**Pitt Hopkins Syndrome** (PTHS) is a genetic disorder that leads to developmental delay, breathing distinct facial features, and low muscle tone. One gene associated with PTHS is TCF4, a transcription factor, which is important for cell differentiation of neural crest cells [1]. Yet, the mechanisms of how TCF4 functions in muscle tissue development are unclear. The overall goal is to determine if TCF4 is required for the differentiation of muscle tissue cells during embryonic development

My **objective** is to explore how TCF4 mediates cell differentiation during muscle tissue development. My **hypothesis** is that deletion of the TCF4 will inhibit the process of cell differentiation in cells found in muscle tissue. *Mus musculus* will be used as the model organism because it is often used as a model to study human muscle disease and muscle tissue development due to its similar muscular structures and function.

**Aim #1: Identify TCF4 mutations that affect cell differentiation processes in muscle tissue**

**Approach:** I will first use BLAST to identify homologs of TCF4. Clustal Omega will be used to align TCF4 mice homolog protein sequences in order to identify well-conserved amino acid regions on the TCF4 gene. Using CRISPR Cas9, I will mutate the amino acids and will screen for low muscle tone through the use of cryosectioning in order to understand how this mutation affects muscle tissue cells. **Hypothesis:** I hypothesize that the mutant mice will result in lower muscle tone, meaning that the well-conserved regions are associated with muscle cell development and differentiation processes. **Rationale:** This will allow me to confirm that cell differentiation processes are related to TCF4 knockout in muscle tissue cells.

**Aim # 2: Identify new genes important for muscle development in TCF4 mutants**

**Approach:** I will perform RNA sequencing to determine differentially expressed genes using muscle tissue cells from wild type and TCF4 mutant mice The differentially expressed genes will be sorted using Gene Ontology to determine which biological processes are most affected by TCF4 knockout. I will expect these genes to be associated with terms related to muscle development and muscle tissue cell differentiation. I will validate these genes by using CRISPR Cas9 to create mutant mice knockouts of the identified genes and screen for the lower muscle tone phenotype. **Hypothesis:** I hypothesize that TCF4 knockout will result in differentially expressed genes crucial for cell differentiation and cell fate determination of muscle tissue. **Rationale:** By evaluating expression levels of these genes, I will be able to identify genes affected by the lack of TCF4. Their GO terms will reveal if they are related to muscle tissue cell differentiation processes and muscle function.

**Aim # 3: Identify new TCF4 interacting proteins involved in muscle tissue development**

**Approach:** Using cells from wild type and TCF4 mutant mice from my first aim, I will use co-Immunoprecipitation (TAP-tag) and mass spectrometry to identify new proteins involved in muscle tissue development. These proteins will then be sorted using GO terms. **Hypothesis:** TCF4 is a transcription factor that may regulate proteins involved in muscle tissue development. **Rationale:** Identifying new proteins that interact with TCF4 can reveal new roles specifically in muscle tissue 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/>