Targets

- Nucleic Acids Research published EBCall in 2013
- Bioinformatics

Introduction

Cancer develops as the result of the accumulation of somatic mutations and clonal selection of cells with mutations that confer a selective advantage on the cell. Understanding the forces that shaped the evolutionary history of a tumor, the mutations that are responsible for its growth, the rate at which mutations are occurring, or how much genetic diversity is likely present in the tumor, requires accurate variant calling, particularly at low variant allele frequency (Williams et al. 2016; Bozic, Gerold, and Nowak 2016; Williams et al. 2018). Accurate variant identification is also critical in optimizing the treatment regime for an individual patients disease (J. Ding et al. 2012; E. R. Mardis 2012; X. Chen et al. 2013; Borad et al. 2014; Findlay et al. 2016). Low frequency mutations present a significant problem for current mutation calling methods because their signature in the data is difficult to distinguish from the noise introduced by Next Generation Sequencing (NGS), and this problem increases as sequencing depth increases.

Methods for identifying true somatic mutations - i.e. variant calling - from NGS data are an active area of research in bioinformatics. The earliest widely used somatic variant callers aimed specifically at tumors, Mutect1 and Varscan2, used a combination of heuristic filtering and a model of sequencing errors to identify and score potential variants, setting a threshold for that score designed to balance sensitivity and specificity (D. C. Koboldt et al. 2012; Cibulskis et al. 2013). Subsequent research gave rise to a number of alternate variant calling strategies including haplotype based callers (Garrison and Marth 2012), joint genotype analysis (SomaticSniper, JointSNVMix2, Seurat, and CaVEMan,MuClone)(D. E. Larson et al. 2012; Roth2012a; Christoforides et al. 2013; D. Jones et al. 2016; Dorri et al. 2019), allele frequency based analysis (Strelka, MuTect, LoFreq, EBCall, deepSNV, LoLoPicker, and MuSE)(Saunders et al. 2012; Wilm et al. 2012; Shiraishi2013b;Gerstung2012; Carrot-Zhang and Majewski 2017; Fan et al. 2016), and a mixture of ensemble and deep learning methods (MutationSeq, SomaticSeq, SNooPer, and BAYSIC). All of these methods have varying levels of complexity, and some are focused on specific types of data. The one thing they all have in common is that they either implicitly or explicitly assume that the probability of a mutation occuring at a give site is proportional to the overall mutation rate, and the same at every site in the genome.

Single nucleotide substitions, i.e. simple mutations, arise in tumors at a rate and at genomic locations driven by two main processes. The first is the spontaneous accumulation of mutations that occurs in all dividing tissues, and has

a characteristic mutation signature that describes the probability of mutation in a given genomic context (Nik-Zainal et al. 2012; Ludmil B Alexandrov et al. 2015; Lee-Six et al. 2018). The second, and far more complex, process is the accumulation of mutations through exposure to mutagens or degradation - via mutation or deletion - of cellular machinery responsible for the identification and repair of damage or replication errors. Many mutagens and DNA repair mechanism defects also have highly specific mutation signatures, such that they can be identified by observing the mutations in the tumor (Alexandrov et al. 2013; Helleday, Eshtad, and Nik-Zainal 2014; Nik-Zainal et al. 2016; Kandoth et al. 2013; L. B. Alexandrov et al. 2016).

Here we present our method ....

Results

Precision - Recall

- Slightly worse in 100X WGS, and slightly better in 500X whole exomes
- Signature has small effect, similar to the way it does in ROC
- Is there an analytic argument for why this trend will continue. At 1000X will we do even better?
- this may go away as I am currently frustrated by trying to compute/understand this.

Origin of sensitivity and specificity differences

- Everything comes down to the number of variants with low alternate read count.
- This is a complicated function of sequencing depth, evolutionary history, mutation rate.

Implementation

We implement our model on top of the MuTect 1.1.7 output. MuTect1 and MuTect2 both report the log likelihood ratio of two models, one with the variant and one without, which we can directly convert to posterior odds in favor of a mutation. Other variant callers have probability models that could be converted to use the mutation signature prior, but MuTect's is most directly accessible. We use MuTect 1.1.7 rather than MuTect2 because MuTect2 also does haplotype calling and realignment, making it difficult to use with simulated data (i.e. MuTect2 does local realignment after mutations are spiked and sometimes loses true mutations as a result). We chose to run MuTect with an initial probability sufficiently low to ensure that nearly every potential variant was

evaluated and assigned a log likelihood ratio in order to have the largest possible range of true and false positive/negative variants to evaluate the performance of our algorithm. However, no sensible analysis would include exceptionally low likelihood variants, so in our results we show result ony for those potential variants which have a log likelihood ratio (TLOD) > 4, which implies log posterior odds of -2.3, i.e. very small. This adjustment does not change the results, it just makes the analysis easier and more meaningful. The algorithm processes a whole genome simulation consisting of 53 million potential variants in 2400 seconds, of which 1600 seconds are consumed reading the data into R, and 800 seconds collecting genomic contexts from the reference genome. For a whole exome with 2.3M potential variants the run time is 142 seconds, with 56 seconds to read the data and 33 seconds to collect the contexts. The portion of the algorithm that actually computes the prior is a trivial fraction of the whole process. If integrated into an already existing variant caller which is already walking the reference genome it should add no significant processing time.

Sensitivity and specificity in simulated data

In order to describe the operating characteristics of our score as a classifier compared to MuTect, we simulated NGS reads and called variants six tumor-normal pairs as described in Methods. We made three 100X whole genomes and three 500X whole exomes, with three differnent mutation spectra. Differences in performance between our method and MuTect are driven by two main factors; the concentration of the data generating mutation signature, and the fraction of the total mutations in the tumor that are at low frequency and thus near the threshold for calling.

The fraction of positive calls that are false positives grows as the threshold used to call variants goes down. In such cases precision-recall curves give a better sense of the risk/reward tradeoff between the methods in an actual variant calling situation.

Convergence of the prior to simulated target distributions.

In both whole genome whole exome simulations, the estimated mutation spectrum is very close to the simulated spectrum (Supplementary Figure1 and Figure 3). We ranked all mutations called by MuTect by their TLOD score from highest to lowest, and computed the Kullback-Leibler divergence between the prior and the target distribution as each new mutation was observed (Figure 2). In our simulations, which have high read depth, the prior converges to the target well before all mutations passed by MuTect are evaluated. The quality of the estimate increases with the number of mutations and will likely be suboptimal for low depth sequence with a small number of high confidence mutations. Convergence is

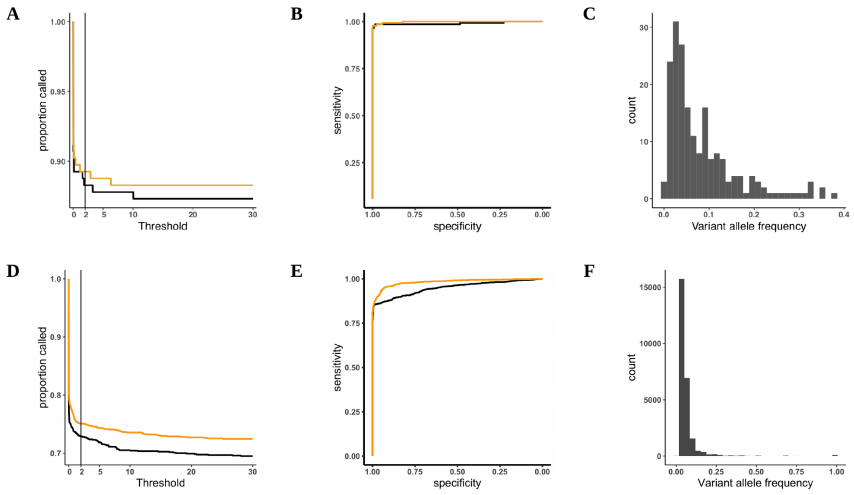

Figure 1: Sensitivity in simulated tumors. A-C) Whole exome simulation. D-F) Whole genome simulation

faster and the prior moves closer to the target distribution the more concentrated the simulated signature is. - this is what we would expect.

Sensitivity in real data

We examined two real tumor datasets in which variants had been validated by deep targeted resequencing (M. Griffith et al. 2015; W. Shi et al. 2018). M. Griffith et al. (2015) performed whole genome sequencing of an acute myeloid leukemia to a depth of ~312X, called variants with seven different variant callers and validated over 200,000 variants by targeted re-sequencing to a depth of ~1000X. This led to a platinum set of variant calls containg 1,343 SNVs. We obtained BAM files from this experiment and called variants using MuTect 1.1.7, then compared the sensitivity of the calls between MuTect and our method (Figure 1A). At any relevant threshold our method is slightly more sensitive than MuTect. MuTect is unable to recover 100% of the calls due to hueristic filtering and other differences between MuTect and the other variant callers used.

W. Shi et al. (2018) performed multi-region sequencing of 6 breast tumors to evaluate the effects of variant calling and sequencing depth on estimates of tumor heterogeneity, validating 1,385 somatic SNVs. As with the leukemia we obtained BAM files for this experiment and compared our method to raw MuTect calls (Figure 1B). We again find that our method is more sensitive than MuTect across the full range of relevant thresholds.

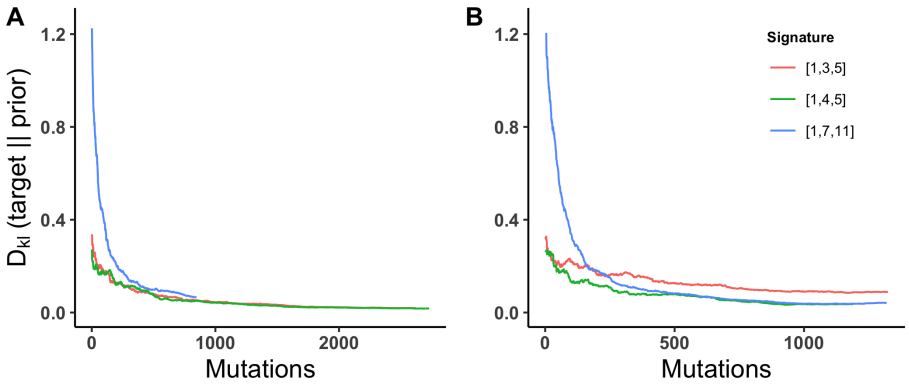

Figure 2: Convergence of the prior to simulated target mutation signatures. The prior distribution converges quickly to the target distribution, and after 200-300 mutations is as close as it will get in both A) WGS simulations and B) Whole exome simulations

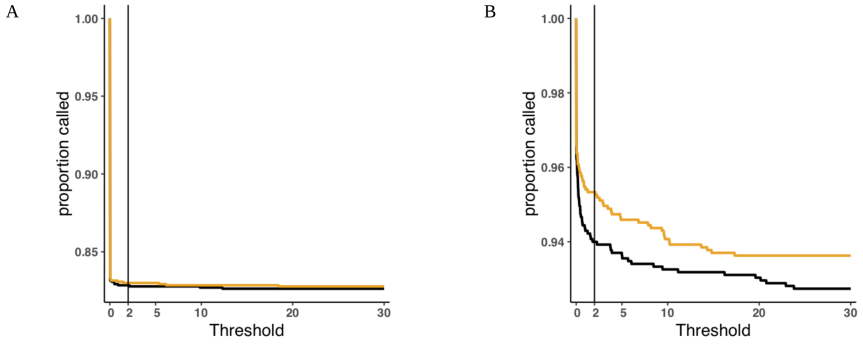

Figure 3: Sensitivity in real tumors. A) AML31 platinum SNV calls (M. Griffith et al. 2015). B) Validated SNV in 6 breast cancers(W. Shi et al. 2018).

Discussion

- Relevance to germline mutations (Rahbari et al. 2016), and somatic mutation in healthy tissue (Lee-Six et al. 2018)
- Relevance to deep learning, should at least be a feature.
- Standalone package, but approach really should be integrated into callers
- Computational efficiency if integrated
- Applicability to other algorithms for somatic variant calling
- Why are false negative rates important?
- heterogeneity
- selection inference
- rare but druggable variant identification
- Caveat: Need for better real tumor validation sets. Focus on false negatives as well as false positives. The aml31 paper gets alot of them, but if they had for instance just used mutect to identify any potential variant that passed all other heuristic filters they would have a better sense of false negative rates.
- Caveat: evolution of mutational spectrum [Rubanova2018a]

Methods

Algorithm

MuTect computes the probability of a mutation from reference allele r to base m as a function of base calls b, estimated allele frequencies f, and per base error probabilities e. The probability that a given base is correctly called can be written as

$$P(b_i \mid e_i, r, m, f) = \begin{cases} f\frac{e_{b_i}}{3} + (1-f)(1-e_{b_i}) & b_i = r \\ f(1-e_{b_i}) + (1-f)\frac{e_{b_i}}{3} & b_i = m \\ \frac{e_{b_i}}{3} & otherwise. \end{cases}$$

Now consider two models for the data. Model M_0 in which there are no variants at a site, and M_f^m where allele m is present at allele fraction f. Assuming reads are independent the likelihood of the model given the data is

$$\mathcal{L}(M_f^m) = P(\{b_i\} \mid \{e_{b_i}\}, r, m, f) = \prod_{i=1}^{d} P(b_i \mid e_{b_i}, r, m, f)$$

and the probability of M_f^m can be written

$$P(m, f \mid \{b_i\}, \{e_{b_i}\}, r) = \mathcal{L}(M_f^m)\frac{P(m, f)}{P(\{b_i\} \mid \{e_{b_i}\}, r)}.$$

We can also express this probability in terms of the model M_0

$$1 - P(m, f \mid \{b_i\}, \{e_i\}, r) = \mathcal{L}(M_0) \frac{1 - P(m, f)}{P(\{b_i\} \mid \{e_{b_i}\}, r)}.$$

Taking the log of the ratio of the two previous equations gives the log odds in favor of M_f^m, and some cancellation yields

$$LOD_T(m, f) = \log_{10} \left(\frac{\mathcal{L}(M_f^m) P(m, f)}{\mathcal{L}(M_0^m)(1 - P(m, f))} \right).$$

A classifier for variants is constructed by selecting an odds threshold δ_T and labeling variants satisfying the condition

$$LOD_T(m, f) = \log_{10} \left(\frac{\mathcal{L}(M_f^m) P(m, f)}{\mathcal{L}(M_0^m)(1 - P(m, f))} \right) \geq \log_{10} \delta_T$$

as true variants, and rejecting them otherwise. Note that the expression for $LOD_T(m, f)$ can be further factorized as the sum of the log-likelihood ratio of the two models and the log odds of the prior for M_f^m. Current variant callers calculate this prior by assuming the allele and its frequency are independent, and that $f \sim \mathrm{U}(0, 1)$, so that $P(f) = 1$. If all substitutions are equally likely, then $P(m) = \mu/3$ where $\mu = 3 \times 10^{-6}$, the estimated per-base mutation rate in tumors. Given these assumptions the log prior odds is a constant, and the classifier can be re-written as

$$LOD_T(m, f) = \log_{10} \left(\frac{\mathcal{L}(M_f^m)}{\mathcal{L}(M_0^m)} \right) \geq \log_{10} \delta_T - \log_{10} \left(\frac{P(m)}{1 - P(m)} \right) \geq \theta_T.$$

If $\delta_T = 2$, i.e the odds in favor of M_f^m is 2, then $\theta_T = 6.3$, and this is the threshold implemented in MuTect 1.

Mutect 2 was released with a threshold of 5.3, implying odds much less than 1.(I have this calculation somewhere). This is made safer by using our method?

The conditional probability that a mutation to allele m will occur given a specific genomic context C, $P(m \mid C)$ can be computed from the empirical data in Figure **??**, but $P(M \mid C)$ can not be. Using Bayes rule we can rewrite$P(m \mid C)$ as

$$P(m \mid C) = P(C \mid m) \frac{P(m)}{P(C)}.$$

Now $P(C \mid m)$ is the mutation spectrum of the tumor, $P(m) = \mu$, and $P(C)$ can be estimated as the frequency of context C in the genome. The new expression for the log odds is

$$LOD_T(m, f) = \log_{10}\left(\frac{\mathcal{L}(M_f^m)P(m \mid C)}{\mathcal{L}(M_0^m)(1 - P(m \mid C))}\right).$$

Variant allele frequency distribution

The allele frequency spectrum of a particular tumor is determined by intrinsic factors including mutation rate and the action of natural selection. In a neutrally evolving tumor the allele frequency distribution is $M(f) \approx 1/f$ (Bozic, Gerold, and Nowak 2016), which creates a roughly decreasing exponential shape on $[0, 1]$ for allele frequency. In this case we are interested in a distribution that has a realistic shape, while also providing a sufficient number of low frequency variants to challenge the algorithm. We chose a Beta(1,6) distribution for WGS simulations at 100X depth, where 20% of variants have frequency between .017 and .057 and 50% are less than .1. For WES simulations at 500X we chose a Beta(2,40) distribution where 20% of variants have frequency between .01 and .025 and 50% are less than .05.

Simulated tumors spectra

We simulated tumors with three different mutation spectra. Each is an equal mixture of three COSMIC signatures as described in Ludmil B Alexandrov et al. (2015) and downloaded from https://cancer.sanger.ac.uk/cosmic/signatures. We used mutation signatures 1, 7, and 11 to represent a highly concentrated mutation signature, signatures 1, 4, and 5 to represent intermediate concentration, and 1,3, and 5 to represent a diffuse mutation signature. - We selected mutations according to these signatures from a set of previously reported cancer mutations derived from the combined TCGA and PCAWG databases.

Simulated bam files

We simulated 100X whole genome and 500X exome normal reads from the GRCH38 reference genome with VarSim/art (Mu et al. 2015), and aligned them to GRch38 with BWA (H. Li and Durbin 2009), both with default parameters. Variants were spiked to create tumors with Bamsurgeon with default parameters (Ewing et al. 2015), and called with MuTect 1.1.7 (Cibulskis et al. 2013) with the following parameters:

```
java -Xmx24g -jar $MUTECT_JAR --analysis_type MuTect --reference_sequence $ref_path \
        --dbsnp $db_snp \
        --enable_extended_output \
        --fraction_contamination 0.00 \
        --tumor_f_pretest 0.00 \
```

```
--initial_tumor_lod -10.00 \
--required_maximum_alt_allele_mapping_quality_score 1 \
--input_file:normal $tmp_normal \
--input_file:tumor $tmp_tumor \
--out $out_path/$chr.txt \
--coverage_file $out_path/$chr.cov
```

. Variants identified by MuTect are labelled as to whether they pass all MuTect filters, pass all filters *other* than the evidence threshold `tlod_f_star`, or fail to pass any filter other than `tlod_f_star`. Variants that pass all filters or fail only `tlod_f_star` are then passed to {method} for prior estimation and rescoring.

Supplementary Figures

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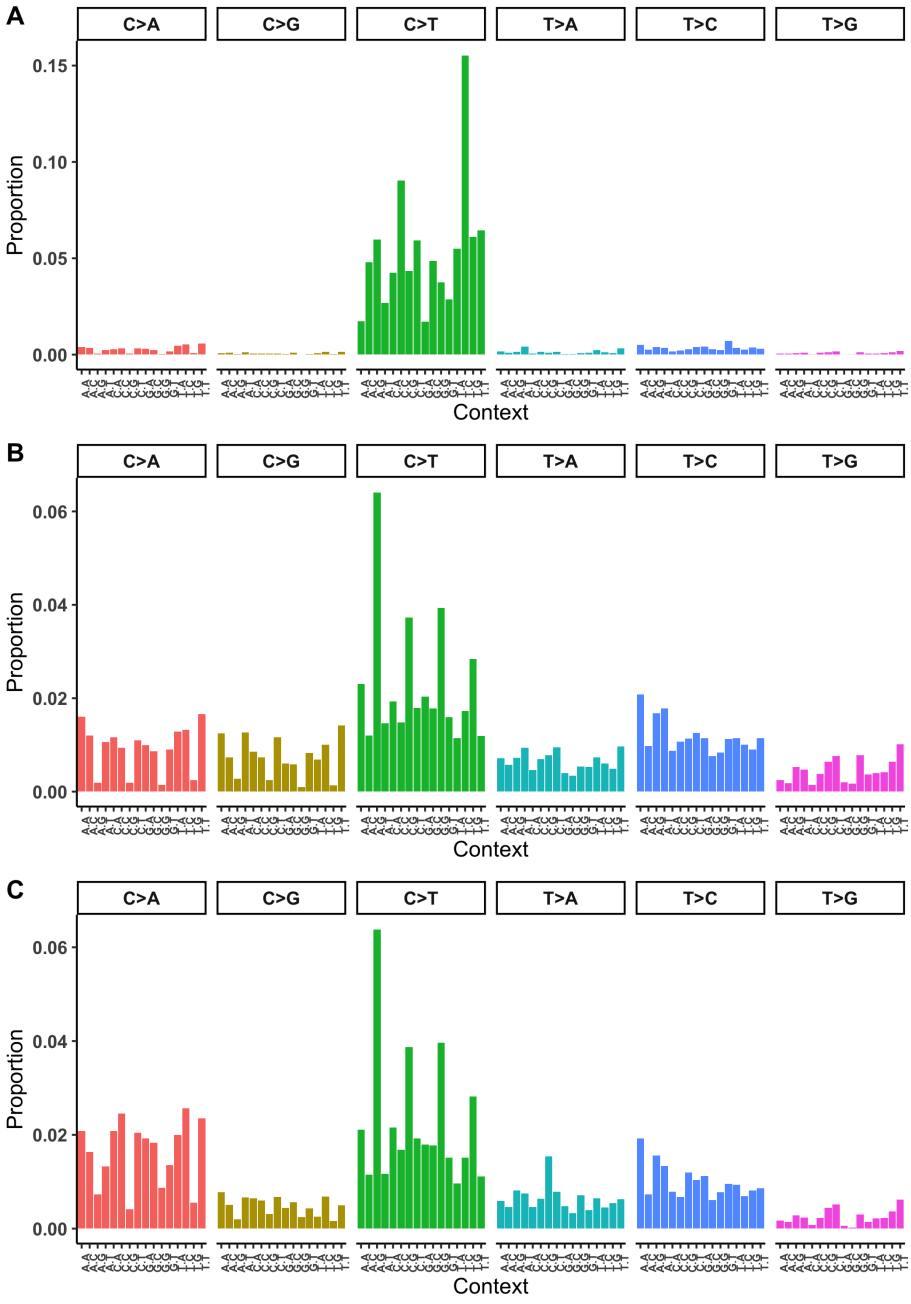

Figure 4: The signatures used to simulate both whole genome and whole exomes A) Equal combination of COSMIC signatures 1, 7, and 11 representing a highly concentrated signature of the type that might be observed in a melanoma. B) Equal combination of COSMIC signatures 1, 3, and 5 representing an intermediate level of concentration typical of a breast tumor. C) Equal combination of COSMIC signatures 1, 4, and 5 representing a diffuse signature typical of a lung tumor.

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