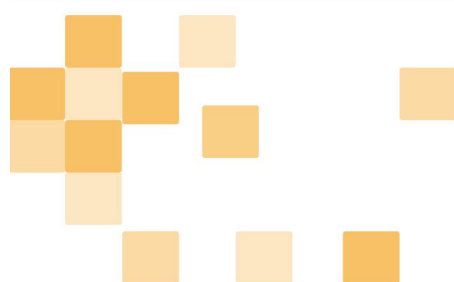
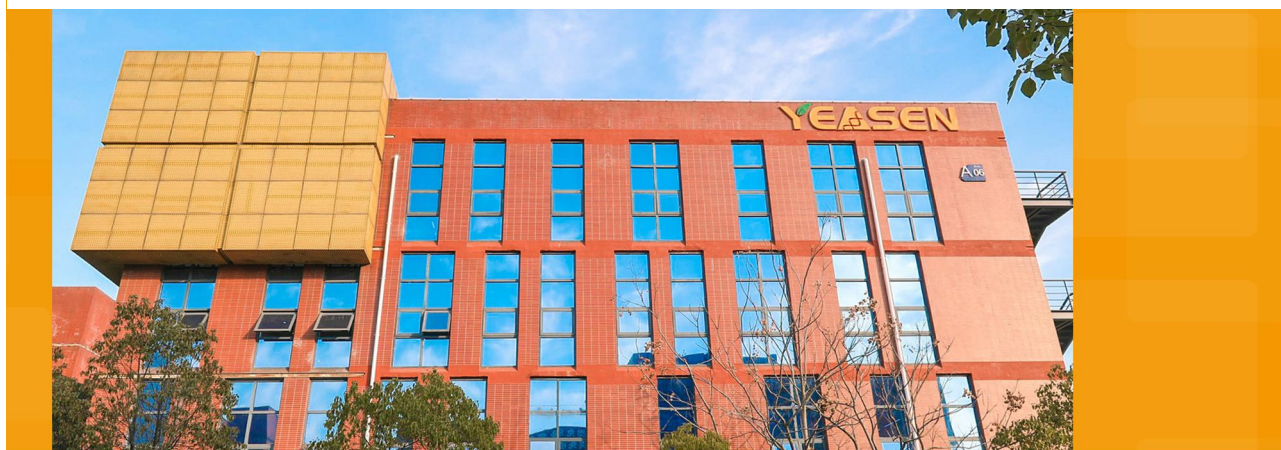


# Hieff NGS™ Ultima Pro DNA Library Prep Kit for Illumina®

Cat#12201



## INSTRUCTION FOR USE

Yeasen Biotechnology (Shanghai) Co., Ltd.



Contents

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Product Information

Product Name	Cat#	Specification
Hieff NGS™ Ultima Pro DNA Library Prep Kit for Illumina®	12201ES24	24 T
	12201ES96	96 T

Product Description

Hieff NGS™ Ultima Pro DNA Library Prep Kit for Illumina® is a new generation library construction kit specially developed and designed for the Illumina® high-throughput sequencing platform. On the basis of the previous generation of library construction kit, this product shows higher efficiencies in end repair, dA-tailing, and adapter ligation than the previous versions. The high-fidelity enzyme significantly improves the uniformity and fidelity of amplification. The kit is compatible with most DNA sample types, including standard genomic DNA from animals/plants/microorganisms, FFPE samples, cfDNA, and ChIP DNA.

- Suitable for 500 pg-500 ng of DNA, including FFPE, cfDNA etc.
- With an industry-leading library conversion rate of more than 70%.
- Multi-sample validation for superior library and sequencing data.
- Strict batch stability.

Product Components

Component Number and Name		12201ES24	12201ES96
12201-A	Endprep Buffer 2.0	144 μL	576 μL
12201-B	Endprep Enzyme	96 μL	384 μL
12201-C	Ligation Enhancer	720 μL	3×960 μL
12201-D	Novel T4 DNA Ligase	120 μL	480 μL
12201-E	Canace™ Pro Amplification Mix	600 μL	4×600 μL
12201-F	Primer Mix	120 μL	480 μL

Shipping and Storage

The product is shipped with dry ice and can be stored at -20°C for one year.

Cautions

1 Operation

- 1.1 For your safety and health, please wear lab coats and disposable gloves for operation. This product is for research use ONLY!
- 1.2 Thaw components at room temperature. Once the components are thawed, mix thoroughly by vortexing, spin the tube briefly and place them on ice for later use.
- 1.3 When preparing the reaction solution of each step, it is recommended to use a pipette to mix well or gently shake. Vigorous shaking may cause a decrease in library output.
- 1.4 It is highly recommended to use filtered pipet tips to avoid cross-contamination. Be sure to change pipet tips when processing different samples.
- 1.5 It is recommended to perform each reaction step in a thermocycler with a heated lid. The thermocycler should be preheated to the set temperature before use.
- 1.6 Improper operation is likely to cause carry-over contaminations through aerosols, impacting the experiment’s accuracy. It is highly recommended to divide the experiment environment into the pre-PCR and post-PCR regions, with separate sets of devices and disposables in each area. Perform routine cleaning for each area by wiping the surfaces with 0.5% sodium hypochlorite or 10% bleach.

2 DNA Fragmentation

- 2.1 This kit is compatible with either mechanically fragmented DNA or enzymatically fragmented DNA.

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2.2 The kit is compatible with 500 pg-500 ng of input DNA. It is highly recommended to use high-quality input DNA with A260/A280 = 1.8-2.0. Table 1 lists the recommended amount of Input DNA.

Table 1. The recommended amount of Input DNA.

Application	Sample type	Input DNA
WGS	Complex genome	50 ng-500 ng
Targeted capture sequencing	Complex genome	10 ng-500 ng
WGS, Targeted sequencing	FFPE DNA	50 ng-500 ng
Targeted sequencing	cfDNA/ctDNA	≥500 pg
WGS	microbial genomes	≥1 ng
WGS (PCR-free)	High-quality input DNA	≥50 ng

Note: When the input DNA is with poor quality or DNA size selection is required, the input DNA amount should be increased accordingly.

2.3 “Input DNA” specifically refers to the DNA samples ready for end repair/dA tailing.

2.4 A beads-purification/size-selection step is recommended post fragmentation if the input DNA sample contains high concentrations of salts like the metal-chelating agent. The salts might impact the efficiencies of the following reactions, including end repair and dA-tailing. Please elute the DNA samples in TE Buffer instead of sterilized ultra-pure water for fragmentation if using the mechanical fragmentation method. If using the enzymatic fragmentation method without performing beads clean-up or size-selection before proceeding to library preparation, please ensure that the stop buffer used doesn’t contain exceeding metal-chelating agent. Otherwise, please clean-up or size select the fragmented samples and elute them in TE buffer or sterilized ultra-pure water (≤50 μL) before proceeding to library preparation.

3 Adapter Ligation

3.1 Yeasen provides the following barcoded adapters and index primers developed for the Illumina platform: 48 Barcoded Adapters: Hieff NGS® Complete Adapter Kit for Illumina®, Set 1~Set 4 (Cat#12615~Cat#12618); 96 Single Index Primers: Hieff NGS® 96 Single Index Primers Kit for Illumina®, Set 1~Set 2 (Cat#12611-Cat#12612); 384 Unique Dual Index (UDI) Primers: Hieff NGS® Stubby UDI Primer Kit for Illumina® (Cat#12404-Cat#12407).

3.2 The adapters’ quality and concentration will directly affect the ligation efficiency and the library yield. Too much adapter favors adapter dimer formation while too little adapter reduces ligation rate and library yield. Table 2 lists the recommended adapter: insert molar ratios for different amounts of DNA input.

Table 2 Recommended adapter: insert molar ratios for 500 pg-1 μg Input DNA

Input DNA	Adapter: Input DNA molar ratio	Input DNA	Adapter: Input DNA molar ratio
1 μg	10:1	50 ng	100:1
500 ng	20:1	25 ng	200:1
250 ng	40:1	1 ng	200:1
100 ng	100:1	500 pg	400:1

Note: Input DNA molarity (pmol)≈ Input DNA mass (ng)/ [0.66 × Input DNA average length(bp)].

4 Bead-based Clean-Up and Size-Selection

- 4.1 DNA size-selection can be performed before end repair/dA-tailing, after adapter ligation, or after amplification.
- 4.2 It is recommended to perform size-selection right after adapter ligation if the input DNA amount is more than 50 ng; otherwise, please perform size-selection after amplification.
- 4.3 The Ligation Enhancer contains a high concentration of PEG, which may cause a significant impact on accurate size-selection. Thus, if size-selection is to be performed right after adapter ligation, it is strongly recommended to add a beads clean-up step before the size-selection. Size selection step can be performed directly if it is performed before the end repair/dA-tailing or after the library amplification.
- 4.4 The magnetic beads should be equilibrated at room temperature before use, otherwise the yield will decrease and the effect will be affected.
- 4.5 Please thoroughly mix the beads before each use by vertexing or pipetting up-and-down.
- 4.6 Please make sure not to take the beads when transferring the supernatant, even trace amounts of the beads may impact the

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following reactions.

4.7 The 80% ethanol used for magnetic bead rinsing should be freshly prepared, otherwise it will affect the recovery efficiency.

4.8 For accurate size-selection, it is recommended to start with a volume of more than 100 µL. If less, it is recommended to bring the volume up to 100 µL with ultra-pure water.

4.9 The magnetic beads should be dried at room temperature before the product is eluted. Insufficient drying will easily cause residual ethanol to affect subsequent reactions; excessive drying will cause the magnetic beads to crack and reduce the purification yield. Normally, drying at room temperature for 3-5 min is enough to allow the beads to fully dry.

4.10 If needed, the purified or size-selected DNA samples eluted in TE buffer can be stored at 4°C for 1-2 weeks or at -20°C for about a month.

5 Library Amplification

5.1 Whether or not to perform library amplification depends on the amount of DNA input, types of the adapters, the sequencing data applications, etc. The amplification step is required if using partial adapters. When using full-length adapters, if the input DNA<200 ng, it is recommended to perform amplification; otherwise, amplification is not necessary.

5.2 Amplification cycle numbers should be strictly controlled. Insufficient amplification may lead to low library yield; Over-amplification may introduce increased bias, errors, duplicated read, and chimeric products. Table 3 lists the recommended cycle numbers to obtain 100 ng or 1000 ng of library yield.

Table 3 The recommended number of cycles to generate 100 ng or 1,000 ng of library yield

Input DNA (ng)	Number of cycles required to generate	
	100 ng	1,000 ng
1000	0	1-2*
500	0	2 - 4
250	0 - 2*	4 - 6
100	2 - 3*	6 - 8
50	3 - 5	7 - 9
10	7 - 9	11 - 13
5	9 - 11	13 - 14
1	13 -15	17 - 19

Note: \* If partial adapter is used, an additional 1-3 cycles are needed. If size-selection is performed before amplification, choose the higher end of the recommended cycle numbers.

6 Library Quality Analysis

6.1 The constructed libraries’ quality is generally analyzed by measuring the concentrations and size distributions.

6.2 Libraries’ concentrations can be measured by fluorescent-based methods such as Qubit® and PicoGreen® or qPCR

6.3 It is NOT recommended to use absorbance-based quantification methods such as NanoDrop®.

6.4. It is recommended to use qPCR method for library quantification: fluorescent-based methods such as Qubit® and PicoGreen® can’t differentiate the incomplete dsDNA structures (inserts with no adapter or with only one of the ends ligated with adapter) from the complete libraries. The qPCR method will only amplify and measure the complete libraries with both ends ligated with adapters (the sequencable libraries), thus providing a more accurate measurement for loading.

6.5 The libraries’ size distribution can be analyzed using Agilent Bioanalyzer or other devices based on the principles of capillary electrophoresis or micro-control flow.

Instructions

1 Required Materials

1.1 Purification beads: Hieff NGS® DNA Selection Beads (Cat#12601), AMPure XP Beads (Cat#A63880), or equivalent products.

1.2 DNA size analysis: Agilent Technologies 2100 Bioanalyzer or equivalent devices.

1.3 DNA Adapter: Hieff NGS® Complete Adapter Kit for Illumina®, Set 1(Cat#12615); Hieff NGS® Complete Adapter Kit for

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Illumina®, Set 2 (Cat#12616); Hieff NGS® Complete Adapter Kit for Illumina®, Set 3(Cat#12617); Hieff NGS® Complete Adapter Kit for Illumina®, Set 4(Cat#12618); or equivalent products.

1.4 Other materials: ethanol, sterilized ultra-pure water, TE Buffer (10 mM Tris-HCl, pH 8.0-8.5; 1 mM EDTA), Eppendorf tubes, PCR tubes, magnetic stands, thermocyclers, etc.

2 OperationFlowchart



Figure 1 Flowchart of library construction using Ultima Pro DNA library prep kit for Illumina

3 Operation Steps

3.1 End Repair/dA-Taling

During this step, the input DNA fragments will be blunted, 5’ phosphorylated, and dA-tailed at the 3’ ends.

3.1.1 Thaw the reagents mentioned in Table 4. Invert to thoroughly mix the reagents and place them on ice for later use.

3.1.2 Assemble the reagents in Table 4 on ice:

Table 4 The reaction system for End Repair/ dA-Tailing

Components	Volume (μL)
Fragmented DNA	x
Endprep Buffer 2.0	6
Endprep Enzyme	4
ddH <sub>2</sub> O	Up to 60

3.1.3 Mix thoroughly by vortexing or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.

3.1.4 Place the PCR tube in a thermocycler and run the reaction programs mentioned in Table 5.

Table 5 The reaction programs for End Repair/dA-Tailing

Temperature	Time
Hot lid 105°C	On
30°C	20 min
72°C	20 min
4°C	Hold

3.2 Adapter Ligation

During this step, the dA-tailed templates will be ligated with Illumina® adapters.

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- 3.2.1 Dilute the adapter to the appropriate concentration according to Table 2.
- 3.2.2 Thaw the reagents mentioned in Table 6. Mix thoroughly and place them on ice for later use.
- 3.2.3 Add the following reagents to the product of step 3.1:

Table 6 The reaction system for Adapter Ligation

Components	Volume (μL)
dA-tailed DNA (Product from step 3.1)	60
Ligation Enhancer	30*
DNA Adapter	5**
Novel T4 DNA Ligase	5

**Note:** \*The Ligation Enhancer is viscous. Please mix thoroughly by inverting or vertexing and briefly spin down before use.  
\*\*The concentration of the Adapter that comes with this kit is 15 μM, which is the same as most commercialized adapters currently available. Please dilute the adapters according to Table 2 and make the volume up to 5 μL with ultra-pure water.

Example of Adapter-adding Calculations: How much adapters should be added when starting with 100 ng of input DNA with an average length of 300 bp?

- **Step 1,** Calculate the molarity of the input DNA. Formula: Input DNA molarity (pmol)≈ Input DNA mass (ng)/ [0.66 × Input DNA average length (bp)]; Input DNA molarity(pmol)=100 ÷ (0.66×300) = 0.5 pmol;
- **Step 2,** Calculate the molarity of the adapters to be added. Find the recommended adapter: insert molar ratios in Table 2; According to the table, the recommended adapter: insert molar ratio for 100 ng input DNA is 100:1, so the molarity of the adapters=100×0.5 pmol=50 pmol;
- **Step 3,** Calculate the volume of the adapters to be added. Adapter concentration=15 μmol/L (If using other adapters, please refer to their concentrations accordingly); Adapter volume =adapter molarity (50 pmol) ÷ adapter concentration (15 μmol/L) = 3.34 μL (Note:15 μmol/L=15 pmol/μL)
- **In Summary,** the adapters to be added is 3.4 μL, make up the volume to 5 μL with 1.6 μL of ultra-pure water (Note: The maximum volume of the adapter is 5 μL).

- 3.2.4 Mix thoroughly by vortexing at low speed or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.
- 3.2.5 Place the PCR tube in a thermocycler and run the reaction programs mentioned in the Table 7.

Table 7 The reaction programs for Adapter Ligation

Temperature	Time
Hot lid 105°C	Off
20°C	15 min
4°C	Hold

Note: If low ligation efficiency is observed for low input DNA, the ligation time can be doubled for better performance.

3.3 Clean-up or Size-selection post Adapter Ligation

This step is to clean-up or size-select the product from the previous step (step 3.2) with magnetic beads to remove unligated adapters, adapter dimers, or other unusable templates.

3.3.1 Clean-up of Adapter-ligated DNA

- 1) Equilibrate the Hieff NGS® DNA Selection Beads at room temperature (~ 30 min). Prepare 80% ethonal.
- 2) Resuspend the beads thoroughly by vortexing or shaking the bottle.
- 3) Add 60 μL Hieff NGS® DNA Selection Beads (0.6×, Beads:DNA=0.6:1) to the adapter ligation product. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min.
- 4) Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), aspirate the supernatant and discard.
- 5) Keep the tube in the magnetic stand, add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads, and incubate at room temperature for 30 sec. Aspirate the ethanol and discard.
- 6) Repeat step 5) once for a total of two washes.
- 7) Remove residual ethanol with a 10 μL - pipette tip. Keep the tube in the magnetic stand, open the lid and air-dry the beads until cracks just appear (about 5 min).

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8) Remove the tube from the magnetic stand and elute the DNA:

- If the product does not need to be size selected, add 21 μL ddH<sub>2</sub>O directly. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min. Spin the tube briefly and place it on magnetic stand. When the solution is clear (about 5 min), transfer 20 μL of supernatant to a new PCR tube carefully without touching the magnetic beads.
- If the product needs to be size selected, add 102 μL ddH<sub>2</sub>O directly. Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min. Spin the tube briefly and place it on magnetic stand. When the solution is clear (about 5 min), transfer 100 μL of supernatant to a new PCR tube carefully without touching the magnetic beads.

Note: If the purified product needs to be stored, it can be eluted with TE Buffer.

3.3.2 Size Selection of Adapter-ligated DNA

- 1) Equilibrate the Hieff NGS® DNA Selection Beads at room temperature for at least 30 min. Prepare 80% ethanol.
- 2) Resuspend the beads thoroughly by vortexing or shaking the bottle.
- 3) Based on the targeted sizes, add the first round of beads to the 100 μL purified DNA templates according to Table 8. Mix thoroughly by vortexing or pipetting 10 times.

Table 8 Recommended Beads:DNA ratios for beads-based size selection

Inserted DNA library size	150 - 250 bp	200-300 bp	300-400 bp	400-500 bp
Final DNA library size	250-350 bp	350-450 bp	450-550 bp	550-650 bp
Volume ratio in the 1 <sup>st</sup> round (Beads:DNA)	0.80×	0.70×	0.60×	0.55×
Volume ratio in the 2 <sup>nd</sup> round (Beads:DNA)	0.20×	0.20×	0.20×	0.15×

Note: "×" in the table indicates the volume of DNA sample. For example, if the insert length of the library is 250 bp and the sample DNA volume is 100 μL, the volume of magnetic beads used in the first round of sorting is 0.7×100 μL=70 μL; the volume of magnetic beads used in the second round of sorting is 0.20× 100 μL=20 μL. The recommended bead volume in the table is for the adapter-ligated DNA. If size selection procedure is performed before ligation, please refer to the manufactural protocols of Hieff NGS® DNA Selection Beads (Cat#12601).

- 4) Incubate at room temperature for 5 min.
- 5) Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), transfer the supernatant to a new PCR tube.
- 6) Add the second round of selection beads to the sample from step 5) according to Table 8. Mix thoroughly by vortexing or pipetting up and down at least 10 times.
- 7) Incubate at room temperature for 5 min.
- 8) Spin the tube briefly and place it onto magnetic stand. When the solution is clear (about 5 min), aspirate the supernatant and discard.
- 9) Keep the tube in the magnetic stand. Add 200 μL of freshly prepared 80% ethanol to rinse the magnetic beads and incubate at room temperature for 30 sec. Aspirate the ethanol and discard.
- 10) Repeat step 9) once for a total of two washes.
- 11) Remove residual ethanol with a 10 μL - pipette tip. Keep the tube in the magnetic stand, open the lid and air-dry the selection beads until cracks just appear (about 5 min).
- 12) Remove the tube from the magnetic stand. Add 21 μL ddH<sub>2</sub>O and mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 min.
- 13) Spin the tube briefly and place it on magnetic stand. When the solution is clear (about 5 min), transfer 20 μL of supernatant to a new PCR tube carefully without touching the magnetic beads.

3.4 Library Amplification

In this step, the adapter-ligated DNA is amplified with PCR amplification after cleanup or size selection.

3.4.1 Thaw the reagents mentioned in Table 9. Mix thoroughly and place them on ice for later use.

3.4.2 Add the following reagents to the product of step 3.3:

Table 9 Reaction system for PCR amplification

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Components	Volume (μL)
Adapter Ligated DNA (Product from step 3.3)	20
Canace® Pro Amplification Mix	25
Primer mix	5

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3.4.3 Mix thoroughly by vortexing or pipetting several times. Spin the reaction solution briefly to the bottom of the tube.  
3.4.4 Place the PCR tube to a thermocycler and run the reaction programs in Table 10.

Table 10 Reaction programs for PCR amplification

Temperature	Time	<u>cycles</u>
98°C	1 min	1
98°C	10 sec	Refer to Table 3
60°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	Hold	-

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3.5 Cleanup and Size-Selection of PCR product

The cleanup steps refer to 3.3.1. Hieff NGS® DNA Selection Beads (0.9×, Beads:DNA=0.9:1) is used to purify the PCR product.  
If size selection is needed, please refer to 3.3.2.

3.6 DNA Library Quality Control

The quality of the constructed DNA library can be evaluated by concentration detection and size distribution detection. For details, please refer to Note 6.

3.7 Examples

3.7.1 Figure 3 showed the size distribution (analyzed with Agilent 2100 DNA 1000 Chip) of the libraries that is constructed with 100 ng of FFPE input DNA (without size-selection) using the Hieff NGS® Ultima Pro DNA Library Prep Kit for Illumina®.

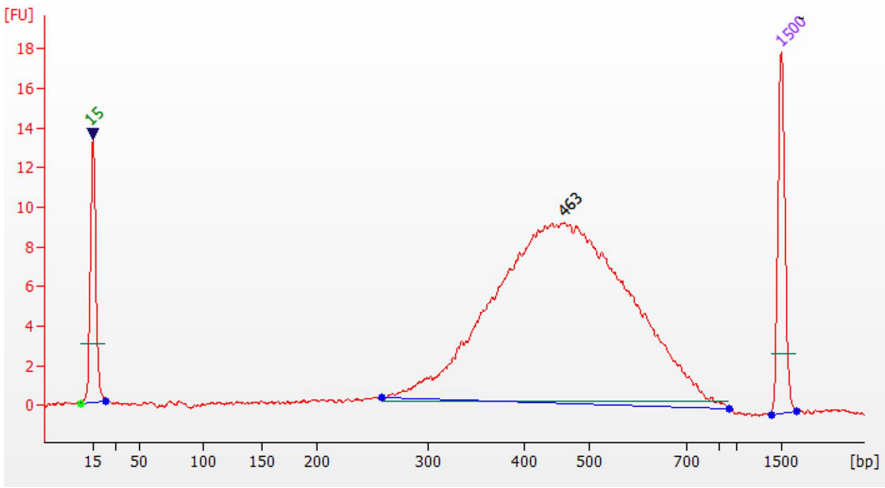


Figure 2 Ultima Pro DNA library prep kit used for the library construction with FFPE samples (without size-selection)

3.7.2 Figure 3 showed the size distribution (analyzed with Agilent 2100 DNA 1000 Chip) of the libraries that is constructed with 10 ng of cfDNA using the Hieff NGS® Ultima Pro DNA Library Prep Kit for Illumina®.

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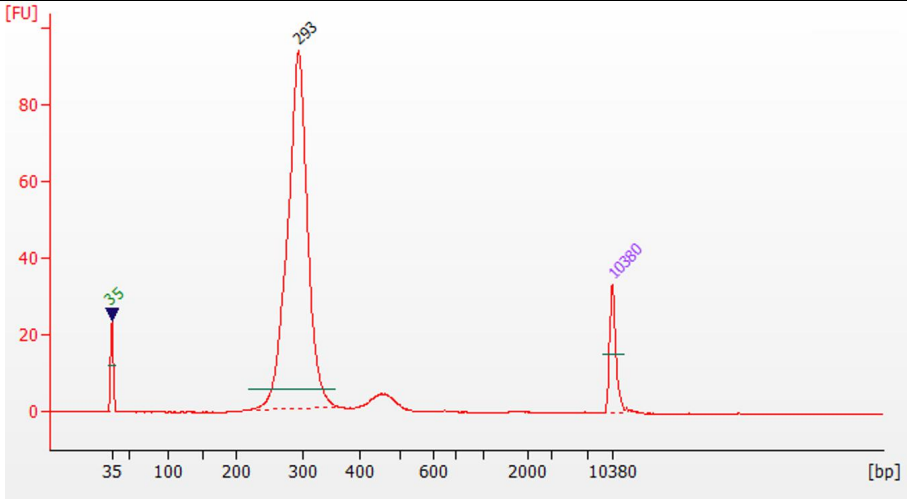


Figure 3 Ultima Pro DNA library prep kit used for the library construction of cfDNA samples

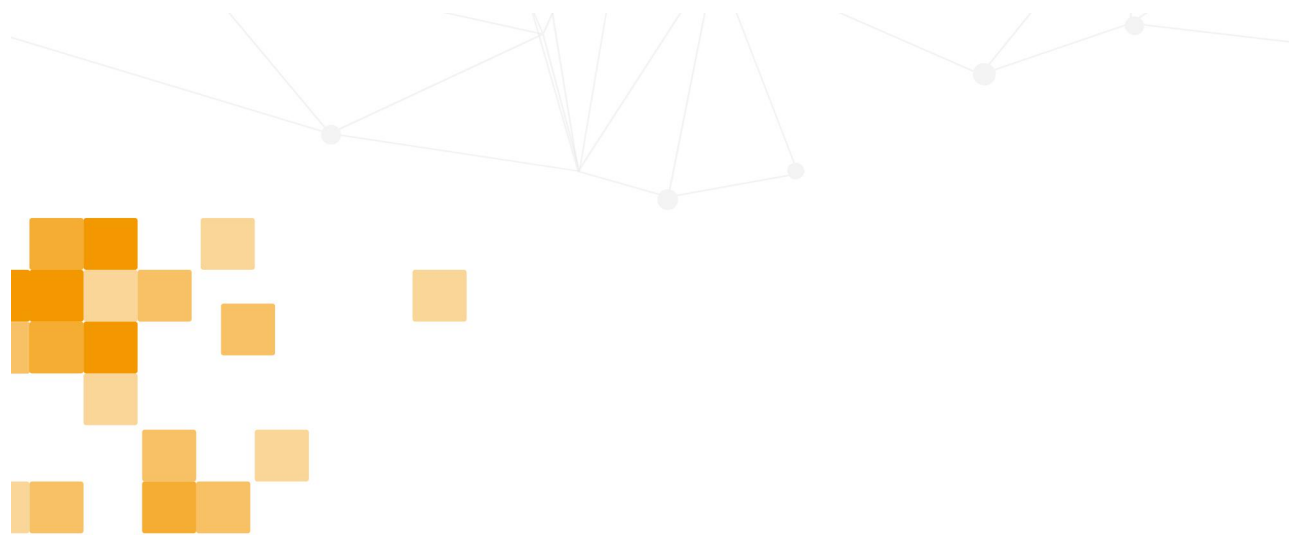
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