

Single Molecule Investigations of Tn5 Transposase – DNA Interactions

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Single DNA molecule micromanipulations provide a unique means for the study of protein – DNA interactions. Here we report the development of a single molecule Tn5 transposase DNA binding and synaptic complex formation assay. The method utilizes a long DNA tether where biotin is bound to one end of the molecule while dioxigenin is bound to the other end. Streptavidin-coated magnetic beads and anti-dioxigenin functionalized glass or polystyrene beads tether the single molecules of DNA for manipulation. Magnetic tweezers are used to extend and contract the DNA tether. A 51 kb DNA tethering molecule containing a 1.3 kb Tn5 transposon has been constructed. This tether is used to study Tn5 transposase – DNA interactions. Initial results of the Tn5 transposase's interactions with non-specific and specific DNA are discussed. Salt affects on the binding of the transposase to DNA and other transposases are described. A model describing the observed results is presented.