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DNA Library Prep Kit for Illumina Sequencing

07/20

(Catalog # K1475-12 Rxns; Store at Multiple Temperatures)

I. Introduction:

BioVision's DNA Library Prep Kit for Illumina Sequencing is used to efficiently convert (2 ng-500 ng) of DNA fragments into fully compatible libraries for Illumina Next Generation Sequencing (NGS) platforms. The sample preparation step involves the preparation of a DNA library by fragmentation, end repair, adenylation, ligation with adapter sequences and amplification. The DNA library is then loaded onto the chips for amplification and sequencing. The presence of adapter sequences allows the hybridization of the libraries to the sequencing chips and provides a universal priming site for sequencing primers. **BioVision's DNA Library Prep Kit** contains index adapters needed for running multiple samples on a single Illumina flow cell as well as the magnetic beads for purification, which is required for the reaction cleanup after ligation and PCR steps. The entire procedure can be completed within 2 hours.

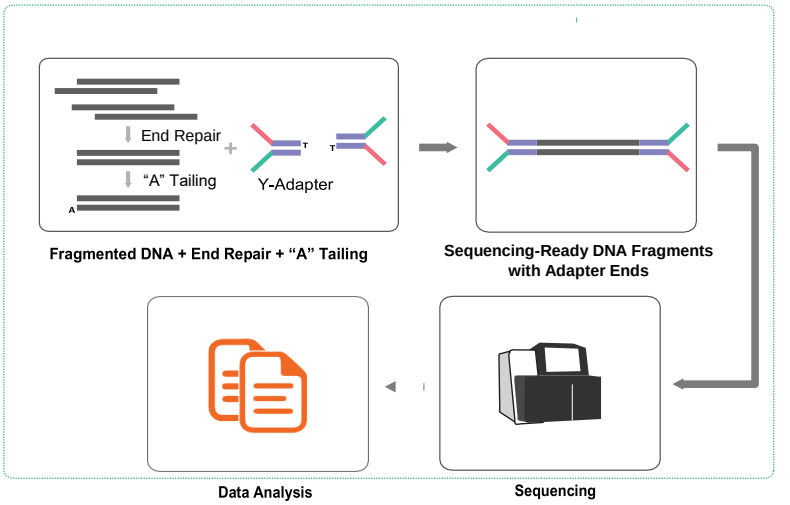

Figure 1. DNA Library Preparation for Illumina Sequencing Workflow

II. Applications:

- Library preparation for Whole Genome Sequencing, ChIP-Seq, Amplicon Sequencing and cDNA Sequencing

III. Key Features:

- High quality Illumina compatible libraries
- Minimal hands on time and short 2 hour library preparation workflow
- Wide range of input amounts (2 ng-500 ng) and sample types, including amplicons and fragmented DNA

IV. Sample Types:

Purified fragmented DNA (recommended size range of 200-500 bp) or PCR amplicons

V. Shipping and Storage Conditions:

The kit is shipped in a gel pack. All reagents except the Magnetic Purification Beads and the Ligation Enhancer should be stored at -20°C upon arrival. The Magnetic Purification Beads and Ligation Enhancer should be kept at 4°C. Avoid repeated freeze-thaw cycles. The kit reagents are stable for 12 months if stored properly.

VI. Kit Contents:

Components	K1475-12 (12 Rxns)	Part Number
10X End Repair Buffer	40 µl	K1475-12-1
End Repair Enzyme Mix	12 µl	K1475-12-2
Ligation Enhancer	75 µl	K1475-12-3
Ligation Buffer	120 µl	K1475-12-4
Ligation Enzyme Mix	55 µl	K1475-12-5
Index Adapters	12 x 5 µl	K1475-12-6
Magnetic Purification Beads	2 x 1.5 ml	K1474-12-7
PCR Primer Mix	70 µl	K1475-12-8
2X PCR MasterMix	330 µl	K1474-12-9
Nuclease-free water	1 ml	K1474-12-10

VII. User Supplied Reagents and Equipment:

- 80% Ethanol
- 10 mM Tris Buffer, pH 8.5
- Purified Fragmented DNA
- Magnetic rack/stand for magnetic beads
- PCR tubes
- 1.5 ml microcentrifuge tubes
- Thermal cycler

VIII. DNA Library Prep Protocol:

Input DNA: 2 ng-500 ng of purified fragmented DNA (recommended size,200 bp-500 bp) or PCR amplicons.

A. End Repair:

1. Briefly vortex the **10X End Repair Buffer**. Mix the **End Repair Enzyme Mix** tube by inverting the tube several times.
2. Add the following components to a new PCR tube. Keep the **End Repair Enzyme Mix** on ice while adding to the reactions.

End Repair Reaction Mixture	
Input DNA	2 ng-500 ng (in µl)
10X End Repair Buffer	3 µl
End Repair Enzyme	1 µl
Nuclease-free water	26-DNA volume µl
Total Reaction Volume	**30 µl**

3. Mix thoroughly by pipetting. Briefly spin to collect all the liquid at the bottom of the tube.
4. Place on the preprogrammed thermal cycler and run the End Repair program.

End Repair Program
Preheated lid 100°C, Volume 30 µl
20°C for 10 min
65°C for 10 min
Hold at 10°C for 10 min

5. Once the sample has reached 10°C, proceed to protocol B, **Adapter Ligation** immediately.

B. Adapter Ligation:

1. Dilute the Index Adapters (in the table shown below) according to input DNA amount using nuclease-free water.

Input DNA amount (ng)	**Adapter Dilution**
< 5 ng	1:30
5 ng-99 ng	1:10
100 ng-500 ng	No dilution required

2. Add the following components to the End Repair reaction mixture. Briefly vortex the **Ligation Buffer**. Mix the **Ligation Enzyme Mix** tube by inverting the tube several times. Keep the Ligation Enzyme Mix on ice while adding to reactions. If multiple samples are being prepared at the same time, use a different **Index Adapter** for each sample.

Adapter Ligation Reaction Mixture	
End Repair Reaction Mixture (from step A)	30 µl
Ligation Enhancer	5 µl
Ligation Buffer	9 µl
Ligation Enzyme Mix	4 µl
Index Adapter	2.5 µl
Total Reaction Volume	**50.5 µl**

3. Mix thoroughly by pipetting. Briefly spin to collect all liquid at the bottom of the tube.
4. Place on the preprogrammed thermal cycler and run the Adapter Ligation program. The heated lid should be off or set to 25°C.

Adapter Ligation Program:
Heated lid off, Volume 50 µl
20°C for 15 min

5. Proceed to Step C, Clean Up Adapter-ligated DNA immediately.

C. Clean Up Adapter-ligated DNA:

Follow **protocol C1:** For size selection protocol (recommended for fragmented gDNA ≥10 ng).

Follow **protocol C2:** For non-size selection protocol (recommended for fragmented gDNA < 10 ng or PCR amplicons).

If your magnetic rack or stand is not suitable for use with PCR tubes or plates, use a pipette to transfer the Adapter Ligation Reaction Mixture into an appropriate tube or plate before proceeding.

C1: Dual Size Selection:

1. Bring **Magnetic Purification Beads** to room temperature (RT).
2. Prepare 420 µl of fresh 80% ethanol for each sample.
3. Vortex **Magnetic Purification Beads** until fully homogenized.
4. Add 40 µl **Magnetic Purification Beads** to each Adapter Ligation Reaction Mixture and mix thoroughly by pipetting.
5. Incubate at RT for 10 min.
6. Place on a **magnetic stand** without shaking for 2 min or until the liquid is clear.
7. Carefully transfer 80 µl of the supernatant to a new tube/well. Discard the beads.
8. Add 100 µl of **Magnetic Purification Beads** to each tube and mix thoroughly by pipetting.
9. Incubate at RT for 10 min.
10. Place on a magnetic stand without shaking for 2 min or until the liquid is clear.
11. With a pipette, remove and **discard all of the supernatant** from each tube/well.
12. Wash two times with freshly prepared 80% Ethanol as follows:
    a. Add **200 µl 80% Ethanol** to each tube/well.
    b. Incubate on the magnetic stand for 30 seconds.
    c. Remove and discard all supernatant from each tube/well.
    d. Remove residual 80% Ethanol with a pipette if necessary.
13. Allow the beads to dry for 2-5 min on the magnetic stand (glossy appearance disappears, but before cracks appear).
14. Remove the tube or plate from the magnetic stand.
15. Add 21.5 µl **Tris Buffer** to each tube/well and mix thoroughly to fully resuspend the beads.
16. Incubate at RT for 2 min.
17. Place on a magnetic stand and wait for the liquid to clear (30 sec).
18. Transfer 20 µl of the supernatant to a new PCR tube or plate.
19. Proceed to **protocol D, PCR Amplification**.

C2: No Size Selection:

1. Bring **Magnetic Purification Beads** to RT.
2. Prepare 420 µl of fresh 80% ethanol for each sample.
3. Vortex **Magnetic Purification Beads** until fully homogenized.
4. Add 55 µl **Magnetic Purification Beads** to Adapter Ligation Reaction Mixture and mix thoroughly by pipetting.
5. Incubate at RT for 10 min.
6. Place on a **magnetic stand** without shaking for 2 min or until the liquid is clear.
7. With a pipette, remove and discard all of the supernatant from each tube/well.
8. Wash two times with freshly prepared **80% Ethanol** as follows:
    a. Add **200 µl 80% Ethanol** to each well.
    b. Incubate on the magnetic stand for 30 sec.
    c. Remove and discard all supernatant from each well.
    d. Remove residual 80% Ethanol with a pipette if necessary.
9. Allow the beads to dry for 2-5 min on the magnetic stand (glossy appearance disappears, but before cracks appear).
10. Remove tube or plate from the magnetic stand.
11. Add 21.5 µl **Tris Buffer** to each well and mix thoroughly to fully resuspend the beads.
12. Incubate at RT for 2 min.
13. Place on a magnetic stand and wait for the liquid to clear (30 sec).
14. Transfer 20 µl of the supernatant to a new PCR tube or plate.
15. Proceed to **protocol D, PCR Amplification**

D. PCR Amplification:

1. Add the following components to the purified adapter ligated DNA sample. Mix the **2X PCR MasterMix** tube by inverting the tube several times or flicking the side of the tube. Do not vortex. Keep 2X PCR MasterMix on ice while adding to reactions.

Library Amplification Mixture	
Purified Adapter Ligated DNA (from step C)	20 µl
PCR Primer Mix	5 µl
2X PCR MasterMix	25 µl
Total Reaction Volume	50 µl

2. Place the reactions on the preprogrammed thermal cycler and run the Library Amplification program. Refer to the table below for the recommended cycle number.

Library Amplification Program	
Preheated lid 100°C, Volume 50 µl	
98°C for 30 sec	
98°C for 10 sec	Cycle x times
60°C for 30 sec	
72°C for 30 sec	
72°C for 5 min	
Hold at 10°C	

Amount of Input DNA	Recommended No. of PCR Cycles
500 ng	5-6
250 ng	6-7
100 ng	7-8
50 ng	8-9
10 ng	9-10
2 ng	10-12

3. Proceed to step **E, Clean Up Amplified DNA Libraries** immediately.

E. Clean Up Amplified DNA Libraries:

If your magnetic rack or stand is not suitable for use with PCR tubes or plates, use a pipette to transfer the Library Amplification Mixture into an appropriate tube or plate before proceeding.

1. Bring **Magnetic Purification Beads** to RT.
2. Prepare 420 µl of fresh 80% ethanol for each sample.
3. Vortex **Magnetic Purification Beads** until fully homogenized.
4. Add 55 µl **Magnetic Purification Beads** to each well and mix thoroughly.
5. Incubate at RT for 10 min.
6. Place on a magnetic stand without shaking for 2 min or until the liquid is clear.
7. With a pipette, remove and discard all of the supernatant from each well.
8. Wash two times with freshly prepared **80% Ethanol** as follows:
    a. Add 200 µl 80% Ethanol to each well.
    b. Incubate on the magnetic stand for 30 sec.
    c. Remove and discard all supernatant from each well.
    d. Remove residual 80% Ethanol with a pipette if necessary.
9. Allow the beads to dry for 2-5 min on the magnetic stand (glossy appearance disappears, but before cracks appear).
10. Remove the tube or plate from the magnetic stand.
11. Add 21.5 µl **Tris Buffer** to each well and mix thoroughly to fully resuspend the beads.
12. Incubate at RT for 2 min.
13. Place on a magnetic stand and wait for the liquid to clear (30 sec).
14. Transfer 20 µl of the supernatant to a new PCR tube or plate.
15. Analyze libraries with a Qubit fluorometer and Agilent 2100 Bioanalyzer to confirm quantity, size distribution and quality prior to sequencing.

F. Adapter and Index Sequences:

Adapter Name	Index	Full Adapter Sequence
YA001	ATCACG	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CATCACGATCTCGTATGCCGTCTTCTGCTTG
YA002	CGATGT	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC CGATGTATCTCGTATGCCGTCTTCTGCTTG
YA003	TTAGGC	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC TTAGGCATCTCGTATGCCGTCTTCTGCTTG
YA004	TGACCA	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC TGACCAATCTCGTATGCCGTCTTCTGCTTG
YA005	ACAGTG	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC ACAGTGATCTCGTATGCCGTCTTCTGCTTG
YA006	GCCAAT	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGCCAATATCTCGTATGCCGTCTTCTGCTTG
YA007	CAGATC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCAGATCATCTCGTATGCCGTCTTCTGCTTG
YA008	ACTTGA	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CACTTGAATCTCGTATGCCGTCTTCTGCTTG
YA009	GATCAG	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGATCAGATCTCGTATGCCGTCTTCTGCTTG
YA010	TAGCTT	GATCGGAAGAGCACACGTCTGAACTCCAGT CACTAGCTTATCTCGTATGCCGTCTTCTGCTTG
YA011	GGCTAC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGGCTACATCTCGTATGCCGTCTTCTGCTTG
YA012	CTTGTA	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCTTGTAATCTCGTATGCCGTCTTCTGCTTG

IX. Related Products:

Product Name	Cat. No.	Size
Magnetic Beads for DNA Purification	M1502	5 ml
Cell & tissue genomic DNA extraction Kit	K1442	100 Preps
Mammalian Cell Genomic DNA Isolation Kit	K967	100 preps
PCR DNA extraction Kit	K1444	100 Preps
Gel and PCR DNA Purification Kit	K1455	50 Preps
RobustReady™ PCR Mix	M1130	200, 1000 Rxns
Breeze™ PCR Mix	M1134	200 Rxns
Tissue Advance™ PCR Kit	M1145	100 Preps
FireStart™ DNA Polymerase	M1149	250, 1000 Units
Advance™ DNA Polymerase	M1151	250, 1000 Units
T4 DNA Ligase (5 u/µl)	9101	250 Units
Link-FAST™ 5 Minutes DNA Ligation Kit	K902	50 Rxns
DNA Quantification Assay Kit (Fluorometric)	K539	200, 2000 Assays
EasyRNA™ Cell/Tissue RNA Mini Kit	K1337	50, 250 Preps

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