Proliferation-dependent positioning of individual centromeres in the interphase nucleus of human lymphoblastoid cell lines

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Running title: nuclear positioning of centromeres.

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Supplementary Material
**Complements on EVF calculations**

**EVF to nucleoli:**
For this EVF, the nucleoli are taken as reference structures, but as centromeres were sometimes observed inside nucleoli, the distance map was built by ordering distances to the nucleolar border and assigning negative values when points are located inside the nucleoli. This can also be explained the following way:
- When the point is located outside the nucleoli, the EVF is the volume of the nucleolus plus the nucleoplasmic volume closer to the nucleolus than the point, divided by the nuclear volume.
- When the point is located inside the nucleoli, the EVF is the volume that is further from the nucleolar border than the point divided by the nuclear volume.

**EVF to nuclear border and nucleoli:**
The distance map was built again by ordering the distances, taking for each point the minimal value between the distance to the nuclear border and the distance to the nucleolar border (which can be negative, as above). As described for the EVF to nucleoli, two cases must be distinguished:
- When the point is located outside the nucleoli: if we call $d_{nl}$ the distance to the nearest nucleolar border, $d_n$ the minimal distance to the nuclear border, and we define $d = \min(d_{nl}, d_n)$, then the EVF is given by $EVF = \frac{V_{nl} + V_{1} + V_{2}}{V_n}$, where $V_{nl}$ is the nucleolar volume, $V_n$ is the nuclear volume, $V_{1}$ is the nucleoplasmic volume lying at a distance to nucleoli below $d$ and $V_{2}$ is the nucleoplasmic volume lying at a distance to the nuclear border below $d$.
- When the point is located inside the nucleoli, the EVF is given by $EVF = \frac{V_{1}}{V_n}$, where $V_{1}$ is the nucleoplasmic volume lying at a distance to the nucleolar border above $d$ and $V_n$ is the nuclear volume.
Table S1:

| Chr  | Sequence                  | Label       | T(°C) |
|------|---------------------------|-------------|-------|
| 1    | $^5$'AaAcTgCtGCgTgAtGt$^3$ | 3’Cy5       | 70    |
| 5    | $^5$'AaAcTgCtCTgCgAtGt$^3$ | 5’Alx488    | 70    |
| 8    | $^5$GaAaTgTtCaGcAcAgTt$^3$ | 5’Cy5       | 65    |
| 10   | $^5$'AaTtGgCcCcTtGaGc$^3$ | 3’dig       | 65    |
| 11   | $^5$'AgGgTrTcAgAgCtGcTc$^3$ | 5’Alx488    | 65    |
| 13   | $^5$'AcCcAgCcAaAcGtGt$^3$ | 5’Alx488    | 65    |
| 16   | $^5$'AaCaAgAcAaAcTcGtTc$^3$ | 3’dig       | 65    |
| 17   | $^5$'tGaTtGaGtGaCtCaC$^3$ | 5’Cy5       | 65    |
| 18   | $^5$'GtGtGcCcTcAaCtAaAg$^3$ | 3’dig       | 65    |
| 22   | $^5$'AtTaGaGcCcTgAaAgCa$^3$ | 5’Alx488    | 72    |

Small letters indicate Locked Nucleic Acids (LNA) modifications, dig is for digoxygenin, Alx488 is for AlexaFluor488. The washing temperature is indicated.

Table S2:

| BAC name | CHORI name  | Chromosome | begin-end                      |
|----------|-------------|------------|--------------------------------|
| 1-155    | RP11-243J18 | Chr 1      | 155629618-155733278            |
| 1-158    | RP11-520H16 | Chr 1      | 158874920-159053664            |
| 1-205    | RP11-6B6    | Chr 1      | 205511288-205671057            |
| 1-208    | RP11-459K23 | Chr 1      | 208809356-208926210            |
| 7-38     | RP11-121A8  | Chr 7      | 38218703-38390318              |
| 17-63    | RP11-489G5  | Chr 17     | 62950110-63144909              |
| 18-44    | RP11-91K12  | Chr 18     | 43993745-44160574              |

This table displays the names currently used in our group, the names according to the Children's Hospital Oakland Research Institute (CHORI), the chromosome number and the localization of the BACs (number refer to the positions of the first and last nucleotides in the human genome assembly, version GRCh37.p13).
Figure S1: Cumulative distributions of nuclear volumes (A), nucleolar volumes (B), and nucleoli number (C) for cycling and quiescent cells. Mean values and standard deviations were computed from experimental images corresponding to results shown in figure 1.
Figure S2: Positioning pattern of centromere from chromosomes 8 and 22 in GM06990 cells. For each chromosome, the three different cumulative distributions are shown for cycling (linear) and quiescent (dotted) cells.
Fig. S3: Influence of the cell cycle on centromere nuclear positioning. Cumulative distributions of EVF computed from the nuclear border (A) and from the nucleoli (B) and from both the nuclear border and nucleoli (C) are shown for three centromeres, as indicated. In each case, data are split between quiescent cells (red), G1 cells (blue), and early (green) and late (purple) replicating cells. Data were obtained with GM06990 cells.
Figure S4: Graphic map of euclidean distances and EVF values calculated from different reference structures
Euclidean distance map (A-C) are used to calculate the three types of EVF (D-F). A and D: EVF toward the nuclear border. B and E: EVF toward the nucleoli. C and F: EVF toward the nucleoli and nuclear border. The nucleolar border is shown in yellow on B, C, E and F. Otherwise colors are used to represent distances, as indicated in the calibration bars on the right, black being used for short distances and white for long ones.
Fig. S5: Scheme depicting the calculation mode for the orientation measurement. Images correspond to maximal projections of 3 images from a z-stack, before (A and C) and after (B and D) segmentation. The color legend is indicated. The complete CT corresponds to the red and blue part. A and B display the orientation toward the periphery, C and D the orientation toward the nucleoli. Measurements are performed in 3D using an euclidean distance map from the reference structure.