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Breeze: an integrated quality control and data analysis application for high-throughput drug screening

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Abstract

Summary: High-throughput screening (HTS) enables systematic testing of thousands of chemical compounds for potential use as investigational and therapeutic agents. HTS experiments are often conducted in multi-well plates that inherently bear technical and experimental sources of error. Thus, HTS data processing requires the use of robust quality control procedures before analysis and interpretation. Here, we have implemented an open-source analysis application, Breeze, an integrated quality control and data analysis application for HTS data. Furthermore, Breeze enables a reliable way to identify individual drug sensitivity and resistance patterns in cell lines or patient-derived samples for functional precision medicine applications. The Breeze application provides a complete solution for data quality assessment, dose–response curve fitting and quantification of the drug responses along with interactive visualization of the results.

Availability and implementation: The Breeze application with video tutorial and technical documentation is accessible at https://breeze.fimm.fi; the R source code is publicly available at https://github.com/potdarswapnil/Breeze under GNU General Public License v3.0.

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

1 Introduction

Advances in automated liquid dispensing, assay miniaturization, systems biology as well as *ex vivo* disease models have boosted the development of high-throughput cell-based functional testing platforms. Thousands of conventional cell lines have been screened with hundreds of compounds in several large-scale projects, such as the Cancer Cell Line Encyclopedia (Barretina *et al.*, 2012), Genomics of Drug Sensitivity in Cancer (Yang *et al.*, 2013), Cancer Therapeutics Response Portal (Seashore-Ludlow *et al.*, 2015) and Genentech Cell Line Screening Initiative (Haverty *et al.*, 2016). Similar drug testing efforts have been applied on primary cell models to generate individualized drug profiles for drug repurposing, patient stratification and for the identification of potential drug combinations (Kodack *et al.*, 2017; Lee *et al.*, 2018; Pemovska *et al.*,

2013, 2015; Saeed *et al.*, 2017). One common end-point in cell-based drug testing is cell viability and/or toxicity readouts generated over multiple concentrations in microwell plates (96-, 384- and 1536-well formats), where plate layout and placement of controls play an important role to minimize the risk of experimental errors influencing data quality (Mpindi *et al.*, 2015). Therefore, quality control (QC) process is required to ensure compliant and reproducible drug testing readouts from the assay. The dose–response curve-fitting process enables direct translation of the raw cell viability measurements based on several intensity scoring technologies to clinically interpretable dose values. The fitted dose–response curve is then used to summarize and quantify the observed response into a single metric, such as IC50 and EC50 dose or as an absolute area under the curve (AUC) or drug sensitivity score (DSS; Yadav *et al.*, 2015). To date, there are several, freely available analysis tools
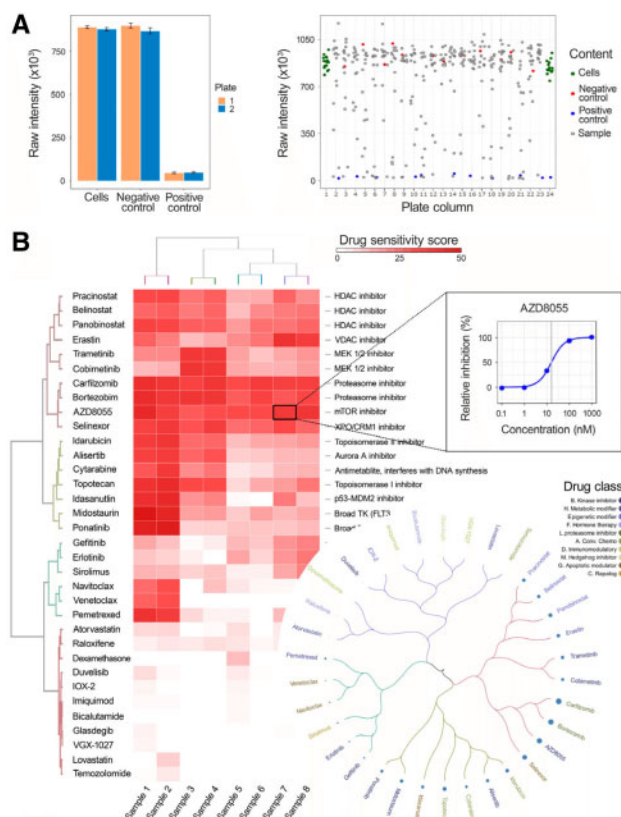
Fig. 1. Example outcome plots of Breeze application. (**A**) The technical quality is visualized by listing QC metrics such as Z' and SSMD, along with bar plots, plate heat maps and scatterplot of signal intensities of plate wells help in identifying distribution of controls and pinpoint outliers. (**B**) Drug quantification visualization in form of interactive heatmap and circular plots. The heatmap shows efficacies of the drugs (higher in red and lower in white) across different samples along with drug names and potential drug targets. Hovering over the heatmap shows individual dose–response curves of specific drugs

available for handling the drug screening data, such as cellHTS (https://www.dkfz.de/signaling/cellHTS/) (Boutros *et al.*, 2006), HTS Navigator (Fourches *et al.*, 2014), Knime (https://knime.com), PharmacoGx (Smirnov *et al.*, 2016) as well as commercial solutions such as Dotmatics (https://dotmatics.com) and Genedata Screener (https://genedata.com). Although these are useful resources for drug testing data analysis, we believe there is a room for improvement in the alignment of the data flow process through QC, dose–response curve fitting, multiparametric scoring and interactive visualizations. Breeze is an easy to use publicly available tool, which includes comprehensive plate QC statistics with a diverse collection of drug quantification metrics and interactive visualization options, which offers the users the ability to perform comparison of drug response profiles across multiple samples (Fig. 1). Systematic use of standardized data quality processing and drug quantification methods enables direct comparison of responses from a large number of studies (Mpindi *et al.*, 2016).

2 Materials and methods

2.1 Data submission and processing
The Breeze application allows multiple data input formats, which need to comprise of drug names, concentration ranges and phenotypic measurements. These measurements can both be provided as raw data or pre-calculated percent inhibition (PI) values. In case, when the raw data are provided, PI for each data point is calculated based on the values of positive and negative controls on the corresponding plate. Detailed description of input data format is given in

technical documentation. A template of the data input structure is available for download to facilitate data processing and analysis.

2.2 Quality control
Common technical errors in HTS assays include spatial plate variability and/or striping due to dispensing errors as well as edge effects due to uneven evaporation of the plate edges. Hence, assessing and quantifying the potential errors of the raw data is a crucial first step of the analysis. The standard QC metrics Z' (Zhang, 1999) and SSMD (Zhang, 2007) explore the distribution of the positive and negative control wells (Chen *et al.*, 2016). However, those metrics may not capture all spatial plate effects and hence Breeze generates a comprehensive table of different metrics along with several visualizations. The QC table includes parameters such as Z', SSMD, signal/background ratio, SD, coefficient of variation and central tendency of controls (Supplementary Fig. S1). The QC visualizations in Breeze include interactive plate heatmaps (Supplementary Fig. S3), scatterplots and barplots (Fig. 1A and Supplementary Figs S1, S2 and S4–S6).Visualizations are helpful in interpretation and spotting technical problems such as issues in dispensing cells, drugs, reagents on culture conditions, edge effects, striping, patterning as well as observing signal window, performance, and distribution of compounds and controls, variations across plates and outliers.

2.3 Curve fitting
Curve fitting is an important part of the dose–response data analysis and involves arranging the PI values at each point of the concentration range and fitting these points using four-parameter logistic curve (Findlay and Dillard, 2007; Vølund, 1978). In order to quantify the quality of curve fitting, a standard error of the estimate is calculated for each dose–response curve. The resulting curve fit images (Fig. 1B, top-right) and the fitting parameters are exported in the Excel files.

2.4 Quantification of drug responses
Breeze offers several possibilities to summarize dose–response relationship into a single metric including IC50, EC50, AUC and DSS. The DSS scoring metric is adding normalization to standard AUC (Yadav *et al.*, 2015). This standardization facilitates the correlation of drug sensitivity and resistance testing results across several studies. The results are depicted by interactive visualizations such as heatmaps and bar plots (Fig. 1B and Supplementary Fig. S7). The heatmap provides a comprehensive overview of the data based on distance matrix methods such as Pearson, Euclidean, Manhattan, Spearman and so on. The bar plots list the top-responding drugs in a sample (Supplementary Fig. S8), while the circular tree correlates drug response patterns among group of samples (Supplementary Fig. S9). The user can also upload the DSS of the reference/control screen, to calculate differential response of drugs, between samples and control.

2.5 Tutorial and feedback
Breeze is implemented using R and PHP and hosted with Apache HTTP Server. To facilitate its usage, a step-by-step video tutorial and example input data are available on the website. The user may leave their comments or suggestions using a feedback form. Breeze source code is provided at https://github.com/potdarswapnil/Breeze, to run analyses independently and potentially extend functionality.

3 Conclusion

The Breeze application facilitates a quick and robust analysis of drug testing data by integrating systematic QC procedures and drug response quantification to a standard metric that enables method comparison across several studies. The interactive visualizations, intuitive graphics and easily exportable results provide a framework for reproducible and quality processing of drug testing data. Breeze is a unique computational environment, which provides extensive

functionality in terms of QC, summary metrics and visualization of different aspects of HTS experiments.

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