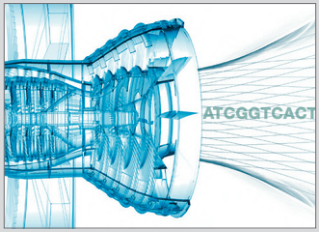

JetSeq™ ER and Ligation Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

JetSeq™ ER and Ligation Kit

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GENERAL INFORMATION

1. KIT CONTENTS

Cap Color	JetSeq ER and Ligation Kit Reagents	Volume
	End-repair Buffer, 5x	960 µL
	ER Enzyme Mix	576 µL
	Ligation Buffer, 5x	288 µL
	Ligase	192 µL
	DEPC-treated Water	1.8 mL (x2)

2. DESCRIPTION

The success of next-generation sequencing is dependent upon the precise and accurate processing of the input DNA. This requires high-quality library preparation using a coordinated series of standard molecular biology reactions whilst maintaining high yields during the intermediate purification steps.

The JetSeq™ ER and Ligation Kit is designed to generate high-quality next-generation sequencing (NGS) libraries suitable for sequencing on Illumina instruments. The kit contains all of the enzymes and buffers necessary for end-repair, A-tailing and ligation in convenient master mix formulations. It offers a streamlined workflow and flexibility where users are free to use adapters of their choice. Other advantages of JetSeq™ ER and Ligation Kit includes:

- Range of DNA input: 0.5 to 3 µg
- Increased speed: adapter-ligated libraries in under 2 hours
- Improved convenience: simpler protocol increases reproducibility

By combining end-repair and A-tailing in one unique step, the JetSeq™ ER and Ligation Kit is able to reduce total NGS library preparation time and minimize the variability caused by additional handling, as well as the risk of contamination or material loss.

Please read this manual carefully to familiarize yourself with the JetSeq™ ER and Ligation Kit protocol before starting.

3. STORAGE

When stored under the recommended conditions and handled correctly, full activity of reagents is retained until the expiry date indicated on the outer box label.

The kit components should be stored at -20 °C. It is recommended that the user avoid repeated freeze-thaw cycles.

4. SAFETY INFORMATION

When working with chemicals, always wear suitable personal protective equipment, including lab coat, gloves and safety glasses.

For detailed information, please consult the material safety data sheets available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The JetSeq™ ER and Ligation Kit is compatible with Illumina® sequencing platforms and is particularly indicated for whole-genome sequencing libraries preparation of samples which can be prone to PCR bias.

This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

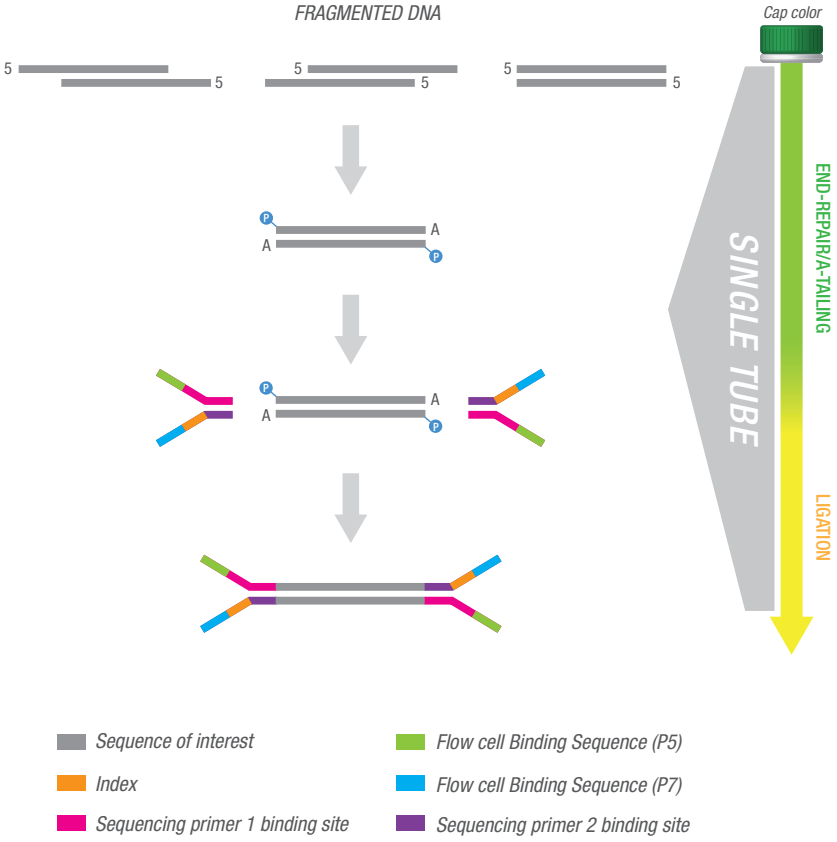

Fig. 1 Workflow for JetSeq™ ER and Ligation Kit

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Oligonucleotide adapters
- 10 mM Tris-HCl (pH 8.0)
- 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl
- Thermal cycler or heat block
- Vortex mixer
- Equipment for the determination of DNA concentration such as Nanodrop™, Qubit™ or equivalent
- Equipment for the determination of DNA size distribution such as Tapestation™, Bioanalyzer or equivalent
- Reagents and equipment for the purification and size selection of DNA fragments such JetSeq™ Clean or AMPure® XP beads (with magnetic device)
- DNase-free plastic ware (0.2 mL tubes, 96-well plates, pipette tips…)
- Molecular grade water
- Freshly prepared 70% ethanol

7. IMPORTANT NOTES

7.1 Recommended DNA preparation method

The most important prerequisite for any NGS library preparation is high-quality DNA. Sample handling and DNA isolation procedures are therefore critical to the success of the procedure. Residual traces of proteins, salts or other contaminants could degrade the DNA or decrease the efficiency of the enzymatic activities necessary for optimal library preparation.

Depending on the sample, we recommend one of the following extraction kits:

- ISOLATE II Genomic DNA Kit (BIO-52066) for the preparation of genomic DNA from fresh tissues and cells
- ISOLATE II Plant DNA Kit (BIO-52069) for isolation of genomic DNA from plants

For more DNA extraction kits, please refer to our ISOLATE II selection tool (www.bioline.com/isolate).

7.2 Recommendations for DNA fragmentation

DNA can be fragmented using one of the following methods:

- Mechanical fragmentation (acoustic, sonication, nebulization)
- Enzymatic fragmentation

To ensure optimal fragmentation of the DNA use the recommended parameters given by the fragmentation solution manufacturer. Check the fragmented DNA to verify that the desired size distribution has been obtained.

7.3 Recommendations for adapters

JetSeq ER and Ligation kit is compatible with adapters used in library preparations to be sequenced with Illumina sequencing platforms. Any purchased or custom adapters compatible with TA-ligation on double-stranded DNA can be used.

Adapters can be annealed following the established protocols and diluted as described in section 8.2.1.

If a new batch of custom adapters is purchased, we suggest dissolving them at a concentration of 100 µM in 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl.

Mix the same volumes of the two adapter stocks to be annealed (e.g. 15 µL of Universal adapter and 15 µL of Indexed adapter).

On a thermocycler, incubate the adapter mixture at 95 °C for 5 minutes, then cool it down ramping 0.5 °C until the temperature of 4 °C is reached.

The annealed adapters should be aliquoted to minimize freeze and thaw cycles and can be stored at -20 °C.

7.4 Recommendations for bead-based clean-up and size selection

DNA fragments and libraries can be cleaned up and/or size selected using paramagnetic beads.

For these applications, JetSeq Clean beads are recommended (www.bioline.com/jetseq clean). Alternatively, AMPure XP beads or similar can be used.

Conditions and beads volumes listed in the protocol below are valid for both JetSeq Clean and AMPure XP. Beads from any other source should be used following the parameters given by the manufacturer.

7.5 Recommendations for quality control throughout the library preparation

Quality of input DNA and DNA libraries can be assessed using Tapestation™, Bioanalyzer or equivalent.

8. PROTOCOL

8.1 End-Repair

Remove the end-repair reagents (green cap) and the DEPC-treated water (blue cap) from storage (-20 °C) and allow them to thaw on ice. Briefly vortex and spin down each reagent before use.

1. Prepare reaction on ice using the volumes shown in table 1 and mix by pipetting up and down.

   Caution: the ER buffer is very viscous. Care should be taken to ensure adequate mixing of end-repair reaction.

Table 1. End-repair reaction mix

Cap Color	Reagent	Quantity
	Fragmented DNA	500 ng - 3 µg
	End-repair buffer, 5x	10 µL
	ER enzyme mix	6 µL
	DEPC-treated water	up to 50 µL

2. Incubate for 30 min at 20 °C then 5 min at 72 °C.
   If a thermocycler is used, we suggest setting the heated lid at 85 °C
3. Cool down at 4 °C or transfer the reaction tube on ice.

8.2 Adapter Ligation

8.2.1 Preparation of Adapter Solution

Prepare an Adapter Solution by diluting adapter stock in a 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl buffer according to Table 2.

Table 2. Recommended adapter concentration for varying starting amounts of DNA input.

DNA Input Amount	Adapters Concentration	Adapter:Insert Molar Ratio*
3 µg	50 µM	10:1
1 µg	25 µM	15:1
500 ng	12.5 µM	15:1

*Adapter:insert molar ratio calculations are based on DNA fragments of 180bp. Users are advised to use this table as guideline to optimize the adapter:insert molar ratio for DNA Input values different from the ones shown in this table.

8.2.2 Adapter Ligation Set-up

Remove the ligation reagents (yellow cap) from storage (-20 °C) and allow them to thaw on ice. Briefly vortex and spin down each reagent before use.

1. Using the end-repair reaction from section 8.1, prepare an adapter-ligation mix by assembling the following reagents on ice (Table 3). Ensure optimal mixing by pipetting up and down.

Table 3. Ligation reaction mix

Cap Color	Reagent	Quantity
	ER reaction from section 8.1	50 µL
	Ligation Buffer, 5x	3 µL
	Adapters (concentration as required, see table 2)	5 µL
	Ligase	2 µL
	DEPC-treated water	5 µL
	Total	65 µL

*Ligation buffer, adapter, ligase and water can be premixed on ice and added in a single pipetting step.

2. Incubate for 15 min at 20 °C.
3. Proceed to post ligation clean-up.

NOTE: we recommend performing the clean-up step immediately after ligation. However, if the user needs to stop after ligation without cleaning-up, it is suggested to inactivate the ligase incubating the mix at 65 °C for 10 min, and then to store it overnight at -20 °C. The clean-up step can be completed on the following day without affecting the quality or the yield of the library.

8.3 Post-ligation Clean-up

This step is crucial to remove un-ligated adapters and adapter-dimers from the library. This protocol has been optimized using JetSeq Clean beads and AMPure XP beads. Users are advised to optimize the clean-up conditions when working with different beads.

NOTE: Equipment and reagents are not provided, see section 6.

Please find below a suggested protocol for post ligation clean-up using JetSeq Clean beads.	
1.	Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex beads thoroughly to ensure homogenous resuspension.
2.	Perform a 0.8x bead-based clean-up by adding 52 µL of homogenous JetSeq Clean beads to each adapter ligated DNA sample. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
3.	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
4.	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the clear supernatant without disturbing the beads.
5.	Continue to keep the tube(s)/plate in the magnetic stand and add 200 µL of 70% ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
6.	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min, and remove the ethanol.
7.	Repeat wash (step 5 to 6).
8.	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use P20 or P10 pipettes and tips to aspirate small volumes of residual ethanol.
9.	Leave the lids open and dry the beads at room temperature for 3-5 min or until the residual ethanol has completely evaporated. IMPORTANT: Do not over-dry the beads as this will decrease yield. The bead pellet is dry when the appearance of the surface changes from shiny to matt.
10.	Remove tube(s)/plate from the magnetic stand. Add 32 µL of 10 mM Tris-HCl pH 8.0 to the bead pellet, mix well by pipetting up and down. Incubate for 3 min at room temperature. Place tube(s)/plate back on the magnetic stand for 2-3 min or until the solution is clear
11.	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
12.	Perform a second 1x bead-based clean-up by adding 30 µL of homogenous JetSeq Clean beads to the supernatant collected from step 11. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
13.	Repeat steps 3 to 9 (capture and wash).
14.	Remove tube(s)/plate from the magnetic stand. Add 32 µL of 10mM Tris-HCl pH 8.0 to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 3 min at room temperature. Place tube(s)/plate back on the magnetic stand for 2-3 min or until the solution is clear.
15.	Remove 30 µL of the solution and transfer to a fresh tube(s)/plate. Discard the beads.

SAFE STOPPING POINT: Adapter-ligated DNA can be stored at -20 ºC up to 1 week.

8.4 Library Analysis

8.4.1 Library Quality

Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment. This is to ensure the absence of adapter-dimers and to verify the library size distribution.

8.4.2 Library Quantification

A rigorous quantification of the purified DNA library is critical for obtaining high quality reads from the chosen sequencing platform. For accurate measurement we recommend using the **JetSeq Library Quantification kit**. This is particularly important when quantifying an end-repair and ligation only library, since this is the only way to accurately quantify fragments correctly ligated to both adapters.

General Information

A TECHNICAL SUPPORT AND TROUBLESHOOTING

For technical assistance or more information on this product, please email us at tech@bioline.com

B ASSOCIATED PRODUCTS

Product	Size	Cat. #
ISOLATE II Genomic DNA Kit	50 prep	BIO-52066
ISOLATE II Plant DNA Kit	50 prep	BIO-52069
JetSeq Library Quantification Hi-ROX Kit	500 Reactions	BIO-68028
JetSeq Library Quantification Lo-ROX Kit	500 Reactions	BIO-68029
JetSeq Clean	50 mL	BIO-68031

C PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace any product that does not conform to the specifications free of charge. This warranty limits Bioline liability to only the replacement of the product.

D TRADEMARK AND LICENSING INFORMATION

JetSeq™ was developed jointly by OGT and Bioline.
JetSeq™ (Bioline Reagents Ltd), Illumina®, Qubit® (ThermoFisher Scientific); AMPure™ (BeckmanCoulter Inc.), Tapestation™

Ordering Information

Product	Size	Cat. #
JetSeq Flex DNA Library Preparation Kit	96 Reactions	BIO-68027
JetSeq ER & Ligation Kit	96 Reactions	BIO-68026

PM0518V1.2

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