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

Catalog Nos. D6420, D6420-PSB, D6420-PSC, D6420-PSD

Guide for Preparing More than 96 Samples

Notice

One *Quick*-16S™ Plus NGS Library Prep Kit (V3-V4) (96 rxns) can support the preparation of up to 96 samples for sequencing on the Illumina MiSeq® platform. The kit comes with Index Primer Plate A, which contains Index Primers ZT501-ZT508 and Index Primers ZT701-ZT712. To prepare more than 96 samples (up to 384 samples), multiple kits with different, unique Index Primer Sets must be ordered as described in the section below.

The *Quick*-16S™ Plus NGS Library Prep Kit (V3-V4) (24 rxns) cannot be used alongside the *Quick*-16S™ Plus NGS Library Prep Kit (V3-V4) (96 rxns), as the Index Primers overlap.

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	D6420	Index Primer Set A
97-192	D6420 & D6420-PSB	Index Primer Sets A & B
193-288	D6420, D6420-PSB, & D6420-PSC	Index Primer Sets A, B, & C
289-384	D6420, D6420-PSB, D6420-PSC, & D6420-PSD	Index Primer Sets A, B, C, & D

Protocol

For each group of up to 96 samples, use a single *Quick*-16S™ Plus NGS Library Prep Kit (V3-V4) 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, and samples 289-384 to Plate 4.

1. Follow the protocol in **Section 1: 1-Step PCR** as written in the *Quick*-16S™ Plus NGS Library Prep Kit (V3-V4) Instruction Manual (found on the D6420 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:

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Plate 1, Samples 1-96

V3-V4 Index Primer Set A: Index Primers ZT501-ZT508 and Index Primers ZT701-ZT712

Index Primers V3F ZT5xx		Index Primers V4R ZT7xx											
		ZT701	ZT702	ZT703	ZT704	ZT705	ZT706	ZT707	ZT708	ZT709	ZT710	ZT711	ZT712
		1	2	3	4	5	6	7	8	9	10	11	12
ZT501	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89*
ZT502	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90*
ZT503	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91*
ZT504	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92*
ZT505	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93*
ZT506	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94*
ZT507	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	**POS****
ZT508	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 Index Primer Set B: Index Primers ZT501-ZT508 and Index Primers ZT713-ZT724

Index Primers V3F ZT5xx		Index Primers V4R ZT7xx											
		ZT713	ZT714	ZT715	ZT716	ZT717	ZT718	ZT719	ZT720	ZT721	ZT722	ZT723	ZT724
		1	2	3	4	5	6	7	8	9	10	11	12
ZT501	A	S97	S105	S113	S121	S129	S137	S145	S153	S161	S169	S177	S185
ZT502	B	S98	S106	S114	S122	S130	S138	S146	S154	S162	S170	S178	S186
ZT503	C	S99	S107	S115	S123	S131	S139	S147	S155	S163	S171	S179	S187
ZT504	D	S100	S108	S116	S124	S132	S140	S148	S156	S164	S172	S180	S188
ZT505	E	S101	S109	S117	S125	S133	S141	S149	S157	S165	S173	S181	S189
ZT506	F	S102	S110	S118	S126	S134	S142	S150	S158	S166	S174	S182	S190
ZT507	G	S103	S111	S119	S127	S135	S143	S151	S159	S167	S175	S183	**POS***
ZT508	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 Index Primer Set C: Index Primers ZT509-ZT516 and Index Primers ZT701-ZT712

Index Primers V3F ZT5xx		Index Primers V4R ZT7xx											
		ZT701	ZT702	ZT703	ZT704	ZT705	ZT706	ZT707	ZT708	ZT709	ZT710	ZT711	ZT712
		1	2	3	4	5	6	7	8	9	10	11	12
ZT509	A	S193	S201	S209	S217	S225	S233	S241	S249	S257	S265	S273	S281
ZT510	B	S194	S202	S210	S218	S226	S234	S242	S250	S258	S266	S274	S282
ZT511	C	S195	S203	S211	S219	S227	S235	S243	S251	S259	S267	S275	S283
ZT512	D	S196	S204	S212	S220	S228	S236	S244	S252	S260	S268	S276	S284
ZT513	E	S197	S205	S213	S221	S229	S237	S245	S253	S261	S269	S277	S285
ZT514	F	S198	S206	S214	S222	S230	S238	S246	S254	S262	S270	S278	S286
ZT515	G	S199	S207	S215	S223	S231	S239	S247	S255	S263	S271	S279	**POS***
ZT516	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 189-384

V3-V4 Index Primer Set D: Index Primers ZT509-ZT516 and Index Primers ZT713-ZT724

Index Primers V3F ZT5xx		Index Primers V4R ZT7xx											
		ZT713	ZT714	ZT715	ZT716	ZT717	ZT718	ZT719	ZT720	ZT721	ZT722	ZT723	ZT724
		1	2	3	4	5	6	7	8	9	10	11	12
ZT509	A	S289	S297	S305	S313	S321	S329	S337	S345	S353	S361	S369	S377
ZT510	B	S290	S298	S306	S314	S322	S330	S338	S346	S354	S362	S370	S378
ZT511	C	S291	S299	S307	S315	S323	S331	S339	S347	S355	S363	S371	S379
ZT512	D	S292	S300	S308	S316	S324	S332	S340	S348	S356	S364	S372	S380
ZT513	E	S293	S301	S309	S317	S325	S333	S341	S349	S357	S365	S373	S381
ZT514	F	S294	S302	S310	S318	S326	S334	S342	S350	S358	S366	S374	S382
ZT515	G	S295	S303	S311	S319	S327	S335	S343	S351	S359	S367	S375	**POS***
ZT516	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.

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2. Pooling by Equal Volume. Add 50 µl of **PCR Inactivation Solution** into a new microcentrifuge tube. Pool equal volumes (**2 µl**) of PCR products from each well of all plates from Section 1 into the tube and mix well. Skip any wells that were used for qPCR standards.

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 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.