

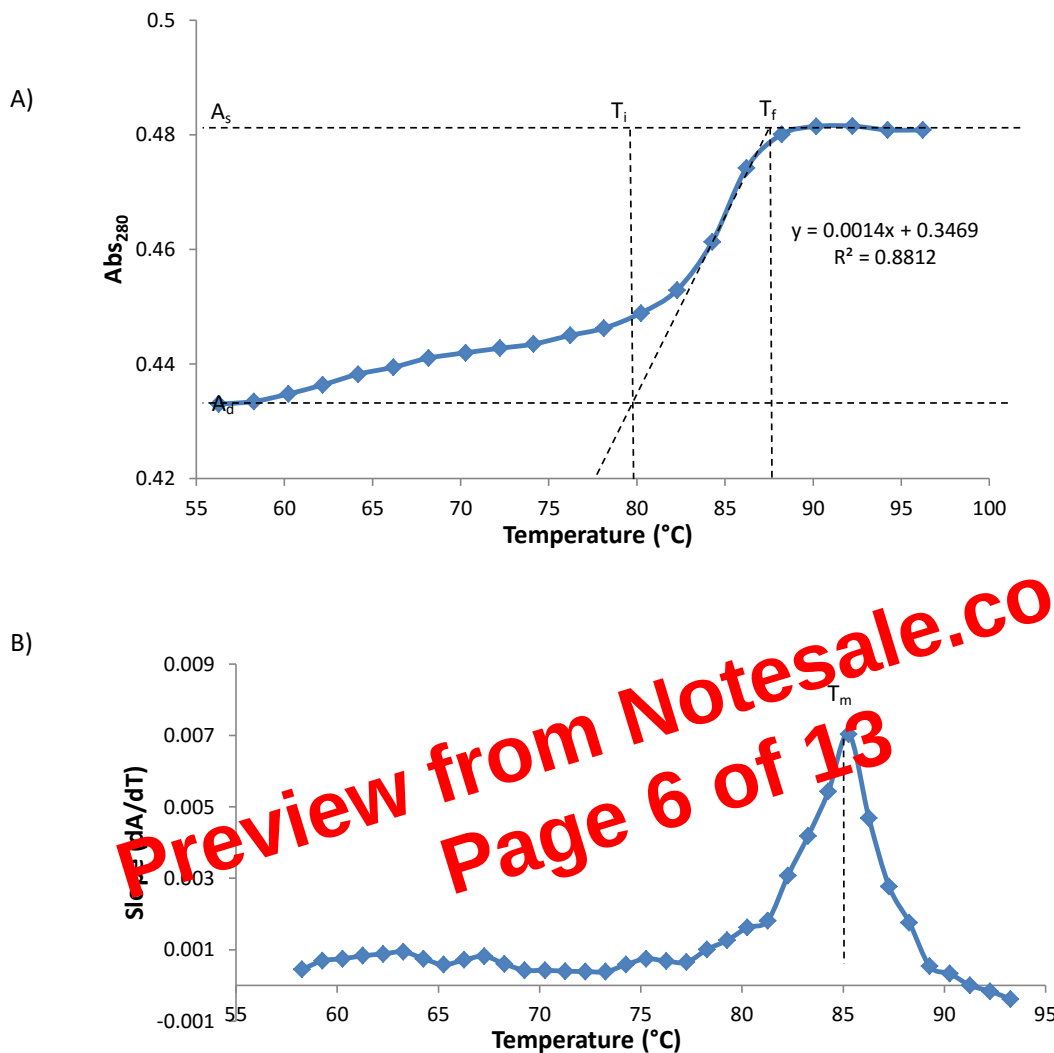


Figure 4: DNA melting curve of prepared *E. coli* DNA. 60 mg of *E. coli*, strain B gram negative bacterium suspended in 4 mL of cold saline-EDTA (0.15 M NaCl in 0.1 M ethylene-diamine tetraacetate – EDTA, pH 8.0) was added to 375 μ L of 25% sodium dodecyl sulfate (SDS) and incubated at 60°C for 10 minutes. 0.725mL of 6.0M NaClO₄ and 5.0 mL of chloroform:isoamyl alcohol (24:1, v/v) was added. The solution was then mixed and centrifuged at 12,000 xg after balancing. 10 mL of 70% ethanol was added over the aqueous phase and mixed gently for precipitation of the DNA. The DNA was retrieved with a glass rod and then immersed in a microtube of 1 mL of 70% EtOH and then let to drain for 10 minutes. The crude DNA was then dissolved in 2 mL of 15mM citrate buffer (sonicated) at pH 7.0 in a 15 mL centrifuge tube. The

Lane 1 from Figure 5 shows that undigested plasmid 57 is in the nicked formation. This means that the plasmid may be damaged or in the process of being replicated at the time of isolation. The nicked DNA migrates slowly mimicking a DNA fragment longer than its linear form, which can be seen in lane 1. The band is also relatively thin, if it were thick it then supercoil conformation would be more plausible. The digestions for EcoRI and HindIII were complete. Figure 5 shows that in lane 4 for EcoRI there was 1 band and in lane 2 for HindIII there were 2 bands. For full digestion there would have to be 3 bands to signify all 3 cuts have been made (2 by HindIII and 1 by EcoRI) which is what is seen in lane 3 so the digestion is complete

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