SYNTHETIC GENOMICS®

NGS Library Construction Kit User Guide

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Product Information

Kit Components

NGS Library Construction Kit Box A (Part number BX2000-08MA)				
Component	**Quantity**	**Cap color**	**Volume**	**Storage Temperature**
End-Repair and Adenylation Buffer	1 vial	Clear	120 µL	–20°C
End-Repair and Adenylation Enzyme Blend		Clear	24 µL	
Ligase Enzyme Blend		Orange	380 µL	
Amplification Master Mix		Red	96 µL	
Amplification Primer Mix		Red	16 µL	
Nuclease-free Water		Blue	1.5 mL	
Resuspension Buffer		Green	1 mL	
Barcodes (1–8)	8 vials	Yellow	5 µL (each)	

NGS Library Construction Kit Box B (Part number BX2000-08MB)				
Component	**Quantity**	**Cap color**	**Volume**	**Storage Temperature**
Paramagnetic DNA Purification Beads	1 vial	Amber	1. 3 mL	4°C

Required materials not included

- 10–50 ng (per reaction) of fragmented DNA in up to 32 µL nuclease-free water
- Ethanol, 70% and 100% (room temperature)
- DNA fragmentation instrument (e.g. Covaris® System S2, E210)

  Note: Enzymatic fragmentation methods are not recommended as they may introduce cleavage bias.
- PCR 8-well tube strips (e.g. VWR, Cat. No. 93001-120) or 1.7-mL microcentrifuge tubes (e.g. VWR, Cat. No. 87003-294)
- 96-well PCR plate non-skirted (e.g. Phenix Research, Cat. No. MPS-499 or similar)
- 96-well library storage and pooling plate (e.g. Fisher Scientific, Cat. No. AB-0765 or similar)
- Adhesive PCR plate seal (e.g. BioRad, Cat. No. MSB1001)
- Magnetic Stand (e.g. Thermo Fisher, Cat. No. 12321D or similar)
- 96-well plate compatible with magnetic stand (e.g. Alpaqua®, Cat. No. A000350)
- Thermocycler with heated lid
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Vortex instrument

NGS Library Construction Kit guidance and recommendations

- Use kit within 6 months of receipt.
- Some buffers include DTT which may precipitate after freezing. If a precipitate is observed it can removed by vortexing the thawed buffer for 1–2 minutes. Buffer performance will not be affected after resuspension.
- Use pipettes that have been accurately calibrated as pipetting errors can negatively impact kit performance.
- Thaw barcodes on ice. Do not heat barcodes above room temperature (20°C to 25°C) as this will adversely impact NGS library construction.
- This kit uses a single index barcode system. If you are using fewer than 8 reactions, ensure that barcodes are properly balanced during the adapter ligation step to avoid registration failures during your sequencing run. See "Appendix B: Sequence information" on page 17 for more information and guidelines.
- Best results are obtained using high quality DNA for library preparation (260/280 nm ratio of 1.8–2.0). The use of nicked or degraded DNA may result in library preparation failure. Ensure that the input sample does not contain contaminating RNA, nucleotides and single-stranded DNA.
- Mechanical or acoustic DNA fragmentation methods that randomly break up DNA into fragments less than 800 bp in size are compatible with this kit. We typically shear to sizes of ~300 bp on average when constructing libraries using this kit. Note that enzymatic methods of fragmentation are not recommended as they may introduce cleavage bias that will lead to suboptimal results.
- Barcodes and primers are included with this kit. Use of included primers and barcodes is highly recommended to avoid errors due to primer design. If you will be designing your own primers, see "Appendix B: Sequence information" on page 17 for primer and adapter sequences.

Overview

Introduction

The the NGS Library Construction Kit is designed to simplify next generation sequencing library construction. Using the reagents provided in this kit and a standardized protocol, a bar-coded next generation sequencing library can be reliably made from 10–50 ng of fragmented input DNA in about 2 hours. The resulting multiplexed DNA library is compatible for sequencing using Illumina® platforms. To streamline library construction, a 1-step end repair and adenylation method simplifies workflow to shorten hands-on time. Magnetic DNA purification beads are conveniently included to enable rapid sample cleanup. To further reduce the amount of hands on time required, this NGS library construction chemistry has been automated on the BioXp™ 3200 System. For further information on performing next generation library preparation on the BioXp system, contact us at **techservices@sgidna.com** or contact your local SGI-DNA representative.

The six key steps involved in preparing library DNA for sequencing are (see Figure 1):

1. DNA isolation and fragmentation
2. End repair and adenylation
3. Adapter ligation
4. Reaction clean-up
5. Library amplification and reaction clean-up
6. Library normalization and pooling

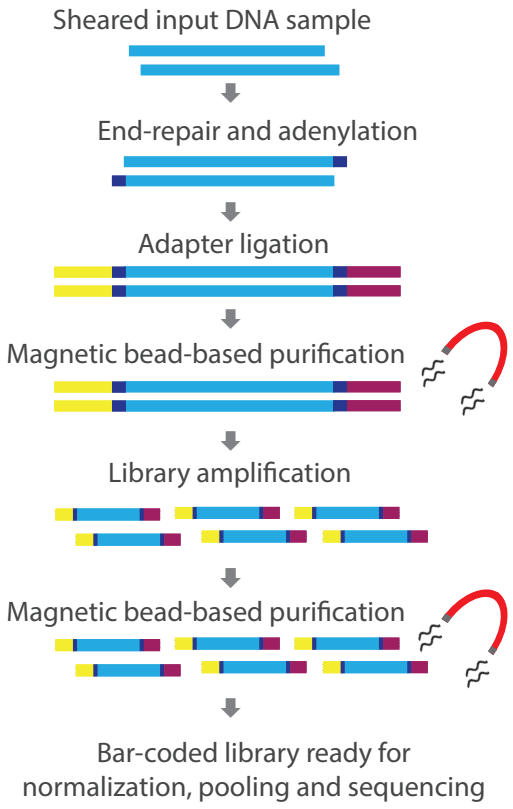

Figure 1. NGS Library Construction Kit Workflow. Starting with sheared genomic DNA, the NGS library construction kit provides all of the key reagents to enable the construction of a barcoded NGS library ready for normalization, pooling and sequencing. Library construction can be fully automated using the BioXp™ 3200 System, an automated version of this library construction chemistry.

Key Features

This NGS Library Construction Kit contains the necessary material to take the your purified and fragmented DNA through preparation and amplification resulting in a NGS library that is read to load onto flow cells for sequencing.

Key features of SGI-DNA NGS Library Construction chemistry
• Streamlined, simplified workflow • 1-step end repair and adenylation for reduced hands-on time • Barcodes provided for construction of multiplex libraries • Magnetic beads included for reaction cleanup • Ready-to-run libraries in about 2 hours starting with 10–50 ng of input DNA • Walk-away automated library construction on the BioXp™ 3200 system

Protocol

Before starting

If this is your first time using this kit, please read through the entire protocol and review "NGS Library Construction Kit guidance and recommendations" on page 5 before starting. Prior to starting NGS library construction, be certain that you have the additional materials not supplied with the kit.

Starting material

The NGS Library Construction Kit enables the creation of an Illumina-compatible NGS library and has been optimized and validated using 10–50 ng of fragmented genomic DNA. See "Appendix C: Frequently asked questions" on page 19 if your input material is outside this range.

Shear NGS library construction input DNA to an average size of 300–800 bp.

Handling kit components

- Allow components to thaw on ice. Keep enzyme-containing components on ice at all times and promptly return to −20°C after use. Do not allow Barcodes to be exposed to temperatures higher than room temperature.
- After thawing, briefly centrifuge all tubes. Place kit components on ice during use. Nuclease-free Water and Resuspension Buffer can be stored at room temperature.
- The DTT in previously frozen buffers may form a precipitate after thawing. If a precipitate forms, vortex the tube for 1 minute or until the precipitate is in solution. Kit performance is not adversely affected once the precipitate is in solution.
- Allow the magnetic beads used for reaction cleanup to come to room temperature and vortex until homogenous prior to use.

End-repair and adenylation

This step will repair single stranded overhangs and add adenosines to sheared fragments.

Required materials provided in the kit

- End-Repair and Adenylation Buffer
- End-Repair and Adenylation Enzyme Blend
- Nuclease-free Water

Materials and equipment supplied by the user

- 10–50 ng of fragmented DNA in up to 32 µL of nuclease-free water
- 96-well PCR plate (recommended) or microfuge tubes
- Adhesive 96-well PCR plate seal
- Microcentrifuge
- Thermocycler with heated lid
- Ice bucket with ice

Procedure

1. For each fragmented DNA sample, add the following reagents on ice to a nuclease-free 96-well PCR plate, PCR strip tube or microfuge tube in the order shown. Mix gently to make a homogenous solution:

Component	Volume
Nuclease-free Water	32–χ µL
Fragmented DNA (10 ng – 50 ng)	χ µL
End-Repair and Adenylation Buffer	15 µL
End-Repair and Adenylation Enzyme Blend	3 µL
Total	**to 50 µL**

2. If you are using a 96-well plate, apply an adhesive PCR plate seal. Place the sealed 96-well plate or microfuge tubes in a thermocycler. Program and start the thermocycler using the following conditions:

22°C	20 minute	1 Cycle
72°C	20 minutes	1 Cycle
4°C	as necessary	1 Cycle

3. Proceed to Adapter Ligation Step.

Adapter ligation

In this step, the barcode adapters are ligated onto the repaired and adenylated DNA fragments. There are 8 different Barcode Adapters provided with this kit. If you will be multiplexing fewer than 8 samples, see "Appendix B: Sequence information" on page 17 for guidelines on how to select barcodes to ensure that the barcodes are properly balanced.

Required materials provided in the kit

- Ligase Enzyme Blend
- Barcodes 1–8
- Nuclease-free Water
- Resuspension Buffer
- Paramagnetic DNA Purification Beads

Materials and equipment supplied by the user

- Thermocycler with heated lid
- 50 µL of End Repaired and Adenylated DNA (from completed procedure "End-repair and adenylation" on page 9)
- 70% Ethanol

Procedure

1. Thaw Ligase Enzyme Blend at room temperature. Vortex the thawed blend for 5–10 seconds. Do not centrifuge as this may cause components of the mix to separate, affecting performance. Place the tube on ice.
2. Prepare adapter dilutions, if necessary, using the recommendations in the following table. Use 2.5 µL of adapter for each sample. Perform adapter dilutions with Nuclease-free Water, depending on input amount and starting adapter concentration.

Amount of input DNA	Barcode adapter dilution
10 ng	1:40
100 ng	1:8.3

3. Combine the following reagents in a 96-well PCR plate or microfuge tube and mix thoroughly using a pipette.

Component	Volume
End Repaired and Adenylated DNA (from "End-repair and adenylation" on page 9)	50 µL
Ligase Enzyme Blend	47.5 µL
Barcode	2.5 µL
Total	**100 µL**

Note: Mix the reaction well as the Ligase Enzyme Blend is very viscous. Optimal mixing may be attained by pipetting reactions up and down 15 times; visually inspect samples to confirm proper mixing.

4. If you are using a 96-well plate, seal the reaction plate with an adhesive PCR plate seal. Incubate 96-well plates or capped microfuge tubes in a thermocycler for 15 minutes at 22°C.
5. Add 50 µL of Paramagnetic DNA Purification Beads to each sample and mix well.

   Note: Thoroughly resuspend the vial of magnetic beads before use.
6. Incubate the plate or tubes at room temperature for 5 minutes to allow DNA to bind to the paramagnetic beads.
7. Place the reactions in a magnetic stand for 5 minutes at room temperature to collect the beads.
8. Using a pipette, carefully collect and discard the supernatant. Do not disturb the pellet.
9. Without removing the samples from the magnetic stand, add 200 µL of freshly prepared 70% ethanol to each pellet.
10. Incubate at room temperature for 30 seconds. Carefully collect and discard the ethanol. Do not disturb the pellet.
11. Repeat steps 9 and 10 to perform a total of 2 ethanol washes. For the final wash, ensure that all ethanol is removed.
12. Remove the samples from the magnetic stand. Allow samples to dry at room temperature for 5 minutes or until the bead pellet is visibly dry.

    Note: Residual ethanol can interfere with the elution of DNA from the paramagnetic beads. Be sure that no ethanol remains before proceeding.
13. Resuspend the dry paramagnetic beads with 52 µL of Resuspension Buffer. Mix well to completely resuspend the beads.
14. Incubate samples at room temperature for 5 minutes to elute the DNA.
15. Place the reactions in a magnetic stand for 5 minutes at room temperature to collect the beads.
16. With the samples still in the magnetic stand, transfer 50 µL of the supernatant to a new well of the 96-well plate or a clean microfuge tube. In this step, the sample is contained in the supernatant. DO NOT DISCARD THE SUPERNATANT. Remove the 96-well PCR plate from the magnetic stand and set aside.
17. To the 50 µL of supernatant collected above , add 40 µL of Paramagnetic DNA Purification Beads and mix well.

    Note: Thoroughly resuspend the vial of magnetic beads before use.
18. Incubate for 5 minutes at room temperature.
19. Place the reactions in a magnetic stand for 5 minutes at room temperature to collect the beads.
20. Using a pipette, carefully collect and discard the supernatant. Do not disturb the pellet.
21. Without removing the samples from the magnetic stand, add 200 µL of freshly prepared 70% ethanol to each pellet.
22. Incubate at room temperature for 30 seconds. Carefully collect and discard the ethanol. Do not disturb pellet.
23. Repeat steps 21 and 22 to perform a total of 2 ethanol washes. For the final wash, ensure that all ethanol is removed.

24. Remove the samples from the magnetic stand. Allow samples to dry at room temperature for 5 minutes or until bead pellets are visibly dry.

    Note: Residual ethanol can interfere with the elution of DNA from the paramagnetic beads. Be sure that no ethanol remains before proceeding.

25. Resuspend the dry paramagnetic beads with 22 µL of Resuspension Buffer. Mix well to completely resuspend the beads.
26. Incubate resuspended samples at room temperature for 5 minutes to elute the DNA.
27. Place the reactions in a magnetic stand for 5 minutes at room temperature to collect the beads.
28. With the samples still in the magnetic stand, transfer 20 µL of supernatant to a new well of the 96-well plate or a clean microfuge tube. In this step, the sample is contained in the supernatant. DO NOT DISCARD THE SUPERNATANT. Remove the 96-well PCR plate from the magnetic stand and set aside.
29. Proceed to PCR Amplification or store samples at −20°C.

PCR Amplification

In this step, the DNA fragments containing the barcode adapters are amplified by PCR to generate sufficient material for sequencing. Excessive rounds of PCR can introduce library bias. Follow the recommended number of cycles in the procedure.

Required materials provided in the kit

- Amplification Master Mix
- Amplification Primer Mix
- Resuspension Buffer
- Nuclease-free Water
- Paramagnetic DNA Purification Beads

Materials and equipment supplied by the user

- Thermocycler with heated lid
- 96-well PCR plate or microfuge tubes
- 70% Ethanol, freshly prepared (room temperature)
- Magnetic stand
- 20 µL of adapter-ligated DNA (from completed procedure "Adapter ligation" on pages 10–12)

Procedure

1. For each sample, combine the following reagents in a 96-well PCR plate or microfuge tube. Keep all reaction components on ice. Mix thoroughly by gently pipetting up and down.

Component	Volume
Adapter Ligated DNA (from completed "Adapter ligation" procedure on page 12)	20 µL
Nuclease-free Water	16 µL
Amplification Master Mix	12 µL
Amplification Primer Mix	2 µL
Total	**50 µL**

2. If you are using 96-well plates, apply an adhesive plate seal.
3. Place the sealed plate or capped microfuge tubes in a thermocycler. The number of cycles depends on the amount of input DNA. Program and start the thermocycler using the following conditions:

Initial denaturation	98°C	2 minutes	1 Cycle
Amplification	98°C	30 seconds	See the following table for cycle number guidance
	65°C	30 seconds	
	72°C	60 seconds	
Final extension	72°C	4 minutes	1 Cycle

Use the following guidance for programming the amplification cycle number.

Amount of input DNA	Number of PCR cycles
10 ng	10–12
50–100 ng	5–7

4. After amplification add 40 µL of Paramagnetic DNA Purification Beads to each well or tube. Mix well.

   Note: Thoroughly resuspend the vial of magnetic beads before use.

5. Place reactions in a magnetic stand for 5 minutes at room temperature to collect beads.
6. Using a pipette, carefully collect and discard the supernatant. Do not disturb the pellet. Some liquid may remain in wells.
7. Without removing the samples from the magnetic stand, add 200 µL of freshly prepared 70% ethanol to each pellet. Incubate the plates or tubes at room temperature for 30 seconds. Carefully collect and discard the ethanol. Do not disturb the pellet.
8. Repeat the previous step to perform a total of 2 ethanol washes. For the final wash, ensure that all ethanol is removed.

9. Remove the samples from the magnetic stand and let dry at room temperature for 5 minutes or until the bead pellet is visibly dry.

   Note: Residual ethanol can interfere with the elution of DNA from the paramagnetic beads. Ensure that no ethanol remains before proceeding.

10. Resuspend the dry paramagnetic beads with 21 µL of Resuspension Buffer. Mix thoroughly to completely resuspend the beads.
11. Incubate the samples at room temperature for 5 minutes to elute the DNA
12. Place reactions in a magnetic stand at room temperature for 5 minutes to collect the beads.
13. Without removing the samples from the magnetic stand, use a pipette to collect and transfer 20 µL of the amplified library to a new well of a 96-well plate or a clean microfuge tube.
14. The resulting libraries may be analyzed by agarose gel electrophoresis or on an Agilent Bioanalyzer or similar microfluidic electrophoresis system. We recommend qPCR to quantify libraries prior to normalization for clustering.
15. After analysis and normalization, the library is ready for cluster generation. Proceed to cluster generation or seal with an adhesive PCR plate seal (if you are using a 96-well plate) and store at −20°C.

Appendix

Appendix A: Expected results

Example of Agilent Bioanalyzer library validation

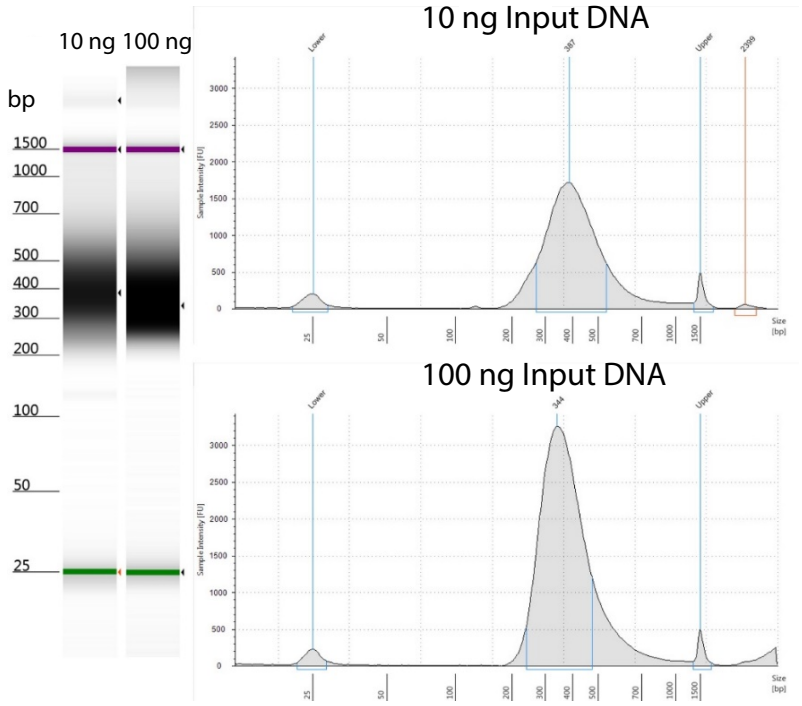

Figure 2. Reliable library preparation from a range of input DNA. Starting with sheared genomic DNA, the NGS library construction kit provides all of the key reagents to enable the construction of a barcoded NGS library ready for normalization, pooling and sequencing. Library construction can be fully automated using the BioXp™ 3200 System, an automated version of this library construction chemistry. To evaluate the quality of the libraries, each sample was run on an Agilent 2100 Bioanalyzer using a High Sensitivity DNA Chip. Both the resulting Bioanalyzer gel image and electropherograms are shown. Mean size distribution between 344–387 is consistent with the expected size due to the ligation of adapters to the input DNA. The size distribution suggests that highly representative libraries were produced from both 10 ng and 100 ng of input DNA.

Appendix B: Sequence information

Oligonucleotide sequences

Item	Sequence
Barcode Adapter 1	**P5** 5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCGATCT
	P7 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGGTCTTCTGCTTG
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT

Table 1. NGS Library Construction Kit oligonucleotide sequences. The top row shows the sequence of Adapter 1. All other Adapters contain the same core sequence with the exception of the boxed sequence. The boxed sequence is the barcode sequence used to differentiate the Barcode Adapters (see the following table for barcode sequences). Primers 1 and 2 are the sequences of the oligonucleotides used to amplify the library.

Barcode Adapter sequences

Barcode	Sequence
1	GCCAAT
2	CTTGTA
3	CGATGT
4	CAGATC
5	ACAGTG
6	GTGAAA
7	AGTCAA
8	ATGTCA

Table 2. Barcode adapter sequences.

Suggested barcode combinations to use with <8 samples

Good index combination example						
Barcode 1	G	C	C	A	A	T
Barcode 2	C	T	T	G	T	A

Bad index combination example						
Barcode 3	C	G	A	T	G	T
Barcode 4	C	A	G	A	T	C
Barcode 5	A	C	A	G	T	G
	X			X		

X = signal missing in one color channel

Table 3. Suggested Barcode combinations for multiplexing fewer than 8 samples. Illumina sequencing instruments use a green laser to read G/T bases and a red laser to read A/C bases. At each cycle at least one of the two nucleotides for each color channel need to be read to ensure proper registration of a barcode index. When performing multiplex sequencing, it is important to maintain color balance for each base of the index read to prevent sequencing failures as a result of registration failures. Use this table to select specific barcode combinations for different multiplex levels to help prevent registration failures.

Location and index of adapter sequences

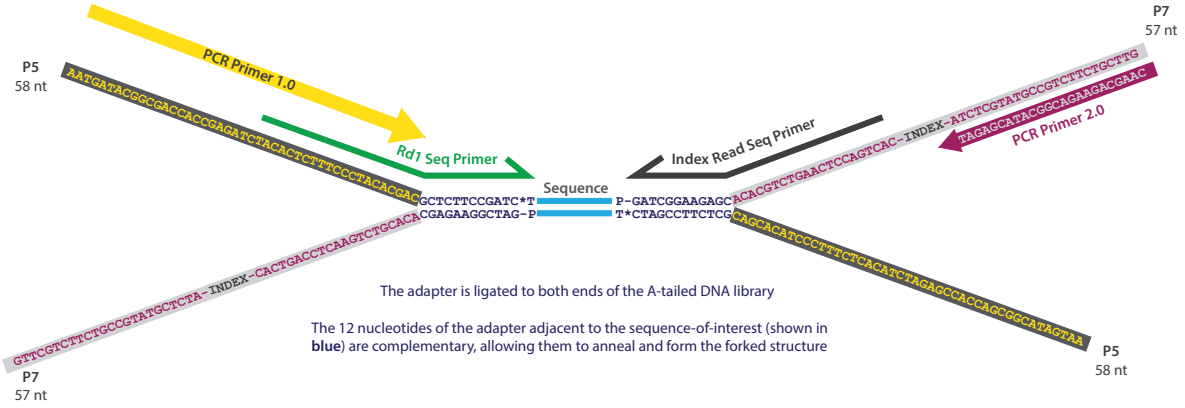

Figure 3. Location of index and adapter sequences in final library. Index and adapter sequences used to identify your template DNA are shown. The last 12 nt of the adapters are complementary, allowing them to anneal and form the forked structure. The adapter is ligated to both ends of the A-tailed DNA library.

Appendix C: Frequently asked questions

1. What are the recommended applications for the SGI-DNA NGS Library Construction Kit?
   - Whole-genome shotgun sequencing
   - Plasmid sequence verification
2. What are the input DNA requirements?

   The protocol provided here has been validated for library construction of 10–50 ng of appropriately fragmented dsDNA. Lower and higher amounts of input material can be used, but modifications to the adapter concentration and the number of cycles for library amplification may need to be empirically determined.
3. What are the major steps in library construction?
   - End repair and A-tailing, which produces end repaired 5′ phosphorylated, 3′-dA-tailed, dsDNA fragments
   - Adapter ligation, during which dsDNA adapters with 3′-dTMP overhangs are ligated to 3′-dA-tailed library fragments
   - Library amplification, which employs high-fidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends
4. Are there safe stopping points in the library construction process?

   The library construction process, from end repair and A-tailing to final, amplified library can be performed in about 2 hours. Stopping points are indicated in the protocol.
5. What are your Covaris® shearing recommendations?
   - If you are performing a cleanup between shearing and end repair, shear in 10 mM Tris-HCl (pH 8 or 8.5) + 1 mM EDTA
   - If you are not performing a cleanup between shearing and end repair, shear in 10 mM Tris-HCl (pH 8 or 8.5) + 0.1 mM EDTA
   - Never shear DNA in water
6. What if I am unsure of whether there is or what the concentration of EDTA is in my sample?

   Perform column or bead-based purification or buffer exchange and resuspend in 10 mM Tris-HCl, pH 8.0-8.5.
7. Is enzymatic fragmentation compatible with the NGS Library Construction Kit?

   Mechanical shearing is highly recommended. Enzymatic shearing is not always completely random and may introduce sequencing bias. We recommend *mechanical* shearing to ensure the best possible sequencing results.
8. Can I assess the outcome of my fragmentation before proceeding into library preparation?

   While it is possible to remove aliquots of the fragmentation reaction product for analysis in the integrated fragmentation/library construction workflow, it is most productive to assess the outcome of fragmentation once the entire workflow has been completed for the following reasons:
   - It is difficult and disruptive to process low-volume aliquots in a way that is fully representative of the final library.
   - Fragmentation profiles for low-input samples (1–10 ng) may not be informative, even when high sensitivity assays are used.

9. What adapters should I use with this kit and at what concentration?
    - Adapters provided in the NGS Library Construction Kit are 6 nt single-index adapters similar to those used in TruSeq® technology.
    - The ligation efficiency is robust for adapter:insert molar ratios from 10:1 to >200:1. High adapter:insert ratios are beneficial for low-input and challenging samples. For <10 ng input, dilute the adapter 1:5 and use 2.5 uL.
10. What QC testing is performed on Adapters?
    - Optimal library construction efficiency, conversion rate: yield of adapter-ligated library (before amplification) by qPCR as a % of input DNA
    - Minimal levels of adapter-dimer formation, modified library construction protocol designed to measure adapter dimer with high sensitivity (0–2%)
    - Nominal levels of barcode cross-contamination, sequenced by MiSeq®: % correct insert to total insert by each barcode, 115,000–500,000 each. Total level of contamination for any barcode is ~0.1–0.5%, measuring chemical cross-contamination and adapter "cross-talk".
11. What is the proportion of fragmented DNA that is converted to adapter-ligated molecules?
    - The proportion of fragmented DNA that is successfully converted to adapter-ligated molecules decreases as input is reduced.
    - When starting end repair/A-tailing with >10 ng fragmented genomic DNA, 5–35% of input DNA is typically recovered as adapter-ligated molecules. This workflow contains an additional bead-based cleanup step prior to library amplification and likely results in a lower yield of adapter-ligated molecules.
12. How many bead-based clean-up steps are required after adapter-ligation?

    Two bead-based clean-up steps are included after adapter-ligation and a third clean-up step is implemented after PCR amplification.
13. What enzyme is used for amplification?

    A low-bias, high-efficiency, high-fidelity DNA Polymerase is used for PCR.
14. How do I know if I overamplified my library?

    In library amplification reactions primers are typically depleted before dNTPs. When DNA synthesis can no longer take place due to substrate depletion, subsequent rounds of DNA denaturation and annealing result in the separation of complementary DNA strands, followed by imperfect annealing to non-complementary partners. This presumably results in the formation of so-called "daisy chains" or "tangled knots", comprising large assemblies of improperly annealed, partially double-stranded, heteroduplex DNA. These species migrate slower and are observed as secondary, higher molecular weight peaks during the electrophoretic analysis of amplified libraries.
15. What are the consequences of overamplification?

    Excessive library amplification can result in unwanted artifacts such as PCR duplicates, chimeric library inserts, amplification bias and heteroduplex formation. It is generally best to limit the extent of library amplification as much as possible, while ensuring that sufficient material is generated for quality control and sequencing.
16. How do I assess the quality of my library after preparation?
    - Library size distribution, and the absence of primer dimers and/or over-amplification products should be confirmed by means of an electrophoretic method.
    - qPCR-based quantification of libraries prior to pooling for target capture or sequencing. qPCR-based quantification of adapter-ligated libraries (prior to library amplification) is not routinely done, but can provide useful data for protocol optimization and troubleshooting.

Technical Services: For technical assistance, contact customer support at **techservices@sgidna.com**