Copy number calculation for QPCR:

A serial dilution of linearized plasmid DNA is used to generate a standard curve for QPCR. Knowing the size of the plasmid that contains the gene of interest one can calculate the number of grams/molecule also known as copy number as follows:

Weight in Daltons (g/mol) = (bp size of plasmid+insert)(330 Da X 2 nucleotide/bp)

Ex. g/mol=(5950 bp)(330 Da X 2 nucleotide/bp)= 3927000 g/mol

Hence: (g/mol)/Avogadro's number $6.02214199 \times 10^{23)}$= g/molecule = copy number

Ex. 3927000g/mol/Avogadro's number $6.02214199 \times 10^{23}$) = 6.52×10^{-18} g/molecule.

Knowing the copy number for a plasmid and the concentration of the plasmid that is added to each PCR reaction, the precise number of molecule in that reaction can be determined as follows:

Concentration of plasmid (g/µl)/copy number

Ex. (3×10^{-7} g/µl) / (6.52×10^{-18}grams/molecules) = 4.6×10^{10} molecules/µl

Having calculated the number of molecules in a µl of linearized plasmid solution, a series of dilutions can be made for subsequent amplification allowing one to generate a standard curve. For the standard curve, the copy number of the unknown samples can then be derived.

The copy numbers of the standards used to generate a curve are listed below.

4.60E+05
4.60E+04
4.60E+03
4.60E+02
4.60E+01
4.60E+00