WHICH REFERENCE GENES ARE MORE SUITABLE FOR AML CELL LINES IN RT-qPCR STUDIES?

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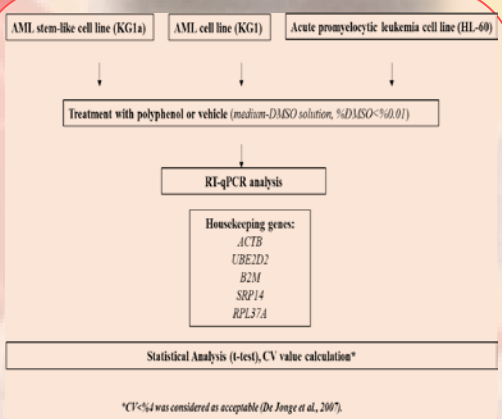
Introduction

Quantitative real-time reverse transcription-polymerase chain reaction (RT-qPCR) is a powerful technique in the diagnosis and molecular research. However, to obtain accurate and reliable results, the selection of suitable housekeeping genes is important. Selected reference genes should be compatible with the cell line or tissue and maintain stability under different experimental conditions with low coefficient variation (CV<0.04). Since housekeeping genes can vary according to the cell or tissue types, identifying the most suitable genes for each cell line is significant. This study aims to identify the most suitable housekeeping gene combinations for AML cell lines. For this purpose, we used three different AML cell lines which were AML cancer stem-like KG1a cell line (FAB 0), its parental and relatively more mature KG1 cell line (FAB1), and acute promyelocytic leukemia cell line HL-60 (FAB3). Five genes (ACTB, UBE2D2, B2M, SRP14, and RPL37A), recommended as the most stable genes for leukemia, were evaluated according to Coefficient Variation (CV) values. We propose different combinations of genes for each cell line studied.

Keywords: Acute myeloid leukemia, Cancer stem cells, Housekeeping gene, Real-time PCR

Material & Methods



Results

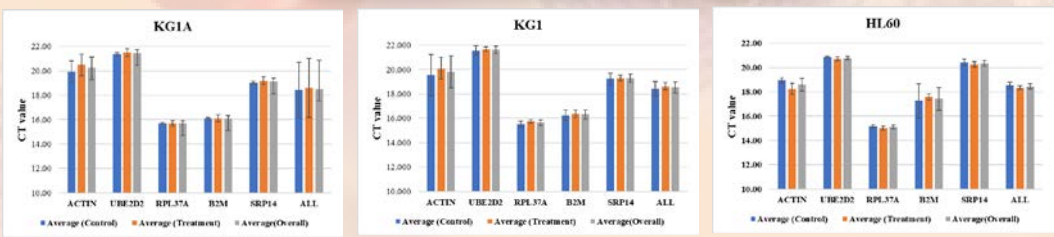


Fig. 1. RT-PCR average CT values of polyphenol-treated, control, all groups of KG1a, KG1, and HL-60 cell lines.

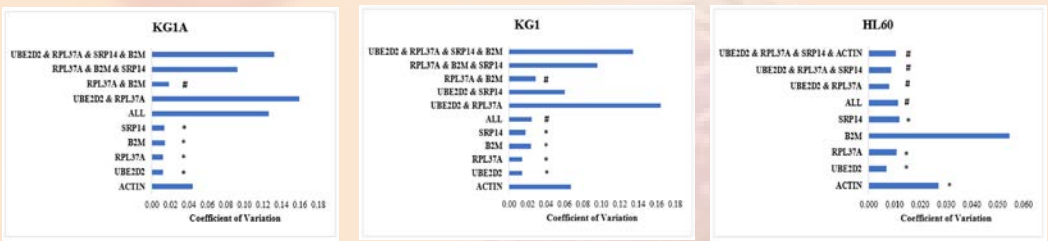


Fig. 2. Coefficient variation values of housekeeping genes and their combinations for KG1a, KG1, and HL-60 cell lines (*CV<0.04 for individual genes; # CV<0.04 for combinations.

Conclusion

According to the results, suitable genes were selected as in the below:

- ✓**KG1a and KG1 cell lines**: UBE2D2, B2M, SRP14 and RPL37A (CV<0.04) were individually suitable.
- ✓**KG1a cell line**: B2M & RPL37A (CV=0.018) was detected as the most suitable combination.
- ✓**KG1 cell line**: ACTB & UBE2D2 & B2M & SRP14 & RPL37A (CV=0.025) and B2M & RPL37A (CV=0.029) were the most useful housekeeping combinations.
- ✓**HL-60 cell line**: All individual genes except B2M displayed high stability. However, all combinations (UBE2D2 & RPL3A; UBE2D2 & RPL3A & SRP14; UBE2D2 & RPL3A & SRP14 & ACTIN; ACTB & UBE2D2 & B2M & SRP14 & RPL37A, CV <%4) can be used for the cell line.

References

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