SOFTWARE TOOL ARTICLE

TIDDIT, an efficient and comprehensive structural variant caller for massive parallel sequencing data [version 1; peer review: 2 approved with reservations]

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Abstract
Reliable detection of large structural variation (> 1000 bp) is important in both rare and common genetic disorders. Whole genome sequencing (WGS) is a technology that may be used to identify a large proportion of the genomic structural variants (SVs) in an individual in a single experiment. Even though SV callers have been extensively used in research to detect mutations, the potential usage of SV callers within routine clinical diagnostics is hindered by high computational costs, usage of non-standard output format, and limited support for the various sequencing platforms and libraries. Another well known, but not well-addressed problem is the large number of benign variants and reference errors present in the human genome that further complicates analysis. Here we present TIDDIT, a time efficient variant caller, that uses discordant read pairs as well as the depth of coverage and split reads to detect and classify a large spectrum of SVs. As part of the software suite, TIDDIT also includes a database functionality that enables filtering for rare variants and reduces the number of false positive calls and background noise. Benchmarked against five state-of-the-art SV callers, TIDDIT performs at an equal/superior level while using only 2 CPU hours per sample. Thanks to its speed, sensitivity, flexibility and ability to easily detect variants on a wide range of WGS library types, TIDDIT solves many of the problems that are currently hindering the utilization of WGS for SV calling in clinical settings.

Keywords
Variant calling, Whole genome sequencing, Structural variation

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Introduction
Genomic structural variants (SVs) are defined as large genomic rearrangements and consist of inversion and translocation events, as well as deletions and duplications. SVs have been shown to be both the direct cause and a contributing factor in many different human genetic disorders, as well as in more common phenotypic traits. In genetic diagnostics, current techniques such as FISH and microarray studies have limited resolution, and the information obtained often needs to be complemented by additional methods for a correct interpretation. Previous studies have shown that whole genome sequencing can be used to successfully identify and characterize structural variants in a single experiment.

The advent of systems like the Illumina HiSeqX allows researchers to sequence Whole Human Genomes (WHG) at high (i.e. 30X) coverage and at a relatively low cost (i.e. 1000S per WHG). The ability to produce large amounts of data at an unprecedented speed has initiated a flourishing of computational tools that are able to identify (i.e. call) structural variants and/or chromosomal breakpoints (i.e. the exact position in the chromosome at which SV takes place). These tools are commonly called variant callers, or simply, callers. Variant callers generally require an alignment file (in BAM format) as input and try to identify differences between the reference genome and the donor/patient genome. To detect structural variants, callers generally use heuristics based on different signals in the WGS data. These signals include discordant pairs, read-depth, and split-reads. Some callers try to reconstruct the patient sequence by applying either local or global de novo assembly techniques. Depending on the size, variant type, and characteristics of the sequencing data, the most suitable method for detecting a variant will differ.

Thanks to the ability to produce high quality sequencing data at a relatively low cost, as well as the potential to detect any variant from a single experiment, whole exome sequencing and whole genome sequencing could be highly useful in clinical diagnostics, especially to study rare disease causing variants. However, to avoid high validation costs, highly precise, yet sensitive callers are needed. To further complicate the situation, an abundance of sequencing platforms and library preparation methods are available. Sequencing data generated from these different sources have different properties, such as read length and coverage. As an example, it has been shown that large insert mate pair libraries are well suited to detect SVs, mainly due to the ability to span repetitive regions and complex regions that act as drivers of structural variation and due to the sensitivity derived from a large physical span coverage compared to small insert size sequencing coverage.

Here we present a new variant caller, TIDDIT. The name highlights the ability to detect many different types of SVs; including but not restricted to translocations, inversions, deletions, interspersed duplications, insertions and tandem duplications. TIDDIT utilizes discordant pairs and split reads to detect the genomic location of structural variants, as well as the read depth information for classification and quality assessment of the variants. By integrating these WGS signals, TIDDIT is able to detect both balanced and unbalanced variants. Finally, TIDDIT supports multiple paired-end sequencing library types, characterized by different insert-sizes and pair orientations.

To simplify the search for rare disease causing variants, TIDDIT is distributed with a database functionality dubbed SVDB (Structural Variant DataBase). SVDB is used to create structural variant frequency databases. These databases are queried for rare disease causing variants, as well as variants following certain inheritance patterns. Utilizing the database functionality, the analysis of rare variants may be prioritized, thus speeding up the diagnosis of rare disease causing variants. To our knowledge, no available caller provides such an extensive framework to call and evaluate rare disease causing structural variants.

Methods
Implementation

Detection of structural variants. TIDDIT requires a coordinate-sorted BAM file as input. There are two phases; in the first phase, coverage and insert size distribution are computed from the BAM file. These data will be used in the subsequent phase. In the second phase, TIDDIT scans the BAM file for discordant pairs and split reads and uses these signals to detect and classify structural variants. These two signals are pooled together by redefining each signal as two genomic coordinates \( S = (p_1, p_d) \), for a split read the \( p_1 \) position is given to the position of the primary alignment, and the \( p_d \) position is given to the position of the supplementary alignment of that read. On the other hand, for discordant pairs, the \( p_1 \) position is given to the the read having the smallest genomic coordinate, and the \( p_d \) position is given to the position of the read that has the largest genomic coordinate. A read pair is deemed to be discordant if the reads map to different contigs, or if the distance exceeds a threshold distance, \( T_d \). By default, \( T_d \) is set to three times the standard deviation plus the average value of the insert size distribution. Every time a SV signal is identified, TIDDIT switches from reading-mode to discovery-mode. As soon as the signal \( S = (p_1, p_d) \) is identified, the set \( D \) is initialized:

\[
D = \{(p_1, p_d)\}
\]

At this point TIDDIT searches for other evidence in the neighborhood. Every time a new signal is identified it is added to set \( D \). The construction of this set is halted only when no other signal is identified in \( W \) consecutive positions. In more detail, if \((p_l1) = c_i \) (i.e., \( p_l1 \) is a position \( j \) on chromosome \( c_i \)), then \( D \) will contain the following signals:

\[
D = \{(p_1, p_d) : p_1 > c_i \land \exists (p'_1, p'_d) : k \neq z \land p'_1 > c_i \land p'_d < p_1 + W\}
\]

The first condition guarantees that \( D \) will contain only signals found after position \( c_i \), i.e. the position of the first signal within the set \( D \). The existential clause guarantees that the set \( D \) will not contain any signal that is too far away from the signals within the set \( D \). \( D \) is obtained by reading the ordered BAM file and populating a data structure with all the detected discordant pairs and split reads. If no signal is identified after reading \( W \) positions from the last position containing a read added to \( D \), the discovery phase is closed.
TIDDIT also records information about local coverage and read orientation while constructing $D$. Once $D$ is computed, TIDDIT partitions it into distinct sets $D_i, D_2, \ldots, D_k$ such that:

$$\forall i, j \in \{1, \ldots, k\}: |D_i \cap D_j| = 0$$

if $u = \min\{p_i^j : p_i^j \in D^j\}$

then $D^i = \{(p_i^1, p_i^2) : (p_i^1, p_i^2) \in D \land (\exists (p_i^j, p_i^k) : k \neq z \land p_i^j > u_i \land p_i^k - W < p_i^j < p_i^k + W)\}$

In other words, $D$ is divided into non overlapping partitions, $D_i$ requiring that all $p_i$ positions form a cluster with properties analogous to the $p_j$ positions. Once $D$ is divided into partitions, TIDDIT checks if any partition represents a structural variant or if it is only noise. In the case of it being a structural variant, TIDDIT tries to associate the identified signal with a specific type of variation. A set $D_i$ is discarded (i.e. the SV is not reported) if the number of pairs forming the set (i.e. the cardinality of the set) is below a given threshold. This threshold is used to control the sensitivity and specificity of TIDDIT. In general, the number of discordant pairs is dependent on multiple factors, and may vary considerably throughout the genome. Therefore the user may need to fine tune the required number of discordant pairs based on the downstream analysis. All callable structural variants in $D$ are reported, and thereafter, $D$ is discarded. TIDDIT will then return to read mode, starting with the next available read pair.

**Classification of structural variants.** TIDDIT identifies candidate variations using discordant pairs and split reads. To determine the variant type, TIDDIT analyses read orientation, as well as the coverage across the region of the first reads, second reads, and the region in between. TIDDIT characterizes three levels of coverage: low, normal and high. If $C$ is the average coverage computed over the entire genome sequence, then the coverage across a region, $C$ is deemed normal if it is satisfying the following condition:

$$P \leftarrow - \text{The ploidy of the organism}$$

$$1 - \frac{0.7}{P} \leq \frac{C}{C} \leq \frac{0.7}{P} + 1$$

If the coverage across a region is lower than normal, it is classified as low coverage. Likewise, if the coverage is higher than normal coverage, it is classified as a high coverage region. The patterns of the variants detected by TIDDIT are represented in Figure 1.

In a deletion event (Figure 1A) a region is absent in the patient genome but present in the reference. When aligned to the reference, the read pairs flanking the deleted region will have a larger insert size than what is expected based on the library insert size distribution. Moreover, split reads will be formed in such a way that one part of the read is aligned to one side of the breakpoint, and another part of the read will be aligned to the other side of the deletion. Furthermore, the coverage of the lost region will be lower than expected. Therefore, TIDDIT will classify a variant as a deletion if the region flanked by the discordant pairs/split reads is classified as a low coverage region.

In an interspersed copy number event (Figure 1B), an extra copy of a region within the genome (the red sequence in Figure 1B) is positioned distant from the original copy. In this case, there will be read pairs and reads bridging the duplicate copy and the distant region. When aligned to the reference, the read pairs will appear to have unexpectedly large insert size, and the reads will appear split across the reference. Thus, TIDDIT will detect these signals. Furthermore, the coverage of the copied region will be higher than expected. By scanning the coverage of the regions where the reads of the discordant pairs are located, TIDDIT will find which region has an extra copy. The interspersed duplication is reported as two events, an intrachromosomal translocation between the duplicated region and the position of the copy, and a duplication across the duplicated region.

In a tandem duplication event (Figure 1C), the extra copy is positioned adjacent to the original copy. Since the distance between the segments is small, there will be pairs where one read is located at the end of the original copy, and the other read is located at the front of the duplicate copy (Figure 1C). When aligned to the reference, the insert size of these read pairs will be as large as the tandem copy itself (Figure 1C). Similarly, there will be reads bridging the two copies. These reads will appear split when mapped to the reference. Furthermore, the orientation of these read pairs and split reads will be inverted. Moreover, the coverage across the entire genomic region will be higher than expected. Thus to classify tandem duplications, TIDDIT will search for sets of discordant pairs/split reads that have inverted read pair orientation as well as high coverage between the read pairs.

In an inversion event (Figure 1D), the sequence of a genomic region is inverted. When aligning the sequencing data the insert size of the read pairs bridging the breakpoints will appear to be larger than expected. Furthermore, both read pairs will get the same orientation, such as reverse/reverse or forward/forward. TIDDIT employs the read pair orientation in order to identify inversions. Given that the inversion is large enough, TIDDIT will find the pairs bridging the breakpoints of the inversion, and will classify the variant as an inversion if the orientation of the discordant pairs are forward/forward or reverse/reverse orientation, and if the orientation of the primary/secondary alignments are forward/reverse, reverse/forward.

In an interchromosomal translocation event (Figure 1E), the first read and second read will map to different contigs (Figure 1E). Reads bridging the translocated segment will appear split between these two contigs. Any read pair mapping to two different contigs is counted as a discordant pair, and any set of signals mapping to different contigs will be classified as an interchromosomal translocation. Intrachromosomal translocation events are similar. They are balanced events, where a genomic region has been translocated to another location within the same chromosome. When aligned to the reference region, these variants will give rise to signals where one read is mapping to the translocated region, and the other read
**Figure 1.** TIDDIT detects all types of SVs based on discordant pairs and split reads. Thereafter, the read orientation and coverage is used for classification of the SV. Deletions (A) are characterized by low coverage across the deleted region (shown in red), and normal orientation. Interspersed duplications (B) are characterized by high coverage across the duplicated region (shown in red), but normal coverage across the other regions affected by the SV. Tandem duplications (C) are characterized by high coverage across the duplicated region (shown in red), the entire duplicated region is found between the discordant pairs/split reads (shown in red), the read pair orientation is inverted compared to the expected library orientation. Inversions (D) are characterized by the orientation of the read pairs (red-purple and red-blue) bridging the inverted region (shown in red). When aligning these discordant pairs to the reference, the reads of the discordant pairs will have the same orientation, forward/forward or reverse/reverse. For split reads, the orientation of the secondary alignment will differ from the primary alignment. Lastly, translocations (E) are characterized by the read pairs bridging the translocated region (red) and its surroundings (blue and purple). Either these regions belong to another contig than the translocated region, or to the same contig but, in contrast to interspersed duplications, the coverage across the translocated region is normal.
mapping to the region where the translocated region is positioned. This will give rise to pairs having larger insert size than expected. However, since there is no change in copy number, the coverage will be normal across the discordant pairs. The orientation of the reads forming the discordant pairs will depend on whether the translocated region is inserted in its original orientation, or if it is inverted relative to its original orientation. Thus the read pairs may retain the standard library orientation, but the orientation could also be inverted. Therefore, intrachromosomal translocations are classified by scanning for discordant pairs having either forward/reverse or reverse/forward orientation and normal coverage in the region between those reads.

**Filtering of structural variant calls.** For each called variation, several statistical values are calculated. They serve two purposes: to provide more information and to filter out noise. In the former case, statistics are employed to understand the structure of the variant and to relate it to the rest of the genome. In the latter case, filters are employed to improve the precision of TIDDIT. TIDDIT utilizes four complementary filters: Expected links, Few links, Unexpected coverage, and Smear. These heuristics are used to set the FILTER column of the VCF generated by TIDDIT.

The main goal of Expected links is to filter variants caused by random events such as contamination or sequencing errors. It uses a statistical model to compute the expected number of discordant pairs using the library insert size, read length, ploidy of the organism and coverage across the region affected by the structural variant. A variant that is defined by less than 40% of the expected number of pairs will fail the Expected links quality test, and is set to Expected links in the FILTER column of the VCF. The statistical model supports variants that are called using discordant pairs, hence for calls based on split reads exclusively, the number of split reads divided by coverage and ploidy is used as an estimate of the expected number of split reads.

Few links aims to filter out calls that are caused by reference errors and misalignment of repetitive regions. As mentioned previously, a variant is defined as a set of positions, \( D_{\text{var}} = \langle p, p' \rangle \). In order to compute the Few links filter, for each \( D_{\text{var}} \), TIDDIT creates another set called \( D_{\text{pair}} \), containing spurious read pairs. Spurious reads pairs are pairs that belong to the interval identified by \( D_{\text{var}} \) but whose mates align to a different chromosome from the one where the pairs form \( D_{\text{var}} \). In other words, TIDDIT checks if a genomic location where a SV can be called is linked to multiple other events. In this case the suspicion is that the called SV is the consequence of a repetitive element. If the fraction of spurious read pairs is too high, the variant within \( D_{\text{var}} \) is considered unreliable, and thus its filter flag is set to Few links. The fraction of spurious read pairs is considered too high if the following formula holds true:

\[
P \leftarrow \text{The ploidy of the organism} \quad \frac{|D_{\text{pair}}|}{|D_{\text{pair}}| + |D_{\text{var}}|} > 0.8 \quad \frac{p}{P}
\]

Smear is a filter designed to remove variants called due to large repetitive regions. In these regions, the split reads and discordant pairs will map in a chaotic manner, hence these regions may appear to be affected by large structural variation. These calls are recognized by searching for variants were the regions of the first read and its mate overlap, or where the regions of the primary and secondary alignment overlap. If this is the case, the variants will fail the Smear test. The filter flag of variants that fail this test is set to Smear.

Lastly, Unexpected coverage also aims to filter out calls caused by reference errors and misalignments, but unlike the Few links, it employs coverage information. The Unexpected coverage filter uses the coverage across the region of the \( p_{\text{var}} \) signals as well as the region of \( p_{\text{var}} \) to determine the quality of a variant call. If the coverage of any of these regions is 10 or more times higher than the average library coverage, the variant will fail the Unexpected coverage test, and its FILTER column is set to Unexpected coverage.

Any variant that passes these filters is set to PASS, those that fail are set according to the filter that rejected the variant. By removing all variants that did not pass this quality control, the precision of TIDDIT improves considerably.

**Structural variant database**

Using the structural variant frequency database (SVDB) software, the user may compare variants found in different samples and annotate the VCF files with the frequency of each variant. The frequency database is built with multiple VCF files containing structural variant information. The VCF files may be generated using any caller that reports structural variants according to the VCF standard. By removing high frequency variants from the VCF file, rare variants may be detected. The database could also perform trio analysis and filter out variants following a certain frequency pattern within a family.

The database is an SQLite database. It contains one entry per input variant. These entries describe the genomic position, variant type, and sample id of the variant. Moreover, each variant is given a unique index number. These SQLite databases may then be used either directly to provide frequency annotation, or they may be exported to a VCF file.

**Export.** The variants of the SQLite structural variant database may be exported to a VCF file. The VCF file is a standard structural variant VCF file, generated by clustering similar variants within the SQLite database, and reporting each cluster as a single VCF entry. The INFO field of each VCF entry contains four custom tags. The FRQ tag describes the frequency of the variant, the OCC tag describes the total number of samples carrying the variant, the NSAMPLES tag describes the total number of samples within the database, and lastly, the variant tag describes the position and id of each clustered variant.

The clustering of the variants is performed using one out of two methods, either an overlap based clustering method or DBSCAN.
**Annotation.** The main purpose of the structural variant frequency database is to query it and use it for frequency annotation. The frequency database is queried using a VCF file. All variants within the VCF file will be annotated with the frequency of that variant within the frequency database. The database used for querying may either be an SQLite database, an exported VCF file, or a multi-sample VCF file such as the thousand genome structural variant VCF.

If an SQLite database is chosen, two separate algorithms are available, overlap or DBSCAN. If DBSCAN is chosen, all variants of the SQLite database and the query VCF are clustered using DBSCAN. Thereafter, the frequency of each query variant is set to the number of separate samples represented in the cluster of that query variant.

**Overlap based clustering.** The most critical part when building and querying the database is to determine if two SVs represent the same event or not. When using the overlap based clustering algorithm, two interchromosomal variants are considered equal if the distance between their breakpoints does not exceed a certain distance threshold. This distance is set to 5 kilobases (kb) as default. However, the user may change it to suit any kind of data. Secondly, for intrachromosomal variants fulfilling the breakpoint distance criteria, the overlap O is computed and used to determine if the variants are similar enough. For a given chromosome, to compute O each variant \( \text{var} \) is regarded as an ordered set of genomics coordinates:

\[
\text{var} = \{ i, i + 1, ..., j - 1, j \}
\]

The overlap parameter \( O \) is defined as the cardinality of the intersection of two variants, divided by the cardinality of the union of the same overlapping variants:

\[
O = \frac{\text{var}_i \cap \text{var}_j}{\text{var}_i \cup \text{var}_j}
\]

Where \( \text{var}_i \) and \( \text{var}_j \) are two overlapping variants (\( O \) equals to 0 if the variants are not overlapping). The default threshold value of the overlap parameter is 0.6.

The database software extracts the variant type from the ALT field. By default, variants of different type will not be considered equal even if the overlap is high enough. When constructing the database, the variants of the VCF file are transferred into an SQLite database. This database can then be queried directly using the previously described procedure. The database could also be exported into a VCF file.

When exporting the database, variants from different patients overlap in complex patterns and form chains where not all variants in one chain are overlapping according to the user set parameters. Consider three variants, \( \text{var}_A, \text{var}_B \) and \( \text{var}_C \) as an example:

The overlap between variants \( \text{var}_A \) and \( \text{var}_B \) as well as \( \text{var}_A \) and \( \text{var}_C \) satisfies the overlap settings, \( O \). However, the overlap between \( \text{var}_C \) and \( \text{var}_B \) is not big enough:

\[
\frac{\text{var}_C \cap \text{var}_B}{\text{var}_C \cup \text{var}_B} \geq O \land \frac{\text{var}_B \cap \text{var}_C}{\text{var}_B \cup \text{var}_C} \geq O \land \frac{\text{var}_A \cap \text{var}_C}{\text{var}_A \cup \text{var}_C} < O
\]

To resolve these cases, all the variants, \( V \) found in all patients are divided into sets \( \chi \) based on the overlap threshold, \( O \) such that

\[
\forall i,j \in 1,...,k : \left| \chi_i \cap \chi_j \right| = 0
\]

\[
V_i \in \chi_i \exists j \, V_j \in \chi_j \text{ s.t.} \quad \frac{\text{var}_i \cap \text{var}_j}{\text{var}_i \cup \text{var}_j} \geq O
\]

Each \( V \) in \( \chi \) may not have a high enough overlap with all \( V_i \) in \( \chi_i \), instead the variants in \( \chi \) may form complex chain-like patterns of overlap. To resolve these chains of variants, each \( \chi \) is divided further into subsets of variants, \( \chi_i \), such that

\[
\chi_i \cup \chi_i \cup ... \cup \chi_i = \chi
\]

Each set \( \chi_i \) is represented by one single variant, \( V'_i \). And for each variant \( V_i \) in \( \chi_i \), the overlap threshold of \( V'_i \) is satisfied.

The sub clusters \( \chi_i \) are computed by defining two sets, \( A \) and \( B \).

\[
A = B = \chi
\]

\( V'_i \) is picked at random from \( A \), and added to \( \chi_i \). Thereafter, \( V'_i \) is tested for overlap against each variant within \( B \). A variant \( V_i \) in \( B \) will be added to \( \chi_i \) if it satisfies the overlap threshold. Once the overlap is calculated between \( V'_i \) and each \( V_i \) in \( B \), \( A \) is redefined as \( A \setminus \chi_i = A \), thereafter \( V'_i \) is printed as a database entry, and the frequency of \( V'_i \) is set to the number of patients carrying a variant found in \( \chi_i \). If the set \( A \) is non-empty, a new \( \chi_i \) is initiated and another \( V'_i \) is sampled from \( A \), and the described process is repeated again.

**DBSCAN clustering.** The second clustering algorithm used by SVDB is DBSCAN. DBSCAN requires two parameters, epsilon and minPTS. At default epsilon is set to 500 bases, and minPTS is set to 2 samples. However, these parameters may be changed by the user.

The DBSCAN clustering is performed by dividing each chromosome and variant type into separate sub-databases; thereafter, a 2-dimensional coordinate system is defined for each sub-database. For intrachromosomal variants, the x coordinate of this plane corresponds to the start position of the variant, and the y coordinate within the plane corresponds to the end position of the variant.

Interchromosomal translocations involve two chromosomes, so when clustering these variants, the contig id is sorted according to lexicographic order. Out of the two contigs involved in the
rearrangement, the contig ordered first is set to the x axis, and the contig last in order is set to be the y axis. Thereafter, each variant is added to the plane as described for intrachromosomal variants. This procedure is repeated for any possible chromosome pair, and each variant type on each chromosome pair.

Once each plane is defined, the variants within each separate plane are clustered using DBSCAN.

**Operation**

**Detection of structural variants.** TIDDIT requires a coordinate sorted BAM file as input, and may be run in two separate modes; variant calling or coverage computation. The coverage computation mode is used to compute the coverage across the entire genome, and returns a BED file as output. The variant calling mode is run to analyse SV across the entire genome. All the detected SVs are returned in a single VCF file.

**System requirements.** TIDDIT has been tested on a large number of datasets; in general, TIDDIT will perform variant calling in less than 2 hours(3) using a single CPU core, and 2 gigabytes of RAM memory or less. The time consumption and memory usage is mainly dependent on the coverage and quality of the input data.

TIDDIT has been tested on Linux as well as Apple macOS. TIDDIT is easy to install and requires only standard c++ libraries. TIDDIT is installed using cmake (https://cmake.org) and make (https://www.gnu.org/software/make/).

**Downstream analysis.** TIDDIT generates a VCF file containing structural variant calls across the entire genome. Initially, these calls may be filtered based on the quality filters described in the implementation section, as well as the SVDB software, using either internal samples or an external dataset such as thousand genomes structural variants (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/integrated_sv_map/). The TIDDIT output VCF is compatible with any tool which supports VCF, hence there’s a large number of tools available for further analysis of the variant calls. Typically, the VCF file is annotated using software such as VEP or snpEFF. Tools such as VCFtools and BEDtools may be used to remove low quality calls, or filter calls within specific genomic regions.

**Results**

The performance of TIDDIT was evaluated using simulated, as well as public datasets containing a large number of validated variants of different size and type. The performance of TIDDIT was compared to current top of the line callers, including Lumpy, Delly, CNVnator, Manta, and Fermikit. These are well known callers that have been tested throughout numerous projects.

Three separate datasets were used to evaluate TIDDIT. First, a simulated dataset generated using Simseq and SVsim (https://github.com/gregoryfaust/svsim) was used. Next, two public large scale sequencing datasets were used, namely the NA12878 sample, and the HG002 sample. Truth sets of validated variants were found for each of the public datasets. A detailed description of the benchmarking of TIDDIT is given in Supplementary File 1.

**Performance of TIDDIT on simulated variants**

To test the performance of the callers, the tools were run on the simulated dataset. The dataset consists of four separate samples, one per variant type. The coverage of these simulated samples was set to 25X, and the read length and insert size was set to 100 and 350 bp, respectively. For each variant type (deletions, duplications, inversions, translocations), 6000 variants were simulated. Hence, the simulated dataset contains 24000 known variants of different type. The results are presented in Table 1. For each caller the sensitivity and precision was computed. The sensitivity differs between different callers and variant types. **Table 1** shows that Fermikit is the caller with the lowest sensitivity. TIDDIT, however, is consistently the caller with the highest sensitivity. It is important to notice how the high sensitivity of TIDDIT is coupled with extremely high precision. In other words, TIDDIT is not only able to call all the real variation in the dataset, but it is also one of the tools least affected by false positives. For instance, looking more closely at the translocations in **Table 1** we can see that TIDDIT has the best trade-off between sensitivity and precision, revealing the highest sensitivity out of all callers, and precision that was only 0.02 lower than Delly and Manta.

**Performance of TIDDIT with NA12878 and HG002**

Lastly, to complement the benchmarking on the simulated dataset, and to test TIDDIT on a more diverse set of sequencing data, the same callers were run on the NA12878 and HG002 samples. The results are presented in **Table 2**. We computed both sensitivity and precision, in three size intervals. Events 0–100 bp in size are usually not considered structural variation, but they were kept as a size interval anyway since some callers also detect these variants.

**Table 1. Sensitivity and precision of the structural variant callers on a simulated dataset consisting of 6000 variants of each variant type.** The variants were simulated using SVsim and Simseq.

<table>
<thead>
<tr>
<th>Caller</th>
<th>Sensitivity</th>
<th>Precision</th>
<th>Sensitivity</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deletions</td>
<td>Duplications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIDDIT</td>
<td>0.96</td>
<td>0.99</td>
<td>0.96</td>
<td>0.99</td>
</tr>
<tr>
<td>CNVnator</td>
<td>0.9</td>
<td>0.92</td>
<td>0.86</td>
<td>0.91</td>
</tr>
<tr>
<td>Delly</td>
<td>0.94</td>
<td>1</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>Fermikit</td>
<td>0.41</td>
<td>1</td>
<td>0.33</td>
<td>1</td>
</tr>
<tr>
<td>Lumpy</td>
<td>0.95</td>
<td>0.97</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>Manta</td>
<td>0.95</td>
<td>1</td>
<td>0.95</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Inversions</th>
<th>Translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIDDIT</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>Delly</td>
<td>0.94</td>
<td>1</td>
</tr>
<tr>
<td>Fermikit</td>
<td>0.35</td>
<td>1</td>
</tr>
<tr>
<td>Lumpy</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Manta</td>
<td>0.95</td>
<td>1</td>
</tr>
</tbody>
</table>

Page 8 of 18
Table 2. The sensitivity (S) and precision (P) of six structural variant callers with the public NA12878 and HG002 samples. These public datasets contain validated deletions of various sizes.

<table>
<thead>
<tr>
<th>Size(bp)</th>
<th>NA12878</th>
<th>HG002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S  P</td>
<td>S  P</td>
</tr>
<tr>
<td>0–100</td>
<td>0.68 0.7</td>
<td>0.62 0.11</td>
</tr>
<tr>
<td>100–1000</td>
<td>0.95 0.5</td>
<td>0.96 0.17</td>
</tr>
<tr>
<td>≥ 1000</td>
<td>0.1 0.09</td>
<td>0.41 0.45</td>
</tr>
</tbody>
</table>

Table 2 shows how the fraction of unique hits within the data contains high quality deletion calls, these non-overlapping calls are not necessarily incorrect. With medium (i.e., 100–1000 bp) and small (i.e., ≤ 100 bp) size variants the performance of the callers differed greatly. For these variants, Manta had the highest sensitivity, while TIDDIT had the highest precision.

Moving on to the HG002 sample, it was found that most variant callers performed worse than with the NA12878 sample. No variant caller produced any significant number of true positive calls in the range of 0–100 bp. TIDDIT had the highest sensitivity on variants larger than 100 bases, and was one of the most precise callers (Table 2).

Evaluation of the database functionality. The performance of the database functionality was evaluated by building databases of different sizes. These databases were built by randomly sampling individuals from a set of 209 that were sequenced through the thousand genomes project. These individuals are listed in Supplementary File 1.

Figure 2 shows how the fraction of unique hits within the database gets lower as the size of the database increases, therefore improving the ability to find unique variants for new patients. A unique hit is defined as a variant that has only been found in the query itself. Already, a relatively small database filtered out a significant amount of variants. On average, a sample queried against a database consisting of only 10 samples contain about 25% unique variants. Still, a larger database filters out more variants. Each query sample was found to contain 7.5% (i.e., ≥250) unique structural variants when filtered against a database containing 200 samples. Since each caller reports a relatively large fraction of false positives (Table 2), the frequency database is necessary to reduce the number of variants. Moreover the frequency database can be used to filter out recurring technical errors connected to the library preparation, sequencing chemistry and alignment of the sequencing data.

CPU hour consumption of the structural variant callers. Each caller except Fermikit was run on a single core of an Intel Xeon E5-2660 CPU. Fermikit was run using 16 Intel Xeon E5-2660 CPU cores.

<table>
<thead>
<tr>
<th>Caller</th>
<th>CPU hour consumption on SV calling</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIDDIT</td>
<td>NA12878 1 1 1</td>
</tr>
<tr>
<td>CNVnator</td>
<td>2 1 1 1</td>
</tr>
<tr>
<td>Delly</td>
<td>45 2 7</td>
</tr>
</tbody>
</table>
As the number of samples within the database increase, the number of unique variants per sample decrease. The structural variant databases may also be used to benchmark different tools and settings, as well as to compare the SVs within a family or population. As an example, the database functionality was used to study differences between populations sequenced through the thousand genomes project\(^{25}\). Three different populations were selected: Han Chinese from Beijing (CHB), Japanese from Tokyo (JPT), and Yoruba from Ibadan (YRI). In total, 25 samples were analysed; 10, 5, and 10 samples from the CHB, JPT, and YRI populations, respectively. All of these samples were analysed using TIDDIT. The similarity of the samples was determined by creating one database per sample, and querying each sample against each database. The fraction of similar SVs was determined by computing the number of common/similar SVs divided by the total number of SVs in that sample. A more detailed description of the analysis and the selected samples is given in Supplementary File 1. It was found that the three populations are distinct based on their SVs. Furthermore, the CHB and JPT populations are relatively similar compared to the YRI population (\textbf{Figure 3}). The CHB population appears to be more homogeneous than the other two populations, with the exception of one individual which appears to be similar to the JPT individuals. On the other hand, the YRI population appears to be most diverse population, and is divided into two clear subpopulations.

**Discussion**

**Variant calling**

Six structural variant callers were benchmarked on simulated data generated by simseq (Table 1), and two public datasets, including NA12878 and HG002 (Table 2). Compared to the other callers, TIDDIT performs well on SV larger than 1000 bp (Table 1, Table 2). TIDDIT is able to identify large structural variants in many experimental setups: low or high coverage, short or long fragment size (i.e., Paired End or Mate Pair) (Table 1, Table 2). Furthermore, TIDDIT has a good balance between sensitivity and precision. Despite being one of the most sensitive tools, it is also one of the most precise tools. These two characteristics (high sensitivity and high precision) in conjunction with a low computational demand makes TIDDIT one of the most capable tools available today for the identification of large SVs greater than 1 kb from WGS data. TIDDIT does not perform well on small variants (Table 2), however TIDDIT performs really well on large variants, especially balanced variants (Table 1). Since TIDDIT is efficient, produces high quality variant calls, and performs well in multiple settings, TIDDIT could be a great addition to WGS pipelines.

**Benchmarking**

Even though the presented benchmarking is extensive, it is not fully complete. The public datasets lack large enough truth sets for balanced variants and duplications. Since the callers perform differently on different variants (Table 1), it would be of value to benchmark the callers against a more varied set of variants. Moreover, the variants of these truth sets are generally small. For instance, the median size of the deletions of NA12878 is about 250 bases, which is smaller than the traditional size of structural variation.\(^3\)

**Database functionality**

The Human genome contains a large amount of repetitive regions, and each individual carries a large number of structural variants.\(^{34,35}\) Due to these reasons, the number of detected structural variants per samples is generally high. Thus, finding a rare disease-causing variant among such a large number of common variants is difficult and time consuming. TIDDIT is distributed together with structural variant database software. This software package uses structural variant VCF files to construct variant frequency databases. The annotation provided by these databases is then used to filter common variants as well as reference errors.

By filtering out high frequency variants from the VCF file, rare disease causing variants can easily be detected (\textbf{Figure 2}). Moreover, the database could be constructed to follow variants that all samples have in common, such as an inherited disease variant within a family, or known disease causing variants, as well as to search for variants following a certain inheritance pattern, or to compare the SVs.
of different populations (Figure 3). The structural variant databases could also be used to benchmark different software tools, settings, and library preparation methods. The database functionality can be used for results obtained from any tool that generates a valid VCF file as format.

Conclusions
TIDDIT is an efficient and comprehensive structural variant caller, supporting a wide range of popular sequencing libraries. Not only does TIDDIT have the functionality of a structural variant caller, it also has a set of functions that helps the user perform further analysis of the bam file. These functions include depth of sequencing coverage analysis and structural variant database functionality. By utilizing these functions, TIDDIT could either perform advanced analysis on its own or be used to perform a wide range of tasks within a variant analysis pipeline. TIDDIT has already been employed in many studies and demonstrated its potential not only with the commonly used Nextera mate pair libraries from Illumina\(^{7,18,36}\) but also with the TrueSeq Nano and PCR-free Paired End libraries\(^7\).

Software availability
Latest source code: https://github.com/J35P312/TIDDIT

Archived source code as at the time of publication: http://doi.org/10.5281/zenodo.439517\(^8\)

License: GNU General Public License version 3.0 (GPLv3)

Figure 3. A comparison of SV calls between twenty five individuals, belonging to the JPT, CHB and YRI populations. These individuals were sequenced through the thousand genomes project. The heatmap is coloured based on the similarity between individuals, and shows that the populations can be distinguished based on their SVs.

Author contributions
JE, FV and DN wrote the code. PO investigated callers and datasets used for benchmarking. AL, DN, FV, and JE designed the TIDDIT algorithm and the functional specification of TIDDIT. JE wrote the benchmarking scripts and performed the benchmarking. All authors participated in the writing of the article.

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the Swedish Research Council [2012-1526 to AL]; the Marianne and Marcus Wallenberg foundation [2014.0084 to AL]; the Swedish Society for Medical Research [S14-0210 to AL]; the Stockholm County Council; the Harald and Greta Jeansson Foundation; the Ulf Lundahl memory fund through the Swedish Brain Foundation; the Nilsson Ehle donations and the Erik Rönngberg Foundation.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments
The authors would like to acknowledge support from Science for Life Laboratory, the National Genomics Infrastructure (NGI), and Uppmax for providing assistance in massive parallel sequencing and computational infrastructure.
Supplementary material

Supplementary File 1: Supplementary methods. A description of the benchmarking of the SV callers.

Click here to access the data.

Supplementary File 2: SV simulation pipeline. The pipeline used to generate the simulated SV datasets.

Click here to access the data.

References

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benchmark structural variant calls. BMC Genomics. 2016; 17: 64.


Data Source
The paper has already two reviewers and they have addressed many of the issues that I would have raised. My main concern however is that the interpretation of the results depend highly on the accuracy of the reference used and that the human reference is still incomplete (non-haplotypic), error-ridden, and evolving. It is unclear how the tool will adapt to the future when large number of accurate haplotypic whole genome references will become available. What makes it even worse is that the software is built on a group of intuitively justifiable heuristics with many hyper-parameters hard-coded. However, all the other tools it competes against also suffer from these problems and the genomics community has been strangely oblivious of these foundational problems.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Partly

Competing Interests: No competing interests were disclosed.
**Reviewer Expertise:** Genomics and Computational Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

01 June 2017

**Reviewer Report**

https://doi.org/10.5256/f1000research.12049.r22619

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**Giuseppe Narzisi**  
New York Genome Center, New York, NY, USA  
**Wayne Clarke**  
New York Genome Center, New York, NY, USA

This paper describes the implementation of a new structural variant caller, called TIDDIT, that uses multiple forms of evidence to call structural variants (deletions, duplications, inversions, and translocations) in whole genome sequencing experiments. In addition to structural variant calling, TIDDIT includes database functionality that helps reduce errors and makes it adaptable to diverse applications (e.g. rare and \textit{de novo} variant detection).

Structural variant calling is an area of research that is in strong need for more reliable and sensitive bioinformatics methods. TIDDIT has the potential to be a valuable open source tool and, overall, we think that the method warrants publication but some further refinement is necessary. We hope that the authors will address the following major and minor comments:

**Major Comments:**

1. The authors list the current problems in the field as computational costs, non-standard output formats, and limited support for different sequencing platforms/library types. We don't believe they have adequately described the tools they compare against with respect to these limitations. For example, both CNVnator and Manta have reasonable computational costs (2 and 3 core hours respectively), while Manta outputs VCF and does not list any platform/library type as a known limitation of their tool. A better description of how their work solves these limitations compared to other tools is required. Alternatively, they could shift focus towards the novelty of the database functionality.

2. Generally, there is sufficient information provided for interpretation. However, there are some cases where further explanations are required. First, Manta is compared against for both the simulated data and NA12878 but is subsequently dropped from analysis of HG002 with no explanation. The authors should provide an explanation for this either in the text or as a footnote to the figures. Second, there is no description of the clustering method selected for the evaluation of database functionality (overlap based and DBSCAN clustering). The two clustering methods are described only from a technical stand point without providing details on how selection would affect
the output or what use cases might be appropriate for each method.

3. While the results generally support the conclusions made, the language used tends to overstate the differences between the tested methods: “TIDDIT is consistently the caller with the highest sensitivity”, “The high sensitivity of TIDDIT is coupled with extremely high precision”, “Despite being one of the most sensitive tools, it is also one of the most precise tools”. For example, while it is true that TIDDIT is the caller with highest sensitivity in the simulated datasets (table 1), the margin of victory is often quite low (only 0.01 in deletions and duplications – with lower precision than Manta, the next most sensitive caller). The authors should adjust the language used in the paper to provide a more truthful and honest description of the real performance of the tool as reported in the tables. Significant better performance is indeed achieved for simulated translocations but the improvements on the other classes of variants seem to be limited.

4. We found the description for resolving the chain-like pattern of overlaps in the “overlap based clustering” to be quite confusing and hard to follow as currently described in the paper. The authors should state more clearly the problem. Is this a specific issue that has not been properly addressed by the community so far? Also, it is usually helpful to explain some of the complexity in interval analysis by including figures that elucidate the details of the process.

Minor Comments:

1. The methods used to generate the simulated data is described well enough, however, in this case making the simulated data sets available would also have been practical and would facilitate reproducing the results independently. The code is easily installed and provides sufficient documentation via the help options to get started with using the tool.

2. Simulated structural variants seem to be created at random locations, however real variants tend to happen in a non-random fashion along the genome, in particular around repetitive sequences. It is important to emphasize in the paper the limitations of the simulated data, which may also explain partially why sensitivity on real data is significantly worse than in the simulated experiment.

3. The caption for Figure 2 seems particularly long for such a simple figure and many of the details given in the figure caption would be better placed in the main text.

4. Equations in the paper should be numbered.

5. On the equation on page 3, the variable $W$ is used to indicate the number of consecutive (base pair) positions used to halt the construction of a set. This value seems to play a significant role in partitioning the genome to identify structural variants. However, there is no information on what value is used and whether it is a user parameter.

6. On page 6: “These calls are recognized by searching for variants [were -> where] the regions of the first read and its mate overlap, or where the regions of the primary and secondary alignment overlap.”

7. The authors report TIDDIT’s system requirements to be only 2 hours using a single CPU and 2 GB of RAM. However, these numbers are uninformative without reporting also the amount of data (e.g., sequence coverage, number of reads, etc.) that was used to test the tool.

Is the rationale for developing the new software tool clearly explained?
Partly

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Partly

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Partly

Competing Interests: Giuseppe Narzisi has previously co-authored papers with Francesco Vezzi, who is one of the authors of the paper in question, but not in the last 3 years.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

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Author Response 22 Jun 2017

Jesper Eisfeldt, Karolinska Institutet, 171 76 Stockholm, Sweden

Hello there!
Thanks for taking your time to reviewing, and sorry for taking such a long time to respond! We agree with your comments, and we have incorporated them in the manuscript:

Major Comments:
1. We now focus more on the database functionality, but we also mention the number of callers that combine low computational cost and the ability to detect all types of SV is limited.

2. Manta does not support variant calling on data having read orientation reverse-forward, hence it was excluded from the HG002 dataset which is sequenced using a mate-pair library. We have now added a short description of the two samples, and also a statement why manta was dropped from the analysis of HG002.
We wanted to use the mate-pair data of HG002 sample mainly to show that TIDDIT performs well on both standard paired-end data and mate-pair data.

3. Thanks, we have adjusted the language to make it more neutral.

4. We have rewritten the overlap based clustering section completely, now it contains an example of such complex cluster of variants; as well as a more practical explanation of the algorithm.

Minor Comments:
1. Due to the large size of the files we decided not to upload the simulated data. Potentially, we could generate the data and make it available on request during a limited time. We are happy to hear that the code is easily installed!

2. That’s true, the simulated variants were randomly positioned throughout the genome. We have added some comments to the benchmark subsection of the discussion section where we comment on this. The main reasons we simulated the variants that way was to reduce the risk of any selection bias, as well as to simplify the simulation. We agree that the most truthful simulation would include more realistically positioned variants, and perhaps even a large number of known disease causing SV.

3. We summarized the caption

4. Thanks, we added numbering to the equations!

5. We added some details on how W is set; in short, we found it through benchmarking on about 350 WGS samples of patients carrying known SV of clinical relevance, which were sequenced using a variety of libraries. However, since we cannot make the patient data publicly available we cannot present our internal benchmarking in the paper.

7. We got the statistics from running TIDDIT on the NA12878 sample, we have now added some more detail to that statement.

Thanks again for reviewing!
Regards
Jesper on behalf of the authors

**Competing Interests:** No competing interests were disclosed.