Skyline QuaSAR Quantitative Statistics

QuaSAR is a program that aids in the Quantitative Statistical Analysis of Reaction Monitoring Experiments. It was designed to quickly and easily convert processed SRM/MRM-MS data into calibration curves, determine limits of detection and quantification, calculate mean and coefficient of variation for all transitions of each peptide in a set of samples, as well as determine the peptide analyte concentration in unknown samples. The resulting output files, consisting of *.csv tables and *.pdf figures, can be readily used for further statistical analyses or reported as output for reports or publications.

In order to use QuaSAR, two files must be provided: a **Skyline Report File** containing peak areas that Skyline measured and a **Concentration Map File** listing the analyte concentrations of the samples**.** This tutorial covers how to export the Skyline Report file and how to create the Concentration Map File in Microsoft Excel.

Getting Started

To start this tutorial, download the following ZIP file:

https://skyline.gs.washington.edu/tutorials/QuaSAR.zip

Extract the files in it to a folder on your computer, like:

C:\Users\brendanx\Documents

This will create a new folder:

C:\Users\brendanx\Documents\QuaSAR

The zip file contains the following files:

`QuaSAR_Tutorial.sky`: A Skyline document containing sample data for QuaSAR.
`QuaSAR_Tutorial.skyd`: Contains extracted chromatogram data for the Skyline document.

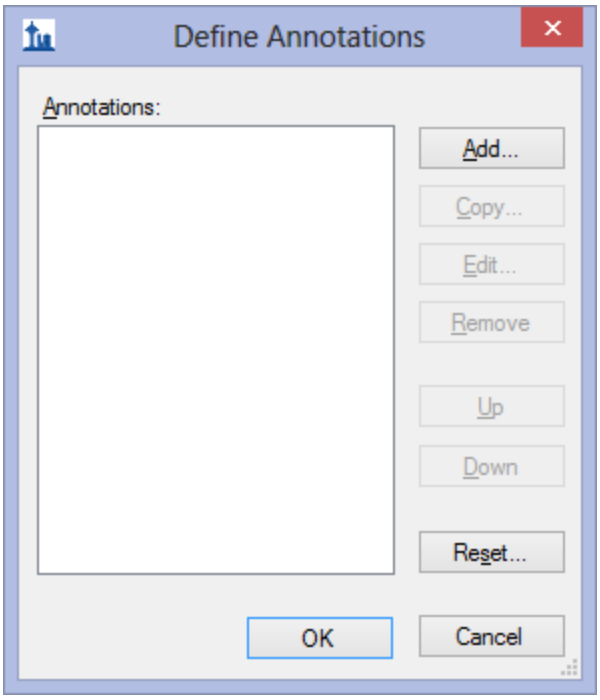
Open the provided file `QuaSAR_Tutorial.sky` in Skyline.

Annotating samples with concentration information

Skyline allows you to associate additional information with the replicates in the document by defining custom annotations.

To create an annotation, perform the following steps:

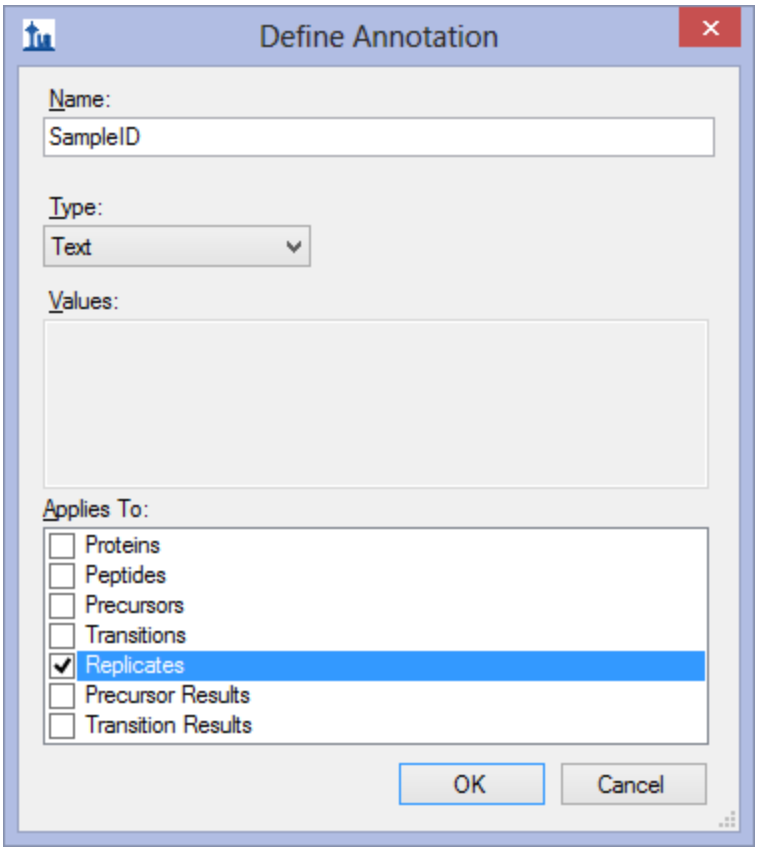
- On the **Settings** menu, click **Annotations**.
- Press the **Edit List** button to bring up the **Define Annotations** form.



The first annotation that will be required by QuaSAR is called “SampleID”, which will indicate what material was used for a particular replicate. To create this annotation, do the following:

- Click the **Add** button in the **Define Annotations** form.
- Enter ‘SampleID’ in the **Name** field of the **Define Annotation** form.
- Leave ‘Text’ as the value in the **Type** dropdown list.
- Check the **Replicates** checkbox in the **Applies To** list.

The **Define Annotation** form should look like:

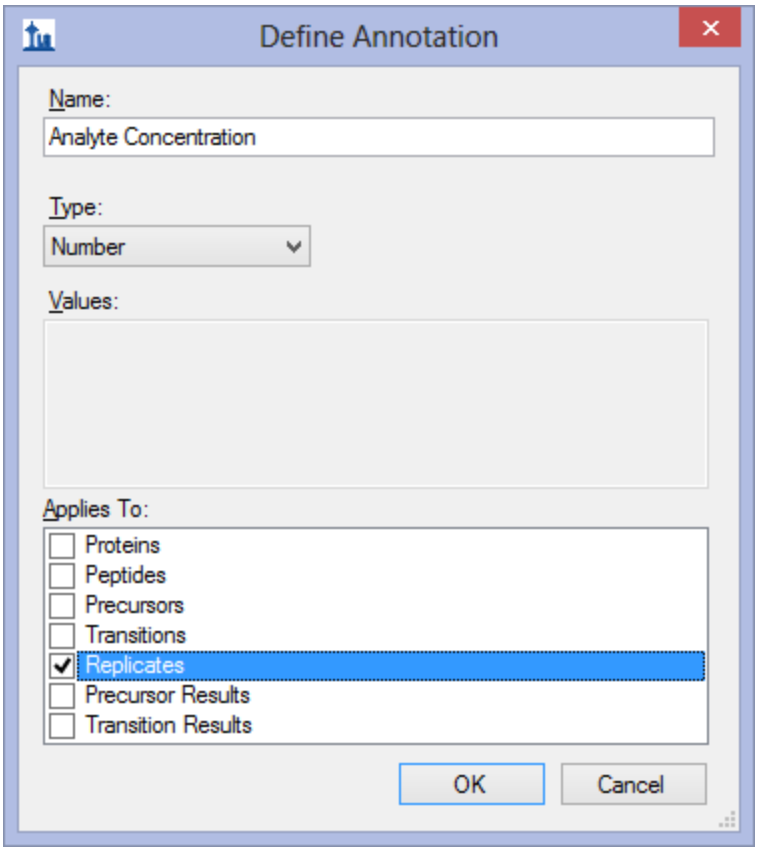


- Click the **OK** button.

The second annotation that will be required by QuaSAR is called “Analyte Concentration”. To create this annotation, do the following:

- Click the **Add** button in the **Define Annotations** form.
- Enter ‘Analyte Concentration’ in the **Name** field of the **Define Annotation** form.
- Choose ‘Number’ in the **Type** dropdown list.
- Check the **Replicates** checkbox in the **Applies To** list.

The **Define Annotation** form should look like:

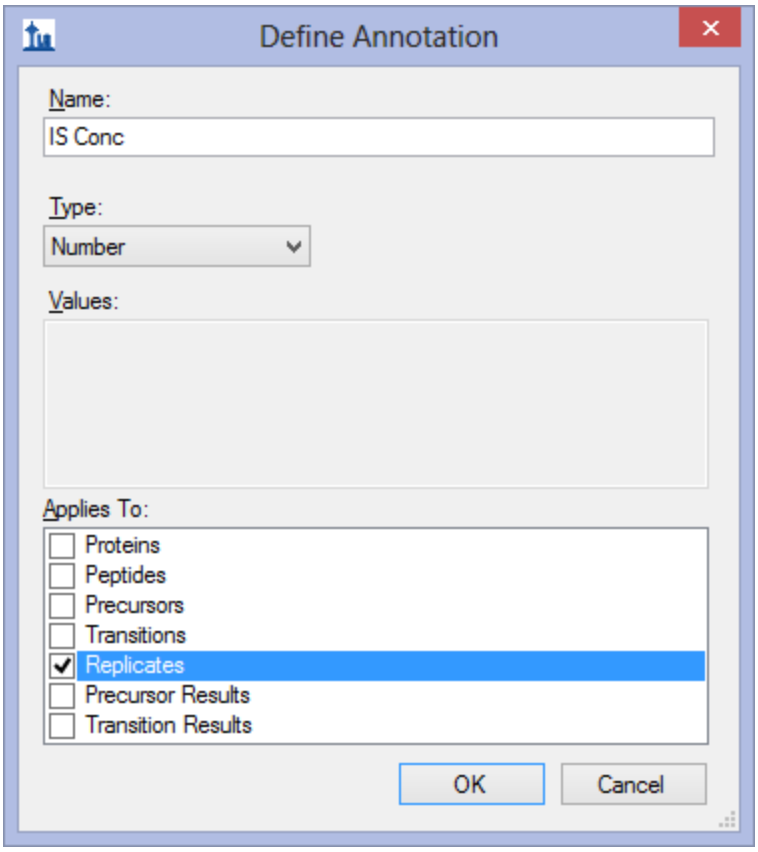


- Click the **OK** button.

The second annotation that will be required by QuaSAR is called “IS Conc” for internal standard concentration. To create this annotation, do the following:

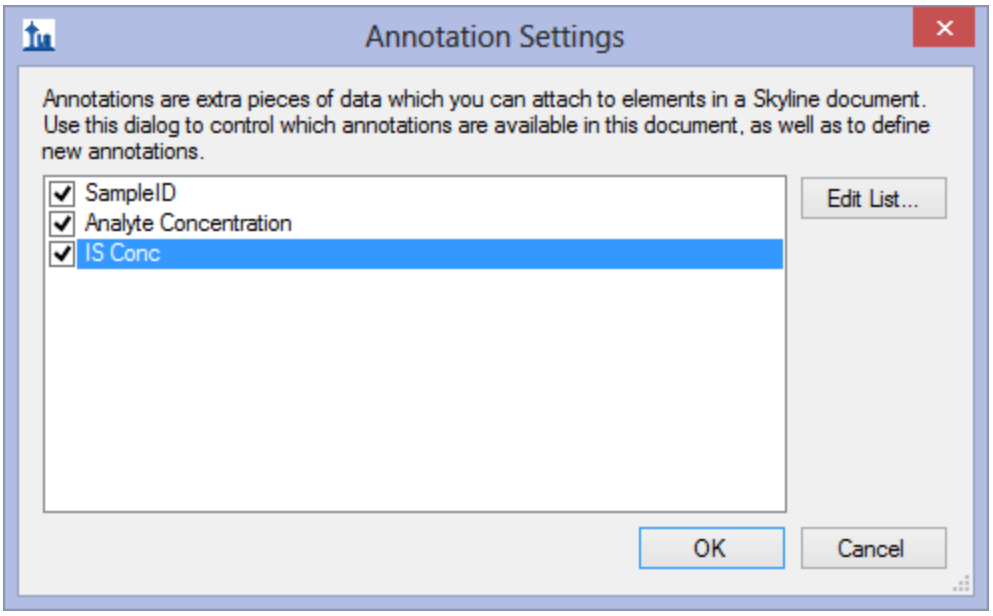
- Click the **Add** button in the **Define Annotations** form.
- Enter ‘IS Conc’ in the **Name** field of the **Define Annotation** form.
- Choose ‘Number’ in the **Type** dropdown list.
- Check the **Replicates** checkbox in the **Applies To** list.

The **Define Annotation** form should look like:



- Click the **OK** button.
- Click the **OK** button in the **Define Annotations** form.

The **Annotation Settings** form will show the list of the annotations that you have just defined, but you must check the checkboxes in the list in order to be able to use these annotations in your current Skyline document. The **Annotation Settings** form should look like the one shown below:



- Click the **OK** button.

Editing annotation values in Skyline is done using the Results Grid. To bring up the Results Grid do the following:

- On the **View** menu, click **Results Grid** (Alt-2).

The **Results Grid** will show you chromatogram peak areas and other measured results for the currently selected peptide, or transition. This amount of data might be distracting while typing in the annotation values, so be sure that one of the proteins is selected in the **Targets** tree view.

The **SampleID** column should be filled in with the single letter found in the replicate name. Use this table to fill in the **Analyte Concentration** values:

SampleID	A	B	C	D	E	F	G	H	I	J
Analyte Concentration	0	.001	.004	.018	.075	.316	1.33	5.62	23.71	100

All of the samples in this experiment have an **IS Conc** value of 10**.**

If you see the main Skyline window flashing as you type in the **Results Grid**, you may want to allow Skyline to change rows in the grid without activating the corresponding replicate chromatogram graph, by doing the following:

- Right-click in the **Results Grid**, and click **Synchronize Selection**.

The completed grid should look like this:

Results Grid

Replicate Name	SampleID	Analyte Concentration	IS Conc
9-1_Site56B_A1_CalCurve_run...	A	0	10
9-1_Site56B_A2_CalCurve_run...	A	0	10
9-1_Site56B_A3_CalCurve_run...	A	0	10
9-1_Site56B_A4_CalCurve_run...	A	0	10
9-1_Site56B_B1_CalCurve_run...	B	0.001	10
9-1_Site56B_B2_CalCurve_run...	B	0.001	10
9-1_Site56B_B3_CalCurve_run...	B	0.001	10
9-1_Site56B_B4_CalCurve_run...	B	0.001	10
9-1_Site56B_C1_CalCurve_run...	C	0.004	10
9-1_Site56B_C2_CalCurve_run...	C	0.004	10
9-1_Site56B_C3_CalCurve_run...	C	0.004	10
9-1_Site56B_C4_CalCurve_run...	C	0.004	10
9-1_Site56B_D1_CalCurve_run...	D	0.018	10
9-1_Site56B_D2_CalCurve_run...	D	0.018	10
9-1_Site56B_D3_CalCurve_run...	D	0.018	10
9-1_Site56B_D4_CalCurve_run...	D	0.018	10
9-1_Site56B_E1_CalCurve_run...	E	0.075	10
9-1_Site56B_E2_CalCurve_run...	E	0.075	10
9-1_Site56B_E3_CalCurve_run...	E	0.075	10
9-1_Site56B_E4_CalCurve_run...	E	0.075	10
9-1_Site56B_F1_CalCurve_run_...	F	0.316	10
9-1_Site56B_F2_CalCurve_run_...	F	0.316	10
9-1_Site56B_F3_CalCurve_run_...	F	0.316	10
9-1_Site56B_F4_CalCurve_run_...	F	0.316	10
9-1_Site56B_G1_Calcurve_run...	G	1.33	10
9-1_Site56B_G2_CalCurve_run...	G	1.33	10
9-1_Site56B_G3_CalCurve_run...	G	1.33	10
9-1_Site56B_G4_CalCurve_run...	G	1.33	10
9-1_Site56B_H1_CalCurve_run...	H	5.62	10
9-1_Site56B_H2_CalCurve_run...	H	5.62	10
9-1_Site56B_H3_CalCurve_run...	H	5.62	10
9-1_Site56B_H4_CalCurve_run...	H	5.62	10
9-1_Site56B_I1_CalCurve_run_...	I	23.71	10
9-1_Site56B_I2_CalCurve_run_...	I	23.71	10
9-1_Site56B_I3_CalCurve_run_...	I	23.71	10
9-1_Site56B_I4_CalCurve_run_...	I	23.71	10
9-1_Site56B_J1_CalCurve_run_...	J	100	10
9-1_Site56B_J2_CalCurve_run_...	J	100	10
9-1_Site56B_J3_CalCurve_run_...	J	100	10
9-1_Site56B_J4_CalCurve_run_...	J	100	10

Record: |< < 40 of 40 > >| Filter:

Creating the concentration map file

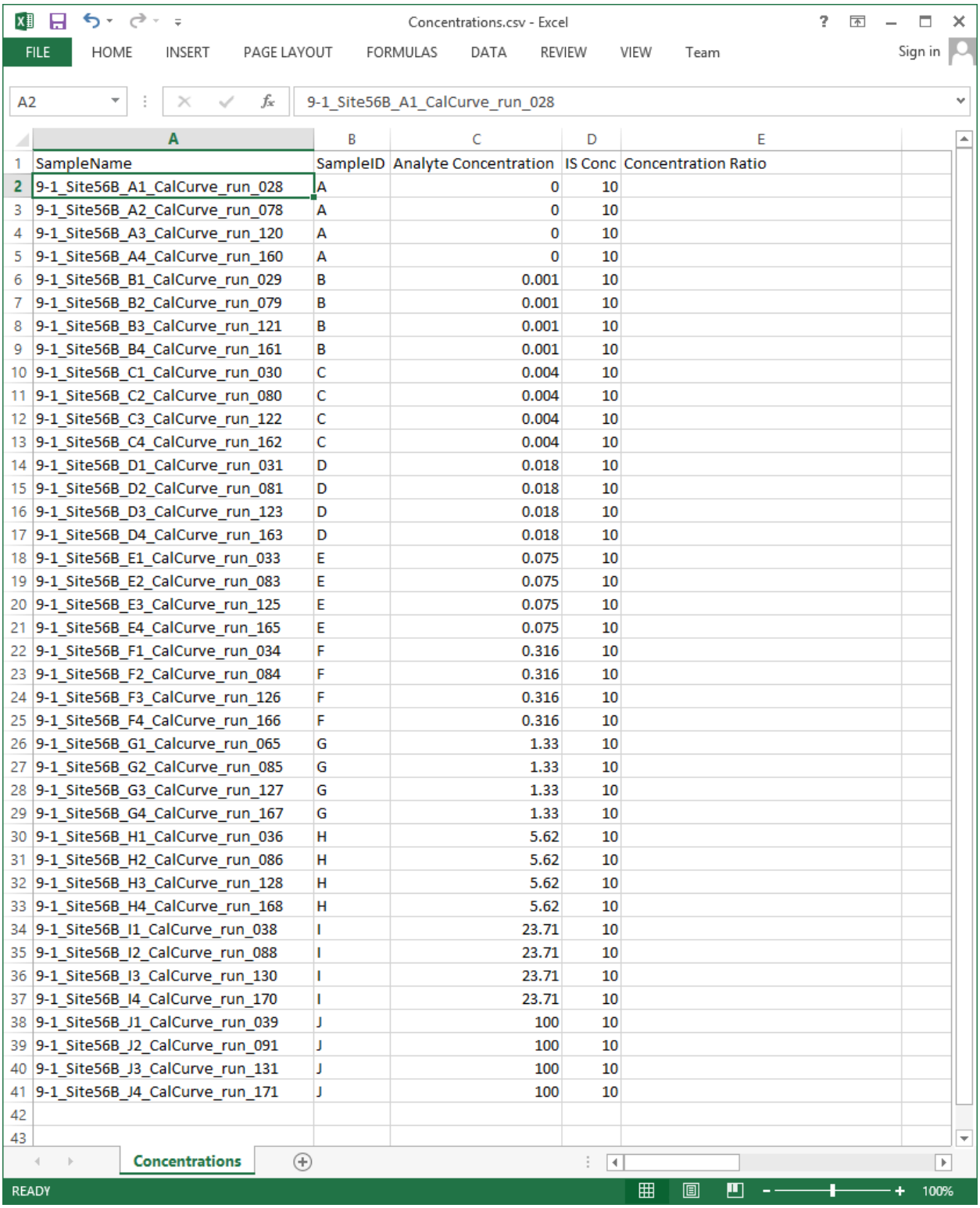
To create the concentration map file required by QuaSAR, perform the following steps:

- Using a spreadsheet program such as Microsoft Excel, create a new spreadsheet with the following values in the first row:

SampleName	SampleID	Analyte Concentration	IS Conc	Concentration Ratio

- In the **Results Grid** in Skyline, click and drag to select all of the rows and columns of data.
- Press **Ctrl-C** to copy the data to the clipboard.
- In Excel, paste the clipboard data into the second row.
- Save this file to Concentration.csv in the folder you created for this tutorial with **Save as type** set to 'CSV (Comma delimited) (*.csv)

The spreadsheet should look something like this:



	A	B	C	D	E
1	SampleName	SampleID	Analyte Concentration	IS Conc	Concentration Ratio
2	9-1_Site56B_A1_CalCurve_run_028	A	0	10	
3	9-1_Site56B_A2_CalCurve_run_078	A	0	10	
4	9-1_Site56B_A3_CalCurve_run_120	A	0	10	
5	9-1_Site56B_A4_CalCurve_run_160	A	0	10	
6	9-1_Site56B_B1_CalCurve_run_029	B	0.001	10	
7	9-1_Site56B_B2_CalCurve_run_079	B	0.001	10	
8	9-1_Site56B_B3_CalCurve_run_121	B	0.001	10	
9	9-1_Site56B_B4_CalCurve_run_161	B	0.001	10	
10	9-1_Site56B_C1_CalCurve_run_030	C	0.004	10	
11	9-1_Site56B_C2_CalCurve_run_080	C	0.004	10	
12	9-1_Site56B_C3_CalCurve_run_122	C	0.004	10	
13	9-1_Site56B_C4_CalCurve_run_162	C	0.004	10	
14	9-1_Site56B_D1_CalCurve_run_031	D	0.018	10	
15	9-1_Site56B_D2_CalCurve_run_081	D	0.018	10	
16	9-1_Site56B_D3_CalCurve_run_123	D	0.018	10	
17	9-1_Site56B_D4_CalCurve_run_163	D	0.018	10	
18	9-1_Site56B_E1_CalCurve_run_033	E	0.075	10	
19	9-1_Site56B_E2_CalCurve_run_083	E	0.075	10	
20	9-1_Site56B_E3_CalCurve_run_125	E	0.075	10	
21	9-1_Site56B_E4_CalCurve_run_165	E	0.075	10	
22	9-1_Site56B_F1_CalCurve_run_034	F	0.316	10	
23	9-1_Site56B_F2_CalCurve_run_084	F	0.316	10	
24	9-1_Site56B_F3_CalCurve_run_126	F	0.316	10	
25	9-1_Site56B_F4_CalCurve_run_166	F	0.316	10	
26	9-1_Site56B_G1_Calcurve_run_065	G	1.33	10	
27	9-1_Site56B_G2_CalCurve_run_085	G	1.33	10	
28	9-1_Site56B_G3_CalCurve_run_127	G	1.33	10	
29	9-1_Site56B_G4_CalCurve_run_167	G	1.33	10	
30	9-1_Site56B_H1_CalCurve_run_036	H	5.62	10	
31	9-1_Site56B_H2_CalCurve_run_086	H	5.62	10	
32	9-1_Site56B_H3_CalCurve_run_128	H	5.62	10	
33	9-1_Site56B_H4_CalCurve_run_168	H	5.62	10	
34	9-1_Site56B_I1_CalCurve_run_038	I	23.71	10	
35	9-1_Site56B_I2_CalCurve_run_088	I	23.71	10	
36	9-1_Site56B_I3_CalCurve_run_130	I	23.71	10	
37	9-1_Site56B_I4_CalCurve_run_170	I	23.71	10	
38	9-1_Site56B_J1_CalCurve_run_039	J	100	10	
39	9-1_Site56B_J2_CalCurve_run_091	J	100	10	
40	9-1_Site56B_J3_CalCurve_run_131	J	100	10	
41	9-1_Site56B_J4_CalCurve_run_171	J	100	10	
42					
43					

- Close the **Results Grid** in Skyline by clicking the red X in its upper right corner.

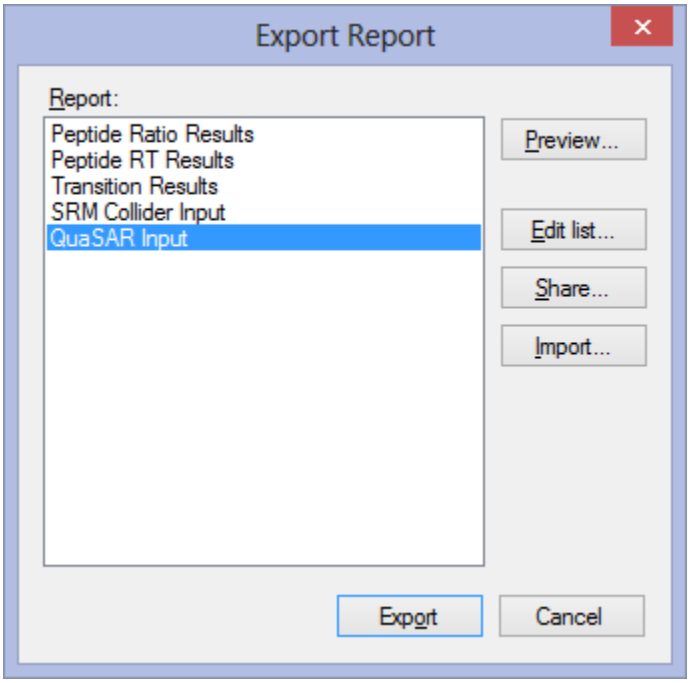
NOTE: In a future version, QuaSAR will accept a single report from Skyline which incorporates the concentration map information you just entered with the peak are information you are about to export. The current version of QuaSAR was designed before support for replicate annotations was added to Skyline in version 1.4.

Exporting QuaSAR input data

To create the Skyline report required by QuaSAR, you will use the built in custom report template installed by default with Skyline. To export this report for the tutorial document, do the following:

- On the **File** menu choose **Export** and then click **Report**
- Select **QuaSAR Input** in the report list.

The **Export Report** form should look like:



- Click the **Export** button.

Skyline will give the option to name the file which defaults to "QuaSAR_Tutorial.csv".

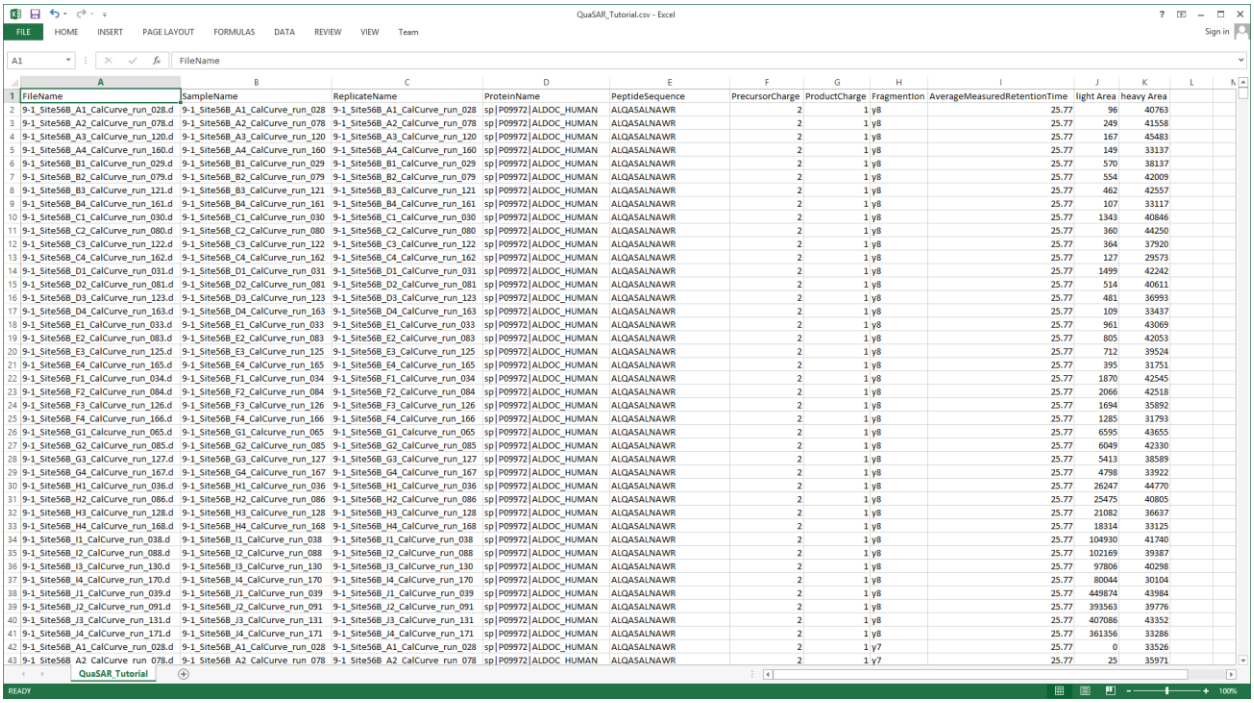
- To accept this report name, click **Save**.

If properly exported, the final .csv Skyline Report file will already be correctly formatted and contain columns with the following headers (with **exact** spelling, capitalization and spaces)

`FileName`
`SampleName`

ProteinName
ReplicateName
PeptideSequence
PrecursorCharge
ProductCharge
FragmentIon
AverageMeasuredRetentionTime
light Area
heavy Area

This is what the exported file will look like if you open it in Excel:



FileName	SampleName	ReplicateName	ProteinName	PeptideSequence	PrecursorCharge	ProductCharge	FragmentIon	AverageMeasuredRetentionTime	light Area	heavy Area
9-1_Site56B_A1_CalCurve_run_028.d	9-1_Site56B_A1_CalCurve_run_028	9-1_Site56B_A1_CalCurve_run_028	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	96	40763
9-1_Site56B_A2_CalCurve_run_078.d	9-1_Site56B_A2_CalCurve_run_078	9-1_Site56B_A2_CalCurve_run_078	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	249	41558
9-1_Site56B_A3_CalCurve_run_120.d	9-1_Site56B_A3_CalCurve_run_120	9-1_Site56B_A3_CalCurve_run_120	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	167	45483
9-1_Site56B_A4_CalCurve_run_160.d	9-1_Site56B_A4_CalCurve_run_160	9-1_Site56B_A4_CalCurve_run_160	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	149	33137
9-1_Site56B_B1_CalCurve_run_029.d	9-1_Site56B_B1_CalCurve_run_029	9-1_Site56B_B1_CalCurve_run_029	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	570	38137
9-1_Site56B_B2_CalCurve_run_079.d	9-1_Site56B_B2_CalCurve_run_079	9-1_Site56B_B2_CalCurve_run_079	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	554	42009
9-1_Site56B_B3_CalCurve_run_121.d	9-1_Site56B_B3_CalCurve_run_121	9-1_Site56B_B3_CalCurve_run_121	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	462	42557
9-1_Site56B_B4_CalCurve_run_161.d	9-1_Site56B_B4_CalCurve_run_161	9-1_Site56B_B4_CalCurve_run_161	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	107	33117
9-1_Site56B_C1_CalCurve_run_030.d	9-1_Site56B_C1_CalCurve_run_030	9-1_Site56B_C1_CalCurve_run_030	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	1343	40846
9-1_Site56B_C2_CalCurve_run_080.d	9-1_Site56B_C2_CalCurve_run_080	9-1_Site56B_C2_CalCurve_run_080	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	360	44250
9-1_Site56B_C3_CalCurve_run_122.d	9-1_Site56B_C3_CalCurve_run_122	9-1_Site56B_C3_CalCurve_run_122	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	364	37920
9-1_Site56B_C4_CalCurve_run_162.d	9-1_Site56B_C4_CalCurve_run_162	9-1_Site56B_C4_CalCurve_run_162	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	127	29573
9-1_Site56B_D1_CalCurve_run_031.d	9-1_Site56B_D1_CalCurve_run_031	9-1_Site56B_D1_CalCurve_run_031	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	1499	42242
9-1_Site56B_D2_CalCurve_run_081.d	9-1_Site56B_D2_CalCurve_run_081	9-1_Site56B_D2_CalCurve_run_081	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	514	40611
9-1_Site56B_D3_CalCurve_run_123.d	9-1_Site56B_D3_CalCurve_run_123	9-1_Site56B_D3_CalCurve_run_123	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	481	36993
9-1_Site56B_D4_CalCurve_run_163.d	9-1_Site56B_D4_CalCurve_run_163	9-1_Site56B_D4_CalCurve_run_163	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	109	33437
9-1_Site56B_E1_CalCurve_run_033.d	9-1_Site56B_E1_CalCurve_run_033	9-1_Site56B_E1_CalCurve_run_033	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	961	43069
9-1_Site56B_E2_CalCurve_run_083.d	9-1_Site56B_E2_CalCurve_run_083	9-1_Site56B_E2_CalCurve_run_083	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	805	42053
9-1_Site56B_E3_CalCurve_run_125.d	9-1_Site56B_E3_CalCurve_run_125	9-1_Site56B_E3_CalCurve_run_125	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	712	39524
9-1_Site56B_E4_CalCurve_run_165.d	9-1_Site56B_E4_CalCurve_run_165	9-1_Site56B_E4_CalCurve_run_165	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	395	31751
9-1_Site56B_F1_CalCurve_run_034.d	9-1_Site56B_F1_CalCurve_run_034	9-1_Site56B_F1_CalCurve_run_034	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	1870	42545
9-1_Site56B_F2_CalCurve_run_084.d	9-1_Site56B_F2_CalCurve_run_084	9-1_Site56B_F2_CalCurve_run_084	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	2066	42518
9-1_Site56B_F3_CalCurve_run_126.d	9-1_Site56B_F3_CalCurve_run_126	9-1_Site56B_F3_CalCurve_run_126	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	1694	35892
9-1_Site56B_F4_CalCurve_run_166.d	9-1_Site56B_F4_CalCurve_run_166	9-1_Site56B_F4_CalCurve_run_166	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	1285	31793
9-1_Site56B_G1_CalCurve_run_065.d	9-1_Site56B_G1_CalCurve_run_065	9-1_Site56B_G1_CalCurve_run_065	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	6595	43655
9-1_Site56B_G2_CalCurve_run_085.d	9-1_Site56B_G2_CalCurve_run_085	9-1_Site56B_G2_CalCurve_run_085	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	6049	42330
9-1_Site56B_G3_CalCurve_run_127.d	9-1_Site56B_G3_CalCurve_run_127	9-1_Site56B_G3_CalCurve_run_127	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	5413	38589
9-1_Site56B_G4_CalCurve_run_167.d	9-1_Site56B_G4_CalCurve_run_167	9-1_Site56B_G4_CalCurve_run_167	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	4798	33922
9-1_Site56B_H1_CalCurve_run_036.d	9-1_Site56B_H1_CalCurve_run_036	9-1_Site56B_H1_CalCurve_run_036	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	26247	44770
9-1_Site56B_H2_CalCurve_run_086.d	9-1_Site56B_H2_CalCurve_run_086	9-1_Site56B_H2_CalCurve_run_086	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	25475	40805
9-1_Site56B_H3_CalCurve_run_128.d	9-1_Site56B_H3_CalCurve_run_128	9-1_Site56B_H3_CalCurve_run_128	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	21082	36637
9-1_Site56B_H4_CalCurve_run_168.d	9-1_Site56B_H4_CalCurve_run_168	9-1_Site56B_H4_CalCurve_run_168	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	18314	33125
9-1_Site56B_I1_CalCurve_run_038.d	9-1_Site56B_I1_CalCurve_run_038	9-1_Site56B_I1_CalCurve_run_038	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	104930	41740
9-1_Site56B_I2_CalCurve_run_088.d	9-1_Site56B_I2_CalCurve_run_088	9-1_Site56B_I2_CalCurve_run_088	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	102169	39387
9-1_Site56B_I3_CalCurve_run_130.d	9-1_Site56B_I3_CalCurve_run_130	9-1_Site56B_I3_CalCurve_run_130	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	97806	40298
9-1_Site56B_I4_CalCurve_run_170.d	9-1_Site56B_I4_CalCurve_run_170	9-1_Site56B_I4_CalCurve_run_170	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	80044	30104
9-1_Site56B_J1_CalCurve_run_039.d	9-1_Site56B_J1_CalCurve_run_039	9-1_Site56B_J1_CalCurve_run_039	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	449874	43984
9-1_Site56B_J2_CalCurve_run_091.d	9-1_Site56B_J2_CalCurve_run_091	9-1_Site56B_J2_CalCurve_run_091	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	393563	39776
9-1_Site56B_J3_CalCurve_run_131.d	9-1_Site56B_J3_CalCurve_run_131	9-1_Site56B_J3_CalCurve_run_131	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	407086	43352
9-1_Site56B_J4_CalCurve_run_171.d	9-1_Site56B_J4_CalCurve_run_171	9-1_Site56B_J4_CalCurve_run_171	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y8	25.77	361356	33286
9-1_Site56B_A1_CalCurve_run_028.d	9-1_Site56B_A1_CalCurve_run_028	9-1_Site56B_A1_CalCurve_run_028	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y7	25.77	0	33526
9-1_Site56B_A2_CalCurve_run_078.d	9-1_Site56B_A2_CalCurve_run_078	9-1_Site56B_A2_CalCurve_run_078	sp|P09972|ALDOC_HUMAN	ALQASALNAWR	2	1	y7	25.77	25	35971

Using QuaSAR

To bring up the QuaSAR web site from Skyline, do the following:

- On the **Tools** menu, click **QuaSAR**.

You also can navigate a web browser to the GenePattern web page:

http://genepattern.broadinstitute.org/gp/pages/index.jsf?lsid=QuaSAR

Unless you have already signed in to the GenePattern web site, this will bring you to the GenePattern sign-in page, and you will need to sign in to GenePattern. If you do not have a GenePattern ID then click the "Click to Register" link to register for an account before you can sign in.

You will now be on the QuaSAR module page. To specify the **Skyline Report File** and **Concentration Map File** you just created for QuaSAR to use, do the following:

- In the **skyline file** field, click the **Choose File** button to invoke the file navigator.
- Locate and select the `QuaSAR_Tutorial.csv` file that you exported from Skyline.
- Click **OK** to accept your choice of file.
- In the **concentration file** field, click the **Choose File** button to invoke the file navigator.
- Locate and select the `Concentrations.csv` file that you saved from Excel.
- Click **OK** to accept your choice of file.
- In the **title** field, enter 'Tutorial'.

For this tutorial you will leave the rest of the options in their default settings. If you wish to learn more about the many options provided by QuaSAR, click the **documentation** link in the top right of the module page.

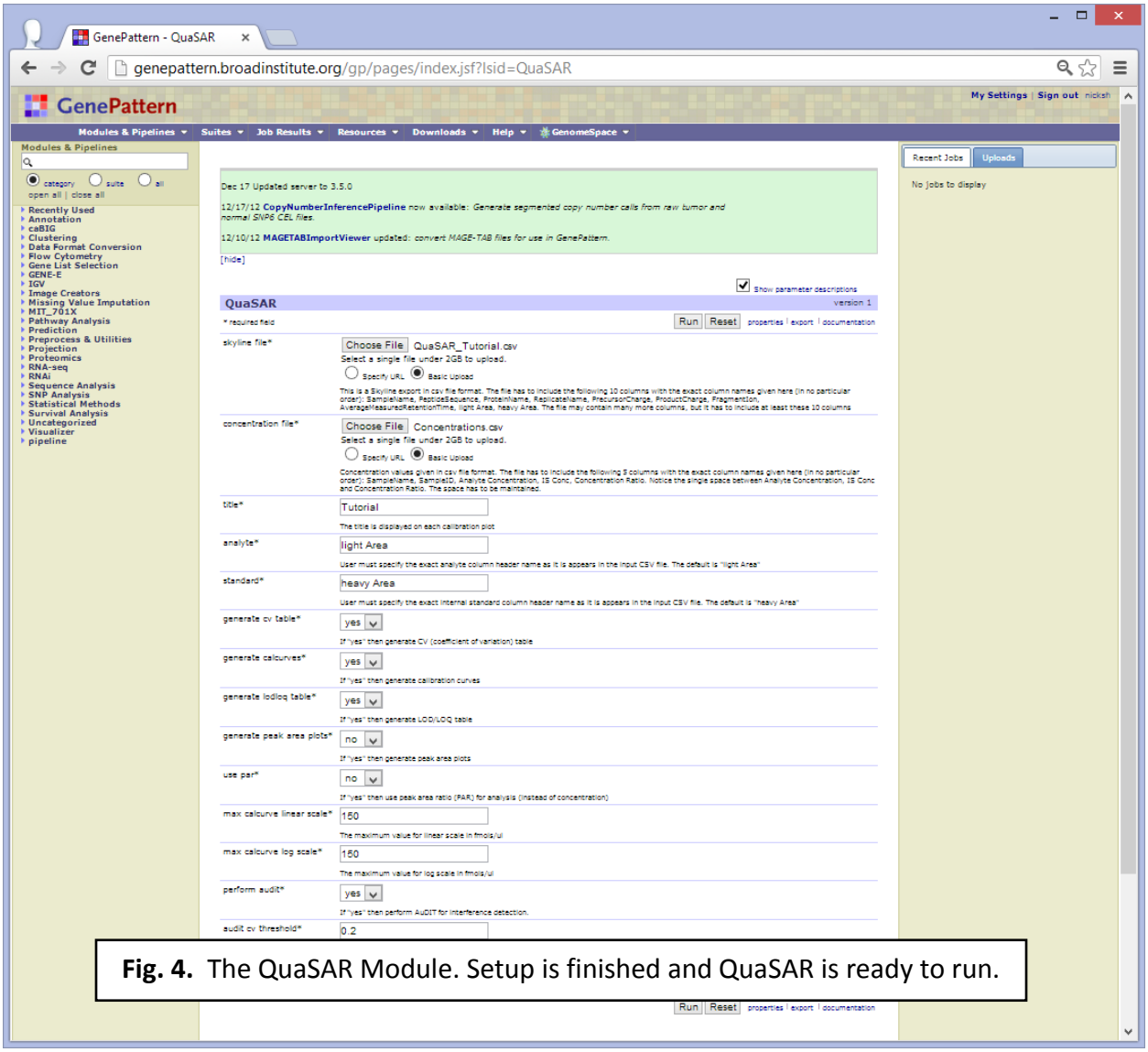
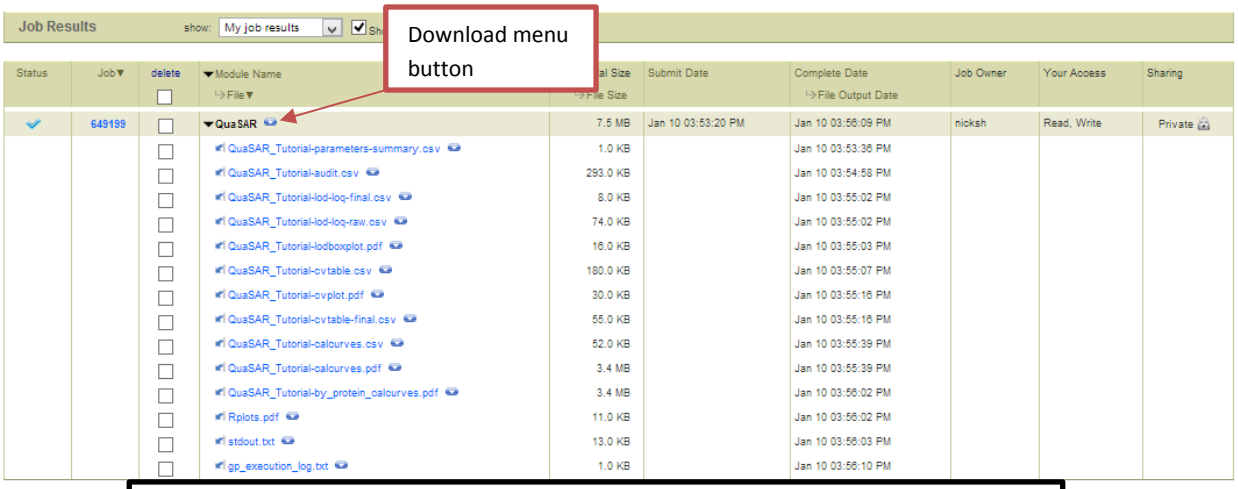


Fig. 4. The QuaSAR Module. Setup is finished and QuaSAR is ready to run.

- Click the **Run** button.

This will take you to a new page where it should indicate that your QuaSAR request has been submitted. If you wish to be notified by email when QuaSAR is finished select the email notification box in the right corner of the QuaSAR bar. It will take approximately 5 minutes for QuaSAR to finish.

Once QuaSAR has finished, you will see a list of files on the same page **(Fig. 5).** These files represent the output from QuaSAR. You can view these files by clicking on them.



The QuaSAR output screen after finishing the tutorial. The button for the dropbox menu that contains the **download** option is indicated.

To download the output files from the QuaSAR module, click the small blue arrow next to "QuaSAR" on the module title. This will reveal a drop box. In the drop box there will be an option to download. Click on **download** and all the QuaSAR output files will be automatically zipped up into one single file and downloaded onto your computer.