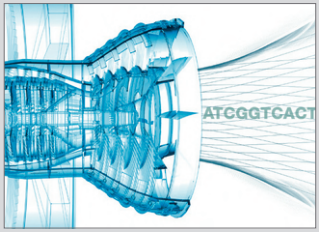

JetSeq™ Flex
DNA Library Preparation Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

JetSeq™ Flex DNA Library Preparation Kit

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GENERAL INFORMATION

1. KIT CONTENTS

Cap Color	JetSeq Flex DNA Library Preparation Reagents	Volume
	End-Repair Buffer, 5x	960 µL
	ER Enzyme Mix	576 µL
	Ligation Buffer, 5x	288 µL
	Ligase	192 µL
	PCR Buffer, 10x	480 µL
	DNA Polymerase	192 µL
	DEPC-treated Water	1.8 mL (x4)

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™ Flex 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. It offers a streamlined workflow and flexibility where users are free to use adapters of their choice. Other advantages of JetSeq™ Flex DNA Library Preparation Kit includes:

- Wide range of DNA input: 1 ng-1 µg fragmented DNA
- Increased speed: simpler protocol executed under 3 hours (excluding quality control tests)
- Convenient safe-stopping point

By combining end-repair and A-tailing in one unique step, the JetSeq Flex 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.

Please read this manual carefully to familiarize yourself with the JetSeq Flex DNA Library Preparation 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™ Flex DNA Library Preparation Kit is compatible with Illumina® library preparation workflows for a wide range of NGS applications, including: targeted sequencing (capture), whole genome sequencing, de novo sequencing, whole exome sequencing and ChIP sequencing.

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

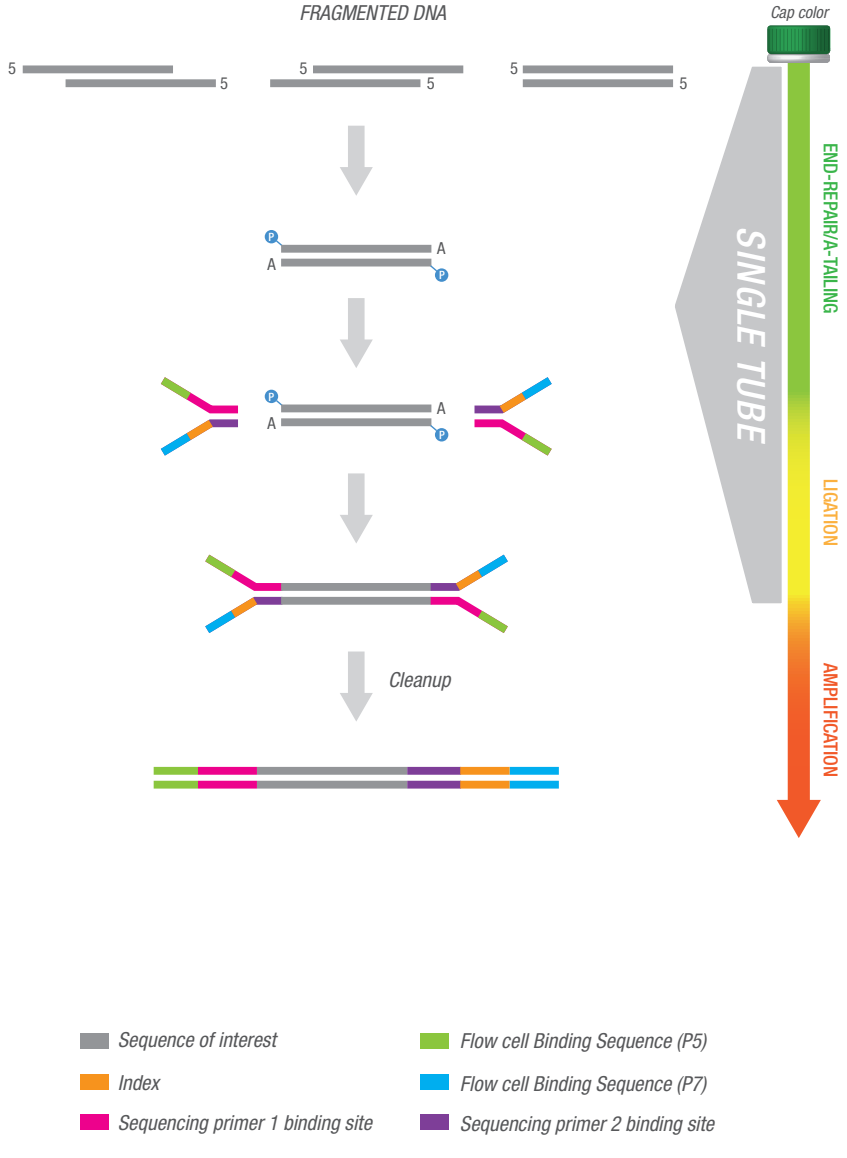


Fig. 1 Workflow for JetSeq™ Flex DNA Library Preparation Kit

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Oligonucleotide adapters (concentration depending on input, see Section 8.2.1)
- PCR primers for NGS library amplification compatible with the adapter system (please consult Section 7.3 for primer sequences).
- 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 as 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 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 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
- 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 (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 Primers for Library Amplification

This kit does not include primers for Library Amplification (Section 8.4).
If amplifying libraries prepared using Illumina Universal adapters, please use the following primer sequences:

- F: 5'-AAT GAT ACG GCG ACC ACC GA-3'
- R: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

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 below and mix by pipetting up and down.

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

   Table 1. End-repair reaction

Cap Color	Reagent	Quantity
	Fragmented DNA	1 ng - 1 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 recommend 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

1. Prepare an Adapter Solution by diluting adapter stocks 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 input DNA.

DNA Input Amount ‡	Adapters Concentration	Adapter:Insert Molar Ratio*
1 µg	30 µM	15:1
100 ng	10 µM	50:1
50 ng	10 µM	100:1
10 ng	4 µM	200:1
1 ng	1.5 µM	750:1

‡Users are advised to use this table as a guideline to optimize the Adapter:Insert molar ratio for DNA Input values different from the ones shown in this table.*Adapter:Insert molar ratio calculations are based on DNA fragments of 150 bp.

When different sizes of DNA insert are used we suggest to modify the Adapter Concentration according with the formula:

$$[Adapter_n] = \frac{[Adapter_{150}] \times 150}{n}$$

Where [Adapter$_n$] is the required new Adapter Concentration, [Adapter$_{150}$] is the Adapter Concentration recommended here for 150 bp inserts, and *n* is the size of the new DNA insert.

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 the tube containing Ligation buffer and spin down each reagent before use.

1. Using the end-repaired 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. Adapter Ligation Reaction Mix

Cap Color	Reagent	Volumes
	End-repair reaction from section 8.1	50 µL
	Ligation buffer, 5x	3 µL
	Adapters (concentration as required, see table 2)	5 µL
	Ligase	2 µL
	Nuclease-free water	5 µL
	Total	**65 µL**

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

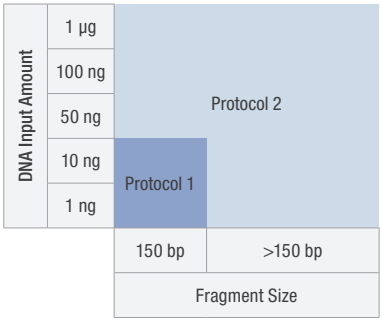
2. Incubate for 15 min at 20 °C.
3. Proceed to post ligation clean-up.

NOTE: we recommend performing the clean-up step immediately after ligation. However, if the user intends to stop after ligation without clean-up, it is suggested to inactivate the ligase by incubating the mix at 65 °C for 10 min, and then to store the adapter-ligated DNA overnight at -20 °C. The clean-up step can be continued 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. Double-sided size selection is optional, however it is not recommended to perform it if insert fragments shorter than 150 bp are used or the starting material is less than 50 ng. Please refer to Table 4 to determine which post-ligation clean up protocol is suggested for your libraries. Follow "Protocol 1" for post-ligation clean-up without double-sided size selection and "Protocol 2" for double-sided size selection. These protocols have been optimized using JetSeq Clean beads and AMPure XP beads. Users are advised to optimize the clean-up conditions when working with different beads.

Table 4. Recommended post-ligation clean up protocols based on Insert size.



8.3.1 Protocol 1 - Clean-up of Adapter-ligated DNA

To ensure an effective removal of un-ligated adapters and adapter-dimers we highly recommend the following procedure composed of two clean-up rounds.

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

1. Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex beads thoroughly to ensure beads are homogenously suspended.
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 supernatant without disturbing the beads.
5. 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.
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 10-20 µL 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 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.
11. Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.
12. Perform a second 0.8x bead-based clean-up by adding 24 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 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 on a 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 °C up to 1 week.

8.3.2 Protocol 2 - Double-sided Size Selection of Adapter-ligated DNA

Refer to table 5 for the recommended beads amount to be added in the double-sided size selection. The appropriate volume of beads is calculated relative to the starting volume of 65 µL for both the first and second cut.
Note: Equipment and reagents are not provided, see section 6.

Table 5. Recommended bead ratios to be used for double-sided size selection, using starting material in the range of 130 - 580 bp in size. Volumes are based on a 65 µL Adapter ligated reaction sample.

Insert Size	130 – 179 bp	180 – 229 bp	230 – 279 bp	280- 429 bp	430 – 580 bp
First cut ratio (vol.)	0.4x (26 µL)	0.4x (26 µL)	0.3x (19.5 µL)	0.2x (13 µL)	0.2x (13 µL)
Second cut ratio (vol.)	0.3x (19.5 µL)	0.2x (13 µL)	0.2x (13 µL)	0.2x (13 µL)	0.1x (6.5 µL)

1. Allow JetSeq Clean beads to equilibrate at room temperature for at least 30 min. Vortex beads thoroughly to ensure beads are homogenously suspended.

2. Perform the first cut by adding the appropriate volume 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 transfer the clear supernatant to fresh tube(s)/plate. **Do not discard supernatant!** Discard the beads containing unwanted large fragments.
5. Perform the second cut by adding the appropriate volume of homogenous JetSeq Clean beads the supernatant from step 4. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 min.
6. Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 min).
7. Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads. **Do not discard beads!**
8. Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% ethanol to each tube. IMPORTANT: Always use freshly prepared 70% ethanol.
9. Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
10. Repeat wash (step 8 to 9).
11. 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.
12. Leave the lids open and dry the beads at room temperature for 2-3 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.
13. 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.
14. 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 °C up to 1 week.

OPTIONAL: If possible, users are recommended to check quality and concentration of the adapter-ligated DNA on a Bioanalyzer (or equivalent). This will confirm the absence of adapter-dimers and help to determine a sufficient number of cycles to be used for library amplification. Please note that the concentration of purified adapter-ligated DNA may not be detectable when less than 10 ng of input DNA was used due to purification yields and sensitivity of the detection method.

8.4 Library Amplification (if required)

8.4.1 Primer Mix Preparation

Prepare the amplification Primer Mix by diluting the primers in nuclease-free water to a final concentration of 2.5 µM each. Store at -20 °C and thaw on ice before use.

NOTE: The amplification primers should be compatible with the adapter system used (for Illumina compatible primers please consult section 7.3).

8.4.2 PCR Set-Up

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

1. Assemble the following reaction on ice using the volumes shown in Table 5. Ensure optimal mixing by pipetting up and down.

Table 6. Library Amplification Reaction

Cap Color	Reagent	Volumes
	Purified adapter-ligated library from section 8.3	30 µL
	PCR buffer, 10x	5 µL
	Primer Mix (2.5 µM each)	5 µL
	DNA polymerase	2 µL
	Nuclease-free water	8 µL

2. Place the tube in a thermocycler and perform the PCR using the following cycling conditions:

Table 7. Cycling conditions

Temperature	Time	Cycles
98 °C	3 min	1
98 °C	30 sec	See table 7 and 8
65 °C	30 sec	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

NOTE: The following guidelines are based on amplification with JetSeq DNA polymerase and the primer mix (P5 and P7 primer sequences). Further optimisation of PCR cycle number may be required.

Table 8. Recommended number of PCR cycles to obtain approximately 100 ng of amplified library from 0.5-200 ng of purified adapter-ligated DNA (Section 8.3).

Amount of adapter-ligated DNA after post ligation clean-up (Section 8.3)	Estimated number of PCR cycles
<200 ng	1-2
20-40 ng	5-6
6-19 ng	6-7
2-5 ng	10-11
<1 ng	13-14

Table 9. Suggested number of PCR cycles required to obtain approximately 100 ng of amplified library from different input DNA (Section 8.1).

Input DNA into end-repair reaction (Section 8.1)	Estimated number of PCR cycles
1 µg	1
100 ng	5-6
50 ng	6-7
10 ng	9-10
1 ng	13-14

8.5 Post-Amplification Clean-Up

Please find below a suggested protocol for post amplification clean-up using JetSeq Clean beads.

1. Allow JetSeq Clean beads to equilibrate at room temperature for 30 min. Vortex beads thoroughly to ensure homogenous resuspension.
2. Perform a 1x (Protocol 1) or 0.8x (Protocol 2) bead-based clean-up by adding 50 and 40 ul of homogenous JetSeq Clean beads respectively to each library amplification. Mix well by pipetting up and down. 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 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 10 µL or 20 µL 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.
10. 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.
11. Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.

    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 quantification and sequencing on MiniSeq, MiSeq, NextSeq and HiSeq platforms and can be pooled if necessary. When loading the library into the flow cell we recommend following the manufacturer's instructions.

8.6 Library Analysis

8.6.1 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. If adapter-dimers or primer-dimers are observed it is recommended to perform another clean-up round of the library, in order to remove these unwanted products. Please refer to Fig.2a and 2b for an example of libraries prepared with JetSeq Flex DNA Library Preparation Kit following the ligation clean-up protocol 1 and 2, respectively.

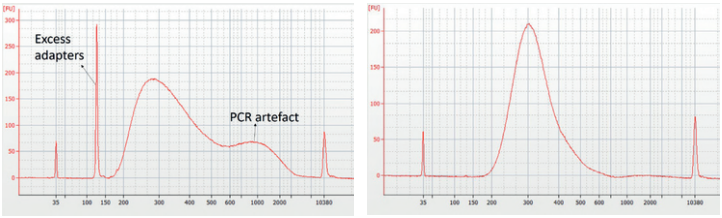


Fig. 2a. Examples of good (right) and bad (left) libraries prepared with JetSeq Flex kit with 10 ng of genomic DNA sheared to ~180 bp fragment size following post-ligation clean-up protocol 1.

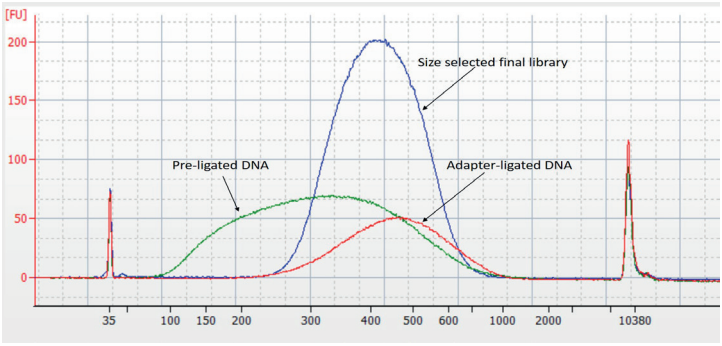


Fig. 2b. Examples of libraries prepared with JetSeq Flex kit with 100 ng of genomic DNA sheared to ~300 bp fragment size following post-ligation clean-up protocol 2.

8.6.2 Library Quantification

A rigorous quantification of the purified DNA library is critical for ensuring high quality sequencing reads. For accurate measurement, we recommend the use of the qPCR-based JetSeq Library Quantification Kit.

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
ISOLATE II PCR and Gel Kit	50 prep	BIO-52059
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), HiSeq™, MiSeq™, MiniSeq™, NextSeq™ (Illumina Inc.); Qubit® (ThermoFisher Scientific); AMPure™ (Beckman Coulter 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

PM0618V1.3

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