Sequence Analysis

ABRA: improved coding indel detection via assembly based re-alignment

Lisle E. Mose[1,*], Matthew D. Wilkerson[1,2], D. Neil Hayes[1,3], Charles M. Perou[1,2] and Joel S. Parker[1,2,*]

[1]Lineberger Comprehensive Cancer Center, [2]Department of Genetics, [3]Department of Internal Medicine, Division of Medical Oncology, Multidisciplinary Thoracic Oncology Program, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

Associate Editor: Dr. Michael Brudno

ABSTRACT

Motivation: Variant detection from next generation sequencing (NGS) data is an increasingly vital aspect of disease diagnosis, treatment and research. Commonly used NGS variant analysis tools generally rely upon accurately mapped short reads in order to identify somatic variants and germline genotypes. Existing NGS read mappers have difficulty accurately mapping short reads containing complex variation (i.e. more than a single base change), thus making identification of such variants difficult or impossible. Insertions and deletions (indels) in particular have been an area of great difficulty (Mills et al., 2011; O'Rawe et al., 2013). Indels are frequent and can have substantial impact on function which makes their detection all the more imperative (1000 Genomes Project Consortium, 2010; Mills et al., 2011).

Results: We present ABRA, an Assembly Based Re-Aligner, which uses an efficient and flexible localized de novo assembly followed by global realignment to more accurately re-map reads. This results in enhanced performance for indel detection as well as improved accuracy in variant allele frequency estimation.

Availability: ABRA is implemented in a combination of Java and C/C++ and is freely available for download at https://github.com/mozack/abra.

Contact: lmose@unc.edu; parkerjs@email.unc.edu

Supplementary Information: Supplementary material is available at *Bioinformatics* online.

1 INTRODUCTION

A number of realignment or assembly methods have been proposed to overcome the alignment errors and reference bias that limit indel detection. Short Read Micro Aligner locally realigns reads to regionally assembled variant graphs (Homer and Nelson, 2010). Pindel uses a pattern growth approach to detect indels (Ye et al., 2009). Dindel realigns reads to candidate haplotypes and uses a Bayesian method to call indels up to 50bp in length (Albers et al., 2011). The Genome Analysis Toolkit (GATK)'s IndelRealigner seeks to minimize the number of mismatching bases via

local realignment (Depristo et al., 2011). Whole genome de novo assembly approaches include Fermi (Li, 2012) and Cortex Var (Iqbal et al., 2012). SOAPIndel performs localized assembly and calling on regions containing reads where only one half of a paired read is mapped (Li et al., 2012). CREST uses soft clipped reads and localized assembly to identify somatic structural variants (Wang et al., 2010). TIGRA utilizes targeted assembly to produce contigs from putative breakpoints (Chen et al., 2013). Additional proprietary localized assembly methods have been developed by Complete Genomics (Carnevali et al., 2012) and Foundation Medicine (Frampton et al., 2013).

Our newly developed tool called ABRA accepts a BAM file as input and produces a realigned BAM file as output, allowing flexibility in selection of variant calling algorithms and other downstream analysis. Global realignment allows reads that are unaligned or improperly mapped to be moved to a correct location. ABRA detects variation that is not present in the original read alignments and improves allele frequency estimates for variation that is present. ABRA can be used to enhance both germline and somatic variant detection and works with paired end as well as single end data.

2 METHODS

The ABRA algorithm consists of localized region assembly, contig building, alignment of assembled contigs and read realignment.

Localized assembly of reads is done on small genomic regions of size two kilobases or smaller. For exome or targeted sequencing, these regions roughly correspond to capture targets. For each region, a De Bruijn graph of k-mers is assembled from the input reads (Pevzner et al., 2001). K-mers containing low quality or ambiguous bases are filtered and k-mers that do not appear in at least two distinct reads are pruned from the graph, reducing the impact of sequencing errors on the assembly process.

After initial pruning of the assembled graph, the graph is traversed to build contigs longer than the original read length. There is no smoothing of the graph to remove low frequency variation as we are interested in detecting such variation. All non-cyclic paths through the graph are traversed. In cases where a cycle in the graph is observed for a given region, that region is iteratively reassembled using increasing k-mer sizes until the cycle no longer exists or a configurable maximum k-mer size is reached. As currently implemented, detection of local insertions is limited to less than

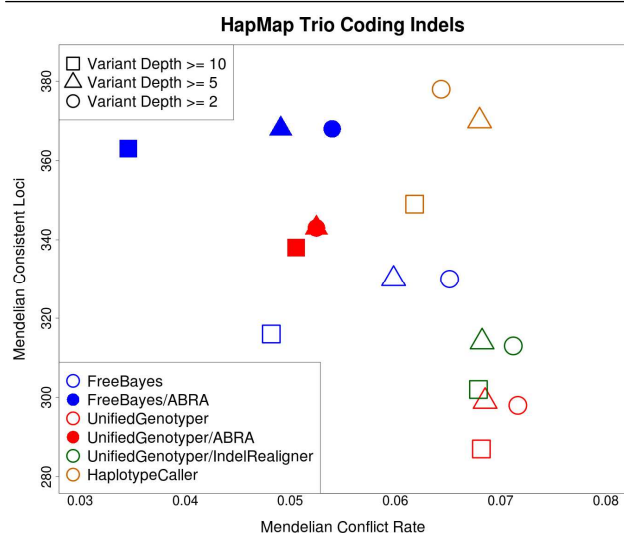---

[*]To whom correspondence should be addressed.

Fig. 1. Mendelian consistent loci and Mendelian conflict rates for Freebayes and UnifiedGenotyper both pre and post ABRA. UnifiedGenotyper results with GATK Local Alignment around Indels as well as HaplotypeCaller results are also shown for comparison. Shapes in this figure represent variant depth while color/shading represent caller and realignment method.

maximum k-mer size. Larger insertions of sequence from another location in the genome are likely to be aligned elsewhere and not included in local assembly, thus limiting detection of insertions as the size approaches read length.

Assembled contigs for all regions are aligned to the reference genome. We currently use bwa mem (Li, 2013) for contig alignment. Chimerically aligned contigs are combined when appropriate (in cases of longer indels). Redundant sequence is removed as well as sequence not varying from the original reference. The result is used as the basis for an alternate reference.

The original reads are mapped to the alternate reference using a non-gapped alignment. Reads that unambiguously align more closely to the alternate than the original reference are modified to reflect the updated alignment information in the context of the original reference.

Typical ABRA runtime for a human whole exome of depth 150X on a machine with 8 cores is roughly 2 hours using less than 16GB of RAM.

3 RESULTS

3.1 HapMap Trio

ABRA was applied to exome target regions of a CEPH Hapmap trio of three individuals sequenced to 50x as part of the Illumina Platinum Genomes project and aligned using bwa mem. Variants were called with and without ABRA using Freebayes (Garrison and Marth, 2012) and UnifiedGenotyper (DePristo et al., 2011). The GATK's HaplotypeCaller was used to call variants without ABRA and the GATK's IndelRealigner was applied to UnifiedGenotyper input. Coding indels with variant allele frequency of 20% or greater are used in this germline evaluation. ABRA enables an increase in the number of Mendelian consistent loci (MCL) detected and a decrease in Mendelian conflict rate (MCR) with either Freebayes or UnifiedGenotyper (Figure 1). The Freebayes/ABRA combination yields a decrease in MCR compared to HaplotypeCaller while remaining competitive in number of MCL detected. Pre/post ABRA concordance for Mendelian consistent SNP loci is greater than 99%. While we anticipate that ABRA will also provide improved performance in non-coding regions, this has not yet been explored.
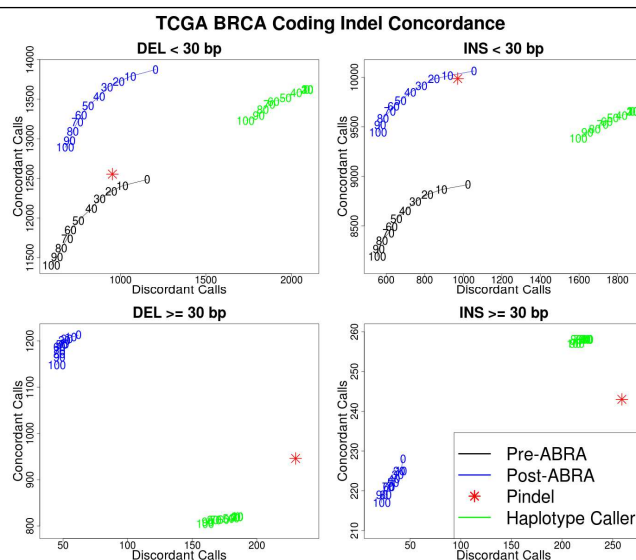

Fig. 2. Concordance / discordance with TIGRA assembled contigs for predicted calls from FreeBayes (pre and post ABRA), Pindel and Haplotype Caller. Indels within the ranges enabled by ABRA are evaluated (deletions up to 2000bp and insertions up to the read length). The numbers in the figure represent a cutoff point for variant quality scores as reported in the respective caller's VCF output. A small number of pre-ABRA deletions longer than 30bp and 0 pre-ABRA insertions longer than 30bp are called. FreeBayes currently does not use reads partially overlapping an insert as supporting evidence, which may impact post-ABRA sensitivity for longer insertions.

3.2 TCGA Tumor and Normal Data

We applied ABRA to 100 normal exomes from the Breast (BRCA) cohort of The Cancer Genome Atlas project (TCGA Network, 2012) using bwa (Li and Durbin, 2009) for the initial alignments. Germline variants were called both with and without ABRA using FreeBayes. We also called germline variants using Haplotype-Caller and Pindel for comparison purposes. To evaluate these calls in the absence of ground truth, we assembled predicted calls for all methods using TIGRA and aligned the resulting contigs with BLAT (Kent et al., 2002). ABRA increased concordance with the TIGRA/BLAT results while maintaining a low discordance rate (Figure 2). Further, ABRA generated estimated allele frequencies closer to 50 and 100%, which is expected in a diploid individual (see Supplementary Material). We next compared pre and post ABRA somatic variant calls on 750 TCGA BRCA normal/tumor exome pairs. Strelka (Saunders et al., 2012) and UNCeqR (Wilkerson et al., in press) were used for somatic calling. Improved detection of somatic mutation was observed in the post ABRA calls (see Supplementary Material).

4 CONCLUSION

ABRA improves upon NGS read alignments, providing enhanced performance in detection of indels as well as greater accuracy in variant allele frequency estimation. ABRA accepts BAM files as input and outputs realigned BAM files, allowing flexibility in downstream analysis. ABRA can be used with a variety of variant callers for both germline and somatic variant calling.

REFERENCES

1000 Genomes Project Consortium. (2010) A map of human genome variation from population-scale sequencing. *Nature.* **467**, 1061–1073.

Albers C.A., *et al.* (2010) Dindel: accurate indel calls from short-read data. *Genome Res.*, **21**, 961–973.

Chen K., *et al.* (2014) TIGRA: A Targeted Iterative Graph Routing Assembler for breakpoint assembly. *Genome Res.*, **24**, 310-317.

Carnevali P., *et al* l. (2011) Computational techniques for human genome resequencing using mated gapped reads. *J. Comput. Biol.*, **19**, 279–292.

Depristo M.A., *et al.* (2003) A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.*, **43**, 491–498.

Frampton G.M., *et al.* (2013) Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nature Biotechnology*, **31**, 1023-1031.

Garrison E., Marth G. (2012) Haplotype-based variant detection from short-read sequencing. *arXiv*, **1207**, 3907.

Homer N., Nelson S.F. (2010) Improved variant discovery through local re-alignment of short-read next-generation sequencing data using SRMA. *Genome Biol.*, **11**, R99.

Iqbal Z., *et al* (2012) De novo assembly and genotyping of variants using colored de bruijn graphs. *Nat. Genet.*, **44**, 226–232.

Kent W.J., (2002) BLAT--the BLAST-like alignment tool. *Genome Res.*, **12**, 656–664.

Li H. (2012) Exploring single-sample SNP and INDEL calling with whole-genome de novo assembly. *Bioinformatics*, **28**, 1838-1844.

Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*, **1303**, 3997.

Li H. and Durbin R. (2009) Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, **25**, 1754-1760.

Li S., *et al.* (2013) SOAPindel: efficient identification of indels from short paired reads. *Genome Res.*, **23**, 195-200

Mills R.E., *et al.* (2011) Natural genetic variation caused by small insertions and deletions in the human genome. *Genome Res.*, **21**, 830–839.

O'Rawe J., *et al.* (2013) Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing. *Genome Med.*, **5**, 28.

Pevzner P.A., *et al.* (2001) An Eulerian path approach to DNA fragment assembly. *Proc. Natl. Acad. Sci.*, **98**, 9748-9753.

Saunders C.T., et al (2012) Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics*, **28**, 1811–1817.

TCGA Network. (2012) Comprehensive molecular portraits of human breast tumours. *Nature*, **490**, 61-70.

Wilkerson M.D., et al (2012) Detecting patient mutomes by integrating DNA and RNA sequencing. *Submitted*

Ye K., et al (2009) Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*, **25**, 2865-2871.