gBlock control for multiple amplicons:

To begin designing your positive control, align your primer sequences with the target to identify the amplicon:

Example:

Forward: CAGCAGCCATTCAAGCAATGC

Reverse : GGTGGAGACCTAATTGGGCTGATTAG

Probe: TATCGGCGATATCGGTTTCATCCTCG

>NC\_012920.1 Homo sapiens mitochondrion, complete genome

TTATCAGTCTCTTCCCCACAACAATATTCATGTGCCTAGACCAAGAAGTTATTATCTCGAACTGACACTGAGCCACAACCCAAACAACCCAGCTCTCCCTAAGCTTCAAACTAGACTACTTCTCCATAATATTCATCCCTGTAGCATTGTTCGTTACATGGTCCATCATAGAATTCTCACTGTGATATATAAACTCAGACCCAAACATTAATCAGTTCTTCAAATATCTACTCATCTTCCTAATTACCATACTAATCTTAGTTACCGCTAACAACCTATTCCAACTGTTCATCGGCTGAGAGGGCGTAGGAATTATATCCTTCTTGCTCATCAGTTGATGATACGCCCGAGCAG**ATGCCAACACAGCAGCCATTCAAGCAATGCTATACAACCGTATCGGCGATATCGGTTTCATCCTCGCCTTAGCATGATTTATCCTACACTCCAACTCATGAGACCCACAACAAATAGCCCTTCTAAACGCTAATCCAAGCCTCACCCCACTACTAGGCCTCCTCCTAGCAGCAGCAGGCAATCAGCCCAATTAGGTCTCCACCCCTGACTCCC**CTCAGCCATAGAAGGCCCCACCCCAGTCTCAGCCCTACTCCACTCAAGCACTATAGTTGTAGCAGGAATCTTCTTACTCATCCGCTTCCACCCCCTAGCAGAAAATAGCCCACTAATCCAAACTCTAACACTATGCTTAGGCGCTATCACCACTCTGTTCGCAGCAGTCTGCGCCCTTACACAAAATGACATCAAAAAAATCGTAGCCTTCTCCACTTCAAGTCAACTAGGACTCATAATAGTTACAATCGGCATCAACCAACCACACCTAGCATTCCTGCACATCTGTACCCACGCCTTCTTCAAAGCCATACTATTTATGTGCTCCGGGTCCATCATCCACAACCTTAACAATGAACAAGATATTCGAAAAATAGGAGGACTACTCAAAACCATACCTCTCACTTCAACCTCCCTCACCATTGGCAGCCTAGCATTAGCAGGAATACCTTTCCTCACAGGTTTCTACTCCAAAGACCACATCATCGAAACCGCAAACATATCATACACAAACGCCTGAGCCCTATCTATTACTCTCATCGCTACCTCCCTGACAAGCGCCTATAGCACTCGAATAATTCTTCTCACCCTAACAGGTCAACCTCGCTTCCCCACCCTTACTAACATTAACGAAAATAACCCCACCCTACTAAACCCCATTAAACGCCTGGCAGCCGGAAGCCTATTCGCAGGATTTCTCATTACTAACAACATTTCCCCCGCATCCCCCTTCCAAACAACAATCCCCCTCTACCTAAAACTCACAGCCCTCGCTGTCACTTTCCTAGGACTTCTAACAGCCCTAGACCTCAACTACCTAACCAACAAACTTAAAATAAAATCCCCACTATGCACATTTTATTTCTCCAACATACTCGGATTCTACCCTAGCATCACA

Copy the amplicon plus a few flanking bases. You do not need to order the entire gene sequence.

**ATGCCAACACAGCAGCCATTCAAGCAATGCTATACAACCGTATCGGCGATATCGGTTTCATCCTCGCCTTAGCATGATTTATCCTACACTCCAACTCATGAGACCCACAACAAATAGCCCTTCTAAACGCTAATCCAAGCCTCACCCCACTACTAGGCCTCCTCCTAGCAGCAGCAGGCAATCAGCCCAATTAGGTCTCCACCCCTGACTCCC**

If you need to design a single control with multiple amplicons you will want to include a unique ~20bp random nucleotide spacer between each amplicon and add a 30bp random spacer to each end of the final gBlock so shift the priming regions away from the termini. This can help to improve reproducibility if there is a small amount of gBlock degradation over time.

(30bp flank #1) –Amplicon 1 – (20bp spacer#1) – amplicon 2 – (20bp spacer#2) – amplicon 3 – (30bp flank #2)

Some of the random spacers we have used successfully before are listed below. Any sequence that has 45-55% g/c content and that lacks repeats, hairpins, and poly base runs should be ok to use.

20bp sequences:

Accgagttgcccgttaaagt

Agatccgatgcgtaaccgtt

Aatggcgtccagttgtcaca

Accaatggctttccgagatg

30bp sequences:

tgcatgatctacgtgcgtcacatgcagtac

cactagctcagattcagtagaccgctgttg

tagtaatgcagacacttgcggtccatctcg

agctgtcagcactactaacttgcggtcagt