

Zymo-Seg[™] SPLAT DNA Library Kit

High-quality library prep solution for challenging DNA samples

Highlights

- · Superior Genomic Precision: SPLAT (Splinted Ligation Adapter Tagging) technology ligates adapters directly onto DNA, preserving information and capturing more SNPs, INDELs, and variants with high accuracy.
- Versatile Sample Handling: Easily prepare high-quality libraries from a variety of sample types, such as genomic DNA, FFPE-derived DNA, and cfDNA.
- · Efficient and Simple Workflow: Prepare DNA samples into NGSready libraries with a 2-step workflow in as little as 3 hours.

Catalog Numbers: D5464



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







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Product Contents

Zymo-Seq™ SPLAT DNA Library Kit	D5464 (12 Preps.)	Storage Temperature
Denaturation Buffer	24 µL	-20°C
Adapter 1	18 µL	-20°C
Adapter 2	18 µL	-20°C
Ligation PreMix	324 μL	-20°C
Amplification PreMix	300 µL	-20°C
Zymo-Seq™ UDI Primer Set (1-12)¹	20 µL/Index	-20°C
Select-a-Size MagBead Concentrate	300 µL	4°C
Select-a-Size MagBead Buffer	10 mL	4°C
DNA Wash Buffer (concentrate) ²	6 mL	Room Temp.
DNA Elution Buffer	4 mL	Room Temp.
DNase/RNase-Free Water	1 mL	Room Temp.
Instruction Manual	1	-

¹ The provided **Zymo-Seq™ UDI Primer Set** (Indexes 1-12) contains 12 pre-mixed unique dual index barcode primers in 1.5 mL tubes. See **Appendix C** on pg. 15 for primer specifications, index sequences, and multiplexing considerations.

² The **DNA Wash Buffer** is supplied as a concentrate. See **Buffer Preparation** on pg. 4 for directed amounts of ethanol to be added upon first use. Cap bottle tightly after each use to prevent ethanol evaporation.

Specifications

- **Sample Input Type:** Sonicated genomic DNA (average size of 300-600 bp), sonicated FFPE-derived DNA (average size of 300-600 bp), cfDNA, etc.
- Minimum Input: 10 ng
- Maximum Input: 500 ng
- Input Quality: For optimal results, input DNA should be free of RNA contamination and enzymatic inhibitors with A260/A280 and A260/A230 values of ≥1.8. DNA with lower purity ratios (A260/A280 & A260/A230) should first be treated with the DNA Clean & Concentrator™ (Cat. No. D4013) prior to processing. DNA should be suspended in water, DNA Elution Buffer, TE buffer, or another similar low-salt buffer.
- Equipment Required: Sonication device for DNA input >600 bp in average size, thermal cycler(s) with temperature-adjustable lids, microcentrifuge for 0.2 mL PCR tubes, magnetic stand for 0.2 mL PCR tubes (e.g., PCR Strip MagStand, Cat. No. 3DP-1002).
- Total Processing Time: ~3 hours
- Hands-On Time: ~1.5 hours
- 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. Please see Appendix D for sequencing considerations and recommendations.
- **Barcode Sequences:** Available in **Appendix C** on pg. 15. Sequences are also available for download <u>here</u> (USA only) or by visiting the Documents section of the D3008 and D3096 product pages at <u>www.zymoresearch.com</u>.

Product Description

The **Zymo-Seq™ SPLAT DNA Library Kit** is a versatile solution for DNA library preparation from a wide variety of sample types. By applying the unique, single-stranded library preparation approach of splinted ligation adapter tagging (SPLAT)¹, DNA libraries can be prepared from genomic DNA, cell-free DNA, and even FFPE-derived DNA with ease. Additionally, the SPLAT technology allows for direct ligation of adapters onto the native ends of each DNA fragment, simultaneously eliminating the need for end-repair and ensuring preservation of all original nucleotides. This feature allows for increased sequencing performance by revealing more SNPs, INDELs, and variants across the entirety of the DNA fragment.

The **Zymo-SeqTM SPLAT DNA Library Kit** easily generates library from 10 ng to 500 ng of pre-fragmented DNA input in just two simple steps: (a) ligation of the single-stranded DNA with the unique splinted adapters concurrently and (b) amplification of the libraries via PCR using unique dual indexes. By utilizing a straightforward workflow that is efficient and user-friendly, the **Zymo-SeqTM SPLAT DNA Library Kit** allows for generation of high-quality libraries ready for DNA sequencing in as little as 3 hours.

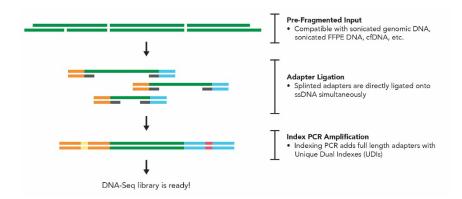


Figure 1. Overview of the Zymo-Seq SPLAT DNA Library Kit workflow. The unique single-stranded library preparation begins with denaturation and simultaneous ligation of adapters onto either end of the DNA fragment. The adapterized samples are then indexed and amplified via PCR, allowing for sequenceready libraries in as little as 3 hours.

¹ Raine, et al. Nucleic Acids Research. 2017; 45 (6): e26

Protocol

Section 1: Before Starting

Buffer Preparation

- ✓ Add 24 mL of 100% ethanol (26 mL of 95% ethanol) to the 6 mL DNA Wash Buffer (concentrate). Initial and mark date of ethanol addition to the bottle.
- ✓ Prepare the Select-a-Size MagBeads by adding 300 µL of the Selecta-Size MagBead Concentrate to the 10 mL Select-a-Size MagBead Buffer bottle. Resuspend by pipetting up and down and/or vortexing. Store at 4°C−8°C.

Recommendations and Best Practices

- ✓ Set the thermal cycler lids to the specified temperatures for each program.
- ✓ All components that are stored at -20°C should be thawed and kept on ice unless otherwise stated. Return to -20°C after each use. Avoid multiple freeze-thaws by making aliquots as necessary.
- ✓ Mix each component well before use by pipetting up and down, flicking, inverting, or very gently vortexing. Centrifuge briefly to collect all contents potentially caught on the sides or caps of the tubes before using.
- ✓ The Ligation Premix is very viscous. Mix well just before pipetting to ensure it is homogenous for best results.
- ✓ Allow the Select-a-Size MagBeads to equilibrate to room temperature for at least 30 minutes before use.

Sample Preparation and Considerations

Genomic DNA

We recommend sonicating intact, genomic DNA to an average fragment size of 300-600 bp before beginning the protocol. Due to differences in genomic DNA size and quality between samples, some optimization of the sonication may be necessary. Please follow the instructions and best practices of the sonication device being used for optimal results. We recommend quantifying the fragmented genomic DNA before proceeding to accurately calculate input amount.

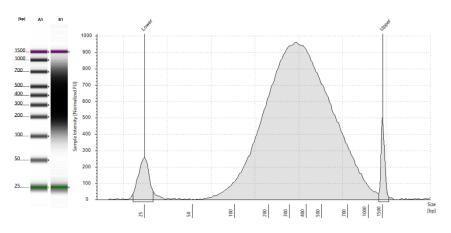


Figure 2. Sonicated genomic DNA suitable for the Zymo-Seq SPLAT DNA Library Kit. Agilent 4200 TapeStation[®] HS D1000 gel (left) and electropherogram (right) of genomic NA12878 DNA that was sonicated to approximately 385 bp in average size. A1 is the molecular weight marker and B1 is the sonicated NA12878 DNA. The DNA was originally extracted from GM12878 cell line using the *Quick-DNA* Miniprep Plus Kit (Cat. No. D4068).

Sample Preparation and Considerations (continued)

FFPE-derived DNA

We recommend sonicating FFPE-derived DNA to an average fragment size of 300-600 bp before beginning the protocol. Due to differences in FFPE size and quality between samples, some optimization of the sonication may be necessary. Please follow the instructions and best practices of the sonication device being used for optimal results. We recommend quantifying the fragmented FFPE DNA before proceeding to accurately calculate the input amount.

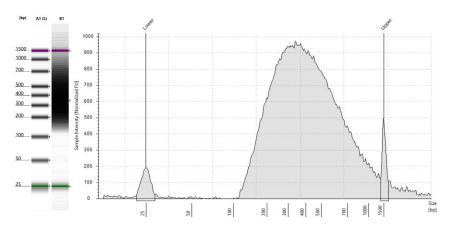


Figure 3. Sonicated FFPE-derived DNA suitable for the Zymo-Seq SPLAT DNA Library Kit. Agilent 4200 TapeStation® HS D1000 gel (left) and electropherogram (right) of FFPE-derived DNA that was sonicated to approximately 435 bp in average size. A1 is the molecular weight marker and B1 is the sonicated FFPE-derived DNA. The DNA was originally extracted from breast cancer FFPE tissue using the *Quick*-DNA FFPE Miniprep Kit (Cat. No. 3067).

Sample Preparation and Considerations (continued)

Cell-free DNA

We recommend inputting cell-free DNA (cfDNA) as direct input, no sonication is necessary. As the cfDNA will typically have mononucleosome, di-nucleosome, and potentially tri-nucleosome peaks, the overall average DNA size is sufficient for high-quality library preparation with this kit. Please proceed to **Section 2** on pg. 8 to begin library preparation.

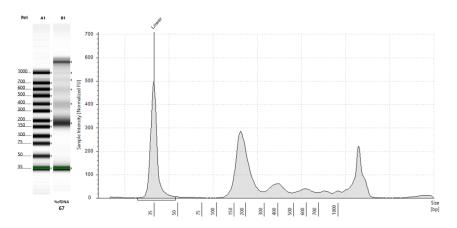


Figure 4. Cell-free DNA suitable for the Zymo-Seq SPLAT DNA Library Kit. Agilent 4200 TapeStation Cell-Free DNA ScreenTape gel (left) and electropherogram (right) of cfDNA after purification. A1 is the molecular weight marker and B1 is the purified cfDNA. The cfDNA was originally extracted from 1 mL of isolated plasma from a healthy donor using the MAGicBead™ cfDNA Isolation Kit (Cat. No. D4086).

Section 2: Adapter Ligation

Before Starting:

✓ Create the following **Denaturation Program** (lid temperature set to 105°C) for a 20 µL sample volume.

Step	Temperature	Time
1	95°C	Hold

✓ On a separate thermal cycler, create the following Adapter Ligation Program (lid temperature set to 45°C) for a 50 µL sample volume. If only one thermal cycler is available, save both programs and leave the lid open after the Denaturation Program is complete to help cool it faster.

Step	Temperature	Time
1	37°C	Hold
2	37°C	1 hour
3	4°C	Hold

- ✓ Thaw the Denaturation Buffer, Adapter 1, and Adapter 2 on ice. Thaw the Ligation PreMix at room temperature.
 - 1. Start the **Denaturation Program** to ensure the thermal cycler is fully heated to 95°C (lid temp 105°C) before Step 5.¹
 - 2. Mix the following components in a 0.2 mL PCR tube for 20 µL total:

Component	Volume
Input DNA (10 – 500 ng)	×μL
Denaturation Buffer	2 µL
DNase/RNase-Free Water	18 - Χ μL
Total Volume	20 µL

- 3. Mix the entire reaction thoroughly by pipetting or flicking. Centrifuge briefly.
- 4. Incubate the sample on ice for 2 minutes.

¹ If another thermal cycler is available, start the **Adapter Ligation Program** to ensure it is fully heated up to 37°C (lid temp 45°C) before Step 9.

- 5. Place samples into the pre-heated **Denaturation Program** and heat for 2 minutes. Immediately return the tube to ice and incubate for 2 minutes.²
- 6. For DNA inputs <50 ng, dilute both **Adapter 1** and **Adapter 2** 6-fold with **DNA Elution Buffer** just before proceeding.

DNA Input	Adapter Dilution
10-49 ng	1:6
50-500 ng	None

Example: 1.5 µL Adapter 1 + 7.5 µL DNA Elution Buffer = 9 µL of 1:6 Diluted Adapter 1

- Mix the Ligation PreMix well at room temperature just before the next step by pipetting up and down, flicking, inverting, or very gently vortexing for <u>at least 30 seconds</u>. Centrifuge briefly.
- Add the following components to the sample on ice and mix completely by pipetting up and down 15-20 times or until visually homogenous³:

Component	Volume
Reaction from Section 2, Step 5	20 µL
Adapter 1	1.5 µL
Adapter 2	1.5 µL
Ligation PreMix	27 µL
Total Volume	50 µL

- Place the sample into the pre-heated thermal cycler running the Adapter Ligation Program. Skip to Step 2 to perform the adapter ligation for 1 hour.⁴
- 10. After the **Adapter Ligation Program** is complete, add 50 μL of **DNA Elution Buffer** to the 50 μL sample for 100 μL total.
- Follow the clean-up protocol in Appendix A on pg. 11 using 100 μL of Select-a-Size MagBeads. Elute the purified ligated sample in 15 μL of DNA Elution Buffer and transfer into a new 0.2 mL PCR tube.

This is a safe stopping point. Purified DNA can be safely stored at ≤-20°C overnight or over the weekend. Otherwise, continue to Section 3.

² If using only one thermal cycler, start the **Adapter Ligation Program** and leave the lid open to help cool. Ensure the thermal cycler is fully pre-heated to 37°C before Step 9.

³ Use the stock concentration of **Adapter 1** and **Adapter 2** if input ≥50 ng. Use a 1:6 dilution if input <50 ng.

⁴ During the ligation program, take the **Select-a-Size MagBeads** out of the 4°C storage to allow the beads to equilibrate to room temperature for at least 30 minutes.

Section 3: Index PCR Amplification

Before Starting:

- ✓ Thaw the **Zymo-Seq™ UDI Primers** and **Amplification PreMix** on ice.
- ✓ Create the following Index PCR Program (lid temperature set to 105°C) for a 50 µL sample volume.

Step	Temperature	Time	Recommended N (based on original s	
1	98°C	3 min	Genomic DNA, cfDNA	FFPE DNA
2	98°C	20 sec	500 ng = 3-4 cycles	500 ng = 5-8 cycles
3	60°C	30 sec	100 ng = 4-5 cycles 50 ng = 5-6 cycles	100 ng = 6-9 cycles 50 ng = 7-10 cycles
4	72°C	30 sec	10 ng = 6-7 cycles	10 ng = 8-11 cycles
5	72°C	1 min		
6	4°C	Hold		

1. Add the following components in a 0.2 mL PCR tube on ice:

Component	Volume
Adapter-ligated DNA	15 µL
Zymo-Seq™ UDI Index Primers	10 µL
Amplification PreMix	25 µL
Total Volume	50 µL

- 2. Mix the entire reaction thoroughly by pipetting or gently vortexing. Centrifuge briefly.
- 3. Place the sample in the thermal cycler and run the **Index PCR Program** for the appropriate number of cycles.^{1,2}
- Follow the clean-up protocol in Appendix A on pg. 11 using 50 μL of Select-a-Size MagBeads. Elute the purified library in 20 μL of DNA Elution Buffer and transfer into a new tube.

This is the final library. Libraries can be safely stored overnight at 4°C or for months at -20°C.

¹ Some optimization of the number of PCR cycles may be necessary depending on sample type and quality. In general, FFPE libraries may require more cycles.

² During the PCR, take the **Select-a-Size MagBeads** out of the 4°C storage to allow the beads to equilibrate to room temperature for at least 30 minutes.

Appendices

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

Before Starting:

- ✓ 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 vigorously shaking or vortexing the Select-a-Size MagBeads until homogenous.
 - 1. Add the recommended volume of **Select-a-Size MagBeads** to each sample. Mix thoroughly by pipetting until homogenous. Incubate for 5 minutes at room temperature.
 - 2. Place the sample on a magnetic stand for 3 minutes, or until the beads have fully separated from the solution. Without dislodging the bead pellet, aspirate slowly and discard the supernatant.
 - 3. While the sample is still on the magnetic stand, add 200 µL of DNA Wash Buffer without disturbing the bead pellet and incubate for 30 seconds. Aspirate slowly and discard the supernatant without dislodging the bead pellet. Repeat this step for a total of 2 washes.
 - 4. While the sample is still on the magnetic stand, keep the tube cap open to air-dry the beads. After 1 minute, aspirate any residual **DNA Wash Buffer** that has collected at the bottom of the tube. Continue to air-dry until the bead pellet <u>appears matte without cracking</u>. See Figure 5 on the next page for an image reference.
 - 5. Remove the sample from the magnetic stand. Add the indicated volume of **DNA Elution Buffer** to the beads and mix thoroughly by pipetting up and down until homogenous. Incubate for 5 minutes at room temperature.
 - 6. Place the sample on the magnetic stand for 1-2 minutes or until the eluate is clear. Transfer the eluate to a new 0.2 mL PCR tube for each sample.

Appendix A: Select-a-Size MagBead Clean-Up Protocol (Continued)

Further Information on Bead Pellet Air-Dry

The optimal air-dry time can vary depending on the humidity and temperature. Optimally dried beads should appear matte without cracking (e.g., the tube in the middle in Figure 5 below).

Start with 2 minutes of air-dry time and adjust the time as needed to achieve optimally dried beads. Wash buffer carryover from insufficiently dried beads and/or overdried beads may reduce nucleic acid recovery.



Figure 5. Examples of over-dried, optimally dried, and under-dried Select-a-Size MagBeads. Overdried beads are cracked and flakey, resembling dried mud. Under-dried beads are glossy and wet, like saturated mud. Optimally dried beads appear damp, but lack gloss.

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 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 D1000 or High Sensitivity D1000 tapes on TapeStation[®] for optimal library characterization. If adapter dimers are present, they will form an approximately 120-150 bp band. Yields will vary depending on the total quantity and quality of sample input DNA. The following **Figures 6, 7,** and **8** are examples of typical libraries from sonicated genomic DNA, sonicated FFPE DNA, and cfDNA inputs prepared with the **Zymo-Seq[™] SPLAT DNA Library Kit**, respectively.

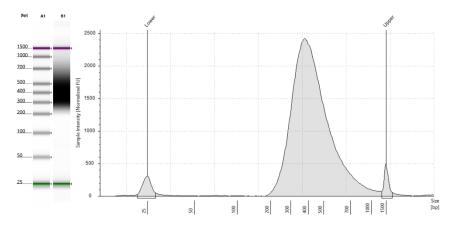
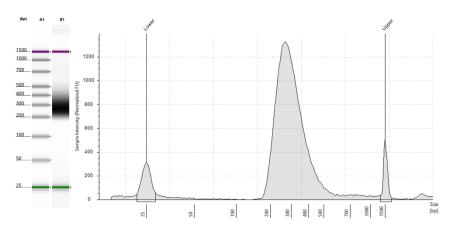
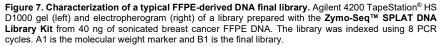


Figure 6. Characterization of a typical genomic DNA final library. Agilent 4200 TapeStation[®] HS D1000 gel (left) and electropherogram (right) of a library prepared with the **Zymo-Seq™ SPLAT DNA** Library Kit from 100 ng of sonicated NA12878 genomic DNA. The library was indexed using 5 PCR cycles. A1 is the molecular weight marker and B1 is the final library.

Appendix B: Library Quantification and Characterization (continued)





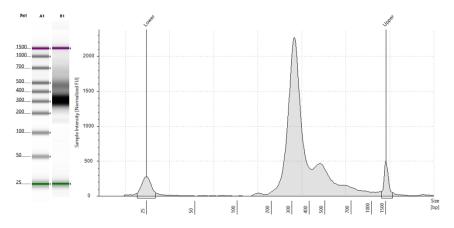


Figure 8. Characterization of a typical cfDNA final library. Agilent 4200 TapeStation[®] HS D1000 gel (left) and electropherogram (right) of a library prepared with the **Zymo-Seq™ SPLAT DNA Library Kit** from 10 ng of cfDNA derived from a donor with colorectal cancer. The library was indexed using 6 PCR cycles. A1 is the molecular weight marker and B1 is the final library.

Appendix C: Unique Dual Index Primer Set

Indexes in the **Zymo-SeqTM UDI Primer Set (Indexes 1-12)** are dispensed in 1.5 mL tubes (D3008). Indexes come as pre-mixes, and the forward and reverse primers are provided at 5 μ M total concentration (2.5 μ M each).

If multiplexing samples together, use a primer set only once per lane. To ensure color balance during multiplexing, use the **Zymo-Seq™ UDI Primers** sequentially (e.g., if pooling 2 samples, use UDIs #1-2. If pooling 4 samples, use UDIs #1-4).

Forward Primer Sequence (i5)¹

5'-AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTC TTTCCCTACACGACGCTCTTCCGATCT-3'

Reverse Primer Sequence (i7)²

5'-CAAGCAGAAGACGGCATACGAGAT<u>NNNNNNN</u>GTGACTGGAG TTCAGACGTGTGCTCTTCCGATCT-3'

Index (Fwd + Rev)	i5 Bases in Adapter/ for MiSeq, HiSeq 2000/2500, and NovaSeq v1.0 ³	i5 Bases for iSeq, NextSeq, HiSeq 3000/3500, and NovaSeq v1.5 ⁴	i7 Bases in Adapter	i7 Bases for all sample sheets
UDI_01	AGCGCTAG	CTAGCGCT	AACCGCGG	CCGCGGTT
UDI_02	GATATCGA	TCGATATC	GGTTATAA	TTATAACC
UDI_03	CGCAGACG	CGTCTGCG	CCAAGTCC	GGACTTGG
UDI_04	TATGAGTA	TACTCATA	TTGGACTT	AAGTCCAA
UDI_05	AGGTGCGT	ACGCACCT	CAGTGGAT	ATCCACTG
UDI_06	GAACATAC	GTATGTTC	TGACAAGC	GCTTGTCA
UDI_07	ACATAGCG	CGCTATGT	CTAGCTTG	CAAGCTAG
UDI_08	GTGCGATA	TATCGCAC	TCGATCCA	TGGATCGA
UDI_09	CCAACAGA	TCTGTTGG	CCTGAACT	AGTTCAGG
UDI_10	TTGGTGAG	CTCACCAA	TTCAGGTC	GACCTGAA
UDI_11	CGCGGTTC	GAACCGCG	AGTAGAGA	TCTCTACT
UDI_12	TATAACCT	AGGTTATA	GACGAGAG	CTCTCGTC

^{1, 2} The NNNNNNN sequences correspond to the "Bases in Adapter" columns in the table.

^{3, 4} The "NovaSeq v1.0" and "NovaSeq v1.5" refer to v1.0 and v1.5 reagent chemistry, respectively.

Appendix D: 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).

Sequencing Parameters:

Libraries generated with this workflow are suitable for any read length, but increased read lengths may require greater amounts of adapter trimming for the shorter library fragments. For most applications, 150 base pairedend (PE) reads are enough to generate substantial amounts of high-quality data for genome-wide coverage.

To estimate the necessary number of reads per library we recommend using the following Lander/Waterman equation¹:

Number of Reads = (Desired Coverage) × (Haploid Genome Length) / (Read Length)

Please note that this equation assumes that reads will be distributed evenly across the genome and contain no overlap between reads. Therefore, this is helpful for estimating the number of reads necessary, however actual coverage may vary in practice due to factors such as variability in library preparation and clustering efficiency during sequencing.

We also recommend using the Illumina[®] Sequencing Coverage Calculator to help determine the number of lanes/flow cells necessary for the desired sequencing instrument and parameters.² Please contact Illumina[®] technical support for more information and recommendations.

Adapter Trimming:

Libraries should be trimmed to remove the following adapter sequences:

Read 1: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA Read 2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

¹ Lander, Waterman. Genomics. 1988; 2(3): 231-239

² Please refer to the following link for the Illumina® Sequencing Coverage Calculator: https://support.illumina.com/downloads/sequencing_coverage_calculator.html

Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq™ SPLAT DNA Library Kit	D5464	12 preps

Individual Kit Components	Catalog No.	Amount
Zymo-Seq™ UDI Primer Sets	D3008 D3096	12 indexes 96 indexes
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24 D4006-2-48	6 mL 24 mL 48 mL
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10	1 mL 4 mL 10 mL
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6	1 mL 4 mL 6 mL
DNA/RNA Shield™	R1100-50 R1100-250	50 mL 250 mL
<i>Quick</i> -DNA™ Miniprep Plus Kit	D4068T D4068 D4069	10 preps 50 preps 200 preps
<i>Quick</i> -DNA™ FFPE Miniprep Kit	D3067	50 preps
MAGicBead™ cfDNA Isolation Kit	D4086 D4086-Custom	2 mL x 50 preps Custom Input Size
DNA Clean & Concentrator®-5	D4013 (capped) D4014 (capped)	50 preps 200 preps
PCR Strip MagStand	3DP-1002	1 unit

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Sample to Sequencer in a Single Day



Sample Collection

Nucleic acid stabilization DNA/RNA Shield™ (Cat. No. R1100-50)

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DNA isolation from any sample type Quick-DNA[™] Miniprep Plus Kit (Cat. No. D4068) Quick-DNA[™] FFPE Miniprep Kit (Cat. No. D3067) MAGicBead[™] cfDNA Isolation Kit (Cat. No. D4086)



DNA-Seq Library Prep Kit

Library prep from any DNA sample Zymo-Seq[™] SPLAT DNA Library Kit (Cat. No. D5464)

↓ NGS-Ready Libraries

In as little as 4 hours

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