Mutoscope: sensitive detection of somatic mutations from deep amplicon sequencing

Shawn E. Yost1, Hakan Alakus2,3, Hiroko Matsui2,3, Richard B. Schwab4,5, Kristen Jepsen2,3, Kelly A. Frazer2,3,4,6,7 and Olivier Harismendy2,3,4,6,*

1Bioinformatics Graduate Program, 2Department of Pediatrics, 3Rady Children’s Hospital, 4Moores UCSD Cancer Center, 5Department of Medicine, 6Clinical and Translational Research Institute and 7Institute for Genomic Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA, USA

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ABSTRACT

Summary: We present Mutoscope, a sequencing analysis pipeline specifically developed for the identification of somatic variants present at low-allelic fraction from high-throughput sequencing of amplicons from matched tumor-normal specimen. Using datasets reproducing tumor genetic heterogeneity, we demonstrate that Mutoscope has a higher sensitivity and generates fewer false-positive calls than tools designed for shotgun sequencing or diploid genomes.


Contact: oharismendy@ucsd.edu

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1 INTRODUCTION

The accurate detection of somatic mutations in tumors is critical for precise diagnostic and selection of targeted therapies (Boyd, 2013), but the low-allelic fraction frequently encountered in heterogeneous or poor cellularity clinical specimens renders this task challenging. In current clinical assays, amplicons covering the exons of 10–100 cancer genes are amplified via polymerase chain reaction-based or analogous approaches and sequenced at high depth to identify mutations. Although experimental (Hiatt et al., 2009), can hinder the detection of mutations. Despite high coverage depth, the error rate resulting from systematic sequencing bias (Harismendy et al., 2009), can hinder the detection of mutations. Although experimental (Hiatt et al., 2013) or analytical (McKenna et al., 2010) methods, or comparison with the normal DNA (Cibulskis et al., 2013; Koboldt et al., 2012) can mitigate this effect, most analysis strategies were developed for sequencing of random shotgun DNA fragments, and thus do not take into account systematic errors specific to amplicon sequencing. In amplicon sequencing, loci are covered by reads with identical genomic starting positions, and because the error rate increases along the length of the read (Fig. 1a), a variable consensus error rate exists over the target (Fig. 1b). Analytical strategies specifically designed for amplicon sequencing have the potential to enhance the mutation detection accuracy of current clinical assays, especially at low-allelic fraction.

Here, we present Mutoscope, a software dedicated to the detection of mutations at low-allelic fraction from amplicon sequencing of matched tumor-normal samples pairs. Mutoscope determines the amplicon of origin for each read and measures the specific experimental error rate from sequencing the normal DNA. The mutations in the tumor are then identified by comparison with the error rate using a binomial statistics and classified as germ line or somatic by comparison with the normal DNA. A set of filters adapted to amplicon sequencing then eliminates false-positive calls. We used two experimental datasets, a mixture of 8 normal DNA (MIX) and a set of 80 tumor-normal spiked-in (TNS) pairs derived from 38 different normal germ line DNA samples to measure the performance of the approach in comparison with other mutation callers.

2 METHODS

Data generation: The data from the MIX sample or used in the preparation of the TNS pairs was generated using microdroplet polymerase chain reaction amplification (Harismendy et al., 2011) of 1736 amplicons from 47 genes clinically actionable for breast cancer (Supplementary Methods), followed by high-throughput sequencing of 151 nt long paired-end reads on a MiSeq sequencer (Illumina, San Diego, CA, USA) resulting in 981-fold average coverage depth. The data are available through the Short Reads Archive (SRA) at the NCBI (SRA067609 and SRA067610).

Analysis principle: Mutoscope aligns the reads to the genome using BWA-SW algorithm (Li and Durbin, 2009). Multi-mapping reads, reads with a low Smith–Waterman score, or not aligning to the specified amplicons are removed. Mutoscope then determines the amplicon of origin for each read and measures the error rate using the normal DNA sequencing, stratified by the major drivers of sequencing errors: nucleotide, position in the read and read type (forward or reverse). The allelic fraction of the mutation is compared with this error rate using a binomial test for significance; the mutations are then classified as germ line or somatic using a Bayesian likelihood method. Finally, Mutoscope filters out false-positive variants using, for example, read group bias, low-average mutant allele quality or predicted false positive from non-specific amplification.

Benchmarking: The benchmarking was performed using ROC package (Sing et al., 2005). The prediction score used for the classification corresponded to the binomial P-value for Mutoscope, somatic P-value for VarScan (Koboldt et al., 2012), tumor Fstar LOD score for MuTect (Cibulskis et al., 2013) and VCF quality score for LoFreq, GATK and Illumina MiSeq Reporter (McKenna et al., 2010; Wilm et al., 2012). All false-negative prediction scores were set to 0. All benchmarked tools relied on the same alignment performed by Mutoscope, except for Illumina MiSeq reporter that performs its own alignment. Whenever allowed, each tool was run without extensive previous filtering to strictly compare the accuracy of the mutation detection. Complete methods are available as Supplementary Methods.
3 RESULTS

We benchmarked Mutascope against other mutation callers using sequencing data generated from a mixture of 8 normal DNA samples with known genotypes (MIX sample) resulting in ‘somatic mutations’ at variable allelic fraction. The classification of the 162 somatic mutations at low-allelic fraction (0.01–0.1) by Mutascope was more accurate to detect mutations at an allelic fraction of 0.1 rather than 0.01 (Fig. 1e), and in the former case, its performance was comparable with MuTect and superior to VarScan or LoFreq.

Finally, we tested the effect of the empirical filters applied by each tool after the classification. These filters are important to eliminate false positives resulting from unpredictable sources of error and not accounted for by the statistical model. Although Mutascope’s filters, such as the read group bias and non-specific amplification, are specifically compatible with amplicons sequencing, we adjusted the parameters of the other tools to ensure a fair comparison, such as strand bias and minimum alternate allele frequency filters. When applied to the mutations at low-allelic fraction in the MIX samples, these filters increase the sensitivity and positive predictive value (Fig. 1f and Supplementary Discussion). The set of high-confidence filters from MuTect affects the sensitivity the most. This observation highlights synergies between Mutascope’s two core statistical components: the experimentally driven mutation detection (binomial test) and tumor-normal comparison (Fisher test) resulting in a superior performance.

Therefore, by design, Mutascope specifically optimizes the mutation detection and filtering for deep amplicon sequencing. The resulting higher accurate detection of somatic mutations at low-allelic fraction increases utility in cancer molecular diagnostics.

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REFERENCES


