

Efficient high throughput scNMT profiling with the VIAFLO 384

Introduction

Single-cell nucleosome occupancy, methylome and transcriptome sequencing (scNMT-seq) is an innovative, high throughput technology offering simultaneous insights into nucleosome occupancy, the methylome and the transcriptome at a single-cell level. This multi-omics approach enables the user to understand both the genetic state of cells and the molecular mechanisms underlying differentiation, transcriptional regulation and disease.

In 2022, Cerrizuela *et al.* published an optimized protocol for high throughput, cost effective and scalable scNMT-seq. This article provides a comprehensive overview of the scNMT-seq library preparation workflow, highlighting the use of the VIAFLO 384 handheld electronic pipette for enhancing throughput, precision and reproducibility.

Key benefits:

- **High throughput:** The VIAFLO 384 enables users to pipette 384 samples simultaneously. This significantly increases speed, precision and reproducibility.
- **Reagent efficiency:** The VIAFLO 384 enables pipetting volumes from 0.5 µl to 1250 µl to allow miniaturized scNMT-seq protocols. This ensures minimal reagent consumption to reduce overall experimental costs.
- **Versatility:** The VIAFLO 384 uses interchangeable heads that can be easily swapped by the user to provide 384, 96 and 24 channel pipetting across various volume ranges, supporting a wide range of tasks across many plate formats.
- **Magnetic module:** The MAG module's vertical magnet movement enables magnetic bead handling at the press of a button to avoid unnecessary manual interventions.
- **Magnetic beads:** INTEGRA offers MAGFLO™ magnetic beads for NGS library preparation or PCR purification, reducing processing costs, while increasing the reproducibility and quality of DNA purification and size selection.
- **All-in-one solution:** INTEGRA's user-friendly set-ups enable efficient liquid and magnetic bead handling on a single platform.

Overview: How INTEGRA Biosciences' 384 channel electronic pipette aids in high throughput scNMT profiling



This application note showcases the protocol for scNMT-seq library preparation using the VIAFLO 384, following the publication of Cerrizuela *et al.*

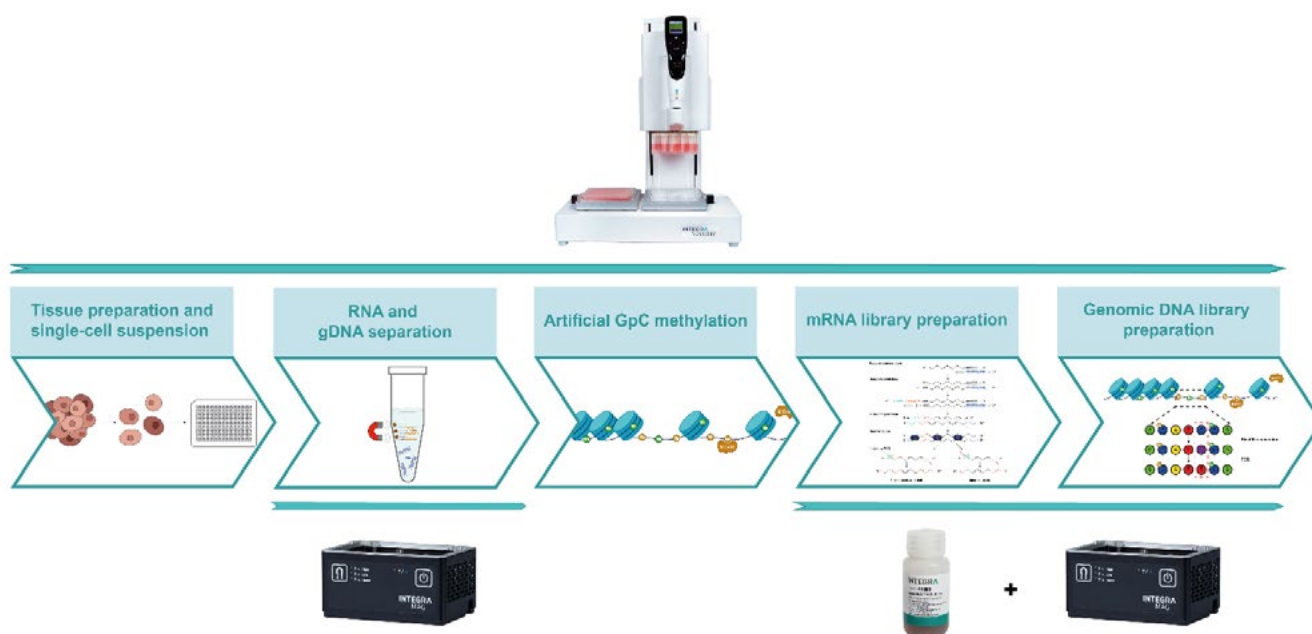


Figure 1: Schematic representation of the scNMT-seq library preparation workflow. The top row shows the use of the VIAFLO 384 and MAG module for each step of the workflow. The center row is a schematic representation of each step. The bottom row shows which steps use magnetic beads, such as MAGFLO NGS beads.

Step-by-step procedure

This scNMT-seq workflow for up to 384 samples consists of the following steps:

- 1) Tissue preparation and single cell suspension
- 2) RNA and gDNA separation
- 3) Artificial GpC methylation
- 4) mRNA library preparation, following the SMART-seq3 protocol
- 5) Genomic DNA library preparation

The scNMT-seq protocol takes advantage of high throughput liquid handling devices, such as the INTEGRA VIAFLO 384 and MAG modules. For detailed information about volumes and incubation times, please refer to the reference publication.

1. Tissue preparation and single cell suspension

Objective: Isolate single cells from tissue or cell culture.

When isolating single cells, different parameters must be considered depending on the users' starting material. Factors like extracellular matrix composition or cell-cell junctions play important roles in the structural integrity of tissues and 3D cell structures, and must be disrupted to allow proper cell dissociation. Different starting material can be processed with different commercially available kits. INTEGRA offers liquid handling solutions for these kits, such as the lightweight, single channel VIAFLO electronic pipettes for various volumes ranging from 0.5-5,000 μ l.

Once single cells are in suspension, techniques such as FACS can be used to sort living cells into 384 well plates.

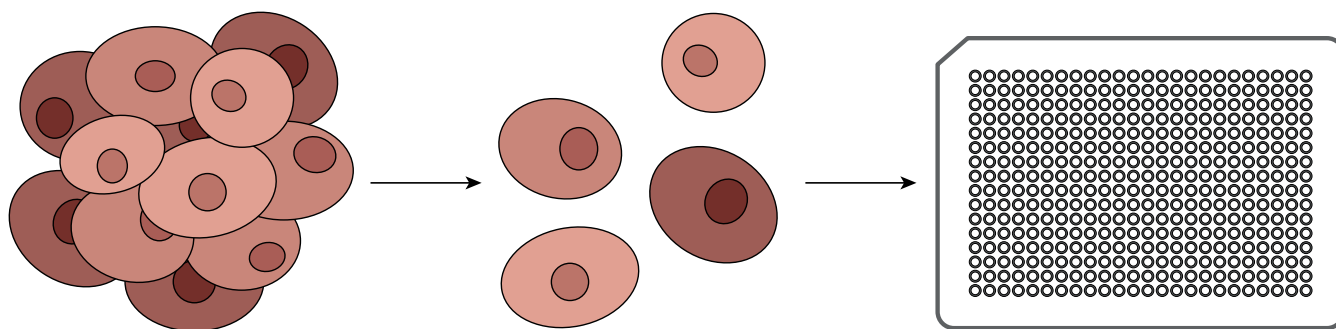


Figure 2: Schematic representation of tissue preparation and single cell suspension, showing a group of cells being dissociated and sorted into a 384 well plate.

2. mRNA and gDNA separation

Objective: Isolate mRNA and genomic DNA.

mRNA must be separated from gDNA to analyze the transcriptome of sample cells. mRNA has a poly-(A) tail, which can be captured using complementary oligo(dT)-coated magnetic beads with minimal gDNA contamination. For this, magnetic beads coated in streptavidin are incubated with biotinylated oligo(dT). This procedure consists of multiple incubations and washes, which require capture and release of magnetic beads. The prepared beads are then added to each sample to immobilize the mRNA and separate it from the gDNA in the supernatant. INTEGRA's MAG module initiates bead collection and release using vertical magnet movements at the push of a button. This strong magnetic module is compatible with 384 well PCR plates and the VIAFLO 384, simplifying magnetic bead separation compared to manual processing.

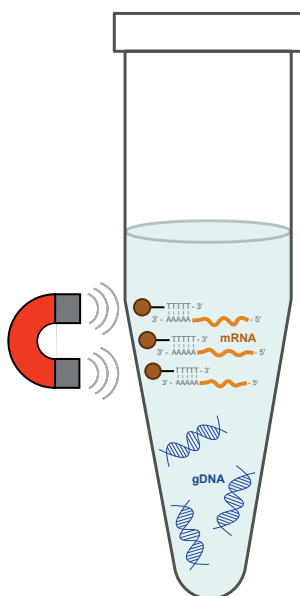


Figure 3: Schematic representation of mRNA and gDNA separation. mRNA (orange) is captured using magnetic beads (brown) coated with oligo-T sequences. These beads are captured with a magnetic field, and separated from the aqueous solution containing the gDNA (dark blue).

3. Artificial GpC methylation

Objective: GpC methylation to assess chromatin accessibility.

Investigating nucleosome occupancy without interfering with endogenous DNA methylation relies on introducing artificial DNA methylation at GpC sites using GpC methyltransferase (M.CviPI). This DNA modification is distinct from the naturally occurring CpG DNA methylation in mammals, and is only introduced to free GpC sites that are not bound by nucleosomes.

The VIAFLO 384 equipped with a 0.5-12.5 μ l, 384 channel head can be used to enable fast, precise and simultaneous transfer of 1 μ l of GpC methyltransferase master mix into 384 wells containing samples. This ensures that all reactions are started at the same time, and limits technical variability between reactions. After desulfonation of the DNA, methylated regions can be identified, revealing endogenous DNA methylation at CpG sites and nucleosome-free regions at GpC sites. This elegant approach results in the simultaneous identification of DNA methylation and nucleosome occupancy from single cells.

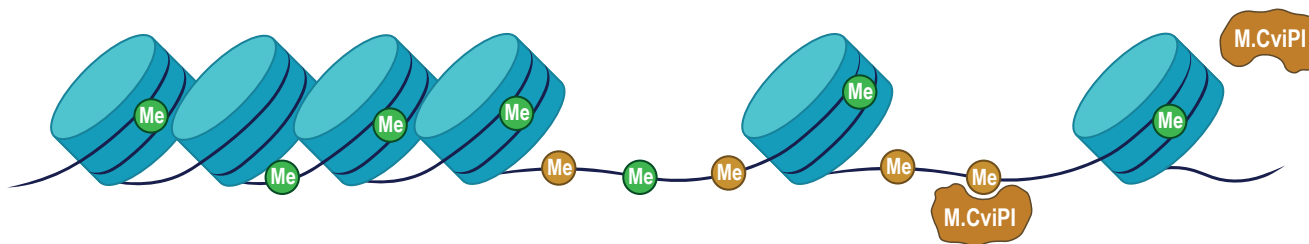


Figure 4: Schematic representation of artificial GpC methylation. DNA wrapped around histones (blue) is naturally methylated at CpG sites (green). Methylation at the GpC sites (orange circle) is introduced using the M.CviPI enzyme (orange shape) that can only access free DNA not wrapped around histones.

4. mRNA library preparation

Objective: Generate an mRNA library for sequencing using the SMART-seq3 protocol.

To analyze a transcriptome, mRNA can be reverse transcribed (RT) into cDNA, amplified via PCR, and purified using a VIAFLO 384 equipped with a 0.5-12.5 µl, 384 channel head and solid-phase reversible immobilization (SPRI) magnetic beads. INTEGRA's MAG module and MAGFLO magnetic beads offer efficient DNA size selection and PCR clean-up. The purified cDNA can then be normalized using INTEGRA's ASSIST PLUS pipetting robot, a versatile, automated liquid handler. For more information, please see automated DNA normalization for NGS library prep.

The next steps follow the library preparation according to the SMART-seq3 protocol. This takes advantage of the tagmentation technology, which enables simultaneous fragmentation and tagging of cDNA. This can be performed with the VIAFLO 384 to ensure reactions start simultaneously. SDS (<0.4 µl) should be performed with a low volume liquid dispenser, followed by indexing PCR and purification using magnetic beads. INTEGRA's MAGFLO NGS magnetic beads can be used for precise and adjustable size selection. They provide equivalent results to gold standard beads, while maintaining set ratios, such as the bead to sample ratios of x0.75, x0.65 and x1.0 used in the SMART-seq3 protocol. To further simplify the workflow, the VIAFLO 384 can be combined with the MAG module. Finally, the libraries are normalized and pooled before sequencing.

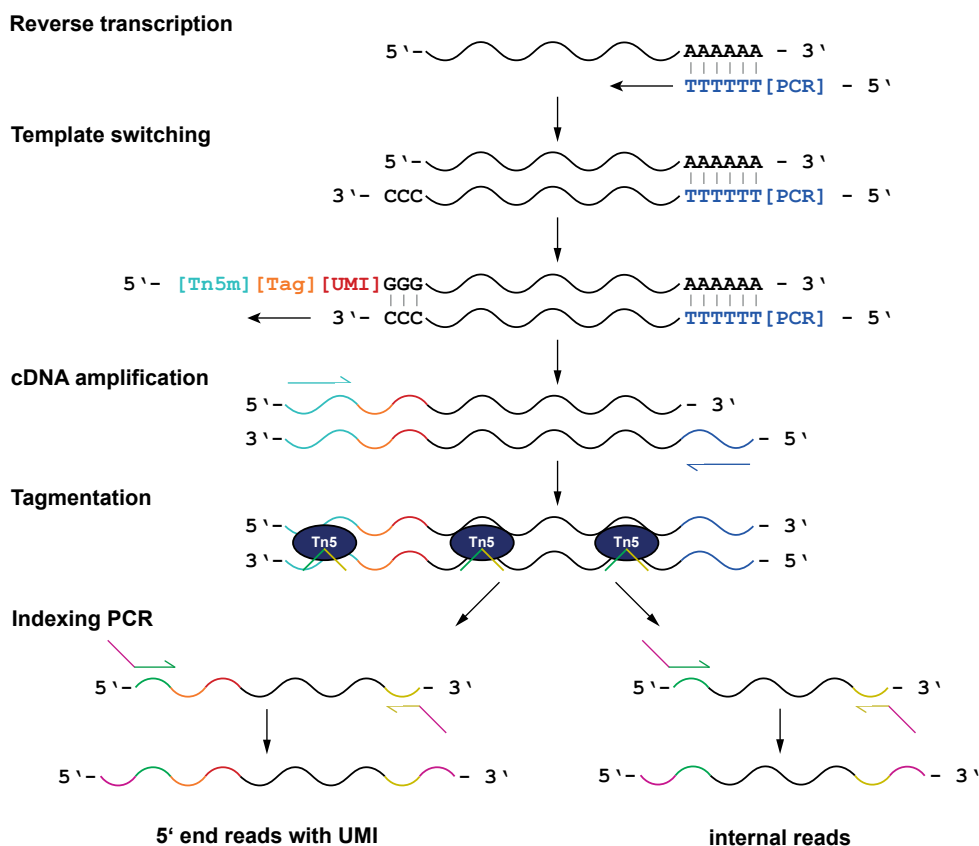


Figure 5: A step-by-step representation of mRNA library preparation using the SMART-seq3 protocol.

5. Genomic DNA library preparation

Objective: Prepare DNA for methylation analysis.

This step performs bisulfite sequencing on the artificially and naturally methylated gDNA. Artificial methylation at GpC sites will reveal nucleosome-free gDNA, while the natural methylation at CpG sites carries valuable information on the cell's epigenetic state. During this section of the protocol, most pipetting steps fall in the 3-33.3 μ l range. Therefore, a VIAFLO 384 equipped with a 2-50 μ l, 384 channel pipetting head can be used in combination with a MAG module. A few steps require volumes below 0.5 μ l, which can be performed with a low volume liquid handler.

The gDNA must first be purified using magnetic beads, such as INTEGRA's MAGFLO NGS beads for size selection. The purified gDNA is then treated for bisulfite conversion, magnetic bead clean-up and desulfonation. This is followed by exonuclease treatment, first and second strand synthesis, final library amplification and index PCR. After most steps, tedious magnetic bead clean-ups are required, which can be simplified using the previously referenced PCR clean-up set-up. Finally, the libraries are normalized and pooled for sequencing.

Partial plates: Programs can be adapted at any time to a different number of samples, giving laboratories flexibility to meet current and future demands.

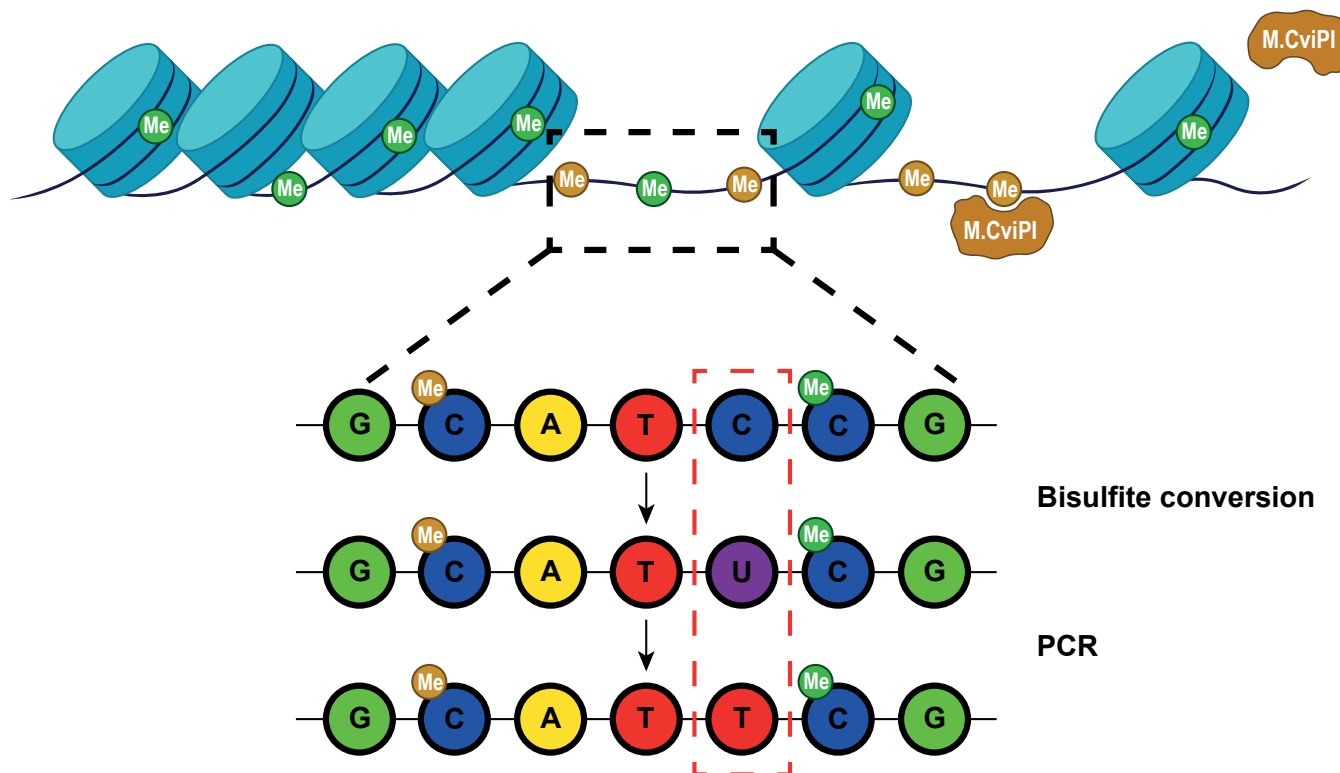


Figure 6: Schematic representation of bisulfite sequencing. The top row represents DNA wrapped around histones (blue) that are naturally methylated at CpG sites (green). Further DNA methylation is introduced at GpC sites (orange circle) using the M.CviPI enzyme (orange shape). Below, the stepwise transition of unmethylated C to U to T is shown.

Conclusion

INTEGRA's VIAFLO 384 provides a versatile solution for cutting-edge molecular biology applications requiring precise pipetting in a high throughput setting. Combining this instrument with the MAG module and MAGFLO magnetic beads results in an all-in-one package for sequencing protocols, providing a reliable foundation for automating

scNMT-seq workflows. Most tedious pipetting steps can be processed faster and more precisely with the VIAFLO 384 than any other handheld pipette, while maintaining the simplicity that makes it accessible to virtually any lab user.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6031	VIAFLO 384 channel handheld electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6131, 6136	384 channel pipetting heads (12.5 µl, 50 µl)	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6455	12.5 µl sterile, filter GRIPTIPS®	https://www.integra-biosciences.com/en/gripts/automation-gripts
INTEGRA Biosciences	6465	125 µl sterile, filter GRIPTIPS®	https://www.integra-biosciences.com/en/gripts/automation-gripts
INTEGRA Biosciences	4505	ASSIST PLUS pipetting robot	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6337	150 ml reservoir, individually sealed, sterile, polypropylene	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	4900	MAG module for magnetic bead separation	https://www.integra-biosciences.com/en/modules/mag-and-heatmag
INTEGRA Biosciences	4908	Adapter for 384 well PCR plates (MAG/HEATMAG)	https://www.integra-biosciences.com/en/modules/mag-and-heatmag
INTEGRA Biosciences	7000, 7002, 7004	MAGFLO™ NGS, 1-500 ml	https://www.integra-biosciences.com/en/ngs-purification/magflotm-pcr

Contact us:

