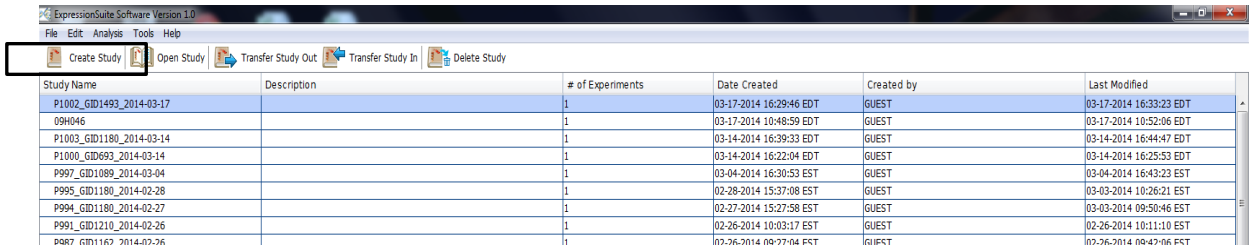
How to analyze qPCR data using ExpressionSuite Software

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

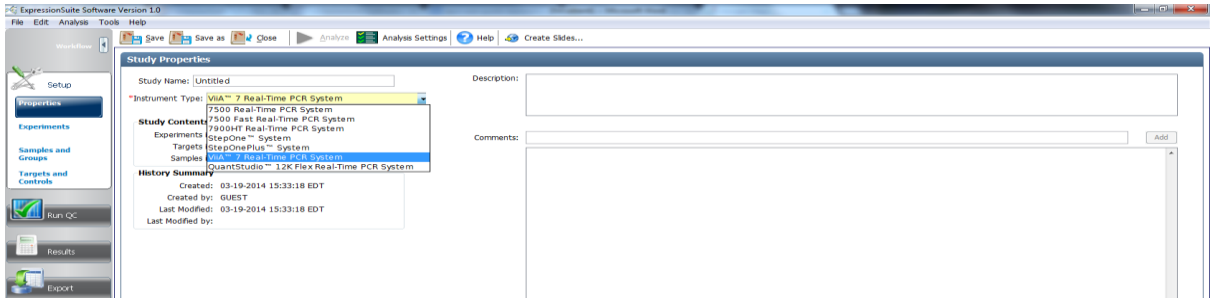
http://www.lifetechnologies.com/ca/en/home/global/forms/expressionsuite-software-registration.html

ExpressionSuite uses the comparative Cτ method (ΔΔCτ) 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.

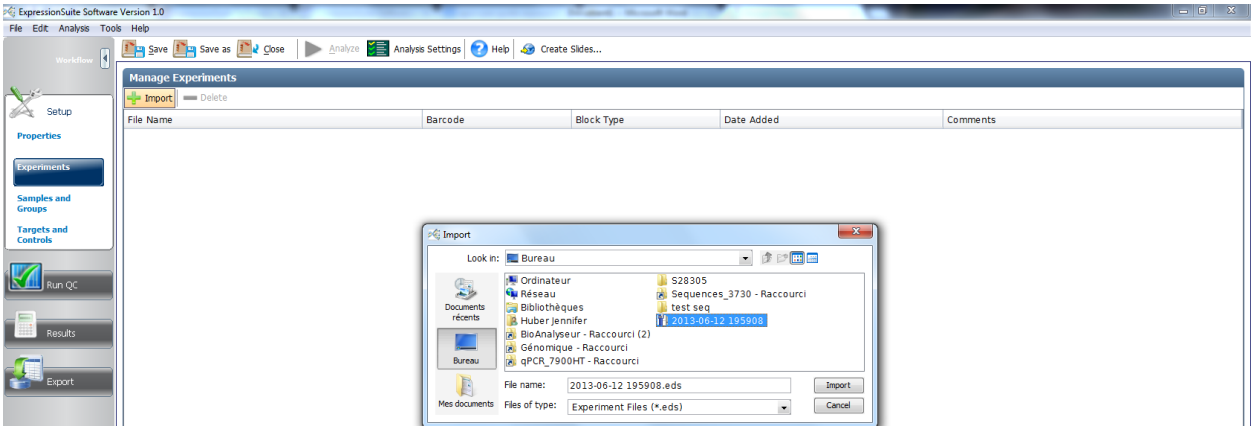
1. On the ExpressionSuite's main menu, click on **Create Study**.



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

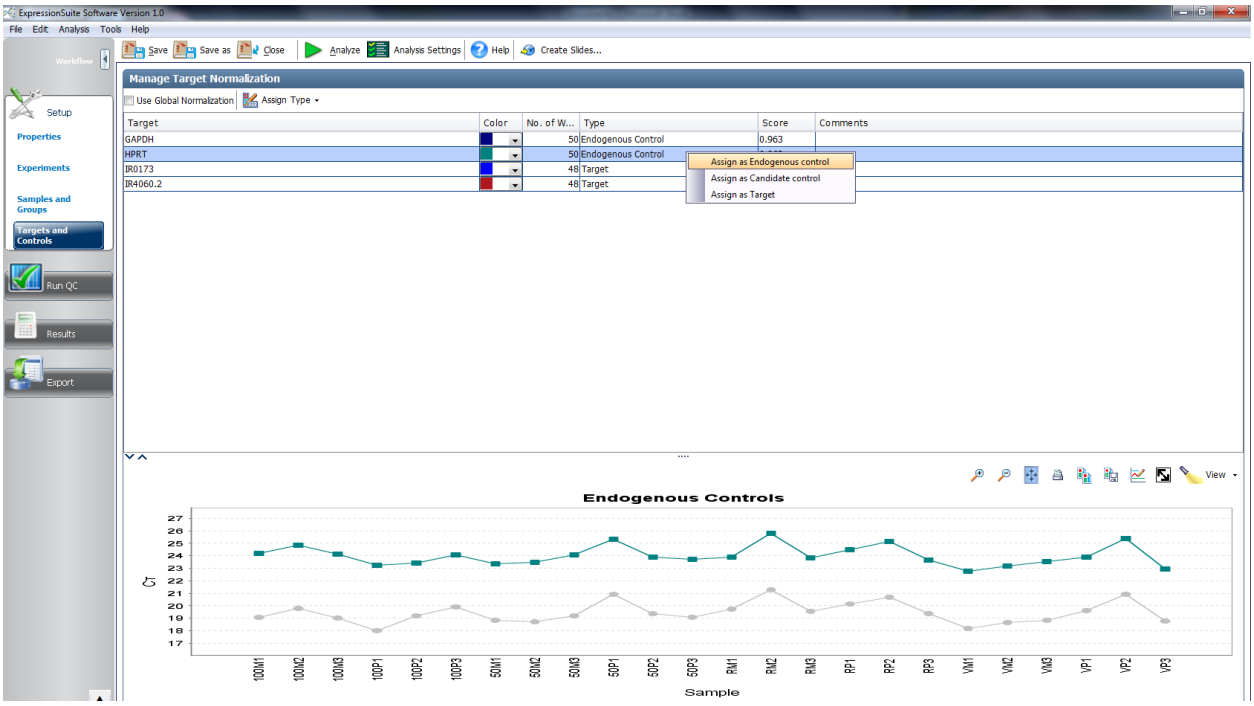


3. In the left menu, click on **Experiments** and **Import** to import one or more projects.

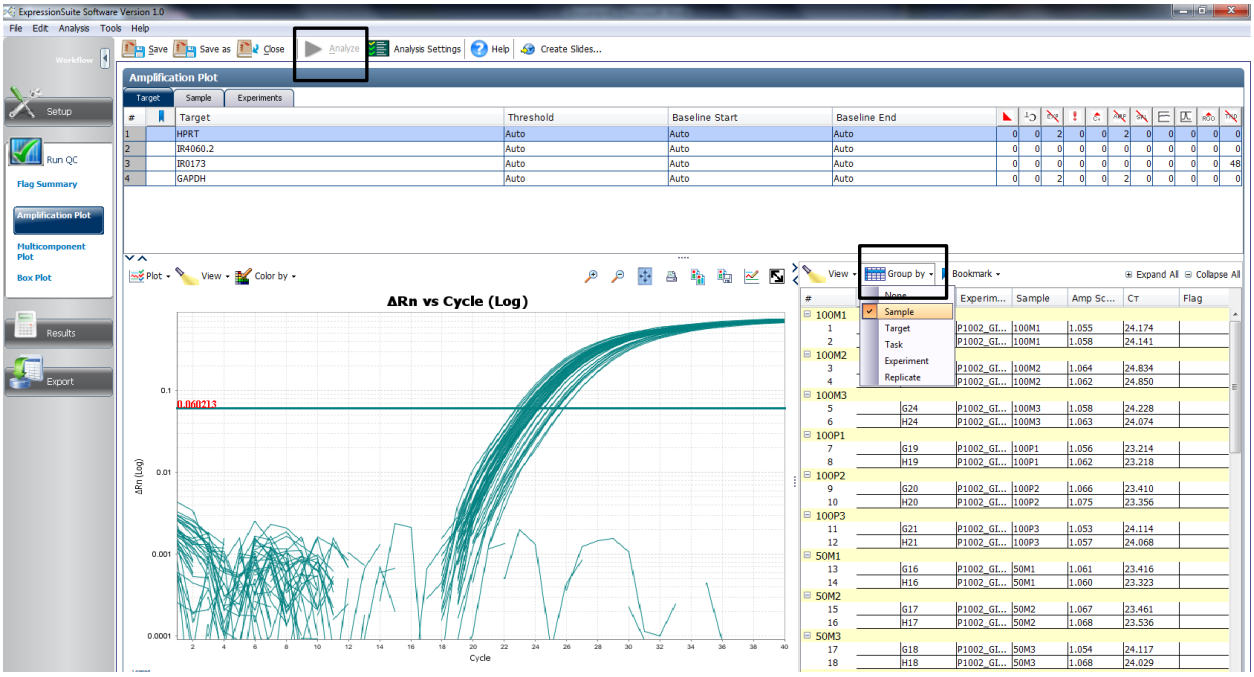


4. In the left menu, click on **Samples** and **Groups** if you assign biological replicates.

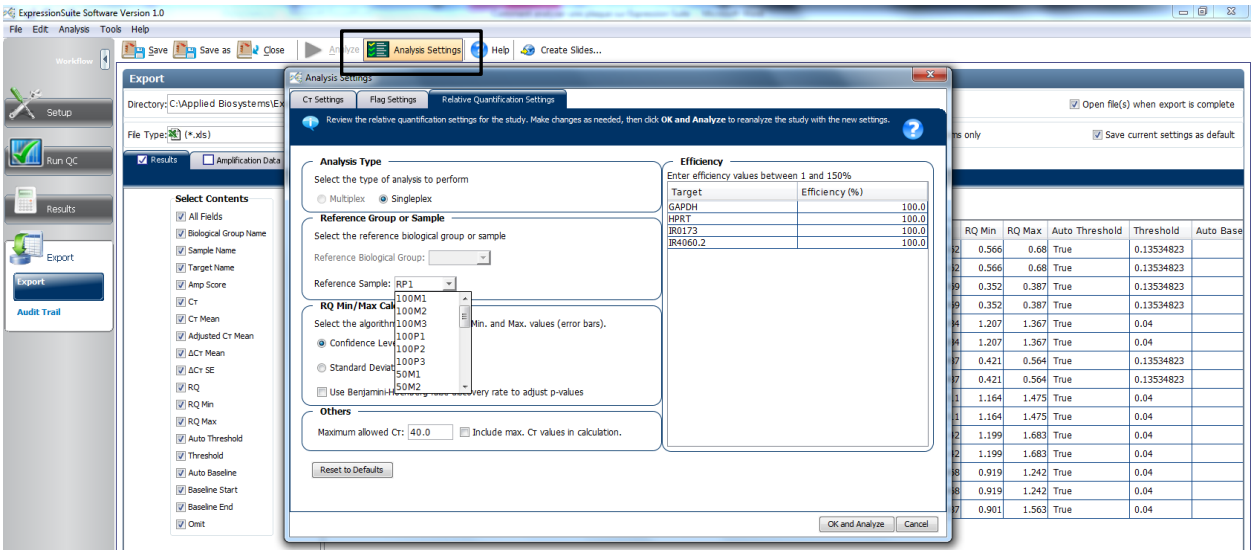
5. In the left menu, click on **Targets** and **Controls** to assign your genes (Targets) and your endogenous controls by right-clicking under the Type column.



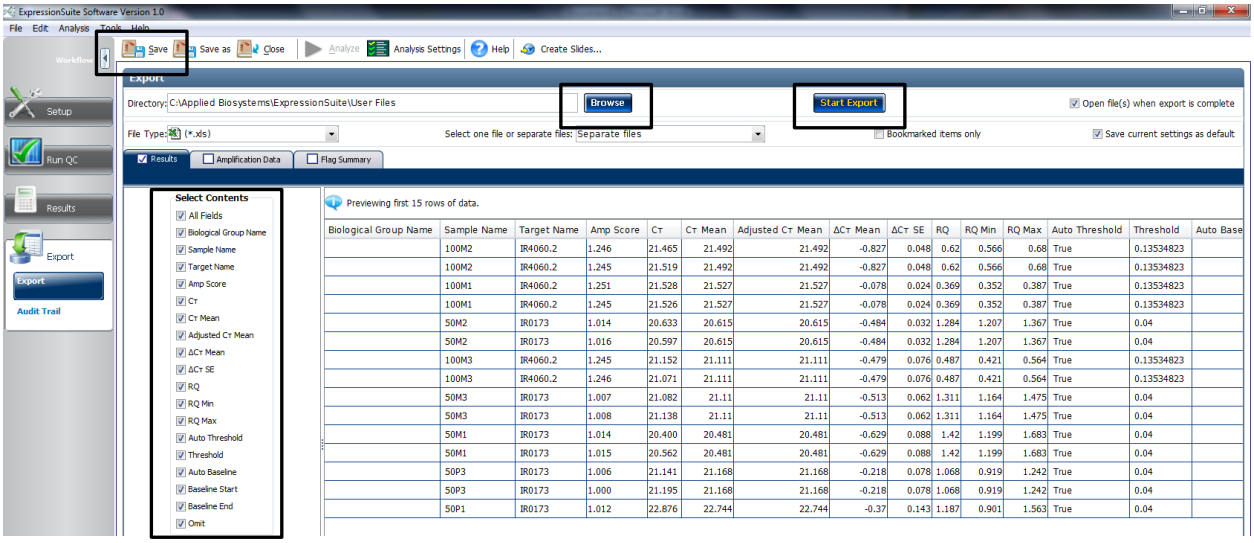
6. In the left menu, click **Run QC** and **Amplification Plot** and then click **Analyze**. Afterwards click on **Group by** and select **Sample**. You can then check your technical duplicates/triplicates, which should not exceed more than 0.3 Ct between each replicate.



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.



Biological Group Name	Sample Name	Target Name	Amp Score	Cт	Cт Mean	Adjusted Cт Mean	ΔCт Mean	ΔCт 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	21.11	21.11	-0.513	0.062	1.311	1.164	1.475	True	0.04	
	50M3	IR0173	1.008	21.138	21.11	21.11	-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	