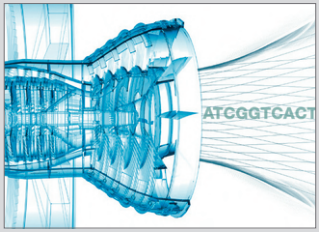

JetSeq™
DNA Library Preparation Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

JetSeq™ DNA Library Preparation Kit

TABLE OF CONTENTS

GENERAL INFORMATION

1. KIT CONTENTS

Cap Color	JetSeq DNA LIBRARY PREPARATION Reagents	Volume	
	Step 1: ER Buffer	160 µL	Box 1
	Step 1: ER Enzyme Mix	96 µL	
	Step 2: Ligation Buffer	48 µL	
	Step 2: Adaptor A	80 µL	
	Step 2: Adaptor B	80 µL	
	Step 2: Ligase	32 µL	
	Step 3: PCR Buffer	80 µL	
	Step 3: Primer Mix	80 µL	
	Step 3: DNA Polymerase	32 µL	
	Step 4: PCR Buffer	80 µL	
	Step 4: Primer	16 µL	
	Step 4: DNA Polymerase	32 µL	
	Nuclease Free Water	1.8 mL	

Cap Color	JetSeq DNA LIBRARY PREPARATION Index Set	Volume	
	Index 1	20 µL	Box 2
	Index 2	20 µL	
	Index 3	20 µL	
	Index 4	20 µL	
	Index 5	20 µL	
	Index 6	20 µL	
	Index 7	20 µL	
	Index 8	20 µL	
	Index 9	20 µL	
	Index 10	20 µL	
	Index 11	20 µL	
	Index 12	20 µL	
	Index 13	20 µL	
	Index 14	20 µL	
	Index 15	20 µL	
	Index 16	20 µL	

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 of sheared DNA using a coordinated series of standard molecular biology reactions whilst maintaining high yields during the intermediate purification steps.

The JetSeq™ DNA Library Preparation Kit is designed to generate high-quality next generation sequencing (NGS) libraries suitable for sequencing on Illumina MiSeq™, NextSeq™ or HiSeq™ instruments. The kit contains all of the enzymes and buffers necessary for end-repair, A-tailing, ligation and amplification in convenient master mix formulations as well as 16 barcoded adapters that can be used for single or multiplex reads.

- Low input: 0.01-3 µg fragmented DNA
- Increased speed: sequencing ready library in under 3 hours
- Improved confidence: simpler protocol improves reproducibility
- Improved quality: maximum coverage from all sample types
- Maximum convenience: all-in-one kit

By combining end-repair and A-tailing in one unique step, the JetSeq™ DNA Library Preparation 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.

Please read this manual carefully to familiarize yourself with the JetSeq™ DNA Library Preparation protocol before starting (also available on www.bioline.com/jetseq).

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™ DNA Library Preparation Kit is designed for Illumina® library construction workflows for a wide range of NGS applications, including: targeted sequencing (capture), whole genome sequencing, de novo sequencing, whole exome sequencing and ChIP sequencing.

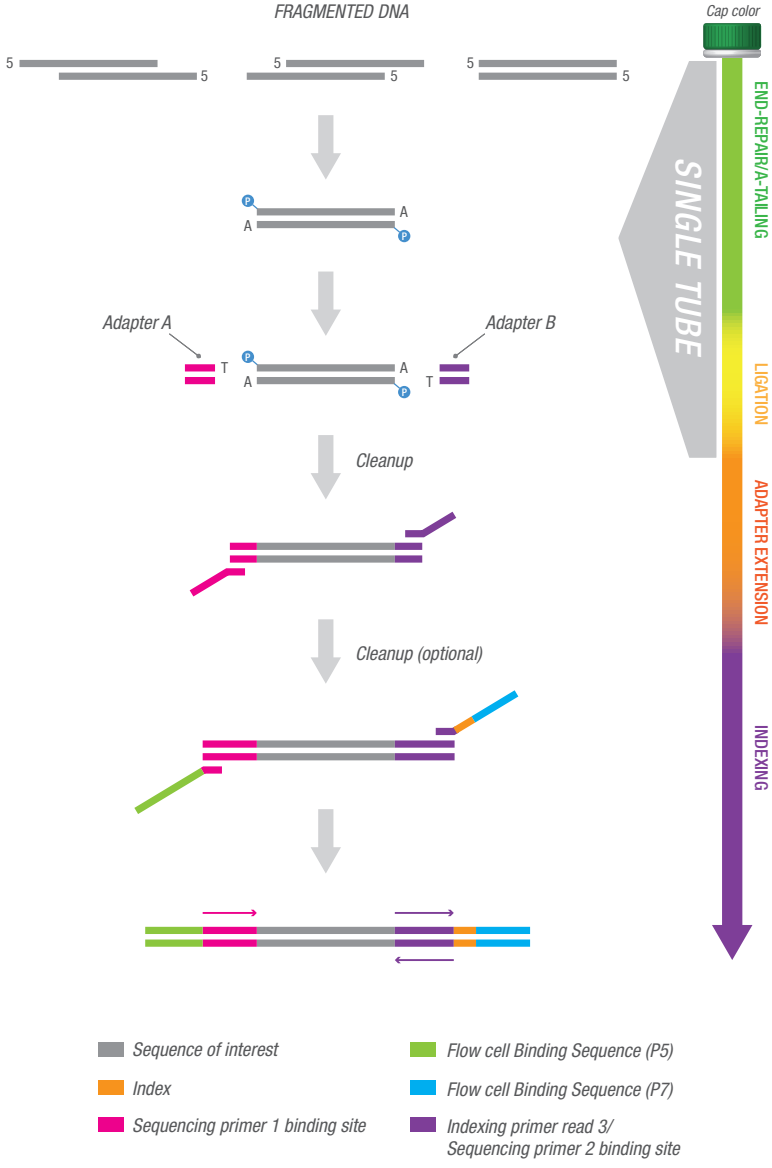

Fig. 1 Workflow for JetSeq™ DNA Library Preparation Kit

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- PCR equipment: Thermal cycler.
- Equipment for the determination of DNA concentration such as Nanodrop™, Qubit™, Tapestation™, Bioanalyzer or equivalent.
- Equipment for the determination of DNA size distribution such as Tapestation™, Bioanalyzer or equivalent.
- Equipment for the purification and size selection of DNA fragments such as AMPure™, Dynabeads™, SPRI™ beads or other equivalent column-based systems.

7. IMPORTANT NOTES

7.1. DNA preparation and quality control

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 experiment. Residual traces of proteins, salts or other contaminants will degrade the DNA or decrease the efficiency of the enzymatic activities necessary for optimal library preparation.

7.1.1 Recommended genomic DNA preparation method

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 FFPE RNA/DNA Kit (BIO-52087) for the preparation of genomic DNA from FFPE tissue samples.
- 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).

7.1.2 Recommendations for DNA fragmentation

DNA can be fragmented using one of the following methods:

- Mechanical fragmentation (acoustics, sonication, nebulization).
- Enzymatic fragmentation.

To ensure complete fragmentation of the DNA that is needed for library preparation, only use the recommended parameters given in the manufacturer's instructions. Check the fragmented DNA to ensure a correct size distribution is obtained.

8. PROTOCOL

8.1 End-repair

Remove the "Step 1" reagents (green cap) and the nuclease free water (blue cap) from storage (-20 °C) and allow them to thaw on ice.

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

Table 1. End-repair reaction mix

Cap Color	Reagent	Quantity
	Fragmented DNA	0.01 - 3 µg
	Step 1: ER buffer	10 µL
	Step 1: ER enzyme mix	6 µL
	Nuclease free water	up to 50 µL

2. Incubate for 30 min at 20 °C then 30 min at 72 °C.
3. Transfer the reaction tube on ice (4 °C).

8.2 Adaptor ligation

Remove the “Step 2” reagents (yellow cap) from storage (-20 °C) and allow them to thaw on ice.

1. Using the end-repair reaction from section 8.1 assemble the following reagents on ice. Mix by pipetting up and down.

Table 1. Adaptor ligation reaction mix

Cap Color	Reagent	Volumes
	End-repair reaction from section 8.1	50 µL
	Step 2: Ligation Buffer	3 µL
	Step 2: Adaptor A	5 µL
	Step 2: Adaptor B	5 µL
	Step 2: Ligase	2 µL
	Total	**65 µL**

Table 2. Recommended adaptor volumes for varying starting amounts of DNA

Starting DNA	1x (1–3 µg)	1x (0.5–0.99 µg)	1x (250–499 ng)	1x (100–249 ng)	1x (50–99 ng)	1x (1–49 ng)
DNA sample	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL
Step 2: Ligation Buffer	3 µL	3 µL	3 µL	3 µL	3 µL	3 µL
Step 2: Adaptor A	5 µL	1.5 µL	1 µL	0.5 µL	0.25 µL	≤0.125 µL*
Step 2: Adaptor B	5 µL	1.5 µL	1 µL	0.5 µL	0.25 µL	≤0.125 µL*
Step 2: Ligase	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL
Nuclease-free H2O	0 µL	7 µL	8 µL	9 µL	9.5 µL	9.75 µL
TOTAL	65 µL	65 µL	65 µL	65 µL	65 µL	65 µL

*Note: Lower starting amounts may need further optimization for optimal ligation.

2. Incubate for 15 min at 20 °C.
3. Clean-up and size select the adaptor-ligated library. It is important at this stage to remove unwanted adaptor-dimers.
   Note: Equipment and reagents are not provided, see section 6

Please find below a suggested protocol for post ligation clean-up using AMPure XP beads.

a)	Use only room temperature AMPure XP beads.
b)	Mix the reagent well so that the reagent appears homogeneous and consistent in color.
c)	Add 117 µL of homogenous AMPure XP beads to each adaptor ligated DNA sample (in either 1.5 mL LoBind tubes or 0.2 mL LoBind tubes). Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
d)	Put the tube in the magnetic stand and wait for the solution to clear (which should take approximately 3–5 min).
e)	Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
f)	Continue to keep the tube in the magnetic stand/rack whilst adding 500 µL (or 200 µL) of 70% ethanol to each tube.
g)	Let the tube sit for 1 min to allow any disturbed beads to settle, and remove the ethanol.
h)	Repeat wash (step f and step g).
i)	After the second wash seal the tube or plate and centrifuge briefly (260 x g for 30 sec).
j)	Return the tube to the magnetic stand/rack and wait 1 min. Remove any remaining ethanol using a P20 pipette and tip, being careful not to touch the bead pellet.
k)	Dry the samples on a 37 °C heat block for 3–5 min or until the residual ethanol completely evaporates.
l)	IMPORTANT: Do not over-dry as this will decrease yield.
m)	Note: Bead pellet is dry when the appearance of the surface changes from shiny to matt.
n)	Add 32 µL nuclease-free water directly to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 3 min at room temperature. Centrifuge briefly to consolidate the sample and place on a magnetic stand/rack for 2–3 min or until the solution is clear.
o)	Remove 30 µL of the supernatant and transfer to a fresh LoBind tube. The beads can be discarded at this time.

4. Assess the quality and concentration of the cleaned up adaptor-ligated DNA
- Confirm the DNA library size distribution and the absence of adaptor-dimers on a Bioanalyzer, Tapestation or equivalent. An increase of 58 bp should be measured following the ligation of the adaptors.
- Determine concentration of the purified adaptor-ligated DNA using Nanodrop, Qubit or equivalent.

5. The purified DNA can be stored at -20 °C.

8.3 Adaptor extension (PCR 1)

Remove the “Step 3” reagents (orange cap) from storage (-20 °C) and allow them to thaw on ice.

1. Assemble the following reaction on ice using the quantities shown below. Mix by pipetting up and down.

Table 3. PCR 1 reaction mix

Cap Color	Reagent	Volumes
	Purified adaptor-ligated library from 8.2.4	1-20 ng
	Step 3: PCR buffer	5 µL
	Step 3: Primer Mix	5 µL
	Step 3: DNA polymerase	2 µL
	Nuclease free water	up to 50 µL

2. Run the PCR using the following conditions.

Table 4. PCR 1 cycling conditions

Temperature (°C)	Time	Cycles
98 °C	3 min	1
98 °C	30s	See table 5
65 °C	30s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

Table 5. Number of cycles recommended according to the amount of purified adaptor-ligated DNA used

DNA quantity in PCR1	Number of PCR cycles
>20 ng	5
9-20 ng	6
4-8 ng	7
2-3 ng	8
1-1.9 ng	9
<1 ng	10 or more

Note: It is not recommended to perform >10 cycles as this will increase the percentage of duplicates.

3. If target selection or DNA capture is used, please proceed from here before going to step 8.3, section 4.

4. Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment. This is to ensure the absence of adaptor-dimers. If adaptor-dimers are observed it is recommended that a clean-up of the adaptor extension (PCR 1) is performed in order to remove these unwanted products.

Please find below a suggested protocol for post adaptor extension (PCR1) clean-up using AMPure XP beads.	
a)	Use only room temperature AMPure XP beads.
b)	Mix the reagent well so that the reagent appears homogeneous and consistent in color.
c)	Add 90 µL of homogenous AMPure XP beads to each adaptor ligated DNA sample (in either 1.5 mL LoBind tubes or 0.2 mL LoBind tubes). Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
d)	Put the tube in the magnetic stand and wait for the solution to clear (which should take approximately 3–5 min).
e)	Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
f)	Continue to keep the tube in the magnetic stand/rack whilst adding 500 µL (or 200 µL) of 70% ethanol to each tube.
g)	Let the tube sit for 1 min to allow any disturbed beads to settle, and remove the ethanol.
h)	Repeat wash (step f and step g).
i)	After the second wash seal the tube or plate and centrifuge briefly (260 x g for 30 sec).
j)	Return the tube to the magnetic stand/rack and wait 1 min. Remove any remaining ethanol using a P20 pipette and tip, being careful not to touch the bead pellet.
k)	Dry the samples on a 37 °C heat block for 3–5 min or until the residual ethanol completely evaporates.
l)	IMPORTANT: Do not over-dry as this will decrease yield.
m)	Note: Bead pellet is dry when the appearance of the surface changes from shiny to matt.
n)	Add 32 µL nuclease-free water directly to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 3 min at room temperature. Centrifuge briefly to consolidate the sample and place on a magnetic stand/rack for 2–3 min or until the solution is clear.
o)	Remove 30 µL of the supernatant and transfer to a fresh LoBind tube. The beads can be discarded at this time.

5. Determine the PCR product concentration using a Nanodrop, Qubit or equivalent.
   Note: If the samples are not to be used immediately, store at -20 °C.

8.4 Adaptor completion and indexing (PCR 2)

Remove the “Step 4” reagents (purple cap) from storage (-20 °C) and allow them to thaw on ice.

1. Prepare the following reaction mix on ice using the quantities shown below. Mix by pipetting up and down.

Table 6. Adaptor completion and indexing (PCR 2) reaction mix

Cap Color	Reagent	Quantity
	PCR product from 8.3.5	0.5-5 ng**
	Index (1-16)	5 µL
	Step 4: PCR buffer	5 µL
	Step 4: Primer	1 µL
	Step 4: DNA polymerase	2 µL
	Nuclease free water	Up to 50 µL

** if target selection or DNA capture is used the DNA may be at a too low concentration to be measured. In this case we would suggest to use 14 µL of the enriched fraction.

2. Run the PCR with the following cycling conditions:

Table 7. Adaptor completion and indexing (PCR 2) cycling conditions

Temperature (°C)	Time	Cycles
98 °C	3 min	1
98 °C	30s	9**
65 °C	30s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

** if target selection or DNA capture is used the DNA may be at a too low concentration to be measured. In this case we would suggest to use 20 cycles.

3. Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment. This is to ensure the absence of adaptor-dimers.

- If adaptor dimers are observed it is recommended to remove these unwanted products by size selection using a suitable clean-up and size selection equipment and reagents.
  Note: Equipment and reagents are not provided, see section 6
- If no adaptor dimers are detected perform only a clean-up of the product of PCR 2 using a suitable clean-up and size selection equipment and reagents.
  Note: Equipment and reagents are not provided, see section 6

Please find below a suggested protocol for post indexing clean-up using AMPure XP beads.

a)	Use only room temperature AMPure XP beads.
b)	Mix the reagent well so that the reagent appears homogeneous and consistent in color.
c)	Add 90 µL of homogenous AMPure XP beads to each adaptor ligated DNA sample (in either 1.5 mL LoBind tubes or 0.2 mL LoBind tubes). Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
d)	Put the tube in the magnetic stand and wait for the solution to clear (which should take approximately 3–5 min).
e)	Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
f)	Continue to keep the tube in the magnetic stand/rack whilst adding 500 µL (or 200 µL) of 70% ethanol to each tube.
g)	Let the tube sit for 1 min to allow any disturbed beads to settle, and remove the ethanol.
h)	Repeat wash (step f and step g).
i)	After the second wash seal the tube or plate and centrifuge briefly (260 x g for 30 sec).
j)	Return the tube to the magnetic stand/rack and wait 1 min. Remove any remaining ethanol using a P20 pipette and tip, being careful not to touch the bead pellet.
k)	Dry the samples on a 37 °C heat block for 3–5 min or until the residual ethanol completely evaporates.
l)	IMPORTANT: Do not over-dry as this will decrease yield.
m)	Note: Bead pellet is dry when the appearance of the surface changes from shiny to matt.
n)	Add 32 µL nuclease-free water directly to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 3 min at room temperature. Centrifuge briefly to consolidate the sample and place on a magnetic stand/rack for 2–3 min or until the solution is clear.
o)	Remove 30 µL of the supernatant and transfer to a fresh LoBind tube. The beads can be discarded at this time.

When comparing the products of PCR 1 (adaptor extension) and PCR 2 (adaptor completion and indexing reaction), an increase of approximatively 70 bp of the DNA size should be observed.

4. Determine the PCR product concentration using Nanodrop, Qubit or equivalent. For accurate measurement we recommend the JetSeq Library Quantification Kit.

5. The DNA library is ready for sequencing on MiSeq, NextSeq and HiSeq platforms and can be pooled if necessary. When loading the library in the sequencing machine we recommend following the manufacturer's instructions.

Appendix A: Adaptor indexes

The nucleotide sequences for the 16 indexes provided are detailed in the table below.

Index Number	Sequence
1	AACGTGAT
2	AAACATCG
3	AGTGGTCA
4	ACCACTGT
5	GATAGACA
6	GTGTTCTA
7	TGGAACAA
8	TGGTGGTA
9	ACATTGGC
10	CAGATCTG
11	CATCAAGT
12	AGTACAAG
13	AGATCGCA
14	GACTAGTA
15	GGTGCGAA
16	TGAAGAGA

Appendix B: Low multiplexing guidelines

Illumina platform such as MiSeq and HiSeq use a red laser to sequence A/C and a green laser to sequence G/T. To ensure accurate registration of the index read, both a red and green signal must be present at each cycle. It is also important to maintain color balance where possible.

If pooling less than eight samples in the final sequencing pool we suggest using the following index combinations

Number of samples in pool	Index
1	Any index
2	2 & 6
3	Option A: 4, 6 & 7 Option B: 1, 11 & 16
4	Option A: 2, 6, 10 & 14 Option B: 9, 12, 15 & 16
6	Option A: 1, 2, 4, 6, 7 & 8 Option B: 2, 8, 9, 12, 15 & 16
8	Option A: 1-8 Option B: 9-16

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 FFPE RNA/DNA Kit	50 prep	BIO-52087
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

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's 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), HiSeq™, MiSeq™, NextSeq™ (Illumina Inc.); Qubit® (ThermoFisher Scientific); Dynabeads™ (Dynal Inc.); AMPure™ (Backman Coulter Inc.)

Ordering Information

Product	Size	Cat. #
JetSeq DNA Library Preparation Kit	16 Reactions	BIO-68025

USA
email: info.us@bioline.com
Order Toll Free: +1 888 257 5155

United Kingdom
email: info.uk@bioline.com
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Australia
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