MethylBERT: A Transformer-based model for read-level DNA methylation pattern identification and tumour deconvolution

Yunhee Jeong[1†], Clarissa Gerhäuser[1], Guido Sauter[2], Thorsten Schlomm[3], Karl Rohr[4], Pavlo Lutsik[1,5†]

[1]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, Heidelberg, Germany
[2]Universitätsklinikum Hamburg Eppendorf, Institut für Pathologie, Martinistraße 52, 20246 Hamburg, Germany
[3]Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany, Germany
[4] Biomedical Computer Vision Group, BioQuant, IPMB, Heidelberg University, Im Neuenheimer Feld 267, Heidelberg, Germany
[5] Department of Oncology, Catholic University (KU) Leuven, Leuven, Belgium

[†] Corresponding authors. Yunhee Jeong (e-mail: y.jeong@dkfz-heidelberg.de); Pavlo Lutsik, (e-mail: pavlo.lutsik@kuleuven.be)

Abstract

DNA methylation (DNAm) is a key epigenetic mark that shows profound alterations in cancer. Read-level methylomes enable more in-depth DNAm analysis due to the broad coverage and preservation of rare cell-type signals, compared to array-based data such as 450K/EPIC array. Here, we propose MethylBERT, a novel Transformer-based model for read-level methylation pattern classification. MethylBERT identifies tumour-derived sequence reads based on their methylation patterns and genomic sequence. Using the calculated classification probability, the method estimates tumour cell fractions within bulk samples and provides an assessment of the model precision. In our evaluation, MethylBERT outperforms existing deconvolution methods and demonstrates high accuracy regardless of methylation pattern complexity, read length and read coverage. Moreover, we show its potential for accurate non-invasive early cancer diagnostics using liquid biopsy samples. MethylBERT represents a significant advancement in read-level methylome analysis. It will increase the accuracy of tumour deconvolution and enhance circulating tumour DNA studies.

Introduction

DNA methylation (DNAm) refers to a methyl group added to nucleotide bases in the DNA. Aberrant methylation patterns at CpGs reflect epigenetic heterogeneity in tumours [1]. Therefore, DNAm data has been widely used for estimating tumour purity to examine the tumour epigenetic landscape relevant to clinical outcomes, tumour diagnosis and phenotypic characteristics [2–4].

DNAm can be profiled by sequencing methods such as whole genome bisulfite sequencing (WGBS) [5] or reduced representation bisulfite sequencing (RRBS) [6], as well as microarray methods like Infinium 450K/EPIC array [7]. High-quality sequencing-based profiling delivers sequence reads over a broad range of genomic coverage and preserves single-molecule signals of rare cell populations [8]. Sequencing-based data is even more crucial in circulating tumour DNA (ctDNA) analysis, which facilitates non-invasive early diagnosis, prognosis and treatment response monitoring for cancer patients [9–11]. Nevertheless, most tumour deconvolution methods have been developed for array-based DNAm profiles because of the intuitive application of classical linear algebraic algorithms to matrices of average DNA methylation levels (beta-values) [12]. Moreover, our analysis showed that existing sequencing-based deconvolution methods do not perform better than array-based methods implying that they fail to fully exploit the advantages of sequencing data for accurate inference [13].

To overcome these limitations, we propose MethylBERT, a novel deep learning method for read-level methylation pattern identification and tumour deconvolution based on Bidirectional Encoder Representations from Transformers (BERT). MethylBERT uses a pre-trained BERT model to encode read-level methylomes and classifies sequencing reads into tumour or normal cell types. The estimated posterior probability of cell types is used for tumour purity estimation by applying Bayes' theorem and maximum likelihood estimation. Along with purity estimation, MethylBERT provides the model precision based on the Fisher information and estimation adjustment taking the region-wise tumour purity into account. To our knowledge, MethylBERT is the first Transformer-based deep learning method for tumour deconvolution using sequencing-based DNAm data (Supplementary Table 1).

We have thoroughly evaluated MethylBERT and compared it with existing methods, which demonstrated the remarkable performance of MethylBERT in read-level methylation pattern classification and tumour deconvolution. In our evaluation, we not only investigate the performance of MethylBERT but also analyse what the model actually learns via pre-training using reference genome sequences. Moreover, we suggest using the Fisher information to estimate the precision of the deconvolution model. This gives guidance about how accurate as the estimated tumour purity is, which represents essential information for analysing bulk samples without ground-truth tumour purity. Finally, we also propose using MethylBERT as a non-invasive method for early diagnosis of cancers based on ctDNA analysis.

Results

MethylBERT overview

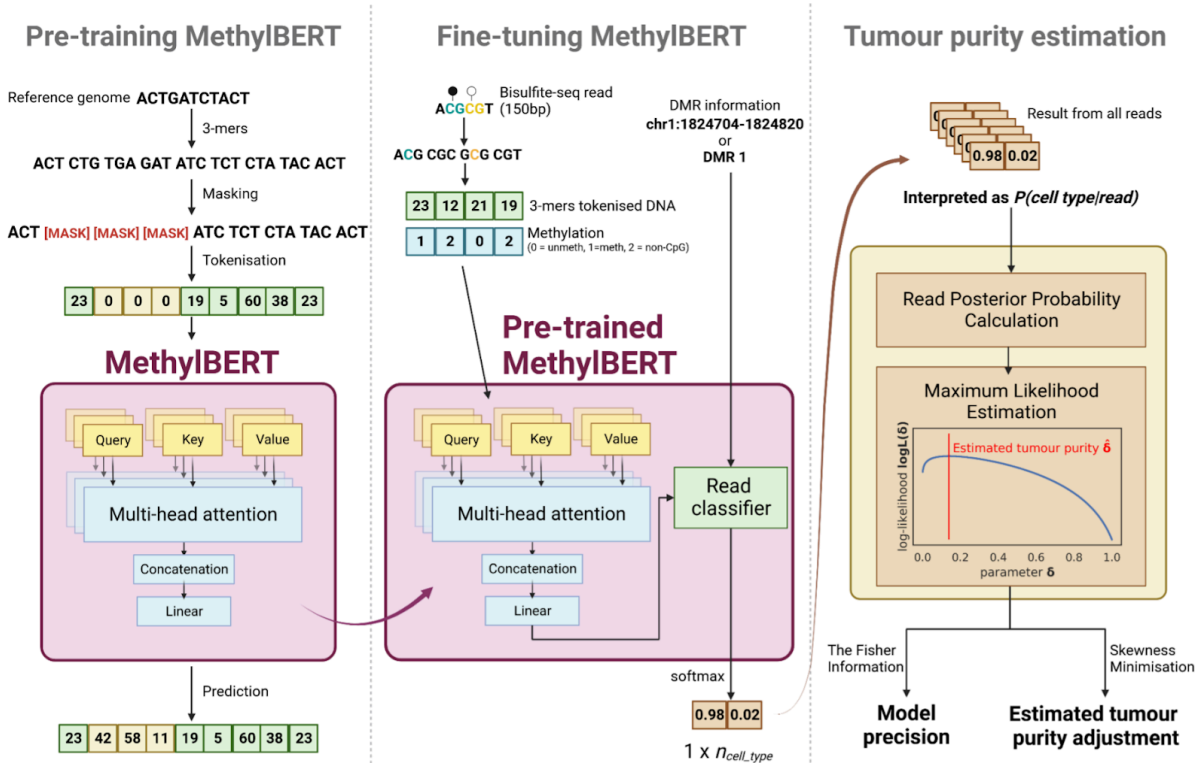

Figure 1. MethylBERT overview. The main three steps are separated by dotted lines.

MethylBERT includes three main steps (Figure 1). First, the MethylBERT model is pre-trained with a reference genome processed into 3-mer sequences. After pre-training, the MethylBERT model is fine-tuned to learn the read-level methylation pattern classification task. The final output converted with a softmax function is interpreted as the posterior probability $P(c_j|r_i)$ of the cell type c_j given a read r_i. In our case, the cell type is either tumour (*T*) or normal (*N*). Classification is performed by assigning the cell type with a higher posterior probability.

For the final tumour purity estimation, we apply Bayes' theorem to compute the probability $P(r_i|c_j)$ in the likelihood function using $P(c_j|r_i)$ assuming that every read has an equal marginal probability. Afterwards, the tumour purity is determined by maximum likelihood estimation. The estimated tumour purity can also be adjusted based on the skewness of the region-wise tumour ratio. The adjustment is particularly useful when the analysed bulks have a very high or low ratio of tumour-derived reads. A detailed description of the three steps of MethylBERT is provided in Methods.

MethylBERT classifies complex read-level methylation patterns

We simulated read-level methylomes in different scenarios to evaluate the robustness of the MethylBERT read classification with respect to the pattern complexity (the details are in Methods). We compared our method with CancerDetector [14] and DISMIR [15] as well as a baseline method implemented using a hidden Markov model (HMM) designed to classify read-level methylation patterns into cell types.

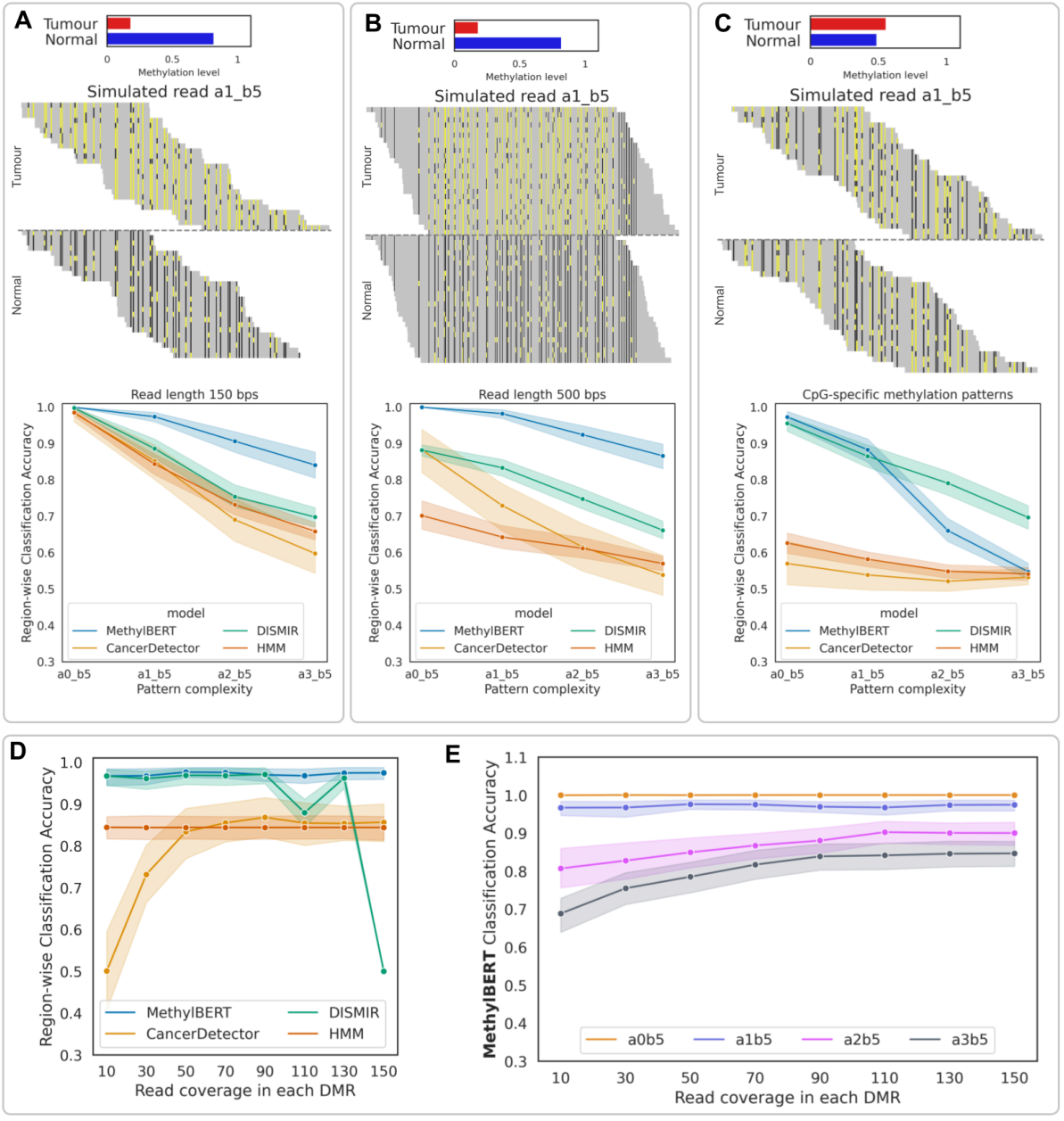

Figure 2. Read-level methylome classification results. **A-C.** An example of simulated read-level methylation patterns for tumour and normal cell types and the classification accuracy comparison across different complexities (A) with read length 150 bps, (B) with read length 500 bps, and (c) with CpG-specific methylation patterns. Yellow and black on each read show methylated and unmethylated CpGs, whereas grey represents other bases. Sequence reads for two cell types are divided by a dotted line in the middle. Region-wise methylation levels in tumour and normal cell types are shown in the histogram. **D.** Read-level methylome classification accuracy comparison for different read coverages. **E.** MethylBERT read-level methylome classification accuracy for different read coverages and pattern complexities.

In our first scenario, we simulated different complexities of 150 bps read-level methylomes from a beta-binomial distribution. Our simulation algorithm generates more complex methylation patterns with an increasing α value of the beta distribution (Supplementary Figure 2A and B). For all complexities of methylation patterns, MethylBERT outperformed the other three methods in the classification task (Figure 2A). All four methods yielded the highest accuracy and the lowest deviation of accuracy values over DMRs for the simplest methylation patterns (a0_b5). Although the accuracy decreases with increasing complexity for all methods, MethylBERT still classified reads more accurately than the other methods in each case. We further simulated longer read-level methylation patterns with 500 bp length. In this case, the reads cover both a DMR and non-DMRs on the genome resulting in more complicated methylation patterns, because most DMRs are shorter than 500 bps (Supplementary Figure 2C). With the 500bps of simulated reads, MethylBERT again performed better than the other methods regardless of the complexity (Figure 2B). It likewise achieved more precise classification for simpler complexity keeping the lowest deviation of accuracy at the complexity a0_b5. On the other hand, for the same complexity level, CancerDetector and HMM performed worse and had a higher deviation of accuracy in the results with 500 bp reads than in the results with 150 bp reads. Overall, the deep learning methods MethylBERT and DISMIR yielded better classification results for the 500 bp read simulation.

Neighbouring CpGs usually have a consistent methylation pattern, but the innate inclination of methylation for each CpG and erroneous methylation patterns in cancer are non-trivial factors [16,17]. Thus, we conducted the same evaluation for the extreme scenario where only CpG-specific methylation patterns differ between tumour and normal whereas region-specific methylation levels are almost identical (Methods, Supplementary Figure 2D). CancerDetector and HMM could not classify reads into cell types despite the clear pattern difference in the case of a0_b5 and a1_b5, whereas MethylBERT performed best with the exception of highly noisy patterns in a2_b5 and a3_b5 (Figure 2C). These results demonstrate that MethylBERT is capable of detecting tumour-specific methylation patterns not misled by the average methylation level in a region.

Read coverage is another important factor that can affect read-level methylome analysis. Many deconvolution algorithms pre-select regions based on the minimum number of CpGs and read coverage within the region so that sufficient methylomes can be used for deconvolution [13]. Hence, we simulated read-level methylomes with variable read coverage in DMRs. Again, MethylBERT achieved the best methylation pattern classification performance (Figure 2D). MethylBERT shows an accuracy value above 0.95 even for coverage 10 where the sample means cannot represent the population mean well (Supplementary Figure 2E). On the other hand, CancerDetector could not perform accurate read classification for read coverages below 50. We also performed the MethylBERT read classification analysis for every combination of read coverages and complexities (Figure 2E). MethylBERT keeps a high accuracy regardless of the coverage for the complexity a0_b5 and a1_b5. However, the accuracy converges at the highest value when the read coverage is higher than 100 for the complexity a2_b5 and a3_b5.

MethylBERT pre-training allows the model to understand sequence features

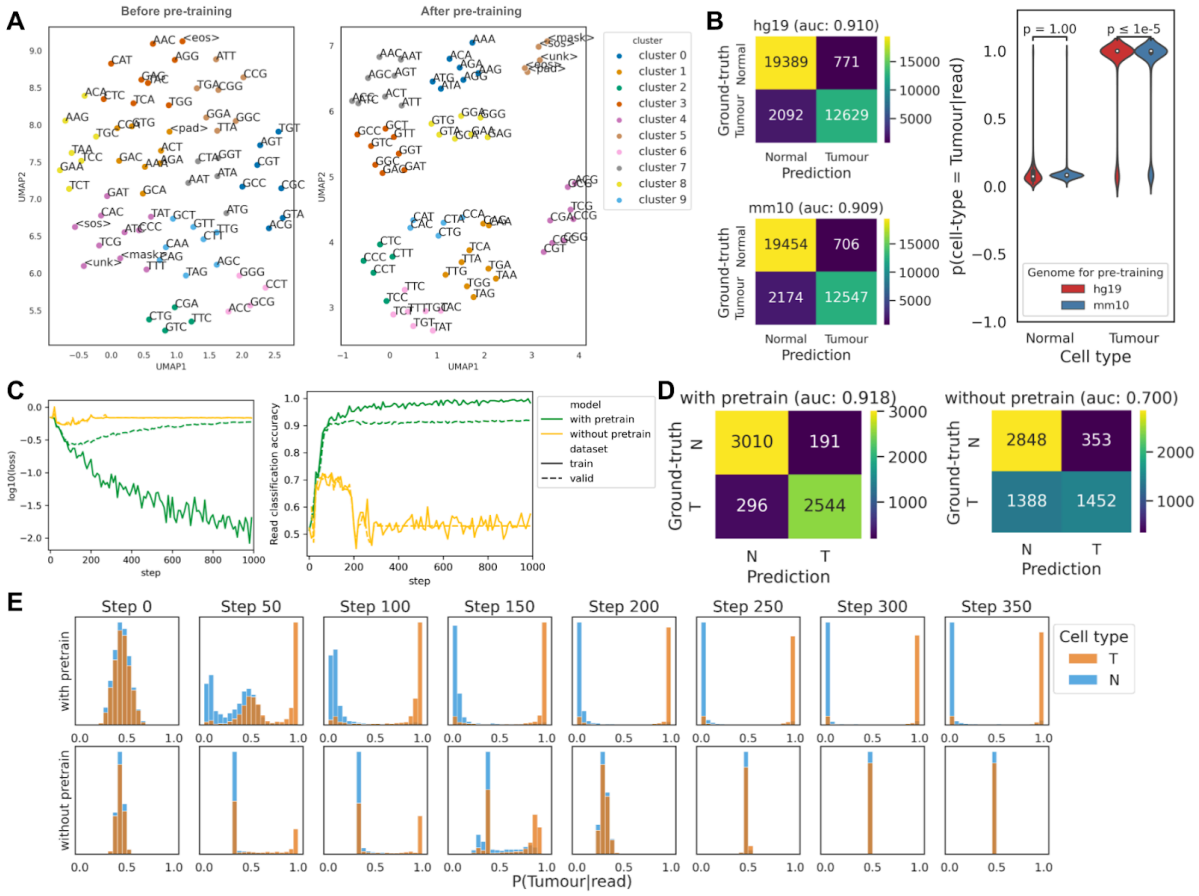

Figure 3. Impact of pre-training in MethylBERT. **A.** UMAP plot of 3-mer token embeddings before and after pre-training. The clusters were made via k-means clustering. **B.** Confusion matrix of read classification results by the model pre-trained with human genome hg19 (top left) and with mouse genome mm10 (bottom left). Distribution of *P(cell type=Tumour|read)* in both cell types calculated by the two pre-trained models (right). P-values in the violin plot were calculated using paired t-test statistics. **C.** Training (solid line) and validation (dotted line) curves of MethylBERT with and without pre-training (green and yellow). Both graphs are plotted every 10 steps. **D.** Confusion matrix of read classification results by the MethylBERT model with and without pre-training calculated at the step when each model achieved the best validation performance. **E.** Histogram of *P(cell type=Tumour|read)* in tumour and normal reads (orange and blue each) calculated by MethylBERT with and without pre-training (top and bottom).

Bidirectional pre-training is a pivotal feature of the BERT model [18], alleviating the restricted choice of a model architecture. It was shown by Ji et al. [19] that the pre-trained BERT model can be successfully fine-tuned for various DNA sequence analyses such as promoter region prediction. Clark et al. [20] have carefully described what kind of information BERT learns during pre-training in natural language processing. Yet, the efficacy of BERT pre-training on DNA sequences is still poorly understood.

We have primarily found that pre-training enables the BERT model to understand the mutual relationships between DNA 3-mers (Figure 3A). Even though any information about CpGs or methylation has not been provided, BERT distinguishes 3-mer tokens including “CG” from

other tokens (Cluster 4). This might be driven by the repetitive “CG” patterns especially occurring in CpG islands. In addition, the pre-trained BERT model is able to associate paired DNA nucleotides with each other (A-T and C-G). UMAP2 embedding divides the 3mers tokens into two groups: those that start with C/T and the others starting with A/G. Each cluster is composed of tokens whose first nucleotide is the same and whose last nucleotide makes a nucleotide pair. For example, in cluster 7, all tokens start with A and end with C/T. We hypothesise that BERT pre-training identified the nucleotide pairs because of Chargaff’s second parity rule that the amount of two nucleotides in a pair is approximately equal in a single DNA strand. Special tokens (e.g. <unk> or <mask>) also make up a separate cluster from the other DNA 3-mer tokens only after pre-training.

We have also evaluated the influence of pre-training on the identification of tumour methylation patterns. For this, we compared the performance of read classification when the MethylBERT model was pre-trained and when it was not pre-trained. The comparison was done by utilising diffuse large B cell lymphoma (DLBCL) and non-neoplastic B cell samples. In order to avoid the additional influence carried by the dominant promoter hypermethylation in the tumour, we selected 50 DMRs where tumour cells are hypermethylated and hypomethylated, respectively (Supplementary Figure 3A and B). Both MethylBERT models with and without pre-training gradually decrease the loss value during the early steps of fine-tuning (Figure 3C). However, when MethylBERT is not pre-trained, the loss value increases again after 100 steps and the accuracy eventually converges around 0.5. The confusion matrix of classified reads also shows that pre-trained MethylBERT achieves far more accurate classification results (Figure 3D).

Figure 3E shows the change in the probability $P(cell\ type\ =\ Tumour|read)$ during MethylBERT fine-tuning. When MethylBERT is pre-trained with the reference genome, the probability distribution of tumour reads and normal reads start separating from each other at step 50. Over further steps, the model yields higher probability values of correct cell types for the reads. For instance, at step 350, $P(cell\ type\ =\ Tumour|read)$ of normal reads are close to 0 and the probability of tumour reads are close to 1. On the other hand, MethylBERT without pre-training could distinguish some tumour reads from normal reads until step 150, but afterwards the $P(cell\ type\ =\ Tumour|read)$ distribution of two cell types becomes indistinguishable. According to the correlation between the estimated probability and methylation level of reads, both models classify the reads mainly based on the methylation level at step 50 (Supplementary Figure 3C). However, only the pre-trained model can overcome the bias and, in the following steps, keep the accuracy high without $P(cell\ type\ =\ Tumour|read)$ not being strongly correlated with the methylation level.

Pre-training on an entire reference genome requires a long training time, so the cross-species applicability of the pre-trained MethylBERT model will augment the utility to analyse various samples. For this reason, we have investigated the discrepancy in fine-tuning performance between pre-trained models with human (hg19) and mouse (mm10) genomes. The classification results of DLBCL and non-neoplastic B cell reads show that both human and mouse genomes are eligible as pre-training data for human cancer analysis (Figure 3B). The read classification area under the curve (AUC) score calculated in the validation set shows a difference below 0.001 between the two reference genomes. The distribution of calculated $P(cell\ type\ =\ Tumour|read)$ also does not significantly differ in

non-neoplastic B cell reads. However, in the DLBCL reads, the two sets of probability values still have a p-value below 1.0×10^{-5} for paired t-test statistics.

These results clearly demonstrate that pre-training is a vital step enabling the MethylBERT model to understand the major sequence features of the DNA. Furthermore, the MethylBERT fine-tuning can prevent bias towards read-wise methylation levels only when pre-training is performed. Finally, the cross-species applicability of the pre-training broadens the range of available samples for MethylBERT analysis.

MethylBERT deconvolutes mixed tumour methylomes and accurately estimates tumour purity

We next evaluated the tumour purity estimation performance of MethylBERT using *in silico*-generated pseudo-bulk samples. The samples were obtained by mixing reads randomly sampled from DLBCL and non-neoplastic B cell samples with controlled proportions. For the comparison, we benchmarked MethylBERT against CancerDetector [14], DISMIR [15] and Houseman's method [21]. Houseman's method performed best in diverse deconvolution experiments in our previous benchmarking study [13]. Although DISMIR features a procedure for informative genomic region selection for deconvolution, it could not make reasonable estimates with self-selected regions. Therefore, we applied DISMIR on the same DMRs used for the other methods and this is referred to as 'DISMIR_dmr' in the following. For the MethylBERT results, we present the performance from both models with and without estimation adjustment (described in Methods) to study the impact of the adjustment.

MethylBERT outperformed other methods with respect to the absolute error between the ground-truth and estimated tumour purity (Figure 4A and B). Although Houseman's method achieved better performance for the bulks with a high tumour purity, it could not accurately estimate the purity when the ground-truth value is low. On the contrary, CancerDetector performed better for the bulk samples where the tumour was a minor cell type. However, MethylBERT maintained its high accuracy for both low and high tumour purities.

To further improve the accuracy of tumour purity estimation, MethylBERT employs estimation adjustment based on the distribution of region-wise (local) estimated tumour purity (Methods). This is conceptually equal to the 'removal of confounding factors' in CancerDetector [14], as it also copes with outlier regions where the estimated proportion of tumour-derived reads is different from the majority. MethylBERT finds the optimal mapping of local estimates to reduce the skewness, whereas CancerDetector iteratively removes regions that are outside of the standard deviation. Therefore, we have specifically compared the MethylBERT estimation adjustment and CancerDetector removal of confounding factors using the same $P(cell\ type|read)$ values (Figure 4C). MethylBERT estimation adjustment outperformed CancerDetector removal of confounding factors in terms of median absolute error between the ground truth and estimated purities. Although CancerDetector removal of confounding factors cannot improve the estimated values when the tumour is the major cell

type, MethylBERT is able to make a better adjustment regardless of ground-truth tumour purity (Figure 4D).

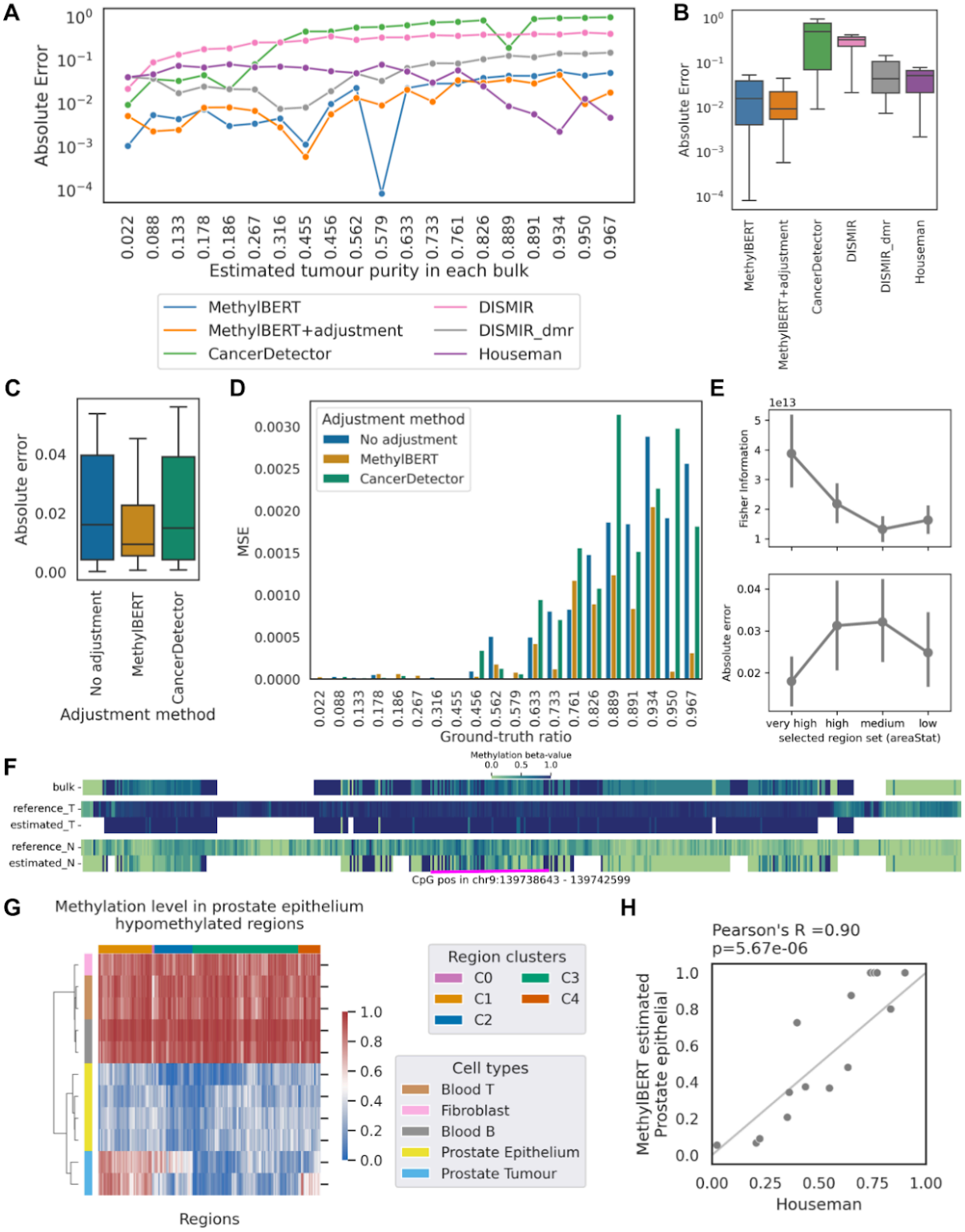

Figure 4. Tumour deconvolution and the estimation adjustment results for DLBCL pseudo-bulk samples and lymph node metastasis samples from prostate cancer patients. **A-B.** Absolute error between the ground-truth and estimated tumour purity. (A) Bulks are sorted according to the tumour purity on the x-axis in the line plot. (B) In the box plot, the middle bar, bottom and top edges of the box

plot indicate the median, first quantile and third quantile values, respectively. **C-D.** Performance comparison of different estimation adjustment methods. (C) The boxplot presents the absolute error. (D) The histogram shows the mean squared error between the ground-truth and the estimated tumour purity for pseudo-bulks sorted by the ground truth. **E.** The Fisher Information values and the mean absolute error calculated for the pseudo-bulk samples with four different region sets. Dots and lines indicate the mean value and the confidence interval in each region set. **F.** Estimated CpG-wise methylation beta-values of bulk 8 within DMR 14 (chr9:139738643-139742599) compared with the reference tumour and normal methylation patterns (bottom four rows). The first row shows the original beta-values of bulk 8 in DMR 14. The complex methylation patterns in the normal cell type (chr9:139740205-139740700) are indicated by the magenta line. **G.** Methylation level comparison between prostate tumour, normal prostate epithelium, T cell, B cell, and fibroblasts in the 250 regions provided by the normal cell type atlas [22]. The regions were clustered using the hierarchical clustering algorithm. **H.** Correlation between the MethylBERT-estimated prostate epithelium proportion (without tumour reference data) and Houseman's method-estimated tumour purity (with tumour reference data) for the lymph node samples acquired from hormone-sensitive metastatic prostate cancer patients.

In real application scenarios, ground-truth tumour purity is not available for evaluation. Therefore, as a measure of model estimates quality, MethylBERT provides the precision of the tumour purity estimation using the Fisher information. When multiple experiments are performed by MethylBERT, users can obtain information on which experiment is more likely to make a more precise estimation by comparing the Fisher information indicating the precision of likelihood estimation models [23]. Here, we show how the Fisher information can be used for determining the best DMRs. For this, we split the 100 DMRs ordered by the region quality (indicated by areaStat value as explained in Methods) into four groups: very high, high, medium, and low, meaning that a higher areaStat value represents higher methylation difference and more CpGs within the region. Both tumour purity and the Fisher information were calculated in respective groups by MethylBERT (Figure 4E). The result shows that the mean absolute error and the estimation quality measured by the Fisher information are anti-correlated. Hence, the Fisher information from MethylBERT can be used to analyse the quality of maximum likelihood estimation when other information for the quality evaluation is not available.

In addition to the accurate inference of mixture proportions, MethylBERT can reconstruct the sample-specific tumour and normal cell-type methylation patterns, in both bulk and read-level formats. For example, we compared the MethylBERT-estimated tumour and normal cell-type methylation beta-values for a pseudo-bulk sample to the reference beta-values obtained from the training data (Figure 4F). At the level of single CpGs within one region, MethylBERT successfully reconstructed the methylation beta-values of the reference profile, although the methylation patterns in chr9:139740205-139740700 show a high complexity in the normal cell type. The estimated beta-values were also more similar to the correct reference cell-type profile according to the hierarchical clustering result (Supplementary Figure 5A). Moreover, we found that MethylBERT successfully dissected tumour and normal methylation patterns over DMRs in all pseudo-bulk samples (Supplementary Figure 5B and D). In order to make sure that the result is not influenced by dominant tumour hypermethylation over the selected DMRs, we tested it again with 100 regions where half is tumour hypermethylated and the other half is hypomethylated regions. MethylBERT still successfully dissected cell type-specific methylation patterns in these tests (Supplementary Figure 5C and E).

When MethylBERT is used for practical applications, it could be the case that tumour reference sequencing data is not available. Thus, using lymph node samples collected from hormone-sensitive metastatic prostate cancer patients, we evaluated MethylBERT's applicability to analysing tumour bulk samples without cancer-derived reference data. Instead of tumour reference data, we used the normal cell type atlas which includes blood cells and prostate epithelium cells [22]. We hypothesised that the estimation of prostate epithelium cell fraction should align with the tumour purity in lymph node samples because of tissue invasion and metastasis, which is a well-known cancer hallmark [24]. Therefore, we evaluated the MethylBERT-estimated prostate epithelial cell proportions compared to the prostate tumour purity estimated using prostate cancer reference data (as described in Methods). Despite the methylation level difference in some regions between normal prostate epithelium and prostate tumour samples (region clusters C1 and C4 in Figure 4G), the prostate-derived read proportion estimation by MethylBERT without tumour reference data shows a strong positive correlation with the prostate tumour purity estimated by other deconvolution methods [21,25–27] trained with tumour reference data (Figure 4H and Supplementary Figure 4). These results present a practical use case of MethylBERT combined with the normal cell type atlas [22] even when the reference data is partially available.

MethylBERT enables accurate circulating tumour DNA analysis by detecting rare tumour signals

Besides bulk tumour deconvolution, precise sequencing-based tumour deconvolution is required in ctDNA analysis due to the extremely low quantity of tumour-derived DNA found in liquid biopsies at early disease stages (<5%). Hence, we evaluated MethylBERT as an early cancer detection algorithm for blood plasma samples and compared its performance to other previous methods.

To validate whether MethylBERT can detect a very low percentage of tumour-specific signals, we further generated 10 pseudo-bulks by mixing reads from non-neoplastic B-cell and DLBCL samples with a tumour ratio smaller than 1%. MethylBERT achieved a lower median absolute error than the other methods (Figure 5A). All methods can estimate tumour purities proportional to the ground-truth values with the exception of DISMIR applied with its own selected regions but MethylBERT shows the highest correlation between two values with p-value < 0.01 (Figure 5B). Therefore, we demonstrated that MethylBERT is sufficiently sensitive for ctDNA analysis compared to the currently available methods.

As an application to real ctDNA samples, we used targeted BS-seq data collected from 14 healthy donors and 40 colorectal cancer (CRC) patients in five different stages (GSE149438) (Supplementary Figure 6A). The estimated tumour purity significantly differs between healthy donors and tumour patients later than stage I (Figure 5C). This implies that MethylBERT can be instrumental for ctDNA tumour diagnosis in some early stages (II-III) of CRC patients. The median value of estimated tumour contents has an explicit discrepancy between the healthy donors and all stages of CRC patients.

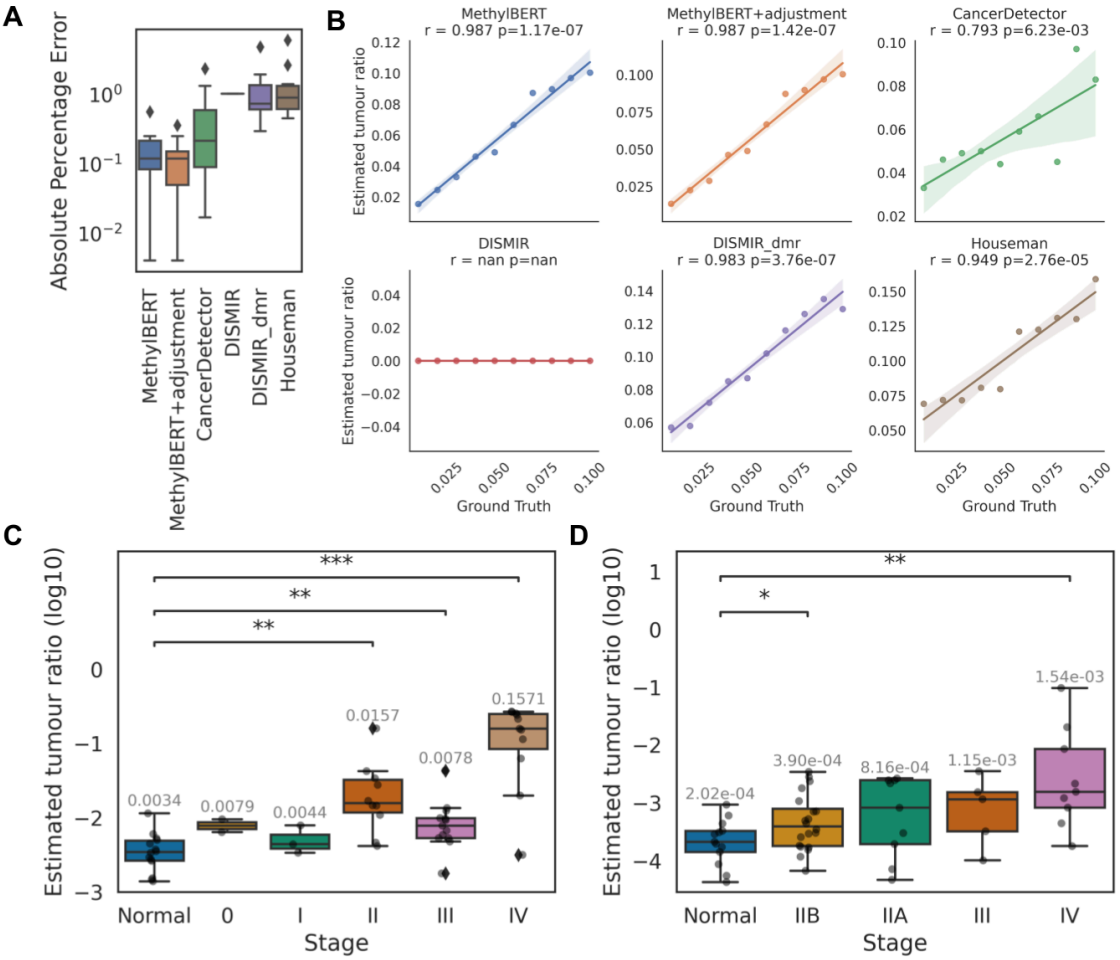

Figure 5. ctDNA tumour deconvolution results. **A.** Deconvolution of simulated pseudo-bulk samples with a low percentage of tumour DNA. Distribution of absolute percentage error values in each method. **B.** Comparison of ground-truth and estimated tumour purities in each method. For each comparison, Spearman's correlation is given with a respective p-value. **C-D.** Tumour deconvolution results in ctDNA samples (C) from CRC patients and (D) PDAC patients. Both analyses include healthy donors as well. The median of estimated tumour purities in each stage is written at the top of the box plot. Two-sided Mann-Whitney-Wilxcoxon test p-values between each stage and healthy donors are denoted with stars. '*', '**', and '***' mean p-value ≤ 0.05, ≤ 0.01, and ≤ 0.001, respectively.

From the same dataset, we also collected 44 pancreatic ductal adenocarcinoma (PDAC) patients in four different stages (IIA, IIB, III and IV) and conducted the same analysis using MethylBERT (Supplementary Figure 6B and Figure 5D). PDAC is widely recognised as one of the trickiest cancer types to be identified during the early stages, which is also confirmed by the original analysis in this dataset [28]. The median tumour purity value estimated by MethylBERT is higher in every stage of PDAC cohorts than in healthy donors. Among the early-stage patients, stage IIB showed a statistically significant difference from the healthy donors. Overall, in both CRC and PDAC ctDNA analyses, all early-stage cancer patient samples except for CRC stage II have a median value lower than 0.01 demonstrating the necessity of sensitive sequencing-based tumour deconvolution models in ctDNA methylation analysis.

Discussion

DNA methylation undergoes profound changes during tumorigenesis [1,29] resulting in highly specific methylation patterns in tumour cells. Sequencing data is particularly valuable in this respect, since it offers DNAm patterns at single-molecule resolution, broad genomic coverage and the preservation of rare cell-type signals.

Aiming to fully utilise the potential of sequencing-based data, we developed MethylBERT, a new Transformer-based model for read-level tumour methylation pattern identification. Based on the estimated posterior probability by the MethylBERT model, the tumour purity of each bulk sample can be inferred by maximum likelihood estimation. The estimated purity can be adjusted for a more accurate inference by considering region-wise tumour purity estimation.

In the benchmarking using simulated read-level methylation patterns with different scenarios, MethylBERT achieved the best performance regardless of methylation pattern complexity, read length and read coverage. Our deconvolution benchmarking results demonstrate not only the remarkable performance of MethylBERT in varying experiments but also provide in-depth analyses of BERT pre-training and the dynamics of the estimated posterior probability during model training. Moreover, MethylBERT could distinguish some early-stage cancer patients from healthy donors using blood plasma samples. This assures the applicability of MethylBERT as an early tumour diagnosis model. We are convinced that MethylBERT will become a valuable tool in the field of cancer research, extending the usability of various types of DNA methylation sequencing data. MethylBERT is agnostic with respect to the source of read-level methylation data, and can be applied to both solid tumour tissues and blood plasma samples as well as non-cancerous bulk methylomes, unlike previous methods that focused on only specific sample types [14,15,30].

In the future, different extensions of MethylBERT are possible. The current version only infers the tumour purity within bulk samples, but, with further modifications of the model, deconvolution of multiple or unknown cell types within a bulk could be accomplished. Furthermore, we are currently working on a computationally less demanding version of the MethylBERT model with a reduced number of parameters. This work is particularly important for long-read sequencing data analysis [31,32]. Based on the results of read-level methylation classification with 500bp-long simulated reads, we believe that MethylBERT can handle differentially methylated patterns being a part of a read-level sequence, and will be able to provide accurate and robust analyses of tumour-specific methylation patterns in long-read sequencing data.

Methods

MethylBERT model

The aim of MethylBERT is to classify sequence reads with their CpG methylation patterns and sequence into dichotomous cell-type-related classes, e.g., tumour or normal. Applied to a set of genomic regions, it also provides a global maximum likelihood estimate of cell-type proportions for the two classes, e.g., tumour purity in the tumour-normal deconvolution scenario.

BERT model

The basis of MethylBERT is the Bidirectional Encoder Representations from Transformers (BERT) model which has achieved groundbreaking progress in various natural language processing studies [33,34]. Transformers [35] in the BERT model take the attention mechanism resembling human cognitive attention and map given query (Q), key (K) and value (V) sequences to an output sequence. Transformers particularly use scaled dot-product attention [36], where the dot-product of query and key sequences are scaled by the inverse square root of the dimension of key vectors:

$$Attention(Q,\ K,\ V)\ =\ softmax(\frac{QK^T}{\sqrt{d_k}})V.$$

The scale factor enables the model to avoid an extremely large magnitude of multiplied value which especially occurs with a long sequence input. Multi-head attention which concatenates H multiple attentions calculated from the weighted query, key and value sequences is a major advantage of Transformers in learning different projections of these three sequences:

$$A_i\ =\ Attention_i(QW^Q{}_i,\ KW^K{}_i,\ VW^V{}_i),$$

$$Multi-head\ attention(Q,\ K,\ V)\ =\ Concatenation(A_1,\ldots,A_H)W^O$$

where A_i refers to an attention layer. In every attention layer, given query, key and value matrices are projected using the parameter matrices $W^Q{}_i$, $W^K{}_i$ and $W^V{}_i$. Then, multi-head attention is created as a concatenation of all H attention layers projected using another parameter matrix W^O.

We modified the BERT model to process DNA sequence fragments and sequential methylation patterns (Supplementary Figure 1). Originally, BERT needs three types of input embeddings: token embeddings, segment embeddings and position embeddings. In MethylBERT, we replaced the token embeddings assigned for tokenised words with DNA token embeddings. The segment embeddings indicate the sentence label of each token and are used for NSP. Instead, for MethylBERT, we created methylation embeddings for encoded methylation patterns. The position embeddings were also used in MethylBERT to guide the position of tokens in the sequencing read.

Pre-training MethylBERT

As described above, BERT requires long pre-training to learn the general context of input data and avoids heavily engineered task-specific architectures for every specific task. We use the Masked Language Model (MLM) for pre-training while the original BERT method performs pre-training of the model via both MLM and next sentence prediction (NSP) [18]. NSP is known to make the BERT model understand semantic dependencies across sentences [37], however, since we focus on the methylation pattern of unpaired reads, NSP was disregarded for MethylBERT pre-training. The overall pre-training scheme to use 3-mers for MLM is inspired by DNABERT [19].

We split the hg19 genome into 510bp segments and generated a 3-mer sequence of each segment, and the 3-mer segment is referred to as a token. While Ji et al. randomly sampled the read length between 5 and 510 with a certain probability for DNABERT [19], we used a fixed value of the sequence length 510 since it did not make a major performance change. For pre-training, we only applied a masked language model (MLM) by randomly masking 15% of 3-mers. Three masking schemes were employed following the original BERT paper [18]: 80% of selected 3-mers were masked with [MASK] token, 10% were replaced with another randomly chosen token, and the rest was unchanged. We masked left and right tokens together with the selected token in order to prevent a biased model predicting the masked token from neighbouring tokens. 3-mers tokens have 69 labels in total including five special tokens. A categorical cross-entropy loss L was calculated over all tokens $t \in \{1 \ldots T\}$ for every step of the model optimisation:

$$L_{pre-training} = -\sum_{t=1}^{T}\sum_{l=1}^{69} m^t y^t_l \, log(\hat{y}^t_l)$$

where y^t_l and $\hat{y}^t_l$ refer to as one-hot encoded value of label l in token t and corresponding logit value calculated by the MethylBERT model. m^t is a binary value indicating whether token t is masked or not.

We pre-trained the MethylBERT model for 120k steps including 10k warm-up steps and 20k decrease steps at the end of the training with a learning rate of 4e-4. The batch size was set to 256 and the gradient was accumulated over 4 steps. In the network architecture, the hidden layer had a size of 768 and the baseline model had 12 layers with 12 attention heads. However, since the smaller size BERT models performed similarly to the baseline model in the read classification analysis using simulated data, we used 6 layers of the MethylBERT model for 500 bps read analysis (Supplementary Figure 7). AdamW optimiser [38] was used with decay rate 0.01, β_1=0.9 and β_2=0.98 values. Since reference genomes do not have methylation patterns, we filled the methylation embeddings up with zeros during pre-training.

Fine-tuning for read-level methylation pattern classification

For fine-tuning, the encoder network in MethylBERT encodes a read-level reference DNA sequence and CpG methylation patterns (Supplementary Figure 1). Although reference DNA sequences do not contain any tumour-specific genetic information, they carry the exact position of a read in a given region as well as sequence features associated with specific methylation patterns. This information is necessary for the model to learn which CpGs are more likely to show tumour-specific signals. Previous studies also have pointed out that integrated information of DNA sequences and methylation can improve modelling [15,39].

DNA sequence fragments from reads are processed into 3-mer sequences as described in the previous section. The input length was reduced to 150 due to the shorter sequencing read length. In order to represent CpG methylomes, three numbers were used for methylation pattern encoding: 0 for unmethylated CpGs, 1 for methylated CpGs, and 2 for non-CpGs. CpG methylation patterns were assigned to the 3-mers where the cytosine of CpG is located in the middle.

The encoder part of the MethylBERT network takes DNA, methylation and position embeddings, and generates an encoded vector of $sequence_length \times 768$ dimension. This vector is concatenated with DMR information embedded into a space of dimension $sequence_length$, and the following the cell-type classifier calculates the posterior probabilities of cell types using the concatenated vectors. The DMR label is provided as information to determine region-wise tumour-specific methylome profile. The cell type with the highest posterior probability is determined as the classification outcome.

The MethylBERT model was fine-tuned over 600 or 1000 steps with a learning rate of 4e-4. We used the same optimisation scheme as for pre-training but applied the cross-entropy loss on the cell-type label for read-level methylome classification:

$$L_{fine-tuning} = -\sum_{r=1}^{R} \sum_{c \in \{T, N\}} m_{r,c} \log \frac{exp(x^{c}_{r})}{\sum_{c' \in \{T, N\}} exp(x^{c'}_{r})}$$

where x^{c}_{r} is the final activation for the cell type c (T=tumour, N=normal) for each read r. m^{c}_{r} is a binary value from one-hot encoded cell-type label c. The logits are normalised with a softmax function before the cross-entropy loss is calculated.

Tumour purity/fraction estimation

Since the proportions of the tumour and non-tumour compartments sum up to one, we used a single-parameter likelihood function to estimate the best tumour purity $\hat{\delta}$ from collected reads $\{r_1, \ldots, r_N\}$:

$$L(\delta) = \prod_{i=1}^{N} [\delta P(r_i | cell\ type = Tumour) + (1 - \delta) P(r_i | cell\ type = Normal)],$$

$$\hat{\delta} = argmax_{\delta}\, L(\delta).$$

The MethylBERT model calculates only the posterior probabilities of cell types given a read, so Bayes' theorem is applied to calculate the posterior probability of a read assuming that every read has the same marginal probability:

$$P(r_i|cell\ type = T) \propto P(cell\ type = T|r_i)P(cell\ type = T)^{-1}.$$

The prior probability of the cell types is calculated from the training dataset used for fine-tuning. For less complex likelihood computation and the Fisher information calculation, we use the log-likelihood function for maximum likelihood estimation. We employ a grid-search algorithm to find the optimal parameter $\hat{\delta}$ increasing the δ value by 0.0001 from zero to one.

As shown in Supplementary Figure 8A, the ratio of tumour-derived reads in DMRs does not have a symmetric distribution when tumour-normal cell types do not have an equal proportion. This is also shown by the negative correlation between the ground-truth tumour purity and the skewness of region-wise tumour purities (Supplementary Figure 8B). However, estimating the tumour purity only using the log-likelihood function above assumes that tumour-derived reads are equally distributed in every DMR. Therefore, we propose an adjustment of estimated tumour purity to take the region-wise tumour purities into account.

In a symmetric distribution, the skewness value is zero, thus we find a mapping which minimises the skewness of region-wise tumour purities to adjust the tumour purity. Let $\overline{W} = \{W_1, ..., W_K\}$ be parameters of the mapping for the estimated tumour purity in K regions, $\overline{\delta} = \{\delta_1, ..., \delta_K\}$. Assuming that the DMR k includes N reads, $\{r^k_1, ..., r^k_N\}$, the region-wise tumour purity δ_k is calculated as:

$$\delta_k = argmax_{\delta} \prod_{r^k_i \in \{r^k_1, ..., r^k_N\}} [\delta P(r^k_i|cell\ type = Tumour) + (1 - \delta)P(r^k_i|cell\ type = Normal).$$

The skewness of region-wise tumour purities can be calculated via the adjusted Fisher-Pearson standardised moment coefficient:

$$G_1(\overline{\delta}) = \frac{m_3(\overline{\delta})\sqrt{K(K-1)}}{m_2(\overline{\delta})^{3/2}(K-2)},\ m_i(\overline{\delta}) = \frac{1}{K}\sum_{i=1}^{K}(\delta_i - \mu)^i$$

where μ is the sample mean of region-wise tumour purites, $\frac{1}{K}\sum_{i=1}^{K}\delta_i$. Therefore, the mapping parameters $\overline{W}$ are optimised to minimise the skewness of region-wise tumour purities as follows:

$$\widehat{W} = argmin_W\, G_1(\overline{W} \circ \overline{\delta}) = argmin_W\, G_1(\{W_1\delta_1, ..., W_K\delta_K\})$$

where $\overline{W} \circ \overline{\delta}$ refers to the element-wise multiplication of two vectors $\overline{W}$ and $\overline{\delta}$. The expectation-maximisation (EM) algorithm is used to find the optimal mapping parameters $\widehat{W}$. Once the best mapping is found, we assume that the distribution of region-wise estimates is symmetric and determine the final estimation of tumour purity as the mean value of mapped region-wise tumour purities:

$$\hat{\delta} = \frac{1}{K}\widehat{W}^T\overline{\delta}.$$

The Fisher information indicates the amount of information about a model parameter carried by observed variables. In other words, the Fisher information is equivalent to an estimate of the model precision [23]. It is calculated as the variance of the derivative of the log-likelihood function with respect to the model parameter:

$$FI(\delta) = Var[\frac{\partial}{\partial\delta} logL(\delta)].$$

When the tumour purity adjustment is applied, the Fisher information cannot be calculated for the final estimation but in each region. Therefore, in this case, MethylBERT provides the Fisher information values as many as the number of selected DMRs.

Data preparation

Diffuse large B cell lymphoma WGBS data

Diffuse large B cell lymphoma (DLBCL) and non-neoplastic B-cell whole genome bisulfite sequencing data were downloaded from the Gene Expression Omnibus (GEO) database with the accession number GSE137880 [40]. 8 samples from two DLBCL patients and 8 non-neoplastic B cell samples from two donors were used for the experiments. From both cell types, 4 samples were assigned for the training and validation dataset and the rest were used for creating pseudo-bulks. All downloaded FastQ files were aligned with hg19 reference genome by Bismark 0.22.3 [41] after trimming using Trim Galore 0.6.6 [42], then duplicated reads were removed using picard Mark Duplicates 1.141. We followed the whole pipeline specified in our previous benchmarking study [13]. For Houseman's method, the read-level methylomes were converted into an array shape using Methrix [43].

CRC and PDAC BS-seq data

To train each MethylBERT model for ctDNA analysis derived from CRC and PDAC patients, we downloaded single-cell BS-seq of CRC cells with the GEO accession number GSE97693 [44] and WGBS of PDAC tissue with GSE63123 [45]. Only part of the CRC samples (341 cells) were downloaded as a training dataset. Similarly, we utilised only a subset of the PDAC data set involving seven samples collected from primary tumours in three different patients. During the data processing, we used TrimGalore 0.6.6 for adaptor trimming and quality control. Afterwards, the samples were aligned with the hg19 reference genome using Bismark 0.22.3. Duplicate removal was done by picard Mark Duplicates 1.141.

CRC, PDAC and control healthy ctDNA blood plasma data

For ctDNA experiments, we downloaded targeted BS-seq of plasma samples from gastrointestinal cancer patients and controlled healthy donors with the GEO accession number GSE149438 [28]. The downloaded dataset includes samples from 46 healthy donors, 74 PDAC patients, and 40 CRC patients in different cancer stages. We only used 44 PDAC patients whose cancer stage is clarified for the experiment. 32 healthy plasma samples were used to fine-tune the MethylBERT model and the rest was used as a comparison group in the tumour diagnosis analyses. We processed the data via trimming using TrimGalore, alignment to hg19 using Bismark and duplicate removal using picard Mark

Duplicates. The samples were aligned with paired-end mode and the unmapped reads were re-aligned with single-end mode.

DMR calling

Selecting informative regions with tumour-specific signals is vital in tumour purity estimation. For MethylBERT, we pre-selected differentially methylated regions (DMRs) to collect reads presenting informative methylation patterns. Tumour-specific DMRs were called by comparing tumour samples to non-tumour samples and the DSS package was used for the calling [46]. Parameters were set up as follows: delta value 0.2, P-value threshold 0.05, minimum number of CpGs 4, minimum length 50 bps and distance to merge 50 bps. In all analyses, we picked the top 100 DMRs based on the highest areaStat score. DSS performs a Wald test to identify differentially methylated loci and the areaStat score is calculated by summing all the test statistics up within each DMR. Therefore, a higher value of areaStat is likely to secure a larger number of CpGs in the region that are significantly differentially methylated between tumour and normal cell types.

Read-level methylome simulation

Simulated read-level methylomes were used to mimic different scenarios of tumour-specific signals for the evaluation of methylation pattern classification. We selected 100 CpG islands with the highest number of CpGs as the regions where reads are sampled. For each region, two mean methylation levels need to be assigned for tumour and normal cell types. We sampled a tumour mean methylation value d_i from a beta distribution whose β parameter is fixed to 5 and assigned $1 - d_i$ to a normal mean methylation value. Then, read-level methylation patterns were sampled from a binomial distribution with the probability $1 - d_i$ and d_i for normal and tumour cell types, respectively. The entire mechanism of sampling a read-level methylome with *K* CpGs, $\overline{m}^T = \{m^T_1, \ldots, m^T_K\}$ for tumour cell type and $\overline{m}^N = \{m^N_1, \ldots, m^N_K\}$ for normal cell type, is described as follows:

$$d_i \sim Beta(\alpha, \beta = 5),$$

$$\overline{m}^T \sim Binomial(n = K, p = d_i),$$

$$\overline{m}^N \sim Binomial(n = K, p = 1 - d_i)$$

where α and β are two shape parameters of the beta distribution, and n and p are the number of trials and the success probability of one trial in the binomial distribution.

Four different α values, 0.1, 1.0, 2.0 and 3.0, were used in the beta distribution to model different complexities of methylation patterns. Since a larger alpha value increases the

variance of the beta distribution with a fixed beta value, it is more likely that a lower tumour methylation value d_i will be sampled. This makes a higher methylation value $1 - d_i$ assigned to normal cell type. The smaller gap between d_i and $1 - d_i$ forms more complex methylation patterns between tumour and normal cell types (Supplementary Figure 2).

When CpG-specific methylation patterns are sampled (Supplementary Figure 2D), d_i and $1 - d_i$ are assigned to the odd and even indices of CpGs. Therefore, in this simulation, the average methylation level between tumour and normal cell types do not significantly differ, but still have cell type-specific methylation patterns.

Evaluation of read classification performance

The read classification performance evaluation includes an HMM-based algorithm. We designed the HMM to take methylation patterns as an observation, thus the observation has two categories: methylated and unmethylated CpGs. The hidden state is also a two-categorical variable with the assumption of whether the CpG is differentially methylated or not between tumour and normal cell types.

Although CancerDetector [14] and DISMIR [15] do not explicitly mention 'read classification', CancerDetector calculates $P(read|cell\ type = Tumour)$ whereas DISMIR has a 'd-score' that quantifies the chance that a given read is derived from tumour cells as interpreted by the authors. Therefore, we conducted read classification for those methods by assigning every read to the tumour class when the probability or d-score > 0.5.

In Figure 2D-E and Supplementary Figure 2, we evaluated MethylBERT read classification with different read coverage of the training set and the results show that MethylBERT can classify reads accurately in the case that the sample mean cannot represent the population mean well due to the low coverage. The standard error of the mean (SEM) was used for measuring how likely sample means of simulated read-level methylation patterns represent the real population mean. The SEM was calculated over the average methylation level of reads in each region.

Prostate epithelium proportion analysis for the lymph node sample acquired from prostate cancer patients

We collected 15 lymph node samples from hormone-sensitive metastatic prostate cancer patients and generated both WGBS and 450k array data. The 450k data were processed following [47], while the WGBS data were processed following the DLBCL data preprocessing. For the array-based deconvolution methods [21,25–27], in-house prostate tumour and epithelial cell reference (as described in [47]) and additional cell types acquired from GEO (with the accession numbers GSE35069, GSE86258, GSE74877, GSE71837,

GSE49667, and GSE87797) were used: monocytes, B cells, T cell, natural killer cells, granulocytes, fibroblasts, endothelium, and mesenchymal stromal cells.

MethylBERT was trained with samples collected from the normal cell type atlas [22] not using any tumour reference data. Instead of tumour reference data, we used four prostate epithelium samples, whereas we used two blood T cell, two blood B cell, and one colon fibroblast samples for the normal cell-type label considering the composition of normal lymph node bulks. For DMRs, we used the top 250 hypomethylated regions in prostate epithelium cells provided by the atlas. After the fine-tuning, we performed prostate epithelium proportion estimation with the WGBS data from the lymphoma samples.

Data availability

All data sets used in the study are downloaded from Gene Expression Omnibus (GEO). DLBCL WGBS data was downloaded with the accession number GSE137880, and ctDNA blood plasma samples (targeted BS-seq) were downloaded with the accession number GSE149438. The colorectal cancer (scBS-seq) and pancreatic cancer (WGBS) samples were downloaded with the accession numbers GSE97693 and GSE63123, respectively. The lymph node WGBS data from prostate cancer patients are available upon request.

Code availability

The read-level methylation simulation code is available at https://github.com/CompEpigen/methylseq_simulation. The MethylBERT code and package are available at https://github.com/CompEpigen/methylbert and https://pypi.org/project/methylbert/.

Acknowledgements

We acknowledge extensive discussions from Verena Wolf (Saarland University), Christoph Plass (German Cancer Research Center), Lisa Barros de Andrade e Sousa, and Marie Piraud (Helmholtz AI).

References

1. *Guo, M., Peng, Y., Gao, A., Du, C., and Herman, J.G. (2019). Epigenetic heterogeneity in cancer. Biomark. Res. 7, 23.*

2. *Zhao, L., Wu, X., Zheng, J., and Dong, D. (2021). DNA methylome profiling of circulating tumor cells in lung cancer at single base-pair resolution. Oncogene 40, 1884–1895.*

3. *Meyer, B., Clifton, S., Locke, W., Luu, P.-L., Du, Q., Lam, D., Armstrong, N.J., Kumar, B., Deng, N., Harvey, K., et al. (2021). Identification of DNA methylation biomarkers with potential to predict response to neoadjuvant chemotherapy in triple-negative breast cancer. Clin. Epigenetics 13, 226.*

4. *Zhang, W., Feng, H., Wu, H., and Zheng, X. (2017). Accounting for tumor purity improves cancer subtype classification from DNA methylation data. Bioinformatics 33, 2651–2657.*

5. *Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., Nery, J.R., Lee, L., Ye, Z., Ngo, Q.-M., et al. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462, 315–322.*

6. *Meissner, A., Gnirke, A., Bell, G.W., Ramsahoye, B., Lander, E.S., and Jaenisch, R. (2005). Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. Nucleic Acids Res. 33, 5868–5877.*

7. *Dedeurwaerder, S., Defrance, M., Calonne, E., Denis, H., Sotiriou, C., and Fuks, F. (2011). Evaluation of the Infinium Methylation 450K technology. Epigenomics 3, 771–784.*

8. *Shu, C., Zhang, X., Aouizerat, B.E., and Xu, K. (2020). Comparison of methylation capture sequencing and Infinium MethylationEPIC array in peripheral blood mononuclear cells. Epigenetics Chromatin 13, 51.*

9. *Yan, Y.-Y., Guo, Q.-R., Wang, F.-H., Adhikari, R., Zhu, Z.-Y., Zhang, H.-Y., Zhou, W.-M., Yu, H., Li, J.-Q., and Zhang, J.-Y. (2021). Cell-Free DNA: Hope and Potential Application in Cancer. Front. Cell Dev. Biol. 9, 639233.*

10. *Duffy, M.J., and Crown, J. (2022). Circulating Tumor DNA as a Biomarker for Monitoring Patients with Solid Cancers: Comparison with Standard Protein Biomarkers. Clin. Chem. 68, 1381–1390.*

11. *Yang, Y., Zhang, T., Wang, J., Wang, J., Xu, Y., Zhao, X., Ou, Q., Shao, Y., Wang, X., Wu, Y., et al. (2022). The clinical utility of dynamic ctDNA monitoring in inoperable localized NSCLC patients. Mol. Cancer 21, 117.*

12. *Gervin, K., Salas, L.A., Bakulski, K.M., van Zelm, M.C., Koestler, D.C., Wiencke, J.K., Duijts, L., Moll, H.A., Kelsey, K.T., Kobor, M.S., et al. (2019). Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. Clin. Epigenetics 11, 125.*

13. *Jeong, Y., de Andrade E Sousa, L.B., Thalmeier, D., Toth, R., Ganslmeier, M., Breuer, K., Plass, C., and Lutsik, P. (2022). Systematic evaluation of cell-type deconvolution pipelines for sequencing-based bulk DNA methylomes. Brief. Bioinformatics 23.*

14. *Li, W., Li, Q., Kang, S., Same, M., Zhou, Y., Sun, C., Liu, C.-C., Matsuoka, L., Sher, L., Wong, W.H., et al. (2018). CancerDetector: ultrasensitive and non-invasive cancer detection at the resolution of individual reads using cell-free DNA methylation sequencing data. Nucleic Acids Res. 46, e89.*

15. *Li, J., Wei, L., Zhang, X., Zhang, W., Wang, H., Zhong, B., Xie, Z., Lv, H., and Wang, X. (2021). DISMIR: Deep learning-based noninvasive cancer detection by integrating DNA sequence and methylation information of individual cell-free DNA reads. Brief. Bioinformatics 22.*

16. *Affinito, O., Palumbo, D., Fierro, A., Cuomo, M., De Riso, G., Monticelli, A., Miele, G., Chiariotti, L., and Cocozza, S. (2020). Nucleotide distance influences co-methylation between nearby CpG sites. Genomics 112, 144–150.*

17. *Paska, A.V., and Hudler, P. (2015). Aberrant methylation patterns in cancer: a clinical view. Biochem Med (Zagreb) 25, 161–176.*

18. *Devlin, J., Chang, M.-W., Lee, K., and Toutanova, K. (2018). BERT: Pre-training of deep bidirectional transformers for language understanding. arXiv.*

19. *Ji, Y., Zhou, Z., Liu, H., and Davuluri, R.V. (2021). DNABERT: pre-trained Bidirectional Encoder Representations from Transformers model for DNA-language in genome. Bioinformatics 37, 2112–2120.*

20. *Clark, K., Khandelwal, U., Levy, O., and Manning, C.D. (2019). What Does BERT Look At? An Analysis of BERT's Attention. arXiv.*

21. *Houseman, E.A., Accomando, W.P., Koestler, D.C., Christensen, B.C., Marsit, C.J., Nelson, H.H., Wiencke, J.K., and Kelsey, K.T. (2012). DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 13, 86.*

22. *Loyfer, N., Magenheim, J., Peretz, A., Cann, G., Bredno, J., Klochendler, A., Fox-Fisher, I., Shabi-Porat, S., Hecht, M., Pelet, T., et al. (2023). A DNA methylation atlas of normal human cell types. Nature 613, 355–364.*

23. *Fujita, K., Okada, K., and Katahira, K. (2022). The Fisher information matrix: A tutorial for calculation for decision making models.*

24. *Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell 100, 57–70.*

25. *Benelli, M., Romagnoli, D., and Demichelis, F. (2018). Tumor purity quantification by clonal DNA methylation signatures. Bioinformatics 34, 1642–1649.*

26. *Zhang, Z., Wiencke, J.K., Kelsey, K.T., Koestler, D.C., Christensen, B.C., and Salas, L.A. (2022). HiTIMED: hierarchical tumor immune microenvironment epigenetic deconvolution for accurate cell type resolution in the tumor microenvironment using tumor-type-specific DNA methylation data. J. Transl. Med. 20, 516.*

27. *Qin, Y., Feng, H., Chen, M., Wu, H., and Zheng, X. (2018). InfiniumPurify: An R package for estimating and accounting for tumor purity in cancer methylation research. Genes Dis. 5, 43–45.*

28. *Kandimalla, R., Xu, J., Link, A., Matsuyama, T., Yamamura, K., Parker, M.I., Uetake, H., Balaguer, F., Borazanci, E., Tsai, S., et al. (2021). EpiPanGI Dx: A Cell-free DNA Methylation Fingerprint for the Early Detection of Gastrointestinal Cancers. Clin. Cancer Res. 27, 6135–6144.*

29. *Liu, Y., Gu, Y., Su, M., Liu, H., Zhang, S., and Zhang, Y. (2019). An analysis about heterogeneity among cancers based on the DNA methylation patterns. BMC Cancer 19, 1259.*

30. *Zheng, X., Zhao, Q., Wu, H.-J., Li, W., Wang, H., Meyer, C.A., Qin, Q.A., Xu, H., Zang, C., Jiang, P., et al. (2014). MethylPurify: tumor purity deconvolution and differential methylation detection from single tumor DNA methylomes. Genome Biol. 15, 419.*

31. *Logsdon, G.A., Vollger, M.R., and Eichler, E.E. (2020). Long-read human genome sequencing and its applications. Nat. Rev. Genet. 21, 597–614.*

32. *Gouil, Q., and Keniry, A. (2019). Latest techniques to study DNA methylation. Essays Biochem. 63, 639–648.*

33. *Aum, S., and Choe, S. (2021). srBERT: automatic article classification model for systematic review using BERT. Syst. Rev. 10, 285.*

34. *Koroteev, M.V. (2021). BERT: A Review of Applications in Natural Language Processing and Understanding. arXiv.*

35. *Vaswani, A., Shazeer, N., Parmar, N., Uszkoreit, J., Jones, L., Gomez, A.N., Kaiser, L., and Polosukhin, I. (2017). Attention is all you need. arXiv.*

36. *Luong, M.-T., Pham, H., and Manning, C.D. (2015). Effective Approaches to Attention-based Neural Machine Translation. arXiv.*

37. *Shi, W., and Demberg, V. (2019). Next Sentence Prediction helps Implicit Discourse Relation Classification within and across Domains. In Proceedings of the 2019 Conference on Empirical Methods in Natural Language Processing and the 9th International Joint Conference on Natural Language Processing (EMNLP-IJCNLP) (Stroudsburg, PA, USA: Association for Computational*

Linguistics), pp. 5789–5795.

38. *Loshchilov, I., and Hutter, F. (2017). Decoupled Weight Decay Regularization. arXiv.*

39. *Angermueller, C., Lee, H.J., Reik, W., and Stegle, O. (2017). DeepCpG: accurate prediction of single-cell DNA methylation states using deep learning. Genome Biol. 18, 67.*

40. *Do, C., Dumont, E.L.P., Salas, M., Castano, A., Mujahed, H., Maldonado, L., Singh, A., DaSilva-Arnold, S.C., Bhagat, G., Lehman, S., et al. (2020). Allele-specific DNA methylation is increased in cancers and its dense mapping in normal plus neoplastic cells increases the yield of disease-associated regulatory SNPs. Genome Biol. 21, 153.*

41. *Krueger, F., and Andrews, S.R. (2011). Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics 27, 1571–1572.*

42. *Krueger, F., James, F., Ewels, P., Afyounian, E., Weinstein, M., Schuster-Boeckler, B., Hulselmans, G., and Sclamons (2023). FelixKrueger/TrimGalore: v0.6.10 - add default decompression path. Zenodo.*

43. *Mayakonda, A., Schönung, M., Hey, J., Batra, R.N., Feuerstein-Akgoz, C., Köhler, K., Lipka, D.B., Sotillo, R., Plass, C., Lutsik, P., et al. (2021). Methrix: an R/Bioconductor package for systematic aggregation and analysis of bisulfite sequencing data. Bioinformatics 36, 5524–5525.*

44. *Adossa, N., Khan, S., Rytkönen, K.T., and Elo, L.L. (2021). Computational strategies for single-cell multi-omics integration. Comput. Struct. Biotechnol. J. 19, 2588–2596.*

45. *McDonald, O.G., Li, X., Saunders, T., Tryggvadottir, R., Mentch, S.J., Warmoes, M.O., Word, A.E., Carrer, A., Salz, T.H., Natsume, S., et al. (2017). Epigenomic reprogramming during pancreatic cancer progression links anabolic glucose metabolism to distant metastasis. Nat. Genet. 49, 367–376.*

46. *Park, Y., and Wu, H. (2016). Differential methylation analysis for BS-seq data under general experimental design. Bioinformatics 32, 1446–1453.*

47. *Gerhauser, C., Favero, F., Risch, T., Simon, R., Feuerbach, L., Assenov, Y., Heckmann, D., Sidiropoulos, N., Waszak, S.M., Hübschmann, D., et al. (2018). Molecular Evolution of Early-Onset Prostate Cancer Identifies Molecular Risk Markers and Clinical Trajectories. Cancer Cell 34, 996-1011.e8.*