MERCURIUS™
BRB-seq kits
for blood transcriptomics

High quality
High throughput
Low cost

3’ mRNA sequencing of blood RNA samples
with integrated globin depletion

Alithea Genomics

Stratech

stratech.co.uk/alithea

BRB-SEQ overview

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

The two central aspects of our technology are the use of the ***BRB-seq™ oligos*** **and custom globin blockers** (Fig. 1).

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. Using *BRB seq™* oligos with different barcodes enables molecular "tagging" of individual RNA samples.

With the ***Blood BRB-seq™*** **kits**, we also provide custom-designed globin blockers which prevent globin mRNA from being tagged, resulting in clean globin-depleted RNA sequencing data.

After the initial tagging and blocking steps, all samples are pooled and processed in parallel in one single tube for the remainder of the workflow (Fig. 2).

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.

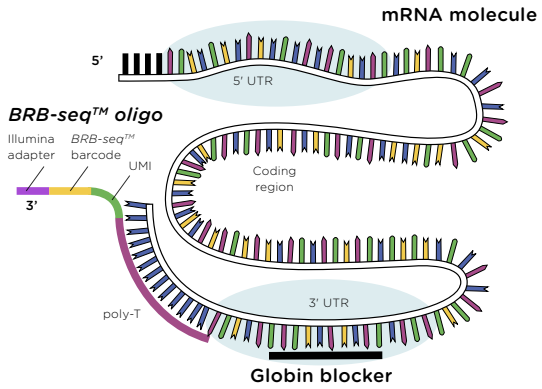

Fig. 1
Structure of the BRB-seq™ oligos, globin blockers and the target mRNA molecule.

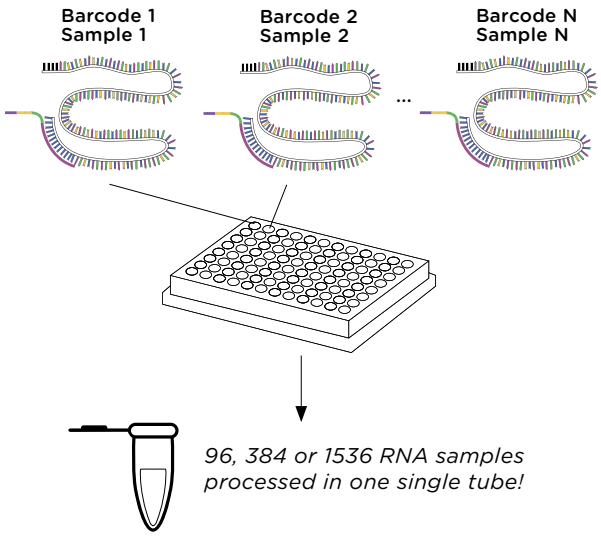

Fig. 2
Each plate position corresponds to a barcode and a sample. This is how we know what barcode corresponds to which sample when all samples together in one tube.

ADVANTAGES OF BRB-SEQ

HIGHLIGHTS

Fast	10x fewer manual steps
Cost-efficient	10x less reagent consumption
All-in-one	Globin depletion is integrated in the workflow at now extra steps
Robust	reduced technical variability by processing 96 samples as one
Accurate	PCR duplicates are removed by using unique molecular identifiers (UMI)

***BRB-seq™* enables large blood transcriptomics projects based on RNA-seq**.
Pooling and processing samples in a single tube translates into a **significant reduction in reagent consumption and manual effort as compared to other RNA-seq alternatives** (Fig. 3).

Moreover, our integrated depletion approach provides a convenient and efficient way to deplete globin genes without a significant impact on experimental budget and labor costs.

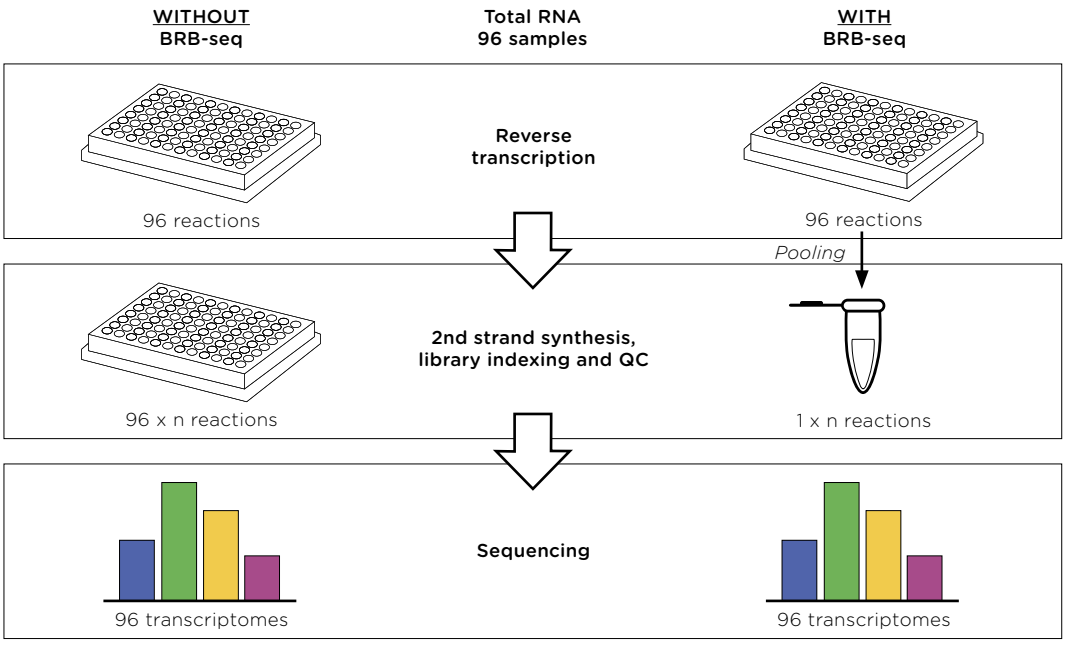

Fig.3: Comparison between the standard RNA-seq workflow and BRB-seq.

LARGE SCALE BLOOD 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 (counts per million) thresholsd (Fig. 4).

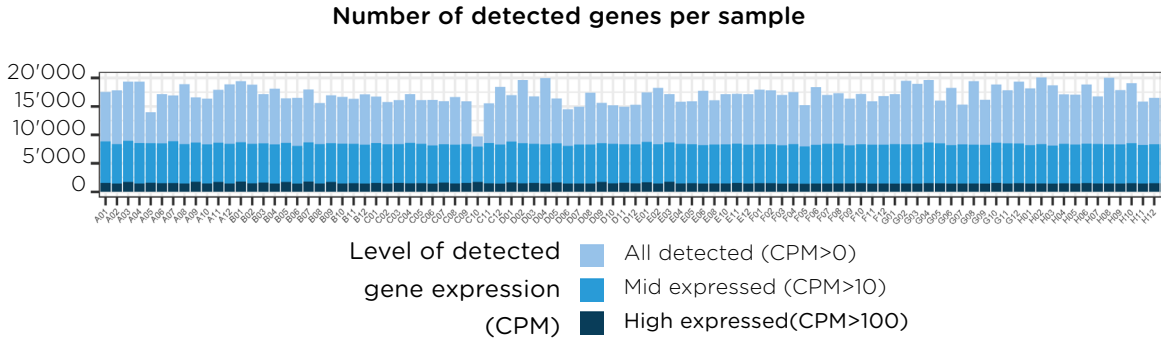

Fig.4
Sample plot generated by using the MERCURIUS™ BRB-seq kits pipeline. Number of detected genes for different CPM thresholds, i.e. a gene is considered detected only if the number of attributed reads is above the CPM threshold. The library was sequenced at on average 3.1 M reads/sample (96 samples).

An important aspect of the ***Blood BRB-seq™* kits** is the high-quality globin-depleted data. Our results show that our globin depletion strategy provides efficien (Fig. 5a) and specific (Fig. 5b) reduction of globin genes in the resulting sequencing data.

Fig.5a

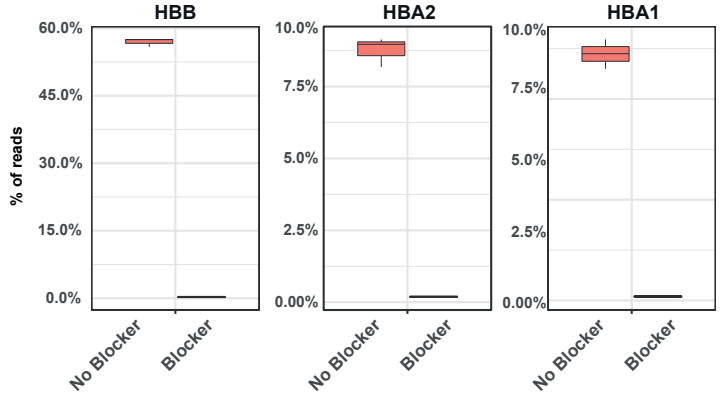

Fig.5b

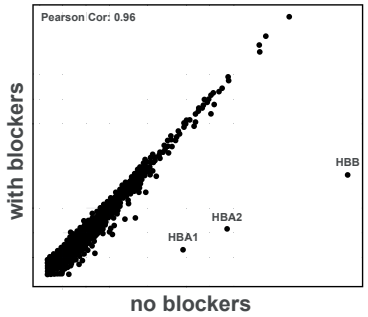

Fig.5
Our benchmarking results showing a) depletion levels for the three globin genes HBB, HBA1 and HB2 and b) correlation between the same samples with and without globin depletion.