

HTpcrA: A webtool for the analysis and visualization of qPCR data

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Abstract

Single-cell qPCR is now a routinely used method to measure the expression of a selected number of genes within single cells. A typical experiment will generate 96 cells, for each of which, the expression of 96 genes will be measured. This can also be combined with index sorting where the intensities of the surface markers used to sort the cells is also recorded.

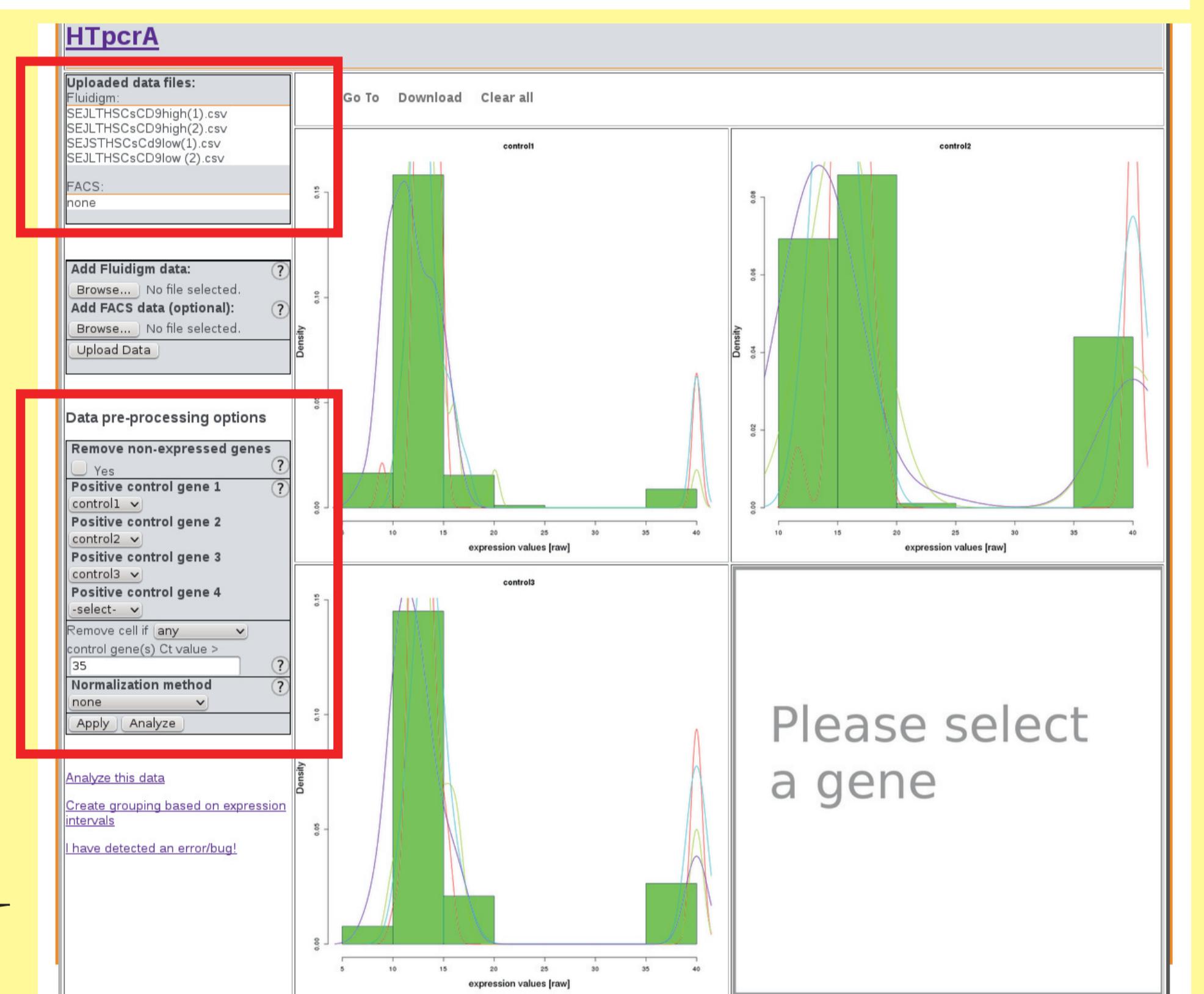
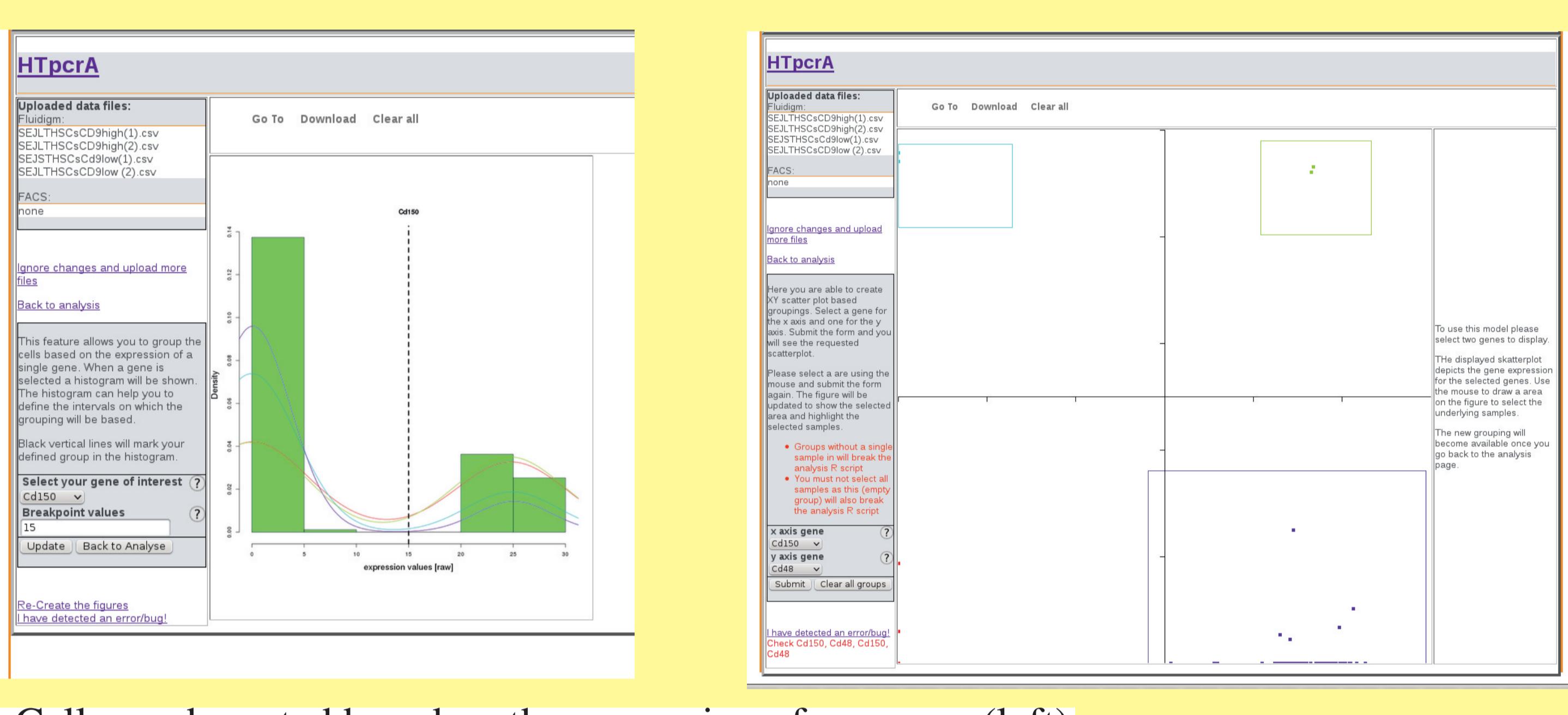
At the moment there is some debate as to how this data should be handled and analyzed, but many of these methods are not accessible to bench scientists who wish to try and process their own data.

We present High-throughput PCR Analysis (HTpcrA), a feature-rich webtool designed to up-load and analyze Biomark Fluidigm data. The tool is intuitive to use and many analysis methods are available to the user. The tool will also join the expression and index sorting data allowing one to be compared to the other. All results are downloadable, including figures and scripts.

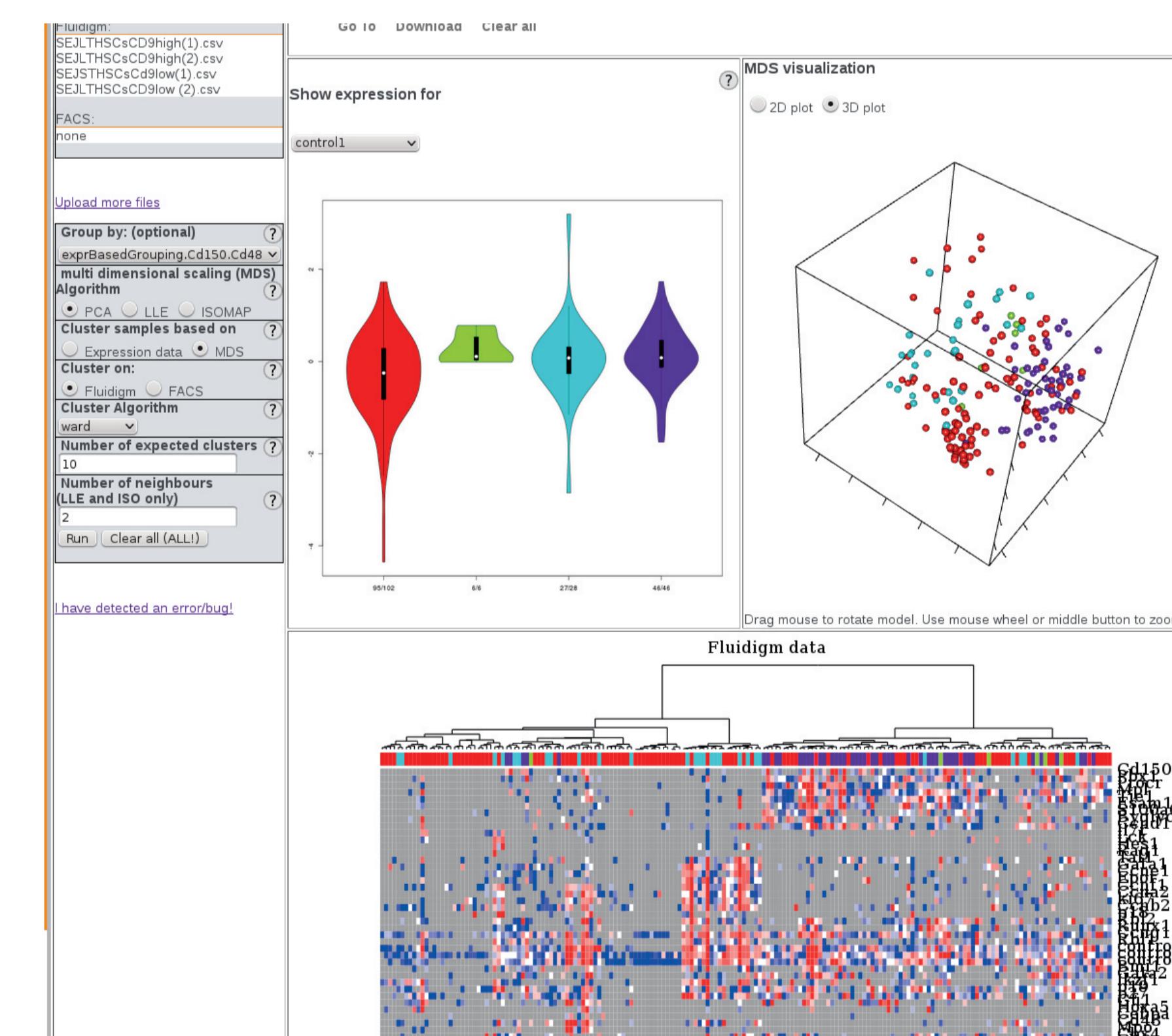
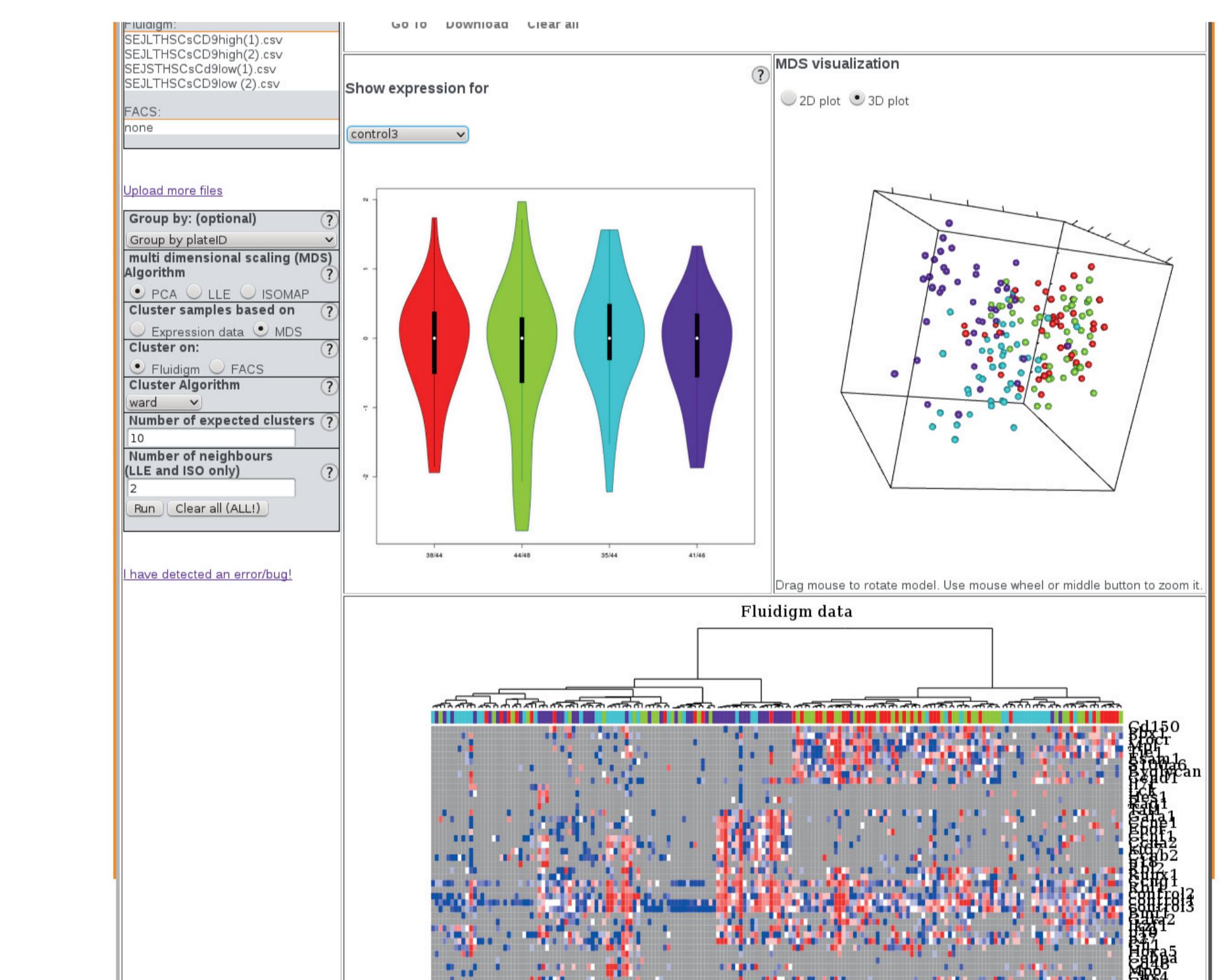
You have generated
a lot of qPCR data using
the Fluidigm platform.
E.g. 200 samples with 48 genes per sample
in separate csv data files.

(1) File Upload and pre-processing

Preprocessing involves the inspection of house-keeping genes on which basic filtering criteria can be applied. The histograms will also allow the identification of outlier samples and plates.



(2) Expression based grouping



The analysis section allows you to cluster and perform MDS based on expression or FACS data. Expression levels of genes/markers and the quality of the grouping can be accessed using expression summary or per sample data and the colored (3D) MDS.

The MDS 3D result can be rotated and scaled directly in the browser using WebGL.

The heatmap is a scalable vector graphics and therefore very flexible, and of publication quality.

All result files can be downloaded from the server including analysis scripts, pre-processed data, all figures, and the 3D model as functional web page.

The analyzed data has been published in [1].

Your data will not be saved on the server.

The HTpcrA server is accessible at
<http://stemsybio.bmc.lu.se/HTPCR/>



Used Technology: Perl Catalyst, R, Java script, HTML, WebGL, methods described in [2]

[1] The tetraspanin CD9 affords high-purity capture of all murine hematopoietic stem cells. Karlsson et. al. CellRep 2013

[2] Data exploration, quality control and testing in single-cell qPCR-based gene expression experiments. McDavid 2013 Bioinformatics