

xGen™ INDEXING PRIMERS

OVERVIEW

Use the **xGen Indexing Primers** to perform indexing PCR on next generation sequencing (NGS) libraries that contain TruSeq™-compatible truncated adapters ligated to the library molecules. Be sure to refer to the library prep kit protocol you are using for any additional instructions before using these primers.

Primer formats

xGen Indexing Primers are available as four different deliverables:

- **Tubes.** To be arrayed into an 8x12 plate. Combinatorial dual index, barcode length of 8 nucleotides.
 - xGen CDI Primers, 96 rxn
- **Single Use Plates.** Individual single-use (96-well) plates with a pierceable seal. Unique dual index, barcode length of 8 nucleotides. Each well contains one specific index pair for indexing one sample.
 - xGen UDI Primers, 16 rxn
 - xGen UDI Primers Plate 1, 8 nt
 - xGen UDI Primers Plate 2, 8 nt
- **Single Use Plate Bundles.** Groups of either 384, 768, or 1536 primer pairs. All are single use (96-well) plates, with a pierceable seal. Designed for high plexity workflows with a 10 nt barcode to allow proper edit distance between sample indexes. Each well contains one specific index pair for indexing one sample.
 - xGen UDI 10 nt Primer Plates 1–4
 - xGen UDI 10 nt Primer Plates 1–8
 - xGen UDI 10 nt Primer Plates 1–16
- **Bulk Primers.** Like the bundles above, but available in bulk quantities (2 nmol of each primer), delivered in a 96-well deep-well plate. Groups of either 384, 768, or 1536 primer pairs. Designed for both high plexity and high-throughput workflows with a 10 nt barcode to allow the proper edit distance between sample indexes. Each well contains one specific index pair in bulk format.
 - xGen UDI 10 nt Primer Plates 1–4, 2 nmol
 - xGen UDI 10 nt Primer Plates 1–8, 2 nmol
 - xGen UDI 10 nt Primer Plates 1–16, 2 nmol

Low level multiplexing

Low level multiplexing, also known as low-plexity multiplexing (i.e., mixing up to 12 samples), is a compromise cost-to-coverage option that may have inadequate diversity due to the limited number of barcodes available. Because each position in a set of index sequences needs to include at least one A or C base, and at least one G or T base—and ideally a balance of both—barcode sequences in low level multiplexing should be carefully chosen to result in both red and green color channels. It is not recommended to mix indexes between products, unless you can verify 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 Indexing Primers 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.

DIRECTIONS FOR USE

1. Thaw the xGen Indexing Primers at room temperature before use, then keep on ice during use.
2. After thawing, briefly vortex the plates (or tubes, if using CDI primers) to mix, then centrifuge to collect the liquid in the bottom of the well before breaking the seal.
3. For Plates: When preparing the indexing PCR reaction, pierce the seal of the plate using a pipette tip, then directly pipette the required volume of primers.
4. Prepare the PCR Master Mix as instructed in the library prep protocol you are using, then add the designated volume of primers.

Sequencing and analysis

To fill out index sequences in the sample sheet, use the IDT Master Index List file, found under 'Resources' on the [xGen NGS Adapters & Indexing Primers web page](#).

Plating CDI primers for indexing

The recommended pattern for arraying each CDI primer on a sample plate is indicated in Figure 1.

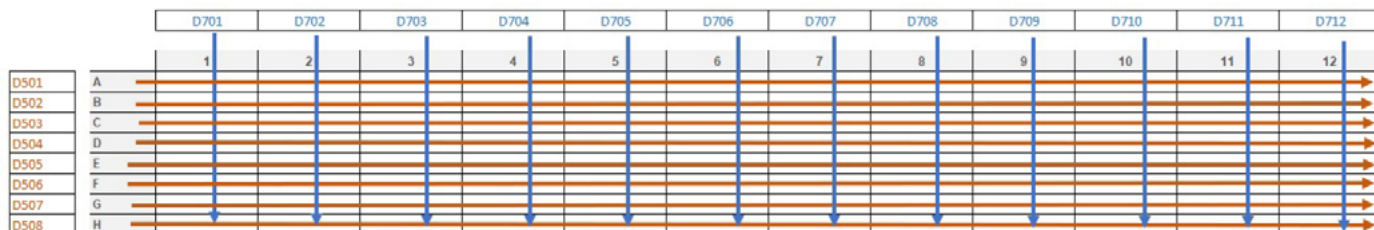


Figure 1. The recommended array pattern for CDI primers on a sample plate.

PLATE LAYOUTS

10005975–xGen™ 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

10005922–xGen™ UDI Primers Plate 1, 8 nt

	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

10008052–xGen™ UDI 10 nt Primer Plates 1–4 and 10008055 - xGen™ UDI 10 nt Primer Plates 1–4, 2 nmol

xGen UDI 10 nt Primers 1–96 (and 2 nmol)

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

xGen UDI 10 nt Primers 97–192 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	98	99	100	101	102	103	104	105	106	107	108
B	109	110	111	112	113	114	115	116	117	118	119	120
C	121	122	123	124	125	126	127	128	129	130	131	132
D	133	134	135	136	137	138	139	140	141	142	143	144
E	145	146	147	148	149	150	151	152	153	154	155	156
F	157	158	159	160	161	162	163	164	165	166	167	168
G	169	170	171	172	173	174	175	176	177	178	179	180
H	181	182	183	184	185	186	187	188	189	190	191	192

xGen UDI 10 nt Primers 193–288 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	194	195	196	197	198	199	200	201	202	203	204
B	205	206	207	208	209	210	211	212	213	214	215	216
C	217	218	219	220	221	222	223	224	225	226	227	228
D	229	230	231	232	233	234	235	236	237	238	239	240
E	241	242	243	244	245	246	247	248	249	250	251	252
F	253	254	255	256	257	258	259	260	261	262	263	264
G	265	266	267	268	269	270	271	272	273	274	275	276
H	277	278	279	280	281	282	283	284	285	286	287	288

xGen UDI 10 nt Primers 289–384 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	290	291	292	293	294	295	296	297	298	299	300
B	301	302	303	304	305	306	307	308	309	310	311	312
C	313	314	315	316	317	318	319	320	321	322	323	324
D	325	326	327	328	329	330	331	332	333	334	335	336
E	337	338	339	340	341	342	343	344	345	346	347	348
F	349	350	351	352	353	354	355	356	357	358	359	360
G	361	362	363	364	365	366	367	368	369	370	371	372
H	373	374	375	376	377	378	379	380	381	382	383	384

10008053–xGen™ UDI 10 nt Primer Plates 1–8 and **10008056**–xGen™ UDI 10 nt Primer Plates 1–8, 2 nmol
 [Includes plates 1-4, see above for layouts]

xGen UDI 10 nt Primers 385–480 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	385	386	387	388	389	390	391	392	393	394	395	396
B	397	398	399	400	401	402	403	404	405	406	407	408
C	409	410	411	412	413	414	415	416	417	418	419	420
D	421	422	423	424	425	426	427	428	429	430	431	432
E	433	434	435	436	437	438	439	440	441	442	443	444
F	445	446	447	448	449	450	451	452	453	454	455	456
G	457	458	459	460	461	462	463	464	465	466	467	468
H	469	470	471	472	473	474	475	476	477	478	479	480

xGen UDI 10 nt Primers 481–576 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	481	482	483	484	485	486	487	488	489	490	491	492
B	493	494	495	496	497	498	499	500	501	502	503	504
C	505	506	507	508	509	510	511	512	513	514	515	516
D	517	518	519	520	521	522	523	524	525	526	527	528
E	529	530	531	532	533	534	535	536	537	538	539	540
F	541	542	543	544	545	546	547	548	549	550	551	552
G	553	554	555	556	557	558	559	560	561	562	563	564
H	565	566	567	568	569	570	571	572	573	574	575	576

xGen UDI 10 nt Primers 577–672 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	577	578	579	580	581	582	583	584	585	586	587	588
B	589	590	591	592	593	594	595	596	597	598	599	600
C	601	602	603	604	605	606	607	608	609	610	611	612
D	613	614	615	616	617	618	619	620	621	622	623	624
E	625	626	627	628	629	630	631	632	633	634	635	636
F	637	638	639	640	641	642	643	644	645	646	647	648
G	649	650	651	652	653	654	655	656	657	658	659	660
H	661	662	663	664	665	666	667	668	669	670	671	672

xGen UDI 10 nt Primers 673–768 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	673	674	675	676	677	678	679	680	681	682	683	684
B	685	686	687	688	689	690	691	692	693	694	695	696
C	697	698	699	700	701	702	703	704	705	706	707	708
D	709	710	711	712	713	714	715	716	717	718	719	720
E	721	722	723	724	725	726	727	728	729	730	731	732
F	733	734	735	736	737	738	739	740	741	742	743	744
G	745	746	747	748	749	750	751	752	753	754	755	756
H	757	758	759	760	761	762	763	764	765	766	767	768

10008054–xGen™ UDI 10 nt Primer Plates 1-16 and **10008057**–xGen™ UDI 10 nt Primer Plates 1–16,
 2 nmol [Includes plates 1-8, see above for layouts]

xGen UDI 10 nt Primers 769–864 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	769	770	771	772	773	774	775	776	777	778	779	780
B	781	782	783	784	785	786	787	788	789	790	791	792
C	793	794	795	796	797	798	799	800	801	802	803	804
D	805	806	807	808	809	810	811	812	813	814	815	816
E	817	818	819	820	821	822	823	824	825	826	827	828
F	829	830	831	832	833	834	835	836	837	838	839	840
G	841	842	843	844	845	846	847	848	849	850	851	852
H	853	854	855	856	857	858	859	860	861	862	863	864

xGen UDI 10 nt Primers 865–960 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	865	866	867	868	869	870	871	872	873	874	875	876
B	877	878	879	880	881	882	883	884	885	886	887	888
C	889	890	891	892	893	894	895	896	897	898	899	900
D	901	902	903	904	905	906	907	908	909	910	911	912
E	913	914	915	916	917	918	919	920	921	922	923	924
F	925	926	927	928	929	930	931	932	933	934	935	936
G	937	938	939	940	941	942	943	944	945	946	947	948
H	949	950	951	952	953	954	955	956	957	958	959	960

xGen UDI 10 nt Primers 961–1056 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	961	962	963	964	965	966	967	968	969	970	971	972
B	973	974	975	976	977	978	979	980	981	982	983	984
C	985	986	987	988	989	990	991	992	993	994	995	996
D	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008
E	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020
F	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032
G	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044
H	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056

xGen UDI 10 nt Primers 1057–1152 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068
B	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080
C	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092
D	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104
E	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116
F	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128
G	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140
H	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152

xGen UDI 10 nt Primers 1153–1248 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164
B	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176
C	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188
D	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200
E	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212
F	1213	1214	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224
G	1225	1226	1227	1228	1229	1230	1231	1232	1233	1234	1235	1236
H	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248

xGen UDI 10 nt Primers 1249–1344 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260
B	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270	1271	1272
C	1273	1274	1275	1276	1277	1278	1279	1280	1281	1282	1283	1284
D	1285	1286	1287	1288	1289	1290	1291	1292	1293	1294	1295	1296
E	1297	1298	1299	1300	1301	1302	1303	1304	1305	1306	1307	1308
F	1309	1310	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320
G	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330	1331	1332
H	1333	1334	1335	1336	1337	1338	1339	1340	1341	1342	1343	1344

xGen UDI 10 nt Primers 1345–1440 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1345	1346	1347	1348	1349	1350	1351	1352	1353	1354	1355	1356
B	1357	1358	1359	1360	1361	1362	1363	1364	1365	1366	1367	1368
C	1369	1370	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380
D	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390	1391	1392
E	1393	1394	1395	1396	1397	1398	1399	1400	1401	1402	1403	1404
F	1405	1406	1407	1408	1409	1410	1411	1412	1413	1414	1415	1416
G	1417	1418	1419	1420	1421	1422	1423	1424	1425	1426	1427	1428
H	1429	1430	1431	1432	1433	1434	1435	1436	1437	1438	1439	1440

xGen UDI 10 nt Primers 1441–1536 (and 2 nmol)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1441	1442	1443	1444	1445	1446	1447	1448	1449	1450	1451	1452
B	1453	1454	1455	1456	1457	1458	1459	1460	1461	1462	1463	1464
C	1465	1466	1467	1468	1469	1470	1471	1472	1473	1474	1475	1476
D	1477	1478	1479	1480	1481	1482	1483	1484	1485	1486	1487	1488
E	1489	1490	1491	1492	1493	1494	1495	1496	1497	1498	1499	1500
F	1501	1502	1503	1504	1505	1506	1507	1508	1509	1510	1511	1512
G	1513	1514	1515	1516	1517	1518	1519	1520	1521	1522	1523	1524
H	1525	1526	1527	1528	1529	1530	1531	1532	1533	1534	1535	1536

Technical support: applicationsupport@idtdna.com

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