High Throughput Gene Expression Analysis of Arabidopsis thaliana using Quantitative RT-PCR

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Abstract
Quantitative RT-PCR is a powerful tool for analysis of differential gene expression. Although the technology has been widely used for RNA quantification of individual genes, its application for high throughput gene expression screening is limited. We have developed a walk-away automated protocol for Quantitative RT-PCR using the Primer Library for Arabidopsis thaliana, which is designed and manufactured using Sigma-Genosys' Custom Oligo Program. For gene expression studies, the entire process was automated from primer library plate dilutions through quantitative analysis and melt curve analysis on the ABI PRISM 7700. — The entire process was automated from primer library plate dilutions through quantitative analysis and melt curve analysis on the ABI PRISM 7700.

Materials
Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich. Plant samples were obtained through a donation from the Donald Danforth Plant Science Center.

Methods
Human cells were grown in Dulbecco's Modified Eagle's Medium, 10% Fetal Bovine Serum, 4 mM L-glutamine, and 1 mM Sodium Pyruvate to about 90% confluence. The medium was discarded and 3 ml of Lysis solution was added to the dish for lysis. After the cells were lysed, the lysed cells were filtered and purified using the GenElute™ Total RNA Purification Kit. The SYBR® Green Quantitative RT-PCR Kit was used for all PCR reactions.

Conclusions
The primer library for Arabidopsis genes is suitable for expression analysis of defense-related study with high efficiency and reproducibility.

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Table 1. Gene Expression Analysis

Table 2. Gene Response in Pathogen Infection

Figure 1: Aliquots of Human Total RNA Samples

Figure 2: Melt Curve Analysis of Human Total RNA Samples

Figure 3: Real-time Quantification of Defense-response Genes

Figure 4: Melt Curve Analysis of Human Total RNA Samples

Figure 5: Real-time Quantification of Defense-response Genes

Figure 6: Quantitative RT-PCR of Defense-response Gene Expression

Figure 7: Melt Curve Analysis of Human Total RNA Samples

Figure 8: Quantitative RT-PCR of Defense-response Gene Expression

Figure 9: Melt Curve Analysis of Human Total RNA Samples

Figure 10: Quantitative RT-PCR of Defense-response Gene Expression

Figure 11: Melt Curve Analysis of Human Total RNA Samples

Figure 12: Quantitative RT-PCR of Defense-response Gene Expression

Figure 13: Melt Curve Analysis of Human Total RNA Samples

Figure 14: Quantitative RT-PCR of Defense-response Gene Expression