Recommended strategies for unique dual index designs

Aug 11, 2017

Unique dual indexing is a known mitigation for filtering index-hopped reads seen in downstream analyses when unexpected index combinations are assigned to data sets during the de-multiplexing step. This bulletin describes best practices and considerations when designing custom unique dual indexes. For more information about index hopping refer to the index hopping white paper.

A number of strategies can be employed to develop unique dual indexes. Some of the more common designs are tandem, tandem complement, and distinct (Figure 1). All three approaches provide distinct index combinations such that no samples share an i7 or i5 index sequence with other samples within the library pool.

Figure 1. Examples of the most common unique dual index designs.

Tandem Design (same index on either side of insert)

----AGCGCTAG----------Insert----------AGCGCTAG----
----TCGCGATC----------Insert----------TCGCGATC----

Tandem Complement Design (reverse complement of same index)

----AGCGCTAG----------Insert----------CTAGCGCT----
----TCGCGATC----------Insert----------GATCGCGA----

Distinct Design (unrelated index sequences)

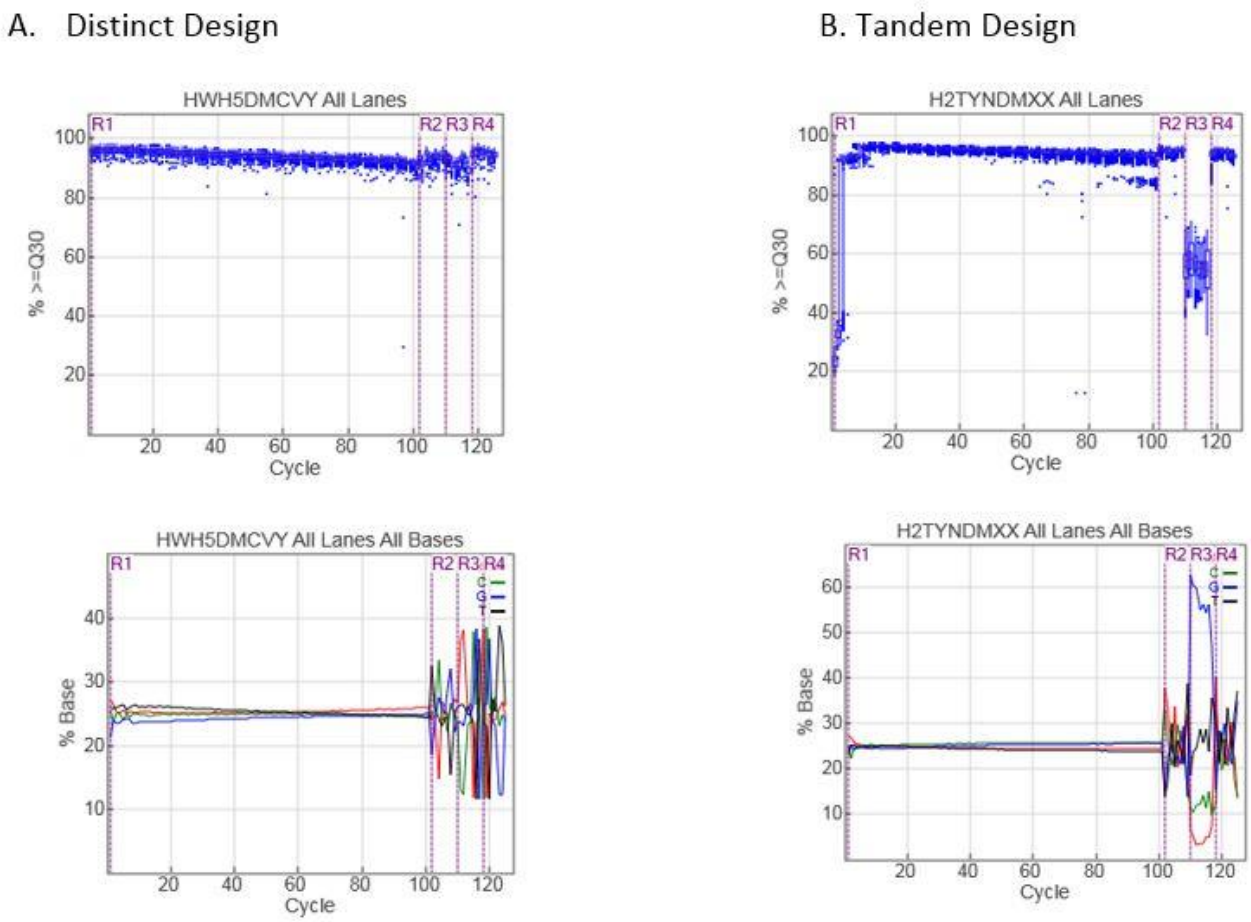
----AGCGCTAG----------Insert----------CGTCTGCG----
----TCGCGATC----------Insert----------GCAGACGC----

Recent testing and evaluation of the different design strategies has identified a variable effect on sequencing quality associated with the use of tandem or tandem complement designs. We want to share the observed effect and provide a recommendation for avoiding the tandem design strategy.

Impact of tandem designs on i5 Read

Below are examples of two flow cells, each containing a specific type of library prep adapter design (Figure 2). Compared are the distinct design (eg, the TruSeq adapters) and a tandem design. In these experiments, tandem designs exhibit lower quality (Q30 < 75%) and the phenotype ‘T overcall / A undercall’ resulting in poor de-multiplexing for the Index 2 or i5 Read.

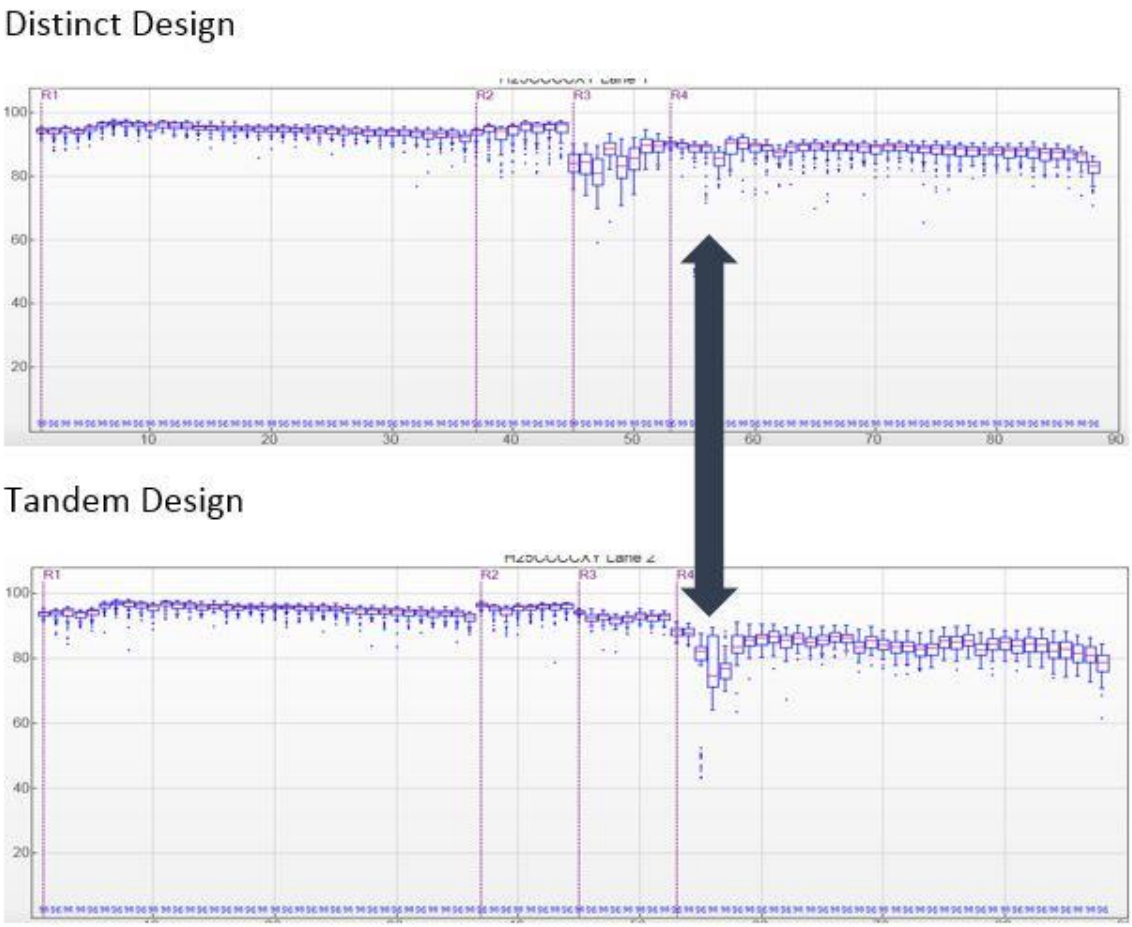
Figure 2. Sequencing performance comparison of distinct and tandem unique index designs.



Impact of tandem designs on Read 4

Below are lanes from the same flow cell where tandem complement and distinct library prep designs were run using the same oligo paired in alternative combinations, either as a tandem complement or a distinct combination (Figure 3).

Figure 3. Comparison of Read 4 performance of distinct and tandem unique index designs.



In Figure 2, the region highlighted by the arrow shows an unevenness in the Q30 scores of the tandem paired indexes not seen with the distinct pairing design. The level of unevenness is variable and has minimal impact on the data. This phenotype may be seen when using tandem designs. It could be considered cosmetic in many customers' workflows.

Due to the reduced Q30 scores of the i5 Index Read and the Read 4 unevenness found in tandem designed indexes, **we recommend customers use unique indexes (distinct pairing) in their index designs going forward.**

Illumina has entered into a partnership with Integrated DNA Technologies (IDT) to create distinct unique dual index designs, the **IDT for Illumina - TruSeq UD Indexes.**

The IDT Illumina partnership is creating 2 initial products using the distinct design principles and the partnership will continue to build out the index portfolio using this strategy.

Indexes Available for pre-order

Catalog # 20020590 - IDT for Illumina – TruSeq DNA UD Indexes (24 Indexes, 96 Samples)
Catalog # 20020591 - IDT for Illumina – TruSeq RNA UD Indexes (24 Indexes, 96 Samples)

For additional information, email Technical Support at techsupport@illumina.com

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