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Development of tools for in situ hybridization in human embryonic stem cells

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Our laboratory uses human embryonic stem cells (hESC) to understand how embryonic cells differentiate into the cells of the placenta. The long term goal of my research is to develop in situ hybridization methods and use them to determine where genes are being expressed within differentiating hESC colonies. I focused my research on two genes that code for transcription factors, OCT 4 and GATA 2. OCT 4 is required to maintain stem cells in an undifferentiated state. GATA 2 is thought to be one of the key transcription factors that promote stem cell differentiation into syncytiotrophoblast, a placental cell type. The first step in in situ hybridization is the development of a labeled RNA probe. I designed primers to amplify a 214bp portion of the GATA2 gene sequence by PCR, and then cloned the PCR product into E. coli bacteria. I confirmed the presence and orientation of the insert by using restriction digestion and DNA sequencing. I used bacteria already transformed with a 182bp OCT4 probe sequence to create a riboprobe. I grew up the bacteria overnight, lysed them, and purified the plasmid DNA. I linearized the plasmid with a restriction enzyme, purified it, and transcribed the DNA to RNA, incorporating digoxygenin (DIG) labeled nucleotides. In the near future, I will utilize the same method to transcribe the GATA2 probe, and use both RNA probes to perform in situ hybridizations on hESC.