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Generating GBX1 anitbodies: A useful tool in determining developmental mechanisms regulated by GBX1

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Inactivation of *Gbx1* in mice results in a locomotor phenotype specifically affecting hind-limb motion. GBX1 is a DNA-binding transcription factor that regulates the expression of its direct target genes. We are trying to create antibodies, which can be used as a reagent to identify target genes directly regulated by GBX1, in addition, these antibodies will be used for immunohistochemical analysis. The results from these studies will provide insight into the clarification of regulatory mechanisms controlling locomotion in mammals. In order to generate antibodies to GBX1, we will generate protein, which can be used elicit an immune response in chickens. To do this we have cloned the *Gbx1* open reading frame (ORF) into the pBluescript II KS (+/-) vector, which allows for DNA sequencing. We then transformed the construct into DH5α cells. After sequencing, the *Gbx1* ORF was subcloned into the protein expression vector pRSET B and transformed into BL21 bacterial cells. Protein expression was induced using Isopropyl β-D-1-thiogalactopyranoside (IPTG). GBX1 protein will then be analyzed by SDS-PAGE and Western blot analysis. Upon confirmation of protein expression, GBX1 will be purified and used to elicit an immune response in chickens to generate GBX1 antibodies. Once antibodies have been generated, characterization will be carried out by analyzing the antibodies using Western blot analysis and immunohitochemistry.