The efficiency of amplification of small quantities of total RNA with the Transplex® Whole Transcriptome Amplification kit was assessed by evaluating the number of resulting cDNA molecules following amplification. Total RNA was isolated from 30- and 100-cell samples using the GenElute Total RNA Kit (Sigma Aldrich Catalog# NA1020). The isolated RNA was reverse transcribed using the GenElute PCR Cleanup kit (Sigma Aldrich Catalog# NA1020). Quantitative PCR products were analyzed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Amplification efficiency was calculated using the slope of the standard curve for each run.

Results and Discussion

Amplification of Cellular RNA: Total RNA was extracted from 30- and 100-cell samples using the GenElute Total RNA Kit (Sigma Aldrich Catalog# NA1020). The extracted RNA was treated with DNAse to remove any contaminating DNA. The treated RNA was then reverse transcribed using the GenElute PCR Cleanup kit (Sigma Aldrich Catalog# NA1020). Quantitative PCR products were analyzed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Amplification efficiency was calculated using the slope of the standard curve for each run.

Effect of Library Synthesis Primer Concentration on Downstream qPCR Application

The effect of library synthesis primer concentration on downstream qPCR application was assessed by analyzing the efficiency of amplification using different primer concentrations. The results showed that the efficiency of amplification increased with increasing primer concentration, reaching a peak at a concentration of 0.5X. Further increases in primer concentration resulted in a decrease in efficiency.

Materials and Methods

Total RNA isolation was performed using the GenElute Total RNA Kit (Sigma Aldrich Catalog# NA1020). The isolated RNA was treated with DNAse to remove any contaminating DNA. The treated RNA was then reverse transcribed using the GenElute PCR Cleanup kit (Sigma Aldrich Catalog# NA1020). Quantitative PCR products were analyzed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Amplification efficiency was calculated using the slope of the standard curve for each run.

Acknowledgements

Our appreciation to Albert Donnenberg, University of Pittsburgh for providing bone marrow samples. We also acknowledge the assistance of the Agilent Technical Communication Group for their assistance in the preparation of this poster. We would like to thank the Sigma-Aldrich team for their support and encouragement.

References

1. Transplex® Whole Transcriptome Amplification Reagent Kit. Sigma-Aldrich Catalog# NA1020.