EntroGen's Internal Quality Control Assay enables accurate measurement of DNA input to complement mutation detection assays

Amelia Stanco and Gary Potikyan

Introduction

Optimal template DNA concentration is a critical component of qPCR-based mutation detection kits. Too much DNA may result in loss of assay specificity or inaccurate measurement due to the presence of PCR inhibitors. Too little DNA may result in reduced assay sensitivity.

The Internal Quality Control Assay (cat. no. IQCA-RT50) is designed for measurement of DNA input and can be utilized to determine optimal amounts of DNA sample for assessment using our mutation detection kits. Here we demonstrate that EntroGen's IQCA can be applied to accurately adjust DNA sample concentration for use in the Colorectal Cancer (cat. no. CRC-RT48) Mutation Detection Panel.

Product Application

IQCA contains all necessary reagents for the amplification of DNA samples by qPCR with the exception of the DNA template. As an example of its utility, IQCA was applied towards reaction 1 of the CRC assay (detection of KRAS codon 12, 13, and 117 mutations). Amplification of 108 DNA samples isolated from formalin fixed, paraffin embedded tumors with CRC reaction 1 before IQCA correction is depicted in Figure 1. Of these FFPE DNA samples, 40.7% (44/108) are outside of the optimal internal control (IC; 26-29) C_T range for the CRC assay.

In order to reduce sample quality failure rate, IQCA was used to determine the amplifiable quantity of each sample. The amount of sample loaded into the CRC assay was adjusted using a simple formula that takes into account the resulting IQCA C_T value of each sample. Amplification of 108 FFPE DNA samples with CRC reaction 1 after IQCA correction is shown in Figure 2. Of these FFPE DNA samples, only 2.78% (3/108) are outside of the optimal IC C_T range for the CRC assay. Two of these samples have an IQCA VIC C_T value of >32.

Figure 1. FFPE DNA sample amplification with CRC reaction 1 before IQCA assay correction.

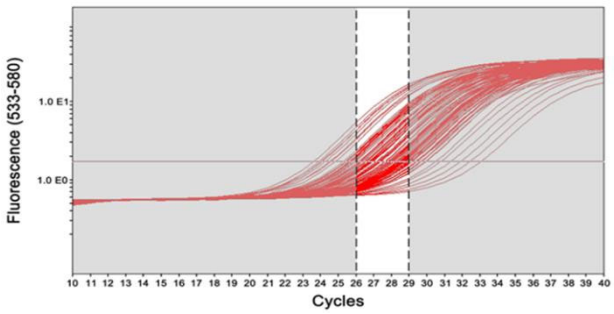
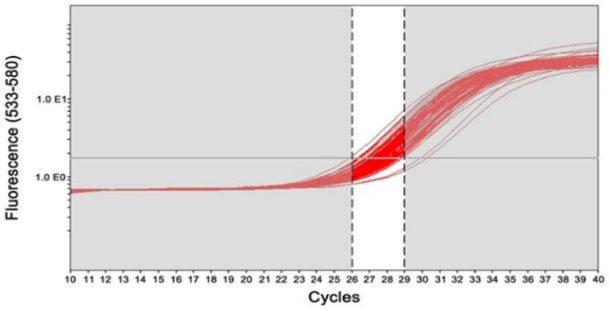


Figure 2. FFPE DNA sample amplification with CRC reaction 1 after IQCA assay correction.



Of note, it may not be possible to bring DNA samples with an IQCA VIC C_T value of >32 into CRC assay range due to sample volume limitations. The third sample is likely out of range due to pipetting error.

Conclusions

Here we demonstrate substantial improvement in the alignment of DNA sample concentration to qPCR-based mutation detection kit parameters. Our results show that adjusting sample input volume using the IQCA drastically reduces the need for re-runs due to sample quality issues (>40% sample quality failure rate without vs <3% with IQCA).