

Biotechnologies towards Sustainable Development in Malaysia

Zarina Zainuddin

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Chapter 11

Important considerations in qRT-PCR

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Introduction

Reliability of qRT-PCR data is essential, where linearity, precision, specificity, limitation of detection and quantification must be taken into account (Vaerman *et al.*, 2004). To achieve biologically meaningful results, it is important to implement several procedures and precautions to minimize experimental error. These procedures can be incorporated into a protocol at many stages, including sampling, total RNA isolation, and design of the internal reference. The following strategies must be considered carefully when optimising the specificity, sensitivity, and reproducibility of the reactions.

Sample size and collection

Ensuring replicate samples are of a similar size, by sampling similar tissue volumes or weights, is the first stage of reducing experimental error. However, this can be difficult due to the nature of biological samples (Huggett *et al.*, 2005). Materials should be harvested from ≥ 3 biological replicates for statistical analysis, frozen immediately in liquid nitrogen, and stored at -80°C to minimize RNA degradation (Udvardi *et al.*, 2008).