Enhancing BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay data with the Jumpcode Genomics DepleteX™ Mitochondrial DNA Depletion Kit

Introduction

The BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay facilitates the detection of differentially expressed genes with remarkable accuracy across distinct samples at a single-cell level. It relies on a randomized approach of gene detection using all captured mRNA transcripts. This common approach can present challenges in single-cell RNA-seq (scRNA-seq) if there is a high abundance of captured mitochondrial (mito) transcripts in the single-cell assay. Importantly, this contamination can result in unnecessary sequencing of mito gene information that may be undesired. Traditional approaches to mitigate mito gene information in scRNA-seq data often involve post-sequencing computational filtration of these mito genes. Here we present an alternative approach that can be used prior to sequencing that can enhance assay performance. We showcase the use of the Jumpcode Genomics DepleteX™ Mitochondrial DNA Depletion Kit with the BD Rhapsody™ WTA Assay to remove unwanted mito transcript information during library preparation before sequencing, without compromising data quality.

Targeted approach to remove unwanted mito content

The Jumpcode Genomics DepleteX™ Mitochondrial DNA Depletion Kit employs CRISPR technology with a custom-designed mito guide set (detailed in Table 1), which removes unwanted mito gene amplicons with high specificity from BD Rhapsody™ WTA Assay libraries of human samples. This results in the removal of an uninformative portion of transcriptomic reads from libraries prior to sequencing, thereby potentially improving sequencing capacity by reallocating reads toward more biologically relevant genes.

Highlights

- Mitochondrial mRNA reduced to as low as 0.2% of the total number of RNA molecules.*
- Compatible with both single-cell and single-nuclei RNA-seq
- No off-target effects indicated by high correlation (R^2>0.99) of gene expression between mito-depleted and undepleted samples.*
- No significant changes in cell composition and total cell numbers between mito-depleted and undepleted samples*
- Simple and flexible workflow to remove mitochondrial genes.

*Data from PBMCs; some variability expected with different cell types.

Table 1: The mitochondrial genes targeted by the DepleteX™ Mitochondrial DNA Depletion Kit.

Mitochondrial gene list		
MT1E	MT-CO2	MT-ND4
MT2A	MT-CO3	MT-ND4L
MT-ATP6	MT-CYB	MT-ND5
MT-ATP8	MT-ND1	MTRNR2L12
MTCH1	MT-ND2	MTRNR2L8
MT-CO1	MT-ND3	

BD

We generated single-cell and single-nuclei whole transcriptome mRNA libraries from cryopreserved human PBMCs using the BD Rhapsody™ System mRNA Whole Transcriptome Analysis Library Preparation Protocol.[1] Next, we followed the DepleteX™ Kit protocol as described in the Jumpcode Genomics DepleteX™ Mito Depletion Kit user manual[2] to deplete mito genes from the libraries (Figure 1, workflow). Libraries with or without mito depletion were generated from the same samples, sequenced to the same read depth and analyzed with the BD Rhapsody™ Sequence Analysis Pipeline 2.0. It is noteworthy to mention that we have tested mito depletion in two different steps of library preparation after the RPE PCR step (Figure 1, Step 3) and after the index PCR step (Figure 1, Step 4). Depletion of mito genes at both steps showed very comparable performance without generating any observable bias in WTA molecule detection. Therefore, users can determine which step works best for them to implement mito depletion during their library preparation protocol.

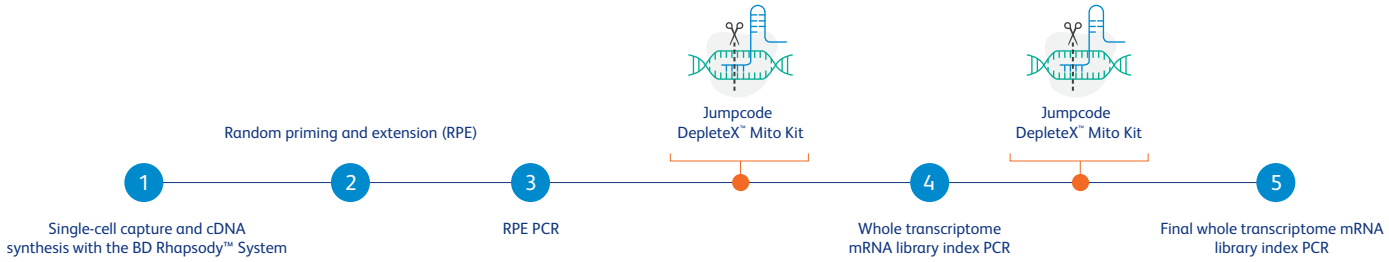

Figure 1. Overview of the DepleteX™ Mitochondrial DNA Depletion Kit workflow on the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay workflow. Data shown in this technote have been generated by integrating the DepleteX™ Mitochondrial DNA Depletion Kit workflow after index library preparation (Step 4). An alternative approach is to utilize the kit after RPE PCR (Step 3).

Results

Efficiency of DepleteX™ Mitochondrial DNA Depletion Kit in enhancing informative reads

There was a substantial reduction in the percentage of mito molecules in the samples treated with the DepleteX™ Mitochondrial DNA Depletion Kit when compared to the undepleted sample. Specifically, mito molecules decreased from 33.29% to 4.78% in the nuclei samples and from 11.94% to 0.22% in the cell samples (Figure 2A). Importantly, the reduction in mito molecules is observed for each mitochondrial gene as shown in Figure 2B, where we show a reduction or complete elimination of the specific mitochondrial genes targeted by the DepleteX™ Mitochondrial DNA Depletion Kit.

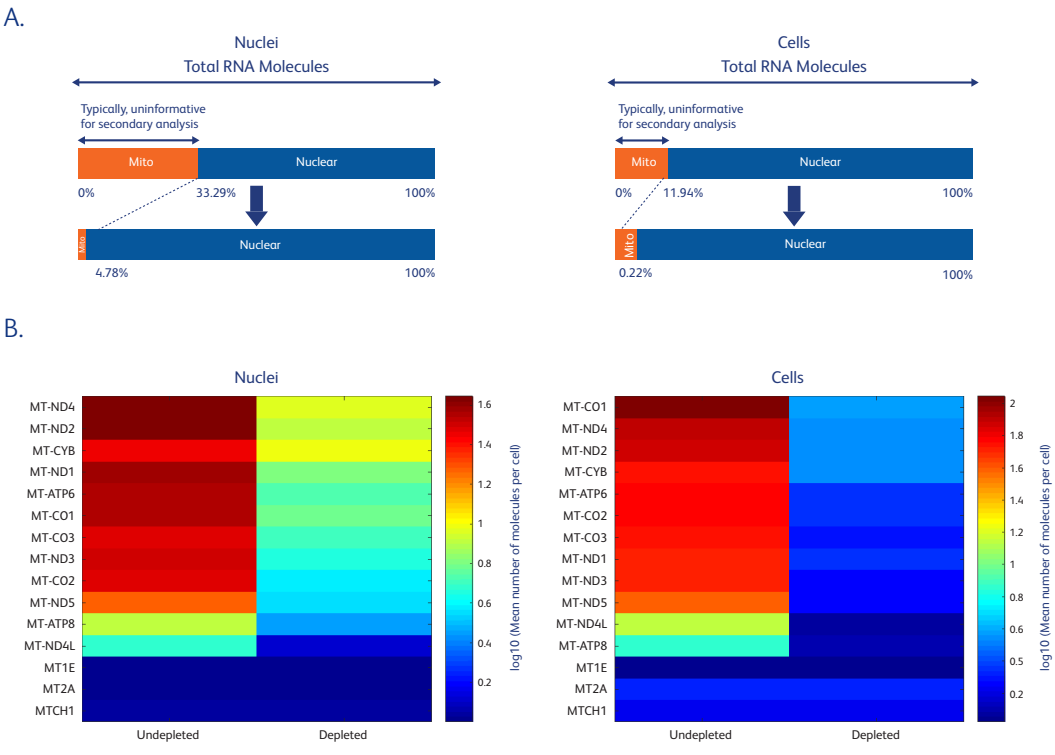

Figure 2. Reduction in the percentage of mito molecules after depletion with the DepleteX™ Mitochondrial DNA Depletion Kit. **A)** PBMC nuclei (left) and cell samples (right) were examined to compare the fraction of mito molecules in mRNA derived from the standard BD Rhapsody™ WTA Assay against their mito-depleted counterparts processed using the DepleteX™ Mitochondrial DNA Depletion Kit. The bar shows the distribution of total RNA molecules from a typical single-cell sequencing run pre and post depletion with the undesired mito portion distinctly highlighted in orange. **B)** Heatmaps illustrating the log10(median number of molecules per cell) for the mitochondrial genes in mito depleted and undepleted samples. Pairwise comparisons are shown for each of the 15 out of 17 mito target genes on PBMC nuclei (left) and cell samples (right). The remaining two mito targets were undetectable in these samples. Blue, lower expression; red, higher expression.

Mito-depletion faithfully maintains biological insights from data

To assess the potential effects of the DepleteX™ Mitochondrial DNA Depletion Kit on the overall gene expression landscape, a correlation plot of the entire transcriptome without the targeted mito genes was generated for the depleted versus undepleted samples. A correlation of R^2>0.99 was observed (Figure 3) indicating no discernible impact on the gene expression profiles of the remaining non-mito genes. In addition, the percent composition of cell types between mito-depleted and undepleted samples was also unchanged (Figure 4). Together, analysis of WTA gene expression and cell sample composition suggest that there are no discernible biological alterations after the application of the DepleteX™ Mitochondrial DNA Depletion Kit.

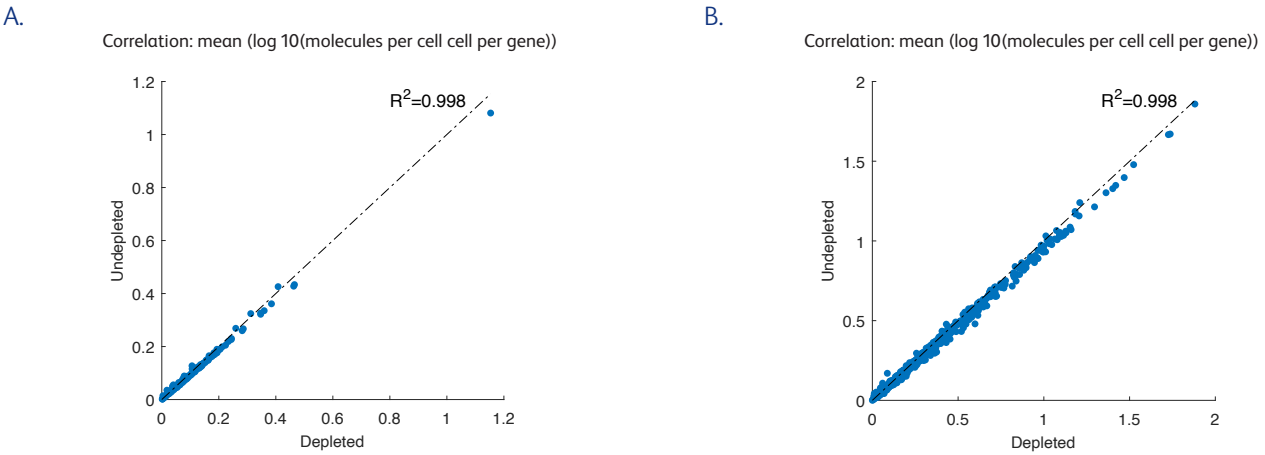

Figure 3. High gene expression correlation with no off-target effects with the DepleteX™ Mitochondrial DNA Depletion Kit. Gene expression correlation plots were generated for depleted and undepleted PBMC nuclei **(A)** and cell **(B)** samples. Here, the mito-depleted sample is represented on the x-axis and the undepleted sample on the y-axis, underscoring the kit's effectiveness in maintaining gene expression profiles between depleted and undepleted samples.

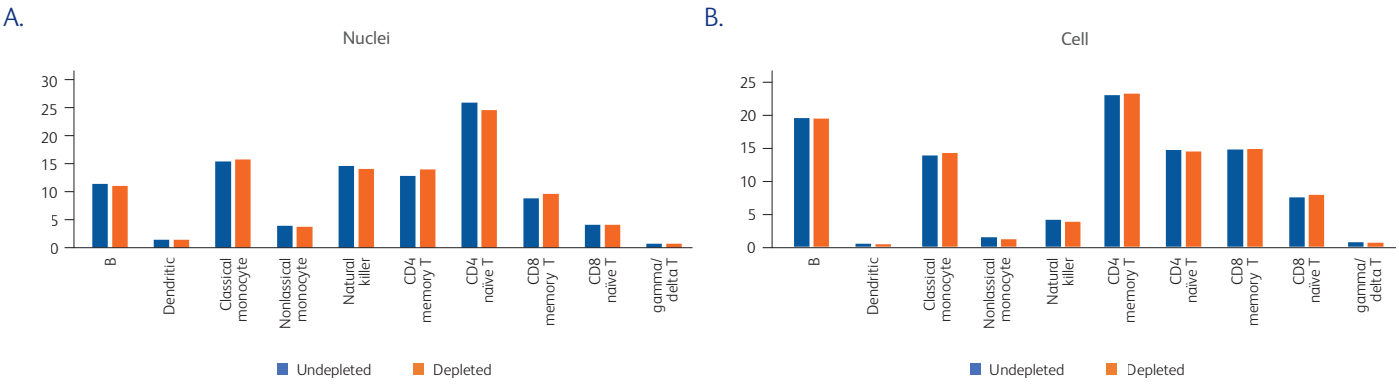

Figure 4. Cell type composition remains unchanged. Bar plots were generated for % of cell types for mito-depleted and undepleted nuclei **(A)** and cells **(B)** from PBMC samples and show that mito-depletion did not perturb cell type calls.

Conclusion

The Jumpcode Genomics DepleteX™ Mitochondrial DNA Depletion Kit selectively depletes the majority of mito transcripts from the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay data without introducing any change to the transcriptional profiles of non-mito genes, attesting to the kit's unparalleled specificity. The integration of the DepleteX™ Mitochondrial DNA Depletion Kit with the BD Rhapsody™ WTA Assay provides a solution for researchers looking to enrich their sample's non-mitochondrial transcriptomic information prior to sequencing.

Ordering information

Companion Products	
Description	Cat. No.
BD Rhapsody™ HT Xpress Package	666625
BD Rhapsody™ Scanner	633701
BD Rhapsody™ WTA Amplification Kit	633801
BD Rhapsody™ cDNA Kit	633773
BD Rhapsody™ 8-Lane Cartridge	666262
BD Rhapsody™ Enhanced Cartridge Reagent Kit	664887
DepleteX™ Mitochondrial DNA Depletion Kit	KIT2002

Additional resources

For any additional questions, contact **scomix@bdscomix.bd.com** or access the BD Rhapsody™ HT Xpress System and Scanner protocols online at **bdbiosciences.com/htxpress**.

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