PHIAL version 1.0
NMED-NT65480A/PI65480

Requirements

1. R software package (version 2.14 or higher) – available for free at:
   http://www.r-project.org/
   [Note: PC users may have to add R to the PATH. See http://cran.r-project.org/doc/manuals/R-admin.html]
2. R Packages (to install locally, use *install.packages()* and then *library()* functions in a local version of R – see "R Setup"):
   optparse (http://cran.r-project.org/web/packages/optparse/index.html)
   gplots (http://cran.r-project.org/web/packages/gplots/index.html)
   Nozzle.R1 (http://cran.r-project.org/web/packages/Nozzle.R1/index.html)
3. Patient data (see "Details On Inputs")

R Setup
(Please follow the instructions at http://www.r-project.org/ to install a local version of R on your computer if not already done.)

1. Load R in a local terminal window:

   ```
   wm19e-787:PHIAL_demo eliezer$ R
   ```

2. Install <u>all three</u> required packages (see above) using the *install.packages()* function. A screenshot example is provided for installing the Nozzle.R1 package:

   ```
   > install.packages("Nozzle.R1")
   ```

   You may be prompted to select a mirror to download the package and can choose the location geographically closest to you.

3. Load each package library into R with the *library()* function:

   ```
   > library(Nozzle.R1)
   > library(gplots)
   > library(optparse)
   ```

   You have now successfully set up a local version of R to run PHIAL.

4. Exit R as follows:

   ```
   > q()
   Save workspace image? [y/n/c]: n
   wm19e-787:PHIAL_demo eliezer$
   ```

Moving Data
Copy the PHIAL_demo folder from the provided CD to your computer hard drive before running PHIAL.

Details On Inputs
Patient Data
The wrapper software requires a "patient_data.txt" file that must list required parameters in the following format (see the provided examples for each of the three demonstration cases):

Parameter	Comments
individual	The study ID for the individual being analyzed (no spaces)
tumor_type	The tumor type for ClinicalTrials.gov link generation (e.g. "Colon", "Prostate"; no spaces)

output_dir	The output directory desired for PHIAL (Default: same folder as input sources)
mutation_data	An annotated MAF file with mutations (see http://www.broadinstitute.org/oncotator/ for desired file format)
indel_data	An annotated MAF file with insertion/deletions (see http://www.broadinstitute.org/oncotator/ for desired file format)
rearrangement_data	An annotated dRanger rearrangements file output (see examples for desired file format)
copynumber_data	A segmented copy number profile for the individual (see examples for seg.txt file format)

The input files listed in "patient_data.txt" must be provided as tab-delimited text files in the formats of the respective analysis tool (e.g. Oncotator annotated MAF).

Please see the provided example folders (e.g. patient_demo_1) which have all necessary files.

Databases
All necessary databases for running PHIAL are provided with the demonstration package in the databases folder. The "databases.txt" file should be provided to the wrapper script to point to the relevant databases.

Running PHIAL
PHIAL is provided as an executable RScript to be run from the command line and requires the R software package. A wrapper script is provided for ease of use.

From within the PHIAL folder, execute the command:

```
Rscript PHIAL_wrapper.R -i <patient_data> -d <databases>
```

Example:

```
wm19e-787:PHIAL_demo eliezer$ Rscript PHIAL_wrapper.R -i ./patient_demos/patient_demo_2/
phial_patient_demo_2_input.txt -d ./databases/databases.txt
```

(More examples are below as well)

Depending on the number of alterations present in an individual patient's exome, a PHIAL run may take seconds to minutes.

A sample run:

```
[1] "Loading patient data"
[1] "Loading input databases"
[1] "Scoring somatic alterations"
[1] "Scoring somatic mutations"
[1] "Scoring somatic indels"
[1] "Scoring copy number alterations"
[1] "Scoring rearrangements"
[1] "Merging data sets"
[1] "Making PHIAL gel"
[1] TRUE
[1] "Creating Nozzle HTML report"
[1] "Somatic PHIAL completed."
MBR19E-787:PHIAL_demo eliezer$
```

Output Files
There are seven output files generated by PHIAL, which are placed in the output directory:

1. <individual>_complete_muts_indels_scna_detailed.txt = Complete listing of ranked alterations with the maximum amount of details about each alteration.

2. <individual>_complete_muts_indels_scna.txt = Complete listing of ranked alterations with less details for quicker review (e.g. in an online report).

3. <individual>_investigate_clinical_relevance_high.txt = The highest scoring alterations.

4. <individual>_investigate_clinical_relevance_low.txt = The next highest scoring set of alterations.

5. <individual>_investigate_biological_relevance.txt = Biologically relevant alterations as determined by PHIAL.

6. <individual>_phial_gel.png = PHIAL patient gel PNG image.

7. <individual>_cancer_genome_report.html = HTML report of the PHIAL interpretation for the patient's exome. ***By opening this HTML file in an Internet browser, one can review the patient PHIAL report*** (works best with Safari or Chrome)***.***

Examples
Three patient cases are provided as examples in the patient_demos folder, which include the requisite mutations, insertion/deletion, copy number alteration, and rearrangement input files. To run any of these demos from within the PHIAL_demo folder, execute the following from the command line:

```
Rscript PHIAL_wrapper.R -i ./patient_demos/patient_demo_1/phial_patient_demo_1_input.txt
-d ./databases/databases.txt

Rscript PHIAL_wrapper.R -i ./patient_demos/patient_demo_2/phial_patient_demo_2_input.txt
-d ./databases/databases.txt

Rscript PHIAL_wrapper.R -i ./patient_demos/patient_demo_3/phial_patient_demo_3_input.txt
-d ./databases/databases.txt
```

A reminder that for these demonstrations, all output files (including the HTML report) are written to the individual patient's folder.

Questions, Comments, Feedback?
We are continually updating and improving PHIAL, incorporating new functionality and attempting to make it as user-friendly as possible. If you have any questions, comments, feedback, or suggestions, please do not hesitate to contact us as:

phial-help@broadinstitute.org

Disclaimer
PHIAL is a research tool only. It cannot be used exclusively to make medical decisions and is not intended for that purpose. It currently can only be used by editors and reviewers confidentially.