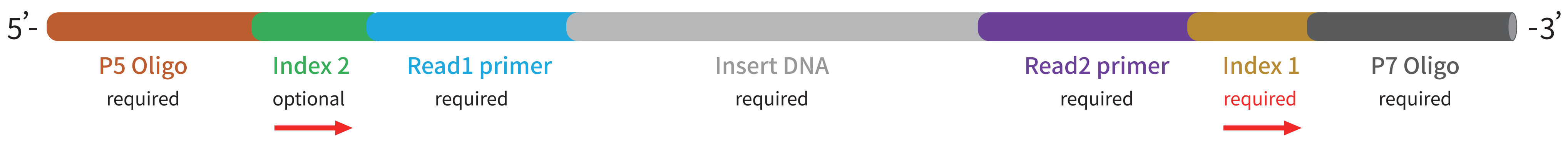
Index filling guidelines

Library Structure


5'- -3'

P5 Oligo required

Index 2 optional

Read1 primer required

Insert DNA required

Read2 primer required

Index 1 required

P7 Oligo required

- The order of elements in your library should be the same as they are in the picture above.
- P5 and P7 Oligos are used for binding your library to the flow cell. They should be the same as Illumina sequences. Sequencing primers (Read 1 and Read 2) are used for sequencing your DNA insert and index. Please let your TS know if your library contains non-standard Illumina sequences (or if you are not sure).

Sites	Standard Illumina sequences
P5 Oligo sequence (P5→P7, 5'→3')	AATGATACGGCGACCACCGAGATCTACAC
P7 Oligo sequence (P5→P7, 5'→3')	ATCTCGTATGCCGTCTTCTGCTTG
Read1 Primers (P5→P7, 5'→3')	ACACTCTTTCCCTACACGACGCTCTTCCGATCT TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTTCAGAGTTCTACAGTCCGACGATC
Read2 Primers (P5→P7, 5'→3')	AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC CTGTCTCTTATACACATCTCCGAGCCCACGAGAC AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCG TGGAATTCTCGGGTGCCAAGGAACTCCAGTCAC

Required index orientation

- Index sequences are used for demultiplexing. Index 1 (i7 index) is required, and index 2 (i5 index) is optional.
- The correct orientation of the indexes that should be filled in your sample information form or SIF is P5→P7, 5'→3', as the red arrows show in the picture above:
- For i7 index, please refer to "i7 bases for sample sheet" in your library preparation guide. For i5 index, please select "i5 Bases for Sample Sheet NovaSeq 6000 with v1.0 reagent kits".

We use Illumina V1.5 reagents for NovaSeq sequencing (workflow B/reverse complement) but our demultiplexing software requires the index sequence in V1.0 orientation (workflow A/forward).

Index Name	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases in Adapter	i5 Bases for Sample Sheet NovaSeq 6000 with v1.0 reagent kits, MiSeq, HiSeq 2000/2500, NextSeq 2000 (Sample Sheet v2)	i5 Bases for Sample Sheet iSeq, NovaSeq 6000 with v1.5 reagent kits, MiniSeq, NextSeq 500/550, HiSeq 3000/4000/X, NextSeq 2000 (Sample Sheet v1)
UDP0001	CGCTCAGTTC	GAACTGAGCG	TCGTGGAGCG	TCGTGGAGCG	CGCTCCACGA
UDP0002	TATCTGACCT	AGGTCAGATA	CTACAAGATA	CTACAAGATA	TATCTTGTAG
UDP0003	ATATGAGACG	CGTCTCATAT	TATAGTAGCT	TATAGTAGCT	AGCTACTATA

⚠ NOTE

- The length of index sequences is limited to a maximum of 8bp. If the read length of index is more than 8bp, please contact your sales representative or TS.
- Please ensure that you provide your indexes accurately and in the correct orientation. Failure to do so may result in additional charges.
- It is stated in our quotation terms that "18. For partial lane sequencing project, after the sequencing work is started, if the sequencing is ruined due to the wrong information provided by the customer (including but not limited to the index information of the library, library type of the sample), the customer shall pay $6,000 to Novogene for the loss."
- This index filing instructions apply to both NovaSeq S4 as well as HiSeq samples.

If you have any questions about which indexes to provide, please let us know – our team will be happy to review them with you.