ExpressPlex™ HT Library Prep Kit FAQs

What applications are recommended for the ExpressPlex HT library preparation kit?

The ExpressPlex HT library preparation kit is recommended for high throughput 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.

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.

An automated, liquid-handling platform is required for accurate library preparation using the ExpressPlex HT kit. Manual processing of the plates is not recommended. Liquid handlers with 96- or 384-tip heads are recommended for fastest processing. seqWell has successfully processed ExpressPlex HT plates on the Tecan Fluent and SPT Apricot platforms. For more information, please contact support@seqwell.com.

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

Yes. The ExpressPlex HT 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 HT kit is for high throughput applications. Each assay-ready plate can process 384 samples. Up to 1536 samples can be processed per kit. Four separate kits are available and can be multiplexed together, enabling up to 6,144 samples in a single sequencing run.

If I processed <384 samples, can I reuse the remaining reagents?

The kit is designed as a single-use, assay ready high throughput solution. If fewer than 384 samples are to be prepared at one time, seqWell recommends use of our 96-well ExpressPlex Library prep kit that allows for flexible batch sizes.

How many total combinations are commercially available?

seqWell offers a total of 6144 index combinations in the ExpressPlex HT Library Prep Kit.

Can I automate the ExpressPlex HT Library Prep Kit?

The ExpressPlex HT Library Prep Kit is meant to be automated and is highly amenable to most automation platforms. We do not recommend processing samples with this kit manually. Please contact support@seqwell.com for more guidance.

What type of plates come in the kit?

The reagents are plated in skirted Bio-Rad Hard-shell 384-well plates.

DNA Input:

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

The recommended input range for the ExpressPlex HT Kit is 8 - 40 ng (2 - 10 ng/µl). The kit uses 4 µl of purified DNA sample that is diluted 1:1 during the reaction set up. Use of less than 8 ng of samples is not recommended due to increased risk of failure.

Why is the input DNA diluted during the reaction set up?

To save on tips and to have a more streamlined automated workflow, the input DNA is first mixed into the Indexing Reagent before both are transferred into the Ready Reaction plate, leaving some DNA behind in the Indexing Plate.

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 HT library preparation kit performs optimally with 8 ng of dsDNA per reaction (after factoring in the 1:1 dilution of 16ng input that occurs during the reaction setup), 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 HT libraries?

ExpressPlex HT 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 from the region of interest.

What quantification methods are recommended for plasmids and PCR products?

The ExpressPlex HT 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 HT 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 to 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 flow cell 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 HT libraries are sequenced using the same primers as Nextera® libraries. ExpressPlex HT 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 HT libraries?

ExpressPlex HT 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 HT libraries is diverse, and PhiX is not required for sequencing ExpressPlex HT libraries. However, it is recommended to use a 1 - 2% of PhiX spike-in to serve as an internal sequencing quality control.

Compatibility:

What is the compatibility of ExpressPlex HT libraries?

There is 1 i7 index set per ExpressPlex HT library prep kit with 16 different i5's arranged over 4 384-well plates (1536 Reactions). The sets are designed so their i5 base composition is color-matched and compatible within each set. Sets A-D can be sequenced together for multiplexing up to 6,144 in a single sequencing run.

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

ExpressPlex HT indices have not been compared to all commercially available indices sets. However, within the seqWell's product line, ExpressPlex HT indices overlap with purePlex indices. Some purePlex libraries will not be compatible with ExpressPlex HT libraries. Reach out to support@seqwell.com for more assistance.

What is the compatibility of the ExpressPlex HT 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 HT libraries.

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

Please see the links in the product web page resources section to the ExpressPlex HT 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

How do I demultiplex my samples?

While up to 6,144 available barcodes can be multiplexed on one run, there may be limitations to demultiplexing on an Illumina instrument. If using a sample sheet to demultiplex on an Illumina instrument, attempting to do this for more than 1,536 samples may cause errors. We recommend demultiplexing large numbers of samples off-instrument. If you need assistance on how to demultiplex more than 1,536 samples at a time, please contact us at support@seqwell.com for guidance.

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