

Nov 20, 2020

◆ Protocol for Use with Large Insert Libraries (470-520 bp) (NEB #E7120)

Forked from Protocol for Use with Standard Insert Libraries (370-420 bp) (NEB#E7120)

New England Biolabs¹

¹New England Biolabs



ABSTRACT

This protocol details how to construct Large Insert libraries from start to finish using NEBNext reagents.

The corresponding NEB manual is here: https://www.neb.com/-/media/nebus/files/manuals/manuale7120.pdf and this protocol relates to section 2.

Overview

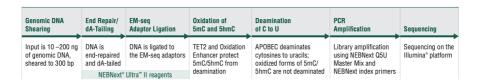


Figure 1. NEBNext Enzymatic Methyl-seq Kit Workflow.

The Enzymatic Methyl-seq kit (EM-seq) for Illumina contains all the components needed to make libraries that are enzymatically modified to detect 5-methylcytosines (5mC) and 5-hydroxymethylcytosines (5hmC).

Figure 1 is an overview of the EM-seq workflow. Firstly, a library is made by ligating EM-seq adaptor to sheared end repaired/dA-tailed genomic DNA. This is followed by two sets of enzymatic conversion steps to differentiate cytosines from 5mC and 5hmC. Finally, libraries are PCR amplified before sequencing.

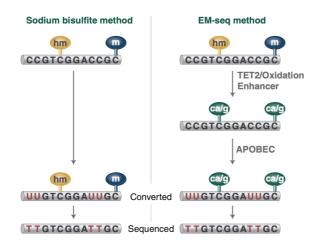


Figure 2. Overview of Sodium Bisulfite Conversion and EM-seq.

Figure 2 shows a comparison of the sodium bisulfite and EM-seq methods. Sodium bisulfite treatment of DNA results in the deamination of cytosines into uracils, however the modified forms of cytosine (5mC and 5hmC) are

not deaminated. Therefore, the preference of bisulfite to chemically deaminate cytosines enables the methylation status of cytosines to be determined. When bisulfite treated DNA is PCR amplified, uracils are replaced by thymines and the 5mC/5hmC are replaced by cytosines. Once sequenced, unmethylated cytosines are represented by thymines and 5mC and 5hmC are represented by cytosines. By comparing sequences to non-converted genomes the appropriate methylation status can be assessed.

Enzymatic Methyl-seq is a two step enzymatic conversion process to detect modified cytosines. The first step uses TET2 and an oxidation enhancer to protect modified cytosines from downstream deamination. TET2 enzymatically oxidizes 5mC and 5hmC through a cascade reaction into 5-carboxycytosine [5-methylcytosine (5mC) Þ5-hydroxymethylcytosine (5hmC) Þ 5-formylcytosine (5fC) Þ 5- carboxycytosine (5caC)]. This protects 5mC and 5hmC from deamination. 5hmC can also be protected from deamination by glucosylation to form 5ghmc using the oxidation enhancer. The second enzymatic step uses APOBEC to deaminate C but does not convert 5caC and 5ghmC. The resulting converted sequence can be analyzed like bisulfite-treated DNA. Typical aligners used to analyze data include but are not limited to Bismark and BWAMeth.

The workflow described in the NEBNext Enzymatic Methyl-seq Kit is user-friendly and enables methylation detection from inputs ranging between 10 ng–200 ng. EM-seq converted DNA is more intact than bisulfite-converted DNA, resulting in libraries with longer sequencing reads, reduced GC bias and more even genome coverage.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction of indexed libraries and sequenced on an Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the Custom Solutions department at NEB. Please contact Custom@neb.com for further information.

EXTERNAL LINK

https://www.neb.com/protocols/2019/03/28/protocol-for-use-with-large-insert-libraries-470-520-bp-e7120

DOI

dx.doi.org/10.17504/protocols.io.bfu2jnye

EXTERNAL LINK

PROTOCOL CITATION

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FORK FROM

Forked from Protocol for Use with Standard Insert Libraries (370-420 bp) (NEB#E7120), Isabel Gautreau

KEYWORDS

dna preparation, elution, large insert libraries

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Nov 20, 2020

OWNERSHIP HISTORY



PROTOCOL INTEGER ID

36474

MATERIALS TEXT

MATERIALS

⋈ NEBNext Enzymatic Methyl-seq Kit − 96 rxns New England

Biolabs Catalog #E7120L

Required Materials Not Included:

- Covaris® S2 instrument or other fragmentation equipment
- PCR strip tubes
- Recommended: Formamide (Sigma #F9037-100 ml) or optional 0.1 N NaOH. Formamide is preferred. If using NaOH, please see FAQ on NEB #E7120 FAQ page.
- 80% Ethanol
- 0.1X TE, pH 8.0
- Nuclease-free Water
- Magnetic rack/stand, such as NEBNext Magnetic Separation Rack (NEB #S1515S)
- PCR machine
- Bioanalyzer®, TapeStation® and associated consumables or other fragment analyzer

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

ABSTRACT

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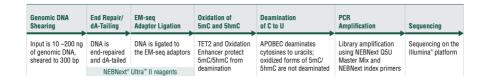


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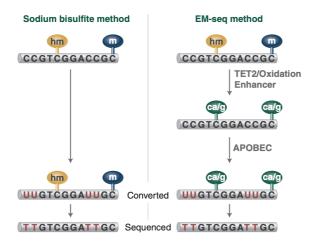


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Enzymatic Methyl-seq is a two step enzymatic conversion process to detect modified cytosines. The first step uses TET2 and an oxidation enhancer to protect modified cytosines from downstream deamination. TET2 enzymatically oxidizes 5mC and 5hmC through a cascade reaction into 5-carboxycytosine [5-methylcytosine (5mC) Þ5-hydroxymethylcytosine (5hmC) Þ 5-formylcytosine (5fC) Þ 5- carboxycytosine (5caC)]. This protects 5mC and 5hmC from deamination. 5hmC can also be protected from deamination by glucosylation to form 5ghmc using the oxidation enhancer. The second enzymatic step uses APOBEC to deaminate C but does not convert 5caC and 5ghmC. The resulting converted sequence can be analyzed like bisulfite-treated DNA. Typical aligners used to analyze data include but are not limited to Bismark and BWAMeth.

The workflow described in the NEBNext Enzymatic Methyl-seq Kit is user-friendly and enables methylation detection from inputs ranging between 10 ng-200 ng. EM-seq converted DNA is more intact than bisulfite-converted DNA, resulting in libraries with longer sequencing reads, reduced GC bias and more even genome coverage.

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DNA Preparation (Section 2.1)

1 DNA Preparation

Starting materials is 10 ng-200 ng DNA

Combine 10 ng-200 ng of genomic DNA with control DNA, CpG methylated pUC19 (lilac) and unmethylated lambda DNA (lilac) in $50 \, \mu l$ made up with 0.1X TE pH8. The amount of control DNA added is dependent on the number of reads required.

Citation: New England Biolabs (11/20/2020). Protocol for Use with Large Insert Libraries (470âÂÂ520 bp) (NEB #E7120). https://dx.doi.org/10.17504/protocols.io.bfu2jnye

If checking library quality on a MiSeq $^{\circledR}(2-4 \text{ M} \text{ reads per library})$ prior to deep sequencing on NovaSeq $^{\circledR}$, HiSeq $^{\circledR}$ or NextSeq $^{\circledR}$ (100–500 M reads per library) then the amount of controls spiked to the sample DNA is higher than what is required for direct deep sequencing. Having higher ng of control DNA for samples that are sequenced on a MiSeq ensures that there are enough control reads to accurately call cytosine conversion. We recommend this for users who are inexperienced with next generation sequencing library preparation. For libraries sequenced to a depth of 2–4 M paired end reads, approximately 5,000 x 76 base paired end reads of unmethylated lambda and 500 x 76 base paired end reads of CpG methylated pUC19 are needed to give enough reads for accurate conversion estimates. If these same libraries are sequenced to a higher depth of 200–400 M reads per library, then the number of reads associated with the controls would be in vast excess, 500,000 for unmethylated lambda and 50,000 for pUC19.

Recommended Control Inputs:

- Pre-sequencing on MiSeq prior to deep sequencing on NovaSeq, HiSeq or NextSeq: spike in 1 μl of 0.1 ng/μl pUC19 control DNA (lilac) and 1 μl of 2 ng/μl unmethylated lambda DNA (lilac) per 10-200 ng sample DNA.
- Direct Sequencing on NovaSeq, HiSeq or NextSeq: Dilute the pUC19 (lilac) and the unmethylated lambda control (lilac) 1:100 using 0.1X TE, pH 8.0. Spike in 1 μl diluted pUC19 (0.001 ng) control DNA and 1 μl diluted unmethylated lambda DNA (0.02 ng) per 10–200 ng sample DNA

2 Shearing DNA

The combined $\[\]$ **50 µl genomic DNA** and control DNAs are fragmented to an average insert size of 350–400 bp (470–520 bp final Illumina library). Fragmentation can be done using a preferred fragmentation device such as a Covaris instrument. Enzymatic fragmentation is not recommended as this may result in the removal of methylation marks.

Transfer the **50** µl sheared DNA to a new PCR tube for End Prep.

DNA does not need to be cleaned up or size selected before End Prep.

End Prep of Sheared DNA (Section 2.2)

3 On ice, mix the following components in a sterile nuclease-free PCR tube:

COMPONENT	VOLUME
Fragmented DNA	50 μl
(green) NEBNext Ultra II End Prep Reaction Buffer	7 μΙ
(green) NEBNext Ultra II End Prep Enzyme Mix	3 µl
Total Volume	60 µl



Set a $\Box 100 \ \mu l$ or $\Box 200 \ \mu l$ pipette to $\Box 50 \ \mu l$ and then pipette the entire volume up and down at least 10 times to mix thoroughly.

Perform a quick spin to collect all liquid from the sides of the tube.

It is important to mix well. The presence of a small amount of bubbles will not interfere with the performance.

5 Place in a thermocycler with the heated lid set to \geq 8 75 °C or on, and run the following program:

```
© 00:30:00 at § 20 °C
© 00:30:00 at § 65 °C
Hold at § 4 °C
```

Ligation of EM-seq Adaptor (Section 2.3)

6 3

§ On ice , add the following components directly to the ☐60 µl End Prep reaction mixture and mix well:

COMPONENT	VOLUME
(red) NEBNext EM-seq Adaptor	2.5 μΙ
(red) NEBNext Ligation Enhancer	1 µl
(red) NEBNext Ultra II Ligation Master Mix	30 μΙ
Total Volume	93.5 µl

Ligation Enhancer and Ligation Master Mix can be mixed ahead of time and is stable for at least 8 hours at & 4 °C . We do not recommend adding adaptor to a premix in the adaptor ligation step. Premix adaptor and sample and then add the other ligation reagents.

7

Set a $\Box 100 \ \mu I$ or $\Box 200 \ \mu I$ pipette to $\Box 80 \ \mu I$ and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

CAUTION: The Ligation Master Mix is viscous. Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance.

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Incubate at § 20 °C for © 00:15:00 in a thermocycler with the heated lid off.

Clean-Up of Adaptor Ligated DNA (Section 2.4)

9 Vortex Sample Purification Beads to resuspend.

10

Add 110 µl of resuspended NEBNext Sample Purification Beads to each sample. Mix well by pipetting up and down at least 10 times.

Be careful to expel all of the liquid out of the tip during the last mix.

11

Incubate samples on bench top for at least $\, \odot \, 00:05:00 \,$ at $\, \, \vartheta \,$ Room temperature $\, . \,$

- 12 Place the tubes against an appropriate magnetic stand to separate the beads from the supernatant.
- After © 00:05:00, or when the solution is clear, carefully remove and discard the supernatant.

Be careful not to disturb the beads that contain DNA targets

CAUTION: DO NOT discard the beads

- 14 Add **200** µl of 80% freshly prepared ethanol to the tubes while in the magnetic stand.
- 15

Incubate at & Room temperature for © 00:00:30 before carefully removing and discarding the supernatant.

Be careful not to disturb the beads that contain DNA targets.

16



Repeat the ethanol wash once for a total of two washes.

Be sure to remove all visible liquid after the second wash using a p10 pipette tip.

17 Air dry the beads for up to 2 minutes while the tubes are on the magentic stand with the lid open.

CAUTION: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

- Remove the tubes from the magnetic stand. Elute the DNA target from the beads by adding $29 \, \mu l$ of Elution Buffer (white).
- 19

Mix well by pipetting up and down 10 times. Incubate for at least © 00:01:00 at & Room temperature.

If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

20 (II

Place the tube on the magnetic stand. After \bigcirc **00:03:00**, or whenever the solution is clear, transfer

■28 µl of the supernatant to a new PCR tube.

SAFE STOPPING POINT: Samples can be stored overnight at $~ \mbox{\$ -20 °C}$.

Oxidation of 5-Methylcytosines and 5-Hydroxymethylcytosines (Section 2.5)

21 Prepare **TET2 Buffer**. Use option A if you have E7120S/E7120G (24 Reactions/G size) and option B if you have E7120L (96 reactions).

The TET2 Reaction Buffer Supplement is a powder. Centrifuge before use to ensure it is at the bottom of the tube.

Option A: E7120S/E7120G

- 1. Add 100 µl of TET2 Reaction Buffer to one tube of TET2 Reaction Buffer Supplement and mix well.
- 2. Write date on tube.

Option B: E7120L

- 1. Add 400 µl of TET2 Reaction Buffer to one tube of TET2 Reaction Buffer Supplement and mix well.
- 2. Write date on tube.

The reconstituted buffer should be stored at § -20 °C and discarded after 4 months.

22

& On ice, add the following components directly to the \square 28 μ 1 EM-seq adaptor ligated DNA (from step 20).

COMPONENT	VOLUME
(yellow) TET2 Reaction Buffer (TET2 Reaction Buffer plus reconstituted TET2 Reaction Buffer Supplement)	10 µl
(yellow) Oxidation Supplement	1 µl
(yellow) DTT	1 µl
(yellow) Oxidation Enhancer	1 µl
(yellow) TET2	4 µl

Mix thoroughly by vortexing, centrifuge briefly.

For multiple reactions, a master mix of the reaction components can be prepared before addition to the sample DNA. 5mC/5hmC oxidation is initiated by the addition of the Fe(II) solution to the reaction in the next step.

23

Dilute the [M] 500 Milimolar (mM) Fe(II) Solution (yellow) by adding 11 µl to 1249 µl of water.

Use the solution immediately, do not store it. Discard after use.

Combine Diluted Fe(II) Solution and EM-seq DNA with Oxidation Enzymes (from Step 22).

COMPONENT	VOLUME
EM-seq DNA (from step 22)	45 μl
Diluted Fe(II) Solution (from step 23)	5 μΙ
Total Volume	50 μΙ

Mix thoroughly by vortexing or by pipetting up and down at least 10 times, centrifuge briefly.

24



25



Transfer the samples to ice and add $\square 1 \mu l$ of Stop Reagent (yellow).

COMPONENT	VOLUME
(yellow) Stop Reagent	1 μΙ
Total Volume	51 μΙ

Mix thoroughly by vortexing or by pipetting up and down at least 10 times and centrifuge briefly.

26



Incubate at § 37 °C for © 00:30:00 then at § 4 °C in the thermocycler with the heated lid set to ≥ § 45 °C or on.

SAFE STOPPING POINT: Samples can be stored overnight at either § 4 °C in the thermocycler or at & -20 °C in the freezer.

Clean-Up of TET2 Converted DNA (Section 2.6)

Vortex Sample Purification Beads to resuspend.

28



Add 90 µl of resuspended NEBNext Sample Purification Beads to each sample. Mix well by pipetting up and down at least 10 times.

Be careful to expel all of the liquid out of the tip during the last mix.

29

9

Incubate samples on bench top for at least @00:05:00 at \$ Room temperature.

30 Place the tubes against an appropriate magnetic stand to separate the beads from the supernatant.

5m

31 After © 00:05:00, or when the solution is clear, carefully remove and discard the supernatant.

Be careful not to disturb the beads that contain DNA targets

CAUTION: DO NOT discard the beads.

32



Add 200 µl of 80% freshly prepared ethanol to the tubes while in the magnetic stand. Incubate at

 δ **Room temperature** for \bigcirc **00:00:30**, then carefully remove and discard the supernatant.

Be careful not to disturb the beads that contain DNA targets.

33



Repeat the previous wash once for a total of two washes.

Be sure to remove all visible liquid after the second wash using a p10 pipette tip.

34 Air dry the beads for up to 2 minutes while the tubes are on the magnetic stand with the lid open.

CAUTION: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

Remove the tubes from the magnetic stand. Elute the DNA target from the beads by adding $\Box 17 \ \mu l$ of Elution Buffer (white).

36

Mix well by pipetting up and down 10 times. Incubate for at least 1 minute at 8 Room temperature.

If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

37



Place the tube on the magnetic stand. After © 00:03:00, or whenever the solution is clear, transfer

16 μl of the supernatant to a new PCR tube.

SAFE STOPPING POINT: Samples can be stored overnight at -20°C.

Denaturation of DNA (Section 2.7)

38

The DNA can be denatured using either Formamide or 0.1 N Sodium Hydroxide.

Use option A for denaturing using Formamide and option B for denaturing using 0.1 N Sodium hydroxide.

Option A: Formamide (Recommended)

- 2. Add **4 μl Formamide** to the **16 μl of oxidized DNA**. Vortex to mix or by pipetting up and down at least 10 times, centrifuge briefly.
- 3. Incubate at § 85 °C for © 00:10:00 in the pre-heated thermocycler with the heated lid on.
- 4. Immediately place § On ice.
- 5. Proceed immediately to the next section.

Option B: Sodium Hydroxide (Optional, see FAQ about preparing NaOH)

- 1. Prepare freshly diluted 0.1 N NaOH.
- 2. Pre-heat thermocycler to $\ \mbox{\em §}\ \mbox{\bf 50 °C}$ with the heated lid set to $\mbox{\em \geq }\ \mbox{\em §}\ \mbox{\bf 60 °C}$ or on.
- 3. Add **4 μl 0.1 N NaOH** to the **16 μl**. Vortex to mix or by pipetting up and down at least 10 times, centrifuge briefly.
- 4. Incubate at \$50 °C for \$\igcup 00:10:00\$ in the pre-heated thermocycler with the heated lid set to ≥ \$60 °C or on.
- 5. Immediately place on ice.
- 6. Proceed immediately to the next section.

Deamination of Cytosines (Section 2.8)

39

COMPONENT	VOLUME
Nuclease-free water	68 µl
(orange) APOBEC Reaction Buffer	10 μΙ
(orange) BSA	1 μΙ
(orange) APOBEC	1 μΙ
Total Volume	100 μΙ

For multiple reactions, a master mix of the reaction components can be prepared before addition to the denatured DNA.

40



Mix thoroughly by vortexing or by pipetting up and down at least 10 times, centrifuge briefly.

41



Incubate at § 37 °C for ⊙ 03:00:00, then at § 4 °C in a thermocycler with the heated lid set to ≥ § 45 °C or on.

SAFE STOPPING POINT: Samples can be stored overnight at either § 4 °C in the thermocycler or at & -20 °C in the freezer.

Clean-Up of Deaminated DNA (Section 2.9)

42

CAUTION: The Sample Purification Beads behave differently during the APOBEC clean-up. After the bead washes, do not overdry the beads as they become very difficult to resuspend.

Vortex Sample Purification Beads to resuspend.



Add 100 µl of resuspended NEBNext Sample Purification Beads to each sample. Mix well by pipetting up and down at least 10 times.

13

Be careful to expel all of the liquid out of the tip during the last mix.

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44	
	Incubate sample on bench top for at least $ \odot 00:05:00 $ at $ \& Room temperature $.
45	Place the tubes against an appropriate magnetic stand to separate the beads from the supernatant.
46	After © 00:05:00 , or when the solution is clear, carefully remove and discard the supernatant.
	Be careful not to disturb the beads that contain DNA targets. CAUTION: DO NOT discard the beads.
47	
	Add 200 µl of 80% freshly prepared ethanol to the tubes while in the magnetic stand. Incubate at
	§ Room temperature for $© 00:00:30$, then carefully remove and discard the supernatant.
	Be careful not to disturb the beads that contain DNA targets.
48	
	Repeat the wash once for a total of two washes.
	Be sure to remove all visible liquid after the second wash using a p10 pipette tip.
49	Air dry the beads for up to 90 seconds while the tubes are on the magnetic stand with the lid open.
	CAUTION: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

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■21 µl of Elution Buffer (white).

Remove the tubes from the magnetic stand. Elute the DNA target from the beads by adding



Mix well by pipetting up and down 10 times. Incubate for at least 1 minute at 8 Room temperature.

If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

52



Place the tube on the magnetic stand. After © 00:03:00 , or whenever the solution is clear, transfer

■20 µl of the supernatant to a new PCR tube.

SAFE STOPPING POINT: Samples can be stored overnight at \$ -20 °C.

PCR Amplification (Section 2.10)

53 § On ice , add the following components to the ■20 µl of deaminated DNA from the previous section:

COMPONENT	VOLUME
EM-seq Index Primer*, **	5 μΙ
(blue) NEBNext Q5U Master Mix	25 µl
Total Volume	50 μΙ

^{*} Refer to section 3 in the manual for barcode pooling guidelines. (https://www.neb.com/-



Mix thoroughly by vortexing or by pipetting up and down at least 10 times, centrifuge briefly.

55



Place the tube in a thermocycler and perform PCR amplification using the following cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	
Annealing	62°C	30 seconds	4-8*
Extension	65°C	60 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	-

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[/]media/nebus/files/manuals/manuale7120.pdf)
** EM-seq primers are supplied in tubes in #E7120S or as a 96 Unique Dual Index Primer Pairs Plate in #E7120L

*Cycle Recommendations:

- 10 ng DNA input: 8 cycles
- 50 ng DNA input: 5-6 cycles
- 200 ng DNA input: 4 cycles

SAFE STOPPING POINT: Samples can be stored overnight at either $~\delta$ 4 °C in the thermocycler or at $~\delta$ -20 °C in the freezer.

Clean-Up of Amplified Libraries (Section 2.11)

56 Vortex Sample Purification Beads to resuspend.

57

Add **90** µl of water to each sample. Mix well by pipetting up and down at least 10 times.

Add **91 μl of resuspended NEBNext Sample Purification Beads** to each sample. Mix well by pipetting up and down at least 10 times.

Be careful to expel all of the liquid out of the tip during the last mix.

59

- 60 Place the tubes against an appropriate magnetic stand to separate the beads from the supernatant.
- After © 00:05:00, or when the solution is clear, carefully remove and discard the supernatant.

Be careful not to disturb the beads that contain DNA targets.

CAUTION: DO NOT discard the beads.

62

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Add 200 µl of 80% freshly prepared ethanol to the tubes while in the magnetic stand. Incubate at

§ Room temperature for © 00:00:30, then carefully remove and discard the supernatant.

Be careful not to disturb the beads that contain DNA targets.

63



Repeat the wash once for a total of two washes.

Be sure to remove all visible liquid after the second wash using a p10 pipette tip.

Air dry the beads for up to © 00:02:00 while the tubes are on the magnetic stand with the lid open.

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CAUTION: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

- Remove the tubes from the magnetic stand. Elute the DNA target from the beads by adding
 - **■21** µl of Elution Buffer (white) or
 - ■21 μl of TE 10 mM Tris, 0.1 mM EDTA, pH 8.0) or low TE (for long term storage).



Mix well by pipetting up and down 10 times. Incubate for at least © 00:01:00 at & Room temperature.

If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic stand.

67



Place the tube on the magnetic stand. After © 00:03:00, or whenever the solution is clear, transfer

20 μl of the supernatant to a new PCR tube.

SAFE STOPPING POINT: Samples can be stored overnight at $8 - 20 \, ^{\circ}\text{C}$.

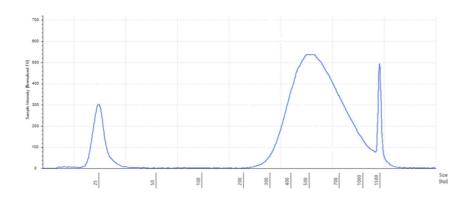
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Use a Bioanalyzer or TapeStation to determine the size distribution and concentration of the libraries.

A typical EM-seq library would have the following TapeStation trace.

50 ng of NA12878 genomic DNA.



 $69 \quad \text{Sequence using the preferred Illumina platform. 2 x 100 base reads or 2 x 150 base reads for large insert libraries. } \\$