ACTIVE MOTIF®

Enabling Epigenetics Research

TIP-ChIP™ Assay Kit

Catalog No. 53211

(Version A3)

Revision	Date	Description of Change
A2	January, 2026	Corrected error in PIXUL® sonication parameters on page 13
A3	February, 2026	Corrected Index Primer and Barcode Tables on pages 17, 20, and 21
		Updated step 4 of Cell Sample Fixation Protocol on page 7

Contents

Overview

TIP-ChIP (Tagmented, Indexed, and Pooled ChIP-Seq) is a streamlined cost-effective method for performing ChIP-Seq on up to 96 samples in a single tube reaction, with lower input and reduced variability than traditional Chromatin Immunoprecipitation.

TIP-ChIP is a high-resolution, two-step chromatin immunoprecipitation (ChIP) epigenetic technique designed to precisely map protein–DNA interactions genome-wide from individual samples that are indexed and pooled to enable high-throughput ChIP from 300,000 to 1 million cells per sample.

This method is especially useful to perform ChIP in multiple samples in the same experiment. Because samples in TIP-ChIP are pooled together per target, there are fewer steps – only one immunoprecipitation (IP) per target as opposed to IPs per sample in traditional ChIP.

ChIP-Seq is a well-established technique for mapping protein-DNA interactions and histone modifications genome-wide; however, there are some limitations to ChIP-Seq including the large sample input requirement of 1 to 10 Million cells, time-consuming and cumbersome protocol, difficult sample chromatin preparation, one sample processed per reaction, and batch effects.

TIP-ChIP overcomes many of these limitations by combining samples into one reaction per IP. By pooling samples, 300,000 to 1 million cells are needed per sample, and only 8 samples are needed to pool for one IP. Additionally, TIP-ChIP does not rely on traditional chromatin preparation, and instead utilizes Tn5 transposase to incorporate unique barcodes per sample. This streamlines the assay workflow, improves data quality, and eliminates batch effects.

Product	Format	Catalog No.
TIP-ChIP™ Assay Kit	1 × 96 rxns	53211

Kit Components and Storage

Reagents	Quantity	Storage
TIP-ChIP Assembled Transposomes Plate	1 × 96-well	-20°C
Hypotonic Quench	1.16 mL	4°C
5X TC Tag Buffer	1.10 mL	-20°C
10X PBS	220 µL	4°C
Digitonin (1%)	55 µL	-20°C
10% Tween 20	55 µL	RT
Chromatin Opening Buffer	4.8 mL	RT
SPRI Beads	1.2 mL	4°C
Releasing Buffer	1.2 mL	RT
Protease Inhibitor Cocktail (PIC)	300 µL	-20°C
Tagmentation Stop Solution	475 µL	4°C
5M NaCl	60 µL	RT
Proteinase K	42 µL	-20°C
RNase A	40 µL	-20°C
BSA (10 mg/mL)	195 µL	-20°C
Blocker	195 µL	-20°C
Blocking Reagent AM1	195 µL	-20°C
ChIP Buffer without SDS	10.74 mL	RT
ChIP Buffer	21.6 mL	RT
LiCl Buffer	21.6 mL	RT
Wash Buffer AM1	21.6 mL	RT
TE, pH 8.0	28.9 mL	RT
Elution Buffer AM4	1.2 mL	RT
ChIP Filtration Column	12 columns	RT
TIP-ChIP Column	24 columns	RT
TIP-ChIP DNA Binding Buffer	7.2 mL	RT
TIP-ChIP DNA Wash Buffer	1.92 mL	RT
DNA Purification Elution Buffer	720 µL	RT
Protein A Agarose Beads	1.92 mL	4°C
Q5 Polymerase NGS MM	660 µL	-20°C
AM qPCR Dye	6.6 µL	-20°C
AM i5-001 Primer	31.25 µL	-20°C
AM i5-002 Primer	31.25 µL	-20°C
AM i5-003 Primer	31.25 µL	-20°C

Reagents	Quantity	Storage
AM i5-004 Primer	31.25 µL	-20°C
AM i5-005 Primer	31.25 µL	-20°C
AM i7-001 Primer	31.25 µL	-20°C
AM i7-002 Primer	31.25 µL	-20°C
AM i7-003 Primer	31.25 µL	-20°C
AM i7-004 Primer	31.25 µL	-20°C
AM i7-005 Primer	31.25 µL	-20°C
Stop Solution	20 mL	RT
Diversi-Phi Indexed PhiX	10 µL	-20°C
AM qPCR Standard 1	325 µL	-20°C
AM qPCR Standard 2	325 µL	-20°C
AM qPCR Standard 3	325 µL	-20°C
AM qPCR Standard 4	325 µL	-20°C
AbFlex® Histone H3K27ac Antibody (rAb)	10 µL	-20°C
Histone H3K4me3 Antibody (pAb)	10 µL	-20°C
AbFlex® RNA Pol II CTD Phospho Ser2P Antibody (rAb)	20 µL	-20°C

Additional Materials Required

- Thermoshaker with microplate and PCR tube blocks
- Probe Sonicator
- Microcentrifuge
- Centrifuge
- Incubator
- End-over-end rotator
- 1.5 mL low-bind microcentrifuge tubes
- PCR 8-tube strips
- Single-channel pipettes and appropriate tips (including 200 µL wide-bore pipette tips)
- Multi-channel pipettes and appropriate tips
- PCR plate
- PCR plate seal
- Magnetic plate for PCR tubes
- 100% EtOH
- Vortex
- Thermal cycler with heated lid
- Accessories for VWR® Cooling Thermal-Shake Touch (Cat No. 89232-912) Microplate Thermal Block with Lid

Required Materials for Cell Sample Fixation

- Liquid nitrogen
- 10X Cell Fixation Buffer (See below for 10X Cell Fixation Buffer recipe.)
  - 16% Formaldehyde Solution (*e.g.* Pierce™ Catalog No. 28906)
  - 5 M NaCl (e.g. Millipore Sigma Catalog No. S6546-1L)
  - 250 mM EDTA, pH 8.0 (*e.g.* Millipore Sigma Catalog No. 20-158)
  - 1 M HEPES, pH 7.5 (*e.g.* Thermo Fisher Scientific Catalog No. J60717.AP)
- 1X PBS
- Swing bucket centrifuge
- Corning® 96-well Clear Round Bottom TC-treated Microplate
- Multichannel pipette
- Aluminum sealing foil

Recommended Experimental Setup

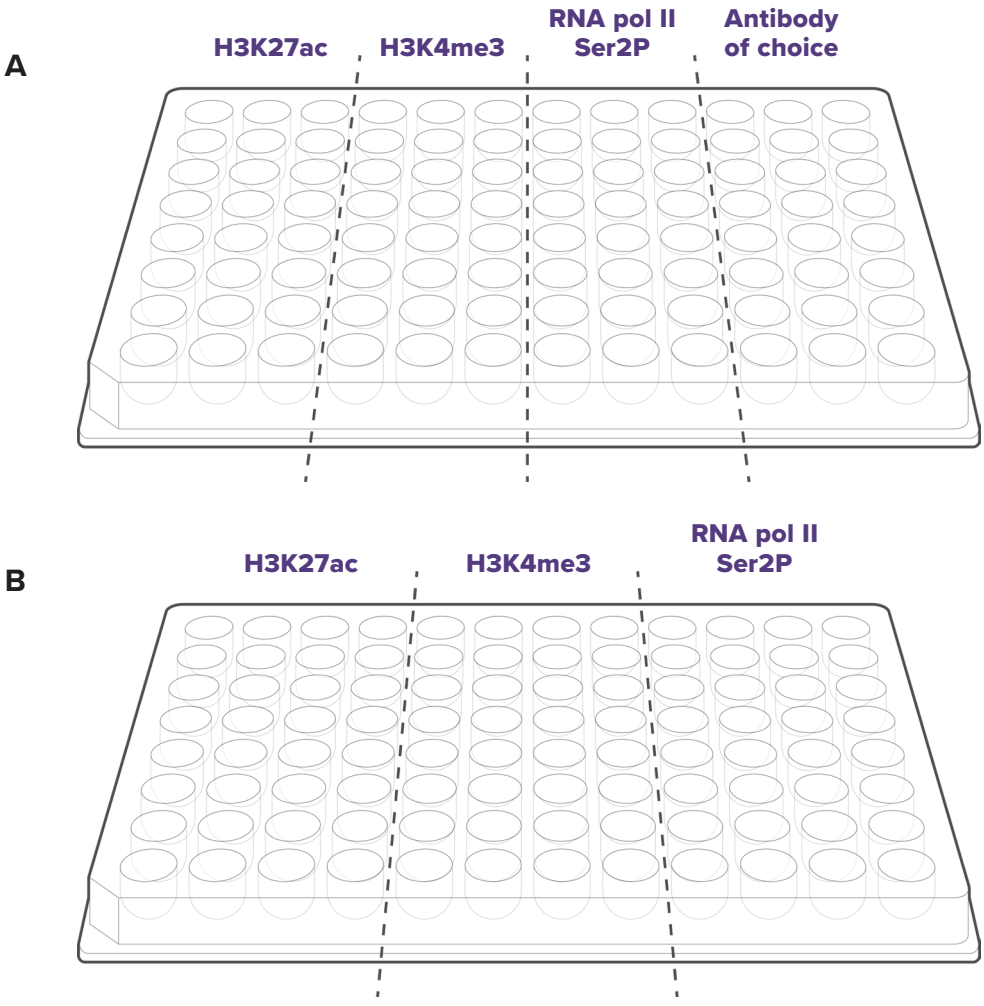
The TIP-ChIP kit is designed to support flexible experimental designs while maximizing the use of the provided antibodies. Each kit includes sufficient antibody for 32 pooled samples per target for the following chromatin marks:

- H3K27ac
- H3K4me3
- RNA Polymerase II Ser2P

To help you get the most value from the kit, we recommend designing your 96-well plate layout around treatment groups in triplicate. This approach increases experimental robustness while ensuring that each antibody provided in the kit can be applied to a meaningful comparison.

Suggested Layouts

With TIP-ChIP there is flexibility in experimental design. For example, plates can be configured to process 24 (layout A) or 32 samples (layout B) per IP for ChIP.



Using Kit Antibodies as Positive Controls

The antibodies provided in this kit are fully validated for TIP-ChIP and may be used as positive controls in cases where an experiment is designed around a user-selected antibody.

Running a kit-supplied antibody alongside an experimental antibody provides a benchmark to confirm that samples were properly fixed and contained sufficient material for the assay, the chromatin was successfully tagmented, and that the overall TIP-ChIP workflow is performing correctly.

Including a positive control antibody provides assurance that the assay is working as expected and helps distinguish antibody-specific performance from issues related to sample preparation or workflow execution.

Optimizing a New Antibody for TIP-ChIP

This kit includes three TIP-ChIP validated antibodies for H3K27ac, H3K4me3, and RNA Pol II Ser2P, but you may also use a ChIP-validated antibody of your choice.

When using a non TIP-ChIP validated antibody, check vendor ChIP validation data. Most suppliers provide an antibody:chromatin ratio (*e.g.*, µg antibody per µg chromatin). Use this as a starting point for the amount of antibody to add into your IP. We typically start with 4 µg antibody per 8 pooled samples.

Note: See **Antibody Validation Guidelines** on **page 23**

Cell Sample Fixation Protocol

For best results Active Motif recommends fixing 300,000 to 1,000,000 cells per well. While less than 300,000 cells will be accepted, there is an increased likelihood of the duplication rate increasing to a point where analysis is not possible. The protocol below is designed for fixing cells in a 96-well round bottom culture plate. A Corning® 96-well Clear Round Bottom TC-treated Microplate (Product No. 3799) is recommended. Cells can either be grown in the plate, or aliquoted into the plate directly before fixation.

	Minimum	Recommended
Histone Modifications	300K	300K-1M
Transcription Factors	1M	1M

Protocol

1. Prepare the **10X Cell Fixation Buffer** using the table below. The formaldehyde should be added to the buffer directly before use.

⚠ Do not prepare more than 4 hours in advance.

10X Cell Fixation Buffer

Below is a recipe for making 1.35 mL of 10X Cell Fixation Buffer, using a convenient 1 mL ampule of Pierce™ 16% Formaldehyde (w/v), Methanol free, Catalog No. 28906. All reagents should be molecular biology grade.

Reagent	Volume	Final Concentration
16% Formaldehyde Solution	928 µL	11%
5 M NaCl	27 µL	100 mM
250 mM EDTA, pH 8.0	5.4 µL	1 mM
1 M HEPES, pH 7.5	67.5 µL	50 mM
Water	322.1 µL	
Total Volume	**1350 µL**	

2. Count cells and check viability. Viability should be at least 80%.
3. Adjust cell concentration so that 100 uL contains the desired amount of cells (3.0×10^6 - 10.0×10^6 cells/mL)

Note: If this is not applicable, ensure that the total volume of each well containing cells is consistent across the plate and does not exceed 110 µL. Adjust with warm or room temperature media if needed.

4. Aliquot 100 µL of cells into each well of a 96-well plate.

For additional help, watch:
Epi Insider: TIP-ChIP Formaldehyde Fixation in a 96-well Plate

5. Add 10 µL (1/10 volume) of the **10X Cell Fixation Buffer** to each well.

⚠ The amount of time the cells are exposed to the fixation buffer is critical, please use a multichannel pipette to carry out this step, and **step 7**, as quickly as possible.

6. Place plate on a nutator at room temperature for 10 minutes.
7. Add 5.5 µL (1/20 volume) **Stop Solution** to each well to stop fixation, pipetting up and down to mix.

⚠ All remaining steps must be performed on ice or in a cold room.

8. Incubate plate on ice for at least 5 minutes.
9. Spin plate in a swing bucket centrifuge at 800 × *g* for 5 minutes at 4°C.
10. Remove supernatant by inverting the plate. For best results, invert the plate quickly and without hesitation to dispose supernatant in one motion.
11. Add 100 µL of cold **1X PBS** to each well. Incubate on ice for 2 minutes.
12. Spin plate in a swing bucket centrifuge at 800 × *g* for 5 minutes at 4°C.
13. Remove supernatant by inverting the plate. For best results, invert the plate quickly and without hesitation to dispose supernatant in one motion.
14. Add 100 µL of cold **1X PBS** to each well.
15. Spin plate in a swing bucket centrifuge at 800 × *g* for 5 minutes at 4°C.

Note: If you have not already, collect liquid nitrogen while plate is spinning.

16. Remove supernatant by inverting the plate. For best results, invert the plate quickly and without hesitation to dispose supernatant in one motion.
17. Seal plate with aluminum sealing foil, we recommend TempPlate® Sealing Foil from USA Scientific.
18. Fill a Styrofoam ice bucket (or any liquid nitrogen safe container) with liquid nitrogen so that the bottom is covered with at least 1 inch of liquid nitrogen.
19. Carefully place plate in liquid nitrogen so that it floats on top for at least 20 seconds. The plate does not need to be completely submerged.
20. Transfer to storage at -80°C until ready to perform protocol.

For additional help, watch:
Epi Insider: TIP-ChIP Formaldehyde Fixation in a 96-well Plate

Experimental Protocol

This protocol uses small reaction volumes and is designed to be performed using a multichannel pipette for consistency and efficiency. To facilitate accurate and rapid pipetting, we recommend pre-aliquoting all small-volume reagents, such as the Hypotonic Quench Solution, Tagmentation Master Mix and Stop Solution, into 0.2 mL PCR tubes or strip tubes prior to beginning the procedure. This setup allows direct multichannel access and reduces pipetting error during time-sensitive steps.

Ensure that you have the correct thermomixer setup for this protocol. A microplate block adapter with a flat bottom is required to properly heat the round-bottom culture plate containing fixed and frozen cells and PCR tube adapter is required for tagmentation. The thermomixer must be capable of reaching 62°C with shaking at 800 rpm.

Additionally, steps requiring shaking at 37°C should be performed using a plate shaker placed inside a 37°C incubator. Prepare both instruments in advance to ensure a smooth and uninterrupted workflow.

Buffer Preparation

Add 12 mL of 100% ethanol to the **TIP-ChIP DNA Wash Buffer** prior to first use.

Day 1 – Tagmentation and Input Generation

Chromatin Opening and Cell Permeabilization

1. Pre-heat a thermomixer with a Microplate Block (flat bottom) to 62°C.
2. Place a plate shaker in a 37°C incubator.
3. Prior to retrieving cells, thaw and add **Protease Inhibitor Cocktail (PIC)** to **Chromatin Opening Buffer**. For every 1 mL of **Chromatin Opening Buffer**, add 10 µL of **PIC**. 60 µL of prepared **Chromatin Opening Buffer** is required per sample, but a minimum volume of 2 mL will need to be prepared for use with a reservoir.
4. Add **Chromatin Opening Buffer** a reservoir.
5. Obtain the 96-well plate containing fixed and frozen pellets from -80°C. Work quickly so cells do not thaw.
6. With a multichannel, add 50 µL of room temperature **Chromatin Opening Buffer** to each well.
7. Seal the plate with a PCR plate seal and place the plate in the 62°C-preheated thermomixer with the Microplate Block (flat bottom), with shaking at 800 rpm for 10 minutes.
8. After 10 minutes, quick spin the plate to collect all the liquid at the bottom, remove the seal and add 12 µL of **Hypotonic Quench**.

It is recommended that the Hypotonic Quench is aliquoted in a 0.2 mL PCR strip tube for easier handling with a multichannel pipette.

9. Re-seal the plate with a new plate seal and place the plate on a plate shaker located in a 37°C incubator. Gently mix the plate for 15 minutes on the lowest setting.

10. During the incubation change the adapter on the thermomixer to a 200 µL PCR tube adapter and pre-heat to 55°C.

11. During the 15-minute incubation, make the Tagmentation Master Mix as follows and keep on ice. For every 8 samples, make 9 samples worth of Tagmentation Master Mix. Once is it prepared, aliquot evenly across 0.2 mL PCR strip tubes such that a multichannel pipette can be used to add it to the plate.

Tagmentation Master Mix

Reagents	Volume
5X TC Tag Buffer	10 µL
10X PBS	1.65 µL
1% Digitonin	0.50 µL
10% Tween 20	0.50 µL
H_2O	24.35 µL
Total Volume	**37 µL**

12. Retrieve the plate from the incubator and centrifuge at 1000 × *g* for 5 minutes at 4°C.

13. During centrifugation, retrieve the **TIP-ChIP Assembled Transposomes Plate** and do a quick spin to collect all the liquid at the bottom. Tear off the number of reactions you are performing. Keep those on ice and return the rest to -20°C.

Note: TIP-ChIP Assembled Transposomes Plate caps might break upon opening. To prevent breakage, cut the desired strip(s) for use with a razor blade and slightly warm up the caps with a gloved hand for easier removal. If the caps break upon tearing, 0.2 mL PCR strip tube caps are compatible for resealing.

14. Following centrifugation, remove seal, then promptly and carefully invert the 96-well plate in one swift, continuous motion over a designated waste to dump the supernatant from all wells simultaneously. Immediately return the plate to the upright position.

15. Using a multichannel pipette, add 37 µL of the Tagmentation Master Mix to each well. Pipette mix and transfer the entire volume to the **TIP-ChIP Assembled Transposomes Plate** containing 13 µL of Tn5 per well.

See **page 21** for plate map.

Note: It is recommended that the Tagmentation Master Mix is aliquoted in a 0.2 mL PCR strip tube for easier handling with a multichannel pipette.

16. Reseal the plate with the caps or PCR strip tube caps, vortex gently to mix and quick spin to pull any liquid to the bottom before incubating.

17. Incubate at 55°C with shaking at 800 rpm for 15 minutes on a thermomixer.

18. After the incubation, quickly spin to bring the liquid to the bottom.
19. With a multichannel, add 4.2 µL of **Tagmentation Stop Solution** to each well and incubate at room temperature for 15 minutes.

Note: It is recommended that the **Tagmentation Stop Solution** is aliquoted in a 0.2 mL PCR strip tube for easier handling with a multichannel pipette.

20. After the incubation, pool a minimum of 8 samples (wells) from the plate into a single tube appropriate for the volume, making sure to gently pipette mix before transferring. All samples in a pool must be profiled with the same antibody, and once pooled, samples cannot be separated or split.
21. Centrifuge the pooled tube at 12,000 × *g* for 3 minutes at 4°C.
22. Gently discard the supernatant making sure not to disturb the pellet.
23. With a single channel pipette, carefully resuspend the pellet in 100 µL of room temperature **Releasing Buffer**, pipetting up and down 30 times to mix.
24. Incubate at room temperature for 30 minutes, gently vortexing the sample every 10 minutes. It is recommended that 25-27 minutes into the 30-minute incubation to begin preparing the probe sonicator (see **Probe Sonication** section below for sonication parameters).
25. During the incubation period prepare your Immunoprecipitation (IP) tubes.

    A. To a new 1.5 mL tube, add 295 µL of cold **ChIP Buffer (without SDS)**.

    B. To the same tube, add 1X PBS. The volume of the 1X PBS will change depending on the amount of antibody used. The volume of the antibody + 1X PBS buffer equals 30 µL.

    C. Leave the tube on ice until chromatin is fully prepared.

Sonication

Choose one of the two options below for sonication.

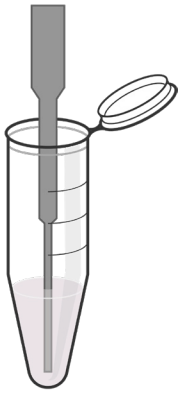
Probe Sonication (Option 1)

After the 30-minute incubation, sonication will be used to release the chromatin from the nuclei. We have validated the following parameters should be used for the Probe Sonicator (Active Motif EpiShear Cat No. 53051; 120-Watt, 20 Hz):

To prevent samples from freezing, place the tubes in a micropipette tip box filled with ice instead of a standard cooling block.

26. After the 30-minute incubation, place the 1.5 mL tube containing the pooled samples on ice in an empty micropipette tip box. If there are any bubbles, remove them prior to sonication.

27. Carefully insert the sonication probe into the tube so that it is fully submerged and positioned at the bottom of the tube. Ensure the probe tip is centered and not touching the sides. This helps minimize foam formation during sonication. To estimate accurate set-up: gently tug at the cap of the 1.5 mL tube. The tube should be positioned snugly; being able to slightly move up and down. It should not be locked into place nor move too much.



28. Sonicate using the following parameters:

1 minute of active sonication

Pulse:
15 seconds ON
30 seconds OFF
Amplification: 20%

3 minutes of total sonication

29. Once sonication is complete, centrifuge the tube at 17,500 × *g* for 15 minutes at 4°C.

30. During the centrifugation, add 5 µL of **PIC** to each IP tube prepared in **step 25**.

31. To the same tube, add the antibody and keep on ice. Select one of the following antibodies per IP.

1.5 µg **AbFlex® Histone H3K27ac Antibody (rAb)**
2 µg **Histone H3K4me3 Antibody (pAb)**
4 µg **AbFlex® RNA Pol II CTD Phospho Ser2P Antibody (rAb)**

32. After centrifugation, carefully transfer 8 µL of the supernatant (~10%) into a new 0.2 mL PCR tube.

33. Transfer the remaining volume (~85 µL) of supernatant to the prepared IP tube containing the antibody, be careful not to disrupt the pellet.

34. Incubate sample on end-over-end rotator at 4°C overnight.

35. To the 0.2 mL PCR tube containing the input from **step 32**, add 1.5 µL of **Proteinase K** and 1.5 µL of **RNase A**.

36. Place your samples in a thermal cycler for an overnight decrosslinking with the volume set to 11 µL and the lid temperature set to 105°C.

PIXUL® Sonication (Option 2, requires PIXUL® Multi-Sample Sonicatior)

Prior to sonication, start circulating PIXUL® to cool it below 15°C.

Transfer 100 µL of your sample into a PIXUL® plate and fill the remaining wells in that column with water. Seal plate and sonicate on the PIXUL® with the following parameters:

2 minutes
Pulse: 50
Burst Rate: 20 Hz
PRF: 1 kHz

Overnight Decrosslinking Parameters:

37°C for 30 minutes
55°C for 4 hours
65°C for 12 hours
65°C hold

⚠ The 65°C hold is so you can continue the assay the following day. This is the end of Day 1. The DNA Purification must be done on the following day, not more than 18 hours later.

Bead Preparation

Note: All centrifugation steps can be performed either at 4°C or room temperature if a chilled centrifuge is not available.

1. Retrieve **Protein A Agarose beads** from 4°C and gently swirl tube or gently pipette mix with a wide bore tip until beads are in solution.
2. Transfer 40 µL of beads per IP into a 1.5 mL tube.

Note: At minimum, 4 reactions worth should be performed so that beads mix properly.

3. Centrifuge the beads at 1,250 × *g* for 3 minutes. Remove supernatant without disturbing beads.
4. To the beads add 120 µL per IP of cold **TE, pH 8.0**.
5. Centrifuge the beads at 1,250 × *g* for 3 minutes. Remove supernatant without disturbing beads.
6. To the beads add:

   A. 32 µL per IP of cold **TE, pH 8.0**
   B. 4 µL per IP of **Blocking Reagent AM1**
   C. 4 µL per IP of **BSA**
   D. 4 µL per IP of **Blocker**

7. Place the tube in an end-over-end rotator at 4°C overnight. Make sure beads are mixing before leaving.

END OF DAY 1

Day 2 – Input purification and ChIP Washes

Input Purification

1. Pre-heat an aliquot of **DNA Purification Elution Buffer** to 37°C, enough for 12.5 µL per sample.
2. Collect de-crosslinked input from thermal cycler.
3. For each sample, add a **TIP-ChIP Column** to a 1.5 mL tube to collect liquid.
4. Directly to the PCR tube containing the input add 5X volume of **TIP-ChIP DNA Binding Buffer** directly (55 µL).
5. Mix and transfer to the **TIP-ChIP Column**.
6. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
7. Add 200 µL of **TIP-ChIP DNA Wash Buffer** (making sure ethanol has been added to the buffer).
8. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
9. Add another 200 µL of **TIP-ChIP DNA Wash Buffer**.
10. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
11. Transfer column to a new 1.5 mL tube and centrifuge at room temperature for 2 minutes at ≥ 10,000 × *g* to remove all residual wash buffer.
12. Transfer column to a new 1.5 mL tube for sample collection.
13. Add 12.5 µL of warmed **DNA Purification Elution Buffer** to column and incubate at room temperature for 1 minute.
14. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
15. Store at 4°C until ready to amplify if amplifying the same day, or ≥ -20°C for long term storage. (-80°C is recommended, if possible)

To increase eluted yield, elution can be performed in two spins, each with 6.25 µL of **DNA Purification Elution Buffer**.

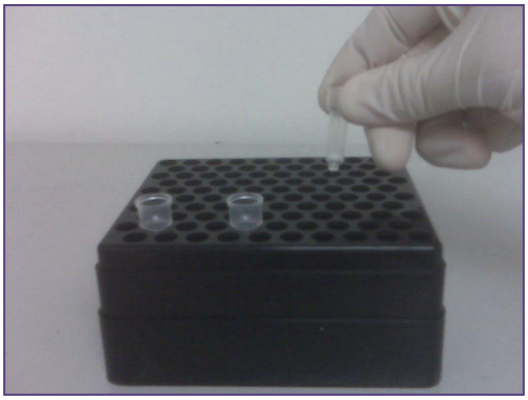
Input Quality Control

1. Take 2 µL of each input and dilute in 8 µL of **DNA Purification Elution Buffer**.
2. Run the diluted input on a Tape Station HSD5000 tape.
3. Look for a peak at ~1000 bp.

ChIP Washes

1. Remove the tube containing the blocked beads and IP tubes from the 4°C incubator and do a quick spin to bring all liquid to bottom of the tube.
2. Using wide bore tips, pipette mix the beads and transfer 40 µL of the beads to each IP.
3. Incubate for 3 hours at 4°C on an end-over-end rotator.

4. Prepare for the washes by setting up one **ChIP Filtration Column** for each sample in a 1 mL pipette box to collect the flow through. Additionally, in a 1.5 mL tube, aliquot 100 µL per sample of the **Elution Buffer AM4** and pre-heat it on a 37°C heat block.



5. Before starting the washes, break off the plug of the **ChIP Filtration Column** so that the liquid can flow through using gravity.
6. After the 3-hour incubation, quickly centrifuge samples, and add 600 µL of the **ChIP Buffer (without SDS)** to each IP sample and transfer entire volume into the **ChIP Filtration Column**.
7. Add 900 µL of **ChIP Buffer** to the column, do not incubate.
8. Add 900 µL of **ChIP Buffer** to the column, incubate for 3 minutes.
9. Add 900 µL of **Wash Buffer AM1**, incubate for 3 minutes.
10. Add 900 µL of **Wash Buffer AM1**, incubate for 3 minutes.
11. Add 900 µL of **LiCl Buffer**, incubate for 3 minutes.
12. Add 900 µL of **LiCl Buffer**, incubate for 3 minutes.
13. Add 900 µL of **TE** Buffer, do not incubate.
14. Add 900 µL of **TE** Buffer, incubate for 3 minutes.
15. Transfer the column into a 1.5 mL microcentrifuge tube. Centrifuge at 1,300 × *g* for 3 minutes to collect any residual wash buffer.
16. Transfer the column to a new 1.5 mL tube for sample collection.
17. Add 50 µL of the pre-warmed **Elution Buffer AM4** and incubate at room temperature for 10 minutes.
18. Centrifuge at 1,300 × *g* for 3 minutes.
19. Add another 50 µL of the pre-warmed **Elution Buffer AM4** for a total of 100 µL and incubate at room temperature for 10 minutes.
20. Centrifuge at 1,300 × *g* for 3 minutes
21. Add 5 µL of **5M NaCl**, 2 µL of **Proteinase K**, and 2 µL of **RNase A** to each sample, pipette mix and transfer the total volume (109 µL) to a new PCR tube.
22. Decrosslink overnight in a thermal cycler with the volume set to 100 µL and the lid temperature set to 105°C.

Decrosslinking Protocol:

A. 37°C for 30 minutes

B. 55°C for 4 hours

C. 65°C for 12 hours

D. 20°C hold

Purification should be performed the following day, no more than 18 hours later.

END OF DAY 2

Day 3 – ChIP DNA Purification and PCR Amplification

IP Purification

1. Pre-heat an aliquot of **DNA Purification Elution Buffer** to 37°C, enough for 12.5 µL per sample.
2. Collect de-crosslinked input from thermal cycler.
3. For each sample, add a **TIP-ChIP Column** to a 1.5 mL tube to collect liquid.
4. Transfer de-crosslinked DNA from PCR tube to a new 1.5 mL tube containing 5X volume of **TIP-ChIP DNA Binding Buffer** (545 µL).
5. Mix and transfer to spin column.
6. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
7. Add 200 µL of **TIP-ChIP DNA Wash Buffer** (making sure ethanol has been added to the buffer).
8. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
9. Add another 200 µL of **TIP-ChIP DNA Wash Buffer**.
10. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
11. Transfer column to a new 1.5 mL tube and centrifuge at room temperature for 2 minutes at ≥ 10,000 × *g* to remove all residual wash buffer.
12. Transfer column to a new 1.5 mL tube for sample collection.
13. Add 12.5 µL of warmed **DNA Purification Elution Buffer** to column and incubate at room temperature for 1 minute.
14. Centrifuge at room temperature for 30 seconds at ≥ 10,000 × *g* (max speed).
15. Store at 4°C until ready to amplify if amplifying the same day, or ≥ -20°C for long term storage. (-80°C is recommended, if possible)

To increase eluted yield, elution can be performed in two spins, each with 6.25 µL of **DNA Purification Elution Buffer**.

PCR Amplification

TIP-ChIP libraries can be amplified using either quantitative PCR (qPCR) to monitor amplification in real time or end-point PCR if qPCR instrumentation is not available. The same PCR master mix is used for both approaches.

If performing qPCR, add **AM qPCR Dye** at a 1:100 dilution to each reaction. Standards are provided to assist in determining the optimal number of cycles. If performing end-point PCR, simply omit the **AM qPCR Dye**, and use the same amplification cycling conditions.

Determining PCR Cycle Number

The amount of material in both input and IP samples will vary depending on the number of pooled samples and the number of cells per sample. Input samples typically contain excess material, so we recommend either diluting the input or amplifying only a portion. The entire volume of the IP should be used.

As a general guideline:

- Input samples usually require 3-8 PCR cycles
- IP samples typically require 10-15 PCR cycles

The ideal amplification range corresponds to signal levels between the 3rd and 4th standard on the qPCR curve. However, amplification beyond the 4th standard is acceptable if the reaction remains in the exponential phase and does not exhibit signs of plateauing.

1. Thaw **AM qPCR Dye**, **AM qPCR Standards**, and **AM Primers**, one i5 and one i7 for each sample to be amplified.

⚠ If samples are to be sequenced together, ensure each sample has a unique i5/i7 combination.

	MiSeq, HiSeq 2000/2500, NovaSeq v1.0 Reagent Kits	iSeq, MiniSeq, NextSeq, HiSeq 3000/4000, NovaSeq v1.5 Reagent Kits
Index Primer	**Sample Sheet**	**Sample Sheet**
AM i5-001 Primer	AGTTGAAT	ATTCAACT
AM i5-002 Primer	ACCGGCCA	TGGCCGGT
AM i5-003 Primer	CTGAACCG	CGGTTCAG
AM i5-004 Primer	GCGTGCTC	GAGCACGC
AM i5-005 Primer	ATACCGTT	AACGGTAT
AM i7-001 Primer	GCAGTCTT	GCAGTCTT
AM i7-002 Primer	TGATCAGT	TGATCAGT
AM i7-003 Primer	GTCGGCAC	GTCGGCAC
AM i7-004 Primer	TGGAAGAG	TGGAAGAG
AM i7-005 Primer	CGGAAGGT	CGGAAGGT

2. Prepare PCR master mix by transferring 27.5 µL per sample of **Q5 Polymerase NGS MM** to a 1.5 mL tube and adding 0.275 µL per sample of SYBR dye (1:100).

3. Aliquot 25 µL of the master mix into a walled PCR plate, one well per sample.
4. Add 6.25 µL of 10 µM i5 primer to each well.
5. Add 6.25 µL of 10 µM i7 primer to each well.
6. Add purified DNA.
7. Add enough water to bring the total reaction volume to 50 µL.
8. Add 25 µL of each qPCR standard to a different row of the walled PCR plate.
9. Seal plate, briefly mix on plate mixer and quick spin to collect liquid to bottom of the plate.
10. Perform qPCR with the following program. Once completed, transfer the samples to new PCR strip tubes for purification.

    72°C for 5 minutes
    98°C for 60 seconds
    98°C for 10 seconds
    65°C for 30 seconds
    72°C for 60 seconds
    – Cycle (see **Determining PCR Cycle Number** on **page 17**)

Library Purification

1. During the PCR amplification, take the **SPRI beads** out of the fridge and let them sit at room temperature.
2. Once PCR reaction is complete, fully resuspend **SPRI beads** by vortexing or pipette mixing. Use immediately after resuspension to avoid beads settling at the bottom of the tube.
3. Perform double-sided **SPRI bead** clean-up with 25 µL bead solution (0.5X sample volume) for right side clean up and 25 µL (1X sample volume) for left side clean up as follows:

    A. Add 25 µL (0.5 volumes) of **SPRI beads** to each tube containing PCR product.
    B. Vortex the tubes briefly to mix.
    C. Incubate the samples at room temperature for 2 minutes.
    D. Apply magnet to sample tubes and wait for bead pellets to form.
    E. Keeping the tubes on the magnet, carefully transfer the supernatant to new PCR tubes, taking care to avoid the bead pellets.
    F. Add 25 µL of **SPRI beads** to each tube containing supernatant.
    G. Vortex the tubes briefly to mix.
    H. Incubate the samples at room temperature for 5 minutes.
    I. Apply magnet to sample tubes and wait for bead pellets to form. Aspirate and discard the supernatant once the beads have all collected to one side.
    J. Keeping the tubes on the magnet and with the magnetic beads still pelleted, add 180 µL fresh 80% EtOH to each well, taking care not to disturb the pellet. Incubate for 30 seconds, aspirate, and repeat with another 180 µL EtOH.

K. Aspirate supernatant completely and air dry the beads until beads are no longer shiny (about 30 seconds).

L. Remove tubes from the magnet and elute DNA by adding in 17.5 µL of **DNA Purification Elution Buffer** to each well.

M. Vortex tubes briefly and incubate for 5 minutes at room temperature.

N. Place the PCR tube strips on the magnet and wait for bead pellet to form.

O. With the tubes still on the magnet, transfer 16.5 µL of eluate to fresh, labeled PCR tubes.

P. TIP-ChIP libraries can be stored at -20°C long-term.

At this stage, libraries are ready for quantification and sequencing. For TIP-ChIP libraries, use the following sequencing parameters:

Index 1: 8 bp
Index 2: 8 bp
Read 1: 75 bp
Read 2: 75 bp

See **Sample Sheet Information** on **page 20** and **TIP-ChIP Sequencing Guildlines** on **page 22**

After adapter trimming, this setup yields paired-end 50 bp reads, which are ideal for downstream TIP-ChIP analysis.

TIP-ChIP libraries are typically low-complexity and should be sequenced with caution to avoid issues with base calling. If sequencing alone (not multiplexed with other diverse libraries), include at least 20% Diversi-Phi Indexed PhiX to increase sequence diversity. For convenience, Active Motif's Diversi-Phi has been included in your kit and can be added directly to your final sequencing library. For detailed instructions on how to add Diversi-Phi, please refer to the product page: **activemotif.com/catalog/1397/diversi-phi-indexed-phix**

Sample Sheet Information

i5 Primer

5´- AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3´

i7 Primer

5´- CAAGCAGAAGACGGCATACGAGAT[i7]GTGACTGGAGTTCAGACGTGTGCTCTTCCGA*T-3´

	MiSeq, HiSeq 2000/2500, NovaSeq v1.0 Reagent Kits	iSeq, MiniSeq, NextSeq, HiSeq 3000/4000, NovaSeq v1.5 Reagent Kits
Index Primer	**Sample Sheet**	**Sample Sheet**
AM i5-001 Primer	AGTTGAAT	ATTCAACT
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AM i5-003 Primer	CTGAACCG	CGGTTCAG
AM i5-004 Primer	GCGTGCTC	GAGCACGC
AM i5-005 Primer	ATACCGTT	AACGGTAT
AM i7-001 Primer	GCAGTCTT	GCAGTCTT
AM i7-002 Primer	TGATCAGT	TGATCAGT
AM i7-003 Primer	GTCGGCAC	GTCGGCAC
AM i7-004 Primer	TGGAAGAG	TGGAAGAG
AM i7-005 Primer	CGGAAGGT	CGGAAGGT

TIP-ChIP Assembled Transposomes Plate Map

Visit activemotif.com/catalog/1402/tip-chip-kit#documents for the TIP-ChIP™ Plate Map with Barcodes.

Well		1	2	3	4	5	6	7	8	9	10	11	12
	Index	5i1	5i2	5i3	5i4	5i5	5i6	5i7	5i8	5i9	5i10	5i11	5i12
A	7i1	7i1, 5i1	7i1, 5i2	7i1, 5i3	7i1, 5i4	7i1, 5i5	7i1, 5i6	7i1, 5i7	7i1, 5i8	7i1, 5i9	7i1, 5i10	7i1, 5i11	7i1, 5i12
B	7i2	7i2, 5i1	7i2, 5i2	7i2, 5i3	7i2, 5i4	7i2, 5i5	7i2, 5i6	7i2, 5i7	7i2, 5i8	7i2, 5i9	7i2, 5i10	7i2, 5i11	7i2, 5i12
C	7i3	7i3, 5i1	7i3, 5i2	7i3, 5i3	7i3, 5i4	7i3, 5i5	7i3, 5i6	7i3, 5i7	7i3, 5i8	7i3, 5i9	7i3, 5i10	7i3, 5i11	7i3, 5i12
D	7i4	7i4, 5i1	7i4, 5i2	7i4, 5i3	7i4, 5i4	7i4, 5i5	7i4, 5i6	7i4, 5i7	7i4, 5i8	7i4, 5i9	7i4, 5i10	7i4, 5i11	7i4, 5i12
E	7i5	7i5, 5i1	7i5, 5i2	7i5, 5i3	7i5, 5i4	7i5, 5i5	7i5, 5i6	7i5, 5i7	7i5, 5i8	7i5, 5i9	7i5, 5i10	7i5, 5i11	7i5, 5i12
F	7i6	7i6, 5i1	7i6, 5i2	7i6, 5i3	7i6, 5i4	7i6, 5i5	7i6, 5i6	7i6, 5i7	7i6, 5i8	7i6, 5i9	7i6, 5i10	7i6, 5i11	7i6, 5i12
G	7i7	7i7, 5i1	7i7, 5i2	7i7, 5i3	7i7, 5i4	7i7, 5i5	7i7, 5i6	7i7, 5i7	7i7, 5i8	7i7, 5i9	7i7, 5i10	7i7, 5i11	7i7, 5i12
H	7i8	7i8, 5i1	7i8, 5i2	7i8, 5i3	7i8, 5i4	7i8, 5i5	7i8, 5i6	7i8, 5i7	7i8, 5i8	7i8, 5i9	7i8, 5i10	7i8, 5i11	7i8, 5i12

Read 1 barcodes	Sample Sheet	Read 2 barcodes	Sample Sheet
5i1	CGTGAT	7i1	ACATCG
5i2	GCCTAA	7i2	CACTGT
5i3	TGGTCA	7i3	ATTGGC
5i4	GATCTG	7i4	ATCAGT
5i5	TCAAGT	7i5	GCGGAC
5i6	CTGATC	7i6	TTTCAC
5i7	AAGCTA	7i7	GGCCAC
5i8	GTAGCC	7i8	CGAAAC
5i9	TACAAG		
5i10	TTGACT		
5i11	GGAACT		
5i12	TGACAT		

TIP-ChIP Sequencing Guildlines

TIP-ChIP libraries are generated by pooling multiple barcoded samples prior to amplification. Because barcoding and pooling occur upstream, individual samples within a library cannot be balanced against each other. Instead, each amplified library (containing many barcoded samples) should be balanced against other libraries based on final DNA concentration before sequencing.

These guidelines are optimized for the Illumina sequencing platform, and we recommend targeting 20–30 million reads per sample to achieve robust coverage.

Sequencing Configuration:

Index 1: 8 cycles
Index 2: 8 cycles
Read 1: 75 cycles*
Read 2: 75 cycles*

*Read 1 and Read 2 may be extended to 76 cycles if the sequencing platform uses a dark cycle for the first base.

Barcode Structure and Trimming Requirements:

The sample barcodes are located in the first 6 bases of both Read 1 and Read 2. Following the barcodes, the next 19 bases correspond to the ME sequence. These regions must be removed before alignment. The first 25 bases of each read should be trimmed prior to downstream analysis.

PhiX Control Requirement:

Because TIP-ChIP libraries are relatively low complexity, sequencing runs should include 20% Diversi-Phi Indexed PhiX Spike-In. Detailed instructions for incorporating Diversi-Phi Indexed PhiX into libraries can be found in the Diversi-Phi Indexed PhiX manual.

Antibody Validation Guidelines

Validating an antibody for TIP-ChIP does not require using a minimum of 8 samples. Instead, 1 sample of 1M cells can be used. In this case, the tagmentation and barcoding of the sample will take place in a 1.5 mL tube rather than a plate. Fixation of the sample also occurs in a 1.5 mL tube rather than a plate.

Only steps 1-18 differ from the plate-base, mutli-sample protocol

Day 1 – Tagmentation and Input Generation

Chromatin opening and cell permeabilization

1. Pre-heat a thermomixer with a 1.5 mL block to 62°C.
2. Place an end-over-end rotator in a 37°C incubator.
3. Prior to retrieving cells, thaw and add **Protease Inhibitor Cocktail (PIC)** to **Chromatin Opening Buffer**. For every 1 mL of **Chromatin Opening Buffer**, add 10 µL of **PIC**.
4. Obtain the 1.5 mL tube containing the fixed and frozen pellet from -80°C and add 50 µL of **Chromatin Opening Buffer** to the tube.
5. Place the plate in the 62°C-preheated thermomixer with the 1.5 mL block, with shaking at 800 rpm for 10 minutes.
6. After 10 minutes, quick spin the tube to collect all the liquid at the bottom and add 12 µL of **Hypotonic Quench**.
7. Place the tube on an end over end rotator located in a 37°C incubator. Rotate for 15 minutes on the lowest setting.
8. During the incubation change the adapter on the thermomixer to a 200 µL PCR tube adapter and pre-heat to 55°C.
9. Prepare the Tagmentation Master Mix, as follows, and keep on ice.

Tagmentation Master Mix

Reagents	Volume
5X TC Tag Buffer	10 µL
10X PBS	1.65 µL
1% Digitonin	0.50 µL
10% Tween 20	0.50 µL
H_2O	24.35 µL
Total Volume	**37 µL**

10. Retrieve the tube from the incubator and spin at 1000 × *g* for 5 minutes at 4°C.
11. During centrifugation, retrieve the **TIP-ChIP Assembled Transposomes Plate** and do a quick spin to collect all the liquid at the bottom. Tear off the number of reactions you are performing. Keep those on ice and return the rest to -20°C.

Note: Tn5 strip-plate caps might break upon opening. To prevent breakage, cut the desired strip(s) for use with a razor blade and slightly warm up the caps with a gloved hand for easier removal. If the caps break upon tearing, 0.2 mL PCR strip tube caps are compatible for resealing.

12. Following centrifugation, carefully remove and discard the supernatant.
13. Add 37 µL of the Tagmentation Master Mix to each well. Pipette mix and transfer the entire volume to the **TIP-ChIP Assembled Transposomes Plate** containing 13 µL of Tn5 per well.
14. Vortex gently to mix and quick spin to pull any liquid to the bottom before incubating.
15. Incubate at 55°C with shaking at 800 rpm for 15 minutes on a thermomixer.
16. After the incubation, quickly spin to bring the liquid to the bottom.
17. Add 4.2 µL of **Stop Solution** and incubate at room temperature for 15 minutes.
18. Transfer the sample into a new 1.5 mL tube and continue with the protocol from step 21 of day 1 of the multi-sample protocol.

Options for Input Normalization

In TIP-ChIP samples are barcoded and then pooled, 90% of the sample is used for ChIP and 10% is used as input for normalization. Therefore input always exists as a barcoded pool.

TIP-ChIP, like traditional ChIP-Seq, uses input for normalization. However, in TIP-ChIP, input can be used in multiple ways to offer advantages, depending on the experimental set up and researcher needs. Options include:

1. No input normalization. Since TIP-ChIP has low background and uses pooled samples (meaning all samples are subjected to the exact same manipulations) technical variation is nearly nonexistent, resulting in clean, interpretable data without input normalization.
2. Normalization for peak calling (as in traditional ChIP-Seq), of which there are three options.
    - **A.** One input pool is used for every sample in the experiment.
    - **B.** Input pools for each sample group (*e.g.* if using multiple cell lines/types in the same experiment, pooled cell-type specific inputs are generated for normalization of the group of samples of each cell type)
    - **C.** Matched input for each sample.
3. Input derived scaling factors for ChIP-Seq normalization. This function replaces the need for spike-in normalization. If each sample does not have the same number of starting cells or if there are global differences in histone PTMs or protein binding, normalization using the input scaling factor will reveal the true biological differences.

Each of these scenarios will be described in more detail below.

No input normalization

Traditional ChIP-Seq has inherently high background. Peak calling algorithms like MACS use input to model the background signal and remove false peaks caused by genomic duplications, sonication or sequencing biases, ensuring that only true antibody-specific enrichment is called as a peak. TIP-ChIP background is low because non-specific chromatin pull down is spread equally across all samples. As background decreases the need for input-based normalization decreases. The lack of input normalization in CUT&RUN and CUT&Tag is based on the same principle.

Normalization for peak calling

Option A: One input pool

In TIP-ChIP inputs are generated from barcoded samples then pooled. After sequencing the pool, sample-specific inputs can be generated by using barcodes to demultiplex the samples. When using one input pool for all samples, demultiplexing is not necessary. The entire input pool is used as an input for each sample.

- Use when all samples in the pool are derived from the same cell line or same tissue type.
- Simplifies data analysis.
- This is the most cost-effective use of input. Sequencing depth for the entire pool is 10-20 million reads.

Option B: Input pools for sample groups

When an experiment includes multiple different cell line or different tissue types and you are doing replicates or have multiple conditions or treatments, it is best practice to use an input that is representative of the sample. As described above all of your barcoded input samples will be pooled in TIP-ChIP. In this case you will use the barcodes to demultiplex. Then you will bioinformatically pool all the inputs from a given cell type into a single BAM file and all the inputs from a different cell type into another BAM file. These cell type-specific input files will be used for peak calling only for the samples of the corresponding cell type.

- Use when your samples are derived from different cell lines or tissue types.
- Sequence to a depth that gives 10-20 million reads per input pool.
- Ensures appropriate removal of cell type specific duplications or cell type specific sample prep biases.

Option C: Matched input for each sample

When an experiment includes multiple primary samples (*e.g.* 96 different human PBMCs) it may be appropriate to use a separate input for each sample. In this case all barcoded input samples are pooled, sequenced and demultiplexed so that input can be used in peak calling for each individual sample.

- Use when samples are biologically distinct
- Sequence to a depth of 10-20 million reads per sample. For example, if there are 48 samples then sequence the input pool to a depth of 480 to 960 million.

Input derived scaling factors for ChIP-Seq normalization

In TIP-ChIP, all barcoded samples undergo the same immunoprecipitation and library preparation steps, minimizing technical variability across samples. Some variation may still occur due to differences in cell numbers resulting from inaccurate cell counting or quality of the starting material. TIP-ChIP uses sequencing of the pooled input to calculate a normalization factor for each sample. This factor can be applied to scale ChIP-Seq data, compensating for differences in input amount.

Additionally, like conventional ChIP, TIP-ChIP does not inherently detect global changes in histone modification levels, such as the reduction of H3K27me3 following EZH2 inhibitor treatment. While standard ChIP-Seq methods address this with spike-in controls, TIP-ChIP instead uses sequencing of the pooled input to calculate a normalization factor. This factor can be applied to scale the ChIP-Seq data and reveal global changes.

$$\textbf{Normalization factor} = \frac{\textbf{ChIP reads for sample}}{\textbf{Input reads for sample}}$$

- Use to achieve quantitative ChIP-Seq results.
- Input for each sample is derived from demultiplexing the input pool.
- The Input encodes the starting mixing ratio of each barcode, acting as a built-in reference for each sample.
- Sequence to a depth of 10-20 million reads per sample. For example, if there are 48 samples then sequence the input pool to a depth of 480 to 960 million.
- No spike-in normalization required, the Input alone corrects for uneven starting amounts and technical variation.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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