

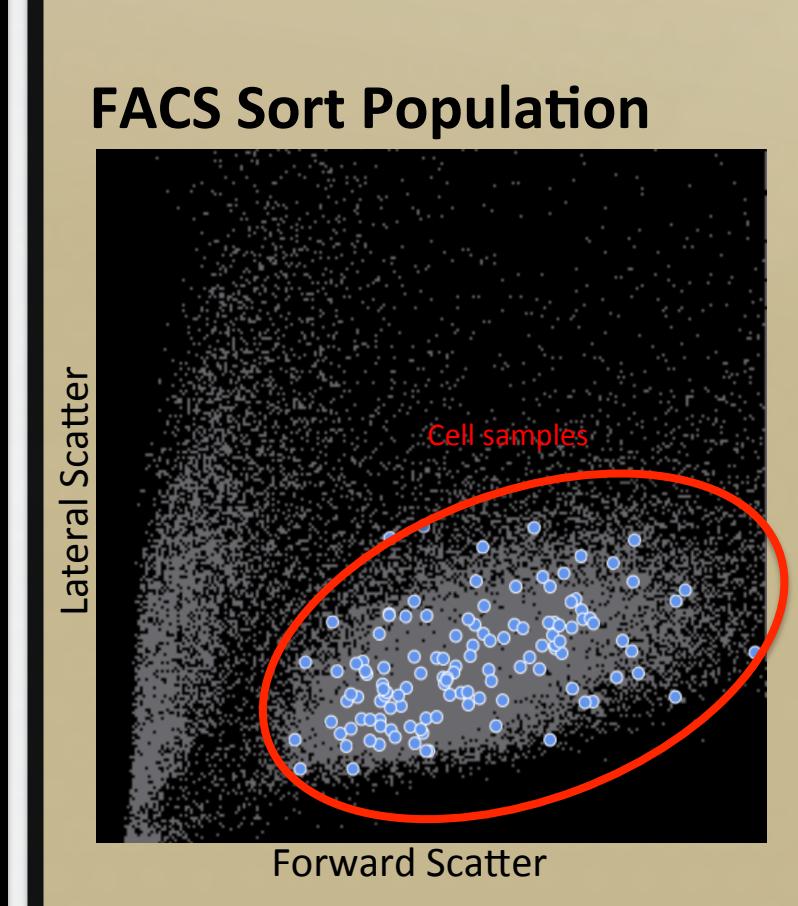


Mitochondrial DNA Copy Count in Cancer

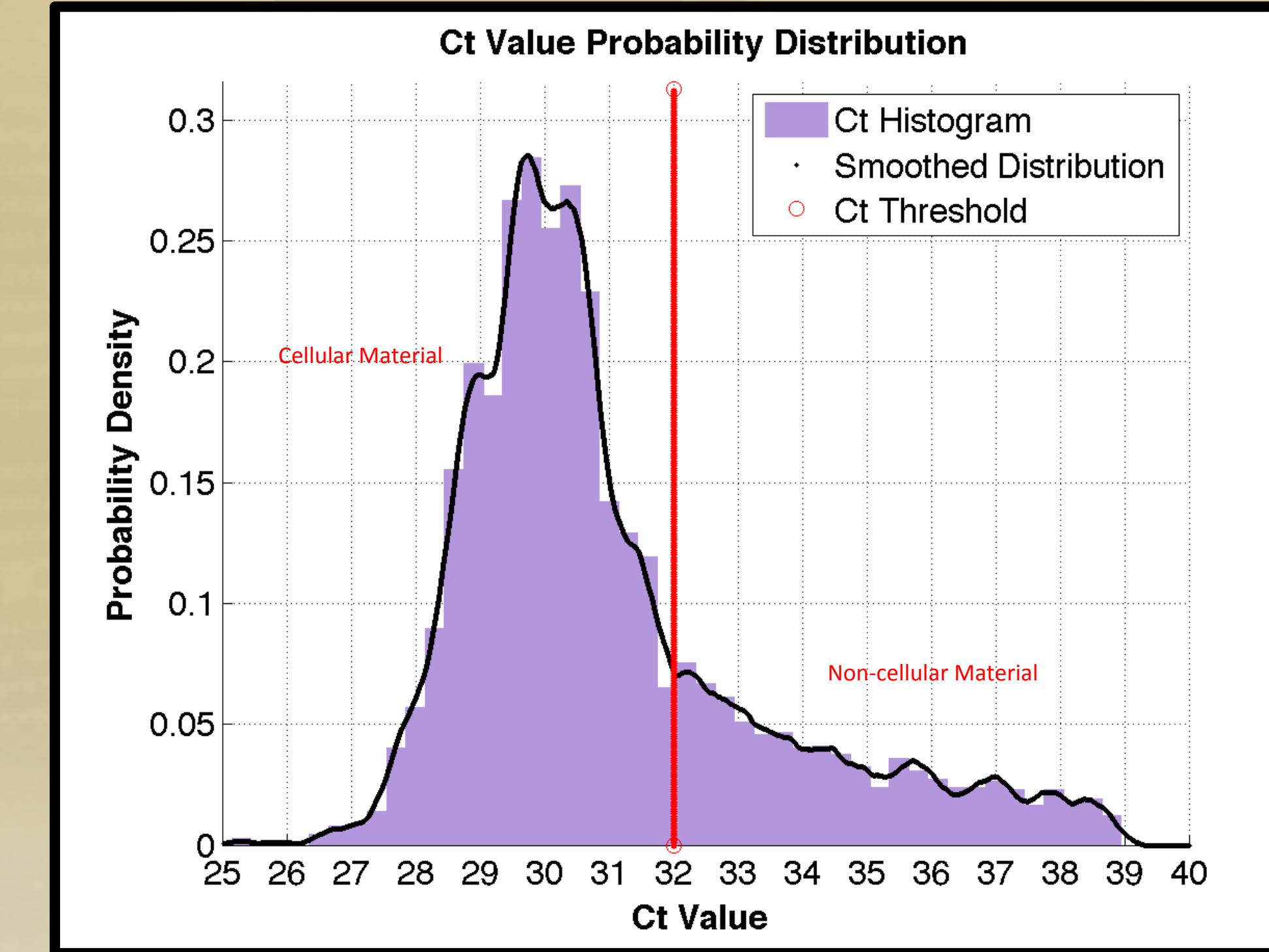
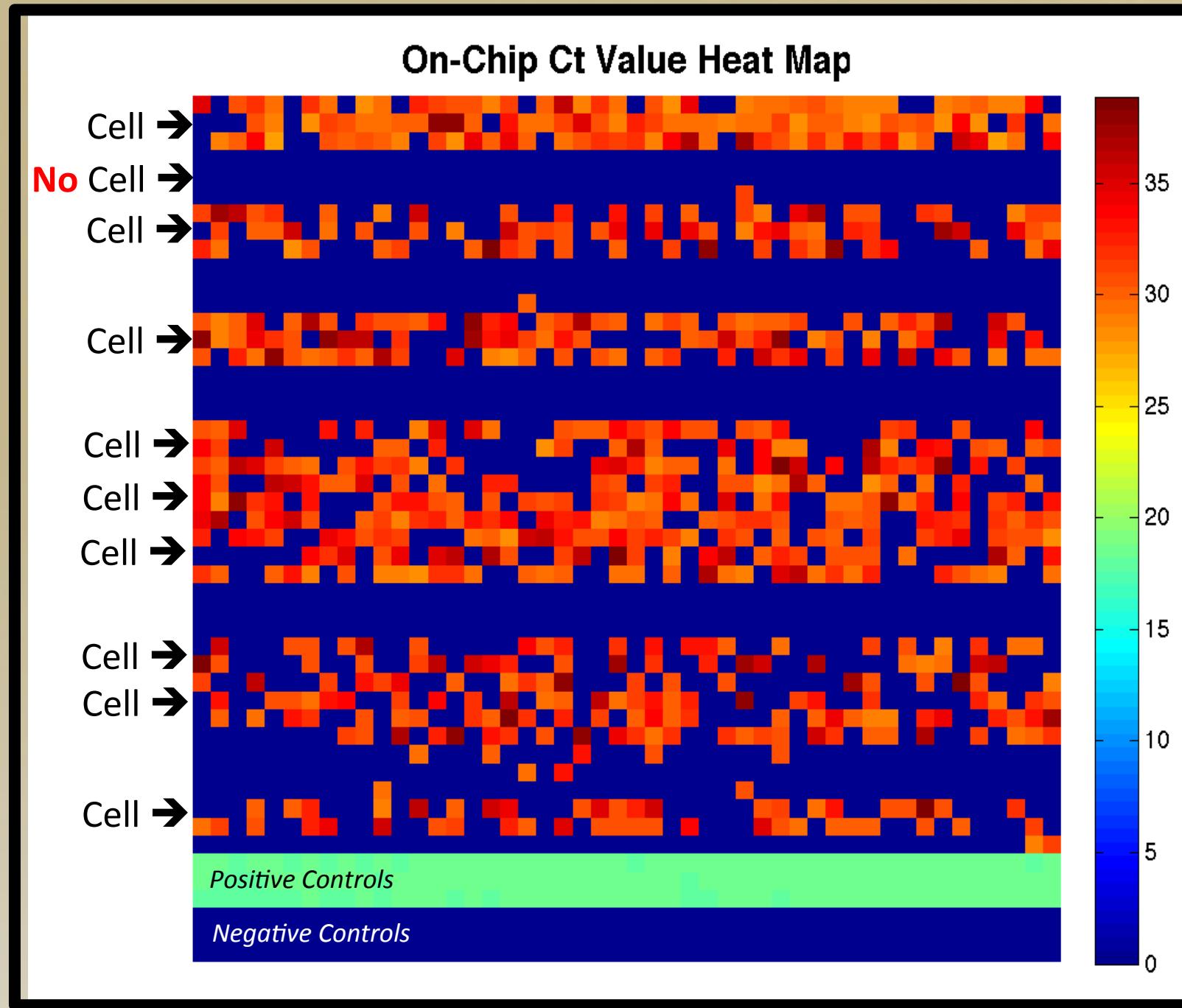
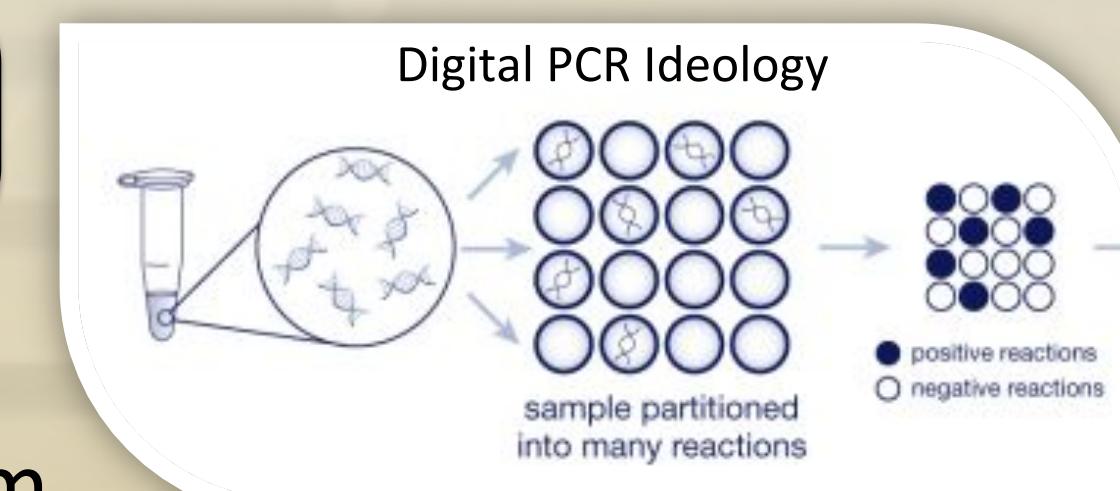
Introduction and Motivation

Human cells lack histones and reparative pathways for mitochondrial DNA (mtDNA), making mtDNA highly prone to oxidative damage. Pathogenic mtDNA mutations may cause over or under replication of mtDNA. For these reasons, it is theorized that mtDNA copy number is a cancer biomarker. This project aims to detect mtDNA copy number alterations at single-cell resolution. Success could not only elucidate underlying mechanisms of mtDNA regulation, but also allow mtDNA copy count to be used for cancer diagnosis and prognosis.

Experimental Design and Methods

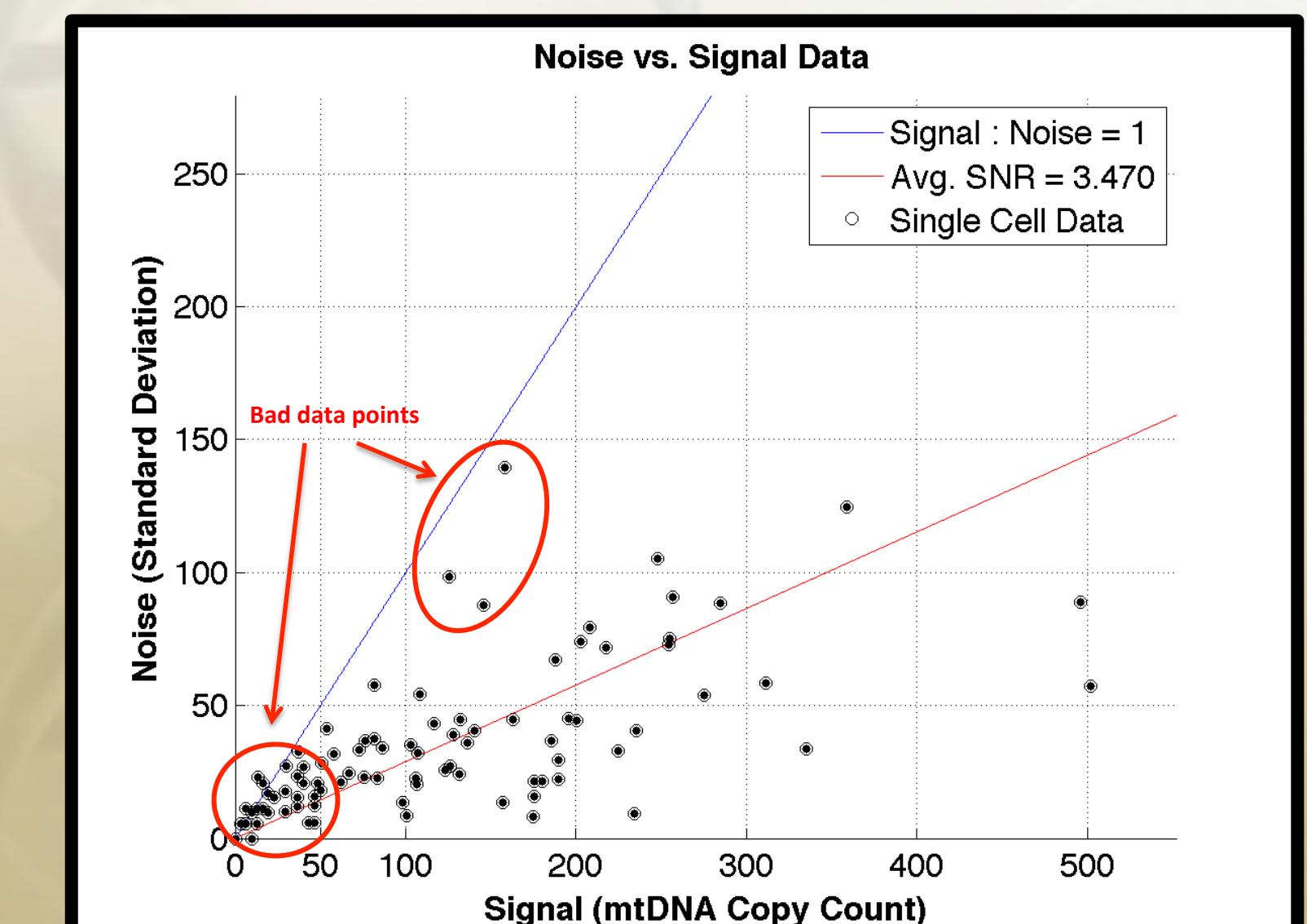


To quantify changes in mtDNA copy number in human cells, K562 cells were cultured with and without ethidium bromide (EtdB). EtdB reduces mtDNA count by inhibiting polymerase gamma. Single K562 cells were sorted using Fluorescence Assisted Cell Sorting (FACS), lysed in Triton-X and loaded into three inlets of a 48.48 Digital Array® Fluidigm Microfluidic Chip. The contents of each inlet was further partitioned into 48 reaction chambers and polymerase chain reaction (PCR) thermo-cycling was performed for 40 cycles. The number of reaction chambers in which DNA amplification reached threshold fluorescence before 32 cycles (Ct threshold) was used to extrapolate mtDNA copy count for each cell.



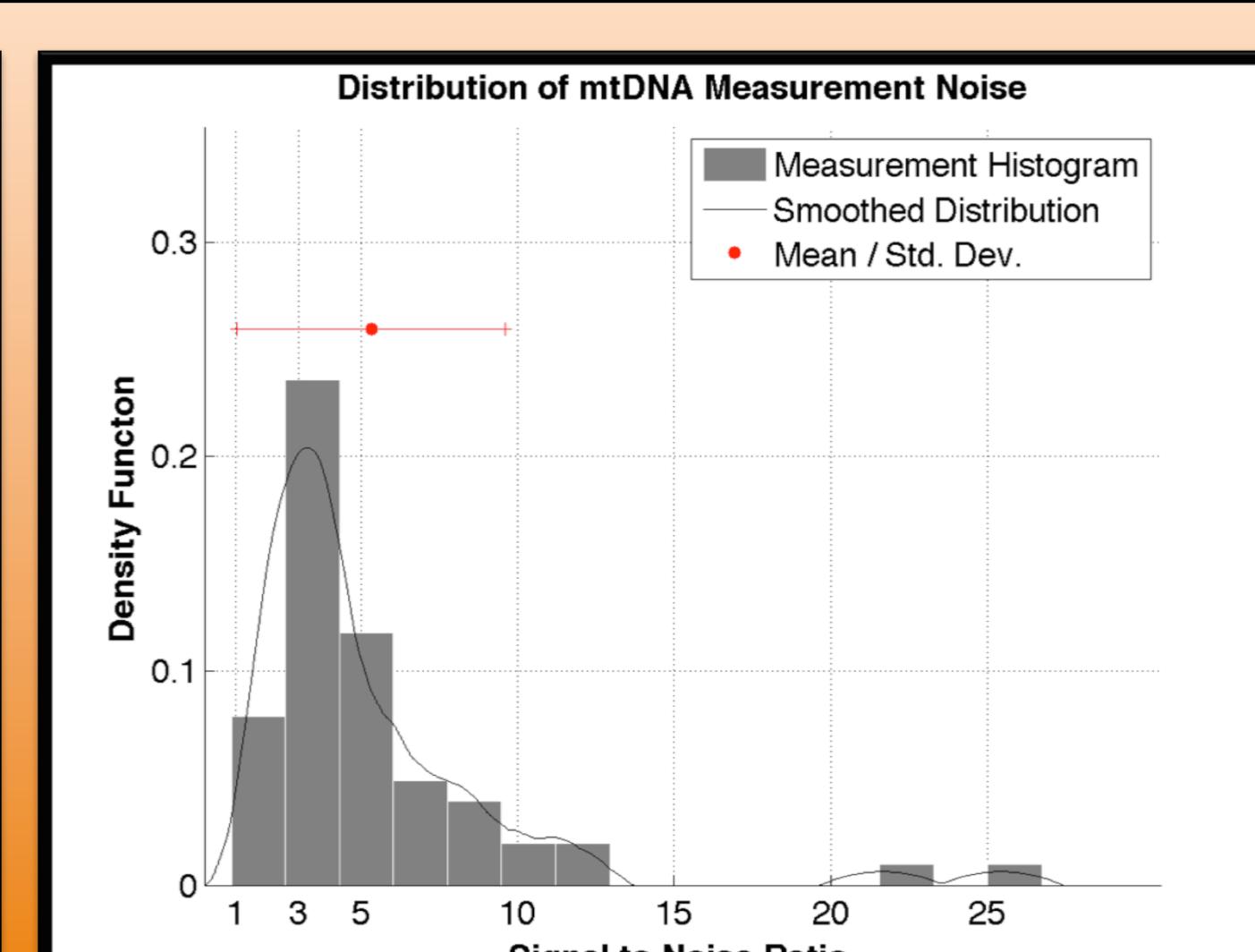
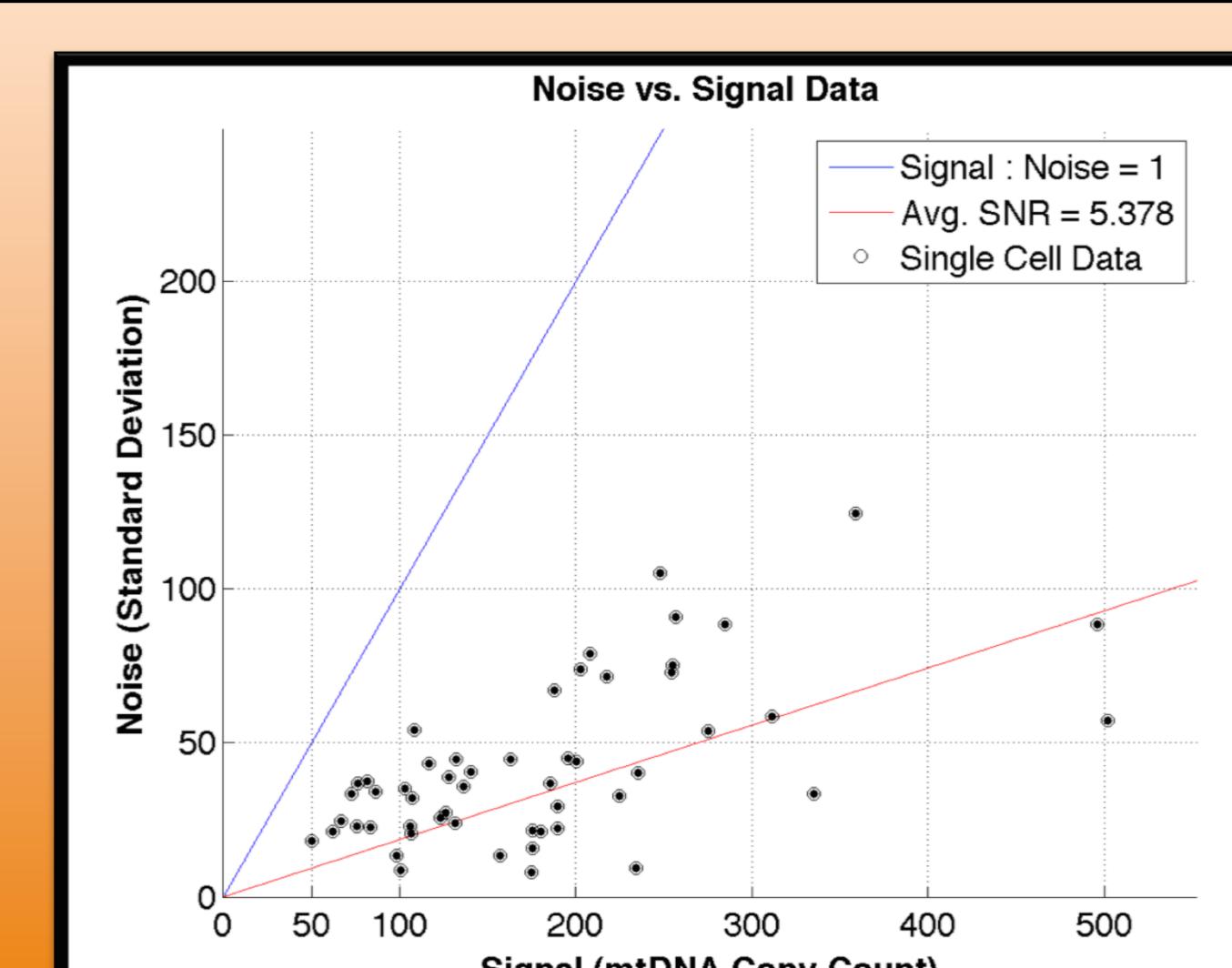
Data Quality and Filtering

Single-cell mtDNA copy count data was filtered based on signal to noise ratio (SNR). High SNR indicates greater confidence in measurement, thus higher quality of data. Samples with $\text{SNR} < 2$ were excluded from further cellular analysis. Samples with mtDNA copy count < 40 were deemed to be noise from amplification of non-cellular material. These data points were also excluded from further mtDNA copy count analysis.



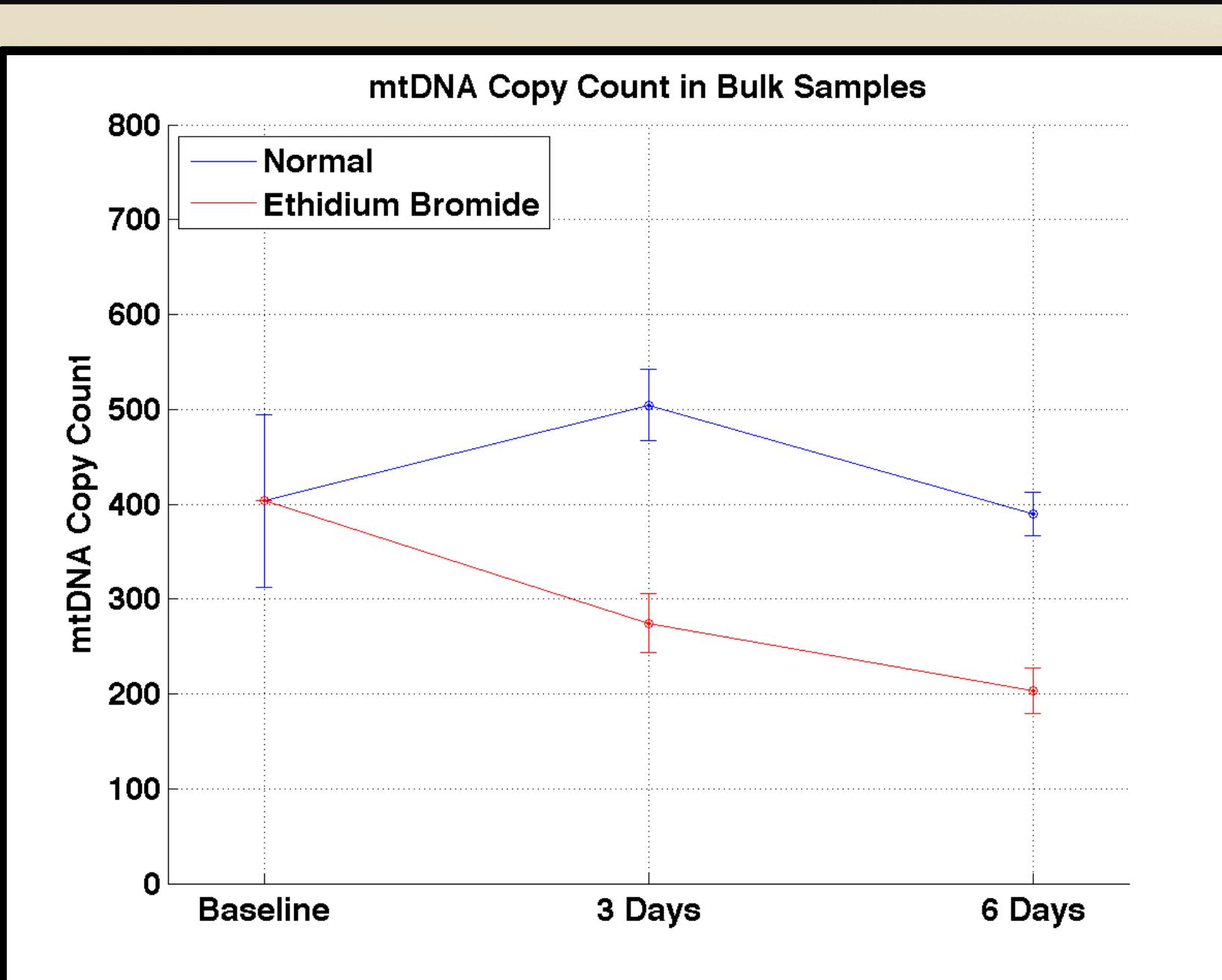
Filtered Data

Data filtering improves SNR from 3.470 to 5.378

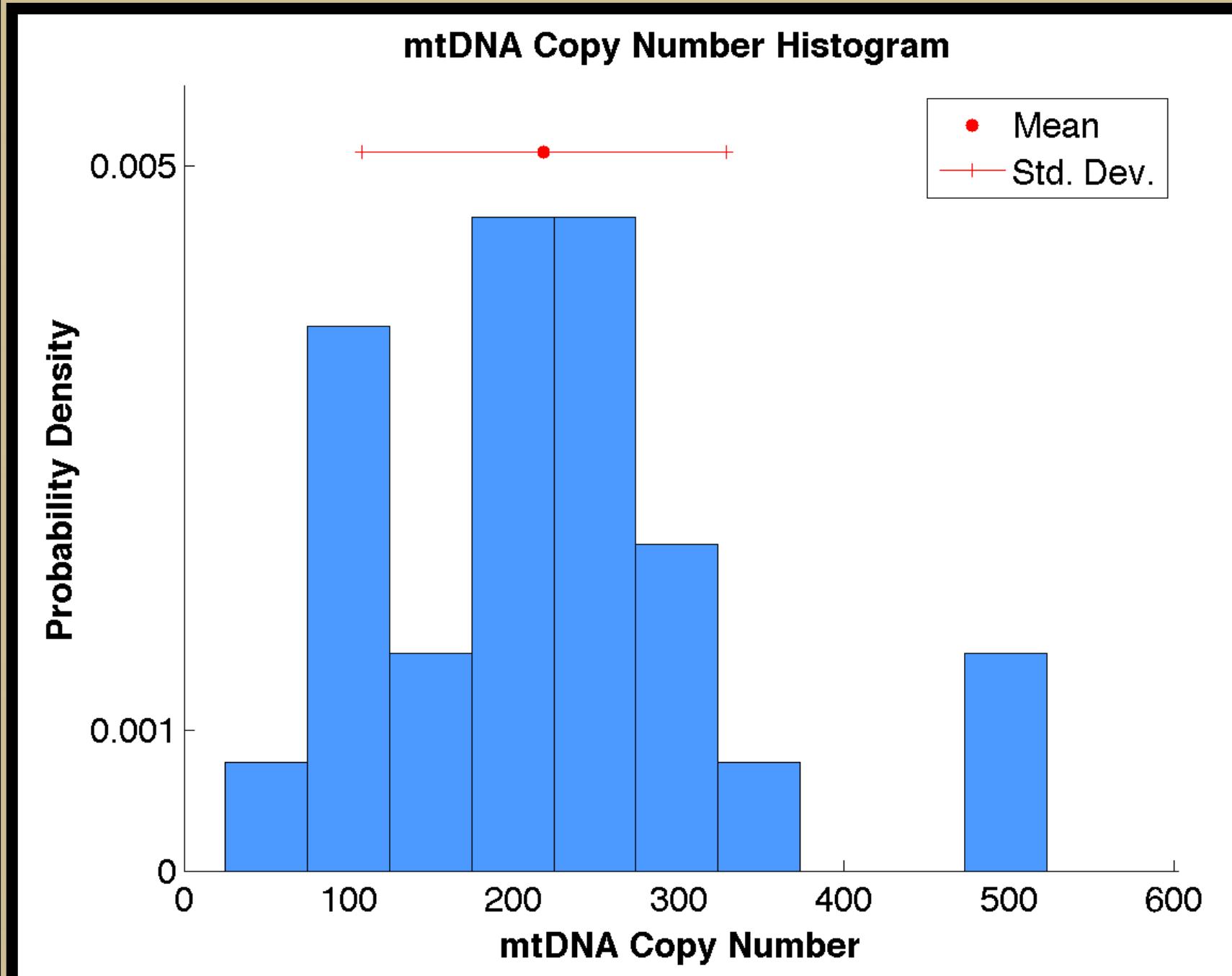


Results

Both single-cell and bulk measurement of mtDNA copy count confirm the hypothesis that ethidium bromide effectively reduces mtDNA copy count in K562 cells. Additionally, extrapolation of mtDNA copy count probability distribution from our data confirms the hypothesis that changes in mtDNA copy count distribution can be detected using single-cell PCR assays.

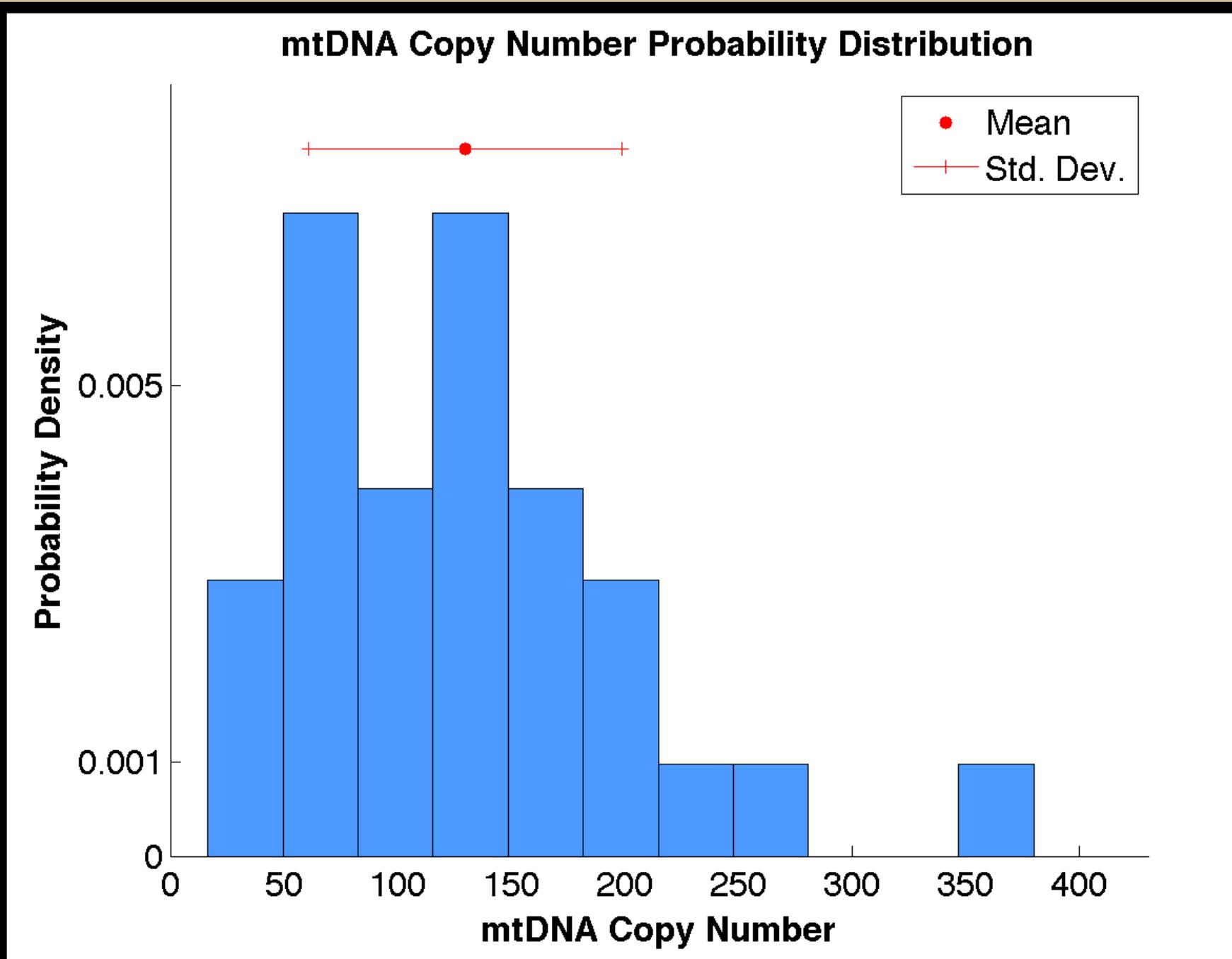


Normal K562 Cells



206 +/- 115 mtDNA genomes

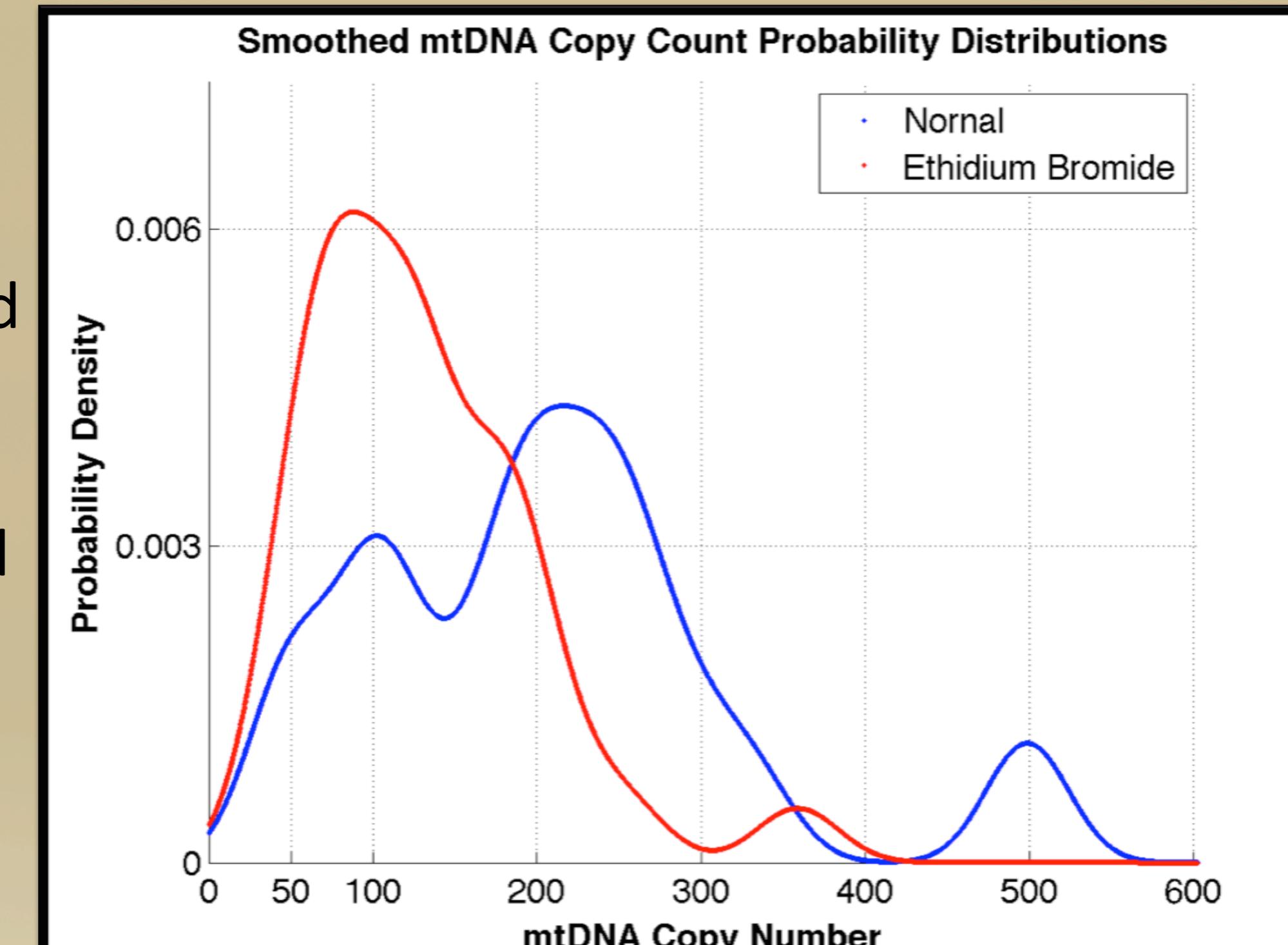
K562 cells in EtdBr for 7 Days



130 +/- 69 mtDNA genomes

Conclusions and Future Direction

The discrepancy in average mtDNA copy count between bulk and single-cell measurements suggests error in either method. This method confounded by incomplete lysis of mitochondria prior to the PCR assay. This problem could be solved by the suspension of single-cells in a more efficient lysis buffer, such as lysozyme. Further, single-cell methods are more prone to contamination, making the delineation of cellular and non-cellular measurements difficult. Despite these sources of error, our results suggest that a single-cell approach may be more accurate and yield more information about mtDNA count distribution than bulk measurement.



References:

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