xGen™ sample indexing products for Ultima Genomics®

Overview

IDT has several solutions available for customers sequencing on an Ultima Genomics platform, depending on which workflow is performed.

When performing WGS, use one of these product families, depending on your samples:

- xGen PCR-Free Adapters for Ultima Genomics
  - For applications with adequate gDNA input to support a PCR-free workflow, or when indexing by ligation is preferred
- xGen Indexing Primers for Ultima Genomics
  - For applications where indexing by PCR is preferred
  - Requires uracil-tolerant PCR mix
  - Requires xGen Stubby Adapter (catalog # 10005974 or 10005924, sold separately)

When performing target enrichment by hybridization capture, use one of these required products:

- xGen HybCap Adapters for Ultima Genomics

Note: Different adapters are required for hybridization capture due to the modifications found on the native Ultima® adapters. Because of this, conditioning of the final library is required before sequencing with the UG Amplification Kit from Ultima Genomics (catalog # 20020007, https://www.ultimagenomics.com/).

When performing sample indexing using the xGen Indexing Primers for Ultima, you must use an uracil-tolerant polymerase for PCR. To satisfy this requirement, we recommend using the UG Amplification Kit noted above.

Note: Identical index sequences are included for products used in WGS, or enrichment.

Product details

All xGen sample indexing products for Ultima are loaded into single-use 96-well plates containing a pierceable seal. The sample barcode has a length of either 15 or 17 nucleotides; check the index list and plate map for details. Each well contains material for indexing one sample.

For WGS (PCR-Free Workflow):

- xGen PCR-Free Adapters for Ultima, 16 rxn
- xGen PCR-Free Adapters for Ultima P1, 96 rxn
- xGen PCR-Free Adapters for Ultima P2, 96 rxn

For WGS (Indexing by PCR Workflow):

- xGen Indexing Primers for Ultima, 16 rxn
- xGen Indexing Primers for Ultima P1, 96 rxn
- xGen Indexing Primers for Ultima P2, 96 rxn
- xGen Indexing Primers for Ultima P3, 96 rxn
- xGen Indexing Primers for Ultima P4, 96 rxn

When performing targeted sequencing by hybridization capture use one of these required products:

- xGen HybCap Adapters for Ultima, 16 rxn
- xGen HybCap Adapters for Ultima Plate 1, 96 rxn
- xGen HybCap Adapters for Ultima Plate 2, 96 rxn
- xGen HybCap Adapters for Ultima Plate 3, 96 rxn
- xGen HybCap Adapters for Ultima Plate 4, 96 rxn

Low-level multiplexing

For an 8-plex, use any column

For more information, go to www.idtdna.com/ContactUs.

Handling & storage

- Store the xGen sample indexing products for Ultima at –20°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.

Library construction for WGS (PCR-Free workflow):

1. Thaw the xGen PCR-Free Adapters for Ultima on ice.

   Important:

   - Use the library construction method appropriate for performing either WGS (PCR-Free or Indexing by PCR), or targeted sequencing and follow the guidance specific to that workflow.
   - Keep the xGen PCR-Free Adapters for Ultima on ice during use.

2. Once thawed, briefly vortex the plate containing the adapters BEFORE breaking its seal, then centrifuge to collect the liquid at the bottom of the wells.
3. Prepare the Ligation Master Mix as instructed in the applicable library prep protocol by adding the appropriate quantity of xGen PCR-Free Adapters for Ultima.

   Tip: The exact amount of adapter is dependent on the protocol and the initial input DNA quantity.

   If needed, use NGS Adapter Buffer (catalog # 10006743) to dilute the adapters.
4. Before plating the adapters into the ligation 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 new pipette tip for each adapter well to avoid cross-contamination of the indexes.
5. Follow the appropriate ligation conditions from the library construction protocol being performed.
6. Return any unused portion of the plate and store at –20°C.
7. Perform the remaining steps on reaction cleanup and library QC before sequencing.

Library construction for WGS (Indexing by PCR)

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

   Important:

   - Use the library construction method appropriate for performing either WGS (PCR-Free or Indexing by PCR), or targeted sequencing and follow the guidance specific to that workflow.
   - Keep the xGen Indexing Primers for Ultima and xGen Stubby Adapter on ice during use.

2. Once thawed, briefly vortex the plate containing the desired primers BEFORE breaking its seal, as well as the tube of xGen Stubby Adapter, then centrifuge to collect the liquid at the bottom of the wells or tube.
3. Prepare the Ligation Master Mix as instructed in the applicable library prep protocol by adding the appropriate quantity of xGen Stubby Adapter.

   Important: The required quantity of xGen Stubby Adapter can be added to the bulk Ligation Master Mix since it is not barcoded. Sample barcoding is done in a subsequent step, using the xGen Indexing Primers for Ultima.

   Tip: The exact amount of adapter is dependent on the protocol and initial input DNA quantity.

   If needed, use NGS Adapter Buffer (catalog # 10006743) to dilute the xGen Stubby Adapter.
4. Use the appropriate ligation conditions from the library construction protocol being performed.

   Important: Return any unused portion of the xGen Stubby Adapter to storage at –20°C.
5. Perform the remaining steps on reaction cleanup and library QC before PCR.
6. Prepare the PCR Master Mix as recommended in the library construction protocol being followed. Before adding the indexing primers to each 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 indexing primer well to avoid cross-contamination of the indexes.
   - We recommend using the UG Amplification Kit available from Ultima Genomics (catalog # 20020007, https://www.ultimagenomics.com) since it already contains the required uracil-tolerant polymerase.

7. Use the appropriate PCR conditions from the library construction protocol being performed.
8. Return any unused portion of the indexing primer plate to storage at –20°C.
9. Perform the remaining steps to perform post-PCR cleanup and library QC before sequencing.

WGS applications

Enzymatic prep

Fragmentation, end-repair, A-tailing

Ligation

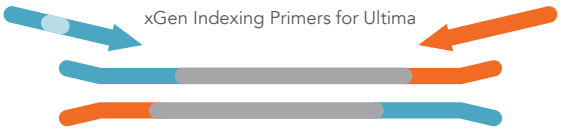
Using either:

- xGen Stubby Adapter [catalog #10005974 (16 rxn) or 10005924 (96 rxn)]

or

- xGen PCR-Free Adapters for Ultima

PCR amplification



[PCR optional, depending input quantity and yields]

Must use Library Amplification Primers and PCR Mix found in UG Amplification Kit (Ultima catalog #20020007)

Using UG Amplification Kit (Ultima catalog #20020007)

Ultima-compatible library

Library construction for targeted (hybridization capture) sequencing

1. Thaw the xGen HybCap Adapters for Ultima and xGen HybCap Lib Amp Primers for Ultima on ice.

   Important:

   - Use the library construction method appropriate for performing either WGS (PCR-Free or Indexing by PCR) or targeted sequencing and follow the guidance specific to that workflow.
   - Keep the xGen HybCap Adapters for Ultima and xGen HybCap Lib Amp Primers for Ultima on ice during use.

2. After thawing, briefly vortex the plate containing the desired xGen HybCap Adapters for Ultima BEFORE breaking its seal, then centrifuge to collect the liquid at the bottom of the wells using a new pipette tip.
3. Prepare the Ligation Master Mix as instructed in the applicable library prep protocol by adding the quantity of xGen HybCap Adapters for Ultima.

   Tip:

   - The optimal amount of adapter is dependent on the protocol and initial input DNA quantity.
   - If needed, use NGS Adapter Buffer (catalog # 10006743) to dilute the adapters.

4. Before plating the adapters into the ligation 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 adapter well to avoid cross-contamination of the indexes.

5. Use the appropriate ligation conditions from the library construction protocol being performed.

   Important: After plating into the ligation reaction, return the xGen HybCap Adapters for Ultima to –20°C storage.

6. Perform post-ligation SPRI cleanup, followed by library QC.

   Important: If PCR is needed to meet the recommended 500 ng yield requirement for hybridization capture, then use the required xGen HybCap Lib Amp Primer Mix for Ultima for amplifying the libraries.

7. Perform the Hybridization Capture workflow up to the Post-Capture PCR step, as outlined in the xGen Hybridization Capture of DNA Libraries Protocol.

   Important: If no additional PCR is needed to increase yields for the hybridization capture workflow, return the xGen HybCap Lib Amp Primer Mix for Ultima back to –20°C storage until needed for the Post-Capture PCR step.

8. For Post-Capture PCR, use the xGen HybCap Lib Amp Primer Mix for Ultima to amplify the libraries.

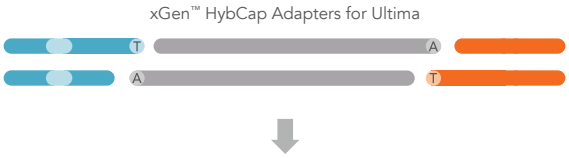
   Important: After Post-Capture PCR, return the xGen HybCap Lib Amp Primers for Ultima back to –20°C storage.

9. A final conditioning of the libraries is required before sequencing; use the library amplification primers and PCR reagents in the UG Amplification Kit from Ultima Genomics (catalog # 20020007, https://www.ultimagenomics.com).
10. Follow the appropriate steps to perform reaction cleanup and final library QC before sequencing.

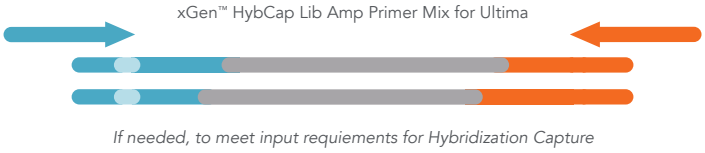
Targeted (hybridization capture) sequencing applications

Enzymatic prep
Fragmentation, end-repair, A-tailing

Ligation



PCR amplification (if needed)

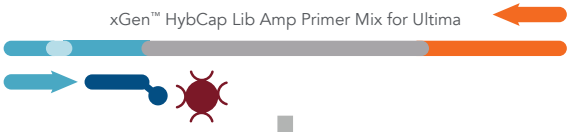


If needed, to meet input requiements for Hybridization Capture

Intermediate library

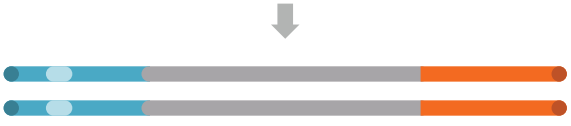
Hybridization capture workflow

On-bead, post-capture PCR



Final library conditioning
After completing Hybridization Capture workflow

Ultima-compatible library



Sequencing and analysis

To view the sequences for each index primer, locate the xGen for Ultima Index List File found in the Resources section for xGen for Ultima Genomics.

Plate layouts

10016830 – xGen PCR-Free Adapters for Ultima, 16 rxn
10016991 – xGen Indexing Primers for Ultima, 16 rxn
10017011 – xGen HybCap Adapters for Ultima, 16 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z0001	Z0009	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	Z0002	Z0010	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
C	Z0003	Z0011	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	Z0004	Z0012	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	Z0005	Z0013	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	Z0006	Z0014	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	Z0007	Z0015	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	Z0008	Z0016	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty

10016841 – xGen PCR-Free Adapters for Ultima P1, 96 rxn
10016992 – xGen Indexing Primers for Ultima P1, 96 rxn
10017012 – xGen HybCap Adapters for Ultima Plate 1, 96 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z0001	Z0009	Z0017	Z0025	Z0033	Z0041	Z0049	Z0057	Z0065	Z0073	Z0081	Z0089
B	Z0002	Z0010	Z0018	Z0026	Z0034	Z0042	Z0050	Z0058	Z0066	Z0074	Z0082	Z0090
C	Z0003	Z0011	Z0019	Z0027	Z0035	Z0043	Z0051	Z0059	Z0067	Z0075	Z0083	Z0091
D	Z0004	Z0012	Z0020	Z0028	Z0036	Z0044	Z0052	Z0060	Z0068	Z0076	Z0084	Z0092
E	Z0005	Z0013	Z0021	Z0029	Z0037	Z0045	Z0053	Z0061	Z0069	Z0077	Z0085	Z0093
F	Z0006	Z0014	Z0022	Z0030	Z0038	Z0046	Z0054	Z0062	Z0070	Z0078	Z0086	Z0094
G	Z0007	Z0015	Z0023	Z0031	Z0039	Z0047	Z0055	Z0063	Z0071	Z0079	Z0087	Z0095
H	Z0008	Z0016	Z0024	Z0032	Z0040	Z0048	Z0056	Z0064	Z0072	Z0080	Z0088	Z0096

10016842 – xGen PCR-Free Adapters for Ultima P2, 96 rxn
10016993 – xGen Indexing Primers for Ultima P2, 96 rxn
10017013 – xGen HybCap Adapters for Ultima Plate 2, 96 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z0097	Z0105	Z0113	Z0121	Z0129	Z0137	Z0145	Z0153	Z0161	Z0169	Z0177	Z0185
B	Z0098	Z0106	Z0114	Z0122	Z0130	Z0138	Z0146	Z0154	Z0162	Z0170	Z0178	Z0186
C	Z0099	Z0107	Z0115	Z0123	Z0131	Z0139	Z0147	Z0155	Z0163	Z0171	Z0179	Z0187
D	Z0100	Z0108	Z0116	Z0124	Z0132	Z0140	Z0148	Z0156	Z0164	Z0172	Z0180	Z0188
E	Z0101	Z0109	Z0117	Z0125	Z0133	Z0141	Z0149	Z0157	Z0165	Z0173	Z0181	Z0189
F	Z0102	Z0110	Z0118	Z0126	Z0134	Z0142	Z0150	Z0158	Z0166	Z0174	Z0182	Z0190
G	Z0103	Z0111	Z0119	Z0127	Z0135	Z0143	Z0151	Z0159	Z0167	Z0175	Z0183	Z0191
H	Z0104	Z0112	Z0120	Z0128	Z0136	Z0144	Z0152	Z0160	Z0168	Z0176	Z0184	Z0192

10016994 – xGen Indexing Primers for Ultima P3, 96 rxn
10017014 – xGen HybCap Adapters for Ultima Plate 3, 96 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z0193	Z0201	Z0209	Z0217	Z0225	Z0233	Z0241	Z0249	Z0257	Z0265	Z0273	Z0281
B	Z0194	Z0202	Z0210	Z0218	Z0226	Z0234	Z0242	Z0250	Z0258	Z0266	Z0274	Z0282
C	Z0195	Z0203	Z0211	Z0219	Z0227	Z0235	Z0243	Z0251	Z0259	Z0267	Z0275	Z0283
D	Z0196	Z0204	Z0212	Z0220	Z0228	Z0236	Z0244	Z0252	Z0260	Z0268	Z0276	Z0284
E	Z0197	Z0205	Z0213	Z0221	Z0229	Z0237	Z0245	Z0253	Z0261	Z0269	Z0277	Z0285
F	Z0198	Z0206	Z0214	Z0222	Z0230	Z0238	Z0246	Z0254	Z0262	Z0270	Z0278	Z0286
G	Z0199	Z0207	Z0215	Z0223	Z0231	Z0239	Z0247	Z0255	Z0263	Z0271	Z0279	Z0287
H	Z0200	Z0208	Z0216	Z0224	Z0232	Z0240	Z0248	Z0256	Z0264	Z0272	Z0280	Z0288

10016995 – xGen Indexing Primers for Ultima P4, 96 rxn
10017015 – xGen HybCap Adapters for Ultima Plate 4, 96 rxn

	1	2	3	4	5	6	7	8	9	10	11	12
A	Z0289	Z0297	Z0305	Z0313	Z0321	Z0329	Z0337	Z0345	Z0353	Z0361	Z0369	Z0377
B	Z0290	Z0298	Z0306	Z0314	Z0322	Z0330	Z0338	Z0346	Z0354	Z0362	Z0370	Z0378
C	Z0291	Z0299	Z0307	Z0315	Z0323	Z0331	Z0339	Z0347	Z0355	Z0363	Z0371	Z0379
D	Z0292	Z0300	Z0308	Z0316	Z0324	Z0332	Z0340	Z0348	Z0356	Z0364	Z0372	Z0380
E	Z0293	Z0301	Z0309	Z0317	Z0325	Z0333	Z0341	Z0349	Z0357	Z0365	Z0373	Z0381
F	Z0294	Z0302	Z0310	Z0318	Z0326	Z0334	Z0342	Z0350	Z0358	Z0366	Z0374	Z0382
G	Z0295	Z0303	Z0311	Z0319	Z0327	Z0335	Z0343	Z0351	Z0359	Z0367	Z0375	Z0383
H	Z0296	Z0304	Z0312	Z0320	Z0328	Z0336	Z0344	Z0352	Z0360	Z0368	Z0376	Z0384

For more information, go to: **www.idtdna.com/ContactUs**

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