Functional analysis of SOX3 binding at the *Dbx1* locus

This thesis submitted in part fulfilment of the requirements for the Degree of Master of Philosophy in the discipline of Biochemistry, School of Molecular and Biomedical Science, at the University of Adelaide

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Declaration

I declare that this thesis does not incorporate, without my acknowledgment, any material previously submitted for a degree or diploma in any other university. To the best of my knowledge this thesis does not contain any material written or published by any other person, except where due reference is made.

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ABSTRACT

Sox3 a members of the SOX transcription factor family, is essential for normal brain development and required for growth of pituitary and hypothalamus. Sox3, as well as Sox2 which is another member in SOXB1 subfamily are widely expressed in neural progenitor cells and show functional redundancy. ChIP-seq data by Bergsland et al, 2011 has identified five putative SOX3 binding sites near/at the *Dbx1* locus. Microarray data from the lab (N. Rogers, unpublished data) has identified *Dbx1* as downregulated in *Sox3* null neural progenitor cells. Together these data suggest that *Dbx1* may be regulated directly by SOX3.

To investigate the possibility that SOX3 regulates the *Dbx1* locus *in vitro*, we performed gel shift assays and Luciferase Reporter Assays to see if SOX3 binds any of the five *Dbx1* regulatory sites. Due to time constraints we were not able to optimize the gel shift assays to obtain any informative results. Secondly, we optimized Luciferase Reporter Assays providing preliminary data suggesting SOX3 may bind at one of the tested *Dbx1* sites. To study the redundancy between *Sox2* and *Sox3*, *Sox2* was also tested in the Luciferase Reporter Assays indicating Sox2 may also regulate the same site as *Sox3* . Due to time constraints, the other three binding sites remain to be analyzed in the future.

The function of Dbx1 is best characterized in the context of the developing neural tube (also known as the spinal cord). To identify how other neural tube marker genes are regulated by *Sox3*, qPCR was performed with some marker genes in Sox3 null E9.5 mouse embryos compared with WT embryos. *Dbx1*, *Pax6*, *Ngn2* and *Olig2* all showed significant decrease in *Sox3* null. Further study of these genes will be required to assess the significant outcome of their down regulation in an *in vivo* context.