xGen Stubby Adapter and UDI Primer Pairs

Guidelines

Delivery

The xGen Stubby Adapter is delivered in a tube at a concentration of 15 µM. The xGen UDI Primer Pairs are delivered in single-use plates with a pierceable seal. Each well of the xGen UDI Primer Pairs plate contains a specific unique dual indexing primer pair at a concentration of 20 µM (10 µM of each i5 and i7 primer) for indexing one sample.

The primer pairs are loaded in the plate 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 Primer Pairs 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 the xGen Stubby Adapter and UDI Primer Pairs at –20°C.
- Thaw the xGen Stubby Adapter on ice before use. Keep on ice during use.
- Thaw the UDI Primer Pairs before use. Keep on ice during use.
- Do not heat the xGen Stubby Adapter above room temperature (15°–25°C).

Directions for use

1. Thaw the tube of Stubby Adapter on ice. Once thawed, vortex mix, then briefly centrifuge to collect material at the bottom of the tube.

   Important! Keep the Stubby Adapter tube on ice while in use.

2. Thaw the indexing primer plate before use. Once thawed, briefly centrifuge the plate with the plastic cover in place to collect material at the bottom of wells.

   Important! Keep the indexing primer plate on ice while in use.

3. Add the quantity of xGen Stubby Adapter to the ligation reaction as determined by your library preparation protocol.

   Note: The optimal amount of adapter is dependent upon your protocol and the amount of input in your library preparation.

   Tip: If you need to dilute the stubby adapter, always use Nuclease-Free Duplex Buffer.

4. Because the xGen Stubby Adapter is methylated, bisulfite conversion can be carried out after ligation (of the adapter), but before library amplification.

5. When preparing the indexing PCR, pierce the seal of the plate using a pipette tip, then directly pipet the required volume of your indexing primers.

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

6. Add the quantity of xGen UDI Indexing Primer Pairs to the indexing PCR reaction as indicated in your library preparation protocol.

7. Return any unused portion of the stubby adapter and indexing primer plate to storage at –20°C.

Sequencing and analysis

Use the xGen Stubby Adapter and indexing primers to construct TruSeq™-compatible NGS libraries with a TA-ligation library prep kit for sequencing on an Illumina sequencer.

More information can be found on our xGen Stubby Adapter and UDI Primers Pairs product page.

Sample index plate layout

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

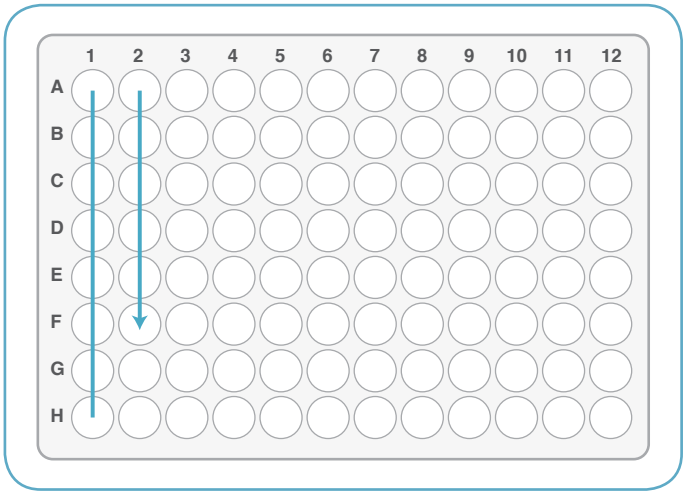

Figure 1. Set up a multiplex run by pooling samples in designated columns.

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