



Technical overview

Kinnex library preparation using Kinnex prep kits

Revio system ICS v13.5

Vega system ICS v1.2

SMRT Link v26.2

PN 103-911-600 Rev 01 | October 2026

Technical overview

Kinnex library preparation using Kinnex prep kits

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Introduction

What is Kinnex?

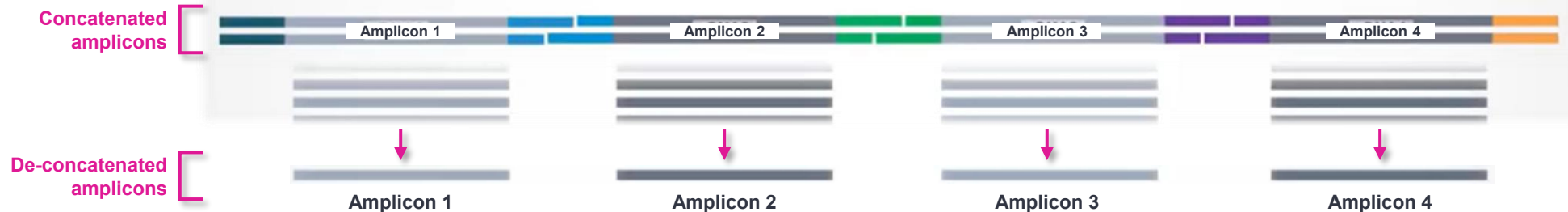
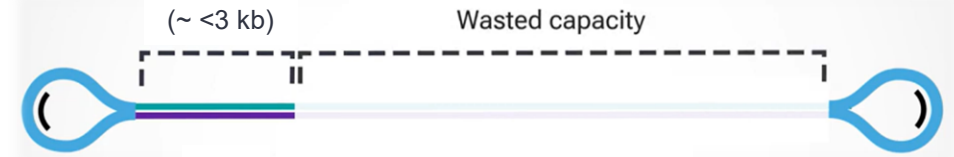
Kinnex is a general amplicon concatenation method for increasing HiFi sequencing data yield

- PacBio **Kinnex kits** are based on the MAS-Seq (Multiplexed Arrays Isoform Sequencing) method¹ originally developed by Al'Khafaji et al. (2023) to concatenate cDNAs into longer fragments suitable for long-read sequencing
- PacBio Kinnex method takes advantage of the disparity between optimal DNA fragment lengths for HiFi sequencing, 15–20 kb, and smaller amplicon sizes (e.g., <3 kb)
- By **concatenating** amplicons into larger fragments, **PacBio Kinnex method increases HiFi data throughput per run** (i.e., per SMRT Cell acquisition)²

Large insert HiFi libraries are ideal for whole genome sequencing...



...but for smaller amplicon sizes, the extra sequencing capacity is wasted



Kinnex method concatenates smaller amplicons into larger fragments to be sequenced with HiFi

→ HiFi reads can be bioinformatically deconcatenated to represent the original amplicon molecules

When is Kinnex concatenation appropriate?

Kinnex concatenation is generally suitable for amplicons with an average insert size <3 kb

- Kinnex concatenation is a method that increases sequencing throughput for **smaller amplicons** (e.g., cDNA or other gDNA amplicons <3 kb)
- The **balance between the amplicon size and the concatenation factor**, as well as additional Kinnex library generation cost, needs to be taken into consideration¹
- HiFi sequencing produces optimal yield for libraries between 15 – 20 kb; therefore, the throughput advantage with the Kinnex concatenation method plateaus for larger amplicon sizes exceeding 3 kb.

You can consider concatenating amplicons using Kinnex kits if:

- ✓ The amplicons have an **average size <3 kb**²
- ✓ The amplicons have molecular ends that are either directly compatible or can be re-amplified to establish **Kinnex compatibility**

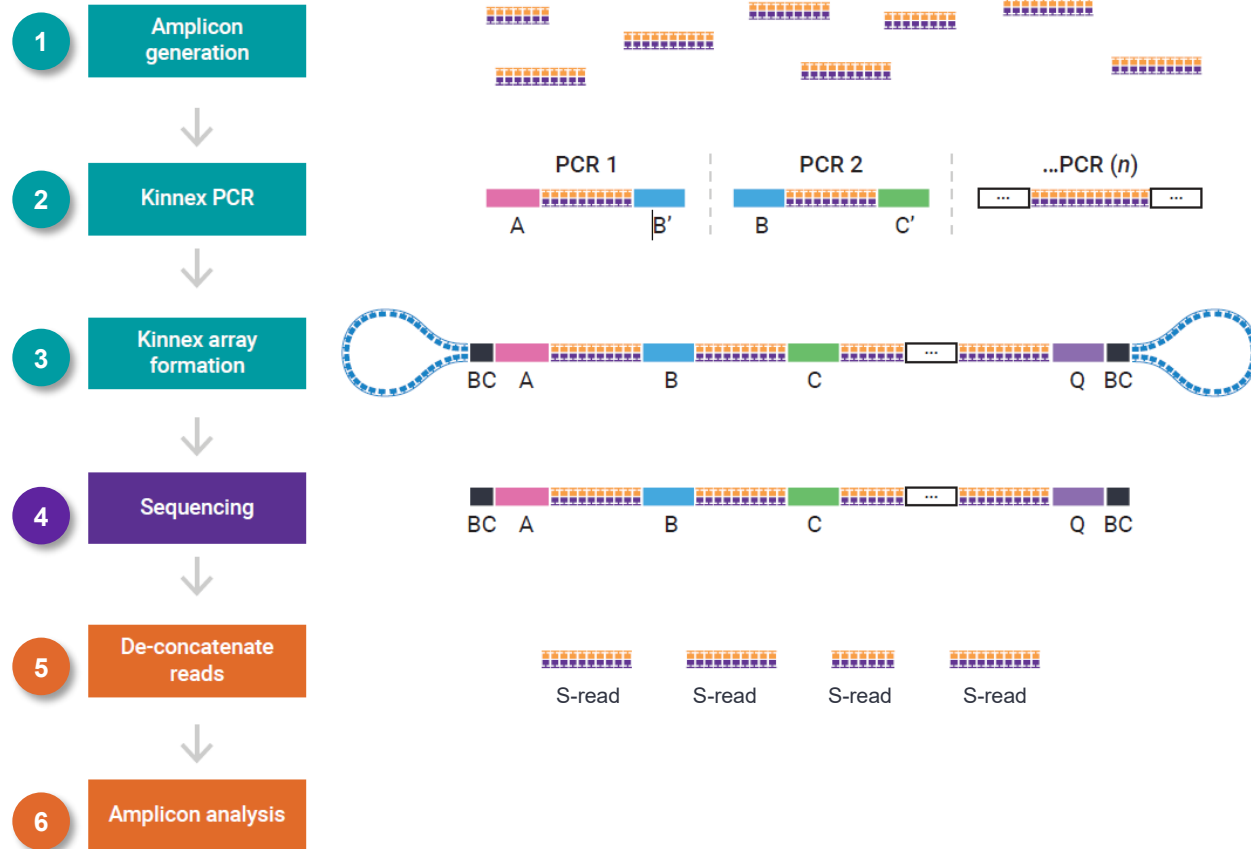
The logo for Kinnex v2.0 features a stylized icon of four interconnected circles to the left of the text "KINNEX v2.0" in a bold, purple, sans-serif font.

Benefits of Kinnex concatenation include:

- **Increased throughput** on PacBio long-read sequencers
- **Retention of HiFi accuracy** levels in conjunction with increased throughput levels
- **No change to secondary analysis** – once HiFi reads are de-concatenated into segmented reads (**S-reads**), the S-reads represent the original, pre-concatenated amplicon and can be analyzed with already-established pipelines.
 - For example, de-concatenated 16S amplicons can be analyzed using the PacBio HiFi-16S-workflow pipeline, while de-concatenated bulk cDNA amplicons can be analyzed using the PacBio Iso-Seq workflow

Kinnex concatenation workflow overview

Key steps in Kinnex concatenation workflow include amplicon generation, Kinnex PCR, and Kinnex array formation



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 workflow key steps¹

- 1. Amplicon generation:** Amplicons are required to have Kinnex-compatible molecular ends (see later section)
 - Once these are generated, choose the appropriate Kinnex kit based on the average amplicon size (or target application) and proceed with Kinnex PCR
- 2. Kinnex PCR:** Run parallel PCR reactions per sample [i.e., 8, 12, or 16 reactions based on the Kinnex kit chosen] using premixed Kinnex primer pairs
 - This step generates amplified DNA products containing **programmable sequences** at both ends
- 3. Kinnex array formation:** Library inserts containing programmable ends are assembled to generate a linear array
 - Ligation of barcoded Kinnex terminal adapters results 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 HiFi sequencing yields
- 4. Sequencing:** 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 on Revio and Vega systems.
- 5. De-concatenate reads:** Once HiFi reads are generated, run SMRT Link **Read Segmentation** data utility to produce segmented reads (**S-reads**) that represent the original unconcatenated amplicons
- 6. Amplicon analysis:** Use SMRT Link or command line tools to perform **downstream secondary analysis** of recovered amplicon segment reads

Experimental design considerations for concatenating amplicons to increase HiFi sequencing data throughput

Kinnex v2.0 workflows enable flexibility for choosing different Kinnex prep kits based on input amplicon average insert size

Kinnex prep kit recommendations for different input amplicon size ranges

- HiFi sequencing produces optimal data yield for inserts between 15–20 kb; therefore, the data throughput advantage with Kinnex concatenation methods plateaus for larger amplicon sizes exceeding 3 kb
- You can consider concatenating amplicons using Kinnex kits if the amplicons have an **average size <3 kb**¹

Input amplicon average insert size ¹	Default recommended Kinnex prep kit	Expected throughput (Segmented reads or ‘S-reads’)
<1 kb	Kinnex 16-fold prep kit (103-836-900)	Revio (SPRQ-Nx): 100 – 150 M Vega (SPRQ-Nx): 80 – 100 M
1 – 2 kb	Kinnex 12-fold prep kit (103-072-100)	Revio (SPRQ-Nx): 70 – 100 M Vega (SPRQ-Nx): 50 – 60 M
1.5 – 3 kb	Kinnex 8-fold prep kit (103-072-000)	Revio (SPRQ-Nx): 50 – 70 M Vega (SPRQ-Nx): 40 – 50 M
>3 kb	Not recommended for Kinnex concatenation	

Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput (102-326-636) provides guidance on selecting an appropriate Kinnex concatenation level based on input amplicon size range

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.

What is Kinnex?

The Kinnex kits are based on the method developed by AlKhafaji et al. (2023). Originally termed multiplexed array isoform sequencing (MAIS-ISO-seq or MAIS-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 MAIS-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 MAIS-Seq method and adjusted the concatenation factors according to different average amplicon sizes (Table 1).

How Kinnex works

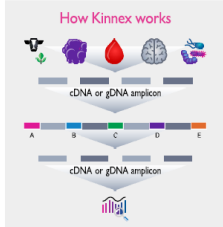


Figure 1. How Kinnex works. Watch the Kinnex animation to learn more.

Average size	Recommended kit	Protocol
200–1,000 bp	Kinnex 16-fold prep kit (103-836-900)	Preparing Kinnex libraries using the Kinnex 16-fold prep kit
1–2 kb	Kinnex 12-fold prep kit (103-072-100)	Preparing Kinnex libraries using the Kinnex 12-fold prep kit
1.5–3 kb	Kinnex 8-fold prep kit (103-072-000)	Preparing Kinnex libraries using the Kinnex 8-fold prep kit
>3 kb	Not recommended for Kinnex concatenation	

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

PacBio

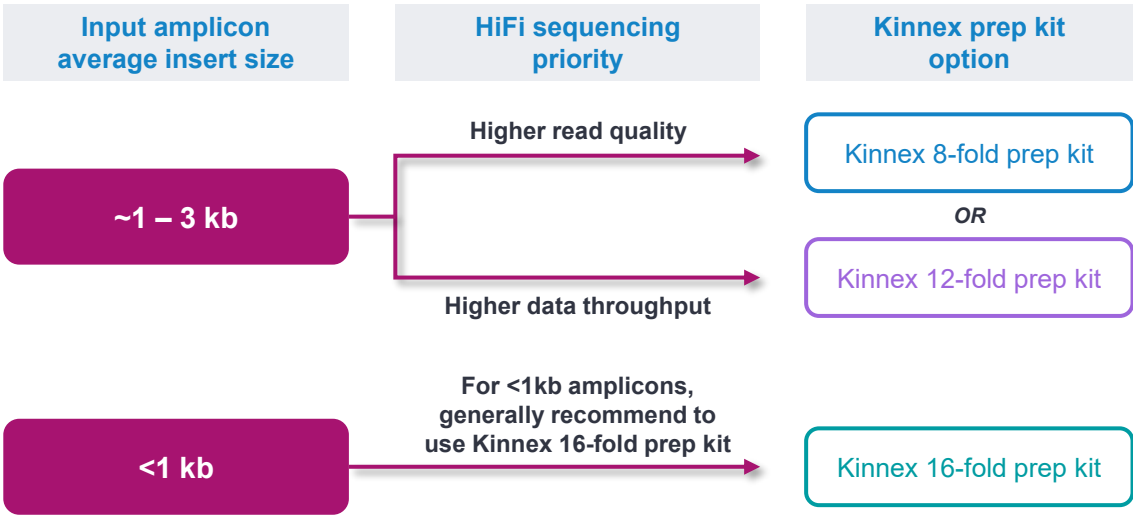
Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput (102-326-636).

Experimental design considerations for concatenating amplicons to increase HiFi sequencing data throughput (cont.)

Users may consider choosing a higher-fold Kinnex prep kit for increased data throughput or a lower-fold Kinnex prep kit for increased read quality

Kinnex prep kit options to achieve either higher data throughput or higher read quality

- Selecting a **higher-fold Kinnex prep kit** can enable **higher data throughput** (i.e., higher number of segmented reads (“S-reads”) per acquisition) [**Note:** The final Kinnex concatenated HiFi library size should not greatly exceed the optimal range of 15 – 20 kb.]
- Selecting a **lower-fold Kinnex prep kit** can enable **higher read quality** (i.e., higher mean HiFi read QV score)



Note: Kinnex concatenated HiFi library size should not greatly exceed ~20 kb

Example:
1.5 kb amplicon Kinnex library prepared with Kinnex 8-fold prep kit versus Kinnex 12-fold prep kit

HiFi sequencing performance results obtained for a 1.5 kb amplicon Kinnex library prepared with Kinnex 8-fold prep kit and Kinnex 12-fold prep kit (Revio system + SPRQ-Nx chemistry)

Kinnex prep kit	P1	HiFi yield	HiFi reads	HiFi read length	HiFi read quality	Q30+ bases	S-reads
Kinnex 12-fold prep kit	69%	147 Gb	7.8 M	18.8 kb	Q34	93.9%	93.4 M
Kinnex 8-fold prep kit	74%	120 Gb	9.5 M	12.6 kb	Q37	95.4%	75.9 M

Annotations: **Higher read quality** points to Q37; **Higher S-read yield** points to 93.4 M.

IMPORTANT: Final data throughput (yield) will depend on **application-specific downstream analysis procedures and quality filter settings used³**

- For some applications using secondary analysis tools that require high read quality, datasets generated with Kinnex 8-fold prep kit may retain a higher yield of useable reads after quality filtering (despite lower raw S-read throughput) compared to datasets generated with Kinnex 12-fold prep kit

Experimental design considerations for concatenating amplicons to increase HiFi sequencing data throughput (cont.)

Use the recommended input amplicon DNA quantity for Kinnex concatenation to generate sufficient amounts of final library material for HiFi sequencing

Input amplicon mass requirements for Kinnex library preparation

- Refer to Kinnex Procedure & checklist library prep protocol [documentation](#) for the latest recommended amplicon input mass requirements

Kinnex prep kit	Procedure & checklist protocol reference	Recommended input amplicon length range	Recommended input amplicon DNA quantity	Expected Kinnex HiFi library size	# of supported acquisitions per library
Kinnex 8-fold prep kit	Procedure & checklist – Preparing Kinnex libraries using the Kinnex 8-fold prep kit (103-920-900)	1,500 – 3,000 bp	35 ng of Kinnex-compatible amplicons for input into Kinnex PCR step	~12 – 20 kb	>8 acquisitions for Revio SPRQ/SPRQ-Nx chemistry >8 acquisitions for Vega SPRQ-Nx chemistry >2 acquisitions for Vega v1 chemistry
Kinnex 12-fold prep kit	Procedure & checklist – Preparing Kinnex libraries using the Kinnex 12-fold prep kit (103-921-000)	1,000 – 2,000 bp	35 ng of Kinnex-compatible amplicons for input into Kinnex PCR step	~12 – 20 kb	>8 acquisitions for Revio SPRQ/SPRQ-Nx chemistry >8 acquisitions for Vega SPRQ-Nx chemistry >2 acquisitions for Vega v1 chemistry
Kinnex 16-fold prep kit	Procedure & checklist – Preparing Kinnex libraries using the Kinnex 16-fold prep kit (103-885-300)	<1 kb	- For cDNA amplicons generated via template-switch oligo (TSO) method (e.g., 10x Genomics): >15 ng of cDNA amplicons for input into TSO PCR step and 25 ng of post-TSO removal amplicons for input into Kinnex PCR step - For other amplicon types and cDNA synthesized without TSO method: 25 ng of Kinnex-compatible amplicon DNA/cDNA for input into Kinnex PCR step	~10 – 17 kb	>4 acquisitions for Revio SPRQ/SPRQ-Nx chemistry >4 acquisitions for Vega SPRQ-Nx chemistry >1 acquisitions for Vega v1 chemistry

Experimental design considerations for concatenating amplicons to increase HiFi sequencing data throughput (cont.)

Amplicons must be generated or amplified to have Kinnex-compatible molecular ends before proceeding with Kinnex library prep procedures¹

Establishing Kinnex-compatible molecular ends

- To be compatible with Kinnex workflows, **amplicons must be generated with sequence-defined ends (Kinnex handles)** as depicted in the underlined portion in the figure below
- Optional barcodes** (e.g., 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** (e.g., Kinnex bulk Iso-Seq cDNA amplicons, Kinnex 16S amplicons, 10x Single Cell Gene Expression libraries) 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 prep²

For using Kinnex 8-fold prep kit (103-072-000) for 8-fold concatenation:

- Procedure & checklist – Preparing Kinnex libraries using the Kinnex 8-fold prep kit ([103-920-900](#))
- Technical overview – Kinnex library preparation using Kinnex prep kits (TBD)

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

- Procedure & checklist – Preparing Kinnex libraries using 16S rRNA amplicons ([103-921-000](#))
- Technical overview – Kinnex library preparation using Kinnex prep kits (TBD)

For using Kinnex 16-fold prep kit (PN: 103-836-900) for 16-fold concatenation:

- Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit ([103-885-300](#))
- Technical overview – Kinnex library preparation using Kinnex prep kits (TBD)

¹ Refer to *Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput* ([102-326-636](#)) for guidance on how to establish Kinnex-compatible molecular ends with custom amplicon sample types and for general Kinnex concatenation troubleshooting tips.

² 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



Kinnex product configuration overview

Kinnex library prep kits for concatenating amplicons for HiFi sequencing

Kinnex kits offer scalable, cost-effective solutions for increasing amplicon HiFi sequencing data throughput



Kinnex 8-fold prep kit

(103-072-000)

[12 rxn]

8-fold concatenation

Kinnex PCR 8-fold kit + Kinnex concatenation kit 2.0

Sample multiplexing:

Up to 12-plex with the Iso-Seq express 2.0 kit¹ or 48-plex when combined with all four Kinnex barcoded adapters

Suitable amplicon size range:

1.5 – 3 kb²

Example application:

Full-length RNA sequencing
Full-length 16S rRNA sequencing

PacBio protocol references:



Procedure & checklist – Preparing Kinnex libraries using the Kinnex 8-fold prep kit ([103-920-900](#))



Procedure & checklist – Preparing Kinnex-compatible 16S rRNA amplicons ([103-885-200](#))



Procedure & checklist – Preparing Kinnex compatible cDNA using Iso-Seq express 2.0 kit ([102-396-000](#))



Kinnex 12-fold prep kit

(103-072-100)

[12 rxn]

12-fold concatenation

Kinnex PCR 12-fold kit + Kinnex concatenation kit 2.0

Sample multiplexing:

Up to 384-plex 16S amplicons or 1,536-plex when combined with all four Kinnex barcoded adapters

Suitable amplicon size range:

1 – 2 kb²

Example application:

Full-length RNA sequencing
Full-length 16S rRNA sequencing

PacBio protocol references:



Procedure & checklist – Preparing Kinnex libraries using the Kinnex 12-fold prep kit ([103-921-000](#))



Procedure & checklist – Preparing Kinnex-compatible 16S rRNA amplicons ([103-885-200](#))



Procedure & checklist – Preparing Kinnex compatible cDNA using Iso-Seq express 2.0 kit ([102-396-000](#))



Kinnex 16-fold prep kit

(103-836-900)

[12 rxn]

16-fold concatenation

Kinnex PCR 16-fold kit + Kinnex concatenation kit 2.0

Sample multiplexing:

Up to 4-plex using Kinnex barcoded adapters

Suitable amplicon size range:

<1 kb²

Example application:

Single-cell RNA sequencing

PacBio protocol reference:



Procedure & checklist – Preparing Kinnex libraries using the Kinnex 16-fold RNA kit ([103-885-300](#))

Third-party vendor protocol reference:



10x Chromium single cell cDNA generation user guides (refer to [10x Genomics Support](#))

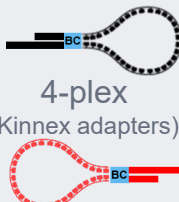
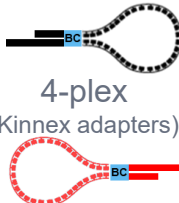
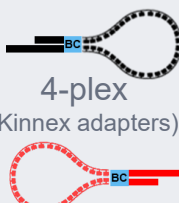
¹ PacBio Iso-Seq express 2.0 kit part number is 103-071-500.

² See *Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput* ([102-326-636](#)).

³ Updated *Procedure and checklist* uses a new part number but there are no other major changes to overall Kinnex library prep workflow aside from using the new Kinnex concatenation kit 2.0.

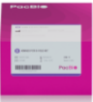
Kinnex library prep kit product configurations and features

Kinnex kit products are compatible with Vega and Revio systems with SPRQ-Nx chemistry¹

	Kinnex protocol	Kinnex prep kit	Concatenation factor	Sample multiplexing capacity			# segmented reads (S-reads) per SMRT Cell acquisition	SMRT Link support
				Amplicon multiplexing	Library multiplexing	Total multiplexing capacity		
Kinnex 8-fold	Preparing Kinnex libraries using the Kinnex 8-fold prep kit (103-920-900) 	Kinnex 8-fold prep kit (103-072-000) 12 rxn 	8-fold	N-plex (sample-dependent) Example: 12-plex for cDNA amplicon generation using Iso-Seq express 2.0 kit	 4-plex (Kinnex adapters)	N x 4-plex Example: 48-plex for Kinnex bulk RNA analysis	40 – 50 M (Vega + SPRQ-Nx) 50 – 70 M (Revio + SPRQ-Nx)	SMRT Link v26.2+ Demux BC Read seg. Iso-Seq
Kinnex 12-fold	Preparing Kinnex libraries using the Kinnex 12-fold prep kit (103-921-000) 	Kinnex 12-fold prep kit (103-072-100) 12 rxn 	12-fold	N-plex (sample-dependent) Example: 384-plex for 16S amplicon generation using indexed PCR primers	 4-plex (Kinnex adapters)	N x 4-plex Example: 1,536-plex for Kinnex 16S rRNA analysis	50 – 60 M (Vega + SPRQ-Nx) 70 – 100 M (Revio + SPRQ-Nx)	SMRT Link v26.2+ Demux BC Read seg. Iso-Seq
Kinnex 16-fold	Preparing Kinnex libraries using the Kinnex 16-fold prep kit (103-885-300) 	Kinnex 16-fold prep kit (103-836-900) 12 rxn 	16-fold	N-plex (sample-dependent) Example: Single-plex for single-cell cDNA amplicon generation using 10x kits	 4-plex (Kinnex adapters)	N x 4-plex Example: 4-plex for Kinnex single-cell RNA analysis	80 – 100 M (Vega + SPRQ-Nx) 100 – 150 M (Revio + SPRQ-Nx)	SMRT Link v26.2+ Demux BC Read seg. Single-cell Iso-Seq

Kinnex library prep kit product components

Kinnex kit products share the same Kinnex concatenation kit 2.0 and are compatible with existing standalone amplicon generation products

Kinnex protocol		Kinnex prep kit	Kinnex v2.0 prep kit bundle included components				Optional products are compatible with all Kinnex prep kits	Example OPTIONAL products [Not included in Kinnex prep kit bundles but required for application-specific amplicon generation]
Kinnex 8-fold	Preparing Kinnex libraries using the Kinnex 8-fold prep kit (103-920-900)	Kinnex 8-fold prep kit (103-072-000) 12 rxn	 Kinnex PCR 8-fold kit 103-071-600 (12 rxn)	 Kinnex concatenation kit 2.0 103-836-400 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300		For bulk cDNA amplicon generation:  Iso-Seq express 2.0 kit 103-071-500 (24 rxn)
	Preparing Kinnex libraries using the Kinnex 12-fold prep kit (103-921-000)	Kinnex 12-fold prep kit (103-072-100) 12 rxn	 Kinnex PCR 12-fold kit 103-071-700 (12 rxn)	 Kinnex concatenation kit 2.0 103-836-400 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300		For 16S amplicon generation: 16S gene amplification PCR primers (customer-supplied) ¹ 
	Preparing Kinnex libraries using the Kinnex 16-fold prep kit (103-885-300)	Kinnex 16-fold prep kit (103-836-900) 12 rxn	 Kinnex PCR 16-fold kit 103-836-300 (12 rxn)	 Kinnex concatenation kit 2.0 103-836-400 (12 rxn)	 Kinnex capture beads kit 103-076-000 (12 rxn)	 Elution buffer 101-633-500	 SMRTbell cleanup beads 102-158-300	For single-cell cDNA amplicon generation: 10x Genomics Chromium Single Cell 3' or 5' reagent kits (or other PacBio Compatible Partner products ²)

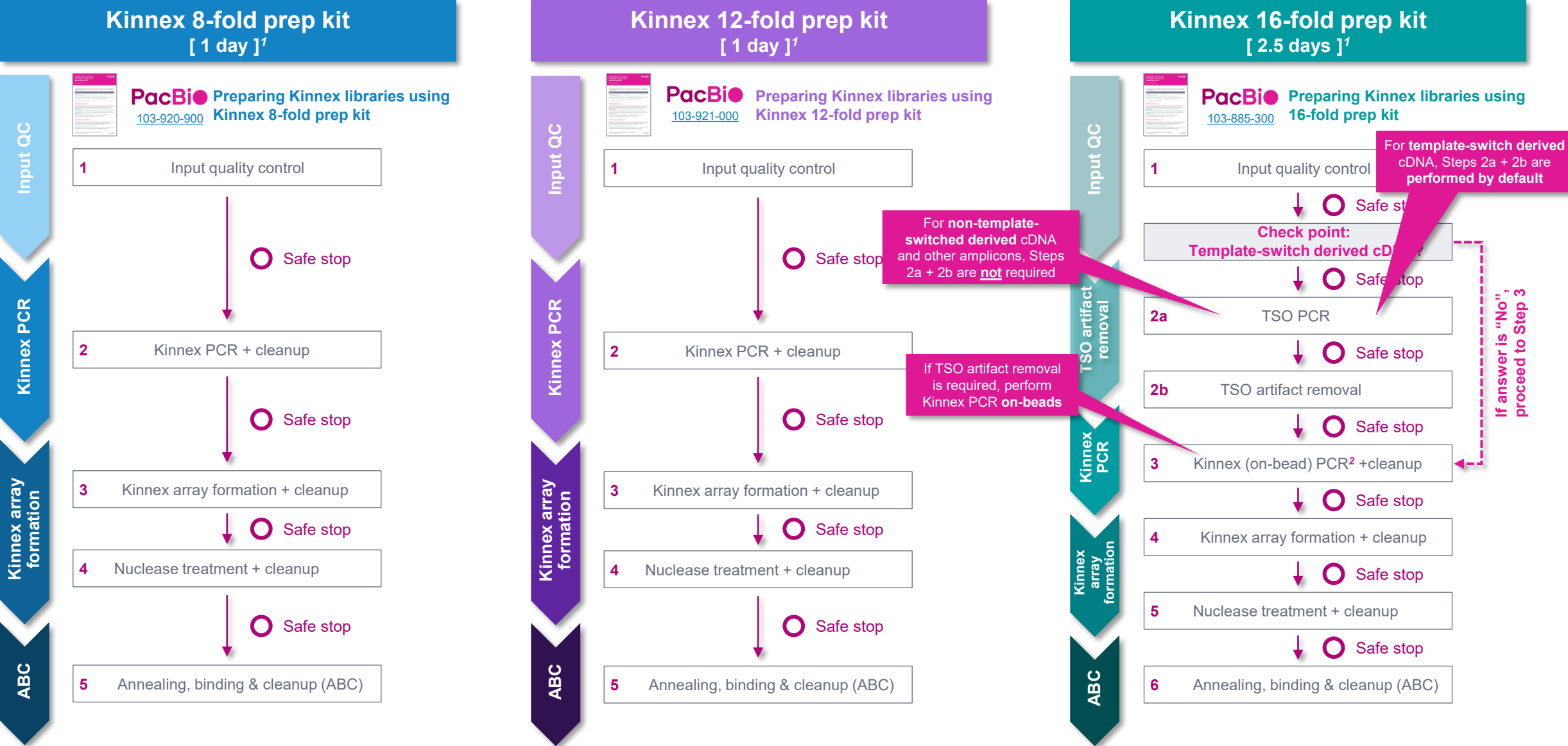
¹ Refer to *Procedure & checklist – Preparing 16S rRNA amplicons for Kinnex* ([103-885-200](#)) for recommended 16S gene-specific forward and reverse PCR primer sequences to order.
² Refer to specific *Application or Technical Note* literature for compatibility with third-party products such as ArgenTag and Parse Biosciences single-cell kits.



Kinnex library preparation workflow overview

Kinnex v2.0 8-fold/12-fold/16-fold library prep kit workflow comparison overview

All three Kinnex v2.0 prep kits share a consolidated workflow for Kinnex PCR and Kinnex array formation – but Kinnex 16-fold prep kit workflow includes an upstream TSO removal step required for processing template switch-derived cDNA amplicon samples



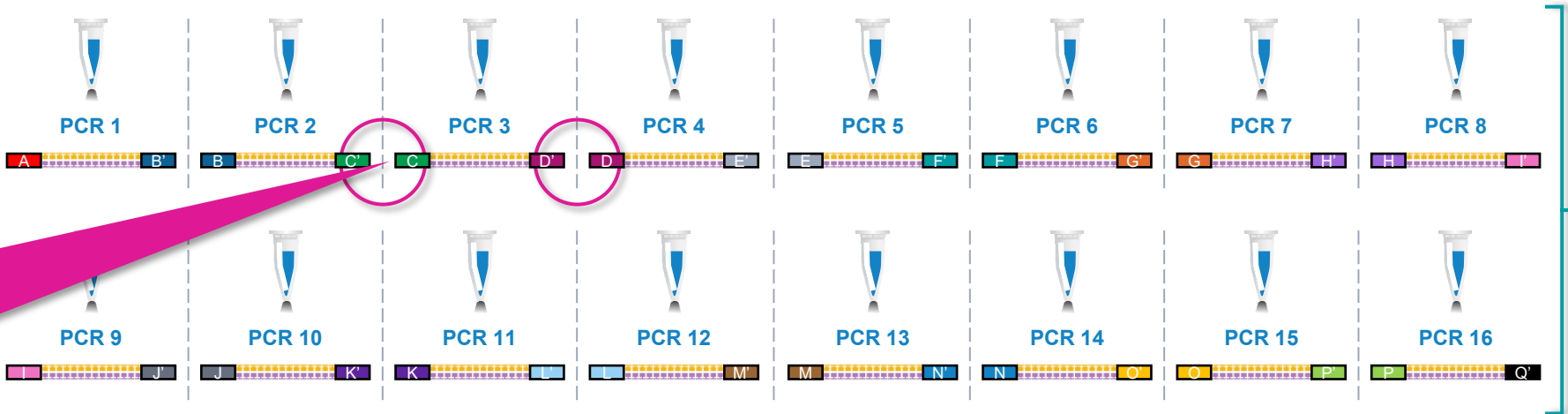
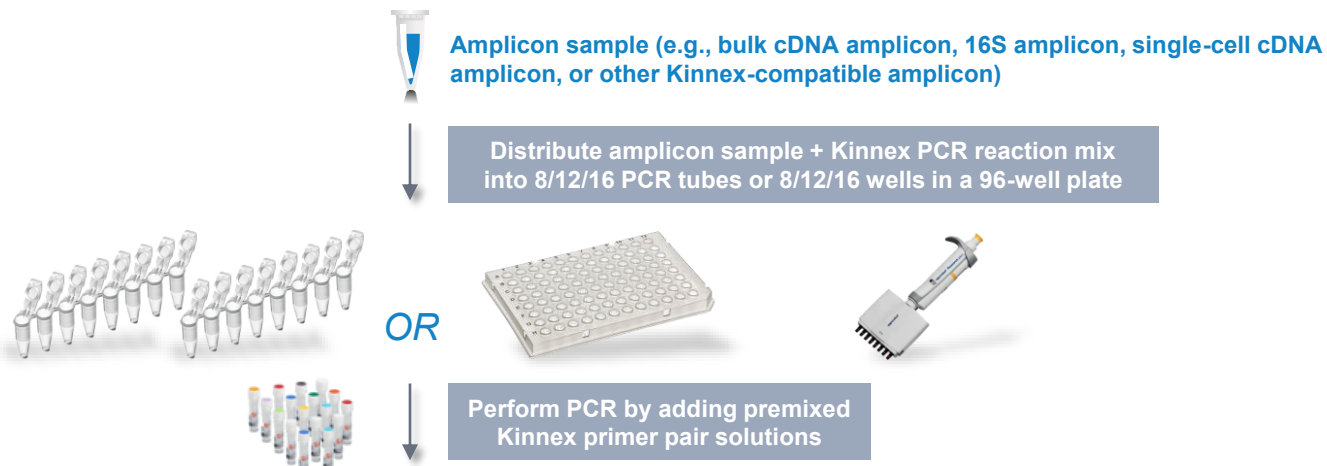
¹ Workflow times shown are for processing up to 12 Kinnex libraries.
² If TSO artifact removal is required, perform Kinnex PCR reaction on-beads; if TSO artifact removal is not required, perform Kinnex PCR reaction off-beads.

Kinnex PCR step overview

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

Kinnex prep kit	Concatenation factor
Kinnex 8-fold prep kit	8-fold
Kinnex 12-fold prep kit	12-fold
Kinnex 16-fold prep kit	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

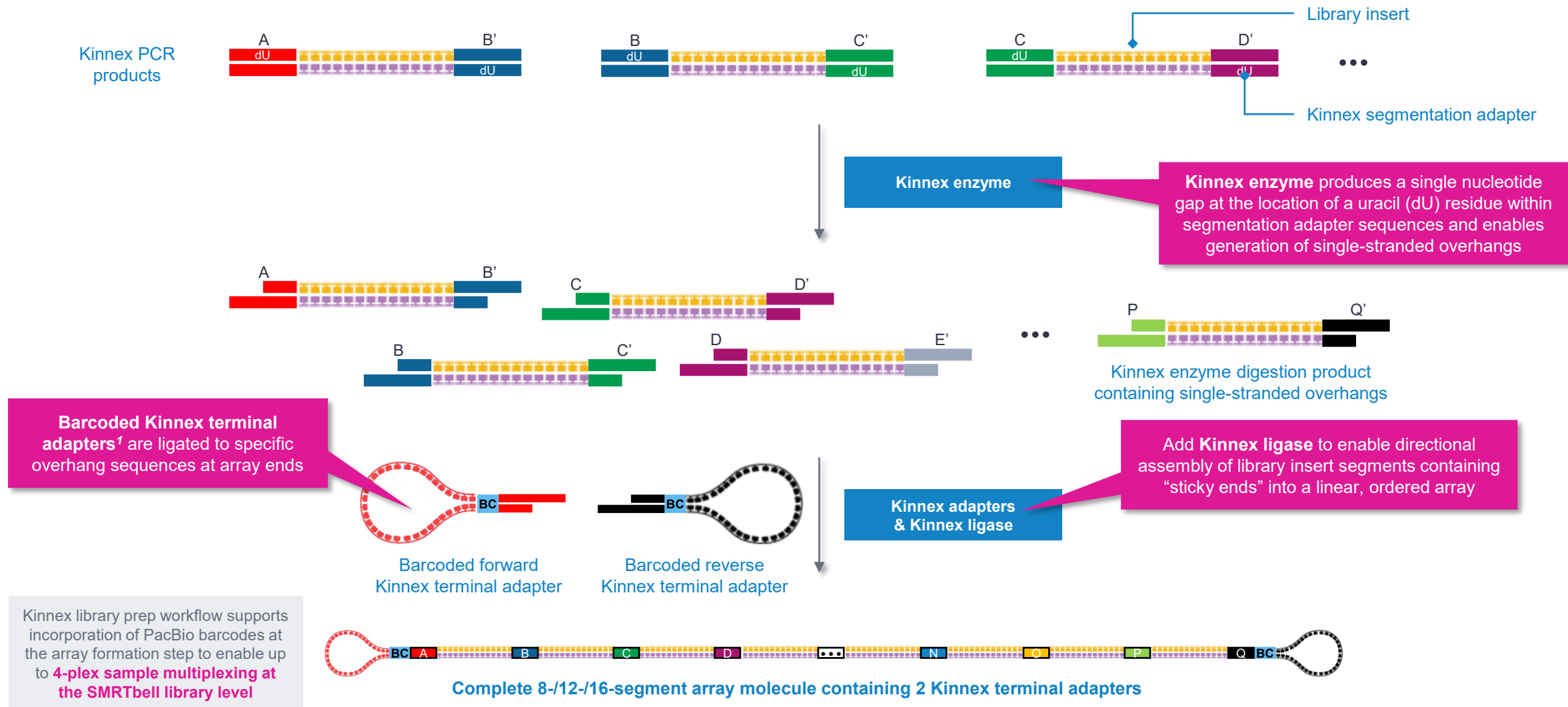
Example Kinnex PCR setup using Kinnex 16-fold prep kit (for 16-fold concatenation)

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

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

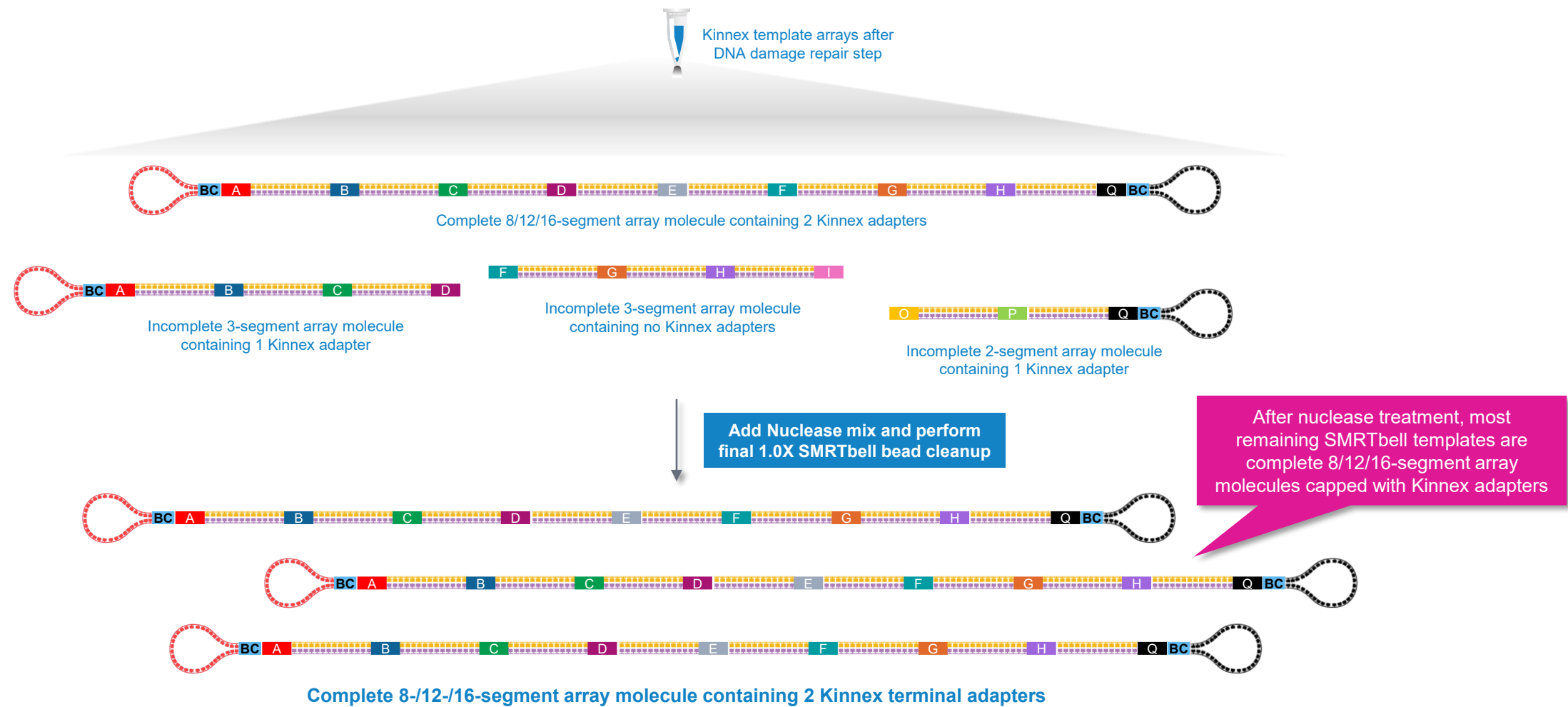
Kinnex array formation step overview

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



Nuclease treatment + cleanup step overview

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





Kinnex sequencing preparation workflow overview

Sample setup and run design recommendations for Kinnex libraries

Key sample setup and run design setup parameters for Revio and Vega systems (SMRT Link v26.2)

Workflow	Key setup parameters	Vega system v1.2+ (SPRQ-Nx) recommended settings	Revio system v13.5+ (SPRQ-Nx) recommended settings
Sample setup 	Library type	Kinnex	Kinnex
	Primer	Kinnex sequencing primer	Kinnex sequencing primer
	Polymerase kit	Vega SPRQ polymerase kit	Revio SPRQ polymerase kit
	Concentration on plate	80 – 130 pM	130 – 160 pM
Run design   Runs	Use Adaptive Loading	N/A	YES
	Application Type²	Kinnex full-length RNA; or Kinnex 16S rRNA; or Kinnex single-cell RNA	Kinnex full-length RNA; or Kinnex 16S rRNA; or Kinnex single-cell RNA
	Library Type³	Kinnex	Kinnex
	Movie Acquisition Time	24 hrs	24 hrs
	Add Analysis	YES	YES
	Analysis workflow	Read segmentation data utility or other application-specific analysis workflow	Read segmentation data utility or other application-specific analysis workflow
	Segmentation adapter set	MAS-Seq Adapter v1 (MAS16); or MAS-Seq Adapter v2 (MAS12); or MAS-Seq Adapter v3 (MAS8)	MAS-Seq Adapter v1 (MAS16); or MAS-Seq Adapter v2 (MAS12); or MAS-Seq Adapter v3 (MAS8)

Refer to **Procedure & checklist protocol documentation** for the latest sample setup (ABC¹) recommendations for Kinnex libraries

¹ ABC = Anneal sequencing primer / Bind polymerase / Clean up bound complexes



² **Note:** A “Kinnex generic” run design application type option for running Kinnex custom amplicon libraries will be available in a future version of SMRT Link.

³ **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.

Sample setup and run design recommendations for Kinnex libraries (cont.)

Key sample setup and run design setup parameters for Revio and Vega systems (SMRT Link v26.2)

Workflow	Key setup parameters	Vega system v1.2+ (SPRQ-Nx) recommended settings	Revio system v13.5+ (SPRQ-Nx) recommended settings
Sample setup 	Library type	Kinnex	Kinnex
	Primer	Kinnex sequencing primer	Kinnex sequencing primer
	Polymerase kit	Vega SPRQ polymerase kit	Revio SPRQ polymerase kit
	Concentration on plate	80 – 130 pM	130 – 160 pM
Run design 	Use Adaptive Loading	N/A	YES
	Application Type ²	Kinnex full-length RNA; or Kinnex 16S rRNA; or Kinnex single-cell RNA	Kinnex full-length RNA; or Kinnex 16S rRNA; or Kinnex single-cell RNA
	Library Type ³	Kinnex	Kinnex
	Movie Acquisition Time	24 hrs	24 hrs
	Add Analysis	YES	YES
	Analysis workflow	Read segmentation data utility or other application-specific analysis workflow	Read segmentation data utility or other application-specific analysis workflow
Runs 	Segmentation adapter set	MAS-Seq Adapter v1 (MAS16); or MAS-Seq Adapter v2 (MAS12); or MAS-Seq Adapter v3 (MAS8)	MAS-Seq Adapter v1 (MAS16); or MAS-Seq Adapter v2 (MAS12); or MAS-Seq Adapter v3 (MAS8)

Refer to **Procedure & checklist protocol documentation** for the latest sample setup (ABC¹) recommendations for Kinnex libraries

¹ ABC = Anneal sequencing primer / Bind polymerase / Clean up bound complexes

NOTE: SMRT Link v26.2 dropdown menu does not contain a “Kinnex generic” application type option²

To set up a Kinnex generic (custom) amplicon library run design in SMRT Link v26.2, users can perform the following:

1. Select “Kinnex 16S rRNA” application type option as a placeholder.
2. Manually specify the correct **Segmentation adapter set** used
 - e.g., select “MAS-Seq Adapter v3 (MAS8)” if Kinnex 8-fold prep kit was used for Kinnex library construction
3. Manually **specify any other analysis options if applicable**
 - **Note:** By default, **Add Analysis = YES** and **Analysis workflow = Read segmentation** *if* “Kinnex 16S rRNA” application type is specified)

² **Note:** A “Kinnex generic” run design application type option for running Kinnex custom amplicon libraries will be available in a future version of SMRT Link.

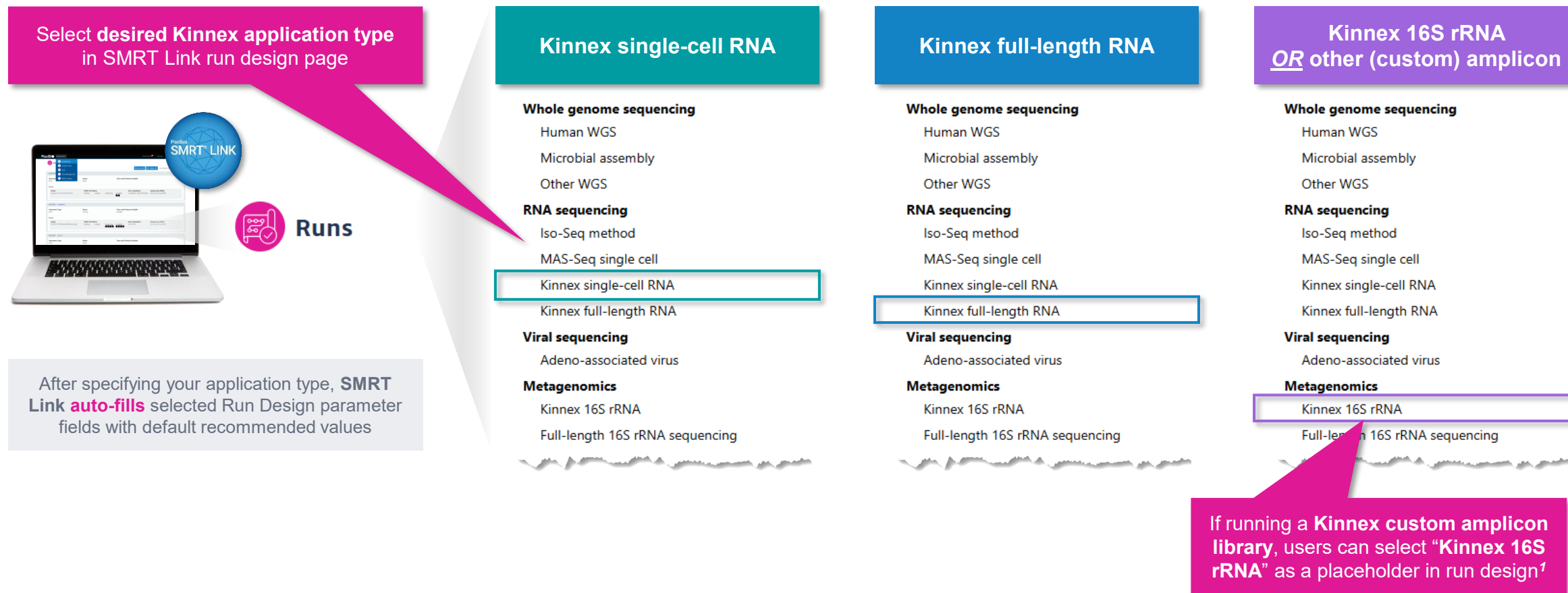
³ **Note:** SMRT Link Run design **Library Type** field determines which adapter finding algorithm is used during post-primary analysis. When sequencing a Kinnex library sample, if ‘Standard’ library type is mistakenly selected instead of ‘Kinnex’ then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.

Example run design setup for application-specific Kinnex libraries

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



v26.2



Example run design setup for application-specific Kinnex libraries (cont.)

Sample and run information



v26.2

Kinnex single-cell RNA

Kinnex full-length RNA

Kinnex 16S rRNA
OR other (custom) amplicon

▼ Plate 1, Well A01: Kinnex single-cell RNA library demo	▼ Plate 1, Well A01: Kinnex full-length RNA library demo	▼ Plate 1, Well A01: Kinnex 16S rRNA library demo
Application Required Kinnex single-cell RNA	Application Required Kinnex full-length RNA	Application Required Kinnex 16S rRNA
Plate Well Required Plate 1, Well A01	Plate Well Required Plate 1, Well A01	Plate Well Required
Well Name Required	Well Name Required	Well Name Required
Well Comment	Well Comment	Well Comment
Library Type Required Kinnex	Library Type Required Kinnex	Library Type Required Kinnex
Insert Size (bp) Required 15000	Insert Size (bp) Required 16000	Insert Size (bp) Required 17000
Library Concentration (pM) Required	Library Concentration (pM) Required	Library Concentration (pM) Required
Movie Acquisition Time (hours) 24	Movie Acquisition Time (hours) 24	Movie Acquisition Time (hours) 24

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

Default Library Type = **Kinnex** (instead of Standard or AAV)¹

If running a **Kinnex custom amplicon library**, users can select “**Kinnex 16S rRNA**” as a placeholder in run design

Default Movie Time = **24 hrs**

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



Library Type field determines which adapter finding algorithm is used during post-primary analysis¹

Forward and reverse Kinnex terminal adapters have different structures

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.

Example run design setup for application-specific Kinnex libraries (cont.)

Sample indexing (barcoding) information

Kinnex single-cell RNA

Kinnex full-length RNA

Kinnex 16S rRNA
OR other (custom) amplicon

Samples

Sample is indexed ☒ YES ☐ NO

Indexes Required

MAS SMRTbell Barcoded Adapters (v2)

Biosample names Required

Interactively From a File

Sample is indexed ☒ YES ☐ NO

Indexes Required

MAS SMRTbell Barcoded Adapters (v2)

Biosample names Required

Interactively From a File

Sample is indexed ☒ YES ☐ NO

Indexes Required

MAS SMRTbell Barcoded Adapters (v2)

Biosample names Required

Interactively From a File

Sample is indexed field is automatically set to YES for all Kinnex application types

Default Indexes FASTA for Kinnex libraries = MAS SMRTbell Barcoded Adapters (v2)

Complete Kinnex library molecule containing barcoded Kinnex terminal MAS adapters¹ 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 ID
☐ bcM0001--bcM0001
☐ bcM0002--bcM0002
☐ bcM0003--bcM0003
☐ bcM0004--bcM0004

Included Barcodes

Filter...

Barcode ID	Bio Sample ID
------------	---------------

Barcode Selector and Sample Name Editor

Available Barcodes

Filter...

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

Included Barcodes

Filter...

Barcode ID	Bio Sample ID
☐ 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.

Example run design setup for application-specific Kinnex libraries (cont.)

Data options

Kinnex single-cell RNA

Data Options

Include Base Kinetics

☐ YES ☒ NO

Consensus Mode

☒ MOLECULE ☐ STRAND

Assign Data To Project

General Project

Default = NO for Include Base Kinetics

Default Consensus Mode = MOLECULE¹

Kinnex full-length RNA

Data Options

Include Base Kinetics

☐ YES ☒ NO

Consensus Mode

☒ MOLECULE ☐ STRAND

Assign Data To Project

General Project

Kinnex 16S rRNA
OR other (custom) amplicon

Data Options

Include Base Kinetics

☐ YES ☒ NO

Consensus Mode

☒ MOLECULE ☐ STRAND

Assign Data To Project

General Project

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

Example run design setup for application-specific Kinnex libraries (cont.)



v26.2

Analysis options

Kinnex single-cell RNA

Kinnex full-length RNA

Kinnex 16S rRNA OR other (custom) amplicon

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

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

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

Analysis Options

Add Analysis ☒ YES ☐ NO

Analysis Name Required Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required Read Segmentation

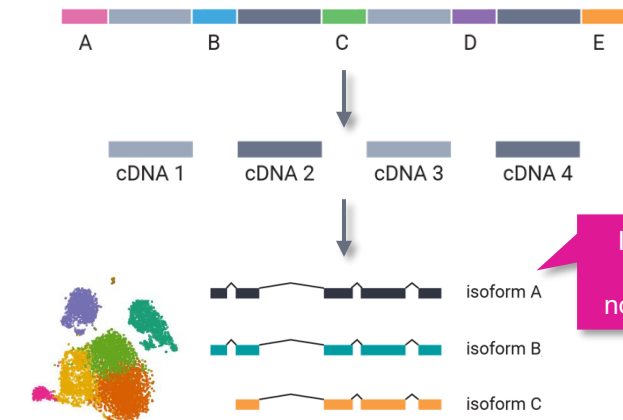
Segmentation Adapter Set MAS-Seq Adapter v2 (MAS12)

Advanced Parameters

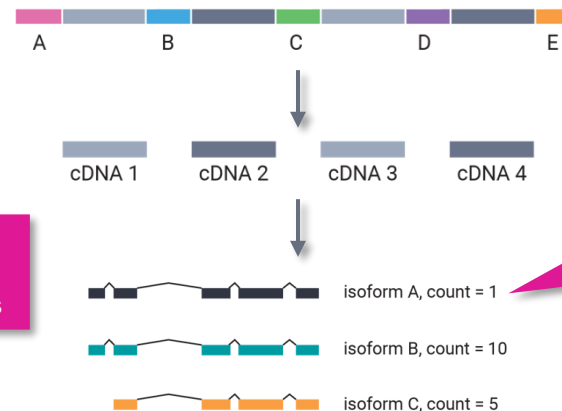
Analysis Workflow is automatically filled in

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

Read Segmentation and Single-Cell Iso-Seq

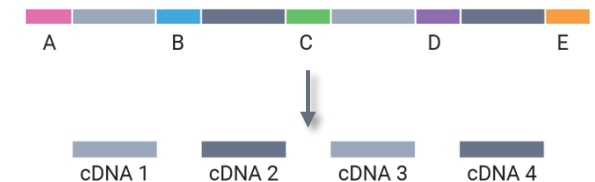


Read Segmentation and Iso-Seq



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

Read Segmentation



Example run design setup for application-specific Kinnex libraries (cont.)



v26.2

Analysis options

Kinnex single-cell RNA

Analysis Options

Default Segmentation Adapter Set =
MAS-Seq Adapter v1 (MAS16) for
Kinnex single-cell RNA application type

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

Default Segmentation Adapter Set =
MAS-Seq Adapter v3 (MAS8) for
Kinnex full-length RNA application type

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
OR other (custom) amplicon

Analysis Options

Default Segmentation Adapter Set =
MAS-Seq Adapter v2 (MAS12) for
Kinnex 16S rRNA application type

Segmentation Adapter Set

MAS-Seq Adapter v2 (MAS12)

Advanced Parameters

IMPORTANT!

If needed, manually specify the correct
Segmentation Adapter Set that
corresponds to the Kinnex library prep
concatenation method actually used

Kinnex PCR products

Kinnex array formation

Complete array molecule containing concatenated library insert segments

Kinnex library prep kit used	Segmentation Adapter Set to specify in SMRT Link v26.2 run design Analysis Options
Kinnex 8-fold prep kit (103-072-000)	MAS-Seq Adapter v3 (MAS8)
Kinnex 12-fold prep kit (103-072-100)	MAS-Seq Adapter v2 (MAS12)
Kinnex 16-fold prep kit (103-836-900)	MAS-Seq Adapter v1 (MAS16)

PacBio

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Example run design setup for application-specific Kinnex libraries (cont.)

Analysis options

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

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

Specify Primer Set used for cDNA amplification

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.

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'

Forward (F) PCR primer (Barcoded Iso-Seq primer bcXX²)

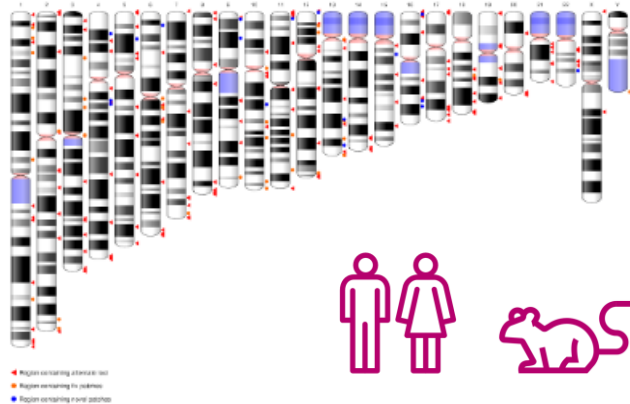
Reverse (R) PCR primer (Iso-Seq cDNA amplification primer)

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)



Example run design setup for application-specific Kinnex libraries (cont.)



Analysis options

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

Reference Set Required Human Genome hg38, with Gencode v39 annotations

Kit Type ☒ 10X 3' KIT ☐ 10X 5' KIT

Advanced Parameters

Specify Kit Type used for single-cell cDNA generation

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

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

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

OR

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

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

Kinnex full-length RNA

Analysis Options

Add Analysis ☒ YES ☐ NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set MAS-Seq Adapter v1 (MAS16)

Primer Set Required 10x Chromium single cell 3' cDNA

Reference Set Required Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples ☒ Pool reads and cluster together

Advanced Parameters

Specify how to perform read clustering for barcoded samples

Pool reads and cluster together

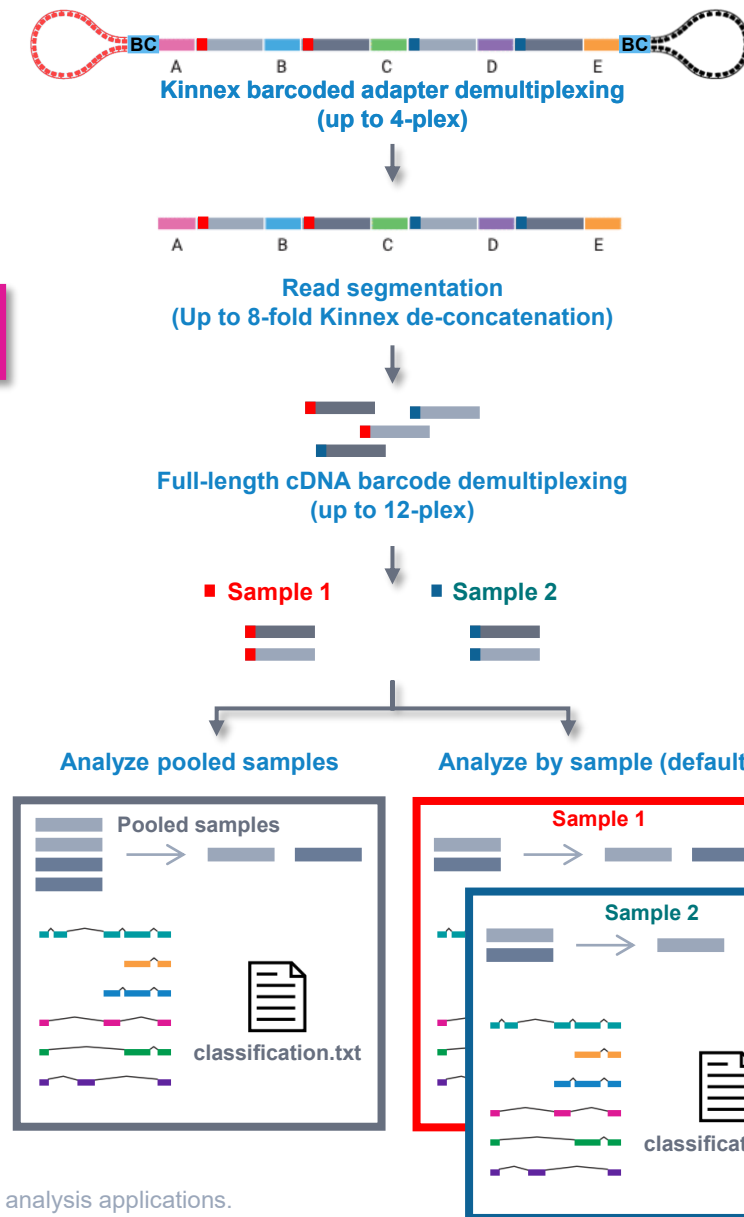
-- select --

Cluster reads separately

Pool reads and cluster together

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 v26.2





Kinnex example sequencing performance

Kinnex example performance – Revio system v13.5 + SPRQ-Nx

Kinnex libraries show excellent HiFi sequencing performance across multiple SMRT Cell uses that meets or exceeds Revio system specifications for every use¹

Kinnex library type									
Kinnex 8-fold prep kit	Use	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	P1	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex full-length RNA, UHRR ²	1	137.1	9.5	14.4	27	84.4	72.1 M	1.83 kb	50 – 70 M S-reads per acquisition
	2	135.2	9.3	14.5	29	68.8	71.0 M	1.83 kb	
	3	116.2	7.9	14.6	29	60.2	61.2 M	1.83 kb	
Kinnex 12-fold prep kit	Use	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	P1	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex 16S rRNA, 384-plex ATCC MSA1002 ³	1	170.5	8.8	19.3	33	78.8	103.4 M	1.61 kb	70 – 100 M S-reads per acquisition
	2	168.2	8.7	19.4	35	65.4	102.1 M	1.61 kb	
	3	139.8	7.2	19.5	36	50.3	84.9 M	1.61 kb	
Kinnex 16-fold prep kit	Use	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	P1	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex single-cell 3' RNA (10x) ⁴	1	153.7	10.9	14.1	29	74	164 M	906 bp	100 – 150 M S-reads per acquisition
	2	143.0	10.0	14.2	29	71	153 M	908 bp	
	3	127.3	8.9	14.4	30	61	136 M	909 bp	

¹ **Note:** HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yields are based on high-quality samples prepared following best practices. Reduced HiFi yield on Use 2 and/or Use 3 is typically caused by lower P1 loading efficiency.

² Kinnex full-length RNA UHRR libraries were prepared using Kinnex 8-fold prep kit and run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 140 pM on-plate loading concentration).

³ Kinnex full-length 16S rRNA, ATCC MSA1002 libraries were prepared with Kinnex 12-fold pre kit and run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 120 pM OPLC).

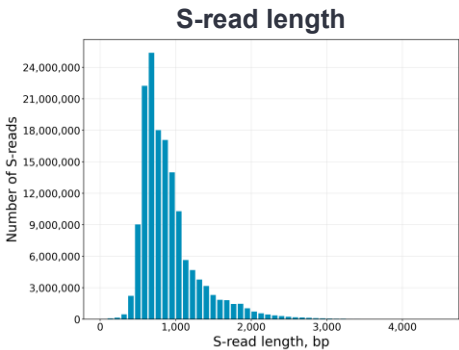
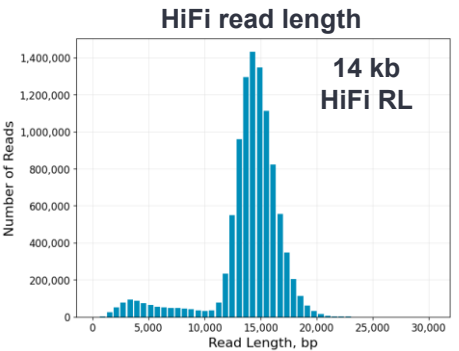
⁴ Kinnex single-cell 3' RNA libraries were prepared with Kinnex 16-fold pre kit and run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 160 pM OPLC).

⁵ See *Revio specifications sheet* ([102-326-552](#)) for expected HiFi yield performance range for different library insert sizes and applications.

Kinnex example performance – Revio system v13.5 + SPRQ-Nx (cont.)

Kinnex single-cell RNA

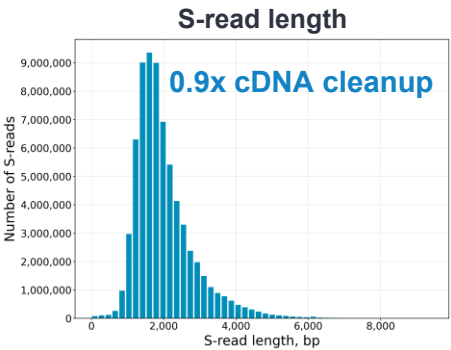
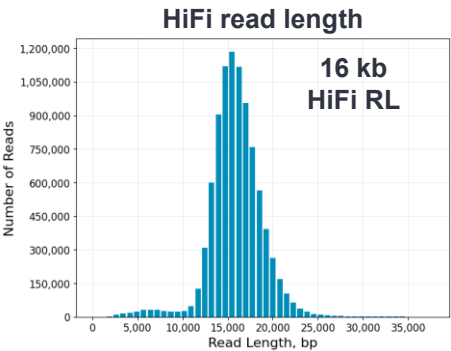
Kinnex 16-fold prep kit



Value	Analysis Metric
10,030,881	Reads
149,910,947	Segmented reads (S-reads)
906	Mean length of S-reads
88.24 %	Percent of reads with full arrays
14.94	Mean array size (concatenation factor)

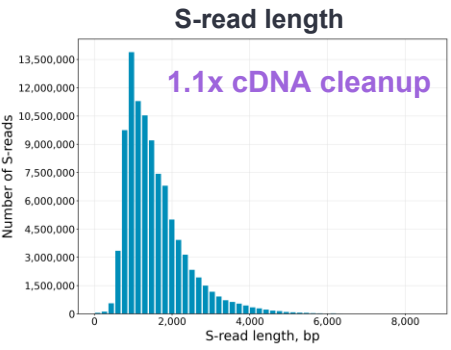
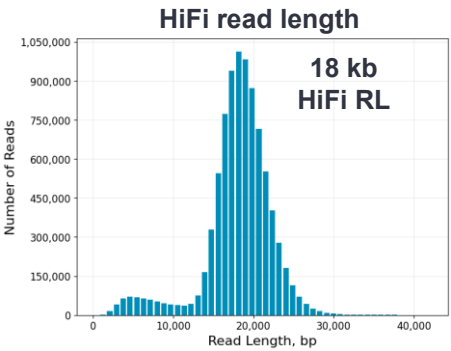
Kinnex full-length RNA

Kinnex 8-fold prep kit



Value	Analysis Metric
9,044,421	Reads
69,407,271	Segmented reads (S-reads)
2,002	Mean length of S-reads
92.67 %	Percent of reads with full arrays
7.67	Mean array size (concatenation factor)

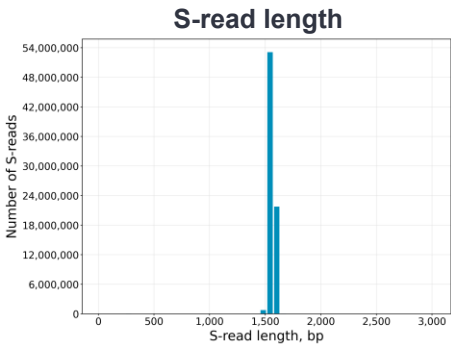
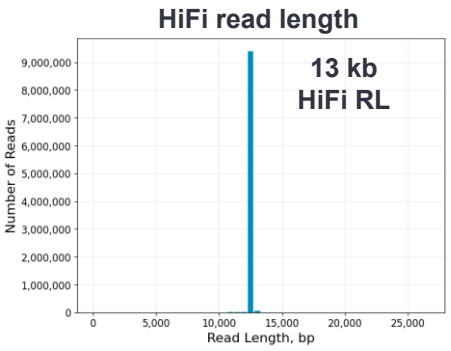
Kinnex 12-fold prep kit



Value	Analysis Metric
8,742,074	Reads
96,912,559	Segmented reads (S-reads)
1,566	Mean length of S-reads
87.77 %	Percent of reads with full arrays
11.09	Mean array size (concatenation factor)

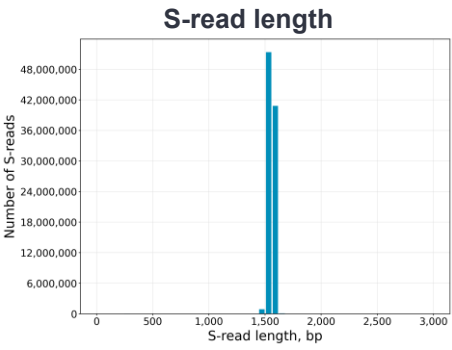
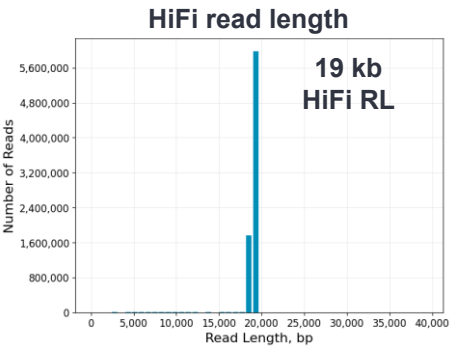
Kinnex 16S rRNA

Kinnex 8-fold Prep kit



Value	Analysis Metric
9,541,465	Reads
75,949,260	Segmented reads (S-reads)
1,560	Mean length of S-reads
97.77 %	Percent of reads with full arrays
7.96	Mean array size (concatenation factor)

Kinnex 12-fold prep kit



Value	Analysis Metric
7,860,184	Reads
93,421,796	Segmented reads (S-reads)
1,560	Mean length of S-reads
96.94 %	Percent of reads with full arrays
11.89	Mean array size (concatenation factor)

Kinnex example performance – Vega system v1.2 + SPRQ-Nx

Kinnex libraries show excellent HiFi sequencing performance that meets or exceeds Vega system specifications¹

Kinnex library type								
Kinnex 8-fold prep kit	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	Loading level	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex full-length RNA, UHRR ²	99.9	6.5	15.3	34	63%	47.9 M	1.98 kb	40 – 50 M S-reads per acquisition
Kinnex 12-fold prep kit	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	Loading level	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex 16S rRNA, 384-plex ATCC MSA1001, 1003 ³	99.9	5.2	19.3	34	67%	60.6 M	1.61 kb	50 – 60 S-reads per acquisition
Kinnex 16-fold prep kit	HiFi yield (Gb)	HiFi reads (M)	HiFi length (kb)	HiFi read QV	Loading level	Segmented reads per acquisition	Mean S-read length	Product specifications ⁵
Kinnex single-cell 3' RNA (10x) ⁴	99.9	7.1	14.1	29	67%	108.1 M	894.9 bp	80 – 100 M S-reads per acquisition

¹ **Note:** HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yields are based on high-quality samples prepared following best practices.

² Kinnex full-length RNA UHRR libraries were run on a Vega system with SPRQ-Nx chemistry (24-hour acquisition time; 100 pM on-plate loading concentration).

³ Kinnex full-length 16S rRNA, ATCC MSA1001 + MSA 1003 libraries were run on a Vega system with SPRQ-Nx chemistry (24-hour acquisition time; 80 pM on-plate loading concentration).

⁴ Kinnex single-cell 3' RNA libraries (4-plex) were prepared with Kinnex 16-fold pre kit and run on a Vega system with SPRQ-Nx chemistry (24-hour acquisition time; 130 pM OPLC).

⁵ See **Vega specifications sheet** ([102-326-625](#)) for expected HiFi yield performance range for different library insert sizes and applications.



Technical documentation & applications support resources



Technical resources for Kinnex library preparation, sequencing & data analysis

Kinnex library preparation literature & other resources

- **General Kinnex library preparation**
 - Procedure & checklist – Preparing Kinnex libraries using Kinnex 8-fold prep kit ([103-920-900](#))
 - Procedure & checklist – Preparing Kinnex libraries using Kinnex 12-fold prep kit ([103-921-000](#))
 - Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit ([103-885-300](#))
 - Technical overview – Kinnex library preparation using Kinnex prep kits ([103-911-600](#))
 - Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit ([103-916-000](#))
 - Technical overview – Kinnex library preparation using Kinnex 12-fold prep kit ([103-916-100](#))
 - Technical overview – Kinnex library preparation using Kinnex 16-fold prep kit ([103-916-200](#))
- **Application-specific Kinnex library preparation**
 - Application note – Kinnex full-length RNA kit for isoform sequencing ([102-326-591](#))
 - Application note – Kinnex 16S rRNA kit for full-length 16S sequencing ([102-326-601](#))
 - Application note – Kinnex single-cell RNA for single-cell isoform sequencing ([102-326-549](#))
 - Procedure & checklist – Preparing Kinnex compatible cDNA using Iso-Seq express 2.0 kit ([102-396-000](#))
 - Procedure & checklist – Preparing 16S rRNA amplicons for Kinnex ([103-885-200](#))

Data analysis resources

- **General data analysis**
 - SMRT Link software installation guide [[Link](#)]
 - SMRT Link user guide [[Link](#)]
 - SMRT Tools reference guide [[Link](#)]
- **Application-specific Kinnex data analysis**
 - 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](#))

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

Posters & publications

- **Kinnex bulk RNA applications**

- Ma, J. et al. (2026) Long-read RNA sequencing improves isoform and splicing outlier detection in whole blood from rare disease trios. MedRxiv [[Link](#)]
- Wissel, D. et al. (2026) A systematic benchmark of high-accuracy PacBio long-read RNA sequencing for transcript-level quantification. Genome Biology [[Link](#)]
- Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology [[Link](#)]
- Pardo-Palacios, F. et al. (2024) Systematic assessment of long-read RNA-seq methods for transcript identification and quantification. Nature Biotech [[Link](#)]
- Pardo-Palacios, F., et al., (2024) SQANTI3: curation of long-read transcriptomes for accurate identification of known and novel isoforms. Nature Methods [[Link](#)]
- Schertzer, M.D. et al. (2023) Cas13d-mediated isoform-specific RNA knockdown with a unified computational and experimental toolbox. Nature comm [[Link](#)]

- **Kinnex 16s rRNA applications**

- Anderson, M.A.J. et al. (2025) Unlocking River Biofilm Microbial Diversity: A Comparative Analysis of Sequencing Technologies. Molecular Ecology Resources [[Link](#)]
- PacBio PAG poster (2025) – High-resolution microbiome species profiling at scale with the Kinnex kit for full-length 16S rRNA sequencing [[Link](#)]
- PacBio ASM Microbe poster (2024) - Microbiome species profiling at scale with the Kinnex kit for full-length 16S rRNA sequencing [[Link](#)]
- PacBio PAG poster (2024) – Increasing the throughput of full-length 16S sequencing with Kinnex kits [[Link](#)]
- Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology [[Link](#)]
- Notario, E. et al. (2023) Amplicon-based microbiome profiling: from second- to third-generation sequencing for higher taxonomic resolution. Genes [[Link](#)]

- **Kinnex single-cell RNA applications**

- Mantri, M. et al. (2026) Single-cell splice isoform usage reveals distinct axes of cellular identity and senescence. BioRxiv. [[Link](#)]
- Wissel, D. et al. (2025) A systematic benchmark of high-accuracy PacBio long-read RNA sequencing for transcript-level quantification. Genome Biology [[Link](#)]
- Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology [[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](#)]

Webinars

- PacBio webinar (2025) – Detection and quantification of transcript isoforms using high-depth Kinnex full-length RNA sequencing [[Link](#)]
- 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 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 datasets¹

Application	Dataset	Data type	PacBio system
Kinnex full-length RNA (Kinnex 8-fold library)	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
Kinnex 16S rRNA (Kinnex 12-fold library)	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
Kinnex single-cell RNA (Kinnex 16-fold library)	Homo sapiens - PBMC 10x Chromium Single Cell 5' and 3' libraries [Link]	HiFi long read	Sequel II & Revio systems
	Homo sapiens - HG002 (10x 5') [Link]	HiFi long read	Revio system



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Revision history (description)	Version	Date
Initial release.	01	October 2026

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APPENDIX 1: Example Kinnex applications and workflows

Default Kinnex library prep workflow recommendations based on input amplicon average insert size

Kinnex workflows enable flexibility for choosing different Kinnex library prep kits based on different input amplicon average size ranges¹

Input amplicon average insert size ¹	Default recommended Kinnex prep kit	Expected throughput (Segmented reads or ‘S-reads’)	Kinnex application notes
<1 kb	Kinnex 16-fold prep kit (103-836-900)	Revio (SPRQ-Nx): 100 – 150 M Vega (SPRQ-Nx): 80 – 100 M	• Default kit for Kinnex single-cell RNA and short custom amplicons <1 kb
1 – 2 kb	Kinnex 12-fold prep kit (103-072-100)	Revio (SPRQ-Nx): 70 – 100 M Vega (SPRQ-Nx): 50 – 60 M	• Default kit for most Kinnex bulk RNA applications that enables higher data throughput and better recovery of shorter (< 1kb) transcripts
1.5 – 3 kb	Kinnex 8-fold prep kit (103-072-000)	Revio (SPRQ-Nx): 50 – 70 M Vega (SPRQ-Nx): 40 – 50 M	• Default kit for Kinnex 16S rRNA applications • Optional kit for Kinnex bulk RNA applications when retaining largest transcripts is the priority
>3 kb	Not recommended for Kinnex concatenation		

Default Kinnex library prep workflow recommendations based on HiFi sequencing application

Default Kinnex workflow recommendations provide a starting point for achieving an optimal balance between HiFi sequencing data quality and overall throughput

Kinnex application	Default recommended Kinnex prep kit	Expected throughput (Segmented reads or 'S-reads')	Kinnex application notes
Single-cell RNA	Kinnex 16-fold prep kit (103-836-900)	Revio (SPRQ-Nx): 100 – 150 M Vega (SPRQ-Nx): 80 – 100 M	- Perform single-cell cDNA amplicon generation using 10x Genomics system or other PacBio Compatible Partner products¹ - Kinnex 16-fold library prep workflow uses unified Kinnex concatenation kit 2.0 and reduces hands-on time for 10x single-cell RNA applications
Bulk rRNA	Kinnex 12-fold prep kit (103-072-100)	Revio (SPRQ-Nx): 70 – 100 M Vega (SPRQ-Nx): 50 – 60 M	- Perform cDNA amplicon generation using Iso-Seq express 2.0 kit + 1.1x cDNA cleanup² 1.1x cDNA cleanup + Kinnex 12-fold library prep workflow provides higher S-read yield and better recovery of <1 kb transcripts - Can optionally consider using Kinnex 8-fold prep kit (+ 0.9x cDNA cleanup) for Kinnex bulk RNA applications when retaining largest transcripts is the priority
16S rRNA	Kinnex 8-fold prep kit (103-072-000)	Revio (SPRQ-Nx): 50 – 70 M Vega (SPRQ-Nx): 40 – 50 M	- Perform 16S amplicon generation using Kinnex-compatible barcoded 16S primers³ - Kinnex 8-fold library prep workflow generally provides higher read quality and generates more usable reads after downstream data filtering using 16S analysis tools
Custom amplicons	Insert size-dependent	Depends on selected Kinnex library prep method	- Generally, recommend to use Kinnex 16-fold prep kit for amplicons <1 kb; Kinnex 12-fold pre kit for amplicons 1 – 2 kb; and Kinnex 8-fold prep kit for amplicons 1.5 – 3 kb - For overlapping amplicon size ranges, can select appropriate Kinnex library prep kit based on consideration of HiFi sequencing performance priorities (i.e., higher read quality versus higher throughput)

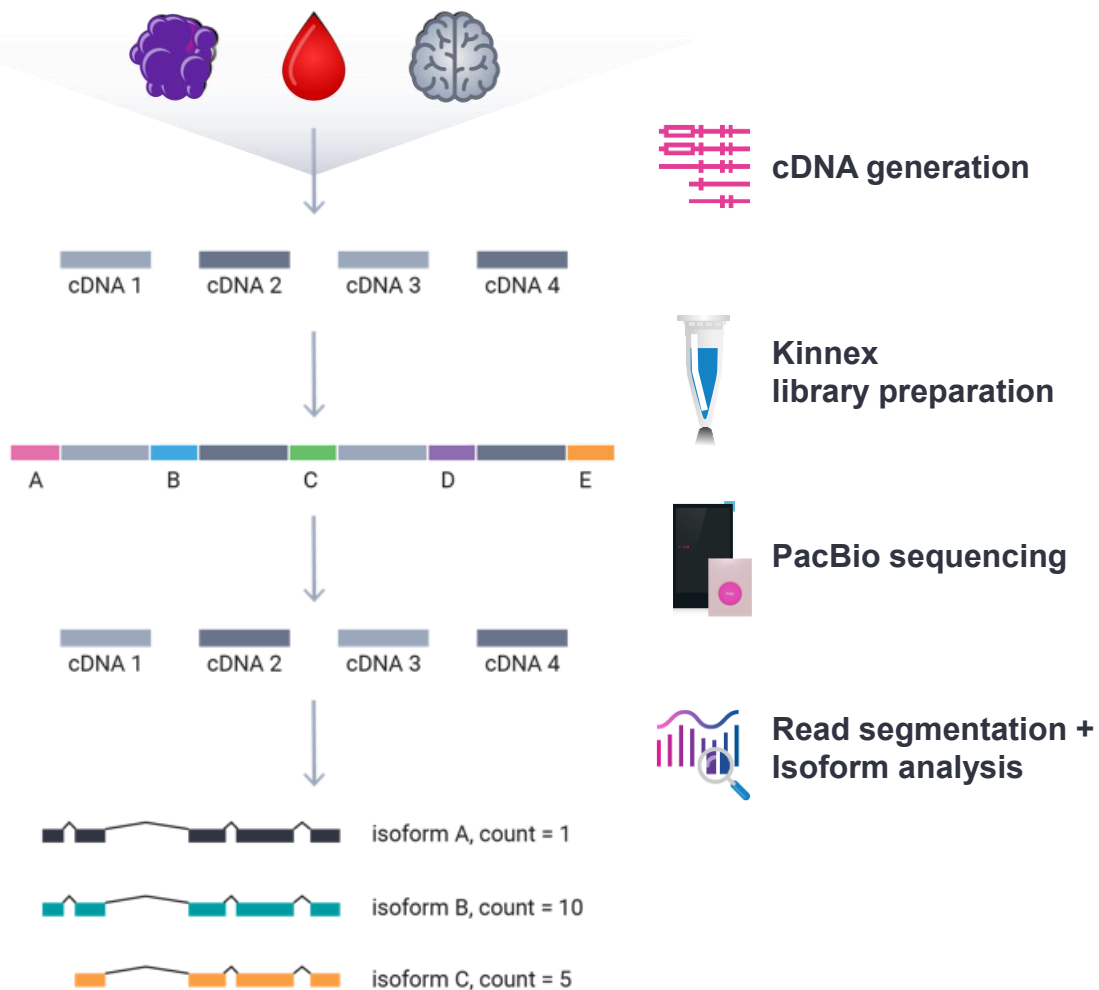
¹ Refer to specific [Application Note](#) literature for compatibility with third-party products such as ArgenTag and Parse Biosciences single-cell kits.

² Refer to *Procedure & checklist – Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit* ([102-396-000](#)).

³ Refer to *Procedure & checklist – Preparing 16S rRNA amplicons for Kinnex* ([103-885-200](#)) for recommended 16S gene-specific forward and reverse PCR primer sequences to order.

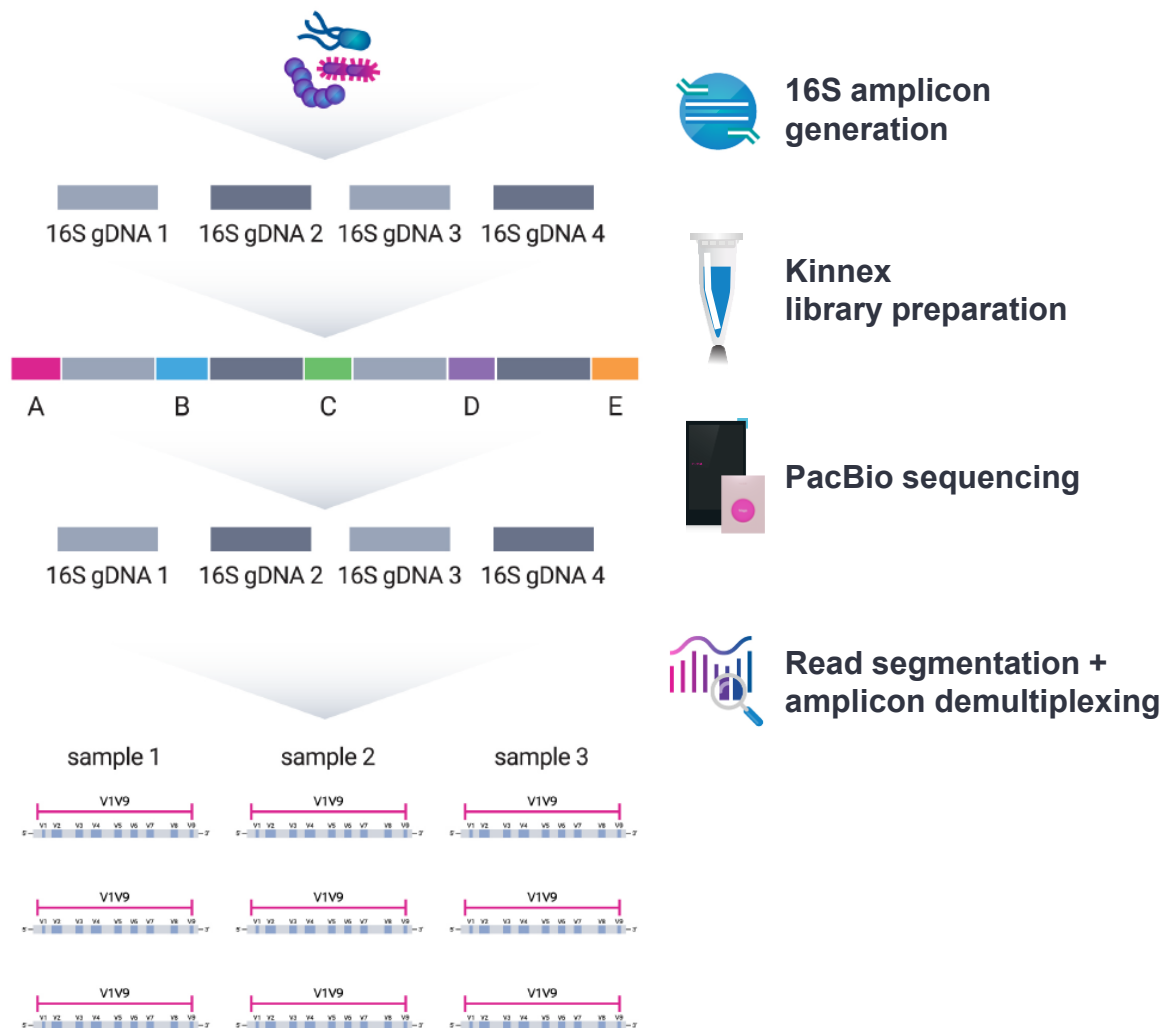
42

Kinnex workflow for bulk RNA sequencing applications



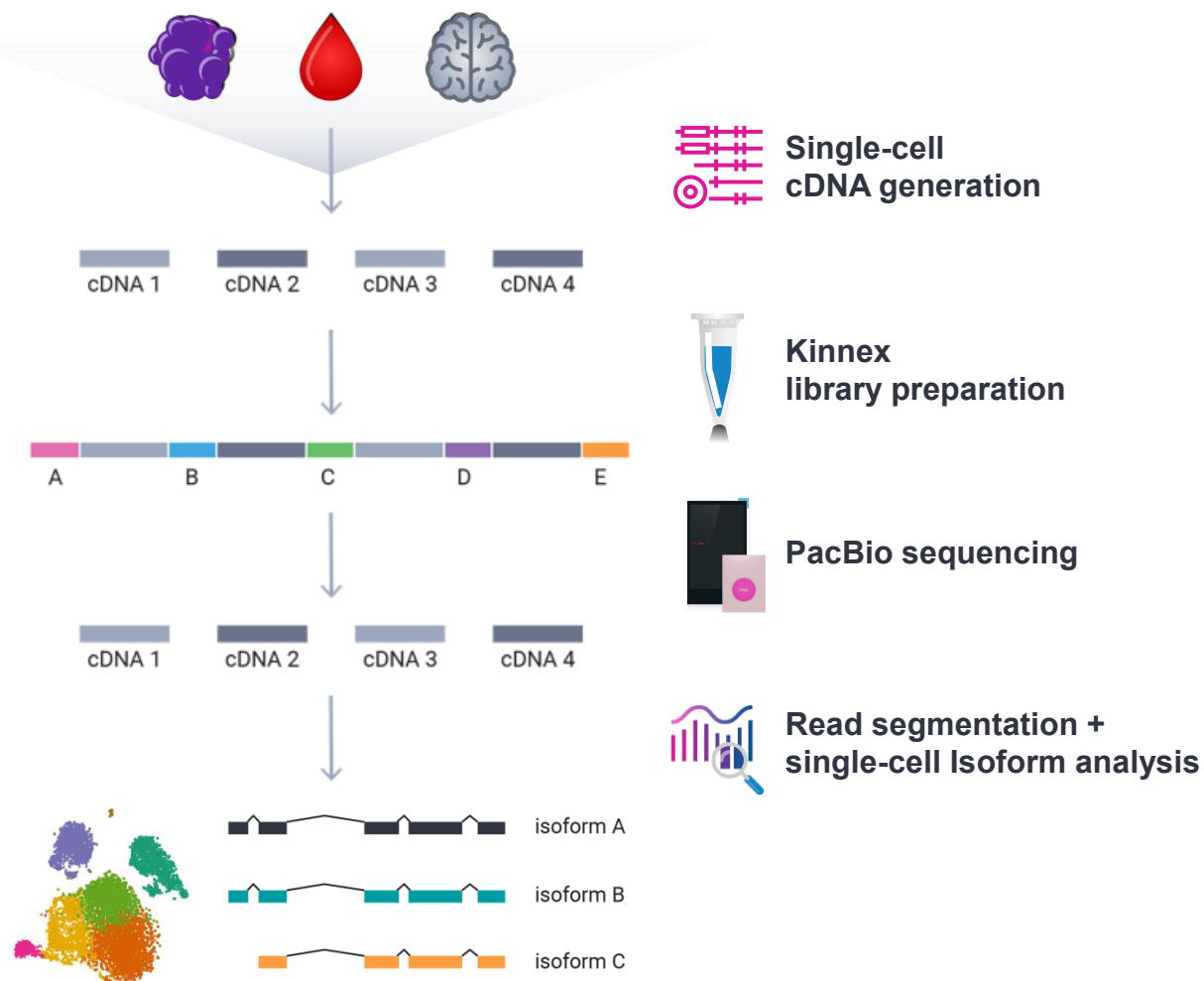
- 300 ng total RNA (RIN ≥ 7) input for generating up to 12-plex barcoded cDNA amplicons using **Iso-Seq express 2.0 kit (103-071-500)**
- 1-day Kinnex library preparation using **Kinnex 8-fold prep kit** or **Kinnex 12-fold prep kit¹**
- SMRT Link Run Design support for 'Kinnex full-length RNA' application type with auto-analysis (read segmentation + isoform analysis)
- SMRT Link Iso-Seq isoform-classification software to identify novel genes and isoforms with abundance information

Kinnex workflow for 16S rRNA sequencing applications



- Generate 16S amplicons (up to 384-plex) using user-supplied barcoded 16S PCR primers (obtained from third-party oligo synthesis provider)
- 1-day Kinnex library preparation using **Kinnex 8-fold prep kit** or **Kinnex 12-fold prep kit**
- 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 PacBio GitHub tools or other custom 16S analysis pipeline

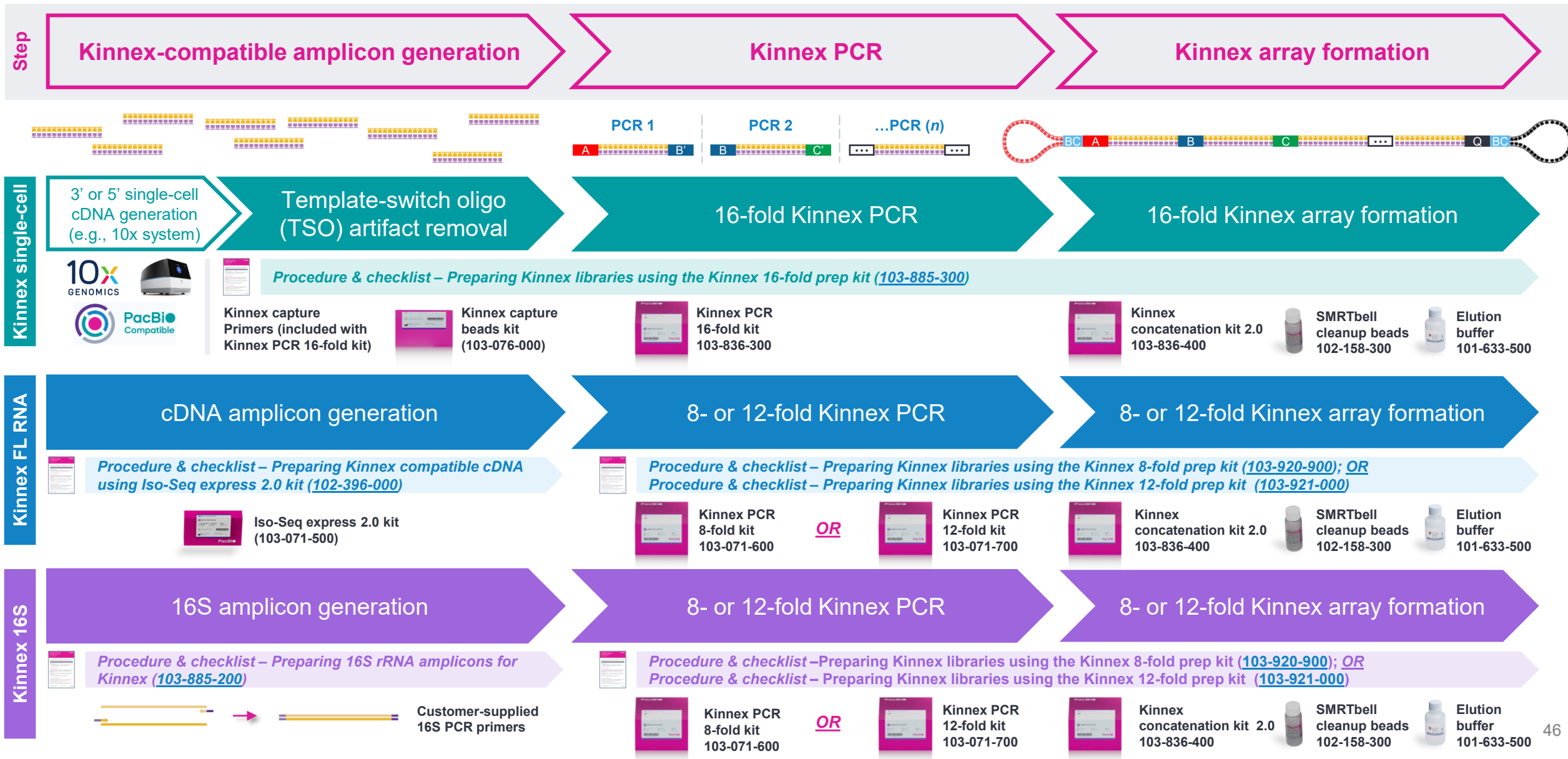
Kinnex workflow for single-cell RNA sequencing applications



- Single-cell cDNA amplicon generation using 10x Chromium Single Cell (3' or 5') reagent kits or other PacBio Compatible products¹ (3,000 to 20,000 target cell recovery)
- ≥ 15 ng 10x single-cell cDNA input for Kinnex library preparation (2.5 days) using **Kinnex 16-fold prep 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*)

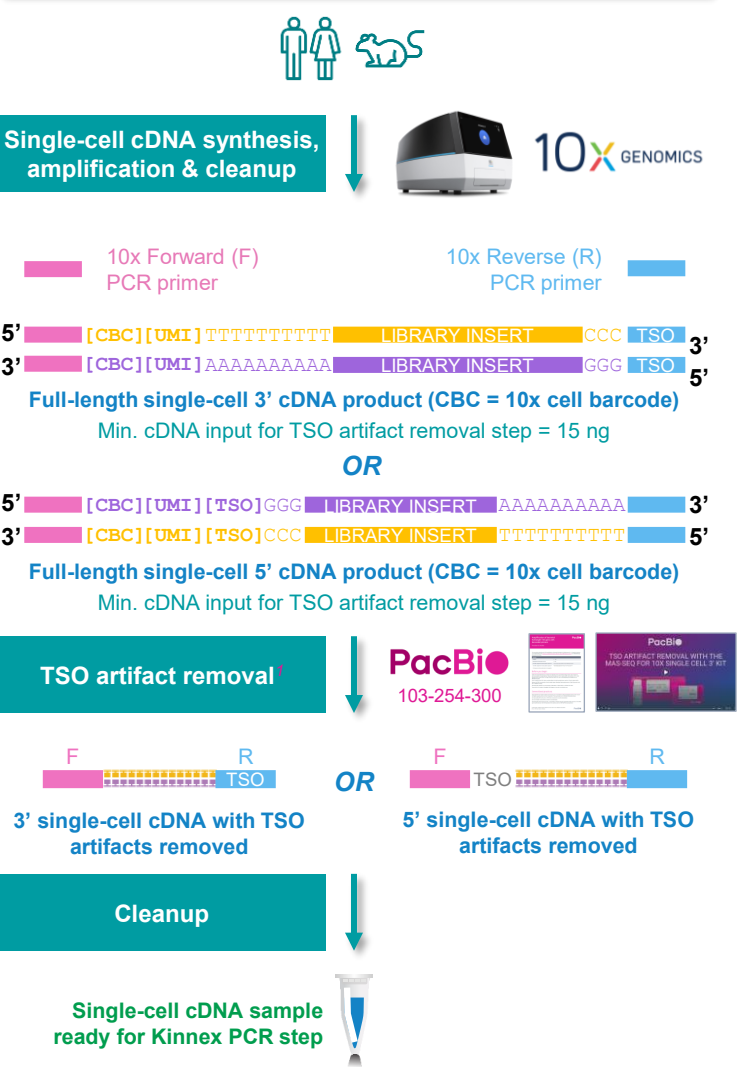
Example Kinnex library preparation workflows for bulk RNA, 16S rRNA and single-cell RNA sequencing applications

Kinnex workflows enable flexibility for choosing different Kinnex prep kits based on project-specific needs



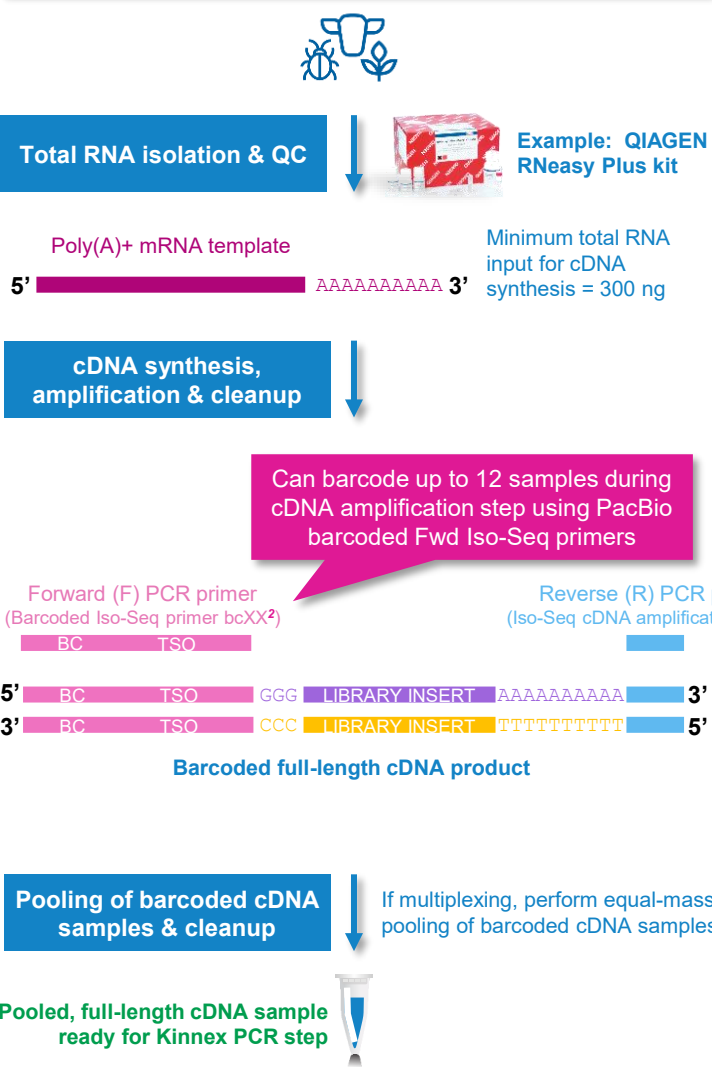
Example Kinnex-compatible amplicon generation workflows for bulk RNA, 16S rRNA and single-cell RNA sequencing applications

Single-cell cDNA sample preparation



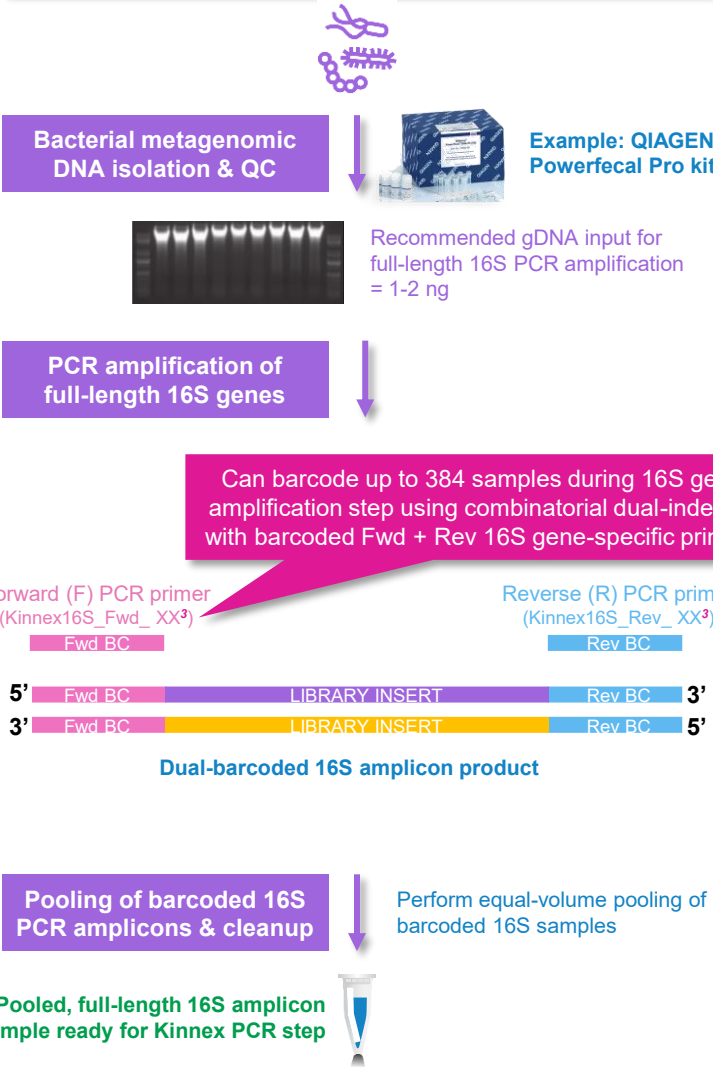
¹ Video tutorial is available: See *TSO artifact removal demo for PacBio MAS-Seq for 10x Single Cell 3' kit* [Click to view the online video: [Link](#)]

Full-length RNA sample preparation



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

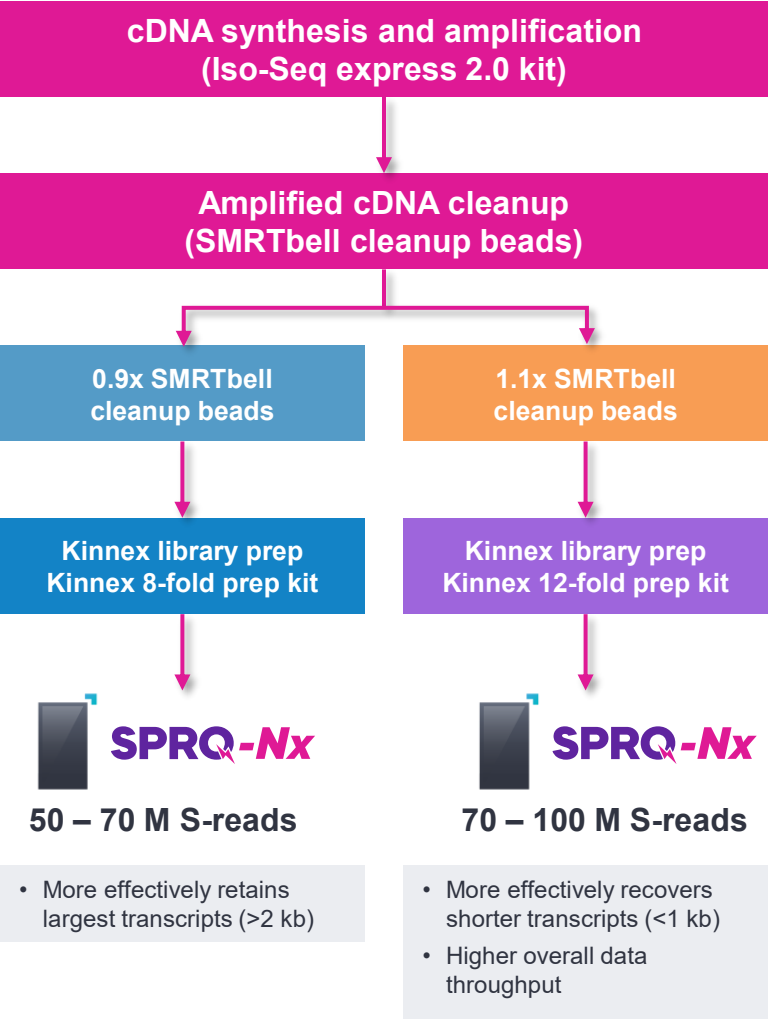


³ Can use 12 different 16S barcoded Forward PCR primers + 32 different 16S barcoded Reverse PCR primers to multiplex up to 384 samples per library prep Rx

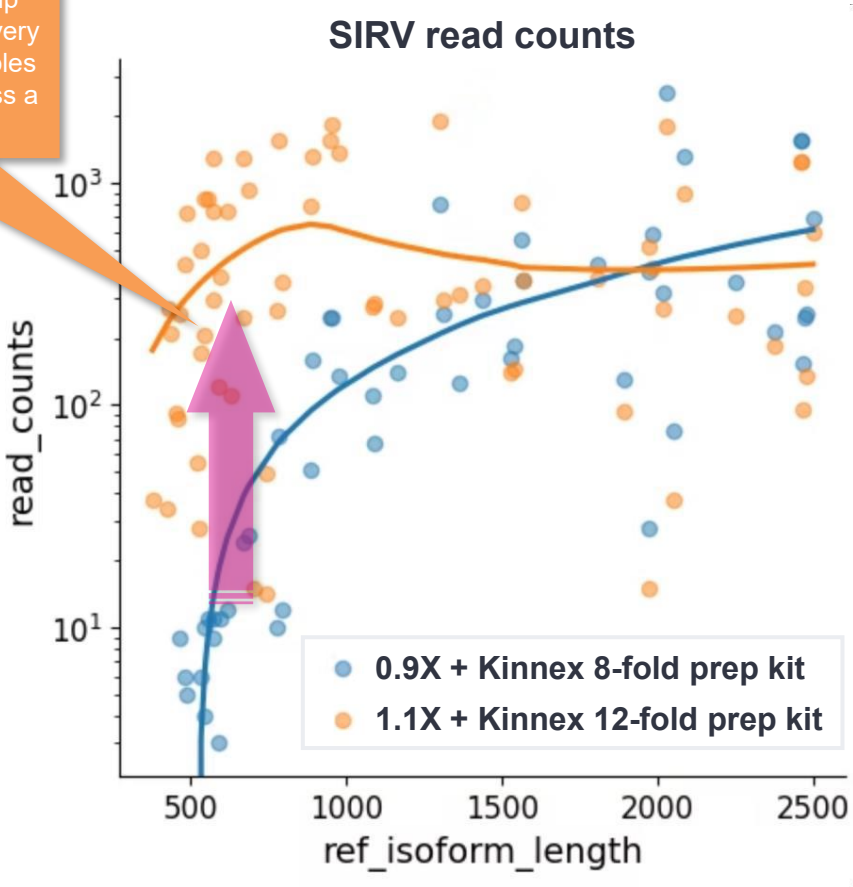
Comparison of 0.9x vs. 1.1x SMRTbell bead cDNA cleanup for Kinnex bulk RNA sequencing

1.1x SMRTbell bead cDNA cleanup + Kinnex 12-fold library prep workflow provides higher data throughput and better recovery of shorter transcripts for Kinnex bulk RNA applications compared to 0.9x SMRTbell bead cDNA cleanup + Kinnex 8-fold library prep workflow

Example Kinnex bulk RNA workflow



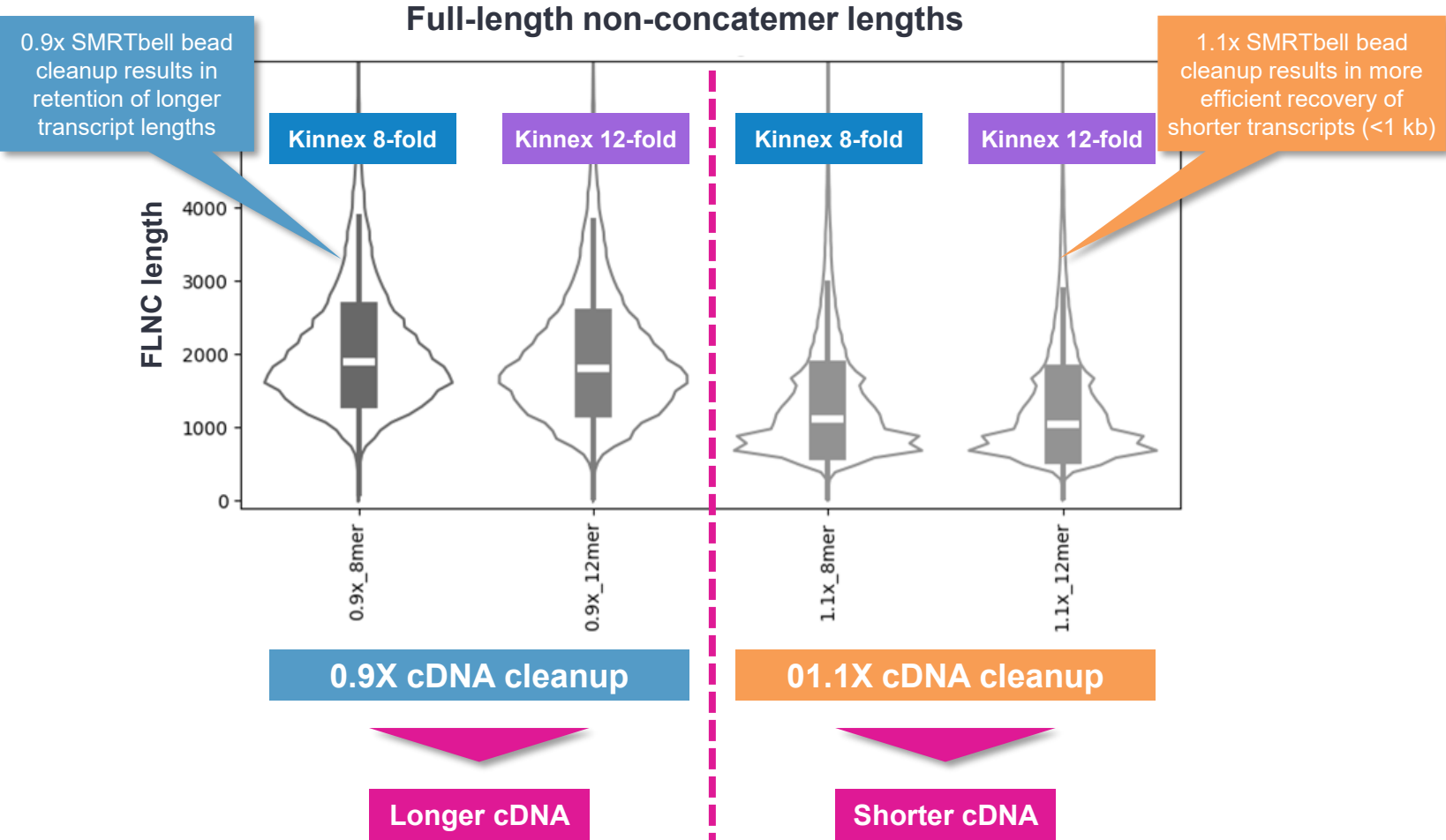
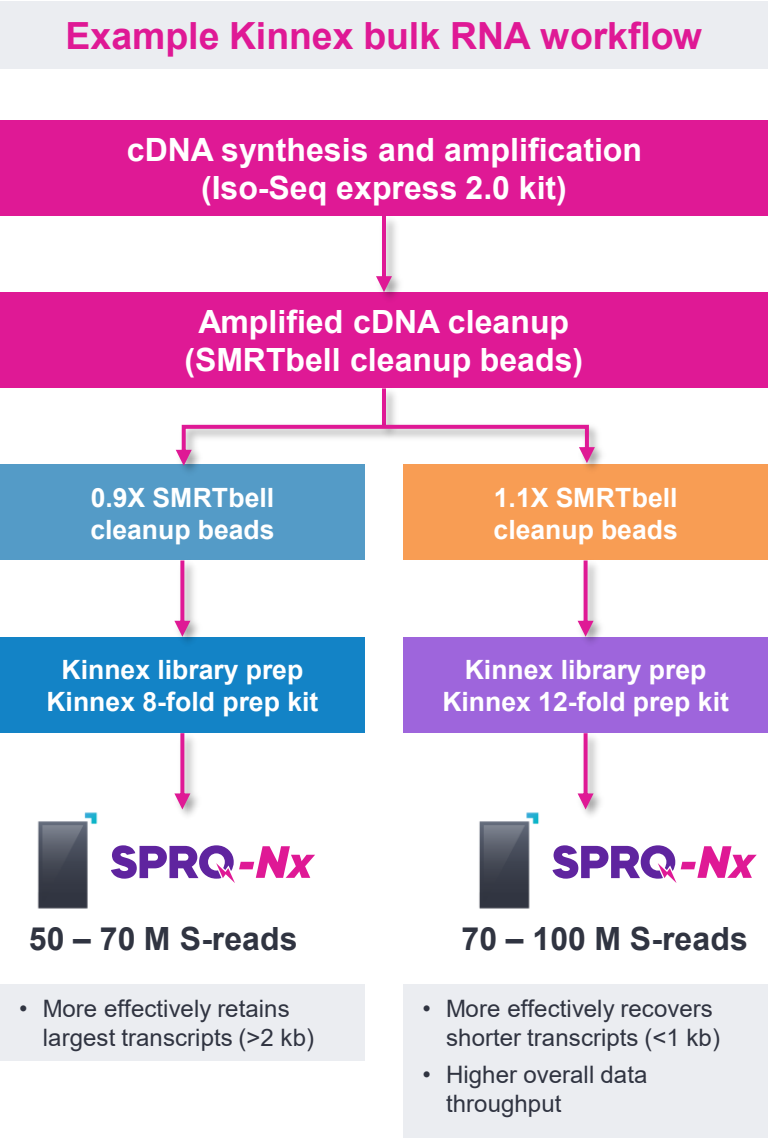
1.1x SMRTbell bead cleanup results in more efficient recovery of <1 kb transcripts and enables improved quantification across a broader size range



Comparison of transcript counts obtained for spike-in RNA variant (SIRV) control samples prepared using 0.9X versus 1.1X SMRTbell bead cleanup procedures during post-cDNA amplification step in *Procedure & checklist – Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit* (102-396-000). SIRV-Set 2 (Iso Mix E0) (Lexogen Cat. No. 050) contains 69 isoform sequences in equimolar ratios, which originate from 7 genes.

Comparison of 0.9x vs. 1.1x SMRTbell bead cDNA cleanup for Kinnex bulk RNA sequencing

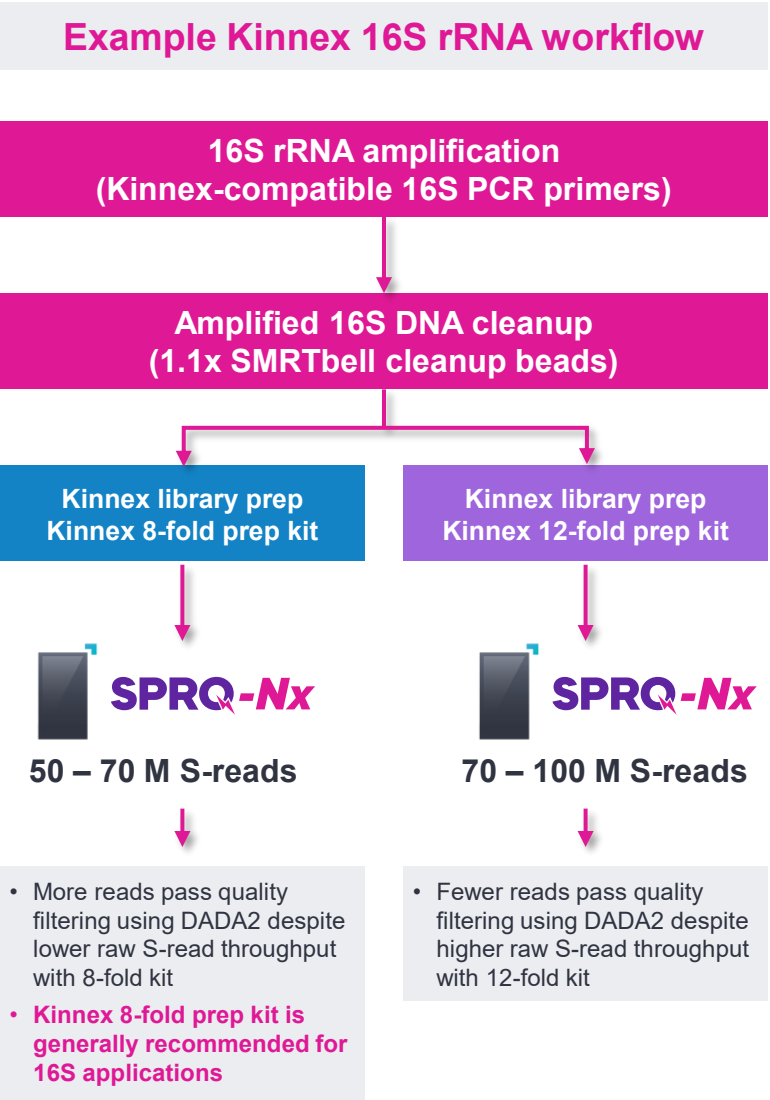
1.1x SMRTbell bead cDNA cleanup + Kinnex 12-fold library prep workflow provides higher data throughput and better recovery of shorter transcripts for Kinnex bulk RNA applications compared to 0.9x SMRTbell bead cDNA cleanup + Kinnex 8-fold library prep workflow



- **1.1x SMRTbell bead cDNA cleanup + Kinnex 12-fold prep kit workflow** provides **higher S-read throughput** and **better recovery of <1 kb transcripts** for Kinnex bulk RNA applications
- Can optionally consider using **0.9x SMRTbell bead cDNA cleanup + Kinnex 8-fold prep kit workflow** for Kinnex bulk RNA applications when retaining the **largest** transcripts is the priority

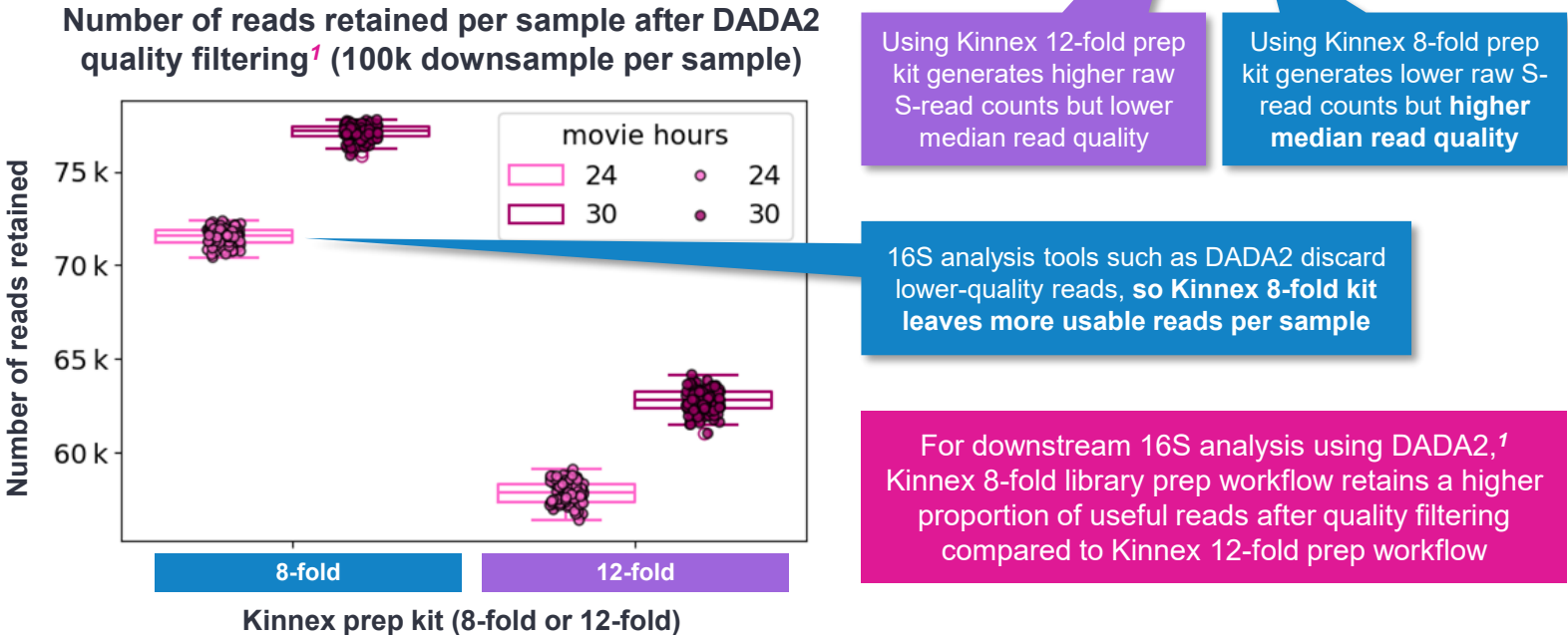
Comparison of Kinnex 8-fold prep kit versus 12-fold prep kit for Kinnex 16S rRNA sequencing

Kinnex 8-fold prep kit provides higher data quality that is suitable for downstream 16S metagenomics analysis tools



Example HiFi sequencing metrics for 16S rRNA libraries (384-plex) prepared with Kinnex 8-fold prep kit vs. Kinnex 12-fold prep kit

Sample	Movie time (hrs)	P1 (%)	HiFi Yield (Gb)	HiFi reads	Read quality (median)	Q30+ BQ (%)	S-reads per acquisition
16S_12mer	24	69.14	147.9	7,860,184	Q34	93.9%	93,421,796
16S_8mer	24	74.96	120.4	9,541,465	Q37	95.4%	75,949,260
16S_12mer	30	65.52	151.2	8,028,963	Q35	94.4%	95,454,790
16S_8mer	30	70.03	123.8	9,811,370	Q40	96.4%	78,112,438



Comparison of number of reads retained after DADA2 quality filtering. Kinnex 16S rRNA libraries (384-plex) were prepared with Kinnex 8-fold prep kit or Kinnex 12-fold prep kit and run on a Revio system with SPRO-Nx chemistry using 24 or 30 movie acquisition times.

¹ Refer to [HiFi-16S-workflow](#) documentation available on [PacBio GitHub](#) for details about quality filtering steps when processing HiFi full-length 16S data into high-quality amplicon sequence variants (ASVs) using QIIME 2 and DADA2.



APPENDIX 2: PacBio compatible Kinnex library preparation workflows



Preparing Kinnex single-cell libraries with ArgenTag kits

Preparing Kinnex single-cell libraries with ArgenTag kits: Getting started



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 – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits [ArgenTag]

Technical documentation describing the workflow for preparing PacBio Kinnex single-cell libraries with single-cell cDNA generated using ArgenTag Single-Cell RNA kit for Long-Read Sequencing.

Procedure & checklist – Preparing Kinnex libraries using Kinnex 8-fold prep kit ([103-920-900](#)) or Kinnex 12-fold prep kit ([103-921-000](#)) [PacBio]

Technical documentation containing PacBio
SMRTbell library construction details.

Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit (103-916-000) or Kinnex 12-fold prep kit (103-916-100) [PacBio]

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 ArgenTag Single-Cell RNA kit for Long-Read Sequencing

Technical note – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits ([ArgenTag](#)) describes the workflow for preparing PacBio Kinnex single-cell libraries with single-cell cDNA generated using ArgenTag Single-Cell RNA kit for Long-Read Sequencing

Technical Note

Single-Cell isoform Sequencing with ArgenTag and PacBio® Kinnex™ kits

ArgenTag PacBio

Overview

This technical note outlines the experimental conditions for preparing PacBio Kinnex libraries using cDNA generated with the ArgenTag Single-Cell RNA kit for Long-Read Sequencing.

ArgenTag cDNA is compatible with both the Kinnex full-length RNA kit (8-fold concatenation) and the Kinnex 16S rRNA kit (12-fold concatenation). The choice of kit depends on the experimental goal: the 8-fold kit maximizes read length, while the 12-fold kit maximizes sequencing throughput.

ArgenTag Single-Cell Kit for Long-Read Sequencing

PacBio Kinnex 8-fold

Kinnex full-length RNA kit
For maximized read-length

PacBio Kinnex 12-fold

Kinnex 16S rRNA kit
For maximized number of reads

Figure 1: PacBio Kinnex kit options compatible with the ArgenTag Single-Cell RNA Kit for Long-Read Sequencing.

Required materials and equipment

- ArgenTag Single-Cell kit (cat #AT001-SD2) for Long-Read Sequencing and third party materials described in section 3 of the protocol [1].
- For the cDNA cleanup (step 8.C), we recommend AMPure® PB beads (PacBio cat #100-265-900).
- Reagents and equipment from the selected Kinnex protocol [2, 3], starting from the Kinnex PCR step.
- Note that the TSO artifact removal step is not required. ArgenTag cDNA is inherently TSO artifact-free, streamlining library preparation to ~5 hours.

ArgenTag single-cell long cDNA

Kinnex PCR

Kinnex array

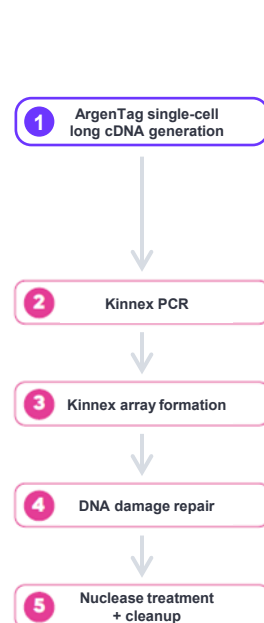
DNA damage repair

Nuclease treatment and cleanup

Figure 2: Library preparation workflow for generating Kinnex libraries from ArgenTag single-cell barcoded long cDNA. Steps include Kinnex PCR, array formation, DNA damage repair, and nuclease treatment and cleanup.

ArgenTag PacBio

argenitag.com - 1



ArgenTag Technical note
Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits ([ArgenTag](#))



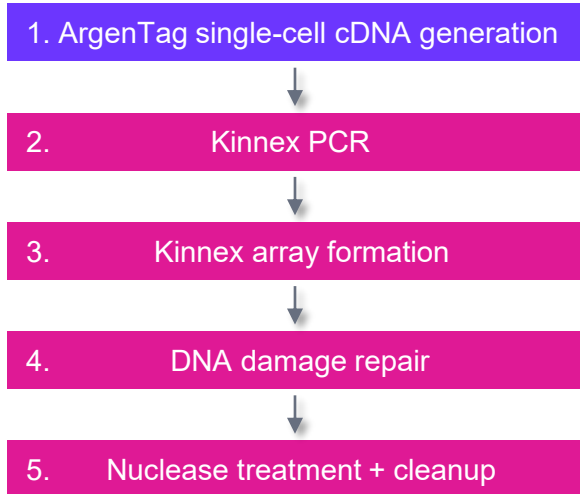
PacBio Procedure & checklist
Preparing Kinnex libraries using Kinnex 8-fold prep kit ([103-920-900](#))
Or
Preparing Kinnex libraries using Kinnex 12-fold prep kit ([103-921-000](#))

Overview

- ArgenTag Technical note ([ArgenTag](#)) outlines the experimental conditions for preparing PacBio Kinnex libraries using cDNA generated with the [ArgenTag Single-Cell RNA kit for Long-Read Sequencing](#)
- ArgenTag cDNA is compatible with both the [PacBio Kinnex 8-fold prep kit](#) and the [Kinnex 12-fold prep kit](#)
- The choice of Kinnex prep kit depends on the experimental goal:
 - Kinnex 8-fold kit maximizes read length**, while the **Kinnex 12-fold kit maximizes sequencing throughput**

Workflow overview for preparing Kinnex single-cell libraries with ArgenTag Single-Cell RNA kit for Long-Read Sequencing

Follow ArgenTag ArgenTag Single-Cell RNA kit procedure¹ to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems

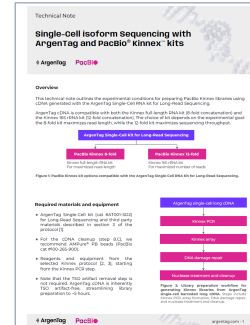


ArgenTag single-cell cDNA generation procedure overview¹

Technical note – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits

[[ArgenTag](#)]





ArgenTag single-cell cDNA generation procedure key steps

- Single-cell cDNA material first needs to be generated as described in the ArgenTag protocol¹
Procedure & checklist – ArgenTag Single-Cell kit for Long-Read Sequencing handbook



Procedure & checklist – ArgenTag Single-Cell kit for Long-Read Sequencing handbook [[ArgenTag](#)]

- Use the generated cDNA as input into the **PacBio Kinnex 8-fold prep protocol** ([103-920-900](#)) or **Kinnex 12-fold prep protocol** ([103-921-000](#)) starting at **Step 3: Kinnex PCR**.

ArgenTag single-cell cDNA generation workflow¹ (Step 1)

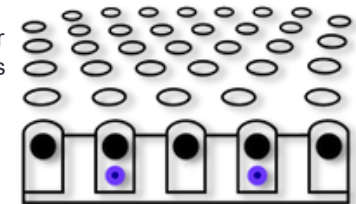
1: Load the ArgenTag chip

Pipette your cell suspension onto the ArgenTag microwell chip. Cells will settle into individual microwells by gravity.



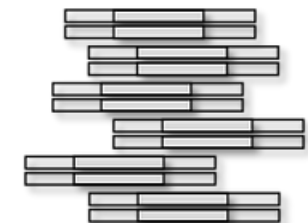
2: Capture and barcode

Add the barcoded magnetic beads to pair with the cells in microwells. Lyse the cells in place and recover the beads with the poly-A mRNA.



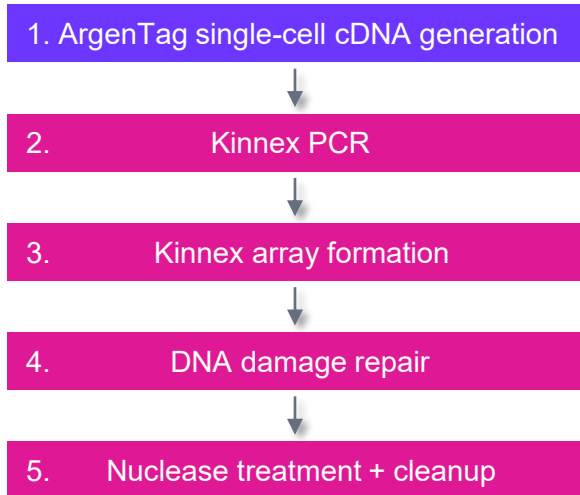
3: Reverse transcribe and amplify

Perform the reverse transcription with the magnetic beads, and amplify to generate full-length, barcoded cDNA.



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](#))



PacBio Kinnex library construction procedure¹

Procedure & checklist – Preparing Kinnex libraries using Kinnex 8-fold prep kit ([103-920-900](#)) or Kinnex 12-fold prep kit ([103-921-000](#))

PacBio

Preparing Kinnex[®] libraries using the Kinnex 8-fold prep kit¹
Procedure & checklist

Overview

This document describes the workflow for constructing Kinnex[®] 8-fold libraries from single-cell cDNA samples using the Kinnex 8-fold prep kit. The workflow is designed to be performed on a single-cell cDNA sample. The generation of the library is dependent on the Kinnex[®] 8-fold prep kit and the Kinnex 8-fold prep kit.

Overview

Step	Time
1. Preparing the library	1 day for 8-fold libraries
2. Preparing the library	1 day for 12-fold libraries

Number of expected libraries per library

Kit	Number of expected libraries per library
Kinnex 8-fold prep kit	100
Kinnex 12-fold prep kit	100

Kit

Kit	Kit
Kinnex 8-fold prep kit	Kinnex 8-fold prep kit
Kinnex 12-fold prep kit	Kinnex 12-fold prep kit

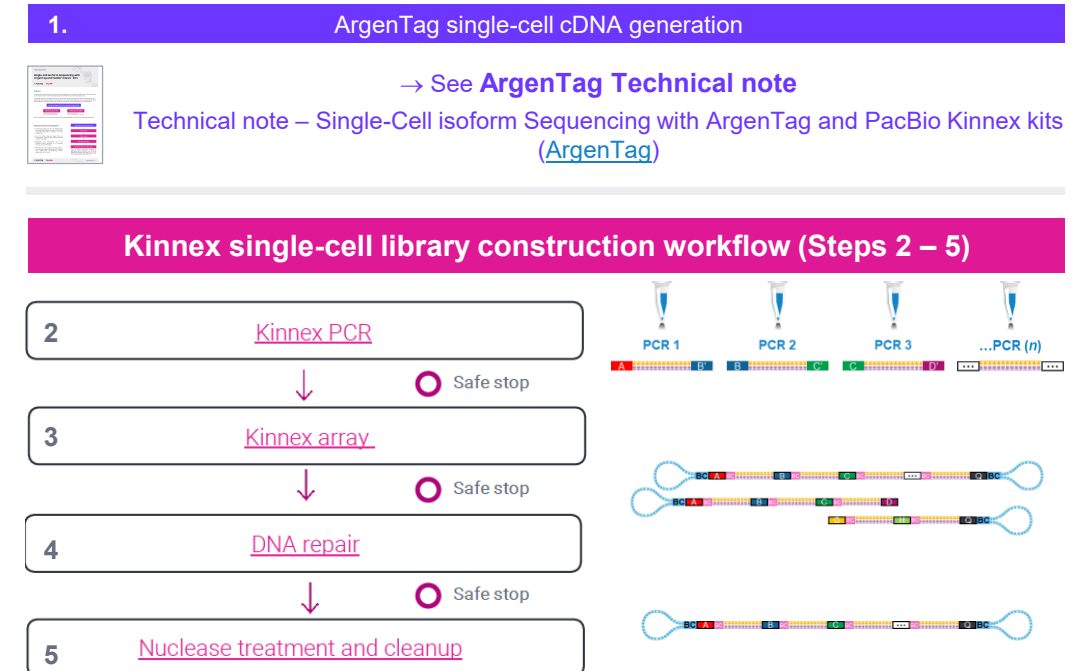
Expected library size (bp)

Kit	Expected library size (bp)
Kinnex 8-fold prep kit	1,000 – 5,000 bp
Kinnex 12-fold prep kit	1,000 – 5,000 bp

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Kinnex single-cell library prep procedure key steps

- Following cDNA QC per the ArgenTag user manual: Ideal cDNA profile should have an average peak above 1,200 bp with fragments in the 1,000–5,000 bp range exceeding 30% of total molecules
- Proceed directly to Kinnex PCR step using 35 ng input for 8-fold concatenation using **Kinnex 8-fold prep kit** ([103-920-900](#)) or 12-fold concatenation using **Kinnex 12-fold prep kit** ([103-921-000](#))
- Library preparation then continues according to the respective PacBio Kinnex protocol
- Following sample setup ABC, prepare libraries for sequencing using SMRT Link Run Design using same parameters as standard Kinnex libraries

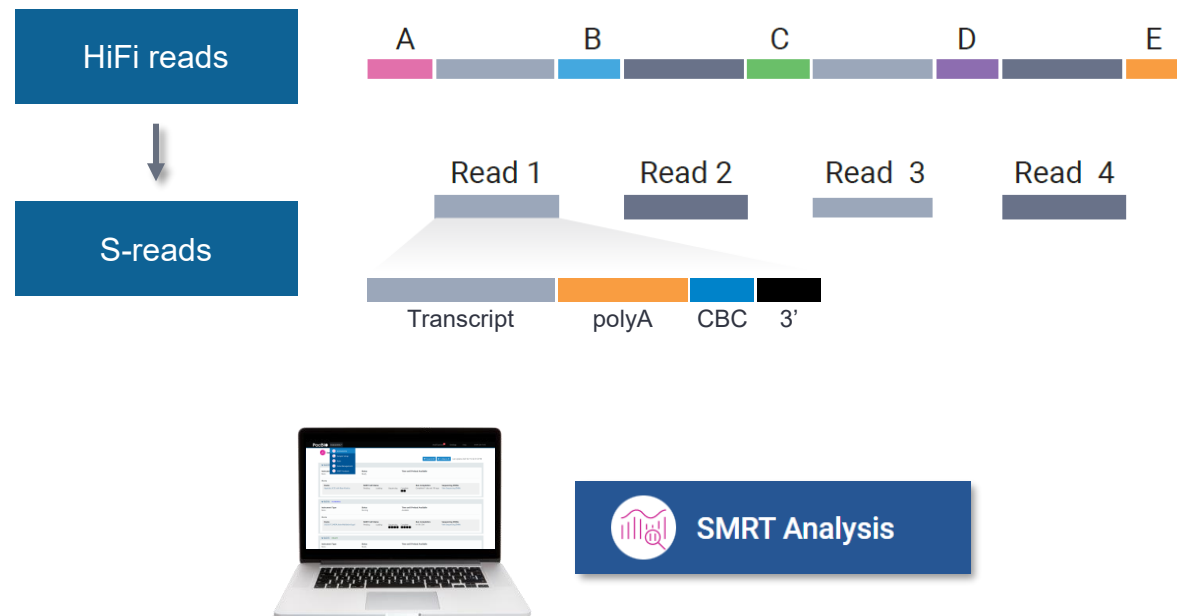


SMRT Link informatics and secondary analysis recommendations for Kinnex single-cell libraries generated with ArgenTag Single-Cell RNA kit

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

- 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 ArgenTag single-cell cDNA barcode information
- S-reads can be demultiplexed and analyzed using methods described in **Technical note – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits** [[ArgenTag](#)]¹

SMRT Link read segmentation workflow



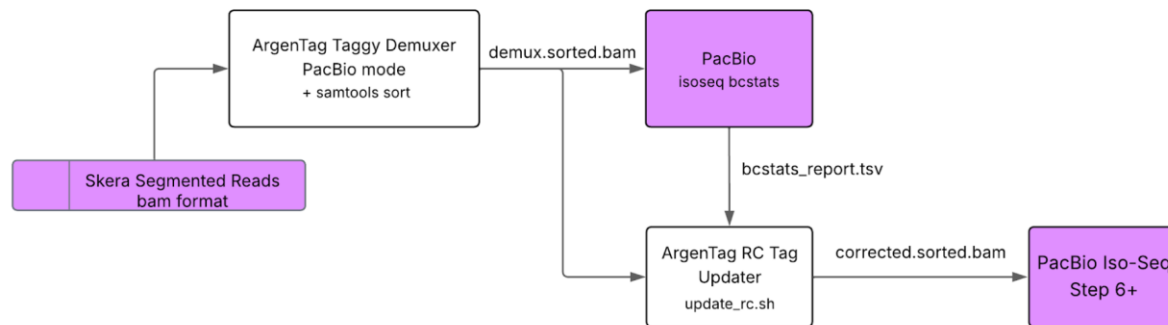
SMRT Link informatics and secondary analysis recommendations for Kinnex single-cell libraries generated with ArgenTag Single-Cell RNA kit (cont.)

Segmented reads (S-reads) can be demultiplexed and analyzed using ArgenTag + PacBio tools

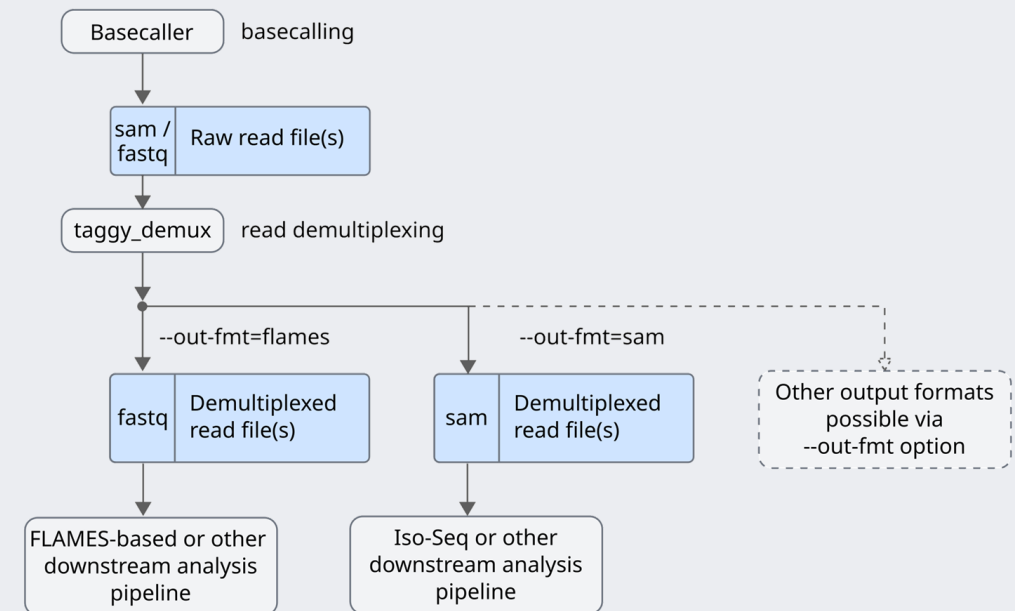
- S-reads can be demultiplexed and analyzed using the bioinformatics pipeline described below¹:

AT taggy demux + update_rc.sh + PB Iso-Seq pipeline description

- Input:** S-reads (.bam)
- Steps:** AT Taggy Demuxer, + update_rc.sh +, PB Iso-Seq workflow (step 6+), Pigeon.
- Output:** GTF, gene and isoform classification, gene- and isoform-level count matrix, isoform classification.

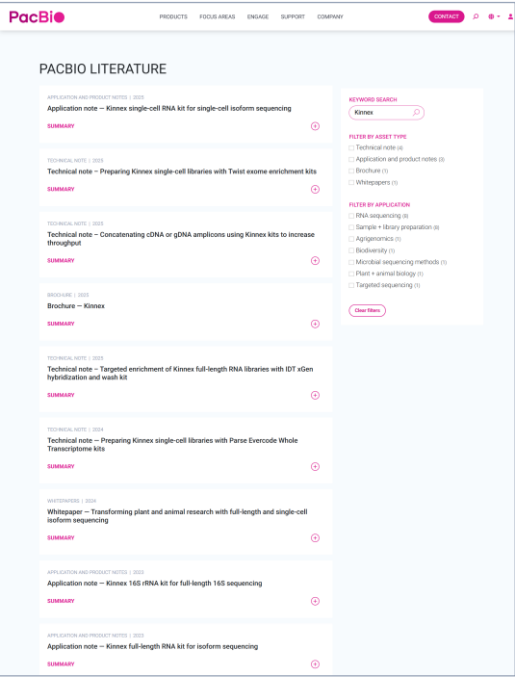
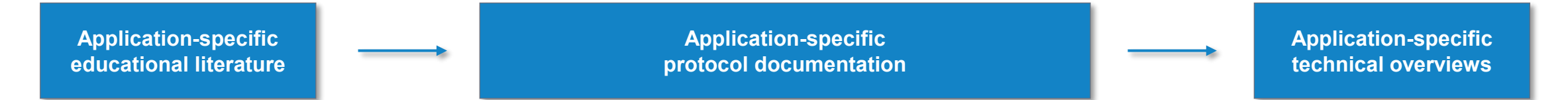


ArgenTag single-cell read demultiplexing workflow



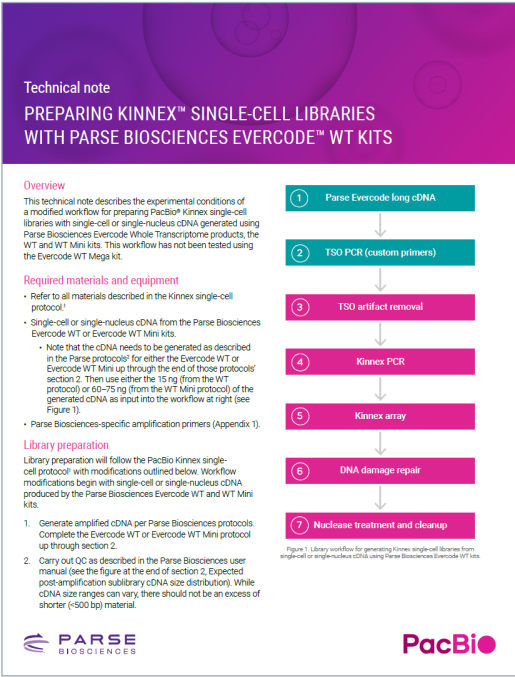
Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

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



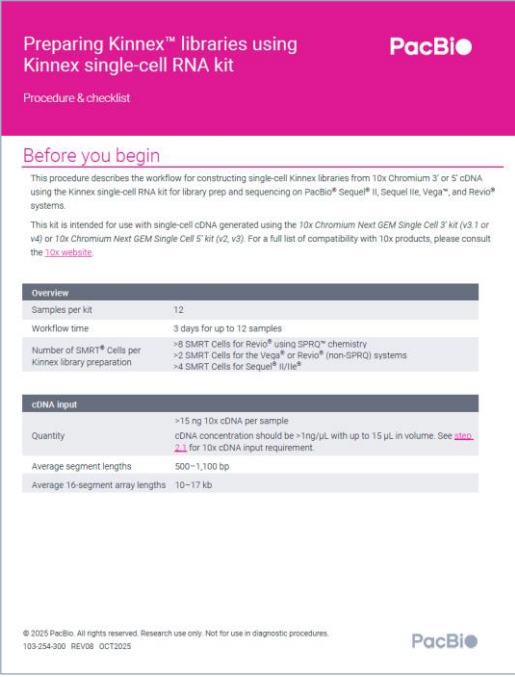
PacBio literature website [Link]

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



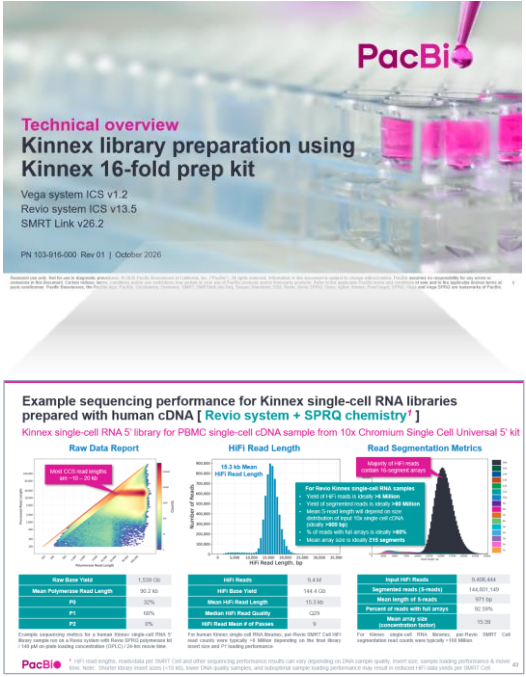
Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610) [PacBio]

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 – Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.



Technical overview – Kinnex library preparation using Kinnex 16-fold prep kit (103-916-200)

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 protocols² 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 protocol¹ 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.

- Generate amplified cDNA per Parse Biosciences protocols. Complete the Evercode WT or Evercode WT Mini protocol up through section 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.

```
graph TD; 1[1 Parse Evercode long cDNA] --> 2[2 TSO PCR (custom primers)]; 2 --> 3[3 TSO artifact removal]; 3 --> 4[4 Kinnex PCR]; 4 --> 5[5 Kinnex array]; 5 --> 6[6 DNA damage repair]; 6 --> 7[7 Nuclease treatment and cleanup];
```

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

Preparing Kinnex Single-Cell Libraries with Parse Evercode Whole Transcriptome Kits (102-326-610)

Preparing Kinnex Libraries using Kinnex 16-fold Prep Kit (103-885-300)

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]

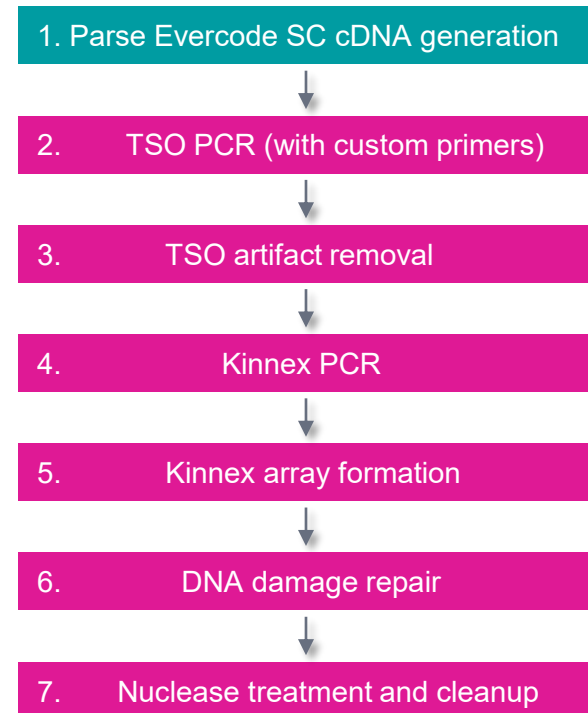
Overview

- Parse whole transcriptome kits use split-pool combinatorial barcoding to enable scale-up of single cell projects to millions of cells or nuclei¹
- 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 Procedure & checklist
Preparing Kinnex libraries using Kinnex 16-fold prep kit ([103-885-300](#))

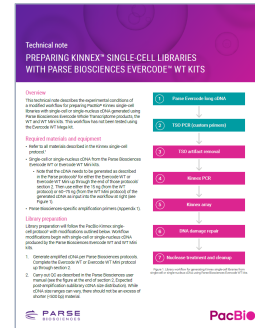
Workflow overview for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits

Follow Parse Bioscience whole transcriptome kit procedure¹ to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems



Parse single-cell cDNA generation procedure overview¹

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



Parse single-cell cDNA generation procedure key steps

- Single-cell cDNA material first needs to be generated as described in the Parse protocols¹ for either the **Evercode WT** or **Evercode WT Mini kit** up through the end of those protocols' **Section 2**.



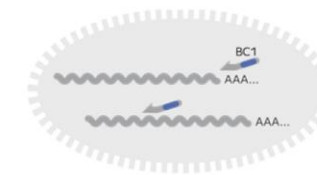
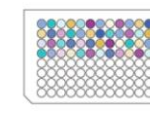
Evercode WT User Guides
[UMWT3300](#) or [UMWT3100](#)

- 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 16-fold prep kit protocol** (103-885-300) starting at **Step 2: TSO PCR (with custom primers²)**.

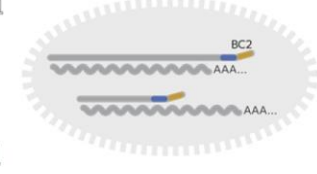
Parse Evercode single-cell cDNA generation workflow¹ (Step 1)

Section 1: In situ cell / nuclei barcoding

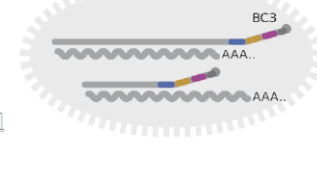
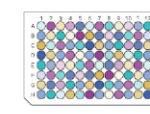
Barcoding round 1



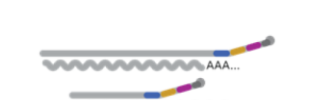
Barcoding round 2



Barcoding round 3

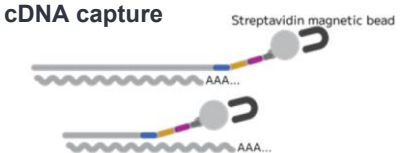


Lysis and Sublibrary Generation

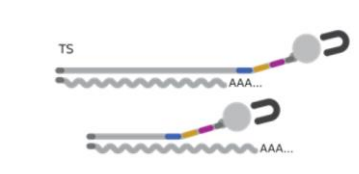


Section 2: cDNA capture and amplification

cDNA capture



cDNA template switch



cDNA amplification

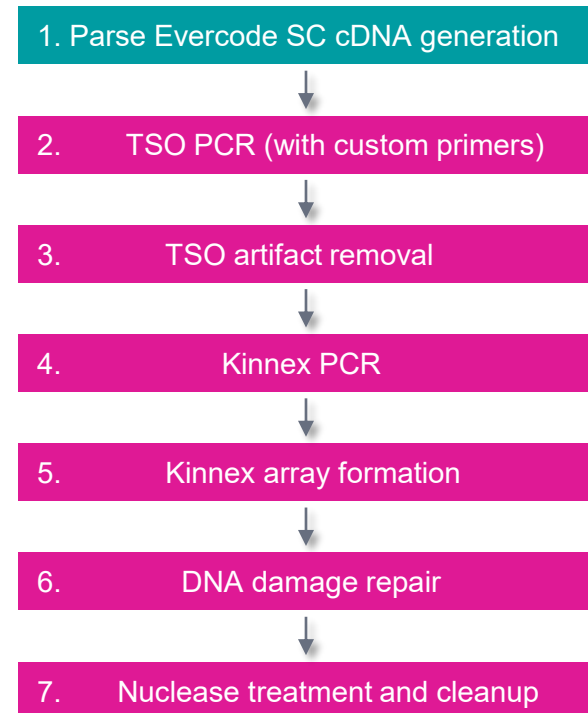


¹ For single-cell cDNA generation procedure details, refer to **Evercode WT User Guides** ([UMWT3300](#) or [UMWT3100](#)) available from the [Parse Biosciences website](#).

² Perform **TSO PCR (Step 2)** with Parse Biosciences-specific amplification primers (see Appendix 1 in **Parse Technical note** [102-326-610](#)).

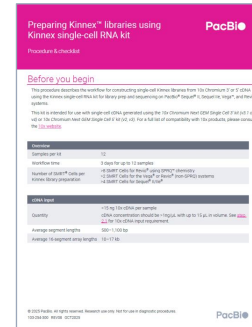
Workflow overview for preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (cont.)

Parse single-cell cDNA samples may be used for Kinnex library construction by following *PacBio Procedure & checklist – Preparing Kinnex libraries using the Kinnex 16-fold prep kit* ([103-885-300](#))



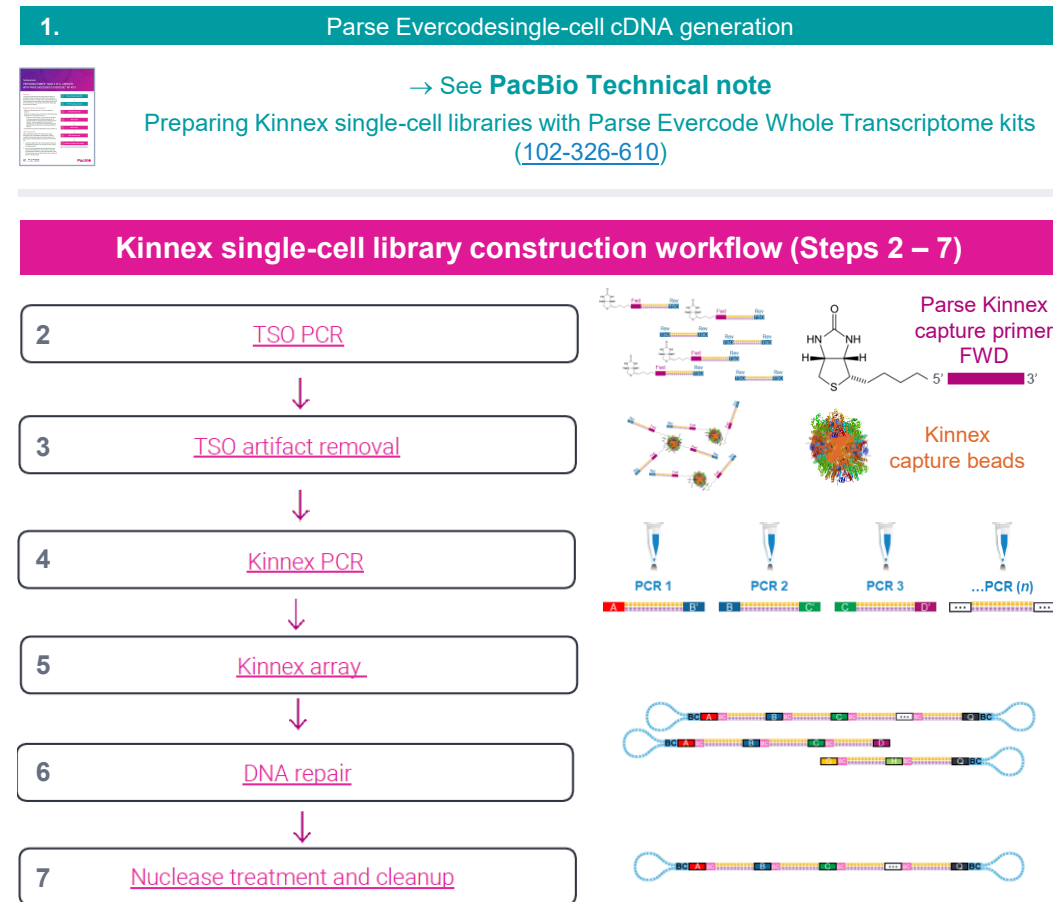
PacBio Kinnex library construction procedure¹

Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit ([103-885-300](#))



Kinnex SC library prep procedure key steps

- Follow **PacBio Kinnex 16-fold prep kit protocol** ([103-885-300](#)) (**Steps 2 – 7**) with modifications:
- 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 16-fold prep kit 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 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 16-fold pre kit protocol (step 5.21 in [103-885-300](#)).

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

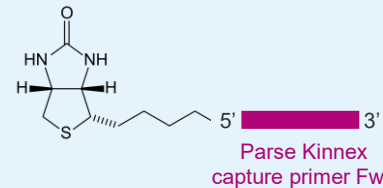
Order custom TSO PCR amplification primers as described below from your preferred oligo vendor

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 16-fold prep kit (103-836-900)

Parse Kinnex forward capture primer

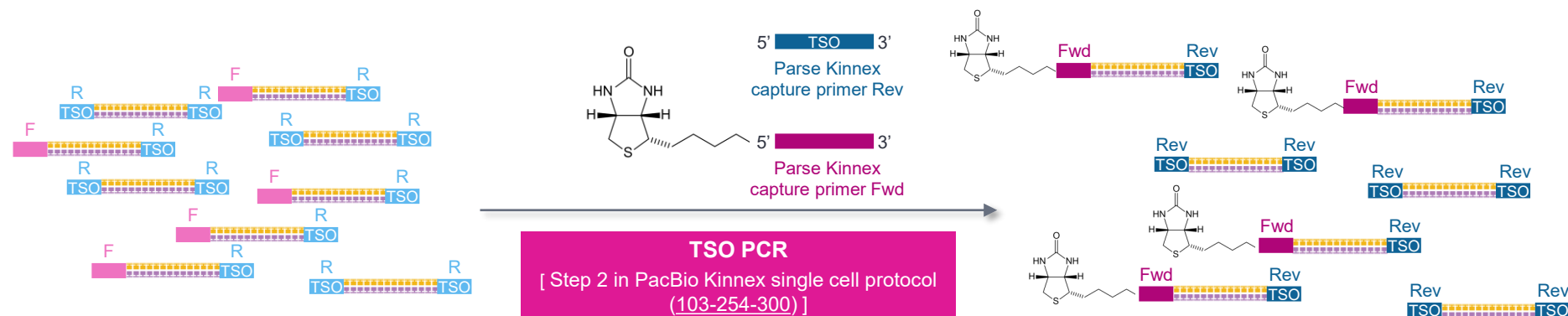
/5Biosg/AA/ideoxyU/CTACACGACGCTCTT
CCGATCTCAGACGTGTGCTCTTCCGATC



Parse Kinnex reverse capture primer

AAGCAGTGGTATCAACGCAGAG

5' TSO 3'
Parse Kinnex
capture primer Rev



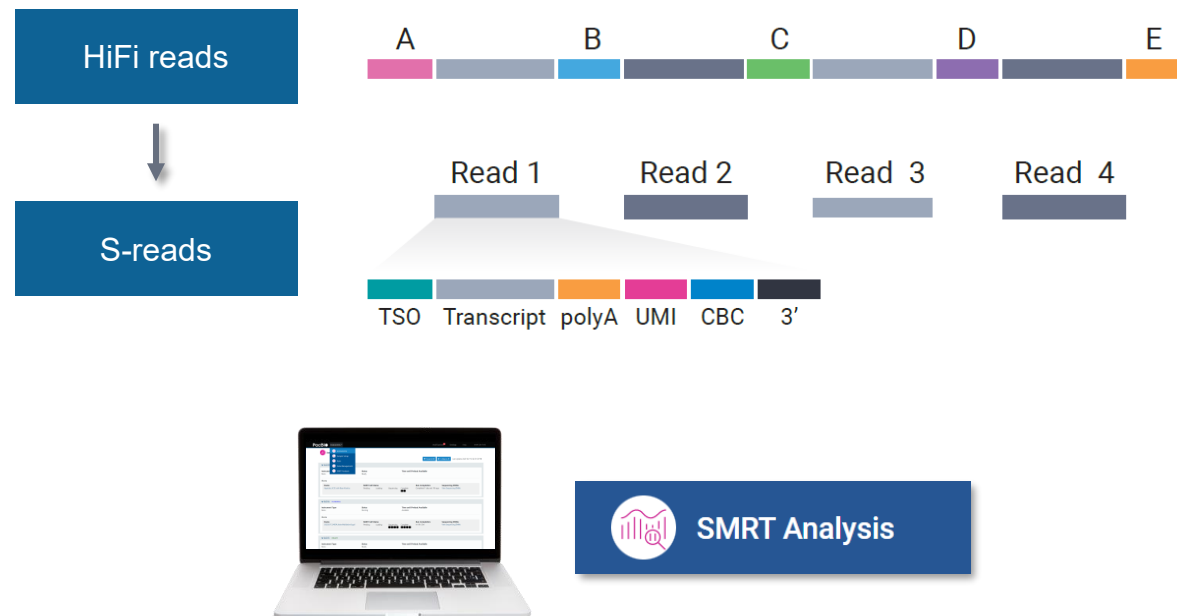
TSO PCR step in Kinnex single-cell RNA procedure uses a modified PCR primer (Parse Kinnex capture primer Fwd) to incorporate a biotin tag into desired cDNA products with the correct structure.¹

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

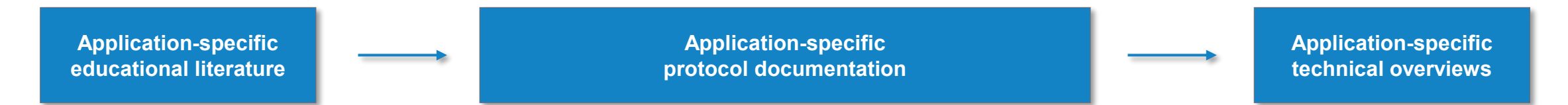
- Note: The SMRT Link single-cell Iso-Seq workflow does not currently support analysis of Parse Biosciences data.
- Instead, Read Segmentation can be run in SMRT Link (or skera in the command line) to generate segmented reads (S-reads).
- The S-reads will contain the original single-cell or single-nucleus 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 guide² (login required) for secondary analysis recommendations using command line and community tools.

SMRT Link read segmentation workflow



Preparing Kinnex single-cell libraries with Twist exome enrichment kits

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



PacBio LITERATURE

Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing

Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits

Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput

Brochure – Kinnex

Technical note – Targeted enrichment of Kinnex full-length RNA libraries with iOT aden hybridization and wash kit

Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcription kits

Whitepaper – Transforming plant and animal research with full-length and single-cell isoform sequencing

Application note – Kinnex 16S RNA kit for full-length 16S sequencing

Application note – Kinnex full-length RNA kit for isoform sequencing

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

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*. 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	Twist 100031: 2 nm Twist 100032: 12 nm Twist 100033: 96 nm
OR	
Twist RNA Exome Panel	Twist 10143: 2 nm Twist 10144: 12 nm Twist 10146: 96 nm
Twist Universal Blockers	Twist 100856: 2 nm Twist 100876: 12 nm Twist 100787: 96 nm
Twist Standard Hyb and Wash Kit v2 with Amp Mix	Twist 105559: 2 nm Twist 105560: 12 nm Twist 105561: 96 nm

* The 10x cDNA primer is included in 10x Single Cell Gene Expression kits. If stock lot 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.

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.

Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit 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 SMART® Cells per Kinnex library preparation	>8 SMART® Cells for Revio® using SPRQ™ chemistry >2 SMART® Cells for the Vega® or Revio® (non-SPRQ) systems >4 SMART® 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 link .
Average segment lengths	500–1,100 bp
Average 16-segment array lengths	10–17 kb

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103-254-300 REV08 OCT2025

Procedure & checklist – Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

Technical overview
Kinnex library preparation using Kinnex 16-fold prep kit

Vega system ICS v1.2
Revio system ICS v13.5
SMRT Link v26.2

PN 103-916-200 Rev 01 | October 2026

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

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

Raw Data Report

HiFi Read Length

Read Segmentation Metrics

Raw Data Report	HiFi Read Length	Read Segmentation Metrics
Raw Reads: 1,100,000	HiFi Reads: 1,100,000	HiFi Reads: 1,100,000
Mean Read Length: 1,100 bp	Mean Read Length: 1,100 bp	Mean Read Length: 1,100 bp
QV: 40	QV: 40	QV: 40
Percent of Reads with 100% QV: 99.9%	Percent of Reads with 100% QV: 99.9%	Percent of Reads with 100% QV: 99.9%

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103-254-300 REV08 OCT2025

Technical overview – Kinnex library preparation using Kinnex 16-fold prep kit (103-916-200)

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 libraries¹. 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.

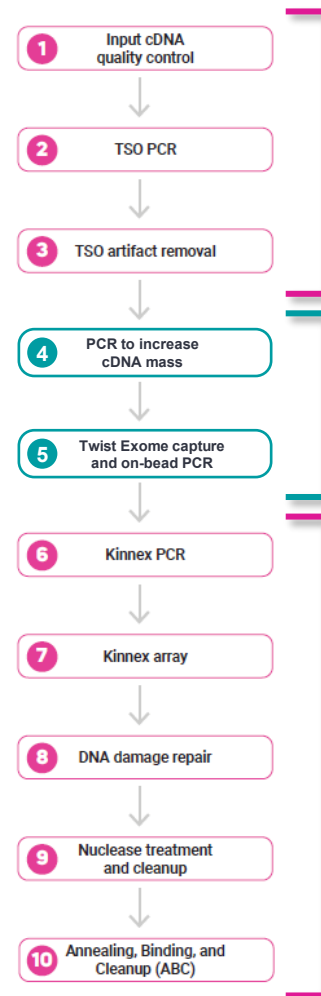
Single-cell cDNA generation	
Single-nuclei cDNA library	Refer to single-cell vendor (10x, Parse)
10x cDNA primer*	10x 2000089

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	Twist 102031: 2 rxn Twist 102032: 12 rxn Twist 102033: 96 rxn
OR	
Twist RNA Exome Panel	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 16-fold prep kit ([103-885-300](#))



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]



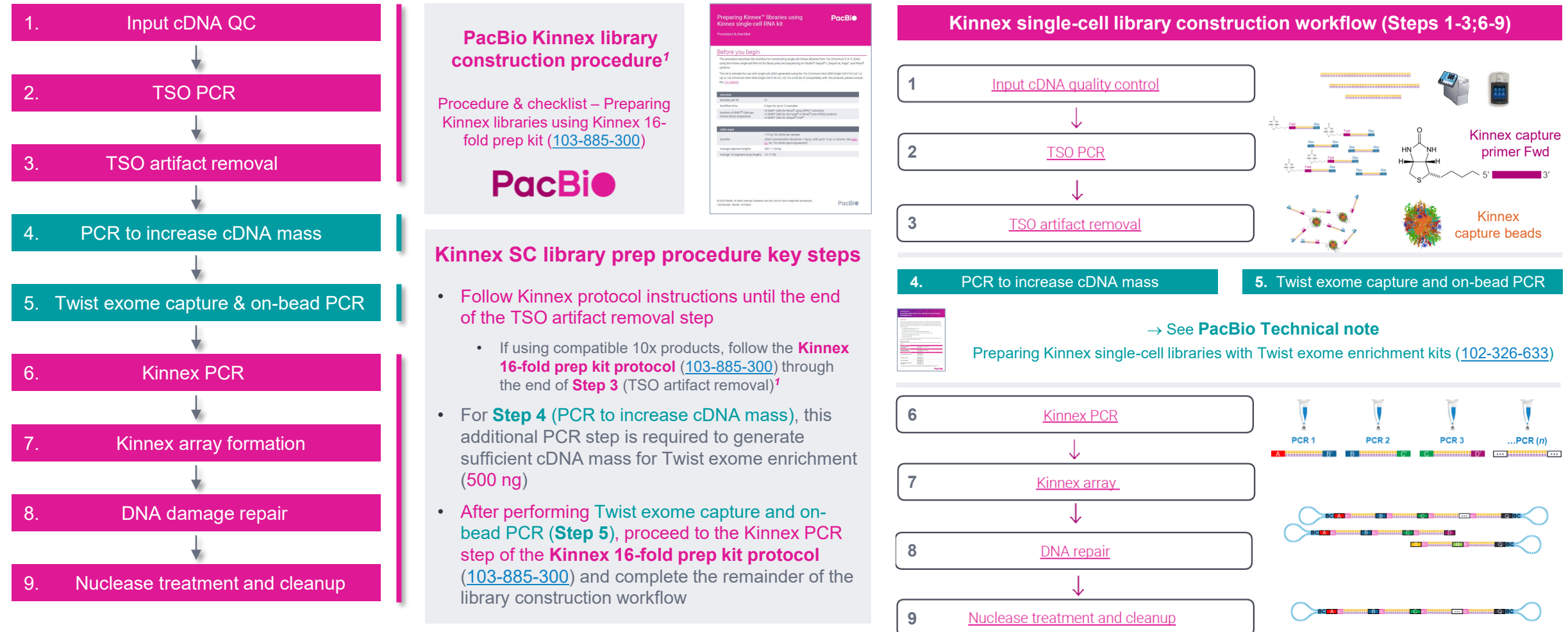
PacBio Procedure & checklist
Preparing Kinnex libraries using Kinnex 16-fold prep kit ([103-885-300](#))

Overview

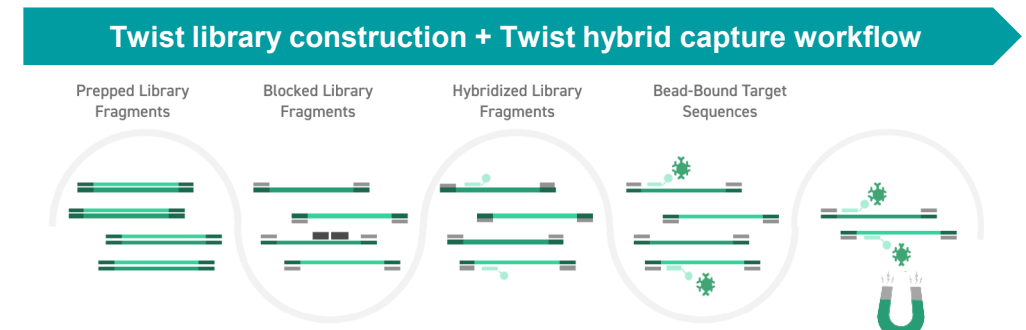
- The purpose of using Twist exome enrichment is to enrich for spliced mRNA in single-nuclei libraries¹
- This workflow has been tested on *10x Single Cell 3' Gene Expression* (v3.1) libraries, but is also compatible with single-nuclei libraries generated with the following:
 - *10x Single Cell Gene 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

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



Follow PacBio Kinnex library prep protocol and modified Twist target enrichment procedure¹ to generate single-cell hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems



- **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 16-fold prep kit protocol** ([103-885-300](#)) and complete the remainder of the library construction.

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 primer¹ 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 oligos² 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/

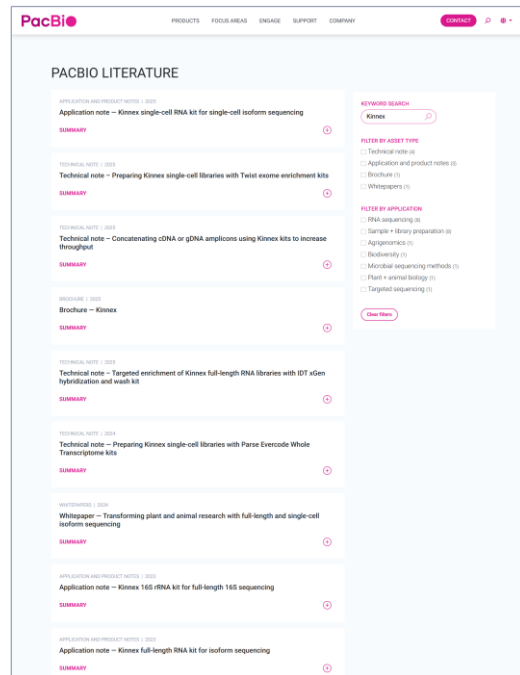
¹ The 10x cDNA primer is **included** in 10x Single Cell Gene Expression kits. If stock kit primers are not available, follow the recommendations described here for custom ordering (see Appendix 1 in *Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits* ([102-326-633](#))).

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 capture kits

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

Application-specific educational literature

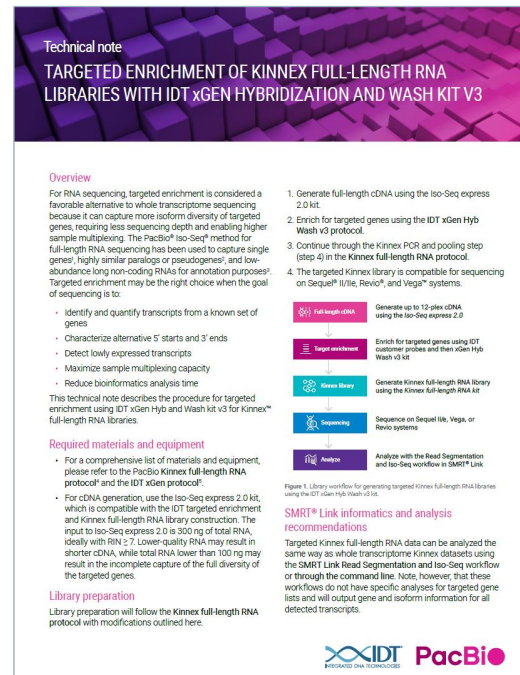


PacBio literature website [Link]

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



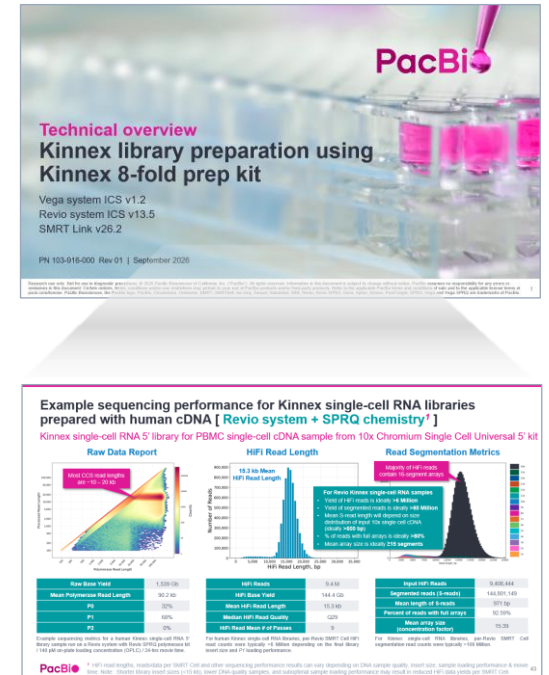
Application-specific protocol documentation



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

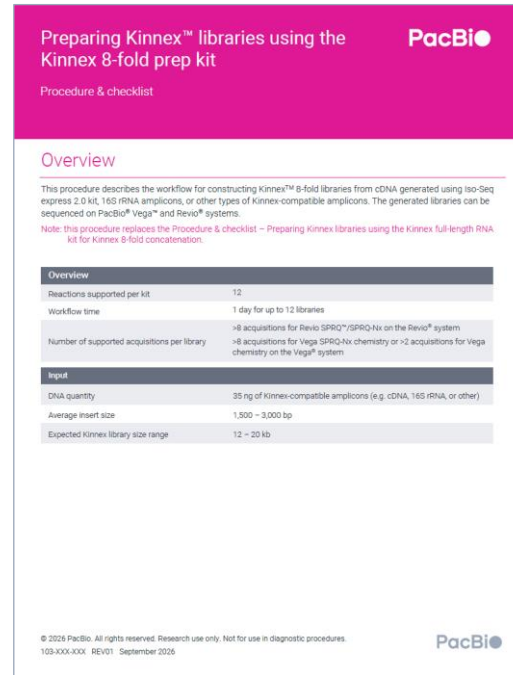
Technical documentation describing the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for preparing Kinnex full-length RNA libraries for PacBio sequencing.

Application-specific technical overviews



Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit (103-916-000) or Kinnex 12-fold prep kit (103-916-100) [PacBio]

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.



Procedure & checklist – Preparing Kinnex libraries using Kinnex 8-fold prep kit (103-920-900) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

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 genes¹, highly similar paralogs or pseudogenes², and low-abundance long non-coding RNAs for annotation purposes³. 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 protocol⁴ and the IDT xGen protocol⁵.

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.

Generate up to 12-plex cDNA using the Iso-Seq express 2.0 kit.

Enrich for targeted genes using the IDT xGen Hyb Wash v3 protocol.

Continue through the Kinnex PCR and pooling step (step 4) in the Kinnex full-length RNA protocol.

The targeted Kinnex library is compatible for sequencing on Sequel® II/IIe, Revio®, and Vega™ systems.

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

Sequence on Sequel IIe, Vega, or Revio systems

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

PacBio

```
graph TD; 1[1 Input RNA quality control] --> 2[2 cDNA synthesis + cleanup]; 2 --> 3[3 cDNA amplification + cleanup]; 3 --> 4[4 Target enrichment with IDT xGen kit]; 4 --> 5[5 Kinnex PCR]; 5 --> 6[6 Kinnex array formation + cleanup]; 6 --> 7[7 Nuclease treatment + cleanup]; 7 --> 8[8 Annealing, binding and cleanup (ABC)];
```

PacBio Procedure & checklist
Preparing Kinnex compatible cDNA using Iso-Seq express 2.0 kit ([102-396-000](#))

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 Kinnex 8-fold prep kit ([103-920-900](#))

Overview

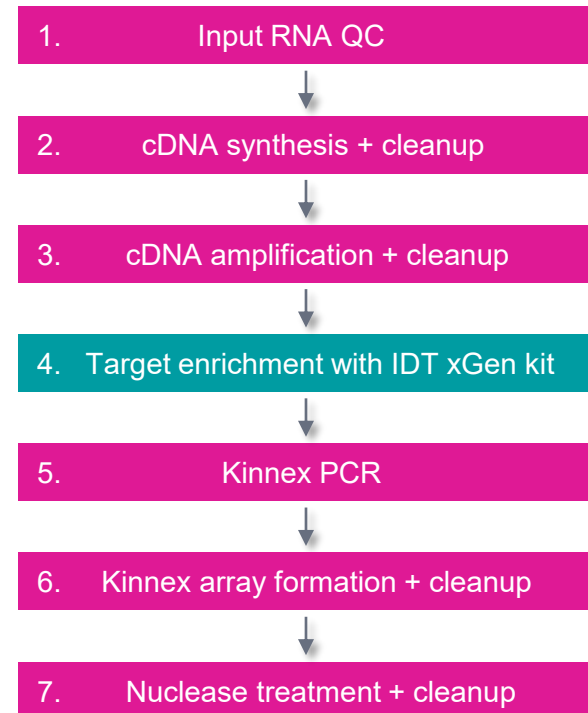
- For RNA sequencing, **targeted enrichment** may be the right choice when the goal of sequencing is to:
 - Identify and quantify transcripts from a known set of genes
 - Characterize alternative 5' starts and 3' ends
 - Detect lowly expressed transcripts
 - Maximize sample multiplexing capacity
 - Reduce 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](#)).

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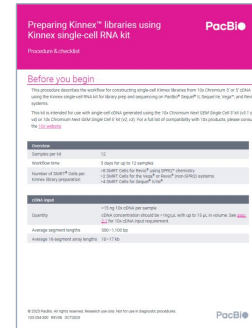
Workflow overview for targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3

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



PacBio Kinnex library construction procedure¹

Procedure & checklist – Preparing Kinnex libraries using the Kinnex 8-fold prep kit ([103-920-900](#))



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 generally follow the **Kinnex 8-fold prep kit protocol** with the following additional steps:
 - Generate full-length cDNA using the Iso-Seq express 2.0 kit by following **Steps 1-3 in the Iso-Seq cDNA synthesis protocol** ([102-396-000](#)).
 - 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 (Step 2) in the Kinnex 8-fold prep kit** ([103-920-900](#)) protocol and complete the remainder of the library construction workflow

Bulk Iso-Seq cDNA amplicon generation workflow

1. [Input RNA quality control](#)



2. [cDNA synthesis + cleanup](#)



3. [cDNA amplification + cleanup](#)



4. **Target enrichment with IDT xGen kit**



→ See **PacBio Technical note**

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



Kinnex bulk RNA library construction workflow

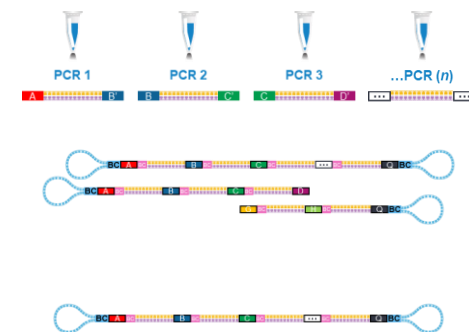
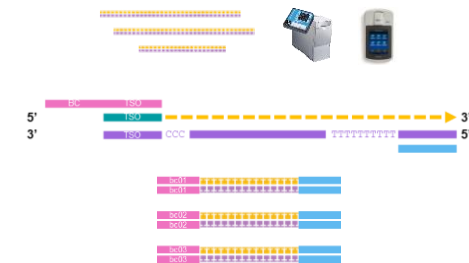
5. [Kinnex PCR](#)



6. [Kinnex array formation + cleanup](#)

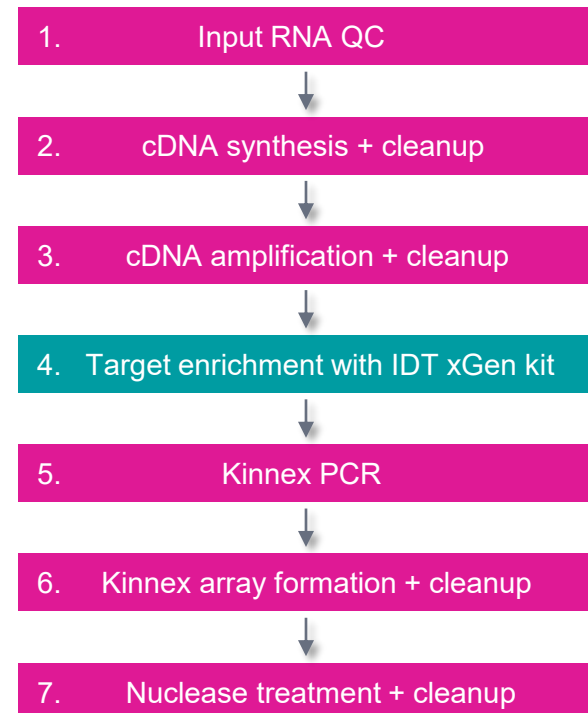


7. [Nuclease treatment + cleanup](#)



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



IDT xGen hybrid capture procedure overview¹

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



IDT xGEN hybrid capture procedure key steps¹

- Add Blocker Master Mix to each cDNA sample (500 ng²) 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).

Add blocking oligos



xGen blocking oligo

Blocked cDNA sequences

Hybridize targets to capture probes

xGen Hyb Probes

Hybridized cDNA sequences

Incubate with magnetic streptavidin beads

Streptavidin bead

Bead-bound target sequences

Isolate targets with magnet



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).

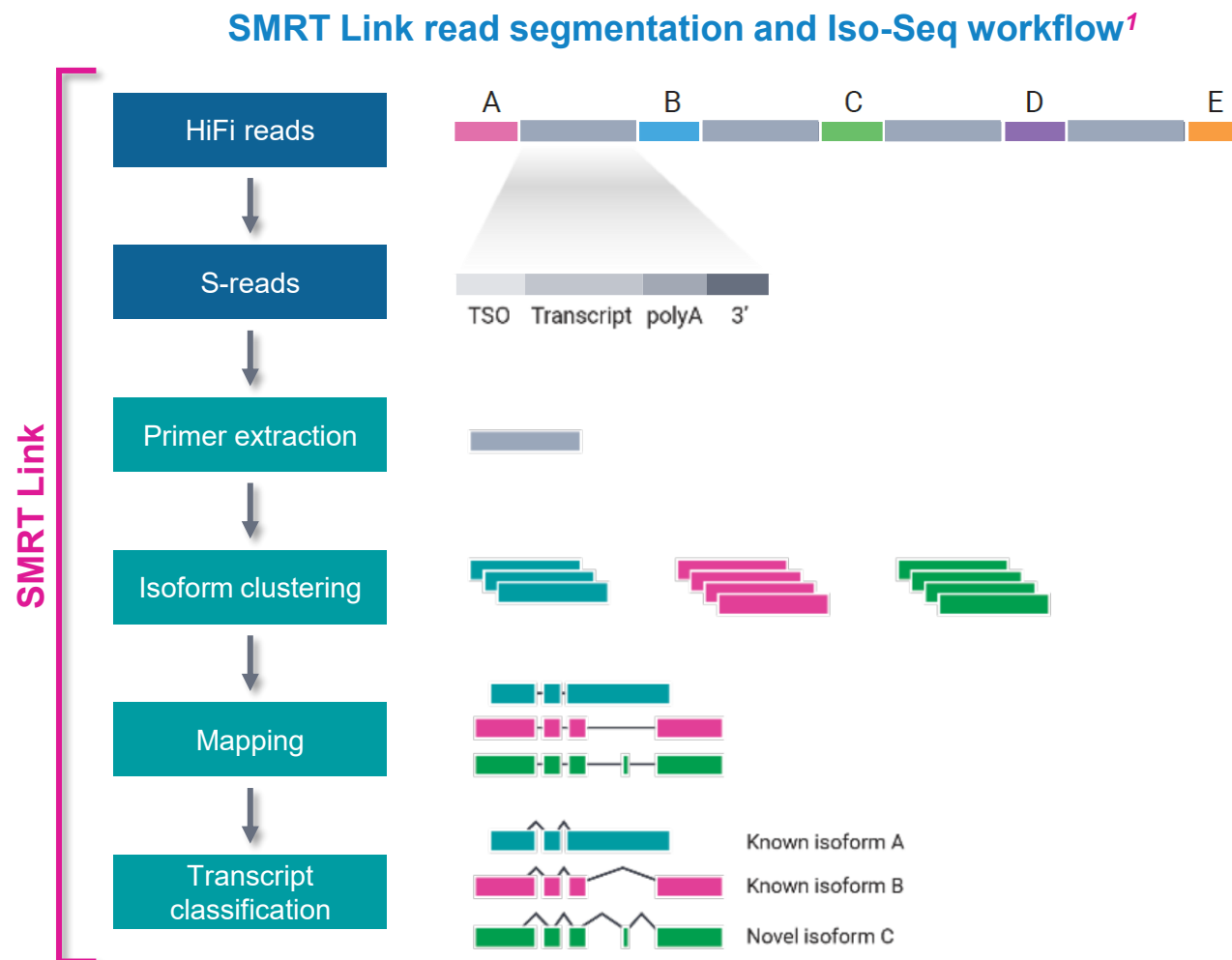
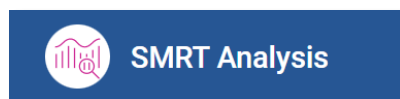
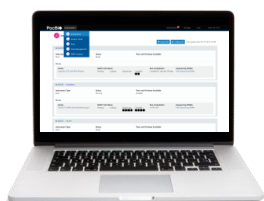
² 100 ng to 6 µg cDNA inputs can be used for short hybridizations (1-2 hrs). Generally, we recommend not to exceed 2.5 µg total input for 1-hr hybridization time for very large panels (>30 Mb).

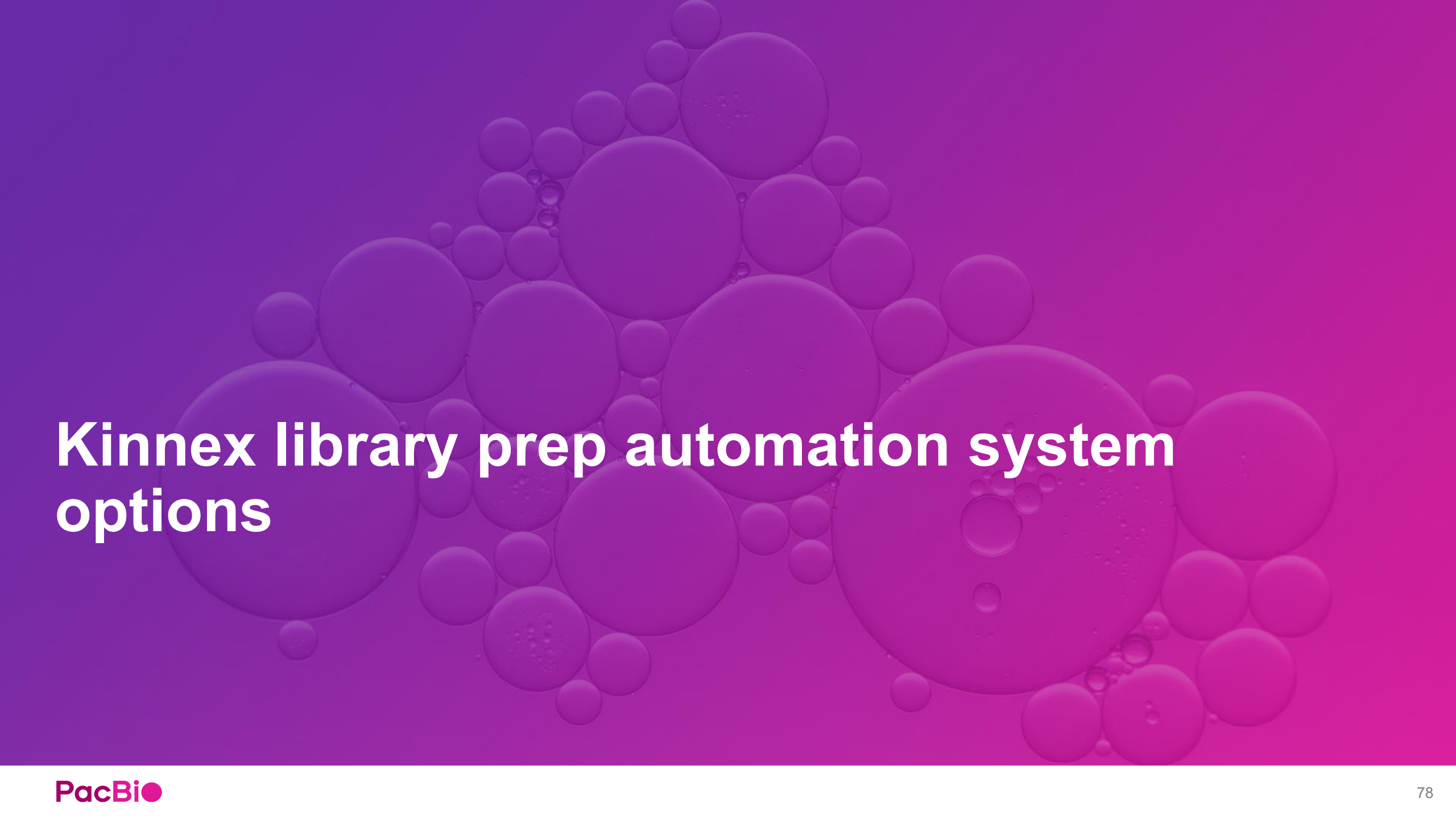
³ This procedure supports short hybridization times of only 1 hr as well as the standard 4- or 16-hr incubations. We recommend users empirically test the shorter hybridization time for their workflows.

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.





Kinnex library prep automation system options

Automated Kinnex full-length RNA library preparation with the SPT Labtech firefly platform

SPT firefly automated Kinnex workflow improves reproducibility and scalability in large-scale transcriptomics studies by reducing manual steps in library preparation¹

Automated walk-away workflow and processing with the SPT Labtech firefly system enables:

- **Reduced hands-on time** – Manual pipetting decreased by up to 80%, allowing researchers to focus on data analysis and experimental design
- **Improved consistency** – User-to-user and day-to-day variation is minimized
- **Scalability** – Generation of high-quality, full-length transcript data is enabled at higher throughput to support large-scale genomics initiatives



Automated Kinnex library preparation with the Opentrons OT-2 system

Kinnex PCR workflow step for Kinnex 8-fold/12-fold/16-fold prep kits can be automated on the Opentrons OT-2 system¹

Opentrons OT-2 is a high-precision, open-source liquid handler designed to be easy for scientists to use

- OT-2 has a user-friendly, plug and play graphical interface to design protocols compatible with any ANSI/SBS compliant or automation friendly labware.
- OT-2 base model includes an Opentrons GEN2 Single-Channel Pipette; you can also use up to two pipettes at the same time.

