Guidelines for Color-Balanced Loading of seqWell Libraries on Illumina Sequencers with XLEAP Chemistry

For seqWell library preparation products that use combinatorial dual-indexing (CDI) for multiplexing of samples at the plate level, it is important to adhere to color balancing requirements on indexing reads needed to ensure optimal performance on Illumina sequencing instruments.

The guidelines below provide recommended best practices for utilizing seqWell products with CDI indexing, particularly when pooling ≤ 4 plates into a single pool for sequencing. Affected seqWell products relevant to this advisory are ExpressPlex 2.0 (96-well), ExpressPlex 2.0 (384-well), and AgriPrep.

Please note that these guidelines are only necessary for the batching plates when using the XLEAP-SBS chemistry on certain Illumina instruments:

NovaseqX
NovaSeq X Plus
NextSeq 1000 (if upgraded to XLEAP compatibility)
NextSeq 2000 (if upgraded to XLEAP compatibility)

Each of these kits will include an additional tube of our PhiRx Indexed Control for spiking into low plexity sequencing runs. This is a sequencing control spike-in reagent that adds diversity to both the library reads and barcode reads. It was optimized for XLEAP's color-balancing requirements and allows for demultiplexing of previously unidentifiable reads. More information about our PhiRx Indexed Control, such as the user guide and FAQs, can be found here: https://seqwell.com/phirx-color-balancing/

We recommend the use of PhiRx; however, if this is not possible, please refer to the guidelines outlined in the tables on the following page for index set combinations that provide the most effective XLEAP color balancing.

Further details on XLEAP-specific color-balancing requirements can be found here: https://knowledge.illumina.com/instrumentation/novaseq-x-x-plus/instrumentation-novaseq-x-x-plus-reference_material-list/000008422

ExpressPlex 2.0 (96-well) & AgriPrep:

Recommended to use **Set 1000** as a base set with the following guidelines based on plexity of plates:

Sequencing System	Plexity (Number of Plates)	Combinations in Index Sets
NextSeq 1000/2000 Systems, NovaSeq X/X Plus Systems (XLEAP reagent)	5~16	Choose any combination with Set 1000 and add other plates as needed.
	4	**The following combination is recommended:**
		Set 1000
	3	**One of the following combinations:**
		Set 1000: 1001, 1002 and 1004
		Set 1000: 1001, 1003 and 1004
	2	**The following combination is recommended:**
		Set 1000: 1001 and 1004
	1	Not recommended by Illumina, demux by i7 only

ExpressPlex 2.0 (384-well):

Recommended to use **Any Set** as a base set with the following guidelines based on plexity of plates:

Sequencing System	Plexity (Number of Plates)	Combinations in Index Sets
NextSeq 1000/2000 Systems, NovaSeq X/X Plus Systems (XLEAP reagent)	2~16	Choose any combination with Set A/B/C/D - 1000 **OR** 3000 and add other plates as needed.
	1	**One of the following plates are recommended:**
		Set A/B/C/D -1000
		Set A/B/C/D - 3000

If you already have purchased the Illumina XLEAP reagents and different sets of the affected seqWell products, please contact the seqWell Support Team at support@seqwell.com for guidance.

We appreciate your continued use of seqWell's products and strive to deliver an unmatched customer experience. Our commitment to quality and partnership will ensure that our users can confidently continue their research while maintaining compatibility with Illumina's XLEAP reagents.

For any questions or to obtain a quote for ordering product, please contact:

- sales@seqwell.com
- support@seqwell.com
- Or call 1-855-737-9355