


How to analyze qPCR data using ExpressionSuite Software

ExpressionSuite is a qPCR analysis software freely available on the web from Life Technologies website (for PC only) to the following address:

<http://www.lifetechnologies.com/ca/en/home/global/forms/expressionsuite-software-registration.html>

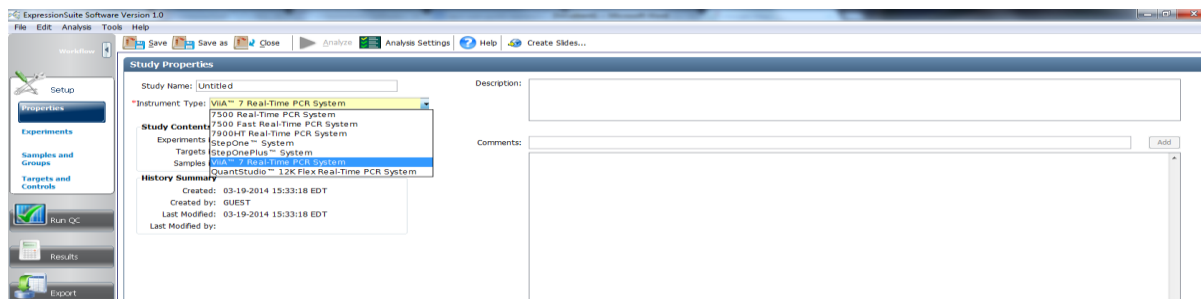
ExpressionSuite uses the comparative Ct method ($\Delta\Delta C_t$) to quickly and accurately quantify the relative expression of genes in a large number of samples. The software allows you to analyze a project prepared on one or more plates with several endogenous controls; gives you the RQ values and standard deviation; adjusts the efficiency levels of your assays and several other statistical and graphical features. You can also export your data into Excel.

1. On the ExpressionSuite's main menu, click on **Create Study**.

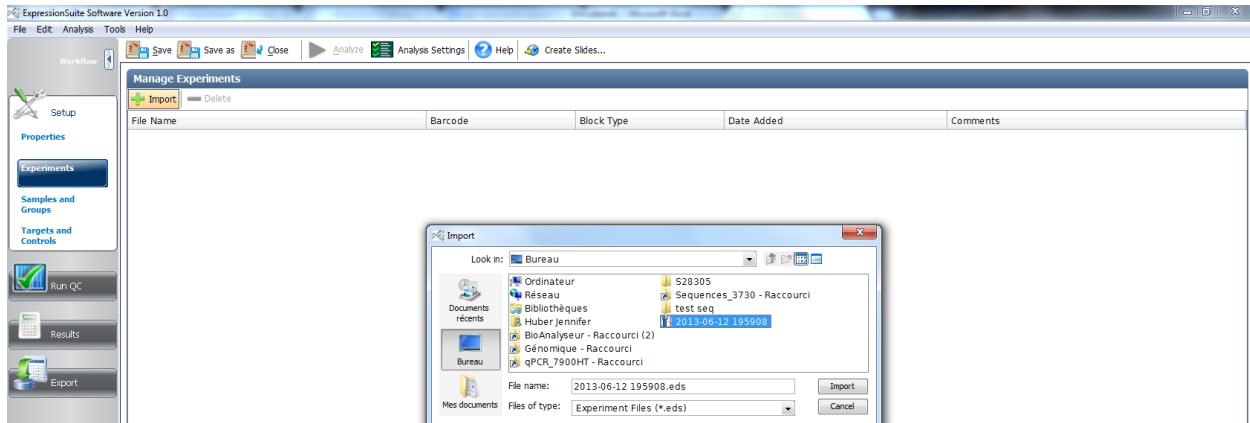


Study Name	Description	# of Experiments	Date Created	Created by	Last Modified
P1002_GID1493_2014-03-17		1	03-17-2014 16:29:46 EDT	GUEST	03-17-2014 16:33:23 EDT
09H046		1	03-17-2014 10:48:59 EDT	GUEST	03-17-2014 10:52:06 EDT
P1003_GID1180_2014-03-14		1	03-14-2014 16:39:33 EDT	GUEST	03-14-2014 16:44:47 EDT
P1000_GID693_2014-03-14		1	03-14-2014 16:22:04 EDT	GUEST	03-14-2014 16:25:53 EDT
P997_GID1089_2014-03-04		1	03-04-2014 16:30:53 EST	GUEST	03-04-2014 16:43:23 EST
P995_GID1180_2014-02-28		1	02-28-2014 15:37:08 EST	GUEST	03-03-2014 10:26:21 EST
P994_GID1180_2014-02-27		1	02-27-2014 15:27:58 EST	GUEST	03-03-2014 09:50:46 EST
P991_GID1210_2014-02-26		1	02-26-2014 10:03:17 EST	GUEST	02-26-2014 10:11:10 EST
0987_GM1163_2014-02-26		1	02-26-2014 09:27:04 EST	GUEST	02-26-2014 09:47:06 EST

2. Name your project. Click on **Instrument Type** and select Viia7.

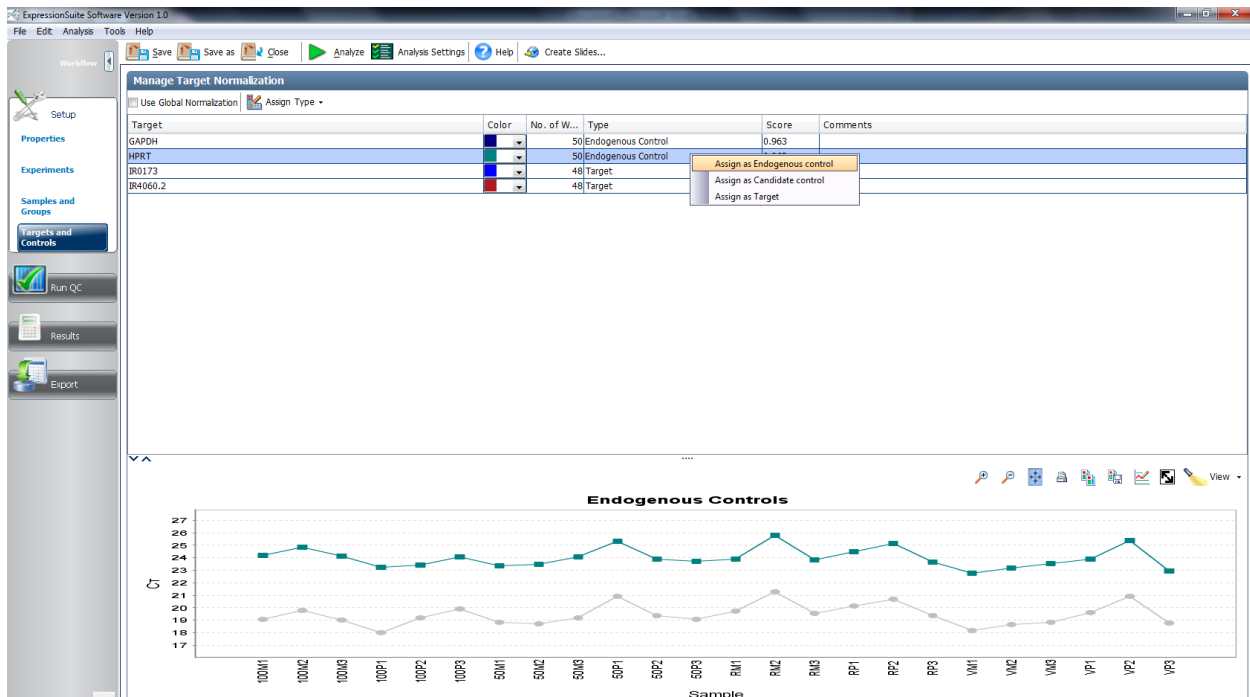


3. In the left menu, click on **Experiments** and **Import** to import one or more projects.

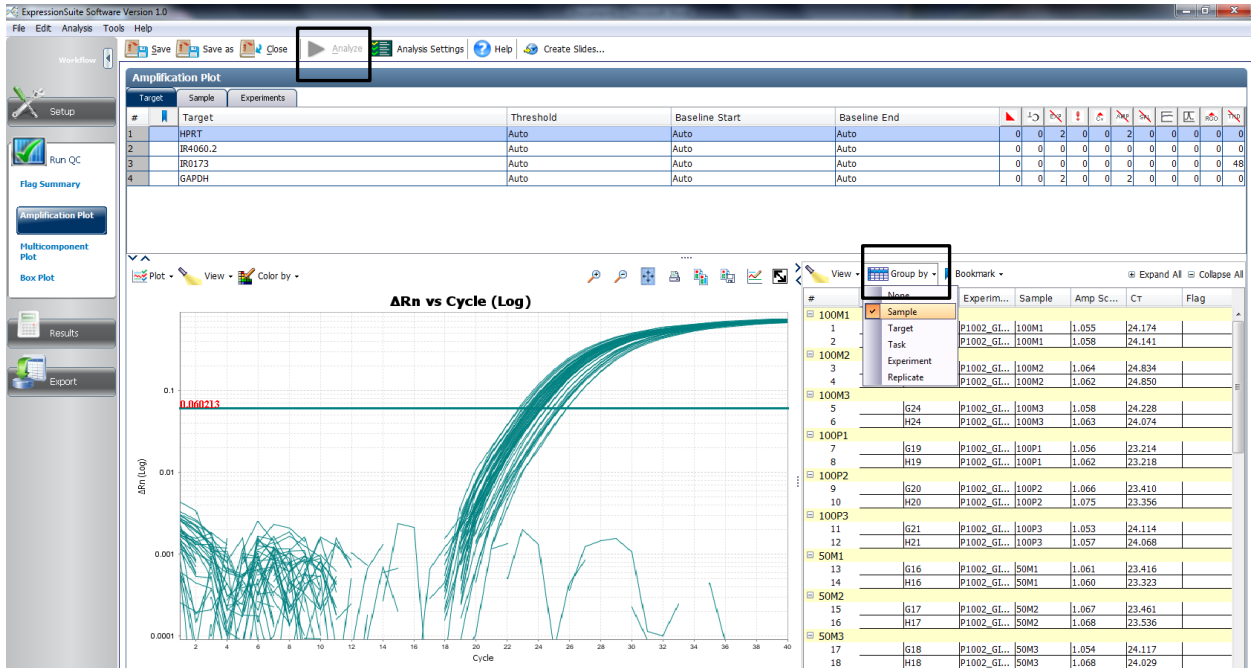


4. In the left menu, click on **Samples** and **Groups** if you assign biological replicates.

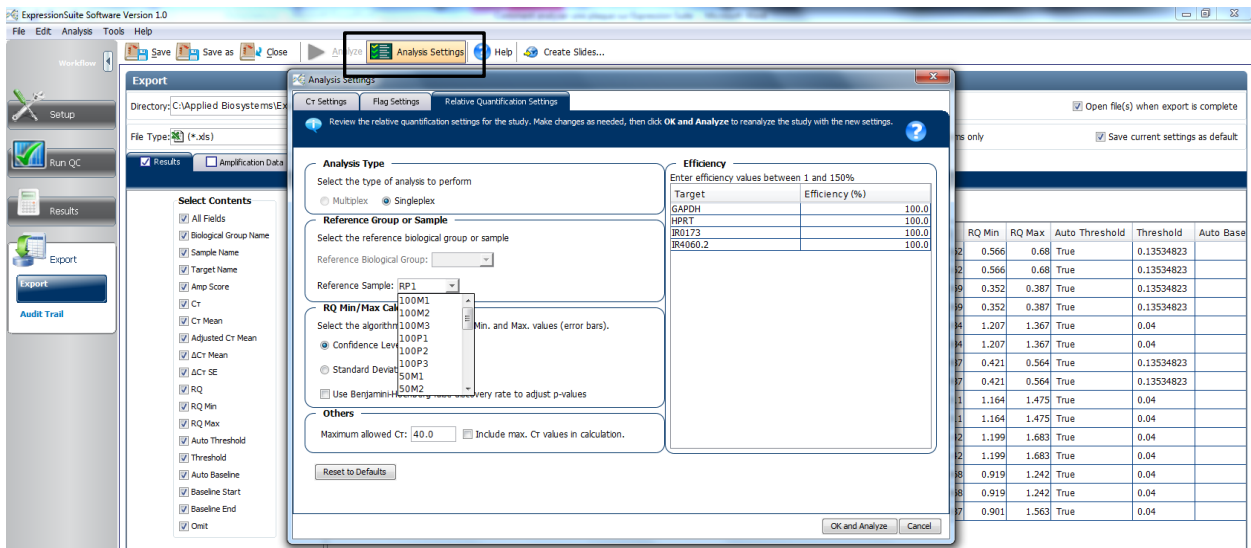
5. In the left menu, click on **Targets** and **Controls** to assign your genes (Targets) and your endogenous controls by right-clicking under the Type column.



6. In the left menu, click **Run QC** and **Amplification Plot** and then click **Analyze**. Afterwards click on **Group by** and select **Sample**. You can then check your technical duplicates/triplicates, which should not exceed more than 0.3 Ct between each replicate.



7. In the left menu, click on **Export**, **Export** and **Analysis Settings**. In the **Relative Quantification Settings** tab, you can determine your reference sample and modify certain statistical parameters. You can also change the % of efficiency of each of your tests. It is recommended for an assay to have a slope between 3.0 and 3.6, which corresponds to an efficiency of 90 and 110 % respectively. Click **Ok and Analyze**.



8. Click **Save**. Choose the folder where you want to export your Excel file by clicking on **Browse**. You can select (check) which values you want to include (Sample Name, Target Name, RQ, RQ min, etc...) then click **Start Export**. Your Excel file will open automatically.

Directory: C:\Applied Biosystems\ExpressionSuite\User Files

File Type: *.xls

Select one file or separate files: Separate files

Results Amplification Data Flag Summary

Previewing first 15 rows of data.

Biological Group Name	Sample Name	Target Name	Amp Score	Ct	Ct Mean	Adjusted Ct Mean	Δ Ct Mean	Δ Ct SE	RQ	RQ Min	RQ Max	Auto Threshold	Threshold	Auto Base
100M2	IR4060.2		1.246	21.465	21.492	21.492	-0.827	0.048	0.62	0.566	0.68	True	0.13534823	
100M2	IR4060.2		1.245	21.519	21.492	21.492	-0.827	0.048	0.62	0.566	0.68	True	0.13534823	
100M1	IR4060.2		1.251	21.528	21.527	21.527	-0.078	0.024	0.369	0.352	0.387	True	0.13534823	
100M1	IR4060.2		1.245	21.526	21.527	21.527	-0.078	0.024	0.369	0.352	0.387	True	0.13534823	
50M2	IR0173		1.014	20.633	20.615	20.615	-0.484	0.032	1.284	1.207	1.367	True	0.04	
50M2	IR0173		1.016	20.597	20.615	20.615	-0.484	0.032	1.284	1.207	1.367	True	0.04	
100M3	IR4060.2		1.245	21.152	21.111	21.111	-0.479	0.076	0.487	0.421	0.564	True	0.13534823	
100M3	IR4060.2		1.246	21.071	21.111	21.111	-0.479	0.076	0.487	0.421	0.564	True	0.13534823	
50M3	IR0173		1.007	21.082	21.111	21.111	-0.513	0.062	1.311	1.164	1.475	True	0.04	
50M3	IR0173		1.008	21.138	21.111	21.111	-0.513	0.062	1.311	1.164	1.475	True	0.04	
50M1	IR0173		1.014	20.400	20.481	20.481	-0.629	0.088	1.42	1.199	1.683	True	0.04	
50M1	IR0173		1.015	20.562	20.481	20.481	-0.629	0.088	1.42	1.199	1.683	True	0.04	
50P3	IR0173		1.006	21.141	21.168	21.168	-0.218	0.078	1.068	0.919	1.242	True	0.04	
50P3	IR0173		1.000	21.195	21.168	21.168	-0.218	0.078	1.068	0.919	1.242	True	0.04	
50P1	IR0173		1.012	22.876	22.744	22.744	-0.37	0.143	1.187	0.901	1.563	True	0.04	