GladiaTOX: global assessment of dose-response indicators in toxicology

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Vincenzo Belcastro*, Stephane Cano, Diego Marescotti, Stefano Acali, Ignacio Gonzalez-Suarez, Florian Martin, Filipe Bonjour, Nikolai V. Ivanov, Manuel C. Peitsch, and Julia Hoeng

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

* Corresponding author - vincenzo.belcastro@contracted.pmi.com

Introduction and Objectives

GladiaTOX R package is an open-source (GPL-2), flexible solution to high-content screening (HCS) data processing and reporting in biomedical research. GladiaTOX takes advantage of ToxCast pipeline (tcpl) core functionalities (Filer et al.).

GladiaTOX major extensions over tcpl are

- Provide web-service solution to fetch raw data
- Computes severity score
- Export ToxPi formatted files (Marvel et al., Reif et al.)
- Export quality control (QC) reports
- Export data processing reports

Package submitted to Bioconductor

Contact: vincenzo.belcastro@contracted.pmi.com

Software package written in R - freely available at:
git clone --branch revbioinf https://github.com/pmpsa-hpc/GladiaTOX.git

Material

Normal human bronchial epithelial (NHBE) cells were purchased from Lonza (Catalog no. CC-2540, Lonza, Cologne, Germany). The donor was a 60-year-old Caucasian male with no history of smoking. The cells were maintained in a humidified incubator at 37°C and 5% CO2 and cultured in bronchial epithelial cell medium (Bullet Kit CC 3170, Lonza) according to the vendor's recommendations.
Cells were exposed to a range of harmful and potentially harmful constituents of tobacco smoke in a concentration-dependent manner. A number of toxicity endpoints were measured via HCS (Marescotti et al.).

Toxicant	Vehicle	Doses (µM)					
		1	2	3	4	5	6
Acid derivatives							
Acrylamide	*Water*	10000	5000	2500	1250	1.2E-01	4.0E-02
Aromatic amines							
O-Anisidine	*Ethanol*	10000	5000	1000	200	7.0E-05	6.0E-07
Phenols							
Phenol	*Ethanol*	5000	2000	1000	500	2.4E-01	1.6E-02
M-Cresol	*Ethanol*	5000	2000	1000	500	5.0E-02	3.0E-04
O-Cresol	*Ethanol*	5000	2000	1000	500	7.5E-04	9.0E-07
P-Cresol	*Ethanol*	5000	2000	1000	500	1.5E-01	8.0E-04
PAHs							
Naphthalene	*Ethanol**	5000	2500	1250	625	2.0E-02	8.0E-05
Metals/ elements							
Mercury	*Ethanol*	160	120	80	40	4.0E-05	8.0E-06
Nickel	*Water*	2000	1000	200	100	3.4E-05	1.4E-05
Arsenite	*Water*	50	25	12.5	6.25	1.7E-04	3.0E-05
Selenite	*Water*	500	250	200	125	3.5E-04	2.0E-05

Table 1. *List of chemicals and concentrations used in NHBE cells. (*: vehicle was 1.75% EtOH + 0.25% dimethyl sulfoxide [DMSO]).*

References

Filer, D.L., et al. tcpl: the ToxCast pipeline for high-throughput screening data. Bioinformatics 2017;33(4):618-620.

Fourches, D., et al. HTS navigator: freely accessible cheminformatics software for analyzing high-throughput screening data. Bioinformatics 2014;30(4):588-589.

Marescotti, D., et al. High Content Screening Analysis to Evaluate the Toxicological Effects of Harmful and Potentially Harmful Constituents (HPHC). Journal of visualized experiments : JoVE 2016(111).

Marvel, S.W., et al. ToxPi Graphical User Interface 2.0: Dynamic exploration, visualization, and sharing of integrated data models. BMC bioinformatics 2018;19(1):80.

Reif, D.M., et al. Endocrine profiling and prioritization of environmental chemicals using ToxCast data. Environmental health perspectives 2010;118(12):1714-1720.

Methods

GladiaTOX fits three models (Figure 1A) for each dose-response series in the experiment. Minimal effective concentrations (MEC) are then identified as the intersection of the fitted curve with the noise band (grey band in Figure 1B).

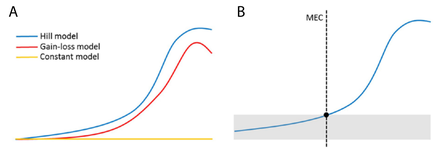

Figure 1. Example of model fitting and MEC. *(A) The three models fitted by the GladiaTOX package. (B) Identification of MEC values. The MEC (black dot) value is the concentration (x-axis) at which the fitted model (blue line) intersects the cutoff (grey band, median absolute deviation of vehicle responses).*

The noise band is computed as three times the baseline median absolute deviation of vehicle responses.

Severity scores are toxicological indicator values. For each chemical, the severity score is computed with the following formula:

$$-log10\left(\frac{1}{ne}\sum_{e} MEC_e\right)$$

where *ne* is the number of tested endpoints, *e* is a generic endpoint, and MEC*e* is the MEC for endpoint *e*. The severity score expresses the average impact of chemicals across all tested endpoints. Larger values correspond to higher chemical toxicity. Severity scores are then scaled in the interval [0, 1].

Results (1) - QC

Raw data heatmaps and positive control dose-response plots are two QC plots useful for diagnostics. Figure 2 shows an heatmap of data quantification values (Figure 2A) before normalization. Figure 2B shows the response of the positive control for the DNA damage assay. The plate in the example has passed QC, as at least one concentration has response values beyond the noise band.

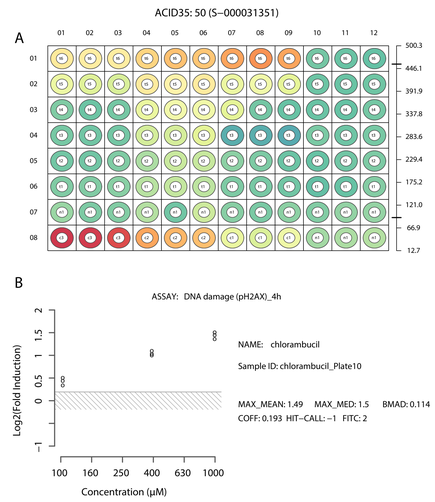

Figure 2. Example of QC output. *(A) Image of raw data heatmap of a plate. Plate title contains the assay component identification number (ACID35), the plate identification number (50), and the plate name (S-000031351). Plate dimension is 8x12. Letters and numbers in each well indicate the well type (t: treatment, c: positive control, n: neutral control or vehicle), and the concentration index (from low to high), respectively. Row 8 displays the values of the assay positive control (chlorambucil in the example). (B) Positive control dose-response plot. Plotted values are normalized against the vehicle (log2 fold change). The grey band indicates vehicle variability. The right panel reports additional information such as noise band margin (COFF).*

ACID: Assay component ID;
MAX_MEAN: Max mean response value;
MAX_MED: Max median response value;
BMAD: Baseline median absolute deviation;
COFF: Cutoff response value;
HIT-CALL: 1 if active, 0 if inactive; -1 if NA;
FITC: Fitting category.

Results (2) - Processing and Reporting

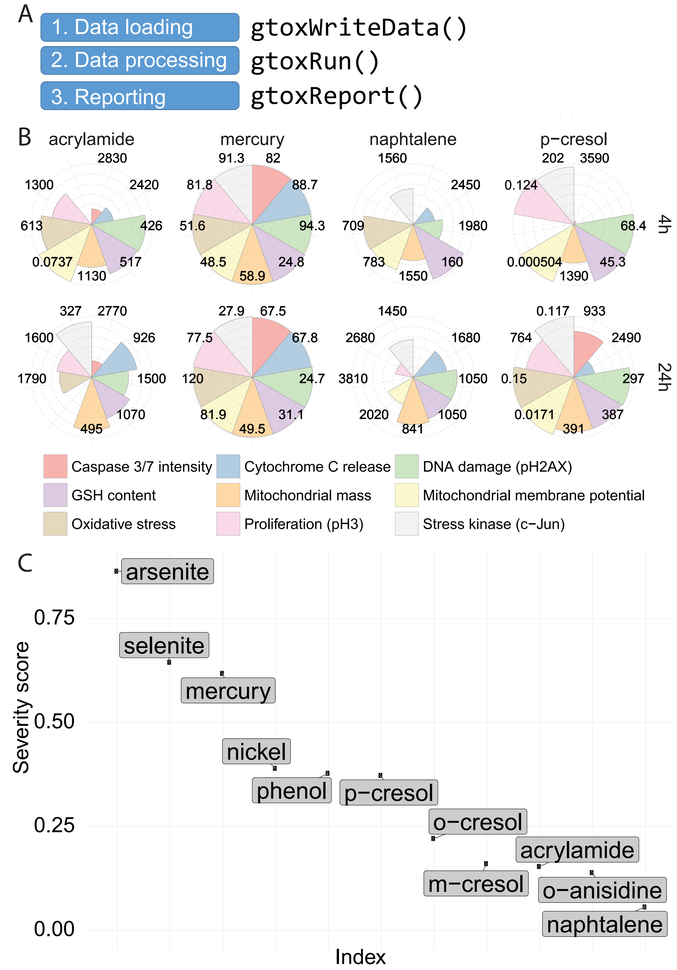

Figure 3: Processing workflow and reporting plots. *(A) Simplified analysis workflow with package function calls. (B) MEC plot. A circular plot is shown for each chemical and endpoint. Numbers indicate MEC values (uM). Filled slices indicate higher toxicity (low MEC). MECs for only four chemicals are reported, at 4 and 24 hours. (C) Severity score plot. On the y-axis, chemicals are sorted according to severity score values. Higher scores are associated with higher toxicity.*

Conclusions

The GladiaTOX package, with its suite of functionalities (Figure 3A), represents an all-in-one, open-source, flexible solution to store, process, and report HCS data in biomedical research.

Lower MECs are associated with higher toxicity (see Figure 3B). The MEC average impact is then summarized as a severity score (Figure 3C) that represents the overall impact of chemicals across multiple endpoints (arsenite has the highest score in the above-described example).

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