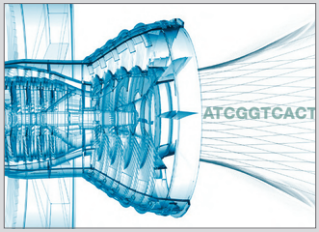

JetSeq™ Clean

Product Manual

BIOLINE

A Meridian Life Science® Company

JetSeq™ Clean

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1. KIT CONTENTS

Product Number	Description
BIO-68030	JetSeq Clean - 5 mL
BIO-68031	JetSeq Clean - 50 mL
BIO-68032	JetSeq Clean - 500 mL

2. DESCRIPTION

JetSeq™ Clean is an efficient Next Generation Sequencing (NGS) library preparation clean-up system based on paramagnetic beads technology. It is designed for purification and size selection of DNA fragments in the library construction process for NGS.

With its simple, three-step protocol, JetSeq Clean removes salts, primers, primer-dimers and dNTPs, while DNA fragments are selectively bound to the magnetic particles; highly purified DNA is eluted with low salt elution buffer or water and can be used directly for downstream applications. The protocol can be adapted to your current liquid handling workstation (e.g. Beckman, Hamilton, Tecan, Caliper, PerkinElmer, Agilent and Eppendorf) utilizing your current protocol as well as being performed manually.

Features:

- Designed for Next-Generation Sequencing library preparation clean-up and size selection
- Ideal for (left-sided and double-sided) size selection for Next-Generation Sequencing library preparation
- High recovery of fragmented DNA, NGS libraries and amplicons greater than 100 bp
- Efficiently removes unincorporated dNTPs, primers, primer dimers and other contaminants
- No centrifugation or filtration

JetSeq Clean can be used in the following applications:

- Next Generation Sequencing library clean-up
- Next Generation Sequencing size selection
- PCR and qPCR clean-up
- Fragment analysis
- Restriction enzyme clean-up

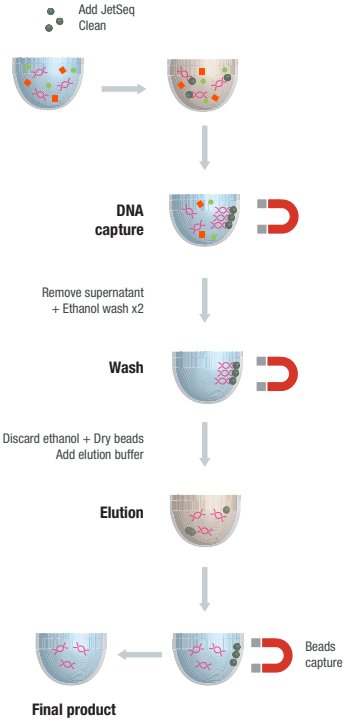
3. Materials and Equipment to be supplied by User

- 384-well, 96-well plates of 1.5 mL tubes, DNase-free
- Magnetic Separation Device
- Pipettes/Multichannel pipettor
- Multichannel disposable reservoirs
- 70% ethanol (IMPORTANT: freshly prepare ethanol solution every time, from non-denatured alcohol)
- Nuclease-free water or Elution Buffer (10mM Tris-HCl pH 8.0)

4. Storage

Upon reception, store JetSeq Clean at 4 °C to 8 °C. Do not freeze as this will damage the beads.

CLEAN-UP/LEFT-SIDED SIZE SELECTION



DOUBLE-SIDED SIZE SELECTION

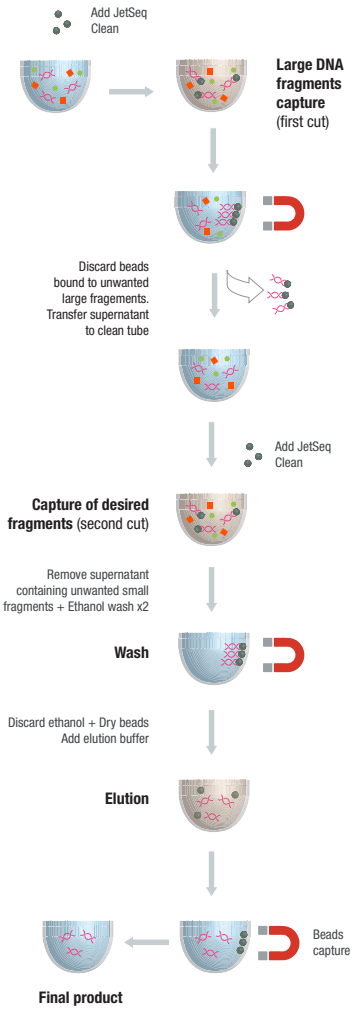


Fig. 1 Workflow for JetSeq™ Clean

5. PROTOCOLS

5.1 JetSeq Clean - Clean-up Protocol

The following protocol describes an efficient procedure to clean-up DNA from enzymatic reactions where nucleotides, unbound oligos and DNA fragments shorter than 100 bp will be removed. If a specific range of fragment sizes is required, please refer to the Left-Sided Size Selection and Double-Sided Size Selection Protocols for more detail.

1. IMPORTANT: Allow JetSeq Clean beads to equilibrate at room temperature. Vortex the JetSeq Clean beads reagent thoroughly to fully resuspend the magnetic beads prior to usage.

2. Measure the sample(s) reaction volume in the tube/96/348-well plate. Determine if transferring the sample(s) to a processing tube/96/348-well plate is required.

3. Perform a 1.8x bead-based clean-up by adding the following volumes of JetSeq Clean beads to the samples.

Table 1. Clean-up sample to JetSeq Clean volumes

Sample Format	Sample Volume (µL)	JetSeq Clean (µL)
Tube	50	90
	100	180
	150	270
96-well plate	10	18
	20	36
	50	90
384-well plate	5	9
	7	12.6
	10	18

4. Pipette up and down at least 10 times. Incubate at room temperature for 5 minutes.

5. Place the tube/96/348-well plate on a magnetic stand to separate the JetSeq Clean beads. Incubate at room temperature until the JetSeq Clean beads are completely cleared from solution.

6. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

7. Add 500/200/30 µL 70% ethanol to each tube/well.

8. Incubate at room temperature for 1 minute. It is not necessary to resuspend the JetSeq Clean beads.

9. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

10. Repeat steps 7-9 for a second 70% ethanol wash step.

11. Leave the tube/96/348-well plate on the magnetic stand for 3 minutes to air dry the JetSeq Clean beads. Remove any residue liquid with a pipette.

    Note: It is important to dry the JetSeq Clean beads before elution. Residual ethanol may interfere with downstream applications. 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.

12. Remove the tube/96/348-well plate from magnetic stand.

13. Elute the sample in an appropriate volume of Elution Buffer (see section 3) or molecular biology grade water. Mix well by pipetting up and down 10 times or vortex for 30 seconds.

14. Incubate at room temperature for 2-3 minutes.

15. Place the tube/96/348-well plate onto a magnetic stand to separate the JetSeq Clean beads. Incubate at room temperature until the JetSeq Clean beads are completely cleared from solution.

16. Transfer the cleared supernatant containing cleaned-up DNA to a new nuclease-free tube/96/348-well microplate and close the lid/seal with non-permeable sealing film.

17. Use the eluted material for the desired downstream applications.

5.2 JetSeq Clean - Left-Sided Size Selection

The size of DNA fragments binding to JetSeq Clean beads is based on the volumetric ratio of bead suspension to sample. Generally, decreasing the bead:sample ratio will decrease the binding efficiency of smaller DNA fragments, while increasing the ratio will progressively increase the binding efficiency of shorter fragments to the beads.

Modifying this ratio allows to control the size of DNA fragments binding to the beads, ensuring the recovery of DNA fragments of desired size.

1. IMPORTANT: Allow JetSeq Clean beads to equilibrate at room temperature. Vortex the JetSeq Clean beads reagent thoroughly, to fully resuspend the magnetic beads prior to usage.

2. To perform a left-sided size selection, add the required volume of JetSeq Clean beads:

   Volume of Sample x Recommended Beads Ratio = Volume of JetSeq Clean beads

   For example, if the sample volume is 65 µL and 0.8x ratio is required,

   65 (µL) x 0.8 = 52 µL of JetSeq Clean beads is needed.

   The size range of DNA fragments recovered with left-sided size selection is dependent on the ratio (volume) of JetSeq Clean beads added to the sample. Table 2 is a guideline for the purification of NGS libraries prepared using JetSeq™ Flex DNA Library Preparation Kit with JetSeq Clean beads. It is recommended to optimize the bead:sample volumetric ratio if libraries

are prepared using different manufacturer reagents. For example, if the DNA sample is dissolved in TE buffer and requires size selection prior to NGS library preparation, the ratio required to obtain the desired fragments is expected to be higher than described below.

Table 2. Left-Sided Size Selection beads to sample ratios

Fragments to be selected	Recommended beads:sample ratio
>100 bp	0.8x
>120 bp	0.6x
>150 bp	0.5x
>180 bp	0.4x
>250 bp	0.3x
>400 bp	0.2x

3. Pipette up and down at least 10 times. Incubate at room temperature for 5 minutes.

4. Place the tube/96/348-well plate in the magnetic stand at room temperature to separate the JetSeq Clean beads and wait until the JetSeq Clean beads are completely cleared from the solution.

5. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

6. Add 500/200/30 µL 70% ethanol to each sample.

7. Incubate at room temperature for 1 minute. It is not necessary to resuspend the JetSeq Clean beads.

8. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

9. Repeat steps 6-8 for a second 70% ethanol wash step.

10. Leave the tube/96/348-well in the magnetic stand for 3 minutes to air dry the JetSeq Clean beads. Remove any residue liquid with a pipette.

    Note: It is important to dry the JetSeq Clean beads before elution. Residual ethanol may interfere with downstream applications. 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.

11. Remove the tube/96/348-well plate from the magnetic stand.

12. Elute the sample in an appropriate volume of Elution Buffer or molecular biology grade water (see section 3). Mix well by pipetting up and down at least 10 times or vortex for 30 seconds.

13. Incubate at room temperature for 2-3 minutes.

14. Place the tube/96/348-well plate in the magnetic stand to separate the JetSeq Clean beads. Incubate at room temperature until the JetSeq Clean beads are completely cleared from solution.

15. Transfer the cleared supernatant containing size-selected DNA to a new nuclease-free tube/96/348well-plate or microplate and close the lid/seal with non-permeable sealing film.

16. Use the eluted material for the desired downstream applications or store it at the conditions recommended by the specific protocol followed.

5.3 JetSeq Clean - Double-Sided Size Selection

Double-sided size selection consists of two cuts: “first cut” and “second cut”. Generally, unwanted large fragments will be excluded at the first cut, followed by the removal of small fragments at the second cut. During the first cut, unwanted large fragments are bound to the beads and discarded, leaving small desired fragments in the supernatant. A fresh volume of beads is then added to the supernatant during the second cut to bind the desired fragments. Depending on the beads:sample ratio, shorter unwanted fragments will not bind the beads and will be washed away.
Note that the volume of the beads added to the second cut is calculated relative to the sample volume at the beginning of the size selection process.

Size selection can be applied at various stages in the NGS library construction workflows i.e. after fragmentation, after adapter ligation and after library amplification. JetSeq Clean beads may be employed in any of these steps, for both effective selection and exclusion of fragment sizes.

1. IMPORTANT: Allow JetSeq Clean beads to equilibrate at room temperature. Vortex the JetSeq Clean beads reagent thoroughly to fully resuspend the magnetic beads prior to usage.

   Table 3 is a guideline for the purification of NGS libraries prepared using JetSeq™ Flex DNA Library Preparation Kit with JetSeq Clean beads. It is recommended to optimize the bead:sample volumetric ratio if libraries are prepared using different manufacturer reagents.

Table 3. Double-Sided Size Selection beads to sample ratios

Fragment Size	First cut ratio	Second cut ratio
250 - 300 bp	0.4x	0.3x
300 - 350 bp	0.4x	0.2x
350 - 400bp	0.3x	0.2x
400 - 500 bp	0.2x	0.2x
600 - 700 bp	0.2x	0.1x

2. Perform the first cut by adding the required volume of JetSeq Clean beads to the sample. Pipette up and down at least 10 times. Incubate at room temperature for 5 minutes.

3. Place the tube/96/348-well in the magnetic stand at room temperature to separate the JetSeq Clean beads and wait until the JetSeq Clean beads are completely cleared from the solution.

4. Keeping the tube/plate in the magnetic stand, carefully remove and transfer the clear supernatant to clean tube/96/348-well plate. Do not discard supernatant! Discard the beads containing unwanted large fragments.

5. Perform the second cut by adding appropriate volume of homogenous JetSeq Clean beads the supernatant from step 4. Mix well by pipetting up and down at least 10 times or vortex for 30 seconds. Incubate at room temperature for 5 minutes.

6. Place the tube/96/348-well in the magnetic stand at room temperature to separate the JetSeq Clean beads and wait until the JetSeq Clean beads are completely cleared from the solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

8. Add 500/200/30 µL 70% ethanol to each sample.

9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the JetSeq Clean beads.

10. Aspirate and discard the cleared supernatant. Do not disturb the JetSeq Clean beads.

11. Repeat steps 8-10 for a second 70% ethanol wash step.

12. Leave the tube/96/348-well plate in the magnetic stand for 3 minutes to air dry the JetSeq Clean beads. Remove any residue liquid with a pipette.

    Note: It is important to dry the JetSeq Clean beads before elution. Residual ethanol may interfere with downstream applications. 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 the tube/96/348-well plate from the magnetic stand.

14. Elute the sample in an appropriate volume of Elution Buffer (see section 3). Mix well by pipetting up and down 10 times.

15. Incubate at room temperature for 2-3 minutes.

16. Place the tube/96/348-well plate in the magnetic stand to separate the JetSeq Clean beads. Incubate at room temperature until the JetSeq Clean beads are completely cleared from solution.

17. Transfer the cleared supernatant containing size-selected DNA to a new nuclease-free tube/96/348-well plate or microplate and close the lid/seal with non-permeable sealing film.

18. Use the eluted material for the desired downstream applications or store it at the conditions recommended by the specific protocol followed.

General Information

A Troubleshooting

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact Bioline Technical Support (tech@bioline.com).

Low yield

Cause	Solution
Low input material	Increase the number amplification cycles for PCR
Smaller product size	Small DNA fragments normally give lower yield
Ethanol residue	During the drying step, remove any liquid from bottom of the well
Old ethanol used	Ensure to use freshly made ethanol prior to clean up or size selection
Beads loss during the procedure	Increase magnetization time. Aspirate slowly
DNA remains bound to beads	Increase elution volume and resuspend in Elution Buffer or molecular biology grade water thoroughly
Incomplete resuspension of the beads during elution	Vortex or pipet up and down to fully resuspend the beads. Ensure adequate volume of the Elution Buffer to cover the JetSet Clean beads completely during the elution step
Over-drying of beads	Reduce the amount of time for drying the beads and do not dry the beads at 37°C heat-block
Nucleases contamination	Ensure to work DNase-free, to prevent DNA loss
Improper storage of the kit	Ensure the kit is within the expiration date and stored properly from the date of receipt

Primer/adapter carryover

Cause	Solution
Insufficient wash of the beads	Wash the beads one more time with 70% ethanol
Supernatant not completely removed	Ensure to remove all the supernatant
Beads:sample ratio is not optimal	Optimize beads:sample volumetric ratio

Undesired fragment sizes being selected

Cause	Solution
Incorrect beads:sample volumetric ratio	Ensure that the correct volume of JetSeq Clean beads solution is added to the first cut, and the volume of beads needed for the second cut is calculated relative to the volume of the DNA at the start of the size selection procedure
Carryover of beads from first cut to second cut	Optimization of beads:sample volumetric ratio may be needed if samples are prepared using other manufacturer kits as the buffers may have different binding properties
Insufficient mixing of sample and JetSeq Clean beads	Increase magnetization time. Care should be taken not to aspirate beads when transferring solution

Problems in downstream applications

Cause	Solution
Salt carryover	Freshly prepared 70% ethanol must be used at room temperature
Ethanol carryover	Ensure the beads are completely dried before elution and pipette residual ethanol out carefully from the bottom and/or side of the well

B ASSOCIATED PRODUCTS

Product	Size	Cat. #
JetSeq Flex DNA Library Preparation Kit	96 Reaction	BIO-68027
JetSeq ER & Ligation Kit	96 Reaction	BIO-68026
JetSeq DNA Library Preparation Kit	16 Reaction	BIO-68025
JetSeq Library Quantification Hi-ROX Kit	500 Reaction	BIO-68028
JetSeq Library Quantification Lo-ROX Kit	500 Reaction	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 liability to only the replacement of the product.

D TRADEMARK AND LICENSING INFORMATION

JetSeq™ (Bioline Reagents Ltd).

Ordering Information

Product	Size	Cat. #
JetSeq Clean	5 mL	BIO-68030
JetSeq Clean	50 mL	BIO-68031
JetSeq Clean	500 mL	BIO-68032

PM0717V1.0

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