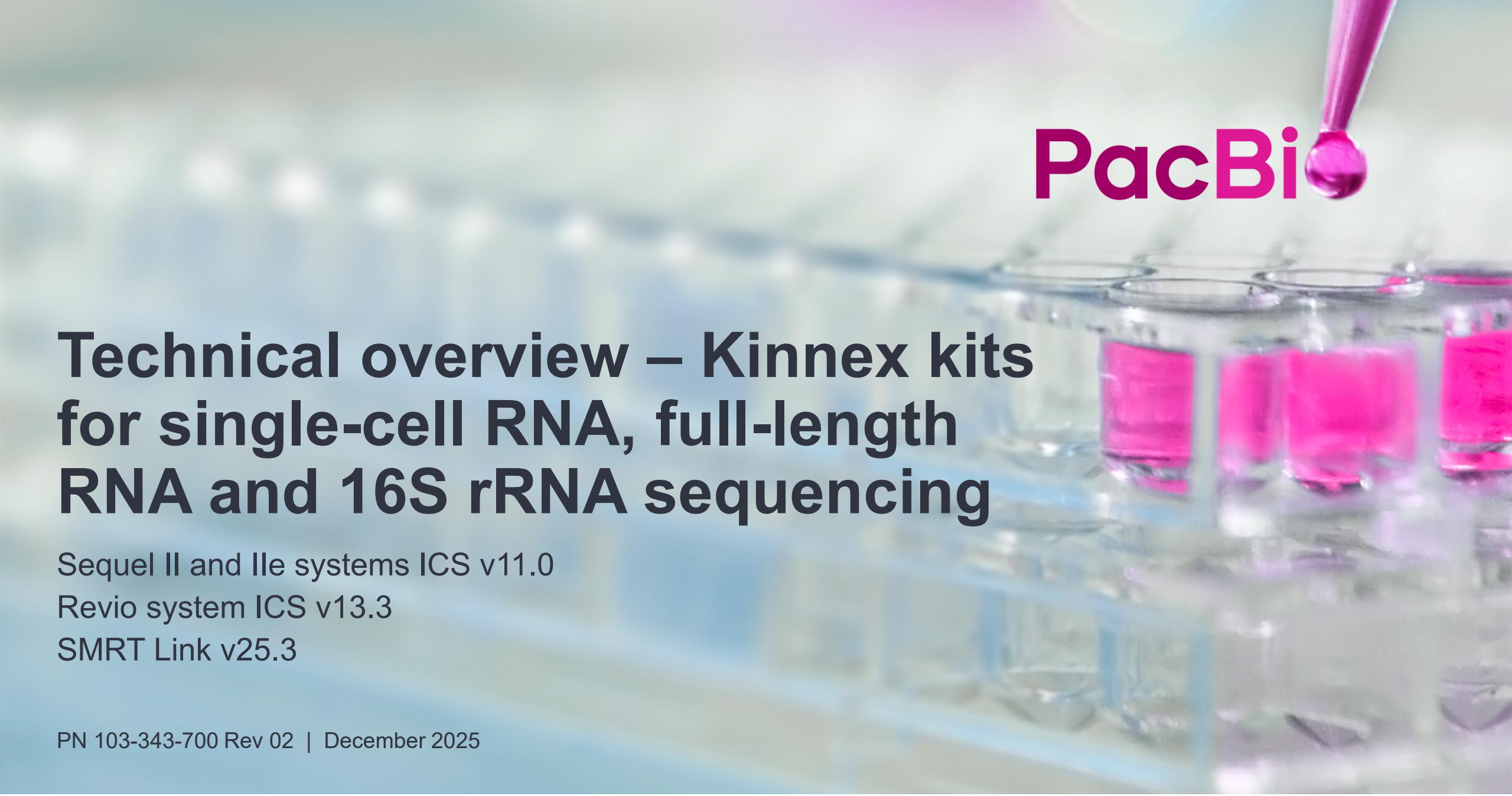


Technical overview – Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing

Sequel II and IIe systems ICS v11.0
Revio system ICS v13.3
SMRT Link v25.3

PN 103-343-700 Rev 02 | December 2025

Technical overview

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

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
8. APPENDIX 1 – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput
9. APPENDIX 2 – PacBio compatible Kinnex library preparation workflows
    i. Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits
    ii. Preparing Kinnex single-cell libraries with Twist exome enrichment kits
    iii. Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3
10. APPENDIX 3 – SMRT Link run design procedure for Sequel IIe system

PacBio

Kinnex product introduction

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

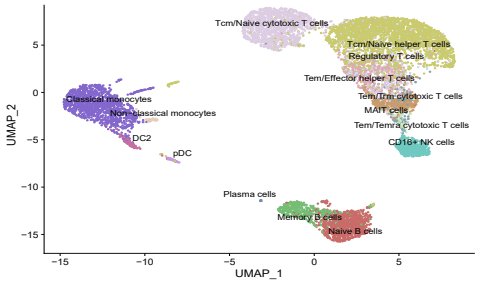
Kinnex kits offer scalable, cost-effective RNA sequencing solutions

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

Single-cell
RNA sequencing

4-plex using
Kinnex barcoded adapters
[16-fold concatenation]

100-120M reads – Revio system
50-60M reads – Vega system
30-40M reads – Sequel IIe system



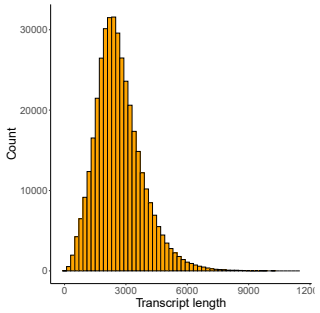
Identify cell type-specific isoform expression

Kinnex full-length RNA kit
(103-072-100)

Full-length
RNA sequencing

12-plex with the Iso-Seq express 2.0 kit[1] or 48-plex when combined with all four Kinnex barcoded adapters
[8-fold concatenation]

50-60M reads – Revio system
20-30M reads – Vega system
16-20M reads – Sequel IIe system



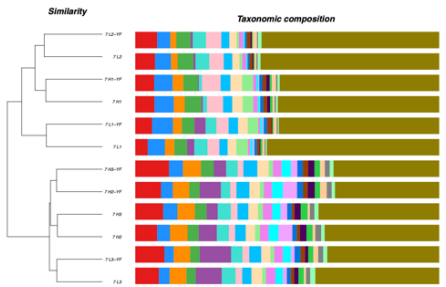
Full-length transcripts from 1–10 kb for bulk RNA samples

Kinnex 16S rRNA kit
(103-072-000)

Full-length 16S rRNA
for species identification

384-plex 16S amplicons or 1,536-plex when combined with all four Kinnex barcoded adapters
[12-fold concatenation]

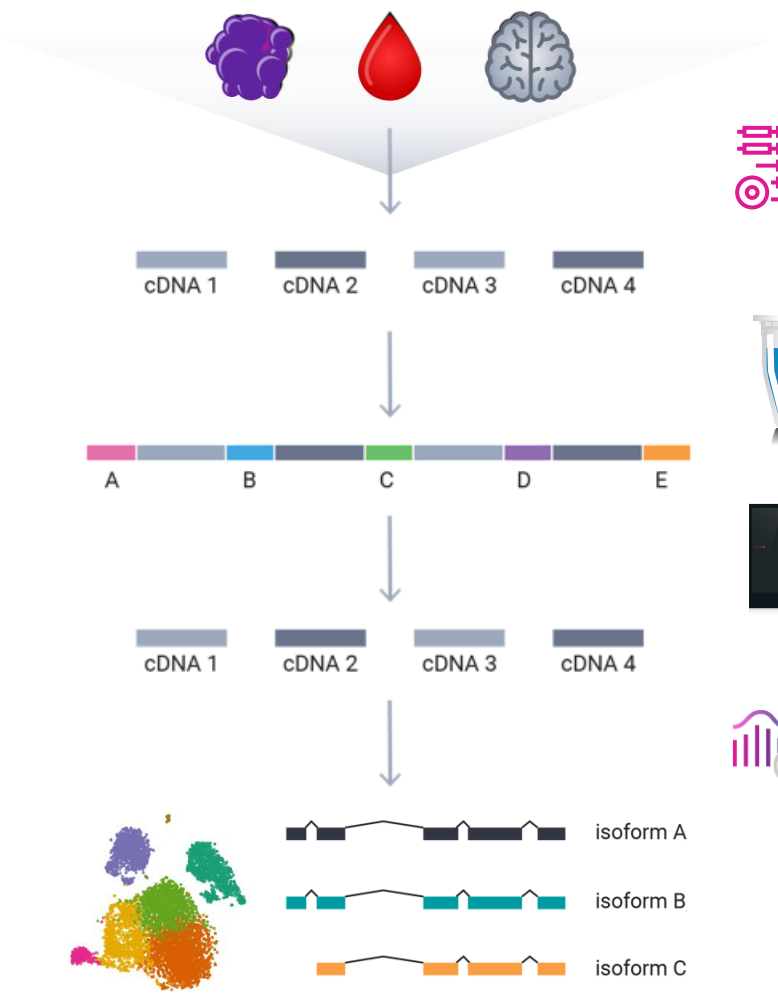
70-80M reads – Revio system
30-40M reads – Vega system
20-25M reads – Sequel IIe system



Taxonomic profiling for human, animal, and environmental samples

[1] PacBio Iso-Seq express 2.0 kit part number is 103-071-500.

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



Single-cell cDNA generation

Kinnex library preparation

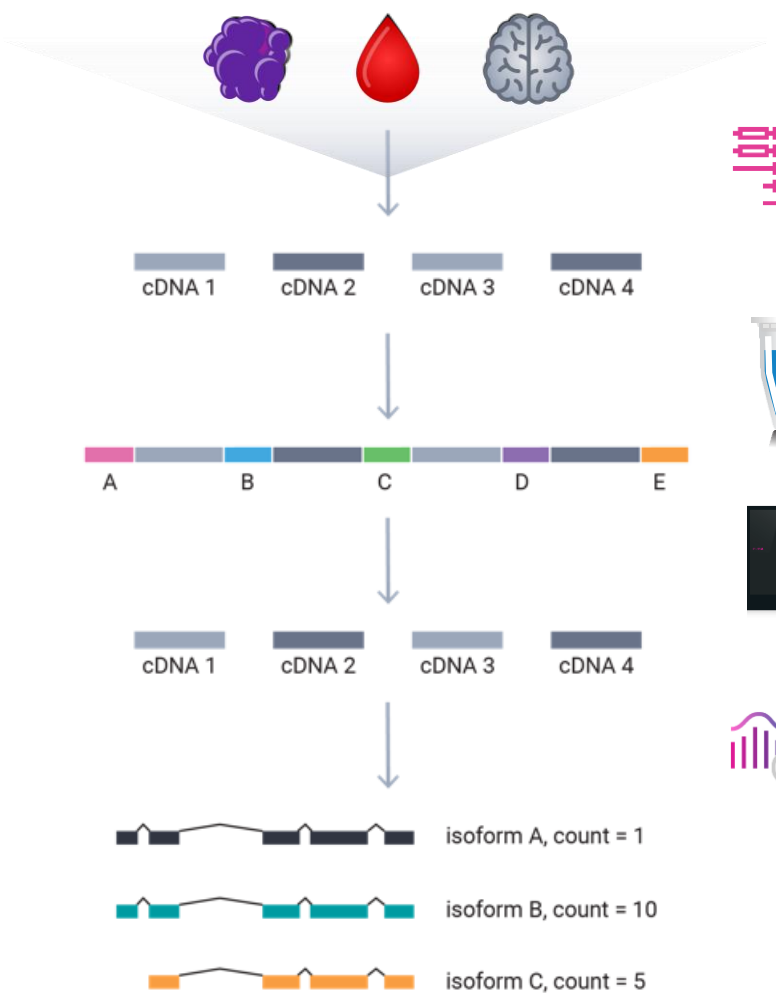
PacBio sequencing

Read segmentation + single-cell Isoform analysis

- 10x Chromium Single Cell 3’ kit (v3.1) and 5’ kit (v2)[1]
- 15–75 ng cDNA input
- 3,000 to 20,000 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)
- 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] Kinnex single-cell RNA protocol is compatible with single-cell cDNA from (1) 10x Chromium Single Cell Universal 3’ or 5’ kit; Single Cell Next GEM (v3.1 3’ or v2 5’); (2) 10x Visium HD Spatial Gene Expression kit; (3) Parse Evercode WT or WT mini kit; or (4) ArgenTag Single-Cell RNA Library Kit. See APPENDIX 2 – PacBio compatible Kinnex library preparation workflows.

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



cDNA generation

Kinnex library preparation

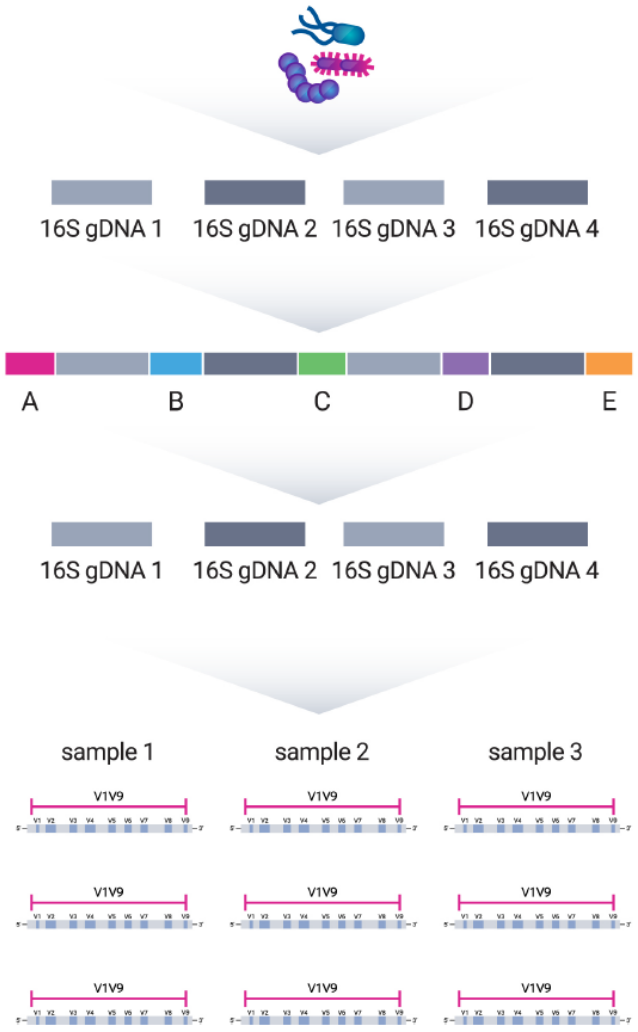
PacBio sequencing

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)[1]
- SMRT Link Run Design support for 'Kinnex full-length RNA' application type with auto-analysis (read segmentation + isoform analysis)
- SMRT Link Iso-Seq isoform-classification software to identify novel genes and isoforms with abundance information

[1] See ***Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit*** (102-326-632) for modified workflow for performing targeted enrichment using IDT xGen Hyb and Wash kit v3 for preparing PacBio Kinnex full-length RNA libraries.

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)
- 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

Kinnex product configuration overview

Kinnex product design overview and supported applications

Kinnex kits utilize the MAS-Seq concatenation method[1] to increase throughput on PacBio long-read systems

Step

Library insert generation → **Kinnex PCR** → **Kinnex array formation**

Supports both Kinnex PCR and Kinnex array formation steps

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

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

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

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

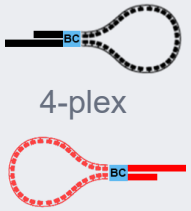
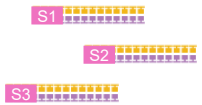
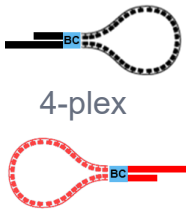
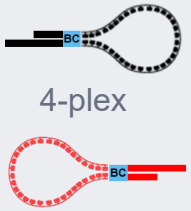
103-071-700

PacBio

[1] Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology. 42:582. [Link]

Kinnex bundle kit product features

Kinnex bundle kit products are compatible with Sequel II/IIe, Vega 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 IIe system) **50 – 60 M** (Vega system) **100 – 120 M** (Revio system)	**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**	**16 – 20 M** (Sequel IIe system) **20 – 30 M** (Vega system) **50 – 60 M** (Revio system)	**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**	**20 – 25 M** (Sequel IIe system) **30 – 40 M** (Vega system) **70 – 80 M** (Revio system)	**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 bundle kit products are compatible with Sequel II/IIe, Vega and Revio systems

	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[1]

Kinnex single-cell RNA (2 days)

PacBio 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
4. Kinnex PCR + cleanup
   - Safe stop

Day 1 (~6 hrs)

Kinnex PCR

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)

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
4. Kinnex PCR + cleanup
   - Safe stop

Day 1 (~5 hrs)

Kinnex PCR

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)

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
4. Kinnex PCR + cleanup
   - Safe stop

Day 1 (~3 hrs)

Kinnex PCR

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

10x Forward (F) PCR primer

10x Reverse (R) PCR primer

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

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

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

OR

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

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

F R TSO

3' single-cell cDNA with TSO artifacts removed

OR

F TSO R

5' single-cell cDNA with TSO artifacts removed

Cleanup

Single-cell cDNA 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]

Full-length RNA sample preparation

Total RNA isolation & QC

Example: QIAGEN RNeasy Plus kit

Poly(A)+ mRNA template

5' AAAAAAAAAA 3'

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

Forward (F) PCR primer (Barcoded Iso-Seq primer bcXX[2])

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

BC TSO

5' BC TSO GGG LIBRARY INSERT AAAAAAAAAA 3'
3' BC TSO CCC LIBRARY INSERT TTTTTTTTTT 5'

Barcoded full-length cDNA product

Pooling of barcoded cDNA samples & cleanup

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

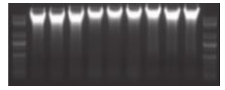
Pooled, full-length cDNA sample ready for Kinnex PCR step

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

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

Forward (F) PCR primer (Kinnex16S_Fwd_ XX[3])

Reverse (R) PCR primer (Kinnex16S_Rev_ XX[3])

Fwd BC Rev BC

5' Fwd BC LIBRARY INSERT Rev BC 3'
3' Fwd BC LIBRARY INSERT Rev BC 5'

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

[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)

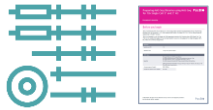
MAS-Seq for 10x 3’ concatenation kit (102-407-900)

MAS capture beads kit (102-428-400)

TSO PCR	SMRTbell bead cleanup	TSO artifact removal	SMRTbell bead cleanup	~3 hrs
1.0 hr	20 min	1.0 hr	20 min	

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	~3 hrs
1.0 hr	20 min	1.0 hr	20 min	

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	~3 hrs
1.3 hrs	20 min	1.0 hr	20 min	

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

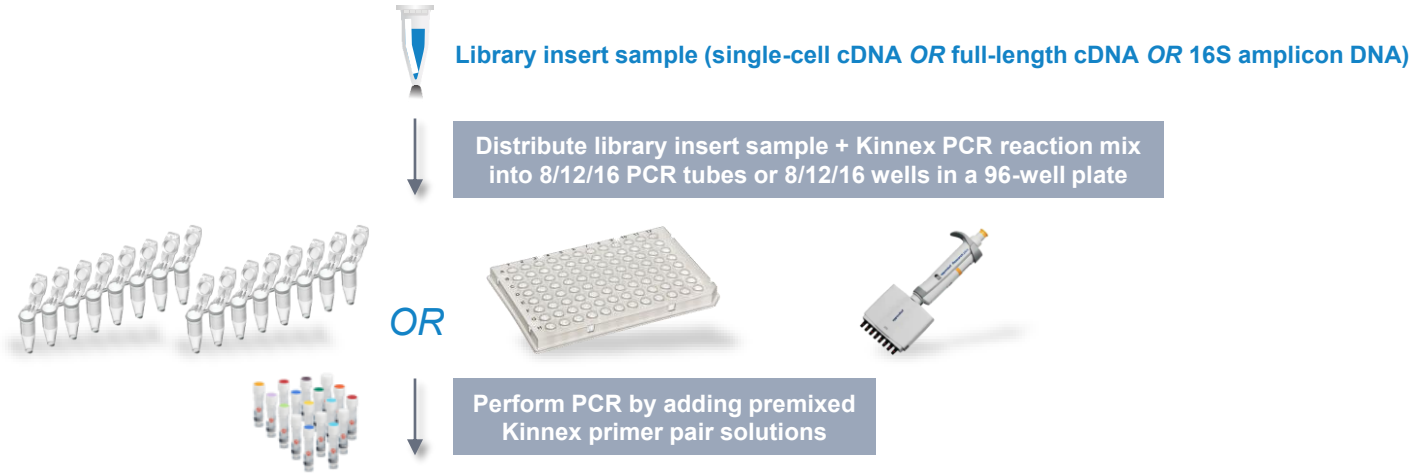
PCR amplification of F-L 16S genes	Pooling of barcoded 16S PCR amplicons	SMRTbell bead cleanup	~2 hrs
1.3 hrs	10 min	20 min	

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



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

Application-specific Kinnex PCR 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)

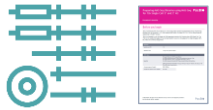
Kinnex PCR (16-mer) → SMRTbell bead cleanup → ~2 hrs

1.5 hrs | 20 min

Step	Temperature	Duration	Cycles
Initial Denaturation	98˚C	3 min	1
Denaturation	98˚C	20 s	
Annealing	68˚C	30 s	9
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)

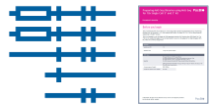
Kinnex PCR (16-mer) → SMRTbell bead cleanup → ~2 hrs

1.5 hrs | 20 min

Step	Temperature	Duration	Cycles
Initial Denaturation	98˚C	3 min	1
Denaturation	98˚C	20 s	
Annealing	68˚C	30 s	9
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 → ~2 hrs

1.5 hrs | 20 min

Step	Temperature	Duration	Cycles
Initial Denaturation	98˚C	3 min	1
Denaturation	98˚C	20 s	
Annealing	68˚C	30 s	9
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 → ~1.5 hrs

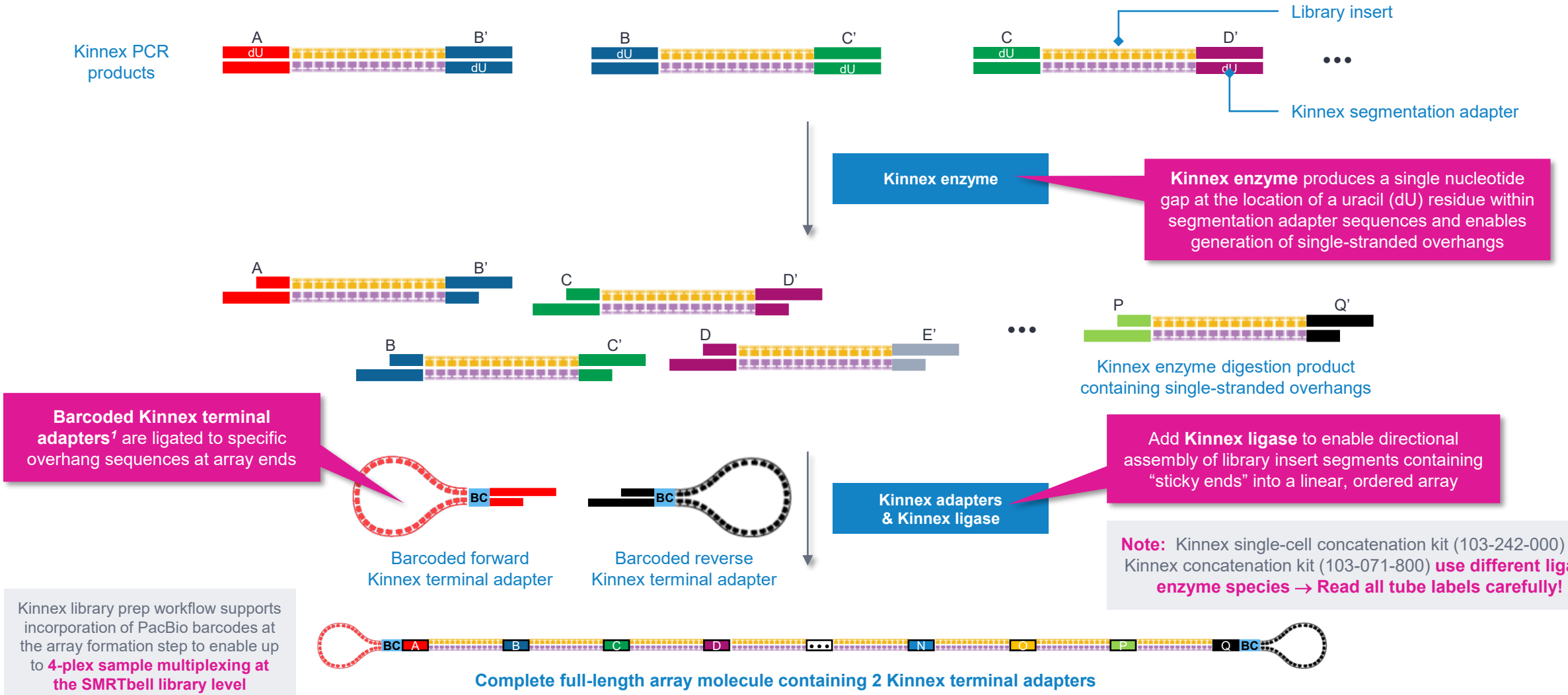
1.0 hr | 20 min

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

Kinnex PCR thermal cycler program

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

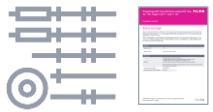


[1] Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-bcM0004) are available for Kinnex array formation step. **Note:** Kinnex concatenation workflow is not compatible with standard SMRTbell adapters from SMRTbell prep kit 3.0 and is also not compatible with SMRTbell barcoded adapter plate 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
30 min	1 hour	20 min	30 min	20 min	1 hour	20 min

~4.5 hrs → Final library

Kinnex single-cell

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

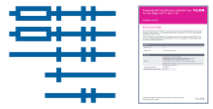
Kinnex single-cell RNA concatenation kit (103-242-000)

Kinnex digestion	Ligation	SMRTbell bead cleanup	DNA damage repair	SMRTbell bead cleanup	Nuclease treatment	SMRTbell bead cleanup
30 min	1 hour	20 min	30 min	20 min	1 hour	20 min

~4.5 hrs → 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
1 hour	30 min	20 min	15 min	20 min

~3 hrs → 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
1 hour	30 min	20 min	15 min	20 min

~3 hrs → Final library

Kinnex digestion + ligation performed as a single reaction instead of two separate sequential reactions and SMRTbell bead cleanup is not required afterwards

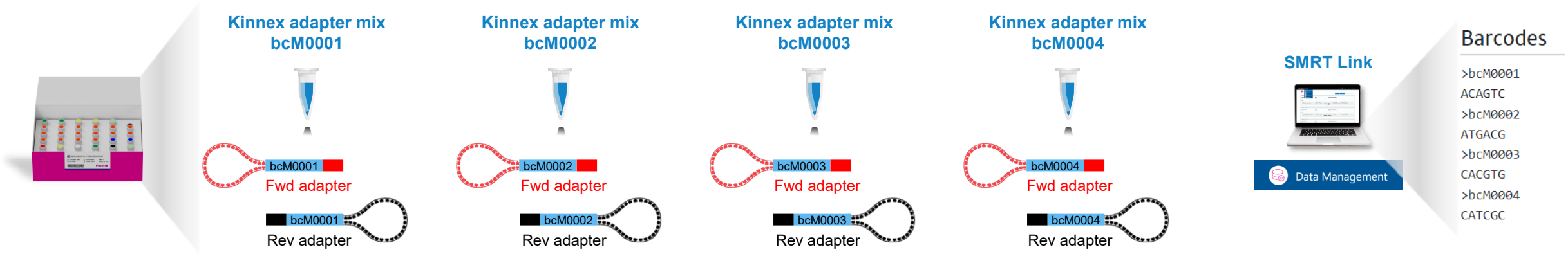
→ Kinnex full-length RNA & 16S rRNA protocols are more **streamlined**

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

Kinnex SMRTbell adapter design

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

- Kinnex adapters contain barcode sequences[1] to enable (optional) sample multiplexing at the SMRTbell library level (up to 4-plex)
  - Forward and reverse Kinnex adapter pairs are pre-mixed in Kinnex concatenation kits
  - Kinnex concatenation kits contain a total of 4 barcoded Kinnex adapter mixes (bcM0001-bcM0004) to enable multiplexing of up to 4 samples per SMRT Cell



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.

Kinnex SMRTbell adapter design (cont.)

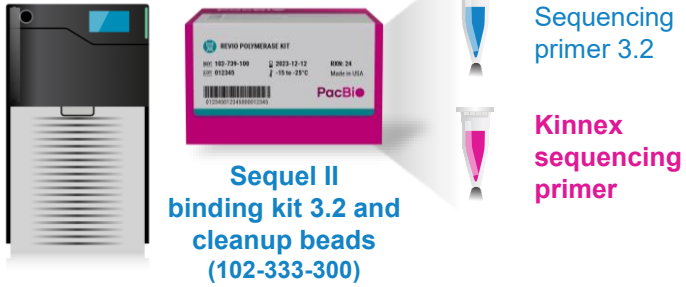
Kinnex terminal adapters use a new design that enables improved HiFi sequencing performance

- Kinnex adapters enable longer polymerase read length performance and improved sample loading efficiency
  - → Improved overall HiFi data yield

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

Annealed & bound Kinnex library molecule

- Kinnex adapter design requires a different sequencing primer (Kinnex sequencing primer[1]) for annealing reaction

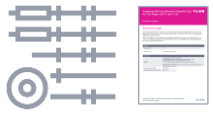


[1] If needed, Kinnex sequencing primer may be provided free-of-charge to any customers that are still using older Sequel II binding kits or older Revio polymerase kits that do not already contain the primer.

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

Kinnex digestion	Ligation	SMRTbell cleanup	DNA damage repair	SMRTbell cleanup	uclease ent	SMRTbell bead cleanup
IN: 10 µg of pooled MAS PCR products		**OUT: ≥5 µg of MAS array products**	**IN:** 5 µg of MAS array products			**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	SMRTbell bead cleanup	TSO removal	SMRTbell bead cleanup
IN: 15 ng, 60-75 ng or 75 ng of 10x 3’ or 5’ cDNA	**OUT: ~200 ng – 1 µg of amplified products**	**IN:** 150 ng – 1 µg of TSO PCR products	**OUT: ≥50 ng of TSO artifact-depleted cDNA**

Kinnex PCR

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

Kinnex array formation

Kinnex digestion	Ligation	SMRTbell cleanup	DNA damage repair	SMRTbell cleanup	Nuclease treatment	SMRTbell bead cleanup
IN: 5 µg of pooled Kinnex PCR products				**OUT: 80% recovery from Kinnex digestion Rx input**	**IN:** 40 µL of DDR reaction products	**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	SMRTbell bead cleanup	cDNA amplification	SMRTbell bead cleanup
IN: 300 ng of total RNA per sample			**OUT: ≥100 ng of amplified cDNA per sample**

Kinnex PCR

Kinnex PCR	SMRTbell bead cleanup
IN: 55 ng of amplified cDNA (single or multiplex)	**OUT: 6-12 µg of amplified products after pooling**

Kinnex array formation

Kinnex digestion / Ligation	DNA damage repair	SMRTbell cleanup	Nuclease treatment	SMRTbell bead cleanup
IN: 5 µg of pooled Kinnex PCR products		**OUT: 80% recovery from Kinnex digestion Rx input**	**IN:** 40 µL of DDR reaction products	**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	SMRTbell bead cleanup	Pool barcoded 16S PCR amplicons	SMRTbell bead cleanup
IN: 1-2 ng of bacterial gDNA per sample	**OUT: 100-300 ng of PCR products per sample**		

Kinnex PCR

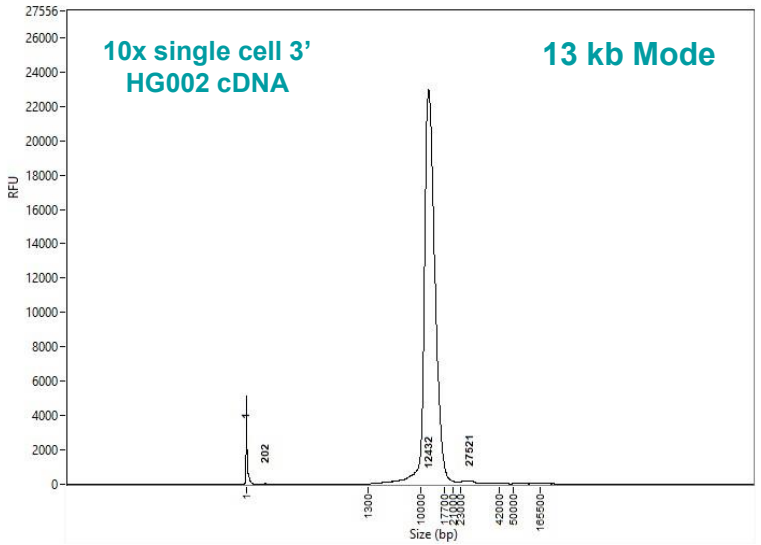
Kinnex PCR	SMRTbell bead cleanup
IN: 35 ng of pooled 16S amplicons	**OUT: 5-9 µg of amplified products after pooling**

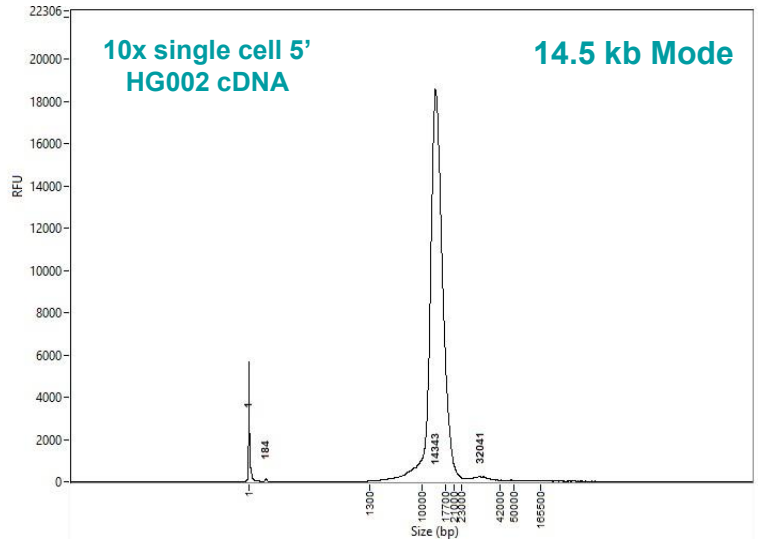
Kinnex array formation

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

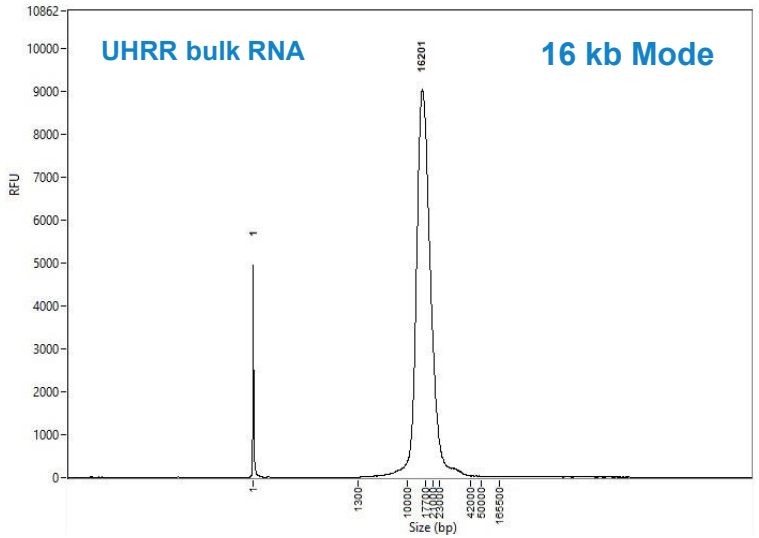
Example Femto Pulse DNA sizing QC results for final Kinnex SMRTbell libraries

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

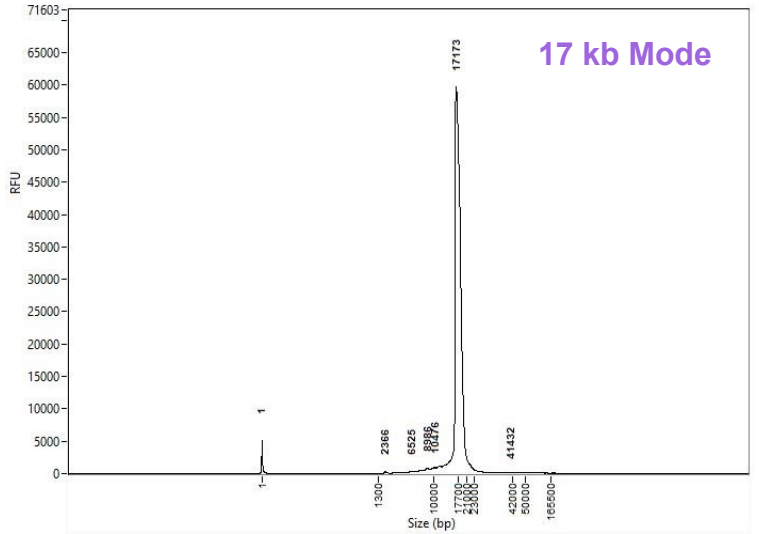




Kinnex full-length RNA
(8-fold target concatenation)



Kinnex 16S rRNA
(12-fold target concatenation)



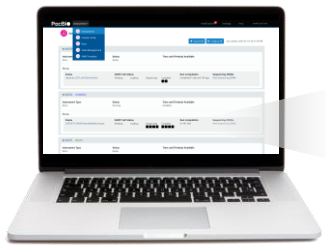
Kinnex library type	Kinnex library size range
Kinnex single-cell RNA	~10 – 17 kb
Kinnex full-length RNA	~12 – 16 kb
Kinnex 16S rRNA	~17 – 18 kb

Kinnex sequencing preparation workflow overview

Sample Setup & Run Design recommendations for Kinnex libraries

Follow SMRT Link run design instructions to prepare Kinnex libraries for sequencing

Select **desired Kinnex application type** in SMRT Link run design page



Runs

After specifying your application type, **SMRT Link auto-fills** selected 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

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

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

SMRT Link module	Key setup parameters	Sequel IIe system recommended settings	Vega system recommended settings	Revio system recommended settings
Sample setup	Library type	Kinnex	Kinnex	Kinnex
	Primer	Kinnex sequencing primer	Kinnex sequencing primer	Kinnex sequencing primer
	Binding/Polymerase kit	Sequel II binding kit 3.2	Vega polymerase kit	Revio SPRQ polymerase kit
	Concentration on plate	40 – 60 pM	130 – 160 pM	130 – 160 pM
Runs → Run design	Use Adaptive Loading	YES	N/A	YES
	Library Type[1]	Kinnex	Kinnex	Kinnex
	Movie Acquisition Time	30 hrs	24 hrs	24 hrs
	Add Analysis	YES	YES	YES

[1] **Note:** SMRT Link Run design **Library Type** field determines which adapter finding algorithm is used during post-primary analysis. 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 and Vega systems

Sample and run information

Kinnex single-cell RNA

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

Application (Required)	Kinnex single-cell RNA
Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	15000
Library Concentration (pM) (Required)	
Movie Acquisition Time (hours)	24

Kinnex full-length RNA

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

Application (Required)	Kinnex full-length RNA
Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	16000
Library Concentration (pM) (Required)	
Movie Acquisition Time (hours)	24

Kinnex 16S rRNA

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

Application (Required)	Kinnex 16S rRNA
Plate Well (Required)	Plate 1, Well A01
Well Name (Required)	
Well Comment	
Library Type (Required)	Kinnex
Insert Size (bp) (Required)	17000
Library Concentration (pM) (Required)	
Movie Acquisition Time (hours)	24

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

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

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 and Vega systems (cont.)

Sample indexing (barcoding) information

Kinnex single-cell RNA

Samples

Default **Indexes** FASTA = **MAS adapter indexes**

Sample is indexed YES NO

Indexes Required: MAS adapter indexes

Biosample names Required: Interactively | From a File

Kinnex full-length RNA

Samples

Sample is indexed YES NO

Indexes Required: MAS adapter indexes

Biosample names Required: Interactively | From a File

Kinnex 16S rRNA

Samples

Sample is indexed YES NO

Indexes Required: MAS adapter indexes

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

Filter...

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

Included Barcodes

Filter...

Barcode	Bio Sample

Barcode Selector and Sample Name Editor

Available Barcodes

Filter...

Barcode
bcM0003--bcM0003
bcM0004--bcM0004

Included Barcodes

Filter...

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
```

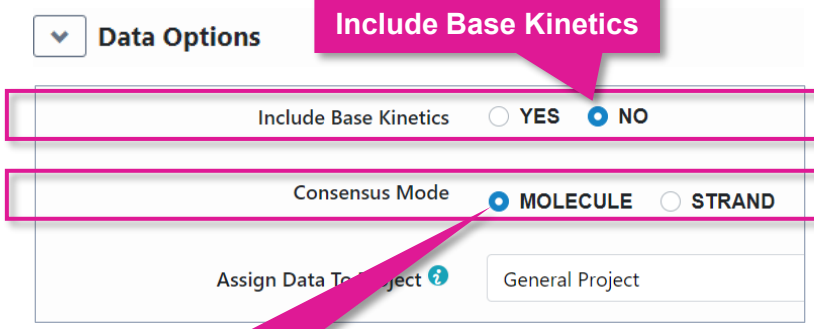
[1] Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-bcM0004) are available for Kinnex array formation step. Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

SMRT Link Run Design procedure for Revio and Vega systems (cont.)

Data options

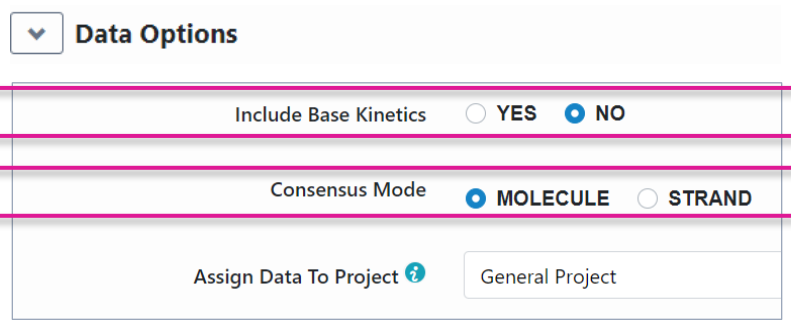
Kinnex single-cell RNA

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

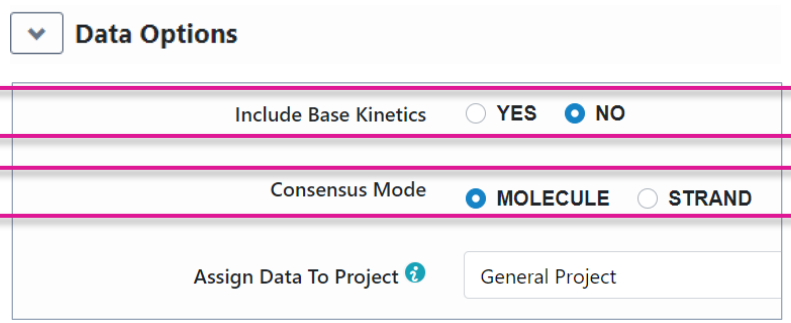


Default **Consensus Mode = MOLECULE**[1]

Kinnex full-length RNA



Kinnex 16S rRNA



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 analysis options

Kinnex single-cell RNA

Analysis Options

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

Analysis Workflow is automatically filled in

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

Advanced Parameters

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

Advanced Parameters

Kinnex 16S rRNA

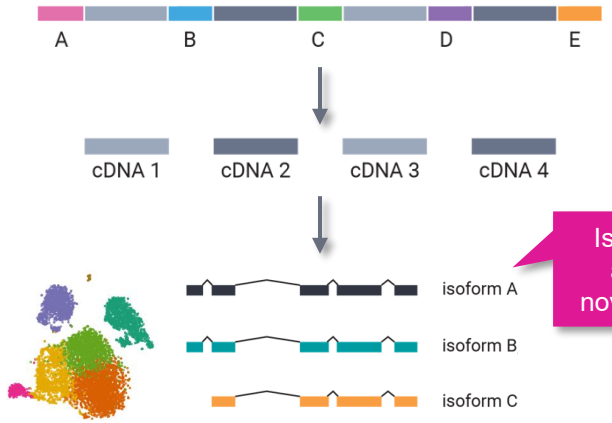
Analysis Options

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)

Advanced Parameters

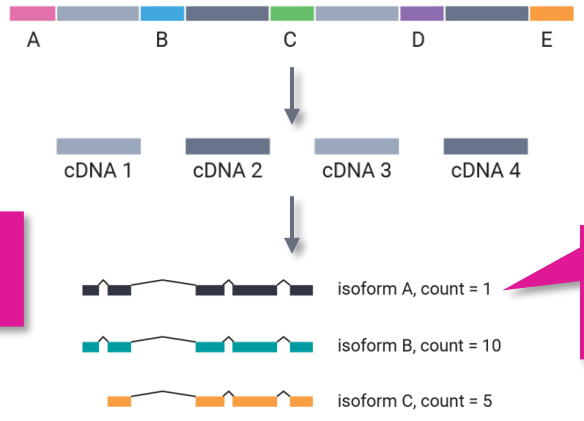
Split arrayed HiFi reads at adapter positions, generating segmented reads (S-reads)

Read Segmentation and Single-Cell Iso-Seq



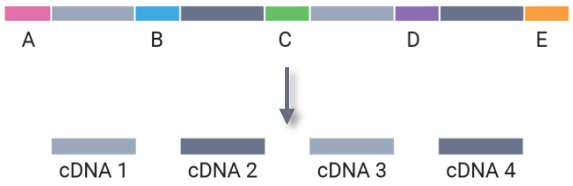
Isoform-classification analysis to identify novel genes & isoforms

Read Segmentation and Iso-Seq



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

Read Segmentation



SMRT Link Run Design analysis options (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

Advanced Parameters

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

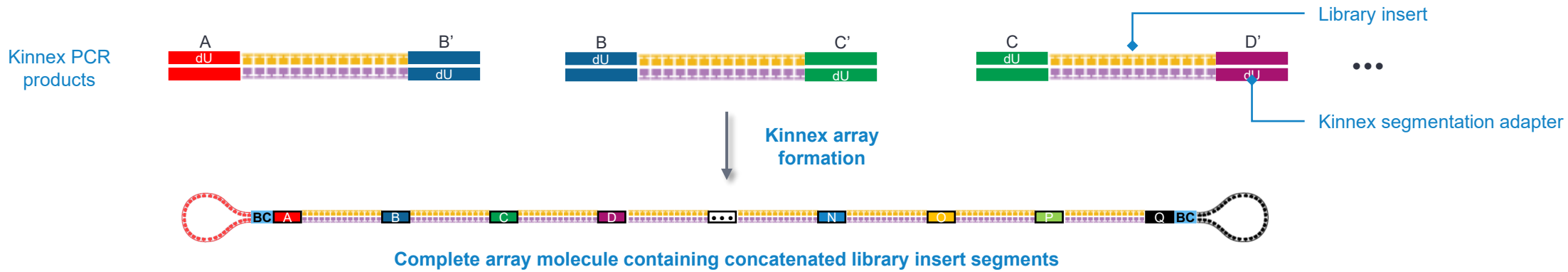
Advanced Parameters

Kinnex 16S rRNA

Analysis Options

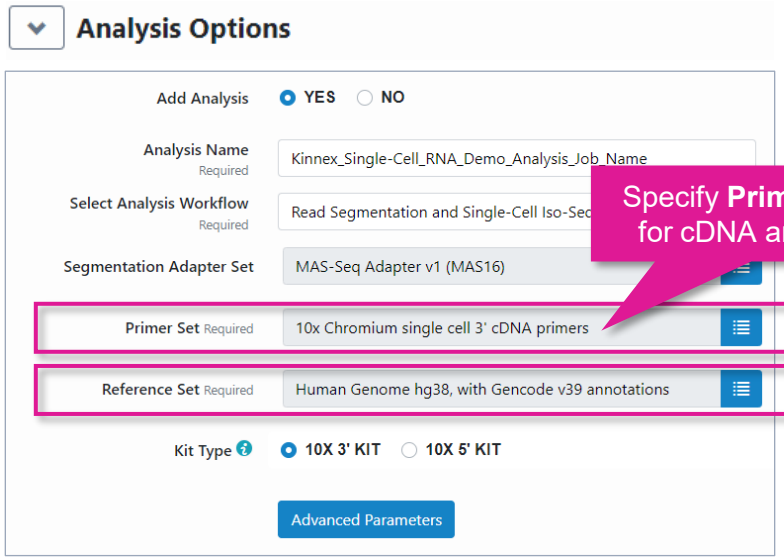
Add Analysis	YES / NO
Analysis Name Required	Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name
Select Analysis Workflow Required	Read Segmentation
Segmentation Adapter Set	MAS-Seq Adapter v2 (MAS12)

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



SMRT Link Run Design analysis options (cont.)

Kinnex single-cell RNA



Specify **Primer Set** used for cDNA amplification

10x Forward (F) PCR primer

10x Reverse (R) PCR primer

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 Chromium single cell 3' cDNA primers'

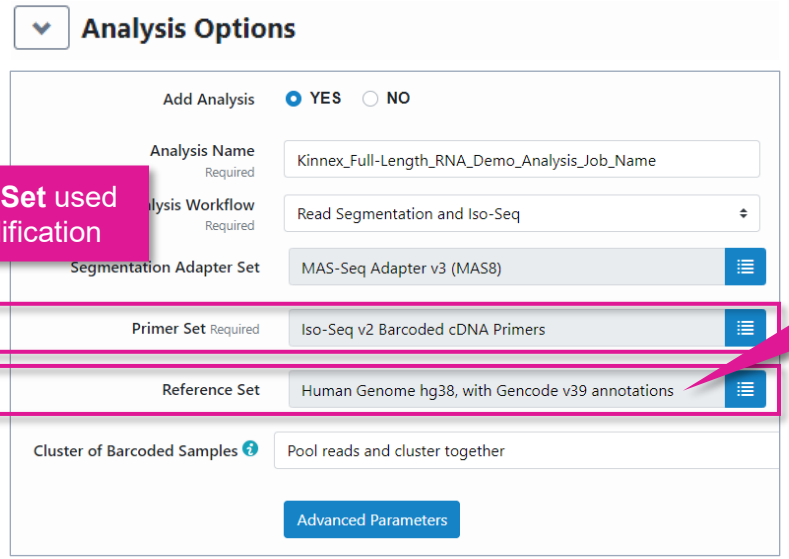
OR

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 Chromium single cell 5' cDNA primers'

Kinnex full-length RNA



Forward (F) PCR primer (Barcoded Iso-Seq primer bcXX[2])

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

5' BC TSO GGG LIBRARY INSERT AAAAAAAAAA 3'

3' BC TSO CCC LIBRARY INSERT TTTTTTTTTT 5'

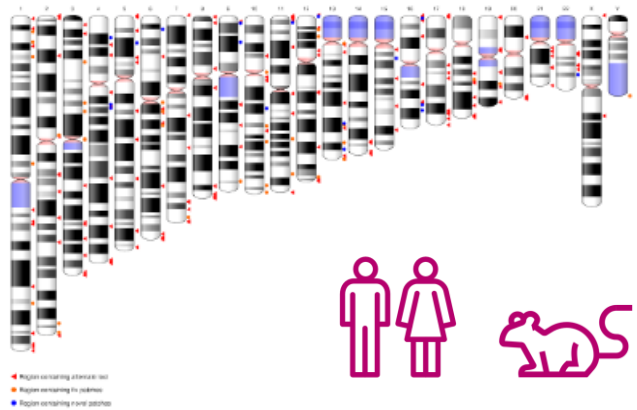
For Kinnex full-length RNA analysis, select 'Iso-Seq v2 Barcoded cDNA primers'

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

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

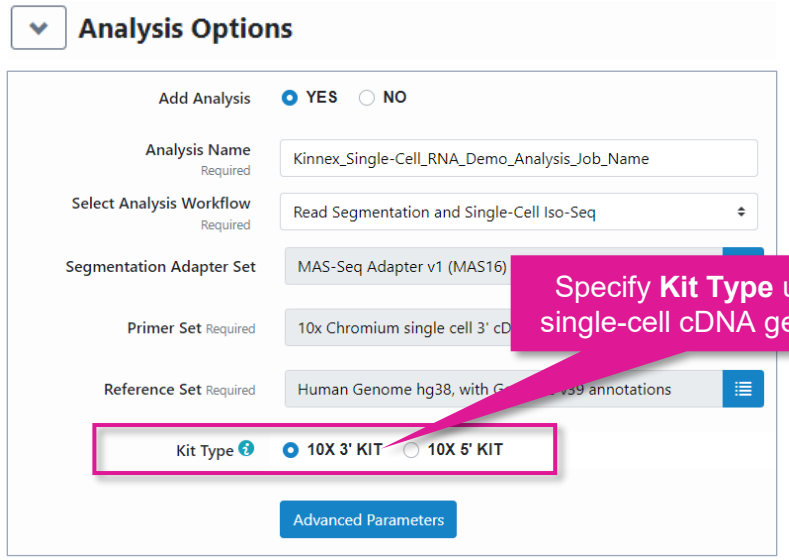
- Human Genome hg38, with Gencode v39 annotations
- Mouse Genome mm39, with Gencode vM28 annotations

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



SMRT Link Run Design analysis options (cont.)

Kinnex single-cell RNA



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

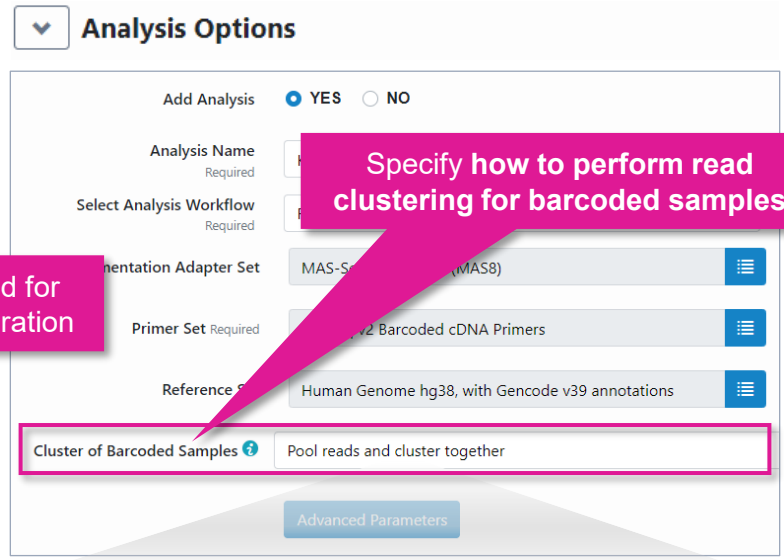
Specification of Kit Type (10x 3' Kit or 10x 5' Kit) **determines which set of 10x barcode sequences to use**, and also affects **UMI and single-cell barcode design settings**[1]

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

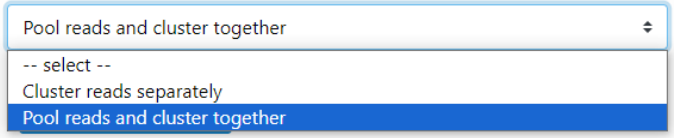
OR

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

Kinnex full-length RNA

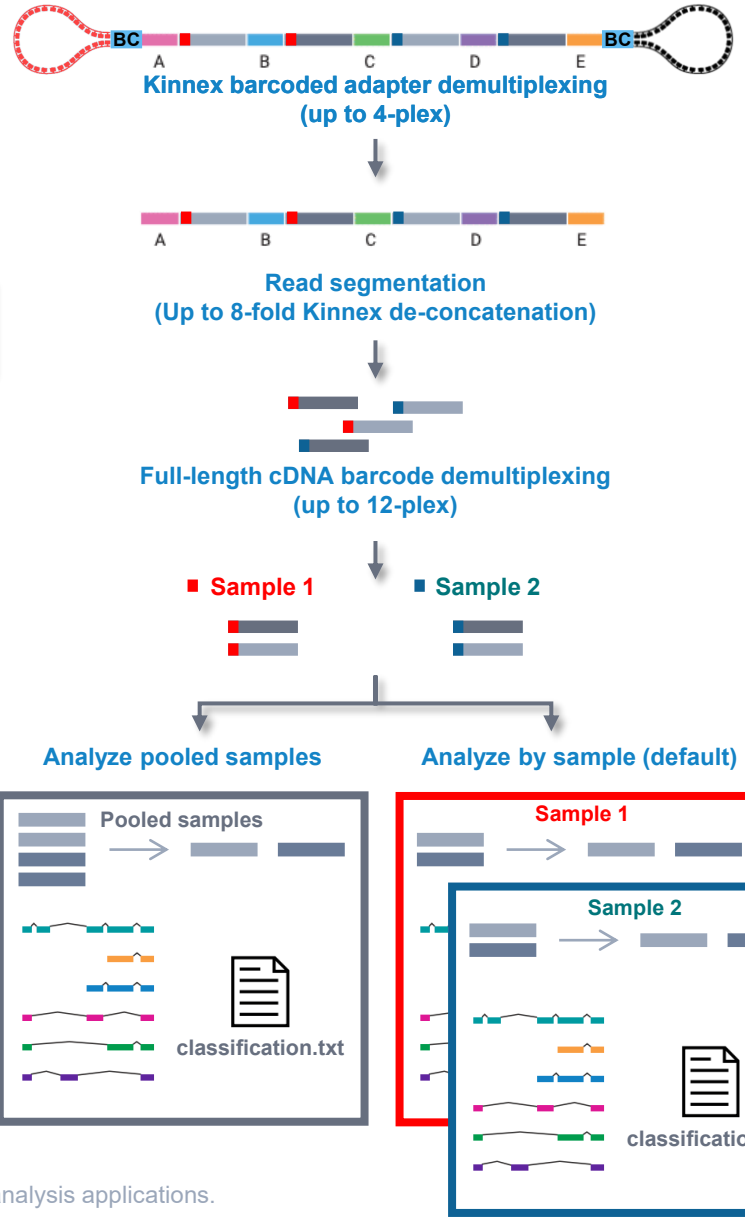


Specify **how to perform read clustering for barcoded samples**



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



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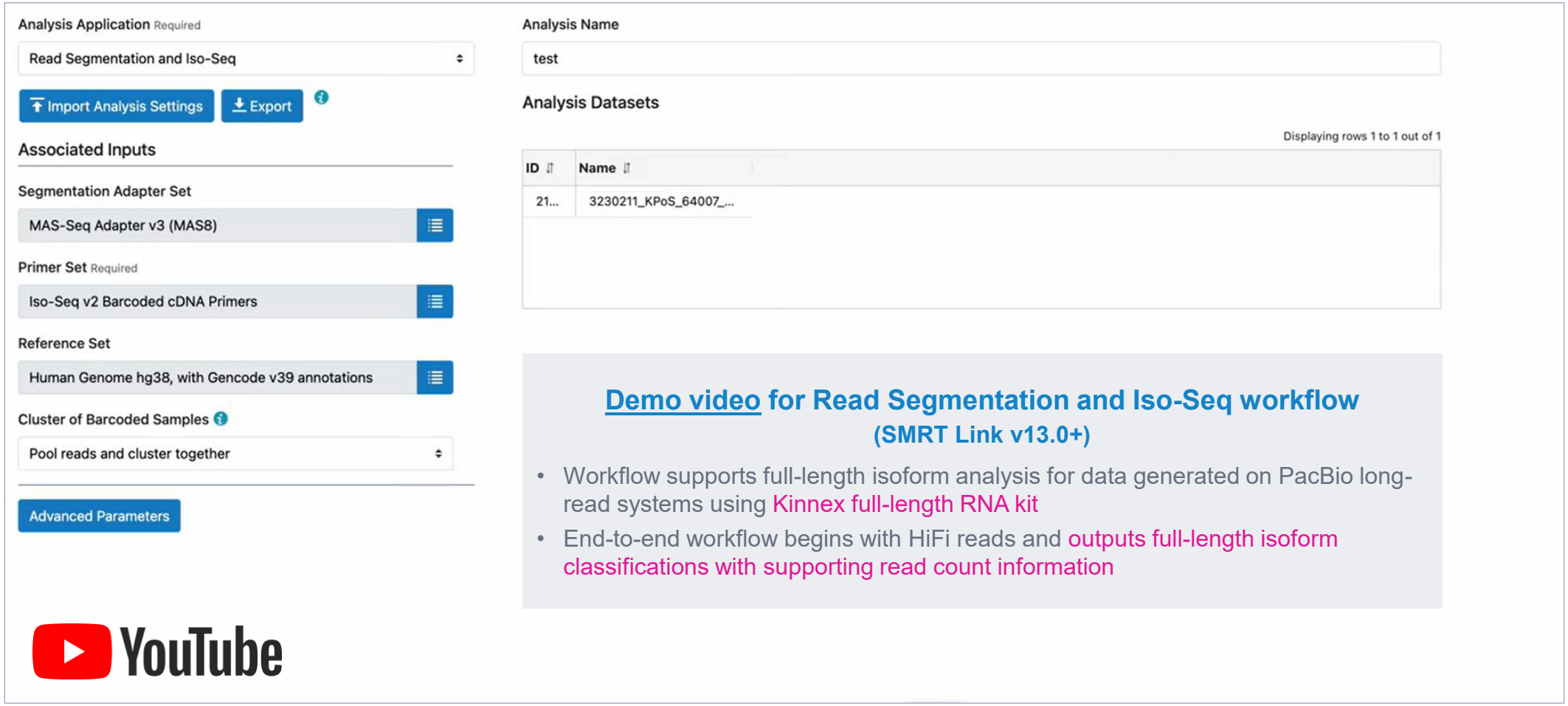
[1] See ***SMRT Link User Guide*** documentation for detailed descriptions of advanced parameters for Iso-Seq / Single-Cell Iso-Seq analysis applications.

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

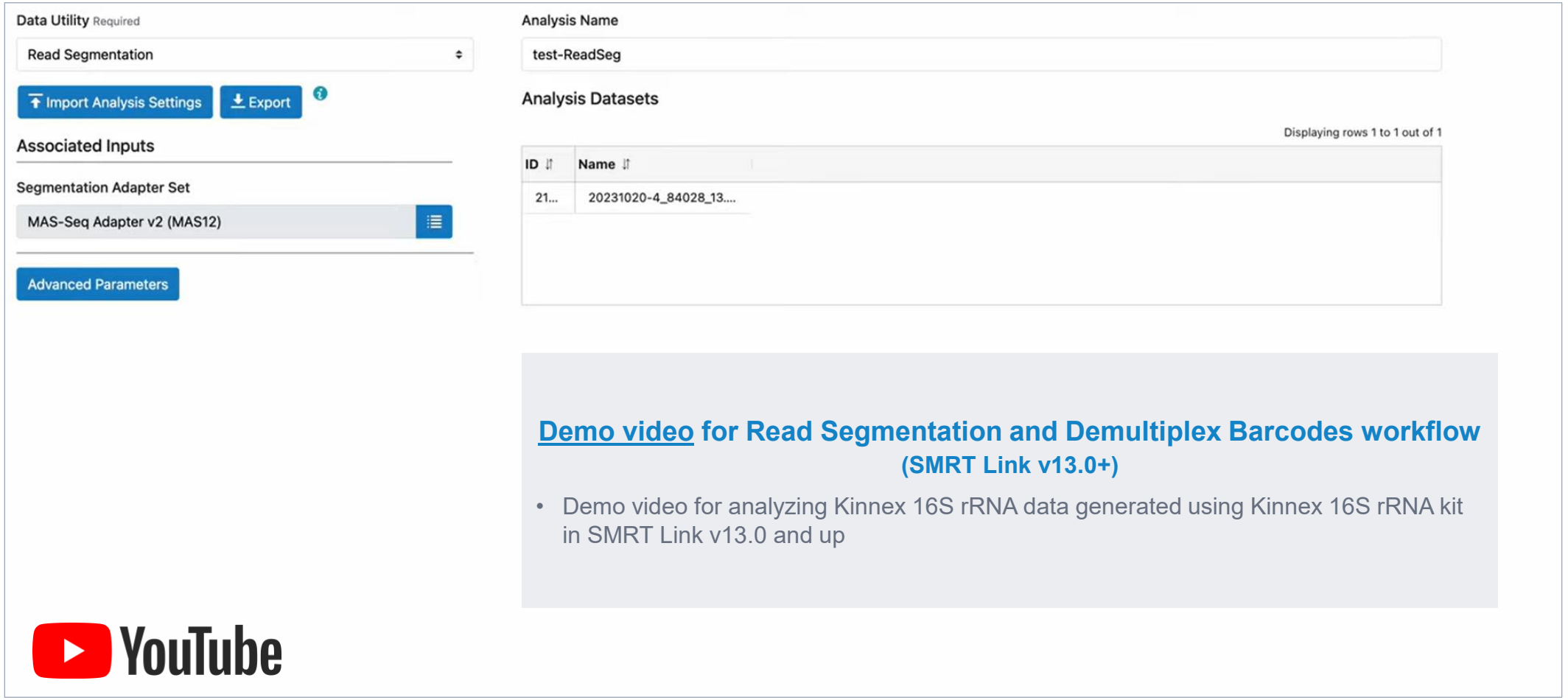


Demo video for Read Segmentation and Iso-Seq workflow
(SMRT Link v13.0+)

- Workflow supports full-length isoform analysis for data generated on PacBio long-read 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

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



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 long-read systems.

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples
Number of SMRT® Cells per Kinnex library preparation	>8 SMRT Cells for Revio® using SPRQ™ chemistry >2 SMRT Cells for the Vega® or Revio® (non-SPRQ) systems >4 SMRT Cells for Sequel® II/IIe®

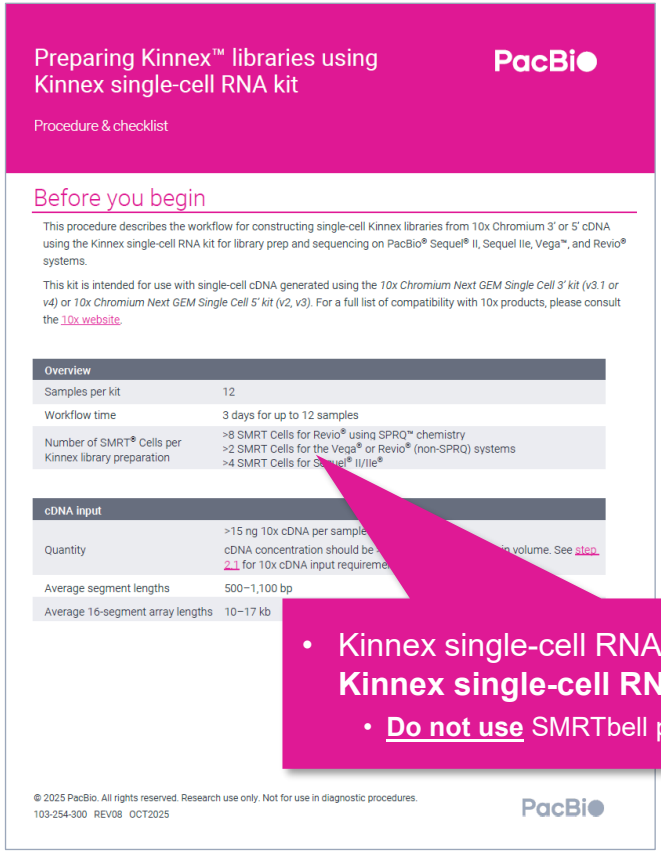
cDNA input	
Quantity	>15 ng 10x cDNA per sample cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,100 bp
Average 16-segment array lengths	10–17 kb

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

Concatenation factor

16-fold

Kinnex library template (~10 – 17 kb) containing 16 concatenated single-cell cDNA segments


Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

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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 for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, 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 v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples
Number of SMRT® Cells per Kinnex library preparation	>8 SMRT Cells for Revio® using SPRQ™ chemistry >2 SMRT Cells for the Vega® or Revio® (non-SPRQ) systems >4 SMRT Cells for Sequel® II/IIe®

cDNA input	
Quantity	>15 ng 10x cDNA per sample cDNA concentration should be … volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,100 bp
Average 16-segment array lengths	10–17 kb

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103-254-300 REV08 OCT2025

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- 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 third-party single-cell platforms & 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	**40-60 pM (Sequel II/IIe system)** **130-160 pM (Vega/Revio system)**	85 pM (Sequel II/IIe system) 225 pM (Revio system)
Run time	Sequel II/Iie system: 30hr movie with adaptive loading Revio/Vega system: 24hr movie with adaptive loading	
S-read yield	~30-40 M reads (Sequel II/IIe system) 50-60 M reads (Vega system) ~100-120 M reads (Revio system)	

Kinnex single-cell RNA experimental design considerations

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

	Sequel II and IIe systems	Vega system	Revio system + SPRQ chemistry
Experimental goal	Characterize alternative splicing in single cells / cell types		
Transcript reads per SMRT Cell	30-40 M reads	50-60 M reads	100-120 M reads
Sample multiplexing[1]	Not recommended	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 – 10,000 cells for running a single (non-multiplexed) sample on one Vega SMRT Cell	<10,000 cells per sample if multiplexing 2 samples per Revio SMRT Cell (2-plex) 10,000 – 20,000 cells per sample if running a single (non-multiplexed) sample on 1–2 Revio SMRT Cells
Expected coverage	Obtain 3,000 – 10,000 unique reads/single cell	Obtain 3,000 – 10,000 unique reads/single cell	Obtain up to ~20,000 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 cDNA from **(1)** 10x Chromium Single Cell Universal 3' or 5' kit; Single Cell Next GEM (v3.1 3' or v2 5'); **(2)** 10x Visium HD Spatial Gene Expression kit; **(3)** Parse Evercode WT or WT mini kit; or **(4)** ArgenTag Single-Cell RNA Library Kit		
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

5' [CBC][UMI]TTTTTTTTTT 3'
3' AAAAAAAAAA 5'

Poly(A)+ mRNA template

Contains Kinnex PCR handle 1

10x GENOMICS

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

5' [CBC][UMI]TTTTTTTTTT CCC 3'
3' AAAAAAAAAA GGG TSO 5'

Template Switch Oligo (TSO)

10x GENOMICS

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

5' [CBC][UMI]TTTTTTTTTT CCC 3'
3' AAAAAAAAAA GGG TSO 5'

10x Forward PCR Primer

10x GENOMICS

2nd-strand synthesis & cDNA amplification

5' [CBC][UMI]TTTTTTTTTT CCC TSO 3'
3' GGG TSO 5'

TSO
10x Reverse PCR Primer

10x GENOMICS

Cleanup

Contains Kinnex PCR handle 2

5' [CBC][UMI]TTTTTTTTTT CCC TSO 3'
3' [CBC][UMI]AAAAAAAAAA GGG TSO 5'

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

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

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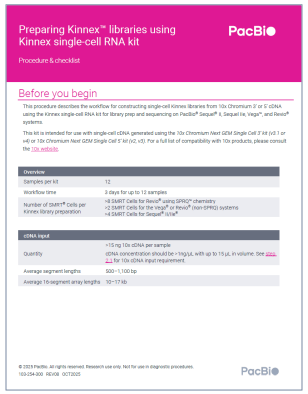

Procedure & checklist (103-254-300)

Kinnex library prep, sequencing & analysis

F R TSO

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)

PCR 1 | PCR 2 | PCR 3… | …PCR (*n*)

A B' | B C' | C D' | … …

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

Pooling & Kinnex array formation

BC A B C … Q BC
BC A B C D
O P Q BC

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

DNA damage repair & nuclease treatment

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

Purified polymerase-bound 16-segment Kinnex library

Long-read sequencing & data analysis

Sequel II/IIe, Vega 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

I. 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

II. 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

III. 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

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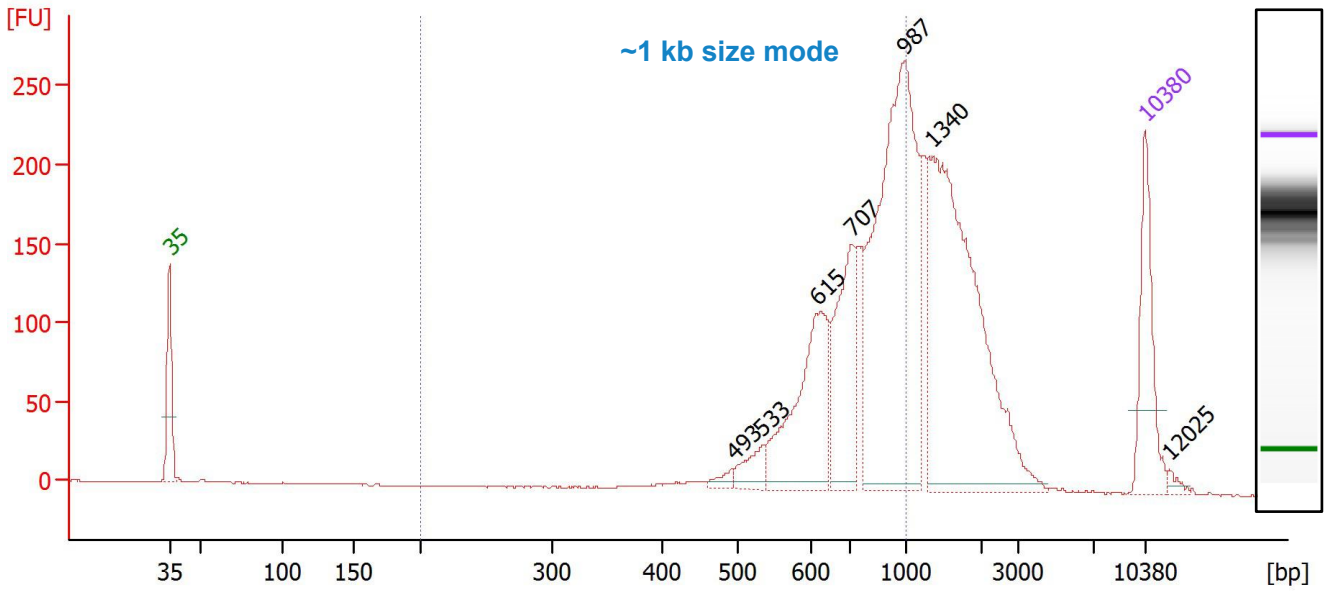
Example Kinnex single-cell RNA library preparation QC results

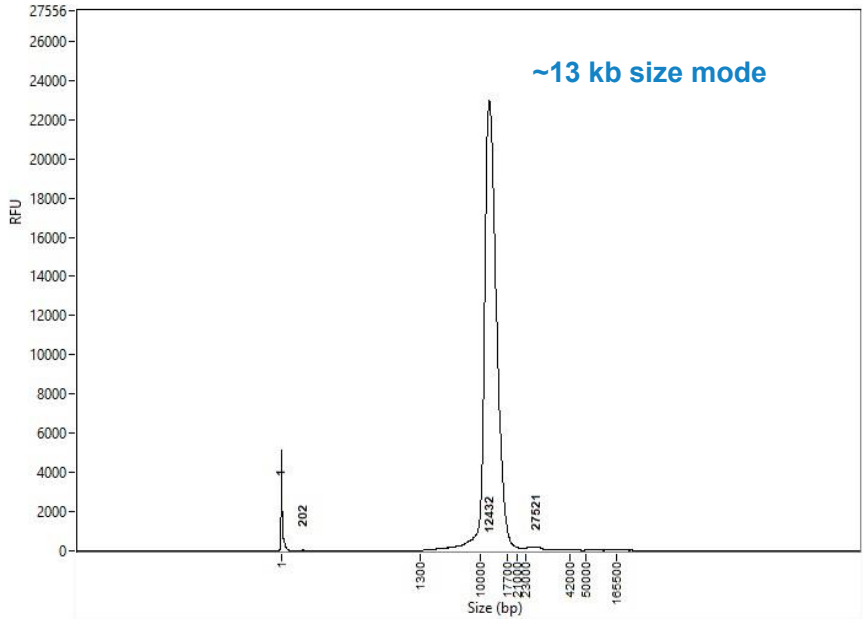
Kinnex single-cell 3’ RNA library prepared with human cDNA

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 the following number of SMRT Cells:

- >8 SMRT Cells for Revio using SPRQ chemistry
- >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems
- >4 SMRT Cells for Sequel II/IIe systems

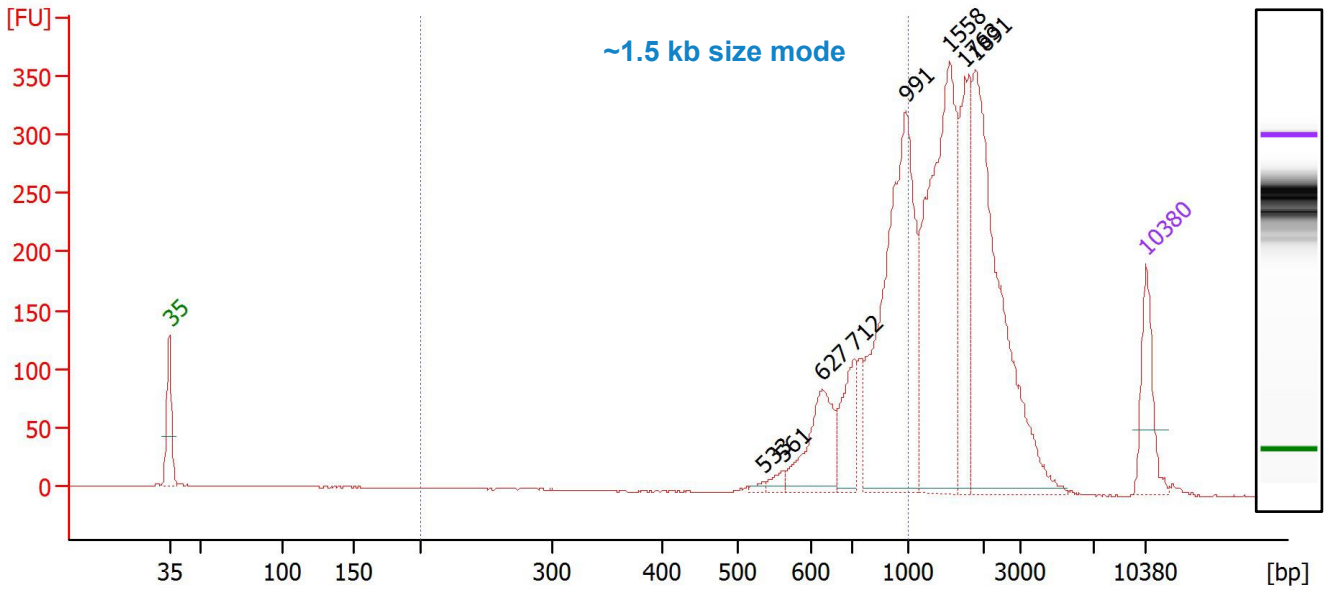
10x single cell 3’ cDNA input	15 ng
cDNA input for Kinnex array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1100 ng (22.0%)

[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.)

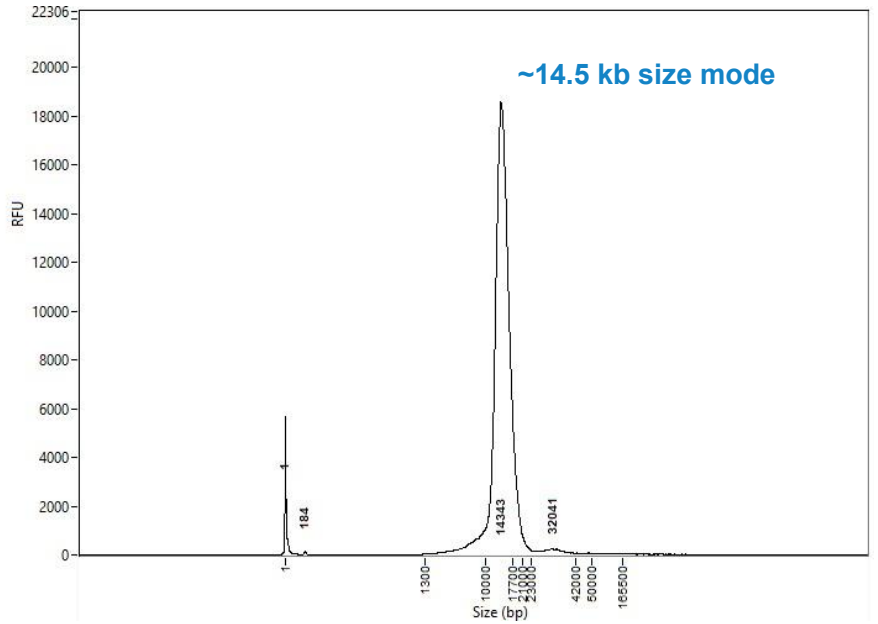
Kinnex single-cell 5’ RNA library prepared with human cDNA

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 the following number of SMRT Cells:

- >8 SMRT Cells for Revio using SPRQ chemistry
- >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems
- >4 SMRT Cells for Sequel II/IIe systems

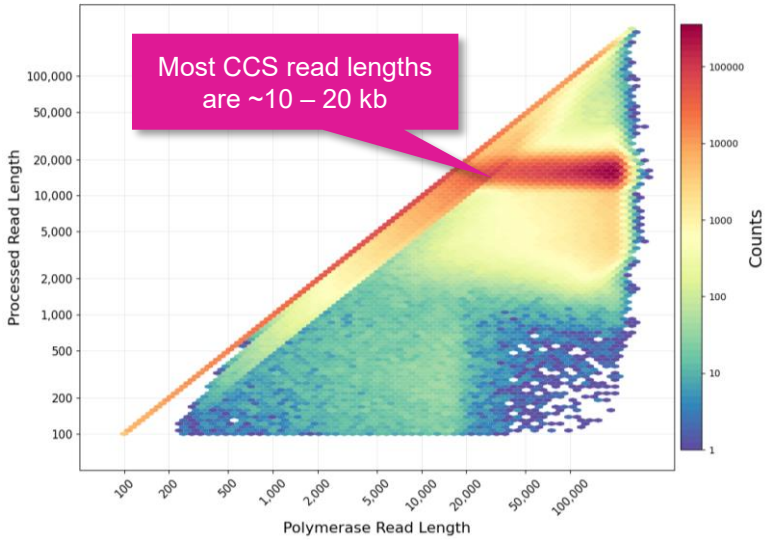
10x single cell 5’ cDNA input	15 ng
cDNA input for Kinnex array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1008 ng (20.2%)

[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 + SPRQ chemistry[1]]

Kinnex single-cell RNA 5’ library for PBMC single-cell cDNA sample from 10x Chromium Single Cell Universal 5’ kit

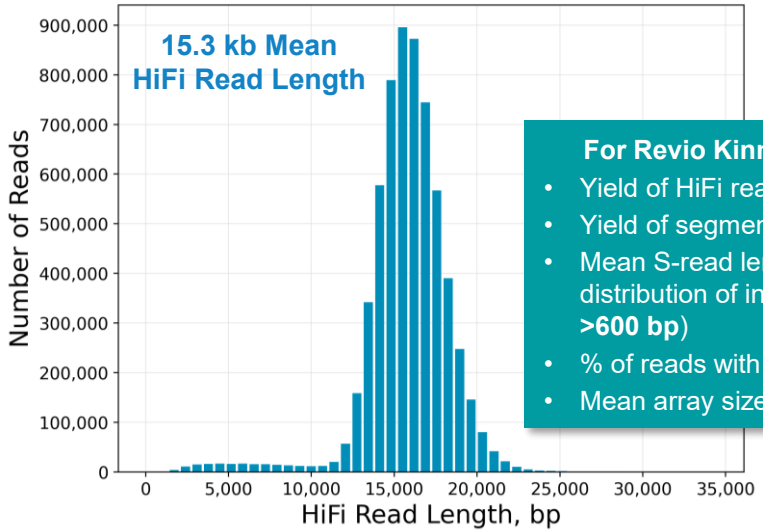
Raw Data Report



Raw Base Yield	1,539 Gb
Mean Polymerase Read Length	90.2 kb
P0	32%
P1	68%
P2	0%

Example sequencing metrics for a human Kinnex single-cell RNA 5’ library sample run on a Revio system with Revio SPRQ polymerase kit / 140 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

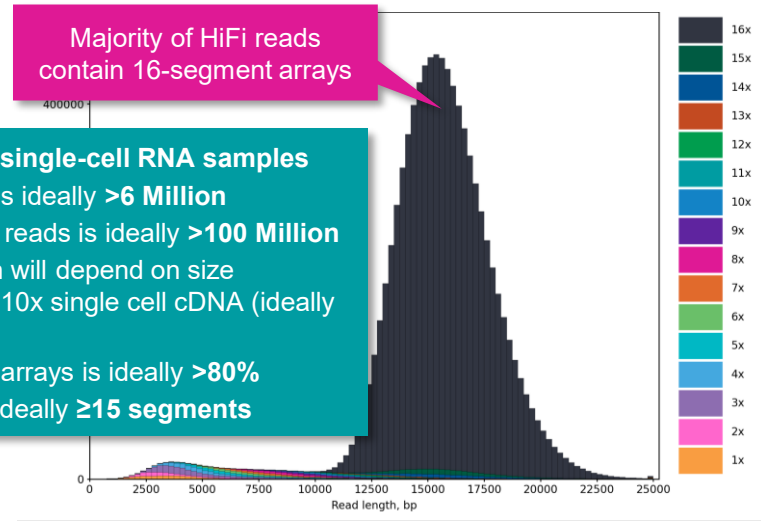
HiFi Read Length



HiFi Reads	9.4 M
HiFi Base Yield	144.4 Gb
Mean HiFi Read Length	15.3 kb
Median HiFi Read Quality	Q29
HiFi Read Mean # of Passes	9

For human Kinnex single-cell RNA libraries, per-Revio SMRT Cell HiFi read counts were typically >6 Million depending on the final library insert size and *P1* loading performance.

Read Segmentation Metrics



For Revio Kinnex single-cell RNA samples

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>100 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	9,408,444
Segmented reads (S-reads)	144,801,149
Mean length of S-reads	971 bp
Percent of reads with full arrays	92.59%
Mean array size (concentration factor)	15.39

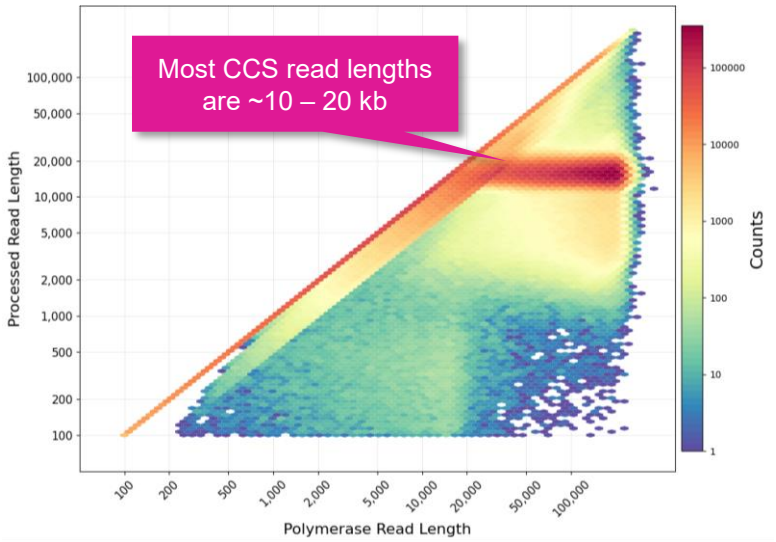
For Kinnex single-cell RNA libraries, per-Revio SMRT Cell segmentation read counts were typically >100 Million.

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

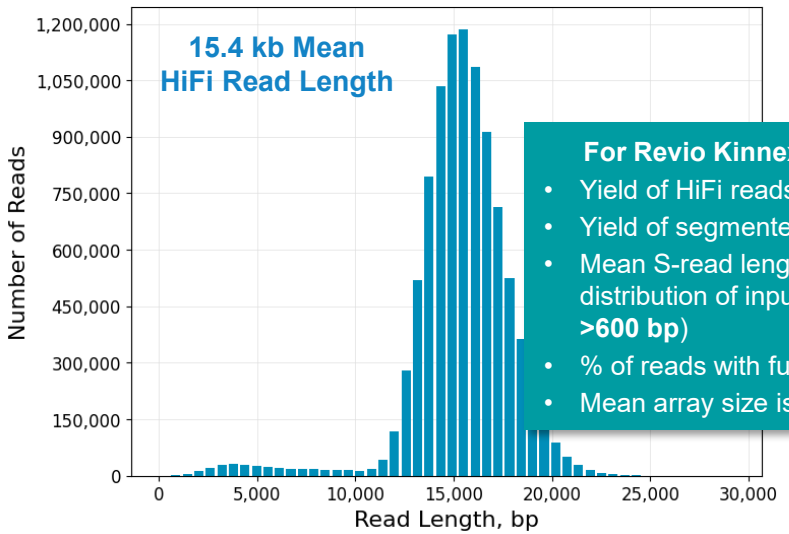
Example sequencing performance for Kinnex single-cell RNA libraries prepared with human cDNA [Revio system + SPRQ chemistry[1]] (cont.)

Kinnex single-cell RNA 3' library for PBMC single-cell cDNA sample from 10x Chromium Single Cell Universal 3' kit

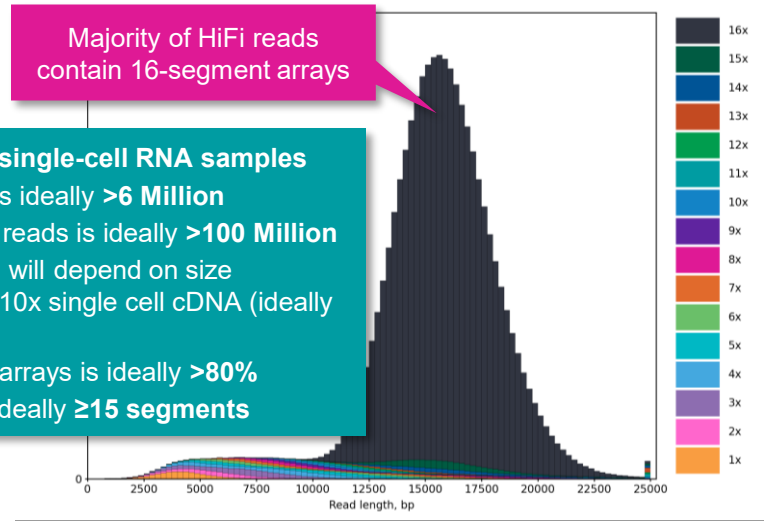
Raw Data Report



HiFi Read Length



Read Segmentation Metrics



For Revio Kinnex single-cell RNA samples

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>100 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	1,570 Gb
Mean Polymerase Read Length	86.5 kb
P0	27%
P1	72%
P2	0%

Example sequencing metrics for a human Kinnex single-cell RNA 3' library sample run on a Revio system with Revio SPRQ polymerase kit / 140 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

HiFi Reads	9.6 M
HiFi Base Yield	148.1 Gb
Mean HiFi Read Length	15.4 kb
Median HiFi Read Quality	Q29
HiFi Read Mean # of Passes	8

For human Kinnex single-cell RNA libraries, per-Revio SMRT Cell HiFi read counts were typically >6 Million depending on the final library insert size and *P1* loading performance.

Input HiFi Reads	9,605,565
Segmented reads (S-reads)	149,894,918
Mean length of S-reads	963 bp
Percent of reads with full arrays	92.24%
Mean array size (concentration factor)	15.61

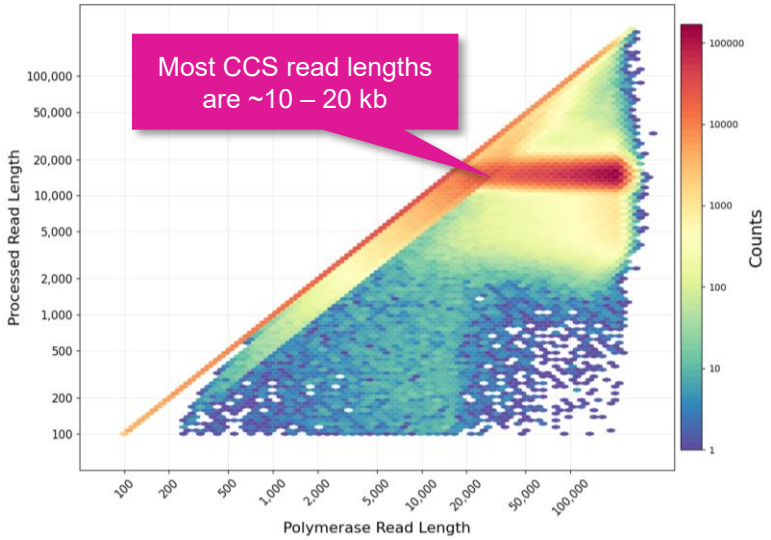
For Kinnex single-cell RNA libraries, per-Revio SMRT Cell segmentation read counts were typically >100 Million.

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

Example sequencing performance for Kinnex single-cell RNA libraries prepared with human cDNA [Vega system[1]]

Kinnex single-cell RNA 3' library for PBMC single-cell cDNA sample from 10x Chromium Single Cell Universal 3' kit

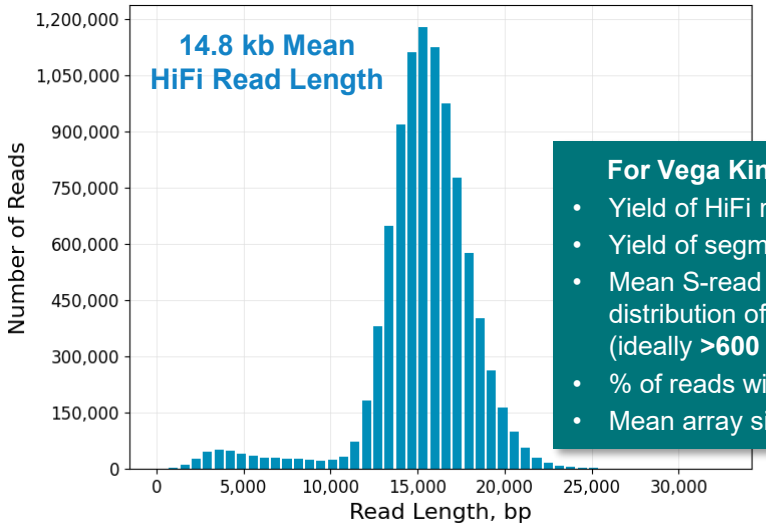
Raw Data Report



Mean Polymerase Read Length	94.1 kb
Loading Level	71%

Example sequencing metrics for a human Kinnex single-cell RNA 3' library sample run on a Vega system with Vega polymerase kit / 150 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

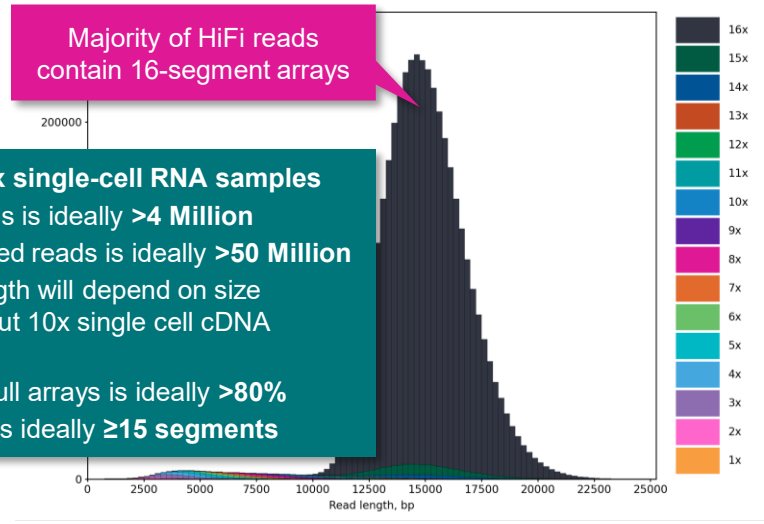
HiFi Read Length



HiFi Reads	4.4 M
HiFi Base Yield	64.9 Gb
Mean HiFi Read Length	14.8 kb
Median HiFi Read Quality	Q29
HiFi Read Mean # of Passes	9

For human Kinnex single-cell RNA libraries, per-Vega SMRT Cell HiFi read counts were typically >4 Million depending on the final library insert size and sample loading performance.

Read Segmentation Metrics



For Vega Kinnex single-cell RNA samples

- Yield of HiFi reads is ideally **>4 Million**
- Yield of segmented reads is ideally **>50 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	4,372,029
Segmented reads (S-reads)	68,570,669
Mean length of S-reads	924 bp
Percent of reads with full arrays	93.78%
Mean array size (concentration factor)	15.68

For Kinnex single-cell RNA libraries, per-Vega SMRT Cell segmentation read counts were typically >50 Million.

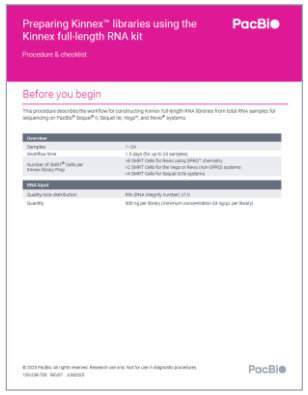
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

PacBio

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 long-read systems



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	1.5 days (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	>8 SMRT Cells for Revio using SPRQ™ chemistry >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems >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)

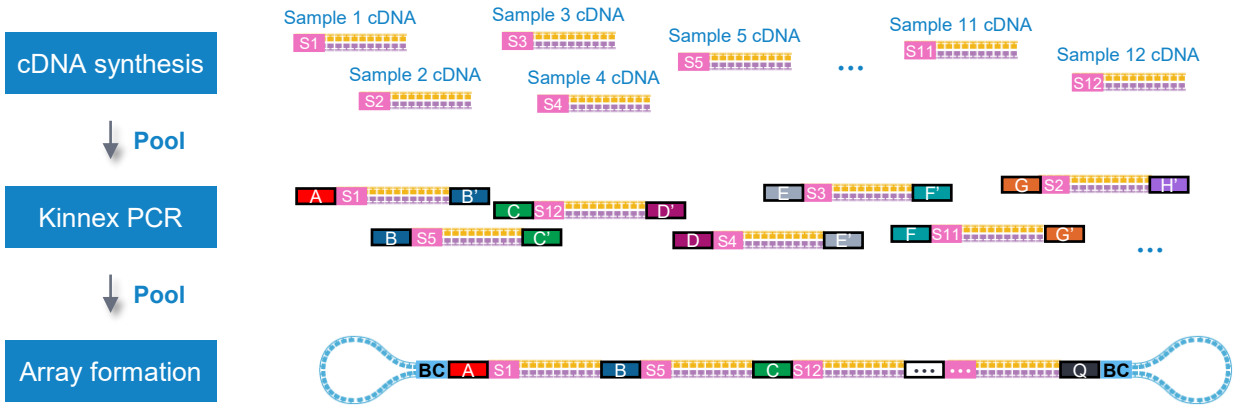
- Kinnex full-length RNA library prep protocol uses **Kinnex full-length RNA kit** and **Iso-Seq express 2.0 kit[1]**
  - **Do not use** SMRTbell prep kit 3.0 with this protocol

Kinnex full-length RNA library barcoding options

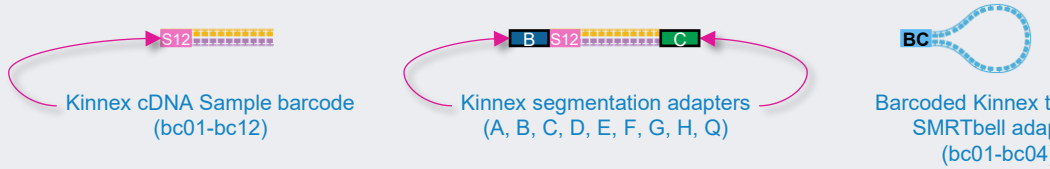
Kinnex full-length RNA library preparation procedure supports **up to 48-plex** sample multiplexing through combined use of:

→ **12 different barcoded cDNA amplification PCR primers**

→ **4 different barcoded Kinnex terminal SMRTbell adapters**



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



[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	
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

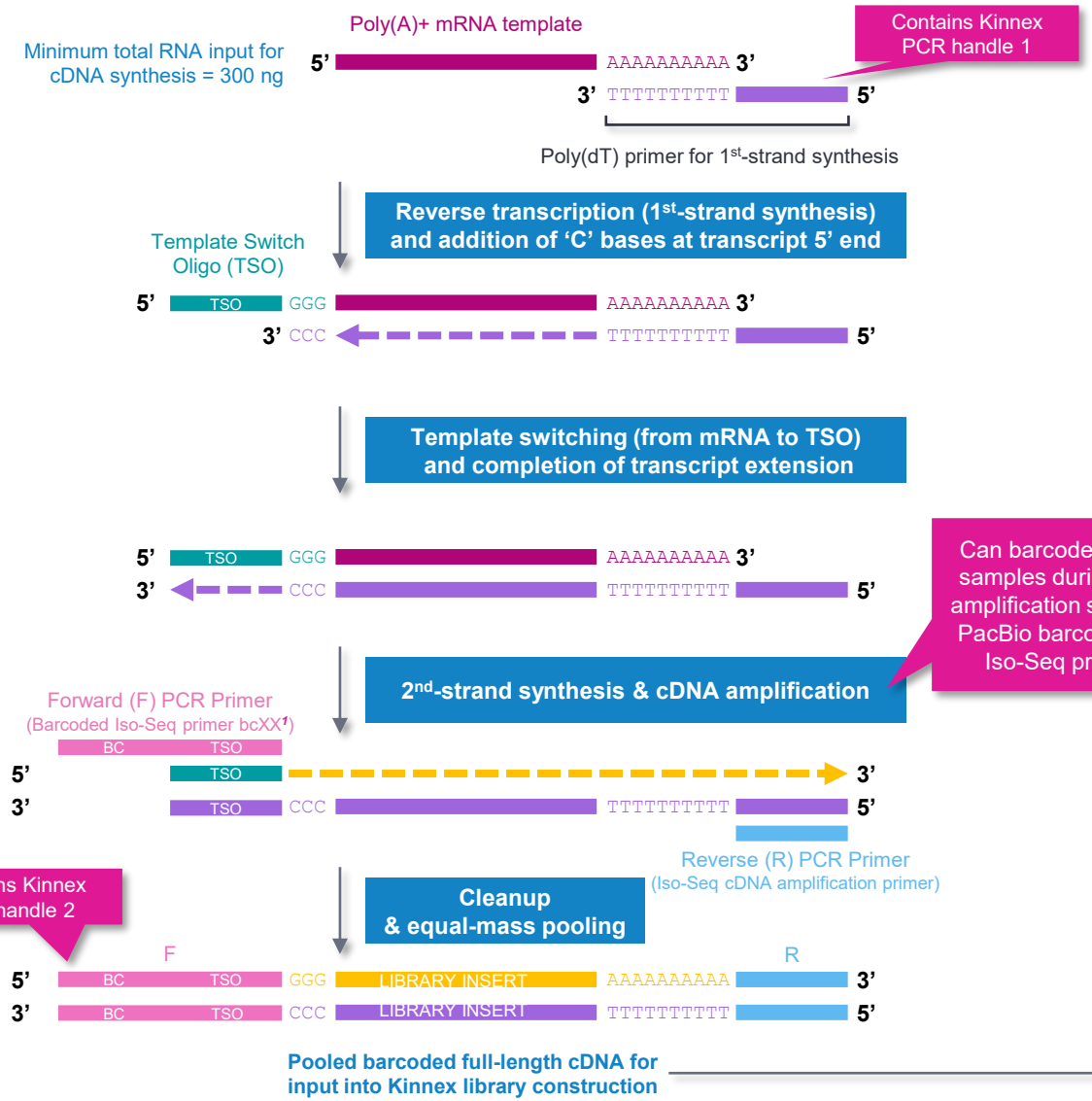
Kinnex full-length RNA application use case recommendations for PacBio systems

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)	
	Vega system: Up to 3 samples per Vega SMRT Cell (3-plex)	**Vega system:** Up to 6 samples per Vega SMRT Cell (6-plex)	
	Revio system + SPRQ: Up to 6 samples per Revio SMRT Cell (6-plex)	**Revio system + SPRQ:** Up to 12 samples per Revio SMRT Cell (12-plex)	
Expected data throughput (per SMRT Cell)	**Sequel II/IIe system:** Up to 20 M reads divided by *N* samples		
	Vega system: Up to 30 M reads divided by *N* samples		
	Revio system + SPRQ: Up to 60 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.

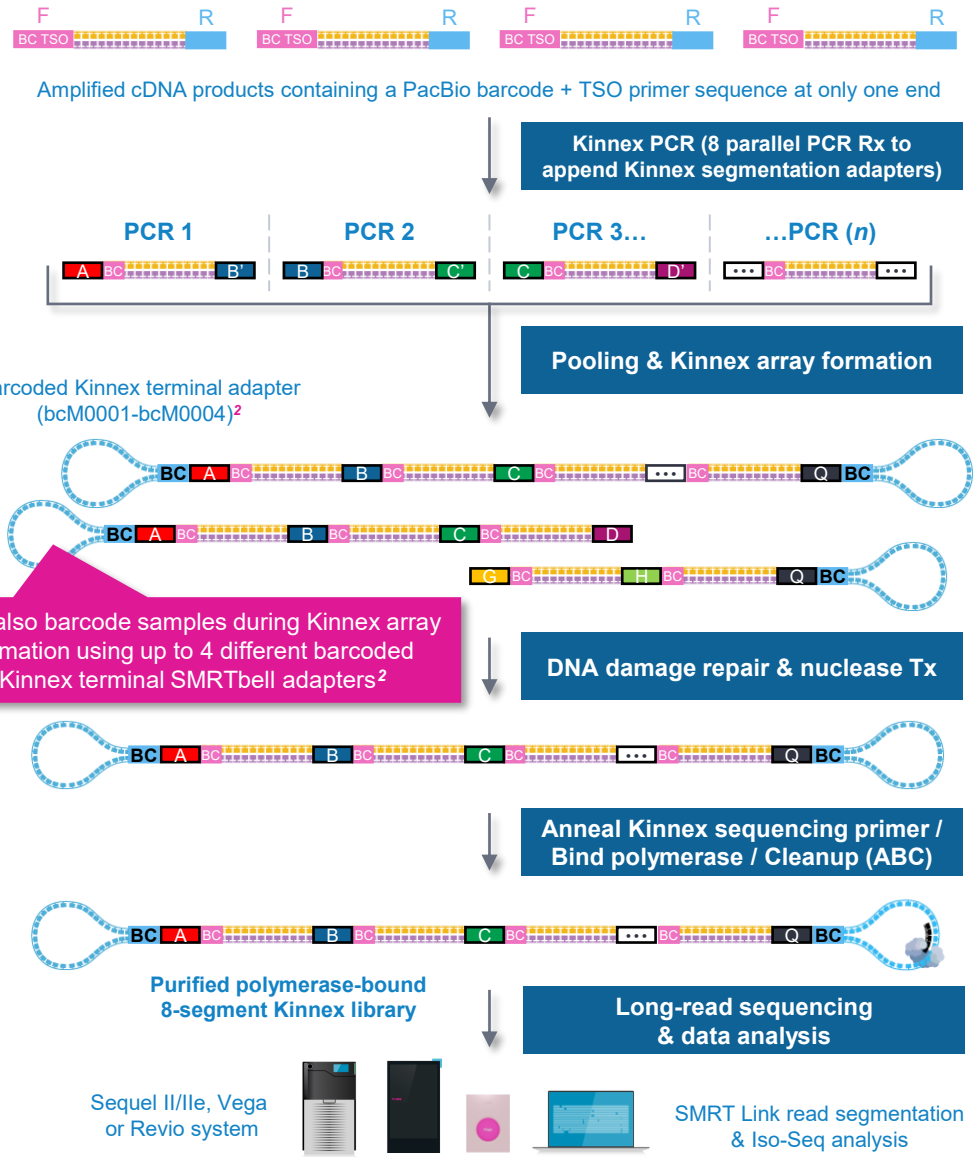
Kinnex full-length RNA method overview

Full-length cDNA synthesis & amplification



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

Kinnex library prep, sequencing & 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

I. 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**

II. 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**

III. 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

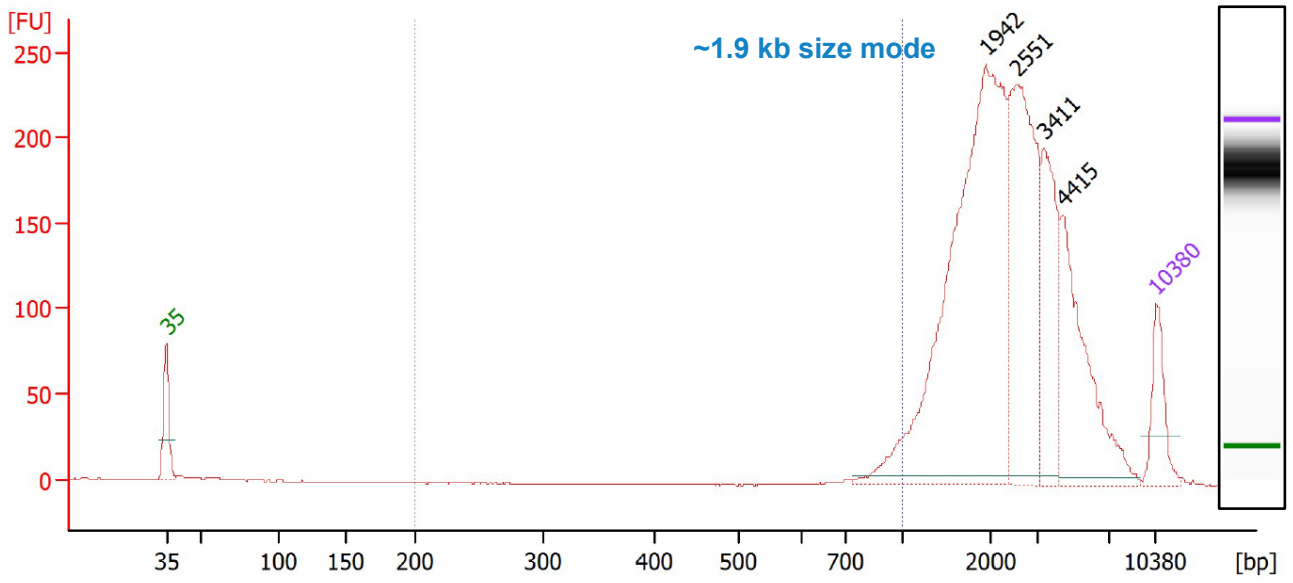
Final SMRTbell bead cleanup

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

Example Kinnex full-length RNA library preparation QC results

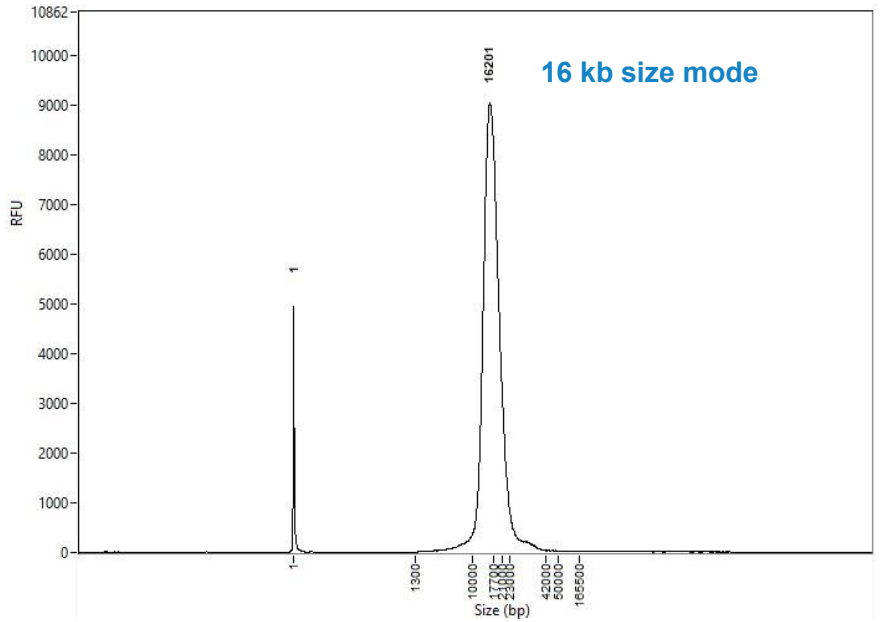
Kinnex full-length RNA library prepared with human UHRR total RNA

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 the following number of SMRT Cells:

- >8 SMRT Cells for Revio using SPRQ chemistry
- >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems
- >4 SMRT Cells for Sequel II/IIe systems

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%)

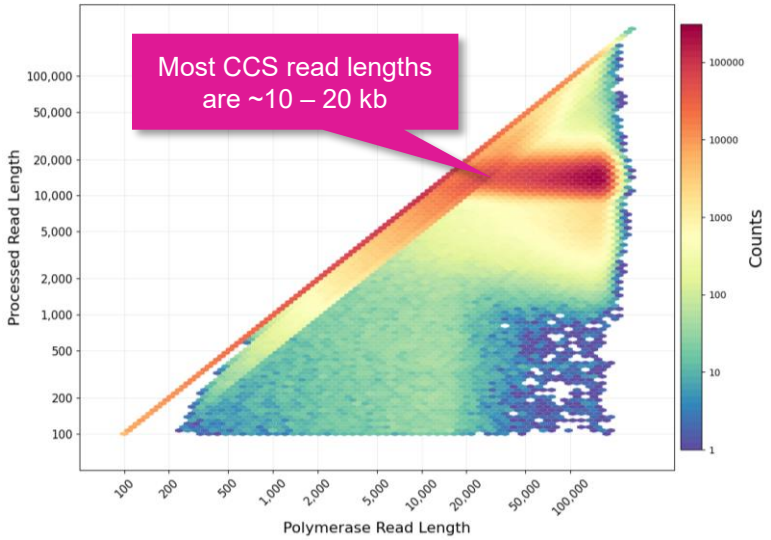
[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~25% when using UHRR total RNA samples for Kinnex full-length RNA library construction.

PacBio

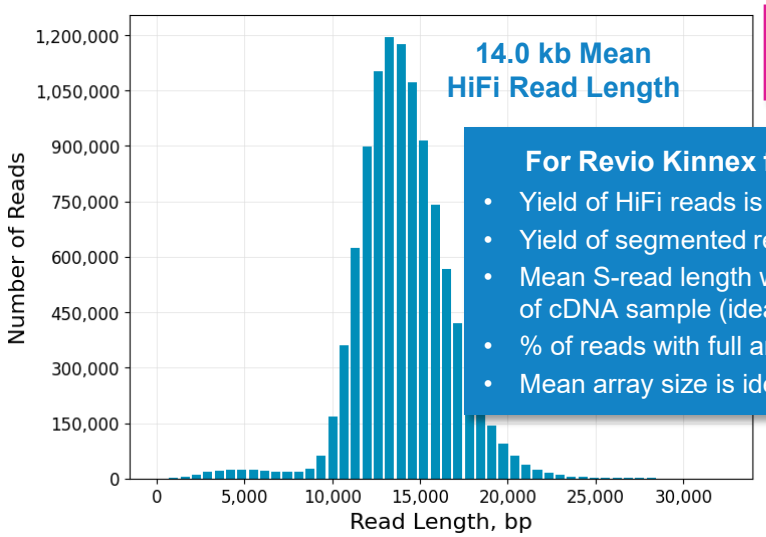
Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA [Revio system + SPRQ chemistry[1]]

Kinnex full-length RNA library for universal human reference RNA (UHRR) sample

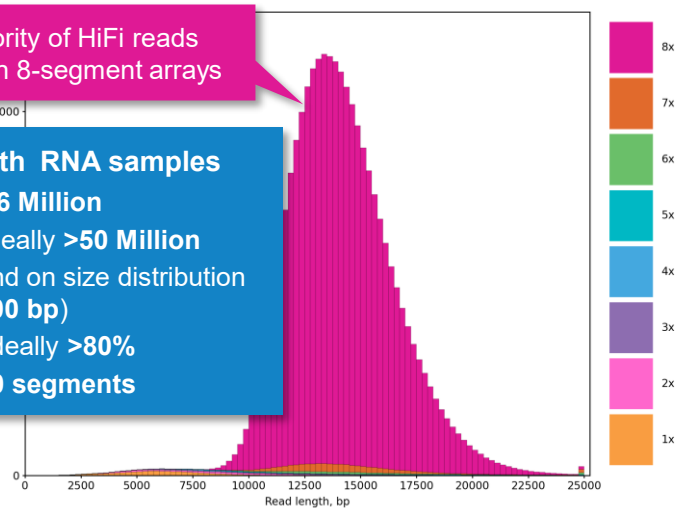
Raw Data Report



HiFi Read Length



Read Segmentation Metrics



For Revio Kinnex full-length RNA samples

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>50 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**

Raw Base Yield	1,442 Gb
Mean Polymerase Read Length	71.9 kb
P0	20%
P1	80%
P2	1%

Example sequencing metrics for a Universal Human Reference RNA (UHRR) Kinnex full-length RNA library sample run on a Revio system with Revio SPRQ polymerase kit / 160 pM on-plate loading concentration (OPLC) / 24-hrs movie time.

HiFi Reads	10.4 M
HiFi Base Yield	146.6 Gb
Mean HiFi Read Length	14.0 kb
Median HiFi Read Quality	Q32
HiFi Read Mean # of Passes	7

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

Input HiFi Reads	10,404,037
Segmented reads (S-reads)	81,012,128
Mean length of S-reads	1,758 bp
Percent of reads with full arrays	94.55%
Mean array size (concentration factor)	7.79

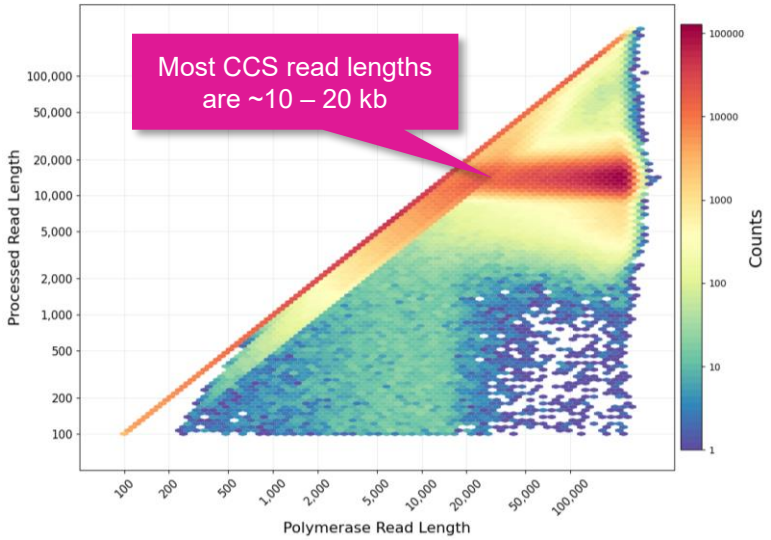
For UHRR Kinnex libraries, per-Revio SMRT Cell segmentation read counts were typically >50 Million.

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

Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA [Vega system[1]]

Kinnex full-length RNA library for universal human reference RNA (UHRR) sample

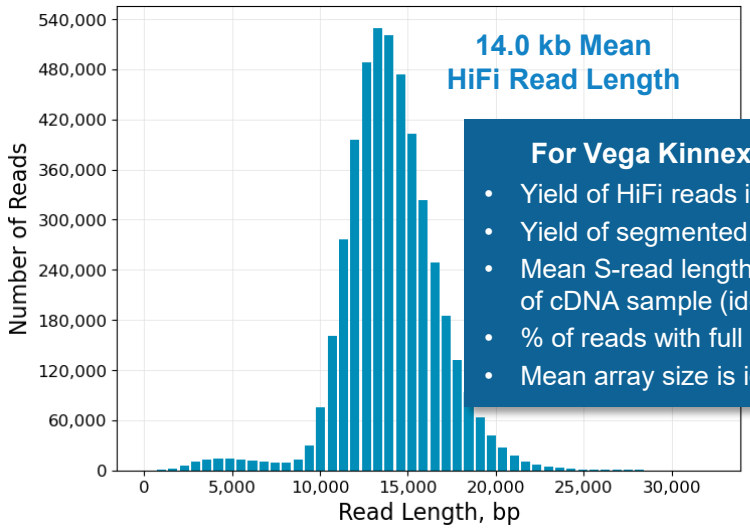
Raw Data Report



Mean Polymerase Read Length	95.3 kb
Loading level	61%

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

HiFi Read Length



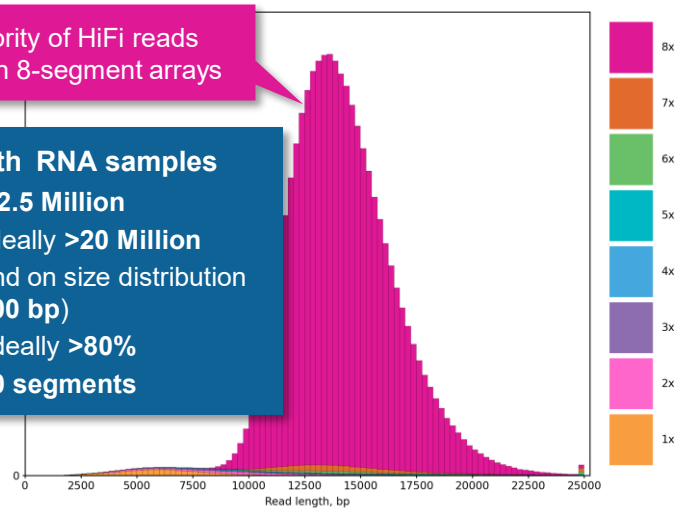
For Vega Kinnex full-length RNA samples

- Yield of HiFi reads is ideally **>2.5 Million**
- Yield of segmented reads is ideally **>20 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**

HiFi Reads	4.6 M
HiFi Base Yield	65.0 Gb
Mean HiFi Read Length	14.0 kb
Median HiFi Read Quality	Q35
HiFi Read Mean # of Passes	11

For UHRR Kinnex full-length RNA libraries, per-Vega SMRT Cell HiFi read counts were typically >2.5 Million depending on the final library insert size and sample loading performance.

Read Segmentation Metrics



Input HiFi Reads	4,642,616
Segmented reads (S-reads)	35,917,435
Mean length of S-reads	1,759 bp
Percent of reads with full arrays	94.14%
Mean array size (concentration factor)	7.74

For UHRR Kinnex libraries, per-Vega SMRT Cell segmentation read counts were typically >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, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may result in reduced HiFi data yields per 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 long read systems

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

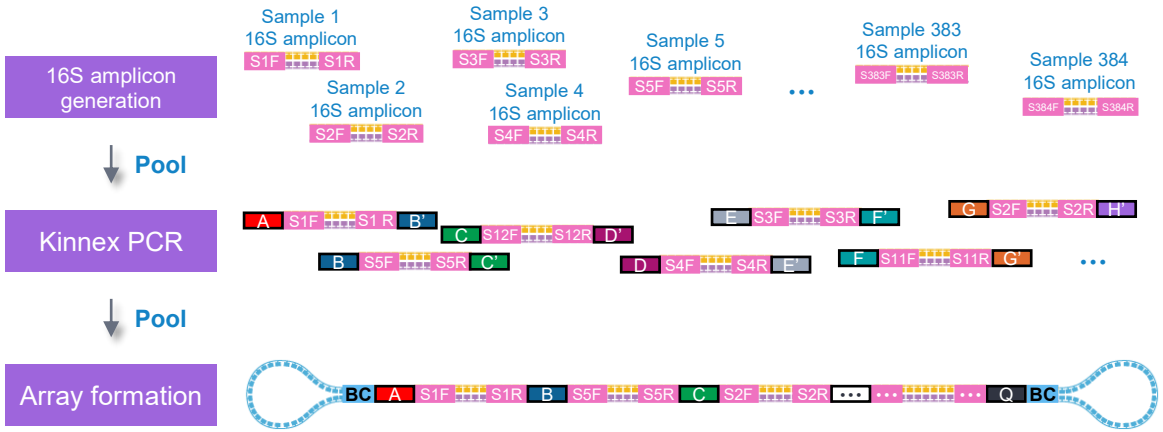
Overview	
Samples	1–384
Workflow time	1.5 days for up to 96 samples
Number of SMRT Cells per library Prep	>8 SMRT Cells for Revio using SPRQ™ chemistry >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems >10 SMRT Cells for Sequel II/IIe
Metagenomic DNA mass	Total genomic DNA of 1–2 ng per metagenomic sample

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

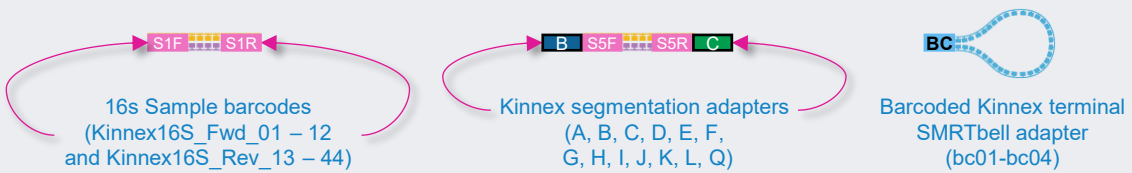
Kinnex full-length 16s rRNA library barcoding options

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



[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		Vega system		Revio system + SPRQ chemistry	
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 1,152 samples per Vega SMRT Cell (1,152-plex)		Up to 1,536 samples per Revio SMRT Cell (1,536-plex)	
Expected coverage per sample[2]	**96-plex**	**208 K**	**96-plex**	**416 K**	**96-plex**	**729 K**
	192-plex	**104 K**	**192-plex**	**208 K**	**192-plex**	**364 K**
	384-plex	**52 K**	**384-plex**	**104 K**	**384-plex**	**182 K**
	768-plex	26 K	**768-plex**	**52 K**	**768-plex**	**91 K**
	1,152-plex	17 K	**1,152-plex**	**34 K**	**1,152-plex**	**60 K**
	1,536-plex	13 K	1,536-plex	26 K	**1,536-plex**	**45 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, Vega and Revio systems are expected to achieve 20–25 million, 30-40 million and 70–80 million 16S sequences, respectively. For most 16S analysis applications, typically aim for ~30-50 K reads/sample.

PacBio

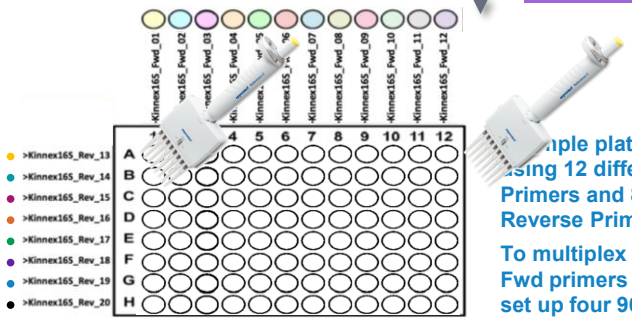
Kinnex 16S rRNA method overview

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]



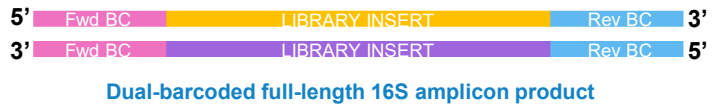
Can barcode up to 384 samples during 16S gene amplification step using PacBio barcoded Fwd and Rev primers[1]

…mple plate layout for 96-plex PCR design …sing 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

Contains Kinnex PCR handle 1

Equal-volume pooling & cleanup

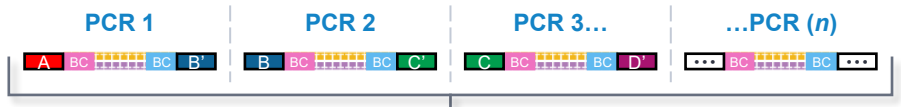
Contains Kinnex PCR handle 2

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

Kinnex library prep, sequencing & analysis

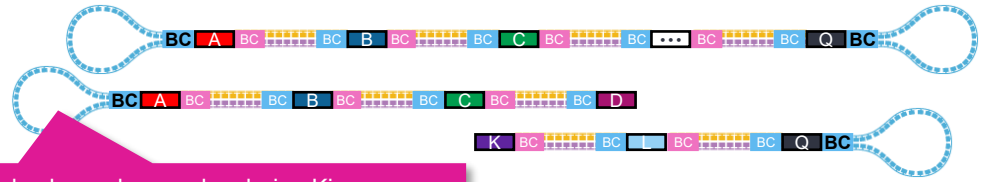
Amplified 16S products containing a different PacBio barcode at each end (Fwd BC ≠ Rev BC)

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



Pooling & Kinnex array formation

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



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

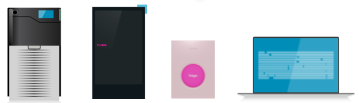
DNA damage repair & nuclease Tx

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

Purified polymerase-bound 12-segment Kinnex library

Long-read sequencing & data analysis

Sequel II/IIe, Vega 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

I. 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

II. 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**

III. 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

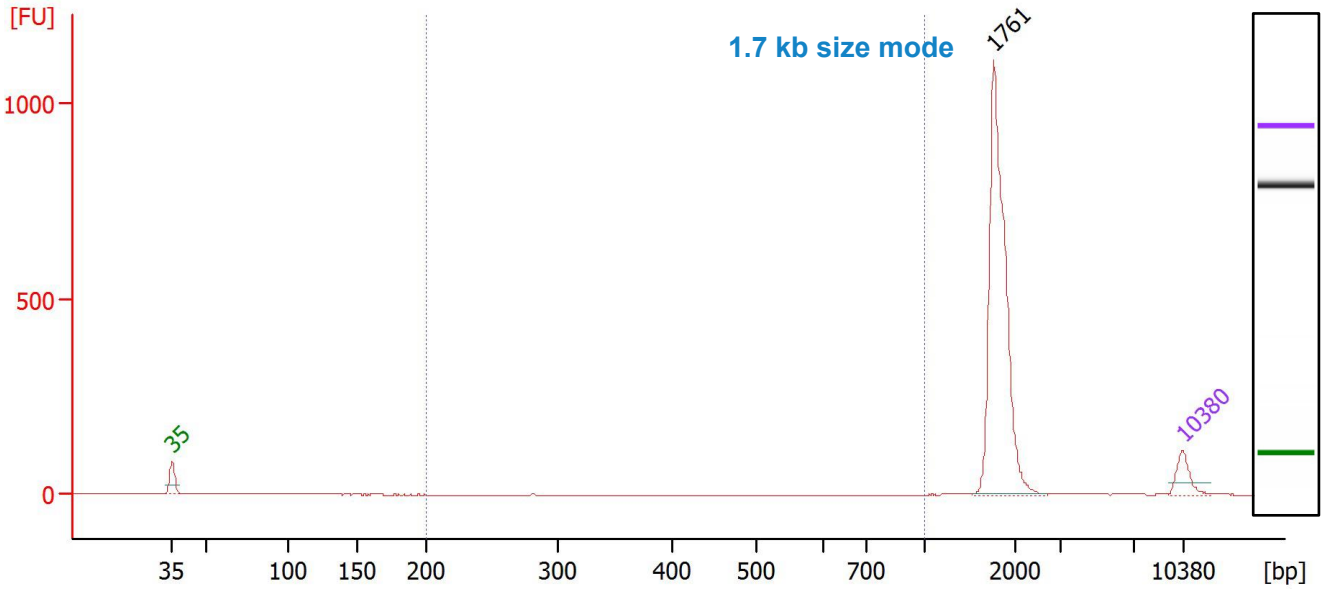
Final SMRTbell bead cleanup

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

Example Kinnex 16S rRNA library preparation QC results

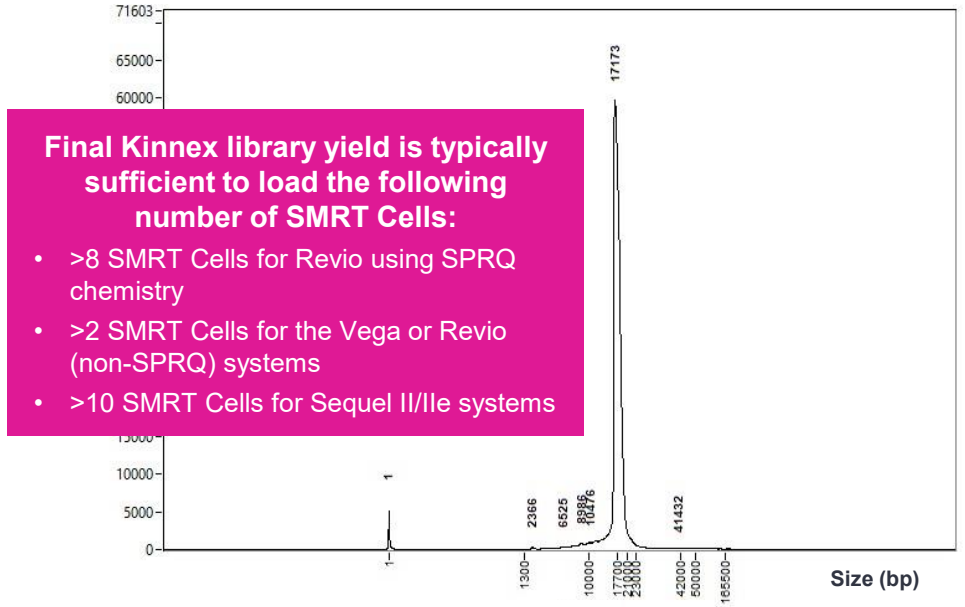
Kinnex full-length 16S RNA library prepared from mock microbial community genomic DNA

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



Final Kinnex library yield is typically sufficient to load the following number of SMRT Cells:

- >8 SMRT Cells for Revio using SPRQ chemistry
- >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems
- >10 SMRT Cells for Sequel II/IIe systems

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

Example sample multiplexing design for 1,536-plex 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**

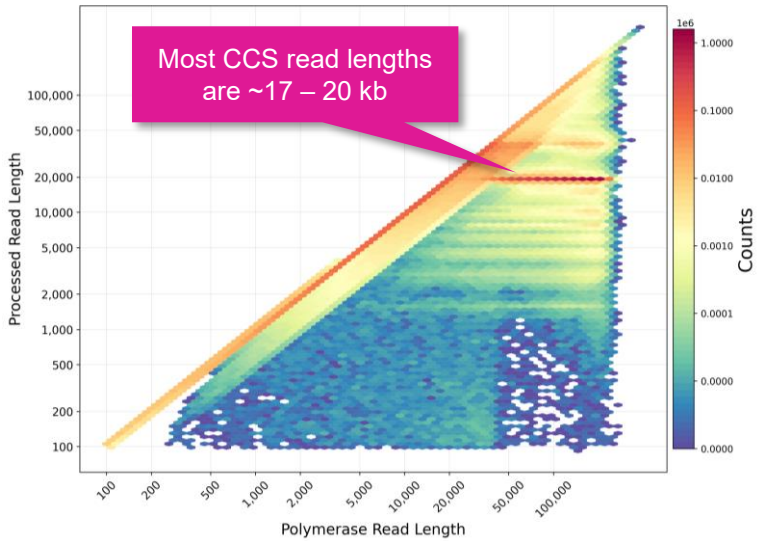
gDNA input for 16S PCR	1.1 ng
16S amplicon DNA input Kinnex PCR products for Kinnex array formation	6000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1080 ng (18%)

[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 + SPRQ[1]]

Kinnex full-length 16S library for mock microbial community gDNA sample mixture (384-plex)

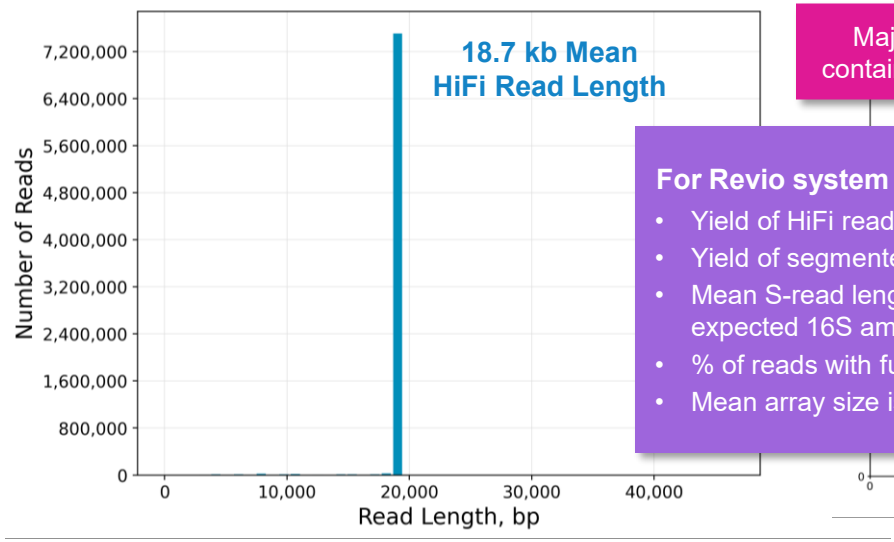
Raw Data Report



Raw Base Yield	1,400 Gb
Mean Polymerase Read Length	80.5
P0	30%
P1	69%
P2	3%

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

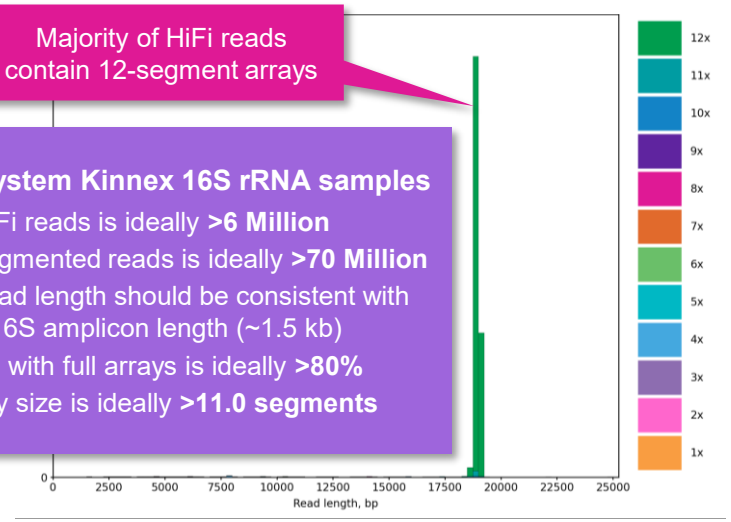
HiFi Read Length



HiFi Reads	7.7 M
HiFi Base Yield	144.5 Gb
Mean HiFi Read Length	18.7 kb
Median HiFi Read Quality	Q34
HiFi Read Mean # of Passes	7

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

Read Segmentation Metrics



For Revio system Kinnex 16S rRNA samples

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>70 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	7,713,616
Segmented reads (S-reads)	91,255,042
Mean length of S-reads	1,558 bp
Percent of reads with full arrays	96.16%
Mean array size (concentration factor)	11.83

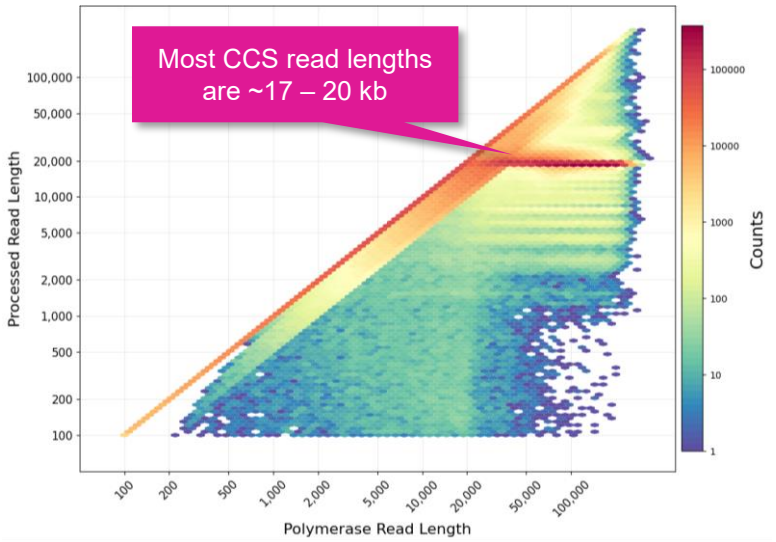
For Kinnex 16S rRNA libraries, per-Revio SMRT Cell segmentation read counts were typically >70 Million.

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

Example sequencing performance for Kinnex 16S rRNA libraries prepared from mock microbial community genomic DNA [Vega system[1]]

Kinnex full-length 16S library for mock microbial community gDNA sample mixture (384-plex)

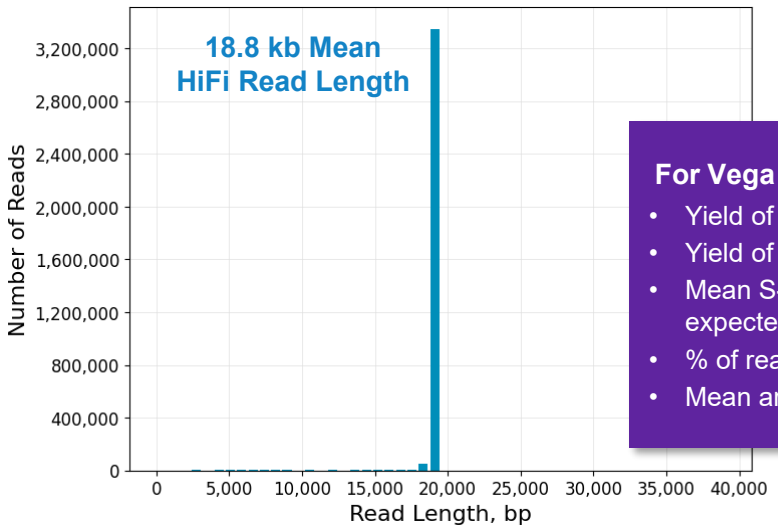
Raw Data Report



Mean Polymerase Read Length	64.1
Loading level	67%

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

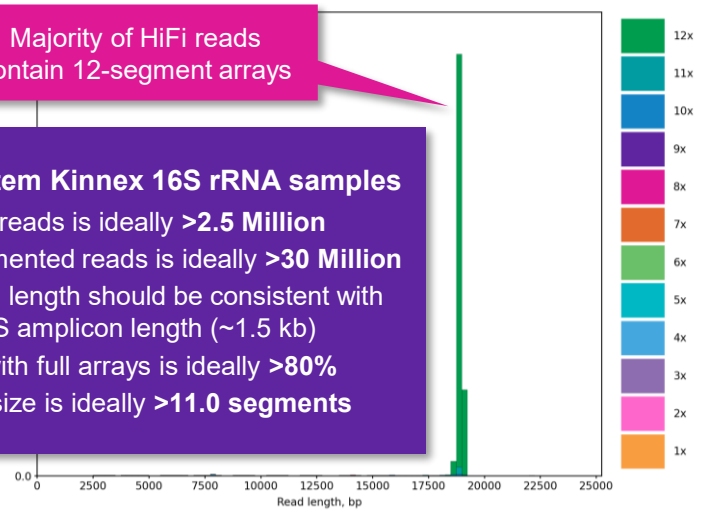
HiFi Read Length



HiFi Reads	3.5 M
HiFi Base Yield	65.0 Gb
Mean HiFi Read Length	18.8 kb
Median HiFi Read Quality	Q31
HiFi Read Mean # of Passes	6

For Kinnex 16S rRNA libraries, per-Vega SMRT Cell HiFi read counts were typically >2.5 Million depending on the final library insert size and sample loading performance.

Read Segmentation Metrics



For Vega system Kinnex 16S rRNA samples

- Yield of HiFi reads is ideally **>2.5 Million**
- Yield of segmented reads is ideally **>30 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	3,464,764
Segmented reads (S-reads)	41,088,694
Mean length of S-reads	1,558 bp
Percent of reads with full arrays	95.86%
Mean array size (concentration factor)	11.86

For Kinnex 16S rRNA libraries, per-Vega SMRT Cell segmentation read counts were typically >30 Million.

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

Sample Name	*P1* % [Revio system]	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 sample (*P1*) loading on PacBio long-read systems

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

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 Kinnex full-length RNA troubleshooting guide (103-552-100)
- SMRT Link Kinnex single-cell troubleshooting guide (103-516-100)
- SMRT Link MAS-Seq troubleshooting guide (102-994-400)
- 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

- Wissel, D. et al. (2025) A systematic benchmark of high-accuracy PacBio long-read RNA sequencing for transcript-level quantification. BioRxiv [Link]
- Deng, E. et al. (2025) Systematic evaluation of single-cell RNA-seq analyses performance based on long-read sequencing platforms. J Advanced Res [Link]
- Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology. [Link]
- Schertzer, M.D. et al. (2023) Cas13d-mediated isoform-specific RNA knockdown with a unified computational and experimental toolbox. Nature comm [Link]

Webinars

- PacBio video (2024) – Kinnex explained – how concatenating smaller amplicons increases throughput for PacBio HiFi sequencing [Link]
- PacBio PRISM webinar (2024) – Let's stick together – exploring PacBio Kinnex kits [Link]
- PacBio video (2024) – Increasing the throughput of full-length 16S and RNA-Seq with Kinnex kits [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 and Vega 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	Vega system
	Homo sapiens – Universal human reference RNA (UHRR) [Link]	HiFi long read	Revio system – SPRQ chemistry
	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
	Homo sapiens – Brain [Link]	HiFi long read	Revio system

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

Example PacBio data sets

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

APPENDIX 1: Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput

Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput

Kinnex concatenation is a general method that can increase sequencing throughput for smaller amplicons[1]

Benefits of Kinnex concatenation

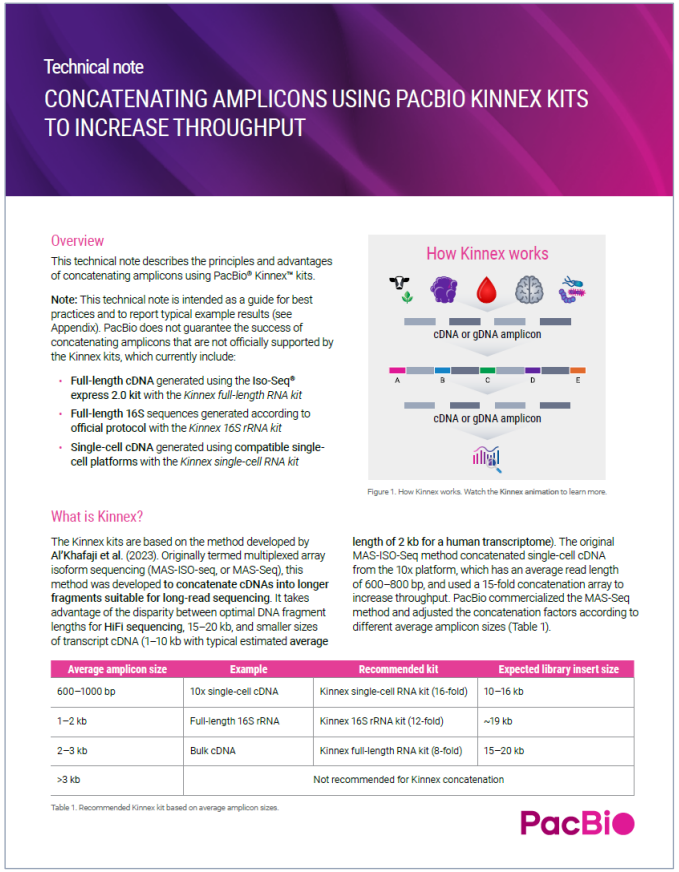
- Increased throughput on PacBio long-read sequencers
- Retained HiFi accuracy despite throughput increase
- No change to secondary analysis – once reads are deconcatenated into S-reads, the S-reads represent the original, pre-concatenated amplicon and can be analyzed with established pipelines.

When is Kinnex concatenation appropriate?

- The balance between the amplicon size and the concatenation factor, as well as additional Kinnex library generation cost, needs to be taken into consideration.
- HiFi sequencing produces optimal yield for inserts between 15–20 kb; therefore, the throughput advantage plateaus for larger amplicon sizes exceeding 3 kb.
- You can consider concatenating amplicons using Kinnex kits if:
  - The amplicons have an average size between 200 bp – 3 kb
  - The amplicons have molecular ends that are either directly compatible or can be re-amplified to establish Kinnex compatibility

Recommended Kinnex kit based on average amplicon sizes.

Average amplicon size	Example	Recommended Kinnex kit	Expected Kinnex library size
600 – 1000 bp	10x single-cell cDNA	Kinnex single-cell RNA kit (16-fold)	10 – 16 kb
1 – 2 kb	Full-length 16S	Kinnex 16S rRNA kit (12-fold)	~19 kb
2 – 3 kb	Bulk cDNA	Kinnex full-length RNA kit (8-fold)	15 – 20 kb
>3 kb	**Not recommended for Kinnex concatenation**		


Technical note

CONCATENATING AMPLICONS USING PACBIO KINNEX KITS TO INCREASE THROUGHPUT

Overview

This technical note describes the principles and advantages of concatenating amplicons using PacBio® Kinnex™ kits.

Note: This technical note is intended as a guide for best practices and to report typical example results (see Appendix). PacBio does not guarantee the success of concatenating amplicons that are not officially supported by the Kinnex kits, which currently include:

- **Full-length cDNA** generated using the **Iso-Seq® express 2.0 kit** with the *Kinnex full-length RNA kit*
- **Full-length 16S** sequences generated according to **official protocol** with the *Kinnex 16S rRNA kit*
- **Single-cell cDNA** generated using **compatible single-cell platforms** with the *Kinnex single-cell RNA kit*

How Kinnex works

cDNA or gDNA amplicon

A B C D E

cDNA or gDNA amplicon

Figure 1. How Kinnex works. Watch the Kinnex animation to learn more.

What is Kinnex?

The Kinnex kits are based on the method developed by **Al'Khafaji et al.** (2023). Originally termed multiplexed array isoform sequencing (MAS-ISO-seq, or MAS-Seq), this method was developed **to concatenate cDNAs into longer fragments suitable for long-read sequencing**. It takes advantage of the disparity between optimal DNA fragment lengths for **HiFi sequencing**, 15–20 kb, and smaller sizes of transcript cDNA (1–10 kb with typical estimated **average length of 2 kb for a human transcriptome**). The original MAS-ISO-Seq method concatenated single-cell cDNA from the 10x platform, which has an average read length of 600–800 bp, and used a 15-fold concatenation array to increase throughput. PacBio commercialized the MAS-Seq method and adjusted the concatenation factors according to different average amplicon sizes (Table 1).

Average amplicon size	Example	Recommended kit	Expected library insert size
600–1000 bp	10x single-cell cDNA	Kinnex single-cell RNA kit (16-fold)	10–16 kb
1–2 kb	Full-length 16S rRNA	Kinnex 16S rRNA kit (12-fold)	~19 kb
2–3 kb	Bulk cDNA	Kinnex full-length RNA kit (8-fold)	15–20 kb
>3 kb	Not recommended for Kinnex concatenation		

Table 1. Recommended Kinnex kit based on average amplicon sizes.

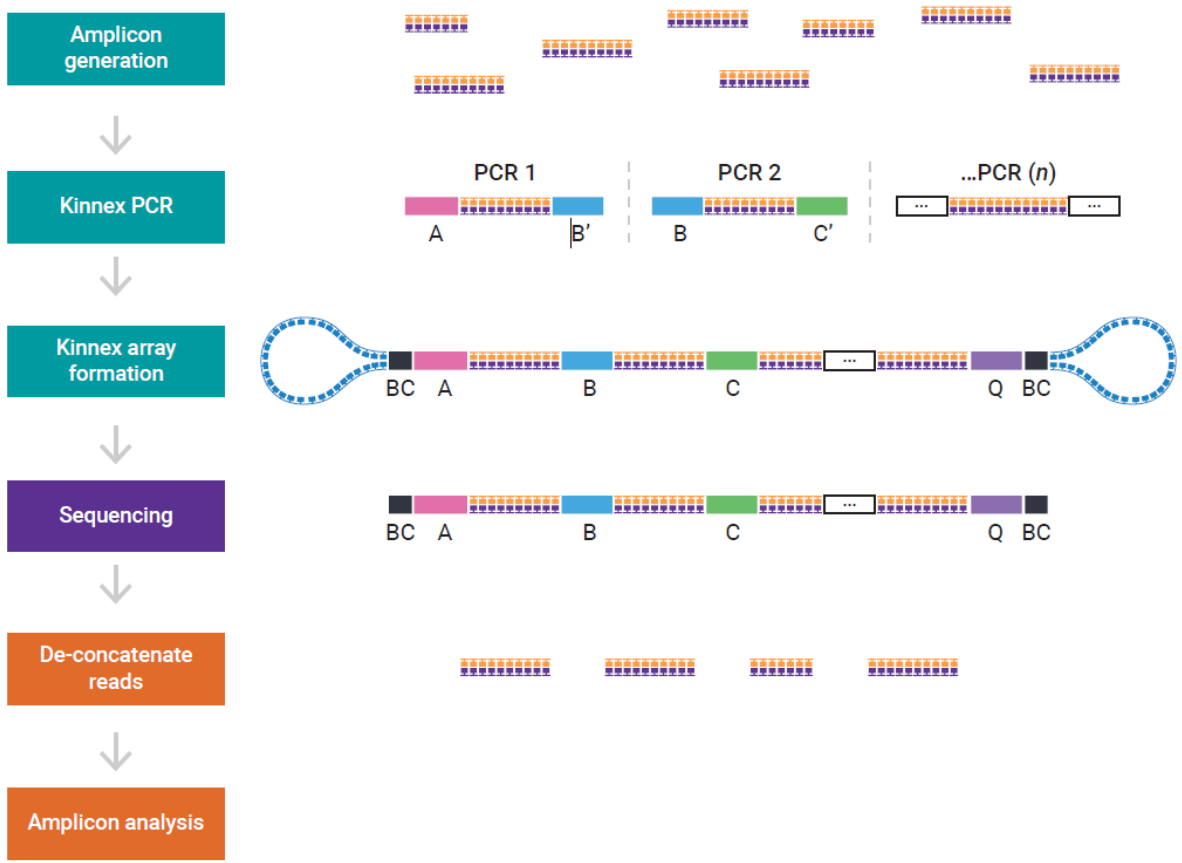
PacBio

Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput (102-326-636)

[1] Refer to ***Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput*** (102-326-636) for an overview of the general procedure for concatenating amplicons using PacBio Kinnex kits.

Kinnex concatenation workflow overview

Follow Kinnex library prep protocol documentation for specific details on concatenating cDNA or gDNA amplicons using Kinnex kits



Kinnex concatenation workflow. Amplicons must be generated or amplified to have Kinnex-compatible molecular ends before continuing to the Kinnex PCR and array formation step. Kinnex libraries should be sequenced with the appropriate sequencing chemistry and run configurations. Once de-concatenated using Read Segmentation in SMRT Link, the individual amplicons can be analyzed using amplicon-specific workflows.

Kinnex concatenation procedural notes[1]

- To establish Kinnex compatibility, amplicons are required to have Kinnex-compatible molecular ends (see next section)
  - Once these are generated, choose the appropriate Kinnex kit based on the recommended concatenation factor listed in Table on previous slide and proceed with Kinnex PCR
- The **Kinnex PCR** steps consist of parallel PCR reactions per sample [i.e., 8, 12, or 16 reactions based on the Kinnex kit chosen] using premixed Kinnex primer pairs
  - The resulting PCRs generate amplified DNA products containing programmable sequences at both ends
- In the **Kinnex array formation** step, library inserts containing programmable ends are assembled to generate a linear array.
  - Further, the addition of barcoded Kinnex terminal adapters result in the formation of complete, full-length array SMRTbell templates along with partial arrays.
  - Subsequent nuclease treatment removes partial arrays to retain only full array SMRTbell templates for achieving optimal sequencing yield.
- **Note:** Kinnex terminal adapters are different from standard SMRTbell adapters and hence require the Kinnex sequencing primer (103-179-000) during the "Annealing, Binding, and Cleanup (ABC)" step for optimal sequencing results.
- Once HiFi reads are generated, **Read Segmentation** will produce the segmented reads (S-reads) that represent the original unconcatenated amplicons, which can be used for further analysis.

[1] Refer to the relevant Kinnex library prep protocols and Kinnex technical overview training documentation for specific details regarding Kinnex PCR and Kinnex array formation steps.

Establishing Kinnex-compatible molecular ends

Amplicons must be generated or amplified to have Kinnex-compatible molecular ends before proceeding with Kinnex library prep procedure

- To be compatible with the Kinnex workflow, amplicons must be generated with sequence-defined ends as depicted in the underlined portion in the figure below
- Optional barcodes (such as sample indices, UMIs and single cell barcodes) should be placed internally between the Kinnex handles and the amplicon-specific primers.
- Kinnex handles may be present already in certain amplicons, such as 10x Single Cell Gene Expression libraries or the Kinnex 16S amplicons, or can be added by PCR amplification, such as for Parse Evercode single-cell libraries

Kinnex FWD primer	CTACACGACGCTCTTCCGATCT – [optional barcodes] – [amplicon specific FWD primers]
Kinnex REV primer	AAGCAGTGGTATCAACGCAGAG – [optional barcodes] – [amplicon specific REV primers]

Example 1. Kinnex 16S forward and reverse primer sequences

Kinnex 16S FWD01	CTACACGACGCTCTTCCGATCT – GATCGAGTCA – AGRGTTYGATYMTGGCTCAG
Kinnex 16S REV13	AAGCAGTGGTATCAACGCAGAG – TCATCGACGT – RGYTACCTTGTTACGACTT

Example 2. Iso-Seq express 2.0 forward and reverse primer sequences

IsoSeqX bc01 FWD	CTACACGACGCTCTTCCGATCT – ACTACAC – GCAATGAAGTCGCAGGGTTGGG
IsoSeqX REV	AAGCAGTGGTATCAACGCAGAGTAC

Schematic for Kinnex-compatible primers. Kinnex handles (5' to 3') are shown in black underline and must be present at the ends of the amplicons to be compatible with Kinnex concatenation. Optional barcodes can be included internally. Amplicon-specific primers (and optional internal barcode sequences) must be designed to avoid strong secondary structures in the context of Kinnex handles.

Resources for Kinnex library prep[1]

For using Kinnex full-length RNA kit (PN: 103-072-000) for 8-fold concatenation:

- Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)
- Technical overview – Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)

For using Kinnex 16S rRNA kit (PN: 103-072-100) for 12-fold concatenation:

- Procedure & checklist – Preparing Kinnex libraries using 16S rRNA amplicons (103-238-800)
- Technical overview – Kinnex library preparation using Kinnex 16S rRNA kit (103-344-800)

For using Kinnex single-cell RNA kit (PN: 103-072-200) for 16-fold concatenation:

- Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)
- Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit (103-344-600)

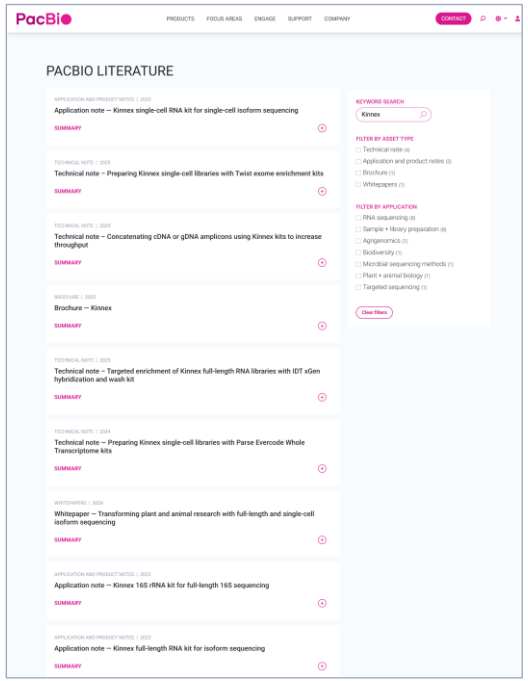
[1] Refer to the relevant Kinnex library prep protocols and Kinnex technical overview training documentation for specific details on concatenating cDNA or gDNA amplicons using Kinnex kits

APPENDIX 2: PacBio compatible Kinnex library preparation workflows

Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

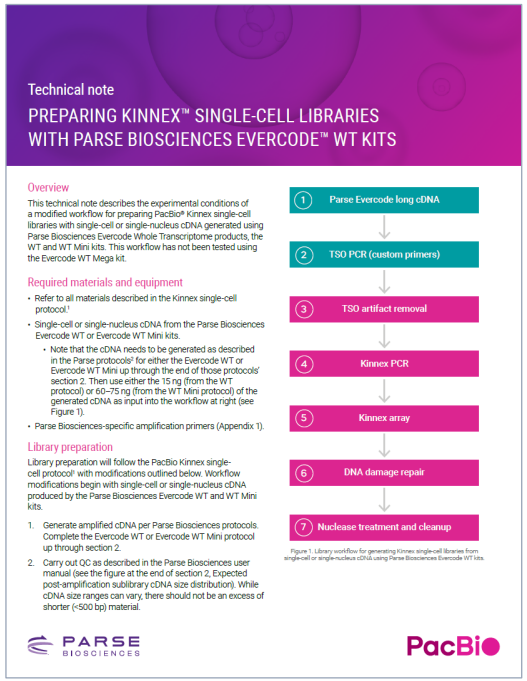
Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits: Getting started

Application-specific educational literature → **Application-specific protocol documentation** → **Application-specific technical overviews**



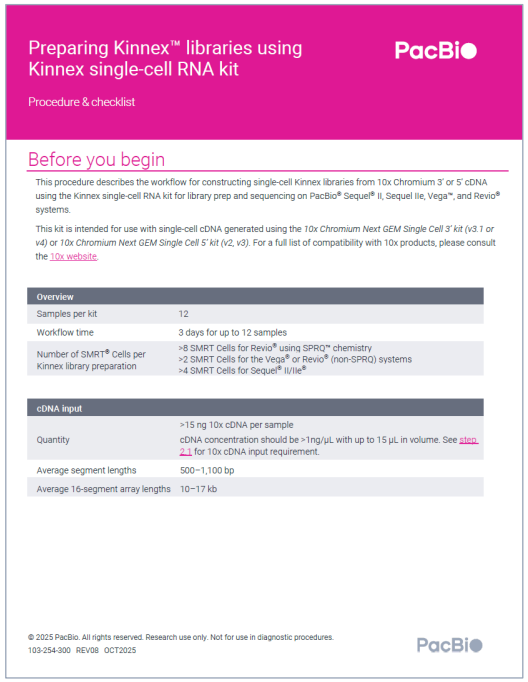
PacBio literature website [Link]

Application-specific brochures, informational guides and other product literature containing best practices recommendations for library preparation and data analysis workflows.



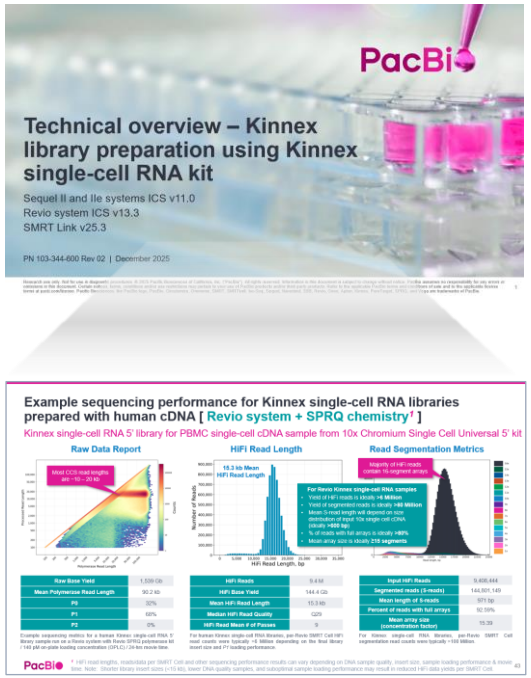
Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610) [PacBio]

Technical documentation describing a modified workflow for preparing PacBio Kinnex single-cell libraries with single-cell or single-nucleus cDNA generated using Parse Biosciences Evercode Whole Transcriptome products.



Procedure & checklist – Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

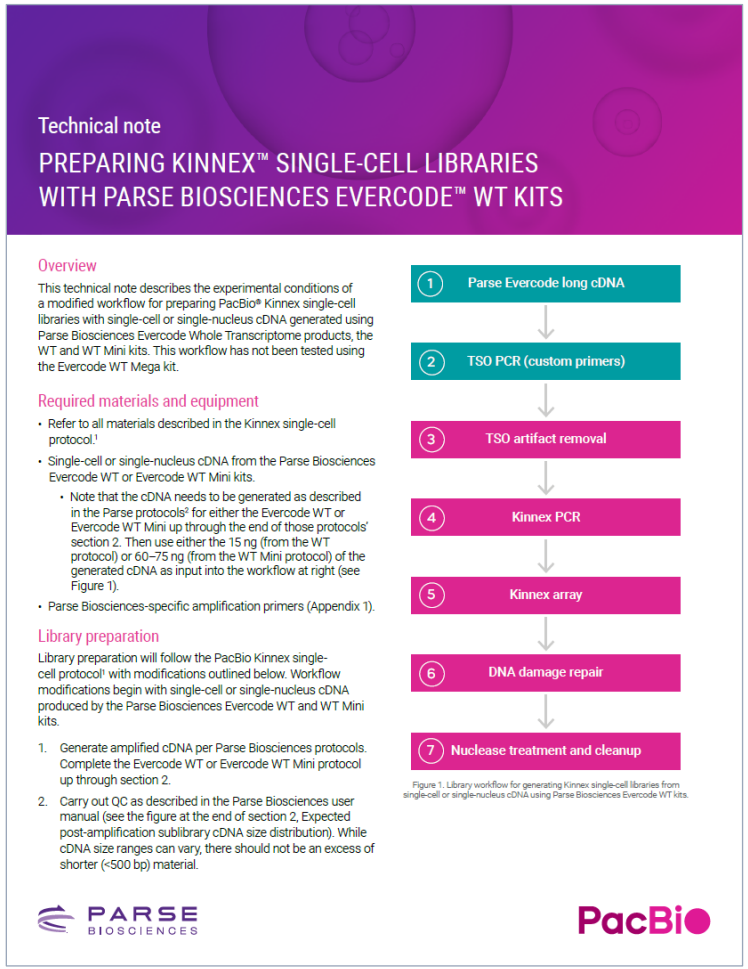


Technical overview: Kinnex library preparation using Kinnex single-cell RNA kit (103-344-600)

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

Technical reference for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610) describes a modified workflow for preparing PacBio Kinnex single-cell libraries with single-cell or single-nucleus cDNA generated using Parse Biosciences Evercode Whole Transcriptome products



Technical note

PREPARING KINNEX™ SINGLE-CELL LIBRARIES WITH PARSE BIOSCIENCES EVERCODE™ WT KITS

Overview

This technical note describes the experimental conditions of a modified workflow for preparing PacBio® Kinnex single-cell libraries with single-cell or single-nucleus cDNA generated using Parse Biosciences Evercode Whole Transcriptome products, the WT and WT Mini kits. This workflow has not been tested using the Evercode WT Mega kit.

Required materials and equipment

- Refer to all materials described in the Kinnex single-cell protocol.[1]
- Single-cell or single-nucleus cDNA from the Parse Biosciences Evercode WT or Evercode WT Mini kits.
  - Note that the cDNA needs to be generated as described in the Parse protocols[2] for either the Evercode WT or Evercode WT Mini up through the end of those protocols' section 2. Then use either the 15 ng (from the WT protocol) or 60–75 ng (from the WT Mini protocol) of the generated cDNA as input into the workflow at right (see Figure 1).
- Parse Biosciences-specific amplification primers (Appendix 1).

Library preparation

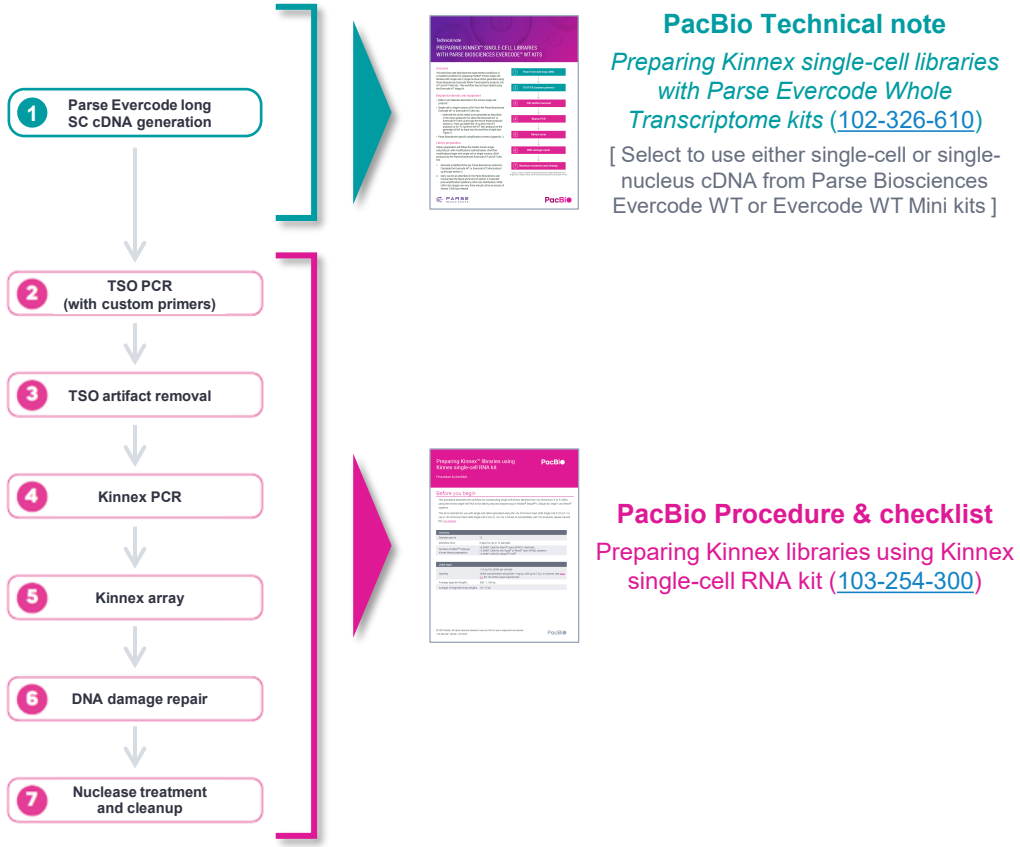
Library preparation will follow the PacBio Kinnex single-cell protocol[1] with modifications outlined below. Workflow modifications begin with single-cell or single-nucleus cDNA produced by the Parse Biosciences Evercode WT and WT Mini kits.

1. Generate amplified cDNA per Parse Biosciences protocols. Complete the Evercode WT or Evercode WT Mini protocol up through section 2.
2. Carry out QC as described in the Parse Biosciences user manual (see the figure at the end of section 2, Expected post-amplification sublibrary cDNA size distribution). While cDNA size ranges can vary, there should not be an excess of shorter (<500 bp) material.

Figure 1. Library workflow for generating Kinnex single-cell libraries from single-cell or single-nucleus cDNA using Parse Biosciences Evercode WT kits.

PARSE BIOSCIENCES

PacBio



PacBio Technical note

Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610)

[Select to use either single-cell or single-nucleus cDNA from Parse Biosciences Evercode WT or Evercode WT Mini kits]

PacBio Procedure & checklist

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

Overview

- Parse whole transcriptome kits use split-pool combinatorial barcoding to enable scale-up of single cell projects to millions of cells or nuclei[1]
- This Kinnex library prep workflow has been tested with single-cell or single-nucleus cDNA generated using Parse Biosciences Evercode WT and WT Mini kit whole transcriptome products
- This workflow has not been tested using the Evercode WT Mega kit

PacBio

1 Learn more about how Parse technology uniquely labels cells without needing to isolate individual cells at Parse Biosciences website.

Workflow overview for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

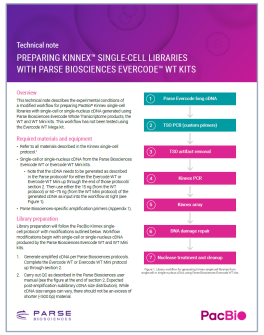
Follow Parse Bioscience whole transcriptome kit procedure[1] to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems

1. Parse Evercode SC cDNA generation
2. TSO PCR (with custom primers)
3. TSO artifact removal
4. Kinnex PCR
5. Kinnex array formation
6. DNA damage repair
7. Nuclease treatment and cleanup

Parse single-cell cDNA generation procedure overview[1]

Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610)

PacBio



Parse single-cell cDNA generation procedure key steps

- Single-cell cDNA material first needs to be generated as described in the Parse protocols[1] for either the **Evercode WT or Evercode WT Mini kit** up through the end of those protocols' **Section 2**.

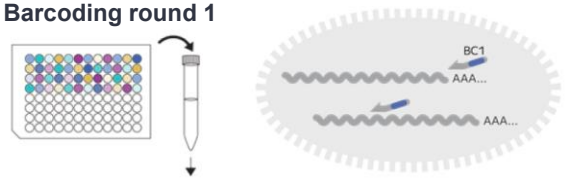
Evercode WT User Guides
UMWT3300 or UMWT3100

- Use either 15 ng (from the WT protocol) or 60–75 ng (from the WT Mini protocol) of the generated cDNA as input into the **PacBio Kinnex single-cell library prep protocol** (103-254-300) starting at **Step 2: TSO PCR (with custom primers[2])**.

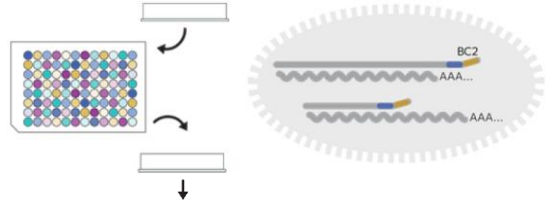
Parse Evercode single-cell cDNA generation workflow[1] (Step 1)

Section 1: In situ cell / nuclei barcoding

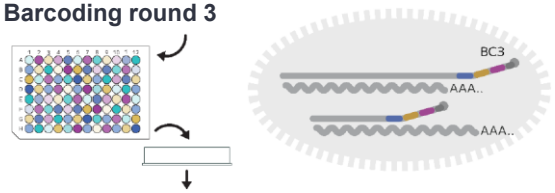
Barcoding round 1



Barcoding round 2



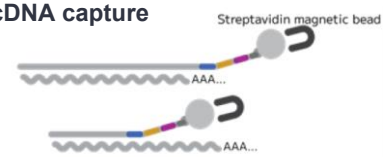
Barcoding round 3



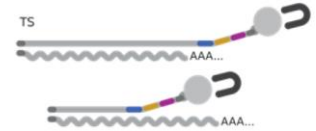
Lysis and Sublibrary Generation

Section 2: cDNA capture and amplification

cDNA capture



cDNA template switch



cDNA amplification

PacBio

1 For single-cell cDNA generation procedure details, refer to **Evercode WT User Guides** (UMWT3300 or UMWT3100) available from the Parse Biosciences website.
2 Perform TSO PCR (Step 2) with Parse Biosciences-specific amplification primers (see Appendix 1 in **Parse Technical note** 102-326-610).

Workflow overview for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (cont.)

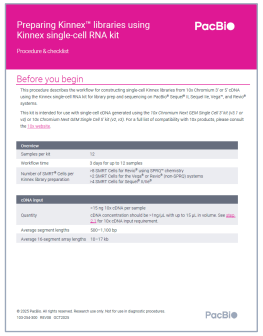
Parse single-cell cDNA samples may be used for Kinnex library construction by following PacBio *Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit* (103-254-300)

1. Parse Evercode SC cDNA generation
2. TSO PCR (with custom primers)
3. TSO artifact removal
4. Kinnex PCR
5. Kinnex array formation
6. DNA damage repair
7. Nuclease treatment and cleanup

PacBio Kinnex library construction procedure[1]

Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

PacBio



Kinnex SC library prep procedure key steps

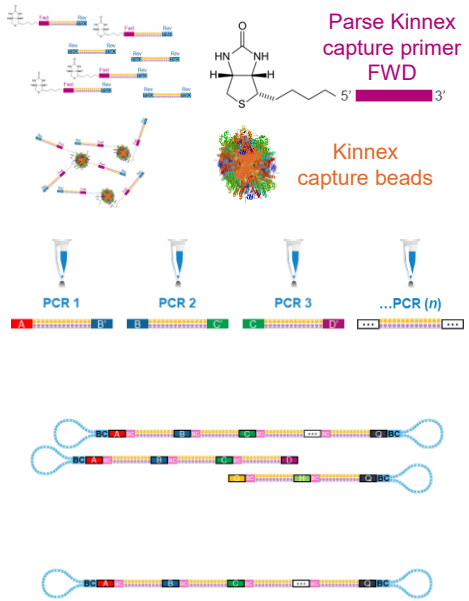
- Follow **PacBio Kinnex single cell protocol** (103-254-300) (**Steps 2 – 7**) with modifications below:
  - Generate amplified single-cell cDNA per Parse Biosciences protocols. Complete the**Evercode WT or Evercode WT Mini protocol** up through **Section 2**.
  - Carry out cDNA quantification and sizing QC as described in the Parse Biosciences user manual.
  - Begin **Kinnex single-cell protocol from step 2 (TSO PCR)** with the following modification: Follow step 2.1 and use 5 µL of **Parse Biosciences-specific amplification primers** as described in Appendix 1 instead of Kinnex 5' or 3' capture primer mixes.[1]
  - After running TSO PCR program, proceed with the subsequent Kinnex workflow from cleanup with 1.5X SMRTbell cleanup beads in the TSO PCR step through the rest of the protocol.[2]

1. Parse Evercodesingle-cell cDNA generation

→ See **PacBio Technical note**
Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610)

Kinnex single-cell library construction workflow (Steps 2 – 7)

2 TSO PCR
↓
3 TSO artifact removal
↓
4 Kinnex PCR
↓
5 Kinnex array
↓
6 DNA repair
↓
7 Nuclease treatment and cleanup



PacBio

1 This replacement is critical to adapt the Parse library termini to function with the Kinnex concatenation workflow.
2 Subsequent QC and library quantification can be carried out as described in the Kinnex single-cell protocol (step 7.21 in 103-254-300).

TSO PCR amplification primer oligo recommendations for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

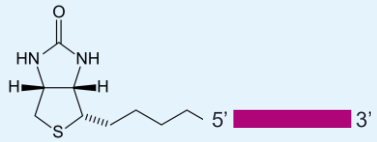
Order custom TSO PCR amplification primers as described below from your preferred oligo vendor

Parse Biosciences-specific primers required for TSO PCR amplification step

- Order custom primers from your preferred oligo vendor
- An example of IDT code is shown at right
- Primers should be HPLC-purified
- Order these primers and reconstitute in TE buffer (10 mM Tris HCl pH 8, 0.1 mM EDTA).
- Use 10 µM solutions of each of these primers in place of the yellow-capped Kinnex 3’ capture primer mix or the red-capped Kinnex 5’ capture primer mix in the Kinnex single-cell RNA kit (103-072-200)

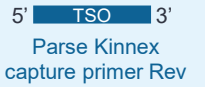
Parse Kinnex forward capture primer

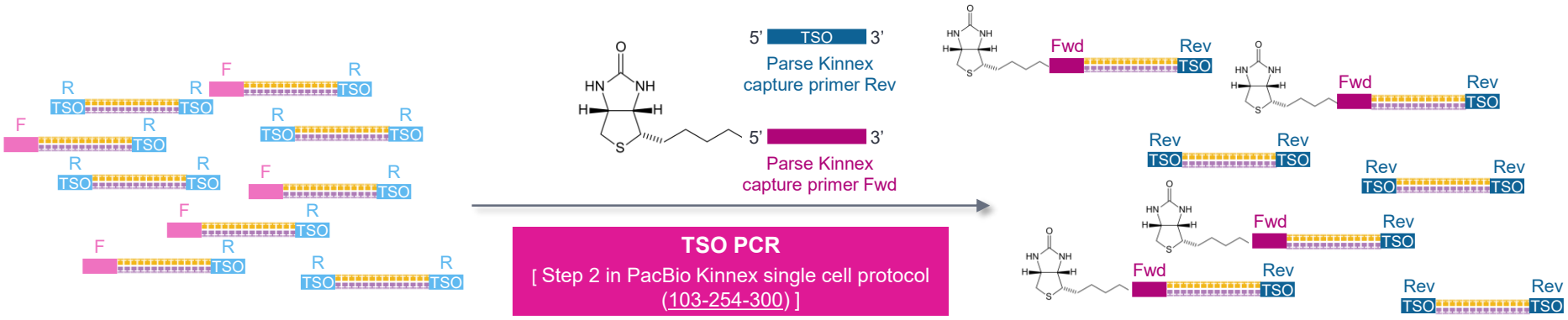
/5Biosg/AA/ideoxyU/CTACACGACGCTCTT
CCGATCTCAGACGTGTGCTCTTCCGATC



Parse Kinnex reverse capture primer

AAGCAGTGGTATCAACGCAGAG





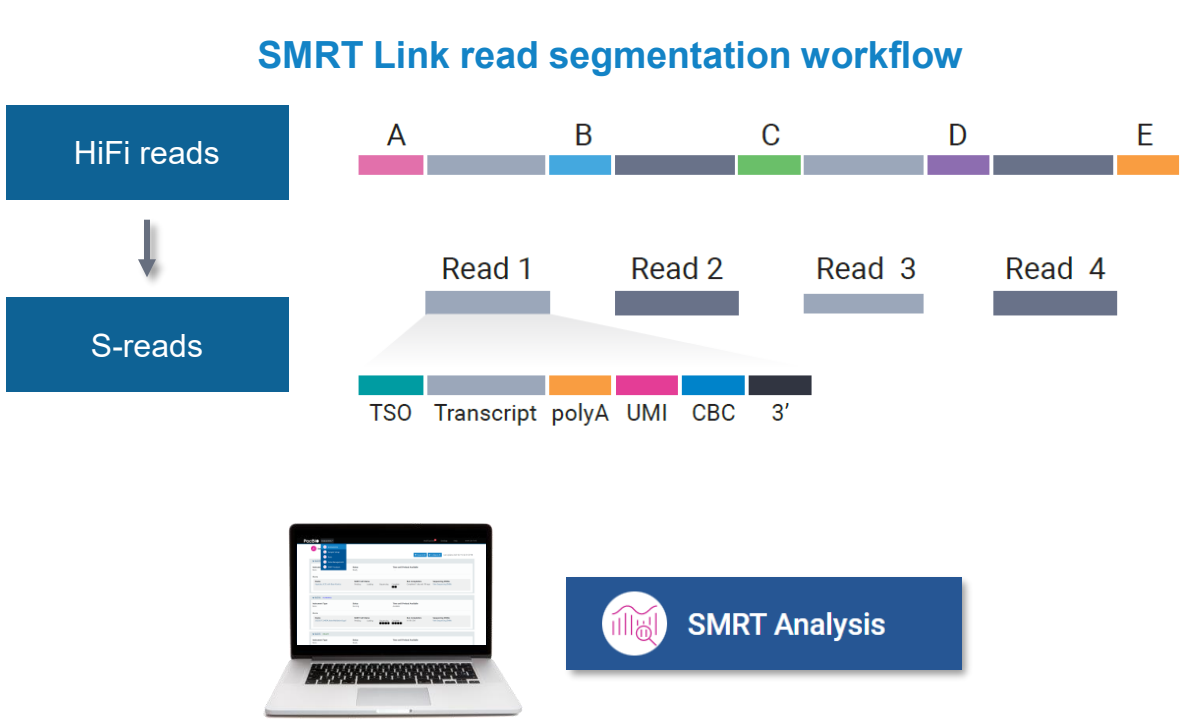
TSO PCR step in Kinnex single-cell RNA procedure uses a modified PCR primer (Parse Kinnex capture primer Fwd) to incorporate a biotin tag into desired cDNA products with the correct structure.[1]

[1] The subsequent TSO artifact removal step (Step 3) in the **Kinnex single-cell protocol** (103-254-300) uses streptavidin-coated Kinnex capture beads to pull down and enrich for biotin-tagged cDNA products with the correct structure.

SMRT Link informatics and secondary analysis recommendations for Kinnex single-cell libraries generated with Parse Evercode Whole Transcriptome kits

Read Segmentation can be run in SMRT Link (or skera in the command line) to generate segmented reads

- Note: The SMRT Link single-cell Iso-Seq workflow does not currently support analysis of Parse Biosciences data.
- Instead, Read Segmentation can be run in SMRT Link (or skera in the command line) to generate segmented reads (S-reads).
- The S-reads will contain the original single-cell or single-nucleus cDNA-containing barcode information. Refer to Rebboah et al. (2021) (section Preprocessing of LR-Split-seq data)[1] and Parse Biosciences long-read data processing guide[2] (login required) for secondary analysis recommendations using command line and community tools.

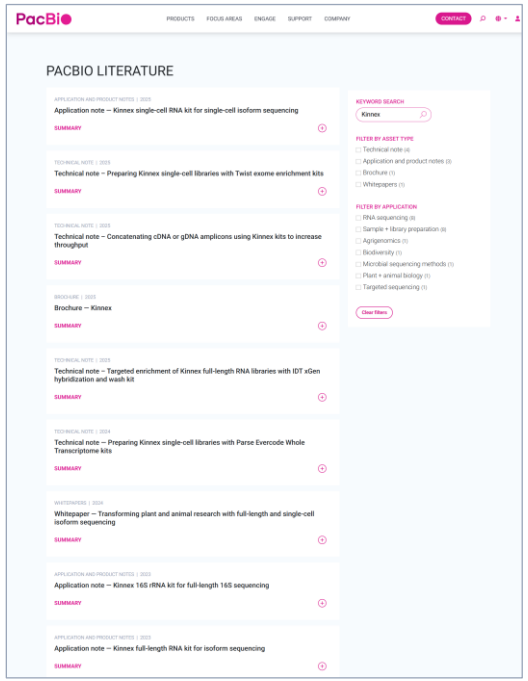


1 Rebboah, E., et al. (2021). Mapping and modeling the genomic basis of differential RNA isoform expression at single-cell resolution with LR-Split-seq. Genome biology, 22, 1-28 [Link].
2 Workflow-for-Processing-Long-Read-Data-with-the-Parse-Biosciences-Pipeline. Parse Biosciences long-read data processing guide.

Preparing Kinnex single-cell libraries with Twist exome enrichment kits

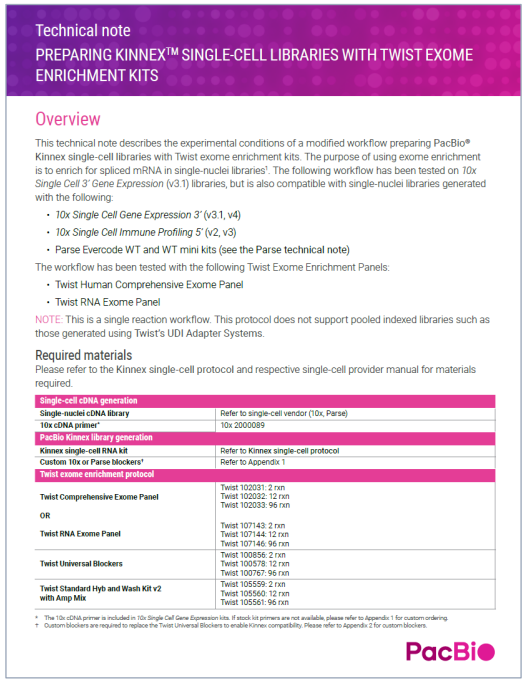
Preparing Kinnex single-cell libraries with Twist exome enrichment kits: Getting started

Application-specific educational literature → **Application-specific protocol documentation** → **Application-specific technical overviews**



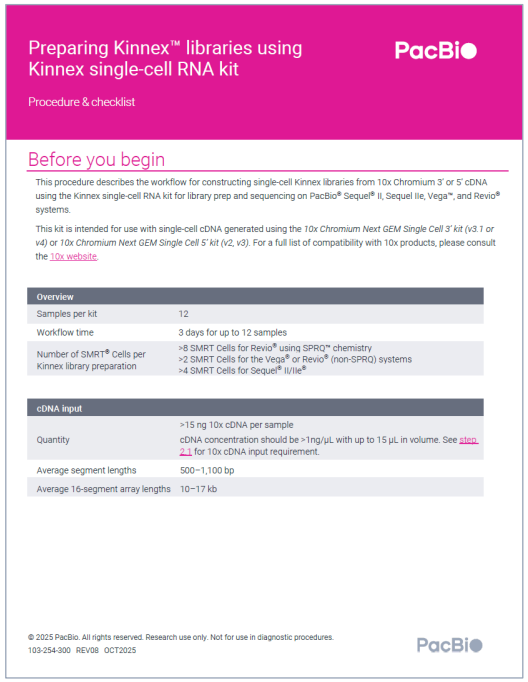
PacBio literature website [Link]

Application-specific brochures, informational guides and other product literature containing best practices recommendations for library preparation and data analysis workflows.



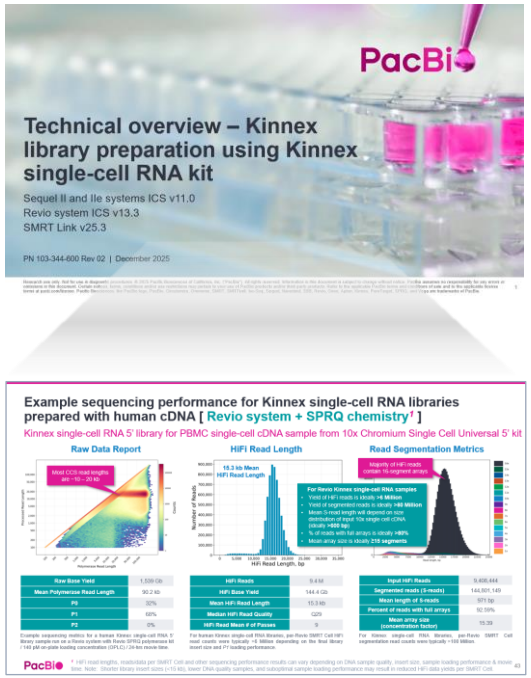
Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633) [PacBio]

Technical documentation containing a modified version of Twist Bioscience hybrid capture target enrichment protocol for generating enriched cDNA libraries for PacBio sequencing.



Procedure & checklist – Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

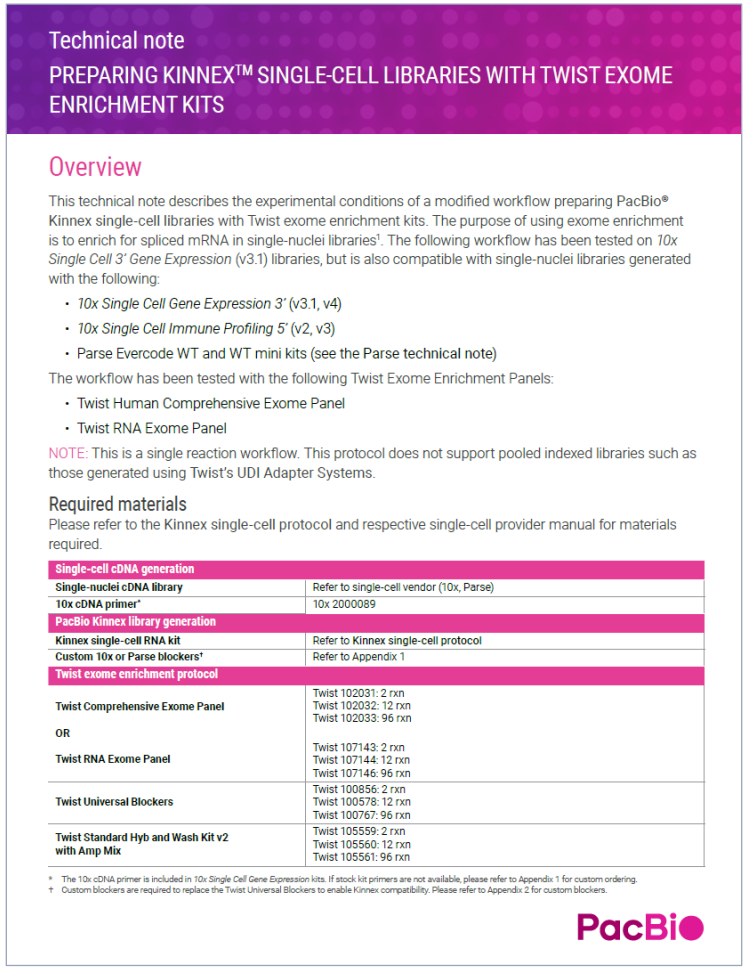


Technical overview: Kinnex library preparation using Kinnex single-cell RNA kit (103-344-600)

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

Technical reference for preparing Kinnex single-cell libraries with Twist exome enrichment kits

Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633) describes a modified workflow for preparing PacBio Kinnex single-cell libraries with Twist exome enrichment kits



Technical note

PREPARING KINNEX™ SINGLE-CELL LIBRARIES WITH TWIST EXOME ENRICHMENT KITS

Overview

This technical note describes the experimental conditions of a modified workflow preparing PacBio® Kinnex single-cell libraries with Twist exome enrichment kits. The purpose of using exome enrichment is to enrich for spliced mRNA in single-nuclei libraries[1]. The following workflow has been tested on *10x Single Cell 3' Gene Expression* (v3.1) libraries, but is also compatible with single-nuclei libraries generated with the following:

- *10x Single Cell Gene Expression 3'* (v3.1, v4)
- *10x Single Cell Immune Profiling 5'* (v2, v3)
- Parse Evercode WT and WT mini kits (see the Parse technical note)

The workflow has been tested with the following Twist Exome Enrichment Panels:

- Twist Human Comprehensive Exome Panel
- Twist RNA Exome Panel

NOTE: This is a single reaction workflow. This protocol does not support pooled indexed libraries such as those generated using Twist's UDI Adapter Systems.

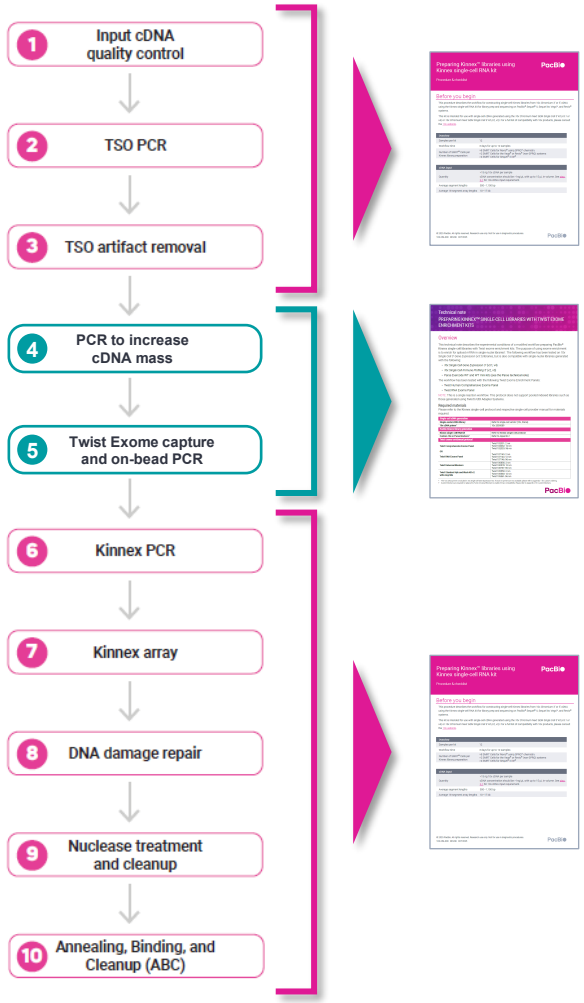
Required materials

Please refer to the Kinnex single-cell protocol and respective single-cell provider manual for materials required.

Single-cell cDNA generation	
Single-nuclei cDNA library	Refer to single-cell vendor (10x, Parse)
10x cDNA primer*	10x 2000089
PacBio Kinnex library generation	
Kinnex single-cell RNA kit	Refer to Kinnex single-cell protocol
Custom 10x or Parse blockers†	Refer to Appendix 1
Twist exome enrichment protocol	
Twist Comprehensive Exome Panel **OR** **Twist RNA Exome Panel**	Twist 102031: 2 rxn Twist 102032: 12 rxn Twist 102033: 96 rxn Twist 107143: 2 rxn Twist 107144: 12 rxn Twist 107146: 96 rxn
Twist Universal Blockers	Twist 100856: 2 rxn Twist 100578: 12 rxn Twist 100767: 96 rxn
Twist Standard Hyb and Wash Kit v2 with Amp Mix	Twist 105559: 2 rxn Twist 105560: 12 rxn Twist 105561: 96 rxn

* The 10x cDNA primer is included in *10x Single Cell Gene Expression* kits. If stock kit primers are not available, please refer to Appendix 1 for custom ordering.
† Custom blockers are required to replace the Twist Universal Blockers to enable Kinnex compatibility. Please refer to Appendix 2 for custom blockers.

PacBio



PacBio Procedure & checklist

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

PacBio Technical note

Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633)

[Select to use either Twist Human Comprehensive Exome Panel or Twist RNA Exome Panel]

PacBio Procedure & checklist

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

Overview

- The purpose of using Twist exome enrichment is to enrich for spliced mRNA in single-nuclei libraries[1]
- This workflow has been tested on *10x Single Cell 3' Gene Expression (v3.1)* libraries, but is also compatible with single-nuclei libraries generated with the following:
  - 10x Single Cell Gene Expression 3' (v3.1, v4)
  - 10x Single Cell Immune Profiling 5' (v2, v3)
  - Parse Biosciences Evercode WT and WT mini whole transcriptome kits (see Parse Technical note 102-326-610)
- The workflow has been tested with the following Twist Exome Enrichment Panels:
  - Twist Human Comprehensive Exome Panel
  - Twist RNA Exome Panel
- **NOTE:** This is a single reaction workflow. This protocol **does not** support pooled indexed libraries such as those generated using Twist's UDI Adapter Systems

PacBio

[1] Hardwick, S. A., Hu, W., Joglekar, A., et al. (2022). Single-nuclei isoform RNA sequencing unlocks barcoded exon connectivity in frozen brain tissue. Nature Biotechnology, 40(7), 1082-1092. https://doi.org/10.1038/s41587-022-01231-3.

Workflow overview for preparing Kinnex single-cell libraries with Twist exome enrichment kits

Follow PacBio Kinnex library prep protocol and modified Twist target enrichment procedure to generate single-cell hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

1. Input cDNA QC
2. TSO PCR
3. TSO artifact removal
4. PCR to increase cDNA mass
5. Twist exome capture & on-bead PCR
6. Kinnex PCR
7. Kinnex array formation
8. DNA damage repair
9. Nuclease treatment and cleanup

PacBio Kinnex library construction procedure[1]

Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)

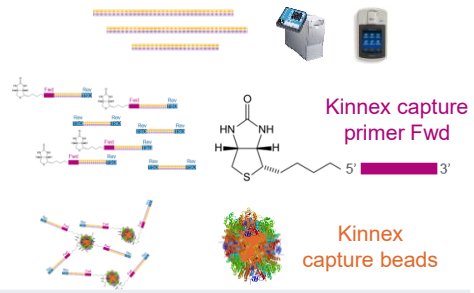
PacBio

Kinnex SC library prep procedure key steps

- Follow Kinnex protocol instructions until the end of the TSO artifact removal step
  - If using compatible 10x products, follow the **Kinnex single-cell library protocol** (103-254-300) through the end of **Step 3** (TSO artifact removal)[1]
- For **Step 4** (PCR to increase cDNA mass), this additional PCR step is required to generate sufficient cDNA mass for Twist exome enrichment (500 ng)
- After performing Twist exome capture and on-bead PCR (**Step 5**), proceed to the Kinnex PCR step of the **Kinnex single-cell library protocol** (103-254-300) and complete the remainder of the library construction workflow

Kinnex single-cell library construction workflow (Steps 1-3;6-9)

1 Input cDNA quality control
2 TSO PCR
3 TSO artifact removal

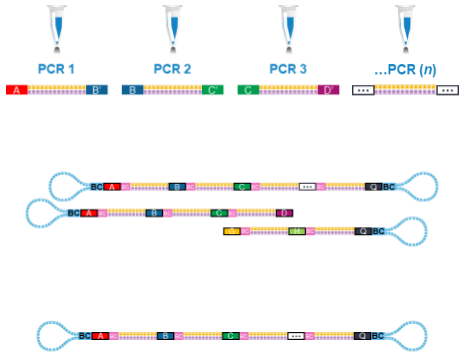


4. PCR to increase cDNA mass

5. Twist exome capture and on-bead PCR

→ See **PacBio Technical note**
Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633)

6 Kinnex PCR
7 Kinnex array
8 DNA repair
9 Nuclease treatment and cleanup



PacBio

[1] **Note:** If using compatible Parse Biosciences products, follow ***Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits*** (102-326-610) until the end of Step 3 (TSO artifact removal).

Workflow overview for preparing Kinnex single-cell libraries with Twist exome enrichment kits (cont.)

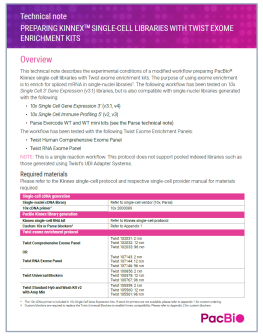
Follow PacBio Kinnex library prep protocol and modified Twist target enrichment procedure[1] to generate single-cell hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

1. Input cDNA QC
2. TSO PCR
3. TSO artifact removal
4. PCR to increase cDNA mass
5. Twist exome capture & on-bead PCR
6. Kinnex PCR
7. Kinnex array formation
8. DNA damage repair
9. Nuclease treatment and cleanup

Modified Twist hybrid capture procedure overview[1]

Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633)

PacBio



Twist library construction + Twist hybrid capture workflow

Twist exome capture & on-bead PCR procedure key steps[1]

- **Step 5.1: Twist exome capture setup**
  - Concentrate amplified cDNA sample (generated previously in Step 4) with 1.5X SMRTbell cleanup beads and resuspend in 12 µL of hybridization blocker solution.
- **Step 5.2: Twist probe hybridization**
  - Prepare probe solution and incubate the hybridization reaction at 70°C for 16 hours in a thermal cycler with the lid at 85°C.
- **Step 5.3: Bind hybridization capture reaction to Streptavidin beads**
  - Prepare streptavidin beads and bind targets by adding hybridization reaction solution to the beads. Incubate for 5 minutes at 68°C. Agitation is not required. Do not vortex.
- **Step 5.4: On-bead PCR amplification of Twist enriched library**
  - Prepare PCR mix and keep sample on ice until the thermal cycler lid has heated to 105°C. After PCR amplification is complete, perform cleanup with 1.5X SMRTbell cleanup beads and evaluate DNA quantification QC using Qubit assay.
- If sample quantity is acceptable (25 ng is required), proceed to the Kinnex PCR step of the **Kinnex single-cell protocol** (103-254-300) and complete the remainder of the library construction.

[1] The hybridization capture workflow described here is a modification of the **Twist Target Enrichment Standard Hybridization v2 Protocol** (DOC-001273).

PCR amplification primer and hybridization blocker oligo recommendations for preparing Kinnex single-cell libraries with Twist exome enrichment kits

For optimal library prep yields and on-target hybrid capture performance, order custom primers as described below from your preferred oligo vendor

10x cDNA primer[1] required for PCR amplification step to increase cDNA mass [Step 4]

- Order custom primers from your preferred oligo vendor.
- Standard desalt primers are acceptable.
- For each FWD and REV primer, add 10 µL of 10 µM to the PCR reaction to achieve a final concentration of 1 µM.

10x cDNA primer FWD

CTACACGACGCTCTTCCGATCT

10x cDNA primer REV

AAGCAGTGGTATCAACGCAGAG

Custom 10x or Parse blocker oligos[2] required for preparing samples for Twist hybridization capture reaction [Step 5.1]

- Order custom primers from your preferred oligo vendor.
- An example of IDT code is shown here.
- Blocker oligo primers should be HPLC-purified.
- Dilute blocker oligos to 10 µM and add 2 µL of each FWD and REV blocker.

10x Custom Blocker FWD

CTACACGACGCTCTTCCGATCT/3SpC3/

10x Custom Blocker REV

AAGCAGTGGTATCAACGCAGAG/3SpC3/

Parse Custom Blocker FWD

CTACACGACGCTCTTCCGATCTCAGACGTGTGCTCTTCCGATC/3SpC3/

Parse Custom Blocker REV

AAGCAGTGGTATCAACGCAGAG/3SpC3/

PacBio

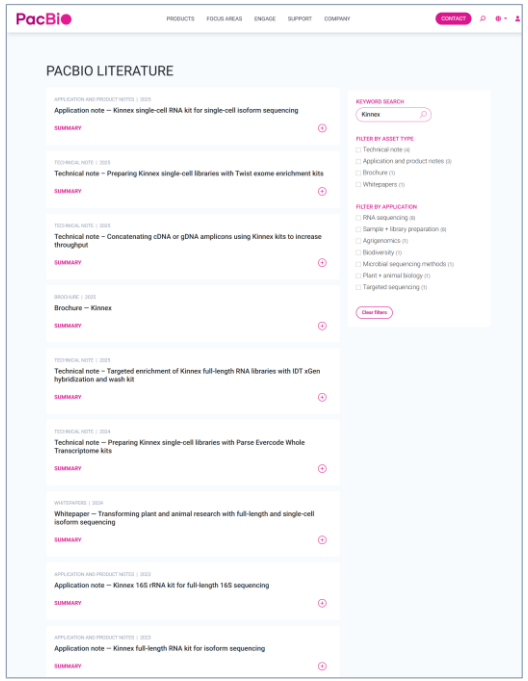
[1] The 10x cDNA primer is **included** in 10x Single Cell Gene Expression kits. If stock kit primers are not available, follow the recommendations described here for custom ordering (see Appendix 1 in ***Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits*** (102-326-633)).

[2] Custom blockers are required to replace the Twist Universal Blockers to enable Kinnex compatibility.

Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3

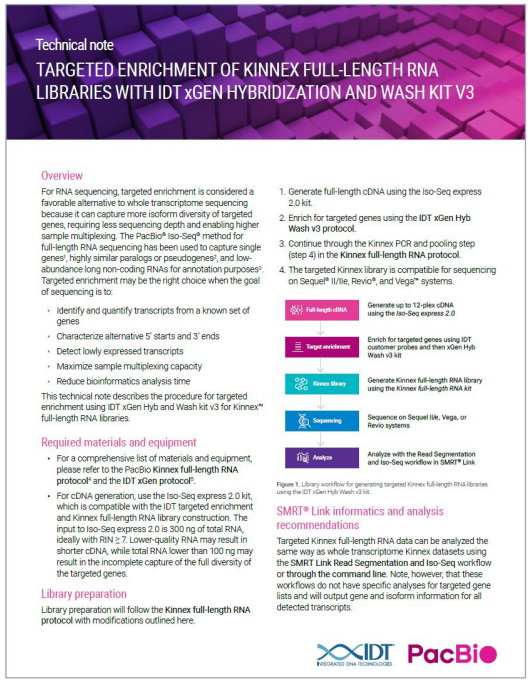
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3: Getting started

Application-specific educational literature → **Application-specific protocol documentation** → **Application-specific technical overviews**



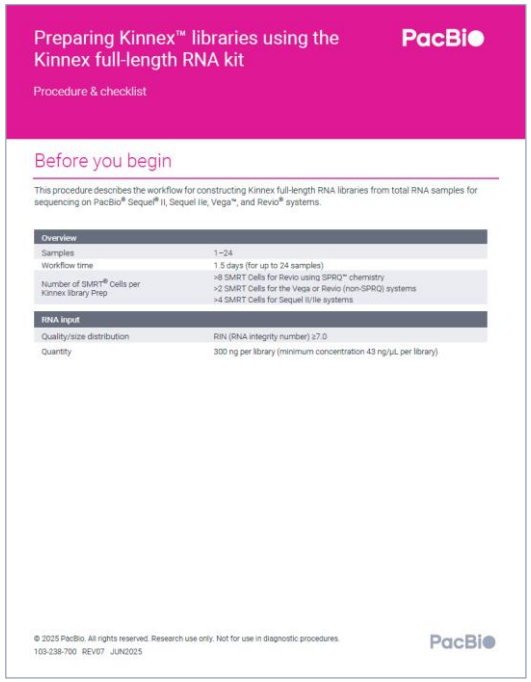
PacBio literature website [Link]

Application-specific brochures, informational guides and other product literature containing best practices recommendations for library preparation and data analysis workflows.



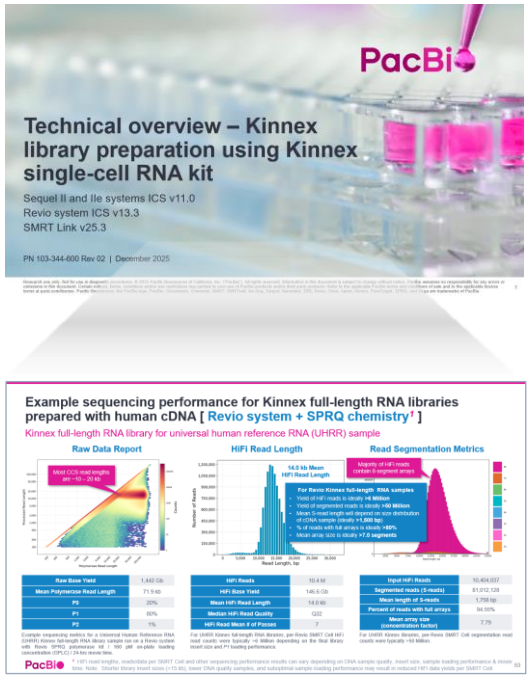
Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632) [PacBio]

Technical documentation describing the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for preparing Kinnex full-length RNA libraries for PacBio sequencing.



Procedure & checklist – Procedure & checklist – Preparing Kinnex libraries using Kinnex full-length RNA kit (103-238-700) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

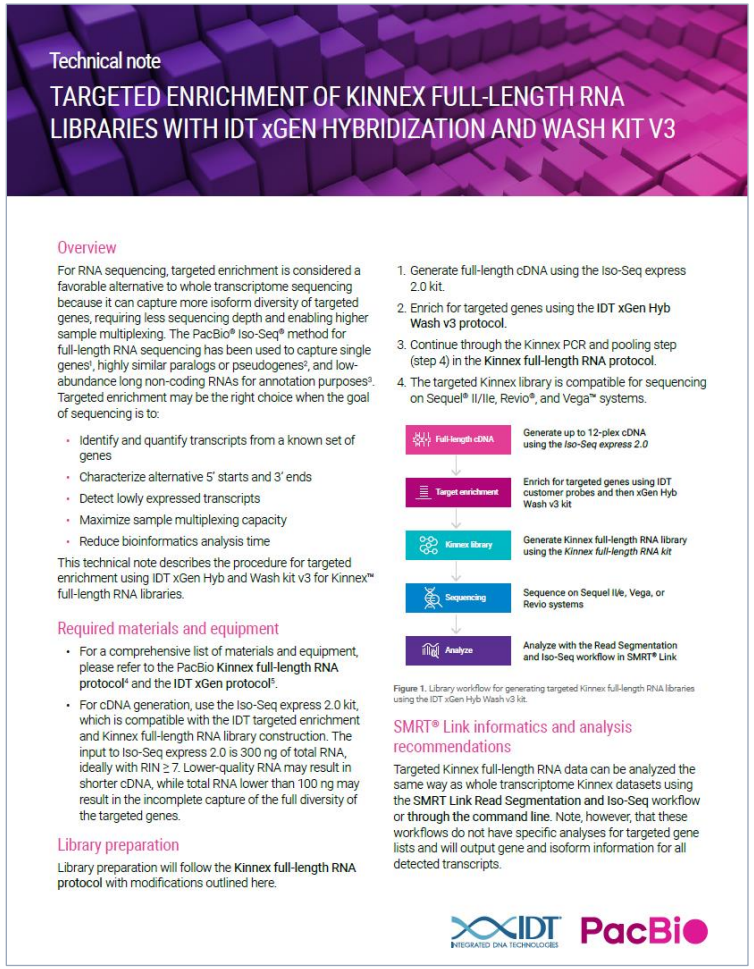


Technical overview: Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)

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

Technical reference for targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3

Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit (102-326-632) describes the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for Kinnex full-length RNA libraries



Technical note

TARGETED ENRICHMENT OF KINNEX FULL-LENGTH RNA LIBRARIES WITH IDT xGEN HYBRIDIZATION AND WASH KIT V3

Overview

For RNA sequencing, targeted enrichment is considered a favorable alternative to whole transcriptome sequencing because it can capture more isoform diversity of targeted genes, requiring less sequencing depth and enabling higher sample multiplexing. The PacBio® Iso-Seq® method for full-length RNA sequencing has been used to capture single genes[1], highly similar paralogs or pseudogenes[2], and low-abundance long non-coding RNAs for annotation purposes[3]. Targeted enrichment may be the right choice when the goal of sequencing is to:

- Identify and quantify transcripts from a known set of genes
- Characterize alternative 5' starts and 3' ends
- Detect lowly expressed transcripts
- Maximize sample multiplexing capacity
- Reduce bioinformatics analysis time

This technical note describes the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for Kinnex™ full-length RNA libraries.

Required materials and equipment

- For a comprehensive list of materials and equipment, please refer to the PacBio **Kinnex full-length RNA protocol**[4] and the **IDT xGen protocol**[5].
- For cDNA generation, use the Iso-Seq express 2.0 kit, which is compatible with the IDT targeted enrichment and Kinnex full-length RNA library construction. The input to Iso-Seq express 2.0 is 300 ng of total RNA, ideally with RIN ≥ 7. Lower-quality RNA may result in shorter cDNA, while total RNA lower than 100 ng may result in the incomplete capture of the full diversity of the targeted genes.

Library preparation

Library preparation will follow the **Kinnex full-length RNA protocol** with modifications outlined here.

1. Generate full-length cDNA using the Iso-Seq express 2.0 kit.
2. Enrich for targeted genes using the **IDT xGen Hyb Wash v3 protocol**.
3. Continue through the Kinnex PCR and pooling step (step 4) in the **Kinnex full-length RNA protocol**.
4. The targeted Kinnex library is compatible for sequencing on Sequel® II/IIe, Revio®, and Vega™ systems.

Full-length cDNA — Generate up to 12-plex cDNA using the *Iso-Seq express 2.0*

Target enrichment — Enrich for targeted genes using IDT customer probes and then xGen Hyb Wash v3 kit

Kinnex library — Generate Kinnex full-length RNA library using the *Kinnex full-length RNA kit*

Sequencing — Sequence on Sequel IIe, Vega, or Revio systems

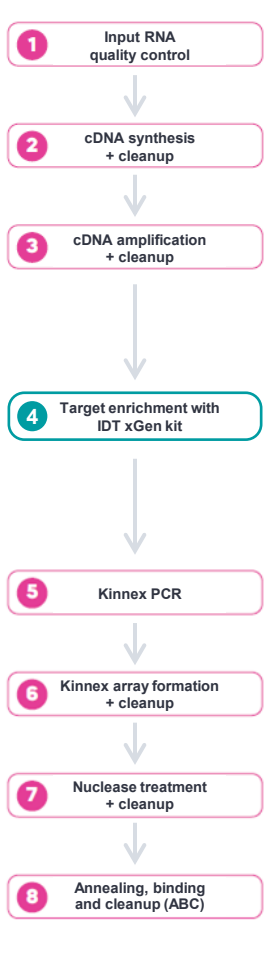
Analyze — Analyze with the Read Segmentation and Iso-Seq workflow in SMRT® Link

Figure 1. Library workflow for generating targeted Kinnex full-length RNA libraries using the IDT xGen Hyb Wash v3 kit.

SMRT® Link informatics and analysis recommendations

Targeted Kinnex full-length RNA data can be analyzed the same way as whole transcriptome Kinnex datasets using the **SMRT Link Read Segmentation and Iso-Seq** workflow or **through the command line**. Note, however, that these workflows do not have specific analyses for targeted gene lists and will output gene and isoform information for all detected transcripts.

IDT INTEGRATED DNA TECHNOLOGIES PacBio



PacBio Procedure & checklist

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

PacBio Technical note

Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632)[1]

PacBio Procedure & checklist

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

Overview

- For RNA sequencing, **targeted enrichment** may be the right choice when the goal of sequencing is to:
  - Identify and quantify transcripts from a known set of genes
  - Characterize alternative 5' starts and 3' ends
  - Detect lowly expressed transcripts
  - Maximize sample multiplexing capacity
  - Reduce bioinformatics analysis time
- ***Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3*** (102-326-632) describes the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for Kinnex full-length RNA libraries

PacBio

[1] For step-by-step IDT hybridization capture workflow details, refer to the **IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol** (RUO24-3081_001).

Workflow overview for targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3

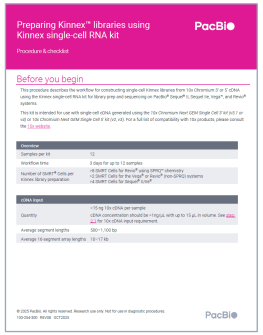
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

1. Input RNA QC
2. cDNA synthesis + cleanup
3. cDNA amplification + cleanup
4. Target enrichment with IDT xGen kit
5. Kinnex PCR
6. Kinnex array formation + cleanup
7. Nuclease treatment + cleanup

PacBio Kinnex library construction procedure[1]

Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

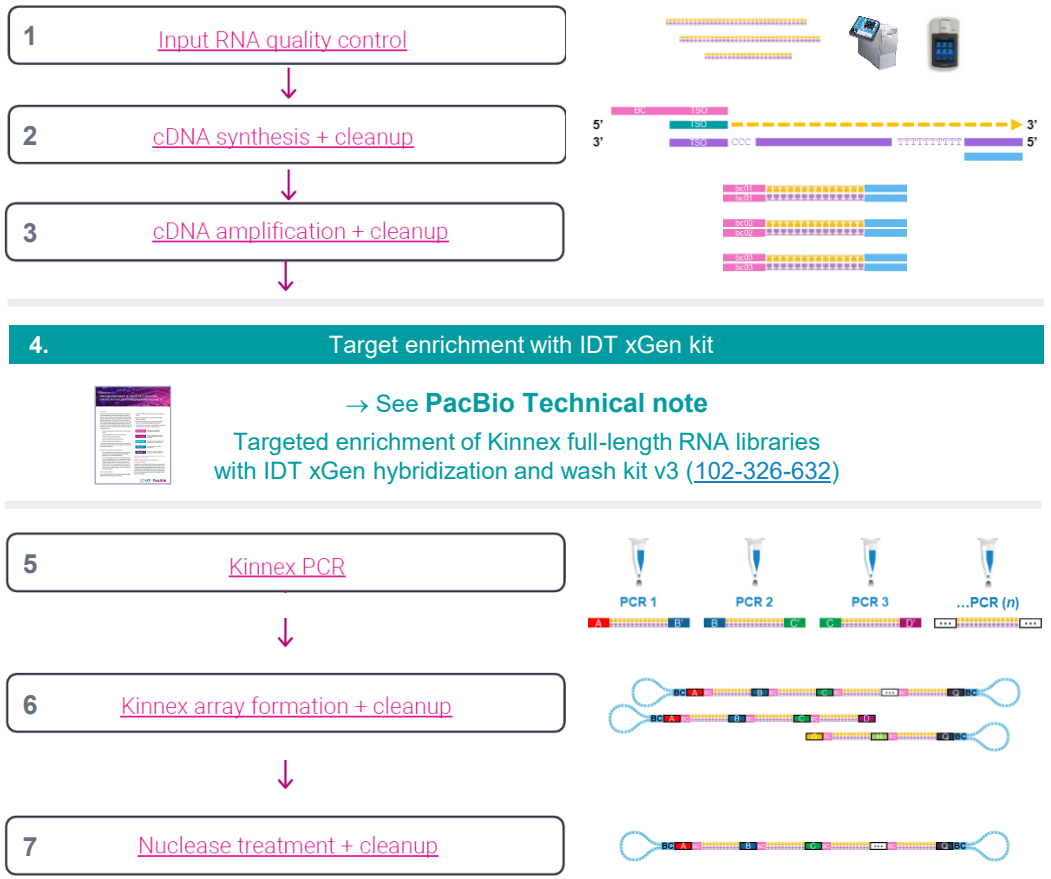
PacBio



Kinnex full-length RNA library prep procedure key steps

- For cDNA generation, use the Iso-Seq express 2.0 kit with 300 ng of total RNA input, ideally with RIN ≥ 7.[1]
- Library preparation will follow the **Kinnex full-length RNA protocol** (103-238-700) with modifications outlined here:
  - Generate full-length cDNA using the Iso-Seq express 2.0 kit by following **Steps 1-3** in the Kinnex full-length RNA protocol.
  - Enrich for targeted genes using the **IDT xGen Hyb Wash v3 protocol** (RUO24-3081_001; also see **IDT xGen Technical note** (102-326-632)).
  - Continue through the **Kinnex PCR step** in the Kinnex full-length RNA protocol (page 13 Step 4) and complete the remainder of the library construction workflow

Kinnex full-length RNA library construction workflow (Steps 1-3;5-7)



[1] Lower-quality RNA may result in shorter cDNA, while total RNA lower than 100 ng may result in the incomplete capture of the full diversity of the targeted genes.

Workflow overview for targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (cont.)

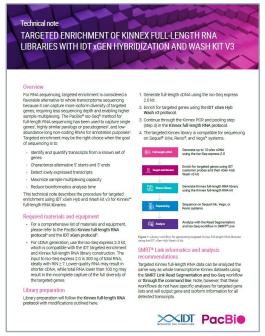
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

1. Input RNA QC
2. cDNA synthesis + cleanup
3. cDNA amplification + cleanup
4. Target enrichment with IDT xGen kit
5. Kinnex PCR
6. Kinnex array formation + cleanup
7. Nuclease treatment + cleanup

IDT xGen hybrid capture procedure overview[1]

Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632)

PacBio



IDT xGEN hybrid capture procedure key steps[1]

- Add Blocker Master Mix to each cDNA sample (500 ng[2]) and dry down
- Add Hybridization Master Mix to each sample well and incubate for desired hybridization time.[3]
- Prepare hybridization wash buffer and capture beads within 2 hours before use
- Add (streptavidin) capture beads to sample (30 min)
- Perform 3 rounds of washing with wash buffers
- Perform post-capture PCR reaction + cleanup
- Perform DNA quantification QC using Qubit assay and DNA sizing QC using Agilent TapeStation or other equivalent system

IDT xGEN hybrid capture workflow

IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol (RUO24-3081_001).

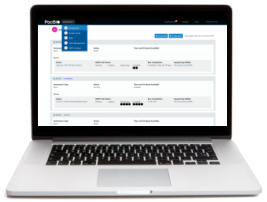
1 For hybridization capture workflow details, refer to the **IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol** (RUO24-3081_001).
2 100 ng to 6 µg cDNA inputs can be used for short hybridizations (1-2 hrs). Generally, we recommend not to exceed 2.5 µg total input for 1-hr hybridization time for very large panels (>30 Mb).
3 This procedure supports short hybridization times of only 1 hr as well as the standard 4- or 16-hr incubations. We recommend users empirically test the shorter hybridization time for their workflows.

PacBio

SMRT Link informatics and secondary analysis recommendations for targeted Kinnex full-length RNA data

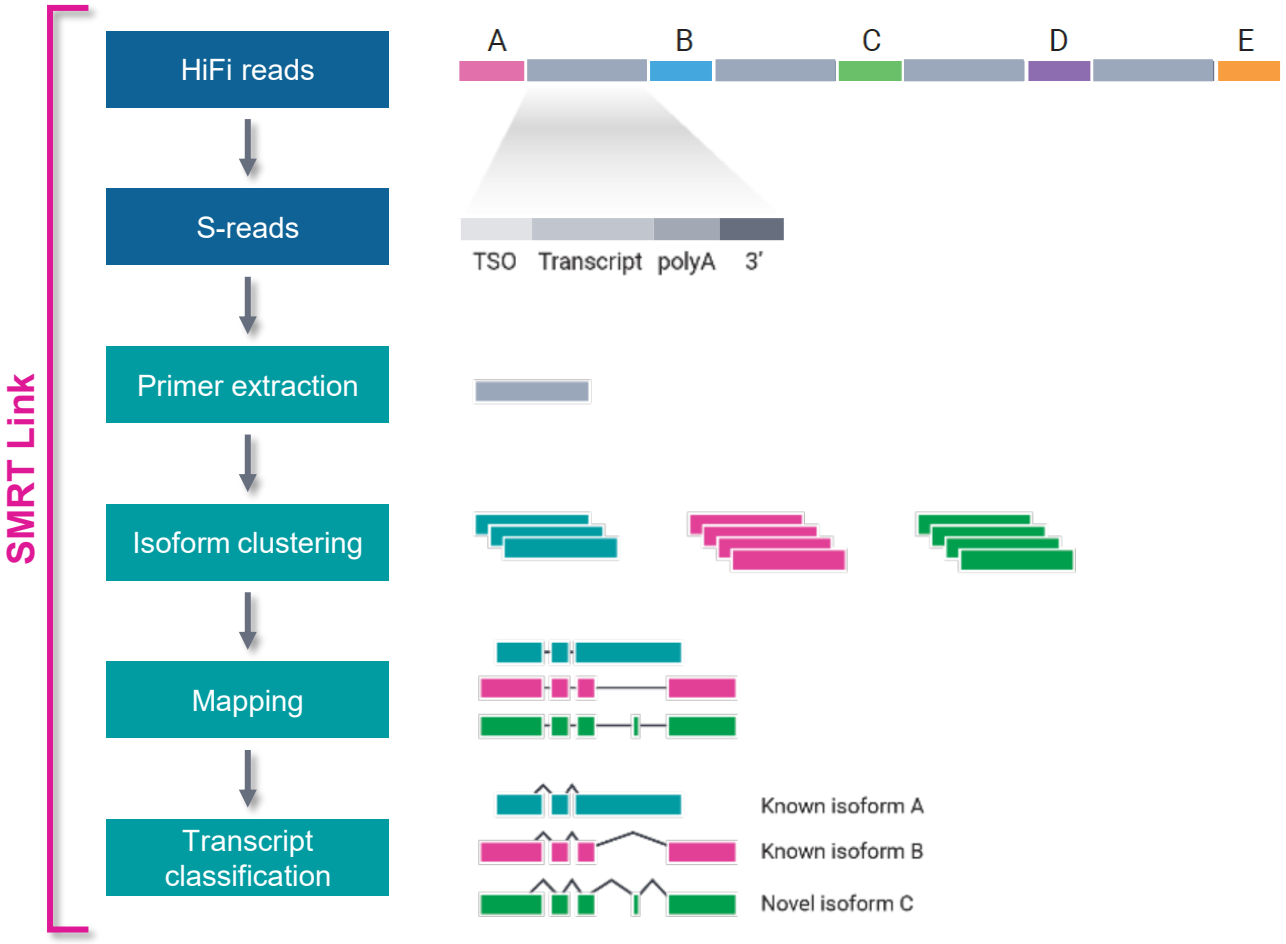
Use SMRT Link Read Segmentation and Iso-Seq application to perform isoform-classification analysis to identify novel genes & isoforms with abundance information

- Targeted Kinnex full-length RNA data can be analyzed the same way as whole transcriptome Kinnex datasets using the SMRT Link Read Segmentation and Iso-Seq workflow or through the command line.
- Note, however, that these workflows do not have specific analyses for targeted gene lists and will output gene and isoform information for all detected transcripts.



SMRT Analysis

SMRT Link read segmentation and Iso-Seq workflow[1]



[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for Read Segmentation and Iso-Seq analysis application.

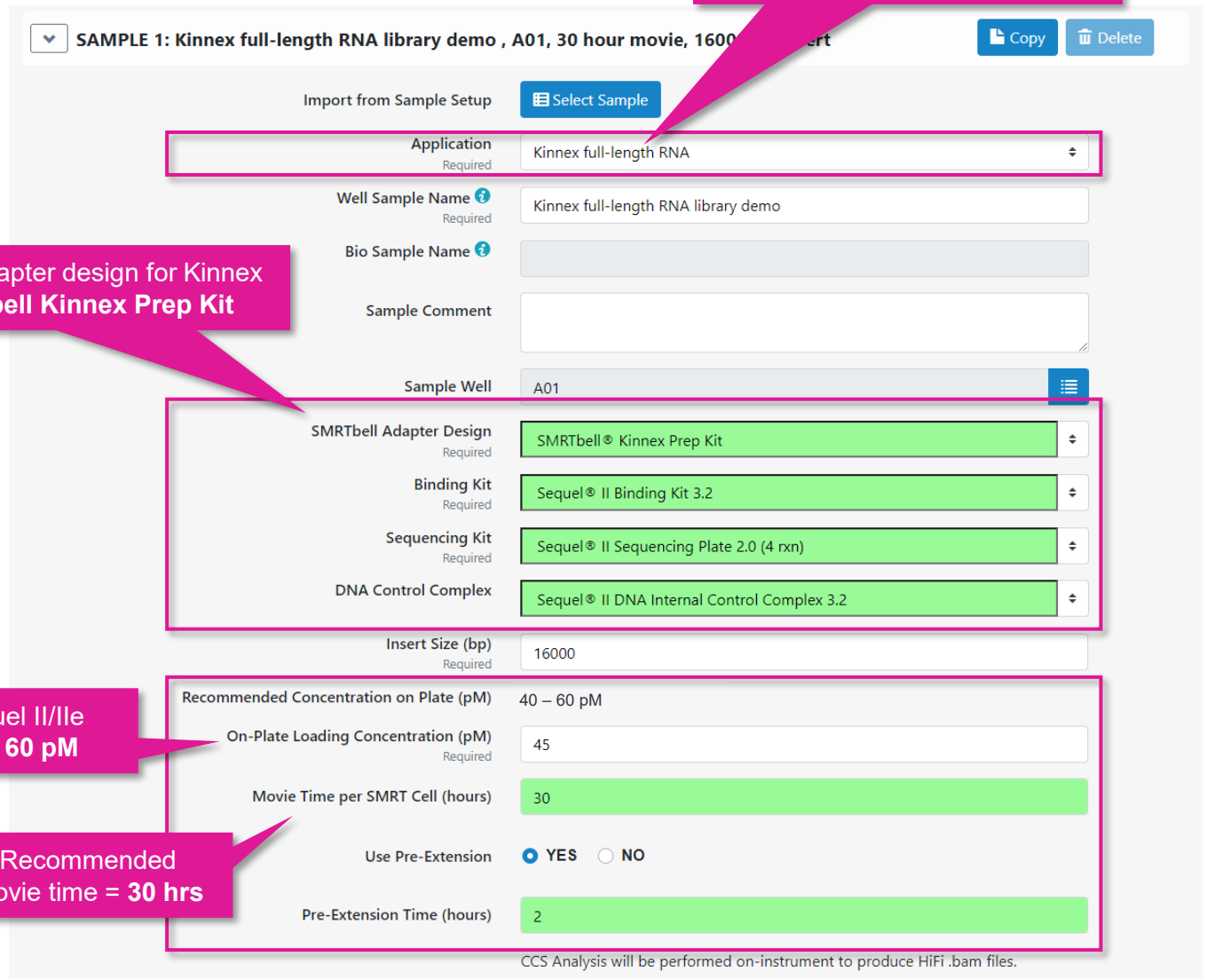
APPENDIX 3: SMRT Link run design procedure for Sequel IIe system

SMRT Link Run Design procedure for Sequel IIe system

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]



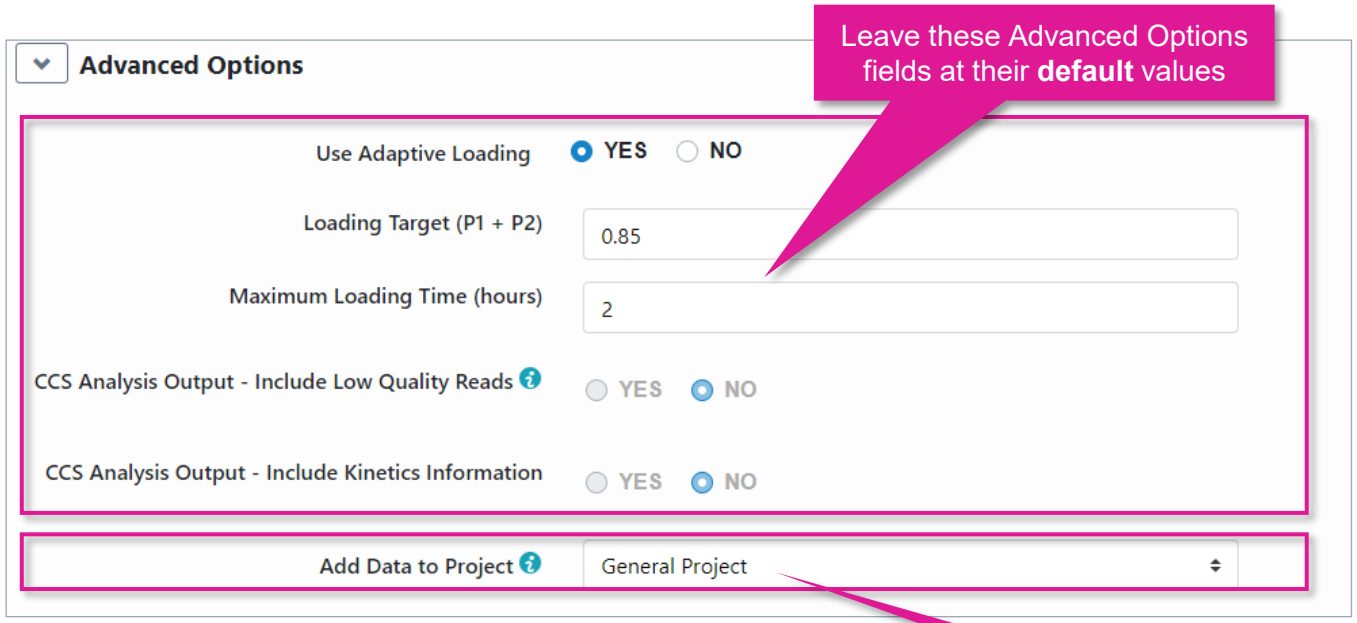
Example sample information entered into a Sequel IIe system run design worksheet for a Kinnex full-length RNA library sample.

[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 IIe system (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



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

SMRT Link Run Design procedure for Sequel IIe system (cont.)

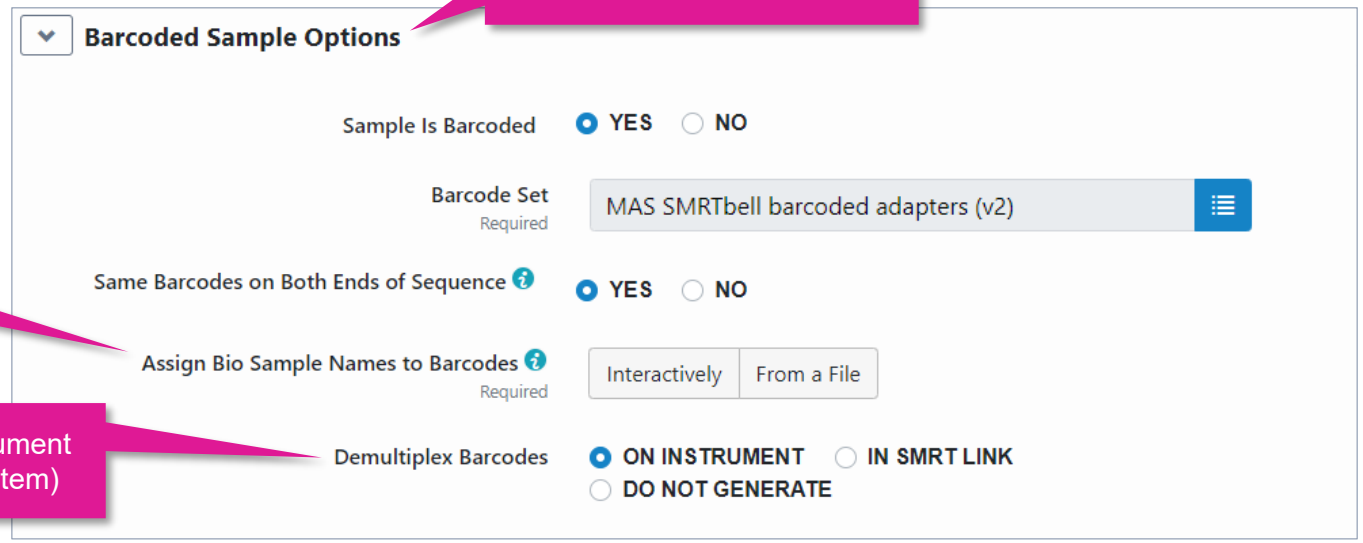
Barcoded sample options

- 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)



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

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Revision history (description)	Version	Date
Updated example Kinnex sequencing performance data for PacBio long-read systems (Revio system + SPRQ chemistry and Vega system). Also added information about PacBio compatible Kinnex library preparation workflows using third-party kit products.	02	December 2025