xGen™ Normalase™ Indexing Primers

Overview

Use the xGen™ Normalase™ Indexing Primers to perform indexing PCR on next generation sequencing (NGS) libraries that contain TruSeq™-compatible truncated adapters ligated to the library molecules. After Normalase indexing PCR and its subsequent cleanup, the libraries are ready for the Normalase workflow. The sample index length for both UDI and CDI Normalase primers is 8 nucleotides. Refer to the xGen Normalase Module protocol for additional instructions before using these primers.

Primer formats

xGen Normalase Indexing Primers are available as three different deliverables:

- Normalase CDI Primers. Tubes, to be arrayed into 8x12 plate by the end user—including a separate tube of Reagent R6.
  - xGen Normalase CDI Primers, 96 rxn
- Normalase "Plates" (1, 2, 3, or 4). Individual single-use (96-well) plates with a pierceable seal—including a separate tube of Reagent R7. Each well contains one specific index pair for indexing one sample.
  - xGen UDI Primers Plate 1
  - xGen UDI Primers Plate 2
  - xGen UDI Primers Plate 3
  - xGen UDI Primers Plate 4
- Normalase "Sets" 1–4. Distinct combinations of four single-use (96-well) plates with a pierceable seal. Each set contains 384 unique primer combinations. Purchasing all sets (1–4) provides 1536 unique primer pairs, with Reagent R7 tubes for each plate. Each well contains one specific index pair for indexing one sample.
  - Set 1: Plates 1, 2, 3, and 4
  - Set 2: Plates 5, 6, 7, and 8
  - Set 3: Plates 9, 10, 11, and 12
  - Set 4: Plates 13, 14, 15, and 16

Handling and storage

1. Store the xGen Normalase Indexing Primers at –20°C.
2. If any material remains unused, carefully reseal 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 Normalase 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: For indexing PCR preparation , pre-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 shown in Table 1.

Important: Add the primers directly to the sample by first adding reagent R7 (or R6) to the Master Mix, then dispensing 26 µL of Master Mix into each well.

Table 1. PCR Master Mix reaction recipe.

Indexing PCR component	Volume (µL)
NGS Library (after ligation and cleanup)	20
Normalase Primer Pairs • UDI Primers: use 4 µL from the desired well • CDI Primers: use 2 µL from each of the two index tubes	4
Reagent R7, if using UDI primers Reagent R6, if using CDI primers	1
2x PCR Master Mix	25
Total volume:	50

5. Adjust the PCR cycling conditions, as follows:
   a. Add at least one extra cycle when using Normalase primers as compared to conventional indexing primers.
   b. Add a 1-minute final extension step at the temperature specified by the polymerase used for library construction.

Sequencing and analysis

To fill out index sequences in the sample sheet, use the IDT Master Index List file located on the xGen™ NGS Adapters & Indexing Primers web page.

Prepare the plate

Use the recommended pattern for arraying each index on a sample plate, as indicated in Figure 1.

Use 2 µL of each index, when adding to the sample plate, to achieve a total of 4 µL of primer addition, as indicated in Table 1.

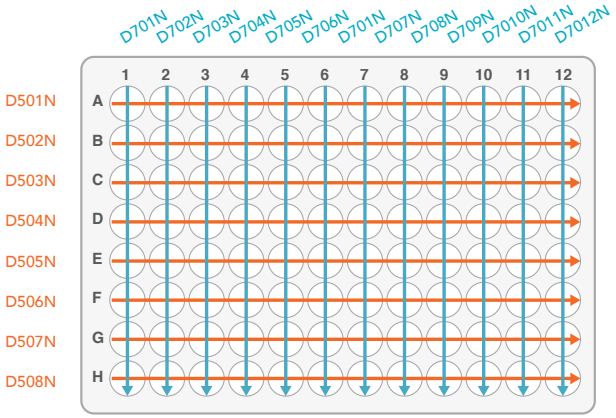

Figure 1. The recommended array pattern for CDI primers on a sample plate.

Plate layouts

10009795 - xGen™ Normalase™ UDI Primers Set 1 (Normalase UDI Index Plates 1–4: 10009796, 10009797, 10009798, 10009799)

10009796 - xGen™ Normalase™ UDI Primers Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU001	SU009	SU017	SU025	SU033	SU041	SU049	SU057	SU065	SU073	SU081	SU089
B	SU002	SU010	SU018	SU026	SU034	SU042	SU050	SU058	SU066	SU074	SU082	SU090
C	SU003	SU011	SU019	SU027	SU035	SU043	SU051	SU059	SU067	SU075	SU083	SU091
D	SU004	SU012	SU020	SU028	SU036	SU044	SU052	SU060	SU068	SU076	SU084	SU092
E	SU005	SU013	SU021	SU029	SU037	SU045	SU053	SU061	SU069	SU077	SU085	SU093
F	SU006	SU014	SU022	SU030	SU038	SU046	SU054	SU062	SU070	SU078	SU086	SU094
G	SU007	SU015	SU023	SU031	SU039	SU047	SU055	SU063	SU071	SU079	SU087	SU095
H	SU008	SU016	SU024	SU032	SU040	SU048	SU056	SU064	SU072	SU080	SU088	SU096

10009797 - xGen™ Normalase™ UDI Primers Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU097	SU105	SU113	SU121	SU129	SU137	SU145	SU153	SU161	SU169	SU177	SU185
B	SU098	SU106	SU114	SU122	SU130	SU138	SU146	SU154	SU162	SU170	SU178	SU186
C	SU099	SU107	SU115	SU123	SU131	SU139	SU147	SU155	SU163	SU171	SU179	SU187
D	SU100	SU108	SU116	SU124	SU132	SU140	SU148	SU156	SU164	SU172	SU180	SU188
E	SU101	SU109	SU117	SU125	SU133	SU141	SU149	SU157	SU165	SU173	SU181	SU189
F	SU102	SU110	SU118	SU126	SU134	SU142	SU150	SU158	SU166	SU174	SU182	SU190
G	SU103	SU111	SU119	SU127	SU135	SU143	SU151	SU159	SU167	SU175	SU183	SU191
H	SU104	SU112	SU120	SU128	SU136	SU144	SU152	SU160	SU168	SU176	SU184	SU192

10009798 - xGen™ Normalase™ UDI Primers Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU193	SU201	SU209	SU217	SU225	SU233	SU241	SU249	SU257	SU265	SU273	SU281
B	SU194	SU202	SU210	SU218	SU226	SU234	SU242	SU250	SU258	SU266	SU274	SU282
C	SU195	SU203	SU211	SU219	SU227	SU235	SU243	SU251	SU259	SU267	SU275	SU283
D	SU196	SU204	SU212	SU220	SU228	SU236	SU244	SU252	SU260	SU268	SU276	SU284
E	SU197	SU205	SU213	SU221	SU229	SU237	SU245	SU253	SU261	SU269	SU277	SU285
F	SU198	SU206	SU214	SU222	SU230	SU238	SU246	SU254	SU262	SU270	SU278	SU286
G	SU199	SU207	SU215	SU223	SU231	SU239	SU247	SU255	SU263	SU271	SU279	SU287
H	SU200	SU208	SU216	SU224	SU232	SU240	SU248	SU256	SU264	SU272	SU280	SU288

10009799 - xGen™ Normalase™ UDI Primers Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU289	SU297	SU305	SU313	SU321	SU329	SU337	SU345	SU353	SU361	SU369	SU377
B	SU290	SU298	SU306	SU314	SU322	SU330	SU338	SU346	SU354	SU362	SU370	SU378
C	SU291	SU299	SU307	SU315	SU323	SU331	SU339	SU347	SU355	SU363	SU371	SU379
D	SU292	SU300	SU308	SU316	SU324	SU332	SU340	SU348	SU356	SU364	SU372	SU380
E	SU293	SU301	SU309	SU317	SU325	SU333	SU341	SU349	SU357	SU365	SU373	SU381
F	SU294	SU302	SU310	SU318	SU326	SU334	SU342	SU350	SU358	SU366	SU374	SU382
G	SU295	SU303	SU311	SU319	SU327	SU335	SU343	SU351	SU359	SU367	SU375	SU383
H	SU296	SU304	SU312	SU320	SU328	SU336	SU344	SU352	SU360	SU368	SU376	SU384

10009800 - xGen™ Normalase™ UDI Primers Set 2 (Normalase UDI Index Plates 5–8)

X91096-5–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU385	SU393	SU401	SU409	SU417	SU425	SU433	SU441	SU449	SU457	SU465	SU473
B	SU386	SU394	SU402	SU410	SU418	SU426	SU434	SU442	SU450	SU458	SU466	SU474
C	SU387	SU395	SU403	SU411	SU419	SU427	SU435	SU443	SU451	SU459	SU467	SU475
D	SU388	SU396	SU404	SU412	SU420	SU428	SU436	SU444	SU452	SU460	SU468	SU476
E	SU389	SU397	SU405	SU413	SU421	SU429	SU437	SU445	SU453	SU461	SU469	SU477
F	SU390	SU398	SU406	SU414	SU422	SU430	SU438	SU446	SU454	SU462	SU470	SU478
G	SU391	SU399	SU407	SU415	SU423	SU431	SU439	SU447	SU455	SU463	SU471	SU479
H	SU392	SU400	SU408	SU416	SU424	SU432	SU440	SU448	SU456	SU464	SU472	SU480

X91096-6–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU481	SU489	SU497	SU505	SU513	SU521	SU529	SU537	SU545	SU553	SU561	SU569
B	SU482	SU490	SU498	SU506	SU514	SU522	SU530	SU538	SU546	SU554	SU562	SU570
C	SU483	SU491	SU499	SU507	SU515	SU523	SU531	SU539	SU547	SU555	SU563	SU571
D	SU484	SU492	SU500	SU508	SU516	SU524	SU532	SU540	SU548	SU556	SU564	SU572
E	SU485	SU493	SU501	SU509	SU517	SU525	SU533	SU541	SU549	SU557	SU565	SU573
F	SU486	SU494	SU502	SU510	SU518	SU526	SU534	SU542	SU550	SU558	SU566	SU574
G	SU487	SU495	SU503	SU511	SU519	SU527	SU535	SU543	SU551	SU559	SU567	SU575
H	SU488	SU496	SU504	SU512	SU520	SU528	SU536	SU544	SU552	SU560	SU568	SU576

X91096-7–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU577	SU585	SU593	SU601	SU609	SU617	SU625	SU633	SU641	SU649	SU657	SU665
B	SU578	SU586	SU594	SU602	SU610	SU618	SU626	SU634	SU642	SU650	SU658	SU666
C	SU579	SU587	SU595	SU603	SU611	SU619	SU627	SU635	SU643	SU651	SU659	SU667
D	SU580	SU588	SU596	SU604	SU612	SU620	SU628	SU636	SU644	SU652	SU660	SU668
E	SU581	SU589	SU597	SU605	SU613	SU621	SU629	SU637	SU645	SU653	SU661	SU669
F	SU582	SU590	SU598	SU606	SU614	SU622	SU630	SU638	SU646	SU654	SU662	SU670
G	SU583	SU591	SU599	SU607	SU615	SU623	SU631	SU639	SU647	SU655	SU663	SU671
H	SU584	SU592	SU600	SU608	SU616	SU624	SU632	SU640	SU648	SU656	SU664	SU672

X91096-8–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU673	SU681	SU689	SU697	SU705	SU713	SU721	SU729	SU737	SU745	SU753	SU761
B	SU674	SU682	SU690	SU698	SU706	SU714	SU722	SU730	SU738	SU746	SU754	SU762
C	SU675	SU683	SU691	SU699	SU707	SU715	SU723	SU731	SU739	SU747	SU755	SU763
D	SU676	SU684	SU692	SU700	SU708	SU716	SU724	SU732	SU740	SU748	SU756	SU764
E	SU677	SU685	SU693	SU701	SU709	SU717	SU725	SU733	SU741	SU749	SU757	SU765
F	SU678	SU686	SU694	SU702	SU710	SU718	SU726	SU734	SU742	SU750	SU758	SU766
G	SU679	SU687	SU695	SU703	SU711	SU719	SU727	SU735	SU743	SU751	SU759	SU767
H	SU680	SU688	SU696	SU704	SU712	SU720	SU728	SU736	SU744	SU752	SU760	SU768

10009811 - xGen™ Normalase™ UDI Primers Set 3 (Normalase UDI Index Plates 9–12)

X91096-9–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU769	SU777	SU785	SU793	SU801	SU809	SU817	SU825	SU833	SU841	SU849	SU857
B	SU770	SU778	SU786	SU794	SU802	SU810	SU818	SU826	SU834	SU842	SU850	SU858
C	SU771	SU779	SU787	SU795	SU803	SU811	SU819	SU827	SU835	SU843	SU851	SU859
D	SU772	SU780	SU788	SU796	SU804	SU812	SU820	SU828	SU836	SU844	SU852	SU860
E	SU773	SU781	SU789	SU797	SU805	SU813	SU821	SU829	SU837	SU845	SU853	SU861
F	SU774	SU782	SU790	SU798	SU806	SU814	SU822	SU830	SU838	SU846	SU854	SU862
G	SU775	SU783	SU791	SU799	SU807	SU815	SU823	SU831	SU839	SU847	SU855	SU863
H	SU776	SU784	SU792	SU800	SU808	SU816	SU824	SU832	SU840	SU848	SU856	SU864

X91096-10–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU865	SU873	SU881	SU889	SU897	SU905	SU913	SU921	SU929	SU937	SU945	SU953
B	SU866	SU874	SU882	SU890	SU898	SU906	SU914	SU922	SU930	SU938	SU946	SU954
C	SU867	SU875	SU883	SU891	SU899	SU907	SU915	SU923	SU931	SU939	SU947	SU955
D	SU868	SU876	SU884	SU892	SU900	SU908	SU916	SU924	SU932	SU940	SU948	SU956
E	SU869	SU877	SU885	SU893	SU901	SU909	SU917	SU925	SU933	SU941	SU949	SU957
F	SU870	SU878	SU886	SU894	SU902	SU910	SU918	SU926	SU934	SU942	SU950	SU958
G	SU871	SU879	SU887	SU895	SU903	SU911	SU919	SU927	SU935	SU943	SU951	SU959
H	SU872	SU880	SU888	SU896	SU904	SU912	SU920	SU928	SU936	SU944	SU952	SU960

X91096-11–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU961	SU969	SU977	SU985	SU993	SU1001	SU1009	SU1017	SU1025	SU1033	SU1041	SU1049
B	SU962	SU970	SU978	SU986	SU994	SU1002	SU1010	SU1018	SU1026	SU1034	SU1042	SU1050
C	SU963	SU971	SU979	SU987	SU995	SU1003	SU1011	SU1019	SU1027	SU1035	SU1043	SU1051
D	SU964	SU972	SU980	SU988	SU996	SU1004	SU1012	SU1020	SU1028	SU1036	SU1044	SU1052
E	SU965	SU973	SU981	SU989	SU997	SU1005	SU1013	SU1021	SU1029	SU1037	SU1045	SU1053
F	SU966	SU974	SU982	SU990	SU998	SU1006	SU1014	SU1022	SU1030	SU1038	SU1046	SU1054
G	SU967	SU975	SU983	SU991	SU999	SU1007	SU1015	SU1023	SU1031	SU1039	SU1047	SU1055
H	SU968	SU976	SU984	SU992	SU1000	SU1008	SU1016	SU1024	SU1032	SU1040	SU1048	SU1056

X91096-12–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1057	SU1065	SU1073	SU1081	SU1089	SU1097	SU1105	SU1113	SU1121	SU1129	SU1137	SU1145
B	SU1058	SU1066	SU1074	SU1082	SU1090	SU1098	SU1106	SU1114	SU1122	SU1130	SU1138	SU1146
C	SU1059	SU1067	SU1075	SU1083	SU1091	SU1099	SU1107	SU1115	SU1123	SU1131	SU1139	SU1147
D	SU1060	SU1068	SU1076	SU1084	SU1092	SU1100	SU1108	SU1116	SU1124	SU1132	SU1140	SU1148
E	SU1061	SU1069	SU1077	SU1085	SU1093	SU1101	SU1109	SU1117	SU1125	SU1133	SU1141	SU1149
F	SU1062	SU1070	SU1078	SU1086	SU1094	SU1102	SU1110	SU1118	SU1126	SU1134	SU1142	SU1150
G	SU1063	SU1071	SU1079	SU1087	SU1095	SU1103	SU1111	SU1119	SU1127	SU1135	SU1143	SU1151
H	SU1064	SU1072	SU1080	SU1088	SU1096	SU1104	SU1112	SU1120	SU1128	SU1136	SU1144	SU1152

10009812 - xGen™ Normalase™ UDI Primers Set 4 (Normalase UDI Index Plates 13–16)

X91096-13–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1153	SU1161	SU1169	SU1177	SU1185	SU1193	SU1201	SU1209	SU1217	SU1225	SU1233	SU1241
B	SU1154	SU1162	SU1170	SU1178	SU1186	SU1194	SU1202	SU1210	SU1218	SU1226	SU1234	SU1242
C	SU1155	SU1163	SU1171	SU1179	SU1187	SU1195	SU1203	SU1211	SU1219	SU1227	SU1235	SU1243
D	SU1156	SU1164	SU1172	SU1180	SU1188	SU1196	SU1204	SU1212	SU1220	SU1228	SU1236	SU1244
E	SU1157	SU1165	SU1173	SU1181	SU1189	SU1197	SU1205	SU1213	SU1221	SU1229	SU1237	SU1245
F	SU1158	SU1166	SU1174	SU1182	SU1190	SU1198	SU1206	SU1214	SU1222	SU1230	SU1238	SU1246
G	SU1159	SU1167	SU1175	SU1183	SU1191	SU1199	SU1207	SU1215	SU1223	SU1231	SU1239	SU1247
H	SU1160	SU1168	SU1176	SU1184	SU1192	SU1200	SU1208	SU1216	SU1224	SU1232	SU1240	SU1248

X91096-14–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1249	SU1257	SU1265	SU1273	SU1281	SU1289	SU1297	SU1305	SU1313	SU1321	SU1329	SU1337
B	SU1250	SU1258	SU1266	SU1274	SU1282	SU1290	SU1298	SU1306	SU1314	SU1322	SU1330	SU1338
C	SU1251	SU1259	SU1267	SU1275	SU1283	SU1291	SU1299	SU1307	SU1315	SU1323	SU1331	SU1339
D	SU1252	SU1260	SU1268	SU1276	SU1284	SU1292	SU1300	SU1308	SU1316	SU1324	SU1332	SU1340
E	SU1253	SU1261	SU1269	SU1277	SU1285	SU1293	SU1301	SU1309	SU1317	SU1325	SU1333	SU1341
F	SU1254	SU1262	SU1270	SU1278	SU1286	SU1294	SU1302	SU1310	SU1318	SU1326	SU1334	SU1342
G	SU1255	SU1263	SU1271	SU1279	SU1287	SU1295	SU1303	SU1311	SU1319	SU1327	SU1335	SU1343
H	SU1256	SU1264	SU1272	SU1280	SU1288	SU1296	SU1304	SU1312	SU1320	SU1328	SU1336	SU1344

X91096-15–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1345	SU1353	SU1361	SU1369	SU1377	SU1385	SU1393	SU1401	SU1409	SU1417	SU1425	SU1433
B	SU1346	SU1354	SU1362	SU1370	SU1378	SU1386	SU1394	SU1402	SU1410	SU1418	SU1426	SU1434
C	SU1347	SU1355	SU1363	SU1371	SU1379	SU1387	SU1395	SU1403	SU1411	SU1419	SU1427	SU1435
D	SU1348	SU1356	SU1364	SU1372	SU1380	SU1388	SU1396	SU1404	SU1412	SU1420	SU1428	SU1436
E	SU1349	SU1357	SU1365	SU1373	SU1381	SU1389	SU1397	SU1405	SU1413	SU1421	SU1429	SU1437
F	SU1350	SU1358	SU1366	SU1374	SU1382	SU1390	SU1398	SU1406	SU1414	SU1422	SU1430	SU1438
G	SU1351	SU1359	SU1367	SU1375	SU1383	SU1391	SU1399	SU1407	SU1415	SU1423	SU1431	SU1439
H	SU1352	SU1360	SU1368	SU1376	SU1384	SU1392	SU1400	SU1408	SU1416	SU1424	SU1432	SU1440

X91096-16–PLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1441	SU1449	SU1457	SU1465	SU1473	SU1481	SU1489	SU1497	SU1505	SU1513	SU1521	SU1529
B	SU1442	SU1450	SU1458	SU1466	SU1474	SU1482	SU1490	SU1498	SU1506	SU1514	SU1522	SU1530
C	SU1443	SU1451	SU1459	SU1467	SU1475	SU1483	SU1491	SU1499	SU1507	SU1515	SU1523	SU1531
D	SU1444	SU1452	SU1460	SU1468	SU1476	SU1484	SU1492	SU1500	SU1508	SU1516	SU1524	SU1532
E	SU1445	SU1453	SU1461	SU1469	SU1477	SU1485	SU1493	SU1501	SU1509	SU1517	SU1525	SU1533
F	SU1446	SU1454	SU1462	SU1470	SU1478	SU1486	SU1494	SU1502	SU1510	SU1518	SU1526	SU1534
G	SU1447	SU1455	SU1463	SU1471	SU1479	SU1487	SU1495	SU1503	SU1511	SU1519	SU1527	SU1535
H	SU1448	SU1456	SU1464	SU1472	SU1480	SU1488	SU1496	SU1504	SU1512	SU1520	SU1528	SU1536

Technical support: applicationsupport@idtdna.com

> For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

> SEE WHAT MORE WE CAN DO FOR **YOU** AT **WWW.IDTDNA.COM**.

Doc ID: RUO22-0724_001 04/22

INTEGRATED DNA TECHNOLOGIES