Department of Bioinformatics and Computational Biology

Technische Universität München

Master's Thesis in Bioinformatics

Variation of HERV elements in the KORA cohort

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Variation von HERV elementen in der KORA Kohorte

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2 Data

2.1 HERV annotation

HERV annotation was pertained from RepeatMasker[1] repeat library. RepeatMasker is a tool that screens DNA sequences against a library interspersed repeats and low complexity DNA sequences. It generates an annotation of identified repeats and masks them in the query sequence.

The track representing all identified elements from the RepeatMasker library for human genome hg19 was downloaded from the UCSC genome browser download section (`http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/rmsk.txt.gz`). It contains a total of 5298130 occurrences of repeats. Each entry consists of 17 values. Not in order these are repeat name the repeat class and family, as well as the chromosome, strand, and the genomic start and end position of the repeat occurence. Furthermore it contains the position in the whole sequence, that is known for the identified repeat, that is covered by the occurrence. It also describes the quality of the alignment of the repeat sequence to the annotated position using the Smith Waterman alignment score[2] and the number of base mismatches, deletions and insertions per thousand base pairs. Finally there is an indexing field used to speed aup chromosme range queries and the first digit of the id field in the RepeatMasker output file. The first 10 lines of the track are shown in table 2.1.

To extract HERV elements the annotation was filtered on different columns generating three sets variable size. Multiple HERV sets were created as an atempt to cover different possible definitions of HERV elements.

This work only discerned between different HERV element types by defining different HERV sets. Therefore, within each set annotations, whose genomic positions overlap or are directly adjacent, were merged into one element.

A first set, HERV set 1 (HERV S1) was constructed by extracting all elements that contained "ERV" in the repeat name column. This set The resulting 42508 annotations condensed to 35589 elements after merging. The elements have a mean width of 949 bp and cover a total of 33.8 Mbp, which is ca 1.04% of the human genome. The distribution of element lengths in HERV S1 is shown in Figure 2.1

Alternatively filtering the annotation for "ERV" in the superfamily column leads to 696689 annotations. HERV set 2 (HERV S2) contains all endogenous retroviral sequences found in the human genome. After merging overlapping and adjacent annotations this led to 633323 elements. Their mean width is 415 bp and they make up to 262.8 Mbp or ca 8.13% of the human genome.

A third set "HERV set 3" (HERV S3) was constructed by filtering the repeat name column for "HERV", which resulted in 21361 annotations. As this set contains only elements

bin	swScore	milliDiv	milliDel	milliIns	genoName	genoStart	genoEnd	genoLeft	strand	repName	repClass	repFamily	repStart	repEnd	repLeft	id
585	1,504	13	4	13	chr1	10,000	10,468	$-2.49 \cdot 10^8$	+	(CCCTAA)n	Simple_repeat	Simple_repeat	1	463	0	1
585	3,612	114	270	13	chr1	10,468	11,447	$-2.49 \cdot 10^8$	-	TAR1	Satellite	telo	−399	1,712	483	2
585	437	235	186	35	chr1	11,503	11,675	$-2.49 \cdot 10^8$	-	L1MC	LINE	L1	−2,236	5,646	5,449	3
585	239	294	19	10	chr1	11,677	11,780	$-2.49 \cdot 10^8$	-	MER5B	DNA	hAT-Charlie	−74	104	1	4
585	318	230	38	0	chr1	15,264	15,355	$-2.49 \cdot 10^8$	-	MIR3	SINE	MIR	−119	143	49	5
585	203	162	0	0	chr1	16,712	16,749	$-2.49 \cdot 10^8$	+	(TGG)n	Simple_repeat	Simple_repeat	1	37	0	6
585	239	338	148	0	chr1	18,906	19,048	$-2.49 \cdot 10^8$	+	L2a	LINE	L2	2,942	3,104	−322	7
585	652	346	85	42	chr1	19,947	20,405	$-2.49 \cdot 10^8$	+	L3	LINE	CR1	3,042	3,519	−970	8
585	270	331	7	27	chr1	20,530	20,679	$-2.49 \cdot 10^8$	+	Plat_L3	LINE	CR1	2,802	2,947	−639	9
585	254	279	47	39	chr1	21,948	22,075	$-2.49 \cdot 10^8$	+	MLT1K	LTR	ERVL-MaLR	15	142	−453	1

Table 2.1: First ten rows of the RepeatMasker annotation on hg19

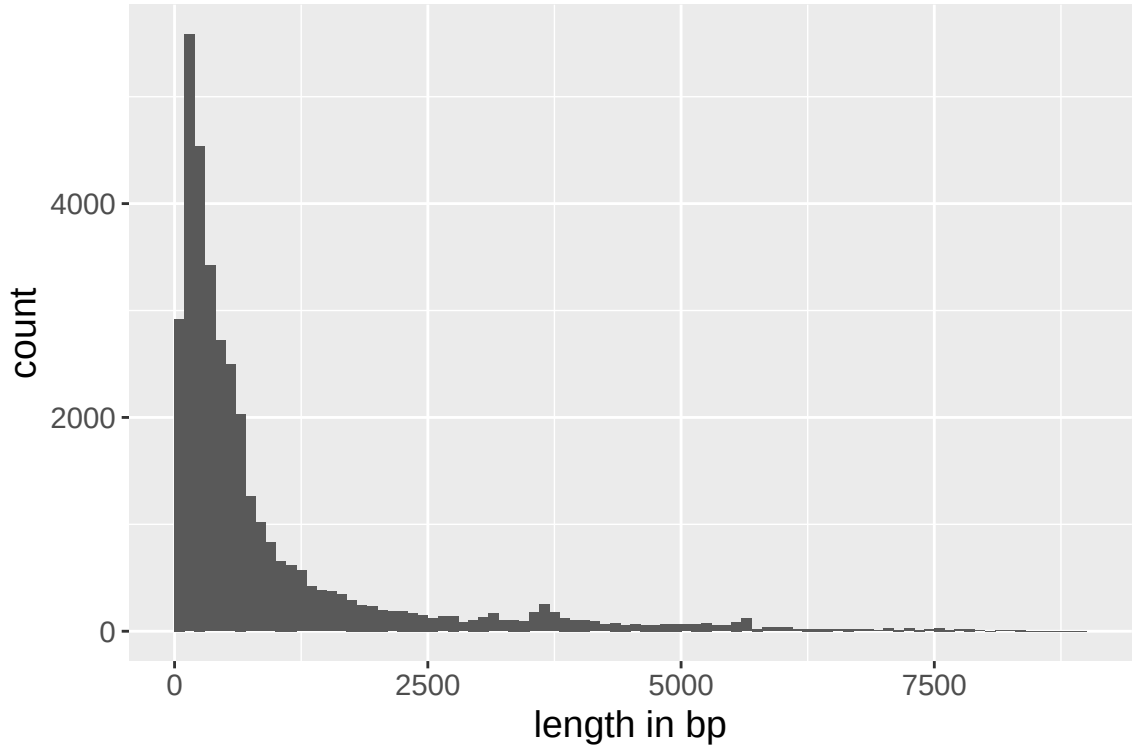

Figure 2.1: Length distribution of elements in HERV S1

that are explicitely named "HERV", it is my hope that might contain only endogenous retroviral elements, that were inserted directly into the human genome. Merging overlapping and adjacent annotations resulted in 15403 elements with an average width of 1468 bp and a combined length of 22.6 Mbp.

2.2 KORA

The expression, methylation and genotype data used in this work were generated by the platform for Cooperative Health Research in the Region of Augsburg - short KORA. It contains health surveys as well as examinations of individuals of German nationality living in the area of Augsburg, Bavaria. The objective of KORA is to track changes in health conditions over a long period in order to identify and examine the causes, effects and development of chronical diseases.

The data comes from the KORA F4 Survey, which was conducted from 2006 to 2008 and comprised samples of 3080 individuals. F4 is a follow up study to the survey S4 performed from 1999 to 2001 and containing 4261 individuals.

All measurements were performed on whole blood samples. Houseman blood counts[] describing the composition of different cell types for each individual are available.

Not all essays are available for all samples. Therefore different analyses were performed on varying sets of indiviudals according to availability of the required data types. A diagram of the which samples were available for each essay can be seen in figure 2.2.

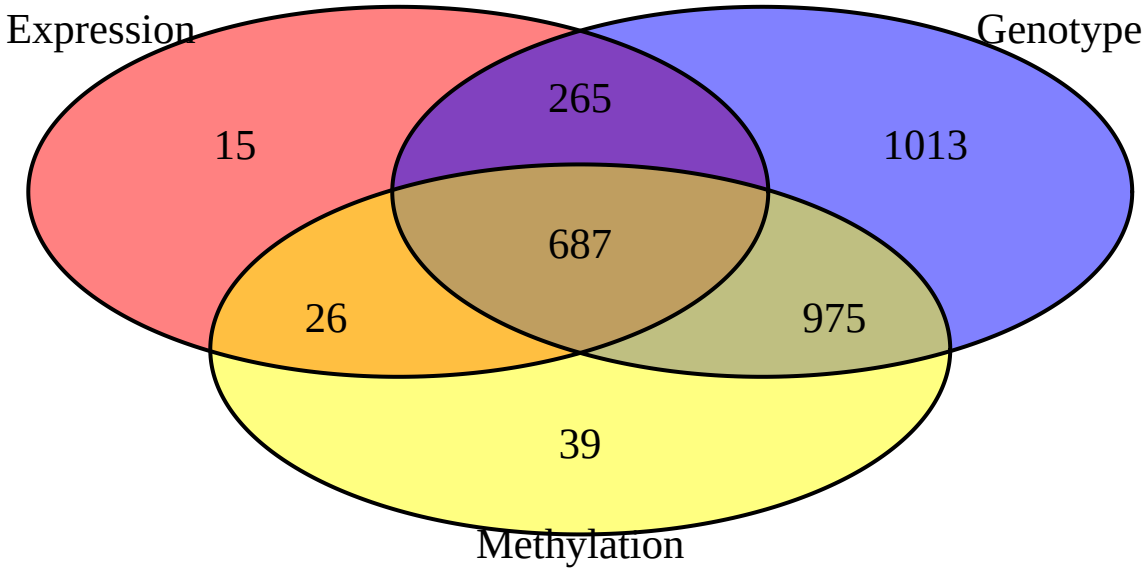

Figure 2.2: Number of samples with genotype, expression and methylation measurements in KORA F4 Survey

2.2.1 Expression

The expression data was generated using the HumanHT-12 v3.0 Gene Expression BeadChip. The chip can measure expression values for 49576 probes. However only 47864 probes represent an actual genomic location.

Measurements for 993 individuals are available from the KORA F4 survey. The comprise values for a total of 48803 probes per sample. Probes that do not map to a genomic location were excluded in all analyses, leaving 47864 probes. Of these 29521 are annotated to total of 19288 genes.

To not lose information, especially in hERV regions that are usuallly sparesly annotated with genes, probes without genes were not discarded and most analyses were performed on probe level or only partially abstracting to gene level.

2.2.2 Methylation

DNA methylation was measured using the Infinium HumanMethylation450K BeadChip, which interrogates methylation levels at 485577 genomic locations.

Methylation data was available for 1727 individuals and 485512 sites, which make up all 'cg' and 'ch' probe type probes.

2.2.3 Genotypes

Genotyping was performed with the Affymetrix Axiom array. The Iluminus calling algorithm was used for genotype calling and missing values were imputed using the IMPUTE2 software[3]. SNPs were filtered at IMPUTE value of 0.4.

After excluding all SNPs with a minor allele frequency of less than one percent measurements of 9533127 SNPs for 3788 individuals were available.

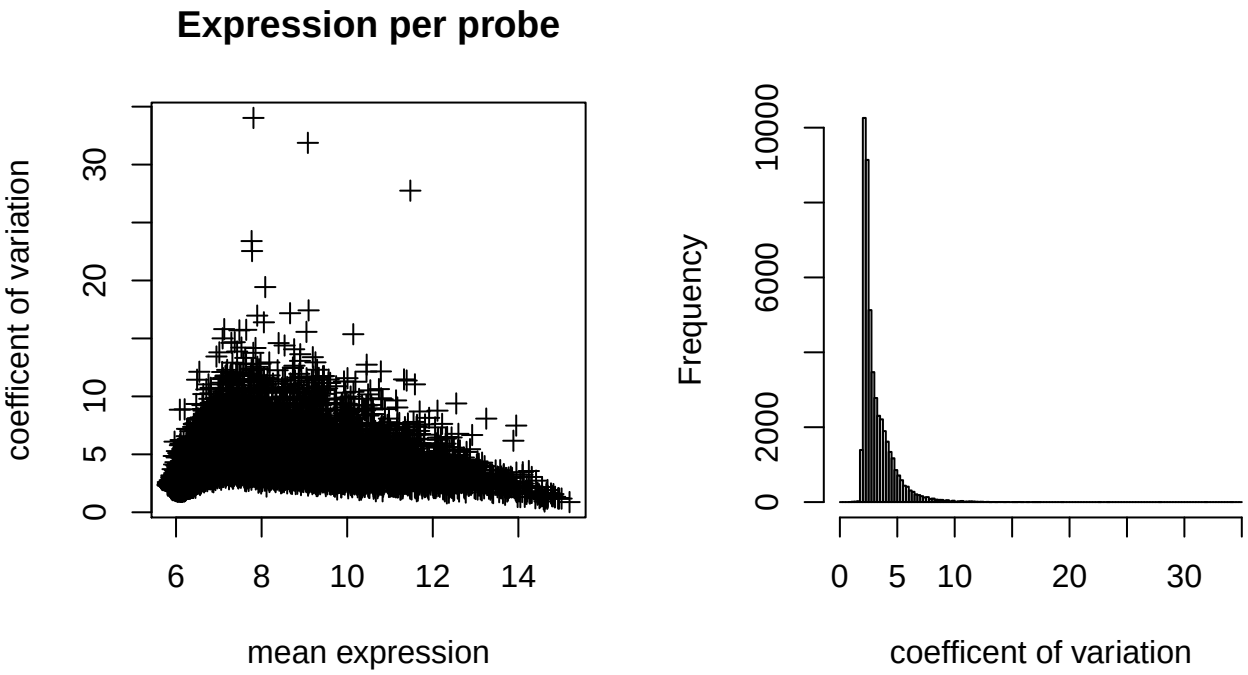

Figure 2.3: Coefficient of expression variance over 993 individuals

2.2.4 Covariates

Several covariates were known for each sample. These were age, sex, body mass index (BMI) and wide blood cell count, as well as experimental factors like storage time and RNA integrity number (RIN).

2.2.5 Methylation quantitative trait loci

Previously process methylation quantitative trait loci (meQTL) data was used. The data set contained a total of xxxxxx significantly associated cpg-snp pairs. xxxx distinct cpg-sites and xxxxxx snps were part of at least one meQTL. xxxx pairs consisted of cpgs and snps on the same chromosome, while xxxxx association were between different chromosomes.

2.3 Transcription factor binding

Transcription factor binding sites were obtained from two publicy available sources:

First was the third version of the track "Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs"[4] downloaded from the UCSC genome browser download section (`http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeRegTfbsClustered/wgEncodeRegTfbsClusteredWithCellsV3.bed.gz`).

It combines 690 high quality ENCODE ChIP-seq data sets, which were processed with the Factorbook motif discovery and annotation pipeline[4]. The pipeline uses the tools MEME-ChIP[5] and FIMO[6] from the MEME software suite and merges discovered

motifs with known motifs from Jaspar[7] and TransFac[8] using machine learning methods and manual curation.

The track contains a total of 438044 distinct peaks for 161 transcription factors in 91 cell types. For our analyses we filtered and combined the peaks for 23 blood related cell types. This leaves a total of 2173371 peaks for 125 transcription factors.

The second source was the ReMap project[9]. It combines 395 publicy available ChIP-seq data sets covering 132 different transcription factors across 83 cell lines. ReMap uses Bowtie2[] to map reads to the human genome and the tool MACS[] for peak calling. The finished data set was downloaded from the ReMap website(`http://tagc.univ-mrs.fr/remap/download/All/filPeaks_public.bed.gz`).

It contains xxxxx peaks. After filtering for 19 blood related cell types a of xxxx peaks of xxx different transcription factors remained.

Combining both filtered data sets lead to a total of xxxxx peaks of xxx transcription factors.

2.4 Chromatin states

Chromatin state annotations were downloaded from Roadmap Epgenomics Core 15-state model`http://egg2.wustl.edu/roadmap/data/byFileType/chromhmmSegmentations/ChmmModels/coreMarks/jointModel/final`. The Model provides a whole genome chromatin state annotation of 200 bp wide windows to the following 15 states: Active Transcription Start Site (TSS), Flanking Active TSS, Transcription at gene 5' and 4', Strong transcription, Weak transcription, Genic enhancers, Enhancers, ZNF genes & repeats, Heterochromatin, Bivalent/Poised TSS, Flanking Bivalent TSS/Enhancer, Bivalent Enhancer, Repressed PolyComb, Weak Repressed PolyComb and Quiescent/Low. The model is available for 127 diverse cell lines.

It was generated using ChromHMM v1.10[10] on the chromatin marks H3K4me1, H3K4me3, H3K27me3, H3K9me3, and H3K36me3. ChromHMM is based on a multivarate Hidden Markov Model.

In this work the annotations for 27 blood related cell lines were used. To get the distribution of states for a single feature or a set of features the different annotations for the different cell lines were weighted according to the houseman counts and summed up.

3 Methods

3.1 Overlaps

The task of whether HERV elements contained any annotated elements like expression probes, genes, cpg sites and transcription factor binding sites (tfbs) was performed using funtion "findOverlaps" from the Bioconductor package "GenomicRanges"[11].

Features were considered to be of interest for the analysis of an HERV element, if they overlapped by at least one base pair.

Features were also filtered for the HERV elements and their 1kb or 2kb up- and downstream regions.

3.2 Data normalization

Expression and methylation values were corrected for available covariates by calculating the residual matrix. A linear model containing the cell compositions and the first 20 principal components was used for methylation.

Expression residuals were calculated using a linear model of age, sex, RNA integrity number (RIN), plate and storage time.

3.3 eQTL/eQTM calculation

Expression quantitative trait loci (eQTL) and expression quantitative trait methylation were calculated using the Bioconductor package MatrixEQTL[12]. MatrixEQTL tests for association of SNP-transcript pairs. It offers two modes of modeling the effect of the genotype on transcription levels:

When setting the parameter "$useModel = modelLINEAR$", as was done in this work, an additive linear model is used. The association ism modeled as simple linear regression and the absolute value of the sample correlation is used as test statistic.

Alternatively when choosing "$modelAnova$" for the paramter, the effect is modeled with ANOVA model. In this case the test statistic is the squared sample correlation.

After calculating the test statistics the p-values for the all pairs that pass a defined significance threshold are calculated. These are corrected multiple testing using a Benjamini-Hochberg procedure, adapted for not recording all p-values.

MatrixEQTL is very performant because it manages to reduce the calculation of the test statistic to one single large matrix multiplication by cleverly transforming the genotype and transcription variables.

MatrixEQTL also allows to include covariates in the QTL calculation. As the expression and methylation values used are residuals and therefore already consider covariates this option is not used. Furthermore MatrixEQTL can differentiate between cis- and trans-interactions. The maximal distance to consider a pair on the same chromosome as cis was set to 50kb.

The threshold for significant cis-QTLs during calculation was set to 10e-6 and 10e-8 for trans.

3.4 Functional Analysis of Gene Sets

In multiple analyses functional Gene Ontology enrichments were performed.

First a set of all GO annotations with any evidence code for gene symbols was retrieved from the Bioconductor package AnnotationDbi[13].

Then a hypergeometric test[14] for overreprensentation is performed on a set of genes of interest. For most enrichments a custom backround set of genes specific to the analysis is given. Finally the p-values for overrepresented GO terms were adjusted for multiple testing using the Holm method[15].

3.5 Gaussian Graphical Models

4 Results

4.1 Normalized Data

The distribution of the quantile normalized expression values and the expression residuals over all probes and samples can be seen in figure **??**. As expected the residuals follow a normal distribution. This is important for the calculation of the Gaussian graphical models, as the normal distribution of the data is one of base assumptions[].

4.2 HERV region features

In this section I will describe the expression probes that overlap with any HERV element and the cpgs and SNPs that lie within any HERV element and/or their flanking regions. The results for the set of all endogenous retroviral elements, HERV set 2, without flanking regions are described in detail. The results for the other sets and including flanking regions will be shown in tables or in the supplementary data.

4.2.1 Expression

A total of 2343 expression probes overlap directly with at least one of 2271 HERV elements from HERV S2. This means ca 4.50% of all expression probes overlap with HERV elements and expression measurements are known for parts of ca 0.37% of all HERV elements.

The mean expression of the probes overlapping with HERV elements, as shown in figure 4.1 is on average lower than for all probes (figure 2.3). The coefficent of variance, however, shows a very similar pattern for the whole set of probes and the considered subset.

Of these 2343 probes 510 were annotated to one of 449 different genes. I performed a GO enrichment for the biological process ontology on these genes with the set of all genes with available expression data as background. The two most significantly enriched terms are defense response (GO:0006952, $p = 6.17e - 05$) and innate immune response (GO:0045087, $p - value = 2.97e - 04$), but after correcting for multiple testing no terms are significantly enriched.

Term ID	Term	p	fdr
GO:0098542	defense response to other organism	$2.97 \cdot 10^{-7}$	$2.35 \cdot 10^{-3}$
GO:0006952	defense response	$3.95 \cdot 10^{-7}$	$3.12 \cdot 10^{-3}$
GO:0009593	detection of chemical stimulus	$4.94 \cdot 10^{-7}$	$3.91 \cdot 10^{-3}$
GO:0050907	detection of chemical stimulus involved in sensory perception	$5.09 \cdot 10^{-7}$	$4.03 \cdot 10^{-3}$
GO:0050911	detection of chemical stimulus involved in sensory perception of smell	$7.91 \cdot 10^{-7}$	$6.26 \cdot 10^{-3}$
GO:0045087	innate immune response	$9.45 \cdot 10^{-7}$	$7.47 \cdot 10^{-3}$
GO:0007606	sensory perception of chemical stimulus	$1.78 \cdot 10^{-6}$	$1.41 \cdot 10^{-2}$
GO:0050906	detection of stimulus involved in sensory perception	$2.31 \cdot 10^{-6}$	$1.83 \cdot 10^{-2}$
GO:0007608	sensory perception of smell	$2.71 \cdot 10^{-6}$	$2.14 \cdot 10^{-2}$
GO:0051607	defense response to virus	$5.08 \cdot 10^{-6}$	$4.02 \cdot 10^{-2}$
GO:0006955	immune response	$6.06 \cdot 10^{-6}$	$4.79 \cdot 10^{-2}$

Table 4.1: Significantly enriched GO biological process terms among genes overlapping with HERV S2.

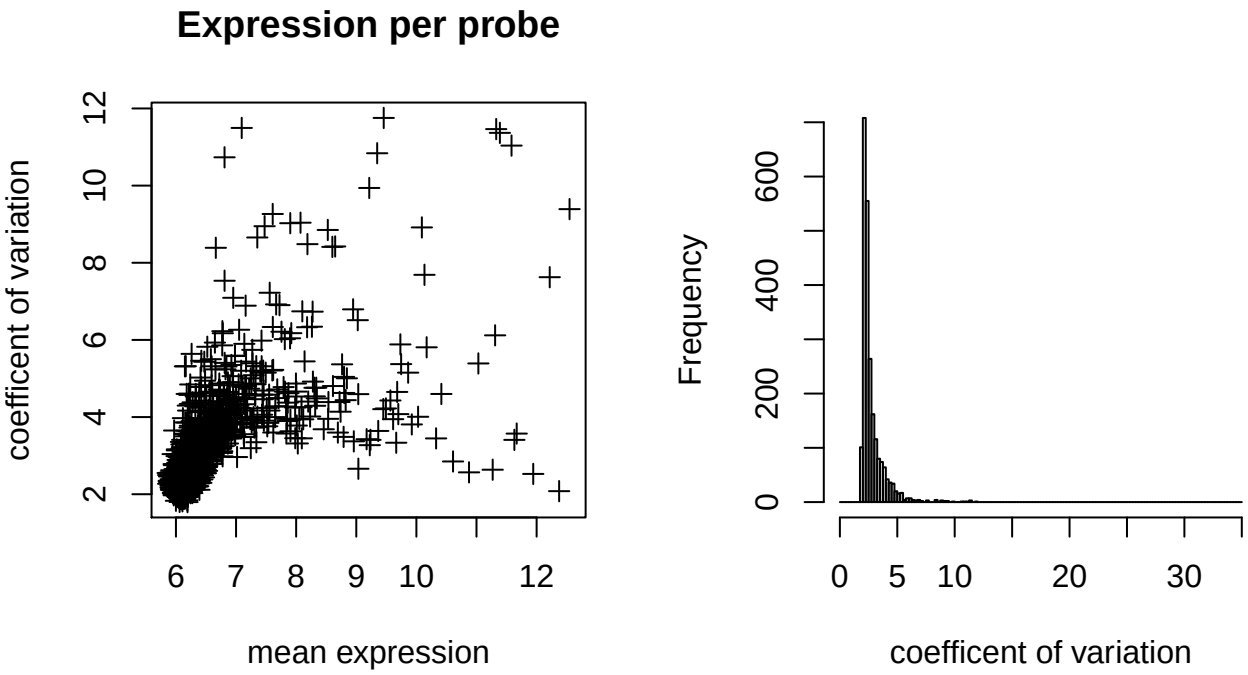

Figure 4.1: Coefficient of expression variance over 993 individuals and 2343 expression probes overlapping with HERV S2.

Set	S1	S1.1kb	S1.2kb	S2	S2.1kb	S2.2kb	S3	S3.1kb	S3.2kb
Pairs	239	516	822	2,368	6,393	12,457	165	265	440
HERVs	219	475	745	2,271	5,954	11,361	146	238	391
Probes	239	349	548	2,343	4,709	8,044	165	215	311
Genes	21	73	144	449	1,207	2,400	15	44	79

Table 4.2: Number of expression probes overlapping with different HERV sets and flanking regions. "Pairs" is the total number of overlaps occurring, "HERVs" is the number of distinct HERV elements that have an overlap with any of the expression probes, Probes describes the number of distinct expression probes that overlap with the HERV elements or their flanking regions, "Genes" is the number of distinct Genes that are annotated to these probes.

4.2.2 Methylation

17077 CpG sites, equaling 3.52% of all measured CpGs, were found within HERV S2 and 12871 distinct HERV elements (2.10%) contain at least one interogated CpG site.

The variance of the measurements of these sites, which is shown in figure **??**, is lower on average.

The number of CpG sites within all HERV sets and including flanking regions is shown in table 4.3

Set	S1	S1.1kb	S1.2kb	S2	S2.1kb	S2.2kb	S3	S3.1kb	S3.2kb
Pairs	1,587	6,785	12,050	17,077	$1.13 \cdot 10^5$	$2.45 \cdot 10^5$	1,152	3,481	6,438
HERVs	973	3,470	4,910	12,871	62,316	$1.03 \cdot 10^5$	614	1,568	2,440
CpGs	1,587	4,497	7,790	17,077	78,466	$1.39 \cdot 10^5$	1,152	2,671	4,404

Table 4.3: Number of CpGs overlapping with different HERV sets and flanking regions. "Pairs" is the total number of overlaps occurring, "HERVs" is the number of distinct HERV elements that have an overlap with any of the expression probes, "CpGs" is the number of distinct CpG sites that lie within the HERV elements or their flanking regions.

4.2.3 Genotypes

A total of 890780 the considered SNPs are located within elements of HERV S2. This constitutes 9.34% of all SNPs. These SNPs are found in 330744 distinct HERV elements. Therefore, 53.99% contain at least one SNP.

The results for all sets and flanking regions are shown in table 4.4

Set	S1	S1.1kb	S1.2kb	S2	S2.1kb	S2.2kb	S3	S3.1kb	S3.2kb
Pairs	$1.25 \cdot 10^5$	$4.48 \cdot 10^5$	$6.4 \cdot 10^5$	$8.91 \cdot 10^5$	$5.29 \cdot 10^6$	$9.64 \cdot 10^6$	89,066	$2.09 \cdot 10^5$	$3.23 \cdot 10^5$
HERVs	21,805	37,679	31,805	$3.31 \cdot 10^5$	$5.73 \cdot 10^5$	$5.78 \cdot 10^5$	10,139	13,225	13,289
SNPs	$1.25 \cdot 10^5$	$2.64 \cdot 10^5$	$3.72 \cdot 10^5$	$8.91 \cdot 10^5$	$3.31 \cdot 10^6$	$4.79 \cdot 10^6$	89,066	$1.54 \cdot 10^5$	$2.05 \cdot 10^5$

Table 4.4: Number of SNPs overlapping with different HERV sets and flanking regions. "Pairs" is the total number of overlaps occurring, "HERVs" is the number of distinct HERV elements that have an overlap with any of the expression probes, "SNPs" is the number of distinct considered SNPs that lie within the HERV elements or their flanking regions.

4.2.4 Chromatin states

Data in /storage/groups/groups_epigenreg/users/julian.schmidt ...

4.3 eQTLs

Associations were calculated for 156.4 millions cis acting SNP-expression probe pairs and 456.1 billion possible pairs in trans.

812147 of cis-pairs were significantly associated with p-values of 1e-6 or less. These significant eQTLs have a false discovery rate of less than 1.93e-4. A total of 551728 distinct SNPs and 4903 expression probes were part of at least one cis-eQTL. 4145 of these probes are annotated to 3552 different genes. The remaining 758 can not be assigned to a specific gene.

There were a total of 1511235 significant trans-eQTL with an p-value of less than 1e-8 and a FDR of less than 3.02e-4. These are made up by 229332 distinct SNPs and 21338 expression probes. 11505 of the probes found in at least one trans-eQTL are annotated to 9389 different genes, while 9767 probes are not assignet to a gene.

4.4 eQTMs

4.5 meQTLs

4.6 HERV realated regulatory networks

4.6.1 Data collection

5 Discussion

6 Conclusion

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