

1 **Genome-wide analysis of repetitive elements** 2 **associated with gene regulation**

3 **Repetitive elements and gene regulation**

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23 ABSTRACT

24 Nearly half of the human genome is made up of transposable elements (TEs) and
 25 evidence supports a possible role for TEs in gene regulation. Here, we have integrated
 26 publicly available genomic, epigenetic and transcriptomic data to investigate this
 27 potential function in a genome-wide manner. Results show that although most TE
 28 classes are primarily involved in reduced gene expression, Alu elements are associated
 29 with up regulated gene expression. This is consistent with our previously published
 30 work which showed that intronic Alu elements are capable of generating alternative
 31 splice variants in protein-coding genes, and further illustrates how Alu elements can
 32 alter protein function or gene expression level. Furthermore, non-coding regions were
 33 found to have a great density of TEs within regulatory sequences, most notably in
 34 repressors. Our exhaustive analysis of recent datasets has extended and updated our
 35 understanding of TEs in terms of their global impact on gene regulation, and indicates
 36 a significant association between repetitive elements and gene regulation.

37 **Keywords:** Transposable elements/Epigenetic/Gene expression/Gene ontology/Regulatory
 38 elements.

39

40 INTRODUCTION

41 Repetitive elements are similar or identical DNA sequences present in multiple copies
 42 throughout the genome. The majority of the repetitive sequences in the human genome are

43 derived from transposable elements (TEs) [1, 2] that can move within the genome,
 44 potentially giving rise to mutations or altering genome size and structure. Typical eukaryotic
 45 genomes contain millions of copies of transposable elements (TEs) and other repetitive
 46 sequences. TEs fall into two major classes: those moving/replicating via a copy and paste
 47 mechanism and an RNA intermediate (retrotransposons) and those moving via direct cut
 48 and paste of their DNA sequences (DNA transposons). Retrotransposons can be subdivided
 49 into two groups: Those with long terminal repeats (LTRs), and those without LTRs (non-
 50 LTRs). Human LTR elements are related to endogenous retroviruses (HERVs), which along
 51 with similar elements account for nearly 8% of the human genome [3]. Non-LTR
 52 retrotransposons include two sub-types: autonomous long interspersed elements (LINEs)
 53 and non-autonomous short interspersed elements (SINEs), which are dependent on
 54 autonomous elements for their replication; both LINEs and SINEs are widespread in
 55 eukaryotic genomes. LINE-1 (long interspersed element 1) and Alu elements are two TEs
 56 that belong to non-LTR retrotransposons, which account for approximately one-quarter of
 57 the human genome [1].

58 A number of existing studies have shown that TEs can influence host genes by providing
 59 novel promoters, splice sites or post-transcriptional modification to re-wire different
 60 developmental regulatory and transcriptional networks [4-6]. TEs tend to regulate gene
 61 expression through several mechanisms [6-9]. For example, the expression levels of protein
 62 coding genes containing repetitive elements are significantly associated with the number of
 63 repetitive elements in those genes in rodents [7]. L1 family repeats show a stronger negative

64 correlation with expression levels than the gene length [10], and the presence of L1
65 sequences within genes can lower transcriptional activity [11]. Moreover, TEs have been
66 shown to influence gene expression through non-coding RNAs, resulting in the reduction or
67 silencing of gene expression [12]. For example, the expression of long intergenic non-
68 coding RNAs (lincRNAs) was strongly correlated with HERVH transcriptional regulatory
69 signals [13]. Past studies have found that TEs have contributed to nearly half of the active
70 regulatory elements of the human genome [14], by altering gene promoters and creating
71 alternative promoters and enhancers to regulate gene activity [15-17]. According to previous
72 research, 60% of TEs in both human and mouse were located in intronic regions and all TE
73 families in human and mouse can exonize, supporting the view that TEs may create new
74 genes and exons by promoting the formation of novel or alternative transcripts [18, 19]. The
75 association between repetitive elements and RNAs has also been investigated. For example,
76 Alu elements in lincRNAs can lead to *STAU1* mediated mRNA decay by duplexing with
77 complementary Alu elements in the 3'UTRs of mRNAs [20], and the insertion of TEs may
78 also drive the evolution of lincRNAs and alter their biological functions [13].

79

80 In this paper, TEs in the human genome were analyzed using genome-wide datasets
81 associated with gene regulation. These datasets enabled an assessment of the association of
82 TEs with chromatin states, as marked by histone modification within six human cell lines,
83 lincRNAs, Gene Ontology (GO) enrichment, as well as overall transcriptome profiles.
84 Whilst our analysis is limited to a general comparison of repeat families, as opposed to

specific repeat elements, we found clear associations between repeat families and gene regulation, both within regulatory regions and in the generation of splice variants.

RESULTS

The distribution of repetitive elements in the human genome

Initially, we compared the distribution of repetitive elements in the human genome. We found many repetitive elements overlapped with gene models from the human RefSeq gene datasets, and their distributions with respect to components of the gene model are shown in Fig 1. Most repetitive elements were found in non-coding intervals such as 5'UTR introns, CDS introns, 3'UTR introns and intergenic regions. In regards to clade-specific repeats, these were found more often in introns and intergenic regions than in 3'UTR exons or 5'UTR exons (Fig 1).

Fig 1. Distribution of repetitive elements overlapping with different human gene regions.

Gene regions are shown on the x-axis and the y-axis shows the percentages of the genomic regions containing repetitive elements. Human-specific repeats were those annotated with "Homo sapiens" or "primates" as their origin (see Table S6 for the list of human-specific repeat classes). The remaining repetitive regions were categorized as shared repeats.

Role of transposable elements in gene regulation by chromatin states.

Based on previous studies, TE-derived sequences can provide transcription factor binding

105 sites, promoters and enhancers, and insulators/silencers [5, 21, 22]. To look for enrichment of
 106 TEs within regulatory elements, we looked at the proportions of nucleotides with a TE in each
 107 of the six defined regulatory elements [23] as they appear in different components of the gene
 108 model. This represents the probability of a given nucleotide within an regulatory element
 109 (RE) being from a transposable element (TE), i.e. $p(\text{TE}|\text{RE})$ (see Methods for details) across
 110 the set of genic regions (Fig 2A). Confidence intervals for the pairwise differences in
 111 $p(\text{TE}|\text{RE})$ are shown in Fig 2B, and these reveal that for all regulatory elements, TEs are more
 112 sparsely distributed across regulatory elements within CDS exons than across regulatory
 113 elements in all other genic regions. Likewise, regulatory elements in the 3'UTR were more
 114 sparsely populated with TEs in comparison to those in other regions, with the sole exception
 115 of Active Promoters in the 5'UTR, Conversely, Intergenic Polycomb Repressed Regions were
 116 enriched for TEs in comparison to these elements in other components of gene models.
 117 Finally, Intergenic Insulators were also found to be enriched for TEs in comparison to
 118 Insulators in all other components of gene models, except for those in 5'UTRs.

119 **Fig 2. Analysis of the co-occurrence of Transposable Elements and Regulatory**

120 **Elements across multiple genomic regions** A) Probability estimates are those of an

121 individual base within each region being part of a Regulatory Element, i.e. $p(\text{RE})$, or a

122 Transposable Element within a Regulatory Element, i.e. $p(\text{TE}|\text{RE})$. Error bars indicate ± 1

123 Std. Error as calculated on the logit-transformed values. B) $1 - \alpha$ Confidence Intervals for

124 the difference between logit-transformed probabilities $p(\text{TE}|\text{RE})$, adjusted for multiple

125 comparisons at the level $\alpha = 0.05/6$ within each RE (60). Intervals highlighted in red are

126 those do not contain zeros and are indicative of a significant difference between the two
127 values.

128 **Different classes of transposable elements and their associations with chromatin state.**

129 In order to systematically characterize the role of different repeat classes within the defined
130 regulatory elements, the distribution of regulatory elements within specific classes of TEs
131 were investigated, using the estimates of $p(\text{RE}|\text{TE})$ (See Methods). Out of all six TE classes
132 investigated (Alu, L1, L2, LTR, MIR and DNA), L1 elements were consistently found with
133 the lowest probability of a nucleotide also belonging to a regulatory element (Fig 3A). It
134 was also clear that regulatory elements had the highest probability of being exapted as Weak
135 Enhancer and Polycomb Repressed Regions compared to the other elements. Confidence
136 intervals were used to perform pair-wise comparisons on the probability of containing an
137 RE for each TE type. No difference was found between ancestral and recent L1 elements for
138 any RE (Fig 3B), and L1s were confirmed as containing a significantly lower proportion of
139 their content as an RE in comparison to all other TEs. The notable exceptions to this were
140 Polycomb Repressed Regions, where little difference was found in their rate of occurrence
141 between any TE types, beyond comparative enrichment in MIR elements compared to L1s.
142 MIR elements were more likely to contain a strong enhancer than all other elements, except
143 L2 and DNA elements. DNA elements were also more likely to contain an Insulator than
144 Alu elements, as well as the previously mentioned L1 elements.

Fig 3. Analysis of the occurrence of Regulatory Elements within specific classes of

Transposable Elements. A) Probability estimates are for an individual base within each

type of element belonging to each of the regulatory elements, i.e. $p(\text{RE}|\text{TE})$. Error bars

indicate ± 1 Std. Error as calculated on the logit-transformed values. B) $1 - \alpha$ Confidence

Intervals for the difference between logit-transformed probabilities $p(\text{RE}|\text{TE})$, adjusted for

multiple comparisons at the level $\alpha = 0.05/6$ within each RE (60). Intervals highlighted in

red are those do not contain the zero and are indicative of a significant difference between

the two values

Are Regulatory Elements containing TEs abundantly present in long intergenic non-coding

RNAs?

TEs are a source of endogenous small RNAs in animals and plants, and endogenous small

RNAs are considered to be functionally significant in gene regulation [24]. Furthermore, it

is well known that many Alu elements have inserted into long non-coding RNAs and

mRNAs, which can cause mRNA decay via short imperfect base-pairing [25]. We expanded

this to see whether different classes of TEs had any significant associations with non-coding

RNA, especially lincRNAs.

Unsurprisingly, we found that TEs consistently made up a lower proportion of nucleotides

in CDS-exons across all regulatory elements, when compared to CDS-introns, lincRNA

exons and lincRNA introns (Fig 4). An additional enrichment for TEs in Active Promoters

within lincRNA introns was also observed in comparison to all other regions investigated in

this stage of the analysis. Weak Promoters in both lincRNA introns and CDS introns also

showed TE enrichment compared to both types of exonic regions. The observation that >30% of nucleotides from many of the regulatory elements were derived from TEs was also quite striking. In particular, the observation that lincRNA exonic regions contained the highest RE density for Polycomb Repressed Regions (Fig 4), with a nearly a third of these nucleotides being derived from TEs, suggests that the presence of transposable elements in lincRNA exons may be strongly linked to gene regulation.

Fig 4. Analysis of the co-occurrence of Transposable Elements and Regulatory Elements across non-coding regions A) Probability estimates for an individual base within each type of non-coding region being part of a Regulatory Element $p(\text{RE})$ or a Transposable Element within each Regulatory Element $p(\text{TE}|\text{RE})$. Error bars indicate ± 1 Std. Error as calculated on the logit-transformed values. B) $1 - \alpha$ Confidence Intervals for the difference between logit-transformed probabilities $p(\text{TE}|\text{RE})$, adjusted for multiple comparisons at the level $\alpha = 0.05/6$ within each RE (60). Intervals highlighted in red show significant pairwise differences (confidence intervals do not cross the 0 difference value).

Associations of TEs with gene model features and gene expression

Next, we summarized the overall distribution of transposable elements within various components of the gene model, by finding genes containing TEs across single or multiple components (Fig S1, Table 1), and genes containing one or more types of TEs (Fig S2, Table 2). We further examined the relationship between gene length and which components of a gene contain a TE (Fig S3), as well as the relationship between gene length and the presence of a specific type of TE (Fig S4), using a Wilcoxon Test (Tables S2 & S3) in both

cases. We found that only genes with TEs in the 3'UTR or within multiple genic regions showed a bias towards longer length, whilst for TEs exclusively within the proximal promoter or 5'UTR there was a bias towards shorter genes (Fig S3; Table S2). When assessing the relationship between gene length and the presence of a specific TE class, the length of genes with Alu, L2 or MIR elements alone were very similar to genes with no TE, whilst L1 and LTR elements showed a bias towards shorter genes, and the presence of multiple elements biased towards longer genes (Fig S4; Table S3).

Table 1: Total counts of elements within each genomic region, along with the number of genes with Transposable Elements in one region only.

Region	Total	Elements in Single Regions	Proportion
Proximal Promoter	9462	4835	0.511
5'UTR	4913	1528	0.311
CDS	655	131	0.200
3'UTR	5640	1766	0.313

Table 2: Total counts of each TE element, along with how many are found in isolation, i.e. in genes with no other elements.

Element Type	Total	Found in Isolation	Proportion
Alu	9460	2199	0.232
L1	5111	539	0.105
L2	5863	687	0.117
LTR	4089	363	0.089
MIR	7638	1423	0.186

199 **Effects on the probability of a gene being detected as expressed due to the presence of** 200 **a TE across the different component of the gene model**

201 As chromatin states are not always indicative of changes in transcriptional activity, we
202 investigated any effects on human gene expression due to the presence of specific TE
203 classes within each of the four regulatory regions, i.e., Proximal Promoter, 5'UTR, CDS and
204 3'UTR. However, as TEs are far less frequent in CDS regulatory regions with the vast
205 majority co-occurring with other TEs (Figure S1), the subsequent analysis instead focused
206 on the other three regions. Six human tissue transcriptome datasets (adipose, brain, kidney,
207 liver, skeletal muscle and testes tissue) were selected from the Illumina BodyMap2 dataset
208 for this analysis, and global patterns of gene expression were investigated based on the
209 presence or absence of each TE within each of these three genic regions.

210 The weighted bootstrap method was applied to both the probability of a gene being detected
211 as expressed (Fig 5A), and to the overall expression levels for those genes detected as
212 expressed (Fig 5B). This revealed that Alu elements are commonly associated with a higher
213 probability of expression when located in either the 5'UTR or the 3'UTR across the
214 majority of tissues. In contrast to the presence of an Alu, the presence of L1 elements in the
215 Proximal Promoter showed a negative impact on the probability of a gene being detected as
216 expressed in 3 of the 6 tissues, with the remaining tissues being directionally consistent and
217 quite likely to be Type II errors (Supplementary Fig S6A).

218 **Fig 5. Effects of the presence of each TE in each genomic region.** A) Confidence
 219 Intervals for the difference in the probability of a gene being detected as expressed due to
 220 the presence of each TE in each genic region. B) Confidence Intervals for the difference in
 221 mean log2(TPM) counts. For both A) and B), Confidence Intervals were obtained using the
 222 weighted bootstrap and are $1 - \alpha/m$ intervals, where $\alpha = 0.05$ and $m = 90$ as the total
 223 number of intervals presented. Dots represent the median value from the bootstrap
 224 procedure, whilst the vertical line indicates zero. Intervals which do not contain zero are
 225 coloured red, and indicate a rejection of the null hypothesis, $H_0: \Delta\theta = 0$, where θ represents
 226 the parameter of interest.

227 **Effects on the levels of gene expression due to the presence of a TE in each component**
 228 **of the gene model.**

229 Again using the weighted bootstrap approach to minimize any influence of co-occurring
 230 elements, the presence of an Alu in the 5'UTR was found to be associated with increased
 231 expression levels in five of the six tissues investigated (Fig 5B). Similarly, Alu elements in
 232 the Proximal Promoter were associated with increased expression in two of the tissues. Alu
 233 elements in 3'UTR were associated with elevated expression levels in the Kidney sample
 234 only. The presence of ion

235 elements showed varying degrees of reduced gene expression across the tissues when
 236 located in the 3'UTR only. It was also noted that whilst strongly controlling the family-wise
 237 Type-I error rate (FWER), the adjusted confidence intervals will result in an increase in the

238 Type-II error rate where true differences are not able to be detected. As such, the point at
239 which the confidence intervals would include zero was found and taken as a proxy for the p-
240 value. Confidence intervals based on these p values to an FDR of 0.05 are shown in
241 Supplementary Figure S6 with the p values given in Supplementary Table S4. It is clear
242 from this additional approach that the role of TEs such as L1 elements in Proximal
243 Promoters and 3'UTRs, LTR elements in 5'UTRs and many of the elements in the 3'UTR
244 may have been considerably understated in this more conservative approach.

245 **Analysis of genes with exapted or exonized TEs**

246 TEs may influence gene expression in different ways, thus we evaluated the possible
247 functional effects of repetitive elements in the human genome, the six primary repeat classes
248 were mapped to the human genome (<http://www.repeatmasker.org>). Genic regions
249 (annotated using Gene Ontology) that overlapped with TEs were analyzed to assess the
250 association of TEs with different gene functions.

251

252 The three fundamental GO categories are: cellular component, molecular function and
253 biological process. Enrichment information for each GO category is listed in Supplementary
254 Table S5. We discovered that for the biological process category (Fig 6, Table S5a), the
255 predominant types of annotation were related to regulatory processes involving metabolic
256 processes. This was consistent with the annotations for the cellular component terms, which
257 were predominantly for intracellular/cytoplasmic structures (Fig S7, Table S5b). The
258 molecular function terms had functions mainly associated with binding (Fig S8, Table S5c).

259 Using this same method, we also found that genes with protein coding exons containing
260 Alus were enriched for the GO term “intracellular non-membrane-bounded organelle”.
261 Interestingly, these exonization/exaptation events were found associated with splice variants
262 when incorporating Alu sequences (Table S6 & Fig S9, S10).

263 **Fig 6. Enrichment of GO terms of genes containing TEs in promoter, 5'UTR and**
264 **3'UTR regions.**

265 Enrichment of GO terms of genes containing TEs in “Biological Process”. Genes containing
266 different types of repetitive elements in the proximal promoter regions are labeled as
267 "Promoter with Repeats", and Genes containing repetitive elements in UTR regions are
268 labeled as "5/3UTR with Repeats". Genes named “Combined Repeats” are the combined
269 data from 3 regions we mentioned above. The darker the color, the greater the GO term
270 enrichment as determined by FDR.

271 Moreover, according to our analysis of TEs and alternative splicing data, we found that
272 2.98% of alternatively spliced transcripts contained TEs within protein coding exons (Table
273 3). Alu and MIR were more likely to be involved in alternative splicing and exonization,
274 which is consistent with previous studies showing that exonization of SINEs occurred in
275 primates [26]. Based on our study, LINEs may also have contributed to these splice variant
276 activities. This shows that exonization of TEs could potentially increase the coding and
277 regulatory versatility of the transcriptome.

Table 3. The number of repeats in protein coding regions (CDS-exon) with alternative splicing. Repeats were counted only if they overlapped CDS-exon regions by at least 25 bps.

Repeat Class							
	Alu	MIR	L1	L2	LTR	ERV	All repeats
TEs in CDS-exon containing AS	329	224	186	213	229	15	1,402

DISCUSSION

In this work, we have primarily analyzed the distribution of various classes of transposable elements, and their association with regulatory elements (active chromatin) and gene expression in the human genome. Based on the analysis of the TE distributions in genic regions and corresponding gene expression patterns, the presence of some TEs was found to be associated with changes in gene expression. Further, gene function as defined by GO term analysis differed depending on the TE insertion site within the gene. Finally, we looked at TEs present in ncRNAs, specifically lincRNAs, and found that repetitive elements were present at higher levels in lincRNAs than coding exons.

Considering the association between the location of TEs in genes, we found that genes had a greater proportion of sequence originating from TEs in the 5' and 3'UTRs compared to coding exons (Fig 2). This is not surprising considering the potential adverse effect of TE insertion in a protein coding sequence, but it is also relevant with respect to the known

295 regulatory functions within the UTRs [27, 28]. The repeat content for 5'UTR introns was
296 comparable to other types of introns, but this may be significant in the context of
297 transcriptional repression, where genes with shorter 5' UTR introns are expressed at higher
298 levels [29, 30].

299 Furthermore, the presence of TEs in genomic regions that can be epigenetically modified to
300 regulate transcription through active chromatin [31], was consistent with our findings that
301 TEs have a potential role as regulators of gene expression. From our results (Fig 2), we
302 found that some functional regions of active chromatin contained higher percentages of TEs,
303 especially Polycomb Repressed Regions and Weak Enhancers. This fits with the existing
304 theory of epigenetic silencing of TEs [31], since TEs in Polycomb Repressed Regions
305 would inevitably be silenced, and was also consistent with the high repeat content of
306 5'UTRs, which are also known to regulate gene expression [32]. Furthermore, it has been
307 shown that TEs in 3'UTRs are associated with lower transcript abundance [33], and with the
308 clear exception of Alu elements, we have presented further evidence of this. This suggests
309 that exaptation of repetitive elements into regulatory regions is most often associated with
310 repression of gene expression. The general theme of TEs having a role in transcriptional
311 repression was further supported with lincRNAs, which are known to regulate gene
312 expression through epigenetic mechanisms [34] and competition with transcription factors
313 [25]. In our analysis, lincRNA exons were found to be clearly enriched for Polycomb
314 Repressed Regions whilst the abundance of TEs within these regions was relatively
315 consistent with both CDS and lincRNA introns (Fig 4). However, this overall enrichment

for repressed regions is consistent with previous research that lincRNAs containing TEs can reduce gene expression in many tissues and cell lines [13]. As different repeat classes were also found to be present at different levels in active chromatin or in specific regulatory regions, such as Polycomb Repressed Regions (Fig 3), this suggests a function with respect to gene expression.

Figures 5B and S6B summarize our findings with regard to gene expression, and indicate that Alu elements in 3'UTR, 5'UTR and proximal promoter regions are commonly associated with increased gene expression. Taken in addition with the increased probability of expression due to the presence of an Alu in the 5'UTR and 3'UTR (Fig 5A), these results support previous reports showing TEs such as Alus can be exapted as transcription factor binding sites [35-37], but are in contrast with reports concerning the direction of expression for human genes. We also found genes containing L1 elements were associated with decreasing gene expression (Fig 5), and that L1 elements were less prevalent in regulatory elements or active chromatin, when compared to other repeat classes (Fig 3). This makes intuitive sense as most L1 elements in the human genome are 5' truncated and lack promoter content compared to Alu elements [38]. This is also consistent with a previous study showing that highly and broadly expressed housekeeping genes can be distinguished by their TE content, with these genes being enriched for Alus and depleted for L1s [39]. LTRs were found associated with repression of gene expression, which is in contrast to previous work that implicated LTRs as alternative promoters [40]. Anecdotally, it has been shown that an LTR in the first intron of the equine TRPM gene suppresses gene expression

337 by acting as an alternative poly-A site [41], and the insertion of LTRs in introns has been
 338 associated with premature termination of transcription [42], supporting the results presented
 339 here. L2 and MIR are ancient TE families conserved among mammals, and are regarded as
 340 inactive or fossil TE elements [43]. However, these TEs showed a level of association with
 341 reduced gene expression when located in 3'UTRs (Fig 5), which is also consistent with a
 342 previous finding on their ability to impact the evolution of gene 3' ends by containing cis-
 343 elements for modified polyadenylation [44].

344 In addition to potentially altering gene expression by insertion into regulatory elements, TEs
 345 may also be associated with specific functional characteristics of expressed protein coding
 346 genes. When we examined the functional annotation of repeat containing genes, we found
 347 that some functions were over-represented (Table S5). Perhaps the most interesting of these
 348 associations was that genes with Alu insertions were found to contribute to coding exons
 349 through alternative splice variants. One explanation of this observation is that Alu-induced
 350 alternative transcripts may result in nonsense mediated decay of alternative transcripts [45].
 351 Two examples of alternatively spliced genes of this type with implications for human
 352 disease are *DISC1* and *NOS3* (Table S6 and Fig S9 & S10). *DISC1* alternative transcripts
 353 are known to contribute to increased risk of schizophrenia [46, 47] and *NOS3* transcript
 354 variants are associated with cardiovascular disease phenotypes [48, 49]. Based on previous
 355 research, nearly 4% of protein-coding sequences include transposable elements, and one-
 356 third of them are Alu insertions [50]. Therefore, Alu exonization in protein-coding genes
 357 may play an important role in modifying gene expression.

358 In conclusion, while there are many publications implicating TEs in the regulation of
 359 individual genes, our work clarifies some previous uncertainties and resolves some
 360 contradictions, confirming that this role of TEs is significant across the genome. In general,
 361 most TEs would appear to be strongly associated with repression of gene expression, either
 362 through the 5'UTR or perhaps as components of lincRNA exons. However, the presence of
 363 Alus in 3'UTR and proximal promoter regions may act to increase gene expression. These
 364 results are consistent with some previous published research [10] and provide a new
 365 understanding of how repeats are associated with epigenetic regulation of gene expression.
 366 Finally, while exapted TEs may contribute to the generation of transcripts that undergo
 367 nonsense mediated decay as part of gene regulation, we speculate that they may also provide
 368 an opportunity for alternative splicing and novel exaptation. TEs therefore are important
 369 agents of change with respect to the evolution of gene expression networks.

370

371 **MATERIAL AND METHODS**

372 **Theoretical framework and methods**

373 We constructed pipelines to analyze the distribution of repetitive elements in different parts
 374 of the human genome. Repetitive elements overlapping with protein coding regions, non-
 375 coding regions and regulatory elements were identified. GO term over-representation and
 376 expression analyses were carried out for repetitive elements overlapping with protein-
 377 coding regions. The pipelines and related materials are described below.

378 **Tools used to develop pipelines for repetitive element analysis**

The identification and classification of TEs from the human genome was conducted by developing a pipeline with Perl, R [51], and BEDTools [52]. Perl was used to extract information from different datasets. R was used to build graphs to illustrate the repeat distribution in different genic regions, the identification of repetitive elements with respect to functional elements, GO term over-representation analysis and expression analysis of TEs. BED format file intersection was used to extract the overlapping regions between different datasets, with a lower limit of 25-bps. The UCSC Genome Browser [53, 54] was used to download genome sequence data and genome annotations including RefSeq genes. RSEM [55] was adopted to assemble RNA-Seq reads into transcripts and estimate their abundance (measured as transcripts per million (TPM)). Plots were generated using ggplot2 in R [56].

Datasets

Genomes and annotations. NCBI's Human genome and its annotation datasets (RefSeq hg19) [57] were downloaded from the UCSC Genome Browser [23, 53]. A total of 37,697 human RefSeq transcripts were merged into 18,777 genes by taking the longest transcript(s) that represented each distinct gene locus. Repetitive elements were downloaded from the RepeatMasker (<http://www.repeatmasker.org>) track of the UCSC Genome Browser. All repetitive sequence intervals were also de-duplicated to deal with potential overlapping repeat annotations. Overall, there were 5,298,130 human repetitive elements which represented approximately 1.467Gb in the human genome.

398 **Regulatory element datasets from six human cell lines.** The regulatory element datasets
 399 from six human cell lines were downloaded from the UCSC Genome Browser. Each cell
 400 line dataset contained the annotation of six regulatory elements: 1) Active Promoters, 2)
 401 Weak Promoters, 3) Strong Enhancers, 4) Weak Enhancers, 5) Insulators and 6) Polycomb
 402 Repressed Regions. These regulatory element annotations were derived from different
 403 chromatin states that have been marked by histone methylation, acetylation and histone
 404 variants H2AZ, PolIII, and CTCF [23].

405 **Gene expression datasets from six human tissues.** Human RNA-seq data from the
 406 Illumina bodyMap2 transcriptome (Paired End reads only)
 407 (<http://www.ebi.ac.uk/ena/data/view/ERP000546>) dataset was used to measure the
 408 association between TEs and the expression levels of genes containing TEs in six tissues.

409 **The distribution of repetitive elements in the human genome.** To assess how human TEs
 410 were distributed in genes, we compared different genic regions containing TEs. Based on
 411 Repbase [58, 59] annotations identified by RepeatMasker (<http://www.repeatmasker.org>),
 412 repeat elements in human were divided into two categories: human-specific repeats, and
 413 repeats shared with different species. Human-specific repeats were those annotated with
 414 “Homo sapiens” or “primates” as their origin (See Table S6 for the list of human-specific
 415 repeat classes), whilst those remaining were categorized as shared repeats. Intergenic
 416 regions as well as the exons and introns within 5’UTR, CDS and 3’UTR regions from
 417 RefSeq genes [60] were then compared with these different categories of repeats. Next, we

418 generated the summarised distributions of repetitive elements overlapping these regions by
 419 calculating the proportions of bases belonging to repetitive elements within each of the
 420 combined sets of regions, i.e.:

$$proportion = \frac{total\ length\ of\ repeats\ in\ defined\ regions\ (bps)}{total\ length\ of\ defined\ regions\ (bps)} \quad (1)$$

421 The code repository for the above can be found at
 422 <https://github.com/UofABioinformaticsHub/RepeatElements>.

423 **The occurrence of transposable elements within regulatory regions.** We further explored
 424 the association between any TE and the regulatory elements defined above, by calculating
 425 the proportion of nucleotides within each of the five sets of genic regions (5'UTR, CDS-
 426 exon, CDS-intron, 3'UTR and Intergenic) that were part of a regulatory element for each of
 427 the six human cell lines. The proportion of nucleotides that were TEs within each regulatory
 428 element were also calculated for each genic region. All proportions were subsequently
 429 transformed using the logit function for model fitting across tissues (Table S1) using the
 430 model

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (2)$$

431 where y_{ijk} is the logit transformed proportions representing $p(\text{TE}|\text{RE})$ across each genic
 432 region i , each regulatory element j and tissue k , such that μ is the overall mean, α_i is the
 433 effect due to each genic region, β_j is the effect due to each regulatory element with $(\alpha\beta)_{ij}$
 434 representing any changes not accounted for in the first two terms. Tests for normality and
 435 homoscedasticity were performed using the Shapiro-Wilk test and Levene's test
 436 respectively. Where violations of homoscedasticity were found robust standard errors were

437 obtained using the sandwich estimator [61]. Confidence Intervals for pairwise comparisons
438 were obtained as implemented in the R package *multcomp* [62] in order to control the Type
439 I error at $\alpha=0.05$ across the entire set of comparisons.

440 A specific TE analysis was then performed using six of the major human TE classes based
441 on the Repbase classification system: Alu, L1, L2, MIR, LTR and DNA. L1 elements were
442 further resolved into either ancestral (L1M, L1PB and L1PA subfamilies) or recent/clade-
443 specific (L1HS subfamily), based on their Repbase annotations. The proportions of
444 nucleotides within each TE type that were also regulatory elements were calculated giving
445 tissue-specific estimates of $p(\text{RE}|\text{TE})$. Proportions were again transformed using the logit
446 function, and the same analysis as above was performed.

447 **Quality control and preprocessing of the gene expression data in different human**
448 **tissues.** RNA-seq reads of six human tissues were first assessed using FastQC software
449 (www.bioinformatics.babraham.ac.uk), to provide an overview of whether the raw RNA-
450 Seq data contained any problems or biases before further analysis. Reads with poor-quality
451 bases were trimmed (based on the results of FastQC with MINLEN set to 26) for subsequent
452 data analysis. Table 4 showed the numbers of reads in raw RNA-Seq datasets and the
453 statistics after the QC process by using Trimmomatic-0.32 [63]. Then, we built transcript
454 reference sequences using rsem-prepare-reference [64] from Hg19 human RefSeq genes.
455 The references were then input to rsem-calculate-expression [64] using default parameters
456 for all 6 tissues to obtain TPM based expression values.

Table 4. Description of RNA-Seq datasets and QC results. Reads with poor-quality bases were trimmed using FastQC (with MINLEN set to 26, HEADCROP set to 13 and LEADING set to 15).

	Raw Data (Read Number)	High Quality reads (%)			Low Quality reads (%)
		Both Reads	Forward Only Reads	Reverse Only Reads	
Kidney	8,039,733,700	78.72	9.84	4.87	6.58
Liver	8,004,862,300	81.25	8.11	4.67	5.96
Brain	7,351,304,700	69.31	7.76	12.86	10.06
Testes	8,183,619,900	76.9	9.88	5.51	7.71
Adipose	7,730,007,200	79.88	9.74	4.49	5.89
Skeletal Muscle	8,211,113,900	76.06	12.12	4.87	6.94

Proximal promoter regions were defined as 1,000bp upstream of the gene transcription start sites based on the longest transcripts for each gene. Alu, MIR, L1, L2 and LTR repeat regions were then identified within the proximal promoters, 5'UTR, CDS and 3'UTR regions.

The weighted bootstrap procedure for assessing the effects of a TE in each genic region.

Many genes contain multiple transposable elements, with only a minority of genes containing a single TE (Fig S2). In order to assess any effects on transcription due to the presence of a single TE, a weighted bootstrap approach was devised. For a given TE within each genic region within each individual tissue, the frequencies of co-occurring TEs and combinations of TEs were noted. Uniform sampling probabilities were then used for the set of genes containing a specific TE in a specific region, whilst sampling weights were assigned to genes lacking the specific TE based on TE composition, such that the TE content of the sampled set of reference genes matched that of the test set of genes, based on

the defined categories. Gene length was divided into 10 bins and these were included as an additional category when defining sampling weights. This ensured that two gene sets were obtained for each bootstrap iteration, which were matched in length and TE composition with the sole difference being the presence of the specific TE within each specific genic region (Figure S5). The mean difference in expression level, as measured by log(TPM), and the difference in the proportions of genes detected as expressed were then used as the variables of interest in the bootstrap procedure. The bootstrap was performed on sets of 1000 genes for 10,000 iterations using the proximal promoter as defined above, along with 5'UTR and 3'UTRs. When comparing expression levels, genes with zero read counts were omitted prior to bootstrapping. In order to compensate for multiple testing considerations, confidence intervals were obtained across the $m = 90$ tests at the level $1 - \alpha/m$, which is equivalent to the Bonferroni correction, giving confidence intervals which controlled the FWER at the level $\alpha = 0.05$. Approximate two-sided p-values were also calculated by finding the point at which each confidence interval crossed zero, and additional significance was determined by estimating the FDR on these sets of p-values using the Benjamini-Hochberg method.

Long intergenic non-coding RNAs and TEs. Annotations for 8,196 previously described putative human lincRNAs were downloaded [65] and the distribution of TEs within regulatory elements in lincRNA exons and introns was obtained using the same methods as above. The previously described regression models were then used to analyse this dataset.

494 **Association of functional elements with human repetitive elements.** To demonstrate the
 495 potential functional significance of repetitive elements, the Database for Annotation,
 496 Visualization and Integrated Discovery (DAVID) [66] was used to perform the GO
 497 classification. We first extracted Gene-IDs from overlapping regions between different gene
 498 categories (1000bp proximal promoter, 5'UTR, 3'UTR, and the combination of these 3
 499 regions) and TEs. These gene-lists were then submitted to the DAVID Functional
 500 Classification Tool. We chose the third level of GO terms to describe the over-represented
 501 functional terms for the three datasets and visualized the functional over-representation of
 502 overlapped genes using the R package *heatmap.2*. The p-value was applied in the GO
 503 analysis as the standard index to determine the degree of enrichment. The threshold for
 504 over-represented GO terms was set to an FDR (Benjamini-Hochberg method) less than 0.05.
 505 Protein-coding genes with Alus were also visualised with the UCSC genome browser
 506 (<http://genome.ucsc.edu/>) to compare their mRNA with various gene datasets and
 507 annotations.

508 **Association of alternative splicing and protein coding regions containing TEs.** In order
 509 to assess the relationship between transposable elements and exonization, an
 510 alternative splicing annotation dataset (SIB Alt-splicing) was downloaded from the
 511 UCSC Genome Browser (<http://genome.ucsc.edu/>). These data were generated from
 512 RefSeq genes, Genbank RNAs and ESTs that aligned to the human genome. A total of
 513 46,973 alternatively spliced transcripts were intersected with gene models containing
 514 transposable elements.

515

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519

520 **AUTHOR CONTRIBUTION**

521 DLA and CCW conceived, designed and managed the study. LZ collected the datasets,
522 implemented the analysis pipeline, and analyzed the data. SMP analyzed the data. ZPQ, DFC,
523 ZQH prepared datasets. LZ, DLA and CCW wrote and revised the manuscript. All authors
524 reviewed and approved the final manuscript.

525

526 **CONFLICT OF INTEREST**

527 The author(s) declare that they have no competing interests.

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711

712

Repeat Type Shared Specific

Proportion of bases

0.4
0.2
0.0

5'UTR
exon

5'UTR
intron

CDS
exon

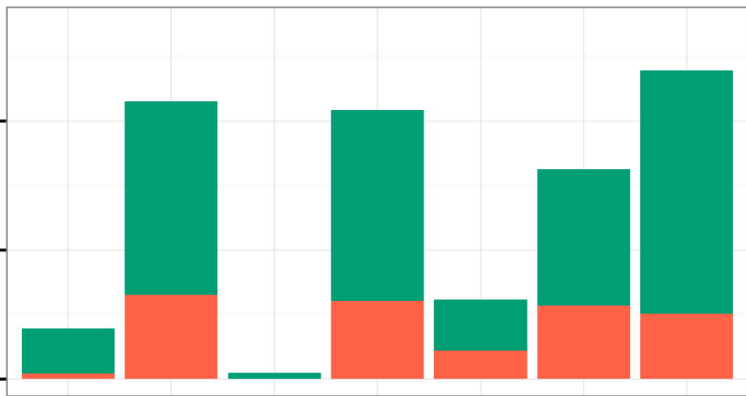
CDS
intron

3'UTR
exon

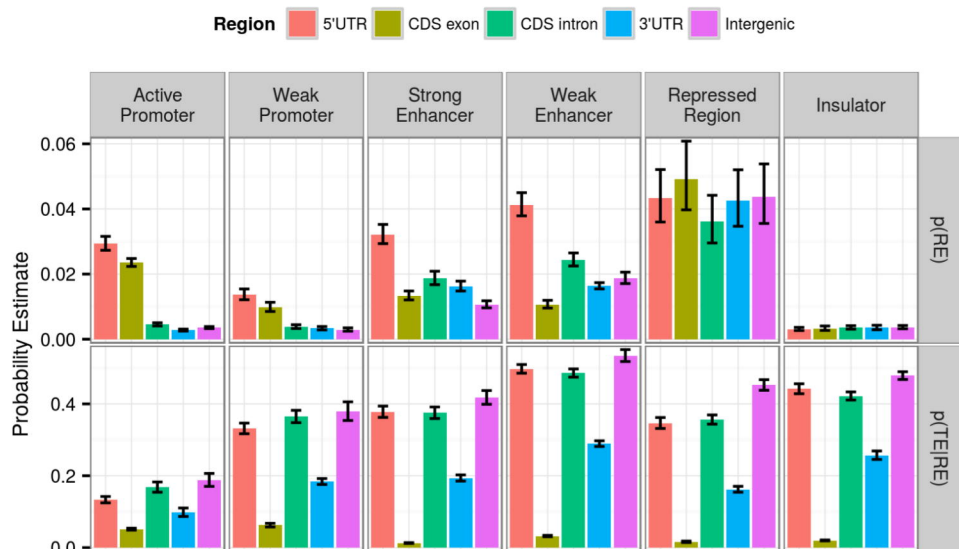
3'UTR
intron

Intergenic

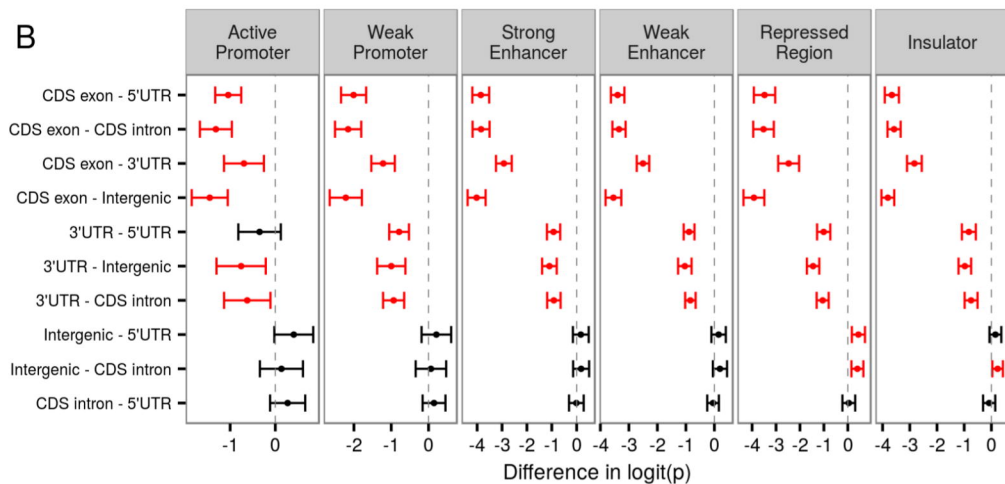
Region



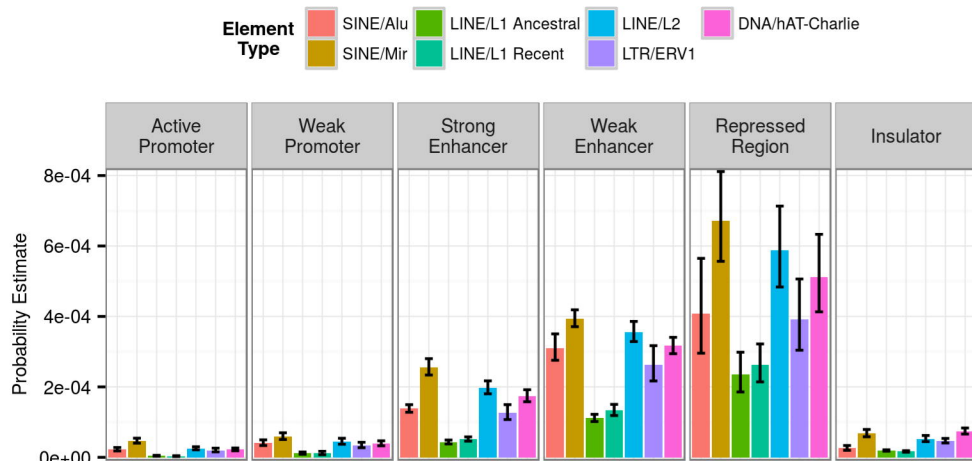
A



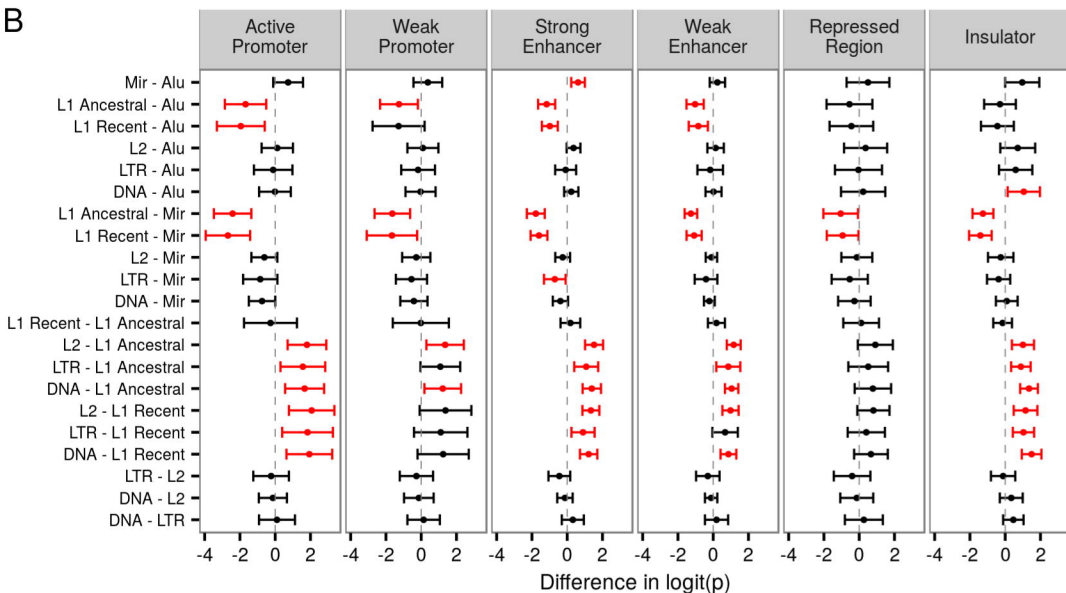
B



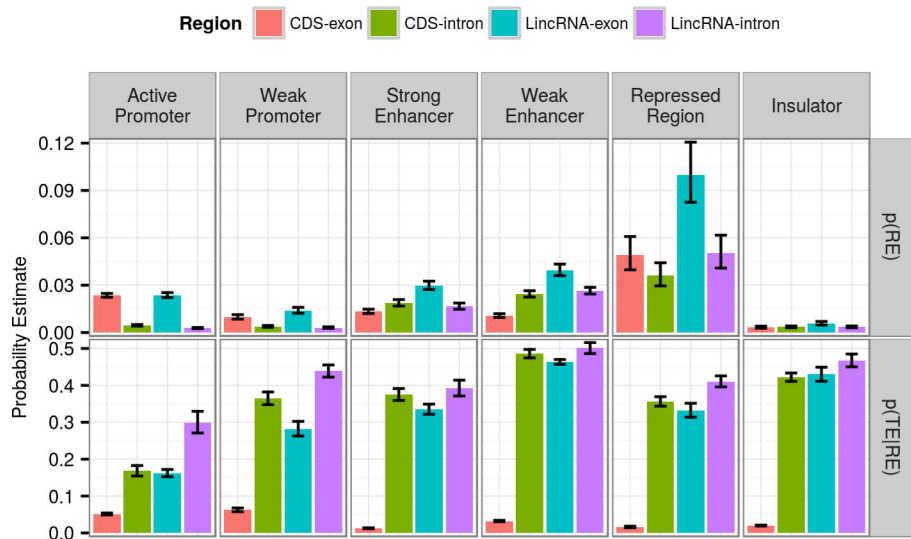
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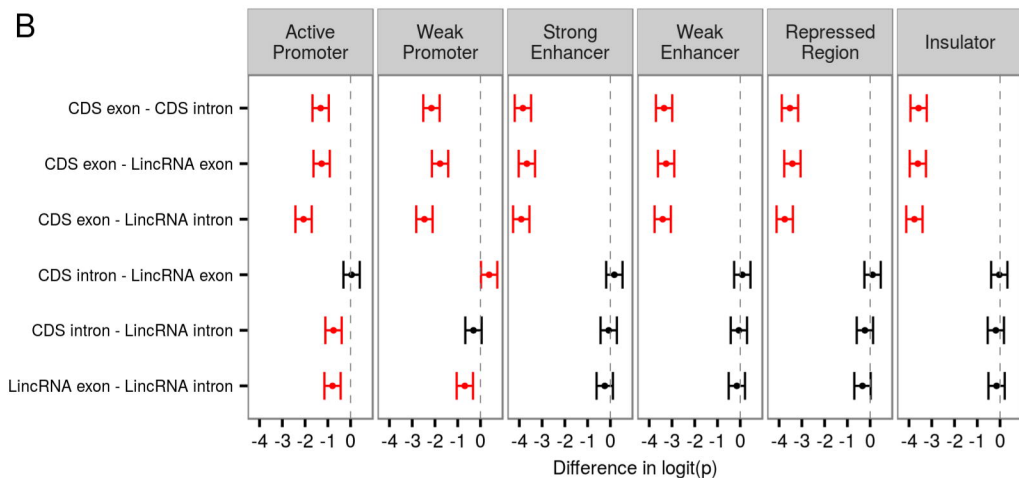
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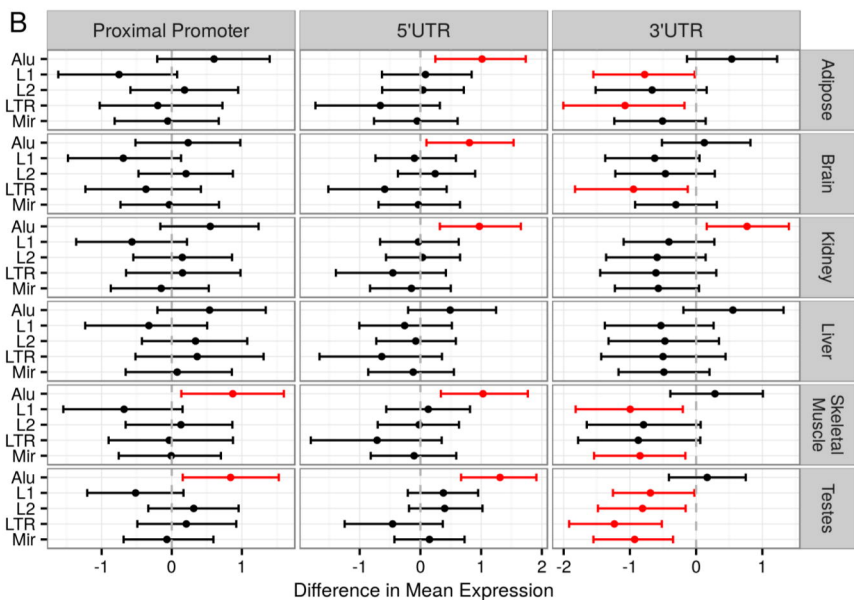
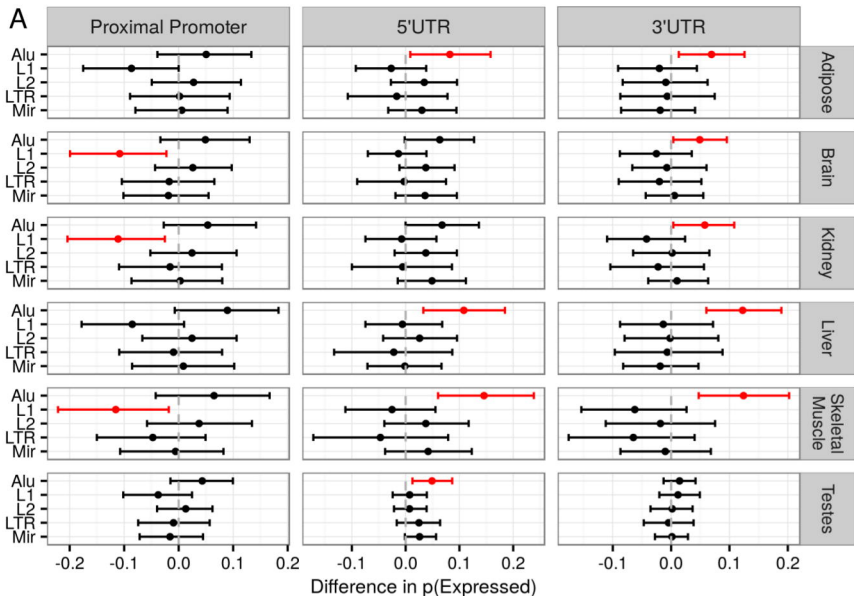


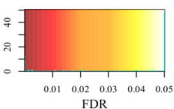
A



B







Biological Process

