Pitt Hopkins Syndrome (PTHS) is a genetic disorder that leads to developmental delay, breathing problems, and distinctive facial features. The under expression of the *TCF4* gene results in the occurrence of PTHS. Loss of *TCF4*, a transcription factor is specifically important for cell differentiation. [1] *TCF4* is involved in the differentiation of neural crest cells, which result in the most common neurological symptoms of PTHS. However, there are not clear symptoms that indicate how the heart and lung organs are affected by TCF4 inactivation. The overall goal is to determine if TCF4 is required for the differentiation of heart and lung cells during embryonic development.

My **objective** is to explore how *TCF4* mediates cell differentiation during heart and lung development. My **hypothesis** is that deletion of the *TCF4*will inhibit the process of cell differentiation in cells found in the heart and lungs, because the interaction with *MATH1* is necessary for proper cell differentiation in these tissues. *MATH1* is a transcription factor that interacts with *TCF4* to initiate proper neuronal development. [2] *Drosophila melanogaster* will be used for this study due to the tremendous amount of heart and lung research conducted using this model organism. It is often used as a model to study lung development, lung cancer, cardiac disease, and heart development. These are processes that are relevant to the focus of this study.

**Aim #1:** Characterize processes of cell differentiation in lung and heart tissue cells with *TCF4* inactivation

**Approach:** I will perform Next-Generation Illumina sequencing in order to compare the effects of *TCF4* inactivation on processes of cell differentiation in the heart and lung tissue cells with cell differentiation in neural crest cells.

**Rationale:** Using Illumina sequencing will highlight if the inactivation of *TCF4* has similar effects on pathways in heart and lung tissues as it does on neural crest cells. This will be compared to the inhibition of *MATH1/TCF4* interactions that occurs as a result of *TCF4* inactivation in neural crest cells.

**Hypothesis:** The inactivation of *TCF4* will inhibit cell differentiation in heart and lung tissue cells. Since *TCF4* is an E-protein, its importance is mainly found through its interactions with other proteins. Hence, the inactivation of *TCF4* will inhibit pathways that are crucial for proper cell differentiation in the heart and lungs.

**Aim # 2:** Identify genes relevant to cell differentiation processes via RNA-seq

**Approach:** I will perform RNA sequencing wild type mice and mice with *TCF4* inactivation throughout processes of cell differentiation in heart and lung tissue. This data will then be analyzed using gene ontology in order to determine the role of *TCF4* in both wild type and mutant fruit flies.

**Rationale:** Genes that are identified via RNA sequencing are possible target proteins for cell differentiation processes carried out through the activation of *TCF4*. This will allow the identification of crucial molecular players that interact with *TCF4* in these processes.

**Hypothesis:** The gene ontology results of RNA-seq will show that *TCF4* is crucial for processes related to cell growth and differentiation in heart and lung tissue cells. Genes that are related to *TCF4* activity*,* which are important for cell differentiation and cell fate determination processes in the heart and lungs, will be identified via the RNA sequencing.

**Aim # 3:** Evaluate *TCF4* protein interactions to infer its role in heart and lung cell differentiation

**Approach:** I will use resources such as String in order to develop a protein interaction network. I will identify proteins with Gene Ontology terms that relate to cell differentiation and cell fate determination. Western blotting will then be used to test interactions between *TCF4* and proteins involved in heart and lung cell differentiation. Western blotting will test proteins present when TCF4 is activated and when TCF4 is absent. This will be done using wild type fruit flies and fruit flies with reduced *TCF4* expression.

**Rationale:** *TCF4* acts as a transcription factor and E-protein, which indicates that it is heavily characterized by its protein interactions. Hence, confirming the types of proteins that TCF4 interacts with will reveal information regarding the role of TCF4 in cell differentiation in heart and lung cells.

**Hypothesis:** *TCF4* is an E-protein so it will bind with primarily other transcription factors in order to form heterodimers that are crucial for processes related to proper heart and lung development.

References:

[1] TCF4 gene - Genetics Home Reference. (n.d.). <https://ghr.nlm.nih.gov/gene/TCF4>

[2] Flora, A. (2007). The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. *NCBI*. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1978485/>