Supplementary Information

for Bauer et al. "DNA Catenation Maintains Structure of Human Metaphase Chromosomes"

Supplementary Methods

Timelapse images of on-chip chromosome digestions with proteinase K were loaded into ImageJ and any timelapse images containing multiple chromsomes were cropped to provide separate timelapse files. A custom ImageJ Java macro was used to carry out morphometry analysis on timelapse images of chromosome digestions in ImageJ:

```
setAutoThreshold("Default");
setAutoThreshold("Default dark");
setAutoThreshold("Default dark");
run("Convert to Mask", "calculate black");
setAutoThreshold("Default dark");
run("Convert to Mask", "calculate black");
run("Open", "stack");
run("Fill Holes", "stack");
run("Analyze Particles...", "size=100-Infinity
      circularity=0.00-1.00 show=Nothing display clear
      summarize stack");
saveAs("Text", "/DataFolder/file.xls");
```

The output from each run was put into Excel & plotted vs. time. The morphology of the chromosomes after 20 minutes' digestion was used to compare between different chromosome samples.

Supplementary Movie Legends

Movie S1– On-chip Chromosome Protease Digestion
Timelapse of proteinase K digestion of a native metaphase chromosome stained with YOYO-1 dye. The chromosomes expand during digestion and retain their canonical 'X' shape.

Movie S2 – DNA Catenation at the Centromere
Using on-chip fludic manipulation to explore chromosome structure after proteinase K digestion. After looking around inside the chip and adjusting illumination, the chromosome is subjected to fluid flow to separate the chromatid bodies. Discrete fibres are visible (e.g. at 1:18) catenating the sister chromatids.

Movie S3 – 3D View of DNA Catenation
Three-dimensional projection of the real-time epifluorescence movie highlights the DNA fibres catenating sister chromatids. The corresponding two-dimensional movie is shown adjacent, with the region shown in 3D indicated (white rectangle) along with the perspective (white arrow).