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

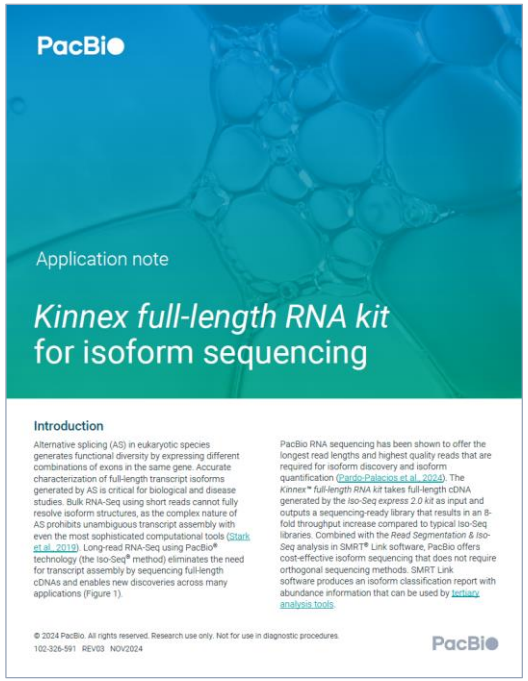
Technical overview

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

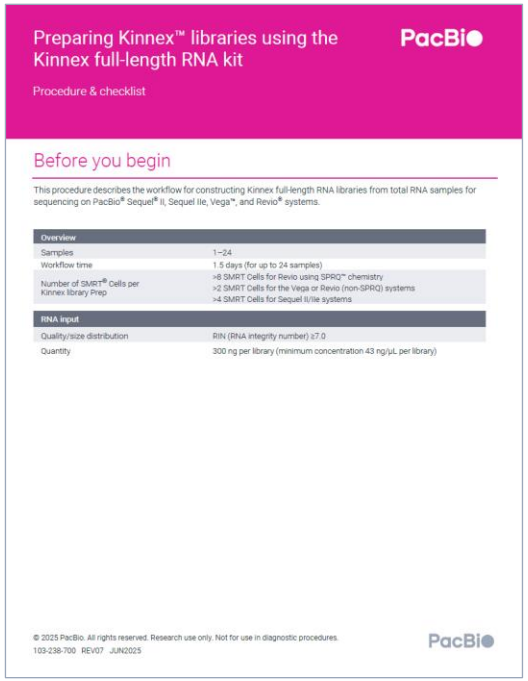
Kinnex library preparation using Kinnex full-length RNA kit: Getting started

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



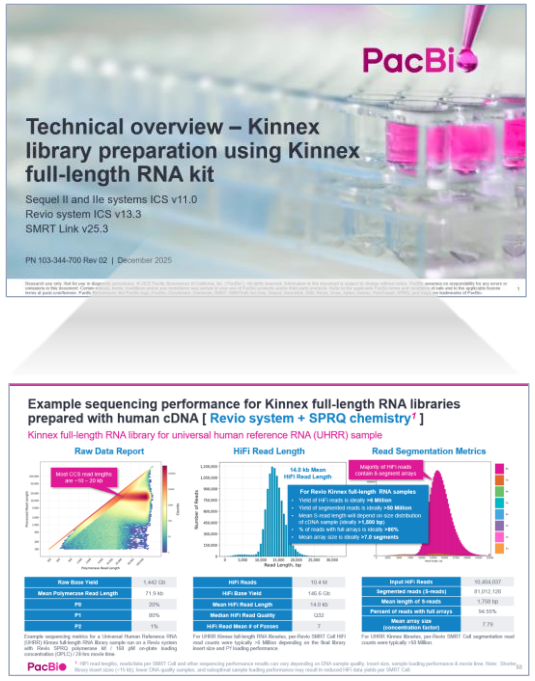
Application note – Kinnex full-length RNA kit for isoform sequencing (102-326-591)

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



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

Technical documentation containing application-specific library preparation protocol details.



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

Data analysis
(SMRT Link)

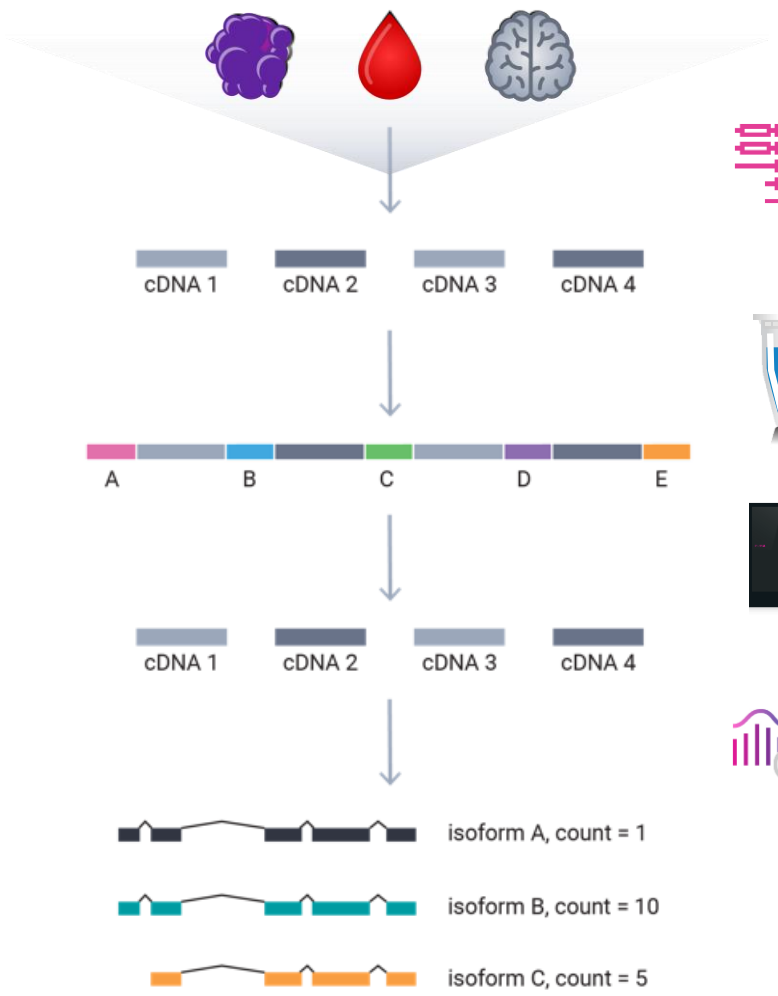
Use SMRT Link Read Segmentation data utility to split arrayed transcript HiFi reads
Use SMRT Link Iso-Seq analysis application to identify novel genes and isoforms with abundance information

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

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



cDNA generation

Kinnex library preparation

PacBio sequencing

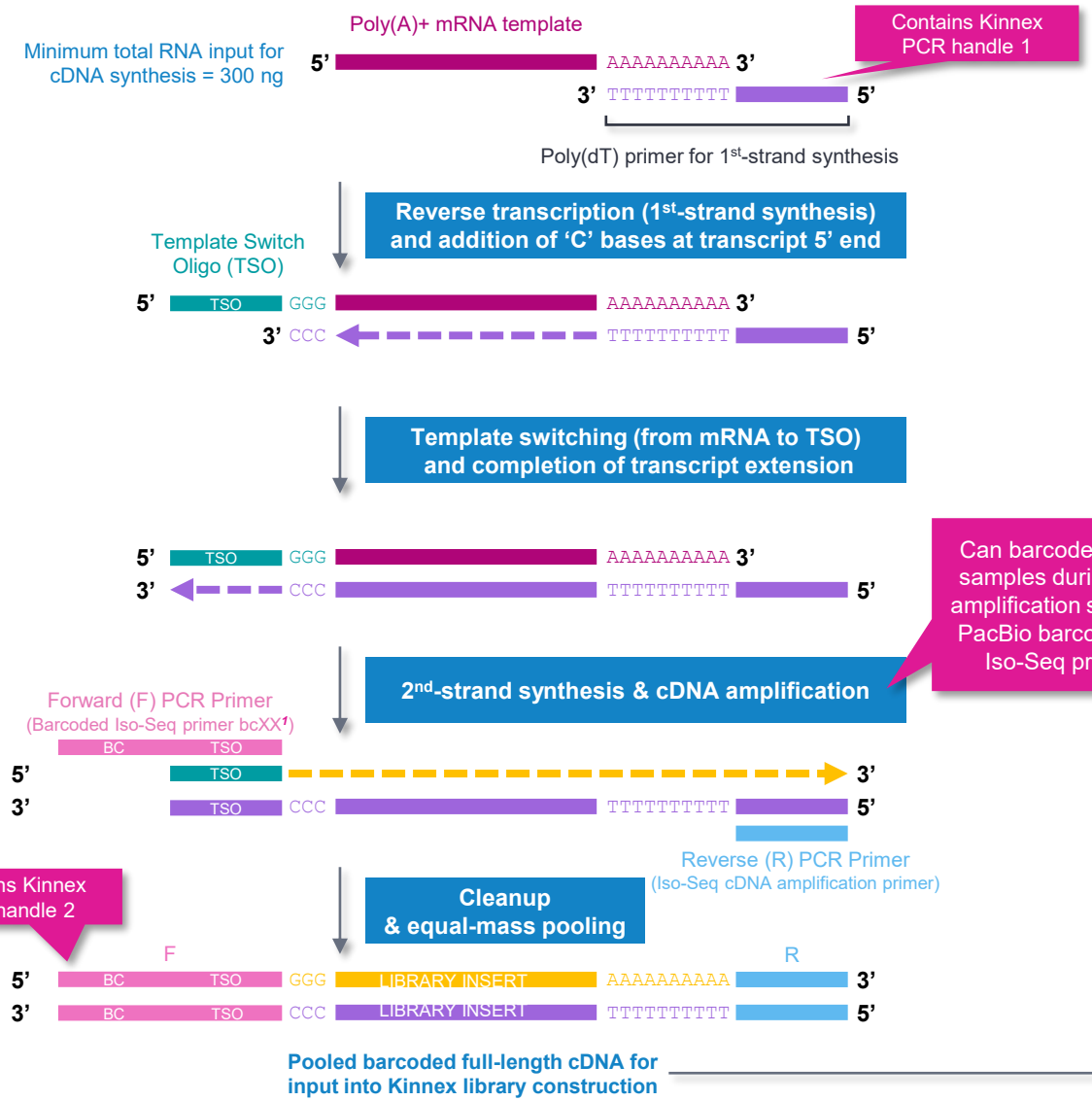
Read segmentation + Isoform analysis

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

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

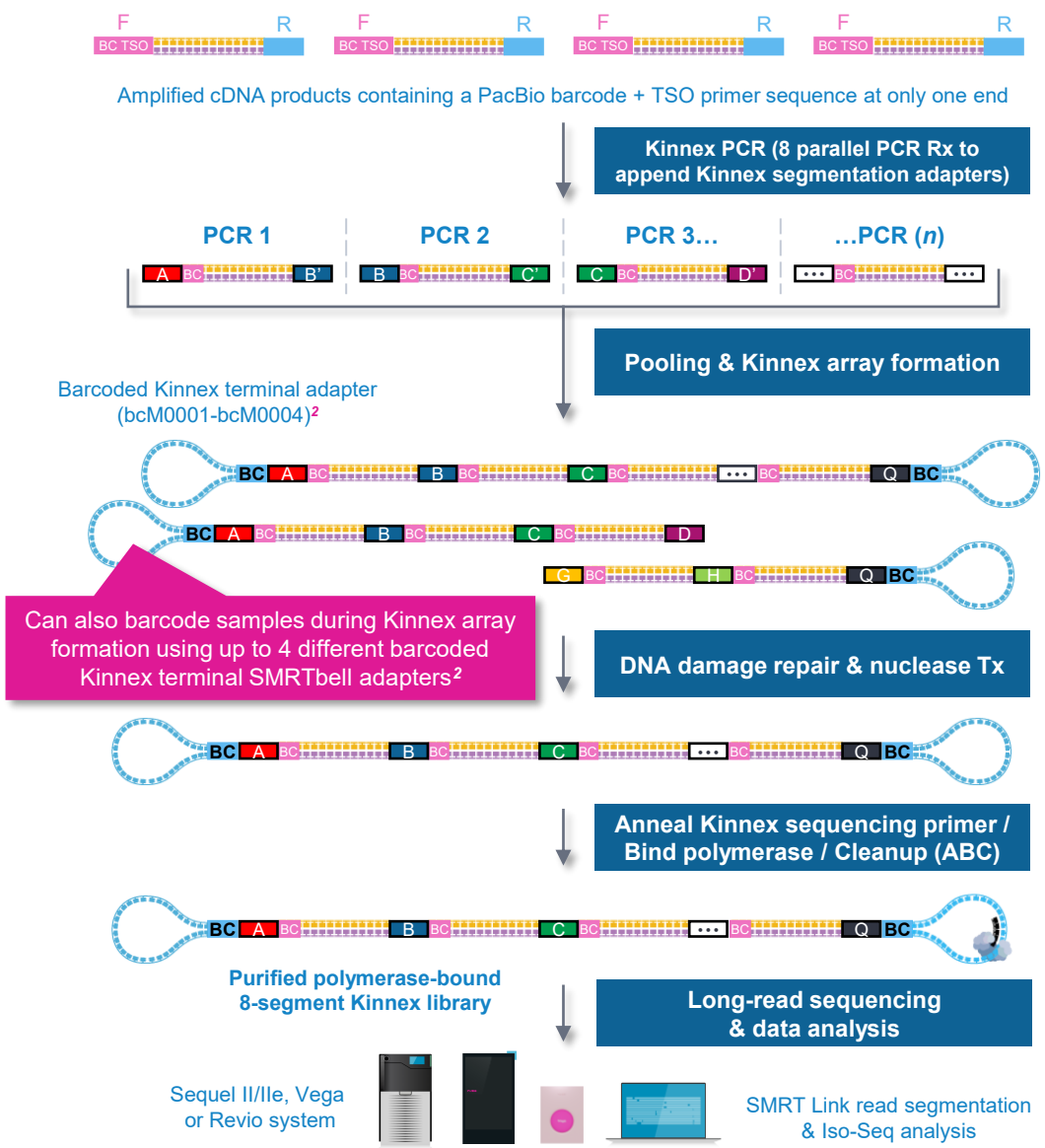
Kinnex full-length RNA method overview (cont.)

Full-length cDNA synthesis & amplification



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

Kinnex library prep, sequencing & analysis



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

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

Kinnex full-length RNA library preparation procedure description

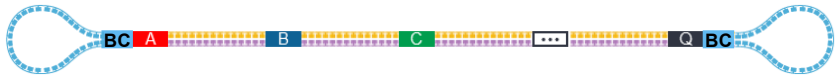
Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700) describes the workflow for constructing Kinnex libraries from total RNA samples using the **Iso-Seq express 2.0 kit** and **Kinnex full-length RNA kit** for sequencing on PacBio long-read systems

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/IIe systems

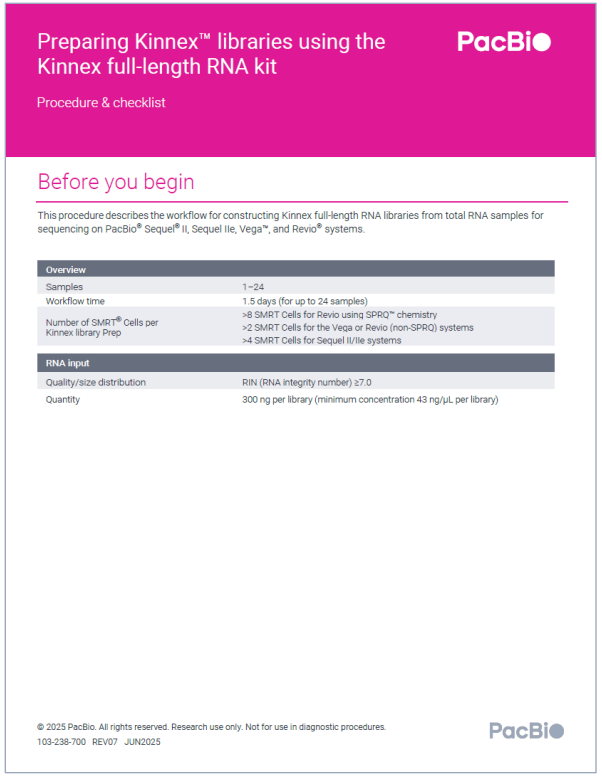
RNA input	
Quality/size distribution	RIN (RNA integrity number) ≥7.0
Quantity	300 ng per library (minimum concentration 43 ng/µL per library)

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

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



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


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

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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

[1] **Note:** Iso-Seq express 2.0 kit (103-071-500) is **not included** in Kinnex full-length RNA bundle kit (103-072-000) and must be purchased separately from PacBio.

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 1st-strand cDNA synthesis and PCR amplification of cDNA products generated from input total RNA.

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.

Iso-Seq express 2.0 kit components

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

Kinnex full-length RNA kit components

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

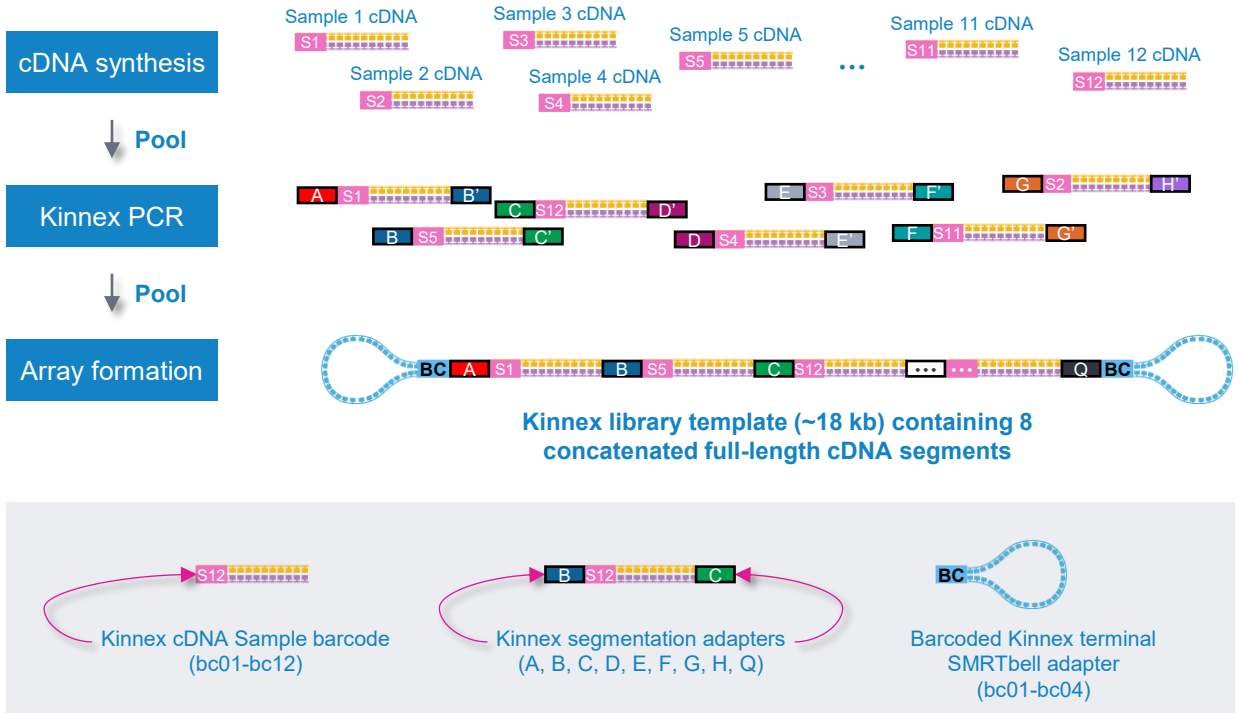
Kinnex full-length RNA library barcoding options for sample multiplexing

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
3. formation” in the workflow.
4. **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[1]	**Sequel II/IIe system:** Up to 2 samples per SMRT Cell 8M (2-plex)	**Sequel II/IIe system:** Up to 3 samples per SMRT Cell 8M (3-plex)	
	Vega system: Up to 3 samples per Vega SMRT Cell (3-plex)	**Vega system:** Up to 6 samples per Vega SMRT Cell (6-plex)	
	Revio system + SPRQ: Up to 6 samples per Revio SMRT Cell (6-plex)	**Revio system + SPRQ:** Up to 12 samples per Revio SMRT Cell (12-plex)	
Expected data throughput (per SMRT Cell)	**Sequel II/IIe system:** Up to 20 M reads divided by *N* samples		
	Vega system: Up to 30 M reads divided by *N* samples		
	Revio system + SPRQ: Up to 60 M reads divided by *N* samples		
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)		
Total RNA input into Kinnex library prep workflow	300 ng total RNA (RIN ≥7) for 1st-strand cDNA synthesis		
SMRT Link data analysis workflows	Read Segmentation and Iso-Seq analysis application with option to "pool reads and cluster together" to get a master isoform classification file with per-sample full-length read counts		
Community data analysis tools	Annotation & quantification: PIGEON, SQANTI3 / Differential analysis: TappAS / Fusion calling: pbfusion / Visualization: SWAN		

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

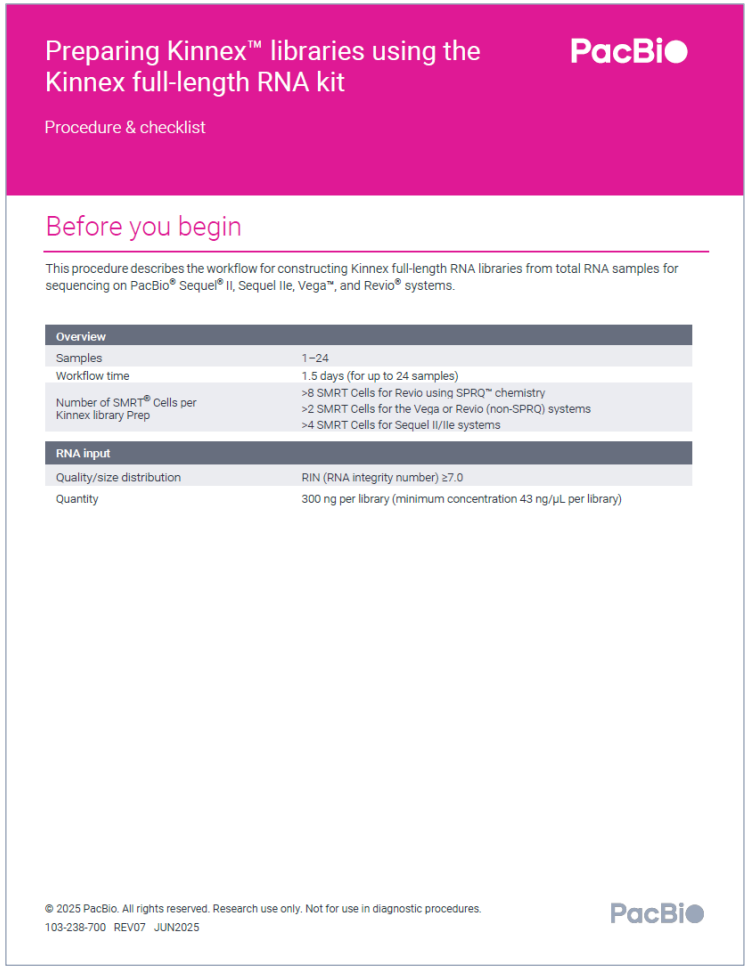
Kinnex full-length RNA library preparation workflow details

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

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PacBio

PacBio Documentation (103-238-700)

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

Kinnex full-length RNA library construction workflow overview

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

	Cleanup	DNA QC	Walk-away time[1]	
1 Input RNA quality control	N/A	Qubit RNA HS assay Bioanalyzer RNA 6000 Nano kit	N/A	
Safe stop				
2 cDNA synthesis + cleanup	1.3X SMRTbell cleanup beads	N/A	1.5 hrs	Day 1 (~5 hrs*)
3 cDNA amplification + cleanup	0.9X SMRTbell cleanup beads	Qubit dsDNA HS assay Bioanalyzer HS DNA assay	1.5 hrs	
Safe stop				
4 Kinnex PCR + cleanup	1.05X SMRTbell cleanup beads	Qubit dsDNA HS assay	1.5 – 2.0 hrs	
5 Kinnex array formation + cleanup	1.0X SMRTbell cleanup beads	N/A	2.0 hrs	Day 2 (~3 hrs*)
Safe stop				
6 Nuclease treatment + cleanup	1.0X SMRTbell cleanup beads	Qubit dsDNA HS assay Femto Pulse gDNA 165 kb kit	0.6 hrs	

[1] **Excludes** hands-on time for setting up enzymatic reaction steps and additional time required to perform DNA sizing QC and DNA concentration QC.

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
	Purple	Iso-Seq RT buffer 103-103-900
cDNA synthesis	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

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

- 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[1]
  - RIN ≥7.0 (ideally ≥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

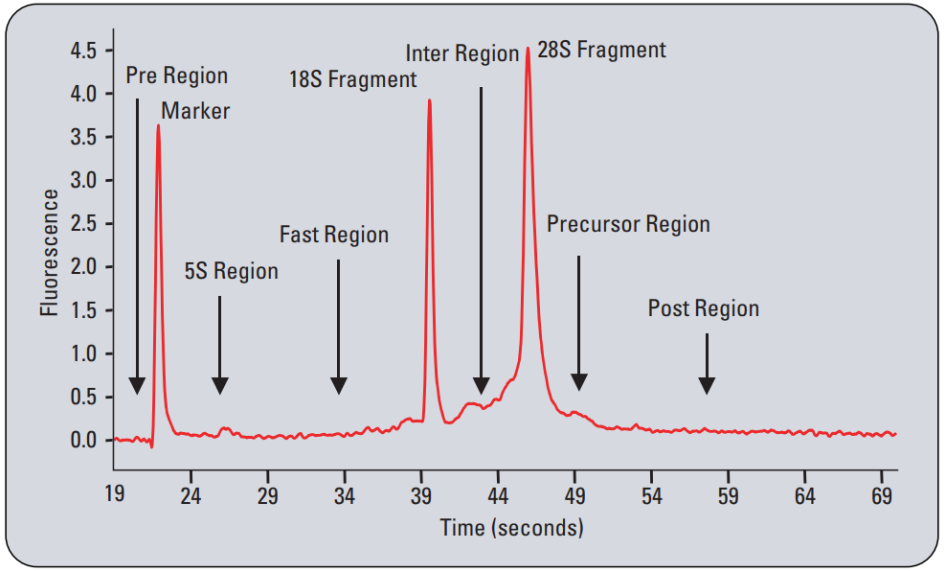
Step 1
procedural summary

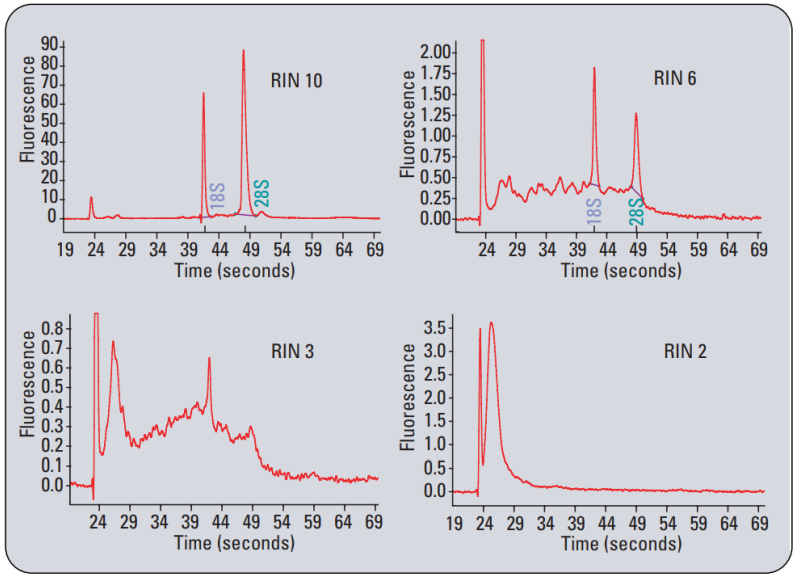
DNA
sizing QC

Total RNA RIN ≥ 7

↓

Proceed to **Step 2**





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)

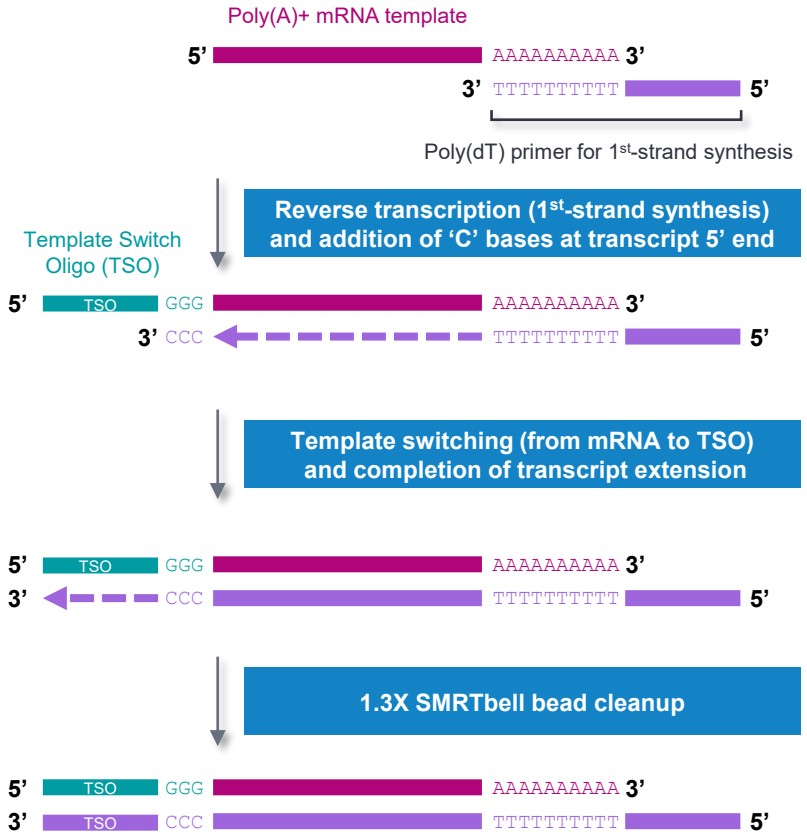
[1] Refer to *Agilent Application Note: RNA Integrity Number (RIN) – Standardization of RNA Quality Control* (5989-1165EN)

cDNA synthesis + cleanup

In this step, total RNA samples are converted to 1st-strand cDNA

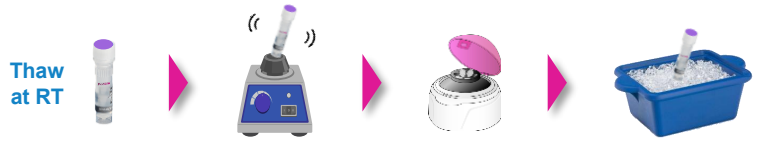
1 Input RNA quality control
↓ Safe stop
2 cDNA synthesis + cleanup
↓
3 cDNA amplification + cleanup
↓ Safe stop
4 Kinnex PCR + cleanup
↓
5 Kinnex array formation + cleanup
↓ Safe stop
6 Nuclease treatment + cleanup

Minimum total RNA input for cDNA synthesis = 300 ng

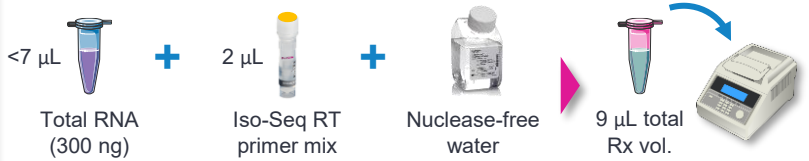


Step 2 procedural summary

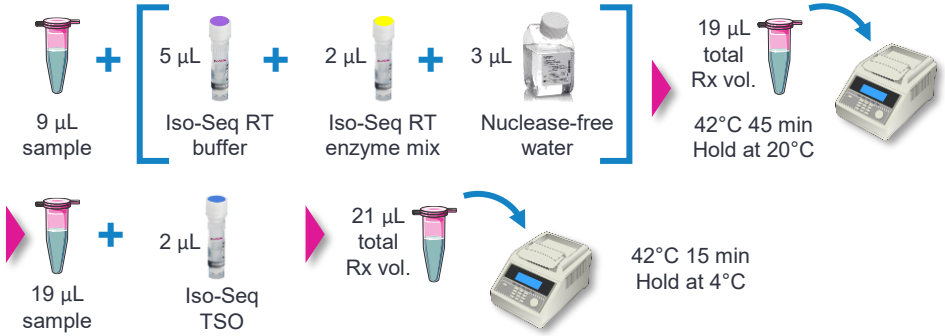
2.1 Thawing reagents for first-strand cDNA synthesis



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

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

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
		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
2.2.1	Total RNA (300 ng)	<7 µL

- 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
		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
2.3.1	Purple	Iso-Seq RT buffer (vortex briefly before use)	5 µL

- 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 µL of elution buffer to the 21 µL reverse transcription and template switching reaction (Section 2.3) for a total volume of 50 µL.
	2.4.2	Add 65 µL (1.3X v/v) of resuspended, room-temperature SMRTbell cleanup beads.
	2.4.3	Mix beads by pipetting 10 times or until evenly distributed

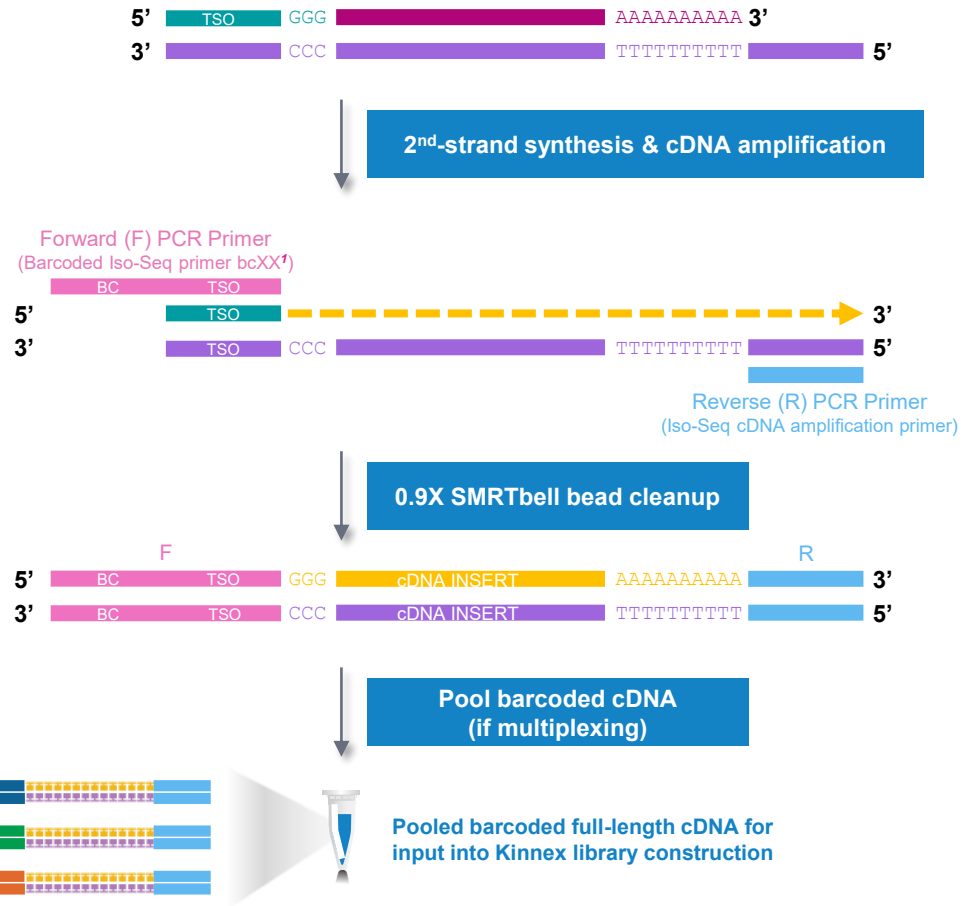
- Perform **1.3X** SMRTbell bead cleanup

cDNA amplification + cleanup

In this step, first-strand cDNA products are PCR-amplified and barcoded using barcoded Iso-Seq primers

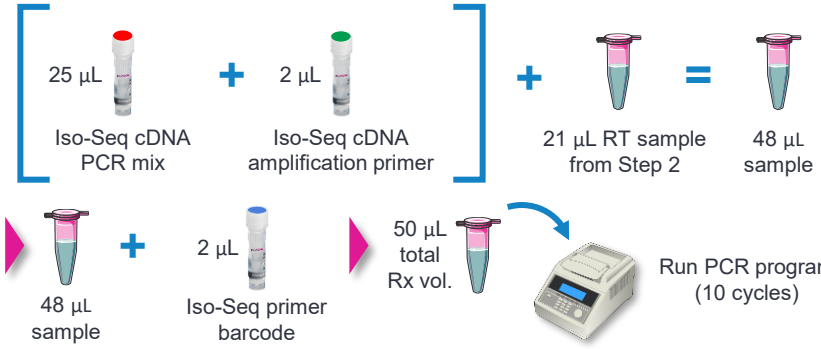
1 Input RNA quality control
↓ Safe stop
2 cDNA synthesis + cleanup
↓
3 cDNA amplification + cleanup
↓ Safe stop
4 Kinnex PCR + cleanup
↓
5 Kinnex array formation + cleanup
↓ Safe stop
6 Nuclease treatment + cleanup

Barcode up to 12 samples during cDNA amplification step using PacBio barcoded Forward Iso-Seq primers[1]

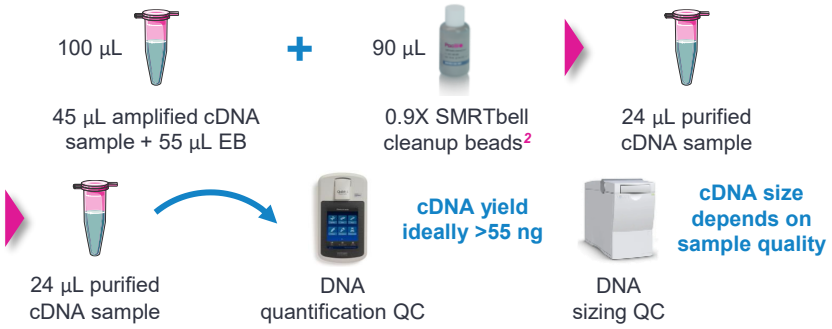


Step 3 procedural summary

3.1 cDNA amplification



3.2 0.9X SMRTbell bead cleanup



3.3 Pooling barcoded cDNA (skip if not multiplexing)

Proceed to **Step 4**

PacBio

[1] Twelve barcoded Iso-Seq primers (Iso-Seq primer bc01–12) are available for cDNA amplification step.
[2] If targeting longer cDNA, 86 µL of SMRTbell cleanup beads (0.86x v/v) can be used.

cDNA amplification + cleanup

Procedural notes

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

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.

✔	Tube color	Components	Volume
	Red	Iso-Seq cDNA PCR mix	25 µL
	Green	Iso-Seq cDNA amplification primer	2 µL
		Total volume	27 µL

	3.1.2	On ice, add 27 µL of reaction mix 3 to the 21 µL of the eluted cDNA (from previous Section 2.4).
	3.1.3	Add 2 µL of the appropriate Iso-Seq primer barcode to each sample for a total volume of 50 µL.

✔	Tube color	Component	Volume
	White	Iso-Seq primer	2 µL

	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.

PCR program	
45 seconds at 98°C	1 cycle
10 seconds at 98°C	10 cycles
15 seconds at 60°C	
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

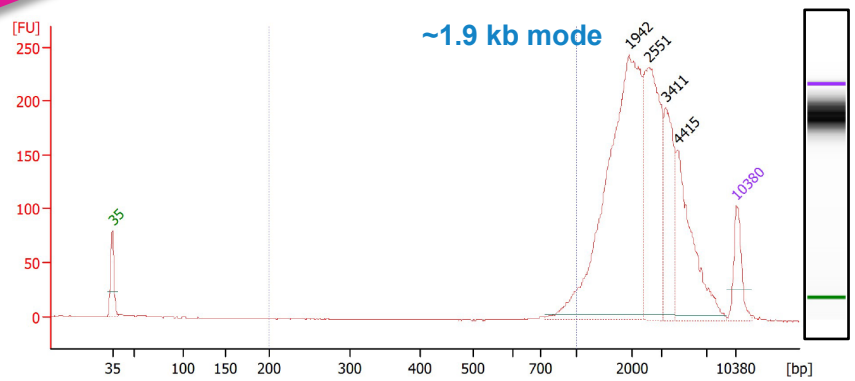
1. Input RNA quality control
2. cDNA synthesis + cleanup
3. cDNA amplification + cleanup
4. Kinnex PCR + cleanup
5. Kinnex array formation + cleanup
6. Nuclease treatment + cleanup

Safe stop

3.2 Cleanup of amplified cDNA using 0.9X SMRTbell Cleanup beads

✔	Step	Instructions
	3.2.1	Add **55 µL of elution buffer** to a new strip tube. Transfer **45 µL of PCR-amplified cDNA** from Section 3.1 into the strip tube containing elution buffer for a **final volume of 100 µL**. Add 90 µL (0.9x v/v) of resuspended, room-temperature SMRTbell cleanup beads. **The correct ratio of beads to sample is critical at this step.** If targeting longer cDNA, 86 µL 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 µL of the supernatant to a new strip tube. Discard the old strip tube with beads.
		Recommended: Measure concentration and size distribution of each cDNA sample.
	3.2.15	• Take a 1 µL aliquot from each strip tube. Dilute each aliquot with 4 µL of elution buffer. • Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. • Dilute 1:4 dilution further to 1.5 ng/µL based on the Qubit reading if needed. • Run 1 µL on an Agilent Bioanalyzer using a High Sensitivity DNA kit.
	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[1]
- Perform DNA concentration QC using Qubit ds DNA HS assay and DNA sizing QC using Bioanalyzer



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

- 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

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

Kinnex PCR

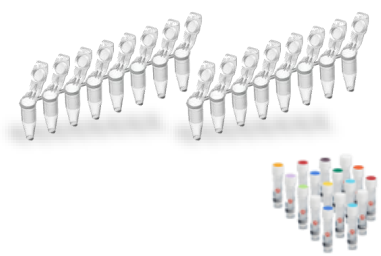
In this step, incorporate programmable Kinnex segmentation adapter sequences into amplified cDNA products

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

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

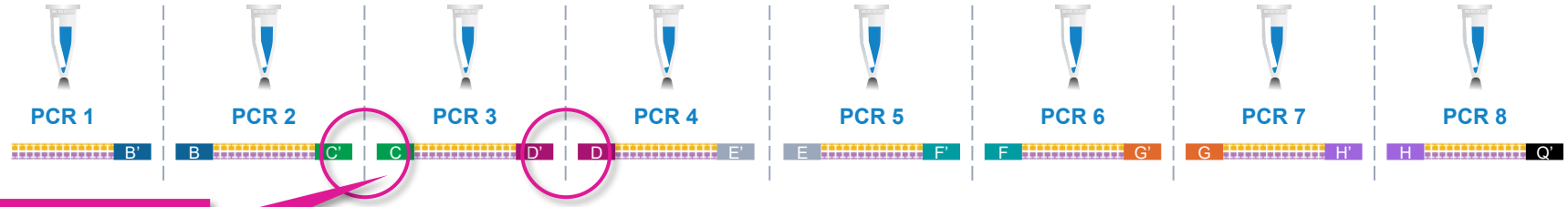
Barcoded cDNA sample after 0.9X SMRTbell bead cleanup (from Step 3)

Distribute barcoded cDNA sample + Kinnex PCR reaction mix into 8 PCR tubes or 8 wells in a 96-well plate



OR

Perform Kinnex PCR by adding premixed Kinnex primer pair solutions



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

Pool 8 Kinnex PCR products together and perform 1.05X SMRTbell bead cleanup

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

PacBio

Kinnex PCR (cont.)

Procedural summary

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

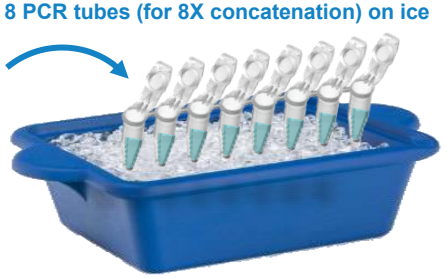
Step 4 procedural summary

4.1 Kinnex PCR

X = 55 (ng) / purified pooled DNA concentration (from Step 3)

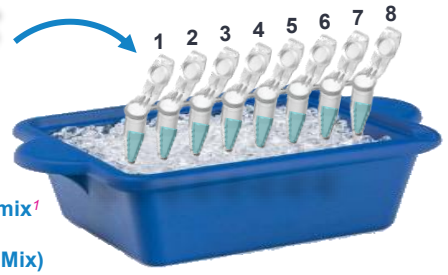
88 - X µL PCR-grade water + 110 µL Kinnex PCR mix + X µL 55 ng of amplified cDNA sample from Step 3 = 198 µL master mix → 198 µL master mix

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



Kinnex primer mix

Add 2.5 µL of Kinnex primer mix[1] into each of 8 PCR tubes (containing 22.5 µL of Master Mix)



Kinnex PCR Rx tube	8X concatenation	Tube color
1 [25 µL Rx volume]	Kinnex primer mix A	Orange
2 [25 µL Rx volume]	Kinnex primer mix B	
3 [25 µL Rx volume]	Kinnex primer mix C	
4 [25 µL Rx volume]	Kinnex primer mix D	
5 [25 µL Rx volume]	Kinnex primer mix E	
6 [25 µL Rx volume]	Kinnex primer mix F	
7 [25 µL Rx volume]	Kinnex primer mix G	
8 [25 µL Rx volume]	Kinnex primer mix HQ	

25 µL PCR Rx vol.

Run Kinnex PCR program (10 cycles)

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

Kinnex PCR 1: 23 µL + Kinnex PCR 2: 23 µL + Kinnex PCR 3: 23 µL + ••• + Kinnex PCR 8: 23 µL → **Pooled Kinnex PCR reactions**: 184 µL total pooled vol.

184 µL Pooled Kinnex PCR reactions + 193 µL 1.05X SMRTbell cleanup beads → 40 µL purified Kinnex PCR sample

Proceed to **Step 5**

[1] 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

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

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
Kinnex PCR mix	110 µL
55 ng of amplified cDNA from Step 3.2.16	X µL
Total volume	198 µL

X = 55 (ng)/purified pooled DNA concentration from step 3.2.16 (single-plex) or step 3.3.3 (multiplex)

*10% overage included

	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.
	4.1.7	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
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

1. Input RNA quality control
2. cDNA synthesis + cleanup
3. cDNA amplification + cleanup
4. Kinnex PCR + cleanup
5. Kinnex array formation + cleanup
6. Nuclease treatment + cleanup

Safe stop

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

✔	Step	Instructions
	4.2.1	Add exactly **23 µL** from each of the 8 PCR reactions into a 1.5 mL tube for a total volume of **184 µL**. 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. **The correct ratio of beads to pooled sample is critical at this step.**
	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.
	4.2.10	Remove residual 80% ethanol: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in the magnetic separation rack until the beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	4.2.11	Remove the tube from the magnetic rack. **Immediately** add **40 µL** of **elution buffer** 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 **room temperature** for **5 minutes** 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 **new tube strip**. 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[1] 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

[1] DNA concentration QC measurements for individual Kinnex PCR reactions prior to pooling are not required.

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

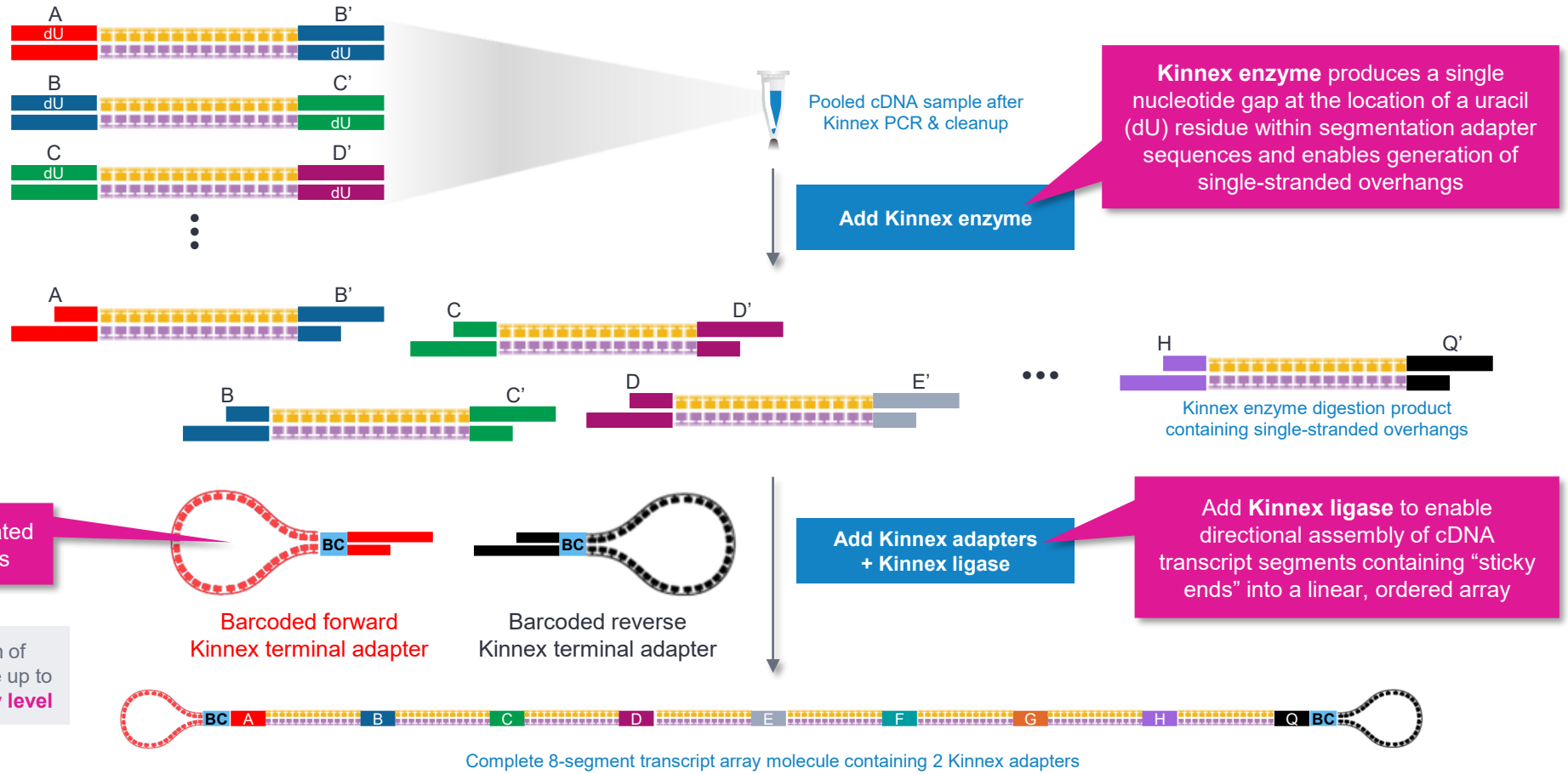
4 Kinnex PCR + cleanup

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[1] are ligated to specific overhang sequences at array ends

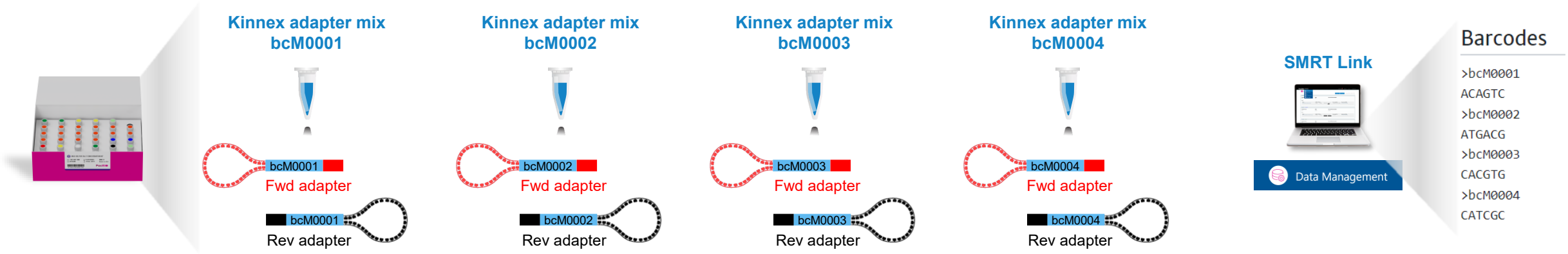
Kinnex library prep workflow supports incorporation of PacBio barcodes at the array formation step to enable up to **4-plex sample multiplexing at the SMRTbell library level**

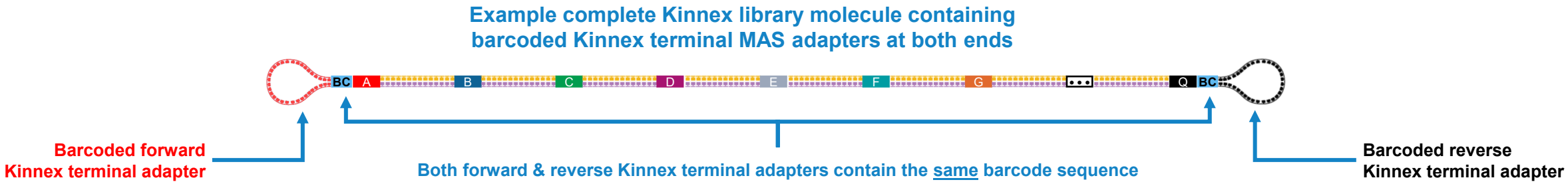
[1] **Note:** Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-M0004) are available for Kinnex array formation step. **Note:** Kinnex concatenation workflow is not compatible with standard SMRTbell adapters from SMRTbell prep kit 3.0 and is also not compatible with SMRTbell barcoded adapter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

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





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

Kinnex array formation (cont.)

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

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

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

Annealed & bound Kinnex library molecule

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

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

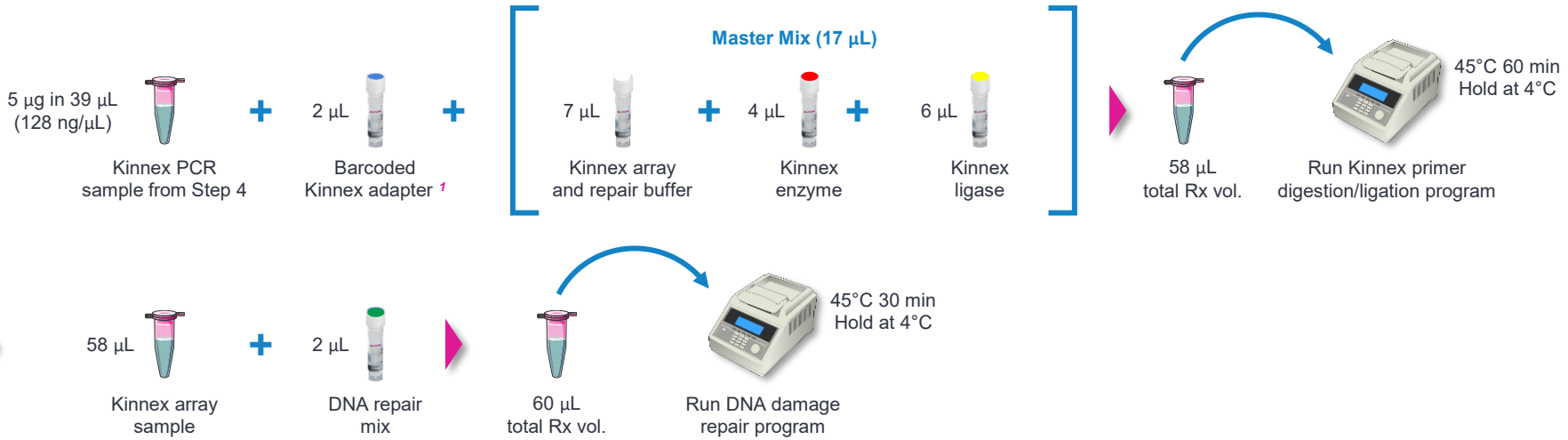
Kinnex array formation (cont.)

Procedural summary

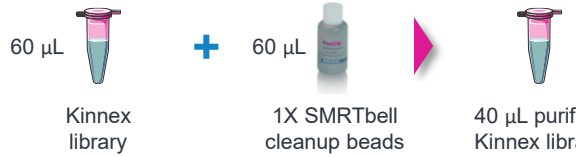
1 Input RNA quality control
↓ Safe stop
2 cDNA synthesis + cleanup
↓
3 cDNA amplification + cleanup
↓ Safe stop
4 Kinnex PCR + cleanup
↓
5 Kinnex array formation + cleanup
↓ Safe stop
6 Nuclease treatment + cleanup

Step 5 procedural summary

5.1 Kinnex array formation



5.2 1X SMRTbell cleanup



Proceed to **Step 6**

1 Kinnex full-length RNA kit bundle (103-072-000) contains four different barcoded Kinnex adapter mixes (bcM0001–bcM0004) → Select one barcode to use per Kinnex library preparation reaction.

Kinnex array formation (cont.)

Procedural notes

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

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 Step 4.2.15, 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.

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.

Step	Temperature	Duration
1	45°C	60 min
2	4°C	Hold

	5.1.3	
	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.

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	

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.

PacBio

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

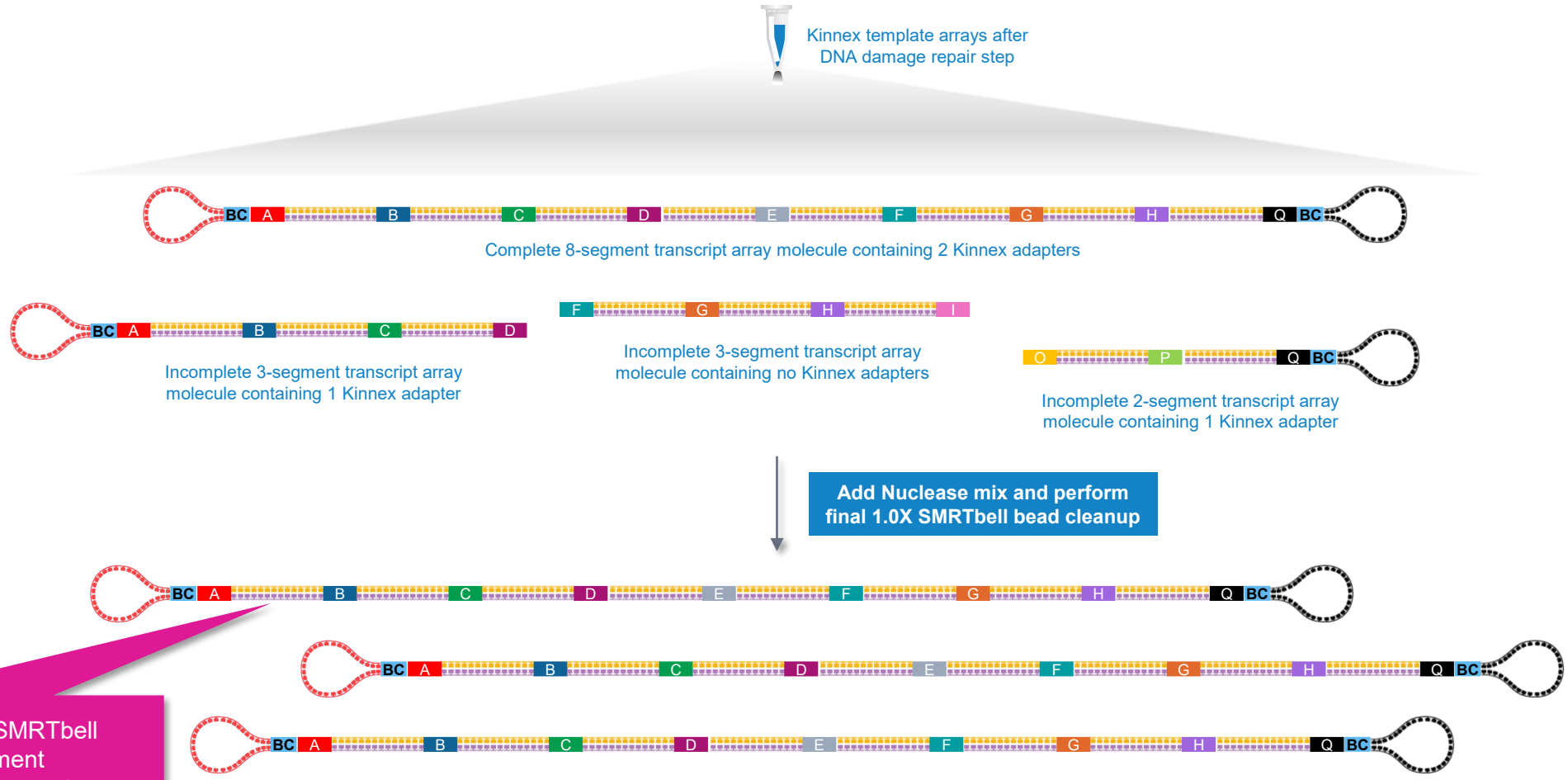
4 Kinnex PCR + cleanup

↓

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

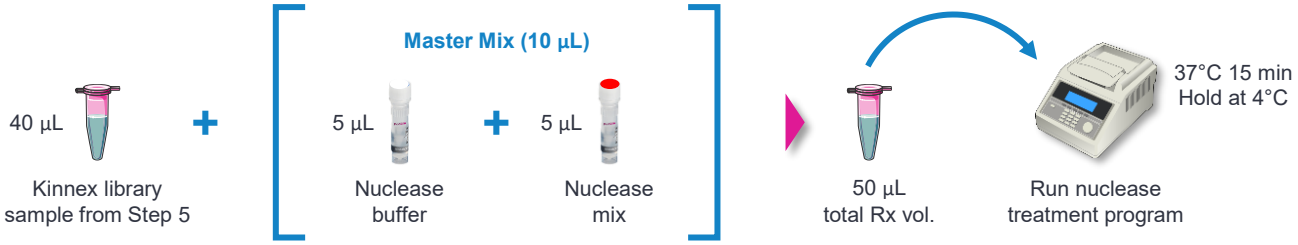
Nuclease treatment & cleanup (cont.)

Procedural summary

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

Step 6 procedural summary

5.1 Nuclease treatment



5.2 1X SMRTbell cleanup

Proceed to **ABC**[1]

[1] Follow **Step 7** instructions in **Procedure & checklist** to prepare the Kinnex SMRTbell library (25 µL) for sequencing on Revio (+SPRQ) or Vega system.

Nuclease treatment & cleanup (cont.)

Procedural notes

1. Input RNA quality control
2. Safe stop
3. cDNA synthesis + cleanup
4. cDNA amplification + cleanup
5. Safe stop
6. Kinnex PCR + cleanup
7. Kinnex array formation + cleanup
8. Safe stop
9. **Nuclease treatment + cleanup**

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	**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.

Step	Temperature	Duration
1	37°C	15 min
2	4°C	Hold

- Perform nuclease treatment for **15 min**

Nuclease treatment & cleanup (cont.)

Procedural notes

1. Input RNA quality control
2. cDNA synthesis + cleanup
3. cDNA amplification + cleanup
4. Kinnex PCR + cleanup
5. Kinnex array formation + cleanup
6. Nuclease treatment + cleanup

Safe stop

6.2 Final cleanup with 1X SMRTbell cleanup beads

✓	Step	Instructions
	6.2.1	Add 50 µL 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 µL, 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.
	6.2.8	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from
	6.2.14	Take a 1 µL 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 pg/µL 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 µL of library to <60 ng/µL**. Using a concentration above 60 ng/µL 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 µg 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 **≤60 ng/ µL** to proceed with sample setup (ABC[1]) [Step 7]
  - → Using a concentration above 60 ng/µL 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 ≥2 SMRT Cells

Kinnex FL RNA

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

Library insert generation

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

IN: 300 ng of total RNA per sample

OUT: ≥100 ng of amplified cDNA per sample

Kinnex PCR

Kinnex PCR → **SMRTbell bead cleanup**

IN: 55 ng of amplified cDNA (single or multiplex)

OUT: 6-12 µg of amplified products after pooling

Kinnex array formation

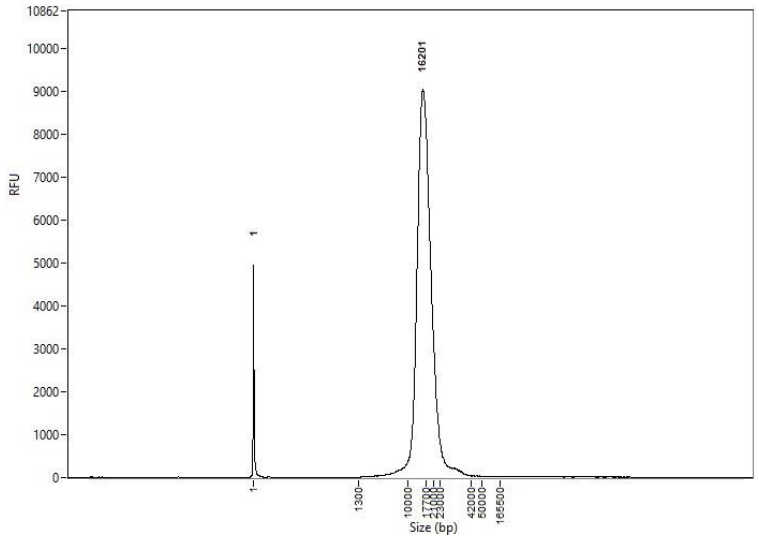
Kinnex digestion / Ligation → **DNA damage repair** → **SMRTbell cleanup** → **Nuclease treatment** → **SMRTbell bead cleanup**

IN: 5 µg of pooled Kinnex PCR products

OUT: 80% recovery from Kinnex digestion Rx input

IN: 40 µL of DDR reaction products

OUT: 10-25% recovery from Kinnex digestion Rx input



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 (%)[1]	1460 ng (24.7%)

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

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

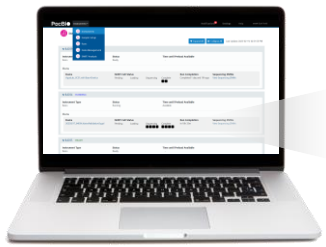
PacBio

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



Runs

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

Kinnex single-cell RNA[1]

- **Whole genome sequencing**
  - Human WGS
  - Microbial assembly
  - Other WGS
- **RNA sequencing**
  - Iso-Seq method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- **Viral sequencing**
  - Adeno-associated virus
- **Metagenomics**
  - Kinnex 16S rRNA
  - Full-length 16S rRNA sequencing

Kinnex full-length RNA[2]

- **Whole genome sequencing**
  - Human WGS
  - Microbial assembly
  - Other WGS
- **RNA sequencing**
  - Iso-Seq method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- **Viral sequencing**
  - Adeno-associated virus
- **Metagenomics**
  - Kinnex 16S rRNA
  - Full-length 16S rRNA sequencing

Kinnex 16S rRNA[2]

- **Whole genome sequencing**
  - Human WGS
  - Microbial assembly
  - Other WGS
- **RNA sequencing**
  - Iso-Seq method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- **Viral sequencing**
  - Adeno-associated virus
- **Metagenomics**
  - Kinnex 16S rRNA
  - Full-length 16S rRNA sequencing

SMRT Link module	Key setup parameters	Sequel IIe system recommended settings	Vega system recommended settings	Revio system recommended settings
Sample setup	Library type		Kinnex	
	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	
	Library Type[1]		Kinnex	
	Movie Acquisition Time	30 hrs	24 hrs	24 hrs
	Add Analysis		YES	

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

SMRT Link Run Design procedure for Revio and Vega systems

Sample and run information

Kinnex single-cell RNA

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

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

Kinnex full-length RNA

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

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

Kinnex 16S rRNA

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

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

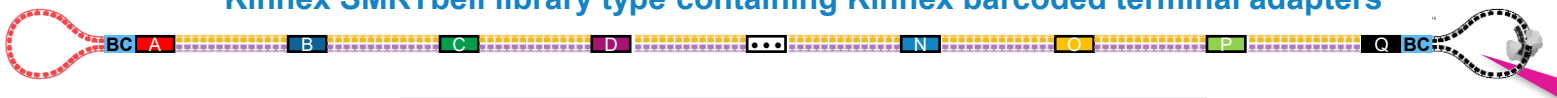
Select **application type** to autofill Library Type & Movie Acquisition Time recommended settings

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

Standard SMRTbell library type containing standard SPK3 barcoded terminal adapters

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

Kinnex SMRTbell library type containing Kinnex barcoded terminal adapters



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

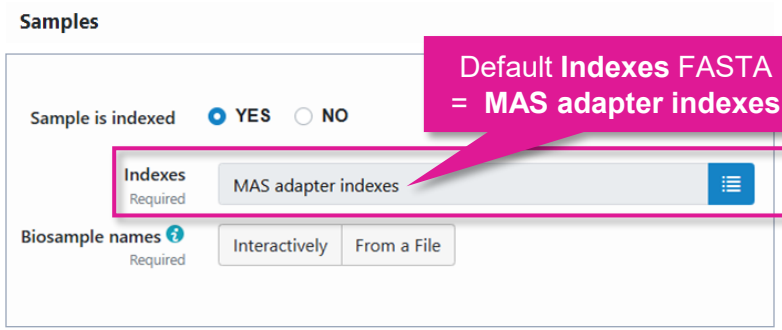
Library Type field determines which adapter finding algorithm is used during post-primary analysis[1]

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

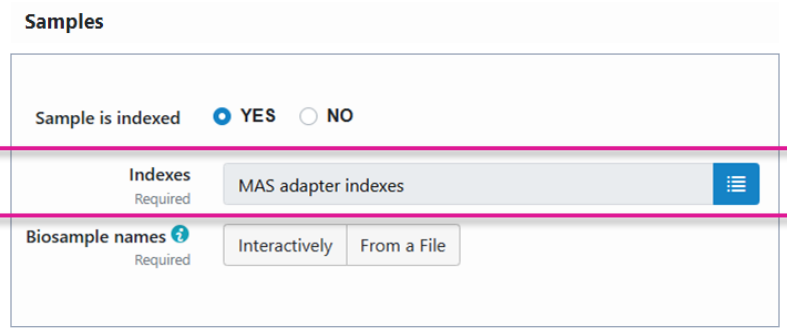
SMRT Link Run Design procedure for Revio and Vega systems (cont.)

Sample indexing (barcoding) information

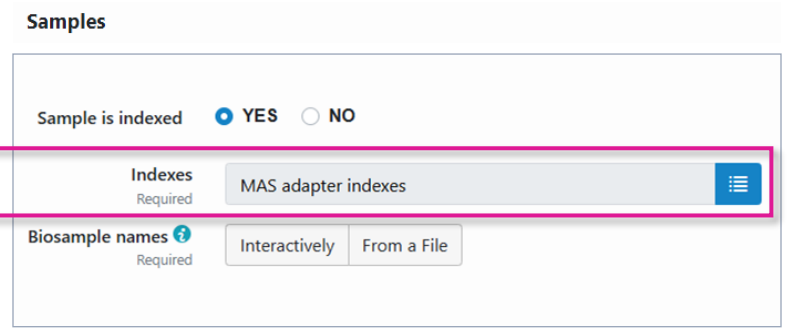
Kinnex single-cell RNA



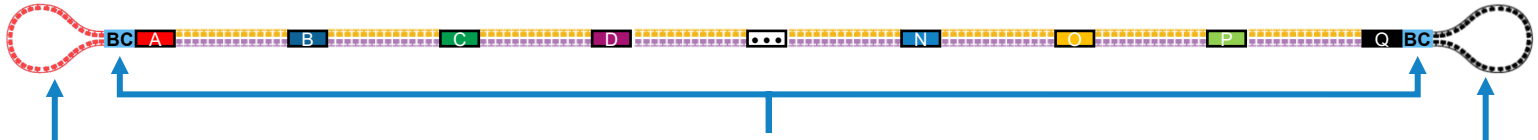
Kinnex full-length RNA



Kinnex 16S rRNA



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

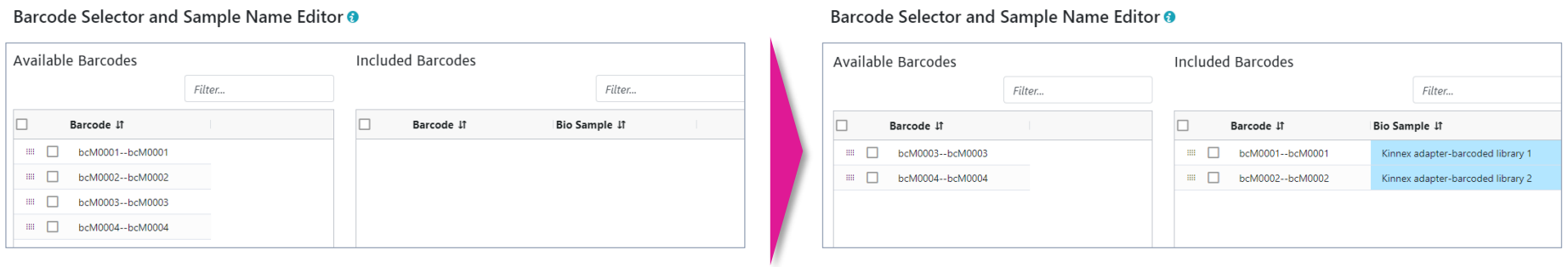


Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

Example interactive biosample name specification



MAS SMRTbell barcoded adapter indexes

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

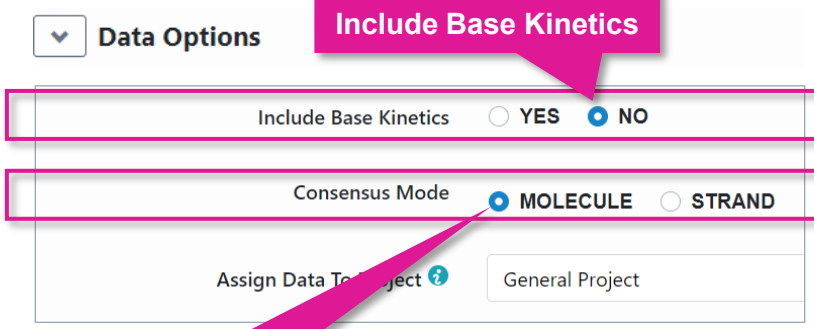
[1] Four barcoded terminal Kinnex adapters (Kinnex adapter bcM0001-bcM0004) are available for Kinnex array formation step. Kinnex adapter barcode sequences can be downloaded from SMRT Link Data Management module.

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

Data options

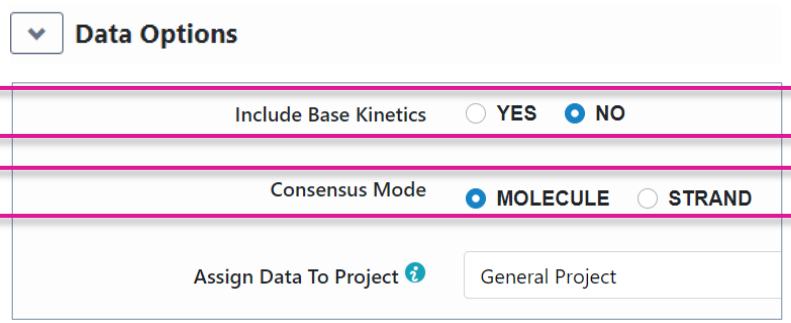
Kinnex single-cell RNA

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

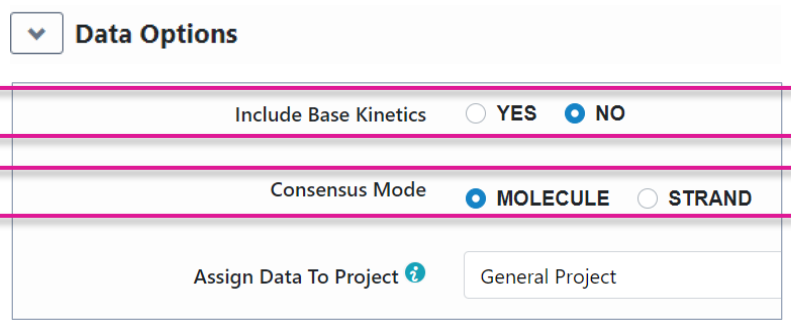


Default **Consensus Mode = MOLECULE**[1]

Kinnex full-length RNA



Kinnex 16S rRNA



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

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

SMRT Link Run Design analysis options

Kinnex single-cell RNA

Analysis Options

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

Analysis Workflow is automatically filled in

Add Analysis	YES / NO
Analysis Name Required	Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Nam
Select Analysis Workflow Required	Read Segmentation and Single-Cell Iso-Seq
Segmentation Adapter Set	MAS-Seq Adapter v1 (MAS16)
Primer Set Required	10x Chromium single cell 3' cDNA primers
Reference Set Required	Human Genome hg38, with Gencode v39 annotations
Kit Type	10X 3' KIT / 10X 5' KIT

Advanced Parameters

Kinnex full-length RNA

Analysis Options

Add Analysis	YES / NO
Analysis Name Required	Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name
Select Analysis Workflow Required	Read Segmentation and Iso-Seq
Segmentation Adapter Set	MAS-Seq Adapter v3 (MAS8)
Primer Set Required	Iso-Seq v2 Barcoded cDNA Primers
Reference Set	Human Genome hg38, with Gencode v39 annotations
Cluster of Barcoded Samples	Pool reads and cluster together

Advanced Parameters

Kinnex 16S rRNA

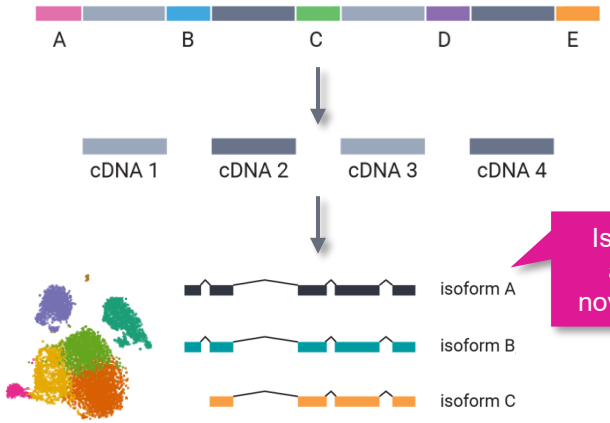
Analysis Options

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

Advanced Parameters

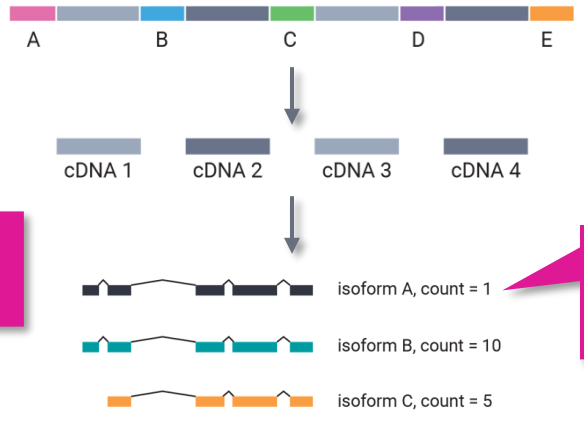
Split arrayed HiFi reads at adapter positions, generating segmented reads (S-reads)

Read Segmentation and Single-Cell Iso-Seq



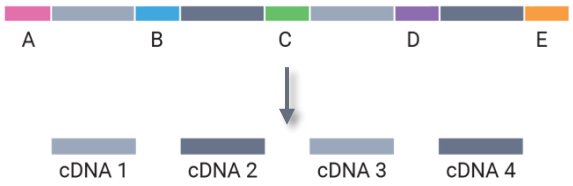
Isoform-classification analysis to identify novel genes & isoforms

Read Segmentation and Iso-Seq



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

Read Segmentation



SMRT Link Run Design analysis options (cont.)

Kinnex single-cell RNA

Analysis Options

Add Analysis	YES / NO
Analysis Name Required	Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name
Select Analysis Workflow Required	Read Segmentation and Single-Cell Iso-Seq
Segmentation Adapter Set	MAS-Seq Adapter v1 (MAS16)
Primer Set Required	10x Chromium single cell 3' cDNA primers
Reference Set Required	Human Genome hg38, with Gencode v39 annotations
Kit Type	10X 3' KIT / 10X 5' KIT

Advanced Parameters

Kinnex full-length RNA

Analysis Options

Add Analysis	YES / NO
Analysis Name Required	Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name
Select Analysis Workflow Required	Read Segmentation and Iso-Seq
Segmentation Adapter Set	MAS-Seq Adapter v3 (MAS8)
Primer Set Required	Iso-Seq v2 Barcoded cDNA Primers
Reference Set	Human Genome hg38, with Gencode v39 annotations
Cluster of Barcoded Samples	Pool reads and cluster together

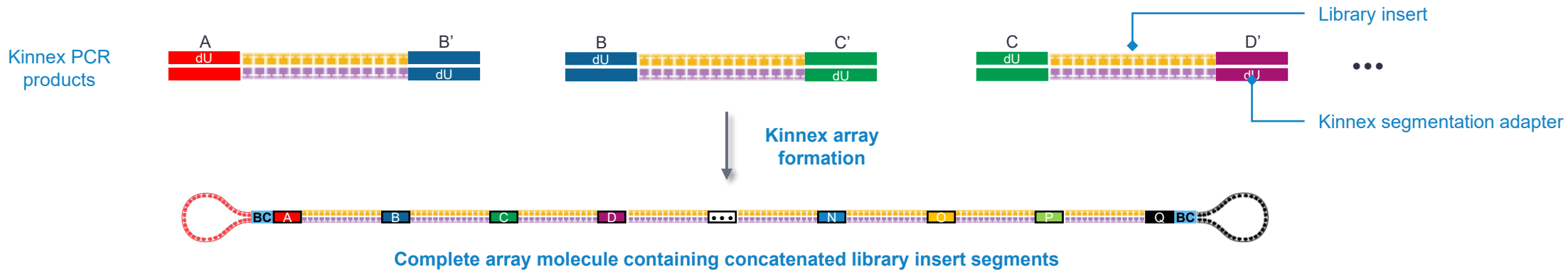
Advanced Parameters

Kinnex 16S rRNA

Analysis Options

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

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



SMRT Link Run Design analysis options (cont.)

Kinnex single-cell RNA

Analysis Options

Add Analysis	YES / NO
Analysis Name (Required)	Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name
Select Analysis Workflow (Required)	Read Segmentation and Single-Cell Iso-Seq
Segmentation Adapter Set	MAS-Seq Adapter v1 (MAS16)
Primer Set (Required)	10x Chromium single cell 3' cDNA primers
Reference Set (Required)	Human Genome hg38, with Gencode v39 annotations
Kit Type	10X 3' KIT / 10X 5' KIT

Advanced Parameters

Specify **Primer Set** used for cDNA amplification

10x Forward (F) PCR primer — 10x Reverse (R) PCR primer

```
5' [CBC][UMI]TTTTTTTTT LIBRARY INSERT CCC TSO 3'
3' [CBC][UMI]AAAAAAAAA LIBRARY INSERT GGG TSO 5'
```

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

OR

```
5' [CBC][UMI][TSO]GGG LIBRARY INSERT AAAAAAAAA 3'
3' [CBC][UMI][TSO]CCC LIBRARY INSERT TTTTTTTTT 5'
```

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

Kinnex full-length RNA

Analysis Options

Add Analysis	YES / NO
Analysis Name (Required)	Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name
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

Forward (F) PCR primer (Barcoded Iso-Seq primer bcXX[2]) — Reverse (R) PCR primer (Iso-Seq cDNA amplification primer)

```
5' BC TSO GGG LIBRARY INSERT AAAAAAAAA 3'
3' BC TSO CCC LIBRARY INSERT TTTTTTTTT 5'
```

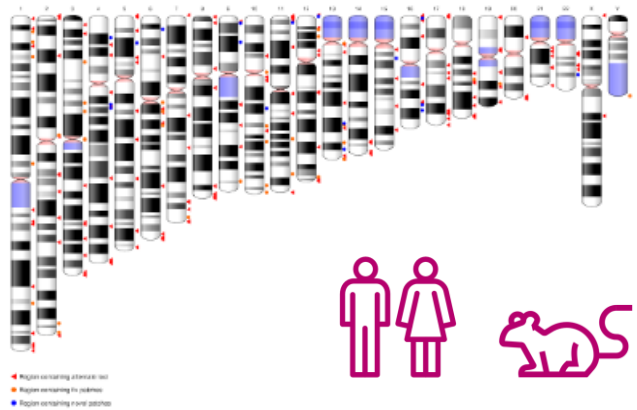
For Kinnex full-length RNA analysis, select 'Iso-Seq v2 Barcoded cDNA primers'

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

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

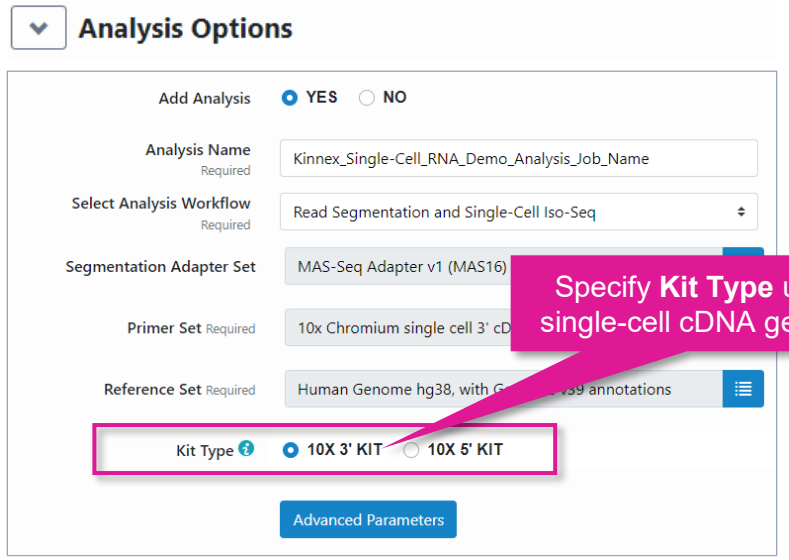
- Human Genome hg38, with Gencode v39 annotations
- Mouse Genome mm39, with Gencode vM28 annotations

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



SMRT Link Run Design analysis options (cont.)

Kinnex single-cell RNA



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

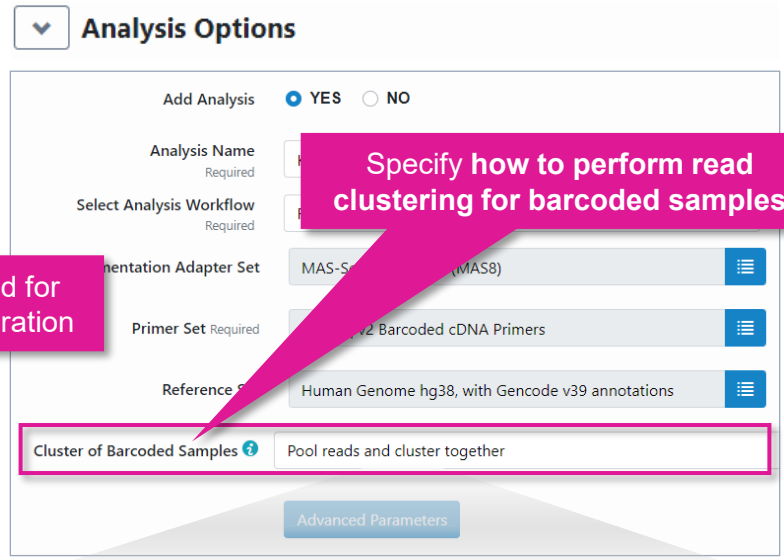
Specification of Kit Type (10x 3' Kit or 10x 5' Kit) **determines which set of 10x barcode sequences to use**, and also affects **UMI and single-cell barcode design settings**[1]

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

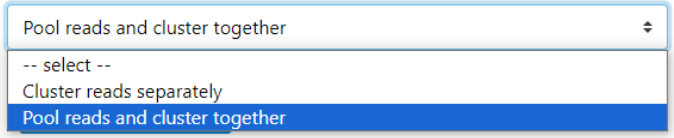
OR

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

Kinnex full-length RNA

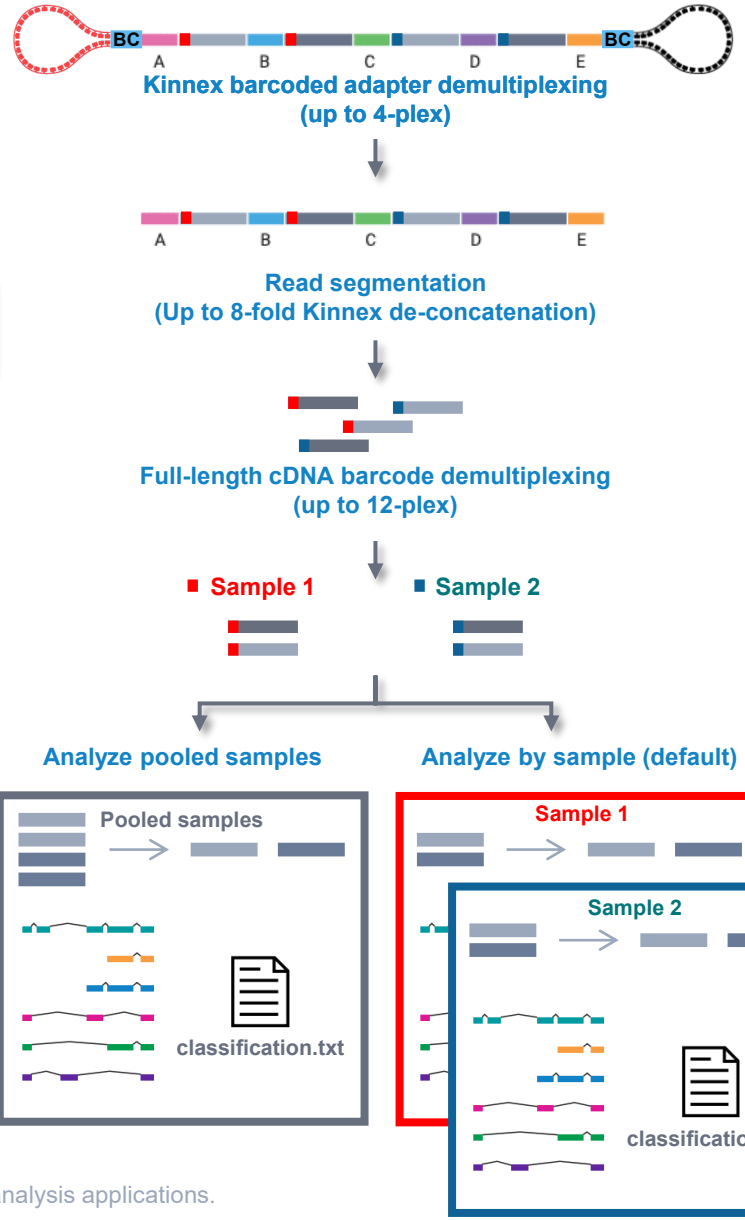


Specify **how to perform read clustering for barcoded samples**



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

Clustering options for Kinnex full-length RNA samples

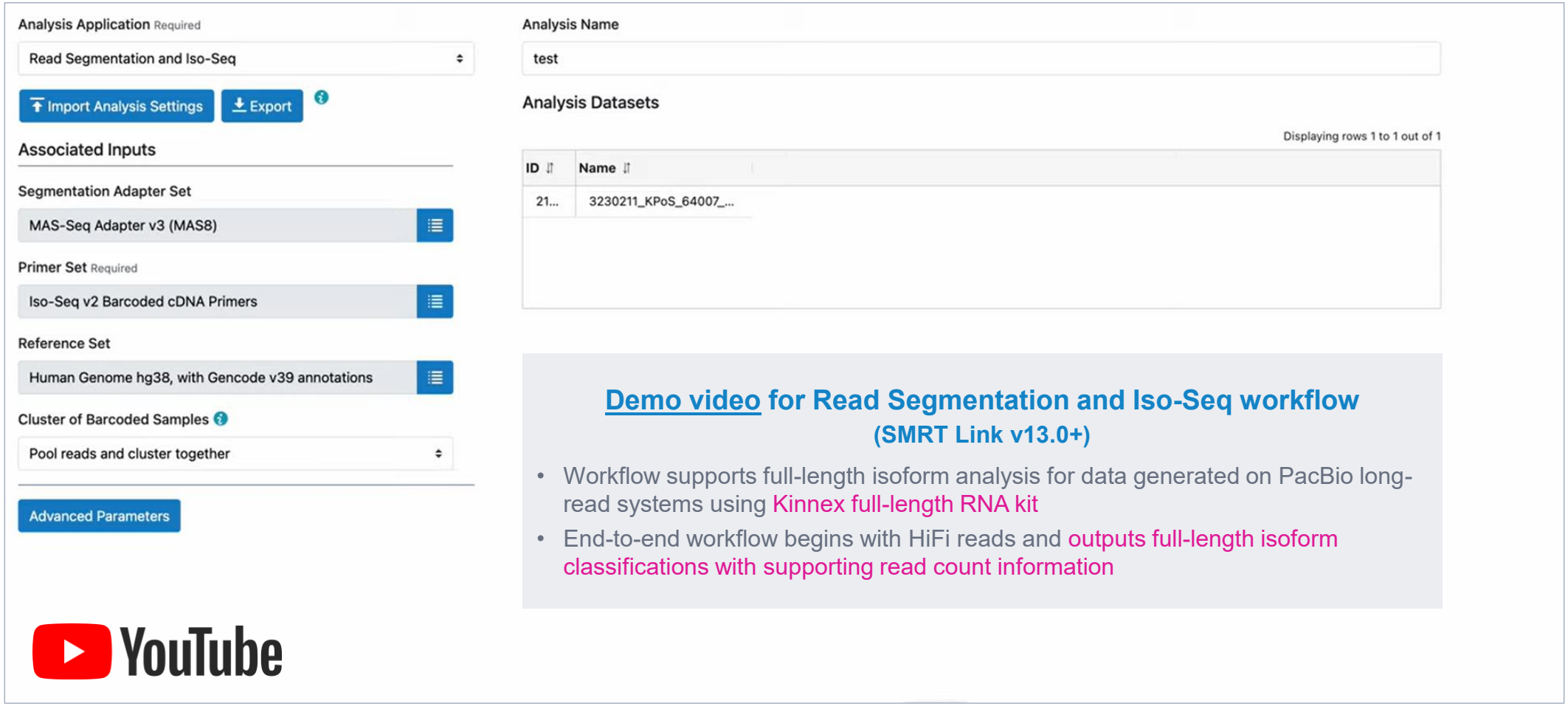


PacBio

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

SMRT Link Read Segmentation and Iso-Seq analysis video demonstration

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



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

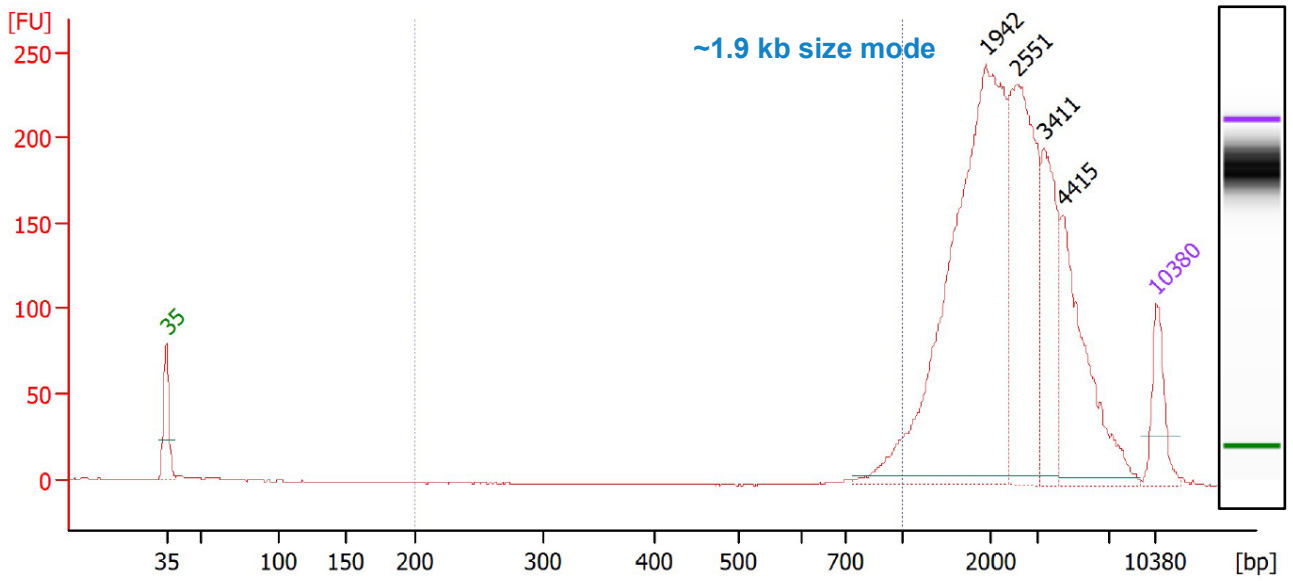
- Workflow supports full-length isoform analysis for data generated on PacBio long-read systems using Kinnex full-length RNA kit
- End-to-end workflow begins with HiFi reads and outputs full-length isoform classifications with supporting read count information

Kinnex full-length RNA example sequencing performance data

Example Kinnex full-length RNA library preparation QC results

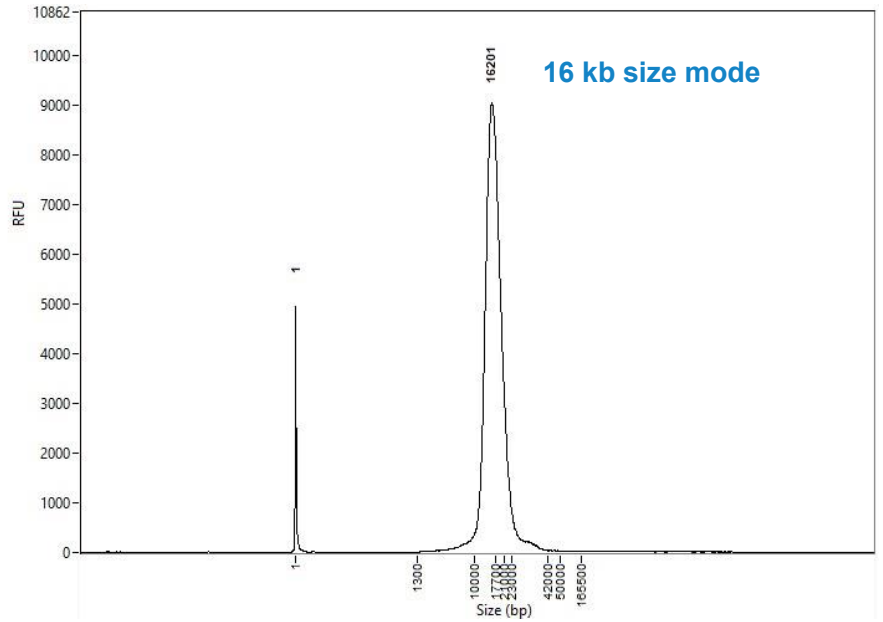
Kinnex full-length RNA library prepared with human UHRR total RNA

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

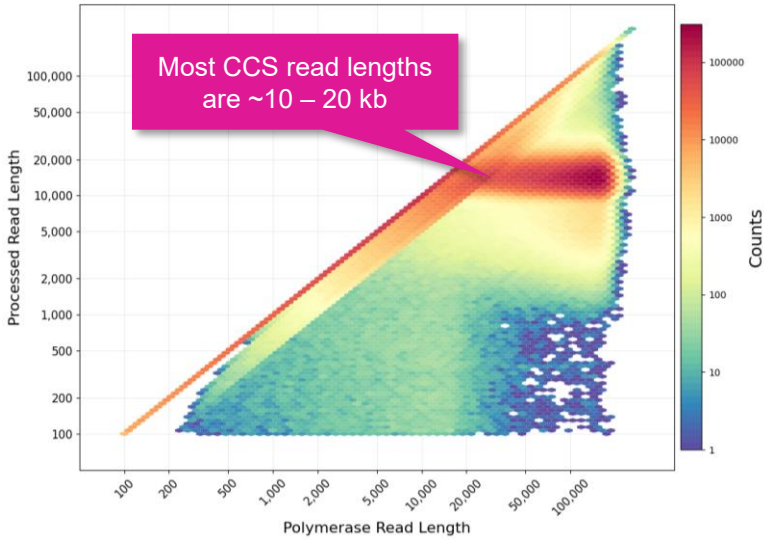
Total RNA input for cDNA synthesis	300 ng
cDNA input for Kinnex array formation	5900 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1460 ng (24.7%)

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

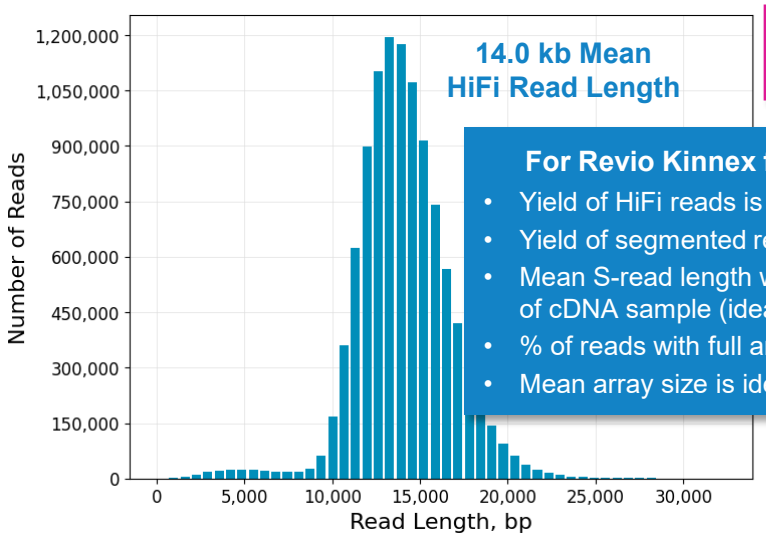
Example sequencing performance for Kinnex full-length RNA libraries prepared with human cDNA [Revio system + SPRQ chemistry[1]]

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

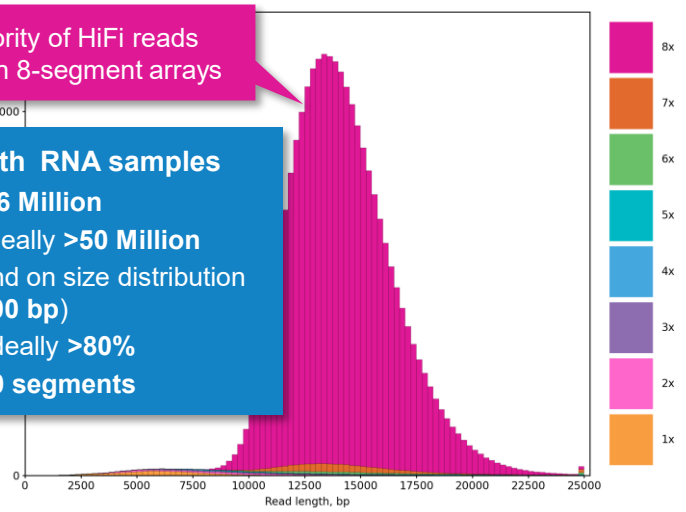
Raw Data Report



HiFi Read Length



Read Segmentation Metrics



For Revio Kinnex full-length RNA samples

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

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

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

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

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

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

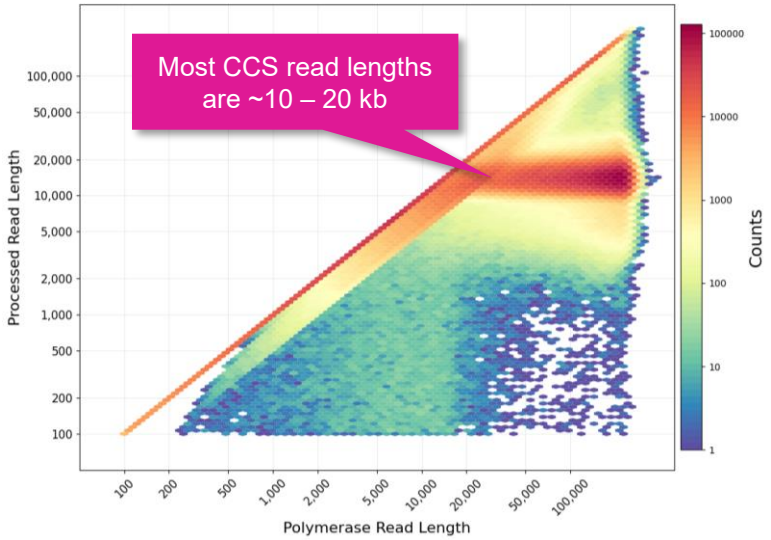
For UHRR Kinnex libraries, per-Revio SMRT Cell segmentation read counts were typically >50 Million.

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

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

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

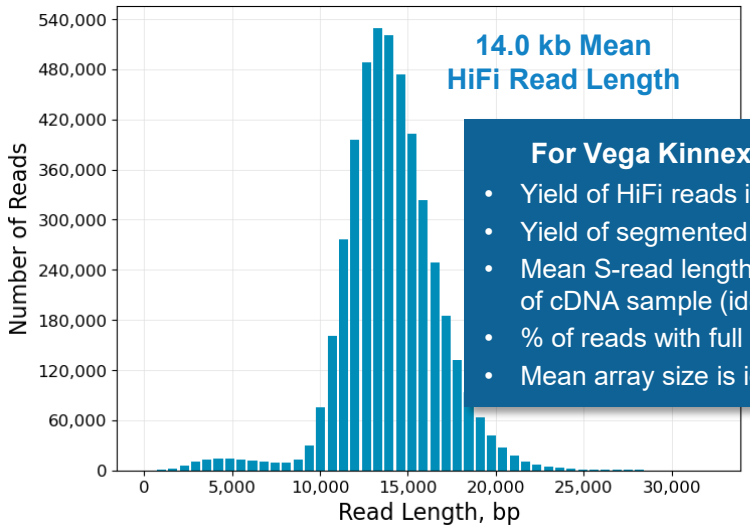
Raw Data Report



Mean Polymerase Read Length	95.3 kb
Loading level	61%

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

HiFi Read Length



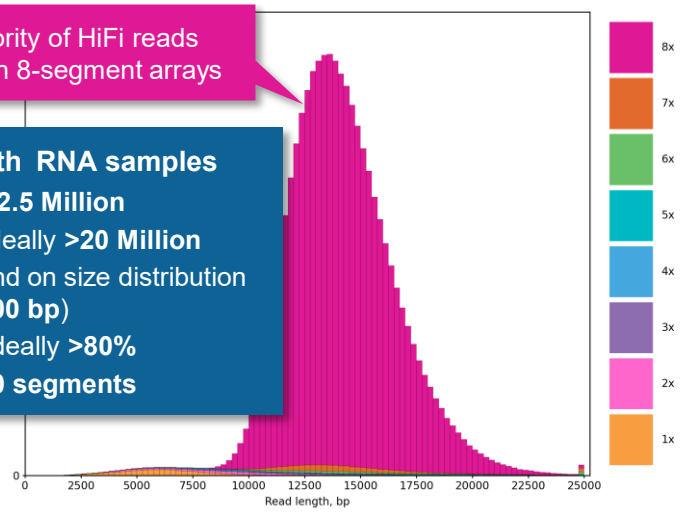
For Vega Kinnex full-length RNA samples

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

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

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

Read Segmentation Metrics



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

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

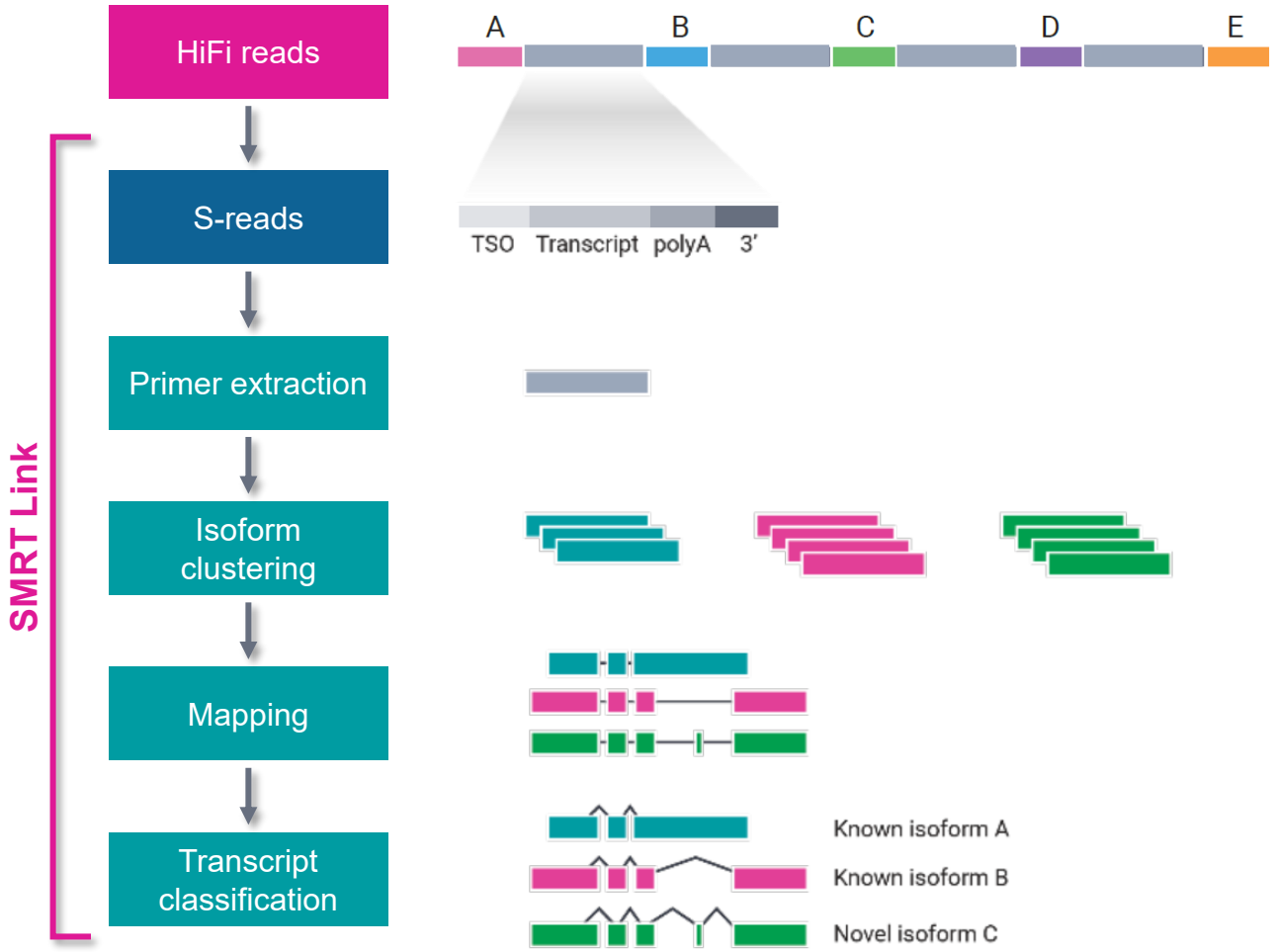
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

Kinnex full-length RNA data analysis workflow

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[1]



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[2]

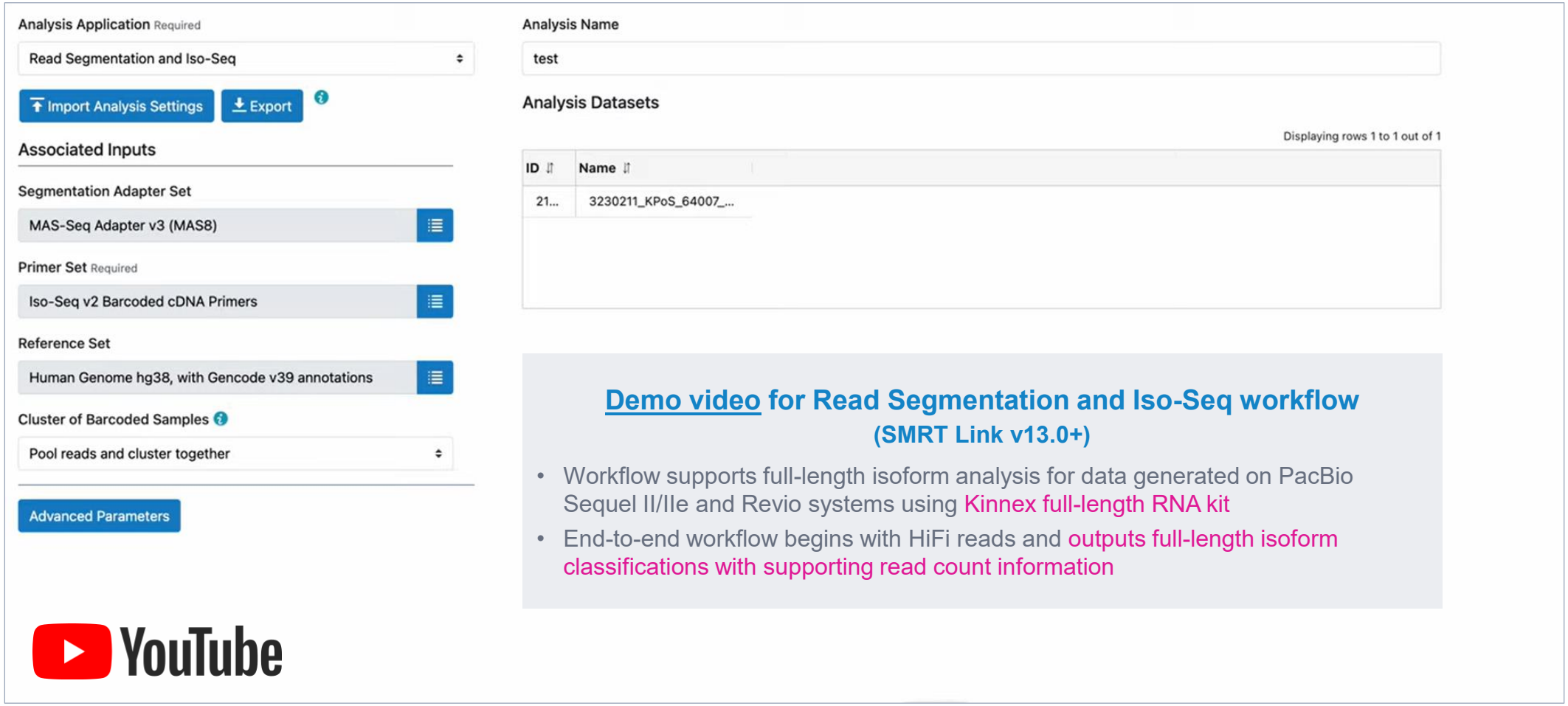
- If an annotation (e.g., Gencode) is provided, isoforms are classified against it using pigeon (the PacBio implementation of SQANTI3) to identify known and novel genes/isoforms
- The Iso-Seq workflow can jointly analyze pooled sample reads to produce a unified isoform annotation with per-sample read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

PacBio

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

SMRT Link Read Segmentation and Iso-Seq analysis video demonstration

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



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

- Workflow supports full-length isoform analysis for data generated on PacBio 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[1]

Analysis recommendations for Iso-Seq data based on reference genome and annotation availability[1]

- 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	• Use the Iso-Seq workflow with preloaded human / mouse annotation to get mapped, unique isoforms with classifications and read count information (FASTA, GFF, TXT).
Model organism with good annotation	• Run Iso-Seq workflow with uploaded reference genome to get mapped, unique isoforms (FASTA, GFF) • Generate pigeon-compliant annotation and use the command line for isoform classification with read count information (TXT)
Non-model organism with genome	• Run Iso-Seq workflow with uploaded reference genome to get mapped, unique isoforms (FASTA, GFF)
No genome	• Run Iso-Seq workflow without reference genome to get unique isoforms (FASTA)

PacBio

[1] See *Application note – Kinnex full-length RNA kit for isoform sequencing* (102-326-591).

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[1]

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

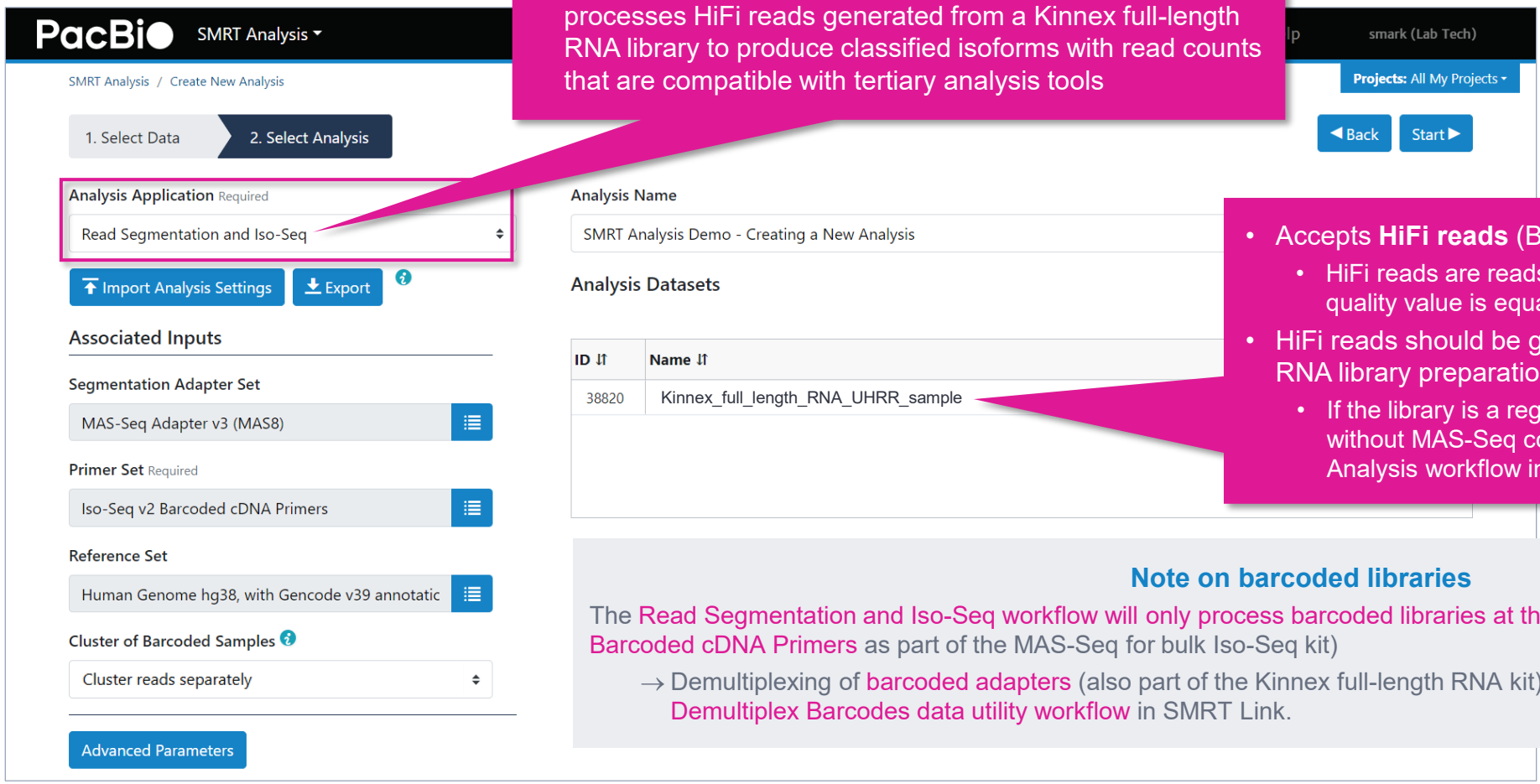
Example application	Human genetics disease studies	Biopharma for identifying highly expressed targets	Plant & animal whole genome annotation
Experimental goal	Isoform discovery and quantification of moderate-to-rare transcripts	Isoform discovery of high expressed transcripts	Comprehensive transcript 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[1]	**Sequel II/IIe system:** Up to 2 samples per SMRT Cell 8M (2-plex)	**Sequel II/IIe system:** Up to 3 samples per SMRT Cell 8M (3-plex)	
	Vega system: Up to 3 samples per Vega SMRT Cell (3-plex)	**Vega system:** Up to 6 samples per Vega SMRT Cell (6-plex)	
	Revio system + SPRQ: Up to 6 samples per Revio SMRT Cell (6-plex)	**Revio system + SPRQ:** Up to 12 samples per Revio SMRT Cell (12-plex)	
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		

[1] See ***Application note – Kinnex full-length RNA kit for isoform sequencing*** (102-326-591).

SMRT Link Read Segmentation and Iso-Seq analysis application setup

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

- **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

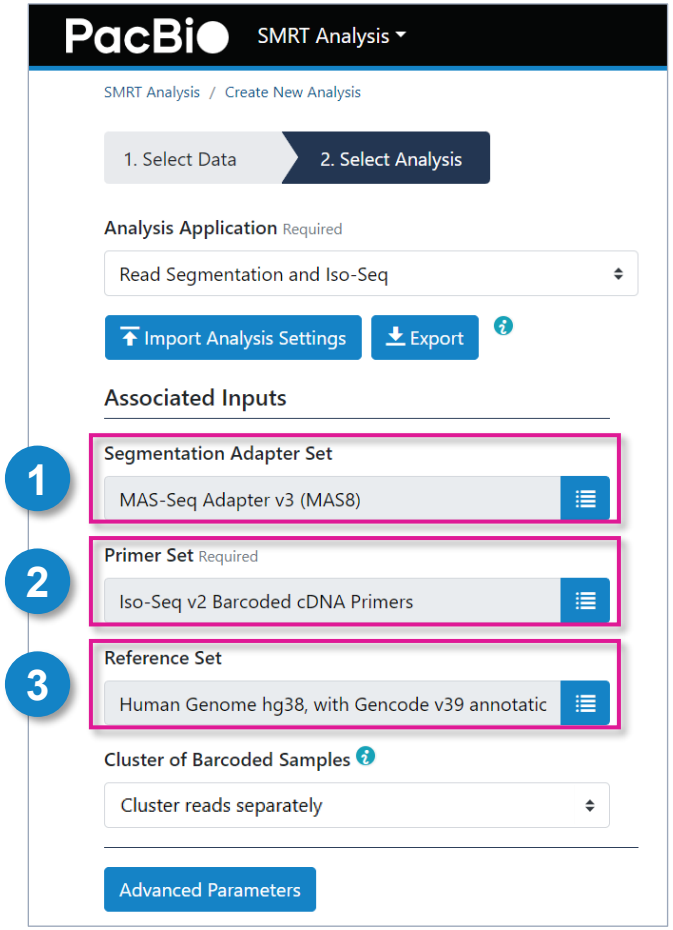
The Read Segmentation and Iso-Seq workflow will only process barcoded libraries at the cDNA level (such as using Iso-Seq v2 Barcoded cDNA Primers as part of the MAS-Seq for bulk Iso-Seq kit)

→ 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.

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

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

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



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 `T`s 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

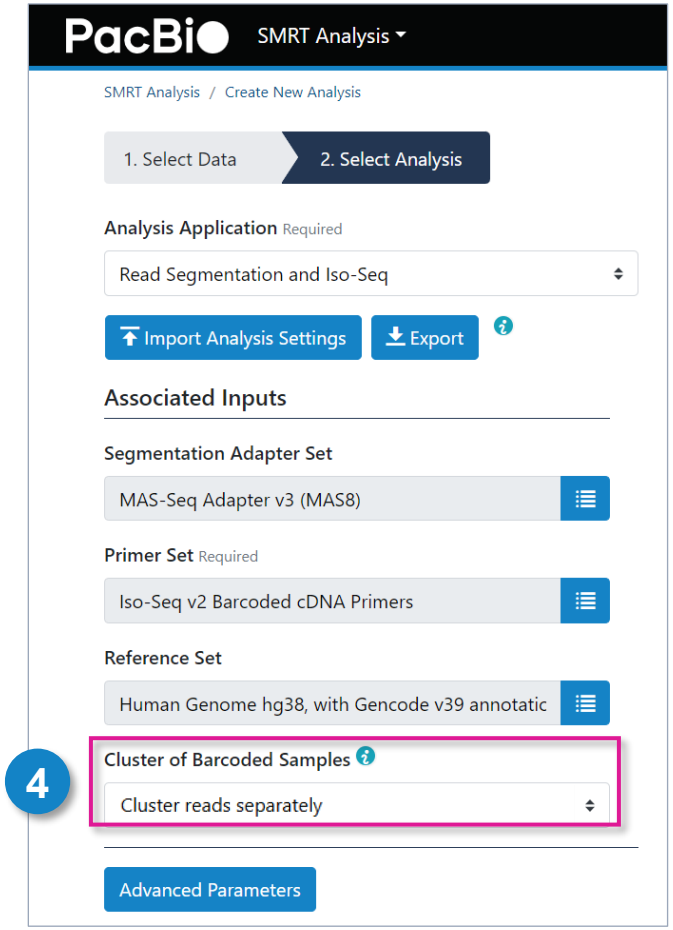
3. Reference Set (Required)

- 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`)

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

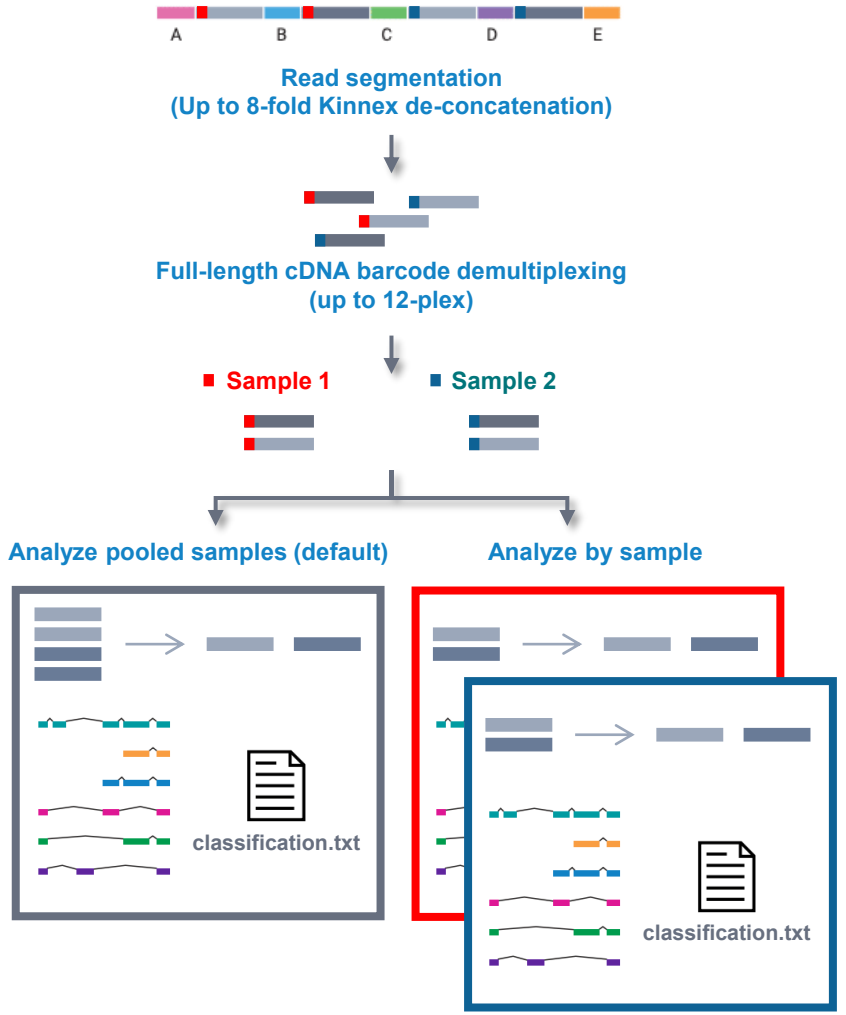
SMRT Link Read Segmentation and Iso-Seq analysis application setup (cont.)

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



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`



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

Example SMRT Link Read Segmentation data utility processing results[1] 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)

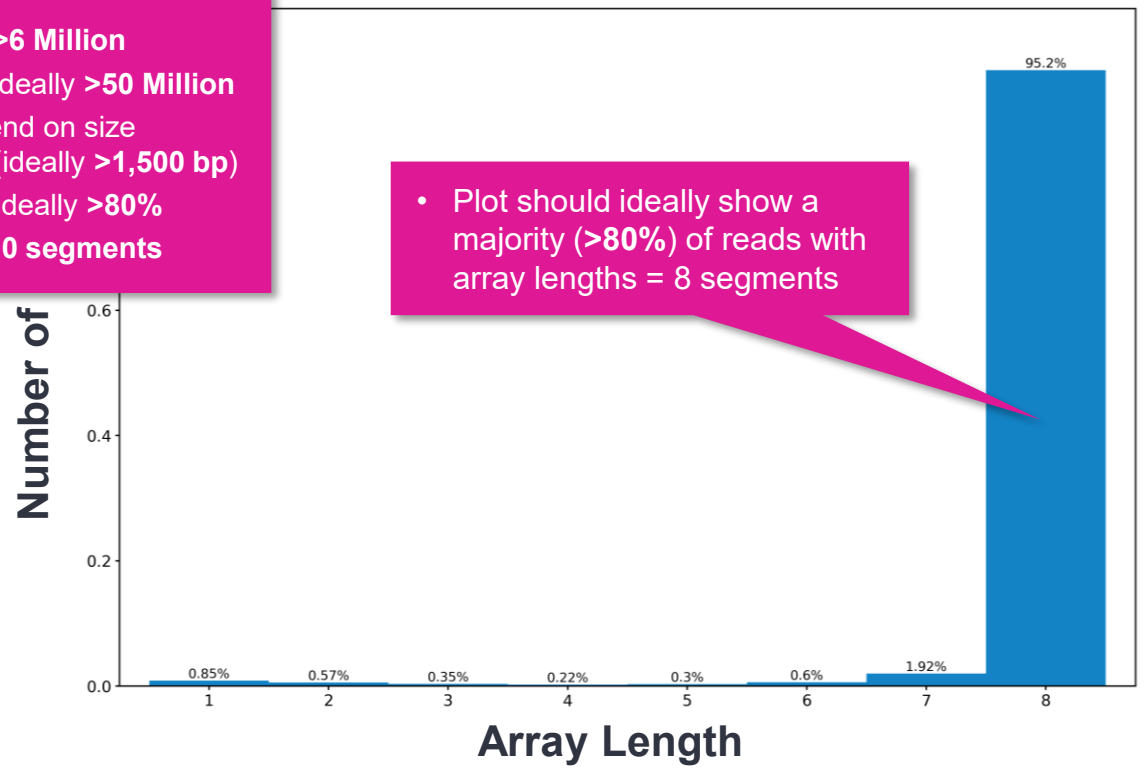
Example Revio system + SPRQ data shown.

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**

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

Segmentation Statistics



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

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

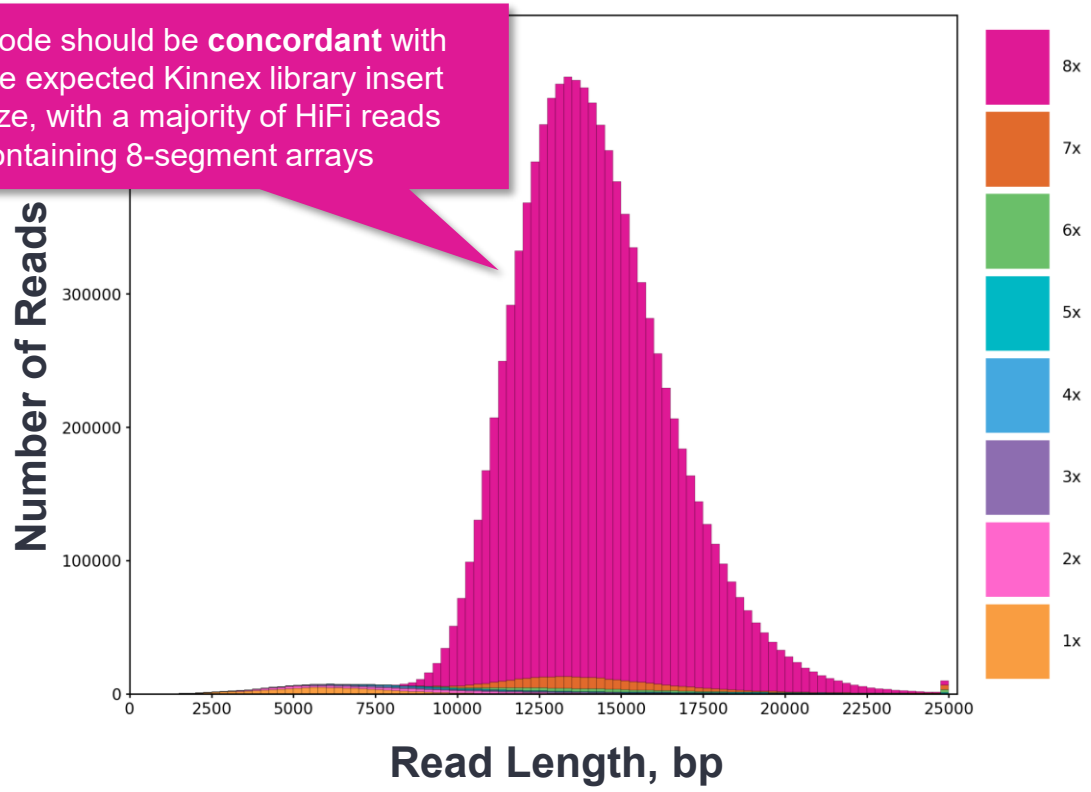
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Refer to ***SMRT Link v13.1 Kinnex single-cell troubleshooting guide*** (103-516-100) for example performance metrics typically achievable with Kinnex libraries under optimal *P1* loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex full-length RNA libraries.

Example SMRT Link Read Segmentation data utility processing results[1] 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

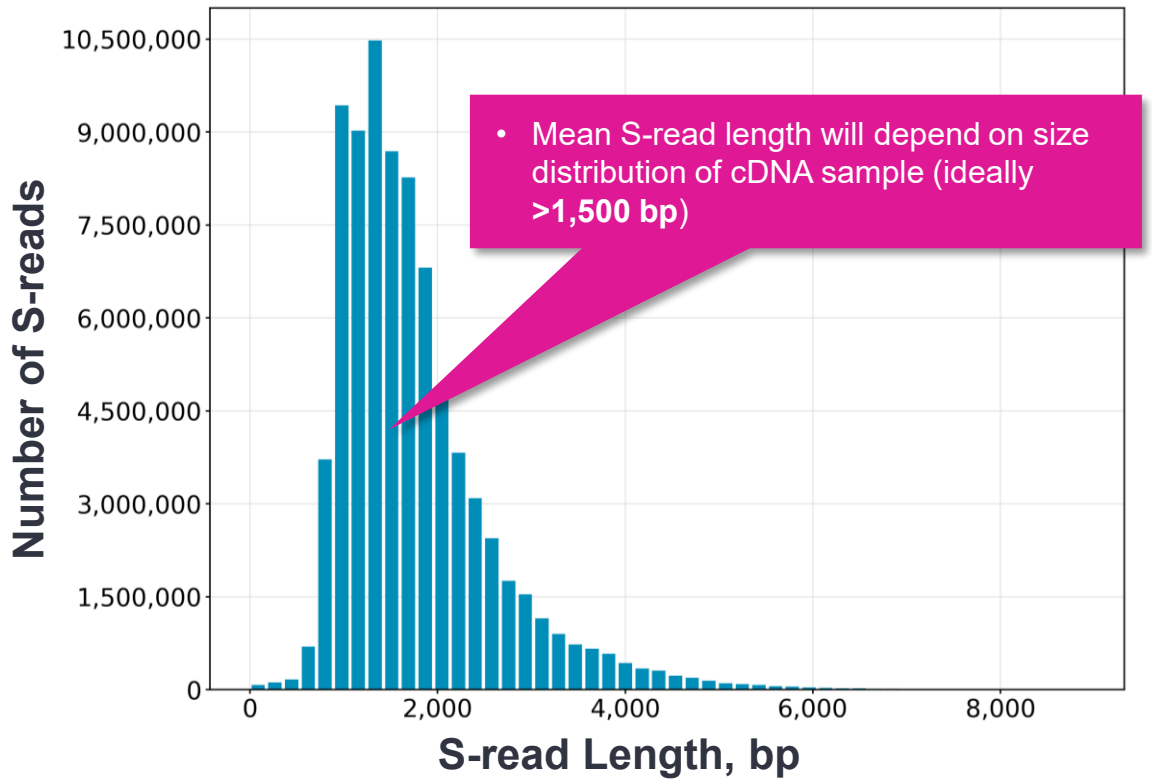
- Mode should be **concordant** with the expected Kinnex library insert size, with a majority of HiFi reads containing 8-segment arrays



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

S-read Length Distribution

- Mean S-read length will depend on size distribution of cDNA sample (ideally **>1,500 bp**)



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

[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, *P1* loading performance & movie time. Note: Refer to ***SMRT Link v13.1 Kinnex single-cell troubleshooting guide*** (103-516-100) for example performance metrics typically achievable with Kinnex libraries under optimal *P1* loading conditions. For Revio system, we recommend aiming for ~60 – 80% *P1* loading for Kinnex full-length RNA libraries.

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-Concatemer Reads with 5’ and 3’ Primers:** Number of nonconcatemer CCS reads with 5’ and 3’ primers detected
- **Non-Concatemer Reads with 5’ and 3’ Primers and Poly-A Tail (FLNC Reads):** Number of non-concatemer CCS reads with 5’ and 3’ primers and polyA tails detected. This is usually the number for full-length, nonconcatemer (FLNC) reads, unless polyA tails are not present in the sample
- **Mean Length of FLNC Reads:** Mean length of the non-concatemer 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

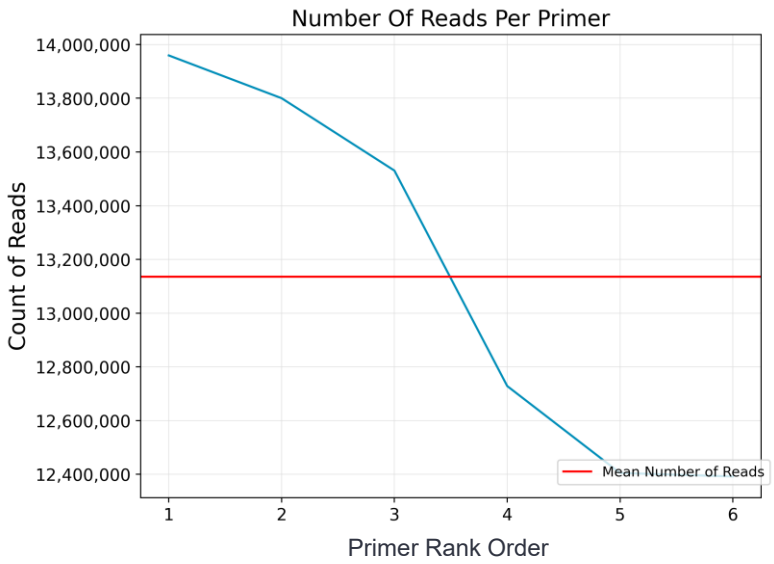
Bio Sample Name	Primer Name	CCS Reads	Mean Primer Quality	Reads with 5' and 3' Primers	Non-Concatamer Reads with 5' and 3' Primers
BioSample_1	IsoSeqX_bc01_5p--IsoSeqX_3p	13,800,150	99.7	13,800,150	13,787,052
BioSample_2	IsoSeqX_bc02_5p--IsoSeqX_3p	13,959,398	99.7	13,959,398	13,937,187
BioSample_3	IsoSeqX_bc03_5p--IsoSeqX_3p	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

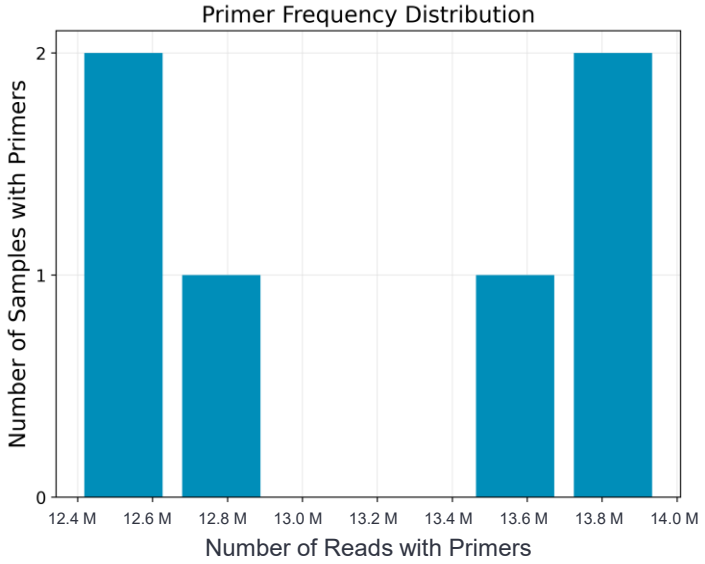
- **Bio Sample Name:** Name of the biological sample associated with the primer
- **Primer Name:** A string containing the pair of primer indices associated with this biological sample
- **CCS Reads:** Number of CCS reads associated with the primer
- **Mean Primer Quality:** Mean primer quality associated with the primer
- **Reads with 5’ and 3’ Primers:** Number of CCS reads with 5’ and 3’ cDNA primers detected
- **Non-Concatemer Reads with 5’ and 3’ Primers:** Number of non-concatemer CCS reads with 5’ and 3’ primers detected
- **Non-Concatemer 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.

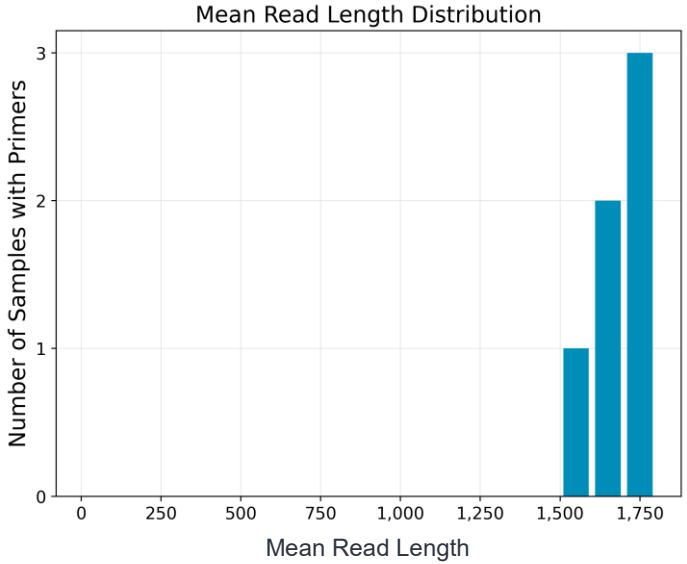
Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

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

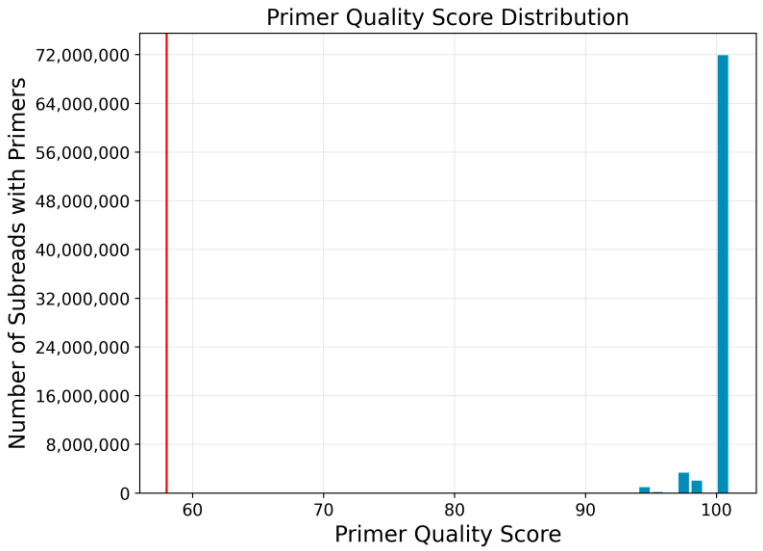
Primer Frequency Distribution: Maps the number of samples with primers by the number of reads with primers

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

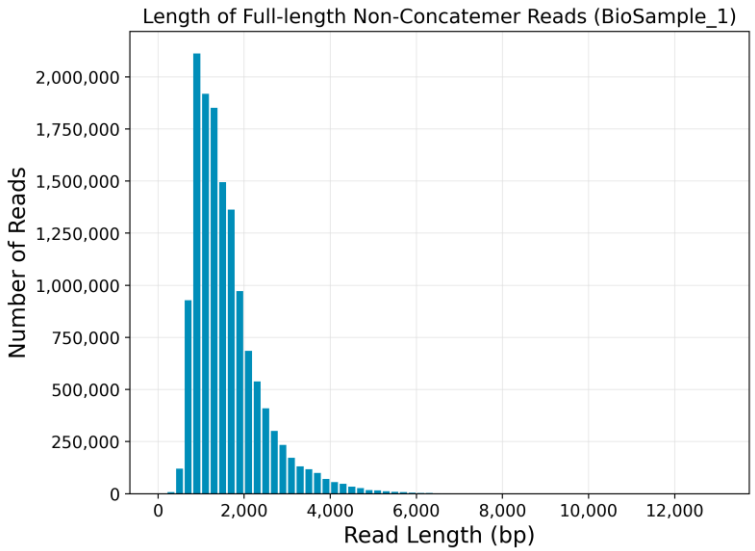
Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

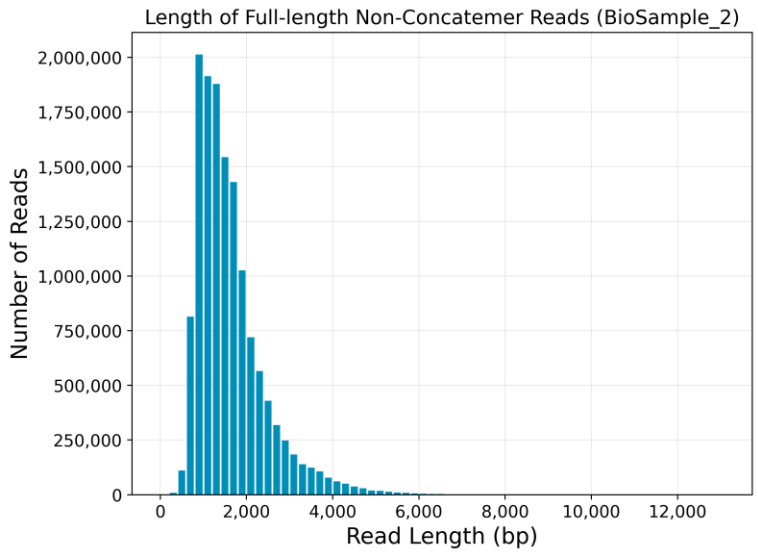
SMRT Link Iso-Seq analysis job report – Read Classification statistics

Primer Quality Scores



Length of Full-length Non-Concatemer Reads





Primer Quality Score Distribution: Histogram of primer scores

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.)

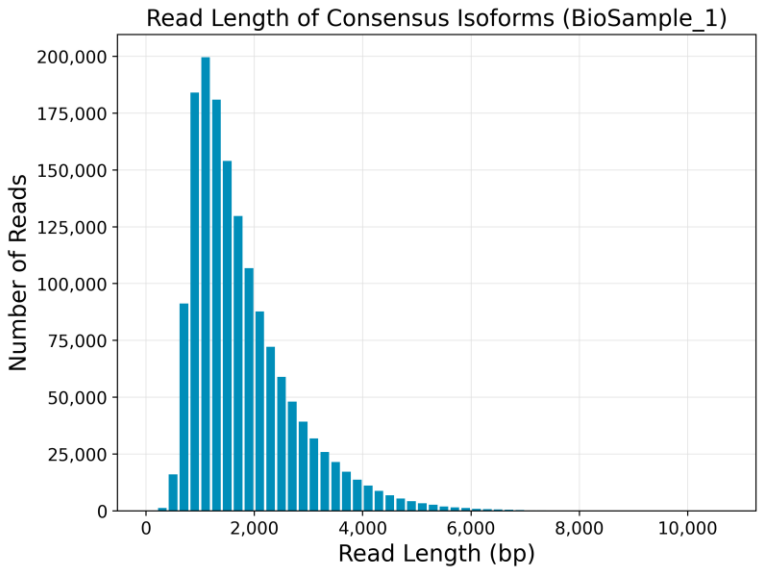
SMRT Link Iso-Seq analysis job report – Transcript Clustering statistics

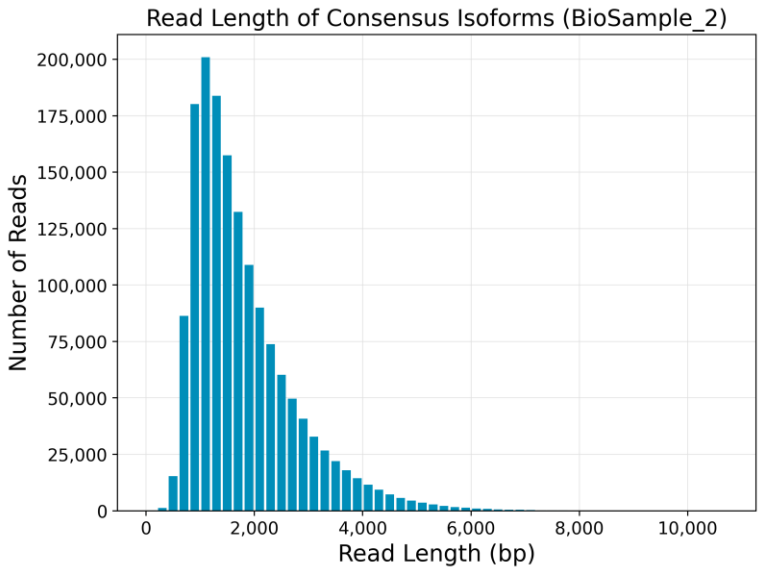
Summary Metrics

Sample Name	Number of High-Quality Isoforms
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)[1]

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

PacBio

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

Example SMRT Link 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

Isoform distributions across structural categories:

- Distribution of the % of isoforms by structural categories

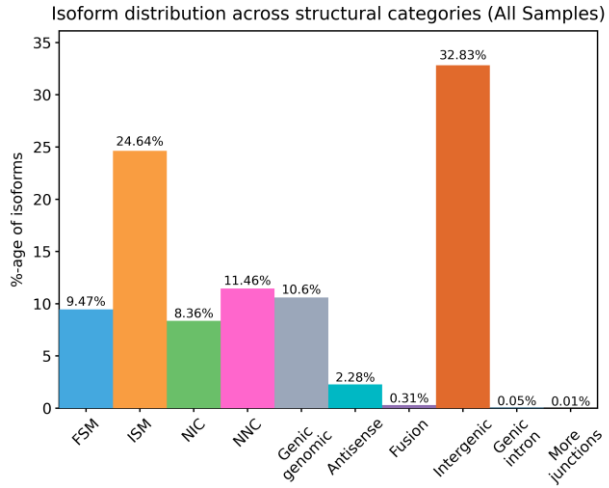
Example Revio system + SPRQ data shown.

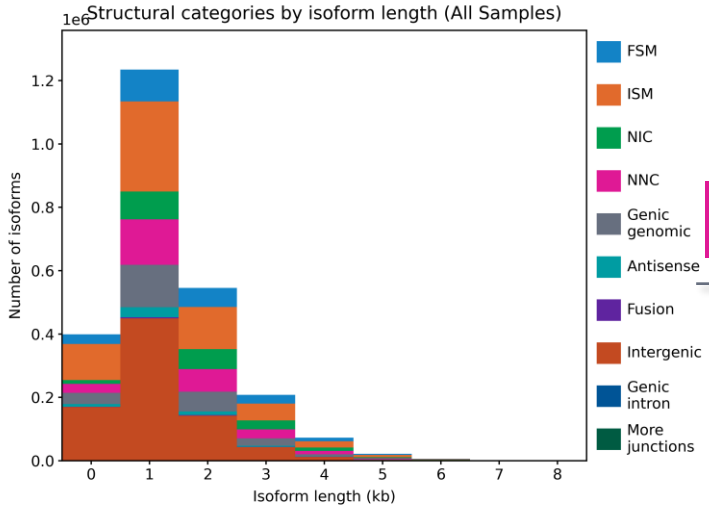
Structural categories by isoform lengths:

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

Example Revio system + SPRQ data shown.

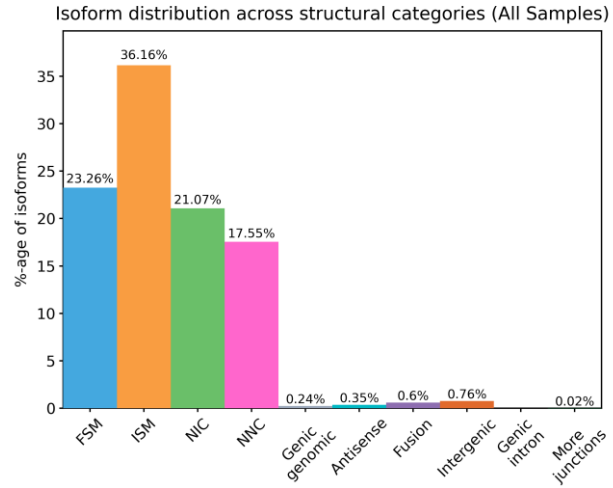
Transcript Classification Plots

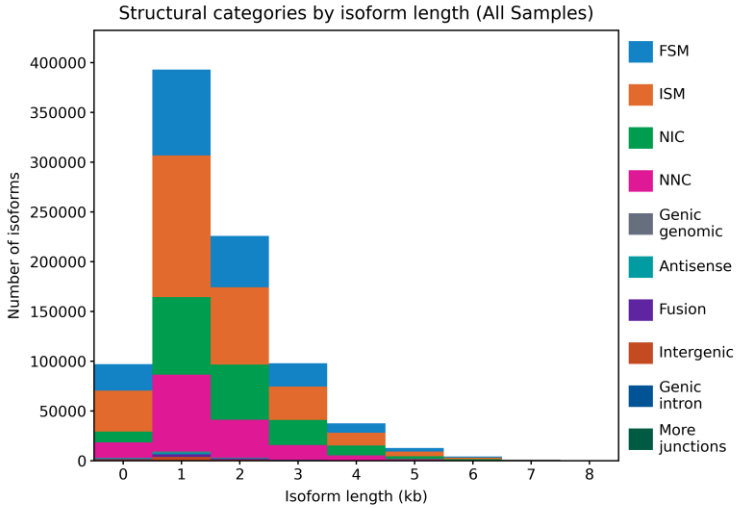




Filter out reads based on the SQANTI3 transcript filtering criteria

Transcript Classification Plots, Filtered





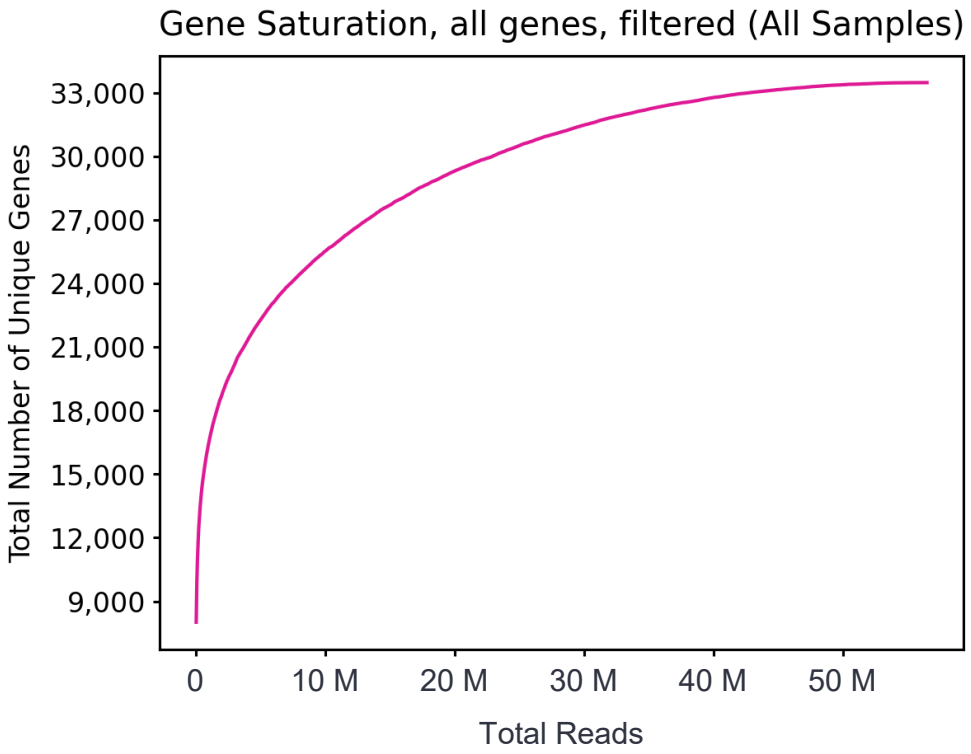
Filter out reads based on the SQANTI3 transcript filtering criteria

PacBio

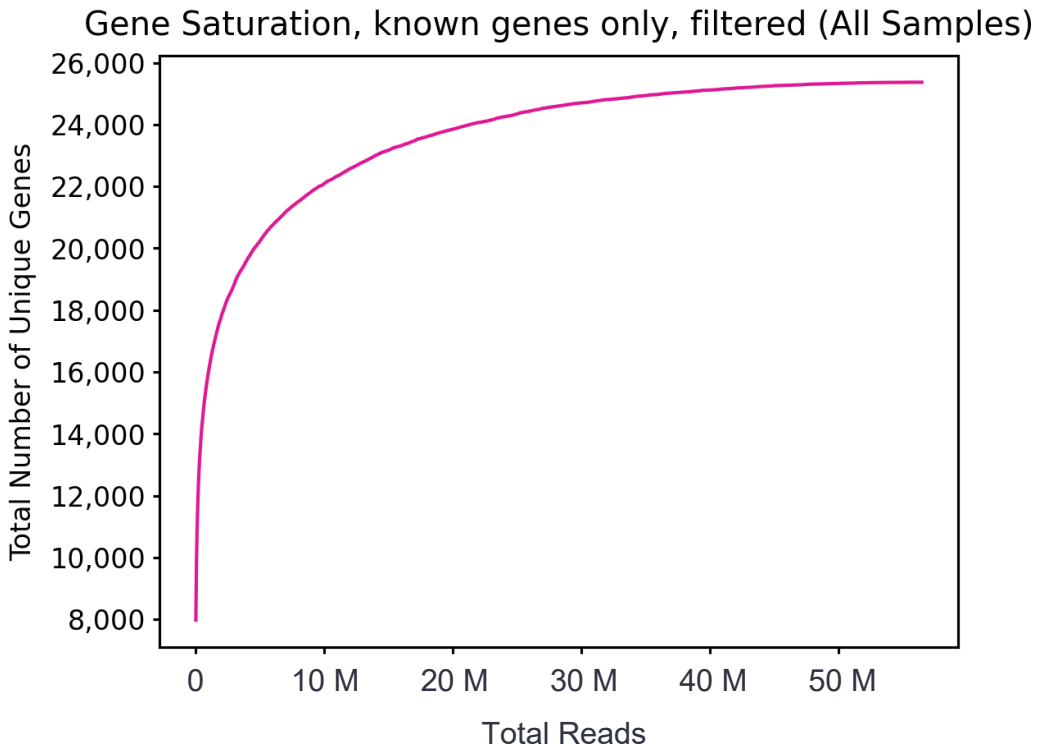
Example SMRT Link Iso-Seq analysis results for Kinnex full-length RNA library prepared with human UHRR sample (cont.)

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

Gene Saturation



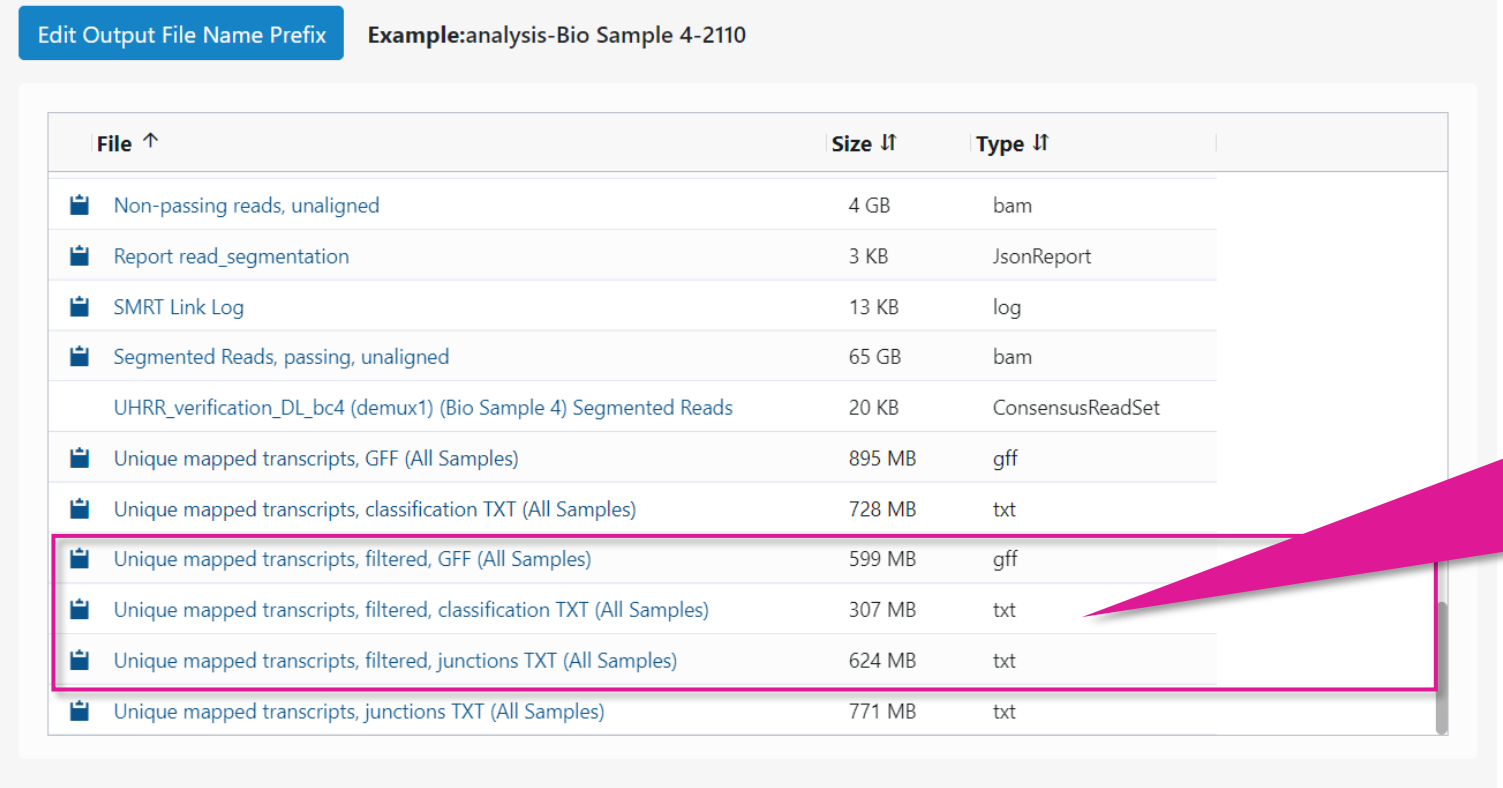
Gene Saturation, all genes, filtered: Saturation plot showing the level of gene saturation for all genes, after filtering out reads based on the SQANTI transcript filtering criteria



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



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 [Link]
Total RNA isolation	Qiagen RNeasy Plus Kits [Link]
	Sigma Spectrum Plant Total RNA Kit [Link]
	iNtRON Easy Spin Total RNA [Link]
	TRIzol Reagent can be used to isolate total RNA from tissues or cells, including lipid-rich and difficult samples [Link]
RNA stabilization & storage	RNALater is an aqueous, nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA [Link]

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°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 ~2.0.
- RNA sample has an OD260/OD230 ratio ≥2.0.
- RNA sample has a RIN number ≥7.0 (ideally recommend ≥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) [Link]	HiFi long read	Vega system
	Homo sapiens – Universal human reference RNA (UHRR) [Link]	HiFi long read	Revio system – SPRQ chemistry
	Homo sapiens – Universal human reference RNA (UHRR) [Link]	HiFi long read	Sequel II & Revio systems
	Homo sapiens – HG002 [Link]	HiFi long read	Revio system
	Homo sapiens – Heart [Link]	HiFi long read	Revio system
	Homo sapiens – Cerebellum [Link]	HiFi long read	Revio system
	Homo sapiens – Brain [Link]	HiFi long read	Revio system

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

Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput

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

Benefits of Kinnex concatenation

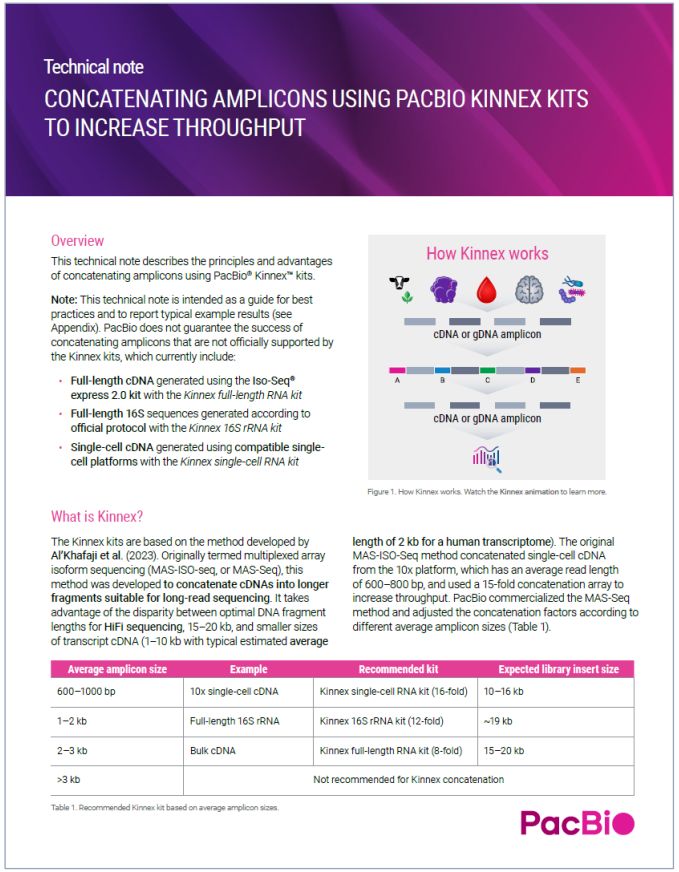
- Increased throughput on PacBio long-read sequencers
- Retained HiFi accuracy despite throughput increase
- No change to secondary analysis – once reads are deconcatenated into S-reads, the S-reads represent the original, pre-concatenated amplicon and can be analyzed with established pipelines.

When is Kinnex concatenation appropriate?

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

Recommended Kinnex kit based on average amplicon sizes.

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


Technical note

CONCATENATING AMPLICONS USING PACBIO KINNEX KITS TO INCREASE THROUGHPUT

Overview

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

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

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

How Kinnex works

cDNA or gDNA amplicon

cDNA or gDNA amplicon

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

What is Kinnex?

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

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

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

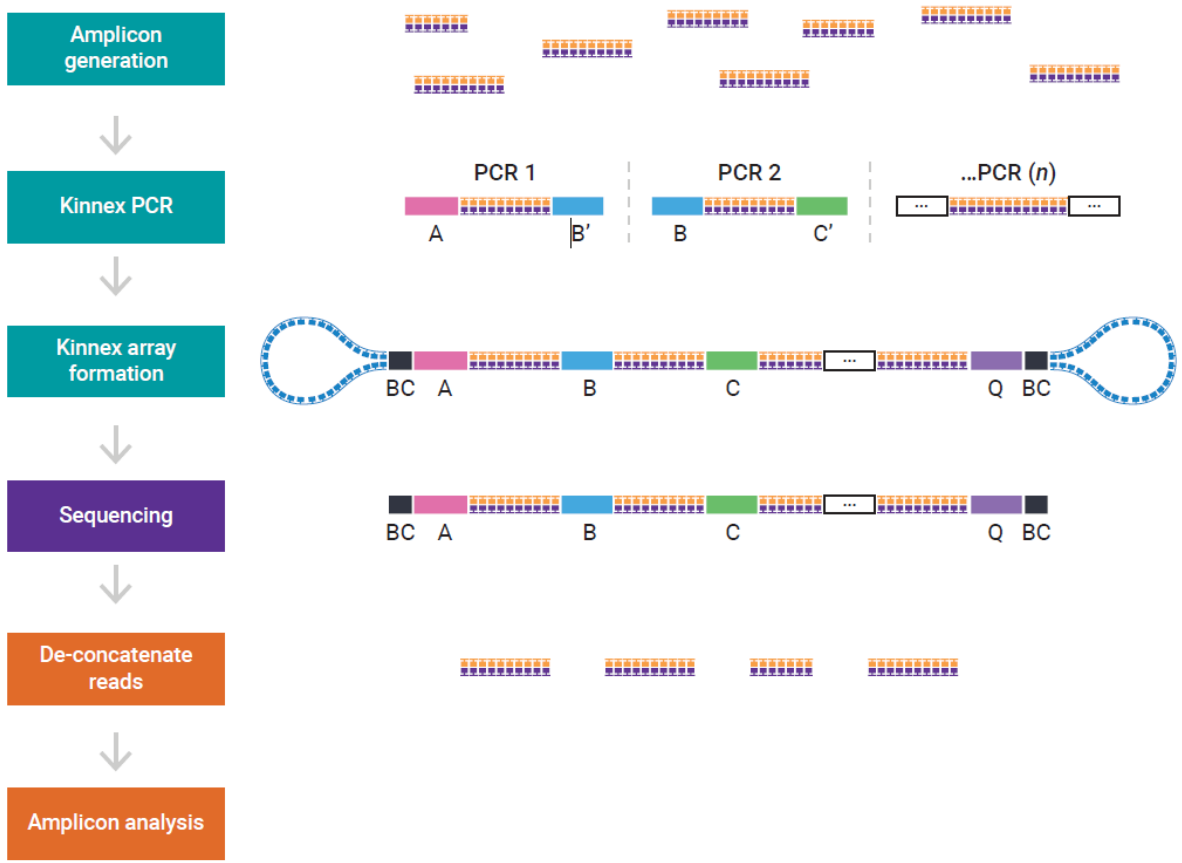
PacBio

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

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

Kinnex concatenation workflow overview

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



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

Kinnex concatenation procedural notes[1]

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

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

Establishing Kinnex-compatible molecular ends

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

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

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

Example 1. Kinnex 16S forward and reverse primer sequences

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

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

IsoSeqX bc01 FWD	CTACACGACGCTCTTCCGATCT – ACTACAC – GCAATGAAGTCGCAGGGTTGGG
IsoSeqX REV	AAGCAGTGGTATCAACGCAGAGTAC

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

Resources for Kinnex library prep[1]

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

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

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

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

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

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

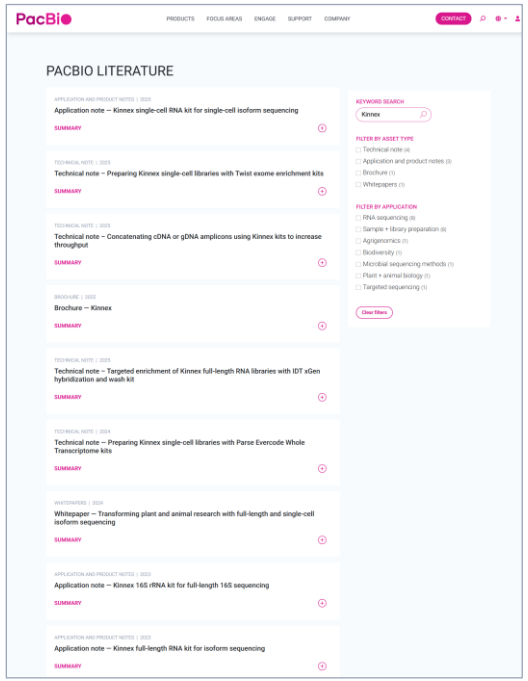
[1] Refer to the relevant Kinnex library prep protocols and Kinnex technical overview training documentation for specific details on concatenating cDNA or gDNA amplicons using Kinnex kits

APPENDIX 2: PacBio compatible Kinnex library preparation workflows

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

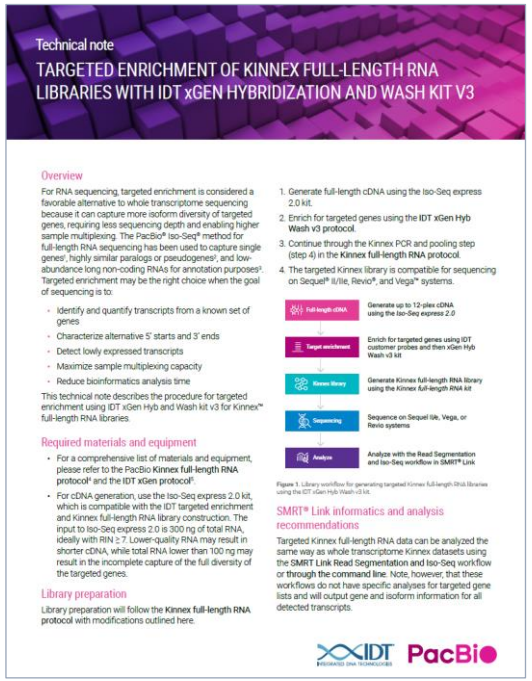
Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3: Getting started

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



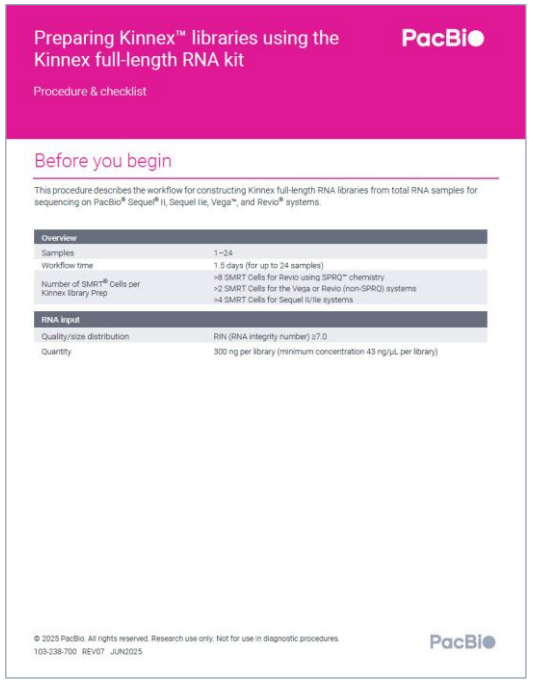
PacBio literature website [Link]

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



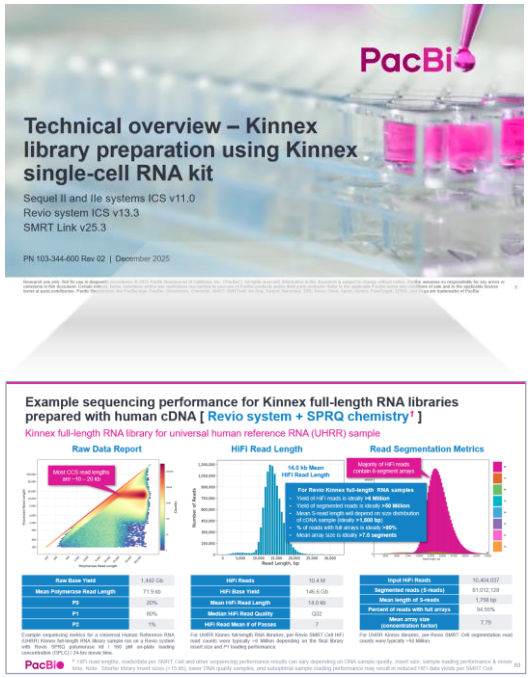
Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit v3 (102-326-632) [PacBio]

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



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

Technical documentation containing PacBio SMRTbell library construction details.

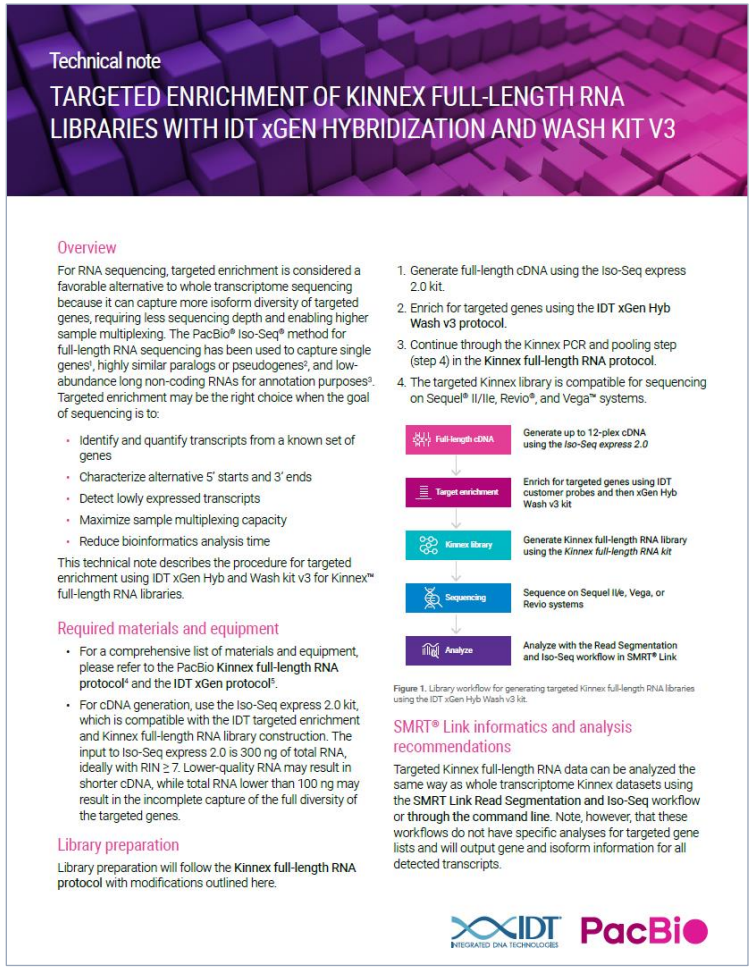


Technical overview: Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)

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

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

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



Technical note

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

Overview

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

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

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

Required materials and equipment

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

Library preparation

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

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

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

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

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

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

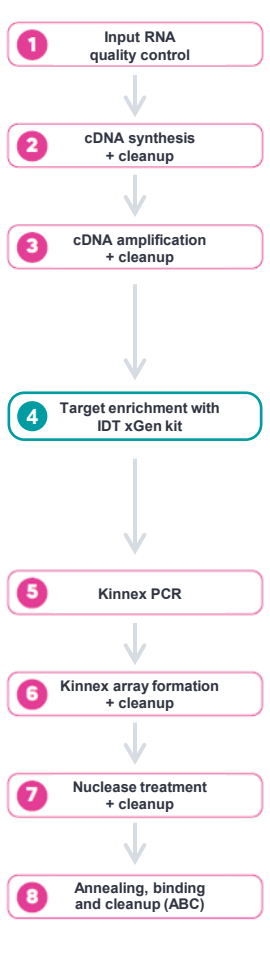
Analyze — Analyze with the Read Segmentation and Iso-Seq workflow in SMRT® Link

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

SMRT® Link informatics and analysis recommendations

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

IDT INTEGRATED DNA TECHNOLOGIES PacBio



PacBio Procedure & checklist

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

PacBio Technical note

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

PacBio Procedure & checklist

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

Overview

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

PacBio

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

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

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

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

PacBio Kinnex library construction procedure[1]

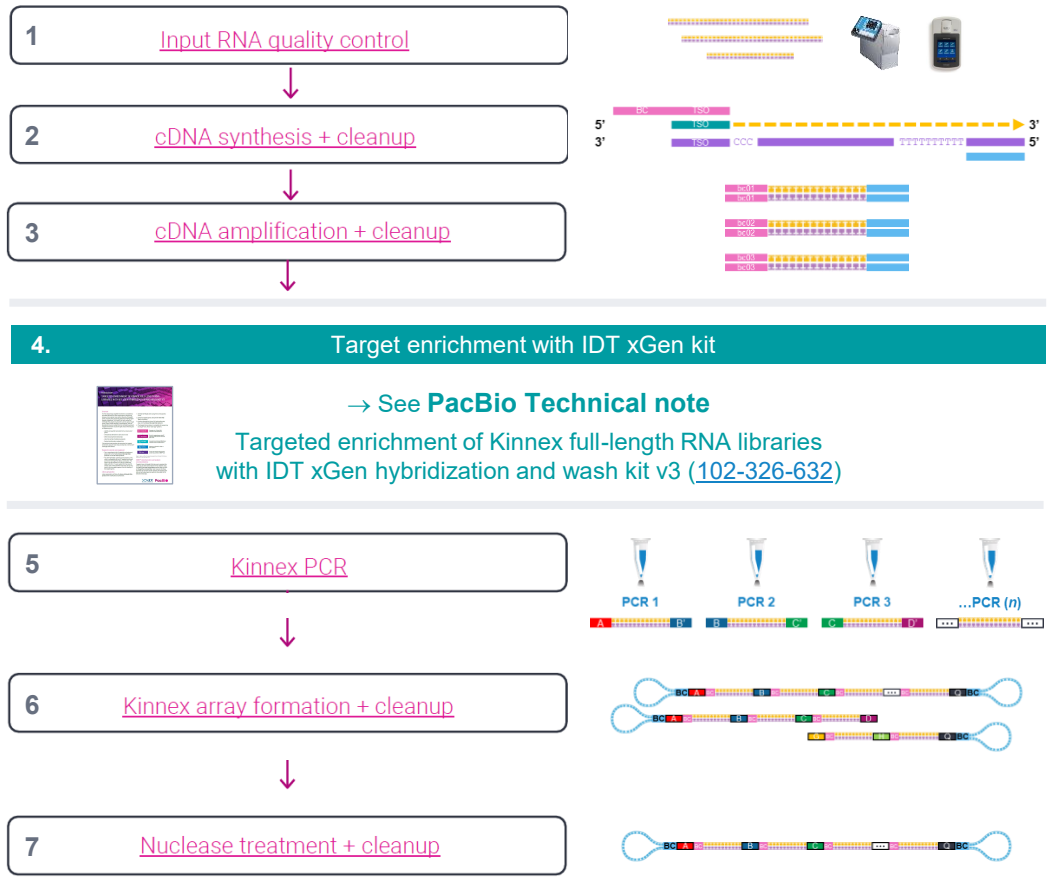
Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit (103-238-700)

PacBio

Kinnex full-length RNA library prep procedure key steps

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

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



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

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

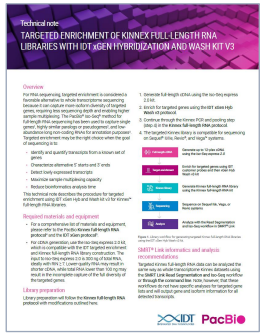
Follow PacBio Kinnex library prep protocol and IDT target enrichment procedure to generate full-length RNA hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

IDT xGen hybrid capture procedure overview[1]

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

PacBio



IDT xGEN hybrid capture procedure key steps[1]

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

IDT xGEN hybrid capture workflow

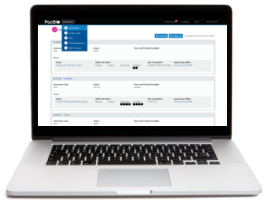
IDT xGen Hybridization and Wash Kit v3 (EAP) for PacBio Targeted Kinnex protocol (RUO24-3081_001).

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

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

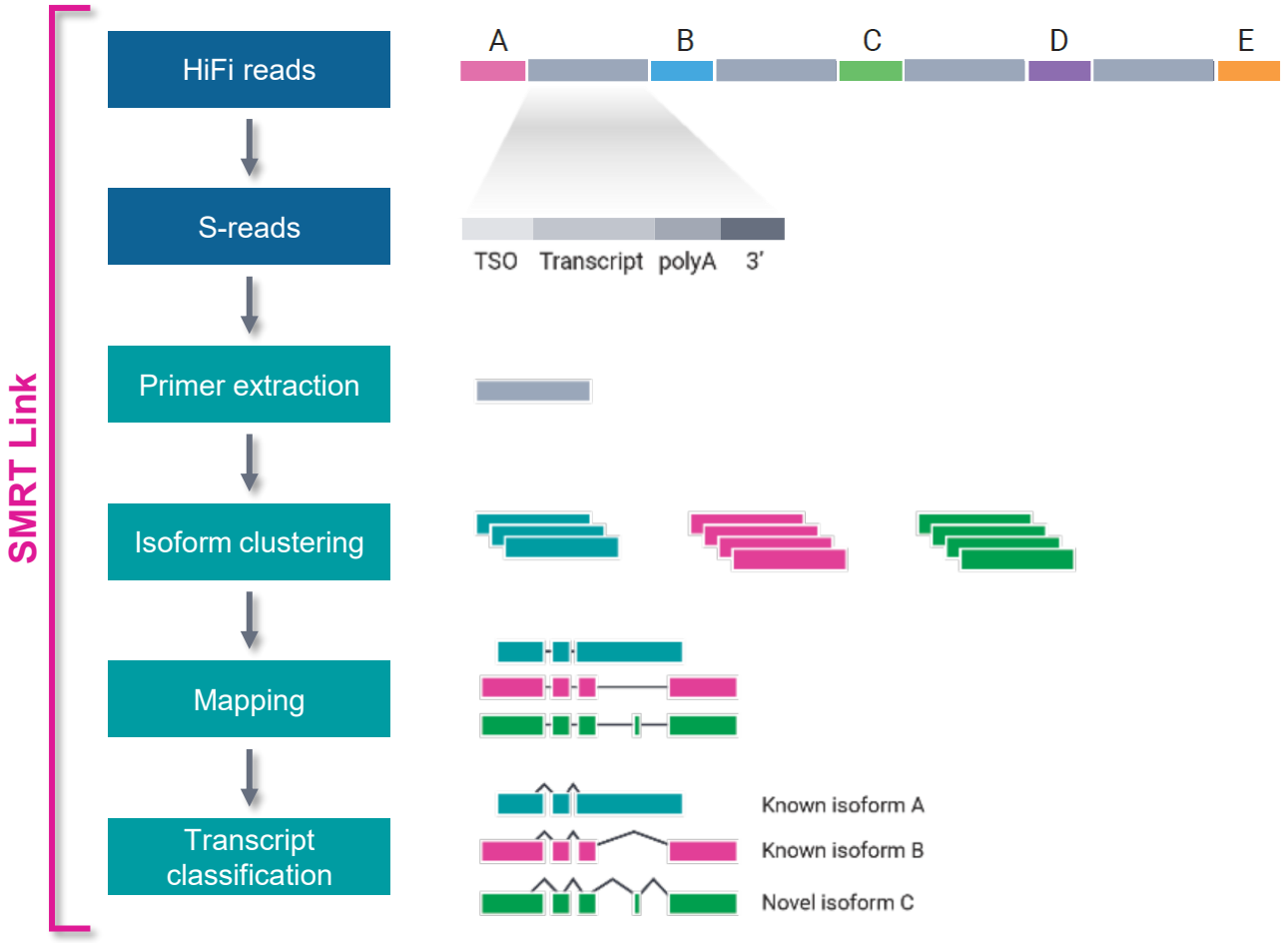
Use SMRT Link Read Segmentation and Iso-Seq application to perform isoform-classification analysis to identify novel genes & isoforms with abundance information

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



SMRT Analysis

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



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

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

SMRT Link Run Design procedure for Sequel IIe system

Sample information and run information

- Select desired Kinnex application from the **Application** field drop-down menu
- The following fields are auto-populated with default recommended values and high-lighted in green:
  - ❑ **SMRTbell Adapter Design**
    - → SMRTbell Kinnex Prep Kit
  - ❑ **Binding Kit**
    - → Sequel II Binding Kit 3.2
  - ❑ **Sequencing Kit**
    - → Sequel II Sequencing Plate 2.0 (4 rxn or 1 rxn)
  - ❑ **DNA Control Complex**
    - → Sequel II DNA Internal Control Complex 3.2
  - ❑ **Movie Time per SMRT Cell**
    - → 30 hrs
  - ❑ **Pre-Extension Time**
    - → 2 hrs

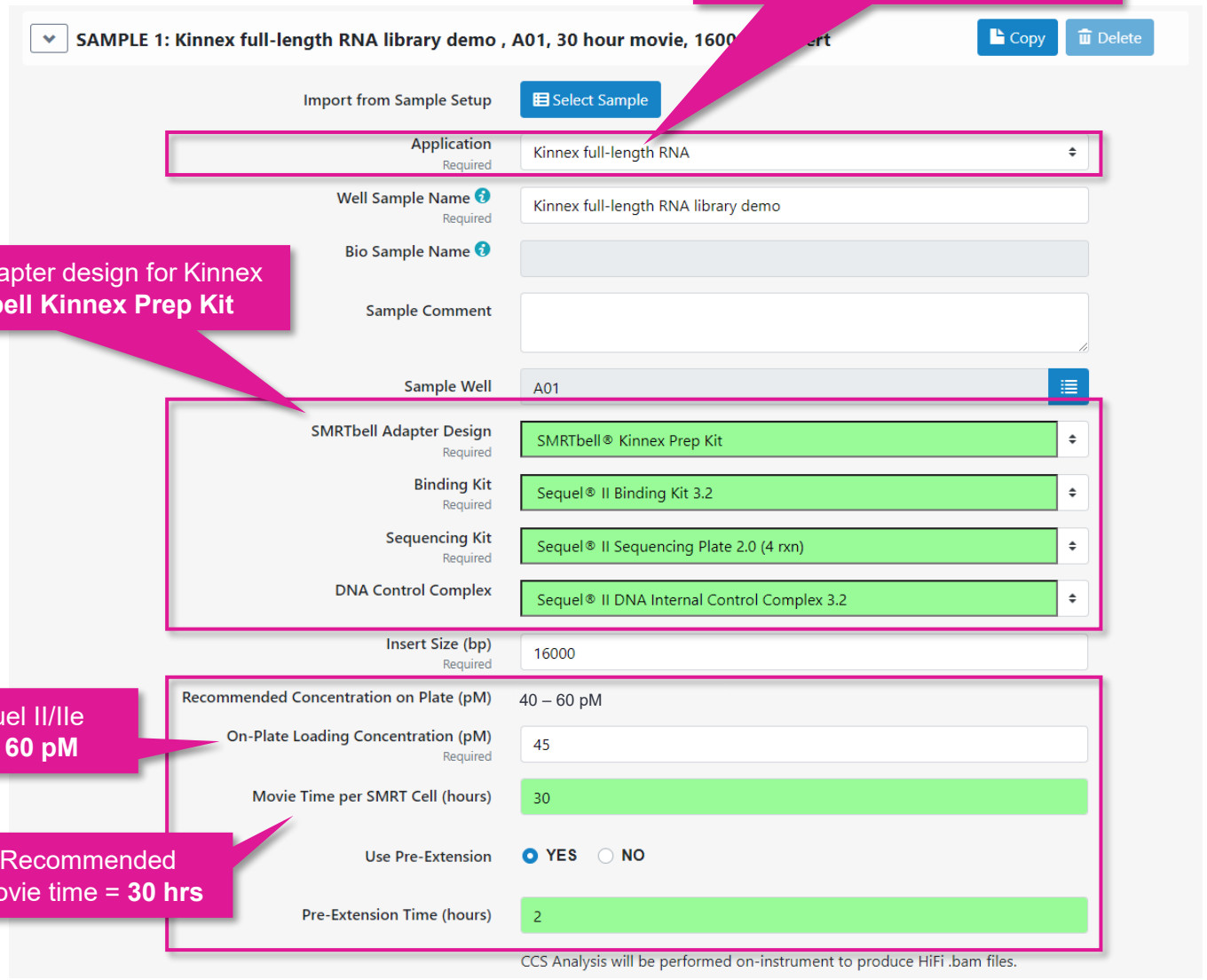
SMRTbell Adapter Design field determines which adapter finding algorithm is used during post-primary analysis[1]

Select desired Kinnex application type from drop-down menu

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

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

Recommended movie time = **30 hrs**



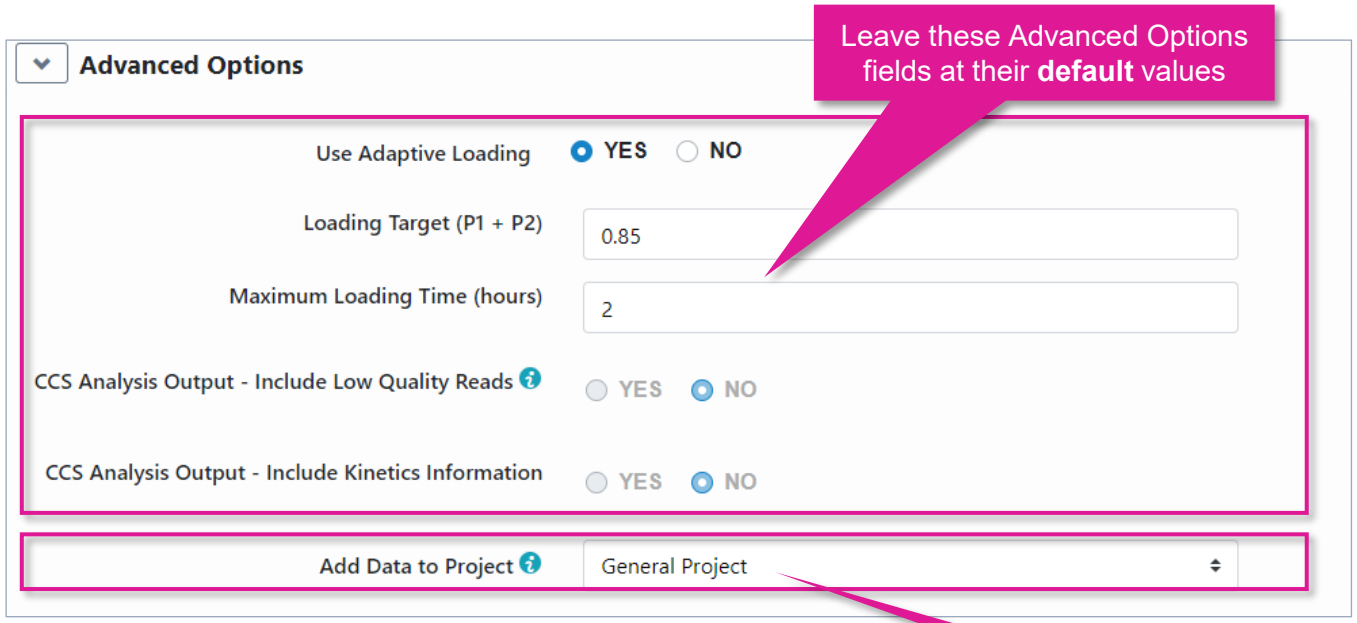
Example sample information entered into a Sequel IIe system run design worksheet for a Kinnex full-length RNA library sample.

[1] **Note:** When sequencing a Kinnex library sample, if 'Overhand-SMRTbell Prep Kit 3.0' is mistakenly selected instead of 'SMRTbell Kinnex Prep kit' in the SMRTbell Adapter Design field, then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.

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

Advanced options

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



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

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

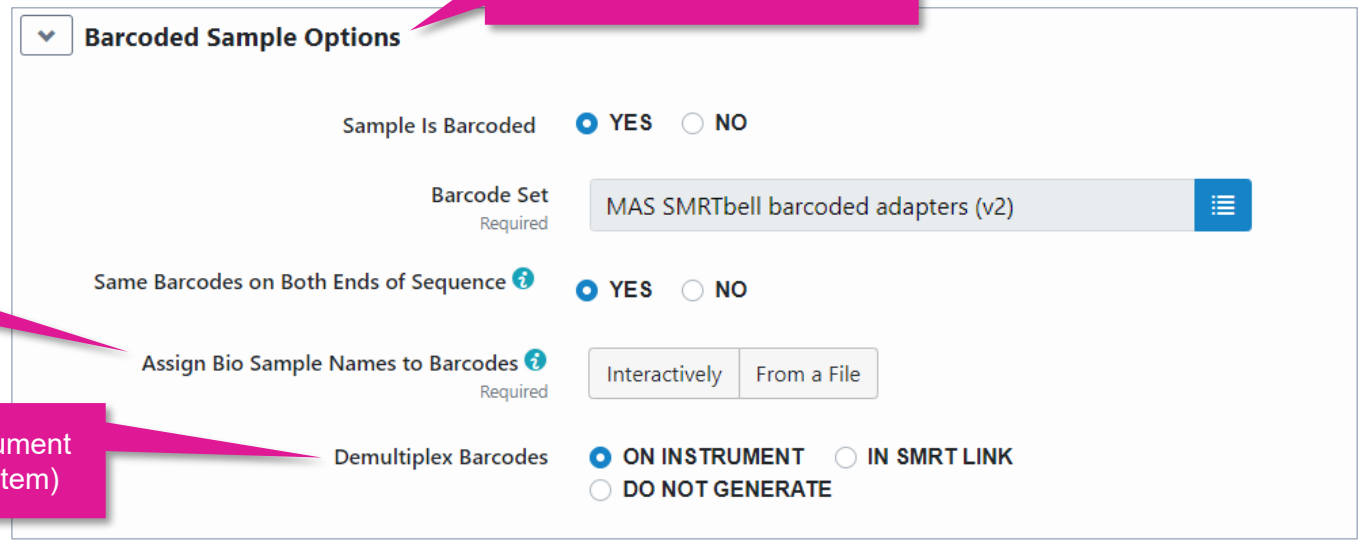
Barcoded sample options

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

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

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

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



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

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Revision history (description)	Version	Date
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