



Guidelines for Color-Balanced Loading of seqWell Libraries on Illumina Sequencers with XLEAP SBS™ 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 1-4 plates into a single pool for sequencing. Affected seqWell products relevant to this advisory are ExpressPlex™, ExpressPlex™ HT, plexWell™, and plexWell™ LP library preparation kits.

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

- Novaseq™X
- NovaSeq X Plus
- NextSeq™ 1000 (if upgraded to XLEAP compatibility)
- NextSeq 2000 (if upgraded to XLEAP compatibility)

The following guidelines adhere to XLEAP-specific color-balancing requirements, which can be reviewed in more detail here: https://knowledge.illumina.com/instrumentation/novaseq-x-x-plus/instrumentation-novaseq-x-x-plus-reference_material-list/000008422

ExpressPlex:

Recommend using **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 HT:

Recommend using **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

plexWell and plexWell LP:

Recommend using **Set B** 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 B and add other plates as needed.
	4	The following combination is recommended:
		Set B
	3	One of the following combinations:
		Set B: X021, X024, and X038 Set B: X021, X038, and X044
	2	The following combination is recommended:
1	Set B - X021 and X038	
	1	Not recommended by Illumina, demux by i7 only

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 and want to ensure our users will be able to confidently continue their research, by ensuring 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