S2C Work-flow

Overview

S2C (Signal to cell Count) is a web application for predicting cell count of a species/organism, from which RNA/DNA was extracted and utilized in a microarray based oligonucleotide hybridization. The web application has been built on the concept that positive control probes account for errors/uncertainties associated with each step of a microarray experiment. Generally, in a microarray experiment, multiple control probes are used. S2C utilizes the signal intensity of these multiple positive controls, based on a multiple linear regression model, and then predicts the cell count associated with each probe/signal intensity present on the microarray chip. This application can also be used with multi-species universal microarray chip for predicting cell counts.

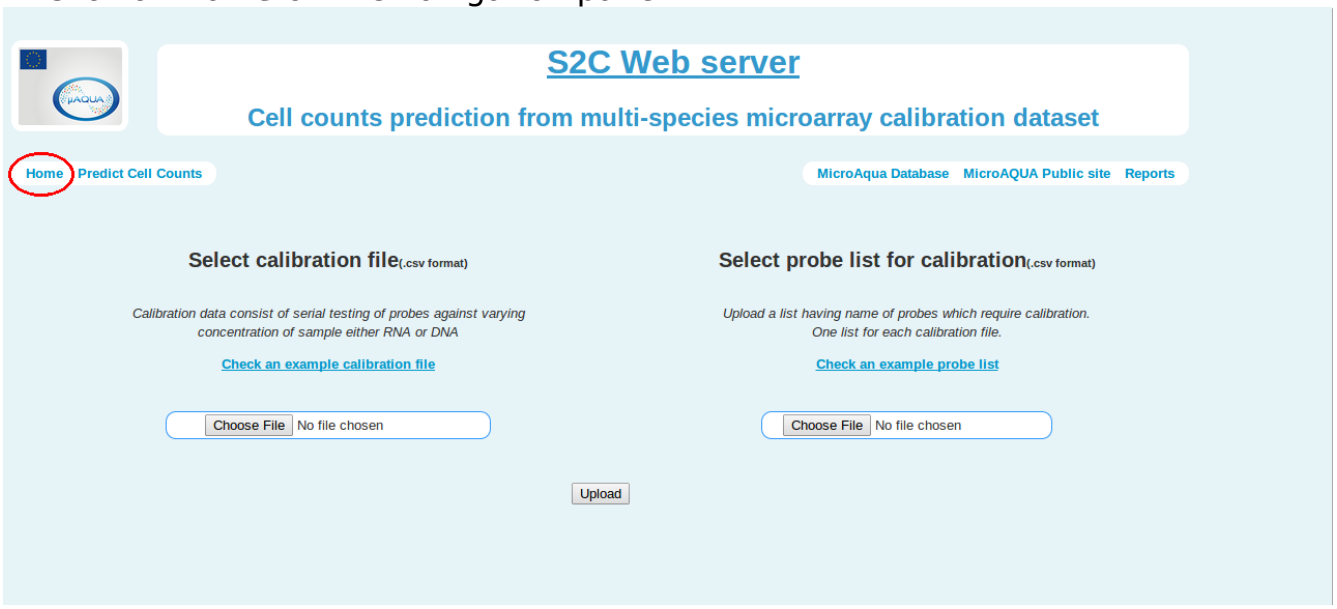
The document briefly describes a work flow of predicting cell count using calibration data. The application has two sections. The first section generates a coefficients file after normalizing the calibration signal intensities. Coefficients are nothing but a list of fitted linear regression model parameter values. The second section predicts the cell count using the coefficients file.

General Structure

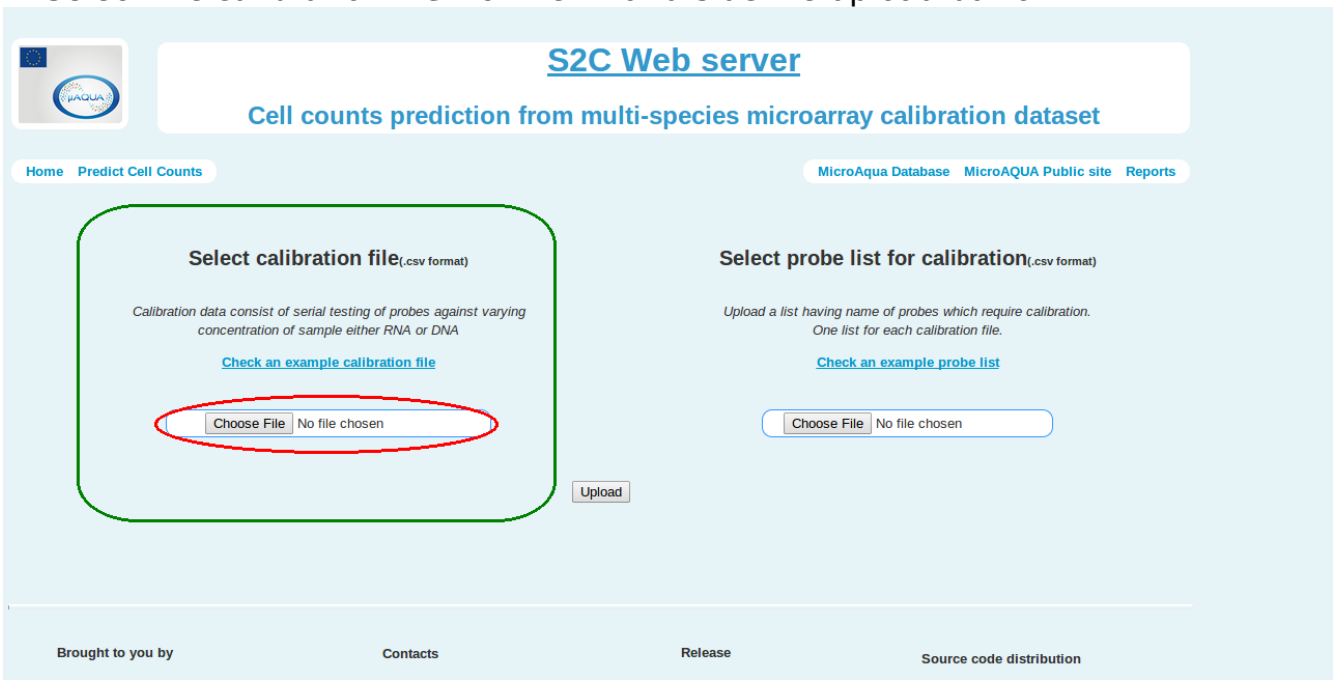
The web page of the application is divided in three sections: Header, body and footer. Header has a general title of the application and a navigation bar. Left side of the navigation bar has links to navigate within the S2C application and right hand side of the panel has links to jump over to different sections of the MicroAQUA project. The body consists of the input and output display units of the application. The footer displays general information related to the application, the MicroAQUA project and contact information.

NOTE: Input file templates are available in the form of a file as down loadable links.

1. Click on *Home* on the navigation panel.

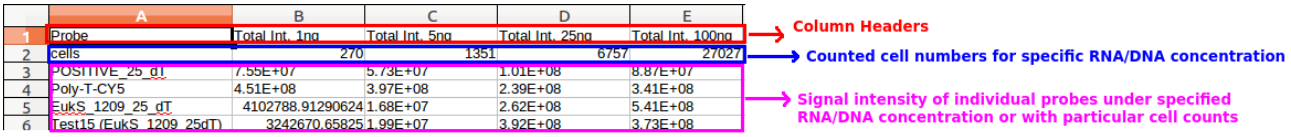


2. Select the *calibration file* from left-hand-side file upload button.



Calibration data is obtained from microarray experiments performed with known concentration of RNA/DNA or cell counts. The calibration data file format looks like

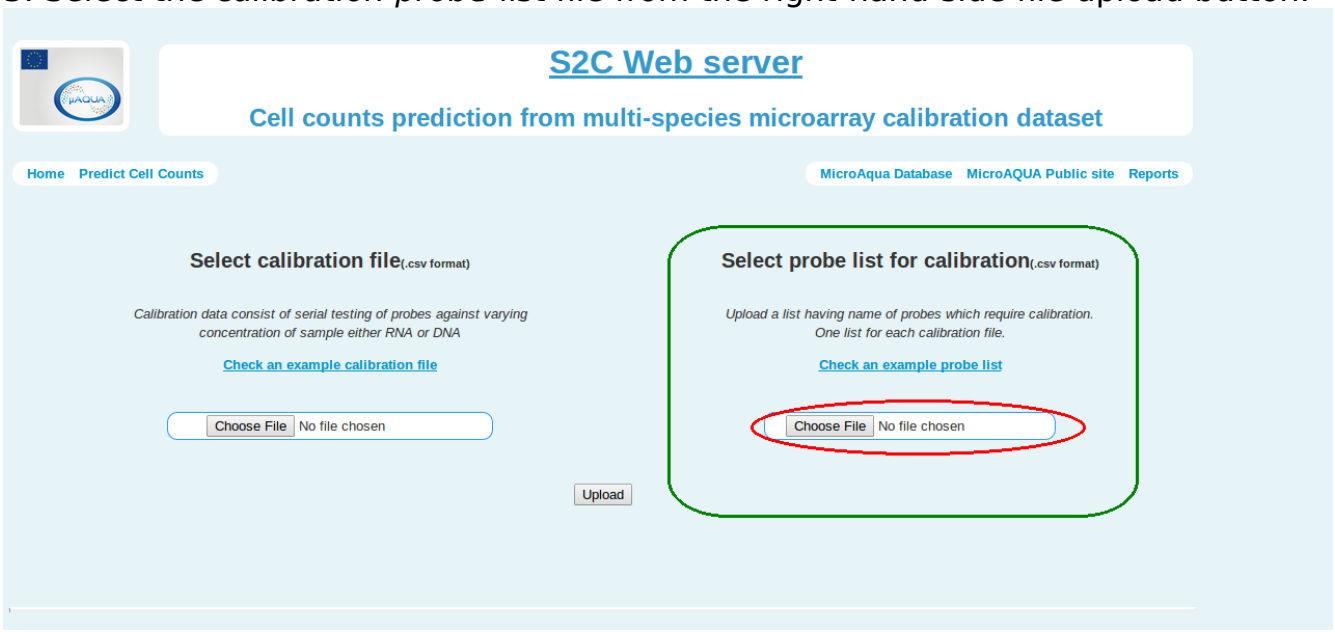
the following image:



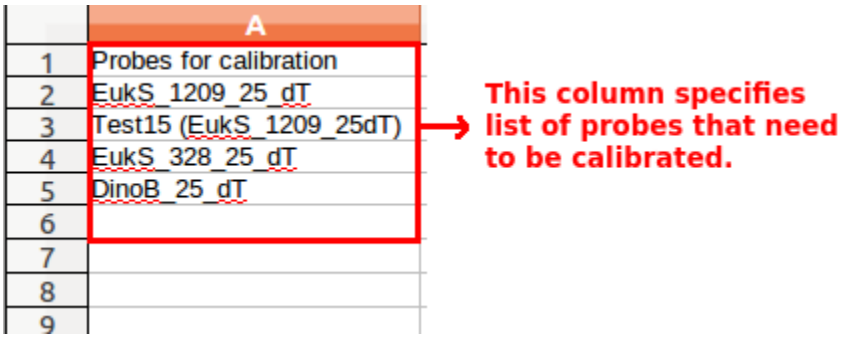
	A	B	C	D	E
1	Probe	Total Int. 1ng	Total Int. 5ng	Total Int. 25ng	Total Int. 100ng
2	cells	270	1351	6757	27027
3	POSITIVE_25_dT	7.55E+07	5.73E+07	1.01E+08	8.87E+07
4	Poly-T-CY5	4.51E+08	3.97E+08	2.39E+08	3.41E+08
5	EukS_1209_25_dT	4102788.91290624	1.68E+07	2.62E+08	5.41E+08
6	Test15 (EukS_1209_25dT)	3242670.65825	1.99E+07	3.92E+08	3.73E+08

Users must strictly follow this data format.

3. Select the *calibration probe* list file from the right-hand-side file upload button.

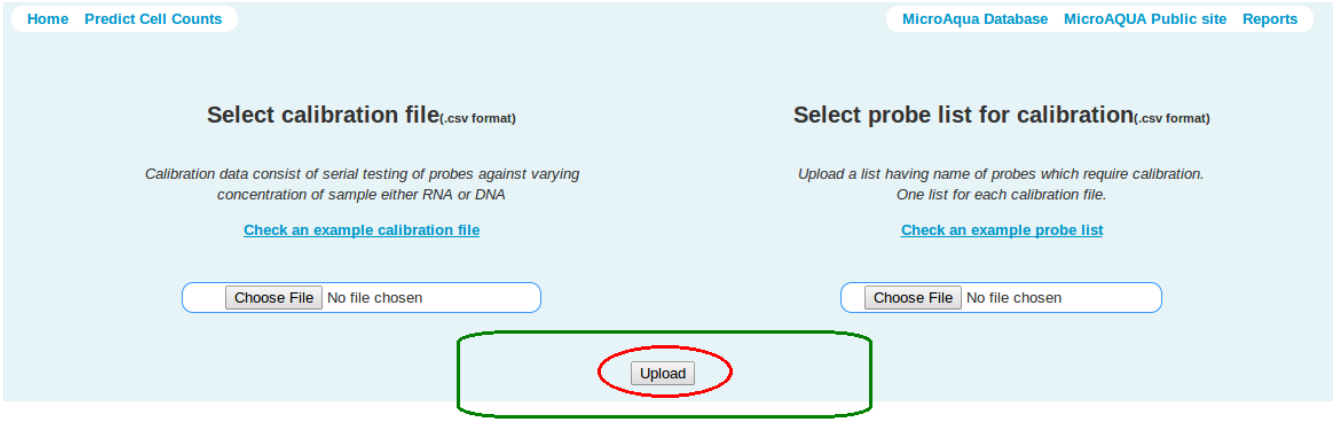


The calibration probe list file contains a list of probes which need to be calibrated for their successful utilization in cell count prediction. The format looks like the following:

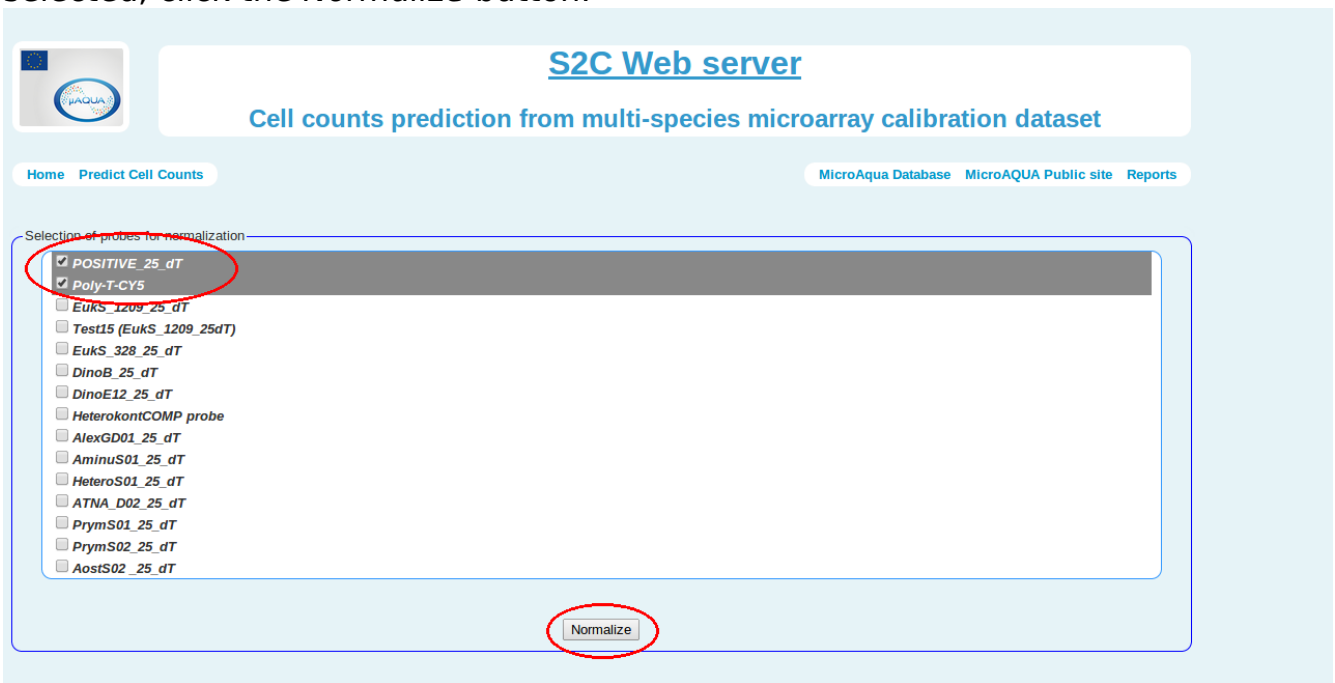


	A
1	Probes for calibration
2	EukS_1209_25_dT
3	Test15 (EukS_1209_25dT)
4	EukS_328_25_dT
5	DinoB_25_dT
6	
7	
8	
9	

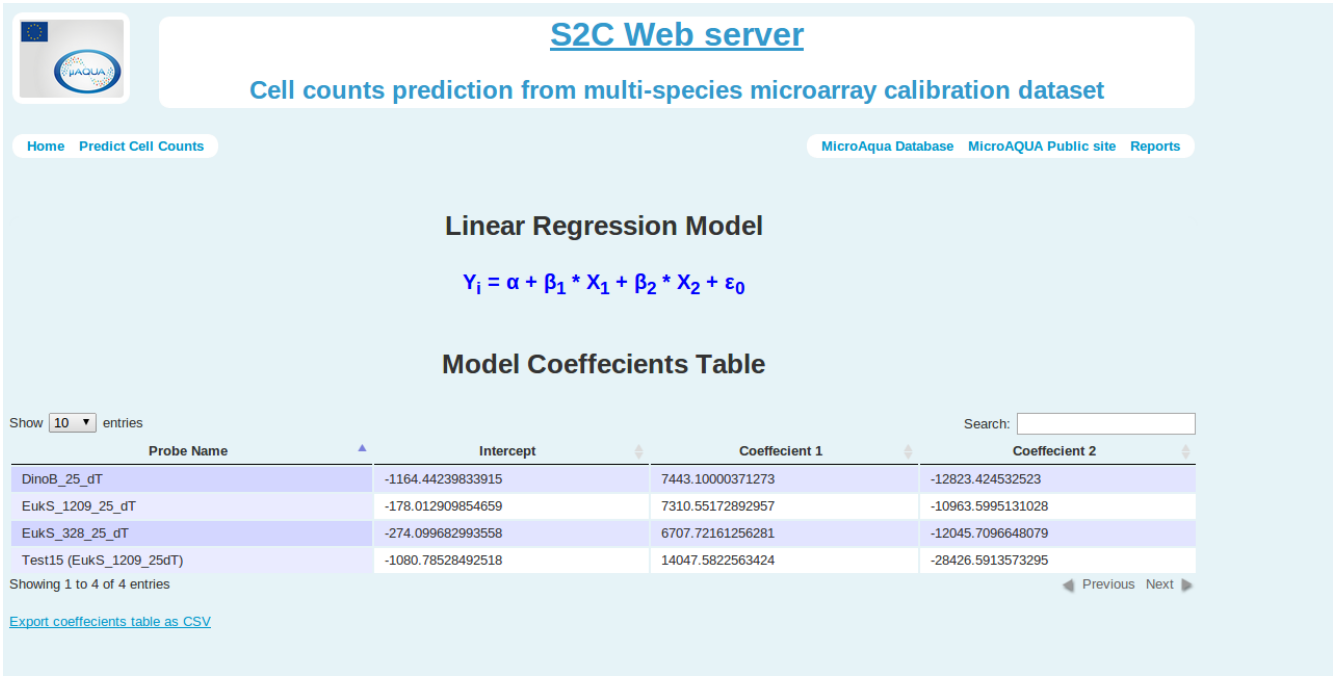
4. Once files are selected, press the *Upload* button.



5. Once the files are uploaded successfully, user will be routed to a new page containing probe list (parsed from the calibration file). The raw calibration signal intensities need to be normalized with positive controls. At this point, the user can select multiple probes for normalization. Once the normalization probes are selected, click the *Normalize* button.



6. The next page which appears, displays a linear model and number of parameters/coefficients($\beta_1 ... \beta_n$), number depends on number of normalization probes selected, obtained after fitting calibration data. This page also displays an exportable coefficient values list calculated for individual calibration probe (uploaded from select calibration probe list file).



S2C Web server

Cell counts prediction from multi-species microarray calibration dataset

Home Predict Cell Counts MicroAqua Database MicroAQUA Public site Reports

Linear Regression Model

$$Y_i = \alpha + \beta_1 * X_1 + \beta_2 * X_2 + \varepsilon_0$$

Model Coeffecients Table

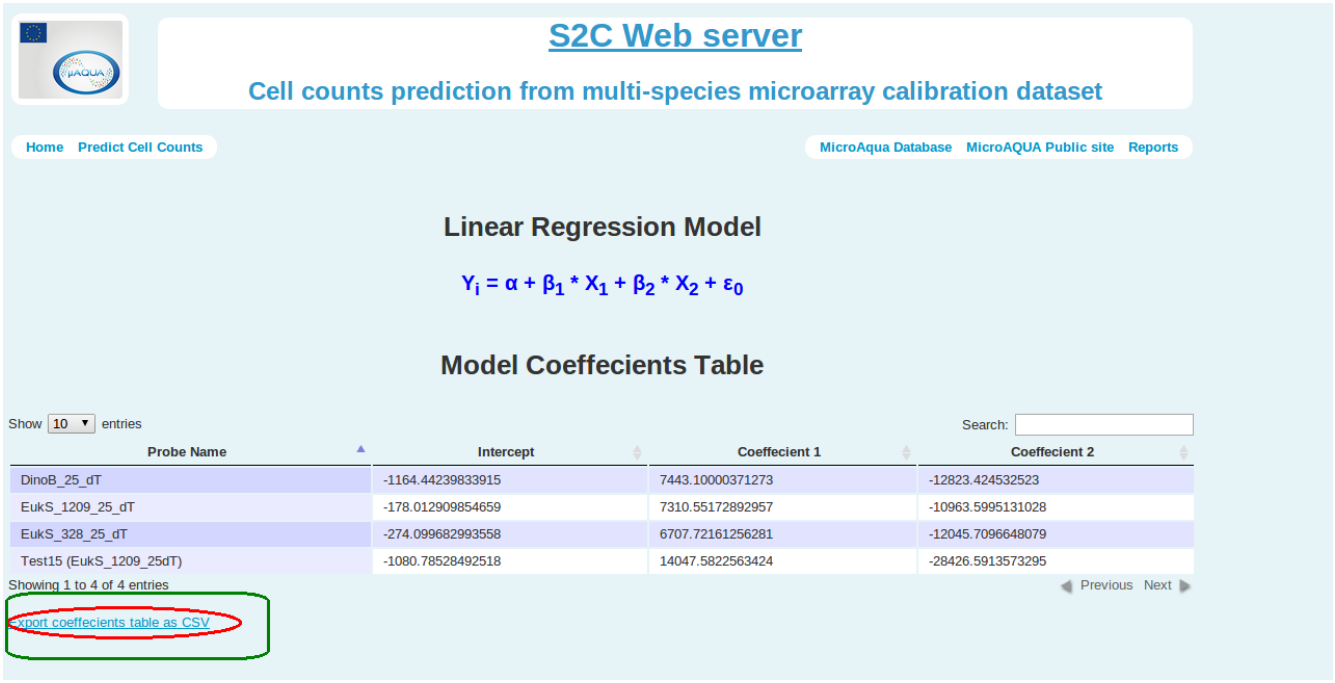
Show 10 entries Search:

Probe Name	Intercept	Coeffecient 1	Coeffecient 2
DinoB_25_dT	-1164.44239833915	7443.10000371273	-12823.424532523
EukS_1209_25_dT	-178.012909854659	7310.55172892957	-10963.5995131028
EukS_328_25_dT	-274.099682993558	6707.72161256281	-12045.7096648079
Test15 (EukS_1209_25dT)	-1080.78528492518	14047.5822563424	-28426.5913573295

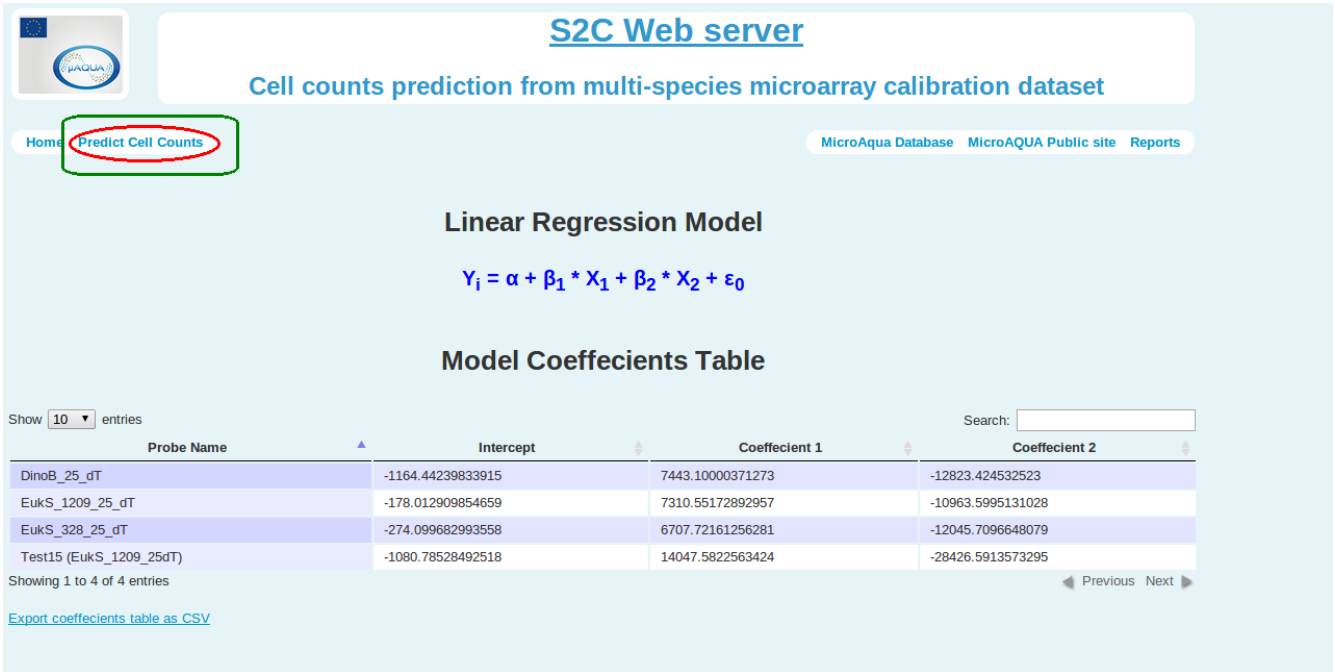
Showing 1 to 4 of 4 entries Previous Next

Export coeffecients table as CSV

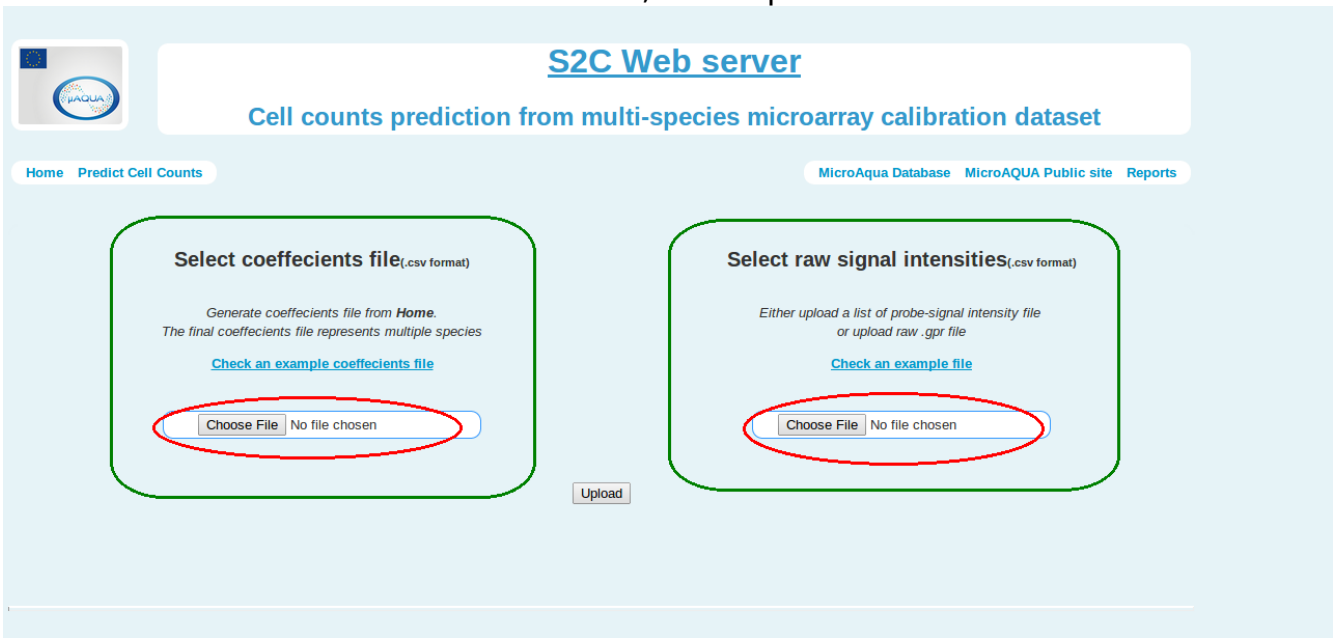
7. The table values can be downloaded from the download link available below the table. Download this file.



8. Once the coefficients file is downloaded, click on *Predict Cell Count* from the navigation panel.

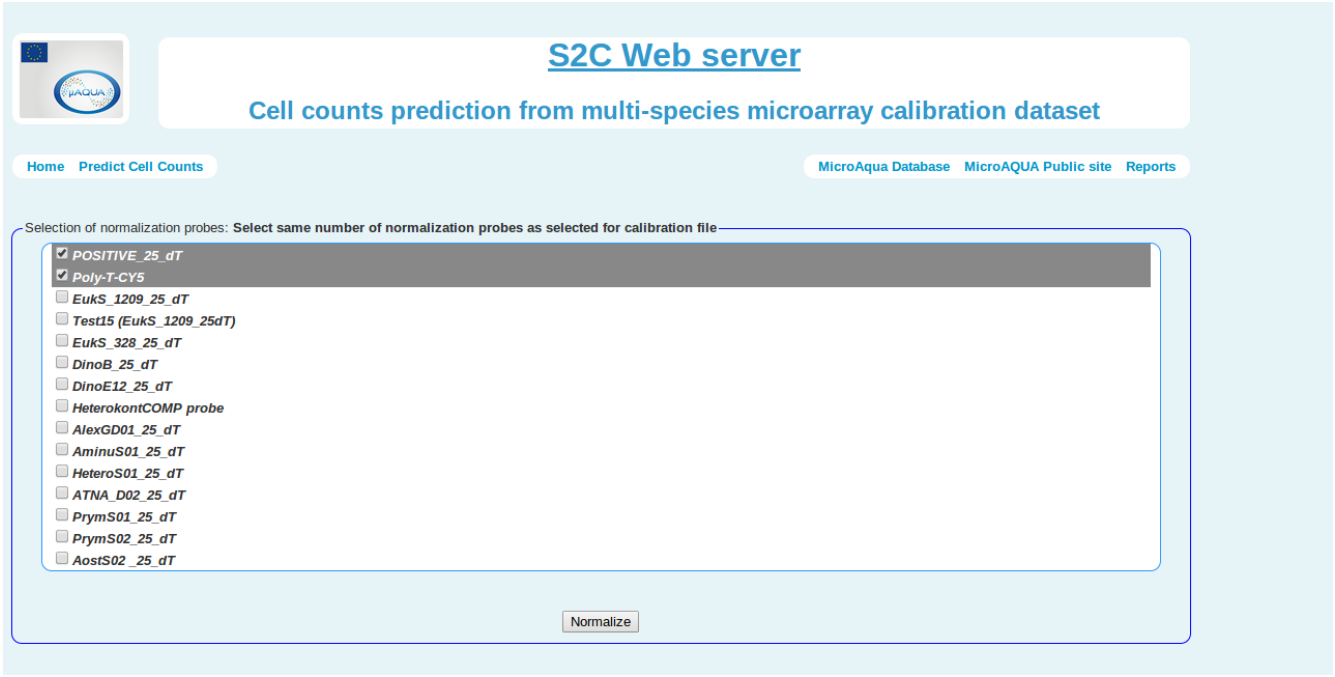


9. This page again has a file upload button. Select the *coefficient file* downloaded from the previous page in the left-hand-side file upload button. The second file upload button is for the Signal Intensity file for which we want to predict cell counts. Once both the files are selected, click Upload.

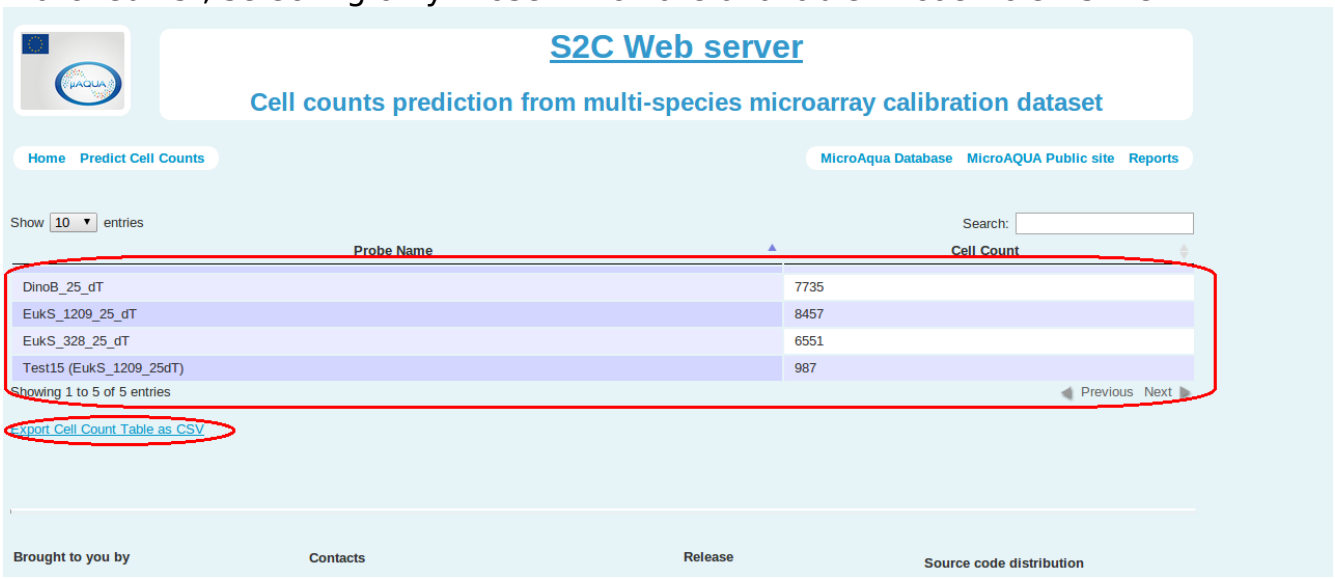


10. On the next page, the probe list appears. Here probes for normalization are selected. The second normalization is for raw signal intensity of the probes just uploaded in the previous page.

NOTE: Select the same probes for normalization used for normalizing calibration data.



11. Once the normalization probes are selected, click on the *Normalize* button. The next resulting page will list the probes with their cell count values. Note the list of probes, its not the same as present in the raw signal intensity files. Its a matched list, selecting only those which are available in coefficients file.



The resulting table can be downloaded from the link available below in .csv file format.