

Materials List for:

# TChIP-Seq: Cell-Type-Specific Epigenome Profiling

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URL: <https://www.jove.com/video/58298>

DOI: [doi:10.3791/58298](https://doi.org/10.3791/58298)

## Materials

Name	Company	Catalog Number	Comments
Protein LoBind tube, 2 mL	Eppendorf	No. 0030108132	For cell lysis
Protein LoBind tube, 1.5 mL	Eppendorf	No. 0030108116	For ChIP and library preparation
DNA LoBind tube, 1.5 mL	Eppendorf	No. 0030108051	For ChIP and library preparation
8-strip PCR tube	BIO-BIK	3247-00	For ChIP and library preparation
SK Mill	TOKKEN	SK-200	Handy cryogenic grinder to make cell powder for fixation
Metal bullet	TOKKEN	SK-100-DLC10	Accessory of SK Mill
2 mL stainless steel tube	TOKKEN	TK-AM5-SUS	An option for cell lysis
2 mL stainless steel tube holder	TOKKEN	SK-100-TL	An option for cell lysis
16% formaldehyde (w/v), methanol-free	Pierce	28906	To fix cells. Prepare 1% solution before use.
Glycine	Nacalai Tesque	17109-35	Prepare 2.5 M stock
D-PBS (-)(1x)	Nacalai Tesque	14249-24	For washing lysate and purified DNA
HEPES	Nacalai Tesque	02443-05	For Lysis buffer 1. Prepare 1 M, pH 7.5 stock.
5 M NaCl, molecular biology grade	Nacalai Tesque	06900-14	For Lysis buffer 1, Lysis buffer 2, ChIP Elution Buffer, and Tris-EDTA-NaCl Buffer
0.5 M EDTA, molecular biology grade	Wako Pure Chemical Industries, Ltd.	311-90075	For Lysis buffer 1, Lysis buffer 2, ChIP Elution Buffer, and Tris-EDTA-NaCl Buffer
Glycerol	Wako Pure Chemical Industries, Ltd.	072-04945	For lysis buffer 1
NP-40	Nacalai Tesque	25223-75	For lysis buffer 1
Triton X-100, molecular biology grade	Nacalai Tesque	12967-32	For Lysis buffer 1
Tris	Nacalai Tesque	35406-91	For Lysis buffer 2, ChIP Elution Buffer, and Tris-EDTA-NaCl Buffer. Prepare 1 M, pH 8.0 stock.
0.1 M EGTA pH neutral	Nacalai Tesque	08947-35	For Lysis Buffer 2
Protease inhibitor cocktail (100x)	Nacalai Tesque	25955-24	To block degradation of protein
RIPA buffer	Thermo Fisher Scientific	89900	For cell lysis and washing
milliTUBE 1 mL AFA Fiber	Covaris	520130	Sonicator tube. Accessory of Focused-ultrasonicator
Focused-ultrasonicator	Covaris	S220 or E220	To digest DNA into adequate size for ChIP-Seq

UltraPure 10% SDS	Thermo Fisher Scientific	15553-027	For ChIP Elution Buffer
RNase A	Nacalai Tesque	30141-14	To purify DNA from lysate
Proteinase K, recombinant, PCR Grade	Sigma-Aldrich	3115887001	To purify DNA from lysate
Ethanol	Wako Pure Chemical Industries, Ltd.	054-07225	Make 70% solution
Monoclonal anti-FLAG M2 antibody produced in mouse	Sigma-Aldrich	F1804	To purify chromatin expressed in cells of interest
Dynabead M-280 Sheep Anti-Mouse IgG	Thermo Fisher Scientific	11201D	This can be used for anti-FLAG IP and anti-H3K4me3 IP
Anti-tri-methyl histone H3 (K4), mouse monoclonal antibody	Wako Pure Chemical Industries, Ltd.	301-34811	Any other antibody that works for ChIP analysis will work
10x Blocking Reagent	Sigma-Aldrich	11096176001	For blocking during affinity purification
Denhardt's solution	Nacalai Tesque	10727-74	For blocking during affinity purification
Glycogen (5 mg/ml)	Thermo Fisher Scientific	AM9510	To purify DNA from lysate
Qubit 2.0 Fluorometer	Thermo Fisher Scientific	Q32866	For quantification of isolated DNA
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851	For quantification of isolated DNA
0.5 mL tube	Axygen	10011-830	For quantification by Qubit
Phenol/chloroform/isoamyl alcohol (25:24:1)	Nacalai Tesque	25970-56	To purify DNA from lysate
AMPure XP beads	Beckman Coulter	A63881	SPRI magnetic beads for library preparation
Metal ice rack	Funakoshi	IR-1	To keep the cell lysate frozen
Sample Cooler	New England Biolabs	T7771S	Helps fix cells with minimal damage
2100 Bioanalyzer	Agilent Technologies	G2939BA	To check the quality of isolated DNA fragments. Another fragment analyzer can be used.
Bioanalyzer 2100 Expert Software	Agilent Technologies	G2946CA	Supplied with the Bioanalyzer
High Sensitivity DNA Kit	Agilent Technologies	5067-4626	To check the quality of the isolated DNA fragments
KAPA LTP Library Preparation Kit	Roche	07961898001	Supplied with 10x KAPA End Repair Buffer, KAPA End Repair Enzyme Mix, KAPA A-Tailing Buffer, KAPA A-Tailing Enzyme, KAPA Ligation Buffer, KAPA DNA Ligase, and PEG/NaCl solution
NEXTflex DNA Barcodes	BIOO Scientific	NOVA-514101	Adapter for library preparation. Supplied with DNA Barcode Adapters and Primer Mix.
KAPA Real-Time Library Amplification Kit	Roche	07959028001	Supplied with 2x KAPA HiFi HS real-time PCR Master Mix, PCR Primer Mix, and Fluorescent Standards
2x KAPA HiFi HotStart ReadyMix	Roche	KM2602	For library preparation. Additionally, this enzyme may be required for the KAPA Real-Time Library Amplification Kit
Buffer EB	Qiagen	19086	10 mM Tris-Cl, pH 8.5 for elution of DNA
386-well qPCR plate	Thermo Fisher Scientific	4309849	For real-time PCR
QuantStudio 7 Flex Real-Time PCR System	Thermo Fisher Scientific	4485701	To quantify DNA

MicroAmp Optical Adhesive Film	Thermo Fisher Scientific	4311971	For real-time PCR
MicroAmp Clear Adhesive Film	Thermo Fisher Scientific	4306311	For plate sealing
End-repair master mix			Combine 1.4 $\mu$ L of 10x KAPA End Repair Buffer, 1 $\mu$ L of KAPA End Repair Enzyme Mix, and 1.6 $\mu$ L of H <sub>2</sub> O
A-tailing master mix			Combine 1 $\mu$ L of KAPA A-Tailing Buffer, 0.6 $\mu$ L of KAPA A-Tailing Enzyme, and 8.4 $\mu$ L of H <sub>2</sub> O
Ligation buffer mix			Combine 2 $\mu$ L of KAPA ligation buffer and 6 $\mu$ L of H <sub>2</sub> O
Real-time PCR master mix			Combine 5 $\mu$ L of 2x KAPA HiFi HS real-time PCR Master Mix, 0.35 $\mu$ L of PCR Primer Mix (10 $\mu$ M each of forward primer AATGATACGGCGACCACCGAG and reverse primer CAAGCAGAAGACGGCATACGAG) and 3.15 $\mu$ L of H <sub>2</sub> O
PCR master mix			Combine 10 $\mu$ L of 2x KAPA HiFi Ready Mix, 0.9 $\mu$ L of PCR Primer Mix, and 0.6 $\mu$ L of H <sub>2</sub> O
Integrative Genomics Viewer	Broad Institute	IGV_2.3.88	Genome browser to visualize sequencing data
DNA oligonucleotide: 5'-GCCTACGCAGGTCTTGCTGAC-3'	Eurofins Genomics		A primer to amplify the promoter region of GAPDH
DNA oligonucleotide: 5'-CGAGCGCTGACCTTGAGGTC-3'	Eurofins Genomics		A primer to amplify the promoter region of GAPDH
SYBR Premix Ex Taq	Takara	RR420L	To quantify the DNA corresponding to the GAPDH promoter region
Thermal Cycler Dice	Takara	TP870	To quantify the DNA corresponding to the GAPDH promoter region