PacBio

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 103-916-000 Rev 01 | October 2026

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Kinnex library preparation using Kinnex 16-fold prep kit

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2. Kinnex 16-fold library preparation workflow
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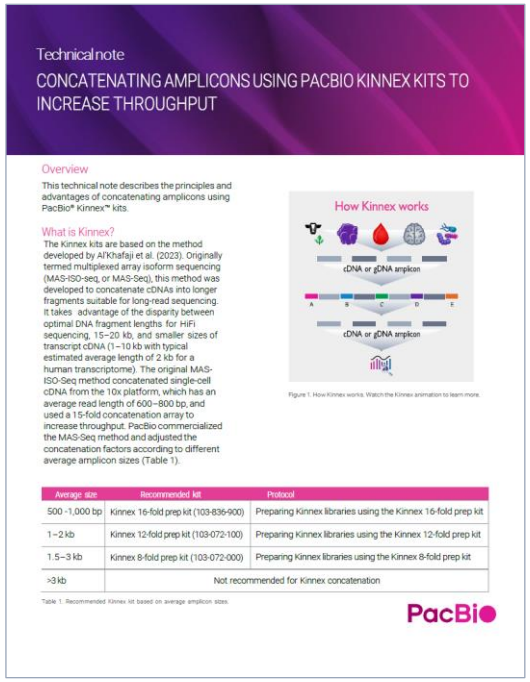
Kinnex library preparation using Kinnex 16-fold prep kit: Getting started

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



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

How Kinnex works

cDNA or gDNA amplicon

Average size	Recommended kit	Protocol
500 -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.

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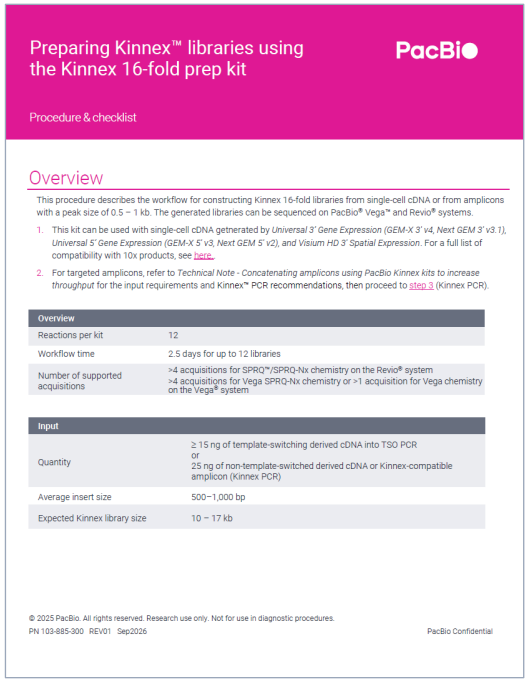
Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput (102-326-636)

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



Preparing Kinnex™ libraries using the Kinnex 16-fold prep kit

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Procedure & checklist

Overview

This procedure describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio® Vega™ and Revio® systems.

1. This kit can be used with single-cell cDNA getnerated by *Universal 3' Gene Expression (GEM-X 3' v4, Next GEM 3' v3.1), Universal 5' Gene Expression (GEM-X 5' v3, Next GEM 5' v2), and Visium HD 3' Spatial Expression*. For a full list of compatibility with 10x products, see here.
2. For targeted amplicons, refer to *Technical Note - Concatenating amplicons using PacBio Kinnex kits to increase throughput* for the input requirements and Kinnex™ PCR recommendations, then proceed to step 3 (Kinnex PCR).

Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio® system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega® system

Input	
Quantity	≥ 15 ng of template-switching derived cDNA into TSO PCR or 25 ng of non-template-switched derived cDNA or Kinnex-compatible amplicon (Kinnex PCR)
Average insert size	500–1,000 bp
Expected Kinnex library size	10 – 17 kb

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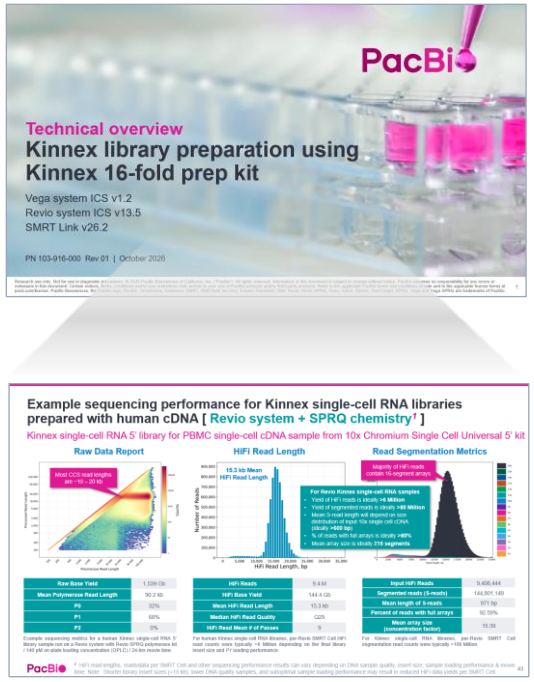
Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300)

Technical documentation containing application-specific Kinnex library preparation protocol details.



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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

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

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

Raw Data Report | HiFi Read Length | Read Segmentation Metrics

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

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

Input HiFi Reads	9,436,444
Segmented reads (5-reads)	144,801,149
Mean length of 5-reads	971 bp
Percent of reads with full arrays	92.59%
Mean array size (concentration factor)	15.39

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

Technical overview presentations describe sample preparation details for constructing concatenated HiFi libraries using Kinnex prep kits. Example sequencing performance data for Kinnex libraries are also summarized.

Kinnex-compatible amplicon generation
(Application-specific procedure)

Follow application-specific protocols to generate amplicons with the required Kinnex PCR handle sequences

↓

Kinnex library preparation
(Kinnex prep kit)

Use Kinnex-compatible amplicons to generate Kinnex library containing arrayed DNA segments

↓

HiFi sequencing
(Revio & Vega systems)

Perform ABC[1] and sequence Kinnex libraries on PacBio long-read systems

↓

Data analysis
(SMRT Link)

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

Use SMRT Link or other tools to perform downstream secondary analysis

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[1] ABC = Anneal primer / Bind polymerase / Clean up bound complex

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Kinnex library concatenation method overview

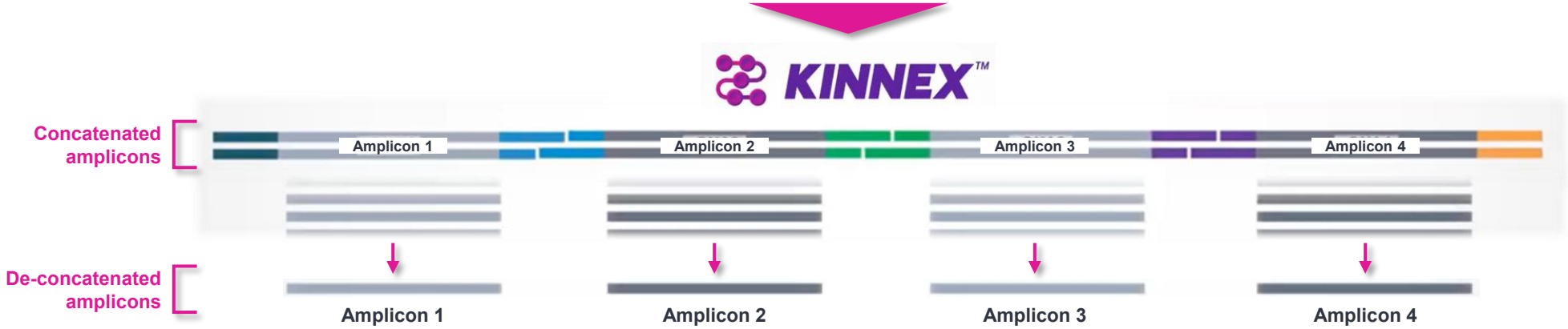
What is Kinnex?

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) method[1] 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 de-concatenated to represent the original amplicon molecules

[1] The original MAS-ISO-Seq method concatenated single-cell cDNA from the 10x platform, which has an average read length of 600–800 bp, and used a 15-fold concatenation array to increase throughput. See Al'Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology. [Link]

[2] Watch the Kinnex animation to learn more about how the PacBio Kinnex method works.

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 consideration[1]
- 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**

KINNEX v2.0

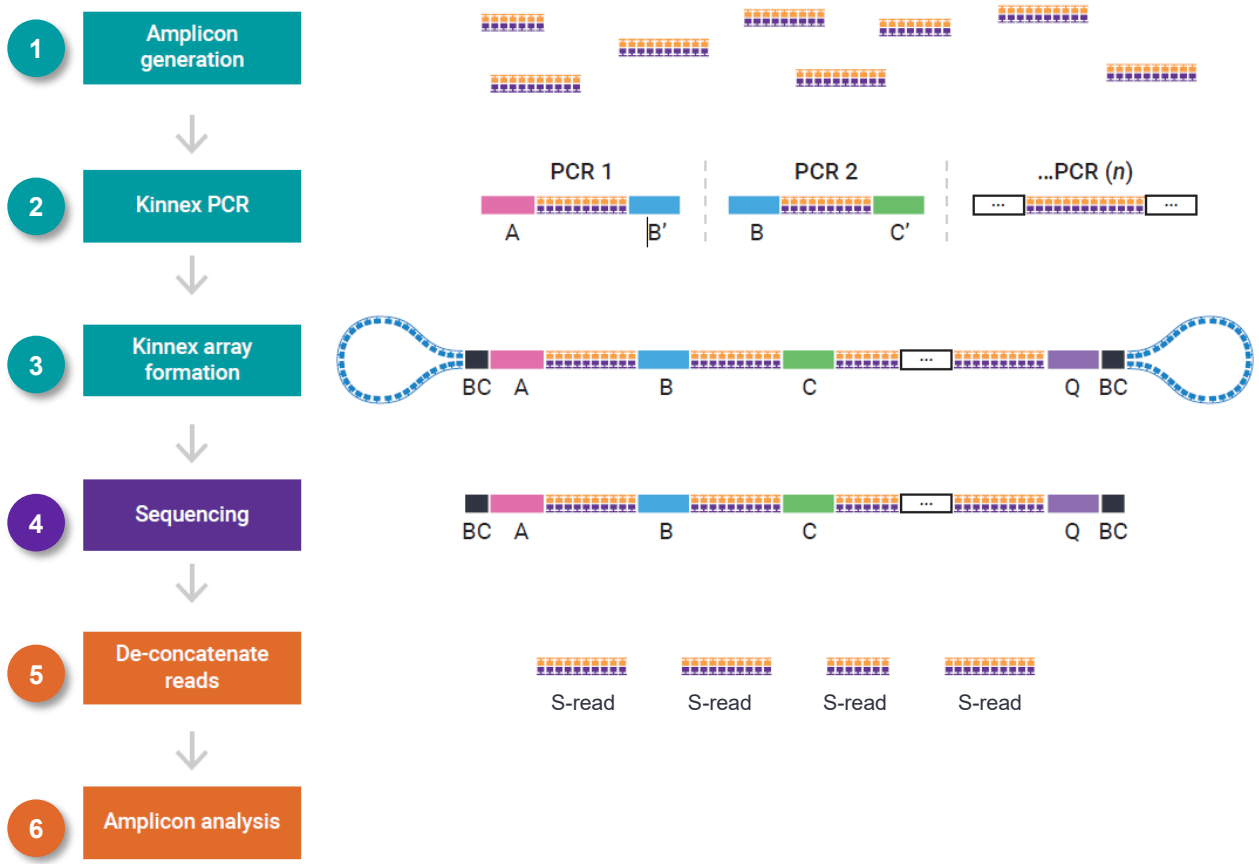
Benefits of Kinnex concatenation include:

- 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

[1] See ***Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput*** (102-326-636) for a description of the principles and advantages of concatenating amplicons using PacBio Kinnex kits
[2] Guidance is based on peak or average insert size distribution. Targeted amplicons over 3 kb are not supported.

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 steps[1]

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

[1] See ***Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput*** (102-326-636) for Kinnex concatenation workflow overview; refer to the relevant Kinnex library prep protocols and Kinnex technical overview training documentation for specific details regarding Kinnex PCR and Kinnex array formation steps.

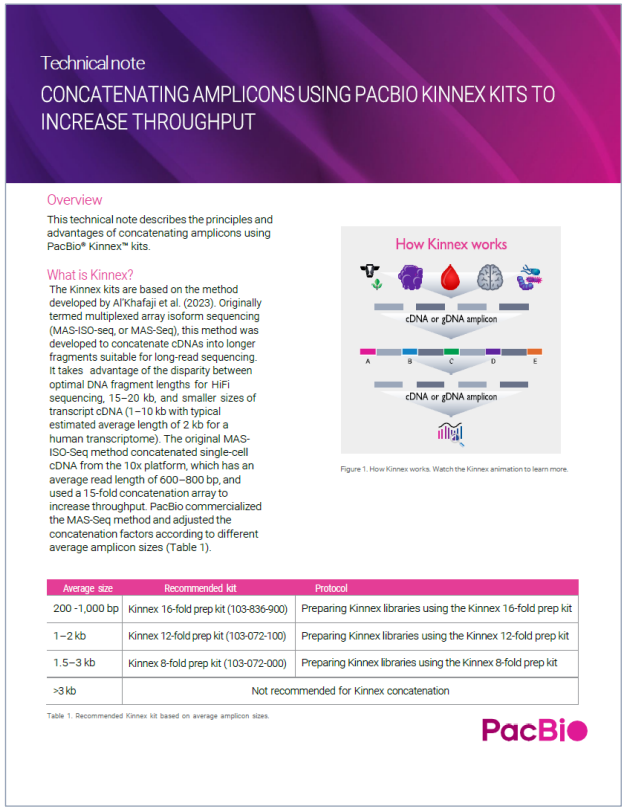
Experimental design considerations for concatenating amplicons to increase HiFi sequencing data throughput

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

How Kinnex works

cDNA or gDNA amplicon

A B C D E

cDNA or gDNA amplicon

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

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.

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

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

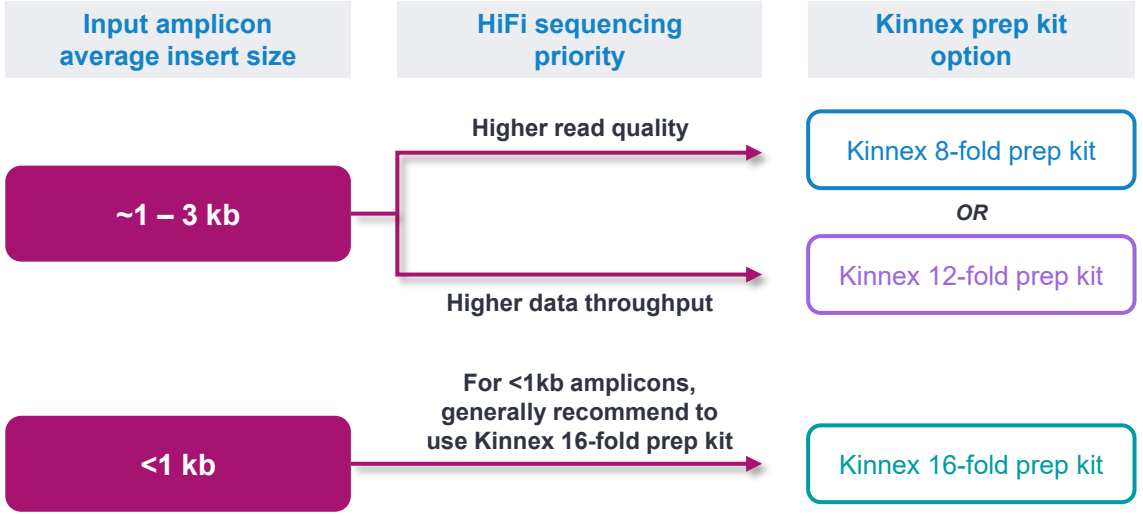
[1] Guidance is based on peak or average insert size distribution. Targeted amplicons over 3 kb are not supported for Kinnex concatenation procedures.

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

Higher read quality

Higher S-read yield

IMPORTANT: Final data throughput (yield) will depend on **application-specific downstream analysis procedures and quality filter settings used**[3]

- 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

[1] Example: For Kinnex 16s rRNA applications using secondary analysis tools that require high read quality (e.g., DADA2), 16S datasets generated with Kinnex 8-fold prep kit may retain a higher yield of reads after 16S 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 procedures[1]

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 prep[2]

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

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 ranges[1]

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**		

[1] Guidance is based on peak or average insert size distribution. Targeted amplicons over 3 kb are not supported. Refer to ***Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput*** (102-326-636) for latest recommendations on Kinnex library prep kit selection based on average amplicon insert size.

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 products[1] • 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 cleanup[2] • 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 primers[3] • 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)

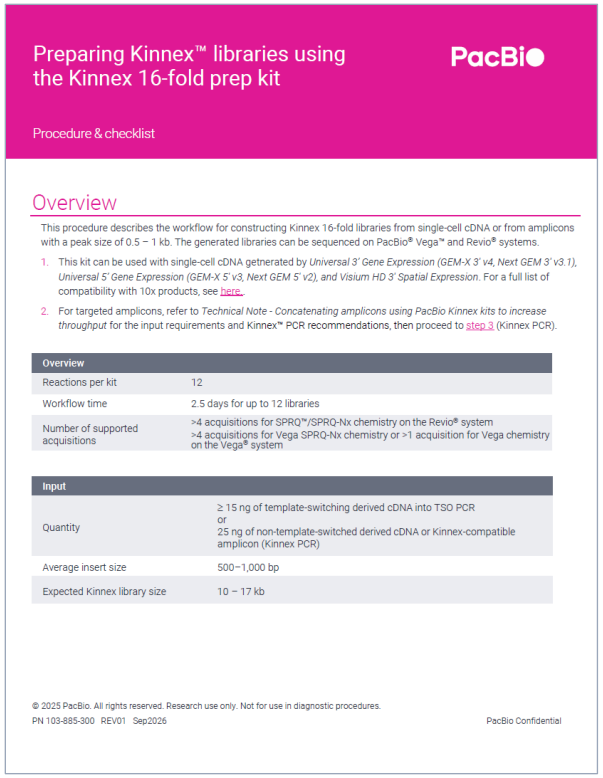
[1] Refer to specific Application Note literature for compatibility with third-party products such as ArgenTag and Parse Biosciences single-cell kits.
[2] Refer to ***Procedure & checklist – Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit*** (102-396-000).
[3] Refer to ***Procedure & checklist – Preparing 16S rRNA amplicons for Kinnex*** (103-885-200) for recommended 16S gene-specific forward and reverse PCR primer sequences to order.

Kinnex 16-fold library preparation procedure description

Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300) describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio Vega and Revio systems.

Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio® system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega® system

Input	
Quantity	≥ 15 ng of template-switching derived cDNA into TSO PCR or 25 ng of non-template-switched derived cDNA or Kinnex-compatible amplicon (Kinnex PCR)
Average insert size	500–1,000 bp
Expected Kinnex library size	10 – 17 kb



Preparing Kinnex™ libraries using the Kinnex 16-fold prep kit

PacBio

Procedure & checklist

Overview

This procedure describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio® Vega™ and Revio® systems.

1. This kit can be used with single-cell cDNA getnerated by *Universal 3' Gene Expression (GEM-X 3' v4, Next GEM 3' v3.1), Universal 5' Gene Expression (GEM-X 5' v3, Next GEM 5' v2), and Visium HD 3' Spatial Expression*. For a full list of compatibility with 10x products, see here.
2. For targeted amplicons, refer to *Technical Note - Concatenating amplicons using PacBio Kinnex kits to increase throughput* for the input requirements and Kinnex™ PCR recommendations, then proceed to step 3 (Kinnex PCR).

Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio® system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega® system

Input	
Quantity	≥ 15 ng of template-switching derived cDNA into TSO PCR or 25 ng of non-template-switched derived cDNA or Kinnex-compatible amplicon (Kinnex PCR)
Average insert size	500–1,000 bp
Expected Kinnex library size	10 – 17 kb

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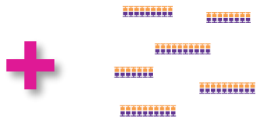
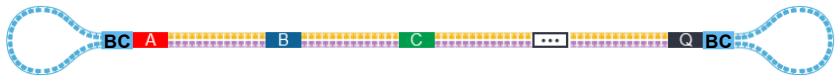
PacBio Documentation (103-885-300)

Kinnex 16-fold prep kit
103-836-900 (12 rxn)



Kinnex-compatible amplicons[1,2]



Kinnex 16-fold library template (~10–17 kb)
Contains 16 concatenated amplicon segments

Refer to **separate** protocols[1,2] for generating Kinnex-compatible amplicons to be used as input for this Kinnex library prep procedure

[1] This kit can be used with single-cell cDNA amplicons generated by Universal 3' Gene Expression (GEM-X 3' v4, Next GEM 3' v3.1), Universal 5' Gene Expression (GEM-X 5' v3, Next GEM 5' v2), and Visium HD 3' Spatial Expression. For a full list of compatibility with 10x products, see 10x Genomics Support Hub.

[2] For other types of targeted amplicons, refer to ***Technical Note – Concatenating amplicons using PacBio Kinnex kits to increase throughput*** (102-326-636) for the input requirements and Kinnex PCR recommendations, then proceed to step 3 (Kinnex PCR).

Kinnex 16-fold library preparation workflow

Use Kinnex 16-fold prep kit to generate 16-segment Kinnex concatenation library

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

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

Kinnex 16-fold prep kit components

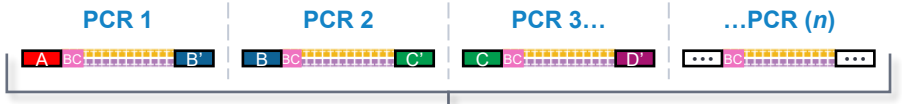
Component		Description
1		**Kinnex PCR 16-fold kit (12 rxn)** • Contains reagents for Kinnex PCR to incorporate segmentation adapters
2		**Kinnex concatenation kit 2.0 (12 rxn)** • Contains reagents for Kinnex array formation and SMRTbell template construction • Includes barcoded Kinnex adapter mixes (bcM0001 – bcM0004)
3		**SMRTbell cleanup beads** • For DNA cleanup
4		**Elution buffer** • For DNA cleanup

Kinnex 16-fold library prep, sequencing & analysis

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

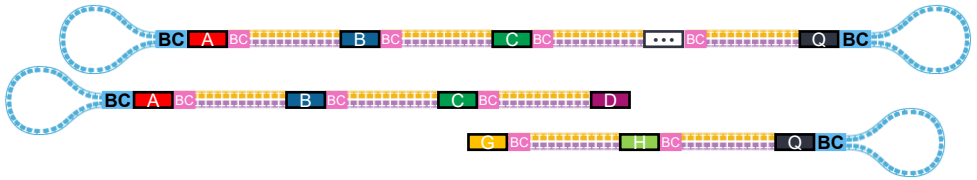
Kinnex-compatible amplicons containing Kinnex handles (☐) + PacBio barcodes (BC)

Kinnex PCR



Kinnex array formation

Barcoded Kinnex terminal adapter (bcM0001-bcM0004)



Nuclease treatment

ABC

Purified polymerase-bound 16-segment Kinnex library

Sequencing

Revio or Vega system

SMRT Link
read segmentation data utility

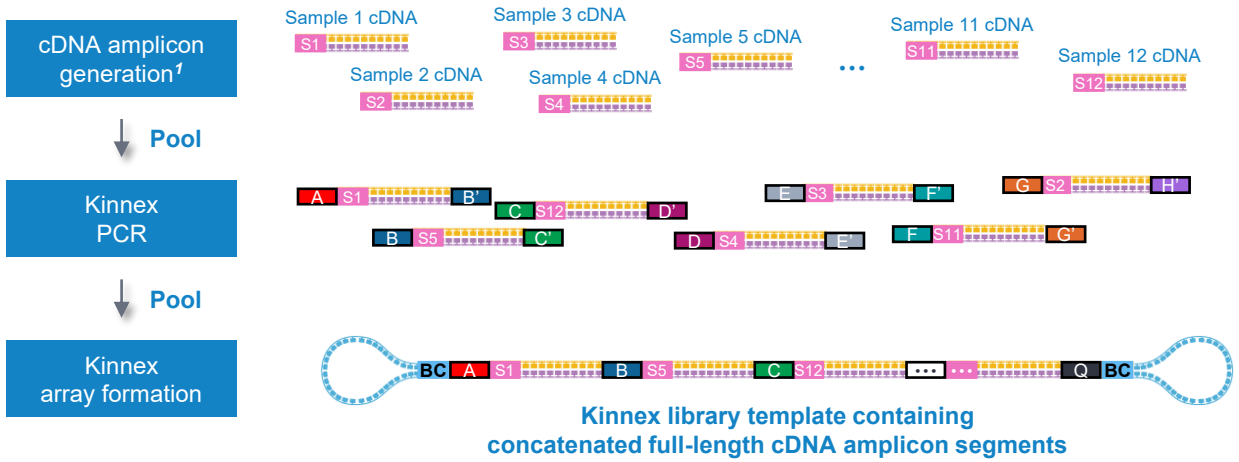
Kinnex library prep barcoding options for sample multiplexing

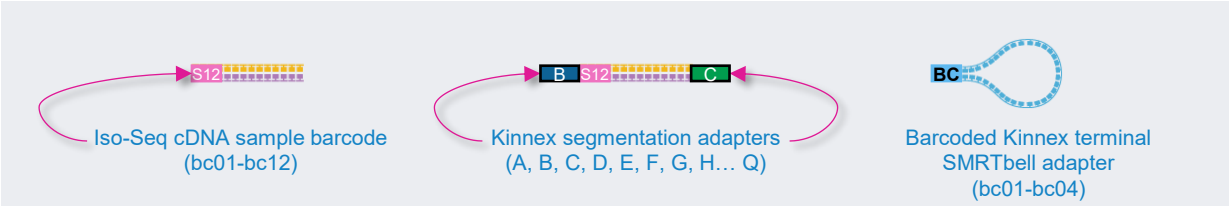
Kinnex library prep kits support sample multiplexing through combined use of amplicon-level sample barcodes + Kinnex library-level barcodes

Kinnex library preparation procedure supports sample multiplexing through combined use of:

- **Amplicon sample barcodes** incorporated during amplicon generation step using, for example, PacBio barcoded Iso-Seq cDNA PCR primers bc01–12[1], PacBio barcoded 16S PCR primers Fwd_01 – 12 + Rev_13 – 44,[2] etc.
- **Kinnex library barcodes** incorporated during Kinnex array formation step using PacBio barcoded Kinnex terminal SMRTbell adapters (bc01 – bc04)[3]

Example 1: Workflow to generate 48-plex Kinnex bulk RNA library using (12) barcoded Iso-Seq cDNA primers + (4) barcoded Kinnex SMRTbell adapters





Kinnex bulk RNA sample multiplexing can be achieved with one of the three following methods:

1. **Barcoded target-specific PCR primers**
   - Use up to **12** different barcoded Iso-Seq primers (bc01–12) in PacBio cDNA generation protocol[1] to amplify samples.
   - Amplified barcoded Iso-Seq cDNA samples can be pooled (up to 12-plex) and brought through a single Kinnex PCR reaction in Kinnex library prep protocol.
2. **Barcoded Kinnex adapters**
   - Use up to **4** different Kinnex barcoded adapters (bc01–04) in Kinnex array formation step in Kinnex library prep protocol workflow.
3. **A combination of the above two approaches** to achieve up to **48-plex**.

[1] To generate Kinnex compatible cDNA refer to protocol, ***Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit*** (102-396-000).
[2] To generate full-length 16S rRNA amplicons refer to protocol, ***Preparing Kinnex-compatible 16S rRNA amplicons*** (103-885-200).
[3] Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

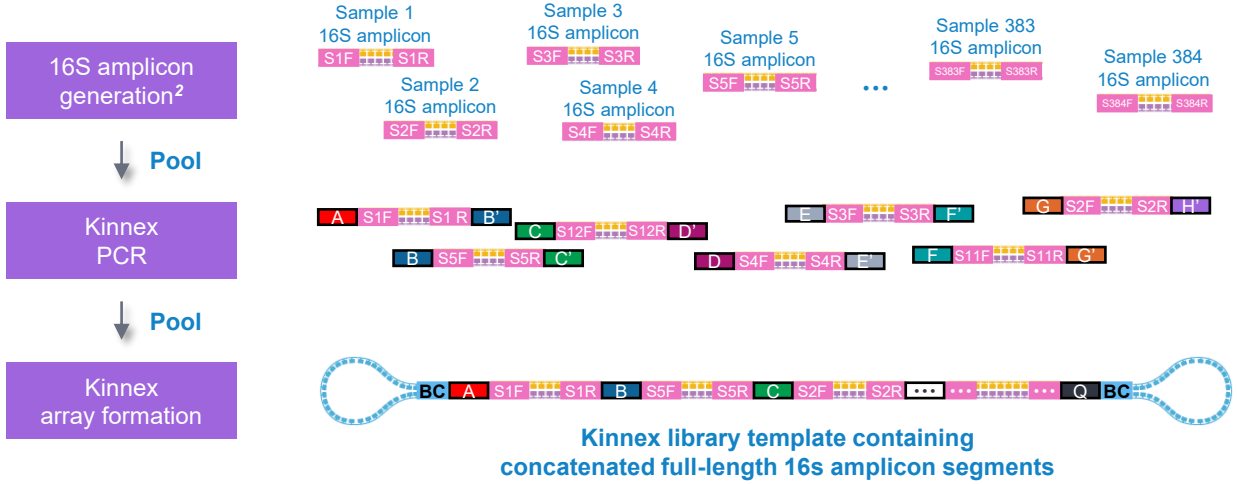
Kinnex library prep barcoding options for sample multiplexing (cont.)

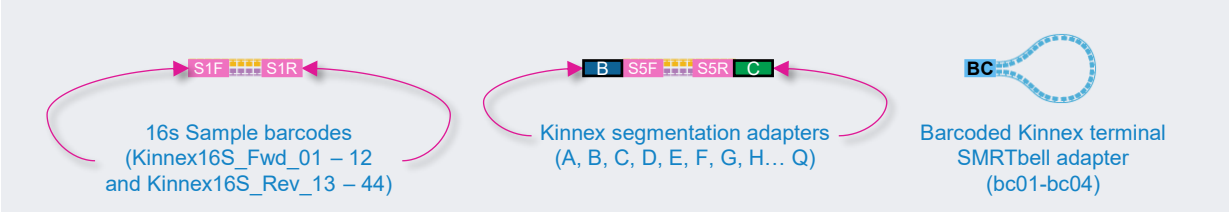
Kinnex library prep kits support sample multiplexing through combined use of amplicon-level sample barcodes + Kinnex library-level barcodes

Kinnex library preparation procedure supports sample multiplexing through combined use of:

- **Amplicon sample barcodes** incorporated during amplicon generation step using, for example, PacBio barcoded Iso-Seq cDNA PCR primers bc01–12[1], PacBio barcoded 16S PCR primers Fwd_01 – 12 + Rev_13 – 44,[2] etc.
- **Kinnex library barcodes** incorporated during Kinnex array formation step using PacBio barcoded Kinnex terminal SMRTbell adapters (bc01 – bc04)[3]

Example 2: Workflow to generate 1,536-plex Kinnex 16s rRNA library using (12) barcoded forward PCR primers + (32) barcoded reverse PCR primers + (4) barcoded Kinnex SMRTbell adapters





Kinnex 16s rRNA sample multiplexing can be achieved with one of the three following methods:

1. **Barcoded target-specific PCR primers**
   - Use up to **12** different barcoded 16S Forward PCR primers (Fwd_01 – 12) + **32** different barcoded 16S Rerverse PCR primers (Rev_13 – 44) in PacBio 16S amplicon generation protocol[2] to amplify samples.
   - Amplified barcoded 16S amplicon samples can be pooled (up to 384-plex) and brought through a single Kinnex PCR reaction in Kinnex library prep protocol.
2. **Barcoded Kinnex adapters**
   - Use up to **4** different Kinnex adapters (bc01–04) in Kinnex array formation step in Kinnex library prep protocol workflow.
3. **A combination of the above two approaches** to achieve up to **1,536-plex**.

[1] To generate Kinnex compatible cDNA refer to protocol, ***Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit*** (102-396-000).
[2] To generate full-length 16S rRNA amplicons refer to protocol, ***Preparing Kinnex-compatible 16S rRNA amplicons*** (103-885-200).
[3] Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

PacBio

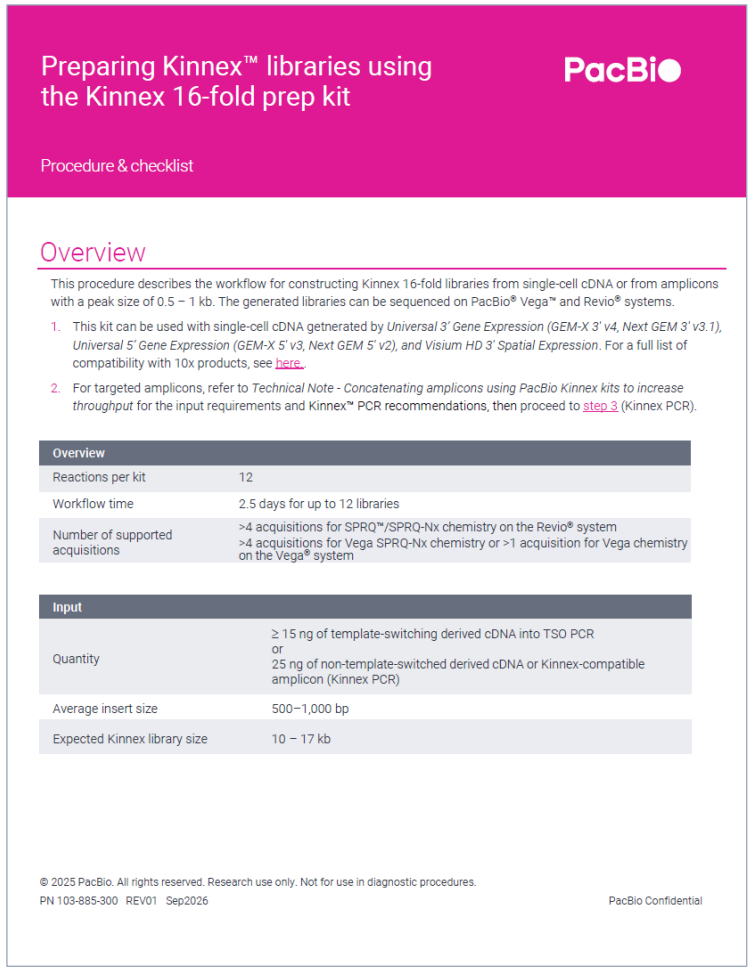
Kinnex 16-fold library preparation workflow

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

Procedure & checklist 103-885-300 describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio Vega and Revio systems.

Procedure & checklist contents

1. Kinnex-compatible amplicon input QC recommendations and general best practices for reagent & sample handling.
2. Enzymatic workflow steps for removal of template-switch oligo (TSO) artifacts from input 10x single cell cDNA samples.[1]
3. Enzymatic workflow steps for construction of 12-segment Kinnex arrays from Kinnex-compatible amplicons.
4. Enzymatic workflow steps for nuclease treatment of Kinnex libraries.
5. Workflow steps for final cleanup of Kinnex libraries using SMRTbell cleanup beads.
6. Sample setup ABC[2] workflow steps to prepare Kinnex libraries for sequencing on Revio and Vega systems.



Preparing Kinnex™ libraries using the Kinnex 16-fold prep kit

PacBio

Procedure & checklist

Overview

This procedure describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio® Vega™ and Revio® systems.

1. This kit can be used with single-cell cDNA getnerated by *Universal 3' Gene Expression (GEM-X 3' v4, Next GEM 3' v3.1), Universal 5' Gene Expression (GEM-X 5' v3, Next GEM 5' v2), and Visium HD 3' Spatial Expression*. For a full list of compatibility with 10x products, see here.
2. For targeted amplicons, refer to *Technical Note - Concatenating amplicons using PacBio Kinnex kits to increase throughput* for the input requirements and Kinnex™ PCR recommendations, then proceed to step 3 (Kinnex PCR).

Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio® system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega® system

Input	
Quantity	≥ 15 ng of template-switching derived cDNA into TSO PCR or 25 ng of non-template-switched derived cDNA or Kinnex-compatible amplicon (Kinnex PCR)
Average insert size	500–1,000 bp
Expected Kinnex library size	10 – 17 kb

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PacBio Documentation (103-885-300)

[1] For template-switch derived input single-cell cDNA, TSO artifact removal step is performed by default. For non-TSO derived single-cell cDNA and other amplicons, TSO artifact removal step is not required.

[2] ABC = Anneal primer / Bind polymerase / Clean up bound complex

Kinnex 16-fold library preparation and sequencing workflow key steps

Kinnex library construction steps can be completed within 1 day[1]

KCAG + QC

Kinnex-compatible amplicon generation[2] — Example: 10x single-cell cDNA

PacBio or other third-party reagents

Refer to appropriate PacBio or other third-party protocol documenation[2,3]

Kinnex library prep

1 Input quality control — 0.5 hrs

Qubit 4 fluorometer system (ThermoFisher Sci. Q33238)
DNA sizing QC tool (Agilent TapeStation or other tool)

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

If answer is "No", proceed to Step 3

2a TSO PCR — 1.5 hrs

↓ Safe stop

2b TSO artifact removal — 1.5 hrs

↓ Safe stop

3 Kinnex (on-bead) PCR — 2 hrs

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

↓ Safe stop

4 Kinnex array formation + cleanup — 2 hrs

↓ Safe stop

5 Nuclease treatment + cleanup — 0.5 hrs

↓ Safe stop

ABC

6 Annealing, binding & cleanup (ABC) — 1 hr

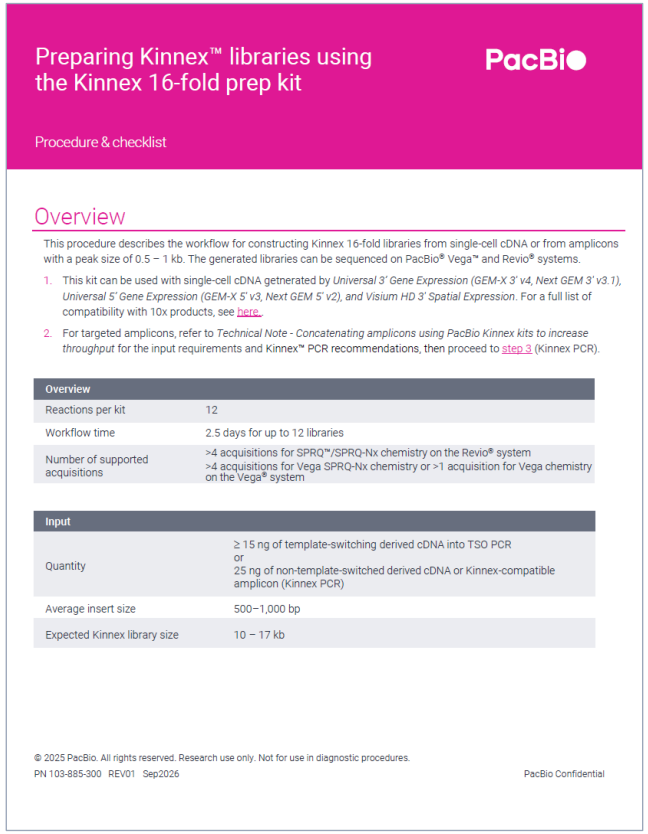
Revio SPRQ polymerase kit (103-496-900) *or*
Vega SPRQ polymerase kit (103-794-300)

1 DAY

1 DAY

Kinnex 16-fold library prep protocol reference

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



Preparing Kinnex™ libraries using the Kinnex 16-fold prep kit

PacBio

Procedure & checklist

Overview

This procedure describes the workflow for constructing Kinnex 16-fold libraries from single-cell cDNA or from amplicons with a peak size of 0.5 – 1 kb. The generated libraries can be sequenced on PacBio® Vega™ and Revio® systems.

1. This kit can be used with single-cell cDNA getnerated by *Universal 3' Gene Expression (GEM-X 3' v4, Next GEM 3' v3.1), Universal 5' Gene Expression (GEM-X 5' v3, Next GEM 5' v2), and Visium HD 3' Spatial Expression*. For a full list of compatibility with 10x products, see here.
2. For targeted amplicons, refer to *Technical Note - Concatenating amplicons using PacBio Kinnex kits to increase throughput* for the input requirements and Kinnex™ PCR recommendations, then proceed to step 3 (Kinnex PCR).

Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio® system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega® system

Input	
Quantity	≥ 15 ng of template-switching derived cDNA into TSO PCR or 25 ng of non-template-switched derived cDNA or Kinnex-compatible amplicon (Kinnex PCR)
Average insert size	500–1,000 bp
Expected Kinnex library size	10 – 17 kb

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[1] Example workflow step times shown are for manually processing up 12 Kinnex libraries using Kinnex 16-fold prep kit.
[2] To generate Kinnex compatible single-cell cDNA, refer to 10x Genomics Support website (or refer to Appendix 2 in this presentation for information about other PacBio Compatible single-cell platforms).
[3] 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 other custom amplicon sample types.

PacBio

General best practices recommendations for preparing Kinnex 16-fold libraries

Reagent and sample handling

Kinnex library prep reagents

- Room temperature is defined as any temperature in the range of 18–23°C for this protocol
- Thaw Kinnex array and repair buffer, nuclease buffer, Kinnex adapter mix, and primers at room temperature
- Briefly vortex reagent buffers and Kinnex adapters prior to use. Enzyme mixes do not require vortexing
- Quick-spin all reagents in a microcentrifuge to collect liquid at tube bottom prior to use
- Keep all temperature-sensitive reagents on ice
- Bring SMRTbell cleanup beads and Qubit 1X dsDNA HS reagents to room temperature for 30 minutes prior to use
- Ensure SMRTbell cleanup beads are thoroughly mixed to a homogenous suspension before accurately pipetting the required volume, as small changes in bead volume can significantly alter the size distribution of your sample
- During bead binding and elution steps, pipette-mixing until beads are distributed evenly in solution
- Mix each reaction by pipetting up and down 10 times or until fully resuspended. Avoid introducing bubbles.
- In Kinnex PCR, keep sample(s) on ice until thermal cycler lid has reached 105°C to avoid digestion of primers by polymerase exonuclease activity
- The workflow takes approximately 2.5 days to complete
    - If a stop is necessary, refer to the workflow for safe stopping points

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

Temperature-sensitive reagents Kinnex PCR 16-fold kit 103-836-300	
Tube color	Reagent
Green	Kinnex 16-fold PCR mix 103-875-600
Yellow	Kinnex 3′ capture primer mix 103-182-400
Red	Kinnex 5′ capture primer mix 103-182-200
Orange	Kinnex primer mix A 103-107-800
	Kinnex primer mix B 103-107-900
	Kinnex primer mix C 103-108-000
	Kinnex primer mix D 103-108-100
	Kinnex primer mix E 103-108-200
	Kinnex primer mix F 103-108-300
	Kinnex primer mix G 103-108-400
	Kinnex primer mix H 103-153-000
	Kinnex primer mix I 103-153-100
	Kinnex primer mix J 103-153-200
	Kinnex primer mix K 103-153-300
	Kinnex primer mix L 103-153-400
	Kinnex primer mix M 103-153-500
	Kinnex primer mix N 103-153-600
	Kinnex primer mix O 103-153-700
	Kinnex primer mix PQ 103-153-800

Temperature-sensitive reagents Kinnex concatenation kit 2.0 103-836-400	
Tube color	Reagent
Red	Kinnex enzyme 103-110-400
Yellow	Kinnex ligase 103-110-500
White	Kinnex array and repair buffer 103-110-300
Green	Kinnex repair mix 103-837-500
Light Purple	Nuclease buffer 103-110-200
Light Green	Nuclease mix 103-110-100
Blue	Kinnex adapter bc01 mix 103-109-600
	Kinnex adapter bc02 mix 103-109-700
	Kinnex adapter bc03 mix 103-109-800
	Kinnex adapter bc04 mix 103-109-900

General best practices recommendations for preparing Kinnex 16-fold libraries (cont.)

Reagent and sample handling

Sequencing prep reagents (for sample setup ABC)

- Once thawed, place reaction buffers and sequencing primer on-ice prior to making master mix. The Loading buffer should be left at room-temperature.
- **Note:** The Loading buffer is light sensitive and should be protected from light when not in use.
- **Keep the following reagents on a cold block or ice:**
  - Sequencing polymerase
  - Sequencing control
- **Bring the following reagents up to room temperature 30 minutes prior to use:**
  - Loading buffer
  - SMRTbell cleanup beads

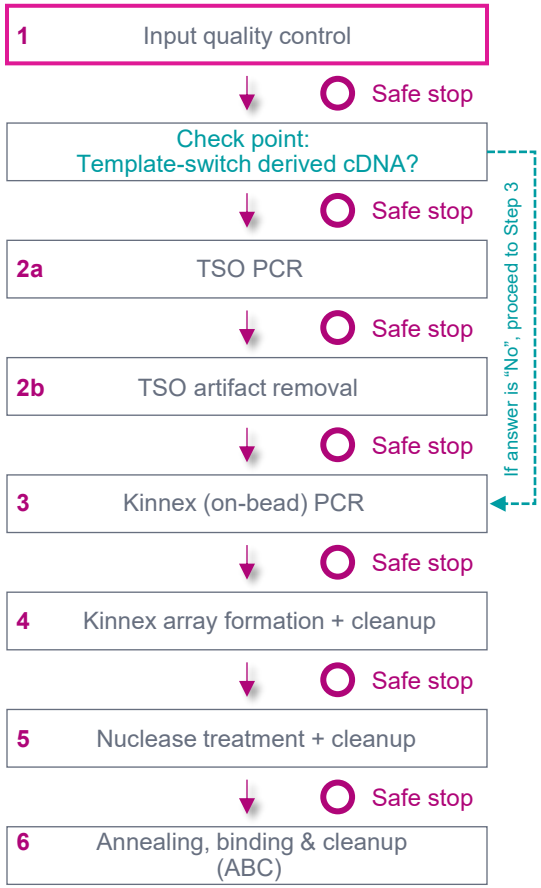
Thaw the following reagents at room temperature:

Component	Tube color
Annealing buffer	Light blue
Kinnex sequencing primer	Light purple
Polymerase buffer	Yellow
Loading buffer	Green
Dilution buffer	Blue

Input quality control

Input quality control is highly recommended before proceeding to the Kinnex PCR step

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



- The recommended input for Kinnex 16-fold PCR are Kinnex-compatible amplicons with an average size distribution between 0.5 to 1 kb
  - Single-cell cDNA or amplicons are expected to have an average fragment size approximately 0.5 – 1 kb after Kinnex PCR (Step 3)
  - 10x Chromium-generated cDNA with a peak size up to 1,500 bp is acceptable prior to beginning this Kinnex library prep procedure
  - **Note:** Input amplicon sizes >1,500 bp may reduce Kinnex concatenation efficiency, which will result in lower library yields.
    - We recommend preparing Kinnex libraries with the Kinnex 12-fold prep kit if your input amplicon sample size mode is ≥1,500 bp.
- **For single-cell cDNA generated *via* a template-switching reverse transcription method:**
  - Minimum input for Kinnex 16-fold library prep is 15 ng of single-cell cDNA amplicons to go into TSO PCR step and 25 ng of post-TSO removal single-cell cDNA amplicons to go into Kinnex PCR
  - For single-cell cDNA from 10x Genomics, follow the best practices in the 10x Chromium user guide up until cDNA amplification, cleanup and QC (refer to 10x Chromium user guide for 5' kits and 3' kits).
  - Input cDNA quality control is highly recommended before proceeding to the Kinnex library prep workflow.
- **For amplicon and cDNA synthesized without a template-switching reverse transcription method:**
  - Proceed directly from step 1 (input quality control) to step 3 (Kinnex PCR) with 25 ng of Kinnex-compatible amplicons as the input.

Step 1
procedural summary

DNA sizing QC

Amplicon QC

Proceed to **Step 2a** if using template-switch derived input single cDNA

Or

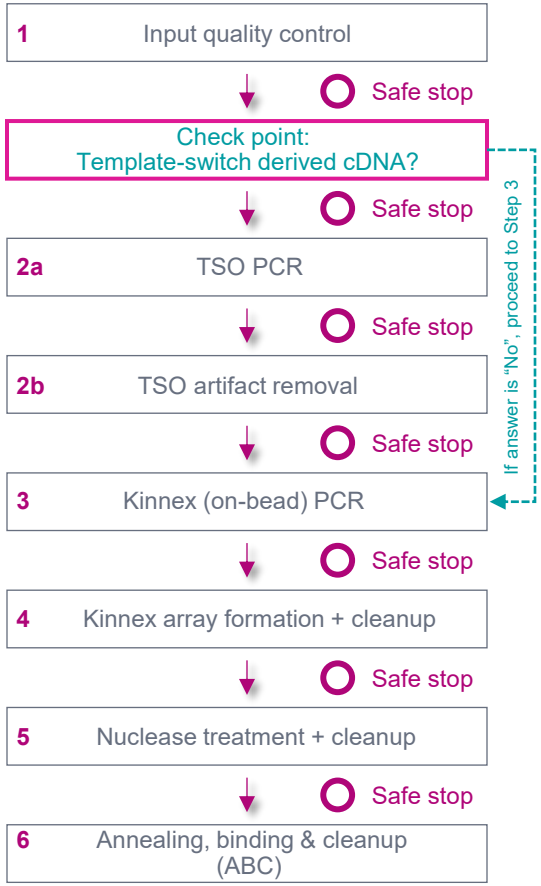
Proceed to **Step 3** if using non-template-switch derived input amplicons

[1] To generate Kinnex-compatible cDNA please refer to protocol, ***Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit*** (102-396-000). To generate Kinnex-compatible 16S rRNA amplicons please refer to protocol, ***Preparing Kinnex-compatible 16S rRNA amplicons*** (103-885-200). To generate other Kinnex-compatible amplicons please refer to the technical note, ***Concatenating amplicons using PacBio Kinnex kits to increase throughput*** (102-326-636) for the input, size, and PCR requirements.

Check point: Template-switch derived cDNA?

TSO PCR and TSO artifact removal steps are required for template-switch derived cDNA

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



- **For input cDNA amplicons generated *via* template-switching method:**
  - Perform TSO PCR (Step 2a) and TSO artifact removal (Step 2b)
  - High-purity cDNA with intact 5′ and 3′ ends are essential for optimal Kinnex library quality, yield, and sequencing performance.
  - Depending on input cDNA quality, these steps may be skipped; however, doing so may reduce library quality and yield and negatively impact sequencing performance.
- **For non-template-switched derived input cDNA and other input amplicon sample types:**
  - TSO PCR (Step 2a) and TSO artifact removal (Step 2b) are not required
  - After completing Input Quality Control (Step 1), proceed directly to Kinnex PCR (Step 3)

[1] To generate Kinnex-compatible cDNA please refer to protocol, ***Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit*** (102-396-000). To generate Kinnex-compatible 16S rRNA amplicons please refer to protocol, ***Preparing Kinnex-compatible 16S rRNA amplicons*** (103-885-200). To generate other Kinnex-compatible amplicons please refer to the technical note, ***Concatenating amplicons using PacBio Kinnex kits to increase throughput*** (102-326-636) for the input, size, and PCR requirements.

TSO PCR & TSO artifact removal

Perform Steps 2 & 3 to remove template switch oligo (TSO) priming artifacts generated during cDNA synthesis

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

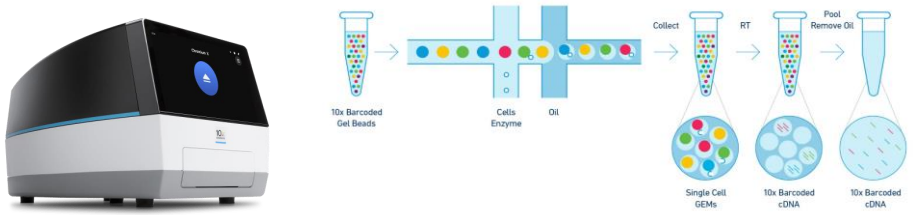
5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

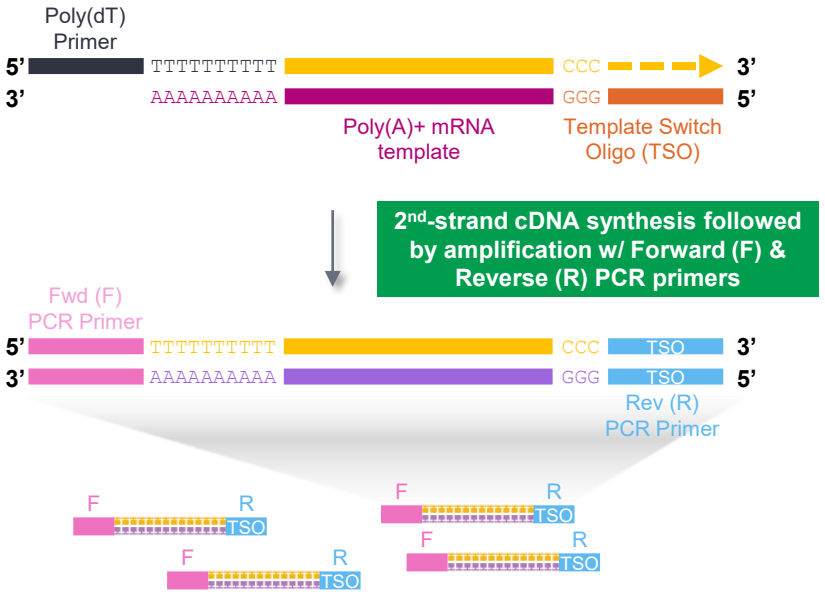
If answer is "No", proceed to Step 3

TSO priming artifacts can occur if the TSO acts as a nonspecific primer on poly(A)+ mRNA



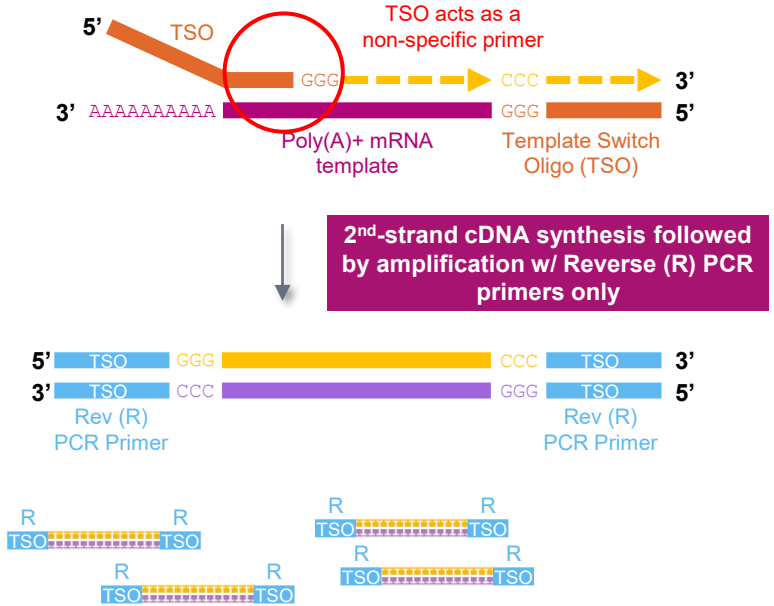
- Up to ~50% of cDNA products from the 10x Chromium single cell cDNA preparation workflow may contain a TSO priming artifact instead of the correct structure

Example synthesis of 10x 3' cDNA products containing correct structure



☑ **Amplified (full-length) 10x cDNA products with correct structure (TSO sequence at one end)**

Example synthesis of 10x 3' cDNA products containing TSO priming artifact



☒ **Amplified (non-full length) 10x cDNA products with TSO priming artifacts (TSO sequences at both ends)**

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TSO PCR & TSO artifact removal (cont.)

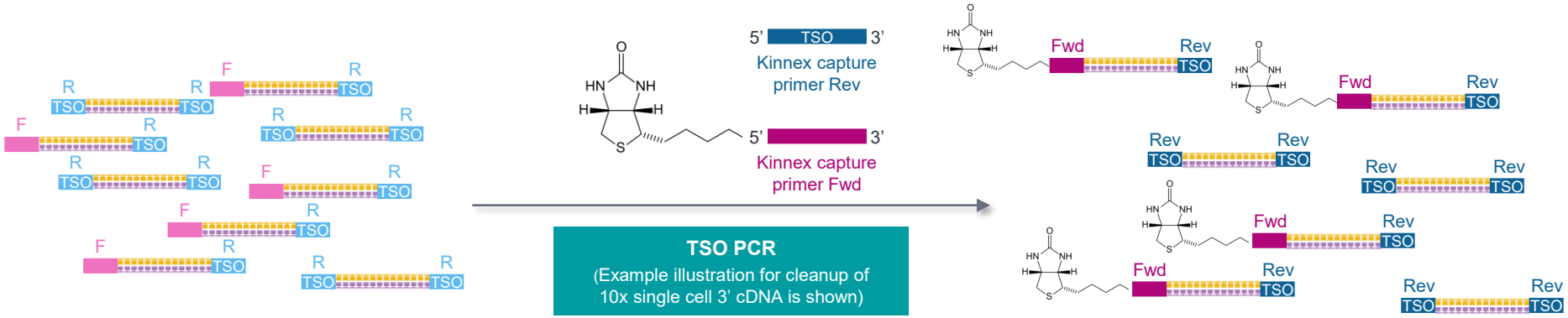
Role of Kinnex capture primers and Kinnex capture beads

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

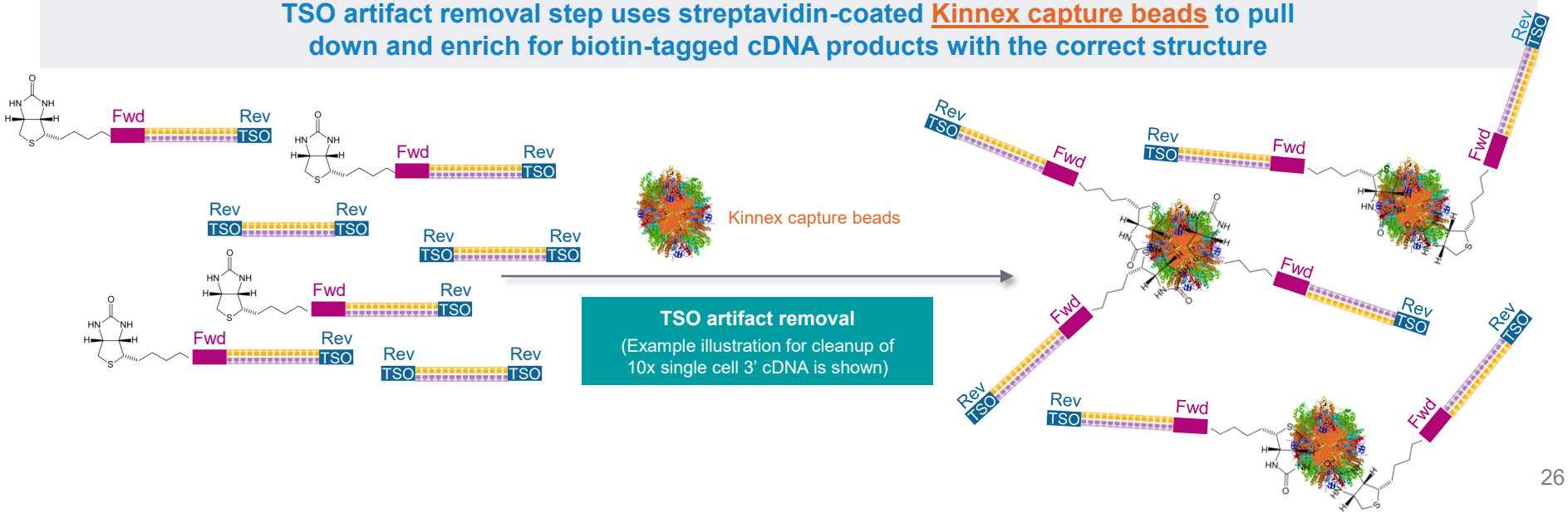
1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

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



TSO artifact removal step uses streptavidin-coated Kinnex capture beads to pull down and enrich for biotin-tagged cDNA products with the correct structure



PacBio

TSO PCR

Procedural summary

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

Step 2a procedural summary

2a.1 – 2a.4 TSO PCR

- Normalize single-cell cDNA sample input to **15 ng** if it is **between 15 ng and 59 ng** using elution buffer
- Normalize single-cell cDNA sample input to **75 ng** if it is **higher than 75 ng** using elution buffer
- For single-cell cDNA amounts **between 60–75ng, proceed without normalizing**
- Select to use ***either*** the **Kinnex 3' *or* 5' capture primer mix** depending on the 10x Genomics single-cell RNA reagent kit used

Up to 15 µL (1.0-1.5 ng/µL in elution buffer or water) Single-cell cDNA sample + 25 µL Kinnex PCR mix +

Select either Kinnex 3' or 5' capture primer mix depending on 10x Genomics kit used

10 µL Kinnex 5' capture primer mix *OR* 10 µL Kinnex 3' capture primer mix

+ Up to 50 µL Nuclease-free water = 50 µL PCR Rx vol.

50 µL PCR Rx vol.

Run TSO PCR program (3 or 5 cycles)

Select TSO PCR program based on cDNA input. Keep sample on ice until thermal cycler lid has heated to 105°C.

TSO PCR program for 15 ng cDNA input

Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	5
3	30 sec	65°C	
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

OR

TSO PCR program for 60-75 ng cDNA input

Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	3
3	30 sec	65°C	
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

2a.5 – 2a.20 1.5X SMRTbell cleanup

50 µL TSO PCR sample + 75 µL 1.5X SMRTbell cleanup beads → 42 µL purified TSO PCR sample

- **Proceed to Step 2b of the protocol if sample quantity is acceptable (between 200 ng – 600 ng)**
- **If yield is >600 ng, proceed to TSO artifact removal step with 600 ng of amplified biotinylated DNA**
- **If yield is <200 ng, repeat TSO PCR with two additional PCR cycles**

Proceed to **Step 2b**

PacBio

TSO PCR

Procedural notes

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

2a. TSO PCR

✔	Step	Instructions
		Normalize sample input to 15 ng if it is between 15 ng and 59 ng using elution buffer. Normalize sample input to 75 ng if it is higher than 75 ng using elution buffer. For input amounts between 60–75ng, proceed without normalizing. Select either the Kinnex 3' or 5' capture primer mix depending on the 10x Genomics kit used. Set up the following PCR reaction on ice (RM1).

Reaction Mix 1 (RM1):

2a.1

✔	Tube color	Component	Volume
	Green	Kinnex 16-fold PCR mix	25 µL
	Red	Kinnex 5' capture primer mix **or**	10 µL
	Yellow	Kinnex 3' capture primer mix	
		10x 5' or 3' cDNA library (1–5 ng/µL)	Up to 15 µL
		Total volume	**50 µL**

2a.2	Pipette-mix **RM1**.
2a.3	Quick spin **RM1** in a microcentrifuge to collect liquid.
2a.4	Select the TSO PCR program based on cDNA input. Keep sample on ice until the thermal cycler lid has heated to 105°C.

TSO PCR program (15 ng input)

Heated lid set at 105°C. Reaction volume set to 50 µL.

Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	
3	30 sec	65°C	5
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

Or TSO PCR program (60-75 ng input)

Heated lid set at 105°C. Reaction volume set to 50 µL.

Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	
3	30 sec	65°C	3
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

- If needed, **normalize** cDNA sample input amounts to 15 ng or 75 ng
- ***IMPORTANT!*** Select either the Kinnex 3' or 5' capture primer mix depending on the 10x Genomics kit used
- Set up TSO PCR reactions **ON ICE**
- PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.
- For **lower** cDNA sample inputs (15 ng), use a **higher** number of PCR cycles (5)

After completing TSO PCR step, perform cleanup with 1.5X SMRTbell cleanup beads and proceed to TSO artifact removal (Step 3) if sample quantity is acceptable (between 200 ng – 600 ng)

- If yield is >600 ng, proceed to the next step with 600 ng of amplified biotinylated DNA
- If yield is <200 ng, repeat TSO PCR with two additional PCR cycles

PacBio

TSO artifact removal

Procedural summary

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

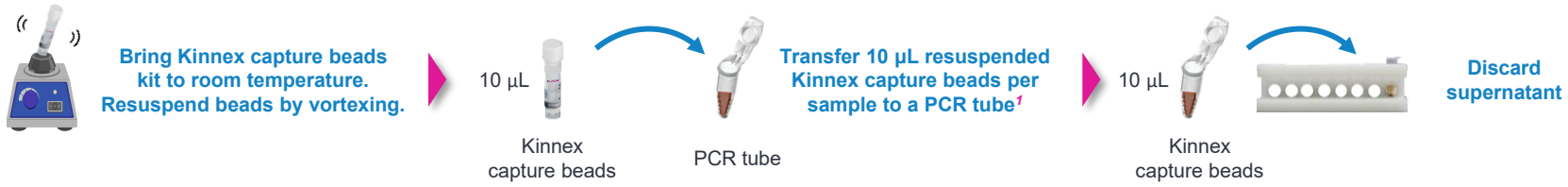
1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

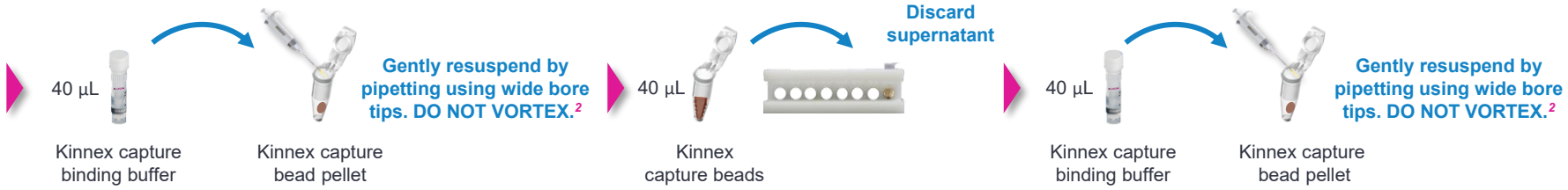
Step 2b procedural summary

2b.1 – 2b.17 TSO artifact removal

2b.1 – 2b.2 Resuspend Kinnex capture beads by vortexing and transfer 10 µL of beads per sample to a PCR tube



2b.3 – 2b.5 Resuspend Kinnex capture beads in Kinnex capture binding buffer (Perform resuspension 2 times)



2b.6 – 2b.8 Add purified TSO PCR sample (containing biotinylated DNA-fragments) from Step 2 to resuspended beads and incubate on rotator

2b.9 – 2b.12 Resuspend Kinnex capture beads/DNA-complex in Kinnex capture washing buffer (Perform wash 2 times)

[1] Scale up the volume of beads if processing multiple samples (with 10% overage). If preparing more than 40 µL of beads, use a 1.5 mL LoBind tube instead of a PCR tube.
[2] Scale up the volume of Kinnex capture binding buffer accordingly if preparing multiple samples.

PacBio

TSO artifact removal

Procedural summary

Step 3 procedural summary (cont.)

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

1 Input quality control
↓ Safe stop

Check point: Template-switch derived cDNA?
↓ Safe stop

2a TSO PCR
↓ Safe stop

2b TSO artifact removal
↓ Safe stop

3 Kinnex (on-bead) PCR
↓ Safe stop

4 Kinnex array formation + cleanup
↓ Safe stop

5 Nuclease treatment + cleanup
↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

2b.1 – 2b.17 TSO artifact removal (cont.)

2b.13 – 2b.14 Resuspend Kinnex capture beads/DNA-complex in nuclease-free water

2b.15 – 2b.17 Resuspend Kinnex capture beads/DNA-complex in elution buffer and evaluate sample concentration

Proceed to **Step 3**

Proceed to Step 3 of the protocol if sample quantity is acceptable (25 ng is required to proceed to Kinnex PCR step).

Note: The **Kinnex capture binding buffer** used in **Steps 2b3 – 2b5** may appear clear or opaque; either appearance is acceptable. Due to its high viscosity, pipette slowly and avoid fully expelling the liquid during mixing to minimize foaming. Excess bubbles may result in lower cDNA recovery. Tap the tube or perform a quick-spin to reduce bubbles, if needed. For multiple samples, scale up the Kinnex capture bead and Kinnex capture binding buffer with a 15% overage in a 1.5mL LoBind tube

TSO artifact removal

Procedural notes

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

2b. TSO artifact removal

✓	Step	Instructions
	2b.1	Bring Kinnex capture beads kit to room temperature. Resuspend the beads by vortexing.
	2b.2	Transfer 10 µL resuspended Kinnex capture beads per sample to a PCR tube.
	2b.3	Add 40 µL Kinnex capture binding buffer per sample and gently resuspend by pipetting. DO NOT VORTEX.
	2b.4	Place the tube on the magnet until the beads separate fully from the solution and **remove the supernatant**.
	2b.5	Remove the tube from magnet rack and resuspend the beads in 40 µL Kinnex capture binding buffer per sample by slow pipetting. DO NOT VORTEX. For multiple samples, distribute 40 µL of the scale-up resuspended Kinnex capture beads into one PCR tube per sample before proceeding to Step 2b.6.
	2b.6	Add 40 µL of the biotinylated DNA-fragments (from Step 2a.18) to the resuspended beads for a final volume of 80 µL. Mix carefully and slowly to avoid foaming of the solution.
	2b.7	Incubate the tube at room temperature for 15 minutes. Quick-spin the tube in a microcentrifuge to collect liquid.
	2b.8	Place the tube on the magnet until the beads separate fully from the solution and remove the supernatant.
	2b.9	Remove the tube from the magnet. Resuspend the Kinnex capture beads/DNA-complex in 80 µL Kinnex capture washing buffer by rigorous pipette mixing until evenly distributed.
	2b.15	Resuspend the capture beads/DNA-complex in 40 µL of elution buffer by vortexing until evenly distributed. IMPORTANT: DNA remains bound to beads, please **keep the bead solution** for subsequent steps.
	2b.16	Evaluate sample concentration: Take a 1 µL aliquot from each tube. Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Yield typically ranges from 10%–40% of the input TSO PCR amount.
	2b.17	Proceed to the next step of the protocol if sample quantity is acceptable. 25 ng of product is required for the Kinnex PCR. Using less than 25 ng may lead to less than 5ug yield from Kinnex PCR

SAFE STOPPING POINT – Store at 4°C

DO NOT FREEZE CAPTURE BEADS/DNA-COMPLEX SOLUTION

- Bring Kinnex capture beads to **room temperature** and resuspend by vortexing

- **Critical step! For all Kinnex capture bead handling steps:** Pipette mix with care and avoid generating bubbles by using **wide bore tips** for mixing (**do not vortex**)
  - When excess bubbles are present, lower cDNA recovery is expected

- **IMPORTANT:** DNA remains bound to beads
  → **Keep** the bead solution for subsequent steps

- **Do not freeze** capture beads/DNA complex solution

After completing TSO artifact removal step, proceed to Kinnex (on-bead) PCR (Step 4) if sample quantity is acceptable (minimum 25 ng)

[1] **Note:** Using less than 25 ng of post-TSO removal amplicon sample for input into Kinnex PCR step may lead to less than 5 µg yield from Kinnex PCR.

Kinnex PCR

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

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

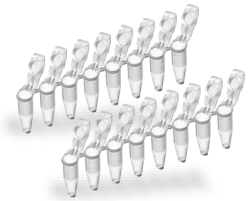
If answer is "No", proceed to Step 3

Step 2 procedural description

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

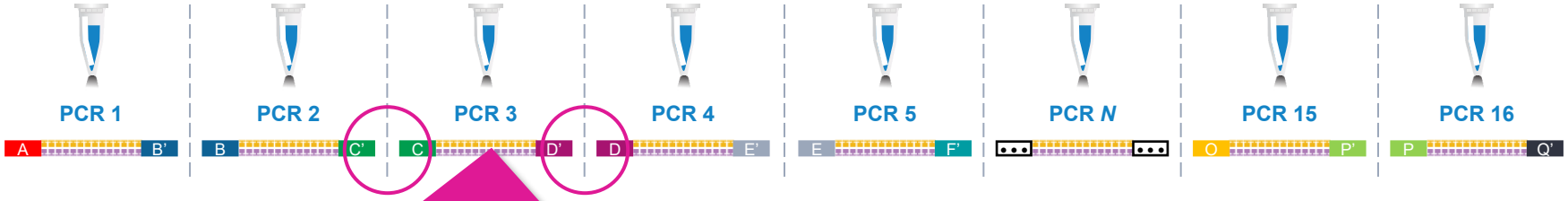
cDNA sample after TSO artifact removal & cleanup (Step 3) or other input amplicon sample

↓ **Distribute input amplicon sample + Kinnex PCR reaction mix into 16 PCR tubes or 16 wells in a 96-well plate**



OR

↓ **Perform Kinnex PCR by adding premixed Kinnex primer pair solutions**



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

↓ **Pool 16 Kinnex PCR products together and perform 1.2X SMRTbell bead cleanup[1]**

Kinnex PCR-amplified amplicon sample ready for Kinnex array formation step

[1] **Note:** Use 1.5X SMRTbell cleanup bead ratio for amplicons <400 bp, or 2X for amplicons <200 bp.

Kinnex PCR (cont.)

Procedural summary

Step 3 procedural summary

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

1 Input quality control
↓ Safe stop

Check point: Template-switch derived cDNA?
↓ Safe stop

2a TSO PCR
↓ Safe stop

2b TSO artifact removal
↓ Safe stop

3 Kinnex (on-bead) PCR
↓ Safe stop

4 Kinnex array formation + cleanup
↓ Safe stop

5 Nuclease treatment + cleanup
↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

3.1 Prepare Kinnex primers premix & 3.2 Kinnex PCR

X = 25 (ng) / purified amplicon concentration (from Step 3)

101 - X µL PCR-grade water + 145 µL Kinnex PCR mix + X µL 25 ng of beads/DNA-complex *or* 25 ng of Kinnex-compatible amplicons = 246 µL master mix → 246 µL master mix

Aliquot 14 µL of Master Mix into each of the 16 PCR tubes (for 16X concatenation) on ice

Kinnex primer mix

Add 2.5 µL of Kinnex primer mix[1] into each of 16 PCR tubes (containing 22.5 µL of Master Mix)

Kinnex PCR Rx tube	12X concatenation	Tube color
1 [25 µL Rx volume]	Kinnex primer mix A	Orange
2 [25 µL Rx volume]	Kinnex primer mix B	
3 [25 µL Rx volume]	Kinnex primer mix C	
4 [25 µL Rx volume]	Kinnex primer mix D	
5 [25 µL Rx volume]	Kinnex primer mix E	
6 [25 µL Rx volume]	Kinnex primer mix F	
7 [25 µL Rx volume]	Kinnex primer mix G	
• • •	• • •	
13 [25 µL Rx volume]	Kinnex primer mix M	
14 [25 µL Rx volume]	Kinnex primer mix N	
15 [25 µL Rx volume]	Kinnex primer mix O	
16 [25 µL Rx volume]	Kinnex primer mix PQ	

16.5 µL PCR Rx vol.

Run Kinnex PCR program (9 cycles)

3.2 Pooling of 16 Kinnex PCR products and 1.2X SMRTbell cleanup[1]

Kinnex PCR 1: 16.5 µL + Kinnex PCR 2: 16.5 µL + Kinnex PCR 3: 16.5 µL + ••• + Kinnex PCR 16: 16.5 µL → **Pooled Kinnex PCR reactions**: 264 µL total pooled vol.

264 µL Pooled Kinnex PCR reactions + 317 µL 1.2X SMRTbell cleanup beads → 21 µL purified Kinnex PCR sample

Proceed to **Step 4**

[1] **Note:** Use 1.5X SMRTbell cleanup bead ratio for amplicons <400 bp, or 2X for amplicons <200 bp.

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Kinnex PCR (cont.)

Procedural notes

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

3. Kinnex (on-bead) PCR

✓	Step	Instructions
	3.1	Thaw primers. The entire volume of primers can be transferred to two 8-tube strips for ease of use with a multi-channel pipette.

PCR tube	Kinnex primers premix	Tube color	PN
1	Kinnex primers premix A	Orange	103-107-800
2	Kinnex primers premix B		103-107-900
3	Kinnex primers premix C		103-108-000
4	Kinnex primers premix D		103-108-100
5	Kinnex primers premix E		103-108-200
6	Kinnex primers premix F		103-108-300
7	Kinnex primers premix G		103-108-400
8	Kinnex primers premix H		103-153-000
9	Kinnex primers premix I		103-153-100
10	Kinnex primers premix J		103-153-200
11	Kinnex primers premix K		103-153-300
12	Kinnex primers premix L		103-153-400
13	Kinnex primers premix M		103-153-500
14	Kinnex primers premix N		103-153-600
15	Kinnex primers premix O		103-153-700
16	Kinnex primers premix PQ		103-153-800

3.2 Set up the following PCR reaction mix in 1.5mL LoBind tube per sample on ice.
Reaction Mix 2 (RM2):

✓	Master mix components	Volume for 16X concatenation*
	PCR-grade water	101−X µL
	Kinnex 16-fold PCR mix	145 µL
	25 ng of **beads/DNA-complex** (from Step 2b.15) or 25 ng of Kinnex-compatible amplicons	X µL
	Total volume	246 µL

X = 25 (ng) / input concentration. If the DNA yield from step 2b.15 is greater than 25 ng, proceed with only 25 ng.
*10% overage included

- Can transfer entire volume of primers to PCR tubes for ease of use with multi-channel pipettes (16 primer mix tubes)

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

PacBio

Kinnex PCR (cont.)

Procedural notes

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

If answer is "No", proceed to Step 3

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

3.1 Kinnex (on-bead) PCR (cont.)

✔	Step	Instructions
	3.3	Pulse vortex to homogenize the **RM2** and quick-spin in a microcentrifuge to collect liquid. Note: It is critical to thoroughly homogenize the RM2 immediately before proceeding to the next step. Inadequate mixing or uneven distribution of the beads/DNA complex may result in low Kinnex library yield.
	3.4	Aliquot **14 µL** of **RM2** into two 8-tube strip PCR tubes on ice (16 tubes total, 14 µL per tube)
	3.5	Add 2.5 µL of the appropriate Kinnex primer mix into each respective PCR tubes from step 3.4 for a total volume of 16.5 µL.
	3.6	Pipette-mix up and down 10 times. Quick-spin to collect all liquid.
	3.7	Run the Kinnex PCR program and keep sample on ice until thermal cycler lid has heated to 105°C. The duration of PCR is approximately 1 hour.

Kinnex PCR program

Heated lid set at 105°C. Reaction volume set to 20 µL.

Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	
3	30 sec	65°C	9
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

Recommended: Quantify each of the 16 tubes individually using the 1× dsDNA HS Kit after the PCR program to ensure consistent PCR yields. Ideally, the %CV across the 16 reactions would be <5%.

- **Critical step! Correct setup of all 16 Kinnex PCR reactions is required** – any missing/incorrect Kinnex primer pairs will result in no/low SMRTbell yield

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

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Kinnex PCR (cont.)

Procedural notes

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

3.8 Pooling of 16 Kinnex PCR products and 1.2x SMRTbell cleanup

✔	Step	Instructions
		Clean up with 1.2X SMRTbell cleanup beads (standard)
		NOTE: use 1.5X SMRTbell cleanup bead ratio for cDNA <400 bp, or 2X for cDNA <200 bp
	3.8	Quick-spin to collect all liquid. Pool the entire volume of all 16 reactions into a single 1.5 mL LoBind tube.
	3.9	Add 317 µL (1.2X v/v) of resuspended, room-temperature SMRTbell cleanup beads to the PCR pool.
	3.10	Pipette-mix the beads until evenly distributed.
	3.11	Quick-spin the 1.5 mL LoBind tube in a microcentrifuge to collect all liquid from the sides of the tubes.
	3.12	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
	3.13	Place the 1.5 mL LoBind tube in a magnetic separation rack until beads separate fully from the solution.
	3.14	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	3.15	Slowly dispense 1 mL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
	3.21	Place the 1.5 mL LoBind tube in a magnetic separation rack until beads separate fully from the solution.
	3.22	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a [illegible] strip. Discard old tube with beads.
	3.23	Evaluate sample concentration. • Take a 1 µL aliquot from each tube, dilute with 9 µL of elution buffer. Using 1 µL of the dilution, measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Yield typically ranges from 5–11 µg.
	3.24	Proceed to the next step of the protocol if sample quantity is acceptable: • 5 µg is recommended • < 5 µg may result in lower library prep yields Do not proceed if less than 3 µg is available

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

- Pool entire volume (16.5 µL) from each Kinnex PCR reaction in a clean 1.5 mL DNA LoBind tube for a total combined volume of 264 µL

- Add **317 µL** of SMRTbell cleanup beads (1.2X)
- Kinnex PCR mix significantly increases stringency of SMRTbell clean up beads, **so accurate pipetting is critical**

- Perform DNA concentration QC using Qubit dsDNA HS kit
- Typical yield of Kinnex PCR products is **5–11 µg**

- Proceed to the next step of the protocol if sample quantity is acceptable (recommended input: 5 µg)
- **Do not proceed if less than 3 µg is available**
- **Note:** If Kinnex PCR yield is less than 3 µg, repeat Kinnex PCR and quantify each individual tube with Qubit 1X dsDNA HS assay for low yield troubleshooting

PacBio

Kinnex array formation

In this step, assemble amplicon DNA "segments" containing programmable ends into a linear array

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

Step 4 procedural description

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

1 Input quality control

Safe stop

Check point: Template-switch derived cDNA?

Safe stop

2a TSO PCR

Safe stop

2b TSO artifact removal

Safe stop

f answer is "No", proceed to Step 3

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

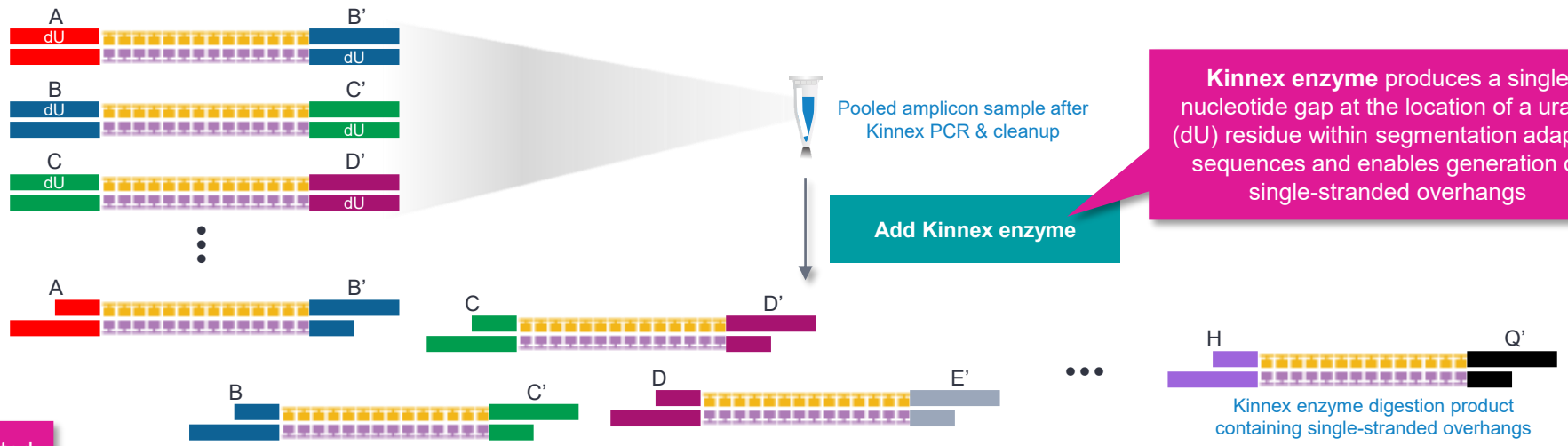
4 Kinnex array formation + cleanup

Safe stop

5 Nuclease treatment + cleanup

(ABC)

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



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

Add **Kinnex ligase** to enable directional assembly of cDNA transcript segments containing "sticky ends" into a linear, ordered array

Complete 16-segment transcript array molecule containing 2 Kinnex adapters

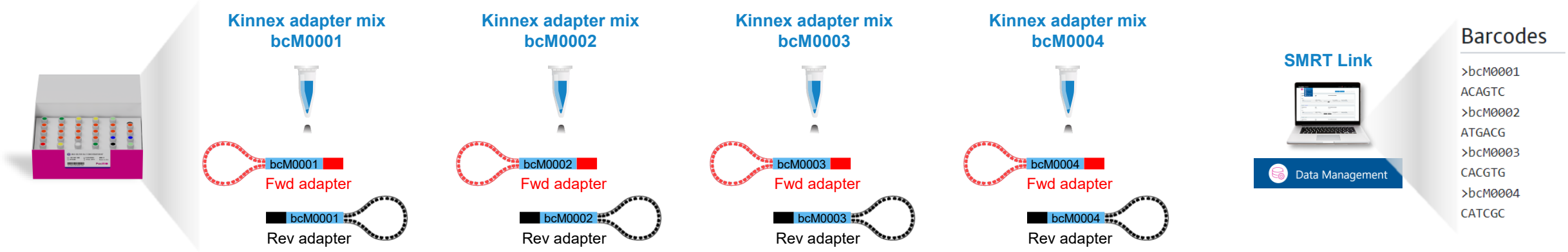
PacBio

[1] **Note:** Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-M0004) are available for Kinnex array formation step. **Note:** Kinnex concatenation workflow is not compatible with standard SMRTbell adapters from SMRTbell prep kit 3.0 and is also not compatible with SMRTbell adapter index plate 96A/B/C/D.

Kinnex array formation (cont.)

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

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



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

Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

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

Kinnex array formation (cont.)

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

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

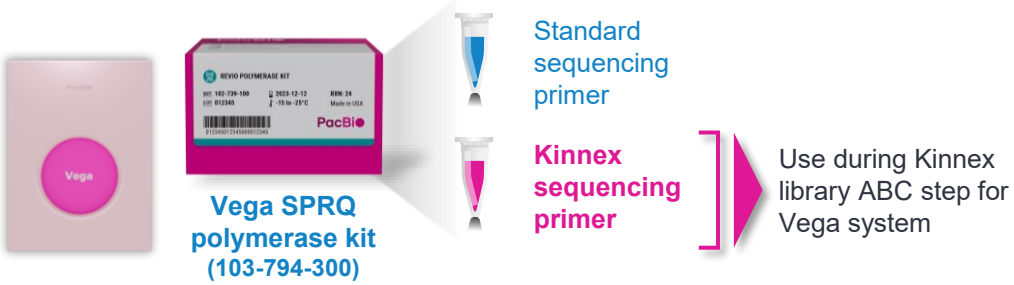
Forward and reverse Kinnex terminal adapters have **different structures**

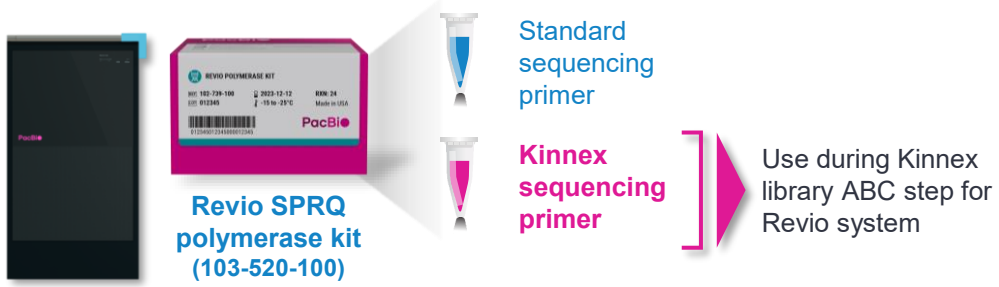
Annealed & bound Kinnex library molecule

Barcoded forward Kinnex terminal adapter

Barcoded reverse Kinnex terminal adapter

- Kinnex adapters require **Kinnex sequencing primer**[1] (instead of Standard sequencing primer) for the annealing reaction during sample setup ABC step





[1] **Note:** Revio SPRQ polymerase kit and Vega SPRQ polymerase kit contain both the Standard sequencing primer and the Kinnex sequencing primer.

Kinnex array formation (cont.)

Procedural summary

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

Step 4 procedural summary

3.1 Kinnex array formation

5 µg in 20 µL (250 ng/µL) Kinnex PCR sample from Step 4 + 2 µL Barcoded Kinnex adapter[1] +

Master Mix (17 µL): 3.5 µL Kinnex array and repair buffer + 4 µL Kinnex enzyme + 6 µL Kinnex ligase

→ 35.5 µL total Rx vol. → Run Kinnex primer digestion/ligation program (37°C 15 min, 45°C 45 min, Hold at 4°C)

→ 35.5 µL Kinnex array sample + 2 µL Kinnex repair mix → 37.5 µL total Rx vol. → Run Kinnex repair program (37°C 15 min, 45°C 15 min, Hold at 4°C)

3.2 1.2X SMRTbell cleanup

→ 37.5 µL Kinnex array sample + 42.5 µL Elution buffer = 80 µL Kinnex array sample → 80 µL Kinnex array sample + 96 µL 1.2X SMRTbell cleanup beads → 40 µL purified Kinnex library

Proceed to **Step 5**

[1] Kinnex concatenation kit 2.0 contains four different barcoded Kinnex adapter mixes (bcM0001–bcM0004) → Select one barcode to use per Kinnex library preparation reaction.

Kinnex array formation (cont.)

Procedural notes

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

1 Input quality control
↓ Safe stop

Check point: Template-switch derived cDNA?
↓ Safe stop

2a TSO PCR
↓ Safe stop

2b TSO artifact removal
↓ Safe stop

3 Kinnex (on-bead) PCR
↓ Safe stop

4 Kinnex array formation + cleanup
↓ Safe stop

5 Nuclease treatment + cleanup
↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

4.1 Kinnex array formation

✓	Step	Instructions
	4.1	In a 0.2 mL PCR tube, add **5 µg** of the Kinnex PCR product Section 3, in 20 µL of volume (250 ng/µl). Dilute with elution buffer going into this step if sample is too concentrated. Using less or more than 5 µg might result in low library recovery and compromised sequencing performance.
		Add **2 µL of Kinnex adapter** (bc01–04; **select one barcode per library preparation**).

✓	Tube color	Component	Volume
	Blue	Kinnex barcoded adapter	2 µL

✓	Step	Instructions
	4.2	Add the following components in the listed order. If processing multiple samples, make a master mix with 10% overage. Pipette mix master mix. **Reaction Mix 3 (RM3):**

✓	Tube color	Component	Volume

✓	Step	Instructions
	4.3	Add **13.5 µL of RM3** to the PCR tube containing sample and Kinnex barcoded adapter for a final volume of 35.5 µL. Pipette-mix and run the Kinnex primer digestion/ligation program.

Kinnex primer digestion/ligation program

Heated lid set at ≥55°C. Reaction volume set to 40 µL.

Step	Time	Temperature
1	15 min	37°C
2	45 min	45°C
3	Hold	4°C

✓	Step	Instructions
	4.4	After running the Kinnex primer digestion/ligation program, add **2 µL of Kinnex repair mix** directly to the Kinnex primer digestion/ligation sample for a final volume of 37.5 µL.

✓	Tube color	Component	Volume
	Green	Kinnex repair mix	2 µL

✓	Step	Instructions
	4.5	Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid.
		Run the Kinnex repair program.

- Recommended input amount to proceed with Kinnex array formation is **5 µg** of Kinnex PCR amplicons (from Step 2)
  - Proceeding with <3 µg is **not recommended** since lower input amounts may lead to insufficient final library yields to enable optimal sequencing results

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

- Add **Kinnex repair mix** to repair nicked / damaged DNA sites within newly formed Kinnex array products

- After running **Kinnex repair program**, perform 1.2X SMRTbell bead cleanup (Step 4.8)

PacBio

Nuclease treatment & cleanup

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

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

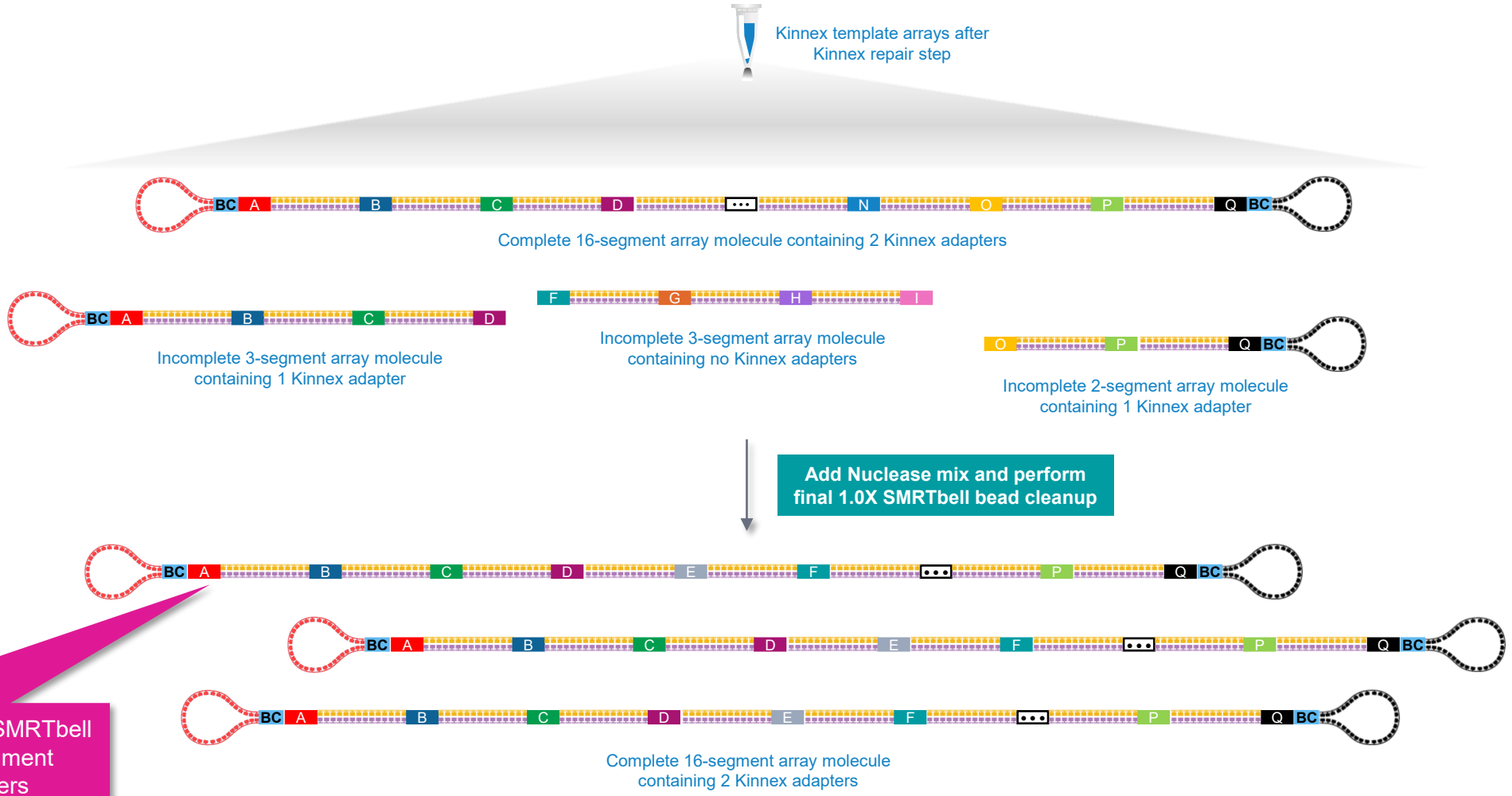
4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

If answer is "No", proceed to Step 3

Step 5 procedural description



- After nuclease treatment, most remaining SMRTbell templates are complete (full-length) 16-segment array molecules capped with Kinnex adapters

PacBio

Nuclease treatment & cleanup (cont.)

Procedural summary

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

1 Input quality control
↓ Safe stop
Check point: Template-switch derived cDNA?
↓ Safe stop
2a TSO PCR
↓ Safe stop
2b TSO artifact removal
↓ Safe stop
3 Kinnex (on-bead) PCR
↓ Safe stop
4 Kinnex array formation + cleanup
↓ Safe stop
5 Nuclease treatment + cleanup
↓ Safe stop
6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

Step 5 procedural summary

4.1 Nuclease treatment

40 µL Kinnex library sample from Step 5 +

Master Mix (10 µL): 5 µL Nuclease buffer + 5 µL Nuclease mix

→ 50 µL total Rx vol. → Run nuclease treatment program (37°C 60 min, Hold at 4°C)

4.2 1.2X SMRTbell cleanup

50 µL Nuclease-treated Kinnex library + 60 µL 1.2X SMRTbell cleanup beads → 25 µL final purified Kinnex library → Final purified Kinnex library → DNA quantification QC → DNA sizing QC

Expect ~5–15% recovery of the starting Kinnex-PCR product

Kinnex 16-fold library size is typically ~10–17 kb

Proceed to **ABC**[1]

[1] Follow **Step 5** instructions in **Procedure & checklist** to prepare the Kinnex SMRTbell library (25 µL) for sequencing on the Revio system or Vega system.

Nuclease treatment & cleanup (cont.)

Procedural notes

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

1 Input quality control

↓ Safe stop

Check point:
Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

5.1 Nuclease treatment

✓	Step	Instructions
	5.1	Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample from the previous step in the order and volume listed below. Reaction Mix 4 (RM4):

✓	Tube color	Component	Volume
	Light purple	Nuclease buffer	5 µL
	Light green	Nuclease mix	5 µL
		Total volume	10 µL

5.2	Pipette-mix RM4.	
5.3	Quick-spin RM4 in a microcentrifuge to collect liquid.	
5.4	Add 10 µL of RM4 to each sample. Total volume should equal 50 µL.	
5.5	Pipette-mix each sample.	
5.6	Quick-spin the strip tube in a microcentrifuge to collect liquid.	
5.7	Run the nuclease treatment program. **Nuclease treatment program** Heated lid set at ≥ 47°C. Reaction volume set to 50 µL.	

Step	Time	Temperature
1	60 min	37°C
2	Hold	4°C

- Perform nuclease treatment for **60 min**

Nuclease treatment & cleanup (cont.)

Procedural notes

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

1 Input quality control

↓ Safe stop

Check point: Template-switch derived cDNA?

↓ Safe stop

2a TSO PCR

↓ Safe stop

2b TSO artifact removal

↓ Safe stop

3 Kinnex (on-bead) PCR

↓ Safe stop

4 Kinnex array formation + cleanup

↓ Safe stop

5 Nuclease treatment + cleanup

↓ Safe stop

6 Annealing, binding & cleanup (ABC)

If answer is "No", proceed to Step 3

5.8 Final cleanup with 1.2X SMRTbell cleanup beads

✔	Step	Instructions
	5.8	Add 60 µL (1.2X v/v) SMRTbell cleanup beads to each sample from the previous step. Pipette-mix the beads until evenly distributed.
	5.9	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
	5.10	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
	5.11	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
	5.12	Slowly remove the cleared supernatant without disturbing the beads. It is recommended to save the supernatant in another tube strip in case of poor DNA recovery.
	5.13	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
	5.14	Repeat the previous step.
	5.15	Remove residual 80% ethanol: • Remove tube strip from the magnetic separation rack. • Quick spin tube strip in a microcentrifuge. • Place tube strip back in a magnetic separation rack until beads separate fully from the solution.
	5.21	Recommended: Evaluate sample concentration and size. • Take a 1 µL aliquot from each tube, dilute with 4 µL of elution buffer. • Using 1 µL of the dilution, measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. **Expect 5-15% recovery of the starting Kinnex-PCR product.** Yield typically ranges from 250–750 ng with 5 µg input. • Recommended: Further dilute each aliquot to 250 pg/µL with the Femto Pulse dilution buffer. Check the final SMRTbell library size distribution with a Femto Pulse system. See Library QC section in the Best practice and Figure 1 in the Appendix for an example.
	5.22	If library concentration exceeds 20 ng/µL, **normalize to 20 ng/µL**, and proceed to ABC with **25 µL** of normalized library. Using a concentration above 20 ng/µL may result in lower loading during sequencing.
	5.23	Proceed to Section 6 to prepare library for sequencing on Vega, Vega SPRQ-Nx or Revio (+SPRQ/SPRQ-Nx chemistry).
	5.24	Store SMRTbell libraries at 4°C if sequencing within the week. Long-term storage should be at -20°C. Minimize freeze-thaw cycles when handling SMRTbell libraries.

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

- Perform **DNA concentration QC** on final purified Kinnex RNA library using a Qubit dsDNA HS assay
  - Typical final SMRTbell library yield from 5 µg of input DNA into Kinnex array formation is **~5 – 15%** – a much higher observed yield might suggest incomplete digestion of partial SMRTbell templates
  - **Troubleshooting tip:** If SMRTbell library yield is higher than expected and sample loading is lower than expected, consider repeating nuclease treatment step

- Perform **DNA sizing QC** on final purified Kinnex library using a Femto Pulse system (expected final library insert size is **~10 – 17 kb**)

- Kinnex final SMRTbell library concentration must be **≤60 ng/ µL** to proceed with sample setup ABC [Step 6]
  → Using a concentration above 20 ng/µL may result in lower loading during sequencing

PacBio

Kinnex 16-fold library prep inputs & expected step yields

Final Kinnex library yield is typically sufficient to support ≥8 SMRT Cell acquisitions on Revio & Vega systems

Kinnex 16-fold prep kit

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

Kinnex PCR

Kinnex PCR → **SMRTbell bead cleanup**

IN: 25 ng of amplicon insert (single or multiplex)

OUT: 5-11 µg of amplified products after pooling

Kinnex array formation

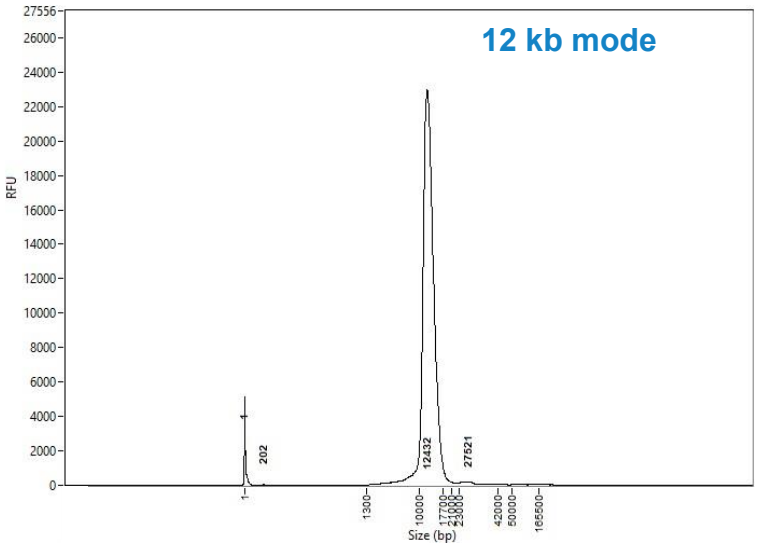
Kinnex digestion / Ligation → **DNA damage repair** → **SMRTbell cleanup** → **Nuclease treatment** → **SMRTbell bead cleanup**

IN: 5 µg of pooled Kinnex PCR products

OUT: 80% recovery from Kinnex digestion Rx input

IN: 40 µL of purified Kinnex repair reaction products

OUT: 5-15% recovery from Kinnex digestion Rx input



Example Femto pulse DNA sizing QC analysis results for final Kinnex 16-fold library generated for a 10x Chromium single-cell 3' cDNA sample prepared from a human cell line (HG002).

Example Kinnex 16-fold library prep yields

Amplicon input for Kinnex 12-fold array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	500 ng (10%)

[1] **Post-nuclease treatment & final cleanup yields typically ranged from ~5% to ~15% when using single-cell 3' cDNA samples for Kinnex 16-fold library construction.**

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

- >8 acquisitions for Revio system with SPRQ-Nx chemistry
- >8 acquisitions for Vega system with SPRQ-Nx chemistry

PacBio

Kinnex sequencing preparation workflow

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	**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
	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)

Runs

Refer to **Procedure & checklist protocol documentation** for the latest sample setup (ABC[1]) 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
	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)

Runs

IMPORTANT!

Refer to **Procedure & checklist protocol documentation** for the latest sample setup (ABC[1]) 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 option**[2]

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

v26.2

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

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

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

Kinnex single-cell RNA

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

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

Viral sequencing
- Adeno-associated virus

Metagenomics
- Kinnex 16S rRNA
- Full-length 16S rRNA sequencing

Kinnex full-length RNA

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

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

Viral sequencing
- Adeno-associated virus

Metagenomics
- Kinnex 16S rRNA
- Full-length 16S rRNA sequencing

Kinnex 16S rRNA *OR* other (custom) amplicon

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

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

Viral sequencing
- Adeno-associated virus

Metagenomics
- Kinnex 16S rRNA
- Full-length 16S rRNA sequencing

If running a **Kinnex custom amplicon library**, users can select "**Kinnex 16S rRNA**" as a placeholder in run design[1]

[1] **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.

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

v26.2

Sample and run information

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]

Default Movie Time = **24 hrs**

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

Standard SMRTbell library type containing standard SPK3 barcoded terminal adapters

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

Kinnex SMRTbell library type containing Kinnex barcoded terminal adapters

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

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

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

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

v26.2

Sample indexing (barcoding) information

Kinnex single-cell RNA

Samples

Sample is indexed: YES / NO

Indexes (Required): MAS SMRTbell Barcoded Adapters (v2)

Biosample names (Required): Interactively | From a File

Kinnex full-length RNA

Sample is indexed: YES / NO

Indexes (Required): MAS SMRTbell Barcoded Adapters (v2)

Biosample names (Required): Interactively | From a File

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 field is automatically set to **YES** for all Kinnex application types

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

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

Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

Example interactive biosample name specification

Barcode Selector and Sample Name Editor

Available Barcodes: Filter...

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

Included Barcodes: Filter...

Barcode	Bio Sample

Barcode Selector and Sample Name Editor

Available Barcodes: Filter...

Barcode
bcM0003--bcM0003
bcM0004--bcM0004

Included Barcodes: Filter...

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

SMRT Link

Data Management

MAS SMRTbell barcoded adapter indexes

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

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

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

SMRT LINK v26.2

Data options

Kinnex single-cell RNA

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

Data Options

Include Base Kinetics: YES / NO

Consensus Mode: MOLECULE / STRAND

Assign Data To Project: General Project

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

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

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

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

Analysis options

v26.2

Kinnex single-cell RNA

Analysis Options

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

Add Analysis: YES / NO

Analysis Name (Required): Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Nam

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

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

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

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

Kit Type: 10X 3' KIT / 10X 5' KIT

Advanced Parameters

Kinnex full-length RNA

Analysis Options

Analysis: YES / NO

Name (Required): Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name

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

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

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

Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Advanced Parameters

Kinnex 16S rRNA *OR* other (custom) amplicon

Analysis Options

Add Analysis: YES / NO

Analysis Name (Required): Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name

Select Analysis Workflow (Required): Read Segmentation

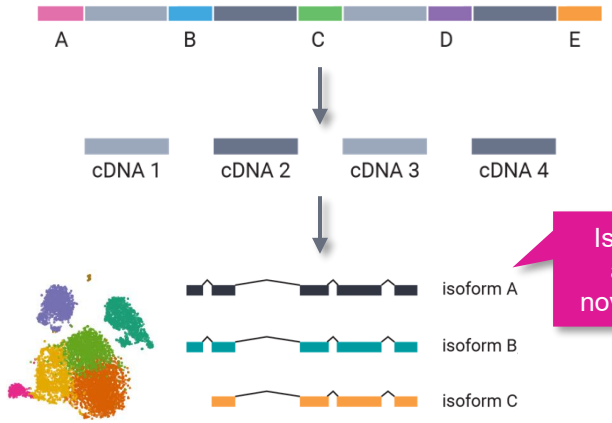
Segmentation Adapter Set: MAS-Seq Adapter v2 (MAS12)

Advanced Parameters

Analysis Workflow is automatically filled in

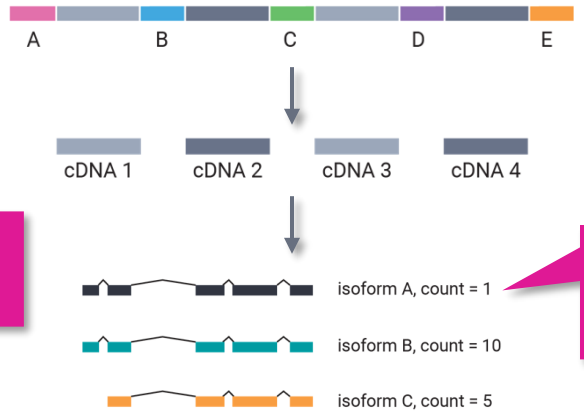
Split arrayed HiFi reads at adapter positions, generating segmented reads (S-reads)

Read Segmentation and Single-Cell Iso-Seq



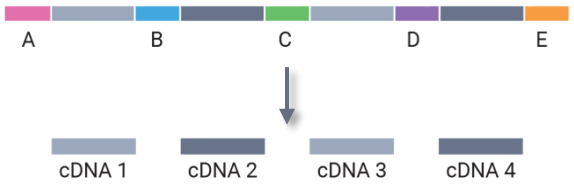
Isoform-classification analysis to identify novel genes & isoforms

Read Segmentation and Iso-Seq



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

Read Segmentation



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

SMRT LINK 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

Add Analysis	
Analysis Name Required	
Select Analysis Workflow Required	Read Segmen… …gle-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

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

Add Analysis	
Analysis Name Required	
Select Analysis Workflow Required	Read Segme… …Seq
Segmentation Adapter Set	MAS-Seq Adapter v3 (MAS8)
Primer Set Required	Iso-Seq v2 Barcoded cDNA Primers
Reference Set	Human Genome hg38, with Gencode v39 annotations
Cluster of Barcoded Samples	Pool reads and cluster together

Advanced Parameters

Kinnex 16S rRNA *OR* other (custom) amplicon

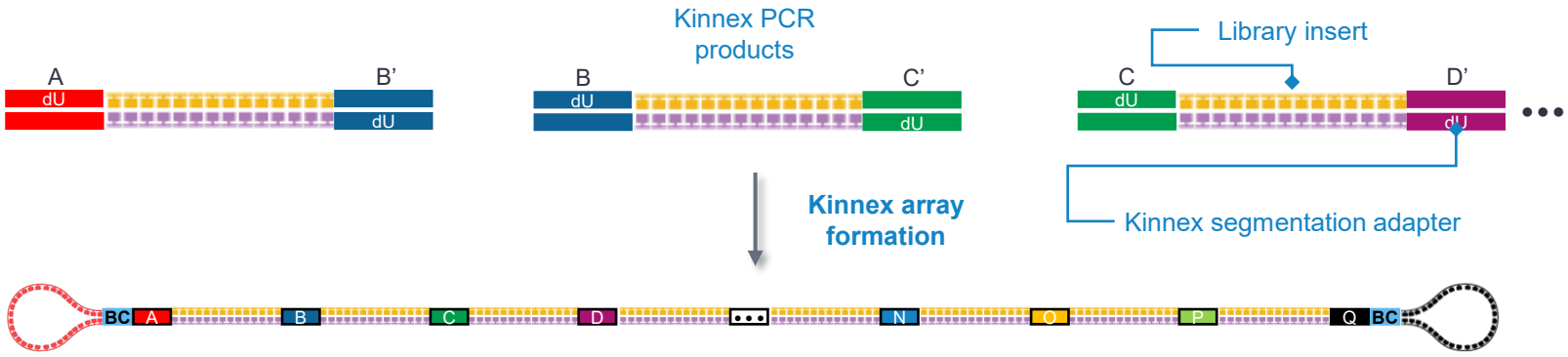
Analysis Options

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

Add Analysis	
Analysis Name Required	
Select Analysis Workflow Required	Read …
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**



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)

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

SMRT LINK v26.2

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

Specify **Primer Set** used for cDNA amplification

10x Forward (F) PCR primer

10x Reverse (R) PCR primer

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

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

OR

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

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

Kinnex full-length RNA

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 **Reference** Set. Default sets are:

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

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

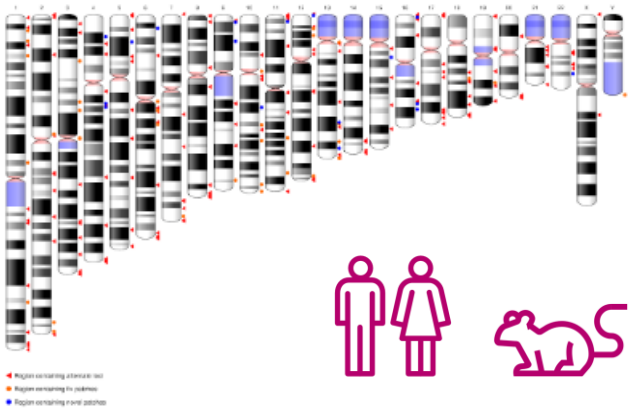
Reverse (R) PCR primer (Iso-Seq cDNA amplification primer)

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

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

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

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



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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' cD

Reference Set (Required): Human Genome hg38, with G 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]TTTTTTTTTT LIBRARY INSERT CCC TSO 3'
3' [CBC][UMI]AAAAAAAAAA LIBRARY INSERT GGG TSO 5'

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

OR

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

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

Kinnex full-length RNA

Analysis Options

Add Analysis: YES / NO

Analysis Name (Required)

Select Analysis Workflow (Required)

mentation Adapter Set: MAS-S (MAS8)

Primer Set (Required): v2 Barcoded cDNA Primers

Reference: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Advanced Parameters

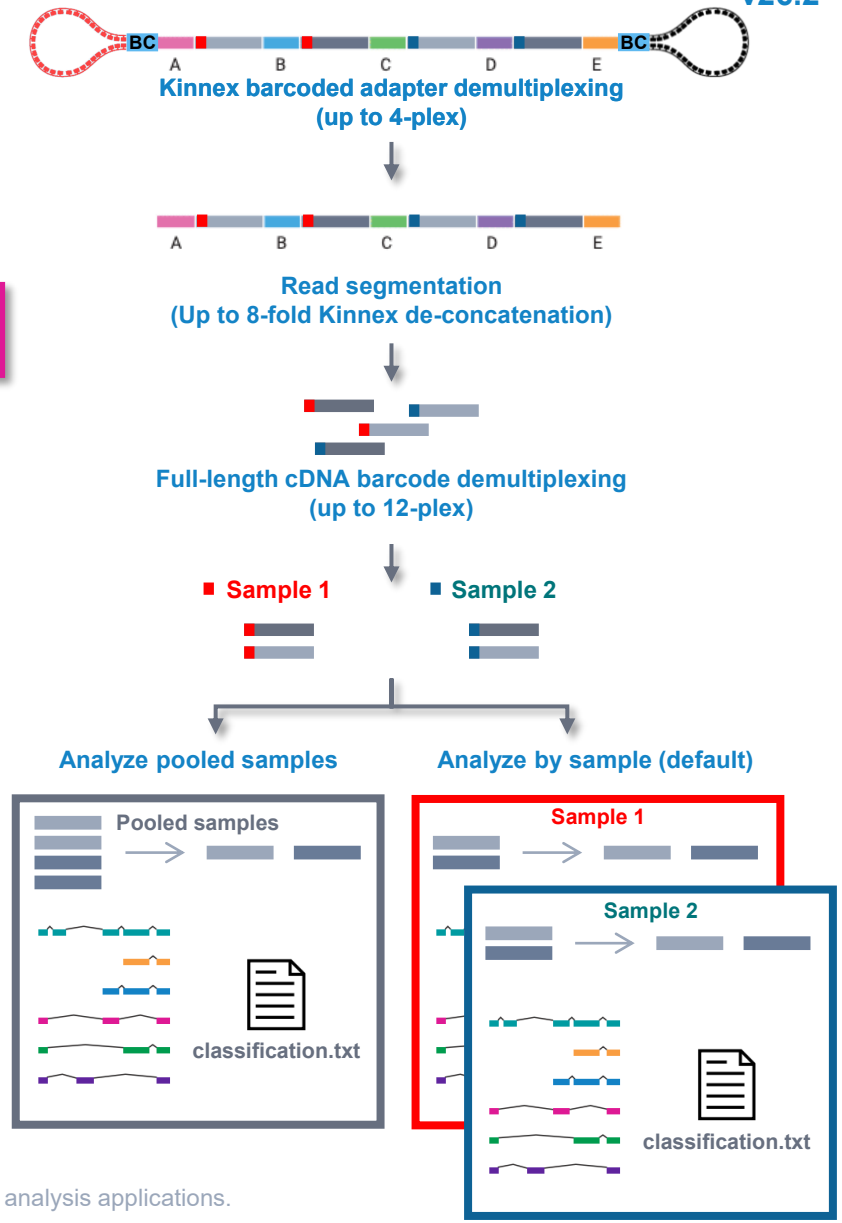
Specify **how to perform read clustering for barcoded samples**

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



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

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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 use[1]

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.

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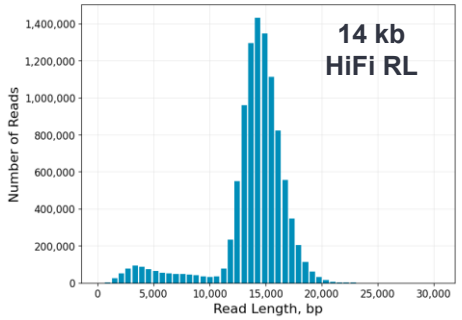
Kinnex example performance – Revio system v13.5 + SPRQ-Nx (cont.)

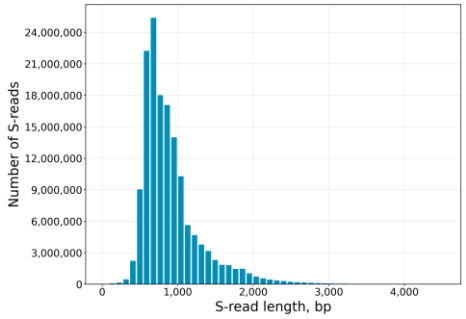
Kinnex single-cell RNA

Kinnex 16-fold prep kit

HiFi read length



S-read length

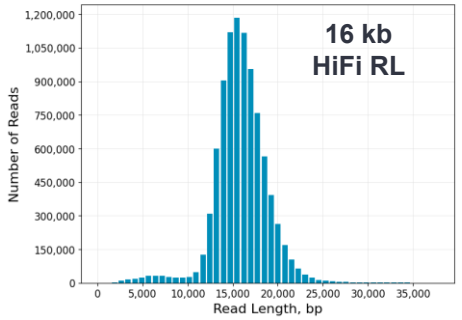


Value	Analysis Metric
10,030,881	Reads
149,910,947	Segmented reads (S-reads)
906	Mean length of S-reads
88.24 %	Percent of reads with full arrays
14.94	Mean array size (concatenation factor)

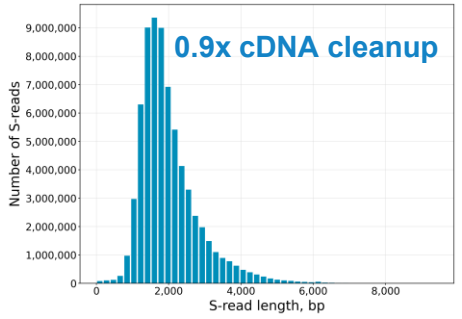
Kinnex full-length RNA

Kinnex 8-fold prep kit

HiFi read length



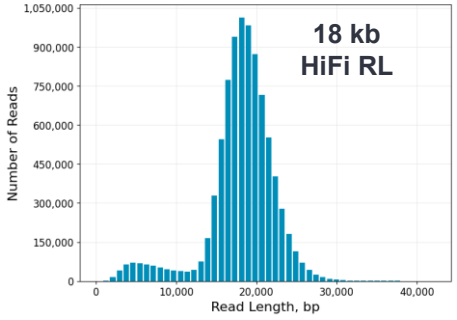
S-read length



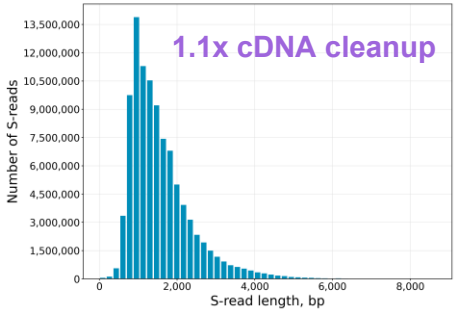
Value	Analysis Metric
9,044,421	Reads
69,407,271	Segmented reads (S-reads)
2,002	Mean length of S-reads
92.67 %	Percent of reads with full arrays
7.67	Mean array size (concatenation factor)

Kinnex 12-fold prep kit

HiFi read length



S-read length

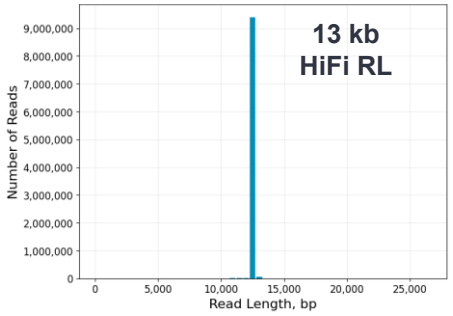


Value	Analysis Metric
8,742,074	Reads
96,912,559	Segmented reads (S-reads)
1,566	Mean length of S-reads
87.77 %	Percent of reads with full arrays
11.09	Mean array size (concatenation factor)

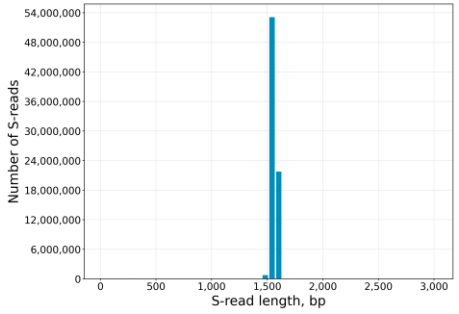
Kinnex 16S rRNA

Kinnex 8-fold Prep kit

HiFi read length



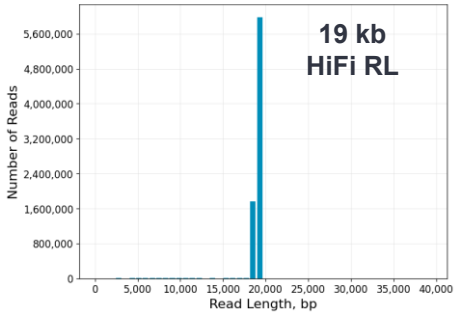
S-read length



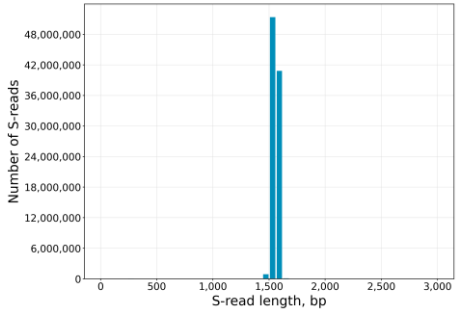
Value	Analysis Metric
9,541,465	Reads
75,949,260	Segmented reads (S-reads)
1,560	Mean length of S-reads
97.77 %	Percent of reads with full arrays
7.96	Mean array size (concatenation factor)

Kinnex 12-fold prep kit

HiFi read length



S-read length



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 specifications[1]

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.

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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 datasets[1]

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

[1] Example PacBio datasets may be downloaded at https://www.pacb.com/connect/datasets/.

www.pacb.com

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

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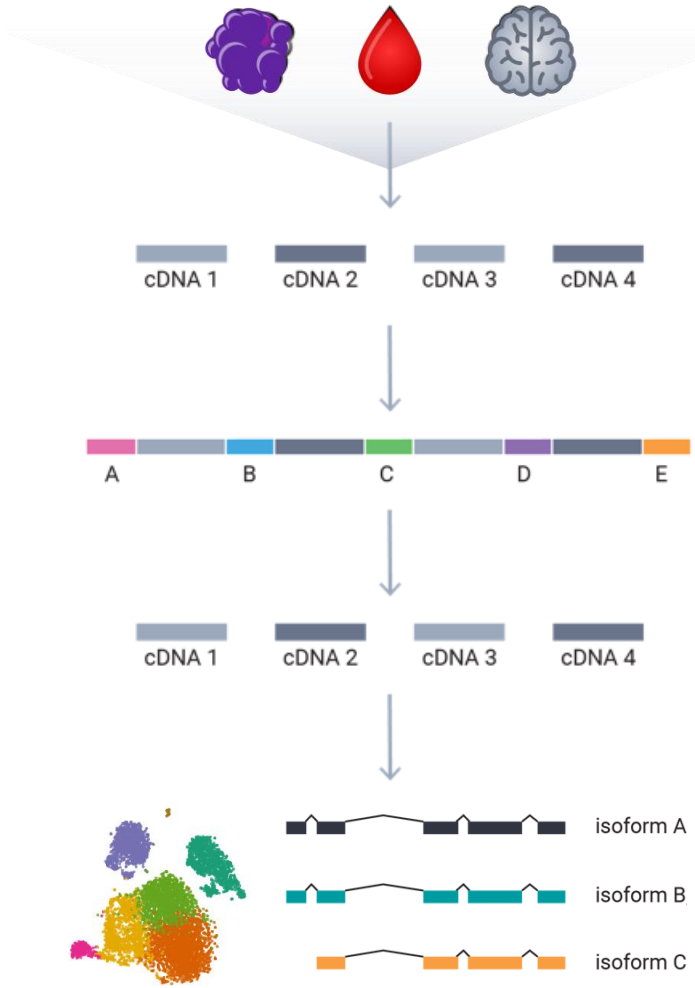
APPENDIX 1: Example Kinnex single-cell RNA workflow

Kinnex single-cell RNA library preparation workflow overview

Kinnex single-cell RNA method overview

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



Single-cell cDNA generation

Refer to 10x Genomics Support website to download 10x Chromium user guides and other documentation[1]

Kinnex library preparation

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

PacBio sequencing

Read segmentation + Single-cell isoform analysis

SMRT Link user guide (103-891-900)

- Single-cell cDNA amplicon generation using 10x Chromium Single Cell (3' or 5') reagent kits or other PacBio Compatible products[1] (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*)

[1] Kinnex single-cell RNA workflow is generally compatible with single-cell cDNA from (1) 10x Chromium Single Cell Universal 3' or 5' kit; Single Cell Next GEM (v3.1 3' or v2 5'); (2) 10x Visium HD Spatial Gene Expression kit; (3) Parse Evercode WT or WT mini kit; or (4) ArgenTag Single-Cell RNA Library Kit. See 10x Genomics Support Hub and APPENDIX 2 – PacBio compatible Kinnex library preparation workflows for more information about other third-party PacBio Compatible products.

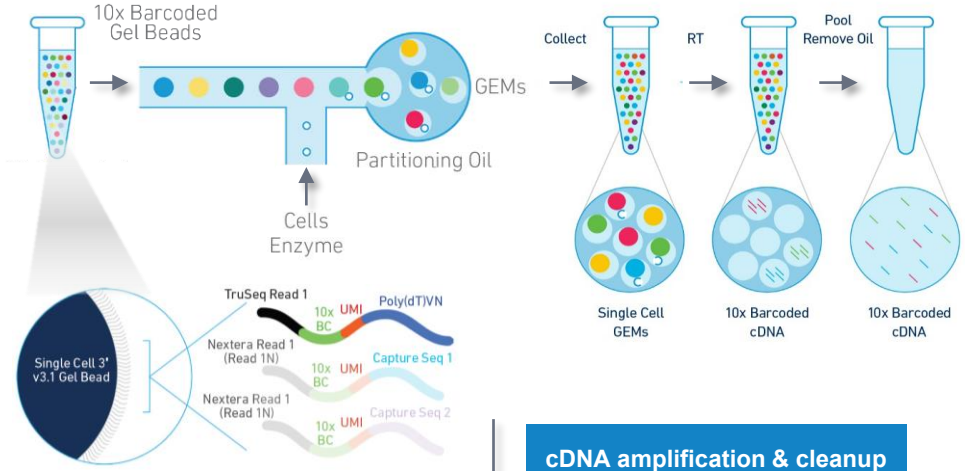
Kinnex single-cell RNA method overview

Single-cell cDNA sample preparation[1]

10x GENOMICS

Chromium Controller/X/iX

Chromium Next GEM Single Cell 5' or 3' Kit



cDNA amplification & cleanup

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

PacBio

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

Kinnex library prep, sequencing & analysis

PacBio

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

10x single-cell cDNA input

Template switch oligo (TSO) artifact removal

cDNA with expected Fwd & Rev primer sequences

Kinnex segmentation adapter incorporation & array formation

16-segment Kinnex array construct containing barcoded SMRTbell adapters at both ends

DNA damage repair & nuclease treatment / ABC[2]

PacBio long-read systems

Sequel IIe, Vega or Revio system

SMRT Link

Read segmentation & single-cell Iso-Seq analysis

[1] Refer to 10x Genomics Support website to download 10x Chromium user guides and other documentation.

[2] ABC = Anneal sequencing primer / Bind polymerase / Complex cleanup

Kinnex single-cell RNA workflow key steps

Single-cell cDNA sample preparation[1]

10x GENOMICS

Poly(dT) primer for 1st-strand synthesis

CBC = 10x cell barcode
UMI = Unique molecular identifier

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

Poly(A)+ mRNA template

Example 10x single cell 3' cDNA workflow is shown here

Samples incorporate a 10x cell barcode during single-cell cDNA synthesis

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

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

Template Switch Oligo (TSO)

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

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

2nd-strand synthesis & cDNA amplification

10x Forward PCR Primer

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

TSO
10x Reverse PCR Primer

cDNA cleanup

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

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

PacBio

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

Kinnex library prep, sequencing & analysis

PacBio

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

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

TSO artifact removal

F R TSO

Kinnex PCR (parallel PCR Rxs to append Kinnex segmentation adapters)

PCR 1	PCR 2	PCR 3…	…PCR (*n*)
A B'	B C'	C D'	… …

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

Pooling & Kinnex array formation

BC A B C … Q BC
BC A B C D
O P Q BC

Samples can be barcoded during Kinnex array formation using up to 4 different barcoded Kinnex SMRTbell adapters[2]

Kinnex array repair & nuclease Tx

BC A B C … Q BC

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

BC A B C … Q BC

Purified polymerase-bound 16-segment Kinnex library

Long-read sequencing & data analysis

Revio or Vega system

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

[1] Refer to 10x Genomics Support website to download 10x Chromium user guides and other documentation.

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

Kinnex single-cell RNA experimental design considerations

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

	Vega system + SPRQ-Nx chemistry	Revio system + SPRQ-Nx chemistry
Experimental goal	Characterize alternative splicing in single cells / cell types	
Expected data throughput (per acquisition)	**Kinnex 16-fold library:** 80 – 100 M reads	**Kinnex 16-fold library:** 100 – 150 M reads
Sample multiplexing[1]	Not recommended	Up to 2 samples per Revio SMRT Cell acquisition (2-plex)
Cell input into 10x Chromium single cell 3' or 5' cDNA generation workflow	3,000 – 10,000 cells for running a single (non-multiplexed) sample on one Vega SMRT Cell acquisition	<10,000 cells per sample if multiplexing 2 samples per Revio SMRT Cell acquisition (2-plex) 10,000 – 20,000 cells per sample if running a single (non-multiplexed) sample on 1–2 Revio SMRT Cell acquisitions
Expected coverage	Obtain 3,000 – 10,000 unique reads/single cell	Obtain up to ~20,000 unique reads/single cell
Single-cell cDNA input into Kinnex library prep workflow	≥15 ng of cDNA from **(1)** 10x Chromium Single Cell Universal 3' or 5' kit; Single Cell Next GEM (v3.1 3' or v2 5'); **(2)** 10x Visium HD Spatial Gene Expression kit; **(3)** Parse Evercode WT or WT mini kit; or **(4)** ArgenTag Single-Cell RNA Library Kit[2]	
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300)	
SMRT Link data analysis workflows	Read Segmentation and Single-cell Iso-Seq Analysis	
Community data analysis tools	Annotation & quantification: SQANTI3 / Differential analysis: TappAS / Fusion calling: pbfusion / Visualization: SWAN	

[1] Kinnex prep kits can support up to 4-plex sample multiplexing per SMRT Cell through the use of four different barcoded Kinnex terminal SMRTbell adapters during Kinnex single-cell RNA library construction.

[2] See 10x Genomics Support Hub and APPENDIX 2 – PacBio compatible Kinnex library preparation workflows for more information about other third-party PacBio Compatible products.

PacBio

General best practices recommendations for preparing Kinnex single-cell RNA libraries

cDNA Input

- Use an optimal input range of 3,000 – 10,000 cells[1] for the 10x Chromium single cell 3' cDNA generation workflow
- Follow the best practices in the **10x Chromium user guide**.
- Input cDNA quality control is highly recommended before proceeding to the Kinnex single-cell RNA workflow

Refer to 10x Genomics Support website to download 10x Chromium user guides and other documentation.

DNA sizing and quantitation QC

- Perform DNA concentration measurements with a **Qubit fluorometer** using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit.

Qubit 4 fluorometer and 1X ds DNA High Sensitivity Assay Kit (Thermo Fisher Scientific)

- Perform DNA sizing measurements with a **Bioanalyzer system** using the High Sensitivity DNA Kit (for input cDNA QC) or with a **Femto Pulse system** using the Genomic DNA 165 kb Kit (for final SMRTbell library QC)

Bioanalyzer 2100 System and High Sensitivity DNA Kit (Agilent Technologies)

Femto Pulse System and Genomic DNA 165 Kit (Agilent Technologies)

[1] **Note:** Cell capture efficiency of the 10x Chromium single cell workflow is **~60%**. For example, to achieve a **target cell recovery** of ~5,000 cells, approximately 10,000 cells can be used for input into the 10x Chromium single cell workflow.

Example Kinnex 16-fold single-cell RNA library prep inputs & expected yields

Final Kinnex library yield is typically sufficient to support ≥8 SMRT Cell acquisitions on Revio & Vega systems

Kinnex single-cell RNA

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

TSO artifact removal

- **TSO PCR** — IN: 15 ng of 10x single-cell 3' or 5' cDNA
- **SMRTbell bead cleanup** — OUT: ~200 ng – 600 ng of amplified products
- **TSO artifact removal** — OUT: 10%–40% of the input TSO PCR amount.

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

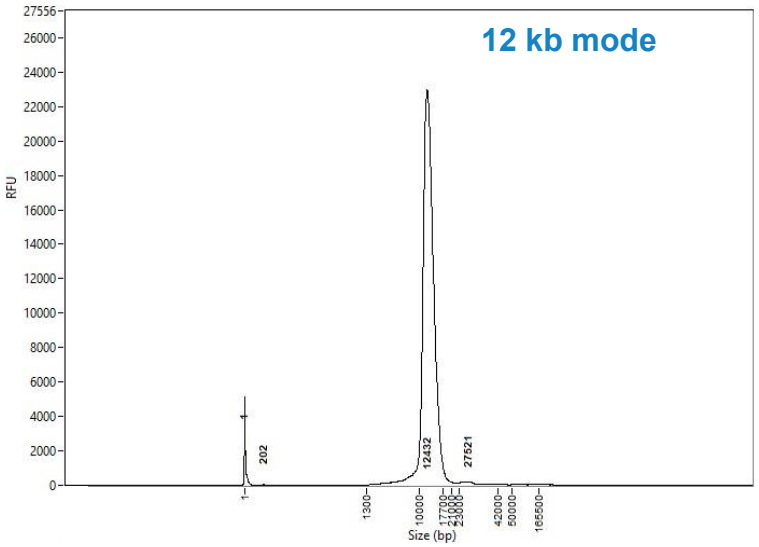
Kinnex PCR

- **Kinnex PCR** — IN: 35 ng of amplified cDNA (single or multiplex)
- **SMRTbell bead cleanup** — OUT: 5-11 µg of amplified products after pooling

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

Kinnex array formation

- **Kinnex digestion / Ligation** — IN: 5 µg of pooled Kinnex PCR products
- **Kinnex repair**
- **SMRTbell cleanup** — OUT: 80% recovery from Kinnex digestion Rx input
- **Nuclease treatment** — IN: 40 µL of purified Kinnex repair reaction products
- **SMRTbell bead cleanup** — OUT: 5-15% recovery from Kinnex digestion Rx input



Example Femto pulse DNA sizing QC analysis results for final Kinnex 16-fold library generated for a 10x Chromium single-cell 3' cDNA sample prepared from a human cell line (HG002).

Example Kinnex 8-fold bulk RNA library prep yields

10x single cell 3' cDNA input	15 ng
Single-cell cDNA amplicon input for Kinnex 16-fold array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	500 ng (10%)

[1] Post-nuclease treatment & final cleanup yields typically ranged from ~5% to ~15% when using single-cell 3' cDNA samples for Kinnex single-cell RNA library construction.

Final Kinnex library yield is typically sufficient to run the following number of SMRT Cell acquisitions:

- >8 acquisitions for Revio system using SPRQ-Nx chemistry
- >8 acquisitions for Vega system using SPRQ-Nx chemistry

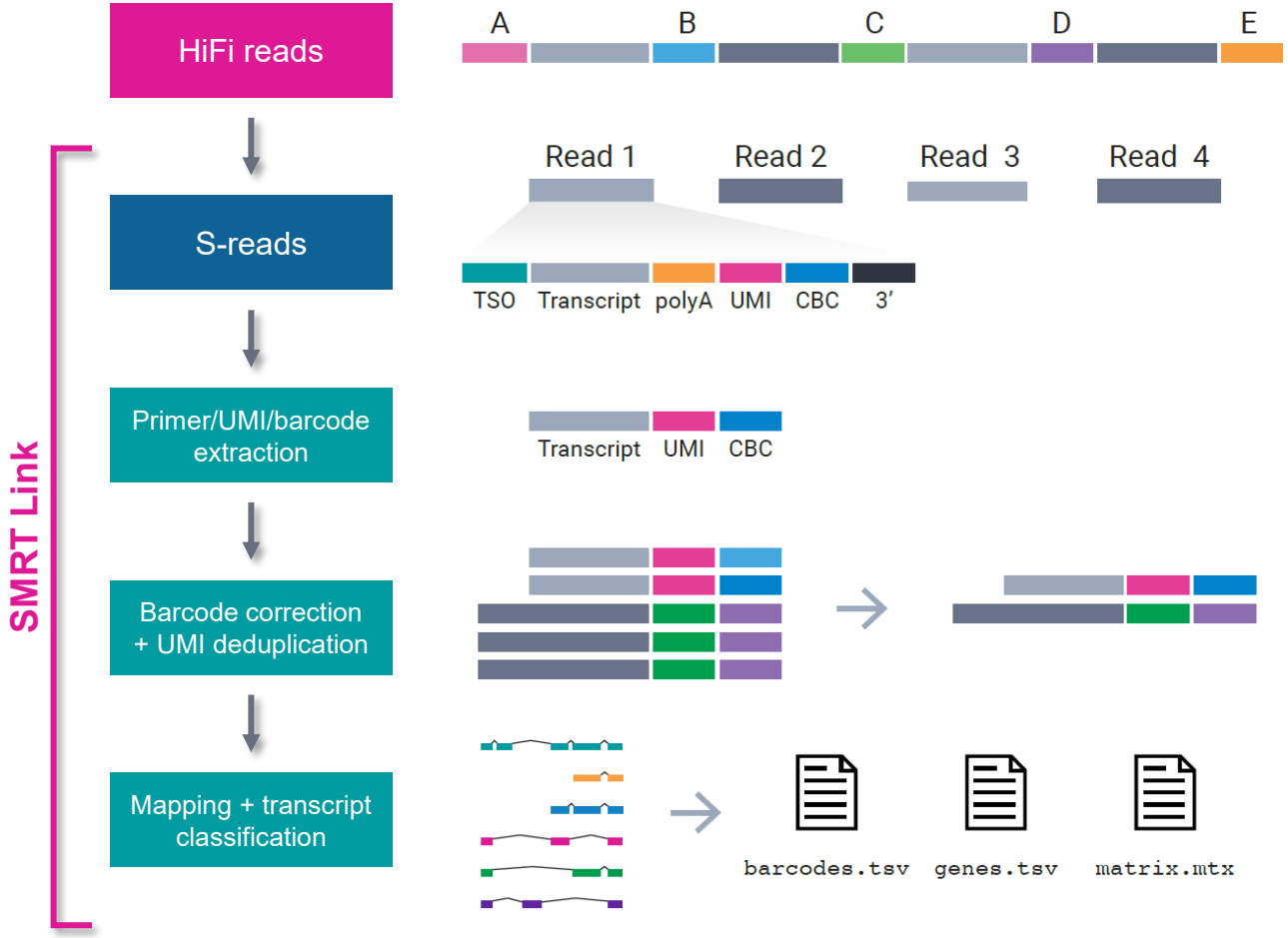
PacBio

Kinnex single-cell RNA data analysis workflow overview

Kinnex single-cell RNA bioinformatics workflow overview

SMRT Link Read Segmentation and Single-cell Iso-Seq workflow processes HiFi reads generated from Kinnex single-cell RNA libraries to to produce classified isoforms with read counts that are compatible with tertiary analysis tools

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



Read segmentation

- HiFi reads are segmented into individual segmented reads (S-reads) that represent the original cDNA sequences

Primer/UMI/barcode extraction

- Primers and polyA tails are removed, but also used to orient the read into 5' – 3' orientation
- Single-cell barcode and UMI information are extracted

Barcode correction & UMI deduplication

- Cell barcodes are corrected given an expected barcode list.
- Real cells — cell barcodes that represent encapsulated single cells (as opposed to ambient RNA) are also identified at this step.
- Reads are then deduplicated based on cell barcodes and UMIs.

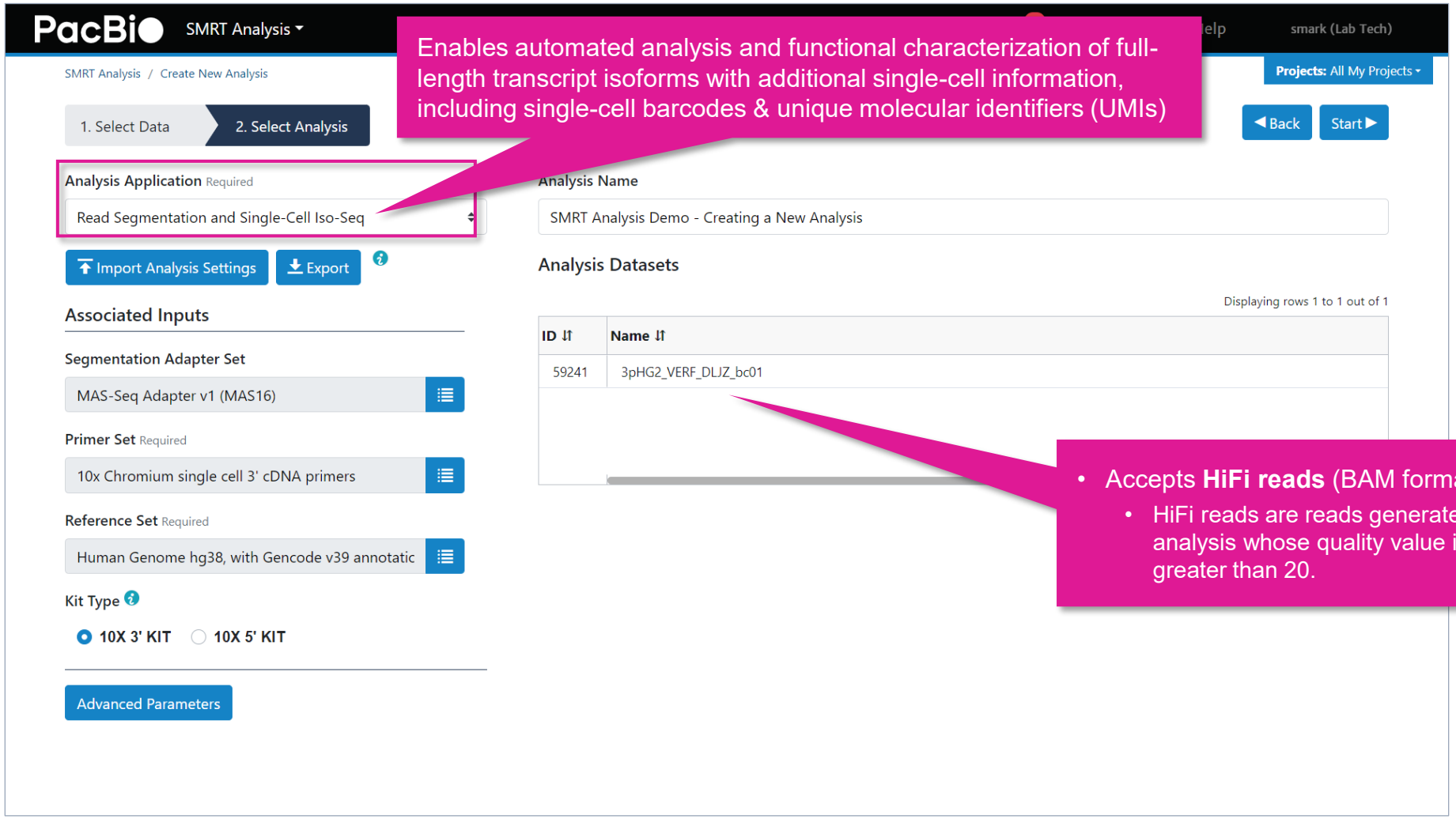
Mapping and transcript classification[2]

- Deduplicated reads are mapped to the reference genome and classified against a transcript annotation (e.g., GENCODE).
- Finally, a gene- and isoform-level single-cell matrix is output for tertiary analysis.

[1] See ***SMRT Link User Guide*** (Documentation) for detailed descriptions of parameter settings for Read Segmentation and Single-Cell Iso-Seq analysis application.
[2] Note: SMRT Link only supports transcript classification for human and mouse samples. Non-human/mouse samples will require customized annotation GTF files to be run *via* the command line.

SMRT Link Read Segmentation and Single-Cell Iso-Seq analysis application setup

Specify **Read Segmentation and Single-Cell Iso-Seq** analysis application type in SMRT Link[1]



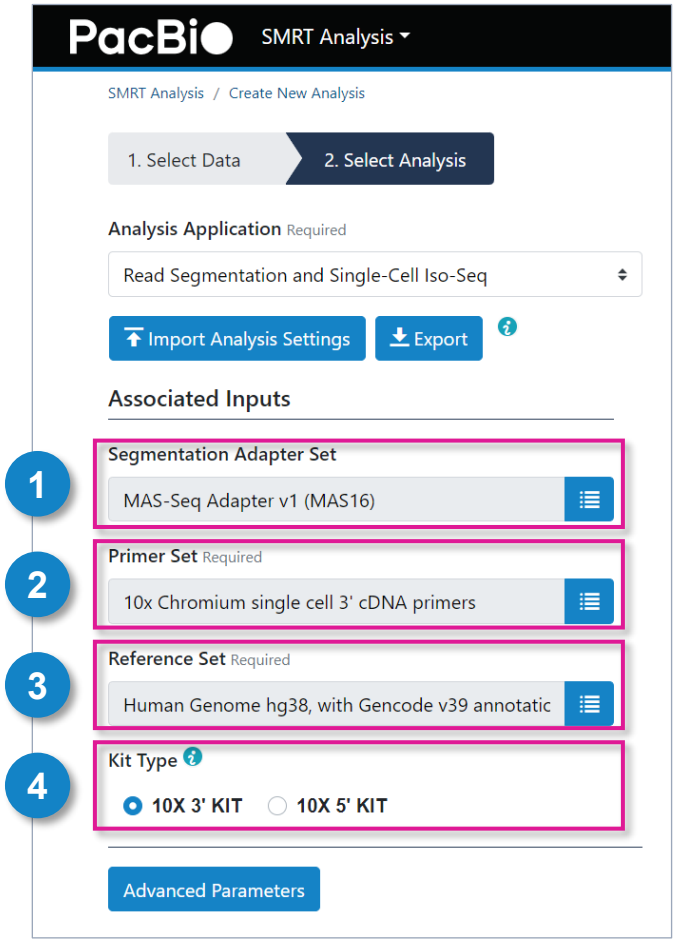
Enables automated analysis and functional characterization of full-length transcript isoforms with additional single-cell information, including single-cell barcodes & unique molecular identifiers (UMIs)

- Accepts **HiFi reads** (BAM format) as input
  - HiFi reads are reads generated with CCS analysis whose quality value is equal to or greater than 20.

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

SMRT Link Read Segmentation and Single-Cell Iso-Seq analysis application setup (cont.)

Specify **Read Segmentation and Single-Cell Iso-Seq** analysis application required associated inputs[1]



1. Segmentation Adapter Set (Required)

- Specify a FASTA file, provided by PacBio, containing segmentation adapters. If you need a custom segmentation adapter set, click Advanced Parameters and use a custom FASTA file formatted as described in the SMRT Link User Guide documentation.

2. Primer Set (Required)

- Specify a primer sequence file in FASTA format to identify cDNA primers for removal. The primer sequence includes the 5' and 3' cDNA primers.
- Primer IDs must be specified using the suffix `_5p` to indicate 5' cDNA primers and the suffix `_3p` to indicate 3' cDNA primers. The 3' cDNA primer should not include the Ts and is written in reverse complement.
- Each primer sequence must be unique.

3. Reference Set (Required)

- Specify one of two default reference genome and annotation sets to align high quality isoforms to, and to collapse isoforms mapped to the same genomic loci. The default sets are `Human_hg38_Gencode_v39` and `Mouse_mm39_Gencode_vM28`.

4. Segmentation Adapter Set (Required)

- Specify the 10x 3' Kit, or 10x 5' Kit. This determines which set of 10x primers and barcode sequences to use, and also affects the UMI and single-cell barcode design settings.

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

Example SMRT Link Read Segmentation data utility processing results[1] for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA

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

Summary Metrics

Value	Analysis Metric
9,709,186	Reads
152,811,229	Segmented reads (S-reads)
928	Mean length of S-reads
94.89 %	Percent of reads with full arrays
15.74	Mean array size (concatenation factor)

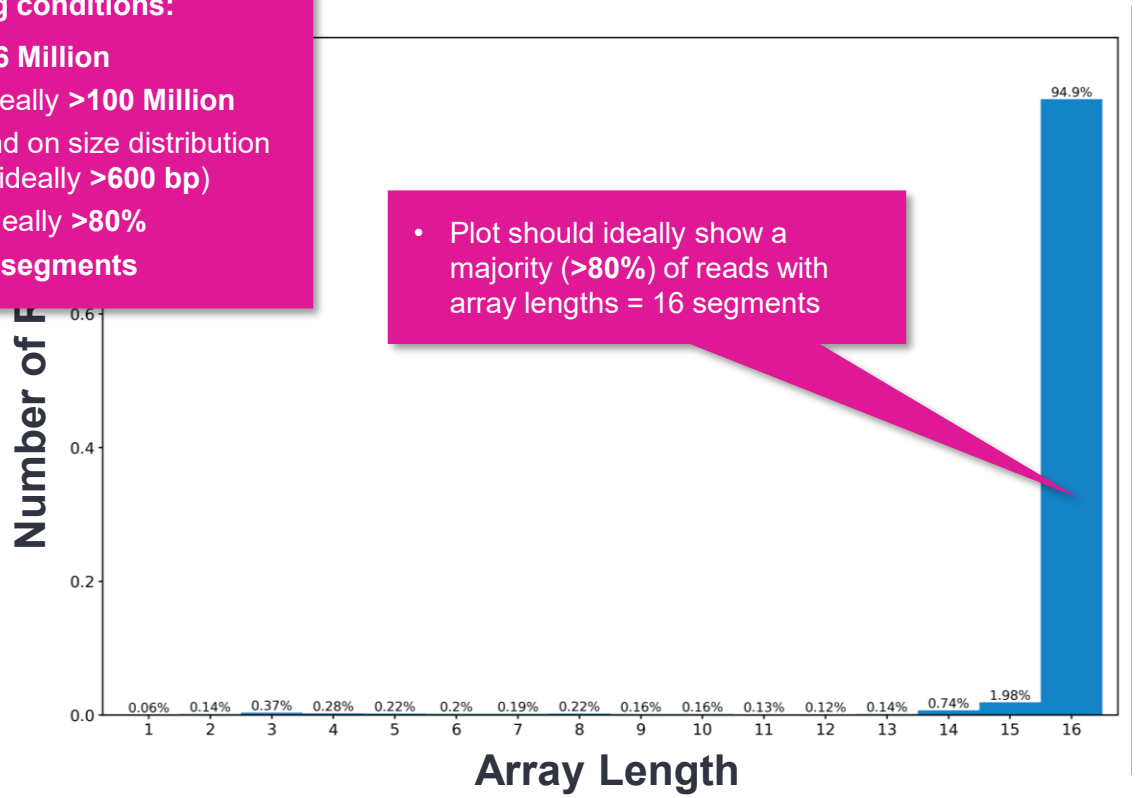
Example Revio system (+SPRQ) data shown.

For Revio Kinnex single-cell RNA libraries under optimal sample loading conditions:

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>100 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

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

Segmentation Statistics



- Plot should ideally show a majority (**>80%**) of reads with array lengths = 16 segments

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

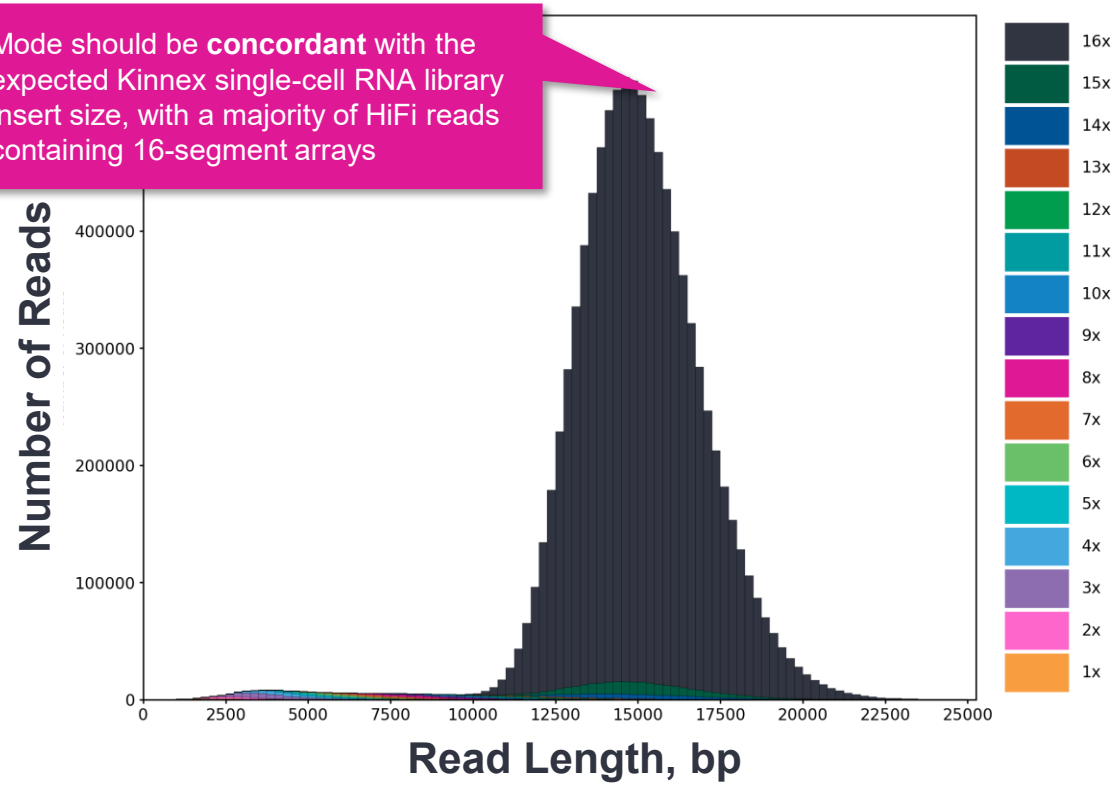
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Refer to **SMRT Link Kinnex single-cell RNA troubleshooting guide** (102-994-400) for example performance metrics typically achievable with Kinnex single-cell RNA single-cell libraries under optimal sample loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex single-cell RNA libraries.

Example SMRT Link Read Segmentation data utility processing results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

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

Length of Reads

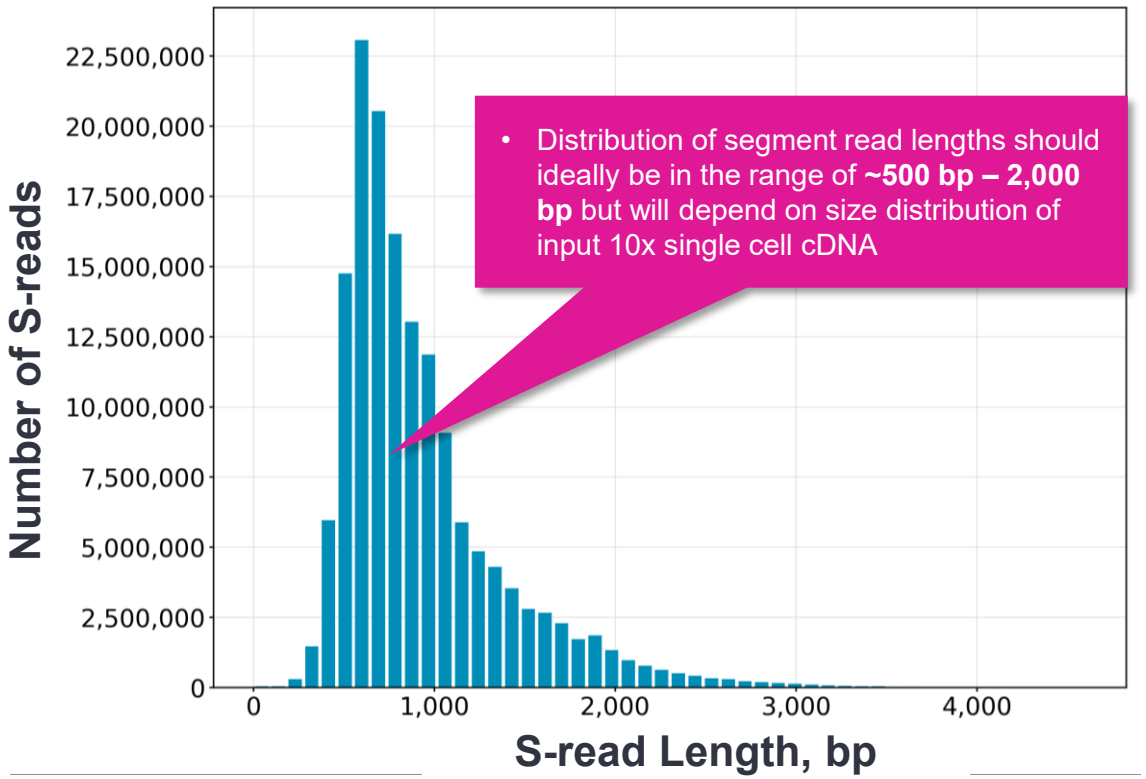
- Mode should be **concordant** with the expected Kinnex single-cell RNA library insert size, with a majority of HiFi reads containing 16-segment arrays



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

S-read Length

- Distribution of segment read lengths should ideally be in the range of **~500 bp – 2,000 bp** but will depend on size distribution of input 10x single cell cDNA



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

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Refer to **SMRT Link Kinnex single-cell RNA troubleshooting guide** (102-994-400) for example performance metrics typically achievable with Kinnex single-cell RNA single-cell libraries under optimal sample loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex single-cell RNA libraries.

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA

SMRT Link Single-Cell Iso-Seq Analysis job report – Read Statistics

Summary Metrics

Value	Analysis Metric
152,811,229	Reads
SEGMENT	Read Type
151,565,748	Reads with 5' and 3' Primers with extracted UMIs and Barcodes
147,805,910	Non-Concatamer Reads with 5' and 3' Primers and Poly-A Tail (FLNC reads)
144,807,139	FLNC Reads with Valid Barcodes
147,466,710	FLNC Reads with Valid Barcodes, corrected
110,681,049	Reads after Barcode Correction and UMI Deduplication

Example Revio system + SPRQ data shown.

- **Reads:** Total number of input reads for analysis.
- **Read Type:** Type of input reads - CCS, SEGMENT, or mixed if there are multiple input data sets with mixed data types.
- **Reads with 5’ and 3’ Primers with extracted UMIs and Barcodes:** The number of reads with 5’ and 3’ cDNA primers detected, and UMI/cell barcode information extracted. Also known as full-length tagged reads (FLT Reads).
- **Non-Concatemer Reads with 5’ and 3’ Primers and Poly-A Tail (FLNC Reads):** The number of non-concatemer reads with 5’ and 3’ primers and polyA tails detected after UMI/cell barcode information has been extracted.
- **FLNC Reads with Valid Barcodes:** Number of full-length non-concatemer reads that include valid single-cell barcodes.
- **FLNC Reads with Valid Barcodes, corrected:** Number of full-length non-concatemer reads that include valid single-cell barcodes, after barcode correction.
- **Reads after Barcode Correction and UMI Deduplication:** Number of deduplicated reads, after barcode correction.

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Refer to **SMRT Link Kinnex single-cell RNA troubleshooting guide** (102-994-400) for example performance metrics typically achievable with Kinnex single-cell RNA single-cell libraries under optimal sample loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex single-cell RNA libraries.

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

SMRT Link Single-Cell Iso-Seq Analysis job report – Cell Statistics

Summary Metrics

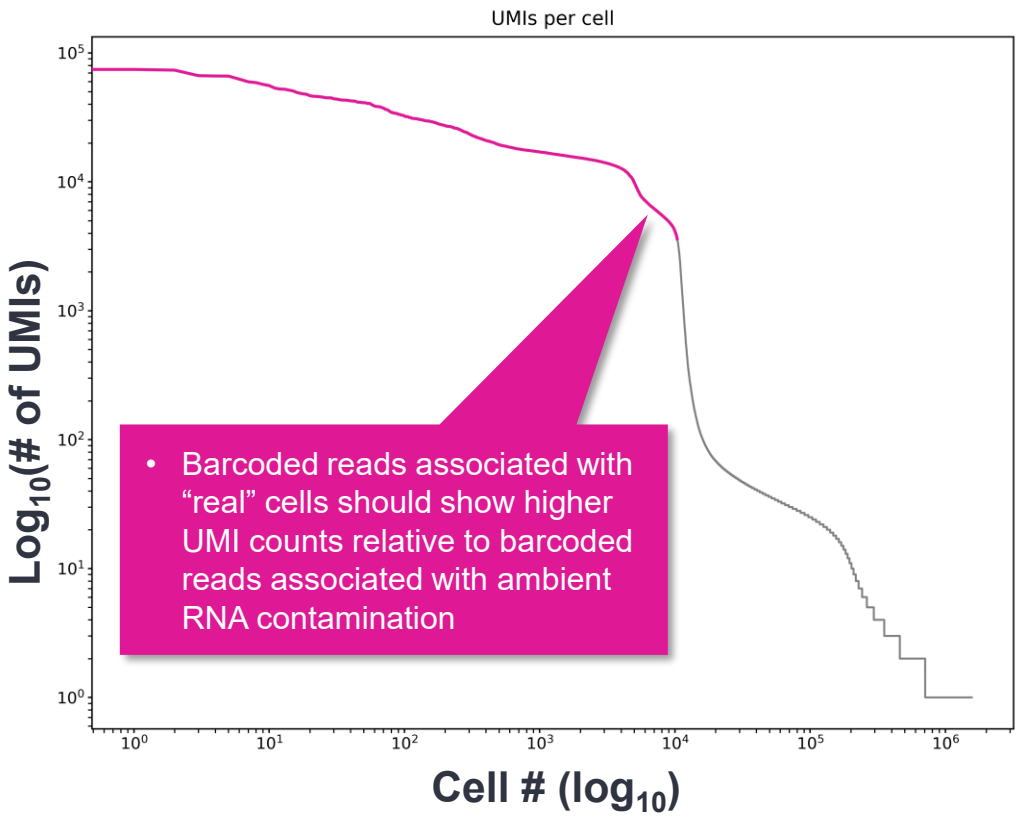
Value	Analysis Metric
10,324	Estimated Number of Cells
90.65%	Reads in Cells
12,978	Mean Reads per Cell
9,194	Median UMIs per Cell

Example Revio system + SPRQ data shown.

- There is no "correct" number of cells – this metric depends on what was specified in the 10x Chromium single cell workflow as the intended target cell recovery

- **Estimated Number of Cells:** The estimated number of cells.
- **Reads in Cells:** The percentage of reads in cells.
- **Mean Reads per Cell:** The mean number of reads per cell.
- **Median UMIs per Cell:** The median number of unique molecular identifiers (UMIs) per cell.

Barcode Rank Plot



- Barcoded reads associated with "real" cells should show higher UMI counts relative to barcoded reads associated with ambient RNA contamination

Displays the distribution of UMI counts and which barcodes were inferred to be associated with cells. The X-axis denotes barcodes ranked in decreasing order by UMI counts mapped to each barcode, and the Y-axis denotes the UMI count for the *N*-th ranked barcode. (Example Revio system + SPRQ data shown.)

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Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Summary Metrics

Value	Analysis Metric
120,736,189	FLNC Reads Mapped Confidently to Genome
79,264,395	FLNC Reads Mapped Confidently to Transcriptome
1,902,966	Total Unique Genes
98,700	Total Unique Genes, filtered
37,474	Total Unique Genes, known genes only
28,955	Total Unique Genes, filtered, known genes only
4,399,569	Total Unique Transcripts
999,042	Total Unique Transcripts, filtered
103,849	Total Unique Transcripts, known transcripts only
93,952	Total Unique Transcripts, filtered, known transcripts only

Example Revio system + SPRQ data shown.

- **FLNC reads mapped confidently to genome:** The number of FLNC reads mapped to the reference genome. This number is calculated first based on the number of deduplicated reads mapped to the genome, then expanded to account for duplicate FLNC reads for each unique molecule.
- **FLNC reads mapped confidently to transcriptome:** The number of FLNC reads mapped to the reference genome in which the read is later associated with a transcript that is classified as one of the following: FSM, ISM, NIC, or NNC.
- **Total unique genes:** The total number of unique genes across all cells.
- **Total unique genes, filtered:** The total number of unique genes, after filtering out reads based on the SQANTI transcript filtering criteria.
- **Total unique genes, known genes only:** The total number of unique genes across all cells in which the gene is annotated in the reference annotation.
- **Total unique genes, filtered, known genes only:** The total number of unique genes (genes annotated in the reference annotation) across all cells, after filtering out reads based on the SQANTI transcript filtering criteria.
- **Total unique transcripts:** The total number of unique transcripts across all cells.
- **Total unique transcripts, filtered:** The total number of unique transcripts across all cells, after filtering out reads based on the SQANTI transcript filtering criteria.
- **Total unique transcripts, known transcripts only:** The total number of unique transcripts across all cells in which the gene the transcript belongs to is annotated in the reference annotation.
- **Total unique transcripts, filtered, known transcripts only:** The total number of unique transcripts across all cells, after filtering out reads based on the SQANTI transcript filtering criteria. Only transcripts associated with known genes (genes annotated in the reference annotation) are included.

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Refer to **SMRT Link Kinnex single-cell RNA troubleshooting guide** (102-994-400) for example performance metrics typically achievable with Kinnex single-cell RNA single-cell libraries under optimal sample loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex single-cell RNA libraries.

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Transcript Summary

Value	Analysis Metric
3,369	Median Genes per Cell
2,476	Median Genes per Cell, known genes only
4,522	Median Transcripts per Cell
2,145	Median Transcripts per Cell, known transcripts only
1,902,966	Total Unique Genes
37,474	Total Unique Genes, known genes only
4,399,569	Total Unique Transcripts
103,849	Total Unique Transcripts, known transcripts only

Filter out reads based on the SQANTI3 transcript filtering criteria[1]

Example Revio system + SPRQ data shown.

Transcript Summary, Filtered

Value	Analysis Metric
1,822	Median Genes per Cell
1,787	Median Genes per Cell, known genes only
2,389	Median Transcripts per Cell
1,839	Median Transcripts per Cell, known transcripts only
98,700	Total Unique Genes
28,955	Total Unique Genes, known genes only
999,042	Total Unique Transcripts
93,952	Total Unique Transcripts, known transcripts only

- **Median genes per cell:** The median number of genes per cell.
- **Median genes per Cell, known genes only:** The median number of unique, known genes (genes annotated in the reference annotation) per input cell.
- **Median transcripts per cell:** The median number of transcripts per cell.
- **Median transcripts per cell, known transcripts only:** The median number of transcripts per cell. Only transcripts associated with known genes are included.
- **Total unique genes:** The total number of unique genes across all cells.
- **Total unique genes, known genes only:** The total number of unique, known genes (genes annotated in the reference annotation) across all cells.
- **Total unique transcripts:** The total number of unique transcripts across all cells.
- **Total unique transcripts, known transcripts only:** The total number of unique transcripts across all cells. Only transcripts associated with known genes are included.

[1] Refer to the ***SMRT Link User Guide*** (Documentation) for further details regarding filtering criteria based on the SQANTI3 software.

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Transcript Classification, filtered (All samples)

Category	Count	CAGE Detected	CAGE Detected, (%)	polyA Detected	polyA Detected, (%)
FSM	167573	103997	62.06%	58617	34.97%
ISM	314765	158530	50.36%	111958	35.56%
NIC	273808	190384	69.53%	109290	39.91%
NNC	514400	345302	67.12%	232608	45.21%
Antisense	17604	2071	11.76%	9938	56.45%
Fusion	16945	10215	60.28%	8019	47.32%
More junctions	282	158	56.02%	127	45.03%
Genic intron	0	0	0.00%	0	0.00%
Genic genomic	18361	9601	52.29%	7788	42.41%
Intergenic	63690	2479	3.89%	43701	68.61%

Example Revio system + SPRQ data shown.[1]

- **Category:** Transcript classification[2] assigned by the classification and filtering tool `pigeon`, based on the SQANTI3 software
- **Count:** The number of transcripts, after filtering out reads based on the SQANTI filtering criteria, in a specific classification
- **CAGE Detected:** The number of transcripts where the transcription start site falls within 50 bp of an annotated CAGE (Cap Analysis of Gene Expression) peak site
- **CAGE Detected, (%):** The percentage of transcripts where the transcription start site falls within 50 bp of an annotated CAGE peak site
- **polyA Motif Detected:** The number of transcripts where a known polyA motif is detected upstream of the transcription end site
- **polyA Motif Detected, (%):** The percentage of transcripts where a known polyA motif is detected upstream of the transcription end site

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[1] **Note:** Unfiltered transcript classification data are also displayed in the Iso-Seq analysis job report.
[2] Refer to the ***SMRT Link User Guide*** (Documentation) for descriptions of transcript classification categories (e.g., FSM – Full splice match, ISM – Incomplete splice match, etc.).

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

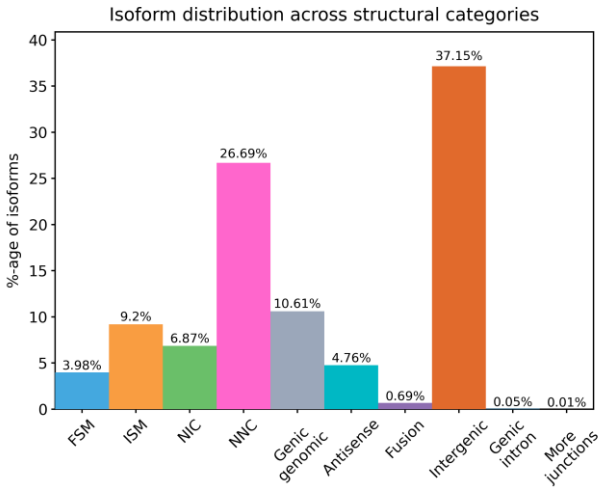
SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Transcript Classification Plots

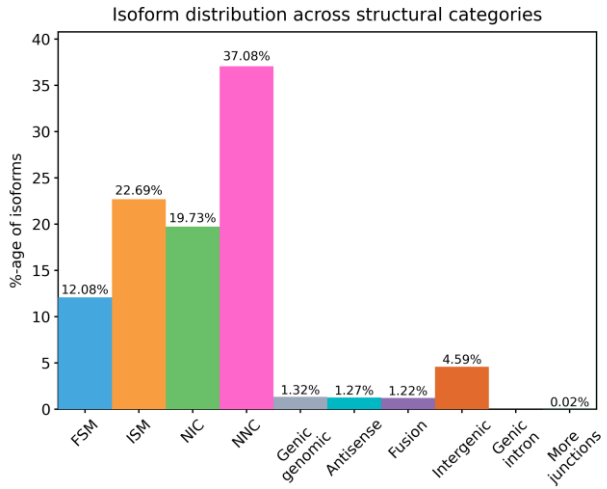
Transcript Classification Plots, Filtered

- **Isoform distributions across structural categories:**
  - Distribution of the % of isoforms by structural categories.

Example Revio system + SPRQ data shown.

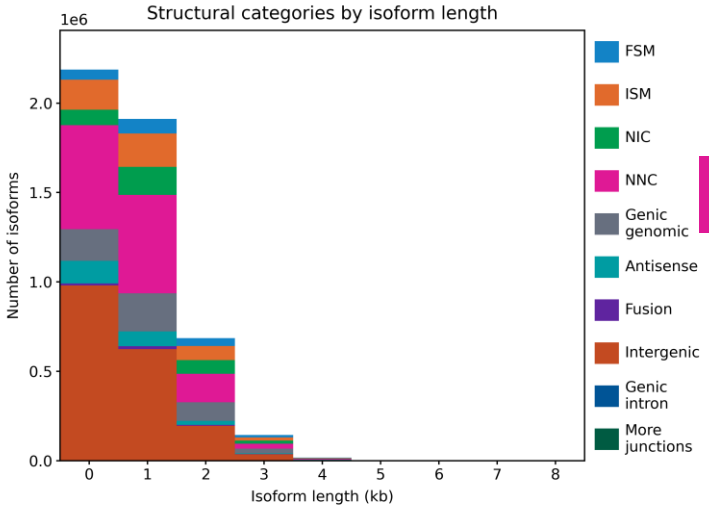


Filter out reads based on the SQANTI3 transcript filtering criteria

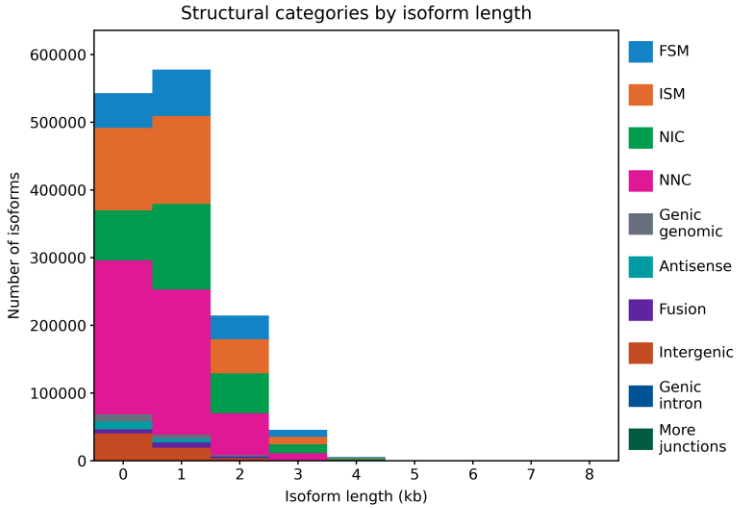


- **Structural categories by isoform lengths:**
  - Histogram display of the number of isoforms by their length in kb and their structural category.

Example Revio system + SPRQ data shown.



Filter out reads based on the SQANTI3 transcript filtering criteria



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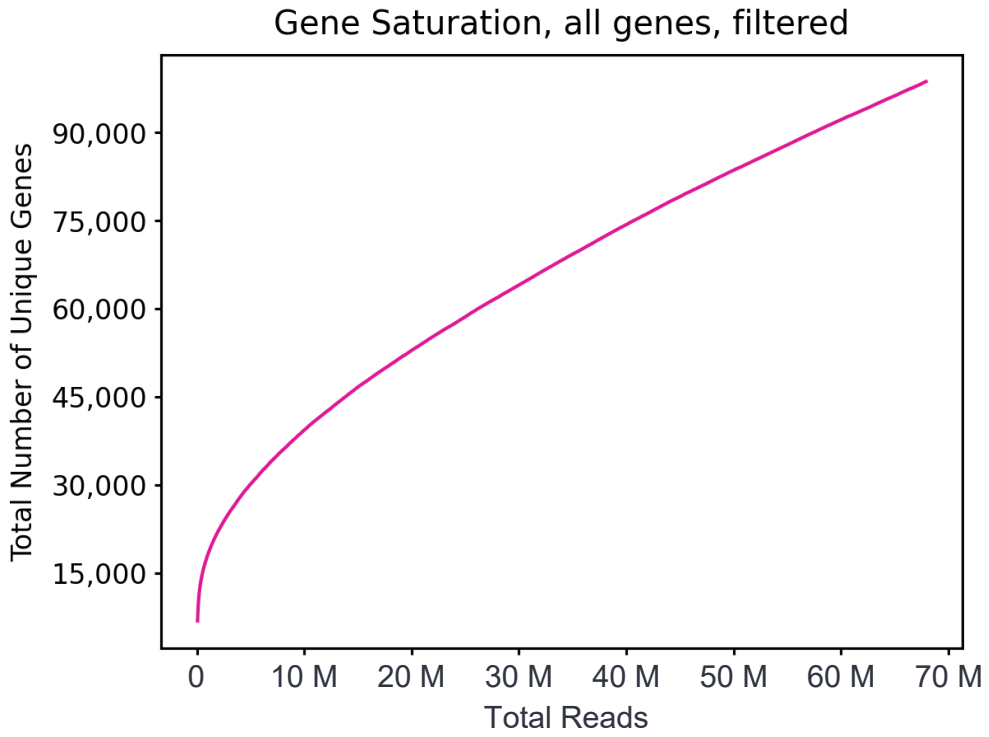
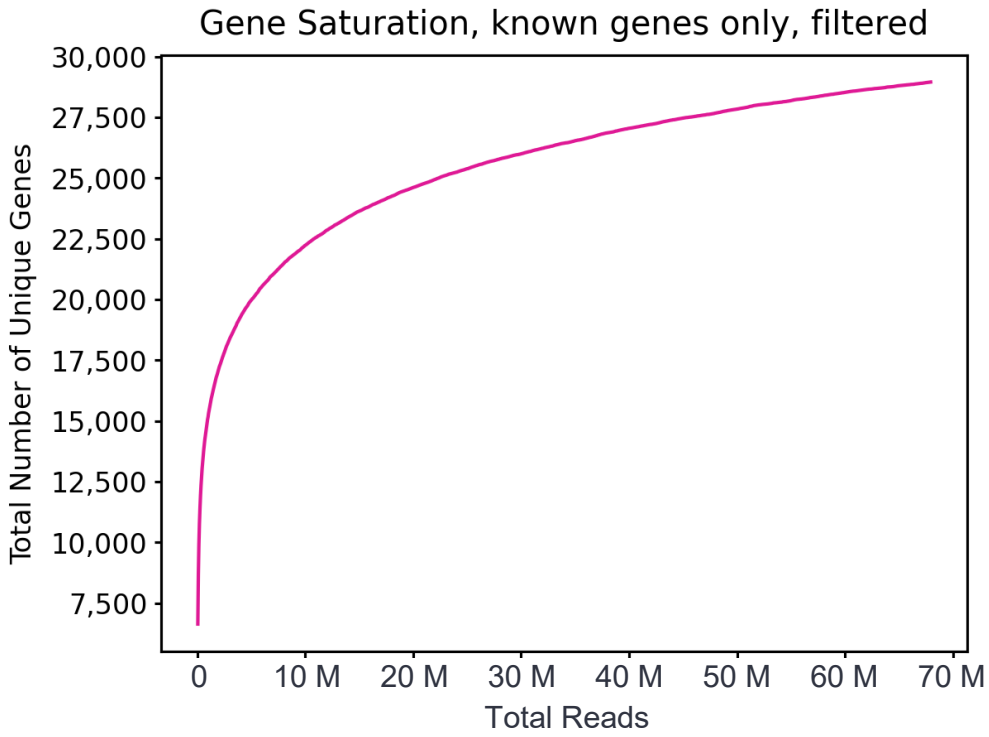
Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Gene Saturation





Gene Saturation, all genes, filtered: Saturation plot showing the level of gene saturation for all genes, after filtering out reads based on the SQANTI transcript filtering criteria

Gene Saturation, known genes only, filtered: Saturation plot showing the level of gene saturation, for unique known genes only (genes annotated in the reference annotation) per cell, after filtering out reads based on the SQANTI transcript filtering criteria

Example Revio system + SPRQ data shown.

Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

File downloads tab

Edit Output File Name Prefix **Example:**analysis-Bio Sample 3-78360

File	Size	Type
Non-passing reads, unaligned	4 GB	bam
Report read_segmentation	3 KB	JsonReport
SMRT Link Log	14 KB	log
Segmented Reads, passing, unaligned	57 GB	bam
Single-cell isoform and gene matrix, tar-gzipped	1 GB	tgz
Unique mapped transcripts, GFF	1 GB	gff
Unique mapped transcripts, classification TXT	788 MB	txt
Unique mapped transcripts, filtered, GFF	481 MB	gff
Unique mapped transcripts, filtered, classification TXT	246 MB	txt
Unique mapped transcripts, filtered, junctions TXT	451 MB	txt
Unique mapped transcripts, junctions TXT	785 MB	txt

Files shown in the File Downloads tab are available on the analysis results page. Additional files are also available on the SMRT Link server in the analysis output directory.

Refer to SMRT Link user guide for descriptions of downloadable output files

- **Key output file!**
- Gzipped file containing Seurat-compatible isoform and gene matrix files

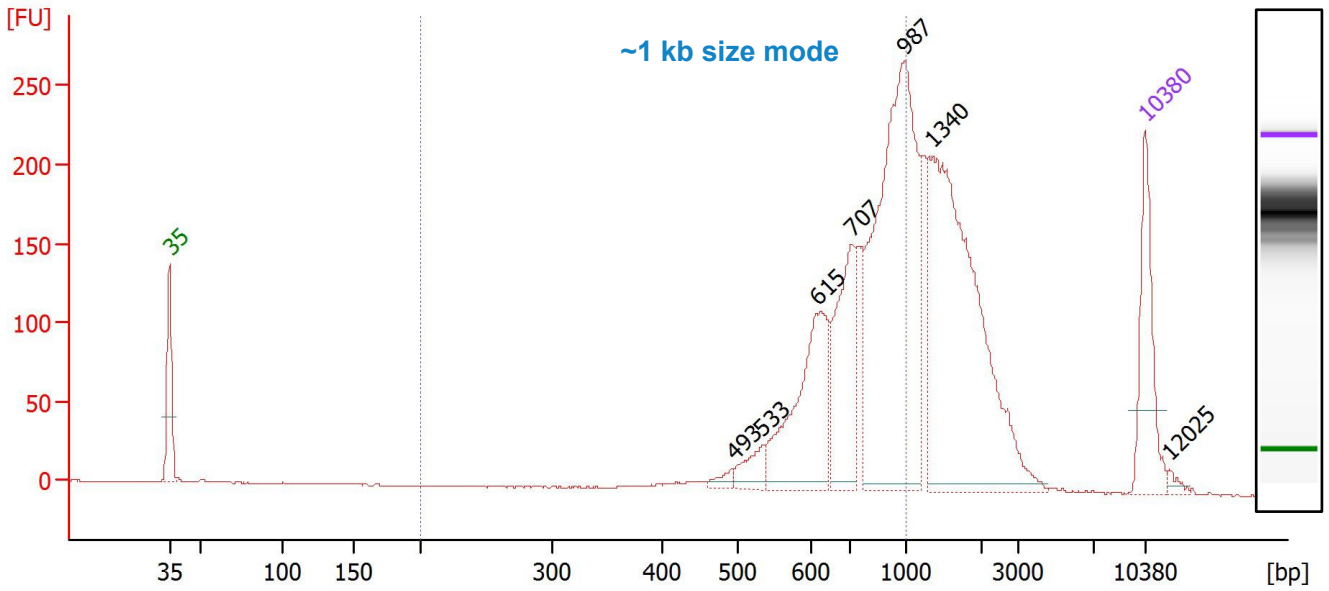
- These files are useful for **visualizing** isoform structures in Integrative Genomics Viewer (IGV) / UCSC genome browser and enable understanding of why an isoform is novel/known, etc.
  - GFF file containing unique mapped transcripts after filtering
  - Text file containing unique mapped transcript classifications against annotations, after filtering
  - Text file containing information about unique mapped transcript junctions, after filtering

Kinnex single-cell RNA library example sequencing performance data

Example Kinnex 16-fold single-cell RNA library preparation QC results

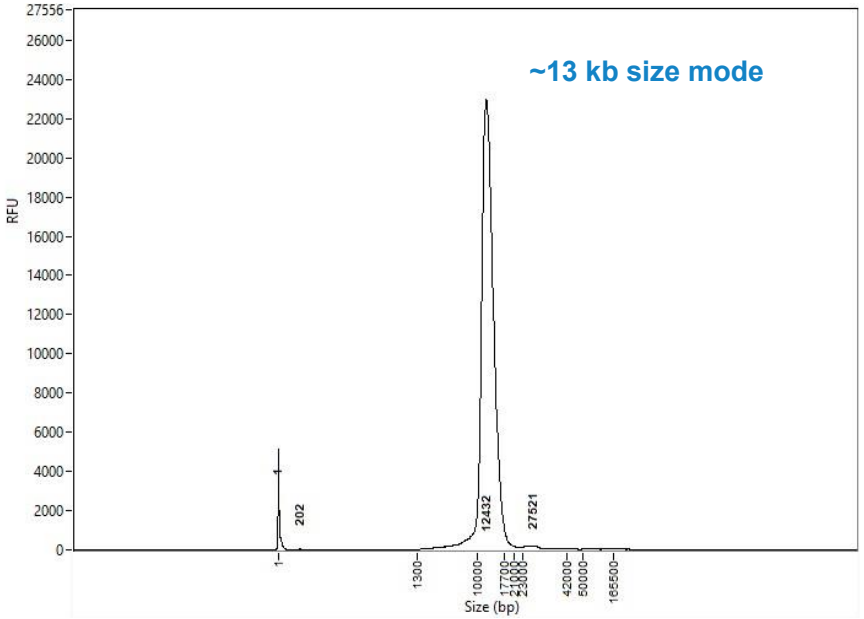
Kinnex single-cell 3’ RNA library prepared with human cDNA

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



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

Final Kinnex single-cell RNA library QC



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

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

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

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

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

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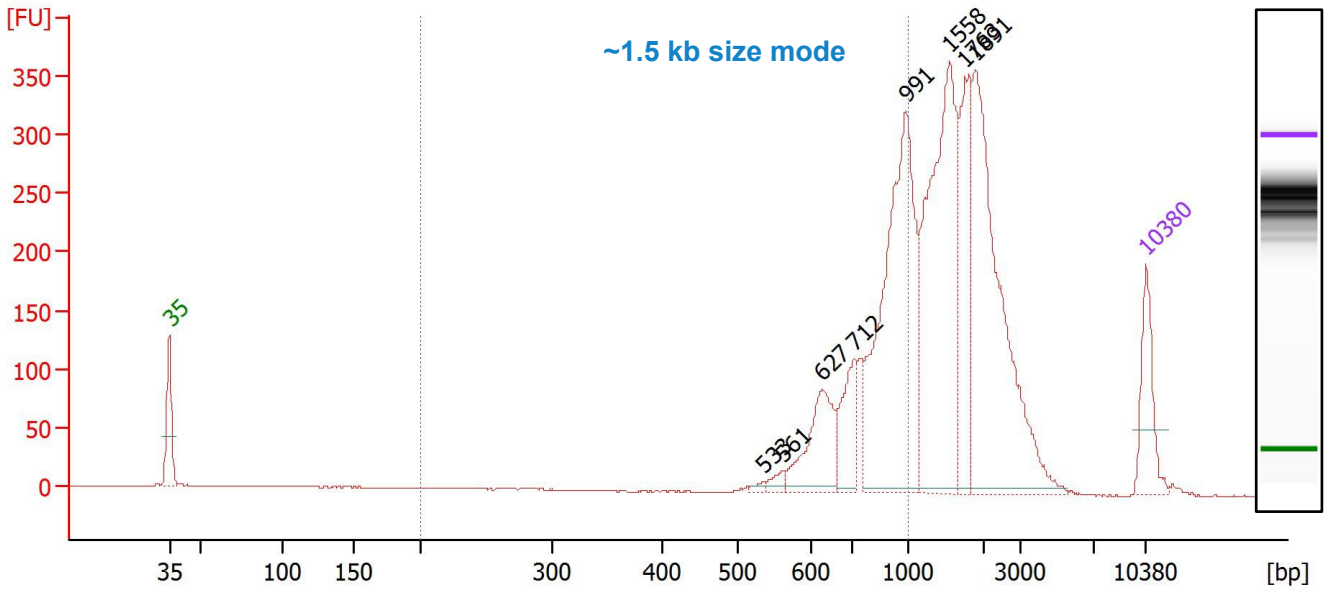
Example Kinnex 16-fold single-cell RNA library preparation QC results (cont.)

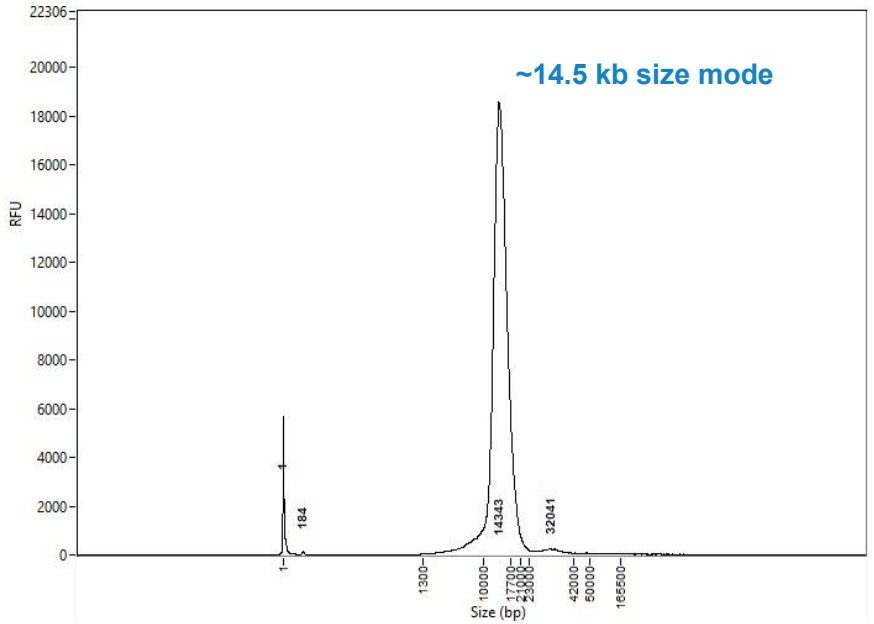
Kinnex single-cell 5’ RNA library prepared with human cDNA

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



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

Final Kinnex single-cell RNA library QC



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

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

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

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

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

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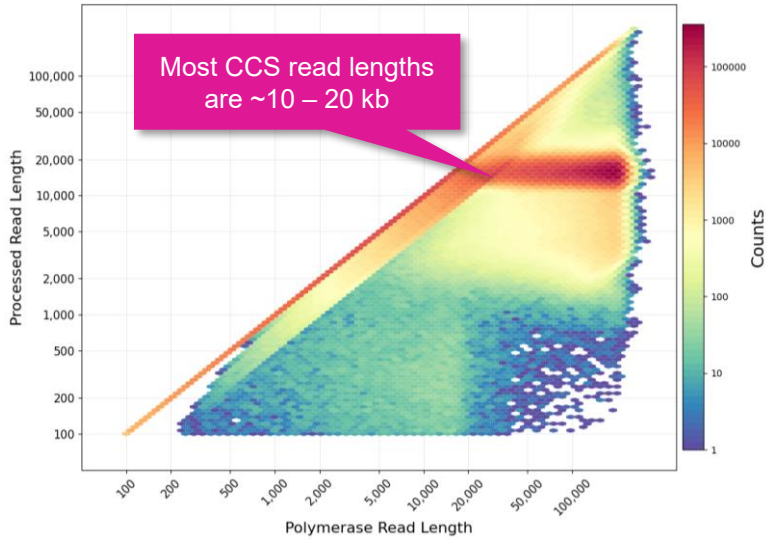
Example Kinnex 16-fold single-cell RNA sequencing results [Revio system[1]]

Kinnex 16-fold single-cell RNA library prepared from PBMC single-cell cDNA isolated using 10x Chromium Single Cell Universal 5' kit

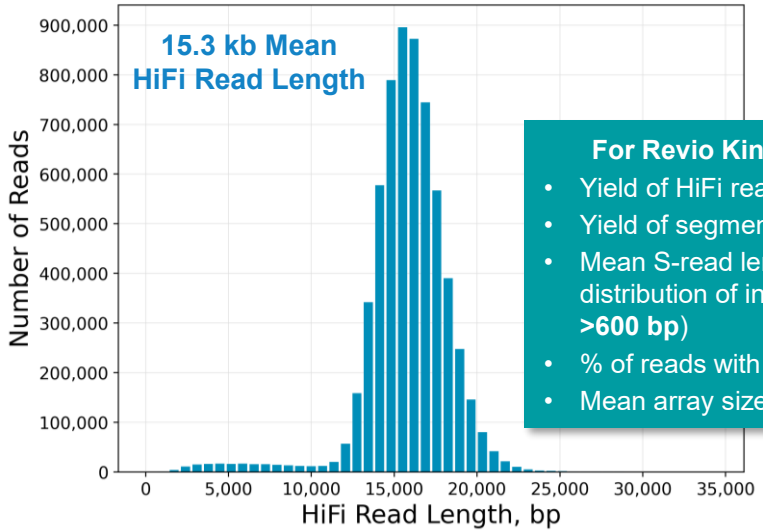
Raw Data Report



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

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

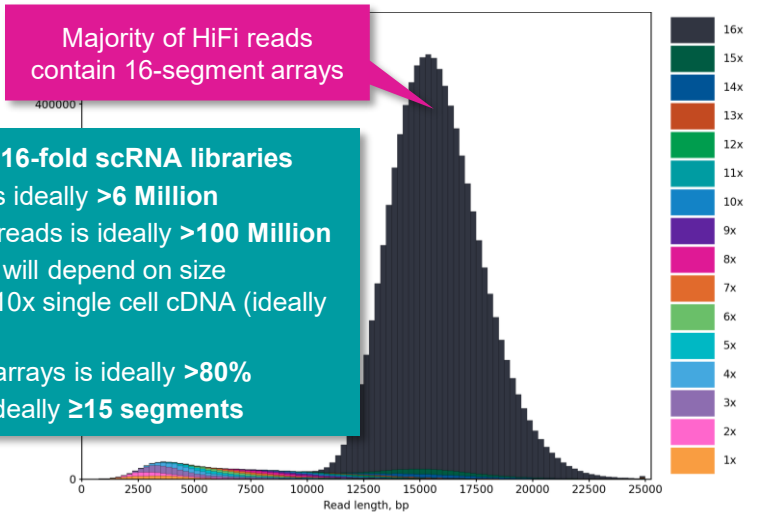
HiFi Read Length



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

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

Read Segmentation Metrics



For Revio Kinnex 16-fold scRNA libraries

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>100 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

Input HiFi Reads	9.4 M
Segmented reads (S-reads)	144.8 M
Mean length of S-reads	971 bp
Percent of reads with full arrays	92.5%
Mean array size (concentration factor)	15.3

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

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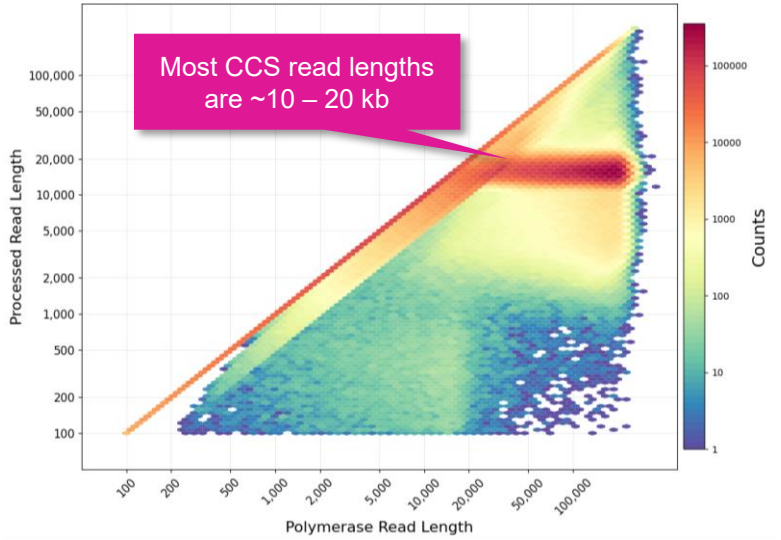
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

Example Kinnex 16-fold single-cell RNA sequencing results [Revio system[1]]

Kinnex 16-fold single-cell RNA library prepared from PBMC single-cell cDNA isolated using 10x Chromium Single Cell Universal 3' kit

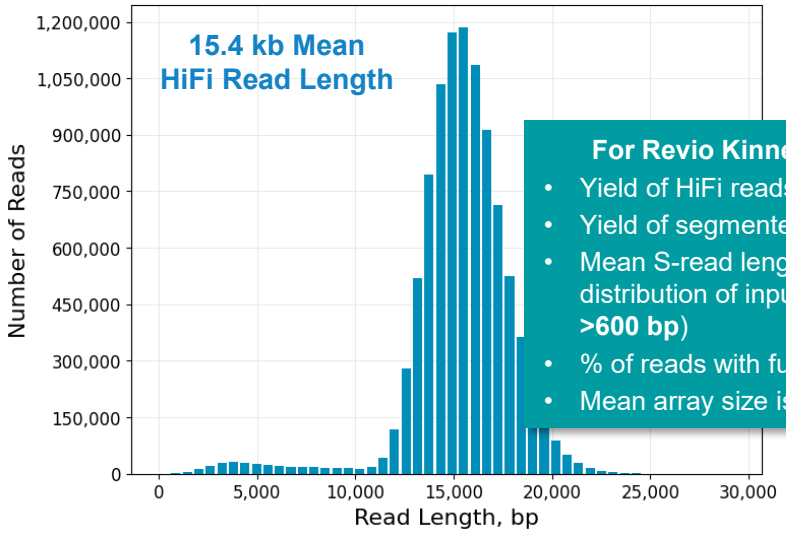
Raw Data Report



Raw Base Yield	1,570 Gb
Mean Polymerase Read Length	86.5 kb
P1	72%

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

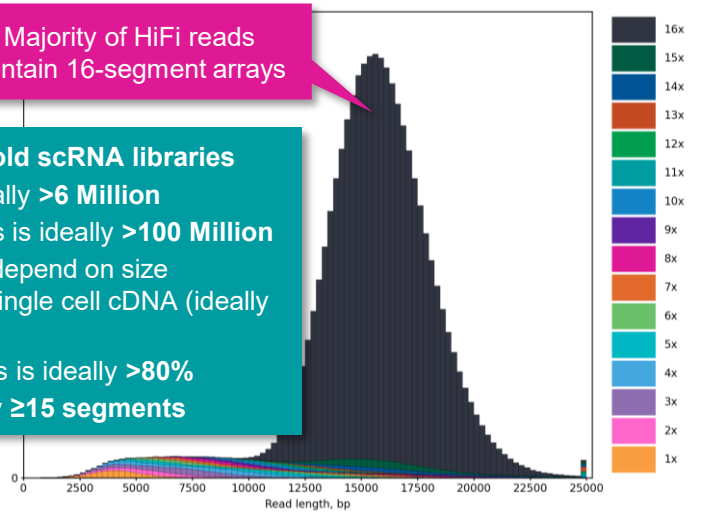
HiFi Read Length



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

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

Read Segmentation Metrics



For Revio Kinnex 16-fold scRNA libraries

- Yield of HiFi reads is ideally **>6 Million**
- Yield of segmented reads is ideally **>100 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

Input HiFi Reads	9.6 M
Segmented reads (S-reads)	149.8 M
Mean length of S-reads	963 bp
Percent of reads with full arrays	92.2%
Mean array size (concentration factor)	15.6

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

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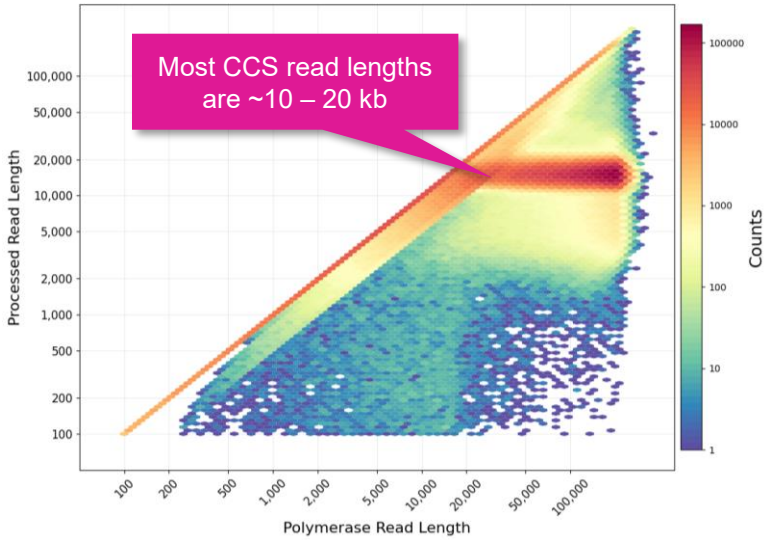
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

Example Kinnex 16-fold single-cell RNA sequencing results [Vega system[1]]

Kinnex 16-fold single-cell RNA library prepared from PBMC single-cell cDNA isolated using 10x Chromium Single Cell Universal 3' kit

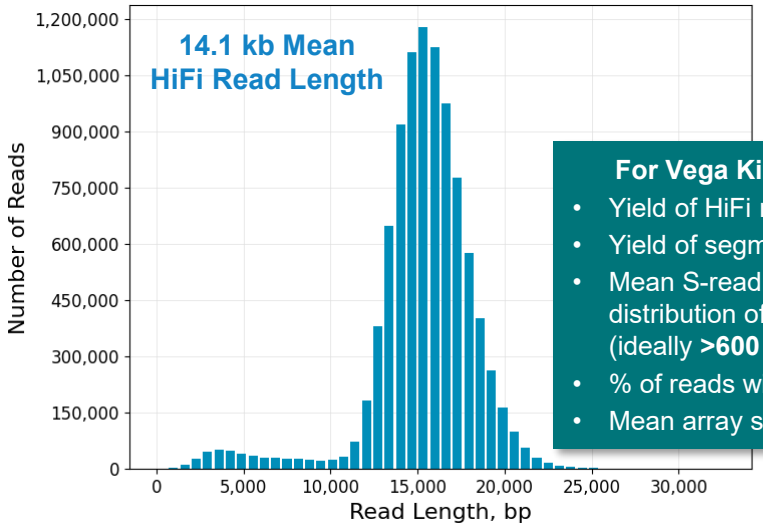
Raw Data Report



Mean Polymerase Read Length	94.7 kb
Loading Level	67%

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

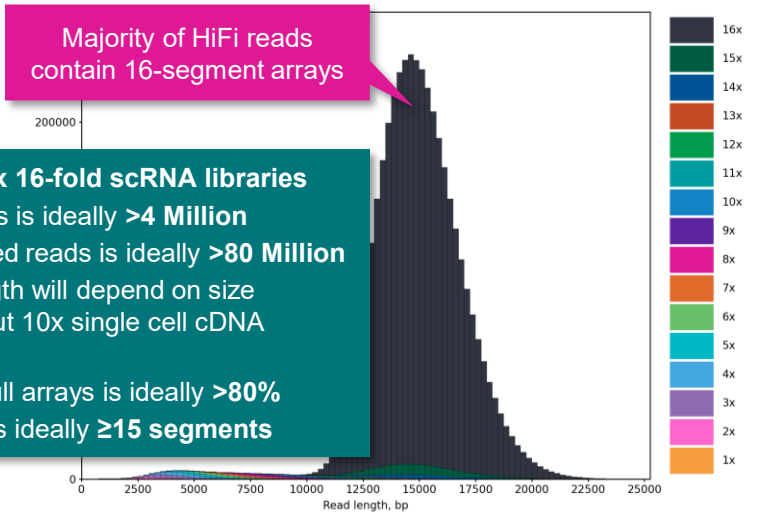
HiFi Read Length



HiFi Reads	7.1 M
HiFi Base Yield	99.9 Gb
Mean HiFi Read Length	14.1 kb
Median HiFi Read Quality	Q29
HiFi Read Mean # of Passes	9

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

Read Segmentation Metrics



For Vega Kinnex 16-fold scRNA libraries

- Yield of HiFi reads is ideally **>4 Million**
- Yield of segmented reads is ideally **>80 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

Input HiFi Reads	7.1 M
Segmented reads (S-reads)	108.1 M
Mean length of S-reads	895 bp
Percent of reads with full arrays	92.0%
Mean array size (concentration factor)	15.3

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

PacBio

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

Technical resources for Kinnex single-cell RNA library preparation, sequencing & data analysis

Technical resources for Kinnex single-cell RNA library preparation, sequencing & data analysis

Single-cell cDNA sample preparation literature & other resources

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

Kinnex library preparation literature & other resources

- Application note – Kinnex single-cell RNA for single-cell isoform sequencing (102-326-549)
- 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 16-fold prep kit (103-916-200)
- Whitepaper – Bulk and single-cell isoform sequencing for human disease research (102-326-576)

Data analysis resources

- Application note – Bioinformatics tools for full length isoform sequencing (102-326-593)
- SMRT Link Kinnex full-length RNA troubleshooting guide (103-552-100)
- SMRT Link Kinnex single-cell troubleshooting guide (103-516-100)
- SMRT Link MAS-Seq troubleshooting guide (102-994-400)
- SMRT Link software installation guide [Link]
- SMRT Link user guide [Link]
- SMRT Tools reference guide [Link]

Technical resources for Kinnex single-cell RNA library preparation, sequencing & data analysis (cont.)

Publications

- 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. BioRxiv [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) - Detecting shared mis-splicing across blood cancers using long-read single-cell 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]

Example PacBio data sets

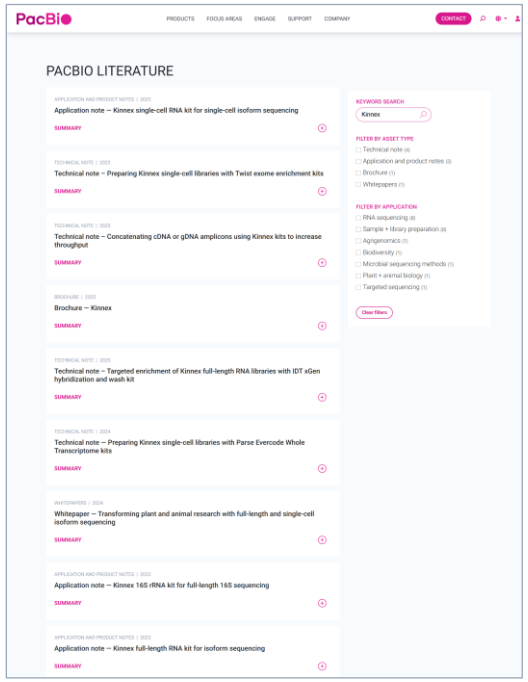
Application	Dataset	Data type	PacBio system
Kinnex single-cell RNA sequencing	Homo sapiens - PBMC 10x Chromium Single Cell 5' and 3' libraries [Link]	HiFi long read	Sequel II & Revio systems
	Homo sapiens - HG002 (10x 5') [Link]	HiFi long read	Revio system

APPENDIX 2: PacBio compatible Kinnex library preparation workflows

Preparing Kinnex single-cell libraries with ArgenTag kits

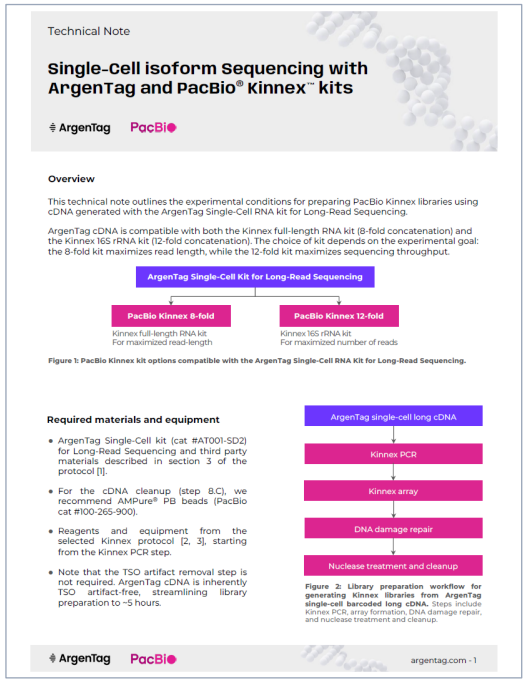
Preparing Kinnex single-cell libraries with ArgenTag kits: Getting started

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



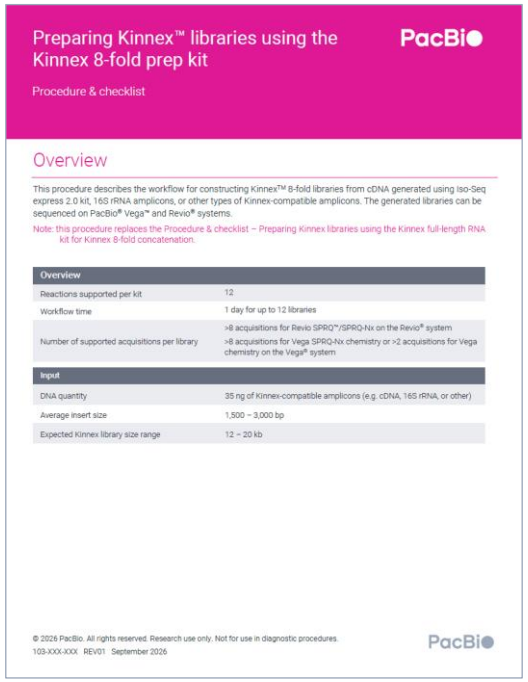
PacBio literature website [Link]

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



Technical note – 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.

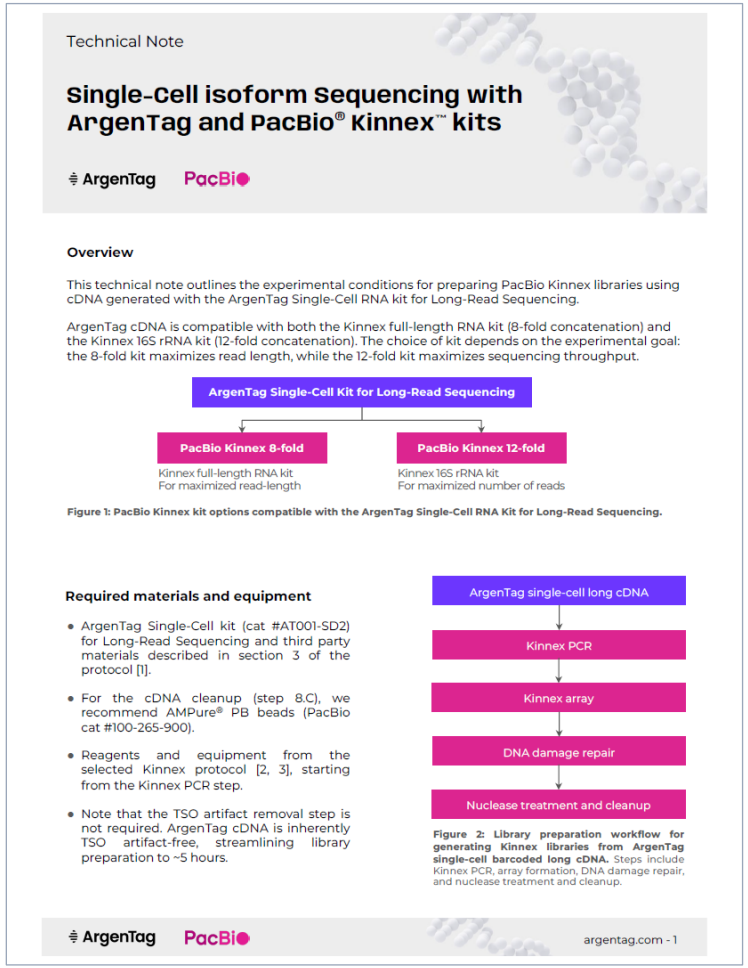
Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit (103-916-000) **or Kinnex 12-fold prep kit** (103-916-100) [PacBio]

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.

PacBio

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.

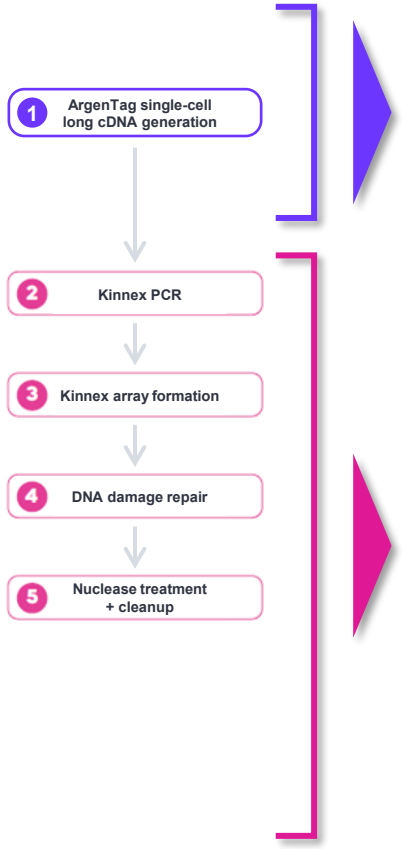
Required materials and equipment

- 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 argentag.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**

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

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

Follow ArgenTag ArgenTag Single-Cell RNA kit procedure[1] to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems

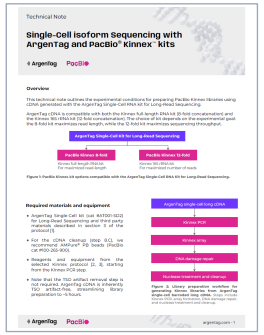
1. ArgenTag single-cell cDNA generation
2. Kinnex PCR
3. Kinnex array formation
4. DNA damage repair
5. Nuclease treatment + cleanup

ArgenTag single-cell cDNA generation procedure overview[1]

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

[ArgenTag]

ArgenTag


Technical Note

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

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 DNA kit (8-fold concatenation) and the Kinnex 12-fold kit. The Kinnex full-length DNA kit is optimized for maximum read length, while the 12-fold kit maximizes sequencing throughput.

ArgenTag Single-Cell RN for Long-Read Sequencing

PacBio Kinnex 8-fold
Kinnex full-length DNA kit
For maximum read length

PacBio Kinnex 12-fold
Kinnex 12-fold kit
For maximum 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 #A4700-1022) for Long-Read Sequencing and third party materials described in section 3 of the protocol [1].
- For the cDNA cleanup (step 3.C), we recommend PacBio Ref® 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. Single-cell cDNA is generated using ArgenTag Single-Cell kit and nuclease treatment and cleanup.

ArgenTag PacBio

ArgenTag single-cell cDNA generation procedure key steps

- Single-cell cDNA material first needs to be generated as described in the ArgenTag protocol[1] ***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**.

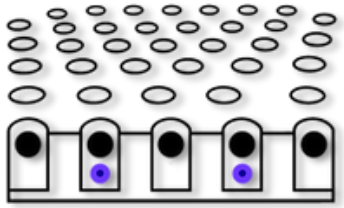
ArgenTag single-cell cDNA generation workflow[1] (Step 1)

1: Load the ArgenTag chip

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

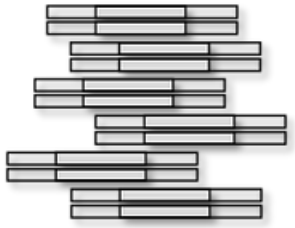
2: Capture and barcode

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.



[1] For single-cell cDNA generation procedure details, refer to ***Procedure & checklist – ArgenTag Single-Cell kit for Long-Read Sequencing handbook*** available from the ArgenTag website.

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

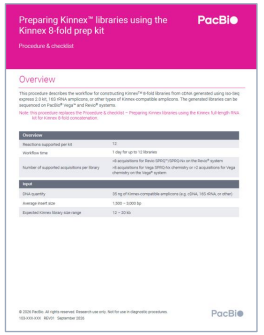
Parse single-cell cDNA samples may be used for Kinnex library construction by following PacBio *Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit* (103-254-300)

1. ArgenTag single-cell cDNA generation
2. Kinnex PCR
3. Kinnex array formation
4. DNA damage repair
5. Nuclease treatment + cleanup

PacBio Kinnex library construction procedure[1]

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

PacBio



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

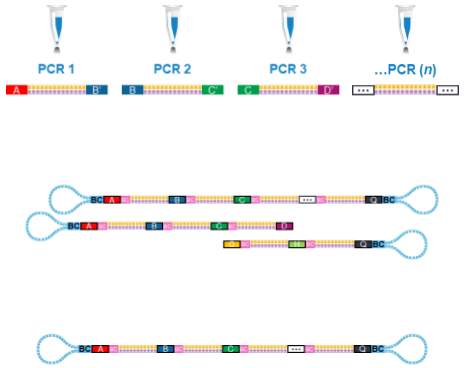
1. ArgenTag single-cell cDNA generation

→ See **ArgenTag Technical note**

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

Kinnex single-cell library construction workflow (Steps 2 – 5)

2 Kinnex PCR
↓ Safe stop
3 Kinnex array
↓ Safe stop
4 DNA repair
↓ Safe stop
5 Nuclease treatment and cleanup

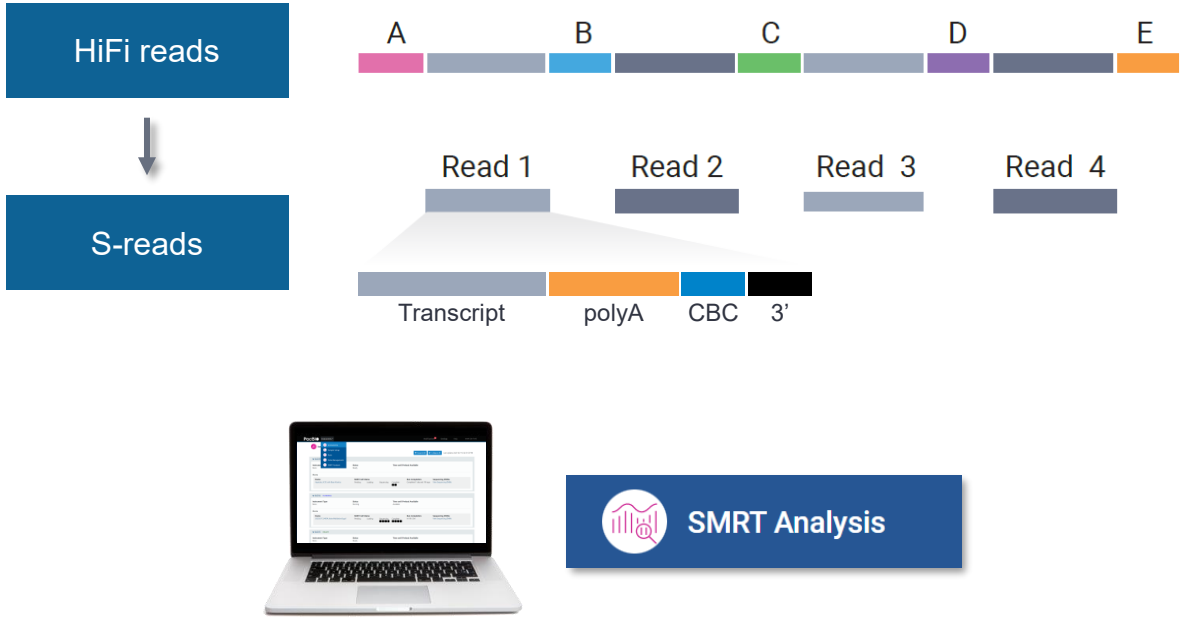


SMRT Link informatics and secondary analysis recommendations for Kinnex single-cell libraries generated with ArgenTag Single-Cell RNA kit

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



[1] Refer to ArgenTag website or ***Technical note – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits*** [ArgenTag] and ***ArgenTag single-cell read demultiplexing pipeline*** website [ArgenTag GitHub] for secondary analysis recommendations using command line and community tools.

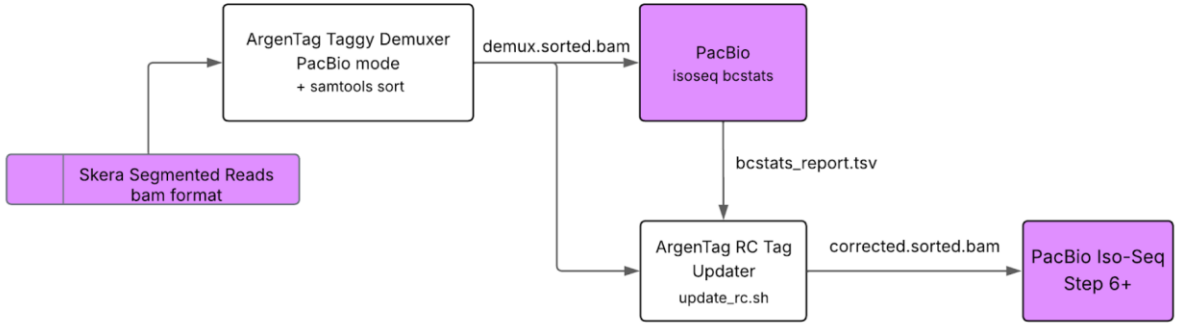
SMRT Link informatics and secondary analysis recommendations for Kinnex single-cell libraries generated with ArgenTag Single-Cell RNA kit (cont.)

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

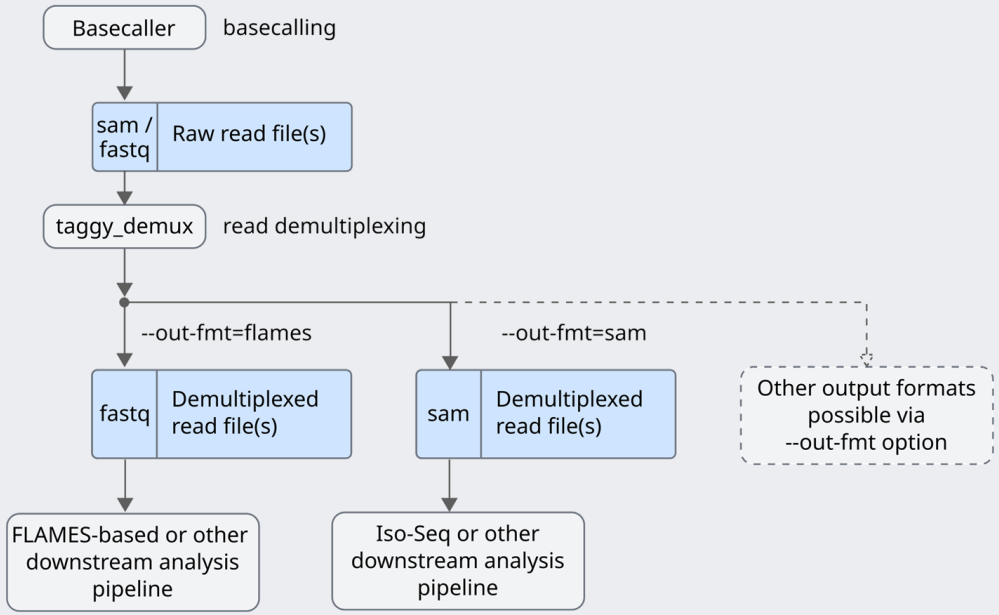
- S-reads can be demultiplexed and analyzed using the bioinformatics pipeline described below[1]:

AT taggy demux + update_rc.sh + PBIso-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

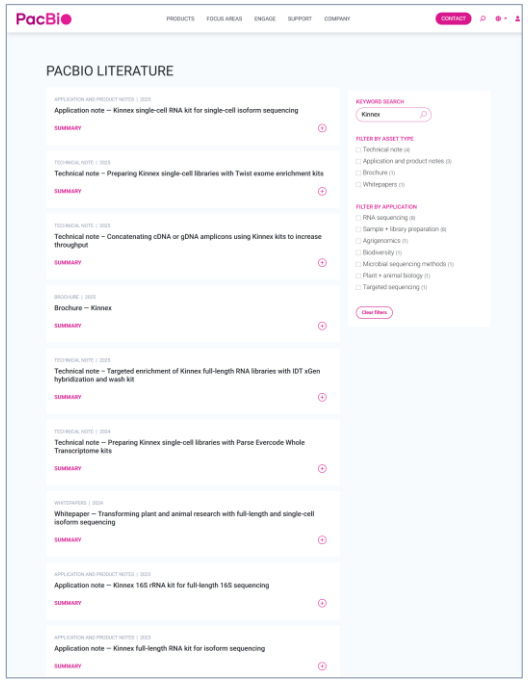


[1] Refer to ArgenTag website or ***Technical note – Single-Cell isoform Sequencing with ArgenTag and PacBio Kinnex kits*** [ArgenTag] and ***ArgenTag single-cell read demultiplexing pipeline*** website [ArgenTag GitHub] for secondary analysis recommendations using command line and community tools.

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

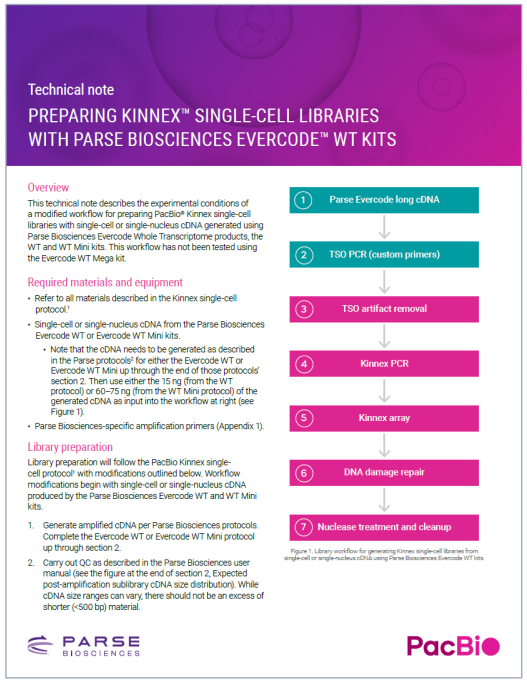
Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits: Getting started

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



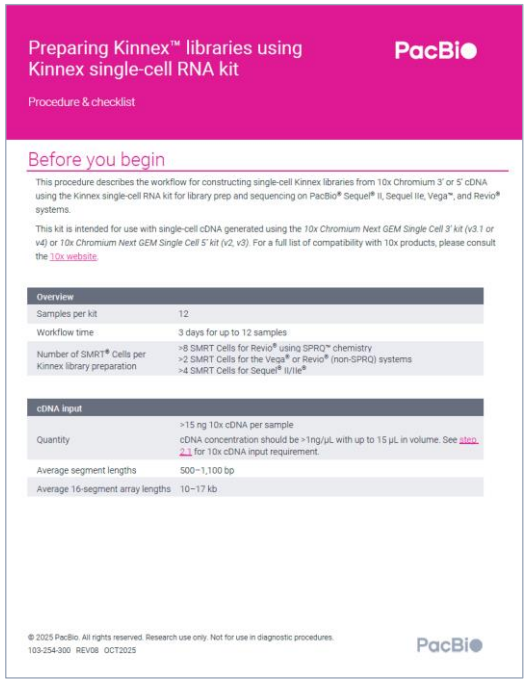
PacBio literature website [Link]

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



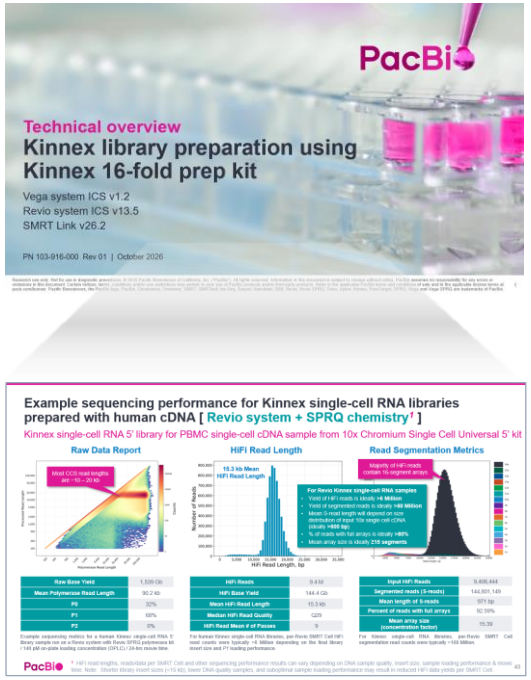
Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits (102-326-610) [PacBio]

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



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

Technical documentation containing PacBio SMRTbell library construction details.



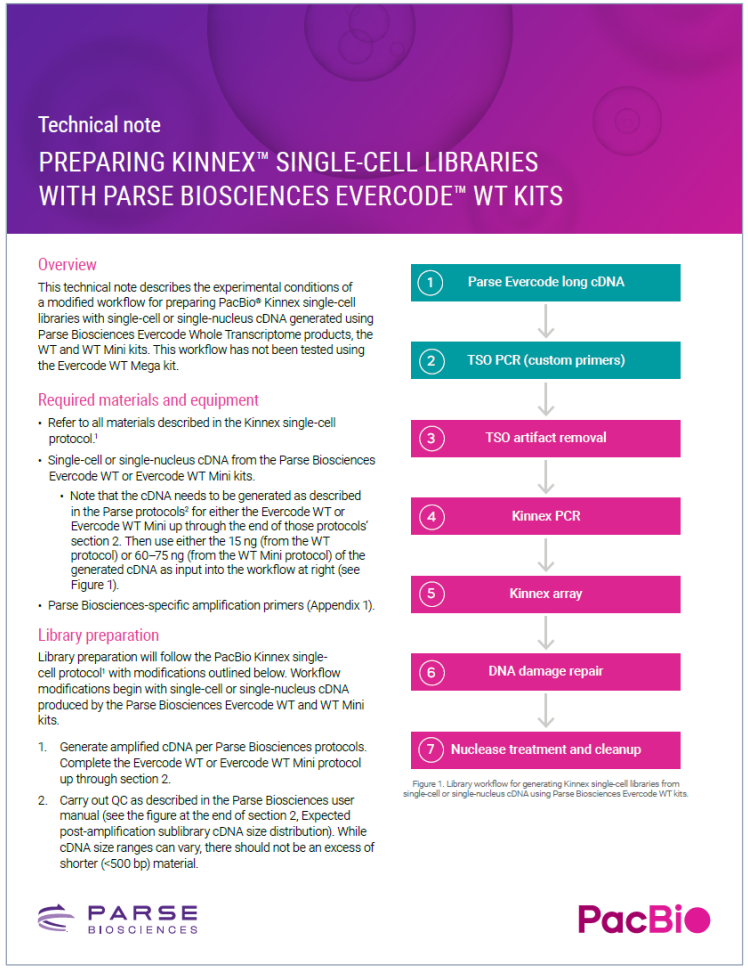
Technical overview – Kinnex library preparation using Kinnex 16-fold prep kit (103-916-200)

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.

PacBio

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

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



Technical note

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

Overview

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

Required materials and equipment

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

Library preparation

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

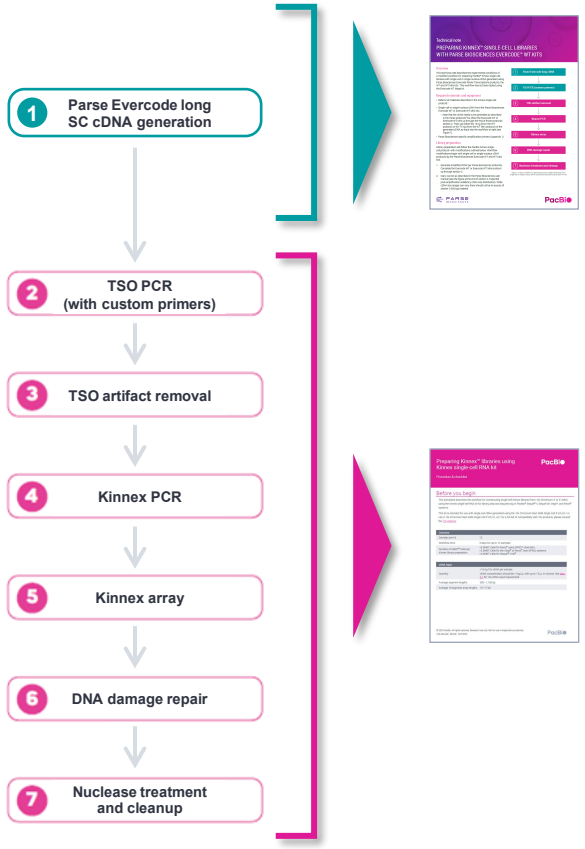
1. Generate amplified cDNA per Parse Biosciences protocols. Complete the Evercode WT or Evercode WT Mini protocol up through section 2.
2. Carry out QC as described in the Parse Biosciences user manual (see the figure at the end of section 2, Expected post-amplification sublibrary cDNA size distribution).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

1 Parse Evercode long cDNA
2 TSO PCR (custom primers)
3 TSO artifact removal
4 Kinnex PCR
5 Kinnex array
6 DNA damage repair
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.

PARSE BIOSCIENCES

PacBio



PacBio Technical note

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

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

PacBio Procedure & checklist

Preparing Kinnex libraries using Kinnex 16-fold prep kit (103-885-300)

Overview

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

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

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

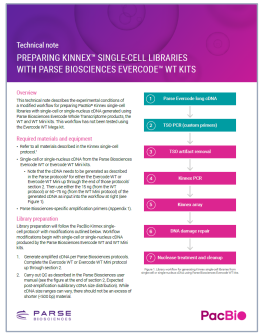
Follow Parse Bioscience whole transcriptome kit procedure[1] to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems

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

Parse single-cell cDNA generation procedure overview[1]

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

PacBio



Parse single-cell cDNA generation procedure key steps

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

Evercode WT User Guides
UMWT3300 or UMWT3100

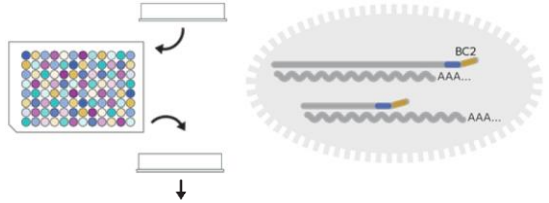
- Use either 15 ng (from the WT protocol) or 60–75 ng (from the WT Mini protocol) of the generated cDNA as input into the **PacBio Kinnex 16-fold prep kit protocol** (103-885-300) starting at **Step 2: TSO PCR (with custom primers[2])**.

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

Section 1: In situ cell / nuclei barcoding

Barcoding round 1

Barcoding round 2

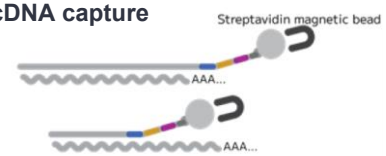


Barcoding round 3

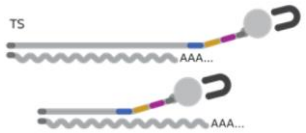
Lysis and Sublibrary Generation

Section 2: cDNA capture and amplification

cDNA capture



cDNA template switch



cDNA amplification

PacBio

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

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

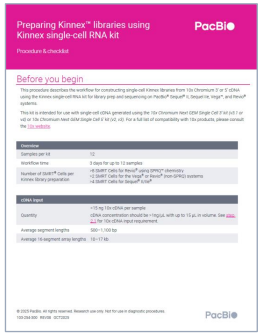
Parse single-cell cDNA samples may be used for Kinnex library construction by following PacBio *Procedure & checklist – Preparing Kinnex libraries using the Kinnex 16-fold prep kit* (103-885-300)

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

PacBio Kinnex library construction procedure[1]

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

PacBio



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]

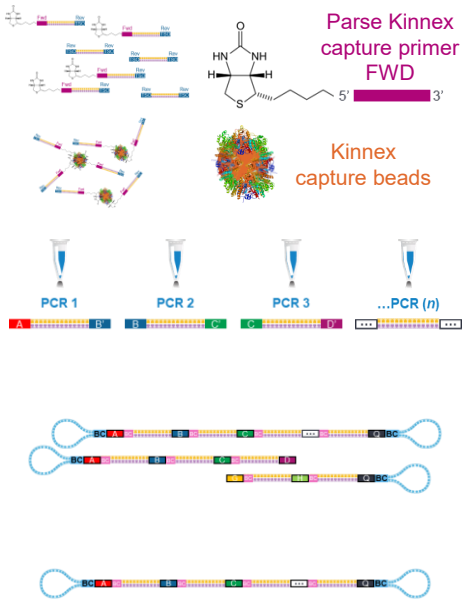
1. Parse Evercodesingle-cell cDNA generation

→ See **PacBio Technical note**

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

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

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



[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

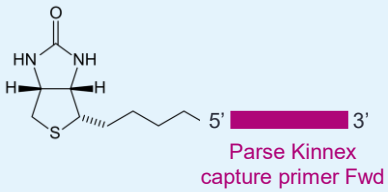
Order custom TSO PCR amplification primers as described below from your preferred oligo vendor

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)

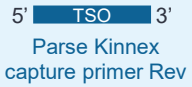
Parse Kinnex forward capture primer

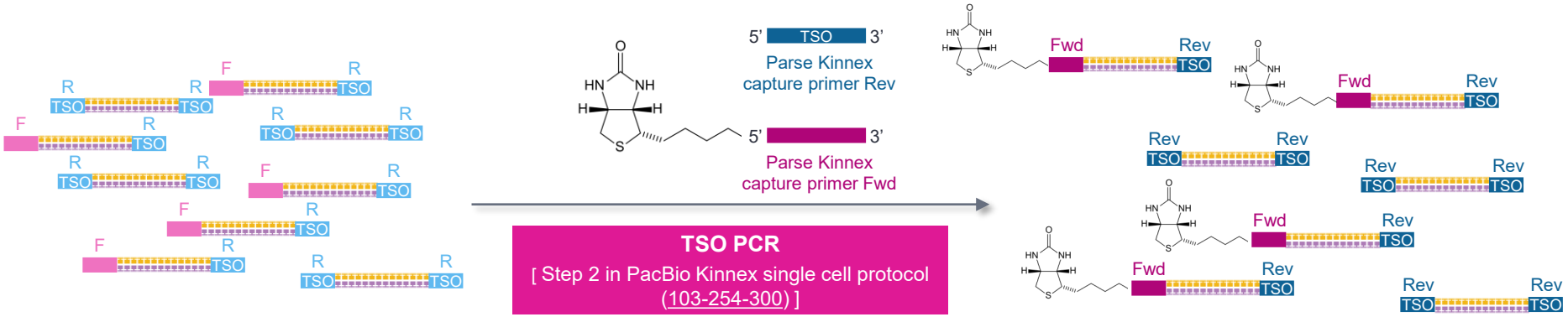
/5Biosg/AA/ideoxyU/CTACACGACGCTCTTCCGATCTCAGACGTGTGCTCTTCCGATC



Parse Kinnex reverse capture primer

AAGCAGTGGTATCAACGCAGAG





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

PacBio

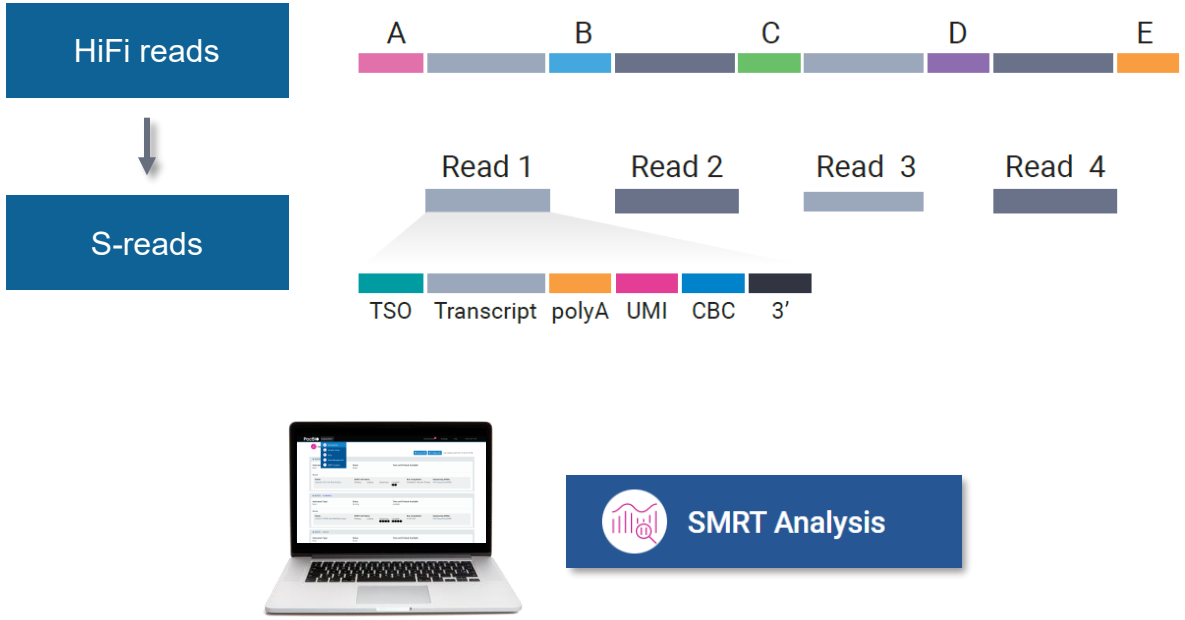
[1] The subsequent TSO artifact removal step (Step 3) in the **Kinnex 16-fold prep kit protocol** (103-885-300) uses streptavidin-coated Kinnex capture beads to pull down and enrich for biotin-tagged cDNA products with the correct structure.

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

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

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

SMRT Link read segmentation workflow

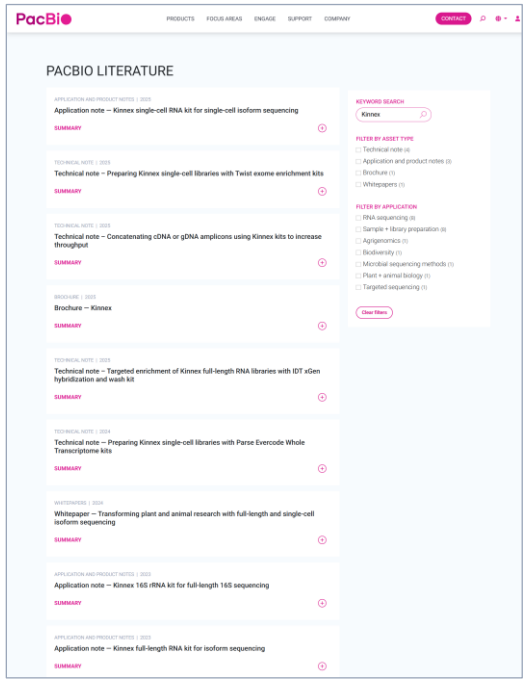


[1] Rebboah, E., et al. (2021). Mapping and modeling the genomic basis of differential RNA isoform expression at single-cell resolution with LR-Split-seq. Genome biology, 22, 1-28 [Link].
[2] Workflow-for-Processing-Long-Read-Data-with-the-Parse-Biosciences-Pipeline. Parse Biosciences long-read data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

Preparing Kinnex single-cell libraries with Twist exome enrichment kits

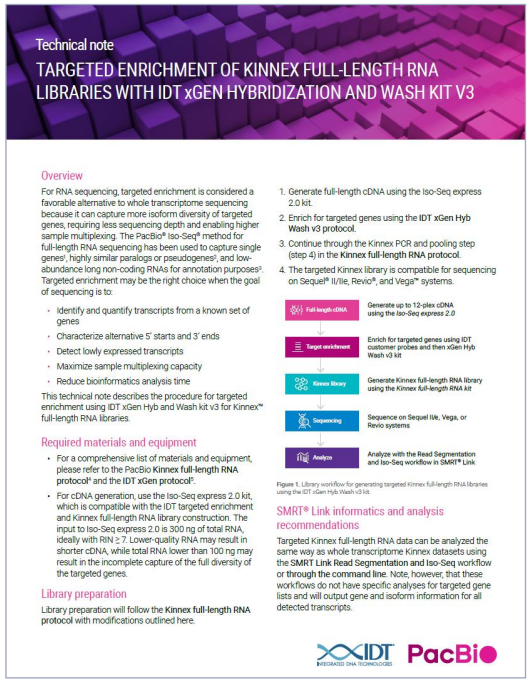
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3: Getting started

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



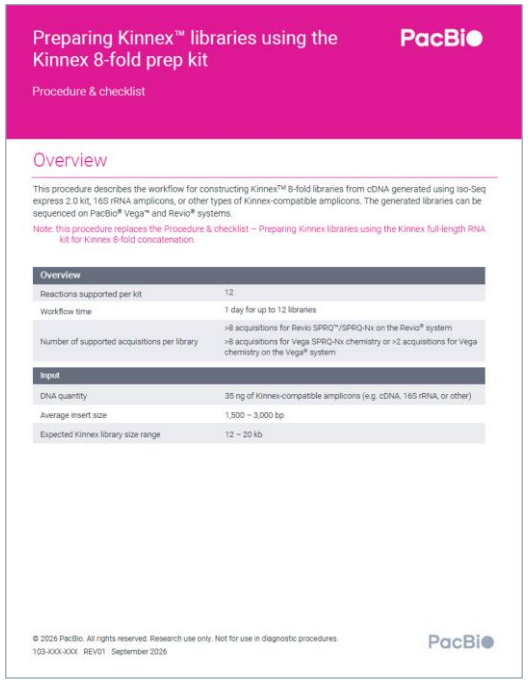
PacBio literature website [Link]

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



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

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



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

Technical documentation containing PacBio SMRTbell library construction details.

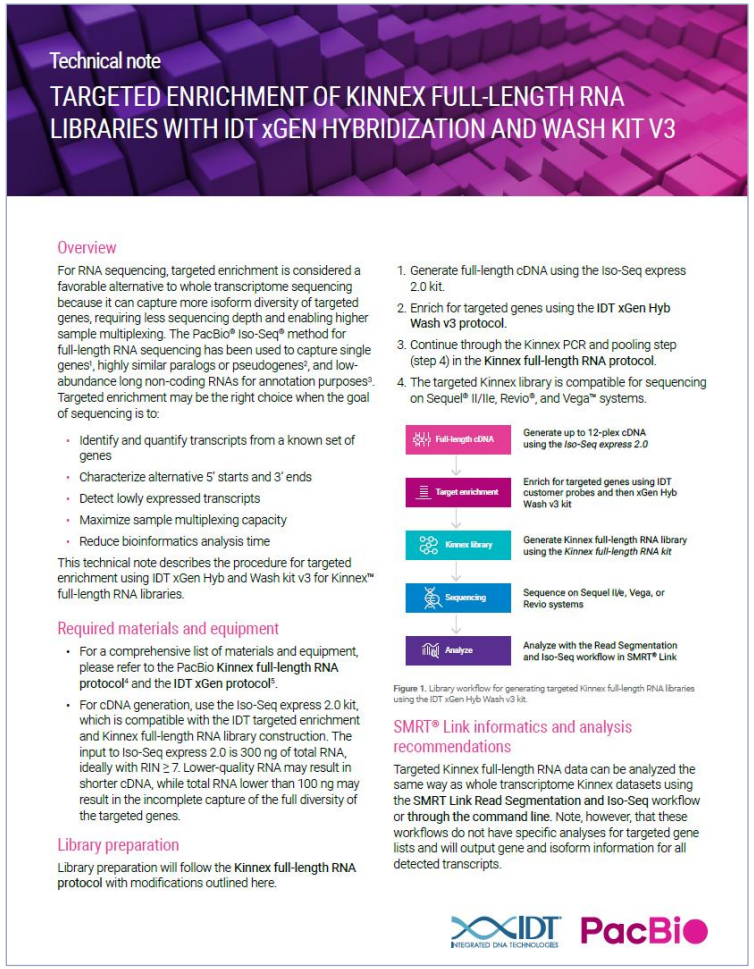
Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit (103-916-000) **or Kinnex 12-fold prep kit** (103-916-100) [PacBio]

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.

PacBio

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

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



Technical note

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

Overview

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

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

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

Required materials and equipment

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

Library preparation

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

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

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

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

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

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

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

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

SMRT® Link informatics and analysis recommendations

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

IDT INTEGRATED DNA TECHNOLOGIES PacBio

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

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

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

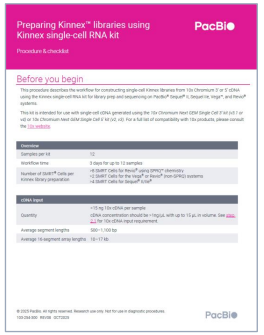
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

PacBio Kinnex library construction procedure[1]

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

PacBio



Kinnex full-length RNA library prep procedure key steps

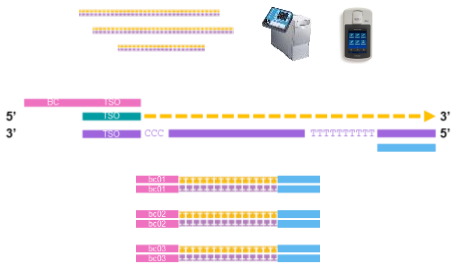
- For cDNA generation, use the Iso-Seq express 2.0 kit with 300 ng of total RNA input, ideally with RIN ≥ 7.[1]
- Library preparation will generally follow the **Kinnex 8-fold prep kit protocol** with the following additional steps:
  - Generate full-length cDNA using the Iso-Seq express 2.0 kit by following **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**

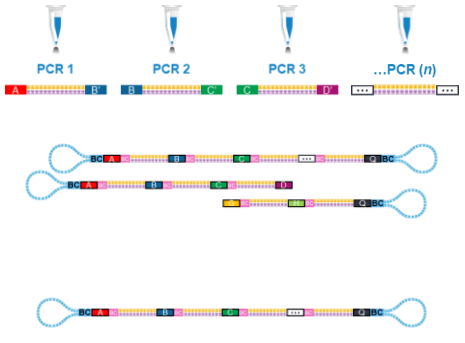
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632)

Kinnex bulk RNA library construction workflow

5 Kinnex PCR

6 Kinnex array formation + cleanup

7 Nuclease treatment + cleanup



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

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

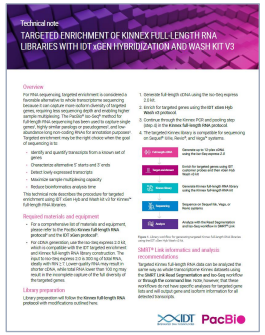
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

IDT xGen hybrid capture procedure overview[1]

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

PacBio

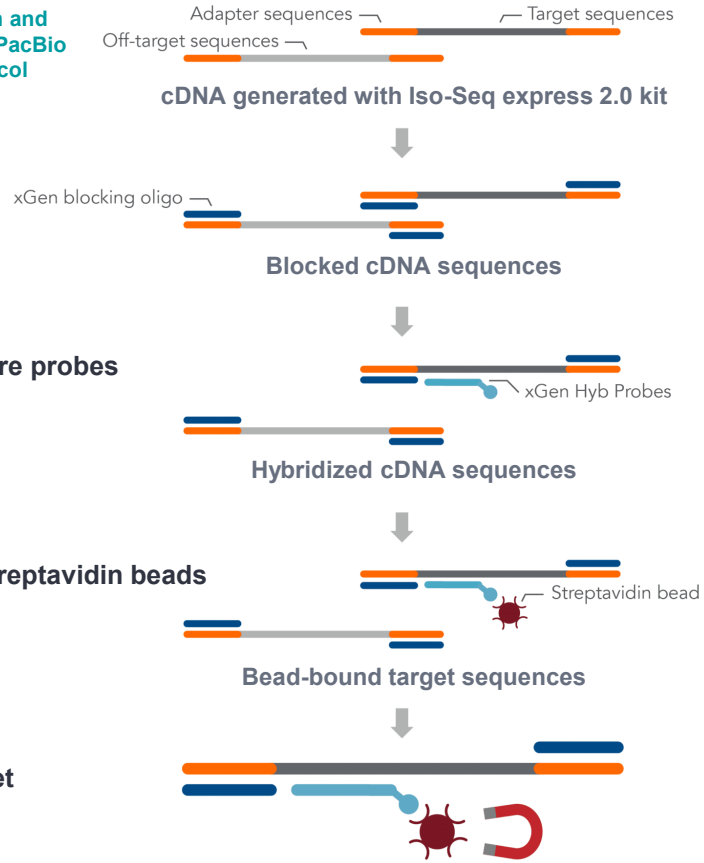


IDT xGEN hybrid capture procedure key steps[1]

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

IDT xGEN hybrid capture workflow

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

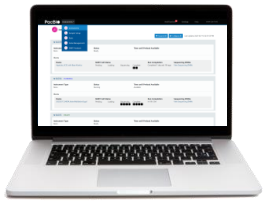


[1] For hybridization capture workflow details, refer to the **IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol** (RUO24-3081_001).
[2] 100 ng to 6 µg cDNA inputs can be used for short hybridizations (1-2 hrs). Generally, we recommend not to exceed 2.5 µg total input for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

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

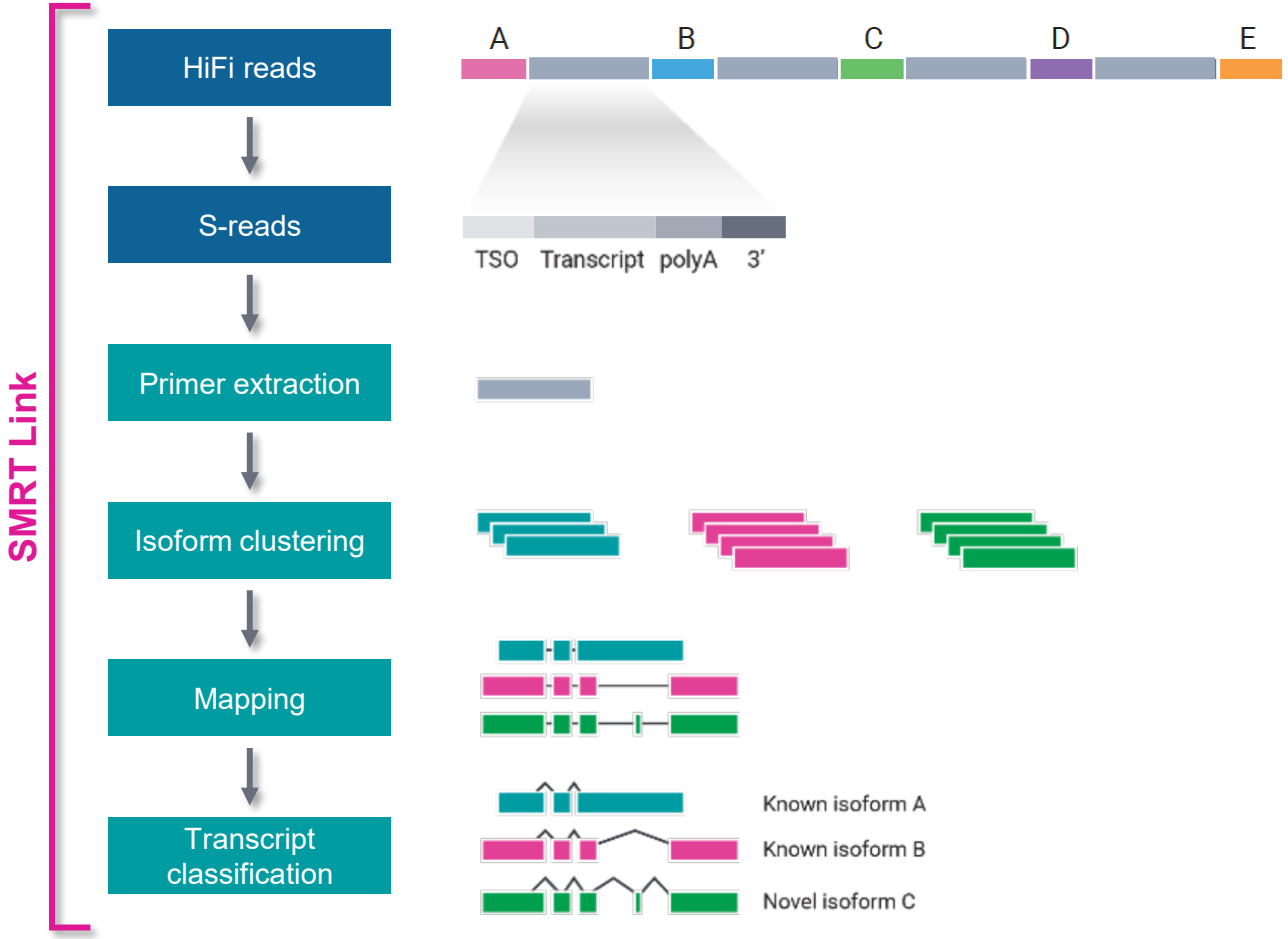
Use SMRT Link Read Segmentation and Iso-Seq application to perform isoform-classification analysis to identify novel genes & isoforms with abundance information

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



SMRT Analysis

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

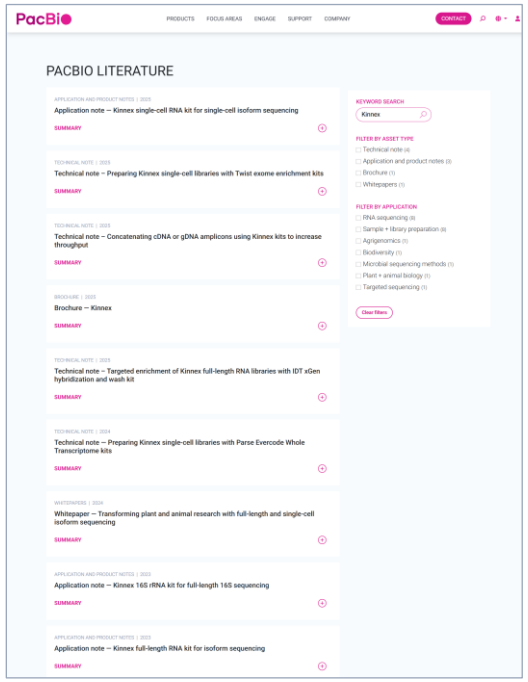


[1] See *SMRT Link User Guide* (Documentation) for detailed descriptions of parameter settings for Read Segmentation and Iso-Seq analysis application.

Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization capture kits

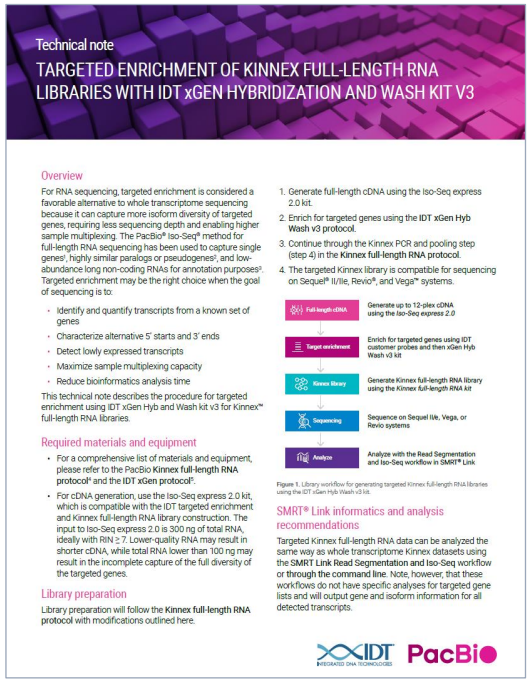
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3: Getting started

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



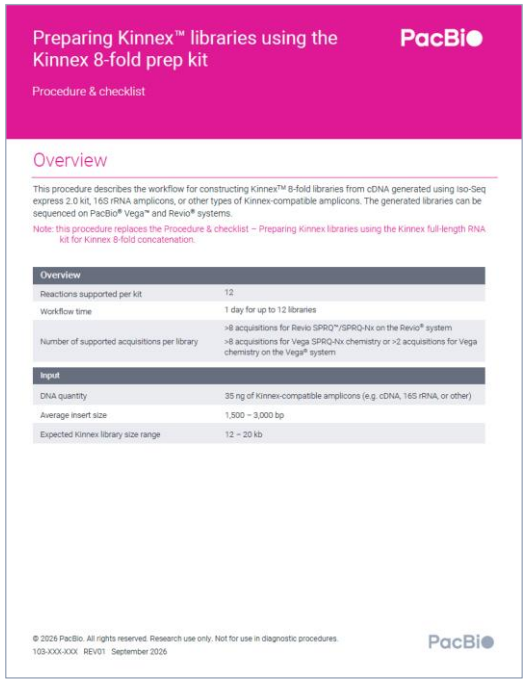
PacBio literature website [Link]

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



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

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



Procedure & checklist – 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.

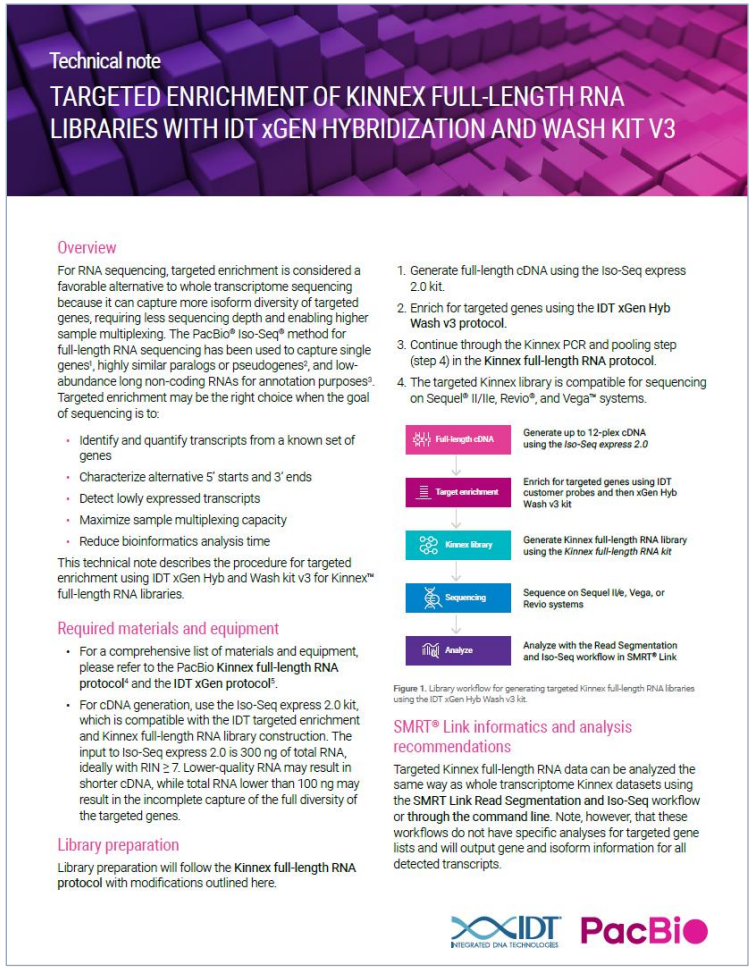
Technical overview – Kinnex library preparation using Kinnex 8-fold prep kit (103-916-000) **or Kinnex 12-fold prep kit** (103-916-100) [PacBio]

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.

PacBio

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

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



Technical note

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

Overview

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

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

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

Required materials and equipment

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

Library preparation

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

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

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

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

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

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

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

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

SMRT® Link informatics and analysis recommendations

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

IDT INTEGRATED DNA TECHNOLOGIES PacBio

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

Or

Preparing Kinnex libraries using Kinnex 12-fold prep kit (103-921-000)

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

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

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

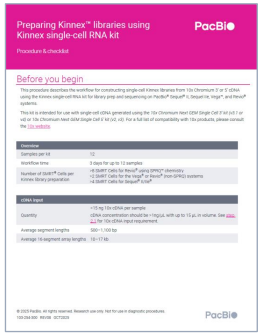
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

PacBio Kinnex library construction procedure[1]

Procedure & checklist – Preparing Kinnex libraries using the Kinnex 8-fold (103-920-900)/12-fold prep kit (103-921-000)

PacBio


Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

PacBio

Procedure & checklist

Before you begin

Kinnex full-length RNA library prep procedure key steps

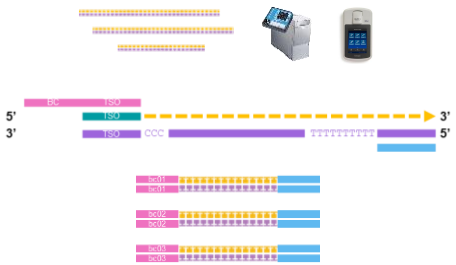
- For cDNA generation, use the Iso-Seq express 2.0 kit with 300 ng of total RNA input, ideally with RIN ≥ 7.[1]
- Library preparation will generally follow the **Kinnex 8-fold/12-fold prep kit protocol** with the following additional steps:
  - Generate full-length cDNA using the Iso-Seq express 2.0 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 (103-920-900) /12-fold prep kit (103-921-000) 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**

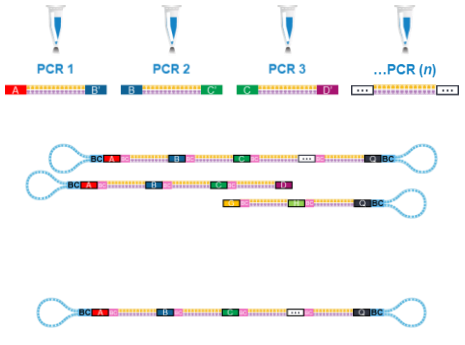
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632)

Kinnex bulk RNA library construction workflow

5 Kinnex PCR

6 Kinnex array formation + cleanup

7 Nuclease treatment + cleanup



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

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

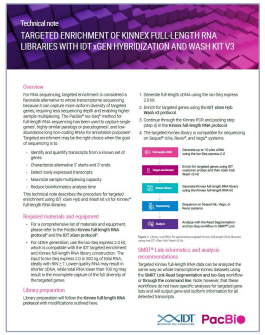
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

IDT xGen hybrid capture procedure overview[1]

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

PacBio

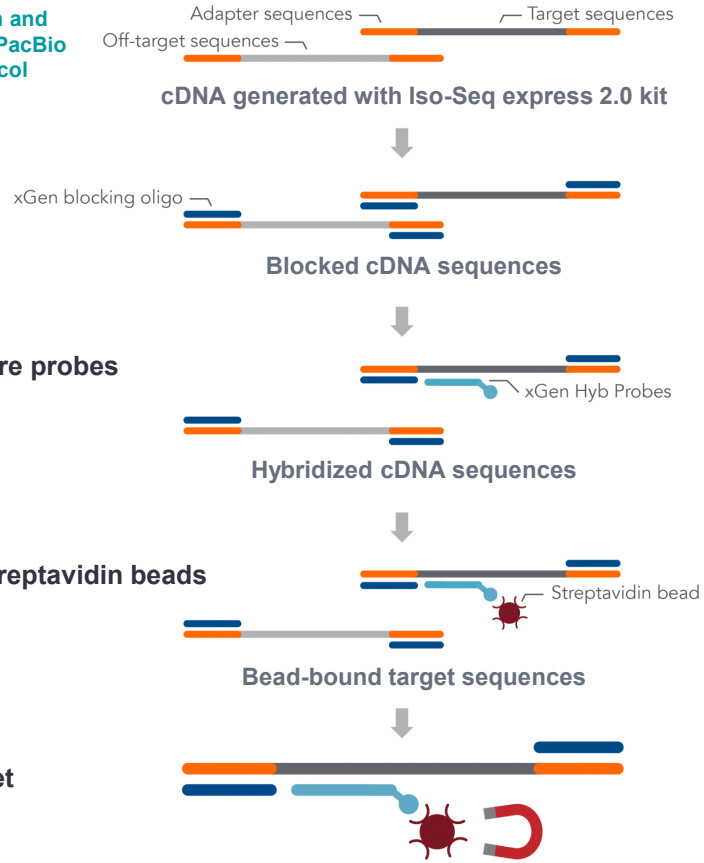

Technical note
TARGETED ENRICHMENT OF KINNEX FULL-LENGTH RNA LIBRARIES WITH IDT xGEN HYBRIDIZATION AND WASH KIT V3

IDT xGEN hybrid capture procedure key steps[1]

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

IDT xGEN hybrid capture workflow

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

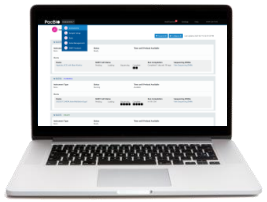


[1] For hybridization capture workflow details, refer to the **IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol** (RUO24-3081_001).
[2] 100 ng to 6 µg cDNA inputs can be used for short hybridizations (1-2 hrs). Generally, we recommend not to exceed 2.5 µg total input for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

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

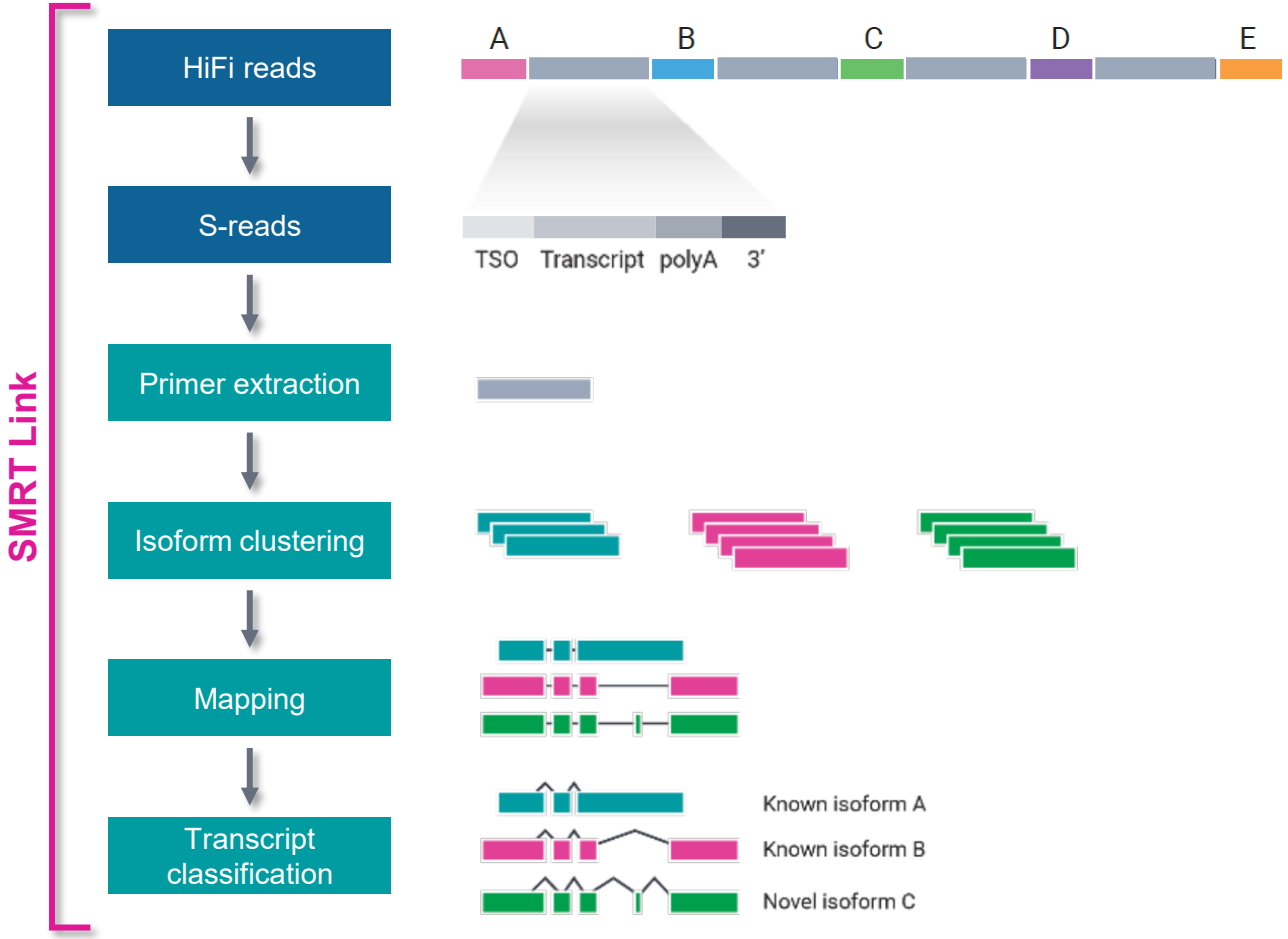
Use SMRT Link Read Segmentation and Iso-Seq application to perform isoform-classification analysis to identify novel genes & isoforms with abundance information

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



SMRT Analysis

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



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

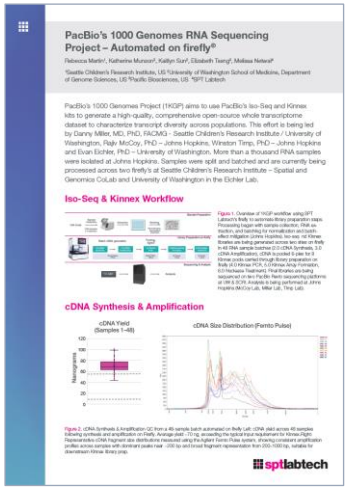
Kinnex library prep automation system options

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 preparation[1]

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


PacBio's 1000 Genomes RNA Sequencing Project – Automated on firefly®

Iso-Seq & Kinnex Workflow

cDNA Synthesis & Amplification

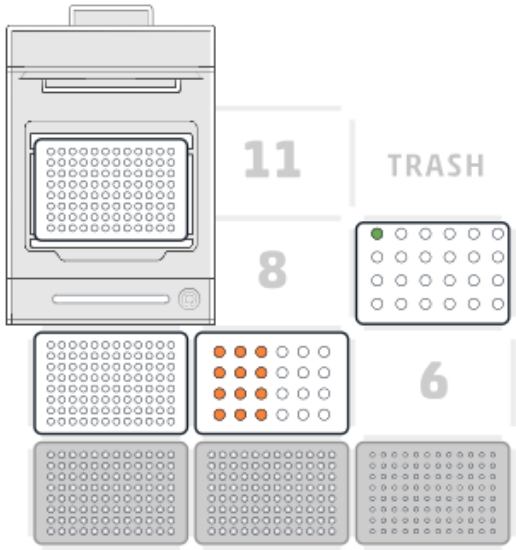
[1] See *PacBio's 1000 Genomes RNA Sequencing Project – Automated on firefly* case study (SPT Labtech).

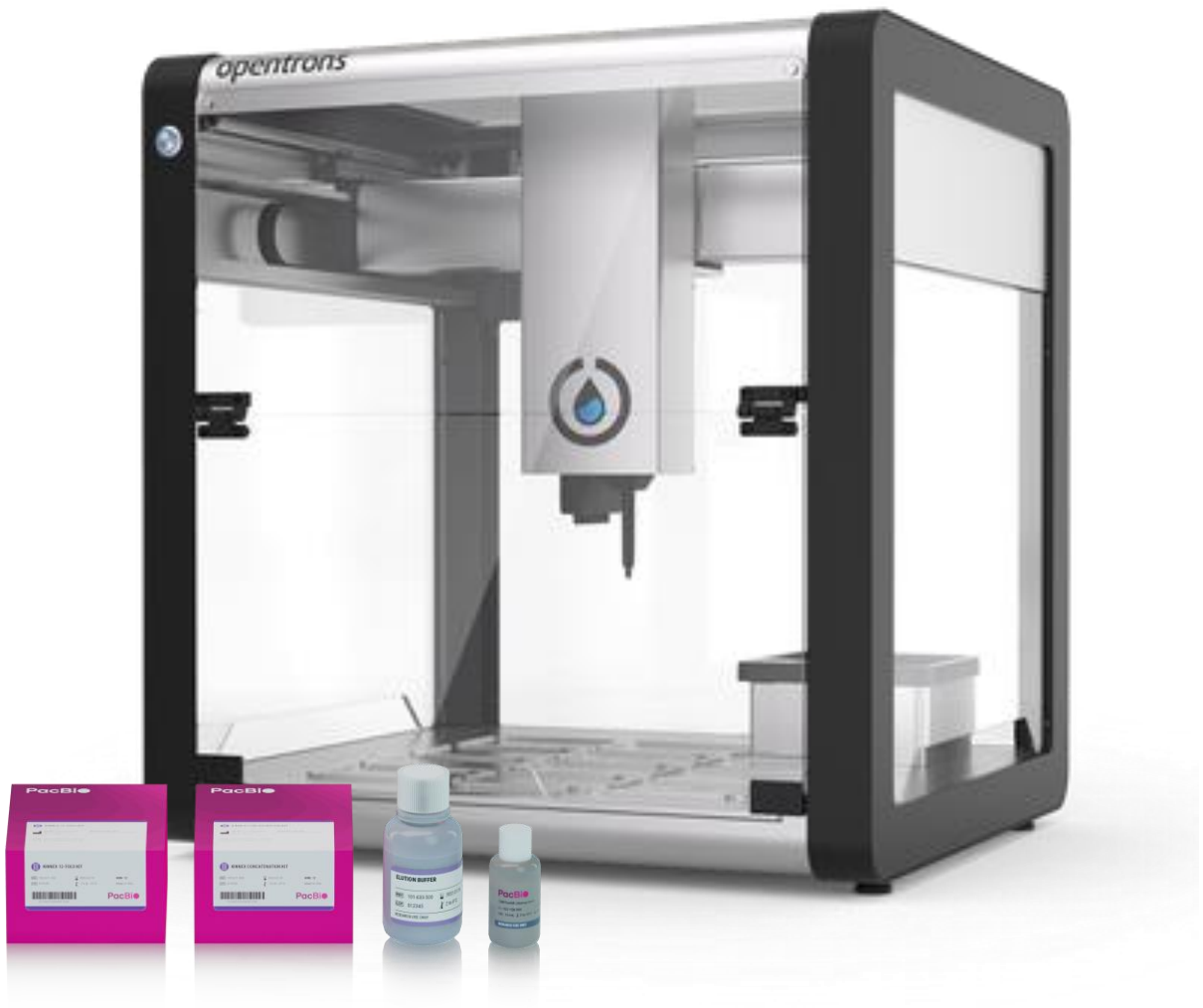
Automated Kinnex library preparation with the Opentrons OT-2 system

Kinnex PCR workflow step for Kinnex 8-fold/12-fold/16-fold prep kits can be automated on the Opentrons OT-2 system[1]

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.





[1] Visit https://library.opentrons.com/p/pacbio_kinnex_pcr to obtain automation protocol for running the Kinnex PCR workflow step on Opentrons OT-2 system.