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

PacBio

Procedure & checklist

Overview

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

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

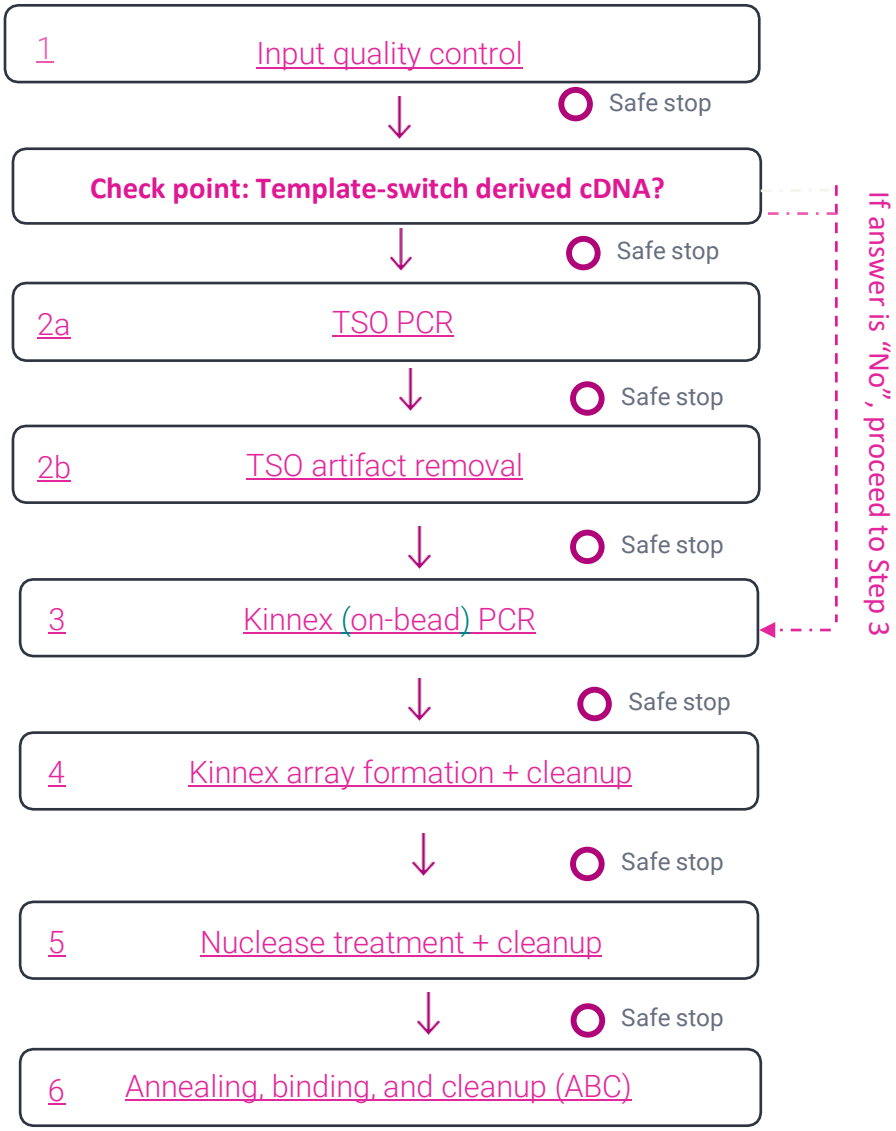
Overview	
Reactions per kit	12
Workflow time	2.5 days for up to 12 libraries
Number of supported acquisitions	>4 acquisitions for SPRQ™/SPRQ-Nx chemistry on the Revio system >4 acquisitions for Vega SPRQ-Nx chemistry or >1 acquisition for Vega chemistry on the Vega system

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

103-885-300 REV01 SEP2026

Workflow



© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Materials and equipment

Recommended cDNA sizing (use one of the following sizing systems)	
Agilent 2100 Bioanalyzer system	Agilent Technologies, Inc. G2939BA
Agilent High Sensitivity DNA Kit	Agilent Technologies, Inc. 5067-4626
4150 or 4200 TapeStation System	Agilent Technologies G2992AA/ G2991BA
High Sensitivity D5000 ScreenTape Assay	Agilent Technologies 5067-5592/5593/5594
D5000 ScreenTape Assay	Agilent Technologies 5067-5588/5589/5590
DNA quantitation	
Qubit fluorometer	ThermoFisher Scientific Q33238
Qubit 1X dsDNA HS assay kit	ThermoFisher Scientific Q33230
Kinnex® library preparation	
Kinnex™ 16-fold prep kit	PacBio® 103-836-900
Sequencing preparation	
Revio® SPRQ™ polymerase kit *or*	PacBio® 103-520-100
Vega SPRQ polymerase kit *or*	PacBio® 103-794-300
Vega™ polymerase kit	PacBio® 103-517-600

Other supplies	
200 Proof ethanol, molecular biology or ACS grade	Any major lab supplier (MLS)
Nuclease-free water, molecular biology grade	Any MLS
8-channel pipettes	Any MLS
Microcentrifuge	Any MLS
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708
Magnetic bead rack	ThermoFisher Scientific 12321D
Magnetic separation rack compatible with 0.2 mL 8-tube strips	V&P Scientific VP 772F4-1
Thermocycler compatible with 0.2 mL 8-tube strips	Any MLS
1.5 mL DNA LoBind tubes	Eppendorf 022431021
0.5 mL DNA LoBind tubes	Eppendorf 022431005
Microcentrifuge	Any MLS

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Before you begin

Single-cell cDNA or Kinnex-compatible amplicon input

Single-cell cDNA or amplicons are expected to have an average fragment size approximately 0.5 – 1 kb after Kinnex PCR. 10x Chromium generated cDNA with a peak size up to 1,500 bp is acceptable prior to beginning.

Note: Input size >1,500 bp may reduce the concatenation efficiency which will result in lower library yields. We recommend preparing Kinnex library with 12-fold prep kit if you have mode at 1,500 bp.

The recommended minimum input for single-cell cDNA generated via template-switching:

- 15 ng for TSO PCR
- 25 ng for Kinnex PCR

For single-cell cDNA from 10x Genomics, follow the best practices in the 10x Chromium user guide up until cDNA amplification, cleanup and QC (refer to 10x Chromium user guide for 5' kits and 3' kits). Input cDNA quality control is highly recommended before proceeding to the Kinnex workflow.

For amplicon and cDNA synthesized without template-switching RT, proceed directly from step 1 (input quality control) to step 3 (Kinnex PCR) with 25 ng as the input.

TSO PCR for cDNA generated via template-switching

For **cDNA generated via template-switching, perform Step 2a (TSO PCR) and Step 2b (TSO artifact removal).** High-purity cDNA with intact 5' and 3' ends are essential for optimal Kinnex library quality, yield, and sequencing performance.

Depending on cDNA quality, these steps may be skipped; however, doing so may reduce library quality and yield and negatively impact sequencing performance.

For **non-template-switched derived cDNA and amplicon, Steps 2a and 2b are not required**. After completing Step 1 (input Quality Control), proceed directly to Step 3 (Kinnex PCR).

Kinnex library QC

Library quality is critical for achieving optimal sequencing results. The Femto Pulse system provides the best resolution for sizing of the kinnex array formation and is recommended for intermediate quality control during the workflow (before the nuclease treatment step), as well as for final library quality assessment. Please see appendix for an example.

- Femto Pulse system, Agilent Technologies, M5330AA
- Genomic DNA 165 kb kit, Agilent Technologies, FP-1002-0275

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Reagent and sample handling

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

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

		Kinnex primer mix PQ 103-153-800

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

The Kinnex capture beads kit is not temperature-sensitive and contains the following contents:

Tube color	Reagent
Clear	Kinnex capture beads 103-144-900
	Kinnex capture binding buffer 103-145-400
	Kinnex capture washing buffer 103-145-500

Anneal, bind, and cleanup

Thaw the following reagents at room temperature:

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

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Bring the following reagents up to room temperature 30 minutes prior to use:

- Loading buffer
- SMRTbell cleanup beads

Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Thermocycler programs

Program thermocycler(s) prior to beginning the protocol for the first time.

1. TSO PCR program (15 ng input)

The TSO PCR program is required for input cDNA generated via template-switching during reverse transcription.

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

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

or TSO PCR program (60-75 ng input)

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

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

2. Kinnex PCR program

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

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

3. Kinnex primer digestion/ligation program

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

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

4. Kinnex repair program

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

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

5. Nuclease treatment program

Heated lid set at ≥47°C. Reaction volume set to 50 µL.

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Workflow steps

1. Input amplicon quality control

- Evaluate the size distribution of the input cDNA or amplicon on Bioanalyzer using high sensitivity DNA kit or on Agilent TapeStation using High Sensitivity D5000 ScreenTape Assay to determine whether it is suitable for the protocol. The input should have a size mode of 500–1,000 bp.
- For cDNA generated via template-switching, at least 15 ng of input is required to proceed with TSO PCR. For example, if using 10x Chromium 3' or 5' single cell cDNA, follow the corresponding 10x Chromium user guide through cDNA amplification, cleanup and QC before continuing with this protocol.
- For targeted amplicons or cDNA synthesized without a template-switching step, proceed directly from step 1 (input quality control) to step 3 (Kinnex PCR) with 25 ng of input.
- To sequence cDNA fragments <400 bp, it is recommended to increase the SMRTbell cleanup concentration to 1.5X during the Kinnex PCR cleanup step.
- To sequence cDNA fragments <200 bp, it is recommended to increase the SMRTbell cleanup concentration to 2X for both the TSO PCR (2a) and Kinnex PCR cleanup (3) steps.

2a. TSO PCR

This PCR step generates biotinylated DNA-fragments to enable removal of TSO priming artifacts generated during cDNA template-switching synthesis (e.g., 10x Genomics Chromium). The figure below illustrates the difference between a correct molecule and a TSO artifact.

cDNA amplification with Kinnex capture primers:

✔	Step	Instructions
		Normalize sample input to 15 ng if it is between 15 ng and 59 ng using elution buffer. Normalize sample input to 75 ng if it is higher than 75 ng using elution buffer. For input amounts between 60–75ng, proceed without normalizing. Select either the Kinnex 3' or 5' capture primer mix depending on the 10x Genomics kit used. Set up the following PCR reaction on ice (RM1).
	2a.1	**Reaction Mix 1 (RM1):**

✔	Tube color	Component	Volume
	Green	Kinnex 16-fold PCR mix	25 µL
	Red	Kinnex 5' capture primer mix **or**	10 µL
	Yellow	Kinnex 3' capture primer mix	

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

10x 5′ or 3′ cDNA library (1–5 ng/µL)	Up to 15 µL
Total volume	**50 µL**

2a.2 Pipette-mix **RM1**.

2a.3 Quick spin **RM1** in a microcentrifuge to collect liquid.

2a.4 Select the TSO PCR program based on cDNA input. Keep sample on ice until the thermal cycler lid has heated to 105°C.

TSO PCR program (15 ng input)

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

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

or TSO PCR program (60–75 ng input)

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

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

Cleanup with 1.5X SMRTbell cleanup beads (standard)

NOTE: if cDNA <200 bp needs to be sequenced, use 2X SMRTbell cleanup bead ratio

2a.5 Add 75 µL (1.5X v/v) of resuspended, room-temperature SMRTbell cleanup beads to each tube of amplified cDNA.

2a.6 Pipette mix the beads until evenly distributed.

2a.7 Quick-spin the tube strip in a microcentrifuge to collect liquid.

2a.8 Incubate at room temperature for 10 minutes to allow DNA to bind beads.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

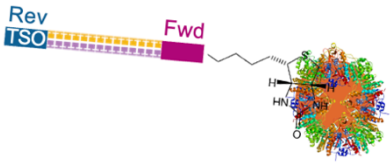
2a.9	Place tube strip in a magnetic separation rack until beads separate fully from the solution.
2a.10	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
2a.11	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
2a.12	Repeat the previous step.
2a.13	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.
2a.14	Remove tube strip from the magnetic rack. **Immediately add 42 µL of elution buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.
2a.15	Quick-spin the tube strip in a microcentrifuge to collect liquid.
2a.16	Incubate at room temperature for 5 minutes to elute DNA.
2a.17	Place tube strip in a magnetic separation rack until beads separate fully from the solution.
2a.18	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a new tube strip. Discard old tube strip with beads.
2a.19	Evaluate sample concentration. Take a 1 µL aliquot from each tube. Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Yield typically ranges from 200–600 ng.
2a.20	Proceed to the next step of the protocol if sample quantity is between 200 – 600 ng. • If the yield is greater than 600 ng, proceed to the next step with 600 ng of amplified biotinylated DNA. • If the yield is less than 200 ng, repeat TSO PCR with two additional PCR cycles.

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

2b.TSO artifact removal

In this step, removal of DNA fragments containing TSO artifacts is performed using Kinnex capture beads. Below is an illustration of Kinnex capture beads binding to the correct cDNA molecule.



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

✔	Step	Instructions
	2b.1	Bring Kinnex capture beads kit to room temperature. Resuspend the beads by vortexing.
	2b.2	Transfer 10 µL resuspended Kinnex capture beads per sample to a PCR tube.
	2b.3	Add 40 µL Kinnex capture binding buffer per sample and gently resuspend by pipetting. DO NOT VORTEX.
	2b.4	Place the tube on the magnet until the beads separate fully from the solution and **remove the supernatant**.
	2b.5	Remove the tube from magnet rack and resuspend the beads in 40 µL Kinnex capture binding buffer per sample by slow pipetting. DO NOT VORTEX. For multiple samples, distribute 40 µL of the scale-up resuspended Kinnex capture beads into one PCR tube per sample before proceeding to Step 2b.6.
	2b.6	Add 40 µL of the biotinylated DNA-fragments (from Step 2a.18) to the resuspended beads for a final volume of 80 µL. Mix carefully and slowly to avoid foaming of the solution.
	2b.7	Incubate the tube at room temperature for 15 minutes. Quick-spin the tube in a microcentrifuge to collect liquid.
	2b.8	Place the tube on the magnet until the beads separate fully from the solution and remove the supernatant.
	2b.9	Remove the tube from the magnet. Resuspend the Kinnex capture beads/DNA-complex in 80 µL Kinnex capture washing buffer by rigorous pipette mixing until evenly distributed.
	2b.10	Place the tube on the magnet until the beads separate fully from the solution and remove the supernatant.
	2b.11	Repeat the step 2b.9.
	2b.12	Repeat step 2b.10.
	2b.13	Remove the tube from the magnet. Resuspend the Kinnex capture beads/DNA complex in 150 µL nuclease free water by pipette mixing until evenly distributed.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

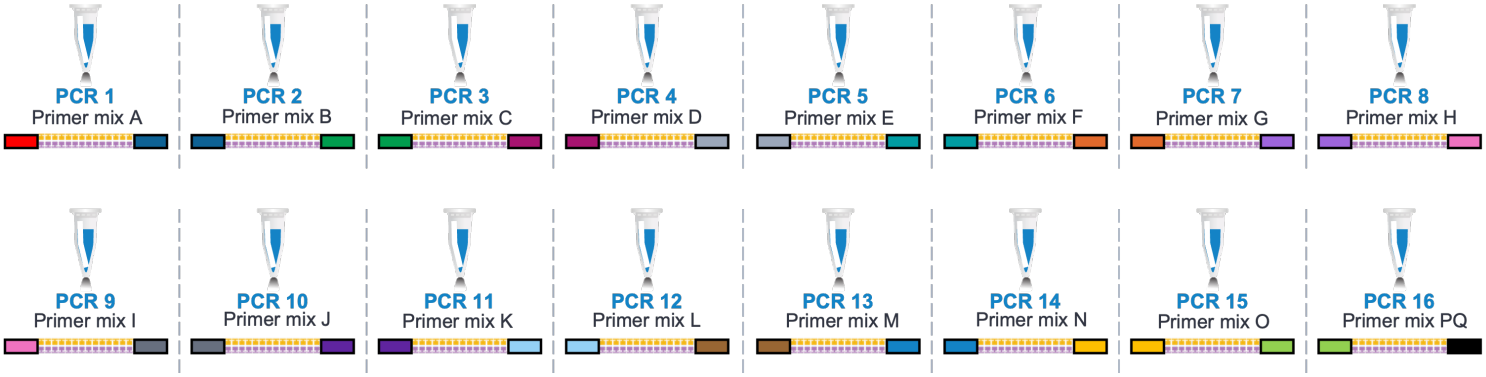
2b.14	Place the tube on the magnet until the beads separate fully from the solution and remove the supernatant.
2b.15	Resuspend the capture beads/DNA-complex in 40 µL of elution buffer by vortexing until evenly distributed. IMPORTANT: DNA remains bound to beads, please **keep the bead solution** for subsequent steps.
2b.16	Evaluate sample concentration: Take a 1 µL aliquot from each tube. Measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Yield typically ranges from 10%–40% of the input TSO PCR amount.
2b.17	Proceed to the next step of the protocol if sample quantity is acceptable. 25 ng of product is required for the Kinnex PCR. Using less than 25 ng may lead to less than 5ug yield from Kinnex PCR.

SAFE STOPPING POINT – Store at 4°C

DO NOT FREEZE CAPTURE BEADS/DNA-COMPLEX SOLUTION

3. Kinnex (on-bead) PCR

Perform 16 parallel cDNA amplification reactions with Kinnex primers to generate DNA fragments containing orientation-specific Kinnex segmentation adapter sequences.



Note: All 16 PCR reactions are required. Missing/incorrect primer pairs will result in no/low library yield.

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

PCR tube	Kinnex primers premix	Tube color	PN
1	Kinnex primers premix A	Orange	103-107-800
2	Kinnex primers premix B		103-107-900
3	Kinnex primers premix C		103-108-000
4	Kinnex primers premix D		103-108-100

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

5	Kinnex primers premix E		103-108-200
6	Kinnex primers premix F		103-108-300
7	Kinnex primers premix G		103-108-400
8	Kinnex primers premix H		103-153-000
9	Kinnex primers premix I		103-153-100
10	Kinnex primers premix J		103-153-200
11	Kinnex primers premix K		103-153-300
12	Kinnex primers premix L		103-153-400
13	Kinnex primers premix M		103-153-500
14	Kinnex primers premix N		103-153-600
15	Kinnex primers premix O		103-153-700
16	Kinnex primers premix PQ		103-153-800

3.2 Set up the following PCR reaction mix in 1.5mL LoBind tube per sample on ice.

Reaction Mix 2 (RM2):

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

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

3.3 Pulse vortex to homogenize the **RM2** and quick-spin in a microcentrifuge to collect liquid.

Note: It is critical to thoroughly homogenize the RM2 immediately before proceeding to the next step. Inadequate mixing or uneven distribution of the beads/DNA complex may result in low Kinnex library yield.

3.4 Aliquot **14 µL** of **RM2** into two 8-tube strip PCR tubes on ice (16 tubes total, 14 µL per tube).

3.5 Add **2.5 µL** of the appropriate Kinnex primer mix into each respective PCR tubes from step 3.4 for a total volume of 16.5 µL.

3.6 Pipette-mix up and down 10 times. Quick-spin to collect all liquid.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

3.7 Run the Kinnex PCR program and keep sample on ice until thermal cycler lid has heated to 105°C. The duration of PCR is approximately 1 hour.

Kinnex PCR program

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

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

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

Clean up with 1.2X SMRTbell cleanup beads (standard)

NOTE: use 1.5X SMRTbell cleanup bead ratio for cDNA <400 bp, or 2.0X for cDNA <200 bp

3.8 Quick-spin to collect all liquid. Pool the entire volume of all 16 reactions into a single 1.5 mL LoBind tube.

3.9 Add 317 µL (1.2X v/v) of resuspended, room-temperature SMRTbell cleanup beads to the PCR pool.

3.10 Pipette-mix the beads until evenly distributed.

3.11 Quick-spin the 1.5 mL LoBind tube in a microcentrifuge to collect all liquid from the sides of the tubes.

3.12 Incubate at room temperature for 10 minutes to allow DNA to bind beads.

3.13 Place the 1.5 mL LoBind tube in a magnetic separation rack until beads separate fully from the solution.

3.14 Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.

3.15 Slowly dispense 1 mL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.

3.16 Repeat the previous step.

3.17 Remove residual 80% ethanol:

- Remove the 1.5 mL LoBind tube from the magnetic separation rack.
- Quick-spin the 1.5mL LoBind tube in a microcentrifuge.
- Place the 1.5 mL LoBind tube back in a magnetic separation rack until beads separate fully from the solution.
- Remove residual 80% ethanol and discard.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

3.18	Remove the 1.5 mL LoBind tube from the magnetic rack. **Immediately add 21 µL of elution buffer** to each tube and resuspend the beads.
3.19	Quick-spin the 1.5 mL LoBind tube in a microcentrifuge.
3.20	Incubate at room temperature for 10 minutes to elute DNA.
3.21	Place the 1.5 mL LoBind tube in a magnetic separation rack until beads separate fully from the solution.
3.22	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a new PCR tube strip. Discard old tube with beads.
3.23	Evaluate sample concentration. • Take a 1 µL aliquot from each tube, dilute with 9 µL of elution buffer. Using 1 µL of the dilution, measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. Yield typically ranges from 5–11 µg.
3.24	Proceed to the next step of the protocol if sample quantity is acceptable: • 5 µg is recommended • < 5 µg may result in lower library prep yields • Do not proceed if less than 3 µg is available Note: If the Kinnex PCR yield is less than 3 µg, repeat Kinnex PCR and quantifying each individual tube with 1X dsDNA HS kit for low yield troubleshooting.

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

4. Kinnex array formation

In this step, treat PCR amplified cDNA fragments with Kinnex enzyme to create single-stranded extensions to enable directional assembly of cDNA segments into a linear array.

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

✔	Tube color	Component	Volume
	Blue	Kinnex barcoded adapter	2 µL

4.2

Add the following components in the listed order.

If processing multiple samples, make a master mix with 10% overage. Pipette mix master mix.

Reaction Mix 3 (**RM3**):

✔	Tube color	Component	Volume
	White	Kinnex array and repair buffer	3.5 µL
	Red	Kinnex enzyme	4 µL
	Yellow	Kinnex ligase	6 µL
		Total volume	**13.5 µL**

4.3

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

Kinnex primer digestion/ligation program

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

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

4.4

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

✔	Tube color	Component	Volume
	Green	Kinnex repair mix	2 µL

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

4.5 Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid.

4.6 Run the Kinnex repair program.

Kinnex repair program

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

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

4.7 Add **42.5 uL of elution buffer** to bring the total volume to 80 uL.

Clean up with 1.2X SMRTbell cleanup beads

4.8 Add 96 µL (1.2X v/v) of resuspended, room-temperature SMRTbell cleanup beads to each sample.

4.9 Pipette-mix the beads until evenly distributed.

4.10 Quick-spin the tube strip in a microcentrifuge to collect liquid.

4.11 Incubate at room temperature for 10 minutes to allow DNA to bind beads.

4.12 Place the tube strip in a magnetic separation rack until beads separate fully from the solution.

4.13 Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.

4.14 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.

4.15 Repeat the previous step.

4.16 Remove residual 80% ethanol:

- Remove the tube strip from the magnetic separation rack.
- Quick-spin the tube strip in a microcentrifuge.
- Place the tube strip back in a magnetic separation rack until beads separate fully from the solution.

Remove residual 80% ethanol and discard.

4.17 Remove the tube strip from the magnetic rack. **Immediately add 41 µL of elution buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.

4.18 Quick-spin the tube strip in a microcentrifuge to collect liquid.

4.19 Incubate at room temperature for 5 minutes to elute DNA.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

4.20	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
4.21	Slowly remove the cleared supernatant without disturbing the beads. Transfer 41 µL of the eluate to a **new PCR strip tube**. Discard old tube with beads.
4.22	Recommended to save aliquot for potential troubleshooting. • Take a 1 µL aliquot from each tube, dilute with 4 µL of elution buffer.

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

5. Nuclease treatment and cleanup

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

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

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

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

Clean up with 1.2X SMRTbell cleanup beads

	5.8	Add 60 µL (1.2X v/v) SMRTbell cleanup beads to each sample from the previous step. Pipette-mix the beads until evenly distributed.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

5.9	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
5.10	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
5.11	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
5.12	Slowly remove the cleared supernatant without disturbing the beads. It is recommended to save the supernatant in another tube strip in case of poor DNA recovery.
5.13	Slowly dispense 200 µL, or enough to cover the beads, of freshly prepared 80% ethanol into each tube. After 30 seconds, remove the 80% ethanol and discard.
5.14	Repeat the previous step.
5.15	Remove residual 80% ethanol: • Remove tube strip from the magnetic separation rack. • Quick spin tube strip in a microcentrifuge. • Place tube strip back in a magnetic separation rack until beads separate fully from the solution. Remove residual 80% ethanol and discard.
5.16	Remove the tube strip from the magnetic rack. Immediately add **26 µL of elution buffer** to each tube and resuspend the beads by pipetting 10 times or until evenly distributed.
5.17	Quick-spin the tube strip in a microcentrifuge to collect liquid.
5.18	Incubate at room temperature for 5 minutes to elute DNA.
5.19	Place the tube strip in a magnetic separation rack until beads separate fully from the solution.
5.20	Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a new tube. Discard old tube strip with beads.
5.21	Recommended: Evaluate sample concentration and size. • Take a 1 µL aliquot from each tube, dilute with 4 µL of elution buffer. • Using 1 µL of the dilution, measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. **Expect 5-15% recovery of the starting Kinnex-PCR product.** Yield typically ranges from 300–750 ng with 5 µg input. • Recommended: Further dilute each aliquot to 250 pg/µL with the Femto Pulse dilution buffer. Check the final SMRTbell library size distribution with a Femto Pulse system. See Library QC section in the Best practice and Figure 1 in the Appendix for an example.
5.22	If library concentration exceeds 20 ng/µL, **normalize to 20 ng/µL**, and proceed to ABC with **25 µL** of normalized library. Using a concentration above 20 ng/µL may result in lower loading during sequencing.
5.23	Proceed to Section 6 to prepare library for sequencing on Vega, Vega SPRQ-Nx or Revio (+SPRQ/SPRQ-Nx chemistry).
5.24	Store SMRTbell libraries at 4°C if sequencing within the week. Long-term storage should be at -20°C. Minimize freeze-thaw cycles when handling SMRTbell libraries.

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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

6. Annealing, binding, and cleanup (ABC)

This step is for preparing the Kinnex library (25 µL) for sequencing on Revio (+SPRQ/SPRQ-Nx), Vega, and Vega SPRQ-Nx. If pooling is being done or a custom volume is to be processed, see Appendix section A1. **The Polymerase kit used will depend on which sequencer or chemistry is being used (see below).**

Kit	PN
Revio SPRQ polymerase kit	103-520-100
Vega polymerase kit	103-517-600
Vega SPRQ polymerase kit	103-794-300

✔	Step	Instructions
	6.1	Prepare the appropriate volume of master mix with 10% overage using the per reaction volumes listed below.

Annealing mix

✔	Tube	Component	Volume
	Light blue	Annealing buffer	12.5 µL
	Light purple	Kinnex sequencing primer	12.5 µL
		Total volume	**25 µL**

6.2	Pipette-mix the Annealing mix and quick spin to collect liquid.
6.3	Add **25 µL** of the Annealing mix to each library. Total volume should equal **50 µL**.
6.4	Pipette-mix each sample and quick spin to collect liquid.
6.5	Incubate at room temperature for **15 minutes**.
6.6	During primer incubation, prepare the polymerase dilution (see below) and store on ice.
6.7	To prepare the polymerase, add the following components to a new microcentrifuge tube on ice. Adjust component volumes for the number of samples being prepared, plus 10% overage.

Polymerase Dilution

✔	Tube	Component	Volume
	Yellow	Polymerase buffer	47 µL
	Purple	Sequencing polymerase	3 µL
		Total volume	**50 µL**

6.8	Pipette mix the **polymerase dilution** and quick-spin to collect liquid.
6.9	Add **50 µL of polymerase dilution** to primer annealed sample. Total volume should equal to **100 µL**.
6.10	Pipette-mix each sample and quick-spin to collect liquid.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

6.11	Incubate at **room temperature for 15 minutes.**
6.12	Proceed immediately to the next step of the protocol to remove excess polymerase.
	Post-binding cleanup with 1X SMRTbell cleanup beads
6.13	Add **100 µL** of resuspended, room-temperature SMRTbell cleanup beads to each sample.
6.14	Pipette-mix the beads until evenly distributed and quick-spin if necessary to collect all liquid from the sides of the tube.
6.15	Incubate at room temperature for 10 minutes to allow DNA to bind beads.
6.16	Place sample on an appropriate magnet and allow beads to separate fully from the solution
6.17	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant. **DO NOT USE EtOH.** Proceed immediately to the elution. It is important not to let the beads dry out.
6.18	Remove sample from the magnet and immediately add Loading buffer to each tube and resuspend the beads by pipette mixing.

	Revio/Vega SPRQ polymerase Kit	Vega polymerase Kit
Loading buffer	25 µL	50 µL

6.19	Quick-spin the samples to collect any liquid from the sides of the tube.
6.20	Incubate at room temperature for 15 minutes to elute DNA.
6.21	Place sample on magnet and allow beads to separate fully from the solution.
6.22	Slowly remove the cleared eluate without disturbing the beads. Transfer eluate to a new tube. Discard the old tube with beads.
6.23	Use **1 µL** of sample to measure DNA concentration with a Qubit fluorometer using the 1x dsDNA HS kit. **Important:** The **Qubit Flex** instrument is not compatible with measuring polymerase-bound library in Loading Buffer because the concentration readings will not be accurate.
6.24	Proceed to the **Loading Calculator** in SMRT Link v26.1 or higher to calculate the final dilution for adding the sample to the sequencing reagent plate. The recommended loading concentration is 130–160 pM.

PROTOCOL COMPLETE

Important: Polymerase-bound libraries can be stored at 4°C for up to 1 month, or at -20°C for up to 6 months prior to sequencing. Polymerase-bound libraries can withstand up to 4 freeze-thaw cycles. Note that the Loading buffer is light sensitive.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Appendix

A1. Annealing, binding, and cleanup (ABC) for custom volumes

This step is for preparing libraries for sequencing on PacBio sequencers. The sequencing polymerase is stable once bound to the HiFi library and can be stored at 4°C for 1 month or at -20°C for at least 6 months. Use the calculations below to determine reagent volumes based on input sample volume:

	SMRTbell library	Annealing buffer	Kinnex sequencing primer	Polymerase dilution
Volume (µL)	x	x/2	x/2	x*2
Example	100	50	50	200

See Section 6 (ABC) for full protocol.

A2. Library QC

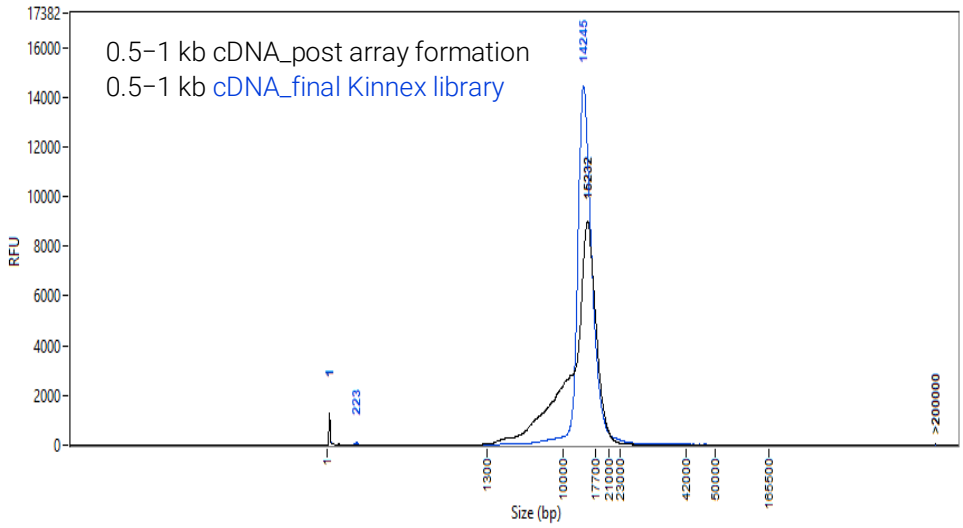


Figure 1. 16-fold post array formation and final library quality control on FEMTO Pulse

APPENDIX COMPLETE

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

FAQs

1. Can I use less than 3 µg of Kinnex PCR input for array formation?

The recommended Kinnex PCR input for array formation is 5 µg. To generate enough of SMRTbells for sequencing, we do not recommend using less than 3 µg of Kinnex PCR product for 16-fold Kinnex array formation.

2. What troubleshooting steps can I take when the library yields are low?

If the library size distribution at step 5.21 is clean and the yield is sufficient for 1-2 movie acquisitions, you may proceed with ABC and sequencing. Low library yield may result from inefficient array formation. We recommend the following troubleshooting steps:

a. Repeat the Kinnex PCR with vigorous master mix mixing. At step 3.7, quantify each of the 16 individual PCR reactions using the Qubit 1X dsDNA HS kit. Ideally, the coefficient of variation (%CV) across the 16 reactions would be less than 5%.

b. Measure the 5-fold diluted aliquot saved at step 4.22 using a Qubit fluorometer with the 1× dsDNA HS Assay Kit. Further dilute the aliquot to 250 pg/µL and assess the array size distribution using a Femto Pulse system. Figure 1 in the Appendix shows an example of an ideal array profile post array formation.

c. For challenging samples, extending the Kinnex repair incubations to 1 hr at 37°C followed by 1 hr at 45°C may help.

Revision history (description)	Version	Date
Initial release	01	September 2026

Research use only. Not for use in diagnostic procedures. ©2026 Pacific Biosciences of California, Inc. ("PacBio"). All rights reserved. Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third-party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at pacb.com/license. Pacific Biosciences, the PacBio logo, PacBio, Circulomics, Omniome, SMRT, SMRTbell, Iso-Seq, Sequel, Nanobind, Revio, Revio SPRQ, Kinnex, PureTarget, SPRQ, Vega and Vega SPRQ are trademarks of PacBio. Reference to third-party companies, products, or services are for informational purposes only and do not imply endorsement, sponsorship, or affiliation.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.