New Cross-linking Protocol (1 X-link/50-60bp)

1. Harvest approx. 6 x 10^8 cells, 4 minutes at 2,200 rpm in tabletop centrifuge (Beckman).

2. Resuspend in 1.5 ml psoralen solution (see 3 for psoralen solution) in a 50mm x 9mm plate. Hexamine Cobolt Chloride (50 mM) was added to the solution after I discovered that my 0 minute induction time point had cleavage.

3. Crosslink with 10 µg/ml trimethylpsoralen (from a 200 µg/ml stock in 100% ethanol) in 50 mM Tris-Cl pH 8.0, 50 mM EDTA.

4. Add psoralen solution 5 min. prior to cross-linking and store cells in the dark.

5. Irradiate 15 min. at 365 nm with the UV spot lamp (UVP longwave UV lamp model B-100 A) at a distance of 6 cm with the cells/plate on ice.

6. Repeat step 5 another 3 or 4 times.

New Reversal of Cross-linking Protocol

1. Irradiate gel with cross-linked DNA in a UV stratalinker at 1200 µjoules.

2. Flip the gel over and irradiate over side with 1200 µjoules in a UV stratalinker.