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# Activity of *LHX2*-associated *cis*-Regulatory Modules During Limb Development

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## INTRODUCTION AND OBJECTIVES:

Limb development occurs along three major axes – proximal-distal, anterior-posterior, and dorsal-ventral. Fibroblast growth factors (FGFs) secreted by the apical ectodermal ridge (AER) and sonic hedgehog (SHH) released from the zone of polarizing activity (ZPA) are responsible for proximal-distal and anterior-posterior development, respectively, and maintain each other through a positive feedback loop. This reciprocal loop is critical for proper limb development. Recently, we identified LIM homeobox 2 (*LHX2*) as an intermediate in FGF-mediated *SHH* expression. There are over 25 conserved regions of non-coding DNA associated with the *LHX2* gene locus that could serve as regulatory modules and targets of FGF signaling. We hypothesize that FGF regulates *LHX2* through at least one of these potential cis regulatory modules (PCRM).

**METHODS:** We selected 10 PCRM with active chromatin marks in the limb and screened for their activity within the *LHX2* expression domain (distal mesoderm subjacent to the AER). Each PCRM was inserted into the pTK-GFP reporter and electroporated into the distal mesoderm of HH20-23 chicken embryo wing buds. PCRM

activity was determined 24 hours later using fluorescence microscopy.

**RESULTS AND CONCLUSIONS:** We found three of the PCRM display activity (CRM (-19), CRM (-2), and CRM (-1)) that overlap *LHX2* expression in the chicken wing bud. One CRM (-19), approximately 130 kb upstream of the *LHX2* locus, is most consistent with the pattern of *LHX2*. Further studies are underway to determine whether these CRM interact with the *LHX2* promoter and whether FGF regulates their activity.

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