Genotyping somatic insertions and deletions

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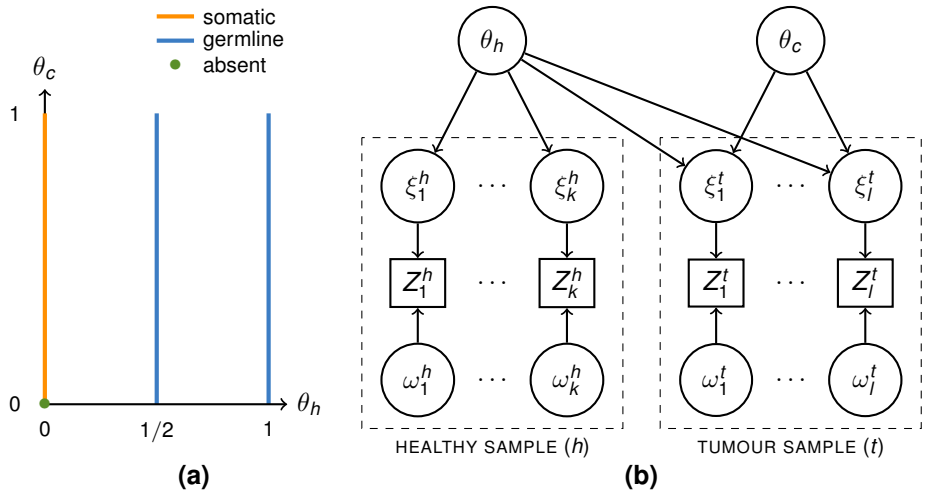
Background. Cancer is a genetic disorder in the first place; somatic mutations in the genome of an originally healthy cell allow for the maintenance of a potentially rapidly proliferating hetergeneous mix of cancer clones. This explains why in the recent past several thousands of cancer/control genome pairs have been sequenced, concerted by global consortia [7]; to date, the cancer genomes sequenced already amount to petabytes of data. The promises this massive pile of data holds for applications in precision oncology are enormous and have the potential to lead to drastically improved diagnosis and selection of therapy protocols.

While calling somatic single nucleotide variants (SNVs) can be done at both high recall and precision (e.g. [1, Mutect]), calling somatic indels has remained difficult and complex. Confounding factors, such as alignment and fragment length uncertainty, can pose substantial challenges already for germline indels. This becomes particularly disturbing for indels of length 30-150 bp (the *NGS twilight zone of indels*). In earlier work, we have shown how to resolve these issues and safely call and genotype substantial amounts of such indels in the frame of population-scale projects [4, 3, 5].

When calling and genotyping somatic indels, where genotyping refers to estimating the allele frequency of variants, cancer heterogeneity and data impurity make another confounding layer of issues. Heterogeneity and impurity of samples imply that estimating the allele frequency of somatic variants requires to appropriately quantify the inherent uncertainties. Only if this complex mix of disturbing factors has been appropriately disentangled, calling somatic indels at sufficient recall and precision is possible. Since, prior to our approach, there have been no methods to call *somatic twilight zone indels*, somatic variant databases are still virtually devoid of this type of genetic variation. Beyond this, genotyping somatic indels has also remained a substantial computational challenge in general.

Results. Here, we present a method, PROSIC (Postprocessing somatic indel calls), based on a Bayesian latent variable model (see Fig. 1) that aids in genotyping somatic indel calls while accounting for the above mentioned confounding factors of impurity, the unknown clonal structure, and alignment and typing uncertainty. Our method requires a list of potential somatic indel calls in VCF format, together with a cancer

and a matched normal BAM file as input. The output then is an annotated VCF where indel calls have been genotyped (by a VAF estimate) and been equipped with a Bayesian type a posteriori probability that the indel is somatic, as derived from the model.



(a) Genotype space. Genotypes need to be estimated for both cancer (θ_c) and control sample (θ_h). While $\theta_h \in \{0, \frac{1}{2}, 1\}$, representing absence, hetero- or homozygosity of the variant, $\theta_c \in [0, 1]$, reflecting that VAF's of somatic variants can cover the whole range due to cancer heterogeneity and impurity. **(b)**: The latent variable model, where $i \in \{1, ..., k\}, j \in \{1, ..., l\}$ index the alignments of the healthy and the cancer sample, respectively. Latent variables representing uncertainties (ω, ξ) and allele frequencies (θ_h, θ_c) are represented by circles; note that θ_h has an influence also on the cancer sample, which addresses impurity. Rectangles represent variables (Z_i^j) that can be immediately observed, such as alignment length and gaps.

We have evaluated our model on simulated data and on the datasets provided by the DREAM challenge (see `https://www.synapse.org/#!Synapse:syn312572`). We demonstrate that we can raise both recall and precision substantially, often achieving quite drastic improvements (more than 30% in recall and 30-40% in precision, reaching precision rates of 85-95%) in comparison to standard, best-practice somatic indel calling workflows provided by gold standard indel discovery methods such as Platypus [6], Pindel [8] and the HaplotypeCaller [2]. We also demonstrate that our tool compares very favorably with best practice pipelines on cancer/control cell line data. Finally, we point out ways how to substantially increase recall in the *somatic indel twilight zone* of 30-150 bp at precison rates of at least 80% which, to the best of our knowledge, is novel. The German Cancer Research Center (DKFZ) has submitted an official proposal that our tool will be integrated into the ICGC somatic indel calling pipelines to postprocess and genotype indel calls arising from the latest TCGA project (`https://tcga-data.nci.nih.gov/tcga/tcgaAbout.jsp`) on more than 2800 matched cancer/control genome pairs.

Conclusions. We present a statistical, latent variable model which allows to estimate allele frequencies of indels in matched cancer/control samples, and to derive Bayesian a posteriori probabilities for the indel calls to be somatic. In this, we take all disturbing data uncertainties, such as sample impurity, cancer heterogeneity, alignment and typing uncertainties into account, which also allows us to make good calls in relatively difficult-to-access regions of the human genome. When applying our model to indel callsets generated by gold standard indel discovery tools, we achieve substantial improvements over current best-practice workflows both in terms of recall and precision. In summary, we are providing a tool that allows to leverage ordinary, well-approved indel callers into high quality somatic indel callers. See `https://github.com/louisdijkstra/somatic-indel-calling` for software.

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