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Letter to the Editor

NUCLEAR DNA CONTENT AND GENOME SIZE OF TROUT AND HUMAN

In their recent paper, Thomas et al. (1) discussed design, resolution, sensitivity, and reproducibility of the National Aeronautics and Space Administration/American Cancer Society flow cytometer. The results of their study demonstrated high stability and sensitivity of the instrument, which is suitable for detection of near-diploid tumor cells. Although the performance of the instrument is impressive, we believe that Thomas et al. made serious errors in calculating the DNA contents of trout and human.

To estimate the DNA content of human cells in picograms of DNA, the authors used trout red blood cell nuclei as the internal reference standard and assumed 2.37 pg of DNA for a trout nucleus. With this value, the DNA contents of human female and male nuclei were estimated to be equal to 3.77 and 3.70 pg of DNA, respectively. The new estimates for trout and human differ drastically from previous ones, which range from 4.9 to 6.3 pg for various species of trout (2–5) and from 6.0 to 7.0 pg for human (5–9). Because trout and human nuclei are often used as internal reference standards to determine genome size in animals and plants (5,10,11), the results published by Thomas et al. (1) need correction to avoid serious mistakes.

Careful reading of the paper showed that the DNA content of trout (the authors did not specify the species) was derived after determining the ratio of mean DNA content of human male to trout to be 1.565. The DNA amount of the human nucleus, 3.70 pg, was calculated with the assumption that there are 6.162×10^9 nucleotides for the human male nucleus and that a mean nucleotide molecular weight of 360 g/mol. We believe these data are not correct.

The ratio of human to trout DNA content determined by Thomas et al. (1) differs significantly from that of other reports. For instance, Vindeløv et al. (12) found that rainbow trout has 80% of human DNA content.

The most recent estimate of the size of the human diploid male genome is 6.294×10^9 nucleotide pairs (13).

Mean nucleotide molecular weight is not 360 (Table 1).

The amount of DNA in a human cell nucleus was incorrectly calculated by multiplying the diploid genome size (in base pairs) by the mean weight of a nucleotide rather than of a nucleotide pair.

By using the data in Table 1, relative weights of nucleotide pairs can be calculated as follows: AT = 615.3830 and GC = 616.3711, bearing in mind that formation of one phosphodiester linkage involves a loss of one H_2O molecule. Further, phosphates of nucleotides in the DNA chain are acidic, so at physiologic pH the H^+ ion is dissociated (15). Provided the ratio of AT to GC pairs is 1:1, and ignoring the presence of modified nucleotides, the mean relative weight of one nucleotide pair is 615.8771. In any case, the error should be smaller than 1%.

The relative molecular weight may be converted to an absolute value by multiplying it by the atomic mass unit (1 u), which equals one-twelfth of a mass of ^{12}C, i.e., 1.660539×10^{-27} kg (16). Consequently, the mean weight of one nucleotide pair would be 1.023×10^{-9} pg, and 1 pg of DNA would represent 0.978×10^9 base pairs. The same conversion factor (0.98×10^9) was proposed by Cavalier-Smith (17), to our knowledge the only correct number provided in the relevant literature. In contrast, Straus (18) and Bennett and Smith (19) reported a lower value (0.965×10^9). The correct formulas for converting the number of nucleotide pairs to picograms of DNA and vice-versa are:

$$\text{genome size (bp)} = (0.978 \times 10^9) \times \text{DNA content (pg)}$$

$$\text{DNA content (pg)} = \text{genome size (bp)}/(0.978 \times 10^9)$$

The current estimates for human female and male diploid genome sizes are 6.406×10^9 bp and 6.294×10^9 bp, respectively (13). By using the conversion formulas given above, diploid human female and male nuclei in G_1 phase of the cell cycle should contain 6.550 and 6.436 pg of DNA, respectively. These values are in line with those of previous reports and differ drastically from those estimated by Thomas et al. (1).

Trout DNA content may be calculated by using the ratio of DNA amount of human versus trout. The ratio of 1.565 determined by Thomas et al. (1) seems too high when compared with other reports (12). The discrepancy might be due to the use of 4′,6-diamidino-2-phenylindole to stain the samples, which binds preferentially to AT-rich DNA (20) and, hence, could result in the biased ratio of DNA amounts (11,21). Provided trout has 80% of human DNA content (12), trout nuclei should contain 5.149–5.240 pg

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Table 1
*Relative Molecular Weights of Nucleotides**

Nucleotide	Chemical formula	Relative molecular weight
2′-deoxyadenosine 5′-monophosphate	$C_{10}H_{14}N_5O_6P$	331.2213
2′-deoxythymidine 5′-monophosphate	$C_{10}H_{15}N_2O_8P$	322.2079
2′-deoxyguanosine 5′-monophosphate	$C_{10}H_{14}N_5O_7P$	347.2207
2′-deoxycytidine 5′-monophosphate	$C_9H_{14}N_3O_7P$	307.1966

*Calculated with the following standard atomic weights as provided by Vocke (14): $A_r(H) = 1.0079$, $A_r(C) = 12.0107$, $A_r(N) = 14.0067$, $A_r(O) = 15.9994$, $A_r(P) = 30.9738$. Standard atomic weights are scaled to nuclide ^{12}C with $A_r(^{12}C) = 12$ and rounded to four decimals.

of DNA. These values agree with the published data and differ significantly from those reported by Thomas et al. (1).

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