FAQs - AgriPrep Library Prep Kit

1. What applications are recommended for the AgriPrep Library Prep Kit?
    - The AgriPrep Library Prep Kit has been optimized for low-coverage whole genome sequencing (lcWGS) of plant and animal genomes for agrigenomics applications such as genotyping-by-sequencing (GBS). Please contact sales@seqwell.com if you are interested in using a similar workflow for other applications that required <=50M clusters (eg. microbial WGS).

2. Is AgriPrep compatible with RNA-seq?
    - AgriPrep has not been validated for use in RNA-seq.

3. Are AgriPrep kits compatible with crude extractions?
    - AgriPrep Library Preparation Kits have only been validated for purified DNA inputs.

4. 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.

5. Are all required adapters, indexes, amplification master mix and amplification primers included in AgriPrep Library Prep Kit?
    - Yes. The AgriPrep kit includes all the indexed adapters, amplification master mix, and amplification primers necessary to make dual-indexed Illumina-compatible libraries.

6. My thermal cycler is incompatible with the full-skirted plates provided in the kit. How do I process my samples?
    - Start off the library preparation with the plates provided. After the reactions are mixed by pipette, seal and centrifuge the plate. Then, transfer the reactions to a plate compatible with your thermal cycler. Seal the new reaction plate and centrifuge again before loading into your thermal cycler. Processing it this way is critical to ensure proper proportions of reagents are used and normalization is successful.

7. How many samples can I batch together?
    - The AgriPrep (96-well) kit allows for batching 16 – 96 samples per assay-ready plate. Batching fewer than 16 samples may be possible for experienced users.

8. If I processed <96 samples of 96-well kit plate, can I reuse the remaining reagents?
    - AgriPrep reagents can be saved if they are as-shipped (i.e. if no DNA or Indexing reagents have been added, and no incubations have been done). If processing <96 samples for your 96-well kit plate, and you would like to preserve the reagents, set the reaction up as described in the User Guide.
    - With a razor blade, cut the seals up to the sample number being processed. Only peel the heat seal from the wells of the Indexing Reagent Plate and Ready Reaction Plate corresponding to the total number of samples that will be processed. Proceed with both sample transfer and Indexing Reagent transfer into the Ready Reaction plate wells in use, pipette mix, seal, and centrifuge. Unseal the plate and transfer the reactions to a new PCR plate. Cover up the used wells to prevent contamination and store remaining reagents at -20°C for subsequent use.

9. How many total index combinations are commercially available?
    - For the AgriPrep Library Prep Kit, up to 1536 index combinations are available off-the-shelf, and up to 3072 index combinations are available via custom ordering. If more than 1536 index combinations are required, please contact sales@seqwell.com.

10. Can I automate the AgriPrep Library Prep Kit?
    - The AgriPrep Library Prep Kit is highly amenable to automation platforms. Please review our ExpressPlex 2.0 (96 & 384-well) Library Preparation Kit Automation Guide document in Kit User Guides section of our website for guidance on automation development and available validated method methods. For access to a validated method or additional guidance, please contact support@seqwell.com.

11. Are the plate seals pierceable with automation?
    - No, the seals are not pierceable. You will need to peel back the seal to access the wells. We have done extensive testing to confirm no contamination issues if plates are properly centrifuged before peeling.

12. What is the recommended DNA input range for the AgriPrep Kit?
    - The recommended input concentration range for the AgriPrep Kit is 2.5 - 50 ng/µl. The kit uses 4 µl of purified DNA sample (10 - 200 ng). The normalization range is 5 - 25 ng/µl (20 - 100 ng total). Use of DNA input concentrations of less than 2.5 ng/µl is not recommended due to increased risk of failure.

13. My samples are all more concentrated than 50 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 (2.5 - 50 ng/µl), optimally aiming for an average of 10 ng/µl. Use of lower or higher DNA concentration may adversely affect sequencing performance.

14. The concentration of DNA sample input is variable. Can the samples still be prepped together?
    - The AgriPrep library prep kit performs optimally with 40 ng of dsDNA per reaction. However, individually normalizing each sample to 10 ng/µl is not necessary as AgriPrep Library Prep Kits are formulated to tolerate up to a 20-fold difference in sample input (10 to 200 ng).

15. What quantification methods are recommended for plasmids and PCR products?
    - The AgriPrep Kit is sensitive to dsDNA concentration outside the recommended range. Fluorometric methods for dsDNA (e.g., Qubit or PicoGreen) are generally more reliable for assaying input samples than spectrophotometric methods (unless supercoiled, see below). Regardless of the quantification methods employed, the purity of the DNA should be considered. There are several contaminants of genomic DNA that can interfere with quantification including protein, plasmid DNA, ssDNA and RNA. The presence of these contaminants inflates the apparent DNA concentration.

16. I see library fragments >1200bp in my AgriPrep purified library that I know will not cluster well on an Illumina flowcell. Does this affect my library quantification and sequencing?
    - Fragments >1200bp do not interfere with clustering on the flowcell or the data sequencing quality. However, they will affect library quantification and thus the optimal loading density (see User Guide to properly adjust library quantification based on fragment analysis, summarized below).
    - Use the TapeStation, Fragment Analyzer, or similar equipment to conduct a region analysis to determine the percentage of the DNA mass that is sequenceable (see User Guide for more details), then multiply this percentage of your DNA in the clusterable range to determine the sequenceable library content.
    - This region should be used for BOTH library size and mass/molarity measurements for loading.

17. Can I QC check my libraries prior to pooling?
    - No, it is not recommended to QC your libraries prior to pooling. The protocol was designed for pooling even volumes of each library right after PCR, then going into a single bead purification, then QCing one final pool. We expect the yield of each individual well to be too low for QC, and may impact your ability to evenly pool and normalize.

18. What is the expected fragment size of the AgriPrep library?
    - The expected fragment size can vary depending on the sample input. Typically, the fragment size of the AgriPrep library is 300 – 2,000 bp. If the input DNA was shorter than 1000 bp, the resulting library fragment size distribution will be shorter than the input DNA.

19. What if I made a mistake in the protocol?
    - To better understand the potential impact and for guidance, please contact support@seqwell.com.

20. 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).
    - To determine the library size, it is critical to employ a region analysis described in the Library Quantification and QC section of the User Guide.

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

22. Can AgriPrep libraries be run on sequencers other than Illumina instruments?
    - All current protocols create libraries with Illumina adapter sequences and are geared and optimized to run on Illumina sequencers. Many users have been successful using conversion kits for a variety of other sequencers. Please reach out to support@seqwell.com for more information.

23. Do I need to spike PhiRx Indexed Control into the final library prior to sequencing?
    - The PhiRx Indexed Control is provided with each kit of AgriPrep for optional use, but is strongly advised. The base composition of AgriPrep libraries is highly diverse, so PhiRx is not required for read diversity, however it can be used at a 1-2% spike-in for an internal sequencing control.
    - If sequencing on XLEAP-SBS chemistry, or sequencing fewer than 4 plates at once, a 5-10% spike-in may be required to mitigate any issues that can occur with color balancing.
    - For more details about the PhiRx, please refer to the PhiRx Indexed Control FAQs found here: https://seqwell.com/resource-category/faqs/

24. What is the compatibility of AgriPrep indexes?
    - There are 4 different i5 index sets per AgriPrep 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.
    - AgriPrep indexes overlap with ExpressPlex 2.0 (96-well) indexes and should not be run together.
    - AgriPrep can be run with ExpressPlex 2.0 (384-well) Sets B-D, NOT Set A.

25. Are the AgriPrep indexes mutually exclusive of other library preparation kit indexes?
    - AgriPrep indexes have not been compared to all commercially available index sets. However, AgriPrep indexes do not overlap with indexes in Illumina's Nextera product line. Within seqWell's product line, AgriPrep Indexes overlap with ExpressPlex 2.0 (96-well) indexes and ExpressPlex 2.0 (384-well) Set A.
    - 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 AgriPrep libraries.

26. Where can indexes for making a sample sheet for the MiSeq and other systems be found?
    - Please find them in the user guide and AgriPrep Index List document in the Index List section on our website. The indexes can be copied directly from the master index list.

27. 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