Hieff NGS™ OnePot Pro DNA Library Prep Kit for Illumina

12205

INSTRUCTIONS FOR USE

Ver. HB221028

CONTENT

Product description

Hieff NGS™ OnePot Pro DNA Library Prep Kit for Illumina is a new generation enzymatic fragmentation-based library prep kit professionally developed for Illumina high-throughput sequencing platform. This set simplifies the operation process compared with the traditional library construction method and also greatly reduces the time and cost by performing fragmentation, end repair and dA-tailing of dsDNA in one reaction. This library prep kit has an excellent library conversion rate and is applicable for samples from all common animals, plants, microorganisms, etc., and also the FFPE samples. This upgraded kit using the latest optimized ligase greatly decreases the self-ligation rate during adapter ligation. Moreover, the introduction of a new high-fidelity polymerase further improves the homogeneity and fidelity of amplification.

Components

Components No.		Components	12205ES24	12205ES96
12205-A	●	Smearase™ Buffer	240 μL	960 μL
12205-B	●	Smearase™ Enzyme	120 μL	480 μL
12205-C	●	Ligation Enhancer	720 μL	3×960 μL
12205-D	●	Novel T4 DNA Ligase	120 μL	480 μL
12205-E	○	Canace™ Pro Amplification Mix	600 μL	4×600 μL
12205-F	○	Primer Mix	120 μL	480 μL

Specifications

Product Type	Library Preparation Kit
Libraries	Fragment Library
Fragmentation method	Enzyme
Input amount	100 pg - 1000 ng
For Use With (Equipment)	Illumina™ Platforms
Sample Type	gDNA
Sequencing Type	Genome & DNA Sequencing
Product Line	DNA library constrction
For Use With (Application)	NGS library preparation
Quantity	/24/96 Reaction

Shipping and Storage

Dry ice shipping. -15°C ~ -25°C storage, valid for one years.

Instructions

1. Required Materials Not Included

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

1.2 DNA quality control: Agilent Technologies 2100 Bioanalyzer or equivalent devices.

1.3 DNA Adapter:such as YEASEN Complete Adapters for illumina platform (Cat#12615~12618); or other types of adapters.

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

2. Workflow

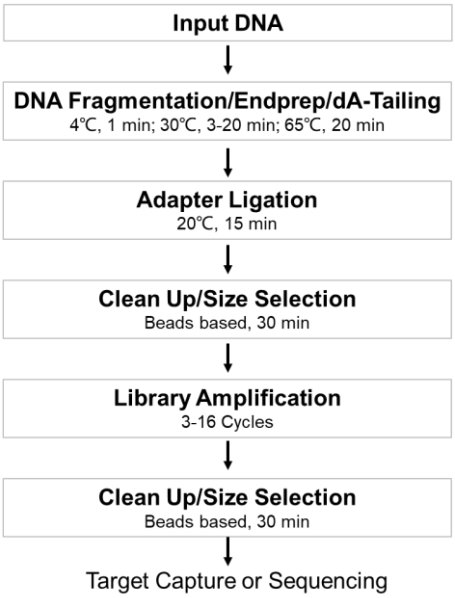

Figure 1. Workflow for OnePot Pro DNA library prep kit for Illumina

3. Operation steps

3.1 DNA Fragmentation/End Repair/dA-Tailing

This step performs genomic DNA samples fragmentation, end-repair and dA-tailing in one reaction.

3.1.1 Thaw the reagents list in Table 3. Invert and mix thoroughly, and place them on ice for later use.

3.1.2 Prepare the reactions on ice according to Table 1.

Table 1. Reaction Assembly for DNA Fragmentation/ End Repair/ dA-Tailing

Regent Name	Volume(μL)
Input DNA	x
Smearase™ Buffer	10
Smearase™ Enzyme	5
TE	Up to 60

3.1.3 Gently mix by pipetting or shaking, Centrifuge briefly to get the solution down.

3.1.4 Place the tube in a thermocycler and set the program according to table 2 to perform DNA fragmentation, end-repair, and dA-tailing reaction.

Table 2. Program setup for DNA Fragmentation/ End Repair/ dA-Tailing

Temperature	Duration
Heat lid to 105°C	On
4 °C	1 min*

30 °C	3-20 min**
65 °C	20 min
4 °C	Hold

Note:

*Pre-set the program to 4℃ to effectively control the fragmentation performance and to avoid over-fragmentation. Please place the reaction tube into the thermocycler after the heat block is cooled to 4℃.

**Please refer to Table3 for the fragmentation of intact genomic DNA. The fragmentation time is recommended to extend by another 2-4 minutes if the input DNA amount is 500-1000 ng. For FFPE DNA samples with uncertain quality, please refer to Table 4.

Table 3. Guideline for choosing fragmentation time for standard DNA

Insert peak size	Fragmentation Time	Modification Range
600 bp	8 min	6-12 min
350 bp	10 min	8-14 min
250 bp	12 min	10-15 min
200 bp	15 min	13-18 min
150 bp	20 min	15-25 min

Table 4. Guideline for choosing fragmentation time for FFPE DNA

Insert peak size	Fragmentation Time	DIN*
250 bp	9-13 min	> 8.0
250 bp	8-11 min	6.5-8.0
250 bp	4-8 min	4.2-6.5
250 bp	3-6 min	2.5-4.2

Note: *DIN is DNA Integrity Number, which a method to define the degree of FFPE DNA degradation by using Agilent 2200. Please refer to figure 2 for details.

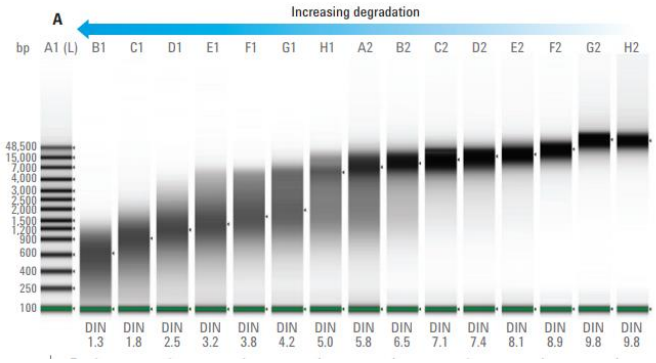

Figure 2. DIN values of DNA samples with different degrees of degradation defined by Agilent 2200

3.2 Adapter Ligation

This step enables the product from step 3.1ligated with special Illumina adapters.

3.2.1 Please refer to table 1 to dilute the adapter to appropriate concentration according to the amount of input DNA.

3.2.2 Thaw the reagents list in table 5, invert and mix thoroughly, and place them on ice for later use.

3.2.3 Add the reagents list in table 6 to step 3.1 PCR tube.

Table 5. Reaction Assembly for Adapter Ligation

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

Note: *The Ligation Enhancer is viscous. Please mix thoroughly by inverting or vortexing and briefly centrifuge before use.

**The concentration of the adapters in this kit is 15 μM, which is consistent with that of most commercialized adapters currently available.

[Example for calculation the volume of Adapter]: How much adapters should be added when Input DNA is 100 ng and Input DNA length is 300 bp?

Step 1: Calculate the number of moles of the input DNA. Formula: Input DNA moles (pmol)≈ Input DNA mass (ng)/ [0.66 × Input DNA average length(bp)]; Input DNA moles (pmol) =100 ÷ (0.66×300) = 0.5 pmol;

Step 2: Calculate the number of moles of the adapters to be added. Query the recommended molar ratios of adapter: input DNA according to table 1 in Note 3. According to table 1, when the input DNA is 100 ng, the recommended molar ratio of adapter to input DNA is 100:1, so the adapter moles =100×0.5 pmol=50 pmol;

Step 3: Calculate the volume of the adapters to be added. Adapter concentration =15 μmol/L (Note: If using other adapters, please refer to their concentrations accordingly); Adapter volume (μL) = adapter moles (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: Please don't add more than 5 μL of adapters).

3.2.4 Gently mix by pipetting or shaking, and centrifuge briefly to get the solution down.

3.2.5 Put the tube into a thermocycler and set up the program according to table 6 to start the adapter ligation reaction.

Table 6. Program setup for Adapter Ligation

Temperature	Duration
Heat lid to 105°C	Off
20°C	15 min
4°C	Hold

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

3.3 Post-ligation Clean-up or Size-selection

This step is to purify or size-select the product in step 3.2 with magnetic beads. The purification can remove residue adapters, adapter dimers, or other unusable products.

Clean-up:

3.3.1 Preparation: take the Hieff NGS™ DNA Selection Beads out of the fridge, and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol freshly.

3.3.2 Thoroughly mix the beads by inverting or vertexing.

3.3.3 Add 60 μL Hieff NGS™ DNA Selection Beads (0.6×, Beads : DNA = 0.6:1) to the tube containing the adapter-ligated product in step 3.2, shack and mix well, and incubate at room temperature for 5 minutes.

3.3.4 Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully remove the liquid.

3.3.5 Keep the tube on the magnetic stand, directly add 200 μL freshly prepared 80% ethanol to the tube. Incubate

at room temperature for 30 seconds and carefully remove the liquid.

3.3.6 Repeat step 5 again.

3.3.7 Keep the tube on the magnetic stand, open the cap and dry the beads until the beads are just cracked (no more than 5 minutes).

3.3.8 Take the tube off the magnetic stand for elution:

1) If size-selection is not required, directly add 21 μL ddH_2O. Thoroughly mix by vertexing or pipetting and incubate at room temperature for 5 minutes. (Note: if the purified product needs to be stored, please elute it in TE Buffer.) Briefly centrifuge and place it on the magnetic stand. After the magnetic beads are completely adsorbed (about 5 min), carefully transfer 20 μL supernatant to a new PCR tube without aspirating the beads.

2) If the size-selection is required, directly add 102 μL ddH_2O. Thoroughly mix by vertexing or pipetting and incubate at room temperature for 5 minutes. (If the purified product needs to be stored, please elute it in TE Buffer.) Briefly centrifuge and place it on the magnetic stand. After the magnetic beads are completely adsorbed (about 5 min), carefully transfer 100 μL supernatant to a new PCR tube without aspirating the beads.

Size-selection:

3.3.1 Preparation: take the Hieff NGS™ DNA Selection Beads out of the fridge, and equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol freshly.

3.3.2 Thoroughly mix the beads by inverting or vertexing.

3.3.3 Based on the targeted insert sizes, add the first round of beads to the 100 μL purified DNA templates according to table 7. Mix by vertexing or pipetting 10 times.

Table 7. Recommended molar ratios of Beads to DNA for size-selections

Targeted insert size	150-250 bp	200-300 bp	300-400 bp	400-500 bp	500-600 bp
Expected final library size	250-350 bp	350-450 bp	450-550 bp	550-650 bp	650-750 bp
First round Beads:DNA ratio	0.80×	0.70×	0.60×	0.55×	0.50×
Second round Beads:DNA ratio	0.20×	0.20×	0.20×	0.15×	0.15×

Note: "×" in the above table refers to the volume of the purified DNA templates. For example, if targeting 250 bp insert size for a DNA sample with 100 μL, then the volume of the first round of the beads is 0.70×100 μL=70 μL, and the volume of the second round of the beads is 0.20×100 μL=20 μL. The recommended ratios mentioned in this table are for adapter-ligated DNA templates. If performing size selection before adapter ligation, please refer to the recommended ratios mentioned in the user manual of Hieff NGS™ DNA Selection Beads (Cat#12601).

3.3.4 Incubate at room temperature for 5 minutes.

3.3.5 Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully transfer the supernatant to a new tube.

3.3.6 Add the second round of beads to the supernatant according to table 9.

3.3.7 Thoroughly mix by vertexing or pipetting 10 times. Incubate at room temperature for 5 minutes.

3.3.8 Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully remove the liquid.

3.3.9 Keep the tube on the magnetic stand, directly add 200 μL freshly prepared 80% ethanol to the tube. Incubate at room temperature for 30 seconds and carefully remove the supernatant.

3.3.10 Repeat step 9 again.

3.3.11 Keep the tube on the magnetic stand, open the cap and dry the beads until the beads are just cracked (no more than 5 minutes).

3.3.12 Take the tube off the magnetic stand, add 21 μL ddH2O. Thoroughly mix by vertexing or pipetting and incubate at room temperature for 5 minutes.

3.3.13 Centrifuge briefly to get the solution down, and place the centrifuge tube on the magnetic rack. After the magnetic beads are completely adsorbed (about 5 min), carefully transfer 20 μL supernatant to a new tube without aspirating the beads.

3.4 Library Amplification

This step can enrich the purified or size-selected products by PCR amplification.

3.4.1 Thaw the reagents list in table 8, invert and mix thoroughly, and place them on ice for later use.

3.4.2 Assemble the following reaction in a sterilized PCR tube.

Table 8. Reaction Assembly for Library Amplification

Components	Volume(μL)
Purified Adapter-Ligated DNA	20
Canace™ Pro Amplification Mix	25
Primer Mix	5

3.4.3 Gently mix by pipetting or shaking, and centrifuge briefly to get the solution down.

3.4.4 Put the tube into a thermocycler and set up the program according to table 9 to start the amplification.

Table 9. Program Setup for Library Amplification

Temperature	Duration	Cycle number
98°C	1 min	1
98°C	10 sec	Refer to table 2 in the Precautions
60°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	Hold	-

3.5 Post-Amplification Clean-up/Size Selection

Operation is same as that in step 3.3.1 (Post-ligation Clean-up). Please use the Hieff NGS™ DNA Selection Beads with 0.9× beads (Beads : DNA = 0.9:1) to purify the amplified libraries.

If size-selection is needed, please directly refer to step 3.3.2 (Post-ligation Size-selection).

3.6 Quality Control of the Final Libraries

The quality of the constructed library is generally evaluated by measuring the concentration and size distribution. For details, please refer to Note 6.

3.7 Reference Example

Digestion 200 ng of Human gDNA (NA12878) samples using the Hieff NGS™ OnePot Pro DNA Library Prep Kit for Illumina, the fragmentation effects are shown in Figure 3, and the final library size distribution are shown in Figure3.

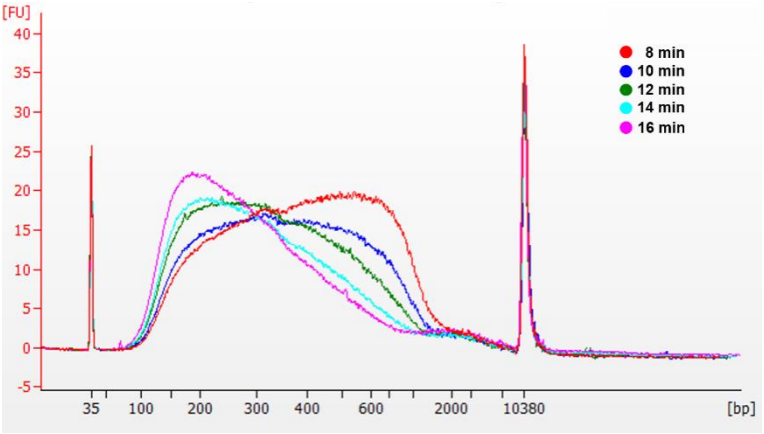

Figure 3 OnePot Pro DNA library prep kit used for the fragmentation of 200 ng Human gDNA (Fragmentation result with various fragmentation time)

Notes

1. Operation

1.1 For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.

1.2 Please thaw the component of the kit at room temperature before use, invert and mix thoroughly, centrifuge briefly and place on ice for later use.

1.3 Please mix the reaction reagents by pipetting or vortexing gently. Vigorous vortexing may impact the library yield.

1.4 To avoid cross-contamination of samples, it is highly recommended to use filter pipette tips and change the pipette tips when drawing different samples.

1.5 It is highly recommended to pre-heat the thermocycler to the reaction temperature set for each reaction step before use.

1.6 Improper operations likely cause aerosol pollution, affecting the accuracy of the experiment. Mandatory physical isolation of PCR reaction system preparation area and PCR product purification area is recommended. Please use special pipette tips and other equipment, and regularly clean each area (wipe with 0.5% sodium hypochlorite or 10% bleach) to ensure the cleanliness of the experimental environment.

1.7 This product is used for research only!

2. DNA Fragmentation

2.1 This kit is compatible with 500 pg - 1 μg of input DNA. High quality input DNA (A260/A280 = 1.8-2.0) is highly recommended.

2.2 High concentration of metal ion chelator or other salt remained in Input DNA may affect subsequent experiments, it is recommended to dilute DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for fragmentation.

2.3 For conventional high-quality genomic DNA, fragmentation time refers to Table 5. This product is compatible with various samples with different GC content and has minimal bias. For the FFPE samples with different degrees of degradation, the fragmentation time refers to Table 6. Customers need to fine-tune the recommended fragmentation time in their own experimental system to achieve the best results.

2.4 The preparation process of fragmentation reaction should be operated on ice to ensure accurate fragmentation effect.

2.5 For FFPE DNA library construction, if the sample quality is poor and the customer is not satisfied with the results,

you can choose our FFPE DNA Repair Reagent (Cat#12606) for FFPE DNA Repair. Please refer to this product instruction for specific usage. This reagent can be performed simultaneously with the fragmentation/end-repair/A-tailing reaction without additional operations.

3. Adapter Ligation

3.1 For the Illumina platform: YEASEN provides Complete Adapter Kit(Cat#12615~Cat#12618); 96 Single Index Primers (Cat#12611~Cat#12612);384 Unique Dual Index (UDI) Primers(Cat#12404~Cat#12407).

3.2 The quality and concentration of the adapter will directly affect the ligation efficiency and the library yield. Too much adapters may cause more adapter dimers while too little adapters reduce ligation efficiency and library yield. The adapter usage recommended for different amounts of DNA input are list in Table 10.

Table 10. Recommended adapter usage 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 mole (pmol)≈ Input DNA mass (ng)/ [0.66 × Input DNA average length(bp)].

4. Beads Clean-up and Size-selection

4.1 DNA size-selection can be performed before end repair/dA-tailing, after adapter ligation, or after library amplification.

4.2 Size-selection is recommended to perform right after adapter ligation if the input DNA amount is ⩾ 50 ng or after library amplification if the input DNA amount is < 50 ng.

4.3 The high concentration of PEG in Ligation Enhancer can have a significant impact on size-selection. Thus, if size-selection is performed right after adapter ligation, a beads clean-up step must be performed first following by size-selection. If size-selection is performed before end repair/dA-tailing or after library amplification, you can directly proceed to size-selection without purification.

4.4 The magnetic beads should be equilibrated to room temperature before use, otherwise the yield and the size-selection performance will be affected.

4.5 Please thoroughly mix the magnetic beads by vortexing or pipetting before each use.

4.6 Do not aspirate the magnetic beads when transferring the supernatant, even trace amounts of beads residue can impact the subsequent library quality.

4.7 Please prepare fresh 80% Ethanol used for rinsing the magnetic beads to ensure the recovery efficiency.

4.8 When performing size-selection, the initial sample volume should be ⩾100 μL. If not, it is recommended to bring the volume up to 100 μL with ultra-pure water to avoid much more pipetting error.

4.9 The beads should be allowed to dry at room temperature before elution. Insufficient drying will easily cause anhydrous ethanol residue to affect subsequent reactions; excessive drying will cause the magnetic beads to crack and further reduce purification yield. Typically, drying for 3-5 minutes at room temperature is sufficient to allow the beads to dry sufficiently.

4.10 The purified or size-selected DNA samples are recommended to store in TE buffer instead of ultra-pure water at 4°C for 1-2 weeks or at -20°C for about 1 month.

5. Library Amplification

The number of amplification cycles should be strictly controlled. Insufficient amplification cycles can lead to low library yield; Over-amplification can lead to a variety of adverse consequences such as increased library preference, increased duplication reads, increased chimeric products, and accumulation of amplified mutations. The number of amplification cycles recommended for 500 pg - 1μg input DNA to obtain 1 μg products are list in table 11.

Table 11. Recommended amplification cycle for 500 pg-1 μg input DNA to abtain 1 μg product

Input DNA (ng)	Number of cycles required to generate
	1 μg
1 μg	3 - 5**
500 ng	4 - 6
200 ng	5 - 7
50 ng	7 - 9
1 ng	13 - 15
500 pg	14 - 16

Note: **The amplification cycles list in above table are used to construct library with high-quality human gDNA. When DNA quality is poor or size-selection is required, library amplification is performed with reference to a higher cycle number.

6. Library Quality Analysis

6.1 The quality of the constructed library is generally evaluated by measuring the concentration and size distribution.

6.2 The library concentration can be measured by methods based on double-stranded DNA fluorescent dyes such as Qubit and PicoGreen or methods based on qPCR absolute quantification.

6.3 Methods based on spectral detection such as NanoDrop are not recommended to library quantification.

6.4 The qPCR method is recommended for library quantification: methods based on double-stranded DNA fluorescent dyes such as Qubit and PicoGreen can not effectively distinguish the products of inserts without double-end adapters, inserts with single-end adapter or other incomplete dsDNA structures. Otherwise, the qPCR method will only amplify the complete libraries with double-end adapters (namely the ready to sequence libraries), thus providing a more accurate quantification method.

6.5 It is recommended to detect the library size-selection by devices based on capillary electrophoresis or microfluidic principles such as Agilent Bioanalyzer 2100.

YEASEN

To enable success of our customers
Together to make a healthier and brighter world

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