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# DNA Methylation Patterns of Line-1 Elements Specific to HC21P in Cancer Cells

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# DNA METHYLATION PATTERNS OF LINE-1 ELEMENTS SPECIFIC TO HC21P IN CANCER CELLS

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To Marvin, my dad, my best friend.
Without you, I would not have been able to finish this journey.

Tell me and I forget
Teach me and I learn
Involve me and I remember

Benjamin Franklin

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ABSTRACT

Long Interspersed Nuclear Elements (LINEs) are retrotransposons. They function by making a cDNA copy, reverse-transcribing it into DNA and reinserting itself back into the genome; thereby, increasing its copy number. Sequencing of the human genome revealed that LINEs (L1) are underrepresented on the short arm of human chromosome 21 (HC21), but it is enriched in full-length copies. A number of previous studies have investigated the DNA methylation patterns of L1s in cancer cells, but these studies are typically done in euchromatic regions. The heterochromatic short arm of HC21 is primarily composed of heterochromatin. However these regions can be targeted for study since they play an important role in chromosome function and a few genes may be concealed in them. Here I investigate DNA methylation patterns in promoter regions of four full-length L1s on the short arm of HC21 in prostate cancer cells with various forms of aggression. I show that the L1s on the short arm are hypomethylated relative to those on the long euchromatic arm. Also the short arm L1s are hypomethylated in prostate cancer cells relative to normal cells, but there is no apparent correlation of methylation level and degree of tumor aggression.

# CHAPTER ONE

## INTRODUCTION

The Human Genome Project identified close to 20,500 genes, but the 10-15% of the genome consisting of heterochromatin was not included (IHGSC, 2004). Heterochromatic regions are highly condensed clusters of tandemly repetitive DNA sequences that are very difficult to sequence and generally do not have coding genes. However, these regions play an important role in chromosome function, including centromeres and telomeres. The tandem repeats, or satellite DNAs, exist as sequence families (Lee et al., 1997). Satellite regions are located on the short (p) arms of all the acrocentric chromosomes (13, 14, 15, 21, and 22). The extensive sequence similarity of these regions allows non-homologous pairing during meiosis (DeCapoa et al., 1973).

The short arm of human chromosome 21 (HC21p) is an ideal model for studying heterochromatin. HC21 is the smallest chromosome in the genome and its p arm provides the highest fraction of heterochromatin of any human chromosome. HC21p is comprised of large clusters of tandem repeats interrupted by islands of low copy number repeats. It includes a number of satellite families such as alphoid, beta, and satellite I as well as interspersed repetitive DNA sequence families (Doering et al., 1993). The two major classes of these repeat families are SINEs (short interspersed nuclear element) and LINEs (long interspersed nuclear element), with LINE-1 (Figure 1) being the most prevalent family of LINEs constituting more than 17% of the total genome (Kazazian, 2004). Because of their persistence for hundreds of millions of years,

LINEs are considered to be the most successful transposable element. As hosts began to develop mechanisms responding to retrotransposition, LINE subfamilies emerged (Boissinot & Furano, 2005). LINE-1 (L1) constitutes 17% of the total genome (Boissinot & Furano, 2005). Within the last 25 million years, five primate-specific L1s have surfaced, from oldest to youngest : L1PA5, L1PA4, L1PA3, LIPA2, AND L1PA1 (Smit et al., 1995). L1PA1 is also human specific and has shown correlation in it's retrotransposition linked to disease (Hancks & Kazazian, 2012).

LINE-1 (L1) elements are 6,000 bp long, include a 5' and 3' UTR, two ORFs that code for all proteins needed for autonomous retrotransposition, and a poly adenylation signal (Feng et al., 1996).

Figure 1. The Structure of an L1 Element (Singer et al., 2010)


*Note*. Two open reading frames; 5' and 3'. UTR = untranslated regions; TSD = target site duplication; pA = polyadenylation tail.

These retrotransposons function by making a cDNA copy, reverse-transcribing it into DNA and reinserting itself back into the genome. This increases the copy number within the genome. This mechanism is known as "target primed reverse transcription" (Kazazian, 2004). L1 reverse transcriptase frequently disengages from the RNA template before completing the cDNA sequences. Therefore, full-length L1s are rare and typically elements in the genome are 5' truncated (Feng et al., 1996). Only full-length L1s are active since truncated elements lack a promoter. Aside from containing multiple transcription factor binding sites, the promoter of L1s

contain a high frequency of CpG sites (Hata & Sakaki, 1997). Methylation of these CpGs is an epigenetic mechanism used to regulate gene expression. As the promoter regions of DNA sequences become saturated with methyl groups in a CpG di-nucleotide context, an indirect relationship with gene expression is seen—increased levels of methylation and decreased gene expression (Klug et al., 2008). This is true of L1s as well. Promoter methylation of L1s has been shown to suppress retrotranspositon of full-length L1s (Hata & Sakaki, 1997). Hypomethylation of L1s is associated with their retrotransposition as well as chromosomal aberration, hypermethylation of tumor suppressor genes, and alternative transcription of oncogenes (Roman-Gomez et al., 2005).

A number of studies suggest that L1s may contribute to the process of X chromosome inactivation. LINEs are twofold enriched on the X chromosome relative to autosomes (Bailey et al., 2000). The most significant increase was found in Xq13: a region containing the X inactivation center (Bailey et al., 2000). Furthermore, many of the L1s on the inactive X chromosome are expressed during X chromosome inactivation (Chow et al., 2010; Hansen, 2003). Prior work in our lab has shown that L1s are underrepresented on the short arm of human chromosome 21; however, there are 20 times more full-length L1s on the short arm than the long arm (Beris, 2003). In another study, analysis of the X chromosome showed that most of these elements were clustered near the X inactivation center. Genomic segments that "escaped" inactivation were reduced in the number of L1s compared to those genes involved in X inactivation. This provided strong, non-random evidence pointing to the L1 elements being signals to propagate X inactivation along the chromosome (Lyon, 1998).

Thus, L1s on HC21p may be involved in establishing and/or maintaining the heterochromatin in this region in a manner analogous to the L1s on the inactive X. I will characterize the DNA methylation patterns of the full-length L1s on HC21p, a region that has not been previously studied.

L1 methylation patterns have been studied frequently in cancer cells, where global patterns of DNA methylation are dramatically different in tumor cells versus normal cells (Bestor et al., 2014). A study of chronic myelogenous leukemia (CML) revealed 74.5% hypomethylation of the DNA in these cells compared to matched normals (Roman-Gomez et al., 2005). In addition, the degree of hypomethylation increases as the clinical stage progresses (Roman-Gomez et al., 2005). Additional studies show that global patterns of LINE methylation may not necessarily correlate with cancer prognosis. One study looked at global DNA methylation of two transposable elements in various types of cancers and found that LINEs showed no correlation in prostate cancer prognosis (Barry et al., 2015). When comparing locus-specific methylation of oral cancer cells versus normal, the two cell types could be differentiated (Pobsook et al., 2011). When this team attempted to do the same technique using global methylation, they did not see differentiation (Pobsook et al., 2011). Similarly, another study looked at various tumor tissue samples and found that LINEs are preferentially methylated in a cell-specific and locus-specific manner (Nüsgen et al., 2015). Another study showed that L1s were globally less methylated than matched normal tissue, but did not find a significant correlation between L1 methylation and clinical prognosis or tumor grade (Ogino et al., 2008). These experiments suggest that locus-specific L1 methylation patterns may give a more accurate approach to distinguishing normal and tumor cells. All this previous work has been done in

euchromatic regions; L1s in heterochromatic regions have not been examined.

This project investigated DNA methylation patterns in the promoter regions of four full-length L1s on heterochromatic HC21p. This allowed me to see if different L1 families show different patterns of methylation. I compared these L1s on the short arm to a control L1 on the euchromatic long arm region. Methylation at all these L1 loci were assayed in normal leukocytes as well as prostate cancer cells from varying stages of tumor progression (respectively: RWPE, LNCaP, PC3, DU145).

# CHAPTER TWO

# MATERIALS AND METHODS

## Introduction

DNA methylation assays require the design of PCR primers that are locus specific. The primers are strategically placed so that they are within the beginning of the L1 promoter and also contain the unique sequences that are alongside it (Tables 1-6). To determine methylation of L1 promoter CpGs, I utilized bisulfite sequencing. If the CpG is methylated, bisulfite treatment will not affect the site. However, if the CpG is unmethylated, C will be converted to a U, resulting in the insertion of thymine during PCR. The PCR products are then cloned into a plasmid vector and transformed into competent E. coli cells. The vectors are recovered using a mini prep kit and sent out for DNA sequencing. Utilizing an online software program, Geneious, we annotated and trimmed bisulfite-treated and normal clones. We compared the consensus sequence of 12 unconverted clones to 12 converted to see changes in methylation via QUMA. In addition to showing how efficient the conversion process is, this online methylation analysis tool gives us a visual pattern of methylation for the specific locus.

PCR was performed on bisulfite-treated (converted) and unconverted DNA cell lines. A second round of PCR was performed for increased specificity. After verification via agarose gel electrophoresis, PCR products were cloned. Mini prep was performed on colonies and sent out for Sanger sequencing. DNA methylation of L1s in prostate cancer was analyzed via four cell lines. These cells were donated from a colleague and former member of the Doering Lab, Dr. Jennifer Doll. Dr. Doll's research investigates the role of obesity and high fat diets in the

progression of prostate cancer. Each cell line used varies in its state of progression. RWPE-1 is a normal human prostate epithelial cell line. It has been immortalized with HPV and therefore p53 and retinoblastoma (Rb) negative. LNCaP is a prostate cancer line that has metastasized to lymph nodes. It is androgen-sensitive and mildly aggressive. PC-3 prostate cancer cells have metastasized to the bone. It is androgen-insensitive and more aggressive. DU145 prostate cancer cells have metastasized to the brain. It too is androgen-insensitive and most aggressive. These are standard cell lines used in prostate cancer research (Alimira et al., 2006). We purchased WAV17 from Coriell Cell Repositories. WAV17 is a somatic (fibroblast) mouse-human hybrid cell line that contains only chromosome 21. White blood cells were also ordered as a control to the body's natural defense system.

**Bisulfite Conversion**

To analyze methylation patterns, 1ug of DNA from each cell line was bisulfite treated (using QIAGEN's EpiTect Plus kit).

**Polymerase Chain Reaction**

Bioline's EPIK Amplification Kit was used for unconverted DNA. Standard protocol was followed with 2ug starting DNA. The QIAGEN PyroMark PCR kit, standard protocol, was used for converted samples with 10ng starting DNA. Primers used for PCR, Tables 1-6, were designed to include part of the unique sequence adjacent to the L1, and part of the conserved sequence within the L1(Tincher, 2016).

**Cloning**

Invitrogen TOPO® TA Cloning Kit for Sequencing used for PCR products with the following variations: 4ul fresh PCR product and increased reaction time of 45 minutes.

**Transformation**

ZYMO Research Premade Mix & Go Competent E. coli cell kit utilized. Standard protocol followed with the following modifications: 6 ul transformation product added to cells; cells incubated for 5 minutes on ice.

**Sequence Analysis**

Sanger sequencing performed by ACGT, INC molecular biology services. Sequences analyzed via Geneious software. Converted sequences were compared to unconverted sequences using the Quantification tool for Methylation Analysis (QUMA). Parameters set included: sequences must be at least 90% identical and have 95% conversion efficiency. Since our cancer cell samples are genomic DNA, QUMA accounted for amplification of LINE-1 sequences on other acrocentric chromosomes by using a strict CpG site parameter. If any CpG sites arose that were not in the reference, or an expected CpG site was missing, those points were not included in data analysis.

Table 1. Locus 1 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| L1PF9_ Uncon | GTAGGACCCTCTGAGCCAGGTGTG | Outside Forward | 61-66 | 761 | - |
| L1PF9_ Con | GTAGGATTTTTTGAGTTAGGTGTG | Outside Forward | 50-55 | 761 | - |
| AF254982.R5_ Uncon | GGAGCCCTTTGTGCCCTATTGTGTAAAAGGA | Outside Reverse | 61-66 | 761 | - |
| AF254982.R5_ Con | AAAACCCTTTATACCCTATTATATAAAAAAA | Outside Reverse | 50-55 | 761 | - |
| L1P.N1_ Uncon | GAGCCAGGTGTGGGATATAGTCTC | Nested Forward | 57-62 | - | 681 |
| L1P.N1_ Con | GAGTTAGGTGTGGGATATAGTTTT | Nested Forward | 54-59 | - | 681 |
| AF254.N1_ Uncon | CTTCTTTGTGATGAATACATTCCTCACAC | Nested Reverse | 57-62 | - | 681 |
| AF254.N1_ Con | CTTCTTTATAATAAATACATTCCTCACAC | Nested Reverse | 54-59 | - | 681 |

Table 2. Locus 2 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| AF254.R7_ Uncon | CTGCTGCCTTTTATTTGGCTATGCCC | Outside Forward | 58-63 | 889 | - |
| AF254.R7_ Con | TTGTTGTTTTTTATTTGGTTATGTTT | Outside Forward | 49-54 | 889 | - |
| L1PF10_ Uncon | CTTACTTGATTCTGGATATTGGGTCCAGCT | Outside Reverse | 58-63 | 889 | - |
| L1PF10_ Con | CTTACTTAATTCTAAATATTAAATCCAACTCT | Outside Reverse | 49-54 | 889 | - |
| AF254.N3_ Uncon | CCCAGAGGTGAAGTCTACAGAGGCAGG | Nested Forward | 62-67 | - | 826 |
| AF254.N3_ Con | TTTAGAGGTGAAGTTTATAGAGGTAGG | Nested Forward | 53-58 | - | 826 |
| L1P.N2_ Uncon | TTGATTCTGGATATTGGGTCCAGCTCTTCCCC | Nested Reverse | 62-67 | - | 826 |
| L1P.N2_ Con | TTAATTCTAAATATTAAATCCAACTCTTCCCC | Nested Reverse | 53-58 | - | 826 |

Table 3. Locus 3 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| L1PF13_ Uncon | GCTGTCTGTCAACCCTTTCTTTGACTAGG | Outside Forward | 56-61 | 658 | - |
| L1PF13_ Con | GTTGTTTGTTAATTTTTTTTTTGATTAGG | Outside Forward | 49-54 | 658 | - |
| AF254.R8_ Uncon | GTTATCCACCATAGTCCTGAAAGTGCTC | Outside Reverse | 56-61 | 658 | - |
| AF254.R8_ Con | ATTATCCACCATAATCCTAAAAATACTC | Outside Reverse | 49-54 | 658 | - |
| L1P.N5_ Uncon | CTTTCTTTGACTAGGAAAGGGAACTCC | Nested Forward | 56-61 | - | 534 |
| L1P.N5_ Con | TTTTTTTTGATTAGGAAAGGGAATTTT | Nested Forward | 46-51 | - | 534 |
| AF254.N4_ Uncon | GTCCTGAAAGTGCTCCAAATGTCC | Nested Reverse | 56-61 | - | 534 |
| AF254.N4_ Con | ATCCTAAAAATACTCCAAATATCC | Nested Reverse | 46-51 | - | 534 |

Table 4. Locus 4 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| L1PF12_ Uncon | TGTTCTGGGAGAACCACTGTTCTC | Outside Forward | 56-61 | 827 | - |
| L1PF12_ Con | TGTTTTGGGAGAATTATTGTTTTT | Outside Forward | 48-53 | 827 | - |
| CT476.R1_ Uncon | CTTCTTTGGAATGTGTGCATACAACTCACC | Outside Reverse | 56-61 | 827 | - |
| CT476.R1_ Con | CTTCTTTAAAATATATACATACAACTCACC | Outside Reverse | 48-53 | 827 | - |
| L1P.N4_ Uncon | GCTGTCAGACAGGGACATTTAAGT | Nested Forward | 54-59 | - | 655 |
| L1P.N4_ Con | GTTGTTAGATAGGGATATTTAAGT | Nested Forward | 46-51 | - | 655 |
| CT476.N1_ Uncon | TAATAACTACACAGAAGCAAACTGGC | Nested Reverse | 54-59 | - | 655 |
| CT476.N1_ Con | TAATAACTACACAAAAACAAACTAAC | Nested Reverse | 46-51 | - | 655 |

Table 5. Locus 5 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| L1PF16_ Uncon | GCCCAACTGTTACCTTGCAGTTTG | Outside Forward | 56-61 | 559 | - |
| L1PF16_ Con | GTTTAATTGTTATTTTGTAGTTTG | Outside Forward | 44-49 | 559 | - |
| CR535.R1_ Uncon | CTTGGATGTGTGATCAAATCTCAATTCC | Outside Reverse | 56-61 | 559 | - |
| CR535.R1_ Con | CTTAAATATATAATCAAATCTCAATTCC | Outside Reverse | 44-49 | 559 | - |
| L1P.N8_ Uncon | GTTTGATCTCAGACTGCTGTGCTA | Nested Forward | 54-59 | - | 502 |
| L1P.N8_ Con | GTTTGATTTTAGATTGTTGTGTTA | Nested Forward | 46-51 | - | 502 |
| CR535.N1_ Uncon | CAGAAGCTGATTCATTATCCTCTTGAG | Nested Reverse | 46-51 | - | 502 |
| CR535.N1_ Con | CAAAAACTAATTCATTATCCTCTTAAA | Nested Reverse | 46-51 | - | 502 |

Table 6. Long Arm Locus 1 Corresponding Primer Names for First and Second Round PCRs and Temperature Gradients Used to Determine Ideal Annealing Temperatures and Amplicon Sizes

| Name | Sequence | Condition | Ideal Temperature Gradient (°C) | Outside Amplicon Size | Nested Amplicon Size |
|---|---|---|---|---|---|
| L1PF14q_ Uncon | CCAGGTGTGGGATATAGTCTCATGGTGC | Outside Forward | 58-63 | 415 | - |
| L1PF14q_ Con | CCAAATATAAAATATAATCTCATAATAC | Outside Forward | 44-49 | 415 | - |
| AL163.R1q_ Uncon | GACCCAACACTCAGTTCTAGCATTTCCTCTC | Outside Reverse | 58-63 | 415 | - |
| AL163.R1q_ Con | GATTTAATATTTAGTTTTAGTATTTTTTTTT | Outside Reverse | 44-49 | 415 | - |
| L1P.N6_ Uncon | CTTCCCAGGTGAGGCAATGC | Nested Forward | 53-58 | - | 268 |
| L1P.N6_ Con | CTTCCCAAATAAAACAATAC | Nested Forward | 48-53 | - | 268 |
| AL163.N1_ Uncon | CCAACACTCAGTTCTAGCATTTCCTCTC | Nested Reverse | 53-58 | - | 268 |
| AL163.N1_ Con | TTAATATTTAGTTTTAGTATTTTTTTT | Nested Reverse | 48-53 | - | 268 |

# CHAPTER THREE

# RESULTS

## Introduction

We looked at patterns of methylation across four different loci. These loci were studied in cancer cells with varying forms of metastasis. For comparison, we used a cell line which only contained HC21, and WBC (Table 7). For each locus studied, I give a brief description of the findings regarding overall % methylation and how uniform it is or not over the positions in the locus (Figures 2-12, Tables 8-18). Then I discuss comparisons between loci, and draw attention to the clearly higher methylation for loci on the long arm versus the short arm and the overall lower methylation in cancer cells versus WBC (Table 7). That trend is there even though there is no clear pattern with regard to severity of the cancer.

### Long Arm Locus 1

When compared to loci on the short arm of HC21, this euchromatic control locus has high levels of methylation across all cell lines, ranging from 92.2%-100%. When comparing data in Table 7, there are no obvious changes in patterns of methylation between cell lines.

**Locus 1.** L1s are highly methylated in the WAV17 and WBC lines (78.9% and 74.4% respectively). And while DU145 prostate cancer would be considered stage three, similar methylation was observed there (81.2%). L1s are known to be hypomethylated genome-wide in cancer cells. It is believed that this instability is what makes the DNA unstable and contributes to "genetic chaos" in the same manner in which the X chromosome is inactivated. Here, we don't

particularly see that to be true. There is no observable trend between cancer aggression and methylation. Some of the CpG sites are also located within the promoter. Promoter sequences have higher percentages of methylation. It can be inferred that this is because promoters contain CpG islands that regulate gene control. However, in this case, there is not much variation between promoter sequences and total.

**Locus 2**. For all cell types, hypomethylation at this locus is less than the euchromatic control. In general, we see that baseline prostate methylation in the normal RWPE-1cell line is less than WBC (41.2% vs. 73.40%). No clear pattern differences were observed (Tincher, 2016).

**Locus 3.** At this locus, methylation was always less than in euchromatic control (Table 7). When we compare Locus 3 of each cell line to the euchromatic locus of the same cell line, we see the following differences: RWPE 65.90%, LNCaP 71.60%, DU145 46.70%, PC3 62.20%. The biggest difference in methylation between cell lines is in LNCaP (71.60%). LNCaP is the least aggressive of the cell lines. There is no observable correlation between methylation and cancer aggression at this locus.

**Locus 4.** Across all prostate cell lines, Locus 4 always has much less than half of its CpG sites methylated. Prostate cell lines overall have lower methylation levels (7.6%, 21.4%, 28.6%, 34.9%) than WBCs at 69.4%. When compared to euchromatic controls, while we see no clear patterns of methylation, it is higher in Long Arm Locus 1 by at least 30% in all cell lines.

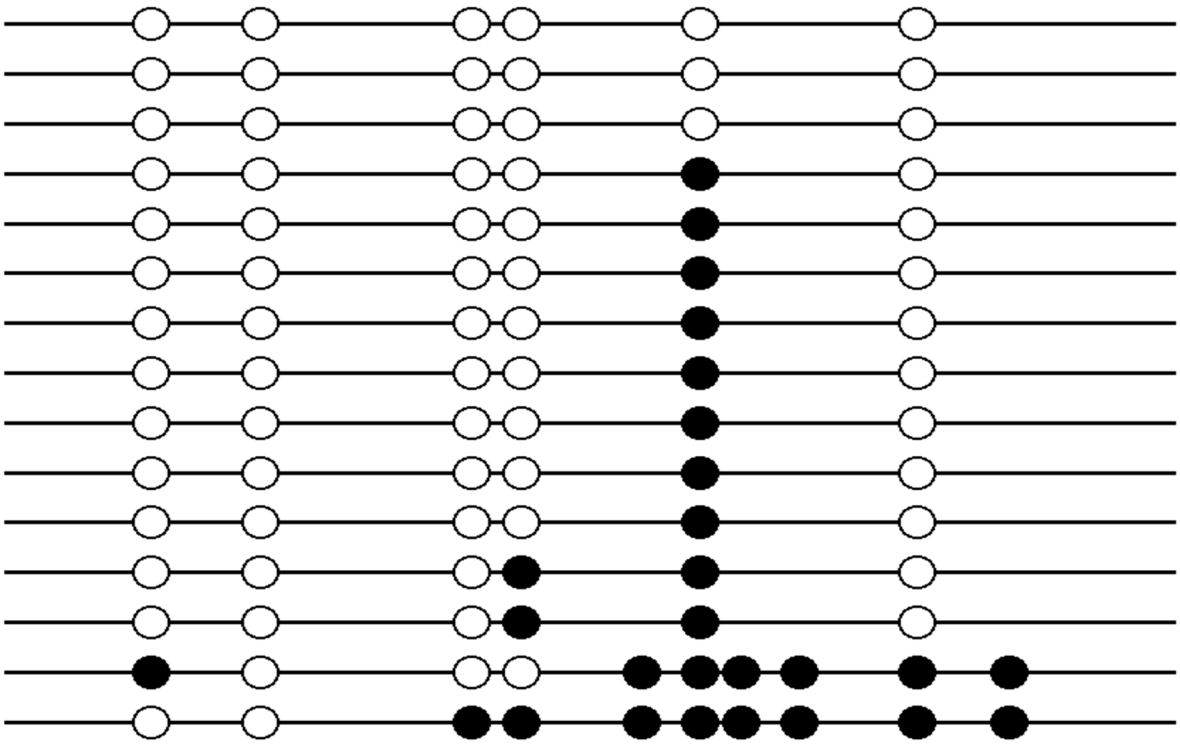
**Locus 5.** A full set of data across all cell lines was unable to be completed. Collected data are shown in Figures 10-12. At this locus WBC are 36.3% methylated and WAV17 is 77.1% methylated. Again, there is substantially lower % methylation in this short arm locus than in the long arm.

Table 7. Total Percent Methylation of L1 Loci By Cell Line

| | WAV17 | WBCs | RWPE-1 | LNCaP | DU145 | PC3 | Placenta |
|---|---|---|---|---|---|---|---|
| Locus 1 Promoter/ Total | 89.6%/ 78.9% | 82.9%/ 74.4% | 41.9% 35.6 | 22.0%/ 6.8% | 89.5%/ 81.2% | 48.0% | 26.6%/ 25.6% |
| Locus 2 | 56.7% | 73.4% | 41.2% | 0.70% | 48.9% | * | 25.0% |
| Locus 3 | 80.0% | 46.2% | 27.6% | 21.4% | 53.3% | 33.7% | 22.1% |
| Locus 4 | 52.4% | 69.4% | 21.4% | 28.6% | 34.9% | 7.60% | 28.6% |
| Locus 5 | 77.1% | 36.3% | 51.6% | No Information Available | | | |
| Long Arm Locus 1 | 99.2% | 97.0% | 93.5% | 93.0% | 100.0% | 95.9% | 92.2% |

*Note*. The values of percent methylation within the amplicon for each given locus across all clones collected from each sample are indicated here. For the loci with amplicons containing one or more CpG dinucleotides in the sequence adjacent to the L1 element, the values are given as total percent methylation within the L1 promoter/total percent methylation within the entire amplicon (promoter/total).
* = No data available.

Figure 2. Locus 3 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Diagram

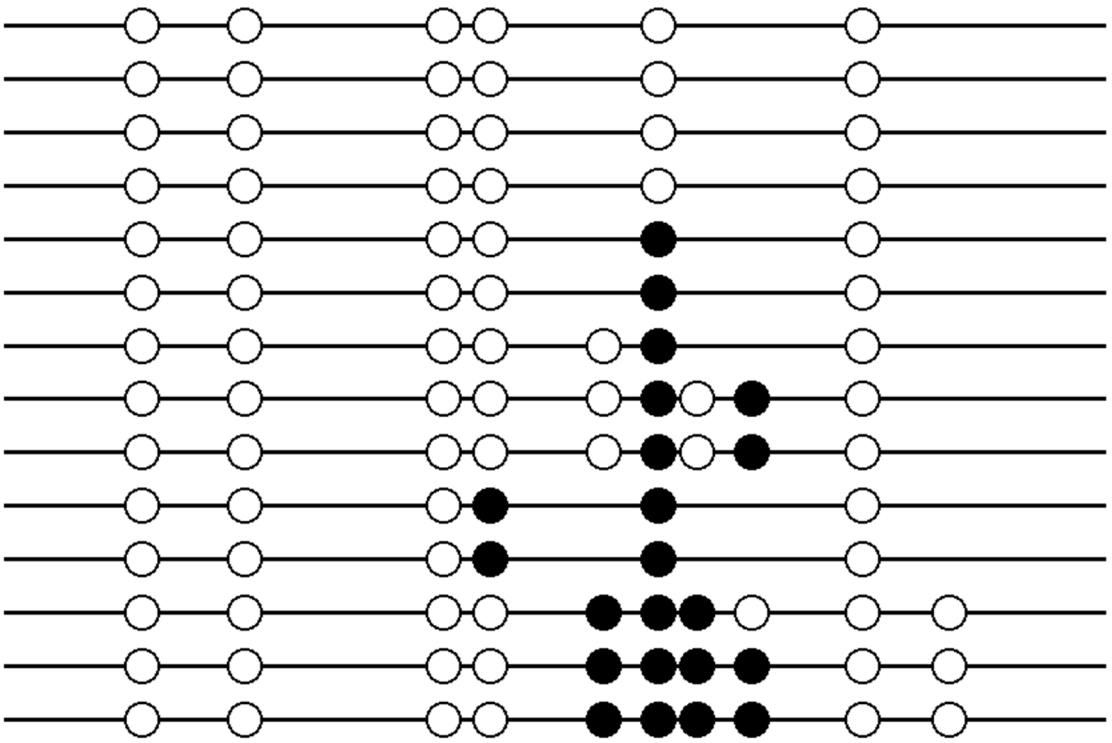


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 8. Locus 3 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 298 | | | | | | | | | | |
| Number of CpGs | 10 | | | | | | | | | | |
| CpG position | 46 | 73 | 136 | 142 | 173 | 182 | 185 | 194 | 224 | 245 | total |
| Number of methylated CpGs | 1 | 0 | 1 | 3 | 2 | 12 | 2 | 2 | 2 | 2 | 27 |
| Number of CpGs | 15 | 15 | 15 | 15 | 2 | 15 | 2 | 2 | 15 | 2 | 98 |
| Ratio of methylated (%) | 6.7 | 0.0 | 6.7 | 20.0 | 100.0 | 80.0 | 100.0 | 100.0 | 13.3 | 100.0 | 27.6 |

Figure 3. Locus 3 LNCAP Converted vs. WAV17 Unconverted QUMA Analysis Diagram

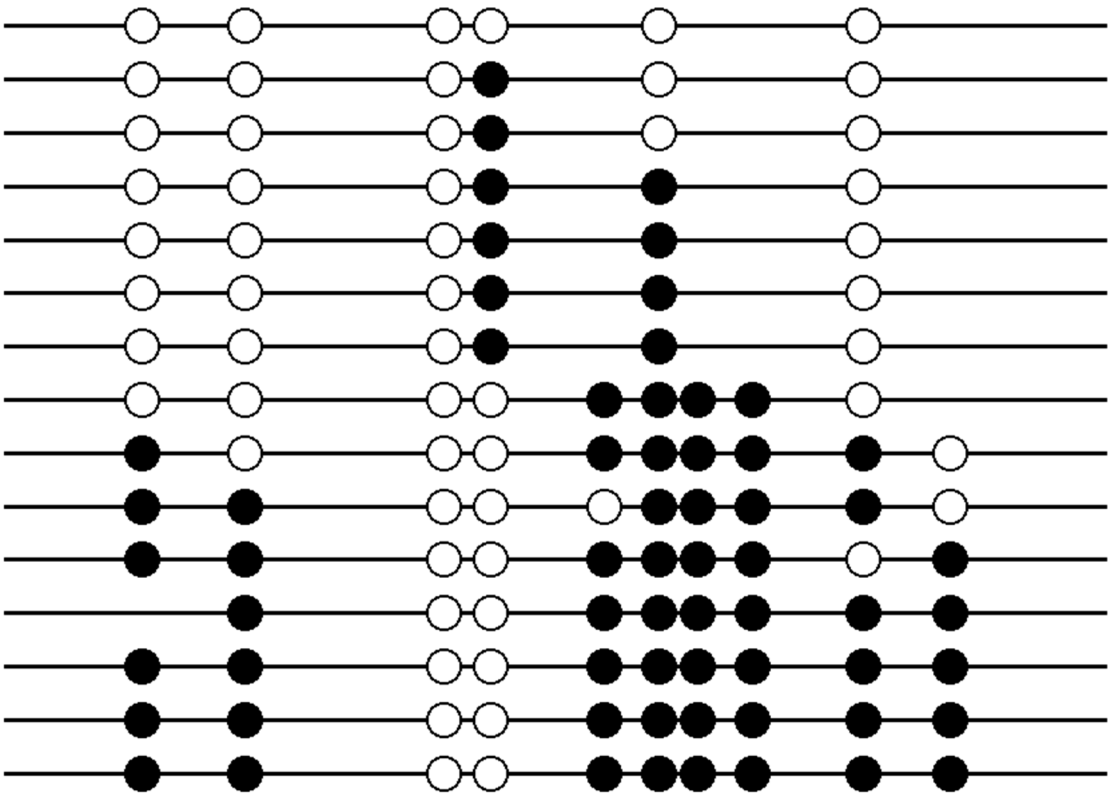


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 9. Locus 3 LNCAP Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 298 | | | | | | | | | | |
| Number of CpGs | 10 | | | | | | | | | | |
| CpG position | 46 | 73 | 136 | 142 | 173 | 182 | 185 | 194 | 224 | 245 | total |
| Number of methylated CpGs | 0 | 0 | 0 | 2 | 3 | 10 | 3 | 4 | 0 | 0 | 22 |
| Number of CpGs | 14 | 14 | 14 | 14 | 6 | 14 | 5 | 5 | 14 | 3 | 103 |
| Ratio of methylated (%) | 0.0 | 0.0 | 0.0 | 14.3 | 50.0 | 71.4 | 60.0 | 80.0 | 0.0 | 0.0 | 21.4 |

Figure 4. Locus 3 DU145 Converted vs. WAV17 Unconverted QUMA Analysis Diagram

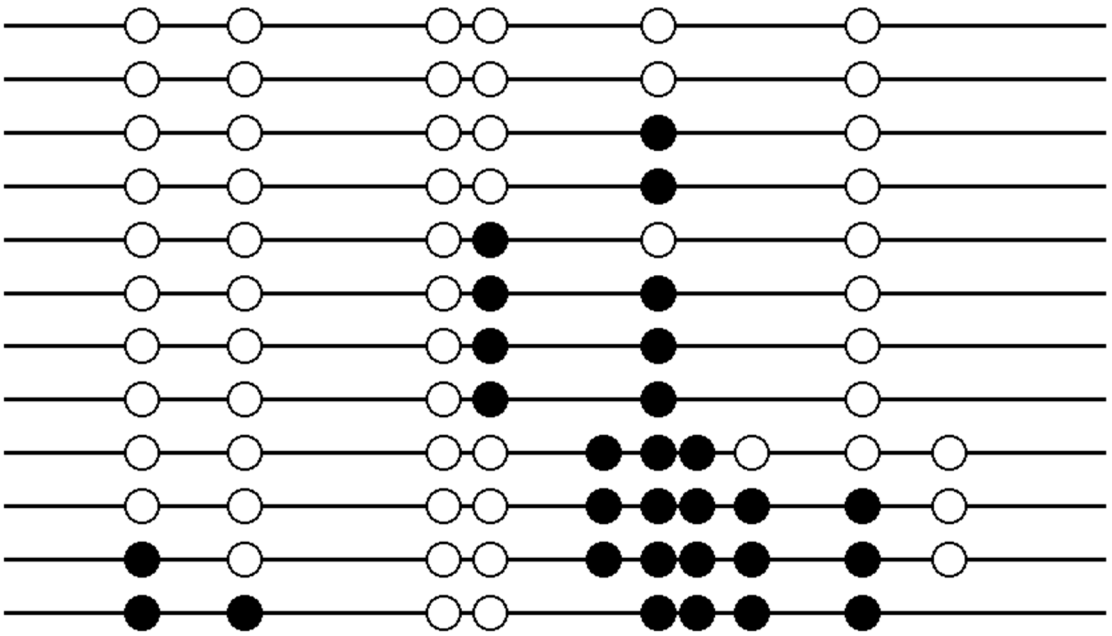


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 10. Locus 3 DU145 Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| Length of target genome sequence | 298 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of CpGs | 10 | | | | | | | | | | |
| CpG position | 46 | 73 | 136 | 142 | 173 | 182 | 185 | 194 | 224 | 245 | total |
| Number of methylated CpGs | 6 | 6 | 0 | 6 | 7 | 12 | 8 | 8 | 6 | 5 | 64 |
| Number of CpGs | 14 | 15 | 15 | 15 | 8 | 15 | 8 | 8 | 15 | 7 | 120 |
| Ratio of methylated (%) | 42.9 | 40.0 | 0.0 | 40.0 | 87.5 | 80.0 | 100.0 | 100.0 | 40.0 | 71.4 | 53.3 |

Figure 5. Locus 3 PC3 Converted vs. WAV17 Unconverted QUMA Analysis Diagram

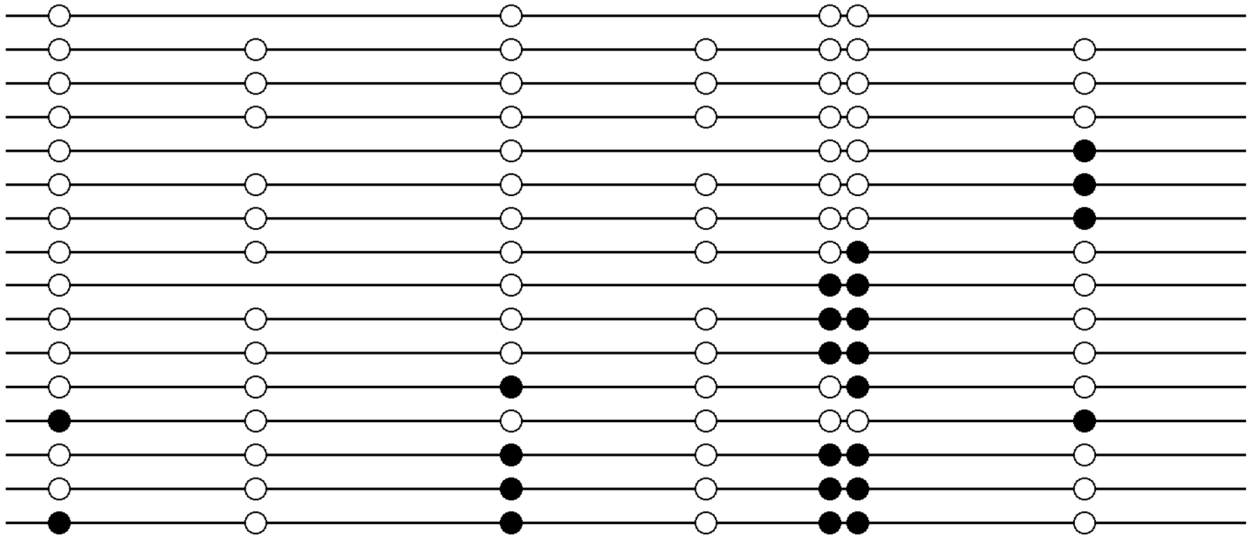


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 11. Locus 3 PC3 Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 298 | | | | | | | | | | |
| Number of CpGs | 10 | | | | | | | | | | |
| CpG position | 46 | 73 | 136 | 142 | 173 | 182 | 185 | 194 | 224 | 245 | total |
| Number of methylated CpGs | 2 | 1 | 0 | 4 | 3 | 9 | 4 | 3 | 3 | 0 | 29 |
| Number of CpGs | 12 | 12 | 12 | 12 | 3 | 12 | 4 | 4 | 12 | 3 | 86 |
| Ratio of methylated (%) | 16.7 | 8.3 | 0.0 | 33.3 | 100.0 | 75.0 | 100.0 | 75.0 | 25.0 | 0.0 | 33.7 |

Figure 6. Locus 4 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Diagram

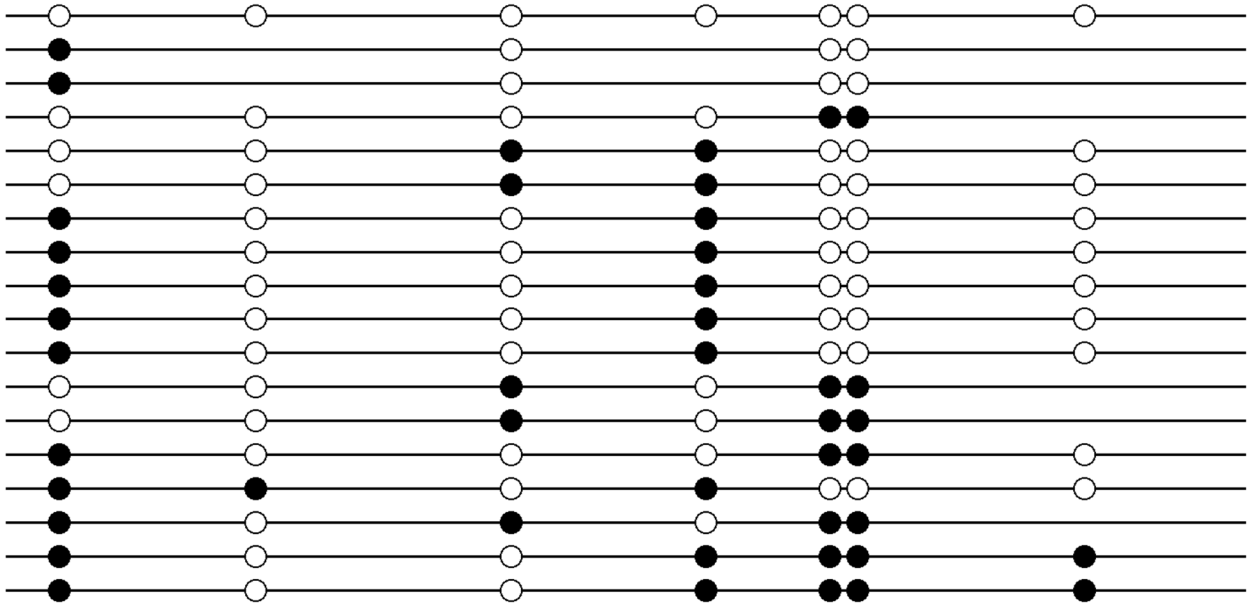


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 12. Locus 4 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 655 | | | | | | | |
| Number of CpGs | 7 | | | | | | | |
| CpG position | 26 | 131 | 271 | 375 | 437 | 442 | 565 | total |
| Number of methylated CpGs | 2 | 0 | 4 | 0 | 6 | 8 | 4 | 24 |
| Number of CpGs | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 112 |
| Ratio of methylated (%) | 12.5 | 0 | 25 | 0 | 37.5 | 50 | 25 | 21.4 |

Figure 7. Locus 4 DU145 Converted vs. WAV17 Unconverted QUMA Analysis Diagram

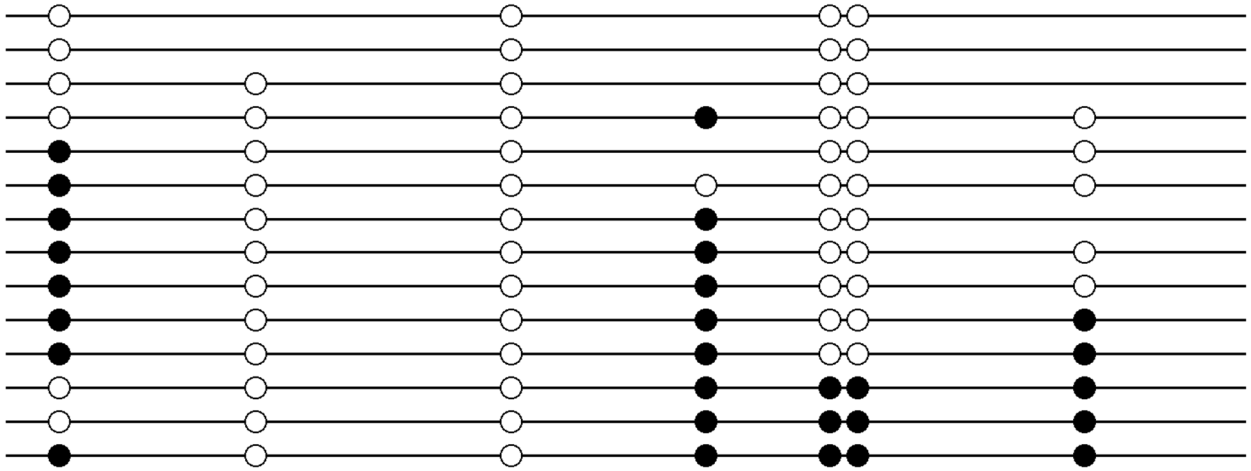


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 13. Locus 4 DU145 Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 655 | | | | | | | |
| Number of CpGs | 7 | | | | | | | |
| CpG position | 26 | 131 | 271 | 375 | 437 | 442 | 565 | total |
| Number of methylated CpGs | 12 | 1 | 5 | 10 | 7 | 7 | 2 | 44 |
| Number of CpGs | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 126 |
| Ratio of methylated (%) | 66.7 | 5.6 | 27.8 | 55.6 | 38.9 | 38.9 | 11.1 | 34.9 |

Figure 8. Locus 4 LNCAP Converted vs. WAV17 Unconverted QUMA Analysis Diagram

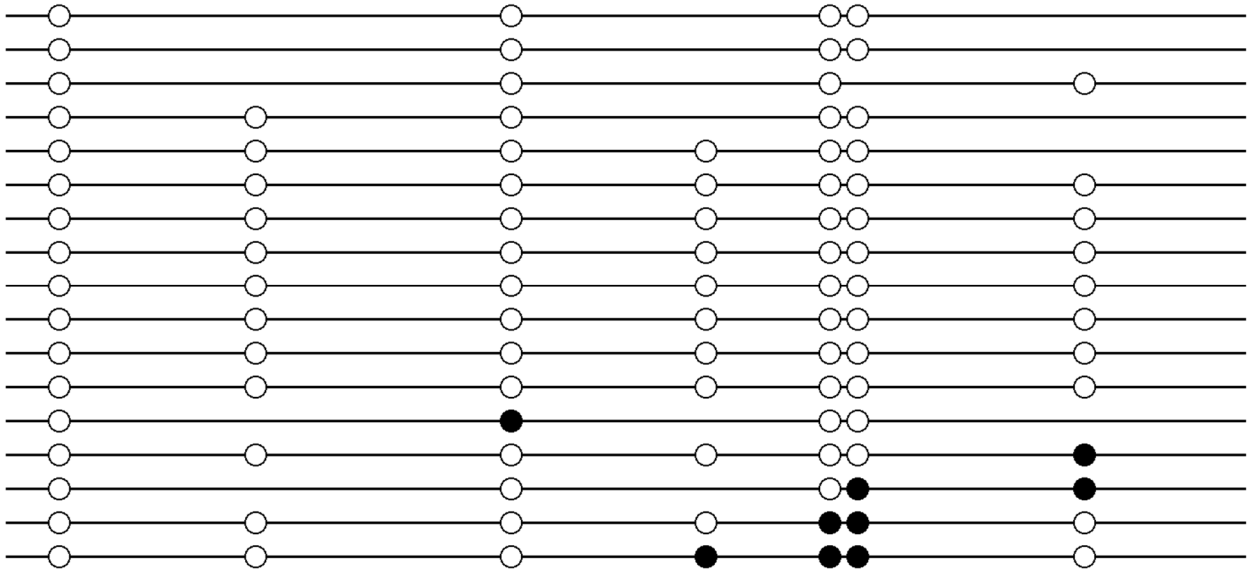


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 14. Locus 4 LNCAP Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 655 | | | | | | | |
| Number of CpGs | 7 | | | | | | | |
| CpG position | 26 | 131 | 271 | 375 | 437 | 442 | 565 | Total |
| Number of methylated CpGs | 8 | 0 | 0 | 9 | 3 | 3 | 5 | 28 |
| Number of CpGs | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 98 |
| Ratio of methylated (%) | 57.1 | 0 | 0 | 64.3 | 21.4 | 21.4 | 35.7 | 28.6 |

Figure 9. Locus 4 PC3 Converted vs. WAV17 Unconverted QUMA Analysis Diagram

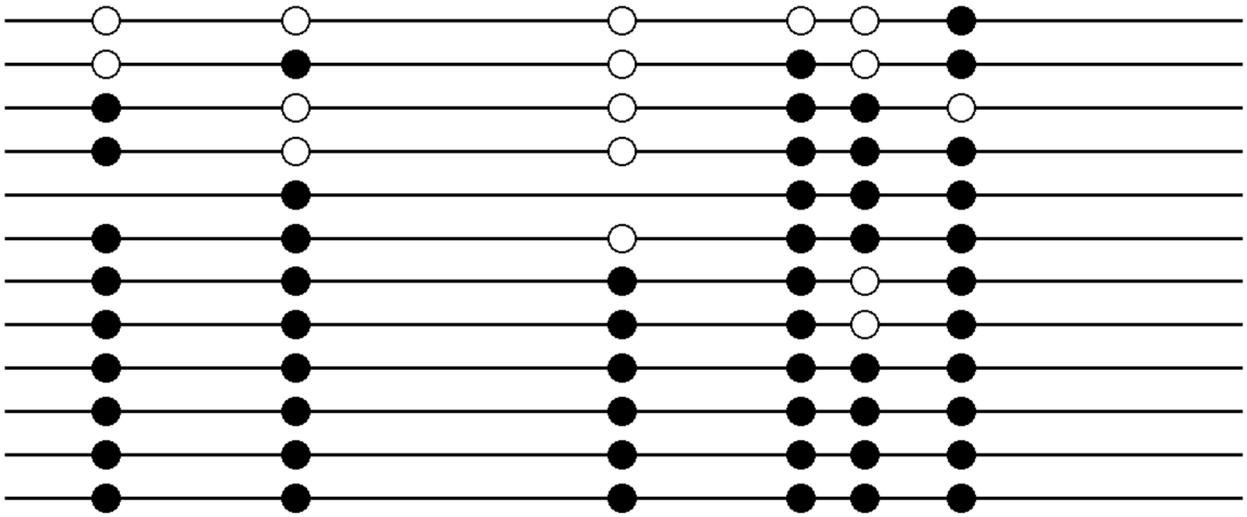


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 15. Locus 4 PC3 Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 655 | | | | | | | |
| Number of CpGs | 7 | | | | | | | |
| Number of bisulfite sequences (used) | 17 | | | | | | | |
| CpG position | 26 | 131 | 271 | 375 | 437 | 442 | 565 | total |
| Number of methylated CpGs | 0 | 0 | 1 | 1 | 2 | 3 | 2 | 9 |
| Number of CpGs | 17 | 17 | 17 | 17 | 17 | 16 | 17 | 118 |
| Ratio of methylated (%) | 0 | 0 | 5.9 | 5.9 | 11.8 | 18.8 | 11.8 | 7.6 |

Figure 10. Locus 5 WAV17 Converted vs. WAV17 Unconverted QUMA Analysis Diagram

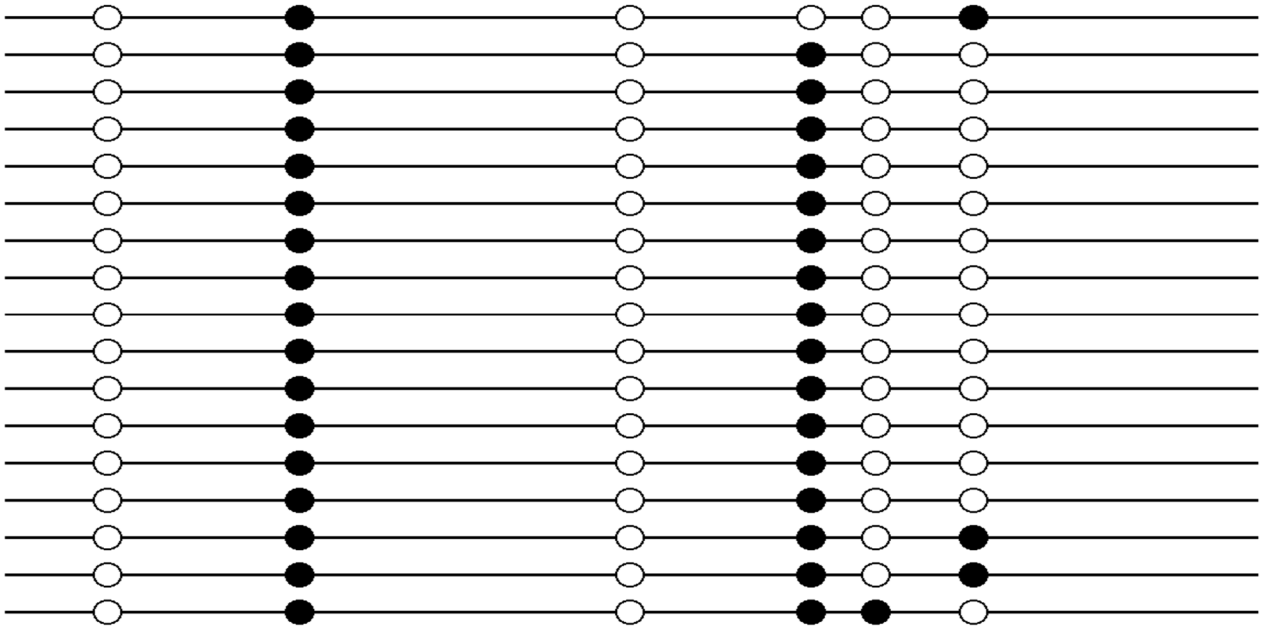


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 16. Locus 5 WAV17 Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 502 | | | | | | |
| Number of CpGs | 6 | | | | | | |
| CpG position | 41 | 117 | 256 | 327 | 345 | 378 | total |
| Number of methylated CpGs | 9 | 9 | 6 | 11 | 8 | 11 | 54 |
| Number of CpGs | 11 | 12 | 11 | 12 | 12 | 12 | 70 |
| Ratio of methylated (%) | 81.8 | 75.0 | 54.5 | 91.7 | 66.7 | 91.7 | 77.1 |

Figure 11. Locus 5 WBC Converted vs. WAV17 Unconverted QUMA Analysis Diagram

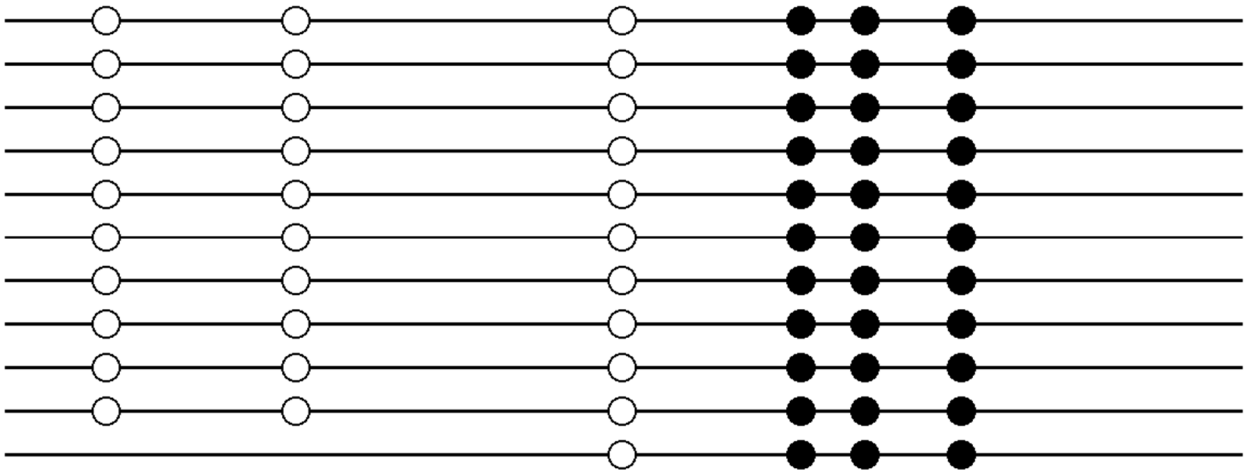


*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 17. Locus 5 WBC Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 502 | | | | | | |
| Number of CpGs | 6 | | | | | | |
| CpG position | 41 | 117 | 256 | 327 | 345 | 378 | total |
| Number of methylated CpGs | 0 | 17 | 0 | 16 | 1 | 3 | 37 |
| Number of CpGs | 17 | 17 | 17 | 17 | 17 | 17 | 102 |
| Ratio of methylated (%) | 0.0 | 100.0 | 0.0 | 94.1 | 5.9 | 17.6 | 36.3 |

Figure 12. Locus 5 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Diagram



*Note*. Open circles are unmethylated CpGs. Closed circles are methylated.

Table 18. Locus 5 RWPE Converted vs. WAV17 Unconverted QUMA Analysis Data Summary

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Length of target genome sequence | 502 | | | | | | |
| Number of CpGs | 6 | | | | | | |
| CpG position | 41 | 117 | 256 | 327 | 345 | 378 | Total |
| Number of methylated CpGs | 0 | 0 | 0 | 11 | 11 | 11 | 33 |
| Number of CpGs | 10 | 10 | 11 | 11 | 11 | 11 | 64 |
| Ratio of methylated (%) | 0.0 | 0.0 | 0.0 | 100.0 | 100.0 | 100.0 | 51.6 |

# CHAPTER FOUR

# DISCUSSION

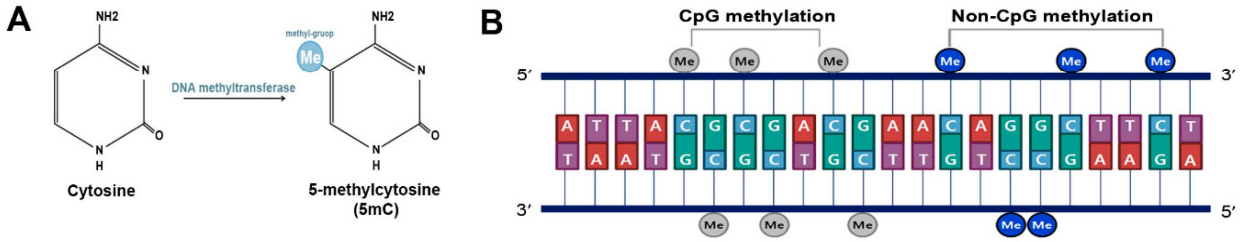
## Introduction

Worldwide cancer is one of the leading causes of death. In 2018, there were 18.1 million new cases. Globally, 9.5 million deaths were related to cancer the same year, and in the next 20 years, these numbers are expected to grow: 29.5 million new cases per year and 16.4 million deaths (National Cancer Institute, 2020). It would seem as if cancer is evolving with man: a race to see who will come out victorious or evolutionarily fitter. Physicians and research scientists alike have spent years devising ways to detect cancer before it grows beyond control. These measures include physical screenings, checking for tumor markers in blood serum, and also genetic testing.

In the early 90's BRCA gene discovery was a game changer. It is now used in a clinical setting. Deletions in this gene have been associated with high risks of developing breast and ovarian cancer (Vande Perre et al., 2018). Since its discovery, scientists have incorporated genetics to identify cancer risks prognosis (Johnson et al., 2002). In this study, we sought such a gene. We wondered if there is a genetic correlation between our gene of interest, LINE-1, and different stages of prostate cancer. Long Interspersed Nuclear Elements (LINES) are retrotransposons. This means they move around, or copy and paste themselves, in various parts of the genome: jumping genes. It is generally assumed that when this gene moves around, it physically causes genomic instability. In addition, a number of tumor suppressor genes have been shown to be silenced by promoter methylation.

To understand my analysis, one must remember that methylation correlates to a gene being “on” or “off”. On the DNA backbone, cytosine bonded to guanine via phosphodiester bonds, which I will refer to as CpG sites henceforth, is the point of methylation (see Figure 13; Jang et al., 2017).

Figure 13. DNA Methyltransferase Adding Methyl Group to Fifth Carbon of Cytosine (A)




*Note*. DNA methylation in a CpG context: methyl group added between phosphodiester bonds of cytosine and guanine (gray). Blue is not CpG methylation because cytosine and guanine hydrogen bonded, but phosphodiester bond is to another nucleotide.

A study evaluating hypomethylated intragenic L1genes showed that genes hosting intragenic L1s were more likely to be repressed in cancer (Aportenwan et al., 2011). Furthermore, repression levels are linked to the degree of L1 hypomethylation. Hypomethylation degree varies by locus and changes throughout initiation and development of cancer. Advanced stages of cancer were directly correlated with higher degrees of hypomethylation. Gene repression and quantity of repressed genes due to L1s may promote cancer progression (Aportenwan et al., 2011). Active L1s (hypomethylated) within genes inhibit the gene’s expression.

**Analysis by Locus**

The correlation between cancer cells and methylation is ongoing. In colorectal cancer, for example, global patterns of hypomethylation are its signature (Hinoue et al., 2012). When compared to the euchromatic region, Long Arm Locus 1, heterochromatic L1s on HC21p were

typically hypomethylated (Figure 18). The prostate cell line L1s are also hypomethylated versus WBC. Even in normal prostate, RWPE-1, WBC is much more methylated. This may be indicative of the fact that prostate baseline methylation is lower. Figures 14-17 compare methylation at each locus of each cell line compared to white blood cells.

Figure 14. Comparison of Percent Methylation Across All Cell Lines for Locus 1

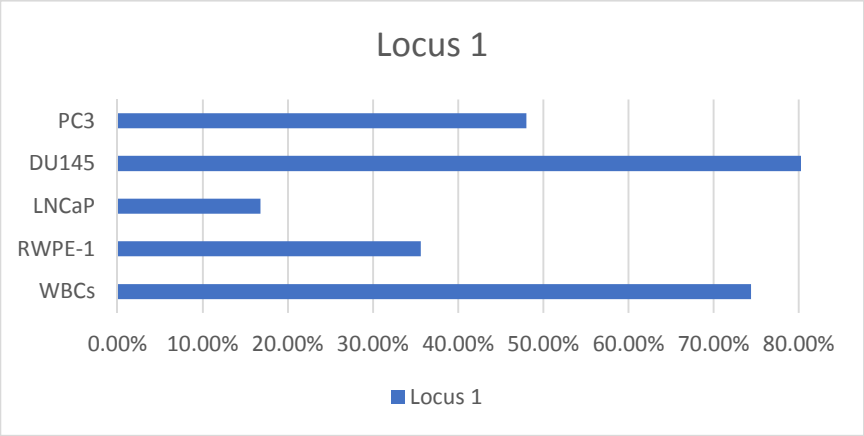



Figure 15. Comparison of Percent Methylation Across All Cell Lines for Locus 2

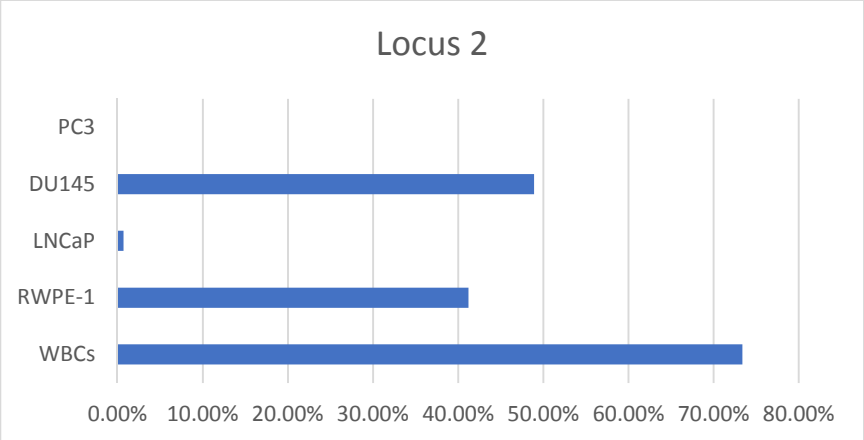


Figure 16. Comparison of Percent Methylation Across All Cell Lines for Locus 3

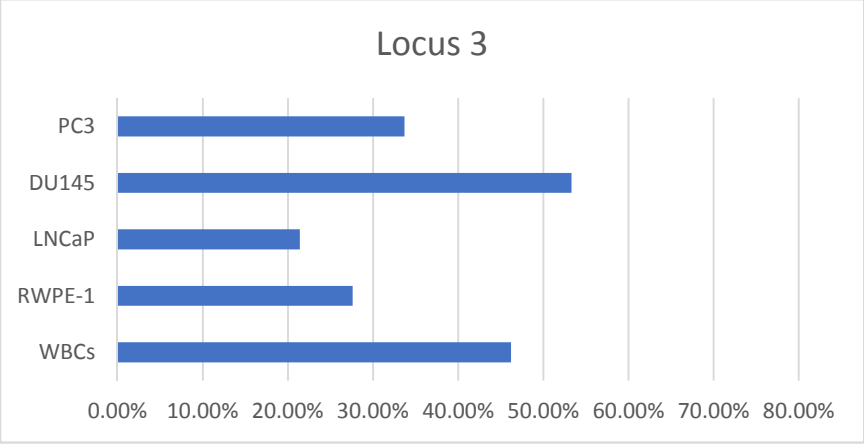
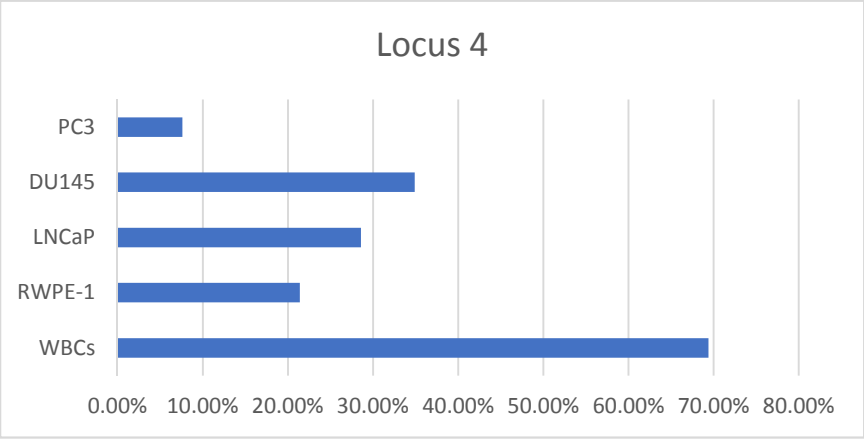


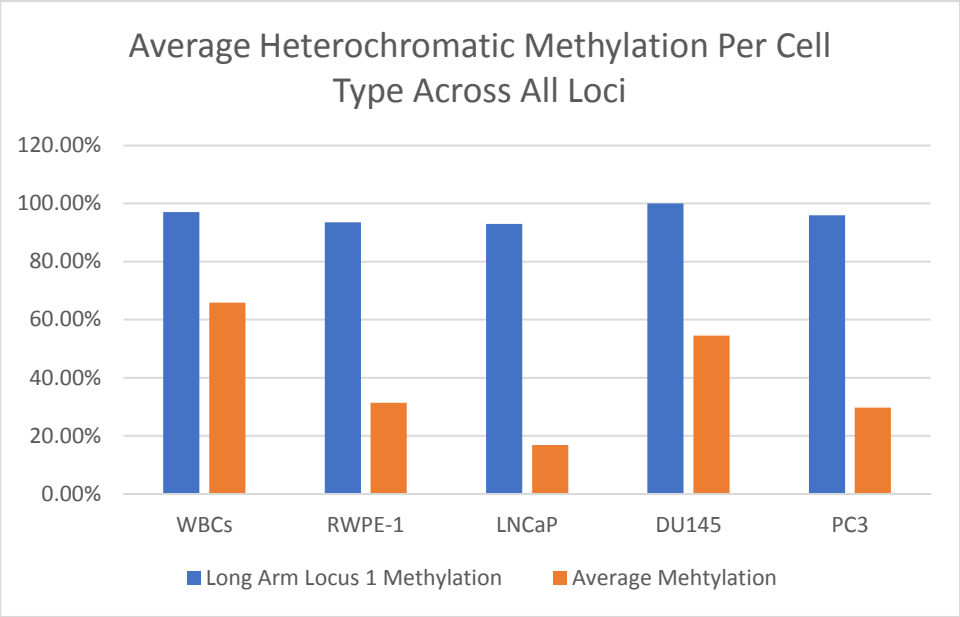

Figure 17. Comparison of Percent Methylation Across All Cell Lines for Locus 4




The functional role of each CpG site is not yet known. We use overall percent methylation to be consistent with other studies. Mayo Clinic did a study on L1s in prostate cancer in euchromatic regions, and was able to show that gene methylation was very homogeneous in different patients. This "pattern" was consistent and did not change during metastasis. Therefore, exploring the methylation of loci is an effective biomarker for cancer detection only (Mahapatra et al., 2012) When we compare L1 methylation as a whole (all loci) across each cell line in these heterochromatic regions to their euchromatic counterparts (Figure

18), there is a clearly lower level of L1 methylation on the p (short/heterochromatic/tightly wound) arm versus the q (long/euchromatic/loosely configured) arm. This supports previous studies suggesting that hypomethylated L1s facilitate heterochromatin formation (Hansen, 2003; Chow et al., 2010). This also supports very recent work indicating that there are substantial regions in heterochromatic regions of the genome that are hypomethylated (Gershman et al., 2022). Overall, Figure 18 reiterates that L1s in cancer cell lines are less methylated than white blood cells.

Figure 18. Comparison of Average Heterochromatic Methylation Per Cell Type to Euchromatic Methylation of Same Cell Type




**Patterns of Cellular Methylation**

In patients with prostate cancer, L1s were significantly less methylated compared to matched normal tissue, although this study did not find a significant correlation between L1 methylation and clinical features such as tumor grade (Ogino et al., 2008). When considering patterns of methylation, Long Arm Locus 1 had none. With such high degrees of methylation,

variation across cell lines was not noticed. There were no clearly observable differences in methylation patterns between normal and cancer cells nor between different cancer aggression stages. When considering which locus would be a good candidate for cancer biomarkers, one would need to explore more options. This would include testing more loci to seek observable correlations and including retrotranspositionally active LINEs.

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# VITA

Dametria Jetters started her humble beginnings from the south side of Chicago. She graduated from Morgan Park High School, then went onto pursue a Bachelors of Science from Chicago State University. While there she excelled in the biology department as a budding research scientist studying apoptotic biochemical pathways. As a first generation college student, she worked hard and became president of the Honors College, a Presidential Scholar, member of the Beta Beta Beta Biological Honors Society, pioneer of the Minority Association of Premedical Students CSU-chapter, department tutor, and member of the Sigma Lambda Gamma Sorority.

After living in New York for a brief time, Dametria Jetters started the Masters of Science program in Loyola's biology department in August 2014, with a concentration on epigenetics.

The next few years she devoted to the unique challenges of raising her family, and then returned to her program. To meet the demand of exigent medical expenses for her family, she worked as a full time chemist, while in school, at Tradebe Treatment and Recycling. This position required that she identify, classify and segregate chemicals with an attempt to recycle as much material as possible. In addition, she had to utilize the material's chemical properties such as solubility, reactivity to water, and flammability to make the best disposal technology decision with regard to State regulations. The market segments served include: educational, industrial, government, research and municipal household hazardous waste collections.

Dametria Jetter's next position was with her current employer: Charles River Laboratories. She started as a research assistant, responsible for performing moderately complex

laboratory work in collaboration with departmental staff and in compliance with SOPs and GLP regulations. This included recording project data and setup of basic laboratory equipment and instrumentation with guidance from senior technical staff. She was eventually promoted to her current position: Senior Research Analyst. In this role, she performs assay transfers and develops method procedures (DNA/RNA purification and quantitation, qPCR, qRT-PCR, etc.) for Molecular Biology based on protocols and in compliance with SOPs and GLP regulations. She also serves as lead/primary technician for both complex and highly complex studies in Molecular Biology disciplines. She develops methods and successfully transfers studies to other members of the group for pre-validation and validation testing. This also requires that she perform special, non-routine projects in the laboratory, as assigned. She develops new techniques, procedures, or scientific innovations that expand the capabilities of the department. In addition, she provides leadership and technical guidance in the laboratory and assists in the coordination of resource planning.