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Applications Note

Genome analysis

ShinyCNV: a Shiny/R application to view and annotate DNA copy number variations

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Abstract

Motivation: Single nucleotide polymorphism (SNP) array is the most widely used platform to assess somatic copy number variations (CNVs) in cancer studies. Many SNP data-based CNV callers are available, however, the false positive rates from automated calling are commonly high, and reported breakpoints can be inaccurate. Manual review for each reported CNV by visualizing the SNP data is important, but is challenging for users lacking computational experience. To address this, we present a Shiny/R application ShinyCNV, an interactive graphical user interface to view and annotate CNVs.

Results: With this application, normalized SNP data, which includes log R ratio (LRR) and B allele frequency, can be plotted against the reported CNVs, and users can visually check the reliability of CNVs *per se* or adjust the incorrectly assigned breakpoints. Further, the interactive LRR spectrum panel within ShinyCNV can facilitate the process to identify commonly affected CNV regions from a group of samples, and to visually check if important focal gains/losses are missing from reported CNVs. ShinyCNV is designed to be intuitive for cancer researchers and can be easily installed for either personal use or deployed on servers to provide online service.

Availability and implementation: ShinyCNV and the tutorial are freely available from https://github.com/gzhmat/ShinyCNV.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

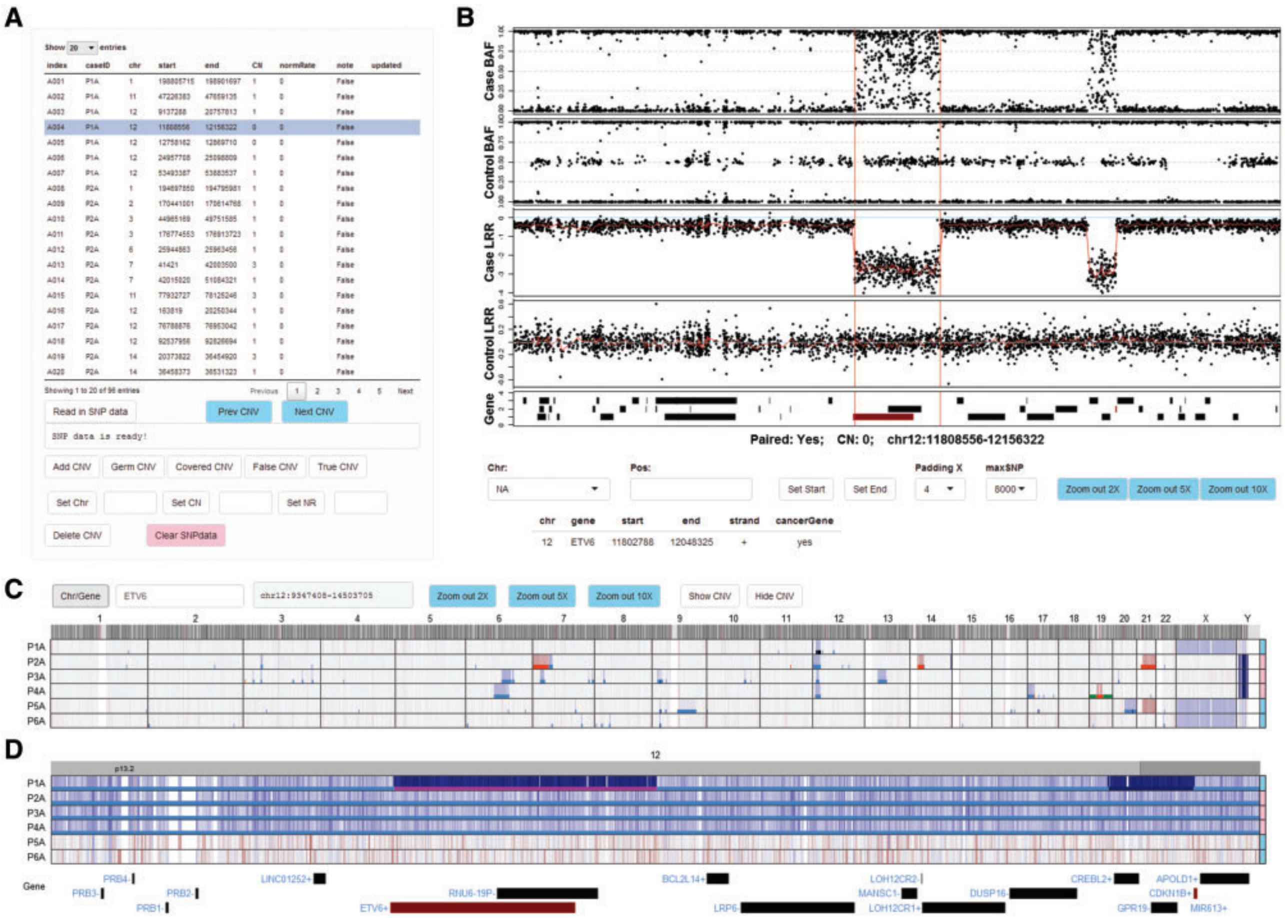
1 Introduction

Somatic copy number variations (CNVs) are important in cancer initiation, progression and relapse (Caren *et al.*, 2010; Mullighan *et al.*, 2007; Mullighan *et al.*, 2009; Weir *et al.*, 2007). In cancer genomic studies, high-density single nucleotide polymorphism (SNP) array has been proven a more systematic and sensitive platform in identifying CNVs compared with conventional cytogenetic karyotyping or fluorescence *in situ* hybridization approaches, especially for focal copy number gains and losses (Mullighan *et al.*, 2007; Zhang *et al.*, 2016). However, the segments detected from various CNV detection software are often false positive or with inaccurate breakpoints due to low quality of SNP data, CNV complexity and/or insufficiently trained segmentation algorithms. To ensure high-quality results, manual review may be required to compare the normalized SNP data and reported CNVs. Several CNV analysis tools have permitted this visual comparison (Li, 2008; Li *et al.*, 2008; Sun *et al.*, 2009), but to our knowledge, none are specifically designed to facilitate this process in a semi-automated manner that allows simple adjustment of breakpoint following data inspection.

Here we present ShinyCNV, an interactive Shiny/R application which takes CNV regions reported from external tools and normalized SNP data (either from Illumina or Affymetrix platform) as input, to help users identify reliable CNVs and adjust inaccurate segment boundaries simply and intuitively.

2 Implementation

ShinyCNV is designed to visualize and annotate CNV analysis result efficiently for users with limited experience in programing.



Show 20 entries

index	caseID	chr	start	end	CN	normRate	note	updated
A001	P1A	1	198805715	198901697	1	0	False	
A002	P1A	11	47226383	47659135	1	0	False	
A003	P1A	12	9137288	20757813	1	0	False	
A004	P1A	12	11808556	12156322	0	0	False	
A005	P1A	12	12758162	12869710	0	0	False	
A006	P1A	12	24957708	25098809	1	0	False	
A007	P1A	12	53493387	53883537	1	0	False	
A008	P2A	1	194697850	194795981	1	0	False	
A009	P2A	2	170441001	170614768	1	0	False	
A010	P2A	3	44965169	49751585	1	0	False	
A011	P2A	3	176774553	176813723	1	0	False	
A012	P2A	6	25944863	25963458	1	0	False	
A013	P2A	7	41421	42003500	3	0	False	
A014	P2A	7	42015620	51084321	1	0	False	
A015	P2A	11	77932727	78125246	3	0	False	
A016	P2A	12	163819	20250344	1	0	False	
A017	P2A	12	76788876	76963042	1	0	False	
A018	P2A	12	92537958	92626694	1	0	False	
A019	P2A	14	20373822	36454920	3	0	False	
A020	P2A	14	36458373	36531323	1	0	False	

Showing 1 to 20 of 96 entries

SNP data is ready!

Paired: Yes; CN: 0; chr12:11808556-12156322

Chr: NA

Padding X: 4

maxSNP: 8000

chr	gene	start	end	strand	cancerGene
12	ETV6	11802788	12048325	+	yes

Chr/Gene: ETV6

chr12:9347408-14503705

Fig. 1. Viewing and annotation of CNVs by ShinyCNV. **A**, Table of CNVs. This table is imported from reported CNVs by OncoSNP (Yau *et al.*, 2010): index, user defined CNV identifier; CN, copy number; normRate (NR), normal sample contamination rate, estimated by OncoSNP and can be marked as 0 if unknown. The CNVs are noted as 'False' initially and need manual review. Any modification of this table would trigger update to a backup file. **B**, B allele frequency (BAF) and log R ratio (LRR) of each probe for case and matched control samples. *X* axis shows the chromosome position. For LRR, the two-copy regions should be around 0, which means normal, and the higher and lower LRR intensity indicates copy number gain and loss, respectively. For BAF, the SNPs should be clustered along *Y* axis at 0, 0.5 and 1 for two-copy regions, while other types of clustering would indicate copy number changes. When a CNV region is selected, BAF and LRR around this region will be plotted for case and control samples, with the reported CNV region marked by two vertical red lines. RefSeq genes are shown at the bottom panel, and the ones reported as consensus cancer genes according to COSMIC database are in red. Detailed gene information can be seen by clicking the gene bars. For CNVs reported with incorrect breakpoints, users can click the BAF/LRR figure (with SNPs around) and the nearest SNP's chromosome coordination will be shown in 'Pos:' input box, and then the selected CNV's start or end position can be updated by clicking 'Set Start' or 'Set End'. Drop-list 'Padding X' is provided to adjust the length of padding regions around the CNV; 'maxSNP' is available to customize the maximum number of SNPs shown in each BAF/LRR panel. The figure can be zoomed in by mouse-swiping in the panel, and zoom out by clicking the 'Zoom out *X' buttons. **C**, Normalized SNP intensity (LRR) spectrum for all the analyzed cases. Chromosomes are labeled on the top and case IDs are at the left side. Cases' genders are annotated at the right side, with pink for female and skyblue for male. Probe intensity is corresponding to DNA copy, which is shown in blue and red to indicate potential copy number loss and gain, respectively. Two buttons are provided to show ('Show CNV') or hide ('Hide CNV') the imported CNVs in different colors: copy-neutral loss of heterozygosity in green, one copy loss in light blue, two-copy loss (0 copy left) in dark blue, one copy gain in red and two or more copy gain in dark red. The selected CNV is highlighted in magenta as shown in Figure 1D. **D**, Zoomed-in view of a cancer gene *ETV6*. Users can zoom in to specific chromosome, region and gene through the input box 'Chr/Gene'. Mouse-swiping zoom in and button supported zoom out are only available within chromosome range. An online tutorial is available at https://github.com/gzhmat/ShinyCNV

To achieve this, this application is developed based on the widely used R environment, and the relied extra packages will be installed automatically during the first run. In general, ShinyCNV is developed by wrapping up the graphics and data-table processing functions in R packages, and the interactive features are provided from Shiny R package.

To accommodate the challenge of presenting millions of SNP probes from dozens of samples on an interactive platform, the visualization is constructed based on the basic R graphic toolkit, and the data tables are mainly processed by 'data.table' and 'dplyr' R packages due to their high efficiency. The normalized SNP intensity is further sampled and smoothed to grant users with interactive and responsive experience, while the visual sensitivity for checking CNVs is maintained as much as possible.

3 Results

To access ShinyCNV, users may download the package including R scripts and reference files from https://github.com/gzhmat/ShinyCNV to their personal computer due to the relatively large size of input SNP data.

3.1 Input data

Two types of input datasets are required to run ShinyCNV:

3.1.1 Normalized SNP data

Currently, Illumina Human Infinium Genotyping array and Affymetrix Genome-Wide SNP6.0 are the two most widely used SNP platforms in cancer research, and both types of data can be accepted by ShinyCNV after normalization.

For the Infinium array, GenomeStudio (Illumina) is freely available to perform normalization based on a set of reference samples (e.g. HapMap samples). Through normalization, two quantitative metrics are generated: one is B allele frequency (BAF), which is interpolated from three known genotype clusters AA (with 0 B allele), AB (with 50% B allele) and BB (with 100% B allele); the other is log R ratio [LRR, log2(Robserved/Rexpected)], where Rexpected is interpolated from the observed allelic ratio compared to the reference genotype clusters (Peiffer *et al.*, 2006; Wang *et al.*, 2007). For tumor samples observed with high hyperdiploidy (chromosome number $\geq$51) or hypodiploidy (chromosome number $\leq$39), it would be beneficial to use internal reference chromosomes (copy number is two) to perform normalization (Pounds *et al.*, 2009). A detailed guide on how to prepare normalized data is available from PennCNV's website http://penncnv.openbioinformatics.org/. System memory required for loading the dataset is linearly correlated with the sample size (Supplementary Fig. S1).

Although the concept of using BAF and LRR in CNV detection is proposed by analyzing SNP data from Infinium platform, the Affymetrix SNP6.0 data can also be converted into this format and a tutorial is available from PennCNV's website too.

3.1.2 Reported CNV regions

To detect initial CNV regions from SNP data, users are open to any CNV callers as long as the segment breakpoint and estimated copy number are available (Supplementary Table S1). For demonstration, we used OncoSNP (Yau *et al.*, 2010) due to its features in detecting somatic CNV by integrating tumor and matched normal control data, and estimating normal sample contamination and intra-tumor heterogeneity etc. More importantly, the input SNP data for OncoSNP is in the same format for ShinyCNV.

3.2 ShinyCNV panels

For demonstration, we used SNP dataset from Illumina Infinium Omni2.5-exome8-v1.3 platform (2 561 003 probes per samples) to assess somatic CNVs in six cases with leukemia.

After uploading the CNV segments into the CNV table panel of ShinyCNV (Fig. 1A), normalized SNP data can be imported conveniently after pointing out the data directory. Through clicking the buttons from CNV table panel, users can perform the following editing: add/delete CNV, annotate CNV (as germline, covered in larger CNV regions, false positive or true somatic) and update CNV (change chromosome, copy number or norm sample contamination rate). Meanwhile, the boundaries of selected CNV in CNV table will be shown in BAF/LRR panel together with normalized SNP intensity for visual check (Fig. 1B). The most common use of BAF/LRR panel is to zoom into the potential breakpoint to update the CNV boundary by mouse-clicking, which is critical if the breakpoint is close to key cancer genes. With CNV table and interactive BAF/LRR figure, it is sufficient to check the reliability of the reported CNVs and correct their breakpoints.

To check CNV spectrum across different samples, whole genome level SNP intensity (here is LRR) is shown at the bottom of ShinyCNV immediately after SNP data imported (Fig. 1C). Users can navigate to any chromosome, gene or genomic region through input box. More conveniently, genomic regions can be zoomed in by mouse-swiping in the figure region to show finer details (Fig. 1D). This panel is not only useful for identifying commonly affected CNV regions, but also for checking if important focal gain/loss is missing from reported CNVs.

4 Conclusion and outlook

ShinyCNV is designed with a clear purpose to visualize and annotate CNVs for users with limited experience in programing. With basic R environment and internet connection, this user-friendly Shiny application will be automatically set up and intuitively applied to visually review the reported CNVs. Since Shiny package was designed to build interactive web applications, it is straightforward to deploy ShinyCNV on servers to provide online service for users.

For now, the default parameters are optimized and encoded in the script to speed up the visualization process for most researchers, while the following versions will have more functionalities and front-end widgets to meet customized requirements based on feedbacks from users. More recently, applying whole genome sequence data to detect CNVs is becoming increasingly common, and ShinyCNV can also be adapted for CNV viewing and annotating once the normalized sequence depth (as LRR) and variant allele frequency (as BAF) are properly prepared.

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