VARiD: A variation detection framework for color-space and letter-space platforms

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VARiD: A variation detection framework for color-space and letter-space platforms

Adrian V. Dalca1,2,*, Stephen M. Rumble3, Samuel Levy4 and Michael Brudno2,5
1Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA, 2Department of Computer Science, University of Toronto, Toronto, ON, Canada, 3Department of Computer Science, Stanford University, Stanford, 4Scripps Genomic Medicine, The Scripps Research Institute, La Jolla, CA, USA and 5Donnelly Centre and the Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, Canada

ABSTRACT

Motivation: High-throughput sequencing (HTS) technologies are transforming the study of genomic variation. The various HTS technologies have different sequencing biases and error rates, and while most HTS technologies sequence the residues of the genome directly, generating base calls for each position, the Applied Biosystem’s SOLiD platform generates di-base-coded (color space) sequences. While combining data from the various platforms should increase the accuracy of variation detection, to date there are only a few tools that can identify variants from color space data, and none that can analyze color space and regular (letter space) data together.

Results: We present VARiD—a probabilistic method for variation detection from both letter- and color-space reads simultaneously. VARiD is based on a hidden Markov model and uses the forward-backward algorithm to accurately identify heterozygous, homozygous and tri-allelic SNPs, as well as micro-indels. Our analysis shows that VARiD performs better than the AB SOLiD toolset at detecting variants from color-space data alone, and improves the calls dramatically when letter- and color-space reads are combined.

Availability: The toolset is freely available at http://compbio.cs.utoronto.ca/varid

Contact: varid@cs.toronto.edu

1 INTRODUCTION

High-throughput sequencing (HTS) technologies are revolutionizing the way biologists acquire and analyze genomic data. HTS machines, such as 454/Roche, Illumina/Solexa and AB SOLiD are able to sequence up to a full human genome per week, at a cost hundreds of fold less than previous methods. The resulting data consists of reads ranging in length between 35 and 400 nt, from unknown locations in the genome. Analysis of these datasets poses an unprecedented informatics challenge due to the sheer number of reads that a single run of an HTS machine can produce, the shortness of the reads, and the various technologies’ different sequencing biases and error rates. The two basic steps in the discovery of variants in the human population from reads coming from any of these technologies are: first, the mapping of reads to a finished (reference) genome, and second the identification of variation by analysis of these mappings.

In the last few years, there have been many approaches proposed for mapping reads from HTS technologies (Campagna et al., 2009; Langmead et al., 2009; Li and Durbin, 2009; Li et al., 2008a, b, 2009; Lin et al., 2008; Rumble et al., 2009 among many others; see Fig. 1. Color-space description: Parts (a) and (b) show the correspondence between di-nucleotides and their color space representation with a translation matrix and the corresponding Finite State Automaton. In part (c), we show the effect of SNPs on the color-space representation of the read, as well as effect of sequencing errors on the trivial translation of the read from color to letter space. The first letter shown in the reads is actually the last letter of the linker, which helps to ‘lock-in’ on one of the four possible translations of a color-space read.

Dalca and Brudno, 2010; Flicek and Birney, 2009 for reviews) that utilize a wide variety of approaches. Compared to this multitude of mapping tools, there have only been a handful of toolsets for single nucleotide polymorphism (SNP) and small (1–5 bp) indel discovery. The main challenge in detecting these variants is using the error rates of the sequencing platform, the potentially incorrect mappings, and the varying coverage to determine the likelihood that a position represents a heterozygous or homozygous variant with respect to some reference genome. We use the term heterozygous to refer to the case when a single donor allele differs from the reference, and homozygous to refer to the case when both donor alleles differ from the reference, and are the same as each other. Tri-allelic SNPs, when the two donor alleles differ from each other and from the reference,
are rare. This variation detection task is further complicated by the different types of errors and data representation methods used by various technologies. For example, while the predominant error type in Illumina sequencing is the misreading of a base pair, in 454/Roche the most common mistake is insertion/deletion errors (mappping quality). Most of the tools for SNP calling analyze one base of the reference genome at a time and do not use adjacent locations to help call SNPs (positions are considered independent).

AB SOLiD’s dibase sequencing presents several unique challenges for SNP and indel identification. While typical, letter-space reads represent the DNA sequences directly as a string of A’s, C’s, G’s and T’s, and the predominant error is the miscall of a color to another, two nucleotides are read at every step of the sequencing process together as one color. Only four dyes are used for the 16 possible dibases (Fig. 1a), and the predominant error is the miscall of a color (colors are usually written as numbers 0–3). Most tools for variation detection (Li et al., 2008a, 2009; Marth et al., 1999) combine a detailed data preparation step, in which the reads are filtered, realigned and often rescored, with a nucleotide or heterozygosity calling step, typically done using a Bayesian framework. The typical parameters considered are the sequencing error rate, the SNP rate in the population (the prior) and the likelihood of misalignment (mapping quality). Most of the tools for SNP calling analyze one base of the reference genome at a time and do not use adjacent locations to help call SNPs (positions are considered independent).

In this section, we introduce our application of a HMM to the process of detecting variation from mapped reads. We begin by describing a simplified version of the model, and then describe the details of the full model and pipeline.

2 ALGORITHMS

In this section, we introduce our application of a HMM to the process of detecting variation from mapped reads. We begin by describing a simplified version of the model, and then describe the details of the full model and pipeline.

2.1 A hidden Markov model for variation detection

An HMM is a statistical model where the states of the system are hidden—that is, not observable directly—and respect a Markov progression. The observables are emissions from the hidden states. For a detailed introduction to HMMs, we refer the reader to Chapter 3 of Durbin et al. (1999). The structure of an HMM is defined in terms of the possible hidden states and the permitted transitions and hidden states and the permitted transitions between these. The model is parameterized by the emissions and transition probabilities. In the context of variation detection, we define the following HMM model (illustrated in Fig. 2):

- **States**: the unknown states in the HMM indicate the possible donor genotypes at each position in the genome. As we will model color-space, as well as letter-space data, and color-space sequencing corresponds to the change between adjacent nucleotides, the HMM will have states that correspond to pairs of consecutive positions. Overall, there are 16 possible states: [AA, AC, AG, AT, CA, ..., TG, TT], illustrated in green in Figure 2a.

- **Transitions**: as each state corresponds to a pair of nucleotides, two adjacent states will overlap by one nucleotide: for example, the state at positions (5, 6) will be followed by the state at positions (6, 7), thus sharing the nucleotide at position 6.

Some tools for color-space SNP calling first map the reads in color space by translating the reference, but then translate the multiple alignment back to nucleotide space in order to call SNPs (Li and Durbin, 2009; Li et al., 2008a). McKernan et al., 2009 describe Corona Lite, a consensus technique where each valid pair of read colors votes for an overall base call. Currently, there are no methods that can simultaneously take full advantage of both color- and letter-space data to call variants—an important, consideration since the advantages and disadvantages of the various platforms are quite disparate. By combining these data sources, it is possible to exploit the strengths of multiple HTS technologies to improve on the accuracy of current SNP callers. Here, we present VARI-D—a probabilistic approach for variant identification from either or both letter- and color-space data simultaneously. We represent both types of data as emissions from a hidden Markov model (HMM), while the underlying genotypes of the sequenced genome are the hidden states. By applying the forward–backward algorithm on the HMM we generate, for every base of the genome, a probability distribution over the possible bases. In our testing, VARI-D performs more accurately than AB’s Corona Lite pipeline for just color-space data, while its ability to incorporate letter-space data allows for more accurate determination of genomic variants using multiple read types, simultaneously.

*Fig. 2. Illustration of the simplified VARI-D HMM. In (a) emissions, states and transitions are illustrated, and in (b) we illustrated in detail how one can transition from one state to the next. Note that Y is shared in the illustration (b), and hence we can only transition from a state ending in a, say, letter A to a state starting with A.*
We show such a computation for the state CC. This example is also described in the text, see Equation (5).

Consequently the transitions are constrained so that states that end with some nucleotide $Y$ can only transition to states that start with the same nucleotide $Y$, thus forcing transitions that obey the overlap between adjacent states (Fig. 2b). Using this constraint and the frequency of each nucleotide, we define our transition probabilities:

$$P(\text{transition } p X|(Z) = \begin{cases} \text{frequency}(X) & \text{if } X = Z \\ 0 & \text{otherwise} \end{cases}$$

For example, the state (TA) will have probability of 0 to transition in any state not starting with A due to our constraint, and the probability of transition to state (AY), where $Y$ is one of \{A, C, G, T\}, is equal to nucleotide $Y$'s frequency.

- **Emissions**: given that the states of the model correspond to the donor genotypes, the emissions are the donor reads at these loci, generated by either letter- or color-space sequencing technologies (Fig. 3). The genotype state at some position $(p, p+1)$ can emit one color and one letter (we arbitrarily choose the second, $p+1$ letter as the emission). As the states overlap, the first nucleotide is emitted by the previous state. Since the emissions are (mapped) reads, and since platforms and mappers are prone to error, a state corresponding to the di-nucleotide CA will emit color 1 with high probability, although it may emit other colors with some error probability $\epsilon$. Similarly, CA will emit the letter A with high probability, but may emit other letters with some error $\xi$. We define the probability of emitting one particular color or letter $\epsilon$ from the state CA as (Fig. 4):

$$P(\text{emission} = c | \text{state} = \text{CA}) = \begin{cases} 1 - 3\epsilon & \text{if } \epsilon = 1 \\ \epsilon & \text{if } \epsilon = 0, 2 \text{ or } 3 \end{cases}$$

$$q(c | \text{CA}) = \begin{cases} 1 - 3\epsilon & \text{if } \epsilon = 1 \\ \epsilon & \text{if } \epsilon = 0, 2 \text{ or } 3 \end{cases}$$

- **Genotyping**: we formulate the problem of variation detection from letter- and color-space sequencing as the problem of finding the maximum likelihood state for each genotype’s position, given the emissions generated by the HMM. To obtain the most likely state at each position we use the forward–backward algorithm. This algorithm first computes, for each state, the probability of being in this state having observed all of the emissions prior to this position (forward probability), all the remaining emissions (backward probability), and the probability of starting in this state if we are to observe the forward and backward probabilities for a specific location, one gets the overall likelihood of each state at that location given all the observed emissions (Fig. 5). We detect variants by comparing the most likely state with the reference nucleotide at this position.

### 2.2 VARiD: algorithm for variation identification

In the previous subsection, we described a simplified HMM for variation detection that can use both color- and letter-space data. This
simple HMM, however, calls only a single nucleotide per position, and cannot detect events such as micro-indels or heterozygous SNPs. In this section, we describe the full VARiD variation identification algorithm, including the expanded HMM utilized to address the above shortcomings, and the use of base and mapping quality values to parameterize the emission probabilities. We also describe the post-processing methods utilized in VARiD to filter some types of spurious calls. A summary of the VARiD pipeline and model is given in Figure 6.

2.2.1 Extensions to the HMM

- **Insertions and Deletions**: In order to detect micro-indels, the model must include gaps in the state definitions. Due to the nature of color-space sequencing, the expanded model needs to maintain the last letter before the current gap was started. For example, the \( A-\rightarrow G \) subsequence, represented by the states \( \{(A-\rightarrow \), \(-\rightarrow \), \(-G\)\}, should emit the color 2 of AG on the last state, which is accomplished by maintaining four gap types, gapA, gapC, gapG and gapT, with the rule that a gapX state can only follow the letter X or another gapX state. Thus, in addition to the 16 basic states there are also 24 gap states: 4 states (X, gapX), 4 states (gapX, gapX), and 16 states (gapX, Y), where X and Y are nucleotides \{A, C, G or T\}, giving a total of 40 states.

![Fig. 5. An example of the resulting probabilities given by the forward-backward algorithm: in this case, the state AT will be most likely and the nucleotide T will therefore be proposed.](image)

- **Heterozygous SNPs**: To allow for heterozygous variant detection, we build an expanded set of states by taking the cross-product of the state space with itself. Each state represents both alleles at a position and thus corresponds to a pair of dibases, e.g. \( (AC/AG) \) or \( (A/TG) \). After expanding the states for indels and diploid states, there are a total of \( 4^2 = 1600 \) states in the HMM. Similar to the transition probabilities above, only a small fraction of the possible transitions are allowed: states where the second nucleotides in the two alleles are A and G, for example, can only transition to states where the first nucleotides are A and G and the transition probabilities in such cases are based on nucleotide frequencies. An example of resulting states and transitions is shown in Figure 7a.

- **Emission probabilities**: While the simple model described above used constant errors \( \epsilon \) and \( \xi \) to parameterize color- and letter-space emissions, respectively, in practice the error rates vary with the position in the read, and most platforms also generate a quality score for each position in the read to indicate the likelihood of error. VARiD can use both of these sources of information, either converting a quality value into an error likelihood (assuming it is on the standard Phred scale) or using pre-specified error likelihoods for every position in a read. In the results presented below we use the second approach, as in our experience with the AB SOLiD data the quality values proved less informative than the read position. The per-position error frequencies are maximum likelihood estimates obtained from the alignments of the color-space reads. For the 454 data, we use a fixed error probability of 0.5%, also inferred from the mappings.

- **First color**: The first color in a color-space read is encoded relative to the last letter of the linker that connects the DNA to the slide. This will cause the first color in a read to be different from the corresponding color in other reads, which are encoded relative to the previous DNA letter. To address this, we ‘translate through’ the first color of the read, thus obtaining the first-sequenced DNA letter, and use this letter as an emission. For example, if a read began ‘T2312…’, it will be converted to

![Fig. 6. A summary of the steps involved in the described pipeline. The purple sections are inputs, outputs or steps performed with previous software. The blue parts illustrate steps described in this manuscript.](image)
We instead supplement the current probabilistic model with a post-allele combination such as red–yellow, blue–green, although the blue–green combination is actually not present in any read. Instead of incorporating a higher order model which would incur complexity costs, we simply check the (generally few) proposed SNPs and disregard cases such as these.

2.2.2 Post-processing

The HMM that VARiD utilizes is memoryless: the information about the specific reads that generated certain letters and colors is not maintained. This leads to the possibility that a valid path through the state space is not supported by any reads. Figure 7b depicts an example that may result in a heterozygous SNP prediction: four counts of red and two counts of blue for the first position, and four yellow, and two green for the second. Red:yellow and blue:green are considered ‘valid’ adjacent color changes that typically support a SNP. In this case, however, there are no individual reads that support the blue:green combination, indicating that this combination is actually unlikely to appear in the genome and hence is unlikely to be a heterozygous variant. While the proper approach to fixing this problem would be to use a higher order HMM, this would be computationally inefficient. We instead supplement the current probabilistic model with a post-processing step, where we verify that a statistically likely fraction of the reads directly support each heterozygous SNP call. This approach is fast, as putative SNPs are rare.

2.2.3 Running time

The running time of the typical forward-backward algorithm is $O(n^2 r t)$, where $n$ is the length of the sequence and $r$ is the number of permitted transitions. When $r < k^2$, where $k$ is the number of states, in the VARiD HMM $k=1600$ and it is necessary to utilize sparse matrix operations to efficiently implement the forward-backward algorithm. Overall, the running time of VARiD is linear in the length of the genome. Furthermore, it is possible to parallelize VARiD over larger intervals by splitting the reference into smaller segments or windows, with the requirement that they be slightly overlapping. The overlapping regions can then be easily reconciled. VARiD required $\sim 4$ min on a single Intel P4 Xeon 3.2GHz machine to predict variants in the 80 kb of the human genome that we analyze in the next section.

### 3 RESULTS

To test VARiD, we utilized the dataset of Harismendy et al. (2009), who sequenced several regions of the human genome, spanning a total of 260 kb, from four individuals (NA17156, NA17275, NA17460 and NA17773), both with the AB SOLiD platform and the 454/Roche Pyrosequencer. To validate the SNP calls, the authors also resequenced 80 kb from the same regions with Sanger sequencing. From the original high-coverage datasets, we generated reduced coverage, randomly selected subsets from the individuals with different degrees of coverage. To analyze the AB SOLiD data we ran the SOLiD System Analysis Pipeline Tool (Corona Lite 4.2.2 with the 35_3 schema) on the color-space data, as well as VARiD with both the AB Pipeline mappings as well as SHRMP (Rumble et al., 2009) mappings, for all of the read subsets. For the 454 data, we ran VARiD and gigabayes (Marth et al., 1999) on the letter-space reads (using Mosaic and SHRMP as the mapping tools). Finally, we tested our prediction pipeline on various color- and letter-space subsets combined. We compared the variants called by each method with the Sanger validation set to compute the following statistics:

- Number of true positive (TP): SNPs that the predictor detects that are also in the validation set;
- Number of false positive (FP): SNPs the predictor calls variant that are not in the validation set;
- Precision: the number of true positives as a fraction of all predictions; $P = TP/(TP + FP)$;
- Recall: the fraction of true positives as a fraction of the validated SNPs; $R = TP/(TP + FN)$;
- $F^*$-measure: the harmonic mean of precision ($P$) and recall ($R$); $F^* = 2 \times P \times R / (P + R)$.

The results of our analysis are illustrated in Figures 8–10, where we present results of color space only, results of letter space only and results for combinations of the two sequence types, respectively.

In Figure 8, we present results from variation identification with VARiD and the Corona Lite SNP caller (http://www.solidssoftwarertools.com/glf/project/mapreads) using the color-space data. We ran VARiD both with the alignments produced...
by the AB pipeline for the Corona caller and with alignments generated by SHRiMP. While the results as a whole demonstrate the difficulty of calling variants from color-space data, even at high coverages, a direct comparison of the two SNP calling pipelines shows that at low-coverage (10x) VARiD outperforms the Corona pipeline when using the same set of mappings generated by AB’s own mapping tool, while at higher coverage VARiD has better precision and worse recall (and a lower F-measure). The VARiD + SHRiMP pipeline has slightly lower precision than Corona and VARiD + AB mapper, but a significantly better recall, leading to a higher F-measure score. The VARiD + SHRiMP pipeline has slightly lower precision than Corona and VARiD + AB mapper, but a significantly better recall, leading to a higher F-measure score. The VARiD + SHRiMP pipeline has slightly lower precision than Corona and VARiD + AB mapper, but a significantly better recall, leading to a higher F-measure score. 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will vary depending on the costs of the platforms and their respective accuracies.

In Figure 10, we consider the various possible coverage combinations between the AB SOLiD data and the 454/Roche. In general, the performance of VARiD on a certain coverage of color-space data can be greatly improved with just a small number of 454 reads. More concretely, comparing at cost we can look at 50x coverage of color space with 5x coverage of 454 data: when combined, we find 84% precision and 77% recall. Looking at the cost equivalent coverage of just 454 data—10x—gives 7–9% lower precision and recall. Similarly, for the cost equivalent coverage of AB SOLiD data—100x—will again perform worse. Combining the data thus shows significant improvement over predicting variation from letter or color space only.

4 DISCUSSION

The various HTS technologies that have emerged in the past few years have different data representations, advantages, biases and features. In this work, we introduced a novel probabilistic framework for variation identification, which can use both letter- and color-space data simultaneously. We have shown in our results that when using only color-space data—a data type for which very few genomic analysis tools exist—the model outperforms the AB SOLiD toolkit Corona Lite, and performs on par with gigaBayes predictions for letter-space data alone. More importantly, when the color- and letter-space data are combined, the VARiD framework allows for a significant performance increase, demonstrating that a method that can take into consideration multiple technologies, combining their different advantages and compensating for their different weaknesses can achieve higher accuracy variant predictions than are possible from any single data type.

ACKNOWLEDGEMENTS

We thank Adrian Dalca Sr for help with the implementation.

Funding: National Sciences and Engineering Research Council (NSERC) of Canada; Mathematics of Information Technology and Complex Systems (MITACS) grant; Life Technologies (Applied Biosystems).

Conflict of Interest: none declared.

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