pBR322 Linking Number Markers

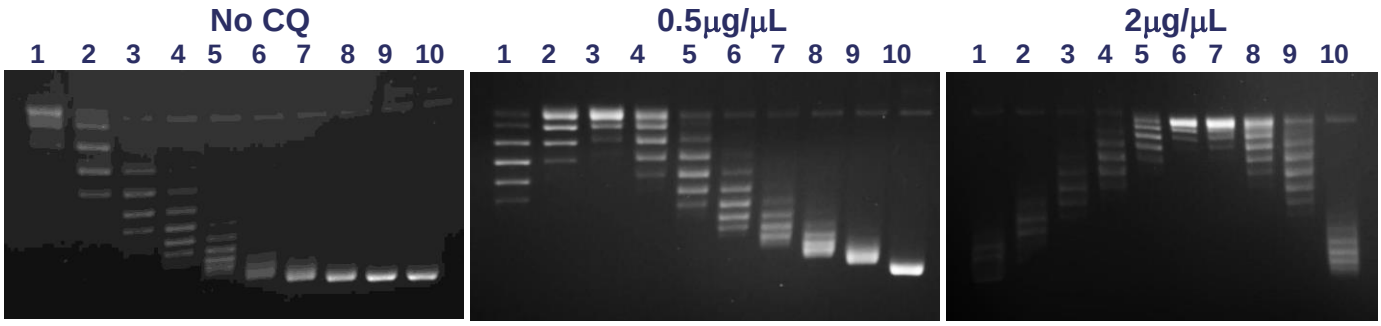
Product Description (Product Numbers LNM001)

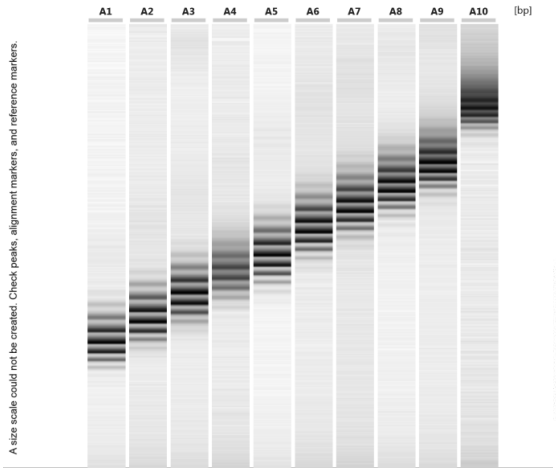
Plasmid pBR322 DNA is produced by the large-scale alkaline-lysis method (Sambrook *et al*., 1989). It is then relaxed with topoisomerase I in the presence of different amounts of ethidium bromide. After extraction to remove the ethidium and the topo I the markers are resuspended in T10E1.
They are shipped on dry ice at a concentration of 1 mg/ml in TE. Store at 4°C or below.
For *in vitro* laboratory research use only.

TE Storage Buffer 10 mM Tris.HCl (pH 7.5), 1 mM EDTA

Analysis on agarose gels

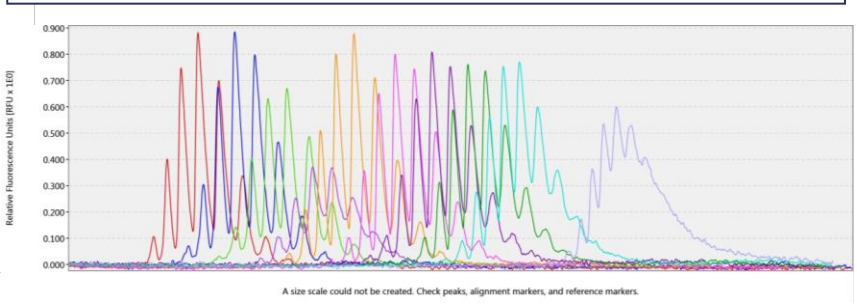
2μl of each marker was loaded onto a 1% agarose gel and run in TAE buffer. Three gels are shown run in the presence of 0, 0.5 and 2 μg/mL chloroquine(CQ) to resolve the different topoisomers. Using the central topoisomer of sample 1 (relaxed pBR322) as a linking number of 0, it is possible to use the different markers to count the linking numbers up to about -23 (negative supercoiling).





Analysis using a Qiaxcel machine

Each marker was loaded onto a Qiaxcel machine and the samples separated. The image on the left shows the visual output from the separation. The image on the right shows the peak profiles from each marker overlaid.



References

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, Plainview, NY.