



Technische Universität München

Department of Bioinformatics and Computational Biology

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Master's Thesis in Bioinformatics

Variation of HERV elements in the KORA cohort

Julian Schmidt

Department of Bioinformatics and Computational
Biology

Technische Universität München

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Kohorte**

Author: Julian Schmidt
Supervisor: Dr. Matthias Heinig
Advisor: Johann Hawe
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1 Introduction

1.1 Regulation of cell functions and Epigenetics

- central dogma of biology
- rising importance of other factors atop DNA → epigenetics
- quick overview epigenetic marks
- chromatin states
- DNA methylation
- snp -> cpg -> expression pattern
- TF -> cpg interaction

1.2 Human endogenous retroviruses

- first humane genome -> "junk DNA"
- ongoing discovery for non-coding regions
- still masking of difficult sequence for many analysis -> repeats
- repeat classes -> ... -> herv
- herv origin - ...-virus like
- herv structure: LTR - pol - env - ... - LTR
- discovered roles of hervs in general regulation/diseases
-

1.3 Effect network analysis

- many bioinformatics methods find correlations, but not direct cause
- attempt to discern direct connections from bigger data webs
- hope to find possible biological mechanisms of gene regulation = path in model
- used approach: Gaussian Graphical models

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 occurrence. 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 up chromosome 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 attempt 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 · 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 · 10 ⁸ | - | TAR1 | Satellite | telo | -399 | 1,712 | 483 | 2 |
| 585 | 437 | 235 | 186 | 35 | chr1 | 11,503 | 11,675 | -2.49 · 10 ⁸ | - | L1MC | LINE | L1 | -2,236 | 5,646 | 5,449 | 3 |
| 585 | 239 | 294 | 19 | 10 | chr1 | 11,677 | 11,780 | -2.49 · 10 ⁸ | - | MER5B | DNA | hAT-Charlie | -74 | 104 | 1 | 4 |
| 585 | 318 | 230 | 38 | 0 | chr1 | 15,264 | 15,355 | -2.49 · 10 ⁸ | - | MIR3 | SINE | MIR | -119 | 143 | 49 | 5 |
| 585 | 203 | 162 | 0 | 0 | chr1 | 16,712 | 16,749 | -2.49 · 10 ⁸ | + | (TGG)n | Simple.repeat | Simple.repeat | 1 | 37 | 0 | 6 |
| 585 | 239 | 338 | 148 | 0 | chr1 | 18,906 | 19,048 | -2.49 · 10 ⁸ | + | L2a | LINE | L2 | 2,942 | 3,104 | -322 | 7 |
| 585 | 652 | 346 | 85 | 42 | chr1 | 19,947 | 20,405 | -2.49 · 10 ⁸ | + | L3 | LINE | CR1 | 3,042 | 3,519 | -970 | 8 |
| 585 | 270 | 331 | 7 | 27 | chr1 | 20,530 | 20,679 | -2.49 · 10 ⁸ | + | Plat_L3 | LINE | CR1 | 2,802 | 2,947 | -639 | 9 |
| 585 | 254 | 279 | 47 | 39 | chr1 | 21,948 | 22,075 | -2.49 · 10 ⁸ | + | MLT1K | LTR | ERV1-MaLR | 15 | 142 | -453 | 1 |

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

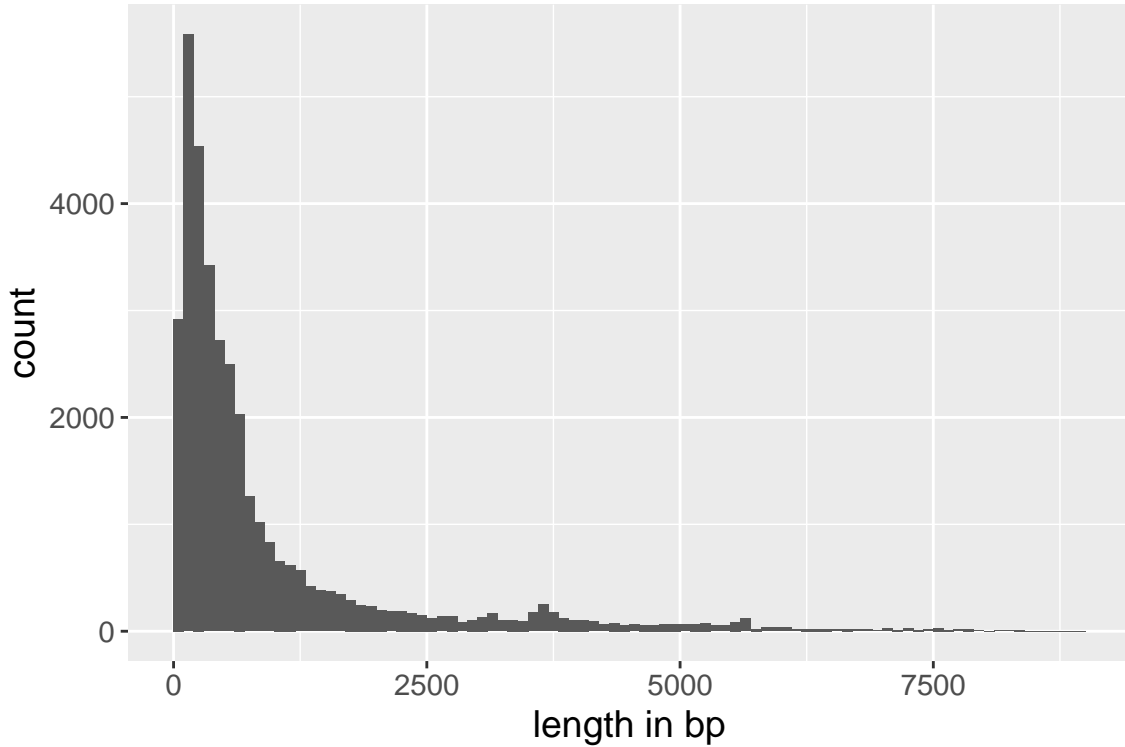


Figure 2.1: Length distribution of elements in HERV S1

that are explicitly 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 individuals 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.

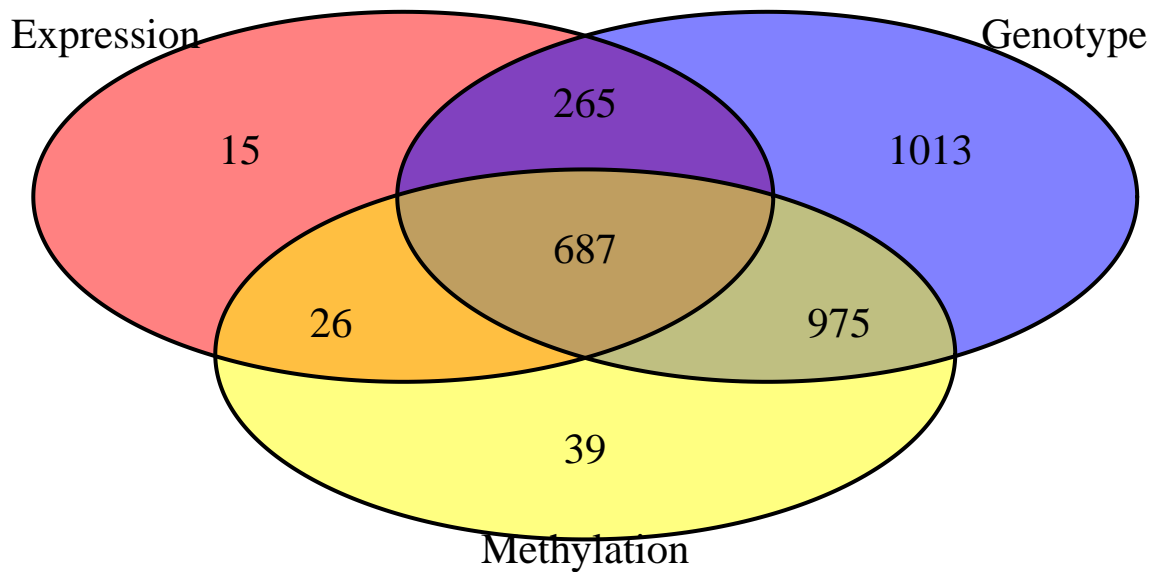


Figure 2.2: Number of samples with genotype, expression and methylation measurements in KORA F4 Survey

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 usually sparsely 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 Illuminus 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.

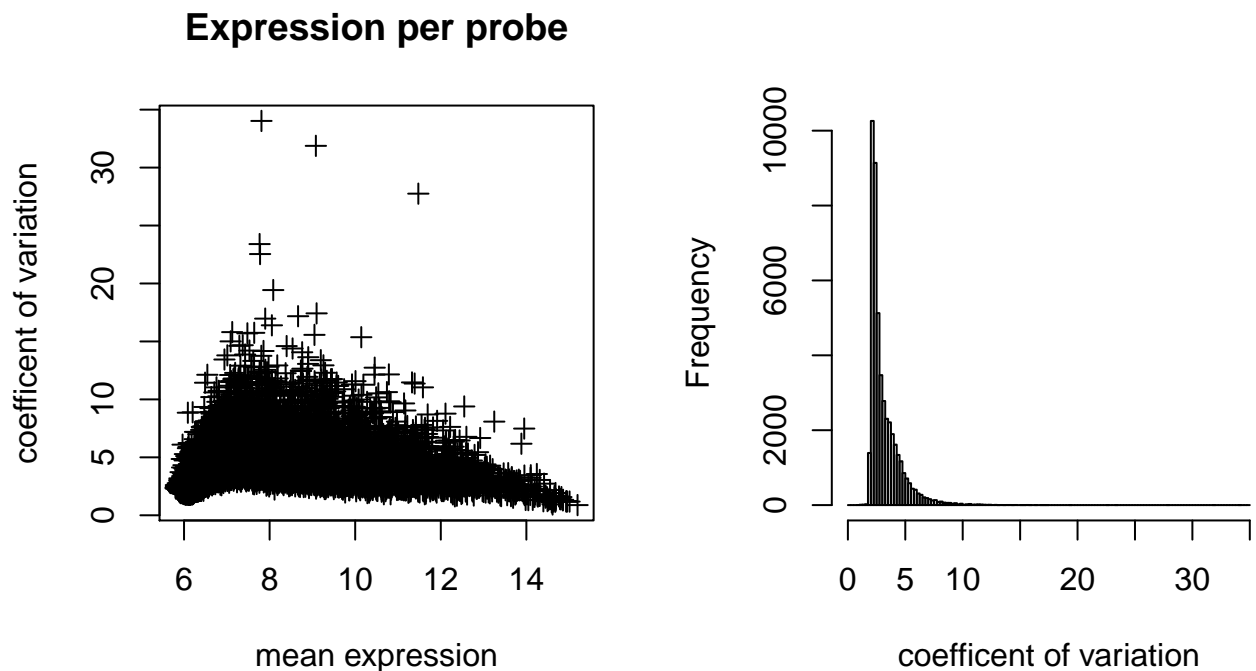


Figure 2.3: Coefficient of expression variance over 993 individuals

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. xxxxx distinct cpg-sites and xxxxxx snps were part of at least one meQTL. xxxxx pairs consisted of cpgs and snps on the same chromosome, while xxxxxx association were between different chromosomes.

2.3 Transcription factor binding

Transcription factor binding sites were obtained from two publicly 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 publicly 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 xxxxx 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 Epigenomics 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 multivariate 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 function "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 is modeled as simple linear regression and the absolute value of the sample correlation is used as test statistic.

Alternatively when choosing "*modelAnova*" for the parameter, 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 overrepresentation is performed on a set of genes of interest. For most enrichments a custom background 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 coefficient 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.

However, when including the 2kb flanking regions of HERV S2 and performing the GO enrichment on the 2400 identified genes, the 11 GO terms shown in table ?? are significantly overrepresented. They are all connected to immune response and the detection of stimuli.

The results for all HERV sets and including flanking regions is shown in table 4.2.

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 interrogated 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

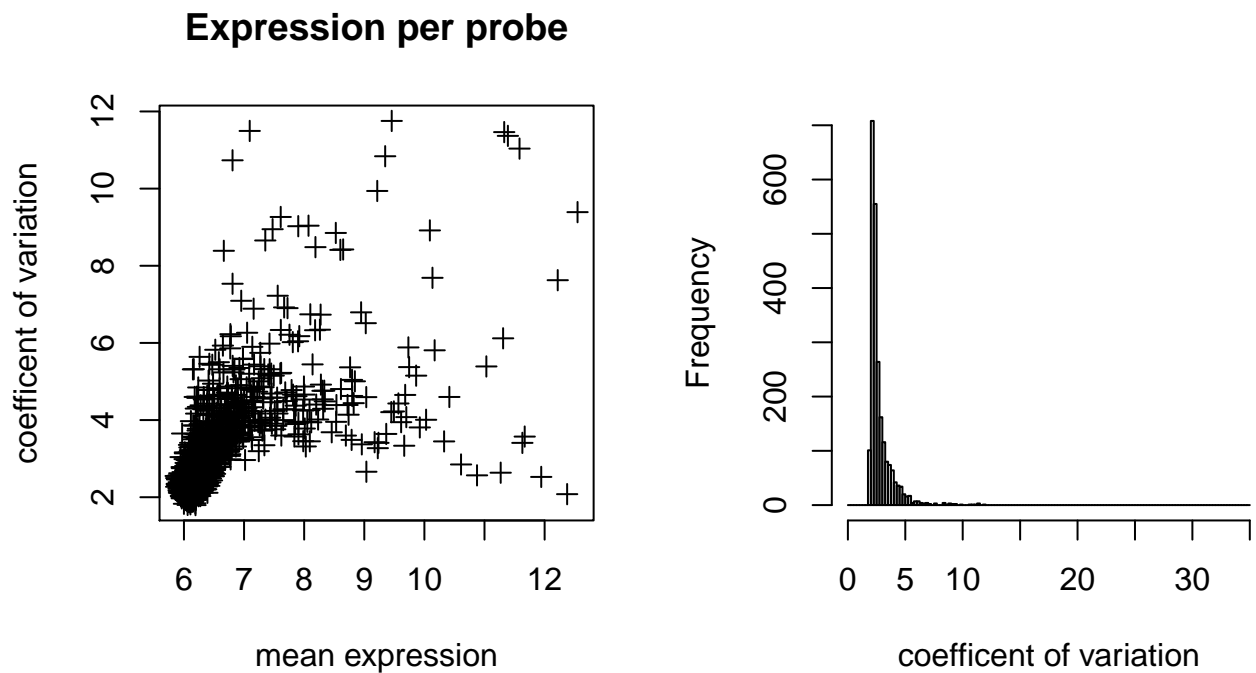


Figure 4.1: Coefficient of expression variance over 993 individuals and 2343 expression probes overlapping with HERV S2.

| 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.

4.2.3 Genotypes

A total of 890780 SNPs that were measured 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.

| 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.

| 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.

4.2.4 Chromatin states

4.3 eQTLs

4.4 eQTMs

4.5 meQTLs

4.6 HERV related regulatory networks

4.6.1 Data collection

5 Discussion

6 Conclusion

References

- [1] R Smit AFA, Hubley and Green P. Repeatmasker open-4.0. 2013-2015.
- [2] T.F. Smith and M.S. Waterman. Identification of common molecular subsequences. *Journal of Molecular Biology*, 147(1):195 – 197, 1981.
- [3] Bryan N. Howie, Peter Donnelly, and Jonathan Marchini. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLOS Genetics*, 5(6):1–15, 06 2009.
- [4] Jie Wang, Jiali Zhuang, Sowmya Iyer, XinYing Lin, Troy W. Whitfield, Melissa C. Greven, Brian G. Pierce, Xianjun Dong, Anshul Kundaje, Yong Cheng, Oliver J. Rando, Ewan Birney, Richard M. Myers, William S. Noble, Michael Snyder, and Zhiping Weng. Sequence features and chromatin structure around the genomic regions bound by 119 human transcription factors. *Genome Res*, 22(9):1798–1812, Sep 2012.
- [5] Philip Machanick and Timothy L. Bailey. Meme-chip: motif analysis of large dna datasets. *Bioinformatics*, 27(12):1696–1697, 2011.
- [6] Charles E. Grant, Timothy L. Bailey, and William Stafford Noble. Fimo: scanning for occurrences of a given motif. *Bioinformatics*, 27(7):1017–1018, 2011.
- [7] Aziz Khan, Oriol Fornes, Arnaud Stigliani, Marius Gheorghe, Jaime A Castro-Mondragon, Robin van der Lee, Adrien Bessy, Jeanne ChÃˆneby, Shubhada R Kulkarni, Ge Tan, Damir Baranasic, David J Arenillas, Albin Sandelin, Klaas Vandepoele, Boris Lenhard, BenoÃ®t Ballester, Wyeth W Wasserman, FranÃ§ois Parcy, and Anthony Mathelier. Jaspas 2018: update of the open-access database of transcription factor binding profiles and its web framework. *Nucleic Acids Research*, 46(D1):D260–D266, 2018.
- [8] V. Matys, O. V. Kel-Margoulis, E. Fricke, I. Liebich, S. Land, A. Barre-Dirrie, I. Reuter, D. Chekmenev, M. Krull, K. Hornischer, N. Voss, P. Stegmaier, B. Lewicki-Potapov, H. Saxel, A. E. Kel, and E. Wingender. Transfac(?) and its module transcompel(?): transcriptional gene regulation in eukaryotes. *Nucleic Acids Res*, 34(Database issue):D108–D110, Jan 2006. 16381825[pmid].
- [9] Aurelien Griffon, Quentin Barbier, Jordi Dalino, Jacques van Helden, Salvatore Spicuglia, and Benoit Ballester. Integrative analysis of public chip-seq experiments reveals a complex multi-cell regulatory landscape. *Nucleic Acids Research*, 43(4):e27, 2015.
- [10] Jason Ernst and Manolis Kellis. Chromhmm: automating chromatin-state discovery and characterization. *Nature Methods*, 9:215 EP –, Feb 2012. Correspondence.
- [11] Michael Lawrence, Wolfgang Huber, Herv   Pag  s, Patrick Aboyoun, Marc Carlson, Robert Gentleman, Martin Morgan, and Vincent Carey. Software for computing and annotating genomic ranges. *PLoS Computational Biology*, 9, 2013.

- [12] Andrey A. Shabalin. Matrix eqtl: ultra fast eqtl analysis via large matrix operations. *Bioinformatics*, 28(10):1353–1358, 2012.
- [13] Hervé Pagès, Marc Carlson, Seth Falcon, and Nianhua Li. *AnnotationDbi: Annotation Database Interface*, 2017. R package version 1.38.1.
- [14] S Falcon and R Gentleman. Using GOstats to test gene lists for GO term association. *Bioinformatics*, 23(2):257–8, 2007.
- [15] Sture Holm. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6(2):65–70, 1979.