plexWell™ 384 Library Preparation Kit
and
plexWell™ 96 Library Preparation Kit
for Illumina® Sequencing Platforms

(Part Nos. PW384, PW096)

User Guide

plexWell 96/384 Library Preparation

Workflow Diagram

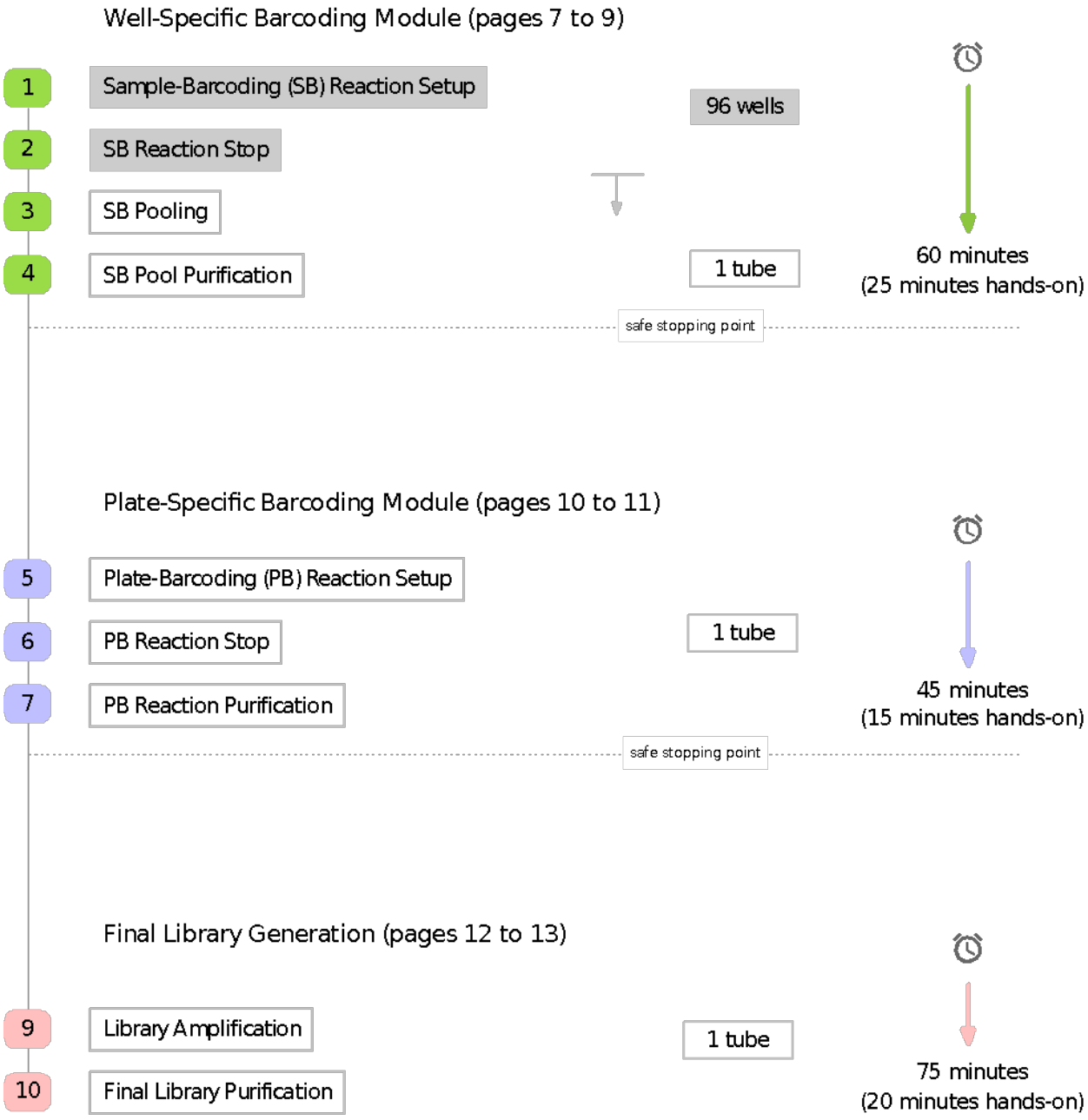

Table 1. Components of plexWell 384 Library Preparation Kit

Components (Box 1 of 2)	Description	Storage Condition	Quantity
Sample Barcode Plate P/N: SBP96	**SBP96 Plate:** Input/assay ready SB reagent in low profile, fully-skirted 96-well red PCR plate	-20°C	4
Pool Barcode Reagent **X007** P/N: PBX007 **X060** P/N: PBX060 **X079** P/N: PBX079 **X089** P/N: PBX089	0.5 ml tube, red cap, 10 µl	-20°C	1 1 1 1
Library Primer Mix P/N: PRM384	0.5 ml tube, natural cap, 24 µl	-20°C	1

Components (Box 2 of 2)	Description	Storage Condition*	Quantity
Coding Buffer (3X) P/N: CB0384	2 ml tube, violet cap, 1.5 ml	Room temperature	2
X Solution P/N: QB0096	2 ml tube, black cap, 1.5 ml	Room temperature	4
MAGwise™ Paramagnetic Beads P/N: MG5000	10 ml tube, white cap, 5 ml	4°C	1

*Upon arrival, store kit components as indicated in the chart above.

Table 2. Components of plexWell 96 Library Preparation Kit

Components (Box 1 of 2)	Description	Storage Condition	Quantity
Sample Barcode Plate P/N: SBP96	**SBP96 Plate:** Input/assay ready SB reagent in low profile, fully-skirted 96-well red PCR plate	-20°C	1
Pool Barcode Reagent X007 P/N: PBX007	0.5 ml tube, red cap, 10 µl	-20°C	1
Library Primer Mix P/N: PRM384	0.5 ml tube, natural cap, 24 µl	-20°C	1

Components (Box 2 of 2)	Description	Storage Condition*	Quantity
Coding Buffer (3X) P/N: CB0384	2 ml tube, violet cap, 1.5 ml	Room temperature	1
X Solution P/N: QB0096	2 ml tube, black cap, 1.5 ml	Room temperature	1
MAGwise Paramagnetic Beads P/N: MG1200	2 ml tube, white cap, 1.2 ml	4°C	1

*Upon arrival, store kit components as indicated in the chart above.

Introduction

plexWell 384 and 96 Library Preparation Kits come in an assay-ready 96-well configuration to streamline high-throughput multiplexed library preparation. Each kit contains sufficient reagents to prepare dual-indexed Illumina-compatible libraries from 384 and 96 individual DNA samples, respectively. Multiple DNA types are suitable input for the kit, ranging in size and complexity from amplicons to bacterial genomic DNA. plexWell libraries are compatible with the Illumina MiSeq, NextSeq, HiSeq and NovaSeq systems.[1]

This multiplexed library preparation procedure is optimized for inputs of 10 ng of purified dsDNA per sample, and typically generates library fragment lengths ranging from 500 – 1,000 bp. The primary advantages and benefits of using the plexWell 96 and 384 Library Preparation Kits are a streamlined 96 sample multiplexed library preparation workflow that tolerates variation in DNA input concentration and greatly saves on labor and consumable costs. Using a plexWell 384 kit, up to four libraries can easily be prepared in 96-sample batches and loaded on the same sequencing run---all in a single day.

plexWell library preparation kits from seqWell utilize proprietary transposase-based reagents to insert barcoded adapters directly into input DNA in two separate steps. In the first barcoding step, different i7-barcoded adapters are inserted into each of the 96 DNA samples in segregated reactions. Next, the i7-barcoded DNA samples are all pooled into a single tube. In the second barcoding step, a single i5-barcoded adapter is inserted into each pool of 96 i7-barcoded DNA samples. Finally, each 96-plex library is amplified in a single PCR reaction using universal library primers (*i.e.*, P5 and P7 primers), making for a highly efficient multiplexed library prep workflow (see Workflow Diagram).

[1] Refer to Illumina technical documentation for specific instructions on how to set up a sequencing run for a Nextera-style dual-indexed library prior to loading a plexWell library on your model of sequencing system.

User-Supplied Reagents, Equipment, Reagents & Consumables, and Thermal Cycler Programs

Reagents

- 100% Ethanol (molecular biology grade)
- Tris-HCl, pH 8.0
- Ultrapure Water (PCR grade)
- PicoGreen® DNA assay (recommended) or other validated dsDNA quantification assay
- KAPA Biosystems HiFi HotStart ReadyMix (KK2602 or KK2601) for library amplification

Equipment & Consumables

- Single-channel pipettors (1-20 µl, 20-200 µl, 100-1,000 µl)
- Multi-channel pipettors (1-10 µl, 10-200 µl)
- Pipette tips (low-retention barrier tips)
- Eppendorf Tubes® (1.5 ml, DNA LoBind Tubes)
- PCR plate seals (must be evaporation-resistant)
- 96-well thermal cycler (compatible with PCR 8-tube strips)
- Magnetic stand for 1.5 ml and 2 ml tubes
- 0.2 ml PCR 8-tube strips and caps/seals
- Benchtop centrifuge to pulse-spin 1.5/2 mL tubes and PCR 8-tube strips
- Plate centrifuge
- Vortex mixer

Thermal Cycler Programs (all with lid-heating on)

- TAG Program: 55°C for 15 minutes; 25°C hold.
- STOP Program: 68°C for 10 min; 25°C hold.
- FILL_AMP Program:
    - 72°C for 10 minutes (fill-in)
    - 95°C for 3 minutes (initial denaturation)
    - 98°C for 30 seconds
    - 64°C for 15 seconds
    - 72°C for 30 seconds

    (12 cycles)
    - 72°C for 3 minutes (final extension)
    - 4°C hold

Before starting procedure:

Measure and adjust input DNA concentration. Assay the DNA concentration of each 96 well plate of samples to be processed by PicoGreen or other validated dsDNA assay. Adjust the average concentration of input DNA across each plate to 2.5 ng/µl (10 ng input) in 10 mM Tris-HCl, pH 8.0. Do not use EDTA-containing solutions (*e.g.*, TE buffer) to dissolve or dilute input DNA because EDTA can inhibit enzymatic activity. *See the Appendix for more detailed information on adjusting input DNA concentration.*

Program thermal cycler(s). For convenience, set-up the thermal cycler programs listed on the previous page before starting.

Pulse-spin kit components. Liquids can condense or shift locations inside containers during shipment or storage. Before using the **SBP96 Plate**, and before dispensing from reagent tubes, pulse-spin in a suitable centrifuge to gather the reagents at the bottom of the well or tube. If the kit components freeze during shipment or storage, thaw, mix and pulse-spin before use.

Equilibrate MAGwise Paramagnetic Beads to room temperature. MAGwise beads can be stored for up to 2 weeks at room temperature or for longer periods at 2 - 8°C. If stored cold, warm at room temperature for 30 minutes before use. Vortex to thoroughly resuspend beads prior to use. To transfer volumes accurately, pipette slowly and do not pre-wet pipette tips.

Check the X Solution for precipitate before use. If a precipitate is visible, incubate at 37°C for 5 minutes (or longer if necessary). Mix gently by inversion until the precipitate dissolves (do not vortex). Note: **X Solution** contains SDS and will precipitate if stored below room temperature. Overly vigorous mixing will cause foaming.

Please note that Coding Buffer is viscous. Store **Coding Buffer (3X)** at room temperature. Pipette slowly and do not pre-wet pipette tips to transfer volumes accurately. While adding **Coding Buffer** to reactions, mix in completely by pipetting up and down several times with the same pipette tip(s) used for addition. Always change pipette tips before adding **Coding Buffer** to different reactions.

Prepare 80% ethanol fresh daily.

Prepare 10 mM Tris-HCl, pH 8.0. Prepare 10 mM Tris-HCl, pH 8.0 from a concentrated stock solution diluted with ultrapure water (both molecular-biology grade). You will need ~100 µl per 96-well sample plate. Do not use EDTA-containing solutions (e.g., TE).

Safe stopping points are indicated in the protocol. For optimal results, proceed directly to the next step unless a safe stopping point is indicated.

Procedure

1. Sample-Barcoding (SB) Reaction Set-up

SB reactions should be set-up at room temperature. If processing more than one plate, complete the set-up of one **SBP96 Plate** (through starting the thermal cycler) before proceeding to the next one.

a. Pulse-spin the **SBP96 Plate**; then remove the seal carefully.

b. Transfer 4 µl of input DNA (approximately 2.5 ng/µl) to each well (one sample per well) of the **SBP96 Plate**. Mix thoroughly and slowly by pipetting (5 times at 4 µl), being careful not to introduce excessive bubbles. Use clean tips for addition of each sample.

c. Carefully pipette 4 µl of **Coding Buffer (3X)** to each well of the **SBP96 Plate**, using new pipette tips for each transfer. Mix thoroughly and slowly by pipetting up and down ten times at 4 µl, being careful not to introduce excessive bubbles.

Useful Tip: *Aliquot 55 µl of **Coding Buffer (3X)** into each well of an 8-tube strip, and then use a multichannel pipettor to transfer 4 µl (and mix) into each SB reaction.*

d. Seal the **SBP96 Plate**, transfer to a thermal cycler, and run the TAG program, below, with lid-heating on:

55°C for 15 minutes;
25°C hold

2. SB Reaction Stop

a. Confirm there is no precipitate in the **X Solution.** Slowly pipette contents of tube 5 times to ensure everything is mixed.

Note: This solution contains SDS and vigorous mixing will cause it to foam. Pipetting slowly and under the surface of the solution will give the best results.

b. Pulse-spin **SBP96 Plate** and then remove seal.

c. Add 6 µl of **X Solution** to each well of the **SBP96 Plate**. Pipette up and down slowly 5 times to mix. Change pipette tips for each addition.

Useful Tip: Aliquot 85 µl of X Solution *to an 8-strip tube, then use a multichannel pipette to transfer 6 µl from the strip to each column of the* SBP96 Plate.

d. Seal SBP96 Plate, pulse-spin, then transfer to a thermal cycler and run the STOP program, below, with lid-heating on:

68°C for 10 minutes
25°C hold

3. SB Pooling (within plate)

a. Pulse-spin SBP96 Plate and then remove seal. Transfer 9 µl of stopped SB reactions from columns 1-12 into an 8-well strip tube, pipetting twice after each dispense to mix after each addition. *Do NOT pool samples from different SBP96 plates together*!

Optional: If bubbles are present after pooling stopped SB reactions in strip tube, use a tabletop centrifuge to remove bubbles prior to proceeding

b. Transfer entire contents (95-108 µl) from each well of column 1 (or strip tube) to a supplied 2 ml tube, pipette twice after each dispense to mix. You will have a total of ~800-860 µl.

Optional: If bubbles are present after pooling use a tabletop centrifuge to remove bubbles prior to proceeding

4. SB Pool Purification

a. Vortex (or vigorously pipet) *room temperature* MAGwise Paramagnetic Beads to ensure that the beads are fully resuspended.

b. Add 850 µl (approximately 1 volume equivalent) of MAGwise to the pooled SB reactions and mix thoroughly by pipetting. Incubate on bench for 5 minutes to allow DNA to bind.

c. Place tube on magnetic stand and let beads settle, 5 minutes. A pellet should form on one side of the tube and the supernatant should be visibly clear after 5 minutes.

d. Remove and discard supernatant with pipette. Be careful not to disturb the pellet.

Useful tip: Use a large pipette tip to remove most of the supernatant and then if necessary, use a smaller one to remove the remaining supernatant.

e. Wash beads with 80% ethanol.
    i. With tube in the magnetic stand, add 1.5 ml of 80% ethanol without disturbing beads. If this volume is insufficient to cover the bead pellet, add a larger volume.
    ii. After ≥30 seconds, remove and discard supernatant, being careful not to disturb pellet, to complete the wash step.

f. Repeat previous step for a total of 2 washes with 80% ethanol.

g. Air dry beads by leaving the tube uncapped on the magnetic stand for 3 minutes. Check to ensure that there is no visible ethanol in the tubes. If ethanol droplets are still visible, air-dry the beads longer (do not dry the pellet longer than 5 minutes total or the DNA recovery will be compromised).

h. Remove the tube from magnetic stand and pipette 25 µl of 10 mM Tris-HCl, pH 8.0 on top of the bead pellet. Pipette the solution along the inner wall of the tube multiple times to thoroughly resuspend the bead pellet.

i. Incubate the tube on the bench for at least 5 minutes to elute the purified SB reaction pool from the beads.

j. Return tube to magnetic stand and allow a bead pellet to reform on the inner wall of the tube (~2 minutes).

k. When the supernatant has cleared completely, carefully transfer 24 µl of DNA eluate to a PCR tube. The transferred eluate contains the purified SB reaction pool.

    Note: *Transfer of some beads with the purified SB reaction pool will not inhibit the Pool Barcode reaction.*

SAFE STOPPING POINT

Proceed immediately to the next step or store the purified SB reaction pool at -20°C.

5. Pool Barcoding (PB) Reaction Setup

a. Add 5 µl of **Pool Barcode (PB) Reagent** to the PCR tube containing the purified SB reaction pool. Pipette up and down five times after dispensing to ensure entire volume of **PB Reagent** is dispensed.

   Note: **PB Reagent** *contains the i5 index. To enable multiplexing on the same sequencing runs, use a different* ***PB Reagent*** *for each SB reaction pool.*

b. Add 14 µl of **Coding Buffer** to the PCR tube containing the purified SB pool. Mix thoroughly by pipetting.

c. Cap the PCR tube containing the PB reaction, transfer to a thermal cycler, and run the TAG program, below, with lid heating on:

   55°C for 15 minutes
   25°C Hold

6. PB Reaction Stop

a. Pulse-fuge PB reaction, then add 20 µl of **X solution** to each PB reaction. Mix thoroughly by pipetting slowly 10 times at 50 µl.

b. Re-cap PB reactions, transfer to a thermal cycler and run the STOP program, below, with lid heating on:

   68°C for 10 minutes
   25°C Hold

7. PB Reaction Purification

a. Pulse-spin stopped PB reaction, then transfer entire contents (~60 µl) of each stopped PB reaction to its own 1.5 ml LoBind tube.

b. Briefly vortex or pipette MAGwise to ensure beads are suspended. Then add 60 µl (1 volume equivalent) of MAGwise to each stopped PB reaction and mix thoroughly by pipetting.

c. Incubate on bench for at least 5 minutes to allow DNA to bind.

d. Place tube in magnetic stand and let beads settle, 3 minutes. A pellet should form on one side of the tube and the supernatant should be visibly cleared after 3 minutes.

e. Remove and discard supernatant with pipette. Be careful not to disturb the pellet.

Useful tip: Use a large pipette tip to remove most of the supernatant and then use a smaller one to remove the remaining supernatant.

f. Wash beads with 80% ethanol.

   i. With tube in the magnetic stand, add 300 µl of 80% ethanol without disturbing beads.
   ii. After ≥30 seconds, remove and discard supernatant, being careful not to disturb pellet.

g. Repeat previous step for a total of 2 washes with 80% ethanol. Use a large pipettor to remove most of the ethanol waste, and then use a smaller pipettor (e.g., P20) to remove the residual ethanol that collects at the bottom of the tube.

h. Air dry beads by leaving the tube uncapped on the magnetic stand for 3 minutes. Check to ensure that there is no visible ethanol in the tubes. If ethanol droplets are still visible, air-dry the beads longer (do not dry the pellet longer than 5 minutes total or the DNA recovery will be compromised).

i. Remove tubes from magnetic stand and resuspend bead pellet in 24 µl of 10 mM Tris-HCl, pH 8.0. Pipette the liquid along the inside of the tube several times to thoroughly resuspend the bead pellet.

j. Incubate on bench for at least 5 minutes to elute the purified DNA from the beads.

k. Return tubes to magnetic stand and allow bead pellet to form on the inner wall of the tube (~2 minutes).

l. When supernatant has cleared completely, carefully transfer 23 µl of DNA eluate to new labelled PCR tube. The transferred supernatant contains the purified PB product.

Note: *Transfer of some beads with the purified PB product will not inhibit library amplification.*

SAFE STOPPING POINT

Proceed immediately to the next step or store the purified PB reaction at -20°C.

8. Library Amplification

a. Add 4 µl of **Library Primer Mix** to the purified PB product.

b. Add 27 µl of Kapa HiFi Hot Start ReadyMix (2X) and mix well by pipetting.

c. Close the PCR tube, and run the FILL_AMP program, below, with lid heating on:

Fill-in:	72°C for 10 min
Initial denaturation:	95°C for 3 min
12 Cycles of:	98°C for 30 seconds
	64°C for 15 seconds
	72°C for 30 seconds
1 Cycle of:	72°C for 3 minutes
	4°C hold

SAFE STOPPING POINT

Proceed immediately to the next step, or freeze the amplified library at -20°C.

9. Library Purification

Following library amplification, it is necessary to remove residual primers and short library fragments. To adjust the size of the final library, please see Appendix B for suggestions on altering MAGwise ratios.

a. After PCR, pulse-spin and transfer the library amplification reaction to a 1.5 ml LoBind tube. Measure the total volume. Note: volumes normally change due to film-loss and evaporation during thermal cycling so it is important to measure the pooled volume.

b. Dilute the library amplification reaction to a final volume of 205 µl with 10 mM Tris-HCl, pH 8.0 and mix thoroughly. Transfer 100 µl to a new 1.5 mL LoBind tube for purification. Set aside 5 µl of unpurified material for a control. Store the remaining 100 µl at -20 C as unpurified material until library QC is complete.

c. Vortex (or vigorously pipette) room temperature MAGwise to ensure beads are completely resuspended.

d. Add 75 µl (0.75 volume equivalent) of MAGwise to the 1.5 ml LoBind tube containing 100 µl of diluted amplified library. Mix thoroughly by pipetting up and down.

e. Incubate on the bench for 5 minutes to allow the DNA to bind.

f. Transfer the 1.5 ml tube to a magnetic stand and let the beads settle completely, approximately 3 minutes. A bead pellet will form along one side of the tube and the supernatant should appear completely clear after 3 minutes.

g. Slowly remove the supernatant with a pipettor and discard. Be careful not to disturb the bead pellet.

h. Wash with 80% ethanol.

    i. With the tube in the magnetic stand, add 500 µl of 80% ethanol without disturbing the beads.
    ii. After 30 seconds, slowly remove and discard supernatant, being careful not to disturb the pellet.

i. Repeat previous step for a total of 2 washes with 80% ethanol. Use a small pipettor (e.g., P20) to remove the residual ethanol after the second wash.

j. Air-dry beads by leaving the tube uncapped on the magnetic stand for 2 minutes. Check to ensure that there are no visible ethanol droplets in the tubes after 2 minute. If ethanol droplets are still visible, air-dry the beads longer (do not dry the pellet longer than 5 minutes total or the DNA recovery may be compromised).

k. Remove tube from magnetic stand and add 32 µl of 10 mM Tris-HCl, pH 8.0. Pipette the liquid along the inside of the tube several times to thoroughly resuspend the bead pellet.

l. Incubate for 5 minutes on the bench to elute the purified library from the magnetic beads.

m. Return tubes to magnetic stand and allow bead pellet to form on the inner wall of the tube (~ 2 minutes).

n. When the supernatant has cleared completely, carefully transfer 28 µl of DNA eluate to a new 1.5 ml LoBind tube. The transferred eluate contains the purified, multiplexed library. The remaining 4 µl of eluate may be used for electrophoretic analysis.

SAFE STOPPING POINT

Store the purified, multiplexed library at -20°C, or proceed directly to library QC.

Library QC

Electrophoretic analysis: Run 3 µl of purified library on a gel along with 5 µl of unpurified amplified library from step 9b. Alternatively, an Agilent Bioanalyzer (High Sensitivity DNA or DNA7500 kits), Tapestation (High Sensitivity D5000 or D5000 kits), or Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit, DNF-474) can be used following the manufacturer's instructions for these instruments. For optimal sequencing results, use a region analysis for fragments of 300-1300 bp to determine the average cluster-able fragment length for size adjustment with SYBR based qPCR. See Figure 1 (below) for representative traces for purified libraries run on the Fragment analyzer, as well as a representative image of size distribution by gel electrophoresis.

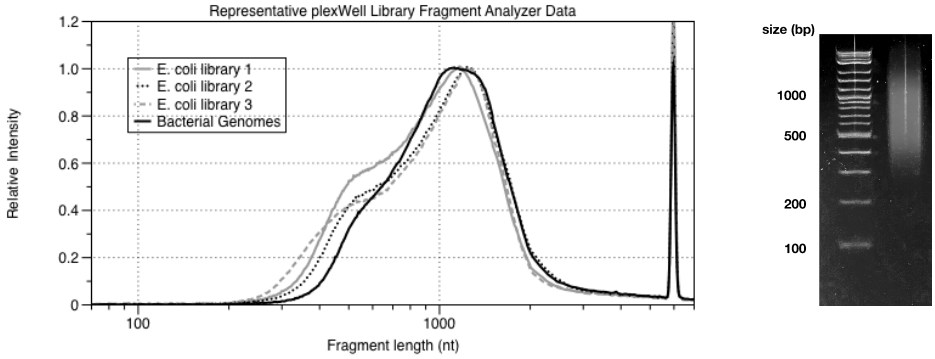

Figure 1. Representative plexWell library traces on the fragment analyzer (left), and gel electrophoresis (2% Invitrogen E-Gel) (right). Libraries on left were generated from samples specified in the legend and purified with 0.75 volume equivalents of MAGwise. Insert size analysis following sequencing (MiSeq v2 chemistry, 2x76) revealed median insert lengths of 350, 340, 275, and 390, respectively.

***Note*:** plexWell library preparation kits sometimes produce fragments around 1500 bp that appear to constitute a large portion of the library on instruments such as the Agilent Bioanalyzer, TapeStation, or Fragment Analyzer. However, these fragments do not appreciably contribute to the library concentration and do not cluster on standard Illumina flow cells. As such it is not necessary to remove them from the library. For sequencing on Illumina patterned flow cells, it may be necessary to remove fragments >1200 bp.

qPCR assay: Use 2 µl of each library for qPCR analysis. Follow kit and instrument documentation for appropriate conditions and dilutions. For KAPA Library Quantification kits, prepare a 1/100,000 dilution of the libraries. Use the average fragment size as determined by electrophoresis to calculate the library concentration.

plexWell libraries are sequenced using the same primers as Nextera® libraries. Refer to Illumina technical documentation for specific instructions on how to set up a sequencing run for a Nextera-style dual-indexed library prior to loading a plexWell library on your model of sequencing system.[2]

[2] The sequencing primers provided in TruSeq v3 Cluster kits are incompatible with Nextera-style libraries, including plexWell libraries. The TruSeq Dual Index Sequencing Primer Box from Illumina is required for sequencing plexWell libraries on older systems, such as the HiSeq 2500, HiSeq 2000, HiSeq 1500, GA*II*x, and HiScanSQ.

Appendix: Adjusting starting sample concentration

plexWell 96 and plexWell 384 kits perform optimally with 10 ng of dsDNA per well, however, individually adjusting each sample to 2.5 ng/µl is not necessary as plexWell library preparation kits are formulated to tolerate up to a 10-fold difference in sample input (3 to 30 ng) within a 96 well plate. To achieve the best library performance, apply a global dilution factor to the input samples in a 96-well plate such that the average DNA concentration across all samples will be 2.5 ng/µl (*i.e.*, average of 10 ng per input sample).

If the method used to produce input DNA for library prep is well-characterized and generates consistent amounts of DNA per sample (*i.e.*, low CV), it may be adequate to assay only several or a few dozen samples from a 96-well plate (*i.e.*, spot-check the DNA concentration using a PicoGreen Assay). However, if the DNA concentration is extremely variable across the samples (*e.g.*, if the concentration difference between any two samples is greater than 10-fold), then outlier samples may need to be individually diluted into the acceptable 10-fold concentration range in order to achieve more uniform read counts across samples.

If the DNA concentration of your input samples cannot easily be confined to a 10-fold range, or, if an average sample concentration of 2.5 ng/µl cannot be easily achieved, consider improving the method used to produce input DNA and assaying samples more rigorously before starting the plexWell library prep procedure.

Important Reminder: Do not use EDTA-containing solutions (*e.g.*, TE buffer) to dissolve or dilute input DNA because EDTA can inhibit enzymatic activity.

Appendix B: *Guidelines for library prep in smaller batches (<96 samples):*

- When making plexWell libraries from fewer than 96 samples, only pool SB reactions from wells that receive input DNA.
- >80 samples; follow the plexWell 96 procedure as written, pooling only SB reactions from wells that receive input DNA
- 64-80 samples; change SB pooling volume to 11 µl per well (instead of 9 µl per well), adjust volume of MAGwise beads for SB pool purification to 1 volume equivalent of the total pool volume.
- 48-60 samples; change SB pooling volume to 14 µl per well (instead of 9 µl per well), adjust volume of MAGwise beads for SB pool purification to 1 volume equivalent of the total pool volume.
- <48 samples; consider running replicates of samples to fill all wells of the **SBP96 Plate**.
- For processing any number of samples <96, also consider using the plexWell Plus 24 Library Preparation Kit from seqWell (P/N: PWP24), featuring a flexible multiplexed workflow for generating libraries in batches of 8 - 24 samples at a time. Enough reagents are provided with the plexWell Plus 24 kit to prepare libraries from 96 individual DNA samples.

Appendix C: Alternate purification conditions

We strongly recommend using the MAGwise purification conditions specified in the user guide for library purification, especially for first-time users. Depending on your application, however, you may wish to bias your library toward larger or smaller insert sizes. This appendix provides some general guidelines for modifying MAGwise purification conditions. **Note:** *In addition to fragment size distribution, other library properties (e.g., library complexity, yield, etc.) are impacted by purification conditions.*

Bead-based size-selection depends on several factors, including the binding conditions and the starting population of fragments. This user guide calls for purifying only half of your total amplified multiplexed library. However, if the fragment size distribution of the library purified under standard conditions (0.75 volume equivalent of MAGwise) does not meet your specific application needs, the remaining 100 µl of unpurified library amplification can be used to optimize size selection. See below for general purification guidelines.

MAGwise for final purification

Adjusting the MAGwise volume equivalent by ±0.04X changes the cut-off by approximately 50-100 basepairs as shown in the table below.*

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Volume Equivalents of MAGwise (added to diluted library amp)	Fragments retained (bp)
0.79	>300
0.75 (recommended)	**>375**
0.71	>475

* **Guidelines only**: Individual results may vary based on the initial size distribution and concentration of the unpurified library.

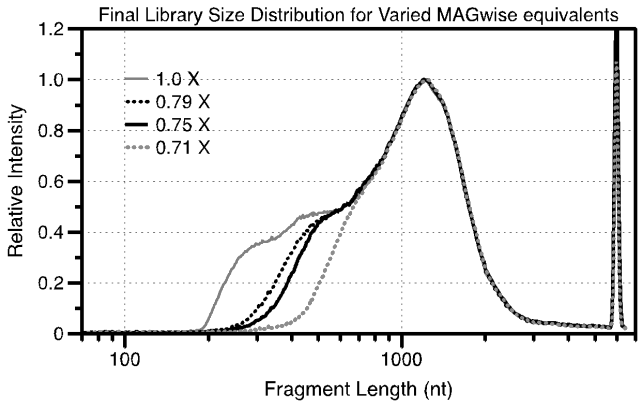

Figure 2 (left). Representative analysis of library sizing (Fragment Analyzer) with varying MAGwise purification conditions.

Experienced User Checklist

- Sample Barcoding
  - Centrifuge SBP96 plate
  - Add 4 µl sample to SBP96 and pipette 5x
  - Add 4 µl coding buffer to SBP96 and pipette 10x
  - Seal plate. Run TAG incubation
  - Add 6 µl X-solution to SB reactions (SBP96 plate) and pipette 10x
  - Seal plate. Run STOP incubation
  - Pool 9 µl from each SB reaction
  - Complete SB purification (1 volume equivalent)
  - Transfer 24 µl of purified SB pool to 0.2 ml PCR tube (PB reaction tube)

- Pool Barcode Reaction
  - Centrifuge Pool Barcode Reagent
  - Add 5 µl Pool Barcode Reagent to 24 µl of purified SB pool and pipette 5x
  - Add 14 µl coding buffer to PB reaction tube and pipette 10x
  - Run TAG incubation
  - Add 20 µl X-solution to PB reaction and pipette 10x
  - STOP incubation
  - Complete PB purification (1 volume equivalent)
  - Transfer 23 µl of purified PB pool to 0.2 ml PCR tube (amplification tube)

- Library Amplification
  - Add 4 µl Library primer mix to amplification tube
  - Add 27 µl of KAPA HiFi HotStart ReadyMix and pipette to mix
  - Run Fill Amp program
  - Complete Library Purification (0.75 volume equivalent)

Technical Assistance

For technical assistance, contact seqWell Technical Support.

Email: support@seqwell.com

Website: https://seqwell.com/products/plexwell-kit/

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