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## **Generation of a Lentiviral-Based RNAi Vector to Study V(D)J Recombination in Primary B Cells**

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Poster Presentation P69

**GENERATION OF A LENTIVIRAL-BASED RNAI VECTOR TO STUDY V(D)J  
RECOMBINATION IN PRIMARY B CELLS**

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B cells are a primary component of the immune system and the body's humoral defense against pathogens through their production of antibodies. The B cell antigen receptor region contains a segment known as Variable Diversity Joining (V(D)J), which undergoes V(D)J recombination during the pro-B cell stage to generate a repertoire of lymphocytes that produce a broad range of antibodies. Many factors affect this process of V(D)J recombination; our focus has been on the histone methyltransferase G9a. We hypothesize that important aspects of V(D)J recombination are in fact regulated by G9a, which has previously been shown to have an impact on gene expression and histone modification in B cells. The goal of this research was to create a lentiviral-based vector that would enable us to easily and efficiently perform RNA interference (RNAi) experiments in primary cells. RNAi is a process that targets a gene of interest, halting translation and therefore expression of the gene. It is used in order to determine the effects of removing a particular gene; in our case, G9a. To make the vector for use in RNAi experiments, we began with the plasmid plenti6. We then removed the poisonous ccd<sub>b1</sub> gene using a polymerase chain reaction method and replaced the Blasticidin gene with Green Fluorescent Protein (GFP). This plasmid will allow us to generate a lentivirus that expresses GFP and eliminates G9a in mouse cells. Future work will utilize this plasmid to synthesize G9a-deleted cells and subsequently, determine the role of G9a in the gene regulation process and V(D)J recombination in B cells.