

GREVE: Genomic Recurrent Event ViEwer to assist the identification of patterns across individual cancer samples

Jean-Baptiste Cazier^{1,*}, Chris C. Holmes^{1,2} and John Broxholme¹¹Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, OX3 7BN and ²Department of Statistics, University of Oxford, 1 South Parks Road, OX1 3TG, Oxford, UK

Associate Editor: Alfonso Valencia

ABSTRACT

Summary: GREVE has been developed to assist with the identification of recurrent genomic aberrations across cancer samples. The exact characterization of such aberrations remains a challenge despite the availability of increasing amount of data, from SNParray to next-generation sequencing. Furthermore, genomic aberrations in cancer are especially difficult to handle because they are, by nature, unique to the patients. However, their recurrence in specific regions of the genome has been shown to reflect their relevance in the development of tumors. GREVE makes use of previously characterized events to identify such regions and focus any further analysis.

Availability: GREVE is available through a web interface and open-source application (<http://www.well.ox.ac.uk/GREVE>).

Received on May 11, 2012; revised on August 3, 2012; accepted on September 2, 2012

1 INTRODUCTION

Genomic aberrations have been the subject of much interest in the past decade with variable degrees of success. Two categories have to be distinguished: exactly matching germline and unique, often somatic, aberrations. There has been much effort to identify and catalogue the former in order to treat them like regular markers such as SNPs (Iafate *et al.*, 2004). The first difficulty lies in the exact characterization of the breakpoints. Furthermore, such inventory is impossible for somatic events that are by definition unique. Still, the recurrence of overlapping regions can indicate a key controlling area, e.g. a small deletion on 9p in adolescent acute lymphoblastic leukemia (Paulsson *et al.*, 2008).

Current approaches are essentially based on either the integration into a general-purpose browser to provide context, but no measure of overlap, or the creation of a heatmap where the copy number itself is used as a metric across all types of events to characterize the recurrence (Cancer Genome WorkBench, <https://cgwb.nci.nih.gov/cgi-bin/heatmap>; Mermel *et al.*, 2011). This single continuous value is then used to construct a score at every location. GREVE is designed to look into further details by allowing the user to define further subgroups such as copy neutral LOH that would be ignored otherwise. Furthermore, GREVE provides a highly configurable interface and specific statistics on recurrent events.

GREVE has been successfully used in numerous cancer studies where the cohort size varied from a handful (Langemeijer *et al.*,

2009; Olsson *et al.*, 2011; Paulsson *et al.*, 2010) to hundreds (Gupta *et al.*, 2008; O'Shea *et al.*, 2009). Highly flexible, GREVE provides the ability to statistically explore a given dataset and to present results in a ready-to-publish format.

2 FEATURES

The purpose of GREVE is to enable a flexible view of aberrations across the genome, or per chromosome, and score their recurrence. Therefore, the default usage is very simple with the input of the sole list of events being sufficient, while it is highly configurable with further optional input to help the inspection.

2.1 Key features

GREVE transforms a list of events into a genomic representation, summarizes and scores their recurrence across samples:

- Read flat or Excel input files containing a list of event per individual and type, with build 35, 36 or 37 location (Fig. 1b).
- Plot all events genome-wide and per chromosome with cytobands. The views can either sort all the events or overlay them with a fixed set of individuals (Fig. 1c).
- Calculate, tabulate and plot recurrence within any given type of events (e.g. Gain, Loss and LOH), score each overlapping segment across multiple statistics (Fig. 1c).
- Generate publication-ready figures in several graphical formats (EPS, PDF, PNG, JPG or TIFF; Fig. 1c).
- Inclusion of key genes on the per-chromosome plot as given in a separate list or known CNV from the Database of Genomic Variants (Iafate *et al.*, 2004).

2.2 Formatting features

Because each study will have varying numbers of individuals and events, the default layout may not be optimal. All positions and colors are available in an optional configuration file.

- Size and color choice for each aberration type.
- Distance between successive events and chromosomes.
- Highlight aberration of certain type (default 'LOH').
- Merger of exactly matching events into a larger block.

3 IMPLEMENTATION

The GREVE web front end is implemented in HTML/PHP as a wrapper around the Python (van Rossum and Drake, 2001)

*To whom correspondence should be addressed.

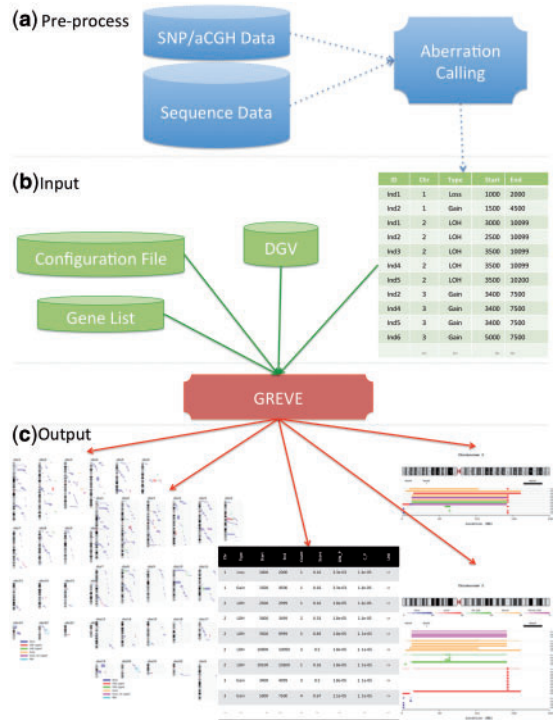


Fig. 1. Usage of GREVE where (a) pre-processing from any source generates a list of events to be used as (b) input together with the optional DGV, Configuration and Gene file. This can generate several output (c): two types of genome-wide views (sorted by aberration type or individual), chromosome view with overlap, gene and labels, as well as the detailed list of overlapping events with corresponding counts and statistics

script engine running on the web server. It requires ImageMagick software (Still, 2005) for figure format conversion from the default Encapsulated PostScript format. The Poisson binomial test is implemented as a wrapper around the *Poibin* R-package (Hong et al., 2011). The web interface allows the upload of all necessary files and a convenient way to select filters and options. It then outputs ready-to-publish figures as well as overlapping details. Examples and frequently asked questions are available on the website.

All the options in the engine software are available through the graphical interface. However, to allow batch processing and further analysis, the source code is available on the website. This should allow specific extensions to match any given project such as subgrouping of individuals (Purdie et al., 2009, 2010). The large demo analysis with 709 events across 30 samples takes 7s on an AMD64 3.0GHz processor with 64Gb of RAM to process with overlaps and scores.

3.1 Input files

Only the list of events with corresponding sample labels and type is necessary to run GREVE. It can be generated from the output of various aberration callers from SNP or CGH array as well as sequencing data with eventual post-processing (Fig. 1a and b). Further optional flags, filters and files can be provided to add information or tailor the presentation:

- The list of events with location, sample labels and type of aberration.

- An optional gene list with name and position.
- An optional configuration file allows further tailoring of the figures without the need to modify the program.

3.2 Output

The result of the analysis is composed of figures and tables (Fig. 1c):

- Genome-wide and chromosome view of the events in all formats.
- Details of the overlap of events across each chromosome are available directly in the interface as a table or in a separate file.
- The counts and proportion of overlap reflect the comparison to a control set where no somatic event would be expected.
- The Poisson binomial *P*-value tests the probability of a type occurring at the same location depending on the individual proportion on a chromosome (C_P) or genome-wide (GW_P).

ACKNOWLEDGEMENTS

The authors thank K. Paulsson, E. Domingo, R. Ramamoorthy, K. Purdie and B. Wright for their suggestions and feedback.

Funding: J.-B.C. was supported by Cancer Research UK and a Wellcome Trust Core grant (075491/Z/04).

Conflict of Interest: none declared.

REFERENCES

- Gupta, M. et al. (2008) Novel regions of acquired uniparental disomy discovered in acute myeloid leukemia. *Genes Chromosomes Cancer*, **47**, 729–739.
- Hong, Y. (2011) On computing the distribution function for the sum of independent and non-identical random indicators. *Technical Reports*. Vol. 11–12, Department of Statistics, Virginia Tech, VA.
- Iafraite, A.J. et al. (2004) Detection of large-scale variation in the human genome. *Nat. Genet.*, **36**, 949–951.
- Langemeijer, S. et al. (2009) Acquired mutations in *TET2* are common in myelodysplastic syndromes. *Nat. Genet.*, **41**, 838–842.
- Mermel, C. et al. (2011) GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol.*, **12**, R41.
- Olsson, L. et al. (2011) Clonal evolution through loss of chromosomes and subsequent polyploidization in chondrosarcoma. *PLoS One*, **9**, e24977.
- O’Shea, D. et al. (2009) Regions of acquired uniparental disomy at diagnosis of follicular lymphoma are associated with both overall survival and risk of transformation. *Blood*, **113**, 2298–2301.
- Paulsson, K. et al. (2008) Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: unexpected similarities with pediatric disease. *Proc. Natl. Acad. Sci. USA*, **105**, 6708–6713.
- Paulsson, K. et al. (2010) Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukaemia. *Proc. Natl. Acad. Sci. USA*, **107**, 21719–21724.
- Purdie, K.J. et al. (2009) Single nucleotide polymorphism array analysis defines a specific genetic fingerprint for well-differentiated cutaneous SCCs. *J. Invest. Dermatol.*, **129**, 1562–1568.
- Purdie, K.J. et al. (2010) High-resolution genomic profiling of human papillomavirus-associated vulval neoplasia. *Br. J. Cancer*, **102**, 1044–1051.
- Still, M. (2005) The Definitive Guide to ImageMagick. In van Rossum, G. and Drake, F.L. (eds.) *Python Reference Manual*. (2001), PythonLabs, APress Academic, Virginia, USA.