Learning Molecular and Cell Biology Methods Using Human Cancer Models

Melissa A. Gathman[1,], Razan S. Waliagha[2], Amit Kumar Mitra [3,4]*

[1] Undergraduate Student, Department of Biological Sciences, Auburn University
[2] MS Graduate Student, Department of Drug Discovery and Development, Harrison College of Pharmacy, Auburn University
[3] Assistant Professor, Department of Drug Discovery and Development, Harrison College of Pharmacy
[4] Director of the Center for Pharmacogenomics and Single-Cell Omics Initiative (AUPharmGx), Auburn University

Prostate cancer is the most common non-skin cancer in men in the United States (Zhao, 2021). One form of treatment for prostate cancer is drugs that inhibit cell division (Zhao, 2021). Multiple myeloma is a cancer of the plasma cells (Padala, 2021). Proteosome inhibitors (PIs) are the main form of treatment for MM and often have resistance built against it over time in cancer patients, making these genes an effective target to look at (Padala, 2021).

Many cancers can often develop drug resistance to various treatments to primary therapies such as Docetaxel (Prostate Cancer) and PIs (Multiple Myeloma) and are, therefore, viable subjects to look at specific genetic influences on reactions to certain drugs and to study new therapies (Padala, 2021; Mitra, 2020).

My research goal was to learn and practice techniques in order to validate new therapies against drug-resistant and aggressive forms of cancers. This was split into different sections, including Primer Design, Cell Culture, and Assays/Gels.

For primer design, we selected the genes (PSMB4 and PSMB5) that code for proteasomes that break down proteins and cell waste that naturally builds up were first investigated (Padala, 2021; Mitra, 2020). As proteasome inhibitor drugs are used as treatments against Multiple Myeloma, naturally, we focused on genes that code for proteasomes in order to explore potential targets for genetic therapies or identify any impacts on PI treatment as a result of genetic variation.

Genes were queried on the NCBI database (https://www.ncbi.nlm.gov/gene) to obtain an annotated version in order to determine what is a coding exon in comparison to a non-coding intron. After this, a list of common single nucleotide polymorphisms (SNPs) was compiled from the NCBI's dbSNP database (https://www.ncbi.nlm.gov/gene). We focused on those SNPs that are in an exon using the UCSC Genome Browser (https://genome.ucsc.edu/cgi-bin/hgGateway). Representative examples are provided in Figure 1 and Table 1.

Primers were designed via Primer3 (https://primer3.ut.ee/) to generate amplicons between 250-450bp in length to target the selected SNPs' loci. Primer lengths were between 18-24bp. Additional characteristics of the primers included GC content of ~40%-80% and a melting temperature Max of 63.0°C (Mitra, 2011).

Cell culture was practiced with PC3M, an aggressive prostate cancer cell line, in T-25 and T-75 flasks with F12K media. Media was changed every ~2-3 days until 90% confluent and then split by adding trypsin (4 mL in T-25 and 6-7 mL in T-75), spinning them down via a centrifuge, and placing half in a new flask and freezing, and the other half in another flask to keep incubating. PC3M cells are adherent to the flask. Therefore, in order to split cells, trypsin is used to remove them from the bottom. By being able to do this, cell lines were able to be maintained, which is the first step to doing DNA/RNA extraction, and the various assays and gels needed for drug treatment experiments.

MTT assays are used to assess cytotoxicity assays. First, cells must be around 75%-95% confluent. They are then centrifuged down to a pellet, and 10 µL cells are placed into a microtube and counted using a cell counter. This is used to determine cells to be added in each well to add up to 80 µL per well. After cells are plated, they are incubated for 24 hours, and drug is added. Following 48 hours of incubation with the drug, MTT is added, and an absorbance assay was performed for drug sen-

* Corresponding author: mag0099@auburn.edu

sitivity studies.

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This fellowship supported undergraduate research involved learning many vital techniques and protocols in a lab setting and will hopefully lend to future uses in the experiments mentioned above. Being well-versed in these techniques and protocols is an essential pre-requisite to research on i) developing targeted approaches for cancer treatment, ii) identifying if there are any genetic variations that influence response to these drugs, and iii) predicting which molecular pathways are activated or inhibited following treatment with novel drugs.

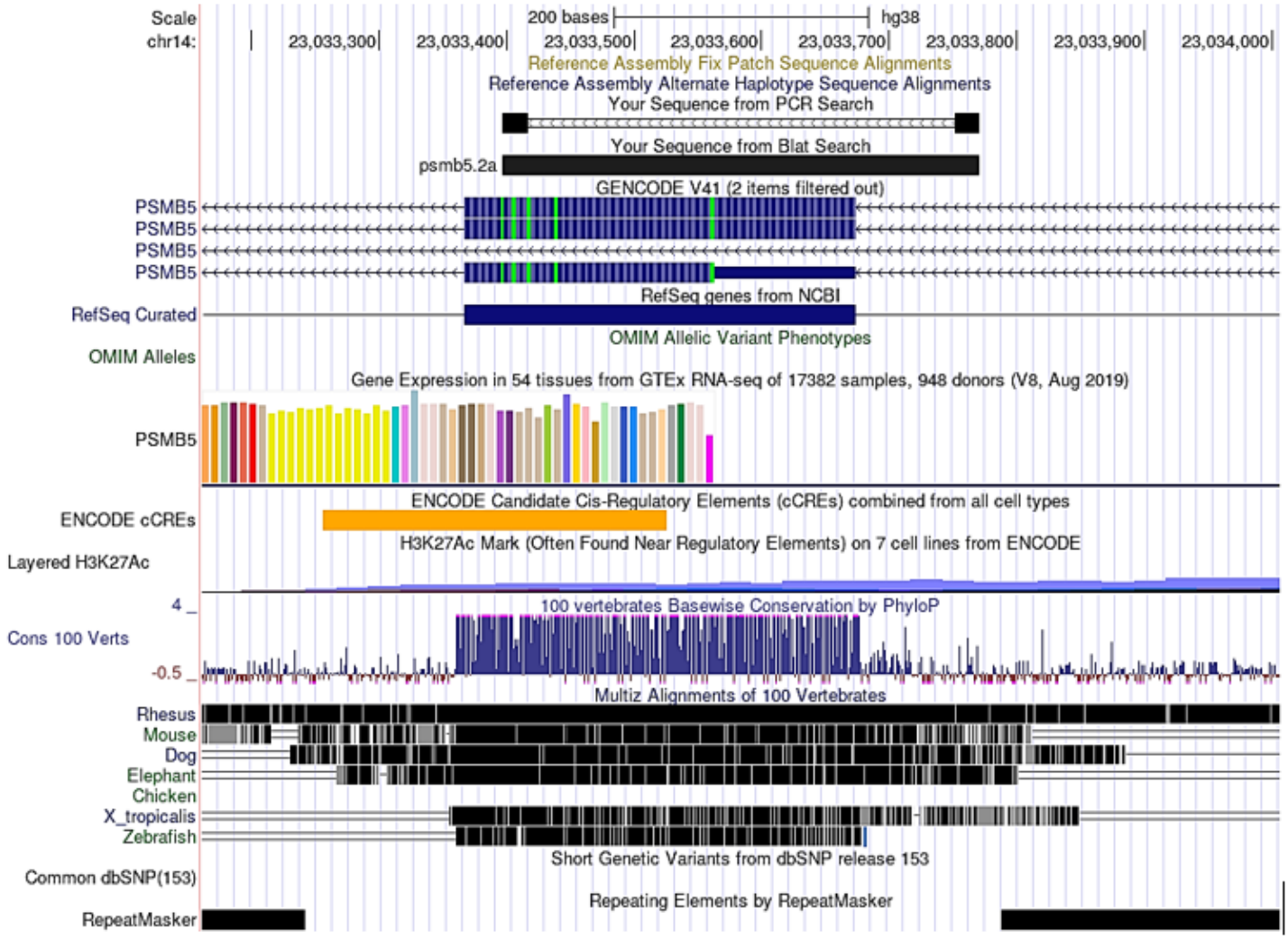

Fig. 1. UCSC Genome Browser image showing PCR primers for the Gene PSMB5 (Exon 2)

Table 1 Primers created using Primer3 for SNP identification using PCR-Sanger Sequencing method.

Gene	Exon	Product Length	Amplicon sequence
PSMD4	1	250bp	AAATGGTCTTTCGCATCTGGACCGCAAACT CCGGTCAGAAATGGTTTCGCATGGCtcttcttttg gaggcgtgcttgccagcagtcaaaatggctccattccggaatagatttat aggaagtgaagctgtgacggcgaggcgttgcccggcctatctttgctag gcgttctcagaattagttctttctgcccacactagacatggcgcttgccag cgtgttggagagaccgctaccggtgaaccagcgcgggtttttcggactt gggggtcgtgcagatctgctggatctaggtccagggagtctcagtgatg gtctgagcctggccgcgccaggctggggtgtcccaaagagccaggaa tcgaaatgcttcatggaacaaccaccctggccttcaaggtgtggagcca gcccccttgccaggctgagtactgaacgcccgcgacttgcctggcctc cagcCTGACCGGAGTTTGCGGT
PSMD4	2	422bp	GAGATGTGCTGGGTTTGGATATGGTGCCC ATGGACAGCCCCGAGATGTGCTGGGTTTG GATtgatgcaaaaagaagtatGtaggagtgtttttgtggtcttatgt ggcctgttttgtgttttcctctgatcttaacagttccgccatggagtcata gttgcagctgactccagggctacagcgggtgcttacattgcctccca gacggtgaagaaggtgatagagatcaacccatacctgctaggcacc atggctgggggcgcagcggattgcagcttctgggaacggctgttgg ctcggcaatgtcgaatctatgagcttcgaaataaggaacgcatctctg tagcagctgcctccaaactgcttgccaacatggtgtatcagtacaaag gcatGGGGCTGTCCATGGGCACCAT
PSMB5	2	375bp	GAGATGTGCTGGGTTTGGAT ATGGTGCCCATGGACAGCCCC GAGATGTGCTGGGTTTGGATtgatgcaaaaagaa gtatgtaggagtgtttttgtggtcttatgtggcctgttttgtgttttcctctg atcttaacagttccgccatggagtcatagttgcagctgactccagggc tacagcgggtgcttacattgcctcccagacggtgaagaaggtgatag agatcaacccatacctgctaggcaccatggctgggggcgcagcgg attgcagcttctgggaacggctgttggctcggcaatgtcgaatctatg agcttcgaaataaggaacgcatctctgtagcagctgcctccaaactg cttgccaacatggtgtatcagtacaaaggcatGGGGCTGTC CATGGGCACCAT

Statement of Research Advisor

Ms. Gathman's research in the lab as an undergraduate research fellow involved extensive orientation to standard cell and molecular biology techniques. She has shown gradual progress over the past year. We are confident that she will be able to contribute to genomics, pharmacogenomics and translational research in industry or academia, whichever career path she chooses to pursue.

-Amit Kumar Mitra, Department of Drug Discovery, Harrison College of Pharmacy

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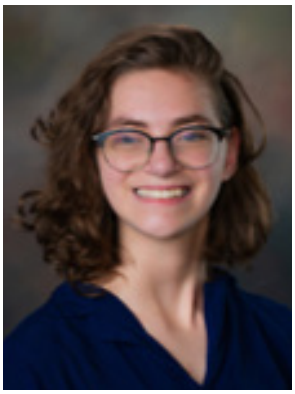
Authors Biography



Melissa A. Gathman is a senior-year student pursuing a B.S. degree in Genetics at the College of Science and Mathematics at Auburn University. She has spent time learning a variety of important techniques necessary to succeed as a lab researcher.

Razan S. Waliagha, MS is a Student Pharmacist and Pharmacogenomics Oncology Graduate at Harrison College of Pharmacy at Auburn University. She is skilled in Good Laboratory Practice (GLP), Medical Terminology, Molecular and Cell Biology techniques, Pharmacology, and Clinical Pharmacology.

Dr. Amit K. Mitra is an Assistant Professor at the Harrison College of Pharmacy at Auburn University, and Director of the AU Center for Pharmacogenomics and Single-Cell Omics Initiative (AU-PharmGx). His research focuses on investigating genomic and pharmacogenomic factors underlying variations in drug sensitivity, as well as predicting secondary drug candidates in aggressive human cancers such as relapsed/refractory multiple myeloma and lethal forms of prostate cancer.