NEXTFLEX® UNIQUE DUAL INDEX BARCODES

DNA-SEQ · RNA-SEQ · BARCODES · METAGENOMICS · AMPLICON PANELS · TARGET CAPTURE · EPIGENETICS · SMALL RNA-SEQ

Mitigate Index Hopping & Spread of Signal on Patterned Flow Cells with the NEXTFLEX® Unique Dual Index Barcodes

The NEXTFLEX® unique dual index barcodes are barcoded adapters for sequencing on Illumina® platforms that provide unprecedented data security in sequencing applications. Increased mis-assignment of indexes has been shown to occur on Illumina® sequencing instruments that feature a patterned flow cell and exclusion amplification technology. The NEXTFLEX® unique dual index barcodes are designed to specifically mitigate the index hopping or spread of signal phenomenon associated with Illumina® platforms that utilize a patterned flow cell. Index mis-assignment can lead to increased false positive rates, which are especially detrimental to sensitive applications. Multiplexing with NEXTFLEX® unique dual index barcodes drastically increases processing capacity while reducing costs by allowing the user to pool multiple libraries in a single flow cell lane.

Illumina® sequencing instruments that utilize a patterned flow cell and exclusion amplification technology is known to suffer from increased levels of sample mis-assignment. The use of NEXTFLEX® unique dual index barcodes prevents such mis-assigned reads from appearing in final data sets allowing for the highest assurance of data integrity.

KEY FEATURES

- Unique dual index adapters for multiplexing up to 96 Illumina® sequencing libraries
- Mitigate index hopping or spread of signal that can occur during sequencing
- Decreases level of mis-assigned reads in sequencing data
- Compatible with both paired-end and single-read Illumina® sequencing

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Samples Demultiplexed via Unique 8nt i7 Index

Amplicon

Index	1	2	3	4	5	6	7	8
1	99.84	0.02	0.02	0.04	0.02	0.02	0.07	0.08
2	0.03	99.82	0.02	0.06	0.03	0.02	0.21	0.09
3	0.03	0.03	99.86	0.07	0.02	0.03	0.11	0.1
4	0.02	0.03	0.02	99.58	0.02	0.02	0.09	0.09
5	0.03	0.03	0.03	0.09	99.85	0.04	0.13	0.09
6	0.02	0.02	0.01	0.05	0.02	99.83	0.08	0.06
7	0.01	0.02	0.01	0.02	0.01	0.01	99.15	0.04
8	0.03	0.04	0.02	0.09	0.03	0.03	0.16	99.46

Samples Demultiplexed via Unique 8nt i7 Index & Unique 8nt i5 Index

Amplicon

Index	1	2	3	4	5	6	7	8
1	100	0	0	0	0	0	0	0
2	0	99.9	0	0	0	0	0	0
3	0	0.01	100	0	0	0.01	0	0
4	0	0	0	100	0	0	0	0
5	0	0	0	0	100	0	0	0
6	0	0	0	0	0	99.9	0	0
7	0	0	0	0	0	0	100	0
8	0	0	0	0	0	0	0	100

Figure 1. NEXTFLEX® unique dual index Barcodes decrease index mis-assignment on the Illumina® HiSeq® 4000 platform. A set of libraries prepared using NEXTFLEX® unique dual index barcodes was sequenced on the HiSeq® 4000 sequencing platform. The numbers indicate percentage of correct insert reads assigned to index sequences. The resultant data was demultiplexed twice: first by taking only the unique I7 index into account (left panel), and second by taking both unique I7 and I5 indexes into account (right panel). By assessing both the unique I7 and I5 indexes, sequence mis-assignment was drastically decreased.

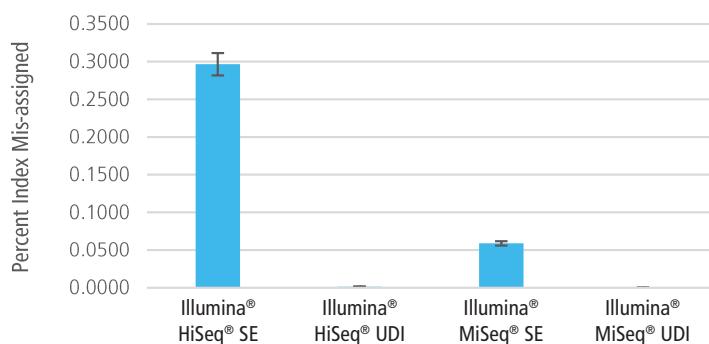## Unique Dual Index vs. Single Index on Illumina® HiSeq® & MiSeq® Platforms

Figure 2. NEXTFLEX® Unique Dual Index Barcodes increase confidence in sequencing data. The data generated on the HiSeq® 4000 platform showed a dramatic reduction in the percentage of mis-assigned reads, and the data generated on the MiSeq® platform also showed a reduction. The results indicate that the NEXTFLEX® unique dual index barcodes greatly reduce the amount of mis-assigned reads in data sets, and that this occurs at a higher rate on the instruments that use exclusion amplification chemistry and patterned flow cells.

ORDERING INFORMATION

Catalog #	Kit Name	Quantity
NOVA-514150	NEXTFLEX® Unique Dual Index Barcodes (96 barcodes)	192 rxns

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