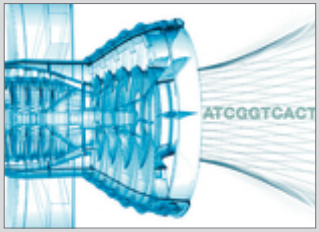


# JetSeq™ Flex
# DNA Library Preparation Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

# JetSeq™ Flex DNA Library Preparation Kit

## TABLE OF CONTENTS



## 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 |
| | Nuclease-Free 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.

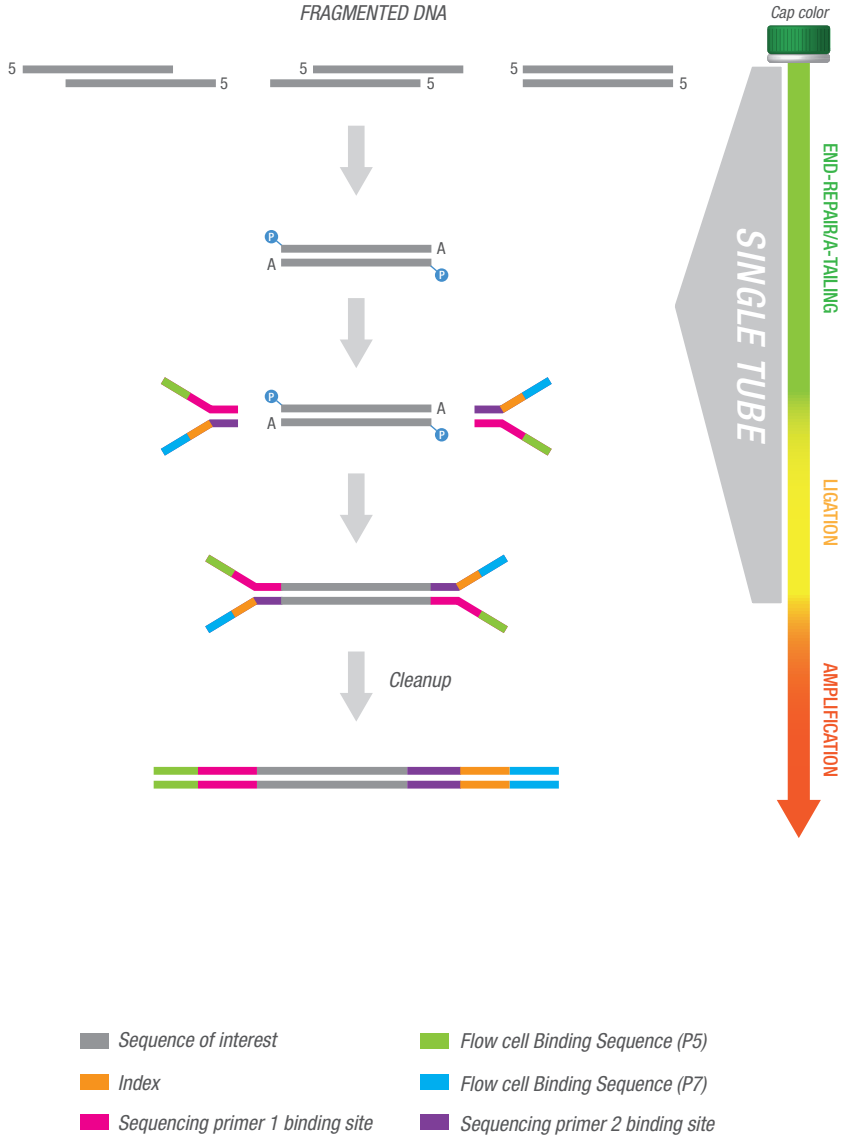


**Fig. 1 Workflow for JetSeq™ Flex DNA Library Preparation Kit**

# 6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Oligonucleotide adapters
- PCR primers for NGS library amplification compatible with the adapter system
- 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
- 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™XP beads, magnetic device and 80% ethanol
- DNase-free plastic ware (0.2 mL tubes, 96-well plates, pipette tips…)
- Molecular grade water

# 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 could degrade the DNA or decrease the efficiency of the enzymatic activities necessary for optimal library preparation.

## 7.2 Recommended 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 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).

## 7.3 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 only use the recommended parameters given by the manufacturer. Check the fragmented DNA to ensure the desired size distribution has been obtained.

## 7.4 Recommendation 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 nuclease-free 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 |
| | Nuclease-free 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 DNA input.**

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

*Adapter:insert molar ratio calculations are based on DNA fragments of 150 bp. 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. 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 unligated adapters and adapter-dimers from the library. To ensure this we highly recommend the following procedure composed of two clean-up rounds.

*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. | |
|---|---|
| 1. | Allow AMPure XP 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 AMPure XP beads to each adapter-ligated DNA sample. 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 80% ethanol to each tube. IMPORTANT: Always use freshly prepared 80% 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 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 0.8 x bead-based clean-up by adding 24 µL of homogenous AMPure XP beads to the supernatant collected from step 11. Mix well by pipetting up and down. Incubate at room temperature for 5-15 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. 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 supernatant 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 the quality and concentration of the adapter-ligated DNA on a Bioanalyzer (or equivalent) prior to the PCR enrichment step in order to confirm the absence of adapter-dimers and to determine a sufficient number of cycles for library amplification has been used. 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

### 8.4.1 Primer Mix Preparation

1. Prepare the Primer Mix by diluting the primers in nuclease-free water to the 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.*

### 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 4. Ensure optimal mixing by pipetting up and down.

**Table 4. 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 5. Cycling conditions**

| Temperature | Time | Cycles |
|---|---|---|
| 98 °C | 3 min | 1 |
| 98 °C | 30 sec | See table 6 and 7 |
| 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 6. 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) | Number of PCR cycles |
|---|---|
| ~200 ng | 1-2 |
| ~15 ng | 5-6 |
| ~2 ng | 10-11 |
| ~0.5 ng | 12-13 |

**Table 7. Suggested number of PCR cycles required to obtain approximately 100 ng of amplified library from different DNA input (Section 8.1).**

| Input DNA into end-repair reaction (Section 8.1) | Estimated number of PCR cycles |
|---|---|
| 1 µg | 1-2 |
| 100 ng | 6-7 |
| 10 ng | 11-12 |
| 1 ng | 13-14 |

## 8.5 Post-Amplification Clean-Up

| Please find below a suggested protocol for post amplification clean-up using AMPure XP beads. | |
|---|---|
| 1. | Allow AMPure XP beads to equilibrate at room temperature for 30 min. Vortex beads thoroughly to ensure homogenous resuspension |
| 2. | Perform a 1x bead-based clean-up by adding 50 µL of homogenous AMPure XP beads to each library amplification sample. Mix well by pipetting up and down. Incubate at room temperature for 5-15 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 80% ethanol to each tube. IMPORTANT: Always use freshly prepared 80% 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 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 the library size distribution. If adapter-dimers or primer-dimers are observed it is recommended that another clean-up round of the library is performed in order to remove these unwanted products. Please refer to Fig.2 for an example of libraries prepared with JetSeq Flex DNA Library Preparation Kit following the current protocol.

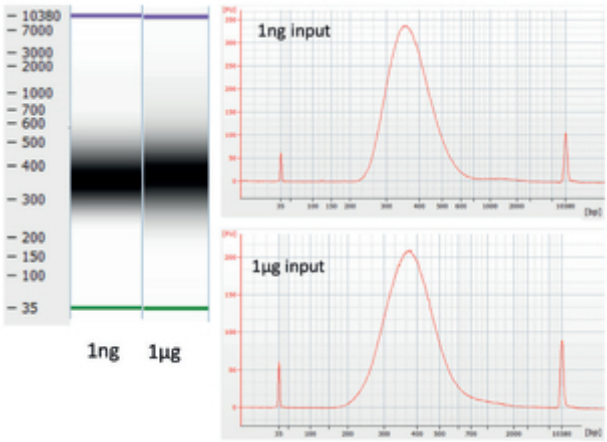


**Fig. 2 Examples of libraries prepared with JetSeq Flex kit with 1 ng and 1 µg of genomic DNA.**

### 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 **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 DNA Library Preparation Kit | 16 Reactions | BIO-68025 |

## 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 |

PM0317V1.1

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

**France**
email: info.fr@bioline.com
Tel: +33 (0)1 42 56 04 40

**United Kingdom**
email: info.uk@bioline.com
Tel: +44 (0)20 8830 5300

**Australia**
email: info.au@bioline.com
Tel: +61 (0)2 9209 4180

**Germany**
email: info.de@bioline.com
Tel: +49 (0)3371 68 12 29

**Singapore**
email: info.sg@bioline.com
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