



# Technical overview – Kinnex library preparation using Kinnex full-length RNA kit

Sequel II and IIe systems ICS v11.0

Revio system ICS v13.3

SMRT Link v25.3

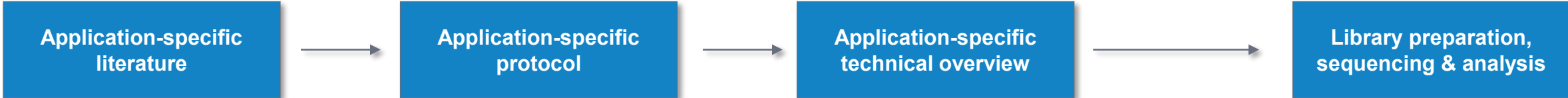
PN 103-344-700 Rev 02 | December 2025

# Technical overview

## Kinnex library preparation using Kinnex full-length RNA kit

1. Kinnex full-length RNA method overview
2. Kinnex full-length RNA library preparation workflow details
3. Kinnex full-length RNA sequencing preparation workflow details
4. Kinnex full-length RNA example sequencing performance data
5. Kinnex full-length RNA data analysis workflow overview
6. Technical documentation & applications support resources
7. APPENDIX 1 – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput
8. APPENDIX 2 – PacBio compatible Kinnex library preparation workflows
  - i. Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3
9. APPENDIX 3 – SMRT Link run design procedure for Sequel IIe system

# Kinnex library preparation using Kinnex full-length RNA kit: Getting started



**PacBio**

Application note

## Kinnex full-length RNA kit for isoform sequencing

**Introduction**

Alternative splicing (AS) in eukaryotic species generates functional diversity by expressing different combinations of exons in the same gene. Accurate characterization of full-length transcript isoforms generated by AS is critical for biological and disease studies. Bulk RNA-Seq using short reads cannot fully resolve isoform structures, as the complex nature of AS prohibits unambiguous transcript assembly with even the most sophisticated computational tools (Sarkis et al., 2019). Long-read RNA-Seq using PacBio® technology (the Iso-Seq® method) eliminates the need for transcript assembly by sequencing full-length cDNAs and enables new discoveries across many applications (Figure 1).

PacBio RNA sequencing has been shown to offer the longest read lengths and highest quality reads that are required for isoform discovery and isoform quantification (Caldes et al., 2024). The Kinnex™ full-length RNA kit takes full-length cDNA generated by the Iso-Seq express 2.0 kit as input and outputs a sequencing-ready library that results in an 8-fold throughput increase compared to typical iso-Seq libraries. Combined with the Read Segmentation & Iso-Seq analysis in SMRT Link software, PacBio offers cost-effective isoform sequencing that does not require orthogonal sequencing methods. SMRT Link software produces an isoform classification report with abundance information that can be used by [Isoform analysis tools](#).

© 2024 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures. 102-326-591 REV03 NOV2024 **PacBio**

## Application note – Kinnex full-length RNA kit for isoform sequencing (102-326-591)

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

**PacBio**

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

Procedure & checklist

**Before you begin**

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

Overview	
Samples	1–24
Workflow time	1.5 days (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	~8 SMRT Cells for Revio using SPRQ™ chemistry ~2 SMRT Cells for the Vega or Revio (non-SPRQ) systems ~4 SMRT Cells for Sequel II/e systems

RNA input	
Quality/race distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/μL per library)

© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures. 103-238-700 REV07 JUN2025 **PacBio**

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

Technical documentation containing application-specific library preparation protocol details.

**PacBio**

## Technical overview – Kinnex library preparation using Kinnex full-length RNA kit

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

PN 103-344-700 Rev 02 | December 2025

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

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

Raw Data Report	HiFi Read Length	Read Segmentation Metrics
<p>Raw Read Yield: 4,402 GB</p> <p>Mean Polymerase Read Length: 71,430 bp</p> <p>PI: 96%</p> <p>PI: 80%</p>	<p>HiFi Reads: 50.4 M</p> <p>HiFi Read Length: 442,410 bp</p> <p>Mean HiFi Read Length: 14,010 bp</p> <p>Median HiFi Read Quality: Q30</p>	<p>Segmented HiFi Reads: 10,863,007</p> <p>Segmented Reads (≥ 100bp): 11,912,108</p> <p>Mean Length of Segments: 5,750 bp</p> <p>Percent of Reads with Full Entry: 84.0%</p> <p>Mean Entry Size (Concatenation Ratio): 7.25</p>

**PacBio** <sup>1</sup> HiFi read lengths, contiguity, and other sequencing performance results are only dependent on RNA sample quality, read size, sample handling performance & device flow. Other sequencing performance metrics like mean HiFi quality, accuracy, and throughput are sample handling performance dependent and not dependent on RNA sample quality.

## Technical Overview – Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)

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

**cDNA synthesis & amplification**  
(Iso-Seq express 2.0 kit)

300 ng input total RNA per sample  
RIN (RNA integrity number) ≥7.0  
Sample multiplexing options available

**Kinnex library preparation**  
(Kinnex PCR 8-fold kit + Kinnex concatenation kit)

Use amplified cDNA to generate Kinnex library containing 8-segment array  
Sample multiplexing up to 48-plex supported

**SMRT sequencing**  
(Sequel IIe, Vega & Revio systems)

Perform ABC<sup>1</sup> and sequence Kinnex libraries on PacBio long-read systems

**Data analysis**  
(SMRT Link)

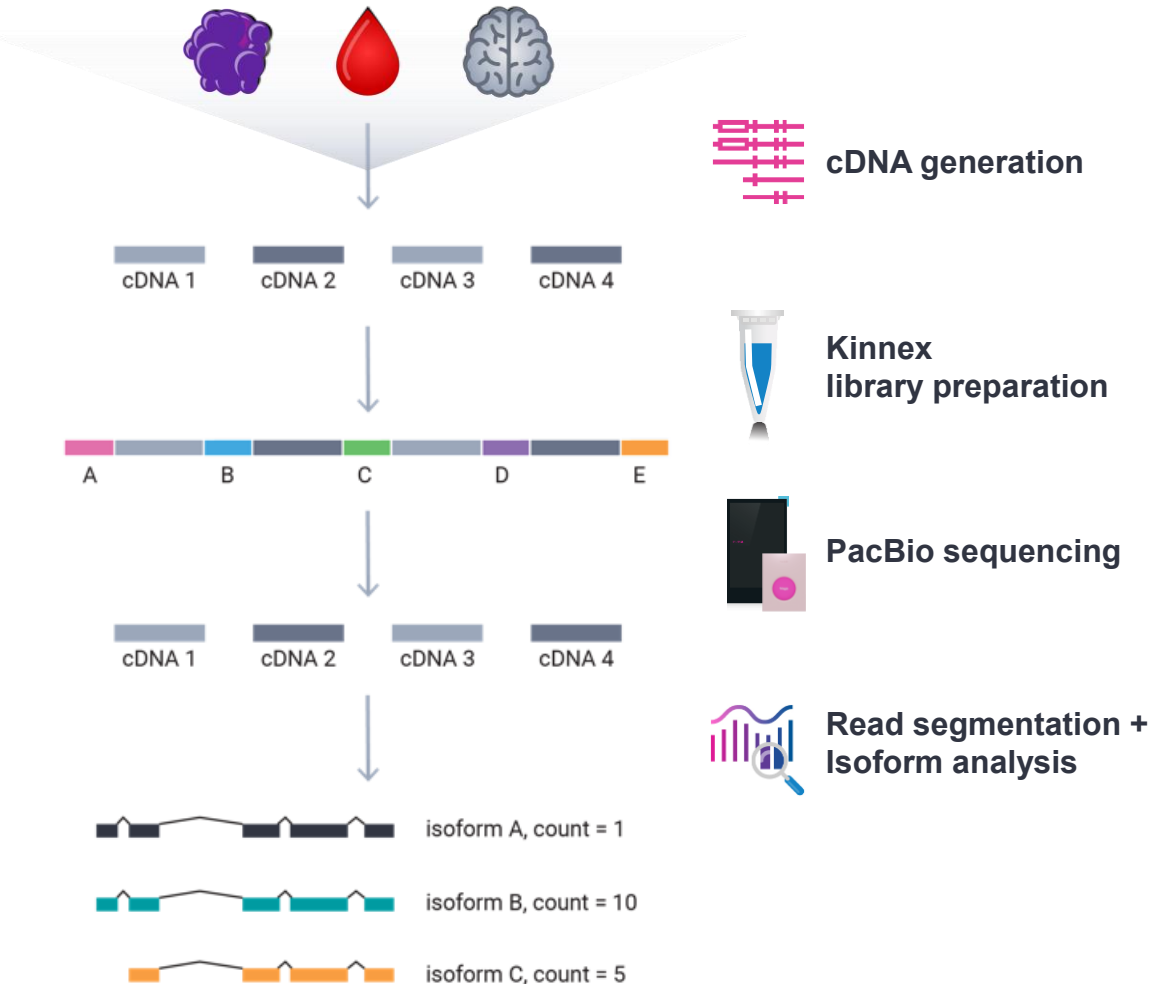
Use SMRT Link Read Segmentation data utility to split arrayed transcript HiFi reads  
Use SMRT Link Iso-Seq analysis application to identify novel genes and isoforms with abundance information



# Kinnex full-length RNA method overview

# Kinnex full-length RNA method overview

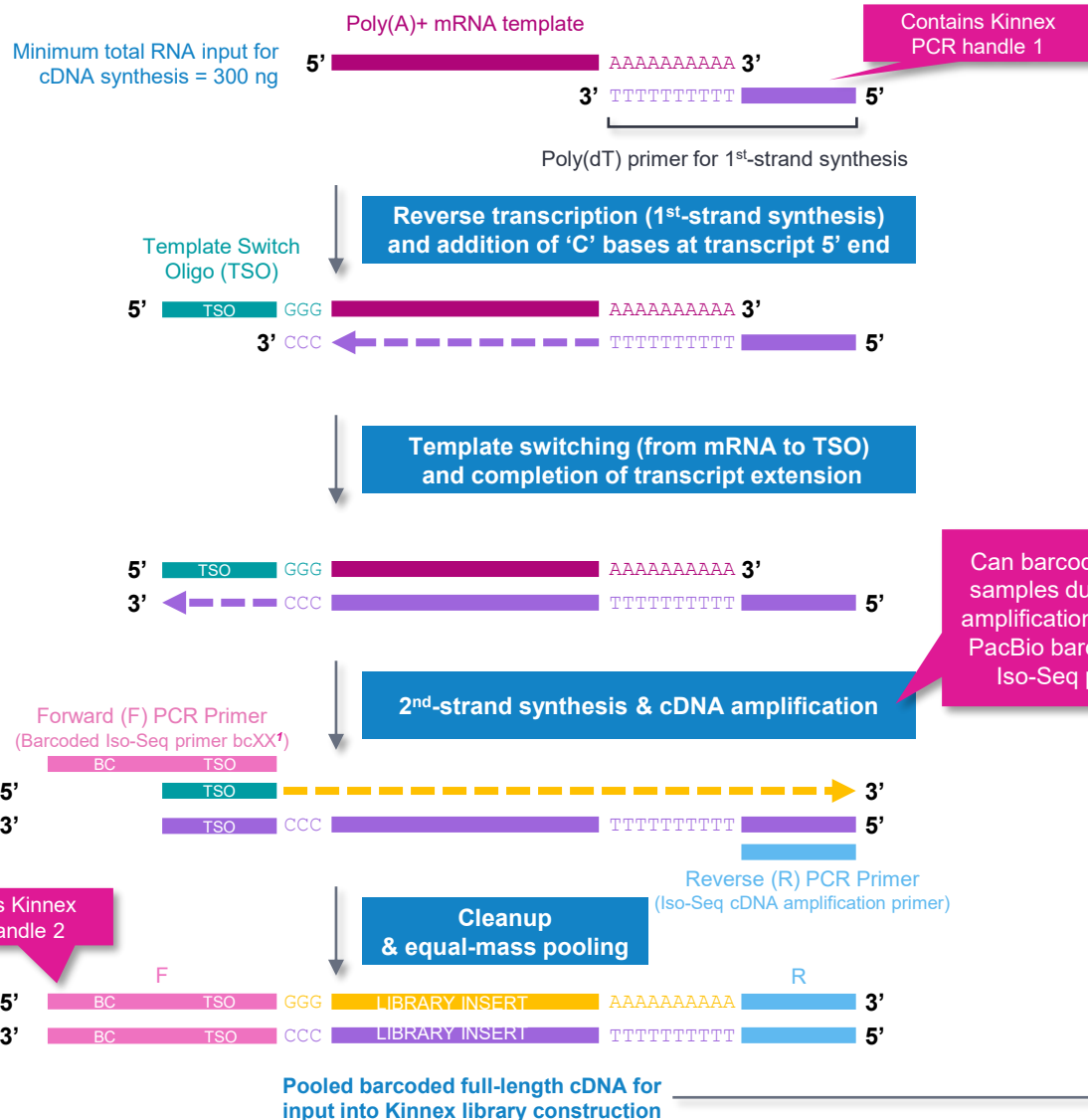
Use Kinnex full-length RNA kit to perform high-accuracy, full-length isoform sequencing with PacBio long-read systems



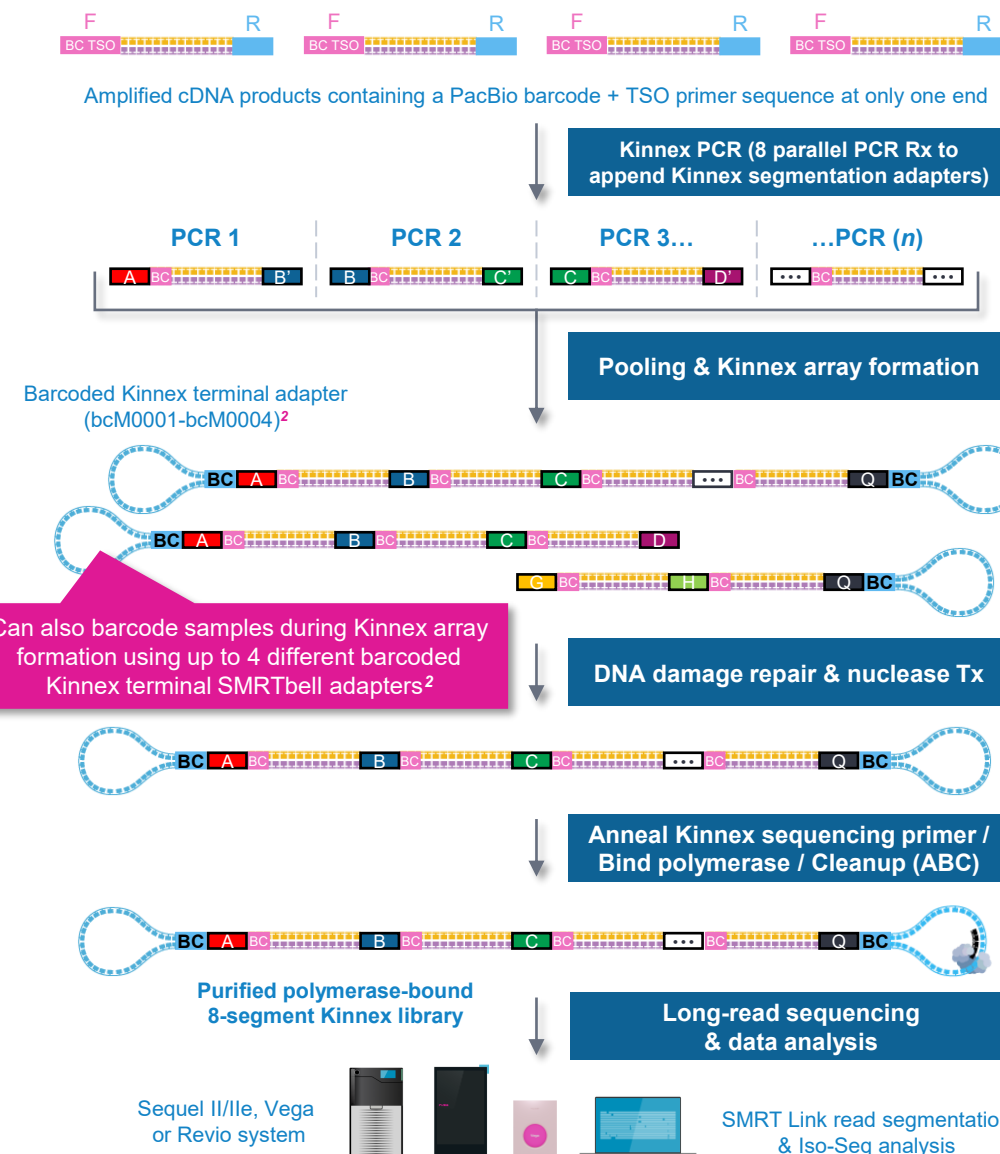
- Input 300 ng total RNA, RIN  $\geq 7$
- Generate up to 12-plex barcoded cDNA using **Iso-Seq express 2.0 kit (103-071-500)**
- 2-day Kinnex library preparation using **Kinnex full-length RNA kit (103-072-000)<sup>1</sup>**
- SMRT Link Run Design support for 'Kinnex full-length RNA' application type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

# Kinnex full-length RNA method overview (cont.)

## Full-length cDNA synthesis & amplification



## Kinnex library prep, sequencing & analysis



<sup>1</sup> Twelve barcoded Iso-Seq primers (Iso-Seq primer bc01–12) are available for cDNA amplification step.

<sup>2</sup> Kinnex adapter barcode sequences can be downloaded from [SMRT Link Data Management module](#).

# Kinnex full-length RNA library preparation procedure description

Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit ([103-238-700](#)) describes the workflow for constructing Kinnex libraries from total RNA samples using the **Iso-Seq express 2.0 kit** and **Kinnex full-length RNA kit** for sequencing on PacBio long-read systems

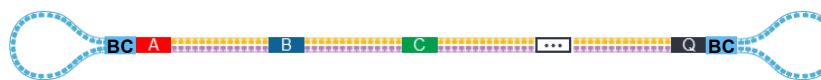
Overview	
Samples	1–24
Workflow time	1.5 days (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	>8 SMRT Cells for Revio using SPRQ™ chemistry >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems >4 SMRT Cells for Sequel II/Ile systems
RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/μL per library)



**Kinnex full-length RNA kit**  
103-072-000 (12 rxn)



**Iso-Seq express 2.0 kit<sup>1</sup>**  
103-071-500 (24 rxn)



**Kinnex full-length RNA library template (~12–16 kb)**  
Contains 8 concatenated full-length cDNA segments

PacBio

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

Procedure & checklist

---

Before you begin

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

Overview	
Samples	1–24
Workflow time	1.5 days (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	>8 SMRT Cells for Revio using SPRQ™ chemistry >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems >4 SMRT Cells for Sequel II/Ile systems
RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/μL per library)

© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures. 103-238-700 REV07 JUN2025

PacBio

PacBio [Documentation \(103-238-700\)](#)

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








# Kinnex full-length RNA kit bundle and Iso-Seq express 2.0 kit components

Kinnex full-length RNA kit bundle and Iso-Seq express 2.0 kit provide full support for Kinnex library prep workflow

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

Includes Iso-Seq Express template switching oligo, barcoded cDNA PCR Primers, and other reagents needed for performing 1<sup>st</sup>-strand cDNA synthesis and PCR amplification of cDNA products generated from input total RNA.





### Iso-Seq express 2.0 kit components

Component	Description
1 	<b>Iso-Seq RT buffer</b> • For 1 <sup>st</sup> -strand cDNA synthesis
2 	<b>Iso-Seq RT primer mix</b> • For 1 <sup>st</sup> -strand cDNA synthesis
3 	<b>Iso-Seq RT enzyme mix</b> • For 1 <sup>st</sup> -strand cDNA synthesis
4 	<b>Iso-Seq template switch oligo</b> • For 1 <sup>st</sup> -strand cDNA synthesis
5 	<b>Iso-Seq cDNA PCR mix</b> • Enzyme nucleotide mix for cDNA amplification
6 	<b>Iso-Seq cDNA amplification primer</b> • Reverse primer for cDNA amplification
7 	<b>Iso-Seq primers (bc01 – bc12)</b> • Barcoded forward primers (bc01 – bc04) for cDNA amplification

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

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 full-length RNA libraries.

### Kinnex full-length RNA kit components

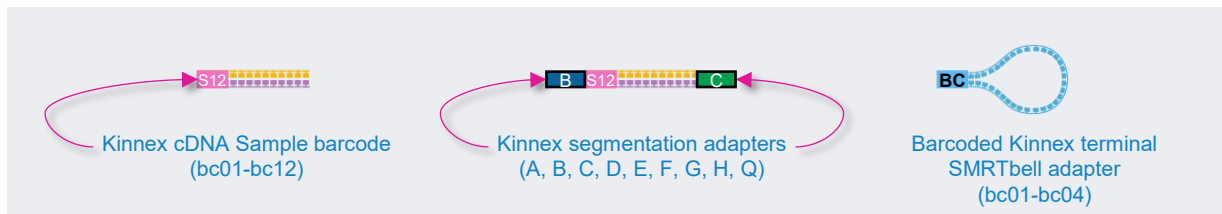
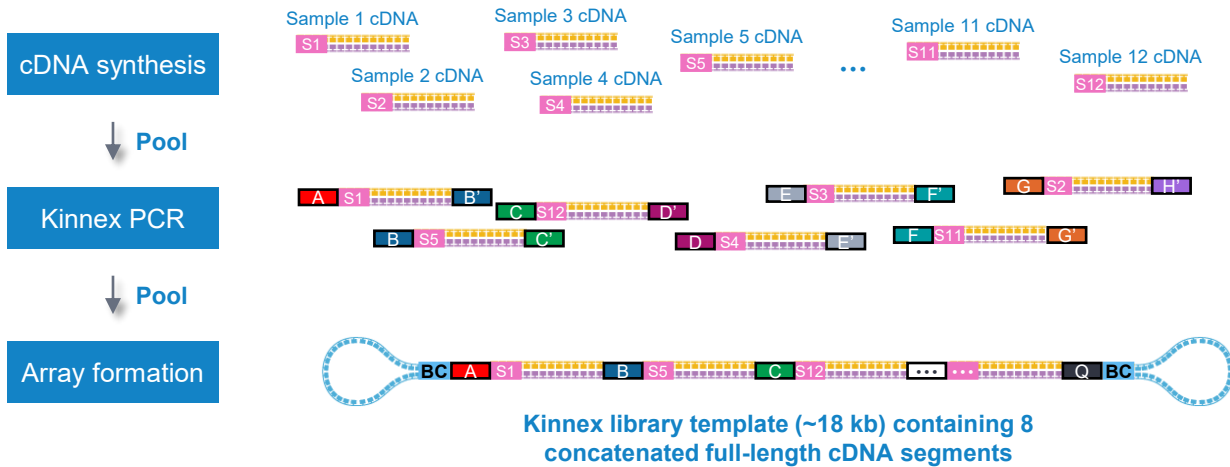
Component	Description
1 	<b>Kinnex PCR 8-fold kit (12 rxn)</b> • Contains reagents for Kinnex PCR to incorporate segmentation adapters
2 	<b>Kinnex concatenation kit (12 rxn)</b> • Contains reagents for Kinnex array formation and SMRTbell template construction • Includes barcoded Kinnex adapter mixes (bcM0001 – bcM0004)
3 	<b>SMRTbell cleanup beads</b> • For DNA cleanup
4 	<b>Elution buffer</b> • For DNA cleanup

# Kinnex full-length RNA library barcoding options for sample multiplexing

Kinnex full-length RNA library preparation procedure supports up to 48-plex sample multiplexing

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

- 12 different barcoded cDNA amplification PCR primers (bc01 – bc12)
- 4 different barcoded Kinnex terminal SMRTbell adapters (bc01 – bc04)



## Multiplexing best practices

Sample multiplexing can be achieved with one of the three following methods:

1. **Barcoded cDNA primers** using Iso-Seq primers bc01–12 in step 3 of the protocol. To multiplex, use the Iso-Seq cDNA amplification primer in combination with Iso-Seq primers bc01–12 to amplify samples. After SMRTbell cleanup, Iso-Seq samples can be pooled and brought through a single Kinnex PCR reaction. Each barcoded primer is sufficient for 2 reactions, with the Iso-Seq kit supporting a total of 24 reactions.
2. **Barcoded adapters** using Kinnex adapters bc01–04. In this case, use barcoded adapters at step 5 “Kinnex array formation” in the workflow.
3. **A combination of the above two approaches** to achieve 48-plex.

**Note:** If not performing multiplexing, the same Iso-Seq primer barcodes and Kinnex adapter barcodes are still used, but without pooling.

# Kinnex full-length RNA experimental design considerations

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

Example application	Human genetics disease studies	Biopharma for identifying highly expressed targets	Plant & animal whole genome annotation
Experimental goal	Isoform discovery and quantification of moderate-to-rare transcripts	Isoform discovery of high expressed transcripts	Comprehensive transcript annotation in a species
Example study design	Disease vs. normal tissues with multiple replicates	Disease cohort with >20+ samples	Plant or animal with multiple tissue types
Target depth of coverage per sample	10 M reads per sample	5 M reads per sample	5 M reads per sample
Sample multiplexing <sup>1</sup>	<b>Sequel II/IIe system:</b> Up to 2 samples per SMRT Cell 8M (2-plex)	<b>Sequel II/IIe system:</b> Up to 3 samples per SMRT Cell 8M (3-plex)	
	<b>Vega system:</b> Up to 3 samples per Vega SMRT Cell (3-plex)	<b>Vega system:</b> Up to 6 samples per Vega SMRT Cell (6-plex)	
	<b>Revio system + SPRQ:</b> Up to 6 samples per Revio SMRT Cell (6-plex)	<b>Revio system + SPRQ:</b> Up to 12 samples per Revio SMRT Cell (12-plex)	
Expected data throughput (per SMRT Cell)	<b>Sequel II/IIe system:</b> Up to 20 M reads divided by <i>N</i> samples		
	<b>Vega system:</b> Up to 30 M reads divided by <i>N</i> samples		
	<b>Revio system + SPRQ:</b> Up to 60 M reads divided by <i>N</i> samples		
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit ( <a href="#">103-238-700</a> )		
Total RNA input into Kinnex library prep workflow	300 ng total RNA (RIN ≥7) for 1 <sup>st</sup> -strand cDNA synthesis		
SMRT Link data analysis workflows	Read Segmentation and Iso-Seq analysis application with option to “pool reads and cluster together” to get a master isoform classification file with per-sample full-length read counts		
Community data analysis tools	Annotation & quantification: PIGEON, SQANTI3 / Differential analysis: TappAS / Fusion calling: pbfusion / Visualization: SWAN		



# Kinnex full-length RNA library preparation workflow details

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

Procedure & checklist [103-238-700](#) describes the workflow for constructing Kinnex libraries from total RNA samples using the **Iso-Seq express 2.0 kit** and **Kinnex full-length RNA kit** for sequencing on PacBio long-read systems

## Procedure & checklist contents

1. **Total RNA input QC recommendations** and **general best practices** for reagent & sample handling.
2. Enzymatic workflow steps for **cDNA synthesis and amplification**.
3. Enzymatic workflow steps for **construction of 8-segment Kinnex arrays** from amplified cDNA.
4. Enzymatic workflow steps for **DNA damage repair & nuclease treatment** of Kinnex libraries.
5. Workflow steps for **final cleanup of Kinnex SMRTbell libraries** using SMRTbell cleanup beads.
6. **Sample setup ABC<sup>1</sup>** workflow steps to prepare Kinnex SMRTbell libraries for sequencing on Revio (+SPRQ) and Vega systems.

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

Procedure & checklist

---

### Before you begin

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

Overview	
Samples	1–24
Workflow time	1.5 days (for up to 24 samples)
Number of SMRT® Cells per Kinnex library Prep	>8 SMRT Cells for Revio using SPRQ™ chemistry >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems >4 SMRT Cells for Sequel II/Ile systems

RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/μL per library)

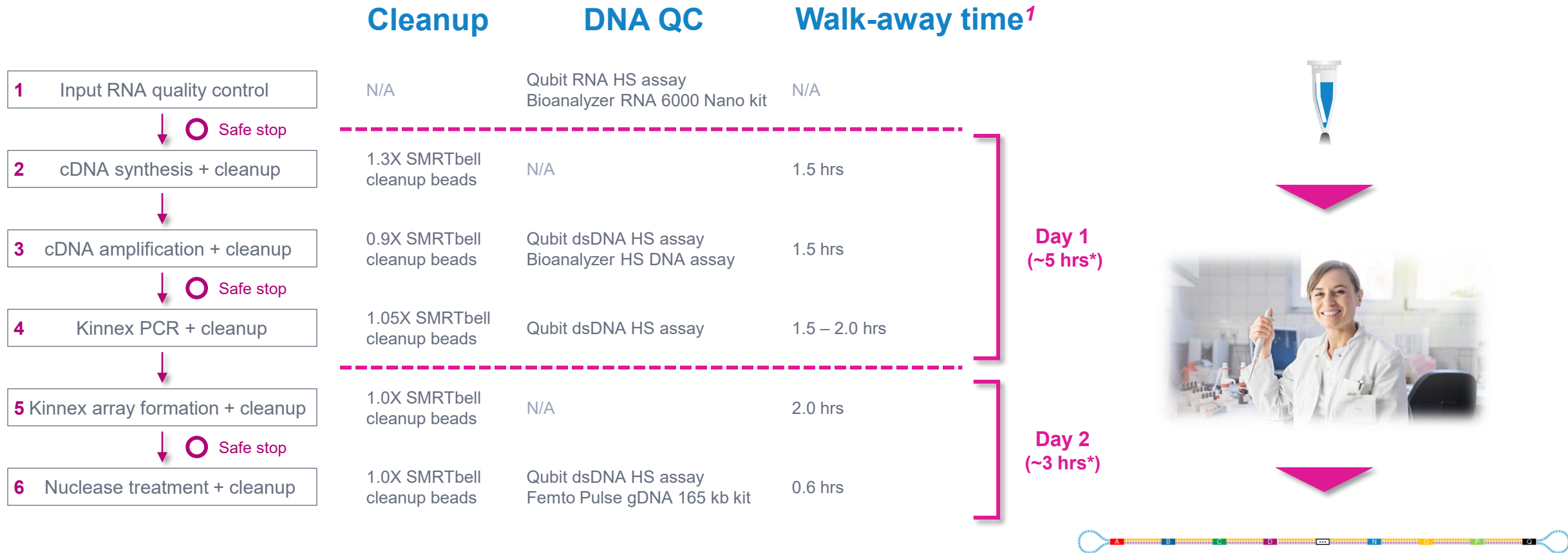
© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.  
103-238-700 REV07 JUN2025

**PacBio**

PacBio [Documentation](#) ([103-238-700](#))

# Kinnex full-length RNA library construction workflow overview

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



# General best practices recommendations for preparing Kinnex full-length RNA 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.
- Take care to accurately pipette SMRTbell cleanup beads because small changes in volume can significantly alter the size distribution of your sample.
- Equilibrate the SMRTbell cleanup beads at room temperature for 30 mins prior to use.
- In cDNA amplification and Kinnex PCR, **keep sample(s) on ice until thermal cycler lid has reached 105°C** to avoid digestion of primers by polymerase exonuclease activity.
- Ensure that the DNA damage repair mix is stored at -20°C to avoid poor library performance.**
- This workflow takes ~1.5 days to complete.
  - If a stop is necessary, refer to the workflow for **safe stopping points**.

Temperature-sensitive reagents		
Iso-Seq express 2.0 kit 103-071-500		
	Tube color	Reagent
cDNA synthesis	Purple	Iso-Seq RT buffer 103-103-900
	Orange	Iso-Seq RT primer mix 103-104-000
	Yellow	Iso-Seq RT enzyme mix 103-104-100
	Red	Iso-Seq cDNA PCR mix 103-104-200
	Blue	Iso-Seq Express TSO 2.0 103-104-300
	Green	Iso-Seq cDNA amplification primer 103-104-400
		Iso-Seq primer bc01 103-104-500
		Iso-Seq primer bc02 103-104-600
		Iso-Seq primer bc03 103-104-700
		Iso-Seq primer bc04 103-104-800
		Iso-Seq primer bc05 103-104-900
		Iso-Seq primer bc06 103-105-000
	White	Iso-Seq primer bc07 103-105-100
		Iso-Seq primer bc08 103-105-200
		Iso-Seq primer bc09 103-105-300
		Iso-Seq primer bc10 103-105-400
		Iso-Seq primer bc11 103-105-500
		Iso-Seq primer bc12 103-105-600

Temperature-sensitive reagents	
Kinnex PCR 8-fold kit 103-071-600	
Tube color	Reagent
Green	Kinnex PCR mix 103-107-700
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 HQ 103-108-500

Temperature-sensitive reagents	
Kinnex concatenation kit 103-071-800	
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	DNA repair mix 103-110-000
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 full-length RNA libraries

## Reagent and sample handling

### Sequencing prep reagents (for sample setup ABC)

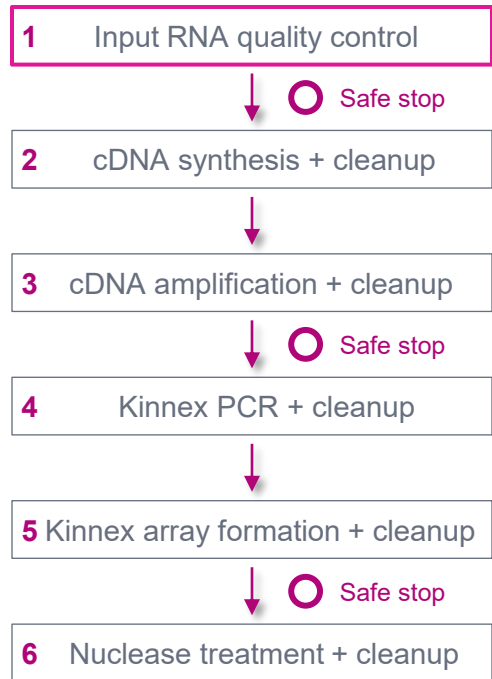
- Once thawed, place reaction buffers and sequencing primer on-ice prior to making master mix. The Loading buffer should be left at room-temperature.
- **Note:** The Loading buffer is **light sensitive** and should be protected from light when not in use.
- **Keep the following reagents on a cold block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

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

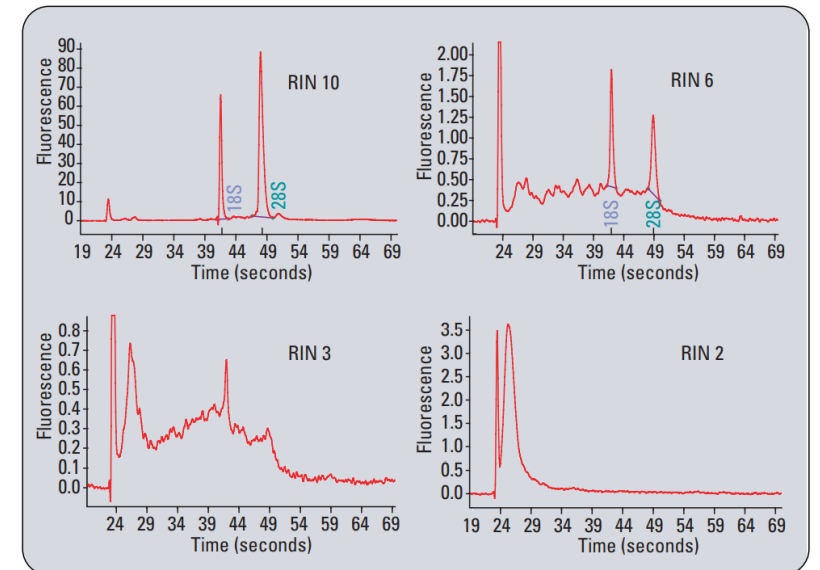
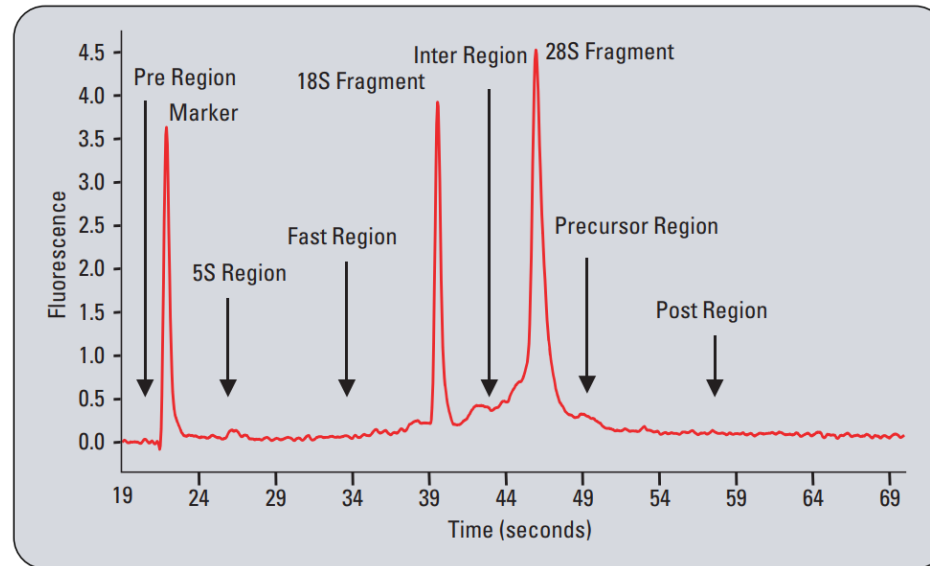
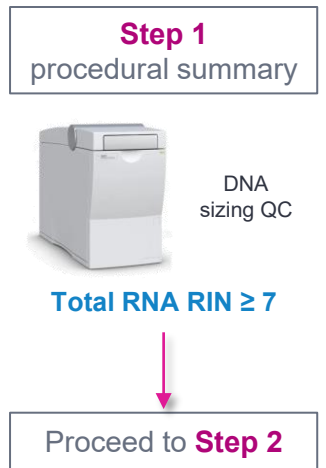
# Input RNA quality control

Input RNA quality control is highly recommended before proceeding to the MAS-Seq library prep workflow



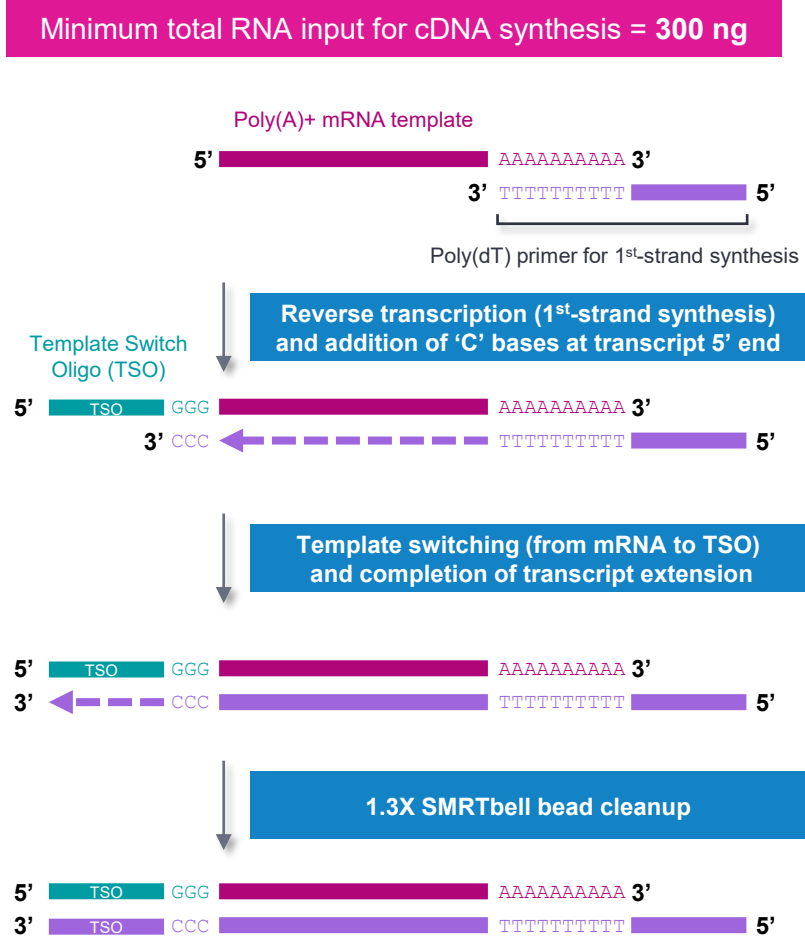
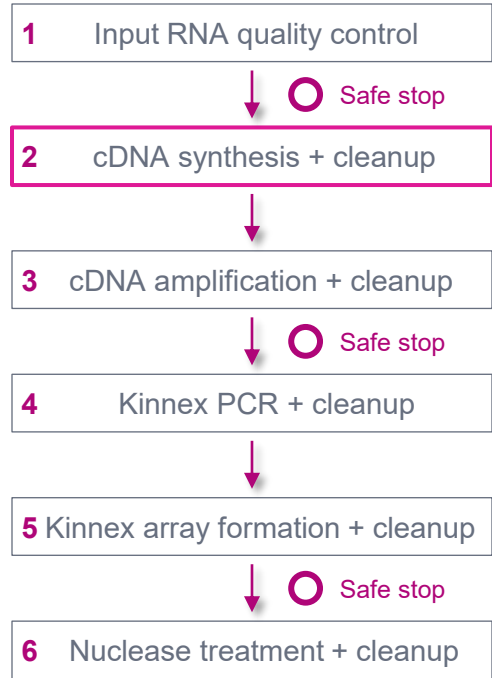
**Left:** Bioanalyzer electropherogram detailing the regions that are indicative of RNA quality. **Right:** Sample electro-pherograms corresponding to different RNA Integrity Number (RIN) scores. Samples range from intact (RIN 10), to degraded (RIN 2). Images from Agilent Application Note: RNA Integrity Number (RIN) – Standardization of RNA Quality Control ([5989-1165EN](#))

- 300 ng of total RNA per library (minimum concentration 43 ng/μL per library) is required for this procedure
- Sample QC of input total RNA samples should be assessed by measuring RNA Integrity Number (RIN) using a Bioanalyzer 2100 instrument (Agilent Technology) with RNA 6000 Nano kit<sup>1</sup>
  - RIN  $\geq 7.0$  (ideally  $\geq 8.0$ ) is sufficient for Kinnex full-length RNA protocol
  - Samples with RIN  $< 7.0$  can be processed, but risk of significant underperformance or even failure is greatly increased
- RIN score (1 to 10) is related to ratio of the area under 28s and 18s fragment peaks and also takes into account signal intensity above baseline in the Inter-Region and Fast Region since this is where degradation products appear
- Higher RIN numbers are correlated with better overall sample quality and lower degradation



# cDNA synthesis + cleanup

In this step, total RNA samples are converted to 1<sup>st</sup>-strand cDNA



## Step 2 procedural summary

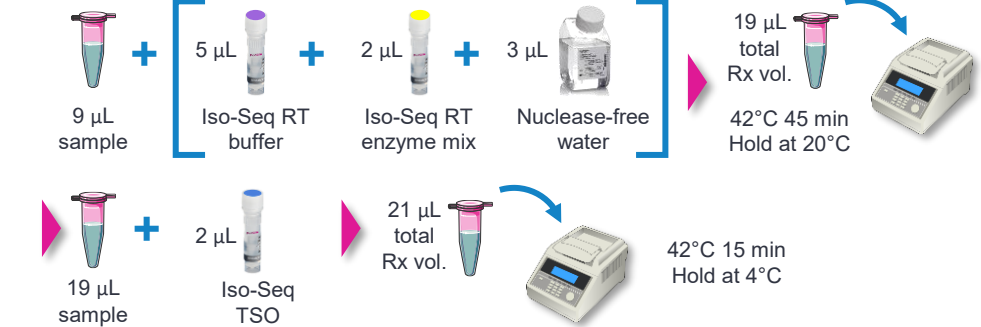
### 2.1 Thawing reagents for first-strand cDNA synthesis



### 2.2 Primer annealing for first-strand cDNA synthesis



### 2.3 Reverse transcription and template switching



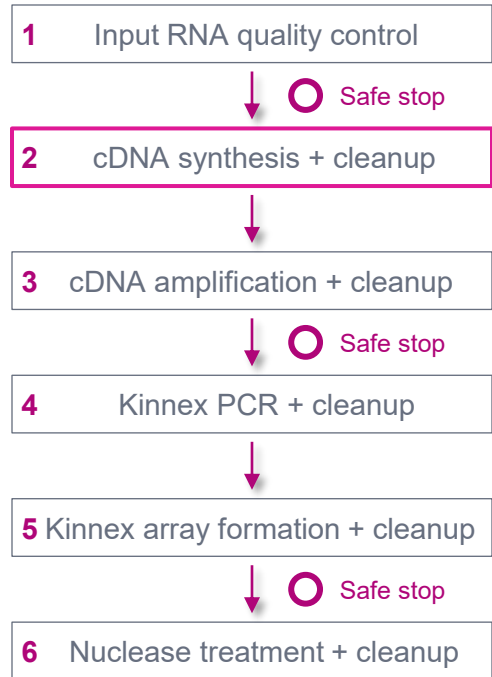
### 2.4 1.3X SMRTbell bead cleanup



Proceed to **Step 3**

# cDNA synthesis + cleanup (cont.)

## Procedural notes



### 2.1 Thawing reagents for first-strand synthesis

Step	Instructions
2.1.1	Quick-spin the Iso-Seq RT enzyme mix in the microcentrifuge to collect liquid, then place on ice. Thaw the following components at room temperature, briefly vortex to mix, then quick-spin to collect liquid and place on ice.

Tube color	Reagent
Orange	Iso-Seq RT primer mix

• After thawing specified reagents **on ice**, perform a quick spin to collect liquid, then **place on ice**

### 2.2 Primer annealing for first-strand synthesis

Step	Instructions
2.2.1	For each RNA sample to be processed, prepare reagent mix 1 on ice by adding the following components to each tube in the PCR strip tube.

Components	Volume
Total RNA (300 ng)	<7 $\mu$ L

• Set up primer annealing reaction mix **on ice** and then transfer to thermal cycler for incubation

### 2.3 Reverse transcription and template switching

Step	Instructions
2.3.1	For each RNA sample, prepare reagent mix 2 on ice by adding the following components in the order and volume listed below. Adjust component volumes for the number of samples being prepared, plus 10% overage.

Tube color	Components	Volume
Purple	Iso-Seq RT buffer (vortex briefly before use)	5 $\mu$ L

• Set up reverse transcription reaction mix **on ice** and then transfer to thermal cycler for incubation

### 2.4 1.3X SMRTbell bead cleanup

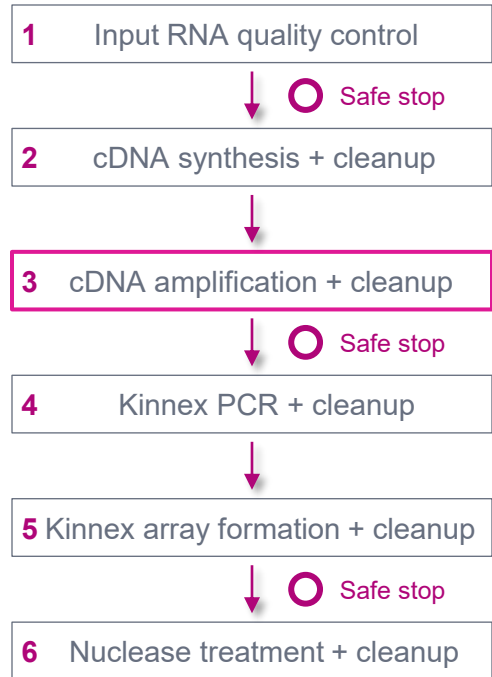
Step	Instructions
2.4.1	For each sample, add 29 $\mu$ L of elution buffer to the 21 $\mu$ L reverse transcription and template switching reaction ( <a href="#">Section 2.3</a> ) for a total volume of 50 $\mu$ L.
2.4.2	Add 65 $\mu$ L (1.3X v/v) of resuspended, room-temperature SMRTbell cleanup beads.
2.4.3	Mix beads by pipetting 10 times or until evenly distributed.

• Perform **1.3X** SMRTbell bead cleanup



# cDNA amplification + cleanup

## Procedural notes



### 3.1 cDNA amplification

Step	Instructions														
3.1.1	For each sample, prepare reaction mix 3 on ice by adding the following components in the order and volume listed below. Adjust component volumes for the number of samples being prepared plus 10% overage. Pipette mix master mix. Iso-Seq primer bc01-12 will be added to each sample individually and should not be added to the master mix.														
	<table border="1"><thead><tr><th>Tube color</th><th>Components</th><th>Volume</th></tr></thead><tbody><tr><td>Red</td><td>Iso-Seq cDNA PCR mix</td><td>25 <math>\mu</math>L</td></tr><tr><td>Green</td><td>Iso-Seq cDNA amplification primer</td><td>2 <math>\mu</math>L</td></tr><tr><td></td><td>Total volume</td><td>27 <math>\mu</math>L</td></tr></tbody></table>	Tube color	Components	Volume	Red	Iso-Seq cDNA PCR mix	25 $\mu$ L	Green	Iso-Seq cDNA amplification primer	2 $\mu$ L		Total volume	27 $\mu$ L		
Tube color	Components	Volume													
Red	Iso-Seq cDNA PCR mix	25 $\mu$ L													
Green	Iso-Seq cDNA amplification primer	2 $\mu$ L													
	Total volume	27 $\mu$ L													
3.1.2	On ice, add 27 $\mu$ L of reaction mix 3 to the 21 $\mu$ L of the eluted cDNA (from previous <a href="#">Section 2.4</a> ).														
3.1.3	Add 2 $\mu$ L of the appropriate Iso-Seq primer barcode to each sample for a total volume of 50 $\mu$ L.														
	<table border="1"><thead><tr><th>Tube color</th><th>Component</th><th>Volume</th></tr></thead><tbody><tr><td>White</td><td>Iso-Seq primer</td><td>2 <math>\mu</math>L</td></tr></tbody></table>	Tube color	Component	Volume	White	Iso-Seq primer	2 $\mu$ L								
Tube color	Component	Volume													
White	Iso-Seq primer	2 $\mu$ L													
3.1.4	Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid.														
3.1.5	Run the thermal cycler program below with the lid temperature set to 105°C. Keep sample on ice until thermal cycler lid has heated to 105°C.														
	<table border="1"><thead><tr><th colspan="2">PCR program</th></tr></thead><tbody><tr><td>45 seconds at 98°C</td><td>1 cycle</td></tr><tr><td>10 seconds at 98°C</td><td></td></tr><tr><td>15 seconds at 60°C</td><td>10 cycles</td></tr><tr><td>3 minutes at 72°C</td><td></td></tr><tr><td>5 minutes at 72°C</td><td></td></tr><tr><td>Hold at 4°C</td><td></td></tr></tbody></table>	PCR program		45 seconds at 98°C	1 cycle	10 seconds at 98°C		15 seconds at 60°C	10 cycles	3 minutes at 72°C		5 minutes at 72°C		Hold at 4°C	
PCR program															
45 seconds at 98°C	1 cycle														
10 seconds at 98°C															
15 seconds at 60°C	10 cycles														
3 minutes at 72°C															
5 minutes at 72°C															
Hold at 4°C															

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

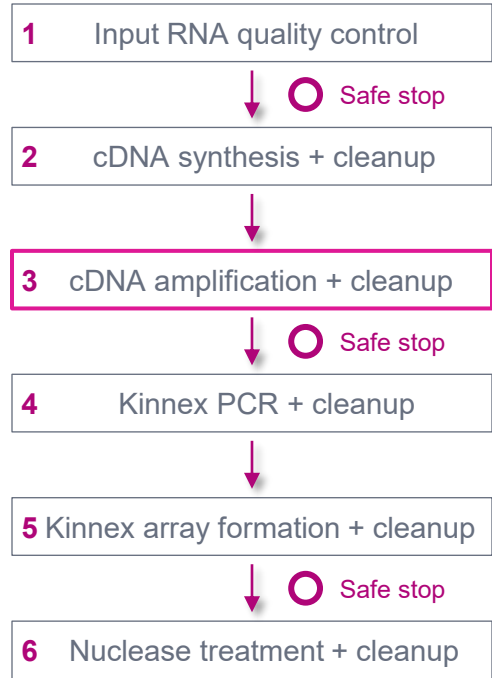
- Set up cDNA amplification reaction mix on ice
- Do not add Barcoded Forward Iso-Seq primers to master mix reaction

- Add desired Barcoded Forward Iso-Seq primer (select one of bc01-bc12) to each individual cDNA amplification reaction

- After setting up reactions on ice, add PCR reactions to thermal cycler after the lid has preheated to 105°C to avoid digestion of primers by polymerase exonuclease activity

# cDNA amplification + cleanup (cont.)

## Procedural notes



### 3.2 Cleanup of amplified cDNA using 0.9X SMRTbell Cleanup beads

Step	Instructions
3.2.1	Add 55 $\mu$ L of elution buffer to a new strip tube. Transfer 45 $\mu$ L of PCR-amplified cDNA from Section 3.1 into the strip tube containing elution buffer for a final volume of 100 $\mu$ L. Add 90 $\mu$ L (0.9x v/v) of resuspended, room-temperature SMRTbell cleanup beads. <b>The correct ratio of beads to sample is critical at this step.</b> If targeting longer cDNA, 86 $\mu$ L of SMRTbell cleanup beads (0.86x v/v) can be used.
3.2.2	Mix beads by pipetting 10 times or until evenly distributed.
3.2.14	Slowly pipette off the cleared supernatant without disturbing the beads. Transfer 24 $\mu$ L of the supernatant to a new strip tube. Discard the old strip tube with beads. <b>Recommended: Measure concentration and size distribution of each cDNA sample.</b>
3.2.15	<ul style="list-style-type: none"> <li>Take a 1 <math>\mu</math>L aliquot from each strip tube. Dilute each aliquot with 4 <math>\mu</math>L of elution buffer.</li> <li>Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit.</li> <li>Dilute 1:4 dilution further to 1.5 ng/<math>\mu</math>L based on the Qubit reading if needed.</li> <li>Run 1 <math>\mu</math>L on an Agilent Bioanalyzer using a High Sensitivity DNA kit.</li> </ul>
3.2.16	The expected recovery after cDNA amplification SMRTbell clean-up is >80 ng. A minimum of 55 ng of total cDNA is recommended to proceed with Kinnex PCR (Step 4). If less than 55 ng but more than 25 ng is recovered, proceed with Kinnex PCR but expect lower yields. Do not proceed with less than 25 ng.

• Perform **0.9X** SMRTbell bead cleanup<sup>1</sup>

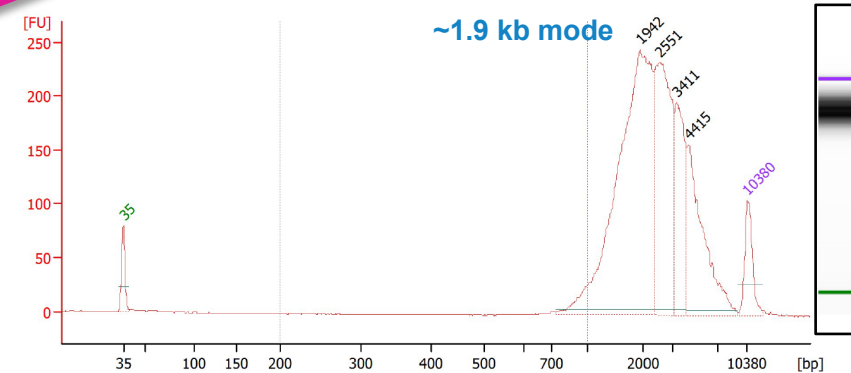
• Perform DNA concentration QC using Qubit ds DNA HS assay and DNA sizing QC using Bioanalyzer

• Expected yield of purified cDNA product is **>80 ng**  
 • Minimum cDNA amount needed to proceed with Kinnex PCR = **55 ng** (do not proceed with <25 ng)

### 3.3 Pooling barcoded cDNA (skip if not multiplexing)

Step	Instructions
3.3.1	Using the concentration reading from the Qubit fluorometer, pool an equal mass of each barcoded cDNA sample for a total mass of 55 ng. Store any remaining purified, amplified barcoded cDNA at 4°C for future use.
3.3.2	Quick-spin the tube strip in a microcentrifuge to collect liquid.
3.3.3	Proceed to next step of the protocol.

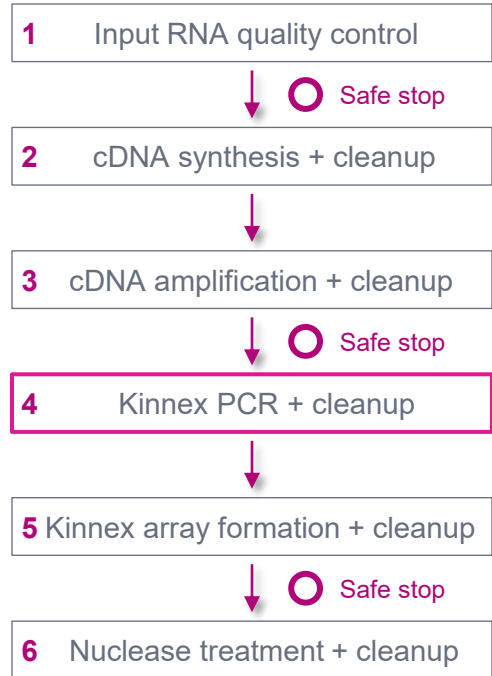
• If multiplexing, perform **equal-mass pooling** of each barcoded cDNA sample after 0.9X SMRTbell bead cleanup



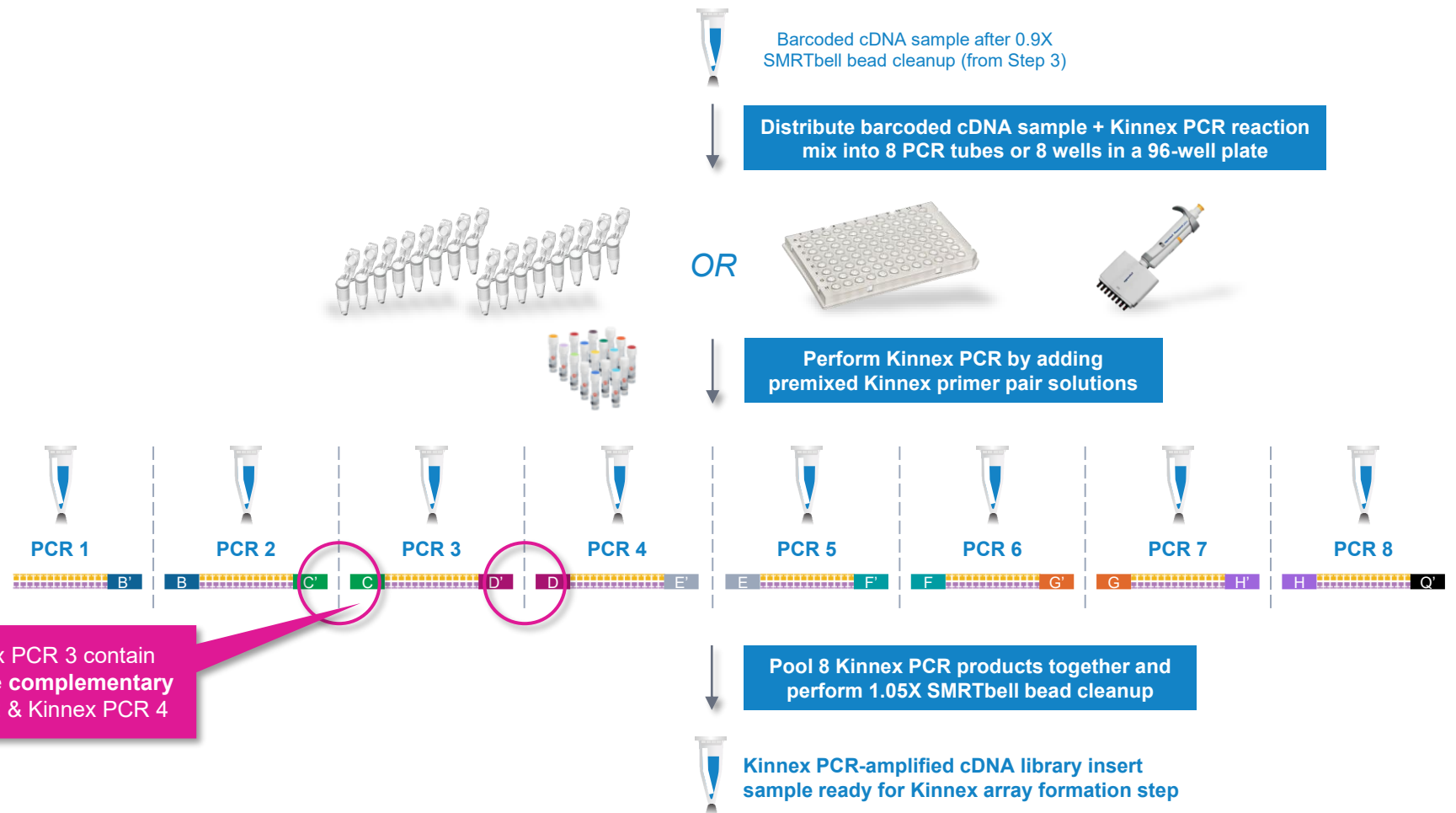
Example Bioanalyzer DNA sizing QC analysis results for amplified full-length cDNA generated from a universal human RNA reference (UHRR) total RNA sample.

# Kinnex PCR

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



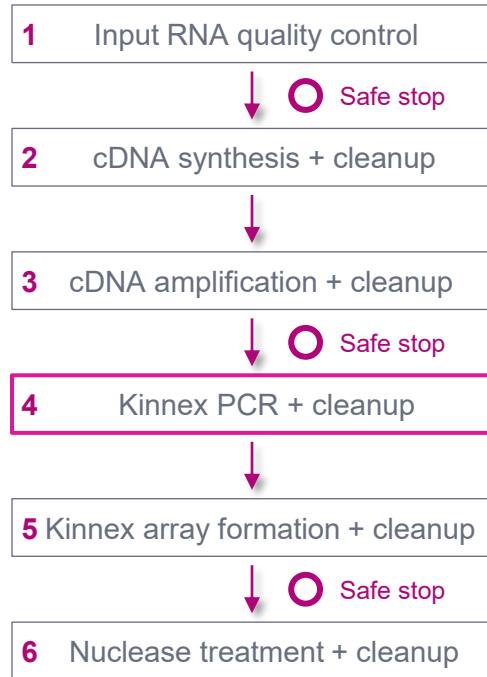
Set up 8 parallel Kinnex PCR reactions per sample with premixed Kinnex primers to generate amplified cDNA products containing programmable sequences at both ends



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

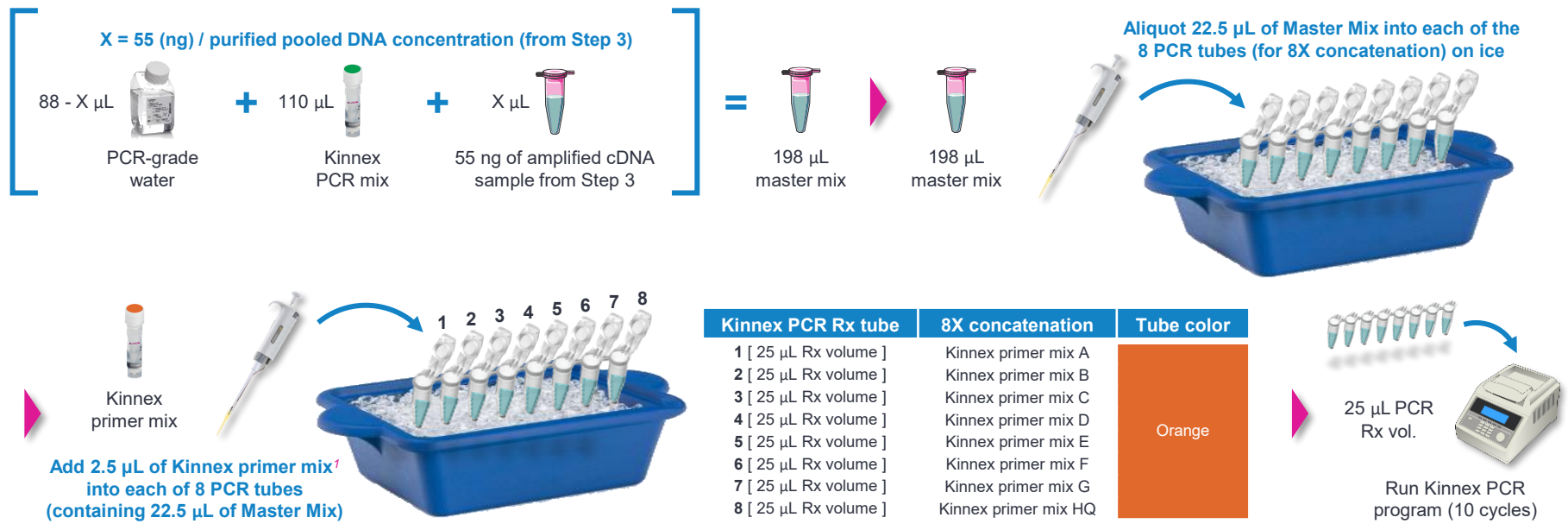
# Kinnex PCR (cont.)

## Procedural summary

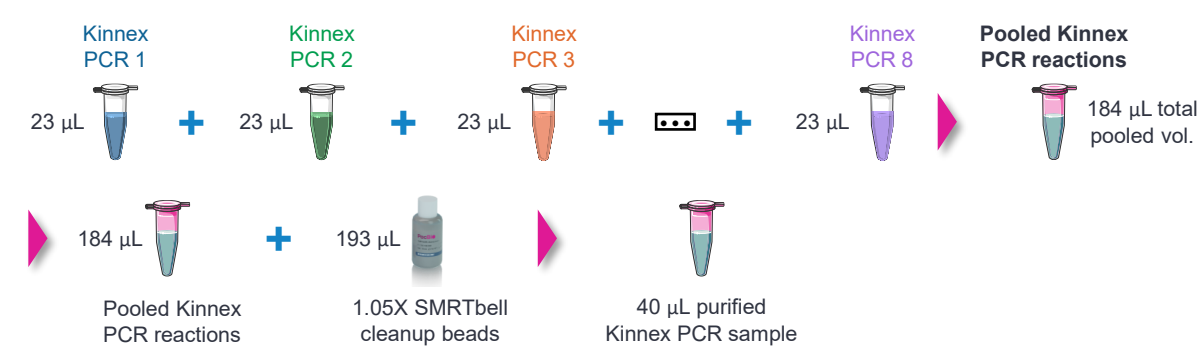


### Step 4 procedural summary

#### 4.1 Kinnex PCR



#### 4.2 Pooling of 8 Kinnex PCR products and 1.05X SMRTbell cleanup

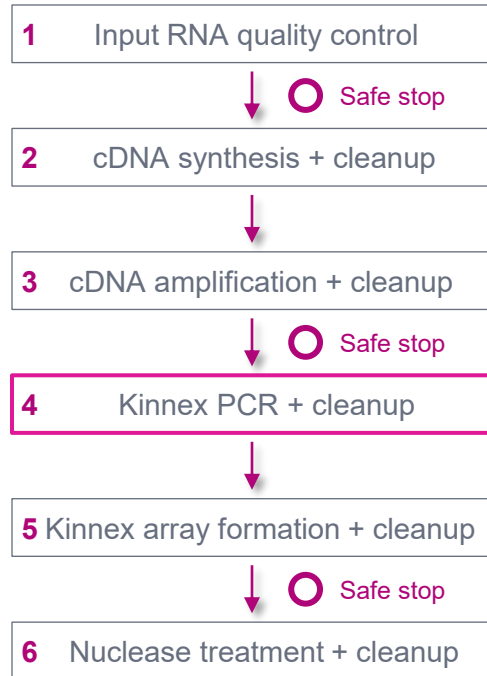


Proceed to **Step 5**

<sup>1</sup> Kinnex PCR 8-fold kit (103-071-600) contains eight different Kinnex primer mixes (Kinnex primer mix A/B/C/D/E/F/G/HQ).

# Kinnex PCR (cont.)

## Procedural notes



### 4.1 Kinnex PCR

✓ Step Instructions

4.1.1 Thaw primers. The entire volume of primers can be transferred to an 8-tube strip for ease of use with a multi-channel pipette.

	8X concatenation	Tube color	PN
1	Kinnex primer mix A	Orange	103-107-800
2	Kinnex primer mix B		103-107-900
3	Kinnex primer mix C		103-108-000
4	Kinnex primer mix D		103-108-100
5	Kinnex primer mix E		103-108-200
6	Kinnex primer mix F		103-108-300
7	Kinnex primer mix G		103-108-400
8	Kinnex primer mix HQ		103-108-500

4.1.2 Briefly vortex to mix, then quick-spin to collect liquid and place the primer mixes on ice.

4.1.3 Thaw the following components, briefly vortex to mix, then quick-spin to collect liquid and place on ice. Add the components **on ice** in a LoBind tube.

Master mix components	Volume for 8X concatenation*	
PCR-grade water	88-X µL	X = 55 (ng)/purified pooled DNA concentration from <a href="#">step 3.2.16</a> (single-plex) or <a href="#">step 3.3.3</a> (multiplex) *10% overage included
Kinnex PCR mix	110 µL	
55 ng of amplified cDNA from <a href="#">Step 3.2.16</a>	X µL	
Total volume	198 µL	

4.1.4 Aliquot 22.5 µL of Master Mix 1 into each of the 8 PCR tubes (for 8X concatenation) **on ice**.

4.1.5 Add 2.5 µL of Kinnex primer mix into each of 8 PCR tubes from step 4.1.4.

4.1.6 Thoroughly mix by pipetting up and down 10 times. Quick-spin to collect all liquid.

Set up the thermal cycler program listed below with the lid set to 105°C. **Keep sample(s) on ice until the lid is heated to 105°C.**

The duration of PCR is approximately 1 hour.

Step	Temperature	Duration	Cycles
4.1.7 Initial denaturation	98°C	3 min	1
Denaturation	98°C	20 s	

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

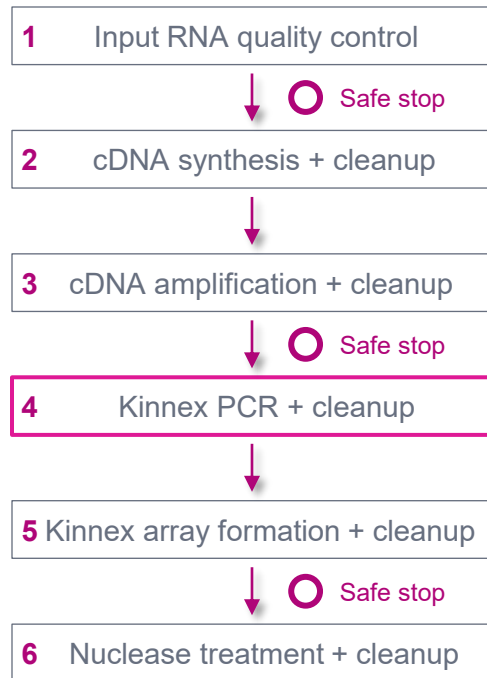
- Set up Kinnex PCR reactions **ON ICE**
- PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.

- **Critical step! Correct setup of all 8 Kinnex PCR reactions is required** – any missing/incorrect MAS primer pairs will result in no/low SMRTbell yield

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

# Kinnex PCR (cont.)

## Procedural notes



### 4.2 Pooling of 8 Kinnex PCR products and 1.05X SMRTbell cleanup

✓ Step	Instructions
4.2.1	Add exactly <b>23 μL</b> from each of the 8 PCR reactions into a 1.5 mL tube for a total volume of <b>184 μL</b> . An equal volume of each PCR product is necessary for efficient array assembly.
4.2.2	Add 193 μL (1.05X v/v) of resuspended, room-temperature SMRTbell cleanup beads to a tube of pooled Kinnex PCR amplicon. <b>The correct ratio of beads to pooled sample is critical at this step.</b>
4.2.3	Pipette-mix the beads until evenly distributed.
4.2.4	Quick-spin the tube in a microcentrifuge to collect liquid.
4.2.9	Repeat the previous step.
	Remove residual 80% ethanol:
4.2.10	<ul style="list-style-type: none"><li>Remove the tube from the magnetic separation rack.</li><li>Quick-spin the tube in a microcentrifuge.</li><li>Place the tube back in the magnetic separation rack until the beads separate fully from the solution.</li><li>Remove residual 80% ethanol and discard.</li></ul>
4.2.11	Remove the tube from the magnetic rack. <b>Immediately</b> add <b>40 μL</b> of <b>elution buffer</b> to the tube and resuspend the beads by pipetting 10 times or until evenly distributed.
4.2.12	Quick-spin the tube in a microcentrifuge to collect liquid.
4.2.13	Incubate at <b>room temperature</b> for <b>5 minutes</b> to elute DNA.
4.2.14	Place tube in a magnetic separation rack until beads separate fully from the solution.
4.2.15	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a <b>new tube strip</b> . Discard old tube with beads.
4.2.16	Make a 1:10 dilution of the sample in elution buffer and measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Typical yield is 6–18 μg.

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

- Pool **exactly 23 μL** from each Kinnex PCR reaction in a clean 1.5 mL DNA LoBind tube<sup>1</sup> for a total combined volume of 184 μL

- Add **exactly 193 μL** of SMRTbell cleanup beads (1.05X)
- Kinnex PCR mix significantly increases stringency of SMRTbell clean up beads, **so accurate pipetting is critical**

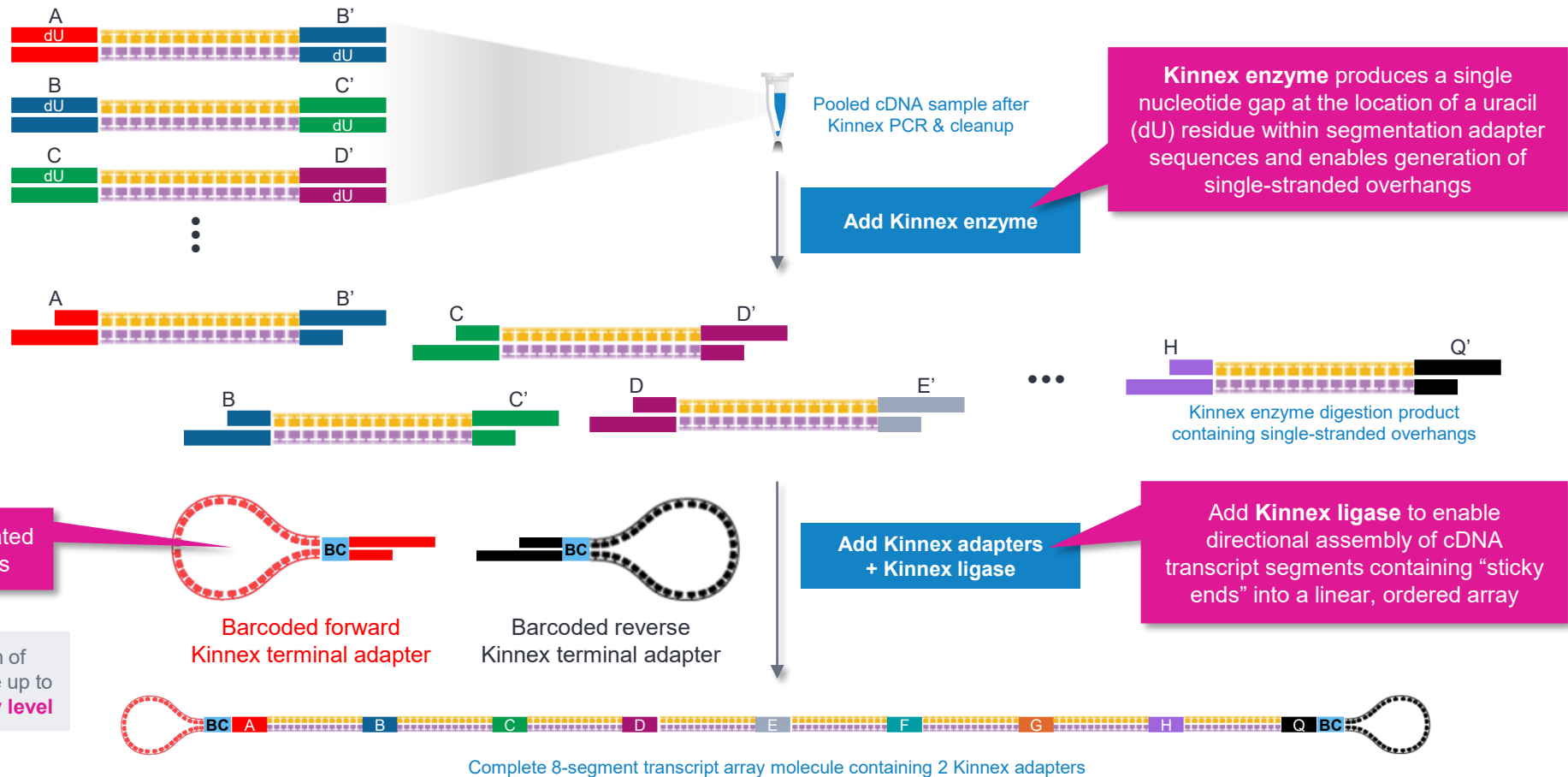
- Perform DNA concentration QC to verify there is sufficient yield of Kinnex PCR products (min. 4 μg) to proceed to Kinnex array formation step

# Kinnex array formation

In this step, assemble cDNA transcripts (“segments”) containing programmable ends into a linear array

- 1 Input RNA quality control
- ↓ ○ Safe stop
- 2 cDNA synthesis + cleanup
- ↓
- 3 cDNA amplification + cleanup
- ↓ ○ Safe stop
- 4 Kinnex PCR + cleanup
- ↓
- 5 **Kinnex array formation + cleanup**
- ↓ ○ Safe stop
- 6 Nuclease treatment + cleanup

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



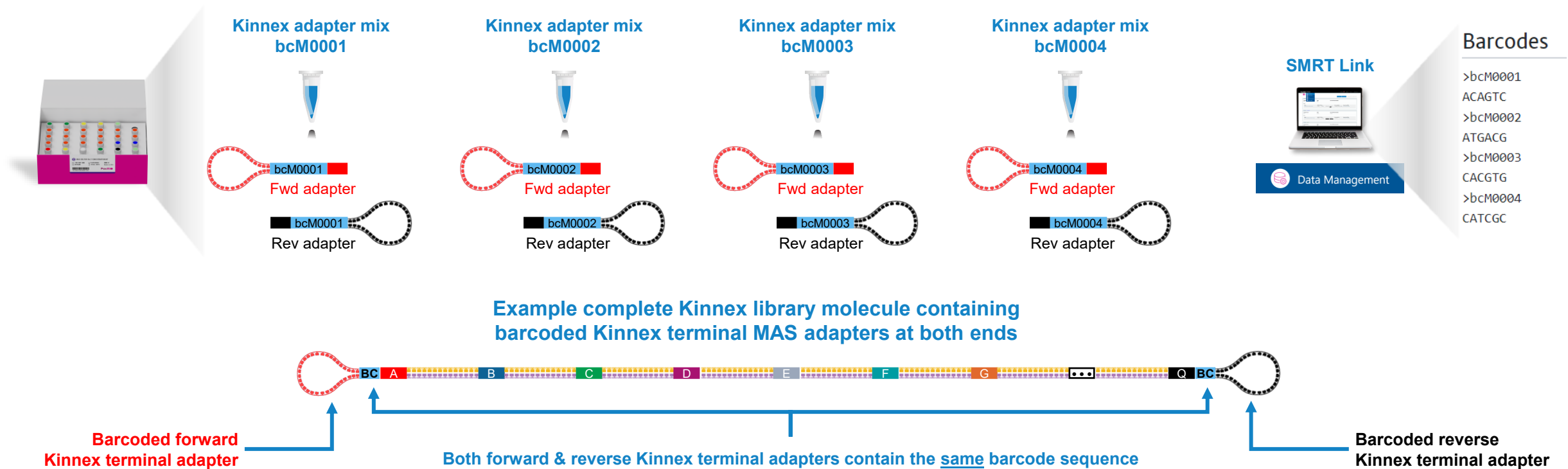
Barcoded Kinnex terminal adapters<sup>1</sup> are ligated to specific overhang sequences at array ends

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 array formation (cont.)

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

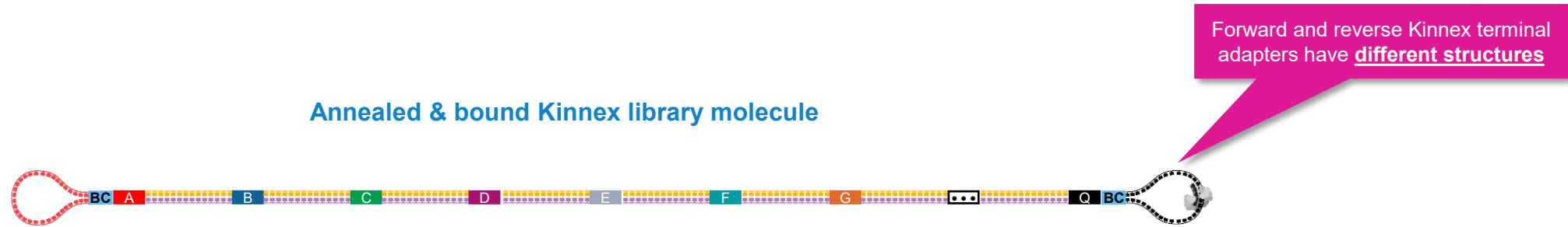
- Kinnex adapters contain **barcode sequences<sup>1</sup>** to enable (optional) sample multiplexing at the SMRTbell library level (**up to 4-plex**)
  - Forward and reverse Kinnex adapter pairs are pre-mixed in Kinnex concatenation kits
  - Kinnex concatenation kits contain a total of **4 barcoded Kinnex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



# 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

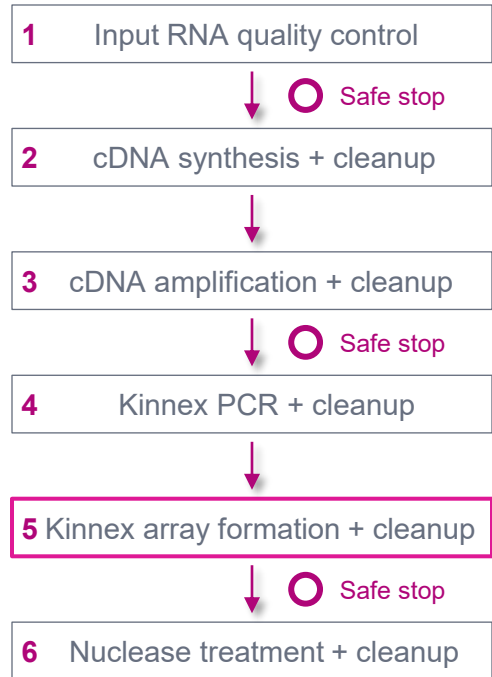


- Kinnex adapter design requires a **different sequencing primer (Kinnex sequencing primer<sup>1</sup>)** for annealing reaction



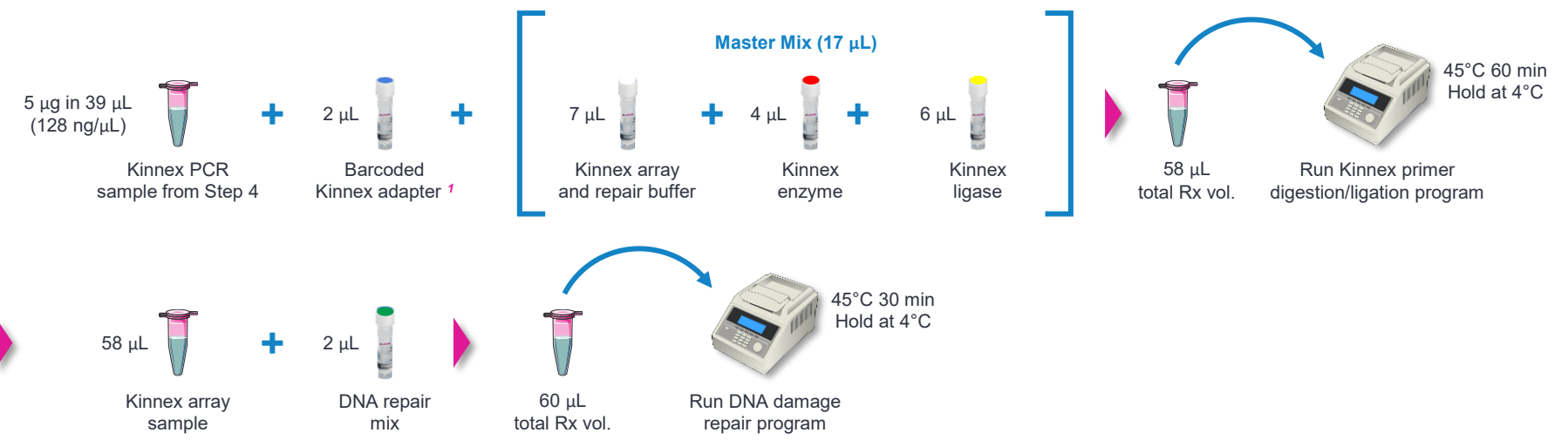
# Kinnex array formation (cont.)

## Procedural summary

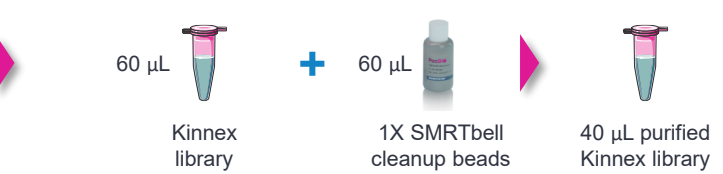


### Step 5 procedural summary

#### 5.1 Kinnex array formation



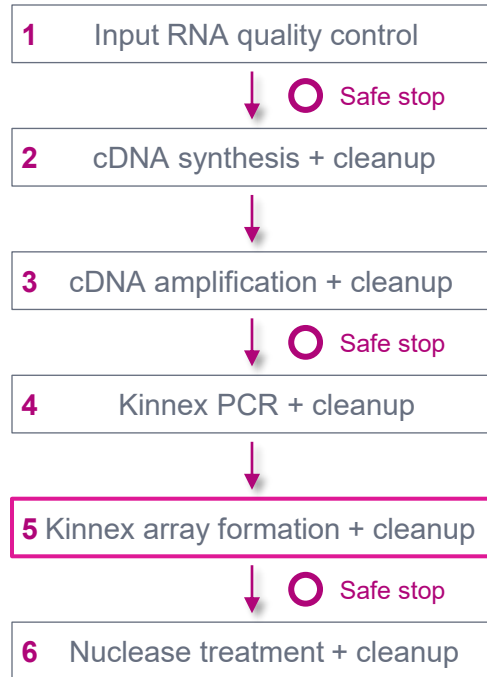
#### 5.2 1X SMRTbell cleanup



Proceed to **Step 6**

# Kinnex array formation (cont.)

## Procedural notes



## 5.1 Kinnex array formation

Step	Instructions									
5.1.0	Place the DNA damage repair mix on ice at all times and immediately return the DNA damage repair mix back to the freezer (-20°C) after use. Improper storage and handling of the DNA damage repair mix may result in poor library performance and should not be used for subsequent reactions.									
5.1.1	In a 0.2 mL PCR tube, add 5 µg of sample from <a href="#">Step 4.2.15</a> , in 39 µL of volume (128 ng/µL). Dilute with elution buffer going into this step if the sample is too concentrated. Add 2 µL of Kinnex adapter bc01-04 (select one barcode per library preparation). Note: if combining multiple barcoded Kinnex libraries for sequencing, make sure each library uses one of the 4 different Kinnex barcoded adapters.									
	<table border="1"><thead><tr><th>Tube color</th><th>Components</th><th>Volume</th></tr></thead><tbody><tr><td>Blue</td><td>Kinnex adapter bc</td><td>2 µL</td></tr></tbody></table>	Tube color	Components	Volume	Blue	Kinnex adapter bc	2 µL			
Tube color	Components	Volume								
Blue	Kinnex adapter bc	2 µL								
5.1.2	Add the following components in the listed order. If processing multiple samples, make a master mix with 10% overage. Pipette mix master mix.									
	Add 17 µL of master mix to the PCR tube containing sample and Kinnex adapter. Pipette-mix and run the Kinnex primer digestion/ligation program with the lid set to 55°C.									
5.1.3	<table border="1"><thead><tr><th>Step</th><th>Temperature</th><th>Duration</th></tr></thead><tbody><tr><td>1</td><td>45°C</td><td>60 min</td></tr><tr><td>2</td><td>4°C</td><td>Hold</td></tr></tbody></table>	Step	Temperature	Duration	1	45°C	60 min	2	4°C	Hold
Step	Temperature	Duration								
1	45°C	60 min								
2	4°C	Hold								
5.1.4	After running the Kinnex primer digestion/ligation program, add 2 µL of DNA repair mix directly to the Kinnex primer digestion/ligation sample. <table border="1"><thead><tr><th>Tube color</th><th>Components</th><th>Volume</th></tr></thead><tbody><tr><td>Green</td><td>DNA repair mix</td><td>2 µL</td></tr></tbody></table>	Tube color	Components	Volume	Green	DNA repair mix	2 µL			
Tube color	Components	Volume								
Green	DNA repair mix	2 µL								
5.1.5	Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid. Run the DNA Damage Repair Program with the lid set to >55°C.									
5.1.6	<table border="1"><thead><tr><th>Step</th><th>Temperature</th><th>Duration</th></tr></thead></table>	Step	Temperature	Duration						
Step	Temperature	Duration								

• **IMPORTANT!** Place DNA damage repair mix on ice at all times and immediately return the DNA damage repair mix back to the freezer (-20°C) after use

- Improper storage and handling of the DNA damage repair mix may result in poor library performance and should not be used for subsequent reactions

• Recommended input amount to proceed with Kinnex array formation is 5 µg of Kinnex PCR amplicons (from Step 4)

- 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

• Run Kinnex primer digestion/ligation program with heated lid set to 55°C.

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

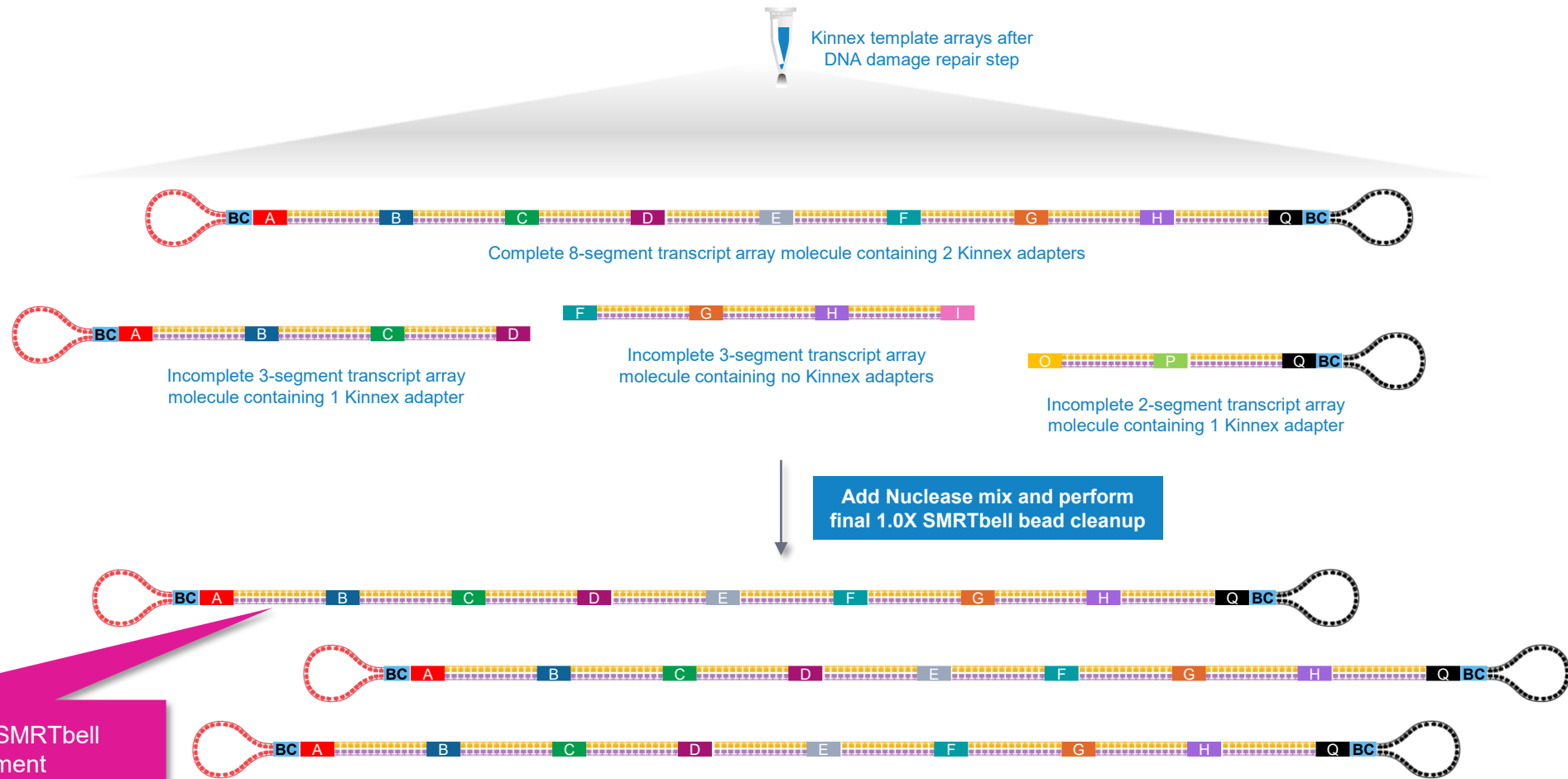
• After DNA Damage Repair step, perform **1X SMRTbell bead cleanup** at room temp. (**Step 5.2**)

- Add 1X v/v (60 µL) of resuspended, room temperature SMRTbell cleanup beads to each sample.

# Nuclease treatment & cleanup

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

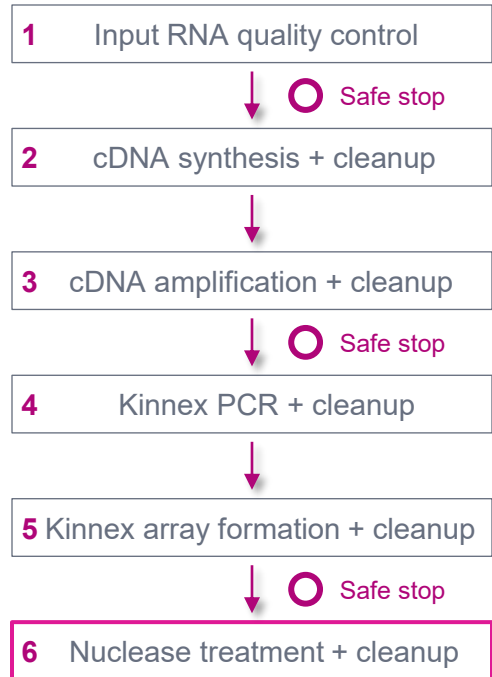
- 1 Input RNA quality control  
↓ ○ Safe stop
- 2 cDNA synthesis + cleanup  
↓
- 3 cDNA amplification + cleanup  
↓ ○ Safe stop
- 4 Kinnex PCR + cleanup  
↓
- 5 Kinnex array formation + cleanup  
↓ ○ Safe stop
- 6 Nuclease treatment + cleanup



• After nuclease treatment, most remaining SMRTbell templates are complete (full-length) 8-segment transcript array molecules capped with Kinnex adapters

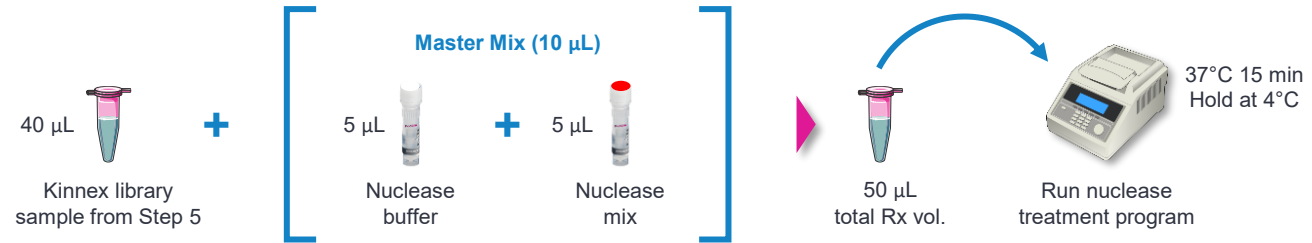
# Nuclease treatment & cleanup (cont.)

## Procedural summary

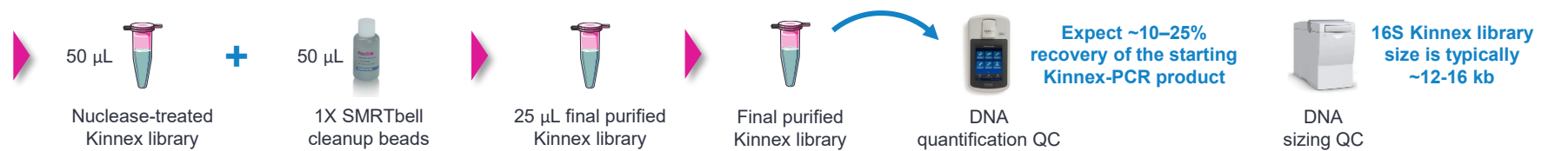


### Step 6 procedural summary

#### 5.1 Nuclease treatment



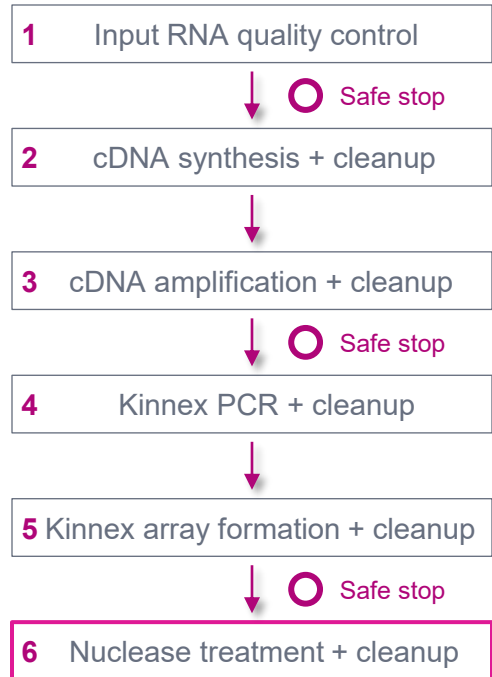
#### 5.2 1X SMRTbell cleanup



Proceed to **ABC<sup>1</sup>**

# Nuclease treatment & cleanup (cont.)

## Procedural notes



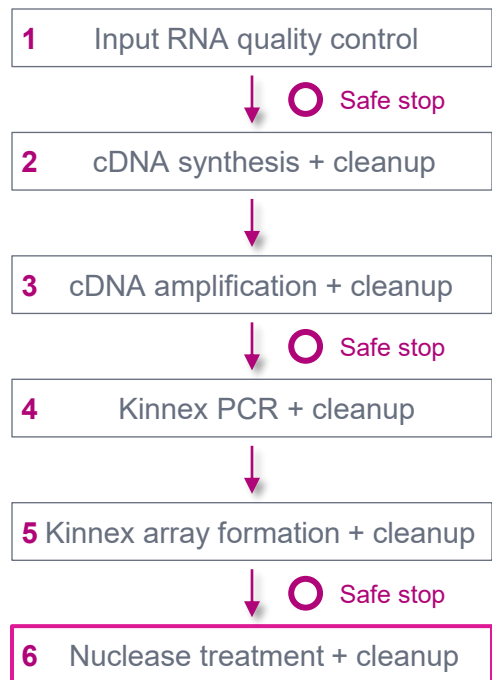
## 6.1 Nuclease treatment

✓ Step	Instructions															
	Add the following components listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. Pipette mix master mix. For individual preps, add components directly to each sample from the previous step in the order and volume listed below.															
6.1.1	<table border="1"><thead><tr><th colspan="3">Nuclease master mix</th></tr><tr><th>Tube</th><th>Component</th><th>Volume</th></tr></thead><tbody><tr><td>Light purple</td><td>Nuclease buffer</td><td>5 µL</td></tr><tr><td>Light green</td><td>Nuclease mix</td><td>5 µL</td></tr><tr><td colspan="2">Total volume</td><td>10 µL</td></tr></tbody></table>	Nuclease master mix			Tube	Component	Volume	Light purple	Nuclease buffer	5 µL	Light green	Nuclease mix	5 µL	Total volume		10 µL
Nuclease master mix																
Tube	Component	Volume														
Light purple	Nuclease buffer	5 µL														
Light green	Nuclease mix	5 µL														
Total volume		10 µL														
6.1.2	Add 10 µL of Nuclease Master mix to each sample. The total volume should be 50 µL.															
6.1.3	Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid.															
6.1.4	Run the nuclease treatment program with the lid set to >47°C. <table border="1"><thead><tr><th>Step</th><th>Temperature</th><th>Duration</th></tr></thead><tbody><tr><td>1</td><td>37°C</td><td>15 min</td></tr><tr><td>2</td><td>4°C</td><td>Hold</td></tr></tbody></table>	Step	Temperature	Duration	1	37°C	15 min	2	4°C	Hold						
Step	Temperature	Duration														
1	37°C	15 min														
2	4°C	Hold														

- Perform nuclease treatment for 15 min

# Nuclease treatment & cleanup (cont.)

## Procedural notes



### 6.2 Final cleanup with 1X SMRTbell cleanup beads

✓ Step	Instructions
6.2.1	Add 50 $\mu\text{L}$ SMRTbell cleanup beads to each sample from the previous step. Pipette-mix the beads until evenly distributed.
6.2.2	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
6.2.3	Incubate at room temperature for 10 minutes to allow DNA to bind the beads.
6.2.4	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
6.2.5	Slowly remove the cleared supernatant without disturbing the beads and discard the supernatant.
6.2.6	Slowly dispense 200 $\mu\text{L}$ , or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
6.2.7	Repeat the previous step.
	Remove residual 80% ethanol:
6.2.8	<ul style="list-style-type: none"><li>Remove the tube strip from the magnetic separation rack.</li><li>Quick-spin the tube strip in a microcentrifuge.</li><li>Place the tube strip back in a magnetic separation rack until beads separate fully from</li></ul>
6.2.14	Take a 1 $\mu\text{L}$ aliquot from each tube. Make a 1:5 dilution of the sample in elution buffer and measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Calculate the total mass. Expect 10-25% recovery of the starting Kinnex-PCR product.
	Recommended: Further dilute each aliquot to 250 $\text{pg}/\mu\text{L}$ with the Femto Pulse dilution buffer. Measure the final SMRTbell library size distribution with a Femto Pulse system.
6.2.15	If required, normalize 25 $\mu\text{L}$ of library to <math><60 \text{ ng}/\mu\text{L}</math>. Using a concentration above 60 $\text{ng}/\mu\text{L}</math> into ABC will result in poor sequencing performance.$
6.2.16	Proceed to Section 7 to prepare library for sequencing with Vega or Revio (+SPRQ chemistry). or For sequencing on Revio (non-SPRQ) or Sequel II/e, proceed to SMRT Link Sample Setup for preparing samples.
6.2.17	Store SMRTbell libraries at 4°C if sequencing within two weeks. Store long-term at -20°C.



- Perform **DNA concentration QC** on final purified Kinnex RNA library using a Qubit dsDNA HS assay
  - Typical final SMRTbell library yield from 5  $\mu\text{g}$  of input DNA into Kinnex array formation is **~10 – 25%** – 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 full-length RNA library using a Femto Pulse system (expected final library insert size is **~12 – 16 kb**)

- Kinnex full-length RNA final SMRTbell library concentration must be  **$\leq 60 \text{ ng}/\mu\text{L}$**  to proceed with sample setup (ABC<sup>1</sup>) [ Step 7 ]

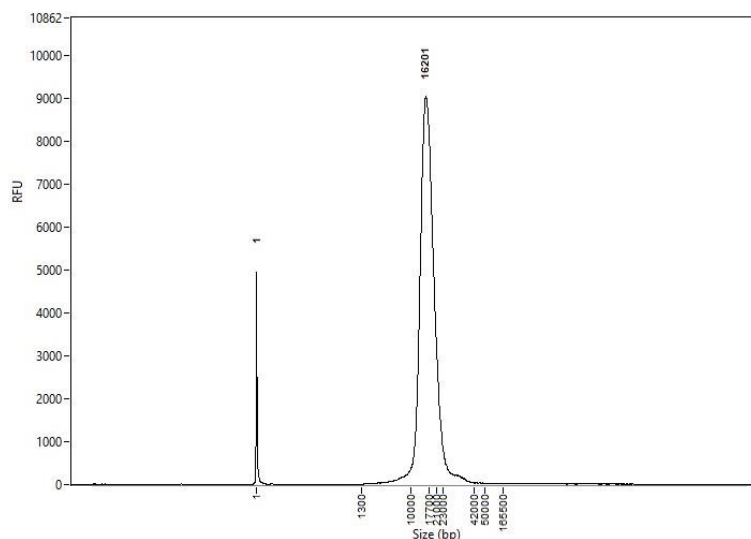
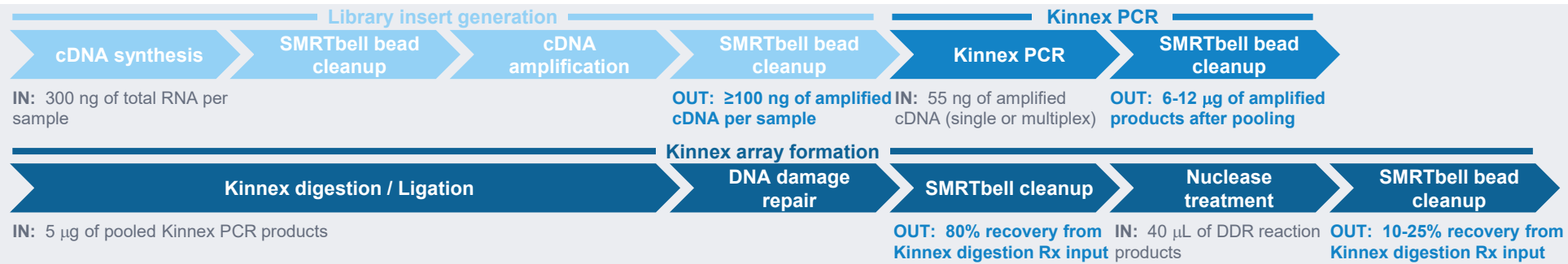
→ Using a concentration above 60  $\text{ng}/\mu\text{L}$  will result in lower loading during sequencing

# Kinnex full-length RNA library prep inputs & expected step yields

Final Kinnex library yield is typically sufficient to load  $\geq 2$  SMRT Cells

Kinnex FL RNA

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



Example Femto pulse DNA sizing QC analysis results for final Kinnex full-length RNA library prepared with human universal human reference RNA (UHRR) total RNA sample.

## Example Kinnex full-length RNA library prep yields

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

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

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

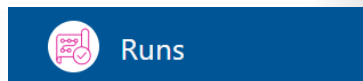
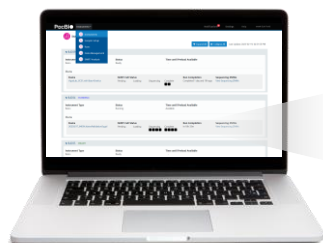
- >8 SMRT Cells for Revio using SPRQ chemistry
- >2 SMRT Cells for the Vega or Revio (non-SPRQ) systems
- >4 SMRT Cells for Sequel II/IIe systems

# **Kinnex full-length RNA sequencing preparation workflow details**

# Sample Setup & Run Design recommendations for Kinnex libraries

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

Select desired Kinnex application type in SMRT Link run design page



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

## Kinnex single-cell RNA<sup>1</sup>

### 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<sup>2</sup>

### 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<sup>2</sup>

### Whole genome sequencing

- Human WGS
- Microbial assembly
- Other WGS

### RNA sequencing

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

### Viral sequencing

- Adeno-associated virus

### Metagenomics

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

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

# SMRT Link Run Design procedure for **Revio and Vega systems**

## Sample and run information

### Kinnex single-cell RNA

### Kinnex full-length RNA

### Kinnex 16S rRNA

▼ 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 <small>Required</small>	Kinnex single-cell RNA	Application <small>Required</small>	Kinnex full-length RNA	Application <small>Required</small>	Kinnex 16S rRNA
Plate Well <small>Required</small>	Plate 1, Well A01	Plate Well <small>Required</small>	Plate 1, Well A01	Plate Well <small>Required</small>	Plate 1, Well A01
Well Name <small>Required</small>		Well Name <small>Required</small>		Well Name <small>Required</small>	
Well Comment		Well Comment		Well Comment	
Library Type <small>Required</small>	Kinnex	Library Type <small>Required</small>	Kinnex	Library Type <small>Required</small>	Kinnex
Insert Size (bp) <small>Required</small>	15000	Insert Size (bp) <small>Required</small>	16000	Insert Size (bp) <small>Required</small>	17000
Library Concentration (pM) <small>Required</small>		Library Concentration (pM) <small>Required</small>		Library Concentration (pM) <small>Required</small>	
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 = Kinnex library type (instead of Standard or AAV)<sup>1</sup>

### 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<sup>1</sup>

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

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

## Sample indexing (barcoding) information

### Kinnex single-cell RNA

### Kinnex full-length RNA

### Kinnex 16S rRNA

**Samples**

Sample is indexed  YES  NO

**Indexes** Required: MAS adapter indexes

**Biosample names** Required: Interactively | From a File

**Samples**

Sample is indexed  YES  NO

**Indexes** Required: MAS adapter indexes

**Biosample names** Required: Interactively | From a File

**Samples**

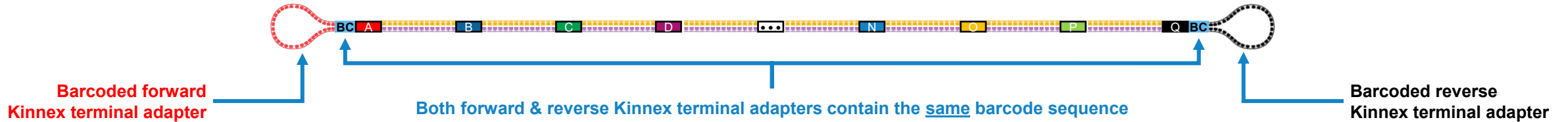
Sample is indexed  YES  NO

**Indexes** Required: MAS adapter indexes

**Biosample names** Required: Interactively | From a File

Default Indexes FASTA = MAS adapter indexes

Example complete Kinnex library molecule containing barcoded Kinnex terminal MAS adapters<sup>1</sup> at both ends



Example interactive biosample name specification

#### Barcode Selector and Sample Name Editor

Available Barcodes	Included Barcodes				
<input type="checkbox"/> bcM0001--bcM0001 <input type="checkbox"/> bcM0002--bcM0002 <input type="checkbox"/> bcM0003--bcM0003 <input type="checkbox"/> bcM0004--bcM0004	<table border="1"> <thead> <tr> <th>Barcode ID</th> <th>Bio Sample ID</th> </tr> </thead> <tbody> <tr> <td> </td> <td> </td> </tr> </tbody> </table>	Barcode ID	Bio Sample ID		
Barcode ID	Bio Sample ID				

#### Barcode Selector and Sample Name Editor

Available Barcodes	Included Barcodes						
<input type="checkbox"/> bcM0003--bcM0003 <input type="checkbox"/> bcM0004--bcM0004	<table border="1"> <thead> <tr> <th>Barcode ID</th> <th>Bio Sample ID</th> </tr> </thead> <tbody> <tr> <td>bcM0001--bcM0001</td> <td>Kinnex adapter-barcoded library 1</td> </tr> <tr> <td>bcM0002--bcM0002</td> <td>Kinnex adapter-barcoded library 2</td> </tr> </tbody> </table>	Barcode ID	Bio Sample ID	bcM0001--bcM0001	Kinnex adapter-barcoded library 1	bcM0002--bcM0002	Kinnex adapter-barcoded library 2
Barcode ID	Bio Sample ID						
bcM0001--bcM0001	Kinnex adapter-barcoded library 1						
bcM0002--bcM0002	Kinnex adapter-barcoded library 2						

SMRT Link



#### MAS SMRTbell barcoded adapter indexes

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

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

## Data options

### Kinnex single-cell RNA

### Kinnex full-length RNA

### Kinnex 16S rRNA

▼ Data Options

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

Include Base Kinetics  YES  NO

Consensus Mode  MOLECULE  STRAND

Assign Data To Project

Default Consensus  
Mode = **MOLECULE**<sup>1</sup>

▼ Data Options

Include Base Kinetics  YES  NO

Consensus Mode  MOLECULE  STRAND

Assign Data To Project

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

▼ Data Options

Include Base Kinetics  YES  NO

Consensus Mode  MOLECULE  STRAND

Assign Data To Project

# SMRT Link Run Design analysis options

## Kinnex single-cell RNA

## Kinnex full-length RNA

## Kinnex 16S rRNA

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

Analysis Options

Analysis Options

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

Primer Set Required

Reference Set Required

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

[Advanced Parameters](#)

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

Primer Set Required

Reference Set

Cluster of Barcoded Samples

[Advanced Parameters](#)

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

[Advanced Parameters](#)

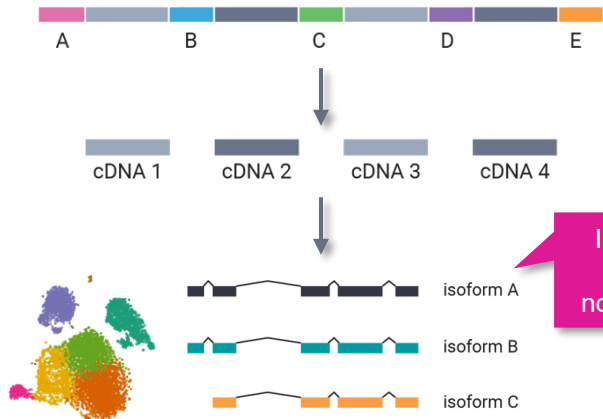
Analysis Workflow is automatically filled in

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

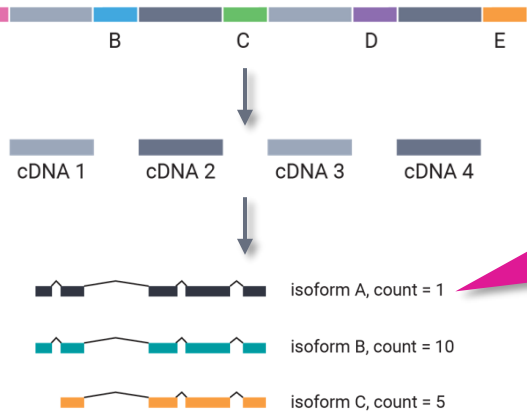
### Read Segmentation and Single-Cell Iso-Seq

### Read Segmentation and Iso-Seq

### Read Segmentation



Isoform-classification analysis to identify novel genes & isoforms



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

# SMRT Link Run Design analysis options (cont.)

## Kinnex single-cell RNA

**Analysis Options**

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

Primer Set Required

Reference Set Required

Kit Type ?  10X 3' KIT  10X 5' KIT

[Advanced Parameters](#)

## Kinnex full-length RNA

**Analysis Options**

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

Primer Set Required

Reference Set

Cluster of Barcoded Samples ?

[Advanced Parameters](#)

## Kinnex 16S rRNA

**Analysis Options**

Add Analysis  YES  NO

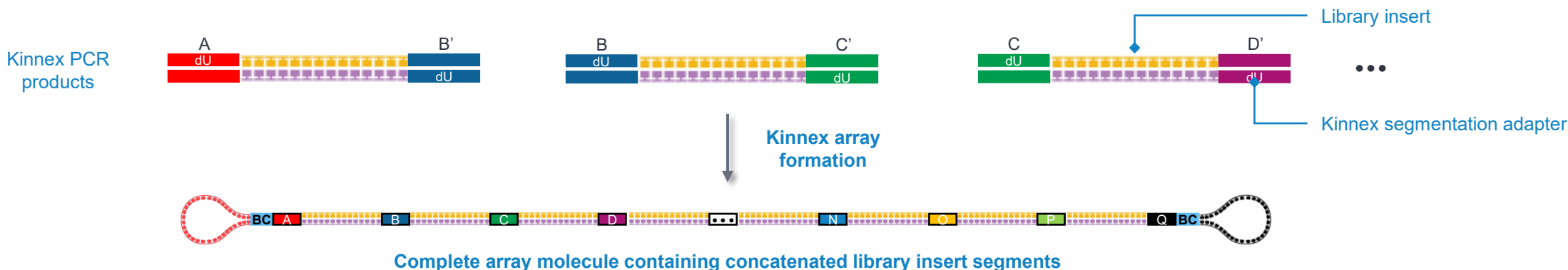
Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set

[Advanced Parameters](#)

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



# SMRT Link Run Design analysis options (cont.)

## Kinnex single-cell RNA

## Kinnex full-length 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

**Analysis Options**

Add Analysis  YES  NO

Analysis Name Required: Kinnex\_Full-Length\_RNA\_Demo\_Analysis\_Job\_Name

Analysis Workflow Required: Read Segmentation and Iso-Seq

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

Primer Set Required: Iso-Seq v2 Barcoded cDNA Primers

Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples  Pool reads and cluster together

Advanced Parameters

Specify Primer Set used for cDNA amplification

Specify Reference Set. Default sets are:

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

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



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

OR

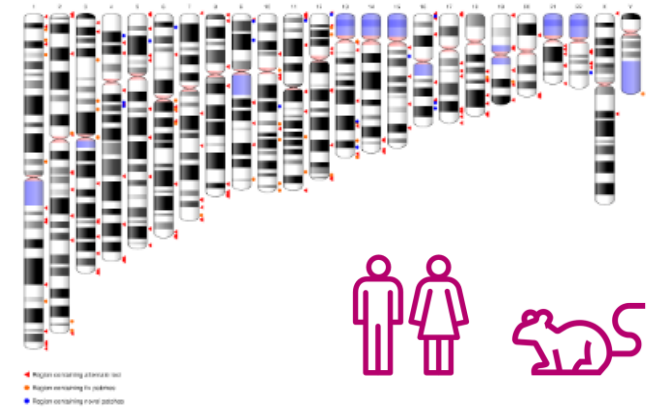


For Kinnex single-cell 5' RNA analysis, select '10x Chromium single cell 5' cDNA primers'



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

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



# SMRT Link Run Design analysis options (cont.)

## Kinnex single-cell RNA

**Analysis Options**

Add Analysis  YES  NO

Analysis Name Required: Kinnex\_Single-Cell\_RNA\_Demo\_Analysis\_Job\_Name

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

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

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

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

Kit Type  10x 3' KIT  10x 5' KIT

Advanced Parameters

Specify Kit Type used for single-cell cDNA generation

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

5' [CBC][UMI]TTTTTTTTT LIBRARY INSERT CCC TSO 3'  
 3' [CBC][UMI]AAAAAAAAA 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: [blank]

Select Analysis Workflow Required: [blank]

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS8)

Primer Set Required: 10x 2 Barcoded cDNA Primers

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

Cluster of Barcoded Samples  Pool reads and cluster together

Advanced Parameters

Specify how to perform read clustering for barcoded samples

Pool reads and cluster together

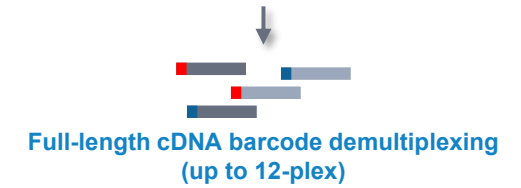
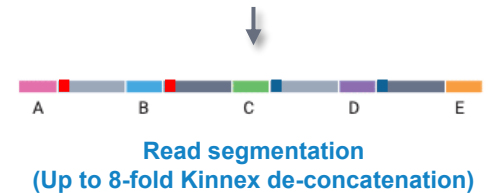
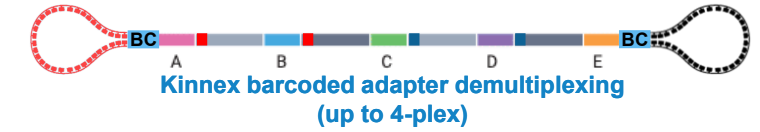
-- select --

Cluster reads separately

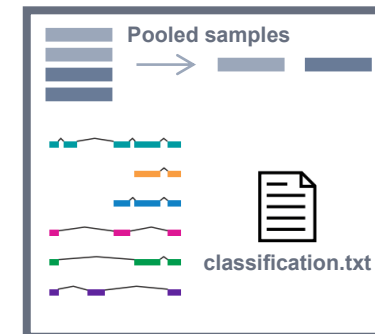
Pool reads and cluster together

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

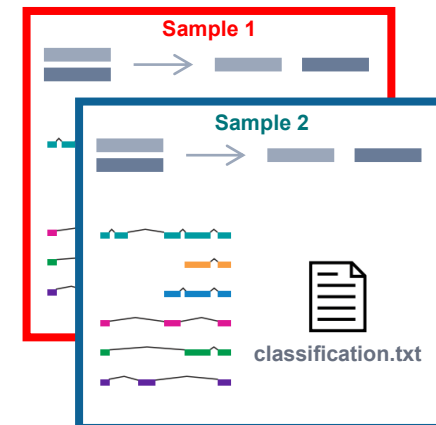
## Clustering options for Kinnex full-length RNA samples



### Analyze pooled samples



### Analyze by sample (default)



# SMRT Link Read Segmentation and Iso-Seq analysis video demonstration

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

**Analysis Application** Required

Read Segmentation and Iso-Seq

Import Analysis Settings Export

**Associated Inputs**

**Segmentation Adapter Set**

MAS-Seq Adapter v3 (MAS8)

**Primer Set** Required

Iso-Seq v2 Barcoded cDNA Primers

**Reference Set**

Human Genome hg38, with Gencode v39 annotations

**Cluster of Barcoded Samples**

Pool reads and cluster together

Advanced Parameters

**Analysis Name**

test


**Analysis Datasets**

Displaying rows 1 to 1 out of 1

ID	Name
21...	3230211_KPoS_64007_...

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

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



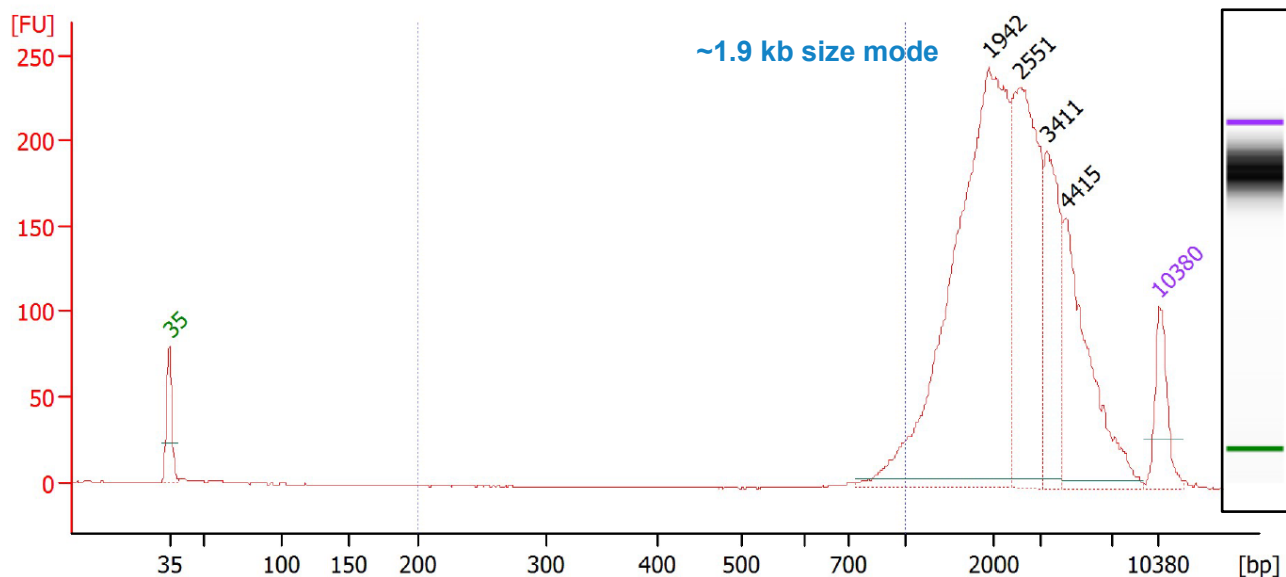


# Kinnex full-length RNA example sequencing performance data

# Example Kinnex full-length RNA library preparation QC results

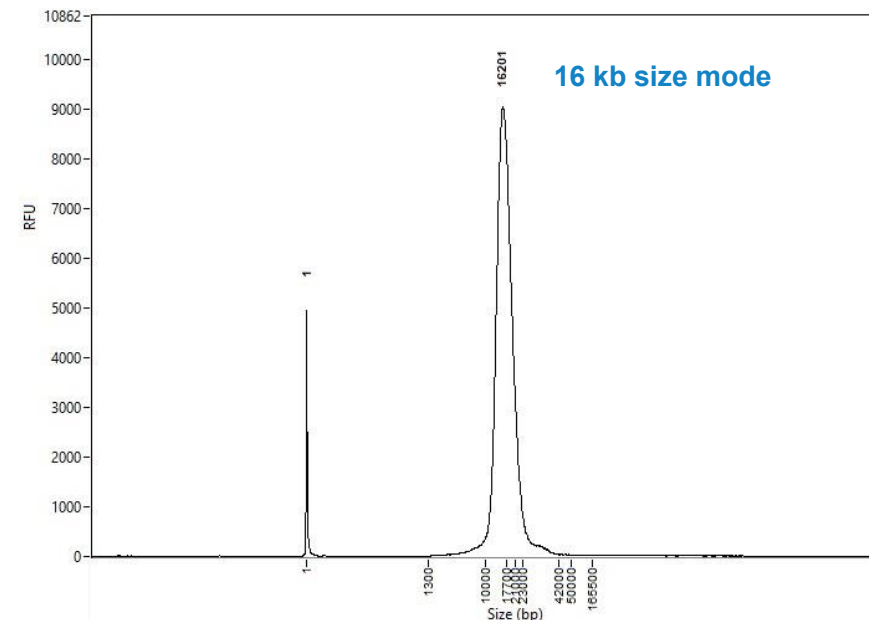
Kinnex full-length RNA library prepared with human UHRR total RNA

## Amplified full-length cDNA QC



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

## Final Kinnex full-length RNA library QC



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

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

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

Total RNA input for cDNA synthesis	300 ng
------------------------------------	--------

cDNA input for Kinnex array formation	5900 ng
---------------------------------------	---------

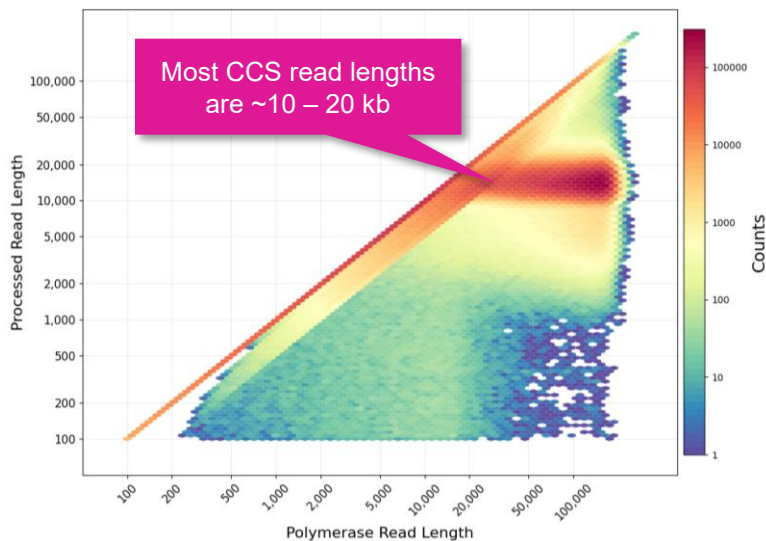
Post-nuclease treatment & final library cleanup yield (%) <sup>1</sup>	1460 ng (24.7%)
--	-----------------

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

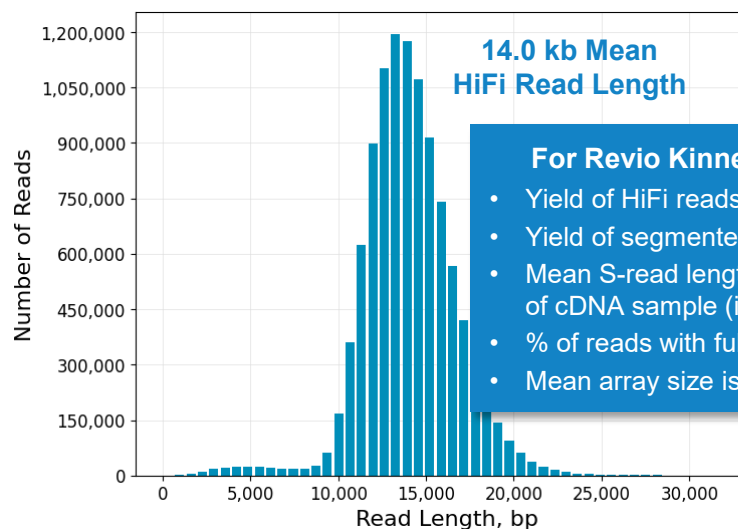
# Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA [ Revio system + SPRQ chemistry<sup>1</sup> ]

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

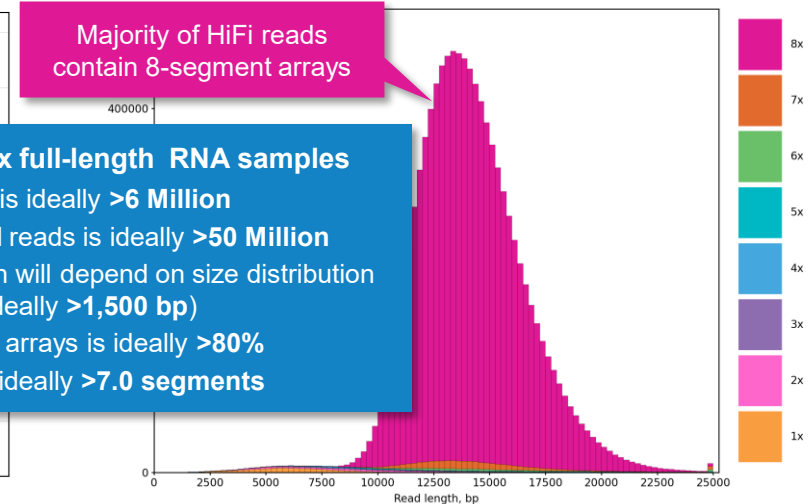
## Raw Data Report



## HiFi Read Length



## Read Segmentation Metrics



**For Revio Kinnex full-length RNA samples**

- Yield of HiFi reads is ideally >6 Million
- Yield of segmented reads is ideally >50 Million
- Mean S-read length will depend on size distribution of cDNA sample (ideally >1,500 bp)
- % of reads with full arrays is ideally >80%
- Mean array size is ideally >7.0 segments

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

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

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

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

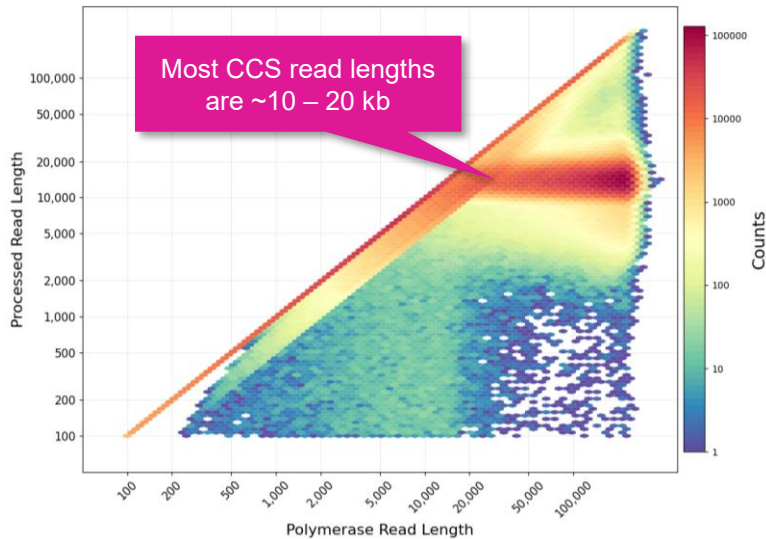
Input HiFi Reads	10,404,037
Segmented reads (S-reads)	81,012,128
Mean length of S-reads	1,758 bp
Percent of reads with full arrays	94.55%
Mean array size (concentration factor)	7.79

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

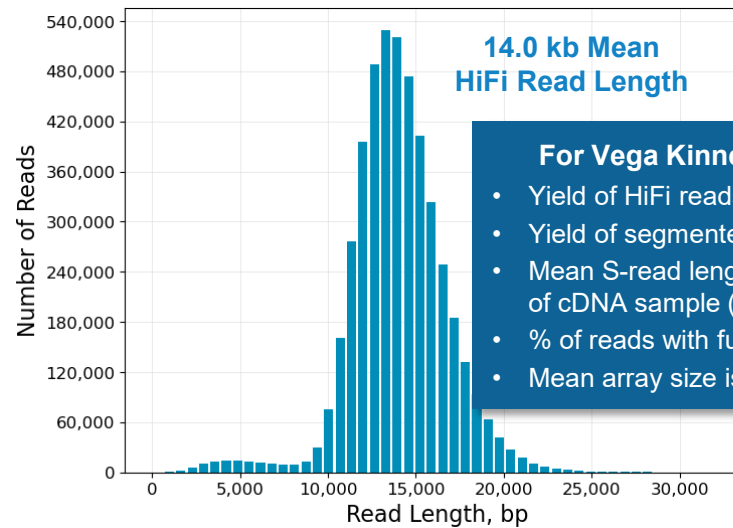
# Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA [ Vega system<sup>1</sup> ]

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

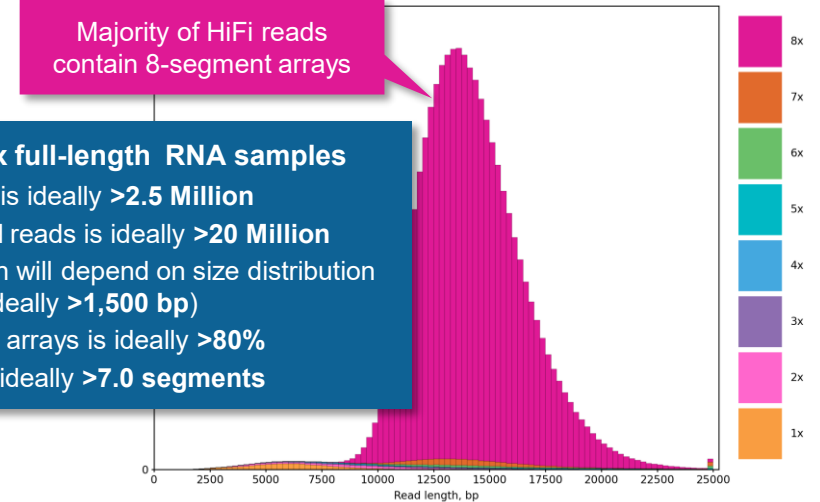
## Raw Data Report



## HiFi Read Length



## Read Segmentation Metrics



**For Vega Kinnex full-length RNA samples**

- Yield of HiFi reads is ideally >2.5 Million
- Yield of segmented reads is ideally >20 Million
- Mean S-read length will depend on size distribution of cDNA sample (ideally >1,500 bp)
- % of reads with full arrays is ideally >80%
- Mean array size is ideally >7.0 segments

Mean Polymerase Read Length	95.3 kb
Loading level	61%

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

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

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

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

For UHRR Kinnex libraries, per-Vega SMRT Cell segmentation read counts were typically >20 Million.

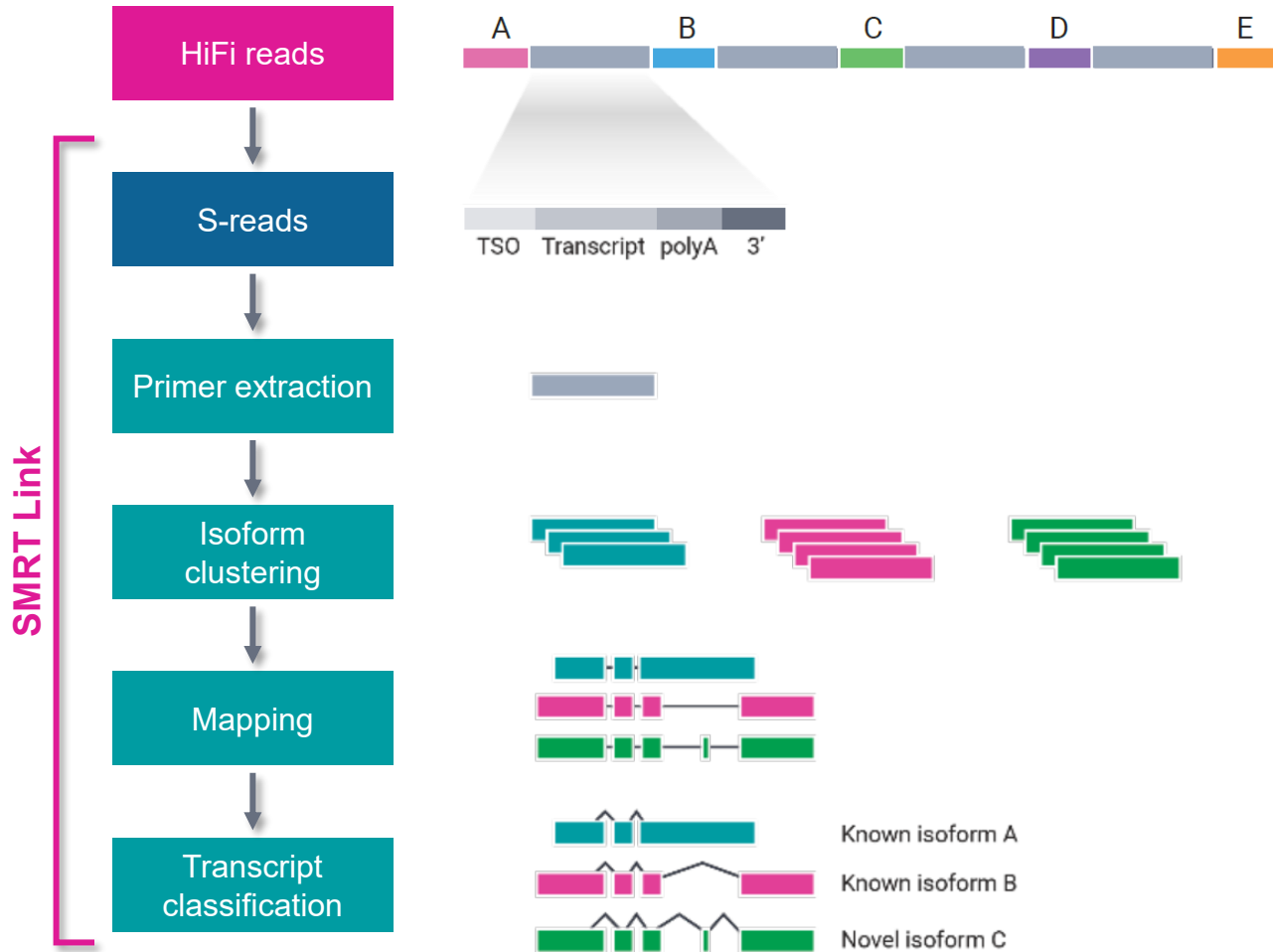


# Kinnex full-length RNA data analysis workflow overview

# Kinnex full-length RNA bioinformatics workflow overview

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

## SMRT Link read segmentation and Iso-Seq workflow<sup>1</sup>



## Read segmentation

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

## Primer extraction

- Primers and polyA tails are removed, but also used to orient the read into 5' → 3' orientation

## Isoform clustering

- FLNC reads are clustered by their sequencing similarity to produce isoform consensus sequences
- This step is the last step of Iso-Seq analysis if no genome is provided

## Mapping

- If a genome is provided, isoform consensus sequences from the previous step are mapped and further collapsed by their exonic structures to produce isoforms as GFF files for visualization

## Transcript classification<sup>2</sup>

- If an annotation (e.g., Gencode) is provided, isoforms are classified against it using pigeon (the PacBio implementation of SQANTI3) to identify known and novel genes/isoforms
- The Iso-Seq workflow can jointly analyze pooled sample reads to produce a unified isoform annotation with per-sample read counts, both raw and normalized as counts per million (CPM)

# SMRT Link Read Segmentation and Iso-Seq analysis video demonstration

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

**Analysis Application** Required

Read Segmentation and Iso-Seq

Import Analysis Settings | Export

**Associated Inputs**

**Segmentation Adapter Set**

MAS-Seq Adapter v3 (MAS8)

**Primer Set** Required

Iso-Seq v2 Barcoded cDNA Primers

**Reference Set**

Human Genome hg38, with Gencode v39 annotations

**Cluster of Barcoded Samples**

Pool reads and cluster together

Advanced Parameters

**Analysis Name**

test


**Analysis Datasets**

Displaying rows 1 to 1 out of 1

ID	Name
21...	3230211_KPoS_64007_...

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

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



# Kinnex full-length RNA bioinformatics workflow recommendations

SMRT Link Read Segmentation and Iso-Seq workflow common considerations and recommendations for analysis of Kinnex full-length RNA data<sup>1</sup>

## Analysis recommendations for Iso-Seq data based on reference genome and annotation availability<sup>1</sup>

- With SMRT Link v13, the **Read segmentation and Iso-Seq workflow** analysis application supports **human and mouse** reference genomes and annotations to produce classified isoforms with read counts.
- If working with **other organisms**, see table below for analysis recommendations

Available reference or annotation	Analysis workflow recommendation
Human or mouse	<ul style="list-style-type: none"><li>• Use the Iso-Seq workflow with preloaded human / mouse annotation to get mapped, unique isoforms with classifications and read count information (FASTA, GFF, TXT).</li></ul>
Model organism with good annotation	<ul style="list-style-type: none"><li>• Run Iso-Seq workflow with uploaded reference genome to get mapped, unique isoforms (FASTA, GFF)</li><li>• Generate <a href="#">pigeon-compliant annotation</a> and use the command line for isoform classification with read count information (TXT)</li></ul>
Non-model organism with genome	<ul style="list-style-type: none"><li>• Run Iso-Seq workflow with uploaded reference genome to get mapped, unique isoforms (FASTA, GFF)</li></ul>
No genome	<ul style="list-style-type: none"><li>• Run Iso-Seq workflow without reference genome to get unique isoforms (FASTA)</li></ul>

# Kinnex full-length RNA bioinformatics workflow recommendations (cont.)

SMRT Link Read Segmentation and Iso-Seq workflow common considerations and recommendations for analysis of Kinnex full-length RNA data<sup>1</sup>

## Sequencing depth recommendations for Iso-Seq data based on experimental goals and study design

Example application	Human genetics disease studies	Biopharma for identifying highly expressed targets	Plant & animal whole genome annotation
Experimental goal	Isoform discovery and quantification of moderate-to-rare transcripts	Isoform discovery of high expressed transcripts	Comprehensive transcript annotation in a species
Example study design	Disease vs. normal tissues with multiple replicates	Disease cohort with >20+ samples	Plant or animal with multiple tissue types
Target depth of coverage per sample	10 M reads per sample	5 M reads per sample	≤5 M reads per tissue (of same species)
Sample multiplexing <sup>1</sup>	<b>Sequel II/IIe system:</b> Up to 2 samples per SMRT Cell 8M ( <b>2-plex</b> )	<b>Sequel II/IIe system:</b> Up to 3 samples per SMRT Cell 8M ( <b>3-plex</b> )	
	<b>Vega system:</b> Up to 3 samples per Vega SMRT Cell ( <b>3-plex</b> )	<b>Vega system:</b> Up to 6 samples per Vega SMRT Cell ( <b>6-plex</b> )	
	<b>Revio system + SPRQ:</b> Up to 6 samples per Revio SMRT Cell ( <b>6-plex</b> )	<b>Revio system + SPRQ:</b> Up to 12 samples per Revio SMRT Cell ( <b>12-plex</b> )	
SMRT Link data analysis workflows	Read Segmentation and Iso-Seq analysis application with option to “pool reads and cluster together” to get a master isoform classification file with per-sample full-length read counts		

# SMRT Link Read Segmentation and Iso-Seq analysis application setup

Specify **Read Segmentation and Iso-Seq** analysis application in SMRT Link<sup>1</sup>

**• Read Segmentation and Iso-Seq** analysis application processes HiFi reads generated from a Kinnex full-length RNA library to produce classified isoforms with read counts that are compatible with tertiary analysis tools

**• Accepts HiFi reads** (BAM format) as input

- HiFi reads are reads generated with CCS analysis whose quality value is equal to or greater than 20
- HiFi reads should be generated using the Kinnex full-length RNA library preparation protocol ([103-238-700](#))
- If the library is a regular (non-Kinnex) Iso-Seq monomer library without MAS-Seq concatenation, use the SMRT Link Iso-Seq Analysis workflow instead

**Note on barcoded libraries**

The **Read Segmentation and Iso-Seq** workflow will only process barcoded libraries at the cDNA level (such as using **Iso-Seq v2 Barcoded cDNA Primers** as part of the MAS-Seq for bulk Iso-Seq kit)

→ Demultiplexing of **barcoded adapters** (also part of the Kinnex full-length RNA kit) should first be performed by running the **Demultiplex Barcodes** data utility workflow in SMRT Link.

ID ¶¶	Name ¶¶
38820	Kinnex_full_length_RNA_UHRR_sample

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

Specify Read Segmentation and Single-Cell Iso-Seq analysis application required associated inputs<sup>1</sup>

**PacBio** SMRT Analysis

SMRT Analysis / Create New Analysis

1. Select Data 2. Select Analysis

Analysis Application Required

Read Segmentation and Iso-Seq

Import Analysis Settings Export

Associated Inputs

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

2 Primer Set Required  
Iso-Seq v2 Barcoded cDNA Primers

3 Reference Set  
Human Genome hg38, with Gencode v39 annotatic

Cluster of Barcoded Samples  
Cluster reads separately

Advanced Parameters

## 1. Segmentation Adapter Set (Default = MAS-Seq Adapter v3 (MAS8))

- 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) (Default = Iso-Seq v2 Barcoded cDNA Primers)

- 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. (See the [SMRT Link User Guide](#) for example Iso-Seq v2 Barcoded cDNA Primer IDs and sequences)
- 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` annotations
- Alternatively, choose other reference genomes (but not with annotations) that were custom-uploaded to SMRT Link
- The Reference Set can be left blank. If blank, the workflow will stop after the isoform clustering step (`isoseq cluster`)

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

Specify **Read Segmentation** and **Single-Cell Iso-Seq** analysis application required associated inputs<sup>1</sup>

**PacBio** SMRT Analysis

SMRT Analysis / Create New Analysis

1. Select Data 2. Select Analysis

Analysis Application Required

Read Segmentation and Iso-Seq

Import Analysis Settings Export

Associated Inputs

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 annotatic

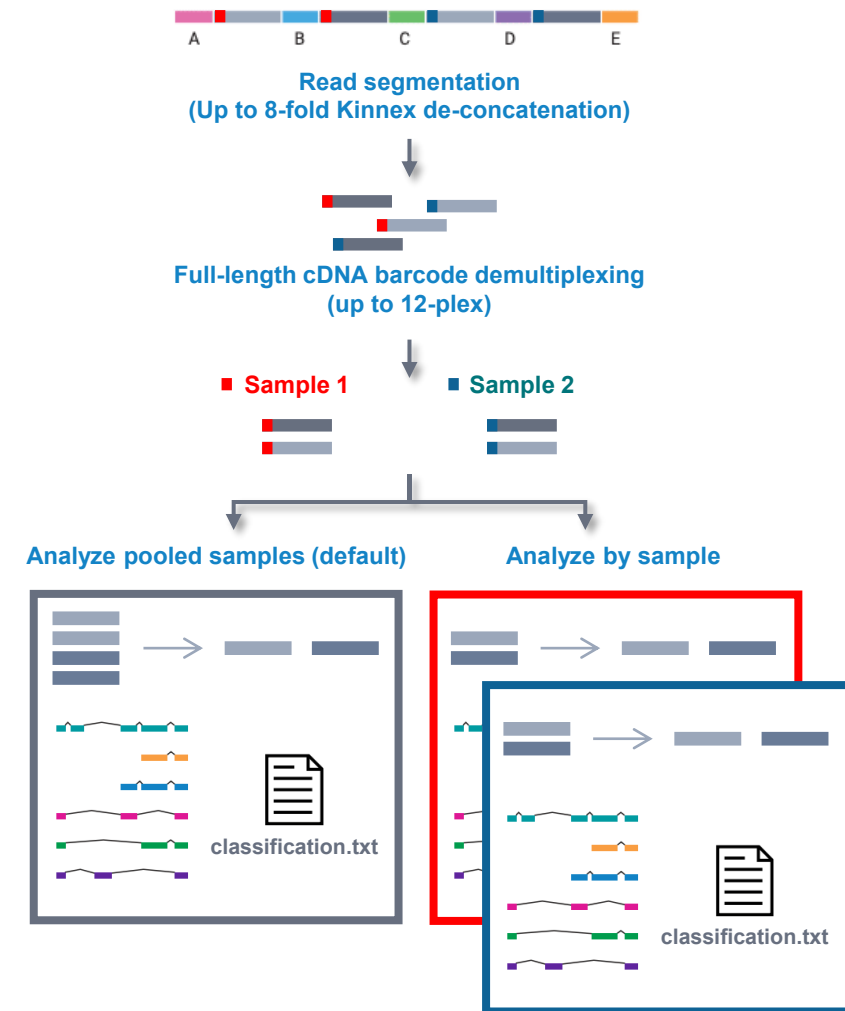
**4** Cluster of Barcoded Samples

Cluster reads separately

Advanced Parameters

## 4. Cluster of Barcoded Samples

- This option specifies barcoded samples that were barcoded at the cDNA level, where the (barcoded) cDNA primers are specified in the Primer Set option. This option does **not** address libraries that were barcoded using barcoded adapters
- Specify whether all FLNC reads will be pooled for clustering, then demultiplexed based on pooled result. **Note:** This setting does **not** apply to non-barcoded samples
- Specify **Pool reads and cluster together** if barcoded samples are from the **same** species, but different tissues, or samples of the same genes but different individuals. The samples are clustered with **all** barcodes pooled
- Specify **Cluster reads separately** if barcoded samples are from different species. The samples are clustered **separately** by barcode
- In either case, the samples on the results page are automatically named BioSample\_1 through BioSample\_N



# Example SMRT Link Read Segmentation data utility processing results<sup>1</sup> for Kinnex full-length RNA library prepared with human UHRR sample

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

### Summary Metrics

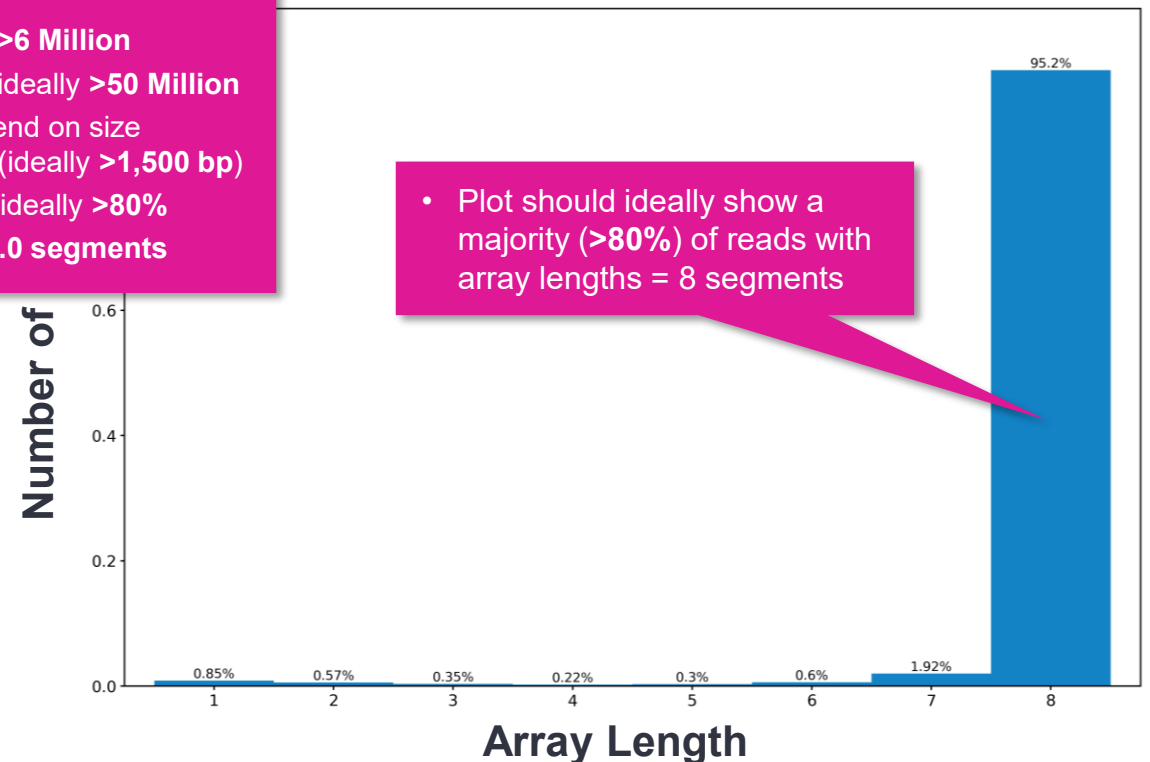
Value	Analysis Metric
10,404,037	Reads
81,012,128	Segmented reads (S-reads)
1,758.004	Mean length of S-reads
94.55 %	Percent of reads with full arrays
7.79	Mean array size (concatenation factor)

Example Revio system + SPRQ data shown.

For Revio system with optimal sample *P1* loading:

- Yield of HiFi reads is ideally >6 Million
- Yield of segmented reads is ideally >50 Million
- Mean S-read length will depend on size distribution of cDNA sample (ideally >1,500 bp)
- % of reads with full arrays is ideally >80%
- Mean array size is ideally >7.0 segments

### Segmentation Statistics



- Plot should ideally show a majority (>80%) of reads with array lengths = 8 segments

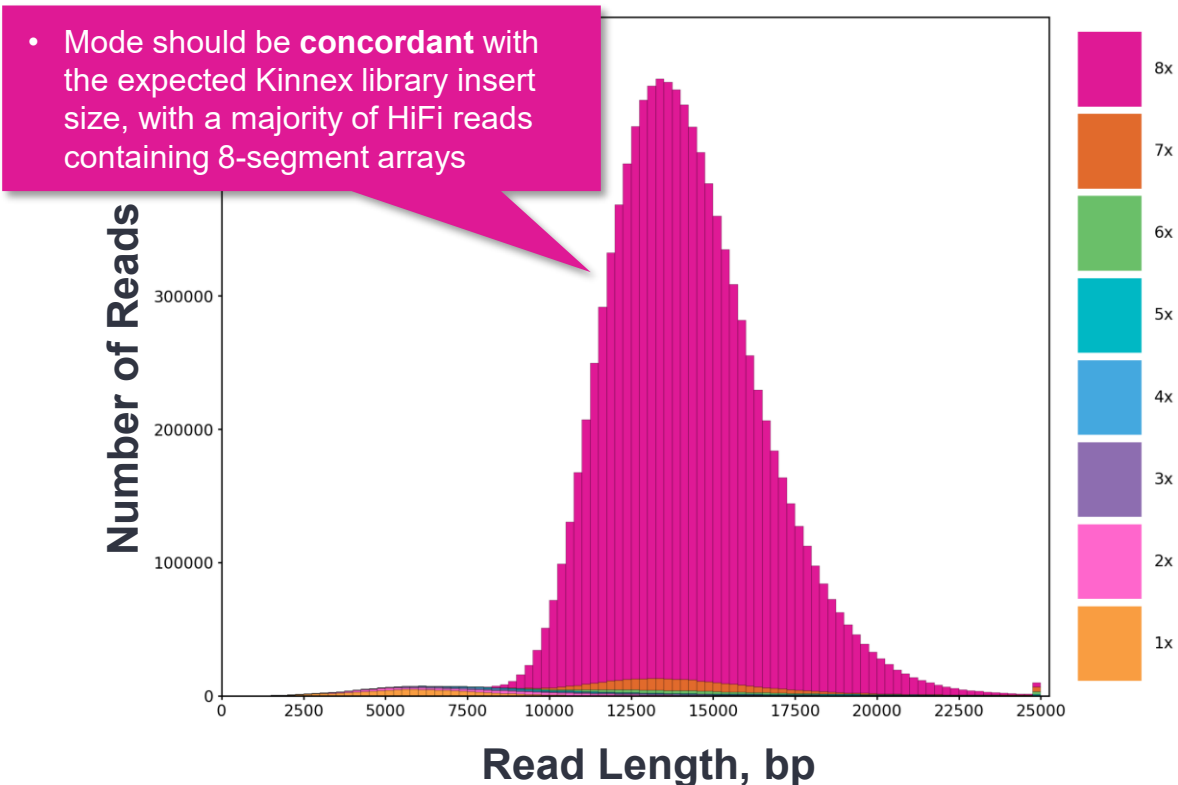
- **Reads:** Number of input arrayed HiFi reads
- **Segmented reads (S-reads):** Number of generated S-reads
- **Mean length of S-reads:** Mean read length of generated S-reads
- **Percent of reads with full arrays:** Percentage of input HiFi reads containing all adapter sequences in the order listed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

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

# Example SMRT Link Read Segmentation data utility processing results<sup>1</sup> for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

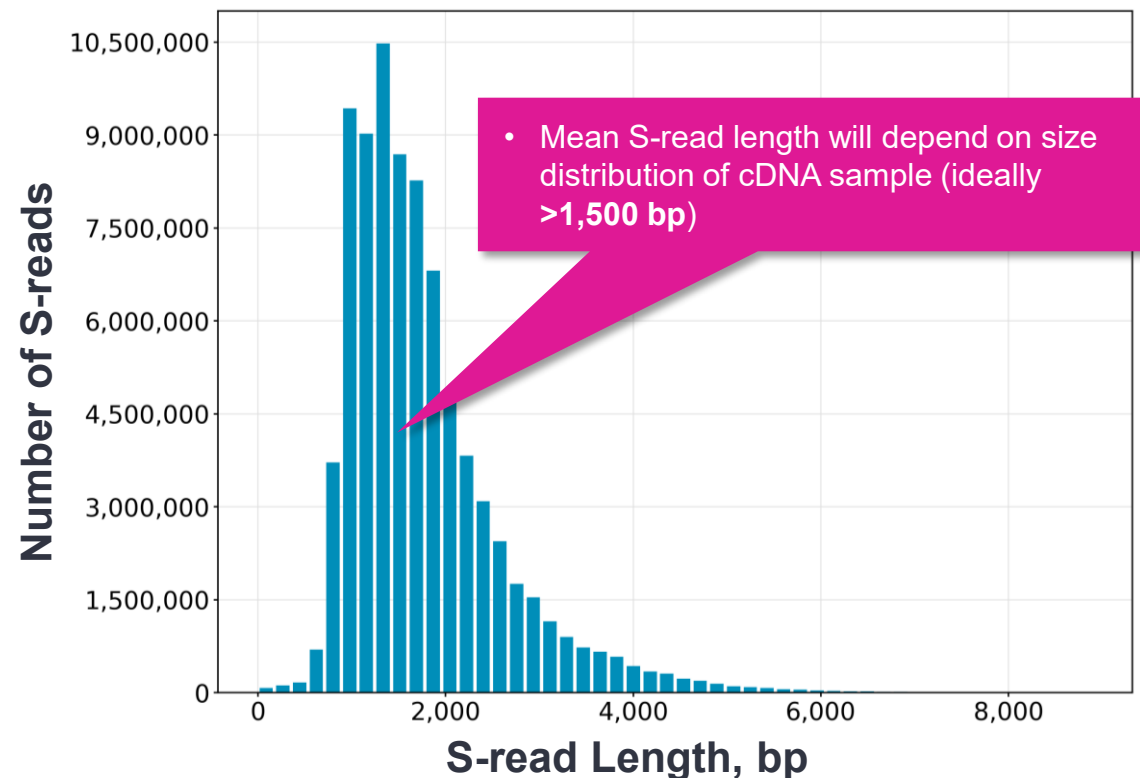
SMRT Link Read Segmentation data utility job report – Length of Reads and S-read Length Distribution

### Length of Reads



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

### S-read Length Distribution



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

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample

## SMRT Link Iso-Seq analysis job report – Read Classification statistics

### Summary Metrics

Value	Analysis Metric
81,012,128	Reads
78,815,334	Reads with 5' and 3' Primers
78,699,872	Non-Concatamer Reads with 5' and 3' Primers
78,639,000	Non-Concatamer Reads with 5' and 3' Primers and Poly-A Tail (FLNC Reads)
1,643	Mean Length of FLNC Reads
6	Unique Primers
13,135,889	Mean Reads per Primer
13,959,398	Max. Reads per Primer
12,391,746	Min. Reads per Primer
2,196,794	Reads without Primers
96.85%	Percent Bases in Reads with Primers
97.28%	Percent Reads with Primers

- **Reads:** Total number of CCS reads
- **Reads with 5' and 3' Primers:** Number of CCS reads with 5' and 3' cDNA primers detected
- **Non-Concatamer Reads with 5' and 3' Primers:** Number of nonconcatamer CCS reads with 5' and 3' primers detected
- **Non-Concatamer Reads with 5' and 3' Primers and Poly-A Tail (FLNC Reads):** Number of non-concatamer CCS reads with 5' and 3' primers and polyA tails detected. This is usually the number for full-length, nonconcatamer (FLNC) reads, unless polyA tails are not present in the sample
- **Mean Length of FLNC Reads:** Mean length of the non-concatamer CCS reads with 5' and 3' primers and polyA tails detected
- **Unique Primers:** Number of unique primers in the sequence
- **Mean Reads per Primer:** Mean number of CCS reads per primer
- **Max. Reads per Primer:** Maximum number of CCS reads per primer
- **Min. Reads per Primer:** Minimum number of CCS reads per primer
- **Reads without Primers:** Number of CCS reads without a primer
- **Percent Bases in Reads with Primers:** Percentage of bases in CCS reads in the sequence data that contain primers
- **Percent Reads with Primers:** Percentage of CCS reads in the sequence data that contain primers

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

## SMRT Link Iso-Seq analysis job report – Read Classification statistics

### Primer Data

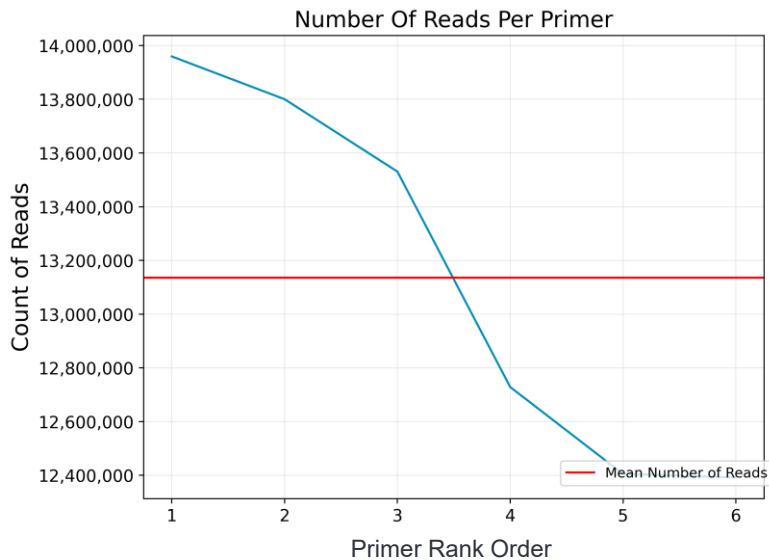
Bio Sample Name ↑	Primer Name ¶	CCS Reads ¶	Mean Primer Quality ¶	Reads with 5' and 3' Primers ¶	Non-Concatamer Reads with 5' and 3' Primers ¶
BioSample_1	IsoSeqX_bc01_5p--IsoSeqX_3p	13,800,150	99.7	13,800,150	13,787,052
BioSample_2	IsoSeqX_bc02_5p--IsoSeqX_3p	13,959,398	99.7	13,959,398	13,937,187
BioSample_3	IsoSeqX_bc03_5p--IsoSeqX_3p	12,728,468	99.7	12,728,468	12,707,044
BioSample_4	IsoSeqX_bc04_5p--IsoSeqX_3p	12,404,920	99.7	12,404,920	12,386,009
BioSample_5	IsoSeqX_bc05_5p--IsoSeqX_3p	12,391,746	99.7	12,391,746	12,372,742
BioSample_6	IsoSeqX_bc06_5p--IsoSeqX_3p	13,530,652	99.7	13,530,652	13,509,838
UNASSIGNED	No Primer	2,196,794	0.0	0	0

- **Bio Sample Name:** Name of the biological sample associated with the primer
- **Primer Name:** A string containing the pair of primer indices associated with this biological sample
- **CCS Reads:** Number of CCS reads associated with the primer
- **Mean Primer Quality:** Mean primer quality associated with the primer
- **Reads with 5' and 3' Primers:** Number of CCS reads with 5' and 3' cDNA primers detected
- **Non-Concatamer Reads with 5' and 3' Primers:** Number of non-concatemer CCS reads with 5' and 3' primers detected
- **Non-Concatamer Reads with 5' and 3' Primers and Poly-A Tail:** Number of non-concatemer CCS reads with 5' and 3' primers and polyA tails detected. This is usually the number for full-length, non-concatemer (FLNC) reads, unless polyA tails are not present in the sample.

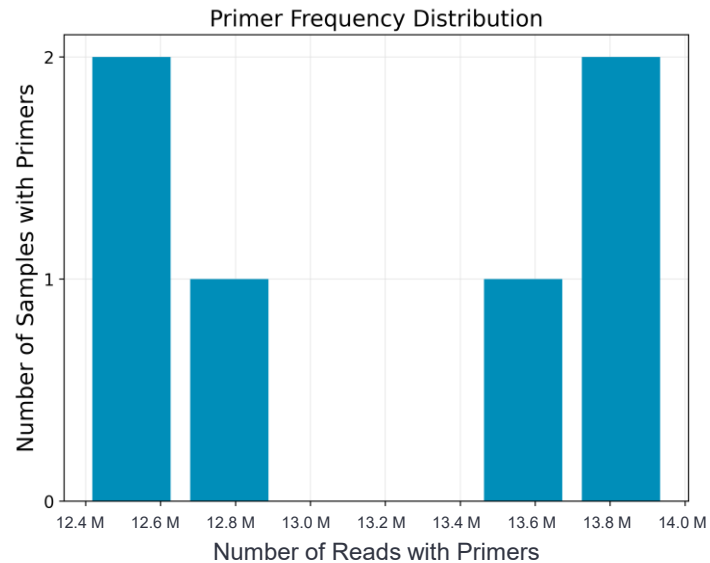
# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

SMRT Link Iso-Seq analysis job report – Read Classification statistics

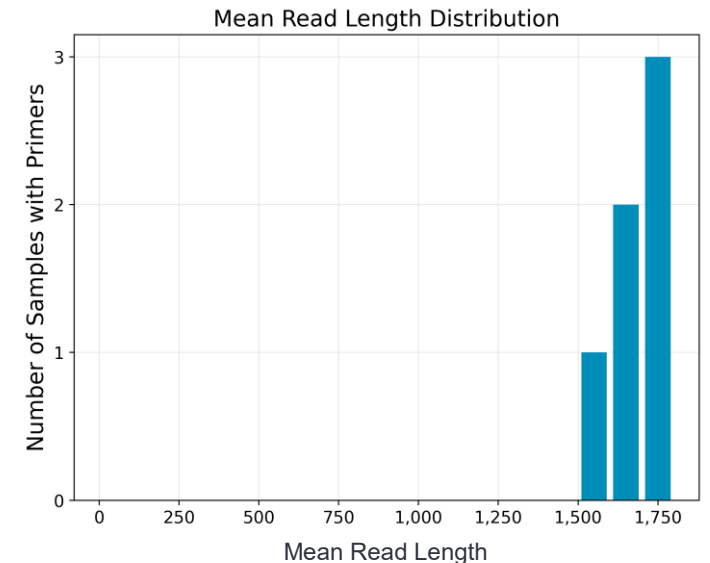
## Primer Read Statistics



**Number Of Reads Per Primer:** Maps the number of reads per primer, sorted by primer ranking



**Primer Frequency Distribution:** Maps the number of samples with primers by the number of reads with primers

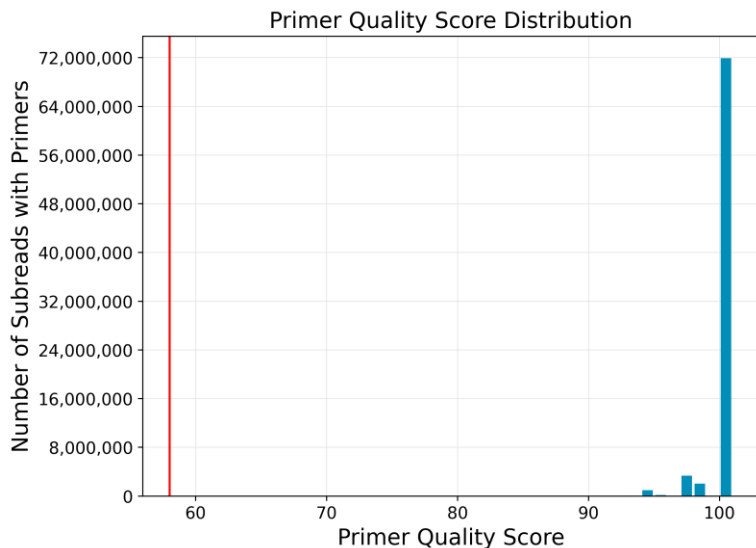


**Mean Read Length Distribution:** Maps the read mean length against the number of samples with primers

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

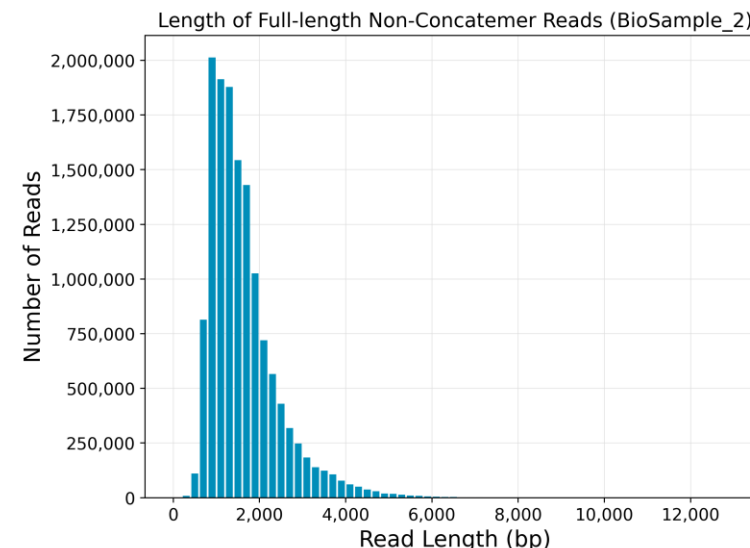
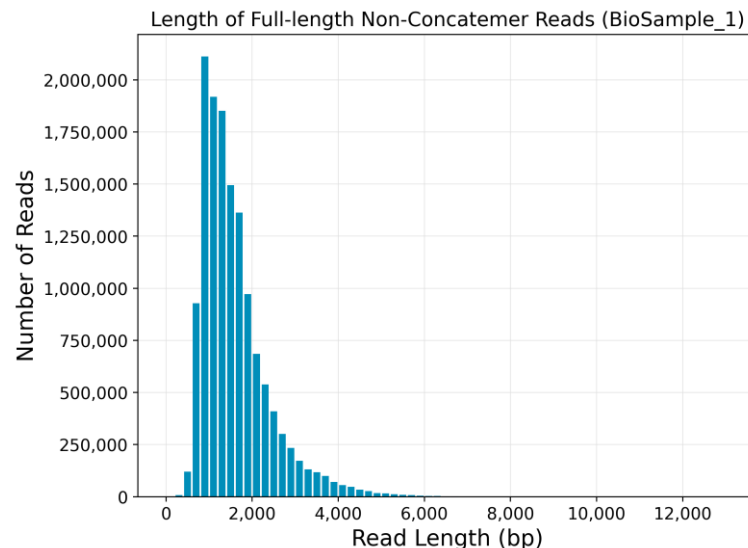
SMRT Link Iso-Seq analysis job report – Read Classification statistics

## Primer Quality Scores



**Primer Quality Score Distribution:** Histogram of primer scores

## Length of Full-length Non-Concatemer Reads



**Length of Full-Length Non-Concatemer Reads:** Per-sample histograms of the read length distribution of non-concatemer CCS reads with 5' and 3' primers and polyA tails detected

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

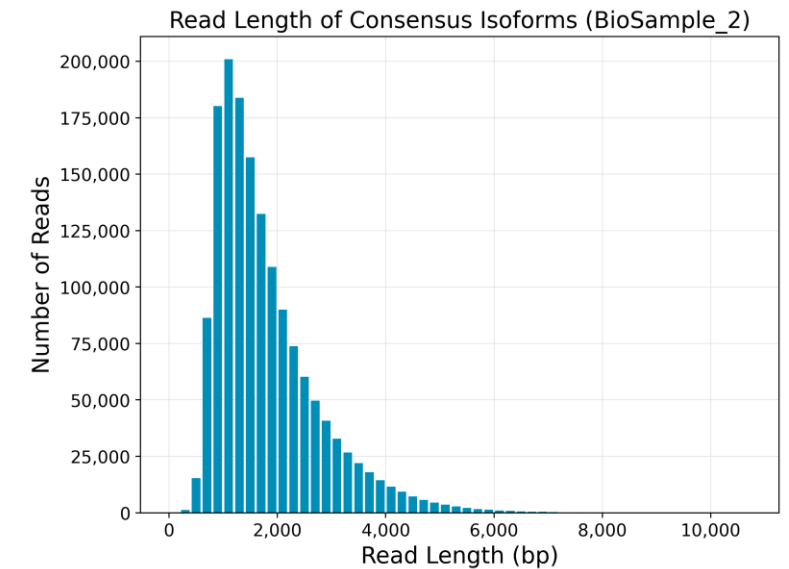
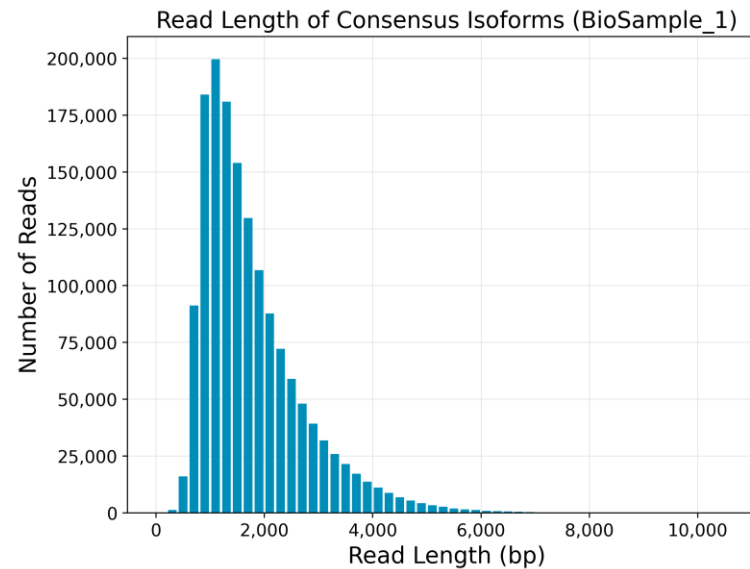
## SMRT Link Iso-Seq analysis job report – Transcript Clustering statistics

### Summary Metrics

Sample Name <sup>IT</sup>	Number of High-Quality Isoforms <sup>IT</sup>
BioSample_1	1,531,163
BioSample_2	1,549,686
BioSample_3	1,447,494
BioSample_4	1,449,777
BioSample_5	1,442,229
BioSample_6	1,521,973
All Samples	2,930,150

- **Sample Name:** Sample name for which the following metrics apply
- **Number of High-Quality Isoforms:** Number of consensus isoforms that have an estimated accuracy **above** the specified threshold

### Length of Consensus Isoforms



- **Length of Consensus Isoforms:** Per-sample histograms of the consensus isoform lengths and the distribution of isoforms exceeding a read length cutoff. Also includes a single histogram plot for all samples.

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

SMRT Link Iso-Seq analysis job report – Transcript Mapping and Classification statistics

## Summary Metrics (All samples)

Sample Name	Genes	Genes, filtered	Known Genes, filtered	Isoforms	Isoforms, filtered	Known isoforms, filtered	Novel isoforms > 1TPM, filtered
BioSample_1	403,607	25,116	21,839	829,922	236,783	64,884	8,731
BioSample_2	405,157	25,172	21,978	840,868	240,486	65,292	8,933
BioSample_3	375,913	24,601	21,629	778,487	223,422	64,022	7,682
BioSample_4	369,570	24,459	21,607	781,424	231,051	63,712	9,386
BioSample_5	367,898	24,382	21,574	778,483	230,618	63,540	9,473
BioSample_6	393,111	24,862	21,827	827,709	241,390	64,789	9,234

- **Sample Name:** Sample name for which the following metrics apply
- **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 isoforms:** The total number of unique isoforms across all cells
- **Total unique isoforms, filtered:** The total number of unique isoforms across all cells, after filtering out reads based on the SQANTI transcript filtering criteria.

# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

## SMRT Link Iso-Seq analysis job report – Transcript Mapping and Classification statistics

### Transcript Classification, filtered (All samples)<sup>1</sup>

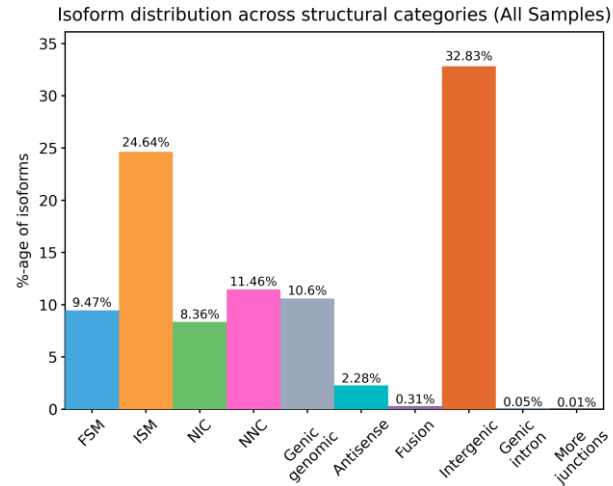
Category IT	Count IT	CAGE Detected IT	CAGE Detected, (%) IT	polyA Detected IT	polyA Detected, (%) IT
FSM	202364	91165	45.05%	108319	53.52%
ISM	314609	40144	12.75%	191810	60.96%
NIC	183274	114701	62.58%	96830	52.83%
NNC	152653	92299	60.46%	82649	54.14%
Antisense	3074	599	19.48%	1890	61.48%
Fusion	5204	2937	56.43%	2921	56.12%
More junctions	135	76	56.29%	82	60.74%
Genic intron	0	0	0.00%	0	0.00%
Genic genomic	2059	802	38.95%	1177	57.16%
Intergenic	6606	509	7.70%	5297	80.18%

- **Category:** Transcript classification<sup>2</sup> 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

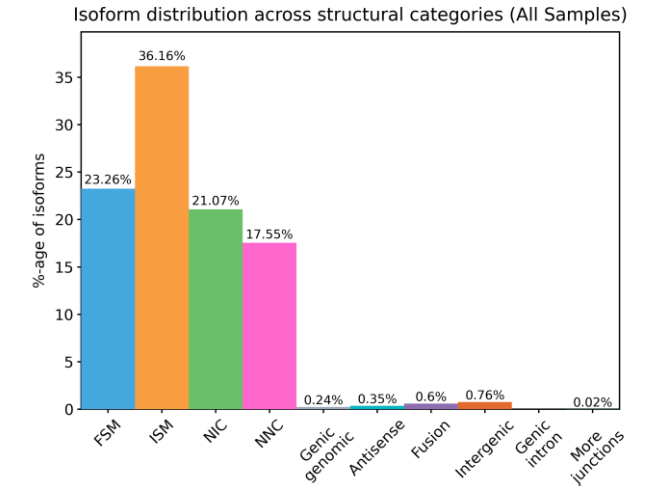
# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

## SMRT Link Iso-Seq analysis job report – Transcript Mapping and Classification statistics

### Transcript Classification Plots



### Transcript Classification Plots, Filtered



#### Isoform distributions across structural categories:

- Distribution of the % of isoforms by structural categories

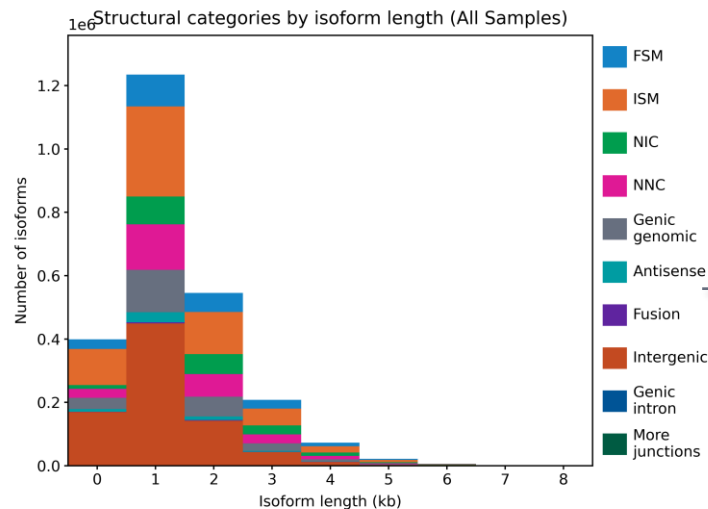
Example Revio system + SPRQ data shown.

Filter out reads based on the **SQANTI3** transcript filtering criteria

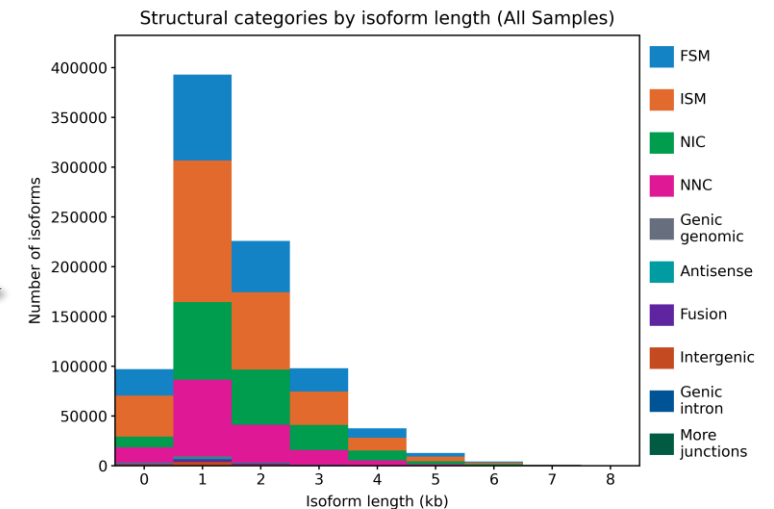
#### Structural categories by isoform lengths:

- Histogram display of the number of isoforms by their length in kb and their structural category

Example Revio system + SPRQ data shown.



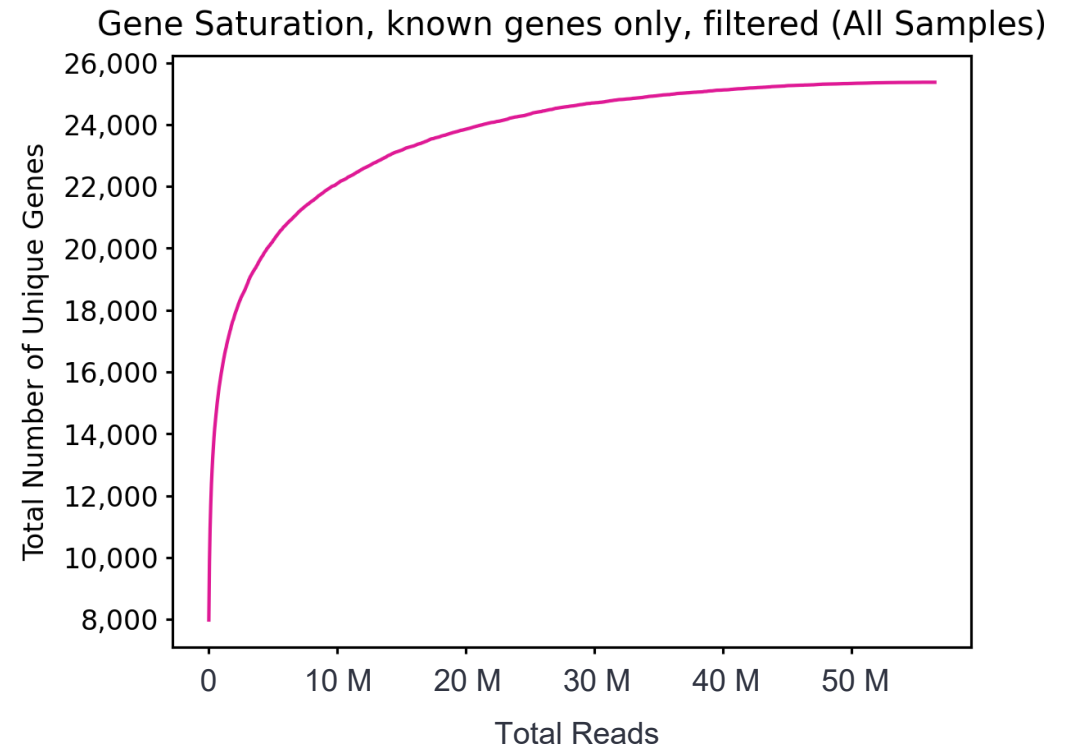
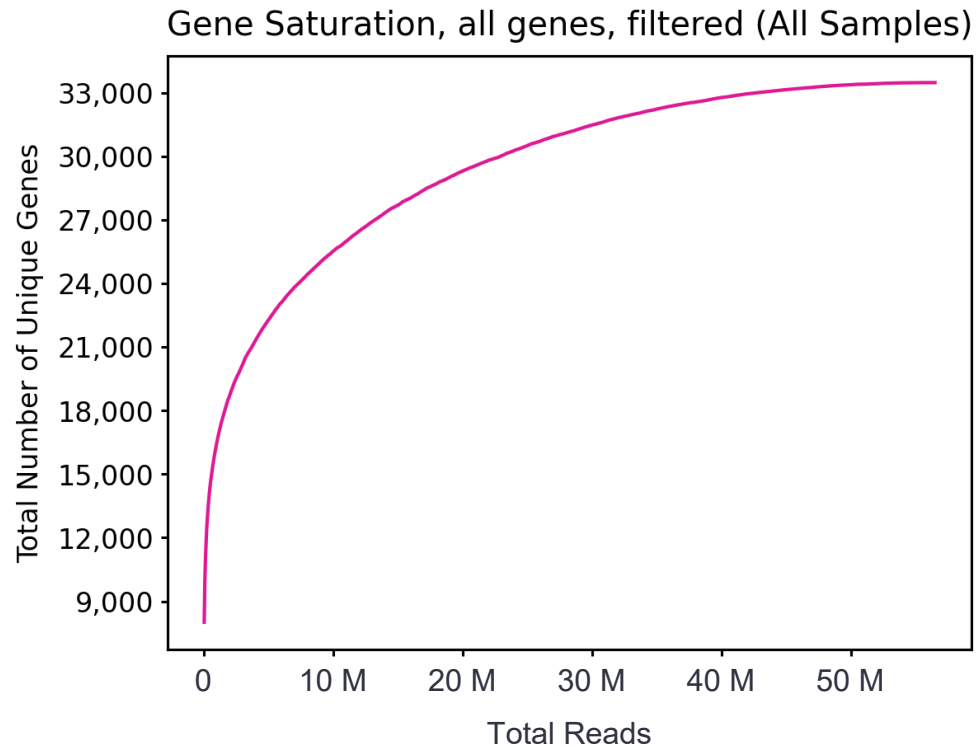
Filter out reads based on the **SQANTI3** transcript filtering criteria



# Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

SMRT Link Iso-Seq analysis job report – Transcript Mapping and Classification 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 SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

## File Downloads tab

Edit Output File Name Prefix Example:analysis-Bio Sample 4-2110

File ↑	Size ↓	Type ↓
Non-passing reads, unaligned	4 GB	bam
Report read_segmentation	3 KB	JsonReport
SMRT Link Log	13 KB	log
Segmented Reads, passing, unaligned	65 GB	bam
UHRR_verification_DL_bc4 (demux1) (Bio Sample 4) Segmented Reads	20 KB	ConsensusReadSet
Unique mapped transcripts, GFF (All Samples)	895 MB	gff
Unique mapped transcripts, classification TXT (All Samples)	728 MB	txt
Unique mapped transcripts, filtered, GFF (All Samples)	599 MB	gff
Unique mapped transcripts, filtered, classification TXT (All Samples)	307 MB	txt
Unique mapped transcripts, filtered, junctions TXT (All Samples)	624 MB	txt
Unique mapped transcripts, junctions TXT (All Samples)	771 MB	txt

Refer to [SMRT Link user guide](#) for descriptions of downloadable output 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

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.



# **Technical documentation & applications support resources**

# Technical resources for Kinnex full-length RNA library preparation, sequencing & data analysis

## RNA sample preparation resources

- Note:** The products below have not been tested or validated by PacBio but are listed here as examples of third-party kits used by other PacBio customers for isolating total RNA for PacBio RNA sequencing (Iso-Seq) applications

Kit type	Product name
mRNA isolation	Ambion Poly(A) Purist MAG Kit [ <a href="#">Link</a> ]
	Qiagen RNeasy Plus Kits [ <a href="#">Link</a> ]
Total RNA isolation	Sigma Spectrum Plant Total RNA Kit [ <a href="#">Link</a> ]
	iNtRON Easy Spin Total RNA [ <a href="#">Link</a> ]
	TRIzol Reagent can be used to isolate total RNA from tissues or cells, including lipid-rich and difficult samples [ <a href="#">Link</a> ]
RNA stabilization & storage	RNALater is an aqueous, nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA [ <a href="#">Link</a> ]

# Technical resources for Kinnex full-length RNA library preparation, sequencing & data analysis

## RNA sample preparation resources (cont.)

Some important considerations to bear in mind when isolating total RNA for Iso-Seq analysis include the following:

- RNA sample has not been exposed to high temperatures (e.g.:  $>65^{\circ}\text{C}$  for 1 hour can cause a detectable decrease in sequence quality) or pH extremes ( $<6$  or  $>9$ ).
- RNA sample has an OD260/OD280 ratio  $\sim 2.0$ .
- RNA sample has an OD260/OD230 ratio  $\geq 2.0$ .
- RNA sample has a RIN number  $\geq 7.0$  (ideally recommend  $\geq 8.0$ ).
- RNA sample has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes are not RNA damaging, but do avoid ethidium bromide.
- RNA sample does not contain denaturants (e.g., guanidinium salts or phenol) or detergents (e.g., SDS or Triton-X100).
- RNA sample does not contain carryover contamination from the original organism / tissue (e.g., heme, humic acid, polyphenols, etc.).
- Only use RNase-free water supplied in the reagent kit or other suppliers.
- Make aliquots of the RNA sample and TSO to avoid excessive freeze-thaw cycles.
- Thaw RNA samples and TSO on ice before use – DO NOT leave on the benchtop.
- Avoid excessive pipetting and vortexing when working with RNA.
- **Note:** RNA samples should only be shipped with dry ice.

# Technical resources for Kinnex full-length RNA library preparation, sequencing & data analysis (cont.)

## Kinnex full-length RNA library preparation literature & other resources

- Application note – Kinnex full-length RNA kit for isoform sequencing ([102-326-591](#))
- Procedure & checklist – Preparing Kinnex libraries using Kinnex full-length RNA kit ([103-238-700](#))
- Technical overview – Kinnex kits for single-cell RNA, full-length RNA and 16S rRNA sequencing ([103-343-700](#))
- Technical overview – Kinnex library preparation using Kinnex full-length RNA kit ([103-344-700](#))
- Video tutorial – SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits [ [Link](#) ]
- Whitepaper – Bulk and single-cell isoform sequencing for human disease research ([102-326-576](#))

## Data analysis resources

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

## Publications

- 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](#) ]
- 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](#) ]

# Technical resources for Kinnex full-length RNA library preparation, sequencing & data analysis (cont.)

## 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](#) ]

## Example PacBio data sets

Application	Dataset	Data type	PacBio system
Kinnex full-length RNA sequencing	Homo sapiens – Universal human reference RNA (UHRR) [ <a href="#">Link</a> ]	HiFi long read	Vega system
	Homo sapiens – Universal human reference RNA (UHRR) [ <a href="#">Link</a> ]	HiFi long read	Revio system – SPRQ chemistry
	Homo sapiens – Universal human reference RNA (UHRR) [ <a href="#">Link</a> ]	HiFi long read	Sequel II & Revio systems
	Homo sapiens – HG002 [ <a href="#">Link</a> ]	HiFi long read	Revio system
	Homo sapiens – Heart [ <a href="#">Link</a> ]	HiFi long read	Revio system
	Homo sapiens – Cerebellum [ <a href="#">Link</a> ]	HiFi long read	Revio system
	Homo sapiens – Brain [ <a href="#">Link</a> ]	HiFi long read	Revio system



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

# Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput

Kinnex concatenation is a general method that can increase sequencing throughput for smaller amplicons<sup>1</sup>

## Benefits of Kinnex concatenation

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

## When is Kinnex concatenation appropriate?

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

Recommended Kinnex kit based on average amplicon sizes.

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

Technical note  
**CONCATENATING AMPLICONS USING PACBIO KINNEX KITS TO INCREASE THROUGHPUT**

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

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

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

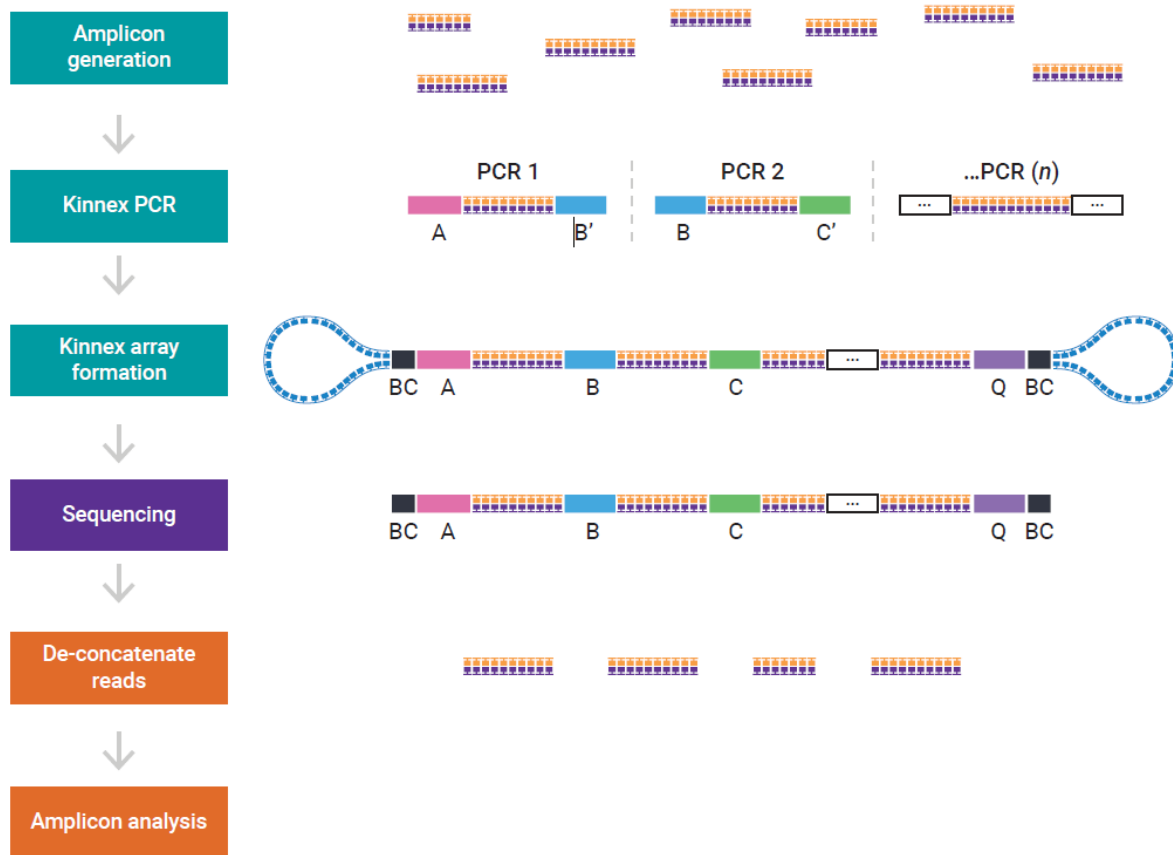
How Kinnex works

**What is Kinnex?**  
The Kinnex kits are based on the method developed by Al'Khafaji et al. (2023). Originally termed multiplexed array isomorph 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).

**Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput**  
[\(102-326-636\)](https://www.pacb.com/technical-note/concatenating-amplicons-using-kinnex-kits-to-increase-throughput/)

# Kinnex concatenation workflow overview

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



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

## Kinnex concatenation procedural notes<sup>1</sup>

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

# Establishing Kinnex-compatible molecular ends

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

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

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

## Example 1. Kinnex 16S forward and reverse primer sequences

Kinnex 16S FWD01 CTACACGACGCTCTTCCGATCT - GATCGAGTCA - AGRGTTYGATYMTGGCTCAG  
Kinnex 16S REV13 AAGCAGTGGTATCAACGCAGAG - TCATCGACGT - RGYTACCTTGTACGACTT

## 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<sup>1</sup>

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

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

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

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

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

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



# **APPENDIX 2: PacBio compatible Kinnex library preparation workflows**

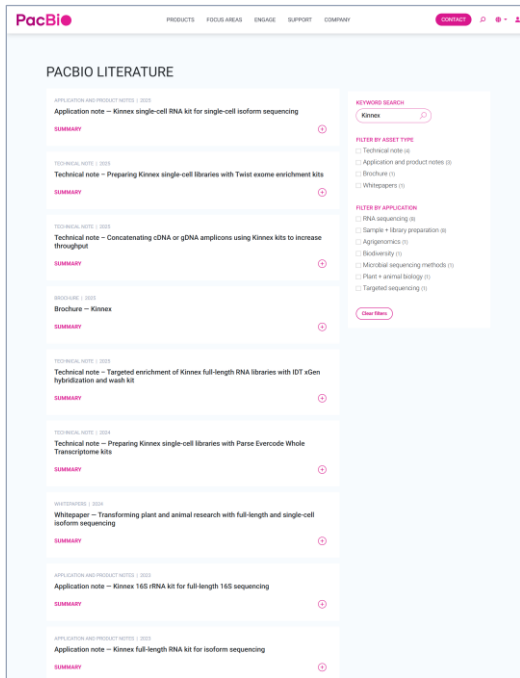
# Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3

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

Application-specific educational literature

Application-specific protocol documentation

Application-specific technical overviews



### 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, highly similar paralogs or pseudogenes, and low-abundance long non-coding RNAs for annotation purposes. 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 and the IDT xGen protocol.
- 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 RN 2.7. Lower-quality RNA may result in shorter cDNA, while total RNA lower than 100 ng may result in the incomplete capture of the full diversity of the targeted genes.

**Library preparation**

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

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

**Library workflow:**

- Generate up to 12-plex cDNA using the Iso-Seq express 2.0 kit.
- Enrich for targeted genes using IDT customer-provided and then xGen Hyb Wash v3 kit.
- Generate Kinnex full-length RNA library using the Kinnex full-length RNA kit.
- Sequence on Sequel II/Hiie, Vega, or Revio systems.
- Analyze with the Read Segmentation and Iso-Seq workflow in SMRT Link.

**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.

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

**© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures. 103-238-700 REV07 JUN2025**

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

Procedure & checklist

**Before you begin**

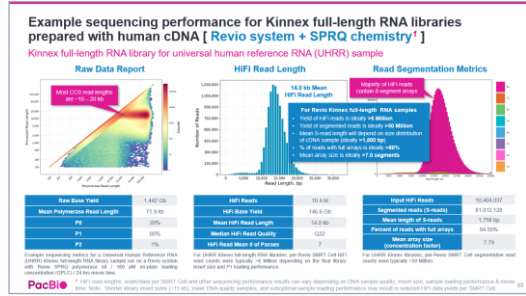
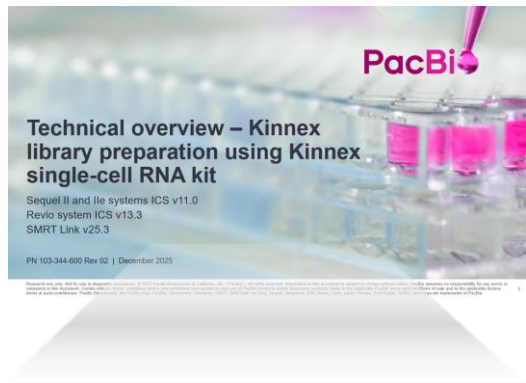
This procedure describes the workflow for constructing Kinnex full-length RNA libraries from total RNA samples for sequencing on PacBio® Sequel II, Sequel Hi, Vega™, and Revio™ systems.

Overview	
Samples	1–24
Workflow time	1.5 days (for up to 24 samples)
Number of SMRT™ Cells per Kinnex library Prep	≥8 SMRT Cells for Revio using SPRQ™ chemistry ≥2 SMRT Cells for the Vega or Revio (non-SPRQ) systems ≥4 SMRT Cells for Sequel II/Hiie systems

**RNA Input**

Quality/size distribution	RNA (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/μL per library)

**© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures. 103-238-700 REV07 JUN2025**



**PacBio literature website [Link]**

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

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

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

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

Technical documentation containing PacBio SMRTbell library construction details.

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

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

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

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

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

**Overview**

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

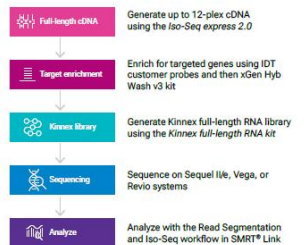
This technical note describes the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for Kinnex® full-length RNA libraries.

**Required materials and equipment**

- For a comprehensive list of materials and equipment, please refer to the PacBio Kinnex full-length RNA protocol<sup>4</sup> and the IDT xGen protocol<sup>5</sup>.
- 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**


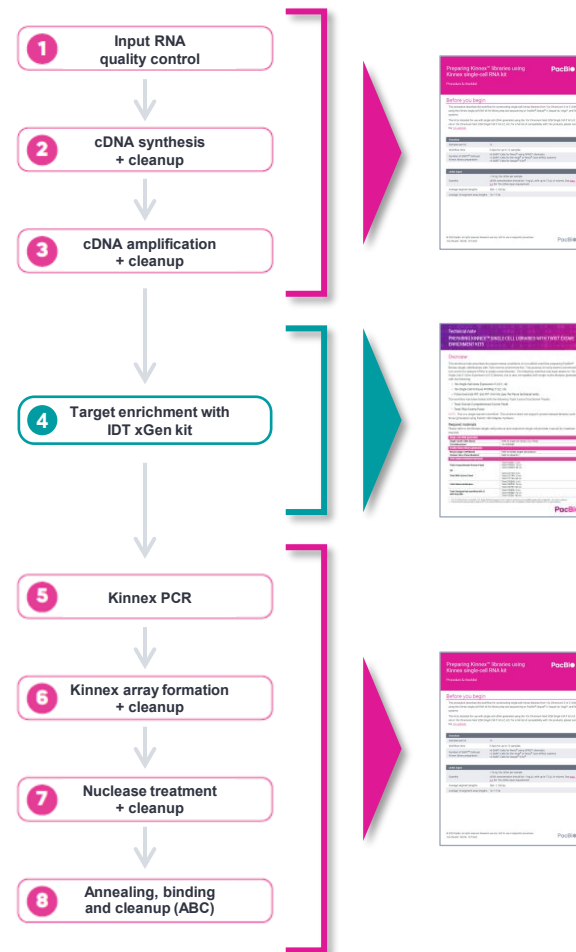
Library preparation will follow the Kinnex full-length RNA protocol with modifications outlined here.



**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.

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

**PacBio Technical note**  
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 ([102-326-632](#))<sup>1</sup>

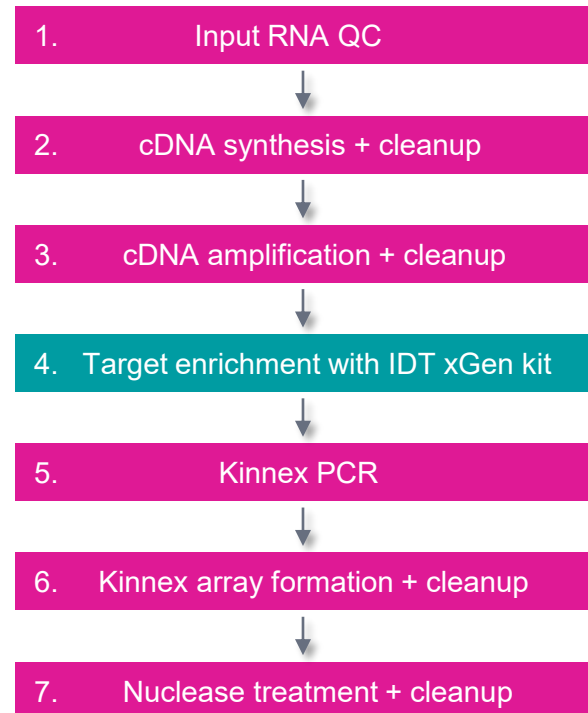
**PacBio Procedure & checklist**  
Preparing Kinnex libraries using the Kinnex full-length RNA kit ([103-238-700](#))

## Overview

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

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

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



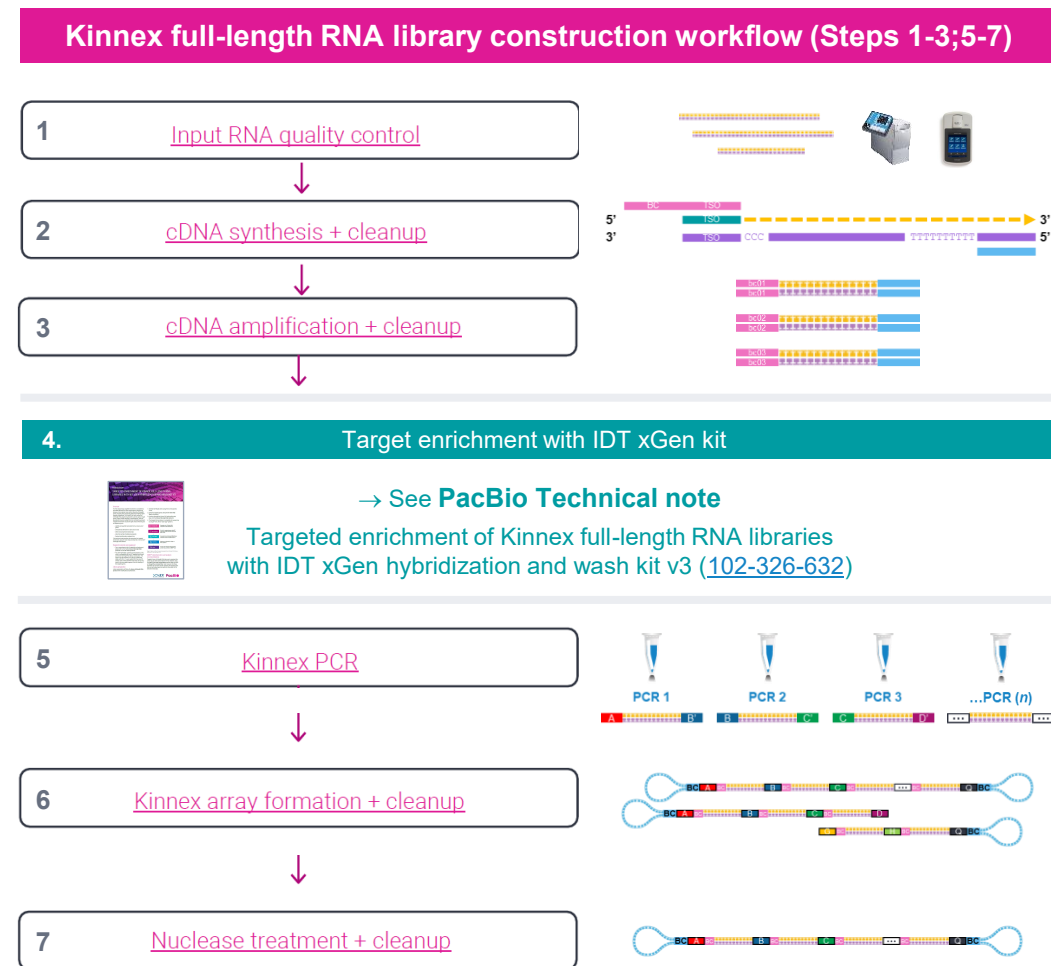
**PacBio Kinnex library construction procedure<sup>1</sup>**

Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit ([103-238-700](https://www.pacb.com/knowledge-base/kinnex-full-length-rna-kit/))



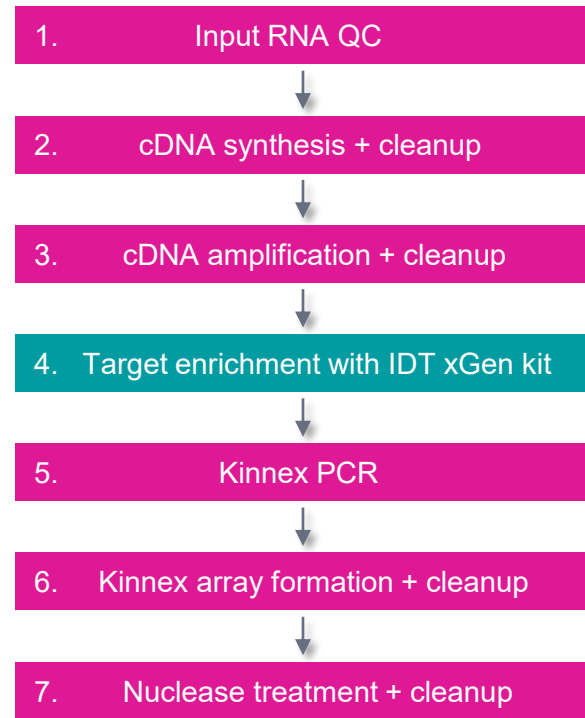
**Kinnex full-length RNA library prep procedure key steps**

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



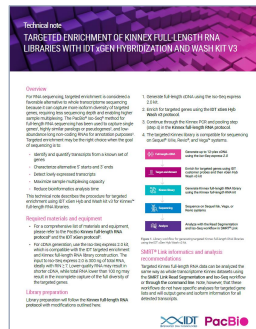
# Workflow overview for targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (cont.)

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



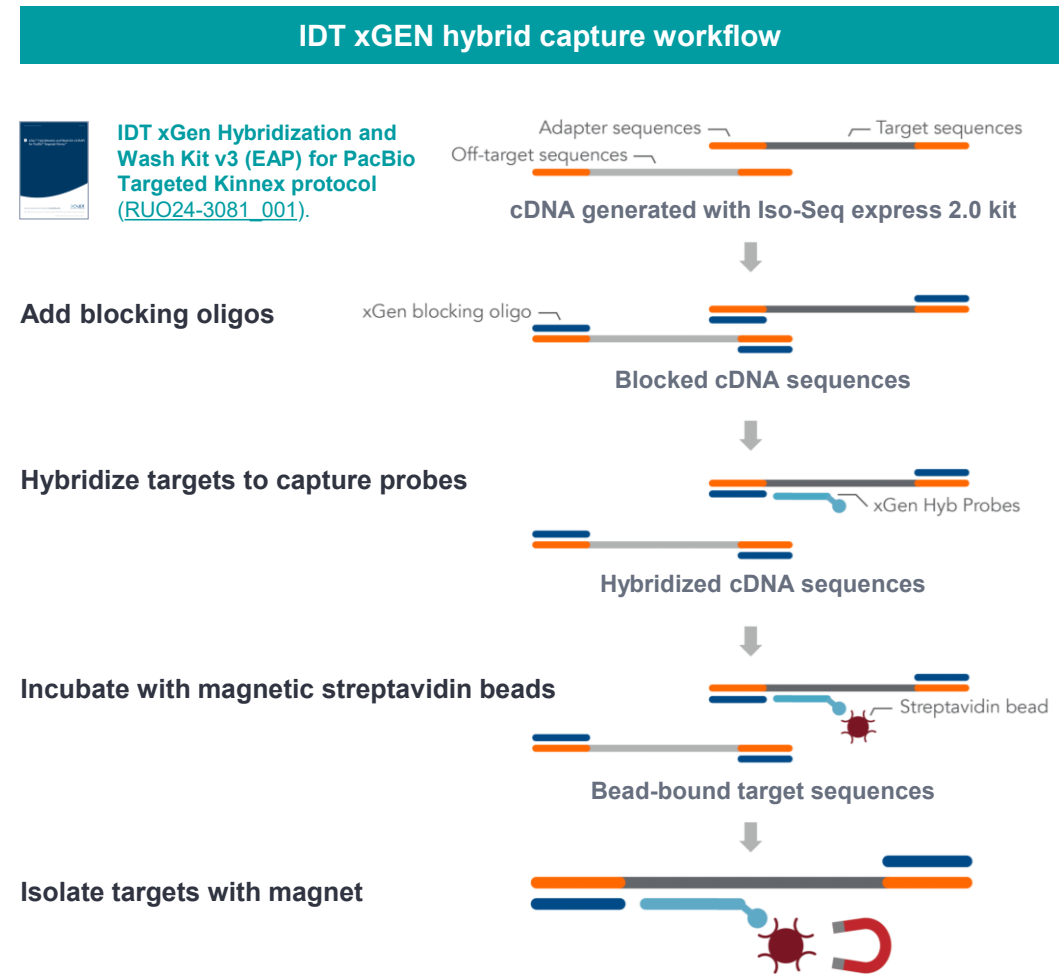
**IDT xGen hybrid capture procedure overview<sup>1</sup>**

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



**IDT xGEN hybrid capture procedure key steps<sup>1</sup>**

- Add Blocker Master Mix to each cDNA sample (500 ng<sup>2</sup>) and dry down
- Add Hybridization Master Mix to each sample well and incubate for desired hybridization time.<sup>3</sup>
- 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



<sup>1</sup> For hybridization capture workflow details, refer to the **IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol** ([RUO24-3081\\_001](#)).  
<sup>2</sup> 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).  
<sup>3</sup> 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.

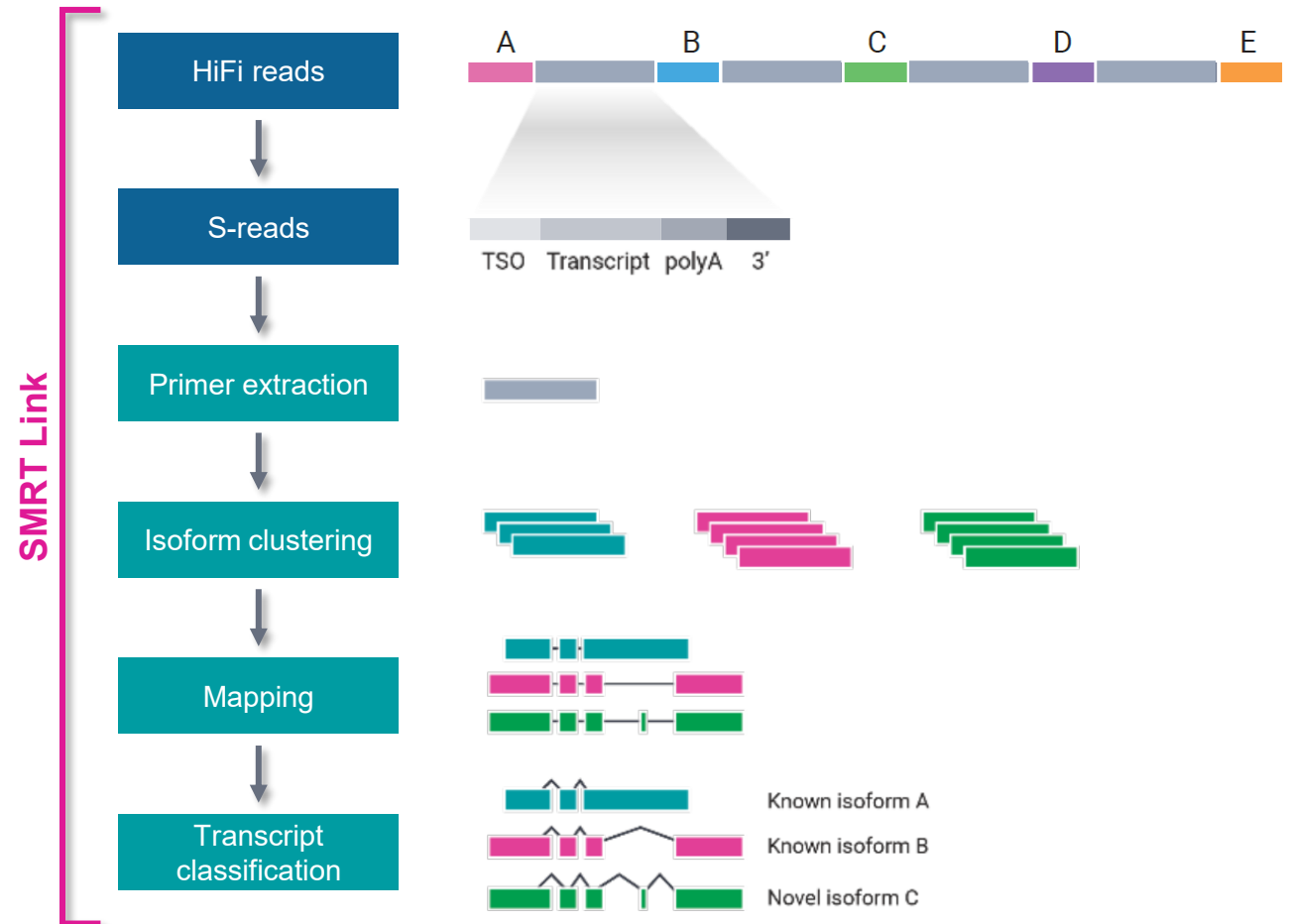
# SMRT Link informatics and secondary analysis recommendations for targeted Kinnex full-length RNA data

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 Link read segmentation and Iso-Seq workflow<sup>1</sup>





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

# SMRT Link Run Design procedure for Sequel IIe system

## Sample information and run information

- Select desired **Kinnex application** from the **Application** field drop-down menu
- The following fields are **auto-populated** with default recommended values and high-lighted in **green**:

- SMRTbell Adapter Design**

→ SMRTbell Kinnex Prep Kit

- Binding Kit**

→ Sequel II Binding Kit 3.2

- Sequencing Kit**

→ Sequel II Sequencing Plate 2.0 (4 rxn or 1 rxn)

- DNA Control Complex**

→ Sequel II DNA Internal Control Complex 3.2

- Movie Time per SMRT Cell**

→ 30 hrs

- Pre-Extension Time**

→ 2 hrs

**SMRTbell Adapter Design** field determines which adapter finding algorithm is used during post-primary analysis<sup>1</sup>

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

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

Recommended movie time = **30 hrs**

Select desired Kinnex application type from drop-down menu

SAMPLE 1: Kinnex full-length RNA library demo , A01, 30 hour movie, 16000 bp insert

Copy Delete

Import from Sample Setup Select Sample

Application Required Kinnex full-length RNA

Well Sample Name Required Kinnex full-length RNA library demo

Bio Sample Name

Sample Comment

Sample Well A01

SMRTbell Adapter Design Required SMRTbell® Kinnex Prep Kit

Binding Kit Required Sequel® II Binding Kit 3.2

Sequencing Kit Required Sequel® II Sequencing Plate 2.0 (4 rxn)

DNA Control Complex Sequel® II DNA Internal Control Complex 3.2

Insert Size (bp) Required 16000

Recommended Concentration on Plate (pM) 40 – 60 pM

On-Plate Loading Concentration (pM) Required 45

Movie Time per SMRT Cell (hours) 30

Use Pre-Extension YES NO

Pre-Extension Time (hours) 2

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

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

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

## Advanced options

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

Advanced Options

Use Adaptive Loading  YES  NO

Loading Target (P1 + P2)

Maximum Loading Time (hours)

CCS Analysis Output - Include Low Quality Reads  YES  NO

CCS Analysis Output - Include Kinetics Information  YES  NO

Add Data to Project

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

Can specify to use a different Project folder

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

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

## Barcoded sample options

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

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

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

Can leave most of these fields at their default values

Barcoded Sample Options

Sample Is Barcoded  YES  NO

Barcode Set Required MAS SMRTbell barcoded adapters (v2)

Same Barcodes on Both Ends of Sequence ?  YES  NO

Assign Bio Sample Names to Barcodes ? Required

Demultiplex Barcodes  ON INSTRUMENT  IN SMRT LINK  
 DO NOT GENERATE

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



[www.pacb.com](http://www.pacb.com)

Revision history (description)	Version	Date
Added procedural summary illustrations to clarify Kinnex library prep protocol steps, updated example Kinnex sequencing performance data for PacBio long-read systems (Revio system + SPRQ chemistry and Vega system) and incorporated information about PacBio compatible RNA sequencing workflows using third-party kit products.	02	December 2025

Research use only. Not for use in diagnostic procedures. © 2025 Pacific Biosciences of California, Inc. (“PacBio”). All rights reserved. Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third-party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at [pacb.com/license](http://pacb.com/license). Pacific Biosciences, the PacBio logo, PacBio, Circulomics, Omniome, SMRT, SMRTbell, Iso-Seq, Sequel, Nanobind, SBB, Revio, Onso, Apton, Kinnex, PureTarget, SPRQ, and Vega are trademarks of PacBio.