plexWell™ FAQs

Applicable to plexWell 96, plexWell 384, and plexWell LP 384 Kits

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1 All Kits

A. General

What is the best way to reach technical support?

Please email support@seqwell.com or call the main seqWell number, +1-855-737-9355 and follow the prompts for support.

Are all required adapters, indices, and amplification primers included in plexWell 96, and plexWell 384 library preparation kits?

Yes. All plexWell kits include all the indexed adapters and amplification primers necessary to make dual-indexed Illumina®-compatible libraries. The Sample Barcode (i7 index) is added via the Sample Barcode Reagent, and the Pool Barcode (i5) is added via the Pool Barcode Reagent.

Can the plexWell workflow be automated?

Yes. Contact support@seqwell.com for assistance with automation.

Are plexWell libraries compatible with downstream target capture?

plexWell libraries have not been tested extensively in target capture workflows. plexWell libraries have full-length, Illumina Nextera®-style adapters with 8-nt i5 and i7 indices. As such, they should be compatible with downstream target capture when combined with appropriate (Nextera-style) adapter and index blockers during hybridization.

B. Reagents, Consumables and Equipment

Are any additional reagents, consumables, or equipment needed?

User-supplied reagents, consumables and equipment needed to prepare plexWell libraries are listed in all plexWell User Guides (available at: https://seqwell.com/resources/). A brief summary is included below:

User-supplied reagents:

- KAPA HiFi HotStart ReadyMix
- 10 mM Tris-HCl, pH 8.0
- ultra-pure water
- molecular biology-grade ethanol
- reagents for DNA quantification (e.g. PicoGreen™)
- reagents for library quantification (e.g. KAPA Library Quantification Kit) and fragment length determination
- Illumina® sequencing kit

User-supplied consumables:

- 1.5 mL and 2.0 mL DNA LoBind® tubes
- PCR tubes and strip tubes with strip caps
- pipette tips
- plate seals

Equipment:

- Vortex mixer
- plate centrifuge
- mini centrifuge
- Single- and multi-channel pipettes
- magnet for MAGwise™ bead-based purification
- thermocycler compatible with low profile, fully-skirted Bio-Rad hard-shell PCR plates
- Electrophoretic system for assessing library fragment size distribution (e.g. BioAnalyzer, TapeStation, or Fragment Analyzer)
- equipment for assessing DNA and library concentration (fluorometer and/or qPCR instrument)
- Illumina sequencing system

Can I use PB and SB reagents from different kits interchangeably?

No. SB and PB reagents supplied in different kits are formulated differently. A letter designation is used to indicate compatible reagents. For example, SBW and PBW reagents or SBX and PBX reagents work together, whereas SBW and PBX reagents do not.

How long are the plexWell™ adapters?

The combined length of adapters and indices are 135 bp.

What is the sequence of the plexWell™ adapters?

plexWell libraries use Nextera® adapter sequences. Use the following sequence for adapter trimming: CTGTCTCTTATACACATCT.

The transposase adapter sequences are:

- 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG (Read 1)
- 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (Read 2)

Can I use a different polymerase for library amplification?

KAPA HiFi HotStart ReadyMix has been validated with plexWell library preparation kits. Other DNA amplification reagents and reaction conditions might produce adequate library yields, but may introduce bias leading to uneven coverage.

Do I need special sequencing primers or sequencing reagents?

plexWell libraries are sequenced with the same primers as Nextera libraries. plexWell libraries are compatible with iSeq™, MiniSeq™, MiSeq™, NextSeq™, HiSeq® and NovaSeq™ sequencing systems. Note that the sequencing primers provided in TruSeq® v3 Cluster kits are not compatible with Nextera-

style libraries, including plexWell libraries. Consequently, 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, GAIIx, and HiScan® SQ system.

Can I purchase individual reagents without purchasing a kit?

X-Solution, Coding Buffer (3X), and MAGwise™ Paramagnetic Beads are available as standalone products. Contact sales@seqwell.com for volume-based pricing. Other reagents may be purchased through a customized kit option. Inquire at sales@seqwell.com.

Which magnets are recommended for the bead cleanups?

We recommend the DynaMag-2 magnet from ThermoFisher, but other appropriate magnets may be used. Bead settling times will vary depending on the strength of the magnet used. For magnets other than the DynaMag-2, visually confirm that the supernatant has cleared before proceeding.

My thermocycler doesn't hold fully skirted low-profile plates. What do I do?

Set up the SB reaction as described in the user guide. Pipette to mix. Seal the plate and centrifuge. Unseal the plate and carefully transfer the reactions to a PCR plate that fits in your thermocycler.

C. Sample Types

What sample types can I use?

plexWell™ works well with any high-quality, double-stranded DNA with fragment sizes >500 bp; from amplicons to mammalian genomic DNA. Contact sales@seqwell.com to identify the plexWell kit most suitable for your sample type and application.

Is there a plexWell library prep for ultra-low inputs?

Currently, the lowest-input plexWell kit recommends an average input of 10 ng. For specific use cases, contact support@seqwell.com to see if a protocol modification may meet your needs.

Do plexWell kits work with degraded DNA samples?

plexWell works well with high-quality, double-stranded DNA with fragment sizes >500 bp. Kits have not been validated for FFPE or other highly degraded DNA samples.

Are plexWell kits compatible with crude extractions?

plexWell Library Preparation Kits have only been validated for purified DNA inputs.

Does the plexWell technology support sequencing of human DNA?

Yes. All plexWell Library Preparation Kits will produce high-quality libraries from human genomic DNA.

I have samples with different genome sizes that need different amounts of coverage. Can I still use plexWell kits?

plexWell kits are specifically designed to produce an even read distribution for each sample within a pool. For samples needing different amounts of sequencing data (e.g. same genome size but different coverage, or different genome size and same average coverage), pool samples needing similar numbers of reads together in the same pool.

My samples are amplicons. Will the entire amplicon be covered evenly?

The depth of coverage near the termini of PCR products will be lower than in other regions. PCR primers should be designed to hybridize 75 – 100 bases upstream and downstream of the region of interest to ensure adequate depth of coverage across the entire amplicon. For long amplicons (>2 kb), best results will be achieved when PCR primers are 250 – 500 bases upstream and downstream of the region of interest.

My amplicons are shorter than 1 kb. Can I still use plexWell library preparation?

Yes. For amplicons ≥1 kb, follow the standard protocol in the User Guide. For amplicons ≥400 but less than 1 kb, alter the final library purification conditions to retain smaller fragments. Typically, a volume equivalent of MAGwise™ Paramagnetic Beads in the range of 0.85 to 1 will be appropriate. For amplicons <400 bp, several factors should be considered when using plexWell library preparation. Please contact support@seqwell.com for tailored recommendations for your project.

Can I use plexWell kits for mRNA-Seq RNA-Seq of ribodepleted RNA?

Yes, but you will first have to convert your RNA to cDNA. Assuming an average cDNA input of 10 ng (and all inputs in the range of 3 – 30 ng), cDNA samples can be processed using the regular plexWell protocol.

D. Sample and library QC

What method(s) are recommended for determining the concentration of input DNA?

Methods specifically designed for the quantification of dsDNA (i.e. those based on the use of a rib depleted fluorescent intercalating dye such as PicoGreen™), are highly recommended. UV or visible spectrophotometric methods, especially those using a NanoDrop® instrument, have not been validated and are frequently inaccurate. A fluorescent, dsDNA-specific method is also recommended for in-process optional QC steps within the plexWell workflow.

My samples are all more concentrated than the average specified for the plexWell kit I want to use. Can I use them as is?

No. First apply a single global dilution factor (GDF) to all samples to bring all samples within the average concentration range for the kit. SB Reagents and PB Reagents supplied in different plexWell kits are optimized for the specified average input. Significant deviation from the average input will result in library fragment distributions that are too short (too little DNA) or too long (too much DNA).

Does the plexWell protocol have in-process QC steps?

plexWell User Guides contain recommendations for optional in-process QC steps. All kits include an optional in-process QC after SB purification (and expected DNA concentrations at this stage in the workflow). If you obtain results that fall outside the expected range, please contact support@seqwell.com for recommendations on how to proceed.

Which method(s) are recommended for library QC?

Library QC entails the determination of (i) library concentration (in a molar, rather than mass-based unit), and (ii) the average distribution of library fragments.

A qPCR-based method is recommended for library quantification. Apply the size adjustment as described in the qPCR protocol.

An electrophoretic system such as the Agilent TapeStation, Bioanalyzer, or Fragment Analyzer is recommended library fragment size determination. Perform a smear analysis of fragments in the range of 250 –1500 bp (or the range specified in your plexWell User Guide) to calculate the average fragment length. An agarose gel with appropriate DNA ladder may also be used to determine the average library fragment length.

If I am only planning to sequence only one plexWell pool, can I skip the PB step?

No. In addition to adding the i5 index, the PB step adds the P5 adapter sequence that is required for clustering on Illumina® flow cells.

Can I adjust the final purification step to retain or remove a larger proportion of small fragments?

Yes. MAGwise™ Paramagnetic Beads works in the same way as other SPRI bead reagents methods such as the AMPure XP reagent. Using a higher volume equivalent of beads will result in the retention of more of the small fragments. Using a lower volume equivalent of beads will remove more small fragments. An increase of 0.05 volume equivalents will decrease the length of retained fragments by approximately 75 – 100 bp.

I want to remove the large fragments from my library. How can I accomplish this?

Any validated SPRI bead or gel-based method for size selection may be used. We have not performed a thorough assessment of sequencing performance with size-selected libraries and are unable to recommend specific bead ratios for size selection. Incorporation of a double-sided size selection typically reduces library yield and complexity, and should only be considered if the potential benefits outweigh the potential reduction in sequencing data quality.

E. Library quality

My library fragments are long. Can I still sequence them?

plexWell™ libraries sometimes produce longer fragments, especially as compared to library prep methods that employ mechanical shearing. Generally speaking, longer fragments (>1000 bp) do not impact library quantification results, and do not cluster efficiently on sequencers. As such, they are not well represented in sequencing data. Provided that the majority of library fragments are shorter than 1000 bp, the presence of longer library fragments is generally not a concern.

During electrophoretic analysis, my libraries displayed a broad smear with a high molecular weight tail. Is this a problem? How do I determine the average fragment size?

This is typical for plexWell libraries. High molecular weight material generally contributes little to the actual library concentration. Perform a smear analysis in the range of 25 – 1500 bp to determine the average fragment size of your library. This number should be used for any size adjustments during library quantitation.

I am concerned about a highly multiplexed PCR. Will this lead to chimera formation?

Multiplex PCR can and does lead to chimera formation. The amplification procedures in plexWell kits have been optimized to minimize chimera formation during the later stages of PCR as primers are depleted. The rate of chimera formation between any two samples is typically <0.02% in pools of 96 samples. For specific concerns regarding your application, contact support@seqwell.com for suggestions on how to further reduce chimera formation.

F. Sequencing and Data Analysis

Do you provide Illumina® Sample Sheet templates?

A complete list of barcodes (indices) for all plexWell kits may be found in the plexWell Master Index List at: https://seqwell.com/resources/.

Contact Illumina technical support for guidance on how to create a Sample Sheet for your sequencer, and/or for assistance with loading custom kit information in BaseSpace.

How do I add plexWell Library Preparation Kits to BaseSpace?

Please contact Illumina technical support for assistance with BaseSpace.

Can multiple plexWell™ libraries (made using different plexWell kits) be sequenced in the same sequencing lane?

plexWell libraries derived from different seqWell library kits can be sequenced together as long as a different i5 index has been used. For convenience, the PB reagents are named with a letter and 3-digit number. If the number is the same, the i5 index is identical. For example, libraries made with PBW014 and PBZ014 cannot be sequenced together, but libraries made with PBW014 and PBZ012 can be sequenced together.

Can plexWell libraries be multiplexed with other library types in the same sequencing lane?

plexWell libraries should be sequenced using paired-end sequencing (dual indexing), with 8-nt index reads. plexWell libraries can be sequenced with other library types in a single lane, as long as none of those libraries contain the same index combinations.

How many samples can I sequence in a single sequencing run?

Commercially available PB allow for sequencing from 96 to 1,920 samples at a time. We continue to expand our PB sets, with a view toward enabling thousands of samples to be sequenced simultaneously. Please inquire if you have a high-volume project that requires a very high degree of multiplexing.

Do I have to perform paired-end (dual-indexed) sequencing if I only used one PB reagent (i5 index) on the run?

No. If a plexWell library with only one i5 index is included in a sequencing run, best practice is to sequence it as though it were a single (i7) index library.

Sequencing in dual-index mode for a single i5 index results in a low-diversity read 3 (i5 index) which can lead to poor read quality and demultiplexing issues. If a single i5 index has been used and the sequencing run was done in dual-index mode, consider demultiplexing on the basis of the i7 index only

Does the plexWell technology support UDIs?

Unique Dual Indexing is currently not supported.

Are plexWell libraries prone to index hopping?

plexWell libraries are not prone to index hopping. Index hopping typically occurs when there is free indexed adapter present on the flow cell. The sequential transposition used in the plexWell workflow limits the carryover of any free adapter. In an experiment designed to specifically assess the degree of index hopping, plexWell libraries showed an index hopping rate of <0.01%.

2 plexWell™ 96 and 384 Kits

plexWell 384 and plexWell LP384 have identical components. Can these reagents be used interchangeably? How do I know which kit to order?

The plexWell LP 384 kit contains more PB reagent, Library Primer Mix, and MAGwise™ Paramagnetic Beads than the plexWell 384 kit, but the reagent compositions are the same. plexWell LP 384 reagents can be used in conjunction with either the plexWell LP 384 or plexWell 96 or 384 protocols. Key differences between the protocols are the starting sample concentration and volume, pooling volume, and default level of plexing per pool. If both the plexWell 384 and plexWell LP 384 protocols are used routinely, purchase the plexWell LP 384 kit.

Can I process <96 samples at a time with a plexWell 96 or 384 kit?

All 96-well plexWell assay-ready kits are validated using all 96 SB reagents simultaneously. For processing less than 96 samples, refer to the Appendix of the User Guide. For additional guidance contact support@seqwell.com.

If I processed <96 samples, can I reuse the remaining SB reagents?

The SB reagents can be saved if they are as-shipped (i.e. if no DNA or buffers have been added, and no incubations have been done). If you are processing <96 samples and would like to preserve the SB reagents, set the SB reaction up as described in the User Guide, but add sample and Coding Buffer only to the appropriate wells of the SB plate. Pipette to mix. Seal the SBX plate, then centrifuge. Unseal the plate and transfer the reactions to a new PCR plate. Reseal the SBX plate and store remaining SB reagents at -20°C for subsequent use.

I ordered multiple plexWell 384 index sets. Do I need to match the SBX and PBX lot numbers?

No. All PBX reagents are compatible with all SBX reagents. It is not necessary to segregate Box 1 and Box 2 components for specific Box 3 reagents.

What is the volume of SB reagent in the SBX96 plate?

Each well of the SBX96 plate contains 4 µl of reagent.

Is more than one PB reagent (i5 index) available for plexWell 96 kits? Can I order two plexWell 96 kits and sequence the libraries in the same lane?

No. All plexWell 96 kits contain the same PB reagent (PBX007). If you need to sequence more than 96 samples in a single lane, purchase a plexWell 384 or LP 384 kit. In cases where <384 samples have to be processed, contact sales@seqwell.com to explore options for a customized kit.

The concentration of my input DNA samples is variable. Can I still use them?

Yes. For plexWell™ 96 and 384 kits, the average DNA concentration across all samples should be adjusted to 2.5 ng/µl by applying a global dilution factor (GDF). However, individual samples can have a higher or lower concentration. For example, if the average concentration across all your samples is 25 ng/µl before starting library prep, you would apply a 10-fold dilution factor to all your samples, to obtain a final average sample input concentration of 2.5 ng/µl. For optimal results, keep the concentration of all samples within a 10-fold range, such that the input falls in the range of 3 – 30 ng (0.75 – 7.5 ng/µl), with an average concentration of 2.5 ng/µl. Nevertheless, it is not necessary, nor recommended, to dilute every sample to exactly 2.5 ng/µl. To understand how to calculate and apply a global dilution factor, contact support@seqwell.com.

What are the expected library QC metrics?

Libraries generated with a plexWell 96 or 384 kit should have an average fragment length in the range of 500 – 850 bp, depending on sample type and average input, and a final concentration >25 nM.

What is the expected duplication rate for libraries generated with a plexWell 96 or 384 kit?

Duplication rate is impacted by sample complexity, library complexity, sequencing depth and sequencing length. plexWell 384 specifications were derived from paired-end (2 x 150 bp) sequencing of libraries made from 10 ng of *E. coli* genomic DNA. For an analysis based on 1 million paired reads (clusters), the duplication rate should be ≤10%.

3 plexWell™ LP 384 kit

plexWell 384 and plexWell LP384 have identical components. Can these reagents be used interchangeably? How do I know which kit to order?

The plexWell LP 384 kit contains more PB reagent, Library Primer Mix, and MAGwise™ Paramagnetic Beads than the plexWell 384 kit, but the reagent compositions are the same. plexWell LP 384 reagents can be used in conjunction with either the plexWell LP 384 or plexWell 96 or 384 protocols. Key differences between the protocols are the starting sample concentration and volume, pooling volume, and default level of plexing per pool. If both the plexWell 384 and plexWell LP 384 protocols are used routinely, purchase the plexWell LP 384 kit.

Is there a 96-reaction version of the plexWell LP 384 kit?

A plexWell LP 96 kit can be provided for pilot projects, demonstrations, or through a custom kit order. Contact sales@seqwell.com to find out how to order a plexWell Low Pass 96 kit.

Can I process <96 samples at a time with the LP 384 kit?

The standard plexWell LP384 protocol is designed for pools of 48 samples, but assumes that 96 samples will be processed at once. For pools of 48 samples, set the SB reaction up as described in the User Guide, but add sample and Coding Buffer only to the appropriate wells of the SB plate. Prior to thermocycling, pulse-fuge the SBX plate. Unseal, then transfer the contents of the DNA-containing wells to a new PCR plate. Reseal and store the original SB plate at -20°C. Place the new PCR plate in the thermocycler for the TAG reaction. For processing of pools <48, refer to the appendix in the User Guide appendix or contact support@seqwell.com.

If I processed <96 samples, can I reuse the remaining SB reagents?

The SB reagents can be saved if they are as-shipped (i.e. if no DNA or buffers have been added, and no incubations have been done). If you are processing <96 samples and would like to preserve the SB reagents, set the SB reaction up as described in the User Guide, but add sample and Coding Buffer only to the appropriate wells of the SB plate. Pipette to mix. Seal the SBX plate, then centrifuge. Unseal the plate and transfer the reactions to a new PCR plate. Reseal the SBX plate and store remaining SB reagents at -20°C for subsequent use.

I ordered multiple plexWell LP 384 index sets. Do I need to match the SBX and PBX lot numbers?

No. All PBX reagents are compatible with all SBX reagents. It is not necessary to segregate Box 1 and Box 2 components for specific Box 3 reagents.

What is the volume of SB reagent in the SBX96 plate?

Each well of the SBX96 plate contains 4 µl of reagent.

The concentration of my input DNA samples is variable. Can I still use them?

Yes. For plexWell™ 96 and 384 kits, the average DNA concentration across all samples should be adjusted to 1.67 ng/µl by applying a global dilution factor (GDF). However, individual samples can have a higher or lower concentration. For example, if the average concentration across all your samples is 17 ng/µl before starting library prep, you would apply a 10-fold dilution factor to all your samples, to

obtain a final average sample input concentration of 1.7 ng/µl. For optimal results, keep the concentration of all samples within a 5-fold range, such that the input falls in the range of 5 – 25 ng (0.83 – 4.17 ng/µl), with an average concentration of 1.67 ng/µl. Nevertheless, it is not necessary, nor recommended, to dilute every sample to exactly 1.67 ng/µl. To understand how to calculate and apply a global dilution factor, contact support@seqwell.com.

What are the expected library QC metrics?

Libraries generated with a plexWell LP 384 kit should have an average fragment length in the range of 500 – 850 bp, depending on sample type and average input, and a final concentration >20 nM.

What is the expected duplication rate for libraries prepared with a plexWell LP 384 kit?

Duplication rate is impacted by sample complexity, library complexity, sequencing depth and sequencing length. plexWell LP384 kit specifications were derived from paired-end (2 x 150 bp) sequencing (on a NovaSeq system) of libraries made from 10 ng of human genomic DNA. For an analysis based on 10 million paired reads (clusters), the duplication rate should be ≤10%. Due to the nature of patterned flow cells, the duplication rate may vary depending on loading efficiency. Customers have sequenced human samples to an average depth of 4X, with resulting duplication rates <20%.

Revision History

Revision. no.	Revision date	Revision details
v20210106	6-Jan-2021	First version