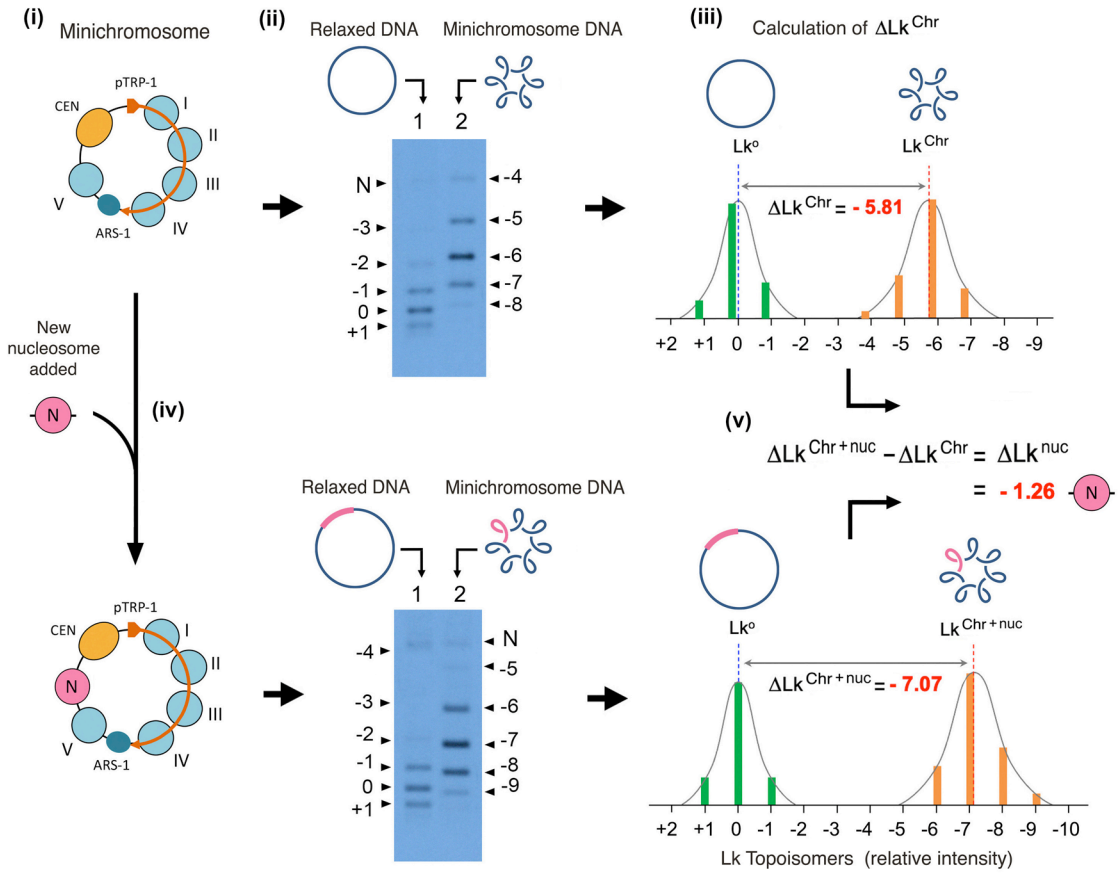
Extended Data

Nucleosomal DNA has topological memory

Joana Segura, Christoforos Nikolaou, Joaquim Roca



Extended Data Fig. 1. Experimental layout to calculate the ΔLk restrained by nucleosomes *in vivo*. In a circular minichromosome **(i)**, the DNA linking number difference constrained by its chromatin elements (ΔLk^{Chr}) is calculated by comparing, via gel electrophoresis **(ii)**, the Gaussian distribution of Lk topoisomers of the DNA relaxed *in vitro* (lane 1) with that of the minichromosome DNA fixed *in vivo* (lane 2). The Lk mean of the relaxed DNA (Lk^0) and that of the minichromosome DNA (Lk^{Chr}) are determined by plotting the intensities of their corresponding Lk topoisomers along a scale of ΔLk units **(iii)**. ΔLk^{Chr} is the distance in Lk units between the two means (Lk^0-Lk^{Chr}=ΔLk^{Chr}). Upon adding a new nucleosome to such minichromosome **(iv)**, the process is repeated to calculate $\Delta Lk^{Chr+nuc}$. The resulting difference between ΔLk^{Chr} and $\Delta Lk^{Chr+nuc}$ equals ΔLk^{nuc}, the ΔLk restrained by the new nucleosome **(v)**. ΔLk^{nuc} is about -1.26 for most nucleosomes, as previously reported by Segura *et al* (2018).

a

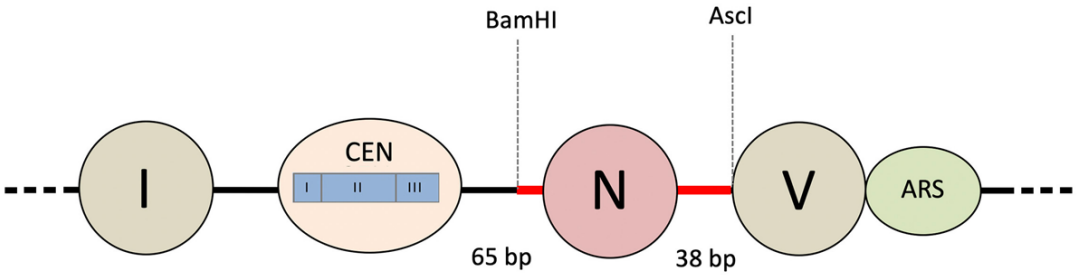
Adaptor with BamH1 cohesive-end ligatable but not recutable

```
5′      *GGACGATTACAGCTACGTG      3′
3′      TCCTGCTAATGTCGATGCACCTAG  5′
```

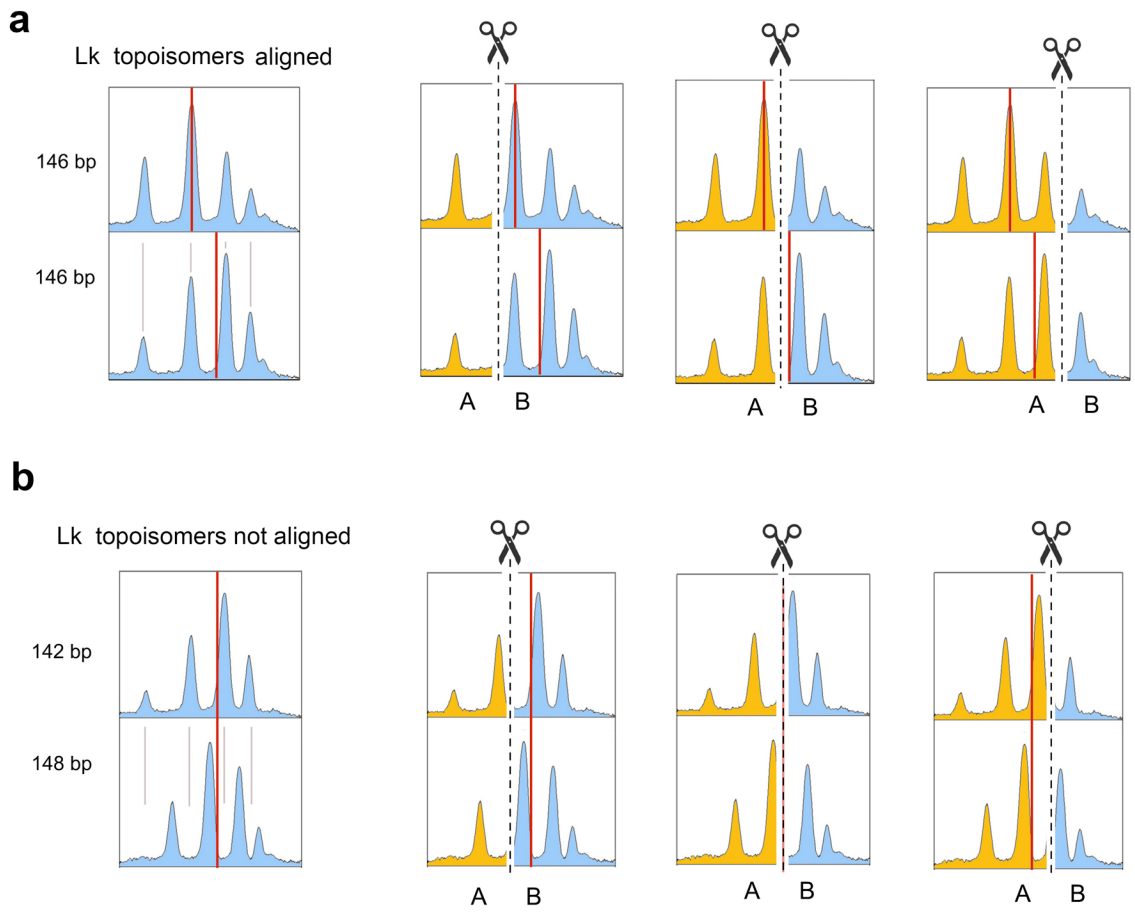
Adaptor with Asc1 cohesive-end ligatable but not recutable

```
5′      *CGTTCGGATCCGTTTAAACGTGAAGAGGTAACTCAT     3′
3′      TGCAAGCCTAGGCAAATTTGCACTTCTCCATTGAGTAGCGC 5′
```

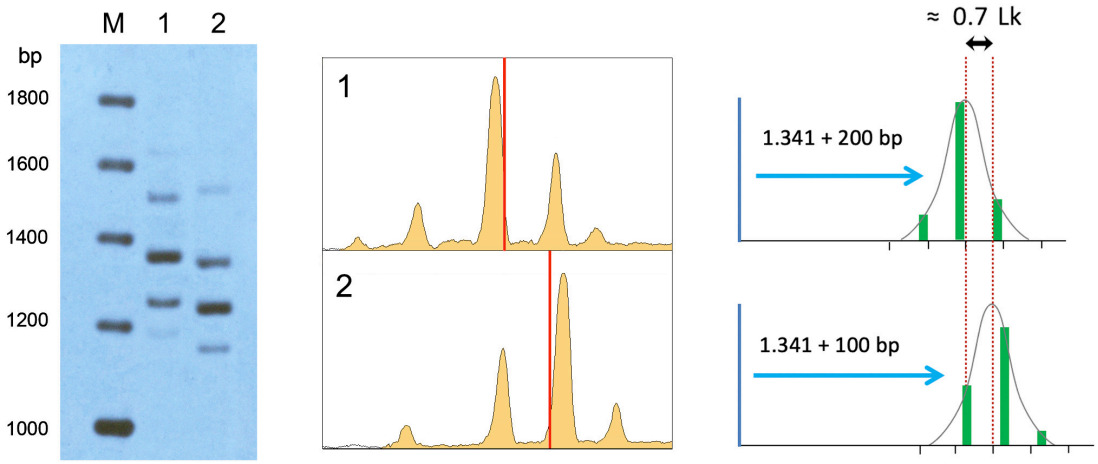
b



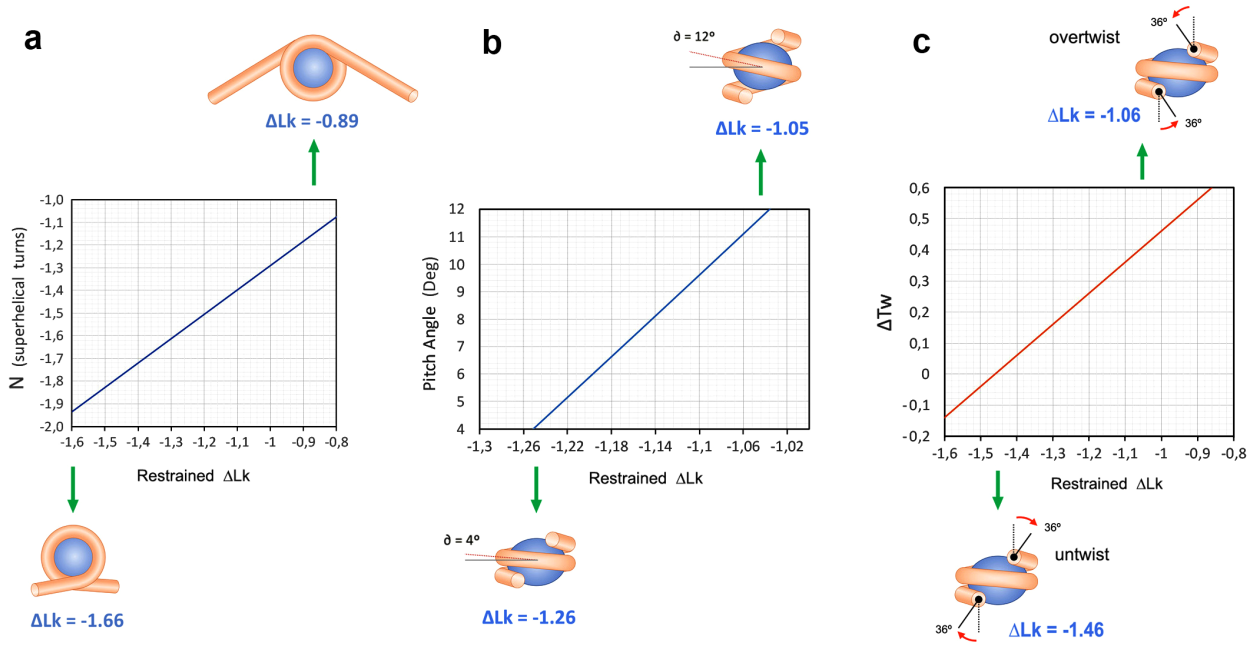
Extended Data Fig. 2. Insertion of the nucleosome library into YCp1.3. a, DNA adaptors ligated to A-tailed nucleosome DNA fragments to generate *BamH1* and *Asc1* cohesive ends. **b,** Position of the nucleosome library between nucleosome V and CEN2 of YCp1.3. The lengths (bp) of the resulting linker DNA segments are indicated.



Extended Data Fig. 3. Effect of aligned and non-aligned Lk distributions when conducting Topo-seq. a, Example of two Lk distributions with different Lk mean (red lines), but with aligned topoisomers, since the DNA fragments of the nucleosome library have equal length (146 bp). Note that, whatever the position of a splitting cut (3 examples depicted), the partition of DNA intensities (coloured areas A and B) always denotes that the two distributions have different Lk mean. **b**, Example of two Lk distributions with equal Lk mean (red lines) but with not aligned topoisomers because the DNA fragments of the nucleosome library have different lengths (142 and 148 bp). In this case, depending on the position of the splitting cut, the partition of DNA intensities does not always denote that the two distributions have equal Lk mean.



Extended Data Fig. 4. Correlation of DNA length with the electrophoretic velocity of Lk distributions. Left, the gel shows a marker of linear DNAs (M) and the Lk distributions of two relaxed DNA molecules differing in 100 bp in length. These molecules are the YCp1.3 minichromosome DNA (1.341 bp) with an insert of 100 bp (lane 1) and 200 bp (lane 2). Note that the size of these inserts encloses the length variability of the nucleosome library (144±33bp). Middle, densitometry of lanes 1 and 2 indicating the position of the Lk means (red lines). Right, the distance of the Lk means of these DNAs differing in 100 bp was equivalent to 0.7 Lk units, thus 0.007 Lk/bp.



Extended Data Fig. 5. Effect of nucleosome DNA wrapping (ΔWr) and twisting (ΔTw) on the restrained ΔLk. a, Plot of restrained ΔLk as a function of the number of DNA super-helical turns (N) wrapped in the nucleosome. ΔLk values are calculated as ΔTw+ΔWr, where ΔTw=+0.2 and ΔWr=N(1–sin 4°). **b,** Plot of restrained ΔLk as a function of the pitch angle (∂) of the super-helical turns wrapped in the nucleosome. ΔLk values are calculated considering ΔTw=+0.2 and ΔWr=1.56(1–sin ∂). **c,** Plot of restrained ΔLk as a function of the DNA twist (ΔTw) restrained by the nucleosome. ΔLk values are calculated considering ΔWr=1.56(1–sin 4°). Nucleosome DNA geometries are modelled for the indicated ΔLk.