Illustration of telomere length measurement by Telometric

Supplementary material to:
Telomere measurement tools: Telometric produces biased estimates of telomere length

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Telomere Restriction Fragment (TRF) measurement is accomplished by estimating telomere fragment size through their comparison against molecular weight markers. Analysis begins by recovering optical density (OD) data at each position (i) down a gel lane. ODi is proportional to the amount of telomere at position i. Using the molecular weight marker, each position is converted to a molecular weight (MWi) allowing telomere abundance intensity to be plotted against MW. When MW is plotted on a linear scale, as is done in Telometric, there are progressively fewer MWi per unit MW at larger MW because distance travelled on the gel is a non-linear function of MW. The appendix to "Telometric's User Manual" (available online, see supplementary excel file) explains how this is dealt with: "Since the telomere length spacing may be non-uniform, a second data set of relative frequencies is generated using linear interpolation at uniformly spaced TL intervals." In other words, Telometric plots these data evenly distributed, e.g. one ODi for every 100 bp. At larger MW this requires interpolation because due to gel image resolution, an ODi may not exist at each 100bp. For instance, when two neighboring positions on the gel are at MW's 10,300 and 10,600, Telometric creates two interpolated data points between the existing data points at 10,400 and 10,500. In this way, this algorithm adds telomeres to the distribution that do not exist. Conversely, when three neighboring pixels are e.g. at MW's 1,000, 1,050 and 1,100 bp than the pixel at 1,050 is omitted from the data as Telometric only records OD data at every 100bp. As mentioned in the Telometric manual, it uses these modified uniformly spaced data to calculate telomere length, instead of the original data read from the gel image directly. This is the single error causing the biased estimates.

To illustrate the consequences of this error, we here compare data obtained using Telometric or an image analysis program that obtains data directly from the gel image without further data treatment (ImageJ). We created a sample gel that includes an actual TRF smear repeated over 9 lanes (see supplemental excel file). In each lane (A - I) the TRF smear begins at a slightly lower molecular weight to illustrate how the bias in Telometric measurements is dependent on telomere length. Notice for example that between 38,000 and 39,000 bp the gel resolution is such that there are only two neighboring data position on the gel (see supplementary material excel file, Image J data), but Telometric's interpolation

algorithm turns this into 10 positions (one optical density at each 100 bp between 38 and 39 kb; see supplementary material excel file, Telometric data). Overall this results in Telometric calculating a variable number of positions which changes substantially when the smear is shifted to higher MW's, from 39 to 354 positions in our example gel. On the contrary, ImageJ calculates a constant 225 positions in each lane when analyzing the same gel. Thus the calculation includes the non-existent telomeres added through the interpolation (when MW distance >100bp), and excludes the omitted telomeres (when MW distance < 100 bp). This is clearly illustrated in the supplementary file when the exact same telomere restriction fragment electrophoresis distribution is repeated across a gel but the molecular weight where the smear begins is shifted to a lower molecular weight in each lane (see supplementary excel file). The Σ(ODi) for each lane should be the same, and this is the case when using ImageJ. However, when Telometric is used the Σ(ODi) changes (from ~3000 to ~100,000) because of deletion and creation of new telomere signal. Consequently, estimates of telomere length obtained using Telometric always overestimate telomere length, and this bias increases with increasing telomere length. In our example, this bias reached over 60% even at moderate telomere lengths (Fig.1).

A separate, but another troubling problem is that Telometric does not repeatably obtain the same OD data from a gel image. In our sample gel image, notice that since the exact same smear is repeated it should yield the same OD data for each lane. This is the case for the OD data obtained with Image J, but Telometric gives different OD data for each lane, decreasing systematically from one side of the gel to the other (see supplementary materials excel file). The decrease is uniform across a smear, and hence does not affect the TRF estimates that Telometric produces, but we mention it as another issue to resolve in case someone takes up our suggestion to develop Telometric into a usable program.