Application Note

Affordable, high throughput bulk RNA barcoding and sequencing plate preparation with the VIAFLO 384

Introduction

3' mRNA-seq is an effective tool for measuring gene expression in an unbiased and quantitative manner. However, existing technologies are too laborious and expensive to be applied to large scale projects with numerous samples. Alithea Genomics has developed a novel, proprietary technology called bulk RNA barcoding and sequencing (BRB-seq), which enables the streamlined preparation of 3' mRNA-seq libraries for hundreds of RNA samples in a single tube. The cornerstone of this technology is the use of BRB-seq oligos, which are synthetic DNA oligonucleotides.

BRB-seq oligos prime the reverse transcription (RT) reaction, during which the unique molecular identifier (UMI) and the sample-specific barcode are integrated into the synthesized complementary DNA (cDNA) strand. The use of BRB-seq oligos enables the molecular 'tagging' of individual RNA samples. After this initial tagging step, all samples (up to 384) can be pooled and processed simultaneously in a single tube for the rest of the workflow.

Alithea Genomics provides kits that contain all the reagents necessary to perform BRB-seq on different sample types, including purified RNA, whole-blood RNA, and cell/organoid lysates.

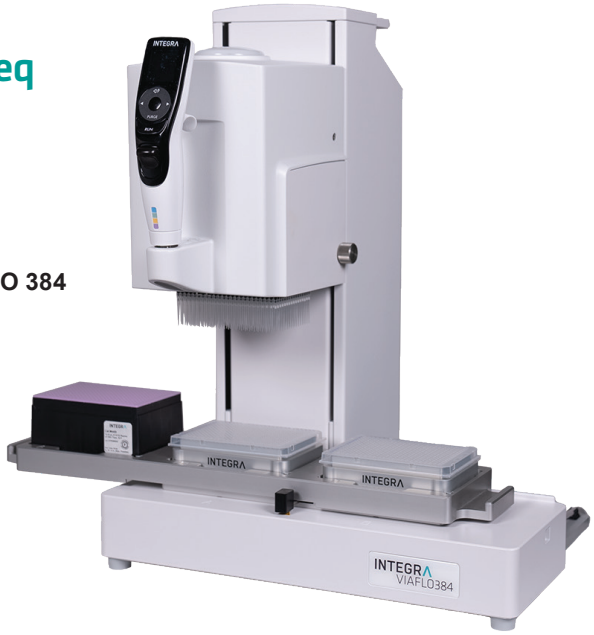
The combination of the BRB-seq kits with the VIAFLO 96 and VIAFLO 384 systems provides a convenient, efficient and reliable workflow for massively multiplexed RNA-seq experiments.

Key benefits:

- The BRB-seq workflow requires a very precise pipetting system, such as the VIAFLO 96 or VIAFLO 384, for simultaneous transfer of small volumes of 96 or 384 samples from one plate to another.
- The 96 and 384 channel pipetting heads are interchangeable on the VIAFLO 384 handheld electronic pipette, providing the user the flexibility to transfer 96 or 384 samples in a single step.
- Processing thousands of samples for BRB-seq can be drastically accelerated by using the VIAFLO 96 or VIAFLO 384 handheld electronic pipettes.
- High quality GRIPTIPS provide a perfect tip seal. This guarantees that all tips on the VIAFLO 96 or VIAFLO 384 are at exactly the same height and will never fall off accidentally. This ensures increased accuracy and precision for liquid handling steps.

Overview: How to prepare samples for BRB-seq

Early-stage multiplexing is possible because of the highly optimized sets of barcoded primers (BRB-seq oligos), which are added during the RT reaction (**Figure 1**).



VIAFLO 384

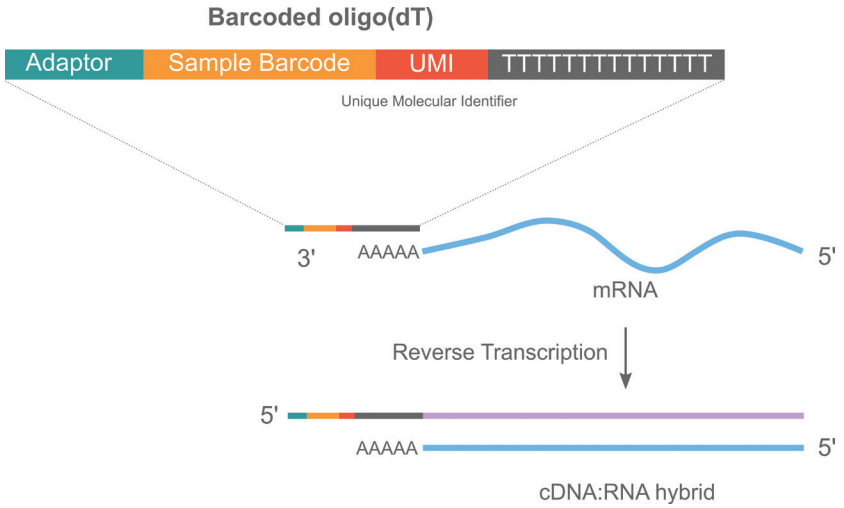


Figure 1: The barcoded oligo-deoxythymidine (dT) is attached at the RT step.

Step by step procedure:

Either the VIAFLO 96 or VIAFLO 384 can be used for the main pipetting steps during sample preparation for BRB-seq. The instruments are equipped with a 96 or 384 channel pipetting head (0.5-12.5 µl) and a three position stage (with positions A, A/B, and B). 12.5 µl Sterile, Filter GRIPTIPS are used. Pre-washed cells (in a 96 well or 384 well Sample plate) are lysed with 10 or 5 µl cell lysis buffer (respectively). Customized VIALINK programs are provided for the cell lysate transfers and pooling steps (**Figure 2**, steps 2, 3 and 7) in 384 well plates. The custom VIALINK programs for 96 well plates are available in the download section.

Overview of the programs:

- **Program 1:** Transfer the cell lysates to the Stock plate – Cell lysate transfer
- **Program 2:** Transfer the cell lysates to the Barcode plate – Barcoding
- **Program 3:** Pooling of samples after RT reaction – Pooling

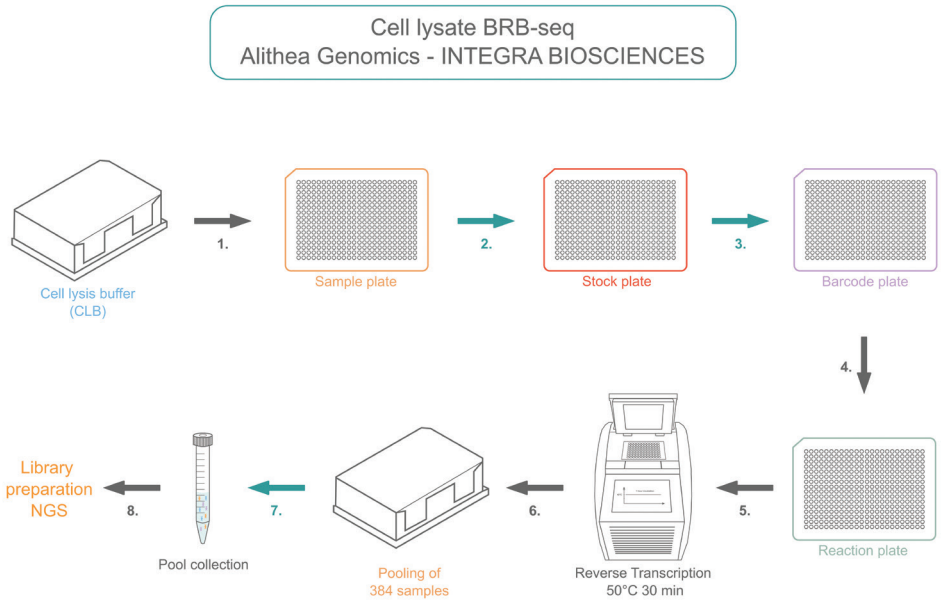


Figure 2: The sample preparation workflow for cell lysate BRB-seq of 384 samples (green arrows represent the transfer steps performed on the VIAFLO 384).

1. Cell lysate transfer

STEP: Transfer of cell lysates to the Stock plate.

HOW TO: Load a full box of 12.5 µl Sterile, filter GRIPTIPS onto the VIAFLO 384. Slide the three position stage to the left so that it is in the second position (A/B-B). Launch the custom program '384_LYS_STORAGE'. A prompt on the pipette screen informs the user that the Sample plate (384 well cell culture plate, Corning) should be placed on position A/B, and the Stock plate – a new 384 well PCR plate (Eppendorf) placed on 384 well cooling block – on position B (**Figure 3**). The pipetting head should then be lowered and the pipette tips should be placed into the individual wells of the Sample plate. The pipette aspirates 7 µl of cell lysate from the wells of the Sample plate and dispenses it to the wells of the Stock plate. When the dispense step is finished, if more samples are to be transferred, the pipette will notify the user that the pipette tips need to be changed and the program restarted. Alternatively, if there are no further samples to transfer, the pipette will notify the user that the program is finished. The Stock plate can then be kept at -80 °C for later analysis or used immediately for continuation of the workflow.

TIPS:

- The aluminum 384 (or 96) well PCR cooling block keeps the samples or reagents chilled.
- The Z-heights are defined in the programs to ensure optimal tip immersion depth.

Figure 3: The three position stage of the VIAFLO 384 set up for the cell lysate transfer step.

2. Barcoding

STEP: Transfer of cell lysates to the Barcode plate.

HOW TO: Load a full box of new 12.5 µl Sterile, Filter GRIPTIPS onto the VIAFLO 384. Move the three position stage to the second position (A/B-B). Select the custom program '384_LYS_OLIGOS'. The instrument will display the prompt that the Stock plate (on a 384 well cooling block) needs to be placed on position A, and the Barcode plate (on a 384 well cooling block) on position B. The pipetting head with the tips should then be lowered into the individual wells of the Stock plate (**Figure 4**). The VIAFLO 384 aspirates 10 µl of the cell lysate from each well of the Stock plate, and dispenses it to the Barcode plate that contains the optimized barcoded oligos. After the dispense step, if new cell lysates are to be transferred to new Barcode plates, the pipette informs the user that the pipette tips need to be changed. Alternatively, if there are no further samples to transfer, the pipette informs the user that the program is finished.

After adding the cell lysate to the barcodes, 10 µl of RT mix should be added manually with a 16 channel (12 channel in the case of 96 well plate) VIALFO pipette from PCR strip tubes (Starlab). The Reaction plate containing the cell lysates, the barcodes, and the RT mix needs to be transferred to a thermal cycler and incubated at 50 °C for 30 min.

Figure 4: The three position stage of the VIAFLO 384 set-up for the barcoding step.

3. Pooling

STEP: Pooling of samples after RT reaction.

HOW TO: After incubation, the barcoded cDNA samples can be pooled together. Load a full box of new 12.5 µl Sterile, Filter GRIPTIPS, slide the three position stage to the second position (A/B-B), and start the custom program '384_POOLING'. The pipette tells the user to place the Reaction plate (on the cooling block) on position A, an INTEGRA 150 ml automation-friendly reservoir on position B, and to lower the pipetting head with the tips into individual wells of the reaction plate (**Figure 5**). The pipette then aspirates 10 µl solution from each well, and dispenses it to the reservoir.

Figure 5: The three position stage of the VIAFLO 384 set-up for the pooling step.

4. Collecting the pool

STEP: Transferring the pool to a vessel.

HOW TO: The pool can be transferred to a 15 ml centrifuge tube (Greiner Bio-one) for further precleaning. This process can be easily executed with INTEGRA's PIPETBOY acu 2. The pool is ready for subsequent library preparation and next generation sequencing.

Results

Sample results obtained using either the BRB-seq™ kit, or the Cell lysate BRB-seq™ kit, and the VIAFLO 384 channel handheld electronic pipettes are shown below.

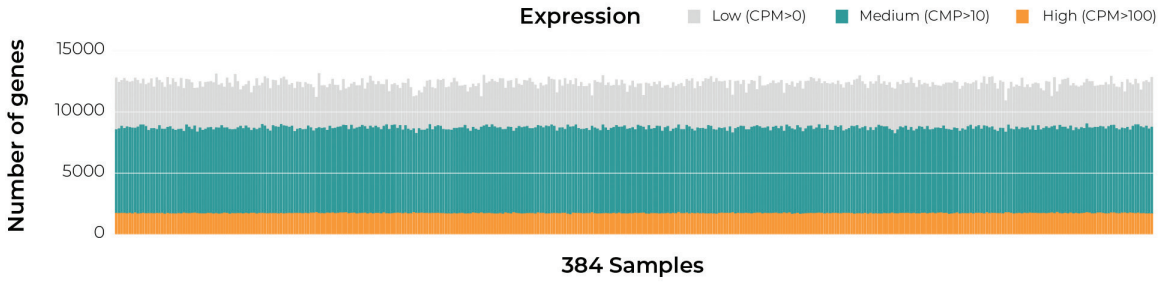


Figure 6: Distribution of the number of detected genes across 384 samples processed in one tube, using Alithea's MERCURIUS™ BRB-seq kit. The library was sequenced at an average of 1 million reads per sample.

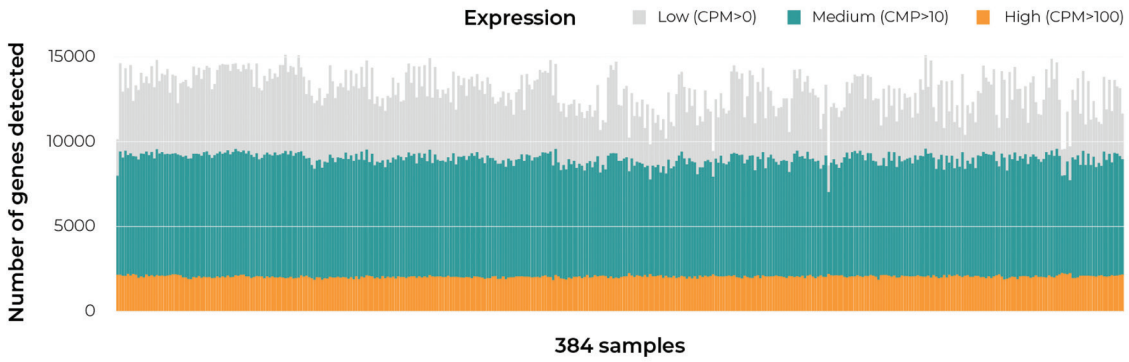


Figure 7: Distribution of the number of detected genes across 384 samples processed in one tube, using Alithea's MERCURIUS™ cell lysates BRB-seq kit. The library was sequenced at an average of 1.5 million reads per sample.

Conclusion

- 'Large scale' and 'high throughput' are often synonyms of bulky, expensive, and fully automated liquid handling solutions. By combining Alithea Genomics' BRB-seq technology with the VIAFLO 96 or VIAFLO 384, we demonstrate that there is an easier way to achieve a reliable, ultra-high throughput and cost-efficient RNA-seq workflow.
- With BRB-seq, up to 384 RNA or cell lysate samples can be processed in a single tube, right from the beginning of the library preparation workflow. With the VIAFLO 96 and 384 systems, the large number of pipetting steps can be condensed into handful of semi-automated plate-to-plate transfers.
- Setting the Z-height and the Tip Align on the VIAFLO 96 and VIAFLO 384 minimizes pipetting variability.
- Thanks to the compact benchtop design, both the VIAFLO 96 and VIAFLO 384 can fit into most laminar flow hoods, or on a small bench.
- Large scale RNA-seq is becoming an important tool that biotech, pharma and academic institutes can use to discover and develop new drugs. Performing this large-scale RNA-seq using BRB-seq and the VIAFLO 96 or VIAFLO 384 drastically reduces the turnaround times and operational costs.
- Using the BRB-seq kits on the VIAFLO 96 or VIAFLO 384 offers a convenient, compact and cost-effective solution to unlock the power of large scale RNA-seq.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA	6001	VIAFLO 96 base unit	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6031	VIAFLO 384 base unit	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6101	96 Channel Pipetting Head 0.5-12.5 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6131	384 Channel Pipetting Head 0.5-12.5 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6250	PCR 96 Well Cooling Block	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA	652270	Sapphire microplate, 96 well, PP	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6255	PCR 384 Well Cooling Block	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	155000	PIPETBOY acu 2	https://www.integra-biosciences.com/global/en/pipette-controllers/pipetboy-acu-2
INTEGRA	4641	VIAFLO 16 channel 0.5-12.5 µl pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo
INTEGRA	4632	VIAFLO 12 channel 0.5-12.5 µl pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo
INTEGRA	6455	12.5 µl Sterile, Filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA	3415	12.5 µl Pre-sterilized, Filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide

INTEGRA	6338	150 ml, Sterile, Polypropylene Automation Friendly Reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
Greiner Bio-One	188271	Tube, 15 ml, PP	https://shop.gbo.com/en/switzerland/products/bioscience/tubes-beakers/15ml-cellstar-polypropylene-tube/188271.html
STARLAB	I1402-3700	0.2 ml 8-Strip Non-Flex PCR Tubes	https://www.starlabgroup.com/GB-en/product/0.2-ml-8-strip-non-flex-pcr-tubes-natural-individually-attached-flast-caps-xtra-clear-i1402-3700.html
EPPENDORF	0030128575	Eppendorf twin.tec® PCR Plate 96	https://www.eppendorf.com/ch-de/eShop-Produkte/Spitzen-Reaktionsgef%C3%A4%C3%9Fe-und-Platten/Platten/Eppendorf-twintec-PCR-Plates-p-0030128575
EPPENDORF	0030128508	Eppendorf twin.tec® PCR Plate 384	https://www.eppendorf.com/ch-de/eShop-Produkte/Spitzen-Reaktionsgef%C3%A4%C3%9Fe-und-Platten/Platten/Eppendorf-twintec-PCR-Plates-p-0030128508
Greiner Bio-One	655180	Cellculture microplate, 96 well, PS, F-bottom	https://shop.gbo.com/en/switzerland/products/bioscience/cell-culture-products/cellstar-cell-culture-microplates/96-well-cell-culture-microplates-clear/655180.html
Corning	3701	384 well clear flat bottom polystyrene TC-treated microplate	https://ecatalog.corning.com/life-sciences/b2b/CH/en/Microplates/Assay-Microplates/384-Well-Microplates/Corning%C2%AE-384-well-Clear-Polystyrene-Microplates/p/3701?pagePath=p/3701
ALITHEA GENOMICS		MERCURIUS™ BRB-seq Library preparation kits for Illumina®	https://alitheagenomics.com/products/mercurius-brb-seq-kit
ALITHEA GENOMICS		MERCURIUS™ Cell lysate BRB-seq Library preparation kits for Illumina®	https://alitheagenomics.com/products/cell-lysate-brb-seq-kits

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