

# GeXP Quantitative Analysis Method

## A Tutorial

# Three major steps

1. Optimize the amount of KAN<sup>r</sup> RNA used in GeXP reactions for a particular multiplex.
2. Perform GeXP multiplex reactions with a serial two-fold dilution of Reference RNA\* and the optimized amount of KAN<sup>r</sup> RNA to establish standard curves for each gene in the multiplex.

*Perform the first two steps one time for each multiplex on each GeXP System.*

3. Use the GeXP Quant Tool to analyze experimental data with the standard curve for that multiplex.

*\* The Reference RNA should be a mixture of the control and experimental samples that will be tested with the multiplex. This is the same RNA used to optimize reverse primer concentrations.*

# 1. Optimize the amount of KAN<sup>r</sup> RNA for a particular multiplex

## Purpose:

The GeXP quantitative analysis method utilizes individual standard curves for each gene in the multiplex. Each standard curve is generated from the relative signal level of a gene normalized to the reaction control gene, KAN<sup>r</sup>, for each concentration of RNA.

To effectively use KAN<sup>r</sup> as a reaction control gene, the amount of KAN<sup>r</sup> RNA in the reaction must be optimized such that the KAN<sup>r</sup> peak is in the median signal range of the multiplex.

# Optimize the amount of KAN<sup>r</sup> RNA in GeXP reactions

- Dilute KAN<sup>r</sup> RNA (stock = 5 ng/ul) over a series of eight 2-fold dilutions
- The final amount of KAN<sup>r</sup> RNA used in each reaction will range from 50 pg to 6400 pg.
  - Note: A broader range may be necessary for some multiplexes.
- Add each dilution of KAN<sup>r</sup> RNA to a RT reaction that contains ~20 ng\* reference RNA per reaction.

*\* Use the optimal amount of Reference RNA; the same amount that will be used when testing experimental samples.*

# Two-fold Serial Dilutions of KAN<sup>r</sup> RNA

Dilution	1	2	3	4	5	6	7	8
Amount of KAN <sup>r</sup> RNA per reaction (pg)	6400	3200	1600	800	400	200	100	50
Volume from previous dilution (uL)	12.8*	25	25	25	25	25	25	25
Volume of diluent# (uL)	37.2	25	25	25	25	25	25	25
Final Concentration (pg/uL)	1280	640	320	160	80	40	20	10
Mixed volume transferred to the next series (uL)	25	25	25	25	25	25	25	0
Final Volume (uL)	25	25	25	25	25	25	25	50

\* Original GeXP KAN<sup>r</sup> RNA with RI (BCI A21041), 5 ng/uL

# Diluent: RNA Storage Solution (preferred) or DNase-RNase-Free H<sub>2</sub>O

**Use 5 uL diluted KAN<sup>r</sup> RNA per reaction for each dilution**

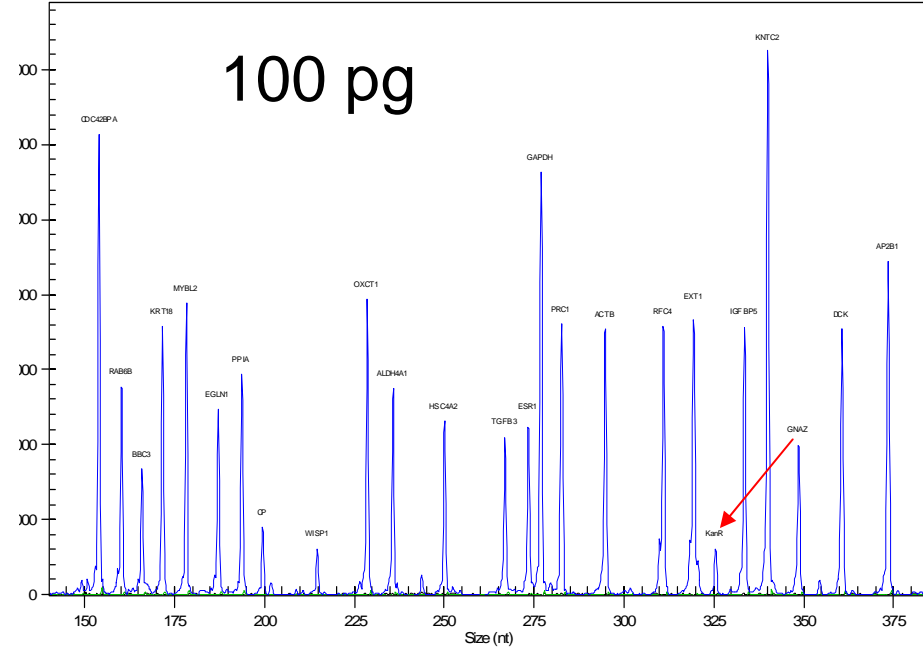
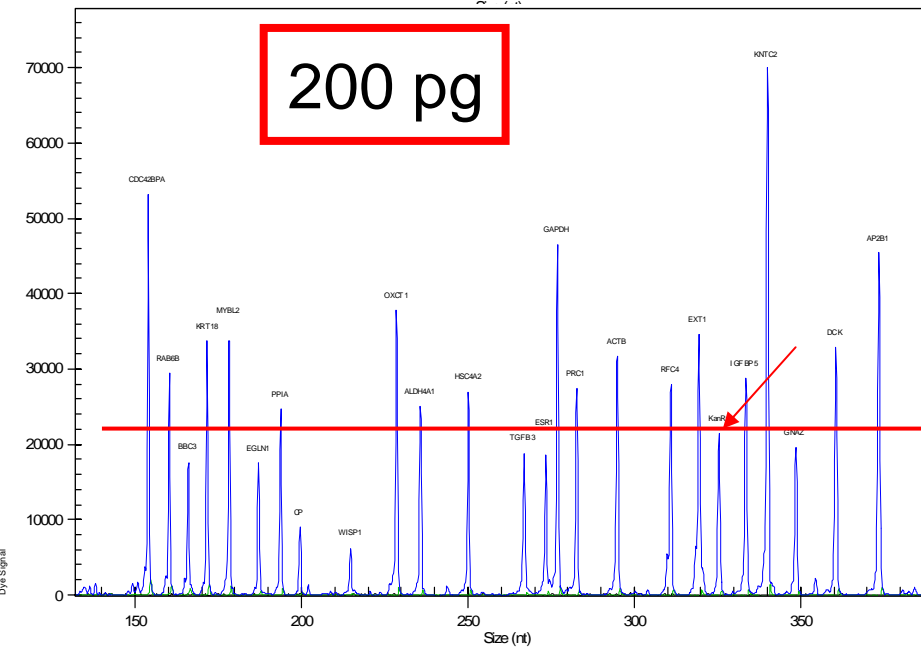
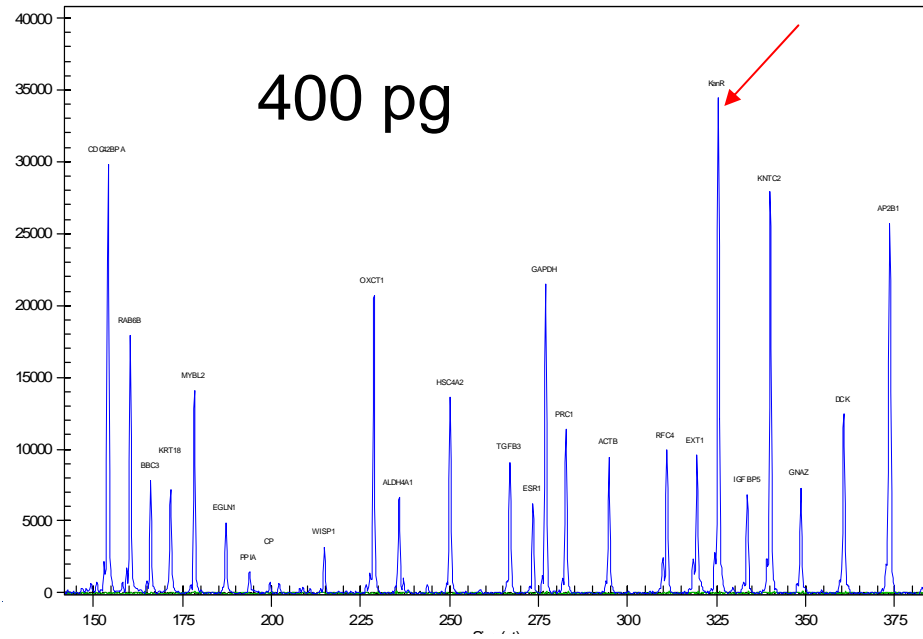
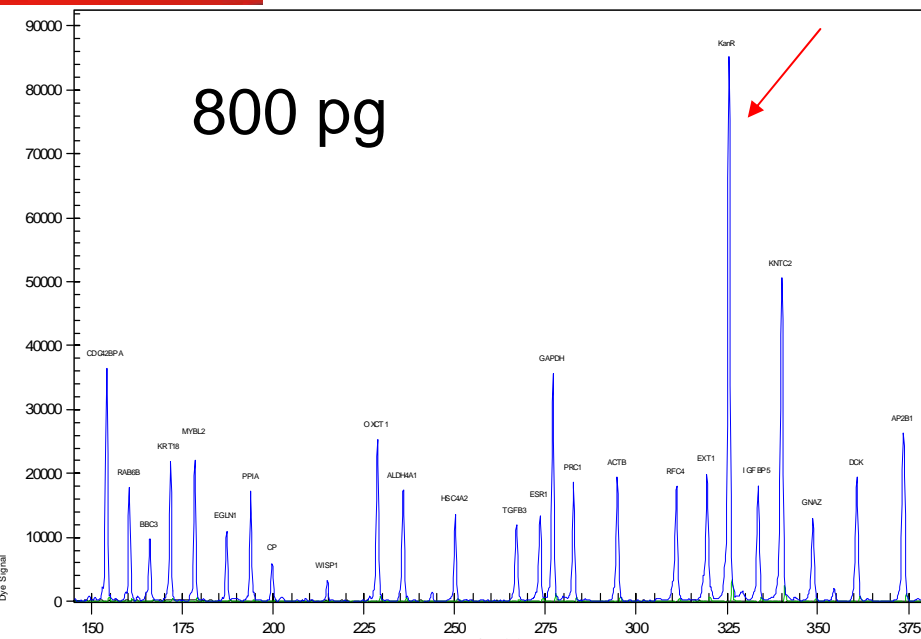
# Important

Accurate dilution is crucial for the quality of a standard curve.

Make sure that:

1. Volume transferred is accurate
2. Every dilution is fully mixed before transferring the next aliquot
3. A fresh barrier tip is used for each dilution

# Example of KAN<sup>r</sup> RNA titration result



## Results of the KAN<sup>r</sup> Titration

- Choose the KAN<sup>r</sup> RNA concentration that gives a KAN<sup>r</sup> peak height that is roughly equivalent to the median peak height of the multiplex.
  - The previous slide demonstrates that 200 pg KAN<sup>r</sup> RNA generated a median peak height and this concentration is optimal for this particular multiplex.
- A particular concentration of KAN<sup>r</sup> is acceptable as long as it produces a peak height of KAN<sup>r</sup> that falls within the peak heights of the multiplex and it is within range (370 - 120,000 RFU in analyzed data) for all samples.

## Results of the KAN<sup>r</sup> Titration *continued*

- Once an optimal KAN<sup>r</sup> concentration for a multiplex is determined, that **exact** same concentration of KAN<sup>r</sup> RNA should be used for all of the experiments with this multiplex in order to use the the standard curve method with GeXP quantitative analysis.
  - Recommendation: Make a stock dilution of the KAN<sup>r</sup> RNA in RNA Storage Solution (Ambion), aliquot and freeze at -80°C for future use with the multiplex.

## 2. Establish standard curves for each gene within a multiplex using Reference RNA

- The GeXP quantitative analysis method uses a standard curve to calculate the **gene expression quantitation (GEQ)** value which corresponds to the relative signal level for each gene in each sample. The GEQ value is then used by the software to calculate a normalized gene expression value for each gene in each sample.
- It is recommended that the standard curve be generated using a series of increasing concentrations of a Reference RNA, preferably from 1 ng to 500 ng. The standard curve can consist of a broader range of RNA concentrations if necessary.
  - Note: The relative signal values for each gene in each sample must fall within the range of the standard curve to generate accurate GEQ values.

# Generate standard curve with 1 ng to 500 ng of Reference RNA

- Perform a series of GeXP RT-PCR reactions with increasing amounts of Reference RNA.
  - Use an optimized multiplex
  - Use the optimized amount of KAN<sup>r</sup> RNA in each reaction
  - The standard curve for each gene in the multiplex is derived from this RNA titration
- The Reference RNA should be a mixture of control and experimental samples.

# Naming standards and experimental samples

Name the samples with the format below so they are properly sorted by eXpress Profiler and recognized by GeXP Quant Tool:

Standards: **Std**<space>###ng

Std 001ng	Std 031.3ng
Std 002ng	Std 062.5ng
Std 003.9ng	Std 125ng
Std 007.8ng	Std 250ng
Std 015.6ng	Std 500ng

Experimental: **U**<space>###

U 001	or	U Normal_01
U 010		U Tumor_11
U 100		U Control_05

*U stands for unknown GEQ value*

Std 1	Std 16	Std128
Control1	Tumor2	

# RT reaction for standard curve

- The optimized amount of KAN<sup>r</sup> RNA per reaction is built into the RT master mix.
  - Make a 4X stock solution of the optimized KAN<sup>r</sup> RNA in RNA Storage Solution, aliquot and freeze for future use with this multiplex
  - 5 uL of the stock solution is added to the RT master mix for each reaction
- Prepare a series of 2-fold dilutions from the Reference RNA
  - Total Reference RNA used per reaction ranges from 1 ng to 500 ng
- 5 uL of each RNA dilution is added directly to the reaction well and then 15 uL of the RT master mix is added
  - Perform reactions in triplicate or quadruplicate for each RNA concentration

## Two-fold Serial dilutions of Reference RNA

Dilution	1	2	3	4	5	6	7	8	9	10
Amount per reaction (ng)	500	250	125	62.5	31.3	15.6	7.8	3.9	2	1
Volume from previous dilution (ul)	50*	25	25	25	25	25	25	25	25	25
Volume from diluent <sup>#</sup> (ul)	0	25	25	25	25	25	25	25	25	25
Mixed volume transferred to the next series (ul)	25	25	25	25	25	25	25	25	25	0
Final Concentration (ng/ul)	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.2
Final volume (ul)	25	25	25	25	25	25	25	25	25	50

\* Reference RNA

# Diluent: RNA Storage Solution (preferred) or DNase-RNase-Free H<sub>2</sub>O

**Use 5 uL of diluted Reference RNA per reaction**

## RT Standard Reaction

<b>Components</b>	uL per rxn	Master Mix
<i>Template-free Zone</i>	X 1	X 40
DNase RNase Free H <sub>2</sub> O	3	120
GeXP 5X RT Buffer	4	160
Reverse Plex	2	80
Reverse Transcriptase	1	40
<i>Template-addition Zone</i>		
<b>Diluted</b> KAN <sup>r</sup> RNA	5	80
* Individual dilutions of Reference RNA	5*	-
<b>Total</b>	<b>20</b>	

Perform RT thermal cycling as usual

## Standard PCR Reaction

Components	uL per rxn
<i>Template-free Zone</i>	
25 mM MgCl <sub>2</sub>	4
GeXP 5X PCR Buffer	4
Forward Plex	2
Taq DNA Polymerase	0.7
<i>Template-addition Zone</i>	
RT reaction (cDNA)	9.3
<b>Total</b>	<b>20</b>

Perform PCR thermal cycling as usual

# Perform Standard GeXP CE Separation

Pre-dilute every PCR reaction with the same dilution factor that was used for the primer optimization.

Run Frag-3 method as usual.

# Modified Fragment Analysis

Fragments separated on the GeXP system are analyzed by a set of **modified** GeXP Fragment Analysis Parameters in order to call all possible peaks, including smallest ones.

**Slope threshold = 1**

**Peak height threshold = 0**

# Important

- Increasing amounts of RNA will cause the peak height to be maximized. This is expected. However, each peak should be intact and not split at the apex.
- A single over-ranged peak that splits into two peaks at the apex (called as two peaks) must be excluded from further analysis.

## Perform the following steps as usual:

- Check for current or separation anomalies.
- Exclude controls (NTC, RT minus) and wells with anomalies from further analysis.
- Apply Exclusion Filters
- Export GeXP Fragment Analysis data to eXpress Analysis
- Set up a new analysis in eXpress Analysis
- Perform Plate Setup and Binning
- Proceed to GeXP Normalization

# Use KAN<sup>r</sup> as the Normalization Gene

The screenshot shows the 'eXpress Analysis' software interface. The 'Normalize Peaks' dialog box is open, with the following settings:

- Multiplex: HuBreastCancer
- Normalization Gene: Kan(r)
- Display zero valued expression levels
- Display normalized values
- Genes: A list of genes with checkboxes, all of which are checked. The genes listed are BC000689, hGAPDH, Kan(r), L27560, NM\_000096, NM\_000125, NM\_000127, NM\_000224, and NM\_000436.

On the left side of the interface, there are three buttons: 'Normalize Peaks', 'Report View', and 'Report View'. The 'Report View' button is highlighted with a red arrow pointing to a callout box.

The main graph displays 'Expression' on the y-axis and 'Treatments' on the x-axis. The treatments are Std 001ng, Std 002ng, Std 004ng, Std 008ng, Std 016ng, Std 032ng, Std 064ng, Std 128ng, Std 256ng, and Std 512ng. The graph shows multiple lines representing different genes. A red arrow points to the line for Kan<sup>r</sup>, which is a straight line, indicating it is the normalization gene.

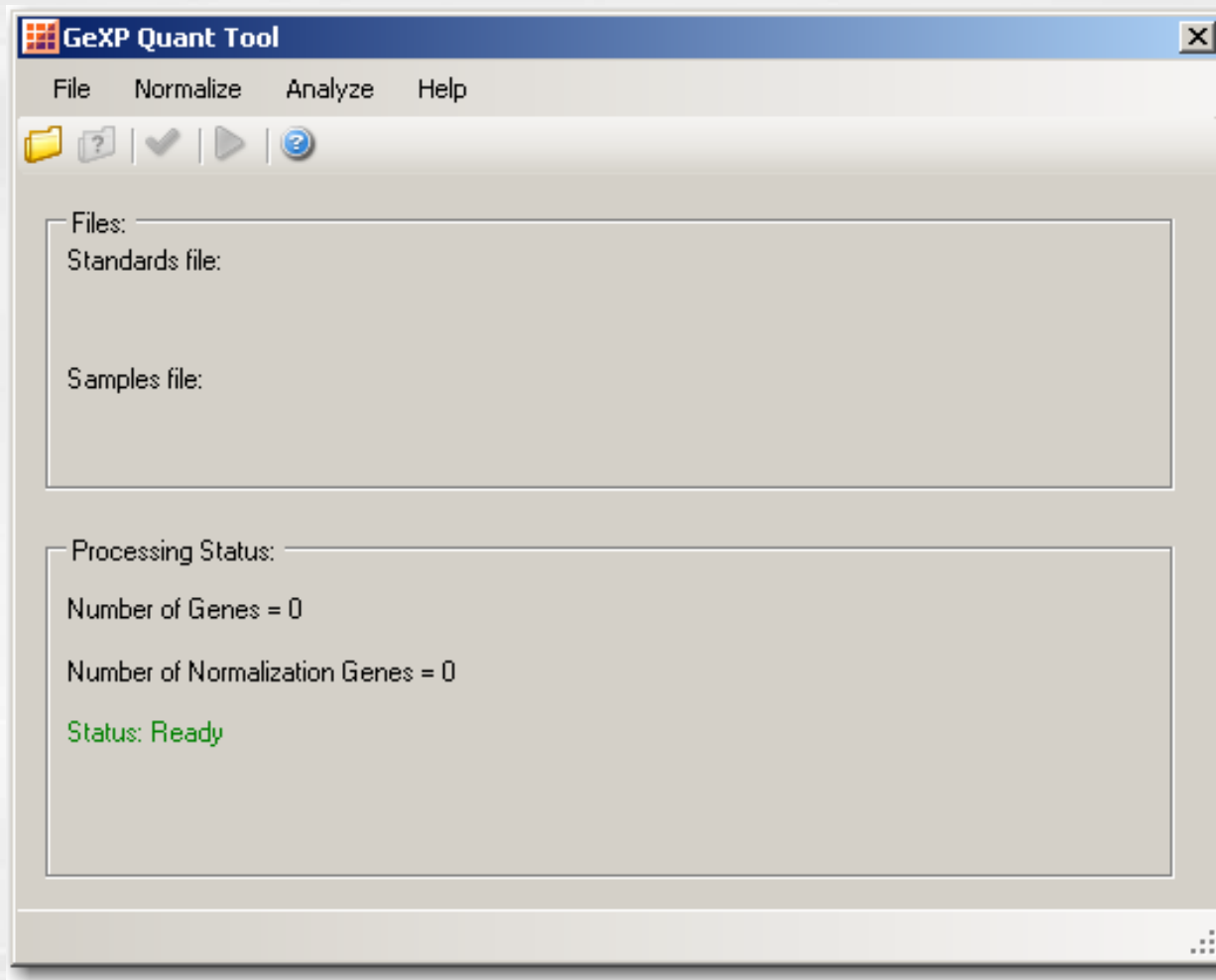
Four callout boxes provide instructions:

1. Select Kan(r) as Normalization Gene
2. Select Display normalized values
3. Click Select All
4. Click on Report View

A text box at the bottom of the graph states: 'Kan<sup>R</sup> is the straight line'.

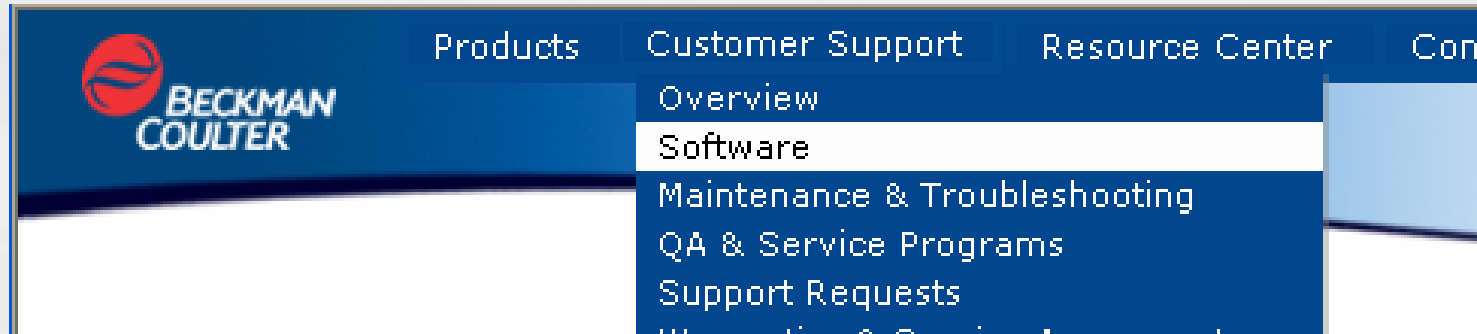


# GeXP Quant Tool



Download this software tool from the Beckman Coulter website.

# Download the GeXP Quant Tool



Go to : [www.beckmancoulter.com](http://www.beckmancoulter.com)

Select "Software" from the Customer Support menu.

From the software download page (shown on next slide):

- Select "GeXP" for step 2
- Select "GeXP Quant Tool" for step 3
- Click the [Download] button

**Step 1 Please Select a Category:****Step 2 Choose a Product:**  
  
  
  
**Step 3 Choose the Software or Demo or Manual:****Step 4 Select a language:**

## • [Download the GeXP Quant Tool](#)

Printer Friendly 

The GeXP Quant Tool automates the quantitative analysis of data obtained from GeXP eXpress Profiler reports.

This application calculates standard curves from the standards in the standards report, determines the Gene Expression Quantitation (GEQ) values for the samples in the sample report, and normalizes the GEQ values based on the selection of one or more reference (housekeeping) genes.

The GeXP Quant Tool output is a Microsoft Excel workbook with worksheets, containing the calculation formulas and results, for each gene in the analysis.

**For research use only; not for use in diagnostic procedures.**

### [Install the GeXP Quant Tool](#)

You may receive one or more of the following security warnings during the software installation:

"Do you want to run or save this file?": Select "Run".

"Do you want to run this software?": Select "Run".

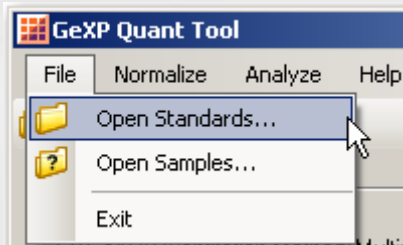
"Do you want to install this application?": Select "Install".

### **System Requirements:**

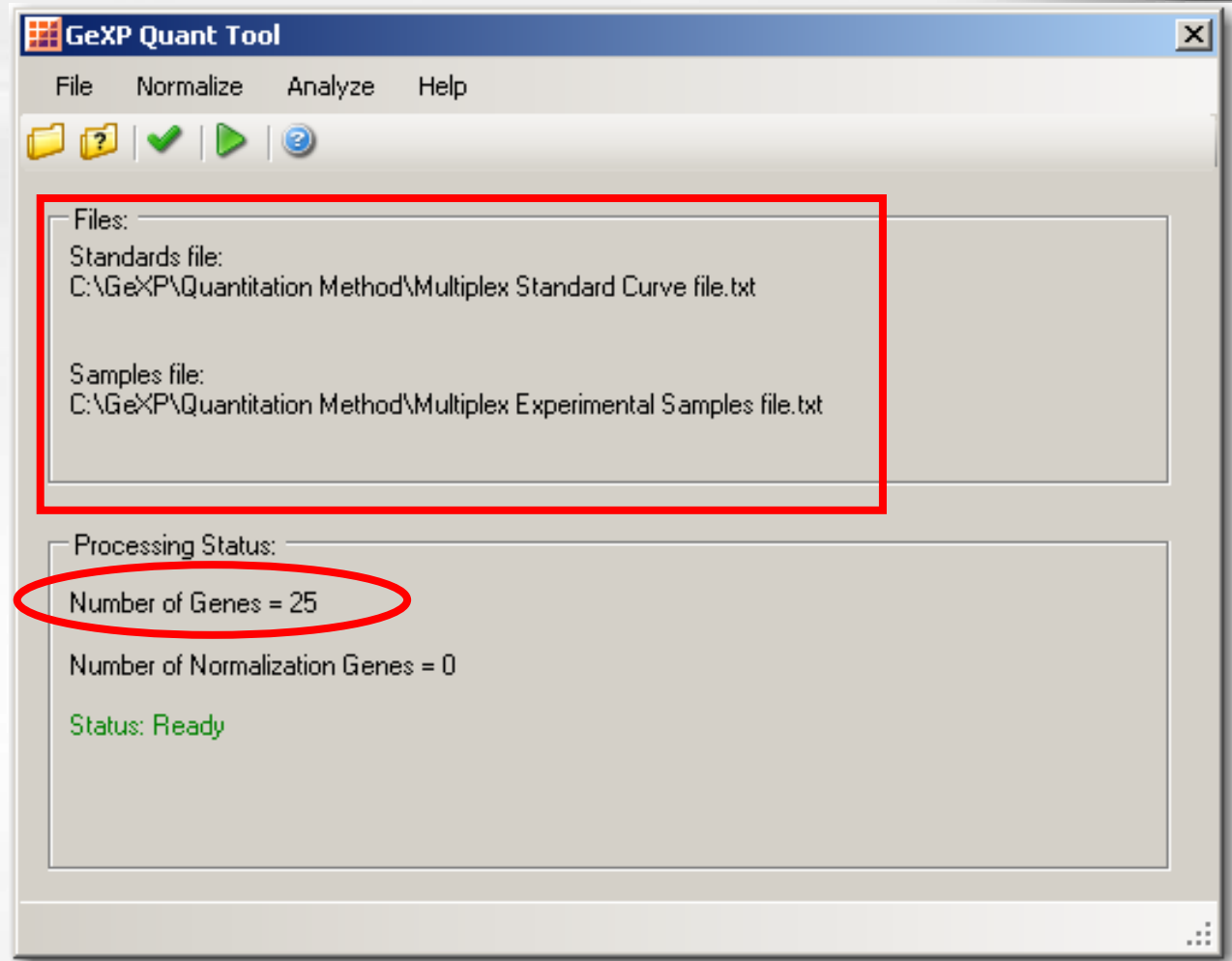
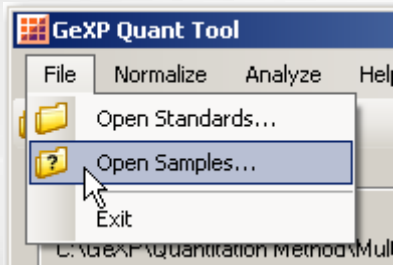
- Microsoft\* Windows\* XP
- Microsoft Excel\* 2003 or 2007

# Loading Data Files

## 1. Load Standards



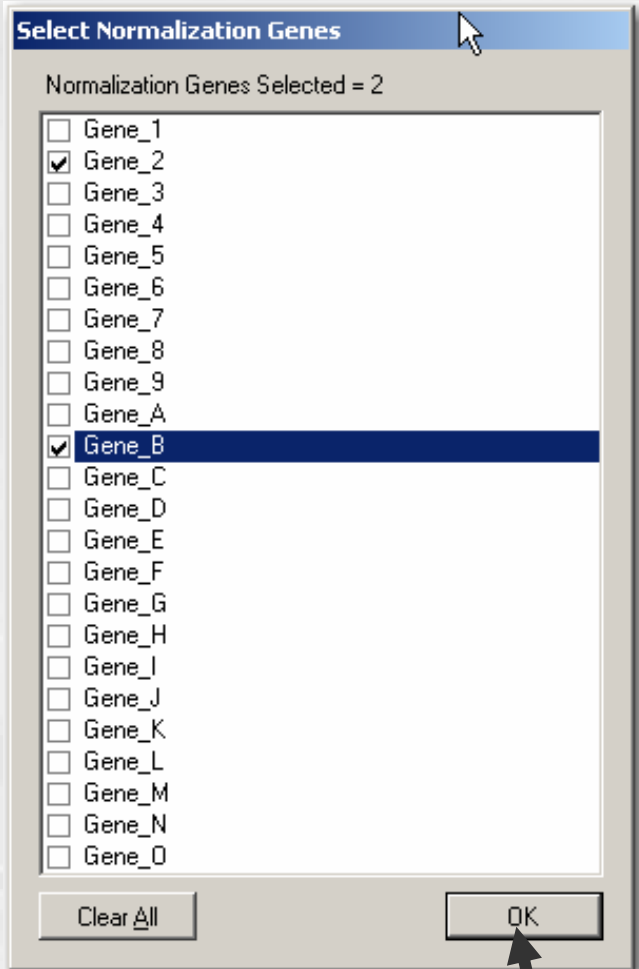
## 2. Load Samples



The standard curve must be loaded each time GeXP Quant Tool is opened.

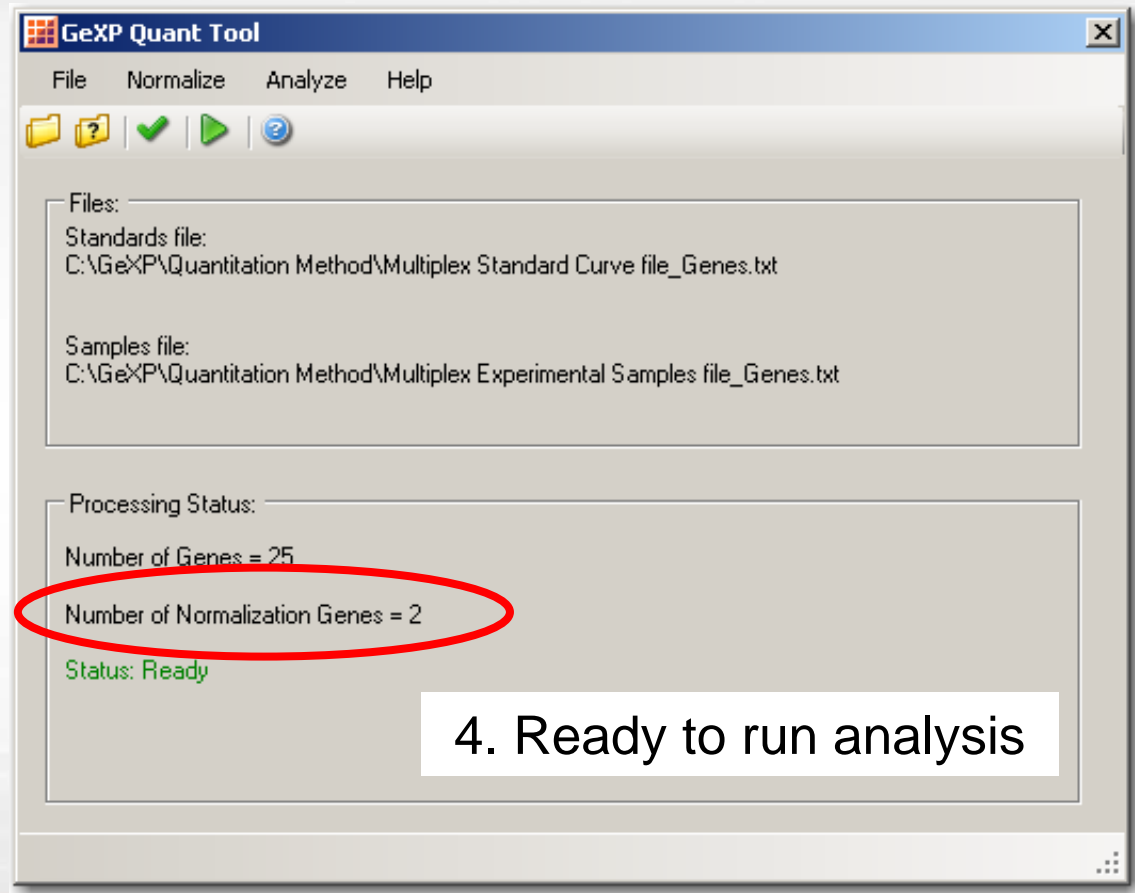
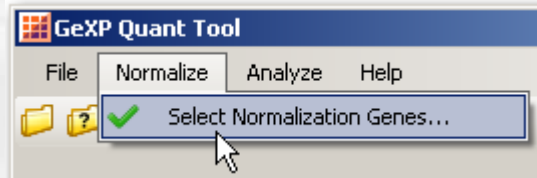
# Choose Normalization Gene(s)

## 2. Choose genes



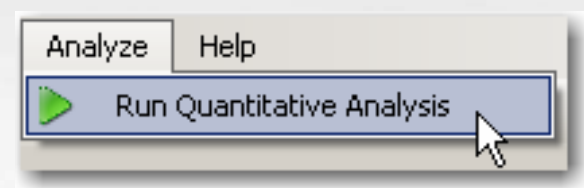
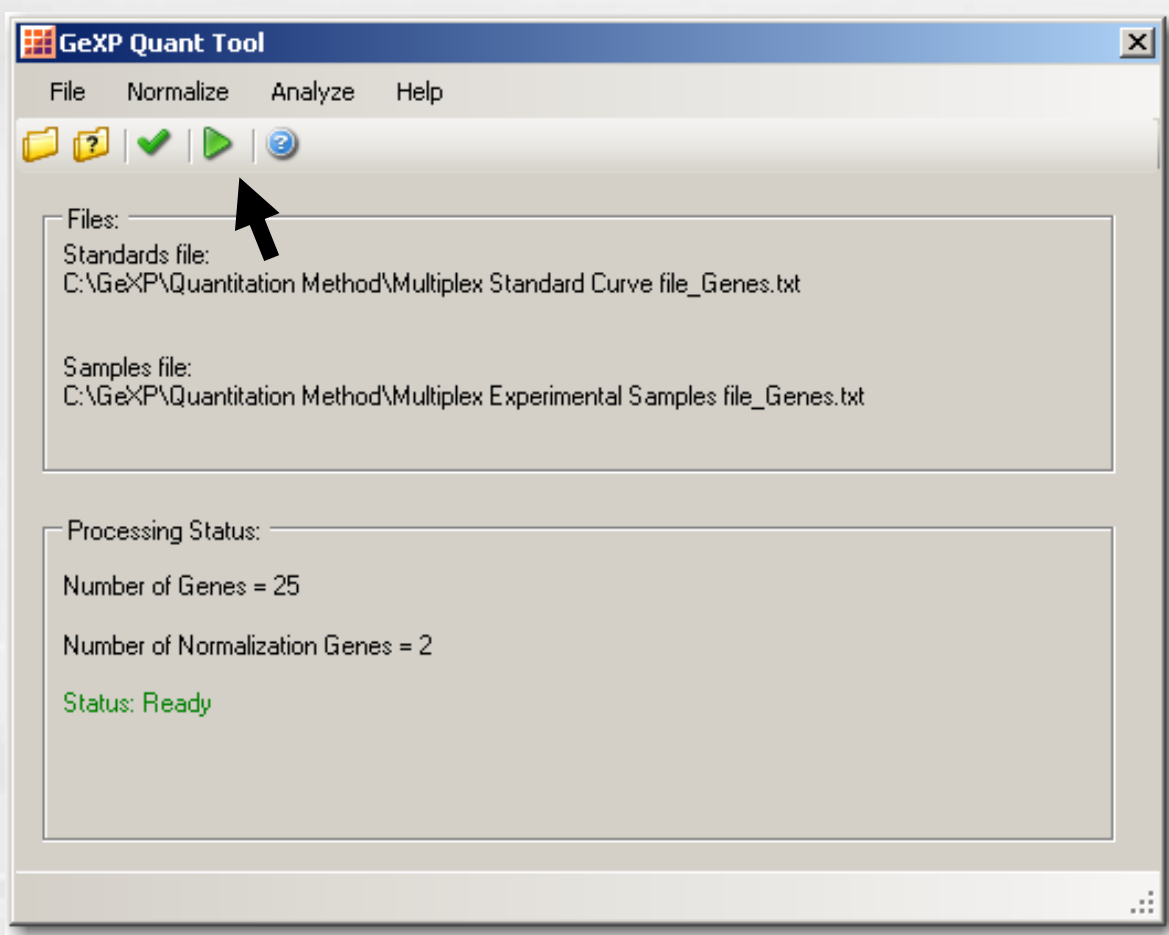
3.

1.

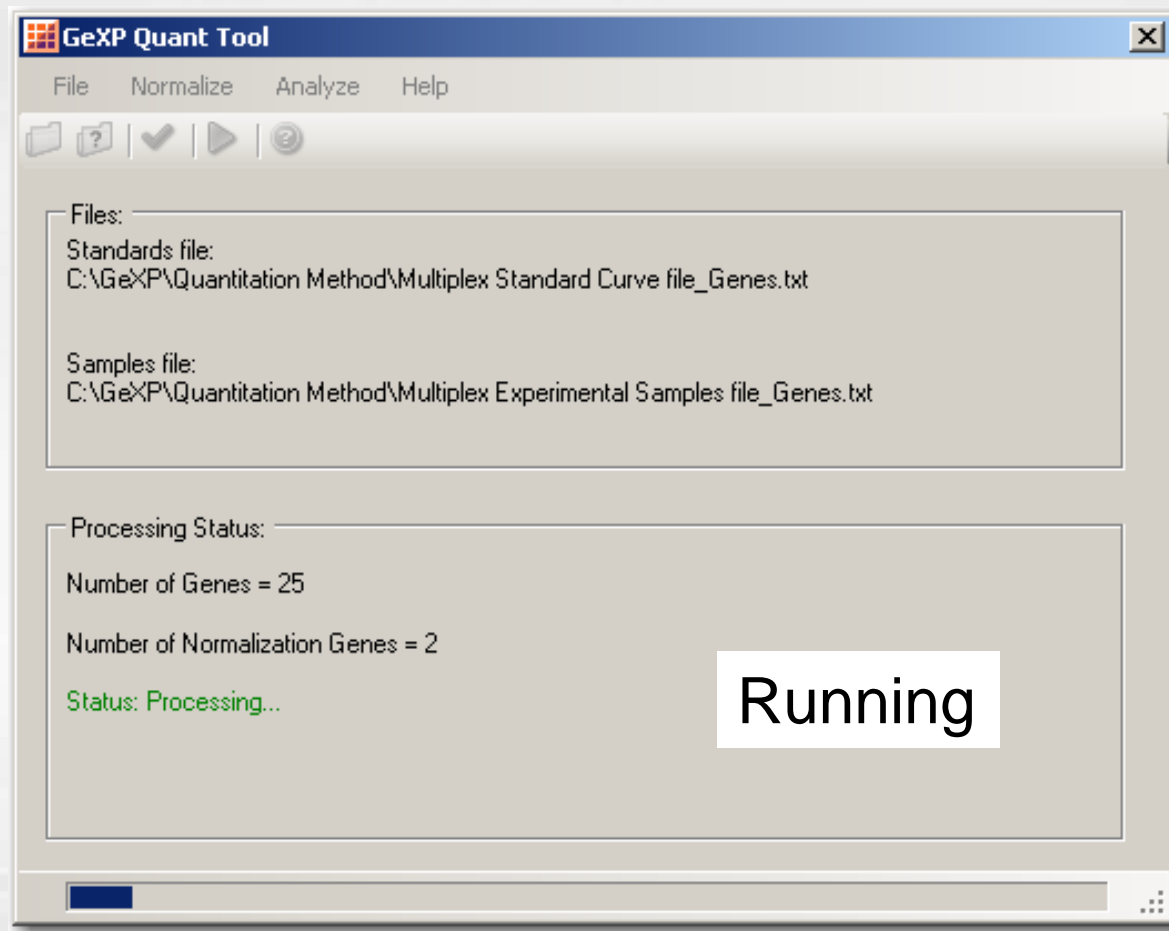


4. Ready to run analysis

# Analyze Data Files



# Analyzing Data Files



# EXCEL Workbook

## Summary Page

	A	B	C	D	E	F	G	H	I
1	<b>GeXP Quant Tool</b>	(version 1.0.3079.26088)							
2	Analysis generated on: 06/18/2008 5:52:15 PM								
3	Analysis created from standards file: C:\GeXP\Quantitation Method\Multiplex Standard Curve file_Genes.txt								
4	Analysis created from samples file: C:\GeXP\Quantitation Method\Multiplex Experimental Samples file_Genes.txt								
5									
6	<b>Normalization Genes</b>								
7	Gene_2								
8	Gene_B								
9									
10	<b>Genes</b>	<b>Curve Fit (R2)</b>							
11	Gene_1	1.0000							
12	Gene_2	0.9998							
13	Gene_3	1.0000							
14	Gene_4	0.9992							
15	Gene_5	0.9999							
16	Gene_6	0.9997							
17	Gene_7	0.9999							
18	Gene_8	0.9985							
19	Gene_9	0.9998							
20	Gene_A	0.9997							
21	Gene_B	0.9999							
22	Gene_C	0.9999							
23	Gene_D	0.9919							
24	Gene_E	0.9998							
25	Gene_F	0.9998							
26	Gene_G	0.9998							
27	Gene_H	0.9996							
28	Gene_I	0.9996							
29	Gene_J	0.9999							

All genes and curve fit values are listed here.

# Description Page

Explains each set of data found on the gene worksheet.

Gene:													
Standards:													
Concentration (ng)	Mean	Std Dev	% CV		Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Replicate #7	Replicate #8	Rep
Standard concentrations, in nanograms, decoded from the standard names.	Statistical mean, standard deviation and percent coefficient of variation for the standard replicates.				The relative signal level of each standard replicate loaded from the standards report.								
Curve Fit(R2)	The R2 value for the standard curve for this gene.												
Samples:													
Sample names.	Statistical mean, standard deviation and percent coefficient of variation for the sample replicates.				The relative signal level of each sample replicate loaded from the samples report.								
GEOs:													
Sample names.	Statistical mean, standard deviation and percent coefficient of variation for the GEQ replicates.				The GEQ values derived from the sample replicates interpolated from the standard curve.								

# Gene Worksheet

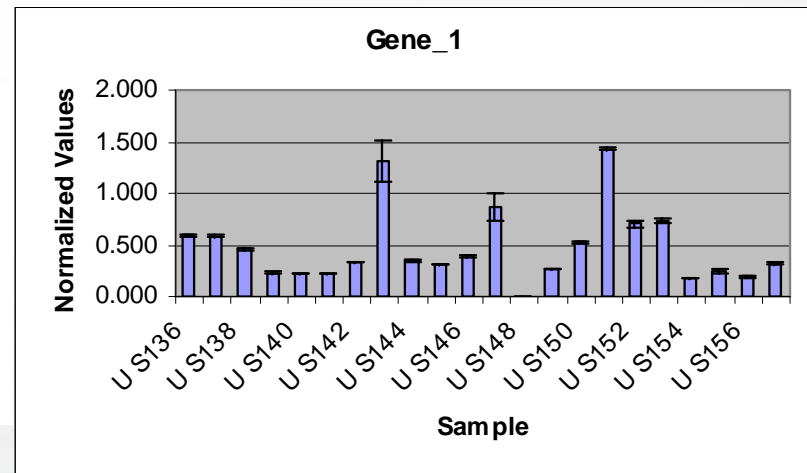
Gene: Gene 1											
Standards:											
Concentration (ng)	Mean	Std Dev	% CV	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #10	Least Squares Fit: 3rd order polynomial		
1.562	0.079	0.012	15.2	0.066	0.095	0.076		0.045137395	9.944918087	12.80002909	0
3.125	0.154	0.018	11.8	0.134	0.150	0.178		0.088249156	0.692802723	1.230988785	#N/A
6.25	0.376	0.014	3.6	0.364	0.369	0.395		0.999955157	1.262695819	#N/A	#N/A
12.5	0.672	0.033	4.8	0.704	0.639			44598.35544	6	#N/A	#N/A
25	1.095	0.086	7.9	1.012	1.214	1.060		213322.9516	9.566404387	#N/A	#N/A
50	1.696	0.047	2.7	1.704	1.748	1.635					
100	2.545	0.064	2.5	2.552	2.619	2.463					
200	3.872	0.084	2.2	3.914	3.946	3.755					
400	5.664	0.127	2.2	5.615	5.539	5.839					
Concentration minimum	0.079										
Concentration maximum	5.664										
Curve Fit(R2)	1.0000										
Samples:											
U S136	1.271	0.009	0.7	1.284	1.263	1.265					
U S137	1.448	0.027	1.9	1.414	1.449	1.481					
U S138	1.400	0.027	1.9	1.433	1.401	1.367					
U S139	1.099	0.004	0.4	1.096	1.105	1.095					
U S140	0.723	0.004	0.5	0.721	0.719	0.728					
U S141	1.074	0.014	1.3	1.086	1.081	1.054					
U S142	1.497	0.014	0.9	1.516	1.494	1.482					
U S143	0.253	0.029	11.5	0.294	0.228	0.238					
U S144	0.846	0.020	2.4	0.840	0.825	0.874					
U S145	0.546	0.004	0.8	0.543	0.543	0.552					
U S146	1.460	0.018	1.3	1.458	1.483	1.438					

Up to 10 replicates of the same sample can be accommodated by GeXP Quant Tool.

# Gene Worksheet continued

	Mean	Std Dev	% CV		Replicate #1	Replicate #2	Replicate #3
66	<b>GEQs Normalized to Gene(s): Gene_2 Gene_B</b>						
67	0.591	0.004	0.7		0.592	0.595	0.585
68	0.587	0.010	1.6		0.574	0.589	0.596
69	0.458	0.015	3.3		0.459	0.476	0.440
70	0.227	0.006	2.6		0.225	0.236	0.222
71	0.224	0.003	1.1		0.221	0.225	0.228
72	0.231	0.002	0.7		0.233	0.231	0.229
73	0.335	0.001	0.2		0.335	0.335	0.334
74	1.316	0.202	15.3		1.591	1.113	1.243
75	0.351	0.011	3.0		0.353	0.337	0.362
76	0.310	0.009	3.0		0.319	0.297	0.312
77	0.395	0.008	2.0		0.384	0.399	0.402
78	0.871	0.136	15.6		1.052	0.724	0.837

The mean GEQ normalized values are used to create a bar chart on the worksheet. These values are the final quantitation values and are used to calculate fold change between samples.



# Fold Change calculation

Fold change =

Treated<sub>GEQnorm value</sub> / Control<sub>GEQnorm value</sub>

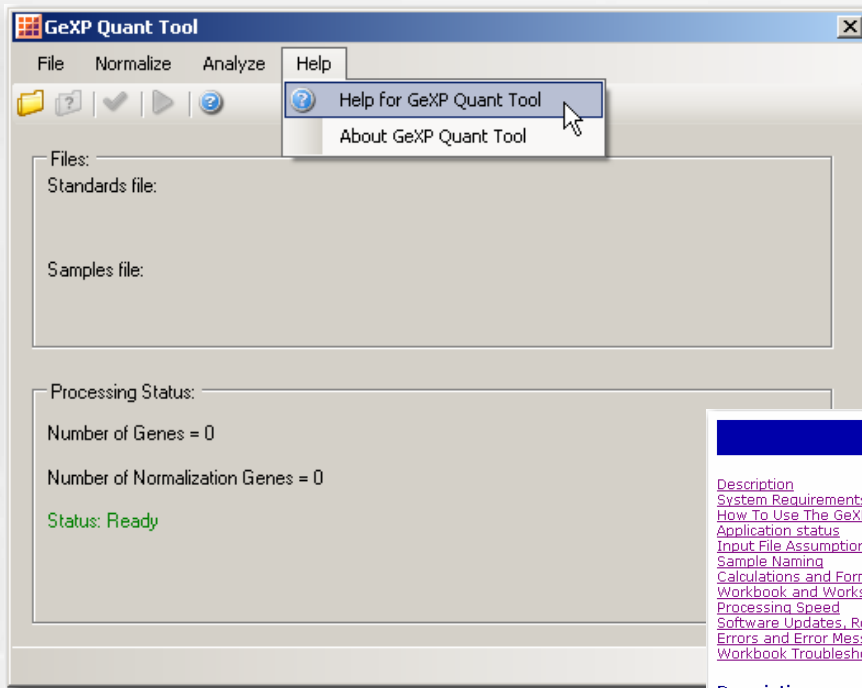
OR

Tumor<sub>GEQnorm value</sub> / Normal<sub>GEQnorm value</sub>

The GEQ normalized value can be derived from either one reference gene or a geometric mean of multiple reference genes, depending on how many reference genes are selected during GeXP Quant Tool analysis.

# Troubleshooting

# Online Instructions and Troubleshooting



## Help for GeXP Quant Tool

[Description](#)  
[System Requirements](#)  
[How To Use The GeXP Quant Tool](#)  
[Application status](#)  
[Input File Assumptions And Limitations](#)  
[Sample Naming](#)  
[Calculations and Formulas](#)  
[Workbook and Worksheet Contents](#)  
[Processing Speed](#)  
[Software Updates, Rollback and Uninstall](#)  
[Errors and Error Messages](#)  
[Workbook Troubleshooting Guide](#)

### Description:

The GeXP Quant Tool automates the quantitative analysis of data obtained from GeXP eXpress Profiler reports.

This application calculates standard curves from the standards in the standards report, determines the Gene Expression Quantitation (GEQ) values for the samples in the sample report, and normalizes the GEQ values based on the selection of one or more reference (housekeeping) genes.

The GeXP Quant Tool output is a Microsoft Excel workbook with one worksheet, containing the calculation formulas and results, for each gene in the analysis. The workbook also contains a summary worksheet, description worksheet and a normalization values worksheet.

**For Research Use Only; not for diagnostic use**

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### System Requirements:

- Operating system: Microsoft Windows XP
- Spreadsheet application: Microsoft Excel 2003 or 2007

## Data is outside the range of the standard curve

GeXP Quant Tool (version 1.0.3079.26088)	
Analysis generated on: 06/18/2008 5:52:15 PM	
Analysis created from standards file: C:\GeXP\Quantitation Method\Multiplex Standard Curve file_Genes.txt	
Analysis created from samples file: C:\GeXP\Quantitation Method\Multiplex Experimental Samples file_Genes.txt	
<b>Normalization Genes</b>	
Gene_2	
Gene_B	
<b>Genes</b>	
	<b>Curve Fit (R2)</b>
Gene_1	1.0000
Gene_2	0.9998
Gene_3	1.0000
Gene_4	0.9992
Gene_5	0.9999
Gene_6	0.9997
Gene_7	0.9999
Gene_8	0.9985
Gene_9	0.9998
Gene_A	0.9997
Gene_B	0.9999
Gene_C	0.9999

Red = One or more samples on that gene page is outside the range of the standard curve.

## Standard Curve for Gene\_4

	A	B	C	D
1	<b>Gene: Gene_4</b>			
2	<b>Standards:</b>			
3	Concentration (ng)	Mean	Std Dev	% CV
4	1.562	0.100	0.012	12.0
5	3.125	0.154	0.012	7.6
6	6.25	0.250	0.023	9.1
7	12.5	0.333	0.004	1.1
8	25	0.554	0.070	12.7
9	50	0.884	0.067	7.6
10	100	1.313	0.041	3.2
11	200	2.187	0.028	1.3
12	400	3.185	0.166	5.2
13	Concentration minimum	0.100		
14	Concentration maximum	3.185		
15				
16	Curve Fit(R2)	0.9992		

**Range of the average signal level (relative to KAN<sup>r</sup>)  
for the standard curve of Gene\_4.**

## Sample Values for Gene\_4

### Range of Standard Curve

	A	B
1	<b>Gene: Gene_4</b>	
13	Concentration minimum	0.100
14	Concentration maximum	3.185

$$0.047 < 0.100$$

Therefore this data point is below the range of this standard curve.

18	<b>Samples:</b>			
19	U S136	0.160	0.010	6.2
20	U S137	0.827	0.024	2.9
21	U S138	0.975	0.025	2.5
22	U S139	0.340	0.009	2.5
23	U S140	0.320	0.003	0.8
24	U S141	0.464	0.011	2.5
25	U S142	0.540	0.006	1.1
26	U S143	0.180	0.013	7.4
27	U S144	0.279	0.010	3.5
28	U S145	0.208	0.005	2.2
29	U S146	0.405	0.007	1.7
30	U S147	0.126	0.016	12.4
31	U S148	#DIV/0!	#DIV/0!	#DIV/0!
32	U S149	0.238	0.005	2.0
33	U S150	0.176	0.009	5.1
34	U S151	0.047	0.007	15.7
35	U S152	0.115	0.004	3.5
36	U S153	0.113	0.002	1.7
37	U S154	0.199	0.010	4.8
38	U S155	0.467	0.014	3.0
39	U S156	0.149	0.008	5.3
40	U S157	0.338	0.004	1.1

## Side Note

### Range of Standard Curve

	A	B
1	<b>Gene: Gene_4</b>	
13	Concentration minimum	0.100
14	Concentration maximum	3.185

ND in eXpress Analysis Report  
= no data for this sample

Therefore no values to  
compute for this sample.

18	<b>Samples:</b>			
19	U S136	0.160	0.010	6.2
20	U S137	0.827	0.024	2.9
21	U S138	0.975	0.025	2.5
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38	U S155	0.467	0.014	3.0
39	U S156	0.149	0.008	5.3
40	U S157	0.338	0.004	1.1

## Data is outside the range of the standard curve

Specific replicates or entire samples, for a particular gene, cannot be accurately quantified with Quant Tool if they are outside the range of the standard curve.

- Delete the out-of-range replicate data from the eXpress Profiler report (in Excel)
- Replace any deletion with '**FR**' (no replicate)
  - Note: If replicates are deleted and **FR** is not inserted, the values will be viewed as zeros by Quant Tool. This can affect quantitation.
- Save as a text file (.txt)
- Reanalyze the data with Quant Tool and the standard curve

## Data is outside the range of the standard curve

If a normalization gene is red on the Summary page, rerun the Quant Tool analysis without that gene as a normalization gene.

- Alternatively, replace out-of-range data points from the eXpress Analysis report and replace with **FR** (no replicate). Reanalyze the data.

If many genes and/or samples have out-of-range data, the standard curve should be expanded and all data re-analyzed with the new standard curve.

## Note on Editing Quant Tool Worksheets

Use caution when manually editing the worksheets generated by the GeXP Quant Tool.

- Deleting an outlier sample replicate value from the worksheet may propagate a zero value, rather than the intended deletion.
- This will add a zero value to the GEQ and normalized GEQ replicates' mean, causing incorrect quantitation results.

When deleting a standard, delete **all** of the cells associated with that standard-- the concentration, the statistics and the replicates.

**Important:** Do not delete the entire row, since this may also delete the standard curve formula cells.

# Summary

1. Optimize the amount of KAN<sup>r</sup> RNA in GeXP reactions

**Once optimized, the exact same concentration of KAN<sup>r</sup> RNA should be used for all of the experiments of a multiplex.**

2. Establish standard curves with Reference RNA

**Important: The accurate dilution is crucial for the quality of a standard curve. Make sure that 1) transferred volume is accurate, 2) every dilution is fully mixed, and 3) use a fresh barrier tip for each dilution.**

3. Run experimental samples with optimized KAN<sup>r</sup> RNA concentration.

# Summary

4. Use eXpress Analysis to obtain signal intensity relative to KAN<sup>r</sup> for standards and samples.
5. Use **GeXP Quant Tool** to calculate normalized GEO values from the standard curve.
6. Calculate fold change between samples.

**Please contact your Beckman Coulter Representative with questions, comments or concerns.**