Additional file 1 – C(t) value and DNA quantity

The formula below describes the inverse relationship between ALU C(t) value and input DNA. Let \( \tau \) and \( c \) be amplification process-specific constants denoting the DNA quantity detection threshold and the method’s efficiency. The efficiency is a composite of the amplification efficiency and background levels. Further, let \( h \) denote DNA quantity (in genome equivalents), and \( C(t) \) denotes the corresponding number of amplification cycles needed to enlarge \( h \) to a DNA quantity exceeding \( \tau \). That is,

\[
C(t) = \min\{i \geq 1: hc^i \geq \tau\}. \tag{1}
\]

The approximate formula for the relation between \( C(t) \) and DNA quantities is obtained from (1) after replacing the inequality with equality,

\[
hc^{C(t)} = \tau. \tag{2}
\]

Taking logarithm with base 10 on both sides of (2) and rearranging terms yields,

\[
C(t) = (\log_{10} \tau - \log_{10} h)/\log_{10} c
= \alpha - \beta \log_{10} h \tag{3}
\]

This formula shows the inverse relationship between DNA quantity and \( C(t) \) value, allowing us to use ALU \( C(t) \) value as a surrogate for DNA quantity in our study.

We designed a supplementary experiment that would allow us to estimate the quantity of DNA for each tumor analyzed given the surrogate ALU \( C(t) \) value. This is accomplished through the use of a TaqMan PCR reaction (C-LESS-C1)[11], which recognizes a DNA strand that does not contain cytosines, and hence is able to amplify the total amount of DNA (bisulfite-converted or unconverted) in a PCR reaction well. The supplementary experiment is based on the analysis of two serially diluted sets of DNA samples: (1) an unconverted peripheral blood leukocyte (PBL) DNA sample of known concentration for use as a standard curve in a C-LESS PCR reaction, and (2) a
bisulfite-converted M.SssI-DNA sample used in ALU PCR reactions of the C-CFR MLH1 DNA methylation analyses. In order to convert the C-LESS PCR standard curve from the PBL sample into an ALU standard curve for the C-CFR MLH1 samples, the dilution series of bisulfite-converted M.SssI-DNA sample (set 2 above) was analyzed using both C-LESS and ALU MethyLight PCR reactions.

First, we used the serially diluted set of unconverted PBL DNA sample of known concentration to estimate the C-LESS C(t) value as a function of the known concentration (463.2 ng DNA undiluted, as measured by Nanodrop-based A260 absorbance technology) and relative number of copies (1024, 256, 64, 16, 4, and 1 for dilutions: undiluted, 1:4, 1:16, 1:64, 1:256, 1:1024). We re-wrote the best-fit linear equation to predict ng of unconverted DNA (on the log10 scale) as a function of C-LESS C(t) value. Since the C-LESS reaction amplifies both template strands of unconverted genomic DNA but only one strand of bisulfite-converted DNA, we expect that bisulfite-converted DNA will amplify one PCR cycle later than that of unconverted DNA. Thus, to estimate the number of haploid bisulfite-converted DNA for the M.SssI-treated DNA sample, we multiply the amount of DNA (in ng) for each sample quantitated from the C-LESS standard curve by the PCR efficiency, and divide by 0.0033 (1 haploid genome = 0.0033 ng DNA). We calculated the estimated C-LESS PCR efficiency in this experiment to be 1.905. Next, we generated a regression of the ALU C(t) value on the estimated number of haploid genomes (on the log10 scale) for the dilution series for the M.SssI-treated DNA sample (dilutions of 1:729, 1:243, 1:81, 1:27, 1:9, 1:3, 1:1). The final step was to calibrate the dilution set from the 28 C-CFR plates to the same reaction run coincidently with C-LESS. We estimated a regression function of the ALU C(t) level from the reaction run coincidently with the C-LESS reaction as a function of the average ALU C(t) level
estimated across the 28 C-CFR plates. Thus, by knowing the $ALU \ C(t)$ level of a tumor in the C-CFR samples, we could then estimate the number of haploid genomes.