Construction of a Luciferase Reporter Vector for Characterizing the Expression of Plcd4 driven By Zbtb24

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B-cell lymphoma constitutes 90% of non-Hodgkin's lymphoma, the seventh most common type of cancer in North America. Our lab previously identified Zbtb24, a zinc finger transcription factor, as a potential tumor suppressor in B-cell lymphoma via whole-genome CRISPR/Cas9 screening. Previous searches for Zbtb24's tumor suppression mechanisms via chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-sequencing (RNA-seq) identified Plcd4, a phospholipase C enzyme, as a potential transcription target of Zbtb24. We hypothesize that Plcd4 is a direct transcription target of Zbtb24. To test this hypothesis, Zbtb24 binding motifs were mapped in the Plcd4 promoter region, and subsequent cloning of this region into a luciferase reporter vector was conducted using restriction enzyme cloning. Cloning of the Cdca7 promoter region, a known transcription target of Zbtb24, was also conducted as a positive control for Zbtb24-driven gene expression in a future luciferase assay. The pGL3 basic luciferase reporter vector was used as the cloning backbone and dual restriction enzymes within the multicloning site were chosen. Restriction recognition sites were designed in primers to amplify mouse Plcd4 and Cdca7 genes using polymerase chain reaction (PCR). Restriction-digested amplicons were ligated into the backbone and transformed into competent cells via bacterial transformation. Colonies were then screened to confirm correct constructs. At this time, screening for the constructs is in progress and is anticipated to yield the correct constructs. Once obtained, these constructs will be used to perform a dual luciferase assay, which along with other results would validate if Plcd4 is a direct transcription target of Zbtb24. Supported by NIH R25ES020721.