FAQs - purePlex™ Library Prep Kit

1. What applications are recommended for the purePlex library preparation kit?
    - The purePlex library preparation kit is recommended for synthetic construct sequencing (amplicons, plasmids, etc.), low-pass whole genome sequencing, whole small genome sequencing (<50 Mb), single cell RNA-seq (scRNA-seq), and metagenomics/microbiome sequencing.

2. How many samples can be processed with a single kit?
    - A single purePlex kit provides enough reagents to process up to 96 samples.

3. Are all required adapters, indices, and amplification primers included in purePlex library preparation kit?
    - Yes. The purePlex kit includes all the indexed adapters and amplification primers necessary to make dual-indexed Illumina-compatible libraries. It is worth noting that the i7 index is added by the i7-Tagging Reagent (i7-TR) and the i5 index is added by the i5-Tagging Reagent (i5-TR).

4. Are any additional reagents, consumables, or equipment needed?
    - Reagents: KAPA HiFi HotStart ReadyMix; 10 mM Tris-HCl, pH 8.0, ultra-pure water, ethanol, reagents for DNA and library QC (PicoGreen), and Illumina sequencing kits.

    - Consumables: 1.5 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 1.5 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.

5. Are MAGwise purification beads the same as other purification beads?
    - MAGwise purification beads are similar to many other commercially available purification beads. The purePlex kit has been validated using MAGwise purification beads, thus it is recommended to use the included MAGwise purification beads for optimal performance. However, other SPRI

purification beads should perform similarly and can be utilized/implemented with optimization.

6. The MAGwise purification beads were accidentally stored at the wrong temperature. Can it still be used?
    - To better understand the potential impact and for guidance, contact support@seqwell.com. Short term storage of MAGwise at room temperature (<2 weeks) or -20°C (<2 days) does not appreciably alter performance for most applications.

7. Coding buffer and X-solution were accidentally stored at the wrong temperature. Can they still be used?
    - Yes. Equilibrate the coding buffer and X-solution to room temperature by removing them from the refrigerator and/or defrosting them. Before use, ensure they are homogenous. Coding Buffer should be vortexed then pulse-fuged. X-solution should be checked for precipitate. If precipitate is present in the X-solution, warm to 37°C, then invert or pipet to mix. Do NOT vortex X-Solution. Store remaining Coding Buffer and X-Solution at room temperature.

8. Is there flexibility in pooling for purePlex library preparation kit?
    - Yes. The purePlex library preparation kit workflow has been optimized and validated for 24 samples per pool, with columns 1-3 in pool A, columns 4-6 in pool B, columns 7-9 in pool C and columns 10-12 in pool D. However, because both the i7 and i5 tagging steps are performed on individual samples, the purePlex kit allows flexibility in pooling and batch sizes. Please see the user guide for alternate pooling strategies and recommendations for best practices.

9. The concentration of DNA sample input is variable. Can the samples still be prepped together?
    - The purePlex library preparation kit performs optimally with 25 ng of dsDNA per well, however, individually adjusting each sample to 5 ng/µl is not necessary as purePlex library preparation kits are formulated to tolerate up to a 10-fold difference in sample input (5 to 50 ng).

10. Can a different index combination of i7-Tagging Reagent and i5-Tagging Reagent for the purePlex library preparation kit be used?
    - No. The purePlex library preparation kit has been optimized for the specific combination of i7-TR and i5-TR. Alternative combinations may affect auto-

normalization, fragment length, library concentration and complexity. However, incorrect combinations of i7-i5 tagging reagent are likely to generate sequence-able library and depending on the sequencing application, it is possible to move forward with the library preparation and sequencing without impacting the sequencing results.

11. Can a different polymerase be used for library amplification?
    - KAPA HiFi HotStart ReadyMix is the only DNA polymerase that has been validated with the purePlex library preparation kit. Alternative polymerases and amplification conditions may produce adequate library yield; however, this could lead to uneven coverage.

12. Are special sequencing primers or sequencing reagents needed?
    - purePlex libraries are sequenced using the same primers as Nextera® libraries. purePlex 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 purePlex libraries. Consequently, the TruSeq Dual Index Sequencing Primer Box from Illumina is required for sequencing purePlex libraries on older systems, such as the HiSeq 2500, HiSeq 2000, HiSeq 1500, GAIIx, and HiScanSQ.

13. How many samples can be multiplexed in a single lane?
    - The kit has 384 UDIs available, so up to 384 samples can be multiplexed in a single lane.

14. Where can indices for making a sample sheet for the MiSeq, and other systems be found?
    - Please see the links on the seqWell website under resources to find them in the user guide and master index list. The indices can be copied directly from the master index list.

15. What adapter sequence should be used for adapter trimming?
    - The purePlex kit uses the same sequence as Nextera for adapter trimming, which is CTGTCTCTTATACACATCT. Additionally, the sequences for the adapter tagging are:
        - Read 1: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
        - Read 2: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG