SUPPLEMENTARY PROTOCOL

1. KAS-seq with mammalian cell cultures

Labeling and DNA isolation

1. Prepare 500 mM N_3-kethoxal stock solution using DMSO. Then prepare the labeling medium by diluting the N_3-kethoxal solution into pre-warmed (37 ˚C) cell culture medium to a final concentration of 5 mM. It is critical to pre-warm the medium to facilitate N_3-kethoxal dissolution.

2. Incubate 1-5 million cells in the labeling medium for 10 min at 37 ˚C. For adhesive cells, apply the labeling medium to the cell culture directly in dishes and incubate cells for 10 min at 37 ˚C. For suspension cells, suspend cells in the labeling medium and incubate them for 10 min at 37 ˚C.

3. Harvest cells after the incubation. Isolate total DNA from cells by using PureLink genomic DNA mini kit (Thermo K182001). Elute DNA by using 50 μL 25 mM K_3BO_3 (pH 7.0). **[Stop point 1]**

Biotinylation and purification

4. Prepare the click reaction mixture as the follows.

2 μg total DNA diluted in 85 μL of 25 mM K_3BO_3 (pH 7.0)
10 μL 10× PBS
5 μL 20 mM DBCO-PEG$_4$-biotin (DMSO solution, Sigma 760749)

Incubate the mixture at 37 ˚C for 1.5 h with shaking at 500 rpm to facilitate the “click” reaction.

5. Add 5 μL RNase A (Thermo 12091039) to the reaction mixture. Incubate the mixture at 37 ˚C for 15 min with shaking at 500 rpm.

6. Purify DNA from the reaction mixture by DNA Clean & Concentrator kit (Zymo D4043). Elute DNA by using 50 μL 25 mM K_3BO_3 (pH 7.0). **[Stop point 2]**

Enrichment of N_3-kethoxal-modified DNA

7. Dilute 1 μg biotinylated DNA in 100 μL 25 mM K_3BO_3 (pH 7.0). Fragment the DNA by sonication using Bioruptor Pico under 30s-on /30s-off setting for 30 cycles. The size of fragmented DNA ranges from 150-350 bp.

8. Save 5 μL of the fragmented DNA as input. Use the remaining 95 μL for enrichment.

9. Wash 10 μL Dynabeads Myone Streptavidin C1 (Thermo 65001) 3 times with 50 μL 1× binding and wash buffer (5 mM Tris-HCl pH 7.4, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20). Re-suspend pre-washed beads in 95 μL 2× binding and wash buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 2 M NaCl, 0.1% Tween-20).

10. Mix the beads with 95 μL fragmented DNA from step 8. Incubate the mixture at room temperature for 15 min with gentle rotation. After incubation, place the mixture on a magnetic rack to remove the supernatant and wash beads 5 times with 100 μL 1× binding and wash buffer.

11. Re-suspend the beads in 15 μL nuclease-free water and heat it at 95 ˚C for 10 min to elute enriched DNA. Then put the mixture on a magnetic rack, and transfer the supernatant to a new labeled tube.

12. In parallel with the previous step, heat the saved input at 95 ˚C for 10 min. Then, put both the input and the eluted samples on ice immediately. **[Stop point 3]**

Library preparation and sequencing

13. Perform library construction by using the Accel-NGS Methyl-seq DNA library kit (Swift 30024). Perform PCR amplification for 7-8 cycles for the input samples and 12-14 cycles for the enriched samples. The library size should range from 200-500 bp.

14. Sequence libraries on Illumina platforms by using single-end mode, aiming to get 30 million reads per sample.

DNAs can be stored at -20 ˚C at the noted stop points if needed.

2. KAS-seq with mouse liver

Labeling and DNA isolation

1. Homogenize mouse liver tissue to a cell suspension in ice-cold PBS by using a dounce homogenizer or a pellet pestle.

2. Spin the cell suspension at 100 g for 15 seconds to sediment and remove potential large tissue pieces at the bottom of the tube.

3. Spin the cell suspension at 800 g for 5 min. Remove the supernatant and save the cell pellet at the bottom of the tube for labeling.

4. Suspend 1-5 million cells in the labeling medium for 10 min at 37 ˚C (refer to step 1 in the protocol for mammalian cell cultures for labeling medium preparation).

5. Isolate total DNA from cells by using PureLink genomic DNA mini kit (Thermo K182001). Elute DNA by using 50 μL 25 mM K_3BO_3 (pH 7.0). **[Stop point 1]**

Perform Biotinylation and purification, Enrichment of N_3-kethoxal-modified DNA, Library preparation and sequencing according to the protocol for mammalian cell cultures.

3. Low-input KAS-seq

Labeling and DNA isolation

1. Label 1,000, 5,000, or 10,000 cells according to the protocol for bulk mammalian cell cultures.

2. Isolate DNA from cells by using the Quick-DNA Microprep Plus kit (Zymo D4074). Elute DNA by using 43 μL 25 mM K_3BO_3 (pH 7.0).

Biotinylation and purification

3. Prepare the click reaction mixture as follows.

42.5 μL DNA in 25 mM K_3BO_3 (pH 7.0)
5 μL 10× PBS
2.5 μL 20 mM DBCO-PEG_4-biotin (DMSO solution, Sigma 760749)

Incubate the mixture at 37 ˚C for 1.5 h with shaking at 500 rpm to facilitate the "click" reaction.

4. Add 2.5 μL RNase A (Thermo 12091039) to the reaction mixture. Incubate the mixture at 37 ˚C for 5 min.

5. Purify DNA from the reaction mixture by DNA Clean & Concentrator kit (Zymo D4043). Elute DNA by using 25 μL 25 mM K_3BO_3 (pH 7.0).

Tagmentation and enrichment

6. Perform tagmentation on biotinylated DNA by using Tagmentation DNA Enzyme (Illumina 15027865). For 1,000 cells, 23.5 μL DNA was mixed with 1.5 μL enzyme and 25 μL Tagmentation DNA Buffer (Illumina 15027866). For 5,000 cells, 23 μL DNA was mixed with 2 μL enzyme and 25 μL Tagmentation DNA Buffer. For 10,000 cells, 20 μL DNA was mixed with 5 μL enzyme and 25 μL Tagmentation DNA Buffer.

7. Incubate the tagmentation mixture at 37 ˚C for 30 min with shaking at 500 rpm.

8. Purify DNA from the reaction mixture by DNA Clean & Concentrator kit (Zymo D4043). Elute DNA by using 55 μL 25 mM K_3BO_3 (pH 7.0).

9. Save 5 μL of the tagmented DNA as input. Use the remaining 50 μL for enrichment.

Enrichment of N_3-kethoxal-modified DNA

10. Wash 5 μL Dynabeads Myone Streptavidin C1 (Thermo 65001) 3 times with 25 μL 1× binding and wash buffer (5 mM Tris-HCl pH 7.4, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20). Re-suspend pre-washed beads in 50 μL 2× binding and wash buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 2 M NaCl, 0.1% Tween-20).

11. Mix the beads with 50 μL fragmented DNA from step 9. Incubated the mixture at room temperature for 15 min with gentle rotation. After incubation, place the mixture on a magnetic rack to remove the supernatant and wash beads 5 times with 100 μL 1× binding and wash buffer.

PCR amplification and library sequencing

12. Adjust the volume of input to 20 μL by adding 15 μL nuclease-free water to the 5 μL input saved in step 9. Re-suspend the washed beads in 20 μL nuclease-free water.

13. Prepare the PCR reaction mix as follows:

20 μL input DNA or beads suspension
2.5 μL i5 index primer (Illumina 20027213)
2.5 μL i7 index primer (Illumina 20027213)
25 μL NEBNext Ultra II Q5 Master Mix (NEB M0544S)

14. Perform PCR reaction in a thermocycler as follows:

1 cycle:	*5 min*	*72 ˚C*
	10 min	*95 ˚C*
15 cycles:	*10 sec*	*98 ˚C*
	30 sec	*60 ˚C*
	1 min	*72 ˚C*

15. Remove the beads from the enriched samples by using a magnetic rack. Purify the library by using MinElute PCR purification kit (Qiagen 28804). The library size should range from 200-600 bp.

16. Sequence libraries on Illumina platforms by using single-end mode with 30 million reads per sample.