Fast Approximate Inference of Transcript Expression Levels from RNA-seq Data

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Abstract

Motivation: The mapping of RNA-seq reads to their transcripts of origin is a fundamental task in transcript expression estimation and differential expression scoring. Where ambiguities in mapping exist due to transcripts sharing sequence, e.g. alternative isoforms or alleles, the problem becomes an instance of non-trivial probabilistic inference. Bayesian inference in such a problem is intractable and approximate methods must be used such as Markov chain Monte Carlo (MCMC) and Variational Bayes. Standard implementations of these methods can be prohibitively slow for large datasets and complex gene models.

Results: We propose an approximate inference scheme based on Variational Bayes applied to an existing model of transcript expression inference from RNA-seq data. We apply recent advances in Variational Bayes algorithmics to improve the convergence of the algorithm beyond the standard variational expectation-maximisation approach. We apply our algorithm to simulated and biological datasets, demonstrating that the increase in speed requires only a small trade-off in accuracy of expression level estimation.

Availability: The methods were implemented in R and C++, and are available as part of the BitSeq project at https://code.google.com/p/bitseq/. The methods will be made available through the BitSeq Bioconductor package at the next stable release.

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1 Introduction

RNA-seq is a technology with the potential to identify and quantify all mRNA transcripts in a biological sample (Mortazavi et al., 2008). Some of these transcripts come from different isoforms or alleles of the same genes or from closely related homologous genes, and consequently they may share much of their primary sequence. Current RNA-seq technologies generate short

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reads that must be aligned to the genome or transcriptome in order to quantify expression levels. In some cases the observed reads may originate from different transcripts and there may be few reads that are useful to distinguish very closely related isoforms or alleles. It is therefore a challenging statistical problem to uncover the expression level of closely related transcripts using RNA-seq technology.

Probabilistic latent variable models, in particular mixture models (Li et al., 2010; Katz et al., 2010; Li and Dewey, 2011; Turro et al., 2011; Glaus et al., 2012; Trapnell et al., 2013; Nariai et al., 2013) are a promising class of methods for inferring transcript expression levels from RNA-seq data. Such models can be used to deconvolve the signal in the read data, assigning reads to alternative, pre-defined transcripts according to their probability of originating from each. The term mixture model derives from the interpretation of the data as being derived from a mixture of different transcripts, the mixture components, with each read originating from one component. Although reads originate from only one component they may map to multiple related components, resulting in some ambiguity in their assignment. Transcript expression levels are model parameters (mixture component proportions) that have to be inferred from the mapped read data. Due to their probabilistic nature these models can fully account for multiple mapping reads, complex biases in the sequence data, sequencing errors, alignment quality scores and prior information on the insert length in paired-end reads. Mixture models have been successfully applied to infer the proportion of different gene isoforms or allelic variants in a particular sample (Katz et al., 2010; Turro et al., 2011), for improving overall gene expression estimates (Li et al., 2010; Li and Dewey, 2011) and for transcript-level differential expression calling (Glaus et al., 2012; Trapnell et al., 2013).

Inference in latent variable models such as these can be carried out by Maximum Likelihood (ML) or Bayesian parameter estimation. In ML the choice of parameters that maximises the data likelihood is obtained through a numerical optimisation procedure. In the case of mixture models a popular choice of algorithm is the Expectation Maximisation (EM) algorithm, as first applied to this model and expressed sequence data by Xing et al. (2006) and later to RNA-seq data for example by Li et al. (2010) among others. For Bayesian inference the most popular approach is Markov chain Monte Carlo (MCMC) and for the case of mixture models a Gibbs sampler is most often used (Katz et al., 2010; Glaus et al., 2012). An advantage of Bayesian inference is that one obtains a posterior probability over the model parameters rather than just a point estimate. This provides a level of uncertainty in the inferred transcript expression levels as well as information about the covariation between estimates for closely related transcripts. The uncertainty information can be usefully propagated into downstream analysis of the data, e.g. calling differentially expressed transcripts from replicated experiments (Glaus et al., 2012).

A Bayesian method, BitSeq, was recently proposed in which inference was carried out using a collapsed Gibbs sampler (Glaus et al., 2012). The method was shown to perform well, especially for the task of inferring the relative expression of different gene isoforms and for ranking transcripts according to their probability of being differentially expressed between conditions. However, for typical modern RNA-seq datasets with hundreds of millions of read-pairs the Gibbs sampler can be inconveniently slow, creating a computational bottleneck in applying a Bayesian approach. As the volume of data continues to grow and gene models are becoming more complex as more alternative transcripts are discovered, more efficient inference algorithms are required so that Bayesian methods can be used to provide practical computational tools for RNA-seq data processing.

An alternative approach to Bayesian inference is to use deterministic approximate infer-
Figure 1: Graphical model of the RNA-seq mixture problem. Given a known Transcriptome $T$ and some observed reads $R$, the inference problem is for $\theta$ through the latent variables $Z$.

ference algorithms such as Variational Bayes (VB) (reviewed by Bishop, 2006). While MCMC algorithms are attractive due to their asymptotic approximation guarantees, VB often provides a much faster method to obtain a good approximation to the posterior distribution. For models where Gibbs sampling can be applied there is typically a closely related VB Expectation Maximisation (VBEM) algorithm. In this contribution we show how VB can be used to massively speed up inference in the BitSeq model for transcript expression-level inference. We show that the mean transcript expression levels and associated posterior distributions are very close to those obtained with MCMC. We use a recent formulation of VB (Hensman et al., 2012) which is shown to provide a greater speed up when compared to a more standard VBEM algorithm. Our new algorithm is implemented in the most recent version of the BitSeq, allowing the method to be applied to much larger RNA-seq datasets in equal computing time.

Recently, an alternative method for variational approximation in the same problem was proposed using a standard VBEM algorithm (Nariai et al., 2013). The assumptions made in our approximation are similar, though the empirical comparisons herein show that our proposed method outperforms theirs in terms of computation time and required memory with negligible differences in accuracy. The improvement in terms of reduced computational cost can be seen as a result of our adoption of a novel VB method. Furthermore we investigate the effects of the variational assumption in this problem, and compare empirically to results using the gold standard, MCMC.

2 Methods

Our probabilistic model of RNA-seq follows Stage 1 of Glaus et al. (2012). We summarise our notation in Table 1. The probabilistic model is shown using standard directed graphical notation in Figure 1. Here we have focussed on the mixture part of the analysis, assuming that the model which associates reads to transcripts (i.e. $p(r_n | T_m)$) is known. Following BitSeq (Glaus et al., 2012), we compute this part of the model a priori, with parameters estimated from uniquely aligned reads.

We consider RNA-seq assays independently, computing an approximate posterior for the transcript proportions $\theta$ in each assay. Subsequent analysis such as differential expression can be done using the estimated distributions of each assay.
The generative model

Transcript fragment proportions

The generative model for an RNA-seq assay is as follows. We assume that the experiment consists of a pile of RNA fragments, where the abundance of fragments from transcript $T_m$ in the assay is $\theta_m$. Fragments are then sequenced in these proportions, so that the prior probability of any fragment corresponding to transcript $T_m$ is $\theta_m$. Introducing a convenient allocation vector $z_{nm}$ for each read, we can write

$$ p(Z|\theta) = \prod_{n=1}^{N} \prod_{k=1}^{K} \theta_k^{z_{nm}}, \quad (1) $$

where $z_{nm} \in \{0, 1\}$ is a binary variable which indicates whether the $n$th fragment came from the $m$th transcript ($z_{nm} = 1$) and is subject to $\sum_{m=0}^{M} z_{nm} = 1$. We use $Z$ to represent the collection of all allocation vectors.

We note that both $\theta$ and $Z$ are variables to be inferred, with $\theta$ the main object of interest. $\theta$ can be transformed later into some more convenient measure, for instance reads per kilobase of length per million sequenced reads (RPKM) [Mortazavi et al. 2008], though it is more convenient from a probabilistic point of view to work with $\theta$ directly.

The variables $Z$ are sometimes known in the machine learning literature as latent variables. Whilst they are not of interest directly, inference in these variables is essential in order to infer $\theta$.

Read generation model

An important part of the model is to realise the likelihood $p(r_n|T_m)$, the probability of generating the $n$th read from the $m$th transcript.

Writing the collection of all reads as $R = \{r_n\}_{n=1}^{N}$, the likelihood of a set of alignments $Z$ is

$$ p(R|T, Z) = \prod_{n=1}^{N} p(r_n|T_m)^{z_{nm}}, \quad (2) $$

where $T_m$ represents the $m$th transcript, $T$ represents the transcriptome.
The values of \( p(r_n|T_m) \) for all alignments can be computed before performing inference in \( \theta \) since we are assuming a known transcriptome. For paired-end reads, the mates originate from single fragment and their likelihood is inferred jointly, denoting \( r_n = (r^{(1)}_n, r^{(2)}_n) \). The likelihood of alignment is computed as

\[
P(r_n|T_m) = P(l|T_m)P(p|l, T_m) \prod_{i=1,2} P(r^{(i)}_n|seq_{mlp_i}),
\]

where \( l \) is the length of a fragment, \( p \) is its position and \( seq_{mlp} \) denotes underlying reference sequence. The fragment length distribution can be pre-defined or inferred empirically. The position likelihood, \( P(p|l,T_m) \), can either assume uniform read distribution or account for read distribution biases using an empirical model. The last term, \( \prod_{i=1,2} P(r^{(i)}_n|seq_{mlp_i}) \) describes the probability of observed read sequences based on quality scores and base discrepancy between read and reference. For detailed description of the alignment likelihood estimation please refer to (Glaus et al., 2012).

Identifying noisy reads

Our model is similar to previous work (Glaus et al., 2012), but does not contain a variable identifying reads as belonging to a ‘noise’ class. To circumvent the explicit formulation of a model with this variable, we introduce a ‘noise transcript’ which we append to the list of known transcripts. The generative probability of any read from this transcript, \( p(r_n|T_0) \), is again calculated according to the model described in (Glaus et al., 2012). Due to the conjugate relationships between the variables in our model and those in (Glaus et al., 2012), the models are the same, subject to a slight reformulation of the prior parameters.

Prior over \( \theta \)

The final part of our model is to specify some prior belief in the vector \( \theta \). To make our approximations tractable, it is necessary to use a conjugate prior, which in this case is a Dirichlet distribution

\[
p(\theta) = \frac{\Gamma(\hat{\alpha}^o)}{\prod_{m=1}^{M} \Gamma(\alpha^o_m)} \prod_{m=1}^{M} \theta^o_m - 1
\]

with \( \hat{\alpha}^o = \sum_{m=1}^{M} \alpha^o_m \). \( \alpha^o_m \) represents our prior belief in the values of \( \theta_m \), and we use a weak but proper prior \( \alpha^o_m = 1; m = 0 \ldots M \). A priori, we assume that the concentrations are all equal, but with large uncertainty.

2.2 Approximate inference

We are interested in computing the posterior distribution for the mixing proportions, \( p(\theta | R, T) \propto \sum_Z p(R | T, Z)p(Z | \theta)p(\theta) \). For very small datasets, it is possible to perform exact Bayesian inference in the our model, however for any realistically sized problem, exact inference is impossible due to the combinatorial size of the number of possible solutions. Our proposed solution is to use a collapsed version of Variational Bayes.

Variational Bayes involves approximating the posterior probability density of all the model parameters with another distribution \( q \),

\[
q(\theta, Z) \approx p(\theta, Z|R, T).
\]
The approximation is optimised by minimising the Kullback-Leibler (KL) divergence between \( q(\theta, Z) \) and \( p(\theta, Z| R, T) \). To make the VB approach tractable, some factorisations need to be assumed in the approximate posterior. In the case of the current model, we need to assume that the posterior probability of the transcript proportions factorises from the alignments:

\[
q(\theta, Z) = q(\theta)q(Z). \tag{6}
\]

Further factorisations in \( q(Z) \) occur due to the simplicity of the model, revealing \( q(Z) = \prod_{n=1}^{N} q(z_n) \).

We write the approximate distribution for \( q(Z) \) using the parameters \( \phi_{nm} \):

\[
q(Z) = \prod_{n=1}^{N} \prod_{m=1}^{M} \phi_{nm}^{z_{nm}}. \tag{7}
\]

We need not to introduce parameters for \( q(\theta) \) since it will arise implicitly in our derivation in terms of \( \phi \).

**The objective function**

Approximate inference is performed by optimisation: the parameters of the approximating distribution are changed so as to minimise the KL divergence. Whilst the KL divergence is not computable, it is possible to derive a lower bound on the marginal likelihood, maximisation of which minimises the KL divergence (see e.g. [Bishop, 2006]). Here we derive a lower bound which is dependent only on the parameters of \( q(Z) \), with the optimal distribution for \( q(\theta) \) arising implicitly for any given \( q(Z) \).

First we construct a lower bound on the conditional log probability of the reads \( R \) given the transcript concentrations \( \theta \) and the known transcriptome \( T \):

\[
\ln p(R | T, \theta) = \ln \int p(R | Z, T)p(Z | \theta) \, dZ \\
\geq \mathbb{E}_{q(Z)} \left[ \ln p(R | Z, T) + \ln p(Z | \theta) - \ln q(Z) \right] \\
\geq \sum_{n=1}^{N} \sum_{m=1}^{M} \phi_{nm} \left( \ln p(r_n | T_m) + \ln \theta_m - \ln \phi_{nm} \right) \\
= \mathcal{L}_1(\theta), \tag{8}
\]

where the first line follows from Jensen’s inequality in a similar fashion to standard VB methods. We have denoted this conditional bound \( \mathcal{L}_1(\theta) \), which is still a function of \( \theta \). In order to generate a bound on the marginal likelihood, \( p(R | T) \), we need to remove this dependence on \( \theta \) which we do in a Bayesian fashion, by substituting \( \mathcal{L}_1(\theta) \) into the following Bayesian marginalisation:

\[
p(R | T) = \int p(R | T, \theta)p(\theta) \, d\theta \\
\geq \int \exp\{\mathcal{L}_1(\theta)\}p(\theta) \, d\theta. \tag{9}
\]
Solving this integral and taking the logarithm gives us our final bound which equates to

\[
\ln p(R \mid T) \geq \mathcal{L} = \sum_{n=1}^{N} \sum_{m=1}^{M} \phi_{nm} \left( \ln p(r_n \mid T_m) - \ln \phi_{nm} \right) \\
+ \ln \Gamma(\hat{\alpha}^o) - \ln \Gamma(\hat{\alpha}^o + N) - \sum_{m=1}^{M} \left( \ln \Gamma(\alpha_m^o) - \ln \Gamma(\alpha_m^o + \hat{\phi}_m) \right),
\]

where \( \hat{\phi}_m = \sum_{n=1}^{N} \phi_{nm} \) and we also have that the approximate posterior distribution for \( \theta \) is a Dirichlet distribution with parameters \( \alpha_m^o + \hat{\phi}_m \).

### 2.3 Optimisation

Having established the objective function as a lower bound on the marginal likelihood, all that remains is to optimise the variables of the approximating distribution \( q(Z, \theta) \). The dimensionality of this optimisation is rather high and potentially rather difficult. Optimisation in standard VB is usually performed by an EM like algorithm, which performs a series of convex optimisations in each of the factorised variables alternately. In our formulation of the problem, we only need to optimise the parameters of the distribution \( q(Z) \), which we do by a gradient-based method. Taking a derivative of (10) with respect to the parameters \( \phi \) gives

\[
\frac{\partial \mathcal{L}}{\partial \phi_{nm}} = \ln p(r_n \mid T_m) - \ln \phi_{nm} + \psi(\alpha_m^o + \hat{\phi}_m),
\]

where \( \psi \) is the digamma function. To avoid constrained optimisation we re-parameterise \( \phi \) as \( \gamma \):

\[
\phi_{nm} = \frac{e^{\gamma_{nm}}}{\sum_{m'=1}^{M} e^{\gamma_{nm'}}}
\]

and it is then possible to optimise the variables \( \gamma \) using a standard gradient-based optimiser.

### 2.4 Geometry

Information geometry concerns the interpretation of statistical objects in a geometric fashion. Specifically, a class of probability distributions behaves as a Riemannian manifold with curvature given by the Fisher information. Amari (1998) showed that the direction of the steepest descent on a such a manifold is given by the natural gradient:

\[
\tilde{\nabla} \mathcal{L} = G^{-1} \nabla \mathcal{L}.
\]

Where \( G \) is the Fisher information matrix. Since we are performing optimisation of the distribution \( q(Z) \), we can make use of the natural gradient in computing a search direction. For our problem, we assume that the \( N \times M \) matrix \( Z \) has been transformed into a \( NM \) vector, and the Fisher information corresponding to \( \gamma_{nm}, \gamma_{n'm'} \) is given by

\[
G[m, n, m', n'] = \begin{cases} 
\phi_{nm} - \phi_{nm}, & \text{if } n = n' \text{ and } m = m' \\
-\phi_{nm}, & \text{if } n = n' \text{ but } m \neq m' \\
0, & \text{otherwise.}
\end{cases}
\]

We note that this structure is block-diagonal, and that each block can be easily inverted using the Sherman-Morrison identity, giving an analytical expression for \( G^{-1} \), and thus making the
natural gradient very fast to compute. This differentiates our method from previous natural gradient-based methods for VB (Honkela et al., 2010), along with our use of the collapsed method.

The optimisation of the variational parameters then proceeds as follows. Following random initialisation, a unit step is taken in the natural gradient direction. Subsequent steps are subject to conjugate gradients (see Honkela et al., 2010). If the conjugate gradient step should fail to improve the objective we revert to a VBEM update, which is guaranteed to improve the bound. For more details, see Hensman et al. (2012).

2.5 Truncation

The optimisation described above has $N \times M$ free parameters for optimisation, one to align each read to each transcript. However, for most read-transcript pairs, $p(r_n | T_m)$ will be negligibly small. We follow (Glaus et al., 2012) in truncating the values of $p(r_n | T_m)$ to zero for reads which do not suitably align. Examining the objective function (10) we see that we can also set $\phi_{nm}$ to zero for these truncated alignments (using the convention that $0 \ln(0) = 0$) and thus also $\gamma_{nm} = -\infty$ for the same. This truncation dramatically reduces the computational load of our algorithm, reducing the dimensionality of the optimisation space as well as reducing the number of operations needed to compute the objective.

2.6 The approximate posterior

Having fitted our model, we may wish to propagate the posterior distribution through a second set of processing, for example to identify differential expressed transcripts. This was known as stage 2 in Glaus et al. (2012). Whilst it may be desirable to solve both stages together in a Bayesian framework, the size of the problem generally forbids this, therefore we propose the use of either a moment-matching or sampling procedure to propagate $q(\theta)$ through further analysis. The approximate posterior $q(\theta)$ is a Dirichlet distribution, whose marginals have the following useful properties:

$$E[\theta_m] = \frac{\alpha^o_m + \hat{\phi}_m}{\hat{\alpha}^o + N},$$

$$\text{var}[\theta_m] = (\alpha^o_m + \hat{\phi}_m)(\hat{\alpha}^o + N - \alpha^o_m - \hat{\phi}_m)C,$$

$$\text{cov}[\theta_m, \theta'_m] = -(\alpha^o_m + \hat{\phi}_m)(\alpha^o_m + \hat{\phi}_m')C,$$

with $C = (\hat{\alpha}^o + N)^{-2}(\hat{\alpha}^o + N + 1)^{-1}$.

This approximate posterior is somewhat inflexible, in that it cannot express arbitrary covariances between the transcripts. This arises from the factorising assumption amongst the assignment of reads to transcripts: reads are assigned independently in the variational method and their dependence cannot be modelled. This is reflected in the results section where we show empirically that the VB approximation leads to an underestimation of the variance. Nonetheless, this simplifying assumption leads to reasonable levels of accuracy, and gives significant benefit in terms of speed increase.

3 Results and Discussion

We evaluate the accuracy of our inference approach using both synthetic and real data. The synthetic data enables comparison against known ground truth, whilst for the real data where
Table 2: The $R^2$ correlation coefficient of estimated expression levels and ground truth on synthetic data with uniform read distribution. Three different expression measures were used: absolute transcript expression, relative within-gene transcript expression and gene expression. Comparison includes sites with at least 1 read per transcript for transcript expression, either 10 or 100 reads per gene for within-gene transcript expression and at least 1 read per gene for gene expression.

the ground truth is unknown, we compare our VB method with a very long run of MCMC. We then return to the synthetic data in a comparison of differential expression analysis using the BitSeq pipeline.

3.1 Inference accuracy and performance on synthetic data

The synthetic data analysed here was used in (Glaus et al, 2012) where the MCMC inference algorithm implemented in BitSeq was compared to three other transcript expression estimation methods. The reads were uniformly sampled from transcripts, with abundances based on real RNA-seq data. For more details regarding synthesis of the data, please refer to (Glaus et al, 2012).

The expression is evaluated in 3 different measures: transcript expression, transcript within-gene relative proportions and gene expression. Comparison of our proposed BitSeq-VB inference method, BitSeq version 0.6.0, Cufflinks version 2.1.1 (Trapnell et al, 2010) and TIGAR version from 10.6.2013 (Nariai et al, 2013) is presented in Table 2.

We see that in each measure, the variational approximation to the posterior performs almost as well as the MCMC implementation. TIGAR performs comparably to both BitSeq methods, though the differences are small. We refer the reader to Nariai et al (2013) for a comparison of TIGAR with competing methods including RSEM.

On this relatively small dataset with 10 million simulated reads, the computational cost is significant for BitSeq and TIGAR. The MCMC version of BitSeq required 503 minutes, and TIGAR required 509 minutes. It is perhaps against the conventional wisdom that the Gibbs sampling procedure should be faster than TIGAR’s variational method, and these differences may be due in part to the implementation (BitSeq uses C++, and TIGAR uses Java), though we find the Gibbs procedure to be efficient in the next section also. We used single threaded mode for BitSeq as TIGAR does not provide explicit parallelisation option and seems to be using only one CPU.

The variational version of BitSeq, using the contemporary collapsed procedure defined above, takes significantly less time than either the BitSeq-MCMC method or TIGAR’s VB at only 21 minutes. This represents a substantial difference that makes the approach attractive in circumstances where results are demanded quickly.

Whilst Cufflinks has the lowest computational requirements of all the methods, Table 2 shows that this comes at the cost of a serious lack of performance.

| Expression | Cutoff | BitSeq VB | BitSeq MCMC | Cuff. 2.1.1 | TIGAR |
|------------|--------|-----------|-------------|-------------|-------|
| Transcript| 1      | 0.994     | 0.994       | 0.826       | 0.998 |
| Relative   | 10     | 0.941     | 0.945       | 0.829       | 0.944 |
| Relative   | 100    | 0.961     | 0.963       | 0.897       | 0.963 |
| Gene       | 1      | 0.994     | 0.994       | 0.838       | 0.999 |
Table 3: Comparison of runtime and memory requirements for MCMC, VB and alternative VB implementation in TIGAR. Smaller, synthetic, data was analysed on single CPU, while 4 CPUs were used for the real data consisting of 100m reads. Analysis was done on computing node with Intel Xeon X5690, 3.47GHz CPU with 12.3MB cache.

|                | Synthetic (10m reads) | Real (100m reads) |
|----------------|-----------------------|-------------------|
|                | time (mins) | memory (GB) | time (mins) | memory (GB) |
| BitSeq VB      | 21          | 2.4          | 310         | 26.4        |
| BitSeq MCMC    | 503         | 0.6          | 1769        | 8.5         |
| TIGAR          | 509         | 8.2          | n/a         | ~80         |

3.2 Real data

The RNA-seq reads were downloaded from Short Read Archive [NCBI, 2010], experiment SRX110318, run SRR387661, generated by the ENCODE consortium [Djebali et al., 2012]. Library extracted from cytosol of human bone marrow tissue affected by leukemia (K562) was sequenced by Illumina Genome Analyzer II, generating 124.8 million read pairs, 76 bp long. We mapped the reads using Bowtie 2.0.6 [Langmead and Salzberg, 2012] to a reference transcriptome using 140869 known coding sequences from Ensembl human cDNA, release 70 [Flicek et al., 2013]. 98.8 million reads were mapped to the reference, with 5 mappings per read on average.

Our main potential concern in using the variational method is the quality of approximation to the posterior. Figure 2 shows a comparison of the variational posterior with a ground truth computed by MCMC. We conclude that the VB method consistently provides very accurate estimates of the posterior mean across the whole range of expression levels. The estimates of posterior variance are less consistent: for a fraction of transcripts the variances are underestimated, sometimes rather severely. The VB method seems to estimate only the Poisson variance of random sampling of reads which underestimates the true variance of the transcript expression levels.

The runtime and memory requirements necessary for the analysis of this data are presented in Table 3. In this case, both inference methods were used in multi threaded setting facilitating 4 CPUs of the computing node with Intel Xeon 3.47GHz CPUs. Please note, both times include the same pre-processing stage which estimates likelihood for each alignment while accounting for non-uniform read distribution bias, which takes 162 minutes. If we subtract this time, then the actual convergence time for VB is significantly lower with 2.5 hours when compared to collapsed MCMC at 26.8 hours. The memory requirements of our VB inference implementation were three times as high as for MCMC, but still proved feasible.

The memory requirements of TIGAR prohibited its use on this data set. Extrapolating linearly, we estimate that TIGAR would require 80GB of system memory to run, which is an infeasible resource for most practitioners. Indeed, [Nariai et al., 2013] demonstrated their algorithm on data sets no larger than 4.5 million reads. For comparison, at the time of writing the Illumina website lists the HiSeq 2500 machine as capable of producing 3 billion reads in a single run.

We conclude that the novel variational method proposed here significantly outperforms the other methods in terms of computational time, and performs very well in estimating the mean of the posterior. If estimation of the expression level is all that is required, then it
Figure 2: A comparison of the first two moments of the approximate posterior expression in counts per transcript: (a) posterior mean ($R^2$ correlation is 0.999) (b) posterior standard deviation: the VB method significantly under-estimates the posterior variance ($\sigma^2$). Shading represents the number of transcripts in each region.

would seem that the VB method suffices. However, downstream methods which make use of uncertainty in the transcript quantification (such as the differential expression analysis proposed in BitSeq) may suffer from the poor approximation in terms of posterior variance.

### 3.3 Convergence comparison

We further investigate convergence properties of MCMC and VB in terms of mean expression. We use subset of data described in previous section restricted to 8713 transcripts of chromosome 19. As the true expression is unknown, we use long run of MCMC as a ground truth for mean expression estimates. Running the inference methods for certain number of iterations, we record the run time and calculate Root Mean Square Error (RMSE) of estimated expression.

The convergence of the variational methods and the Gibbs sampling procedures is shown in Figure 3. For completeness, we include a standard implementation of VB (similar to Nariai et al. 2013), but using the BitSeq model (denoted VBEM). It is straightforward to derive this from our description above as discussed in Hensman et al. (2012).

Our implementation of VB converges first in about 2 minutes. Surprisingly, some runs of collapsed MCMC converge to better estimates even faster than standard VB, which takes around 10 minutes. However, as MCMC is a stochastic method, an estimate that is consistently better than the results obtain by VB can be obtained after 900 minutes.

### 3.4 Using approximate posteriors in differential expression analysis

We have shown that the variational method performs well in estimating the mean of the transcript expression, but struggles with the variance. Here we investigate the effects of this on differential expression analysis.

In order to compare to a ground truth, we return to the synthetic data consisting of two conditions with two replicates each. The variation of expression within replicates was based...
Figure 3: Convergence comparison of Collapsed MCMC with standard VB algorithm and VB with Fletcher-Reeves conjugate gradient optimisation. Expression estimates obtained by very long run of MCMC are used as a ground truth and average root mean square error over 10 runs was calculated, two standard deviations are used as error bars. The VB methods with several randomised initial conditions showed negligible differences in convergence.

Figure 4: Comparison of ROC curves for DE analysis of synthetic data using BitSeq with MCMC posterior, BitSeq with approximate posterior (VB) and Cufflinks. Transcripts were split into three equal-sized groups based on average true read count: $[1, 3.75)$, $[3.75, 26.38)$, above 26.38. The ROCs are averaged over 5 independent analyses with different transcripts being differentially expressed, with two standard deviations as error bars. Using BitSeq with MCMC inference yields better and more stable performance.

on biological variance observed in real data as described by Glaus et al. (2012). Expression of one third of transcripts was changed in one of the conditions, with fold change being uniformly selected from interval $[1.5, 3.5]$.

We use expression estimates obtained by MCMC and VB inference methods in combination with BitSeq differential expression analysis procedure. For comparison, we also compare against alternative approach using Cuffdiff (Trapnell et al., 2013). Figure 4 shows ROC char-
Figure 5: Kernel density estimate of transcripts’ Probability of Positive Log Ratio obtained by BitSeq differential expression analysis. Using expression estimates from VB inference method results in more extreme values of PPLR.

acteristics of the different approaches for transcripts grouped into three groups based on initial mean expression. We can see that using MCMC expression estimates on average outperforms the use of VB estimates in terms of True Positive Rate.

BitSeq differential expression analysis estimates the Probability of Positive Log Ratio (PPLR) for each transcript. PPLR close to 1 signifies high probability of up-regulation, whereas values close to 0 mean high probability of down-regulation. The PPLR is then used for ranking transcripts in terms of differential expression likelihood and selecting significant differences. In Figure 5 we show smoothed distribution of transcripts’ PPLR produced by BitSeq when used with either MCMC or VB expression estimates. Due to the underestimation of variance in the VB inference approach, the resulting PPLR tends to more extreme values in terms of differential expression likelihood.

4 Conclusion

We have presented a variational method for inference in the transcript deconvolution problem of RNA-seq. Building on previous work in BitSeq, we have presented a fast approximate inference method. For the datasets under consideration, the mean of the posterior was well estimated by the approximate method, which indicates that it may be suitable where this is of primary interest, and time and computational resources are limited.

We have compared out method both Gibbs sampling in the same model and to the TIGAR approach. We conclude that the TIGAR implementation requires large amounts of memory, and does not offer a significant improvement over Gibbs sampling in terms of time. Further, TIGAR may not be applicable for large datasets where memory requirements are prohibitive. Also note, TIGAR at the moment does not provide means of accounting for read distribution bias and assumes uniform read distribution. While this is not a problem on simulated data with reads sampled uniformly, for real datasets accounting for these biases is desired.

We have also investigated the effect of the approximation on the calling of differentially expressed transcripts. We show that the variational approximation does not work as well as Gibbs sampling, though it does offer some improvement over the Cufflinks/Cuffdiff method.

We conclude that for maximum effectiveness, Gibbs sampling of the posterior distribution is the most effective method. The BitSeq sampler runs in approximately the same time as the variational TIGAR method (which has previously been shown to be comparable to other methods such as RSEM).

As well as being powerful, the Gibbs method is extremely applicable to parallel computing. With multiple cores or clustered machines available, parallel MCMC chains can be run to
obtain a linear speed up in compute time as the number of processing units increases, once
the burn-in period is complete. Our VB method can also benefit from parallel processing, in
that one of the expensive computations – taking logarithms and exponents to convert between
\( \phi \) and \( \gamma \) can be parallelised. Some preliminary runs show good speed-up for this method on
a multiple-core machine, where this loop can be tightly parallelised.

Finally, we suggest some courses of future action. The fast and consistent convergence of
the VB method mean that it may be useful for a ‘quick look’ examination of the data, a check
to run before the Gibbs sampler is run. Further, since it provides an excellent approximation
to the mean of the posterior, it could be used to e.g. reduce the burn-in time for the Gibbs
sampler, or as the initial stage of a more sophisticated approximating technique.

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