INTEGRATED DNA TECHNOLOGIES

Easily-Designed Standard Curves for qPCR

Creating artificial templates using gBlocks® Gene Fragments

Sequence-verified, dsDNA gBlocks Gene Fragments, are a great alternative to single-stranded oligonucleotides for creating long, custom DNA sequences. Provided in lengths of 125–2000 bp, these fragments function exactly like a double-stranded PCR products in cloning applications, while offering all of the sequence flexibility of custom, chemically synthesized DNA. Include necessary sequence overlaps or restriction sites for isothermal assembly or cloning into any plasmid, for production of additional high-fidelity template. Read about these and other dsDNA applications, see www.idtdna.com/gblocks.

Generate multiple standard curves from a single template

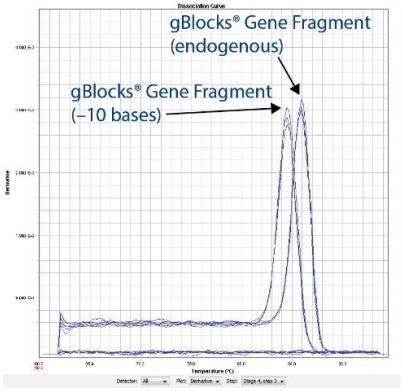
gBlocks Gene Fragments are uniquely advantageous for incorporating multiple control amplicon sequences into a single double-stranded construct. Using them as multi-control templates lowers cost to a level that is comparable to ordering individual oligos on a per assay basis. It also provides some unique benefits when performing qPCR experiments in the lab:

- When performing multiplex experiments, combining control templates onto a single construct means less pipetting and, thus, less experimental variability. Each assay on that construct will have exactly the same amount of template available, providing for more accurate comparisons between those assays.
- For singleplex reactions, you need only make one set of dilutions. Those dilutions can then be used for all the assays represented on that construct. Again, this reduces the chances for pipetting error, and saves time diluting multiple, distinct templates.

When designing gBlocks Gene Fragments with multiple targets, some researchers choose to separate each sequence with several intervening T bases. However, do not add more than 9 T bases between sequence elements, as this will interfere with the synthesis of your gBlocks fragment.

Use dsDNA fragments to detect contamination

Another benefit of dsDNA fragments for qPCR is the ability to quickly generate artificial sequences that can be distinguished from wild-type sequences. In Figure 1, an artificial construct that is 10 bp shorter than the wild-type sequence (LIMK1(–10)) can be distinguished by performing melt curve analysis using intercalating dye–based assays, such as with SYBR® Green. This is very useful if you have concerns about possible contamination by wild-type DNA.



Hs LIMK1 endogenous amplicon sequence

GAACATCATCCACCGAGACCTCAACTCCCACA
ACTGCCTGGTCCGCGAGAACAAGAATGTGGTG
GTGGCTGACTTCGGGCTGGCGCGTCTCATGGT
GGACGACGAAGACT

Hs LIMK1 (–10) sequence

GAACATCATCCACCGAGACCTCAACTCCCACA
ACTGCCAACAAGAATGTGGTGGTGGCTGACTT
CGGGCTGGCGCGTCTCATGGTGGACGACGAAG
ACT

Figure 1. Artificial control sequences can identify contaminating DNA. In this example, which uses a SYBR® Green dye–based assay, the artificial LIMK1(–10) sequence is easily distinguished from a wild-type sequence by the lower peak on this melt curve analysis.

For more information about gBlocks Gene Fragments, go to www.idtdna.com/gblocks.