Identifying the role of PEP-C in HCT116 Colon Cancer Cells

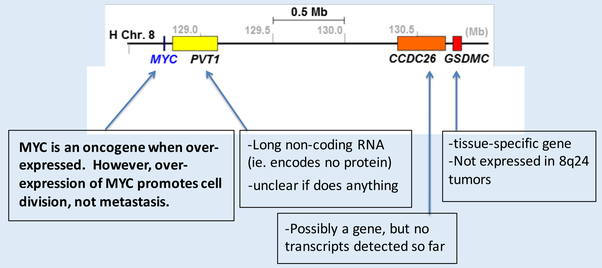
K. Moua and Y. Marahrens
Department of Genetics, Cell Biology, and Development, University of Minnesota, Twin Cities

Purpose:

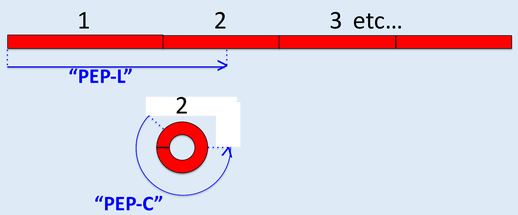
To determine the importance of Pep-C by creating a frameshift mutation that destroys PEP-C but not PEP-L. CRISPR-Cas9 will be utilized to derive the null allele in PEP-C by cleaving a few base pairs from the exon and repairing the chromosome through Non-Homologous End Joining (NHEJ).

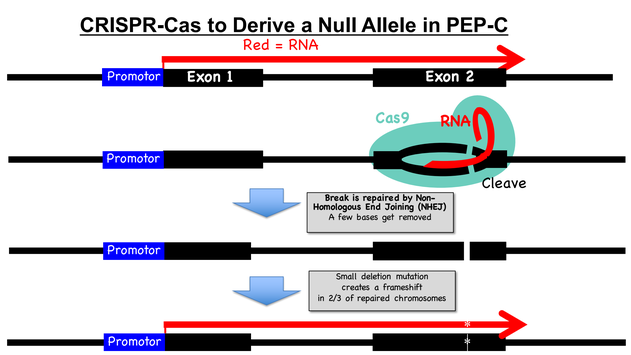
Background:

Duplication of the 8q24.21 chromosomal region has been associated with 25-30% of multiple human cancers across nearly all tumor types (Tseng et. al., 2014). Specifically, the myelocytomatosis (MYC) oncogene is located on 8q24.21 and promotes cell division when overexpressed. It lays adjacent to and transcribes the non-coding RNA gene PVT1.



Previous studies discovered that the excision of PVT1 reduces tumorigenicity, suggesting the existence of a second oncogene within the region. Additionally, PVT1 exon 1 and exon 2 encode proteins PEP-L and PEP-C respectively. PEP-C is produced by a circular exon 2 through back-splicing and has an open reading frame ~50,000 times more abundance than linear PVT1 RNA.





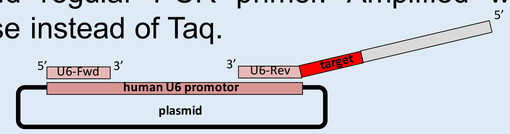
Experimental Design:

1. Design a 125-nucleotide "ultramer" containing 1) sequence to PCR amply the human U6 promoter and 2) PEP-C (PVT exon 2) target sequence for cleavage by CRISPR/Cas9. In addition: order PCR primer to PCR amplify the human U6 promoter (U6-Fwd):

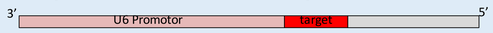
Ultramer: 5'-AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCT ATTTCTAGCTCTAAAAC**GCTGAGCGCCGGATGGAACG**CGGTGTTTCGTCCTTTCCACAAG-3'

U6-Fwd: GAGGGCCTATTTCCCATGATTCC

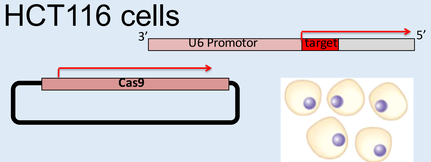
2. PCR amplify the human U6 promoter using an Ultramer PCR primer and regular PCR primer. Amplified with Herculase polymerase instead of Taq.



3. Purified PCR product with "Quiaquick" kit



4. Lipofect the PCR product with a plasmid expressing Cas9 protein into HCT116 cells



5. Grew transfected cells at a low concentration to form colonies. Then used cloning cylinders to obtain isolate clonal cells. For each colony, some of the cells were frozen while the rest were made into DNA.

6. PCR amplifed 48 clonal cell lines.
7. Utilized Surveyor technique to detect minor small mismatches.

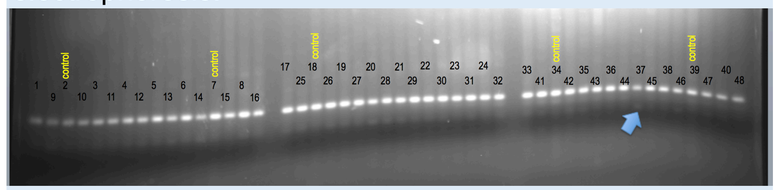
PVT1 Exon 2 Sequence:

GCCTGATCTTTTGGCCAGAAGGAGATTAAAAAGATGCCCCTCAAGATGGCTGTGCCTGTCAGCTGCATGGAGCTTCG
TTCAAGTATTTTCTGAGCCTGATGGATTTACAGTGATCTTCAGTGGTCTGGGGAATAACGCTGGTGGAACCATGCAC
TGGAATGACACACGCCCGGCACATTTCAGGATACTAAAAGTGGTTTTAAGGGAGGCTGTGGCTGAATGCCTCATGGA
TTCTTACAGCTTGGATGTCCATGGGGGACGAAGGACTGCAGCTGGC**TGA**GAGGGTTGAGATCTCTGTTTACTTAGAT
CTCTGCCAACTTCCTTTGGGTCTCCCTATGGAATGTAAGACCCCGACTCTTCCTGGTGAAGCATCTG**ATG**CACGTTC
CATCCGGCGCTCAGCTGGGCTTGAG¶

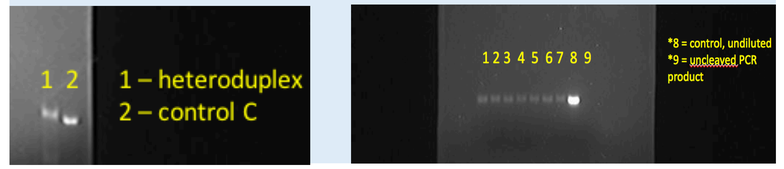
Purple is translated in PEP-C and PEP-L. Green is translated in PEP-C but not PEP-L. Blue arrows indicate cleavage sites

Results:

PCR amplification and gel electrophoresis failed to show differences between the cell lines and the HCT control. In consulting other researchers working with CRISPR/Cas9, we discovered that minor losses in bases (1-3 base losses) would not be detected through agarose electrophoresis.



Results from an additional surveyor technique conducted failed to identify differences between the cell lines.



Future Direction:

Currently, the Marahrens lab has decided to continue the experiment by lipofecting the HCT116 cells with two CRISPR/Cas9 targeting constructs instead of one – as conducted in this experiment. The following two ultramers have been constructed:

U6-Rev-Exon2-Circ1 (=Guide1)
AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTT
CTAGCTCTAAAACG**CTGAGCGCCGGATGGAACGCG**GTGTTTCGTCCTTTCCACAAG

U6-Rev-Exon2-Circ2 (=Guide 2)
5'
AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTT
CTAGCTCTAAAACT**CCGGCGCTCAGCTGGGCTTCG**GTGTTTCGTCCTTTCCACAAG

References:

Barrangou, R., & van der Oost, J. (2013). CRISPR-Cas systems. *RNA-mediated Adaptive*.
Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nat. Protocols*, *8*(11), 2281–2308.
Ran, F. A., Hsu, P. D., Lin, C.-Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., … Zhang, F. (2013). Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. *Cell*, *154*(6), 1380–1389.
Tseng, Y.-Y., Moriarity, B. S., Gong, W., Akiyama, R., Tiwari, A., Kawakami, H., … Bagchi, A. (2014). PVT1 dependence in cancer with MYC copy-number increase. *Nature*, *512*(7512), 82–86.
Wang, H., Yang, H., Shivalila, C. S., Dawlaty, M. M., Cheng, A. W., Zhang, F., & Jaenisch, R. (2013). One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. *Cell*, *153*(4), 910–918.