Manual

Westburg NGS DNA Library Prep Kit

WB 9024
WB 9096

Version 3.1 10/2018

CONTENTS

INTRODUCTION

Despite the tremendous advances in Next Generation Sequencing (NGS) technology, library preparation is still one of the main limiting factors in the NGS workflow. This is mainly due to the mechanical fragmentation and cleanup steps in existing methods, which cause a substantial loss of time and sample and limit the possibilities to automate the workflow.

The Westburg NGS DNA Library Prep Kit replaces mechanical with fully enzymatic fragmentation, while maintaining a low sequence bias similar to shearing. Furthermore, the procedure reduces the number of cleanup steps, saving cost and maximizing library yield. Both fragmentation and library prep are carried out in a single tube, which facilitates automation. The unique production process of the fragmentation mix guarantees lot-to-lot consistency and enables perfect reproducibility within a validated (automated) workflow.

Compatibility

- Whole Genome Sequencing
- Whole Exome Sequencing/Hybridization-Capture
- PCR-free Sequencing
- FFPE Sample Preparation

Sequencing of cfDNA or other fragmented DNA samples

Once validated, the same procedure can be used for cfDNA or other fragmented DNA samples using the Westburg NGS NoFrag Library Prep Kit. No need to implement a whole new procedure when working with various types of samples.

INTRODUCTION

Outline of procedure

In the first step, DNA is enzymatically cut into smaller fragments. The median fragment size can be adjusted to the requirements of your application by modifying reaction time and conditions. The DNA fragments generated are immediately end-repaired and A-tailed during the same incubation. In case the input material consists of shorter DNA fragments, < 1kb, we recommend the use of the NoFrag version of the Westburg NGS DNA Library Prep Kit. Without intermediate cleanup the sample is now ready for ligation of adapters specific for your Illumina sequencing platform.

When working with DNA samples of 100 ng or less it is recommended to perform library amplification with the Westburg NGS DNA Library Amplification Mix. This is a high-fidelity PCR master mix with a robust amplification performance across a wide range of GC percentages, minimizing sequencing bias introduced by PCR.

After library prep and optional amplification, reaction cleanup and removal of adapter-dimers is achieved with magnetic beads. Also this step can be easily automated.

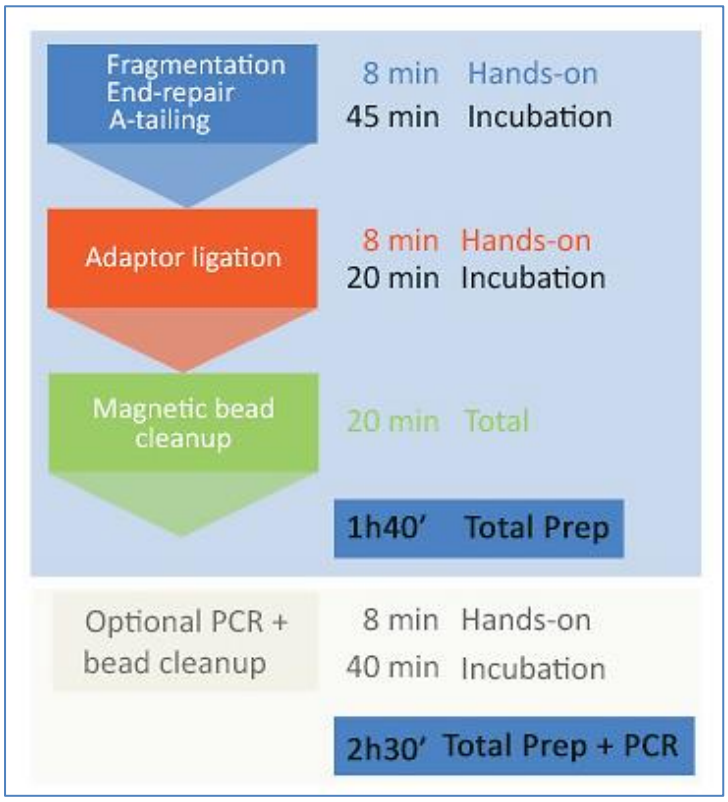

Figure 1. Protocol outline

KIT COMPONENTS

Westburg NGS DNA Library Prep Kit

Cat. No.	WB 9024	WB 9096
Size	**24 rxns**	**96 rxns**
Fragmentation Mix (5X)	240 µl	1.100 µl
Fragmentation Buffer (10X)	250 µl	1.000 µl
Enhancer Buffer	250 µl	1.000 µl
DNA Ligase	240 µl	1.100 µl
Rapid Ligation Buffer (5X)	500 µl	2.200 µl

IMPORTANT: Enzyme mixes and their corresponding reaction buffer are paired per kit for optimal performance. Do not mix between different kits.

Westburg NGS DNA Library Amplification Mix

Optional, to be ordered separately

Cat. No.	WB 9100
Size	**96 Rxns**
Amplification Master Mix (2X)	240 µl
Primer Mix (10 µM)	250 µl

Storage

All reagents must be stored at -15°C to -25°C.

To be supplied by the user

Agencourt® AMPure® XP or SPRI Beads for bead-based library purification	
80% ethanol (ACS grade), freshly prepared in nuclease free water	
Nuclease-free water	
10mM Tris-HCl buffer pH 8.0	
Indexed adapters	
PCR tubes or plates	Westburg Amplistar plastics
Low DNA-binding pipet filter tips	Westburg TripleA filter tips
Low DNA-binding tubes	Westburg U-tube 1.5 mL
Thermocycler	
Magnetic rack for magnetic bead separation	
Capillary electrophoresis device, Agilent® Bioanalyzer or similar device	

IMPORTANT CONSIDERATIONS

Before you start

- The first time, read the entire protocol before you start so you are well prepared and have all required reagents and materials ready for use.
- In order to achieve optimal results, it is important to accurately follow the protocol.
- Plastics must be certified sterile and nuclease-free. Clean the bench surface and pipettes with RNase Away® (ThermoFisher Scientific) to eliminate nuclease and DNA.
- Enzyme-based DNA fragmentation is not just influenced by reaction time and temperature but also by the type of input DNA. We recommend optimizing the reaction time using the same DNA sample, or at least very similar samples.
- **Allow reagents and buffers to thaw completely on ice.** If used too soon the solution volume may not be sufficient and reagent concentrations may be incorrect.
- After thawing the reagents keep them on ice during the entire protocol. Master mixes should also be prepared and kept on ice.
- **Do not vortex the Fragmentation mix or the DNA ligase**. Instead, mix by tapping the tube gently with your finger. Other components can be mixed by briefly vortexing.
- When preparing master mixes for multiple samples calculate 10% extra volume to compensate for pipetting loss.
- Indexed adapters are not included. The kit is compatible with all Illumina indexed adapters. We recommend to use the TruSeq® LT or HT adapters, which can be ordered either directly from Illumina or as custom oligos for NGS from Integrated DNA Technologies (IDT). These adapter sequences are listed in Appendix III.

Input DNA requirements

- Best results are obtained using high-purity DNA samples that are free of residual salts, proteins, detergents or other contaminants. Use a commercially available purification kit that delivers this DNA purity.
- Use 1 - 1000 ng purified DNA as input.
- It is very important that the input DNA concentration is accurately determined, especially when it is less than 100ng DNA. Fluorometric methods (Qubit, Picogreen) are recommended.
- The kit is compatible with DNA samples dissolved in water, 10 mM Tris, 0.1X TE or Buffer EB (QIAGEN elution buffer). DNA is dissolved in 1X TE should be purified upfront using AMPure XP beads (Appendix I) or the fragmentation protocol should be adjusted, see Appendix II.

Before you start

It is essential to optimize DNA fragmentation time if you are using this kit for the first time, in order to get the desired fragment size.

Fragmentation time optimization

The optimization should be carried out using DNA samples that are similar to the actual experimental samples with respect to the amount, source and purification method.

Optimization should be repeated if the DNA amount, source or purification method changes.

For initial optimization we recommend testing 3 time points, as listed in Table 1 on page 8. Fine-tuning may be required if precise fragment size is critical.

For input DNA less than 10 ng reaction time can be shortened by adding 2.5 µl of Enhancer in a 50 µl reaction and incubate with a fragmentation time of 10 min. This will result in a fragment peak size centred around 350 bp.

The size range of the fragmented DNA can be evaluated immediately if the input DNA amount is >100ng. Use 1.8X Agencourt® AMPure® XP beads to purify the fragmented DNA and elute in 10 µl of Tris Buffer or water. Then use the BioAnalyzer® High Sensitivity kit or a similar kit to determine the size distribution.

PROTOCOL

A. Fragmentation, end repair and dA tailing

1a. Fragmentation time optimization

(skip this step if you have already optimized fragmentation time)

Create three programs in a thermocycler as shown in the table below. For program step 2 select three fragmentation times from Table I depending on DNA input amount and desired fragment size. Set the temperature of the heated lid to ~70°C.

Step	Temp.	Time
Pre-cooling	4°C	
1	4°C	1 min
2	32°C	See Table 1
3	65°C	30 min
4	4°C	Hold

Table I: Optimization of fragmentation time

	Initial fragmentation times (minutes)			
Input	**250 bp**	**350 bp**	**450 bp**	**550 bp**
10 ng DNA	20-24-28	13-16-19	11-14-17	7-10-13
100 ng DNA	13-16-19	7-10-13	6-8-10	5-6-7
1000 ng DNA	11-14-17	6-8-10	5-6-7	3-4-5

Proceed with master mix preparation in protocol step 2 on page 9.

1b. Setup for experimental samples

(skip this step if you are optimizing fragmentation time)

Set up the following program in a thermocycler. For program step 2 use the time you determined by optimization. Set the temperature of the heated lid to ~70°C.

Step	Temp.	Time
Pre-cooling	4°C	
1	4°C	1 min
2	32°C	Optimal fragmentation time determined in step 1a
3	65°C	30 min
4	4°C	Hold

Proceed with master mix preparation in protocol step 2 on page 9.

PROTOCOL

Note: it is essential to keep the PCR tube on ice during steps 2-4.

2. For each sample prepare the following master mix in a thin-walled PCR tube on ice. Mix well by gentle vortexing:

Prevent the formation of bubbles.

	Input DNA	
Reagents	**1-10 ng**	**>10 ng**
Fragmentation Buffer (10X)	5 µl	5 µl
Purified DNA	X µl	X µl
Enhancer Buffer	2.5 µl	-
Nuclease-free H2O	(32.5-X) µl	(35-X) µl
Total	**40 µl**	**40 µl**

3. Add to the prepared mix from step 2, 10 µl 5X Fragmentation and mix well by low-speed vortexing.

4. Briefly spin the sample tube and immediately transfer it to the pre-chilled thermocycler (4°C).

5. Start the thermocycler program you created in step 1a or 1b.

6. When the program is complete and cycler block has returned to 4°C, remove the samples from the block and place on ice.

Immediately continue to Protocol B. Adapter ligation on page 10.

B. Adapter Ligation

Note that indexed adapters are not included in the kit. The kit is compatible with all Illumina indexed adapters. We recommend to use the TruSeq® LT or HT adapters, which can be ordered either directly from Illumina or as custom oligos for NGS from Integrated DNA Technologies (IDT). These adapter sequences are listed in Appendix III.

Follow the supplier's recommendation for adapter concentration and usage. For optimal ligation use a molar ratio of adapter to insert between 25:1 and 200:1 based on the amount of input DNA and the mean size of the fragmented DNA. See Appendix III for additional adapter guidelines.

7. Equilibrate 0.8X Agencourt® AMPure® XP beads to room temperature for 20–30 minutes for use in step 13.

8. Thaw adapters, mix briefly by tapping or vortexing and pulse-spin to collect the liquid at the bottom of the tube.

9. Add the required volume of DNA adapter, as specified by the adapter supplier, to the PCR tube containing 50 µl of A-tailed fragmented DNA from step 7. This volume is designated "Y". Mix gently by pipetting and cool on ice.

10. Prepare the following ligation master mix on ice in a separate tube and mix well by gentle pipetting or low-speed vortexing:

Reagent	Volume per reaction
5X Ligation Buffer	20 µl
DNA Ligase	10 µl
Nuclease-free H_2O	(20-Y*) µl
Total	**(50-Y*) µl**

*Y is the volume of adapter used in step 9.

11. Add (50 – Y) µl of the ligation master mix to the sample and mix well by pipetting.

12. Incubate the ligation reaction at 20°C for 15 min.

Do not use a thermocycler with a heated lid for this incubation

Immediately proceed to Protocol C on page 11: Ligation Cleanup.

C. Ligation Cleanup

If size selection is required, use your method of choice and follow the corresponding protocol.

13. Transfer 90 µl of ligation sample from step 12 to a fresh 0.5 ml centrifuge tube. Add 80 µl of thoroughly vortexed room-temperature equilibrated AMPure® XP beads slurry to the ligation sample and mix well by gently vortexing for 30 seconds or pipetting up and down 6-8 times.

14. Incubate the mixture for 5 min at room temperature.

15. Pellet the beads on a magnetic stand until the solution is clear.

16. Carefully discard the supernatant without disturbing the separated magnetic beads.

17. Add 200 µl of freshly prepared 80% ethanol to the beads while on the magnetic stand and incubate for 30 seconds. Carefully remove the ethanol solution without disturbing the separated magnetic beads.

18. Repeat the wash (step 17).

19. Remove residual ethanol with a small pipette tip, if necessary with tube on the magnetic stand.

20. Air-dry the beads on the magnetic stand for 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. While ethanol residue will inhibit the sequencing process.

21. Resuspend the dried beads in 28 µl of 10 mM Tris-HCl, pH 8.0 on the table. Mix well by gently vortexing or pipetting up and down

22. Incubate 3 minutes at room temperature with lid open.

23. Pellet the beads on the magnetic stand. Carefully transfer 25 µl of supernatant into a new tube.

If you are not proceeding immediately, store the sample at -20°C.

Library amplification *(optional)*
In case the starting amount of DNA was < 100ng, or when a large quantity of library is needed, an additional high-fidelity amplification step can be performed with the Westburg NGS DNA Library Amplification Mix (Cat. No. WB 9100). Proceed with section D. Library Amplification on page 12.

If no library amplification is needed proceed with section F on page 14.

D. Library Amplification (optional)

24. Before you start, prepare fresh 80% ethanol.

25. Enter the following program into a thermocycler. The cycler's heated lid must be set at 105°C.

Step	Temperature	Incubation time	Cycles
1	98°C	2 min	1
2	98°C	20 sec	6- 12*
3	60°C	30 sec	
4	72°C	30 sec	
5	72°C	1 min	1
6	4°C	Hold	1

* As a general guideline use 6 cycles for 100 ng, 10 cycles for 10 ng, and 12 cycles for 1 ng input DNA.

It is essential to keep the PCR tube on ice during step 26 and 27.

26. Prepare the following PCR reaction master mix in a separate tube on ice and mix well by pipetting.

Reagents	Volume per reaction
Library Amplification Mix	25 µl
Primer Mix	1.5 µl
Total	**26.5 µl**

27. Transfer 23.5 µl of adapter-ligated and purified DNA to a new thin-walled PCR tube. Add 26.5 µl of the master mix from step 26 and gently mix well by pipetting up-and-down 6-8 times.

28. Pulse-spin the sample tube and immediately transfer to the thermal cycler and start the cycling program from step 25.

29. During the PCR run, equilibrate 1X Agencourt® AMPure® XP beads to room temperature for 20-30 min.

30. When the PCR is complete and sample block has returned to 4°C, remove samples from block and **immediately proceed to section E. DNA Library Cleanup on page 13.**

E. Library Cleanup after Amplification

31. Transfer 50 µl of the PCR solution from step 30 in a fresh 1.5ml centrifuge tube. Add 50 µl of thoroughly vortexed room-temperature equilibrated AMPure® XP beads slurry and mix well by gently vortexing for 30 seconds or pipetting up and down 6-8 times.

32. Incubate the mixture for 5 min at room temperature.

33. Pellet the beads on a magnetic stand until the solution is clear.

34. Carefully discard the supernatant without disturbing the separated magnetic beads.

35. Add 200 µl of freshly prepared 80% ethanol to the beads while on the magnetic stand and incubate for 30 seconds. Carefully remove the ethanol solution without disturbing the separated magnetic beads.

36. Repeat the wash (step 35).

37. Remove residual ethanol with a small pipette tip if necessary with tube on the magnetic stand.

38. Air-dry the beads on the magnetic stand for 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Ethanol residue will inhibit the sequencing process.

39. Resuspend the dried beads in 32.5 µl of 10 mM Tris-HCl, pH 8.0 on the table. Mix well by gently vortexing or pipetting up and down.

40. Incubate 3 minutes at room temperature with lid open.

41. Pellet the beads on the magnetic stand. Carefully transfer 30 µl of supernatant into a new low DNA binding tube.

Now proceed to section F on page 14.

If you are not proceeding immediately, store the sample at -20°C.

F. Determine library quantity and quality

Your DNA library is now ready, but before it can be used for sequencing, library quantification should be performed using real-time PCR or Qubit fluorometric quantification (not included).

Determine library quality using an Agilent BioAnalyzer or similar capillary electrophoresis device. Check for the intended size distribution of library fragments and for the absence of a peak of adapters or adapter-dimers around 120 bp.

Take into account that adapter ligation adds 120 bp to the library fragment size.

TROUBLESHOOTING

If you encounter any issues, the table below may be helpful to find the cause and a solution.
If you need further assistance, do not hesitate to contact our Scientific Support (scientific.support@westburg.eu or +31 33 495 0094). Please provide a detailed description of the problem and include data and/or pictures if possible.

Low Library Yield

Poor DNA quality	Use only high-purity DNA as input. Contaminants may interfere with the activity of the enzymes in the kit reducing library yield.
RNA contamination	RNA may have been co-purified with genomic DNA. This contaminating RNA affects the accuracy of DNA quantification. Use a high-quality DNA isolation kit that sufficiently removes RNA or treat the DNA sample with RNase A.
Too little input DNA for sequencing without amplification	For direct sequencing without amplification, you need to start with 100ng DNA or more to generate enough library yield. When starting with less than 100 ng: after the adapter ligation step perform library amplification with the Westburg NGS DNA Library Amplification Mix (Cat. no. WB 9100)

Unwanted peaks seen after capillary electrophoresis

Short peaks at 60 - 120 bp	These are adapter and adapter-dimer peaks caused by incomplete adapter depletion after library prep. Adapter-dimers will reduce the capacity of the flow cell for the library fragments. Use Agencourt® AMPure® XP Beads to remove free adapters and adapter-dimers. A small contamination of the library with adapter-dimers is acceptable.
DNA fragment size before adapter ligation is not right	The enzymatic fragmentation conditions have not been chosen properly to get the desired fragment size. Increase or decrease reaction time in steps of 2-4 minutes until you get the desired size range.
Larger library fragments after adapter ligation and amplification	If you see a shift in fragment size of more than the expected 120bp after adapter ligation and PCR enrichment, this can be caused by over-amplification of the DNA library. Reduce the number of amplification cycles to a point where library size is correct and yield is still sufficient.

TROUBLESHOOTING

Unwanted peaks seen after capillary electrophoresis *(continued)*

Library fragment size *after* adapter ligation is not correct	**Adapter ligation adds 60 bp to both ends of the DNA fragments, which should be seen in capillary electrophoresis as a shift of 120bp of all library fragments. If this shift is not or only partially seen, adapter ligation efficiency has been too low. Be sure to use the correct amount of starting DNA and the corresponding parameters and incubation times in the protocol for end-repair, A-addition and ligation. Exact adapter sizes can be found in the documentation from the library adapter supplier.**

APPENDIX I. INPUT DNA CLEANUP: REMOVAL OF EDTA AND DIVALENT CATIONS

If the DNA sample is not in one of the recommended buffers (water, 10 mM Tris, 0.1X TE or Buffer EB) you should first purify the DNA using AMPure® XP beads, as described below. Alternatively, use the protocol for fragmentation of samples in 1X TE (Appendix II).

1. If the volume of the sample is below 50 µl, add nuclease-free water to the sample to get a total of 50 µl.

2. Add 90 µl of thoroughly vortexed room-temperature equilibrated AMPure® XP beads slurry to the 50 µl sample and mix well by pipetting. If the DNA sample is more than 50 µl, scale up the volume of AMPure® XP beads accordingly to achieve a 1.8X ratio of beads to DNA.

3. Incubate the mixture for 5 min at room temperature.

4. Pellet the beads on a magnetic stand until the solution is clear.

5. Carefully discard the supernatant without disturbing the separated magnetic beads.

6. Add 200 µl of freshly prepared 80% ethanol to the beads while on the magnetic stand and incubate for 30 seconds. Carefully remove the ethanol solution without disturbing the separated magnetic beads.

7. Repeat the wash (step 6).

8. Remove residual ethanol with a small pipette tip if necessary with tube on the magnetic stand.

9. Air-dry the beads on the magnetic stand for 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Ethanol residue will inhibit the sequencing process.

10. Thoroughly resuspend the dried beads in 45 µl of 10 mM Tris-HCl, pH 8.0 on the table. Mix well by gently vortexing or pipetting up and down.

11. Incubate 3 minutes at room temperature with lid open.

12. Pellet the beads on the magnetic stand. Carefully transfer 42.5 µl of supernatant into a new low DNA binding tube.

13. Determine the concentration of the purified DNA using a fluorometric method such as Qubit or Picogreen.

APPENDIX II. FRAGMENTATION, END REPAIR & dA-TAILING OF DNA IN 1X TE

1. Set up the following program in a thermocycler. Set the temperature of the heated lid to ~70°C if possible:

Step	Temp.	Time
Pre-cooling	4°C	
1	4°C	1 min
2	32°C	5-35 min*
3	65°C	30 min
4	4°C	Hold

***The fragmentation time should be optimized for different amounts of input DNA, see page 8**

It is essential to keep the PCR tube on ice during steps 2-5.

2. For each sample prepare the following master mix in a thin-walled PCR tube on ice. Mix well by gentle vortexing:

Prevent the formation of air bubbles.

	Input DNA:	
Reagents	**1-10 ng**	**>10 ng**
Fragmentation Buffer (10X)	5 µl	5 µl
Purified DNA	X µl	X µl
Enhancer Buffer	5 µl	2.5 µl
Nuclease-free H2O	(30-x) µl	(32.5-X) µl
Total	**40 µl**	**40 l**

3. Add to the prepared mix from step 2, 10 µl 5X Fragmentation and mix well by low-speed vortexing.

4. Briefly spin the sample tube and immediately transfer it to the pre-chilled thermocycler (4°C). Resume the cycling program from step 1.

5. When the program is complete and cycler block has returned to 4°C, remove the samples from the block and place on ice. **Immediately continue to the Part B. Adapter Ligation, page 10.**

APPENDIX III. ADAPTER GUIDELINES

The Westburg NGS DNA Library Preparation kit does not include indexed adapters, necessary to be able to sequence the libraries on Illumina Sequencers. However, the kit is compatible with all Illumina indexed adapters.

We recommend to use the TruSeq® LT or HT adapters, which can be ordered either directly from Illumina or as custom oligos for NGS usage from Integrated DNA Technologies (IDT). These adapter sequences are listed below.

TruSeq® HT indexed adapters (Dual indexing):

D501–D508 Adapters

AATGATACGGCGACCACCGAGATCTACAC**[i5]**ACACTCTTTCCCTACACGACGCTCTTCCGATCT

	i5 Bases for Sample Sheet	i5 Bases for Sample Sheet
i5 Index Name	MiSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
D501	TATAGCCT	AGGCTATA
D502	ATAGAGGC	GCCTCTAT
D503	CCTATCCT	AGGATAGG
D504	GGCTCTGA	TCAGAGCC
D505	AGGCGAAG	CTTCGCCT
D506	TAATCTTA	TAAGATTA
D507	CAGGACGT	ACGTCCTG
D508	GTACTGAC	GTCAGTAC

D701–D712 Adapters

GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**[i7]**ATCTCGTATGCCGTCTTCTGCTTG

i7 Index	i7 Bases for Sample Sheet
D701	ATTACTCG
D702	TCCGGAGA
D703	CGCTCATT
D704	GAGATTCC
D705	ATTCAGAA
D706	GAATTCGT
D707	CTGAAGCT
D708	TAATGCGC
D709	CGGCTATG
D710	TCCGCGAA
D711	TCTCGCGC
D712	AGCGATAG

APPENDIX III. ADAPTER GUIDELINES (continued)

TruSeq® LT Indexed adapters (Single Indexing):

TruSeq Universal Adapter
5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

TruSeq Index Adapters (Index 1–27)
Index numbers 17, 24, and 26 are reserved.
Index sequences are 6 bases as underlined.

TruSeq Adapter, Index 1
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 2
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 3
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTTAGGCATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 4
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 5
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 6
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 7
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 8
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTTGAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 9
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 10
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAGCTTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 11
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGCTACATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 12
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 13
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTCAACAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 14
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTTCCGTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 15
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATGTCAGAATCTCGTATGCCGTCTTCTGCTTG

APPENDIX III. ADAPTER GUIDELINES (continued)

TruSeq Adapter, Index 16
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACCCGTCCCGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 18
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTCCGCACATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 19
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGAAACGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 20
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGGCCTTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 21
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTTTCGGAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 22
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGTACGTAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 23
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACGAGTGGATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 25
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTGATATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 27
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCCTTTATCTCGTATGCCGTCTTCTGCTTG

Adapter ligation guidelines

- Per Library, 2 adapters are needed.
  - When working with the TruSeq® LT adapters, use the Universal Forward adapter and an Indexed adapter of choice.
  - When working with the TruSeq® HT adapters, any combination of a D50x and a D7xx adapter can be used
- In general, the concentration of the adapter stock solutions is 15µM.
- For libraries prepared from >100ng input, use 2.5µl of each adapter (5µl adapter mix in total)
- For libraries prepared from 1-100ng input, use 2.5µl of a 1:10 dilution of each adapter (5µl 1:10 diluted adapter mix in total).

Alternatively, when the adapter concentration is unknown, please use the following recommendation: Based on a fragment size of 200bp, the adapter-to-insert ratio can be optimised as follows:

Input amount	1µg ----------> 1ng
Adapter/insert ratio	25:1----------> 200:1

Legal information about adapter sequences

APPENDIX IV. HYB-CAPTURE RECOMMENDATIONS

Integration of Westburg NGS DNA Library Prep kit in Hybridization Capture workflows

A. Roche® Nimblegen SeqCap™

General SeqCap™ Protocol:

1. Library Preparation & cleanup
2. Library Amplification & cleanup
3. Mix amplified libraries (for multiplex sample capture only)
4. Hybridization Sample libraries and probe pools
5. Wash & recovery of captured DNA
6. Amplification captured DNA & cleanup
7. Sequencing

How to use Westburg NGS DNA Library Prep kit:

1. Prepare libraries with the Westburg NGS DNA Library Prep kit and Indexed adapters.
   Set up fragmentation reaction to achieve DNA fragments of ±200bp.
2. Follow Westburg NGS DNA Library Prep protocol until section C, step 23 (Ligation Cleanup).
3. Proceed with SeqCap™ protocol, starting with Library amplification

B. Agilent® SureSelect™

I. Agilent® SureSelect™ XT

This system uses post-capture indexing. During the library preparation, non-indexed adapters are ligated. After capture, libraries are indexed, using indexing primers during the post-capture amplification.

General SureSelect™ XT protocol:

1. Library Preparation & cleanup (fragmentation by mechanical shearing)
2. Library Amplification & cleanup
3. Hybridization of DNA library samples to capture library
4. Capture & recovery of hybridized libraries
5. Amplification captured libraries with indexing primers
6. Pool indexed, target enriched libraries
7. Sequencing

How to use the Westburg NGS DNA Library Prep kit

1. Prepare libraries with the Westburg NGS DNA Library Prep kit
   - *Set up fragmentation reaction to achieve DNA fragments of 150 - 200bp*
   - *Use the SureSelect™ XT Adapter Oligo Mix (brown capped tube from SureSelect™ XT reagent kit)*
     - *When starting with 1µg input DNA, use 10µL undiluted Adapter Oligo Mix*
     - *When starting with 200ng input DNA, use 10µL of 1:10 diluted (in nuclease free water) Adapter Oligo Mix*
2. Follow Westburg NGS DNA Library Prep protocol until section C, step 23 (Ligation cleanup)
3. Proceed with SureSelect™ XT protocol, starting with library amplification.
   a. *Use the following reaction setup volumes for the library amplification:*

APPENDIX IV. HYB-CAPTURE RECOMMENDATIONS

Reagent	Volume per reaction
Nuclease free water	11 µL
SureSelect primer (brown cap)	1.25 µL
SureSelect ILM Indexing Pre-capture PCR Reverse Primer (clear cap)	1.25 µL
5x Herculase II Reaction Buffer (clear cap)	10 µL
100mM dNTP Mix (green cap)	0.5 µL
Herculase II Fusion DNA Polymerase (red cap)	1 µL
Total	**25 µL**

b. Add 25µL of the PCR reaction mixture to each purified DNA library sample (25µL).
c. Follow PCR protocol as outlined in the SureSelect™ XT manual.
i. Use 4-6 cycles of amplification when starting from 1µg input DNA
ii. Use 10 cycles of amplification when starting from 200ng input DNA

II. Agilent® SureSelect™ XT2

This system uses pre-capture indexing. During the library preparation, full length indexed adapters are ligated, similarely to the Roche Nimblegen or IDT XGEN approach. As a result, Agilent® SureSelect™ XT2 is compatible with any Illumina® sequencing compatible library.

General SureSelect™ XT2 protocol:

1. Library Preparation & cleanup
    - Fragmentation by mechanical shearing
    - Ligation of indexed adapters
2. Library Amplification & cleanup
3. Pool indexed libraries
4. Hybridization of DNA library samples to capture library
5. Capture & recovery of hybridized libraries
6. Amplification captured libraries
7. Sequencing

How to use the Westburg NGS DNA Library Prep kit

1. Prepare libraries with the Westburg NGS DNA Library Prep kit
    - *Set up fragmentation reaction to achieve DNA fragments of 150 - 200bp*
    - *Use the SureSelect™ XT2 Pre-capture Indexed Adapter solution for the adapter ligation (use the index of choice)*
        - *When starting with 1µg input DNA, use 5µL undiluted Pre-capture Indexed Adapter solution*
        - *When starting with 100ng input DNA, use 5µL of 1:5 diluted (in nuclease free water) Pre-capture Indexed Adapter solution.*

2. Follow Westburg NGS DNA Library Prep protocol until section C, step 23 (Ligation cleanup)
3. Proceed with SureSelect™ XT2 protocol, starting with library amplification.
    - When starting with 1µg of Input DNA, use 5 cycles of amplification.
    - When starting with 100ng of Input DNA, use 8 cycles of amplification

APPENDIX IV. HYB-CAPTURE RECOMMENDATIONS

C. Integrated DNA Technologies® XGEN™

- The XGEN™ hybridization capture protocol starts from prepared Illumina® sequencing compatible libraries.
- When working with this system, prepare the libraries, following the Westburg NGS DNA Library Prep protocol, including library amplification and cleanup.
    - Set up the fragmentation reaction to achieve DNA fragments between 150 – 300bp.
- After library amplification, cleanup and quantification, continue with the XGEN™ hybridization capture protocol for the capture of the DNA libraries.

QUALITY CONTROL

All kit components have been subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans. Please contact techsupport@westburg.eu for the Material Safety Data Sheet of the product.

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