EPIGENE: genome wide transcription unit annotation using a multivariate probabilistic model of histone modifications

Anshupa Sahu[1,2], Na Li[2,3], Ilona Dunkel[2], Ho-Ryun Chung[1,2*]

[1]Philipps University of Marburg, Institute for Medical Bioinformatics and Biostatistics, 35037 Marburg, Germany

[2]Max Planck Institute for Molecular Genetics, Otto-Warburg-Laboratory, 14195 Berlin, Germany

[3]Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, 510623 Guangzhou, China

*Correspondence: ho.chung@staff.uni-marburg.de

Abstract

Background

Understanding transcriptome is critical for explaining functional as well as regulatory roles of genomic regions. Current methods for the identification of transcription unit (TU) uses RNA-seq which, however, requires large quantities of mRNA limiting the identification of inherently unstable TUs e.g. for miRNA precursors. This problem can be resolved by chromatin based approaches due to a correlation between histone modifications and transcription.

Results

Here we introduce EPIGENE, a novel chromatin segmentation method for the identification of active TUs using transcription associated histone modifications. Unlike existing chromatin segmentation approaches, EPIGENE uses a constrained, semi-supervised multivariate hidden markov model (HMM) that models the observed combination of histone modifications using a product of independent Bernoulli random variables, to identify active TUs. Our results show that EPIGENE can identify genome-wide TUs unbiasedly. EPIGENE predicted TUs showed an enrichment of RNA Polymerase II in transcription start site and gene body indicating that they have been transcribed. Comprehensive validation with existing annotations revealed that 93% of EPIGENE TUs can be explained by existing gene annotations and 5% of EPIGENE TUs in HepG2 can be explained by microRNA annotations. EPIGENE outperforms existing RNA-Seq based approaches in TU prediction precision across human cell lines. Finally, we identify 381 novel TUs in K562 and 43 novel cell-specific TUs all of which are supported by RNA Polymerase II data.

Conclusions

We demonstrate the applicability of HMM to identify genome-wide active TUs and provides valuable information about unannotated TUs. EPIGENE is an open-source method and is freely available at: https://github.com/imbeLab/EPIGENE .

Keywords

Transcription, epigenetics, histone modifications, hidden markov model, transcript identification

1. Background

Transcription unit (TU) represents the transcribed regions of genome which generates protein-coding genes as well as regulatory non-coding RNAs like microRNA. Accurate identification of TUs is important to better understand the transcriptomic landscape of the genome. With the rapid development of low-cost high-throughput sequencing technologies, RNA sequencing (RNA-seq) has become the major tool for genome-wide TU identification. As a result, popular TU prediction tools such as AUGUSTUS [1], Cufflinks [2], StringTie [3], Oases [4] use RNA-seq data. Though RNA-seq based TU prediction can be considered the state-of-the-art method to annotate the genome, its main drawback lies in the dependence on relatively high quantities of target RNAs. This is problematic for accurate identification of inherently unstable TUs like primary miRNA. This shortcoming of RNA-Seq can be partly alleviated by chromatin-based approaches [5,6], due to the association between histone modifications and transcription.

Eukaryotic DNA is tightly packaged into macromolecular complex called chromatin, which consists of repeating units of 147 DNA base pairs (bp) wrapped around an octamer of four histones H2A, H2B, H3, and H4 called the nucleosome. Post-

translational modifications (PTM) to histones in the form of acetylation, methylation,

phosphorylation and ubiquitination, play an important role in the transcriptional

process. These PTMs are added, read and removed by so called writers, readers and

erasers. In this way nucleosomes serve as signalling platforms [7] that enable the

localized activity of chromatin signalling networks partaking in transcription and other

chromatin-related processes [8]. Indeed, it has been shown that histone modifications

are correlated to the transcriptional status of chromatin [9,10]. For example, H3K4me3

and H3K36me3 are positively correlated with transcription initiation [11,12] and

elongation [13] and are considered as transcription activation marks, whereas

H3K9me3 and H3K27me3 [11,14], are considered as repressive marks as they are

commonly found in repressed regions. Therefore, it is reasonable to assume that

histone modifications profiles can be used to identify cell-type-specific TUs. Given a

deluge of cell-type-specific epigenome data available through many consortia, such

as ENCODE [15], NIH Roadmap Epigenomics [16], DEEP [17], Blueprint [18],

CEEHRC [19] and IHEC [20], a highly robust TU annotation pipeline based on

epigenome markers becomes feasible.

Currently many computational approaches such as ChromHMM [21], EpicSeg [22],

chroModule [23], GenoSTAN [24] etc., have been developed that use histone

modifications as an input to provide a genome annotation. These chromatin

segmentation approaches use a variety of mathematical models with most prominent

one being hidden markov models (HMM). HMMs are a powerful tool for chromatin

state identification based on histone modifications, due to their assumption that a

combination of histone modifications is generated by an underlying hidden chromatin

state emitting a combination of histone modifications according to a particular

probability distribution.

Based on the training, these HMMs can be classified as: (1) unsupervised (methods

like ChromHMM, EpicSeg and GenoSTAN), that do not include prior biological

information and require user to interpret and annotate the learned states based on

existing knowledge about functional genomics. (2) supervised (methods like

chroModule), that relies on a set of positive samples to train on and consequently

yields predictions that reflect the properties of the training set. Although these

approaches annotate genome modules such as promoter, enhancer, transcribed

regions etc, they fail to identify active TUs as they do not constrain the chromatin state

sequence to begin with a transcription start site (TSS) and end with a transcription

termination site (TTS).

To address these shortcomings, we developed a semi-supervised HMM, EPIGENE

(EPIgenomic GENE), which is trained on the combinatorial pattern of IHEC class I

epigenomes (H3K27ac, H3K4me1, H3K4me3, H3K36me3, H3K27me3 and

H3K9me3) that are indicative of active transcription to infer the hidden "transcription

unit state". The emission probabilities represent the probability of a histone mark

occurring in a TU state and the transition probabilities capture the topology of TU

states. The HMM comprises of TU states and background states. The transcription

start site (TSS), exons (first, internal and last exon), introns (first, internal and last

intron) and transcription termination site (TTS) are referred to as the TU states. As,

every TU begins with a TSS state, proceeds through intragenic states like exon and

intron and terminates with a TTS state, a background state can only be reached from

a TTS state and a TSS state can only be reached from a background or TTS (in case

of genes occurring in close proximity to each other) state.

In the forthcoming sections, we describe the method, validate the predicted EPIGENE

transcription units with existing annotations, RNA-Seq and ChIP-seq evidence,

compare the performance of EPIGENE to existing RNA-Seq based TU prediction methods within and across cell lines and show that EPIGENE outperforms state-of-art RNA-Seq based approaches in prediction resolution and precision. In summary, EPIGENE yields predictions with a high resolution and provides a pre-trained model that can robustly be applied across samples.

2. Results and discussion

2.1 Schematic overview of EPIGENE

EPIGENE uses a multivariate HMM (shown in Figure 1A (ii)), which allows the probabilistic modelling of the combinatorial presence and absence of multiple IHEC class I histone modifications. It receives a list of aligned ChIP and control reads for each histone modification, which are converted into presence or absence calls across the genome using normR (see Materials and Methods section 4.5). By default, TU states are analysed at 200 bp non-overlapping intervals called bins. The HMM comprises of 14 TU states and 3 background states where each transcription unit state captures individual elements of gene such as TSS, exons, introns and TTS. The transition probability of transcription unit states were trained in a supervised manner using GENCODE annotations [25] and their emission probabilities were trained on a highly confident set of GENCODE transcripts [25] which showed an enrichment for RNA Polymerase II in K562 cell line (see Materials and Methods section 4.7). The transition and emission probabilities of background states were trained in an unsupervised manner (see Materials and Methods section 4.7). The HMM outputs a vector where each bin is assigned to a TU or background state, which is further refined to obtain active TUs (see Figure 1B).

2.2 Validation with existing gene annotations and RNA-Seq

We validate the predicted transcription units with existing gene annotations and RNA-

Seq evidence, for this we combined the EPIGENE predictions (24,571 TUs) and RNA-

Seq predictions that was obtained from Cufflinks (32,079 TUs) and StringTie (101,656

TUs; refer Table 2-4 in Supplementary file A1 for summary statistics) to generate a

consensus TU set. This consensus TU set comprises of 24,874 TUs, which were then

overlaid with GENCODE and CHESS gene annotation [25,26] (Figure 2). We find that

93% of EPIGENE TUs can be explained by existing gene annotations. We additionally

identified 14,797 (11,584: annotated, 3213: unannotated) RNA-Seq-exclusive TUs

and 1304 (718: annotated, 586: unannotated) EPIGENE-exclusive TUs, of which 65%

of EPIGENE and 31% of RNA-Seq unannotated predictions show enrichment of RNA

Polymerase II. Additional details about RNA Polymerase II enrichment in the

consensus TU set can be seen in Supplementary table S1.

2.3   Histone modifications and RNA Polymerase II occupancy

The correctness of predicted transcription units was estimated in K562, due to the

availability of matched RNA Polymerase II and RNA-Seq profiles. We predicted

24,571 TUs in K562 cell line, majority of which showed typical gene characteristics,

with high enrichment of H3K27ac, H3K4me3 and H3K36me3 in TSS and gene bodies

(Figure 3A).

It is already known that eukaryotic transcription is regulated by phosphorylation of RNA

Polymerase II carboxy-terminal domain in serine 2, 5 and 7. The signal for serine 5

and 7 is strong at promoter region where as signal for serine 2 and 5 phosphorylation

is strong at actively transcribing regions [27]. Therefore, we incorporated RNA

Polymerase II evidence in all the forthcoming analyses. Genome wide RNA

Polymerase II profile for K562 cell line was obtained using four antibodies that capture

RNA Polymerase II signal at transcription initiation and gene bodies. The enrichment

of RNA Polymerase II in predicted TUs was computed using normR [28] (see Materials

and Methods section 4.5). The predicted TUs were classified as: high and low RPKM

based on mRNA levels (threshold = upper quartile). Figure 3B shows the distribution

of RNA Polymerase II enrichment in both the classes of predicted TUs. We observe a

significant proportion of predicted TUs (78%) show a positive enrichment score

indicating the biological correctness of our predictions. We also come across 24

unannotated TUs that report an enrichment score above 0.5 but have a reduced or no

RNA-Seq evidence.

2.4 Method comparison

Currently multiple approaches exist for predicting TU that rely on RNA-Seq evidence.

We compare the performance of EPIGENE with two existing RNA-Seq based

transcript prediction approaches, Cufflinks and StringTie, both of which are known to

predict novel TUs in addition to annotated TUs. The method comparison was

performed in two stages: within cell type and cross cell type comparison using RNA

Polymerase II enrichment as performance indicator (see Materials and methods

section 4.8). The confusion matrix defining the true positives (TP), true negatives (TN),

false positives (FP) and false negatives (FN) can be seen in Figure 4A.

2.4.1 Within cell type comparison

For this comparison, we use the ChIP-seq profile of RNA Polymerase II in K562 cell

line that was obtained using PolIIS5P4H8 antibody, due to its ability to identify RNA

Polymerase II occupancy in TSS and actively transcribed regions.

As, evident from Figure 4B and 4C, EPIGENE outperforms both the RNA-Seq based

approaches and reports a higher AUC (PRC: 0.81, ROC: 0.82) in both the curves

compared to Cufflinks (PRC: 0.59, ROC: 0.64) and StringTie (PRC: 0.75, ROC: 0.79).

The above analysis was repeated for varying resolutions (50,100 and 500 bp); the

AUC reported for varying resolution can be seen in Figure 4D. As observed in the

figure, Cufflinks achieve a lower AUC compared to StringTie and EPIGENE, which is

likely due to the usage of the RABT assembler which results in large number of false

positives [29].

EPIGENE reports a higher AUC than StringTie for varying RNA Polymerase II

resolutions, this can be due to (1) the usage of RNA Polymerase II enrichment as a

performance measure might lead to a ChIP-seq biasness towards EPIGENE, which is

also a ChIP-seq based approach. This results in more true positives compared to

RNA-Seq based approaches, or (2) RNA-mapping artefacts that results in more false

positives than EPIGENE. Therefore, we examined the precision, sensitivity and

specificity values for EPIGENE, Cufflinks and StringTie and found that the increased

AUC for EPIGENE is due to spurious read mappings of RNA-Seq that results in higher

false positives in StringTie and Cufflinks. Figure S2 (included in Supplementary file

A1) shows an example of Cufflinks and StringTie TU that was identified due to

spurious read mapping. This TU exactly overlaps with a repetitive sequence that

occurs in four chromosomes (chromosome 1, 5, 6, X).

2.4.2 Cross cell type comparison

In order to evaluate the performance of EPIGENE across cell types, we applied K562-

trained models to samples from different cell types. We compared the approaches on

three different datasets provided by the ENCODE [15] and DEEP [17,30] consortium:

1. IMR90: lung fibroblast cells with 6 histone modifications,1 RNA Polymerase II,

two control experiments (one each for RNA Polymerase II and histone

modifications) and one RNA-Seq obtained from ENCODE,

2. HepG2_1 and HepG2_2: hepatocellular carcinoma with 6 histone

modifications, one control experiment and one RNA-Seq obtained from DEEP

where two replicates per histone modification and RNA-Seq are available, RNA

Polymerase II ChIP and control experiments obtained from ENCODE.

As shown in Figure 5 A, B and C, K562-trained EPIGENE models consistently achieve

a higher prediction accuracy, outperforming Cufflinks and StringTie.

2.5 EPIGENE identifies transcription units with negligible RNA-Seq evidence

Previous analyses (see section 2.3 and 2.4) indicated the presence of transcription

units with RNA Polymerase II evidence and reduced or no RNA-Seq evidence. Here

we evaluate these transcription units within and across cell lines by: (1) identifying cell-

type specific transcription units that show gene characteristics but lack RNA-Seq

evidence, and (2) looking for the presence of microRNAs that were not identified by

RNA-Seq.

2.5.1 EPIGENE identifies cell-type specific transcription units

We create a consensus set of transcription units by overlaying the EPIGENE

predictions from K562, HepG2 and IMR90. This consensus TU set comprised of

18,248 TUs, of which ~78% showed an enrichment for RNA-Polymerase II. We

identified 10,233 differential TU, of which 8047 were exclusive to cell lines (K562:

4247, IMR90: 2545, HepG2: 1255; see Figure S3 in Supplementary file A1). We

additionally identified 43 highly confident cell-specific TUs (K562: 24, IMR90: 17,

HepG2: 2; additional details in Supplementary table S2) which lacked RNA-Seq

evidence but showed typical characteristics of a TU, with RNA Polymerase II

enrichment at TSS and transcribing regions, H3K4me3 and H3K27ac enrichment at

the TSS and H3K36me3 enrichment in gene body. An example of one such TU can

be seen in Figure 5D.

2.5.2 Identifying microRNAs that lack RNA-Seq evidence

MicroRNAs are small (~22 bp), evolutionarily conserved non-coding RNAs [31,32] derived from large primary microRNAs (pri-miRNA), that are processed to ~70 bp precursors (pre-miRNA) and consequently to their mature form by endonucleases [33,34]. They regulate various fundamental biological processes such as development, differentiation or apoptosis by means of post-transcriptional regulation of target genes via gene silencing [35,36] and are involved in human diseases [37]. Due to the unstable nature of primary microRNA, traditional identification approaches relying on RNA-Seq are challenging. Here, we investigate the presence of primary microRNA that lack RNA-Seq evidence across cell lines. We create a consensus TU set (used in section 2.2) for individual cell lines (K562, HepG2 and IMR90) and overlaid them with miRbase annotations [38] to obtain potential primary microRNA TUs. We identified 655 EPIGENE TUs (5% of total EPIGENE TUs common in both replicates) that can be explained by miRbase annotations. We observe that majority of these are supported by RNA-Seq and Polymerase II evidence (Figure 6A and Figure S4 Supplementary file A1). We additionally identify 2 primary microRNA TUs in HepG2 cell line, which showed an enrichment for H3K4me3 in promoters, H3K36me3 in gene body and RNA Polymerase II in TSS and transcribing regions; and lacked RNA-Seq evidence. One of these transcription units overlaps with a microRNA cluster located between RP-11738B7.1 (lincRNA) and NRF1 gene (see Figure 6B).

2.6 Discussion

In this work, we introduced EPIGENE, a semi-supervised HMM that identifies active TUs using histone modifications. EPIGENE comprises of TU and background sub-models. The TU sub-model was trained in a supervised manner on predefined training sets, while the background was trained in an unsupervised manner. This semi-

supervised approach captures (1) the biological topology of active TUs, and (2)

probability of occurrence of histone modifications in different parts of a TU.

We first showed that majority of the predicted TUs can be explained by existing gene

annotations, histone modifications and RNA Polymerase II. A quantitative comparison

with RNA-Seq reveals the presence of TUs with RNA-Polymerase II enrichment but

negligible RNA-Seq evidence. Considering RNA-Polymerase II as true transcription

indicator, we compared the performance of EPIGENE with two RNA-Seq based

approaches Cufflinks and StringTie. Based solely on the AUC of PRC and ROC curve

as performance measure, EPIGENE achieves a superior performance than RNA-Seq

based approaches. We further showed that EPIGENE can be reliably applied across

different cell lines without the need for re-training and accomplishes a superior

performance than RNA-seq based approaches.

We examine other performance scores like precision, sensitivity and specificity values,

and observe that the high AUC of EPIGENE is due to RNA Seq mapping artefacts that

result in high number of false positive in Cufflinks and StringTie. We further evaluate

the presence of differentially identified TUs in K562, HepG2 and IMR90 cell line that

lack RNA-Seq evidence. The results suggest the presence of cell line exclusive

transcripts that lack RNA-Seq evidence. We additionally identify microRNAs that lack

RNA-Seq evidence due to their labile nature. All of the aforementioned TUs show an

enrichment of RNA Polymerase II in TSS and gene body indicating that they have

been transcribed.

It is important to note that EPIGENE does not differentiate between functional and

non-functional units of a TU (exons and introns) as the association between histone

modifications and alternative splicing is yet to be elucidated [39]. However, EPIGENE

identifies active TUs with greater precision as shown in section 2.4 and in the example

regions presented in this work. The accuracy of EPIGENE predictions depends on the

sequencing depth of the input histone modifications, therefore, high quality ChIP-seq

profiles of histone modifications should be used to obtain confident transcription unit

annotation.

Altogether, the superior performance within and across cell lines, identification of TUs

especially primary microRNAs lacking RNA-Seq evidence as well as interpretability

makes EPIGENE a powerful tool for epigenome based gene annotation.

3. Conclusion

With increasing efforts in the direction of epigenetics, many consortia continue to

provide high quality genome-wide maps of histone modifications but determining the

genome-wide transcriptomic landscape using this data has remained unexplored so

far. Extensive evaluations in this work demonstrated the superior accuracy of

EPIGENE over existing transcript annotation methods based on true transcription

indicators. EPIGENE framework is user-friendly and can be executed by solely

providing binarized enrichments for ChIP-seq experiments, without the need to re-train

the model parameters. The resulting transcript annotations are in good agreement with

RNA-Polymerase II evidence and can be used to provide a cell specific, epigenome-

based gene annotation.

4. Materials and methods

4.1   Library preparation of histone modifications ChIP-seq

313 For K562 cell line presented in this study, ChIP against six core histone modifications,

314 H3K27ac, H3K27me3, H3K4me1, H3K4me3, H3K36me3 and H3K9me3, was

315 performed. The sheared chromatin without antibody (input) served as control. 10×10^6

316 K562 cells were cultured as recommended by ATCC. Chromatin immunoprecipitations

were preformed using the Diagenode auto histone ChIP seq kit and libraries were made using microplex kits according to manufacturer's instructions and 10 PCR cycles.

4.2   Library preparation of RNA Polymerase II ChIP-seq

K562 cells were cultured in IMDM (#21980Gibco) with 10% FBS and P/S. Cells at a concentration of 1.2mio/ml were fixed with 1% Formalin at 37°C for 8min. Nuclei were isolated with a douncer, chromatin concentration was measured and 750µg chromatin per CHIP was used. Samples were sonicated with Biorupter for 33 cycles (3x 11 cycles). Chromatin, antibodies (RNA Pol II Ser2P (H5), RNA Pol II Ser5P (4H8), RNA Pol II Ser7P (4E12) and PolII (8WG16)) and protein G beads were combined and rotated at 4°C. For elution 250µl elution buffer (1% SDS) was used and after reverse crosslinking DNA was isolated by Phenol Chloroform extraction and elute in 1xTE. Final concentration was measured by Qubit. Bioanalyzer was done to check fragment sizes.

4.3   Sequencing and processing of ChIP-seq data

Sequencing for RNA-Polymerase II and histone modifcations was performed on an Illumina Highseq 2500 using a paired end 50-flow cell and version 3 chemistry. The resulting raw sequencing reads were aligned to the genome assembly "hs37d5" with STAR [40] and duplicates were marked using Picard tools [41]. We used *plotFingerprint* which is a part of deepTools [42] to access the quality metrics of for all ChIP-seq experiments.

4.4   Processing of RNA-Seq data

The raw reads from RNA-Seq experiments were downloaded from European Nucleotide Archive (SRR315336, SRR315337 for K562), European Genome Archive

341 (EGAD00001002527 for HepG2) and ENCODE (ENCSR00CTQ for IMR90) and were

342 aligned to the genome assembly "hs37d5" with STAR [40].

4.5   Binarization of enrichment levels

EPIGENE requires the enrichment values of IHEC class I histone modifications in a

binarized data form or a "class matrix" to learn a transcription state model. This was

done by partitioning the mappable regions of the genome of interest into non-

overlapping sub-regions of the same size called bins. In the current setup, the

transcription states are analysed at 200bp resolution, as it roughly corresponds to the

size of a nucleosome and spacer region. Given the ChIP and input alignment files for

each of the histone modifications, the class matrix for multivariate HMM is generated

using the following approach:

1. *Obtaining read counts*: Read counts for all the bins is performed using

bamCount method from R package *bamsignals* [43], with the following

parameter settings: mapqual = 255, filteredFlag = 1024, paired.end = midpoint.

2. *Enrichment calling and binarization*: After having obtained the read counts,

enrichment and binarization for each of the histone modification across all bins

is computed using *enrichR* (binFilter = zero) and *getClasses* (fdr = 0.2) method

from *normR* [28], which uses a negative binomial distribution to perform

enrichment and binarization. This step yields the class matrix that serves as an

input for the multivariate HMM.

4.6   The EPIGENE model

362 EPIGENE uses a multivariate HMM (shown in Figure 1A (ii)) to model the class matrix

363 and identify active transcription units. Class matrix C is a m x n matrix, where, $m =$

364 total number of 200 bp bins, and, n = number of histone modifications. Each entry C_{ij}

365 in the class matrix C corresponds to the binarized enrichment in i-th bin for the j-th

histone modification. The model constitutes k number of hidden states (which is an input parameter of the algorithm), and each row of the class matrix corresponds to a hidden state. The emission probability vector for each hidden state corresponds to the probability with which each histone mark is found for that hidden state. The transition probabilities between the states enables the model to capture the position biases of gene states relative to each other. The emission probabilities of each state represents the probability with which each histone mark occurs in a state. Given this model, the algorithm does the following:

1. Initializes the emission, transition, and initial probabilities.

2. Fits the emission, transition, and initial probabilities using the Baum-Welch algorithm [44].

3. As we are concerned about the most probable sequence of active transcription unit, therefore, the sequence of hidden states is inferred using the Viterbi algorithm [45].

4.7   Training the model parameters

The transition and emission probabilities of the multivariate HMM are trained using GENCODE annotations with the following approach.

1. Bins overlapping gencode transcripts are identified and termed as gencode bins.

2. The gencode bins were categorized as TSS, TTS, 1st, internal and last exon and intron bins, and were subsequently grouped based on transcript IDs.

3. The coverage (in bp) of individual transcription unit component (i.e TSS, 1st exon, 1st intron etc) for each transcript is computed to generate the coverage list, where each entry of the coverage list contains the coverage information (in bp) for individual transcripts.

4. The transition probability of each "transcription unit state" was computed from the coverage list, and the missing probabilities from and to the "background state" are generated in an unsupervised manner.

5. We filtered the gencode transcripts to obtain transcripts that report an enrichment for RNA Polymerase II. This was done by clustering the binarized enrichment values of RNA Polymerase II in TSS and TTS bins of the transcripts and obtaining TSS and TTS bins that reports a high cluster mean for RNA Polymerase II. The emission probability of each "transcription unit state" was computed from class matrix and coverage of these transcripts (coverage computed from Step 2). The missing emission probabilities for the background states are trained in an unsupervised manner.

4.8   Performance evaluation

The performance of EPIGENE and RNA-Seq based transcript prediction approaches is evaluated using RNA Polymerase as performance indicator. This is done by removing assembly gaps in the genomic regions of interest and partitioning the remaining contigs into non-overlapping bins of 200 bps. The actual transcription status of each 200 bp bin was given by the observed binarized RNA Polymerase II enrichment in the bin and the predicted transcription status of the bin for method m, $PT_m(bin)$ is given by:

$$PT_m(bin) = \begin{cases} 1 \ if \ O(bin, P_m) \geq 1 \\ 0 \qquad otherwise \end{cases}$$

where, $O(bin, P_m)$ is the overlap between the bin and method m predictions P_m.

The predictions of EPIGENE and other RNA-Seq based approaches is evaluated by computing the area under curve for Precision-Recall (AUC-PRC) and Receiver Operating Characteristic curve (AUC-ROC) with primary focus on AUC-PRC.

415   Considering a very high class imbalance i.e. $bins_{RNA\ Polymerase\ II^+} \ll$

416    $bins_{RNA\ Polymerase\ II^-}$, the AUC-PRC and AUC-ROC is computed using random

417   sampling as:

418
$$AUC = mean(L_{AUC}) - \left(\frac{stdDev(L_{AUC})}{\sqrt{n}}\right)$$

where, n is the sampling size or number of iterations and L_{AUC} is the list of AUCs

obtained for sampling size n.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Data for ChIP-seq experiments for K562 cell line are available via European

Nucleotide Archive (PRJEB34999). Additional details about other ChIP-seq and RNA-

Seq data used in this work can be found in the Supplementary file A1, Table 1.

EPIGENE code is available at: https://github.com/imbeLab/EPIGENE.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the Else Kröner-Fresenius-Stiftung grant (2016_A105).

Funding for open access charge (2016_A105 to H.C.).

Authors' contributions

The project was conceived by HC. AS performed all the analyses and wrote the

manuscript with inputs from HC. NL performed the ChIP-seq for histone modifcations

in K562. ID performed the ChIP-seq for RNA Polymerase II in K562.

Acknowledgements

441 The authors would like to thank Clemens Thoelken for helpful comments on the

442 manuscript. Many thanks to Sarah Kinkley, Anna Ramisch, Tobias Zehnder and

443 Giuseppe Gallone from MPIMG for their valuable comments and inspiring discussions.

References

1. Stanke M, Morgenstern B. AUGUSTUS: a web server for gene prediction in

eukaryotes that allows user-defined constraints. Nucleic Acids Res. Oxford

University Press; 2005;33:W465–7.

2. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al.

Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts

and isoform switching during cell differentiation. Nat Biotechnol; 2010;28:511–5.

3. Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT, Salzberg SL.

StringTie enables improved reconstruction of a transcriptome from RNA-seq reads.

Nat Biotechnol; 2015;33:290–5.

4. Schulz MH, Zerbino DR, Vingron M, Birney E. Oases: robust de novo RNA-seq

assembly across the dynamic range of expression levels. Bioinformatics. Oxford

University Press; 2012;28:1086–92.

5. Ozsolak F, Milos PM. RNA sequencing: advances, challenges and opportunities.

Nat Rev Genet; 2011;12:87–98.

6. Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, et al. Chromatin

structure analyses identify miRNA promoters. Genes Dev. Cold Spring Harbor

Laboratory Press; 2008;22:3172–83.

7. Turner BM. The adjustable nucleosome: an epigenetic signaling module. Trends

Genet. Elsevier Current Trends; 2012;28:436–44.

8. Perner J, Chung H-R. Chromatin signaling and transcription initiation. Front Life

Sci. Taylor & Francis; 2013;7:22–30.

9. Karlic R, Chung H-R, Lasserre J, Vlahovicek K, Vingron M. Histone modification

levels are predictive for gene expression. Proc Natl Acad Sci. 2010;107:2926–31.

10. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell.

Elsevier; 2007;128:707–19.

11. Barski A, Cuddapah S, Cui K, Roh T-Y, Schones DE, Wang Z, et al. High-

Resolution Profiling of Histone Methylations in the Human Genome. Cell.

2007;129:823–37.

12. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, et al.

Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad

Sci. 2002;99:8695–700.

13. Wagner EJ, Carpenter PB. Understanding the language of Lys36 methylation at

histone H3. Nat Rev Mol Cell Biol. 2012;13:115–26.

14. Beisel C, Paro R. Silencing chromatin: comparing modes and mechanisms. Nat

Rev Genet. 2011;12:123–35.

15. ENCODE Project Consortium TEP. The ENCODE (ENCyclopedia Of DNA

Elements) Project. Science. American Association for the Advancement of Science;

2004;306:636–40.

16. Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A,

Meissner A, et al. The NIH Roadmap Epigenomics Mapping Consortium. Nat

Biotechnol. 2010;28:1045–8.

17. Welcome to DEEP | DEEP. Available from: http://www.deutsches-epigenom-

programm.de/

18. Adams D, Altucci L, Antonarakis SE, Ballesteros J, Beck S, Bird A, et al.

BLUEPRINT to decode the epigenetic signature written in blood. Nat Biotechnol;

2012;30:224–6.

19. Canadian Epigenetics, Environment and Health Research Consortium

(CEEHRC) Network — Epigenomics. Available from: http://www.epigenomes.ca/

20. Welcome to IHEC · IHEC. Available from: http://ihec-epigenomes.org/welcome/

21. Ernst J, Kellis M. ChromHMM: automating chromatin-state discovery and

characterization. Nat Methods; 2012;9:215–6.

22. Mammana A, Chung H-R. Chromatin segmentation based on a probabilistic

model for read counts explains a large portion of the epigenome. Genome Biol.

BioMed Central; 2015;16:151.

23. Won K-J, Zhang X, Wang T, Ding B, Raha D, Snyder M, et al. Comparative

annotation of functional regions in the human genome using epigenomic data.

Nucleic Acids Res; 2013;41:4423–32.

24. Zacher B, Michel M, Schwalb B, Cramer P, Tresch A, Gagneur J. Accurate

Promoter and Enhancer Identification in 127 ENCODE and Roadmap Epigenomics

Cell Types and Tissues by GenoSTAN. Mantovani R, editor. PLoS One. Public

Library of Science; 2017;12:e0169249.

25. Frankish A, Diekhans M, Ferreira A-M, Johnson R, Jungreis I, Loveland J, et al.

GENCODE reference annotation for the human and mouse genomes. Nucleic Acids

Res. Oxford University Press; 2019;47:D766–73.

26. Pertea M, Shumate A, Pertea G, Varabyou A, Breitwieser FP, Chang Y-C, et al.

CHESS: a new human gene catalog curated from thousands of large-scale RNA

sequencing experiments reveals extensive transcriptional noise. Genome Biol.

BioMed Central; 2018;19:208.

27. Komarnitsky P, Cho EJ, Buratowski S. Different phosphorylated forms of RNA

polymerase II and associated mRNA processing factors during transcription. Genes

Dev. 2000;14:2452–60.

28. Johannes Helmuth and Ho Ryun Chung. Introduction to the normR package.

Available from: http://bioconductor.org/packages/release/bioc/vignettes/normr/inst/

doc/normr.html

29. Janes J, Hu F, Lewin A, Turro E. A comparative study of RNA-seq analysis

strategies. Brief Bioinform. Oxford University Press; 2015;16:932–40.

30. Salhab A, Nordström K, Gasparoni G, Kattler K, Ebert P, Ramirez F, et al. A

comprehensive analysis of 195 DNA methylomes reveals shared and cell-specific

features of partially methylated domains. Genome Biol. BioMed Central;

2018;19:150.

31. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of Novel

Genes Coding for Small Expressed RNAs. Science. 2001;294:853–8.

32. Lee RC, Ambros V. An Extensive Class of Small RNAs in Caenorhabditis

elegans. Science. 2001;294:862–4.

33. Bartel DP. MicroRNAs. Cell. Elsevier; 2004;116:281–97

34. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat

Rev Genet; 2004;5:522–31.

35. Carleton M, Cleary MA, Linsley PS. MicroRNAs and Cell Cycle Regulation. Cell

Cycle. Taylor & Francis; 2007;6:2127–32.

36. Plasterk RHA. Micro RNAs in Animal Development. Cell; 2006;124:877–81.

37. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer;

2006;6:857–66.

38. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase:

microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. Oxford

University Press; 2006;34:D140–4.

39. Zhou H-L, Luo G, Wise JA, Lou H. Regulation of alternative splicing by local

histone modifications: potential roles for RNA-guided mechanisms. Nucleic Acids

Res; 2014;42:701–13.

40. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR:

ultrafast universal RNA-seq aligner. Bioinformatics. Oxford University Press;

2013;29:15–21.

41. Wysoker, A., Tibbetts, K., and Fennell T. Picard Tools. 2013. Available from:

http://broadinstitute.github.io/picard/

42. Ramírez F, Dündar F, Diehl S, Grüning BA, Manke T. deepTools: a flexible

platform for exploring deep-sequencing data. Nucleic Acids Res; 2014;42:W187–91.

43. Mammana Alessandro and Helmuth Johannes. Introduction to the bamsignals

package. Available from:

http://bioconductor.org/packages/release/bioc/vignettes/bamsignals/inst/doc/bamsign

als.html

44. Baum LE, Petrie T, Soules G, Weiss N. A Maximization Technique Occurring in

the Statistical Analysis of Probabilistic Functions of Markov Chains. Ann Math Stat.

Institute of Mathematical Statistics; 1970;41:164–71.

45. Viterbi A. Error bounds for convolutional codes and an asymptotically optimum

decoding algorithm. IEEE Trans Inf Theory. 1967;13:260–9.

46. Yuan J, Zhang S, Zhang Y. Nrf1 is paved as a new strategic avenue to prevent

and treat cancer, neurodegenerative and other diseases. Toxicol Appl Pharmacol.

2018;360:273–83.

 List of supplementary files

File name	File format	Title	Description of data
Supplementary_table-S1	.csv	RNA Polymerase II enrichment	RNA Polymerase II enrichment in consensus TU set
Supplementary_table-S2	.csv	Cell specific TUs	Additional details about cell specific TUs that lack RNA-Seq evidence
Supplementary _file_A1	.pdf	Data details and additional results	Details of datasets used and additional results

Figure legends

Figure 1: A. Schematic overview of EPIGENE framework. **B.** An example of EPIGENE

prediction. EPIGENE predictions of METTL4 and NC80 gene, show an enrichment of

H3K27ac and H3K4me3 at TSS (tracks shown in light violet), H3K36me3 in gene body

(tracks shown in green), enhancer mark H3K4me1 few bps upstream or downstream

of TSS (tracks shown in pink), RNA Polymerase II in TSS and gene body (tracks

shown in blue). The predictions also show an absence of repression marks H3K27me3

and H3K9me3 (tracks shown in black). The corresponding RNA-Seq evidence in this

genomic region can be seen in lower most track (track shown in dark pink)

Figure 2: Overlap of EPIGENE predictions with existing gene annotations and RNA-

Seq based predictions

Figure 3: Correctness of EPIGENE predictions. **A.** EPIGENE estimated parameters

for K562 using 17 chromatin states, ranging from 0 (white) to 1 (dark green). **B.**

Distribution of RNA Polymerase enrichment score in EPIGENE predictions,

predictions are divided as: high RPKM (RPKM >= upper quartile) and low RPKM

(RPKM < upper quartile) based on RNA-Seq evidence in predicted transcripts

Figure 4: Performance of EPIGENE compared to existing RNA-Seq based

transcription unit annotation methods: Cufflinks and StringTie. **A.** Contingency matrix

used for method comparison. **B.** Receiver Operating Characteristic curve **C.** Precision-

Recall curve. **D.** Area under ROC and PRC curve for varying RNA Polymerase II

resolution for EPIGENE, Cufflinks and StringTie

Figure 5: A-C. Performance of K562-trained EPIGENE models, Cufflinks and

StringTie across cell lines. **D.** Example of EPIGENE predicted TU that lacks RNA-Seq

evidence (tracks shown in dark pink). The TU was predicted to be active in K562 but

not in HepG2 and IMR90, and is located between pseudogene CASP3P1 and lncRNA

RP5-952N6.1. The TU shows an enrichment of H3K27ac and H3K4me3 at TSS

(tracks shown in light violet), H3K36me3 in gene body (tracks shown in green),

enhancer mark H3K4me1 few bps upstream of TSS (tracks shown in pink), K562 RNA

Polymerase II in TSS and gene body (tracks shown in blue). The TU also show an

absence of repression marks H3K27me3 and H3K9me3 in K562 (tracks shown in

black). We additionally observe the enrichment of repression mark in H3K27me3 in

HepG2 and IMR90 indicating that the region is repressed in both these cell lines

Figure 6: A. Overview of potential primary miRNAs predicted by EPIGENE in HepG2.

B. Example of a TU overlapping a microRNA cluster was predicted by EPIGENE in

HepG2 cell line. This region is located between lincRNA RP11-738B7.1 and gene

NRF1 which was identified as a key player in maintaining cellular homeostasis and

organ integrity [46]. The TU shows an enrichment of H3K27ac and H3K4me3 at TSS

(tracks shown in light violet), H3K36me3 in gene body (tracks shown in green),

enhancer mark H3K4me1 few bps upstream and downstream of TSS (tracks shown

in pink), RNA Polymerase II in TSS (tracks shown in blue). The predictions also show

an absence of repression marks H3K27me3 and H3K9me3 (tracks shown in black)

and RNA-Seq evidence (tracks shown in dark pink).

A

i) Preparing input data

BAM file

normR

Input matrix

	H1	H2	H3	H4	H5	H6
Bin1	0	1	0	1	1	0
Bin2	0	1	0	1	1	0
Bin3	0	1	0	1	1	0
......	0	1	0	1	1	0
......	0	1	0	1	1	0
BinN	0	1	0	1	1	0

ii) Genome annotation

Input matrix

Transition and emission probabilities

TSS 1.exon 1.intron i.exon i.intron last.exon TTS

H3K9me3
H3K27me3
H3K36me3
H3K4me3
H3K27ac
H3K4me1

background

H3K9me3
H3K27me3
H3K36me3
H3K4me3
H3K27ac
H3K4me1

H3K9me3
H3K27me3
H3K36me3
H3K4me3
H3K27ac
H3K4me1

Gene annotations

B

RefSeq Genes		METTL4	NDC80

H3K27ac [0-203]
H3K4me3 [0-319]
H3K36me3 [0-65]
H3K4me1 [0-37]
H3K27me3 [0-14]
H3K9me3 [0-15]

EPIGENE predictions

PolII.8WG16 [0-155]
PolIIS2P.H5 [0-30]
PolIIS5P.4H8 [0-59]
PolIIS7P.4E12 [0-169]
RNA-Seq [0-942]