Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit

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

PN 103-344-600 Rev 02 | December 2025

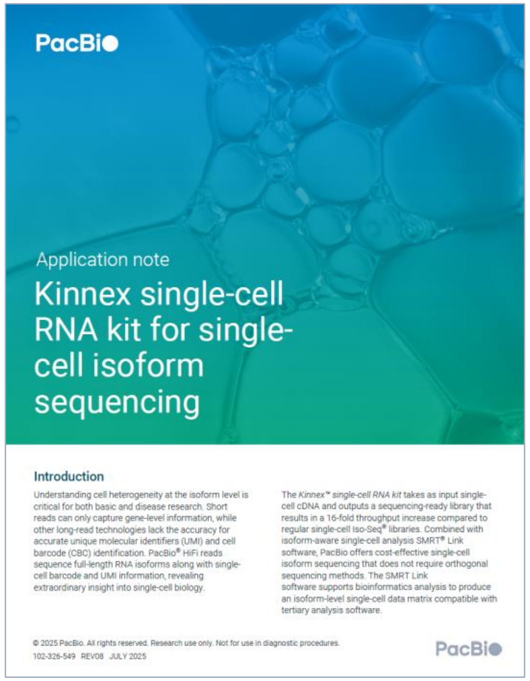
Technical overview

Kinnex library preparation using Kinnex single-cell RNA kit

1. Kinnex single-cell RNA method overview
2. Kinnex single-cell RNA library preparation workflow details
3. Kinnex single-cell RNA sequencing preparation workflow details
4. Kinnex single-cell RNA example sequencing performance data
5. Kinnex single-cell 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. Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits
    ii. Preparing Kinnex single-cell libraries with Twist exome enrichment kits
9. APPENDIX 3 – SMRT Link run design procedure for Sequel IIe system

Kinnex library preparation using Kinnex single-cell RNA kit: Getting started

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



PacBio

Application note

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

Introduction

Understanding cell heterogeneity at the isoform level is critical for both basic and disease research. Short reads can only capture gene-level information, while other long-read technologies lack the accuracy for accurate unique molecular identifiers (UMI) and cell barcode (CBC) identification. PacBio® HiFi reads sequence full-length RNA isoforms along with single-cell barcode and UMI information, revealing extraordinary insight into single-cell biology.

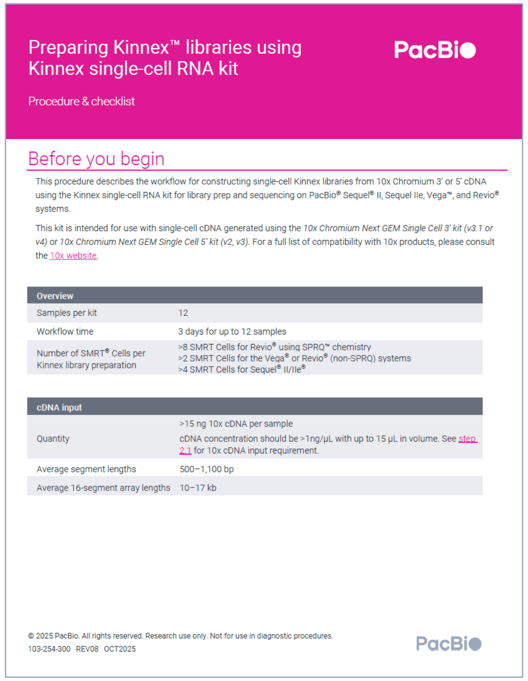
The *Kinnex™ single-cell RNA kit* takes as input single-cell cDNA and outputs a sequencing-ready library that results in a 16-fold throughput increase compared to regular single-cell Iso-Seq® libraries. Combined with isoform-aware single-cell analysis SMRT® Link software, PacBio offers cost-effective single-cell isoform sequencing that does not require orthogonal sequencing methods. The SMRT Link software supports bioinformatics analysis to produce an isoform-level single-cell data matrix compatible with tertiary analysis software.

102-326-549 REV08 JULY 2025

PacBio

Application Note - Kinnex single-cell RNA for single-cell isoform sequencing (102-326-549)

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



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

PacBio

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

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

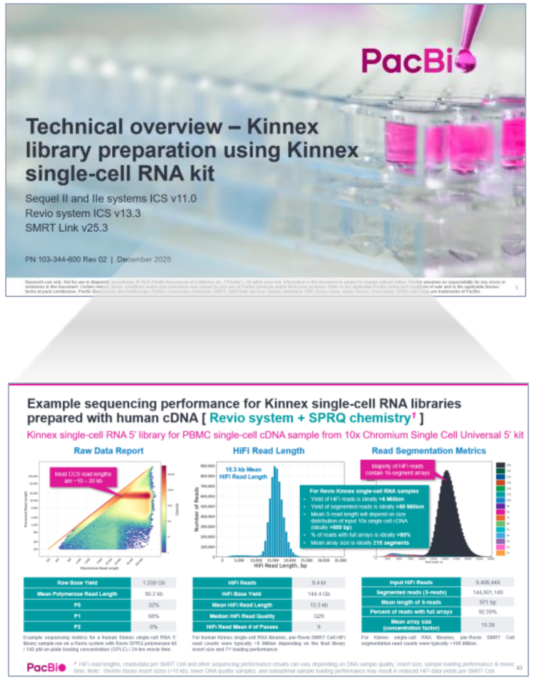
cDNA input	
Quantity	>15 ng 10x cDNA per sample cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,100 bp
Average 16-segment array lengths	10–17 kb

103-254-300 REV08 OCT2025

PacBio

Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (102-254-300)

Technical documentation containing application-specific library preparation protocol details.



PacBio

Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit

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

PN 103-344-600 Rev 02 | December 2025

Example sequencing performance for Kinnex single-cell RNA libraries prepared with human cDNA [Revio system + SPRQ chemistry¹]

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

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

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.

Single-cell cDNA synthesis

Use 10x Genomics Chromium or other system to perform single-cell cDNA synthesis (3,000 – 20,000 cells input)

↓

Kinnex library preparation (Kinnex single-cell RNA kit)

Use ≥15 ng of single-cell cDNA input to generate Kinnex library containing 16-segment array

↓

SMRT sequencing (Sequel IIe, Vega & Revio systems)

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

↓

Data analysis (SMRT Link)

Use Read Segmentation data utility to split arrayed transcript HiFi reads

Use Single-cell Iso-Seq analysis application to identify novel genes and isoforms

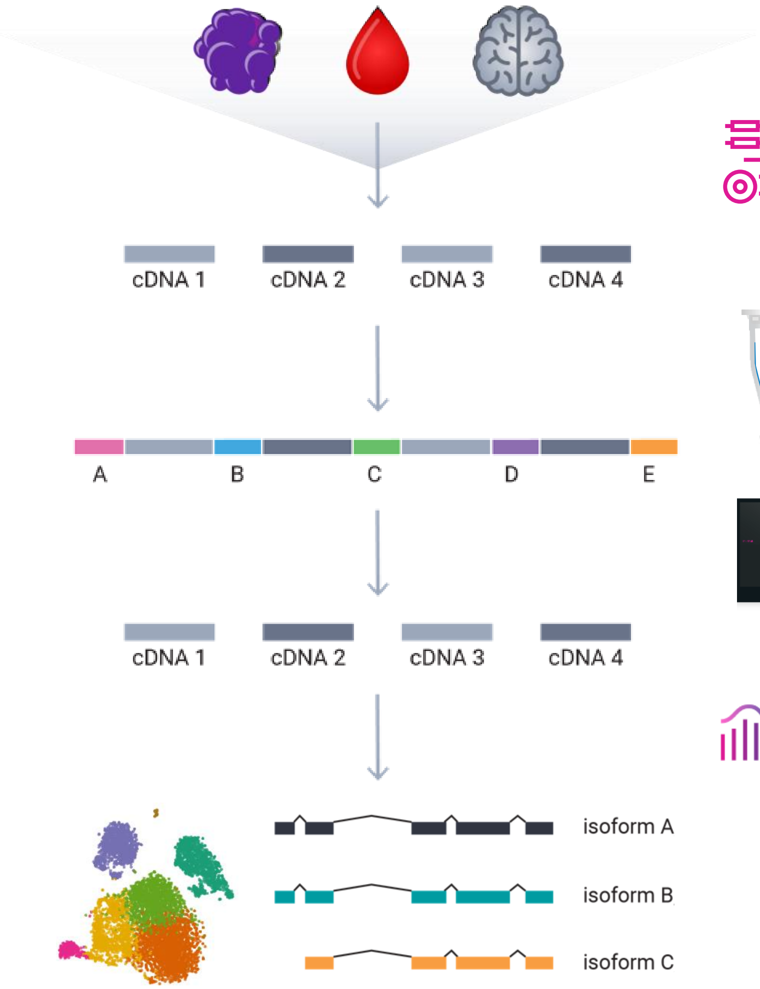
---

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

Kinnex single-cell RNA method overview

Kinnex single-cell RNA method overview

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



Single-cell cDNA generation

Kinnex library preparation

PacBio sequencing

Read segmentation + single-cell Isoform analysis

- 10x Chromium Single Cell 3’ kit (v3.1) and 5’ kit (v2)[1]
- 15–75 ng cDNA input
- 3,000 to 20,000 target cell recovery
- 2-day Kinnex library preparation using Kinnex single-cell RNA kit
- Barcoded Kinnex adapters support up to 4-plex multiplexing
- SMRT Link Run Design support for ‘Kinnex single-cell RNA’ application type option with auto-analysis (read segmentation + single-cell isoform analysis)
- SMRT Link single-cell Iso-Seq isoform-classification software to identify novel genes and isoforms
- Output compatible with tertiary single-cell analysis tools (e.g., *Seurat*, *Scanpy*, *Kana*)

---

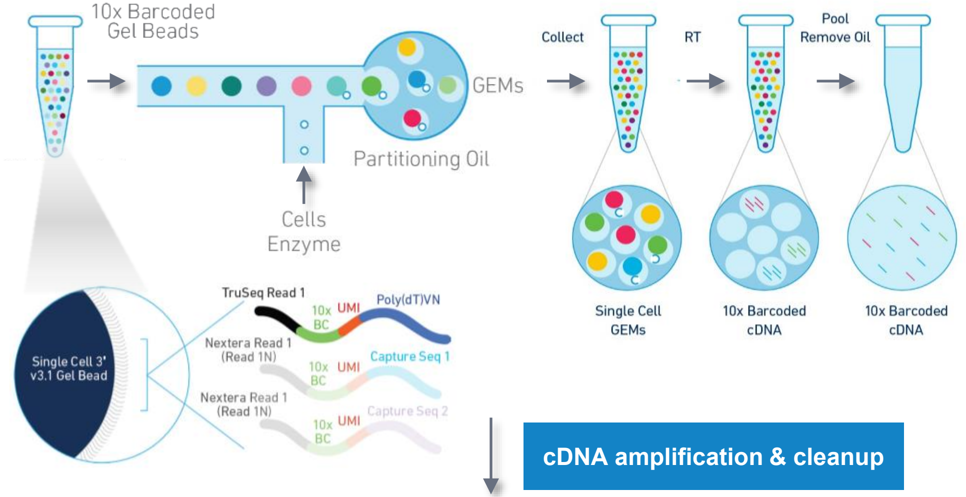
[1] Kinnex single-cell RNA protocol is compatible with single-cell cDNA from (1) 10x Chromium Single Cell Universal 3’ or 5’ kit; Single Cell Next GEM (v3.1 3’ or v2 5’); (2) 10x Visium HD Spatial Gene Expression kit; (3) Parse Evercode WT or WT mini kit; or (4) ArgenTag Single-Cell RNA Library Kit.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

Kinnex single-cell RNA method overview

Single-cell cDNA sample preparation[1]

Chromium Controller/X/iX

Chromium Next GEM Single Cell 5' or 3' Kit



cDNA amplification & cleanup

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


Preparing MAS-Seq libraries using MAS-Seq for 10x Single Cell 3' kit

PacBio

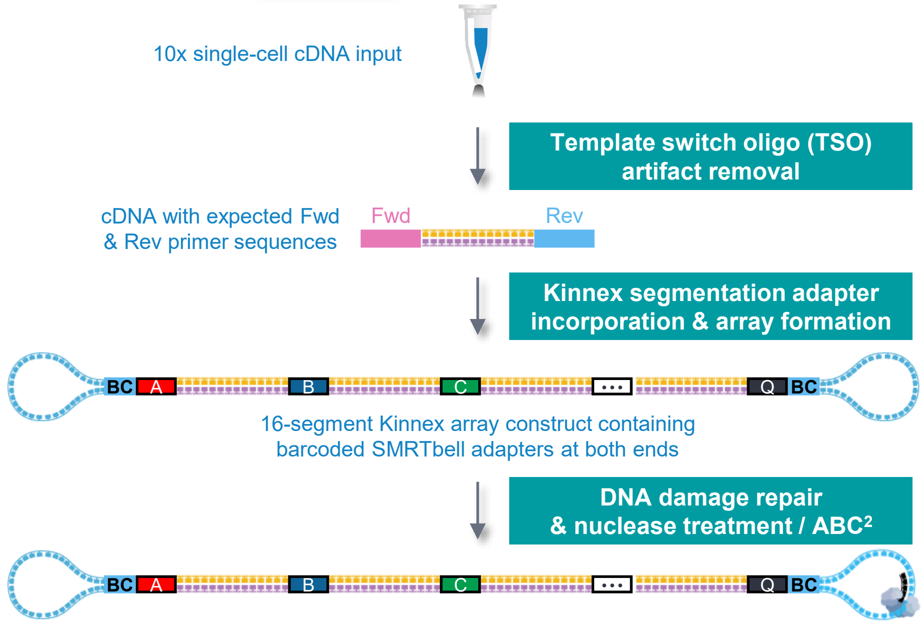
Procedure & checklist

Before you begin

Procedure & checklist
(103-254-300)

Kinnex library prep, sequencing & analysis

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



10x single-cell cDNA input

Template switch oligo (TSO) artifact removal

cDNA with expected Fwd & Rev primer sequences

Kinnex segmentation adapter incorporation & array formation

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

DNA damage repair & nuclease treatment / ABC[2]

PacBio long-read systems

Sequel IIe, Vega
or Revio system

SMRT Link

Read segmentation & single-cell Iso-Seq analysis

---

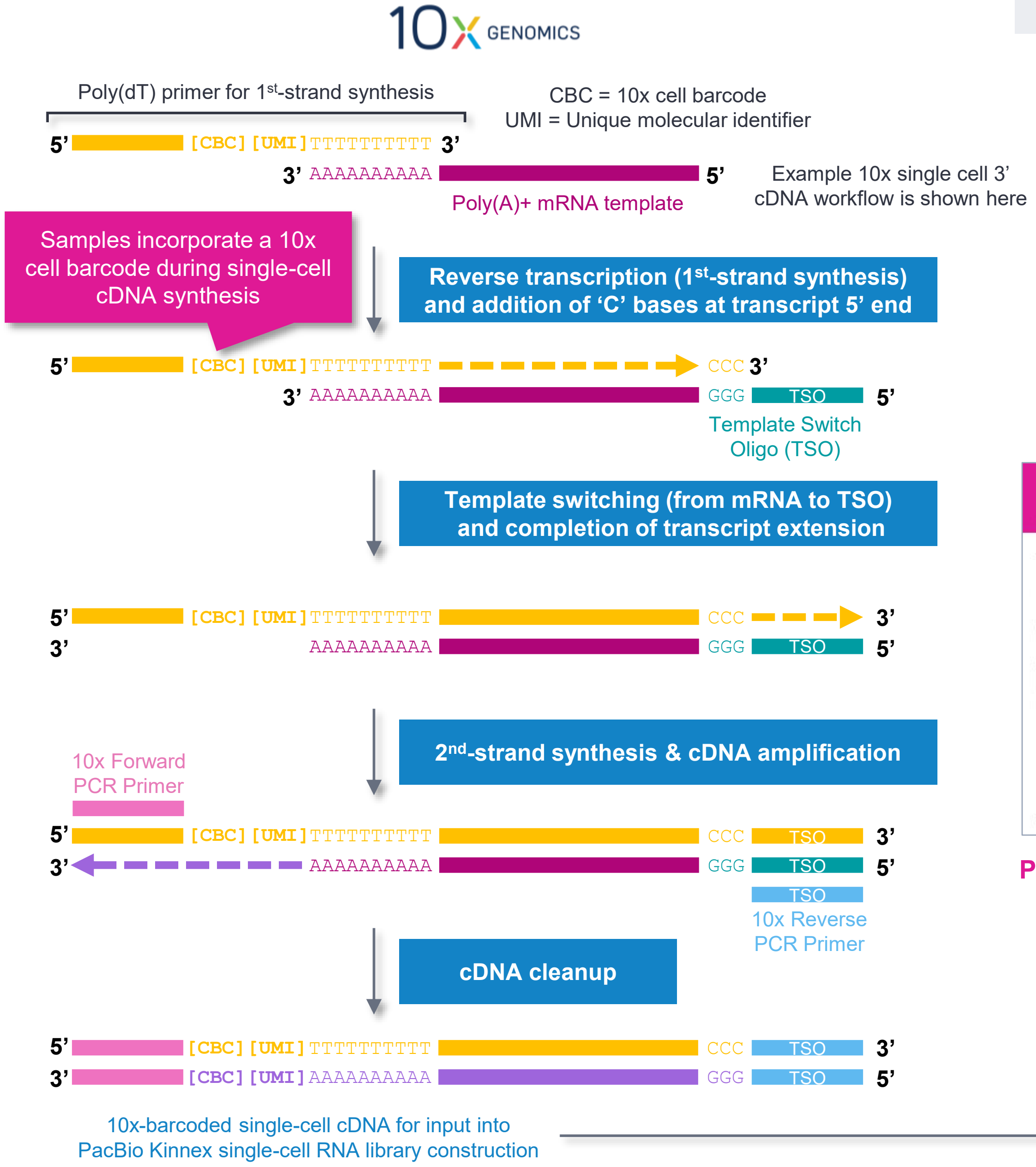
[1] Refer to 10x Genomics Support website to download 10x Chromium user guides and other documentation.

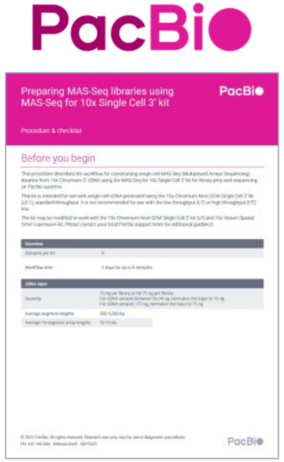
[2] ABC = Anneal sequencing primer / Bind polymerase / Complex cleanup

Single-cell cDNA sample preparation[1]

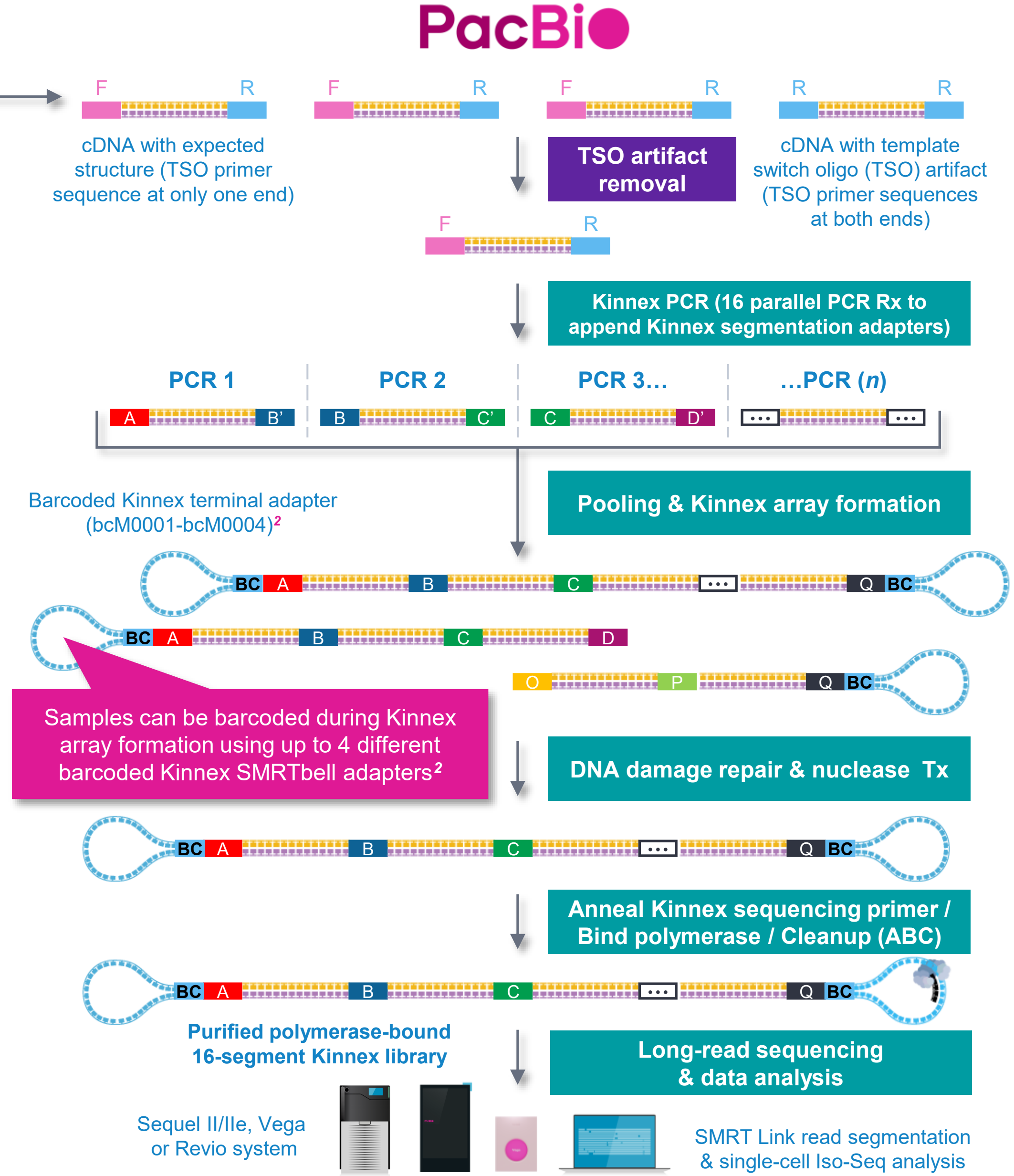
10x & PacBio key protocol steps

Kinnex library prep, sequencing & analysis





Procedure & checklist
(103-254-300)



---

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

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

Kinnex single-cell RNA library preparation procedure description

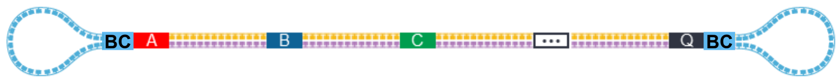
Procedure & checklist – Preparing Kinnex libraries using the Kinnex single-cell RNA kit (103-254-300) describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit (103-072-200) for library prep and sequencing on PacBio long-read systems

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples
Number of SMRT® Cells per Kinnex library preparation	>8 SMRT Cells for Revio® using SPRQ™ chemistry >2 SMRT Cells for the Vega® or Revio® (non-SPRQ) systems >4 SMRT Cells for Sequel® II/IIe®
cDNA input	
Quantity	>15 ng 10x cDNA per sample cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,100 bp
Average 16-segment array lengths	10–17 kb

Kinnex single-cell RNA kit

103-072-200 (12 rxn)

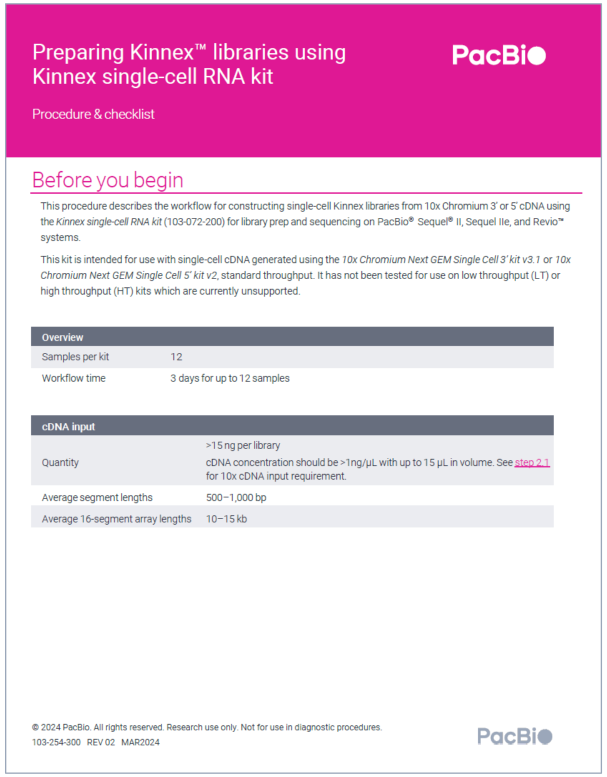
For use with single-cell cDNA generated with 10x Chromium Next GEM Single Cell 3' kit v3.1 or 10x Chromium Next GEM Single Cell 5' kit v2, standard throughput[1]



Kinnex single-cell RNA library template (~10–17 kb)

Contains 16 concatenated full-length cDNA segments

Kinnex single-cell RNA library preparation procedure supports up to 4-plex sample multiplexing through use of 4 different barcoded Kinnex SMRTbell adapters[2]



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the *Kinnex single-cell RNA kit* (103-072-200) for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, and Revio™ systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit v3.1* or *10x Chromium Next GEM Single Cell 5' kit v2*, standard throughput. It has not been tested for use on low throughput (LT) or high throughput (HT) kits which are currently unsupported.

Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples

cDNA input	
Quantity	>15 ng per library cDNA concentration should be >1ng/µL with up to 15 µL in volume. See step 2.1 for 10x cDNA input requirement.
Average segment lengths	500–1,000 bp
Average 16-segment array lengths	10–15 kb

© 2024 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.
103-254-300 REV 02 MAR2024

PacBio Documentation (103-254-300)

- Kinnex full-length RNA library prep protocol uses **Kinnex single-cell RNA kit**
  - → **Do not use** SMRTbell prep kit 3.0 with this protocol

---

[1] This kit is intended for use with single-cell cDNA generated using the 10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4) or 10x Chromium Next GEM Single Cell 5' kit (v2, v3). For a full list of compatibility with 10x products, please consult the 10x website.

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

Kinnex single-cell RNA kit bundle components

Kinnex single-cell RNA kit bundle provides full support for Kinnex library prep workflow

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

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 single-cell RNA libraries from input 10x Chromium Single Cell 5' and 3' cDNA.

Kinnex single-cell RNA kit components

Component		Description
1		**Kinnex capture beads kit (12 rxn)** • Contains reagents for removing template-switch oligo (TSO) artifacts from single-cell cDNA
2		**Kinnex single cell concatenation kit (12 rxn)** • Contains reagents for Kinnex array formation and SMRTbell template construction • Includes barcoded Kinnex adapter mixes (bcM0001 – bcM0004) • Also contains Kinnex capture primer oligos for TSO artifact removal
3		**SMRTbell cleanup beads** • For DNA cleanup
4		**Elution buffer** • For DNA cleanup

Kinnex single-cell RNA experimental design considerations

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

	Sequel II and IIe systems	Vega system	Revio system + SPRQ chemistry
Experimental goal	Characterize alternative splicing in single cells / cell types		
Transcript reads per SMRT Cell	30-40 M reads	50-60 M reads	100-120 M reads
Sample multiplexing[1]	Not recommended	Not recommended	Up to 2 samples per Revio SMRT Cell (2-plex)
Cell input into 10x Chromium single cell 3' or 5' cDNA generation workflow	3,000 – 10,000 cells for running a single (non-multiplexed) sample on one Sequel II SMRT Cell 8M	3,000 – 10,000 cells for running a single (non-multiplexed) sample on one Vega SMRT Cell	<10,000 cells per sample if multiplexing 2 samples per Revio SMRT Cell (2-plex) 10,000 – 20,000 cells per sample if running a single (non-multiplexed) sample on 1–2 Revio SMRT Cells
Expected coverage	Obtain 3,000 – 10,000 unique reads/single cell	Obtain 3,000 – 10,000 unique reads/single cell	Obtain up to ~20,000 unique reads/single cell
Kinnex library prep protocol	Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)		
Single-cell cDNA input into Kinnex library prep workflow	15-75 ng of cDNA from **(1)** 10x Chromium Single Cell Universal 3' or 5' kit; Single Cell Next GEM (v3.1 3' or v2 5'); **(2)** 10x Visium HD Spatial Gene Expression kit; **(3)** Parse Evercode WT or WT mini kit; or **(4)** ArgenTag Single-Cell RNA Library Kit		
SMRT Link data analysis workflows	Read Segmentation and Single-cell Iso-Seq Analysis		
Community data analysis tools	Annotation & quantification: SQANTI3 / Differential analysis: TappAS / Fusion calling: pbfusion / Visualization: SWAN		

---

[1] Kinnex single-cell concatenation kit (103-242-000) can support up to 4-plex sample multiplexing per SMRT Cell through the use of four different barcoded Kinnex terminal SMRTbell adapters during Kinnex single-cell RNA library construction.

Kinnex single-cell RNA library preparation workflow details

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

Procedure & checklist 103-254-300 describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3’ or 5’ cDNA using the **Kinnex single-cell RNA kit** for library prep and sequencing on PacBio long-read systems

Procedure & checklist contents

1. General best practices for reagent & sample handling and 10x single cell cDNA input recommendations.
2. Enzymatic workflow steps for removal of template-switch oligo (TSO) artifacts from input 10x single cell cDNA samples.
3. Enzymatic workflow steps for construction of 16-segment Kinnex arrays from 10x single cell cDNA.
4. Enzymatic workflow steps for DNA damage repair & nuclease treatment of Kinnex single-cell RNA SMRTbell libraries.
5. Workflow steps for final cleanup of Kinnex single-cell RNA 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
Kinnex single-cell RNA kit

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3’ or 5’ cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3’ kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5’ kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

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

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

103-254-300 REV08 OCT2025

PacBio Documentation (103-254-300)

---

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

Kinnex single-cell RNA library construction workflow overview

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

	Cleanup	DNA QC	Walk-away time[1]	
1 Input cDNA quality control	N/A	Qubit HS assay Bioanalyzer HS DNA kit	N/A	
2 TSO PCR	1.5X SMRTbell cleanup beads	Qubit HS assay	1.5 hrs	**Day 1 (~6 – 6.5 hrs*)**
○ Safe stop				
3 TSO artifact removal	1.5X SMRTbell cleanup beads	Qubit HS assay	1.5 hrs	
○ Safe stop				
4 Kinnex PCR	1.5X SMRTbell cleanup beads	Qubit HS assay	1.5 – 2.0 hrs	
○ Safe stop				
5 Kinnex array formation	1.2X SMRTbell cleanup beads	Qubit HS assay	1.5 hrs	
○ Safe stop				
6 DNA damage repair	1.2X SMRTbell cleanup beads	N/A	30 min	**Day 2 (~2 hrs*)**
○ Safe stop				
7 Nuclease Tx + final cleanup	1.2X SMRTbell cleanup beads	Qubit HS assay Femto Pulse gDNA 165 kb kit	1.5 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 single-cell RNA libraries

cDNA Input

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

USER GUIDE

Chromium Next GEM Single Cell 3' Reagent Kits v3.1

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

DNA sizing and quantitation QC

- Perform DNA concentration measurements with a **Qubit fluorometer** using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit.
- Perform DNA sizing measurements with a **Bioanalyzer system** using the High Sensitivity DNA Kit (for input cDNA QC) or with a **Femto Pulse system** using the Genomic DNA 165 kb Kit (for final SMRTbell library QC)

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

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

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

---

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

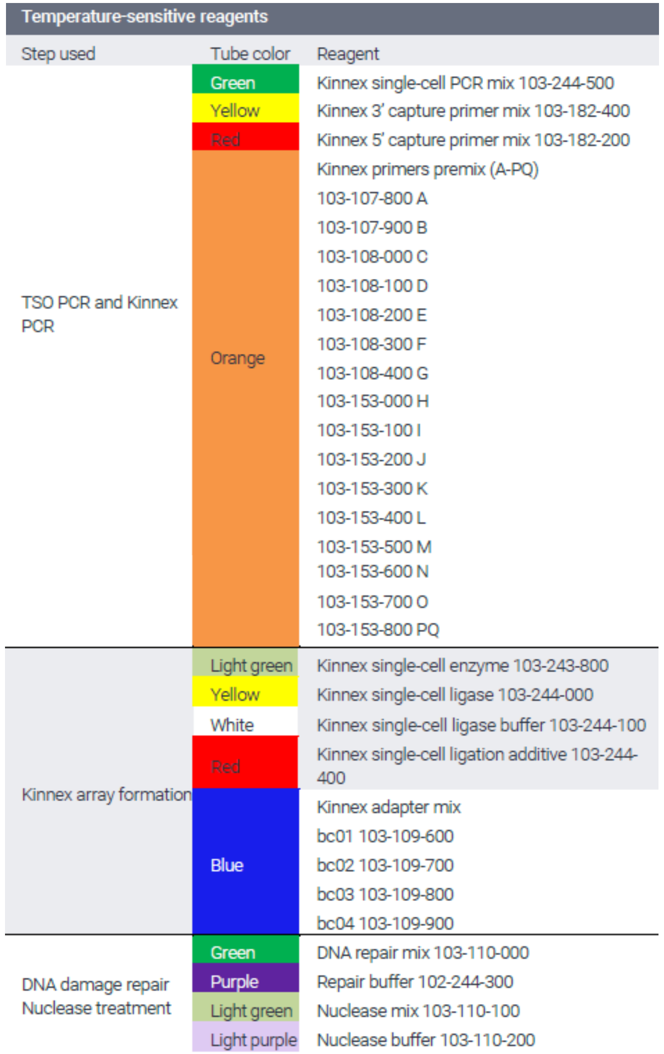
General best practices recommendations for preparing Kinnex single-cell RNA libraries (cont.)

Reagent and sample handling

Kinnex library prep reagents

- Room temperature is defined as any temperature in the range of 18–23°C for this protocol.
- Thaw the repair buffer, nuclease buffer, Kinnex ligase buffer, Kinnex ligation additive, Kinnex adapter mix, and primers at room temperature.
- Briefly vortex reagent buffers and Kinnex adapters prior to use. Enzyme mixes do not require vortexing.
- Quick-spin all reagents in a microcentrifuge to collect liquid at tube bottom prior to use.
- Keep all temperature-sensitive reagents on ice.
- Ensure that the DNA damage repair mix is stored at -20°C to avoid poor library performance.
- **Note:** The Loading buffer is light sensitive and should be protected from light when not in use.
- Bring SMRTbell cleanup beads and Qubit 1X dsDNA HS reagents to room temperature for 30 minutes prior to use.
- Pipette-mix all bead binding and elution steps until beads are distributed evenly in solution.
- Wide-bore pipette tips help to minimize foaming specifically when resuspending Kinnex capture beads.
- Mix all library prep reactions by pipetting up and down 10 times or until fully resuspended.
- Samples can be stored at 4°C at all safe stopping points listed in the protocol.
- 1.5X SMRTbell cleanup is recommended before Kinnex array formation. If the cDNA contains smaller fragments <200 bp, it is recommended to increase the SMRTbell cleanup ratio to 2X.

Temperature-sensitive reagents:



Temperature-sensitive reagents

Step used	Tube color	Reagent
TSO PCR and Kinnex PCR	Green	Kinnex single-cell PCR mix 103-244-500
	Yellow	Kinnex 3' capture primer mix 103-182-400
	Red	Kinnex 5' capture primer mix 103-182-200
	Orange	Kinnex primers premix (A-PQ)
		103-107-800 A
		103-107-900 B
		103-108-000 C
		103-108-100 D
		103-108-200 E
		103-108-300 F
		103-108-400 G
		103-153-000 H
		103-153-100 I
		103-153-200 J
		103-153-300 K
		103-153-400 L
		103-153-500 M
		103-153-600 N
		103-153-700 O
		103-153-800 PQ
Kinnex array formation	Light green	Kinnex single-cell enzyme 103-243-800
	Yellow	Kinnex single-cell ligase 103-244-000
	White	Kinnex single-cell ligase buffer 103-244-100
	Red	Kinnex single-cell ligation additive 103-244-400
	Blue	Kinnex adapter mix
		bc01 103-109-600
		bc02 103-109-700
		bc03 103-109-800
		bc04 103-109-900
DNA damage repair Nuclease treatment	Green	DNA repair mix 103-110-000
	Purple	Repair buffer 102-244-300
	Light green	Nuclease mix 103-110-100
	Light purple	Nuclease buffer 103-110-200

General best practices recommendations for preparing Kinnex single-cell RNA libraries (cont.)

Reagent and sample handling

Sequencing prep reagents (for sample setup ABC)

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

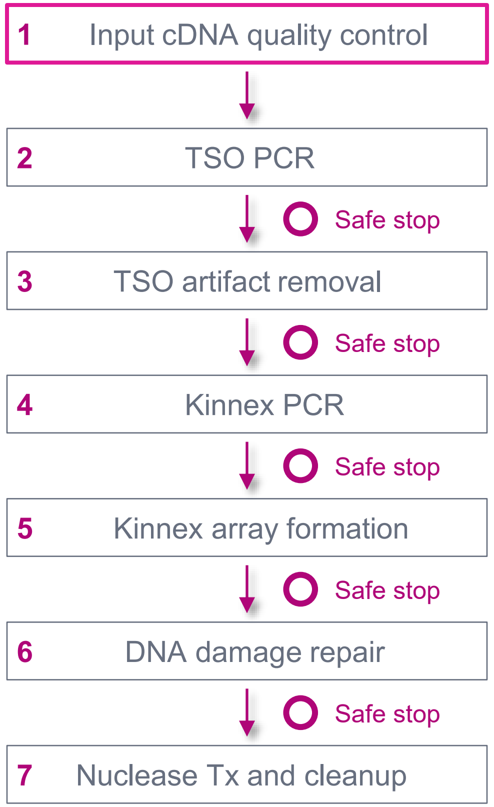
Thaw the following reagents and room temperature:

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

* **Room temperature** (RT) is defined as any temperature in the range of 18 – 23°C for this protocol.

Input cDNA quality control

Input cDNA quality control is highly recommended before proceeding to the Kinnex single-cell RNA library prep workflow

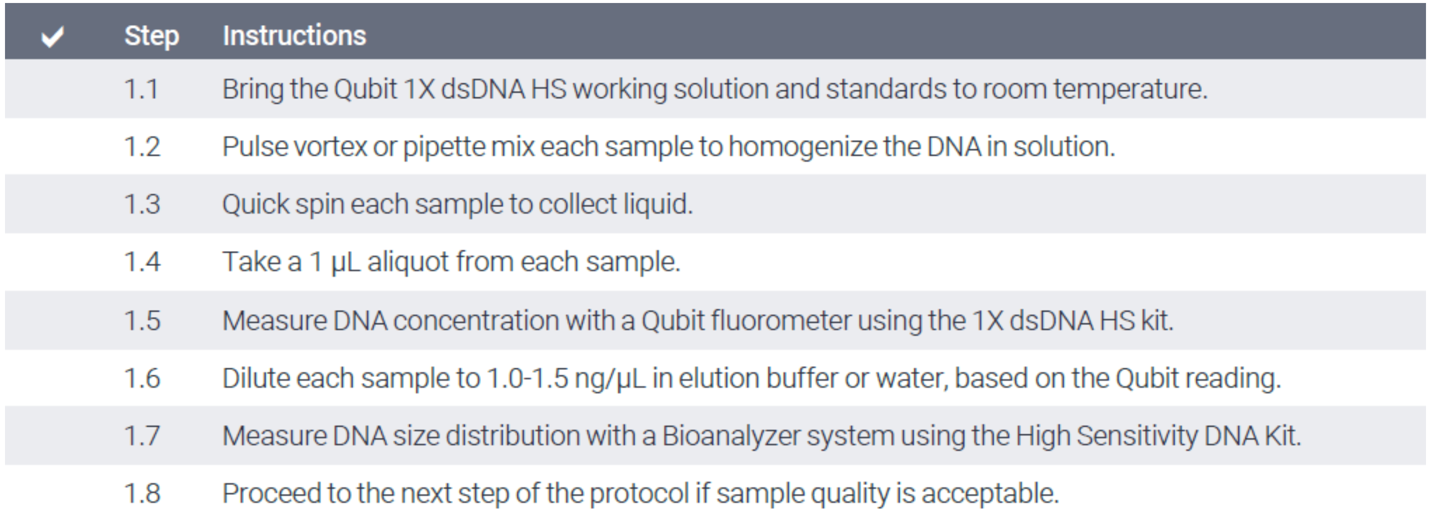

1 Input cDNA quality control
2 TSO PCR
Safe stop
3 TSO artifact removal
Safe stop
4 Kinnex PCR
Safe stop
5 Kinnex array formation
Safe stop
6 DNA damage repair
Safe stop
7 Nuclease Tx and cleanup

- We recommend using an optimal range of 3,000 – 10,000 cells input into 10x Chromium 3' or 5' single cell workflow[1]
- Protocol requires a **minimum of 15 ng** of 10x Chromium single cell cDNA (**maximum of 75 ng** per library)
  - If your cDNA sample amounts are between **16 – 59 ng**, then normalize all samples to 15 ng
  - If your cDNA sample amounts are >**75 ng**, then normalize all samples to 75 ng
  - If your cDNA sample amounts are between **60 – 75 ng**, normalization is not required.
- Evaluate the size distribution of each input cDNA sample to determine whether it is suitable for the protocol (average cDNA fragment size should be between 500 – 1,500 bp)
  - 10x single cell cDNA samples measured with a Bioanalyzer system typically show a peak at ~1 – 1.8 kb

Step 1
procedural summary

Single-cell cDNA size ideally >500 bp

Proceed to **Step 2**



✓	Step	Instructions
	1.1	Bring the Qubit 1X dsDNA HS working solution and standards to room temperature.
	1.2	Pulse vortex or pipette mix each sample to homogenize the DNA in solution.
	1.3	Quick spin each sample to collect liquid.
	1.4	Take a 1 µL aliquot from each sample.
	1.5	Measure DNA concentration with a Qubit fluorometer using the 1X dsDNA HS kit.
	1.6	Dilute each sample to 1.0-1.5 ng/µL in elution buffer or water, based on the Qubit reading.
	1.7	Measure DNA size distribution with a Bioanalyzer system using the High Sensitivity DNA Kit.
	1.8	Proceed to the next step of the protocol if sample quality is acceptable.

---

[1] Refer to 10x Genomics Chromium Single Cell 3' Reagent Kits User Guide – v3.1 (CG000204) or Chromium Single Cell 5' Reagent Kits User Guide – v2 Chemistry Dual Index (CG000331).

Input cDNA quality control (cont.)

Example Bioanalyzer DNA sizing QC results for single cell 3’ cDNA prepared with the 10x Chromium system

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

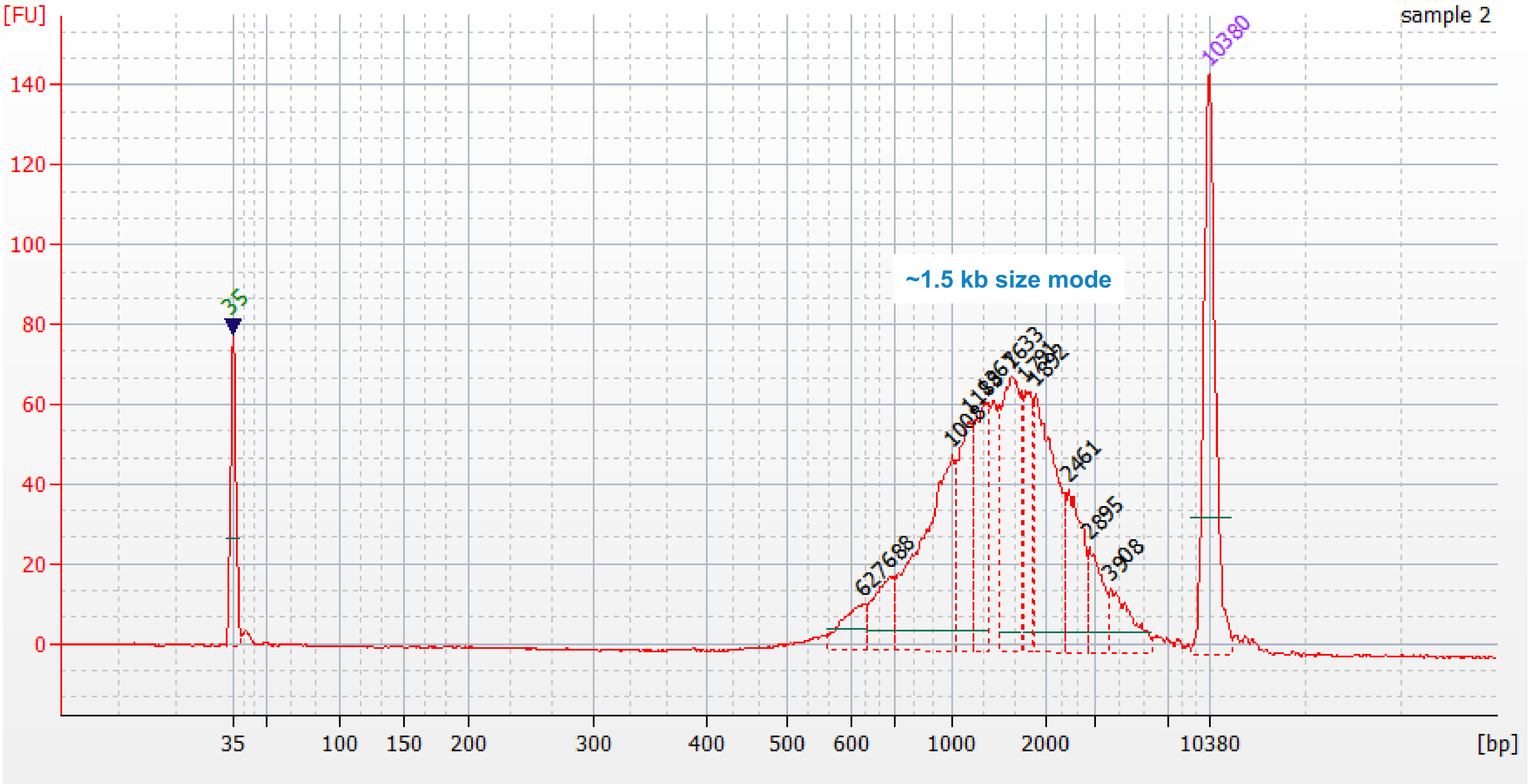
5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

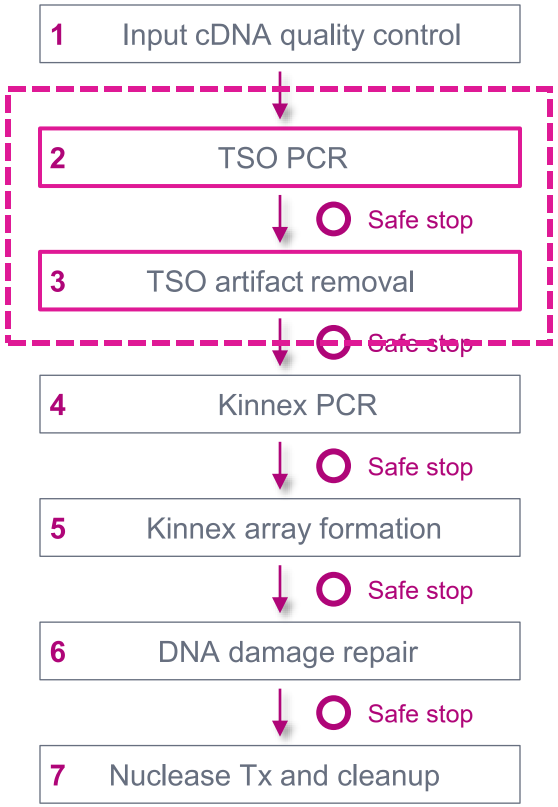
7 Nuclease Tx and cleanup



Bioanalyzer DNA sizing QC analysis results for a 10x Chromium single cell 3’ cDNA sample prepared from a human GM12878 cell line.

TSO PCR & TSO artifact removal

Perform Steps 2 & 3 to remove template switch oligo (TSO) priming artifacts generated during 10x cDNA synthesis


1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ ~~Safe stop~~

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

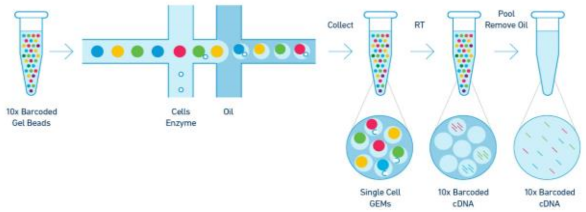
○ Safe stop

6 DNA damage repair

○ Safe stop

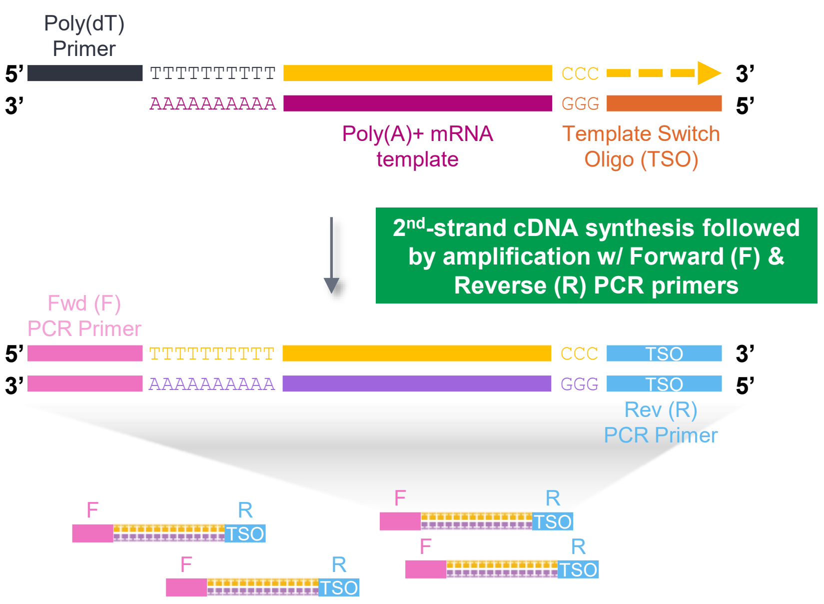
7 Nuclease Tx and cleanup

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



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

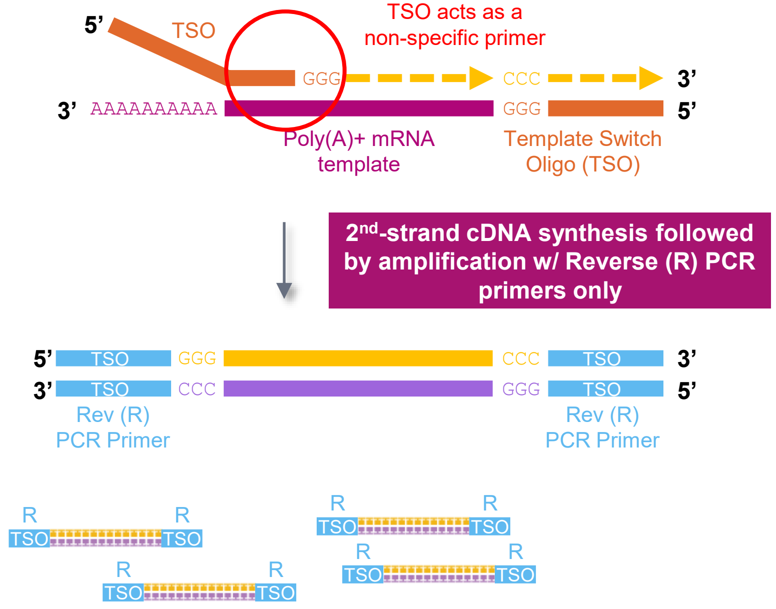
Example synthesis of 10x 3' cDNA products containing correct structure



2nd-strand cDNA synthesis followed by amplification w/ Forward (F) & Reverse (R) PCR primers

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

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



2nd-strand cDNA synthesis followed by amplification w/ Reverse (R) PCR primers only

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

PacBio

TSO PCR & TSO artifact removal (cont.)

Role of Kinnex capture primers and Kinnex capture beads

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

~~○ Safe stop~~

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

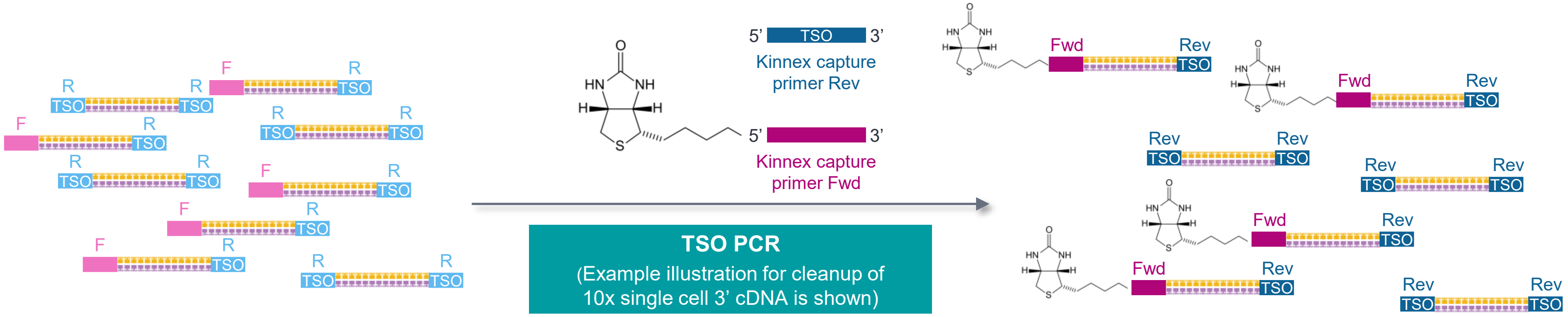
○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

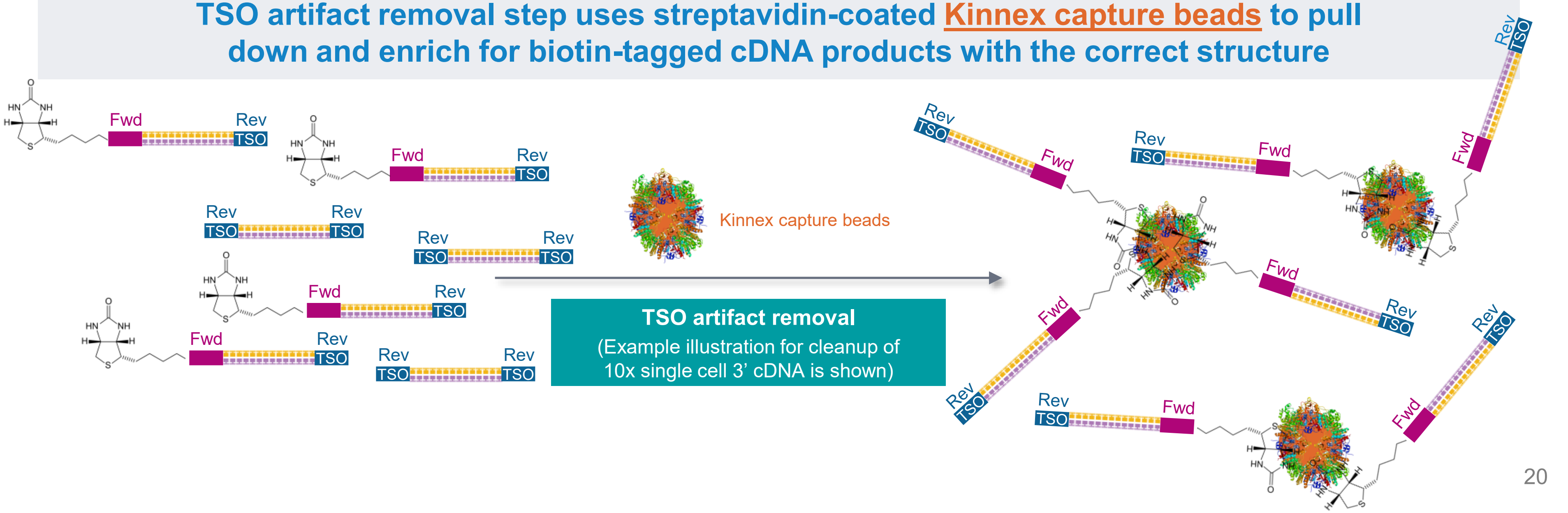
TSO PCR step in Kinnex single-cell RNA procedure uses a modified PCR primer (Kinnex capture primer Fwd) to incorporate a biotin tag into desired cDNA products with the correct structure



TSO PCR

(Example illustration for cleanup of 10x single cell 3' cDNA is shown)

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



TSO artifact removal

(Example illustration for cleanup of 10x single cell 3' cDNA is shown)

TSO PCR

Procedural summary

1 Input cDNA quality control

2 TSO PCR

Safe stop

3 TSO artifact removal

Safe stop

4 Kinnex PCR

Safe stop

5 Kinnex array formation

Safe stop

6 DNA damage repair

Safe stop

7 Nuclease Tx and cleanup

Step 2 procedural summary

2.1 – 2.4 TSO PCR

- Normalize cDNA sample input to **15 ng** if it is **between 15 ng and 59 ng** using elution buffer
- Normalize cDNA sample input to **75 ng** if it is **higher than 75 ng** using elution buffer
- For cDNA amounts **between 60–75ng, proceed without normalizing**

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

TSO PCR program for 15 ng cDNA input

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

OR

TSO PCR program for 60-75 ng cDNA input

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

2.5 – 2.20 1.5X SMRTbell cleanup

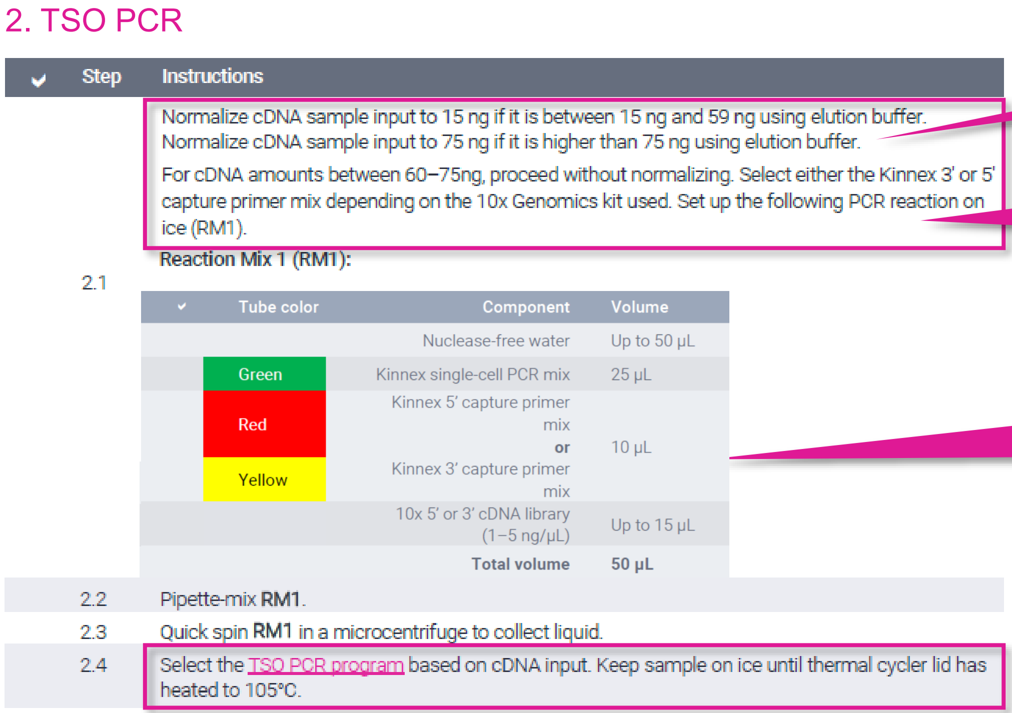
Proceed to the Step 3 of the protocol if sample quantity is acceptable (between 100 ng – 600 ng). If the yield is higher than 600 ng, proceed to TSO artifact removal step with 600 ng of amplified biotinylated DNA.

Proceed to **Step 3**

TSO PCR

Procedural notes

1. Input cDNA quality control
2. TSO PCR
   - ○ Safe stop
3. TSO artifact removal
   - ○ Safe stop
4. Kinnex PCR
   - ○ Safe stop
5. Kinnex array formation
   - ○ Safe stop
6. DNA damage repair
   - ○ Safe stop
7. Nuclease Tx and cleanup



2. TSO PCR

✓	Step	Instructions
	2.1	Normalize cDNA sample input to 15 ng if it is between 15 ng and 59 ng using elution buffer. Normalize cDNA sample input to 75 ng if it is higher than 75 ng using elution buffer. For cDNA amounts between 60–75ng, proceed without normalizing. Select either the Kinnex 3' or 5' capture primer mix depending on the 10x Genomics kit used. Set up the following PCR reaction on ice (RM1). **Reaction Mix 1 (RM1):**
	2.2	Pipette-mix **RM1**.
	2.3	Quick spin **RM1** in a microcentrifuge to collect liquid.
	2.4	Select the TSO PCR program based on cDNA input. Keep sample on ice until thermal cycler lid has heated to 105°C.

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

- If needed, **normalize** cDNA sample input amounts to 15 ng or 75 ng

- ***IMPORTANT!*** Select either the Kinnex 3' or 5' capture primer mix depending on the 10x Genomics kit used

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

- For **lower** cDNA sample inputs (15 ng), use a **higher** number of PCR cycles (5)

TSO PCR program (15 ng input)

Heated lid set at 105°C

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

Or TSO PCR program (60-75 ng input)

Heated lid set at 105°C

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

After completing TSO PCR step, perform cleanup with 1.5X SMRTbell cleanup beads and proceed to TSO artifact removal (Step 3) if sample quantity is acceptable (between 100 ng – 600 ng)

- If the yield is higher than 600 ng, proceed to the next step with 600 ng of amplified biotinylated DNA.

TSO artifact removal

Procedural summary

Step 3 procedural summary

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

○ Safe stop

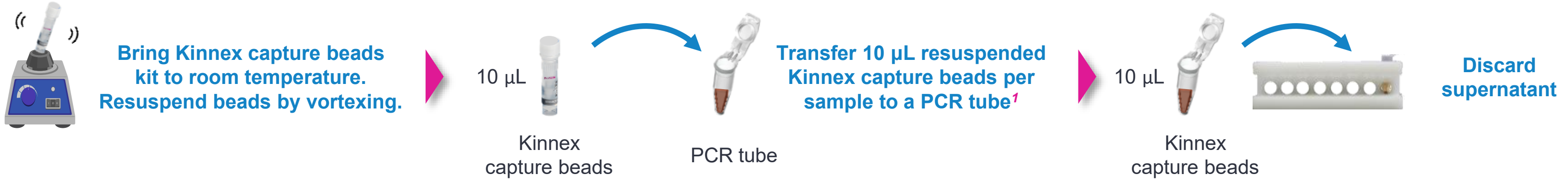
6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

3.1 – 3.21 TSO artifact removal

3.1 – 3.4 Resuspend Kinnex capture beads by vortexing and transfer 10 µL of beads per sample to a PCR tube

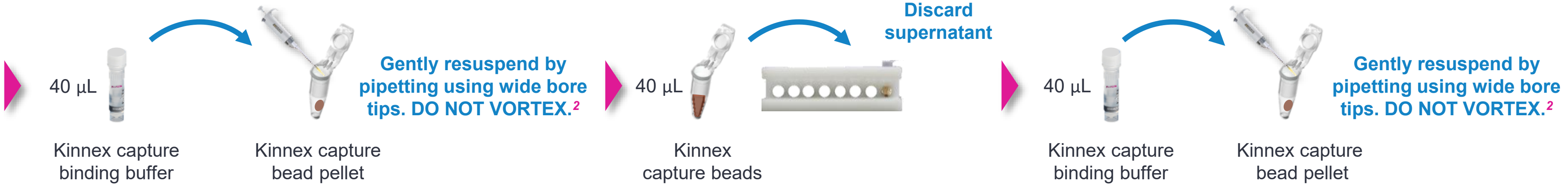


Bring Kinnex capture beads kit to room temperature. Resuspend beads by vortexing.

Transfer 10 µL resuspended Kinnex capture beads per sample to a PCR tube[1]

Discard supernatant

3.5 – 3.7 Resuspend Kinnex capture beads in Kinnex capture binding buffer (Perform resuspension 2 times)

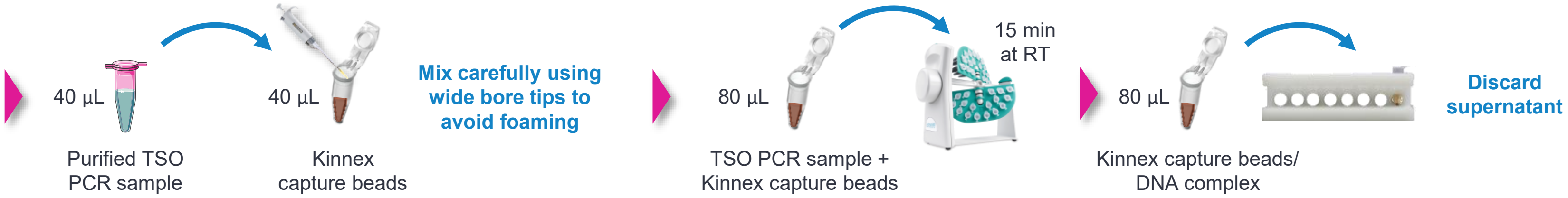


Gently resuspend by pipetting using wide bore tips. DO NOT VORTEX.[2]

Discard supernatant

Gently resuspend by pipetting using wide bore tips. DO NOT VORTEX.[2]

3.8 – 3.10 Add purified TSO PCR sample (containing biotinylated DNA-fragments) from Step 2 to resuspended beads and incubate on rotator



Mix carefully using wide bore tips to avoid foaming

Discard supernatant

3.11 – 3.14 Resuspend Kinnex capture beads/DNA-complex in Kinnex capture washing buffer (Perform wash 2 times)

Pipette-mix thoroughly until evenly distributed

Discard supernatant

[1] Scale up the volume of beads if processing more than 4 samples (with 10% overage). If preparing more than 40 µL of beads, use a 1.5 mL LoBind tube instead of PCR tube.

[2] Scale up the volume of Kinnex capture binding buffer accordingly if preparing more than 40 µL of beads.

TSO artifact removal

Procedural summary

Step 3 procedural summary (cont.)

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

○ Safe stop

6 DNA damage repair

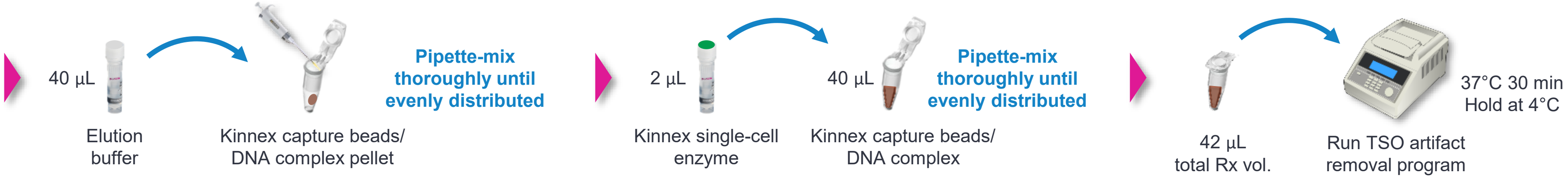
○ Safe stop

7 Nuclease Tx and cleanup

3.1 – 3.21 TSO artifact removal (cont.)

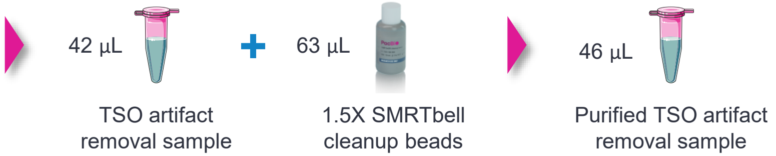
3.15 – 3.16 Resuspend Kinnex capture beads/DNA-complex in nuclease-free water

3.17 – 3.20 Resuspend Kinnex capture beads/DNA-complex in elution buffer, add 2 µL Kinnex single-cell enzyme and run TSO artifact removal program



3.21 Place sample tube on magnet for 1 minute and move supernatant containing library to a fresh tube

3.22 – 3.38 1.5X SMRTbell cleanup



Proceed to Step 4 of the protocol if sample quantity is acceptable (25 ng is required to proceed to Kinnex PCR step).

Proceed to **Step 4**

[1] Scale up the volume of beads if processing more than 4 samples (with 10% overage). If preparing more than 40 µL of beads, use a 1.5 mL LoBind tube instead of PCR tube.

[2] Scale up the volume of Kinnex capture binding buffer accordingly if preparing more than 40 µL of beads.

TSO artifact removal

Procedural notes

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

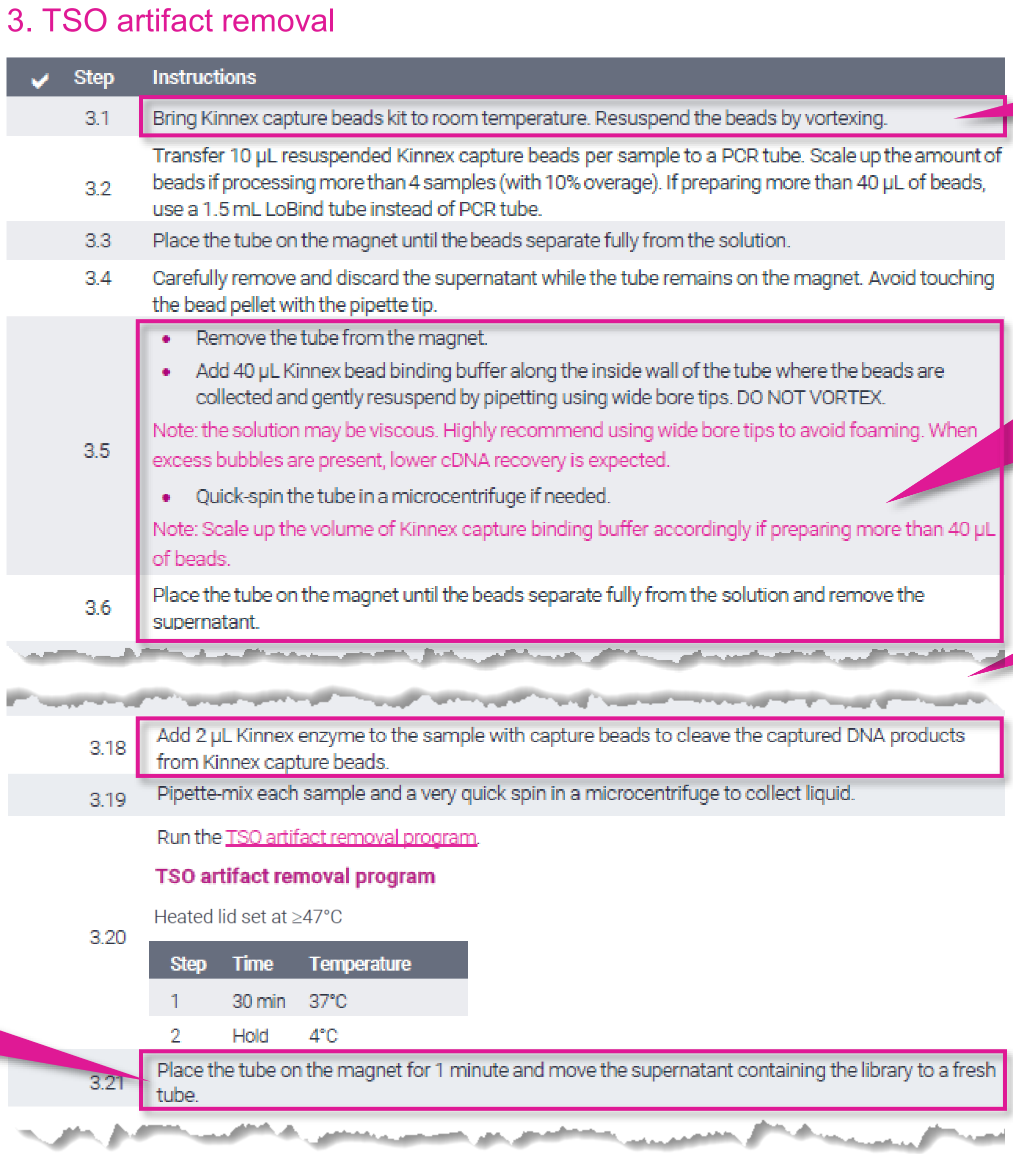
5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup



3. TSO artifact removal

<table>
<tr><th>✓</th><th>Step</th><th>Instructions</th></tr>
<tr><td></td><td>3.1</td><td>Bring Kinnex capture beads kit to room temperature. Resuspend the beads by vortexing.</td></tr>
<tr><td></td><td>3.2</td><td>Transfer 10 µL resuspended Kinnex capture beads per sample to a PCR tube. Scale up the amount of beads if processing more than 4 samples (with 10% overage). If preparing more than 40 µL of beads, use a 1.5 mL LoBind tube instead of PCR tube.</td></tr>
<tr><td></td><td>3.3</td><td>Place the tube on the magnet until the beads separate fully from the solution.</td></tr>
<tr><td></td><td>3.4</td><td>Carefully remove and discard the supernatant while the tube remains on the magnet. Avoid touching the bead pellet with the pipette tip.</td></tr>
<tr><td></td><td>3.5</td><td>

Remove the tube from the magnet.
Add 40 µL Kinnex bead binding buffer along the inside wall of the tube where the beads are collected and gently resuspend by pipetting using wide bore tips. DO NOT VORTEX.

Note: the solution may be viscous. Highly recommend using wide bore tips to avoid foaming. When excess bubbles are present, lower cDNA recovery is expected.

Quick-spin the tube in a microcentrifuge if needed.

Note: Scale up the volume of Kinnex capture binding buffer accordingly if preparing more than 40 µL of beads.</td></tr>
<tr><td></td><td>3.6</td><td>Place the tube on the magnet until the beads separate fully from the solution and remove the supernatant.</td></tr>
<tr><td></td><td>3.18</td><td>Add 2 µL Kinnex enzyme to the sample with capture beads to cleave the captured DNA products from Kinnex capture beads.</td></tr>
<tr><td></td><td>3.19</td><td>Pipette-mix each sample and a very quick spin in a microcentrifuge to collect liquid.</td></tr>
<tr><td></td><td>3.20</td><td>Run the TSO artifact removal program.
TSO artifact removal program
Heated lid set at ≥47°C
<table>
<tr><th>Step</th><th>Time</th><th>Temperature</th></tr>
<tr><td>1</td><td>30 min</td><td>37°C</td></tr>
<tr><td>2</td><td>Hold</td><td>4°C</td></tr>
</table></td></tr>
<tr><td></td><td>3.21</td><td>Place the tube on the magnet for 1 minute and move the supernatant containing the library to a fresh tube.</td></tr>
</table>

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

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

- Add Kinnex enzyme to **cleave** captured cDNA products from Kinnex capture beads

- Keep the **supernatant** after treatment with Kinnex enzyme and placement on the magnet

After completing TSO artifact removal step, perform cleanup with 1.5X SMRTbell cleanup beads and proceed to Kinnex PCR (Step 4) if sample quantity is acceptable (minimum 25 ng)

TSO PCR & TSO artifact removal (cont.)

TSO artifact video demonstration

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

PacBio MAS-Seq TSO artifact removal demo | MAS-Seq for 10x Single Cell 3’ kit

TSO ARTIFACT REMOVAL WITH THE MAS-SEQ FOR 10X SINGLE CELL 3’ KIT

View Video tutorial – PacBio Kinnex single-cell RNA TSO artifact removal demo for Kinnex single-cell RNA kit [YouTube]

Kinnex PCR

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

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

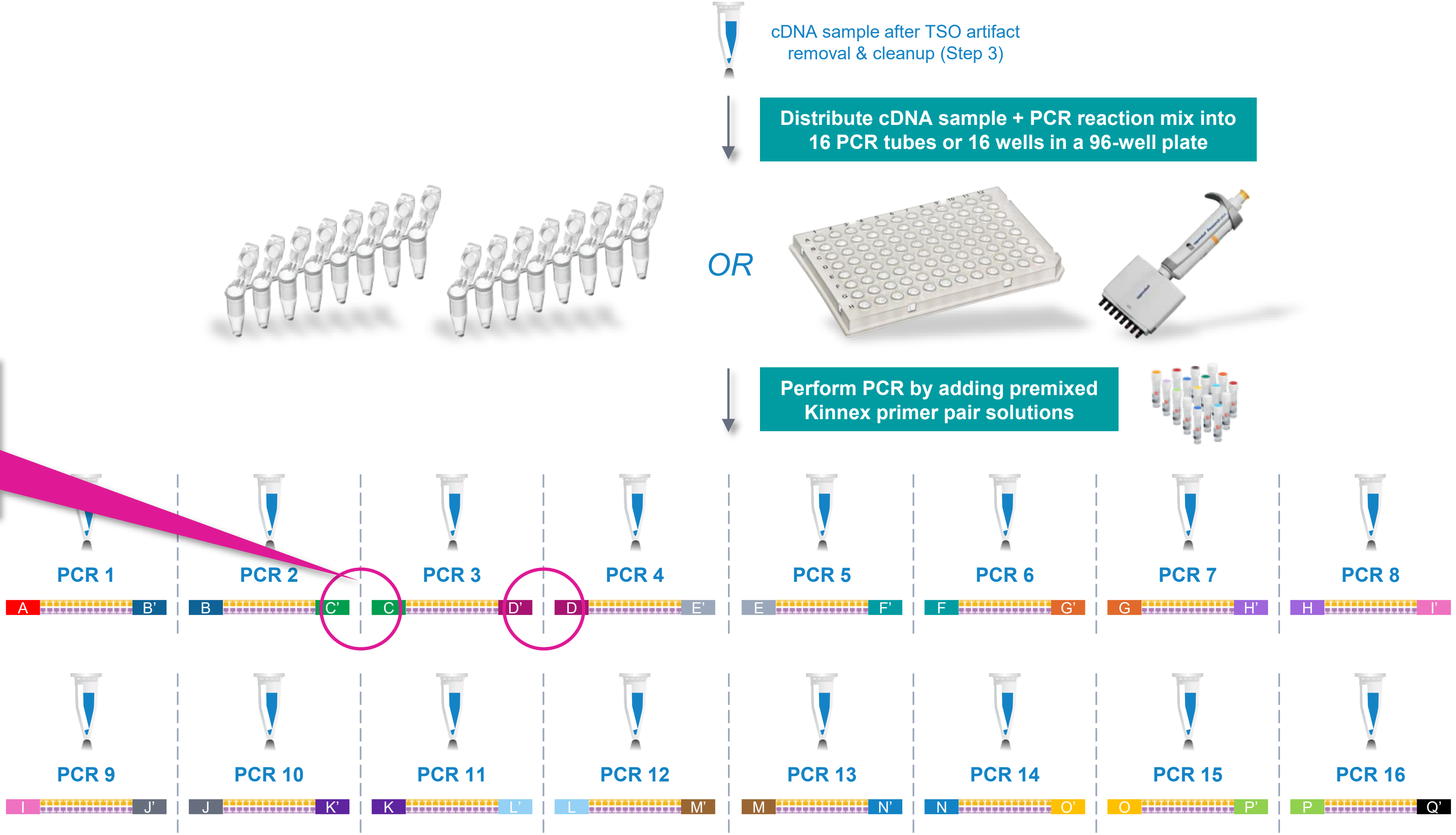
5 Kinnex array formation

○ Safe stop

7 Nuclease Tx and cleanup

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

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



cDNA sample after TSO artifact removal & cleanup (Step 3)

Distribute cDNA sample + PCR reaction mix into 16 PCR tubes or 16 wells in a 96-well plate

OR

Perform PCR by adding premixed Kinnex primer pair solutions

Kinnex PCR (cont.)

Procedural summary

1 Input cDNA quality control

2 TSO PCR

Safe stop

3 TSO artifact removal

Safe stop

4 Kinnex PCR

Safe stop

5 Kinnex array formation

Safe stop

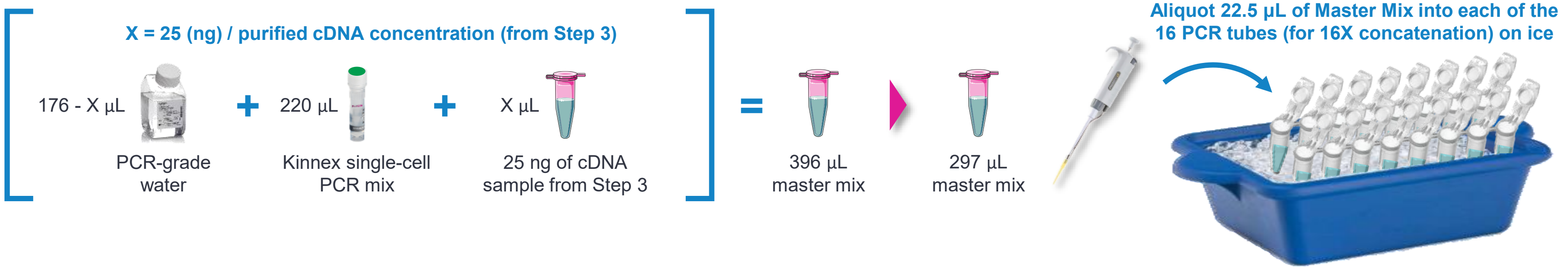
6 DNA damage repair

Safe stop

7 Nuclease Tx and cleanup

Step 4 procedural summary

3.1 Prepare Kinnex primers premix & 3.2 Kinnex PCR

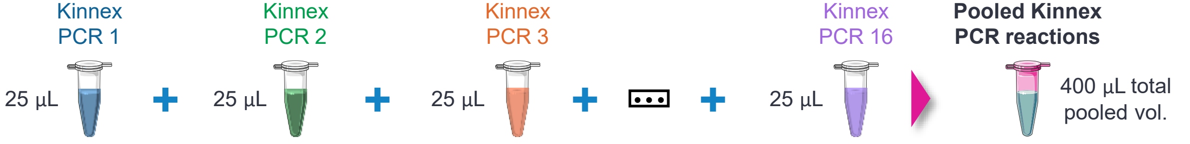


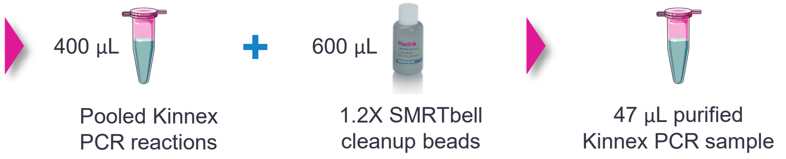
Add 2.5 µL of Kinnex primer mix[1] into each of 16 PCR tubes (containing 22.5 µL of Master Mix)

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

Run Kinnex PCR program (9 cycles)

3.2 Pooling of 16 Kinnex PCR products and 1.1X SMRTbell cleanup





Proceed to **Step 5**

Kinnex PCR (cont.)

Procedural notes

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

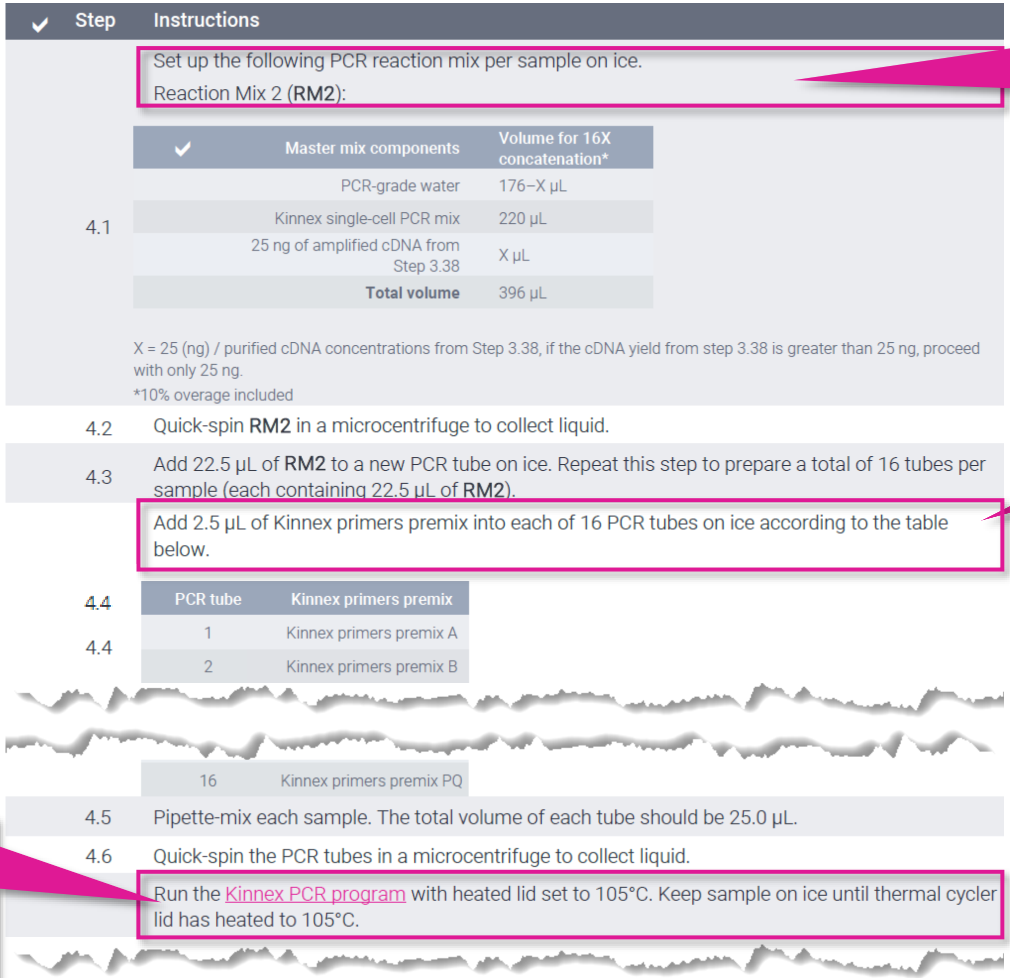
○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

4. Kinnex PCR



✓	Step	Instructions
	4.1	Set up the following PCR reaction mix per sample on ice. Reaction Mix 2 (**RM2**):

✓ Master mix components	Volume for 16X concatenation*
PCR-grade water	176–X µL
Kinnex single-cell PCR mix	220 µL
25 ng of amplified cDNA from Step 3.38	X µL
Total volume	396 µL

X = 25 (ng) / purified cDNA concentrations from Step 3.38, if the cDNA yield from step 3.38 is greater than 25 ng, proceed with only 25 ng.
*10% overage included

Step	Instructions
4.2	Quick-spin **RM2** in a microcentrifuge to collect liquid.
4.3	Add 22.5 µL of **RM2** to a new PCR tube on ice. Repeat this step to prepare a total of 16 tubes per sample (each containing 22.5 µL of **RM2**).
4.4	Add 2.5 µL of Kinnex primers premix into each of 16 PCR tubes on ice according to the table below.

4.4

PCR tube	Kinnex primers premix
1	Kinnex primers premix A
2	Kinnex primers premix B
16	Kinnex primers premix PQ

Step	Instructions
4.5	Pipette-mix each sample. The total volume of each tube should be 25.0 µL.
4.6	Quick-spin the PCR tubes in a microcentrifuge to collect liquid.
	Run the Kinnex PCR program with heated lid set to 105°C. Keep sample on ice until thermal cycler lid has heated to 105°C.

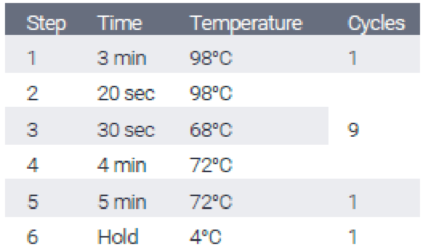
- Set up Kinnex PCR reactions **ON ICE**
- PCR polymerase 3'→5' exonuclease activity negatively impacts amplification yield if prepared at room temp.

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

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

Kinnex PCR program

Heated lid set at 105°C



Step	Time	Temperature	Cycles
1	3 min	98°C	1
2	20 sec	98°C	9
3	30 sec	68°C	
4	4 min	72°C	
5	5 min	72°C	1
6	Hold	4°C	1

- After Kinnex PCR step, pool entire volume of all 16 reactions into a clean 1.5 mL LoBind tube[1] and perform cleanup with 1.5X SMRTbell cleanup beads

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

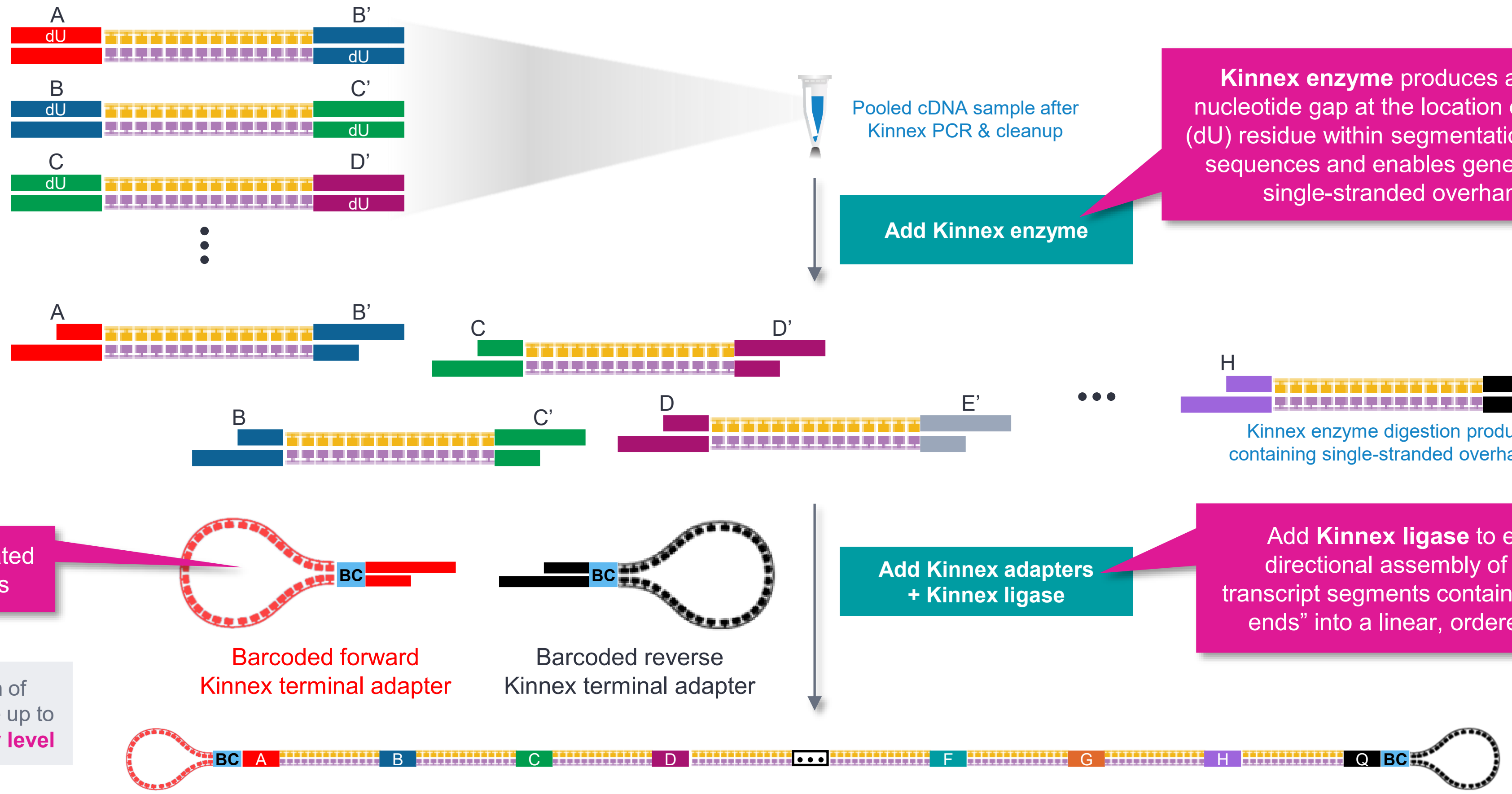
PacBio

Kinnex array formation

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

- **1** Input cDNA quality control
- **2** TSO PCR
  - ○ Safe stop
- **3** TSO artifact removal
  - ○ Safe stop
- **4** Kinnex PCR
  - ○ Safe stop
- **5** Kinnex array formation
  - ○ Safe stop
- **6** DNA damage repair

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



Pooled cDNA sample after Kinnex PCR & cleanup

Add Kinnex enzyme

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

Kinnex enzyme digestion product containing single-stranded overhangs

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

Barcoded forward Kinnex terminal adapter

Barcoded reverse Kinnex terminal adapter

Add Kinnex adapters + Kinnex ligase

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

Complete 16-segment transcript array molecule containing 2 Kinnex adapters

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**

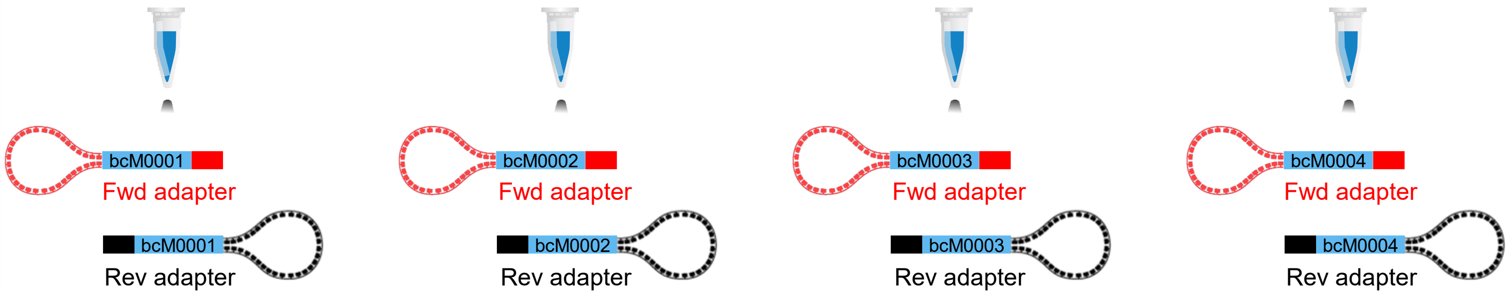
---

[1] **Note:** Single-cell Kinnex single-cell RNA library prep workflow described in this **Procedure & checklist** (103-254-300) 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



Kinnex adapter mix bcM0001

Kinnex adapter mix bcM0002

Kinnex adapter mix bcM0003

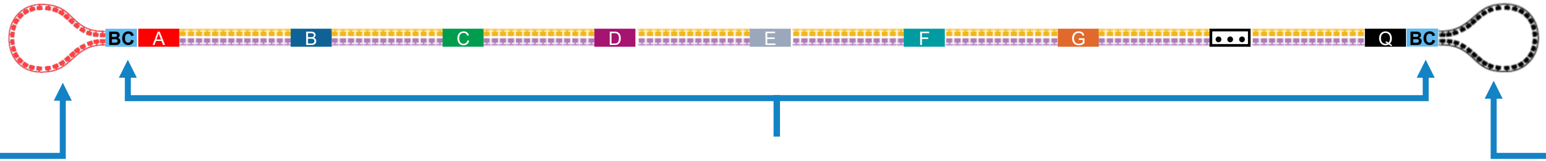
Kinnex adapter mix bcM0004

SMRT Link

Barcodes

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

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



Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

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

Kinnex 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**

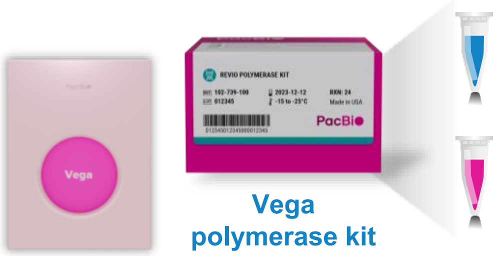
Annealed & bound Kinnex library molecule

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

Sequel II binding kit 3.2 and cleanup beads (102-333-300)

Sequencing primer 3.2

Kinnex sequencing primer



Vega polymerase kit (103-517-600)

Standard sequencing primer

Kinnex sequencing primer

Revio SPRQ polymerase kit (103-520-100)

Standard sequencing primer

Kinnex sequencing primer

---

[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 cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

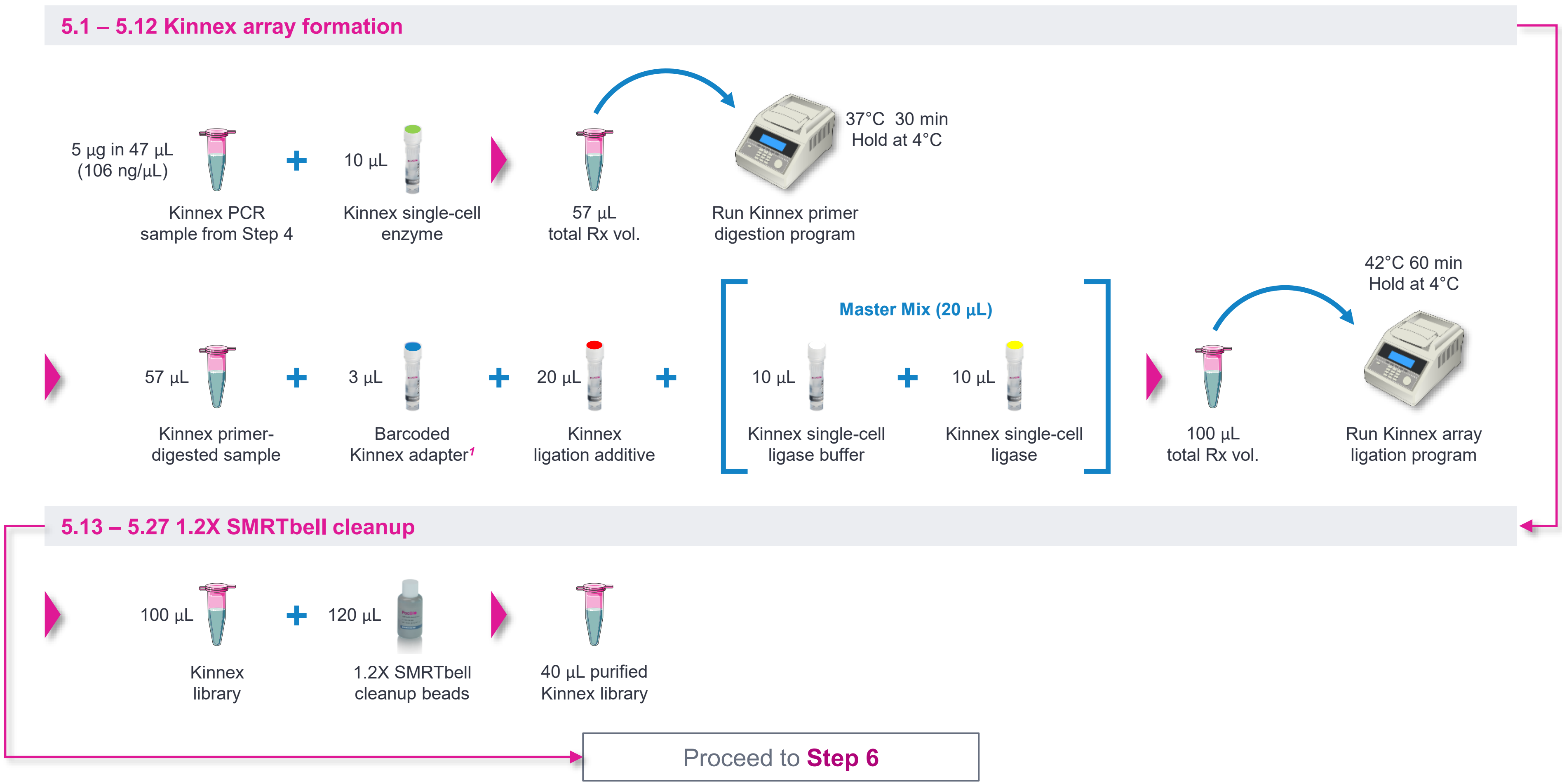
○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

Step 5 procedural summary



5.1 – 5.12 Kinnex array formation

5 µg in 47 µL (106 ng/µL) Kinnex PCR sample from Step 4 + 10 µL Kinnex single-cell enzyme → 57 µL total Rx vol. → Run Kinnex primer digestion program (37°C 30 min, Hold at 4°C)

57 µL Kinnex primer-digested sample + 3 µL Barcoded Kinnex adapter[1] + 20 µL Kinnex ligation additive + Master Mix (20 µL) [10 µL Kinnex single-cell ligase buffer + 10 µL Kinnex single-cell ligase] → 100 µL total Rx vol. → Run Kinnex array ligation program (42°C 60 min, Hold at 4°C)

5.13 – 5.27 1.2X SMRTbell cleanup

100 µL Kinnex library + 120 µL 1.2X SMRTbell cleanup beads → 40 µL purified Kinnex library

Proceed to **Step 6**

PacBio

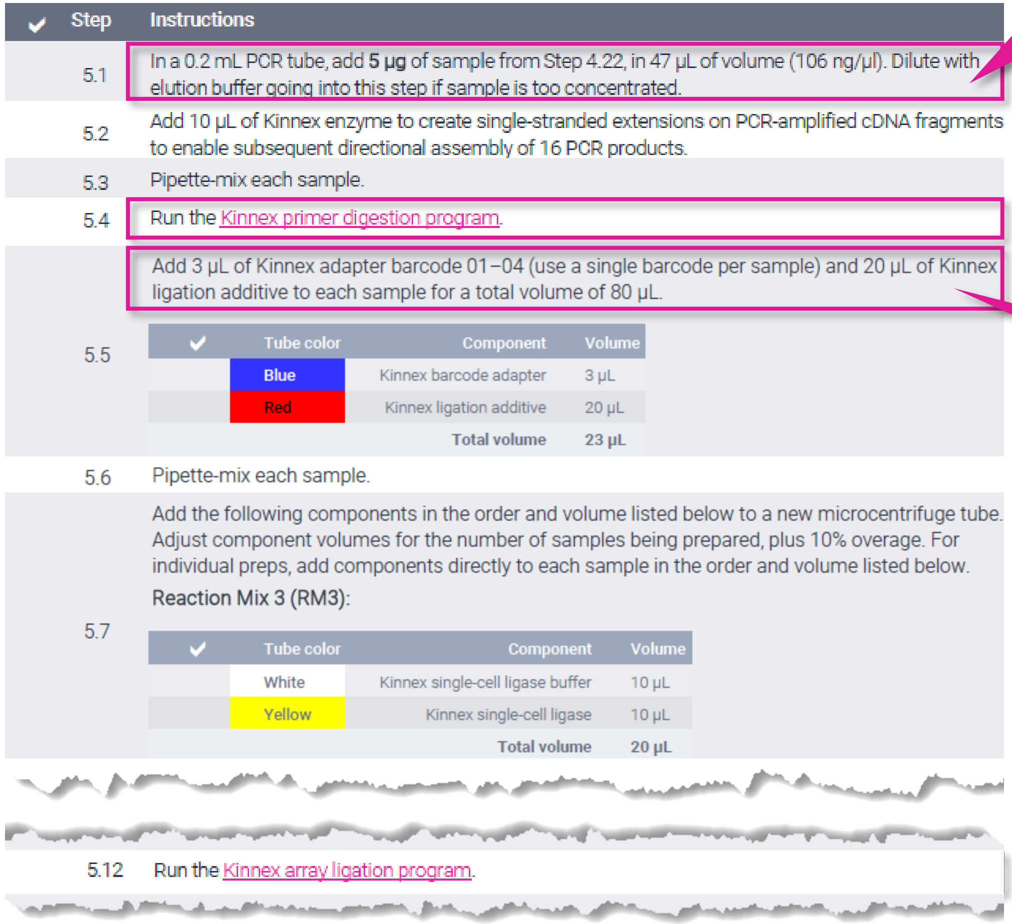
[1] Kinnex single-cell RNA kit bundle (103-072-200) contains four different barcoded Kinnex adapter mixes (bcM0001–bcM0004) → Select one barcode to use per Kinnex library preparation reaction.

Kinnex array formation (cont.)

Procedural notes

- **1** Input cDNA quality control
- **2** TSO PCR
  - ○ Safe stop
- **3** TSO artifact removal
  - ○ Safe stop
- **4** Kinnex PCR
  - ○ Safe stop
- **5** Kinnex array formation
  - ○ Safe stop
- **6** DNA damage repair
  - ○ Safe stop
- **7** Nuclease Tx and cleanup

5. Kinnex array formation



✓	Step	Instructions
	5.1	In a 0.2 mL PCR tube, add **5 µg** of sample from Step 4.22, in 47 µL of volume (106 ng/µl). Dilute with elution buffer going into this step if sample is too concentrated.
	5.2	Add 10 µL of Kinnex enzyme to create single-stranded extensions on PCR-amplified cDNA fragments to enable subsequent directional assembly of 16 PCR products.
	5.3	Pipette-mix each sample.
	5.4	Run the Kinnex primer digestion program.
	5.5	Add 3 µL of Kinnex adapter barcode 01–04 (use a single barcode per sample) and 20 µL of Kinnex ligation additive to each sample for a total volume of 80 µL.

✓	Tube color	Component	Volume
	Blue	Kinnex barcode adapter	3 µL
	Red	Kinnex ligation additive	20 µL
		Total volume	**23 µL**

✓	Step	Instructions
	5.6	Pipette-mix each sample.
	5.7	Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample in the order and volume listed below. Reaction Mix 3 (RM3):

✓	Tube color	Component	Volume
	White	Kinnex single-cell ligase buffer	10 µL
	Yellow	Kinnex single-cell ligase	10 µL
		Total volume	**20 µL**

✓	Step	Instructions
	5.12	Run the Kinnex array ligation program.

- 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

Kinnex primer digestion program

Heated lid set at 47°C

Step	Time	Temperature
1	30 min	37°C
2	Hold	4°C

- ***IMPORTANT:*** Always add Kinnex adapters and Kinnex ligation additive to the sample BEFORE adding Kinnex single-cell ligase
- If combining multiple barcoded Kinnex libraries for sequencing, make sure each library uses one of the 4 different Kinnex barcoded adapters

Kinnex array ligation program

Heated lid set at 52°C

Step	Time	Temperature
1	60 min	42°C
2	Hold	4°C

- After Kinnex array formation step, perform cleanup with 1.2X SMRTbell cleanup beads and proceed to DNA damage repair (Step 6)

DNA damage repair & nuclease treatment

Perform DNA damage repair to repair nicked/damaged DNA sites and perform nuclease treatment to remove incomplete SMRTbell template arrays

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

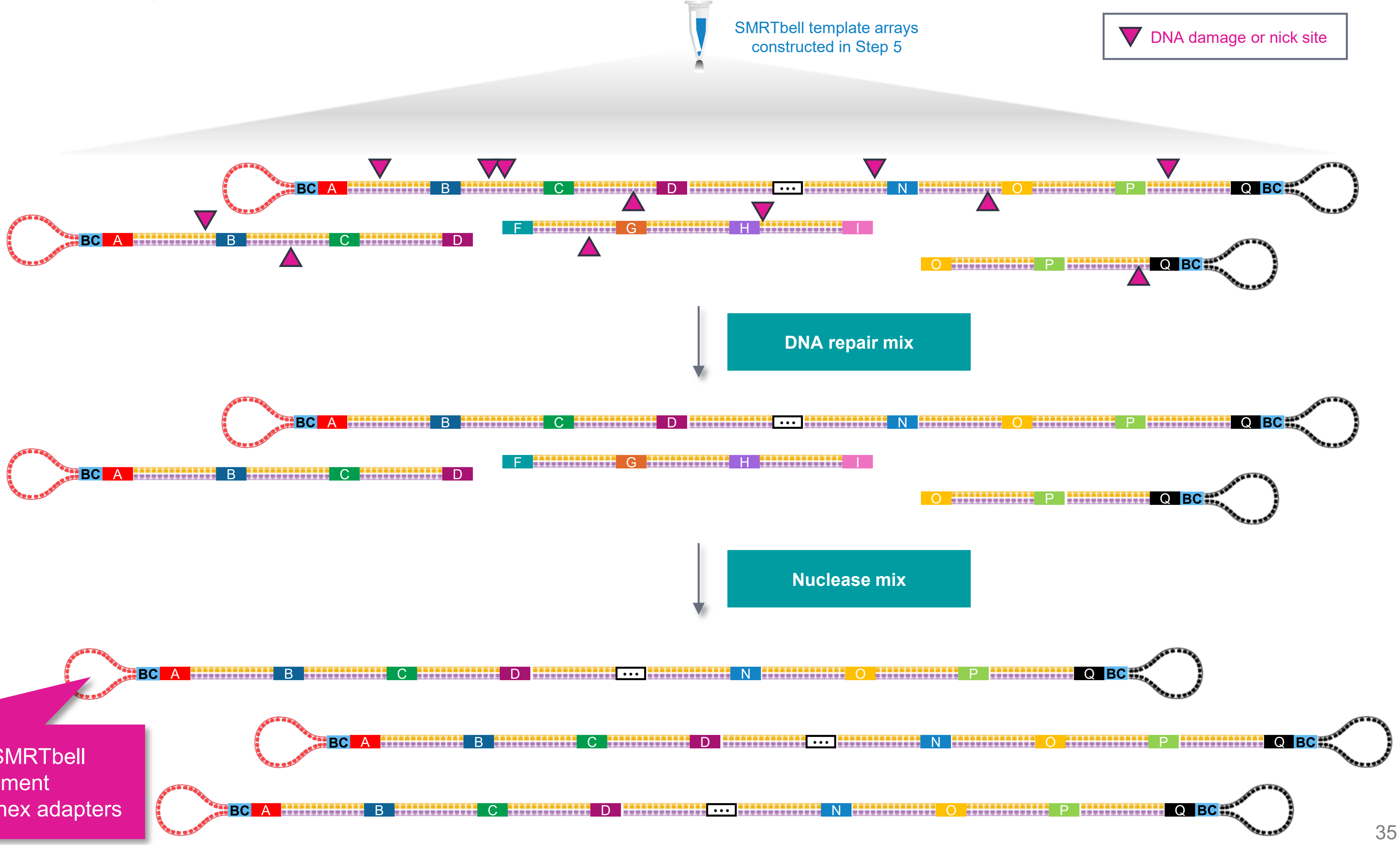
5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup



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

DNA damage repair

Procedural summary

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

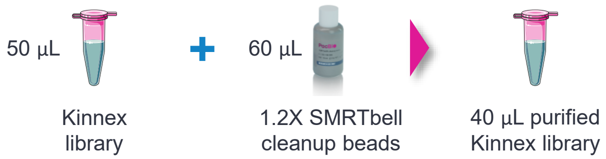
Step 6 procedural summary

6.1 – 6.8 DNA damage repair

42 µL Kinnex array sample + 6 µL Repair buffer + 2 µL DNA repair mix ▶ 50 µL total Rx vol. → Run DNA damage repair program

37°C 30 min
Hold at 4°C

6.9 – 6.22 1.2X SMRTbell cleanup



50 µL Kinnex library + 60 µL 1.2X SMRTbell cleanup beads ▶ 40 µL purified Kinnex library

Proceed to **Step 7**

DNA damage repair

Procedural notes

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

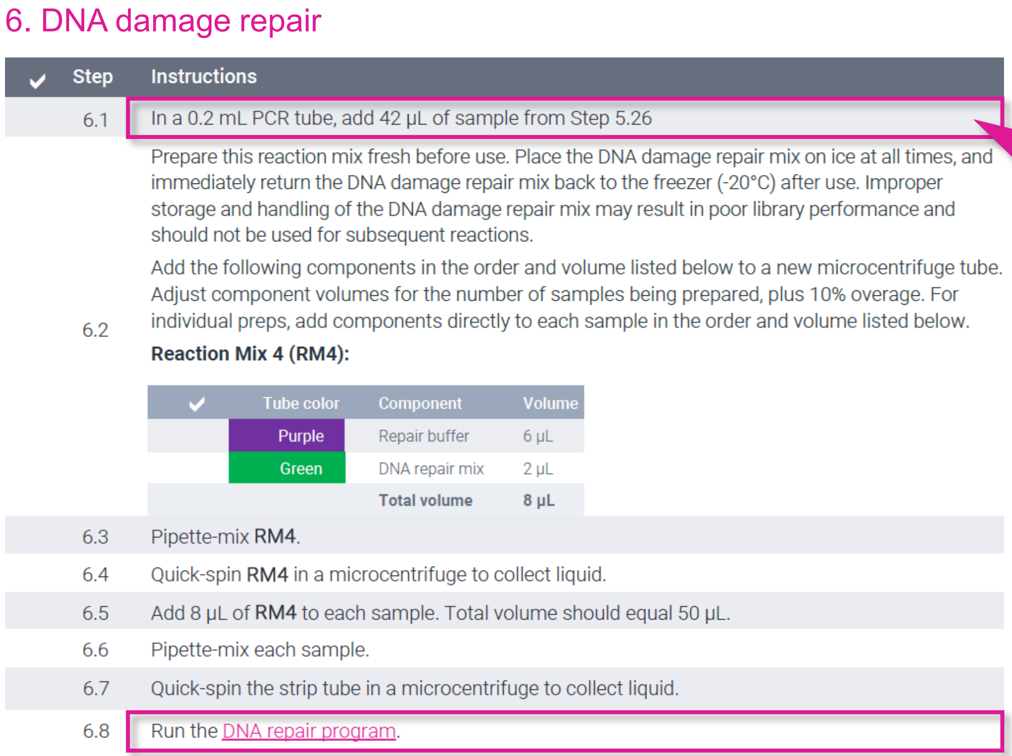
5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

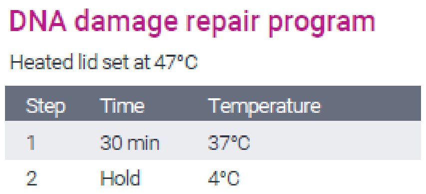


6. DNA damage repair

✓	Step	Instructions
	6.1	In a 0.2 mL PCR tube, add 42 µL of sample from Step 5.26
	6.2	Prepare this reaction mix fresh before use. 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. Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample in the order and volume listed below. **Reaction Mix 4 (RM4):**
	6.3	Pipette-mix **RM4**.
	6.4	Quick-spin **RM4** in a microcentrifuge to collect liquid.
	6.5	Add 8 µL of **RM4** to each sample. Total volume should equal 50 µL.
	6.6	Pipette-mix each sample.
	6.7	Quick-spin the strip tube in a microcentrifuge to collect liquid.
	6.8	Run the DNA repair program.

✓	Tube color	Component	Volume
	Purple	Repair buffer	6 µL
	Green	DNA repair mix	2 µL
		Total volume	**8 µL**

- **DO NOT** proceed with more than 5 µg of Kinnex array input per individual DDR reaction (in a 50 µL reaction volume)
  - Adding more than 5 µg per reaction can result in incomplete digestion of partial (non-full length) SMRTbell template arrays, resulting in low sample loading performance during sequencing



DNA damage repair program

Heated lid set at 47°C

Step	Time	Temperature
1	30 min	37°C
2	Hold	4°C

- After DNA damage repair step, perform cleanup with 1.2X SMRTbell cleanup beads and proceed to Nuclease treatment (Step 7)

Nuclease treatment and cleanup

Procedural summary

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

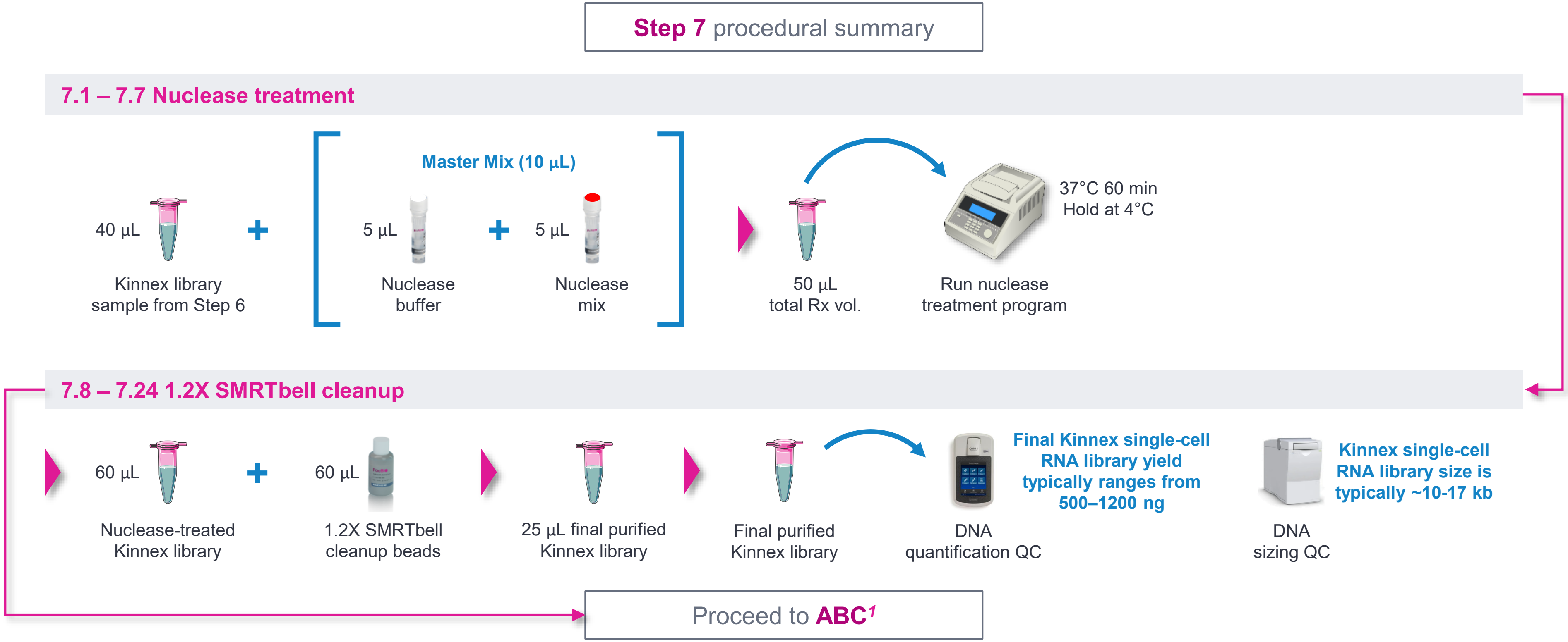
5 Kinnex array formation

○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup



Step 7 procedural summary

7.1 – 7.7 Nuclease treatment

40 µL Kinnex library sample from Step 6 + Master Mix (10 µL) [5 µL Nuclease buffer + 5 µL Nuclease mix] ▶ 50 µL total Rx vol. → Run nuclease treatment program

37°C 60 min
Hold at 4°C

7.8 – 7.24 1.2X SMRTbell cleanup

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

Final Kinnex single-cell RNA library yield typically ranges from 500–1200 ng

DNA sizing QC

Kinnex single-cell RNA library size is typically ~10-17 kb

Proceed to **ABC**[1]

---

[1] Follow **Step 8** instructions in **Procedure & checklist** to prepare the Kinnex SMRTbell library (25 µL) for sequencing on Revio (+SPRQ) or Vega system.

Nuclease treatment and cleanup (cont.)

Procedural notes

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

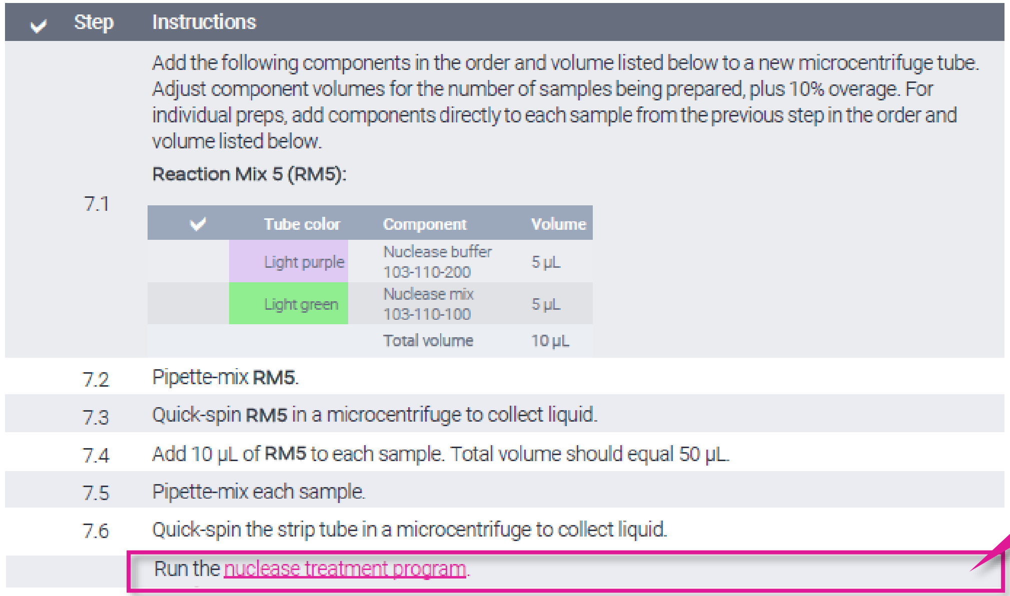
○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

7.1 – 7.7 Nuclease treatment

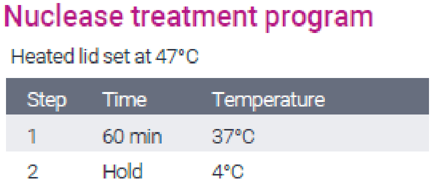


✓	Step	Instructions
	7.1	Add the following components in the order and volume listed below to a new microcentrifuge tube. Adjust component volumes for the number of samples being prepared, plus 10% overage. For individual preps, add components directly to each sample from the previous step in the order and volume listed below. **Reaction Mix 5 (RM5):**
	7.2	Pipette-mix **RM5**.
	7.3	Quick-spin **RM5** in a microcentrifuge to collect liquid.
	7.4	Add 10 µL of **RM5** to each sample. Total volume should equal 50 µL.
	7.5	Pipette-mix each sample.
	7.6	Quick-spin the strip tube in a microcentrifuge to collect liquid.
		Run the nuclease treatment program.

✓	Tube color	Component	Volume
	Light purple	Nuclease buffer 103-110-200	5 µL
	Light green	Nuclease mix 103-110-100	5 µL
		Total volume	10 µL

- **1-hr** nuclease treatment

Nuclease treatment program



Heated lid set at 47°C

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

- After Nuclease treatment step, perform final cleanup with 1.2X SMRTbell cleanup beads (Step 7.8)

Nuclease treatment and cleanup (cont.)

Procedural notes

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

5 Kinnex array formation

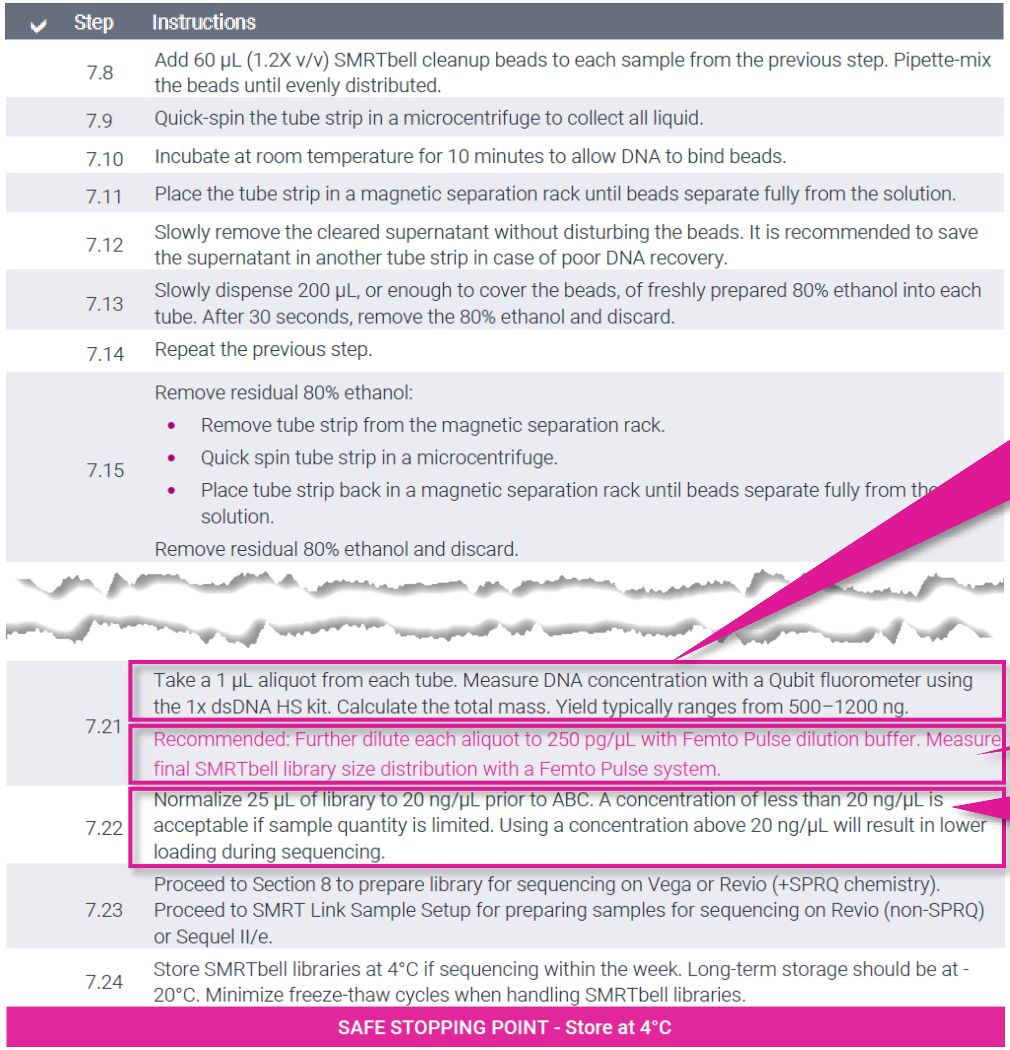
○ Safe stop

6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup

7.8 – 7.24 Final cleanup with 1.2X SMRTbell cleanup beads



✓	Step	Instructions
	7.8	Add 60 µL (1.2X v/v) SMRTbell cleanup beads to each sample from the previous step. Pipette-mix the beads until evenly distributed.
	7.9	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
	7.10	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
	7.11	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
	7.12	Slowly remove the cleared supernatant without disturbing the beads. It is recommended to save the supernatant in another tube strip in case of poor DNA recovery.
	7.13	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
	7.14	Repeat the previous step.
	7.15	Remove residual 80% ethanol: • Remove tube strip from the magnetic separation rack. • Quick spin tube strip in a microcentrifuge. • Place tube strip back in a magnetic separation rack until beads separate fully from the solution. Remove residual 80% ethanol and discard.
	7.21	Take a 1 µL aliquot from each tube. Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Calculate the total mass. Yield typically ranges from 500–1200 ng. Recommended: Further dilute each aliquot to 250 pg/µL with Femto Pulse dilution buffer. Measure final SMRTbell library size distribution with a Femto Pulse system.
	7.22	Normalize 25 µL of library to 20 ng/µL prior to ABC. A concentration of less than 20 ng/µL is acceptable if sample quantity is limited. Using a concentration above 20 ng/µL will result in lower loading during sequencing.
	7.23	Proceed to Section 8 to prepare library for sequencing on Vega or Revio (+SPRQ chemistry). Proceed to SMRT Link Sample Setup for preparing samples for sequencing on Revio (non-SPRQ) or Sequel II/e.
	7.24	Store SMRTbell libraries at 4°C if sequencing within the week. Long-term storage should be at -20°C. Minimize freeze-thaw cycles when handling SMRTbell libraries.
		SAFE STOPPING POINT - Store at 4°C

- 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 the nuclease treatment step

- Perform **DNA sizing QC** on final purified Kinnex single-cell RNA library using a Femto Pulse system (expected final library insert size is **~10 – 15 kb**)

- Normalize 25 µL of library to 20 ng/µL prior to ABC[1]. A concentration of less than 20 ng/µL is acceptable if sample quantity is limited.
  → Using a concentration above 20 ng/µL will result in lower loading during sequencing

---

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

Nucleace treatment and cleanup (cont.)

Example Femto Pulse DNA sizing QC results for Kinnex single-cell RNA library before nuclease treatment and after nuclease treatment & final cleanup

1 Input cDNA quality control

2 TSO PCR

○ Safe stop

3 TSO artifact removal

○ Safe stop

4 Kinnex PCR

○ Safe stop

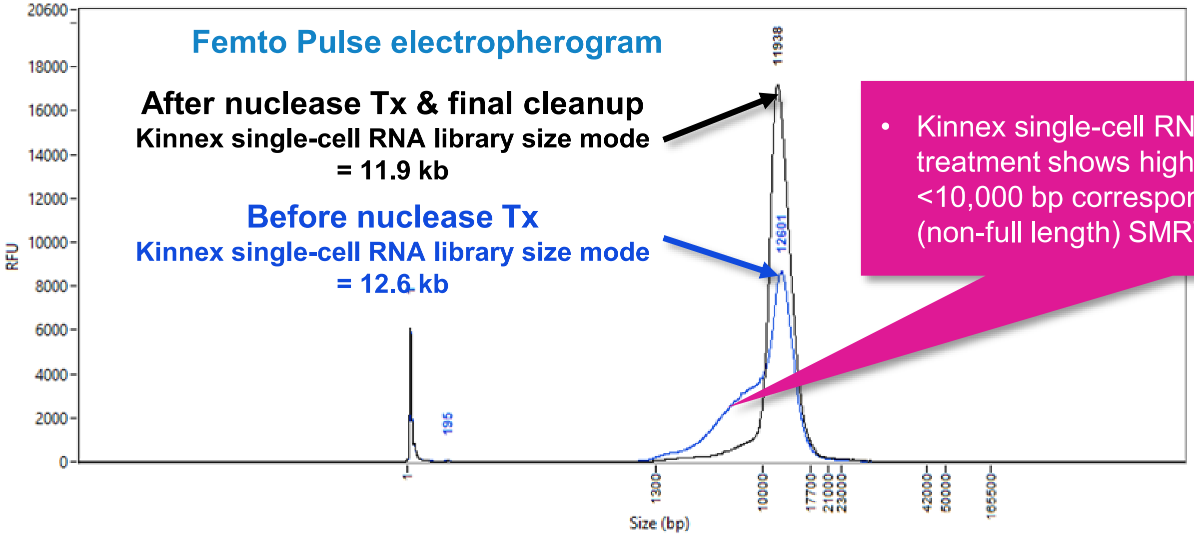
5 Kinnex array formation

○ Safe stop

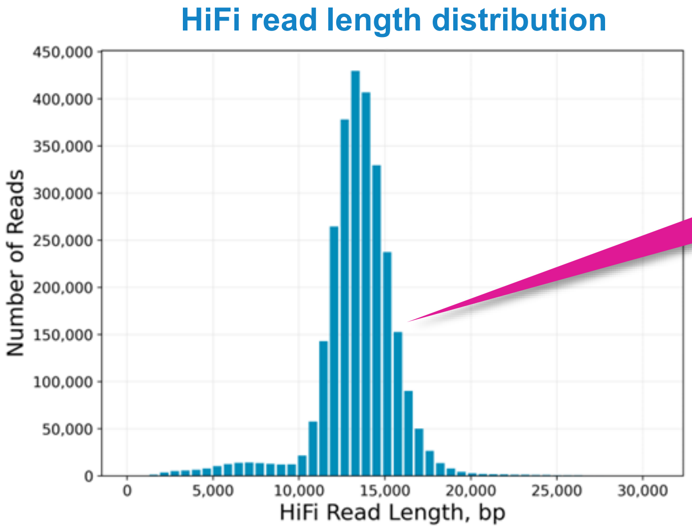
6 DNA damage repair

○ Safe stop

7 Nuclease Tx and cleanup



- Kinnex single-cell RNA library before nuclease treatment shows high amounts of smaller fragments <10,000 bp corresponding mostly to **incomplete** (non-full length) SMRTbell template arrays



- HiFi read length mode is consistent with expected final library insert size

Kinnex single-cell RNA library prep inputs & expected step yields

Final Kinnex library yield is typically sufficient to load ≥2 SMRT Cells

Kinnex single-cell

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

Library insert generation

TSO PCR — **IN:** 15 ng, 60-75 ng or 75 ng of 10x 3' or 5' cDNA

SMRTbell bead cleanup — **OUT: ~200 ng – 1 µg of amplified products**

TSO removal — **IN:** 150 ng – 1 µg of TSO PCR products

SMRTbell bead cleanup — **OUT: ≥50 ng of TSO artifact-depleted cDNA**

Kinnex PCR

Kinnex PCR — **Min. IN:** 50 ng* of TSO artifact-depleted cDNA

SMRTbell bead cleanup — **OUT: ≥10 µg of amplified products after pooling**

Kinnex array formation

Kinnex digestion — **IN:** 5 µg of pooled Kinnex PCR products

Ligation

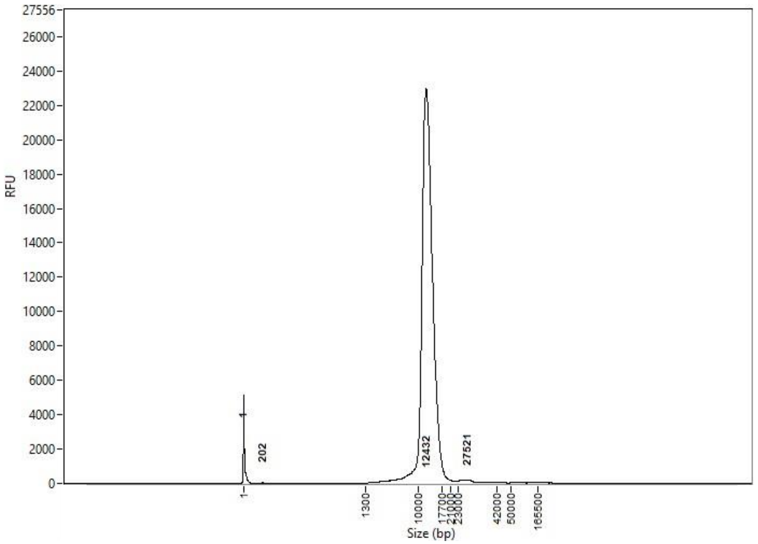
SMRTbell cleanup

DNA damage repair

SMRTbell cleanup — **OUT: 80% recovery from Kinnex digestion Rx input**

Nuclease treatment — **IN:** 40 µL of DDR reaction products

SMRTbell bead cleanup — **OUT: 10-25% recovery from Kinnex digestion Rx input**



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

Example Kinnex single-cell RNA library prep yields

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

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

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

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

Kinnex single-cell RNA sequencing preparation workflow details

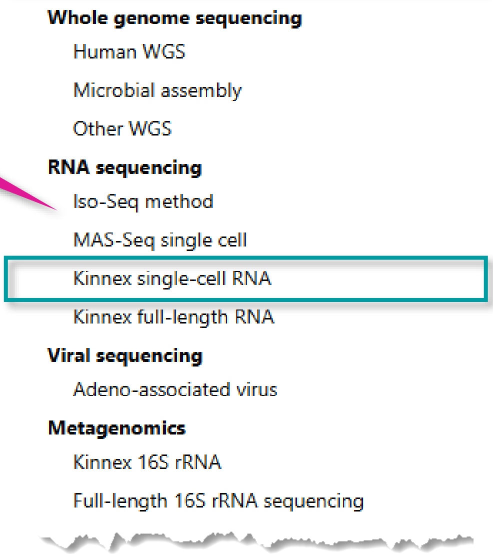
Sample Setup & Run Design recommendations for Kinnex libraries

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

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

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

Kinnex single-cell RNA[1]



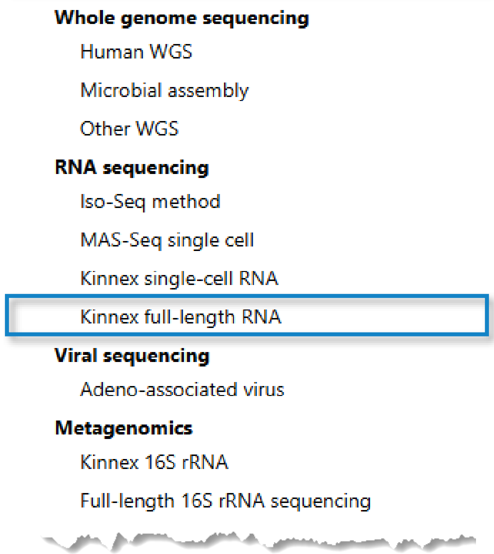
Whole genome sequencing
- Human WGS
- Microbial assembly
- Other WGS

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

Viral sequencing
- Adeno-associated virus

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

Kinnex full-length RNA[2]



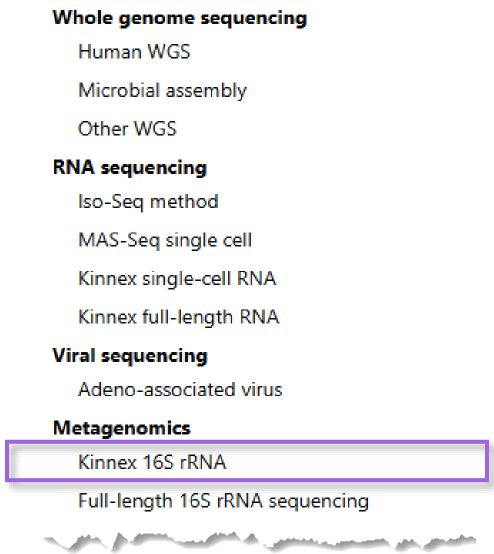
Whole genome sequencing
- Human WGS
- Microbial assembly
- Other WGS

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

Viral sequencing
- Adeno-associated virus

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

Kinnex 16S rRNA[2]



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

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

Viral sequencing
- Adeno-associated virus

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

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

---

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

SMRT Link Run Design procedure for Revio and Vega systems

Sample and run information

Kinnex single-cell RNA

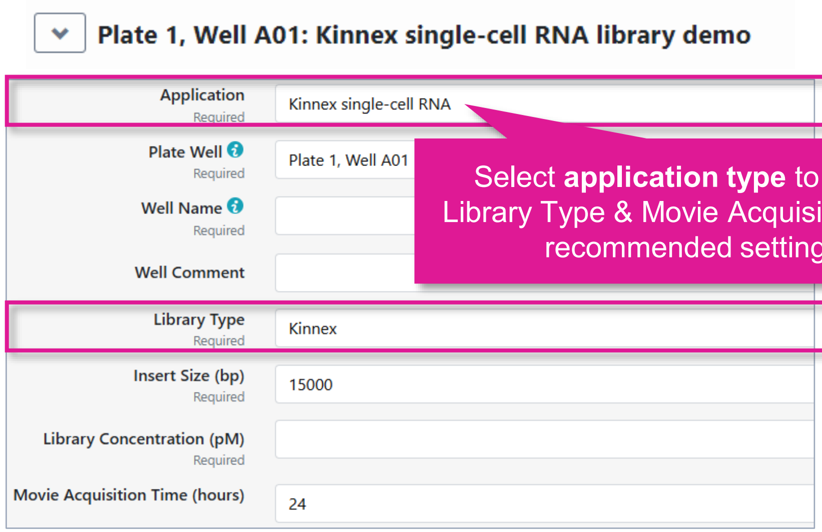


Plate 1, Well A01: Kinnex single-cell RNA library demo

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

Kinnex full-length RNA

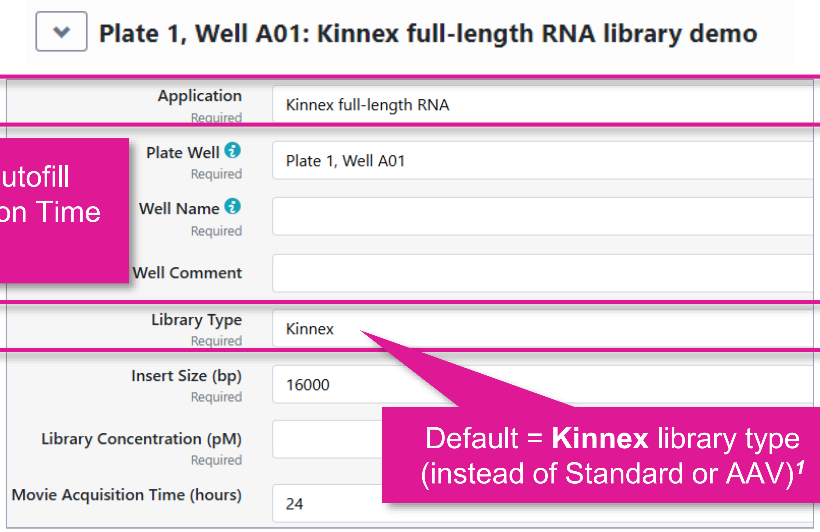


Plate 1, Well A01: Kinnex full-length RNA library demo

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

Kinnex 16S rRNA

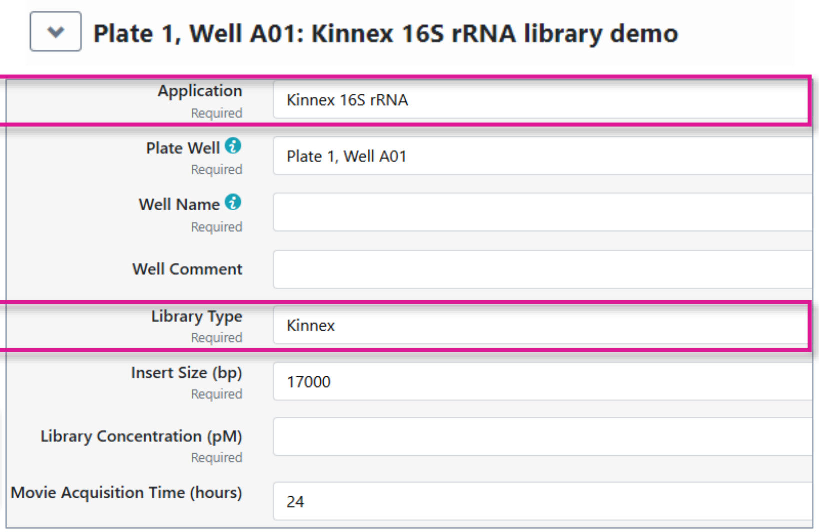


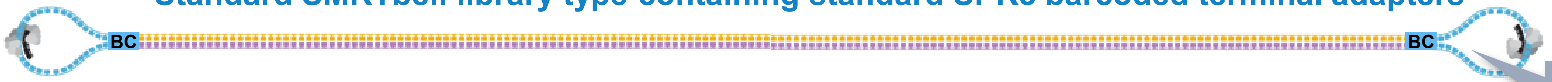
Plate 1, Well A01: Kinnex 16S rRNA library demo

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

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

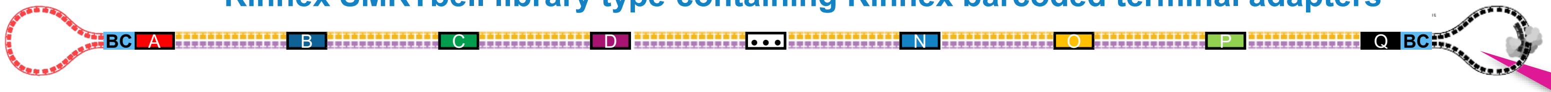
Default = **Kinnex** library type (instead of Standard or AAV)[1]

Standard SMRTbell library type containing standard SPK3 barcoded terminal adapters



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

Kinnex SMRTbell library type containing Kinnex barcoded terminal adapters



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

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

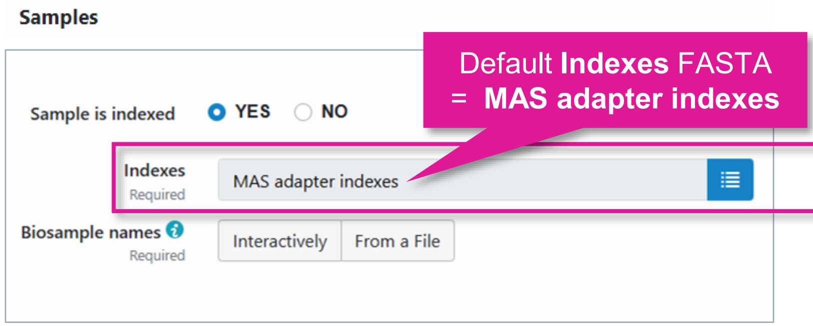
---

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

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

Sample indexing (barcoding) information

Kinnex single-cell RNA



Samples

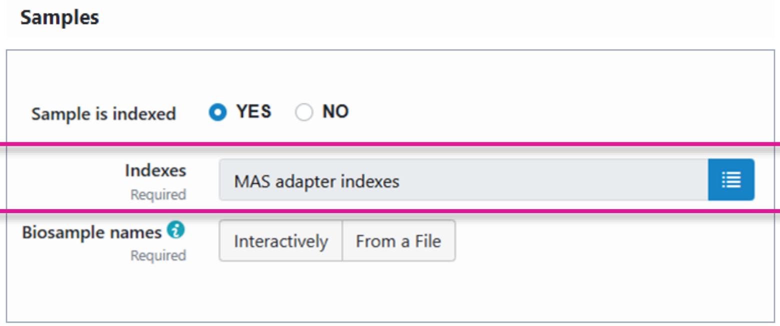
Sample is indexed ● YES ○ NO

Indexes (Required): MAS adapter indexes

Biosample names (Required): Interactively | From a File

Default Indexes FASTA = **MAS adapter indexes**

Kinnex full-length RNA



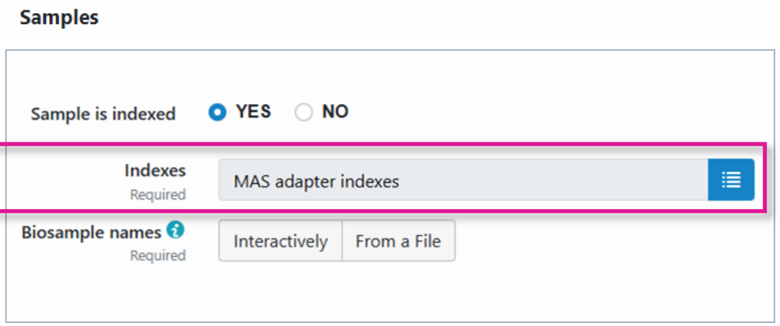
Samples

Sample is indexed ● YES ○ NO

Indexes (Required): MAS adapter indexes

Biosample names (Required): Interactively | From a File

Kinnex 16S rRNA



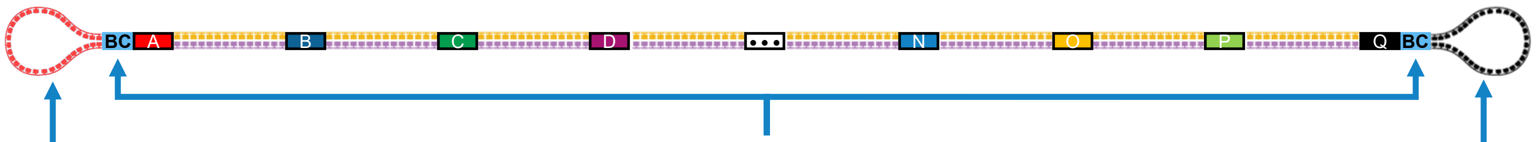
Samples

Sample is indexed ● YES ○ NO

Indexes (Required): MAS adapter indexes

Biosample names (Required): Interactively | From a File

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

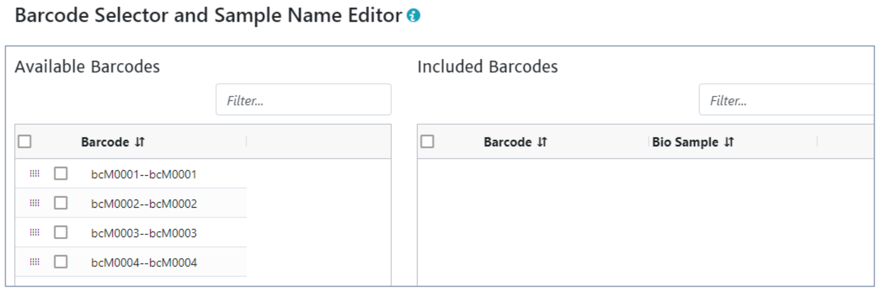


Barcoded forward Kinnex terminal adapter

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

Barcoded reverse Kinnex terminal adapter

Example interactive biosample name specification



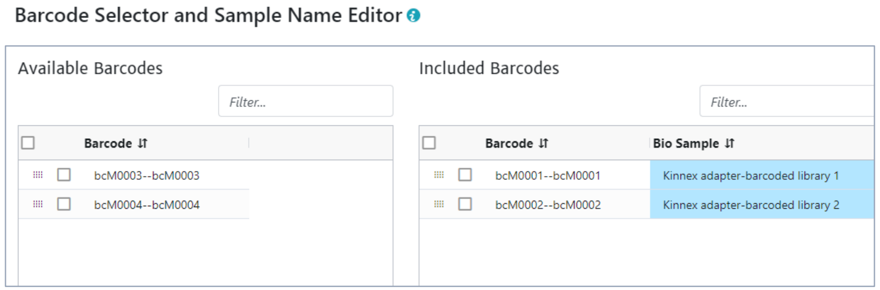
Barcode Selector and Sample Name Editor

Available Barcodes

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

Included Barcodes

☐	Barcode	Bio Sample



Barcode Selector and Sample Name Editor

Available Barcodes

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

Included Barcodes

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

SMRT Link

MAS SMRTbell barcoded adapter indexes

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

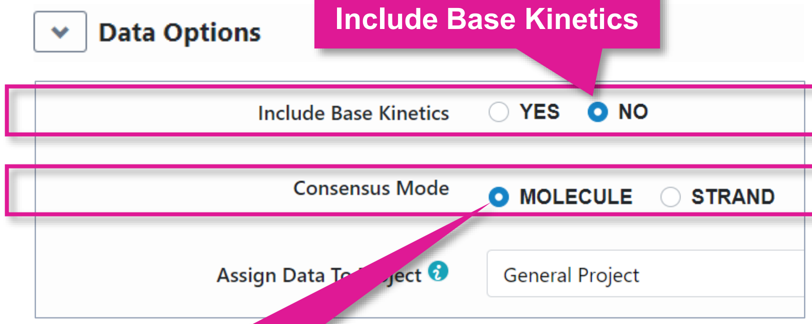
---

[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



Data Options

Include Base Kinetics ○ YES ● NO

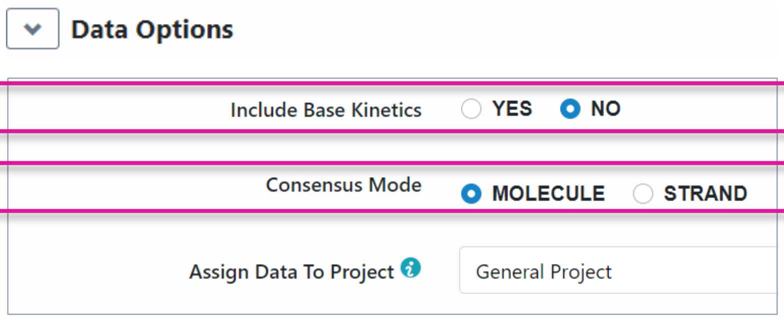
Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

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

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

Kinnex full-length RNA



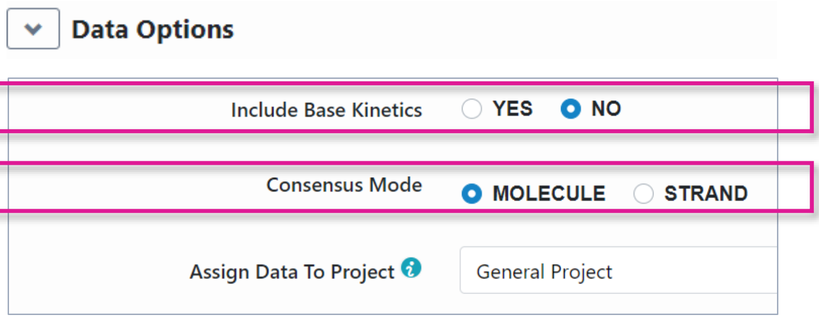
Data Options

Include Base Kinetics ○ YES ● NO

Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

Kinnex 16S rRNA



Data Options

Include Base Kinetics ○ YES ● NO

Consensus Mode ● MOLECULE ○ STRAND

Assign Data To Project: General Project

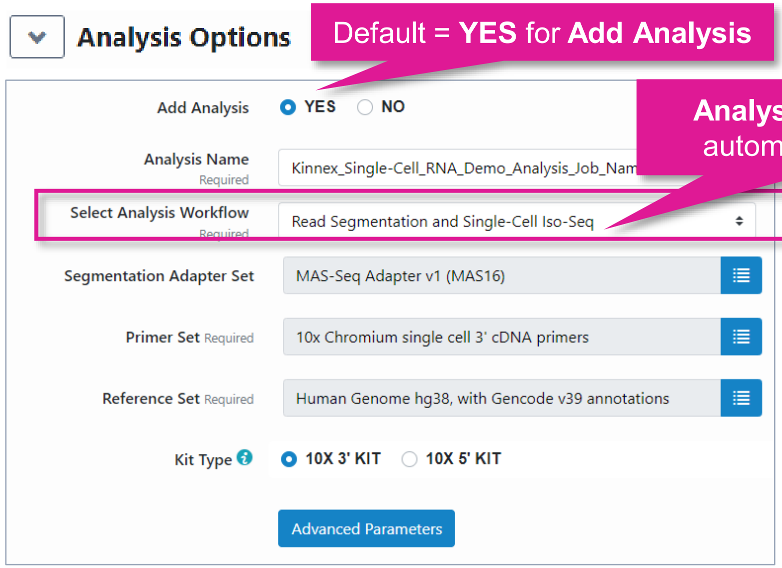
Can leave Include Base Kinetics and Consensus Mode fields at their default settings for Kinnex library samples

---

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

SMRT Link Run Design analysis options

Kinnex single-cell RNA



Analysis Options

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

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Nam

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

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

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

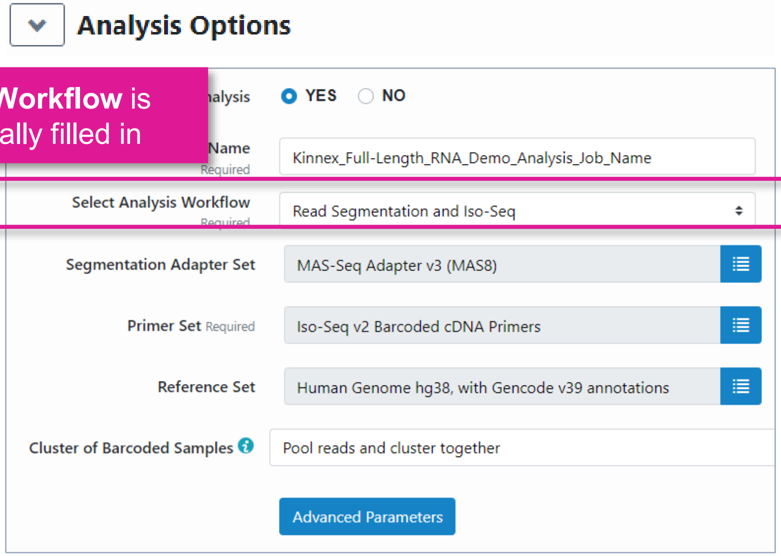
Reference Set Required: Human Genome hg38, with Gencode v39 annotations

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

Advanced Parameters

Analysis Workflow is automatically filled in

Kinnex full-length RNA



Analysis Options

alysis ● YES ○ NO

Name Required: Kinnex_Full-Length_RNA_Demo_Analysis_Job_Name

Select Analysis Workflow Required: Read Segmentation and Iso-Seq

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

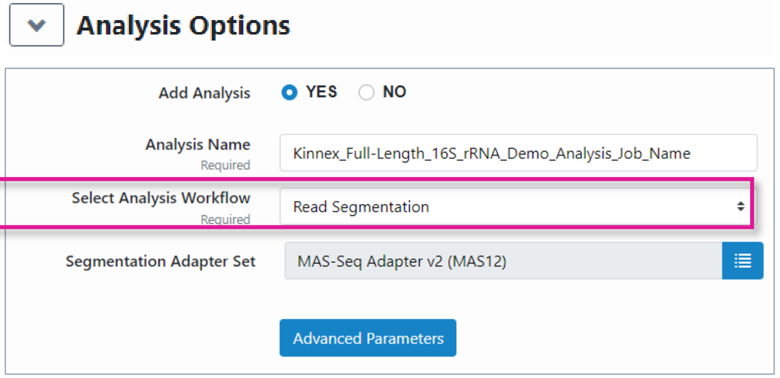
Primer Set Required: Iso-Seq v2 Barcoded cDNA Primers

Reference Set: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

Advanced Parameters

Kinnex 16S rRNA



Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Full-Length_16S_rRNA_Demo_Analysis_Job_Name

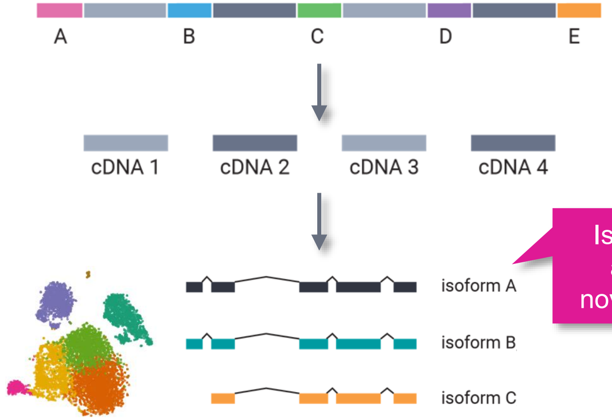
Select Analysis Workflow Required: Read Segmentation

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

Advanced Parameters

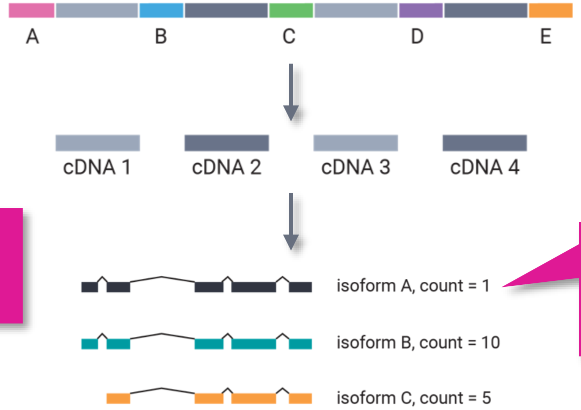
Split arrayed HiFi reads at adapter positions, generating segmented reads (S-reads)

Read Segmentation and Single-Cell Iso-Seq



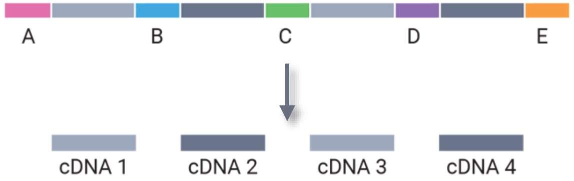
Isoform-classification analysis to identify novel genes & isoforms

Read Segmentation and Iso-Seq



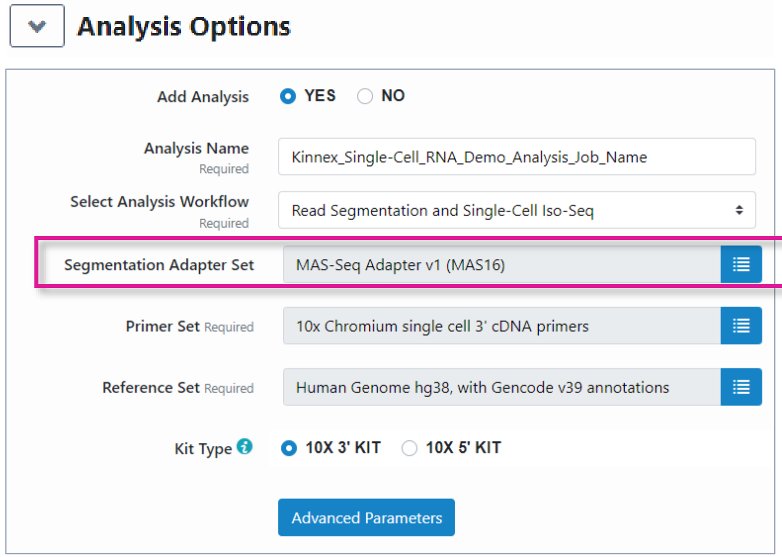
Isoform-classification analysis to identify novel genes & isoforms with abundance information (raw counts & normalized counts per million)

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

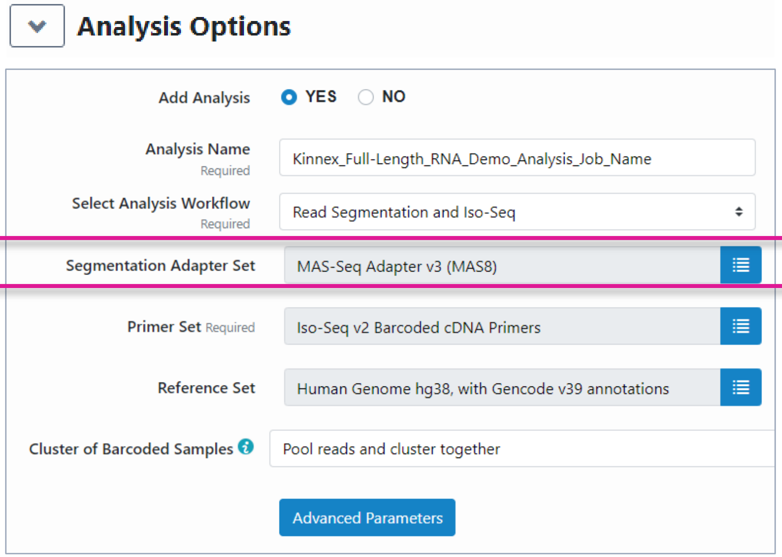
Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

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

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

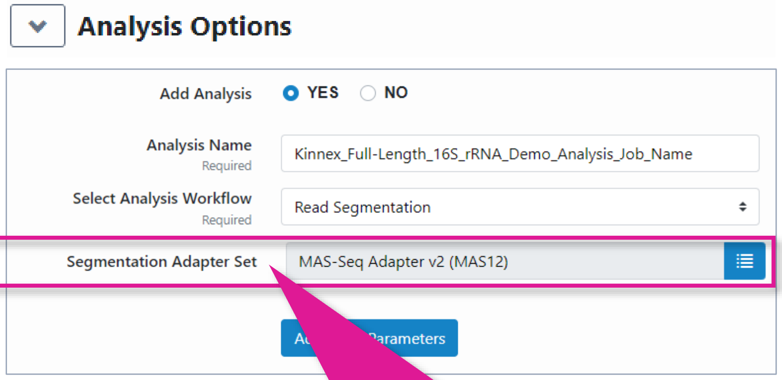
Segmentation Adapter Set: MAS-Seq Adapter v3 (MAS8)

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

Kinnex 16S rRNA



Analysis Options

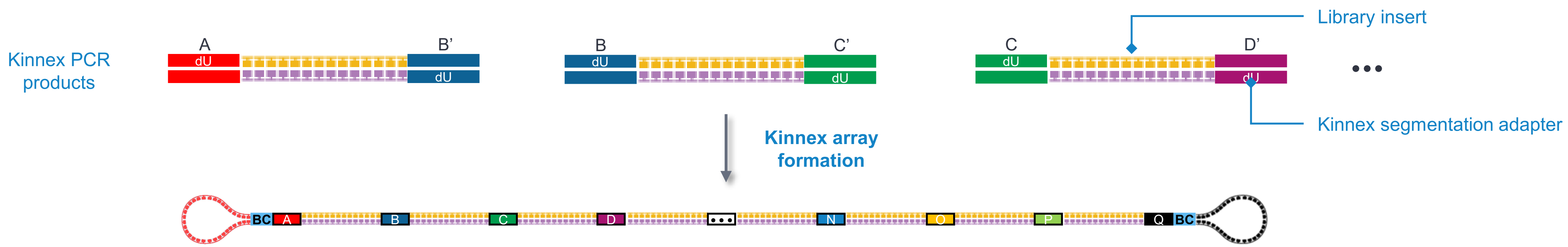
Add Analysis ● YES ○ NO

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)

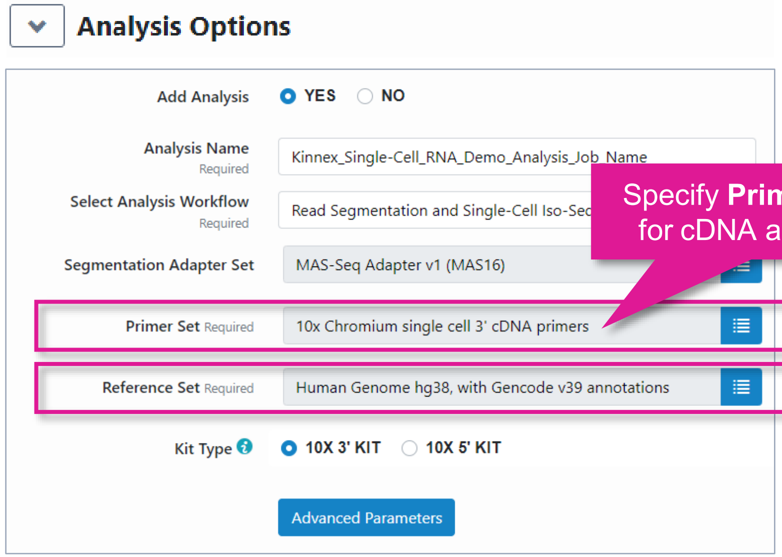
Specify **Segmentation Adapter Set** that corresponds to the Kinnex library concatenation method used



Complete array molecule containing concatenated library insert segments

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

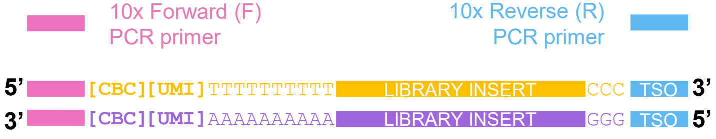
Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

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

Specify **Primer Set** used for cDNA amplification

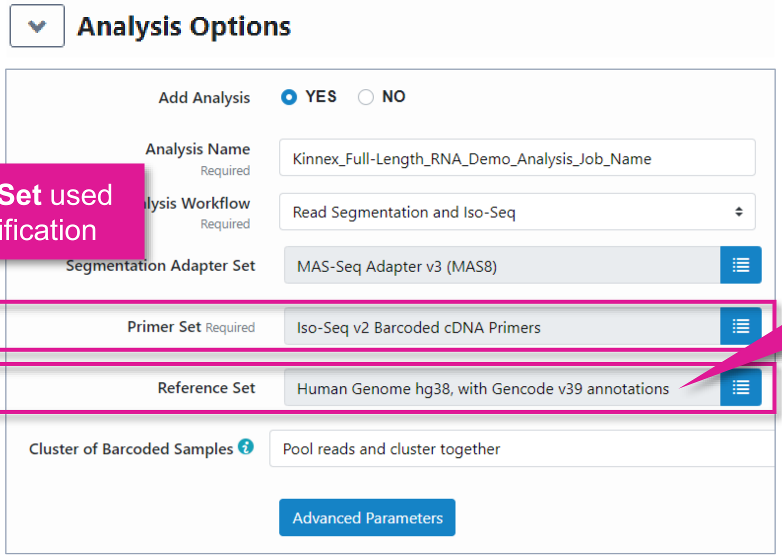


For Kinnex single-cell 3' RNA analysis, select '10x Chromium single cell 3' cDNA primers'

OR

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

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

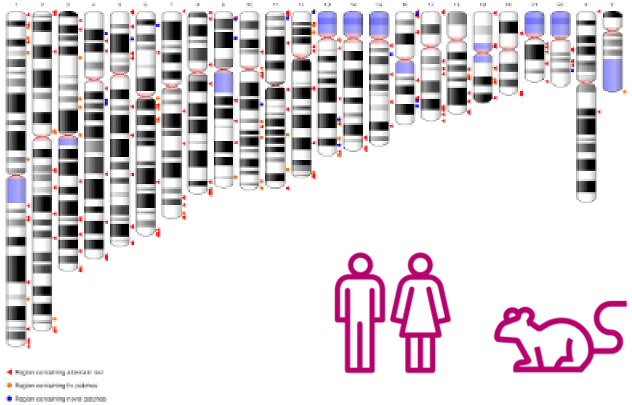
For Kinnex full-length RNA analysis, select 'Iso-Seq v2 Barcoded cDNA primers'

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

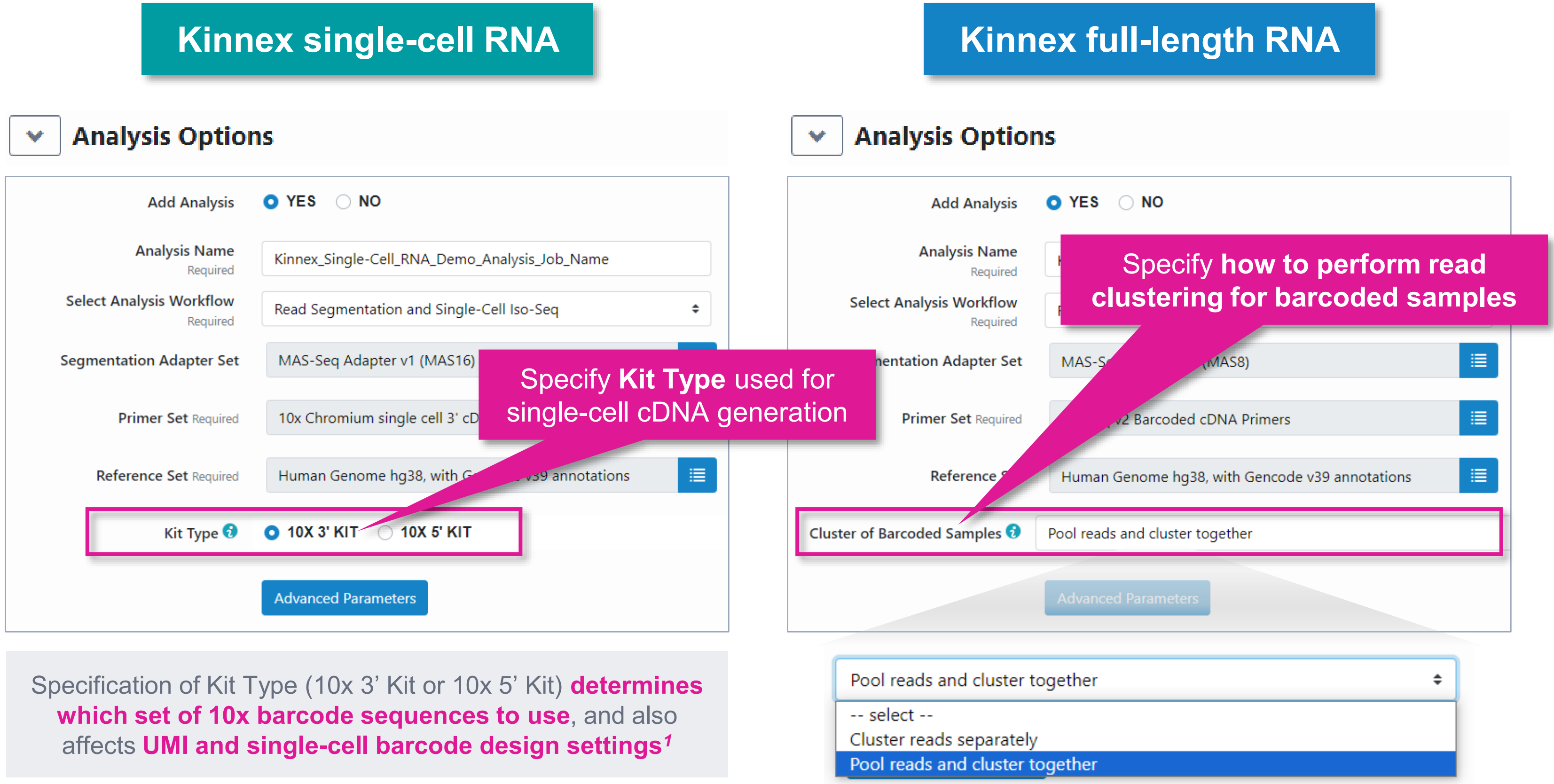
Specify **Reference** Set. Default sets are:

- 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

Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required: Kinnex_Single-Cell_RNA_Demo_Analysis_Job_Name

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

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

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

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

Kit Type ● 10X 3' KIT ○ 10X 5' KIT

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

Kinnex full-length RNA

Analysis Options

Add Analysis ● YES ○ NO

Analysis Name Required

Select Analysis Workflow Required

entation Adapter Set: MAS-S (MAS8)

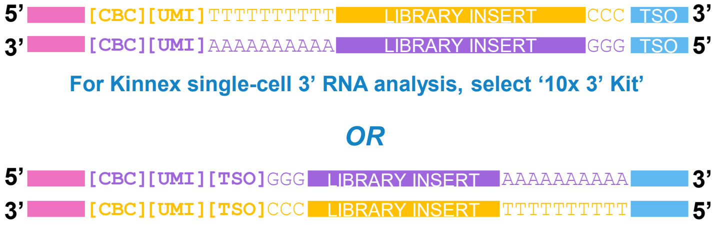
Primer Set Required: v2 Barcoded cDNA Primers

Reference: Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples: Pool reads and cluster together

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

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]



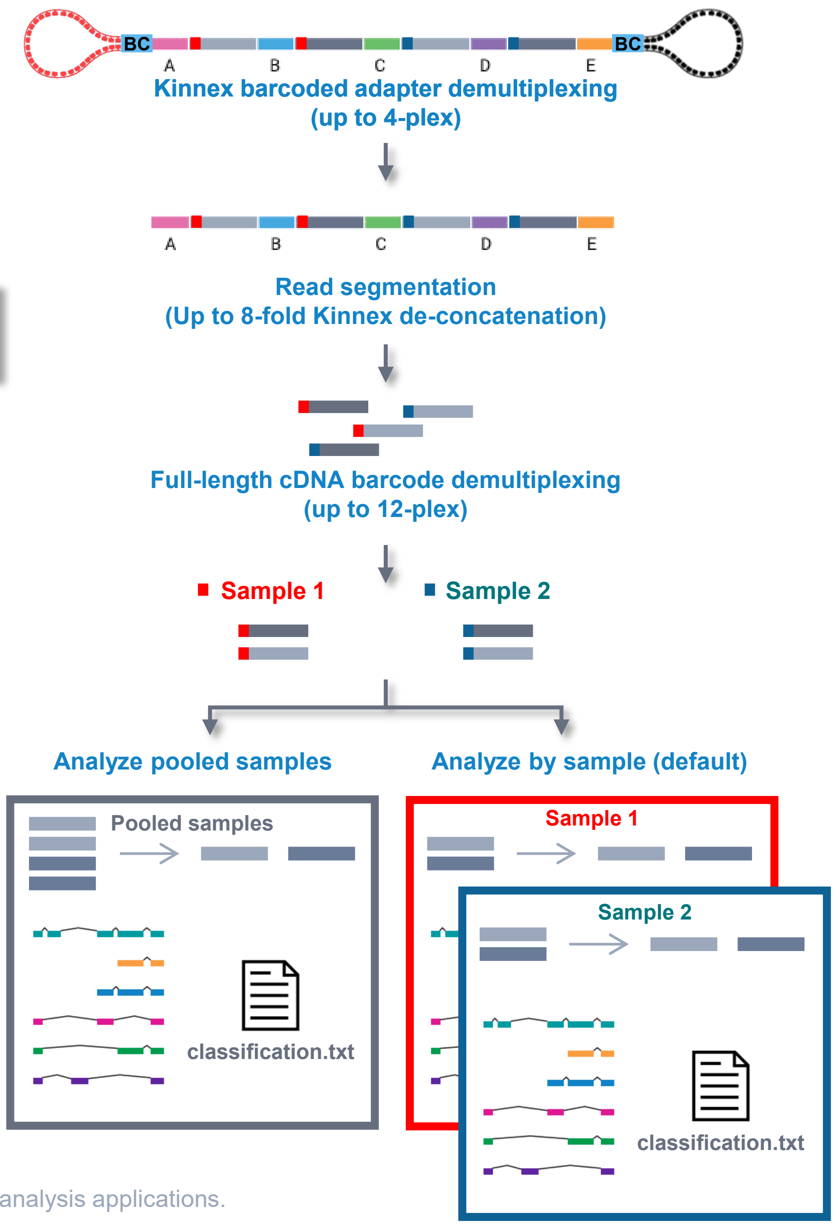
For Kinnex single-cell 3' RNA analysis, select '10x 3' Kit'

OR

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

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



Kinnex barcoded adapter demultiplexing (up to 4-plex)

Read segmentation (Up to 8-fold Kinnex de-concatenation)

Full-length cDNA barcode demultiplexing (up to 12-plex)

Analyze pooled samples

Analyze by sample (default)

---

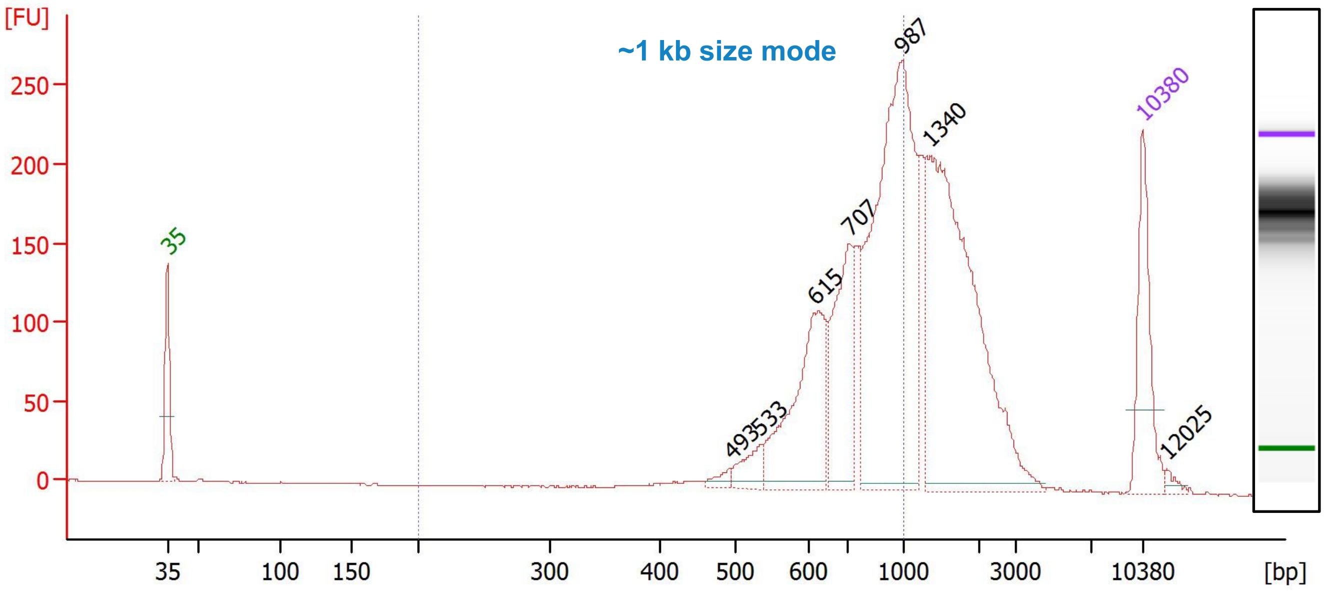
[1] See ***SMRT Link User Guide*** documentation for detailed descriptions of advanced parameters for Iso-Seq / Single-Cell Iso-Seq analysis applications.

Kinnex single-cell RNA example sequencing performance data

Example Kinnex single-cell RNA library preparation QC results

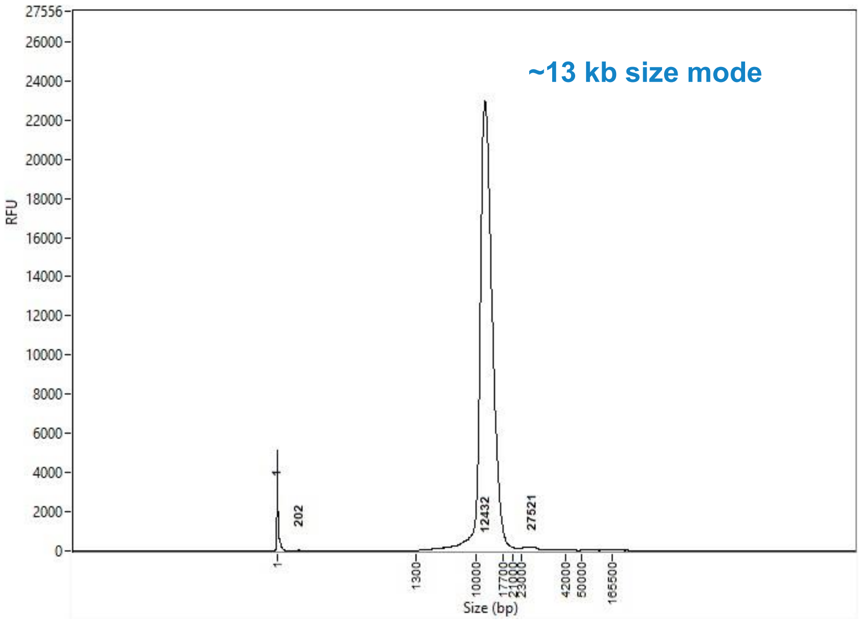
Kinnex single-cell 3’ RNA library prepared with human cDNA

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



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

Final Kinnex single-cell RNA library QC



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

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

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

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

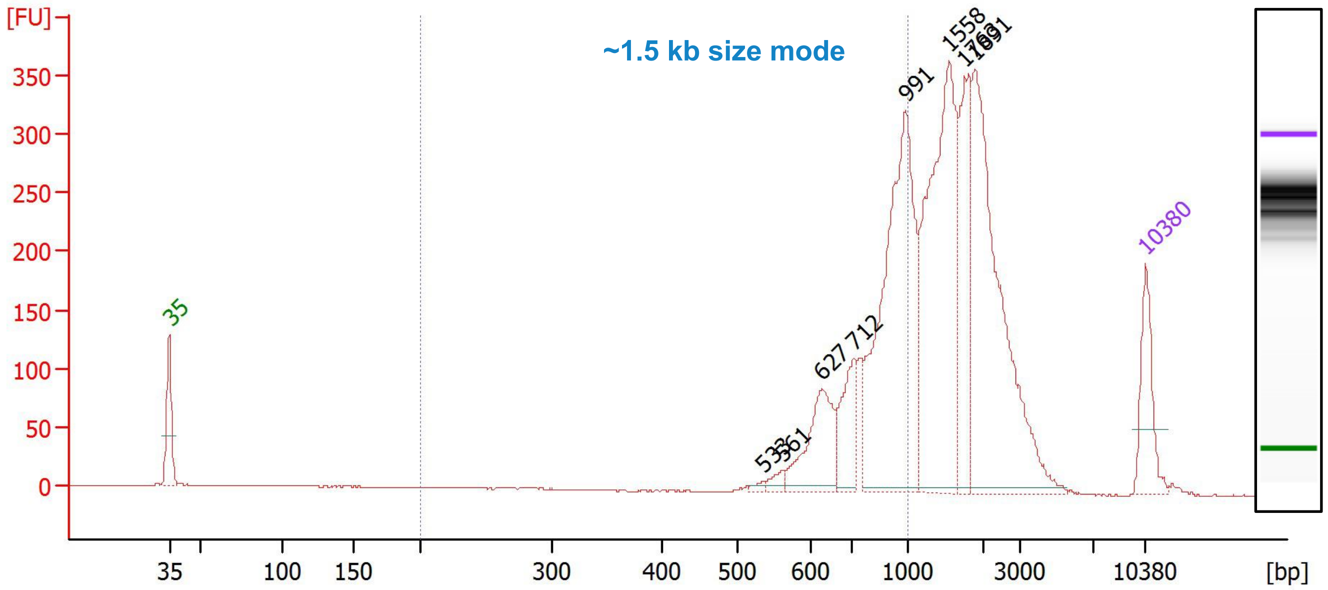
[1] Post-nuclease treatment & final cleanup yields typically ranged from ~10% to ~25% when using single-cell 3’ cDNA samples for Kinnex single-cell RNA library construction.

PacBio

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

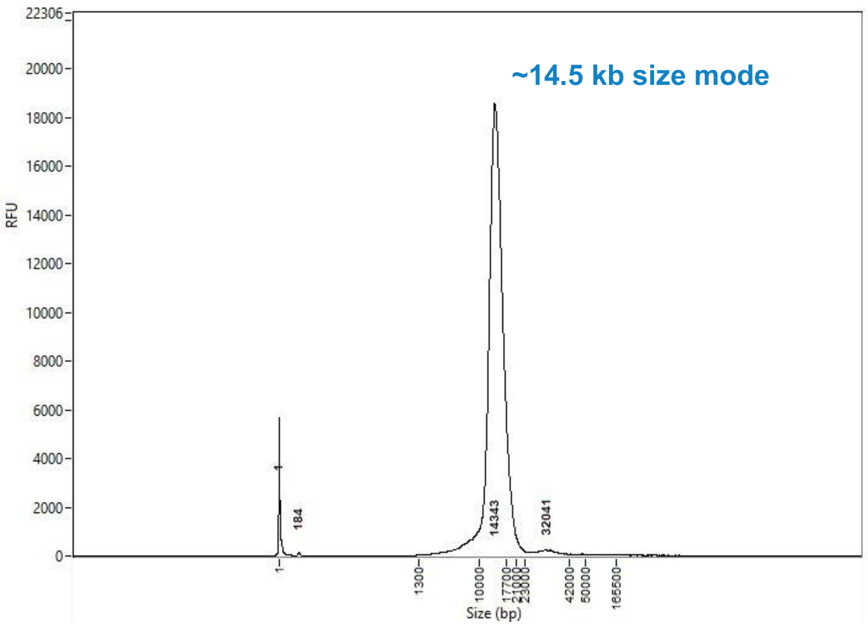
Kinnex single-cell 5’ RNA library prepared with human cDNA

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



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

Final Kinnex single-cell RNA library QC



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

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

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

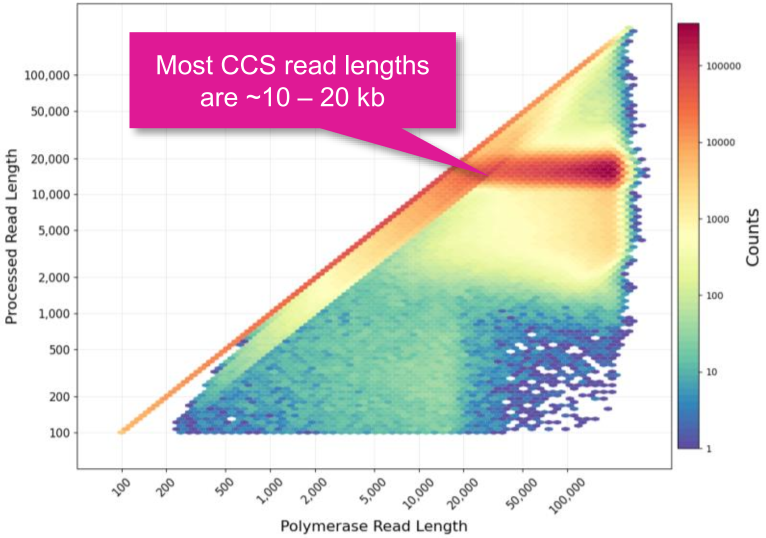
10x single cell 5’ cDNA input	15 ng
cDNA input for Kinnex array formation	5000 ng
Post-nuclease treatment & final library cleanup yield (%)[1]	1008 ng (20.2%)

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

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

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

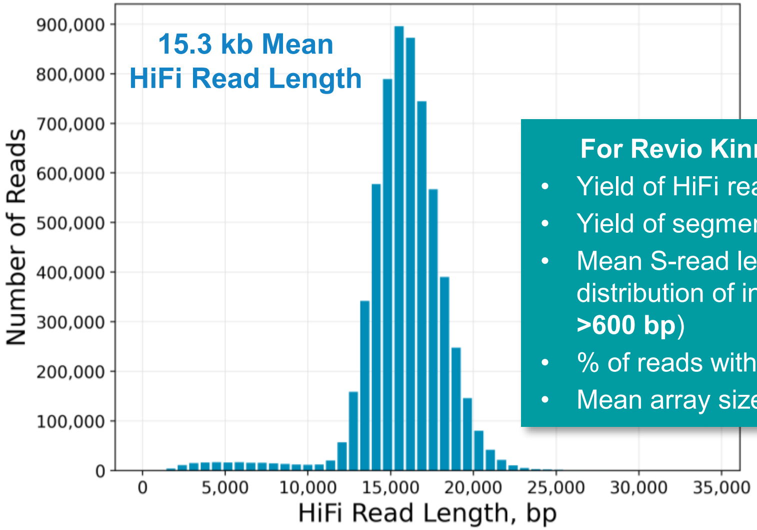
Raw Data Report



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

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

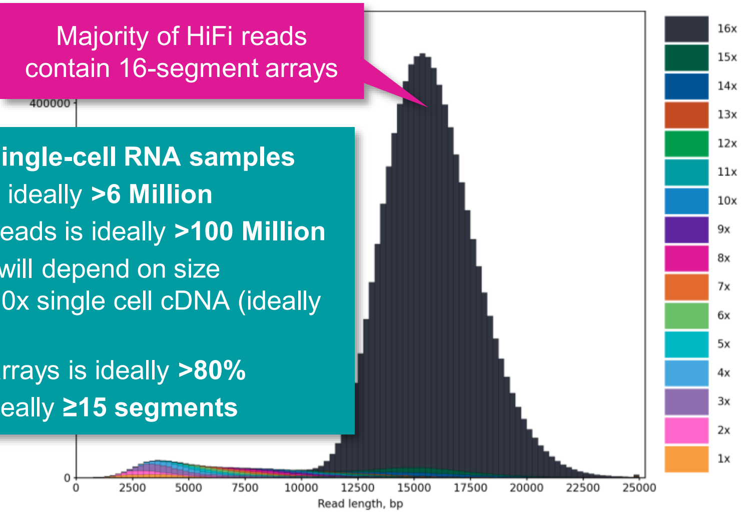
HiFi Read Length



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

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

Read Segmentation Metrics



For Revio Kinnex single-cell RNA samples

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

Input HiFi Reads	9,408,444
Segmented reads (S-reads)	144,801,149
Mean length of S-reads	971 bp
Percent of reads with full arrays	92.59%
Mean array size (concentration factor)	15.39

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

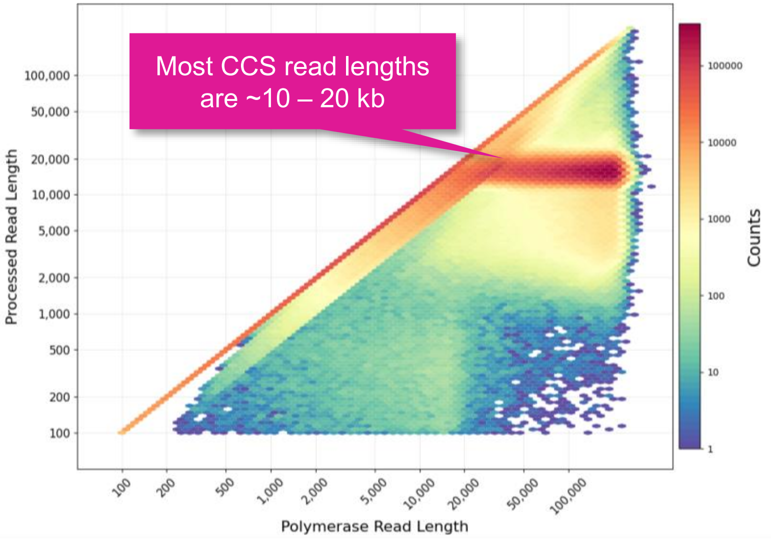
---

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

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

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

Raw Data Report

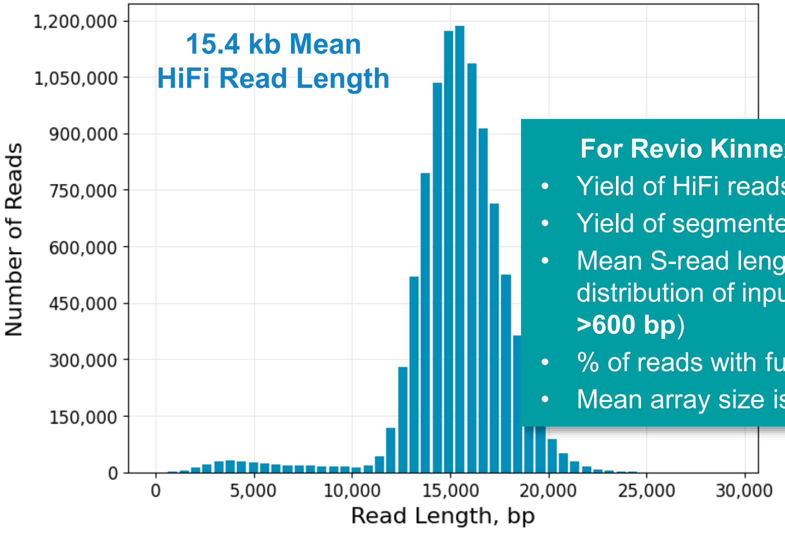


Most CCS read lengths are ~10 – 20 kb

Raw Base Yield	1,570 Gb
Mean Polymerase Read Length	86.5 kb
P0	27%
P1	72%
P2	0%

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

HiFi Read Length

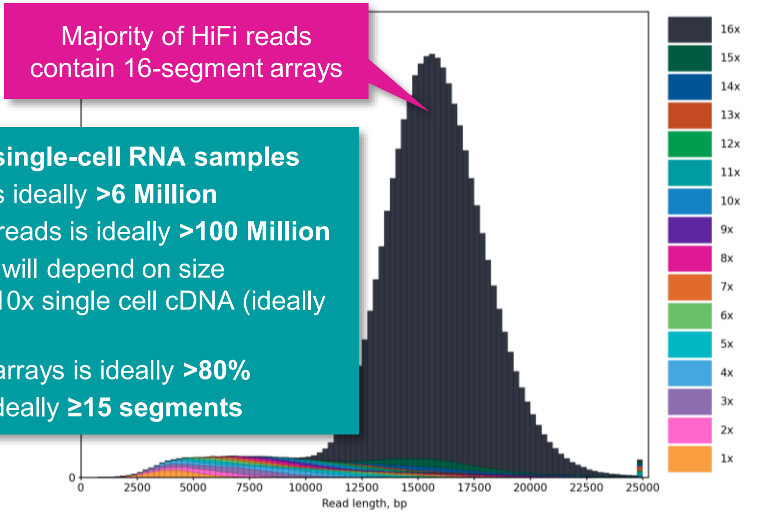


15.4 kb Mean HiFi Read Length

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

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

Read Segmentation Metrics



Majority of HiFi reads contain 16-segment arrays

For Revio Kinnex single-cell RNA samples

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

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

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

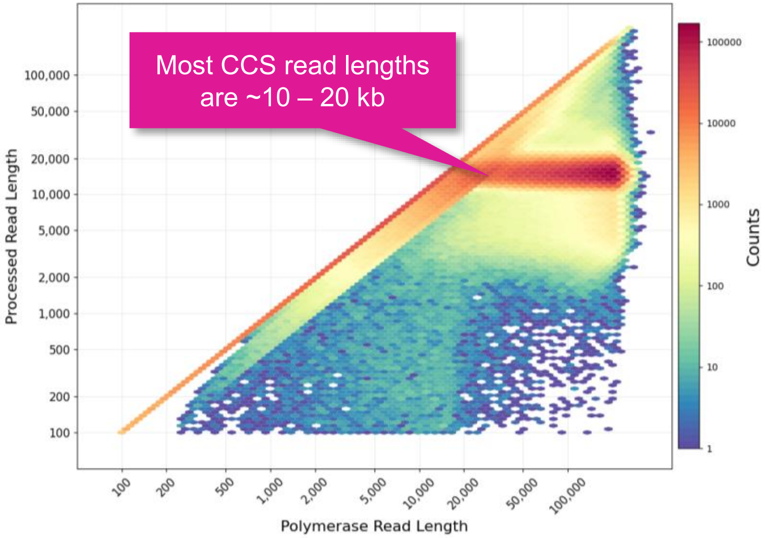
---

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

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

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

Raw Data Report

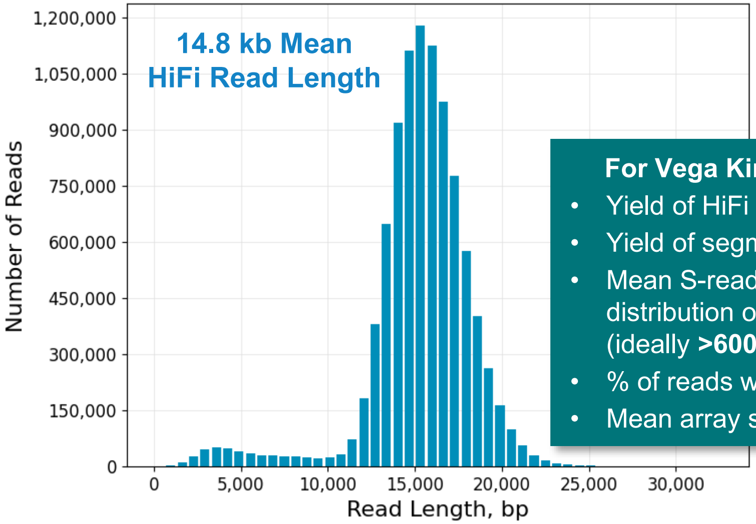


Most CCS read lengths are ~10 – 20 kb

Mean Polymerase Read Length	94.1 kb
Loading Level	71%

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

HiFi Read Length



14.8 kb Mean HiFi Read Length

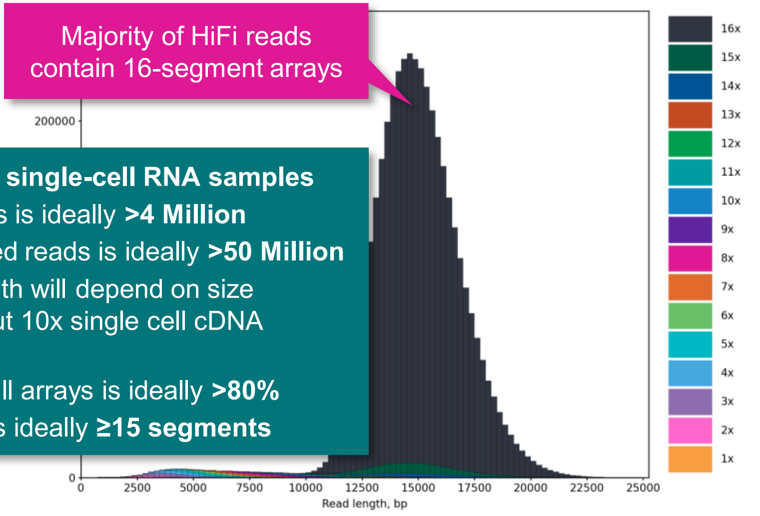
For Vega Kinnex single-cell RNA samples

- Yield of HiFi reads is ideally **>4 Million**
- Yield of segmented reads is ideally **>50 Million**
- Mean S-read length will depend on size distribution of input 10x single cell cDNA (ideally **>600 bp**)
- % of reads with full arrays is ideally **>80%**
- Mean array size is ideally **≥15 segments**

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

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

Read Segmentation Metrics



Majority of HiFi reads contain 16-segment arrays

Input HiFi Reads	4,372,029
Segmented reads (S-reads)	68,570,669
Mean length of S-reads	924 bp
Percent of reads with full arrays	93.78%
Mean array size (concentration factor)	15.68

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

---

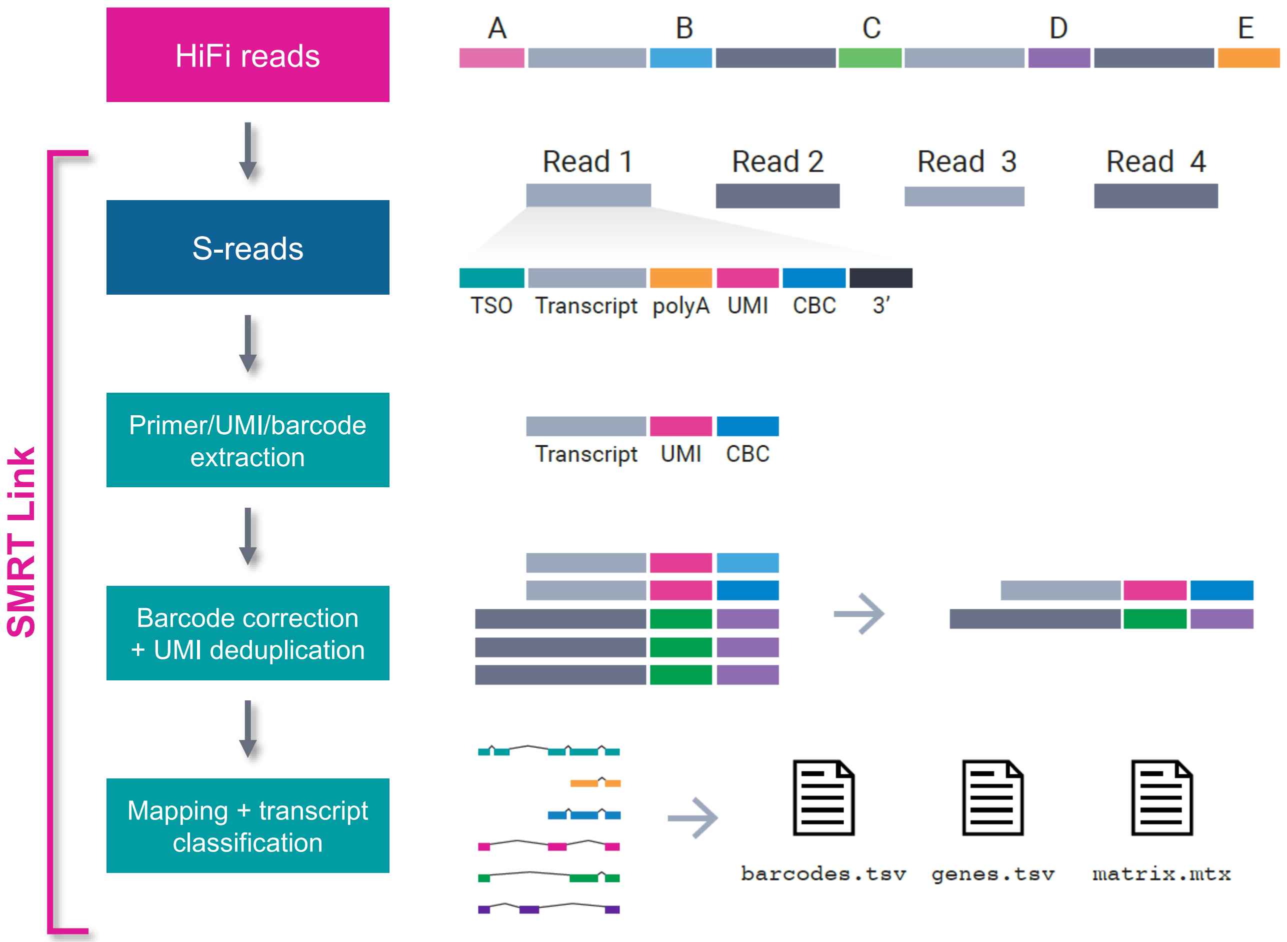
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

Kinnex single-cell RNA data analysis workflow overview

Kinnex single-cell RNA bioinformatics workflow overview

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

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



Read segmentation

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

Primer/UMI/barcode extraction

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

Barcode correction & UMI deduplication

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

Mapping and transcript classification[2]

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

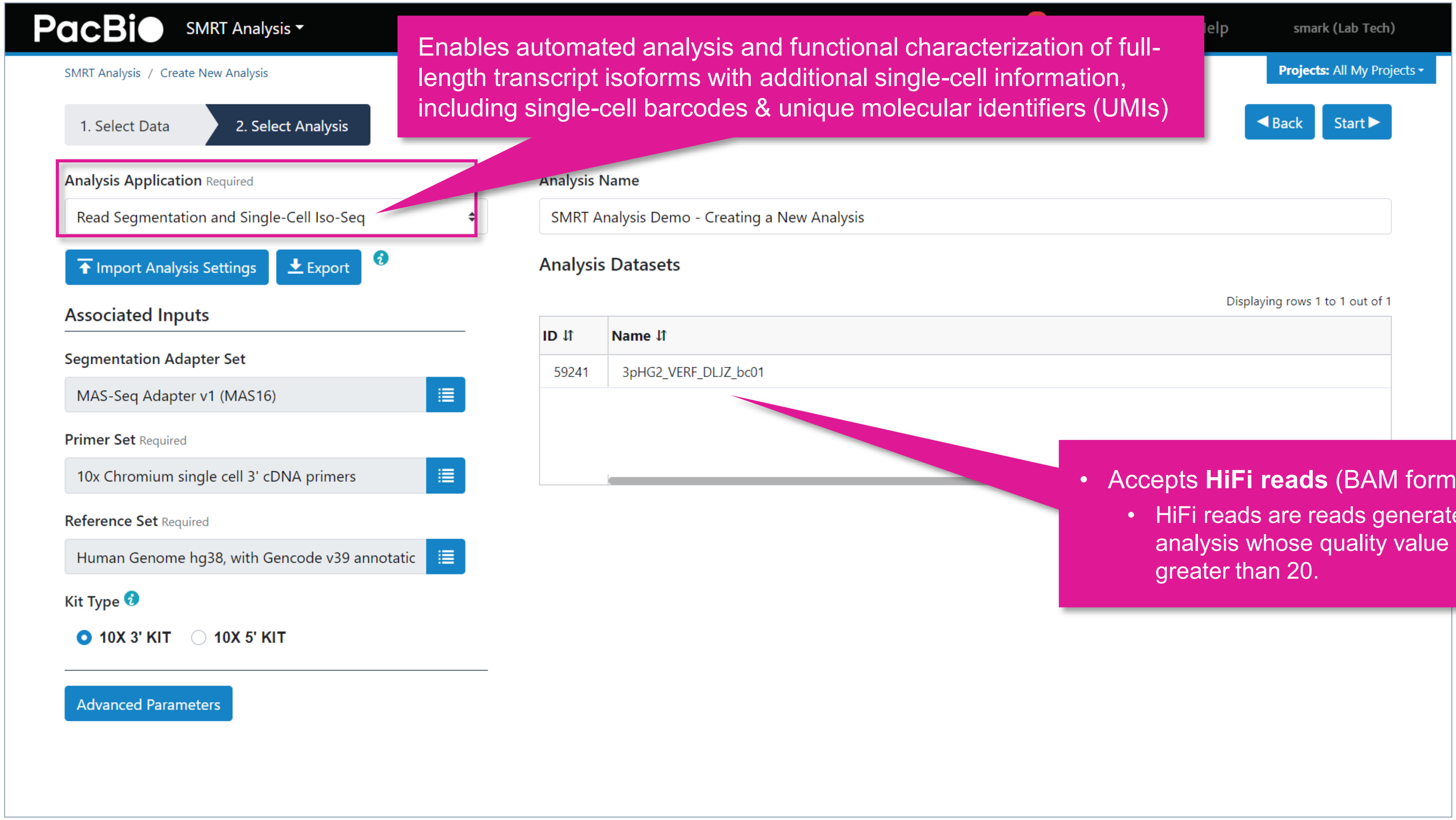
---

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

[2] Note: SMRT Link only supports transcript classification for human and mouse samples. Non-human/mouse samples will require customized annotation GTF files to be run *via* the command line.

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

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



Analysis Application Required: Read Segmentation and Single-Cell Iso-Seq

Analysis Name: SMRT Analysis Demo - Creating a New Analysis

Associated Inputs

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 annotatic

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

Analysis Datasets

Displaying rows 1 to 1 out of 1

ID	Name
59241	3pHG2_VERF_DLJZ_bc01

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

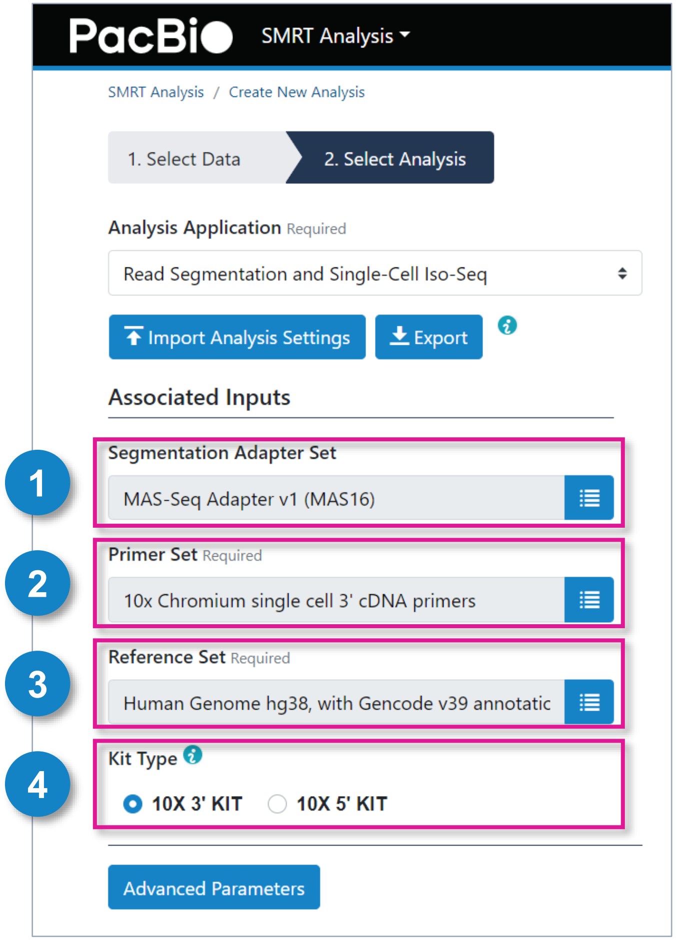
- Accepts **HiFi reads** (BAM format) as input
  - HiFi reads are reads generated with CCS analysis whose quality value is equal to or greater than 20.

---

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

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

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



Analysis Application 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 annotatic

Kit Type: ● 10X 3' KIT ○ 10X 5' KIT

1. Segmentation Adapter Set (Required)

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

2. Primer Set (Required)

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

3. Reference Set (Required)

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

4. Segmentation Adapter Set (Required)

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

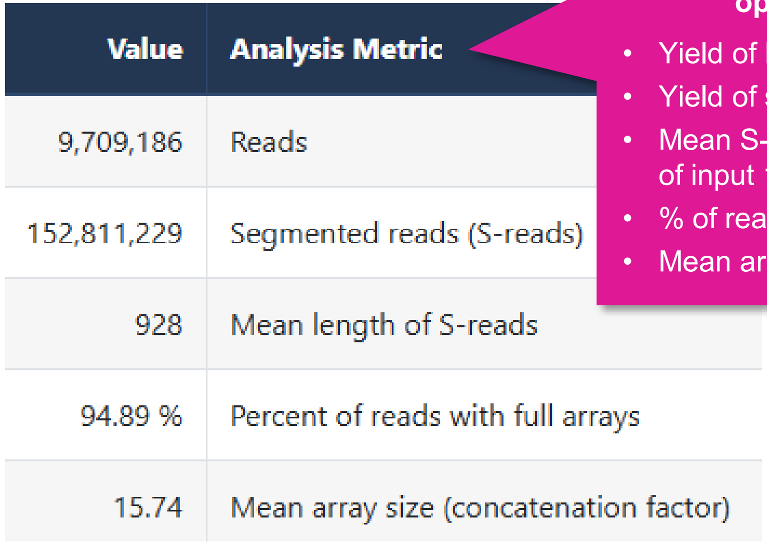
PacBio

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

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

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

Summary Metrics



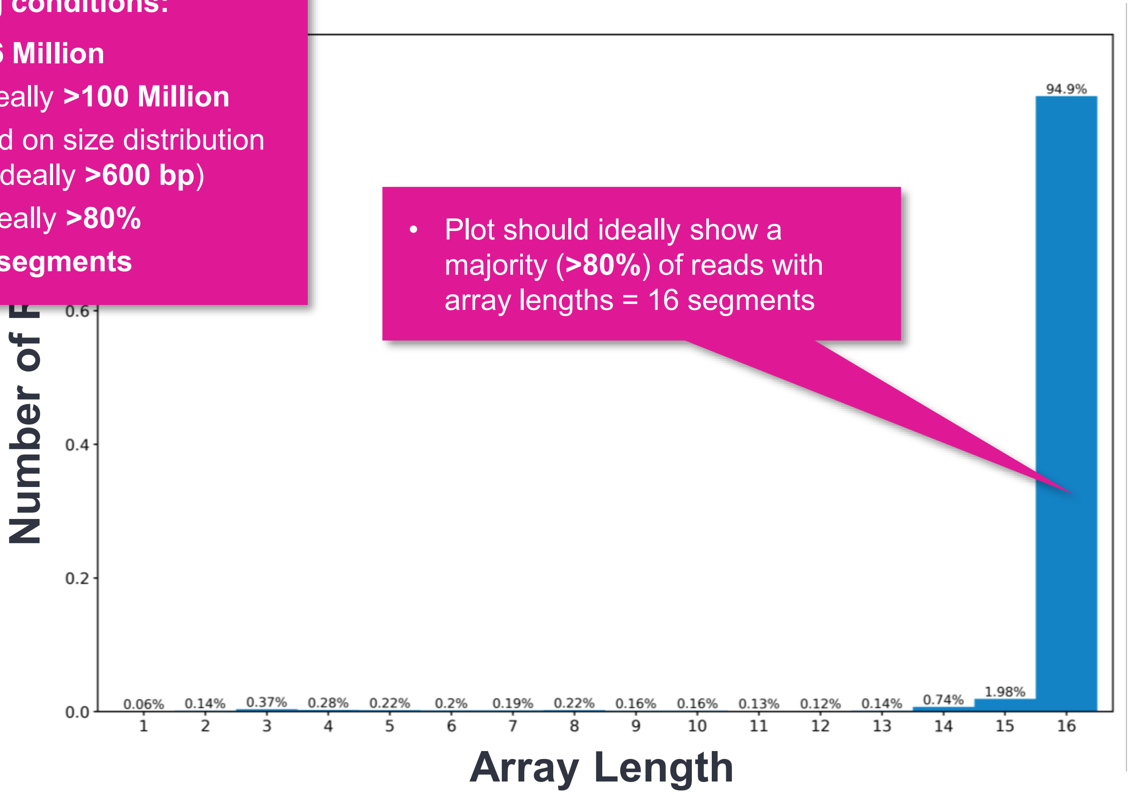
Value	Analysis Metric
9,709,186	Reads
152,811,229	Segmented reads (S-reads)
928	Mean length of S-reads
94.89 %	Percent of reads with full arrays
15.74	Mean array size (concatenation factor)

Example Revio system (+SPRQ) data shown.

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

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

Segmentation Statistics



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

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

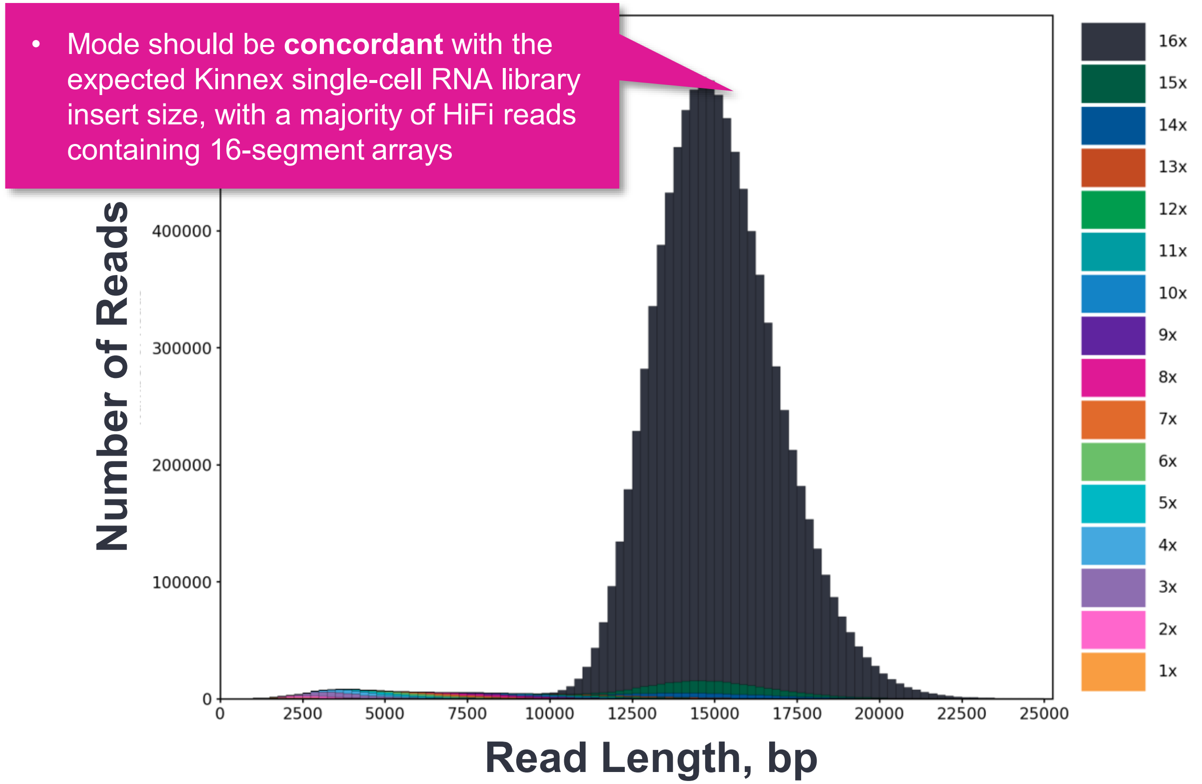
- **Reads:** Number of input arrayed HiFi reads
- **Segmented reads (S-reads):** Number of generated S-reads
- **Mean length of S-reads:** Mean read length of generated S-reads
- **Percent of reads with full arrays:** Percentage of input HiFi reads containing all adapter sequences in the order listed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

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

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

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

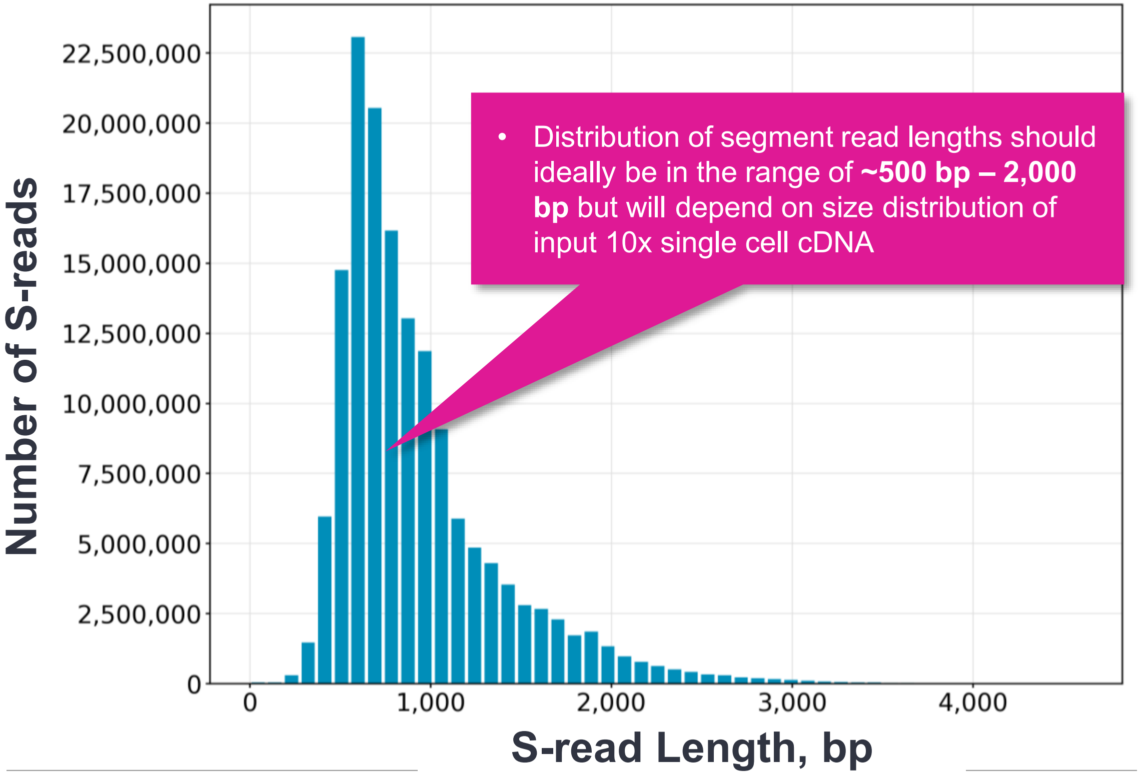
Length of Reads



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

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

S-read Length



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

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

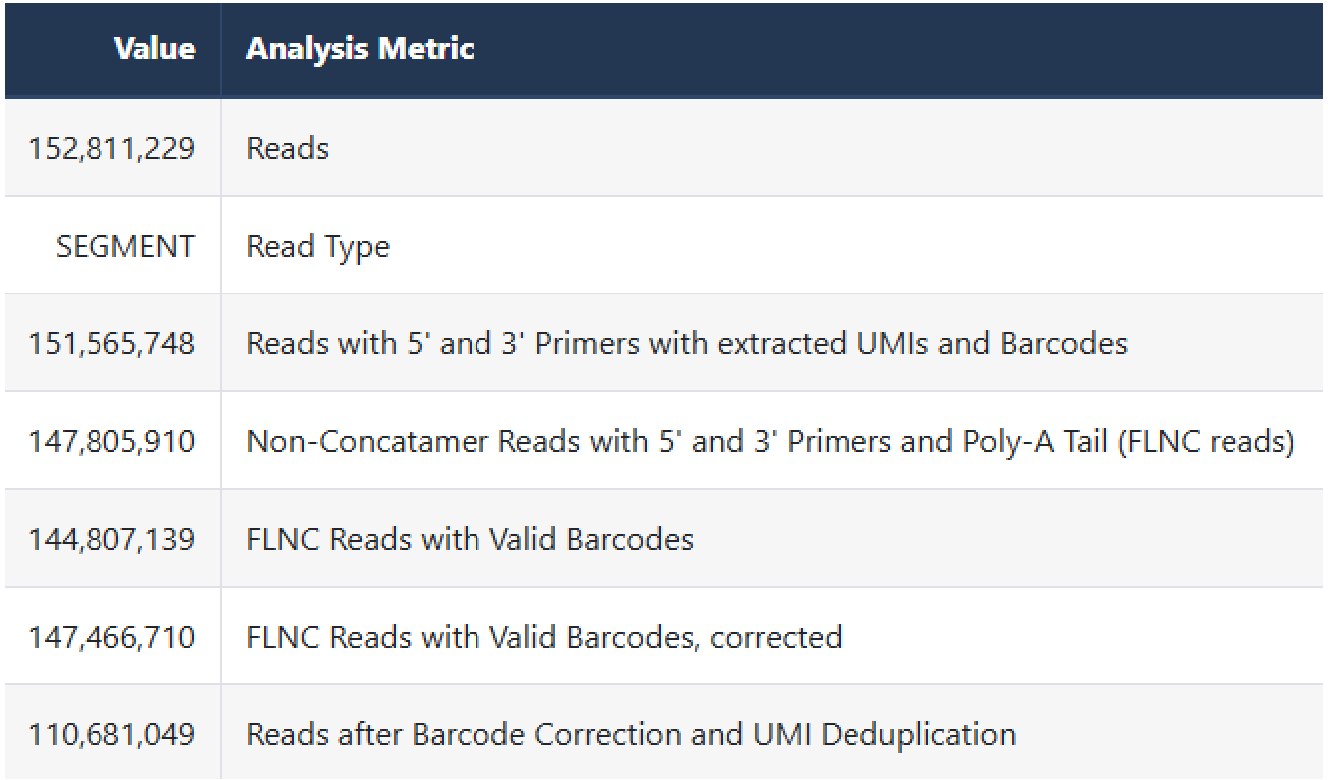
---

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

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

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

Summary Metrics



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

Example Revio system + SPRQ data shown.

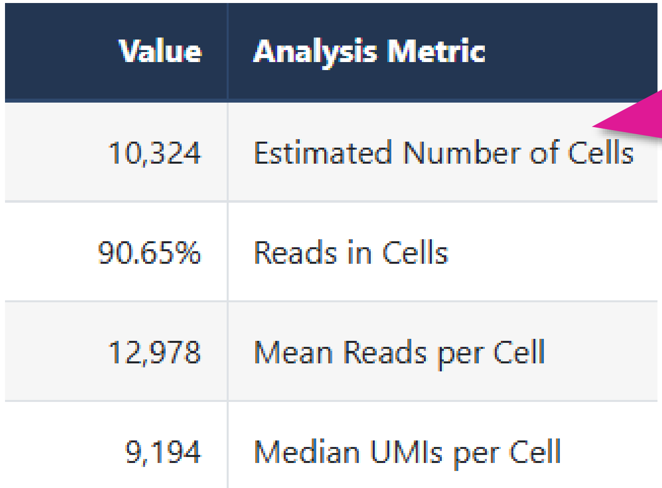
- **Reads:** Total number of input reads for analysis.
- **Read Type:** Type of input reads - CCS, SEGMENT, or mixed if there are multiple input data sets with mixed data types.
- **Reads with 5’ and 3’ Primers with extracted UMIs and Barcodes:** The number of reads with 5’ and 3’ cDNA primers detected,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

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

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

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

Summary Metrics



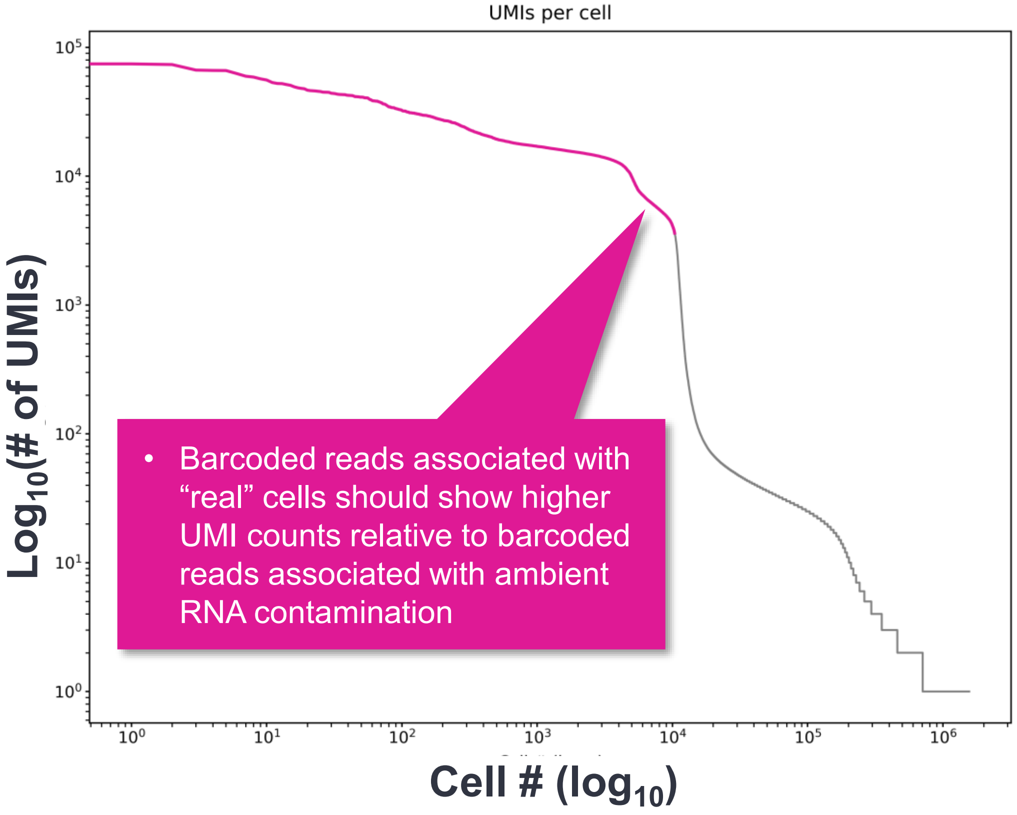
Value	Analysis Metric
10,324	Estimated Number of Cells
90.65%	Reads in Cells
12,978	Mean Reads per Cell
9,194	Median UMIs per Cell

Example Revio system + SPRQ data shown.

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

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

Barcode Rank Plot

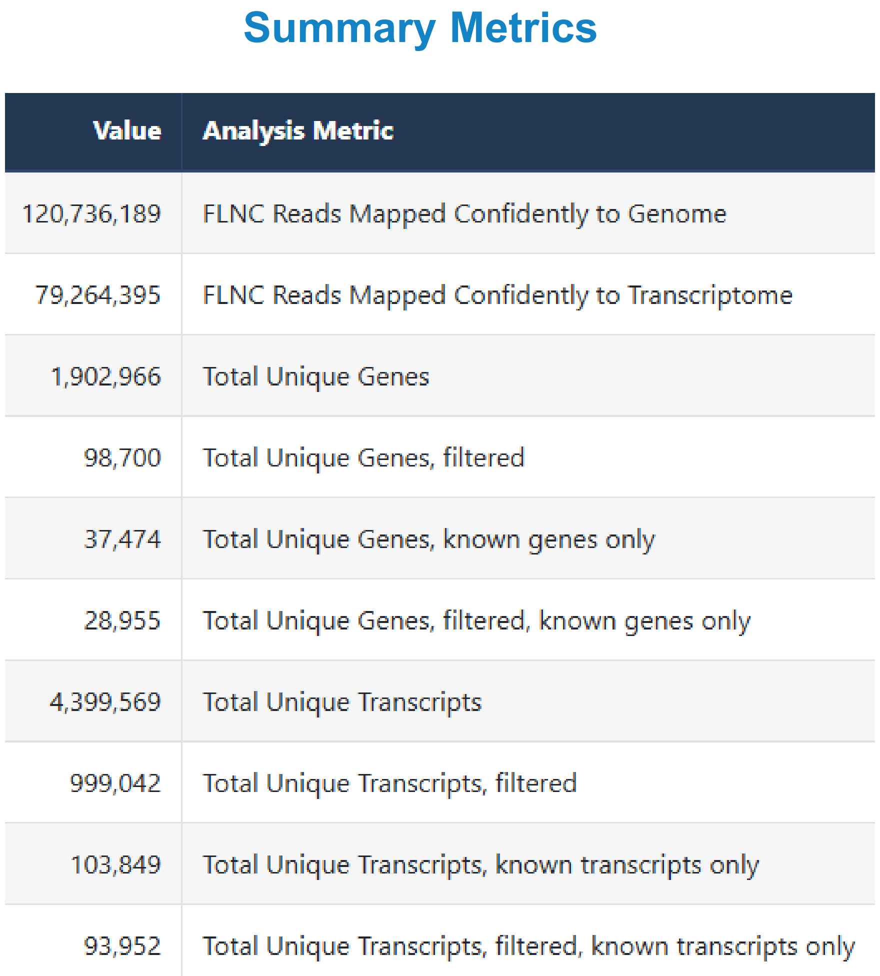


- Barcoded reads associated with “real” cells should show higher UMI counts relative to barcoded reads associated with ambient RNA contamination

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

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

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



Summary Metrics

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

Example Revio system + SPRQ data shown.

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

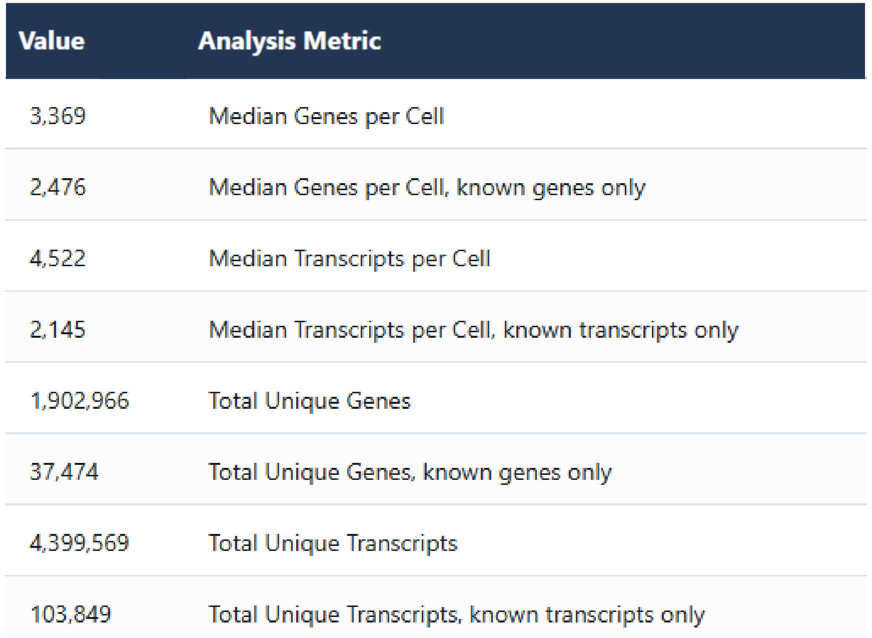
---

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

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

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

Transcript Summary

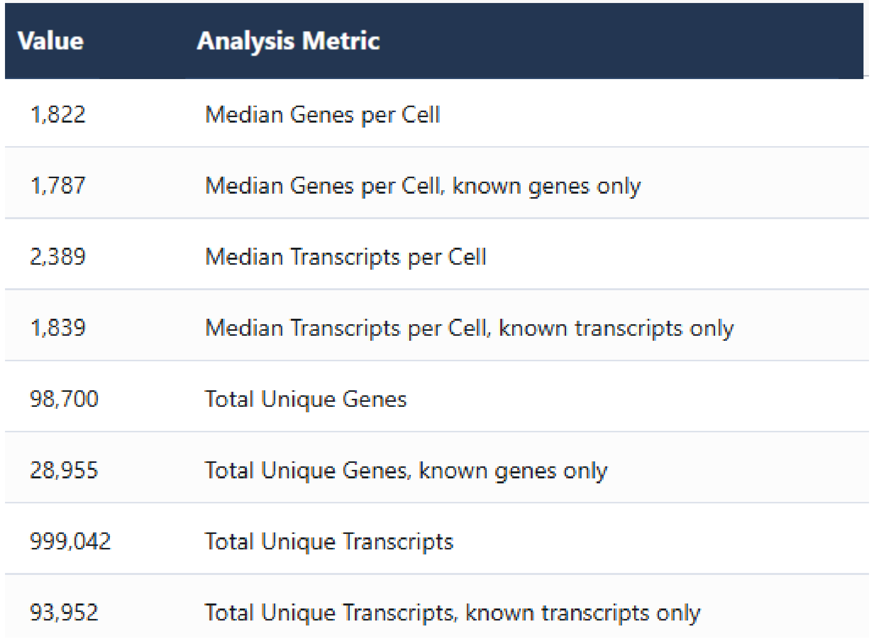


Value	Analysis Metric
3,369	Median Genes per Cell
2,476	Median Genes per Cell, known genes only
4,522	Median Transcripts per Cell
2,145	Median Transcripts per Cell, known transcripts only
1,902,966	Total Unique Genes
37,474	Total Unique Genes, known genes only
4,399,569	Total Unique Transcripts
103,849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

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

Example Revio system + SPRQ data shown.

Transcript Summary, Filtered



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

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

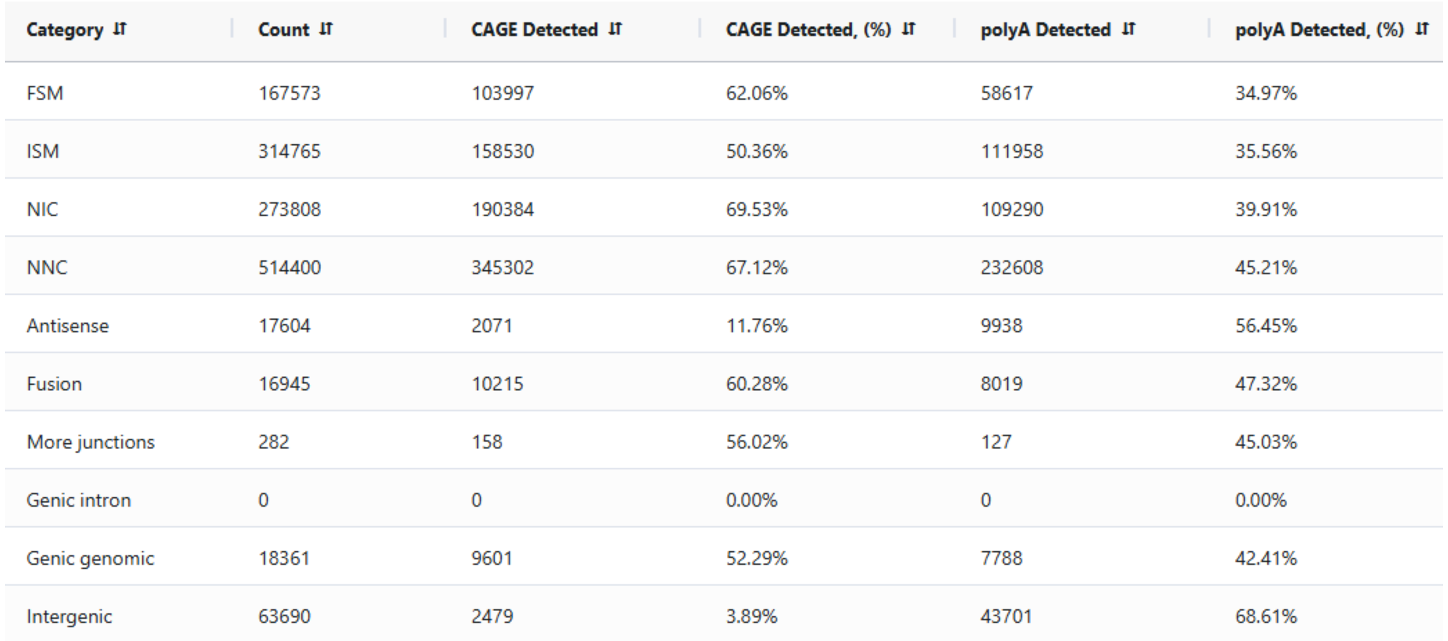
---

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

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

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

Transcript Classification, filtered (All samples)



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

Example Revio system + SPRQ data shown.[1]

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

[1] **Note:** Unfiltered transcript classification data are also displayed in the Iso-Seq analysis job report.

[2] Refer to the ***SMRT Link User Guide*** (Documentation) for descriptions of transcript classification categories (e.g., FSM – Full splice match, ISM – Incomplete splice match, etc.).

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

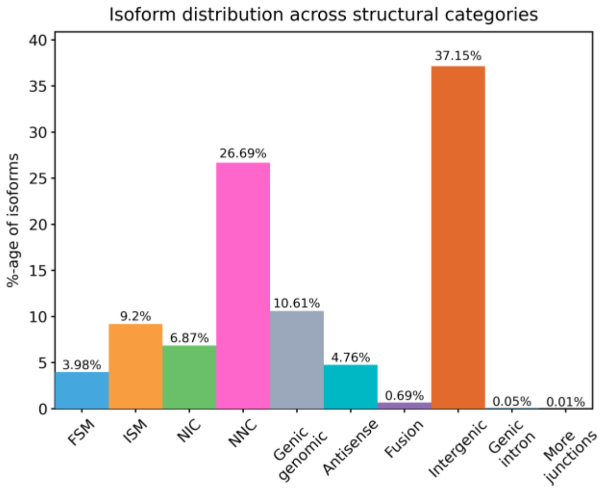
SMRT Link Single-Cell Iso-Seq Analysis job report – Transcript Statistics

Transcript Classification Plots

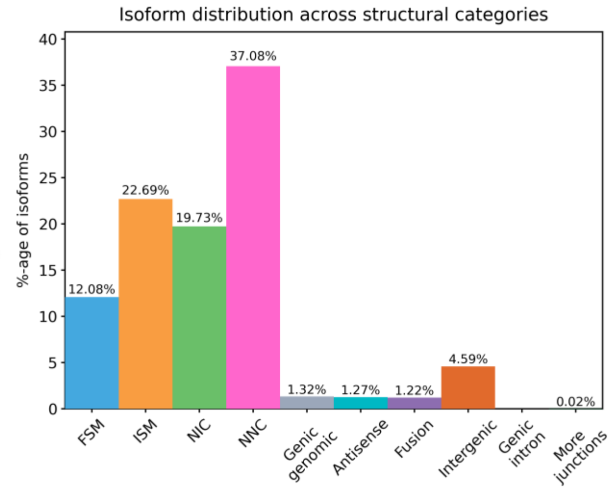
Transcript Classification Plots, Filtered

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

Example Revio system + SPRQ data shown.

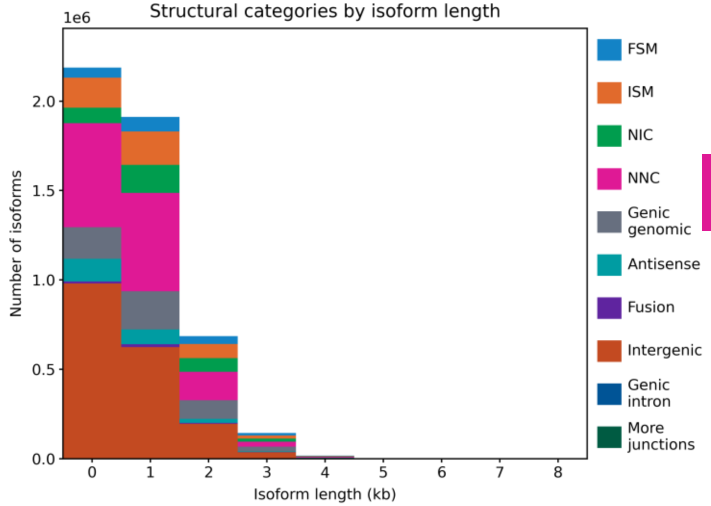


Filter out reads based on the SQANTI3 transcript filtering criteria

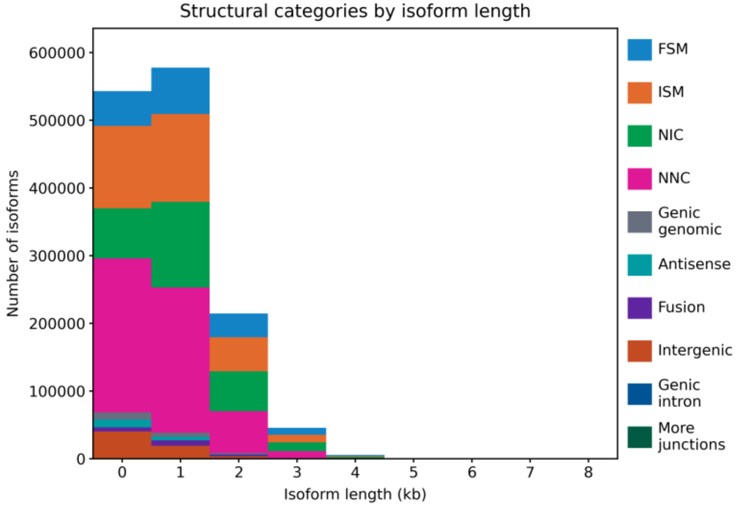


- **Structural categories by isoform lengths:**
  - Histogram display of the number of isoforms by their length in kb and their structural category.

Example Revio system + SPRQ data shown.



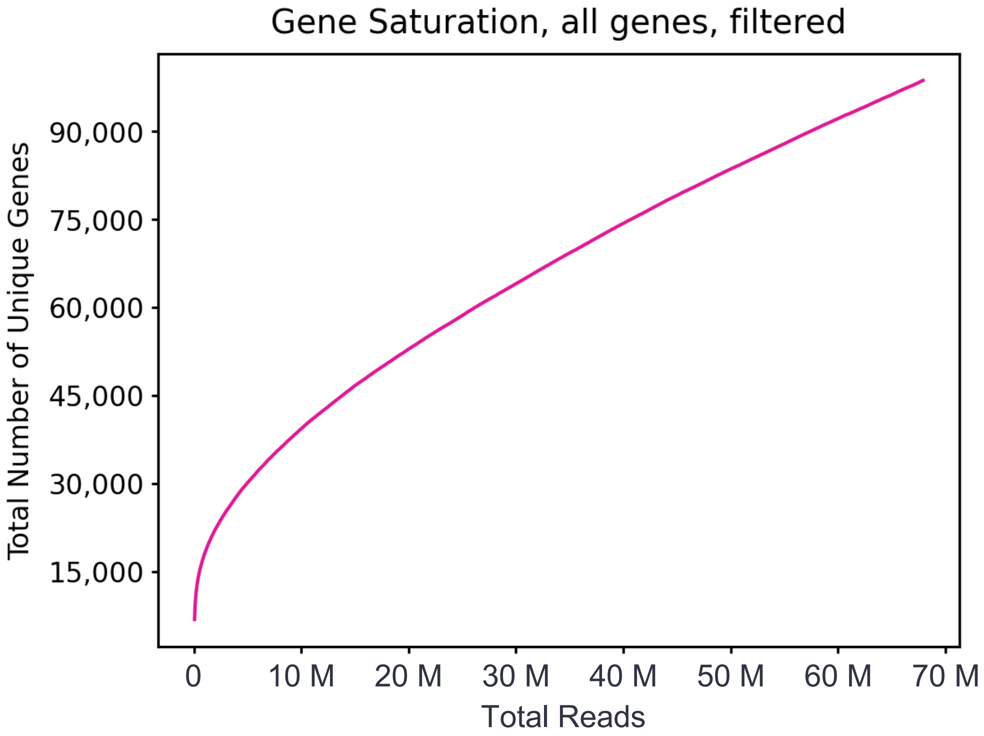
Filter out reads based on the SQANTI3 transcript filtering criteria

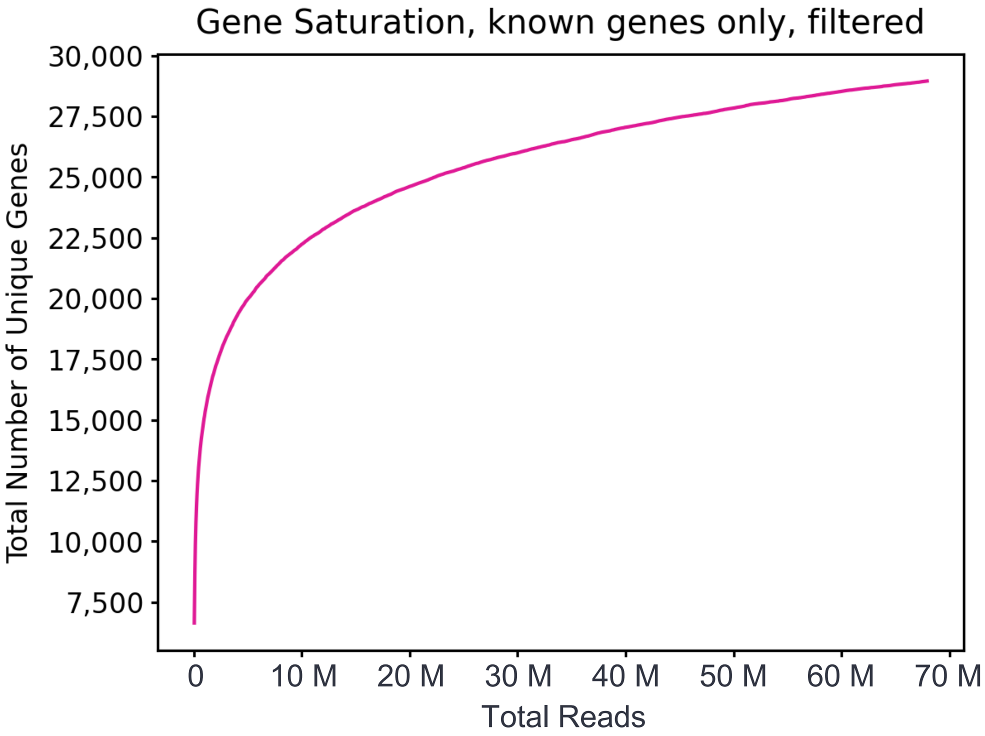


Example SMRT Link Single-Cell Iso-Seq Analysis results for Kinnex single-cell RNA libraries prepared with PBMC single cell cDNA (cont.)

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

Gene Saturation





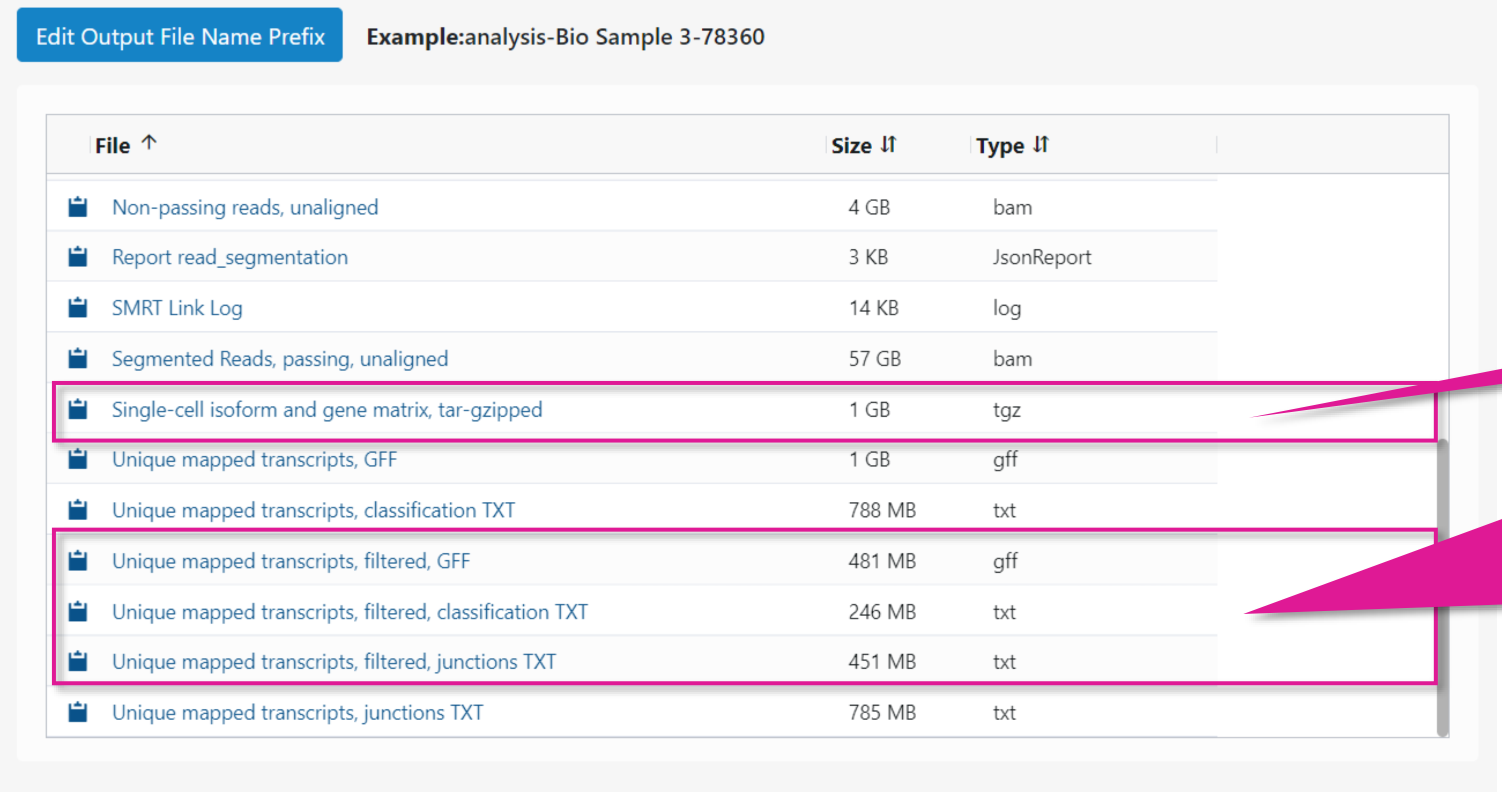
Gene Saturation, all genes, filtered: Saturation plot showing the level of gene saturation for all genes, after filtering out reads based on the SQANTI transcript filtering criteria

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

Example Revio system + SPRQ data shown.

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

File downloads tab



Example:analysis-Bio Sample 3-78360

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

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

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

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

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

Technical documentation & applications support resources

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

Single-cell cDNA sample preparation literature & other resources

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

Kinnex single-cell RNA library preparation literature & other resources

- Application note – Kinnex single-cell RNA for single-cell isoform sequencing (102-326-549)
- Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (102-254-300)
- 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 single-cell RNA kit (103-344-600)
- Video tutorial – PacBio Kinnex single-cell RNA TSO artifact removal demo for Kinnex single-cell RNA kit [Link]
- Video tutorial – SMRT Link Sample Setup and Run Design setup procedure for Kinnex kits [Link]

Data analysis resources

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

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

Publications

- Wissel, D. et al. (2025) A systematic benchmark of high-accuracy PacBio long-read RNA sequencing for transcript-level quantification. BioRxiv [Link]
- Al’Khafaji, A.M. et al. (2024) High-throughput RNA isoform sequencing using programmable cDNA concatenation. Nature biotechnology [Link]
- Deng, E. et al. (2025) Systematic evaluation of single-cell RNA-seq analyses performance based on long-read sequencing platforms. J Advanced Res [Link]

Webinars

- PacBio webinar (2025) - Detecting shared mis-splicing across blood cancers using long-read single-cell RNA sequencing [Link]
- PacBio video (2024) – Kinnex explained – how concatenating smaller amplicons increases throughput for PacBio HiFi sequencing [Link]
- PacBio PRISM webinar (2024) – Let’s stick together – exploring PacBio Kinnex kits [Link]
- PacBio Iso-Seq social club webinar (2022) – TappAS for isoform differential expression analysis [Link]
- PacBio Iso-Seq social club webinar (2022) – Single-cell Iso-Seq applications in cancer and neurological disorders [Link]

Example PacBio data sets

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

PacBio

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

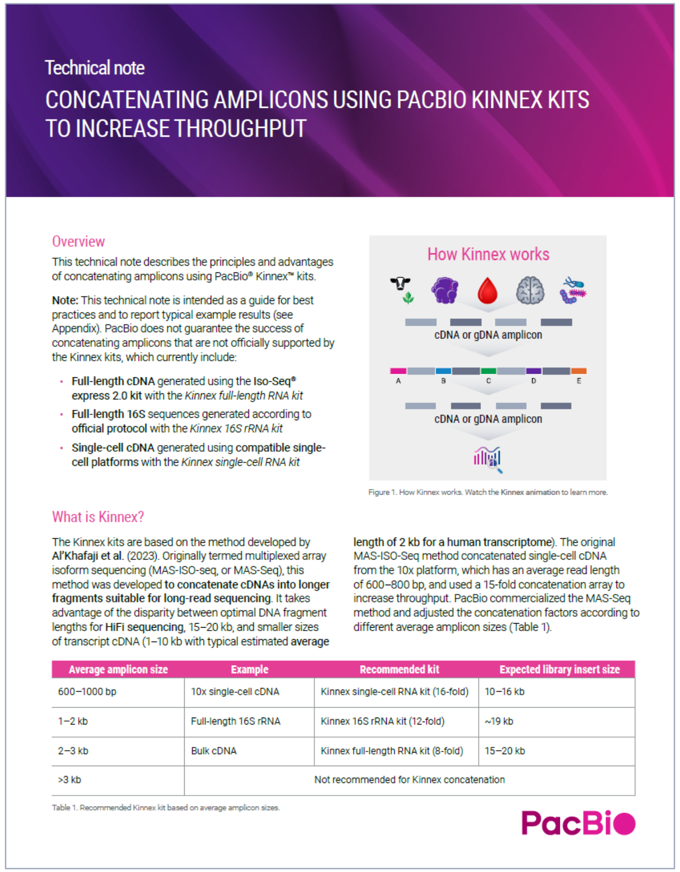
- Increased throughput on PacBio long-read sequencers
- Retained HiFi accuracy despite throughput increase
- No change to secondary analysis – once reads are deconcatenated into S-reads, the S-reads represent the original, pre-concatenated amplicon and can be analyzed with established pipelines.

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*

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.

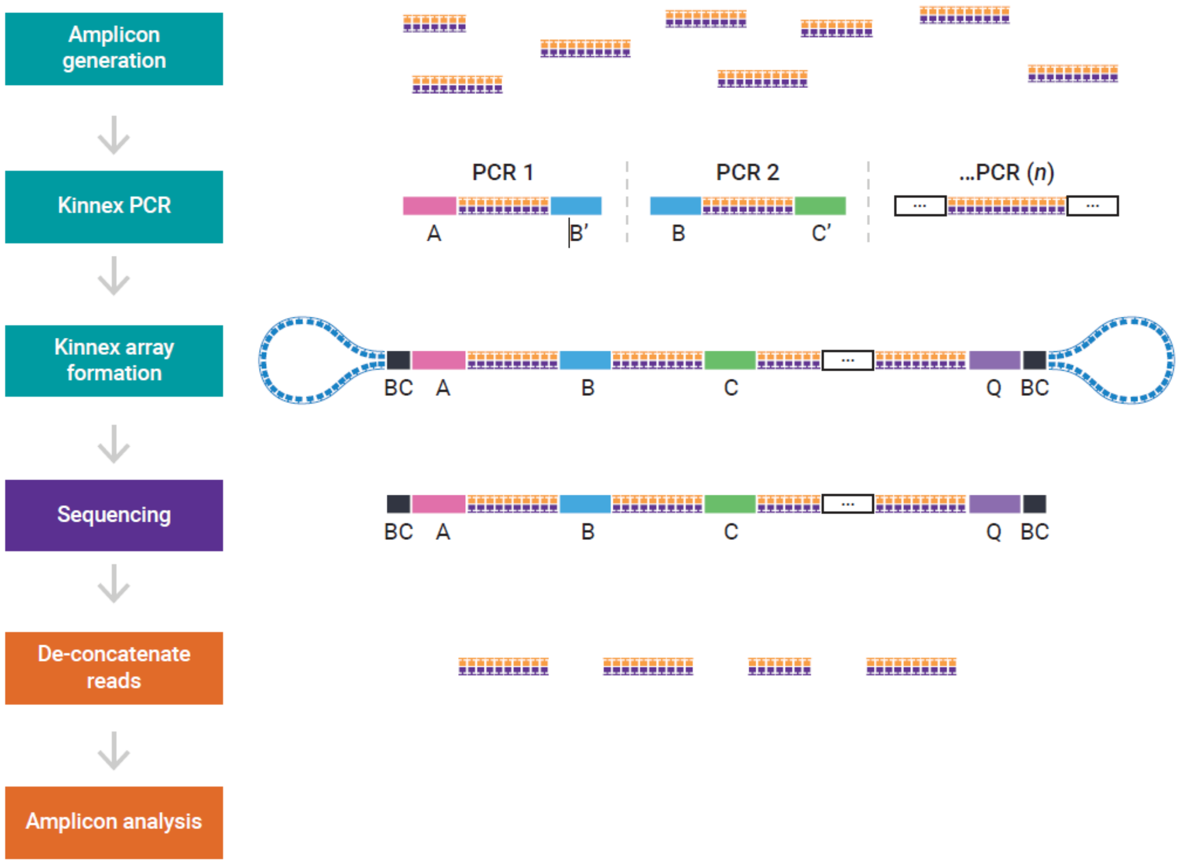
Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput (102-326-636)

---

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

Kinnex concatenation workflow overview

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



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

Kinnex concatenation procedural notes[1]

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

---

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

Establishing Kinnex-compatible molecular ends

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

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

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

Example 1. Kinnex 16S forward and reverse primer sequences

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

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

IsoSeqX bc01 FWD	CTACACGACGCTCTTCCGATCT – ACTACAC – GCAATGAAGTCGCAGGGTTGGG
IsoSeqX REV	AAGCAGTGGTATCAACGCAGAGTAC

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

Resources for Kinnex library prep[1]

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

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

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

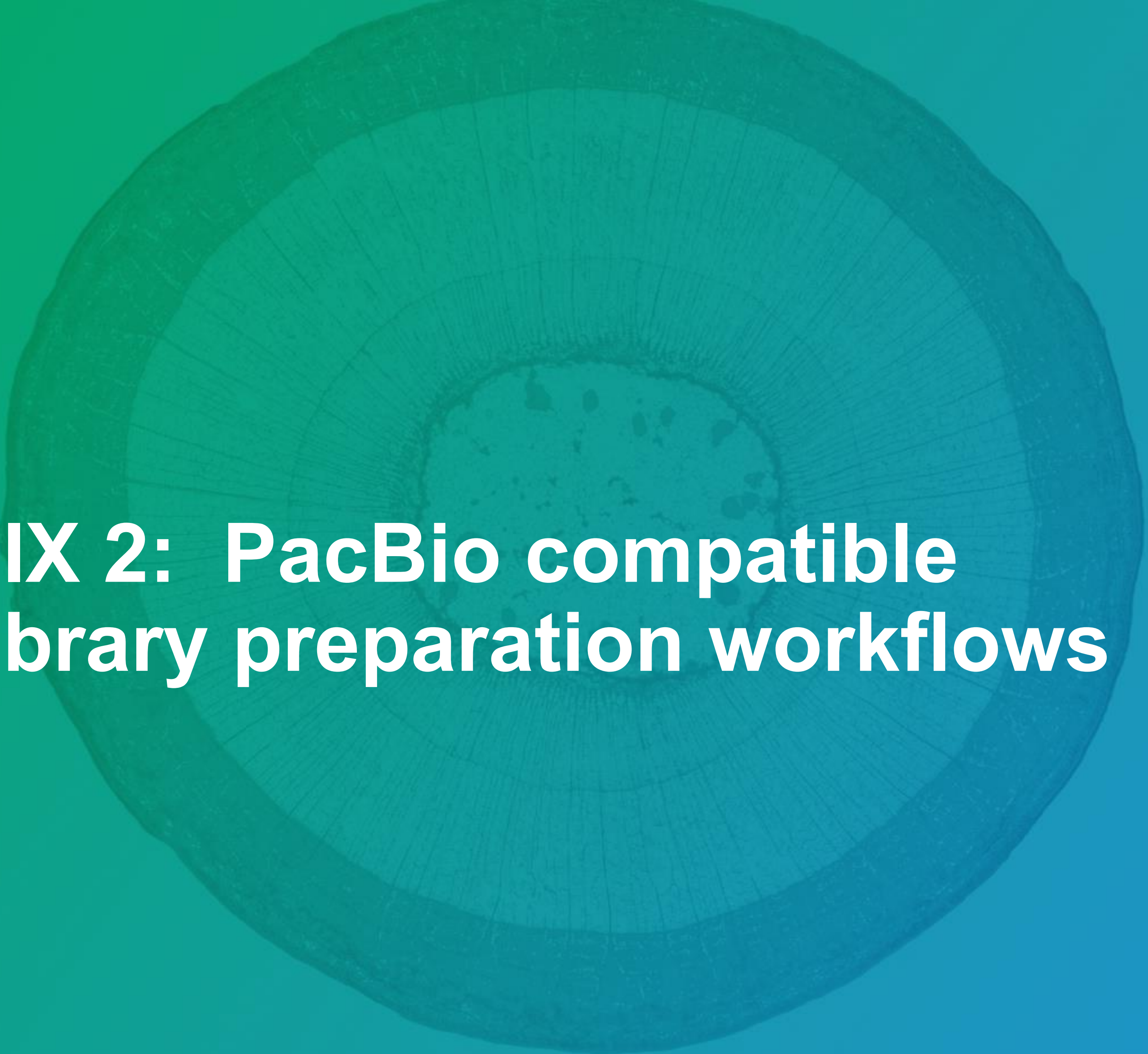
- Procedure & checklist – Preparing Kinnex libraries using 16S rRNA amplicons (103-238-800)
- Technical overview – Kinnex library preparation using Kinnex 16S rRNA kit (103-344-800)

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

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

---

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

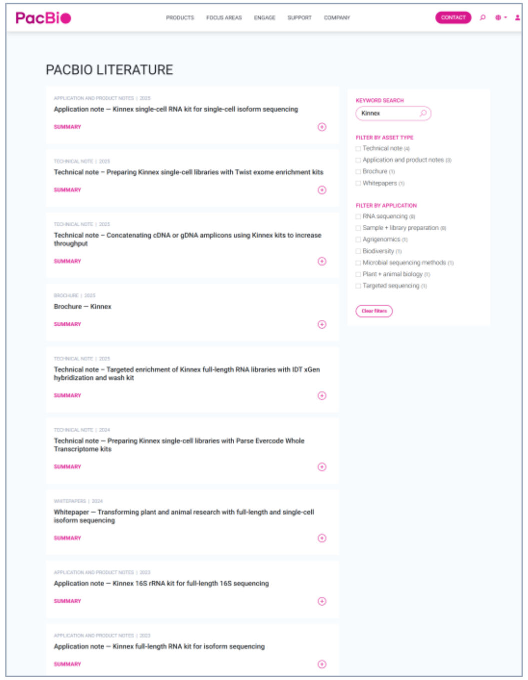


APPENDIX 2: PacBio compatible Kinnex library preparation workflows

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

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

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



PacBio PRODUCTS FOCUS AREAS ENGAGE SUPPORT COMPANY CONTACT

PACBIO LITERATURE

APPLICATION AND PRODUCT NOTES | 2025
Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput
SUMMARY

BROCHURE | 2025
Brochure – Kinnex
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit
SUMMARY

TECHNICAL NOTE | 2024
Technical note – Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits
SUMMARY

WHITEPAPERS | 2024
Whitepaper – Transforming plant and animal research with full-length and single-cell isoform sequencing
SUMMARY

APPLICATION AND PRODUCT NOTES | 2023
Application note – Kinnex 16S rRNA kit for full-length 16S sequencing
SUMMARY

APPLICATION AND PRODUCT NOTES | 2023
Application note – Kinnex full-length RNA kit for isoform sequencing
SUMMARY

KEYWORD SEARCH
Kinnex

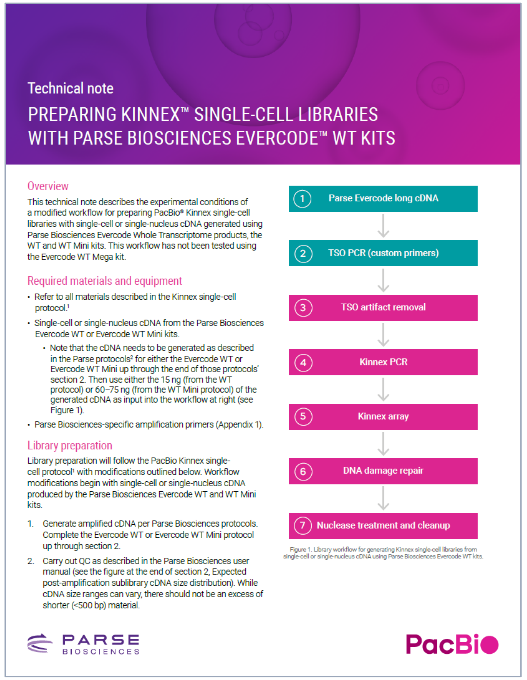
FILTER BY ASSET TYPE
☐ Technical note (4)
☐ Application and product notes (3)
☐ Brochure (1)
☐ Whitepapers (1)

FILTER BY APPLICATION
☐ RNA sequencing (8)
☐ Sample + library preparation (8)
☐ Agrigenomics (1)
☐ Biodiversity (1)
☐ Microbial sequencing methods (1)
☐ Plant + animal biology (1)
☐ Targeted sequencing (1)

Clear filters

PacBio literature website [Link]

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



Technical note

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

Overview

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

Required materials and equipment

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

Library preparation

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

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

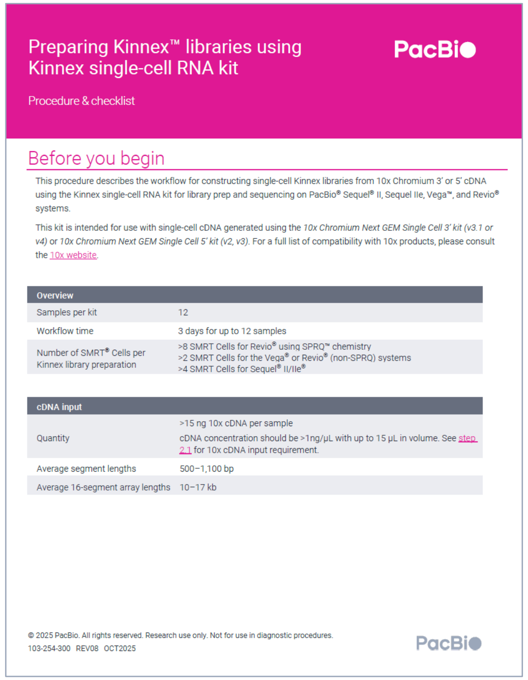
① Parse Evercode long cDNA
② TSO PCR (custom primers)
③ TSO artifact removal
④ Kinnex PCR
⑤ Kinnex array
⑥ DNA damage repair
⑦ Nuclease treatment and cleanup

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

PARSE BIOSCIENCES PacBio

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

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



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

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

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

© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.
103-254-300 REV08 OCT2025

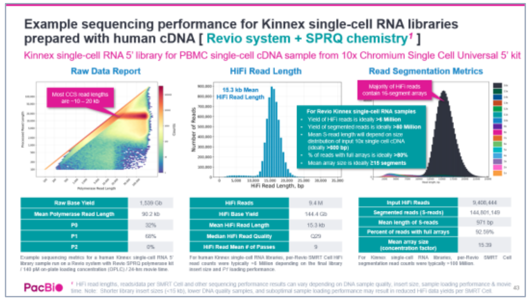
Procedure & checklist – Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300) [PacBio]

Technical documentation containing PacBio SMRTbell library construction details.

Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit

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

PN 103-344-600 Rev 02 | December 2025



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

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

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

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

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

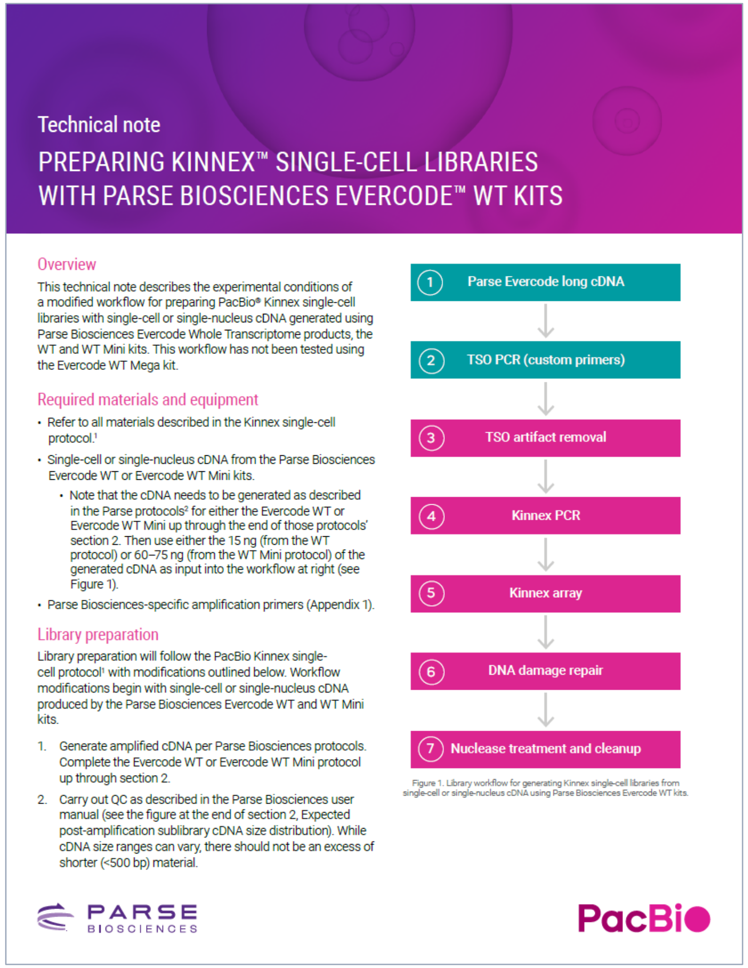
Input HiFi Reads	9,408,444
Segmented reads (S-reads)	144,801,149
Mean length of S-reads	971 bp
Percent of reads with full arrays	92.59%
Mean array size (concentration factor)	15.39

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

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

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

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



Technical note

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

Overview

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

Required materials and equipment

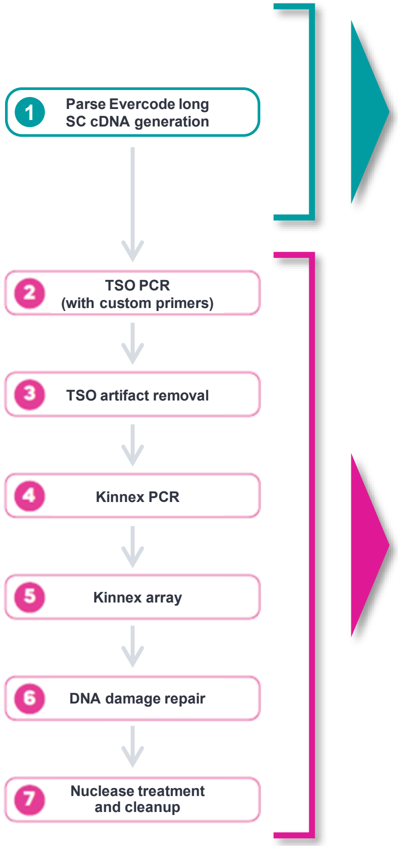
- Refer to all materials described in the Kinnex single-cell protocol.[1]
- Single-cell or single-nucleus cDNA from the Parse Biosciences Evercode WT or Evercode WT Mini kits.
  - Note that the cDNA needs to be generated as described in the Parse protocols[2] for either the Evercode WT or Evercode WT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

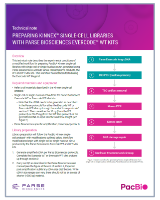
Library preparation

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

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

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

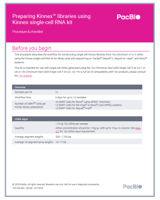




PacBio Technical note

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

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



PacBio Procedure & checklist

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

Overview

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

---

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

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

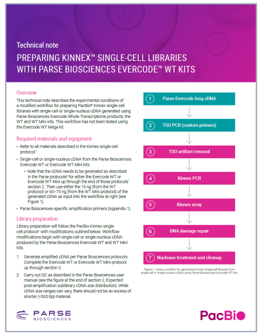
Follow Parse Bioscience whole transcriptome kit procedure[1] to generate single-cell cDNA samples suitable for Kinnex library prep and HiFi sequencing on PacBio long-read systems

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

Parse single-cell cDNA generation procedure overview[1]

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

PacBio



Technical note

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

Overview

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

Required materials and equipment

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

Library preparation

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

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

1 Parse Evercode long cDNA

2 TSO PCR (custom primers)

3 TSO artifact removal

4 Kinnex PCR

5 Kinnex array

6 DNA damage repair

7 Nuclease treatment and cleanup

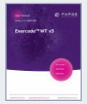
Figure 1. Library workflow for generating Kinnex single-cell libraries from single-cell or single-nucleus cDNA using Parse Biosciences Evercode WT kits.

PARSE BIOSCIENCES

PacBio

Parse single-cell cDNA generation procedure key steps

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



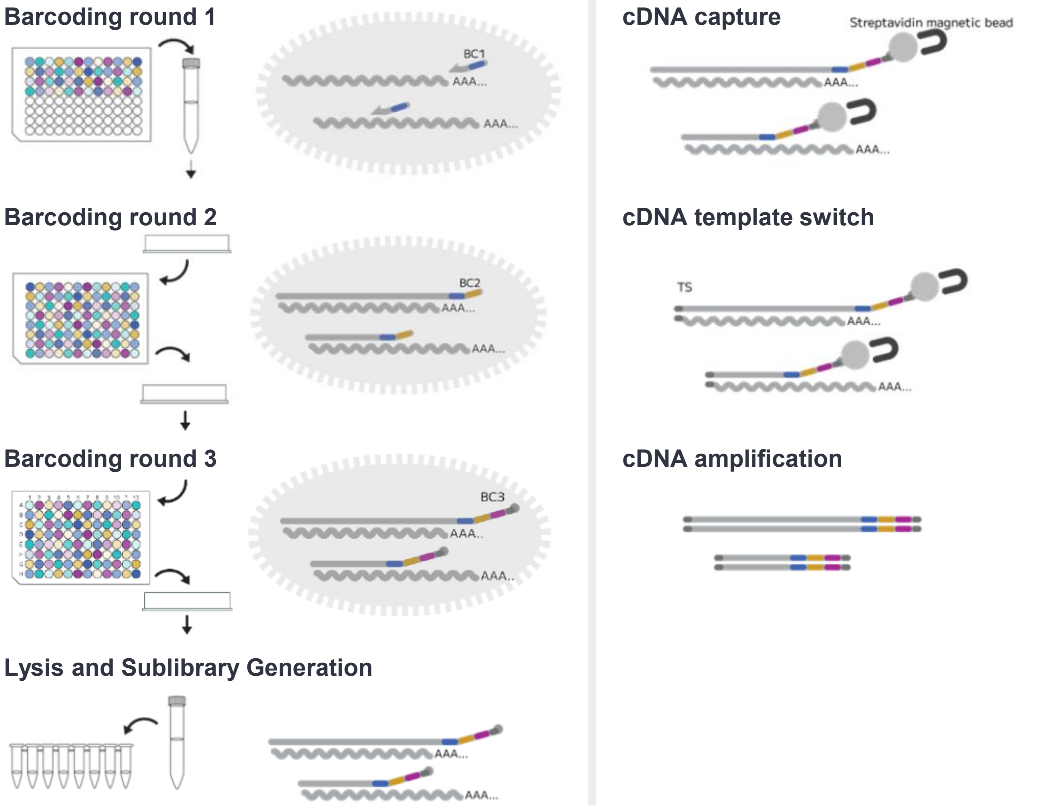
Evercode WT User Guides
UMWT3300 or UMWT3100

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

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

Section 1: In situ cell / nuclei barcoding

Section 2: cDNA capture and amplification



---

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

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

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

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

1. Parse Evercode SC cDNA generation

↓

2. TSO PCR (with custom primers)

↓

3. TSO artifact removal

↓

4. Kinnex PCR

↓

5. Kinnex array formation

↓

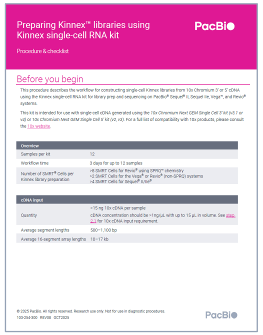
6. DNA damage repair

↓

7. Nuclease treatment and cleanup

PacBio Kinnex library construction procedure[1]

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



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

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

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

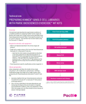
© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.
103-254-300 REV08 OCT2025

PacBio

Kinnex SC library prep procedure key steps

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

1. Parse Evercodesingle-cell cDNA generation



→ See **PacBio Technical note**

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

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

2 TSO PCR

↓

3 TSO artifact removal

↓

4 Kinnex PCR

↓

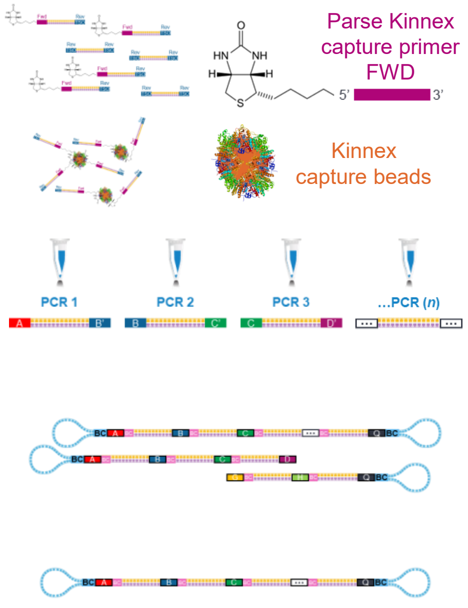
5 Kinnex array

↓

6 DNA repair

↓

7 Nuclease treatment and cleanup



---

[1] This replacement is critical to adapt the Parse library termini to function with the Kinnex concatenation workflow.

[2] Subsequent QC and library quantification can be carried out as described in the Kinnex single-cell protocol (step 7.21 in 103-254-300).

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

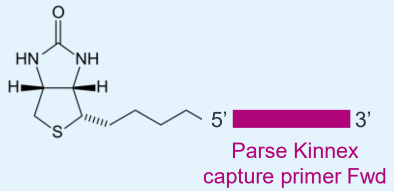
Order custom TSO PCR amplification primers as described below from your preferred oligo vendor

Parse Biosciences-specific primers required for TSO PCR amplification step

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

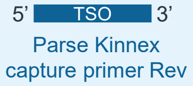
Parse Kinnex forward capture primer

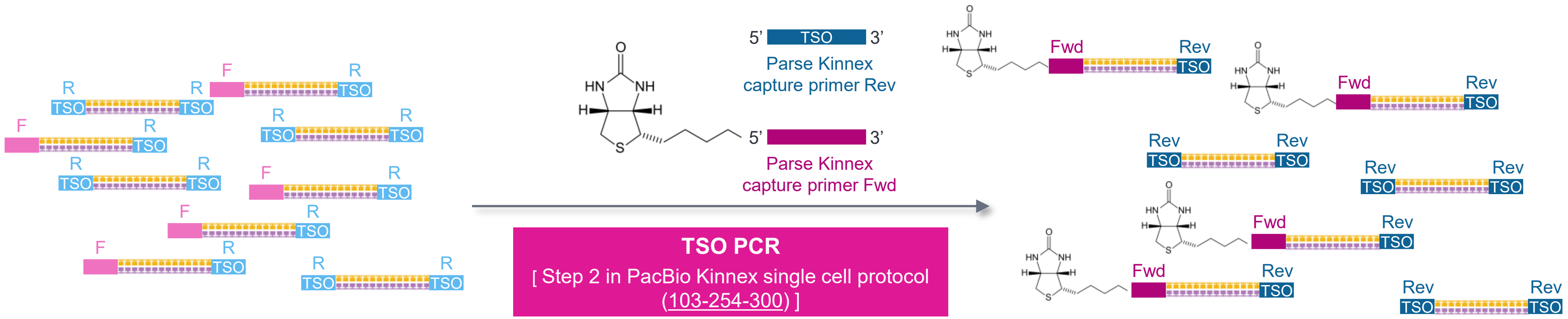
/5Biosg/AA/ideoxyU/CTACACGACGCTCTTCCGATCTCAGACGTGTGCTCTTCCGATC



Parse Kinnex reverse capture primer

AAGCAGTGGTATCAACGCAGAG





TSO PCR

[Step 2 in PacBio Kinnex single cell protocol (103-254-300)]

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

---

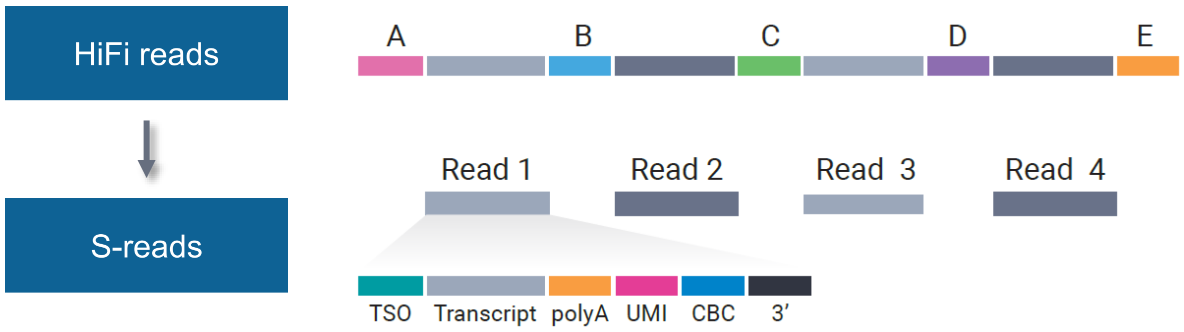
[1] The subsequent TSO artifact removal step (Step 3) in the **Kinnex single-cell protocol** (103-254-300) uses streptavidin-coated Kinnex capture beads to pull down and enrich for biotin-tagged cDNA products with the correct structure.

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

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

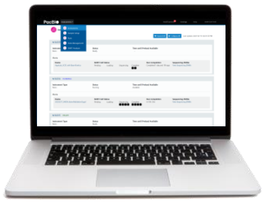
- Note: The SMRT Link single-cell Iso-Seq workflow does not currently support analysis of Parse Biosciences data.
- Instead, Read Segmentation can be run in SMRT Link (or skera in the command line) to generate segmented reads (S-reads).
- The S-reads will contain the original single-cell or single-nucleus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

SMRT Link read segmentation workflow



HiFi reads

S-reads



SMRT Analysis

---

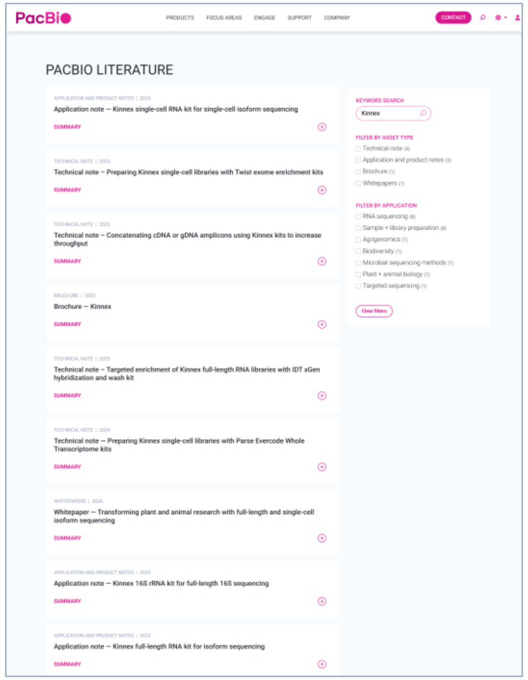
[1] Rebboah, E., et al. (2021). Mapping and modeling the genomic basis of differential RNA isoform expression at single-cell resolution with LR-Split-seq. Genome biology, 22, 1-28 [Link].

[2] Workflow-for-Processing-Long-Read-Data-with-the-Parse-Biosciences-Pipeline. Parse Biosciences long-read data processing guide.

Preparing Kinnex single-cell libraries with Twist exome enrichment kits

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

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



PACBIO LITERATURE

APPLICATION AND PRODUCT NOTES | 2025
Application note — Kinnex single-cell RNA kit for single-cell isoform sequencing
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Preparing Kinnex single-cell libraries with Twist exome enrichment kits
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Concatenating cDNA or gDNA amplicons using Kinnex kits to increase throughput
SUMMARY

BROCHURE | 2025
Brochure — Kinnex
SUMMARY

TECHNICAL NOTE | 2025
Technical note – Targeted enrichment of Kinnex full-length RNA libraries with IDT xGen hybridization and wash kit
SUMMARY

TECHNICAL NOTE | 2024
Technical note — Preparing Kinnex single-cell libraries with Parse Evercode Whole Transcriptome kits
SUMMARY

WHITEPAPERS | 2024
Whitepaper — Transforming plant and animal research with full-length and single-cell isoform sequencing
SUMMARY

APPLICATION AND PRODUCT NOTES | 2023
Application note — Kinnex 16S rRNA kit for full-length 16S sequencing
SUMMARY

APPLICATION AND PRODUCT NOTES | 2023
Application note — Kinnex full-length RNA kit for isoform sequencing
SUMMARY

KEYWORD SEARCH
Kinnex

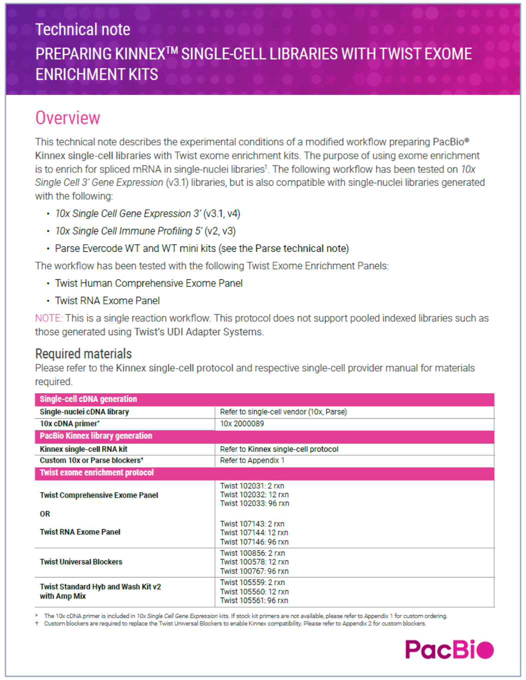
FILTER BY ASSET TYPE
☐ Technical note (4)
☐ Application and product notes (3)
☐ Brochure (1)
☐ Whitepapers (1)

FILTER BY APPLICATION
☐ RNA sequencing (8)
☐ Sample + library preparation (8)
☐ Agrigenomics (1)
☐ Biodiversity (1)
☐ Microbial sequencing methods (1)
☐ Plant + animal biology (1)
☐ Targeted sequencing (1)

Clear filters

PacBio literature website [Link]

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



Technical note

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

Overview

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

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

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

- Twist Human Comprehensive Exome Panel
- Twist RNA Exome Panel

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

Required materials

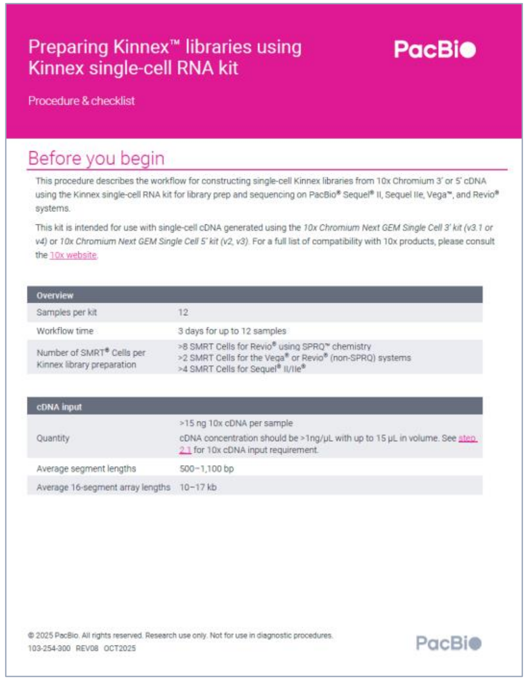
Please refer to the Kinnex single-cell protocol and respective single-cell provider manual for materials required.

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

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

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

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



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

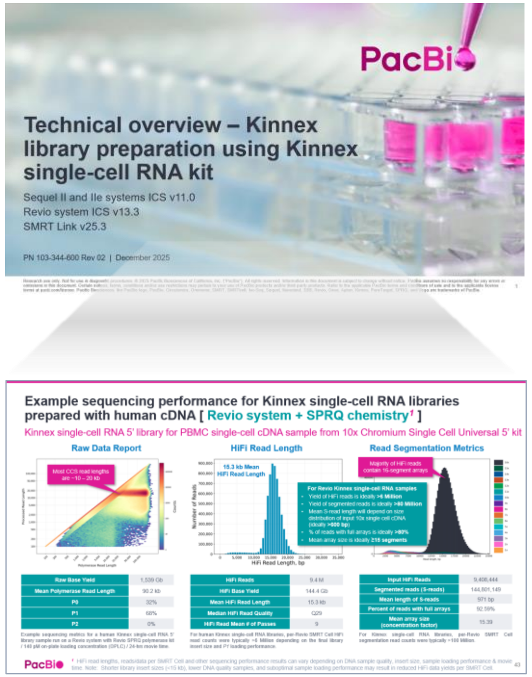
Overview	
Samples per kit	12
Workflow time	3 days for up to 12 samples
Number of SMRT® Cells per Kinnex library preparation	>8 SMRT Cells for Revio® using SPRQ™ chemistry >2 SMRT Cells for the Vega® or Revio® (non-SPRQ) systems >4 SMRT Cells for Sequel® II/IIe®

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

© 2025 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.
103-254-300 REV08 OCT2025

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

Technical documentation containing PacBio SMRTbell library construction details.



Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit

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

PN 103-344-600 Rev 02 | December 2025

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

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

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

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

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

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

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

Input HiFi Reads	9,408,444
Segmented reads (S-reads)	144,801,149
Mean length of S-reads	971 bp
Percent of reads with full arrays	92.59%
Mean array size (concentration factor)	15.39

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

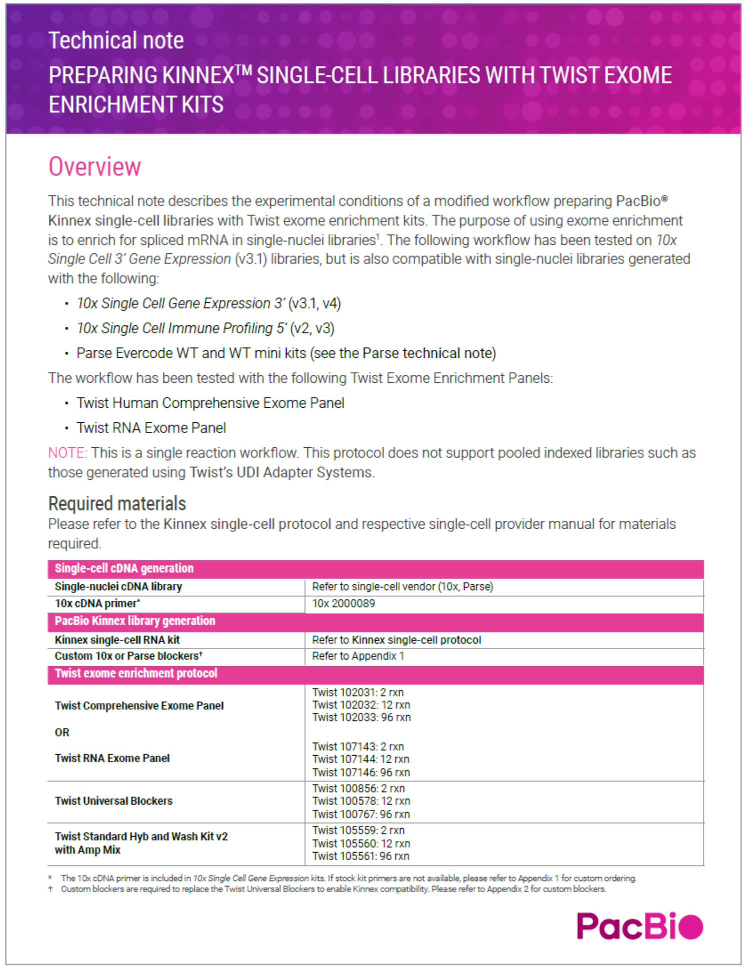
[1] HiFi read lengths, reads/data per SMRT Cell and other sequencing performance results can vary depending on DNA sample quality, insert size, sample loading performance & movie time. Note: Shorter library insert sizes (<15 kb), lower DNA quality samples, and suboptimal sample loading performance may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

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

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

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

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



Technical note

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

Overview

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

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

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

- Twist Human Comprehensive Exome Panel
- Twist RNA Exome Panel

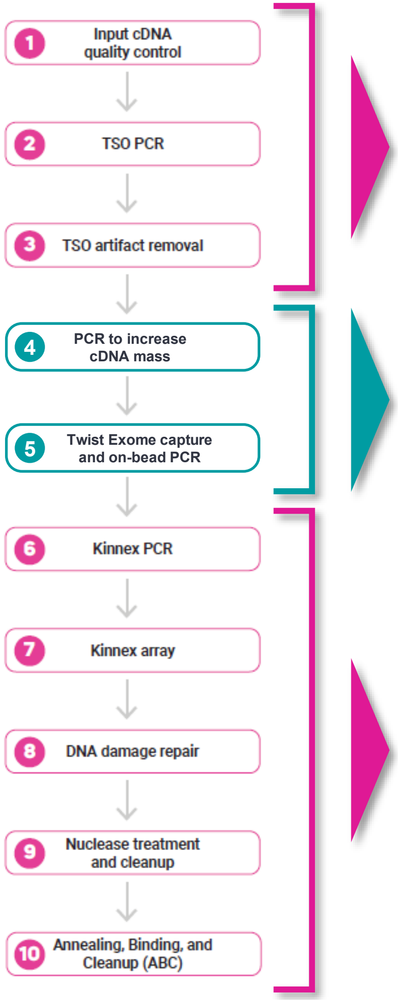
NOTE: This is a single reaction workflow. This protocol does not support pooled indexed libraries such as those generated using Twist's UDI Adapter Systems.

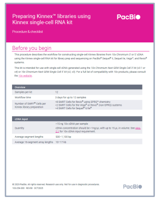
Required materials

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

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

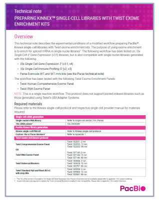
* The 10x cDNA primer is included in *10x Single Cell Gene Expression* kits. If stock kit primers are not available, please refer to Appendix 1 for custom ordering.
† Custom blockers are required to replace the Twist Universal Blockers to enable Kinnex compatibility. Please refer to Appendix 2 for custom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





PacBio Procedure & checklist

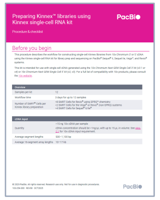
Preparing Kinnex libraries using Kinnex single-cell RNA kit (103-254-300)



PacBio Technical note

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

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



PacBio Procedure & checklist

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

Overview

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

---

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

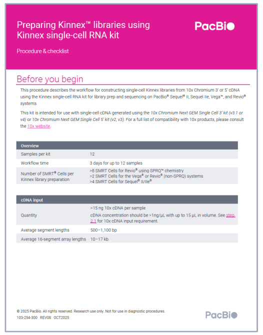
Workflow overview for preparing Kinnex single-cell libraries with Twist exome enrichment kits

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

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

PacBio Kinnex library construction procedure[1]

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



Preparing Kinnex™ libraries using Kinnex single-cell RNA kit

Procedure & checklist

Before you begin

This procedure describes the workflow for constructing single-cell Kinnex libraries from 10x Chromium 3' or 5' cDNA using the Kinnex single-cell RNA kit for library prep and sequencing on PacBio® Sequel® II, Sequel IIe, Vega™, and Revio® systems.

This kit is intended for use with single-cell cDNA generated using the *10x Chromium Next GEM Single Cell 3' kit (v3.1 or v4)* or *10x Chromium Next GEM Single Cell 5' kit (v2, v3)*. For a full list of compatibility with 10x products, please consult the 10x website.

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

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

103-254-300 REV08 OCT2025

Kinnex SC library prep procedure key steps

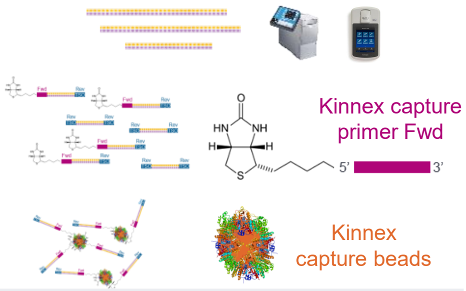
- Follow Kinnex protocol instructions until the end of the TSO artifact removal step
  - If using compatible 10x products, follow the **Kinnex single-cell library protocol** (103-254-300) through the end of **Step 3** (TSO artifact removal)[1]
- For **Step 4** (PCR to increase cDNA mass), this additional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

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

1 Input cDNA quality control

2 TSO PCR

3 TSO artifact removal

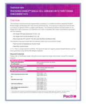


Kinnex capture primer Fwd

Kinnex capture beads

4. PCR to increase cDNA mass

5. Twist exome capture and on-bead PCR



→ See **PacBio Technical note**

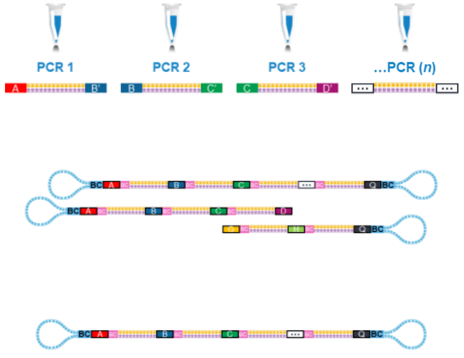
Preparing Kinnex single-cell libraries with Twist exome enrichment kits (102-326-633)

6 Kinnex PCR

7 Kinnex array

8 DNA repair

9 Nuclease treatment and cleanup



---

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

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

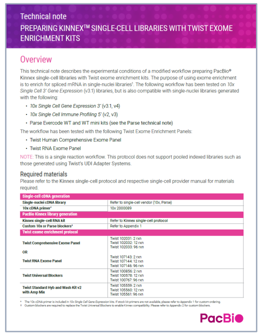
Follow PacBio Kinnex library prep protocol and modified Twist target enrichment procedure[1] to generate single-cell hybrid capture libraries suitable for HiFi sequencing on PacBio long-read systems

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

Modified Twist hybrid capture procedure overview[1]

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

PacBio



Technical note

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

Overview

Twist library construction + Twist hybrid capture workflow

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

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

PacBio

---

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

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

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

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

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

10x cDNA primer FWD

CTACACGACGCTCTTCCGATCT

10x cDNA primer REV

AAGCAGTGGTATCAACGCAGAG

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

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

10x Custom Blocker FWD

CTACACGACGCTCTTCCGATCT/3SpC3/

10x Custom Blocker REV

AAGCAGTGGTATCAACGCAGAG/3SpC3/

Parse Custom Blocker FWD

CTACACGACGCTCTTCCGATCTCAGACGTGTGCTCTTCCGATC/3SpC3/

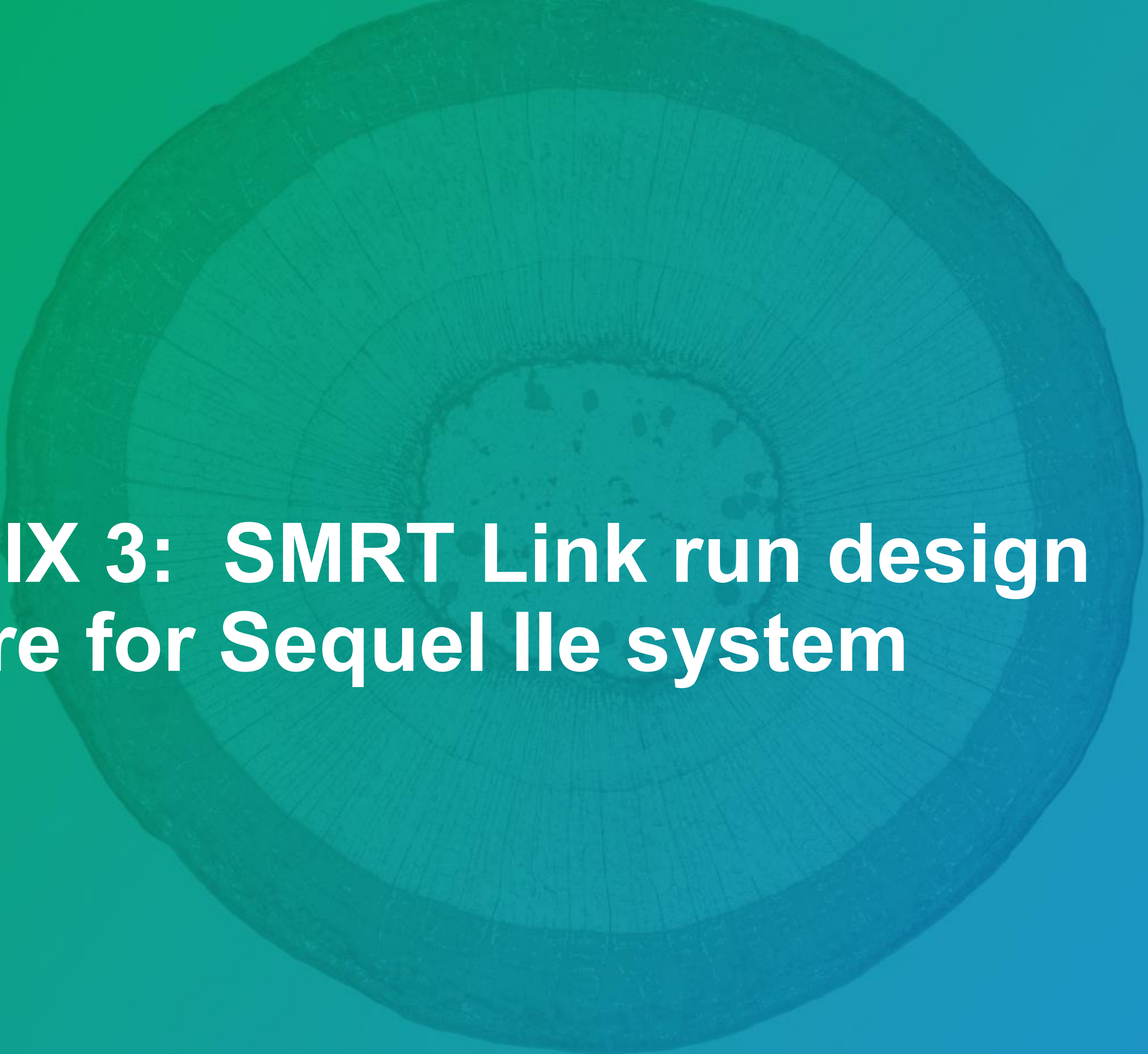
Parse Custom Blocker REV

AAGCAGTGGTATCAACGCAGAG/3SpC3/

---

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

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



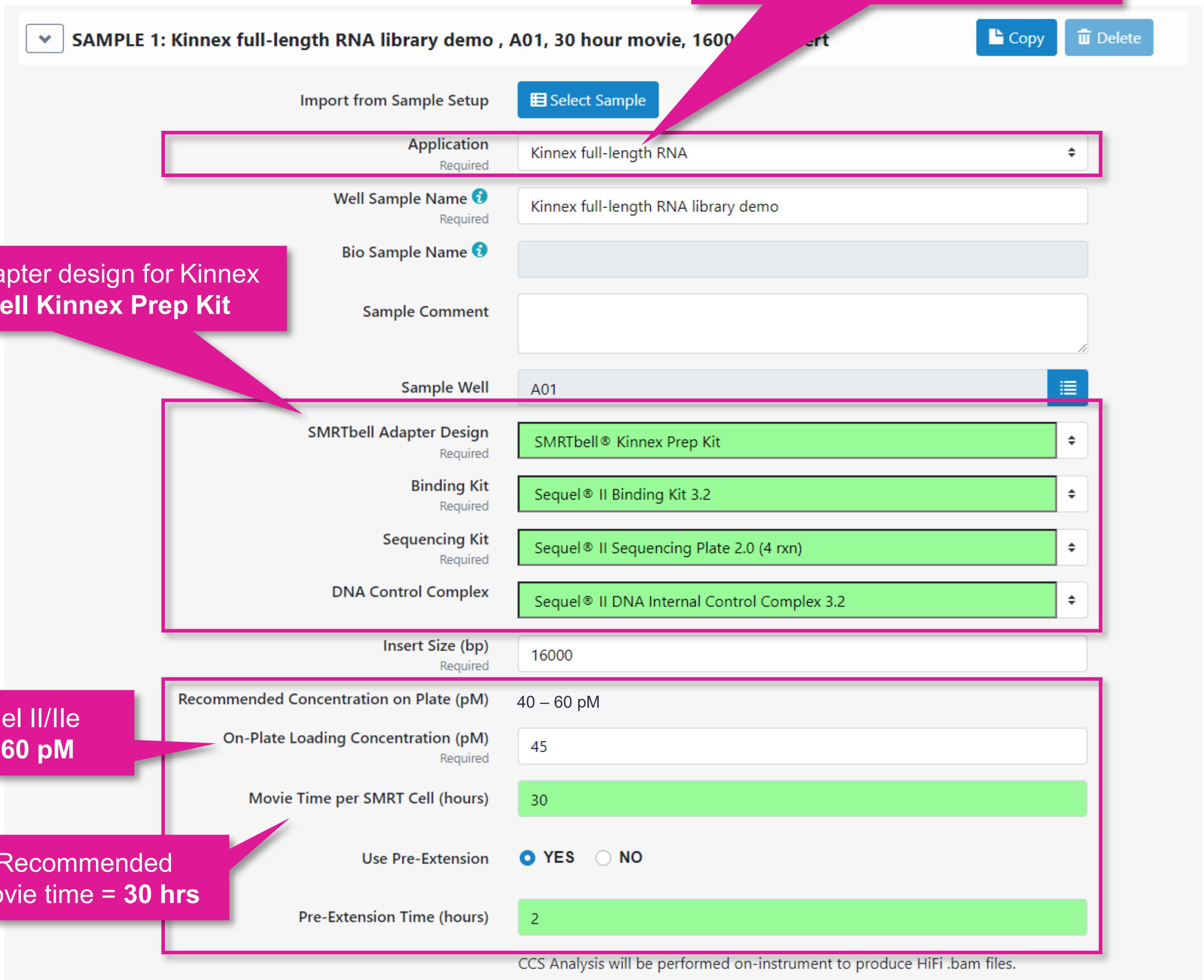
APPENDIX 3: SMRT Link run design procedure for Sequel IIe system

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]



SAMPLE 1: Kinnex full-length RNA library demo , A01, 30 hour movie, 1600 ert

Import from Sample Setup	
Application Required	Kinnex full-length RNA
Well Sample Name Required	Kinnex full-length RNA library demo
Bio Sample Name	
Sample Comment	
Sample Well	A01
SMRTbell Adapter Design Required	SMRTbell® Kinnex Prep Kit
Binding Kit Required	Sequel® II Binding Kit 3.2
Sequencing Kit Required	Sequel® II Sequencing Plate 2.0 (4 rxn)
DNA Control Complex	Sequel® II DNA Internal Control Complex 3.2
Insert Size (bp) Required	16000
Recommended Concentration on Plate (pM)	40 – 60 pM
On-Plate Loading Concentration (pM) Required	45
Movie Time per SMRT Cell (hours)	30
Use Pre-Extension	● YES ○ NO
Pre-Extension Time (hours)	2

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

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.

PacBio

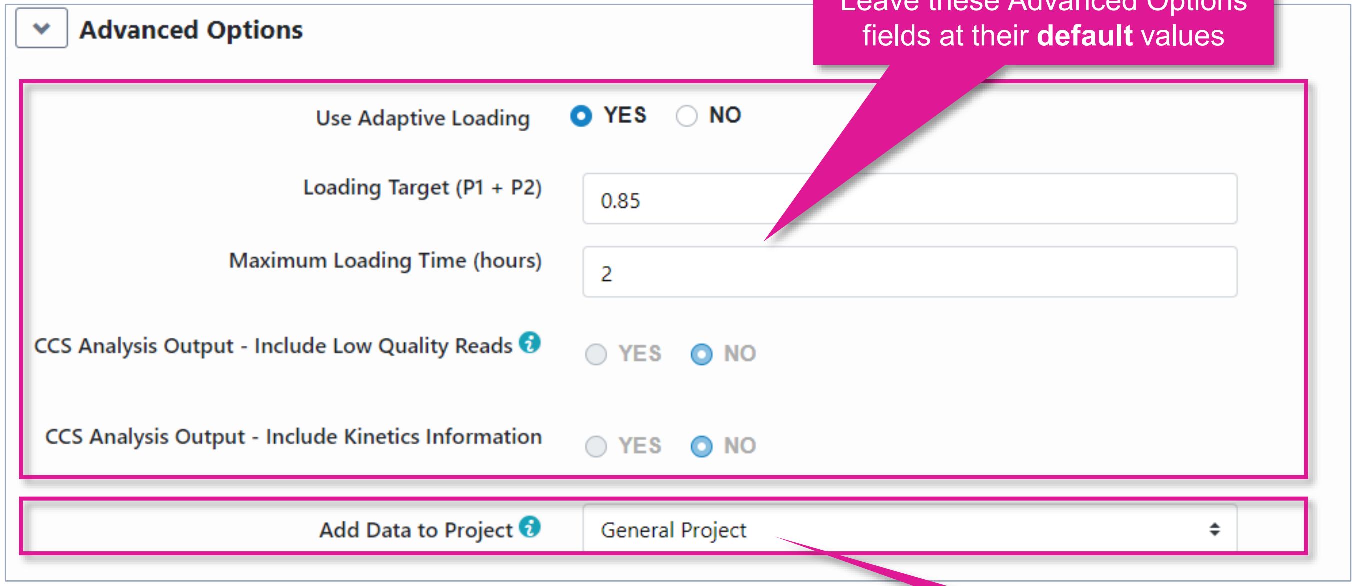
---

[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



Advanced Options

Use Adaptive Loading ● YES ○ NO

Loading Target (P1 + P2): 0.85

Maximum Loading Time (hours): 2

CCS Analysis Output - Include Low Quality Reads ○ YES ● NO

CCS Analysis Output - Include Kinetics Information ○ YES ● NO

Add Data to Project: General Project

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

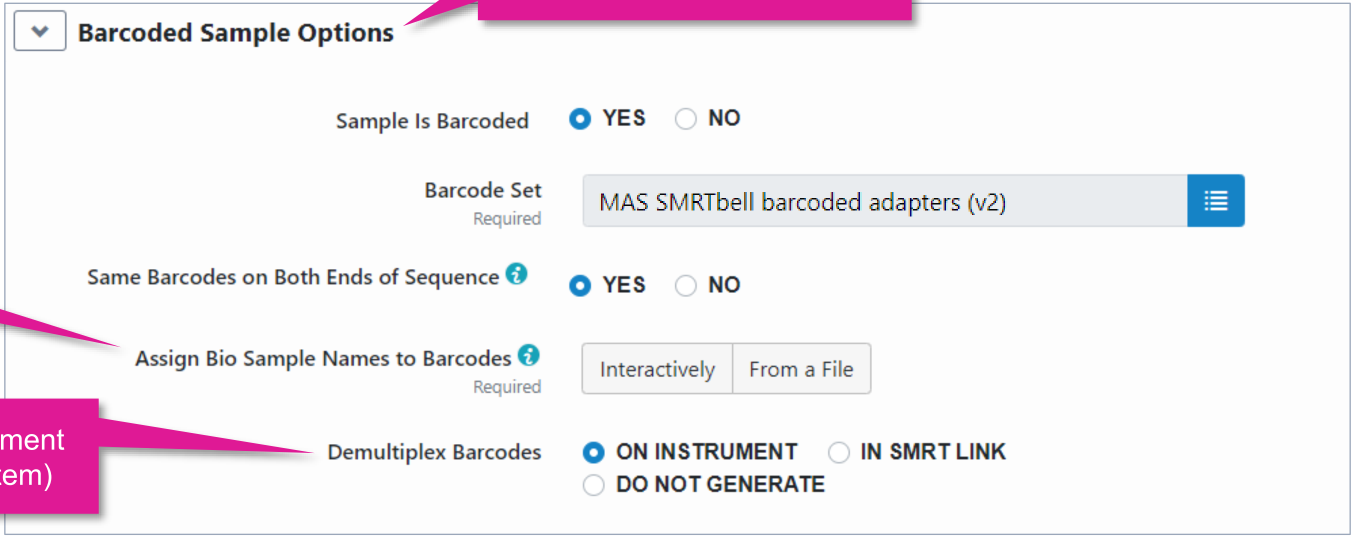
Can specify to use a different Project folder

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

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

Barcoded sample options

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



Barcoded Sample Options

Sample Is Barcoded ● YES ○ NO

Barcode Set (Required): MAS SMRTbell barcoded adapters (v2)

Same Barcodes on Both Ends of Sequence ● YES ○ NO

Assign Bio Sample Names to Barcodes (Required): Interactively | From a File

Demultiplex Barcodes ● ON INSTRUMENT ○ IN SMRT LINK ○ DO NOT GENERATE

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.

www.pacb.com

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