SOP for qPCR standards calculation of concentration to copy number conversion

Standard consists of a:

253 base pair amplicon (double stranded) with a neat concentration of 44.3ng/uL (as determined by Qubit):

1. **Calculate MW of each amplicon**

660 (estimated) MW/bp x 253 = 166980 MW of each dsDNA amplicon

OR can use more accurate conversion of:

(#bp x 607.4)+157.9 = 153830.1 MW of each dsDNA amplicon

1. **Now work out moles per ul:**

44.3 ng/uL = 0.000000443 g/uL (ie 4.43E-08)

Thus: (4.43E-0.8 g/153830.1MW)/uL = moles per uL

= 2.88E-13 moles of amplicon/uL

1. **Then using Avogadro's constant of 6.02214179E+23 amplicons/mole:**

Avogadro's # x 2.88E-13 moles/uL = 1.59768E+11 plasmids/uL

So, the 44.3 ng/uL amplicon solution contains: 1.60E +11 amplicons/uL

Therefore, in the neat there are **1.60E+11 amplicons copies/uL**

So, a series of dilutions of this would be: (this table is using simple bp MW conversion)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| standard dilution | standard conc (ng/ul) | Standard conc in **gram/ul** | moles of amplic/uL | Copy # per ul |
|
| Choose dilutions used | work out from [neat] | convert ng/ul to **g**/ul | x # of bases & bp MW | x by avogadros const. |
|
| 10^0 (neat) | 44.3 | 4.43E-08 | 2.653E-13 | 1.60E+11 |
| 10^-1 | 4.43 | 4.43E-09 | 2.653E-14 | 1.60E+10 |
| 10^-2 | 0.443 | 4.43E-10 | 2.653E-15 | 1.60E+09 |
| 10^-3 | 0.0443 | 4.43E-11 | 2.653E-16 | 1.60E+08 |
| 10^-4 | 0.00443 | 4.43E-12 | 2.653E-17 | 1.60E+07 |
| 10^-5 | 0.000443 | 4.43E-13 | 2.653E-18 | 1.60E+06 |
| 10^-6 | 0.0000443 | 4.43E-14 | 2.653E-19 | 1.60E+05 |
| 10^-7 | 4.43E-06 | 4.43E-15 | 2.653E-20 | 1.60E+04 |
| 10^-8 | 4.43E-07 | 4.43E-16 | 2.653E-21 | 1.60E+03 |
| 10^-9 | 4.43E-08 | 4.43E-17 | 2.653E-22 | 1.60E+02 |