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Generating Gbx2 antibodies: A useful tool in determining developmental mechanisms regulated by GBX2

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The GBX class of homeobox genes is comprised of Gbx1 and Gbx2. Both loss-of-function and gain-of-function studies in mice have shown that Gbx2 is vital for normal anterior hindbrain development of mammalian organisms. To gain more insight into the developmental mechanisms regulated by GBX2, we are generating GBX2 antibodies. To accomplish this, we have subcloned Gbx2 into the pET101/D-TOPO protein expression vector and transformed the construct into BL21(DE3) cells. Protein expression was induced by IPTG. The expressed protein was analyzed by SDS-PAGE as well as Western analysis. Results from the SDS-PAGE and Western analysis suggest that the GBX2 protein is being expressed. Further testing by mass spectroscopy was performed and confirmed that our induced protein is GBX2. Currently, we are using a nickel-chelating resin to bind specifically to the 6X His tagged GBX2 fusion protein in order to purify GBX2. The purified protein will be used to elicit an immune response in chickens to generate the antibodies against GBX2. Generation of GBX2 antibodies will provide an important tool to enhance our knowledge of how GBX2 functions in development. Having these antibodies will allow for cellular localization. In addition, the antibodies will be used in chromatin immunoprecipitation assays, which will allow for the production of a library that contains genes directly regulated by GBX2. The identification of target genes will provide a way to enable the collection of valuable data that will be useful for more long-term research goals involving the specific signaling and genetic pathways in which this transcription factor is involved.