PROTOCOL

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ACCEL-NGS® XL LIBRARY KIT

Compatible with Pacific Biosciences® Platforms

- RS II
- Sequel™ (in development)

Cat. No. 71016

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About This Guide

This guide provides instructions for preparation of long insert templates from double-stranded DNA (dsDNA) using an Accel-NGS XL Library Kit designed for use in place of the Pacific Biosciences (PacBio®) SMRTbell™ Template Prep Kit to achieve subreads 20 kb and greater.

IMPORTANT!

Read the entire Protocol before use, including the Product Information, Kit Contents, Material and Equipment Not Included, and Prepare the DNA Sample, in order to understand how these reagents integrate with the PacBio sequencing reagents.

Product Information

The Accel-NGS XL Library Kit for PacBio is a template preparation kit designed for long-read sequencing on the PacBio sequencing platforms: RS II and Sequel (in development). This kit is designed to work in conjunction with the PacBio DNA Polymerase Binding and MagBead loading kits. Swift's enhanced repair and ligation chemistry enable the consistent production of long subreads exceeding 20 kb in length while simultaneously preventing the formation of adapter dimers.

The Accel-NGS XL Library Kit has been validated with 2-5 µg of high quality DNA sheared using the Covaris® g-TUBE exceeding 20 kb and the Diagenode® Megaruptor exceeding 60 kb.

When sequencing Accel-NGS XL libraries, the polymerase read length equates to the subread length and no CCS reads will be generated. To maximize data output, use the largest fragmentation size feasible for the quality of your sample.

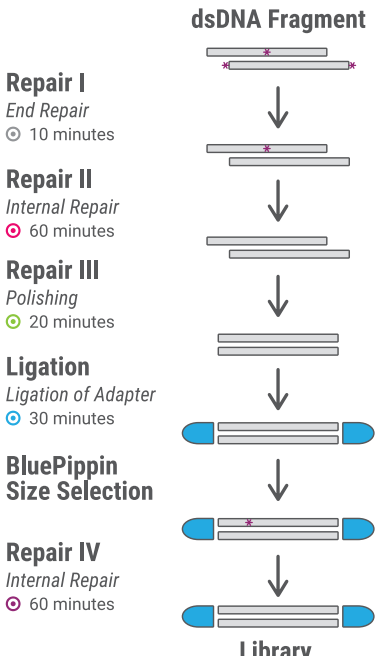
Applications

The Accel-NGS XL Library Kit is suitable for the following applications:

- Whole genome sequencing, including:
  - *De novo* assembly
  - Genome finishing
  - Hybrid sequencing

Accel-NGS XL Library Kit Workflow

Using five incubations in a simple workflow, this protocol repairs both 5´ and 3´ termini and attaches adapter sequences to the ends of fragmented dsDNA. Bead-based AMPure PB clean-ups are used to remove oligonucleotides and small fragments, as well as to change enzymatic buffer composition between steps. Repair I reduces chimeras and palindromic reads by repairing both 5′ and 3′ termini. Repair II repairs internal lesions to increase the read length during SMRT sequencing, and Repair III polishes ends in preparation for the ligation step. **Repair IV is not optional and repairs any damage that occurred during sample manipulation.**



The library prepared using the Accel-NGS XL Library Kit can be readily sequenced on the PacBio RS II platform. Currently, this kit is in development for sequencing on the PacBio Sequel. Contact TechSupport@swiftbiosci.com if you plan to sequence on the Sequel.

Note: When sequencing Accel-NGS XL libraries, the polymerase read length equates to the subread length and no CCS reads will be generated. To maximize data output, use the largest fragmentation size feasible for the quality of your sample.

Perform size selection prior to SMRT sequencing to remove smaller fragments that reduce the loading efficiency of longer inserts on SMRT cells. This purification step will maximize average read length and data output. Following purification, a repair step is performed to maximize read length.

Primer annealing and DNA polymerase binding should be performed according to the "PacBio Template Preparation and Sequencing" guidelines. This kit provides an elution buffer, sequencing primer, and a 10X primer annealing buffer that have been validated for sequencing on the RS II platform. Alternatively, the elution buffer, sequencing primer, and 10X primer annealing buffer supplied by PacBio can be used.

Kit Contents

The Accel-NGS XL Library Kit includes reagents for the preparation of 16 libraries (10% excess volume).

Reagents	Quantity (µl)	Storage (°C)
Buffer W1	104	-20
Enzyme W2	18	-20
Enzyme W3	18	-20
Buffer R1	87	-20
Reagent R2	18	-20
Reagent R3	27	-20
Enzyme R4	35	-20
Enzyme G1	18	-20
Enzyme G2	18	-20
Buffer B1	70	-20
Reagent B2	70	-20
Enzyme B3	44	-20
Buffer B4	900	-20
Buffer P1	87	-20
Reagent P2	18	-20
Reagent P3	27	-20
Enzyme P4	35	-20
Buffer Y1	90	-20
Reagent Y2	35	-20

IMPORTANT!
Place the enzymes on ice, NOT in a cryocooler, for at least 10 minutes to allow enzymes to reach 4 ˚C prior to pipetting.

Reagents	Quantity (mL)	Storage (°C)
PEG NaCl Solution	20.0	Room Temp
Low EDTA TE	0.9	-20
Elution Buffer	1.15	Room Temp

Material and Equipment Not Included

- Qubit® or other fluorometric-based assays for determining DNA concentration
- Pulse Field Gel Electrophoresis (PFGE) for DNA size profile evaluation
- Shearing device, e.g., Covaris g-TUBE, Diagenode Megaruptor
- AMPure PB beads (PacBio)
- Magnetic rack for clean-up steps, e.g., Invitrogen DynaMag™ or Agencourt® SPRIPlate™
- BluePippin Size Selection System (Sage Science)
- MagBead kit v2 (PacBio Cat. No. 100-676-500)
- DNA/Polymerase Binding Kit P6 v2 (PacBio Cat. No. 100-372-700)
- Microfuge
- Programmable thermocycler
- Programmable vortex for controlled vortexing (1000 rpm)
- 0.2 mL LoBind PCR tubes (Eppendorf)
- Aerosol-resistant, low retention pipettes and tips, 2 to 1000 µl
- 200-proof/absolute ethanol (molecular biology-grade)
- Nuclease-free water (molecular biology-grade)

Storage and Usage Warning

Upon receipt, store the Accel-NGS XL Library Kit products at -20 ˚C with the exception of the PEG, Elution Buffer, and TE solutions, which are stored at room temperature.

To maximize use of enzyme reagents when ready to use, remove enzyme tubes from -20 ˚C storage and place on ice, NOT in a cryocooler, for at least 10 minutes to allow enzymes to reach 4 ˚C prior to pipetting. Attempting to pipette enzymes at -20 ˚C may result in a shortage of enzyme reagents.

After thawing reagents to 4 ˚C, briefly vortex (except the enzymes) to mix them well. Spin all tubes in a microfuge to collect contents prior to opening.

IMPORTANT!

- Assemble all reagent master mixes ON ICE and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss.
- Always add reagents to the master mix in the specified order as stated throughout the protocol.

Tips and Techniques

Avoiding Cross-Contamination

To reduce the risk of DNA and library contamination, follow the instructions below:

- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change tips between each sample.

Maximizing Subread Length

Follow the instructions below to maximize DNA quality and subread length:

- Use low-retention microcentrifuge tubes during the entire template preparation process.
- Eliminate vortex or pipette-based mixing as both shear long DNA fragments.
- Do not expose your library to intercalating agents or ultraviolet light.
- Minimize the number of freeze-thaw cycles.
- Store any input DNA in a buffered solution containing a Low EDTA solution (0.1mM), as provided in this kit, to maximize DNA stability.
- Ensure that the DNA's $OD_{260/280}$ and $OD_{260/230}$ ratios are approximately 1.8-2.0 and ~2.0, respectively.
- Ensure that the input DNA is free of any compounds, detergents, or solvents that may be toxic to SMRT sequencing that have carried over from sample purification (phenol, CTAB, SDS, etc.)
- Minimize molecular carryover from cells/tissues of origin (e.g., heme, polyphenols, carbohydrates, etc.).

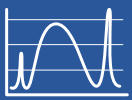
AMPure® PB Purification/Concentration

This protocol has been validated with AMPure PB magnetic beads (PacBio) to facilitate buffer exchange and remove small DNA fragments. It is important that you use specially approved beads appropriate for PacBio SMRT sequencing as regular SPRIselect™ and AMPure XP beads are incompatible. Consider the information below for performing efficient purification/concentration steps:

IMPORTANT!

- Most DNA loss within the workflow will be due to loss during AMPure PB steps. Therefore, if using this protocol for the first time, we strongly recommend that you process a control sample first, and review Appendix, Section A for the AMPure PB Purification/Concentration Protocol.
- Please note that this is a "with bead" protocol, meaning beads added to clean up the Repair I reaction will be retained and re-used throughout subsequent enzymatic reactions and clean-ups. As a result, PEG NaCl is added in place of beads for subsequent clean-up steps.

From Sample to Sequencing



Prepare the DNA Sample

- Assess the quality of your DNA sample
- Fragment the DNA sample and analyze the result
- (Optional) store your DNA for up to 24 hours at 4 ˚C or 2 weeks at -20 ˚C until ready to use

Prepare the Reagent Master Mixes and Ethanol

When instructed in the protocol,

- Place the enzymes on ice for 10 minutes
- Briefly vortex non-enzyme reagents and spin all reagents
- Leave the reagents on ice until ready to use
- Prepare 80% ethanol solution

Prepare the DNA Libraries

- Ensure DNA sample is in Low EDTA TE buffer at a concentration of 100-150 ng/µl
- Follow the library workflow on page 2
- Perform BluePippin size selection
- Perform Repair IV
- Quantify the library

Assemble and Load the PacBio Template Complex

- Primer annealing
- PacBio polymerase binding
- PacBio MagBead-template complex loading

Sequence the DNA library

- RS II
- Sequel (in development)

Prepare the DNA Sample

Input DNA Assessment

Long insert preparation for sequencing on PacBio platforms is sensitive to any un-repairable damage that can cause premature termination of long insert sequencing. As a result, sequencing output will be directly affected by input DNA quality, where poor quality of DNA will minimize the number of achievable long subreads.

Prior to fragmentation, perform the following quality assessments to ensure that the DNA is pure, sheared to the intended molecular weight, and of a high integrity.

- Spectrophotometry (NanoDrop® Spectrophotometer) for evaluation of DNA input purity: We recommend measuring both the $OD_{260/280}$ and $OD_{260/230}$ ratios, as lower than expected results of these metrics is indicative of protein and organic/inorganic contamination, respectively.
- Fluorimetry: PicoGreen® or Qubit fluorimeter for an accurate quantification of DNA input.
- Gel electrophoretic methods: use PFGE for an accurate size determination. Ensure that the desired shear length was attained and that there is no evidence of significant DNA degradation (a significant smear running smaller than the desired size range).

DNA Input Requirements

Depending upon the quality of the DNA sample, approximately 20-50% loss is to be expected as a result of the shearing and concentration process. Therefore, be sure to have sufficient DNA starting material for the long insert template preparation. Using this protocol, increased yields can be achieved due to the simple workflow, high ligation efficiency, and preservation of overall DNA integrity. As a result, the amount of sheared DNA input for long insert template preparation may be reduced accordingly.

This protocol has been validated for input amounts of 2-5 µg. With longer shear lengths, more input DNA will be needed to compensate for the loss in the molar amounts of dsDNA molecules (≥ 30 kb). With smaller shears (20-25 kb), inputs as low as 2 µg of high quality DNA may be sufficient. Please consider several factors when determining input quantity: DNA quality, stringency of the BluePippin size selection, shear size, and percent DNA recovery from AMPure PB steps.

Ensure that your input DNA is diluted in Low EDTA TE at a concentration of 100-150 ng/µL. For more dilute samples, concentrate the DNA by performing a 0.5X AMPure PB clean-up and use a dilution volume targeting the recommended DNA concentration range.

DNA Fragmentation

This protocol is suitable for DNA shear sizes exceeding 20 kb, and has been validated using DNA sheared with both the Diagenode Megaruptor and the Covaris g-TUBE according to manufacturers' recommendation. Consider the important notes below for obtaining an optimal shear quality:

- Depending on the quality of your starting material and the selected method of shearing, you should expect to lose 20-50% of your DNA sample. Therefore, use a sufficient quantity of starting DNA to recover the required 2-5 µg of sheared and concentrated DNA (100-150 ng/µl) for the Repair I reaction.
- Post fragmentation, quantify the sheared DNA using spectrophotometric or fluorometric methods as described previously. In addition, assess size range of the sheared fragments using electrophoretic methods if desired. Note that the quality of sheared DNA is dependent on the starting material quality and size.
- When using 5 µg of sheared DNA, a minimum concentration of 100 ng/µl is required. For more dilute samples, concentrate the DNA by performing an AMPure PB clean-up with an elution volume at the required concentration. For long inserts exceeding 20 kb, we recommend using a 0.5X ratio of the AMPure PB beads to total volume.
- The sheared DNA can be stored for up to 24 hours at 4 ˚C or 2 weeks at -20 ˚C.

Prepare the Reagent Master Mixes and Ethanol

1. To prepare the master mixes, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss.
2. To assemble reagent master mixes for the Repair I, Repair II, and Ligation steps, ensure the reagent vials are at room temperature and enzymes are at 4 ˚C. After thawing reagents, briefly vortex (except the enzymes) to mix them well. Spin all tubes in a microfuge to collect contents prior to opening. Once prepared, master mixes should be stored ON ICE until used.

IMPORTANT!

- Always add reagents in the specified order.
- Pre-mix the master mixes in advance (except Enzyme G1 and Enzyme G2 for Repair III) to ensure the AMPure PB beads do not dry out during the purification/concentration steps, and store on ice until ready to use. For example, while waiting for the completion of Repair I thermocycler program, you may pre-mix Repair II Master Mix, and place it on ice until ready to use.
- For optimal performance of the repair steps, use no more than 5 µg of DNA per library prep reaction. If preparing larger amounts of input DNA, we recommend doubling the amount of DNA input, and carrying out the reactions by doubling each component and total volume.

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3. Prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and nuclease-free water (approximately 1.5 mL will be used per sample).

Prepare the DNA Libraries

Repair I

1. Preset the thermocycler to 37 °C (lid at 40 °C).
2. Transfer the large shear DNA sample to a 0.2 mL PCR tube and adjust the volume of the sample to a final volume of 40 µl using Low EDTA TE, if necessary.
3. Prepare the Repair I Master Mix by adding the reaction components in the specified order listed in the table below.

Reagents	Volume per Sample
Large Sheared DNA in Low EDTA TE	40 µl
Low EDTA TE	12 µl
Buffer W1	6 µl
Enzyme W2	1 µl
Enzyme W3	1 µl
Total Volume	**60 µl**

4. Add the Repair I Master Mix to each sample and mix by gently flicking the tube until thoroughly homogeneous.
5. Spin down the tube using a microfuge, and place in the thermocycler programmed at 37 °C (lid at 40 °C) for 10 minutes during which time you may prepare Repair II Master Mix.
6. Clean up the Repair I reaction using a magnetic rack, AMPure PB magnetic beads (see Material and Equipment Not Included), and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Insert Size	Sample Volume	AMPure PB Bead Volume	PEG NaCl Volume
All Sizes	60 µl	30 µl (ratio: 0.5)	—

IMPORTANT!

- This is a “with bead” protocol, meaning beads added to clean up the Repair I reaction will be retained and re-used throughout subsequent enzymatic reactions and clean-ups. As a result, PEG NaCl is added in place of beads for subsequent clean-up steps.
- Avoid mixing by vortexing or pipetting during clean-ups as both further shear long DNA fragments.

7. Following the Repair I clean-up, while the tube is still on the magnetic rack, carefully remove and discard the supernatant without removing any beads. Proceed immediately to Repair II. DO NOT let the beads dry.

Repair II

8. Add 50 µl of pre-mixed Repair II Master Mix (made in the order listed below) to the beads for each sample.

Reagents	Volume per Sample
Buffer R1	5.0 µl
Reagent R2	1.0 µl
Reagent R3	1.5 µl
Low EDTA TE	40.5 µl
Enzyme R4	2.0 µl
Total Volume	**50.0 µl**

9. Mix by gently flicking the tube until homogeneous, and spin down the tube using a microfuge.
10. Place in the thermocycler set at 37 ˚C (lid at 40 °C) for 60 minutes.
11. Upon completion of the thermocycler program, place the reaction on ice for 1-5 minutes before advancing to the next step (Repair III).

Repair III

12. Pre-set the thermocycler at 20 ˚C (lid heating off).
13. Add 1 µl of Enzyme G1 and Enzyme G2 directly to each sample tube containing Repair II Master Mix.

Reagents	Volume per Sample
Repair II Master Mix	50 µl
Reagent G1	1 µl
Reagent G2	1 µl
Total Volume	**52 µl**

14. Mix by gently flicking the tube until homogeneous, and spin down the tube using a microfuge.
15. Incubate the reaction at 20 ˚C for 20 minutes during which time you may prepare the Ligation Master Mix.
16. Clean up the reaction using a magnetic rack, PEG NaCl solution, and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Insert Size	Sample Volume	AMPure PB Bead Volume	PEG NaCl Volume
All Sizes	52 µl	—	31 µl (ratio: 0.6)

17. Following post-Repair III clean-up, while the tube is still on the magnetic rack, carefully remove and discard the supernatant without removing any beads. Proceed immediately to Ligation. DO NOT let the beads dry.

Ligation

18. Pre-set the thermocycler at 25 ˚C (lid heating off).
19. Add 40 µl of pre-mixed Ligation Master Mix (made in the specified order listed below) to the beads for each sample.

Reagents	Volume per Sample
Buffer B1	4.0 µl
Reagent B2	4.0 µl
Low EDTA TE	29.5 µl
Enzyme B3	2.5 µl
Total Volume	**40.0 µl**

20. Mix by gently flicking the tube until homogeneous, and spin down the tube using a microfuge.
21. Place in the thermocycler set at 25 ˚C (lid heating off) for 30 minutes.
22. Clean up the Ligation reaction using a magnetic rack, PEG NaCl solution, and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Insert Size	Sample Volume	AMPure PB Bead Volume	PEG NaCl Volume	Elution Volume
All Sizes	40 µl	—	18 µl (ratio: 0.45)	25 µl

23. At the end of the clean-up, resuspend the beads in a volume of 25-50 µl of Buffer B4. For downstream BluePippin purification, be sure to adjust your final elution volume to reflect the maximum sample volume permitted per lane (30 µl).
24. Mix by gently flicking the tube until homogeneous, and spin down the tube using a microfuge.
25. Place the tube in a controlled vortexer, apply gentle vortexing (1000 rpm) for 2 minutes to facilitate library elution from the beads.
26. Place the sample tube on a magnetic rack and wait 2 minutes.
27. Carefully transfer the supernatant containing the final library to a clean tube without carrying any beads. This eluate contains your library for size selection.

IMPORTANT!

After library construction, do not treat your final Accel-NGS XL libraries with an exonuclease or end repair reagents supplied by PacBio, as these treatments are incompatible.

BluePippin Size Selection

28. Follow recommendations for BluePippin size selection.

Repair IV

Following the BluePippin size selection, perform a repair. This will maximize read length by repairing any damage that occurred during sample manipulation. The user will need to concentrate the size selected library to a useable volume by using AMPure PB beads (0.5X ratio). See Appendix, Section A for instructions. Elute off of the beads in 30 µl of Elution Buffer.

29. Add 20 µl of the pre-mixed Repair IV Master Mix (in the order listed below) to each 30 µl sample.

Reagents	Volume per Sample
Buffer P1	5.0 µl
Reagent P2	1.0 µl
Reagent P3	1.5 µl
Elution Buffer	10.5 µl
Enzyme P4	2.0 µl
Total Volume	**20.0 µl**

30. Mix by gently flicking the tube until homogeneous, and spin down the tube using a microfuge.
31. Place in the thermocycler set at 37 ˚C (lid at 40 °C) for 60 minutes.
32. Perform an AMPure PB clean-up on the Repair IV reaction using a magnetic rack, AMPure PB magnetic beads, and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Insert Size	Sample Volume	AMPure PB Bead Volume	PEG NaCl Volume
> 20	50 µl	30 µl (ratio: 0.6)	—

33. Elute off of AMPure PB beads in 12 µl of Elution Buffer.

IMPORTANT!

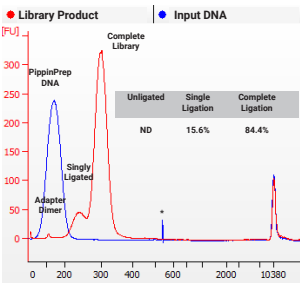
See Appendix, Section B for instructions on the assembly and loading of the PacBio template complex.

Safe Stopping Point

Store freshly prepared libraries at 4 ˚C for up to two days or long term at -20 ˚C. The library is now ready for quantification. Please refer to quality assessment methods outlined on page 7 of this guide.

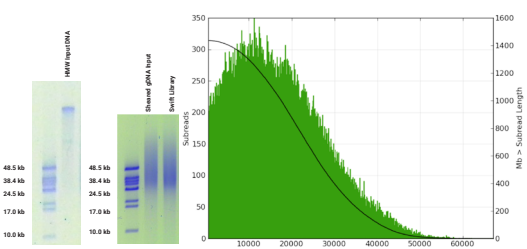
Expected Results

The final library is a heterogeneous mix of ligation-produced DNA species: non-adapted, single adapter ligated, and completely-ligated DNA library molecules. However due to the high ligation efficiency of the Accel-NGS XL Library Kit, ≥ 75% of all molecules represent completely-ligated library molecules that are optimal for SMRTsequencing (See below).



*Ligation efficiency was tested on a Covaris sheared substrate that was size purified to a narrow 150 bp range with the Sage Pippin Prep. 50 ng of substrate was made into library using the Accel-NGS XL Kit with relaxed AM-Pure PB ratios (1.8x) to maximize library retention. No chimeras and minimal adapter dimers are observed. Peak quantification shows an ~85% library conversion rate. *Denotes a non-specific peak*

Below is a representative RS II SMRT cell run from a library prepared with the Accel-NGS XL Kit. The library was prepared from a 40 kb Megaruptor shear of high quality *E. coli* genomic DNA. A 20 to 50 kb size selection was performed on the Sage BluePippin with the additional Swift post-BluePippin repair. The library was loaded onto the RS II at 125 pM concentration. The SMRT cell loaded with a 61.83% P1 productivity. Furthermore, the Accel-NGS XL Library Kit produced high quality contig using the SMRT Portal pipelines.



Accel-NGS XL Kit	Metrics
Polished Contigs	1
Number of Bases	1,440,602,798
Number of Reads	84,178
N50 Read Length	23,224
Mean Subread Length	17,113
Mapped Read Length of Insert	14,553
Average Reference Consensus Concordance	100%

Appendix

Section A: "With-bead" AMPure PB Clean-up/Concentration Protocol

Please use the following protocol for each clean-up step, substituting the correct **Bead Volume**, **PEG NaCl Volume**, and **Elution Volume** based on the table provided for each section.

1. Ensure that the AMPure PB magnetic beads are at room temperature and vortex them to homogenize the suspension thoroughly before use.
2. Add the specified volume of AMPure PB beads (post-Repair I and Repair IV clean-ups/library-concentration step) or PEG NaCl mix (Post Repair III and Ligation clean-ups) to each sample. Mix by flicking the tube to completely suspend contents. **Note:** Be sure to completely resuspend the beads, otherwise significant DNA sample loss will occur.
3. Pulse spin the samples in a tabletop microfuge. DO NOT MIX THE BEADS BY PIPETTING.
4. Allow the DNA to bind the beads. Use controlled vortexing (1000 rpm) for 20 minutes to facilitate DNA binding.
5. After incubation, briefly centrifuge the sample tubes to collect the sample/beads at the bottom of the microtube.
6. Place tubes on a magnetic rack for 5 minutes until the solution appears clear. During the washes, keep sample tubes on the magnet.
7. Gently remove the solution from the tube leaving a few microliters of liquid behind. This will ensure that no beads will be aspirated.
8. Add 200 µl of freshly prepared 80% ethanol solution to the sample while it is still on the magnet, and wash the beads making an effort to wash down all sides of the tube during pipetting.
9. After 30 seconds, remove the ethanol wash from the beads. Be careful not to aspirate any beads.
10. Pipette 200 µl of 80% ethanol to the tube, making the effort to wash down all sides of the tube during pipetting.
11. Gently remove the solution from the tube. Be careful not to aspirate any beads.
12. Briefly spin the tubes to collect any remaining ethanol wash, place the tube on the magnet, and then remove any solution with a fine-tipped pipette. DO NOT LET THE PELLET AIR-DRY.
13. Immediately add the pre-prepared master mix, Buffer B4, Elution Buffer, or Low EDTA TE to each tube first, followed by sample resuspension. DO NOT MIX THE BEADS BY PIPETTING. Completely suspend the beads by either shaking or by gentle flicking. Briefly centrifuge to collect the sample at the bottom of the microtube.
14. If an incubation is the next step, place the tube in the thermocycler at its indicated temperature. If eluting the DNA off of the beads, gently mix the beads at room temperature by controlled vortexing (1000 rpm) for 2 minutes.
15. Put the tube back on a magnetic rack and wait 2 minutes. Gently remove the sample, taking care not to aspirate any beads.

Section B: Primer Annealing and SMRT® Cell Loading

The Accel-NGS XL Library Kit is a template preparation kit for long-read sequencing on the PacBio RS II sequencing platform and is in development for use on the Sequel sequencing platform. Please follow PacBio recommendations for primer annealing and SMRT cell loading.

This kit provides a sequencing primer (Reagent Y2), Elution Buffer, and a 10X primer annealing buffer (Buffer Y1) that have been validated for sequencing on the RS II platform. Alternatively, the sequencing primer, elution primer, and primer annealing buffer supplied by PacBio can be used. Long insert libraries should be loaded using the PacBio DNA/Polymerase Binding Kit P6 v2 (100-372-700) and the PacBio MagBead kit (100-676-500) onto the SMRT Cells.

A sample specific titration for determining an optimal loading concentration is strongly recommended. Loading of templates into zero-mode waveguides (ZMWs) is size dependent with small inserts loading with higher efficiency than larger inserts. Therefore, slightly higher concentrations are required to achieve optimal loading efficiency. Please use the recommended on-plate loading concentrations below as a starting point for optimal SMRT cell loading:

Size Range (kb)	Loading Range (pM)
20-25	100-125
25-30	125-150
30-60	150-350

Please consider library size distribution and molarity as they impact the loading concentration. For larger libraries (≥ 30 kb), we recommend determining the optimal loading concentration by performing a titration with 50 pM increments. For more information on loading conditions, please contact TechSupport@swiftbiosci.com.

For long insert read data acquisition, we recommend 240 or 360 minute movies.

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Notes

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