untitled protocol

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Abstract

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Materials

Topoisomerase I (E.coli) - 500 units M0301L by New England Biolabs

pUC18 50004 by addgene

6X Blue Loading Dye L002 by Gold Biotechnology

Protocol

Add 2 µl of 10×topoisomerase I reaction buffer and 400 ng pUC19 plasmid DNA (Takara, Japan) to each of a series of 1.5-ml microcentrifuge tubes on ice;Adjust volumes with distilled water so that the final reaction volume in each tube, including that of the protein or extract added in step 2, is 20 µl.

Step 1.

Add various amounts of purified RstA or one unit E. coli topoisomerase I protein (NEB, USA) to the tubes, then incubate 10 min at 37°C.

Step 2.

Add 4 µl of 6×loading dye to each tube and load contents on an 0.8% agarose gel. Run gel 2h at 5 to 10 V/cm.

Step 3.

Stain gel with ethidium bromide, destain briefly with water;

Step 4.

Photograph the gel illuminated with a UV transilluminator.

Step 5.