FS Pro DNA Lib Prep Kit for Illumina

RK20261

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Version：N16F23v5.0

Contents

1. Introduction

The FS Pro DNA Lib Prep Kit for Illumina is designed for DNA library preparation for next generation sequencing (NGS) on Illumina® platforms. This kit integrates DNA fragmentation with end repair and dA tailing into one step, and it can be directly followed by adapter ligation without purification. With greatly simplified experimental flow, the entire experiment can be completed within 2 hours. This kit is compatible with DNA samples from different species and different origins. Optimization of reagents and protocol greatly reduced rates of false positives, significantly improving sequencing yield and overall results, including with FFPE DNA samples. This kit is suitable for the construction of PCR-Free libraries (e.g., 100 ng of high-quality genomic DNA).

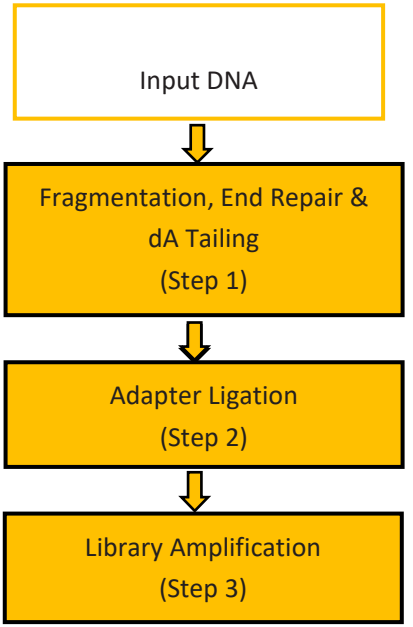


Figure 1. Overall sample preparation workflow.

2. Components

All components should be stored at -20°C. The shelf life of each reagent is one year when stored properly.

Table 1. Components supplied with this product

		Components	8 RXN	24 RXN	96 RXN
Fragmentation, End Repair & dA Tailing	●	FS Pro Buffer I	40 µL	120 µL	480 µL
	●	FS Pro Enzymes I	80 µL	240 µL	960 µL
	●	1X TE Buffer	280 µL	840 µL	1.68 mL
Adapter	●	FS Pro Ligation Buffer I	160 µL	480 µL	1.92 mL
Ligation	●	Ligation Enzymes	40 µL	120 µL	480 µL
Amplification	●	2X PCR Mix	200 µL	600 µL	2.4 mL
	●	10X PCR Primers	40 µL	120 µL	480 µL

3. Storage

Transportation and storage: the FS Pro DNA Lib Prep Kit for Illumina must be stored at -15°C to -25 °C. Because this kit is sensitive to temperature, dry ice or dry ice combined with ice packs should be used for long-distance transportation.

4. Applications

FS Pro DNA Lib Prep Kit for Illumina is suitable for DNA library preparation for NGS. The product includes a fragmentation, end repair and dA-tailing module, an adapter ligation module, and a PCR amplification module. This kit is compatible with various types of DNA template (genomic DNA, FFPE

DNA, etc.) and 1 ng to 1000 ng of input DNA. In summary, the product is recommended to use for the following application fields:

① Whole genome sequencing.

② Exon sequencing and targeted sequencing, including Roche® NimbleGen™ SeqCap™ EZ, Agilent SureSelect, Illumina TruSeq®, IDT X Gen™ Lockdown™ Probes, or other hybridization probes.

③ Metagenomic sequencing.

5. Additional Materials Required

Purification Beads: AFTMag NGS DNA Clean Beads (ABclonal, Cat. No. RK20257).

DNA quality control: Agilent Bioanalyzer, or comparable method to assess the quality of DNA library; ABQubit dsDNA Quantitation Kit (ABclonal, Cat. No. RK30140).

DNA Adapters:

Truncated DNA Adapter Kit for Illumina (ABclonal, Cat. No. RK20294, RK20295, RK20297).

RK20294/RK20297/RK20297 products provide 8 bp single-index truncated adapters.

Dual DNA Adapter 96 Kit for Illumina (ABclonal, Cat. No. RK20287, RK20296).

RK20287/RK20296 products provide 8 bp double-index truncated adapters.

Unique Dual Index for Illumina (ABclonal, Cat. No. RK21622-625).

RK21622-625 products provide truncated adapters with an 8 bp Unique Dual Index at two ends.

Unique Dual Index （with UMI） for Illumina (ABclonal, Cat. No. RK21700-703)

RK21700-703 products provide truncated adapters with an 8 bp Unique Dual Index at two ends and a 3 bp UMI (Unique Molecular Identifier).

Full DNA Adapter Kit for Illumina (ABclonal, Cat. No. RK20292-293, RK20298).

RK21700-703/RK20298 products provide 8 bp single-index full adapters.

Dual Unique UMI Adapters for Illumina (ABclonal, Cat. No. RK21600-602).

RK21600-602 products are full adapters with an 8 bp Unique Dual Index at two ends and a 9bp UMI (Unique Molecular Identifier).

Other Materials: Nuclease-Free Water, 10X TE Buffer (ABclonal, Cat. No. RM20728), 100% ethanol, Vortex mixer, Low adsorption EP tubes, PCR tubes, Magnetic stand, PCR instrument.

6. Notes

6.1 Input DNA

6.1.1 For correctly sized fragmentation, use 1X TE Buffer to dissolve DNA samples (*Important!*).

6.1.2 Input DNA should be quantified using Qubit® or other fluorometric quantification kits.

6.1.3 Impurities in the DNA samples, such as trace amounts of residual RNAs, nucleotides, single-stranded DNAs, and other contaminants, may have an impact on fragment size. If possible, please use 1.8X magnetic beads to purify DNA samples and use 1X TE

Buffer to elute purified DNA samples (*Important!*).

6.2 Fragmentation

6.2.1 Use only 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8,0) when performing fragmentation. Using water will severely shorten fragment size.

6.2.2 If the DNA samples have been dissolved in an EDTA-Free solution, please prepare the reaction system by using 10X TE Buffer (ABclonal, Cat. No. RM20728) according to the following table:

Table 2. Input DNA Fragmentation (EDTA-Free solution)

	Component	Volume
	Input DNA (EDTA-Free solution)	X μL
●	FS Pro Buffer I	5 μL
●	FS Pro Enzymes I	10 μL
	10X TE Buffer	3.5 μL
	Nuclease-Free Water	Up to 50 μL
	Total volume	**50 μl**

6.2.3 If the final concentration of EDTA in the DNA sample is greater than 1 mM, please pretreat the DNA sample using 2.2X magnetic beads and use 1X TE Buffer to elute the purified DNA sample.

6.2.4 It is recommended to use the FFPE DNA QC Kit (ABclonal, Cat. No. RK20229) to treat FFPE DNA samples. For high quality FFPE DNA samples (grade 1-2), follow the recommendations for treating genomic DNA; for FFPE DNA samples (grade 3-5), reduce

fragmentation time to 5-10 minutes.

6.2.5 The fragmentation reaction system is sensitive to temperature; the experiment should be performed on ice. Immediately transfer the prepared reaction to the thermocycler after set-up. Return all components to -15°C to -25°C for storage as soon as possible after use.

6.3 Adapters

6.3.1 This kit contains library construction components and universal PCR primers. Adapter kits need to be purchased separately.

6.3.2 The amount of the adapters used affects ligation efficiency and library yield. Please refer to Table 3 for the recommended adapter concentrations for different DNA inputs.

Table 3. Recommended adapter concentrations for libraries prepared from 5 ng - 1 μg input DNA

Input DNA	Dilution Ratio	Working Concentration
1 μg~50 ng	Undiluted	15 μM
49 ng~25 ng	1:2	7.5 μM
24 ng~10 ng	1:5	3 μM
9 ng~5 ng	1:10	1.5 μM
< 5 ng	1:20	0.75 μM

6.4 Bead-based Purification and Optional Size Selection

6.4.1 The magnetic beads should be equilibrated to room temperature for 30 minutes prior to use to optimize sample recovery and sorting efficiency.

6.4.2 Before use, ensure the magnetic beads are fully resuspended by

vortexing or pipetting up and down several times.

6.4.3 80% ethanol should be freshly prepared.

6.4.4. Inadequate drying may lead to residual ethanol which may affect subsequent experiments. Excessive drying may lead to cracking of magnetic beads and decrease in recovery efficiency.

6.4.5 It is recommended to elute DNA from beads with 1X TE Buffer for stable preservation.

6.4.6 Size selection is an optional step and, if desired, should be performed following the purification step after adapter ligation. It is not recommended to perform size selection after adapter ligation without purification because the ligation system contains a high concentration of PEG.

6.4.8 Size selection inevitably leads to a loss of sample material. If it is required, an input DNA amount greater than 50 ng is recommended.

6.5 Library Amplification

The library amplification step requires strict control of the number of amplification cycles. Insufficient number of amplification cycles leads to decreased library yield; excessive amplification leads to unwanted artifacts. Table 4 shows recommended amplification cycles for libraries prepared to obtain 1 μg library.

Table 4. Recommended cycle numbers for 1 ng–1 μg of input DNA

Input DNA（ng）	Number of cycles required to generate 1 μg library*
1000	2-3
500	3-4
250	4-5
100	5-6
50	6-7
25	7-9
10	9-11
1	13-15

*Note: Add an additional 1-3 cycles when using FFPE DNA samples.

7. Protocol

Step 1. Fragmentation, End Repair & dA Tailing

1.1 Preheat PCR instrument to 32°C.

1.2 Prepare the following solution in a sterile PCR tube on ice (add FS Pro Enzymes I to the reaction system last):

Table 5. Fragmentation and End Repair & dA Tailing Reaction Setup (per sample)

	Component	Volume
	Input DNA (dissolved in 1XTE Buffer)	X μL
●	FS Pro Buffer I	5 μL
●	FS Pro Enzymes I	10 μL
●	1X TE Buffer	Up to 50 μL
	Total volume	**50 μL**

Note: Input DNA is recommended to be dissolved in 1X TE Buffer (10 mM Tris-HCl, 1 mM

EDTA, pH 8, 0). If the DNA sample has been dissolved in an EDTA-Free solution, please prepare the reaction system by using 10X TE Buffer according to the instructions in 6. Notes, 6.2 Fragmentation, 6.22.

1.3 Pipe up and down or vortex to mix, and centrifuge to collect the reaction solution to the bottom of the tube.

1.4 Place the PCR tube on a PCR instrument preheated to 32°C in advance and run the reaction program described in Table 6. Set the temperature of the hot lid to 75°C, and hold at 4°C for no more than 1 hour.

Table 6. Thermal cycler program for Fragmentation, End Repair & dA Tailing

Temperature	Time
32℃	5-25 min (Refer to Table 7)
65℃	30 min
4℃	Hold (< 1h)

Table 7. Recommended fragmentation time for expected-insert size (32℃)

Expected-insert size	Fragmentation time	Adjust fragmentation time
250 bp	20 min	18-25 min
300 bp	15 min	13-17 min
350 bp	10 min	8-12 min
550 bp	5 min	4-6 min

Note: The above recommended time was validated using high-quality human blood gDNA as a template. When using other types of high-quality DNA for library construction, the distribution range of fragmentation products is not much different within the same fragmentation time.

Step 2. Adaptor Ligation

2.1 Equilibrate the AFTMag NGS DNA Clean Beads to room temperature (for at least 30 minutes). Suspend the beads thoroughly by vortexing.

2.2 Dilute the Adapter to appropriate concentration using Low-EDTA TE Buffer according to Table 3.

2.3 Prepare the reaction system described in Table 8 on ice. Mix FS Pro Ligation Buffer I and Ligation Enzymes thoroughly by gently pipetting up and down, briefly centrifuge to collect to the bottom of the tube, and place on ice for use. Working Adapter should be added separately. Mix the prepared ligation reaction system well and centrifuge briefly.

Table 8. Ligation Reaction Setup (per sample)

	Component	Volume
	End Prep Reaction Mix	50 µL
●	FS Pro Ligation Buffer I	20 µL
●	Ligation Enzymes	5 µL
	Working Adapter	5 µL
	Total volume	**80 µL**

2.4 Place the PCR tube on the PCR instrument and run the reaction program described in Table 9. The heated lid setting is off.

Table 9. Thermal cycler program for Ligation Reaction

Temperature	Time
22℃	15 min
4℃	∞

2.5 Pipet 64 µL (0.8X) of beads into 80 µL of the Adapter Ligation products. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

2.6 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, remove the supernatant without disturbing the beads.

2.7 Keeping the PCR tube on the magnetic stand, add 200 µL of 80% ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

2.8 Repeat Step 2.7.

2.9 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 µL pipette. Dry the sample until the residual ethanol has just evaporated.

2.10 Take the tube out of the magnetic stand. Add 22 µL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

2.11 Keeping the PCR tube on the magnetic stand, wait for 2 minutes. Transfer 20 µL of the supernatant to a new PCR tube.

2.12 For product needing size-selection, proceed to size-selection according to 8. Appendix 8.1 Size selection (Optional). For product with no need for size selection, proceed to library amplification directly.

Note: The product resulting from Adapter ligation after purification can be stable for approximately 1- 2 weeks at 4 °C/-20 °C.

Step 3. Library Amplification

FS Pro DNA Lib Prep Kit for Illumina is compatible with a variety of non-index, single-Index and dual-Index Adapters. Please select the library amplification systems described in Table 10, Table 11, Table 12 and Table 13 according to the adapter type. Refer to the Appendix Table for information about all the adapter types.

3.1 Prepare the following PCR Reaction System:

Table 10. PCR Amplification Reaction Setup For Truncated Adapters With Single Index

	Component	Volume
	Adapter-Ligated DNA	20 μL
●	2X PCR Mix	25 μL
○	PCR Index Primer	2.5 μL
●	Universal PCR Primer	2.5 μL
	Total volume	50 μL

Table 11. PCR Amplification Reaction Setup For Truncated Adapters With Combinatorial Dual Index

	Component	Volume
	Adapter-Ligated DNA	20 μL
●	2X PCR Mix	25 μL
●	I5 Primer	2.5 μL
●	I7 Primer	2.5 μL
	Total volume	50 μL

Table 12. PCR Amplification Reaction Setup For Truncated Adapters With Unique Dual Index

	Component	Volume
	Adapter-Ligated DNA	20 µL
●	2X PCR Mix	25 µL
○	UDI Primer	5 µL
	Total volume	50 µL

Table 13. PCR Amplification Reaction Setup For Full-length Adapter

	Component	Volume
	Adapter-Ligated DNA	20 µL
●	2X PCR Mix	25 µL
●	10X PCR Primers	5 µL
	Total volume	50 µL

3.2 Pipet up and down to mix well and centrifuge briefly.

3.3 Place the tube on the thermocycler and run the reaction program described in Table 14. Set the temperature of the heated lid to 105℃. The recommended number of library amplification cycles is shown in Table 4.

Table 14. Thermal cycler program for Library Amplification

Temperature	Time	Cycles
98℃	1 min	1
98℃	10 s	2-15 PCR Cycles
60℃	30 s	
72℃	30 s	
72℃	1 min	1
4℃	∞	1

3.4 Pipet 50 µL (1X) of beads into 50 µL of the Library Amplification products. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

3.5 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, remove the supernatant without disturbing the beads.

3.6 Keeping the PCR tube on the magnetic stand, add 200 µL of 80% ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

3.7 Repeat Step 3.6.

3.8 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 µL pipette. Dry the sample until the residual ethanol has just evaporated.

3.9 Take the tube out of the magnetic stand. Add 32 µL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

3.10 Returning the PCR tube to the magnetic stand, wait for 2 minutes. Transfer 30 μL of the supernatant to a new PCR tube.

Store the final library at -20 °C.

Note: The purified amplification products can be stable for approximately 1- 2 weeks at 4 °C/-20 °C.

8. Appendix

8.1 Size selection (Optional)

Table 14. Size Selection of Library

Expected Insert Size (bp)	380~430	330~380	280~330
Expected library Size (bp)	500~550	450~500	400~450
1st volume ratio (Beads: DNA)	0.6X（60μL）	0.65X（65μL）	0.7X（70μL）
2nd volume ratio (Beads: DNA)	0.15X（15μL）	0.15X（15μL）	0.15X（15μL）

Size selection protocols are optimized for ratios of 0.65X/0.15X.

8.1.1 Adjust product of step 2.11 to 100 μL using Nuclease-Free Water. Pipet 65 μL (0.65X) of beads into the 100 μL of adjusted solution. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

8.1.2 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, transfer the supernatant to a new tube without disturbing the beads.

8.1.3 Pipet 15 μL (0.15X) of beads into the supernatant from 8.1.2. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

8.1.4 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, remove the supernatant without disturbing the beads.

8.1.5 Keeping the PCR tube on the magnetic stand, add 200 µL of 80% ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

8.1.6 Repeat Step 8.1.5.

8.1.7 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 µL pipette. Dry the sample until the residual ethanol has just evaporated.

8.1.8 Take the tube out of the magnetic stand. Add 22 µL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

8.1.9 Returning the PCR tube to the magnetic stand, wait for 2 minutes. Transfer 20 µL of the supernatant to a new PCR tube.

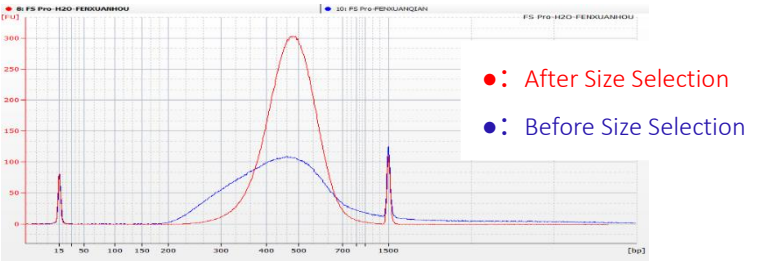


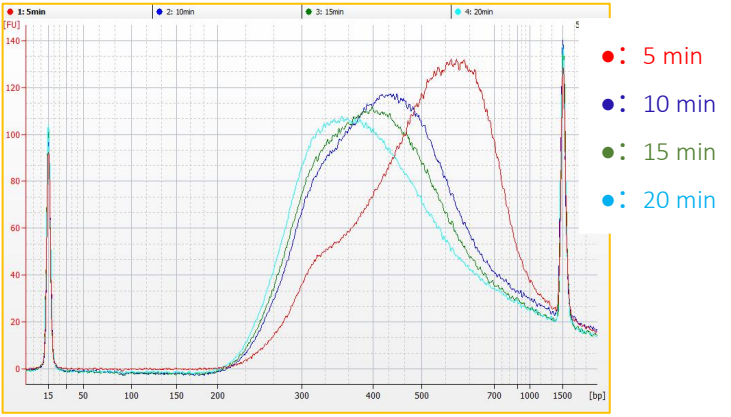
Figure 2. Effect of Size Selection on Library Distribution Peaks

Library construction was performed on two libraries using input DNA of 100 ng FFPE DNA template with this kit. Fragmentation was performed at 32 ℃ for 15 minutes; one library was treated with size selection at 0.65X/0.15X. The final distribution of both libraries is shown in Fig 2 as determined by Bioanalyzer 2100.

8.2 Example Experimental Data

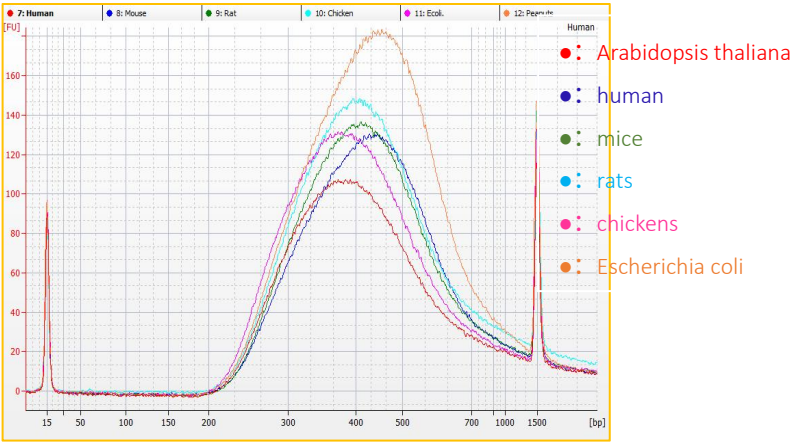
8.2.1 Comparison of Fragmentation Time for Library Preparation.

100 ng human blood gDNA initial template was treated with this kit. Fragmentation was performed 32°C for 5/10/15/20 min, and the distribution of final libraries is shown below.



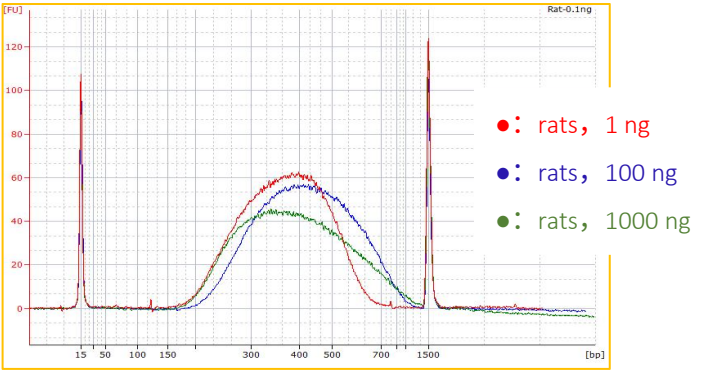
8.2.2 DNA samples from Different Species for Library Preparation

100 ng DNA from humans, rats, mices, chickens, Escherichia coli and peanuts were starting templates for library construction using this kit. Fragmentation occurred at 32°C for 15 min, and the final library distribution libraries are shown below.



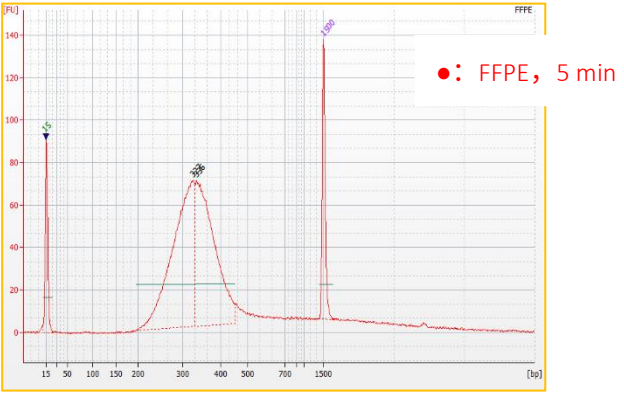
8.2.3 Sample Input Concentrations for Library Preparation.

1 ng, 100 ng, and 1000ng rat DNA template was for library construction with this kit. Fragmentation was performed at 32°C for 15 min, and the distribution of final libraries is shown below.



8.2.4 FFPE DNA Library Preparation.

100 ng FFPE DNA was starting template for library construction using this kit. Fragmentation was performed at 32°C for 5 min, and the final library distribution is shown below.



9. Appendix Table

Table 16. List of adapters for the Illumina platforms

Adapters	Index	UMI/UDI	Cat. NO	Kit
Full	single	×	RK20292	Full DNA Adapter Kit for Illumina Set_C (48 indices)
	single	×	RK20293	Full DNA Adapter Kit for Illumina Set_D (48 indices)
	single	×	RK20298	Full DNA Adapter Kit for Illumina MidiSet (24 indices)
	dual	UMI	RK21600-RK21602	Dual Unique UMI Adapters for Illumina
Truncated	single	×	RK20294	Truncated DNA Adapter Kit for Illumina Set_C (48 indices)
	single	×	RK20295	Truncated DNA Adapter Kit for Illumina Set_D (48 indices)
	single	×	RK20297	Truncated DNA Adapter Kit for Illumina MidiSet (24 indices)
	dual	×	RK20287	Dual DNA Adapter 96 Kit for Illumina
	dual	×	RK20296	Dual DNA Adapter 96 Kit Extended for Illumina
	dual	UDI	RK21622-RK21625	Unique Dual Index for Illumina
	dual	UMI	RK21700-RK21703	Unique Dual Index (with UMI) for Illumina

United States

www.abclonal.com

Address: 500 W. Cummings Park Dr, Woburn, MA 01801, United States

Phone: 888.754.5670, +1 857.259.4898 (Int'l)

Email: service@abclonal.com