qPCR conversions from mass (ng) to copy number using fragment length.

Genomic DNA Standards for the qPCR assay are measured on Qubit with HS fluorometer that is accurate between 0.1 ng – 100ng.

**Chum versus all other salmon: Keta222 is 143 bp fragment**

**Chinook and COHO: ChiCoh1269 is 75 bp fragment**

Chinook standard 0.17 ng/ ul x 2 ul = 0.34 or 4.13 x 10^9

Dilution series goes 4.13 x 10^5 or 10^4

Coho standard 0.15 ng/ul x 2 – 0.30 or 3.65 x 10^9

Dilution series goes 3.65 x 10^5 or 10^4

**Coho versus O.mykiss. mykiss207 is 98 bp fragment**

From Scienceprimer.com

This calculation assumes the average mass of an RNA nucleotide is 340 Daltons (Da), the mass of a DNA nucleotide is 330 Da and the mass of a basepair (bp) in double stranded DNA is 660 Da. This makes the formula weight\* of RNA nucleotides\* 340 g, the formula weight of DNA nucleotides 330 g and the formula weight of the bp's in double stranded DNA 660 g.

Using these numbers, the formula weight of any single or double strand of nucleotides can be estimated by taking the product of the strand length (in bases or bp's) and the average formula weight of the type of nucleotide in the strand.

The inverse of the formula weight is the number of moles of template present in one gram of material.

Using Avogadro's number, 6.022×1023 molecules/mole, the number of molecules of the template per gram can be calculated:

/var/folders/7n/hz4tls550nq535glm9wj3bp400d3b5/T/com.microsoft.Word/WebArchiveCopyPasteTempFiles/molToMolec.png

Finally, the number of molecules or number of copies of template in the sample can be estimated by multiplying by 1×109 to convert to ng and then multiplying by the amount of template (in ng)

This calculator requires the user to input the amount of a template present (in ngs) and the length of the template (in bases or bp's) and with this information the number of copies of the template is calculated.

The formula used is:

/var/folders/7n/hz4tls550nq535glm9wj3bp400d3b5/T/com.microsoft.Word/WebArchiveCopyPasteTempFiles/copies660.png