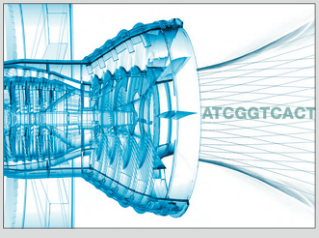

JetSeq™ DNA Library Preparation Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

JetSeq™ DNA Library Preparation Kit

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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: Adapter A	80 µL	
	Step 2: Adapter 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	
	DEPC-treated 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 MiniSeq™, 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.

The JetSeq DNA Library Preparation Kit has been developed and optimized for a variety of DNA sequencing applications, including whole genome sequencing, exome sequencing and targeted sequencing.

- Low input: 1 ng - 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
- Flexible: compatible with target enrichment

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 and material loss.

In the present manual two different protocols are described:

A step-by-step protocol, recommended for all applications, in particular for library preparation protocols that involve enrichment or capture step (Protocol A, section 8.1): quantity and quality of the library is assessed at any step, to ensure optimal results with any enrichment or capture method.

A streamlined protocol, recommended for whole genome sequencing and all the sequencing methods which do not need enrichment step (Protocol B, section 8.2).
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.

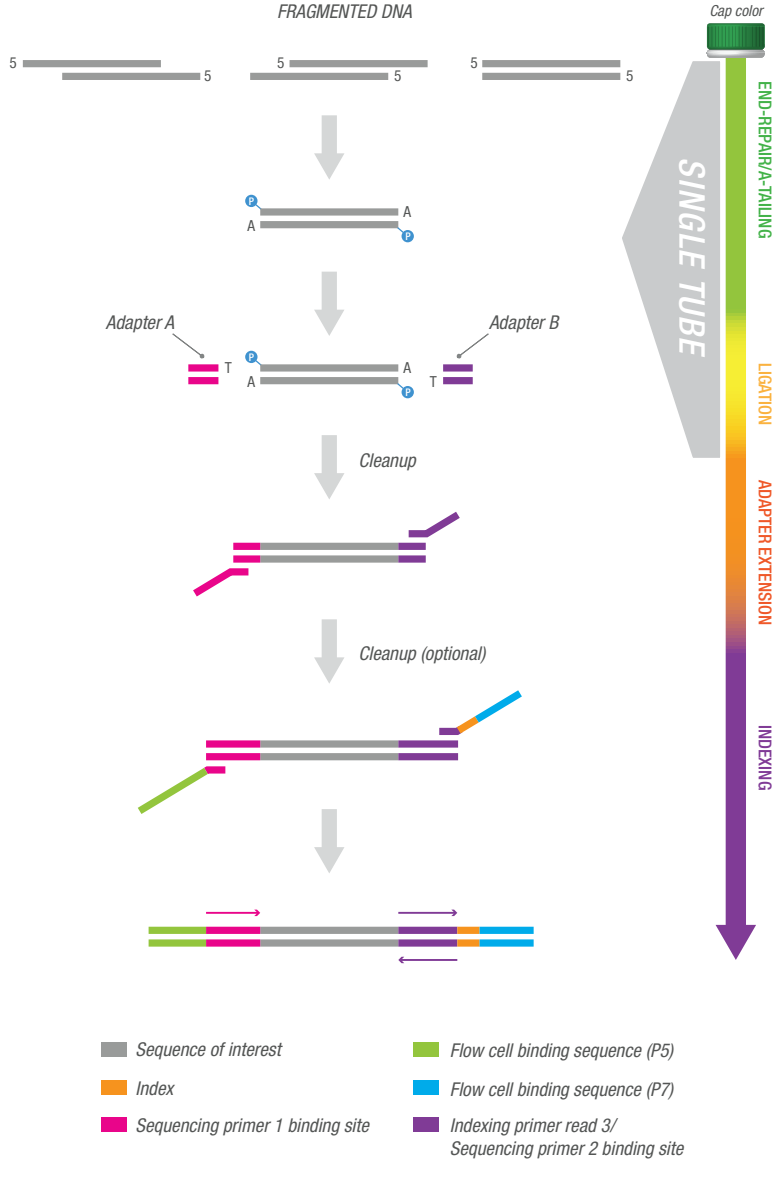

Fig. 1 Principles of JetSeq DNA Library Preparation Kit

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Thermal cycler or heat block
- 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 JetSeq Clean, AMPure™, Dynabeads™, SPRI™ beads or other equivalent column-based (with magnetic device)
- 10 mM Tris-HCl, pH 8.0
- 1 mM Tris-HCl, pH 8.0, 100 µM EDTA, 50 mM NaCl
- Vortex Mixer
- 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 methods

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.

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.
- ISOLATE II PCR and Gel Kit (BIO-52059) for the purification of PCR products and for the isolation of DNA fragments from agarose gels or PCR reactions

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

To ensure the quality of DNA fragmentation 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.

7.3 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.

7.4 Recommendations for quality control throughout the library preparation

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

8. PROTOCOLS

JetSeq DNA Library Preparation Kit can be used following two different protocols, depending on the user requirements. Please refer to Fig.2 for a graphical representation of the two protocols.

Protocol A (target enrichment, section 8.1): it is recommended for the vast majority of DNA library preparations and it is particularly suitable for applications that will include a target enrichment step. The quantification analysis recommended at different stages of the protocol will ensure that the right amount of library is used, independently from the efficiency of each step, n particular of the target enrichment. The suggested starting material is fragmented DNA from 1 ng to 3 µg of input.

Protocol B (section 8.2): this protocol is a streamlined version of the previous one and is not compatible with target enrichment. It is has been optimized to deliver high-quality, high-yield libraries ready for sequencing, in an efficient and reliable way. The suggested starting material is fragmented DNA from 1 ng to 100 ng of input.

For both protocols, all the required enzymes, buffers, adapters, indexes and primers are provided.

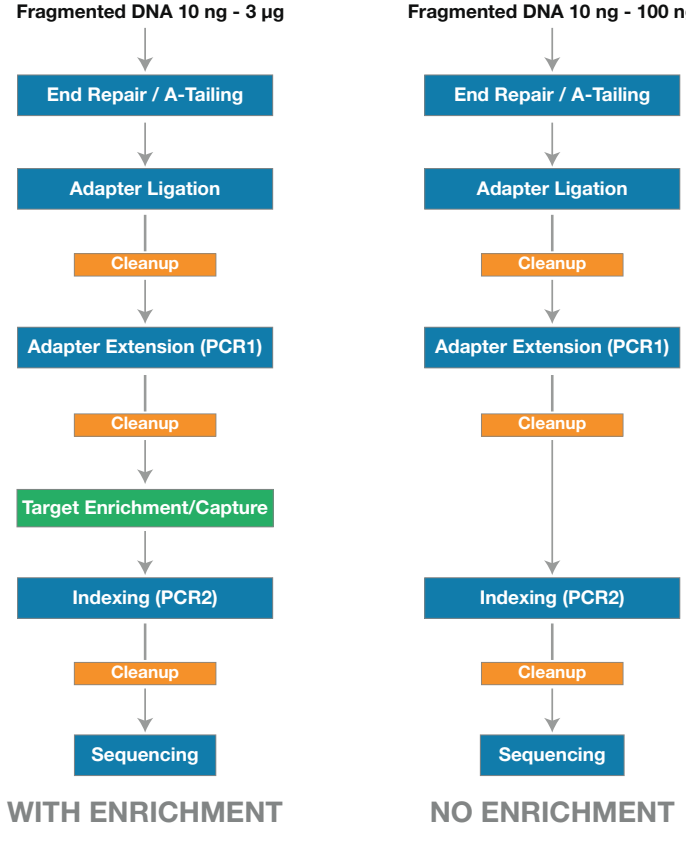

Fig. 2 Protocols A and B workflows. Protocol A includes a target enrichment step, protocol B is a standard, direct protocol.

8.1 Protocol A (target enrichment)

8.1.1 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
	DEPC-treated 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).

8.1.2 Adapter Ligation

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

Preparation of adapter solution

Prepare Adapter A and Adapter B Solutions by diluting adapter stocks in a 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl buffer according to Table 2 for both each of the adapters.

Table 2. Recommended* adapter dilution factors for varying starting amounts of input DNA

Input DNA Amount	Dilution Factor
3-1 µg	no dilution required
0.99-0.5 µg	1:3
499-250 ng	1:5
249-100 ng	1:10
99-50 ng	1:20
49-1 ng	1:40

* Adapter concentrations calculations are based on DNA fragments of 180 bp. Users are advised to use this table as a guideline to optimize the dilution of Adapters to be used for different fragment sizes.

Adapter ligation set-up

1. Using the end-repair reaction from section 8.1.1 assemble the following reagents on ice. Mix by pipetting up and down.

Table 3. Adapter ligation reaction mix

Cap color	Reagent	Volumes
	End-repair reaction from section 8.1.1	50 µL
	Step 2: Ligation buffer	3 µL
	Step 2: Adapter A solution	5 µL
	Step 2: Adapter B solution	5 µL
	Step 2: Ligase	2 µL
	Total	**65 µL**

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

8.1.3 Post-ligation Clean-up

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. All volumes refer to reactions carried out in 0.2 mL tubes or 96-well plates.

a)	Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex thoroughly to ensure beads are homogenously suspended.
b)	Perform a 1.8x bead-based clean-up by adding 117 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.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% freshly prepared ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e and f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10-20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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 at least 10 times. Incubate for 3 min at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	Assess quality and concentration of the cleaned up adapter-ligated DNA: • Confirm the DNA library size distribution and the absence of adapter-dimers on a Bioanalyzer, Tapestation or equivalent. An increase of 58 bp should be measured following the ligation of the adapters. • Determine concentration of the purified adapter-ligated DNA using Nanodrop, Qubit or equivalent.
m)	The purified DNA can be stored at -20 °C up to one week.

8.1.4 Adapter 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 4. PCR 1 reaction mix

Cap color	Reagent	Volumes
	Purified adapter-ligated library from 8.1.3	1-20 ng
	Step 3: PCR buffer	5 µL
	Step 3: Primer mix	5 µL
	Step 3: DNA polymerase	2 µL
	DEPC-treated water	up to 50 µL

2. Run the PCR using the following conditions.

Table 5. PCR 1 cycling conditions

Temperature (°C)	Time	Cycles
98 °C	3 min	1
98 °C	30 s	See table 6
65 °C	30 s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

Table 6. Number of cycles recommended according to the amount of purified adapter-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. **Optional:** Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment. This is to ensure the absence of adapter-dimers. If adapter-dimers are observed it is recommended that a clean-up of the adapter extension (PCR 1) is performed in order to remove these unwanted products.

8.1.5 Post-PCR 1 clean-up

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. All volumes refer to reactions carried out in 0.2 mL tubes or 96-well plates.

a)	Allow JetSeq Clean beads to equilibrate at room temperature for 30 min. Vortex beads thoroughly to ensure homogenous resuspension.
b)	Add 90 µl of homogenous JetSeq Clean beads to each library amplification. Mix well by pipetting up and down. Incubate at room temperature for 5 min.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	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.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e and f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10 µL or 20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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. Incubate for 3 min at room temperature. Place on a magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	Determine the PCR product concentration using a Nanodrop, Qubit or equivalent.
m)	The purified, amplified libraries can be stored at 4 °C for up to two weeks, or at -20 °C for longer periods of time.

If target selection or DNA capture is used, please proceed from here, following the protocol provided by the manufacturer. The enriched, purified libraries will be used as DNA input for the adapter completion and indexing step (PCR 2, section 8.1.6).

8.1.6 Adapter 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 7. Adapter completion and indexing (PCR 2) reaction mix

Cap color	Reagent	Quantity
	PCR product from 8.1.5	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
	DEPC-treated 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 8. Adapter completion and indexing (PCR 2) cycling conditions

Temperature (°C)	Time	Cycles
98 °C	3 min	1
98 °C	30 s	9**
65 °C	30 s	
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. **Optional:** Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment. This is to ensure the absence of unreacted primers and adapter-dimers. If these are observed it is recommended that a clean-up of the adapter completion and indexing (PCR 2) is performed in order to remove these unwanted products.

8.1.7 Post-PCR 2 clean-up

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. All volumes refer to reactions carried out in 0.2 mL tubes or 96-well plates.

a)	Allow JetSeq Clean beads to equilibrate at room temperature for 30 min. Vortex beads thoroughly to ensure homogenous resuspension.
b)	Add 90 µl of homogenous JetSeq Clean beads to each library amplification. Mix well by pipetting up and down. Incubate at room temperature for 5 min.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	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.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e and f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10 µL or 20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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. Incubate for 3 min at room temperature. Place on a magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	Assess quality and concentration of the DNA libraries: • Confirm the DNA library size distribution and the absence of unbound primers or adapter-dimers on a Bioanalyzer, Tapestation or equivalent. When comparing the products of PCR 1 (adapter extension) and PCR 2 (adapter completion and indexing reaction), an increase of approximatively 70 bp of the DNA size should be observed. • Determine the PCR product concentration using Nanodrop, Qubit or equivalent. For accurate measurement we recommend the JetSeq Library Quantification Kit.
m)	The purified, amplified libraries can be stored at 4°C for up to two weeks, or at -20 °C for longer periods of time.

The DNA library is ready for sequencing on MiniSeq, 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.

8.2 Protocol B (no target enrichment)

Protocol B has been optimized to provide a faster, streamlined version of the previous protocol to be used when the target enrichment step is not required. The intermediate DNA fragments obtained after each step are purified using paramagnetic beads and directly used in the following step without the need of quantifying.

8.2.1 End-repair

Remove the “Step 1” reagents (green cap) and the DEPC-treated water (blue cap) from storage (-20 °C) and allow them to thaw on ice.

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

Table 9. End-repair reaction

Cap color	Reagent	Volumes
	Fragmented DNA	1-100 ng
	Step 1: ER buffer	10 µL
	Step 1: 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 recommend setting the heated lid at 85 °C
3. Cool down at 4 °C or transfer the reaction tubes on ice.

8.2.2 Adapter ligation

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

Preparation of adapter solution

Prepare Adapter A and Adapter B Solutions by diluting adapter stocks in a 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl buffer according to Table 2 for each of the adapters.

Table 10. Recommended* adapter dilution factors for varying starting amounts of input DNA.

Input DNA Amount	Dilution Factor
100 – 51 ng	1:10
50 -11 ng	1:20
10 - 1 ng	1:40

* Adapter concentrations calculations are based on DNA fragments of 180 bp. Users are advised to use this table as a guideline to optimize the dilution of Adapters to be used for different fragment sizes.

Adapter ligation set-up

1. Using the end-repaired reaction from section 8.2.1, prepare an adapter-ligation mix by assembling the following reagents on ice. Mix by pipetting up and down.

Table 11. Adapter ligation reaction mix

Cap color	Reagent	Volumes
	End-repair reaction from section 8.2.1	50 µL
	Step 2: Ligation buffer	3 µL
	Step 2: Adapter A solution	5 µL
	Step 2: Adapter B solution	5 µL
	Step 2: Ligase	2 µL
	Total	65 µL

2. Incubate for 15 min at 20 °C.
3. Clean up the adapter-ligated library. It is important at this stage to remove unwanted adapter-dimers.

8.2.3 Post-ligation clean-up

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.***

a)	Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex thoroughly to ensure beads are homogenously suspended.
b)	***For starting DNA input ≥10 ng:*** Perform a 1.8x bead-based clean-up by adding 117 µL of homogenous JetSeq Clean beads to each adapter ligated DNA sample. ***For starting DNA input <10 ng:*** 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.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% freshly prepared ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e to f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10-20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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 at least 10 times. Incubate for 3 min at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	The purified DNA can be stored at -20 °C up to 1 week.

8.2.4 Adapter extension (PCR 1)

1. Remove the “Step 3” reagents (orange cap) from storage (-20 °C) and allow them to thaw on ice.
2. Assemble the following reaction on ice using the quantities show below. Mix by pipetting up and down.

Table 12. Adapter extension (PCR 1)

Cap color	Reagent	Volumes
	Purified adapter-ligated library from 8.2.3	30 µL
	PCR Buffer	5 µL
	Primer Mix	5 µL
	DNA Polymerase	2 µL
	DEPC-treated water	8 µL
	Total	50 µL

1. Run the PCR using the following conditions

Table 13. PCR 1 cycling

Temperature	Time	Cycles
98 °C	3 min	1
98 °C	30 s	See table 6
65 °C	30 s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

Table 14. Number of cycles recommended according to the amount of DNA used

DNA input quantity	Recommended number of PCR cycles
100 – 50 ng	3
10 ng	4
1 ng	6

2. It is recommended that a clean-up of the adapter extension (PCR 1) is performed in order to remove these unwanted products. This step is crucial to remove unwanted adapters and adapter-dimers from the library.

8.2.5 Post adapter extension clean-up

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.***

a)	Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex thoroughly to ensure beads are homogenously suspended.
b)	***For starting DNA input ≥10 ng:*** Perform a 1.8x bead-based clean-up by adding 90 µL of homogenous JetSeq Clean beads to each adapter ligated DNA sample. ***For starting DNA input <10 ng:*** Perform a 1x bead-based clean-up by adding 50 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.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% freshly prepared ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e and f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10-20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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 at least 10 times. Incubate for 3 min at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	The purified, amplified libraries can be stored at 4°C for up to two weeks, or at -20 °C for longer periods of time.

8.2.6 Adapter completion and indexing (PCR 2)

1. Remove the “Step 4” reagents (purple cap) from storage (-20 °C) and allow them to thaw on ice.
2. Prepare the following reaction mix on ice using the quantities shown below. Mix by pipetting up and down.

Table 15. Adapter completion and indexing (PCR 2)

Cap Color	Reagent	Volumes
	PCR product from 8.2.5	30 µL
	Index (1-16)	5 µL
	PCR Buffer	5 µL
	Step 4: Primer	1 µL
	DNA Polymerase	2 µL
	DEPC-treated water	7 µL

1. 1. Run the PCR with the following cycling conditions:

Table 16. Adapter completion and indexing (PCR 2) cycling conditions

Temperature	Time	Cycles
98 °C	3 min	1
98 °C	30 s	See table 9
65 °C	30 s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

Table 17. Number of cycles recommended according to the amount of DNA used

DNA input quantity	Recommended number of PCR cycles
100 – 50 ng	3-4
10 ng	4-5
1 ng	6-7

4. It is crucial to perform post adapter completion and indexing (PCR 2) clean-up in order to remove unbound primers or unwanted adapter-dimers from the library.

8.2.6 Post adapter completion and indexing clean-up

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.***

a)	Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex thoroughly to ensure beads are homogenously suspended.
b)	***For starting DNA input ≥10 ng:*** Perform a 1.8x bead-based clean-up by adding 90 µL of homogenous JetSeq Clean beads to each adapter ligated DNA sample. ***For starting DNA input <10 ng:*** Perform a 1x bead-based clean-up by adding 50 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.
c)	Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
d)	Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
e)	Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% freshly prepared ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
f)	Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
g)	Repeat wash (step e to f).
h)	After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use 10-20 µL tips to aspirate small volumes of residual ethanol.
i)	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.
j)	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 at least 10 times. Incubate for 3 min at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 min or until the solution is clear.
k)	Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
l)	Assess quality and concentration of the DNA libraries • Confirm the DNA library size distribution and the absence of unbound primers or adapter-dimers on a Bioanalyzer, Tapestation or equivalent. When comparing the final libraries with the starting material, an increase of approximatively 120 bp of the DNA size should be observed. • Determine the PCR product concentration using Nanodrop, Qubit or equivalent. For accurate measurement, we recommend the JetSeq Library Quantification Kit.
m)	The purified, amplified libraries can be stored at 4°C for up to two weeks, or at -20 °C for longer periods of time.

The DNA library is ready for sequencing on MiniSeq, 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: Adapter 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
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'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™ (Beckman Coulter Inc.)

Product Manual bioline.com/jetseq

Ordering Information

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

PM0118V1.5

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