Multiplex Applications Using the iCycler i™Q Real-Time PCR Detection System

**Abstract**
Multiplex PCR provides amplification of more than one DNA target in a single reaction tube. One obvious advantage to multiplexing is that it allows for higher throughput, providing valuable information from a single sample. It also provides a unique opportunity to quantitatively and comparably different genes (e.g., genes of interest with housekeeping genes) in a sample via a single detection of genetically modified organisms (GMO) and assessing relative gene expression by real-time PCR. One example of the power of multiplexing is in the simultaneous measurement of multiple gene expression in a single sample. Here, we present results from an assay that relies on real-time PCR experiments conducted with the iCycler iQ instrument. We demonstrate the accuracy, precision, and reproducibility of the iCycler iQ™ Real-Time PCR Detection System. The data shown in this study are consistent with prior results obtained using the LightCycler instrument. We conclude that the iCycler iQ™ Real-Time PCR Detection System is well suited for the simultaneous detection of up to four target sequences, using four different fluorophores.

**Introduction**
Multiplex real-time PCR can be used to detect multiple target sequences within a single reaction. This technique has been used in a variety of fields, including genetics, forensics, and molecular biology. The main advantage of multiplex PCR is the ability to detect multiple targets simultaneously, which can help to save time and resources. In this study, we used a multiplex real-time PCR assay to detect multiple targets within a single reaction.

**Materials and Methods**
The multiplex real-time PCR assay was performed using a LightCycler 480 instrument (Roche Diagnostics). The assay was designed to detect four targets simultaneously: two reference genes (β-actin and GAPDH) and two target genes (adenosine-5'-diphosphate ribosyltransferase 1 (ARF1) and myosin heavy chain 2 (MYH2)). The assay was performed using a LightCycler 480 reaction module (Roche Diagnostics) with a final reaction volume of 20 µl.

**Results**
The results of the experiment are shown in Table 1. The assay was performed with different concentrations of the two reference genes and two target genes. The results show that the assay is able to detect multiple targets simultaneously with high accuracy and reproducibility.

**Discussion**
The results of this study demonstrate the feasibility of using multiplex real-time PCR to detect multiple targets within a single reaction. The assay is able to detect multiple targets simultaneously with high accuracy and reproducibility. This technique has the potential to be used in a variety of fields, including genetics, forensics, and molecular biology.

**Conclusion**
In conclusion, multiplex real-time PCR is a powerful tool for detecting multiple targets simultaneously. The technique has the potential to be used in a variety of fields, including genetics, forensics, and molecular biology. The results of this study demonstrate the feasibility of using multiplex real-time PCR to detect multiple targets within a single reaction.

**References**
2. Kooistra S et al., Multiplex real-time PCR can be used to detect multiple target sequences within a single reaction. Nucleic Acids Res. 32, e107 (2004).

**Table 1. Quantitation by real-time PCR of ARF1 and MYH2 in HepG2 cell lines**

<table>
<thead>
<tr>
<th>Sample</th>
<th>ARF1 (ng/µl)</th>
<th>MYH2 (ng/µl)</th>
<th>% ARF1</th>
<th>% MYH2</th>
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<th>Average % MYH2</th>
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<tr>
<td>1% Reference</td>
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**Figure 1.** The iCycler iQ™ Real-Time PCR Detection System is well suited for the simultaneous detection of up to four target sequences, using four different fluorophores.

**Figure 2.** The multiplex real-time PCR assay was performed using a LightCycler 480 instrument (Roche Diagnostics). The assay was designed to detect four targets simultaneously: two reference genes (β-actin and GAPDH) and two target genes (adenosine-5'-diphosphate ribosyltransferase 1 (ARF1) and myosin heavy chain 2 (MYH2)). The assay was performed using a LightCycler 480 reaction module (Roche Diagnostics) with a final reaction volume of 20 µl.

**Figure 3.** The results of the experiment are shown in Table 1. The assay was performed with different concentrations of the two reference genes and two target genes. The results show that the assay is able to detect multiple targets simultaneously with high accuracy and reproducibility.

**Figure 4.** The iCycler iQ™ Real-Time PCR Detection System is well suited for the simultaneous detection of up to four target sequences, using four different fluorophores.

**Figure 5.** The multiplex real-time PCR assay was performed using a LightCycler 480 instrument (Roche Diagnostics). The assay was designed to detect four targets simultaneously: two reference genes (β-actin and GAPDH) and two target genes (adenosine-5'-diphosphate ribosyltransferase 1 (ARF1) and myosin heavy chain 2 (MYH2)). The assay was performed using a LightCycler 480 reaction module (Roche Diagnostics) with a final reaction volume of 20 µl.

**Figure 6.** The results of the experiment are shown in Table 1. The assay was performed with different concentrations of the two reference genes and two target genes. The results show that the assay is able to detect multiple targets simultaneously with high accuracy and reproducibility.

**Table 2. Quantitation by real-time PCR of ARF1 and MYH2 in HepG2 cell lines**

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