

Quick-16S™ Plus NGS Library Prep Kit (V3-V4, UDI)



Catalog Nos. D6421-PS1, D6421-PS2, D6421-PS3, D6421-PS4, D6421-PS5, D6421-PS6, D6421-PS7, & D6421-PS8

Guide for Preparing More than 96 Samples

Notice

One Quick-16S™ Plus NGS Library Prep Kit (V3-V4, UDI) (96 rxns) can support the preparation of up to 96 samples for sequencing on the Illumina NextSeq® 1000/2000 or Illumina MiSeq® platform. To prepare more than 96 samples (up to 768 samples), multiple kits with different, unique Index Primer Sets must be ordered as described in the section below.

Ordering Guidelines

The table below provides ordering information for customers who would like to prepare more than 96 samples with the Quick-16S™ Plus NGS Library Prep Kit (V3-V4).

Anticipated Number of DNA Samples to Prepare	Recommended Cat #s to Order	Included Primer Sets
1-96	D6421-PS1	Primer Set 1
97-192	D6421-PS1 & D6421-PS2	Primer Sets 1 & 2
193-288	D6421-PS1, D6421-PS2, & D6421-PS3	Primer Sets 1, 2, & 3
289-384	D6421-PS1, D6421-PS2, D6421-PS3, & D6421-PS4	Primer Sets 1, 2, 3, & 4
385-480	D6421-PS1, D6421-PS2, D6421-PS3, D6421-PS4, & D6421-PS5	Primer Sets 1, 2, 3, 4, & 5
481-576	D6421-PS1, D6421-PS2, D6421-PS3, D6421-PS4, D6421-PS5, & D6421-PS6	Primer Sets 1, 2, 3, 4, 5, & 6
577-672	D6421-PS1, D6421-PS2, D6421-PS3, D6421-PS4, D6421-PS5, D6421-PS6, & D6421-PS7	Primer Sets 1, 2, 3, 4, 5, 6, & 7
673-768	D6421-PS1, D6421-PS2, D6421-PS3, D6421-PS4, D6421-PS5, D6421-PS6, D6421-PS7, & D6421-PS8	Primer Sets 1, 2, 3, 4, 5, 6, 7, & 8

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Protocol

For each group of up to 96 samples, use a single Quick-16S™ Plus NGS Library Prep Kit (V3-V4, UDI) with the appropriate group of Index Primers as described in the protocol below. Samples 1-96 are assigned to Plate 1, samples 97-192 to Plate 2, samples 193-288 to Plate 3, samples 289-384 to Plate 4, samples 385-480 to Plate 5, samples 481-576 to Plate 6, samples 577-672 to Plate 7, samples 673-768 to Plate 8.

- Follow the protocol in **Section 1: 1-Step PCR** as written in the Quick-16S™ Plus NGS Library Prep Kit (V3-V4, UDI) Instruction Manual (found on the D6421 Product Page at www.zymoresearch.com). Use the diagrams below to add the appropriate Index Primers to each sample, based on the plate number/sample group:

Plate 1, Samples 1-96

V3-V4 Primer Set 1: Index Primers V3V4_ZB0001-V3V4_ZB0096

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89*
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90*
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91*
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92*
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93*
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94*
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	POS**
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	NEG***

* S89-S94 should be reserved for qPCR standards if absolute quantification is desired.

** POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

*** NEG: A no template control as a negative control.

Plate 2, Samples 97-192

V3-V4 Primer Set 2: Index Primers V3V4_ZB0097-V3V4_ZB0192

	1	2	3	4	5	6	7	8	9	10	11	12
A	S97	S105	S113	S121	S129	S137	S145	S153	S161	S169	S177	S185
B	S98	S106	S114	S122	S130	S138	S146	S154	S162	S170	S178	S186
C	S99	S107	S115	S123	S131	S139	S147	S155	S163	S171	S179	S187
D	S100	S108	S116	S124	S132	S140	S148	S156	S164	S172	S180	S188
E	S101	S109	S117	S125	S133	S141	S149	S157	S165	S173	S181	S189
F	S102	S110	S118	S126	S134	S142	S150	S158	S166	S174	S182	S190
G	S103	S111	S119	S127	S135	S143	S151	S159	S167	S175	S183	POS*
H	S104	S112	S120	S128	S136	S144	S152	S160	S168	S176	S184	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

** NEG: A no template control as a negative control.

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Plate 3, Samples 193-288

V3-V4 Primer Set 3: Index Primers V3V4_ZB0193-V3V4_ZB0288

	1	2	3	4	5	6	7	8	9	10	11	12
A	S193	S201	S209	S217	S225	S233	S241	S249	S257	S265	S273	S281
B	S194	S202	S210	S218	S226	S234	S242	S250	S258	S266	S274	S282
C	S195	S203	S211	S219	S227	S235	S243	S251	S259	S267	S275	S283
D	S196	S204	S212	S220	S228	S236	S244	S252	S260	S268	S276	S284
E	S197	S205	S213	S221	S229	S237	S245	S253	S261	S269	S277	S285
F	S198	S206	S214	S222	S230	S238	S246	S254	S262	S270	S278	S286
G	S199	S207	S215	S223	S231	S239	S247	S255	S263	S271	S279	POS*
H	S200	S208	S216	S224	S232	S240	S248	S256	S264	S272	S280	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

** NEG: A no template control as a negative control.

Plate 4, Samples 289-384

V3-V4 Primer Set 4: Index Primers V3V4_ZB0289-V3V4_ZB0384

	1	2	3	4	5	6	7	8	9	10	11	12
A	S289	S297	S305	S313	S321	S329	S337	S345	S353	S361	S369	S377
B	S290	S298	S306	S314	S322	S330	S338	S346	S354	S362	S370	S378
C	S291	S299	S307	S315	S323	S331	S339	S347	S355	S363	S371	S379
D	S292	S300	S308	S316	S324	S332	S340	S348	S356	S364	S372	S380
E	S293	S301	S309	S317	S325	S333	S341	S349	S357	S365	S373	S381
F	S294	S302	S310	S318	S326	S334	S342	S350	S358	S366	S374	S382
G	S295	S303	S311	S319	S327	S335	S343	S351	S359	S367	S375	POS*
H	S296	S304	S312	S320	S328	S336	S344	S352	S360	S368	S376	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

** NEG: A no template control as a negative control.

Plate 5, Samples 385-480

V3-V4 Primer Set 5: Index Primers V3V4_ZB0385-V3V4_ZB0480

	1	2	3	4	5	6	7	8	9	10	11	12
A	S385	S393	S401	S409	S417	S425	S433	S441	S449	S457	S465	S473
B	S386	S394	S402	S410	S418	S426	S434	S442	S450	S458	S466	S474
C	S387	S395	S403	S411	S419	S427	S435	S443	S451	S459	S467	S475
D	S388	S396	S404	S412	S420	S428	S436	S444	S452	S460	S468	S476
E	S389	S397	S405	S413	S421	S429	S437	S445	S453	S461	S469	S477
F	S390	S398	S406	S414	S422	S430	S438	S446	S454	S462	S470	S478
G	S391	S399	S407	S415	S423	S431	S439	S447	S455	S463	S471	POS*
H	S392	S400	S408	S416	S424	S432	S440	S448	S456	S464	S472	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

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Plate 6, Samples 481-576

V3-V4 Primer Set 6: Index Primers V3V4_ZB0481-V3V4_ZB0576

	1	2	3	4	5	6	7	8	9	10	11	12
A	S481	S489	S497	S505	S513	S521	S529	S537	S545	S553	S561	S569
B	S482	S490	S498	S506	S514	S522	S530	S538	S546	S554	S562	S570
C	S483	S491	S499	S507	S515	S523	S531	S539	S547	S555	S563	S571
D	S484	S492	S500	S508	S516	S524	S532	S540	S548	S556	S564	S572
E	S485	S493	S501	S509	S517	S525	S533	S541	S549	S557	S565	S573
F	S486	S494	S502	S510	S518	S526	S534	S542	S550	S558	S566	S574
G	S487	S495	S503	S511	S519	S527	S535	S543	S551	S559	S567	POS*
H	S488	S496	S504	S512	S520	S528	S536	S544	S552	S560	S568	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

** NEG: A no template control as a negative control.

Plate 7, Samples 577-672

V3-V4 Primer Set 7: Index Primers V3V4_ZB0577-V3V4_ZB0672

	1	2	3	4	5	6	7	8	9	10	11	12
A	S577	S585	S593	S601	S609	S617	S625	S633	S641	S649	S657	S665
B	S578	S586	S594	S602	S610	S618	S626	S634	S642	S650	S658	S666
C	S579	S587	S595	S603	S611	S619	S627	S635	S643	S651	S659	S667
D	S580	S588	S596	S604	S612	S620	S628	S636	S644	S652	S660	S668
E	S581	S589	S597	S605	S613	S621	S629	S637	S645	S653	S661	S669
F	S582	S590	S598	S606	S614	S622	S630	S638	S646	S654	S662	S670
G	S583	S591	S599	S607	S615	S623	S631	S639	S647	S655	S663	POS*
H	S584	S592	S600	S608	S616	S624	S632	S640	S648	S656	S664	NEG**

* POS: The ZymoBIOMICS™ Microbial Community DNA Standard (included in the kit) as a positive control.

** NEG: A no template control as a negative control.

Plate 8, Samples 673-768

V3-V4 Primer Set 8: Index Primers V3V4_ZB0673-V3V4_ZB0768

	1	2	3	4	5	6	7	8	9	10	11	12
A	S673	S681	S689	S697	S705	S713	S721	S729	S737	S745	S753	S761
B	S674	S682	S690	S698	S706	S714	S722	S730	S738	S746	S754	S762
C	S675	S683	S691	S699	S707	S715	S723	S731	S739	S747	S755	S763
D	S676	S684	S692	S700	S708	S716	S724	S732	S740	S748	S756	S764
E	S677	S685	S693	S701	S709	S717	S725	S733	S741	S749	S757	S765
F	S678	S686	S694	S702	S710	S718	S726	S734	S742	S750	S758	S766
G	S679	S687	S695	S703	S711	S719	S727	S735	S743	S751	S759	POS*
H	S680	S688	S696	S704	S712	S720	S728	S736	S744	S752	S760	NEG**

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2. **Pooling by Equal Volume.** Pool equal volumes (**2 µl**) of PCR products from each well of all plates from Section 1 into a new microcentrifuge tube. Skip any wells that were used for qPCR standards. Add ~10 µl of **PCR Inactivation Solution** per 100 µl of library pool and mix well.
3. Follow the protocol in **[Section 3: Final Library Clean-up](#)** as written in the Instruction Manual.
4. Quantify the cleaned library with a fluorometric quantification method that uses dsDNA binding dyes, such as Qubit® as written in **[Section 4: Library Quantification](#)**.
5. The ultra-pure pooled library DNA is now ready for use or storage at ≤-20°C. Refer to platform-specific guidelines for preparation for sequencing. No custom sequencing primers are needed.
 - a. The NextSeq 1000/2000® Reagent Kit P1/P2 (600-cycle) with 40% PhiX spike-in is recommended.
 - b. The MiSeq® Reagent Kit v3 (600-cycle) with 15% PhiX spike-in is recommended. See Appendix F in the Instruction Manual for assistance with sample sheet setup. Remember to set the index size to 10 bp.

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