ZYMO RESEARCH

Epigenetics Made Simple™

Zymo-Seq™ Cell Free DNA WGBS Library Kit

High quality libraries from precious cfDNA samples

Highlights

- **Optimized for small fragment input:** Ideal for small and damaged DNA fragments such as cell-free DNA (cfDNA).
- **Accurate methylation calling:** Direct ligation-based protocol allows for accurate reads and methylation calling of native termini for each DNA fragment.
- **Streamlined and simple workflow:** Prepare robust methyl-seq libraries in as little as 3 steps.

Catalog Numbers:
D5462, D5463

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Zymo-Seq™ Cell Free DNA WGBS Library Kit	D5462 (24 prep)	D5463 (96 prep)	Storage Temperature
Lightning Conversion Reagent[1]	3 x 1.5 mL	15 mL	Room Temp.
M-Binding Buffer	20 mL	80 mL	Room Temp.
M-Wash Buffer (concentrate)[2]	6 mL	24 mL	Room Temp.
L-Desulphonation Buffer	10 mL	40 mL	Room Temp.
DNA Elution Buffer	4 mL	16 mL	Room Temp.
Zymo-Spin™ IC Columns	25	2 x 50	Room Temp.
Collection Tubes	25	2 x 50	Room Temp.
DNA Wash Buffer (concentrate)[3]	6 mL	24 mL	Room Temp.
Select-a-Size MagBead Concentrate	300 µL	2 x 300 µL	4°C
Select-a-Size MagBead Buffer	10 mL	2 x 10 mL	4°C
E coli Non-Methylated Genomic DNA	5 µg/20 µL	5 µg/20 µL	−20°C
Adapter Ligation Buffer 1	48 µL	2 x 96 µL	−20°C
Adapter Ligation Buffer 2[4]	48 µL	2 x 96 µL	−20°C
Adapter Ligation Buffer 3[5]	48 µL	2 x 96 µL	−20°C
Adapter Ligation Master Mix[6]	625 µL	2 x 1.25 mL	−20°C
2X Index PCR Premix	600 µL	2 x 1.2 mL	−20°C
Zymo-Seq™ UDI Primer Set (1-12)[7]	20 µL/Index	-	−20°C
Zymo-Seq™ UDI Primer Plate (1-96)[8]	-	10 µL/Index	−20°C
Instruction Manual	1	1	-

[1] The **Lightning Conversion Reagent** is in a ready-to-use format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.

[2,3] The **M-Wash Buffer** and **DNA Wash Buffer** are supplied as concentrates. See **Buffer Preparation** on pg. 5 for directed amounts of ethanol to be added to each upon first use. Cap bottle tightly after each use to prevent ethanol evaporation.

[4,5,6] The **Adapter Ligation Buffer 2**, **Adapter Ligation Buffer 3**, and **Adapter Ligation Master Mix** reagents are sensitive and should undergo no more than 4 freeze-thaw cycles. Make additional aliquots of each buffer as necessary.

[7,8] The provided **Zymo-Seq™ UDI Primer Set** (Indexes 1-12) (D3008) or **Zymo-Seq™ UDI Primer Plate** (Indexes 1-96) (D3096) contains 12 pre-mixed unique dual-index barcode primers in 1.5 mL tubes or 96 pre-mixed unique dual-index barcode primers in a 96-well plate format respectively. See **Appendix D** for primer specifications, index sequences, and multiplexing considerations.

Specifications

- **Sample Input Material:** Purified cell-free DNA (cfDNA)
- **Minimum Input:** 5 ng
- **Maximum Input:** 10 ng
- **Input Quality:** For optimal results, use at least minimum input of purified cfDNA with no RNA or genomic DNA contamination. cfDNA can be concentrated using the **DNA Clean & Concentrator™** (D4013) prior to processing. cfDNA can be suspended in water, DNA Elution Buffer, or TE buffer.
- **Equipment Required:** Thermal cycler(s) with temperature adjustable lids, microcentrifuge, magnetic stand.
- **Total Processing Time:** ~6 hours
- **Hands-On Time:** ~2 hours
- **Bisulfite Conversion Efficiency:** >99.5% of non-methylated cytosine residues are converted to uracil; >99.5% protection of methylated cytosines.
- **Library Storage:** Libraries eluted in **DNA Elution Buffer** (provided) may be stored at ≤ 4°C overnight or ≤ −20°C for long-term storage.
- **Sequencing Platform Compatibility:** Libraries are compatible with all Illumina sequencing platforms. Recommended: NextSeq®, NovaSeq®.
- **Barcode Sequences:** Available for download here (USA only), or by visiting the Documents section of the D3008 and D3096 product pages at www.zymoresearch.com.

Product Description

The **Zymo-Seq™ Cell Free DNA WGBS Library Kit** provides an optimized and reliable workflow for the preparation of methyl-seq libraries from cell-free DNA (cfDNA). The process is completed in three basic steps: (1) bisulfite conversion using **EZ DNA Methylation-Lightning™** chemistry, (2) direct adapter ligation with innovative splinted adapters, and (3) index PCR amplification. This streamlined workflow has been optimized for use with short, damaged DNA fragments, making whole genome bisulfite sequencing (WGBS) library preparation with cfDNA an efficient process that can be completed in as little as 6 hours.

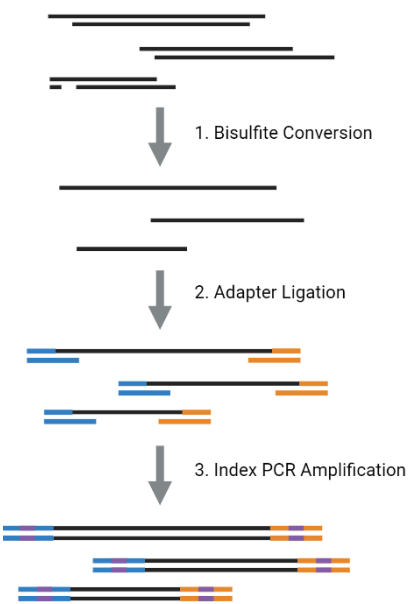

Figure 1. Overview of the Zymo-Seq™ Cell Free DNA WGBS Library Kit protocol. The simple three-step protocol allows users to effortlessly prepare WGBS libraries from cfDNA with no compromise on quality.

The **EZ DNA Methylation-Lightning™** bisulfite conversion is gentle on already short or damaged DNA fragments, resulting in less degradation of the sample compared to other bisulfite conversion chemistries and methods. The bisulfite conversion is completed rapidly while maintaining the integrity of cfDNA.

After bisulfite conversion, DNA remains in a single-stranded conformation, proving more difficult for conventional library preparation methods. This obstacle is circumvented by utilizing unique splinted adapter ligation technology to capture and directly ligate the Illumina-compatible adapters to each end of the bisulfite converted cfDNA rather than performing more laborious second strand synthesis, end repair, and dA tailing steps. These processes incorporate artificial nucleotides to blunt damaged ends or miss them altogether. The direct adapter ligation eliminates this bias by accurately preserving the methylation status of each fragment terminus. This results in faster library preparation as well as more precise methylation calling across the entire DNA fragment.

The splinted adapter ligation technology is also capable of thoroughly capturing small DNA fragments, allowing for library construction from nicked and very short DNA fragments that would otherwise not be viable when using other methods. Libraries can be prepared from a much greater percentage of cfDNA input rather than only the DNA fragments that are of convenient size for traditional library preparation.

Once the adapters have been ligated to the cfDNA, the final step is the amplification and indexing via PCR. The **Zymo-Seq™ UDI Primers** facilitate effortless multiplexing of numerous libraries. After a final clean-up, the cfDNA WGBS libraries are ready for sequencing on any Illumina instrument.

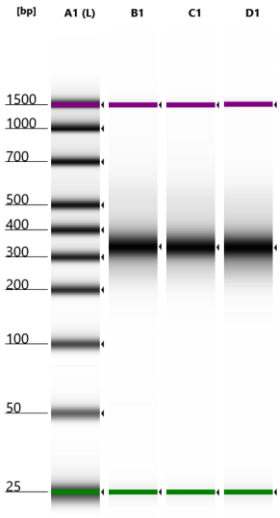

Figure 2. Zymo-Seq™ Cell Free DNA WGBS Libraries prepared from multiple cfDNA samples. Agilent 4200 TapeStation® HS D1000 of libraries prepared using cfDNA extracted from both healthy and cancerous plasma donors[1]. **A1** is a molecular weight marker. **B1** is a library prepared with cfDNA from a 59-year-old healthy plasma donor. **C1** is a library prepared with cfDNA from a 66-year-old lung cancer NSCLC stage IV plasma donor. **D1** is a library prepared with cfDNA from a 69-year-old lung cancer adenocarcinoma stage IV plasma donor. All libraries were prepared using 5 ng purified cfDNA and amplified at 9 index PCR cycles.

[1] cfDNA samples were extracted from 5 mL plasma using the ***Quick*-cfDNA™ Serum & Plasma Kit** (D4076) and concentrated using the **DNA Clean & Concentrator-5™** (D4013).

Protocol

Buffer Preparation

✓ Preparation of the **M-Wash Buffer** concentrate:

1. Add the following volumes of ethanol to the **M-Wash Buffer** concentrate:

M-Wash Buffer	If Using 100% Ethanol	If Using 95% Ethanol
6 mL M-Wash Buffer (D5001-4)	Add 24 mL	Add 26 mL
24 mL M-Wash Buffer (D5002-4)	Add 96 mL	Add 104 mL

2. Initial and mark date of ethanol addition to the bottle.

✓ Preparation of the **DNA Wash Buffer** concentrate:

1. Add the following volumes of ethanol to the **DNA Wash Buffer** concentrate:

DNA Wash Buffer	If Using 100% Ethanol	If Using 95% Ethanol
6 mL DNA Wash Buffer (D4003-2-6)	Add 24 mL	Add 26 mL
24 mL DNA Wash Buffer (D4003-2-24)	Add 96 mL	Add 104 mL

2. Initial and mark date of ethanol addition to the bottle.

✓ Preparation of the **Select-a-Size MagBeads**:

1. Add 300 µL of **Select-a-Size MagBead Concentrate** to each 10 mL **Select-a-Size MagBead Buffer**.
2. Resuspend by pipetting up and down and vortexing. Store at 4°C−8°C.
3. Sample and bead volumes are optimized for **Select-a-Size MagBead** based clean-ups. Recommended volumes in each section will minimize pipetting error

Before Starting:

- ✓ Refer to **Appendix C: *In Situ* Bisulfite Conversion Controls** for considerations regarding the provided ***E. coli* Non-Methylated Genomic DNA** in library preparation and analysis.
- ✓ Components that are stored at −20°C should be thawed and kept on ice unless otherwise stated. Return to −20°C storage after use.
- ✓ Mix each component well before use by pipetting up and down, flicking, inverting, or gently vortexing. Centrifuge briefly to collect all contents potentially caught on the sides or caps of the tubes before using.
- ✓ Avoid multiple freeze-thaws of the **Zymo-Seq™ UDI Primers**. Make additional aliquots as necessary.
- ✓ The **Adapter Ligation Buffer 2**, **Adapter Ligation Buffer 3**, and **Adapter Ligation Master Mix** are very sensitive to freeze-thaw and should only be thawed 4 times maximum. During the first thaw make additional aliquots as necessary to maintain library quality.
- ✓ Before using the **Select-a-Size MagBeads**, allow them to equilibrate to room temperature for 30 minutes.

Section 1: Bisulfite Conversion

Before Starting:

✓ Ensure that the indicated volume of ethanol has been added to the **M-Wash Buffer** (see pg. 5 **Buffer Preparation**).

1. Mix the following components in a 0.2 mL PCR tube[1]:

Component	Volume
Input cfDNA (5 ng – 10 ng)	X µL
E. coli Non-Methylated Genomic DNA (optional)[2]	Y µL
DNase/RNase-Free Water	Up to 20 µL
Total Volume	**20 µL**

2. Add 130 µL of **Lightning Conversion Reagent** to each sample and mix well by pipetting.

3. Centrifuge briefly to ensure there are no droplets in the cap or on the sides of the tube. Place the 0.2 mL PCR tube(s) in a thermal cycler (lid temp 105°C) and perform the following steps:

Temperature	Time
98°C	8 minutes
54°C	60 minutes
4°C	≤ 20 hours (optional)

4. Place a **Zymo-Spin™ IC Column** into a provided **Collection Tube** and add 600 µL of **M-Binding Buffer**.

5. Load the sample (from Step 3) into the **Zymo-Spin™ IC Column** containing the **M-Binding Buffer**. Close the cap and mix by inverting the column several times.

[1] cfDNA inputs >20 µL must be processed using multiple conversion reactions. Replicate reactions can be cleaned using the same column for each by repeating Steps 4-6 up to 5 times.

[2] Using the ***E. coli* Non-Methylated Genomic DNA** (D5016) as a spike-in is highly recommended for determining bisulfite conversion efficiency. If using directly from the tube, the *E. coli* DNA can be spiked in at 2-5% wt of the input cfDNA (e.g., 100-250 pg into 5 ng of cfDNA). If fragmented to 200-300 bp, the *E. coli* DNA can be spiked in at 0.5-1% wt of the input cfDNA (e.g., 25-50 pg into 5 ng of cfDNA). See **Appendix C** for additional information.

6. Centrifuge at full speed (≥ 10,000 x g) for 30 seconds. Discard the flow-through[1].

7. Add 100 µL of **M-Wash Buffer** to the column. Centrifuge at full speed (≥ 10,000 x g) for 30 seconds.

8. Add 200 µL of **L-Desulphonation Buffer** to the column and let stand at room temperature (20-30°C) for 15-20 minutes[2]. After the incubation, centrifuge at full speed (≥ 10,000 x g) for 30 seconds. Discard the flow-through.

9. Add 200 µL of **M-Wash Buffer** to the column. Centrifuge at full speed (≥ 10,000 x g) for 30 seconds. Repeat this wash step for two washes total.

10. Place the column into a 1.5 mL microcentrifuge tube and add 19 µL[3] of **DNA Elution Buffer** directly to the column matrix. Let incubate for 1-5 minutes[4], and then centrifuge at full speed (≥ 10,000 x g) for 30 seconds to elute the cfDNA.

This is a safe stopping point. Bisulfite-converted cfDNA can be safely stored at ≤ −20°C for up to one month.

[1] The capacity of the **Collection Tube** with the column inserted is 800 µL. Empty the **Collection Tube** as necessary to prevent contamination of the column matrix by the flow-through.
[2] Incubation with **L-Desulphonation Buffer** for longer than 20 minutes may result in degradation and lower yield of converted DNA.
[3] Sequential elutions of smaller volumes ≥ 6 µL (e.g., 9.5 µL x 2 for 19 µL total) can help ensure complete elution of all DNA from the column.
[4] Longer incubations of the **DNA Elution Buffer** on the column for up to 5 minutes can ensure greater elution efficiency.

Section 2: Adapter Ligation

Before Starting:

- ✓ Thaw the **Adapter Ligation Buffer 1**, **Adapter Ligation Buffer 2**, and **Adapter Ligation Buffer 3** on ice.
- ✓ Thaw the **Adapter Ligation Master Mix** to room temperature. Once thawed, vortex for at least 30 seconds and invert to mix well.
- ✓ The **Adapter Ligation Buffer 2**, **Adapter Ligation Buffer 3**, and **Adapter Ligation Master Mix** should only be thawed 4 times maximum. Make additional aliquots as necessary upon first thaw.

1. Preheat a thermal cycler to 98°C (lid temp 105°C) and another thermal cycler to 37°C (lid temp 45°C).
   Note: If only a single thermal cycler is available, set to 98°C (lid temp 105°C) initially and change the temperature to 37°C (lid temp 45°C) during the 2-minute return to ice incubation (Step 6). Leave the lid open to help cool.

2. Combine the following on ice in a 0.2 mL PCR tube:

Component	Volume
Bisulfite-converted cfDNA	18 µL
Adapter Ligation Buffer 1	2 µL
Total Volume	**20 µL**

3. Mix entire reaction thoroughly by pipetting or gently vortexing then centrifuge briefly to ensure there are no droplets in the cap or on the sides of the tube.

4. Incubate the tube on ice for 2 minutes.

5. Heat shock by immediately placing the tube at 98°C (lid temp 105°C) for 3 minutes.

6. Immediately return the tube to ice and incubate for at least 2 minutes to fully denature the cfDNA[1].

7. Thoroughly mix the **Adapter Ligation Master Mix** tube by vortexing for at least 30 seconds and inverting several times[2].

[1] If using only one thermal cycler, set the temperature to 37°C (lid temp 45°C) during this incubation so that the temperature is ready by Step 10. Leave the lid open to help cool it faster.
[2] The **Adapter Ligation Master Mix** is very viscous. Mix well after thawing and right before use.

8. Add the following on ice in the order defined below to the tube:

Component	Volume
Denatured cfDNA	20 µL
Adapter Ligation Buffer 2	2 µL
Adapter Ligation Buffer 3	2 µL
Adapter Ligation Master Mix	26 µL
Total Volume	**50 µL**

9. Mix entire reaction thoroughly by pipetting up and down 20-25 times, vortexing, and inverting to ensure complete homogenization[1]. Centrifuge very briefly to ensure there are no droplets in the cap or on the sides of the tube.

10. Incubate the tube at 37°C (lid temp 45°C)[2] for 1 hour in a thermal cycler.

11. After the 1-hour adapter ligation reaction, add 60 µL of **DNA Elution Buffer** to the sample to bring the volume up to 110 µL and mix well by pipetting.

12. Follow the clean-up protocol in **Appendix A** on pg. 13 using 60 µL of **Select-a-Size MagBeads**. Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for 30 minutes prior to use. For elution, resuspend the beads in 15 µL of **DNA Elution Buffer** and aspirate all 15 µL eluate after separation from the beads into a new tube.

This is a safe stopping point. The purified adapter-ligated cfDNA can be safely stored at ≤ −20°C for up to one month.

[1] After addition of the **Adapter Ligation Master Mix**, the reaction will become very viscous. It is possible to mix by pipetting up and down, although additional vortexing and inversion is recommended for complete homogenization.

[2] If using only one thermal cycler, ensure that the temperature has reached 37°C (lid temp 45°C) before starting the incubation. If it is still cooling down, leave the samples on ice until the thermal cycler is ready.

Section 3: Index PCR Amplification

Before Starting:

✓ If utilizing the **Zymo-Seq™ UDI Primer Plate**, wait for the wells to thaw completely before use. Spin down in a plate centrifuge. Pierce the foil with a 10 µL pipette tip, then throw away the tip and use a clean pipette tip to aspirate the primers.

1. Combine the following on ice to a 0.2 mL PCR tube containing the purified adapter-ligated cfDNA[1]:

Component	Volume
Adapter-Ligated cfDNA	15 µL
Zymo-Seq™ UDI Index Primers	10 µL
2X Index PCR Premix	25 µL
Total Volume	**50 µL**

2. Mix entire reaction thoroughly by pipetting or gently vortexing then briefly centrifuge.

3. Perform the following steps in a thermal cycler (lid temp 105°C):

Step	Temperature	Time	Recommended Number of Cycles
1	98°C	3 minutes	
2	98°C	20 seconds	10 ng cfDNA = 8-9 cycles 5 ng cfDNA = 9-10 cycles
3	65°C	30 seconds	
4	72°C	30 seconds	
	Repeat Steps 2-4 for X cycles		
5	72°C	1 minute	
6	4°C	Hold	

This is a safe stopping point. Amplified cfDNA samples can be safely stored overnight at 4°C. Otherwise, continue directly to Step 4 on the next page.

[1] See **Appendix D** for **Zymo-Seq™ UDI Primer** information and multiplexing guidelines.

4. Follow the clean-up protocol in **Appendix A** on pg. 13 using 50 µL of **Select-a-Size MagBeads**. Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for 30 minutes prior to use. For elution, resuspend the beads in 20 µL of **DNA Elution Buffer** and aspirate all 20 µL eluate after separation from the beads.

The eluate is the final library. Libraries can be safely stored for months at ≤ −20°C

Appendices

Appendix A: Select-a-Size MagBead Clean-Up Protocol

Before Starting:

- ✓ Ensure that the indicated volume of ethanol has been added to the **DNA Wash Buffer** (see pg. 5 **Buffer Preparation**).
- ✓ Ensure the **Select-a-Size MagBeads** have been properly prepared before use (see pg. 5 **Buffer Preparation**).
- ✓ Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for 30 minutes prior to use.
- ✓ Resuspend the magnetic particles immediately before use by inverting and/or vortexing the **Select-a-Size MagBeads** until homogenous.

1. Add the indicated volume of **Select-a-Size MagBeads** to the tube. Mix thoroughly by pipetting until homogenous and incubate for 5−10 minutes at room temperature.

    a. If performing clean-up in **Section 2: Adapter Ligation**, add 60 µL of **Select-a-Size MagBeads**.

    b. If performing clean-up in **Section 3: Index PCR Amplification**, add 50 µL of **Select-a-Size MagBeads**.

2. Place the tube on a magnetic stand for 3 minutes, or until the supernatant is clear.

3. Carefully remove the supernatant without disturbing the magnetized bead pellet[1].

4. Without removing from the magnetic stand, add 200 µL of **DNA Wash Buffer** to the tube, incubate for at least 30 seconds, and then remove the supernatant completely without disturbing the magnetized bead pellet. Repeat this wash step for two washes total.

5. Remove the tube from the magnetic stand and centrifuge very briefly. Then return the tube to the magnetic stand, wait for the beads to pellet, and remove any residual **DNA Wash Buffer** with a 10 µL pipette tip.

[1] Avoid aspirating any beads when removing the supernatant.

6. Leave the tube on the magnetic stand and keep the cap open for 2−3 minutes to allow the beads to air dry[1,2].

7. Cap and remove the tube from the magnetic stand. Add the indicated volume of **DNA Elution Buffer** and fully resuspend the beads by pipetting up and down[3]. Incubate for 5 minutes at room temperature.

    a. If performing clean-up in **Section 2: Adapter Ligation**, add 15 µL of **DNA Elution Buffer** to fully resuspend the beads.

    b. If performing clean-up in **Section 3: Index PCR Amplification**, add 20 µL of **DNA Elution Buffer** to fully resuspend the beads.

8. Place the tube back on the magnetic stand for 2 minutes or until the supernatant is clear.

9. Transfer the indicated volume of eluate to a new tube. Discard the beads.

    a. If performing clean-up in **Section 2: Adapter Ligation**, aspirate all 15 µL eluate and transfer to a 0.2 mL PCR tube.

    b. If performing clean-up in **Section 3: Index PCR Amplification**, aspirate all 20 µL eluate and transfer to a new 1.5 mL microcentrifuge tube.

This is a safe stopping point. If moving on to Section 3: Index PCR Amplification, the purified adapter-ligated cfDNA can be safely stored at ≤ −20°C for up to a month. If this is the final clean-up, cfDNA libraries can be safely stored at ≤ −20°C for months.

[1] Do not over dry the beads as this may negatively impact recovery. Beads should remain a matte brown color without cracking.
[2] When performing the clean-up in **Section 2: Adapter Ligation**, the beads are more likely to disperse around the tube and are more susceptible to drying faster than normal.
[3] When performing the clean-up in **Section 2: Adapter Ligation**, the resuspended beads behave differently and will be more challenging to remove from the sides of the tube. Briefly centrifuge tubes if necessary. Take care to ensure the beads are still fully resuspended.

Appendix B: Library Quantification and Characterization

Libraries can be quantified using a preferred method (i.e., NanoDrop®, Qubit®, TapeStation®, etc.). However, quantitative PCR is the recommended method for accurately determining library concentration prior to loading on to the Illumina sequencers.

Libraries should be visualized by using an automated electrophoresis instrument (i.e., Agilent TapeStation®, Agilent Bioanalyzer®, etc.) to determine that the correct library size is present. We recommend running on High Sensitivity tapes/chips for optimal library characterization. If adapter dimers are present, they will form an approximately 130-180 bp band. Yields will vary depending on the total quantity and quality of sample input cfDNA.

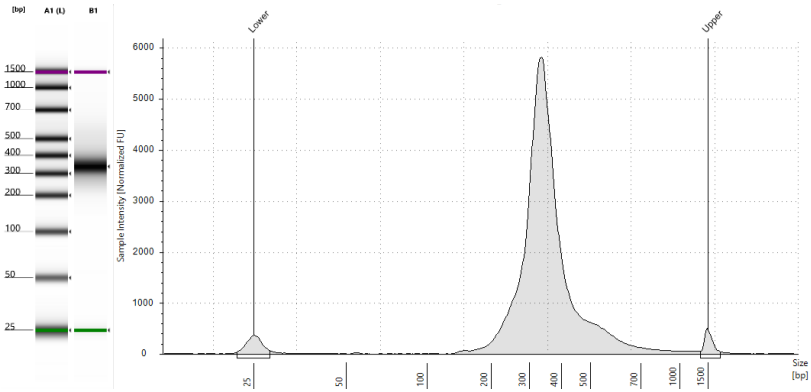

Figure 3. Characterization of a typical Zymo-Seq™ Cell Free DNA WGBS library. Agilent 4200 TapeStation® HS D1000 gel (left) and electropherogram (right) of a library prepared with the Zymo-Seq™ Cell Free DNA WGBS Library Kit from 5 ng healthy male donor cfDNA and indexed using 9 PCR cycles. The kit typically produces libraries with two to three visible peaks. These will correspond to an adapter dimer peak at approximately 130-180 bp which may not be present, a mono-nucleosome peak at approximately 290-350 bp, and a di-nucleosome peak at approximately 480-550 bp. **A1** is a molecular weight marker and **B1** is the final cfDNA WGBS library.

Appendix C: *In Situ* Bisulfite Conversion Controls

The provided ***E. coli* Non-Methylated Genomic DNA** (D5016) can be used *in situ* to determine the bisulfite conversion efficiency. The *E. coli* DNA can be spiked in at different percentages of input cfDNA depending on if it is intact or not. Due to the splinted adapters, the likelihood of each adapter being ligated to either end of a DNA fragment is inversely correlated to fragment size (i.e., the smaller the DNA fragment, the more likely both adapters are to ligate; the larger the DNA fragment, the less likely both adapters are to ligate).

The *E. coli* DNA can be used as-is, however prior fragmentation of the DNA will allow for better coverage. Therefore, we recommend the following amounts of spike-in *E. coli* DNA depending on the situation:

- **Intact *E. coli* DNA:** The *E. coli* DNA comes as ready-to-use intact genomic DNA at high molecular weight. Bisulfite conversion will fragment the DNA somewhat; however, it will still be much larger than the cfDNA input without additional fragmentation. To use directly, spike in at 2-5% wt of input cfDNA (e.g., spiking in 100-250 pg of intact *E. coli* genomic DNA into 5 ng of cfDNA sample). The amount can be adjusted to allow for more coverage.

- **Fragmented *E. coli* DNA (200-300 bp):** For better coverage, the *E. coli* DNA can be fragmented to approximately 200-300 bp in average size prior to use. When fragmented to a smaller size, the spike in amount can be reduced to 0.5-1% wt of input cfDNA (e.g., spiking in 25-50 pg of fragmented *E. coli* genomic DNA into 5 ng of cfDNA sample). The amount can be adjusted to allow for more coverage. Fragmenting the *E. coli* DNA beforehand is recommended and will allow for better coverage, although it is not required for accurate bisulfite conversion efficiency determination.

The bisulfite conversion efficiency can be determined by the percentage of unmethylated cytosines in the aligned *E. coli* reads. The reference genome of *E. coli* strain K-12 substrain MG1655 can be used for alignment and analysis. It can be accessed at the following web address: https://www.ncbi.nlm.nih.gov/genome/167?genome_assembly_id=161521

Appendix D: Unique Dual Index Primer Sets

Indexes in the **Zymo-Seq™ UDI Primer Set (Indexes 1-12)** are dispensed in 1.5 mL tubes (D3008), and the **Zymo-Seq™ UDI Primer Plate (Indexes 1-96)** are dispensed in a single-use foil-sealed 96-well plates (D3096). Indexes come as pre-mixes, and the forward and reverse primers are provided at 5 µM total concentration (2.5 µM each).

The complete index sample sheet is available for download here (USA only), or by visiting the Documents section of the D3008 and D3096 product pages at www.zymoresearch.com.

Primer Sequences:

Forward Primer Sequence (i5):
5′-AATGATACGGCGACCACCGAGATCTACAC**NNNNNNNN**ACACTC
TTTCCCTACACGACGCTCTTCCGATCT-3′

Reverse Primer Sequence (i7):
5′-CAAGCAGAAGACGGCATACGAGAT**NNNNNNNN**GTGACTGGAG
TTCAGACGTGTGCTCTTCCGATCT-3′

UDI Primer Plate (D3096) Setup:

To use UDI primers, pool ≥ 2 libraries in numerical order (down a column not across a row).

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI_01	UDI_09	UDI_17	UDI_25	UDI_33	UDI_41	UDI_49	UDI_57	UDI_65	UDI_73	UDI_81	UDI_89
B	UDI_02	UDI_10	UDI_18	UDI_26	UDI_34	UDI_42	UDI_50	UDI_58	UDI_66	UDI_74	UDI_82	UDI_90
C	UDI_03	UDI_11	UDI_19	UDI_27	UDI_35	UDI_43	UDI_51	UDI_59	UDI_67	UDI_75	UDI_83	UDI_91
D	UDI_04	UDI_12	UDI_20	UDI_28	UDI_36	UDI_44	UDI_52	UDI_60	UDI_68	UDI_76	UDI_84	UDI_92
E	UDI_05	UDI_13	UDI_21	UDI_29	UDI_37	UDI_45	UDI_53	UDI_61	UDI_69	UDI_77	UDI_85	UDI_93
F	UDI_06	UDI_14	UDI_22	UDI_30	UDI_38	UDI_46	UDI_54	UDI_62	UDI_70	UDI_78	UDI_86	UDI_94
G	UDI_07	UDI_15	UDI_23	UDI_31	UDI_39	UDI_47	UDI_55	UDI_63	UDI_71	UDI_79	UDI_87	UDI_95
H	UDI_08	UDI_16	UDI_24	UDI_32	UDI_40	UDI_48	UDI_56	UDI_64	UDI_72	UDI_80	UDI_88	UDI_96

Appendix E: Considerations for Sequencing and Data Analysis

Preparation for Clustering:

Accurate determination of the final library concentration is critical to achieve optimal clustering and sequencing results. For this, we recommend using quantitative PCR (e.g., KAPA® Library Quantification Kit).

Bisulfite conversion reduces the complexity of the library's nucleotide content. Complexity can be increased by loading PhiX or multiplexing with a high diversity library. Optimal PhiX loading will vary based on the sequencer and sequencer software; please contact Illumina technical support for recommendations.

Sequencing Parameters:

Libraries generated with this workflow are suitable for any read length but increased read lengths will require greater amounts of adapter trimming for the shorter library fragments. For most applications, 100 base paired-end (PE) reads are enough to generate substantial amounts of high-quality data for genome-wide coverage. The sequencing depth will be dependent on the genome size, genome coverage, and site coverage required. Generally, aiming for 10X coverage per CpG site is recommended. Sites with more than 10X coverage have a higher reliability in 5mC calling, but certain sites may have less coverage due to gene copy number, variability in library preparation, or clustering efficiency during sequencing. Using 100 bp PE sequencing, we recommend at least 500 million reads for human cfDNA WGBS at 10X CpG coverage, and at least 400 million reads for mouse cfDNA WGBS at 10X CpG coverage.

Adapter Trimming:

Libraries should be trimmed to remove any adapter sequence. No other trimming is required. Use the following sequences to trim the adapters:

Read 1: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
Read 2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Alignment Parameters:

Libraries prepared with this kit are directional. As such, the original-top and original-bottom strands will be represented. We recommend aligning to reference genome hg38 for human cfDNA WGBS, and reference genome mm10 for mouse cfDNA WGBS.

Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq™ Cell Free DNA WGBS Library Kit	D5462 D5463	24 preps. 96 preps.

Individual Kit Components	Catalog No.	Amount
EZ DNA Methylation-Lightning™ Kit	D5030T D5030 D5031	10 rxns 50 rxns 200 rxns
Lightning Conversion Reagent	D5030-1 D5032-1	1.5 mL 15 mL
M-Binding Buffer	D5001-3 D5002-3 D5049-3	20 mL 80 mL 100 mL
M-Wash Buffer (concentrate)	D5001-4 D5002-4 D5007-4	6 mL 24 mL 36 mL
L-Desulphonation Buffer	D5030-5 D5031-5 D5046-5	10 mL 40 mL 80 mL
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-16	1 mL 4 mL 16 mL
Zymo-Spin IC™ Columns	C1004-50 C1004-250	50 pack 250 pack
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 pack 500 pack 1000 pack
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24 D4003-2-48	6 mL 24 mL 48 mL
***E. coli* Non-Methylated Genomic DNA**	D5016	5 µg/20 µL
Zymo-Seq™ UDI Primer Sets	D3008 D3096	12 indexes 96 indexes

Complete Your Workflow

✓ For extraction of high-quality circulating cell-free DNA from up to 10 mL of serum or plasma, up to 5 mL of saliva, and up to 1 mL of amniotic and cerebrospinal fluid:

***Quick*-cfDNA Serum & Plasma Kit**	
Cat. No. D4076	Recover DNA ≥ 100 bp; 50 preps

✓ For extraction of both circulating cell-free DNA and cell-free RNA including protein-bound, exosomal, microRNA, and other small RNA from serum, plasma, and other biological fluid:

***Quick*-cfDNA/cfRNA Serum & Plasma Kit**	
Cat. No. R1072	Recover DNA ≥ 50 bp and RNA ≥ 17 nt; 50 preps

✓ For an all-in-one RRBS library prep kit perfect for DNA methylation profiling at single-nucleotide resolution in CpG-rich regions of the genome including CpG islands, promoters, and gene bodies:

Zymo-Seq RRBS Library Kit	
Cat. No. D5460	24 preps
Cat. No. D5461	48 preps

✓ For epigenetic NGS analysis solutions, contact our Services Department by phone at (949)-679-1190 Ext. 2, by email at services@zymoresearch.com, or on our website at https://www.zymoresearch.com/pages/services:

Next Generation Sequencing Services	
Targeted Bisulfite Sequencing	Evaluate site-specific DNA methylation
Genome-Wide DNA Methylation	RRBS, Methyl-MiniSeq, and Methyl-MaxiSeq
ChIP-Seq Service	Protein/DNA interactions and histone modifications
Human Epigenetic Age	Quantify epigenetic age with Human DNAge
Mouse Epigenetic Age	Quantify biological age across various tissues

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Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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Zymo-Seq™ Cell Free DNA WGBS Library Kit Sales Reference Guide

"High quality libraries from precious cfDNA samples"

Reference Sheet

Overview

Cell free DNA (cfDNA)

- DNA that is found outside of cells circulating in plasma and other bodily fluids
- Biomarker used in non-invasive liquid biopsy
- Currently used in cancer research, prenatal screenings, assessment of transplants
- Typically small and damaged, more difficult to work with

Whole Genome Bisulfite Sequencing (WGBS)

- Detection of methylation modifications at single base resolution using next generation sequencing (NGS)
- cfDNA methylation analysis can reveal tissue of origin and gene regulation → valuable info for cancer research

Currently there is no cfDNA-specific WGBS library prep kit!

Qualifying Questions

- Is your lab working with cell free DNA extracted from liquid biopsy? (i.e., plasma, urine, saliva, stool, amniotic fluid, etc.)
- Are you interested in epigenetic or DNA methylation analysis of the cfDNA?
- Are you interested in WGBS/methyl seq?

Why ZymoSeq™ Cell Free DNA WGBS?

This all-inclusive kit has been developed for WGBS library prep specifically with cfDNA samples.

Innovative protocol makes cfDNA WGBS library prep easier, faster, and less biased than ever.

Ready to sequence libraries is as little as 6 hours! No additional purchases necessary!

✔ **Optimized for small fragment input**
✔ **Accurate methylation calling**
✔ **Streamlined and simple workflow**

Problems Solved

Minimize cfDNA Degradation

- EZ DNA Methylation-Lightning bisulfite conversion is gentle and results in minimal, if any, cfDNA degradation

Difficult Library Prep Made Easy

- Optimized specifically for cfDNA regardless of damage or size

Eliminate Methylation Bias

- No exclusion of DNA ends in library prep
- Consistently reliable sequencing data

Long Workflow Streamlined into 3 Easy Steps

- Reduces time and labor

Sales Tips

Focus on:

Optimized specifically for cfDNA samples

- Lightning bisulfite conversion is gentle on small, damaged cfDNA
- Works with cfDNA of any size and quality

Then…

Captures the entire DNA fragment for sequencing using direct adapter ligation

- Complete sequencing of cfDNA end-to-end
- Less methylation bias than other methods

Then…

All-in-one kit with streamlined workflow

- Includes everything necessary, such as bisulfite conversion reagents, magbeads for clean ups, and unique dual indexing primers
- Simplified into 3 quick steps: (1) Lightning bisulfite conversion, (2) direct adapter ligation, and (3) index PCR amplification

Then…

Protocol is adaptable for other DNA inputs

- Adjustments can be made for other inputs, such as genomic DNA or FFPE-derived DNA
- Contact Zymo Research Technical Support for recommendations regarding your specific sample type

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Zymo-Seq™ Cell Free DNA WGBS Library Kit Sales Reference Guide

"High quality libraries from precious cfDNA samples"

Reference Sheet

Product Comparison

	Zymo-Seq™ Cell Free DNA WGBS Library Kit (D5462, D5463)	**IDT xGen Methyl-Seq DNA Library Prep Kit*** (10009860, 10009824,10009825)	**NEBNext Enzymatic Methyl-Seq Kit** (E7120S, E7120L)
Bisulfite Conversion Reagents	Included	Requires Additional Purchase: • EZ DNA Methylation-Gold Kit	Requires Additional Purchase: • Formamide
Indexing Primers	Included	Requires Additional Purchase: • xGen CDI Primers • xGen UDI Primers	Included
Clean-Up MagBeads	Included	Requires Additional Purchase: • SPRIselect • AMPure XP	Included
Total Workflow Steps	3	5	6
Average Time to Prepare Eight WGBS Libraries	6 hours	8 hours	14 hours

*Formerly known as Swift Accel-NGS Methyl-Seq DNA Library Kit

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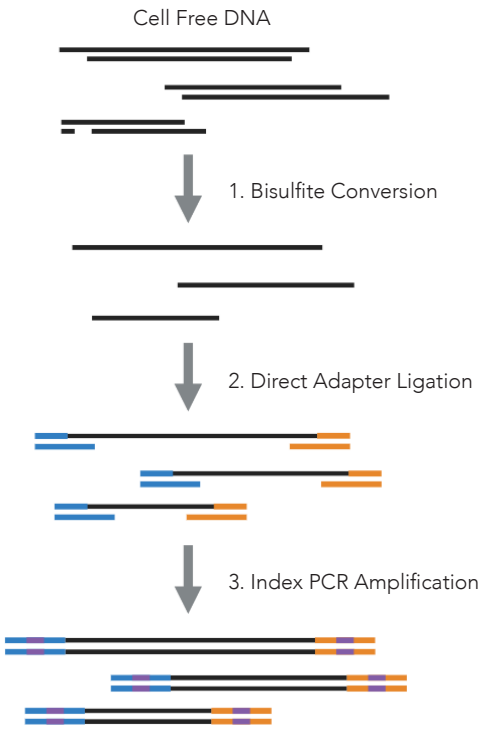
High Quality Libraries from Precious cfDNA Samples

Zymo-Seq™ Cell Free DNA WGBS Library Kit

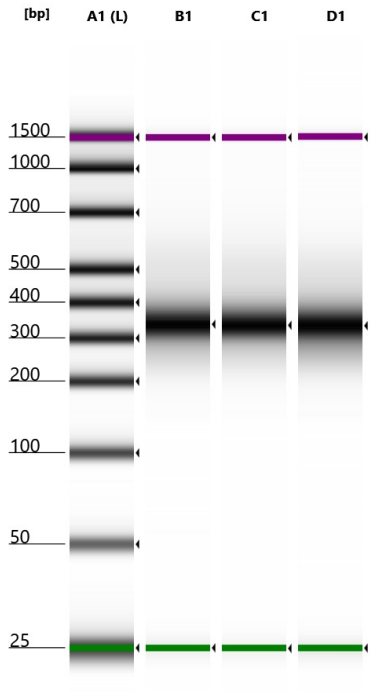
- **Optimized for Small Fragment Input:** Ideal for small and damaged DNA fragments such as cell-free DNA (cfDNA).
- **Accurate Methylation Calling:** Direct ligation-based protocol allows for accurate reads and methylation calling of native termini for each DNA fragment.
- **Simple, Streamlined Workflow:** Prepare robust methyl-seq libraries in as little as 3 steps.

Optimized and Simple Workflow



Overview of the Zymo-Seq™ Cell Free DNA WGBS Library Kit protocol. The cfDNA is first bisulfite converted using optimized conditions for fragmented input. Next, the innovative adapters capture and directly ligate onto any size DNA fragment, thus accurately preserving the methylation status of each terminus. Finally, the adapter ligated cfDNA is indexed and amplified via PCR, and the libraries are ready for sequencing on any Illumina instrument.

High Quality cfDNA Libraries



Zymo-Seq™ Cell Free DNA WGBS libraries prepared from multiple cfDNA samples. Agilent 4200 TapeStation HS D1000 gel of libraries prepared using cfDNA extracted from plasma of healthy and cancerous donors. A1 is the molecular weight marker. B1 was prepared from a healthy 59-year-old donor. C1 was prepared from a lung cancer NSCLC stage IV 66-year-old donor. D1 was prepared from an adenocarcinoma stage IV 69-year-old donor. All libraries were generated using 5 ng input cfDNA and amplified at 9 index PCR cycles.

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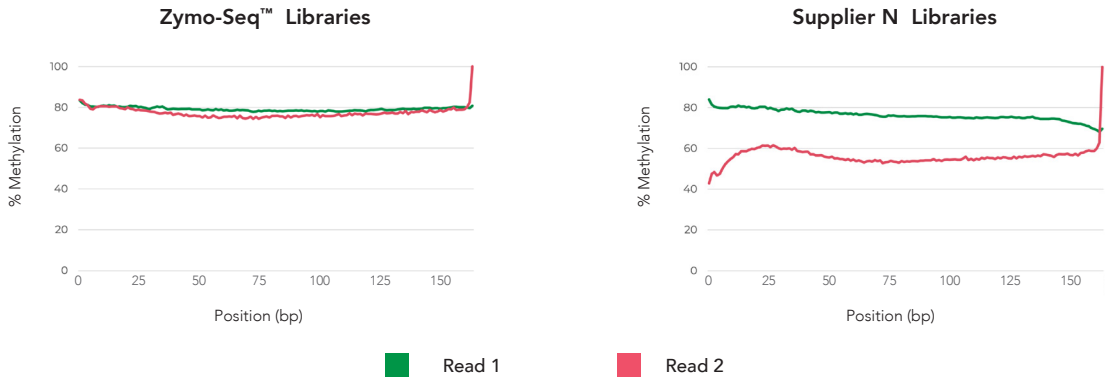
Accurate Methylation Calling Across the Entire Read



Zymo-Seq™ Cell Free DNA libraries minimize library preparation bias commonly found in conventional methods. Unbiased libraries will have consistent methylation levels across the entire read length. Other commercial protocols that include an end-repair step incorporate artificial nucleotides to blunt damaged DNA termini, resulting in significant methylation bias on the 3' end of the DNA fragments. The Zymo-Seq™ Cell Free DNA WGBS Library Kit directly ligates the adapters, eliminating the need for end-repair and thus preserving the integrity of native methylation present on the fragment termini. The Zymo-Seq™ Cell Free DNA library (left) shows consistent CpG methylation across both Read 1 and Read 2 whereas the Supplier N library (right) shows significant bias. The M-Bias plots shown above were generated by plotting the average CpG methylation levels across each position of the mapped read.

Zymo-Seq™ Cell Free DNA WGBS Kit Specifications

Feature	Zymo-Seq™ Cell Free DNA
Sample Type	Cell free DNA, fragmented DNA, gDNA (adaptable)
Equipment	Thermal cycler(s), microcentrifuge, magnetic stand
Reagents	All inclusive
Max UDI	96
Input Amount	> 5ng
Total Assay Time	~6 hours
Hands-On Time	~2 hours
FFPE Compatible	Yes
Compatible Sequencing Platforms	All Illumina instruments. Recommended: HiSeq, NextSeq, NovaSeq

Superior Performance

Metric	Zymo-Seq™ Cell Free DNA
% BS Conversion	99.6%
% Aligned	81.5%
Median Insert Size	158 bp
% CpG Coverage > 5x	81.9%
% Promoter Coverage > 50x	92.1%
% CpG Island Coverage > 50x	92.7%

Library was prepared from 5 ng input cfDNA and sequenced via NovaSeq 6000 with approximately 400M PE sequencing reads. Reads were aligned to hg38 using Bismark and methylation calling was performed with MethylDackel.

Product	Cat. No.	Size
Zymo-Seq™ Cell Free DNA WGBS Library Kit	D5462 D5463	24 preps 96 preps

info@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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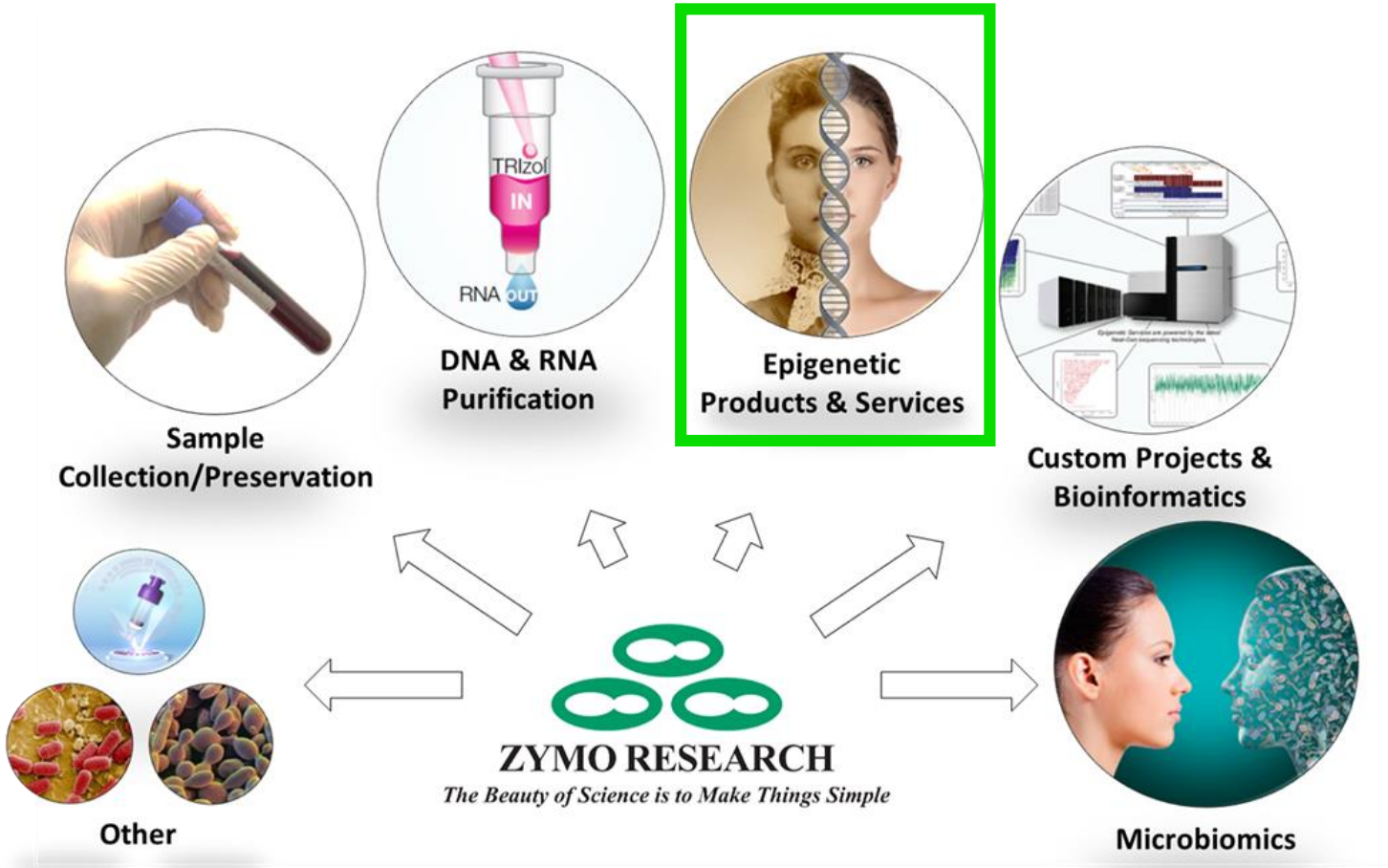
Zymo-Seq Cell Free DNA WGBS Library Kit

Catalog Number: D5462, D5463

What Can Zymo Research Provide?



- ✓ Zymo Research has a strong **IP portfolio** and is a **leading supplier** of many key technologies

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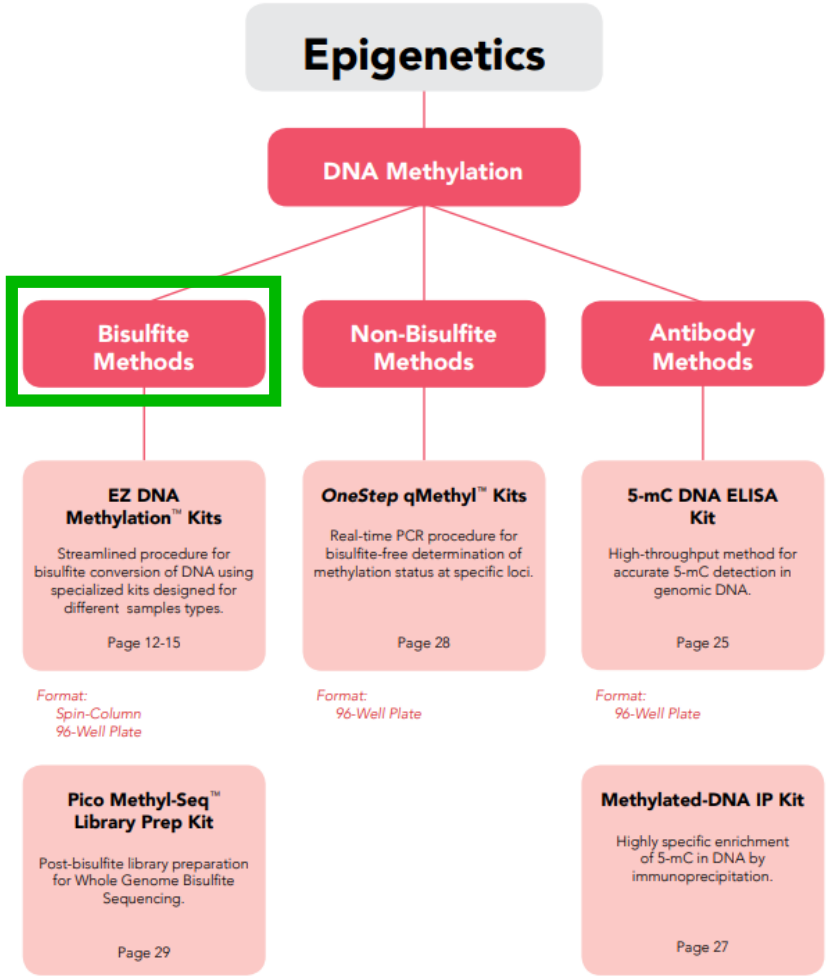
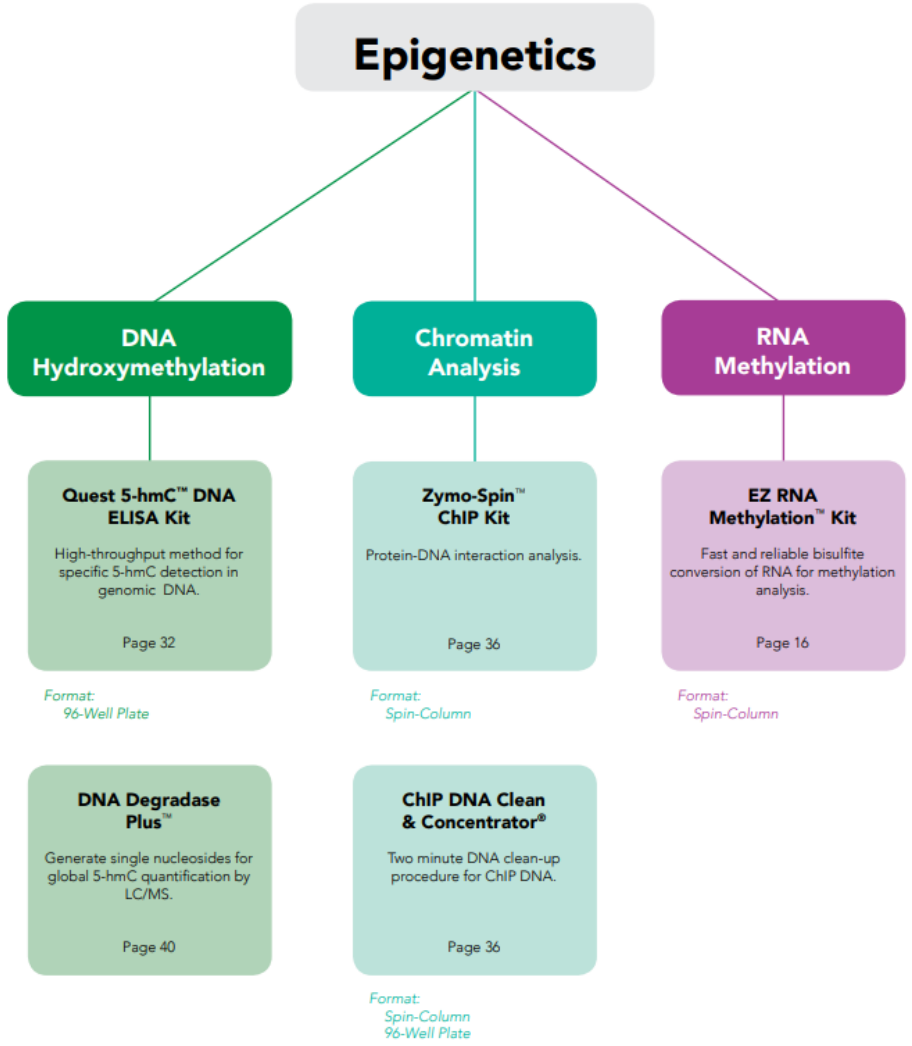
Comprehensive Provider for Epigenetics





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Target Audience & Market Situation

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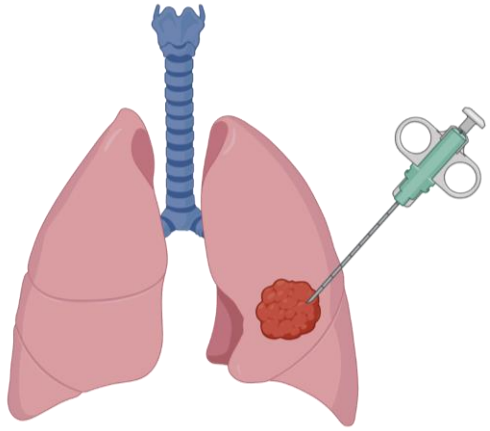
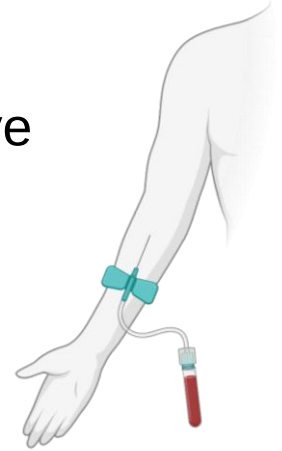
Disease monitoring through biopsies

- Tissue Biopsy
  - Invasive
  - Time-intensive
  - High-risk procedure
  - Painful
  - Localized sample
  - Target needs to be known beforehand



- Liquid Biopsy
  - Typically non-invasive
  - Fast
  - Easy procedure
  - Relatively painless
  - Comprehensive
  - Interrogation of known and unknown targets



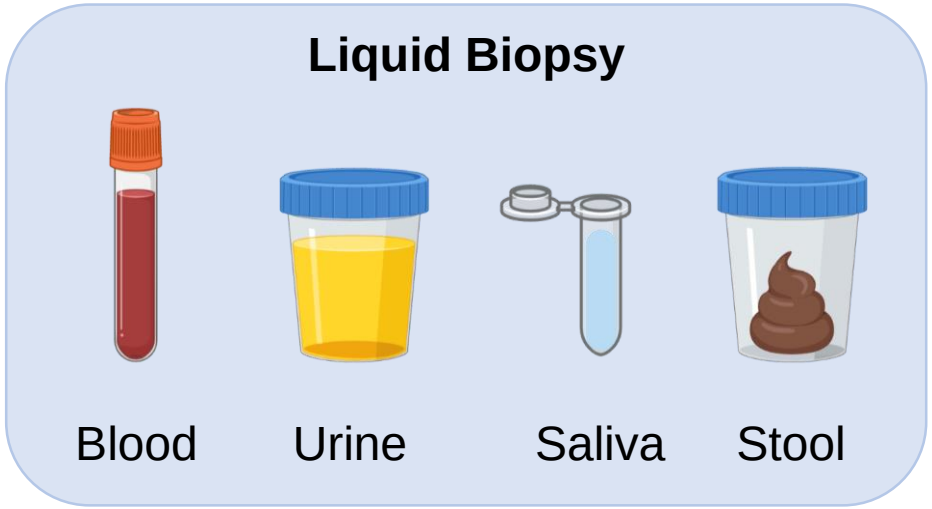
Liquid biopsy can diagnose or track disease progression using biomarkers

ZYMO RESEARCH

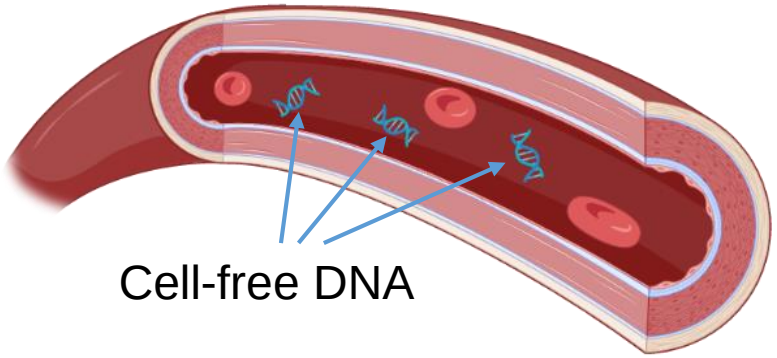
Cell-free DNA as a biomarker for precision medicine

- **Biomarker:** Measurable indicator of health

- What is **cell-free DNA (cfDNA)?**
  - DNA circulating in plasma and other bodily fluids outside of cells



- What are current cfDNA applications?
  - Prenatal screenings (NIPT) (**USD 2.8 billion in 2020)**
  - Cancer diagnosis and monitoring
  - Companion diagnostics
  - Assessment of organ transplants



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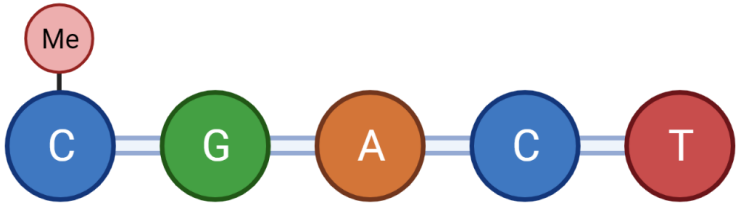
cfDNA carries both genetic and epigenetic information

- ***Genetic***: DNA genome at nucleotide level
  - Abnormal: Mutation, deletion, etc.

- ***Epigenetic***: Dynamic modifications made to the genome that reflect environmental effects
  - Methylation of cytosine in CpG context
  - *Whole genome bisulfite sequencing (WGBS)*



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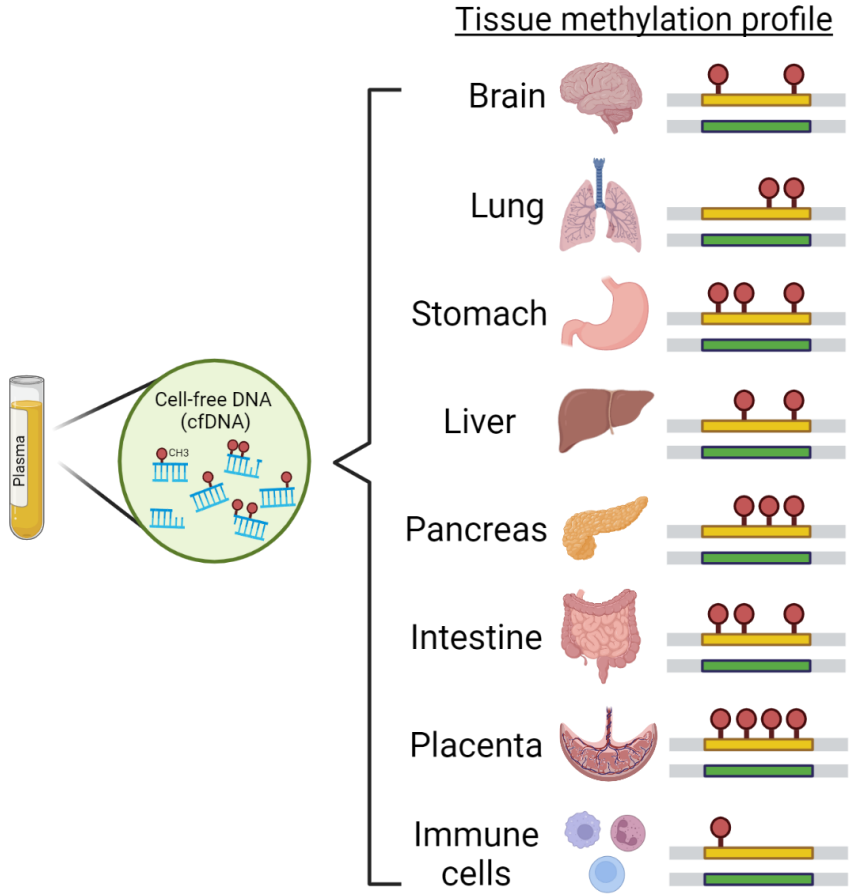
DNA methylation is a more sensitive and precise biomarker

- Methylation occurs early in carcinogenesis
  - Useful for early cancer detection
- Carries tissue of origin information, ideal for screening for specific types of cancer
  - Lung, colon, breast, etc.
- Monitor:
  - Disease progression
  - Treatment options
  - Remission and recurrence



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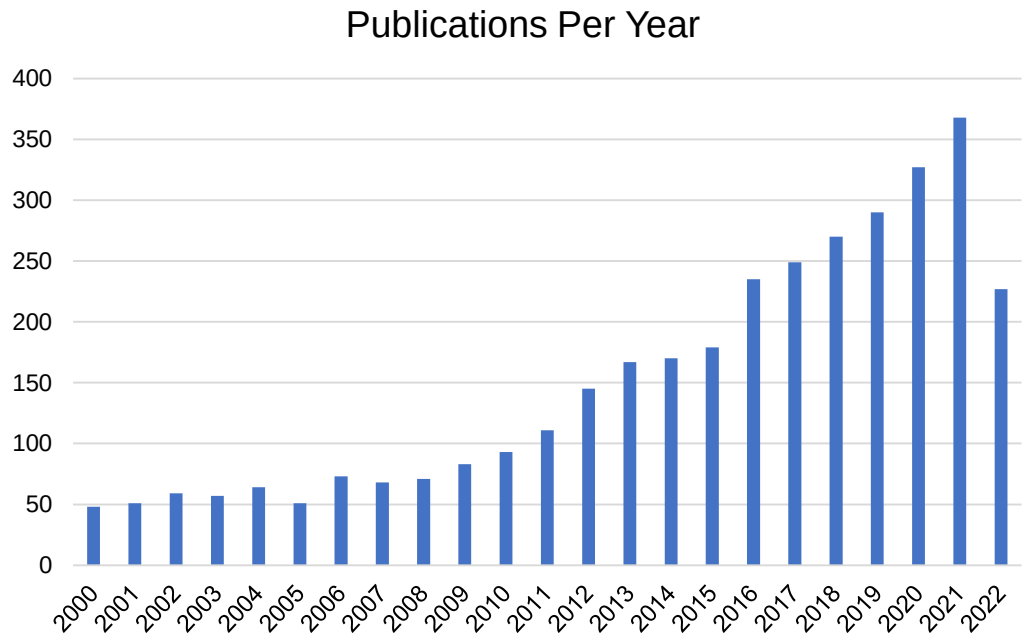
Growing interest in cfDNA methylation analysis



- Since 2000, there have been **3,456 papers** mentioning “cell free DNA methylation”
- Over one third of these publications have occurred since 2019

PubMed search results for “cell free DNA methylation” from years 2000-2022. *PubMed*, NIH National Library of Medicine, National Center for Biotechnology Information. https://pubmed.ncbi.nlm.nih.gov/?term=cell+free+dna+methylation&filter=years.2000-2022

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Where is it being studied?

DANA-FARBER
CANCER INSTITUTE

epi pro colon®
Colon Cancer Screening Blood Test

MGH 1811
MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

epi pro lung

GUARDANT™

FOUNDATION MEDICINE®

JOHNS HOPKINS UNIVERSITY

GRAIL

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Target Audience

Large background of research

- Liquid biopsy
- Oncology
- Clinical R&D
- Academia
- Core/service labs performing NGS sequencing

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Why use WGBS now for cfDNA methylation?

- cfDNA WGBS has become accessible for more groups
  - ***Reduced sequencing costs from Illumina***
  - ***Increased computation power (cloud computation)***
- WGBS covers more regions for discovery
- Easier implementation into epigenomic studies

- However, cfDNA is a very difficult sample type for WGBS library prep
  - Small size
  - Damaged
  - Bisulfite conversion fragmentation
  - Time-consuming workflows

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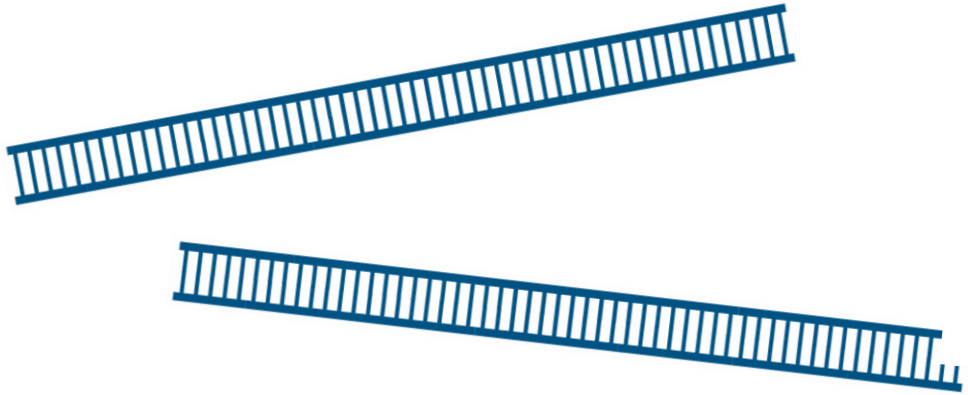
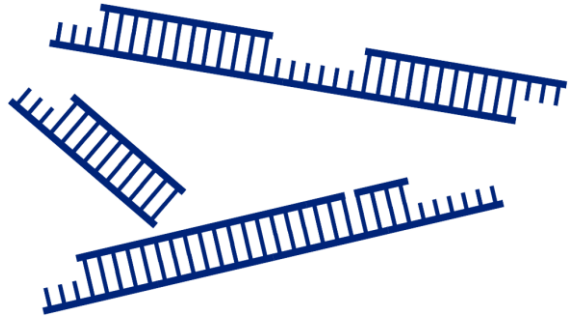
Pain points of using cfDNA in WGBS

- Many methods are not optimized for ***small and damaged fragments***
  - cfDNA: small fragment length (~167 bp), nicked, double- and single-stranded, overhangs on either end of DNA
  - Small fragments are easily excluded from library prep

Typical DNA Input



cfDNA Input



Snyder, Matthew W et al. “Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin.” *Cell* vol. 164,1-2 (2016): 57-68. doi:10.1016/j.cell.2015.11.050
Underhill, Hunter R et al. “Fragment Length of Circulating Tumor DNA.” *PLoS Genet* 12.7 (2016): e1006162. https://doi.org/10.1371/journal.pgen.1006162

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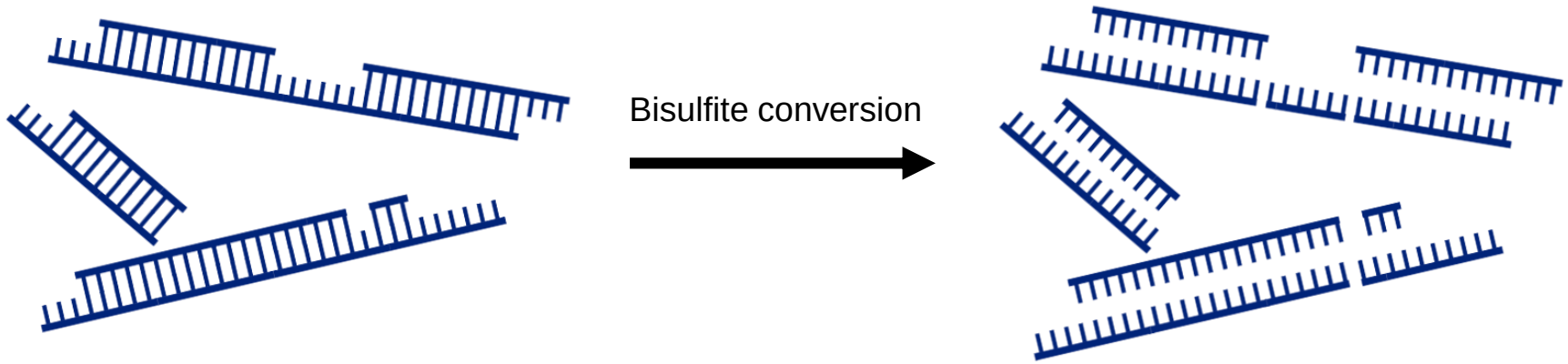
Pain points of using cfDNA in WGBS

- Bisulfite conversion is a harsh chemical process → low pH and high temperatures

- In most cases, ***bisulfite conversion fragments DNA***
  - Loss of sequencing information, less fragments incorporated into library



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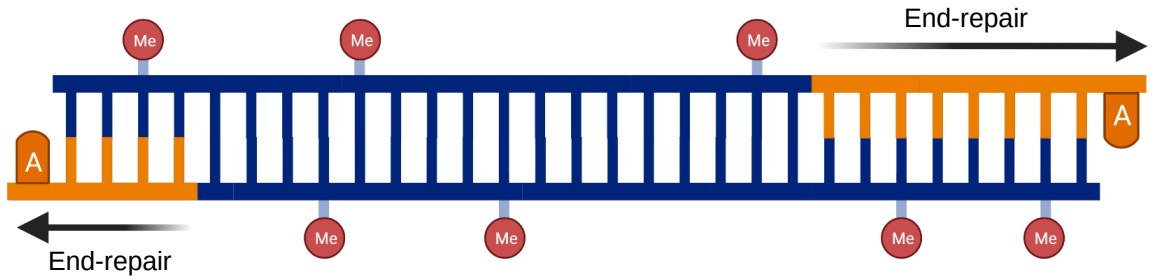
Pain points of using cfDNA in WGBS

- Common adapter strategies ***increase methylation bias and workflow time***

- **End-repair** incorporates artificial nucleotides onto DNA, increasing bias of methylation calling along the read



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Pain points of using cfDNA in WGBS

- Despite the large boom of clinical liquid biopsy samples, ***currently there is no cfDNA-specific WGBS library prep kit!***

- WGBS library prep methods have many faults:

  - **Excessive hands-on time** → difficult to prepare multiple libraries at once

  - **Long, tedious workflows** → easy to make a mistake, difficult to get consistent results

  - **Require additional purchases** → inconvenient and expensive

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We have a solution!

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Zymo-Seq Cell Free DNA WGBS Library Kit

- ✓**Optimized for small fragment input:** Ideal for small and damaged DNA fragments such as cfDNA
- ✓**Accurate methylation calling:** Direct ligation-based protocol allows for accurate reads and methylation calling of native termini for each DNA fragment
- ✓**Streamlined and simple workflow:** Prepare robust methyl-seq libraries in as little as 3 steps

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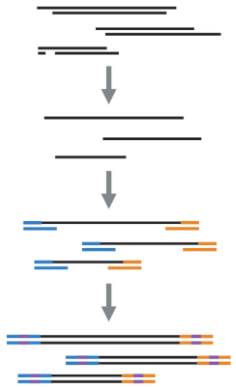
Where does this product fit?

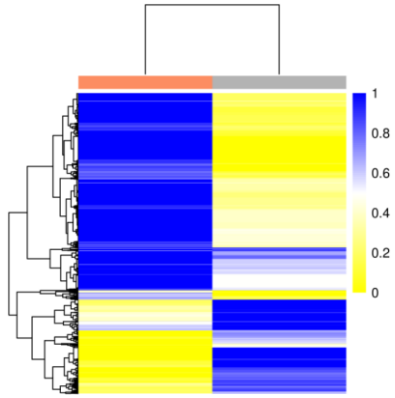
1. Liquid biopsy sample collection

2. cfDNA extraction from liquid biopsy



3. WGBS library preparation

4. Illumina sequencing



5. Bioinformatic analysis

Crucial step in the process → all downstream analysis depends on quality of library prep

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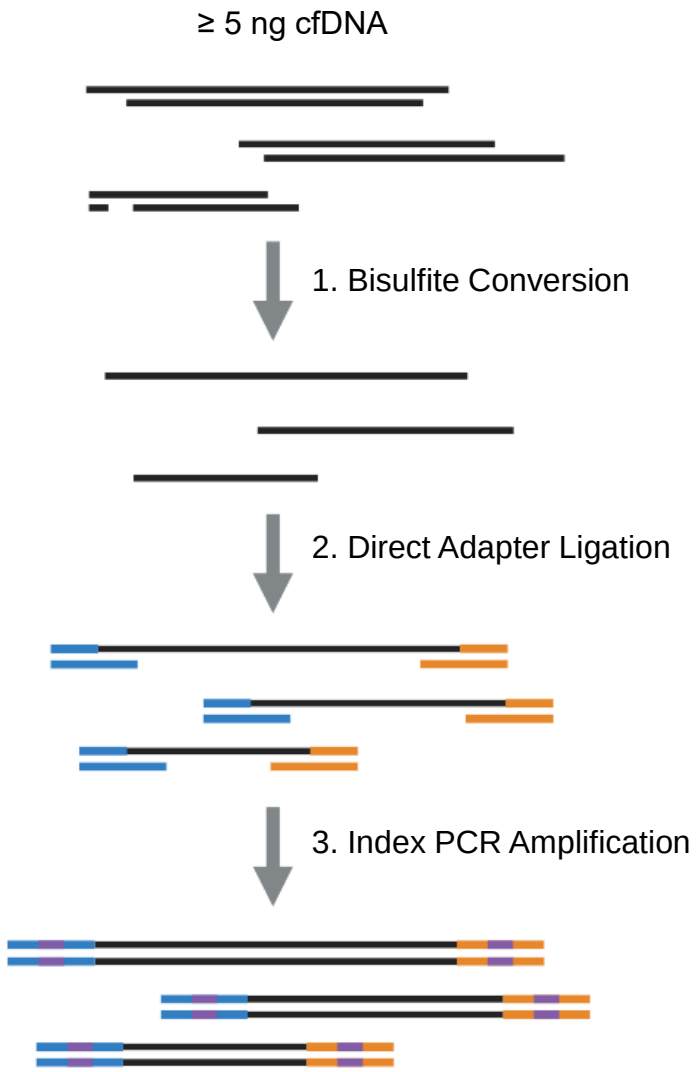
Workflow



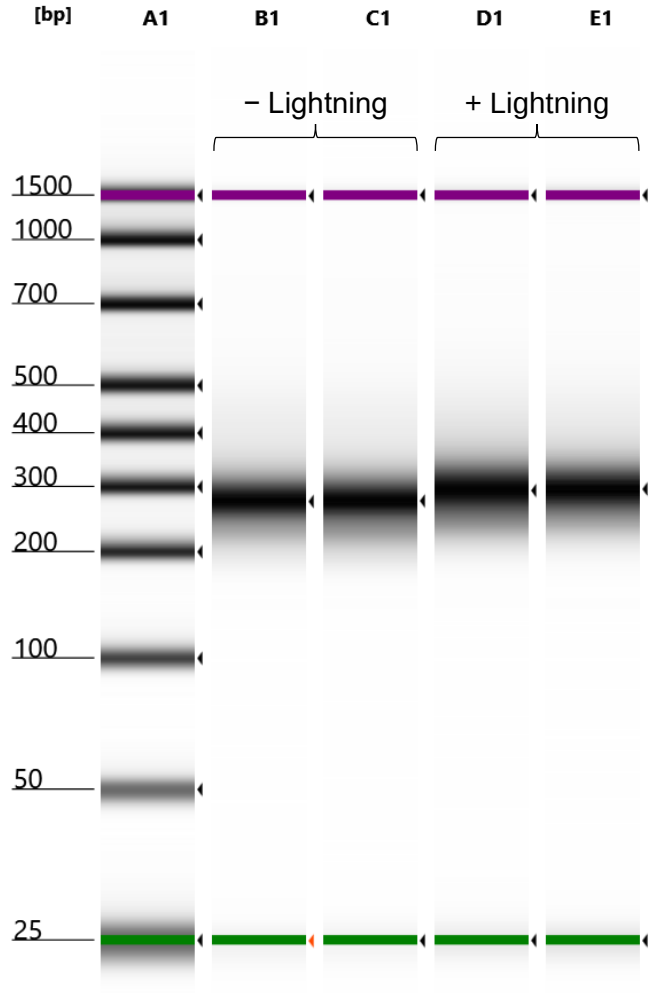
1. cfDNA undergoes gentle bisulfite conversion using **EZ DNA Methylation Lightning**
   - ✓ Reduces potential damage to the sample
2. Splinted adapters capture and directly ligate onto any size DNA fragment
   - ✓ Preserves methylation status of each terminus
3. Adapter-ligated cfDNA is indexed and amplified via PCR
   - ✓ Final libraries are ready for sequencing on any Illumina instrument

ZYMO RESEARCH

Optimized for small fragment input

- **EZ DNA Methylation Lightning Kit** chemistry
  - Minimal sample fragmentation
  - Complete bisulfite conversion in only 90 min
  - Compatible with DNA fragments > 50 bp

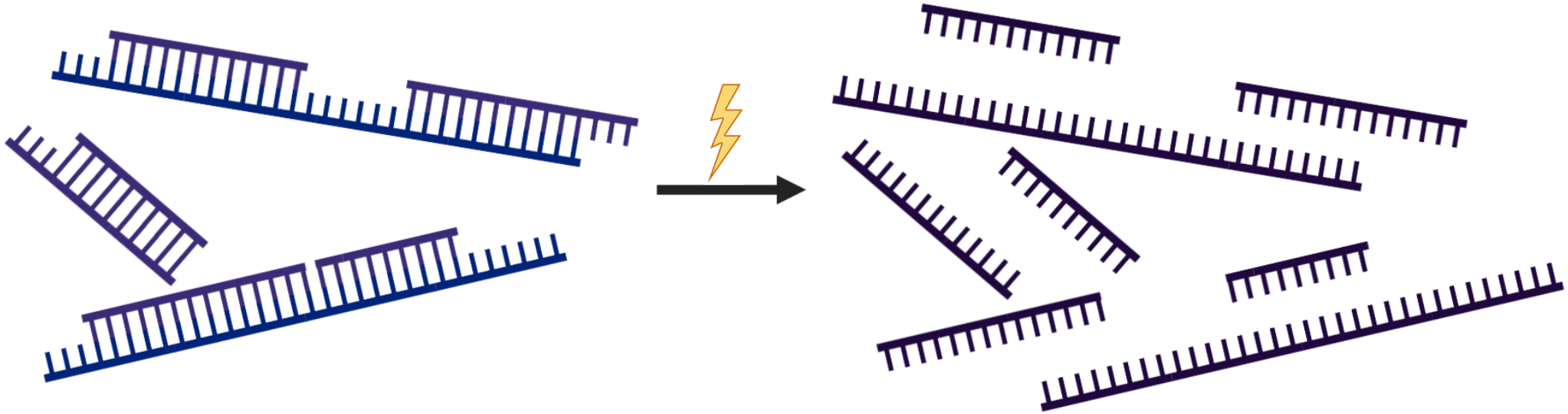
- Gel (right): Libraries prepared with the Zymo-Seq Cell Free DNA WGBS Library Kit with HeLa nucleosomal DNA samples (168 bp). Libraries were prepared with and without the Lightning bisulfite conversion step



ZYMO RESEARCH

Optimized for small fragment input

- Bisulfite conversion changes double-stranded DNA to single-stranded DNA



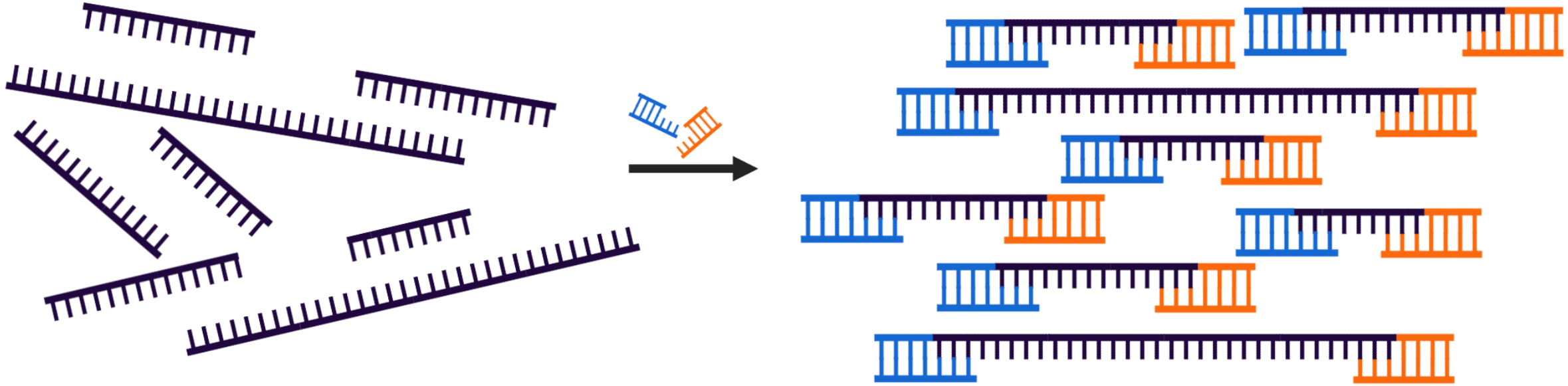
Double-stranded
cfDNA

Bisulfite-converted
cfDNA

ZYMO RESEARCH

Optimized for small fragment input

- Splinted adapters easily capture cfDNA regardless of nicks or damage
- Direct adapter ligation allows each **entire DNA fragment** to be sequenced



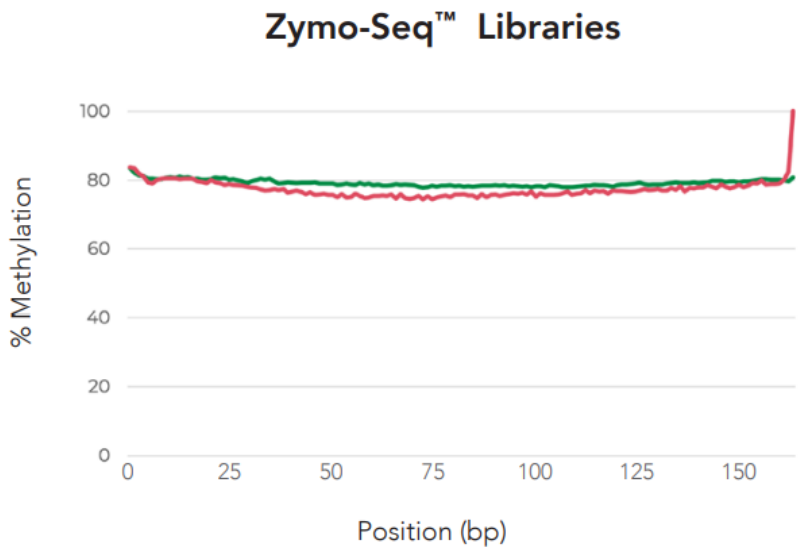
Bisulfite-converted cfDNA

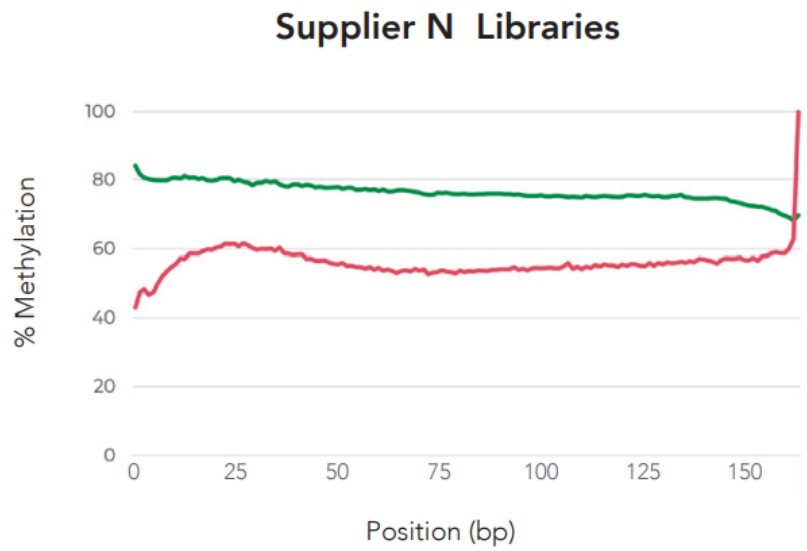
Adapter-ligated cfDNA

ZYMO RESEARCH

Accurate methylation calling

- Unbiased libraries have consistent methylation levels across **Read 1** and **Read 2**
- End-repair steps incorporate artificial nucleotides, resulting in significant methylation bias
- Direct adapter ligation preserves the integrity of native methylation





Read 1 Read 2

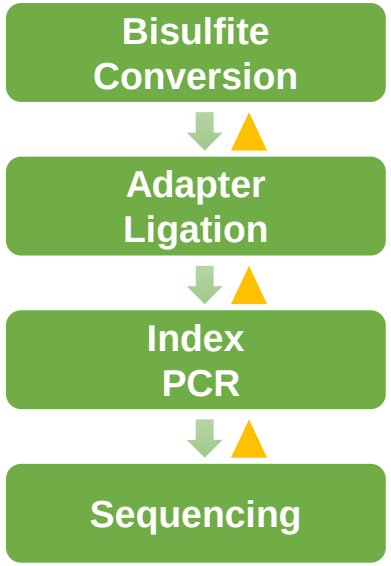
Zymo-Seq libraries have **consistent methylation** and **low bias** → accurate methylation calling

Supplier N libraries have **significant bias** due to end-repair → biased methylation calling

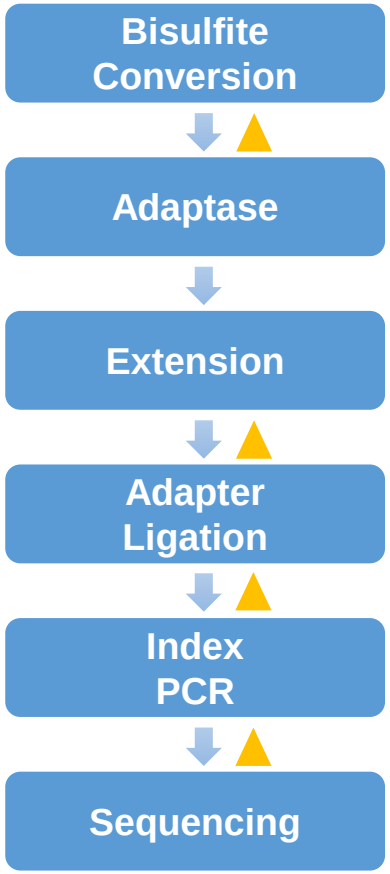
ZYMO RESEARCH

Streamlined and simple workflow

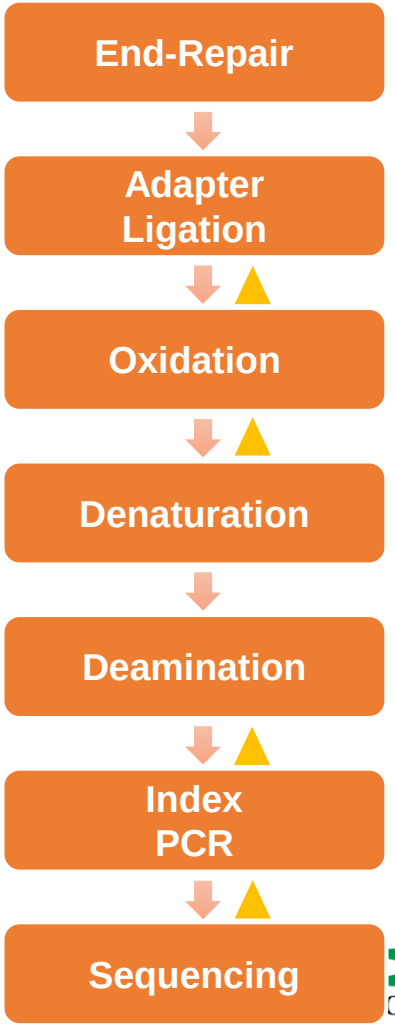
Zymo-Seq Cell Free DNA WGBS Library Kit



IDT xGen Methyl-Seq DNA Library Prep Kit



NEBNext Enzymatic Methyl-Seq Kit



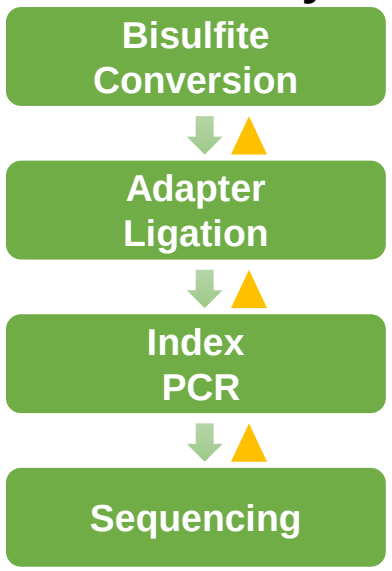
Clean-up

*Formerly known as **Swift Accel-NGS Methyl-Seq DNA Library Kit**

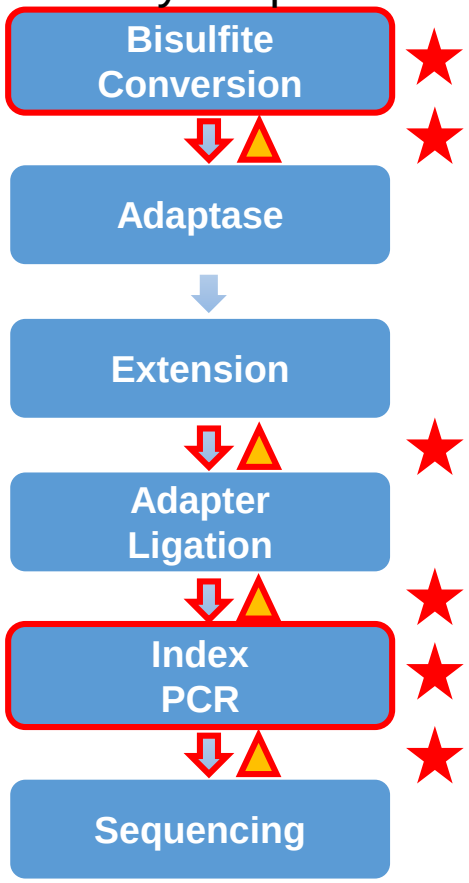
O RESEARCH

Streamlined and simple workflow

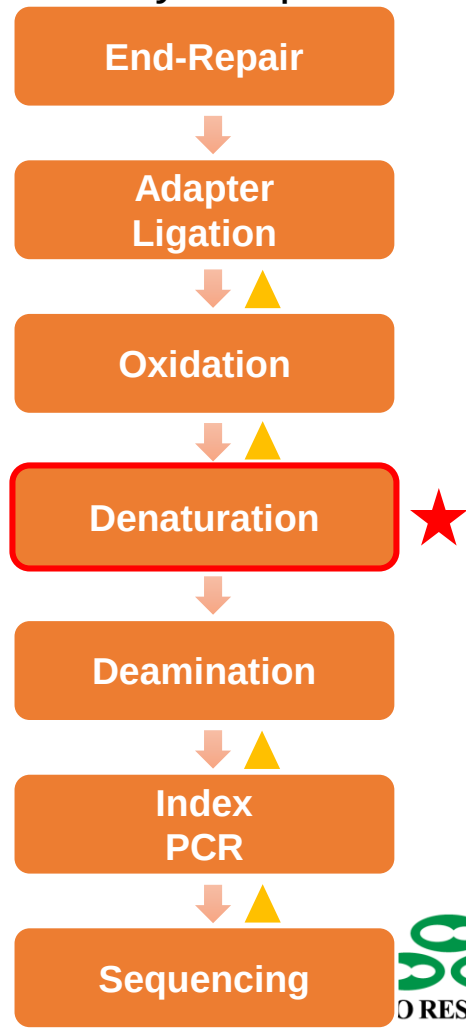
Zymo-Seq Cell Free DNA WGBS Library Kit



IDT xGen Methyl-Seq DNA Library Prep Kit



NEBNext Enzymatic Methyl-Seq Kit



Clean-up

Required material **not** included

*Formerly known as **Swift Accel-NGS Methyl-Seq DNA Library Kit**

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Competitor Comparisons

	Zymo-Seq Cell Free DNA WGBS Library Kit Cat. No. D5462, D5463	IDT xGen Methyl-Seq DNA Library Prep Kit* Cat. No. 10009860, 10009824, 10009825	NEBNext Enzymatic Methyl-Seq Kit Cat. No. E7120S, E7120L
Bisulfite Conversion Reagents	Included	*Requires purchase of:* • EZ DNA Methylation-Gold Kit	*Requires purchase of:* • Formamide
Indexing Primers	Included	*Requires purchase of:* • xGen CDI Primers • xGen UDI Primers	Included
Clean-Up MagBeads	Included	*Requires purchase of:* • SPRIselect • AMPure XP	Included
Total Workflow Steps	3	5	6
Average Time to Prepare 8 Libraries	6 hours	8 hours	14 hours

All-inclusive kit saves the user time AND money!

***Formerly known as **Swift Accel-NGS Methyl-Seq DNA Library Kit**

ZYMO RESEARCH

How to Sell the Zymo-Seq Cell Free DNA WGBS Library Kit

1. Identifying Customers
2. Engaging The Customer
3. Sales Tools
4. Take Home Message
5. Sales Support

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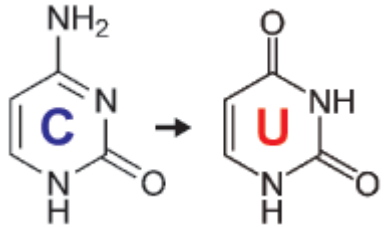
Identifying Customers - Keywords

- Liquid biopsy
  - Plasma
  - Serum
  - Amniotic fluid
  - Cerebrospinal fluid (CSF)
  - Saliva
  - Urine
- Blood collection tubes
  - Anticoagulant (EDTA, citrate, heparin)
  - Streck

- Cell-free DNA (cfDNA)
- Circulating tumor DNA (ctDNA)
- DNA methylation analysis
- Bisulfite (sequencing)
- WGBS, Methyl-Seq
- Epigenetics
- Illumina Sequencing, NGS



ZYMO RESEARCH

Engaging the Customer

1. Verify that they are potential customers (ask questions to help validate a potential customer)
    - *Are you working with cell-free DNA from liquid biopsy?*
    - *Are you looking to do methylation analysis of cell-free DNA?*
    - *Are you currently or planning to perform bisulfite sequencing/methyl-seq/WGBS?*
2. Present the pitch
    - *The Zymo-Seq Cell Free DNA WGBS Library Kit is a streamlined method for preparing high-quality cfDNA libraries for methyl-seq. This all-inclusive kit has been optimized for cfDNA and allows for reproducible libraries with less bias and more accurate methylation calling.*
3. Present key values (reword the 3 highlights)
    - *The protocol has been optimized for short and damaged DNA input, making it perfect for use with cfDNA.*
    - *The direct adapter ligation easily captures cfDNA and results in highly accurate methylation calling across each read.*
    - *The workflow is simple and easy-to-follow, allowing for library preparation in as little as 3 steps.*
4. Close the Meeting
    - *Try it yourself! We have a 100% satisfaction guarantee.*

ZYMO RESEARCH

Potential Customer Objections

I don't want to fragment my cfDNA samples further by performing bisulfite conversion.

- The **EZ DNA Methylation Lightning** chemistry used in the kit is ideal for bisulfite conversion of cfDNA as it results in less fragmentation of DNA

- It has been proven to recover small fragments > 50 bp while still maintaining > 99.5% conversion efficiency

ZYMO RESEARCH

Potential Customer Objections

It's too specific of a kit, I have other types of samples that I want to perform WGBS analysis with.

- The **Zymo-Seq Cell Free DNA WGBS Library Kit's** current protocol has been optimized for cfDNA, however there are many potential applications for the direct adapter ligation technology

- Genomic and FFPE DNA can be used with a modified protocol. Contact Zymo Research Technical Support at (949)-679-1190 Ext. 3 or tech@zymoresearch.com for protocol modifications regarding your specific sample type

ZYMO RESEARCH

Potential Customer Objections

I already have a validated cfDNA WGBS library preparation method.

- This is an all-inclusive kit that features everything that is needed for WGBS library preparation from cfDNA
- Faster turnaround time for preparing sequence-ready WGBS libraries from cfDNA with an easy-to-follow protocol for consistent results
- The **Zymo-Seq Cell Free DNA WGBS Library Kit** has been validated for accurate methylation calling and high coverage, ensuring no compromise on quality of the final libraries

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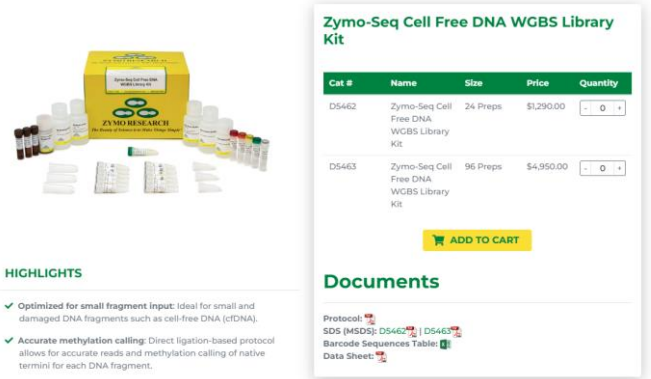
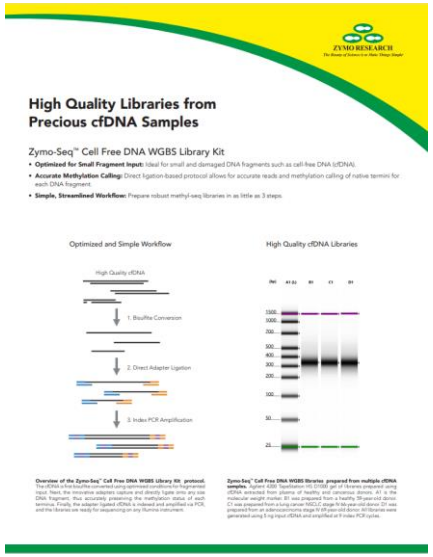
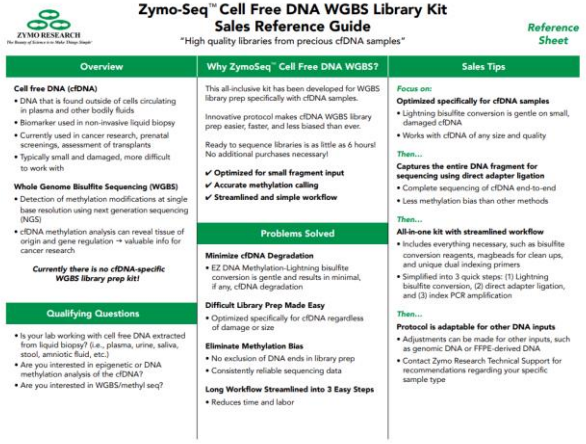
Sales Tools



Product Page



Data Sheet



Sales Reference Guide

Application Note

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Cross-selling

✓ *For cfDNA extraction from serum, plasma, and other biological fluids:*

***Quick*-cfDNA Serum & Plasma Kit**
Cat. No. D4076
50 preps

✓ *For parallel or co-purification of cfDNA and cfRNA from serum, plasma, and other biological fluids:*

***Quick*-cfDNA/cfRNA Serum & Plasma Kit**
Cat. No. R1072
50 preps

✓ *For clean-up and concentration of extracted cfDNA:*

DNA Clean & Concentrator-5
Cat. No. D4013, D4014
50 preps, 200 preps

✓ *For epigenetic NGS analysis solutions:*

Next Generation Sequencing Services	
Targeted Bisulfite Sequencing	Evaluate site-specific DNA methylation
Genome-Wide DNA Methylation	RRBS, Methyl-MiniSeq, and Methyl-MaxiSeq
ChIP-Seq Service	Protein/DNA interactions and histone modifications
Human Epigenetic Age	Quantify epigenetic age with Human DNAge
Mouse Epigenetic Age	Quantify biological age across various tissues

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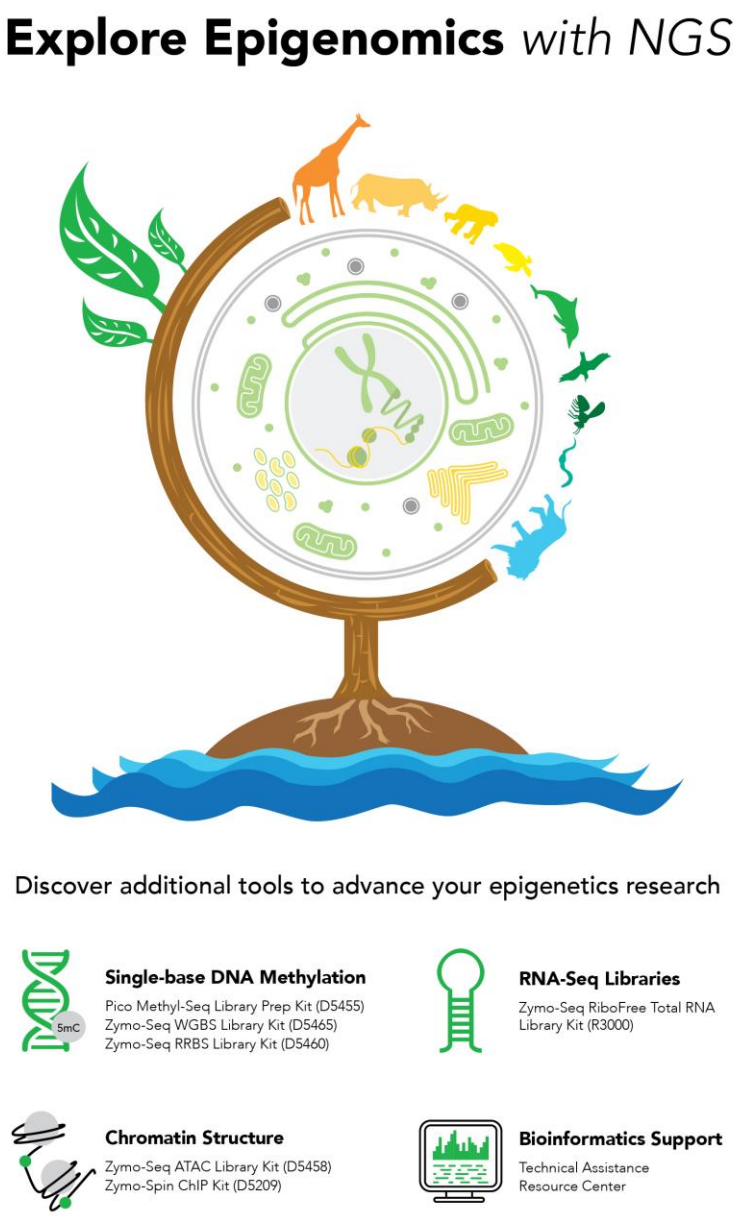
Zymo Research offers a suite of NGS tools to complete the epigenomics picture:

- DNA methylation
- Chromatin analysis
- Transcriptome analysis (RNA-seq)
- Bioinformatics support



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Take Home Message

The Zymo-Seq Cell Free DNA WGBS Library Kit easily produces high-quality libraries from precious cfDNA samples.

- Optimized for cfDNA → accounts for cfDNA characteristics
- Direct adapter ligation → reduces bias and better for capturing cfDNA
- Streamlined protocol → all-inclusive kit that is easy to follow

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Sales Support: Key Contacts for International Support

ZRC Key Contacts

International Orders – INTL Customer Service Team (intlorders@zymoresearch.com). They will be handling all your pricing, ordering, and logistics needs.

Brian Jansen – Account Manager, International Distributors (bjanssen@zymoresearch.com) – Main contact for international distributors.

International Tech Support (techintl@zymoresearch.com) – They can assist with technical inquiries and product training.

Marc E. Van Eden – VP of Business Development (MVanEden@zymoresearch.com) – He can coordinate with you on business related issues.

Sandy Sanchez – Tradeshow Coordinator (ssanchez@zymoresearch.com) – She can assist with tradeshow material requests.

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Sales Support: Key Contacts for International Support

ZRE Key Contacts

Natalie Tritsch – Business Administration Director, Timo Linsenmaier (accounting@zymoresearch.de) – They can assist with any accounting related questions.

Tobias Bräuner (main contact), Phillip Thimm, Simone Kretzschmar – Shipping, Receiving & Customer Service (orders@zymoresearch.de). They will be handling all your pricing, ordering, and logistics needs.

Tamaris Wörner – Tradeshow Coordinator (twoerner@zymoresearch.de) – She can assist with tradeshow material requests.

European Tech Support (tech@zymoresearch.de) – They will handle your technical inquiries and product guidance.

Dr. Thomas Kuri – Managing Director (tkuri@zymoresearch.de) – He can coordinate with you on business related issues.

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Questions?

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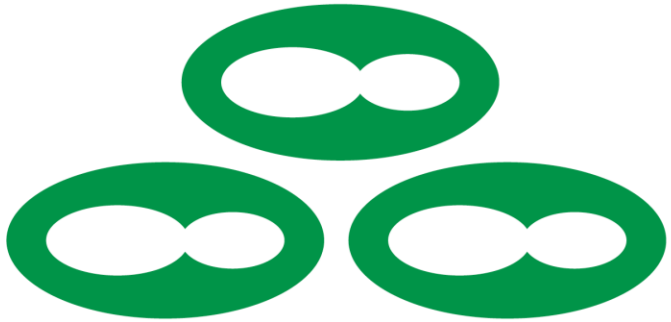

ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®

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 www.zymoresearch.com

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