



Instruction for Use

***AccuNext* CDI Oligos for DNA Library Kit (Illumina)**

AG12529 AG12530 AG12531

Version.V1E1

1. Description

This product is a dual-index PCR primer kit specifically designed for library construction on Illumina high-throughput sequencing platforms. The kit includes both i5 and i7 index primers, enabling the construction of up to 96 unique dual-indexed libraries. It is fully compatible with DNA library preparation workflows, such as our AccuNext DNA Library Prep Kit for Illumina (Code No. AG12535 / AG12536) and AccuNext Short Adapter for DNA Library Kit (Illumina) (Code No. AG12537 / AG12538). All reagents included in this kit undergo rigorous quality control and functional validation, ensuring high success rates in DNA library construction for sequencing.

The constructed library would have a structure shown as below.



2. Kit Information

Kit Name	Cat. No	Specification
<i>AccuNext</i> CDI Oligos for DNA Library Kit (Illumina)	AG 12529	12 rxns
	AG 12530	48 rxns
	AG 12531	96 rxns

3. Transportation and Storage

Storage	Store at -20°C
Transportation	Transport at -20°C Dry Ice or Blue Ice Condition

4. Kit Components

Intended Use	Component Code	AG 12529 (12 rxns)	AG 12530 (48 rxns)	AG 12531 (96 rxns)	Cap Color
i5 Index Primer (D501-D508)	D 501(25µM)	30 µl	30 µl	30 µl	
	D 502(25µM)	-	30 µl	30 µl	●
	D 503(25µM)	-	30 µl	30 µl	●
	D 504(25µM)	-	30 µl	30 µl	●
	D 505(25µM)	-	-	30 µl	●
	D 506(25µM)	-	-	30 µl	●
	D 507(25µM)	-	-	30 µl	●
	D 508(25µM)	-	-	30 µl	●
i7 Index Primer (D701-D712)	D 701(25µM)	5 µl	10 µl	20 µl	●
	D 702(25µM)	5 µl	10 µl	20 µl	●
	D 703(25µM)	5 µl	10 µl	20 µl	●
	D 704(25µM)	5 µl	10 µl	20 µl	●
	D 705(25µM)	5 µl	10 µl	20 µl	●
	D 706(25µM)	5 µl	10 µl	20 µl	●
	D 707(25µM)	5 µl	10 µl	20 µl	●
	D 708(25µM)	5 µl	10 µl	20 µl	●
	D 709(25µM)	5 µl	10 µl	20 µl	●
	D 710(25µM)	5 µl	10 µl	20 µl	●
	D 711(25µM)	5 µl	10 µl	20 µl	●
	D 712(25µM)	5 µl	10 µl	20 µl	●

5. General Guideline

- (1) Concentration of i5 Index Primer/i7 Index Primer is 25 μ M. The volume required for a single library construction may vary depending on the specific library prep kit used.
- (2) For sequencing platforms such as NovaSeq 6000 v1.5 reagents, MiniSeq, NextSeq, HiSeq 3000/4000, the i5 Index Primer sequences must be input as reverse complements in the Sample Sheet or when demultiplexing index sequences. Please refer to Appendix.
- (3) When using different index primers, please be cautious of cross-contamination.
- (4) Before use, allow the product to completely thaw at room temperature (20–25°C). Do not heat to thaw. After thawing, gently mix by pipetting and store on ice temporarily until use. Recommend pairing and plate setup is shown as below.
- (5) The kit enables library differentiation through index combinations. It includes 8 unique i5 index primers and 12 unique i7 index primers. It is recommended to use different combinations for optimal index separation.

For example, with product AG12531, any of the combinations listed in the following table can be selected for dual-indexing.

	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D502	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D503	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D504	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D505	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D506	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D507	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D508	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

6. Sequence Information

i5 Index Primer for Illumina

5' -AATGATACGGCGACCACCGAGATCTACAC *[i5 Index]* ACACTCTTCCCTACACGACGCTCTCCGATCT- 3'

i7 Index Primer for Illumina

5' -CAAGCAGAAGACGGCATAACGAGAT *[i7 Index]* GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT- 3'

[i5 Index] represents the i5 Index sequence of 8 bp length; *[i7 Index]* represents the i7 Index Sequence of 8 bp length

Appendix. Index Sequence Table

Component		Index Sequence	Sample Sheet Input	
			NovaSeq 6000 v1.0 reagents, MiSeq, HiSeq 2000/2500	NovaSeq 6000 v1.5 reagents, MiniSeq, NextSeq, HiSeq 3000/4000
i5 Index Primers	D501	TATAGCCT	TATAGCCT	AGGCTATA
	D502	ATAGAGGC	ATAGAGGC	GCCTCTAT
	D503	CCTATCCT	CCTATCCT	AGGATAGG
	D504	GGCTCTGA	GGCTCTGA	TCAGAGCC
	D505	AGGCGAAG	AGGCGAAG	CTTCGCCT
	D506	TAATCTTA	TAATCTTA	TAAGATTA
	D507	CAGGACGT	CAGGACGT	ACGTCCTG
	D508	GTA CTGAC	GTA CTGAC	GTCAGTAC
i7 Index Primers	D701	CGAGTAAT		ATTACTCG
	D702	TCTCCGGA		TCCGGAGA
	D703	AATGAGCG		CGCTCATT
	D704	GGAATCTC		GAGATTCC
	D705	TTCTGAAT		ATTCAGAA
	D706	ACGAATTC		GAATTCGT
	D707	AGCTTCAG		CTGAAGCT
	D708	GCGCATT		TAATGCCG
	D709	CATAGCCG		CGGCTATG
	D710	TTCGCGGA		TCCGCGAA
	D711	GCGCGAGA		TCTCGCGC
	D712	CTATCGCT		AGCGATAG



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