

Targets

- Bioinformatics
- Plus Comp. Bio
- BMC bioinformatics
- Nature Scientific Reports.
- Genome Biology (published MuSE (Fan et al. 2016))
- Nucleic Acids Research published EBCall in 2013

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).

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 occurring at a give site is proportional to the overall mutation rate, and the same at every site in the genome.

Single nucleotide substitutions, 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
- Working on Data to see how the signature effects this

Implementation

- Justification for using MuTect 1
- Other callers probability model less accessible
- MuTect 2 realignment step made RoC generation tough
- Mutect reports log likelihood ratio, which we convert to log odds
- You can see the reduction in MuTect2 threshold as a lowering of the delta_t or as an order of magnitude increase in the mutation rate.
- The same adjustment can be applied to our method (These might be discussion items)
- Algorithm complexity and speed

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 different mutation spectra. In WES simulations the relatively smaller number of variants, and consequent lower number of very low frequency variants, causes the methods to perform similarly,

but our method is slightly more sensitive and has slightly higher AUROC than raw MuTect scores. The large number of mutations present and at low frequency in whole genome simulations provide a clearer demonstration of the benefits of the method. The portion of the ROC curve for our method is substantially higher than the curve for MuTect, and the MuTect curve is essentially linear, is due to the effect of the prior. The prior is lowering scores of false positive mutations and raising the scores of true positives in this region. (This is super inelegant{bkm}).

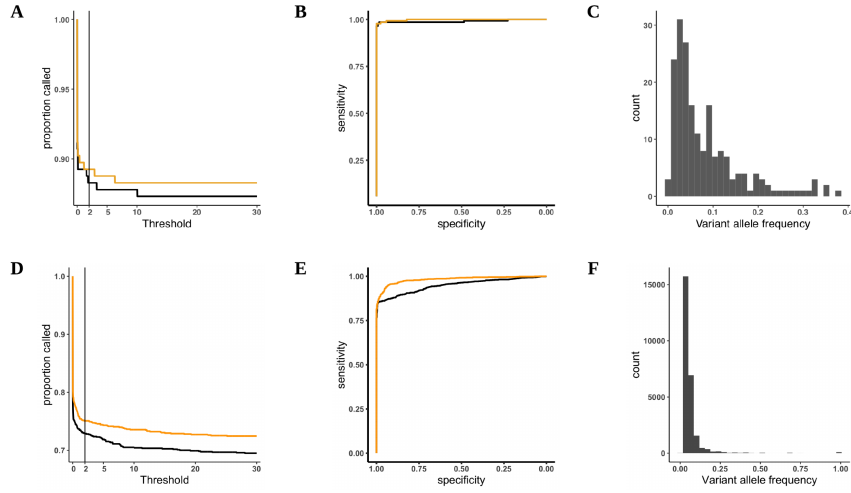


Figure 1: Sensitivity in simulated tumors. A-C) Whole exome simulation. D-F) Whole genome simulation

Convergence of the prior to simulated target distributions.

In both whole genome (Figure 3) and whole exome (supplement) simulations, the estimated mutation spectrum is very close to the simulated spectrum. The conditional probability of mutation at a given site averaged over all sites is $3e-6$ (the $P(m) = \mu$ used by MuTect; important that this is averaged over every site in the genome. The probability here includes estimates of the context content of the genome $P(m | C) = P(C | m) * P(m) / P(C)$), but our method overweights some contexts and underweights others in line with the data generating distribution. (I think I need an exome too. I have the B figure, but need to generate the C figure{bkm}) Supplementary figures for other target distributions? Or a different type of figure than we have here? Or something else? We get what we would expect with other simulated spectra. The prior is as sharp or diffuse as the data generating process.

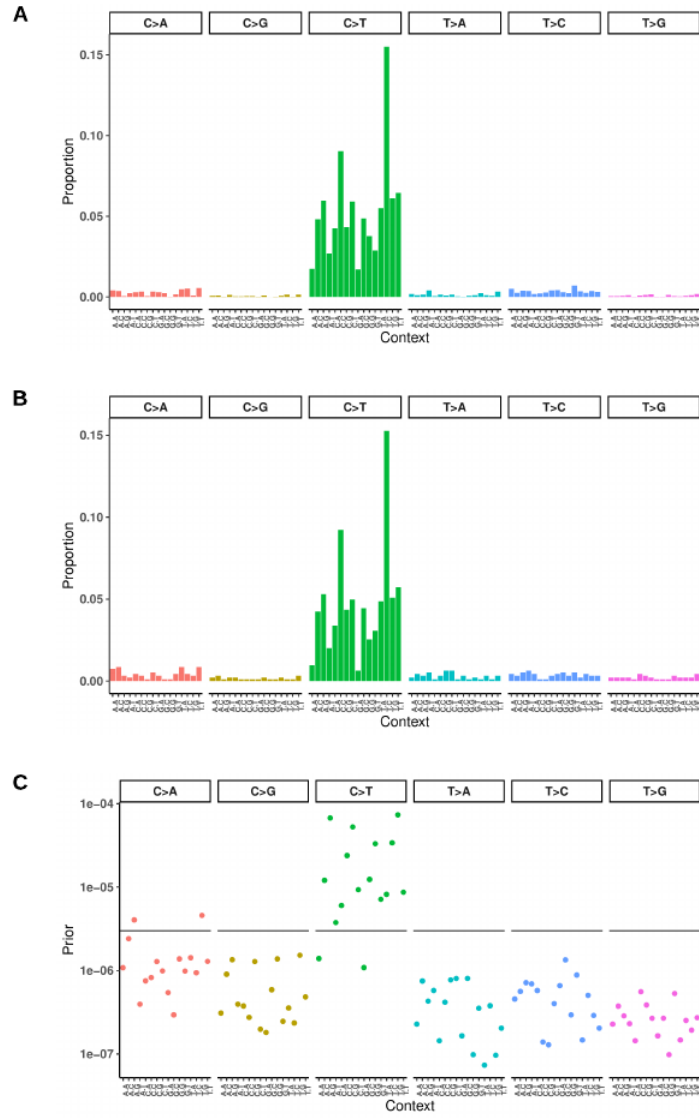


Figure 2: Prior probability of mutation estimated from high confidence calls. A) The simulated mutation spectrum (1,7,11). B) The maximum likelihood estimate of the data generating distribution (Dirichlet). C) The conditional probability of mutation at a site given its genomic context (bar at 3×10^{-6} , the global estimate of mutation rate)

- The performance of the method is always better, but the amount of benefit is directly tied to the concentration of the spectrum

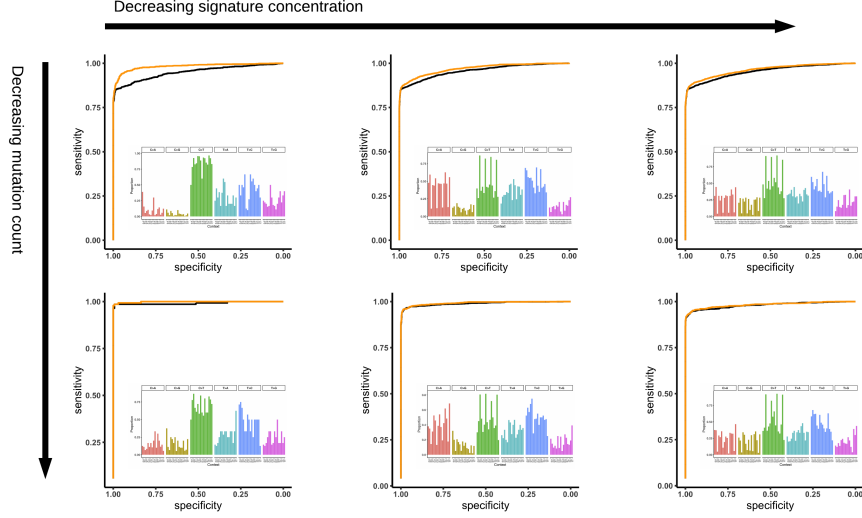


Figure 3: Effect of spectrum concentration on results in WGS. A) 1,7,11 B) 1,3,5 C) 1,4,5

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 $\sim 312\times$, called variants with seven different variant callers and validated over 200,000 variants by targeted re-sequencing to a depth of $\sim 1000\times$. This led to a platinum set of variant calls containing 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 heuristic 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.

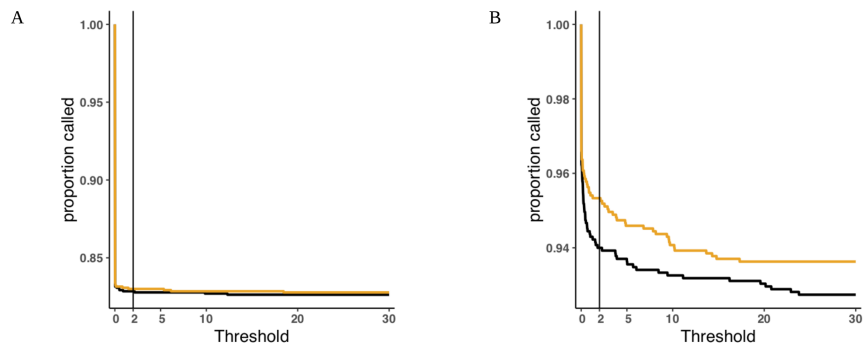


Figure 4: Sensitivity in real tumors. A) AML31 platinum SNV calls (M. Griffith et al. 2015). B) Validated SNV in 6 breast cancers(W. Shi et al. 2018).

Discussion

- Relevance to germline mutations (Rahbari et al. 2016), and somatic mutation in healthy tissue (Lee-Six et al. 2018)
- 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

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

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

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

and the probability of M_f^m can be written

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

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

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

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 $f \sim U(0, 1)$, 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 , $P(m | C)$ can be computed from the empirical data in Figure ??, but $P(M | C)$ can not be. Using Bayes rule we can rewrite $P(m | C)$ as

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

Now $P(C | m)$ 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

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

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.
- The theoretical neutral 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.
- We chose a Beta(1,6) distribution to simulate a roughly neutral evolutionary trajectory while providing a significant fraction of variants in the 1% - 5% range where discrimination is most difficult.
- 20% of variants have frequency between .017 and .057. 50% are less than .1

Simulated tumors spectra

- 100X whole genome and 500X whole exome for each of three signatures
- Real mutations from TCGA and PCAWG
- 1,7,11 UV (Very concentrated at C>T)
- 1,4,5 Tobacco (Slight concentration at C>A and C>T)
- 1,3,5 Breast (diffuse)

Simulated bam files

- 100X normal and 500X exome reads simulated with VarSim/art (Mu et al. 2015) (default parameters?) and aligned with BWA (H. Li and Durbin 2009).(default parameters)
- Variants spiked with Bamsurgeon with default parameters (Ewing et al. 2015).
- Variants called with MuTect 1.1.7 with specific parameters (Cibulskis et al. 2013). (list them, just copy in as code is what I would prefer to see if I was reading the paper).

```
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 rescoreing.

Figures

References

Alexandrov, L. B., Y. S. Ju, K. Haase, P. Van Loo, I. Martincorena, S. Nik-Zainal, Y. Totoki, et al. 2016. “Mutational signatures associated with tobacco smoking in human cancer.” *Science* 354 (6312): 618–22. doi:10.1126/science.aag0299.

Alexandrov, Ludmil B, Philip H Jones, David C Wedge, Julian E Sale, Peter J Campbell, Serena Nik-Zainal, and Michael R Stratton. 2015. “Clock-like mutational processes in human somatic cells.” *Nature Genetics* 47 (12). Nature Publishing Group: 1402–7. doi:10.1038/ng.3441.

Alexandrov, Ludmil B., Serena Nik-Zainal, David C. Wedge, Peter J. Campbell, and Michael R. Stratton. 2013. “Deciphering Signatures of Mutational

Processes Operative in Human Cancer.” *Cell Reports* 3 (1). Cell Press: 246–59. doi:10.1016/j.celrep.2012.12.008.

Borad, Mitesh J., Mia D. Champion, Jan B. Egan, Winnie S. Liang, Rafael Fonseca, Alan H. Bryce, Ann E. McCullough, et al. 2014. “Integrated Genomic Characterization Reveals Novel, Therapeutically Relevant Drug Targets in FGFR and EGFR Pathways in Sporadic Intrahepatic Cholangiocarcinoma.” *PLoS Genetics* 10 (2). doi:10.1371/journal.pgen.1004135.

Bozic, Ivana, Jeffrey M. Gerold, and Martin A. Nowak. 2016. “Quantifying Clonal and Subclonal Passenger Mutations in Cancer Evolution.” *PLoS Computational Biology* 12 (2): e1004731. doi:10.1371/journal.pcbi.1004731.

Carrot-Zhang, Jian, and Jacek Majewski. 2017. “LoLoPicker: detecting low allelic-fraction variants from low-quality cancer samples.” *Oncotarget* 8 (23): 37032–40. doi:10.1101/043612.

Chen, Xiang, Elizabeth Stewart, Anang A. Shelat, Chunxu Qu, Armita Bahrami, Mark Hatley, Gang Wu, et al. 2013. “Targeting Oxidative Stress in Embryonal Rhabdomyosarcoma.” *Cancer Cell* 24 (6). Elsevier Inc.: 710–24. doi:10.1016/j.ccr.2013.11.002.

Christoforides, Alexis, John D. Carpten, Glen J. Weiss, Michael J. Demeure, Daniel D. Von Hoff, and David W. Craig. 2013. “Identification of somatic mutations in cancer through Bayesian-based analysis of sequenced genome pairs.” *BMC Genomics* 14: 302. doi:10.1186/1471-2164-14-302.

Cibulskis, Kristian, Michael S Lawrence, Scott L Carter, Andrey Sivachenko, David Jaffe, Carrie Sougnez, Stacey Gabriel, Matthew Meyerson, Eric S Lander, and Gad Getz. 2013. “Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples.” *Nature Biotechnology* 31 (3). Nature Publishing Group: 213–19. doi:10.1038/nbt.2514.

Ding, J., A. Bashashati, A. Roth, A. Oloumi, K. Tse, T. Zeng, G. Haffari, et al. 2012. “Feature-based classifiers for somatic mutation detection in tumour-normal paired sequencing data.” *Bioinformatics* 28 (2): 167–75. doi:10.1093/bioinformatics/btr629.

Dorri, Fatemeh, Sean Jewell, Alexandre Bouchard-Côté, and Sohrab P. Shah. 2019. “Somatic mutation detection and classification through probabilistic integration of clonal population information.” *Communications Biology* 2 (1). Nature Publishing Group: 44. doi:10.1038/s42003-019-0291-z.

Ewing, Adam D, Kathleen E Houlihan, Yin Hu, Kyle Ellrott, Cristian Caloian, Takafumi N Yamaguchi, J Christopher Bare, et al. 2015. “Combining tumor genome simulation with crowdsourcing to benchmark somatic single-nucleotide-variant detection.” *Nature Methods* 12 (7). Nature Publishing Group: 623–30. doi:10.1038/nmeth.3407.

Fan, Yu, Liu Xi, Daniel S.T. Hughes, Jianjun Zhang, Jianhua Zhang, P. Andrew Futreal, David A. Wheeler, and Wenyi Wang. 2016. “MuSE: accounting for

- tumor heterogeneity using a sample-specific error model improves sensitivity and specificity in mutation calling from sequencing data.” *Genome Biology* 17 (1). Genome Biology: 178. doi:10.1186/s13059-016-1029-6.
- Findlay, John M, Francesc Castro-Giner, Seiko Makino, Emily Rayner, Christiana Kartsonaki, William Cross, Michal Kovac, et al. 2016. “Differential clonal evolution in oesophageal cancers in response to neo-adjuvant chemotherapy.” *Nature Communications* 7. doi:10.1038/ncomms11111.
- Garrison, Erik, and Gabor Marth. 2012. “Haplotype-based variant detection from short-read sequencing,” July. <http://arxiv.org/abs/1207.3907>.
- Griffith, Malachi, Christopher A. Miller, Obi L. Griffith, Kilannin Krysiak, Zachary L. Skidmore, Avinash Ramu, Jason R. Walker, et al. 2015. “Optimizing Cancer Genome Sequencing and Analysis.” *Cell Systems* 1 (3). Elsevier Inc.: 210–23. doi:10.1016/j.cels.2015.08.015.
- Helleday, Thomas, Saeed Eshtad, and Serena Nik-Zainal. 2014. “Mechanisms underlying mutational signatures in human cancers.” *Nature Reviews Genetics* 15 (9). Nature Publishing Group: 585–98. doi:10.1038/nrg3729.
- Jones, David, Keiran M. Raine, Helen Davies, Patrick S. Tarpey, Adam P. Butler, Jon W. Teague, Serena Nik-Zainal, and Peter J. Campbell. 2016. “cgpCaVE-ManWrapper: Simple Execution of CaVEMan in Order to Detect Somatic Single Nucleotide Variants in NGS Data.” *Current Protocols in Bioinformatics* 56 (1). John Wiley & Sons, Ltd: 15.10.1–15.10.18. doi:10.1002/cpbi.20.
- Kandoth, Cyriac, Michael D. McLellan, Fabio Vandin, Kai Ye, Beifang Niu, Charles Lu, Mingchao Xie, et al. 2013. “Mutational landscape and significance across 12 major cancer types.” *Nature* 502 (7471). Nature Research: 333–39. doi:10.1038/nature12634.
- Koboldt, D. C., Q. Zhang, D. E. Larson, D. Shen, M. D. McLellan, L. Lin, C. A. Miller, E. R. Mardis, L. Ding, and R. K. Wilson. 2012. “VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing.” *Genome Research* 22 (3): 568–76. doi:10.1101/gr.129684.111.
- Larson, David E, Christopher C Harris, Ken Chen, Daniel C Koboldt, Travis E Abbott, David J Dooling, Timothy J Ley, Elaine R Mardis, Richard K Wilson, and Li Ding. 2012. “SomaticSniper: identification of somatic point mutations in whole genome sequencing data.” *Bioinformatics (Oxford, England)* 28 (3). Oxford University Press: 311–7. doi:10.1093/bioinformatics/btr665.
- Lee-Six, Henry, Nina Friesgaard Øbro, Mairi S. Shepherd, Sebastian Grossmann, Kevin Dawson, Miriam Belmonte, Robert J. Osborne, et al. 2018. “Population dynamics of normal human blood inferred from somatic mutations.” *Nature* 561 (7724). Nature Publishing Group: 473–78. doi:10.1038/s41586-018-0497-0.
- Li, H., and R. Durbin. 2009. “Fast and accurate short read alignment with Burrows-Wheeler transform.” *Bioinformatics* 25 (14). Oxford University Press:

1754–60. doi:10.1093/bioinformatics/btp324.

Mardis, Elaine R. 2012. “Applying next-generation sequencing to pancreatic cancer treatment.” *Nature Reviews Gastroenterology & Hepatology* 9 (8). Nature Publishing Group: 477–86. doi:10.1038/nrgastro.2012.126.

Mu, J. C., M. Mohiyuddin, J. Li, N. Bani Asadi, M. B. Gerstein, A. Abyzov, W. H. Wong, and H. Y. K. Lam. 2015. “VarSim: a high-fidelity simulation and validation framework for high-throughput genome sequencing with cancer applications.” *Bioinformatics* 31 (9). Oxford University Press: 1469–71. doi:10.1093/bioinformatics/btu828.

Nik-Zainal, Serena, Ludmil B. Alexandrov, David C. Wedge, Peter Van Loo, Christopher D. Greenman, Keiran Raine, David Jones, et al. 2012. “Mutational Processes Molding the Genomes of 21 Breast Cancers.” *Cell* 149 (5): 979–93. doi:10.1016/j.cell.2012.04.024.

Nik-Zainal, Serena, Helen Davies, Johan Staaf, Manasa Ramakrishna, Dominik Glodzik, Xueqing Zou, Inigo Martincorena, et al. 2016. “Landscape of somatic mutations in 560 breast cancer whole-genome sequences.” *Nature* 534 (7605). Nature Publishing Group: 47–54. doi:10.1038/nature17676.

Rahbari, Raheleh, Arthur Wuster, Sarah J Lindsay, Robert J Hardwick, Ludmil B Alexandrov, Saeed Al Turki, Anna Dominiczak, et al. 2016. “Timing, rates and spectra of human germline mutation.” *Nature Genetics* 48 (2). Nature Publishing Group: 126–33. doi:10.1038/ng.3469.

Saunders, Christopher T, Wendy S W Wong, Sajani Swamy, Jennifer Becq, Lisa J Murray, and R Keira Cheetham. 2012. “Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs.” *Bioinformatics (Oxford, England)* 28 (14). Oxford University Press: 1811–7. doi:10.1093/bioinformatics/bts271.

Shi, Weiwei, Charlotte K Y Ng, Raymond S Lim, Lajos Pusztai, Jorge S Reis-Filho, Christos Hatzis, Tingting Jiang, et al. 2018. “Reliability of Whole-Exome Sequencing for Assessing Intratumor Genetic Heterogeneity In Brief Article Reliability of Whole-Exome Sequencing for Assessing Intratumor Genetic Heterogeneity.” *Cell Reports* 25: 1446–57. doi:10.1016/j.celrep.2018.10.046.

Williams, Marc J, Benjamin Werner, Chris P Barnes, Trevor A Graham, and Andrea Sottoriva. 2016. “Identification of neutral tumor evolution across cancer types.” *Nature Genetics* 48 (3). Nature Research: 238–44. doi:10.1038/ng.3489.

Williams, Marc J, Benjamin Werner, Timon Heide, Christina Curtis, Chris P Barnes, Andrea Sottoriva, and Trevor A Graham. 2018. “Quantification of subclonal selection in cancer from bulk sequencing data.” *Nature Genetics* 50 (June). Springer US: 895–903. doi:10.1038/s41588-018-0128-6.

Wilm, Andreas, Pauline Poh Kim Aw, Denis Bertrand, Grace Hui Ting Yeo, Swee Hoe Ong, Chang Hua Wong, Chiea Chuen Khor, Rosemary Petric, Martin Lloyd Hibberd, and Niranjana Nagarajan. 2012. “LoFreq: A sequence-quality

aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets.” *Nucleic Acids Research* 40 (22): 11189–11201. doi:10.1093/nar/gks918.