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Whole Transcriptome Sequencing from Whole Blood with Integrated rRNA and Globin Depletion

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Covaris truCOVER® Globin RNA Depletion Module

Features

- **Superior Depletion Strategy:** the Globin mRNA Depletion Reagent consists of globin-specific oligos that are added directly to the existing rRNA depletion master mix. These oligos effectively 'block' the reverse transcription of globin-associated transcripts. This ultra-efficient approach, for both rRNA and now globin RNA, is in stark contrast with other kits which typically require a separate series of incubation and clean-up steps (see **Figure 1**).
- **Fastest Depletion Reaction:** ribosomal RNA blocking, globin mRNA blocking and reverse-transcription priming all take place in a single 15-minute reaction setup.
- **Reproducible Results, Even with Challenging Samples:** Adaptive Focused Acoustics® (AFA®) mechanical shearing helps deliver consistent insert sizes of RNA across sample qualities, down to a DV_{200} of 30% for FFPE RNA (McCarthy *et al*., 2026), and for whole blood RNA with RIN >5.
- **One Workflow for Blood and FFPE:** same AFA fragmentation settings, amplification cycles, and clean-up ratios for whole blood and FFPE samples, enabling them to be processed in the same run.
- **Broad Input Compatibility:** performance tested with total RNA inputs from 10–500 ng.
- **Automation-Ready Design:** plate-based workflow designed for 24- and 96-sample throughput and for integration with common liquid handling systems.

Benefits and Critical Outcomes for Laboratories

- **Operational Efficiency:** the novel depletion approach eliminates the standalone depletion procedures other workflows require, meaning fewer steps, clean-ups, lost samples, and errors.
- **Ultra-Efficient Depletion:** reduces globin mRNA to below 1% of total reads in every donor and at every input tested.
- **Superior Read Distribution:** affects only globin transcripts with fewer than 0.4% of detected genes differing between libraries prepared with and without the module.
- **Exceptional Transcriptome Clarity and Dynamic Range:** combined ribosomal RNA, mitochondrial rRNA and/or globin mRNA held below 1% in every condition tested to minimize sequencing noise
- **High Reproducibility:** repeatable gene detection at every input tested.

Introduction

RNA sequencing has moved steadily from research into clinical use, with gene fusion detection and differential expression of disease-associated transcripts among its earliest diagnostic applications (Byron *et al*., 2016). Peripheral whole blood is a natural specimen for that transition: it is collected routinely, can be sampled repeatedly, and because none of its components are removed, reflects systemic immune status (Qi *et al*., 2023). Blood transcriptomes are now being mapped at scale (Mishra *et al*., 2026) and used to follow infection and treatment response over time (Sheerin *et al*., 2023), which makes whole transcriptome sequencing (WTS) of whole blood attractive for biomarker discovery, patient stratification, and longitudinal cohorts where repeat tissue sampling is impractical.

That accessibility carries a cost whereby globin transcripts from erythrocytes dominate the blood transcriptome. In 58 matched libraries prepared from the same whole blood specimens, globin accounted for a median of 57% of reads without depletion (range 36–80%), against 0.15–1.62% with depletion included (Sheerin *et al*., 2023). In an undepleted library, most sequencing is spent re-reading a handful of globin transcripts, which results in a considerable waste of laboratory resources and time. In the same study, only 4 of 29 undepleted libraries cleared a 25-million-read threshold, once globin-derived reads were computationally removed. Depleting globin before sequencing also improves technical reproducibility and unmasks thousands of otherwise undetected transcripts (Shin *et al*., 2014).

Globin depletion is already standard practice with multiple chemistries effectively achieving it. In a systematic comparison of four commercial methods, probe hybridization left 0.5% ± 0.6% residual globin and RNase-H digestion 3.2% ± 3.8%, with the enzymatic methods detecting fewer genes and transcripts and introducing 3' coverage bias (Jang *et al*., 2020). A third approach blocks globin during library preparation rather than removing it beforehand; a blocking module benchmarked across 91 blood donors reduced hemoglobin reads from 43% to 8.0%, recovered 1,397 additional expressed genes, and showed no significant differences in enrichment or differential expression with respect to molecular function (Uellendahl-Werth *et al*., 2020).

The practical difference between these approaches lies in what they add to the workflow. Most require a dedicated depletion procedure with its own incubation and clean-up steps, adding time and handling, leading to sample loss from total RNA inputs that are often already limited. The Covaris truCOVER Globin RNA Depletion Module removes that trade-off. A single Globin mRNA Depletion Reagent is added to the depletion master mix already used within the truCOVER Total RNA Library Prep workflow, where it blocks globin transcripts from being converted into library. Ribosomal RNA blocking, globin blocking, and reverse-transcription priming occur in the same reaction setup and incubation, with no additional steps or clean-ups required. This highly efficient workflow enables users to go from RNA sample to library, with industry-leading depletion efficiency, in just over 4 hours. The shorter workflow turnaround leaves laboratory professionals with more time for high-impact transcriptomic analyses, enabled by Covaris truCOVER RNA Library Prep Kit for all sample types, from FFPE tissue to whole blood.

Materials and Methods

RNA Sources and Inputs

Total RNA was isolated from K2EDTA whole blood collected from three unique donors (Research Blood Components) using the Macherey-Nagel NucleoSpin® RNA Blood Midi Kit, which retains RNA from both red and white blood cells. For comparison, RNA was also isolated using the Qiagen QIAamp® RNA Blood Mini Kit, which excludes red blood cell RNA from the eluate and consequentially provides naturally globin-poor material. RNA integrity was assessed on a Bioanalyzer 2100 (Agilent®); all three donors returned RIN values between 9.7 and 10.0.

Whole blood RNA was tested at 10 ng, 100 ng, and 500 ng input; white blood cell RNA (QIAamp extraction, Qiagen) at 10 ng and 100 ng. Each condition was prepared in four technical replicates, except for the 500 ng conditions, which were prepared in duplicate.

AFA Shearing and Library Preparation

RNA was sheared to a 350-nucleotide median target in the 8 AFA-TUBE TPX Strip using an R230 Focused-ultrasonicator (McCarthy *et al*., 2026). Libraries were prepared with the truCOVER Total RNA Library Prep Kit together with the truCOVER Globin RNA Depletion Module (Globin Depletion Module), using Covaris unique dual indexes (UDI) to enable sample pooling and in silico demultiplexing. Control libraries were prepared in parallel from white blood cell RNA following the standard truCOVER workflow, without the Globin Depletion Module, to allow a direct assessment of depletion specificity.

Quantification, Sequencing and Data Analysis

Library concentrations were determined by fluorometric quantification (Qubit™ HS dsDNA, Invitrogen) and fragment size distributions by automated electrophoresis (TapeStation HS D5000, Agilent). Libraries were normalized, pooled at equimolar ratios, and loaded onto an Illumina NextSeq® 2000.

Sequencing reads were processed through the nf-core/rnaseq pipeline, with STAR alignment to the human reference genome. The following post-alignment metrics were evaluated:

- **Globin Depletion:** percentage of reads assigned to the globin gene family.
- **rRNA Depletion:** percentage of reads assigned to cytoplasmic and mitochondrial ribosomal RNA.
- **Depletion Specificity:** transcript content concordance between libraries prepared with and without the Globin Depletion Module.
- **Alignment Rate:** fraction of reads aligning uniquely to the genome.
- **Strand Specificity:** proportion of reads mapping to the antisense strand.
- **Gene Detection:** number of genes detected at TPM >1.

Results and Discussion

Globin Depletion Without a Dedicated Procedure

Adding the globin depletion reagent contributes no additional incubation time, leaving the complete workflow from blood RNA to sequencing-ready library at just over 4 hours. Blood samples processed with the Globin Depletion Module follow the same truCOVER protocol as FFPE samples and can be prepared side by side in the same run. **Figure 1** compares the truCOVER depletion segment against the more intensive alternatives of competing kits, whose depletion-related steps alone require roughly 2 hours of dedicated processing.

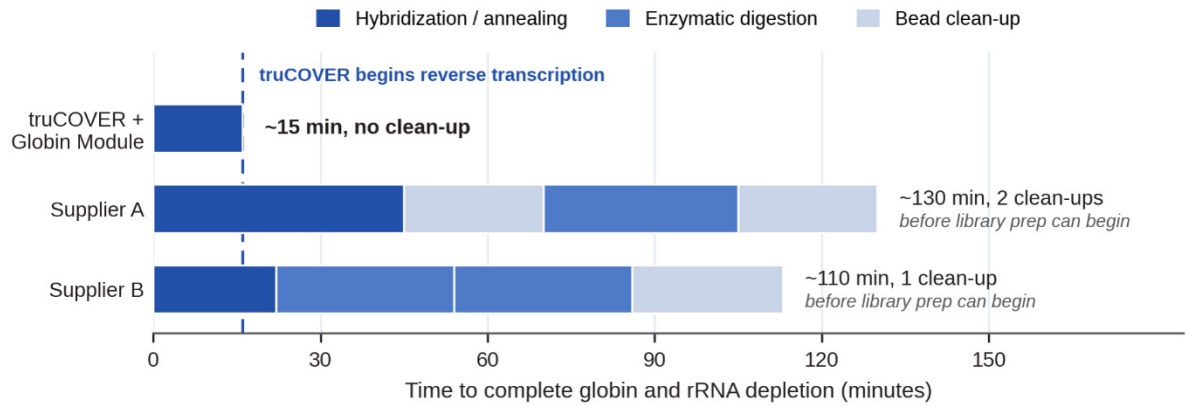

Figure 1. Time to complete globin and ribosomal RNA depletion. The truCOVER Globin RNA Depletion Module performs blocking during the workflow's existing oligo annealing step, in 15 minutes without a dedicated depletion procedure. Supplier A and Supplier B times are taken from published protocols.

Globin and Ribosomal RNA Are Both Held Below 1%

Globin transcript content was calculated as the summed proportion of reads assigned to the globin gene family. Across all three donors and all three input amounts, globin mRNA represented less than 1% of total reads (**Figure 2A**). Depletion efficiency improved with input amount, but even the least favorable condition left more than 98% of the library to informative sequence.

Ribosomal RNA followed the same pattern (**Figure 2B**), with combined cytoplasmic and mitochondrial ribosomal RNA held below 1% of aligned reads in every condition tested.

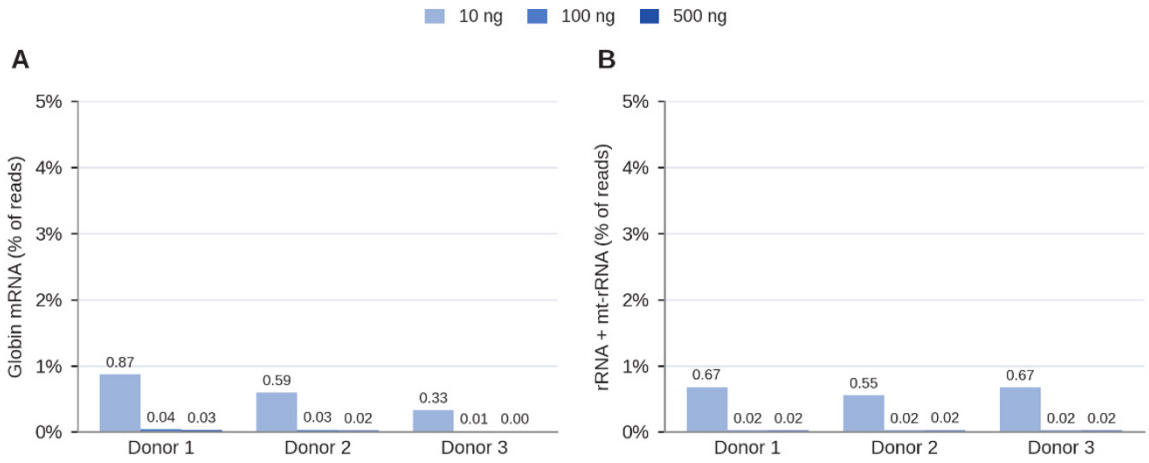

Figure 2. Globin and ribosomal RNA depletion from whole blood RNA. (A) Residual globin mRNA as a percentage of reads. (B) Combined ribosomal and mitochondrial ribosomal RNA as a percentage of reads. Each bar is the mean of four technical replicates, except for the 500 ng conditions, which are the mean of two.

truCOVER reduced globin to below 1% of reads at every input tested. For context, matched globin-depleted libraries in the literature retained 0.15–1.62% globin with a median of 0.32% (Sheerin *et al*., 2023), and a benchmarked blocking module of the same broad approach, the Lexogen QuantSeq Globin Block, left 8.0% (Uellendahl-Werth *et al*., 2020). Cross-study comparison is limited by differences in extraction, library chemistry, and analysis pipeline. The truCOVER result was achieved with no separate depletion protocol, no additional clean-ups, and no added hands-on time.

Published whole blood libraries prepared without depletion carry a median of 57% globin reads (Sheerin *et al*., 2023). At that level, a 25 million read run yields roughly 10.7 million informative reads. At the residual globin levels measured here, nearly all reads are informative, roughly 2.3 times more usable data per run.

Depletion Is Specific to Globin Transcripts

Efficient depletion is only useful if it is also selective, leaving non-globin transcripts to enter the library unimpeded. Libraries were prepared with and without the truCOVER Globin RNA Depletion Module from white blood cell RNA, which contained low levels of background globin to assess depletion selectivity. Any systematic difference in transcript content between the two would indicate an off-target effect.

Transcript abundance was compared between libraries prepared with and without the Globin Depletion Module (**Figure 3**). Expression was highly concordant at both inputs (Pearson r = 0.957 and 0.983 at 10 ng and 100 ng, respectively), and fewer than 0.4% of detected genes differed between workflows, indicating that depletion is confined to the globin transcript family. Independent benchmarking of in-library blocking reached the same conclusion, with enrichment and differential expression analyses showing no significant differences with respect to molecular function between blocked and non-blocked libraries (Uellendahl-Werth *et al*., 2020).

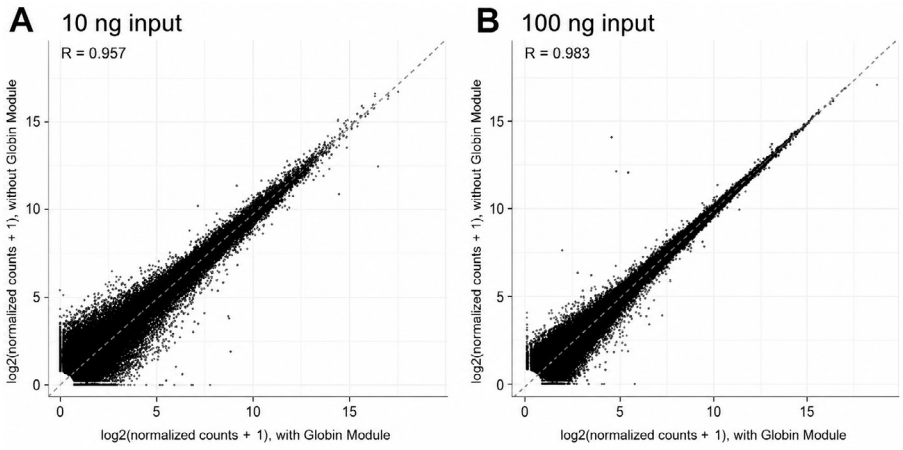

Figure 3. Transcript content concordance between libraries prepared from white blood cell RNA with and without the Globin RNA Depletion Module, at (A) 10 ng and (B) 100 ng input.

Consistent Gene Detection Across a 50-Fold Input Range

Gene detection was high and stable across the input series (**Figure 4**). Detection varied by no more than 11.3% within a donor across the 50-fold input range, and a 10 ng input recovered 88.7–96.0% of the genes detected at 500 ng from the same donor. Repeatability across technical replicates was similarly tight, with coefficients of variation of 0.4–3.3%.

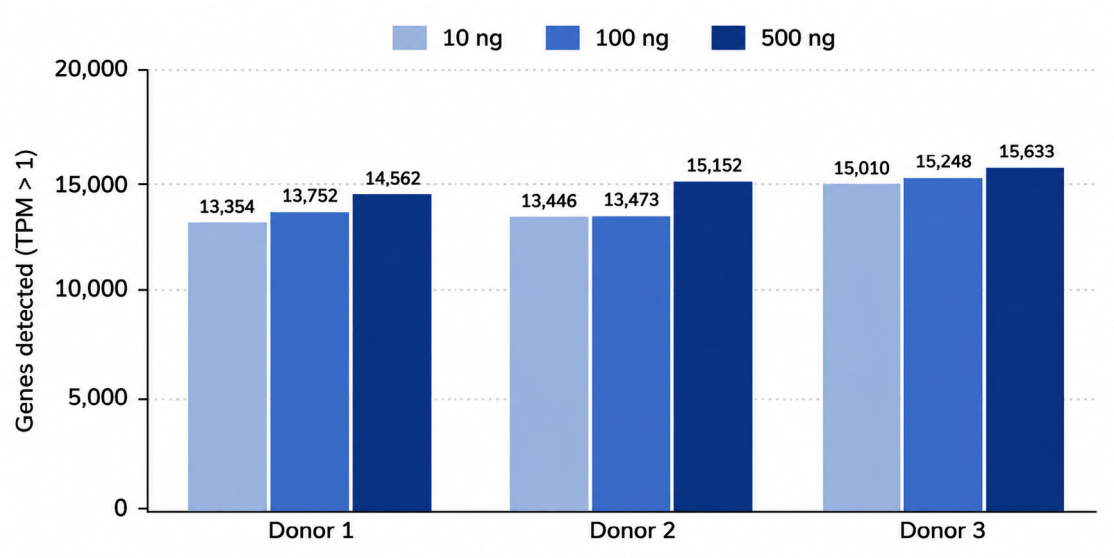

Figure 4. Genes detected at TPM >1 from whole blood RNA across three donors and a 50-fold range of input material. Each bar is the mean of four technical replicates, except for the 500 ng conditions, which are the mean of two.

Sequencing Metrics Are Unchanged

Adding globin depletion did not degrade standard library metrics: 87.9–94.0% of reads aligned uniquely to the genome, and strand specificity for whole blood RNA was maintained at 87.7–90.1%, preserving the orientation information needed to resolve overlapping genes and antisense transcripts. Across all sample types, RNA is sheared to a 350 bp median target, resulting in libraries well matched to 2 x 150 bp sequencing (McCarthy *et al*., 2026).

Conclusion

Globin mRNA is the single largest obstacle to cost-effective whole transcriptome sequencing from whole blood, routinely consuming more than half of a sequencing run and masking the low-abundance transcripts that carry important biological signals. Depletion procedures exist, but they require multiple incubations and clean-ups, with the added time, handling, and sample loss that implies. The Covaris truCOVER Globin RNA Depletion Module reaches the same endpoint inline, blocking globin transcripts during library preparation, enabling users to go from samples to finished libraries in just over 4 hours. Across three donors and a 50-fold input range, residual globin was held below 1% of reads with no off-target depletion and no loss of library quality.

For reference laboratories, contract research organizations and academic core laboratories running blood transcriptomics at scale, the practical results are (1) more usable data per sequencing run, (2) a single truCOVER workflow for both FFPE and whole blood, and (3) a lower complexity workflow that generates libraries from RNA in just over 4 hours.

References

(1) Byron, S. A., *et al*. (2016). Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nature Reviews Genetics, 17: 257–271.

(2) Jang, J. S., *et al*. (2020). Comparative evaluation for the globin gene depletion methods for mRNA sequencing using the whole blood-derived total RNAs. BMC Genomics, 21: 890.

(3) McCarthy, P., *et al*. (2026). Whole transcriptome sequencing from degraded FFPE samples: a fast workflow to deliver robust gene fusion detection. Covaris Technical Note mkt_5557. https://www.covaris.com/wp/wp-content/uploads/resources_pdf/mkt_5557.pdf

(4) Mishra, N., *et al*. (2026). Large-scale analysis of temporal gene expression variation in peripheral blood. Nature Communications, 17: 6992.

(5) Qi, D., *et al*. (2023). Transcriptomic analyses of patient peripheral blood with hemoglobin depletion reveal glioblastoma biomarkers. npj Genomic Medicine, 8: 2.

(6) Sheerin, D., *et al*. (2023). Identification and control for the effects of bioinformatic globin depletion on human RNA-seq differential expression analysis. Scientific Reports, 13: 1859.

(7) Shin, H., *et al*. (2014). Variation in RNA-Seq transcriptome profiles of peripheral whole blood from healthy individuals with and without globin depletion. PLoS ONE, 9(3): e91041.

(8) Uellendahl-Werth, F., *et al*. (2020). A benchmark of hemoglobin blocking during library preparation for mRNA-Sequencing of human blood samples. Scientific Reports, 10: 5630.

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