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Biochemical and Structural Analysis of Ultrabithorax as an Important Developmental Protein in *Drosophila Melanogaster*

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Biochemical and Structural Analysis of Ultrabithorax: An Important Developmental Protein in

Drosophila Melanogaster

Mia Advocate '22 – Sponsor: Doctor Donald Spratt



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Homeodomain transcription factors (TF) are proteins responsible for gene expression. TFs work by binding to cis-regulatory modules that control many important biological pathways and functions. Transcription factor activity is vital in embryonic development. *Drosophila melanogaster* is the primary organism of study in this research as fruit flies share similarities of development and transcriptional processes with humans. *Drosophila* experience rapid maturation met with short lifespans, so fruit flies are ideal organisms to work with in a lab setting. The specific homeodomain of interest in this study is Ultrabithorax (Ubx). Ubx is a Hox protein important in the development of the posterior thoracic and abdominal regions. In *Drosophila* Ubx represses limb formation while aiding in the formation of the wings and halteres. This study conducts a biophysical analysis of Ubx by investigation binding affinity and protein interactions.



Figure 2- Crystal Structure of Ultrabithorax-Extradenticle-DNA Complex. Ubx protein with Exd allows for the increase in selectivity for the affinity of Ubx for a DNA target. Ubx is denoted in red, Exd in cyan, and the DNA in yellow. The Ubx and Exd are bound to opposite sides of the DNA. Image from PDB website.

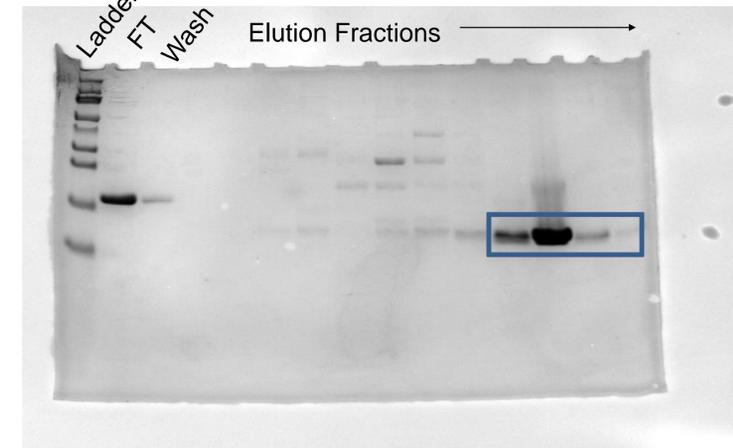


Figure 4- Heparin Purification Samples Run on SDS-PAGE Gel. Samples taken during the Heparin purification step were run on an SDS-PAGE gel to confirm purification of Ubx. The fractions boxed in blue are pure Ubx protein. To purify Ubx AKTA was used and ran through Nickel and Heparin columns.

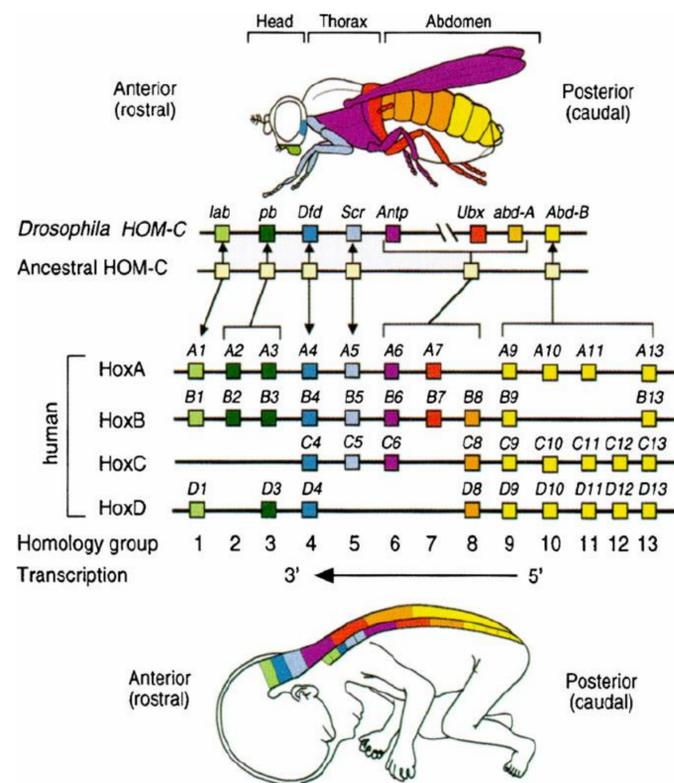


Figure 1- Genomic organization of *Drosophila* homeotic genes and human Hox genes. Each gene is represented by a color box; Ubx is denoted in red. Ultrabithorax is located in the thorax and abdomen regions in *Drosophila* and the prevertebrae of a human fetus. In *Drosophila*, expression of the Hox genes is activated relative to chromosome position, whereas in humans Hox genes encipher homeodomain transcription factors that are found on four different chromosomal locations. Image from Jamila Horabin, 2013.

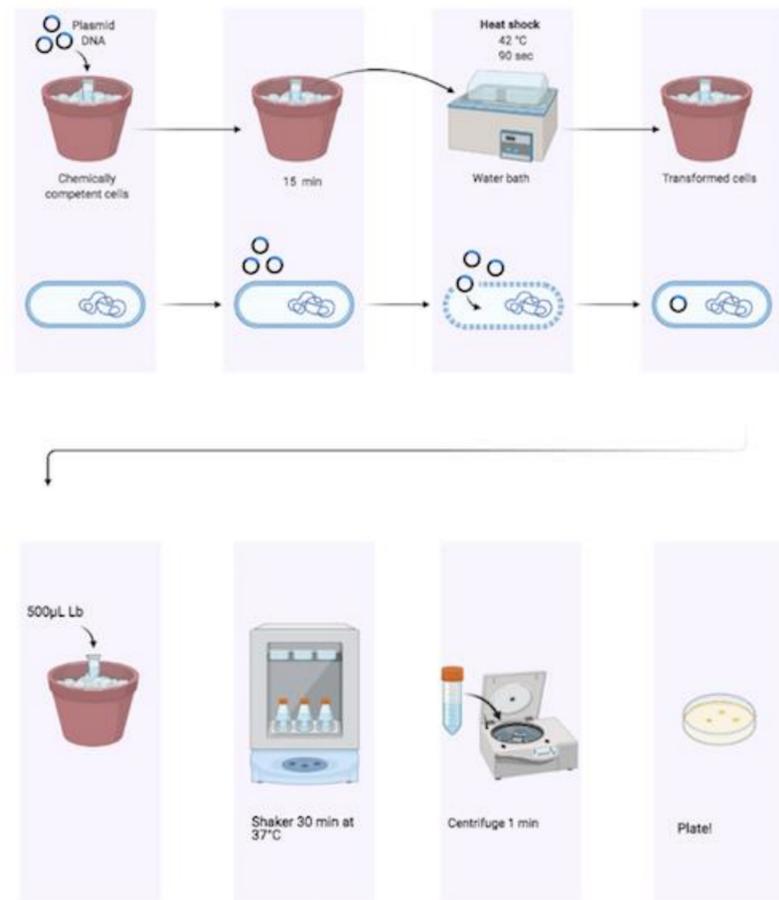


Figure 3- Transformation of Ubx plasmid DNA with Rosetta Cells. Rosetta cells were transferred to an Eppendorf tube with plasmid DNA. A starter culture was prepared from this transformation. Figure was created using BioRender.

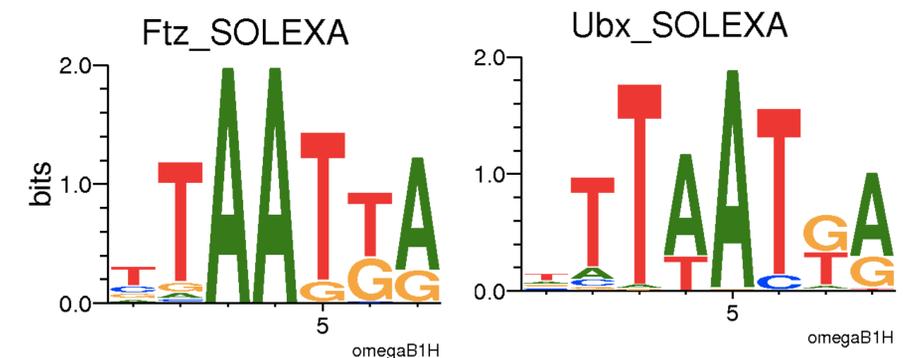


Figure 5- Consensus Sequences of Ftz and Ubx. The DNA oligo used was the Fushi Tarazu (Ftz) consensus sequence. It can be observed that Ftz and Ubx share similar sequencing

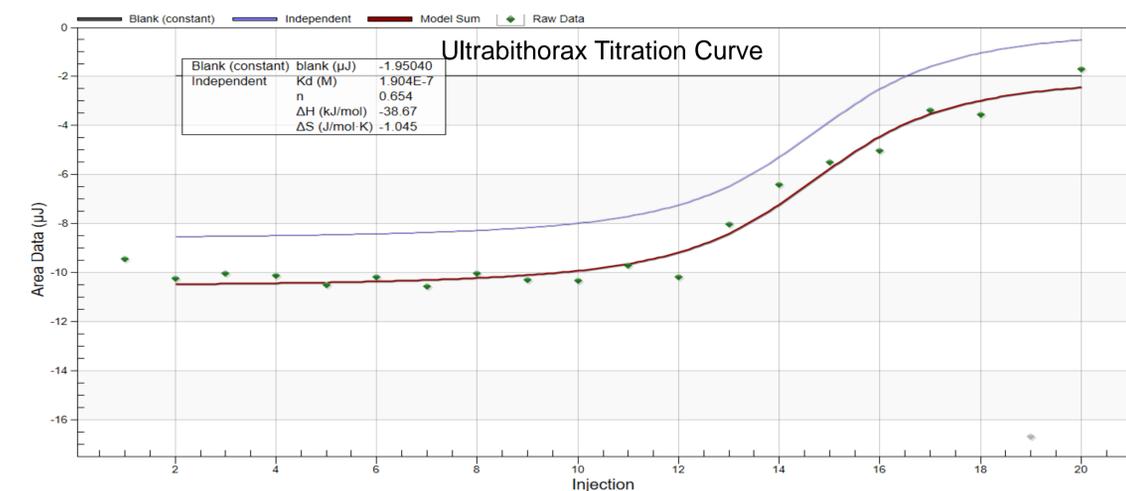


Figure 6-Ultrabithorax Titration Curve. Using the ITC, the dissociation constant for Ubx was able to be observed. The ITC allows for the measurement of enthalpy, which in turn allows us to determine binding affinity of protein to DNA oligo.

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