PCR Concentration Conversions

Ben Shirt-Ediss

Newcastle University UK

Part I
For ssDNA

I use these calculations – they are more general. A hybrid ss-dsDNA complex can be separated, and treated as a ssDNA complex.

1 Mass Concentration to Molar Concentration

I want to convert $ng/\mu l$ to nM.

- In a volume of $1\mu l$, ssDNA at mass concentration $3.42ng/\mu l$ will be present at $3.42ng$.
- The number of moles of ssDNA molecules can be calculated in this mass.
- There is this number of moles in $1\mu l$
- $1\mu l$ is $10^{-6}l$, therefore in 1 litre, there will be this many moles: 10^6 multiplied by the number of moles in $1\mu l$.
- We have a molar concentration of ssDNA (moles per litre).
- To convert a molar concentration to nM, multiply by 10^9. (There are $10^9 nM$ in every M)
- **I assume that the dye FU on the gel is reflective of the mass concentration, and not that it binds just to the dsDNA parts of a complex.**

Mass to Moles of ssDNA Molecules

From the NEB webpage https://nebiocalculator.neb.com/#!/formulas:

$$\text{moles ssDNA} = \frac{\text{mass of ssDNA (g)}}{\text{molecular weight of ssDNA (g/mol)}}$$

$$\text{molecular weight of ssDNA} =$$

$$(\text{number of deoxynucleotide monophosphates of ssDNA} \times \text{average molecular weight of a deoxynucleotide monophosphate}) +$$

$$18.02\text{g/mol}$$

- average molecular weight of a deoxynucleotide monophosphate = 308.97 g/mol, excluding the water molecule removed during polymerization.
- The 18.02 g/mol accounts for the -OH and -H added back to the ends.

$$\text{moles ssDNA} = \frac{ng/10^9}{(nt \times 308.97) + 18.02}$$

Formula Steps

I assume the volume is $1ul$ to do this conversion.

$$ng = ng/\mu l$$

$$\text{moles ssDNA in } 1\mu l = \frac{ng/10^9}{(nt \times 308.97) + 18.02}$$

$$\text{moles ssDNA in } 1l = \text{moles ssDNA in } 1\mu l \times 10^6 = \text{Molar ssDNA concentration}$$

$$nM \text{ ssDNA concentration=Molar ssDNA concentration} \times 10^9$$

Final Formula

$$nM = \left(\frac{(ng/\mu l)/10^9}{(nt \times 308.97) + 18.02} \times 10^6 \right) \times 10^9$$

Simplifying:

$$nM = \left(\frac{(ng/\mu l)}{10^9} \times \frac{1}{(nt \times 308.97) + 18.02} \times 10^6 \right) \times 10^9$$

$$nM = \left(\frac{1}{10^3} \times \frac{(ng/\mu l)}{(nt \times 308.97) + 18.02} \right) \times 10^9$$

$$nM = 10^6 \times \frac{(ng/\mu l)}{(nt \times 308.97) + 18.02} \tag{1}$$

Which is approximately equal to the bp formula below, if $nt = 2bp$

2 Mass Concentration to Molar Concentration

Inverting Eq. 1:

$$nM\,((nt \times 308.97) + 18.02) = 10^6(ng/\mu l)$$

$$ng/\mu l = nM \times \frac{(nt \times 308.97) + 18.02}{10^6}$$

Part II
For dsDNA

3 Mass Concentration to Molar Concentration

$$nM = \frac{ng/\mu l}{g/mol} \times 10^6$$

$$nM = \frac{ng/\mu l}{660 \times bp} \times 10^6$$

If the mass concentration of dsDNA fragments is $ng/\mu l$, and each of these fragments is made of bp base pairs, then this formula gives the nanomolar concentration of these fragments.

Summary: The function from ng/ul mass conc $\{S\}$ to nanomolar conc $[S]$ is:

$$f_1(\{S\}, bp) = \{S\}\frac{10^6}{660 \times bp}$$

4 Molar Concentration to Mass Concentration

$$ng/\mu l = nM\,(g/mol) \times \frac{1}{10^6}$$

$$ng/\mu l = nM\,(660 \times bp) \times \frac{1}{10^6}$$

If the molar concentration of dsDNA fragments is nM, and each of these fragments is made of bp base pairs, then this formula gives the nanograms per microlitre mass concentration of these fragments.

Summary: The function from nanomolar conc $[S]$ to ng/ul mass conc $\{S\}$ is:

$$f_2([S], bp) = [S]\frac{660 \times bp}{10^6}$$