Preparing Kinnex™ libraries using the Kinnex 8-fold prep kit

Procedure & checklist

Overview

This procedure describes the workflow for constructing Kinnex 8-fold libraries from cDNA generated using Iso-Seq® express 2.0 kit, 16S rRNA amplicons, or other types of Kinnex-compatible amplicons. The generated libraries can be sequenced on PacBio® Vega™ and Revio® systems.

Note: This procedure replaces the Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit for Kinnex 8-fold concatenation.

Overview	
Reactions supported per kit	12
Workflow time	1 day for up to 12 libraries
Number of supported acquisitions per library	>8 acquisitions for Revio SPRQ/SPRQ-Nx on the Revio system >8 acquisitions for Vega SPRQ-Nx chemistry or >2 acquisitions for Vega chemistry on the Vega system

Input	
DNA quantity	35 ng of Kinnex-compatible amplicons (e.g. cDNA, 16S rRNA, or other)
Average insert size	1,500 – 3,000 bp
Expected Kinnex library size range	12 – 20 kb

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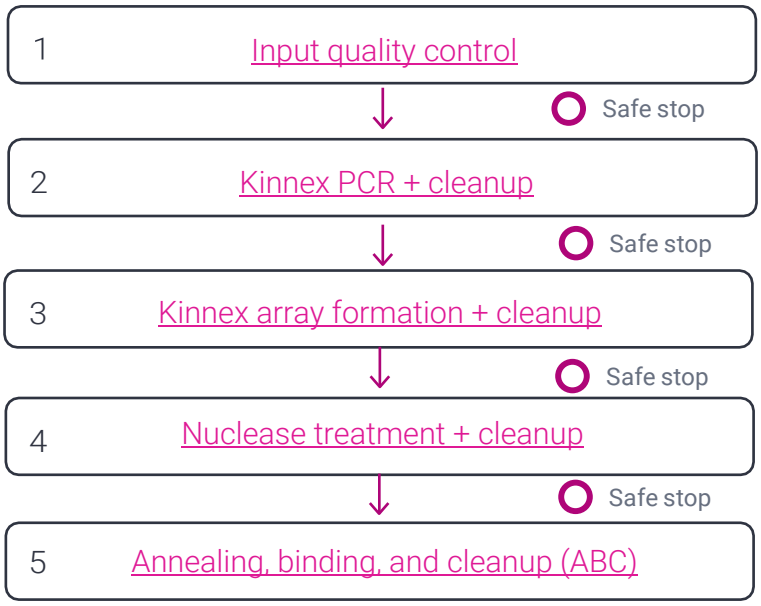
Workflow



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Materials and equipment

Recommended DNA sizing (use one of the following sizing systems)	
2100 Bioanalyzer instrument	Agilent Technologies G2939BA
Agilent High Sensitivity DNA Kit	Agilent Technologies 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	Thermo Fisher Scientific Q33238
Qubit 1X dsDNA HS Assay kit	Thermo Fisher Scientific Q33230
Kinnex™ library preparation	
Kinnex™8-fold prep kit	PacBio® 103-072-000
Sequencing preparation	
Revio® SPRQ™ polymerase kit **or** Vega™ SPRQ™ polymerase kit **or** Vega™ polymerase kit	PacBio® 103-520-100 PacBio® 103-794-300 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 (e.g., Sigma-Aldrich W4502)
8-channel pipettes – P20 & P200	Any MLS
Single-channel pipette – P2, P10, P20, P100 or P200	Any MLS
DNA LoBind tubes, 1.5 mL	Eppendorf 022431021
0.2 mL 8-tube strips	USA Scientific TempAssure 1402-4708
Magnetic separation rack compatible with 0.2 mL 8-tube strips	Any MLS (e.g., V&P Scientific, Inc. VP772F4-1)
Thermal cycler compatible with 0.2 mL 8-tube strips	Any MLS
Microcentrifuge	Any MLS

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Before you begin

Generating Kinnex compatible amplicons

To generate Kinnex compatible cDNA please refer to protocol, **Preparing Kinnex-compatible cDNA using Iso-Seq Express 2.0 kit**.

To generate full-length 16S rRNA amplicons please refer to protocol, **Preparing Kinnex-compatible 16S rRNA amplicons**.

To generate other Kinnex compatible amplicons please refer to the **Technical Note: Concatenating amplicons using PacBio Kinnex kits to increase throughput** for the input, size, and PCR requirements.

The recommended input for Kinnex 8-fold PCR is **35 ng of** Kinnex-compatible cDNA or other types of amplicons with an average size distribution between **1.5–3 kb**.

Smaller cDNA or amplicons may be used if desired. Kinnex 12-fold and 16-fold kits are generally recommended when concatenating smaller amplicons.

Kinnex library size QC

The Agilent Femto Pulse system is recommended when QC of the final library is desired prior to sequencing. See Appendix for an example.

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

Reagent and sample handling best practices

- 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, as small changes in bead volume can significantly alter the size distribution of your sample
- 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

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- The workflow takes approximately 1 day to complete. If a stop is necessary, refer to workflow for safe stopping points.

Temperature-sensitive reagents Kinnex PCR 8-fold kit 103-071-600	
Tube color	Reagent
Green	Kinnex PCR mix 103-107-700
Orange	Kinnex primer mix A 103-107-800
	Kinnex primer mix B 103-107-900
	Kinnex primer mix C 103-108-000
	Kinnex primer mix D 103-108-100
	Kinnex primer mix E 103-108-200
	Kinnex primer mix F 103-108-300
	Kinnex primer mix G 103-108-400
	Kinnex primer mix HQ 103-108-500

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

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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

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.

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Workflow steps

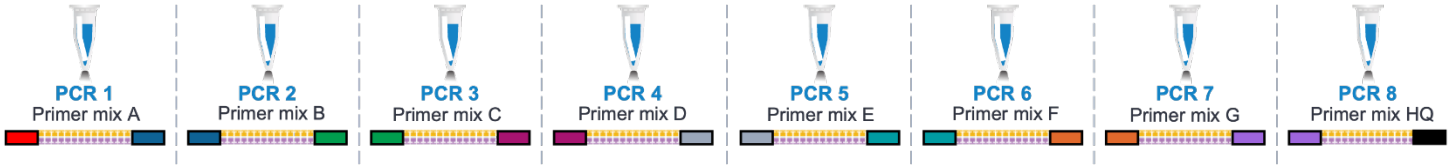
1. Input amplicon quality control

Prior to library preparation, evaluate the size distribution of the input amplicons on Bioanalyzer using high sensitivity DNA kit or on Agilent TapeStation using D5000/High Sensitivity D5000 ScreenTape Assay to determine whether it is suitable for the protocol. The recommended peak or average size should be between 1.5 and 3 kb.

2. Kinnex PCR and pooling

2.1 Kinnex PCR

Perform 8 parallel Kinnex PCR reactions with Kinnex primers to generate DNA fragments containing orientation-specific Kinnex segmentation sequences.



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

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

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

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2.1.3 Thaw the following components, briefly vortex to mix, then quick-spin to collect liquid and place on ice. Add the below components to a LoBind tube, pulse vortex to mix and quick-spin. Place master mix on ice.

Reaction Mix 1 (RM1):

Master mix components	Volume for 8X concatenation*
PCR-grade water	88-X µL
Kinnex PCR mix	110 µL
35 ng of Kinnex-compatible amplicon	X µL
Total volume	198 µL

X = 35 (ng)/input concentration (ng/µL), where the input can be from a single-plex or multiplex sample.
*10% overage included

2.1.4. Aliquot **22.5 µL** of **RM1** into each of the 8 PCR tubes (for 8X concatenation) **on ice.**

2.1.5 Add **2.5 µL** of the appropriate Kinnex primer mix into each respective PCR tube on ice.

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

2.1.7 Run the Kinnex PCR program and keep samples 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 25 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

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

✔	Step	Instructions
	2.2.1	Add exactly **23 µL** from each of the 8 PCR reactions into a 1.5 mL tube for a total volume of **184 µL**.
	2.2.2	Add 193 µL (**1.05X** v/v) of resuspended, room-temperature SMRTbell cleanup beads to a tube of pooled Kinnex PCR amplicon. **The correct ratio of beads to pooled sample is critical at this step.**
	2.2.3	Pipette-mix the beads until evenly distributed.
	2.2.4	Quick-spin the tube in a microcentrifuge to collect liquid.
	2.2.5	Incubate at **room temperature** for **10 minutes** to allow the DNA to bind beads
	2.2.6	Place the tube in a magnetic separation rack until the beads separate fully from the solution.
	2.2.7	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	2.2.8	Slowly dispense **1mL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into the tube. Remove the 80% ethanol and discard.
	2.2.9	Repeat the previous step.
	2.2.10	Remove residual 80% ethanol: • Remove the tube from the magnetic separation rack. • Quick-spin the tube in a microcentrifuge. • Place the tube back in the magnetic separation rack until the beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	2.2.11	Remove the tube from the magnetic rack. **Immediately** add **40 µL** of **elution buffer** to the tube and resuspend the beads by pipetting 10 times or until evenly distributed.
	2.2.12	Quick-spin the tube in a microcentrifuge to collect liquid.
	2.2.13	Incubate at **room temperature** for **5 minutes** to elute DNA.
	2.2.14	Place tube in a magnetic separation rack until beads separate fully from the solution.
	2.2.15	Slowly aspirate the cleared eluate without disturbing the beads. Transfer 40 µL of the eluate to a new PCR tube strip. Discard old tube with beads.
	2.2.16	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 5–9 µg.
	2.2.17	Proceed to the next step of the protocol if sample quantity is acceptable (maximum input: 5 µg). Do not proceed if less than 3 µg is available.

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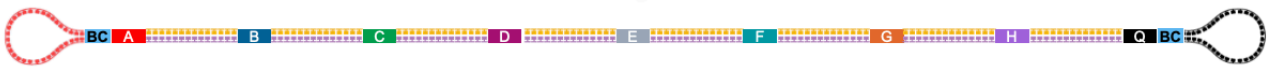
Note: If the Kinnex PCR yield is less than 3 µg, repeat Kinnex PCR and quantifying each individual tube with 1X dsDNA HS kit for low yield troubleshooting.

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

3. Kinnex array formation

3.1 Kinnex array formation

In this step, treat PCR-amplified fragments from Step 2 with Kinnex enzyme, ligase, and barcoded Kinnex terminal adapters to assemble fragments into a linear array.



Complete 8-segment transcript array molecule containing 2 Kinnex adapters

✔	Step	Instructions
	3.1.1	In a 0.2 mL PCR tube, add 5 µg of pooled Kinnex PCR sample from Section 2 in 39 µL of volume (128 ng/µL). Dilute with elution buffer going into this step if the sample is too concentrated. Add 2 µL of Kinnex adapter (bc01–04; select one barcode per library preparation).

Tube color	Components	Volume
Blue	Kinnex barcoded adapter	2 µL

3.1.2 Add the following components in the listed order.

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

Reaction Mix 2 (RM2):

Tube color	Components	Volume
White	Kinnex array and repair buffer	7.0 µL
Red	Kinnex enzyme	4.0 µL
Yellow	Kinnex ligase	6.0 µL
	Total RM2 volume	17 µL

Add **17 µL** of **RM2** to the PCR tube containing sample and Kinnex barcoded adapter for a final volume of 58 µL. Pipette-mix and run the Kinnex primer digestion/ligation program.

3.1.3 Kinnex primer digestion/ligation program

Heated lid set at ≥55°C. Reaction volume set to 60 µL

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

3.1.4	After running the Kinnex primer digestion/ligation program, add 2 µL of Kinnex repair mix directly to the Kinnex primer digestion/ligation sample. **Tube color** / **Components** / **Volume**: Green / Kinnex repair mix / 2 µL
3.1.5	Thoroughly mix by pipetting up and down 10 times and then quick spin to collect all liquid.
3.1.6	Run the Kinnex repair program. **Kinnex repair program** Heated lid set at ≥55°C. Reaction volume set to 60 µL.

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

3.2 1X SMRTbell bead cleanup

Cleanup with 1X SMRTbell cleanup beads

✔	Step	Instructions
	3.2.1	Add **60 µL** (1X v/v) of resuspended, room temperature **SMRTbell cleanup beads** to each sample.
	3.2.2	Pipette-mix the beads until evenly distributed and quick-spin in a microcentrifuge to collect liquid.
	3.2.3	Incubate at room temperature for 10 minutes to allow the DNA to bind the beads.
	3.2.4	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	3.2.5	Slowly remove the cleared supernatant without disturbing the beads. Discard the supernatant.
	3.2.6	Slowly dispense **200 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into each tube. Remove the 80% ethanol and discard.

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	3.2.7	Repeat the previous step.
	3.2.8	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	3.2.9	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. Quick-spin the tube strip in a microcentrifuge to collect liquid.
	3.2.10	Incubate at **room temperature** for **5 minutes** to elute DNA.
	3.2.11	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	3.2.12	Slowly aspirate the cleared eluate without disturbing the beads. Transfer 41 µL of the eluate to a **new PCR strip tube**. Discard old tube with beads.
	3.2.13	Recommended to save aliquot for potential troubleshooting. • Take a **1 µL** aliquot from each tube, dilute with **4µL** of **elution buffer**. **SAFE STOPPING POINT -- Store at 4°C or -20°C for long-term storage**

4. Nuclease treatment & cleanup

4.1 Nuclease treatment

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

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

	4.1.2	Add 10 µL of **RM3** to each sample. The total volume should be 50 µL.
	4.1.3	Thoroughly mix by pipetting up and down 10 times and then quick-spin to collect all liquid.

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4.1.4 Run the nuclease treatment program.

Nuclease treatment program

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

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

4.2 Final cleanup with 1X SMRTbell cleanup beads

✔	Step	Instructions
	4.2.1	Add **50 µL** (1X v/v) of resuspended, room temperature **SMRTbell cleanup beads** to each sample from the previous step. Pipette-mix the beads until evenly distributed.
	4.2.2	Quick-spin the tube strip in a microcentrifuge to collect all liquid.
	4.2.3	Incubate at **room temperature** for **10 minutes** to allow DNA to bind the beads.
	4.2.4	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	4.2.5	Slowly remove the cleared supernatant without disturbing the beads and discard the supernatant.
	4.2.6	Slowly dispense **200 µL**, or enough to cover the beads, of **freshly prepared 80% ethanol** into each tube. Remove the 80% ethanol and discard.
	4.2.7	Repeat the previous step.
	4.2.8	Remove residual 80% ethanol: • Remove the tube strip from the magnetic separation rack. • Quick-spin the tube strip in a microcentrifuge. • Place the tube strip back in a magnetic separation rack until beads separate fully from the solution. • Remove residual 80% ethanol and discard.
	4.2.9	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.
	4.2.10	Quick-spin the tube strip in a microcentrifuge to collect liquid.
	4.2.11	Incubate at **room temperature** for **5 minutes** to elute DNA.
	4.2.12	Place the tube strip in a magnetic separation rack until the beads separate fully from the solution.
	4.2.13	Slowly aspirate the cleared eluate without disturbing the beads. Transfer 26 µL of eluate to a new PCR tube strip. Discard old tube strip with beads.

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4.2.14	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 10-30% recovery of the starting Kinnex-PCR product.** • Recommended: Further dilute each aliquot to 250 pg/µL with the Femto Pulse dilution buffer. Check the final Kinnex library size distribution with a Femto Pulse system (see Figure 1 in the Appendix for an example).
4.2.15	**If required, normalize 25 µL of library to <60 ng/µL**. Using a concentration above 60 ng/µL into ABC will result in poor sequencing performance.
4.2.16	Proceed to Section 5 to prepare library for sequencing with Vega, Vega SPRQ-Nx or Revio (+SPRQ/SPRQ-Nx) chemistry.
4.2.17	Store Kinnex libraries at 4°C if sequencing within the week. Long-term storage should be at -20°C. Minimize freeze-thaw cycles when handling Kinnex libraries.

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

5. 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
	5.1	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**

	5.2	Pipette-mix the Annealing mix and quick spin to collect liquid.
	5.3	Add 25 µL of the Annealing mix to each library. Total volume should equal **50 µL**.
	5.4	Pipette-mix each sample and quick spin to collect liquid.

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5.5 Incubate at **room temperature** for **15 minutes**.

5.6 During primer incubation, prepare the polymerase dilution (see below) and store on ice.

5.7 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**

5.8 Pipette mix the **polymerase dilution** and quick-spin to collect liquid.

5.9 Add **50 µL of polymerase dilution** to primer annealed sample. Total volume should equal **100 µL**.

5.10 Pipette-mix each sample and quick-spin to collect liquid.

5.11 Incubate at **room temperature for 15 minutes.**

5.12 Proceed immediately to the next step of the protocol to remove excess polymerase.

Post-binding cleanup with 1X SMRTbell cleanup beads

5.13 Add **100 µL** of resuspended, room-temperature SMRTbell cleanup beads to each sample

5.14 Pipette-mix the beads until evenly distributed and quick-spin if necessary to collect all liquid from the sides of the tube.

5.15 Incubate at **room temperature** for **10 minutes** to allow DNA to bind beads

5.16 Place sample on an appropriate magnet and allow beads to separate fully from the solution

5.17 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.

5.18 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

5.19 Quick-spin the samples to collect any liquid from the sides of the tube.

5.20 Incubate at **room temperature** for **15 minutes** to elute DNA

5.21 Place sample on magnet and allow beads to separate fully from the solution.

5.22 Slowly aspirate the cleared eluate without disturbing the beads. Transfer eluate to a new tube. Discard the old tube with beads

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5.23	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.
5.24	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.

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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 custom 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 5 for full protocol.

A2. Library QC

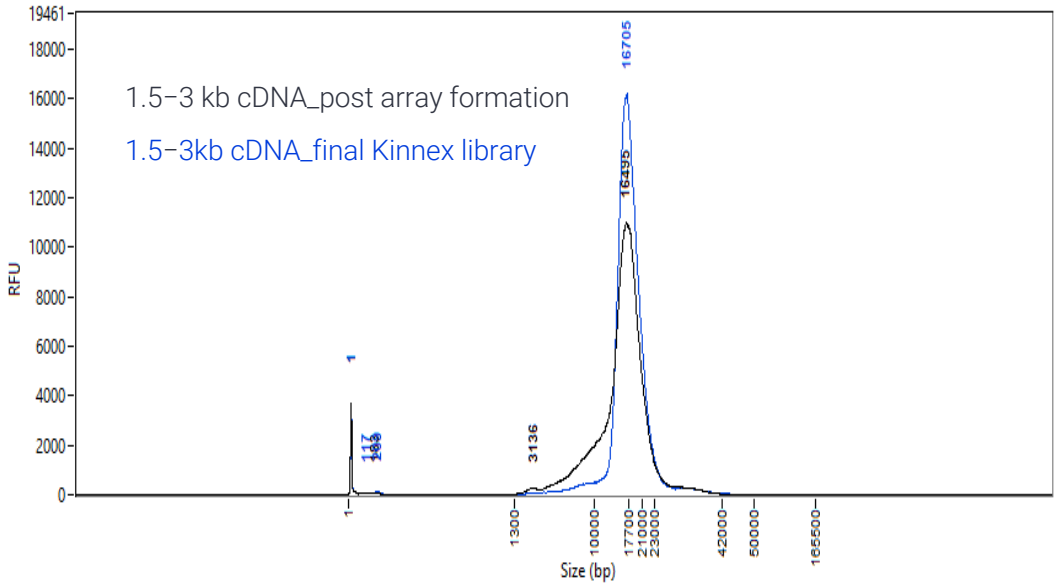

Figure 1. 8-fold array and final library quality control on FEMTO Pulse

APPENDIX COMPLETE

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FAQs

1. My sample has a peak size of 1.5–2 kb. Should I use the Kinnex 8-fold or 12-fold library preparation workflow?

The recommended input peak size is 1.5–3 kb for the Kinnex 8-fold prep kit and 1–2 kb for the Kinnex 12-fold prep kit. Therefore, samples with a peak size of 1.5–2 kb are compatible with either workflow.

Choose the workflow based on your sequencing priority. For example, for 16S rRNA amplicons:

- Use the Kinnex 8-fold prep kit when higher sequencing QV is the priority.
- Use the Kinnex 12-fold prep kit when maximizing sequencing throughput is the priority.

2. Can I use less than 3 µg of Kinnex PCR product for array formation?

The recommended Kinnex PCR input for array formation is **3–5 µg**. Under ideal conditions—when the Kinnex PCR product has the expected yields, profile and no individual PCR reaction has a noticeably lower yield than the others — an aliquot containing as little as **1 µg** of input may be sufficient for two acquisitions using Vega SPRQ-Nx or Revio SPRQ/SPRQ-Nx chemistry.

3. What should I check if my library yield is low?

If the library size distribution is clean at step 4.2.14, you may proceed for sequencing if there is sufficient library for 1-2 acquisitions. Low library yield may result from inefficient array formation. To assess array formation:

1. Measure the 5× diluted aliquot saved at Step 3.2.13 using a Qubit fluorometer with the 1× dsDNA HS Assay Kit.
2. Dilute the aliquot further to 250 pg/µL using Femto Pulse Dilution Buffer.
3. Assess the array size distribution using a Femto Pulse system.

An example of an ideal array profile before nuclease treatment is provided in **Figure 1 of the Appendix**.

4. What happens if I select the wrong segmentation adapter set in SMRT Link analysis?

Selecting the incorrect segmentation adapter set can reduce both the **number of segmented reads** and the **mean array size**. If a segmentation adapter set with a higher concatenation factor than the library was prepared with is selected, the analysis may report **0% of reads with a full array**.

Rerun the analysis using the segmentation adapter set that matches the concatenation factor used during library preparation.

5. What can cause low loading of Kinnex 16S rRNA libraries?

Contaminants carried over from some bacterial genomic DNA samples can adversely affect sequencing performance and result in low library loading.

If low loading is observed, perform an additional cleanup of the final SMRTbell library using either:

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- **3.1×-diluted AMPure® PB** (35% v/v; PN 100-265-900), or
- **PureTarget™ cleanup kit** (PN 103-708-100), following **Step 8 of *Procedure & Checklist – Generating PureTarget Libraries – Manual Protocol*** (PN 103-740-700).

These additional cleanup methods have been shown to effectively remove contaminants that can interfere with sequencing performance.

Revision history (description)	Version	Date
Initial release	01	September 2026
Corrected final volume in step 3.1.3 from 56 µL to 58 µL	02	October 2026

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