JetSeq™ DNA Library Preparation Kit

Bioline

Abstract

This protocol is for the JetSeqTM DNA Library Preparation Kit. The kit is designed to generate high-quality next generation sequencing (NGS) libraries suitable for sequencing on Illumina MiSeqTM, NextSeqTM or HiSeqTM instruments.

Citation: Bioline JetSeq™ DNA Library Preparation Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.fwgbpbw
Published: 14 Nov 2016

Guidelines

1. KIT CONTENTS

Box1:

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

Box 2: JetSeq DNA Library Preparation Index Set (1-16, 20µl each)

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 puri cation steps.

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

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

By combining end-repair and A-tailing in one unique step, the JetSeq™ DNA Library Preparation Kit is able to reduce total NGS library preparation time and minimize the variability caused by additional handling, as well as the risk of contamination.

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

3. STORAGE

When stored under the recommended conditions and handled correctly, full activity of reagents is retained until the expiry date indicated on the outer box label.

The kit components should be stored at -20 °C. It is recommended that the user avoid repeated freeze-thaw cycles.

4. SAFETY INFORMATION

When working with chemicals, always wear suitable personal protective equipment, including lab coat, gloves and safety glasses.

For detailed information, please consult the material safety data sheets available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

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

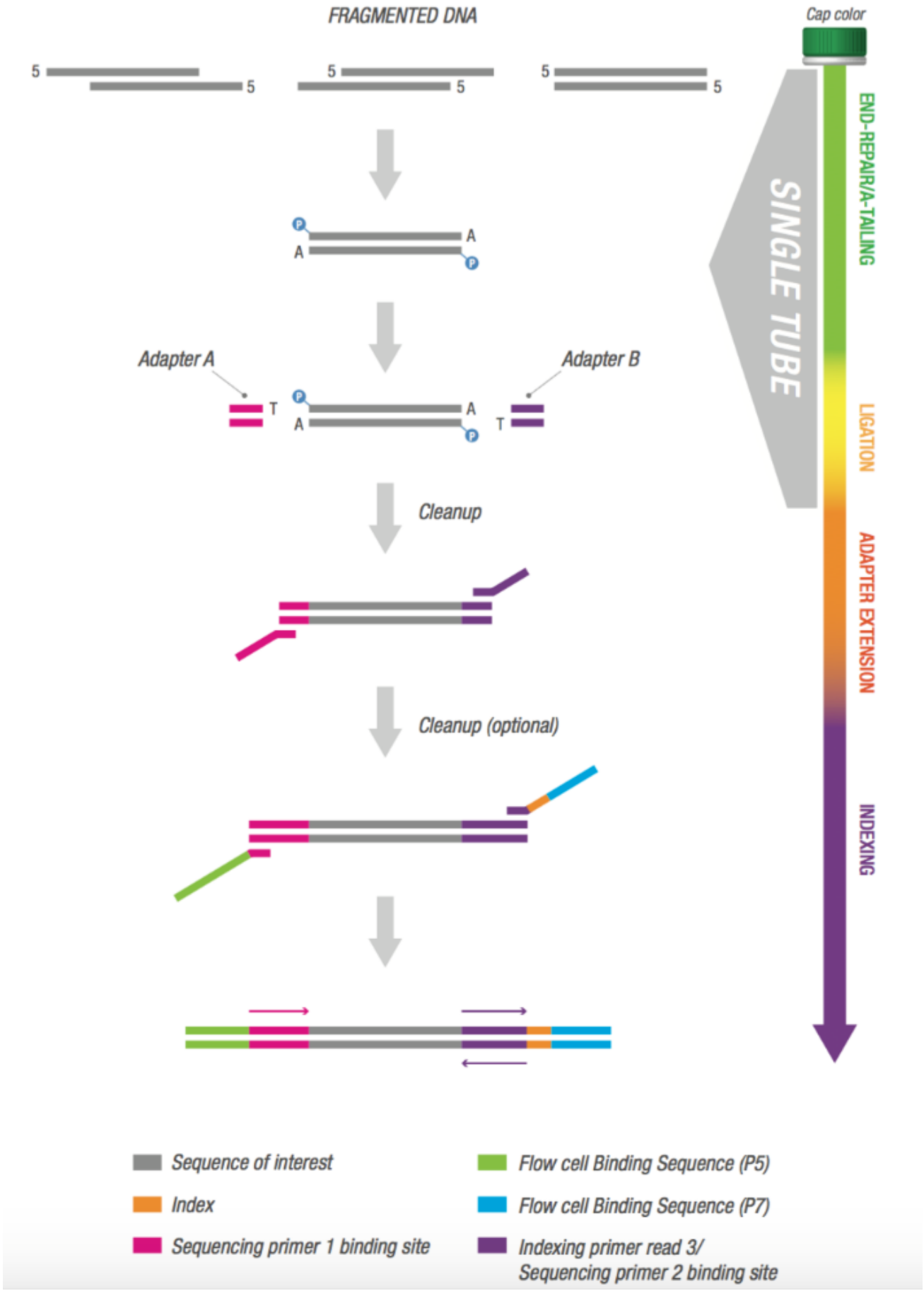

Fig. 1 Workflow for JetSeq™ DNA Library Preparation Kit

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:
PCR equipment: Thermal cycler.
Equipment for the determination of DNA concentration such as Nanodrop™, Qubit™, TapestationTM, Bioanalyzer or equivalent.
Equipment for the determination of DNA size distribution such as Tapestation™, Bioanalyzer or equivalent.
Equipment for the puri cation and size selection of DNA fragments such as AMPure™, Dynabeads™, SPRI™ beads or other equivalent column-based systems.

7. IMPORTANT NOTES

7.1. DNA preparation and quality control

The most important prerequisite for any NGS library preparation is high-quality DNA. Sample handling and DNA isolation procedures are therefore critical
to the success of the experiment. Residual traces of proteins, salts or other contaminants will degrade the DNA or decrease the ef ciency of the enzymatic activities necessary for optimal library preparation.

7.1.1. Recommended genomic DNA preparation method

Depending on the sample, we recommend one of the following extraction kits:

- ISOLATE II Genomic DNA Kit (BIO-52066) for the preparation of genomic DNA from fresh tissues and cells.
- ISOLATE II FFPE RNA/DNA Kit (BIO-52087) for the preparation of genomic DNA from FFPE tissue samples.
- ISOLATE II Plant DNA Kit (BIO-52069) for isolation of genomic DNA from plants.

For more DNA extraction kits, please refer to our ISOLATE II selection tool (www.bioline.com/isolate).

7.1.2. Recommendations for DNA fragmentation

DNA can be fragmented using one of the following methods:

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

To ensure complete fragmentation of the DNA that is needed for library preparation, only use the

recommended parameters given in the manufacturer's instructions. Check the fragmented DNA to ensure a correct size distribution is obtained.

8. PROTOCOL

8.1. End-repair

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

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

Table 1. End-repair reaction mix

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

2. Incubate for 30 min at 20 °C then 30 min at 72 °C.

3. Transfer the reaction tube on ice (4 °C).

8.2. Adaptor ligation

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

1. Using the end-repair reaction from section 7.1 assemble the following reagents on ice. Mix by pipetting up and down.

Table 1. Adaptor ligation reaction mix

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

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

Starting DNA	1x (1–3 µg)	1x (0.5–0.99 µg)	1x (250–499 ng)	1x (100–249 ng)	1x (50–99 ng)	1x (1–49 ng)
DNA sample	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL
Step 2: Ligation Buffer	3 µL	3 µL	3 µL	3 µL	3 µL	3 µL
Step 2: Adaptor A	5 µL	1.5 µL	1 µL	0.5 µL	0.25 µL	≤0.125 µL*
Step 2: Adaptor B	5 µL	1.5 µL	1 µL	0.5 µL	0.25 µL	≤0.125 µL*
Step 2: Ligase	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL
Nuclease-free H20	0 µL	7 µL	8 µL	9 µL	9.5 µL	9.75 µL
TOTAL	65 µL	65 µL	65 µL	65 µL	65 µL	65 µL

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

Appendix A: Adaptor indexes

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

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

Appendix B: Low multiplexing guidelines

Illumina platform such as MiSeq and HiSeq use a red laser to sequence A/C and a green laser to sequence G/T. To ensure accurate registration of the index read, both a red and green signal must be present at each cycle. It is also important to maintain color balance where possible.
If pooling less than eight samples in the final sequencing pool we suggest using the following index combinations

Number of samples in pool	Index
1	Any index

2	2 & 6
3	Option A: 4, 6 & 7 Option B: 1, 11 & 16
4	Option A: 2, 6, 10 & 14 Option B: 9, 12, 15 & 16
6	Option A: 1, 2, 4, 6, 7 & 8 Option B: 2, 8, 9, 12, 15 & 16
8	Option A: 1-8 Option B: 9-16

A TECHNICAL SUPPORT AND TROUBLESHOOTING

For technical assistance or more information on this product, please email us at tech@bioline.com

B ASSOCIATED PRODUCTS

Product	Size	Cat. #
ISOLATE II Genomic DNA Kit	50 prep	BIO-52066
ISOLATE II FFPE RNA/DNA Kit	50 prep	BIO-52087
ISOLATE II Plant DNA Kit	50 prep	BIO-52069
JetSeq Library Quantification Kit	TBD	Please enquire

C PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace any product that does not conform to the specifications free of charge. This warranty limits Bioline’s liability to only the replacement of the product.

D TRADEMARK AND LICENSING INFORMATION

JetSeq™ was developed jointly by OGT and Bioline. JetSeq™ (Bioline Reagents Ltd), HiSeq™, MiSeq™, NextSeq™ (Illumina Inc.); Qubit® (ThermoFisher Scientific); Dynabeads™ (Dynal Inc.); AMPure™ (Backman Coulter Inc.)

Ordering Information

Product	Size	Cat. #

Protocol

End-repair

Step 1.

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

End-repair

Step 2.

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

Table 1. End-repair reaction mix

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

End-repair

Step 3.

Incubate for 30 min at 20 °C (incubation 1/2).

DURATION

00:30:00

End-repair

Step 4.

Incubate for 30 min at 72 °C (incubation 2/2).

DURATION
00:30:00

End-repair

Step 5.

Transfer the reaction tube on ice (4 °C).

Adaptor ligation

Step 6.

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

Adaptor ligation

Step 7.

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

Table 1. Adaptor ligation reaction mix

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

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

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

NOTES

Ben Jackson 03 Nov 2016

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

Adaptor ligation

Step 8.

Incubate for 15 min at 20 °C.

DURATION

00:15:00

Adaptor ligation

Step 9.

Clean-up and size select the adaptor-ligated library. It is important at this stage to remove unwanted adaptor-dimers.

Our suggested protocol for post ligation clean-up using AMPure XP beads:

https://www.protocols.io/view/clean-up-using-ampure-xp-beads-f3ebqje.

NOTES

Ben Jackson 25 Sep 2016

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

Adaptor ligation

Step 10.

Assess the quality and concentration of the cleaned up adaptor-ligated DNA.

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

NOTES

Ben Jackson 03 Nov 2016

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

Adaptor extension (PCR 1)

Step 11.

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

Adaptor extension (PCR 1)

Step 12.

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

Table 3. PCR 1 reaction mix

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

Adaptor extension (PCR 1)

Step 13.

Run the PCR using the following conditions.

Table 4. PCR 1 cycling conditions

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

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

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

NOTES
Ben Jackson 03 Nov 2016

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

Adaptor extension (PCR 1)

Step 14.

Check the quality of the library on a Bioanalyzer, Tapestation or similar equipment.

If target selection of DNA capture is used, **skip this step.**

NOTES
Ben Jackson 26 Sep 2016

This is to ensure the absence of adaptor-dimers. If adaptor-dimers are observed it is recommended that a clean-up of the adaptor extension (PCR 1) is performed in order to remove these unwanted products.

Suggestest protocol for post adaptor extension (PCR1) clean-up using AMPure XP beads in Guidelines.

Ben Jackson 03 Nov 2016

This is to ensure the absence of adaptor-dimers. If adaptor-dimers are observed it is recommended that a clean-up of the adaptor extension (PCR1) is performed in order to remove these unwanted products.

Please see the suggested protocol for post-adaptor extension (PCR1) clean-up using AMPure XP beads.

Adaptor extension (PCR 1)

Step 15.

Determine the PCR product concentration using a Nanodrop, Qubit or equivalent.

NOTES

Ben Jackson 03 Nov 2016

If the samples are not to be used immediately, store at -20 °C.

Adaptor completion and indexing (PCR 2)

Step 16.

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

Adaptor completion and indexing (PCR 2)

Step 17.

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

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

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

NOTES

Ben Jackson 03 Nov 2016

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.

Adaptor completion and indexing (PCR 2)

Step 18.

Run the PCR with the following cycling conditions:

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

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

NOTES

Ben Jackson 03 Nov 2016

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.

Adaptor completion and indexing (PCR 2)

Step 19.

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

- If adaptor dimers are observed it is recommended to remove these unwanted products by size selection using a suitable clean-up and size selection equipment and reagents.

- If no adaptor dimers are detected perform only a clean-up of the product of PCR 2 using a suitable clean-up and size selection equipment and reagents

Please see the [suggested protocol for post indexing clean-up using AMPure XP beads].

NOTES

Ben Jackson 03 Nov 2016

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

Ben Jackson 03 Nov 2016

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

Adaptor completion and indexing (PCR 2)

Step 20.

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

Adaptor completion and indexing (PCR 2)

Step 21.

The DNA library is ready for sequencing on MiSeq, NextSeq and HiSeq platforms and can be pooled if necessary.

ANNOTATIONS

Ben Jackson 26 Sep 2016

When loading the library in the sequencing machine we recommend following the manufacturer's instructions.