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Using cellHTS2 for cell based assays

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cellHTS2: purpose

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- analyze high throughput cell-based assays with low complexity readouts
  e.g. plate reader assays, luminescence assays…
- from raw data to annotated hit list
- data preprocessing, normalization
- data quality assessment
- replicate scoring, annotation
- analysis audit trail

cellHTS2: assay types

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- single color assays
- multi color assays
- activator or inhibitor type assays
- dual way assays
- independent of the instrument, as long as the output is ASCII text
- not coupled to a particular screening library, or to particular organisms

cellHTS2: limitations

Assumes that every well contains the same type of reagents:

the same controls (can be circumvented but becomes very awkward…)

the same library

cellHTS2: publication

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Genome Biol. 2006; 7(7): R66. PMCID: PMC1779553
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Analysis of cell-based RNAi screens

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Abstract

RNA interference (RNAi) screening is a powerful technology for functional

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cellHTS2: structure

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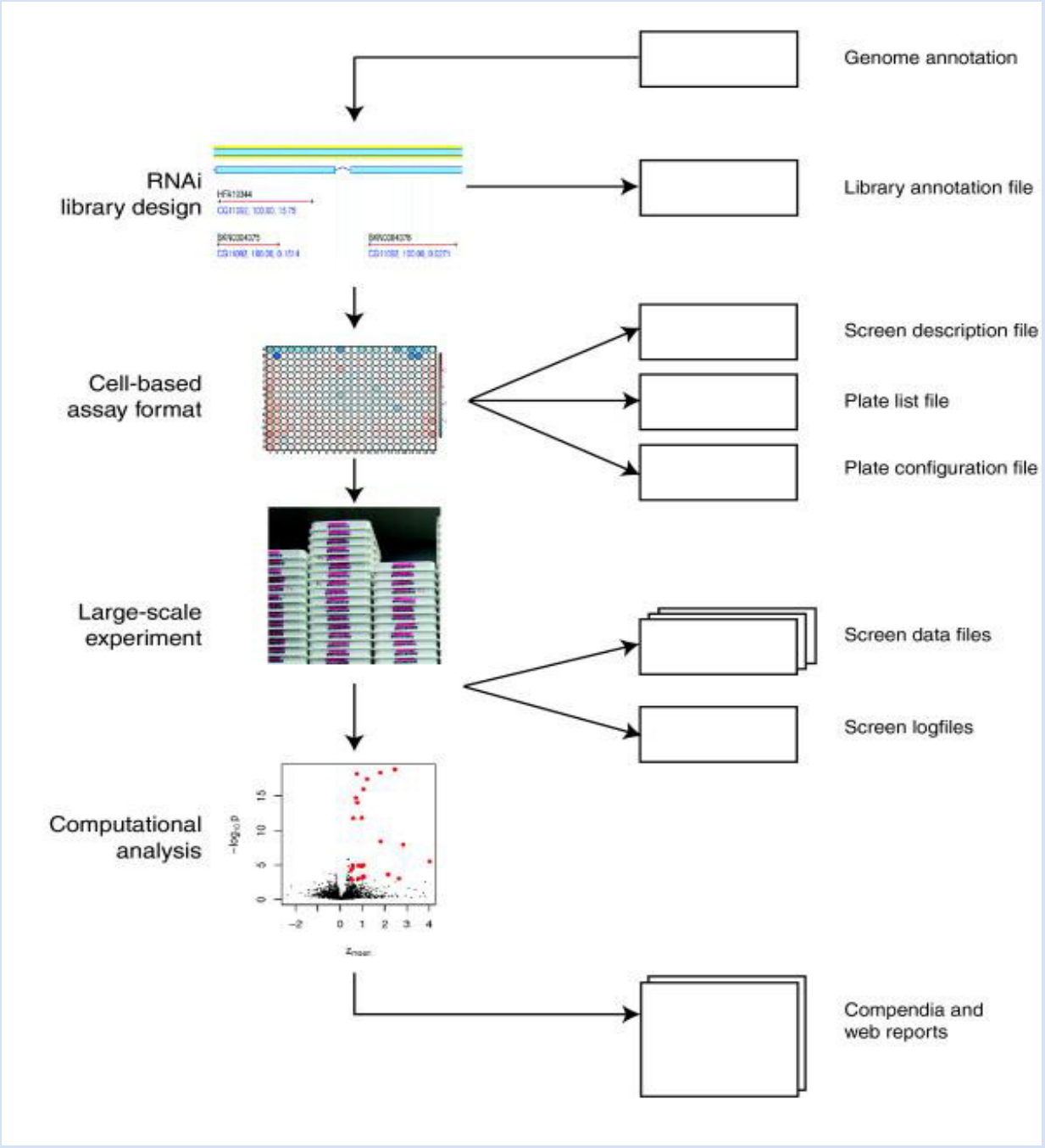

Data structure: screening data

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Numeric values x_{ijk}

- i = wells (e.g. 20,000)
- j = different reporters (e.g. 2)
- k = different assays (e.g. 5)

Metadata about wells

- p_i = plate in which is well i
- r_i = row (within plate) of well i
- c_i = column (within plate) of well I
- siRNA sequence, target gene, ...

Metadata about reporters

- Fluc, Rluc, ...

Metadata about assays k

- replicate number
- different variants of the assay (e.g. Wnt1_LRP6_Frz8, β-Catenin, LRP6ΔE1-4, Wnt1_R-Spondin3, Wnt3a)
- date it was done

Data structure: NChannelSet

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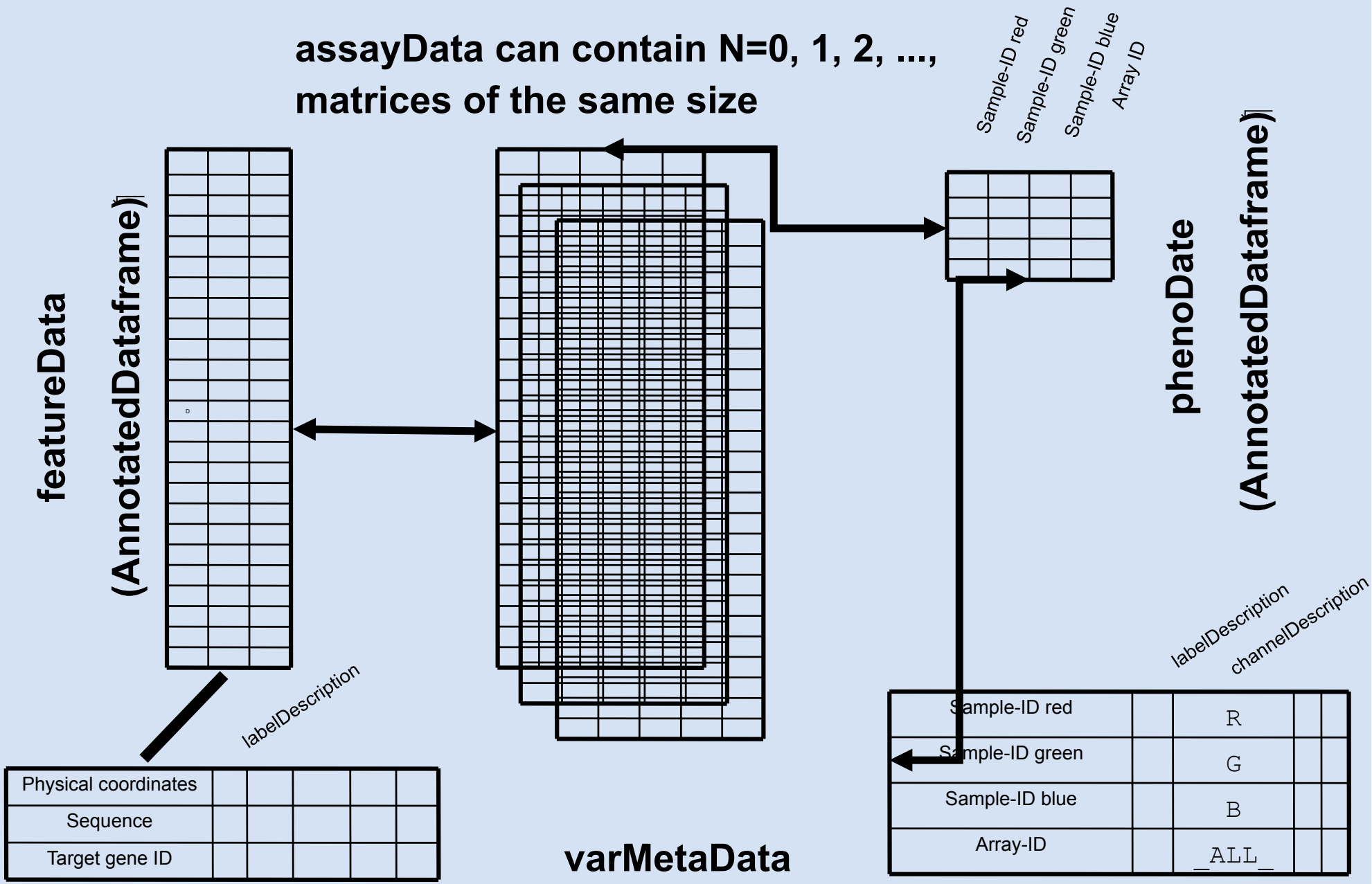

Data import: function *readPlateList()*

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- reads data from most established instruments, but the can handle arbitrary data formats through user-defined import functions

- automatically detects plate layout

- raw data files need to be specified using a plate list file

    columns Filename, Plate, Replicate, (Batch), …

- can read collated data from a single file

Data analysis: four stages

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1) Configuration
   controls, screen design, flagging

2) Annotation
   features, e.g. target genes

3) Normalization
   between and within plates, data transformation

4) Replicate scoring and summarization
   standardization and reduction to a single value per feature (e.g. z-scores)

human readable audit trail in form of an interactive HTML document can be produced at each of these steps

Configuration: function *configure()*

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- screen level: screen description file (MIAME)
- plate level: plate configuration file

  regular expressions as annotation rules

Plate	Well	Content
*	*	sample
*	A0[1-2]	other
*	B01	neg
*	B02	pos

  visualization of plate layouts
  configurationAsScreenPlot()

- flagging: screen log file

Annotation: function *annotate()*

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- description of features in annotation file
- mandatory columns: Plate, Well, GeneID
- optional column GeneSymbol: Human-readable name

Normalization: between plates

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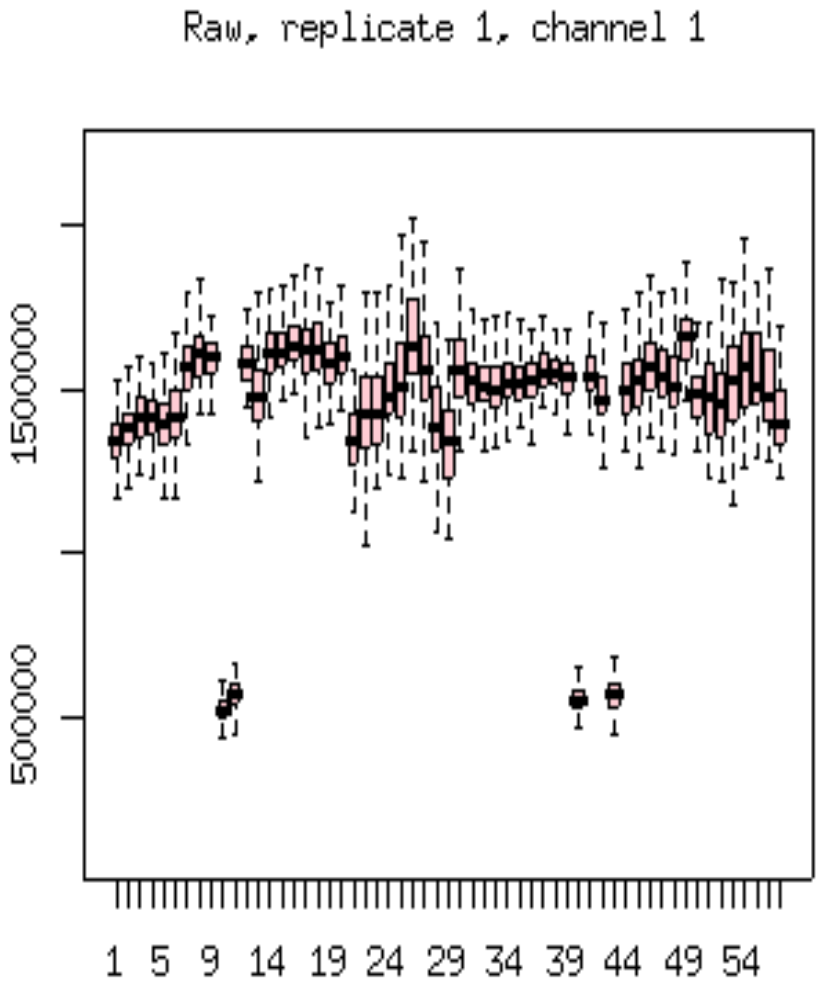

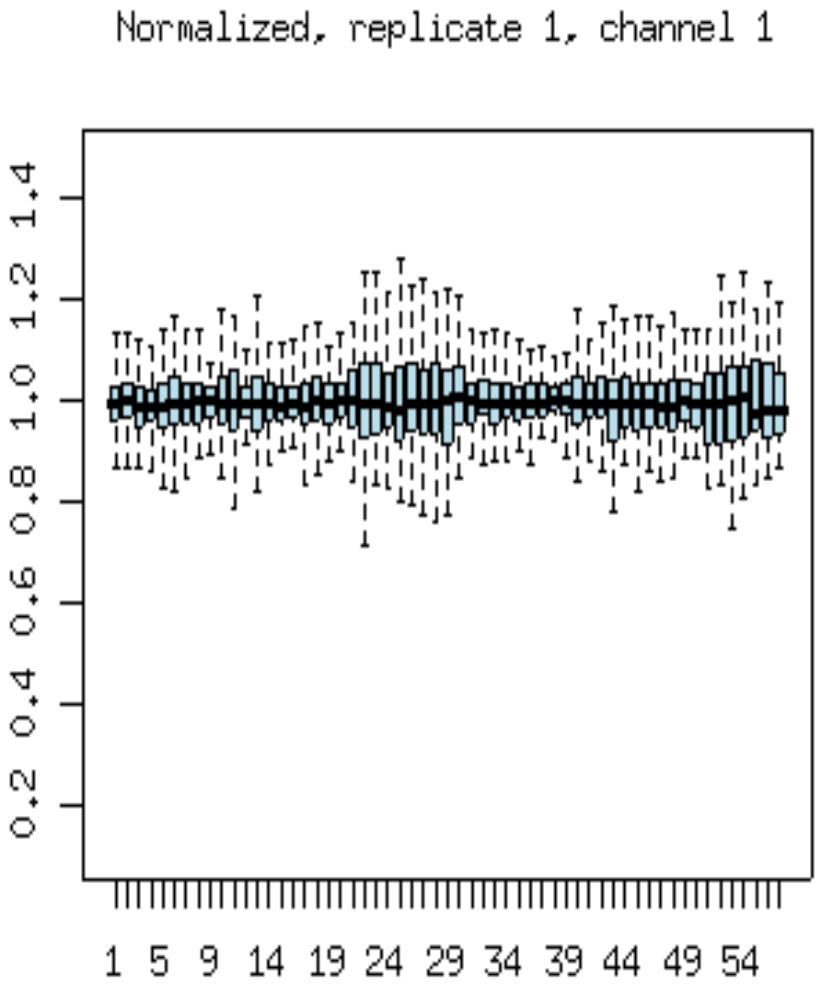

Normalization: *estimating parameters*

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From which data points:

- **Based on the intensities of the controls**

  if they work uniformly well across all plates

- **Based on the intensities of the samples**

  invoke assumptions such as "most genes have no effect", or "same distribution of effect sizes"

Which estimator:

mean *vs* median *vs* shorth

standard deviation *vs* MAD vs IQR

No universally optimal answer, it depends on the data.
In the best case, it doesn't matter.

Normalization: between plates

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- by estimator of location for each plate:
  mean, median, shorth, percent control
- data transformation (optional):
  log or linear scale
- Variance adjustment (optional):
  by plate, by batch, by experiment
- function *normalizePlates()*

Normalization: *spatial within plates*

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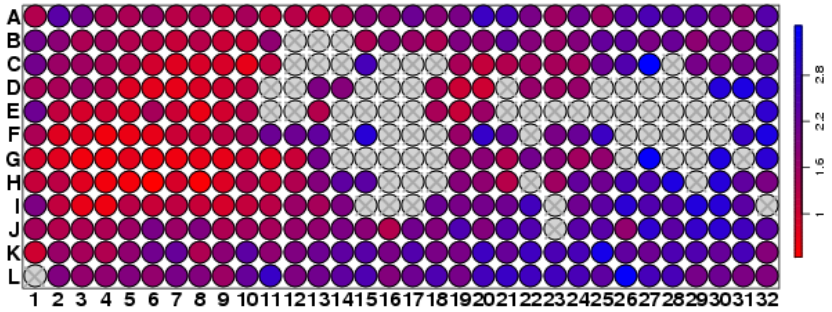
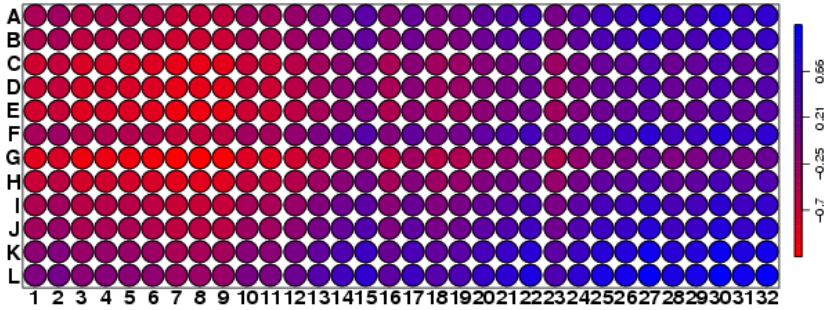
_B_-score:

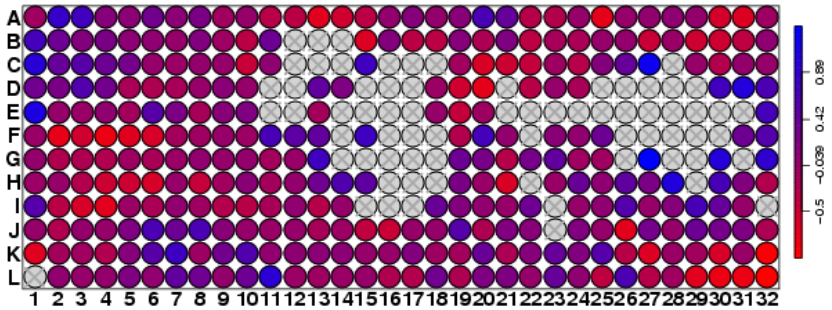
two-way medianpolish

r^{th} row
c^{th} column
i^{th} plate

Malo et al., Nat. Biotech. 2006







Scoring and summarization:

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- *scoreReplicates()*
  z-score, normalized percent inhibition
- *summarizeReplicates()*
  e.g. mean, max, min
- *summarizeChannels()*

HTML report: function *writeReport()*

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report

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