xGEN UDI-UMI ADAPTERS

Guidelines

Delivery

The xGen UDI-UMI and xGen Methyl UDI-UMI Adapters are delivered at a concentration of 15 µM in 96-well plates with a pierceable seal. Each well contains a specific unique dual index (UDI) for indexing one sample.

The adapters are loaded in the plates by column (Figure 1).

Low level multiplexing

Multiplexing, or pooling, involves combining multiple libraries to sequence in the same run. The indexes on the xGen UDI-UMI Adapters are color-balanced in sets of four (1–4, 5–8, 9–12, etc.). Indexes within each group of four are fully color balanced and can be pooled for sequencing. Less than four samples can be multiplexed, but verify color balance before pooling.

Handling and storage

- Store adapters at –20°C.
- Thaw the adapters on ice before use. Keep the adapters on ice during use.
- Do not heat adapters above room temperature (15–25°C).

Directions for use

1. Thaw the adapter plate on ice. Once thawed, briefly centrifuge the plate with the plastic cover in place to collect material at the bottom of wells.

   Important! Keep the xGen UDI-UMI Adapters tube on ice while in use.

2. Pierce seals using a pipette tip and then directly pipet the required volume of your adapters.

   Important! Always use a separate pipette tip for each well to avoid cross contamination of indexes.

3. Add the quantity of xGen UDI-UMI Adapters to the ligation reaction as determined by your library preparation protocol.

   Tips:

   - The optimal amount of adapter is dependent upon your protocol and the amount of input in your library preparation.
   - If you need to dilute the adapter, always use NGS Adapter Buffer (10 mM Tris, 0.1 mM EDTA, and 100 mM NaCl, pH 8.0).
   - Because xGen Methyl UDI-UMI Adapters are methylated, bisulfite conversion can be carried out after ligation (of the adapter), but before library amplification.

4. Return any unused portion of the plate to storage at –20°C.

Sequencing and analysis

Use the xGen UDI-UMI Adapters to construct NGS libraries with a TA-ligation library prep kit for sequencing on an Illumina sequencer.

More information can be found on our xGen UDI-UMI Adapters product page.

UMI details

The UMI (dark blue in image below) is positioned next to the i7 index so that it will sequenced after the i7 index. To sequence the UMI, increase the number of cycles for the i7 index read by 9 cycles.

Note: See the Analysis guidelines for details on use of UMI for error correction.

Tip: Strand-specific libraries constructed using the xGen UDI-UMI Adapters have the insert strandedness flipped (i.e., to the opposite strand) when compared to standard Illumina TruSeq™ adapters. This strand flip must be accounted for in data analysis for strand-specific applications (e.g., stranded RNA-seq, bisulfite-sequencing).

Sample index plate layout

Sample indexes are arrayed in columns, as shown in Figure 1.

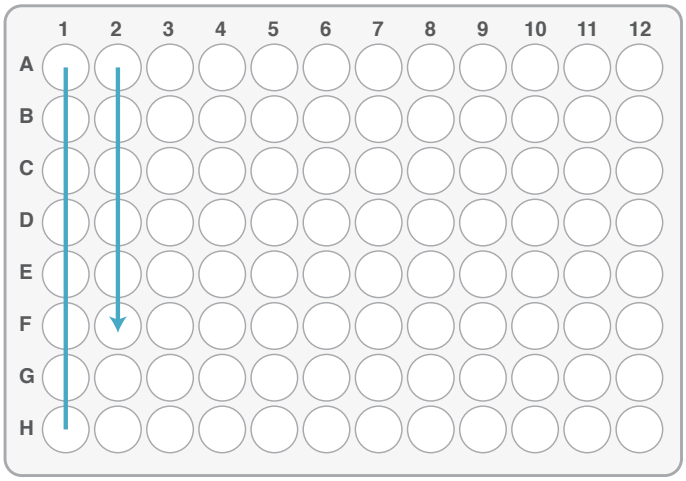

Figure 1. Set up pooling samples for multiplex sequencing in designated columns.

Technical support: applicationsupport@idtdna.com

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