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Technical Overview: Single-Cell Iso-Seq Library Preparation Using SMRTbell Express Template Prep Kit 2.0

Sequel System ICS v8.0 / Sequel Chemistry 3.0 / SMRT Link v9.0
Sequel II Systems ICS v9.0 / Sequel II Chemistry 2.0 / SMRT Link v9.0
Sequel IIe System ICS v10.0 / Sequel II Chemistry 2.0 / SMRT Link v10.0

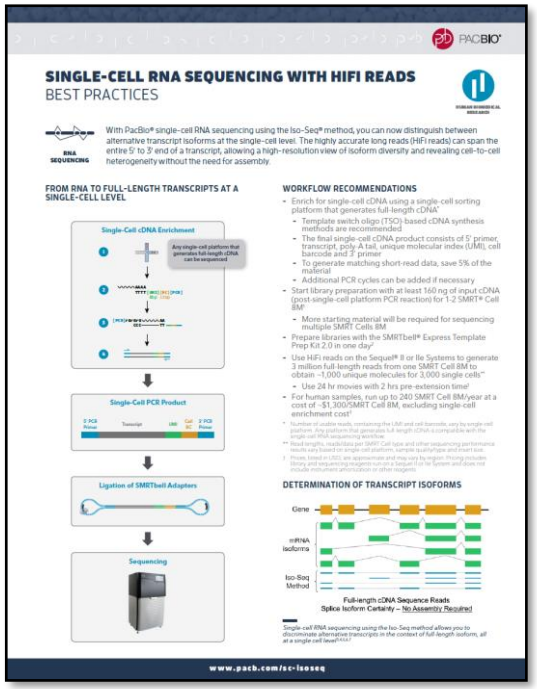
PN 101-925-400 Ver 2021-02-01-A (February 2021)

Single-Cell Iso-Seq Library Preparation Using SMRTbell Express Template Prep Kit 2.0

1. Single-Cell Iso-Seq Workflow Overview & Experimental Design Considerations
2. Single-Cell Iso-Seq Library Sample Preparation Workflow Details
3. Single-Cell Iso-Seq Library Sequencing Workflow Details
4. Single-Cell Iso-Seq Data Analysis Recommendations
5. Single-Cell Iso-Seq Library Example Sequencing Performance Data
6. Technical Documentation & Applications Support Resources

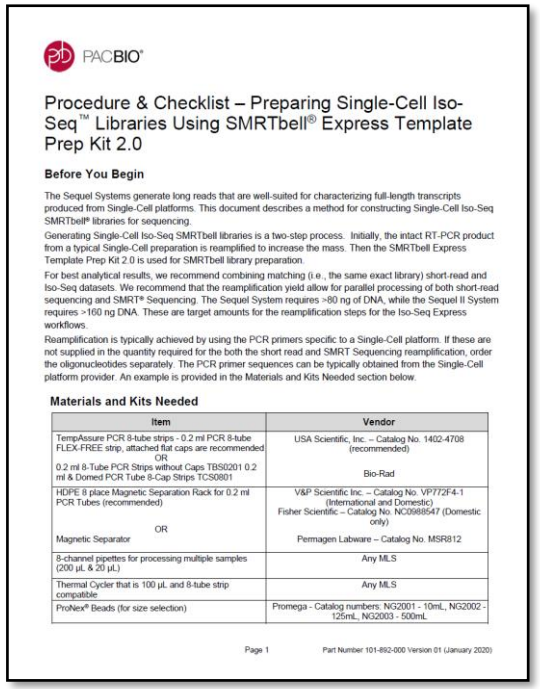
SINGLE-CELL FULL-LENGTH TRANSCRIPT ISOFORM SEQUENCING (scISO-SEQ METHOD): HOW TO GET STARTED

Application-Specific Best Practices Guide → **Application-Specific Procedure & Checklist** → **Application Consumable Bundle Purchasing Guide** → **Library Construction, Sequencing & Analysis**



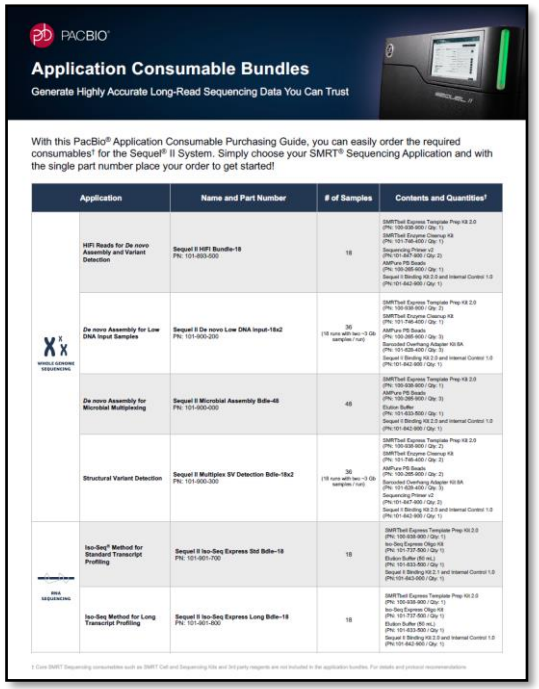
[Application Brief: Single-cell RNA sequencing with HiFi reads - Best Practices](#) (BP109-102020)

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



[Procedure & Checklist – Preparing Single-Cell Iso-Seq™ Libraries Using SMRTbell® Express Template Prep Kit 2.0](#) (101-892-000)

Technical documentation containing sample library construction and sequencing preparation protocol details



[PacBio Application Consumable Bundle Purchasing Guide](#) (PG100-082620)

Purchasing Guide enables users to easily order required consumables needed to prepare a SMRTbell library to run a specific type of application on the Sequel II and IIe Systems*

Single-Cell cDNA Synthesis

Use any third-party single-cell platform to perform cDNA synthesis and amplification

Library Construction (SMRTbell Express TPK 2.0)

Purify Single-Cell Iso-Seq Library Using ProNex Beads

HiFi Sequencing

Generate 1,000 unique reads/single cell for up to 3000 cells per SMRT Cell 8M

Data Analysis

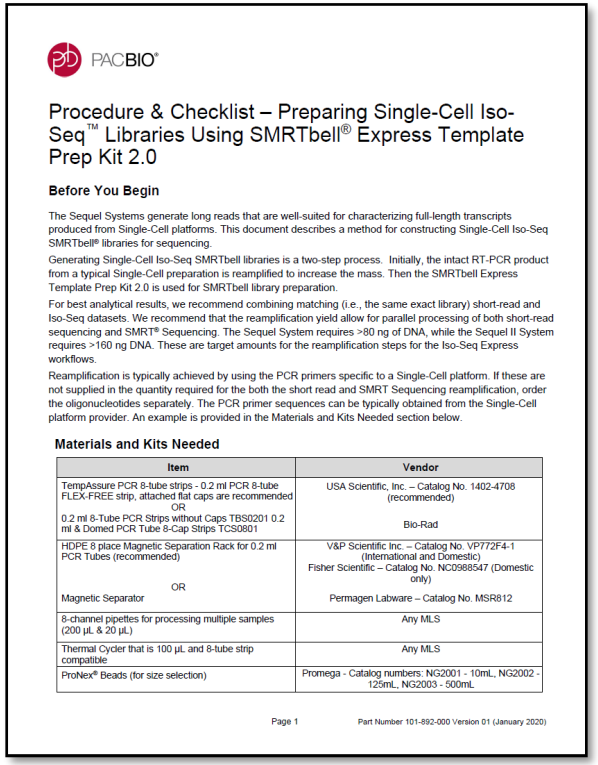
Single-Cell Iso-Seq Analysis Tools ([GitHub](#))

* Application Consumable Bundles include reagents for library construction, primer annealing and polymerase binding. Core PacBio-branded SMRT Sequencing consumables (SMRT Cells, Sequencing Kits & SMRT Oil), plastics and other 3rd-party reagents are not included in the application bundles

Single-Cell Iso-Seq Workflow Overview & Experimental Design Considerations

SINGLE-CELL ISO-SEQ SAMPLE PREPARATION PROCEDURE DESCRIPTION

- Procedure & Checklist – Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0 (PN 101-892-000) PacBio protocol document contains instructions for constructing Single-Cell Iso-Seq SMRTbell libraries for sequencing on the Sequel, Sequel II and Sequel IIe Systems.
- Single-Cell cDNA samples to be used for this procedure are first generated using a third-party single-cell isolation platform.
  - cDNA products from a typical single-cell preparation are initially re-amplified to increase the mass.
  - Following cDNA re-amplification, SMRTbell Express Template Prep Kit 2.0 is then used for SMRTbell library construction.



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Procedure & Checklist – Preparing Single-Cell Iso-Seq™ Libraries Using SMRTbell® Express Template Prep Kit 2.0

Before You Begin

The Sequel Systems generate long reads that are well-suited for characterizing full-length transcripts produced from Single-Cell platforms. This document describes a method for constructing Single-Cell Iso-Seq SMRTbell® libraries for sequencing.

Generating Single-Cell Iso-Seq SMRTbell libraries is a two-step process. Initially, the intact RT-PCR product from a typical Single-Cell preparation is reamplified to increase the mass. Then the SMRTbell Express Template Prep Kit 2.0 is used for SMRTbell library preparation.

For best analytical results, we recommend combining matching (i.e., the same exact library) short-read and Iso-Seq datasets. We recommend that the reamplification yield allow for parallel processing of both short-read sequencing and SMRT® Sequencing. The Sequel System requires >80 ng of DNA, while the Sequel II System requires >160 ng DNA. These are target amounts for the reamplification steps for the Iso-Seq Express workflows.

Reamplification is typically achieved by using the PCR primers specific to a Single-Cell platform. If these are not supplied in the quantity required for the both the short read and SMRT Sequencing reamplification, order the oligonucleotides separately. The PCR primer sequences can be typically obtained from the Single-Cell platform provider. An example is provided in the Materials and Kits Needed section below.

Materials and Kits Needed

Item	Vendor
TempAssure PCR 8-tube strips - 0.2 ml PCR 8-tube FLEX-FREE strip, attached flat caps are recommended OR 0.2 ml 8-Tube PCR Strips without Caps TBS0201 0.2 ml & Domed PCR Tube 8-Cap Strips TCS0801	USA Scientific, Inc. – Catalog No. 1402-4708 (recommended) Bio-Rad
HDPE 8 place Magnetic Separation Rack for 0.2 ml PCR Tubes (recommended) OR Magnetic Separator	V&P Scientific Inc. – Catalog No. VP772F4-1 (International and Domestic) Fisher Scientific – Catalog No. NC0988547 (Domestic only) Permagen Labware – Catalog No. MSR812
8-channel pipettes for processing multiple samples (200 µL & 20 µL)	Any MLS
Thermal Cycler that is 100 µL and 8-tube strip compatible	Any MLS
ProNex® Beads (for size selection)	Promega - Catalog numbers: NG2001 - 10mL, NG2002 - 125mL, NG2003 - 500mL

Page 1 Part Number 101-892-000 Version 01 (January 2020)

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APPLICATIONS

RNA SEQUENCING

Single-Cell Full-Length Transcript Isoform Sequencing (scIso-Seq Method)

SINGLE-CELL ISO-SEQ METHOD FEATURES AND EXPERIMENTAL DESIGN CONSIDERATIONS

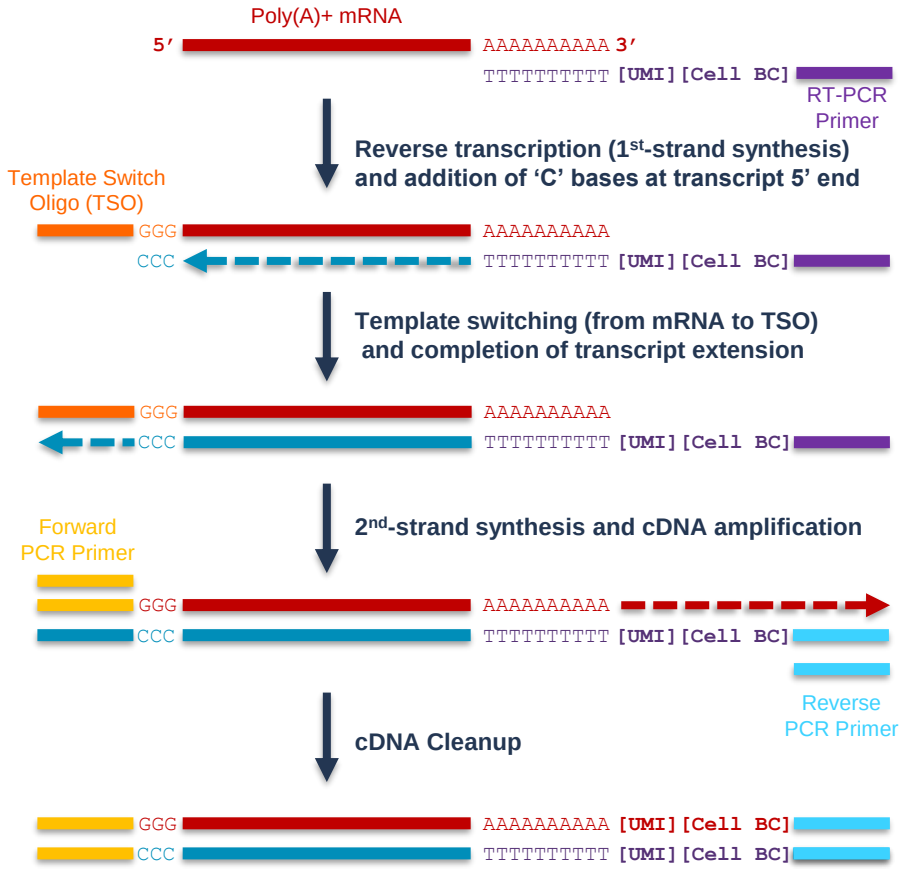
- Generate **full-length transcript isoforms that can be assigned to individual cells** to characterize alternative splicing and cell heterogeneity within tissues.
  - Iso-Seq method uses single-molecule, real-time (SMRT) sequencing technology to produce highly accurate long reads (HiFi reads) using the Sequel, Sequel II and Sequel IIe Systems.
  - Iso-Seq reads can span the entire 5′ to 3′ end of a transcript – *with no assembly required*.
- Although PacBio does not have a specific single-cell partner or system recommendation, in principle, practically **any single-cell platform should be compatible with single-cell Iso-Seq library preparation so long as that platform generates cDNA**.
  - For the Iso-Seq method to achieve full-length cDNAs, it is recommended to use a template-switching oligo (TSO). This is a common technique and is currently used in single-cell platforms and PacBio's current bulk Iso-Seq methods.
- For optimal analytical results, **PacBio recommends combining matching short-read and Iso-Seq datasets** (generated for the same exact single-cell library sample).
  - We recommend that the post-reamplification cDNA yield allow for parallel processing of both short-read sequencing and SMRT Sequencing.
  - The **Sequel System requires >80 ng of cDNA**, while the **Sequel II System requires >160 ng cDNA**. These are target DNA amounts for the PCR re-amplification steps for the Iso-Seq library construction workflow (see Page 4 of the procedure).

SINGLE-CELL ISO-SEQ METHOD FEATURES AND EXPERIMENTAL DESIGN CONSIDERATIONS (CONT.)

- cDNA re-amplification is typically achieved by using the **PCR primers specific to a single-cell isolation platform**.
  - If these are not supplied in the quantity required to generate sufficient cDNA input material for both short-read and SMRT Sequencing library construction, **order additional amounts of primers separately**. (PCR primer oligo sequences can be typically obtained from the single-cell platform provider.)
- A sizing platform such as the Agilent Bioanalyzer is a useful tool for checking the quality of the amplified cDNA; the mean size of a high-quality single-cell SMRTbell library is usually **~1.5 kb with molecules that stretch into the 5–6-kb range**.
- The PacBio single-cell sample prep workflow from cDNA re-amplification to SMRTbell Express library preparation takes **approximately 5 hours** to complete.
- Obtain up to ~3 Million full-length reads with cell barcode and UMI information per Sequel II SMRT Cell 8M
  - 1,000 unique transcript reads / single-cell library prep using 3000 cells input
  - 10,000 unique transcript reads / single cell library prep using 300 cells input

SINGLE-CELL ISO-SEQ WORKFLOW SUMMARY OVERVIEW

Single-Cell cDNA Preparation
(**Third-Party** Single-Cell Isolation System)



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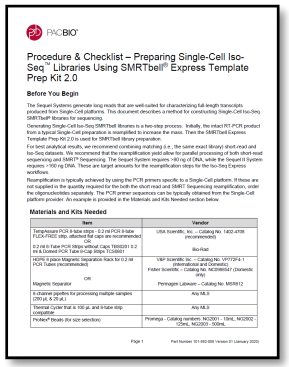
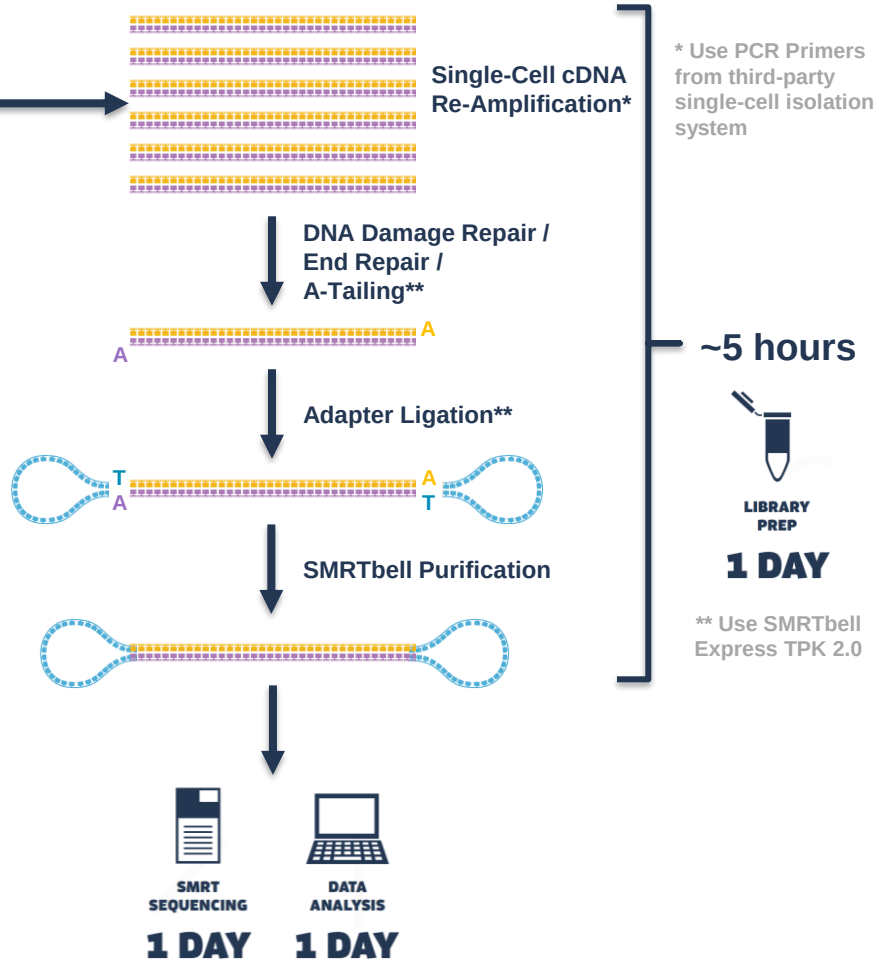

Procedure & Checklist – Preparing Single-Cell Iso-Seq™ Libraries Using SMRTbell® Express Template Prep Kit 2.0

Procedure & Checklist
PN 101-892-000)

Single-Cell SMRTbell Library Preparation
(**PacBio** Sequel, Sequel II and Sequel IIe Systems)

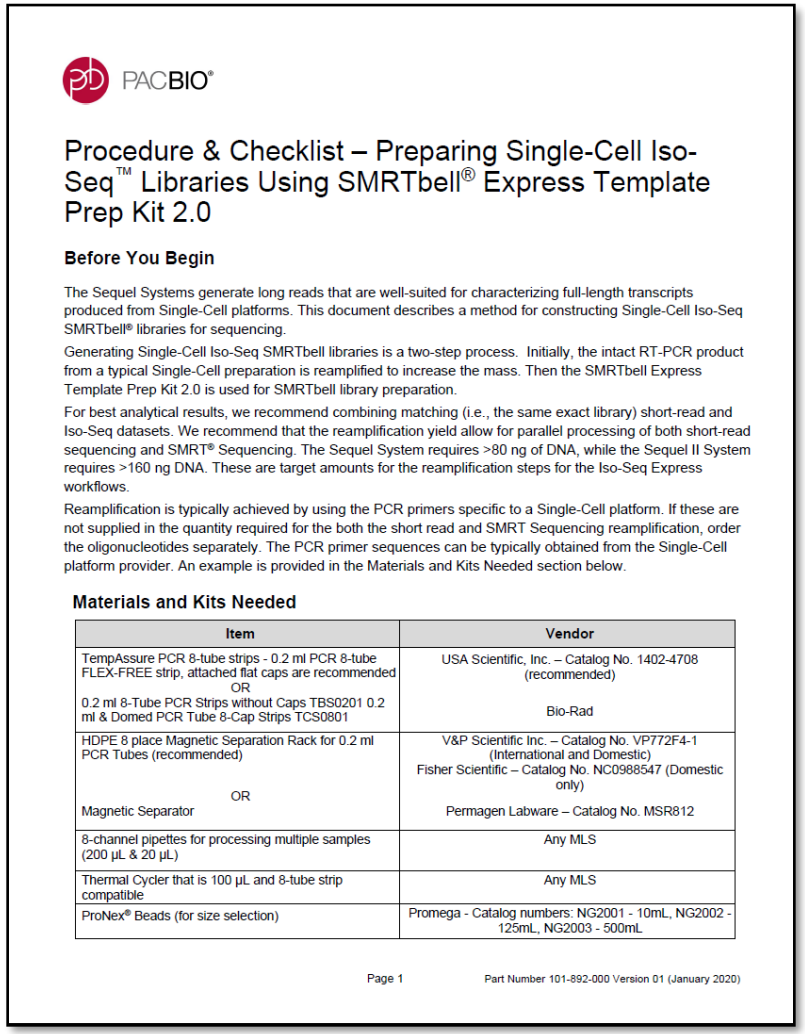


† Pacific Biosciences does not sell a kit for carrying out the Single-Cell RNA Sequencing method. Use of these methods may require rights to third-party owned intellectual property.

Single-Cell Iso-Seq Library Sample Preparation Workflow Details

PROCEDURE & CHECKLIST – PREPARING SINGLE-CELL ISO-SEQ LIBRARIES USING SMRTBELL EXPRESS TEMPLATE PREP KIT 2.0

- This document (PN 101-892-000) contains instructions for constructing Single-Cell Iso-Seq SMRTbell libraries for sequencing on the Sequel, Sequel II and Sequel IIe Systems (Sequel Systems).
- Two-step Single-Cell Iso-Seq sample prep workflow:
  - Intact (un-sheared) RT-PCR products initially generated using a third-party single-cell preparation system are first re-amplified to increase the mass.
  - SMRTbell Express Template Prep Kit 2.0 is then used for SMRTbell library preparation.
- Protocol document contains:
  1. General laboratory best practices and input RNA QC recommendations
  2. Instructions for performing single-cell cDNA re-amplification prior to SMRTbell library construction
  3. Instructions for constructing SMRTbell libraries using amplified single-cell cDNA products and SMRTbell Express Template Prep Kit 2.0
  4. Sample setup guidance for preparing Single-Cell Iso-Seq SMRTbell libraries for sequencing on the Sequel Systems



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Procedure & Checklist – Preparing Single-Cell Iso-Seq™ Libraries Using SMRTbell® Express Template Prep Kit 2.0

Before You Begin

The Sequel Systems generate long reads that are well-suited for characterizing full-length transcripts produced from Single-Cell platforms. This document describes a method for constructing Single-Cell Iso-Seq SMRTbell® libraries for sequencing.

Generating Single-Cell Iso-Seq SMRTbell libraries is a two-step process. Initially, the intact RT-PCR product from a typical Single-Cell preparation is reamplified to increase the mass. Then the SMRTbell Express Template Prep Kit 2.0 is used for SMRTbell library preparation.

For best analytical results, we recommend combining matching (i.e., the same exact library) short-read and Iso-Seq datasets. We recommend that the reamplification yield allow for parallel processing of both short-read sequencing and SMRT® Sequencing. The Sequel System requires >80 ng of DNA, while the Sequel II System requires >160 ng DNA. These are target amounts for the reamplification steps for the Iso-Seq Express workflows.

Reamplification is typically achieved by using the PCR primers specific to a Single-Cell platform. If these are not supplied in the quantity required for the both the short read and SMRT Sequencing reamplification, order the oligonucleotides separately. The PCR primer sequences can be typically obtained from the Single-Cell platform provider. An example is provided in the Materials and Kits Needed section below.

Materials and Kits Needed

Item	Vendor
TempAssure PCR 8-tube strips - 0.2 ml PCR 8-tube FLEX-FREE strip, attached flat caps are recommended OR 0.2 ml 8-Tube PCR Strips without Caps TBS0201 0.2 ml & Domed PCR Tube 8-Cap Strips TCS0801	USA Scientific, Inc. – Catalog No. 1402-4708 (recommended) Bio-Rad
HDPE 8 place Magnetic Separation Rack for 0.2 ml PCR Tubes (recommended) OR Magnetic Separator	V&P Scientific Inc. – Catalog No. VP772F4-1 (International and Domestic) Fisher Scientific – Catalog No. NC0988547 (Domestic only) Permagen Labware – Catalog No. MSR812
8-channel pipettes for processing multiple samples (200 µL & 20 µL)	Any MLS
Thermal Cycler that is 100 µL and 8-tube strip compatible	Any MLS
ProNex® Beads (for size selection)	Promega - Catalog numbers: NG2001 - 10mL, NG2002 - 125mL, NG2003 - 500mL

Page 1 Part Number 101-892-000 Version 01 (January 2020)

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SINGLE-CELL ISO-SEQ WORKFLOW DETAILED OVERVIEW

1. Single-Cell cDNA Synthesis

- Can use any third-party single-cell isolation system that produces full-length cDNA containing cell barcodes and UMIs

2. Single-Cell cDNA Re-Amplification & ProNex Bead Purification

- Follow ***Procedure & Checklist – Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0*** (PN 101-892-000)
- Intact (un-sheared) RT-PCR products initially generated using a third-party single-cell preparation system are re-amplified to increase the mass
- Single-cell library amplification primers for a specific platform may be ordered from any oligo synthesis vendor
- The amount of ProNex beads to use to purify the amplified cDNA depends on the distribution of transcripts produced by the single-cell cDNA preparation and the goal of the experiment.

3. SMRTbell Express TPK 2.0 Library Construction

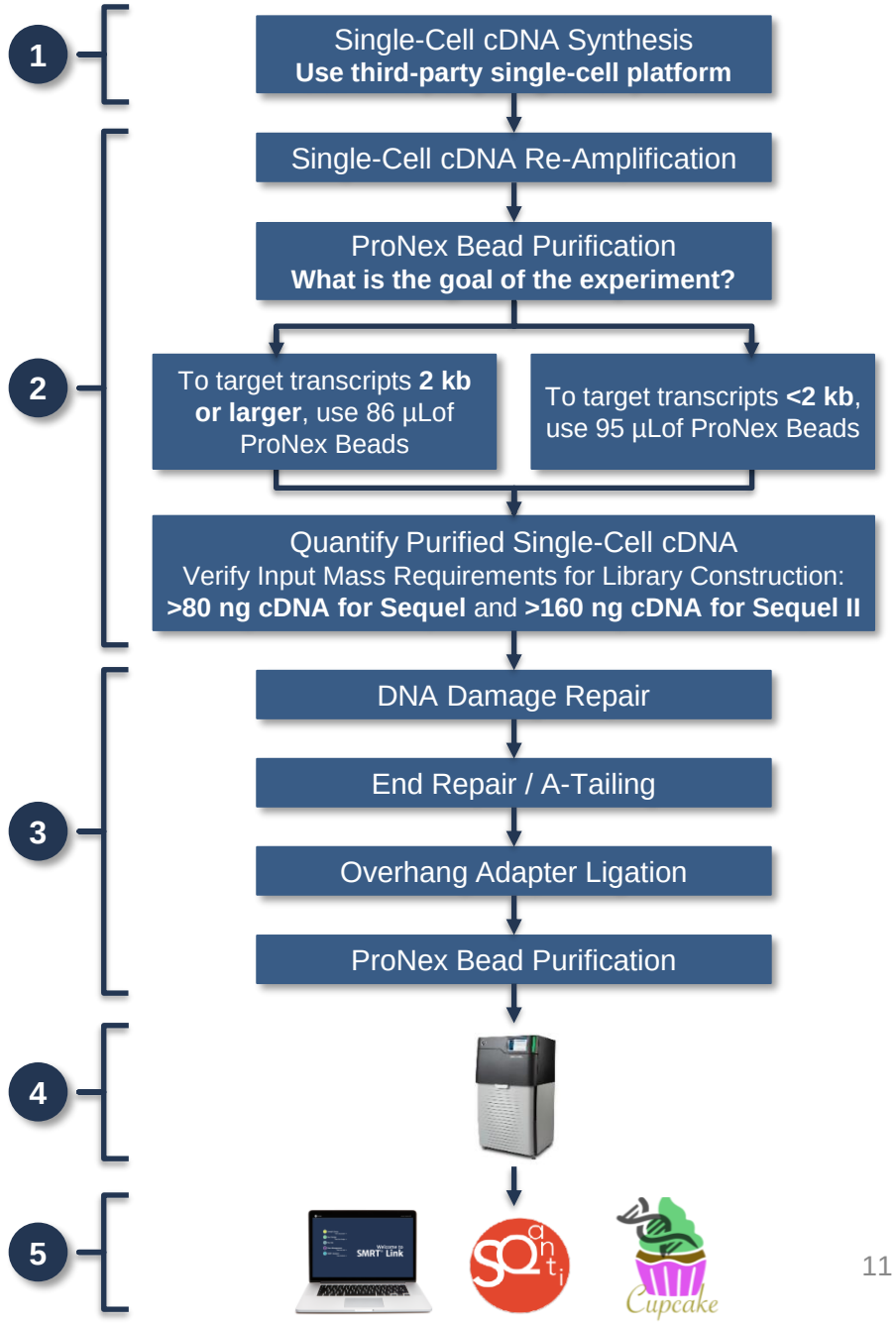
- Single-tube, addition-only reactions
- Typical library yield ≥40%

4. Sequencing Preparation

- Anneal sequencing primer, bind polymerase, perform ProNex bead complex cleanup
- Generate up to ~3 Million full-length single-cell transcript reads per Sequel II SMRT Cell 8M

5. Data Analysis

- De-multiplex barcodes within SMRT Link GUI or on the command line
- Single-cell data analysis can be performed using third-party software such as *SQANTI* and *cDNA_cupcake*



LIST OF REQUIRED EQUIPMENT AND MATERIALS FOR cDNA RE-AMPLIFICATION AND SMRTBELL LIBRARY CONSTRUCTION

ITEM	VENDOR	PROTOCOL STEP(S)
TempAssure PCR 8-tube strips - 0.2 ml PCR 8-tube FLEX-FREE strip, attached flat caps are recommended; ***OR*** 0.2 ml 8-Tube PCR Strips without Caps 0.2 ml & Domed PCR Tube 8-Cap Strips	USA Scientific, Inc. – PN 1402-4708 (recommended) Bio-Rad – PN TBS0201, TCS0801	cDNA Re-amplification; ProNex Bead Purification, SMRTbell Library Construction
HDPE 8 place Magnetic Separation Rack for 0.2 ml PCR Tubes (recommended); **OR** 0.2 mL PCR Strip Magnetic Separator 8 or 12 Strip	V&P Scientific Inc. – PN VP772F4-1 (International and Domestic) or Fisher Scientific – PN NC0988547 (Domestic only) Permagen Labware – PN MSR812	ProNex Bead Purification
ProNex Beads (for size selection)	Promega - PNs: NG2001 - 10mL, NG2002 - 125mL, NG2003 - 500mL	ProNex Bead Purification
Elution Buffer (50 mL)	PacBio PN 101-633-500	ProNex Bead Purification
Ethanol	Any MLS	ProNex Bead Purification
Qubit dsDNA HS Assay Kit	Invitrogen	DNA quantitation QC
Qubit Fluorometer	Invitrogen	DNA quantitation QC
High Sensitivity DNA Kit	Agilent	DNA sizing QC
Bioanalyzer Instrument	Agilent	DNA sizing QC
8-channel pipettes for processing multiple samples (200 µL & 20 µL)	Any MLS	cDNA Re-amplification; ProNex Bead Purification, SMRTbell Library Construction
Thermal cycler that is compatible with 8-tube strips (100 µL volume)	Any MLS	cDNA Re-amplification; SMRTbell Library Construction
SMRTbell Express Template Prep Kit 2.0	Pacific Biosciences	SMRTbell Library Construction
NEBNext High-Fidelity 2X PCR Master Mix (for additional PCR reactions)	NEB PN M0541S	cDNA Re-amplification
Single-Cell cDNA library amplification primers for a specific platform may be ordered from any oligo synthesis company† **Note:** Commercial vendors may have different sequences for their PCR Primers. Please consult your vendor for the specific primer(s) sequence(s).	Single-cell vendor-specific	**Example 1: 10X Chromium Single Cell 3' V2 and V3 PCR Primers** Forward PCR primer (Primer 1): 5'- CTACACGACGCTCTTCCGATCT -3' Reverse PCR primer (Primer 2): 5'- AAGCAGTGGTATCAACGCAGAGT -3' **Example 2: Drop-Seq uses the same sequence for PCR Primer 1 and Primer 2:** 5'- AAGCAGTGGTATCAACGCAGAGT -3' [Macosko, et. al. (2015) Cell. 161:1202-1214]

† Pacific Biosciences does not sell a kit for carrying out the Single-Cell RNA Sequencing method. Use of these methods may require rights to third-party owned intellectual property.

BEST PRACTICES RECOMMENDATIONS FOR PREPARING SINGLE-CELL ISO-SEQ SMRTBELL LIBRARIES

1. Always set your heat blocks or thermocyclers to the appropriate temperature for incubations **before** proceeding with the procedure.
2. ProNex beads:
   a. Equilibrate the ProNex beads at room temperature for 30 mins prior to use.
   b. It is critical to **accurately pipette** ProNex beads because small changes in volume can significantly alter the size distribution of your sample.
3. When performing ProNex bead purification steps, note that 80% ethanol is **hygroscopic** and should be prepared FRESH to achieve optimal results.
4. Using a multi-channel pipettor greatly enhances the ease of processing more than 1 sample.
5. Measure DNA concentration using a **Qubit fluorometer** and **Qubit dsDNA High Sensitivity (HS) Assay Kit** reagents as recommended by the manufacturer.

PREPARATION OF SINGLE-CELL cDNA PCR RE-AMPLIFICATION REACTION

PCR Reaction Preparation and Thermal Cycling Conditions

1. On ice, prepare Reaction Mix 1 by adding the following components in the order listed.

Reaction Mix 1	Volume	✓	Notes
NEBNext® High-Fidelity 2X PCR Master Mix	50 µL		
12 µM PCR Primer 1	2 µL		
12 µM PCR Primer 2	2 µL		
Single-Cell cDNA	≤46 µL		
H_2O	Up to 100 µL		
Total Volume	100 µL		

- Total single-cell (SC) cDNA input mass depends on the specific third-party SC platform and input # of cells used for the SC cDNA preparation.
- PacBio recommends using a **maximum input of ~5000 cells** per SC Iso-Seq library preparation

2. Gently vortex by performing two 2-second pulses and then perform a quick spin to collect all liquid from the sides of the tube.
3. Place the tubes in a thermocycler and run the following program (lid temperature = 105°C):

PCR Program	
45 seconds at 98°C	1 cycle
10 seconds at 98°C	6 cycles*
15 seconds at 62°C	
3 minutes at 72°C	
5 minutes at 72°C	1 cycle
Hold at 4°C	

* Target yield is approximately 200 ng. The number of cycles will vary significantly across systems, number of cells, etc.

- 6 cycles is a recommended starting point.
- If > 500 ng is routinely obtained, **reduce** the number of PCR cycles for future experiments.
- If < 200 ng is routinely obtained **increase** the number of PCR cycles for future experiments.

PURIFICATION OF RE-AMPLIFIED SINGLE-CELL cDNA PRODUCTS

The specific method chosen to purify the re-amplified single-cell cDNA depends on the goal of the experiment and the expected size distribution of transcripts.

- Use **ProNex beads** for purification of amplified cDNA products according to the table below:

GOAL OF EXPERIMENT	PRONEX BEAD VOLUME
1) Single-cell cDNA sample is composed of transcripts centered at ~2 kb or larger; or 2) A reduction in shorter transcripts is desired.	86 µL
1) Single-cell cDNA sample composed primarily of transcripts <2 kb; or 2) No reduction in shorter transcripts is desired.	95 µL

- After purification, perform a sizing QC by running 1 µL of the purified cDNA products on a Bioanalyzer system using a High Sensitivity DNA kit.
- Examining the amplified cDNA on a Bioanalyzer prior to PacBio library construction is an excellent quality control step to ensure that the amplified cDNA material has the expected size distribution.

QUANTITATION OF RE-AMPLIFIED SINGLE-CELL cDNA PRODUCTS

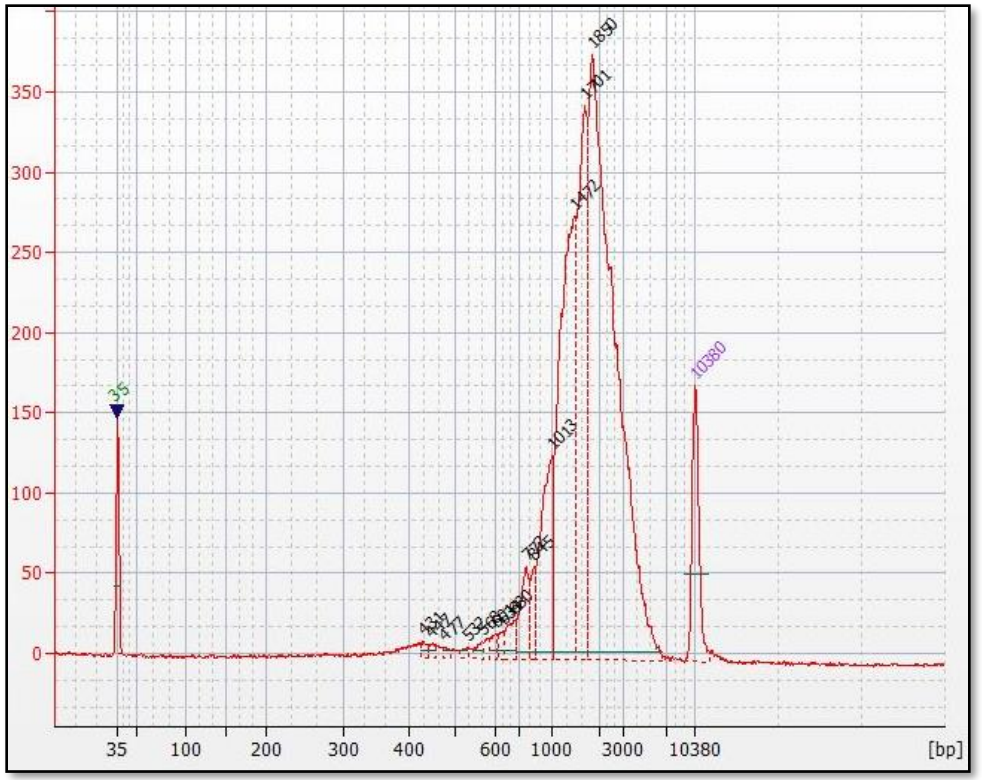
Use a Qubit dsDNA HS assay kit to verify that you have the required mass of purified single-cell cDNA to proceed with SMRTbell library construction

INSTRUMENT	MINIMUM cDNA SAMPLE AMOUNT	RECOMMENDATION FOR SAMPLES WITH LOW YIELD
Sequel System	80 – 500 ng	If total mass is <80 ng (<1.75 ng/µL) → Go to **Appendix 1**
Sequel II and IIe Systems	160 – 500 ng	If total mass is <160 ng (<3.5 ng/µL) → Go to **Appendix 1**

- **Appendix 1: Recommendations for Additional cDNA Amplification by PCR for Samples with a Lower Yield:**
  - The Sequel System and Sequel II and IIe Systems require different amounts (ng) of cDNA for SMRTbell library construction. The Sequel System requires **>80 ng** of cDNA, while the Sequel II and IIe Systems require **>160 ng** of cDNA.
  - If there is not enough DNA to proceed with library construction, refer to **Appendix 1** of the procedure which describes a workflow for enriching cDNA by performing additional PCR cycles.
- Note: PCR over-amplification can result in sub-optimal data.
  - If >500 ng is routinely obtained after cDNA re-amplification, reduce the number of PCR cycles for future experiments.

SIZING QC ANALYSIS OF RE-AMPLIFIED SINGLE-CELL cDNA PRODUCTS

Examining the amplified cDNA on a Bioanalyzer system *prior* to proceeding with PacBio SMRTbell library construction is an excellent quality control step to ensure that the amplified cDNA material has the expected size distribution.



Bioanalyzer sizing QC analysis of a re-amplified single-cell cDNA sample. The re-amplified cDNA sample was purified using 0.95X ProNex beads (95 µL ProNex beads + 100 µL cDNA sample). The average size of the purified single-cell cDNA sample is 1.9 kb

SMRTBELL EXPRESS TEMPLATE PREP KIT 2.0 REAGENT HANDLING RECOMMENDATIONS

- Several reagents in the kits are sensitive to temperature and vortexing
- PacBio highly recommends:
  - Never leaving reagents at room temperature
  - Working on ice at all times when preparing master mixes
  - Finger tapping followed by a quick-spin prior to use

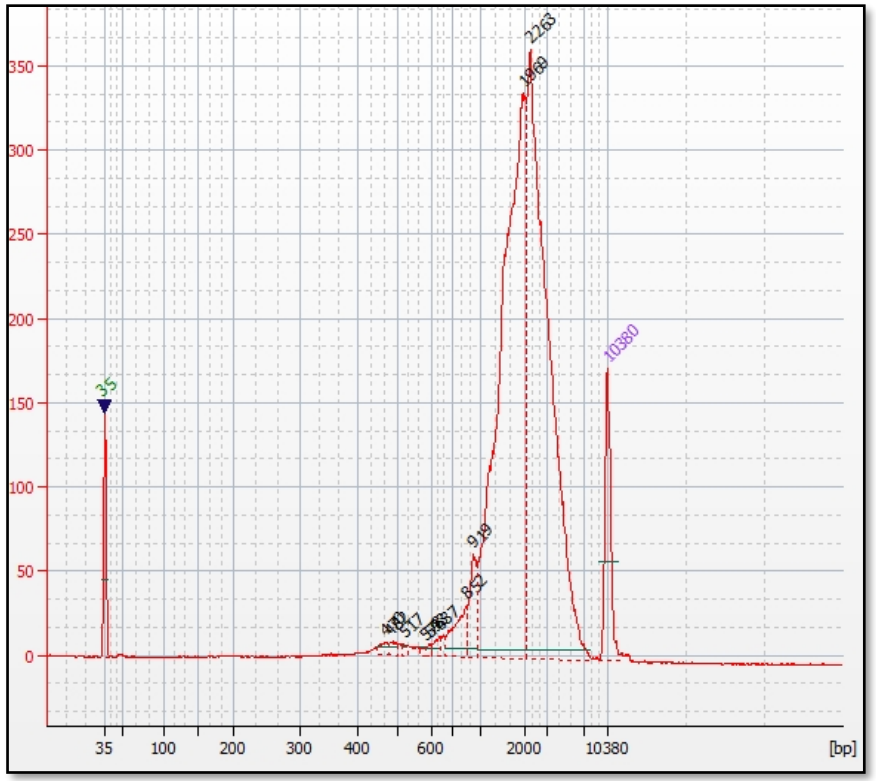
SMRTbell Express TPK 2.0

LIST OF TEMPERATURE-SENSITIVE REAGENTS INCLUDED IN SMRTBELL EXPRESS TPK 2.0.

REAGENT	WHERE USED
DNA Damage Repair Mix v2	DNA Damage Repair
End Prep Mix	End-Repair/A-tailing
Overhang Adapter v3	Ligation
Ligation Mix	Ligation
Ligation Additive	Ligation
Ligation Enhancer	Ligation

SIZING QC ANALYSIS OF FINAL SINGLE-CELL ISO-SEQ SMRTBELL LIBRARIES AFTER PRONEX BEAD PURIFICATION

Characterize the final Single-Cell Iso-Seq SMRTbell library on a Bioanalyzer system *prior* to proceeding with sequencing to ensure that the purified library template material has the expected size distribution.



Bioanalyzer sizing QC analysis of a purified Single-Cell Iso-Seq SMRTbell Express TPK 2.0 library sample. The final Single-Cell Iso-Seq library sample was purified using 1X ProNex beads. The average size of the purified SMRTbell library sample is 2.2 kb

SINGLE-CELL ISO-SEQ SMRTBELL LIBRARY CONSTRUCTION YIELDS

- Overall, SMRTbell library yields in this Single-Cell Iso-Seq workflow are typically **50%** starting from re-amplified single-cell cDNA input into the first enzymatic step (DNA Damage Repair)
- Sufficient amounts of SMRTbell template material can typically be generated using this procedure to run the following numbers of SMRT Cells (per Single-Cell Iso-Seq library preparation reaction):
  - **Sequel System:** ≥3 SMRT Cells 1M
  - **Sequel II and IIe Systems:** ≥1 SMRT Cells 8M

Single-Cell Iso-Seq Library Sequencing Workflow Details

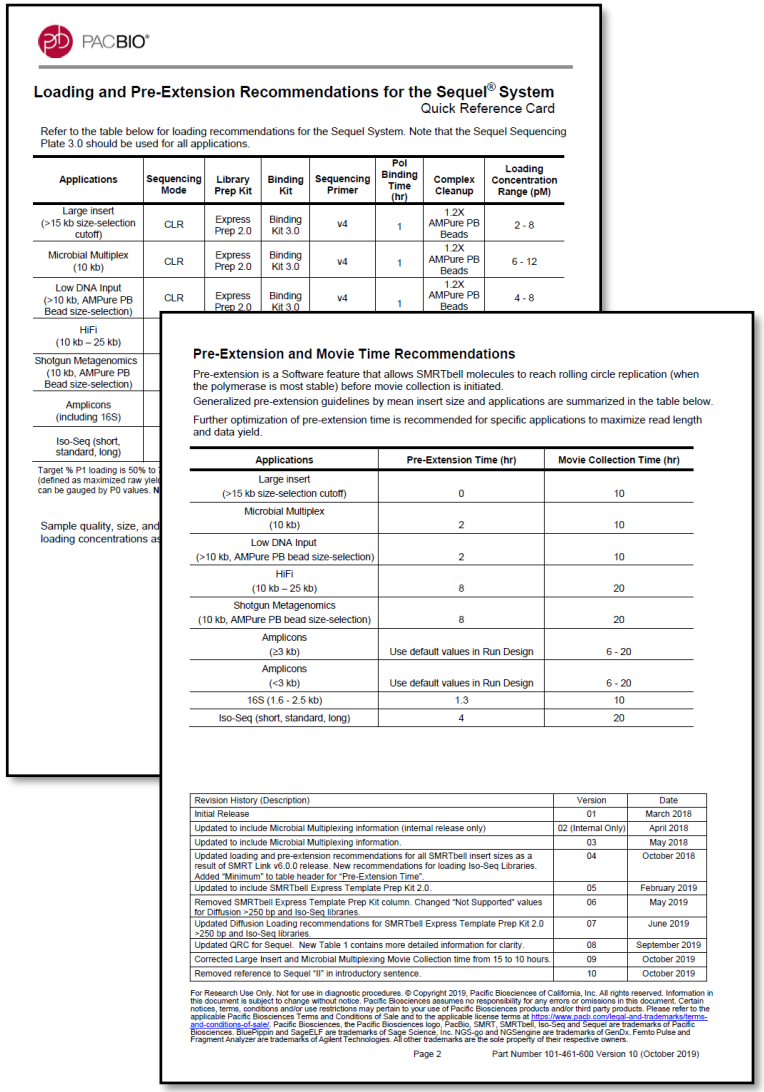
SAMPLE SETUP RECOMMENDATIONS FOR SINGLE-CELL ISO-SEQ LIBRARIES – SEQUEL SYSTEM (CHEMISTRY 3.0)

- Follow **SMRT Link Sample Setup** instructions using the recommendations provided in the Quick Reference Card – Loading and Pre-Extension Time Recommendations for the Sequel System for sequencing bulk Iso-Seq samples.

Applications	Sequencing Mode	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
Iso-Seq (short, standard, long)	CCS	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X ProNex	2 - 8

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
Iso-Seq (short, standard, long)	4	20

* PacBio recommends **Sequel Binding Kit 3.0** for all Iso-Seq Express workflows (Short, Standard, and Long)



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Loading and Pre-Extension Recommendations for the Sequel® System
Quick Reference Card

Refer to the table below for loading recommendations for the Sequel System. Note that the Sequel Sequencing Plate 3.0 should be used for all applications.

Applications	Sequencing Mode	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
Large insert (>15 kb size-selection cutoff)	CLR	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	2 - 8
Microbial Multiplex (10 kb)	CLR	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	6 - 12
Low DNA Input (>10 kb, AMPure PB Bead size-selection)	CLR	Express Prep 2.0	Binding Kit 3.0	v4	1	1.2X AMPure PB Beads	4 - 8
HiFi (10 kb – 25 kb)							
Shotgun Metagenomics (10 kb, AMPure PB Bead size-selection)							
Amplicons (including 16S)							
Iso-Seq (short, standard, long)							

Target % P1 loading is 50% to
(defined as maximized raw yiel
can be gauged by P0 values. N

Sample quality, size, and
loading concentrations as

Pre-Extension and Movie Time Recommendations

Pre-extension is a Software feature that allows SMRTbell molecules to reach rolling circle replication (when the polymerase is most stable) before movie collection is initiated.
Generalized pre-extension guidelines by mean insert size and applications are summarized in the table below.

Further optimization of pre-extension time is recommended for specific applications to maximize read length and data yield.

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
Large insert (>15 kb size-selection cutoff)	0	10
Microbial Multiplex (10 kb)	2	10
Low DNA Input (>10 kb, AMPure PB bead size-selection)	2	10
HiFi (10 kb – 25 kb)	8	20
Shotgun Metagenomics (10 kb, AMPure PB bead size-selection)	8	20
Amplicons (≥3 kb)	Use default values in Run Design	6 - 20
Amplicons (<3 kb)	Use default values in Run Design	6 - 20
16S (1.6 - 2.5 kb)	1.3	10
Iso-Seq (short, standard, long)	4	20

Revision History (Description)	Version	Date
Initial Release	01	March 2018
Updated to include Microbial Multiplexing information (internal release only)	02 (Internal Only)	April 2018
Updated to include Microbial Multiplexing information.	03	May 2018
Updated loading and pre-extension recommendations for all SMRTbell insert sizes as a result of SMRT Link v6.0.0 release. New recommendations for loading Iso-Seq Libraries. Added "Minimum" to table header for "Pre-Extension Time".	04	October 2018
Updated to include SMRTbell Express Template Prep Kit 2.0.	05	February 2019
Removed SMRTbell Express Template Prep Kit column. Changed "Not Supported" values for Diffusion >250 bp and Iso-Seq libraries.	06	May 2019
Updated Diffusion Loading recommendations for SMRTbell Express Template Prep Kit 2.0 >250 bp and Iso-Seq libraries.	07	June 2019
Updated QRC for Sequel. New Table 1 contains more detailed information for clarity.	08	September 2019
Corrected Large Insert and Microbial Multiplexing Movie Collection time from 15 to 10 hours.	09	October 2019
Removed reference to Sequel "II" in introductory sentence.	10	October 2019

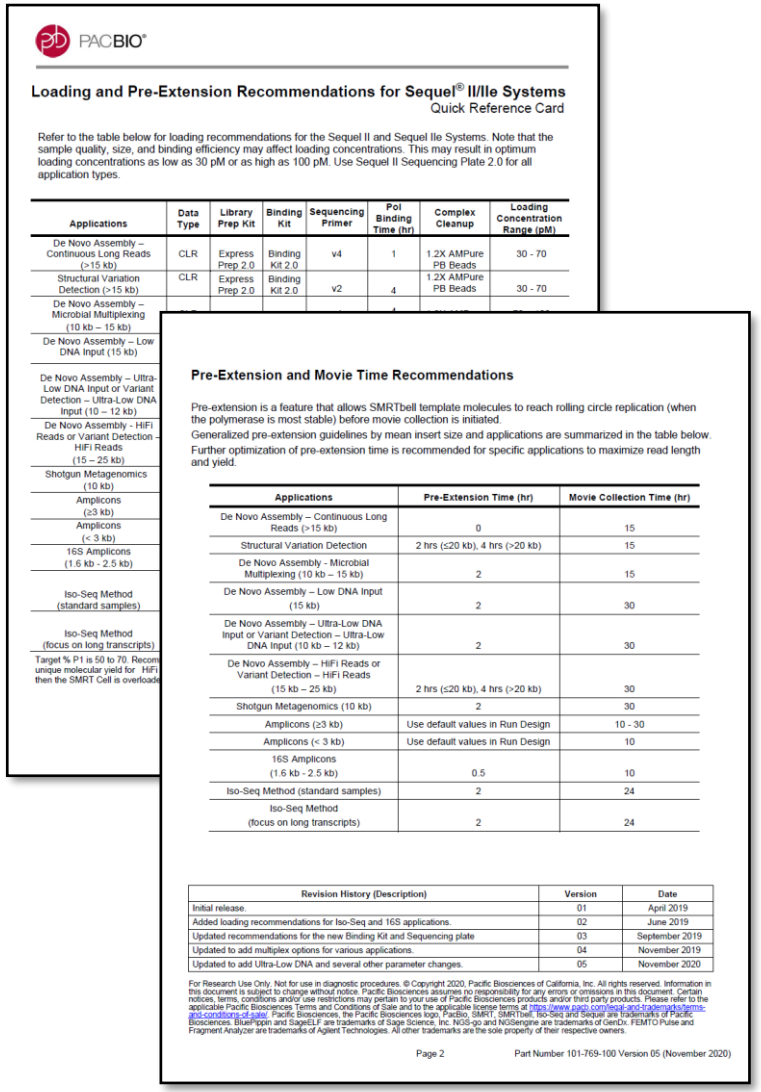
SAMPLE SETUP RECOMMENDATIONS FOR SINGLE-CELL ISO-SEQ LIBRARIES – SEQUEL II AND IIe SYSTEMS (CHEMISTRY 2.0)

- Follow **SMRT Link Sample Setup** instructions using the recommendations provided in the Quick Reference Card – Loading and Pre-Extension Time Recommendations for the Sequel II and IIe Systems for sequencing bulk Iso-Seq samples.
  - For **SMRT Link v10.0** (or higher): Select **'Iso-Seq Method'** from the **Application** field drop-down menu in the SMRT Link Sample Setup and SMRT Link Run Design user interface

Applications	Data Type	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
Iso-Seq Method (standard samples)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X ProNex Beads	40 - 80
Iso-Seq Method (focus on long transcripts)	CCS	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X ProNex Beads	50 - 100

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
Iso-Seq Method (standard samples)	2	24
Iso-Seq Method (focus on long transcripts)	2	24

* PacBio recommends **Sequel II Binding Kit 2.1 for standard** bulk Iso-Seq and Single-Cell Iso-Seq samples. For bulk Iso-Seq and Single-Cell Iso-Seq samples with a focus on **long transcripts**, PacBio recommends **Sequel II Binding Kit 2.0**



PACBIO

Loading and Pre-Extension Recommendations for Sequel® II/IIe Systems
Quick Reference Card

Refer to the table below for loading recommendations for the Sequel II and Sequel IIe Systems. Note that the sample quality, size, and binding efficiency may affect loading concentrations. This may result in optimum loading concentrations as low as 30 pM or as high as 100 pM. Use Sequel II Sequencing Plate 2.0 for all application types.

Applications	Data Type	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
De Novo Assembly – Continuous Long Reads (>15 kb)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Structural Variation Detection (>15 kb)	CLR	Express Prep 2.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	30 - 70
De Novo Assembly – Microbial Multiplexing (10 kb – 15 kb)							
De Novo Assembly – Low DNA Input (15 kb)							
De Novo Assembly – Ultra-Low DNA Input or Variant Detection – Ultra-Low DNA Input (10 – 12 kb)							
De Novo Assembly - HiFi Reads or Variant Detection – HiFi Reads (15 – 25 kb)							
Shotgun Metagenomics (10 kb)							
Amplicons (≥3 kb)							
Amplicons (< 3 kb)							
16S Amplicons (1.6 kb - 2.5 kb)							
Iso-Seq Method (standard samples)							
Iso-Seq Method (focus on long transcripts)							

Target % P1 is 50 to 70. Recom
unique molecular yield for HiFi
then the SMRT Cell is overload

Pre-Extension and Movie Time Recommendations

Pre-extension is a feature that allows SMRTbell template molecules to reach rolling circle replication (when the polymerase is most stable) before movie collection is initiated.
Generalized pre-extension guidelines by mean insert size and applications are summarized in the table below.
Further optimization of pre-extension time is recommended for specific applications to maximize read length and yield.

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
De Novo Assembly – Continuous Long Reads (>15 kb)	0	15
Structural Variation Detection	2 hrs (≤20 kb), 4 hrs (>20 kb)	15
De Novo Assembly - Microbial Multiplexing (10 kb – 15 kb)	2	15
De Novo Assembly – Low DNA Input (15 kb)	2	30
De Novo Assembly – Ultra-Low DNA Input or Variant Detection – Ultra-Low DNA Input (10 kb – 12 kb)	2	30
De Novo Assembly – HiFi Reads or Variant Detection – HiFi Reads (15 kb – 25 kb)	2 hrs (≤20 kb), 4 hrs (>20 kb)	30
Shotgun Metagenomics (10 kb)	2	30
Amplicons (≥3 kb)	Use default values in Run Design	10 - 30
Amplicons (< 3 kb)	Use default values in Run Design	10
16S Amplicons (1.6 kb - 2.5 kb)	0.5	10
Iso-Seq Method (standard samples)	2	24
Iso-Seq Method (focus on long transcripts)	2	24

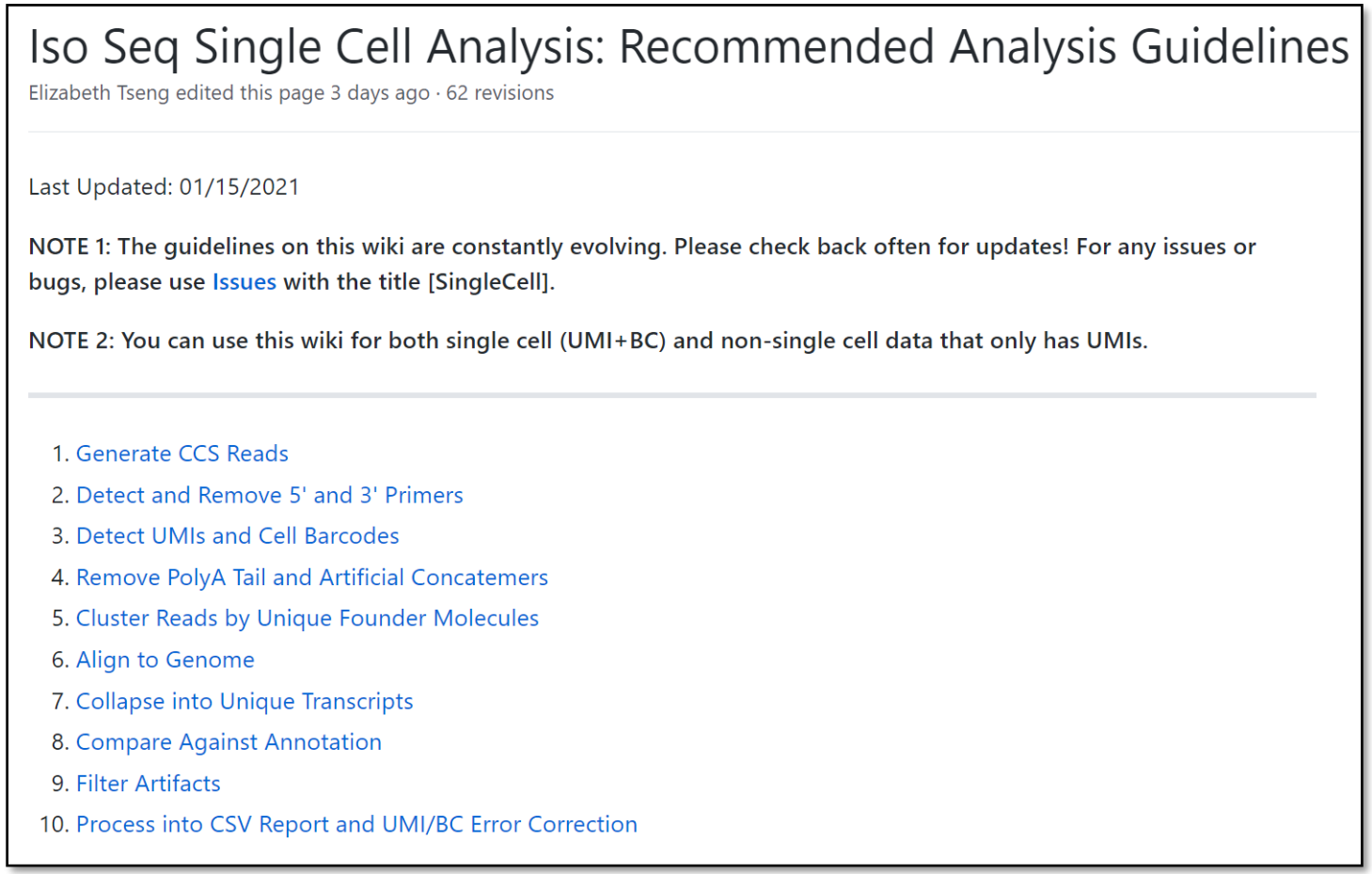
Revision History (Description)	Version	Date
Initial release.	01	April 2019
Added loading recommendations for Iso-Seq and 16S applications.	02	June 2019
Updated recommendations for the new Binding Kit and Sequencing plate	03	September 2019
Updated to add multiplex options for various applications.	04	November 2019
Updated to add Ultra-Low DNA and several other parameter changes.	05	November 2020

Part Number 101-769-100 Version 05 (November 2020)

Single-Cell Iso-Seq Data Analysis Recommendations

SINGLE-CELL ISO-SEQ DATA ANALYSIS RECOMMENDATIONS AND GUIDELINES

Data analysis guidelines for Single-Cell Iso-Seq applications can be found on PacBio's GitHub website.



Iso Seq Single Cell Analysis: Recommended Analysis Guidelines

Elizabeth Tseng edited this page 3 days ago · 62 revisions

Last Updated: 01/15/2021

NOTE 1: The guidelines on this wiki are constantly evolving. Please check back often for updates! For any issues or bugs, please use Issues with the title [SingleCell].

NOTE 2: You can use this wiki for both single cell (UMI+BC) and non-single cell data that only has UMIs.

1. Generate CCS Reads
2. Detect and Remove 5' and 3' Primers
3. Detect UMIs and Cell Barcodes
4. Remove PolyA Tail and Artificial Concatemers
5. Cluster Reads by Unique Founder Molecules
6. Align to Genome
7. Collapse into Unique Transcripts
8. Compare Against Annotation
9. Filter Artifacts
10. Process into CSV Report and UMI/BC Error Correction

Single-Cell Iso-Seq Bioinformatics Tutorial: https://github.com/Magdoll/cDNA_Cupcake/wiki/Iso-Seq-Single-Cell-Analysis:-Recommended-Analysis-Guidelines

STRUCTURAL DIFFERENCES BETWEEN BULK ISO-SEQ *VS.* SINGLE-CELL ISO-SEQ TRANSCRIPT DATA

Bulk Iso-Seq Transcripts

Poly(A+) mRNA

↓

Full-Length 1st-Strand cDNA Synthesis

↓

Full-Length cDNA Amplification

Use Barcoded PCR Primers If Multiplexing Bulk Iso-Seq Samples

Pool Barcoded cDNA if Multiplexing

↓

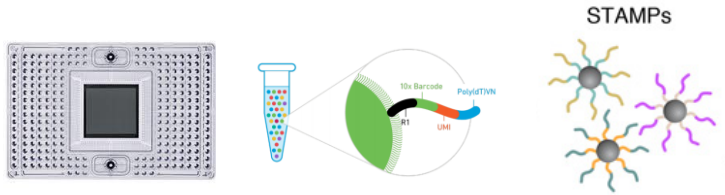
SMRTbell TPK 2.0 Library Construction

↓

SMRT Sequencing

Single-Cell Iso-Seq (scIso-Seq) Transcripts

Any single-cell platform that generates full-length cDNA can be used for sequencing with PacBio

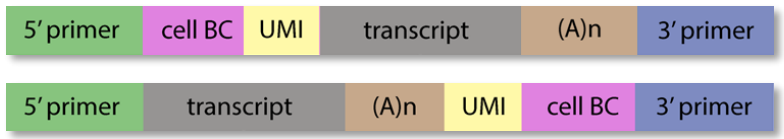


Full-Length cDNA Re-Amplification

Use PCR Primers Specific to single-cell Isolation System

SMRTbell TPK 2.0 Library Construction

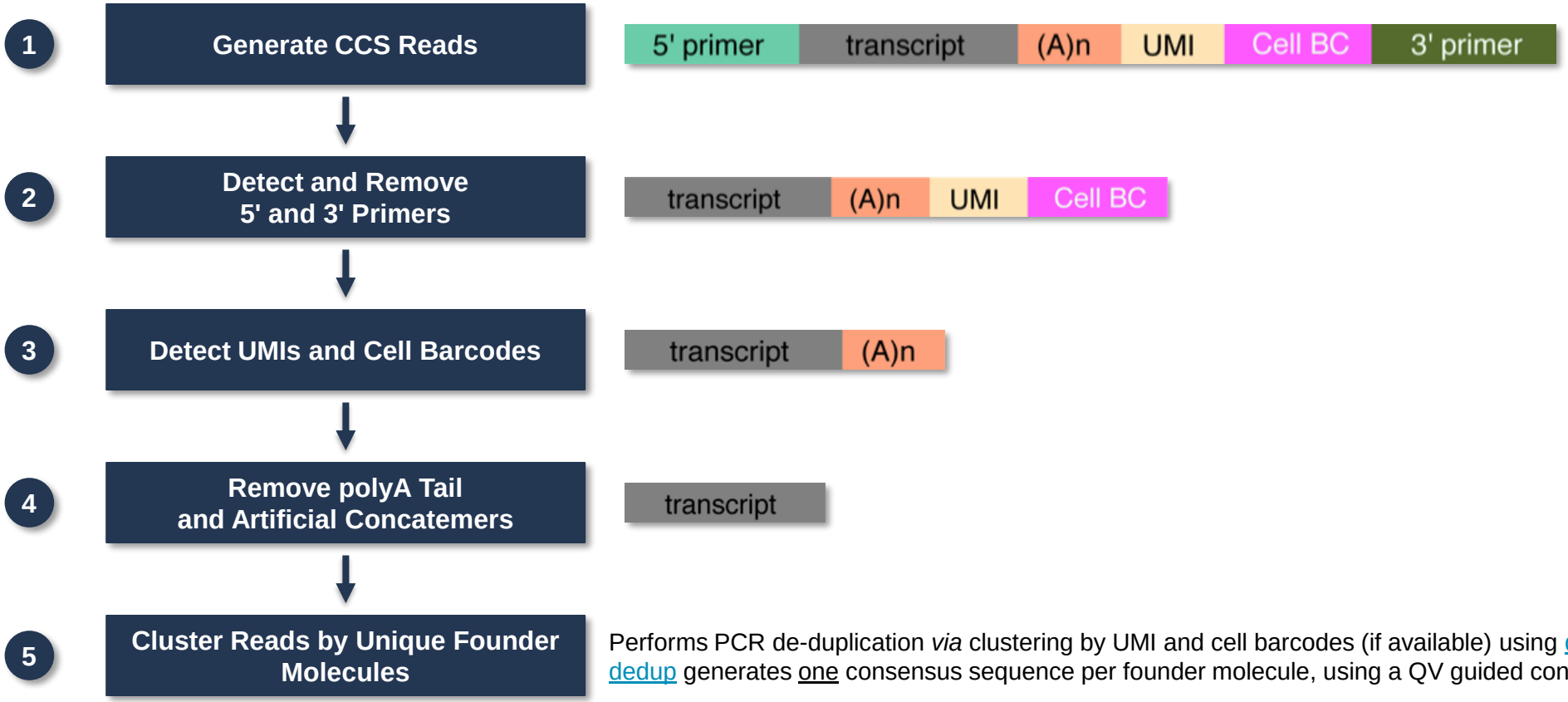
SMRT Sequencing



Transcript structure depends on single-cell platform used

SINGLE-CELL ISO-SEQ DATA ANALYSIS WORKFLOW SUMMARY OVERVIEW

Single-Cell Iso-Seq Bioinformatics Tutorial on Github assumes that the UMIs and cell barcodes (BCs) are on the 3' end (between the polyA tail and 3' primer). The UMIs and BCs can be of any length.

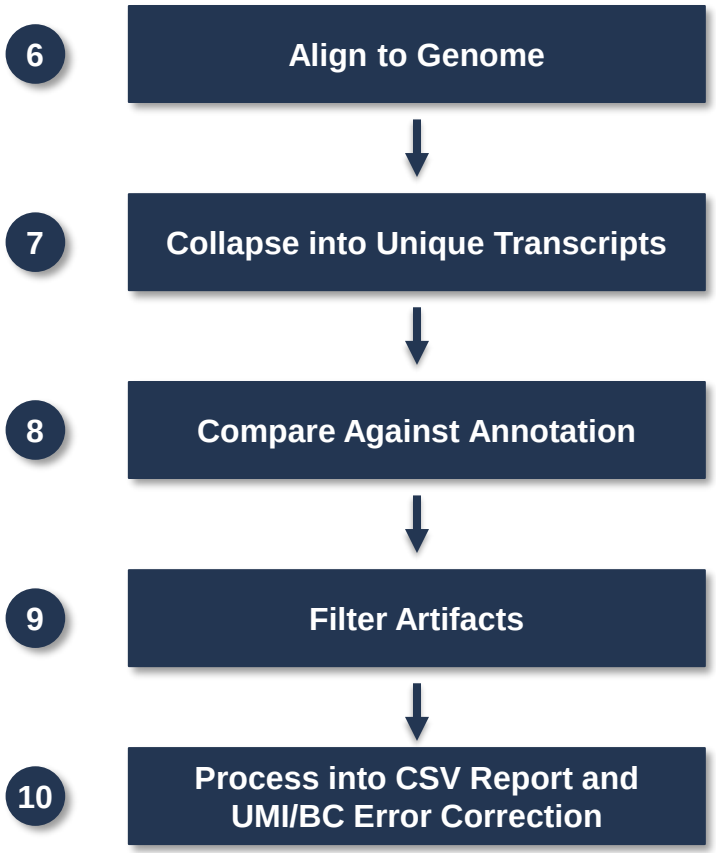


Performs PCR de-duplication *via* clustering by UMI and cell barcodes (if available) using dedup. After deduplication, dedup generates one consensus sequence per founder molecule, using a QV guided consensus approach

See Single-Cell Iso-Seq Bioinformatics Tutorial for data analysis pre-requisites and specific step-by-step instructions: https://github.com/Magdoll/cDNA_Cupcake/wiki/Iso-Seq-Single-Cell-Analysis:-Recommended-Analysis-Guidelines

SINGLE-CELL ISO-SEQ DATA ANALYSIS WORKFLOW SUMMARY OVERVIEW (CONT.)

The following Single-Cell Iso-Seq analysis steps below are **optional**.



Use minimap2 (included in SMRT Analysis), desalt, GMAP, STAR, or BLAT etc. See aligner tutorial on PacBio GitHub website for additional recommendations.

Use Cupcake to collapse mapped FLNC reads into unique transcripts. Read the Cupcake wiki for more details on each script.

Use SQANTI3 to annotate each unique transcript against an annotation. To run SQANTI3, you must provide a GTF annotation (such as GENCODE) and a reference genome FASTA.

OPTIONAL: Can use SQANTI3 to remove cDNA library construction artifacts (e.g., intra-priming artifacts, RT switching artifacts, TSO artifacts, etc.

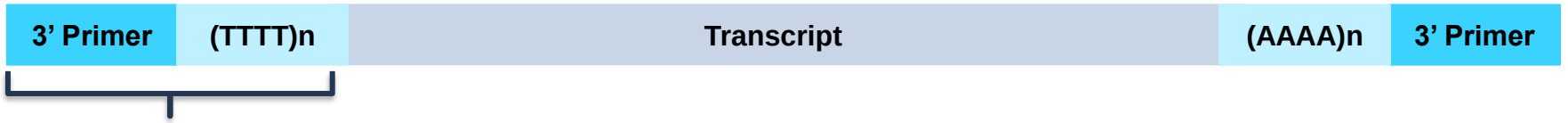
Can use cDNA_Cupcake/single-cell scripts to generate a collated CSV file that links each mapped FLNC read to its classified genes and transcripts based on SQANTI3's output

See Single-Cell Iso-Seq Bioinformatics Tutorial for specific step-by-step instructions: https://github.com/Magdoll/cDNA_Cupcake/wiki/Iso-Seq-Single-Cell-Analysis:-Recommended-Analysis-Guidelines

TYPES OF ISO-SEQ cDNA LIBRARY ARTIFACTS

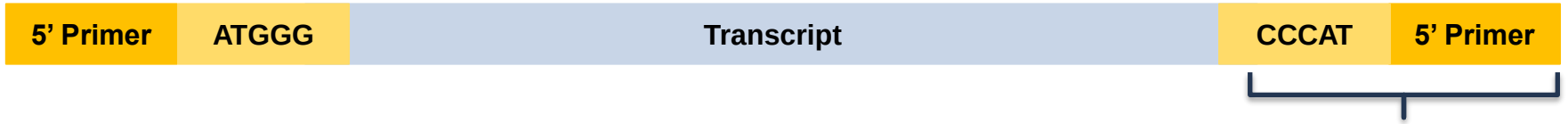
Expected Full-Length Iso-Seq Transcript Structure

RT Priming Artifact



3' – 3' primer artifact can occur if TSO was not added

TSO Priming Artifact



5' – 5' primer artifact can occur if TSO acts as a primer on the RNA

RT Switching Artifact (Genomic View)

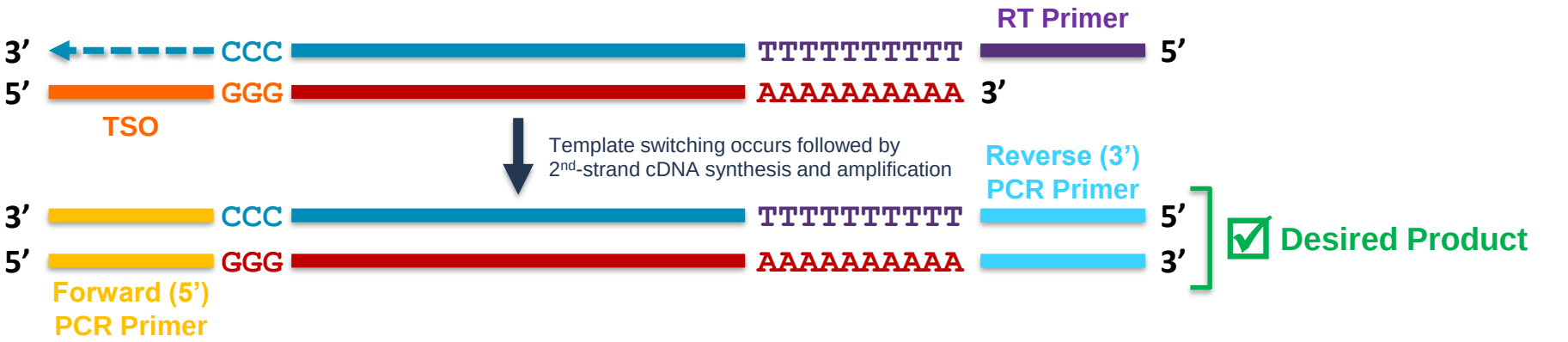
This part of an exon goes missing and when re-mapped to the genome looks like a new intron

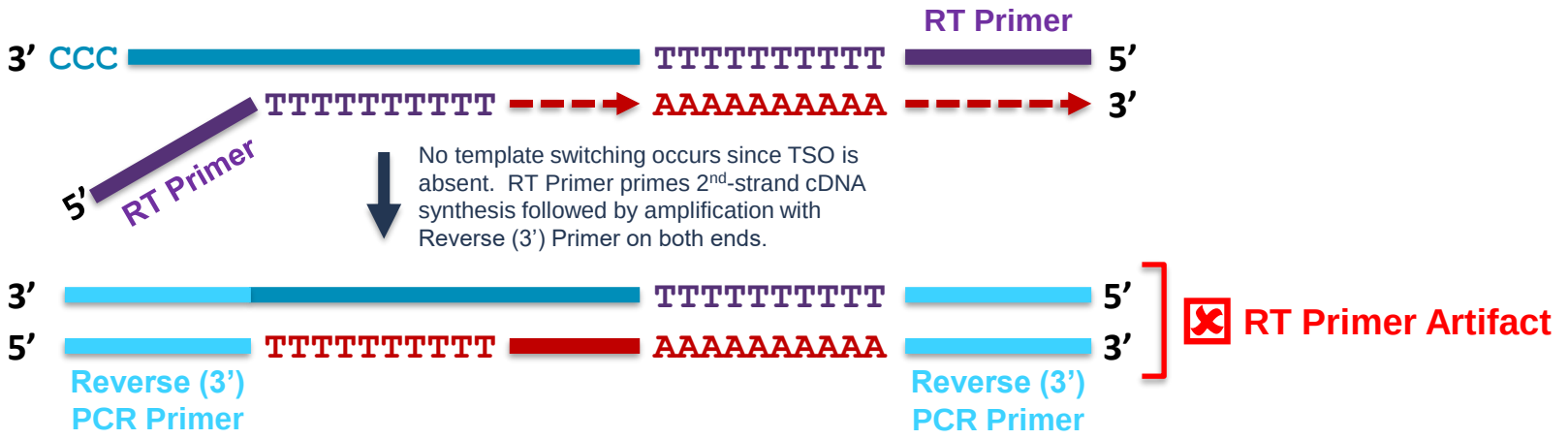
Intra-priming Artifact (Genomic View)

Priming off genomic 'A's results in false 3' end

TYPES OF ISO-SEQ cDNA LIBRARY ARTIFACTS (CONT.)

RT primer (3'p – 3'p) artifacts can occur if TSO was not added

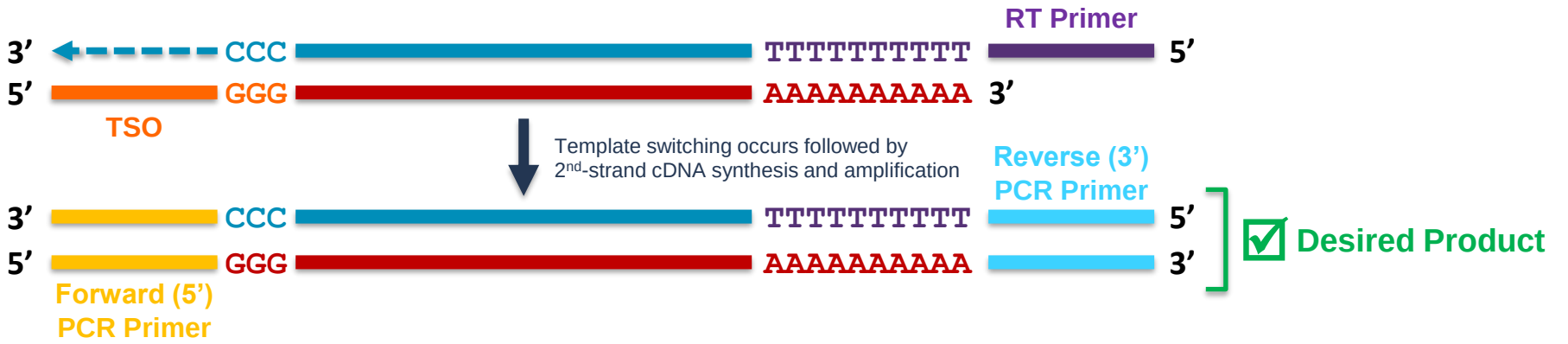


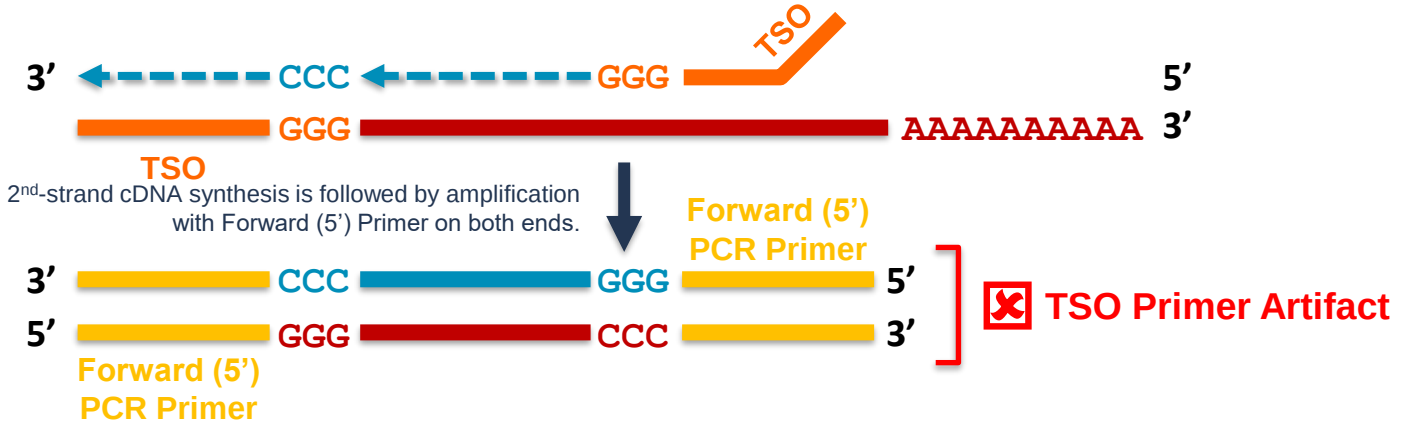



```
@m54019_180901_155009/4784908/ccs
AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTGAGACAGCGTCTCCC
TCTGCCACTGCACTCCAGCCTGGGCGACAGAGCACAAATGTGTCTCAGAAAAAAAAAAGAAAAA
TCCCACTGGAATATCAGTGTCTCAGTTGAGGGT.....GGTTGTTGAGGCACAGAGTTCAAG
CTGCAGTGAGCCATGAAGTCTCTCTTGTATTCCAGCCTGGGTGACAGAGCAAGACCCTGATCAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGTACTCTGCGTTGATACCACTGCTT
```

TYPES OF ISO-SEQ cDNA LIBRARY ARTIFACTS (CONT.)

TSO primer (5'p – 5'p) artifacts can occur if the TSO is acting as a primer on the RNA






```
@m54019_180722_095226/4194667/ccs
AAGCAGTGGTATCAACGCAGAGTACATGGGATGCTTCATCAAGGGCTCTACAACTCACCACAAG
GCTATGTGCTTGGAGACCTTCATCTTTGAGGTTAAATTACTTATTGAGTACTAGATGGTTTTAC
AAAATGTTTTTAGATTTTCTCATCATTTTTTATTCTT.....TCTGCACTGGTTAATTCCTAT
AAGTTCAGTATACAAAGATAGCAATGCCTATTAACAATCTGAATGATACTCTCCCAAACTAAAA
TATTGATTAGGTGCAGAGATACTTTGGAATTAGAAAGACCAAGTCTGAGGTTTAATCCCATGTA
CTCTGCGTTGATACCACTGCTT
```

EXAMPLE LIMA REPORT FOR ISO-SEQ DATA QC ANALYSIS

```
ZMWs input                (A) : 3921897
ZMWs above all thresholds (B) : 1837352 (47%)
ZMWs below any threshold  (C) : 2084545 (53%)

ZMW marginals for (C):
Below min length              : 115 (0%)
Below min score               : 0 (0%)
Below min end score           : 110028 (5%)
Below min passes              : 114 (0%)
Below min score lead          : 0 (0%)
Below min ref span            : 164435 (8%)
Without adapter               : 114 (0%)
Undesired 5p--5p pairs        : 1960565 (94%)
Undesired 3p--3p pairs        : 55601 (3%)
Undesired no hit              : 114 (0%)
```

- Number of input CCS reads for QC analysis
- Number of reads that pass the QC filter **(full-length transcripts)**
- Number of reads that do not pass the QC filter **(non-full-length transcripts)**
- Number of (unusable) non-full-length transcript reads with TSO primer sequence found on both ends

Example LIMA report output indicating that the majority of reads that did not pass QC show a 5'p – 5'p artifact. Such types of 5'p – 5'p artifacts typically result when the TSO is acting as a primer on the input RNA template during first-strand cDNA synthesis.

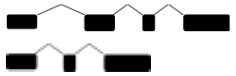
SQANTI: A TOOL FOR CLASSIFYING FULL-LENGTH TRANSCRIPTS

LONG READ DEFINED TRANSCRIPTS
FASTA / FASTQ / GTF

REFERENCE GENOME
FASTA

REFERENCE TRANSCRIPTOME
GTF

Gene A:



OPTIONAL INPUT

- RNA-Seq
- CAGE peak data
- polyA motifs
- tappAS-like functional annotation …

SQANTI3

https://github.com/ConesaLab/SQANTI3

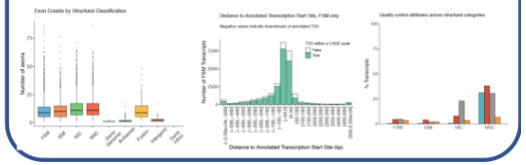
Classification file

ID	Gene	Transcript	Category
PB.1.1	Ctnnd1	ENSMUST00000067232	FSM
PB.1.2	Ctnnd1	novel	NIC
PB.2.1	Novel	novel	Intergenic

Junction file

Junction	Isoform	Splice site	Known
Junction1	PB.1.2	GT-AG	True
Junction2	PB.1.2	GC-AG	True
Junction3	PB.1.2	GT-AF	False

PDF Report

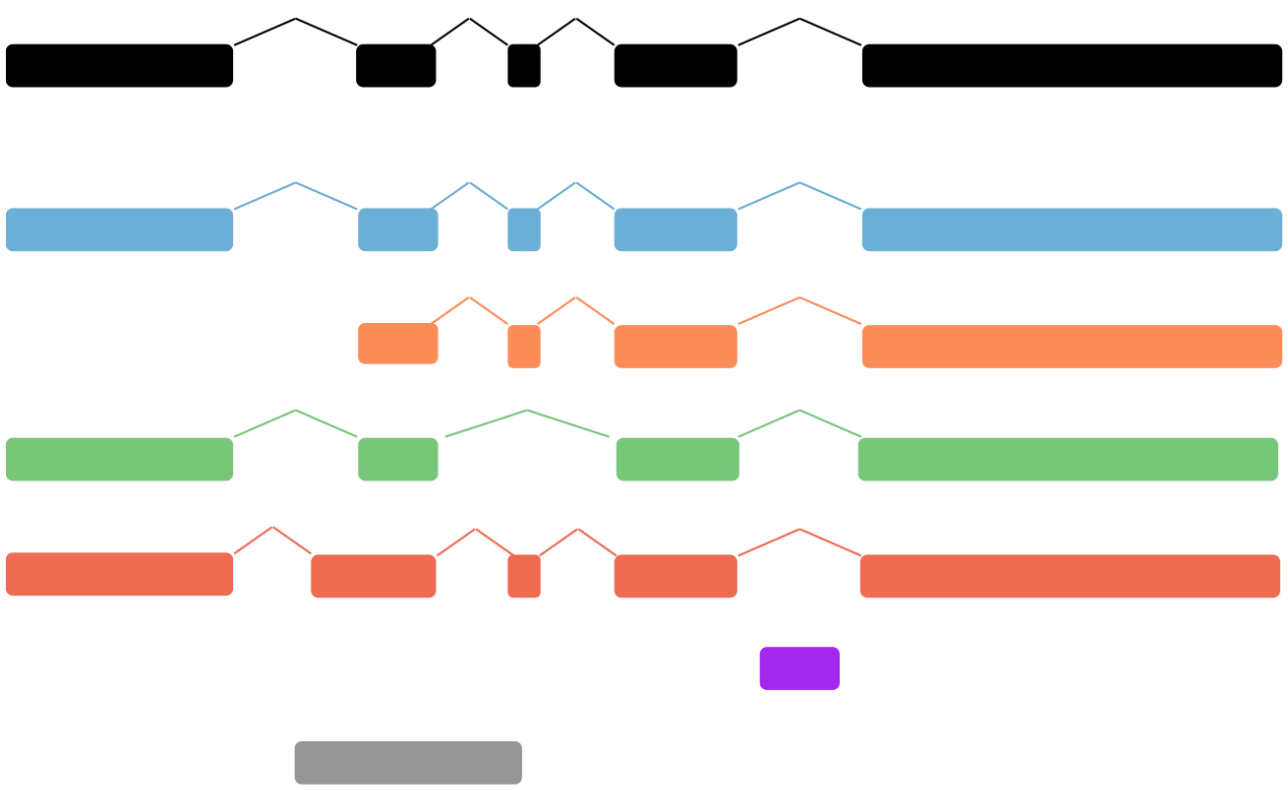


Complementary files

- Genome-corrected transcriptome
- ORF prediction (FASTA)
- CDS-annotated GTF
- tappAS-compatible GFF3

Tardaguila, M. *et al.* (2017) SQANTI: extensive characterization of long read transcript sequences for quality control in full-length transcriptome identification and quantification. Genome Research. 28: 396-411.

SQANTI CLASSIFICATION OF ISO-SEQ TRANSCRIPTS



Reference Transcript

Full Splice Match (FSM)
Matches all splice junctions (SJs) perfectly

Incomplete Splice Match (ISM)
Matches reference the SJs partially

Novel In Catalog (NIC)
Novel isoform with a new combination of known SJs

Novel Not in Catalog (NNC)
Novel isoform with at least one new SJ

Genic Intron
Within an intron

Genic Genomic
Overlaps with introns and exons

Tardaguila, M. *et al.* (2017) SQANTI: extensive characterization of long read transcript sequences for quality control in full-length transcriptome identification and quantification. Genome Research. 28: 396-411.

USING SQANTI AS AN ISO-SEQ DATA QUALITY CONTROL TOOL

What the SQANTI tool is intended for:

- Classification of isoforms against known transcripts
- Classification of splice junctions
- Identification of potential cDNA library artifacts
- Aggregation of supporting evidence for full-length transcripts
- Supporting evidence for functionality (ORFs, Nonsense-mediated mRNA decay (NMD))

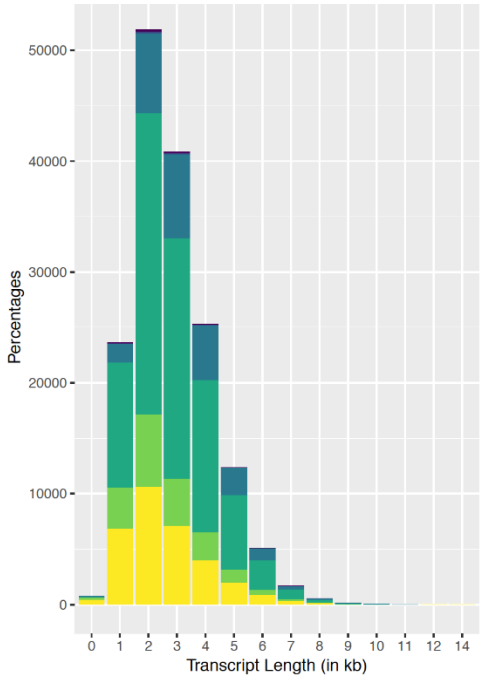
What the SQANTI tool is **NOT** intended for:

- Fixing problems with other parts of the analysis pipeline (e.g., aligner mistakes, low quality sequences, multi-mapping issues)

Single-Cell Iso-Seq Library Example Sequencing Performance Data

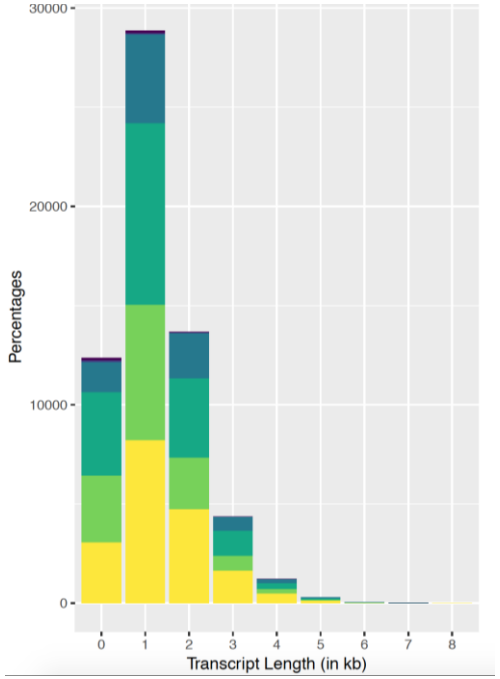
COMPARISON OF cDNA TRANSCRIPT LENGTH DISTRIBUTIONS FOR SINGLE-CELL ISO-SEQ LIBRARY PREPARATIONS *VS.* BULK ISO-SEQ LIBRARY PREPARATIONS

Single-Cell cDNA samples prepared using third-party single-cell isolation platforms tend to produce shorter transcript lengths than bulk cDNA preparations*



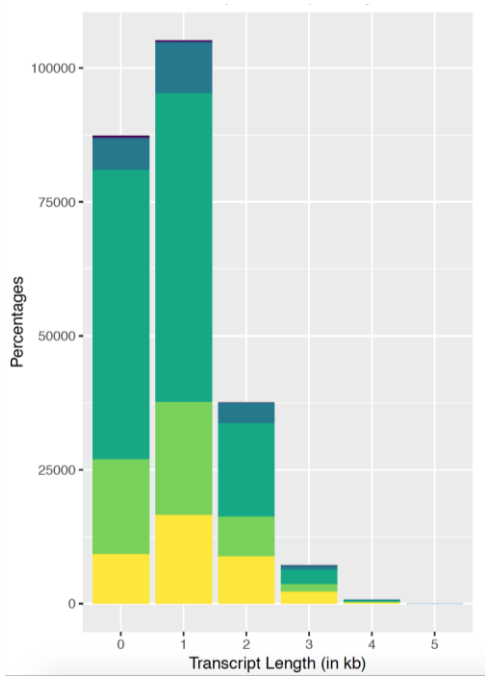
Bulk cDNA Prep
Alzheimer brain

80 – 14,288 bp
(Mean Length: 3.3 kb)



Single-Cell cDNA Prep (Platform 1)
Human Brain Organoid

80 - 8,607 bp
(Mean Length: 1.7 kb)



Single-Cell cDNA Prep (Platform 2)
Human Cell Line

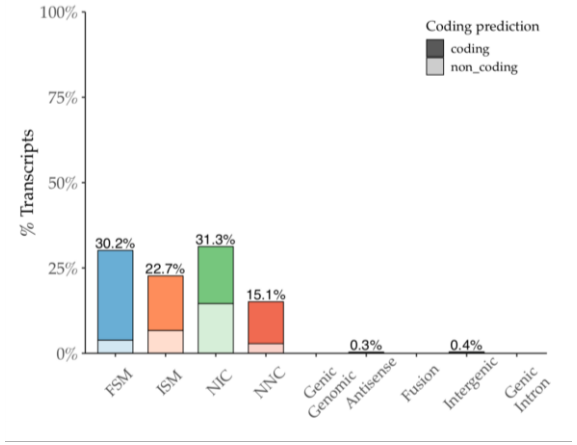
80 – 5,834 bp
(Mean Length: 1.4 kb)

** Read lengths, reads/data per SMRT Cell and other sequencing performance results vary based on sample quality/type and insert size.*

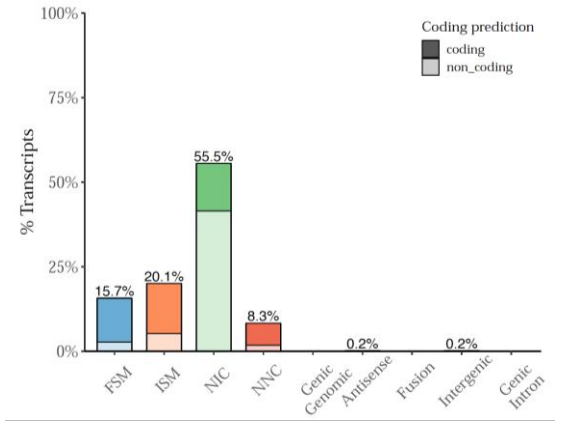
COMPARISON OF ISO-FORM DISTRIBUTIONS ACROSS STRUCTURAL CATEGORIES FOR HUMAN SINGLE-CELL ISO-SEQ SAMPLES

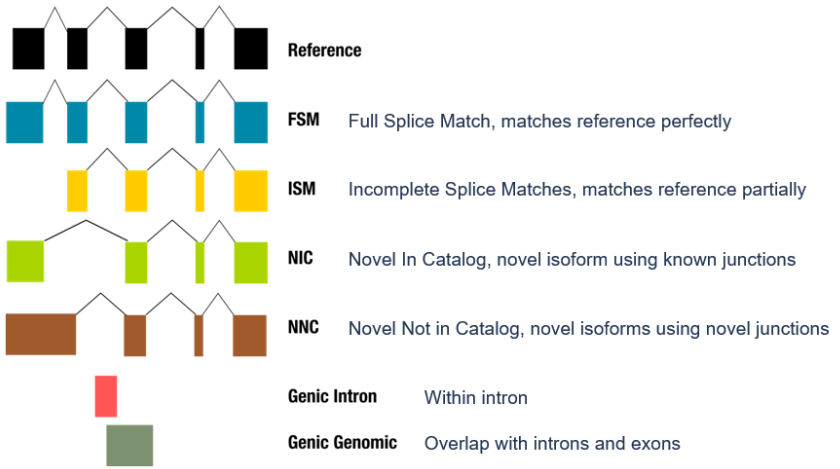
Transcript isoform distribution is highly sample-dependent

Single-Cell, Human Organoid



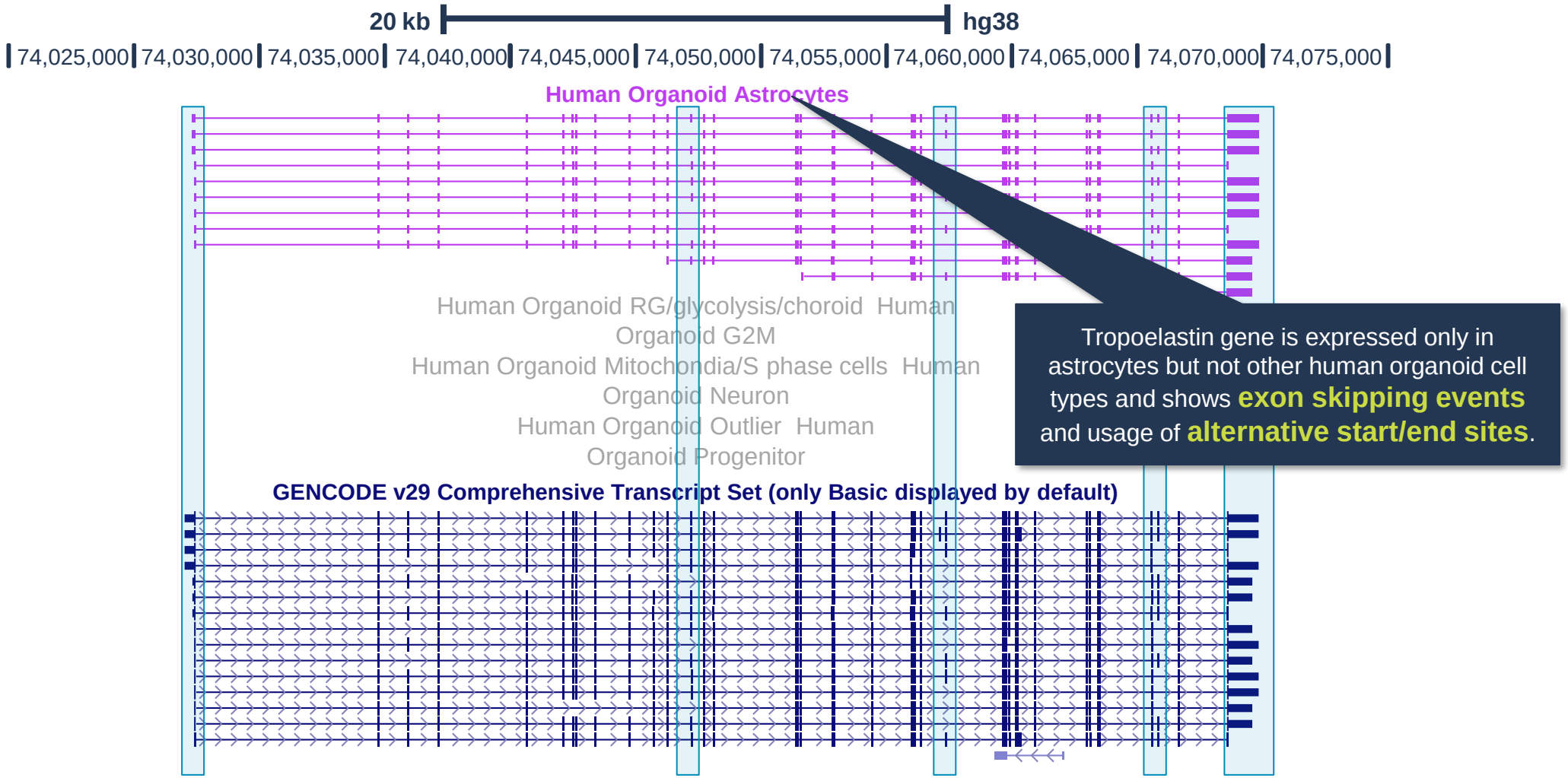
Single-Cell, Human Cell Line





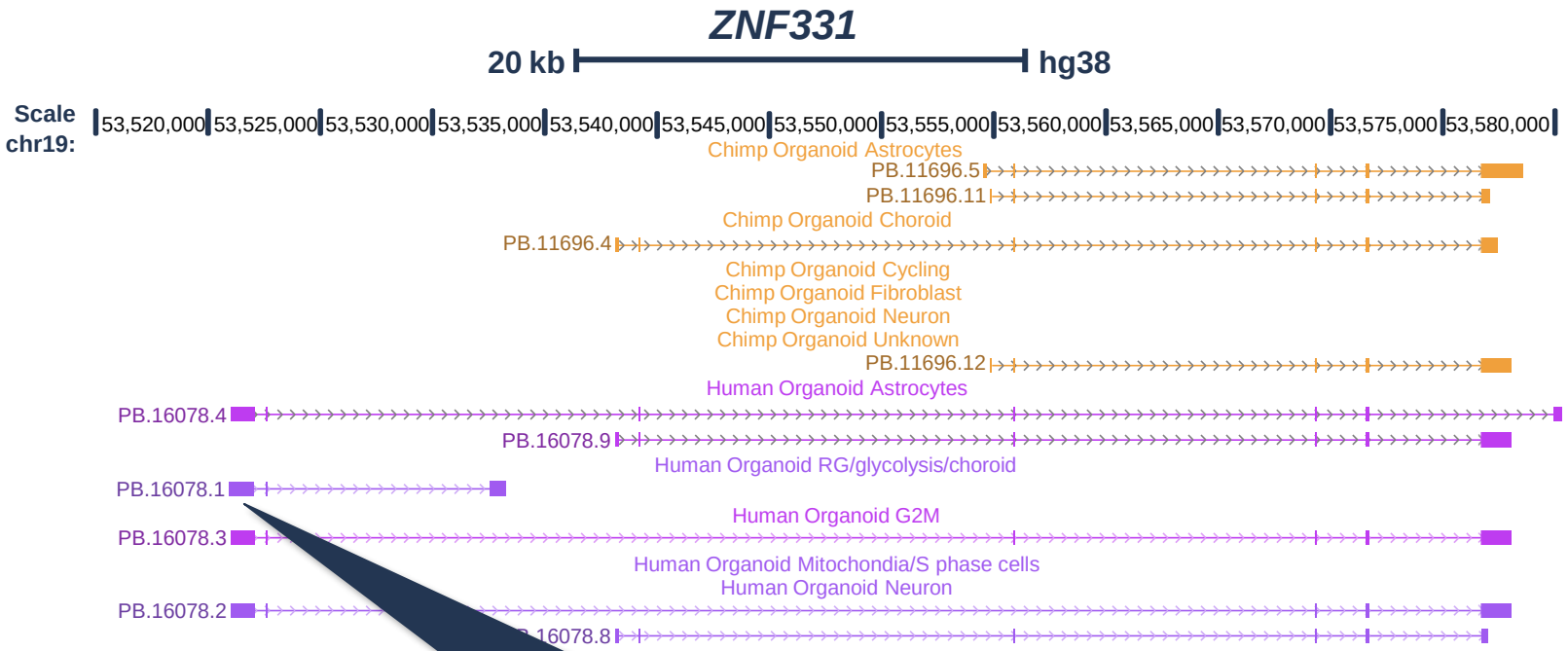
FULL-LENGTH SINGLE-CELL ISO-SEQ METHOD CAN REVEAL CELL-TYPE SPECIFIC ISOFORM EXPRESSION

Example: Multiple tropoelastin isoforms are found in astrocytes but not in other central nervous system (CNS) tissue cell types



FULL-LENGTH SINGLE-CELL ISO-SEQ METHOD CAN REVEAL SPECIES-SPECIFIC ISOFORM EXPRESSION

Example: Alternative transcription start site (TSS) usage for *ZNF331* gene differs in chimp *vs.* human cerebral organoids



Human cerebral organoids show a preference for utilization of an **upstream** transcription start site in the *ZNF331* gene, which may be a contributing factor to the higher expression levels of *ZNF331* in humans vs. chimps.

Technical Documentation & Applications Support Resources

BEST PRACTICES: SINGLE-CELL RNA SEQUENCING (scISO-SEQ ANALYSIS)

Template Preparation with SMRTbell Express Template Prep Kit 2.0

- Enrich for single-cell cDNA using a single-cell sorting platform that generates full-length cDNA*
  - Template switch oligo (TSO)-based cDNA synthesis methods are recommended
  - The final single-cell cDNA product consists of 5' primer, transcript, poly-A tail, unique molecular index (UMI), cell barcode and 3' primer
  - To generate matching short-read data, save 5% of the material
  - Additional PCR cycles can be added if necessary
- Start library preparation with at least 160 ng of input cDNA (post-single-cell platform PCR reaction) for 1-2 SMRT Cell 8M
  - More starting material will be required for sequencing multiple SMRT Cells 8M
- Prepare libraries with the SMRTbell Express Template Prep Kit 2.0 in one day

Sequence on the Sequel, Sequel II or Sequel IIe System

- Use the Sequel II or IIe Systems to generate ~3 million full-length reads from one SMRT Cell 8M to obtain ~1,000 unique molecules for 3,000 single cells**
  - Use 24 hr movies with 2 hrs pre-extension time
- For human samples, run up to 240 SMRT Cell 8M/year at a cost of ~$1,300/SMRT Cell 8M, excluding single-cell enrichment cost†

Data Analysis Solutions with the PacBio Analytical Portfolio

- Analyze HiFi reads which allow accurate single-cell barcode and UMI identification
- Use the single-cell Iso-Seq analysis tools on GitHub to output high-quality, full-length transcript FASTA sequences per UMI, with no assembly required, to characterize transcript variants for each cell

** Number of usable reads, containing the UMI and cell barcode, vary by single-cell platform. Any platform that generates full-length cDNA is compatible with the single-cell RNA sequencing workflow.*

*** Read lengths, reads/data per SMRT Cell type and other sequencing performance results vary based on single-cell platform, sample quality/type and insert size.*

† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II or IIe System and does not include instrument amortization or other reagents.

TECHNICAL DOCUMENTATION AND APPLICATIONS SUPPORT RESOURCES FOR SINGLE-CELL ISO-SEQ LIBRARY PREPARATION, SEQUENCING & DATA ANALYSIS

Sample Preparation Literature

- Application Brief: Application Brief: Single-cell RNA sequencing with HiFi reads - Best Practices (PN BP109-102020)
- Procedure & Checklist – Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0 (PN 101-892-000)
- Quick Reference Card – Loading and Pre-extension Recommendations for the Sequel System (PN 101-461-600)
- Quick Reference Card – Loading and Pre-extension Recommendations for the Sequel II System (PN 101-769-100)
- Overview – Sequel Systems Application Options and Sequencing Recommendations (PN 101-851-300)
- Application Consumable Bundles Purchasing Guide (PN PG100-051320)
- Technical Overview: Single-Cell Iso-Seq Express Library Preparation Using SMRTbell Express Template Prep Kit 2.0 (PN 101-925-400)

Posters

- PacBio AGBT 2020 Poster: A Complete Solution for Full-Length Transcript Sequencing Using the PacBio Sequel II System
- PacBio ENCODE 2019 Poster: Single Cell Isoform Sequencing (scIso-Seq) Identifies Novel Full-length mRNAs and Cell Type-specific Expression

TECHNICAL DOCUMENTATION AND APPLICATIONS SUPPORT RESOURCES FOR SINGLE-CELL ISO-SEQ LIBRARY PREPARATION, SEQUENCING & DATA ANALYSIS (CONT.)

Videos & Webinars

- PacBio ASHG 2020 CoLab: PacBio HiFi reads for comprehensive characterization of genomes and single-cell isoform expression
- PacBio ASHG 2020 Workshop: Single-cell isoform analysis of the nervous system
- LabRoots 2020 Presentation: Single cell gene expression: new insights through the lens of full-length mRNA isoform resolution
- PacBio AGBT 2019 Presentation: Single cell isoform sequencing (scIso-Seq) identifies novel full-length mRNAs

Publications

- Joglekar, A. et al. (2020) Cell-type, single-cell, and spatial signatures of brain-region specific splicing in postnatal development. bioRxiv Preprint. https://doi.org/10.1101/2020.08.27.268730.
- Mincarelli, L. et al. (2020) Combined single-cell gene and isoform expression analysis in haematopoietic stem and progenitor cells. bioRxiv Preprint. https://doi.org/10.1101/2020.04.06.027474.
- Zheng, Y-F. et al. (2020) HIT-scISOseq: High-throughput and High-accuracy Single-cell Full-length Isoform Sequencing for Corneal Epithelium. bioRxiv Preprint. https://doi.org/10.1101/2020.07.27.222349.
- Russell, A. B. et al. (2019) Single-Cell Virus Sequencing of Influenza Infections That Trigger Innate Immunity. Journal of Virology. 93(14):1.
- Gupta, I. et al. (2018) Single-cell isoform RNA sequencing characterizes isoforms in thousands of cerebellar cells. Nature Biotechnology. 36:1197.

TECHNICAL DOCUMENTATION AND APPLICATIONS SUPPORT RESOURCES FOR SINGLE-CELL ISO-SEQ LIBRARY PREPARATION, SEQUENCING & DATA ANALYSIS (CONT.)

Data Analysis Resources

- Single-Cell Iso Seq Analysis Tutorial on GitHub: Recommended scIso-Seq Analysis Guidelines
  - **NOTE:** The Single-Cell Iso-Seq data analysis guidelines presented in this wiki document are **not** officially supported by PacBio.

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