freetlis: an efficient basecaller with calibrated quality scores for Illumina sequencers

Gabriel Renaud, Martin Kircher†, Udo Stenzel and Janet Kelso*
Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, D-04103 Leipzig, Germany
Associate Editor: Alfonso Valencia

ABSTRACT

Motivation: The conversion of the raw intensities obtained from next-generation sequencing platforms into nucleotide sequences with well-calibrated quality scores is a critical step in the generation of good sequence data. While recent model-based approaches can yield highly accurate calls, they require a substantial amount of processing time and/or computational resources. We previously introduced Ibis, a fast and accurate basecaller for the Illumina platform. We have continued active development of Ibis to take into account developments in the Illumina technology, as well as to make Ibis fully open source.

Results: We introduce here freetlis, which offers significant improvements in sequence accuracy owing to the use of a novel multiclass support vector machine (SVM) algorithm. Sequence quality scores are now calibrated based on empirically observed scores, thus providing a high correlation to their respective error rates. These improvements result in downstream advantages including improved genotyping accuracy.

Availability and implementation: Freetlis is freely available for use under the GPL (http://bioinf.eva.mpg.de/freetlis/). It requires a Python interpreter and a C++ compiler. Tailored versions of LIBOCAS and LIBLINEAR are distributed along with the package.

Contact: kelso@eva.mpg.de

Supplementary information: Supplementary data are available at Bioinformatics online.

Received on November 5, 2012; revised on February 8, 2013; accepted on March 1, 2013

1 INTRODUCTION

A crucial step in the Illumina sequencing pipeline is basecalling: the generation of individual nucleotide sequences and associated quality scores, which measure the probability of a sequencing error, from raw intensities. The default basecaller provided by Illumina, Bustard, develops a model from the raw intensities and uses it to perform basecalling.

Alternative basecallers aimed at achieving a better performance than Bustard have been proposed (Whiteford et al., 2009). These basecallers can be divided into those that apply a modeling strategy like Bustard (naiveBayescall, Kao et al., 2009 or see Das and Vikalo, 2012 for a faster implementation) and All your Base (AYB) (Massingham and Goldman, 2012) and those that rely on supervised learning approaches (Ibis, Kircher et al., 2009) or intermediate approaches (Altacyclic, Erlich et al., 2008).

We introduce an update to our basecaller Ibis. FreeIbis replaces the restricted license SVM library with LIBOCAS (Franc and Sonnenburg, 2009), which is released under the GNU Public License. Our results show that freeIbis outperforms the previous version of our software in terms of sequence accuracy. We measured how the decision score of the SVM corresponded to the observed error rate as measured by the number of mismatches for each predicted quality score of control reads to their respective genome. A function approximating this distribution is then used to assign quality scores for individual bases. The resulting scores show a high level of correlation between their observed error rate and the predicted one, thus obviating the need for quality score recalibration as a post-processing step (McKenna et al., 2010). We compare the newest versions of freeIbis and Ibis against the default basecaller for two Genome Analyzer II (GA) runs, a HiSeq run and a MiSeq run. On a set of DNA sequences genotyped using both Sanger and Illumina sequencing technologies, freeIbis provides an improvement in genotype accuracy over the default Illumina basecaller.

2 METHODS

The performance and accuracy of a number of freely available SVM libraries for basecalling were evaluated on a control lane of 51 cycles from a φX174 reference strain (sequence provided by Illumina Inc.) sequenced on a GAII.

An examination of our training data, built using φX174 control sequences, revealed that numerous mislabelled training examples (i.e. intensities representing a certain base but labelled as another) were present and could be attributed to two types of artefacts: genuine sequence errors and divergent bases in the control genome population. To eliminate the effects caused by the latter, a masking procedure for these positions on the genome of the organism used as control was devised. Any training example from a position with a mismatch to a given nucleotide with >10% of its coverage was removed.

As the divergent bases on the φX174 were masked, we sought to measure whether the posterior probabilities of the SVM corresponded with the observed error rate. However, standard implementations of the SVM algorithm do not output posterior probabilities but decisions values for each hyperplane. We implemented a method to convert these values into actual base quality scores (see Supplementary Methods). Alignments were performed using BWA version 0.5.8 a (Li and Durbin, 2009) with default parameters.

3 RESULTS

We compared freeIbis with the masking disabled to the most recent version of Ibis on the aforementioned run containing 200 000 sequences from a φX174 control lane with a high
Fig. 1. Plot of the predicted versus the observed base quality score for control reads. Ideally the base qualities should follow the diagonal line. The root mean square error (RMSE) shows that quality scores predicted using freeIbis have a greater correlation to their observed error rates.

Table 1. Accuracy for each basecaller on a Illumina GAIIx dataset (2 × 126 cycles with 366 135 257 clusters)

| Basecaller   | Training time | Calling time | Mapped (%) | Edit distance |
|--------------|---------------|--------------|------------|---------------|
| Bustard      |               |              | 583 348 201 (83.93%) | 1.379         |
| naiveBayesCall | 591 h       | 658 h        | 578 957 145 (83.34%) | 1.496         |
| AYB          | 394 h         |              | 593 183 967 (85.52%) | 1.076         |
| Ibis         | 19.4 h        | 13.2 h       | 592 929 953 (85.31%) | 1.167         |
| freeIbis     | 21.3 h        | 12.2 h       | 594 095 219 (85.48%) | 1.145         |

The human sequences were mapped to the hg19 version of the human genome. The number of mapped sequences and the average number of mismatches for those were tallied for each method. Time trials were conducted on a machine with 74 GB of RAM and using 8 of the 12 Intel Xeon cores running at 2.27 GHz.

The root mean square error (RMSE) shows that quality scores predicted using freeIbis have a greater correlation to their observed error rates.

4 CONCLUSION

FreeIbis provides substantial improvements in sequence accuracy, quality score calibration and genotyping accuracy over Bustard, and is more computationally efficient than equally accurate model-based methods such as AYB.

ACKNOWLEDGEMENTS

We would like to thank the Bioinformatics Group, the Sequencing and the Population Genetics Group at the Max Planck Institute for Evolutionary Anthropology for providing data and feedback. We are also indebted to Vojtech Franc, Yun Song, Hazel Marsden and Tim Massingham who provided support for use of their software.

Funding: Work was funded by the Max Planck Society.

Conflict of Interest: none declared.

REFERENCES

Das,S. and Vikalo,H. (2012) Onelinecall: fast online parameter estimation and base calling for illumina’s next-generation sequencing. Bioinformatics, 28, 1677–1683.

Erllich,Y. et al. (2008) Alta-cyclic: a self-optimizing base caller for next-generation sequencing. Nat. Methods, 5, 679-682.

Franc,V. and Sonnenburg,S. (2009) Optimized cutting plane algorithm for large-scale risk minimization. J. Mach. Learn. Res., 10, 2157–2192.

Kao,W. et al. (2009) Bayescall: a model-based base-calling algorithm for high-throughput short-read sequencing. Genome Res., 19, 1884.

Kircher,M. et al. (2009) Improved base calling for the illumina genome analyzer using machine learning strategies. Genome Biol., 10, R83.

Li,H. and Durbin,R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25, 1754-1760.

Massingham,T. and Goldman,N. (2012) All your base: a fast and accurate probabilistic approach to base calling. Genome Biol., 13, R13.

McKenna,A. et al. (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res., 20, 1297–1303.

Whiteford,N. et al. (2009) Swift: primary data analysis for the illumina Solexa sequencing platform. Bioinformatics, 25, 2194-2199.