ExpressPlex™ Library Prep Kit FAQs

What applications are recommended for the ExpressPlex library preparation kit?

The ExpressPlex library preparation kit is recommended for synthetic construct sequencing (amplicons, plasmids, etc.).

Are any additional reagents, consumables, or equipment needed?

Reagents: 10 mM Tris-HCl, pH 8.0, ultra-pure water, ethanol, reagents for DNA quantification (PicoGreen), and Illumina sequencing kits.

Consumables: 2 mL LoBind tubes; PCR plate, PCR strip tubes or individual tubes; pipette tips; plate seals or strip caps.

Equipment: Table-top vortex; plate centrifuge; minifuge; appropriate pipettors, magnet (suitable for 2 mL LoBind tube) for MAGwise bead-based purification steps; a thermal cycler, equipment for assessing library size by gel electrophoresis (BioAnalyzer, TapeStation, or Fragment Analyzer, etc.) and library concentration (fluorometer or qPCR instrument), and an Illumina sequencing system.

Are all required adapters, indices, amplification master mix and amplification primers included in ExpressPlex library preparation kit?

Yes. The ExpressPlex kit includes all the indexed adapters, amplification master mix, and amplification primers necessary to make dual-indexed Illumina-compatible libraries.

How many samples can I batch together?

The ExpressPlex kit allows flexible batching. Each assay-ready plate can process up to 96 samples. Up to 384 samples can be processed per kit.

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

The Ready Reaction reagents can be saved if they are as-shipped (i.e. if no DNA or Indexing reagent have been added, and no incubations have been done). If

processing <96 samples and would like to preserve the Ready Reaction reagents, set the reaction up as described in the User Guide, but add sample and Indexing reagent only to the appropriate wells of the Ready Reaction plate. Pipette to mix. Seal the Ready Reaction plate, then centrifuge. Unseal the plate and transfer the reactions to a new PCR plate. Reseal the Ready Reaction plate and store remaining SB reagents at -20°C for subsequent use.

How many total combinations are commercially available?

seqWell offers a total of 1536 index combinations in the ExpressPlex Library Prep Kit. If more than 1536 index combinations are required, please contact sales@seqwell.com.

Does the ExpressPlex Library Prep Kit, 384 Reactions come in a 384-well PCR plate format?

Currently, the ExpressPlex Library Prep Kit, 384 Reactions includes four assay-ready plates in 96-well plate format, four different indexing plates, and a bottle of MAGwise purification beads. If a 384-well plate format is desirable, please contact sales@seqwell.com for better guidance.

Can I automate the ExpressPlex Library Prep Kit?

The ExpressPlex Library Prep Kit is highly amenable to automation platforms. Please contact sales@seqwell.com for better guidance.

DNA Input:

What is the recommended DNA input range for the ExpressPlex Kit?

The recommended input range for the ExpressPlex Kit is 8 - 40 ng (2 - 10 ng/µl). The kit uses 4 µl of purified DNA sample. Use of less than 8 ng of samples is not recommended due to increased risk of failure.

My samples are all more concentrated than 10 ng/µl. Can I use them as is?

seqWell recommends globally diluting samples to bring the average DNA concentration of the samples within our concentration range (2 - 10 ng/µl). Use of lower or higher DNA concentration may adversely affect sequencing performance.

The concentration of DNA sample input is variable. Can the samples still be prepped together?

The ExpressPlex library preparation kit performs optimally with 16 ng of dsDNA per reaction, however, individually normalizing each sample to 4 ng/µl is not necessary as ExpressPlex library preparation kits are formulated to tolerate up to a 5-fold difference in sample input (8 to 40 ng).

What size range of amplicons is suitable for making ExpressPlex libraries?

ExpressPlex library prep is recommended for PCR products >350 bp in length. The efficiency of amplicon tagging is lower for shorter amplicons and within 50 bp of the amplicon termini, so PCR primers should be designed to prime at least 50 bases upstream from the region of interest.

What quantification methods are recommended for plasmids and PCR products?

The ExpressPlex Kit is sensitive to dsDNA concentration outside the recommended range. Fluorometric methods for dsDNA (e.g., PicoGreen, Qubit) are generally more reliable for assaying miniprepped plasmids than spectrophotometric methods. Regardless of the quantification methods employed, the purity of the DNA should be considered. There are several contaminants of plasmids that interfere with quantification including protein, genomic DNA, ssDNA and RNA. The presence of these contaminants inflates the apparent DNA concentration.

I see untagged amplicons or plasmids in my ExpressPlex purified library. Does this affect my library quantification?

qPCR based library quantification will be unaffected. Methods that quantify total DNA content (e.g., PicoGreen, Qubit) will require an adjustment. Use the TapeStation, Fragment Analyzer, or similar equipment to conduct a region analysis in determine the percentage of the DNA mass that is library (see User Guide for more details), then multiply this percentage of your DNA in the clusterable range to determine the library content.

I see untagged amplicons or plasmids in my ExpressPlex purified library. Will unfragmented amplicons or plasmids in purified libraries interfere with sequencing?

Untagged amplicons or plasmids do not interfere with clustering on the flowcell or the data sequencing quality. However, it may affect library quantification and thus

the optimal loading density (see User Guide to properly adjust library quantification).

QC:

What is the expected fragment size of the ExpressPlex library? Can fragments shorter / longer than this size be sequenced?

The expected fragment size of the ExpressPlex library is 400 – 1,000 bp. If amplicons shorter than 1000 bp are used as input into ExpressPlex reactions, the resulting library fragment size distribution will be shorter than the input amplicons.

What if I made a mistake in the protocol?

To better understand the potential impact and for guidance, please contact support@seqwell.com.

Sequencing:

What is the recommended loading concentration?

Please refer to Illumina's instructions for loading concentration. Higher loading concentrations are generally recommended when sequencing on the MiSeq v2/v3 platform if the molarity value of the library is calculated using the PicoGreen assay in conjunction with your average library size (see User Guide for more details).

Are special sequencing primers or sequencing reagents needed?

ExpressPlex libraries are sequenced using the same primers as Nextera® libraries. ExpressPlex libraries are compatible with the iSeq, MiSeq, MiniSeq, NextSeq, HiSeq and NovaSeq sequencing systems. However, the sequencing primers provided in TruSeq v3 Cluster kits are incompatible with Nextera-style libraries, including ExpressPlex libraries. Consequently, the TruSeq Dual Index Sequencing Primer Box from Illumina is required for sequencing ExpressPlex libraries on older systems, such as the HiSeq 2500, HiSeq 2000, HiSeq 1500, GAIIx, and HiScanSQ.

Which Illumina platforms can I use to sequence the ExpressPlex libraries?

ExpressPlex libraries are compatible with the iSeq, MiSeq, MiniSeq, NextSeq, HiSeq and NovaSeq sequencing systems.

Do I need to spike PhiX into the final library prior to sequencing?

Please refer to Illumina's instructions for using PhiX. The base composition of ExpressPlex libraries is diverse, and PhiX is not required for sequencing ExpressPlex libraries. However, it is recommended to use a 1 - 2% of PhiX spike-in to serve as an internal sequencing quality control.

Do I need to sequence with dual-indexing if only one i5 index was used for library preparation?

No. It is only necessary to run in dual-indexing mode if ExpressPlex libraries carry more than one i5 index (e.g., greater than 96 samples). If an ExpressPlex library carrying only one i5 index is loaded on a sequencing run, it is recommended to sequence in the single-index configuration and demultiplex the run with the i7 index.

Compatibility:

What is the compatibility of ExpressPlex 384-plex / 1536-plex libraries?

There are 4 different i5 index sets per ExpressPlex library prep kit (384 Reactions). Sets 1000 – 4000 are designed so their i5 base composition is color-matched and compatible within each set. Sets 1000 – 4000 can be sequenced together for multiplexing up to 1,536 in a single sequencing run.

Are the ExpressPlex indices mutually exclusive of other library preparation kit indices?

ExpressPlex indices have not been compared to all commercially available indices sets. However, within the seqWell's product line, ExpressPlex indices overlap with purePlex Set 1 indices. purePlex Set 2, Set 3 and Set 4 libraries are all compatible with ExpressPlex libraries.

What is the compatibility of the ExpressPlex libraries with other manufacturer's library prep kits?

Given the large number of library prep kits on the market, there is always a risk of barcode collisions between libraries from different manufacturers. Consequently,

seqWell cannot guarantee the compatibility of other manufacturer's libraries with ExpressPlex libraries.

Where can indices for making a sample sheet for the MiSeq, and other systems be found?

Please see the links under resources to find them in the user guide and ExpressPlex master index list on the seqWell website. The indices can be copied directly from the master index list.

What adapter sequence should be used for adapter trimming?

seqWell kits use the same sequence as Nextera for adapter trimming, which is CTGTCTCTTATACACATCT. Additionally, the sequences for the adapter tagmentation are:
Read 1
5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
Read 2
5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG