abm DNA Library Prep Kit for Illumina Sequencing

G947S – 12 reactions
G947L – 48 reactions

Product Description

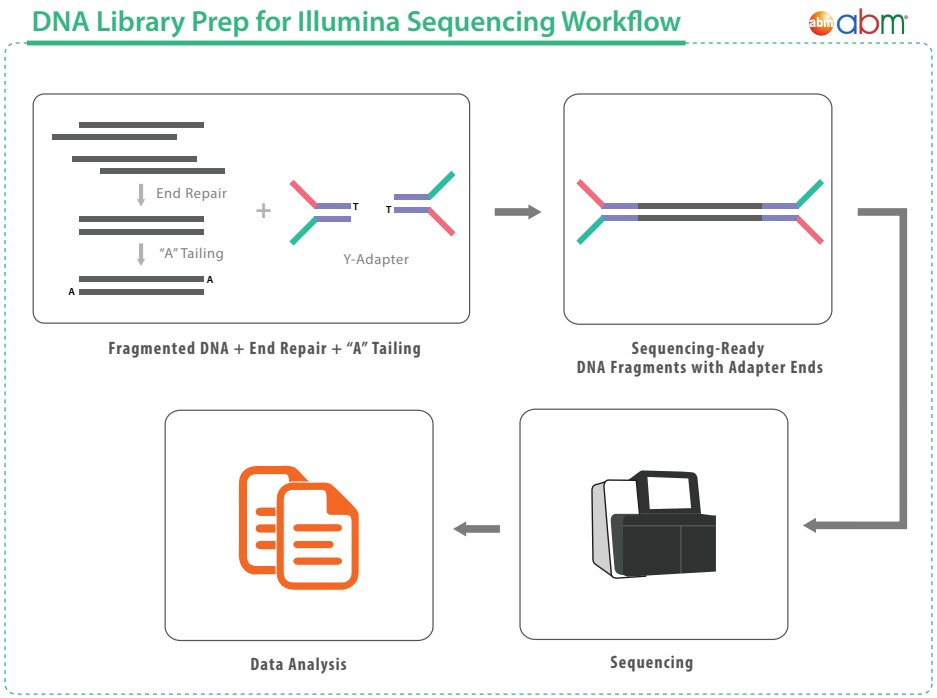
abm's DNA Library Prep kit offers an effective way to convert 2 ng – 500 ng of DNA fragments into fully compatible Illumina libraries. **abm**'s kit includes magnetic purification beads required for reaction cleanup after the ligation and PCR steps, as well as indexes needed for running multiple samples on a single Illumina flow cell. The indexes are premixed to only need one pipetting action. The sequences of the adaptor and indexes are found at the end of this manual. The entire protocol will take approximately 2 hours.

Part No.	Product Components	Volume		Storage Conditions
		G947S	G947L	
G947(S/L) -1	10X End Repair Buffer	1 x 40 µl	4 x 40 µl	-20°C
G947(S/L)-2	End Repair Enzyme Mix	1 x 12 µl	4 x 12 µl	-20°C
G947(S/L)-3	Ligation Enhancer	1 x 75 µl	4 x 75 µl	4°C
G947(S/L)-4	Ligation Buffer	1 x 120 µl	4 x 120 µl	-20°C
G947(S/L)-5	Ligation Enzyme Mix	1 x 55 µl	4 x 55 µl	-20°C
See Section F	Index Adapters	12 x 5 µl each	24 x 5 µl each	-20°C
G947(S/L)-7	PCR Primer Mix	1 x 70 µl	4 x 70 µl	-20°C
G947(S/L)-8	2X PCR MasterMix	1 x 330 µl	4 x 330 µl	-20°C
G947(S/L)-9	Purification Beads	2 x 1.5 ml	8 x 1.5 ml	4°C
RT-0	Nuclease-free H_2O	1 x 1 ml	3 x 1 ml	-20°C
	Size	**12 rxns**	**48 rxns**	

Required Materials Not Supplied with the Kit:

1. 80% Ethanol
2. 10mM Tris buffer, pH8.5
3. Magnetic rack or stand
4. Thermal Cycler

Overview



Protocol

Input DNA: 2 ng – 500 ng of purified fragmented DNA (recommended size range of 200-500 bp) or PCR amplicons.

A. End Repair

1. Briefly vortex the **10X End Repair buffer**. Mix the **End Repair Enzyme Mix** tube by inverting the tube several times.
2. Add the following components to a new PCR tube. Keep the **End Repair Enzyme Mix** on ice while adding to reactions.

End Repair Reaction Mixture	
Input DNA	X µl (2-500 ng)
10X End Repair Buffer	3 µl
End Repair Enzyme Mix	1 µl
Nuclease-free H_2O	26 - X µl
Total Reaction Volume	**30 µl**

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3. Mix thoroughly by pipetting. Briefly spin to collect all liquid at the bottom of the tube.
4. Place on the preprogrammed thermal cycler and run the End Repair program.

End Repair Program:
Preheated lid 100°C, Volume 30 µl
20°C for 10 minutes
65°C for 10 minutes
Hold at 10°C

5. Once the sample has reached 10°C, proceed to **protocol B, Adaptor Ligation** immediately.

B. Adapter Ligation

1. Dilute the Index Adapters according to input DNA amount using nuclease-free water.

Input DNA amounts (ng)	Adapter Dilution
< 5 ng	1:30
5 ng – 99 ng	1:10
100 ng – 500 ng	No dilution required

2. Add the following components to the End Repair reaction mixture. Briefly vortex the **Ligation Buffer**. Mix the **Ligation Enzyme Mix** tube by inverting the tube several times. Keep the **Ligation Enzyme Mix** on ice while adding to reactions. If multiple samples are being prepared at the same time, use a different **Index Adapter** for each sample.

Adaptor Ligation Reaction Mixture	
End Repair Reaction Mixture (from step A)	30 µl
Ligation Enhancer	5 µl
Ligation Buffer	9 µl
Ligation Enzyme Mix	4 µl
Index Adapter	2.5 µl
Total Reaction Volume	**50.5 µl**

3. Mix thoroughly by pipetting. Briefly spin to collect all liquid at the bottom of the tube.
4. Place on the preprogrammed thermal cycler and run the Adaptor Ligation program. The heated lid should be off or set to 25°C.

Adaptor Ligation Program:
Heated lid off, Volume 50 µl
20°C for 15 minutes

5. Proceed to **protocol C, Clean Up Adaptor-ligated DNA** immediately.

C. Clean Up Adapter-ligated DNA

For size selection protocol (recommended for fragmented gDNA >= 10ng), follow **protocol C1**. For non-size selection protocol (recommended for fragmented gDNA < 10 ng or PCR amplicons), follow **protocol C2**. If your magnetic rack or stand is not suitable for use with PCR tubes or plates, use a pipette to transfer the Adapter Ligation Reaction Mixture into an appropriate tube or plate before proceeding.

C1: Dual Size Selection

1. Bring **Purification Beads** to room temperature.
2. Prepare 420ul of fresh 80% ethanol for each sample.
3. Vortex **Purification Beads** until fully homogenized.
4. Add 40 µl **Purification Beads** to each Adaptor Ligation Reaction Mixture and mix thoroughly by pipetting.
5. Incubate at room temperature for 10 minutes.
6. Place on a magnetic stand without shaking for 2 minutes or until the liquid is clear.
7. Carefully transfer 80 µl of the supernatant to a new tube/well. Discard the beads.
8. Add 100 µl **Purification Beads** to each tube and mix thoroughly by pipetting.
9. Incubate at room temperature for 10 minutes.
10. Place on a magnetic stand without shaking for 2 minutes or until the liquid is clear.
11. With a pipette, remove and discard all of the supernatant from each tube/well.
12. Wash two times with freshly prepared 80% Ethanol as follows:
    a. Add 200 µl 80% EtOH to each tube/well.
    b. Incubate on the magnetic stand for 30 seconds.
    c. Remove and discard all supernatant from each tube/well.
    d. Remove residual 80% Ethanol with a pipette if necessary.
13. Allow the bead to dry for 2-5 minutes on the magnetic stand (glossy appearance disappears, but before cracks appear).
14. Remove tube or plate from magnetic stand.
15. Add 21.5 µl Tris Buffer to each tube/well and mix thoroughly to fully resuspend the beads.
16. Incubate at room temperature for 2 minutes.
17. Place on a magnetic stand and wait for the liquid to clear (30 seconds).
18. Transfer 20 µl of the supernatant to a new PCR tube or plate.
19. Proceed to **protocol D, PCR Amplification**.

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C2. No Size Selection

1. Bring **Purification Beads** to room temperature.
2. Prepare 420ul of fresh 80% ethanol for each sample.
3. Vortex **Purification Beads** until fully homogenized.
4. Add 55 µl **Purification Beads** to Adaptor Ligation Reaction Mixture and mix thoroughly by pipetting.
5. Incubate at room temperature for 10 minutes.
6. Place on a magnetic stand without shaking for 2 minutes or until the liquid is clear.
7. With a pipette, remove and discard all of the supernatant from each tube/well.
8. Wash two times with freshly prepared 80% Ethanol as follows:
    a. Add 200 µl 80% EtOH to each well.
    b. Incubate on the magnetic stand for 30 seconds.
    c. Remove and discard all supernatant from each well.
    d. Remove residual 80% Ethanol with a pipette if necessary.
9. Allow the bead to dry for 2-5 minutes on the magnetic stand (glossy appearance disappears, but before cracks appear).
10. Remove tube or plate from magnetic stand.
11. Add 21.5 µl Tris Buffer to each well and mix thoroughly to fully resuspend the beads.
12. Incubate at room temperature for 2 minutes.
13. Place on a magnetic stand and wait for the liquid to clear (30 seconds).
14. Transfer 20 µl of the supernatant to a new PCR tube or plate.
15. Proceed to **protocol D, PCR Amplification.**

D. PCR Amplification

1. Add the following components to the purified adapter ligated DNA sample. Mix the **2X PCR MasterMix** tube by inverting the tube several times or flicking the side of the tube. Do not vortex. Keep **2X PCR MasterMix** on ice while adding to reactions.

Library Amplification Mixture	
Purified Adaptor Ligated DNA (from step C)	20 µl
PCR Primer Mix	5 µl
2X PCR MasterMix	25 µl
Total Reaction Volume	**50 µl**

2. Place the reactions on the preprogrammed thermal cycler and run the *Library Amplification* program. Refer to the table below for the recommended cycle number.

Library Amplification Program:	
Preheated lid 100°C, Volume 50 µl	
98°C for 30 seconds	
98°C for 10 seconds	Cycle x times
60°C for 30 seconds	
72°C for 30 seconds	
72°C for 5 minutes	
Hold at 10°C	

Amount of Input DNA	**Recommended No. of PCR Cycles**
500 ng	5-6
250 ng	6-7
100 ng	7-8
50 ng	8-9
10 ng	9-10
2 ng	10-12

3. Proceed to **protocol E, Clean Up Amplified DNA Libraries** immediately.

E. Clean Up Amplified DNA Libraries

If your magnetic rack or stand is not suitable for use with PCR tubes or plates, use a pipette to transfer the Library Amplification Mixture into an appropriate tube or plate before proceeding.

1. Bring **Purification Beads** to room temperature.
2. Prepare 420ul of fresh 80% ethanol for each sample.
3. Vortex **Purification Beads** until fully homogenized.
4. Add 55 µl **Purification Beads** to each well and mix thoroughly.
5. Incubate at room temperature for 10 minutes.
6. Place on a magnetic stand without shaking for 2 minutes or until the liquid is clear.
7. With a pipette, remove and discard all of the supernatant from each well.
8. Wash two times with freshly prepared 80% Ethanol as follows:
    a. Add 200 µl 80% EtOH to each well.
    b. Incubate on the magnetic stand for 30 seconds.

c. Remove and discard all supernatant from each well.

d. Remove residual 80% Ethanol with a pipette if necessary.

9. Allow the bead to dry for 2-5 minutes on the magnetic stand (glossy appearance disappears, but before cracks appear).
10. Remove tube or plate from magnetic stand.
11. Add 21.5 µl Tris Buffer to each well and mix thoroughly to fully resuspend the beads.
12. Incubate at room temperature for 2 minutes.
13. Place on a magnetic stand and wait for the liquid to clear (30 seconds).
14. Transfer 20 µl of the supernatant to a new PCR tube or plate.
15. Analyze libraries with a Qubit fluorometer and Agilent 2100 Bioanalyzer to confirm quantity, size distribution and quality prior to sequencing.

F. Adaptor and Index Sequences

Adaptor Name	Index	Full Adaptor Sequence
YA001	ATCACG	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CATCACGATCTCGTATGCCGTCTTCTGCTTG
YA002	CGATGT	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC CGATGTATCTCGTATGCCGTCTTCTGCTTG
YA003	TTAGGC	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC TTAGGCATCTCGTATGCCGTCTTCTGCTTG
YA004	TGACCA	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC TGACCAATCTCGTATGCCGTCTTCTGCTTG
YA005	ACAGTG	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC ACAGTGATCTCGTATGCCGTCTTCTGCTTG
YA006	GCCAAT	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGCCAATATCTCGTATGCCGTCTTCTGCTTG
YA007	CAGATC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCAGATCATCTCGTATGCCGTCTTCTGCTTG
YA008	ACTTGA	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CACTTGAATCTCGTATGCCGTCTTCTGCTTG
YA009	GATCAG	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGATCAGATCTCGTATGCCGTCTTCTGCTTG
YA010	TAGCTT	GATCGGAAGAGCACACGTCTGAACTCCAGT CACTAGCTTATCTCGTATGCCGTCTTCTGCTTG
YA011	GGCTAC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGGCTACATCTCGTATGCCGTCTTCTGCTTG
YA012	CTTGTA	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCTTGTAATCTCGTATGCCGTCTTCTGCTTG
YA013	AGTCAA	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CAGTCAAATCTCGTATGCCGTCTTCTGCTTG
YA014	AGTTCC	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CAGTTCCATCTCGTATGCCGTCTTCTGCTTG
YA015	ATGTCA	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CATGTCAATCTCGTATGCCGTCTTCTGCTTG
YA016	CCGTCC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCCGTCCATCTCGTATGCCGTCTTCTGCTTG
YA018	GTCCGC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGTCCGCATCTCGTATGCCGTCTTCTGCTTG
YA019	GTGAAA	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGTGAAAATCTCGTATGCCGTCTTCTGCTTG
YA020	GTGGCC	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGTGGCCATCTCGTATGCCGTCTTCTGCTTG
YA021	GTTTCG	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGTTTCGATCTCGTATGCCGTCTTCTGCTTG
YA022	CGTACG	GATCGGAAGAGCACACGTCTGAACTCCAGT CACCGTACGATCTCGTATGCCGTCTTCTGCTTG
YA023	GAGTGG	GATCGGAAGAGCACACGTCTGAACTCCAGT CACGAGTGGATCTCGTATGCCGTCTTCTGCTTG
YA025	ACTGAT	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CACTGATATCTCGTATGCCGTCTTCTGCTTG
YA027	ATTCCT	GATCGGAAGAGCACACGTCTGAACTCCAGTCA CATTCCTATCTCGTATGCCGTCTTCTGCTTG

ISO 13485: 2003 13485: 2012

All **abm** PCR, RT-PCR, and qPCR products are ISO 13485:2003 and13485:2012 certified as diagnostic grade and in compliance with all regulatory requirements for the design and manufacture of medical devices, as outlined by the International Organization for Standardization (ISO). For technical questions, please email us at technical@abmgood.com or visit our website at www.abmGood.com.

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