16GT: a fast and sensitive variant caller using a 16-genotype probabilistic model

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Abstract

16GT is a variant caller for Illumina whole-genome and whole-exome sequencing data. It uses a new 16-genotype probabilistic model to unify SNP and indel calling in a single variant calling algorithm. In benchmark comparisons with five other widely used variant callers on a modern 36-core server, 16GT ran faster and demonstrated improved sensitivity in calling SNPs, and it provided comparable sensitivity and accuracy for calling indels as compared to the GATK HaplotypeCaller. 16GT is available at https://github.com/aquaskyline/16GT.

Keywords

Variant calling; Bayesian model; SNP calling; Indel calling

Background

Single nucleotide polymorphisms (SNPs) and insertions and deletions (indels) that occur at a specific genome position are interdependent; i.e., evidence that elevates the probability of one variant type should decrease the probability of other possible variant types, and the probability of all possible alleles should sum to 1. However, widely-used tools such as GATK's UnifiedGenotyper [1] and SAMtools [2] use separate models for SNP and indel detection. The model for SNP calling in these two tools is nearly identical: both assume all variants are biallelic (i.e., exactly two haplotypes are present) and use a probabilistic model allowing for 10 genotypes: AA, AC, AG, AT, CC, CG, CT, GG, GT, TT. For indel calling, the GATK UnifiedGenotyper uses a model from the Dindel's variant caller [3], while SAMtools' model is from BAQ [4].

Findings

In order to detect SNPs and indels with a unified approach, we developed a new 16-genotype probabilistic model and its implementation named 16GT. Building on an idea first introduced in Luo et al. [5], 16GT uses an empirically improved model and is the first publicly available implementation. Using X and Y to denote the indels with the highest (X) and second highest (Y) support, we add 6 new genotypes (AX, CX, GX, TX, XX and XY) to the traditional 10-genotype probabilistic model. The six new genotypes include: 1) one homozygous indel (XX); 2) one reference allele plus one heterozygous indel (AX, CX, GX, TX); 3) one heterozygous SNP plus one heterozygous indel (AX, CX, GX, TX, reusing the genotypes in 2); and 4) two heterozygous indels (XY). We exclude the 5 possible combinations AY, CY, GY, TY, YY because X has higher support than Y. By unifying SNP and indel calling in a single variant calling algorithm, 16GT not only runs 4 times faster, but also demonstrates improved sensitivity in calling SNPs and comparable sensitivity in calling indels to the GATK HaplotypeCaller.

Posterior probabilities of these 16 genotypes are calculated using a Bayesian model $P(L|F) \propto P(F|L)P(L)$, where L is an assumed genotype. F refers to the observation of the 6 alleles (A, C, G, T, X, Y) at a given genome position. $P(L)$ is the prior probability of the genotype, $P(F|L)$ is the likelihood of the observed genotype. and $P(L|F)$ is the posterior probability of the genotype. The resulting genotype L_{max} is assigned to the genotype with the highest posterior probability. The distance between the highest posterior probability and the second highest posterior probability is used as a quality metric in 16GT, along with some other metrics introduced by GATK [1].

Calculating the probability of an observation F given the genotype L

To test how well an observation fits the expectation of different genotypes, we use a two-tailed Fisher's Exact Test P and use the resulting p-value as the goodness of fit. When

calculating the likelihood of a homozygous genotype, ideally we expect 100% single allele support from the observation. For example, consider genotype 'AA':

$$P(F|'\mathrm{AA}') = P_{hom}(F_A) \times P_e(F_C, F_G, F_T, F_X, F_Y)$$

where P_e is the probability of an erroneous base call.

For a heterozygous genotype, 50% support is expected for each allele in the genotype, for example consider 'CG':

$$P(F|'CG') = P_{het}(F_C, F_G) \times P_e(F_A,\ F_T, F_X, F_Y)$$

where

$$P_{hom}(F_A) = P\begin{pmatrix} F_A & F \\ (1 - P_{err})F & F \end{pmatrix}$$

$$P_{het}(F_C, F_G) = \sqrt{\prod_{i=C,G} P\begin{pmatrix} F_i & F \\ (0.5 - P_{err})F & F \end{pmatrix}}$$

$$P_e(F_A, F_T, F_X, F_Y) = P\begin{pmatrix} F_A + F_T + F_X + F_Y & F \\ P_{err} \times F & F \end{pmatrix}$$

$$F_s = \sum_{i=1}^{n} f(Q_i, M_i, s) \qquad s\epsilon\{A, C, G, T, X, Y\}$$

where s is the allele type, n is the number of reads supporting allele s, Q_i is the base quality, and M_i is the mapping quality. f is a function describing how s, Q_i and M_i change the observation:

$$f(Q_i, M_i, s) = \alpha \times \beta \times \gamma \begin{cases} \alpha = 0\ if\ M_i = 0 \\ \alpha = 1\ if\ M_i \neq 0 \\ \beta = 0\ if\ Q_i < 10 \\ \beta = 1\ if\ 10 \leq Q_i < 13 \\ \beta = 2\ if\ 13 \leq Q_i < 17 \\ \beta = 3\ if\ 17 \leq Q_i < 20 \\ \beta = 4\ if\ Q_i \geq 20 \\ \gamma = 1\ if\ s\epsilon\{A, C, G, T\} \\ \gamma = 1.375\ if\ s\epsilon\{X, Y\} \end{cases}$$

The possible reasons for an observation that does not match the reference genome are: 1) a true variant; 2) an error generated in library construction; 3) a base calling error; 4) a mapping error; and 5) an error in the reference genome. Reasons 3 and 4 are explicitly

captured in our model. For reasons 2 and 5, we include two error probabilities, P_s for SNP error and P_d for indel error. We define $P_{err}=P_s+P_d$, where P_s and P_d are set to 0.01 and 0.005, respectively. These two values were set empirically based on the observation that SNP errors are more common than indel errors in library construction and in the reference genome.

In addition, most short read aligners use a dynamic programming algorithm to enable gapped alignment, using a scoring scheme that usually penalizes gap opening and extension more than mismatch. Consequently, authentic gaps that occur at an end of a read are more likely to be substituted by a set of false SNPs or alternatively to get trimmed or clipped. Thus, we applied a coefficient γ to weight indel observations more than SNPs, in order to increase the sensitivity on indels.

Calculating the probability of the genotype L

Given 1) a known rate of single nucleotide differences between two unrelated haplotypes; 2) a known rate of single indel differences between two unrelated haplotypes; and 3) a known Transitions to Transversions ratio (Ti/Tv), the 16GT model's prior probabilities are calculated as shown in **Table 1**.

Table 1. P(L), Genotype prior probabilities for a reference allele 'A'. Hom.: homozygous. Het.: heterozygous.

L	Zygosity	Number of SNPs	Number of Indels	Number of Transversions	Prior Probability *P*(*L*)
AA	Hom.	-	-	0	1
GG	Hom.	1	0	2	θ/2*ε*ε
CC, TT	Hom.	1	0	0	θ/2
AG	Het.	1	0	1	θ*ε
AC, AT	Het.	1	0	0	θ
CG, GT	Het.	2	0	1	θ*θ/2*ε
CT	Het.	2	0	0	θ*θ/2
AX	Het.	0	1	0	ω
GX	Het.	1	1	1	ω*θ/2*ε
CX, TX	Het.	1	1	0	ω*θ/2
XX	Hom.	0	1	0	ω/2
XY	Het.	0	2	0	ω*ω/2

Given 1) a known rate θ of single nucleotide differences between two unrelated haplotypes; 2) a known rate ω of single indel differences between two unrelated haplotypes; and 3) a known Transitions to Transversions ratio (Ti/Tv) ε, where transition is expected to occur more frequently than transversion under selective pressure. The default known rates for human genome are: $\theta = 0.001$, $\omega = 0.0001$, $\varepsilon = 2.1$, where $\boldsymbol{\varepsilon}$ is set to the value for human and change between species.

Results

We benchmarked 16GT with GATK UnifiedGenotyper, GATK HaplotypeCaller [1], Freebayes [6], Fermikit [7] and ISAAC [8] using a set of very high-confidence variants developed by the Genome-in-a-bottle (GIAB) project for genome NA12878 [9] (version 2.19, Additional File 1: **Supplementary Note**). The results are shown in **Table 2**.

Table 2. Benchmark comparisons between 16GT and five other variant callers on a dataset from the Genome in a Bottle project consisting of 787M read pairs (53-fold) from genome NA12878. UG: GATK UnifiedGenotyper; HC: GATK HaplotypeCaller. FP: false positive, FN: false negative.

Caller	Time (minutes w/ 36 cores)	SNP						Indel				
		TP	FP				FN	TP	FP			FN
			Total	dbSNP 138	dbSNP 138 %	TP in Omni 2.5			Total	dbSNP 138	dbSNP 138 %	
16GT	121	2,663,179	5,346	4,220	79%	20/20	918	167,549	1,462	944	65%	3,180
UG	29	2,655,608	1,639	563	34%	15/15	8,489	163,839	624	546	88%	6,890
HC	539	2,653,684	419	143	34%	4/4	10,413	168,444	1,232	726	59%	2,285
Freebayes	52	2,655,513	724	353	49%	11/14	8,584	162,505	559	0	0%	8,224
Fermikit	45	2,567,672	2,036	509	25%	9/9	96,425	161,916	1,996	1,076	54%	8,813
ISAAC	63	2,659,438	1,115	586	53%	15/15	4,659	158,642	1,239	710	57%	12,087

For SNPs, 16GT produced the most true positive calls and the fewest false negative calls; i.e. it has the highest sensitivity among all tools. 79% of 16GT's false positive calls were also reported by dbSNP version 138, which is highest among other callers. However, we should point out that the GIAB variant set is biased towards GATK because it was primarily derived from GATK-based analyses, as reported previously [10]. As a less-biased test, we therefore assessed the false positive calls against a set of unbiased calls made by the Illumina Omni 2.5 SNP array **(**Additional File 1: **Supplementary Note)**. Among the 5,346 false positive calls for 16GT, 20 were covered by the Omni array and all 20 (100%) had the correct genotype. Although limited by the small number of measurable alleles in the Illumina Omni 2.5 SNP array, only allowing us to reassess 20 'false positive' calls as true positives, the observation that all 20 genotypes out of the 20 covered alleles are correct suggests that a number of the remaining "false positive" calls are actually correct.

For indels, 16GT produced fewer true positive calls and more false negative calls than HaplotypeCaller, but less than half as many false negative calls as UnifiedGenotyper. 65% of 16GT's false positive indels were covered by dbSNP version 138. Further investigation into the 1,462 false positive indels shows that 981 (67%) of them meet all three of the following criteria: 1) at least three reads supporting the variant; 2) at least one read

supporting both the positive and negative strands, and; 3) in over 80% of the reads that support the variant, there exists no other variant in its flanking 10bp. This suggests that some of these “false positives” might be correct, although further experimental validation would be required to confirm this suggestion. Figure 1 shows three examples where the putative false positive from 16GT is likely to be correct.

Conclusions

16GT is the firstly publicly available implementation using a 16-genotype probabilistic model for variant calling. Compared with local assembly based variant callers, 16GT provides better sensitivity in SNP calling and comparable sensitivity in indel calling. In the future, we will improve 16GT to support somatic variant detection and extend the model to support variant calling in species with more than two haplotypes.

Declarations

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Availability of data and materials

Project name: 16GT

Project homepage: https://github.com/aquaskyline/16GT

Archived version: https://github.com/aquaskyline/16GT/releases/tag/1.0

Operating system: Platform independent

Programming language: C++ and Perl

Other requirements: See GitHub page

License: GPLv3

Any restrictions to use by non-academics: None

Authors' contribution

RL, MCS and SLS conceived the study. RL developed and implemented the 16GT algorithm and benchmarked 16GT with other variant callers. RL, MCS and SLS wrote the paper. All authors have read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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