IMPROVING VARIANT ACCURACY WITH COPY NUMBER VARIANT PIPELINE FOR TARGET SEQUENCING

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INTRODUCTION AND MOTIVATION

Studies comparing human genomes have been shown that more base pairs are altered as a result of structural variants (SVs), including copy number variants (CNVs), than as result of point mutations. Structural variants were first defined as insertions, deletions and inversions greater than 1 kb size. Nevertheless, with the high-throughput sequencing becoming a routine for genome analysis, the spectrum size of SVs and CNVs have been extended to events >50 bp in length [1].

Due to the cost and the complexity of analyzing the amount of data generated by whole-genome sequencing (WGS), targeted capture sequencing including custom gene panels and whole-exome sequencing (WES) has become the predominant approach for genetic diagnostic purposes [2]. These techniques have demonstrated huge power to identify rare and private single-nucleotide variants (SNVs) and small insertions and deletions (INDELs) for genetic diagnostic purposes, however large deletions and duplications are ignored in most of the cases because copy number variation (CNV) identification from target sequencing data is less confident compared to WGS, array comparative genomic hybridization (aCGH) and the multiple ligation probe assay (MLPA) [2, 3].

For next-generation sequencing (NGS) analysis replace theses other methods, a software or a combination of tools for exon CNV detection with high sensitivity, specificity and acceptable false discovery rate is required [4]. With efforts conducted to develop an internal protocol for CNVs detection in TS data, and increase possible genetic case elucidation, the aim of this study was to analyze the performance of state-of-the-art CNV detection methods on targeted next-generation sequencing (NGS) before implementation at our laboratory.

METHODS

High coverage targeted NGS data was generated in Genomika Diagnostics using Qiagen V3 kit. We applied CNV detection algorithms to validate the sensitivity for known CNV events identified by MLPA method. Twelve negative patients for CNV in BRCA1 and five positive patients for deletion in BRCA1 gene confirmed by MLPA were selected to be evaluated by these two softwares: ExomeDepth, and CoNVaDING. This data allowed calculation of the accuracy, precision, sensitivity and specificity for identified deletions.

Quality control were checked using FastQC on the raw sequence data from Illumina Miseq platform. Using python scripts from Qiagen, the FASTQ files were aligned on hg19 genome reference using BWA-MEM and PCR duplicate reads were removed using Picard MarkDuplicates, after that, Unique Molecular Identifier (UMI) sequences are extracted from BAM file for the variant calling. ExomeDepth and CoNVaDING uses BAM files as input, ExomeDepth authors recommend a minimum set of ten controls samples for analysis. In other hand, for CoNVaDING, it is required a minimum set of thirty possible control samples from which the samples with the most similar overall pattern are selected as control samples. We selected random forty-one samples without MLPA confirmation to be the coverage controls in our analysis.

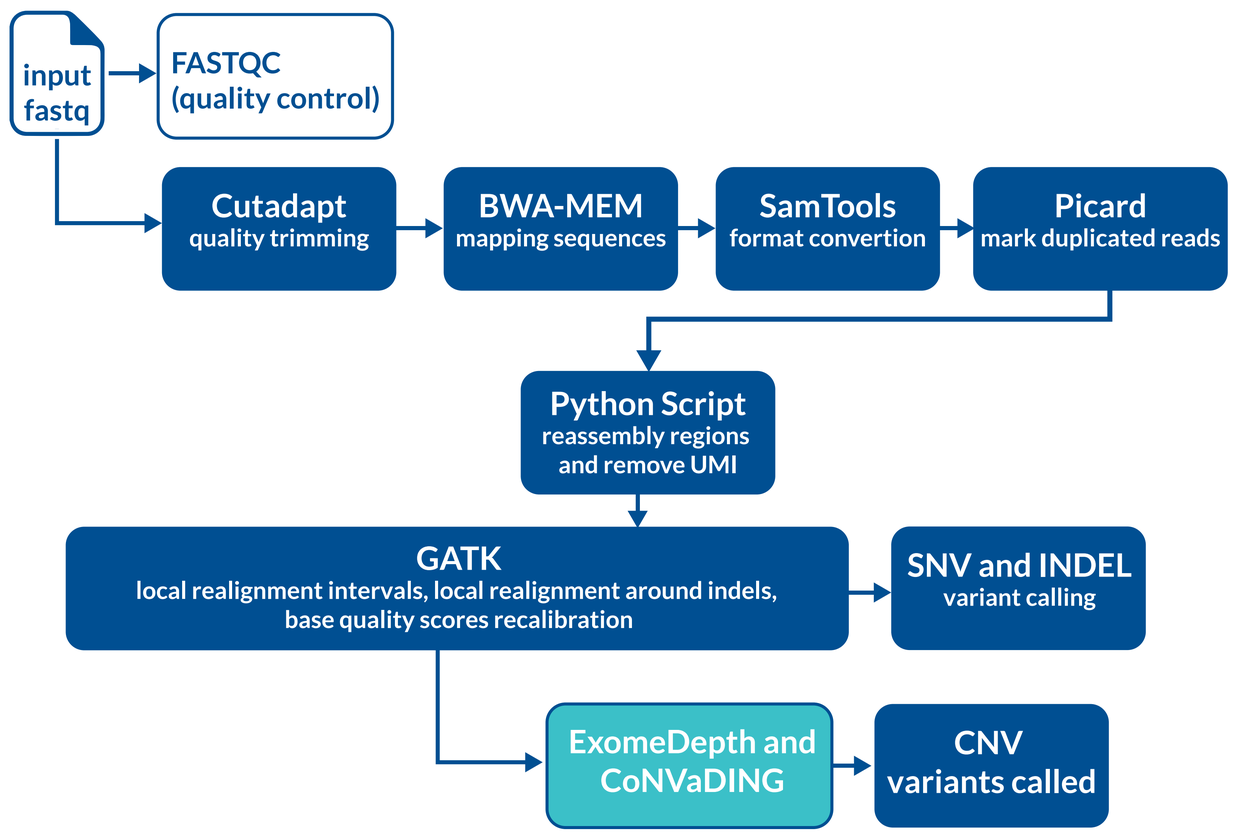
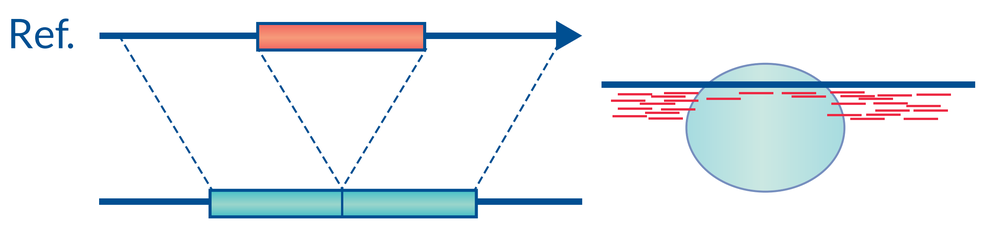
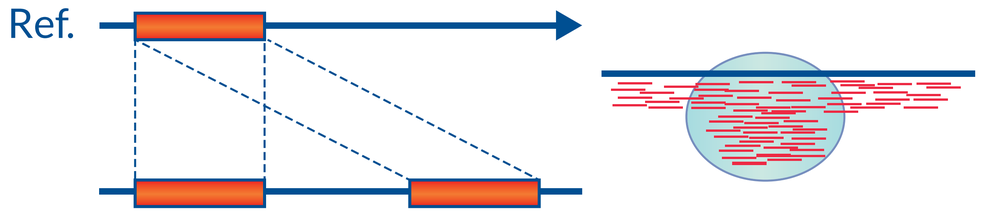


Figure 2: Pipeline workflow for target sequencing variant detection

DELETION



INTERPERSED DUPLICATION



TANDEM DUPLICATION

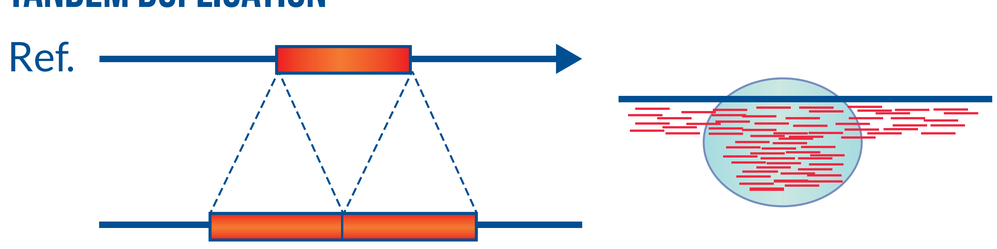


Figure 1: Classes of CNV that can be identified using read depth based softwares available for target sequencing method.

BENCHMARK

Tool	TP	TN	FP	FN	Accuracy	Precision	Sensitivity	Specificity
CoNVaDING	9	11	1	5	76,92%	90%	64,28%	91,67%
ExomeDepth	14	12	0	0	100%	100%	100%	100%

Table 1: Performance evaluation of the CNV calling tools. In this study, we used a collection of statistics derived from a confusion matrix. We counted true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) according to the number of exons (deletions or duplications) called.

CONCLUSION

The current results shows that the ExomDepth CNV caller is reasonable tool for using in production, however, according to best practices in SNV calling variants it is recommended the use of more than one variant calling software, we recommend the same approach for CNV detection. Unfortunately, we couldn't test more CNV calling softwares as VisCap, panelcn.MOPS, DECoN and SeqCNV as we planned to show here. Thereby, the plan is enhance the number of samples used to the benchmark and add to the experiments confirmed examples of large duplications to test more CNV calling tools. Nevertheless, given the results provided by our experiments, ExomeDepth might be a tool for the bioinformatics analysis routine as first step of CNV detection, and then following samples for a confirmation method as MLPA.

REFERENCES

1. Alkan, Can, Bradley P. Coe, and Evan E. Eichler. "Genome structural variation discovery and genotyping". Nature reviews. Genetics 12.5 (2011): 363.

2. Chen, Yong, et al. "SeqCNV: a novel method for identification of copy number variations in targeted next-generation sequencing data". BMC bioinformatics 18.1 (2017): 147.

3. Ellingford, Jamie M., et al. "Validation of copy number variation analysis for next-generation sequencing diagnostics". European Journal of Human Genetics 25.6 (2017): 719-724.

4. Fowler, Anna, et al. "Accurate clinical detection of exon copy number variants in a targeted NGS panel using DECoN." Wellcome open research 1 (2016).

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