➢ Description:

Genome-wide RNAi screening usually adopts hundreds of 96-well or 384-well plates to perform experiments, to study gene functions and discover novel drug targets. However, spatial background noises often blur interpretation of experimental results, which usually have distinct spatial patterns for different plates. Therefore, it is important to identify and correct the spatial background noises when we analyze RNAi screening data. Here, we developed a Software SbacHTS (Spatial background correction for High-Throughput RNAi Screening), for visualization, estimation and correction of spatial background noises of RNAi screening experiment results. It provides a function to assess batch effects across all plates for quality control purpose. SbacHTS can effectively detect and correct spatial background noise leading to higher signal/noise ratio and improved hits discovery for RNAi screening experiments. Besides, input file is as easy as it could be, and no parameters are needed to be tuned or monitored.

➢ Input file format:

PlateDate	PlateNo	WellNo	T1Raw	T2Raw	T3Raw
20090708	Plate00001	A02	0.736439	0.960163	0.870145
20090708	Plate00001	A03	0.722897	0.816592	0.794077
20090708	Plate00001	A04	0.78079	0.752354	0.753819
20090708	Plate00001	A05	0.70115	0.852148	0.780619
20090708	Plate00001	A06	0.860475	0.856264	0.838545
20090708	Plate00001	A07	0.952056	0.919054	0.901847

Note 1. It has to be a csv file whose extension name is ".csv"

Note 2. The first three columns have to be PlateDate, PlateNo, and WellNo, respectively. PlateDate is the specific data for experiments of a plate in the format of "YYYYMMDD". WellNo has to indicate well number for each row of data in the format "LNN" where "L" indicates row name letter and "NN" indicates column name number. PlateNo can be any format is identifiable.

Note 3. The fourth column and beyond should be raw data and have to be more than two column because approaches and methods need replicate date set for analysis.

Note 4. Dataset has to have the first row as column names indicated as above sample.

➢ Available dataset for demonstration of analysis :

In order to demonstrate how to use our application, we provide a built-in dataset for demonstrations and below is the work flow on how to download and perform analysis in our environment.
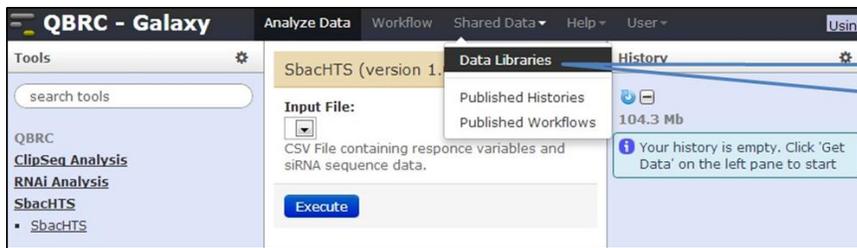
Step 1: Click shared data on the top of the middle and select data libraries tab.
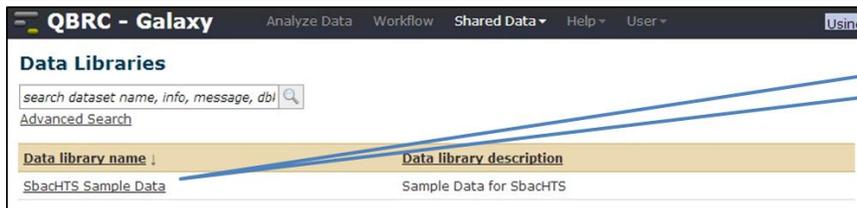
Step 2: Select "SbacHTS_Sample_Data" and click.
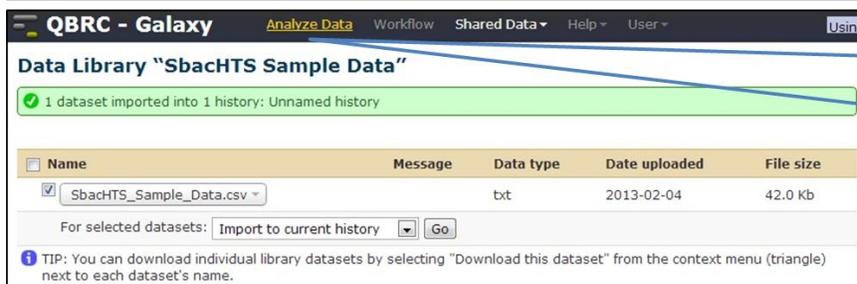
Step 3: Check the box associated with "SbacHTS_Sample_Data.csv" and click "Go". Then click "Analyze data" tab and go back to analysis interface.
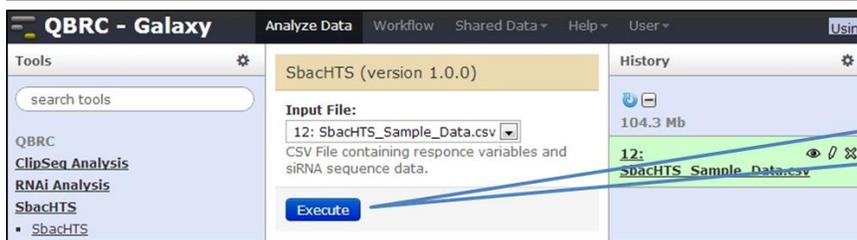
Step 4: Now data is available in the Input File and click "Execute" for demo of analysis
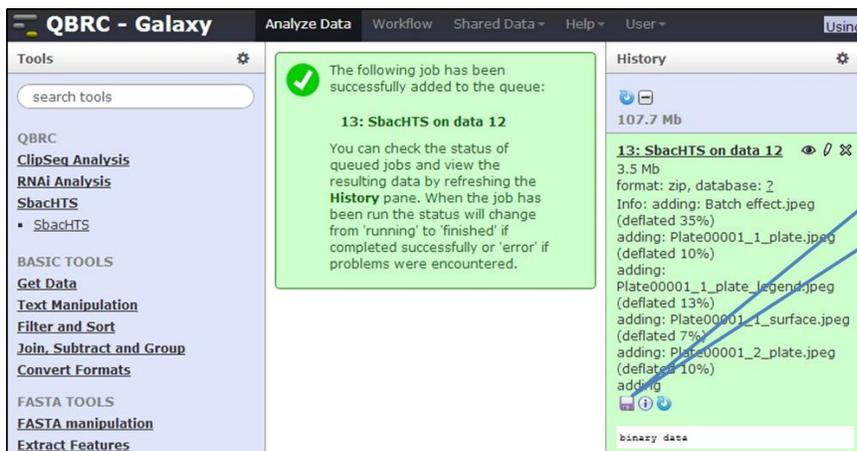
Step 5: When analysis is done, download output results by clicking save icon.

➢ Upload custom data and perform data analysis:

Below is demo of how to upload your own dataset and perform analysis in our application

Step 1: Select "Get Data" → "Upload File"

Step 2: Choose selected file from your computer

Step 3: Click "Execute" button

Step 4: Uploaded file shown here

Step 5: Go back to SbacHTS analysis

Step 6: Click "Execute" button

Step 7: Analysis results shown here for download

➢ Output files:

On our web, we attached a sample data for testing and learning with name "sample_data.csv" and you can download from our web as a learning materials. Based on this sample data, below is demonstration of what output files are available. Analysis is based on our online available data set.
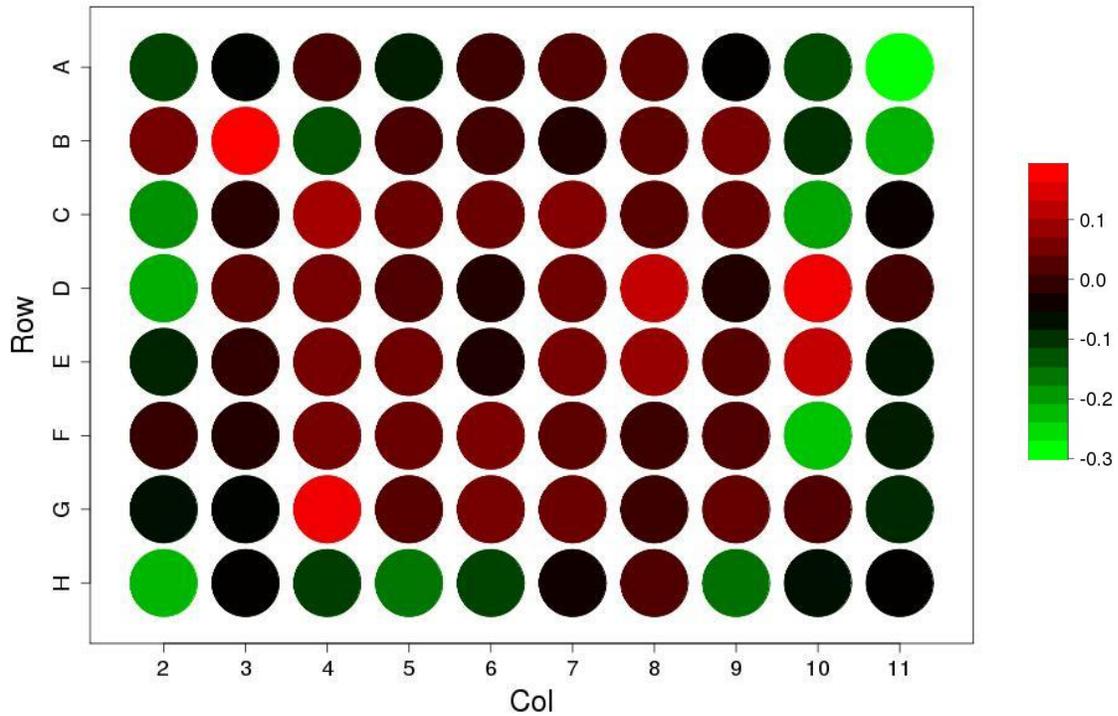
(1). Plate plot of spatial noise: when treating replicate experimental data, after extract mean from each replicate, the remaining part is spatial noise which we molded to fit a spatial pattern for the purpose of removal and correction.
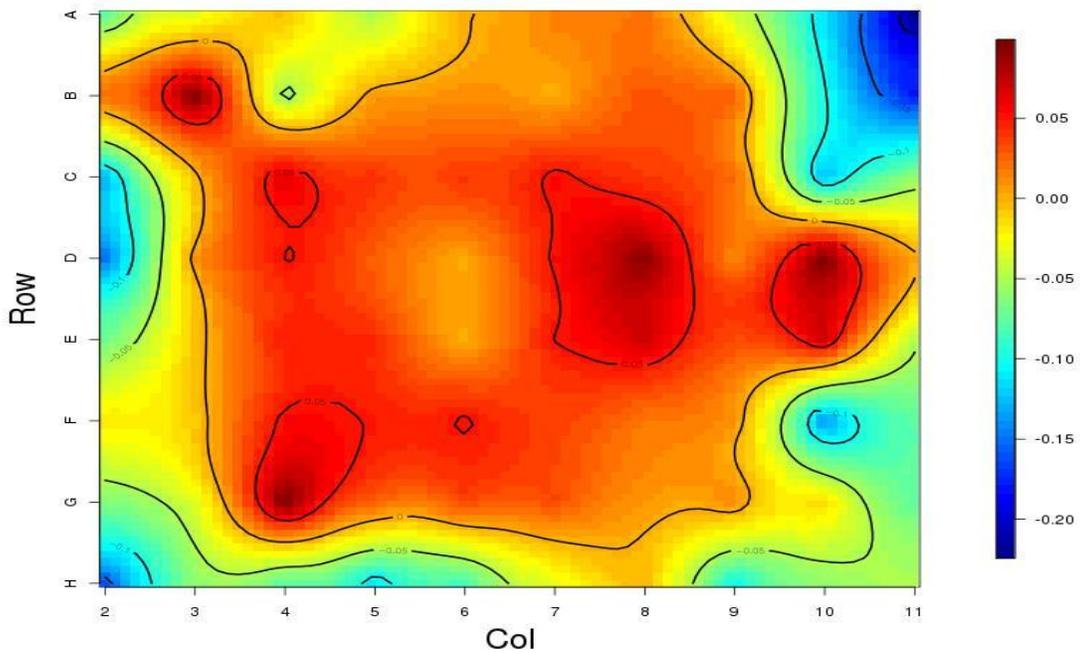
(2). Plot of fitted spatial noise. Based on above data, we fitted the spatial noise using Kriging interpolation and plot the pattern in the form of heat map for best visualization.

plate.date	plate.number	well.number	T1Raw	T2Raw	T3Raw
20090708	Plate00001	A02	0.759705	0.940623	0.866419
20090708	Plate00001	A03	0.747597	0.796472	0.789498
20090708	Plate00001	A04	0.806923	0.731653	0.748386
20090708	Plate00001	A05	0.728718	0.830866	0.774333
20090708	Plate00001	A06	0.889477	0.834402	0.831405
20090708	Plate00001	A07	0.982492	0.896611	0.893854

(3). Corrected data set: after modeling and fitting of spatial noise pattern, we will remove the fitted noise from original data and generate a new data file as below.
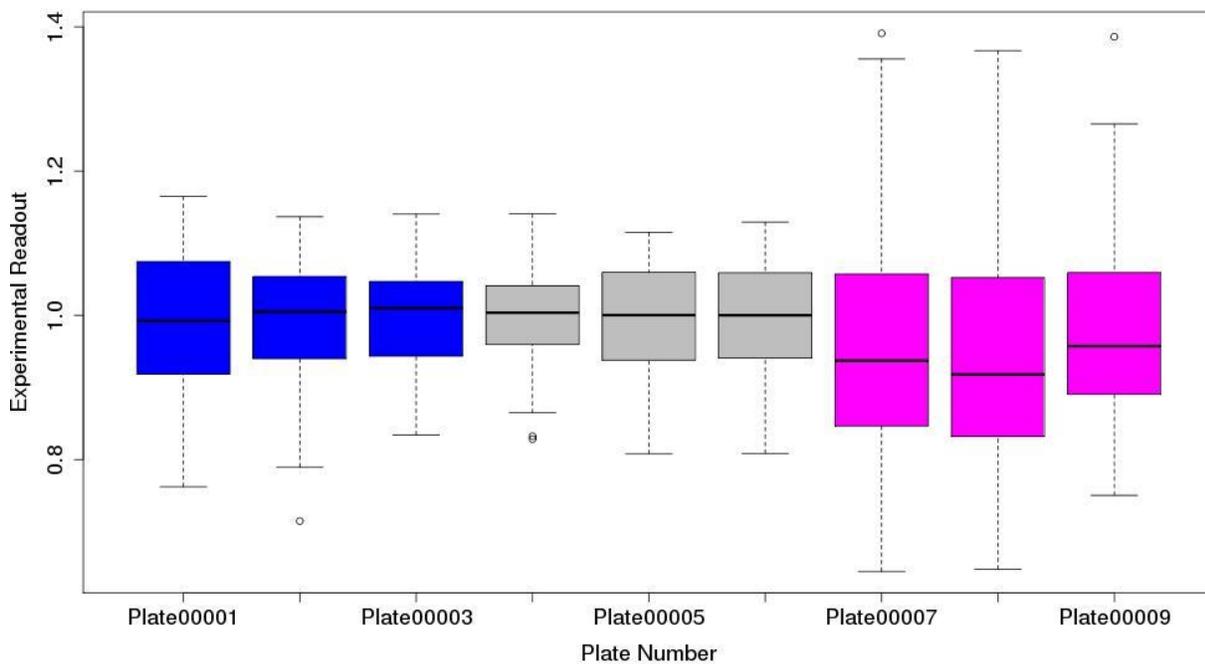
(4). Batch effect plot: if experiments are carried out across different dates, we will provide a plot for visualization of batch effect using corrected data. Plot is as below; different colors indicate different dates on which each individual experiment is being done: