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Real-time qPCR

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We use this protocol and it's working

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Abstract

Real-time qPCR

1 **Prepare cDNA Samples** - Plate cDNA samples on a 96-well qPCR plate.

2 **Prepare Reaction Mix** - Combine the following in each well: - 5 µL Fast SYBR Green Master Mix (4385616, Applied Biosystems) - 3 µL nuclease-free water - 0.5 µL forward primer - 0.5 µL reverse primer - 1 µL cDNA sample

3 **Ensure Technical Replicates** - Plate each sample two to four times to ensure technical replicates.

4 **Prepare Negative Control** - Use a control sample consisting of water with primers and Master Mix as a negative control.

5 **Perform qPCR** - Collect cycle threshold (Ct) values for each well.

6 **Normalize Data** - Normalize Ct values to GAPDH as a housekeeping gene.

7 **Primer Sequences** - Forward (F) primer for Atg7: 5′- GTTCGCCCCCTTTAATAGTGC -3′ - Reverse (R) primer for Atg7: 5′- TGAACTCCAACGTCAAGCGG -3′