

Detection of genomic structural variants from next-generation sequencing data

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Structural variants are genomic rearrangements larger than 50 bp accounting for around 1% of the variation among human genomes. They impact on phenotypic diversity and play a role in various diseases including neurological/neurocognitive disorders and cancer development and progression. Dissecting structural variants from next-generation sequencing data presents several challenges and a number of approaches have been proposed in the literature. In this mini review, we describe and summarize the latest tools – and their underlying algorithms – designed for the analysis of whole-genome sequencing, whole-exome sequencing, custom captures, and amplicon sequencing data, pointing out the major advantages/drawbacks. We also report a summary of the most recent applications of third-generation sequencing platforms. This assessment provides a guided indication – with particular emphasis on human genetics and copy number variants – for researchers involved in the investigation of these genomic events.

Keywords: next generation sequencing, structural variants, copy number variants, statistical methods, whole-exome sequencing, whole-genome sequencing, amplicon sequencing

Introduction

Structural variants (SVs) are genomic rearrangements affecting more than 50 bp. The average SV size detected by the 1000 Genomes Project is 8 kbp (1000 Genomes Project Consortium et al., 2010), whereas a study based on tiling CGH array (Conrad et al., 2010) reports a four times larger value. SVs comprise balanced as well as unbalanced events, namely, variants altering the total number of base pairs in a genome. Thus, SVs include deletions, insertions, inversions, mobile-element transpositions, translocations, tandem repeats, and copy number variants (CNVs).

Several databases – e.g., the Database of Genomic Variants archive which reports structural variation identified in healthy control samples (DGVa¹) – have been created for the collection of SVs data (Lappalainen et al., 2013). Public data resources have been developed with the purpose of supporting the interpretation of clinically relevant variants, e.g., dbVar², or collecting known disease genes (OMIM³) hit by SVs.

Structural variants account for 1.2% of the variation among human genomes while single nucleotide polymorphisms (SNPs) represent 0.1% (Pang et al., 2010). Notably, unbalanced events

¹<http://www.ebi.ac.uk/dgva>

²<http://www.ncbi.nlm.nih.gov/dbvar>

³<http://www.omim.org>

provide 99.8% of the entries reported in dbVar (Lin et al., 2014). CNVs may result in benign polymorphic variations or clinical phenotypes due to gene dosage alteration or gene disruption (Zhang et al., 2009). Though the impact of SVs in human genomics was first recognized by their presence in healthy individuals (Zhao et al., 2013), two models account for their association to human disease. Rare large events (<1%, hundreds kbp) have been related to neurological and neurocognitive disorders (Sebat et al., 2007; Girirajan et al., 2013), whereas multicopy gene families, which are commonly copy number variable, contribute to disease susceptibility.

Next-generation sequencing technologies (NGS) have been revolutionizing genome research [for a survey of NGS tools from quality check to variant annotation and visualization, see Pabinger et al. (2014)] as well as the study of CNVs (Duan et al., 2013; Zhao et al., 2013; Samarakoon et al., 2014; Tan et al., 2014; Alkodsji et al., 2015; Kadalayil et al., 2015) and SVs on the whole (Alkan et al., 2011a), replacing microarrays as the leading platform for the investigation of genomic rearrangement (Pinkel et al., 1998; Snijders et al., 2001; Iafrate et al., 2004; Sebat et al., 2007). NGS platforms are based on various implementations of cyclic-array sequencing (Shendure and Ji, 2008; Shendure et al., 2011). They allow for the sequencing of millions of short (few hundreds bp) DNA fragments (reads) simultaneously and may process a whole human genome in three days at 500-fold less cost than previous methods (Voelkerding et al., 2009; Metzker, 2010).

The 1000 Genomes Project applied methods based on all of the four approaches available for the detection of SVs, reporting false

discovery rates ranging from 10 to 89%, remarkable differences in terms of genomic regions discovered, size range, and breakpoint precision (Mills et al., 2011; Teo et al., 2012).

Overview of the Approaches

Four strategies for the detection of SV signatures that are diagnostic of different rearrangements have been reported in the literature (Figure 1; Table 1).

Read-pair (RP) methods are based on the evaluation of the span and orientation of paired-end reads. Discordant pairs in which the mapping span and/or orientation of the read pairs are inconsistent with the expected insert size are collected. Several classes of SVs can be investigated by means of this approach. Read pairs mapping too far apart are associated to deletions while those found closer than expected are indicative of insertions. Furthermore, orientation inconsistencies can represent inversions and a specific class of tandem duplications.

Read-depth (or read count, RC) approaches assume a random (Poisson or modified Poisson) distribution in mapping depth and investigate the divergence from this distribution to highlight duplications and deletions (Magi et al., 2012). Sequencing of duplicated/amplified regions results in higher read depth while deleted regions show reduced read depth when compared to normal (e.g., diploid) regions.

Split-read (SR) methods allow for the detection of SVs with single base-pair resolution. The presence of a SV breakpoint is investigated on the basis of a split sequence-read signature breaking

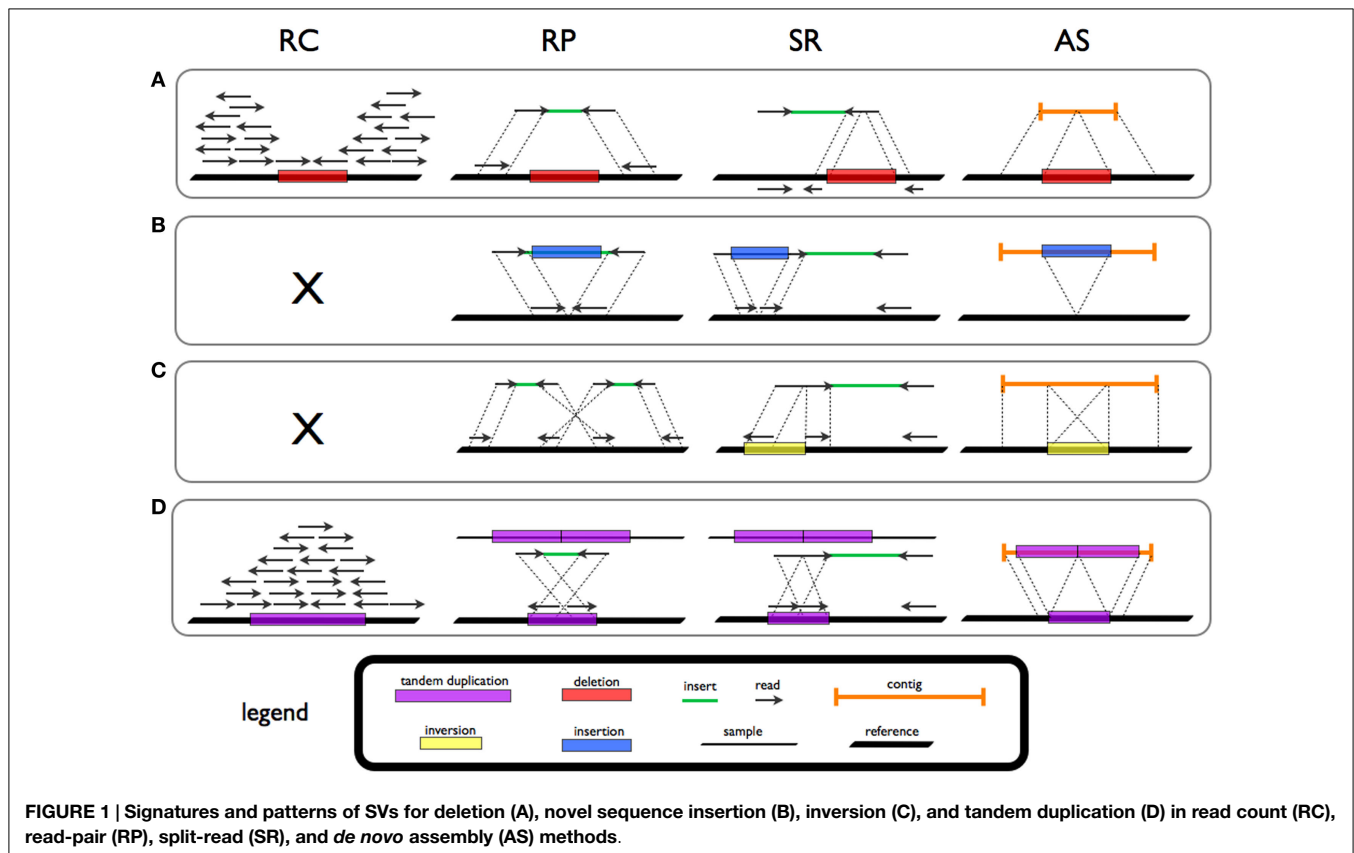


TABLE 1 | A non-exhaustive summary of the tools/algorithms for the investigation of SVs, their input data (WGS, whole-genome sequencing; WES, whole-exome sequencing; CC, custom capture; AMS, amplicon sequencing), and their underlying approach.

Tool/algorithm	Input data	Method	Reference
EXCAVATOR	WES	RC	Magi et al. (2013)
ExomeCNV	WES	RC	Sathirapongsasuti et al. (2011)
CoNIFER	WES	RC	Krumm et al. (2012)
CODEX	WES	RC	Jiang et al. (2015)
XHMM	WES	RC	Fromer et al. (2012)
–	WES/CC	RC	Bansal et al. (2014)
ONCOCNV	AMS	RC	Boeva et al. (2014)
CNVnator	WGS	RC	Abyzov et al. (2011)
SegSeq	WGS	RC	Chiang et al. (2009)
CNAnorm	WGS	RC	Gusnanto et al. (2012)
CNAseq	WGS	RC	Ivakhno et al. (2010)
rSW-seq	WGS	RC	Kim et al. (2010)
cn.MOPS	WGS	RC	Klambauer et al. (2012)
JointSLM	WGS	RC	Magi et al. (2011)
ReadDepth	WGS	RC	Miller et al. (2011)
BIC-seq	WGS	RC	Xi et al. (2011)
PSCC	WGS	RC	Li et al. (2014)
CNV-seq	WGS	RC	Xie and Tammi (2009)
CLEVER	WGS	RP	Marschall et al. (2012)
BreakDancer	WGS	RP	Chen et al. (2009)
VariationHunter	WGS	RP	Hormozdiani et al. (2011)
PEMer	WGS	RP	Korbel et al. (2009)
MoDIL	WGS	RP	Lee et al. (2009)
Gustaf	WGS	SR	Trappe et al. (2014)
Socrates	WGS	SR	Schröder et al. (2014)
Splitread	WGS/WES	SR	Karakoc et al. (2012)
Cortex	WGS	AS	Iqbal et al. (2012)
Magnolya	WGS	AS	Nijkamp et al. (2012)
Tea	WGS	DC	Lee et al. (2012)
RetroSeq	WGS	DC	Keane et al. (2013)
Tangram	WGS	DC	Wu et al. (2014)
Mobster	WGS/WES	DC	Keane et al. (2013)
SVDetect	WGS	RC + RP	Zeitouni et al. (2010)
GASVpro	WGS	RC + RP	Sindi et al. (2012)
CNVer	WGS	RC + RP	Medvedev et al. (2010)
inGAP-sv	WGS	RC + RP	Qi and Zhao (2011)
Pindel	WGS	RP + SR	Ye et al. (2009)
LUMPY	WGS	RP + SR	Layer et al. (2014)
DELLY	WGS	RP + SR	Rausch et al. (2012)
PRISM	WGS	RP + SR	Jiang et al. (2012)
MATE-CLEVER	WGS	RP + SR	Marschall et al. (2013)
NovelSeq	WGS	RP + AS	Hajirasouliha et al. (2010)
HYDRA	WGS	RP + AS	Quinlan et al. (2010)
CREST	WGS	SR + AS	Wang et al. (2011)
SVseq	WGS	RC + SR	Zhang and Wu (2011)
SoftSearch	WGS/WES/CC	RP + SR	Hart et al. (2013)
Genome STRIP	WGS	RP + SR + RC	Handsaker et al. (2011)

Methods designed using WGS data can, in principle, be used with WES data, though with limitations due to the intrinsic sparseness of WES data.

the alignment to the reference. A gap in the read is a marker of a deletion while stretches in the reference reflect insertions.

Theoretically, all forms of structural variation could be investigated by means of *de novo* assembly (AS) methods. *De novo* assembly refers to merging and ordering short fragments to reassemble the original sequence from which the short fragments were sampled (Earl et al., 2011). NGS data intrinsic characteristics, such as (short) read length, limit the use of AS approaches for variant investigation.

Moreover, a specific class of SV, mobile elements (ME) insertions, can be investigated exploiting discordant and clipped (DC) read information.

Read Count Methods

Read count is suitable for the investigation of CNVs. RC methods comprise four steps: RC data preparation, data normalization, SV regions identification, and copy number estimation. Reads mapping to windows/bins of fixed size are counted (Yoon et al., 2009; Magi et al., 2011) and the results are normalized for the mitigation of local GC content and mappability effects.

The correlation between local GC content and read coverage has been detected through the analysis of data from several platforms (Harismendy et al., 2009). Mappability bias is due to repetitive regions within the human genome (Miller et al., 2011).

A segmentation step is necessary to split RC signal into segments characterized by a constant DNA copy number. Algorithms conceived for aCGH data such as the circular binary segmentation (CBS) algorithm (Campbell et al., 2008; Miller et al., 2011) and those based on hidden Markov models (HMM) (Magi et al., 2010) are used with this scope.

Copy number estimation can be tackled by means of two strategies. Both assume that the sequencing process is uniform. Thus, the number of reads mapping to a genomic region is expected to be proportional to the number of times the regions appears in the DNA sample. Three methods (Campbell et al., 2008; Yoon et al., 2009; Magi et al., 2011) estimate DNA copy number of all the detected regions rounding the median RCs (normalized to copy number 2) to the nearest integer, while CNVnator (Abyzov et al., 2011) uses RC signal normalized to the genomic average for the regions of the same length.

A considerable number of methods for the detection of CNV in whole-genome sequencing (WGS) data have been reported in the literature, including CNVnator, CNAnorm, CNAseq, rSW-seq, cn.MOPS, JointSLM, ReadDepth, and BIC-seq (Ivakhno et al., 2010; Kim et al., 2010; Abyzov et al., 2011; Magi et al., 2011; Miller et al., 2011; Xi et al., 2011; Gusnanto et al., 2012; Klambauer et al., 2012). Recently, PSCC (Li et al., 2014) has been compared with SegSeq (Chiang et al., 2009) and ReadDepth (Miller et al., 2011).

CNV Detection from Whole-Exome Data

Due to the costs associated to WGS, the investigation of CNVs using whole-exome sequencing (WES) data is definitely an attractive perspective. Nevertheless, the sparse nature of the target and the non-uniform read-depth among captured regions make CNV detection from WES data awkward with respect to WGS [in particular, regarding the segmentation step as reported in Magi et al. (2013)].

Several tools have been reported in the literature for this purpose including ExomeCNV (Sathirapongsasuti et al., 2011), CoNIFER (Krumm et al., 2012), CNV-seq (Xie and Tammi, 2009), XHMM (Fromer et al., 2012), and recently EXCAVATOR (Magi et al., 2013) and CODEX (Jiang et al., 2015). Notably, the method developed by Bansal and co-workers (Bansal et al., 2014) allows for the analysis of NGS data generated from small subsets of the exome, namely custom capture (CC) data.

Amplicon Sequencing Data

Amplicon sequencing (AMS) techniques have been reported in the literature in particular for clinical applications (Desai and Jere, 2012; Beadling et al., 2013).

Amplicon sequencing data show different biases in respect of WES data (Boeva et al., 2014). Data normalization can be less effective due to the limited number of target regions. Furthermore, protocols involved in the preparation of amplicon libraries result in high depth of coverage at the expense of coverage homogeneity.

The first method designed for the investigation of CNV from AMS data is ONCOCNV. Duplicate sequences are not removed, while RC is performed assigning “each read to only one amplicon region, the one with which the read alignment has the maximum overlap” (Boeva et al., 2014).

Data are then normalized with respect to library size assuming a similar efficiency of PCR amplification for all the targeted regions. GC content and amplicon length biases are corrected by means of a local polynomial regression fitting. Principal component analysis (PCA) is employed to construct a baseline reflecting the technological bias in control samples. The baseline is calculated by means of the first three principal components (calculated from control samples data). In order to define a significant threshold to call a copy number change, the standard deviation of the normalized RCs for each amplicon region is calculated.

This procedure is applied to data from test samples keeping the residuals of the linear regression of normalized RCs over the baseline calculated for the control samples.

Segmentation of the resulting signal profile is performed with CBS method (Venkatraman and Olshen, 2007). A segmentation and clustering approach (SCA) is used to define the copy number state (neutral, gain, or loss) of the segmented regions.

Read-Pair Algorithms

As already mentioned, RP methods, as well as SR approaches, are suitable for the detection of several classes of SV including insertions of novel sequences and inversions. Notably, RP algorithms cannot detect the signatures of novel sequence insertions larger than the average insert size. Several tools based on the detection of SV signatures from *clusters* of read-pairs have been reported in the literature including BreakDancer, VariationHunter, PEMer, and GASV (Chen et al., 2009; Hormozdiari et al., 2009, 2011; Korbelt et al., 2009; Sindi et al., 2009). Remarkably, PEMer can be exploited for the identification of linked insertions (Medvedev et al., 2010).

Clusters can be defined according to two strategies. The standard clustering strategy relies on two parameters: the minimum number of pairs with similar signature and the maximum value of the mean insert size standard deviation for a pair to be considered concordant. The maximum standard deviation value is fixed and events spanning the same locus, resulting in a small value of the insert size standard deviation, may be missed.

Distribution-based approaches, e.g., MoDIL (Lee et al., 2009), exploit the local distribution of all the mappings spanning a particular location on the genome. A read cluster is generated when the local distribution is shifted in respect to the typical insert size distribution. This approach allows for the detection of smaller events (e.g., compared with VariationHunter). The

presence of two superimposed insert size distributions can be also detected, thus allowing for the discrimination of homozygous and heterozygous variants.

In the first implementations of the approach, e.g., BreakDancer (Chen et al., 2009), reads with multiple mappings were discarded. Thus, repetitive regions of the genome (including segmental duplications and copy-number amplifications) could not be investigated. Notably, BreakDancer allows for the identification of inter- and intra-chromosomal translocations. Tools such as MoDIL and VariationHunter or, more recently, CLEVER (Marschall et al., 2012) deal with multiple mapping reads [aligned, for instance, with mrFast (Alkan et al., 2009), Mosaik (Lee et al., 2014), BWA (Li and Durbin, 2010), or Bowtie (Langmead et al., 2009)]. CLEVER uses an insert size-based approach to build a graph with all reads and evaluates SV from maximal cliques. It is particularly well-tuned for the investigation of insertions and deletions of 50–100 bp.

Split-Read Approaches

Though SR methods were conceived for Sanger sequencing reads (Mills et al., 2006), algorithms such as Pindel, Splitread, and Gustaf (Ye et al., 2009; Karakoc et al., 2012; Trappe et al., 2014) use paired-end NGS reads to identify SVs (or indel) events. SR approaches take advantage of one-end anchored reads, namely those pairs in which “one end is anchored to the reference genome and the other end maps imprecisely owing to the presence of an underlying structural variant or indel breakpoint” (Karakoc et al., 2012). SR-based tools can be applied solely to unique reference regions.

Pindel uses pattern growth for optimal matching in target regions, exploiting reads mapped with SSAHA2 [Sequence Search and Alignment by Hashing Algorithm, Ning et al. (2001)], BWA, or Mosaik. It must be stressed that the latest version of Pindel integrates RP to the SR information (Lin et al., 2014). Splitread searches for clusters of split reads using balanced splits as seeds. Splitread can detect, at least in theory, deletions without size limitation, while for insertions the size spectrum depends on the sequencing library. Insertions shorter than the read length can be accurately identified but larger insertions can only be approximately characterized within the insert size (Karakoc et al., 2012). Splitread is suitable for WGS/WES reads aligned using mrsFAST (Hach et al., 2010) to discover indels, SVs, *de novo* events, and pseudogenes.

Recently, Socrates (a SR method designed for cancer genomics) was compared to several tools (Schröder et al., 2014), including BreakDancer, CLEVER, CREST (Wang et al., 2011), DELLY (Rausch et al., 2012), Pindel, and PRISM (Jiang et al., 2012).

Assembly Based Tools

De novo assembly allows – at least in principle – for the detection of all the forms of structural variation but the application of this approach is still challenging due to the limited length of NGS reads (Alkan et al., 2011a; O’Rawe et al., 2015).

AS methods were first exploited for Sanger sequencing data (characterized by read length between 300 and 1000 bp). The original *string graph approach* has been extended to de Bruijn graphs. The Assemblathon competition (Earl et al., 2011) produced a detailed comparison among *de novo* assemblers, including

Phusion2 (Mullikin and Ning, 2003), SGA (Simpson and Durbin, 2010, 2012), Quake (Kelley et al., 2010), the first implementation of SOAPdenovo (Li et al., 2010; Luo et al., 2012), and ALLPATHS-LG (Gnerre et al., 2011), based on simulated data.

Two AS based callers have been reported in the literature for the investigation of SVs. Magnolya (Nijkamp et al., 2012) uses a Poisson mixture model (PMM) for CNV detection from contigs co-assembled from NGS sequencing data. The authors use an overlap-layout-consensus assembler to generate a contig string graph. Contig string graphs are characterized by nodes representing reads and edges representing an overlap. The final form of the graph is produced by transitive reduction – which removes redundant edges – and by unitigging (i.e., collapsing simple paths without branches) (Myers, 2005). In the resulting contig string graph, each node represents a collapsed set of reads called *contig*. Finally, the PMM approach for modeling read count is introduced to estimate the copy number of a contig. Once the model has been corrected for the presence of repetitive regions in the genome and prior knowledge on ploidy has been included, the model with the optimal number of Poisson distributions is selected by means of the lowest Bayesian information criterion. Integer copy numbers can be thus inferred by maximum *a posteriori* estimation. Remarkably, the method can be applied when no reference is available but – as already stressed – it is limited by the short read length typical of NGS platforms.

Cortex uses colored de Bruijn graphs with colors of both edges and nodes representing different samples and, possibly, reference sequences or known variants to assemble NGS reads. “The graph consists of a set of nodes representing words of length k (k -mers). Directed edges join k -mers seen consecutively in the input” (Iqbal et al., 2012). The package includes four algorithms for variant discovery. For example, the *bubble calling* algorithm may be exploited for the detection of variant bubbles in a colored de Bruijn graph from a single diploid individual. It must be stressed that using a reference genome aids the identification of variants while it is indispensable for the investigation of homozygous variant sites. Nevertheless, the sensitivity of the method decreases with the size of the variant. The tool has been extensively tested on human data.

Combined Methods

None of the aforementioned approaches is capable of capturing the full spectrum of SV events with high sensitivity and specificity. RC methods can accurately predict absolute copy numbers but the breakpoint resolution is often inadequate and events such as inversions and novel sequence insertions cannot be detected. On the other hand, RP and SR approaches show low sensitivity in repetitive regions. Several packages combining different approaches for the investigation of SVs have been reported.

Combining RC for the detection of large events and RP for accurate identification of breakpoints can reduce the number of false positive calls [SVDetect (Zeitouni et al., 2010), CNVer (Medvedev et al., 2010), GASVPro (Sindi et al., 2012), and inGAPsv (Qi and Zhao, 2011)]. Genome STRiP (Handsaker et al., 2011) exploits RP, RC, SR, and population-scale patterns to detect genome structural polymorphisms.

Packages implementing RP and (local) AS have been also reported [NovelSeq (Hajirasouliha et al., 2010), HYDRA (Quinlan

et al., 2010)] as well as tools exploiting SR and RC/RP such as SVseq, MATE-CLEVER, and PRISM (Zhang and Wu, 2011; Jiang et al., 2012; Marschall et al., 2013). PRISM was tested on simulated data and compared with Pindel, SVseq, Splitread, and CREST. Notably, DELLY is suitable for detecting copy-number variable deletion and tandem duplication events as well as balanced rearrangements such as inversions or reciprocal translocations (Rausch et al., 2012), while SoftSearch (Hart et al., 2013) is designed for WGS, WES, and CC data. Recently, LUMPY has been shown to be “especially pronounced when evidence is scarce, either due to low coverage data or low variant allele frequency” (Layer et al., 2014). LUMPY is designed to integrate signals rather than refining primary signal with a secondary one. Furthermore, the tool combines different types of evidence from multiple samples.

Detection of Mobile Elements

Mobile elements are repetitive DNA sequences that can change position within the genome (Lander et al., 2001). Due to this intrinsic characteristic, their detection is challenging. Latest estimates suggest that more than half of the human genome is repetitive or repeat-derived (de Koning et al., 2011). Though the DC approach can be ascribed to RP and SR methods, “the mates of the anchoring reads are then mapped to a custom but configurable library of known active ME consensus sequences” (Thung et al., 2014).

Among WGS tools, Tangram (Wu et al., 2014), a tool developed using Mosaik (Lee et al., 2014) alignments (though it may use alignments produced by other mappers), Next-Generation VariationHunter (Hormozdiari et al., 2010), Tea (Lee et al., 2012), RetroSeq eKeane:2013kq, and Mobster (Thung et al., 2014) have been reported in the literature.

Conclusion

Overall, all the approaches discussed are fairly limited with respect to repeated regions of the reference genome (Alkan et al., 2009, 2011b). The complete range of structural DNA variation cannot be investigated with a single tool (Mills et al., 2011), though combined methods may aid the discovery of SV. Three pipelines integrating different tools exploiting WGS data have been reported in the literature (Wong et al., 2010; Lam et al., 2012; Mimori et al., 2013). WES data can be exploited for the investigation of SVs by means of RC, SR, and RP methods – though with limitations due to the intrinsic sparseness of exomic data.

Each method for the detection of SVs shows advantages/drawback. RC methods are particularly well-suited for the investigation of a particular class of SV, namely CNV. Notably, RC can be used to predict absolute copy number. A major drawback of RC tools is the poor breakpoint resolution. Furthermore, they cannot distinguish tandem from interspersed duplications. SR algorithms can accurately predict SV breakpoint (down to single-base resolution) as well as AS methods. Finally, the RP and SR approaches can be applied for the investigation of the widest range of SV classes (i.e., deletions, inversions, novel sequence insertions, tandem duplications), though both cannot be exploited for the calculation of absolute copy number.

The advent of third-generation sequencing (TGS) technology may contribute to overcome these issues (Schadt et al., 2010; Niedringhaus et al., 2011; Pareek et al., 2011; Venkatesan and Bashir, 2011). TGS single-end reads, characterized by read length up to thousands base pairs, may boost AS methods and the application of mapping algorithms allowing for split alignment such as BWA (Li and Durbin, 2010), LAST (Kiełbasa et al., 2011) and BLASR (Chaisson and Tesler, 2012). Though TGS platforms rely on different chemistry, reads produced by platforms, such

as PacBio RS (Kim et al., 2014) and Oxford Nanopore MinION (Bayley, 2015), show similar read length and base-calling accuracy (~85%) (Quail et al., 2012; Quick et al., 2014; Ashton et al., 2015; Chaisson et al., 2015). Recent works have demonstrated that these technologies allow for the investigation of complex repetitive regions of the human genome (Chaisson et al., 2015) as well as the structure of complex antibiotic resistance islands in *Salmonella typhi* (Ashton et al., 2015) and tandem repeats in human bacterial artificial chromosome (Jain et al., 2015).

References

- 1000 Genomes Project Consortium, Abecasis, G. R., Altshuler, D., Auton, A., Brooks, L. D., Durbin, R. M., et al. (2010). A map of human genome variation from population-scale sequencing. *Nature* 467, 1061–1073. doi:10.1038/nature09534
- Abyzov, A., Urban, A. E., Snyder, M., and Gerstein, M. (2011). Cnvnator: an approach to discover, genotype, and characterize typical and atypical cnvs from family and population genome sequencing. *Genome Res.* 21, 974–984. doi:10.1101/gr.114876.110
- Alkan, C., Coe, B. P., and Eichler, E. E. (2011a). Genome structural variation discovery and genotyping. *Nat. Rev. Genet.* 12, 363–376. doi:10.1038/nrg2958
- Alkan, C., Sajjadian, S., and Eichler, E. E. (2011b). Limitations of next-generation genome sequence assembly. *Nat. Methods* 8, 61–65. doi:10.1038/nmeth.1527
- Alkan, C., Kidd, J. M., Marques-Bonet, T., Aksay, G., Antonacci, F., Hormozdiari, F., et al. (2009). Personalized copy number and segmental duplication maps using next-generation sequencing. *Nat. Genet.* 41, 1061–1067. doi:10.1038/ng.437
- Alkods, A., Louhimo, R., and Hautaniemi, S. (2015). Comparative analysis of methods for identifying somatic copy number alterations from deep sequencing data. *Brief. Bioinform.* 16, 242–254. doi:10.1093/bib/bbu004
- Ashton, P. M., Nair, S., Dallman, T., Rubino, S., Rabsch, W., Mwaigwisya, S., et al. (2015). MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat. Biotechnol.* 33, 296–300. doi:10.1038/nbt.3103
- Bansal, V., Dorn, C., Grunert, M., Klaassen, S., Hetzer, R., Berger, F., et al. (2014). Outlier-based identification of copy number variations using targeted resequencing in a small cohort of patients with tetralogy of fallot. *PLoS ONE* 9:e85375. doi:10.1371/journal.pone.0085375
- Bayley, H. (2015). Nanopore sequencing: from imagination to reality. *Clin. Chem.* 61, 25–31. doi:10.1373/clinchem.2014.223016
- Beadling, C., Neff, T. L., Heinrich, M. C., Rhodes, K., Thornton, M., Leamon, J., et al. (2013). Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. *J. Mol. Diagn.* 15, 171–176. doi:10.1016/j.jmoldx.2012.09.003
- Boeva, V., Popova, T., Lienard, M., Toffoli, S., Kamal, M., Le Tourneau, C., et al. (2014). Multi-factor data normalization enables the detection of copy number aberrations in amplicon sequencing data. *Bioinformatics* 30, 3443–3450. doi:10.1093/bioinformatics/btu436
- Campbell, P. J., Stephens, P. J., Pleasance, E. D., O'Meara, S., Li, H., Santarius, T., et al. (2008). Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat. Genet.* 40, 722–729. doi:10.1038/ng.128
- Chaisson, M. J., and Tesler, G. (2012). Mapping single molecule sequencing reads using basic local alignment with successive refinement (blasr): application and theory. *BMC Bioinformatics* 13:238. doi:10.1186/1471-2105-13-238
- Chaisson, M. J. P., Huddleston, J., Dennis, M. Y., Sudmant, P. H., Malig, M., Hormozdiari, F., et al. (2015). Resolving the complexity of the human genome using single-molecule sequencing. *Nature* 517, 608–611. doi:10.1038/nature13907
- Chen, K., Wallis, J. W., McLellan, M. D., Larson, D. E., Kalicki, J. M., Pohl, C. S., et al. (2009). Breakdancer: an algorithm for high-resolution mapping of genomic structural variation. *Nat. Methods* 6, 677–681. doi:10.1038/nmeth.1363
- Chiang, D. Y., Getz, G., Jaffe, D. B., O'Kelly, M. J. T., Zhao, X., Carter, S. L., et al. (2009). High-resolution mapping of copy-number alterations with massively parallel sequencing. *Nat. Methods* 6, 99–103. doi:10.1038/nmeth.1276
- Conrad, D. F., Pinto, D., Redon, R., Feuk, L., Gokcumen, O., Zhang, Y., et al. (2010). Origins and functional impact of copy number variation in the human genome. *Nature* 464, 704–712. doi:10.1038/nature08516
- de Koning, A. P. J., Gu, W., Castoe, T. A., Batzer, M. A., and Pollock, D. D. (2011). Repetitive elements may comprise over two-thirds of the human genome. *PLoS Genet.* 7:e1002384. doi:10.1371/journal.pgen.1002384
- Desai, A. N., and Jere, A. (2012). Next-generation sequencing: ready for the clinics? *Clin. Genet.* 81, 503–510. doi:10.1111/j.1399-0004.2012.01865.x
- Duan, J., Zhang, J.-G., Deng, H.-W., and Wang, Y.-P. (2013). Comparative studies of copy number variation detection methods for next-generation sequencing technologies. *PLoS ONE* 8:e59128. doi:10.1371/journal.pone.0059128
- Earl, D., Bradnam, K., St John, J., Darling, A., Lin, D., Fass, J., et al. (2011). Assemblathon 1: a competitive assessment of de novo short read assembly methods. *Genome Res.* 21, 2224–2241. doi:10.1101/gr.126599.111
- Fromer, M., Moran, J. L., Chambert, K., Banks, E., Bergen, S. E., Ruderfer, D. M., et al. (2012). Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. *Am. J. Hum. Genet.* 91, 597–607. doi:10.1016/j.ajhg.2012.08.005
- Girirajan, S., Dennis, M. Y., Baker, C., Malig, M., Coe, B. P., Campbell, C. D., et al. (2013). Refinement and discovery of new hotspots of copy-number variation associated with autism spectrum disorder. *Am. J. Hum. Genet.* 92, 221–237. doi:10.1016/j.ajhg.2012.12.016
- Gnerre, S., MacCallum, I., Przybylski, D., Ribeiro, F. J., Burton, J. N., Walker, B. J., et al. (2011). High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1513–1518. doi:10.1073/pnas.1017351108
- Gusnanto, A., Wood, H. M., Pawitan, Y., Rabbitts, P., and Berri, S. (2012). Correcting for cancer genome size and tumour cell content enables better estimation of copy number alterations from next-generation sequence data. *Bioinformatics* 28, 40–47. doi:10.1093/bioinformatics/btr593
- Hach, F., Hormozdiari, F., Alkan, C., Hormozdiari, F., Birol, I., Eichler, E. E., et al. (2010). mrsfast: a cache-oblivious algorithm for short-read mapping. *Nat. Methods* 7, 576–577. doi:10.1038/nmeth0810-576
- Hajirasouliha, I., Hormozdiari, F., Alkan, C., Kidd, J. M., Birol, I., Eichler, E. E., et al. (2010). Detection and characterization of novel sequence insertions using paired-end next-generation sequencing. *Bioinformatics* 26, 1277–1283. doi:10.1093/bioinformatics/btq152
- Handsaker, R. E., Korn, J. M., Nemes, J., and McCarroll, S. A. (2011). Discovery and genotyping of genome structural polymorphism by sequencing on a population scale. *Nat. Genet.* 43, 269–276. doi:10.1038/ng.768
- Harismendy, O., Ng, P. C., Strausberg, R. L., Wang, X., Stockwell, T. B., Beeson, K. Y., et al. (2009). Evaluation of next generation sequencing platforms for population targeted sequencing studies. *Genome Biol.* 10, R32. doi:10.1186/gb-2009-10-3-r32
- Hart, S. N., Sarangi, V., Moore, R., Baheti, S., Bhavsar, J. D., Couch, F. J., et al. (2013). Softsearch: integration of multiple sequence features to identify breakpoints of structural variations. *PLoS ONE* 8:e83356. doi:10.1371/journal.pone.0083356
- Hormozdiari, F., Alkan, C., Eichler, E. E., and Sahinalp, S. C. (2009). Combinatorial algorithms for structural variation detection in high-throughput sequenced genomes. *Genome Res.* 19, 1270–1278. doi:10.1101/gr.088633.108
- Hormozdiari, F., Hajirasouliha, I., Dao, P., Hach, F., Yorukoglu, D., Alkan, C., et al. (2010). Next-generation variationhunter: combinatorial algorithms for transposon insertion discovery. *Bioinformatics* 26, i350–i357. doi:10.1093/bioinformatics/btq216
- Hormozdiari, F., Hajirasouliha, I., McPherson, A., Eichler, E. E., and Sahinalp, S. C. (2011). Simultaneous structural variation discovery among multiple paired-end sequenced genomes. *Genome Res.* 21, 2203–2212. doi:10.1101/gr.120501.111
- Iafate, A. J., Feuk, L., Rivera, M. N., Listewnik, M. L., Donahoe, P. K., Qi, Y., et al. (2004). Detection of large-scale variation in the human genome. *Nat. Genet.* 36, 949–951. doi:10.1038/ng1416

- Iqbal, Z., Caccamo, M., Turner, I., Flicek, P., and McVean, G. (2012). De novo assembly and genotyping of variants using colored de bruijn graphs. *Nat. Genet.* 44, 226–232. doi:10.1038/ng.1028
- Ivakhno, S., Royce, T., Cox, A. J., Evers, D. J., Cheetham, R. K., and Tavaré, S. (2010). Cnaseq – a novel framework for identification of copy number changes in cancer from second-generation sequencing data. *Bioinformatics* 26, 3051–3058. doi:10.1093/bioinformatics/btq587
- Jain, M., Fiddes, I. T., Miga, K. H., Olsen, H. E., Paten, B., and Akeson, M. (2015). Improved data analysis for the minion nanopore sequencer. *Nat. Methods* 12, 351–356. doi:10.1038/nmeth.3290
- Jiang, Y., Oldridge, D. A., Diskin, S. J., and Zhang, N. R. (2015). Codex: a normalization and copy number variation detection method for whole exome sequencing. *Nucleic Acids Res.* 43, e39. doi:10.1093/nar/gku1363
- Jiang, Y., Wang, Y., and Brudno, M. (2012). Prism: pair-read informed split-read mapping for base-pair level detection of insertion, deletion and structural variants. *Bioinformatics* 28, 2576–2583. doi:10.1093/bioinformatics/bts484
- Kadalayil, L., Rafiq, S., Rose-Zerilli, M. J. J., Pengelly, R. J., Parker, H., Oscier, D., et al. (2015). Exome sequence read depth methods for identifying copy number changes. *Brief. Bioinform.* 16, 380–392. doi:10.1093/bib/bbu027
- Karakoc, E., Alkan, C., O’Roak, B. J., Dennis, M. Y., Vives, L., Mark, K., et al. (2012). Detection of structural variants and indels within exome data. *Nat. Methods* 9, 176–178. doi:10.1038/nmeth.1810
- Keane, T. M., Wong, K., and Adams, D. J. (2013). Retroseq: transposable element discovery from next-generation sequencing data. *Bioinformatics* 29, 389–390. doi:10.1093/bioinformatics/bts697
- Kelley, D. R., Schatz, M. C., and Salzberg, S. L. (2010). Quake: quality-aware detection and correction of sequencing errors. *Genome Biol.* 11, R116. doi:10.1186/gb-2010-11-11-r116
- Kielbasa, S. M., Wan, R., Sato, K., Horton, P., and Frith, M. C. (2011). Adaptive seeds tame genomic sequence comparison. *Genome Res.* 21, 487–493. doi:10.1101/gr.113985.110
- Kim, K. E., Peluso, P., Babayan, P., Yeadon, P. J., Yu, C., Fisher, W. W., et al. (2014). Long-read, whole-genome shotgun sequence data for five model organisms. *Sci. Data* 1, 140045. doi:10.1038/sdata.2014.45
- Kim, T.-M., Luquette, L. J., Xi, R., and Park, P. J. (2010). rsw-seq: algorithm for detection of copy number alterations in deep sequencing data. *BMC Bioinformatics* 11:432. doi:10.1186/1471-2105-11-432
- Klambauer, G., Schwarzbauer, K., Mayr, A., Clevert, D.-A., Mitterecker, A., Bodenhofer, U., et al. (2012). cn.mops: mixture of poisson for discovering copy number variations in next-generation sequencing data with a low false discovery rate. *Nucleic Acids Res.* 40, e69. doi:10.1093/nar/gks003
- Korbel, J. O., Abyzov, A., Mu, X. J., Carriero, N., Cayting, P., Zhang, Z., et al. (2009). Pomer: a computational framework with simulation-based error models for inferring genomic structural variants from massive paired-end sequencing data. *Genome Biol.* 10, R23. doi:10.1186/gb-2009-10-2-r23
- Krumm, N., Sudmant, P. H., Ko, A., O’Roak, B. J., Malig, M., Coe, B. P., et al. (2012). Copy number variation detection and genotyping from exome sequence data. *Genome Res.* 22, 1525–1532. doi:10.1101/gr.138115.112
- Lam, H. Y. K., Pan, C., Clark, M. J., Lacroute, P., Chen, R., Haraksingh, R., et al. (2012). Detecting and annotating genetic variations using the hugeseq pipeline. *Nat. Biotechnol.* 30, 226–229. doi:10.1038/nbt.2134
- Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., et al. (2001). Initial sequencing and analysis of the human genome. *Nature* 409, 860–921. doi:10.1038/35057062
- Langmead, B., Trapnell, C., Pop, M., and Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 10, R25. doi:10.1186/gb-2009-10-3-r25
- Lappalainen, I., Lopez, J., Skipper, L., Hefferon, T., Spalding, J. D., Garner, J., et al. (2013). Dbvar and dgva: public archives for genomic structural variation. *Nucleic Acids Res.* 41, D936–D941. doi:10.1093/nar/gks1213
- Layer, R. M., Chiang, C., Quinlan, A. R., and Hall, I. M. (2014). Lumpy: a probabilistic framework for structural variant discovery. *Genome Biol.* 15, R84. doi:10.1186/gb-2014-15-6-r84
- Lee, E., Iskow, R., Yang, L., Gokcumen, O., Haseley, P., Luquette, L. J. III, et al. (2012). Landscape of somatic retrotransposition in human cancers. *Science* 337, 967–971. doi:10.1126/science.1222077
- Lee, S., Hormozdiari, F., Alkan, C., and Brudno, M. (2009). Modil: detecting small indels from clone-end sequencing with mixtures of distributions. *Nat. Methods* 6, 473–474. doi:10.1038/nmeth.f.256
- Lee, W.-P., Stromberg, M. P., Ward, A., Stewart, C., Garrison, E. P., and Marth, G. T. (2014). Mosaik: a hash-based algorithm for accurate next-generation sequencing short-read mapping. *PLoS ONE* 9:e90581. doi:10.1371/journal.pone.0090581
- Li, H., and Durbin, R. (2010). Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics* 26, 589–595. doi:10.1093/bioinformatics/btp698
- Li, R., Fan, W., Tian, G., Zhu, H., He, L., Cai, J., et al. (2010). The sequence and de novo assembly of the giant panda genome. *Nature* 463, 311–317. doi:10.1038/nature08696
- Li, X., Chen, S., Xie, W., Vogel, I., Choy, K. W., Chen, F., et al. (2014). Psc: sensitive and reliable population-scale copy number variation detection method based on low coverage sequencing. *PLoS ONE* 9:e85096. doi:10.1371/journal.pone.0085096
- Lin, K., Smit, S., Bonnema, G., Sanchez-Perez, G., and de Ridder, D. (2014). Making the difference: integrating structural variation detection tools. *Brief. Bioinform.* doi:10.1093/bib/bbu047
- Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., et al. (2012). Soapdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* 1, 18. doi:10.1186/2047-217X-1-18
- Magi, A., Benelli, M., Marseglia, G., Nannetti, G., Scordo, M. R., and Torricelli, F. (2010). A shifting level model algorithm that identifies aberrations in array-cgh data. *Biostatistics* 11, 265–280. doi:10.1093/biostatistics/kxp051
- Magi, A., Benelli, M., Yoon, S., Roviello, F., and Torricelli, F. (2011). Detecting common copy number variants in high-throughput sequencing data by using jointslm algorithm. *Nucleic Acids Res.* 39, e65. doi:10.1093/nar/gkr068
- Magi, A., Tattini, L., Cifola, I., D’Aurizio, R., Benelli, M., Mangano, E., et al. (2013). Excavator: detecting copy number variants from whole-exome sequencing data. *Genome Biol.* 14, R120. doi:10.1186/gb-2013-14-10-r120
- Magi, A., Tattini, L., Pippucci, T., Torricelli, F., and Benelli, M. (2012). Read count approach for dna copy number variants detection. *Bioinformatics* 28, 470–478. doi:10.1093/bioinformatics/btr707
- Marschall, T., Costa, I. G., Canzar, S., Bauer, M., Klau, G. W., Schliep, A., et al. (2012). Clever: clique-enumerating variant finder. *Bioinformatics* 28, 2875–2882. doi:10.1093/bioinformatics/bts566
- Marschall, T., Hajirasouliha, I., and Schönhuth, A. (2013). Mate-clever: Mendelian-inheritance-aware discovery and genotyping of midsize and long indels. *Bioinformatics* 29, 3143–3150. doi:10.1093/bioinformatics/btt556
- Medvedev, P., Fiume, M., Dzamba, M., Smith, T., and Brudno, M. (2010). Detecting copy number variation with mated short reads. *Genome Res.* 20, 1613–1622. doi:10.1101/gr.106344.110
- Metzker, M. L. (2010). Sequencing technologies – the next generation. *Nat. Rev. Genet.* 11, 31–46. doi:10.1038/nrg2626
- Miller, C. A., Hampton, O., Coarfa, C., and Milosavljevic, A. (2011). Readdepth: a parallel r package for detecting copy number alterations from short sequencing reads. *PLoS ONE* 6:e16327. doi:10.1371/journal.pone.0016327
- Mills, R. E., Luttig, C. T., Larkins, C. E., Beauchamp, A., Tsui, C., Pittard, W. S., et al. (2006). An initial map of insertion and deletion (indel) variation in the human genome. *Genome Res.* 16, 1182–1190. doi:10.1101/gr.4565806
- Mills, R. E., Walter, K., Stewart, C., Handsaker, R. E., Chen, K., Alkan, C., et al. (2011). Mapping copy number variation by population-scale genome sequencing. *Nature* 470, 59–65. doi:10.1038/nature09708
- Mimori, T., Nariari, N., Kojima, K., Takahashi, M., Ono, A., Sato, Y., et al. (2013). isvp: an integrated structural variant calling pipeline from high-throughput sequencing data. *BMC Syst. Biol.* 7(Suppl. 6):S8. doi:10.1186/1752-0509-7-S6-S8
- Mullikin, J. C., and Ning, Z. (2003). The phusion assembler. *Genome Res.* 13, 81–90. doi:10.1101/gr.731003
- Myers, E. W. (2005). The fragment assembly string graph. *Bioinformatics* 21(Suppl. 2), ii79–ii85. doi:10.1093/bioinformatics/bti1114
- Niedringhaus, T. P., Milanova, D., Kerby, M. B., Snyder, M. P., and Barron, A. E. (2011). Landscape of next-generation sequencing technologies. *Anal. Chem.* 83, 4327–4341. doi:10.1021/ac2010857
- Nijkamp, J. F., van den Broek, M. A., Geertman, J.-M. A., Reinders, M. J. T., Daran, J.-M. G., and de Ridder, D. (2012). De novo detection of copy number variation by co-assembly. *Bioinformatics* 28, 3195–3202. doi:10.1093/bioinformatics/bts601
- Ning, Z., Cox, A. J., and Mullikin, J. C. (2001). Ssaha: a fast search method for large dna databases. *Genome Res.* 11, 1725–1729. doi:10.1101/gr.194201
- O’Rawe, J. A., Ferson, S., and Lyon, G. J. (2015). Accounting for uncertainty in dna sequencing data. *Trends Genet.* 31, 61–66. doi:10.1016/j.tig.2014.12.002

- Pabinger, S., Dander, A., Fischer, M., Snajder, R., Sperk, M., Efreanova, M., et al. (2014). A survey of tools for variant analysis of next-generation genome sequencing data. *Brief. Bioinformatics* 15, 256–278. doi:10.1093/bib/bbs086
- Pang, A. W., MacDonald, J. R., Pinto, D., Wei, J., Rafiq, M. A., Conrad, D. F., et al. (2010). Towards a comprehensive structural variation map of an individual human genome. *Genome Biol.* 11, R52. doi:10.1186/gb-2010-11-5-r52
- Pareek, C. S., Smoczynski, R., and Tretyn, A. (2011). Sequencing technologies and genome sequencing. *J. Appl. Genet.* 52, 413–435. doi:10.1007/s13353-011-0057-x
- Pinkel, D., Seagraves, R., Sudar, D., Clark, S., Poole, I., Kowbel, D., et al. (1998). High resolution analysis of dna copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.* 20, 207–211. doi:10.1038/2524
- Qi, J., and Zhao, F. (2011). ingap-sv: a novel scheme to identify and visualize structural variation from paired end mapping data. *Nucleic Acids Res.* 39, W567–W575. doi:10.1093/nar/gkr506
- Quail, M. A., Smith, M., Coupland, P., Otto, T. D., Harris, S. R., Connor, T. R., et al. (2012). A tale of three next generation sequencing platforms: comparison of ion torrent, pacific biosciences and illumina miseq sequencers. *BMC Genomics* 13:341. doi:10.1186/1471-2164-13-341
- Quick, J., Quinlan, A. R., and Loman, N. J. (2014). A reference bacterial genome dataset generated on the minion™ portable single-molecule nanopore sequencer. *Gigascience* 3, 22. doi:10.1186/2047-217X-3-22
- Quinlan, A. R., Clark, R. A., Sokolova, S., Leibowitz, M. L., Zhang, Y., Hurles, M. E., et al. (2010). Genome-wide mapping and assembly of structural variant breakpoints in the mouse genome. *Genome Res.* 20, 623–635. doi:10.1101/gr.102970.109
- Rausch, T., Zichner, T., Schlattl, A., Stütz, A. M., Benes, V., and Korbel, J. O. (2012). Delly: structural variant discovery by integrated paired-end and split-read analysis. *Bioinformatics* 28, i333–i339. doi:10.1093/bioinformatics/bts378
- Samarakoon, P. S., Sorte, H. S., Kristiansen, B. E., Skodje, T., Sheng, Y., Tjønnfjord, G. E., et al. (2014). Identification of copy number variants from exome sequence data. *BMC Genomics* 15:661. doi:10.1186/1471-2164-15-661
- Sathirapongsasuti, J. E., Lee, H., Horst, B. A. J., Brunner, G., Cochran, A. J., Binder, S., et al. (2011). Exome sequencing-based copy-number variation and loss of heterozygosity detection: Exomecnv. *Bioinformatics* 27, 2648–2654. doi:10.1093/bioinformatics/btr462
- Schadt, E. E., Turner, S., and Kasarskis, A. (2010). A window into third-generation sequencing. *Hum. Mol. Genet.* 19, R227–R240. doi:10.1093/hmg/ddq416
- Schröder, J., Hsu, A., Boyle, S. E., Macintyre, G., Cmero, M., Tothill, R. W., et al. (2014). Socrates: identification of genomic rearrangements in tumour genomes by re-aligning soft clipped reads. *Bioinformatics* 30, 1064–1072. doi:10.1093/bioinformatics/btt767
- Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., et al. (2007). Strong association of de novo copy number mutations with autism. *Science* 316, 445–449. doi:10.1126/science.1138659
- Shendure, J., and Ji, H. (2008). Next-generation dna sequencing. *Nat. Biotechnol.* 26, 1135–1145. doi:10.1038/nbt1486
- Shendure, J. A., Porreca, G. J., Church, G. M., Gardner, A. F., Hendrickson, C. L., Kieleczawa, J., et al. (2011). Overview of dna sequencing strategies. *Curr. Protoc. Mol. Biol.* Chapter 7, Unit 7.1. doi:10.1002/0471142727.mb0701s96
- Simpson, J. T., and Durbin, R. (2010). Efficient construction of an assembly string graph using the fm-index. *Bioinformatics* 26, i367–i373. doi:10.1093/bioinformatics/btq217
- Simpson, J. T., and Durbin, R. (2012). Efficient de novo assembly of large genomes using compressed data structures. *Genome Res.* 22, 549–556. doi:10.1101/gr.126953.111
- Sindi, S., Helman, E., Bashir, A., and Raphael, B. J. (2009). A geometric approach for classification and comparison of structural variants. *Bioinformatics* 25, i222–i230. doi:10.1093/bioinformatics/btp208
- Sindi, S. S., Onal, S., Peng, L. C., Wu, H.-T., and Raphael, B. J. (2012). An integrative probabilistic model for identification of structural variation in sequencing data. *Genome Biol.* 13, R22. doi:10.1186/gb-2012-13-3-r22
- Snijders, A. M., Nowak, N., Seagraves, R., Blackwood, S., Brown, N., Conroy, J., et al. (2001). Assembly of microarrays for genome-wide measurement of dna copy number. *Nat. Genet.* 29, 263–264. doi:10.1038/ng754
- Tan, R., Wang, Y., Kleinstein, S. E., Liu, Y., Zhu, X., Guo, H., et al. (2014). An evaluation of copy number variation detection tools from whole-exome sequencing data. *Hum. Mutat.* 35, 899–907. doi:10.1002/humu.22537
- Teo, S. M., Pawitan, Y., Ku, C. S., Chia, K. S., and Salim, A. (2012). Statistical challenges associated with detecting copy number variations with next-generation sequencing. *Bioinformatics* 28, 2711–2718. doi:10.1093/bioinformatics/bts535
- Thung, D., de Ligt, J., Vissers, L., Stehouwer, M., Kroon, M., de Vries, P., et al. (2014). Mobster: accurate detection of mobile element insertions in next generation sequencing data. *Genome Biol.* 15, 488. doi:10.1186/s13059-014-0488-x
- Trappe, K., Emde, A.-K., Ehrlich, H.-C., and Reinert, K. (2014). Gustaf: detecting and correctly classifying SVs in the NGS twilight zone. *Bioinformatics* 30, 3484–3490. doi:10.1093/bioinformatics/btu431
- Venkatesan, B. M., and Bashir, R. (2011). Nanopore sensors for nucleic acid analysis. *Nat. Nanotechnol.* 6, 615–624. doi:10.1038/nnano.2011.129
- Venkatraman, E. S., and Olshen, A. B. (2007). A faster circular binary segmentation algorithm for the analysis of array cgh data. *Bioinformatics* 23, 657–663. doi:10.1093/bioinformatics/btl646
- Voelkerding, K. V., Dames, S. A., and Durtschi, J. D. (2009). Next-generation sequencing: from basic research to diagnostics. *Clin. Chem.* 55, 641–658. doi:10.1373/clinchem.2008.112789
- Wang, J., Mullighan, C. G., Easton, J., Roberts, S., Heatley, S. L., Ma, J., et al. (2011). Crest maps somatic structural variation in cancer genomes with base-pair resolution. *Nat. Methods* 8, 652–654. doi:10.1038/nmeth.1628
- Wong, K., Keane, T. M., Stalker, J., and Adams, D. J. (2010). Enhanced structural variant and breakpoint detection using svmerge by integration of multiple detection methods and local assembly. *Genome Biol.* 11, R128. doi:10.1186/gb-2010-11-12-r128
- Wu, J., Lee, W.-P., Ward, A., Walker, J. A., Konkel, M. K., Batzer, M. A., et al. (2014). Tangram: a comprehensive toolbox for mobile element insertion detection. *BMC Genomics* 15:795. doi:10.1186/1471-2164-15-795
- Xi, R., Hadjipanayis, A. G., Luquette, L. J., Kim, T.-M., Lee, E., Zhang, J., et al. (2011). Copy number variation detection in whole-genome sequencing data using the bayesian information criterion. *Proc. Natl. Acad. Sci. U.S.A.* 108, E1128–E1136. doi:10.1073/pnas.1110574108
- Xie, C., and Tammi, M. T. (2009). Cnv-seq, a new method to detect copy number variation using high-throughput sequencing. *BMC Bioinformatics* 10:80. doi:10.1186/1471-2105-10-80
- Ye, K., Schulz, M. H., Long, Q., Apweiler, R., and Ning, Z. (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. doi:10.1093/bioinformatics/btp394
- Yoon, S., Xuan, Z., Makarov, V., Ye, K., and Sebat, J. (2009). Sensitive and accurate detection of copy number variants using read depth of coverage. *Genome Res.* 19, 1586–1592. doi:10.1101/gr.092981.109
- Zeitouni, B., Boeva, V., Janoueix-Lerosey, I., Loeillet, S., Legoix-né, P., Nicolas, A., et al. (2010). Svdetect: a tool to identify genomic structural variations from paired-end and mate-pair sequencing data. *Bioinformatics* 26, 1895–1896. doi:10.1093/bioinformatics/btq293
- Zhang, F., Gu, W., Hurles, M. E., and Lupski, J. R. (2009). Copy number variation in human health, disease, and evolution. *Annu. Rev. Genomics Hum. Genet.* 10, 451–481. doi:10.1146/annurev.genom.9.081307.164217
- Zhang, J., and Wu, Y. (2011). Svseq: an approach for detecting exact breakpoints of deletions with low-coverage sequence data. *Bioinformatics* 27, 3228–3234. doi:10.1093/bioinformatics/btr563
- Zhao, M., Wang, Q., Wang, Q., Jia, P., and Zhao, Z. (2013). Computational tools for copy number variation (CNV) detection using next-generation sequencing data: features and perspectives. *BMC Bioinformatics* 14(Suppl. 1):S1. doi:10.1186/1471-2105-14-S11-S1

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