MERCURIUS™
BRB-seq kits

High quality
High throughput
Low cost

3' mRNA sequencing of bulk RNA samples

Alithea Genomics

BRB-SEQ overview

3' mRNA-seq is a great tool for measuring the expression of genes in an unbiased and quantitative way. However, existing solutions are too laborious and expensive to be applied on large-scale projects involving a large number of samples. **Our bulk RNA barcoding and sequencing (BRB-seq¹)** technology enables streamlined preparation of 3' mRNA-seq libraries for hundreds of RNA samples in a single tube.

The central aspect of our technology is the use of the ***BRB-seq™*** **oligos** (Fig. 1), which are synthetic DNA oligonucleotides containing:

- a polyT stretch to capture mRNA molecules
- a sample-specific barcode sequence, optimized for minimal cross-reactivity
- a unique molecular identifier (UMI) that enables digital transcript counting and PCR duplicate removal
- an Illumina adapter sequence for streamlined library preparation

The ***BRB-seq™*** **oligos** prime the reverse transcription reaction, during which the UMI and the sample-specific barcode are integrated into the synthesized cDNA strand. The use of ***BRB-seq™*** **oligos** with different barcodes enables molecular "tagging" of individual RNA samples (Fig. 2).

After this initial tagging step, all samples can be pooled and processed simultaneously in one single tube for the remainder of the workflow.

Currently, **our kits contain 96 *BRB-seq™* barcodes**, which can be used to efficiently process up to 96 RNA samples.

We will **soon launch *BRB-seq™* kits containing 384 and 1536 barcodes**, which can be now for pre-ordered at sales@alitheagenomics.com.
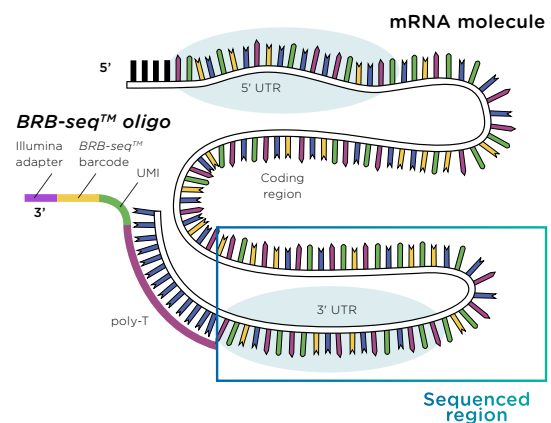

Fig. 1
Structure of the BRB-seq™ oligos and their target mRNA molecule.

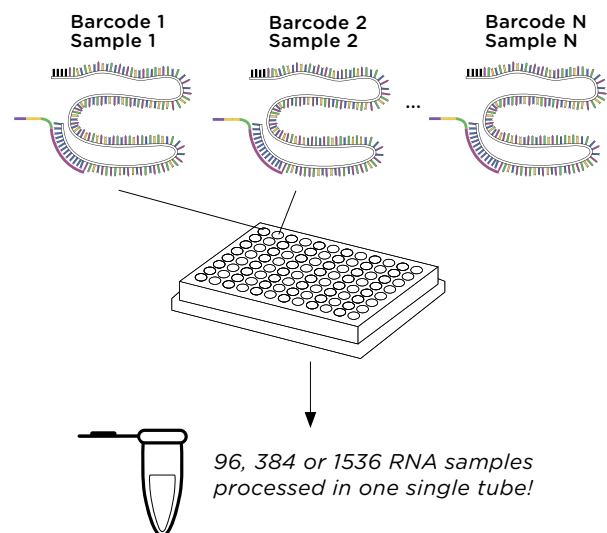*96, 384 or 1536 RNA samples processed in one single tube!*

Fig. 2
Each plate position corresponds to a barcode and a sample. This is how we know what barcode corresponds to which sample when all samples together in one tube.

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¹ Alpern, D., Gardeux, V., et al. BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. Genome Biol 20, 71 (2019).

ADVANTAGES OF BRB-SEQ

HIGHLIGHTS

<u>Fast</u>	10x fewer manual steps
<u>Cost-efficient</u>	10x less reagent consumption
<u>Robust</u>	reduced technical variability by processing 96 samples as one
<u>Accurate</u>	PCR duplicates are removed by using unique molecular identifiers (UMI)

BRB-seq enables **large-scale transcriptomics projects based on RNA-seq**, which would be otherwise technically and financially unfeasible with the other RNA-seq solutions.

Pooling and processing samples in a single tube translates into a **significant reduction in reagent consumption and manual as compared to other RNA-seq alternatives** (Fig. 3).
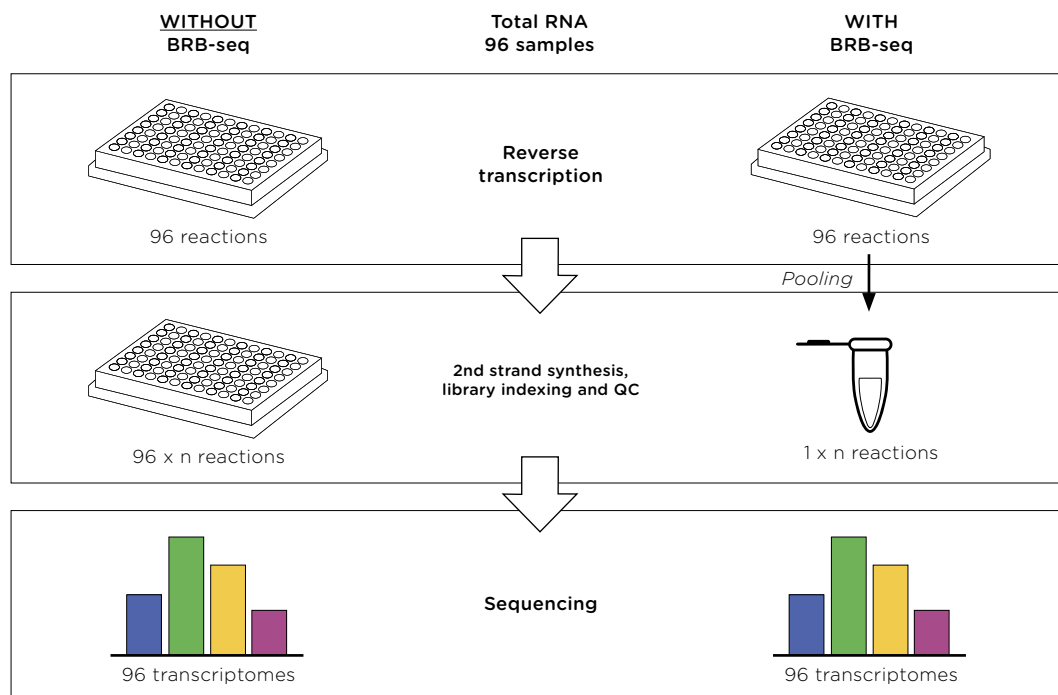

Fig.3: Comparison between the standard RNA-seq workflow and BRB-seq.

LARGE SCALE TRANSCRIPTOMICS MADE POSSIBLE

Following Illumina sequencing, **BRB-seq™ libraries** can be demultiplexed using the provided list of **BRB-seq™ barcodes** (for guidance and info about bioinformatic analysis, contact us at info@ alitheagenomics.com).

Below we report a typical result obtained with a **BRB-seq™ kit**, which shows uniform distribution of the number of detected genes for each sample at different CPM (<u>c</u>ounts <u>p</u>er <u>m</u>illion) thresholsd (Fig. 4).

Number of detected genes per sample

Level of detected gene expression (CPM)

- ▢ All detected (CPM>0)
- ▢ Mid expressed (CPM>10)
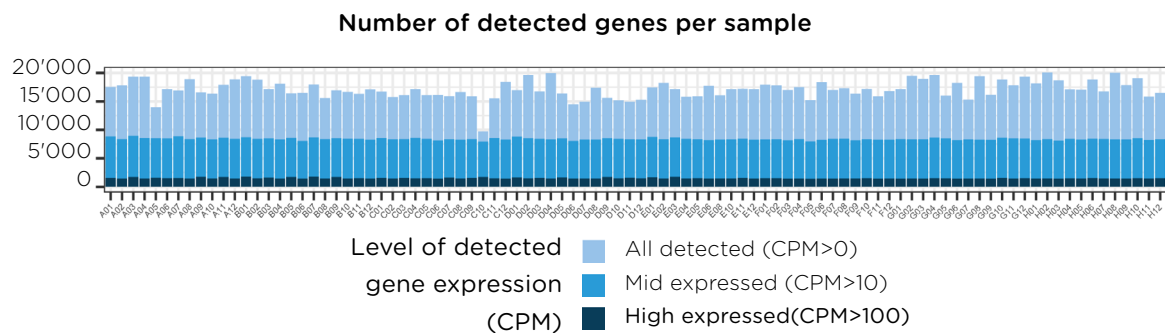- ▢ High expressed(CPM>100)

<u>Fig.4</u>: *Sample plot generated by using the MERCURIUS™ BRB-seq kits pipeline. Number of detected genes for different CPM thresholds, i.e. a gene is considered detected only if the number of attributed reads is above the CPM threshold. The library was sequenced at on average 3.1 M reads/sample (96 samples).*

WHY BRB-SEQ?

"BRB-seq allowed us to plan an experiment with ten times more replicates than usual, and gain insight into the regulation of gene expression in fly embryogenesis".

Prof. Dr. Marc Robinson, University of Lausanne

"BRB-seq provided us with a cost-effective way to quantify transcriptomic changes from a large set of samples."

Dr. Hirokazu Okada, ETH Zurich

"BRB-seq is very cost-effective and it enables accurate comparison of RNA levels among a large number of samples."

Prof. Martin Klingenspor, TUM Munich

SUCCESS STORIES

Below we propose two notable examples that highlight how BRB-seq data contributed to the successful completion of relevant research studies. For a full list of studies that used our service, please visit our website at: alitheagenomics.com/publications

## A stromal cell population that inhibits adipogenesis in mammalian fat depots

Published : Nature 559, 103–108 (2018)
https://doi.org/10.1038/s41586-018-0226-8

In a study published in Nature (2018), scientists from Bart Deplancke's lab at EPFL, Christian Wolfrum's lab at ETHZ, and the Swiss Stem Cell Foundation led by Gianni Soldati, have used a single cell transcriptomics and BRB-seq to characterize, for the first time, the different types of stromal cells that reside within mature fat depots.

Using this approach, the scientists first identified several stromal cell subpopulations in the fat tissue of mice. The research team then isolated these subpopulations and studied their cellular behavior.

Here, BRB-seq was instrumental to obtain and compare the gene expression profile of cell populations obtained and sorted from different human individuals (Fig. 5).

Fig. 5:
Gene expression levels for the most highly expressed genes in three subpopulations of the FACS sorted human adipose stromal cells samples from four individuals. Copyright: Springer Nature (2018)
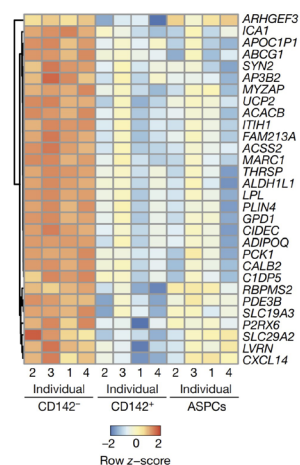
Inter-embryo gene expression variability recapitulates the hourglass pattern of evo-devo

Published : BMC Biol 18, 129 (2020).
https://doi.org/10.1186/s12915-020-00842-z

In another study published in BMC (2020), scientists from the lab of Marc Robinson-Rechavi have used BRB-seq transcriptomics with chromatin immunoprecipitation to measure gene expression and dissect gene regulation mechanisms during embryogenesis in Drosophila melanogaster.

Here, BRB-seq was used to obtain the gene expression profile from 239 embryos collected at 8 different developmental stage (Fig. 6).
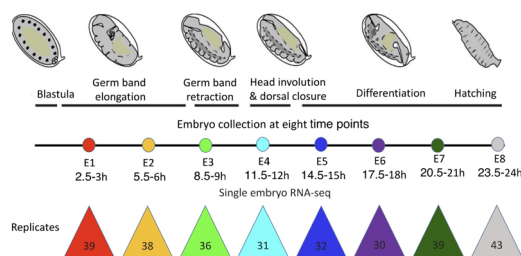

Fig. 6:
Schematic workflow of embryo collection and sample clustering using BRB-seq data. Copyright: Springer Nature (2020)

CONTACT

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guarantee

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technical
support