A computational method for detection of structural variants using Deviant Reads and read pair Orientation: DevRO

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Abstract

Background

Next generation sequencing (NGS) technology has made it possible to perform high-resolution screens for structural variants. Computational methods for detection of structural variants utilize paired-end mapping information, depth of coverage, split reads, or some combination of such data. The available methods are particularly designed to detect structural variants in single genomes or multiple genomes in a pairwise manner. The aim of this study was to develop a bioinformatics pipeline for detection of large structural variants using multiple pooled populations.

Results

Here we describe the method "DevRO", developed to enable identification of structural variants using short insert paired-ends and long-range mate-pairs. DevRO uses paired-end mapping information from both types of libraries for identification of inversions, deletions and duplications followed by read depth information to screen for copy number variants. DevRO can detect structural variants in multiple populations without the need for pairwise comparisons. It uses a combined approach based on (*i*) paired-end mapping and (*ii*) depth of coverage that gives power to the study as compared to traditional methods that are based on either of these. DevRO is also designed to detect deletions in the reference assembly, which is an added functionality as compared to available methods.

Conclusion

We report a bioinformatics pipeline "DevRO" for detection of structural variants using paired-end mapping and depth of coverage methods tested on sequencing reads from multiple pooled rabbit populations. This method is useful when large numbers of populations have been re-sequenced as compared to traditional methods that can detect structural variants in a pairwise manner.

Keywords

Mate pair; paired end mapping; read depth; re-sequencing; rabbit genome; structural variants

Background

In human genetics a focus has been to identify rare structural variants associated with disease whereas in animal genetics as well as in evolutionary biology it is of considerable interest to identify loci under positive selection. Large structural variants (SV) are an important form of genetic variants that frequently underlie phenotypic variation (Andersson, 2013; Weischenfeldt *et al.*, 2013). SV refers to copy or dosage changing variants called copy number variations (CNVs which include deletions, insertions and duplications (Redon *et al.*, 2006)) or dosage neutral variants like inversions and translocations. In humans, approximately 1.2% of a single genome differs from the reference genome as regards CNV genotypes (Pang *et al.*, 2010). The average size of SVs in the human genome is ~8kb and they range from 50bp to large structural events (Alkan *et al.*, 2011). In the recent past, several studies indicated that SVs have been associated with a variety of human diseases (Yang *et al.*, 2013; McCarroll & Altshuler, 2007; Stranger *et al.*, 2007). Whereas in livestock genomes, research in genome-wide CNV identification of various domestic animals showed their importance either in disease association (Olsson *et al.*, 2011), phenotypic changes (Jia *et al.*, 2013; Imsland *et al.*, 2012; Rubin *et al.*, 2010) affecting different traits, association with breed-specific differences in adaptation, health, and production traits (Bickhart *et al.*, 2012) and adaptation to starch rich diet in dogs after domestication (Axelsson *et al.*, 2013).

Before the development of next generation sequencing, structural variants were discovered by either hybridization methods (aCGH) in which the relative probe hybridization intensities differ between two compared genomes (Pinkel *et al.*, 1998) or using Hapmap data and SNP arrays measuring the intensities of probe signals at SNP loci (International HapMap *et al.*, 2010). However, the power was limited because of the size and breakpoint resolution of the predicted SV due to the density of SNP arrays. Sanger sequencing of paired reads was used as an alternative to the above-mentioned methods to detect CNVs, inversions and translocations with high accuracy and resolution at the expense of time and cost. Today next generation sequencing technologies (NGS) can generate a large amount of sequence data for instance by whole genome sequencing at a fraction of the cost and time. Several methods have been developed to enable SV detection from NGS data but each method has some limitations. In general, there are four categories of methods to detect SVs using NGS data 1) Depth of Coverage (DoC); 2) Paired-end mapping (PEM); 3) Split read (SR) and 4) Assembly (AS) based methods (Alkan *et al.*, 2011).

The assumption of depth of coverage based methods (*e.g.* CNVseq and CNVnator) is that the coverage is uniform *i.e.* the number of reads mapped to a region are assumed to follow a Poisson distribution, however these methods are unable to detect inversions and translocations (Abyzov *et al.*, 2011; Xie & Tammi, 2009). Paired-end mapping methods (*e.g.* DELLY and Breakdancer) use the information of paired reads and their orientation. The insert size is used to detect insertions and deletions, although the size of CNVs detected is limited by insert size of the libraries used (Rausch *et al.*, 2012; Chen *et al.*, 2009). Split read methods (*e.g.* Pindel) use the information of anchored reads to identify the breakpoint locations while assembly based methods (*e.g.* SOAPdenovo) are based on a denovo assembly (Li *et al.*, 2010; Ye *et al.*, 2009). Today, several tools have been developed that use a combination of the data available (*e.g* GenomeSTRiP, SVDetect) (Handsaker *et al.*, 2011; Zeitouni *et al.*, 2010).

Most of the available methods either use data from a single sample (CNVnator) or make pairwise comparisons between samples (CNVseq). The complexity arises when there are multiple groups (*e.g.* group1 comprising n populations and group2 comprising m populations) then detection of SVs needs further pairwise comparisons at the downstream level, making it difficult to attribute SVs to a specific group. Another complicating problem arises if the reference genome carries a deletion of a segment or if the resequenced individual/population carries an insertion not present in the reference, because the reads from this region cannot be mapped to the reference genome. This may for instance occur when comparing domestic animals with their wild ancestors, if a DNA segment has been deleted during the domestication process. Clearly, genome sequences from more individuals are needed to define whether a certain SV allele is derived or ancestral.

The aim of this study was to develop a tool based on deviant read and read orientation (DevRO) information using both paired-end mapping and depth of coverage analysis that can detect (*i*) structural variants in multiple populations and (*ii*) detect deletions in a reference assembly compared with individuals carrying a non-deleted allele.

Results and Discussion

Detection of structural variants using whole genome resequencing data

Here we used BAM files (Li *et al.*, 2009) as input to DevRO VariantCaller. The VariantCaller step generated raw variants calls from multiple individuals. In DevRO VariantCaller, we scan the entire genome for PEM signatures (Figure 1) in windows of 1 kb. For each locus we store the information of counts of discordant reads in each population, median mate position for the discordant reads within 2 standard deviations of the mapping distance between mate pair reads, forward and reverse median position of the anchored (singleton) reads and their counts in each population, soft clipped read counts in each population along with forward and reverse strand clipped positions and concordant read counts (Figure 2, Figure S1). This information is further processed at the VariantParser step to calculate the fraction of deviant reads in each group. This information is used to identify loci showing significant differences between groups. The CNVs detected using VariantParser are annotated and scored using the DoC information from paired-end sequencing data.

As a test case we used two data sets. 1) Mate pair (MP) data generated from two wild and two domestic rabbits (average insert size of 4.5 kb and average coverage of 3x) for PEM signatures (Figure 1). 2) Rabbit paired-end (PE) sequencing data from pooled samples of wild and domestic rabbit populations with an average coverage of 10x per population (Carneiro *et al.*, 2014) was used for DoC information. For this particular test case, DevRO uses information of two groups (wild and domestic rabbits) to find SVs with significant frequency differences between the two groups. However, it is not limited to this scenario and can take any two groups (*e.g.* case and control).

This resulted in identification of candidate deletions, duplications (Table S1) and preliminary results of inversions present at a relatively high frequency in either group 1 or group 2. Figure 3 and Figure S2 shows few examples of candidate duplication and deletions predicted by DevRO. Further improvements utilizing for example base quality information of deviant reads, and mapping of breakpoint read sequences on the reference genome will be added to later versions of DevRO.

Condordance with SVDetect and Breakdancer

SVDetect (Zeitouni *et al.*, 2010) is also based on the combined use of PEM and DoC data for detecting SVs in multiple samples. Breakdancer (Chen *et al.*, 2009) is based on the PEM approach for detection of SVs (deletions, inversions and translocations). The main difference between DevRO and these tools is that DevRO, as opposed to SVDetect and Breakdancer, does not require pairwise comparisons of groups (Table 1). We ran SVDetect and Breakdancer using the rabbit mate pair data in order to assess concordance with DevRO results (Table 2). The number of inversions detected was 178 and 281 for SVDetect and DevRO respectively, with 248 overlapping inversions using bedtools feature "reciprocal fraction overlap" of 0.7. Table S2 shows the preliminary list of candidate inversions detected by DevRO not in SVDetect list. One possible reason why SVDetect detected fewer inversions could be it is based on pairwise comparisons as opposed to DevROs group level contrasts. The overlap between SVDetect and DevRO was 500 and 391 for deletions and duplications, respectively (Table 2). However, the overlap between Breakdancer and DevRO showed big differences 13 and 43 for inversions and deletions, respectively. One possible reason for this big difference in deletions could be due to the combined use of PEM and DoC in DevRO while only using PEM in Breakdancer for detection of SVs.

Deletions in the rabbit reference genome

A unique feature with DevRO is that none of the previously described tools are designed to search for deletions present in the reference assembly. This is of particular interest in draft assemblies as well as in domestic animals when the reference genome is based on a domesticated individual, since this makes it challenging to identify regions of the genome that may have been lost during domestication. In rabbits, is based on a domesticated individual, as is the case for all other domesticated species except the chicken. One of the aims with the development of DevRO was to identify deletions present in a reference assembly; such deletions may be due to assembly errors or because the individual used for the assembly carry one or more deletions. In our case, the pileups of singleton reads (anchored reads whose pairs are unmapped) using data from wild rabbits were used to identify candidate loci. These were further narrowed down using the median positions for forward and reverse singletons and soft clipped positions. This resulted in a preliminary candidate list of breakpoints of deletions in the reference genome. These putative breakpoints of deletions were further used to extract the hanging reads. By combining the anchored and hanging read sequences (which represents one mapped read and one unmapped read), BLAT mappings were conducted to the human reference genome in order to investigate whether the unmapped reads corresponded to a human sequence homologous to the rabbit locus as this would indicate that the reference carried a deletion of evolutionary conserved sequences. Further improvements are needed to conduct *de novo* assembly of the singletons and unmapped reads at breakpoints. Further work is also required to experimentally validate these putative deletions.

In order to test DevRO for detection of deletions in reference assembly, we simulated deletions in the rabbit reference assembly (Table S3). Here we deleted ten regions ranging in size from 1 kb to 70 kb on chromosome 1 with repeats (repeat information extracted from UCSC Santa Cruze Browser) flanking the breakpoints or overlapping them. DevRO successfully identified nine of the simulated deletions but was unable to detect one of the regions (Tables S3). This region showed a mappability of 0.008 at the 5' end of the breakpoint indicating a highly repetitive region.

Conclusions

Here we report a bioinformatics pipeline "DevRO" for detection of structural variants using paired-end mapping and depth of coverage analysis and tested it on rabbit data. It has an added routine for detecting deletions present in a reference genome. This method will be useful when large numbers of populations are re-sequenced as compared to other frequenctly used methods it is designed to detect structural variants in pairwise comparisons of groups.

Methods

The input of DevRO is a set of aligned MP or PE reads in SAM/BAM format (Li *et al.*, 2009). All input BAM files from multiple populations are analyzed jointly, to avoid pairwise comparisons in the end.

The pipeline DevRO consists of three modules 1) VariantCaller 2) VariantParser and 3) VariantAnnotate that are used to detect inversions, deletions and duplications when comparing two or more populations as well as deletions in a reference genome seq.

Variant Caller. We used BAM files (Li *et al.*, 2009) from MP data as an input to DevRO VariantCaller. In this step, we scan the genome in windows of 1kb to search for PEM signatures using discordant reads (Figure 1). The discordant or deviant reads have (***I***) abnormal mapping distance in comparison to average mapping distance, the threshold for declaring a PEM as deviant is that it deviates more than three standard deviations from the mean which is a signature for deletion or insertion, (***II***) abnormal relative mapping orientation (in case of duplication or inversion) as shown in Figure 1. The information of the mate orientation strand for the left or right clipped reads to identify breakpoints. Together with counts and position of discordant reads we also recorded the counts of anchored or singleton reads and soft clip reads using bitwise flags and CIGAR (Li *et al.*, 2009) given in alignment files (BAM) as the method described in SAMtools (Li *et al.*, 2009). For duplications, we store all those loci where at least one duplication read signature is observed in any population, and record the information of deviant/discordant and normal read counts for such loci in each population, median positions are calculated using the mates (discordant reads). This step gives us the raw calls for loci with read counts in each SV category for all the populations in question. The following filters were used during this step: mapping quality for discordant reads >=10; same criteria is applied for each SV type for which the VariantCaller is run. Each analysis is done separately for duplications, inversions and deletions. This means that when we are running the script for the duplication scan and we come across inverted loci having no duplication reads then these loci will not be reported in output of duplications call but will be reported in inversion calls. In contrast, if we find inverted reads where we have duplicated reads also, the loci will be stored with the information of read counts for duplications and inversions. The same principle applies for the scan of inversions.

Variant parser. The purpose of this module is to extract only those loci where there is differentiation between the two groups analyzed (domestic and wild rabbits in the data analyzed in this study). The input is the result of Variant Caller raw calls obtained in the above step. In this step we calculate the fraction of abnormal reads in the two groups and only extract the loci where we see a significant difference between the two groups.

The formula for calculating the fraction of reads consistent with duplication in group 1 is as follows:

frac_dup = A1_dup/(A1_dup+A2_dup) , where A1 and A2 represents two groups.

The same type of formula is used to calculate the fraction for all discordant or deviant reads.

Variant Annotate. The predicted CNVs from VariantParser are given as an input to Variant Annotate. We calculate chisq test for each candidate using normalized deviant read counts in each group (A1 and A2) and the total read counts. The purpose of this step is to score and annotate the loci identified in the variant parser step by using pvalue and information of depth of coverage for CNVs using paired-end data (method explained in Carneiro et al., 2014). For each group the average depth is extracted in the predicted regions and an M-value (log2 fold change) is calculated. This step gives CNVs that show significant allele frequency difference between two groups (absolute M-value >=0.7).

For visualization, log2 fold change is plotted for each candidate CNV in R. The breakpoints for inversions are shown using the positions of mate pair. We next annotate the candidates using information for genes, repeats, gaps or custom annotations. Finally, candidate SVs are scored as alpha, beta and gamma by using the following criteria for all SVs:

Alpha: SV with absolute M-values greater than 0.7 from MP and PE data and that fulfill the fraction check, where fraction check is at least 10% of deviant reads or signatures observed.

Beta: Below 10% and above 1% of deviant reads support with or without M-values.

Gamma: without M-value support, with less than 1% deviant reads.

Deletion in the reference genome assembly. For detecting deletions in the reference genome or insertions in the resequenced genome, the genome was scanned as in the VariantCaller step by using pileups of singleton (anchored reads) in non-reference populations with mapping quality greater than 10. Median position is calculated for both forward and reverse singleton reads with counts for discordant reads in each window. If multiple discordant reads are observed, then it is less likely that the singletons are due to deletion in the reference assembly and we discard that window in parser step. We further narrow down the region by making use of median positions of singletons and only allow 0.05% overlap between forward and reverse singletons (if any). Together with this information, soft clips (search for right and left clipped positions at breakpoints) are used to detect breakpoints of deletions in the reference assembly. In order to know what part may have been deleted in the reference genome, we also make use of BLAT to map the unmapped pairs of singletons at breakpoints to the human genome assembly to infer whether a DNA fragment observed in human (and in other species) is homologous to those unmapped reads. The size and expected gene content within the part missing in the reference assembly is inferred.

Datasets. Four rabbits (two domestics and two wild from the *Oryctolagus cuniculus algirus* subspecies) were re-sequenced at 3x coverage using Illumina mate pair (2x50bp) with an average insert of 4.5kb. This dataset was used for paired-end mapping analysis. The depth of coverage analysis was done for the CNVs using the previously published paired-end sequencing data (Carneiro et al., 2014).

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Figures

Figure1. Description of signatures used in the analysis of paired end mapping data. Forward read marked as F and Reverse read marked as R.

Figure 2. **Overview of DevRO SV screen a)** Steps in VariantCaller module; *For detailed workflow see Figure S1 **b)** Steps in VariantParser; ** Group1 and Group2 represents two groups in test data and **c)** VariantAnnotate.

Figure3. Example of Candidate duplication detected by DevRO supported by 16 discordant reads from MP data (a) Mvalue plotted (black dots), with dotted horizontal red line threshold 0.7 **(b)** pvalue plotted with (brown dots) showing the significant difference in depth between group 1 and group2. **(c)** Mean depth plotted for each group in windows of 1kb size using PE data.

Tables

Table1. **Summary of Inversions, Deletions and Duplications detected by SVDetect and DevRO.**

Supplementary Files

Figuere S1. Detailed Flowchart of DevRO VariantCaller module. Discordant reads include inverted reads or duplicated reads or deletion or insertion signatures (see catalog of signatures, Figure 1).

Figure S2. Example of candidate deletions detected using DevRO, group2 (black) containing two pooled populations of rabbits (wilds) and group1 (brown) containing four pooled populations of rabbits (domestic).

(a) Deletion is detected in group2 (deviant reads=12) with significant difference in read depth observed in intergenic region near protein_coding gene (dist=1242), *ARHGEF9* (dist=14541). **(b)** Deletion is detected in group2 (deviant reads=19) with significant difference in read depth observed in intronic region of *SLC44A5* gene.

Table S1. Candidate deletions and duplications predicted by DevRO in each group.

TableS2. **Subset of candidate Inversions detected by DevRO not in SVDetect list**.

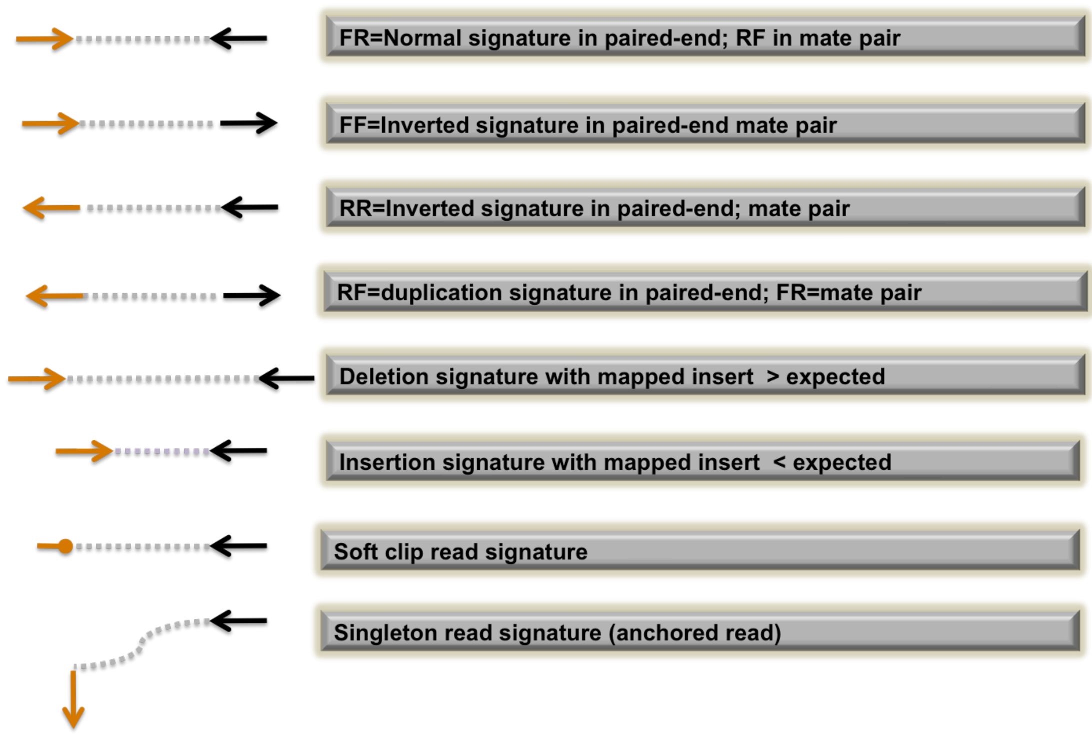


Figure1. Description of signatures used in the analysis of paired end mapping data. Forward read marked as F and Reverse read marked as R.

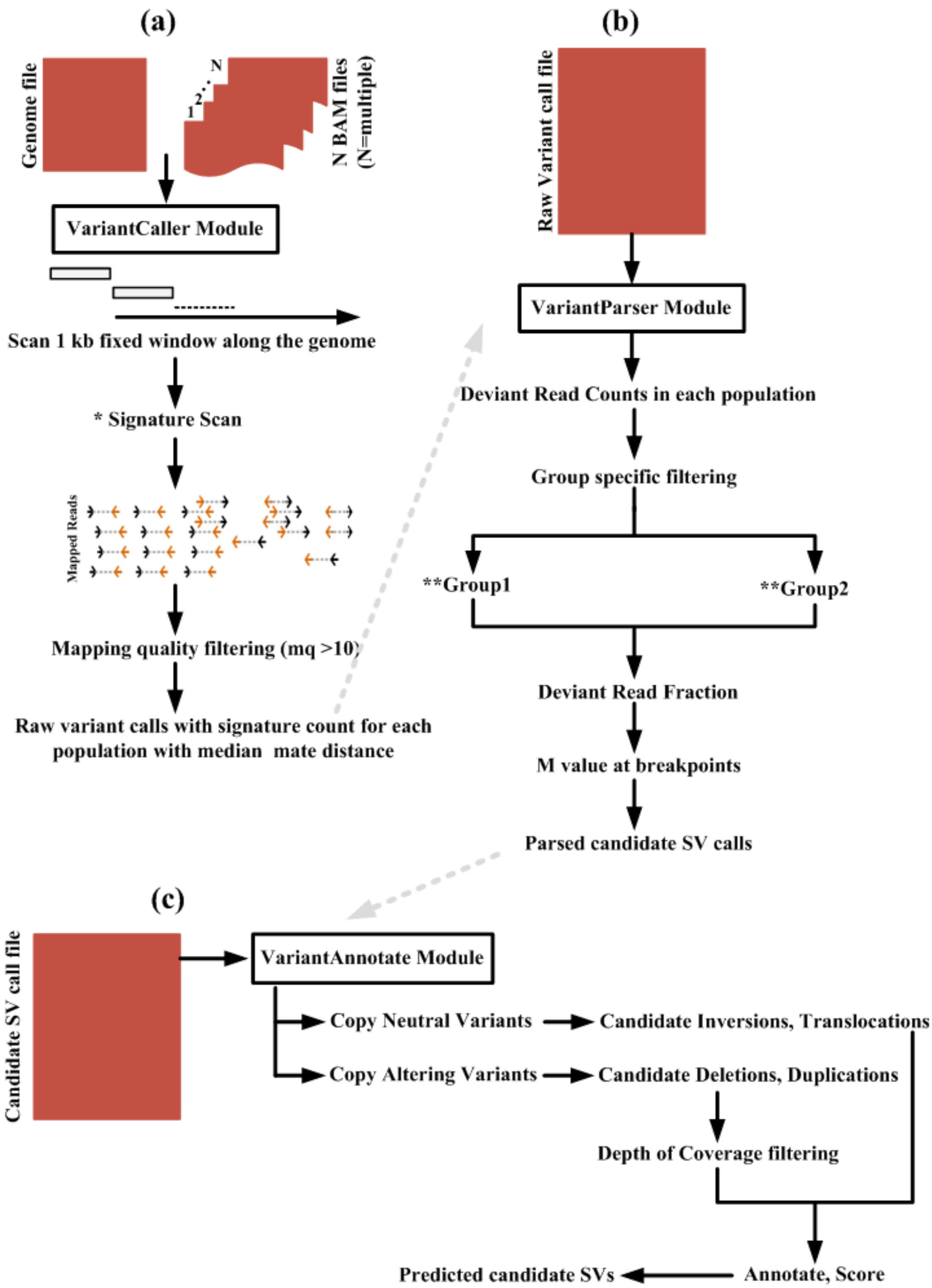


Figure 2. **Overview of DevRO SV screen a)** Steps in VariantCaller module; *For detailed workflow see Figure S1 **b)** Steps in VariantParser; ** Group1 and Group2 represents two groups in test data and **c)** VariantAnnotate.

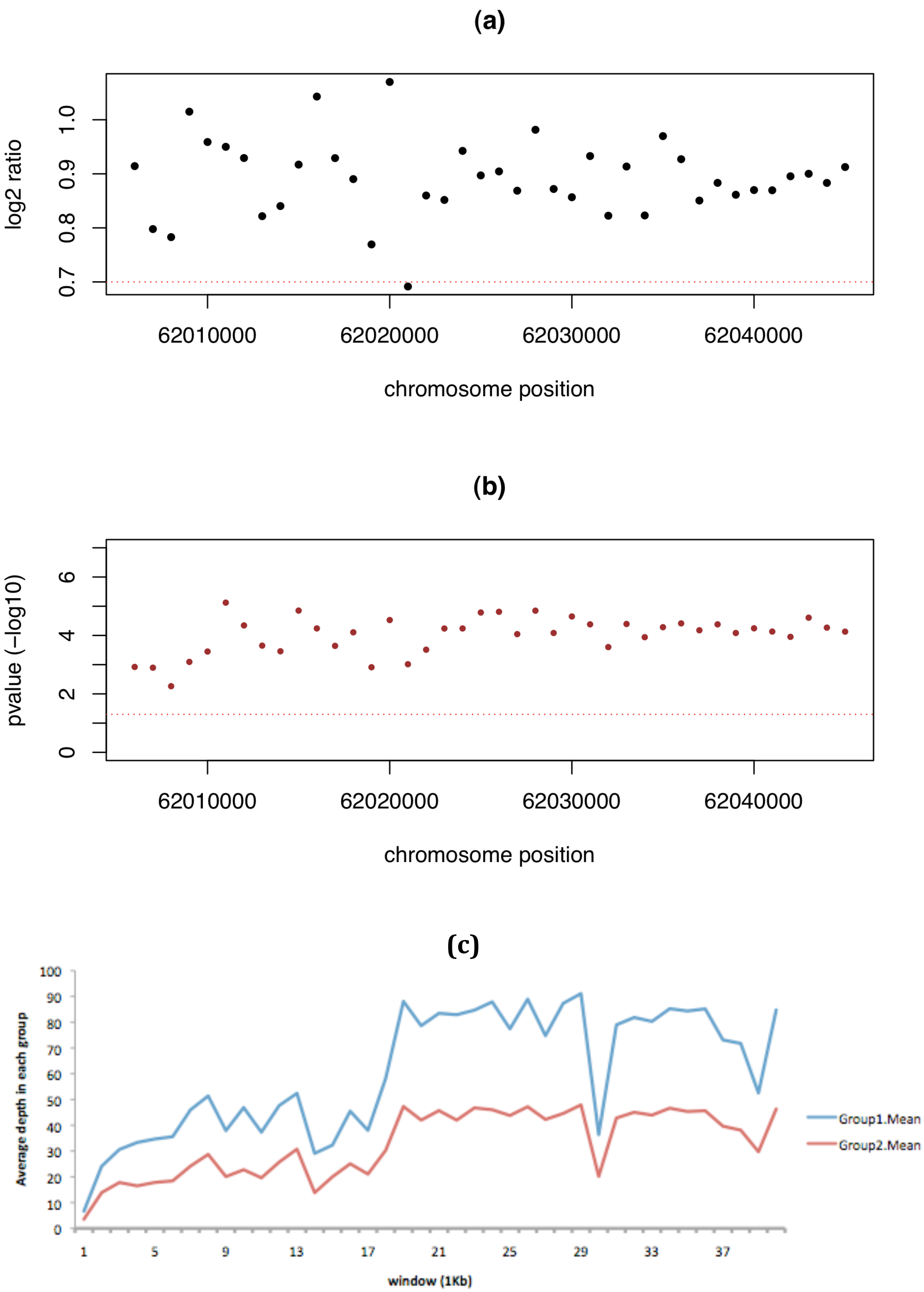


Figure3. Example of Candidate duplication detected by DevRO supported by 16 discordant reads from MP data (a) Mvalue plotted (black dots), with dotted horizontal red line threshold 0.7 **(b)** pvalue plotted with (brown dots) showing the significant difference in depth between group 1 and group2. **(c)** Mean depth plotted for each group in windows of 1kb size using PE data.

Table 1. Summary comparison of DevRO with available SV softwares.

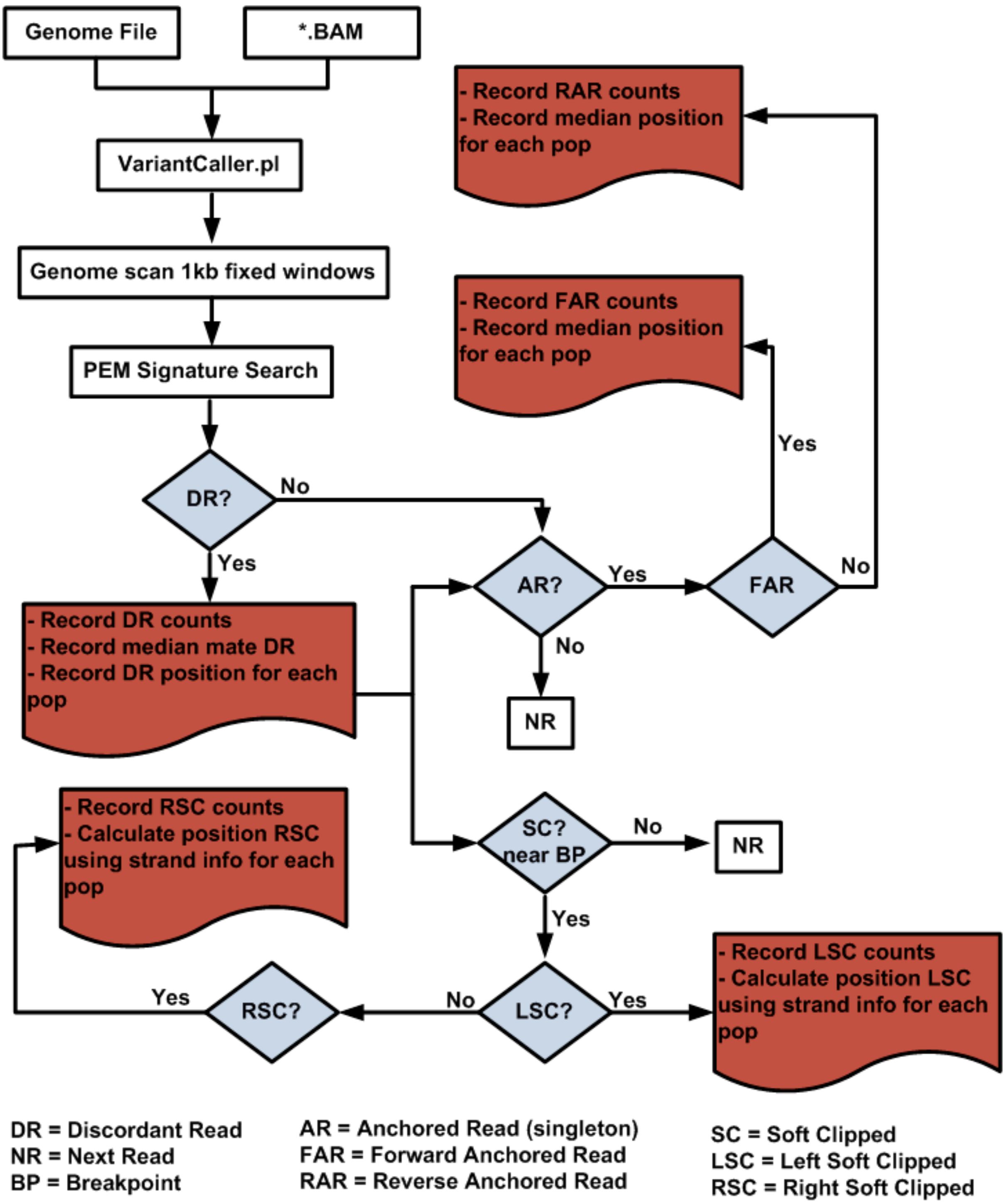
		SVDetect	**Breakdancer**	**DevRO**
Input	*BAM	√	√	√
Output	INV	√	√	√
	INS	√	√	√
	DEL	√	√	√
	DUP	√	–	√
	TRANSL	√	√	√
	Del-Ref	–	–	√
Group-wise Comparisons	Parser	–	–	√
Visualization	Output plots	√	–	√
Methods		PEM+DoC	PEM	PEM+DoC

Table2. Summary of strucrural variants detected by SVDetect, DevRO and Breakdancer tools.

Tool [a]	Inversions	Deletions	Duplications [b]
SVDetect (SVD)	178	702	408
DevRO	281	796	462
Breakdancer (BD)	289	64	NA
OL_SVD-DevRO	248	500	391
OL_BD-DevRO	13	43	NA
OL_SVD-BD	65	62	NA

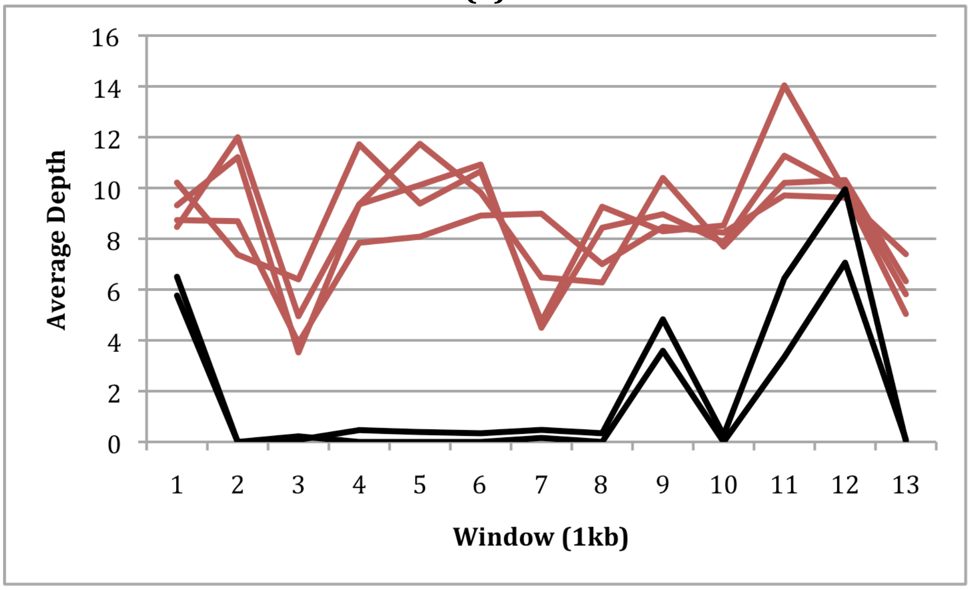
[a] Overlap as "OL", Breakdancer tool as "BD" and SVDetect tool as "SVD"

[b] Breakdancer only predicts inversions and deletions and unknown, Here Unknown not taken into account. NA=not available.



Figuere S1. Detailed Flowchart of DevRO VariantCaller module. Discordant reads include inverted reads or duplicated reads or deletion or insertion signatures (see catalog of signatures, Figure 1).

(a)



(b)

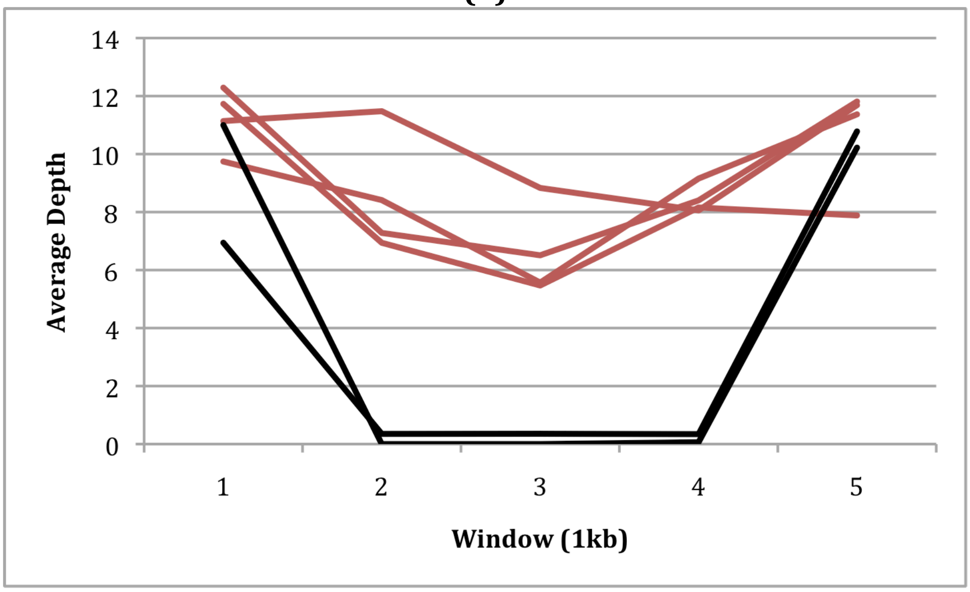


Figure S2. Example of candidate deletions detected using DevRO, group2 (black) containing two pooled populations of rabbits (wilds) and group1 (brown) containing four pooled populations of rabbits (domestic).
(a) Deletion is detected in group2 (deviant reads=12) with significant difference in read depth observed in intergenic region near protein_coding gene (dist=1242), *ARHGEF9* (dist=14541). **(b)** Deletion is detected in group2 (deviant reads=19) with significant difference in read depth observed in intronic region of *SLC44A5* gene.

Table S1. Candidate deletions and duplications predicted by DevRO in each group.

Chr	Start	End	Size (bp)	Type	Group
1	10020939	10024805	3866	Deletion	group2
1	28072850	28076964	4114	Deletion	group2
1	35966681	35970344	3663	Deletion	group1
1	40667085	40676380	9295	Deletion	group2
1	47873948	47875998	2050	Deletion	group2
1	47876478	47878705	2227	Deletion	group2
1	54143394	54146991	3597	Deletion	group2
1	71477307	71480636	3329	Deletion	group2
1	101735057	101740562	5505	Deletion	group2
1	101828749	101832131	3382	Deletion	group2
1	109464153	109467763	3610	Deletion	group2
1	110465935	110469799	3864	Deletion	group2
1	111809164	111812815	3651	Deletion	group2
1	114469995	114477656	7661	Deletion	group2
1	118295065	118300976	5911	Deletion	group2
1	126104457	126106943	2486	Deletion	group2
1	127914075	127917518	3443	Deletion	group2
1	134123231	134126802	3571	Deletion	group2
1	141557162	141568087	10925	Deletion	group2
1	143422101	143428148	6047	Deletion	group2
1	147014667	147018084	3417	Duplication	group1
1	147014896	147018485	3589	Duplication	group1
1	149359205	149363430	4225	Deletion	group2
1	152834092	152837418	3326	Deletion	group2
1	155627655	155632178	4523	Deletion	group2
1	162395864	162400029	4165	Deletion	group2
1	164028470	164035995	7525	Deletion	group2
1	167950071	167955986	5915	Deletion	group2
1	179042360	179051729	9369	Deletion	group2
1	188654581	188662331	7750	Deletion	group2
2	8907804	8911624	3820	Deletion	group2
2	29249655	29251880	2225	Deletion	group2
2	36752580	36760767	8187	Deletion	group2
2	52397532	52405411	7879	Deletion	group2
2	63893771	63905895	12124	Deletion	group2
2	77667693	77670961	3268	Deletion	group2
2	83781069	83790995	9926	Deletion	group2
2	135624242	135629669	5427	Deletion	group2
2	142195529	142196096	567	Duplication	group1
2	151892483	151901166	8683	Deletion	group2
2	169841289	169851166	9877	Deletion	group2
3	1716363	1719832	3469	Deletion	group1

3	72563366	72566806	3440	Deletion	group2
3	97268553	97273216	4663	Deletion	group2
3	112684708	112695199	10491	Deletion	group2
3	126363521	126369602	6081	Deletion	group2
3	142492575	142495252	2677	Duplication	group1
3	145155895	145163627	7732	Deletion	group2
4	18742611	18745883	3272	Deletion	group2
4	28982204	28985227	3023	Deletion	group2
4	55327156	55330354	3198	Deletion	group2
5	20859	24369	3510	Deletion	group2
5	344827	349083	4256	Duplication	group1
5	36303639	36312856	9217	Deletion	group2
6	3363358	3370601	7243	Deletion	group2
6	19228634	19230950	2316	Deletion	group2
7	12981275	12985336	4061	Deletion	group2
7	26803044	26807249	4205	Deletion	group1
7	26803044	26806951	3907	Deletion	group1
7	31718171	31726075	7904	Deletion	group2
7	32507894	32511721	3827	Deletion	group2
7	54843273	54850795	7522	Deletion	group2
7	58958722	58961869	3147	Duplication	group1
7	70261896	70264484	2588	Duplication	group1
7	79489403	79497625	8222	Deletion	group2
7	120402118	120409226	7108	Deletion	group2
7	120749915	120756202	6287	Deletion	group2
7	125173053	125175145	2092	Duplication	group1
7	125386462	125391036	4574	Deletion	group2
7	133758326	133762484	4158	Deletion	group2
7	141972775	141979856	7081	Deletion	group2
7	163088895	163089089	194	Duplication	group1
8	14800377	14803361	2984	Deletion	group1
8	44992852	44995628	2776	Deletion	group2
8	45505862	45509228	3366	Deletion	group2
8	56632623	56640773	8150	Deletion	group2
8	57402830	57408135	5305	Deletion	group2
8	59752725	59758567	5842	Deletion	group2
8	59950628	59954953	4325	Deletion	group2
8	65776341	65780761	4420	Deletion	group2
8	71964830	71969106	4276	Deletion	group2
8	80648555	80655525	6970	Deletion	group2
8	84668351	84671153	2802	Deletion	group2
8	92232111	92239304	7193	Deletion	group2
8	111417475	111419034	1559	Duplication	group2
9	7594534	7599049	4515	Deletion	group1
9	14786168	14793317	7149	Deletion	group2
9	19350787	19352353	1566	Duplication	group1

9	75701994	75706928	4934	Deletion	group2
9	75701994	75705403	3409	Deletion	group2
9	104158641	104161335	2694	Deletion	group2
9	107657364	107664776	7412	Deletion	group2
9	111153173	111160425	7252	Deletion	group2
9	113169200	113170457	1257	Duplication	group1
10	17097046	17100859	3813	Deletion	group1
10	43269463	43276912	7449	Deletion	group2
11	3337013	3340708	3695	Deletion	group2
11	20268944	20276319	7375	Deletion	group2
11	23776245	23782340	6095	Deletion	group2
11	24923632	24931227	7595	Deletion	group2
11	54783931	54788568	4637	Deletion	group2
11	60071092	60075694	4602	Deletion	group2
11	80586769	80593702	6933	Deletion	group2
12	9811249	9819292	8043	Deletion	group2
12	13276045	13283508	7463	Deletion	group2
12	22362392	22366588	4196	Duplication	group2
12	67864698	67868786	4088	Deletion	group2
12	84399800	84402862	3062	Deletion	group2
12	84729252	84737832	8580	Deletion	group2
12	87482467	87491216	8749	Deletion	group2
12	97121922	97125898	3976	Deletion	group2
12	102964911	102972364	7453	Deletion	group2
12	105645122	105653497	8375	Deletion	group2
12	109793243	109801565	8322	Deletion	group2
12	124576676	124582814	6138	Deletion	group2
12	126136891	126141357	4466	Deletion	group2
12	128294878	128296216	1338	Duplication	group1
12	132349837	132354005	4168	Deletion	group2
12	137210541	137218192	7651	Deletion	group2
12	143706074	143708982	2908	Deletion	group2
12	143826021	143831881	5860	Deletion	group2
12	145095491	145096647	1156	Duplication	group1
12	145961895	145967035	5140	Deletion	group2
13	16839514	16844241	4727	Deletion	group2
13	21433995	21441958	7963	Deletion	group2
13	59226582	59234336	7754	Deletion	group1
13	90236204	90239876	3672	Deletion	group2
13	98765308	98769604	4296	Deletion	group2
14	5779535	5782552	3017	Deletion	group2
14	7128562	7132003	3441	Deletion	group2
14	9912909	9919030	6121	Deletion	group2
14	15301386	15304922	3536	Deletion	group2
14	20871985	20879971	7986	Deletion	group2
14	30260260	30266974	6714	Deletion	group2

14	32492338	32493295	957	Duplication	group1
14	38219464	38227796	8332	Deletion	group2
14	48897308	48904413	7105	Deletion	group2
14	113290276	113301431	11155	Deletion	group1
14	116014193	116018905	4712	Deletion	group2
14	116242757	116245446	2689	Deletion	group2
14	120894133	120896921	2788	Deletion	group2
14	124403247	124406724	3477	Duplication	group1
14	124404036	124407175	3139	Duplication	group1
14	131825657	131829899	4242	Deletion	group2
14	132956315	132960054	3739	Deletion	group2
14	137490777	137495281	4504	Deletion	group2
14	146288460	146294205	5745	Deletion	group2
14	150162478	150170001	7523	Deletion	group2
14	151553461	151555787	2326	Deletion	group2
14	151724087	151732320	8233	Deletion	group2
14	153976963	153982969	6006	Deletion	group2
14	154545468	154549225	3757	Deletion	group2
15	26808869	26811837	2968	Deletion	group2
15	30971660	30978614	6954	Deletion	group2
15	30972230	30978614	6384	Deletion	group2
15	40544197	40550341	6144	Deletion	group2
15	61930692	61940459	9767	Deletion	group2
15	63575127	63579020	3893	Deletion	group2
15	63643499	63650867	7368	Deletion	group2
15	65003580	65006277	2697	Duplication	group1
15	72690503	72693247	2744	Deletion	group2
15	78477184	78481021	3837	Deletion	group2
15	80232545	80267536	34991	Duplication	group2
15	80233369	80267734	34365	Duplication	group2
15	82895900	82901437	5537	Deletion	group2
15	86189640	86197057	7417	Deletion	group2
15	96732790	96733582	792	Duplication	group1
15	98159531	98162919	3388	Deletion	group2
15	103898002	103902748	4746	Deletion	group2
16	18033252	18100872	67620	Deletion	group1
16	19590590	19592339	1749	Duplication	group1
16	29964075	30118260	154185	Duplication	group2
16	51032152	51035559	3407	Deletion	group2
16	55597969	55605702	7733	Deletion	group1
17	40281181	40283868	2687	Deletion	group2
17	46968775	46975089	6314	Deletion	group2
17	59950714	59959230	8516	Deletion	group2
17	75224247	75229589	5342	Deletion	group2
17	81264203	81266865	2662	Deletion	group1
18	4310271	4317974	7703	Deletion	group2

18	56156738	56160292	3554	Deletion	group2
18	58650087	58658467	8380	Deletion	group2
19	34305013	34312929	7916	Deletion	group2
X	722954	726552	3598	Deletion	group2
X	1773782	1781304	7522	Deletion	group2
X	20611770	20618218	6448	Deletion	group2
X	41695980	41698714	2734	Duplication	group1
X	42248861	42261352	12491	Deletion	group2
X	44633371	44637590	4219	Deletion	group2
X	74054891	74060468	5577	Deletion	group2
X	76516186	76524291	8105	Deletion	group2
X	86022441	86030686	8245	Deletion	group2
X	96399403	96403985	4582	Deletion	group2
X	96673270	96679147	5877	Deletion	group2
X	108480223	108486844	6621	Deletion	group1

TableS2. Subset of candidate Inversions detected by DevRO not in SVDetect list.

Chromosome	Start	End	Group[a]	Size (bp)
9	18388001	18388750	group2	749
8	729001	729787	group2	786
13	13931001	13931791	group1	790
16	13903001	13903829	group2	828
14	30506001	30506889	group1	888
9	37406000	37406906	group1	906
4	45058000	45058929	group1	929
4	37565000	37565961	group2	961
13	80351000	80351992	group2	992
13	120546000	120547010	group1	1010
14	98774000	98775037	group1	1037
14	86379000	86380041	group1	1041
14	123838000	123839049	group1	1049
16	29365001	29366168	group1	1167
3	22743001	22744190	group1	1189
4	37575000	37576609	group1	1609
14	30518001	30519796	group1	1795
16	27289001	27290858	group2	1857
11	8202001	8203947	group1	1946
3	22682001	22684150	group2	2149
4	35718000	35720375	group2	2375
14	30442001	30444616	group1	2615
2	172979000	172981685	group1	2685
9	48563000	48565730	group1	2730
16	29574001	29576732	group2	2731
3	21551001	21553879	group1	2878
14	98958000	98961458	group2	3458
4	40112000	40115630	group1	3630
3	22216001	22219761	group1	3760
16	52641000	52644941	group2	3941
13	13506001	13510568	group2	4567
4	40116000	40120949	group1	4949
8	43127000	43131968	group1	4968
9	15468001	16079438	group1	611437
16	19914001	20607842	group1	693841
11	37666000	46273752	group2	8607752

[a] Group based on test data representing domestic or wild

Table S3. Simulation Analysis for deletion in the reference assembly

locusID	coordinates[a]	size(bp)	region info[b]	5' mapability[c]	3' mapability[d]	DevRO BP [e]	ReadCounts[f]
region1	chr1:10,974,545-11,004,544	30000	lots of repeats	0.254498332	0.991055897	10974446	32
region2	chr1:20,976,760-20,977,759	1000	simple tandem repeat	0.876914441	0.992240837	20976370	160
region3	chr1:50,989,028-50,991,029	2000	LINES;SINES;simple	0.982284416	0.937495072	50989291	39
region4	chr1:40,252,552-40,257,556	5005	No repeats, only masked ones	0.982258048	0.987218825	40254644	24
region5	chr1:121,020,709-121,027,708	7000	No Repeats	0.996043815	0.995074997	121020479	22
region6	chr1:131,052,703-131,062,702	10000	repeats, LIR,simple	0.994097772	0.93532862	131055431	183
region7	chr1:8,062,089-8,067,088	5000	No overlapping repeat	0.960199177	0.978494121	8066956	23
region8	chr1:28,400,306-28,405,305	5000	LINE on one side of breakpoint	0.980429893	0.000427342	28400443	22
region9	chr1:58,030,839-58,100,838	70000	lots of repeats	0.007576796	0.992102998	NA	NA
region10	chr1:3,029,339-3,032,338	3000	SINE, with no repeats at bp	0.992139649	0.973058135	3029709	46

[a] coordinates of simulated deletions

[b] Information of overlapping repeats at breakpoints (Repeat information extracted from UCSC Santa Cruze Browser)

[c] 5'-1000 weighted mean mapability

[d] 3'+1000 weighted mean mapability

[e] Breakpoint predicted by DevRO for simulated deletions

[f] Number of read counts supporting the simulated deletion