# Calculating CsCl and GB+DNA volumes to reach a target density for a density gradient

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For preparing a CsCl density gradient one typically mixes a high-density solution of CsCl (7.163 M) with an aqueous buffer solution to reach a target density. For DNA separation in SIP studies this is typically 1.725 g $ml^{-1}$. Despite the simplicity of the problem, different formulae appeared in the literature, leading to slightly different end values. Most notably, the famous publication by (Neufeld et al., 2007) uses a constant—1.52—instead of a variable that would represent the density of the mixture. The source of this constant appears to be from the classical book Centrifugal Separations: Molecular and Cell Biology by (Rickwood, 1985), where it is used for a specific case only.

While the practical implications are small so long as one tries to create a solution with a density of 1.725 g $ml^{-1}$ (although the resulting value is still wrong), the deviations become significant the further the target density deviates from this value.

We assume no contraction of volume when mixing CsCl (or CsTFA) with GB because they are both aqueous solutions. If there is a volume contraction then this will have to be determined empirically and corrected for (e.g. such as for ethanol-water mixture).

So assuming no contraction, the formula is simply:

$$\rho_{CsCl} \cdot V_{CsCl} + \rho_{(GB+DNA)} \cdot V_{(GB+DNA)} = \rho_{mix} \cdot V_{mix} \tag{1}$$

Replace $V_{(GB+DNA)}$ with $V_{mix} - V_{CsCl}$

$$\rho_{CsCl} \cdot V_{CsCl} + \rho_{(GB+DNA)} \cdot (V_{mix} - V_{CsCl}) = \rho_{mix} \cdot V_{mix} \tag{2}$$

Open brackets and reorganise sides

$$\rho_{CsCl} \cdot V_{CsCl} + \rho_{(GB+DNA)} \cdot V_{mix} - \rho_{(GB+DNA)} \cdot V_{CsCl} = \rho_{mix} \cdot V_{mix} \tag{3}$$

$$V_{CsCl} \cdot (\rho_{CsCl} - \rho_{(GB+DNA)}) = V_{mix} \cdot (\rho_{mix} - \rho_{(GB+DNA)}) \tag{4}$$

$$V_{CsCl} = V_{mix} \cdot \frac{(\rho_{mix} - \rho_{(GB+DNA)})}{(\rho_{CsCl} - \rho_{(GB+DNA)})} \tag{5}$$

I haven't measured the density of GB but according to my quick calculation it should be around 1.023 g $ml^{-1}$ (I could not determine it precisely because I could not find the density of Tris-HCl). The density of a

DNA sample should be very close to 1, even if it is in TE. Still, even assuming the density of GB+DNA = 1 g ml$^{-1}$ does not change the result by much, so for practical purposes we can even formulate it as follows:

$$V_{CsCl} = V_{mix} \cdot \frac{(\rho_{mix} - 1)}{(\rho_{CsCl} - 1)} \tag{6}$$

The formula in (Neufeld et al., 2007) is for calculating the volume of $V_{GB}$ but it's only a matter of reorganising the equation. In any case, using the example in the paper of a total volume of 6 ml my equation would give: 4.89 ml CsCl solution and 1.11 ml GB + DNA (or 4.86 + 1.14 if you use $\rho = 1.023$ for GB+DNA). This is close to the values of 4.8 + 1.2 given in the paper, but still wrong.

For a direct comparison with (Neufeld et al., 2007), or the book by Rickwood my formula would look like this (assuming $\rho_{(GB+DNA)} = 1$):

$$V_{GB} = V_{mix} \cdot (\rho_{CsCl} - \rho_{mix}) \cdot \frac{1}{(\rho_{mix} - 1)} \tag{7}$$

So instead of $\frac{1}{(\rho_{mix}-1)}$ there is the factor "1.52", which corresponds to a $\rho_{mix} = 1.657895$, but the reason for this is unclear.