xGen™ Stubby Adapter and Indexing Primers

Overview

Use the xGen Stubby Adapter and Indexing Primers products to perform indexing PCR on NGS libraries that require TA-ligation (using the included stubby adapter) for the TruSeq™-compatible library construction workflows that support indexing by PCR. Note that most IDT library prep products already contain stubby adapters as part of the kit contents for the library prep workflow. Refer to xGen NGS Adapters & Indexing Primers for more information and recommendations for selecting library prep kits for indexing solutions. For other commercial library prep workflows, refer to your library prep kit protocol for further instructions prior to using these products.

Product details

xGen Stubby Adapter and Indexing Primers are available in two reaction sizes that are loaded into single-use plates:

- Single-use plates: The adapter and indexing primers are loaded into single-use 96-well plates containing a pierceable seal. The unique dual index (UDI) has a barcode length of 8 nucleotides. Each well contains one specific index pair for indexing one sample.
  - Gen Stubby Adapter-UDI Primers, 16 rxn
  - xGen Stubby Adapter-UDI Primers, 96 rxn

Low-level multiplexing

It is not recommended to mix indexes between products, unless the end-user verifies color-balancing of the barcodes, and sufficient edit distance. If you have specific questions, please reach out to our Scientific Applications Support team at applicationsupport@idtdna.com.

Handling and storage

- Store the xGen Stubby Adapter and Indexing Primers at –20°C
- Do not heat stubby adapters above room temperature (15–25°C)
- If any material remains unused, carefully re-seal the plate with a new adhesive seal to prevent cross-contamination

Important: Do NOT attempt to heat seal the plate again.

Directions for use

1. Thaw the xGen Stubby Adapter and Indexing Primers on ice.

   Important: Keep the xGen Stubby Adapter and Indexing Primers on ice during use.

2. After thawing, briefly vortex both the tube containing the stubby adapter and the plate containing the primers, then centrifuge both tube and plate to collect the liquid at the bottom of the tube and wells. Do this before breaking the seal on the plate.

3. Prepare the ligation master mix as instructed in the library prep protocol by adding the quantity of adapter determined by the library prep protocol being used.

   Tip: The optimal amount of adapter is dependent on the protocol and input DNA quantity going into library prep.

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

4. Before plating the primers into the PCR reaction, pre-pierce the seal of the plate using a pipette tip, then directly pipette the required volume of primers into each reaction.

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

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

Sequencing and analysis

To view the sequences for each index primer, open the IDT Master Index List file, which is also found in the Resources section for xGen NGS Adapters & Indexing Primers.

Plate layouts

10005976–xGen Stubby Adapter-UDI Primers, 16 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
B	2	10	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
C	3	11	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
D	4	12	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
E	5	13	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
F	6	14	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
G	7	15	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*
H	8	16	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*	*empty*

10005921–xGen Stubby Adapter-UDI Primers, 96 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Technical support: applicationsupport@idtdna.com