Quick-16S™ NGS Library Prep Kit

Catalog No. D6400

Guide for Preparing More than 96 Samples

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Notice

One *Quick*-16S™ NGS Library Prep Kit (96 rxns) can support the preparation of up to 96 samples for sequencing on the Illumina MiSeq® platform. The kit comes with Index Primer Set A, which contains Index Primers ZA501-ZA508 and Index Primers ZA701-ZA712. 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. There is no additional charge for the additional needed Index Primer Sets.

The *Quick*-16S™ NGS Library Prep Kit (24 rxns) cannot be used alongside the *Quick*-16S™ NGS Library Prep Kit (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™ NGS Library Prep Kit. Please contact Zymo Research at oemorders@zymoresearch.com to place a custom order for multiple *Quick*-16S™ NGS Library Prep Kits.

Anticipated Number of DNA Samples to Prepare	**Number of *Quick*-16S™ NGS Library Prep Kits to Order**	**Additional Index Primer Sets Needed** *(Please Specify in Order Notes)*
1-96	1	None
97-192	2	Index Primer Set B
193-288	3	Index Primer Set B **AND** Index Primer Set C
289-384	4	Index Primer Set B **AND** Index Primer Set C **AND** Index Primer Set D

Protocol

For each group of up to 96 samples, use a single *Quick*-16S™ NGS Library Prep Kit 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: Targeted Sequence Amplification** and **Section 2: Reaction Clean-up** as written in the *Quick*-16S™ NGS Library Prep Kit Instruction Manual (found on the D6400 Product Page at www.zymoresearch.com).

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2. Follow the protocol in **Section 3: Barcode Addition** as written, except during Step 3. 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

Index Primer Set A: Index Primers ZA501-ZA508 and Index Primers ZA701-ZA712

Index Primers ZA5xx		Index Primers ZA7xx											
		ZA701	ZA702	ZA703	ZA704	ZA705	ZA706	ZA707	ZA708	ZA709	ZA710	ZA711	ZA712
		1	2	3	4	5	6	7	8	9	10	11	12
ZA501	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
ZA502	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
ZA503	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
ZA504	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
ZA505	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
ZA506	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
ZA507	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	**POS***
ZA508	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	**NEG****

* POS: The **ZymoBIOMICS® Microbial Community DNA Standard** should be used as a positive control.

** NEG: A no template control should be used as a negative control.

Plate 2, Samples 97-192

Index Primer Set B: Index Primers ZA501-ZA508 and Index Primers ZB701-ZB712

Index Primers ZA5xx		Index Primers ZB7xx											
		ZB701	ZB702	ZB703	ZB704	ZB705	ZB706	ZB707	ZB708	ZB709	ZB710	ZB711	ZB712
		1	2	3	4	5	6	7	8	9	10	11	12
ZA501	A	S97	S105	S113	S121	S129	S137	S145	S153	S161	S169	S177	S185
ZA502	B	S98	S106	S114	S122	S130	S138	S146	S154	S162	S170	S178	S186
ZA503	C	S99	S107	S115	S123	S131	S139	S147	S155	S163	S171	S179	S187
ZA504	D	S100	S108	S116	S124	S132	S140	S148	S156	S164	S172	S180	S188
ZA505	E	S101	S109	S117	S125	S133	S141	S149	S157	S165	S173	S181	S189
ZA506	F	S102	S110	S118	S126	S134	S142	S150	S158	S166	S174	S182	S190
ZA507	G	S103	S111	S119	S127	S135	S143	S151	S159	S167	S175	S183	**POS***
ZA508	H	S104	S112	S120	S128	S136	S144	S152	S160	S168	S176	S184	**NEG****

* POS: The **ZymoBIOMICS® Microbial Community DNA Standard** should be used as a positive control.

** NEG: A no template control should be used as a negative control.

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Plate 3, Samples 193-288

Index Primer Set C: Index Primers ZB501-ZB508 and Index Primers ZA701-ZA712

Index Primers ZB5xx		Index Primers ZA7xx											
		ZA701	ZA702	ZA703	ZA704	ZA705	ZA706	ZA707	ZA708	ZA709	ZA710	ZA711	ZA712
		1	2	3	4	5	6	7	8	9	10	11	12
ZB501	A	S193	S201	S209	S217	S225	S233	S241	S249	S257	S265	S273	S281
ZB502	B	S194	S202	S210	S218	S226	S234	S242	S250	S258	S266	S274	S282
ZB503	C	S195	S203	S211	S219	S227	S235	S243	S251	S259	S267	S275	S283
ZB504	D	S196	S204	S212	S220	S228	S236	S244	S252	S260	S268	S276	S284
ZB505	E	S197	S205	S213	S221	S229	S237	S245	S253	S261	S269	S277	S285
ZB506	F	S198	S206	S214	S222	S230	S238	S246	S254	S262	S270	S278	S286
ZB507	G	S199	S207	S215	S223	S231	S239	S247	S255	S263	S271	S279	**POS***
ZB508	H	S200	S208	S216	S224	S232	S240	S248	S256	S264	S272	S280	**NEG****

* POS: The **ZymoBIOMICS® Microbial Community DNA Standard** should be used as a positive control.

** NEG: A no template control should be used as a negative control.

Plate 4, Samples 189-384

Index Primer Set D: Index Primers ZB501-ZB508 and Index Primers ZB701-ZB712

Index Primers ZB5xx		Index Primers ZB7xx											
		ZB701	ZB702	ZB703	ZB704	ZB705	ZB706	ZB707	ZB708	ZB709	ZB710	ZB711	ZB712
		1	2	3	4	5	6	7	8	9	10	11	12
ZB501	A	S289	S297	S305	S313	S321	S329	S337	S345	S353	S361	S369	S377
ZB502	B	S290	S298	S306	S314	S322	S330	S338	S346	S354	S362	S370	S378
ZB503	C	S291	S299	S307	S315	S323	S331	S339	S347	S355	S363	S371	S379
ZB504	D	S292	S300	S308	S316	S324	S332	S340	S348	S356	S364	S372	S380
ZB505	E	S293	S301	S309	S317	S325	S333	S341	S349	S357	S365	S373	S381
ZB506	F	S294	S302	S310	S318	S326	S334	S342	S350	S358	S366	S374	S382
ZB507	G	S295	S303	S311	S319	S327	S335	S343	S351	S359	S367	S375	**POS***
ZB508	H	S296	S304	S312	S320	S328	S336	S344	S352	S360	S368	S376	**NEG****

* POS: The **ZymoBIOMICS® Microbial Community DNA Standard** should be used as a positive control.

** NEG: A no template control should be used as a negative control.

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3. Follow the protocol in **Section 4: Library Quantification and Pooling** and **Section 5: Final Library Clean-up** as written in the Instruction Manual.

4. Quantify each cleaned library from **Section 5: Final Library Clean-up** with a fluorometric quantification method that uses dsDNA binding dyes, such as Qubit®.

5. Dilute each library to 4 nM.

6. Mix libraries together based on desired read-assignment ratio. For example, to assign 80% of all reads to Library 1 and 20% of all reads to Library 2, mix 8 µl of Library 1 with 2 µl of Library 2.

7. Sequence the final library on the Illumina MiSeq® platform.