Prostate cancer is one of the most common cancers, with approximately 12.9% of men being diagnosed world-wide. *RASSF1A* (Ras Associated Domain Family 1A), a tumour suppressor, is associated with the disease and functions to regulate cell growth and apoptosis [2]. Many patients exhibit hypermethylated in the promoter region of RASSF1A, leading to the silencing of the gene [1,3,4]. In general, genome wide demethylation is seen with age, yet hypermethylation often occurs in cancer-associated genes [5]. It is well known that the risk of developing prostate cancer increases with age, however, it is unclear *if the hypermethylation of the RASSF1A promoter occurs in aging males.*

My **primary goal** is to identify how the RASSF1A promoter is regulated epigenetically in aging males. My **hypothesis** is that the RASSF1A gene promoter is targeted for hypermethylation with age. My **long term goal** is to better understand the role of hypermethylation plays in regulating RASSF1A in the prostate. I will use Mus musculus as a model systems in my experiments due to the large amount of cancer studies already available that use the system and because they display a similar disease phenotype.

**Aim 1:** Determine the conservation of methylation sites in the RASSF1A promoter.

**Hypothesis:** I hypothesise that the homologs will have very similar conserved regions but the methylation patterns will differ between mammalian species.

**Approach:** I will use Clustal Omega to align the FASTA formatted sequences from each of the RASSF1A homologs. To determine which sites are methylated, I will use bisulfite DNA sequencing. The level of methylation would be based on the number of remaining cytosine residues as unmethylated cytosine’s would be converted to uracil.

**Rationale:** To identify if mammalian homologs share conserved regions when aligned and to establish if they also show the same pattern of hypermethylation.

**Aim 2:** Determine conserved methylation sites in the RASSF1A promoter necessary for proper cell growth throughout development.

**Hypothesis:** Altered conserved methylation sites in the RASSF1A promoter will lead to elevated expression through development.

**Approach:** I would create a CRISPR construct to mutate the conserved methylation sites. The system would be delivered by injection into the prostates of both wt and hypermethylated mutant young and adult male mice. Before and after treatment I would carry out quantitative proteomic analysis using mass spectrometry.

**Rationale:** Conserved methylation sites in the promoter sites in the promoter region of the RASSF1A should uncover necessary sites important to aging males.

**Aim 3:** Determine changes in protein interactions when the RASSF1A promoter is hypermethylated**.**

**Hypothesis:** I hypothesise that RASSF1A protein will not interact with Ras proteins when the RASSF1 promoter is hypermethylated.

**Approach:** I will isolate protein complexes from prostate cells of WT and mutant hypermethylated mice of different age groups using antibody based co-immunoprecipitation using a bait protein for RASSF1A. I would then carry out liquid chromatography mass spectrometry-based identification.

**Rationale:** The Ras oncogene proteins are regulated by RASSF1A. With RASSF1A silenced, it will not be able to function and interact with other proteins as it normally would.

By the end of this study I expect to have a better understanding of the process of increased methylation of the RASSF1 promoter with age and how this hypermethylation affects the resulting proteins interactions. This research will discover more about the changing methylation state of RASSF1A and its promoter during a males aging process. It will also uncover new potential drug targets but identifying protein interactions for the hypermethylated RASSF1A.

[1] Ge, YZ., Xu, LW., Jia, RP. et al. 2014. The association between RASSF1A promoter methylation and prostate cancer: evidence from 19 published studies. *Tumour Biol*. **35**: 3881  
[2] Donninger, H. Vos, M.D. & Clark, G.J. 2007. The RASSF1A tumour suppressor. *Journal of Cell Science*. **120**: 3163-3172.  
[3] Yegnasubramanian, S., Kowalski, J., Gonzalgo, M.L. et al. 2004. Hypermethylation of GpG islands in primary and metastatic human prostate cancer. *Cancer Research*. **64**(6): 1975-1986.

[4] Baylin, S.B. & Herman, J.G. 2000. DNA hypermethylation and tumorigenesis. Epigenetics joins genetics. *Trends in Genetics.* **16**(4): 168-174

[5] Teschendorff, E.A., West, J. & Beck, S. 2013. Age-associated epigenetic drift: implications, and a case of epigenetic thrift? *Human Molecular Genetics.* 22: 7-15