Tissue Type and Gender Effects on DNA Methylation at specific Loci in Mice

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Tėveliams ir Dovilei

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1. Abbreviations

A Actin	alpha actin
AdoMet	adenosylmethionine
Alu	a short DNA stretch originally defined by the action of Alu restriction endonuclease; one of the most abundant mobile primate genome elements
CGI	CpG island
CpG	CG dinucleotide
DMR	differentially methylated region
DNMT	DNA methyltransferase
Dpc	day post coitum
Ed	embryonic day
H19	a gene for non-coding RNA, expressed from maternal allele only
H3Lys9	3rd histone lysine position 9
HDAC	histone deacetylase
IAP	intracisternal A particle

ICF	immunodeficiency, centromere instability, facial anomalies syndrome
ICR	imprinting control region
IGF 2	insulin growth factor 2
LINE-1	LINE-1, long interspersed elements
LFA-1	Lymphocyte function-associated antigen
Lit1	long intronic transcript
mC	methyl cytosine
MyLC	myosin light chain
NESP55	neuroendocrine secretory protein 55
ORF	open reading frame
Peg3	paternally expressed gene 3
PGC	primordial germ cells
SAH	S-adenosylhomocysteine
Snrpn DMR1	small nuclear ribonucleoprotein polypeptide differentially methylated region 1, alternatively Snrpn D1
SVA	a composite repetitive element named after its constitutive elements SINE, VNTR and ALU

TSS	transcription start site
Xa	active X chromosome
Xi	inactive X chromosome

2. Zusammenfassung

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Es sind zum Teil erhebliche Unterschiede der DNS-Methylierung an einzelnen Loci und repetitiven Elementen beim Menschen in verschiedenen Zellen und Geweben beobachtet worden.
Es wurde ebenfalls über mehrere inter-und intrafragmentäre Korrelationsfälle des DNS-Methylierungsmusters berichtet. Um das Ausmaß und die Reproduzierbarkeit solcher Korrelation zu untersuchen analysierten wir die Korrelation der Methylierung zwischen sieben verschiedenen Loci in neun verschiedenen Geweben in einer Population von 100 gesunden sieben Wochen alten CD1 Mäuse. Wir haben hochquantitative Methoden zur genauen Messung der Methylierung bei zwei einzelnen Loci in Promoter des Alpha-Aktin und der leichten Myosin-Kette, an drei unterschiedlich methylierten Regionen der Peg3, SNRPN und LIT1-Genen mit imprinted Loci, und an zwei repetitiven Elementen in den LINE-1 und IAP-LTR-Genen in den verschiedenen Geweben eingesetzt.

In dieser Mäusepopulation haben wir geschlechtsabhängige Methylierungsmuster sowie intergewebliche Korrelation an mehreren Loci im Gehirn und Milz beobachtet. Da die Korrelation zwischen Geweben bisher selten beschrieben wurde, haben wir die Ergebnisse durch erneute Analyse unter Verwendung von SIRPH und Pyrosequenzierung für Milz (intergewebliche Korrelation) und Zungenmuskulatur (geschlechtsassozierte Korrelation) bestätigt, die Korrelationsmuster konnten auch mit diesen Methoden nachgewiesen werden. Diese Experimente sind hier ausführlich geschildert.

Bei ähnlichen Messungen drei Monate später und mit einer anderen, unter ähnlichen Bedingungen gezüchteten Mäusepopulation, konnten keine gewebs- oder geschlechtsabhängige Korrelationsmuster nachgewiesen werden. Hinsichtlich der Größe und Variabilität des Epigenoms sind hier zusätzliche Untersuchungen, die eine größere Anzahl von Loci und eine größere Population umfassen, erforderlich, um Methylierungsmuster eine Systematik zuordnen zu können.

3. Introduction

Waddington first described epigenetics in 1942, advancing the idea that genotype programs phenotypic changes (Feinberg, 2007). Later, many other ideas and experimental evidence began to emerge from it, cleaving the original idea into multiple fields of research. At present, epigenetics defines the broad category of information heritable through cell divisions, and possibly between generations (Rakyan et al., 2001), which is independent of the DNA sequence. What is truly remarkable in the cell, is that over a meter of DNA is not only packed into a nucleus, but this compaction mechanism also allows some areas to be transcribed, while at the same time repressing others (Ducasse and Brown, 2006).

Two main mechanisms known so far to achieve this state of functional compaction are histone modifications and DNA methylation. Histones are instrumental in forming the chromatin structure by winding DNA onto their octamers. Various histone modifications, such as methy-, acety-, phosphorylation, SUMOylation ADP-ribosylation change the structure of the nucleosome and recruit various regulatory complexes (Jenuwein, Allis, 2001; Strahl, Allis, 2000)

The focus of this work is DNA methylation at specific loci in different tissues in a relatively small outbred mice population. Discovered in 1950 in calf thymus (Hotchkiss, 1948), DNA methyation is the covalent attachment of a methyl group to the 5th position of the pyrimidine ring of a cytosine or adenine residue (Fig.1). In eukaryotes it is limited almost exclusively to cytosines, and mammal DNA in somatic cells is methylated mainly in the context of CpG dinucleotides. (Weber and Schuebeler, 2007). The methyl group comes from s-adenosylmethionine, a major methyl group donor that modifies macromolecules (DNA, RNA, proteins) and is a precursor to many vital molecules (dopamine, norepinephrine) (Brosnan et al., 2007). The reaction itself is catalyzed by DNA methyltransferases, or DNMT's. Four are known to date (Brosnan et al., 2007; Katz et al., 2003; listed in more detail in section 1.2.2, page 9). This gives us a source of the process – adenosylmethionine (AdoMet) ; the target – cytosine in a CpG dinucleotide; and the means – DNMT's (Figures 1, 2).

A process usually results in a functional outcome, and in this case the outcomes are a

rainbow of functions, including but not limited to:

- Gene imprinting (Feinberg, 2007),
- Silencing of foreign DNA (Jaenisch and Bird, 2003),
- Repression of gene expression (Feinberg, 2007),

All the above processes link directly to such fundamental themes as ontogenesis, ageing, cancer diagnostic and therapy, the basis of interindividual phenotype differences despite very close genotype and vast intraindividual differences between cells and tissues of the same organism, where genotype is identical.
Yet much more work is still needed to achieve a systemic understanding of how epigenetics influences genesis and phenotype in a given organism, and to determine the degree of influence of various environment factors (Jirtle and Skinner, 2007).

1.2 Methylation – substrates and enzymes

1.2.1 S-Adenosylmethionine (SAM)

Adenosylmethionine, or AdoMet, is the methyl group donor for DNA methylation. The dietary precursors to synthesis of S-adenosylmethionine are B group vitamins, folate, vitamin B-12, choline and methionine, generally referred to as lipotropes. The whole synthesis process is part of one-carbon metabolism, in which S-AdoMet serves as a carbon-group donor in more than 80 reactions; of greater importance are two major reaction groups, namely those of nucleotide synthesis and those of methylation, not only genomic DNA but also proteins, phospholipids, RNA and viral DNA. (Brosnan et al., 2007)
The synthesis of S-adenosylmethionine is facilitated by AdoMet synthetase; the adenosyl portion of ATP is transfered to methionine, as schematically shown in Fig.1. (Percipalle and Visa, 2006). AdoMet exists in two stereoisomeric forms, (S,S)-AdoMet and (R,S)-AdoMet. The S,S enantiomer is biologicaly active, while R,S is an inhibitor of methylases; it constitutes about 3 % of all AdoMet in mouse liver (Percipalle and Visa,

2006). Upon donating the methyl group, S-AdoMet becomes S-adenosylhomocysteine (SAH) (Brosnan et al., 2007). SAH, in turn, acts as an inhibitor for its own synthesis. That is why S-AdoMet:SAH ratio can be used to evaluate the methylation capability of a specific tissue.

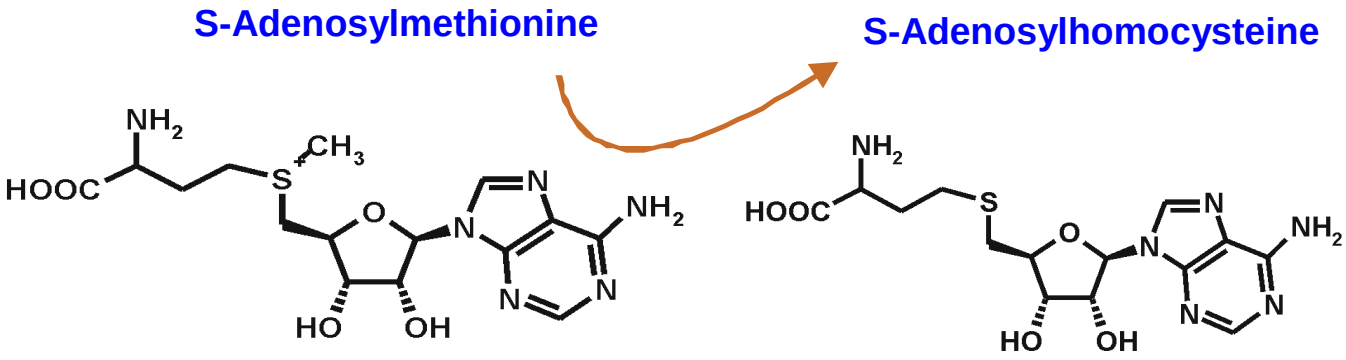


Fig. 1: Simplified scheme of adenosylmethionine synthesis

1.2.2 DNMT's, DNA methyltransferases

Three DNMT's are known in mammals: DNMT1, DNMT2, and DNMT3, named so by discovery timing; all share a number of conserved domains (Weber and Schuebeler, 2005; Attwood et al., 2002).

The first eukaryotic DNA methyltransferase, DNMT1, has been discovered in 1988 and shares a similarity with bacterial methyltransferases. The protein is 1620 amino acids long and has a number of functional domains. Among these, some have been identified that facilitate the protein's import into the nucleus and some that are needed for its association with DNA (Weber and Schuebeler, 2005; Attwood et al., 2002; Goll and Bestor 2005). Since then, many more methyltransferases have been discovered by sequence similarity, in a number of organisms - mice, frogs, and bees to name some. It has been shown that DNMT1 exhibigted 5 to 30 fold greater affinity for hemimethylated DNA, and it was therefore ascribed the maintenance methyltransferase activity. The activity of DNMT1A on unmethylated substrates exceeds that of DNMT3A and DNMT3B, which are supposed to be the main de novo DNA methyltransferases.

DNMT1 is degraded in G0 phase; it also tends to be more concentrated in cycling cells.

Oocytes are an exception, in that they contain high concentrations of DNMT1 in its shortened and degradation-resistant form, the 118 amino acid shorter DNMT1o. This form is bound by annexin V, a phospholipid binding protein, and retained in the cytoplasm of the oocyte and early embryo (Goll and Bestor, 2005). A truncated form of DNMT1 also exists in male germ cells. There have been suggestions DNMT1o is needed to maintain imprinted loci methylation at the eight cell preimplantation stage (Howell et al., 2001).

DNMT2, also discovered by sequence similarity, is found in all organisms that have members of the DNMT1 and DNMT3 families. The protein with all characteristic catalytic methyltransferase motifs is expressed in most mammalian tissues. DNMT2 has been shown to methylate tRNA and also have some weak activity on DNA. Its biological significance is unclear, as both insect and animal knockouts remained viable and without obvious defects (Jeltsch et al., 2006; Goll and Bestor, 2005).

The DNMT3 family has an affinity for unmethylated CG dinucleotdes, which identifies them as de novo DNMTs. Their structural differences led some authors to suggest that DNMT3A is distributive, while 3B is processive (Gowher and Jeltsch, 2002). Knockout experiments have reavealed that DNMT3A null mice live up to four weeks, then die with signs of aganglionic megacolon and azoospermia in males. DNMT3B null exhibited developmental arrest between ED 14.5 and 18.5, and mice defficient in both 3A and 3B underwent developmental arrest at between ED 8.5-9.5 (Okano et al., 1999). Another experiment led to conclude that mice mutant for DNMT3B die at about 9.5 dpc (Chen et al., 2002).

Point mutations in DNMT3B cause ICF syndrome (immunodeficiency, centromere instability, facial anomalies) (Xu et al., 1999). In ICF, demethylation of satelite DNA on chromosomes 1,9, and 16 causes multiple long chromosomal arms; this instability is best observed in T lymphocytes, and accompanied by immunodeficiency – as a result, most patients die of infectious diseases at an early age.

DNMT3L (DNMT3-like) is expressed specifically in germ cells. (Aapola et al., 2000). It participates in establishment of maternal imprints in the oocyte and methylation of dispersed repeated sequences the prospermatogonia. DNMT3L knockouts are viable, but sterile in both sexes. Although DNMT3L has not been shown to participate in DNA methylation directly, its role as as an enhancer of DNMT3A and 3B activity has been

backed by experimental evidence (Fraga and Esteller 2007). DNMT3L is expressed in oocytes after meiotic recombination and prospermatogonia. Its absence does not interfere with oogenesis or early embriogenesis (if the male genome portion is DNMT3L+); male germ cells defficient in DNMT3L proceed to apoptosis via abnormal synapsis or asynapsis. It seems essential for male meiosis, while female meiosis occurs normally in its absence. (Bour'chis and Bestor, 2004).

The reason for male germ cell instability without DNMT may be the awakening and expression of normally silent transposons, such as Line-1 and Iap, which in turn causes chromosomal assymetry and synaptic instability. DNMT3L knockout experiment results are also sex dependent. In females, its deletion results in the absence of maternal methylation imprints, leaving retrotransposons methylated. This leads to biallelic expression of normally silenced genes and eventual embryonic development failure with death until mid-gestation. In males, the methylation of retrotransposons is lost, resulting in their expression, especially Line-1(long interspersed elements) and Iap (intracisternal A particle), which then led to synapsis failure at meiotic prophase and apoptosis before pachytene (Fraga and Esteller, 2007). The paternally methylated DMRs (differentialy methylated regions, often associated with gene promoters and imprinting) stayed methylated, while retrotransposons were not properly inactivated.

DNMT3L and 3A seem to be cooperating in the germ cell line, their function being de novo methylation, namely the establishment of paternal imprints (Kaneda et al., 2004; Chedin et al., 2002).

DNMTs also bind to histone deacetylases (HDACs), and can target them to regions of gene silencing. Histone deacetylation at lysine tails that stick out of the DNA-histone chain is one of the molecular mechanisms associated with silencing. Transcription corepressors, HDACs and chromatin remodeling proteins bind to hypermethylated DNA regions and induce a transcriptionally repressive chromatin conformational state (Ehrlich, 2003).

1.2.3 CpG - quantity; standalone and distribution

The target of methylation in mammalian DNA is cytosine residue at its 5' position. Most mC in mammals is also in the context of 5'CpG3', and enzymatically maintained in this state during cell divisions.

CpG dinucleotides are found in the mouse genome at a frequency which is less than statistically expected. They are found at about 20 % of expected frequency. This could be explained by the tendency of methylcytosine to be deaminated to thymine, a mismatch which is consequently repaired to form a TpG dinucleotide (CpA on complementary strand). At some areas, however, the C and G nucleotide content is about 64 %, while the genome average is 42 %. These areas are known as CpG islands (CGIs). CGIs can be classified according to their position, as associated with a promoter at the 5' end of a gene, and not asociated with known promoters. The two groups are not strictly separated, as about 5 % of promoter-associated CGIs are also due to Alus. Ca. 60 % of human genes are associated with CGI, including all housekeeping genes and about half of tissue-specific genes. In mouse, about 88 % of imprinted genes are associated with CGIs (Attwood et al., 2001). A CGI is usually about 1 kb long, and found at the 5' end of the gene it regulates; it is found in the context of transcriptionally active chromatin. Since their discovery 20 years ago, CpG islands have been a reliable indicator for gene promoters. The CpG islands make up to 2 % of the whole genome. They are associated with promoters and are less methylated than the “loose” non-clustered CG dinucleotides, which are associated with transposable elements (LINE-1 and Alu, for example) - these are largely methylated. Notably, the housekeeping genes are associated with CGI's, while only one out of four tissue-specific genes has one CGI overlapping with its transcription start site (TSS). (43). CpG-islands are originaly defined as an area with a length of more than 200 bp, including a CG-content over 50 % and a ratio of at least observerd CpG/expected CpG ObsCpG/ExpCpG x 0.6. The ratio was calculated according to the following formula:

$$Obs\ /\ Exp\ = \frac{Number\ of\ CpG}{Number\ of\ C \times Number\ of\ G} \times N\,(total\ number\ of\ nucleotide\ sinsequence\ )$$

(Gardiner-Garden et al., 1987). This definition has the drawback that it makes it difficult

to distinguish between CpG-islands and the approximately 1,000,000 Alu copies per haploid genome. The current criterion requires a DNA sequence of at least 500 bp, a GC-content of 55 % and an ObsCpG/ExpCpG ratio higher or equal to 0.65, respectively (Takai et al., 2002; Wang et al., 2004).

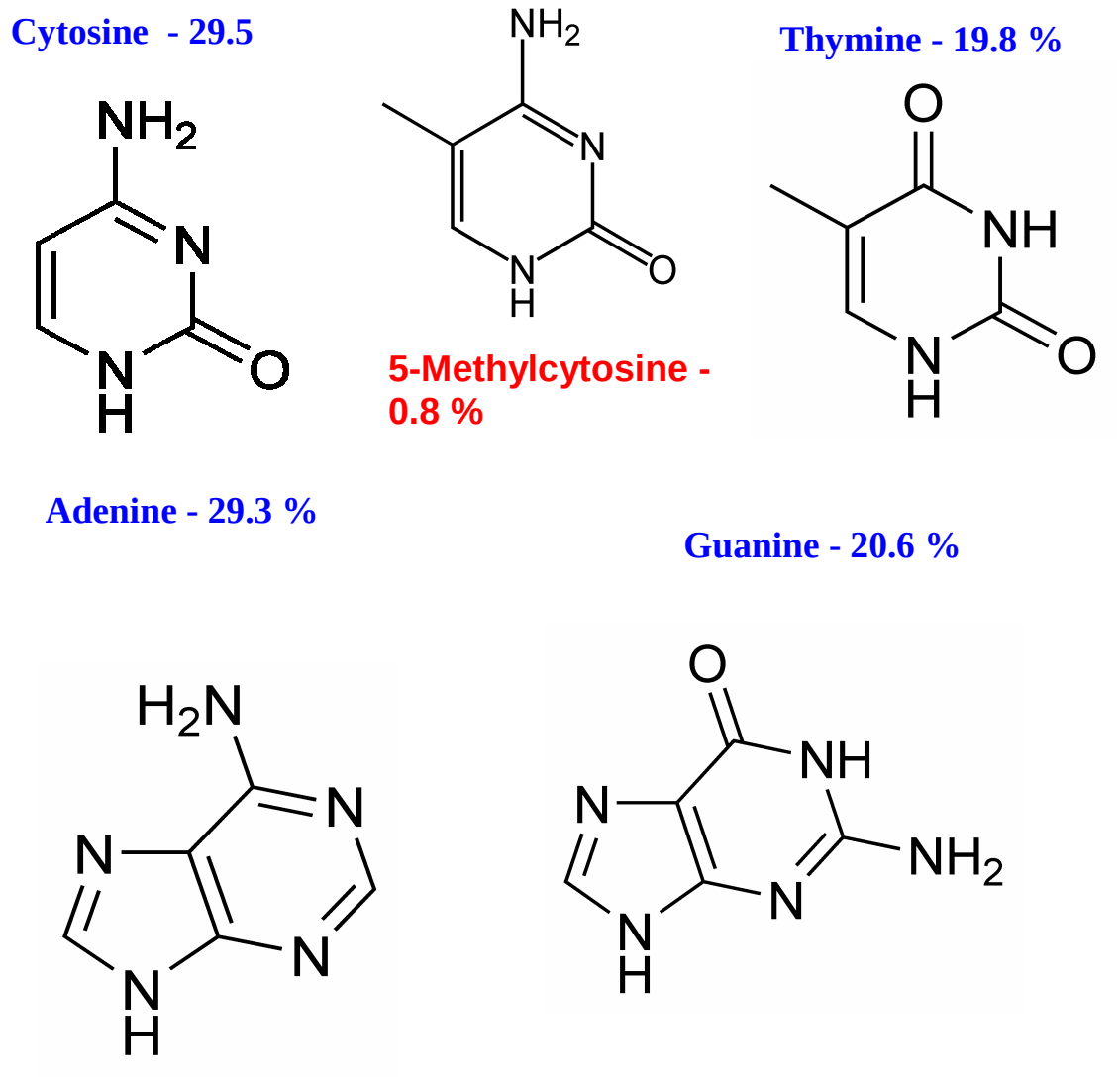


Fig. 2: DNA bases and the relative quantity of methylated cytosines in the genome (Mouse genome database, http://www.informatics.jax.org/, accessed 2011.03.11)

1.3 Methylation – functional aspects

1.3.1 Timing

The dynamics of methylation are different between sexes. Prospermatogonial DNA is de novo methylated during the perinatal period, while oocyte DNA methylation occurs shortly before ovulation; methylation pattern is then quickly erased in the PGCs of the next generation. Paternal germ cell line must thus keep methylation stable through many numbers of divisions, possibly leading to greater epigenetic mutational load in germ cell genomes of older males. This in turn may be associated with greater disease incidence in the resulting offspring. Shortly after fertilization demethylation of the paternal genome is occurs. It is an active process. Maternal genome is then demethylated at a slower pace. Remethylation begins at the blastocyst stage and is different in the embryonic layers (Siedlecki and Zielenkiewicz 2006; Cervoni et al., 1999; Bestor, 2000).

1.3.2 Gene imprinting

In mammals, most autosomal genes are expressed from both paternal and maternal alleles. Imprinting is the process whereby one of the two copies is repressed while the other remains active, as shown by experiments of nuclear transfer in mouse (McGrath and Solter, 1984; Surani et al., 1984), which revealed that same genes behave differently depending upon their parental origin (Bestor, 2004; Doerfler, 2004). Imprinted genes are involved in controlled restriction processes during fetal development, where they are especially important in placental development and growth. Some are suspected to influence behavior, including binding patterns and maternal care. Although a number of lone imprinted genes are known, the majority are clustered and under the control of a single imprinting control region (ICR) (Bestor, 2001; Bird, 2002). The ICR acquires differential methylation in the germ cell stage, and is able to control the expression of all the genes in its cluster. For the standalone-imprinted genes, their promoter is also their ICR. The methylation of imprinted regions is known to be resistant to the genome-wide demethylation occurring after fertilization (Bestor, 2006; Schaefer et al., 2006).

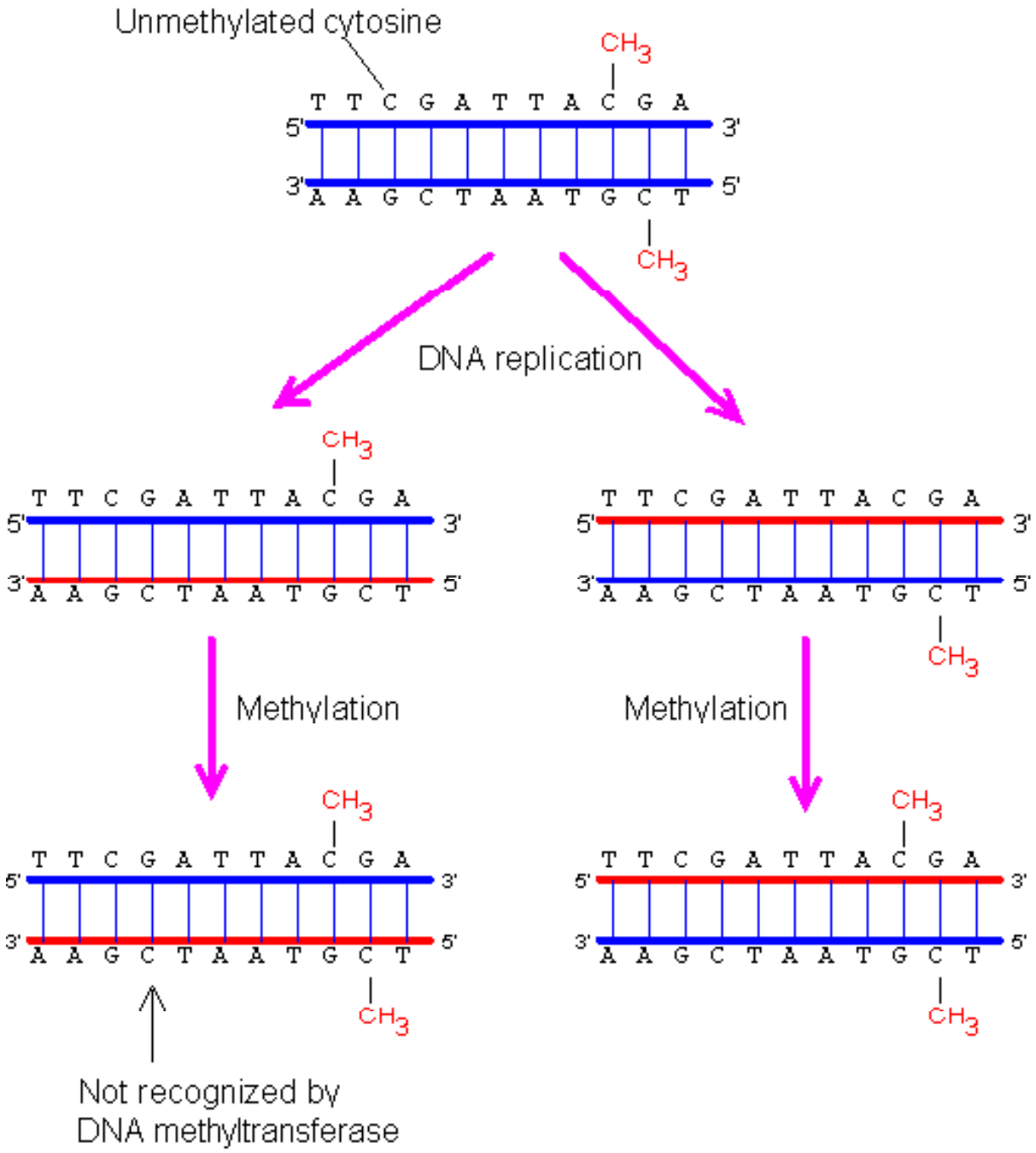


Fig. 3: Mechanism of DNA methylation pattern maintenance. DNMT is only able to methylate CG-sites that are already paired with methylated CG. (http://www.georgiahealth.edu/cancer/people/robertson/research.html, accessed 03.10.2010)

1.3.3 Gene repression

One of the best examples on the repression role of DNA methylation is the Xist locus.The locus that initiates the random X chromosome inactivation, Xist, produces the RNA that inactivates one of the two X chromosomes. On the active chromosome, Xist is not expressed as it is extensively methylated at the 5' region. On the male X, the pattern of Xist methylation is the same as on Xa of females, thus keeping it open to transcription. In essence, methylation has no direct role in establishing X inactivation, but it directly protects the one X chromosome that needs to stay active from it. It also keeps the genes on Xi inactive (Fraga and Esteller, 2007).

Cytosine methylation may inhibit transcription factors from binding to promotors directly, or could influence the binding of macromolecular complex of transcription repressor mSin3a and histone deacetylases, thus possibly influencing chromatin structure, i.e. triggering its condensation. DNA methylation and histone deacetylation display synergy in silencing genes; this silent state is then maintained by dense CpG island Methylation (Toyota and Issa, 1999).

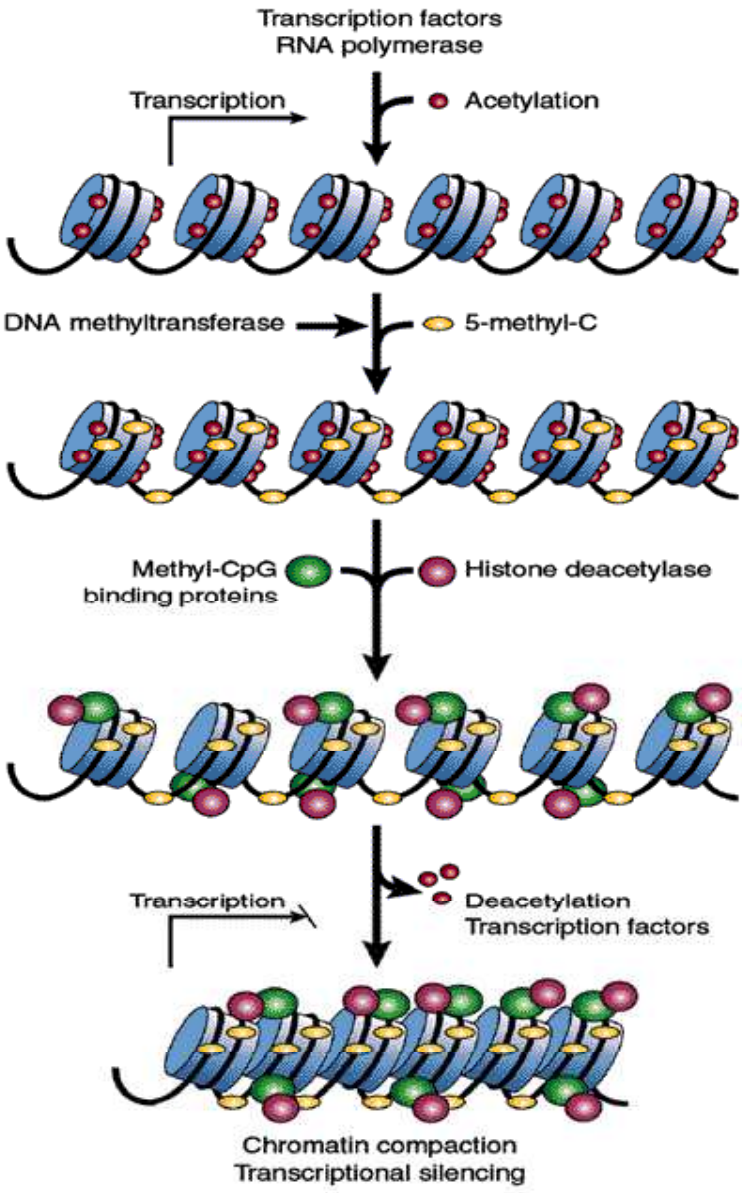


Fig. 4: Silencing of active DNA is mediated by DNA-methyltransferase (DNMT), methyl-CpG binding proteins (MBP) and histone deacetylase (HDAC) (Lee, 1999)

1.4 Known variability

A number of works indicate that DNA methylation varies depending on gene expression intensity and numerous other conditions, such as specific cancers and other diseases.

1.4.1 Differences in health and pathology

Methylation differences have been shown to exist in various pathologic conditions. Rett syndrome, a neurological disorder with early onset, has been traced to mutations in methyl-CpG binding protein 2 gene, which is transcriptional repressor. Its mutations have been identified in up to 90 % sporadic cases of the disease. Also, Dnmt1 levels are high in neurons but it is almost absent in glia. Experimental mice methyl CpG binding protein knockout models showed adverse effects on survival after birth. Microscopic studies of a cell model system revealed methylation to be important for neurite outgrowth. Interestingly, mouse models have reproduced most aspects of the syndrome, including also the special hand wringing behaviors (in this case forepaws) (Shahbazian and Zoghbi, 2002).

Prader-Willi and Angelman syndromes are associated with several genes located on the chromosome 15q11-q13; five of them are expressed only from the paternal chromosome, loss of their function through deletion, uniparental disomy, or imprinting errors causes Prader-Willi syndrome, a neurological disorder with mild developmental delay in association with hyperphagia and obesity later in the course of life. Another two genes are expressed from the maternal chromosome, and loss of their function through one of the mechanisms mentioned above causes Angelmann syndrome with lack of speech, seizures and severe mental retardation. Exact mechanisms of epigenetic regulation factors here is still unknown, however, it is clear that the region can exist in two mutually exclusive states, paternal and maternal, and that these states have markedly different methylation patterns (Buiting et al., 2003; Xin et al., 2001).

Beckwith-Wiedemann syndrome, a congenital condition associated with pre- and

postnatal overgrowth, macroglossia, and anterior abdominal wall defects, has been linked to the chromosomal region 11p15.5, with most common among other causes being an imprinting defect in DMR-Lit1 with loss of methylation on its maternal copy. The locus is an imprinting control region for the subdomain containing about 12 genes. Normally, the maternally derived allele should stay methylated and thus silent. This allele also shows histone H3Lys9 methylation, indicating it as a mechanism for changes on the chromosomal level (El-Maarri et al., 2007).

Complete hydatidiform moles are abnormal pregnancies with embryo absence and degeneration of villi. Mostly only the paternal, or androgenic genome is observed, however one fifth of them show biparental contribution with abnormal hypomethylation in two paternally expressed genes, Snrpn and Peg3, together with hypermethylation of maternally expressed genes NESP55 and H19. Familial studies showed that these defects are traceable to the grandparental generation maternally, possibly pointing to faulty epigenetic reprograming during either oogenesis or postzygotic development (El-Maarri et al., 2003).

In cancer, overall genomic DNA hypomethylation is observed, while certain CpG rich regions tend to be hypermethylated (Fraga and Esteller, 2007; Toyota and Issa, 1999). Global hypomethylation is associated with genomic instability, while regional hypermethylation may lead to loss of gene expression (Eden et al., 1994; Lengauer et al., 1997). Some examples include the estrogen receptor gene in colon mucosa, which becomes increasingly methylated with progressing age and is close to 100 % methylated in colonic cancer, and insulin-like growth factor 2, which is one of the regulating factors in growth of both benign and malignant tissues; its methylation also tends to grow with age and is found to be greater in tumors (Toyota and Issa, 1999).

1.4.2 Gender dependent differences

Probably the most evident difference between sexes is the inactivation of the X chromosome, which accounts for a significant global methylation difference between male and female embryos. At 7,5 days post coitum, female mice embryos were observed to be less methylated than their male counterparts (Prissette et al., 2001) .Even in an individual embryo, paternal and maternal genomes take a dynamically different methylation and demethylation courses (Fig.4) Global analysis data has shown the male genome to be more methylated (Fuke et al., 2004 ; Shimabukuro et al., 2006). The inactive X chromosome is specifically hypermethylated at CpG islands at gene-rich regions while being generally hypomethylated at gene poor regions; the active one is in contrast hypermethylated not at the specific promoter-associated CpG islands but at the bodies of genes. The question deserves deeper and more thorough analysis, as the studies to date differed much on the number of individuals studied, number and sort of tissues taken into account (Eckhardt et al., 2006; El-Maarri et al., 2007; Sandovici et al., 2005).

Differences between male and female lymphocytes have been observed in a study of four different autosomal loci in 134 males and 157 females (Sarter et al., 2005). Three out of four loci were more methylated in males, while the fourth had been observed to be uniformly unmethylated in both sexes. Difference may lie at the very beginning of tissue differentiation, with a study showing female derived ES cells being generally less methylated than male ES cells (Zvetkova et al., 2005).

There are also differences in primordial germ cells, where imprinted regions are more methylated in XY than in XX cells (Durcova-Hills et al., 2004; 2006).

1.4.3 Tissue dependent variation

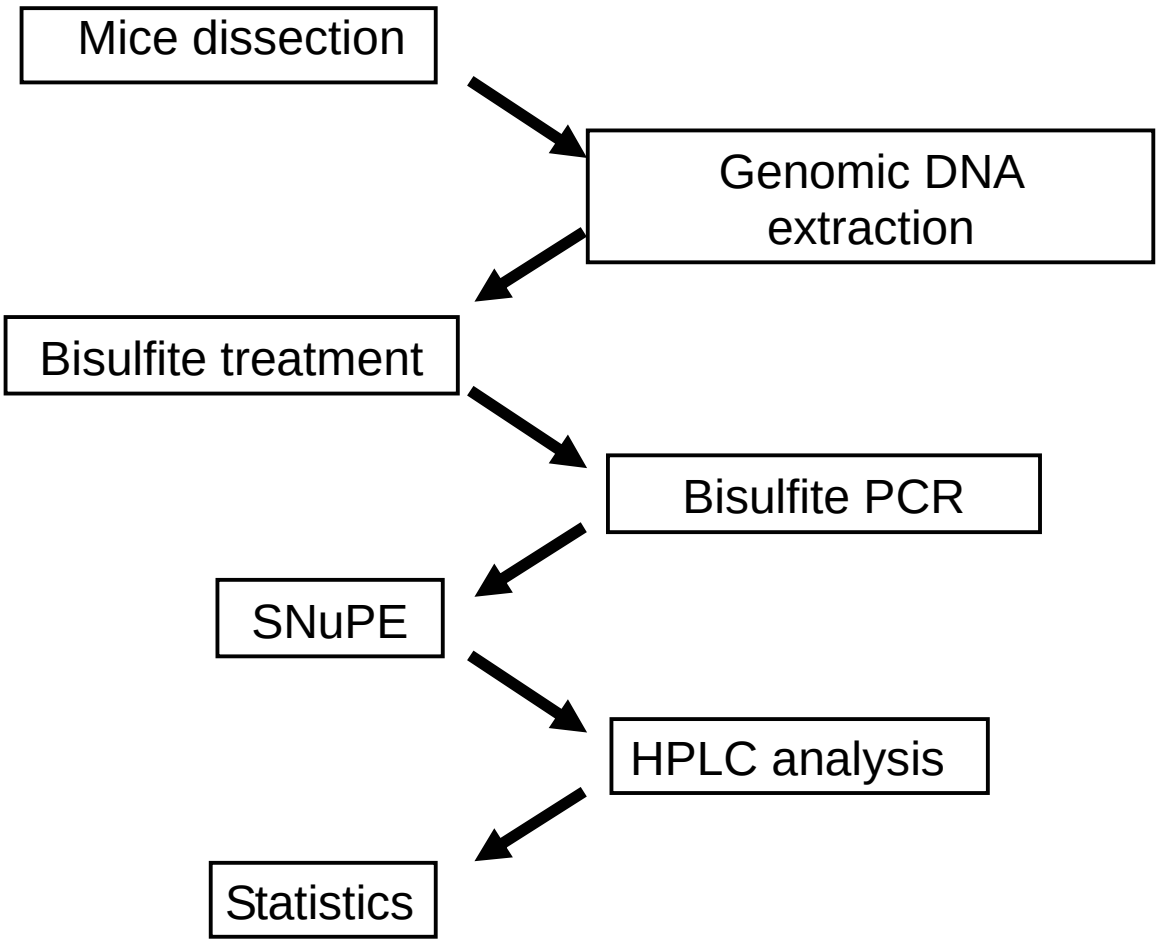
Same loci are methylated differently in various tissues. Repetitive fragments tend to be methylated more in most tissues.

1.4.4 Age dependent variation

Although some genes become more methylated with age, the general trend of the genome is hypomethylation as cellular divisions accumulate. Immortalized cells also show hypermethylation of some genes. Dnmt1, the main maintenance methyltransferase, also decreases in expression with age. A good example of age dependent hypermethylation is the estrogen receptor gene in colonic mucosa, which steadily accumulates methylation with age and is frequently hypermethylated in cancer. Insulin growth factor 2, or IGF2 gene, acquires methylation on the allele that is normally not methylated with age, thus possibly silencing not only the meant to be quiet, but also the necessary active allele. The gene regulates tissue growth and development. Many adult tumors display increased methylation of both alleles, while in young individuals only one of the two is methylated (Lopatina et al., 2002; Toyota and Issa, 1999).

2.Workflow chart.

Tissue samples were used to extract DNA with a standard Qiagen kit, the extracted DNA consequently undergoing bisulfite treatment, PCR and single nucleotide extension steps, so that the level of methylation could be determined using liquid chromatography (HPLC).



3. Aims

This work aims at comparing the level of methylation at different loci in different tissues in a population of 100 outbred mice (50 male and 50 female) grown under the same conditions. More specifically the aims could be stated as:

1- Determine the accurate methylation levels by a highly quantitative assay at the promoters of two single loci (alpha actin and myosin light chain), three differentially methylated regions associated with imprinted loci (Lit1 DMR, SNRPN DMR1 and Peg3 DMR) and two repetitive sequences (Iap and LINE-1) in different mice organs (brain, tongue, skin, lungs, spleen, bone marrow, muscle, testes, and heart);

2- Determine the sex influence on methylation levels

3- Compare the methylation at the above loci between male and females derived samples.

4- Compare the methylation levels between different tissues.

5- Investigate correlation in the level of methylation between different loci in the same tissue (Intra – tissue correlation).

4 Materials and methods

4.1 Tissues

One hundred outbred CD1 mice, 50 male and 50 female, 7 weeks old, have been used for this study.
The following tissue samples and whole organs were taken and frozen immediately in liquid nitrogen: brain, tongue, skin specimen (ears), lungs, spleen, liver, kidney, bone marrow (washed out from lumen of femur with 0.9 % NaCl solution), bone (metaphysis of femur), muscle (femoral extensor and flexor groups, one paw of each individual), testes, and heart.

4.2 Fragments investigated

4.2.1 Single loci

4.2.1.1 Murine MLC1V gene, MyLC

Exon 1 encoding myosin alkali light chain, muscle slow (ventricular) isoform on chromosome 9. (accession n. X12972); Fig.5.
Myosin is the main protein in the contractile apparatus. A myosin molecule consists of four chains, two light and two heavy. Proportions and sort of the heavy and light chain isoforms define the mechanical velocity and power effect of the muscle, which may be as different as balistic jump, run or throw motion or constant postural support. The light chains have a molecular weight of 17000 to 21000 kDa. Both heavy and light chains are subject to tissue and developmental stage specific polymorphisms. (Young et al., 1986). There are a number of different myosin proteins with variety of functions; they differ mostly in their heavy chain. There are three main myosin light chain isoforms, resulting from alternative splicing of its gene and present in various proportions in smooth, skeletal and cardiac muscle; different types of body muscles express different myosin isoforms dependent on their function.

A) Original sequence (accession n. X12972):

1141 acatacaca**C G**tggagcaac tcttctgcac aataaatttg gacaggtaaa ggttagaaaa

1201 gtgtgtacca catgctatac accatagtag gggctggaat gaccaaaggg t**CG**ttcttga

mRNA start

1261 ataactt**CG**c acctgggtgg cccaggtccc ctgatta**[**aga g**CG**aggggtg gtaggatctg

1321 tgttgaggca ccttttcaag tagggagggt ccctggctgt gtgcatggtg ggaggtctct

1381 ggacatttgt atgcattgtc tggtgt**CG**ct gttcagggcc tgtcagtgcc ccagctgggt

1441 ctaggggaag aggctgt**CG**t gtgtcttgtc tctgtggtcc **CG**tttc**CG**gg tgttcacatt

1501 catgtatgtc tgtgtgggtc tgagtgagtg tctgtatatt tatgtctggg tgtttgtatg

1561 cactagtgta tgagtgtctg gatacagcag gcagctgcag tccactcaca gggtcccagg

1621 gtctccagga acaccccaag caca**CG**tggc actccct**CG**a ggactgtcct ccagactagt

1681 tcaacaggct cctttacaa aactcctaag actagtgtct caggttttac acacccacac

1741 t**CG**caca**CGC GCG**cacacac acacacatgt gtgcacacac acatacatac acactggatt

B) Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

1141 atatatata**C G**tggagtaat ttttttgtat aataaatttg gataggtaaa ggttagaaaa

Bisulfite forward primer →

1201 gtgtgtatta tatgttatat attatagtag gggttggaat gattaaaggg t**CG**ttttga

1261 ataattt**CG**t atttgggtgg tttaggtttt ttgattaaga g**CG**aggggtg gtaggatttg

1321 tgttgaggta ttttttaag tagggagggt ttttggttgt gtgtatggtg ggaggttttt

SNuPE primer 1 →

1381 ggatatttgt atgtattgtt tggtgt**CG**tt gtttagggtt tgttagtgtt ttagttgggt

SNuPE primer 2 →

1441 ttaggggaag aggttgt**CG**t gtgttttgtt tttgtggttt **CG**tttt**CG**gg tgtttatatt

1501 tatgtatgtt tgtgtgggtt tgagtgagtg tttgtatatt tatgtttggg tgtttgtatg

1561 tattagtgta tgagtgtttg gatatagtag gtagttgtag tttatttata gggttttagg

Bisulfite reverse primer ←

1621 gtttttagga atattttaag tata**CG**tggt atttttt**CG**a ggattgtttt ttagattagt

1681 ttaataggtt ttttttataa aatttttaag attagtgttt taggttttat atatttatat

1741 t**CG**tata**CG**t g**CG**tatatat atatatatgt gtgtatatat atatatatat atattggatt

Fig. 5: Myosin light chain exon 1, original sequence and sequence after bisulfite processing

4.2.1.2 Alpha-actin gene

Mouse skeletal alpha actin gene (accession n. M12347) (Fig.6). Encodes for a ubiquitous protein in all eukaryotic cells. First discovered as actomyosin by Kuhne in 1861, isolated by Straub, Bonga and Szent-Gyorgyi in 1939-1942. Around 1970, its existence in non-muscle cells has been proven (Pederson and Aebi, 2005). In recent years there has been increasing proof for actin existing in mono- and polymeric forms not only in the cytoplasm, but also in the nucleus of the cells, being closely associated with RNA polymerases. It is a small compact protein of around 43 kDa. In muscles, actin is the rail on which myosin complexes move. In non-muscle cells, it has a variety of functions, including support and stabilization of nuclear matrix and lamina, chromatin remodeling, and being in complex with the known three types of RNA polymerase (Pederson and Aebi 2003). Actin can adopt a great number of forms, such as filaments, sheets, tubes, which makes it the scaffold, roof and floor of many cellular processes such as transcription, chromatin formation, cellular shape, motility, adhesion. Actin is phylogenetically very old, being found in comparable forms among such long diverged species as mammals and fungi (Goodson and Hawse, 2002). Actin defects lead to one of three major forms of myopathy; it is one of the five genes so far linked to the disorder (Clarkson et al., 2004).

A) Original sequence (accession n. M12347:)

361 ggcaggtcag caat**CG**tgtg tccaggtggg cagatctggg gagacctttc aaacaggtaa
421 atcttgggaa gtacagacca g**CG**gtcaaag cagtgacctt tggcccagca cagcccttc**C**
481 **G**tgagccttg gagccagttg ggaggggcag acagctgggg atactctcca tata**CG**gcct
541 ggtc**CG**gtcc tagctacctg ggccagggca gtcctctcct tctttggtca gtgcaggaga
601 cc**CG**gg**CG**gg acccaggctg agaaccagc**C G**aaggaaggg actctagtgc c**CG**acaccca
661 aatatggctt gggaagggca gcaacattct t**CG**ggg**CG**gt gtggggagag ctcc**CG**ggac
mRNA start
721 tatataaaaa cctgtgcaag gggacagg**CG** gtc[aca**CG**ga **CG**taagcctc acttcctacc
781 ct**CG**gcaccc agggcagagt cagagcagca ggtagggtgg aggtggggag ggtgacctgg
841 agacccagca aagaaagcta ttgagccttg gttgtattta gcactgagtt ctggaaattt
901 ctccaaactc acatccagcc cattttgtga ctgggcattt aggatatgcc tgggggtctg

B) Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

361 ggtaggttag taat**CG**tgtg tttaggtggg tagatttggg gagatttttt aaataggtaa
421 attttgggaa gtatagatta g**CG**gttaaag tagtgatttt tggtttagta tagttttttt
Bisulfite forward primer
481 gtgagttttg gagttagttg ggaggggtag atagttgggg atatttttta tata**CG**gttt
541 ggtt**CG**gttt tagttatttg ggttagggta gttttttttt ttttggtta gtgtaggaga
SNuPE primer 1 SNuPE primer 2
601 tt**CG**gg**CG**gg atttaggttg agaattagt**C G**aaggaaggg attttagtgt t**CG**atattta
661 aatatggttt gggaagggta gtaatatttt t**CG**ggg**CG**gt gtggggagag tttt**CG**gat
721 tatataaaaa tttgtgtaag gggatagg**CG** gttata**CG**ga **CG**taagtttt atttttatt
Bisulfite reverse primer
781 tt**CG**gtattt agggtagagt tagagtagta ggtagggtgg aggtggggag ggtgatttgg
841 agatttagta aagaaagtta ttgagttttg gttgtattta gtattgagtt ttggaaattt
901 ttttaaattt atatttagtt tattttgtga ttgggtattt aggatatgtt tgggggtttg

Fig. 6: A fragment of alpha actin gene, original sequence and sequence after bisulfite processing

4.2.2 Differentially methylated regions (DMRs) at imprinted genes

4.2.2.1 Paternally expressed gene 3 (Peg3)

PEG3 is located on chromosome 7, - (Mus musculus, exon 1. Accession n. AF105262; Fig. 7)
and belongs to a group of genes in which loss of methylation has been associated with various neoplastic diseases (Feinberg, 2007). Its human homologue is located on chromosome 19. Peg3 consists of nine exons. A number of repeats and a CpG island is located in its 5' region. The nine-exon transcript is 8.7 kb, ORF contains exons 3 to 9 and is 4.7 kb. It encodes a zinc finger protein, and some experiments indicate its role as a tumour suppressor. During embryogenesis, it is expressed in mesodermal tissues; in adult individuals, a high expression rate is observed in the central nervous system and hypothalamus. Peg3 knockout female mice demonstrate impaired maternal behaviour (Kohda et al., 2001). The gene is expressed in mesodermal tissues of early somites, and later in the gut and hypothalamus. Other tissues with strong expression include tongue and cranial skeleton vertebral cartilage. Less expression occurs in heart and neural tissue (Kuroiwa et al., 1996). In humans, the pattern is completely different, the highest expression occurring in placenta, testes and ovary, while pancreas, prostate, intestine and heart show low expression (Kim et al., 1997). Lack of Peg3 results in growth retardation in affected embryos. Experiments with embryos that had only the paternal genome showed death before gestational day 10, with poor extraembryonic tissue, retarded growth and trophoblast proliferation (Obata et al., 1998).

A) Original Sequence

2641 **G**tcaactc**CG** tgccttgg**CG** ccaagctgtt gccttgacaa cagcagtctg attggcaggg
2701 tgtgggagg**C G**tggtgaggg cccaaag**CG**g ggaatggggt cttggattgg ttagagagga
mRNA start
2761 agctc**CG**cct ctgcagagga ccctgacaag gaggtgtcc**C G**c[agcccttg ctgcaga**CG**c
2821 tggggagtca ggagt**CGCG**g gagga**CG**agc at**CG**gaggag aag**CG**gagag atgtccaccc
2881 tgggctggtg g**CG**c**CG**c**CG**g g**CG**cc**CG**gtt cagtgtgggt gcactagact gc**CG**accctg
2941 gt**CG**gggtgt gtg**CG**tagag tgctgtgctc **CG**ggaggtga gtcagc**CG**gc cacctggctg
3001 ctctgcagca tgcaccctct tagatac**CG**t ctgcagagtt cagatggtgt ttggggtg**CG**
3061 ttgc**CGCG**gg ccaggggg**CG**g cagaccatat ca**CG**gctccc aagggtaact gacaaggctg
3121 cagactg**CG**c ctt**CG**ggaag ggggaatcac ca**CG**gag**CG**g c**CG**tgttgc**C G**cagggatgc
3181 catttaggtg acagggattt aaagtggtat tctataggtc caggcct**CG**g agcctcaggg

Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

Bisulfite forward primer
2641 gttaattt**CG** tgttttgg**CG** ttaagttgtt gtttgataa tagtagtttg attggtaggg
2701 tgtgggagg**C G**tggtgaggg tttaaag**CG**g ggaatggggt tttggattgg ttagagagga
2761 agttt**CG**ttt ttgtagagga ttttgataag gaggtgttt**C G**tagttttg ttgtaga**CG**t
SNuPE primer 1
2821 tggggagtta ggagt**CGCG**g gagga**CG**agt at**CG**gaggag aag**CG**gagag atgtttattt
SNuPE primer 2
2881 tgggttggtg g**CG**t**CG**t**CG**g g**CG**tt**CG**gtt tagtgtggg**t gtattagatt gtCG**attttg
2941 gt**CG**gggtgt gtg**CG**tagag tgttgtgttt **CG**ggaggtga gttagt**CG**gt tatttggttg
3001 ttttgtagta tgtatttttt tagatat**CG**t ttgtagagtt tagatggtgt ttggggtg**CG**
Bisulfite reverse primer
3061 ttgt**CGCG**gg ttaggggg**CG**g tagattatat ta**CG**gttttt aagggtaatt gataaggttg
3121 tagattg**CG**t ttt**CG**ggaag ggggaattat ta**CG**gag**CG**g t**CG**tgttgt**C G**tagggatgt#

Fig. 7: Fragment of paternally expressed gene 3, original sequence and sequence after bisulfite processing

4.2.2.2 Small nuclear ribonucleoprotein N (Snrpn) gene

Snrpn D1 (Mus musculus small nuclear ribonucleoprotein N) gene differentially methylated region1, on chromosome 7, accession n.AF332579, promoter region); Fig.8. Microdeletions and other alterations in this region are observed in patients with Prader–Willi and Angelman syndromes, also in cases of Albright hereditary pseudohypoparathyroidism and osteodystrophy type IA. (Feinberg, 2007) The protein is ribosome associated, found in the spliceosome (Glenn et al., 1997) and responsible for gene splicing, thus having a direct impact on the synthesis of brain proteins, particularly those that function in the hypothalamus (Cassidy et al., 2000). The Snrpn associated DMRs are unmethylated in mice spermatozoa but completely methylated in mature oocytes. The gene contains two differentially methylated regions; - the first DMR includes part of the promoter and transcription start site, and is postulated to inherit a maternal-specific imprint. In mice, this DMR is completely methylated in metaphase II oocytes and unmethylated in sperm. Experiments indicate that oocyte methylation occurs in transition between the non-growing oocyte and metaphase II oocyte, at which stage it is complete.

A) Wild type sequence

67681 aaaaatctgt gtgatgcttg caatcacttg ggagcaattt ttttaaaaaa ttaaatgtat
67741 ttagtaatag gcaattatat ccattattcc agattgacag tgatttttt tttttaatac
67801 a**CG**ctcaaat ttc**CG**cagta ggaatgctca agcattcctt ttggtagctg ccttttggca
67861 ggacattc**CG** gtcagaggga cagagacccc tgcattg**CG**g caaaaatgtg **CG**catgtgca
mRNA start
67921 gccattgcct ggga**CG**catg **CG**tagggagc **CGCGCG**acaa acctgagcca ttg**CG**gca[ag
67981 actag**CG**cag agaggagagg gagc**CG**gaga tgccaga**CG**c ttggttctga ggagtgattt
68041 gcaa**CG**caat ggag**CG**agga aggtcagctg ggcttgtgga ttctagtagt gaaagtgcat

B) Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

Bisulfite forward primer →
67681 aaaaaatttgt gtgatgtttg taattatttg ggagtaattt ttttaaaaaa ttaaatgtat
67741 ttagtaatag gtaattatat ttattatttt agattgatag tgatttttt tttttaatat
SNuPE primer 1 →
67801 a**CG**tttaaat ttt**CG**tagta ggaatgttta agtatttttt ttggtagttg ttttttggta
67861 ggatattt**CG** gttagaggga tagagatttt tgtattg**CG**g taaaaatgtg **CG**tatgtgta
SNuPE primer 2 →
67921 gttattgttt ggga**CG**tatg **CG**tagggagt **CGCGCG**ataa atttgagtta ttg**CG**gtaag

← Bisulfite reverse primer
67981 attag**CG**tag agaggagagg gagt**CG**gaga tgttaga**CG**t ttggttttga ggagtgattt
68041 gtaa**CG**taat ggag**CG**agga aggttagttg ggtttgtgga ttttagtagt gaaagtgtat

Fig. 8: Snrpn fragment, original sequence and sequence after bisulfite processing

4.2.2.3 Mus musculus Lit1

Lit1, or Mus musculus partial Kcnq1 gene encoding for voltage gated potassium channel protein, (accession n.AJ271885, Fig.9) is an antisense-transcript of Kvlqt1, expressed paternally while maternally methylated throughout the developmental stages. Lit1 locus corresponds to a highly conserved region between mouse and human. Loss of maternal allele methylation at its location correlates with Beckwith-Wiedemann syndrome (Yatsuki et al., 2000)

.

A) Wild type sequence

45901 gaacattcCG aaCGgagccc ctcactctca gcattaaaac agctaccaca taacaacaCG
45961 tactccactc actaccttgg tgctggccac acCGggctac aaagctcagg ggtctccaga
46021 ccCGattCGg tttcagctcc agtgCGttct gactCGgccC Ggggtttaga atcCGaaggc
46081 ctgagcCGgt gtcctaggcc actcaccttg ggactCGacC Gacct CGggg ctcaaagggc
46141 ctcaagacca cccctgcttc tgtaagcctg ggccacaaag atgggga CGt ggaCGcaaaa
46201 taCGagaact gagccaCGgc CGtgaaaCGa ggacCGgcCG tgaaaCGagg acCGagcCGt
46261 aactgcaaaa CGaataCGga gccactgCGg caaaaCGaag atggagccca gcCGCGaaag
46321 CGCGgcaCGa atcacctctg cttctggcCG tgagtgcttg cCGCGaggag ggggaggcta
46381 tgatgagCGC Ggccacgcgg acttgCGact tgtgcCGtgc tgactcagag aagaaaccCG
46441 CGctgagaaa aaaaccatac ctaggagaac catgcCGaga aaaagaagCG ctgggaacca
46501 agctgaacag aaaagctctc caagtagaat cacacagagg gaaaagaagC Gtgttgaaga

B) Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

45901 gaatatttCG aaCGgagttt tttattttta gtattaaaat agttattata taataataCG
Bisulfite forward primer
45961 tattttattt attattttgg tgttggttat atCGggttat aaagtttagg ggtttttaga
46021 ttCGattCGg ttttagtttt agtgCGtttt gattCGgttC Ggggtttaga attCGaaggt
46081 ttgagtCGgt gttttaggtt atttattttg ggattCGatC GatttCGggg tttaaagggt
46141 tttaagatta tttttgtttt tgtaagtttg ggttataaag atgggga CGt ggaCGtaaaa
SNuPE primer 1
46201 taCGagaatt gagttaCGgt CGtgaaaCGa ggatCGgtCG tgaaaCGagg atCGagtCGt
46261 aattgtaaaa CGaataCGga gttattgCGg taaaaCGaag atggagttta gtCGCGaaag
SNuPE primer 2
46321 CGCGgtaCGa attatttttg tttttggtCG tgagtgtttg tCGCGaggag ggggaggtta
46381 tgatgagCGC Ggttacgcgg atttgCGatt tgtgtCGtgt tgatttagag aagaaattCG
46441 CGttgagaaa aaaattatat ttaggagaat tatgtCGaga aaaagaagCG ttgggaatta
Bisulfite reverse primer
46501 agttgaatag aaaagtttt taagtagaat tatatagagg gaaaagaagC Gtgttgaaga
46561 aaaattgaga gaatttagta ggttaaaaaa aaatgttgag aagttaagtg gatCGCGtta

Fig. 9: Mouse partial Kcnq gene intron 1, original sequence and sequence after bisulfite processing

4.2.3 Repetitive elements

4.2.3.1 LINE-1 repetitive element

Mus musculus LINE-1 repetitive element (LINE-1), (accession n. D84391; Fig.10).
Multiple sequences of retroviral origin, together with Iap responsible for a large part of the genomic retroviral load. Failure to silence them results in abnormal chromatin structure and early apoptosis. Long interspersed elements (Lines) are mobile genetic sequences that have over time accumulated in the genomes of higher eukaryotes via germline transposition. Lines are a major source of insertional mutagenesis in humans, being involved in both germinal and somatic mutant phenotypes. What makes them particularly interesting, these elements are capable not only of own transposition, but may also take fragments of other genes with them, thus possibly serving as a genome remodeling agent. They are a source of insertional mutagenesis. LINE-1 elements were active throughout mammalian evolution and 17 % of the human genome consists of recognizable copies of LINE-1. Active copies still retropose in the human genome, cause insertional inactivation of human genes and seem to stimulate genomic recombinogenic breaks. LINE-1s also transpose other elements in trans, such as processed pseudogenes, Alu elements and probably also SVA, which are both short interspersed nuclear elements (SINEs) (Weinhold, 2006).

A) Sequence

```
 421 tCGccatctt ggtcCGggac cCGcCGaact taggaaatta gtctgaacag gtgagagggt
 481 gCGccagaga acctgacagc ctctggaaca ggcagaagca cagaggggct gaggcagcac
 541 cctgagtggg cCGgggacag cCGgccacct tcCGgacCGg aggacaggtg ccCGccCGgc
 601 tggggaggCG acctaagcca cagcagcagC GgtCGccatc ttggtcCGgg accCGcCGaa
 661 cttaggaaat tagtctgaac aggtgagagg gtgCGccaga gaacctgaca gcttctggaa
 721 caggCGgaag cacagaggCG ctgaggcagc accctgCGtg ggcCGgggac agcCGgccac
 781 cttcCGgacc agaggacagg tgcccaccCG gctggggagg CGgcctaagc cacagcagca
 841 gCGgtCGcca tcttggtcCG ggaccCGcCG aacttaggaa attagtctga acaggtgaga
 901 gggtgCGcca gagaacctga cagcttctgg aacaggcaga agcacagagg ggctgaggca
 961 gcaccctgtg tgggcCGggg acagcCGgcc accttcCGga cCGgaggaca ggtgcccacc
1021 CGgctgggga ggCGgcctaa gccacagcag cagCGgtCGc catcttggtc cCGggactcc
1081 aaggaactta ggaatttagt ctgcttaagt gagagtctgt accacctggg aactgccaaa
1141 gcaacacagt gtctgagaaa ggtcctgttt tgggccttct tcttCGgcca ggaggaggtc
```

B) Bisulfite Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

```
 421 tCGttatttt ggttCGggat tCGtCGaatt taggaaatta gtttgaatag gtgagagggt
                  Bisulfite forward primer
 481 gCGttagaga atttgatagt ttttggaata ggtagaagta tagaggggtt gaggtagtat
 541 tttgagtggg tCGgggatag tCGgttattt ttCGgatCGg aggataggtg ttCGttCGgt
 601 tggggaggCG atttaagtta tagtagtagC GgtCGttatt ttggttCGgg attCGtCGaa
 661 tttaggaaat tagtttgaat aggtgagagg gtgCGttaga gaatttgata gtttttggaa
 721 taggCGgaag tatagaggCG ttgaggtagt attttgCGtg ggtCGgggat agtCGgttat
 781 ttttCGgatt agaggatagg tgtttattCG gttggggagg CGgtttaagt tatagtagta
 841 gCGgtCGtta ttttggttCG ggattCGtCG aatttaggaa attagtttga ataggtgaga
 901 gggtgCGtta gagaatttga tagtttttgg aataggtaga agtatagagg ggttgaggta
      SNuPE primer 1                                  SNuPE primer 2
 961 gtattttgtg tgggtCGggg atagtCGgtt attttCGga tCGgaggata ggtgtttatt
1021 CGgttgggga ggCGgtttaa gttatagtag tagCGgtCGt tattttggtt tCGggatttt
                                                  Bisulfite reverse primer
1081 aaggaattta ggaatttagt ttgtttaagt gagagtttgt attatttggg aattgttaaa
1141 gtaatatagt gtttgagaaa ggttttgttt tgggtttttt ttttCGgtta ggaggaggtt
```

Fig. 10: Mouse LINE-1 repetitive element, original sequence and sequence after bisulfite processing

4.2.3.2 Intracisternal A-particle gag protein gene, IAP.

Mus musculus clone MIA14 full-length intracisternal A-particle gag protein gene (Iap), accession n. M17551; Fig.11.

Intracisternal A particle - repetitive elements of retroviral origin, found in the whole genome, normaly in silenced state. In mice, its an aggressive retrovirus, normally heavily methylated. Its reactivation causes transcript levels that are 50 to 100-fold higher in compared to normal controls. During global demethylation wave in the preimplantation embryo, the methylation of Iap persists. Specific dynamic paterns of Iap methylation correlate closely with DNMT1 expression (Walsh et al., 1998). There are some division stages at the primordial germ cell level, where genome is both demethylated and dividing. Male germ cells are subject to a few such divisions around embryonic day 13; further divisions are already in the form of densely methylated spermatogonia; these can divide 100 to 150 times; this would be consistent with the idea that methylation is the specific mechanism keeping the retrovirus silent.

A)Sequence

```
  1 tgttgggagc CGCGcccaca ttCGcCGtta caagatggCG ctgacagctg tgttctaagt
 61 ggtaaacaaa taatctgCGc atatgcCGag ggtggttctc tactccatgt gctctgcctt
121 cccCGtgaCG tcaactCGgc CGatgggctg cagccaatca gggagtgaca CGtcctaggC
181 Gaaatataac tctcctaaaa aagggaCGgg gtttCGtttt ctctctctct tgcttcttac
241 actcttgctc ctgaagatgt aagcaataaa gttttgcCGc agaagattct ggtctgtggt
301 gttcttcctg gcCGggCGtg agaaCGCGtc taataacaat tggtgacCGa attcCGggaC
361 Gagaaaaaac tCGggactgg CGcaaggaag atccctcatt ccagaaccag aactgCGggt
```

B) Sequence after bisulfite treatment with pirmer annealing sites, yellow markings showing the binding sites of bisulfite primers, red markings indicating the sites of single nucleotide extension primers:

```
                                                    Bisulfite forward primer
1  tgttgggagt CGCGtttata ttCGtCGtta taagatggCG ttgatagttg tgttttaagt
61 ggtaaataaa taatttgCGt atatgtCGag ggtggttttt tattttatgt gttttgtttt
                                                  SNuPE primer 1
121 tttCGtgaCG ttaattCGgt CGatgggttg tagttaatta gggagtgata CGttttaggC
          SNuPE primer 2
181 Gaaatataat ttttttaaaa aaggggaCGgg gtttCGtttt ttttttttt tgtttttat
                                                  Bisulfite reverse primer
241 atttttgttt ttgaagatgt aagtaataaa gttttgtCGt agaagatttt ggtttgtggt
301 gttttttttg gtCGggCGtg agaaCGCGtt taataataat tggtgatCGa atttCGggat
361 gagaaaaaat tCGggattgg CGtaaggaag atttttatt ttagaattag aattgCGggt
```

Fig. 11: IAP, original sequence and sequence after bisulfite processing

4.3 DNA extraction

Qiagen Dneasy blood and tissue kit was used according to the manufacturer's specifications. Tissue specimens were homogenized in 1,5 ml Eppendorf tubes using a handheld homogenizer. The concentration of DNA was then measured by spectrophotometer.

Approx. 10 mg of spleen and 25 mg of other tissues from each individual were placed into 1.5 ml microcentrifuge tube and suspended in 180 µl buffer ATL, then homogenized with a single use plastic handheld mortar for each sample by grinding until no particles were seen. For more mechanically resistant tissues (bone, cardiac muscle) an electric homogenizer was used.

Then, 20 µl proteinase K was added and the mixture vortexed for 5 to 10 seconds, followed by an overnight-incubation step at 56º C.

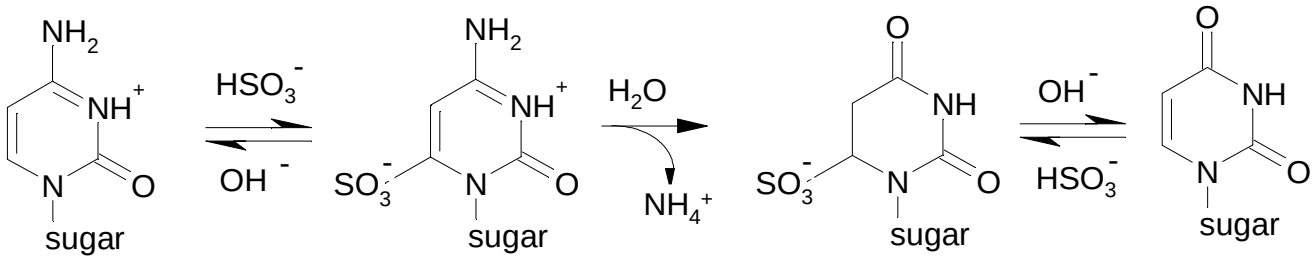
As the lysis is complete, the lysate was vortexed for 15 seconds, then 200 µl buffer AL was added and again mixed by vortexing, followed by 200 µl 96 % ethanol. The mixture is then pipeted (including precipitate if present) into a DNeasy Mini spin column, where it has to be centrifuged at ≥6000 g (8000 rpm) for 1 minute. Then, flow-through and collection tube were discarded.

The column was then placed into a new collection tube, and washed with 500 µl buffer AW1, followed by further centrifugation step at ≥6000 g (8000 rpm) for 1 minute, after which the flow-through and collection tube were again discarded, the column placed into a new collection tube and washed with 500 µl buffer AW2.

The resulting liquid was subjected to centrifugation for 3 min at 20000 g (14000 rpm), after which the collection tube with the flow-through were discarded, the column transferred into the final 1.5 ml microcentrifuge tube and 200 µl buffer AE was pipeted onto the membrane, incubated for 1 min at room temperature and centrifuged at ≥6000 g (8000 rpm) for 1 minute. This step was in some cases repeated twice for maximum DNA yield. DNA concentration was then measured and standard concentration working aliquots prepared.

4.4 Bisulfite conversion

Bisulfite conversion is a multistep reaction that leads to the conversion of cytosine residues to uracil in the presence of sodium bisulfite. Three steps are involved in the reaction, namely sulfonation, deamination and desulfonation. First, cytosines are reversibly sulfonated to cytosine-6-sulfonate. Second, cytosine-6-sulfonate is irreversibly deaminated to form uracil-6-sulfonate; third, uracil-6-sulfonate is then reversibly desulfonated to uracil. Under the right conditions for each step (step 1- low pH and low temperature, step 2 - higher temperature and sodium bisulfite concentration, and step 3 at higher pH), the reaction is highly selective for non-methylated cytosines, and 5-methylcytosines remain unconverted (El Maarri et al., 2004).



I II III

(5´)a-- GTT**C**TG**C**-------**CG**--**C**mG-----G**C**TT**CC**A--

(3´)b-- **C**AAGA**CG**-------G**C**--G**C**m-----**CG**AAGGT--

Denaturation

(5´)a-- GTT**C**TG**C**-------**CG**--**C**mG-----G**C**TT**CC**A--

(3´)b-- **C**AAGA**CG**-------G**C**--G**C**m-----**CG**AAGGT--

Bisulphite treatment

(5´)a-- GTT**U**TG**U**-------**U**G--**C**G-----G**U**TT**CC**A--

(3´)b-- **U**AAGA**U**G-------G**U**--G**C**-----**U**GAAGGT--

Strand specific DNA amplification

(5´)a-- GTT**T**TG**T**-------**T**G--**CG**-----G**T**TT**TT**A-- Upper

(3´)a-- CAAAACA-------AC--GC-----CAAAAAT-- strand

(3´)b-- **T**AAGA**T**G-------G**T**--G**C**-----**T**GAAGGT-- Lower

(5´)b-- ATTCTAC-------CA--CG-----ACTTCCA-- strand

Fig. 12: Bisufite conversion scheme (El Maarri et al., 2004)

The buffers for the bisulfite conversion and bisulfite mix were prepared as per information provided by the manufacturer. The bisulfite mix was reconstituted with 800 µl RNAse free water. The reaction mixture was then prepared as follows: 5 µl of 200 ng/µl DNA, diluted with 15 µl RNAse-free water, 85 µl Bisulfite mix and 35 µl DNA protect buffer. The reaction mixture was then vortexed until it turned blue. Then, the bisulfite reaction was carried out in a thermocycler according to the program listed below:

1. Denaturation at 99 °C for 5 min
2. Incubation at 60 °C for 25 min
3. Denaturation at 99 °C for 5 min
4. Incubation at 60 °C for 85 min
5. Denaturation at 99 °C for 5 min
6. Incubation at 60 °C for 175 min
7. 20 °C pause.

The bisulfite converted product then had to be cleaned from remaining DNA-aggressive reagents.

For this, the PCR tubes were centrifuged briefly, the ensuing supernatant then being transferred to 1.5 ml Eppendorf tubes.

560 µl buffer BL was added. The original protocol calls for additional carrier RNA at this step, however, RNA may be skipped if more than 100 ng DNA is used. Then the reaction mix was vortexed and centrifuged briefly, before transfering it into Epitect spin columns and centrifuging at max speed for 1 min. The flowthrough was then discarded, columns placed back into collection tubes.

Next, 500 µl buffer BW was added and the mixture was centrifuged at max speed for 1 min. Filtrate was again discarded and columns placed back into collection tubes. Then 500 µl buffer BD was added, lids closed, and the reaction mix incubated at ambient temperature for 15 min, followed by centrifugation at max speed for 1 min. Flowthrough again discarded and columns placed back into the tubes.

500 µl buffer BW was then added and centrifuged at max speed for 1 min. Filtrate discarded, columns placed back into tubes. This washing step was repeated twice.

Columns were then placed into new 2 ml collection tubes and centrifuged at max speed for 1 min.

Finally, columns were placed into 1.5 ml microcentrifuge tubes, 20 µl buffer EB (elution step) was added to the center of the membrane, then centrifuged at approx. 15000 g (12000 rpm) for 1 min. The elution step can be repeated with further 20 µl buffer EB to maximize DNA yield.

4.4 Amplification of fragments from bisulfite converted DNA

- Reaction buffer B, ready to use as supplied (SolisBiodyne, Tartu, Estonia)
- MgCl2 100 mM (SolisBiodyne)
- dNTPs (stock concentration 100 mM, working solution 2.5 mM) (Fermentas, Lithuania)
- DNA polymerase (HotFirePol) (SolisBiodyne)
- Molecular biology grade water.
- Primers, stock solution 100 pM, working solution 14 pM (MWG)
- Bisulfite converted DNA.

- PCR amplification: the fragments were amplified in a Biometra T3 thermocycler as follows:

  Step 1: incubate at 95 °C for 15 min

  Step 2: incubate at 92 °C for 30 sec

  Step 3: incubate at X °C for 30 sec (where X is annealing, given in table N.1 with primers used for amplification)

  Step 4: incubate at 72 °C for 1 min; repeat steps 2 to 4 for 34 cycles.

  Step 5: incubate at 72 °C for 10 min for a final elongation step.

  Step 6: incubate at 4 °C until use.

Bisulfite treatment will convert all non-methylated cytosine residues into uracil, the sequence selectivity and specificity for primer annealing is therefore reduced. For this reason, additional precautions concerning primer design and annealing locations need to be made. In designing the primers, care should be taken that the primers do not overlap with CpG dinucleotides. Thus, amplification is independent from the conversion efficiency and methylation status at CpG sites. Primer length should be 25 to 30 nucleotides to insure a robust yet specific annealing. To ensure selective amplification of converted DNA, primers should when possible be located in an originally cytosine-rich region (but not CpGs). Extensive T and A stretches, common for converted DNA, should be avoided in primers to minimize the risk of primer dimere formation. Recommended length of the product is 300 to 500 bp, as DNA depurination and fragmentation occurs.

Tab.1: Primers used for the amplification of bisulfite treated DNA

Fragment	Forward and reverse primers	Product, BP
MylC at 51°C	F: 5'-ATA TTA TAG TAG GGG TTG GAA TGA TTA AAG-3'	410
	R: 5'-CCT ATT AAA CTA ATC TAA AAA ACA ATC CTC-3'	
Alpha Actin at 58°C	F: 5'-GGG GTA GAT AGT TGG GGA TAT TTT T-3'	257
	R: 5'-CCT ACT ACT CTA ACT CTA CCC TAA ATA-3'	
PEG3 at 54°C	F: 5'-TTG ATA ATA GTA GT TGA TTG GTA GGG TGT-3'	395
	R: 5'-ATC TAC AAC CTT ATC AAT TAC CCT TAA AAA-3'	
SNRPN DMR1 at 56°C	F: 5'-AAA TTT GTG TGA TGT TTG TAA TTA TTT GGG-3'	310
	R: 5'-TTT ACA AAT CAC TCC TCA AAA CCA A-3'	
LIT1 at 51°C	F: 5'-GGG TTA TAA AGT TTA GGG GTT TTT AGA TT-3'	470
	R: 5'-AAA CTT TTC TAT TCA ACT TAA TTC CAA AC-3'	
Line1 at 58C°	F: 5'-GTT AGA GAA TTT GAT AGT TTT TGG AAT AGG-3'	620
	R: 5'-TCA AAC ACT ATA TTA CTT TAA CAA TTC CCA-3'	
IAP at 59°C	F: 5'-TTG ATA GTT GTG TTT TAA GTG GTA AAT AAA-3'	210
	R: 5'-AAA ACA CCA CAA ACC AAA ATC TTC TAC-3'	

Amplification procedure:

A master mix* of all reagents except the DNA was prepared, using the following reagent quantities for each sample: 16.4 µl of water, 2.5 µl reaction buffer B, 0.63 µl 100 mM MgCl2, 2.5 µl dNTPs solution, DNA polymerase (the optimal polymerase quantity waried depending on the batch and was adjusted for each batch), 14 pM primer solution, 1 µl.

The ready mix was briefly vortexed, then distributed to PCR strips, followed by addition of 62 ng bisulfite converted DNA, in our case a volume of 2.5 µl. (End volume in each tube was ca. 25 µl), then cycled under the following conditions:

Step 1: 95 °C 15 min
Step 2: 92 °C 30 sec
Step 3: X °C 30 sec (where X is annealing, given in table N.1 together with amplification primer sequences)
Step 4: 72 °C 1 min, then back to step 2 for 34 cycles.

Step 5: 72 °C 10 min, final elongation.
Step 6: 4 °C and pause.

4.5 Amplification product cleanup (exociap)

The amplification products had to be cleaned from all remaining nucleotides and excess primers as these would have interfered with the subsequent single nucleotide extension reaction. An enzyme-based approach was used as described below.
E.Coli exonuclease (ExoI, Fermentas)
Calf intestine alkaline phosphatase (CIAP, Fermentas)
10x EXO-CIAP buffer (Fermentas)
Molecular biology grade water
PCR product from step 3.

PCR product cleanup was done in standard 200 µl PCR tubes using E.Coli exonuclease (ExoI, Fermentas), calf intestine alkaline phosphatase (CIAP, Fermentas), and 10x EXO-CIAP buffer (Fermentas) according to the following procedure:

First, 1.175 µl water was added to the tube, then 0.125 µl EXO, followed by 0.5 µl CIAP and 0.2 µl EXO-CIAP buffer and 5 µl of bisulfite converted DNA; then the samples were incubated as follows in a thermal cycler: 30 min at 37 °C, 20 min at 85 °C, pause at 4 °C.

This method is more expensive than agarose gel based purification of the product, yet enzymatic treatment is more convenient when processing large numbers of samples. However, a highly specific product is a prerequisite for an enzymatic cleanup, whereas in a gel-based purification method it is possible to purify the needed specific band fraction from unwanted unspecific products.

4.6 Single nucleotide primer extension (SNuPE)

- 10x reaction buffer C, supplied ready (SolisBiodyne, Tartu, Estonia)
- TermiPol (SolisBiodyne, Tartu, Estonia)
- ddCTP&ddTTP, 20mMstock, 15 pM working solution. (Fermentas)
- Molecular biology grade water
- MgCl2 25 mM (Solis)
- Exo/CIAP cleaned PCR product from step 4.

Single nucleotide primer extension, (SNuPE) is a prerequisite for HPLC analysis of a given CpG dinucleotide. It is a modification (El-Maarri et al., 2007) of the original method developed by Gonzalgo and Jones (Gonzalgo and Jones, 1997). An oligo just flanking the 5' end of a CpG site, is extended by either a ddCTP or ddTTP, depending on the methylation status of the template. An unmethylated CpG is extended by ddTTP, a methylated CpG by ddCTP. This way the relative content of C and T at a given position can be determined.
The SNuPE reaction was done using primers and temperatures as listed in table 2.
Procedure:
A master mix was prepared with the following reagents (using approx 10 % more volume than needed due to pipeting error):

Reaction buffer C	2 µl
ddCTP&ddTTP (1 mM mix, i.e. 5 µl from stock solution of each diluted by 490 µl water)	2 µl
Water -	8 µl
MgCl2 25mM -	2 µl
TermiPol -	0.2 µl
Primer 15 pM -	2 µl each; end volume 18 µl without DNA.

The master mix was then distributed into PCR strips, followed by addition of 2 µl DNA from the cleaned bisulfite product into each, and the mix was subjected to thermal

cycling under the following conditions:

Step1: incubation at 94 °C for 2 min

Step2: incubation at 92 °C for 30 sec

Step3: incubation at 40 °C for 30 sec

Step4: incubation at 52 °C for 1 min, then back to step 2 for 70 times.

Step4: incubation at 4 °C indefinitely.

The product could then be used directly for HPLC analysis or frozen at -20 °C.

Tab.2: Primers used for the single nucleotide extension step

Fragment	Primer
MylC	1: 5'-GTA TTG TTT GGT GT -3'
	2: 5'-GTG TTT TGT TTT TGT GGT TT-3'
A Actin	1: 5' – GGG ATT TTA GTG TT-3'
	2: 5'- TTT ATT TAG GTT GAG AAT TAG T – 3'
PEG3	1: 5'-GGG GAG TTA GGA GT-3'
	2: 5'-TTT TTT TGT ATT AGA TTG-3'
SNRPN D1	1: 5'-TAG TTA TTG TTT GGG A -3'
	2: 5'-TAG TTG TTT TTT GGT AGG ATA TTT-3'
LIT1	1: 5'- AGA ATT GAG TTA -3'
	2: 5'- ATT ATT TTT GTT TTT GGT – 3'
L1	1: 5'-GTA TTT TGT GTG GGT-3'
	2: 5'-TTT TTT GAG GAT AGG TGT TTA TT-3'
IAP	1: 5'-TAA TTA GGG AGT GAT A-3'
	2: 5'-TAA TTT TTT TAA AAA AGG GA-3'

4.7 HPLC analysis:

- Wave system from Transgenomic, IP RP HPLC column; DNASep (cat. no.DNA 99-3510, Transgenomic), where the stationary phase is alkylated nonporous polystyrene-divinylbenzene-2 µm beads (cat.no.PS/DVD-C18)
- TEAA (triethylamoniumacetate) buffer - ion pairing reagent. (Cat.no.553303)
- Acetonitril (Roth Art.8825.2)
- HPLC-grade water (Merck, cat.no. 1.15333.2500)

High-performance liquid chromatography (HPLC). The SNuPE reaction product was separated and quantified using ion-pair reverse phase HPLC (SNuPE-IP RP HPLC or SIRPH). The original method used radioactively labeled nucleotides and subsequent electrophoresis; the modification used here is based on the possibility to separate different extensions of the same oligo based on retention times for different sequences. This method gives a reliable methylation estimate at selected CpG sites. The extended product is directly loaded on a Wave DNA Analysis System from Transgenomic. As ddTTP is more hydrophobic, it has a longer retention time than ddCTP. If the primers are well optimized, it is possible to quantify multiple CpG site methylation on the same HPLC injection (figure 13). Measuring the height of the peaks and calculating their percentage ratios allows to estimate the methylation percentage at a given CpG site.

Procedure:
20 µl of the SNuPE product from the previous step was used for each injection on the HPLC machine (Wave, Transgenomic), with the oven temperature set to 50 °C, and the elution gradient (proportion of buffers A and B) at flow rate of 0.9 ml/min for 9.3 min as follows:

Tab. 3: HPLC gradient conditions (Wave, Transgenomics)

Step	Time (minutes)	%A (0.1M TEAA)	%B (0.1M TEAA, 25% Acetonitril)
Loading	0.0	90	10
Start gradient	0.1	90	10
Stop gradient	8.1	50	50
Start clean	8.20	0	100
Stop clean	8.7	0	100
Start equilibrate	8.8	90	10
Stop equilibrate	9.3	90	10
Where the start percentage of buffer B in the elution buffer will steadily increase over an 8-min period to reach the "stop gradient" step.			

Calculation of the proportion of methylated CpGs was done according to the following formula: M=[C/(C+T)]*100, where C and T are respectively the peak heights of the ddCTP and ddTTP extended oligonucleotides. Peak heights are then calculated by the Wavemaker software (Fig.13)

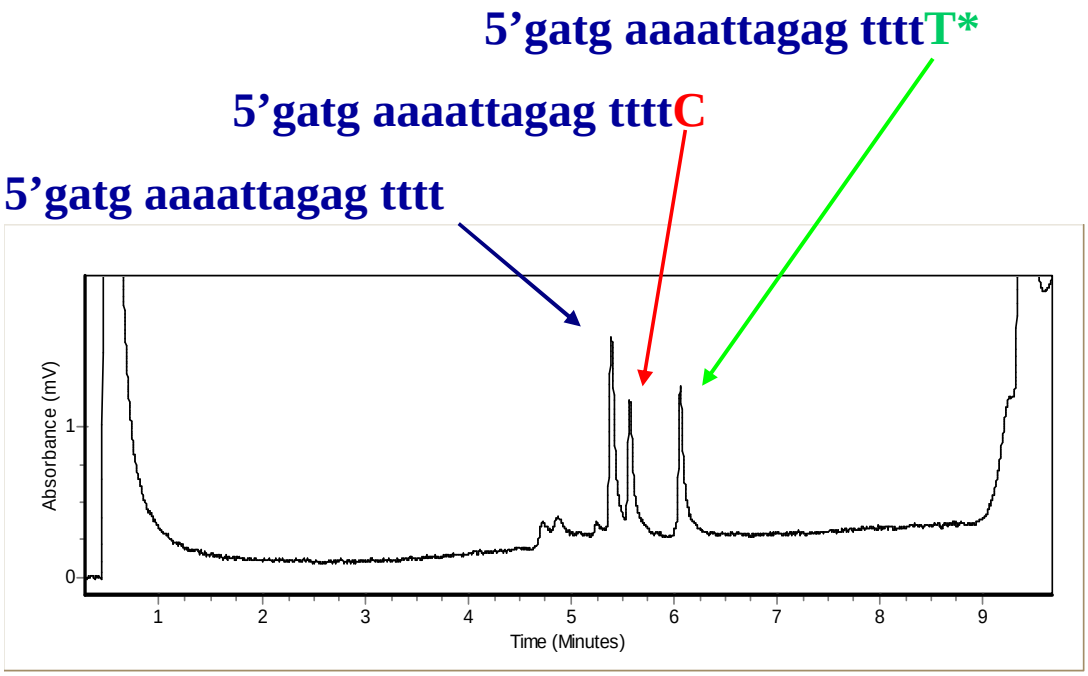


Fig. 13: Scheme of the SNuPE product quantification using HPLC.

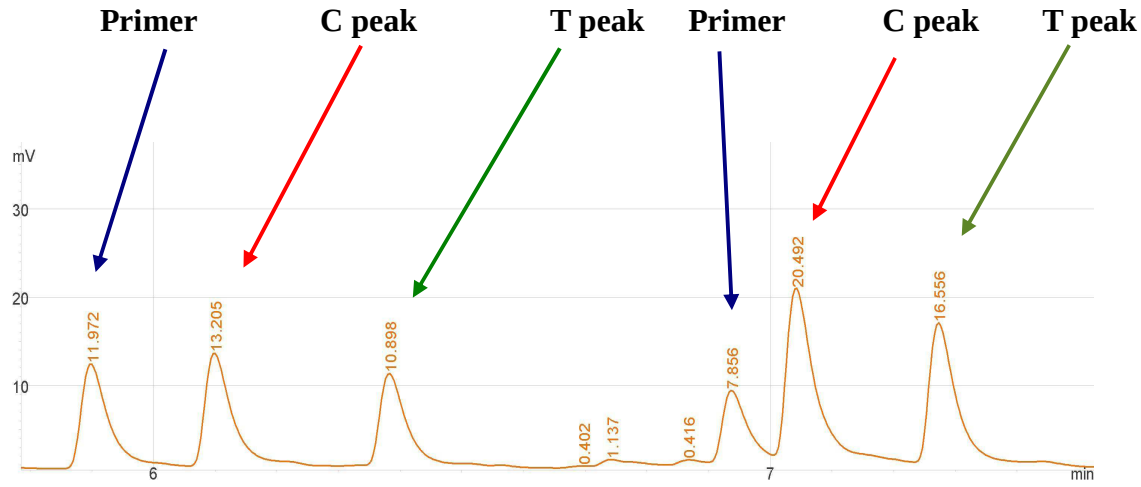


Fig. 14: The reaction could also be multiplexed with up to three primers. Here an example of using two primers at once.

4.8 RT-PCR:

Materials:

- dNTPs: GE Healthcare, 25 mM each.
- RT-enzyme: SuperScript III, Invitrogen.
- Platinum-Taq: Invitrogen
- Rox reference dye: Invitrogen
- Primers: listed in table 4
- Magnesium chloride, 50 mM.
- 10x PCR buffer.
- Real-time cycler: Applied biosystems (ABI) Sequence detection system (SDS) 7700.

Tab.4: Real-time PCR primers used for RNA expression analysis.

Primer	Sequence
Taq-muAactin-F	5'-CTG CCT GAC GGG CAG GT-3'
Taq-muAactin-R	5'-CCG CAG ACT CCA TAC CGA TAA-3'
Taq-muAactin-P-Fam	5'-CGT TTC CGT TGC CCG GAG AC -3'
Taq-muMylc-F	5'-GCT GCA TCA ACT ATG AAG CGT-3'
Taq-muMylc-R	5'-CTG GGC TTC CTG AGA GGC-3'
Taq-muMylc-P-Fam	5'-TGT GAA GCA TAT CAT GGC GAG CTG-3'
Taq-muPBGD-F	5'-CGG CCA CAA CCG CGG AAG AA-3'
Taq-muPBGD-R	5'-GTC TCC CGT GGT GGA CAT AGC AAT GA-3'
Taq-muPBGD-R-Joe	5'-TCG AAT CAC CCT CAT CTT TGA GCC GT-3'

Real-time PCR is based on the detection of fluorescent signals of the amplification. Quantity of the PCR product determines signal strength. For this purpose, an ABI Prism® 7700 SDS machine was used.

Cycling conditions were as follows:

- Incubation at 50˚C for 20 min.
- Incubation at 95˚C for 15 min
- Incubation at 94˚C for 30 sec.
- Incubation at 60˚C for 60 sec.
- Repeat previous steps for 40 cycles.

4.9 Statistical analysis

Pearson correlation and t-test analysis were performed based on the generated methylation data. T-tests were performed to compare differences between males and females at different loci and in different tissues. Uncorrected P values below 0.05 were considered to be significant (α level of 0.05). To correct for multiple testing, Bonferoni correction was used

The Pearson coefficient r was computed for all pairs of methylation variables within one tissue and in the two gender groups separately using the proc corr function from SAS software package (SAS for Windows, v9.1.,SAS Institute Inc.,Cary, NC, USA; http://www.sas.com).

5. Results

5.1 DNA quantification and quality test:

The DNA used in the experiments was extracted from the 9 organs mentioned previously (brain, tongue, skin (ear lobe), lungs, spleen, bone marrow (from lumen of femur), skeletal muscle (from femoral extensor and flexor groups), testes, and heart of 100 mice (50 males, 50 females). A total of 900 units of DNA stock solution were generated. The aliquots were initially loaded on a 1.5 % agarose gel, thus testing the possible level of genomic DNA degradation. An example of this can be seen in Fig.7 below. Once DNA presence was confirmed, spectrophotometric concentration measurement followed.

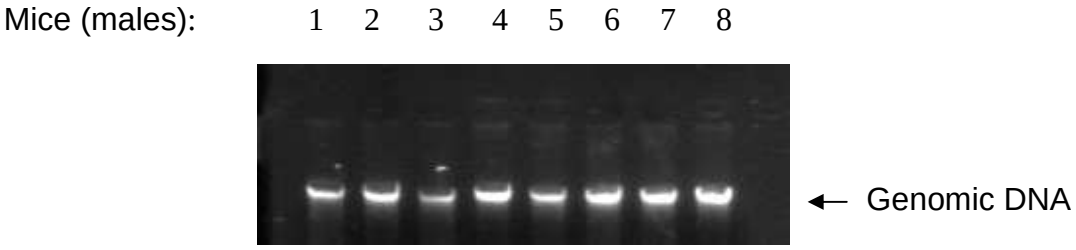
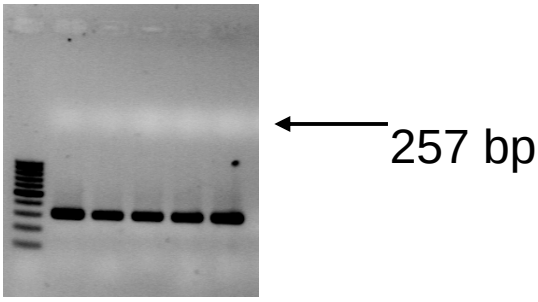


Fig. 15: Genomic DNA samples (extracted from bone marrow) on a 1,5 % agarose gel:

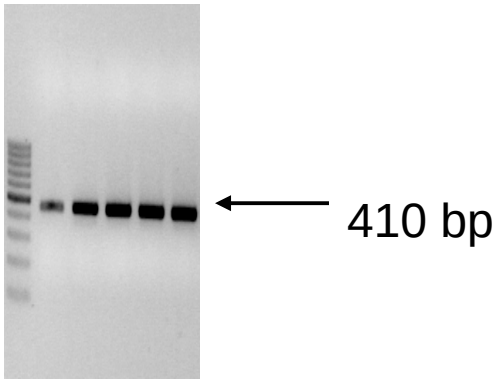
5.2 PCR amplification

The genomic DNA was then subjected to bisulfite treatment (described in detail on p.39-41), and consequently amplified with the region-specific bisulfite primers (Tab. 2, conditions and amplification protocol on p.42-43). Amplification product samples are shown on Fig.8 below. As seen in Fig.8, some products, especially the repetitive elements Iap and LINE-1, have unspecific amplification bands present in the product. This problem was solved by using the second set of primers, namely in the single nucleotide primer extension step (SNuPE), which are highly specific for the sequences targeted.

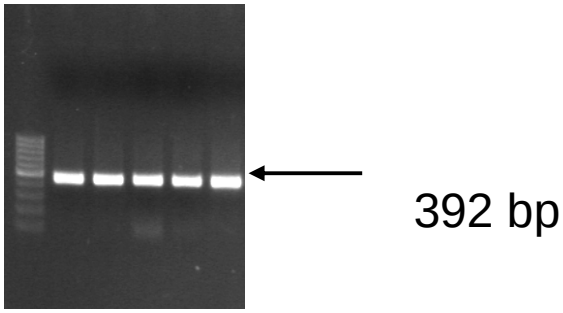
a) Alpha actin



b) Murine MLC1V gene



c) Paternally expressed gene 3, *Peg3*



d) *Snrpn*D

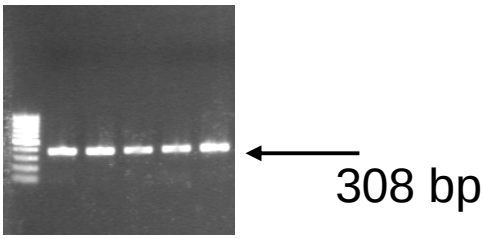


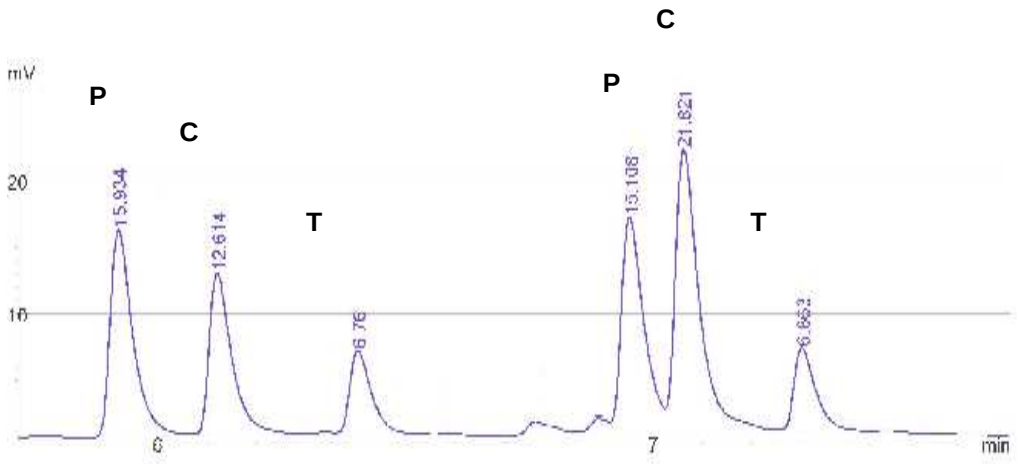
Fig. 16: Bisulfite treated DNA amplification product samples on a 1,5 % agarose gel.

5.3 HPLC chromatograms

The bisulfite amplification product was then subjected to the single nucleotide primer extension protocol (SNuPE; primers listed on Tab.3, reaction conditions listed on p. 45-47). Figure 16 shows some examples of the HPLC chromatograms, the peak height of which is our actual data and mirrors the percentage of CpGs methylated at the given CpG site. Not all CpG sites have been successfully analyzed by HPLC due to multiple preparation steps, each of which could not be performed with 100 % efficiency. The numbers of sites with readable HPLC chromatogram peaks and their raw data (peak height) plus the calculated ddCTP to ddTTP ratio are fully listed in Appendix 2.

As seen in chromatogram examples in figure 16 below, not all chromatograms demonstrated same sharpness of peak separation and distinctive peak height or shape. The peak separation distance is dependent on the sequence of the SNuPE primer used, as different sequences have slightly different melting properties and will therefore react differently to the same conditions on the liquid chromatography column.

Myosin light chain, *MyLC*



Alpha actin

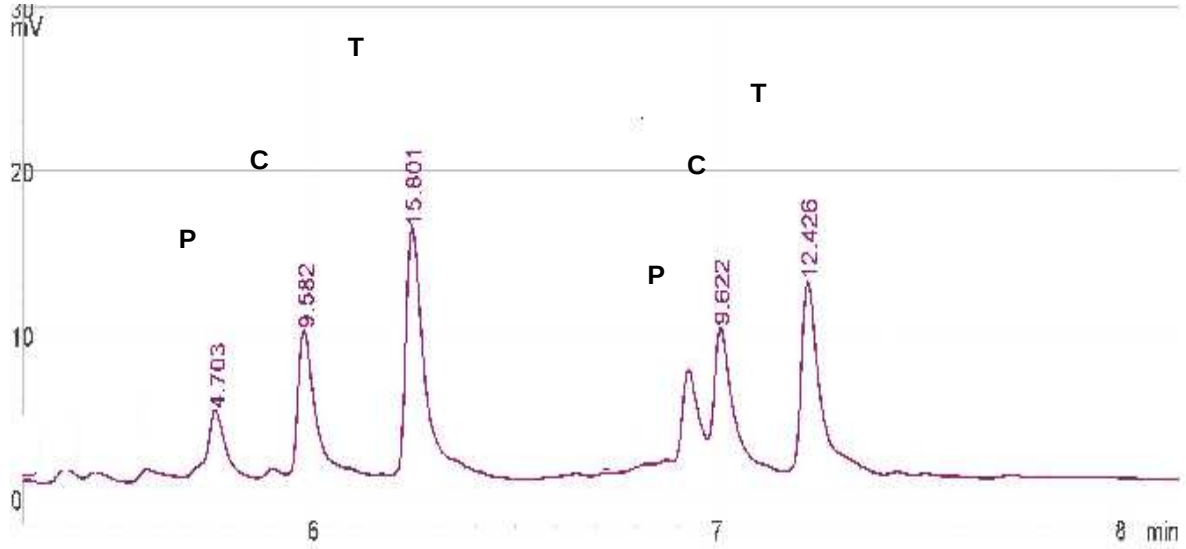


Fig. 17: HPLC chromatogram samples. a) Murine MLC1V gene, MyLC; b) Alpha actin; P-primer, C -ddCTP extended primer, T – ddTTP extended primer.

5.4 The influence of sex on methylation levels: male/female differences

A) Single loci

Two CpG sites have been investigated in myosin light chain gene and two in alpha actin, of 100 mice, 9 organs from each, generating a total of 900 chromatograms and 1800 single cytosine methylation readings. Readable peaks have been obtained for 1696 CpG sites in myosin light chain and 1540 in alpha actin.

Myosin

The CpG sites investigated were located after nucleotide positions 1406 and 1480, accession n.X12972. Initial data indicated males being more methylated in lungs, brain, tongue, and heart. After Bonferoni correction, which was done for the first CpG site in every region studied, none of them were significant enough (Table 5).

Alpha actin

Raw data showed a tendency towards greater methylation in males in lungs , tongue, and muscle, while females tended to be more methylated in brain. After statistical analysis and Bonferoni correction, only the difference in tongue remained significant. (Tab.5-7).

B) Imprinted genes

Peg3

Initial trend showed greater methylation in males in skin, tongue, and muscles, while females were more *methylated in brain*. Statistical analysis after Bonferoni correction left males to be signifficantly more methylated in skin and tongue (Tab.5-7).

Snrpnd1,

Initial trend indicated greater methylation in males in lungs, tongue, and heart, while females leaned toward greater methylation in spleen and bone marrow. Data after bonferoni correction showed males were statistically more methylated in the CpG sites at nucleotide positions 57858 and 57934, accession n.AF332579, in lungs and tongue, and less methylated at position 57858 in bone marrow (Tab. 5-7).

Lit1,

Initial tendency was greater mehylation in males in spleen, lungs, skin, and tongue, while females were more methylated in brain and bone marrow. After Bonferoni correction, males remained more methylated in lungs, tongue and skin, less methylated in brain and in bone marrow.

C)Repeats

Iap

CpG sites at nucleotide positions 170 and 206, accession n. M17551. Uncorrected data suggested males were more methylated in spleen and bone marrow, less methylated in lungs, skin, brain, and heart. After Bonferoni correction, males remained significantly more methylated in bone marrow, but less in lungs and skin (Tables 5 a-c).

LINE-1.

The CpG sites investigated were located at nucleotide positions 965 and 1001, accession n. D84391. Initial results showed males to be more methylated in brain, females in bone marrow, tongue, and heart. After Bonferoni correction, difference remained signifficant in tongue and skeletal muscle (Tables 5 a-c)

Tab. 5: Level of methylation in spleen, lung and skin tissue samples plus male/female T-test and Bonferoni correction; correction shown only for the first positions (first pair of HPLC peaks) analyzed.

		Tissue	Spleen		Lungs		Skin	
			Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
	Regions		Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480
Single loci	MyLC	males	74	80	47	52	66	72
	Acc. n. X12972	females	72	80	45	51	67	71
		T-Test	0.07348109	0.59254373	**0.00469404**	0.32702893	0.18036824	0.05886483
	Bonferroni correction:		1.00E+00		2.96E-01		1.00E+00	
			Pos. 441	Pos. 529	Pos. 441	Pos. 529	Pos. 441	Pos. 529
Imprinted regions	A actin	males	37	50	27	34	26	35
	Acc. n. M12347	females	37	48	25	32	24	34
		T-Test	0.258	0.244	**0.002**	**0.037**	0.058	0.641
	Bonferroni correction:		1.00E+00		1.00E+00		1.00E+00	
			Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922
	PEG3	males	43	49	39	50	45	53
	Acc. n. M12347	females	44	49	38	48	43	50
		T-Test	0.06163219	0.05904186	0.33776207	0.05705342	**0.00001064**	**0.00003568**
	Bonferroni correction:		1.00E+00		1.00E+00		**6.70E-04**	
			Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934
	SNRPN D1	males	42	50	44	56	44	52
	cc. n. AF33257	females	42	51	41	53	44	50
		T-Test	0.43737012	**0.03877697**	**0.00000000**	**0.00049491**	0.12466367	0.06769482
	Bonferroni correction:		1.00E+00		**1.23E-07**		1.00E+00	
			Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348
	LIT1	males	47	39	44	36	46	42
	cc. n. AJ27188	females	47	38	42	35	43	38
		T-Test	**0.01570943**	**0.00418357**	**0.00000041**	0.14552654	**0.00000000**	**0.00000002**
	Bonferroni correction:		9.90E-01		**2.58E-05**		**4.13E-09**	
			Pos. 170	Pos. 206	Pos. 170	Pos. 206	Pos. 170	Pos. 206
Repeats	IAP	males	78	84	77	79	64	60
	Acc. n. M17551	females	77	82	79	81	66	60
		T-Test	0.17646157	**0.00001424**	**0.00000055**	**0.00000300**	**0.00000160**	0.43046263
	Bonferroni correction:		1.00E+00		**3.47E-05**		**1.01E-04**	
			Pos. 965	Pos. 1001	Pos. 965	Pos. 1001	Pos. 965	Pos. 1001
	L1	males	80	84	84	87	85	87
	Acc. n. D84391	females	79	84	84	87	85	88
		T-Test	0.49695	0.90907	0.27722	0.58366	0.59721	0.74366
	Bonferroni correction:		1.00E+00		1.00E+00		1.00E+00	

Tab. 6: Level of methylation in brain, bone marrow, and testicular tissue samples plus male/female T-test and Bonferoni correction; correction shown only for the first positions (first pair of HPLC peaks) analyzed.

		Tissue	Brain		Bone marrow		Testes	
			Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
Single loci	*Regions*		Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480
	MyLC	males	70	80	66	76	72	74
	Acc. n. X12972	females	69	80	65	76		
		T-Test	**0.00894007**	0.28580020	0.39208895	0.99844120		
	Bonferroni correction:		5.63E-01		1.00E+00			
			Pos. 441	Pos. 529	Pos. 441	Pos. 529	Pos. 441	Pos. 529
	A actin	males	46	54	33	39	59	63
	Acc. n. M12347	females	50	57	34	39		
		T-Test	0.056	**0.004**	0.598	0.698		
	Bonferroni correction:		2.41E-01		1.00E+00			
Imprinted regions			Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922
	PEG3	males	44	51	40	47	20	29
	Acc. n. M12347	females	47	54	41	47		
		T-Test	**0.00100214**	**0.00187294**	0.08743775	0.16564843		
	Bonferroni correction:		6.31E-02		1.00E+00			
			Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934
	SNRPN D1	males	45	59	42	54	18	24
	Acc. n. AF332579	females	45	61	43	55		
		T-Test	0.72186344	0.22192811	**0.00006885**	0.09708330		
	Bonferroni correction:		1.00E+00		**4.34E-03**			
			Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348
	LIT1	males	44	38	39	38	20	28
	Acc. n. AJ271885	females	46	41	42	39		
		T-Test	**0.00013058**	**0.00000817**	**0.00001037**	0.05728803		
	Bonferroni correction:		**8.23E-03**		**6.53E-04**			
			Pos. 170	Pos. 206	Pos. 170	Pos. 206	Pos. 170	Pos. 206
Repeats	IAP	males		89	75	81	73	63
	Acc. n. M17551	females		90	73	82		
		T-Test		**0.00116926**	**0.00008632**	0.09224869		
	Bonferroni correction:				**5.44E-03**			
			Pos. 965	Pos. 1001	Pos. 965	Pos. 1001	Pos. 965	Pos. 1001
	L1	males	88	85	72	79	73	71
	Acc. n. D84391	females	88	84	72	80		
		T-Test	0.36289	**0.01975**	0.07199	**0.03683**		

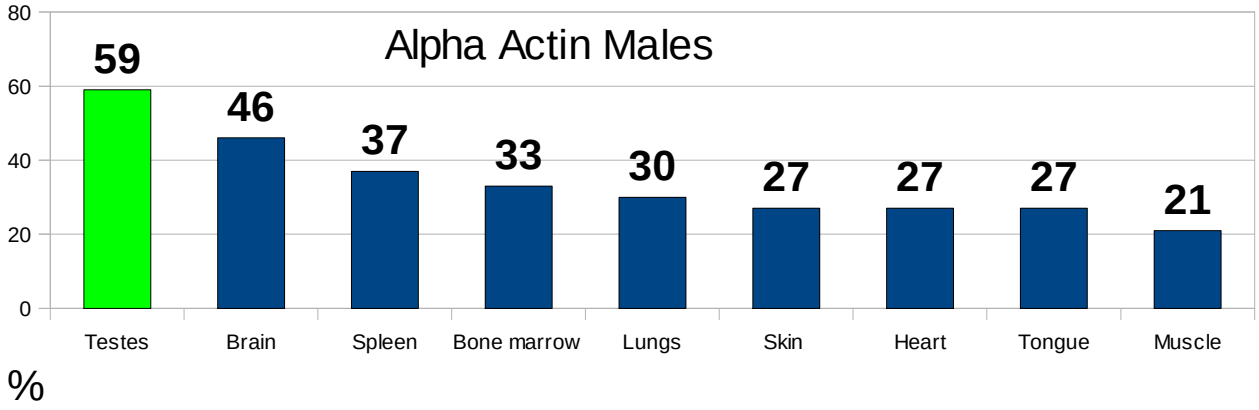
Tab. 7: Level of methylation in tongue, skeletal muscle, and heart muscular tissue samples plus male/female T-test and Bonferoni correction; correction shown only for the first positions (first pair of HPLC peaks) analyzed.

		Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
Single loci	*Regions*	Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480	Pos. 1406	Pos. 1480
	MyLC	66	75	65	75	51	58
	Acc. n. X12972	68	71	66	75	49	56
		0.01676302	**0.00000000**	0.19318411	0.66041539	0.05675236	**0.02870041**
	Bonferroni correction:	1.00E+00		1.00E+00		1.00E+00	
		Pos. 441	Pos. 529	Pos. 441	Pos. 529	Pos. 441	Pos. 529
	A actin	27	37	21	29	27	35
	Acc. n. M12347	23	31	20	30	26	35
		0.000	**0.001**	**0.008**	0.515	0.804	0.598
	Bonferroni correction:	3.35E-0.2		1.00E+00		1.00E+00	
Imprinted regions		Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922	Pos. 2835	Pos. 2922
	PEG3	65	80	51	57	46	58
	Acc. n. M12347	**0.00000000**	67	48	55	47	57
		3.16E-08	**0.00002850**	**0.00145772**	0.10246896	0.72458959	0.34578413
	Bonferroni correction:			9.18E-02		1.00E+00	
		Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934	Pos. 57835	Pos. 57934
	SNRPN D1	50	63	65	75	44	54
	Acc. n. AF332579	43	55	66	75	43	54
		0.00000000	**0.00000000**	0.58944354	0.98802868	**0.04240597**	0.28364517
	Bonferroni correction:	**6.61E-25**		1.00E+00		1.00E+00	
		Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348	Pos. 45216	Pos. 45348
	LIT1	57	47	40	35	34	35
	Acc. n. AJ271885	47	39	41	36	34	35
		0.00000000	**0.00000000**	0.37497445	0.28398094	0.78698771	0.99382305
	Bonferroni correction:	**4.46E-13**		1.00E+00		1.00E+00	
		Pos. 170	Pos. 206	Pos. 170	Pos. 206	Pos. 170	Pos. 206
Repeats	IAP	82	88	62	60	81	88
	Acc. n. M17551	82	88	60	61	80	89
		0.90602758	0.86625529	0.16220050	0.64959605	0.30722528	**0.03667537**
	Bonferroni correction:	1.00E+00		1.00E+00		1.00E+00	
		Pos. 965	Pos. 1001	Pos. 965	Pos. 1001	Pos. 965	Pos. 1001
	L1	85		83	86	81	85
	Acc. n. D84391	86		84	86	82	86
		0.00000000		0.07970	**0.02044**	**0.00016803**	**0.00013**

5.5 Methylation variability at specific loci in different organs

In this part, we investigated the organ specific methylation variations at the studied loci. Complete values and standard deviation are given in Appendix 1.

A) Single loci



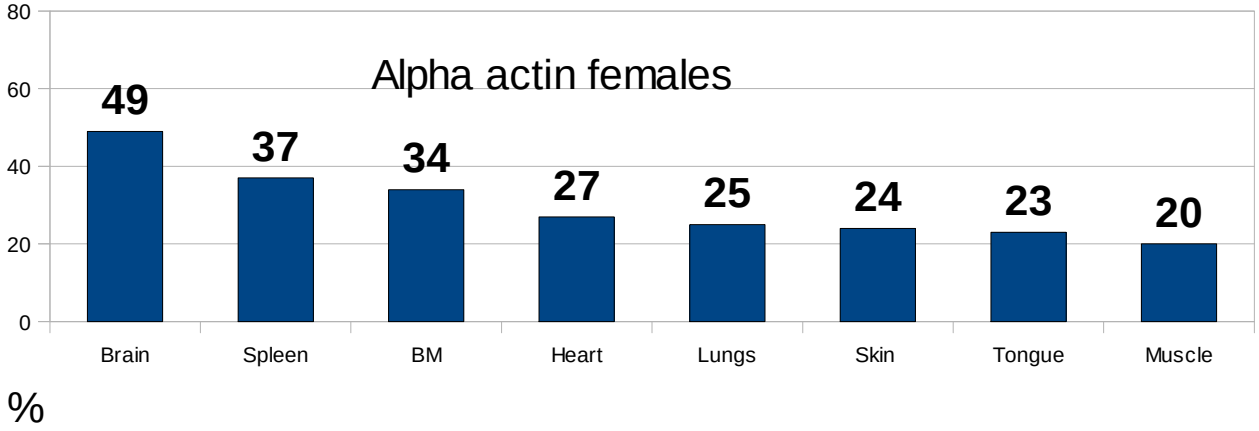


Fig. 18: Alpha actin. Among tissues studied in both sexes, in males, alpha actin was most methylated in brain (46 %), least methylated in skeletal muscle (21 %). Of all the tissues, testis showed the highest methylation level, reaching 59 %. In females, same tendency was observed (brain 49 %, skeletal muscle 20 %).

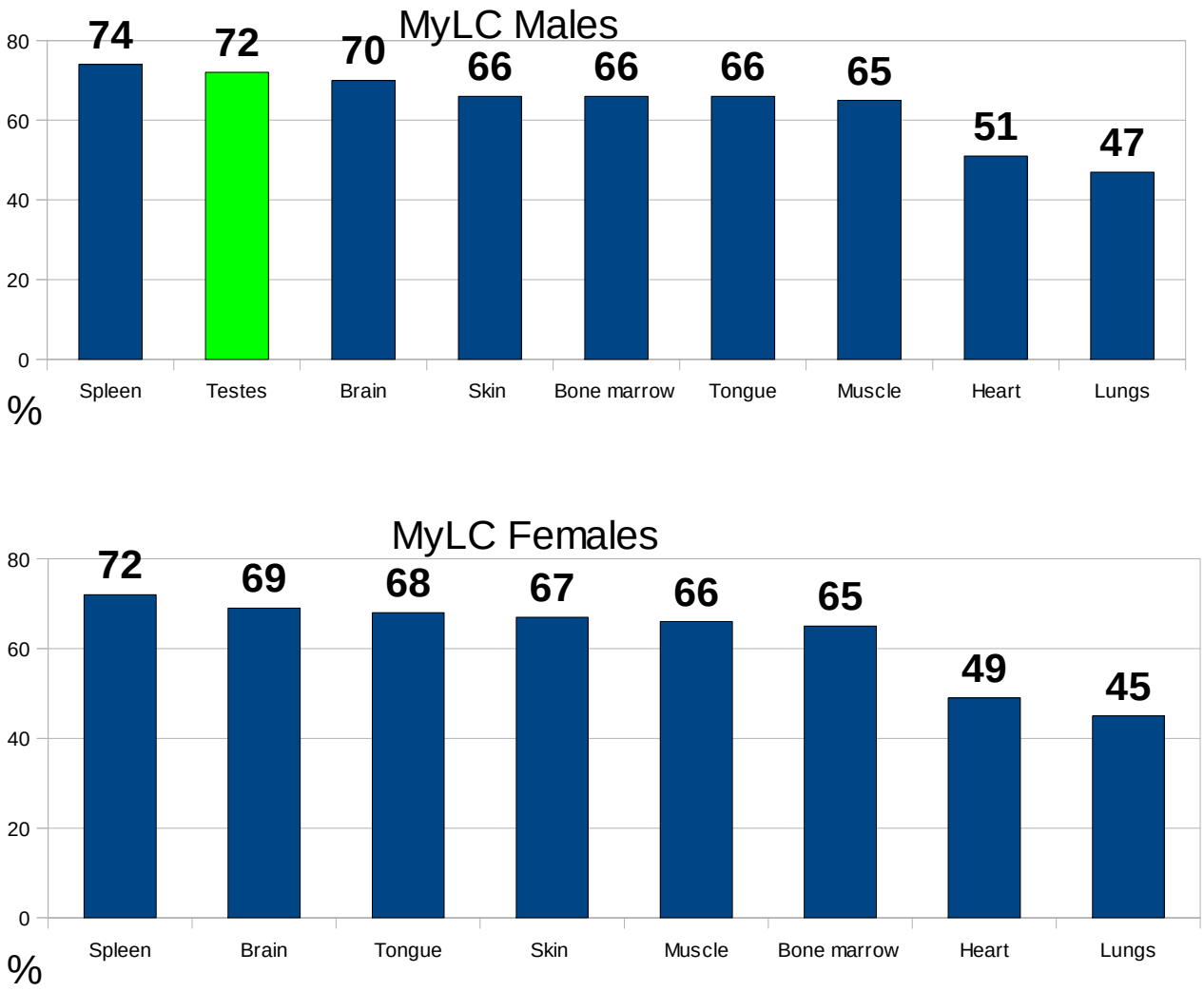


Fig. 19: Myosin light chain: Males are most methylated in spleen (74 %), least methylated in lungs (47 %). Females are also most methylated in spleen (72 %), least methylated in lungs (45 %). Considering that methylation is inversely proportional to gene expression, these results are logical in that if skeletal muscle expresses myosin light chain gene in a high level it should be less methylated there. The relatively low methylation in lungs cannot be simillarly explained.

B)Imprinted genes

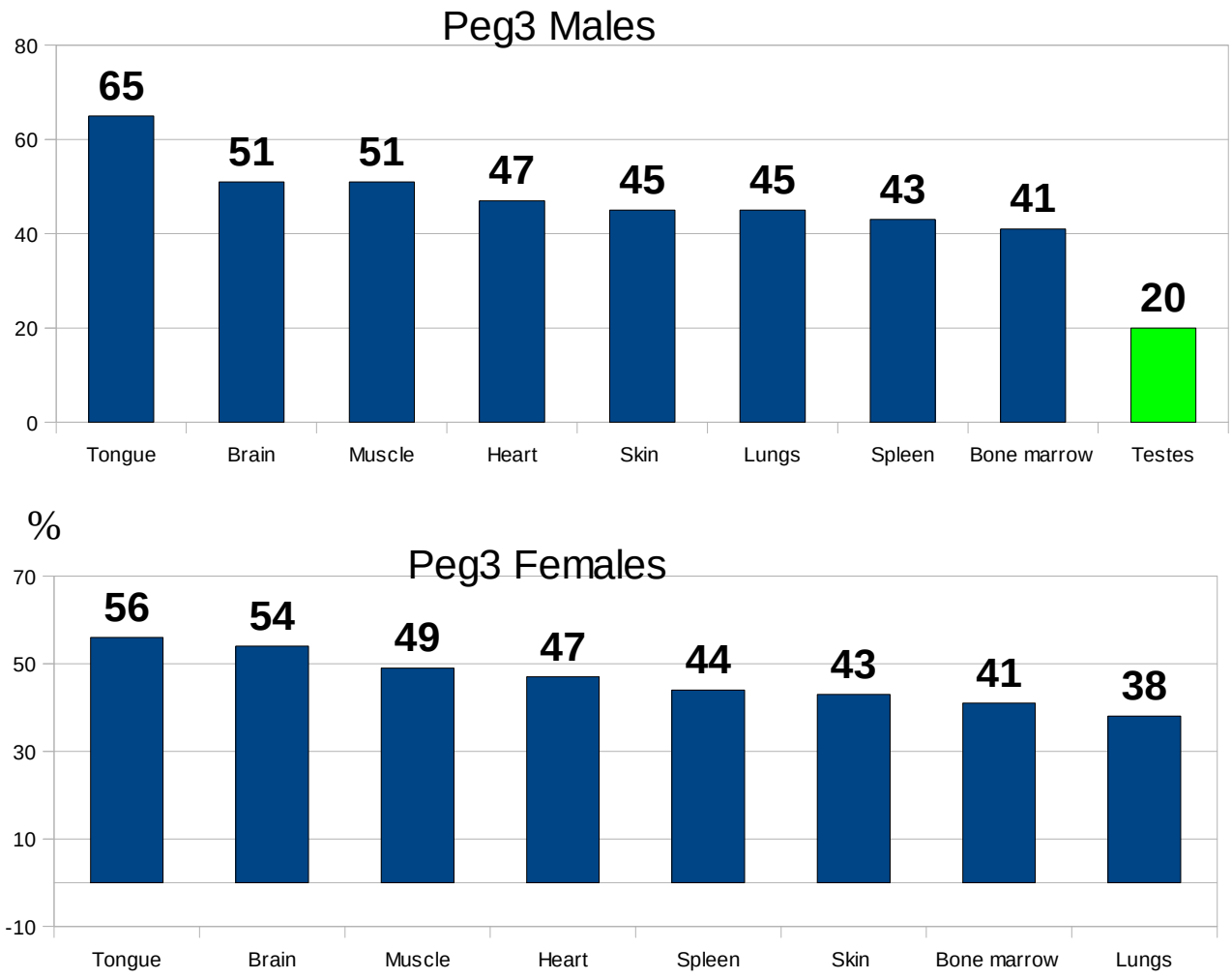


Fig. 20: Peg3: Among the tissues studied, males showed heaviest methylation in tongue (65 %), least methylation in bone marrow (41 %); females were most methylated in tongue (56 %), least methylated in lungs (38 %). Testes showed a signifficant reduction in methylation at this locus due to the imprinting process that leaves it unmethylated in male gametes.

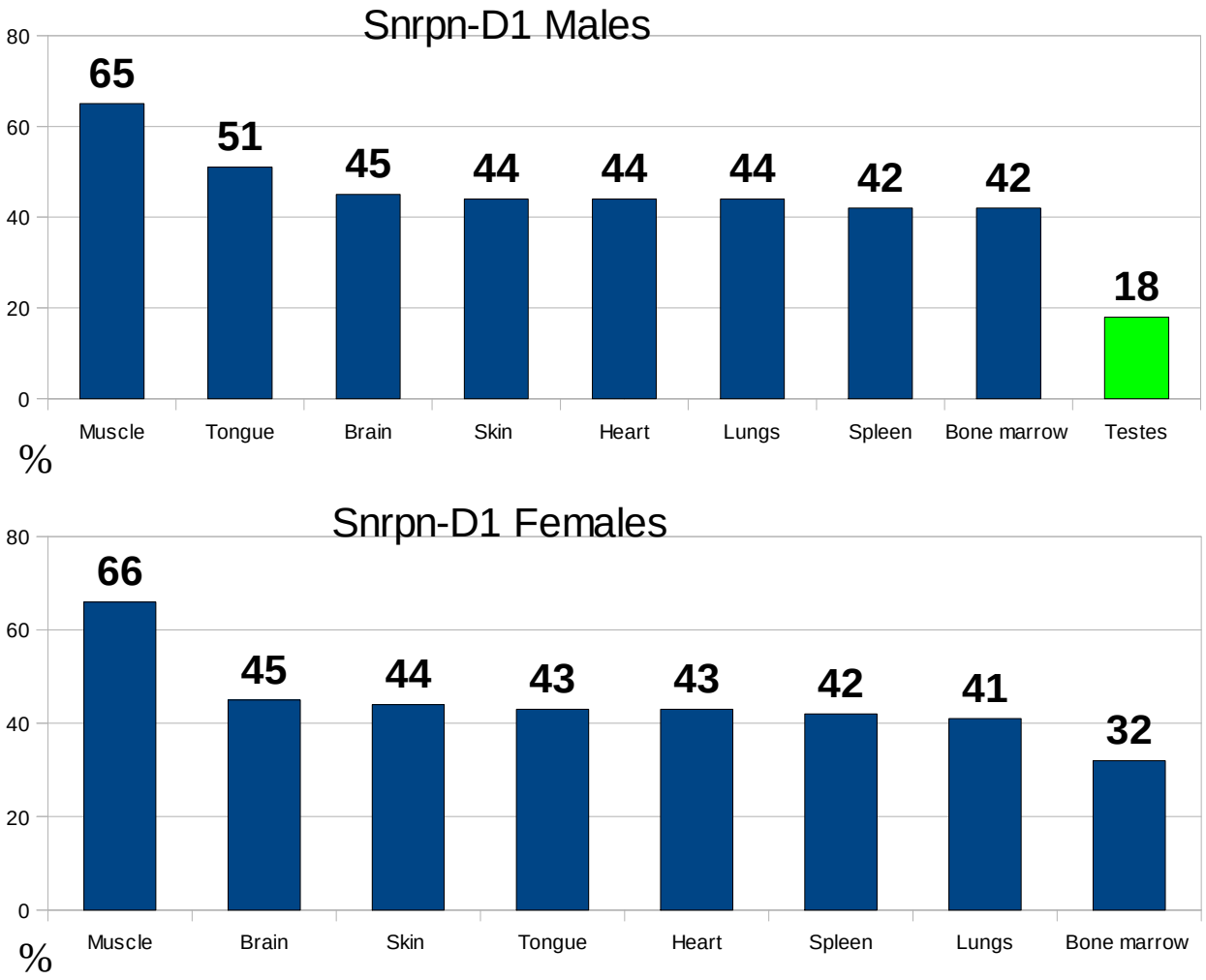
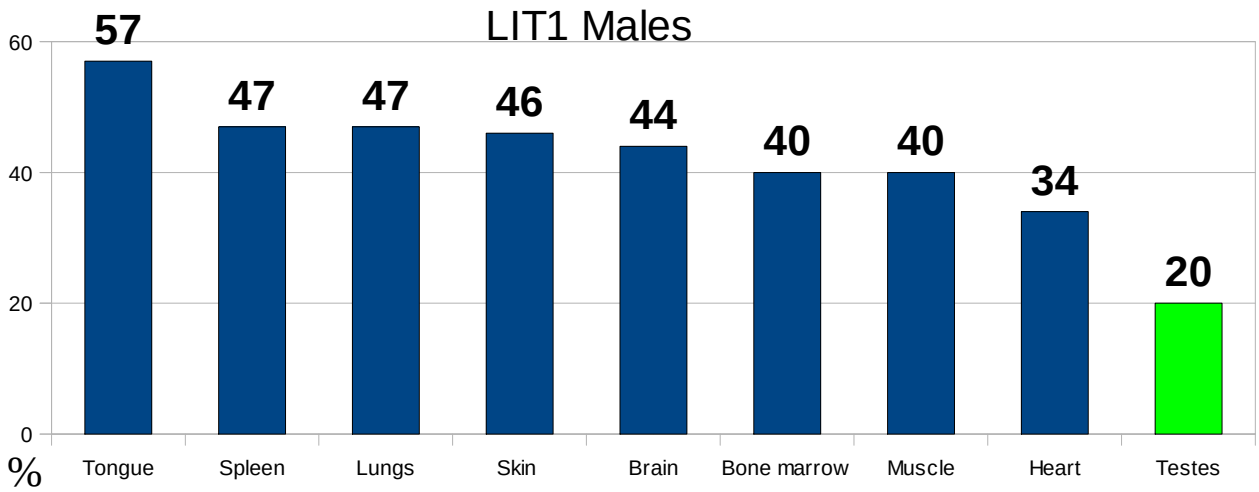


Fig. 21: Snrpn-D1: In males, most methylated in skeletal muscle (65 %) , least methylated in spleen and bone marrow (42 %). In females, most methylated in skeletal muscle (66 %), least in bone marrow (32 %). Again, testis showed very low methylation levels, as imprinting leaves this locus unmethylated in gametes.



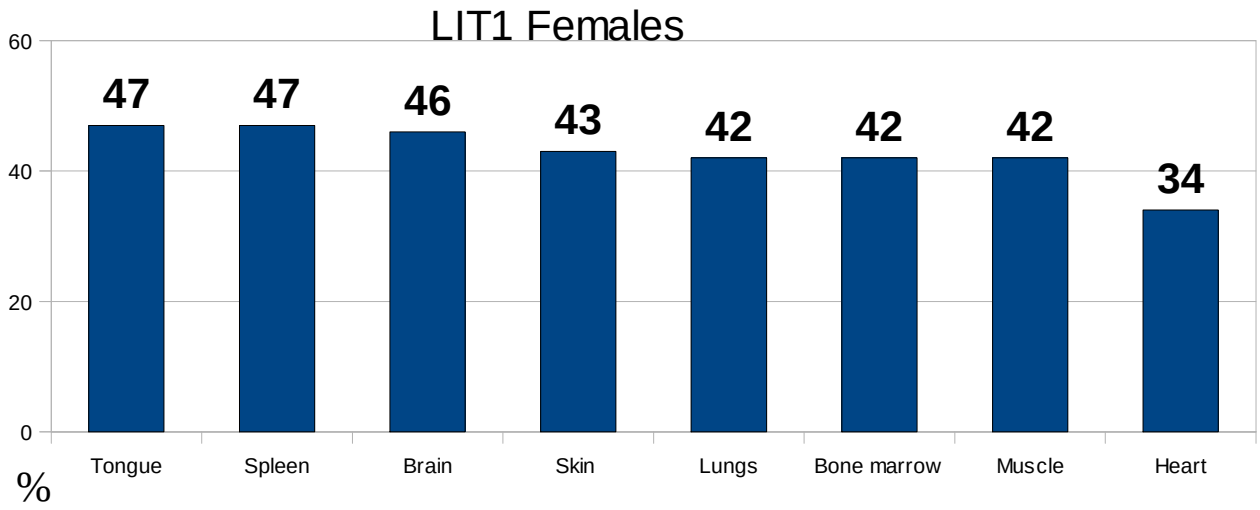
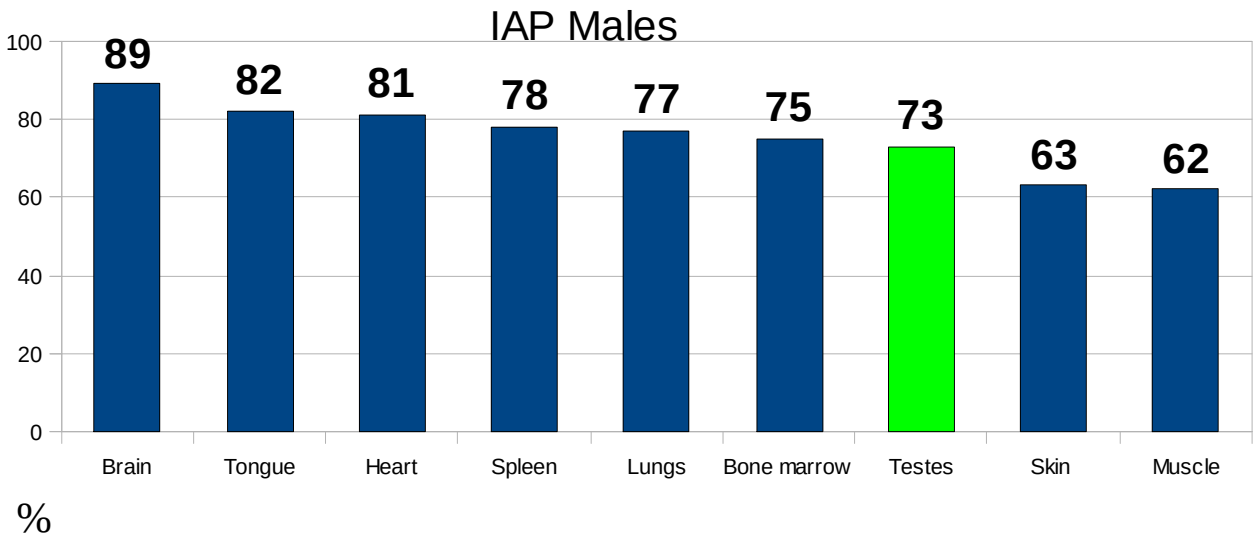


Fig. 22: Lit1: In males, most methylated in tongue (57 %), least methylated in heart muscle (34 %). In females, it is also most methylated in tongue (47 %), least in heart muscle (34 %). Low methylation in testis would again correlate with the imprinting status of this locus.

C)Repeats



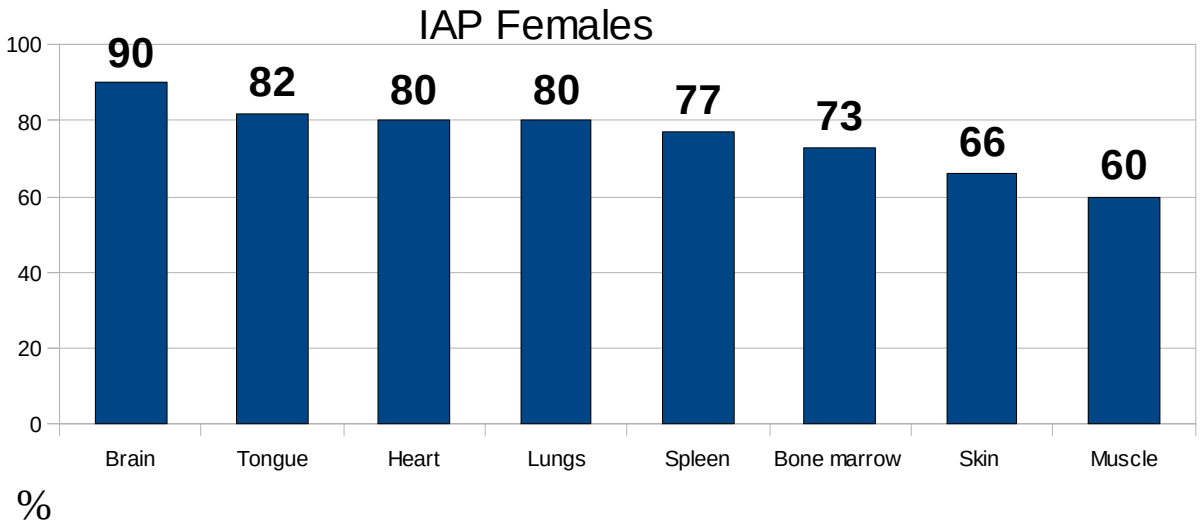


Fig. 23: Iap: highly methylated in most of the tissues analysed. In both males and females, most methylated in brain (89 % and 90 %, respectively), least methylated in skeletal muscle (62 % and 60 %).

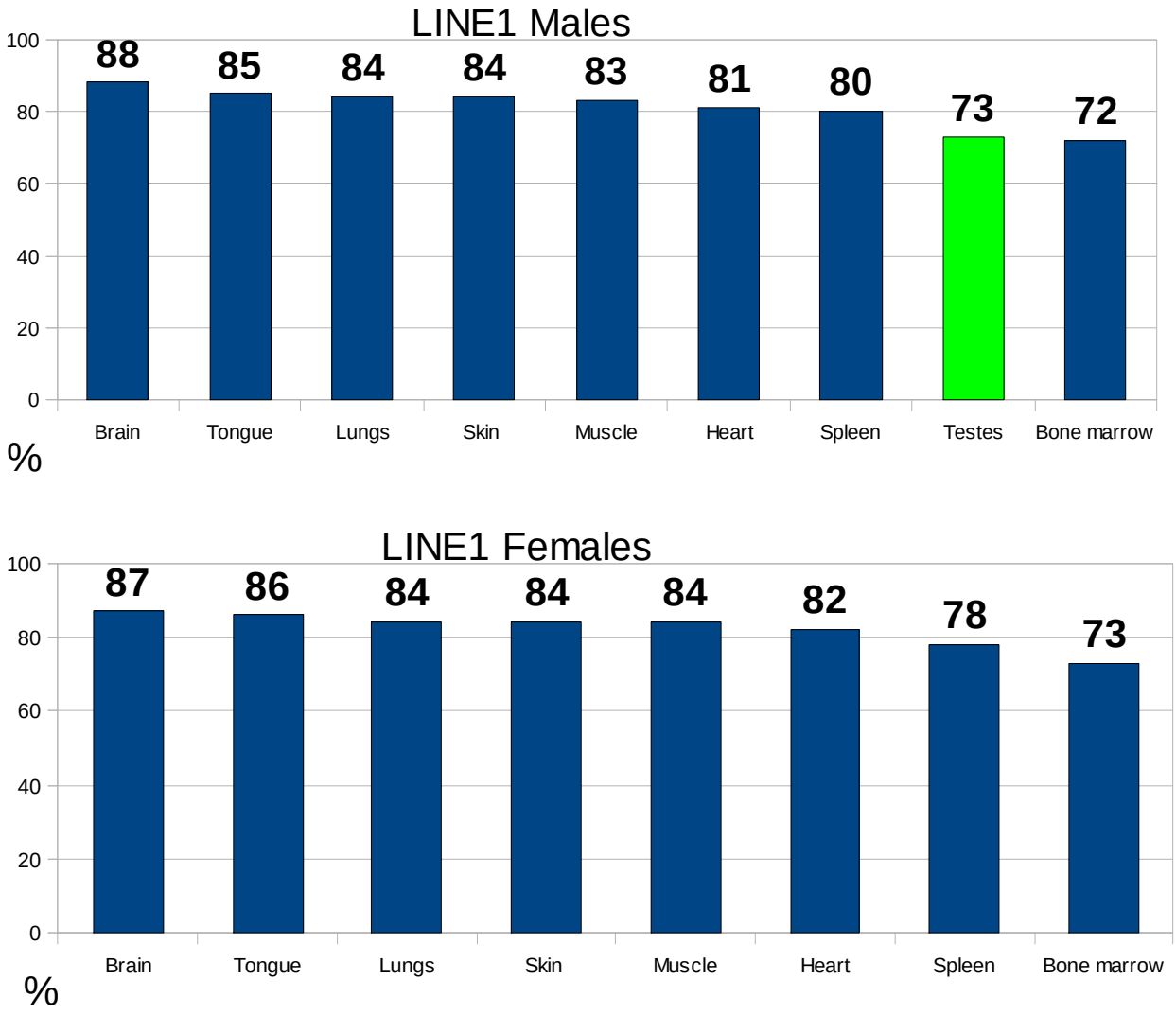


Fig. 24: LINE-1: In males, most methylated in brain (88 %), least methylated in bone marrow (72 %). In females, the same trend is observed, with brain at 87 %, and bone marrow at 73 %. It is to be expected that parasitic repeats are silenced to a significant degree in all tissues.

5.6 Correlation between methylation and RNA expression levels

Quantitative RNA expression analysis was done for the two single copy genes, alpha actin and myosin light chain, in 9 tissues. Liver and kidney have been additionally included in this section. Expression data is presented in the following section. These are different measurements than those presented in sections 4.4 and 4.5; they are, however, comparable. The results presented in the two graphs below are the averages of 3 mice.

A)Alpha actin

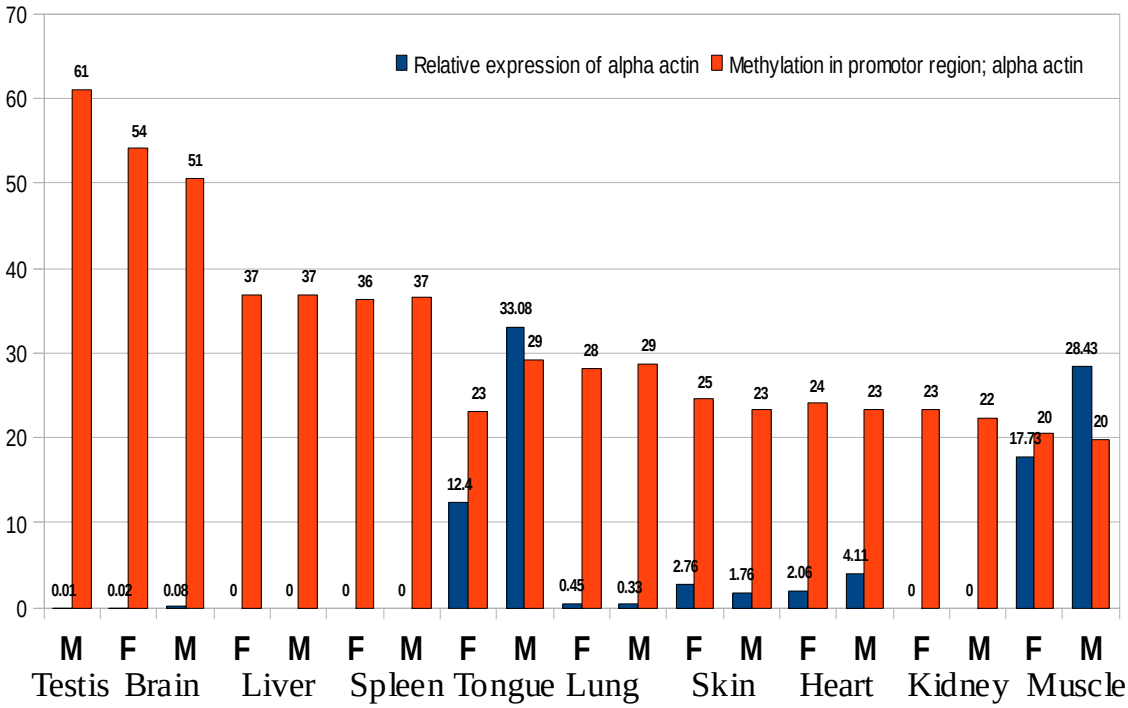


Fig. 25: Relative RNA expression intensity in a given tissue for alpha actin. Methylation is given in percent, expression is relative pyrosequecing data

These results show methylation and expression do not correlate directly. This may be due to the possibility that the expression switch may be programmed on a much larger scale and consist not only of DNA methylation, but also of other factors. Skeletal muscle (femoral and tongue muscles), however, show markedly higher expression; and also selectively lower methylation levels. In tissues with highest methylation levels, testis and brain, no detectable expression was observed. Males show relatively higher expresion in all tissues.

B) Myosin light chain.

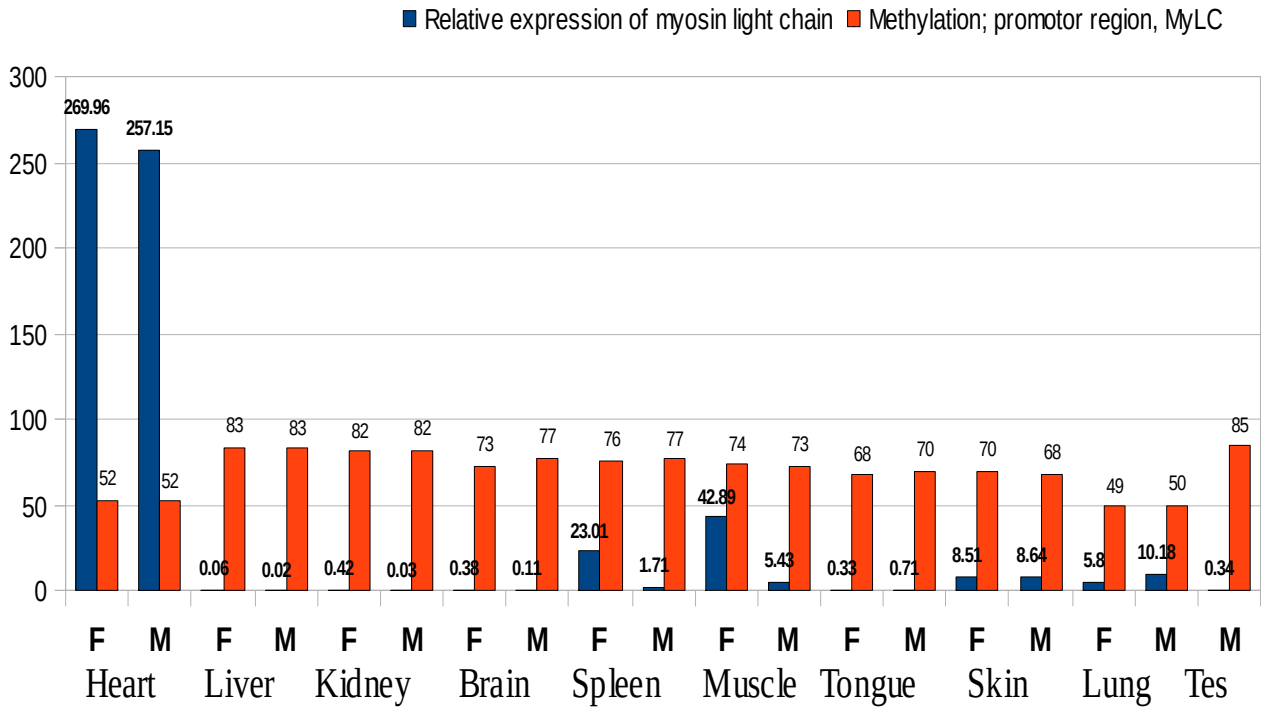


Fig 26: Relative RNA expression intensity in a given tissue for myosin light chain. . Methylation is given in percent, expression is relative pyrosequecing data

Myosin light chain is expressed at high levels in cardiac muscle. Once again, and as at the alpha actin locus, there is no strict correlation between methylation and expression of myosin light chain. The expression of various myosin isoforms and their methylation in heart may be considered more in-depth in the future, as the pattern observed markedly differs from that of other tissues. It is to be noted that highest expression was observed in cardiac muscle, which also shows relatively lower methylation levels.

5.7 Correlation of methylation between different loci within one tissue

Here, we measured DNA methylation values at 6 different loci in 9 different organs of about 100 male and female mice. The methylation data presented below was reanalysed to answer the question if correlation exists between different loci in a given tissue in the level of DNA methylation. For this purpose we used the SAS package program. Several instances of signifficant correlation have been observed; the detailed results are listed in appendix 3. However not all correlations were observed in both male and female group. We consider the strong 'true' correlations are the ones that are detected in both sexes as these are two different groups of samples that could serve as independent replicate for each other; this does not necessary mean that other correlations found in only one sex are not true, as gender specific effects cannot be excluded.

Bone Marrow:

In bone marrow, a strong positive correlation was observed between Myc and LINE-1 in both male and female samples (appendix II).

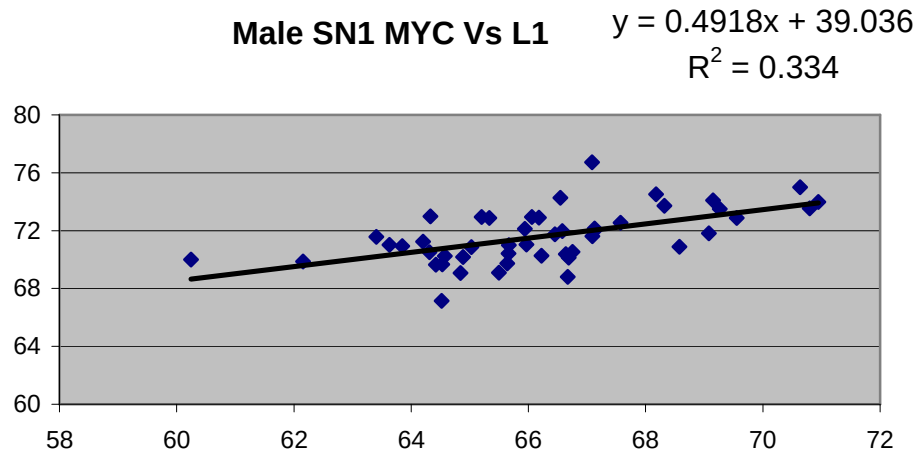


Fig 27: Scatter plot of correlation between SN1 in myosin ligt chain and long interspersed elements in male mice bone marrow, y = 0.4918x+39.036 and R2 = 0.334

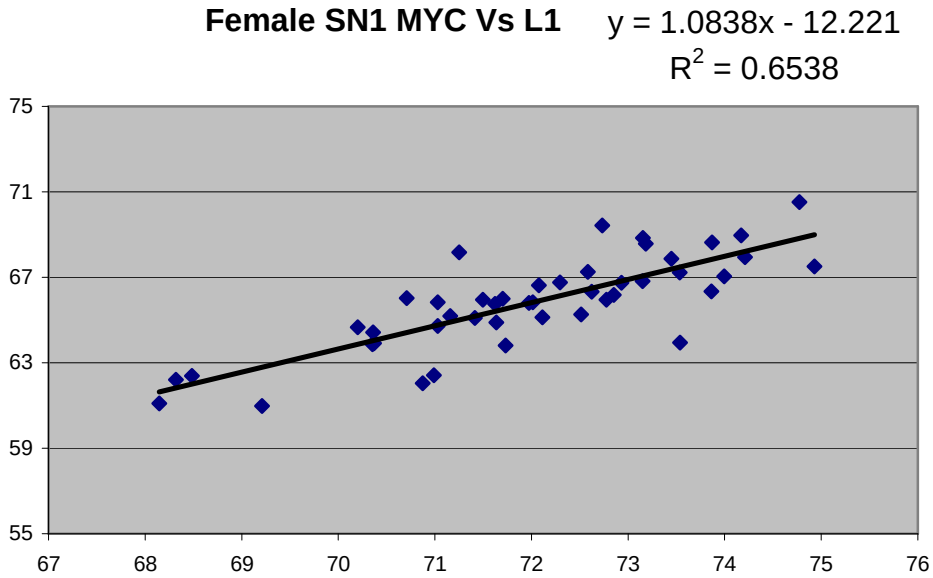


Fig 28: Scatter plot of correlation between SN1 in myosin ligt chain and long interspersed elements in female mice bone marrow, y = 1.0838x-12.221, R2=0.6538

Heart:

Significant correlation was observed in males between Peg3 and Snrpn-D1 loci (at the second CpG: SN2); A positive significance between the same two loci was observed in female samples.

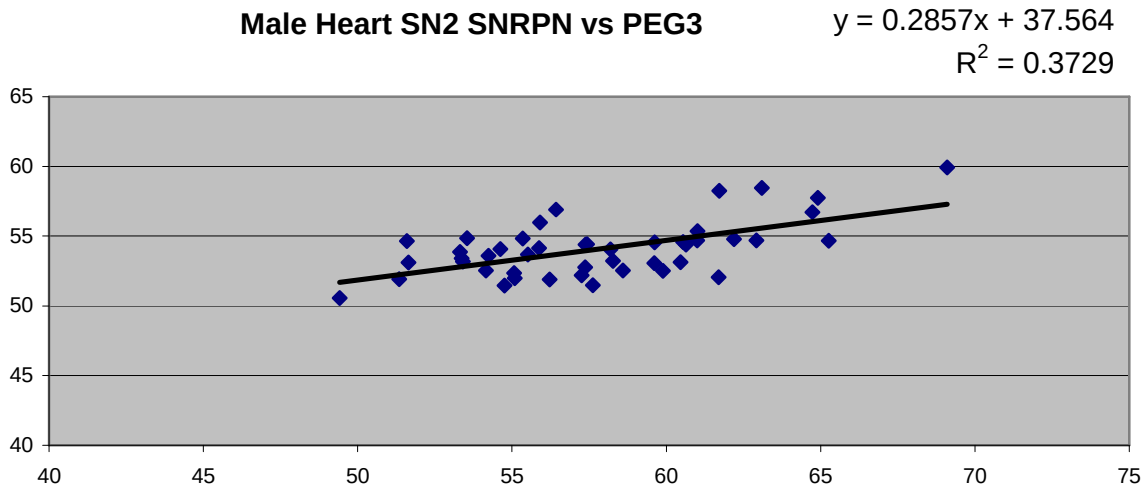


Fig 29: Scatter plot of correlation between SN2 in small nuclear ribonucleoprotein polypeptide differentially methylated region 1, and paternally expressed gene 3 in male mice heart, y = 0.2857x+37.564, R2 = 0.3729

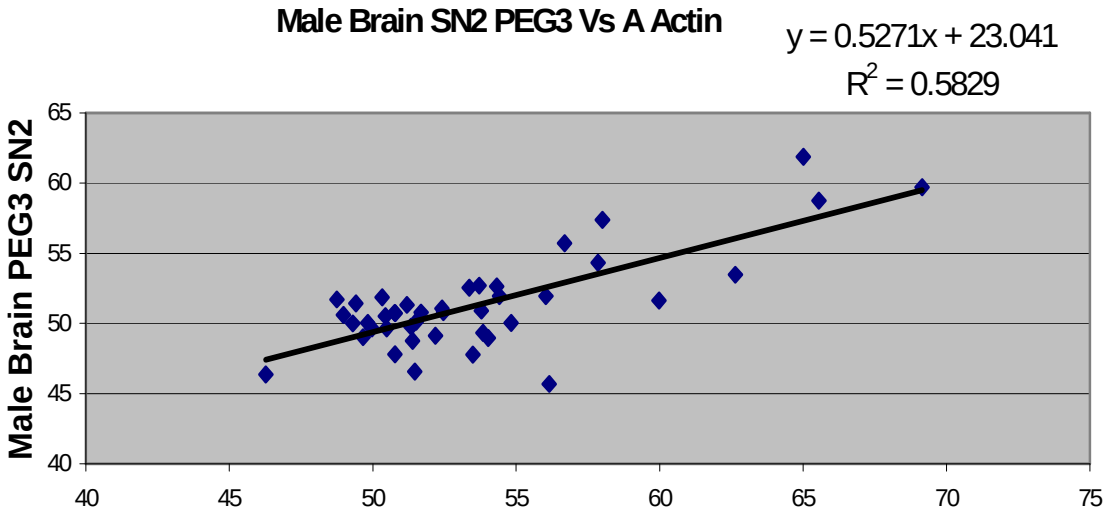


Fig 30: Scatter plot of correlation between SN2 in small nuclear ribonucleoprotein polypeptide differentially methylated region 1, and paternally expressed gene 3 in female mice heart, y =0.2758x+37.824, R2=0.2665

Brain:

In brain tissue, significant correlation could be observed between alpha actin and Peg3 at the second CpG site in both males and females. Similarly significant positive correlation was observed between Peg3 and Snrpn-D1 at the first CpG site (SN1).

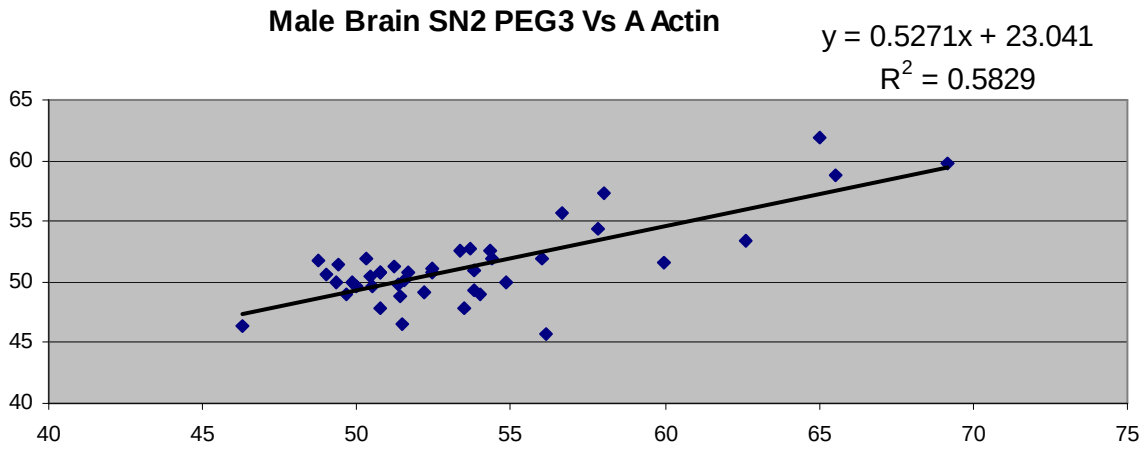


Fig 31: Scatter plot of correlation between SN2 in paternally expressed gene 3 and alpha actin in male mice brain, y = 0.5271x+23.041, R2=0.5829

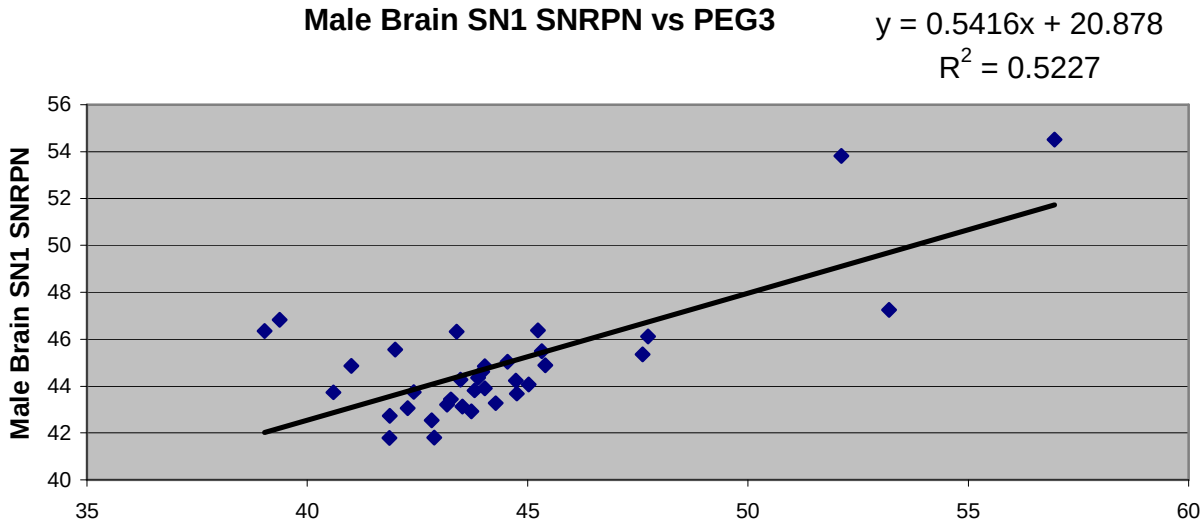


Fig 32: Scatter plot of correlation between SN2 in paternally expressed gene 3 and alpha actin in male mice brain, y =0.5416x+20.878, R2=0.5227

Spleen: I

In spleen tissue samples, the highest degree of correlation was observed simultaneously in males and females. The correlation showed positive significance and are as follows: between Myc and IAP at first CpG site; between Myc and LINE-1 at both studied CpGs; between Snrpn-D1 and IAP at first CpG; between Snrpn-D1 and LINE-1 at first CpG and finally between IAP and LINE-1 at the first CpG.

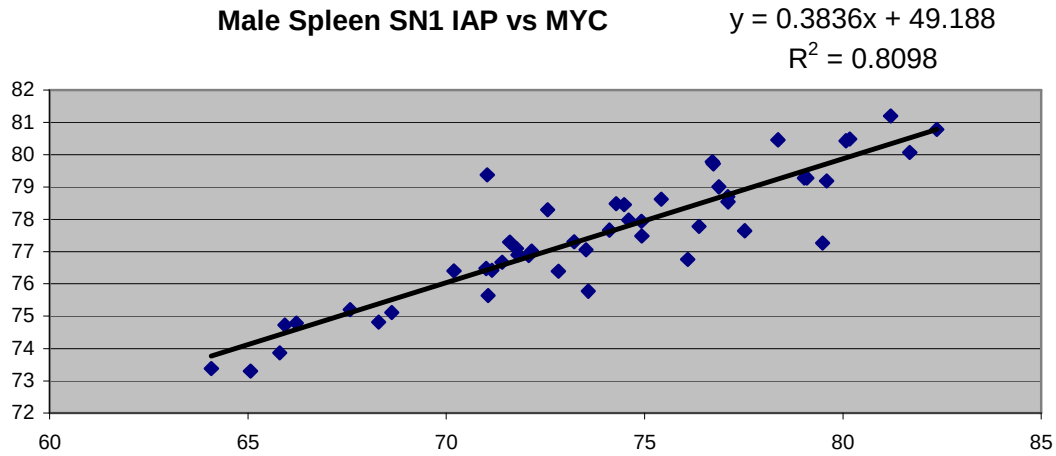


Fig 33: Scatter plot of correlation between SN1 in intracisternal A particle gene and myosin light chain in male mice spleen, y=0.3836x+49.188, R2=0.8098

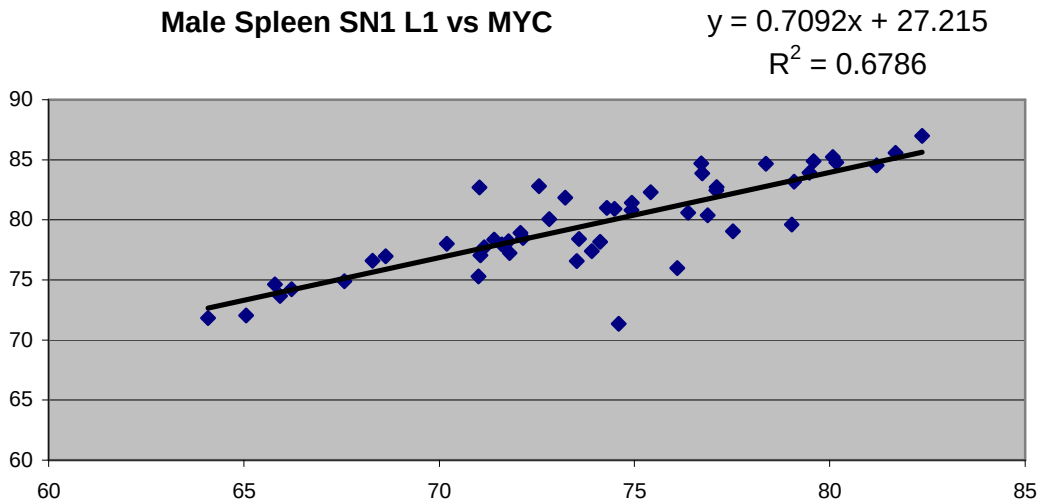


Fig 34: Scatter plot of correlation between SN1 in long interspersed elements and myosin light chain in male mice spleen, y=0.7092x+27.215, R2=0.6786

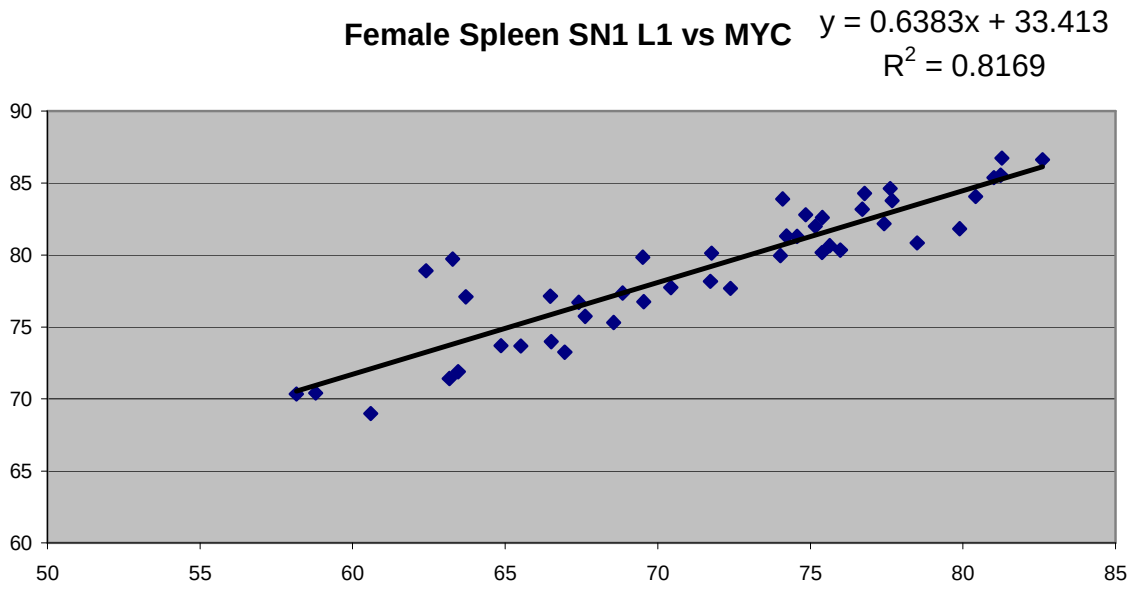


Fig 35: Scatter plot of correlation between SN1 in long interspersed elements and myosin light chain in female mice spleen, y=0.6383x+33.413, R2=0.8169

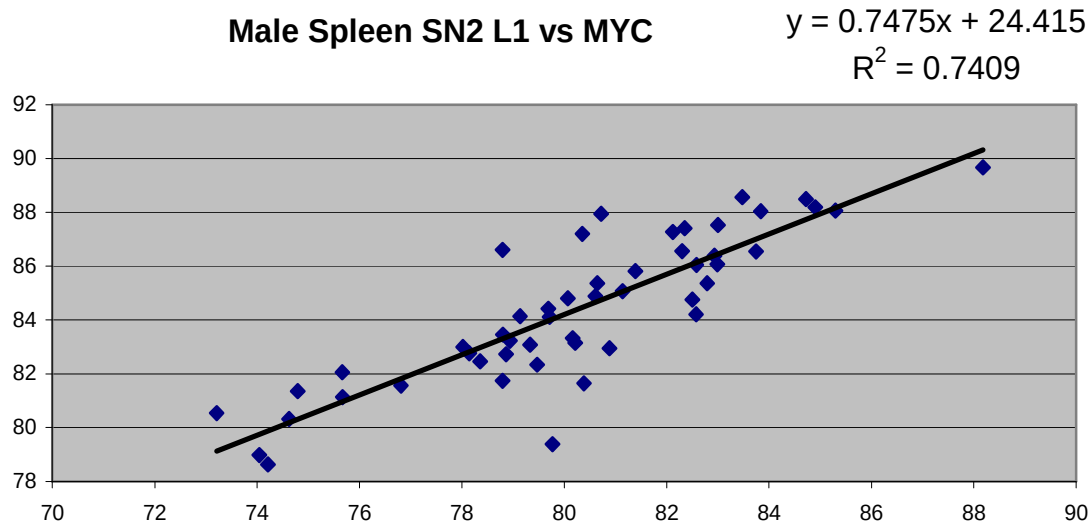


Fig 36: Scatter plot of correlation between SN2 in long interspersed elements and myosin light chain in male mice spleen, y=0.7475x+24.415, R2=0.7409

6 Discussion

Recent methylome studies have shown that DNA methylation exists not only in the CpG context. This is especially true for pluripotent cells (Goodson and Hawse, 2002). However, in this presented work, only methylation in CpG content was investigated. The material consisted of differentiated tissue, with the partial exception of testes, and only CG sites were specifically analyzed in the used approach.

There are differences between somatic tissues. They are comprised of varying proportions of cells in various differentiation states, so that the results are tissue specific patterns of expression and also tissue specific DNA methylation. The questions we raise here are: 1) are there methylation differences between these somatic tissues that are gender specific? 2) Are there methylation differences at CpG sites between different somatic tissues in the same gender? What we are presenting here is a relatively small study as both the number of animals and of gene fragments investigated are limited. However, it comprises genes of some key traits: structural protein encoding genes (myosin and alpha actin), imprinted genes (PEG3, SnrpnD1 and Lit1), and two repetitive elements (LINE-1 and IAP) that are used as surrogate methylation markers to reflect on the overall genome methylation trends.

A) Structural protein encoding genes

Myosin is the main protein in the contractile apparatus. A myosin molecule is composed of two heavy and two light chains. Proportions and sort of the heavy and light chain isoforms define the mechanical velocity and power effect of the muscle. The light chains have a molecular weight of 17 to 21 kDa. Both heavy and light chains are subject to tissue and developmental stage specific isoform changes. There are multiple myosin light chain isoforms, resulting from alternative splicing of its gene and present in various proportions in smooth, skeletal and cardiac muscle; different types of body tissues express different myosin isoforms dependent on their function. The myosin region investigated in this study is the murine MLC1V gene for myosin alkali light chain, exon 1,

a ventricular/slow muscle isoform (Feng 2008; Leoni et al., 2005; Redowicz 2002).
The CpG sites investigated were located after nucleotide positions 1406 and 1480, accession n.X12972 (coding sequence starts at 1365, mRNA at 1298). Initial data indicated males being more methylated in lungs, brain, tongue, and heart. However, after Bonferoni correction for multiple testing, which was done for the first CpG site in every region studied, none of them were significant enough (Table 5). These, then, are but trends, limited by the size of the study.

The difference in heart and tongue between sexes (Tab.5-7) seems clear enough, as these are both muscle tissue dominated organs and both consist of striated muscle. We can well assume that there is a sex dependent difference, as androgen concentration affects myosin subtype predominance, and possibly enacts quantitative changes in the musculature (Redowicz 2002). The difference may exist, however, it may be necessary to discern between individual muscle fiber subtypes to be able to see a more detailed DNA methylation picture. This picture should be dynamic, showing what exactly happens to this methylation as individual cells progress from totipotency to the fully differentiated muscle fibers.

We see there that lungs use myosin in cilia movement. It could well be that there are sex dependent differences in the respiratory pathway epithelium structure, only they were not to be observed with a given method and limited investigated regions. We also have to take into account the heterogeneity of the cells in the probe, not only epithelial. Assuming that CpG methylation reflects repression, muscles responsible for epithelial cilia motion may express this particular myosin isoform. Lungs do stand out between other tissues in myosin, with their value of just 47 %, while other tissues varies between 65 % and 75 % (again, see comparison on page 58).

In addition to the gender differences, the tissues also exhibit distinct differences in myosin methylation. Considering that methylation at these promoter regions is inversely correlated with expression, one would expect higher methylation in tissues where myosin would be less needed. Surprisingly, the least methylation is observed in lungs, while one would expect them to be so in striated muscle dominated tissue, such as

skeletal muscle, heart, and tongue. A gap of ca. 20 % in methylation separates lungs from these tissues. The presence of cardiac, skeletal and tongue muscle on the less methylated side is not surprising. The lungs raise questions in this context-whether this CpG methylation is an isolated random finding, or whether a particular myosin subtype is more actively expressed in the lung, again, possibly in the ciliated epithelial surfaces, as asserted by previous works (Feng 2008). There is also a notable difference between skeletal and cardiac muscle, the latter being almost 20 % less methylated than the former. One has also to consider that methylation at the specific investigated CpGs may not be directly linked to the expression as would be expected.

Summarizing the results, we may say that there are methylation differences in this housekeeping gene, not as evident between sexes, but quite distinct between tissues. For greater functional significance, these results need to be complemented with studies involving a larger sample number, and wider evaluation of larger sequence, not limited to methylation at few CpG sites. A more accurate picture may be possibly obtained by studying not only complete tissues, but also their individual homogeneous sub-cellular populations. These could then possibly be differentiated by expression of specific proteins, in conjunction with their methylation assessment.

Alpha actin gene encodes a small compact protein of around 43 kDa. CpG sites investigated were located directly after nucleotide positions 441 and 529, accession n. M12347 (coding sequence starts at 1785, mRNA at 754). In muscles, actin is the rail on which myosin complexes move. In non-muscle cells, it has a variety of functions, including support and stabilization of nuclear matrix and lamina and chromatin remodeling (McGrath and Solter 1983). Actin can adopt a great number of forms, as filaments, sheets, or tubes, which makes it the scaffold of many cellular processes such as transcription, chromatin formation, cellular shape, motility, and adhesion. It is also phylogenetically very old, being found in comparable forms among such long diverged species as mammals and fungi (Derks et al., 2008).

In alpha actin, raw data showed a tendency towards greater methylation in males in lungs, tongue, and muscle, while females tended to be more methylated in brain,

although this was only seen in one of the two investigated sites (Tab.5-7). After statistical analysis and Bonferoni correction for multiple testing, only the difference in tongue remained significant. (Tab.5-7). RNA expression analysis in alpha actin revealed that in those fragments where its promoter was comparatively less methylated, there was also higher expression, such as tongue and skeletal muscle, whereas stronger methylation correlated with less expression (for example, brain; see alpha actin, brain, primer/site 2 in Tab. 5-7).

We may assume, keeping in line with the hypothesis that greater methylation means less expression, that there are structural differences between male and female tongue muscle. These may be related to the male hormone induced muscle quantitative differences (Redowicz 2002). Though not confirmed, the brain methylation trend remains interesting, showing the possibility of structural differences in the cellular composition of the brain between sexes. This data could have been influenced, as in other tissues, by percentage distribution of different types of cells in the tissue sample. In the brain, this would be for example glial and vascular cells. The difference therefore could be a product of different proportions of these cells between the sexes, while the qualitative cell structure could be identical. Other studies have shown that expression of housekeeping genes, among them also an actin subtype, differs depending on brain area sampled (Wolfrum et al., 1998). Thus, greater uniformity, along with increased accuracy, is needed in the sampling to come to conclusions (Wolfrum et al., 1998). In our work, though brain area sampled was random, there is a trend towards general difference between the sexes.

As we set the sex differences aside and only consider the differences between tissues, the differences of methylation at given sites may be as high as 40 % between testis and skeletal muscle, or 30 % between skeletal muscle and brain, as seen in graph of section 4.5, methylation variability, part c, repeats, on page 62. These results do show, as expected, less methylation and would predict greater expression activity of actin in muscle tissues, such as heart, skeletal muscle and tongue. The skin may fit in this context due to its mechanical properties, which may be traced to actin at the molecular level, being a very basic structural protein.

B) Imprinted genes

In mammals, most autosomal genes are expressed from both paternal and maternal alleles. Imprinting is the process whereby one of the two inherited copies is repressed and the other stays active. This has first been shown in mouse nuclear transfer experiments (Bestor and Bourc'his 2006; Surani and Barton 1983), which showed that same genes might behave differently depending upon their parental origin. (Doerfler 2005; Otsuka et al., 2009). Imprinted genes are involved in controlled growth restriction processes during fetal development, where they are especially important in placental development, and growth in general. Of roughly 30.000 genes expressed in humans, an estimated 74 are imprinted (Ying-Chun et al., 2006).
The functions associated with imprinting to date include tumor suppression, brain development and apoptosis (El-Maarri et al., 2005; Ying-Chun et al., 2006), nurturing behavior and maternal resource availability in mammals (Dvo Kim et al., 2007).

PEG3; paternally expressed gene 3. Two CpG sites in exon 1 were investigated, at nucleotide positions 2835 and 2922 (accession n. AF105262, mRNA starts at 2803). PEG3 consists of nine exons and is ca. 26 kb long. The gene is located on mouse proximal chromosome 7, its human homologue on human chromosome 19. It encodes a zinc finger protein, and some experiments indicate its role as a tumour suppressor (Lucifero et al., 2002). The gene is expressed in mesodermal tissues of early somites, and later in the gut and hypothalamus (Kuroiwa et al., 2009). Later development shows ubiquitous expression in tissues, with predominant expression in brain, testis, ovary and placenta. (El-Maarri et al., 2005). PEG3 has been implicated in regulating development of some brain parts through apoptotic pathways (Broad et al., 2009). PEG3 deficient mice are growth impaired (Meyne and Legator 1980), which also fits to its involvement in numerous cancer lines, including endometrial, ovarial, cervical cancers and glioma (El-Maarri et al., 2005).

Initial trend showed greater methylation in males in skin, tongue, and muscles, while females were more methylated in brain (procentual data plots, page 59). Statistical analysis after Bonferoni correction left males to be significantly more methylated in skin

and tongue (Tab.5-7). Concerning the inter-tissue differences and in accordance to previous works, testes showed less methylation and thus possibly greater expression (El-Maarri et al., 2005); the methylation results from brain tissue, however, were not much different from those of other tissues, for example, the difference between brain, muscle, heart and lung was less than 10 % in the data plot on page 59, while the difference between brain and testes was over 20 %.

Snrpn D1; small nuclear ribonucleoprotein N gene differentially methylated region 1. We analyzed two CpG positions in this gene, nucleotide positions 57858 and 57934 (accession n.AF332579, coding sequence starts at 68049, mRNA at 67979). Initial trend indicated greater methylation in males in lungs, tongue, and heart, while females leaned toward greater methylation in spleen and bone marrow. Data after Bonferoni correction showed males were more methylated in the CpG sites at both positions in lungs and tongue, and less methylated at position 57858 in bone marrow (Table 5). In males, skeletal muscle is the most methylated tissue (65 %), spleen and bone marrow are least methylated (42 %). In females, skeletal muscle is most (66 %), and bone marrow least methylated (32 %). Testis showed very low methylation levels, as imprinting leaves this locus unmethylated in spermatozoa. Overall impression is that skeletal muscle tissue is relatively highly methylated with over 60 % at both loci observed, testes is strongly hypomethylated, with next relatively low methylation tissue being the spleen, and other tissues are relatively even at about 45 %.

The gene is found on chromosome 7 in mouse (Chromosome 15 in humans, (Glenn et al., 1996)). It encodes the Snrpn protein involved in RNA splicing (Rodriguez-Jato et al., 2005). The core gene has 10 exons, transcribes into a 1.4 kb mRNA. Exons 1-3 encode the SNURF protein, 4-10 encode the Snrpn splicosomal protein. Besides the two proteins, the gene also contains a ca. 460 kb long RNA transcript, which results in numerous small RNAs due to variuos splicing. (Suzuki et al., 2009). The Snrpn region contains two alternative upstream promoters and some non-coding exons. There are multiple untranslated upstream exons of unknown function and at least two alternative 5' start sites (Glenn et al., 1997). The sequence is also host to multiple small nucleolar RNAs encoded in the region. The protein has a direct impact on the synthesis of brain

proteins, particularly those that function in the hypothalamus, due to its splicing function (Feinberg 2007). The gene contains two differentially methylated regions and 23 known methylation sites. The first DMR includes part of the promoter and transcription start site, and is postulated to inherit a maternal-specific imprint.

The function of the gene is complex. Experimental deletion data from mouse model shows that a deletion from SNRPN to UBE3A causes hypotonia, growth retardation and partial lethality (Glenn et al., 1997). Loss of these small nucleolar RNAs contributes to Prader-Willi syndrome. Their target molecules remain unknown. SNRPN transcription sense and adhering UBE3A (linked to Angelman syndrome) antisense units serve as host genes for small nucleolar RNAs encoded within them (Glenn et al., 1997).

Lit1, The genomic region of Lit1 corresponds to a highly conserved region between mouse and human. It is an imprinted locus, participating in the expression regulation of KCNQ1a voltage-gated potassium channel protein. CpGs investigated in this region were located at nucleotide positions 45216 and 45348, accession n.AJ271885. Initial tendency was greater mehylation in males in spleen, lungs, skin, and tongue, while females were more methylated in brain and bone marrow. After Bonferoni correction, males remained more methylated in lungs, tongue and skin, less methylated in brain and bone marrow.

LIT1, or long QT intron transcript, codes for an antisense RNA in the region of KvLQT1, a gene encoding a voltage-gated potassium channel. It is transcribed in the opposite direction to the channel gene (Fig.1) The channel gene is about 300 kb long and is expressed from the maternal allele. (Fig.2) The heart is an exception to this, as both paternal and maternal gene copies are expressed (Gaston et al., 2001). Another interesting observation is that in described cases of loss of imprinting of LIT1, demethylation of the maternal allele was always complete. There was also no tumour risk associated with demethylation of LIT1 only, where neighbouring imprinting centres were not affected. However, in tumour tissues, mosaic demethylation patterns have been observed (DeBaun et al., 2002). Our data possibly indicates a more active metabolism in the male brain and bone marrow, pointing to a possible more active

production of the said potassium channel in these tissues. Slight, yet statistically reliable difference in skin and lungs can indicate a slower cellular change rate there in the males, or simply a different cell/connective tissue ratio in the probes. This pilot work provides limited data for few methylation sites of each studied gene, which makes it difficult to reach definitive conclusions.

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difference in skin and lungs can indicate a slower cellular change rate there in the males, or simply a different cell/connective tissue ratio in the probes. This pilot work provides limited data for few methylation sites of each studied gene, which makes it difficult to reach definitive conclusions.

LIT1 is transcribed from centromere to telomere and is expressed from the paternal chromosome. In most patients of BWS, LIT1 expression is abnormal from both alleles, while some demonstrate biallelic expression, indicating relaxation of imprinting (Beckwith 1963). Loss of imprinting in LIT1 correlated completely with its biallelic expression. It is not the only, but the most frequent genetic alteration in BWS, making it a key component in the pathogenesis.

LIT1 methylation abnormalities have been associated with overgrowth and birth defects (Arima et al., 2005). Moreover, other pathologies based on the potassium channel defects are the long QT syndrome, a cardiac muscle defect inherited in a dominant way, and Jervell-Lange-Nielson syndrome, characterized by deafness and cardiac conduction abnormalities (Yatsuki et al., 2000). Another described pathological feature is the midline abdominal wall defect with a frequency of 69 % in patients with abnormal LIT1 methylation (Higashimoto et al., 2003). All this could be related to the LIT1 being in the proximity of a potassium ion channel gene, and the cardiac conductive system running through the septum. Our work also showed LIT1 to be less methylated in cardiac muscle most likely due to more active expression of the region due to its role in ion conduction. LIT1 methylation in BWS patients is also reliably associated with macrosomia (53 % are associated while only 18 % are not)– as potassium channels are some of the more elementary molecular blocks in all cells, their function changes would affect most other cellular processes down the line, which rely on osmosis and ion concentrations for the proper conformation and function of their components.
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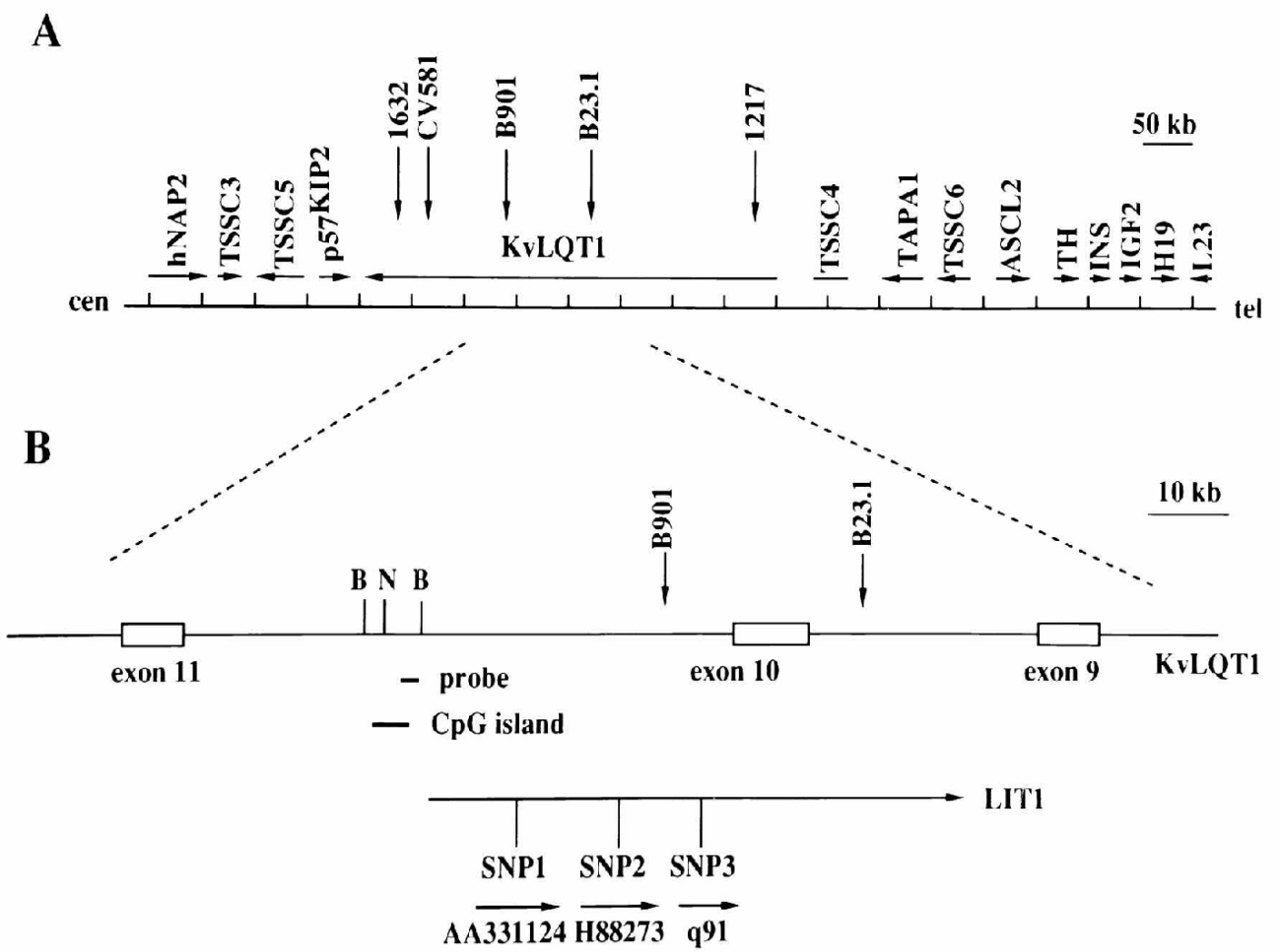

Fig. 37: Location of LIT1 within KvLQT1 (Lister et al., 2000)

C) Repetitive elements

IAP, intracisternal A particle gag protein gene, CpG sites at nucleotide positions 170 and 206 (coding sequence starts at 594), accession n. M17551. Raw data suggested males were more methylated in spleen and bone marrow, less methylated in lungs, skin, brain, and heart. After Bonferoni correction, males remained more methylated in bone marrow, less in lungs and skin (Table 5). The general impression is that as a once active retrovirus, IAP is relatively highly methylated in most tissues, which is not much dependent on the sex of the individual. Comparing to other fragments, such as Snrpn and Lit1, where methylation is mostly under 50 %, IAP reaches heights of well over 70 %, and, in brain, even 90 %.

The mouse genome has up to 1000 copies of IAP. The IAP sequence itself is about 7 kb long (Mays-Hoopes et al., 1983). Although the particle is in some aspects similar to retroviruses (they are collinear with the retroviral RNA and have long terminal repeats), there is no known extracellular form, i.e. no free virus. Therefore, both the version of intracellular evolution to present status and an infectious entry in the past remains possible. The IAP genes present in the mouse genome are not identical, though they are highly homologous.

An experiment as early as 1983 (Feenstra et al., 1986) has shown that the CCGG sequences in the IAP gene are methylated in most tissues (CmCGG) and unmethylated in myeloma, where it is expressed (CCGG), although even myeloma cells do not show full demethylation. Plasmocytoma in the mouse also shows higher IAP expression than other lymphocytes (Dupresoir and Heidmann, 1996). This has little direct relation to our work, as the individuals investigated had no known tumors. IAP is, just as other elements, markedly demethylated in testes. Again, this is proof of epigenetic reprogramming in germ cells and also a link to its possible positive function as a driving factor behind mutagenesis. It would be interesting to see mouse ovary in comparison, whether its results would be similar to testes or whether a lower number of reprogrammed cells would lead to a high methylation levels characteristic of somatic tissues.

Due to the functional properties of the repeat, it can be inactivated by methylation of a few sites; this has been shown in a 1986 experiment (Falzon and Kuff, 1991). Further work indicated that methylation of specific sites had a summation effect in the IAP inactivation. These sites were located in the protein bonding DNAse and endonuclease domains (Lueders et al., 1993).

Due to the nature of the IAP particles, their transcription level controls their transposition, as there is a RNA intermediate. It has also been hypothesized that IAP can become randomly active and cause mutagenesis at the germ cell level during the demethylation waves in their maturation, while staying relatively silent in the grown-up organism (Argeson et al., 1996). This silence is then again in accord with our data

showing high IAP methylation in all examined tissues. IAP expression directly depends on the cellular proliferation rate. This could explain the high IAP methylation in brain and the striking difference between testis and all other tissues. It does not explain well the relatively high methylation levels in kidney and liver, especially the later, which is known for its cellular regeneration abilities. In the testis, IAP expression is limited to specific developmental stages and diminish with further differentiation; the pattern repeated itself over multiple generations and thus seems to be stable (Argeson et al., 1996). Off-time and cell type IAP CpG methylation could be successfully induced with 5-azacytidine, a demethylating agent. The ability of IAP to be activated in specific developmental stages of germ cells might indicate its function, which once a retrovirus acquired to coexist with the host genome, giving it a possibility to generate heritable genetic diversity. Normally, the transposable elements are strictly methylated and their expression either physiologically limited to the given germ cell stages or only achievable by demethylating agents or direct gene manipulation (Gaudet et al., 2004). Demethylating agents in themselves represent a rather clumsy tool, as much more than a single specific target locus is affected. In this, further analysis, understanding and development of exact molecular tools are necessary.

LINE-1: Long interspersed elements (Lines, or LINE-1) are endogenous mobile genetic elements. The CpG sites investigated were located at nucleotide positions 965 and 1001 (coding sequence starts at 1343), accession n. D84391. Initial results showed males to be more methylated in brain, females in bone marrow, tongue, and heart. After Bonferoni correction, difference remained reliable in tongue and cardiac muscle, both for Primer 1 (Table 5). Overall impression is that these elements are strongly inhibited even in tissues where demethylation is genome-wide and systematic, testis reaching a level of over 70 %, more than any other element in our study.

A full LINE-1 element is about 6 kb long, contains two ORF's, first encoding a RNA – binding protein, and second of them encoding a reverse transcriptase (Florl et al., 1999, Takai et al., 2000). ORF1 is a RNA-binding protein functioning as a nucleic acid chaperone; ORF2 is a reverse transcriptase and endonuclease (Cotton et al., 2009). LINE-1 element has a CpG – rich promoter at its 5' end.

Line-1s have accumulated in the eukaryote genome via germline transposition. About 17

% of the human genome consists of LINE-1's, counting roughly 516000 copies in haploid genome, thus making it a very convenient tool for evaluation of quantitative genome methylation at a given site, organ or cell group (R.Scott Hansen, 2003). Greatest portion of them are degenerate and non-functional. Some 30 to 100 of these elements, having assessed their completeness and methylation status, could be active (Takai et al., 2000, Tsutsumi, 2000). Even elements that have lost both reading frame expressional capacity may still function as possible promoter sites, altering the function of genes around them. Again, our data gives only indirect hints to this, as all individuals studied had no known pathological conditions. Only in testes, where the hottest DNA changes of all studied tissues were taking place, was LINE-1 significantly less methylated than in other tissues. This could indirectly be compared to the rapid cellular multiplication in tumors. Microscopic examination showed that uncontrolled LINE-1 transposition results in unstable branched DNA structure (Takai et al., 2000). Active LINE-1s are transcribed into RNA, then reverse-transcribed into cDNA and integrated at another location.

If promoter methylation contributes to the silencing of expression, as seen in previous examples of IAP, this would fit the general higher methylation of repetitive elements. What is interesting is also a statistically reliable sex difference in the methylation of different fragments in the tongue, which could be confirmed despite a rather small population sample. Therefore, we confirmed that differences between sexes exist at given loci. As methylation is not the only epigenetic regulation mechanism and the number of CpG sites investigated was limited, we can only say that differences exist, and leave their exact mapping to further studies.

On the carcinogenic potential of LINE-1: consistent diet deficient in methyl group donors, or choline deficient diet, has been shown to be linked to LINE-1-hypomethylation and the induction of hepatic cancer in rodent experiments (Oki et al., 2008). LINE-1 hypomethylation has been observed as an early change preceding elimination of cancer cell clones in leukemia, a study testing demethylating agent decitabine (Choi et al., 2007). LINE-1 elements have been shown to be transcribed in human teratocarcinoma

cells and to be significantly hypomethylated in urothelial carcinoma cells, with higher-grade tumors displaying a trend towards a more complete demethylation. LINE-1 demethylation has been proved in hepatocellular carcinomas, while it was absent in surrounding liver cirrhosis (Tsutsumi, 2000).

LINE-1 is less methylated in neuroendocrine tumours ($p=0.04$); it was more pronounced in carcinoid than in neuroendocrine tumors. There also were tissue dependent variations, for example, showing that haematologic neoplasia tends to be less hypomethylated than intestinal (Ostertag et al., 2007). The only abdominal organ present was spleen. It does show a tendency towards relatively less methylation, when compared to other tissues (see Line-1 methylation graph under 4.5, Methylation variability at specific loci in different organs). In intestinal neoplasia, LINE-1 hypomethylation is associated with shorter survival times among patients with colon cancer (Srinivasan et al., 2008). Possible mechanisms of this are the genomic instability associated with global genome hypomethylation, transcriptional instability, activation of oncogenes, dormant endogenous viruses and mobile elements, also inflammatory mediators and increased oxidative stress (Srinivasan et al., 2008).
In prostate cancer, LINE-1 hypomethylation has been demonstrated to occur in the early stages of tumoral development, while global hypomethylation followed at a much later, metastatic disease stage, along with even more pronounced LINE-1 hypomethylation (Figueiredo et al., 2009). This insinuates a role of LINE-1 hypomethylation as a possible trigger event. In bladder tumours, hypomethylation of a certain LINE-1 promoter has been proved to induce the transcription of MET-oncogene (a hepatocyte growth factor receptor) in tumour itself and the entire urothelium of the affected bladder (Kuff et al., 1981). This gene is known to exist in a few truncated forms, being active in breast, prostate, colorectal and lung carcinomas. Furthermore, specific LINE-1 element can be targeted by placing one amplification promoter in the element and another in a targeted gene (Kuff et al., 1981). Figures 3 and 4.

Summary on tissue and sex influence on methylation

The end result showed that most of the fragments were less methylated in testes; this coincides with the intensive expression there due to the high cell replication rate. We took the complete organ; were it possible to separate the germ cell population only, these results would be even more different from those of other organs. Brain, where cell division happens at a much slower tempo, displayed higher methylation in most fragments. What we see though is mixture of different cells type like glial and neural tissues together. Neurons and glia would likely demonstrate a different methylation pattern if studied separately.

A good functional correlation can also be seen with alpha actin and muscular tissue; its promoter is comparatively less methylated in tongue, skeletal muscle and heart, where one would also await higher expression. Since these tissue specific differences exists at most loci, it was possible by principle component analysis (using these limited studied loci) to clearly separate most tissues in tissue specific clusters, in both males and females, (figures 18 A and B).

In general, sex has a clear influence on methylation, males appear to be more methylated in muscular tissue, for unclear reasons statistically reliably so only in the tongue, and less methylated in bone marrow. Male hematopoesis may be generally faster than female in mice, which would then correlate with this. Lungs and skin may have to do with different expression levels, male skin being structurally more ”rugged” and lungs having greater volume, and probably slightly different mechanical properties, than their female counterparts. Brain methylation differences between sexes need to be considered in more anatomical and molecular detail. Although differences exist, two CpG sites of one gene are much too little ground to start speculating about general differences. However the sex influence was not equally seen in all tissues, this is clearly illustrated by principle component analysis and non-supervised hierarchical clustering were only tongue and to less extent lung and skin could see a separate clustering of male and female samples.

7. Conclusion

The genome is subject to many regulatory mechanisms, of which methylation is only one. This work serves to show that significant differences exist between closely related organisms, and that sex is one of the determining factors in every organisms particular methylation pattern. We also see that same genes are differently methylated in different organs. Organs are collections of many different cell populations, and greater exactness could be achieved by isolating homogeneous cell populations, which is technically challenging. We also know at this point that DNA methylation pattern of an individual changes during its life. To determine these dynamics, a work of much larger scale is required, involving greater numbers of individuals, DNA from more than one time point in their lives and, as said before, more exact isolation of cell populations. This shall also require methods capable of analyzing multiple CpG sites rapidly and from lesser DNA amounts. The complexity of the problem is further shown by the fact that sex specific methylation results could not be reproduced in another mice population – but were still reproducible in the tissue samples used in the original work. This shows that methylation patterns are possibly dependent on the environmental influences, seasonal variability and other factors. The first group of mice were raised in August/September, the second in December/January; though conditions were close, they may have not been fully identical. The exact CO2 concentration used to kill the mice to harvest tissue samples was not measured; the pathological anaerobic metabolism prior to stop of vital functions may have been different in both groups; exposure to CO2 results in a decrease in alpha-ketoglutarate, which is a cofactor in transforming 5-methylcytosine into 5-carboxylcytosine; between these are the oxidation products which are the intermediate steps in demethylation process. Thus, different levels of CO2 could possibly have caused different methylation product concentration in tissues with active circulation, such as spleen and brain. The SIRPH technique used was very accurate and sensitive, but covered only few methylated spots in the genome. A technique capable of broader analysis and a larger cohort of experimental animals would be needed to visualize broader and general patterns of methylation.

Tables

Figures

10 Appendix 1: Methylation values, complete HPLC data

Tab. 8: Alpha actin methylation data, section 1

Run orde	Mouse N	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	33.29		28.59		39.73	
11	M2	35.61	46.00	27.74	34.62	45.30	
21	M3	35.53	50.28	25.85		42.52	
31	M4	37.88	49.14	28.42	35.36	20.65	
41	M5	39.89	51.91	29.36	39.25	32.00	
51	M6	42.74	49.37	27.92	39.73	27.41	
61	M7	37.74	51.54	29.39	33.79	24.68	
71	M8	37.99	50.51	27.42	36.77	31.09	
3	M9	35.12		27.74		36.32	
13	M10	43.35		28.24	35.54	25.81	
23	M11	35.26	48.33	26.97	33.40	26.34	41.77
33	M12	35.35		28.05		19.64	
43	M13	31.94		26.36	33.39	24.14	
53	M14	34.35	51.14	21.39		22.07	
63	M15	34.74	52.35	27.04	30.74	20.00	
73	M16	36.94	45.60	27.93	29.73	25.92	
5	M17	36.84	52.43	20.89		25.66	32.75
15	M18	38.64	49.55	38.40	37.81	26.76	
25	M19	32.84	48.41	24.96	29.26	35.68	
35	M20	40.46	60.65	30.19	32.41	24.04	
45	M21	43.60		30.12	37.80		
55	M22	34.28	55.61	22.94	32.04		
65	M23	37.36		32.42	34.87	26.77	
75	M24	40.41	51.75	31.49	37.26	26.64	38.17
7	M25	36.55	50.60	25.85	30.10	26.54	42.31
17	M26	35.07	50.77	24.92	30.43	28.58	
27	M27	32.76	54.71	28.51	32.91	24.03	37.24
37	M28	39.12	53.70	29.14	34.40	28.22	
47	M29	32.77	51.70	28.31	31.27	25.86	
57	M30	37.10		27.58	30.65	21.06	
67	M31	38.08	51.24			27.00	
77	M32	32.78	46.91			31.59	
9	M33	39.32	51.21			33.85	
19	M34	34.38				26.07	
29	M35	38.33	51.28			29.52	
39	M36	43.32				25.13	
49	M37	40.98				21.28	
59	M38	39.99				22.67	

Tab. 8: Alpha actin methylation data, section 2

Run order	Mouse N	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	42.35	52.46	32.30	34.25	65.86	69.96
11	M2	42.49	52.42			42.77	46.66
21	M3	43.48	51.52	25.22	47.68	66.62	70.32
31	M4	42.52	49.83	31.92	38.35	64.57	68.74
41	M5	46.02	54.03	33.47	40.41	60.08	63.92
51	M6	46.94	56.15	38.23	39.37	50.68	52.09
61	M7	42.33	50.48	32.26	36.71	25.13	25.23
71	M8	46.01	51.46	29.96	42.55	52.63	57.00
3	M9	50.20	57.86	30.37	36.55	60.31	64.24
13	M10	45.28	53.79	34.92	39.05	59.56	63.51
23	M11	45.38	54.42	30.06	34.26	62.46	65.52
33	M12	43.91	51.23	30.38	38.41	64.47	66.47
43	M13	66.77	65.00	28.74	34.51	61.90	65.76
53	M14	62.28	65.55	30.82	36.50	63.02	65.69
63	M15	45.15	54.83	31.52	35.25	60.80	64.23
73	M16	43.92	54.42	33.65	33.93	59.60	64.96
5	M17	45.25	54.32	34.73	38.52	63.39	66.39
15	M18	70.62	69.16	33.81	41.43	60.88	66.93
25	M19	41.37	50.79	30.69	36.45	64.71	68.53
35	M20	43.60	53.49	36.51	43.45	66.08	69.53
45	M21	41.26	48.98	35.99	36.68	61.81	65.40
55	M22	43.76	51.20	33.75	43.20	57.18	61.35
65	M23	44.94	50.77	34.27	38.46	63.38	67.05
75	M24			36.56	39.36	65.61	68.30
7	M25	46.83	53.36	36.05	40.74	63.76	68.05
17	M26	67.90	62.65	36.45	40.51	60.10	65.56
27	M27	53.83	58.01	30.69	36.94	22.44	24.00
37	M28			38.52	42.68	65.16	68.45
47	M29	42.38	49.67	31.35	38.99	65.65	68.52
57	M30	41.48	49.31	31.26	35.76	65.34	70.18
67	M31	45.30	51.39	33.94	39.31	67.53	71.59
77	M32	39.78	46.27	30.06	34.30	62.29	69.37
9	M33	44.41	51.81	35.62	40.01	64.93	67.17
19	M34	40.50	49.41	32.33	37.04	68.66	71.33
29	M35	45.72	53.84	32.98	42.22	61.85	65.05
39	M36			37.75	43.64	64.57	67.38
49	M37	43.73	52.18	31.38	36.66	71.62	72.72
59	M38	45.57	48.75	34.63	38.88	55.57	59.36

Tab.8: Alpha actin methylation data, section 3

Run orde	Mouse N	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	35.89		17.79	24.34	25.50	
11	M2	28.42		18.76	24.95	26.44	
21	M3	31.66		19.87	29.33	27.01	
31	M4	24.88		20.43	26.07	29.82	
41	M5	27.30	39.68	22.62	29.75	27.00	36.04
51	M6	33.72		24.77	33.32	27.68	32.57
61	M7	36.94		18.93	25.88	27.54	34.69
71	M8	23.35	42.47	18.84	28.02	26.96	
3	M9			17.47	23.43	25.62	32.05
13	M10	25.69		22.10	24.49	25.43	36.26
23	M11	28.43		20.26	25.95	26.76	29.47
33	M12	22.28	47.98	20.27	24.37	24.09	34.99
43	M13	28.21		20.01	26.46	26.65	
53	M14	17.34		23.49	31.01	26.59	37.01
63	M15	18.70		20.26	27.01	23.21	34.24
73	M16	22.62	32.08	22.81		25.31	
5	M17	29.41	36.31	25.19		26.80	37.11
15	M18	31.50		22.95	33.65	29.65	35.66
25	M19	23.16		20.91		27.87	40.02
35	M20	31.27		19.14	38.56	26.86	35.33
45	M21	24.79		23.52		30.72	
55	M22	24.39		19.75		30.29	
65	M23	27.47		25.66		29.76	
75	M24	27.00	42.45	22.68		28.53	
7	M25	26.07	31.24	27.60		30.97	
17	M26	22.24	31.61	18.57	28.53	22.38	
27	M27	21.91	35.36	20.36	100.00	26.10	
37	M28	31.16				26.83	
47	M29	20.18				25.23	
57	M30			16.76		25.22	
67	M31	29.23		26.38	35.01	27.14	
77	M32	22.68		16.19		26.47	
9	M33	23.26	34.68	23.98	38.09	32.61	39.87
19	M34	23.08		20.49	28.46	23.39	
29	M35	25.98		24.31		20.52	
39	M36	27.02		18.08		24.71	
49	M37	23.67	39.56	22.81		23.14	
59	M38	25.74		20.20	32.23	24.79	

Tab.8: Alpha actin methylation data, section 4

Run orde	Mouse N	Spleen		Lungs		Skin		
69	M39	Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2	
79	M40	44.27	54.68			25.82	32.95	44.583
81	M41	40.05	50.15			23.59	32.46	43.6842
82	M42	39.80	48.55	38.38		29.33		45.2819
83	M43	35.43	44.48	42.21		22.79		44.9322
84	M44	31.33	45.75	51.30		24.61		47.9727
85	M45	35.58	46.44	36.99		26.29		
86	M46	29.05	43.34	38.38		22.92		44.9621
87	M47	31.44	46.09	34.95		23.89		41.6809
88	M48	33.17	44.62	32.70		25.28	33.27	44.513
89	M49	33.75	46.87	26.52		23.85	31.15	
90	M50	29.79	42.46	24.72		22.81		42.6045
2	F1	30.94	42.50	26.21		20.46	28.59	33.5301
12	F2	38.63		22.28	28.13	21.88	29.99	65.2991
22	F3	33.45		22.61	30.08	26.58		41.5771
32	F4			21.46	25.63	24.66		56.2994
42	F5	39.06	52.20	23.55	28.05	24.62		67.6637
52	F6	40.50	48.96	23.72	29.76	24.97		23.1718
62	F7	44.80	53.55	25.12	29.45	26.31		56.7696
72	F8	46.39		30.28	35.85	26.37	34.81	46.2235
4	F9	33.95	49.10	21.34		19.89		61.0876
14	F10	39.39		22.36		26.60	36.85	44.5277
24	F11	38.07		28.43	32.49	26.02	36.29	43.2226
34	F12	32.47	50.10	24.94	31.68	26.76	34.70	41.8955
44	F13	38.73	56.01	23.29		23.46		46.6374
54	F14	38.46		27.15		27.67		66.0003
64	F15			23.24	32.68	24.90		42.5319
74	F16	36.67		24.48		18.28		45.0674
6	F17	40.25		31.19	31.62	21.74		56.9827
16	F18	35.04	45.46	25.47		33.88		44.1769
26	F19	38.64	47.63	24.40	35.77	23.70		39.4936
36	F20			23.84	36.95	26.04	38.73	43.8469
46	F21	46.42		24.73	31.76	23.38		46.5218
56	F22	32.28	50.33	20.34	28.77	22.32		38.758
66	F23	42.64		28.24	32.21	21.38	35.53	44.2945
76	F24	34.91		24.53		23.88	33.35	43.2441
8	F25	33.70		21.45	30.29	19.84	30.51	40.172
18	F26	36.94				28.29		68.2387
28	F27	36.46		26.57	41.94	28.19	37.82	46.2426
38	F28	39.0625		28.6761		28.0521	43.5899	66.9201
		34.5537		24.5267	35.1803	8.92691		

Tab.8: Alpha actin methylation data, section 5

Run orde	Mouse N	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	44.58	50.43	39.17	40.02	34.89	37.35
79	M40	43.68	51.33	37.19	38.48	60.20	63.70
81	M41	45.28	51.68	37.57	42.23	69.64	75.67
82	M42	44.93	49.97	34.84	38.90	63.52	69.48
83	M43	47.97	53.71	35.41	49.82	29.37	32.77
84	M44			37.12	44.61	29.03	31.48
85	M45	44.96	50.77	31.89	42.81	50.30	52.89
86	M46	41.68	59.99	32.53	36.96	69.23	71.22
87	M47	44.51	56.68			66.69	70.56
88	M48			29.98	44.58	68.60	71.90
89	M49	42.60	50.33	30.78	64.80	66.29	70.32
90	M50	33.53	56.03			65.13	66.59
2	F1	65.30	66.00	31.84	40.11		
12	F2	41.58	52.20	31.85	36.48		
22	F3	56.30	58.38	30.23	40.32		
32	F4	67.66	64.88	36.71	40.90		
42	F5	23.17	32.05	34.09	37.84		
52	F6	56.77	59.95	37.49	40.01		
62	F7	46.22	54.33	39.54	41.58		
72	F8	61.09	61.10	30.80	34.79		
4	F9	44.53	56.24	34.59	38.57		
14	F10	43.22	56.71	33.73	38.31		
24	F11	41.90	52.34	30.24	37.30		
34	F12	46.64	55.81	34.57	39.30		
44	F13	66.00	62.60	36.83	41.29		
54	F14	42.53	54.73	33.35	35.86		
64	F15	45.07	53.44	33.87	35.81		
74	F16	56.98	59.72	38.50	41.35		
6	F17	44.18	61.08	32.66	40.24		
16	F18	39.49	61.54	36.11	38.69		
26	F19	43.85	50.99	32.39	36.29		
36	F20	46.52	51.72	40.30	47.93		
46	F21	38.76	53.55	26.74	40.23		
56	F22	44.29	51.56	39.36	39.30		
66	F23	43.24	48.80	35.14	41.39		
76	F24	40.17	68.58	30.24	34.29		
8	F25	68.24	67.27	34.36	42.49		
18	F26	46.24	54.47	36.46	39.41		
28	F27	66.9201	66.5016	32.9109	37.6258		
38	F28			29.8615	42.698		

Tab.8: Alpha actin methylation data, section 6

Run order	Mouse No.	Tongue		Muscles		Heart
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1
69	M39	39.18		19.67	35.13	25.80
79	M40	20.44	40.42	23.76		27.83
81	M41	22.56		18.17		25.29
82	M42	26.34		19.08		28.12
83	M43	41.39		22.15		27.11
84	M44	29.67		20.58		23.71
85	M45	26.60	32.29	27.10		28.66
86	M46	25.29		20.55		27.46
87	M47	35.99		22.62		25.54
88	M48	31.30		22.47		31.40
89	M49	24.76		18.55		21.20
90	M50	34.99		26.77		25.49
2	F1	19.76	34.12	20.22	34.97	22.23
12	F2	23.42	28.07	27.88		25.98
22	F3	24.78	30.67	13.09		24.19
32	F4	27.40	37.28	14.80		27.97
42	F5	20.64	30.86	21.85		30.20
52	F6	25.23	27.39	23.15		27.30
62	F7	29.98	34.01	19.35	32.88	26.38
72	F8	20.42	21.36	24.66		27.49
4	F9	23.41	25.05	17.67		19.56
14	F10	27.54		21.84	27.50	27.00
24	F11	20.93	26.40	21.74		41.06
34	F12	22.81	27.40	26.63	40.29	46.91
44	F13	25.33	37.51	22.68	28.92	28.75
54	F14	25.23	29.52	23.28		27.39
64	F15	17.76	28.61	21.93	37.04	24.26
74	F16	22.95	28.11	22.84	28.28	24.85
6	F17	20.22	27.23	20.97	30.28	31.41
16	F18	20.97	34.77	20.20	29.71	24.27
26	F19	23.49	29.62	19.36	30.84	27.06
36	F20	24.27	33.93	21.74		27.76
46	F21	18.01	36.08	14.74	25.46	21.26
56	F22	21.59	24.53	18.76	27.74	25.30
66	F23	21.33		21.40	28.57	29.09
76	F24	24.97	31.35	17.53	26.36	23.72
8	F25	22.50		16.06		21.74
18	F26	23.25		16.00		28.64
28	F27	20.582	30.0203	15.4463	25.1551	28.8001
38	F28	24.3517	39.6167	17.5484		20.7133

Tab.8: Alpha actin methylation data, section 7

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	38.19		30.70		22.56	
58	F30	41.17		23.56	28.92	22.42	
68	F31	36.39	44.28	26.08	33.65	21.17	
78	F32	35.51	48.23	27.85	35.03	25.05	28.04
10	F33			24.03	28.73	33.52	
20	F34	35.26		21.62	32.49	21.82	
30	F35	37.31	48.90	31.49	32.74	23.04	28.35
40	F36	37.64	51.74	24.21	30.41	22.45	
50	F37	40.72		30.88	32.69	21.40	
60	F38	42.71	51.26	25.10		22.51	28.27
70	F39	36.39	52.85	28.77	32.79	25.03	
80	F40	34.23	49.08	28.74	29.32	23.82	
91	F41	38.48	40.67	22.48		23.19	34.25
92	F42	32.01	41.38	25.22	33.45	25.97	35.88
93	F43	36.58	43.70	27.11	29.70	25.63	31.95
94	F44	34.06	44.43	29.82	33.67	24.87	35.15
95	F45	20.65	29.99	25.33	31.76		
96	F46	39.46		27.40	27.67	23.35	35.27
97	F47	30.34		24.45	31.44	20.84	39.91
98	F48	38.18	43.77	24.55	30.68	26.19	
99	F49	32.82	52.65	23.79	38.59	26.28	36.00
100	F50	34.72	44.91	26.36	37.42	25.06	29.58
	Mean males	36.55	49.54	29.66	33.90	26.92	35.06
	SD Males	3.75		5.83		5.57	
	Mean females	37.05	47.55	25.46	32.09	24.18	34.31
	SD Females	4.38		2.88		3.74	
	Mean both	36.79	48.77	27.35	32.79	25.54	34.55
	Control M	27.51	37.44	26.00		26.00	31.00
	Control F	23.00	29.00	25.00	29.00	24.00	29.00

Tab.8: Alpha actin methylation data, section 8

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	50.93	54.45	36.42	40.70		
58	F30	45.27	53.34	30.58	36.69		
68	F31	47.23	55.30	33.41	37.92		
78	F32	40.25	48.35	30.99	41.96		
10	F33	50.64	53.82	32.62	40.60		
20	F34	67.86	65.73	25.12	32.40		
30	F35			31.87	38.68		
40	F36	46.08	54.22	34.59	36.58		
50	F37	62.23	63.53	30.73	37.33		
60	F38	50.62	59.86				
70	F39	69.73	72.12	34.37	38.71		
80	F40	45.80	55.39	31.92	35.24		
91	F41	38.00	44.97	35.76	36.14		
92	F42	48.11	55.60	32.96	36.86		
93	F43	43.87	52.69	37.68	39.55		
94	F44	47.81	54.62	35.97	41.01		
95	F45	43.97	51.06	33.58	38.84		
96	F46	44.24	54.96	33.84	38.20		
97	F47	42.32	51.31	27.45	38.66		
98	F48						
99	F49	55.59	62.49	35.78	42.84		
100	F50	36.62	51.23	39.07	48.16		
	Mean males	46.16	53.59	33.40	39.92	59.12	62.61
	SD Males	7.23		3.00		11.72	
	Mean females	49.02	56.42	33.74	39.11		
	SD Females	10.14		3.31			
	Mean both	47.62	55.04	33.57	39.51		
	Control M			26.00	32.00	29.00	32.00
	Control F	21.00	32.00	25.00	29.00		

Tab.8: Alpha actin methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	18.33	30.36	20.60		25.98	
58	F30	21.09		15.40		28.33	
68	F31	19.72		21.32	54.01	25.66	
78	F32	21.61	31.21	19.31		27.47	
10	F33	26.88		16.71		21.99	32.92
20	F34	20.40	32.82			25.22	
30	F35	28.88		19.17		31.02	38.67
40	F36	18.96	38.23	19.18	32.59	29.90	
50	F37	21.97		14.30		26.52	
60	F38	22.68	31.75	20.88	27.11	27.01	35.11
70	F39	20.97		19.10	33.13	27.24	
80	F40	24.15	38.83	20.05		26.74	
91	F41	24.42		18.00	26.18	27.10	31.93
92	F42	40.92		21.72	30.68	30.43	36.86
93	F43	25.57		15.08		30.65	
94	F44	23.63		24.09	29.36	29.94	
95	F45	23.79		16.61		24.36	
96	F46	25.34		16.77		26.62	
97	F47	25.17		21.43	26.99	23.38	
98	F48	24.44		18.61		27.43	
99	F49	20.99	38.18	20.98		27.60	
100	F50	29.96		21.96	32.67	25.74	
	Mean males	27.19	37.40	21.37	32.25	26.58	35.37
	SD Males	5.30		2.83		2.51	
	Mean females	23.46	31.45	19.69	31.11	27.15	34.68
	SD Females	3.83		3.23		4.46	
	Mean both	25.29	33.21	20.52	31.68	26.87	35.15
	Control M	37.00		24.00	30.00	27.00	
	Control F	25.00	32.00			26.00	38.00

Tab.9: Snrpn D1 methylation data, section 1

Run orde	Mouse N	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	41.91	48.72	45.16		46.54	
11	M2	41.79	49.15	43.95		44.75	
21	M3	39.97	51.31	47.83		43.10	53.11
31	M4	41.06	48.31	43.46		40.92	
41	M5	42.35	50.81	44.77		42.02	
51	M6	43.72	51.35			44.48	
61	M7	41.30	48.56	42.74		45.97	52.67
71	M8	43.30	50.86	45.16			
3	M9	41.27	48.31	46.19		45.50	
13	M10	43.05	50.55	43.30		43.42	
23	M11	41.45	48.78	44.92		44.43	52.30
33	M12	41.93	49.50	43.87		47.67	
43	M13	41.47	48.82	43.72	56.06	45.24	
53	M14	41.55	48.80	45.62		46.48	54.29
63	M15	40.48	47.72	43.09	55.81	43.82	48.26
73	M16	47.95		42.87	55.27		
5	M17	41.65	48.66	44.94		43.22	53.57
15	M18	43.24	50.78	43.93		47.21	
25	M19	42.03	49.82	44.37		46.09	
35	M20	43.35	50.48	44.27	56.81	44.07	
45	M21	43.17	50.86	44.43	57.33		
55	M22	42.29	49.96	42.86	54.17		
65	M23	43.91	52.41	42.99	55.92	47.46	
75	M24	43.69	51.14	42.99		43.86	51.61
7	M25	42.50	49.19	44.08		42.07	
17	M26	44.24	51.26	43.34		39.30	
27	M27	41.28	48.83	42.15	53.85	42.55	51.85
37	M28	42.01	49.16	44.39		46.53	
47	M29	41.76	48.78	42.86	55.82	42.78	52.01
57	M30	42.34	49.74	42.75		43.11	51.76
67	M31	42.64	50.24			39.74	48.50
77	M32	42.07	49.75			45.99	
9	M33	42.58	48.81			43.45	
19	M34	41.80	48.06			45.59	
29	M35	40.08	49.04			44.71	
39	M36	41.26	50.57			45.50	
49	M37	48.02	58.94			42.42	
59	M38	42.98	51.54			40.20	

Tab.9: Snrpn D1 methylation data, section 2

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	42.54	61.78	41.24	54.31	16.12	21.16
11	M2	46.32				29.11	40.46
21	M3					15.68	18.95
31	M4	44.62		41.32		13.61	19.92
41	M5			44.97		16.52	22.11
51	M6	46.35		43.51		24.91	31.55
61	M7	44.28		40.76		34.82	48.55
71	M8	46.83				19.47	32.48
3	M9					15.49	19.95
13	M10	42.92	60.06	40.96		17.18	19.18
23	M11	45.35		40.17	50.78	15.66	21.23
33	M12	45.13		42.03	52.14	16.52	20.23
43	M13	54.51	70.95	42.05		16.11	23.90
53	M14	47.25	63.42	41.10	56.88	14.07	21.46
63	M15	45.56		40.84		14.66	21.38
73	M16	45.49		40.52	56.14	15.83	22.49
5	M17	44.07		41.09	53.45	15.31	21.46
15	M18	53.82		41.73	55.05	15.62	19.54
25	M19	43.27	60.56	42.00		13.80	19.51
35	M20	44.86		40.61	56.09	14.38	17.64
45	M21	43.20	61.07	40.46	53.38	19.39	23.97
55	M22	43.68	60.77	42.93		21.42	23.62
65	M23	44.85		40.07	57.76	13.71	18.05
75	M24			40.57	53.48	13.00	19.28
7	M25	46.38		40.47	54.13	14.66	18.33
17	M26	46.12	61.36	42.73		16.29	19.20
27	M27			41.00	51.18	41.39	51.63
37	M28			41.11	55.38	12.86	15.11
47	M29	41.80	58.61	38.34		12.38	16.46
57	M30	45.05	59.89	38.92	52.22	12.08	17.13
67	M31	43.74	59.16	39.69	57.51	11.58	15.82
77	M32	43.74		39.12	50.96	13.84	18.29
9	M33	42.88	56.52	43.57	53.24	14.53	19.70
19	M34	43.91	57.91	43.15	50.79	10.54	15.33
29	M35	43.43	53.51	43.72	56.15	14.10	20.53
39	M36			41.54	51.15	13.86	19.52
49	M37	42.72	55.77	42.77	53.66	10.83	15.87
59	M38	44.24	62.84	42.61	52.45	17.94	26.19

Tab.9: Snrpn D1 methylation data, section 3

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	53.11	66.07	65.99	76.46	43.31	51.48
11	M2	73.71	61.59	64.04	76.16	42.38	52.54
21	M3	56.39	70.27	63.70	74.48	43.36	52.06
31	M4	50.11	66.19	66.50	74.64	44.29	53.07
41	M5	51.52	64.37	64.89	76.47	44.65	52.36
51	M6	52.72		66.13	76.70	44.14	51.99
61	M7	52.96	64.90	68.08	79.54	43.76	51.46
71	M8	55.69	69.86	63.94	75.68	44.52	54.67
3	M9			63.67	73.48	44.78	52.54
13	M10	53.99	65.88	68.78	79.16	44.78	54.14
23	M11	50.90	66.74	66.82	78.16	44.62	52.50
33	M12	51.30		68.85	77.80	44.39	51.89
43	M13	45.76		66.54	74.38	43.87	52.75
53	M14	48.31	62.10	65.83	76.18	44.51	54.39
63	M15	48.11	61.01	63.64	75.69	44.44	53.18
73	M16	48.44	63.49	64.06	66.10	44.51	53.13
5	M17	52.23	65.10	61.20	75.08	44.41	52.19
15	M18	50.51	62.15	64.53	81.66	45.43	53.40
25	M19	48.44	63.52			44.71	53.22
35	M20	53.13	66.71	64.54	74.80	45.62	53.68
45	M21	48.85				45.26	54.42
55	M22	50.83		63.99	76.13	44.77	57.75
65	M23	47.36	62.90	64.11	70.79	45.64	55.96
75	M24	47.48	59.96	64.50	64.86	45.31	56.92
7	M25	51.27	66.08			43.97	54.83
17	M26	50.00	62.21	65.24	75.37	44.44	54.05
27	M27	46.74	62.69			44.93	54.68
37	M28	52.70		64.92	73.87	44.41	54.38
47	M29	47.27		61.04	75.74	42.96	58.25
57	M30			62.05		40.40	54.55
67	M31	47.10	60.63	66.01	76.64	42.30	54.63
77	M32	45.30	60.05			40.96	53.59
9	M33	50.20	65.38			42.94	50.57
19	M34	58.06		63.69	76.49	42.72	51.92
29	M35	54.88		70.77	73.18	42.39	54.57
39	M36	51.04	63.88	64.66	69.01	42.39	53.85
49	M37	44.48	57.26	62.58		43.79	54.07
59	M38	52.73		66.48	75.88	40.78	54.85

Tab.9: Snrpn D1 methylation data, section 4

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	42.92	51.64			43.40	
79	M40	43.09	50.76			43.71	49.67
81	M41	41.02	51.14			44.81	51.48
82	M42	44.01	48.42			46.19	
83	M43	42.53	50.09			43.22	48.21
84	M44	41.66	48.51			44.35	
85	M45	42.49	49.03			45.26	52.75
86	M46	41.65	49.38			44.15	
87	M47	42.64	50.27	52.57		48.55	55.77
88	M48	42.23	50.61			44.39	51.23
89	M49	42.40	50.49			44.25	
90	M50	39.13	48.84			43.57	53.53
2	F1	41.94	50.39	42.19		41.29	
12	F2	40.95	47.49	41.62		44.69	
22	F3	43.87	51.61	42.04		44.20	
32	F4	40.70	47.88	41.99		43.16	
42	F5	41.86	50.88	42.20		43.62	
52	F6	43.62	51.05	41.04		46.85	
62	F7	41.93	49.88	41.34	54.80	42.41	
72	F8	39.46	49.19	41.67		46.23	
4	F9	42.46	50.40	42.19		43.50	
14	F10	40.53	47.04	42.03	55.11	44.28	
24	F11	42.60	51.65	42.01		43.93	
34	F12	42.12	49.83	41.81	54.83	42.71	
44	F13	52.27	51.66	43.79		46.63	
54	F14	44.28	59.68	43.03		43.69	51.58
64	F15	49.28	51.66	47.21		43.70	
74	F16	39.42	49.11	40.57	54.31	43.15	51.22
6	F17	41.32	48.79			46.59	
16	F18	44.76	53.36	43.51		39.04	
26	F19			46.26		42.46	
36	F20	43.91	51.61	42.70		43.19	
46	F21	40.20	50.38	42.06		42.46	
56	F22	43.79	41.50	40.76		45.65	
66	F23	38.41	51.14	44.41		43.25	48.23
76	F24	44.72	51.97	42.61		43.86	50.45
8	F25	42.41	50.79	43.49		44.37	
18	F26	42.75	54.38	43.20		45.23	
28	F27	43.2009	56.8504	40.7569		44.6255	54.4763
38	F28	41.948	54.5741	41.1441		42.5677	

Tab.9: Snrpn D1 methylation data, section 5

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	43.12	57.06	41.72		34.99	44.80
79	M40	44.36		46.58		18.39	22.96
81	M41	43.82	59.12	43.14	52.46	10.22	16.88
82	M42	41.79	59.64	45.13	55.05	11.35	18.52
83	M43	44.90	59.59			39.45	49.05
84	M44					39.88	49.06
85	M45	43.06	60.28			25.28	31.93
86	M46			40.30	50.61	12.81	19.33
87	M47					17.14	24.77
88	M48					15.27	20.22
89	M49					12.17	16.93
90	M50					15.34	20.58
2	F1	47.65		42.24	51.52		
12	F2	46.08		43.08	51.98		
22	F3	45.80		43.88			
32	F4	44.09	54.56	41.87	49.02		
42	F5	38.92		42.23	53.45		
52	F6	45.50		36.63			
62	F7	42.65	61.90	43.21	54.43		
72	F8	47.65	64.97	42.52	54.00		
4	F9	45.93		42.43	51.35		
14	F10			41.55	51.10		
24	F11	45.80	63.60	42.78	53.06		
34	F12	42.19	60.78	43.28	57.07		
44	F13	49.47		43.86	54.38		
54	F14	49.08		43.84	51.89		
64	F15	44.45		41.32	55.99		
74	F16	47.39	62.19	44.08	57.89		
6	F17	46.57		43.62	59.65		
16	F18			43.79	54.14		
26	F19	43.71		43.46	52.83		
36	F20	44.70	60.80	44.47	57.83		
46	F21			43.21	59.70		
56	F22	46.14		43.34	57.99		
66	F23	47.72		42.51	58.41		
76	F24			42.53	53.51		
8	F25	48.01		45.13			
18	F26	43.20	57.04	41.51	48.68		
28	F27	47.6646	65.6811	42.3298	60.6839		
38	F28			44.0059			

Tab.9: Snrpn D1 methylation data, section 6

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	50.84		65.13	77.09	43.09	53.10
79	M40	48.02	61.00	67.05		44.62	60.39
81	M41	50.75	61.39	66.81	76.47	45.81	59.93
82	M42	47.38	55.91			40.36	
83	M43	47.08	59.72	64.68	77.28	41.04	59.35
84	M44	52.15	64.38	69.91	70.58	41.74	54.78
85	M45	44.57	56.46	64.60	77.33	42.61	56.72
86	M46	49.87	60.63	70.66	78.30	42.66	54.80
87	M47	51.84	63.35			51.85	58.46
88	M48	51.79		65.93	75.92	42.68	55.35
89	M49	46.75	58.18	64.14	75.13	42.21	54.70
90	M50	53.44	66.59	63.25	75.04	42.91	61.37
2	F1	41.87	53.65	66.13	76.42	42.58	54.33
12	F2	45.25	57.16	63.34	75.97	43.76	53.12
22	F3	43.91	56.65			42.54	54.68
32	F4	44.12		70.06	79.14	45.54	56.02
42	F5	41.13	53.71	63.89	73.62	46.57	61.87
52	F6	44.04	54.89	63.21	74.70	43.32	56.49
62	F7			65.13	76.18		
72	F8	44.45	55.90	65.92	76.48	42.40	58.85
4	F9	43.67	52.66	70.32	77.04	44.73	53.51
14	F10	44.30	52.64	67.44	77.99	42.86	50.30
24	F11	43.21	55.21	65.32	75.02	41.82	54.87
34	F12	44.60	53.65	68.28	79.15	50.61	
44	F13	41.97	58.76	69.25	71.12	40.27	53.88
54	F14	43.84	55.39	65.19	78.19	43.18	52.68
64	F15	43.81	52.96	66.54	78.59	42.87	52.72
74	F16	42.82	54.14	64.78	73.50	42.29	53.84
6	F17	44.21	55.74			41.41	51.17
16	F18	41.82				41.46	51.33
26	F19	40.57	56.38			42.21	50.91
36	F20	41.90	52.88			42.26	52.52
46	F21	41.78				42.04	52.81
56	F22	40.73	52.68			41.65	51.03
66	F23	44.53		64.28	74.70	42.26	52.74
76	F24	46.02	61.80	64.60	73.01	42.31	51.61
8	F25	42.24	55.19	67.59	76.79	41.50	51.05
18	F26	40.77	50.60	62.31		41.36	54.28
28	F27	42.0966	53.5429	66.797	80.3952	41.1773	54.2073
38	F28	43.3103	54.3826	63.3248	73.1308	40.6099	50.8385

Tab.9: Snrpn D1 methylation data, section 7

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	40.12	48.82	42.02		42.47	50.49
58	F30	41.72	60.96	41.07		40.39	
68	F31	40.53	49.09	39.42	54.15	43.82	
78	F32	41.80	49.32	40.22		42.97	
10	F33	41.55	48.55	39.87	51.70	43.92	
20	F34	41.05	47.18	40.48	50.63	43.57	
30	F35	42.61	50.20	40.18	50.87	43.89	
40	F36	41.06	48.35	40.20	50.89	44.37	
50	F37	41.01	47.94	39.93	50.11	42.97	
60	F38	42.27	49.98	41.86	55.76	37.50	47.99
70	F39	42.53	51.31	39.67	52.81	46.96	
80	F40	42.86	51.52	40.34	51.98	46.96	
91	F41	40.86	49.56	41.39	58.05	44.47	52.70
92	F42	42.21	50.11	38.43	51.16	44.31	
93	F43	43.44	50.36	39.81	51.68	42.85	
94	F44	44.12	53.43	40.34	51.88	41.99	47.22
95	F45	37.71	50.70	39.72	51.94	39.41	48.69
96	F46	42.90	50.26	40.08	51.03	42.34	50.79
97	F47	43.96	51.18	37.01	52.77	47.90	
98	F48	40.87	47.73	40.35	52.19	43.77	52.42
99	F49	43.26	55.74	39.70		43.76	49.87
100	F50	39.11	51.54	42.01		43.45	49.86
	Mean males	42.38	49.97	44.32	55.67	44.26	51.81
	SD Males	1.56		1.97		2.02	
	Mean femal	42.29	50.79	41.46	52.79	43.63	50.43
	SD Females	2.40		1.80		1.99	
	Mean both	42.34	50.38	42.55	53.66	43.93	51.21
	Total Mean	46.36		48.10		47.57	
	Control M	43.00	51.00	47.00		42.00	
	Control F	43.00	59.00	41.00	50.00	44.00	47.00

Tab.9: Snrpn D1 methylation data, section 8

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	44.52	58.56	43.62	56.76		
58	F30	47.22		45.33			
68	F31	45.44	60.80	42.15	58.59		
78	F32	43.67		46.73			
10	F33	44.33		45.80			
20	F34	46.52	58.39	42.79			
30	F35			44.47			
40	F36			41.81			
50	F37			45.24			
60	F38	43.37					
70	F39	46.20		43.69			
80	F40	42.31	60.31	43.88			
91	F41			42.42	58.64		
92	F42	44.16	60.27	43.20	55.86		
93	F43	39.01					
94	F44	42.52					
95	F45	43.13	58.70				
96	F46						
97	F47						
98	F48			48.38			
99	F49						
100	F50						
	Mean males	44.86	60.00	41.66	53.71	17.83	23.84
	SD Males	2.64		1.73		7.77	
	Mean female	45.08	60.57	43.29	55.01		
	SD Females	2.46		1.77			
	Mean both	44.97	60.23	42.50	54.39		
	Total Mean	52.60		48.44		20.84	
	Control M			45.00		41.00	51.00
	Control F						

Tab.9: Snrpn D1 methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	42.79	57.92	66.48	72.89	41.37	55.76
58	F30	44.87	55.95	63.20	73.22	40.85	57.06
68	F31	40.60	50.34	63.71	69.39	42.03	54.28
78	F32	42.41	54.29			42.69	52.19
10	F33	45.89	59.52			44.06	52.22
20	F34	40.27	51.07			43.68	53.70
30	F35	40.08	53.71			44.18	53.46
40	F36	40.65	55.20			44.01	54.75
50	F37	41.05	54.85			45.66	55.54
60	F38	43.88	53.38			42.50	51.00
70	F39	40.03	49.71			43.95	52.27
80	F40	42.22	57.24			43.02	52.56
91	F41	41.08	57.14	64.16	68.75	57.59	42.75
92	F42	43.15	53.01			44.41	52.33
93	F43	39.00	59.52	65.60	67.24	44.33	53.90
94	F44	44.40	59.19			42.71	52.78
95	F45	42.76	54.20	62.49	75.62	42.54	54.94
96	F46	42.64	57.97	63.13	74.29	43.71	55.42
97	F47	42.82	56.37	66.59	76.51	45.63	55.92
98	F48	37.52	56.62	66.96	77.94	45.31	58.97
99	F49	41.78	53.57	68.11	78.14	44.19	58.38
100	F50	43.10	53.32		76.39	44.92	56.15
	Mean males	50.79	63.02	65.33	75.22	43.77	54.48
	SD Males	4.57		2.25		1.84	
	Mean female	42.57	54.99	65.62	75.21	43.45	53.71
	SD Females	1.79		2.19		2.74	
	Mean both	46.64	58.56	65.45	75.22	43.61	54.10
	Total Mean	52.60		70.34		48.85	
	Control M	42.00	51.00	67.00	78.00	42.00	51.00
	Control F	42.00	51.00	68.00	79.00	41.00	50.00

Tab.10: IAP methylation data, section 1

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	80.59	85.08	83.39	87.01	85.46	88.42
11	M2	74.88	81.13	83.23	87.51	84.72	87.56
21	M3	83.92	86.08	82.49	86.43	83.91	87.28
31	M4	76.61	82.06	83.83	87.31	85.04	87.41
41	M5	84.78	88.04	84.63	88.71	85.21	88.67
51	M6	84.70	87.94	84.27	88.13	84.82	88.33
61	M7	77.24	82.46	83.37	86.55	85.13	87.30
71	M8	78.17	82.72	83.15	86.93	85.78	88.30
3	M9	74.22	80.32	83.13	87.06	85.06	88.00
13	M10	84.66	87.53	83.37	86.98	84.54	87.12
23	M11	74.62	81.35	82.55	87.01	83.85	87.13
33	M12	78.93	84.14	84.25	87.88	84.09	87.50
43	M13	71.83	78.63	82.65	86.32	84.72	87.57
53	M14	78.00	82.99	83.15	87.23	84.72	87.92
63	M15	72.03	78.98	83.63	87.32	84.09	87.01
73	M16	73.67	80.53	83.96	87.24	85.14	87.93
5	M17	75.99	81.75	84.04	87.49	84.65	87.37
15	M18	81.85	86.60	84.04	88.19	84.89	88.62
25	M19	77.72	83.23	82.98	86.98	84.47	87.31
35	M20	81.41	85.81	82.92	87.13	84.22	84.29
45	M21	86.98	89.67	85.20	89.12		
55	M22	76.96	82.76	81.97	86.80		
65	M23	84.88	88.56	83.90	87.61	85.22	88.26
75	M24	80.07	84.81	84.50	88.29	85.27	88.38
7	M25	78.37	83.46	82.80	86.29	84.91	87.76
17	M26	83.88	87.41	83.85	87.36	84.68	87.91
27	M27	76.58	81.65	81.53	85.77	84.04	87.28
37	M28	77.04	82.34	84.28	87.45	85.33	88.45
47	M29	78.50	83.16	81.31	85.48	83.90	86.77
57	M30	77.95	83.32	81.79	85.98	83.84	87.08
67	M31	85.59	88.49			85.11	88.19
77	M32	80.81	84.41			85.86	88.13
9	M33	80.98	84.88			86.16	88.83
19	M34	85.23	88.07			84.39	87.44
29	M35	78.23	83.08			84.77	87.81
39	M36	82.74	86.55			84.50	87.90
49	M37	82.82	87.28			83.57	87.25
59	M38	82.69	86.56			84.21	87.98

Tab.10: IAP methylation data, section 2

Run orde	Mouse N	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	82.46	86.04			85.08	87.71
79	M40	83.18	86.39			75.41	79.74
81	M41	78.41	84.12	84.62	87.13	83.96	86.30
82	M42	80.39	85.36	85.52	88.48	83.58	86.59
83	M43	80.92	85.37	85.98	88.08	84.28	86.28
84	M44	75.29	81.57	85.34	88.03	84.18	86.64
85	M45	79.04	84.20	86.40	88.90	84.54	86.92
86	M46	77.39	82.94	84.34	87.26	84.42	86.16
87	M47	79.61	84.76	86.40	89.23	83.92	86.54
88	M48	84.53	88.19	85.34	88.14	84.20	86.58
89	M49	82.30	87.21	84.38	87.40	86.52	87.62
90	M50	71.36	79.39	84.12	87.11	83.69	85.95
2	F1			83.91	87.25	84.50	88.04
12	F2	71.90	79.46	83.41	86.75	84.84	88.36
22	F3	85.54	88.75	83.23	86.73	84.64	88.07
32	F4	76.76	83.83	84.53	87.70	85.12	88.39
42	F5	82.18	86.59	84.75	87.73	85.17	88.21
52	F6	81.84	86.69	84.34	87.16	84.64	88.09
62	F7	80.12	85.27	84.75	87.00	85.62	88.33
72	F8	73.25	80.36	83.98	86.84	84.69	88.15
4	F9	73.97	80.96	83.26	86.84	84.34	88.16
14	F10	73.69	80.66	83.36	87.48	83.95	87.43
24	F11	82.79	86.47	83.62	87.20	84.60	87.99
34	F12	79.97	84.78	83.44	86.96	84.82	88.03
44	F13	81.31	85.83	85.15	88.16	85.22	88.60
54	F14	84.33	87.88	84.25	87.45	84.32	87.90
64	F15	86.62	89.27	87.09	88.34	84.10	87.40
74	F16	70.35	78.17	84.73	87.67	84.99	88.31
6	F17	75.31	81.37	84.44	87.27	84.50	87.42
16	F18	83.79	87.51	84.12	87.26	84.40	87.39
26	F19	51.76		83.58	87.50	84.72	87.81
36	F20	83.90	87.22	84.50	87.81	84.37	82.90
46	F21	81.29	85.62	83.84	87.14	84.37	87.63
56	F22	79.84	84.48	84.18	87.38	84.63	87.73
66	F23	70.42	78.46	83.26	87.02	83.24	87.06
76	F24	77.75	83.26	84.54	86.91	85.87	87.94
8	F25	76.71	82.43	84.54	87.01	84.44	87.19
18	F26	77.09	83.28	83.54	87.07	84.85	88.24
28	F27	82.60	86.20	83.83	86.94	84.79	87.59
38	F28	77.35	83.04	83.86	87.04	84.73	87.38

Tab.10: IAP methylation data, section 3

Run orde	Mouse N	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	68.99	77.04	83.57	86.91	84.54	87.26
58	F30	84.27	87.97	84.10	87.89	84.84	88.33
68	F31	73.69	80.20	83.88	86.82	85.31	87.91
78	F32	71.42	78.72	84.61	87.78	85.42	88.11
10	F33	75.30	81.13	83.73	87.16	85.28	87.59
20	F34	82.01	86.33	84.02	87.72	84.13	87.32
30	F35	84.62	88.28	84.05	88.23	35.30	88.64
40	F36	77.13	82.74	84.55	87.86	85.42	88.27
50	F37	73.69	80.68	83.31	87.46	83.67	87.28
60	F38	83.19	86.48	83.73	87.39	84.01	87.09
70	F39	86.74	89.29	84.61	88.03	84.81	87.77
80	F40	75.74	81.89	84.47	87.74	84.93	88.04
91	F41	80.17	85.95	83.64	87.22	83.71	85.77
92	F42	77.67	84.08	84.46	88.18	84.32	87.10
93	F43	85.38	89.39	83.52	87.21	86.52	87.82
94	F44	84.06	88.16	84.02	87.52	83.60	86.70
95	F45	78.91	84.12	83.18	87.05	83.13	86.98
96	F46	80.64	85.77	82.85	86.20	84.31	86.45
97	F47	80.34	85.71	83.13	86.23	83.74	85.96
98	F48	78.18	84.45	84.93	87.46	84.72	86.82
99	F49	80.84	86.00	83.79	86.45	84.08	86.07
100	F50	79.73	84.96	83.83	87.05	83.44	86.09
	Mean ma	79.62	84.39	83.81	87.38	84.46	87.28
	SD Male	3.91		1.23		1.49	
	Mean fer	78.47	84.32	84.04	87.30	83.59	87.50
	SD Fema	6.03		0.70		7.00	
	Mean bo	79.05	84.35	83.94	87.34	84.02	87.39
	Total me	81.70		85.64		85.71	
	Control M			83.00	86.00	84.00	88.00
	Control F			84.00	87.00	84.00	87.00

Tab.10: IAP methylation data, section 4

Run orde	Mouse N	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	88.00	84.49	73.98	81.46	82.68	49.63
11	M2	87.56	84.49	71.32		74.78	0.00
21	M3	87.86	84.44	74.99	82.39	66.10	55.49
31	M4	88.29	84.53	72.94	80.64	72.51	52.65
41	M5	88.56	85.51	72.87	80.66	64.74	55.48
51	M6	88.61	85.00	72.90	80.97	67.74	54.45
61	M7	87.77	83.95	73.50	80.20	81.01	49.75
71	M8	87.93	84.68	74.28	82.15	80.37	50.55
3	M9	88.34	85.11	71.57	79.43	66.50	54.43
13	M10	88.00	84.42	71.61	79.56	77.26	50.73
23	M11	87.41	84.48	69.07	77.50	83.27	48.21
33	M12	88.08	84.96	72.14	79.92	63.13	55.87
43	M13	87.70	84.01	70.18	78.41	66.42	54.14
53	M14	87.63	84.47	70.87	78.70	61.18	56.26
63	M15	87.80	84.28	70.00	77.88	63.23	55.19
73	M16	87.52	84.14	69.86	78.44	74.13	51.41
5	M17	88.20	84.68	70.39	78.75	67.38	53.89
15	M18	88.42	85.17	72.13	80.10	74.34	51.86
25	M19	86.11	83.51	74.09	81.35	62.38	56.60
35	M20	87.50	84.48	69.58	78.63	76.10	50.82
45	M21	87.80	84.97	70.26	78.93	69.27	53.26
55	M22	87.23	84.32	73.55	81.65	66.81	55.00
65	M23	87.07	83.86	72.54	80.02	73.97	51.96
75	M24			71.83	79.59	80.12	49.83
7	M25	87.60	84.11	69.66	78.18	68.82	53.18
17	M26	88.61	85.38	72.95	81.20	58.20	58.25
27	M27	88.15	84.80	70.13	77.80	81.67	48.79
37	M28	91.55		71.23	78.65	73.26	51.77
47	M29	87.51	83.66	70.54	77.48	74.39	51.02
57	M30	86.92	83.90	69.65	77.40	65.59	54.13
67	M31	87.56	84.05	69.10	77.63	85.69	47.53
77	M32	88.77	83.90	69.74	77.17	75.55	50.53
9	M33	87.68	83.85	70.98	79.40	81.76	49.27
19	M34	87.99	84.28	71.74	79.61	79.86	49.92
29	M35	88.21	85.57	70.93	78.60	64.58	54.90
39	M36	89.29		71.01	78.60	74.50	51.34
49	M37	87.44	84.27	71.97	79.84	78.11	50.55
59	M38	87.55	83.83	70.51	78.46	67.56	53.73

Tab.10: IAP methylation data, section 5

Run orde	Mouse N	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	87.61	84.19	73.00	80.67	81.06	49.88
79	M40	88.15	84.12	70.88	77.99	72.40	51.86
81	M41	85.00	87.01	68.81	75.72	62.74	54.69
82	M42	85.57	88.30	71.05	77.86	67.61	53.52
83	M43	84.10	86.32	67.14	75.43	81.51	48.06
84	M44	87.12	89.84	72.88	79.79	85.29	48.33
85	M45	84.86	87.16	76.73	83.62	81.12	50.76
86	M46	86.67	88.06	70.42	78.04	70.76	52.45
87	M47	86.74	88.64	72.03	80.37	67.68	54.28
88	M48	86.99	89.13	70.24	77.36	78.79	49.54
89	M49	86.25	88.63	74.52	81.71	82.06	49.89
90	M50	86.09	88.13	73.72	80.22	66.20	54.79
2	F1	88.32	84.22	73.45	81.43		
12	F2	88.64	84.65	70.35	78.95		
22	F3	87.23	84.54	73.54	81.27		
32	F4	87.61	84.36	71.70	79.85		
42	F5	80.93	80.79	70.99	79.36		
52	F6	87.62	84.44	72.01	79.60		
62	F7	88.21	84.55	72.78	80.52		
72	F8	87.46	84.56	70.37	78.47		
4	F9	86.99	84.28	68.32	77.24		
14	F10	87.11	84.64	68.15	77.14		
24	F11	87.95	84.77	72.85	80.92		
34	F12	87.75	84.12	72.51	80.61		
44	F13	88.09	84.43	73.87	80.99		
54	F14	87.37	78.96	70.36	78.16		
64	F15	87.67	84.17	74.17	81.69		
74	F16	87.84	84.30	72.93	80.52		
6	F17	88.59	84.33	86.25	91.09		
16	F18	88.99	85.01	72.30	80.25		
26	F19	88.20	84.71	70.20	78.13		
36	F20	88.00	84.29	72.58	80.36		
46	F21	88.79	84.53	72.08	80.25		
56	F22	88.07	84.25	71.16	79.17		
66	F23	88.04	84.63	69.21	78.46		
76	F24			71.73	79.67		
8	F25	88.25	84.91	73.18	80.48		
18	F26	88.18	85.12	68.48	77.49		
28	F27	88.32	84.61	70.87	78.54		
38	F28	83.53	79.71	72.11	79.76		

Tab.10: IAP methylation data, section 6

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	88.85	85.43	70.71	78.45		
58	F30	87.81	85.28	71.41	80.28		
68	F31	88.01	84.84	71.03	79.27		
78	F32	88.25	84.69	72.73	81.29		
10	F33	88.12	84.57	73.54	81.18		
20	F34	89.19	85.98	73.86	81.41		
30	F35	59.86		71.62	79.29		
40	F36	88.61	84.77	71.97	79.91		
50	F37	87.77	85.17	71.25	80.04		
60	F38	87.81	84.52	80.12	87.43		
70	F39	87.49	84.81	73.15	81.33		
80	F40	87.73	85.09	71.64	79.50		
91	F41	87.23	85.21	71.50	78.54		
92	F42	87.56	85.23	71.03	78.34		
93	F43	86.99	84.29	74.00	80.53		
94	F44	88.18	85.19	74.77	80.91		
95	F45	86.69	84.85	73.15	80.19		
96	F46	89.03	85.39	78.24	84.38		
97	F47	88.22	85.27	72.62	79.64		
98	F48	89.46	85.94	74.21	81.43		
99	F49	88.09	85.55	74.93	80.52		
100	F50	88.22	85.42	72.49	78.83		
	Mean males	87.54	85.21	71.56	79.31	72.84	51.21
	SD Males	1.18		1.84		7.33	
	Mean femal	87.20	84.49	72.57	80.26		
	SD Females	4.20		2.90			
	Mean both	87.37	84.85	72.07	79.79		
	Total mean	86.11		75.93		62.03	
	Control M	85.00	89.00	83.00	87.00	84.00	87.00
	Control F	87.00	85.00	83.00	87.00		

Tab.10: IAP methylation data, section 7

Run orde	Mouse N	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	85.96	89.14	83.99	87.03	81.84	86.11
11	M2	86.25	89.00	83.22	86.35	80.69	84.99
21	M3	85.31	88.28	83.47	85.81	81.44	85.33
31	M4	85.43	88.37	83.70	85.83	79.81	84.32
41	M5	85.59	88.82	83.54	86.67	85.24	87.45
51	M6	86.80	90.25	83.42	86.35	83.40	85.62
61	M7	85.10	87.58	83.64	85.98	83.06	85.06
71	M8	86.22	88.98	83.65	86.58	79.86	83.73
3	M9			83.89	86.52	76.28	82.04
13	M10	86.20	88.64	83.84	86.43	78.48	83.41
23	M11	84.62	87.92	83.99	86.68	78.99	83.87
33	M12	84.35	87.47	83.25	86.66	82.42	85.72
43	M13	83.96	87.11	83.60	85.77	80.17	83.78
53	M14	84.76	87.79	82.79	85.87	80.96	85.35
63	M15	84.94	87.88	83.70	86.49	80.52	84.58
73	M16	84.69	87.82	86.18	87.43	80.60	84.49
5	M17	83.62	86.99	84.27	86.64	81.63	85.68
15	M18	84.34	88.05	82.61	86.52	80.77	85.32
25	M19	83.54	87.07	82.70	85.44	77.91	82.78
35	M20	84.75	87.92	83.16	86.55	79.71	84.09
45	M21	83.78	87.83	84.47	86.83	82.02	86.08
55	M22	84.21	87.77	83.89	86.82	79.32	84.07
65	M23	84.47	88.02	83.70	85.98	81.16	84.91
75	M24	85.18	88.44	83.31	86.02	81.00	85.00
7	M25	84.50	87.80	83.66	86.79	80.07	85.34
17	M26	84.89	88.31	82.77	85.76	80.37	84.86
27	M27	84.17	86.85			78.49	83.33
37	M28	85.60	88.71	82.30	85.82	81.07	84.81
47	M29	84.34	87.38	82.71	85.63	80.40	84.49
57	M30	85.49	88.07	82.67	86.40	79.24	83.69
67	M31	85.50	88.29	83.02	85.78	81.43	85.29
77	M32	85.28	87.70	83.55	85.58	80.26	84.67
9	M33	85.16	83.98	80.11	84.49	84.01	87.77
19	M34	86.14	83.06	84.14	87.17	82.23	86.08
29	M35	85.38	83.44	83.04	85.51	80.12	84.55
39	M36	85.55	83.53	83.53	86.46	81.06	85.02
49	M37	85.12	83.45	83.60	86.17	80.40	84.69
59	M38	85.79	82.44	84.78	84.44	80.44	84.53

Tab.10: IAP methylation data, section 8

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	84.86	82.24	84.08	86.29	81.70	85.07
79	M40	85.11	83.49	83.82	86.15	80.33	84.39
81	M41	84.31	82.99	82.33	85.51	80.20	84.63
82	M42	84.68	83.85	83.47	86.19	80.52	84.91
83	M43	85.08	82.96	82.20	85.09	81.34	84.86
84	M44	85.46	83.61	82.88	86.03	81.28	84.41
85	M45	86.11	84.09	83.78	86.63	80.77	84.54
86	M46	85.81	83.38	84.39	86.42	81.07	84.81
87	M47	84.42	82.98	83.47	86.57	82.27	85.80
88	M48	85.70	83.49	83.26	86.29	80.55	85.03
89	M49	83.85	82.79	81.86	85.54	80.02	84.55
90	M50	86.67	83.35	83.16	85.55	81.36	85.74
2	F1	86.68	85.01	85.17	88.25	82.64	87.40
12	F2	86.54	83.84	83.62	86.91	82.43	86.55
22	F3	85.92	83.72	83.50	86.49	82.42	86.33
32	F4	85.68	83.84	84.15	86.98	83.20	86.89
42	F5	86.92	83.67	84.38	86.34	83.78	87.21
52	F6	86.09	83.80	83.95	86.38	83.28	86.42
62	F7	84.95	82.73	83.93	86.10	82.62	85.78
72	F8	86.31	84.03	82.66	85.80	83.14	86.56
4	F9	86.09	84.64	84.17	87.32	81.81	86.46
14	F10	86.75	84.26	82.58	86.19	81.01	85.52
24	F11	86.92	84.35	83.62	87.02	82.07	85.95
34	F12	87.27	84.52	81.81	85.61	81.69	85.52
44	F13	85.82	83.05	85.18	86.85	82.40	86.01
54	F14	86.36	83.44	84.45	86.47	82.58	86.29
64	F15	86.78	83.49	84.66	87.07	81.58	85.62
74	F16	87.01	84.28	83.26	86.58	82.67	86.52
6	F17	85.57	86.04	84.48	87.34		
16	F18	87.33	84.08	83.77	86.51	81.66	85.38
26	F19	87.22	84.01	83.67	86.80	81.46	85.73
36	F20	87.25	83.90	83.96	86.78	81.41	85.65
46	F21	86.07	83.73	83.89	86.23	80.95	85.06
56	F22	86.30	83.60	83.85	86.37	80.56	84.23
66	F23	87.60	84.76	82.43	85.32	81.54	85.85
76	F24	86.80	83.95	84.74	86.48	81.70	85.18
8	F25	86.19	84.08	83.22	85.90	81.35	85.58
18	F26	87.11	84.21	83.31	86.62	80.79	84.80
28	F27	86.47	84.36	84.00	86.13	81.16	85.45
38	F28	85.18	83.30	83.04	86.04	80.89	84.60

Tab.10: IAP methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	86.61	83.09	83.67	85.72	81.37	85.06
58	F30	86.90	83.88	83.25	86.43	81.65	85.48
68	F31	86.62	84.08	83.03	85.26	81.44	85.38
78	F32			83.65	86.49	82.87	86.54
10	F33	86.40	85.49	85.19	87.00	81.00	84.56
20	F34	86.64	83.78			80.12	84.44
30	F35	86.20	84.17	84.41	87.24	82.39	85.93
40	F36	86.64	83.41	83.54	86.43	82.31	85.56
50	F37	86.34	83.79	83.03	86.51	82.50	86.00
60	F38	85.45	82.87	82.94	85.79	78.15	82.48
70	F39	86.61	83.90	82.46	85.76	81.85	85.31
80	F40	85.04	83.69	83.35	86.08	81.82	85.36
91	F41	84.18	83.72	83.52	86.87	80.75	84.97
92	F42	84.82	83.91	84.62	87.21	82.59	86.17
93	F43	85.69	83.10	83.17	86.14	80.48	84.63
94	F44	85.69	83.03	82.92	86.19	81.80	85.75
95	F45	86.29	83.24	82.68	86.02	82.03	85.79
96	F46	85.95	82.97	83.10	85.83	80.70	84.38
97	F47	85.55	83.20	83.04	85.19	82.26	85.52
98	F48	86.17	84.27	84.92	86.94	83.64	86.21
99	F49	85.48	84.27	84.36	86.76	80.49	84.33
100	F50	85.15	83.80	84.86	86.87	81.77	
	Mean males	85.08	86.31	83.40	86.15	80.77	84.83
	SD Males	0.79		0.87		1.49	
	Mean femal	86.24	83.88	83.70	86.44	81.77	85.59
	SD Females	0.73		0.79		1.01	
	Mean both	85.66	85.10	83.55	86.30	81.26	85.20
	Total mean	85.38		84.92		83.23	
	Control M	85.00	83.00	84.00	87.00	84.00	87.00
	Control F	85.00	83.00	84.00	87.00	83.00	86.00

Tab.11: Line 1 methylation data, section 1

Run orde	Mouse N	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	80.59	85.08	83.39	87.01	85.46	88.42
11	M2	74.88	81.13	83.23	87.51	84.72	87.56
21	M3	83.92	86.08	82.49	86.43	83.91	87.28
31	M4	76.61	82.06	83.83	87.31	85.04	87.41
41	M5	84.78	88.04	84.63	88.71	85.21	88.67
51	M6	84.70	87.94	84.27	88.13	84.82	88.33
61	M7	77.24	82.46	83.37	86.55	85.13	87.30
71	M8	78.17	82.72	83.15	86.93	85.78	88.30
3	M9	74.22	80.32	83.13	87.06	85.06	88.00
13	M10	84.66	87.53	83.37	86.98	84.54	87.12
23	M11	74.62	81.35	82.55	87.01	83.85	87.13
33	M12	78.93	84.14	84.25	87.88	84.09	87.50
43	M13	71.83	78.63	82.65	86.32	84.72	87.57
53	M14	78.00	82.99	83.15	87.23	84.72	87.92
63	M15	72.03	78.98	83.63	87.32	84.09	87.01
73	M16	73.67	80.53	83.96	87.24	85.14	87.93
5	M17	75.99	81.75	84.04	87.49	84.65	87.37
15	M18	81.85	86.60	84.04	88.19	84.89	88.62
25	M19	77.72	83.23	82.98	86.98	84.47	87.31
35	M20	81.41	85.81	82.92	87.13	84.22	84.29
45	M21	86.98	89.67	85.20	89.12		
55	M22	76.96	82.76	81.97	86.80		
65	M23	84.88	88.56	83.90	87.61	85.22	88.26
75	M24	80.07	84.81	84.50	88.29	85.27	88.38
7	M25	78.37	83.46	82.80	86.29	84.91	87.76
17	M26	83.88	87.41	83.85	87.36	84.68	87.91
27	M27	76.58	81.65	81.53	85.77	84.04	87.28
37	M28	77.04	82.34	84.28	87.45	85.33	88.45
47	M29	78.50	83.16	81.31	85.48	83.90	86.77
57	M30	77.95	83.32	81.79	85.98	83.84	87.08
67	M31	85.59	88.49			85.11	88.19
77	M32	80.81	84.41			85.86	88.13
9	M33	80.98	84.88			86.16	88.83
19	M34	85.23	88.07			84.39	87.44
29	M35	78.23	83.08			84.77	87.81
39	M36	82.74	86.55			84.50	87.90
49	M37	82.82	87.28			83.57	87.25
59	M38	82.69	86.56			84.21	87.98

Tab.11: Line 1 methylation data, section 2

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	82.46	86.04			85.08	87.71
79	M40	83.18	86.39			75.41	79.74
81	M41	78.41	84.12	84.62	87.13	83.96	86.30
82	M42	80.39	85.36	85.52	88.48	83.58	86.59
83	M43	80.92	85.37	85.98	88.08	84.28	86.28
84	M44	75.29	81.57	85.34	88.03	84.18	86.64
85	M45	79.04	84.20	86.40	88.90	84.54	86.92
86	M46	77.39	82.94	84.34	87.26	84.42	86.16
87	M47	79.61	84.76	86.40	89.23	83.92	86.54
88	M48	84.53	88.19	85.34	88.14	84.20	86.58
89	M49	82.30	87.21	84.38	87.40	86.52	87.62
90	M50	71.36	79.39	84.12	87.11	83.69	85.95
2	F1			83.91	87.25	84.50	88.04
12	F2	71.90	79.46	83.41	86.75	84.84	88.36
22	F3	85.54	88.75	83.23	86.73	84.64	88.07
32	F4	76.76	83.83	84.53	87.70	85.12	88.39
42	F5	82.18	86.59	84.75	87.73	85.17	88.21
52	F6	81.84	86.69	84.34	87.16	84.64	88.09
62	F7	80.12	85.27	84.75	87.00	85.62	88.33
72	F8	73.25	80.36	83.98	86.84	84.69	88.15
4	F9	73.97	80.96	83.26	86.84	84.34	88.16
14	F10	73.69	80.66	83.36	87.48	83.95	87.43
24	F11	82.79	86.47	83.62	87.20	84.60	87.99
34	F12	79.97	84.78	83.44	86.96	84.82	88.03
44	F13	81.31	85.83	85.15	88.16	85.22	88.60
54	F14	84.33	87.88	84.25	87.45	84.32	87.90
64	F15	86.62	89.27	87.09	88.34	84.10	87.40
74	F16	70.35	78.17	84.73	87.67	84.99	88.31
6	F17	75.31	81.37	84.44	87.27	84.50	87.42
16	F18	83.79	87.51	84.12	87.26	84.40	87.39
26	F19	51.76		83.58	87.50	84.72	87.81
36	F20	83.90	87.22	84.50	87.81	84.37	82.90
46	F21	81.29	85.62	83.84	87.14	84.37	87.63
56	F22	79.84	84.48	84.18	87.38	84.63	87.73
66	F23	70.42	78.46	83.26	87.02	83.24	87.06
76	F24	77.75	83.26	84.54	86.91	85.87	87.94
8	F25	76.71	82.43	84.54	87.01	84.44	87.19
18	F26	77.09	83.28	83.54	87.07	84.85	88.24
28	F27	82.60	86.20	83.83	86.94	84.79	87.59
38	F28	77.35	83.04	83.86	87.04	84.73	87.38

Tab.11: Line 1 methylation data, section 3

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	68.99	77.04	83.57	86.91	84.54	87.26
58	F30	84.27	87.97	84.10	87.89	84.84	88.33
68	F31	73.69	80.20	83.88	86.82	85.31	87.91
78	F32	71.42	78.72	84.61	87.78	85.42	88.11
10	F33	75.30	81.13	83.73	87.16	85.28	87.59
20	F34	82.01	86.33	84.02	87.72	84.13	87.32
30	F35	84.62	88.28	84.05	88.23	35.30	88.64
40	F36	77.13	82.74	84.55	87.86	85.42	88.27
50	F37	73.69	80.68	83.31	87.46	83.67	87.28
60	F38	83.19	86.48	83.73	87.39	84.01	87.09
70	F39	86.74	89.29	84.61	88.03	84.81	87.77
80	F40	75.74	81.89	84.47	87.74	84.93	88.04
91	F41	80.17	85.95	83.64	87.22	83.71	85.77
92	F42	77.67	84.08	84.46	88.18	84.32	87.10
93	F43	85.38	89.39	83.52	87.21	86.52	87.82
94	F44	84.06	88.16	84.02	87.52	83.60	86.70
95	F45	78.91	84.12	83.18	87.05	83.13	86.98
96	F46	80.64	85.77	82.85	86.20	84.31	86.45
97	F47	80.34	85.71	83.13	86.23	83.74	85.96
98	F48	78.18	84.45	84.93	87.46	84.72	86.82
99	F49	80.84	86.00	83.79	86.45	84.08	86.07
100	F50	79.73	84.96	83.83	87.05	83.44	86.09
	Mean males	79.62	84.39	83.81	87.38	84.46	87.28
	SD Males	3.91		1.23		1.49	
	Mean femal	78.47	84.32	84.04	87.30	83.59	87.50
	SD Females	6.03		0.70		7.00	
	Mean both	79.05	84.35	83.94	87.34	84.02	87.39
	Total mean	81.70		85.64		85.71	
	Control M			83.00	86.00	84.00	88.00
	Control F			84.00	87.00	84.00	87.00

Tab.11: Line 1 methylation data, section 4

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	88.00	84.49	73.98	81.46	82.68	49.63
11	M2	87.56	84.49	71.32		74.78	0.00
21	M3	87.86	84.44	74.99	82.39	66.10	55.49
31	M4	88.29	84.53	72.94	80.64	72.51	52.65
41	M5	88.56	85.51	72.87	80.66	64.74	55.48
51	M6	88.61	85.00	72.90	80.97	67.74	54.45
61	M7	87.77	83.95	73.50	80.20	81.01	49.75
71	M8	87.93	84.68	74.28	82.15	80.37	50.55
3	M9	88.34	85.11	71.57	79.43	66.50	54.43
13	M10	88.00	84.42	71.61	79.56	77.26	50.73
23	M11	87.41	84.48	69.07	77.50	83.27	48.21
33	M12	88.08	84.96	72.14	79.92	63.13	55.87
43	M13	87.70	84.01	70.18	78.41	66.42	54.14
53	M14	87.63	84.47	70.87	78.70	61.18	56.26
63	M15	87.80	84.28	70.00	77.88	63.23	55.19
73	M16	87.52	84.14	69.86	78.44	74.13	51.41
5	M17	88.20	84.68	70.39	78.75	67.38	53.89
15	M18	88.42	85.17	72.13	80.10	74.34	51.86
25	M19	86.11	83.51	74.09	81.35	62.38	56.60
35	M20	87.50	84.48	69.58	78.63	76.10	50.82
45	M21	87.80	84.97	70.26	78.93	69.27	53.26
55	M22	87.23	84.32	73.55	81.65	66.81	55.00
65	M23	87.07	83.86	72.54	80.02	73.97	51.96
75	M24			71.83	79.59	80.12	49.83
7	M25	87.60	84.11	69.66	78.18	68.82	53.18
17	M26	88.61	85.38	72.95	81.20	58.20	58.25
27	M27	88.15	84.80	70.13	77.80	81.67	48.79
37	M28	91.55		71.23	78.65	73.26	51.77
47	M29	87.51	83.66	70.54	77.48	74.39	51.02
57	M30	86.92	83.90	69.65	77.40	65.59	54.13
67	M31	87.56	84.05	69.10	77.63	85.69	47.53
77	M32	88.77	83.90	69.74	77.17	75.55	50.53
9	M33	87.68	83.85	70.98	79.40	81.76	49.27
19	M34	87.99	84.28	71.74	79.61	79.86	49.92
29	M35	88.21	85.57	70.93	78.60	64.58	54.90
39	M36	89.29		71.01	78.60	74.50	51.34
49	M37	87.44	84.27	71.97	79.84	78.11	50.55
59	M38	87.55	83.83	70.51	78.46	67.56	53.73

Tab.11: Line 1 methylation data, section 5

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	87.61	84.19	73.00	80.67	81.06	49.88
79	M40	88.15	84.12	70.88	77.99	72.40	51.86
81	M41	85.00	87.01	68.81	75.72	62.74	54.69
82	M42	85.57	88.30	71.05	77.86	67.61	53.52
83	M43	84.10	86.32	67.14	75.43	81.51	48.06
84	M44	87.12	89.84	72.88	79.79	85.29	48.33
85	M45	84.86	87.16	76.73	83.62	81.12	50.76
86	M46	86.67	88.06	70.42	78.04	70.76	52.45
87	M47	86.74	88.64	72.03	80.37	67.68	54.28
88	M48	86.99	89.13	70.24	77.36	78.79	49.54
89	M49	86.25	88.63	74.52	81.71	82.06	49.89
90	M50	86.09	88.13	73.72	80.22	66.20	54.79
2	F1	88.32	84.22	73.45	81.43		
12	F2	88.64	84.65	70.35	78.95		
22	F3	87.23	84.54	73.54	81.27		
32	F4	87.61	84.36	71.70	79.85		
42	F5	80.93	80.79	70.99	79.36		
52	F6	87.62	84.44	72.01	79.60		
62	F7	88.21	84.55	72.78	80.52		
72	F8	87.46	84.56	70.37	78.47		
4	F9	86.99	84.28	68.32	77.24		
14	F10	87.11	84.64	68.15	77.14		
24	F11	87.95	84.77	72.85	80.92		
34	F12	87.75	84.12	72.51	80.61		
44	F13	88.09	84.43	73.87	80.99		
54	F14	87.37	78.96	70.36	78.16		
64	F15	87.67	84.17	74.17	81.69		
74	F16	87.84	84.30	72.93	80.52		
6	F17	88.59	84.33	86.25	91.09		
16	F18	88.99	85.01	72.30	80.25		
26	F19	88.20	84.71	70.20	78.13		
36	F20	88.00	84.29	72.58	80.36		
46	F21	88.79	84.53	72.08	80.25		
56	F22	88.07	84.25	71.16	79.17		
66	F23	88.04	84.63	69.21	78.46		
76	F24			71.73	79.67		
8	F25	88.25	84.91	73.18	80.48		
18	F26	88.18	85.12	68.48	77.49		
28	F27	88.32	84.61	70.87	78.54		
38	F28	83.53	79.71	72.11	79.76		

Tab.11: Line 1 methylation data, section 6

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
58	F30	88.85	85.43	70.71	78.45		
68	F31	87.81	85.28	71.41	80.28		
78	F32	88.01	84.84	71.03	79.27		
10	F33	88.25	84.69	72.73	81.29		
20	F34	88.12	84.57	73.54	81.18		
30	F35	89.19	85.98	73.86	81.41		
40	F36	59.86		71.62	79.29		
50	F37	88.61	84.77	71.97	79.91		
60	F38	87.77	85.17	71.25	80.04		
70	F39	87.81	84.52	80.12	87.43		
80	F40	87.49	84.81	73.15	81.33		
91	F41	87.73	85.09	71.64	79.50		
92	F42	87.23	85.21	71.50	78.54		
93	F43	87.56	85.23	71.03	78.34		
94	F44	86.99	84.29	74.00	80.53		
95	F45	88.18	85.19	74.77	80.91		
96	F46	86.69	84.85	73.15	80.19		
97	F47	89.03	85.39	78.24	84.38		
98	F48	88.22	85.27	72.62	79.64		
99	F49	89.46	85.94	74.21	81.43		
100	F50	88.09	85.55	74.93	80.52		
		88.22	85.42	72.49	78.83		
	Mean males						
	SD Males	87.54	85.21	71.56	79.31	72.84	51.21
	Mean femal	1.18		1.84		7.33	
	SD Females	87.20	84.49	72.57	80.26		
	Mean both	4.20		2.90			
		87.37	84.85	72.07	79.79		
	Total mean						
		86.11		75.93		62.03	
	Control M						
	Control F	85.00	89.00	83.00	87.00	84.00	87.00
		87.00	85.00	83.00	87.00		

Tab.11: Line 1 methylation data, section 7

Run orde	Mouse N	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	85.96	89.14	83.99	87.03	81.84	86.11
11	M2	86.25	89.00	83.22	86.35	80.69	84.99
21	M3	85.31	88.28	83.47	85.81	81.44	85.33
31	M4	85.43	88.37	83.70	85.83	79.81	84.32
41	M5	85.59	88.82	83.54	86.67	85.24	87.45
51	M6	86.80	90.25	83.42	86.35	83.40	85.62
61	M7	85.10	87.58	83.64	85.98	83.06	85.06
71	M8	86.22	88.98	83.65	86.58	79.86	83.73
3	M9			83.89	86.52	76.28	82.04
13	M10	86.20	88.64	83.84	86.43	78.48	83.41
23	M11	84.62	87.92	83.99	86.68	78.99	83.87
33	M12	84.35	87.47	83.25	86.66	82.42	85.72
43	M13	83.96	87.11	83.60	85.77	80.17	83.78
53	M14	84.76	87.79	82.79	85.87	80.96	85.35
63	M15	84.94	87.88	83.70	86.49	80.52	84.58
73	M16	84.69	87.82	86.18	87.43	80.60	84.49
5	M17	83.62	86.99	84.27	86.64	81.63	85.68
15	M18	84.34	88.05	82.61	86.52	80.77	85.32
25	M19	83.54	87.07	82.70	85.44	77.91	82.78
35	M20	84.75	87.92	83.16	86.55	79.71	84.09
45	M21	83.78	87.83	84.47	86.83	82.02	86.08
55	M22	84.21	87.77	83.89	86.82	79.32	84.07
65	M23	84.47	88.02	83.70	85.98	81.16	84.91
75	M24	85.18	88.44	83.31	86.02	81.00	85.00
7	M25	84.50	87.80	83.66	86.79	80.07	85.34
17	M26	84.89	88.31	82.77	85.76	80.37	84.86
27	M27	84.17	86.85			78.49	83.33
37	M28	85.60	88.71	82.30	85.82	81.07	84.81
47	M29	84.34	87.38	82.71	85.63	80.40	84.49
57	M30	85.49	88.07	82.67	86.40	79.24	83.69
67	M31	85.50	88.29	83.02	85.78	81.43	85.29
77	M32	85.28	87.70	83.55	85.58	80.26	84.67
9	M33	85.16	83.98	80.11	84.49	84.01	87.77
19	M34	86.14	83.06	84.14	87.17	82.23	86.08
29	M35	85.38	83.44	83.04	85.51	80.12	84.55
39	M36	85.55	83.53	83.53	86.46	81.06	85.02
49	M37	85.12	83.45	83.60	86.17	80.40	84.69
59	M38	85.79	82.44	84.78	84.44	80.44	84.53

Tab.11: Line 1 methylation data, section 8

Run orde	Mouse N	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	84.86	82.24	84.08	86.29	81.70	85.07
79	M40	85.11	83.49	83.82	86.15	80.33	84.39
81	M41	84.31	82.99	82.33	85.51	80.20	84.63
82	M42	84.68	83.85	83.47	86.19	80.52	84.91
83	M43	85.08	82.96	82.20	85.09	81.34	84.86
84	M44	85.46	83.61	82.88	86.03	81.28	84.41
85	M45	86.11	84.09	83.78	86.63	80.77	84.54
86	M46	85.81	83.38	84.39	86.42	81.07	84.81
87	M47	84.42	82.98	83.47	86.57	82.27	85.80
88	M48	85.70	83.49	83.26	86.29	80.55	85.03
89	M49	83.85	82.79	81.86	85.54	80.02	84.55
90	M50	86.67	83.35	83.16	85.55	81.36	85.74
2	F1	86.68	85.01	85.17	88.25	82.64	87.40
12	F2	86.54	83.84	83.62	86.91	82.43	86.55
22	F3	85.92	83.72	83.50	86.49	82.42	86.33
32	F4	85.68	83.84	84.15	86.98	83.20	86.89
42	F5	86.92	83.67	84.38	86.34	83.78	87.21
52	F6	86.09	83.80	83.95	86.38	83.28	86.42
62	F7	84.95	82.73	83.93	86.10	82.62	85.78
72	F8	86.31	84.03	82.66	85.80	83.14	86.56
4	F9	86.09	84.64	84.17	87.32	81.81	86.46
14	F10	86.75	84.26	82.58	86.19	81.01	85.52
24	F11	86.92	84.35	83.62	87.02	82.07	85.95
34	F12	87.27	84.52	81.81	85.61	81.69	85.52
44	F13	85.82	83.05	85.18	86.85	82.40	86.01
54	F14	86.36	83.44	84.45	86.47	82.58	86.29
64	F15	86.78	83.49	84.66	87.07	81.58	85.62
74	F16	87.01	84.28	83.26	86.58	82.67	86.52
6	F17	85.57	86.04	84.48	87.34		
16	F18	87.33	84.08	83.77	86.51	81.66	85.38
26	F19	87.22	84.01	83.67	86.80	81.46	85.73
36	F20	87.25	83.90	83.96	86.78	81.41	85.65
46	F21	86.07	83.73	83.89	86.23	80.95	85.06
56	F22	86.30	83.60	83.85	86.37	80.56	84.23
66	F23	87.60	84.76	82.43	85.32	81.54	85.85
76	F24	86.80	83.95	84.74	86.48	81.70	85.18
8	F25	86.19	84.08	83.22	85.90	81.35	85.58
18	F26	87.11	84.21	83.31	86.62	80.79	84.80
28	F27	86.47	84.36	84.00	86.13	81.16	85.45
38	F28	85.18	83.30	83.04	86.04	80.89	84.60

Tab.11: Line 1 methylation data, section 9

Run orde	Mouse N	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	86.61	83.09	83.67	85.72	81.37	85.06
58	F30	86.90	83.88	83.25	86.43	81.65	85.48
68	F31	86.62	84.08	83.03	85.26	81.44	85.38
78	F32			83.65	86.49	82.87	86.54
10	F33	86.40	85.49	85.19	87.00	81.00	84.56
20	F34	86.64	83.78			80.12	84.44
30	F35	86.20	84.17	84.41	87.24	82.39	85.93
40	F36	86.64	83.41	83.54	86.43	82.31	85.56
50	F37	86.34	83.79	83.03	86.51	82.50	86.00
60	F38	85.45	82.87	82.94	85.79	78.15	82.48
70	F39	86.61	83.90	82.46	85.76	81.85	85.31
80	F40	85.04	83.69	83.35	86.08	81.82	85.36
91	F41	84.18	83.72	83.52	86.87	80.75	84.97
92	F42	84.82	83.91	84.62	87.21	82.59	86.17
93	F43	85.69	83.10	83.17	86.14	80.48	84.63
94	F44	85.69	83.03	82.92	86.19	81.80	85.75
95	F45	86.29	83.24	82.68	86.02	82.03	85.79
96	F46	85.95	82.97	83.10	85.83	80.70	84.38
97	F47	85.55	83.20	83.04	85.19	82.26	85.52
98	F48	86.17	84.27	84.92	86.94	83.64	86.21
99	F49	85.48	84.27	84.36	86.76	80.49	84.33
100	F50	85.15	83.80	84.86	86.87	81.77	
	Mean ma	85.08	86.31	83.40	86.15	80.77	84.83
	SD Male	0.79		0.87		1.49	
	Mean fer	86.24	83.88	83.70	86.44	81.77	85.59
	SD Fema	0.73		0.79		1.01	
	Mean bo	85.66	85.10	83.55	86.30	81.26	85.20
	Total me	85.38		84.92		83.23	
	Control M	85.00	83.00	84.00	87.00	84.00	87.00

Tab.12: Long intronic transcript methylation data, section 1

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	46.79	38.72	44.78	37.25	49.36	45.12
11	M2	47.32	39.23	46.95	40.72	43.76	43.50
21	M3	48.08	38.82	41.08	35.82	47.99	43.52
31	M4	46.85	39.35	46.59	39.77	42.13	37.55
41	M5	46.94	38.54	46.38	41.06	43.71	39.25
51	M6			43.25	38.62	46.92	42.23
61	M7	44.77	35.34	44.26	37.69	44.04	40.69
71	M8	49.06	40.20	46.54	39.69	34.32	
3	M9	49.13	40.72	45.39	36.17	46.79	42.95
13	M10	49.62	41.49	44.53	29.17	44.57	39.23
23	M11	48.51	39.65	42.38	35.82	45.73	40.10
33	M12	49.09	40.42	45.45	39.45	46.69	41.38
43	M13	50.09	40.54	43.96	29.81	46.40	40.51
53	M14	47.04	38.67	44.12		48.33	43.49
63	M15	45.93	37.32	44.93	30.13	47.32	42.27
73	M16	46.75	38.89	45.42	29.69	45.48	40.87
5	M17	48.18	40.48	44.71	37.09	46.28	40.26
15	M18	48.74	40.74	44.36	37.18	48.74	39.72
25	M19	45.94	37.08	43.60	36.45	44.62	40.29
35	M20	48.44	40.61	43.95	29.35	42.22	36.80
45	M21	47.75	39.47	44.84	36.33		
55	M22	47.92	39.12	44.52	37.36		
65	M23	49.93	41.75	45.28	38.11	47.69	44.63
75	M24	48.87	40.75	44.31	37.98	47.14	42.97
7	M25	47.92	39.25	44.21	38.15	44.08	37.72
17	M26	48.60	39.81	43.03	35.85	42.81	37.67
27	M27	45.91	37.35	40.78	34.65	43.69	37.55
37	M28	48.83	39.79	43.82	38.05	50.91	46.77
47	M29	48.48	39.05	41.92	36.79	44.34	38.31
57	M30	48.00	39.25	43.51	38.37	44.28	39.65
67	M31	47.72	39.26			47.94	43.36
77	M32	48.52	39.50			47.17	47.26
9	M33	48.72	40.10			47.83	43.24
19	M34	46.35	36.93			45.44	41.79
29	M35	47.62	38.52			48.04	43.06
39	M36	48.47	39.13			50.37	44.91
49	M37	48.75	39.22			42.84	39.72
59	M38	48.25	39.71			46.13	42.41

Tab.12: Long intronic transcript methylation data, section 2

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	46.86	37.97			43.21	39.50
79	M40	45.78	37.69			44.77	40.43
81	M41	44.34	35.19	61.93	52.05	47.54	46.01
82	M42	44.33	35.67	52.73		43.91	40.37
83	M43	44.86	35.82	46.72		45.87	42.71
84	M44	47.66	39.02	56.35	53.31	47.65	43.25
85	M45	46.73	38.26	48.29	46.36	48.30	46.61
86	M46	48.11	38.03	50.61	47.16	45.43	42.57
87	M47	45.38	35.63	52.52	47.55	48.66	47.59
88	M48	46.79	37.07	54.12	49.08	44.29	41.84
89	M49	44.53	36.20	54.16	50.88	50.62	
90	M50	43.27	35.79	58.50	50.56	48.00	45.40
2	F1	46.31	37.51	51.58		42.30	37.53
12	F2	45.86	37.91	42.83	35.61	44.11	40.67
22	F3	46.39	37.45	42.37	36.50	44.06	39.80
32	F4	44.99	38.09	43.27	36.42	43.11	38.71
42	F5	47.99	38.58	41.76	36.08	43.08	37.76
52	F6	47.16	38.37	42.55	36.84	45.63	40.56
62	F7	45.80	38.10	43.56	37.76	45.55	44.19
72	F8	45.84	37.65	42.84	39.75	45.86	44.05
4	F9	48.20	38.29	41.44	35.14	43.06	38.68
14	F10			38.63	33.83	46.44	41.84
24	F11	48.39	39.34	38.06	33.89	45.49	40.76
34	F12	48.64	39.08	42.32	35.36	44.55	39.28
44	F13	47.91	39.10			42.83	37.72
54	F14	47.00	41.35	46.38		43.90	39.67
64	F15	46.31	38.42	39.75		40.89	36.60
74	F16	45.72	35.51	39.11	34.05	42.22	38.31
6	F17	47.95	39.06	46.34		42.81	38.81
16	F18	45.31	36.51	42.28	36.75	42.46	29.59
26	F19			43.23	38.53	42.66	37.14
36	F20	47.80	38.76	43.53	37.60	44.30	38.52
46	F21	47.93	38.49	40.53	33.79	43.41	38.17
56	F22	45.67	37.25	44.05	37.31	43.79	40.60
66	F23	46.02	38.43	41.43	36.55	43.53	38.84
76	F24	46.65	38.68	41.10	36.21	46.56	42.06
8	F25	47.97	39.68	43.32	37.20	44.50	39.46
18	F26	44.07	34.81	42.01	35.68	45.02	40.00
28	F27	49.69	35.92	42.75	36.58	42.36	36.63
38	F28	43.03	35.46	43.85	36.93	44.63	31.95
48	F29	45.68	37.20	42.63		43.95	39.33

Tab.12: Long intronic transcript methylation data, section 3

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
58.00	F30	43.37	36.93	42.27	36.36	44.03	31.77
68.00	F31	45.25	36.03	41.37	34.67	41.64	37.51
78.00	F32	45.32	36.33	41.95	35.46	42.69	37.83
10.00	F33	48.50	38.94	43.37	36.02	41.27	37.27
20.00	F34	48.12	38.17	44.10	36.66	42.18	38.26
30.00	F35	47.69	38.02	41.99	36.00	42.17	39.53
40.00	F36	47.20	37.25	41.59	35.34	45.28	39.96
50.00	F37	43.83	35.89	44.21	28.51	44.56	40.40
60.00	F38	48.63	40.10	41.37	36.20	43.09	38.68
70.00	F39	45.27	37.12	41.50	34.88	41.96	37.59
80.00	F40	46.22	37.70	43.24	35.88	41.08	38.57
91.00	F41	49.86	40.27	39.88	34.59	42.63	39.48
92.00	F42	46.64	37.84	42.97	36.58	44.17	40.86
93.00	F43	46.31	37.29	42.00	36.26	42.31	38.47
94.00	F44	47.42	37.99	43.42	36.03	41.08	37.22
95.00	F45	46.78	37.31	42.39	26.52	43.25	32.04
96.00	F46	47.69	37.48	41.10	33.64	41.36	37.44
97.00	F47	44.64	35.03	42.49	26.63	43.60	40.73
98.00	F48	46.30	37.78	41.26	25.07	42.02	37.67
99.00	F49	47.52	38.91	42.63	35.66	43.08	38.28
100.00	F50	44.25	37.40	42.23	36.44	41.69	37.20
	Mean male	47.40	38.72	46.62	39.18	45.92	41.89
	SD Males	1.59		4.80		2.81	
	Mean female	46.61	37.81	42.47	35.18	43.36	38.48
	SD Females	1.58		2.08		1.44	
	Mean both	47.01	38.27	44.33	37.00	44.62	40.11
	Total mean	42.64		40.67		42.37	
	Control M	48.00	40.00	49.00	43.00	49.00	48.00
	Control F	40.00	50.00	43.00	36.00	41.00	36.00

Tab.12: Long intronic transcript methylation data, section 4

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	45.24	38.00	44.93	44.15	11.31	
11	M2	42.99	37.47			35.12	32.09
21	M3	44.55	39.99	38.16		12.17	16.94
31	M4	47.02	39.91	37.31	36.95	15.23	
41	M5	46.33	39.88	39.36	39.25	17.57	
51	M6	43.16	37.60	39.84	41.15	24.84	26.94
61	M7	47.20	39.72	38.41	41.32	58.15	
71	M8	44.34	38.34	36.91		25.85	
3	M9	43.61	39.47	35.34	37.81	12.23	18.63
13	M10	46.63	39.99	36.23	36.87	10.96	
23	M11	44.05	39.08	60.45	33.17	16.25	
33	M12	42.51	36.37	40.14	33.60	13.04	
43	M13	39.49	32.90	42.46	34.75	21.86	
53	M14	39.95	33.14	40.23	39.98	19.90	
63	M15	46.57	40.32	36.64	40.90	22.32	
73	M16	43.93	37.78	41.98	40.84	16.29	
5	M17	39.25	34.85	41.35	31.80	20.38	
15	M18	40.16	36.40	40.89	39.85	12.43	
25	M19	42.57	36.18	31.72	33.48	12.38	
35	M20	40.77	35.68	39.70	32.95	11.15	
45	M21	44.73	37.40	39.79	33.35	15.11	
55	M22	45.64	38.55	41.11	40.22	13.76	
65	M23	39.42	34.76	39.24	38.70		
75	M24			41.56	33.96		
7	M25	42.49	37.21	40.91	40.30		
17	M26	40.47	33.96	41.87	43.92		
27	M27	47.96	43.23	39.46	38.75	41.26	32.15
37	M28			40.11	38.72	11.44	
47	M29	44.33	37.96	35.04	37.47	11.97	
57	M30	45.32	38.22	36.56	28.64	12.01	
67	M31	44.30	36.60	40.12	39.22	13.67	
77	M32	44.33	38.17	38.64	38.55	13.86	
9	M33	45.46	39.11	41.89	40.10	18.78	
19	M34	42.92	36.79	40.50	33.70	12.03	
29	M35	46.70	39.92	35.35	34.94	15.94	
39	M36			39.88	35.22	14.11	
49	M37	45.45	37.79	38.67	32.42	12.70	
59	M38	46.06	39.67	37.70	31.23	23.00	

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Tab.12: Long intronic transcript methylation data, section 5

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	47.29	39.30	28.33	32.76	41.83	36.88
79	M40	47.13	40.20	39.67	43.01	18.21	20.30
81	M41	44.95	37.92	35.96	36.06	11.55	
82	M42	43.66	38.36	41.61	42.51	18.62	
83	M43	44.61	37.79	41.74	45.58	46.33	41.79
84	M44			41.95	44.13	50.47	43.09
85	M45	43.77	37.51	44.88	47.28	29.06	27.22
86	M46	46.48	41.60	39.29	39.33	14.41	
87	M47			39.10		17.28	
88	M48	46.91	41.97	38.82		14.39	
89	M49	45.60	39.53			16.24	18.42
90	M50	47.52	41.79			14.78	16.35
2	F1	45.28	38.58	42.58	37.82		
12	F2	46.91	42.21	41.00	36.09		
22	F3	47.60	40.60	44.22	38.91		
32	F4	44.36	38.31	42.50	35.76		
42	F5	47.20	41.02	43.24	37.19		
52	F6	45.47	41.10	43.19	38.51		
62	F7	48.75	41.72	43.58	38.16		
72	F8	44.76	39.17	42.64	37.58		
4	F9	47.48	41.45	42.55	36.77		
14	F10	45.97	45.38	43.00	37.07		
24	F11	45.50	39.70	45.69	40.55		
34	F12	46.29	39.74	43.77	39.50		
44	F13	46.47	39.43	43.49	37.92		
54	F14	41.93	39.42	44.96	39.51		
64	F15	47.63	41.69	43.24	39.81		
74	F16	45.14	38.89	41.96	37.21		
6	F17	46.37	40.91	42.94	39.27		
16	F18	49.94	48.92	45.11	39.51		
26	F19	48.28	42.66	43.51	38.06		
36	F20	45.50	39.38	42.99	38.85		
46	F21	45.58	42.29	41.81	36.99		
56	F22	47.24	41.77	41.82	38.36		
66	F23	48.64	43.14	44.19	39.78		
76	F24			41.29	37.08		
8	F25	42.32	36.25	39.79	37.77		
18	F26	47.28	39.90	39.84	35.80		
28	F27	43.99	38.37	44.06	38.72		
38	F28			40.24	38.49		

Tab.12: Long intronic transcript methylation data, section 6

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	47.62	40.44	41.89	38.70		
58	F30	46.03	39.29	41.09			
68	F31	44.89	39.51	41.67	38.44		
78	F32	44.15	38.45	40.23			
10	F33	44.88	37.96	37.54	40.92		
20	F34	52.10	46.58	36.26	38.15		
30	F35			38.12	38.43		
40	F36	45.51	38.65	36.39	38.48		
50	F37	40.78	33.98	41.13	39.18		
60	F38	45.94	39.67				
70	F39	44.42	39.50	39.57	40.96		
80	F40	43.71	38.48	41.76	41.34		
91	F41	47.78	39.80	40.35	41.76		
92	F42	47.03	41.16	42.32	40.81		
93	F43	41.80	35.83	41.87	43.92		
94	F44	45.53	40.57	41.71	43.82		
95	F45	45.94	39.25	40.31	41.84		
96	F46	51.58	46.00	37.14	40.63		
97	F47	47.02	41.73	42.42	46.00		
98	F48	49.78	46.45				
99	F49	48.40	46.18	39.74	42.47		
100	F50	47.69	46.51	40.93	43.44		
	Mean males	44.31	38.19	39.61	37.88	19.83	27.57
	SD Males	2.39		4.31		11.22	
	Mean female	46.26	40.81	41.70	39.27		
	SD Females	2.30		2.17			
	Mean both	45.31	39.53	40.67	38.60		
	Total mean	42.42		39.63		23.70	

Tab.12: Long intronic transcript methylation data, section 7

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	74.67	68.09	38.26	39.39	42.54	41.53
11	M2	54.78	45.35			37.55	39.12
21	M3	68.05	53.23	42.48	31.68	40.40	41.22
31	M4	58.33	46.77	41.41	38.66	39.30	39.33
41	M5	63.61	53.09	42.17	38.21		
51	M6	61.71	51.99	41.70	38.08	39.24	39.38
61	M7	59.98	51.44	39.93	38.83	38.67	38.85
71	M8	66.13	53.97	41.31	38.47	35.71	38.69
3	M9			35.91	30.21	37.25	38.00
13	M10	67.04	57.51	38.67	36.72	31.46	33.74
23	M11	60.78	51.82	43.59	42.03	34.17	36.49
33	M12	58.73	48.03	40.96	38.85	33.52	34.83
43	M13		50.41	42.62	40.69	35.30	37.92
53	M14	54.35	41.46	42.68	39.26	34.29	37.87
63	M15	53.38	43.18	41.81	39.01	32.97	36.35
73	M16	49.87	42.33	45.96	27.17	30.62	34.15
5	M17			34.61	22.21	40.76	40.42
15	M18	58.49	46.09	32.43	24.70	35.47	36.74
25	M19	54.37	42.65	41.54	35.87	37.51	37.83
35	M20	54.60	43.41	41.51	39.18	39.89	40.10
45	M21	57.66	47.36	38.86	32.22	38.56	39.43
55	M22	63.60	45.98	39.49	37.12	33.87	42.30
65	M23	62.40	52.87	41.31	34.62	28.80	34.23
75	M24	55.76	41.80	41.88	33.54	28.86	31.80
7	M25	57.29	45.80	32.03	22.87	30.12	31.51
17	M26	53.01	42.06	42.65	40.69	32.28	34.53
27	M27	52.80	41.04		44.96	35.62	37.29
37	M28	63.66	46.56	41.55	33.57	31.29	35.33
47	M29	56.79	48.10	38.34	37.58	23.75	28.47
57	M30			40.26	40.09	25.79	28.74
67	M31	53.34	42.15	43.15	42.10	22.85	25.63
77	M32	52.85	41.40	43.82	33.39	26.99	29.77
9	M33	56.99	45.73	39.06	33.84	30.71	30.25
19	M34	56.81	55.72	45.32	39.14	27.99	28.00
29	M35	67.53	56.52	48.65	34.80	21.87	26.46
39	M36	46.86	39.92	43.45	37.55	27.13	31.17
49	M37	56.04	46.85	43.74	35.86	27.11	29.20
59	M38	39.79	37.94	42.24	39.88	27.35	29.83

Tab.12: Long intronic transcript methylation data, section 8

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	50.28		44.64	36.77	23.31	27.10
79	M40	49.66	40.29	45.92	35.40	31.05	40.29
81	M41	53.64	42.86	36.12	32.08	36.44	32.38
82	M42	52.65	54.77	37.25	16.21	48.86	46.75
83	M43	47.92	46.14	36.62	29.92	38.73	32.03
84	M44	55.87	46.33	43.54	45.31	34.18	31.85
85	M45	50.83	41.91	34.23	32.58	35.38	32.05
86	M46	51.62	42.37	36.83	36.08	37.54	32.88
87	M47	55.95	39.71	32.22	24.35	32.60	31.06
88	M48	56.40	49.15	34.57	29.33	36.94	33.14
89	M49	49.28	40.97	36.97	30.44	35.24	31.92
90	M50	55.47	44.94			47.60	43.90
2	F1	48.42	40.26	35.87	27.10	34.37	37.18
12	F2	45.01	38.13	39.17	29.83	35.44	37.80
22	F3	52.35	43.36	68.30	39.67	34.14	37.89
32	F4	53.55	42.45	46.41	35.93	37.27	38.08
42	F5	43.68	35.16	50.02	38.59	27.27	32.02
52	F6	46.74	37.09	44.79	37.86	31.17	37.84
62	F7	43.94	35.10	42.88	36.94	27.56	31.68
72	F8	48.24	38.42	43.14	39.56	40.78	41.79
4	F9	44.14	35.70	36.35	26.20	35.81	36.55
14	F10	45.33	36.97	39.99	34.13	28.79	29.84
24	F11	48.05	38.66	50.82	42.52	28.41	29.85
34	F12	47.10	38.22	44.26	39.02	28.76	31.96
44	F13	46.42	37.98	45.79	43.73		
54	F14	45.32	38.61	48.81	48.71	34.10	35.11
64	F15	50.60	40.67	42.85	34.03	27.49	31.40
74	F16	48.45	40.32	44.03	40.64		
6	F17	44.86	37.02	40.86	32.81	33.33	36.49
16	F18	46.86	38.86	43.92	39.00	31.80	32.82
26	F19	42.91	34.27			30.56	34.34
36	F20	41.85	33.21	44.09	41.40	30.07	33.63
46	F21	49.28	41.70	42.84	38.71	27.86	32.71
56	F22	44.74	36.11	43.37	41.43	25.49	29.40
66	F23	44.40	39.42	41.78	38.83	30.26	33.66
76	F24	51.25	40.87	41.58	39.82	27.65	30.67
8	F25	48.53	36.56	36.60	27.85	29.70	30.82
18	F26	42.24	34.95	44.91	36.45	30.85	33.65
28	F27	44.87	35.96	42.22	33.81	34.05	36.55
38	F28	50.71	41.69	47.72	46.35	30.32	32.75

Tab.12: Long intronic transcript methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	48.20	39.17	42.92	39.34	26.52	31.81
58	F30	49.01	39.95	41.68	38.79	27.64	33.73
68	F31	47.36	35.20	41.87	33.99	31.00	34.47
78	F32	44.95	37.49			30.57	34.38
10	F33	49.87	39.50	37.49	34.25	36.07	37.46
20	F34	44.64	37.26		37.65	37.20	39.71
30	F35	51.02	39.46	42.00	36.26	37.85	38.06
40	F36	49.51	39.34	40.45	33.78	32.26	34.94
50	F37	48.88	40.89	39.70	32.97	38.77	38.87
60	F38	49.48	39.52	39.62	34.02	35.70	36.41
70	F39	44.79	35.06	41.75	34.52	37.17	37.35
80	F40	47.97	38.81	38.73	30.51	35.82	38.35
91	F41	46.17	39.23	34.65	29.79	39.58	33.23
92	F42	45.32	37.32	32.43	30.48	37.48	31.83
93	F43	45.09	39.74	33.04	31.57	45.02	41.90
94	F44	52.07	44.89	35.73	32.39	38.46	33.67
95	F45	47.00	36.01	31.68	37.24	41.71	36.67
96	F46	55.47	48.89	32.51	28.21	53.04	31.44
97	F47	48.64	44.28	41.08	40.71	37.09	32.20
98	F48	49.01	43.25	38.81	34.03	40.21	37.04
99	F49	43.67	33.94	37.80	36.89	40.98	38.19
100	F50	45.19	38.04	40.38	41.14	43.52	38.43
	Mean males	56.56	46.91	40.30	35.03	33.78	34.94
	SD Males	6.38		3.84		5.94	
	Mean female	47.26	38.70	41.65	36.24	34.10	34.93
	SD Females	3.00		5.95		5.66	
	Mean both	51.72	42.64	40.98	35.63	33.94	34.93

Tab.13: Myosin methylation data, section 1

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	76.37	81.14	45.38	46.87	63.81	73.15
11	M2	67.57	75.67	52.28	52.29	66.68	71.34
21	M3	79.48	82.99	46.69	47.98	63.93	70.32
31	M4	68.29	75.67	48.63	49.84	67.43	71.24
41	M5	80.17	83.84	54.07	55.89	69.08	76.41
51	M6	76.71	80.72	47.30	50.05	66.56	74.43
61	M7	71.81	78.36	45.07	50.56	63.62	69.99
71	M8	74.12	78.86	43.31	50.03	61.81	76.69
3	M9	66.22	74.62	50.38	49.97	67.0931	72.8725
13	M10	78.36	83.00	51.05	51.84	68.4433	73.0447
23	M11	65.80	74.80	45.82	47.10	66.6024	70.3282
33	M12	72.08	79.14	45.95	49.19	66.608	75.1965
43	M13	64.07	74.21	48.61	50.27	68.4934	72.6328
53	M14	70.19	78.02	38.24	50.59	65.0446	72.5621
63	M15	65.06	74.04	46.15	51.19	65.8693	70.4517
73	M16	65.93	73.21	43.0898	47.3127	65.6894	69.3178
5	M17	76.09	78.79	45.8513	49.2692	66.2675	69.764
15	M18	73.23	78.79	47.851	49.2005	67.8219	72.3358
25	M19	71.15	78.94	47.4478	48.6116	64.8098	76.19
35	M20	74.93	81.38	45.7841	45.3064	64.8956	67.5365
45	M21	82.37	88.18	54.7857	55.312		
55	M22	68.63	78.15	47.6208	55.9553		
65	M23	79.59	83.47	43.5617	49.2242	66.2417	73.2391
75	M24	72.82	80.07	45.375	50.463	66.6413	72.4294
7	M25	71.40	78.80	46.2872	47.5565	65.0572	71.1539
17	M26	76.73	82.35	49.343	49.3387	67.7911	68.6024
27	M27	73.52	80.39	45.9456	47.6137	67.339	70.8856
37	M28	71.05	79.47	48.2314	52.2911	62.1071	73.2077
47	M29	72.15	80.21	45.27	47.5516	65.7693	70.2266
57	M30	71.60	80.16	47.9704	53.608	65.9795	68.8202
67	M31	81.69	84.72			67.7996	73.7524
77	M32	74.92	79.69			67.4043	65.902
9	M33	74.29	80.61			65.264	71.5685
19	M34	80.08	85.30			64.9362	68.2333
29	M35	71.77	79.34			69.7918	71.8457
39	M36	77.10	83.74			68.744	75.7735
49	M37	72.56	82.12			66.1598	69.4645
59	M38	71.03	82.30			65.6626	69.3956

Tab.13: Myosin methylation data, section 2

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	77.10	82.58			67.04	71.29
79	M40	79.09	82.94			69.80	74.16
81	M41	73.58	79.72			66.95	72.31
82	M42	76.87	82.79	44.40	51.58	66.37	71.22
83	M43	74.49	80.65	45.19	54.61	67.06	71.55
84	M44	71.00	76.81	47.49	69.08	65.26	70.08
85	M45	77.52	82.57	41.12	55.08	66.64	70.87
86	M46	73.91	80.88	51.05	54.25	64.94	73.78
87	M47	79.03	82.50	51.32	58.26	69.19	76.29
88	M48	81.20	84.90	47.80	57.71	62.67	74.83
89	M49	75.42	80.35	45.55	55.93	61.57	76.40
90	M50	74.60	79.77	43.04	52.54	69.96	75.13
2	F1	81.06	87.58			64.27	73.22
12	F2	63.45	76.55	47.98	47.89	64.40	71.08
22	F3	81.24	82.36	44.69	49.20	68.20	76.61
32	F4	69.54	77.66	42.89	55.08	68.32	72.70
42	F5	77.41	83.16	46.93	52.09	69.35	72.57
52	F6	79.88	83.41	41.56	48.56	63.75	69.83
62	F7	71.76	82.40	47.31	54.00	64.96	72.76
72	F8	66.95	76.17	39.51	48.98	64.09	74.87
4	F9	66.51	77.44	45.62	50.22	68.45	67.15
14	F10	65.51	80.30	46.80	51.52	66.22	67.02
24	F11	74.84	78.82	43.06	45.43	66.01	66.10
34	F12	74.02	81.03	47.84	50.82	65.12	66.18
44	F13	74.22	81.62			62.67	63.55
54	F14			46.95	60.52	62.83	62.93
64	F15	82.61	86.67	37.40	58.18	65.55	70.05
74	F16	58.15	73.66	41.14	50.72	63.95	70.93
6	F17	68.55	76.36	50.07	56.75	69.38	74.12
16	F18	77.68	82.52	45.10	49.41	71.73	74.83
26	F19			46.07	49.47	73.24	75.64
36	F20	74.09	87.25	45.05	45.46	67.31	71.26
46	F21	74.56	81.86	43.71	46.96	68.44	69.63
56	F22	69.51	80.59	46.14022	51.7838	69.75	69.78
66	F23	58.79	72.07	45.78097	53.6503	68.59	72.94
76	F24	70.43	82.16	43.04	49.44	66.68	72.12
8	F25	67.41	77.91	48.73	49.86	66.89	71.73
18	F26	63.71	77.09	44.47	47.25	67.62	72.78
28	F27	75.38	84.29	45.25	48.53	65.50	73.54
38	F28	68.85	80.62	45.24	48.66	68.77	72.86

Tab.13: Myosin methylation data, section 3

Run orde	Mouse No	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	60.59	72.26	51.90	57.85	68.32	71.24
58	F30	76.78	85.12	46.48	53.92	67.56	72.21
68	F31	64.86	73.80	40.61	46.16	67.63	74.00
78	F32	63.17	73.67	40.46	47.74	65.46	71.90
10	F33			50.28	53.19	66.77	69.17
20	F34	75.16	84.95	49.51	50.61	63.69	69.62
30	F35	77.61	82.54	47.18	48.55	67.31	71.83
40	F36	66.48	75.81	47.10	49.72	67.10	69.81
50	F37			43.37	45.44	65.08	68.54
60	F38	76.69	80.81	50.19	58.35	67.84	69.17
70	F39	81.27	83.82	41.15	47.12	63.40	69.43
80	F40	67.62	76.01			66.45	71.87
91	F41	75.37	79.66	43.83	49.40	68.37	72.13
92	F42	72.37	78.84			67.14	69.55
93	F43	81.02	82.25	45.52	52.43	65.92	72.30
94	F44	80.41	82.87	42.24	51.30	66.79	72.52
95	F45	62.40	67.10	45.59	51.14	66.51	70.54
96	F46	75.63	82.41	44.87	51.77	68.05	73.77
97	F47	75.98	84.93	39.72	48.27	66.84	70.34
98	F48	71.72	78.77	40.15	49.13	68.48	72.24
99	F49	78.50	82.67	38.98	46.83	67.30	70.88
100	F50	63.28	71.29	45.07	53.97	67.49	68.75
	Mean male	73.89	80.23	46.93	51.57	66.26	72.05
	SD Males	4.55		3.31		2.01	
	Mean femal	71.80	79.81	44.84	50.73	66.83	71.01
	SD Female	6.65		3.31		2.14	
	Mean both	72.89	80.03	45.80	51.11	66.55	71.52
	Total mea	76.46		48.46		69.04	
	Control M	48.00	50.00	52.00	53.00		
	Control F	49.00	43.00	42.00	50.00	48.00	49.00

Tab.13: Myosin methylation data, section 4

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	73.02	80.77	70.95	79.29	74.54	75.97
11	M2	69.27	78.47			69.19	70.50
21	M3	65.98	74.12	70.64	81.34	68.25	73.28
31	M4	72.61	82.30	66.06	78.37	71.41	74.19
41	M5	74.13	82.50	69.56	73.79	68.90	71.54
51	M6	73.21	81.27	66.18	75.88	69.97	72.12
61	M7	67.87	81.33	69.27	77.17	65.23	72.03
71	M8	66.82	78.23	66.55	73.96	71.71	73.26
3	M9	69.77	80.40	63.41	77.40	69.48	73.10
13	M10	72.98	84.01	67.09	75.90	70.37	74.62
23	M11	68.32	77.91	64.84	74.18	70.87	73.43
33	M12	73.17	81.61	67.13	75.95	68.37	73.89
43	M13	66.12	77.64	64.89	73.39	70.08	74.36
53	M14	64.31	79.54	65.03	74.07	69.99	71.51
63	M15	69.40	79.25	60.24	72.74	68.08	71.91
73	M16	71.27	79.43	62.15	72.76	67.87	67.69
5	M17	72.90	82.06	66.64	75.59	68.60	71.93
15	M18	63.13	77.79	65.94	76.26	68.99	71.39
25	M19	72.00	81.87	69.15	81.34	77.80	77.93
35	M20	70.48	81.08			70.06	73.68
45	M21	72.11	80.33	66.23	74.68	71.06	72.52
55	M22	70.75	79.51	70.80	80.26	65.47	68.50
65	M23	71.02	78.38	67.57	76.09		
75	M24			69.08	81.00		
7	M25	69.52	79.70	64.53	75.24		
17	M26	65.49	77.99	65.20	78.80		
27	M27			66.69	75.61	72.97	75.62
37	M28			64.21	75.65	73.24	75.38
47	M29	72.68	81.26	66.75	77.17	72.77	75.52
57	M30	71.02	79.38	64.42	71.34	71.44	71.79
67	M31	73.29	80.28	65.50	76.46	70.92	72.92
77	M32	73.94	81.77	65.64	75.75	72.57	71.86
9	M33	72.04	81.96	65.66	76.25	76.15	80.49
19	M34	69.97	81.92	66.4547	76.8067	76.77	78.77
29	M35	73.95	81.48	63.8455	75.9144	78.61	77.59
39	M36			63.63	74.85	75.77	77.36
49	M37	70.49	81.74	66.58	75.48	76.68	76.76
59	M38	71.03	82.04	64.31	74.83	73.43	76.49

Tab.13: Myosin methylation data, section 5

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	71.16	81.32	64.33	74.75	73.68	75.96
79	M40	71.93	81.59	68.58	79.21	63.69	68.70
81	M41	71.35	82.07	66.67	75.57	77.20	79.12
82	M42	70.92	82.53	65.97	75.29	72.86	75.13
83	M43	72.65	81.85	64.52	76.28	74.88	76.78
84	M44			65.33	76.98	76.53	79.06
85	M45	71.48	82.10	67.09	79.02	72.70	73.93
86	M46			65.66	76.00	72.90	76.47
87	M47	69.74	79.87	67.14	79.97	73.34	73.71
88	M48			64.58	74.78	72.02	75.54
89	M49	65.28	79.30	68.18	80.25	77.07	79.17
90	M50	64.68	72.08	68.32	78.72	73.28	76.51
2	F1	62.13	76.77	67.87	78.24		
12	F2	68.90	80.15	63.86	73.69		
22	F3	68.58	80.82	67.22	78.44		
32	F4	60.86	74.10	65.99	75.52		
42	F5	66.83	74.89	62.42	74.92		
52	F6	68.94	82.03	65.82	76.05		
62	F7	70.78	81.83	65.95	73.18		
72	F8	66.59	79.06	63.91	75.27		
4	F9	70.06	81.76	62.20	73.25		
14	F10	69.67	78.77	61.10	73.51		
24	F11	72.15	81.97	66.18	76.96		
34	F12	73.38	82.80	65.26	76.81		
44	F13	66.83	75.01	68.63	78.86		
54	F14	68.89	79.13	64.42	73.78		
64	F15	68.98	76.77	68.96	77.41		
74	F16	70.71	80.94	66.74	78.86		
6	F17	65.07	78.49	65.11	76.07		
16	F18	67.99	77.41	66.76	76.93		
26	F19	65.39	80.51	64.67	74.48		
36	F20	72.71	81.30	67.25	77.25		
46	F21	64.01		66.62	77.92		
56	F22	67.87	79.39	65.18316	75.06875		
66	F23	70.02	79.38	60.97543	74.27797		
76	F24			63.82	75.92		
8	F25	68.00	79.77	68.57	78.72		
18	F26	72.06	82.61	62.39	73.37		
28	F27	65.11	79.50	62.04	75.81		
38	F28			65.12	77.16		

Tab.13: Myosin methylation data, section 6

Run order	Mouse No.	Brain	Bone marrow			Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	68.45	79.91	66.02	74.20		
58	F30	71.74	82.68	65.10	74.41		
68	F31	69.65	80.00	64.72	75.16		
78	F32	69.75	79.63	69.42	78.20		
10	F33	73.38	81.52	63.95	78.50		
20	F34	66.50	77.97	66.34	80.91		
30	F35			65.76	73.79		
40	F36	71.95	82.80	65.80	75.97		
50	F37	62.72	75.29	68.16	77.90		
60	F38	70.78	81.35	65.68	70.36		
70	F39	62.62	71.77	66.81	76.82		
80	F40	71.86	80.44	64.88	77.52		
91	F41	69.57	80.04	65.95	78.47		
92	F42	72.57	82.27	65.84	76.12		
93	F43	69.95	82.10	67.04	75.24		
94	F44	69.98	82.38	70.51	81.56		
95	F45	71.98	81.08	68.85	77.26		
96	F46	68.97	80.98	69.09	80.62		
97	F47	73.10	82.36	66.32	77.88		
98	F48	62.50		67.94	78.68		
99	F49	62.94	77.26	67.50	77.55		
100	F50	68.14	80.65	47.51	50.96		
	Mean males	70.31	80.28	66.23	76.42	71.91	74.35
	SD Males	2.89		2.18		3.46	
	Mean female	68.55	79.73	65.49	75.92		
	SD Females	3.32		3.37			
	Mean both	69.39	80.00	65.85	76.16		
	Total mean	74.69		71.01		73.13	
	Control M			47.00	49.00	46.00	47.00
	Control F	54.00	58.00	48.00	51.00		

Tab.13: Myosin methylation data, section 7

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	64.18	72.80	65.99	76.46	58.55	63.72
11	M2	65.02	74.44	64.04	76.16	55.03	60.07
21	M3	66.44	76.48	63.70	74.48	55.92	67.26
31	M4	64.03	78.92	66.50	74.64	53.62	60.37
41	M5	65.55	78.80	64.89	76.47	73.53	76.99
51	M6	62.42	76.22	66.13	76.70	66.94	71.79
61	M7	69.22	79.76	68.08	79.54	68.65	72.82
71	M8	62.73	75.57	63.94	75.68	46.85	54.42
3	M9			63.67	73.48	53.44	56.91
13	M10	65.69	77.87	68.78	79.16	54.64	62.62
23	M11	59.44	76.44	66.82	78.16	51.50	61.22
33	M12	60.30	72.42	68.85	77.80	57.46	62.73
43	M13	61.73	78.52	66.54	74.38	48.34	55.10
53	M14	64.19	77.60	65.83	76.18	54.92	64.27
63	M15	72.62	75.61	63.64	75.69	50.73	56.92
73	M16	65.05	74.74	64.06	75.46	48.14	53.68
5	M17	61.77		61.20	75.08	53.75	58.60
15	M18	69.00	76.27	64.53		53.67	58.34
25	M19	63.20		62.01	69.42	51.58	57.52
35	M20	66.15	77.27	64.54	74.80	49.58	60.08
45	M21	61.76	73.50			50.50	54.42
55	M22			63.99	76.13	52.31	63.51
65	M23	58.88		64.11	70.79	49.89	58.05
75	M24	66.49	73.52	64.50	64.86	46.25	53.64
7	M25	59.55				48.71	54.62
17	M26	67.82	76.87	65.24	75.37	46.79	51.81
27	M27	63.94	80.01			46.59	56.72
37	M28	65.99	72.37	64.92	73.87	47.93	54.60
47	M29	63.20	77.88	61.04	75.74	44.63	51.25
57	M30			62.05		41.80	46.45
67	M31	65.09	76.80	66.01	76.64	46.46	53.59
77	M32	66.49	76.90			50.21	55.69
9	M33	67.92	74.10			58.09	64.01
19	M34	70.07	72.44	63.6855	76.4901	49.32	53.10
29	M35	68.23		70.7687		46.36	52.06
39	M36	72.49	73.91	64.66	69.01	36.47	50.14
49	M37	68.69	73.08	62.58		43.01	48.00
59	M38	72.99	72.57	66.48	75.88	44.70	48.71

Tab.13: Myosin methylation data, section 8

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	66.91	67.91	65.13	77.09	46.69	52.83
79	M40	67.97	71.94	67.05		51.77	60.08
81	M41	68.34	73.50			53.26	61.96
82	M42	70.30	73.70			50.03	66.06
83	M43	72.52	75.68	64.68	77.28	50.91	59.38
84	M44	72.26	69.64	69.91	70.58	51.40	58.38
85	M45	70.75	70.42	64.60	77.33	49.95	59.05
86	M46	69.08	74.57	70.66	78.30	50.54	58.59
87	M47	71.71	74.05	65.93	75.92	51.23	59.79
88	M48	63.86		64.14	62.75	47.05	56.41
89	M49	65.73	70.12	64.14	75.13	50.94	58.32
90	M50	69.15	73.55	63.25	75.04	47.89	56.91
2	F1	64.21	70.21	66.13	76.36	51.42	56.12
12	F2	65.21	67.37	63.34	75.97	51.20	60.72
22	F3	66.57	71.23			44.35	54.63
32	F4	68.33	70.80	70.06	79.14	53.72	60.11
42	F5	68.15	71.03	63.89	73.62	47.51	60.81
52	F6	67.36	68.12	63.21	74.70	45.68	57.32
62	F7	68.23	72.84	65.13	76.18	51.17	60.59
72	F8	66.50	69.48	65.92	76.48	44.96	61.48
4	F9	67.60	69.65	70.32	77.04	52.52	56.56
14	F10	68.84	71.73	67.44	77.99	50.05	55.13
24	F11	64.57	68.51	65.32	75.02	49.34	60.09
34	F12	64.43	68.45	76.25	79.15	49.85	51.76
44	F13	68.32	69.61	69.25	70.83	53.55	57.54
54	F14	69.56	69.80	65.19	78.19	49.52	56.32
64	F15	67.67	69.64	66.54	78.59	48.35	57.26
74	F16	68.78	73.04	64.78	73.50	58.27	61.51
6	F17	68.12	70.74				
16	F18	70.00	72.20				
26	F19	70.06	66.89				
36	F20	70.73	71.43				
46	F21	65.49	75.34				
56	F22	70.86	71.68				
66	F23	70.91	73.67	64.27932	74.7049		
76	F24	70.50	76.63	64.60	73.01		
8	F25	65.15	67.95	67.59	76.79	48.58	54.49
18	F26	71.51	68.46	62.31		46.72	53.49
28	F27	69.22	69.38	66.80	80.40	49.44	59.79
38	F28	66.75	76.82	63.32	73.13	49.69	55.69

Tab.13: Myosin methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	70.08	71.07	66.48	72.89	46.18	56.52
58	F30	68.33	72.02	63.20	73.22	48.71	55.41
68	F31	66.13	67.25	63.71	69.39	49.57	56.10
78	F32	69.97	72.46			49.98	57.15
10	F33					47.11	52.41
20	F34					50.56	57.87
30	F35					49.30	54.52
40	F36					46.02	56.69
50	F37					50.52	57.91
60	F38					52.17	58.87
70	F39					49.29	53.15
80	F40					47.66	52.61
91	F41	63.84	67.26	64.16	68.75	48.28	53.50
92	F42	66.37	69.69			49.35	52.59
93	F43	68.62	71.73	65.60	67.24	50.01	54.34
94	F44	67.17	68.20			49.65	57.03
95	F45	69.06	71.95	62.49	75.62	47.96	53.29
96	F46	70.88	71.32	63.13	74.29	47.25	50.51
97	F47	72.12	71.50	66.59	76.51	45.99	50.95
98	F48	67.01	71.36	66.96	77.94	44.49	48.52
99	F49	63.13	72.31	68.11	78.14	48.02	51.84
100	F50	67.43	70.18	69.06	76.39	50.16	56.42
	Mean males	66.32	74.85	65.19	74.84	51.17	58.27
	SD Males	3.77		2.27		6.34	
	Mean females	67.95	70.74	65.97	75.20	49.15	55.94
	SD Females	2.25		2.88		2.67	
	Mean both	67.09	72.77	65.53	75.00	50.25	57.21
	Total mean	69.93		70.26		53.73	
	Control M	50.00	49.00	67.00	78.00	48.00	49.00
	Control F	51.00	49.00	68.00	79.00	48.00	49.00

Tab.14: Paternally expressed gene 3 methylation data, section 1

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	44.20	49.08	37.79	45.72	44.76	51.38
11	M2	43.45	48.55	39.49	51.33	44.64	
21	M3	49.04	53.78	38.49	49.63	42.15	50.73
31	M4	42.62	48.98	39.85	46.92	38.95	48.17
41	M5	42.84	49.59	40.61	51.11	42.13	52.01
51	M6	42.69	49.13	40.05	50.78	46.37	53.57
61	M7	43.88	49.32	38.80	53.70	40.76	51.32
71	M8	43.15	50.03	38.49	48.06	46.28	
3	M9	42.44	48.05	41.43	55.12	44.60	53.23
13	M10	44.27	49.54	37.30	51.68	42.85	48.74
23	M11	42.83	48.27	39.11	51.35	41.89	49.33
33	M12	44.16	50.15	37.50	47.10	41.88	46.71
43	M13	42.23	48.19	38.35	49.65	46.01	53.47
53	M14	42.55	47.88	41.23	81.77	47.53	54.46
63	M15	42.49	48.74	39.91	51.08	43.05	51.33
73	M16	43.92	48.85	39.66	51.57	42.47	50.85
5	M17	40.82	44.62	38.30	42.22	42.97	50.39
15	M18	43.08	49.29	37.73	48.44	44.36	52.20
25	M19	42.60	47.76	37.17	46.88	44.29	52.35
35	M20	42.80	48.19	37.73	48.65	44.36	51.27
45	M21	43.51	49.63	80.71	50.62		
55	M22	44.97	50.93	37.70			
65	M23	43.40	49.43	38.53		45.48	55.58
75	M24	42.40	48.32	41.27		44.81	53.58
7	M25	42.56	47.25	37.42		40.71	47.89
17	M26	43.39	48.63	37.41		42.66	50.35
27	M27	43.07	48.70	37.60		43.56	50.91
37	M28	41.93	47.40	38.95	53.94	44.19	53.56
47	M29	43.05	48.52	38.21	53.84	42.43	51.24
57	M30	43.49	49.23	37.82		41.28	49.75
67	M31	43.98	50.71	70.12		46.73	57.50
77	M32	42.55	49.05	74.56		44.58	56.52
9	M33	43.54	49.35			43.54	46.64
19	M34	44.06	48.85			45.91	54.64
29	M35	44.34	49.84	68.65		44.34	51.91
39	M36	42.34	48.53			46.30	57.34
49	M37	41.50	48.76	70.48		40.26	49.94
59	M38	43.18	49.97	73.18		45.04	54.75

Tab.14: Paternally expressed gene 3 methylation data, section 2

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	43.34	49.06	55.93		42.42	51.58
79	M40	42.45	48.80			44.27	54.54
81	M41	42.92	46.23	52.40		49.00	55.77
82	M42	41.40	48.09	38.61		45.86	50.96
83	M43	41.81	46.02	46.25		46.27	51.98
84	M44	40.45	47.03			51.21	56.92
85	M45	41.35	47.55			46.16	54.10
86	M46	42.53	47.73	46.77		48.64	57.37
87	M47	40.95	47.36	46.57		48.08	57.01
88	M48	41.95	48.26	44.21		50.52	58.97
89	M49	40.96	46.56	47.17		47.62	59.38
90	M50	41.56	47.97	52.79		45.76	53.83
2	F1	46.75	53.98	38.24	45.68	40.73	47.47
12	F2	46.82	50.42	38.21	43.73	44.13	51.15
22	F3	48.94	55.65	39.37	49.53	41.87	51.40
32	F4	45.75	50.64	36.04	45.79	43.14	51.17
42	F5	46.74	52.26	36.21	47.70	40.96	51.69
52	F6	48.06	54.08	37.04	52.31	45.56	55.93
62	F7	47.01		36.61	52.46	44.25	55.41
72	F8	45.16	50.66	35.82	53.15	41.45	52.40
4	F9	47.33	52.96	37.63	53.60	41.71	47.18
14	F10	48.08	53.52	39.76	69.66	45.93	51.21
24	F11	46.04	51.59	41.06	48.76	41.51	49.79
34	F12	45.27	51.51	38.30	48.62	45.72	53.10
44	F13	43.71	50.23	37.97	73.44	45.09	50.79
54	F14	54.93		39.16	44.23	45.23	53.49
64	F15	43.21	50.23	39.15	52.66	43.58	51.39
74	F16	41.77		39.20	48.06	42.90	50.34
6	F17	42.16	46.96	45.03	79.68	44.41	52.04
16	F18	43.23	48.24	40.46	47.14	43.10	51.12
26	F19			36.91	45.71	42.76	50.18
36	F20	42.85		40.75	50.09	42.39	49.32
46	F21	44.70	49.71	38.47	50.83	42.72	46.92
56	F22	42.31		40.50	49.81	42.35	49.28
66	F23	41.62	46.23	42.22	54.01	44.64	50.78
76	F24	40.73		38.90	47.94	44.83	52.76
8	F25	43.04	47.35	39.52	46.85	44.00	50.56
18	F26	38.71	45.55	36.92	45.34	42.80	51.07
28	F27	41.8701	50.19005	40.19207	48.67804	42.10312	49.3933
38	F28	42.5194	50.10736	42.95611	53.89331	43.27993	49.5975

Tab.14: Paternally expressed gene 3 methylation data, section 3

Run order	Mouse No.	Spleen		Lungs		Skin	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	41.53		37.57	50.58	43.03	50.86
58	F30	43.19	49.87	39.25	50.22	43.37	51.61
68	F31	41.12	47.57	37.54	48.59	41.11	49.82
78	F32	41.04	47.94	40.64		42.23	50.04
10	F33			34.69	37.12	41.06	53.12
20	F34	40.87		38.02	45.80	41.88	50.89
30	F35	42.41	48.28	38.50	48.17	43.30	51.89
40	F36	42.67	47.77	37.13	43.76	42.35	48.71
50	F37	41.38		36.98	45.31	41.34	48.09
60	F38	43.44	50.07	37.09	52.18	41.15	49.27
70	F39	43.09	50.31	36.34	52.14	41.54	49.90
80	F40	41.64	47.84	36.08	42.31	41.49	49.55
91	F41	42.35	46.80	37.85		40.93	49.58
92	F42	41.42	46.37	39.05		42.38	50.51
93	F43	42.37	47.07	37.55	46.91	41.37	48.07
94	F44	43.01	48.03	35.81	44.42	40.41	47.57
95	F45	43.16	46.00			40.93	46.11
96	F46	43.36	54.21	37.15	43.46	39.99	48.46
97	F47	44.52		35.86	42.15	42.20	49.72
98	F48	41.48	46.16	36.21		40.40	49.74
99	F49	41.96	46.97	39.51		41.34	51.02
100	F50	41.99	46.91	37.93		42.70	51.18
Mean males		42.90	48.63	45.28	51.36	44.58	52.74
SD Males		1.34		12.05		2.60	
Mean females		43.69	49.48	38.35	49.83	42.59	50.45
SD Females		2.82		2.02		1.52	
Mean both		43.29	49.00	41.63	50.36	43.57	51.55
Total Mean		46.14		45.99		47.56	
Control M		42.00	49.00	49.00	57.00	39.00	46.00
Control F		43.00	49.00	36.00	49.00	38.00	48.00

Tab.14: Paternally expressed gene 3 methylation data, section 4

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	42.83	50.80	40.49	47.51	17.28	24.75
11	M2	43.39	51.06			33.23	41.67
21	M3	41.76	50.13	41.94		16.66	24.70
31	M4	43.97	50.04	41.02		11.22	
41	M5	42.11	48.98	37.75		19.19	24.90
51	M6	39.03	45.69	38.79		27.57	34.24
61	M7	43.48	49.64	40.62		41.83	53.05
71	M8	39.37	46.58	49.00		24.05	31.49
3	M9	45.37	54.33	42.31		17.17	24.19
13	M10	43.73	50.90	39.15	45.49	16.68	22.78
23	M11	47.61		39.77	47.95	18.01	25.53
33	M12			39.95	48.10	18.03	25.41
43	M13	56.96	61.88	40.81	49.14	16.50	27.93
53	M14	53.20	58.75	41.93	47.53	17.16	25.82
63	M15	42.00	50.03	38.72	44.94	17.73	26.08
73	M16	45.32	51.94	38.62	46.83	18.99	26.68
5	M17	45.02	52.65	41.19	46.84	18.15	25.11
15	M18	52.12	59.70	41.87	46.68	16.39	21.79
25	M19	44.28	50.74	42.03		16.86	24.48
35	M20	41.00	47.78	38.04	47.83	15.96	22.47
45	M21	43.17	50.60	40.66	47.71	21.97	28.73
55	M22	44.75	51.34	40.55	46.96	20.15	27.74
65	M23	44.03	50.75	39.78	46.47	16.41	23.20
75	M24			40.17	46.53	14.83	22.08
7	M25	45.23	52.54	40.84	45.14	14.59	73.02
17	M26	47.73	53.46	39.37		16.09	22.88
27	M27	49.23	57.38	38.56	46.23	49.64	56.74
37	M28			39.34	45.54	11.37	22.99
47	M29	42.88	49.03	38.44	46.21	12.34	22.41
57	M30	44.54	50.01	40.39	46.08	15.73	23.64
67	M31	42.41	48.75	38.01		13.48	21.37
77	M32	40.59	46.37	39.05	45.71	15.19	21.90
9	M33			40.14	46.06	13.89	21.00
19	M34	44.03	51.43	40.30	48.09	12.62	21.45
29	M35	43.26	49.33	39.14	45.86	16.78	23.76
39	M36			38.53	44.43	14.38	21.62
49	M37	41.87	49.14	38.81	44.13	14.12	21.13
59	M38	44.73	51.71	38.80	45.19	21.81	28.74

Tab.14: Paternally expressed gene 3 methylation data, section 5

Run order	Mouse No.	Brain		Bone marrow		Testes	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39	43.52	50.52	39.43		38.40	45.18
79	M40	43.88	49.76	38.18		21.08	28.39
81	M41	43.79	50.77	40.73	45.60	13.89	25.17
82	M42	41.87	49.65	42.29	47.96	15.03	22.77
83	M43	45.40	52.71	42.14	48.90	37.89	55.13
84	M44			43.39	52.02	41.95	50.65
85	M45	42.28	47.81	40.56	46.61	29.94	35.74
86	M46	43.46	51.63	45.33	51.07	16.01	23.44
87	M47	48.12	55.71	42.51		18.12	25.61
88	M48			54.27		16.16	24.04
89	M49	44.10	51.87			14.20	22.61
90	M50	43.50	51.94	38.58	44.24	17.61	26.77
2	F1	50.35	59.24	38.78	44.66		
12	F2	39.56	48.67	41.57	46.84		
22	F3	46.37	53.15	39.97	46.79		
32	F4			38.91	47.31		
42	F5	41.61	50.25	40.38	48.23		
52	F6	52.72	58.01	39.30	46.64		
62	F7	43.73	49.31	40.11	45.93		
72	F8	53.10	59.32	40.01	47.09		
4	F9	45.34	51.79	40.15	45.96		
14	F10	51.26	61.01	40.96	46.72		
24	F11	47.88	54.76	39.78	46.49		
34	F12	46.18	52.91	42.54	49.47		
44	F13	54.48	60.64	41.15	47.74		
54	F14	48.20		41.85	48.05		
64	F15	51.00		41.55	48.26		
74	F16	53.11	57.53	42.16	48.83		
6	F17	45.38		37.38	46.37		
16	F18	52.30	61.74	41.95	48.44		
26	F19	44.50	51.60	40.60	48.07		
36	F20	45.13	52.75	40.21	48.23		
46	F21	54.04		40.60	47.52		
56	F22	50.63	56.49	39.91	45.94		
66	F23	45.85	51.59	39.38	45.37		
76	F24			40.81	47.05		
8	F25	56.24	63.11	41.08	46.33		
18	F26	44.59	52.04	39.82	47.20		
28	F27	56.97	62.96	40.62	48.10		
38	F28			39.82	47.42		
		46.92	53.68	39.71	45.61		

Tab.14: Paternally expressed gene 3 methylation data, section 6

Run order	Mouse No.	Brain		Bone marrow		Testes	
48	F29	Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
58	F30	45.78	53.12	39.70			
68	F31	46.55	53.14	40.60	46.09		
78	F32	46.65	52.04	42.36			
10	F33	41.92	50.34	42.83	46.73		
20	F34	54.76	61.50	42.14	50.01		
30	F35			42.02			
40	F36	44.63	49.80	44.27	54.19		
50	F37	51.34	56.75	41.73			
60	F38	37.62	51.45				
70	F39	48.28		40.14	47.65		
80	F40	39.64	45.76	42.74	47.12		
91	F41	42.84	51.08				
92	F42	43.50	51.06				
93	F43	41.63	49.35				
94	F44	42.83	49.02				
95	F45	44.10	50.76				
96	F46	51.40					
97	F47	56.02					
98	F48	45.99					
99	F49	49.72	58.61				
100	F50	42.14					
	Mean males		51.33	40.67	46.81	19.89	29.04
	SD Males	3.42		2.85		8.63	
	Mean females		54.22	40.76	47.38		
	SD Females	4.87		1.34			
	Mean both		52.69	40.71	47.10		
	Total Mean	26.34		43.91		24.46	
	Control M	45.00	54.00	41.00	48.00	39.00	55.00
	Control F			42.00	50.00		

Tab.14: Paternally expressed gene 3 methylation data, section 7

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
1	M1	77.02		51.98	57.96	47.41	57.62
11	M2	65.27		49.31	54.31	42.22	58.60
21	M3	73.22		46.12	50.48	48.50	61.70
31	M4	62.33		42.72	49.77	50.40	59.61
41	M5	73.61	82.16	45.53	51.48	46.85	55.07
51	M6	60.79		45.49	51.79	47.24	55.09
61	M7	56.86		46.29	53.43	46.19	54.76
71	M8	64.95		52.43	57.60	53.26	65.26
3	M9			50.15	54.54	45.54	54.15
13	M10	65.93		47.89	52.34	45.85	55.88
23	M11	64.99		47.22	51.87	49.49	59.89
33	M12	72.21		48.15	54.02	46.34	56.22
43	M13	65.21		46.17	54.10	46.39	57.37
53	M14	69.96		51.85	58.96	50.74	60.64
63	M15	61.11		49.31	58.77	45.33	53.40
73	M16	55.85		56.94		46.36	60.46
5	M17			54.12		47.74	57.26
15	M18	67.37		50.85		46.36	53.37
25	M19	74.63		53.04		49.44	58.27
35	M20	73.36		52.16	58.97	47.87	55.51
45	M21	58.07		49.88		47.56	57.44
55	M22	52.62		65.97		50.53	64.91
65	M23	52.15		51.24		45.10	55.91
75	M24	63.80		55.46		44.29	56.43
7	M25	73.60		47.69		44.42	55.35
17	M26	67.81		52.22	58.79	47.06	58.19
27	M27	68.90				51.06	61.00
37	M28	72.63		53.27	62.04	47.23	57.38
47	M29	71.33		53.79	58.63	45.00	61.72
57	M30	52.68		54.66		48.13	59.62
67	M31	58.59		50.96		39.84	51.59
77	M32	56.62				45.07	54.23
9	M33	66.39		48.56	56.18	41.80	49.42
19	M34	64.70		52.96	58.32	44.46	51.35
29	M35	63.57		50.19		46.55	60.54
39	M36	53.31				43.48	53.32
49	M37	52.53		50.55		45.20	54.62
59	M38	53.73				44.41	53.54

Tab.14: Paternally expressed gene 3 methylation data, section 8

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
69	M39			53.99	60.64	40.95	51.65
79	M40	56.53		56.26		41.96	
81	M41	70.85		51.14	57.35	56.87	69.10
82	M42	67.70	80.82	52.94		51.38	
83	M43	52.67		57.53	64.33	42.34	
84	M44	72.30		48.23		49.58	62.20
85	M45	71.83	78.15	53.00		49.92	64.73
86	M46	68.47		51.21	55.85	44.51	
87	M47	67.40		48.02		43.99	63.10
88	M48	57.55		54.29	60.97	42.80	61.01
89	M49	68.12	75.33	52.33	61.17	45.60	62.92
90	M50	78.40	85.33	59.71	67.46	48.31	
2	F1	64.36		41.70		59.17	
12	F2	53.76		50.44		54.55	71.29
22	F3	51.50		50.08		46.97	
32	F4	61.65	67.50	53.03		60.44	
42	F5	53.77	63.17	58.10	60.36	53.75	
52	F6	60.84	69.69	47.01		55.94	
62	F7	57.38	67.52	46.68	60.13	43.69	56.26
72	F8	57.03	66.70	49.43	56.74	44.60	
4	F9	52.37	62.89	62.74		47.68	55.96
14	F10	55.83		50.09	55.57	47.59	54.29
24	F11	51.57		54.45		47.35	61.16
34	F12	59.58		47.37	56.05	48.66	
44	F13	64.26		47.70	57.74	45.71	57.31
54	F14	54.15		52.79	58.65	46.41	55.92
64	F15	52.49		47.80		48.31	57.59
74	F16	56.28		41.59	46.46	45.68	59.19
6	F17	56.28		46.35	53.21	40.61	
16	F18	55.79		59.38		44.59	55.38
26	F19	57.55		47.94	53.38	47.14	55.46
36	F20	53.28		51.92	57.99	44.97	53.79
46	F21	59.68		43.74	49.01	45.32	54.79
56	F22	50.60		48.91	54.63	44.08	52.99
66	F23			48.95	55.17	44.11	52.36
76	F24	61.14		46.82	55.36	44.36	52.55
8	F25	60.51		46.85	53.43	44.50	54.42
18	F26			44.74		51.69	64.11
28	F27	57.22		42.11	47.44	48.20	60.61
38	F28	58.55		46.24	54.68	43.61	52.75

Tab.14: Paternally expressed gene 3 methylation data, section 9

Run order	Mouse No.	Tongue		Muscles		Heart	
		Primer 1	Primer 2	Primer 1	Primer 2	Primer 1	Primer 2
48	F29	51.81		45.48	52.71	42.55	55.08
58	F30	56.07		44.92	51.52	49.63	60.19
68	F31	51.41		49.97		43.90	55.08
78	F32	58.49		47.08		48.39	57.40
10	F33		69.58	50.70	57.70	44.47	52.07
20	F34	59.36				46.40	59.53
30	F35			43.45	49.22	48.95	57.59
40	F36			46.56	51.59	49.37	58.33
50	F37			48.91	56.41	48.73	56.67
60	F38	51.43		44.86	50.35	46.70	54.09
70	F39	49.57		47.36	53.64	45.78	54.82
80	F40			48.47	55.78	47.27	56.28
91	F41	55.85		45.60	58.81	47.50	57.31
92	F42	46.14		46.46	55.78	45.34	54.86
93	F43	57.34		51.20	61.19	45.45	56.64
94	F44	60.52		43.34		45.35	57.13
95	F45	51.12		46.69	55.22	46.49	58.36
96	F46	53.51		50.73		42.50	53.21
97	F47	58.02		48.10	53.96	46.00	56.75
98	F48	52.54		55.25	62.24	42.97	62.93
99	F49	48.17		54.49	61.65	48.62	63.54
100	F50	49.42		50.01	56.42	43.51	57.81
Mean males		64.67	80.36	51.39	56.86	46.58	57.81
SD Males		7.51		4.13847		3.25	
Mean females		55.54	66.72	48.66509844		47.11	57.00
SD Females		4.29		4.370357973		3.98	
Mean both		60.31	72.40	49.9827036		46.84	57.42
Total Mean		66.36		52.94631598		52.13	
Control M		46.00	54.00	43		41.00	51.00
Control F		48.00		42	47	40.00	51.00

11 Appendix 2, Correlation of methylation between different loci within one tissue, complete data

Analysed data has been compiled into Excel tables. It is all presented in Tab.14.1-22.2. While the raw data was sorted by the fragment analysed, the end result is sorted by the tissue. In these tables, the Pearson correlation coefficient, mean deviation and number of samples analysed for each sex is presented (males and females were analysed separately).

Tab.15 and 16: Male bone marrow. Correlation observed between: LINE-1-MyLC (both primers)

15

Bone marrow						
Males	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.1183	0.26209	0.28854	0.22727	-0.2189	0.0587
	0.4336	0.082	0.0575	0.1427	0.1534	0.7456
	46	45	44	43	44	33
MYC			0.10046	0.36704	0.09888	0.03857
			0.5165	0.0168	0.5181	0.8312
			44	42	45	33
LIT1					0.04719	0.21029
					0.7638	0.2401
					43	33

16

Bone marrow						
Male	SNRPND1		IAP		L1	
A Actin	SN1	SN2	SN1	SN2	SN1	SN2
	0.31677	0.24223	0.04568	-0.0778	-0.1494	0.09929
	0.0494	0.2332	0.7631	0.6156	0.3162	0.5115
	39	26	46	44	47	46
MYC	0.20285	0.13757	0.25232	0.41157	0.606	0.55107
	0.2219	0.512	0.0907	0.005	<.0001	<.0001
	38	25	46	45	48	48
LIT1	0.12008	0.38968	0.12891	0.23556	0.08899	0.25157
	0.479	0.0491	0.41	0.1332	0.561	0.1037
	37	26	43	42	45	43
PEG3	-0.067	-0.1236	0.24168	0.29867	0.07514	-0.0143
	0.6852	0.5562	0.1097	0.0913	0.6197	0.936
	39	25	45	33	46	34
SNRPND1			0.32027	-0.1201	0.28886	0.41274
			0.0468	0.5589	0.0745	0.0361
			39	26	39	26
IAP					0.2651	0.47932
					0.0686	0.0008
					48	46

Tab.17 and 18: Female bone marrow. Correlation observed between Iap-MyLC (both primers) and Iap-LINE-1 (both primers)

17

Bone marrow						
Female	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.00246	0.09272	0.22446	0.16496	-0.1893	-0.2449
	0.9869	0.5354	0.1251	0.2733	0.2485	0.1563
	47	47	48	46	39	35
MYC			-0.1814	0.27025	0.06298	0.28895
			0.2223	0.0726	0.7033	0.0923
			47	45	39	35
LIT1					-0.4122	0.00323
					0.00091	0.9853
					39	35

18

Bone marrow						
Female	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.1496	0.24452	-0.0864	0.2458	0.0379	0.11163
	0.3506	0.2098	0.5594	0.0922	0.8003	0.455
	41	28	48	48	47	47
MYC	0.30871	0.28016	0.70082	0.59966	0.64942	0.25718
	0.0467	0.1487	<.0001	<.0001	<.0001	0.0776
	42	28	49	48	48	48
LIT1	-0.2146	0.2919	-0.1263	0.58577	-0.2867	0.2059
	0.1779	0.1318	0.3922	<.0001	0.0507	0.1748
	41	28	48	46	47	45
PEG3	0.3457	0.10271	-0.0007	0.09555	0.1694	0.21518
	0.0311	0.6176	0.9965	0.585	0.3093	0.2216
	39	26	39	35	38	34
SNRPND1			0.25365	0.02291	0.28589	0.14074
			0.105	0.9079	0.07	0.4838
			42	28	41	27
IAP					0.7081	0.56472
					<.0001	<.0001
					49	48

Tab.19 and 20: Male brain: Correlation observed between: Peg3-A Actin (both primers), SnrpnD1-A Actin (primer 1), SNPRD1-MyLC (primer 1), SNPRND1-Peg3 (primer 1)

19

Brain						
Male	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.5239	-0.3416	-0.483	-0.2513	0.73014	0.75344
	0.0003	0.025	0.0009	0.0998	<0.0001	<.0001
	43	43	44	44	43	42
MYC	-		0.26833	0.04429	-0.5226	-0.311
			0.0858	0.7806	0.0005	0.0508
			42	42	41	40
LIT1					-0.3189	-0.2512
					0.0396	0.1131
					42	41

20

Brain						
Male	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.75479	0.28035		0.17475	0.11446	0.24946
	<.0001	0.2312		0.2566	0.454	0.0984
	37	20		44	45	45
MYC	-0.7045	-0.3247		-0.1839	-0.0011	-0.0495
	<.0001	0.1625		0.2436	0.09943	0.7526
	37	20		42	43	43
LIT1	-0.7045	-0.3521		0.5157	0.05444	0.23387
	<.0001	0.1279		0.0003	0.7225	0.122
	37	20		44	45	45
PEG3	0.70027	0.41589		0.11895	-0.0189	0.20687
	<.0001	0.0766		0.4589	0.9043	0.1887
	35	19		41	43	42
SNRPND1				-0.1852	0.11408	-0.1532
				0.4344	0.5014	0.5192
				20	37	20

Tab.21 and 22: Female brain: Correlation observed between: A-Actin-MYC (Primer 2), A Actin-Peg3 (primer 2), Peg3-SnrpnD1 (primer 1)

21

Brain								
Female	A Actin		MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2	SN1	SN2
A Actin			-0.4963	-0.6102	-0.2432	-0.0764	0.47698	0.70936
			0.0005	<.0001	0.1074	0.6177	0.0011	<.0001
			45	44	45	45	44	36
MYC					-0.0182	-0.0479	-0.3443	-0.4651
					0.9035	0.7546	0.0191	0.00037
					47	45	46	37
LIT1							0.00625	0.21383
							0.9671	0.2038
							46	37

22

Brain						
Female	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.42094	0.12843		0.04904	-0.0612	0.18368
	0.0118	0.6483		0.749	0.6897	0.2271
	35	15		45	45	45
MYC	-0.2497	0.32187		0.03146	-0.0288	0.09864
	0.142	0.242		0.8374	0.8478	0.5192
	36	15		45	47	45
LIT1	-0.063	-0.1021		0.50545	0.17759	0.26377
	0.7152	0.7174		0.0003	0.2324	0.0732
	36	15		47	47	47
PEG3	0.65868	0.52023		0.40183	0.2783	0.29167
	<.0001	0.0565		0.0137	0.0611	0.0798
	35	14		37	46	37
SNRPND1				0.1494	0.44682	-0.3097
				0.5951	0.0063	0.2614
				15	36	15
IAP						0.30854
						0.0349
						47

Tab.23 and 24: _Male heart: Correlation observed between MyLC-Lit1 (primer 2), MyLC-LINE-1 (primer 1)

23

Heart						
Male	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.21843	-0.0171	0.28205	-0.2249	-0.0407	0.50092
	0.1275	0.9518	0.0496	0.4395	0.7812	0.0005
	50	15	49	14	49	45
MYC			0.48272	14	0.08095	0.11772
			0.0004	0.57195	0.5803	0.4412
			49	<.0001	49	45
LIT1				49	0.46774	0.30551
					0.0008	0.0437
					48	44

24

Heart						
Male	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.27003	-0.0316	0.23942	0.64879	0.13716	0.32901
	0.0606	0.9109	0.094	0.0089	0.3422	0.2311
	49	15	50	15	50	15
MYC	0.23762	-0.0142	0.45165	0.25373	0.55408	0.28047
	0.1002	0.3306	0.001	0.0754	<.0001	0.0485
	49	49	50	50	50	50
LIT1	0.172	0.10334	0.20466	0.17872	0.07015	-0.0833
	0.2424	0.4846	0.1584	0.2192	0.632	0.5691
	48	48	49	49	49	49
PEG3	0.20212	0.50092	-0.1748	-0.176	-0.2328	-0.3188
	0.1683	0.0005	0.2297	0.2474	0.1074	0.0328
	48	45	49	45	49	45
SNRPND1			0.04364	0.06374	-0.0549	-0.1953
			0.7659	0.6635	0.7077	0.1786
			49	49	49	49
IAP					0.48112	0.40294
					0.0004	0.0037
					50	50

Tab.25 and 26: Female heart: Correlation observed between Peg3-SnrpnD1 (primer 2)

25

Heart						
Female	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
	0.07085	0.21108	0.10606	0.22972	0.21344	0.18608
	0.664	0.6881	0.483	0.6202	0.1544	0.6895
	40	6	46	7	46	7
MYC			0.18892	0.10593	-0.1449	0.23444
			0.243	0.5153	0.3723	0.1752
			40	40	40	35
LIT1					-0.0964	0.28502
					0.5238	0.0746
					46	40

26

Heart						
Female	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.02792	-0.218	0.12057	0.04575	0.00425	0.25413
	0.8539	0.6782	0.4143	0.9224	0.9774	0.5824
	46	6	48	7	47	7
MYC	0.00712	0.2248	0.14669	0.18707	-0.0585	0.43557
	0.9657	0.1689	0.3539	0.2355	0.7127	0.0044
	39	39	42	42	42	41
LIT1	0.45314	0.25378	0.09616	0.43176	-0.184	0.12509
	0.0018	0.0925	0.5156	0.0022	0.2156	0.4075
	45	45	48	48	47	46
PEG3	0.3313	0.60354	0.27379	-0.0614	0.3181	0.22766
	0.0262	<.0001	0.0597	0.6991	0.0293	0.1523
	45	40	48	42	47	41
SNRPND1			0.30716	0.25434	0.21125	0.34004
			0.0357	0.0845	0.1588	0.0223
			46	47	46	45
IAP					0.44264	0.26189
					0.0014	0.0722
					49	48

Tab. 27 and 28: Male lungs. Correlation observed between A Actin-LINE-1 (primer2).

27

Lungs						
Male	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.38756	0.37505	0.21278	0.37954	0.16083	-0.0723
	0.0259	0.0709	0.2678	0.0674	0.4136	0.7828
	33	24	29	24	28	17
MYC			0.25173	0.33272	0.11503	0.13431
			0.1796	0.0778	0.545	0.5512
			30	29	30	22
LIT1					0.43567	-0.1034
					0.0182	0.6471
					29	22

28

Lungs						
Male	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.138	0.38214	0.04215	-0.006	0.24929	0.71332
	0.4838	0.3101	0.8158	0.9779	0.1618	<.0001
	28	9	33	24	33	24
MYC	-0.1814	-0.0492	0.10373	0.09022	0.34125	0.4509
	0.3463	0.9	0.5297	0.5849	0.0335	0.004
	29	9	39	39	39	39
LIT1	0.17236	-0.2217	-0.099	0.04416	0.4551	0.31936
	0.3713	0.5664	0.6029	0.8201	0.0115	0.0913
	29	9	30	29	30	29
PEG3	0.49079	-0.4885	-0.0712	-0.0623	0.27137	-0.265
	0.008	0.3256	0.7086	0.7832	0.1469	0.2333
	28	6	30	22	30	22
SNRPND1			-0.3925	0.07454	0.10801	0.35138
			0.0352	0.8489	0.577	0.3538
			29	9	29	9
IAP					-0.0561	-0.2011
					0.7311	0.2134
					40	40

Tab. 29 and 30: Female lungs. No correlation observed.

29

Lungs						
Female	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.13525	-0.164	0.02498	0.13023	0.0086	0.07505
	0.3757	0.3466	0.8676	0.449	0.9537	0.6934
	45	35	47	36	48	30
MYC			0.31851	0.31817	0.0039	0.15224
			0.031	0.04	0.9797	0.3684
			46	42	45	37
LIT1					0.04268	0.41476
					0.7757	0.0132
					47	35

30

Lungs						
Female	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.1999	0.38938	0.01987	-0.2426	-0.0289	-0.0502
	0.1731	0.1102	0.9032	0.2048	0.8439	0.7647
	48	18	40	29	49	38
MYC	-0.1192	0.19114	0.20474	0.0329	-0.1976	0.1702
	0.4353	0.4331	0.2242	0.8489	0.1881	0.2581
	45	19	37	36	46	46
LIT1	-0.1188	0.13552	0.17541	0.00756	-0.0514	0.21585
	0.4265	0.5581	0.2855	0.9661	0.7284	0.1594
	47	21	39	34	48	44
PEG3	0.1781	0.65216	-0.1545	-0.3111	0.05895	0.04791
	0.2259	0.0084	0.3412	0.0831	0.6874	0.7721
	48	15	40	32	49	39
SNRPND1			-0.198	-0.3894	0.28842	-0.1419
			0.2207	0.1688	0.0445	0.5396
			40	14	49	21
IAP					-0.2324	-0.0886
					0.1437	0.5866
					41	40

Tab.31 and 32: Male skeletal muscle: Correlation observed between: MyLC-SnrpnD1 (both primers), LINE-1-MyLC (primer 1)

31

Muscle						
Male	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.0396	0.04384	-0.135	-0.1594	-0.0111	0.29352
	0.8059	0.8503	0.3765	0.4786	0.9444	0.2091
	41	21	45	22	42	20
MYC			0.25266	0.13286	-0.4117	-0.1954
			0.111	0.4398	0.0092	0.3387
			41	36	39	26
LIT1					0.0715	-0.2296
					0.6486	0.2592
					43	26

32

Muscle						
Male	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.0541	0.21014	0.00294	0.18902	-0.0142	-0.4994
	0.7405	0.3479	0.9845	0.3995	0.9244	0.0153
	40	22	46	22	47	23
MYC	0.98645	0.67349	0.05843	-0.1204	0.0623	0.25795
	<.0001	<.0001	0.7132	0.4777	0.6915	0.1179
	41	36	42	37	43	38
LIT1	0.2586	0.04666	-0.0055	-0.2219	0.17751	-0.0706
	0.1071	0.7839	0.9713	0.1383	0.2326	0.6373
	40	37	46	46	47	47
PEG3	-0.3782	-0.1726	-0.3021	0.45033	-0.0259	-0.2214
	0.0192	0.3894	0.0463	0.0162	0.8674	0.2575
	38	27	44	28	44	28
SNRPND1			0.0395	-.0.05784	0	-0.0445
			0.8063	0.7301	1	0.788
			41	38	42	39
IAP					0.18817	-0.0952
					0.2003	0.52
					48	48

Tab.33 and 34: Female skeletal muscle: Correlation observed between MyLC and SnrpnD1 in both primers

33

Muscles						
Female	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.23251	0.36067	0.28008	-0.1772	0.10694	0.4155
	0.2003	0.226	0.0594	0.4303	0.4694	0.0769
	32	13	46	22	48	19
MYC			0.02349	0.0477	0.11813	-0.0694
			0.8985	0.7989	0.5268	0.759
			32	31	31	22
LIT1					0.27192	-0.0269
					0.0708	0.88
					45	34

34

Muscles						
Female	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.03959	0.36067	0.17996	0.36067	0.0418	0.25919
	0.8325	0.226	0.216	0.226	0.7755	0.2324
	31	13	49	13	49	23
MYC	0.86849	1	0.1214	-0.303	0.08005	0.09426
	<.0001	<.0001	0.508	0.0976	0.6632	0.614
	31	31	32	31	32	31
LIT1	-0.035	0.0477	0.00497	-0.0549	0.007	-0.2292
	0.8518	0.7989	0.9739	0.714	0.9632	0.1213
	31	31	46	47	46	47
PEG3	0.17531	-0.0694	0.12122	0.24071	0.12549	0.16762
	0.3541	0.759	0.4118	0.1637	0.3954	0.3358
	30	22	48	35	48	35
SNRPND1			0.01237	-0.303	0.27692	0.09426
			0.9473	0.0976	0.1315	0.614
			31	31	31	31
IAP					0.27989	0.11448
					0.0514	0.4335
					49	49

Tab.35 and 36: Male skin. No correlation observed.

35

Skin						
	MYC		LIT1		PEG3	
Male	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.0426	-0.4358	0.21129	-0.346	0.25877	-0.4871
	0.781	0.2081	0.1686	0.3274	0.0861	0.1534
	45	10	44	10	45	10
MYC			-0.0847	0.25239	-0.0991	0.32961
			0.5714	0.0906	0.5027	0.0253
			47	46	48	46
LIT1					0.47573	0.49004
					0.0007	0.0006
					47	45

36

Skin						
	SNRPND1		IAP		L1	
Male	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.15343	-0.3015	0.09528	0.16048	0.35472	0.42252
	0.3259	0.4681	0.5384	0.6579	0.0181	0.2572
	43	8	44	10	44	9
MYC	-0.1323	0.39836	-0.1356	-0.2107	-0.2653	0.31264
	0.381	0.1016	0.3634	0.1598	0.0715	0.0324
	46	18	47	46	47	47
LIT1	0.47415	0.49975	0.07625	0.23804	0.29484	0.25629
	0.0009	0.0347	0.6145	0.1153	0.0467	0.0892
	46	18	46	45	46	45
PEG3	0.28966	0.21591	0.01471	0.04782	0.01032	0.18245
	0.0509	0.3895	0.9218	0.7579	0.9451	0.2303
	46	18	47	44	47	45
SNRPND1			0.13008	0.03359	0.02845	-0.1403
			0.3944	0.8947	0.8528	0.5911
			45	18	45	17
IAP					0.48588	0.24415
					0.0006	0.106
					46	45

Tab.37 and 38: Female skin. No correlation observed.

37

Skin						
	MYC		LIT1		PEG3	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.05313	0.10294	-0.1617	-0.108	-0.0024	-0.0919
	0.7199	0.6485	0.2724	0.6324	0.9869	0.6841
	48	22	48	22	48	22
MYC			-0.1152	-0.1611	-0.2	0.00862
			0.4257	0.2636	0.1637	0.9526
			50	50	50	50
LIT1					0.47299	0.36335
					0.0005	0.0095
					50	50

38

Skin						
	SNRPND1		IAP		L1	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.26697	0.49811	0.07777	-0.0956	0.11346	-0.426
	0.0666	0.1724	0.5993	0.6722	0.4477	0.048
	48	9	48	22	47	22
MYC	-0.2921	0.14644	-0.0037	0.03165	-0.1364	-0.0753
	0.0396	0.6174	0.9799	0.8273	0.3502	0.6032
	50	14	50	50	49	50
LIT1	0.12371	0.08765	-0.0862	0.04826	0.18852	0.10789
	0.392	0.7657	0.5515	0.7393	0.1945	0.4558
	50	14	50	50	49	50
PEG3	0.2232	0.22768	0.11011	0.23597	0.12275	0.15083
	0.1192	0.4337	0.4465	0.099	0.4008	0.2958
	50	14	50	50	49	50
SNRPND1			0.01702	-0.056	0.22893	-0.056
			0.9066	0.8493	0.1136	0.8491
			50	14	49	14
IAP					-0.0633	0.07123
					0.6656	0.6231
					49	50

Tab.39 and 40: Male spleen. Correlation observed between: MyLC-Iap (primer 1), MyLC-LINE-1 (both primers), SnrpnD1-LINE-1 (primer 1), LINE-1-Iap (primer 1)

39

Spleen						
	MYC		LIT1		PEG3	
Male	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.27323	0.14417	0.26855	0.23212	0.19925	0.21837
	0.0549	0.3878	0.0621	0.1668	0.1699	0.1878
	50	38	49	37	49	38
MYC			-0.0548	-0.1373	0.07083	0.27261
			0.7082	0.3468	0.6287	0.0554
			49	49	49	50
LIT1					0.08962	0.15654
					0.5447	0.2828
					48	49

40

Spleen						
	SNRPND1		IAP		L1	
Male	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.30653	0.19455	0.31087	0.04019	0.4526	0.12419
	0.0341	0.2486	0.0297	0.8133	0.001	0.4576
	48	37	49	37	50	38
MYC	0.41864	0.33787	0.88058	0.35375	0.80881	0.83874
	0.0031	0.0176	<.0001	0.0126	<.0001	<.0001
	48	49	49	49	50	50
LIT1	0.30121	0.24379	-0.0628	0.12932	0.18845	0.07034
	0.0396	0.095	0.6714	0.381	0.1947	0.631
	47	48	48	48	49	49
PEG3	0.0994		0.14103	0.31714	0.17283	0.24514
	0.5062		0.339	0.0264	0.235	0.0862
	47		48	49	49	50
SNRPND1			0.53271	0.26091	0.58886	0.51953
			0.0001	0.0733	<.0001	0.0001
			47	48	48	49
IAP					0.8076	0.44061
					<.0001	0.0015
					49	49

Tab.41 and 42: Female spleen. Correlation observed between MyLC-SnrpnD1 (primer 1), MyLC-Iap (primer 1), MyLC-LINE-1 (both primers), SnrpnD1-Iap (primer 1),SnrpnD1-LINE-1 (primer 1), Iap-LINE-1 (both primers)

41

Spleen						
	MYC		LIT1		PEG3	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.14683	0.226	0.08649	0.03397	0.17778	0.58816
	0.3416	0.2998	0.5767	0.8777	0.2427	0.0032
	44	23	44	23	45	23
MYC			0.27008	0.09327	0.31496	0.48993
			0.0728	0.5422	0.033	0.0018
			45	45	46	38
LIT1					0.13997	-0.0294
					0.3535	0.8628
					46	37

42

Spleen						
	SNRPND1		IAP		L1	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.143	0.19506	0.22573	0.1548	0.19193	0.0636
	0.3603	0.3724	0.1407	0.4915	0.212	0.7731
	43	23	44	22	44	23
MYC	0.6426	0.35418	0.83195	0.38112	0.90428	0.75517
	<.0001	0.017	<.0001	0.0107	<.0001	<.0001
	44	45	45	44	45	45
LIT1	0.02568	-0.0288	0.2536	0.28194	0.27244	0.2739
	0.8655	0.8477	0.0928	0.0637	0.0639	0.0625
	46	47	45	44	47	47
PEG3	0.01631	-0.0086	0.42768	0.24839	0.17962	0.24397
	0.9153	0.9594	0.003	0.1441	0.2323	0.1456
	45	38	46	36	46	37
SNRPND1			0.60696	0.24293	0.61539	0.43357
			<.0001	0.1121	<.0001	0.0023
			44	44	46	47
IAP					0.75218	0.65922
					<.0001	<.0001
					45	44

Tab.43 and 44: Male tongue. Correlation obserwed between Peg3 and SnrpnD1 in primer 1.

43

Tongue						
	MYC		LIT1		PEG3	
Males	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.06528	-0.1215	0.29446	0.50829	0.26265	
	0.6664	0.722	0.0496	0.0915	0.0745	
	46	11	45	12	47	
MYC			-0.3785	0.03756	0.11049	0.95975
			0.0103	0.818	0.4597	0.0096
			45	40	47	5
LIT1					-0.0921	0.66173
					0.5429	0.2238
					46	5

44

Tongue						
	SNRPND1		IAP		L1	
Males	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.31275	0.21698	-0.0234	0.18518	0.31659	
	0.0323	0.4982	0.8758	0.5645	0.0302	
	47	12	47	12	47	
MYC	0.13986	0.28897	-0.0336	0.32032	0.29875	
	0.3485	0.1087	0.8224	0.0412	0.0414	
	47	32	47	41	47	
LIT1	-0.0765	0.29545	0.04873	-0.0647	0.12503	
	0.6134	0.0849	0.7477	0.673	0.4077	
	46	35	46	45	46	
PEG3	0.97609	0.65545	0.17151	-0.0202	0.0334	
	<.0001	0.2298	0.2438	0.9743	0.8198	
	50	5	48	5	49	
SNRPND1			0.17643	0.25059	0.04313	
			0.2303	0.1465	0.7685	
			48	35	49	
IAP					0.24525	
					0.0929	
					48	

Tab.45 and 46: Female tongue. Correlation obserwed between: Lit1 and SnrpnD1 in primer 2.

45

Tongue						
	MYC		LIT1		PEG3	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.11563	0.42615	0.07417	0.17506		0.36341
	0.4716	0.0267	0.6125	0.3462		0.4789
	41	27	49	31		6
MYC			-0.2256	0.34101		-0.4537
			0.1508	0.0271		0.3661
			42	42		6
LIT1						0.2483
						0.5913
						7

46

Tongue						
	SNRPND1		IAP		L1	
Female	SN1	SN2	SN1	SN2	SN1	SN2
A Actin		0.06313	-0.3431	-0.208	-0.3926	
		0.7544	0.017	0.2614	0.0058	
		27	48	31	48	
MYC		0.32142	0.18182	0.26203	0.41362	
		0.0524	0.2552	0.0937	0.0072	
		37	41	42	41	
LIT1		0.59557	-0.1089	-0.0272	-0.1891	
		<.0001	0.4564	0.8512	0.1932	
		45	49	50	49	
PEG3		0.45473		-0.5012		
		0.4416		0.2519		
		5		7		
SNRPND1				-0.0298		
				0.846		
				45		
IAP					0.53072	
					0.0001	
					48	

Tab.47 and 48: Testes: Correlation obserwed between: A Actin-Lit1 (both primers), A Actin-Peg3 (both primers), A Actin-SnrpnD1 (both primers), A Actin-Iap (primer 1), Lit1-Peg3 (both primers), Lit1-SnrpnD1 (both primers), Lit1-Iap (primer 1), SnrpnD1-Peg3 (bothprimers), SnrpnD1-Iap (primer1). Most likely due to general unspeciffic hypomethylation in the testis.

47

Testes						
	MYC		LIT1		PEG3	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	0.14162	0.00307	-0.9444	-0.9263	-0.9676	-0.7591
	0.3478	0.9839	<.0001	<.0001	<.0001	<.0001
	46	46	46	12	50	49
MYC			-0.0941	0.31703	-0.1383	0.00441
			0.5341	0.3154	0.3595	0.9771
			46	12	46	45
LIT1					0.91654	0.92311
					<.0001	<.0001
					46	12

48

Testes						
	SNRPND1		IAP		L1	
	SN1	SN2	SN1	SN2	SN1	SN2
A Actin	-0.967	-0.975	-0.7799	-0.3152	-0.3492	-0.4122
	<.0001	<.0001	<.0001	0.0329	0.0129	0.0029
	50	50	48	46	50	50
MYC	-0.1158	0.0038	0.13959	0.12422	0.18425	0.27494
	0.4433	0.98	0.3662	0.4332	0.2203	0.0644
	46	46	44	42	46	46
LIT1	0.90987	0.94838	0.83128	0.10713	0.38361	0.68777
	<.0001	<.0001	<.0001	0.7539	0.0085	0.0134
	46	12	44	11	46	12
PEG3	0.97609	0.76286	0.76277	0.16785	0.33446	0.27345
	<.0001	<.0001	<.0001	0.2704	0.0176	0.0573
	50	49	48	45	50	49
SNRPND1			0.78227	0.29634	0.35985	0.41107
			<.0001	0.0455	0.0103	0.003
			48	46	50	50
IAP					0.46842	0.03646
					0.0008	0.8099
					48	46

12 Publication

The presented work contributed to an original research publication (article attached in appendix 3):

Measurements of DNA methylation at seven loci in various tissues of CD1 mice. Daugela L, Nüsgen N, Walier M, Oldenburg J, Schwaab R, El-Maarri O. PLoS One. 2012;7(9):e44585. doi: 10.1371/journal.pone.0044585. Epub 2012 Sep 7. PMID:22970256.

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