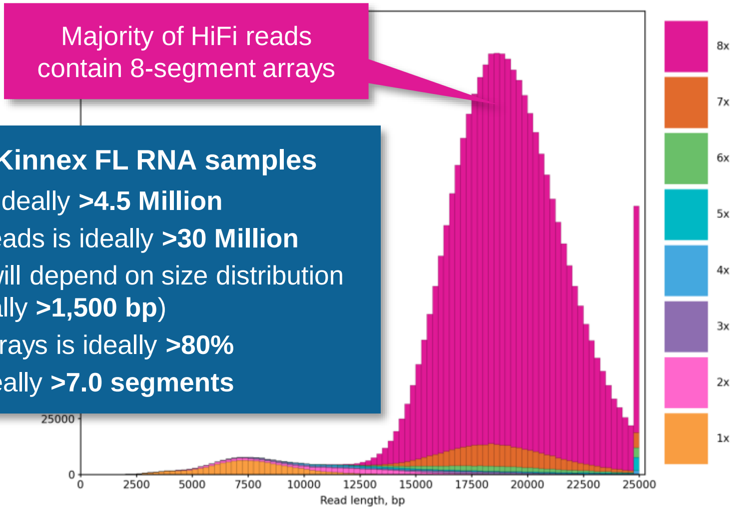
HiFi Read Length


18.41 kb Mean HiFi Read Length

HiFi Reads	5.1 M
HiFi Base Yield	93.47 Gb
Mean HiFi Read Length	18.41 kb
Median HiFi Read Quality	Q28
HiFi Read Mean # of Passes	7

For UHRR Kinnex full-length RNA libraries, per-Revio SMRT Cell HiFi read counts were typically ~5 – 6 Million depending on the final library insert size and *P1* loading performance.

Read Segmentation Metrics


Majority of HiFi reads contain 8-segment arrays

For Revio system Kinnex FL RNA samples

- Yield of HiFi reads is ideally **>4.5 Million**
- Yield of segmented reads is ideally **>30 Million**
- Mean S-read length will depend on size distribution of cDNA sample (ideally **>1,500 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **>7.0 segments**

Input HiFi Reads	5,027,154
Segmented reads (S-reads)	37,216,151
Mean length of S-reads	2,393 bp
Percent of reads with full arrays	85.84%
Mean array size (concentration factor)	7.40

For UHRR Kinnex libraries, per-Revio SMRT Cell segmentation read counts were typically ~30 – 45 Million.

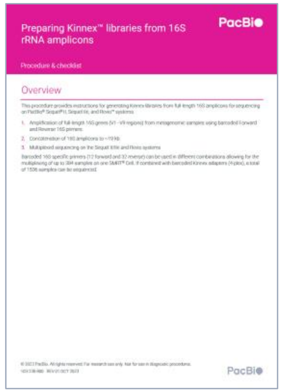
---

[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.

Kinnex 16S rRNA library preparation & sequencing workflow key highlights

Kinnex 16S rRNA library preparation procedure description

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


Preparing Kinnex™ libraries from 16S rRNA amplicons
PacBio
Procedure & checklist
Overview

PacBio Documentation
(103-238-00)

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

Concatenation factor
12-fold

Kinnex PCR 12-fold kit
103-071-700 (12 rxn)

Kinnex concatenation kit
103-071-800 (12 rxn)

SMRTbell cleanup beads
102-158-300

Elution buffer
101-633-500

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

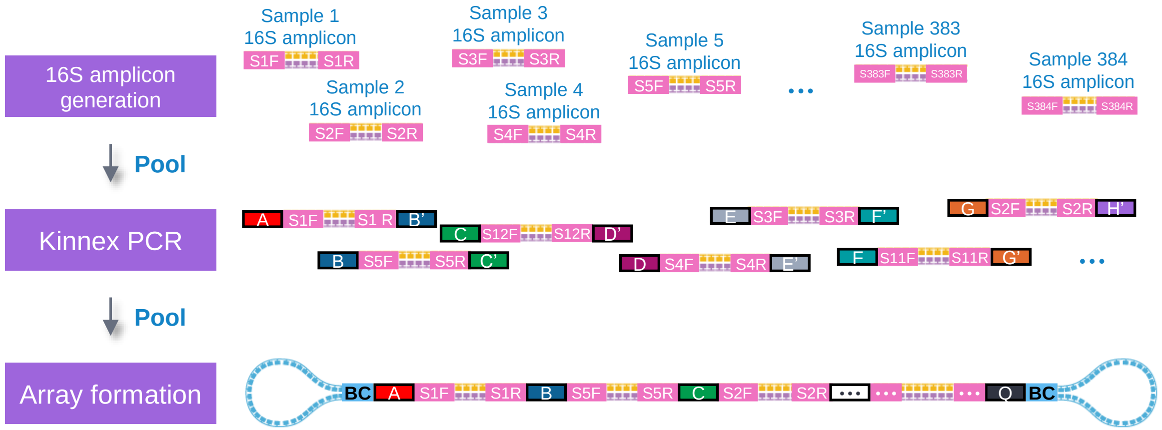
Barcoded 16S-specific primers (12 forward and 32 reverse) can be used in different combinations allowing for the multiplexing of up to 384 samples on one SMRT® Cell. If combined with barcoded Kinnex adapters (4-plex), a total of 1536 samples can be sequenced.

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

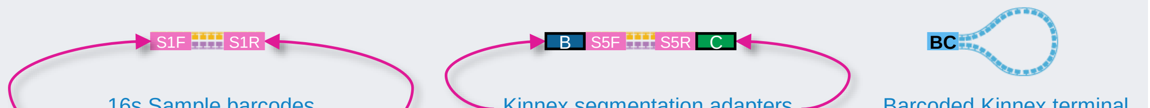
Kinnex full-length 16s rRNA library barcoding options

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]
→ **32 different 16S barcoded Reverse PCR primers**[1]
→ **4 different barcoded Kinnex terminal SMRTbell adapters**



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



16s Sample barcodes (Kinnex16S_Fwd_01 – 12 and Kinnex16S_Rev_13 – 44)

Kinnex segmentation adapters (A, B, C, D, E, F, G, H, I, J, K, L, Q)

Barcoded Kinnex terminal SMRTbell adapter (bc01-bc04)

---

[1] 16S barcoded Forward and Reverse PCR primer oligos (HPLC purified) are not included in Kinnex 16S rRNA kit and must be supplied by users through a third-party oligo vendor.

Kinnex 16S rRNA experimental design considerations

Kinnex 16S rRNA application use case recommendations for PacBio systems

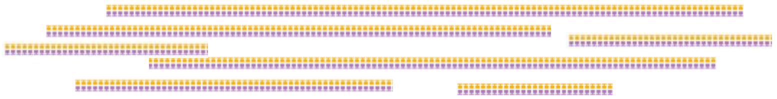
	Sequel II and IIe systems		**Revio system**	
Experimental goal	Determine the microbial diversity (phylogeny and taxonomy) of bacteria in a metagenomic sample			
Sample multiplexing[1]	Up to 384 samples per SMRT Cell 8M (384-plex)		Up to 1,536 samples per Revio SMRT Cell (1536-plex)	
Expected coverage 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.

Kinnex 16S rRNA method overview

Full-length 16S gene amplification

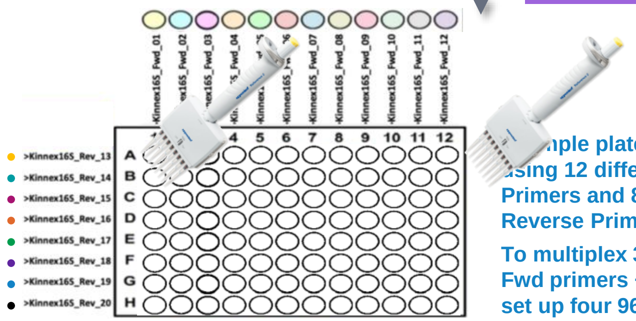


Bacterial gDNA fragments isolated from metagenomic samples

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

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

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



mple 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.

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

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

Dual-barcoded full-length 16S amplicon product

Equal-volume pooling & cleanup

Contains Kinnex PCR handle 1

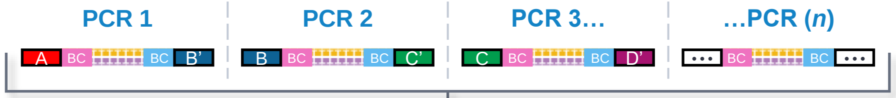
Contains Kinnex PCR handle 2

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

Kinnex library prep, sequencing & analysis

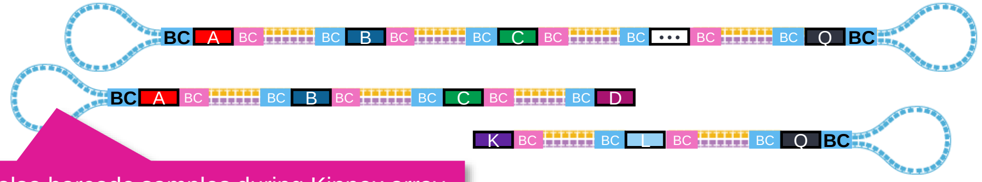
Amplified 16S products containing a different PacBio barcode at each end (Fwd BC ≠ Rev BC)

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



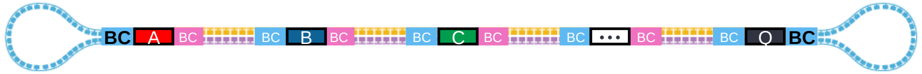
Pooling & Kinnex array formation

Barcoded Kinnex terminal adapter (bc01-bc04)[2]

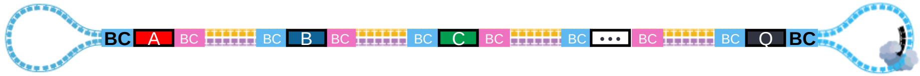


Can also barcode samples during Kinnex array formation using up to 4 different barcoded Kinnex terminal SMRTbell adapters

DNA damage repair & nuclease Tx



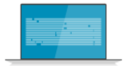
Anneal Kinnex sequencing primer / Bind polymerase / Cleanup (ABC)



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 procedural notes

Library insert generation

PCR amplification of F-L 16S genes

SMRTbell bead cleanup

Pool barcoded 16S PCR amplicons

SMRTbell bead cleanup

PCR amplification of full-length 16S genes

- Customer supplies Kapa PCR mix (HiFi HotStart ReadyMix) and oligos
- Up to **384-plex** can be done at this point using **combinatorial indexing**
- **Set up on ice** and add PCR reaction to thermal cycler **after the lid has preheated to 105°C** to avoid digestion of primers by polymerase exonuclease activity

Kinnex PCR

Kinnex PCR (12-mer)

SMRTbell bead cleanup

Kinnex PCR

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

SMRTbell bead cleanup

- **Pool exactly 23 µL** from each Kinnex PCR reaction for a total combined volume of **276 µL**
- **Add exactly 304 µL** of SMRTbell clean up beads (**1.1X**)
- Kinnex PCR mix significantly increases stringency of SMRTbell clean up beads, so **accurate pipetting is critical**

Kinnex array formation

Kinnex digestion / Ligation

DNA damage repair

SMRTbell bead cleanup

Nuclease treatment

SMRTbell bead cleanup

Kinnex digestion

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

Final SMRTbell bead cleanup

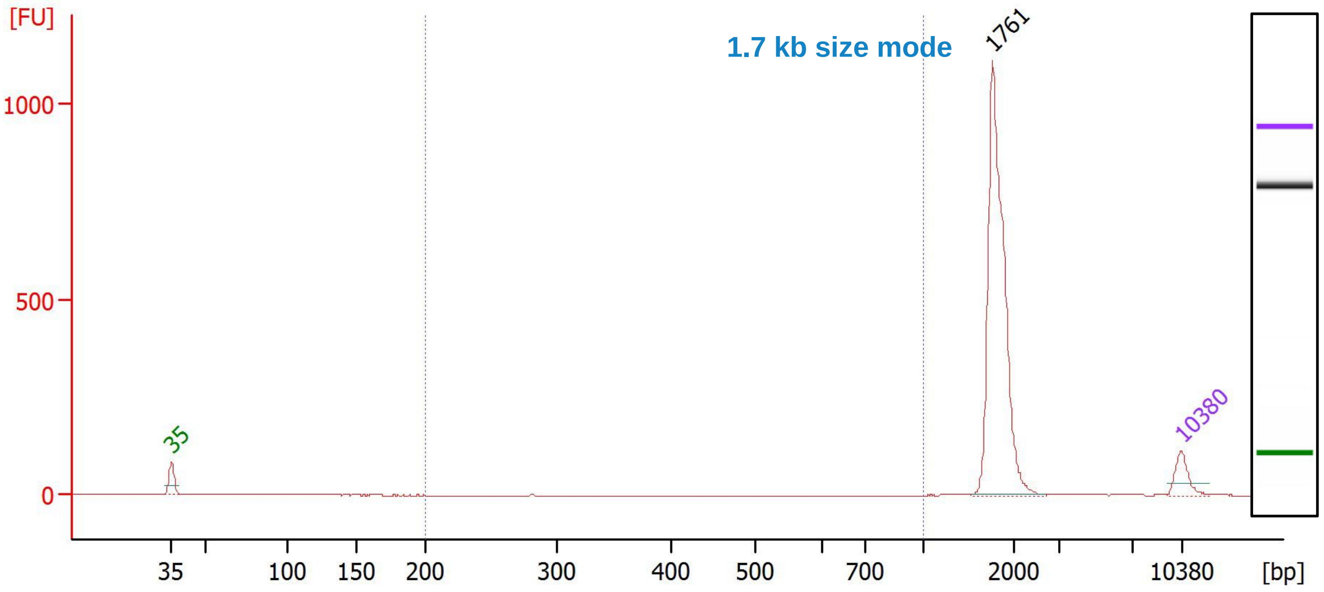
- Perform **1.0X** SMRTbell bead cleanup on final library

Example Kinnex 16S rRNA library preparation QC results

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

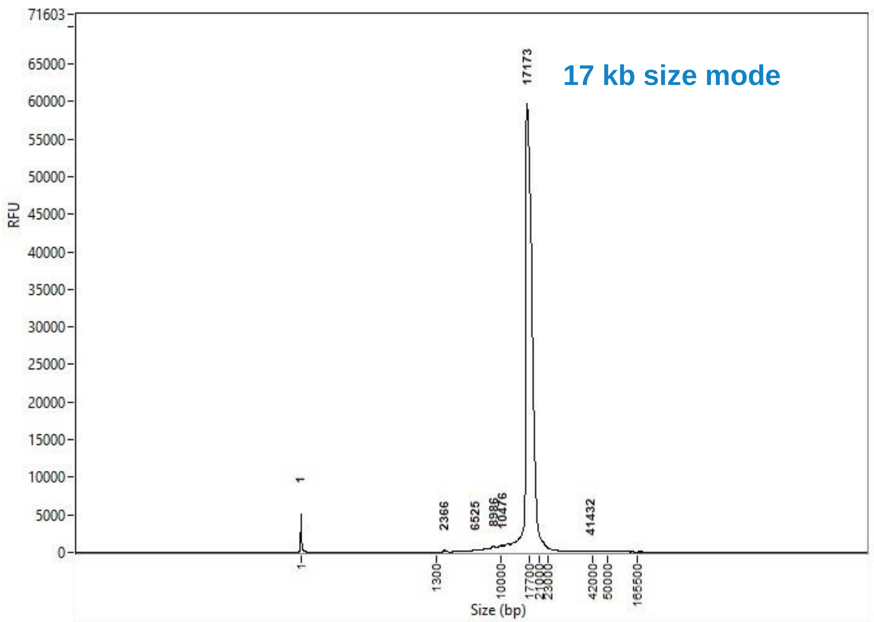
Final Kinnex library yield is typically sufficient to load **≥2 SMRT Cells**

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**

ZymoBIOMICS Fecal Reference
96 barcoded samples

ZymoBIOMICS Gut Microbiome Standard
96 barcoded samples

ATCC MSA-1002 20 Strain Even Mix
96 barcoded samples

ATCC MSA-1003 20 Strain Staggered Mix
96 barcoded samples

X **4 Kinnex barcoded adapters**

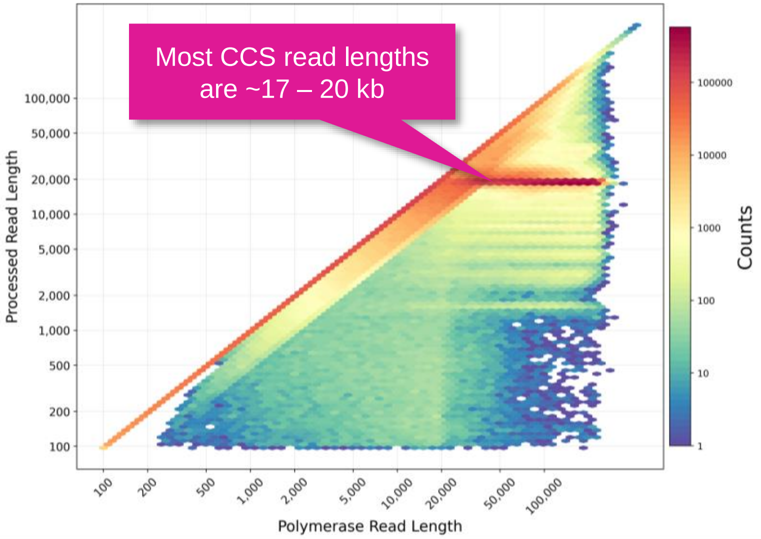
gDNA input for 16S PCR	1.1 ng
16S amplicon DNA input Kinnex PCR products for Kinnex array formation	6000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1080 ng (18%)

[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

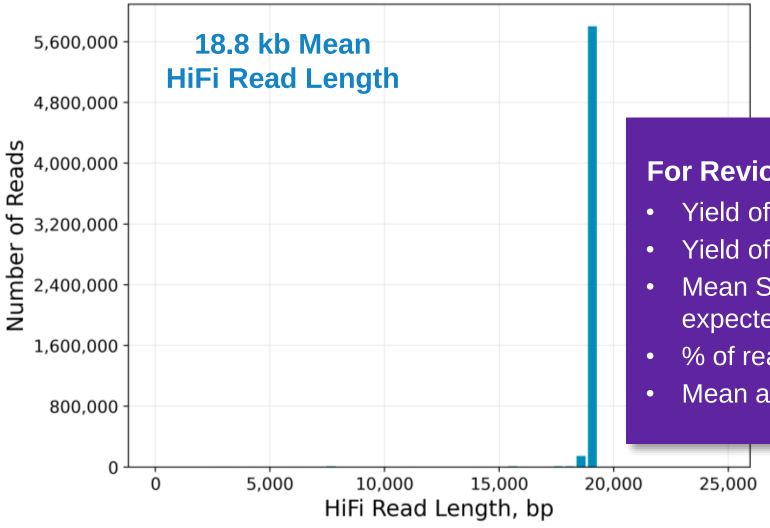


Most CCS read lengths are ~17 – 20 kb

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

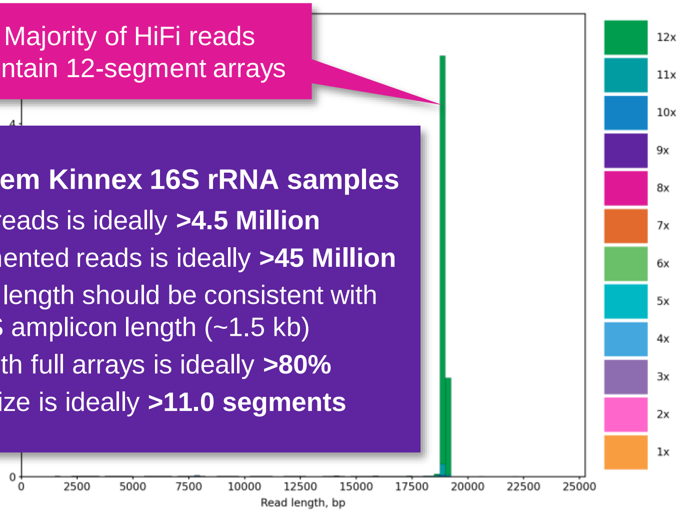


18.8 kb Mean HiFi Read Length

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



Majority of HiFi reads contain 12-segment arrays

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

PacBio

Technical documentation & applications support resources

Technical resources for Kinnex library preparation, sequencing & data analysis

Single-cell cDNA sample preparation literature & other resources

- 10x Genomics Chromium Next GEM Single Cell 3' v3.1 (Single Index) How-to Video [Link]
- 10x Genomics Chromium Single Cell 3' Reagent Kits User Guide – v3.1 (CG000204)
- 10x Genomics Chromium Single Cell 5' Reagent Kits User Guide – v2 Chemistry Dual Index (CG000331)

Kinnex library preparation literature & other resources

- Application note – Kinnex 16S rRNA kit for full-length 16S sequencing (102-326-601)
- Application note – Kinnex full-length RNA kit for isoform sequencing (102-326-591)
- Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing (102-326-549)
- Brochure – Scalable, cost-effective RNA sequencing with PacBio Kinnex kits (102-326-597)
- Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)
- Procedure & checklist – Preparing Kinnex libraries using Kinnex full-length RNA kit (103-238-700)
- Procedure & checklist – Preparing Kinnex libraries from 16s rRNA amplicons (103-238-800)
- Technical overview – Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing (103-343-700)
- Technical overview – Kinnex library preparation for full-length 16S rRNA gene sequencing (103-344-800)
- Technical overview – Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)
- Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit (103-344-600)
- Video tutorial – PacBio Kinnex single-cell RNA TSO artifact removal demo for Kinnex single-cell RNA kit [Link]
- Video tutorial – SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits [Link]
- Whitepaper – Bulk and single-cell isoform sequencing for human disease research (102-326-576)

Technical resources for Kinnex library preparation, sequencing & data analysis (cont.)

Data analysis resources

- Application note – Bioinformatics tools for full length isoform sequencing (102-326-593)
- 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]
- Video tutorial – Read Segmentation and Iso-Seq workflow in SMRT Link: [Link]

Publications and posters

- Schertzer, M.D. et al. (2023) Cas13d-mediated isoform-specific RNA knockdown with a unified computational and experimental toolbox. BioRxiv preprint [Link]
- Al'Khafaji, A.M. et al. (2023) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology. [Link]
- ASM Microbe Poster (2023) – Increasing throughput of full-length 16S sequencing using concatenation [Link]

Webinars

- PacBio webinar (2023) – Understanding clonal evolution using game theory and single-cell long-read isoform analysis [Link]
- PacBio Iso-Seq social club webinar (2022) – Introduction to Iso-Seq method [Link]
- PacBio Iso-Seq social club webinar (2022) – SQANTI3 for isoform classification and annotation [Link]
- PacBio Iso-Seq social club webinar (2022) – TappAS for isoform differential expression analysis [Link]
- PacBio Iso-Seq social club webinar (2022) – Single-cell Iso-Seq applications in cancer and neurological disorders [Link]

Technical resources for Kinnex library preparation, sequencing & data analysis (cont.)

Example PacBio data sets

Application	Dataset	Data type	PacBio system
Kinnex single-cell RNA sequencing	Homo sapiens - PBMC 10x Chromium Single Cell 5' and 3' libraries [Link]	HiFi long read	Sequel II & Revio systems
	Homo sapiens - HG002 (10x 5') [Link]	HiFi long read	Revio system
Kinnex full-length RNA sequencing	Homo sapiens – universal human reference RNA (UHRR) [Link]	HiFi long read	Sequel II & Revio systems
	Homo sapiens – HG002 [Link]	HiFi long read	Revio system
	Homo sapiens – Heart [Link]	HiFi long read	Revio system
	Homo sapiens – Cerebellum [Link]	HiFi long read	Revio 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