StemElite® Gene Expression System

Instructions for Use of Products
B1001 and B1002

StemElite® Gene Expression System

1. Description

The StemElite® Gene Expression System[a,b] is a novel real-time quantitative PCR (qPCR) system for the detection and relative quantification of RNA expression levels associated with the differentiation state or "potency" of cells. This technical manual describes the StemElite® Gene Expression System and provides instructions for use, including reaction setup and data analysis using the Plexor® Analysis Software.

The Plexor® technology takes advantage of the specific interaction between two modified nucleotides to achieve quantitative PCR analysis. One of the PCR primers contains a modified nucleotide (iso-dC) linked to a fluorescent label at the 5´ end. The second PCR primer is unlabeled. The reaction mix includes deoxynucleotides and iso-dGTP modified with the quencher dabcyl. Dabcyl-iso-dGTP is incorporated opposite the iso-dC residue in the primer. Incorporation of the dabcyl-iso-dGTP at this position results in quenching of the fluorescent dye on the complementary strand and a reduction in fluorescence, which allows quantitation during amplification (Figure 1).
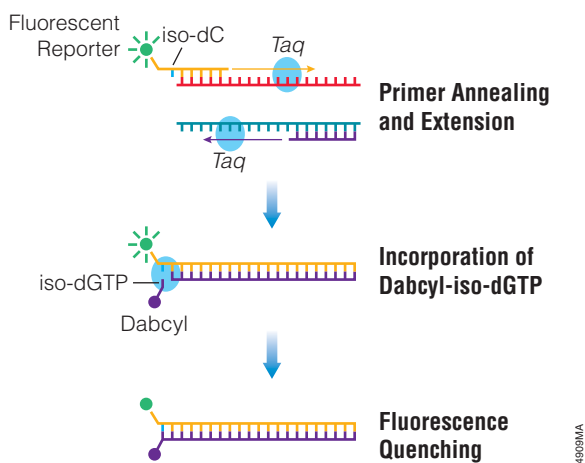
Figure 1. Schematic diagram illustrating the Plexor® real-time PCR process.

With the Plexor® technology, accumulation of product is accompanied by a decrease in fluorescence as shown in Figure 2. The point where the decrease in fluorescence crosses the detection threshold is the quantification cycle, or C_q; samples are compared using this number.

Note: The C_q or quantification cycle, was formerly referred to as threshold cycle, or C_t.
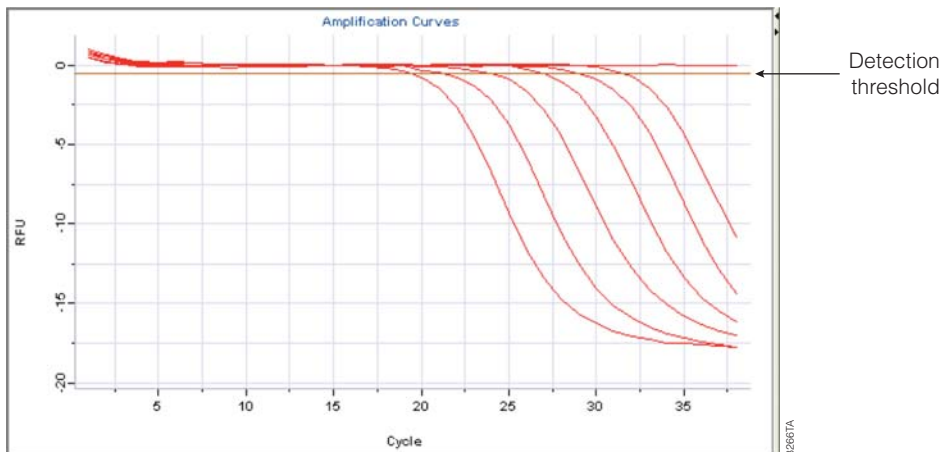
Figure 2. Representative Plexor® amplification curves. The amplification curves show the fluorescence (in relative fluorescence units, RFU) at each cycle of the reaction. The detection threshold is indicated by a horizontal line across the graph. This line is used to determine the quantification cycle (C_q) for the samples.

The Plexor® technology allows the use of a melt curve or dissociation curve to determine the melting temperature (T_m) of the products following amplification (Figure 3). This is useful in assessing the specificity of the reaction.

Figure 3. Representative Plexor® melt curves. Melting temperature is empirically determined by plotting the change in fluorescence (relative fluorescence units) with temperature ($-\Delta$RFU)/ΔT versus temperature and determining the temperature at which the greatest rate of change in fluorescence occurs.

1.   Description (continued)

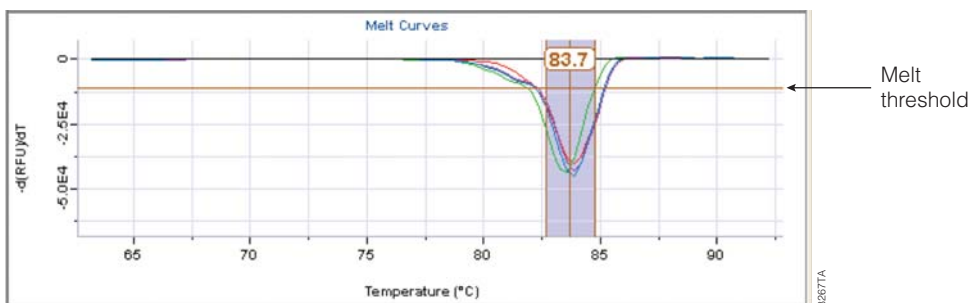Multiplex PCR facilitates simultaneous amplification of multiple targets by incorporating more than one primer pair in a single reaction. The StemElite® Gene Expression System is optimized to quantitatively amplify a two-color duplex. This duplex amplification strategy enables the user to amplify a transcript of interest associated with cellular differentiation, as well as a reference transcript (typically, a housekeeping gene), in a single reaction.

The StemElite® Gene Expression System uses a hot-start chemistry, allowing reaction setup to be performed at room temperature. Additional information on the Plexor® technology is available at: **www.promega.com/plexorresources/**

2.   Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
StemElite® Gene Expression System	**100 reactions**	**B1001**

For in vitro research use only. Not for use in diagnostic procedures. Includes:

*   1 × 1ml   StemElite® 2X Master Mix
*   1 × 1.25ml   Nuclease-Free Water

PRODUCT	SIZE	CAT.#
StemElite® Gene Expression System Plus	**50 reactions**	**B1002**

For in vitro research use only. Not for use in diagnostic procedures. Includes:

*   1 × 1ml   StemElite® 2X Master Mix
*   1 × 1.25ml   Nuclease-Free Water
*   1 GoScript™ Reverse Transcription System, 50 reactions

Storage Conditions: Store all components at −20°C.

StemElite® Primer Pairs (available separately)

PRODUCT	SIZE	CAT.#
StemElite® NANOG/GAPDH Primer Pair (20X)	100µl	B1011
StemElite® SOX2/GAPDH Primer Pair (20X)	100µl	B1021
StemElite® POU5F1/GAPDH Primer Pair (20X)	100µl	B1031
StemElite® LIN28/GAPDH Primer Pair (20X)	100µl	B1041
StemElite® KLF4/GAPDH Primer Pair (20X)	100µl	B1051
StemElite® MYC/GAPDH Primer Pair (20X)	100µl	B1061
StemElite® NPPA/GAPDH Primer Pair (20X)	100µl	B1071
StemElite® MYL7/GAPDH Primer Pair (20X)	100µl	B1081
StemElite® MYL2/GAPDH Primer Pair (20X)	100µl	B1091
StemElite® MYH6/GAPDH Primer Pair (20X)	100µl	B1101
StemElite® MYH7/GAPDH Primer Pair (20X)	100µl	B1111
StemElite® NKX2-5/GAPDH Primer Pair (20X)	100µl	B1121
StemElite® TNNT2/GAPDH Primer Pair (20X)	100µl	B1131
StemElite® TNNI3/GAPDH Primer Pair (20X)	100µl	B1141
StemElite® MEF2C/GAPDH Primer Pair (20X)	100µl	B1151
StemElite® PLN/GAPDH Primer Pair (20X)	100µl	B1161
StemElite® GATA4/GAPDH Primer Pair (20X)	100µl	B1171
StemElite® HNF4A/GAPDH Primer Pair (20X)	100µl	B1301
StemElite® HNF1B/GAPDH Primer Pair (20X)	100µl	B1311
StemElite® PDX1/GAPDH Primer Pair (20X)	100µl	B1321
StemElite® INS/GAPDH Primer Pair (20X)	100µl	B1331
StemElite® FOXA2/GAPDH Primer Pair (20X)	100µl	B1341
StemElite® SOX17/GAPDH Primer Pair (20X)	100µl	B1351
StemElite® GATA6/GAPDH Primer Pair (20X)	100µl	B1361
StemElite® Mus-Nanog/Actb Primer Pair (20X)	100µl	B1371
StemElite® Mus-Sox2/Actb Primer Pair (20X)	100µl	B1381
StemElite® Mus-Pou5f1/Actb Primer Pair (20X)	100µl	B1391
StemElite® Mus-Lin28/Actb Primer Pair (20X)	100µl	B1401
StemElite® Mus-Klf4/Actb Primer Pair (20X)	100µl	B1411
StemElite® Mus-Myc/Actb Primer Pair (20X)	100µl	B1421

For research use only. Not for use in diagnostic procedures.

3. General Considerations

- Use designated work areas and pipettes for pre- and post-amplification steps to minimize the potential for cross-contamination between samples and prevent carryover of nucleic acid from one experiment to the next.

- Wear gloves and change them often.

- Do not open the reactions after amplification is complete. Opening increases the risk of contaminating subsequent reactions with the amplified product.

- Prevent contamination by using aerosol-resistant pipette tips.

4. Instrument Calibration

The StemElite® Gene Expression Primers are provided for use as a two-color duplex reaction. This allows the user to simultaneously amplify a transcript of interest and the reference gene in a single reaction. Each duplex reaction will contain:

Duplex Primer Pairs	Dye Label	Excitation	Emission
Gene of Interest	fluorescein	495nm	520nm
Reference Gene	JOE	520nm	548nm

Before using the StemElite® Gene Expression System, confirm that your real-time PCR instrument is calibrated for the dyes used. Follow the guidelines in the instrument manual to perform the calibration for the appropriate excitation/emission spectra.

5. Preparation of cDNA

Materials to Be Supplied by the User
- nuclease-free reaction tubes
- sterile, aerosol-resistant tips and pipettors
- RNA target sample (DNase-treated)
- 25°C, 45°C and 70°C controlled-temperature heat blocks
- crushed ice

The GoScript™ Reverse Transcription System (Cat.# A5000) is a convenient kit that includes a reverse transcriptase and an optimized set of reagents designed for efficient synthesis of first-strand cDNA in preparation for PCR amplification. Because no cleanup or dilution is necessary following the cDNA synthesis, the product may be added directly to amplification reactions.

For optimal results with the StemElite® Gene Expression System, RNA templates should be free of DNA contamination. This is of particular importance when using amplification assays that are designed within a single exon. To avoid potential amplification of contaminating genomic DNA, the RNA templates should be DNase-treated prior to cDNA synthesis.

Notes:

- RQ1 RNase-Free DNase (Cat.# M6101) can be used for removal of DNA from RNA samples prior to RT-PCR.

- This section outlines the synthesis of cDNA in a 20µl reverse transcription reaction using the GoScript™ Reverse Transcription System (Cat.# A5000). This reaction volume is scalable.

- cDNA prepared as outlined in this section can subsequently be amplified using the StemElite® Gene Expression System (Cat.# B1001) or StemElite® Gene Expression System Plus (Cat.# B1002), which contains the GoScript™ Reverse Transcription System.

Experimental RNA Templates. As a starting point for the detection of RNA at unknown expression levels, we recommend using 100ng of DNase-treated total RNA template per cDNA synthesis reaction. The amount of RNA required to detect the target of interest will depend upon the abundance of the RNA target in each sample. A high-copy-number RNA transcript may be detected in as little as 10pg, while a low-copy-number RNA transcript may require more than 100ng. Up to 1µg of RNA may be used in each reaction.

cDNA Synthesis Negative Control: No Reverse Transcriptase. To test for the presence of contaminating genomic DNA in the RNA templates, a no-reverse transcriptase reaction may be performed. If the DNase treatment of the RNA templates was effective, then no real-time amplification should be observed from the no-reverse transcriptase reaction.

1. Denaturation of RNA and primers.

    a. On ice, combine the RNA template and primers as described below.

Component	Volume Per Reaction
RNA template (DNase-treated)	1−5µl
Oligo(dT)$_{15}$ Primer (500µg/ml)	1µl
Random Primers (500µg/ml)	1µl
Nuclease-Free Water	to a total volume of 7µl

    b. Close each tube tightly. Place the tubes into a preheated 70°C heat block for 5 minutes. Immediately chill on crushed ice for at least 5 minutes. Spin each tube for 10 seconds in a microcentrifuge to collect the condensate and maintain the original volume. Keep the tubes closed and on ice until the reverse transcription reaction mix is added.

5. Preparation of cDNA (continued)

2. Primer annealing and cDNA synthesis.

   a. Prepare the reverse transcription reaction mix by combining the following components of the GoScript™ Reverse Transcription System in a sterile 1.5ml microcentrifuge tube on ice. Prepare sufficient mix to allow 13μl for each cDNA synthesis reaction to be performed. Determine the volumes needed for each component, and combine them in the order listed. Vortex gently to mix, and keep on ice prior to dispensing into the reaction tubes.

Component	Volume Per Reaction
Nuclease-Free Water	4.9μl
GoScript™ 5X Reaction Buffer	4μl
25mM MgCl$_2$	1.6μl
PCR Nucleotide Mix	1μl
Recombinant RNasin® Ribonuclease Inhibitor	0.5μl
GoScript™ Reverse Transcriptase	1μl
	to a total volume of 13μl

   b. Add 13μl of the reverse transcription reaction mix to each RNA/primer tube for a final reaction volume of 20μl.

   c. **Anneal:** Place the tubes in a controlled-temperature heat block equilibrated at 25°C, and incubate for 5 minutes.

   d. **Extend:** Incubate the tubes in a controlled-temperature heat block at 42°C for 45 minutes (this extension incubation time can be extended to up to 2 hours).

   e. **Inactivate Reverse Transcriptase:** The reverse transcriptase must be thermally inactivated prior to amplification. Incubate the reaction tubes in a controlled-temperature heat block at 70°C for 15 minutes to inactivate the reverse transcriptase.

   f. Proceed to Section 6, Preparation of StemElite® Gene Expression Reactions, or store cDNA at −20ºC.

6. Preparation of StemElite® Gene Expression Reactions

Materials to Be Supplied by the User
- real-time PCR instrument and related equipment (i.e., appropriate PCR plates and plate covers)
- sterile, aerosol-resistant pipette tips
- pipettors dedicated to pre-amplification work

The StemElite® Gene Expression System uses a hot-start chemistry, allowing reaction setup to be performed at room temperature. Multiplex amplification is performed using the StemElite® 2X Master Mix and the StemElite® Gene Expression Primers.

Sample cDNA Templates. The sample cDNA may be added directly to the StemElite® Gene Expression reactions; add 5μl of the sample cDNA to each amplification reaction. Alternatively, the sample cDNA may be diluted prior to amplification. As a starting point, dilute the sample cDNA 1:10 in Nuclease-Free Water, then add 5μl of the diluted cDNA to the amplification reaction.

No-Template Control. Include a no-template control (NTC) reaction for each set of reactions. Add 5μl of Nuclease-Free Water to these reactions in place of the cDNA template. There should be no amplification or melt product detected in the NTC reaction.

Negative (No Reverse Transcriptase) cDNA Control. The negative (no reverse transcriptase) cDNA control (Section 5) serves as a negative amplification control during the reaction. If the DNase treatment of the RNA templates was effective, then no real-time amplification should be observed from the no reverse transcriptase reaction.

1. Thaw the StemElite® 2X Master Mix, the StemElite® Gene Expression Primers, and the Nuclease-Free Water. Do not thaw the StemElite® 2X Master Mix and StemElite® Gene Expression Primers at temperatures above room temperature.

   Note: The StemElite® Gene Expression Primers are provided at a 20X concentration.

2. Briefly vortex the StemElite® 2X Master Mix and the StemElite® Gene Expression Primers for 3−5 seconds to mix. Do not centrifuge after vortexing, as this may cause the primers to be concentrated at the bottom of the tube.

3. Determine the number of reactions to be set up. This should include negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does require using a small amount of extra reagent, it ensures that you will have enough PCR master mix for all samples.

4. Prepare the reaction mix by combining the StemElite® 2X Master Mix, the StemElite® Gene Expression Primers and the Nuclease-Free Water, as indicated below. Vortex briefly to mix.

Component	Volume Per Reaction
StemElite® 2X Master Mix	10μl
Nuclease-Free Water	4μl
StemElite® Gene Expression Primers (20X)	1μl

5. Add 15μl of the reaction mix to each of the appropriate wells of an optical-grade PCR plate.

6. Add 5μl of Nuclease-Free Water to the no-template control reactions.

7. Add 5μl of sample cDNA templates to the reaction mix in the appropriate sample wells.

8. Seal the plates with an optical adhesive cover using the plate cover applicator.

9. Centrifuge the plate briefly to collect the contents of the wells at the bottom. The plate is ready for thermal cycling. Protect the plate from extended light exposure or elevated temperatures before cycling. Handle the plate by the edges, and avoid touching the bottom of the plate.

7. Instrument Setup and Thermal Cycling

The general thermal cycling program for the StemElite® Gene Expression System is shown in Table 1.

Table 1. StemElite® Gene Expression System Thermal Cycling Program.

Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
Denaturation:	95°C	5 seconds	40 cycles
Annealing and extension:	60°C	35 seconds	
Dissociation curve:	60°C	15 seconds	1 cycle
	95°C	5 seconds	

For detailed instructions on programming cycling conditions into specific real-time instruments, refer to the appropriate Instrument Setup and Data Analysis Technical Manual, available at: **www.promega.com/plexorresources/**

Section 3 of the Instrument Setup and Data Analysis Technical Manuals describes instrument setup and thermal cycling conditions for cDNA quantitation using Plexor® chemistry.

Once instrument programming is complete, place the PCR plate into the instrument and immediately press "Start" to begin thermal cycling.

Notes:

- Before using the StemElite® Gene Expression System, confirm that your real-time PCR instrument is calibrated for the dyes used. Refer to Section 4 of this technical manual for calibration information.

- Prolonged exposure of the reactions to light or high temperatures before thermal cycling may adversely affect the final results.

8. Data Export

Before the data can be analyzed using the Plexor® Analysis Software, the data must be exported from the real-time detection instrument. For detailed instructions on exporting data from specific real-time instruments, refer to the appropriate Instrument Setup and Data Analysis Technical Manual, available at: **www.promega.com/plexorresources/**

9. Data Import into the Plexor® Analysis Software

For detailed instructions regarding operating system compatibility and software installation, refer to Section 14 of this technical manual. The Plexor® Analysis Software is available free-of-charge on CD-ROM by request. It is also available for download at: **www.promega.com/resources/tools/**

Following data export from the real-time instrument, the data files are imported into the Plexor® Analysis Software. For detailed instructions on importing data from specific real-time instruments into the Plexor® Analysis Software, refer to the appropriate Instrument Setup and Data Analysis Technical Manual, available at:
www.promega.com/plexorresources/

10. Data Analysis with the Plexor® Analysis Software

The Instrument Setup and Data Analysis Technical Manuals describe data analysis procedures for Plexor® Analysis Software. Refer to the Instrument Setup and Data Analysis Technical Manual for detailed instructions regarding the following:

- Sample Definition

- Adjusting the Expected Target Melt Temperature

- Adjusting the Y Axes of the Amplification and Thermal Melt Curves

- Adjusting the Baseline Region and Amplification Threshold Line

In a duplex assay, the Expected Target Melt Temperature must be adjusted for each dye (fluorescein and JOE). Additionally, each transcript of interest (labeled in fluorescein) may have differing Expected Target Melt Temperatures; for example, the Expected Target Melt Temperature of the NANOG amplicon will differ from the Expected Target Melt Temperature of the SOX2 amplicon. If multiple pairs of StemElite® Gene Expression Primers are run on the same plate, the Expected Target Melt Temperature must be specified for each transcript of interest. Plexor® Analysis Software allows designation of up to two Expected T_m regions. In the "Edit" menu select "Use Second Expected T_m Region" or right click in the melt curves window to present that option.

The default Melt Threshold is set at 25% of the signal change for the sample on the plate with the greatest change in signal. In some instances, the sample used to set this threshold may not be typical of all samples on the plate. To adjust the Melt Threshold for each transcript of interest, highlight the samples in the Well Selector. Then in the "Edit" menu, select "Set Melt Threshold From Selected Samples". Alternatively, the Melt Threshold line may be manually adjusted by placing the cursor over the threshold line and dragging the line to the desired location. Changes made to the Melt Threshold line will apply to the entire plate within the same dye channel, including those samples that were not selected. For this reason, each set of samples representing different transcripts of interest should be selected and analyzed separately for best results if the melt peaks vary significantly in amplitude between the sets.

11. Relative Quantitation

11.A. Background

It is often of interest to evaluate the level of a particular transcript in one sample relative to another sample to determine differences in gene expression (1).

Relating Experimental Samples to the Reference Sample. Differences in the level of a particular transcript in an experimental sample (e.g., differentiating cells) relative to a control sample (calibrator, e.g., stem cells) can be evaluated by first calculating a ΔC_q for that transcript:

$$\Delta C_q = (C_q \text{ experimental sample}) - (C_q \text{ control sample})$$

Using the ΔC_q value, a relative quantitation value (expression ratio) for that transcript can be calculated:

$$\text{Relative Quantitation} = E^{-\Delta Cq}$$

where E = PCR efficiency. If reaction efficiency is 100% then E = 2 (product is doubled at each cycle).

Normalizing the Transcript of Interest to the Reference Transcript. Expression levels of a particular gene can be more effectively compared across samples after normalization to a reference transcript (e.g., GAPDH). To achieve this normalization, a ΔC_q is calculated for both the transcript of interest (target) and the reference transcript for each experimental sample relative to the control sample. These values are then used to give the Quant Ratio (normalized Relative Quant, or normalized ratio of change of gene expression):

$$\text{Quant Ratio} = \frac{E_{target}^{-\Delta Cqtarget}}{E_{reference}^{-\Delta Cqreference}}$$

11.B. Performing Relative Quantitation (RQ) Analysis Using the Plexor® Analysis Software

1. **Designate the RQ Group.** In the Well Selector view, select a group of samples that will be analyzed together. This grouping should include the experimental samples, as well as the reference sample; all samples in that designated group will be compared to the reference sample. Click the RQ Group button on the Toolbar to designate all selected samples as belonging to one RQ group.

 Group for RQ analysis

   A group number will appear in the lower right corner of each selected well in this view (Figure 4). This group selection will apply across all dye channels.

   To delete an RQ group: In the Well Selector view, select one or more of the samples in that group, then click the "Delete groups referencing selected well" button on the Toolbar.

 Delete RQ Group

   Note: If any selected well belongs to more than one group, all of these groups will be deleted.
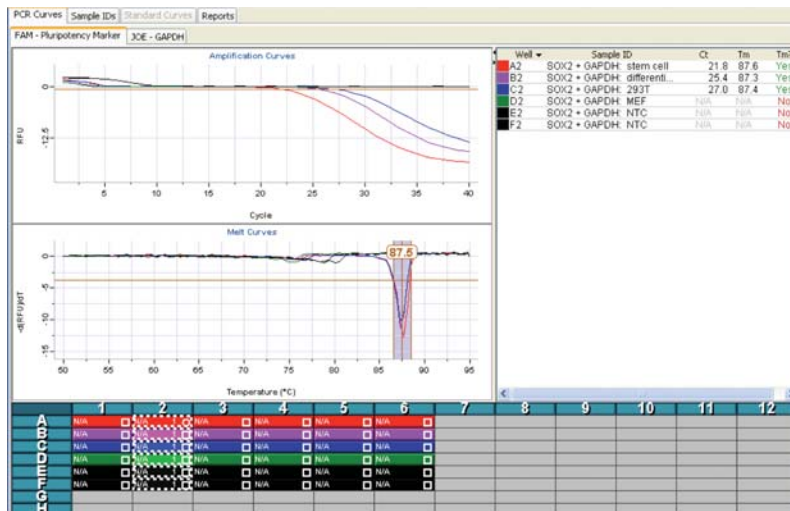
Figure 4. RQ Group and RQ Control designation in the Well Selector view. In this plate, the sample wells that were amplified with the SOX2 + GAPDH primer duplex are selected and designated as RQ Group 1, with the stem cell sample designated as the RQ Control for that group.

2. **Designate the RQ Controls.** Within the RQ group, designate the reference samples by selecting those wells in the Well Selector view and clicking the RQ Control button.

 RQ Control Sample type

Each RQ analysis group should have at least one reference sample. If replicate reference samples are present within a group, confirm that the replicates have similar C_q values. This RQ Control designation will apply across all dye channels.

Note: For purposes of identifying the RQ controls in the RQ report, it may be useful to assign these samples a different color in the Well Selector view.

3. **Initiate RQ Analysis.** Click the "Perform relative quant analysis" button in the Toolbar to start RQ analysis.

 Perform RQ analysis
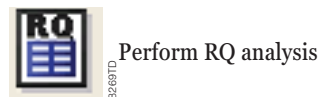
A dialog box will appear (Figure 5). Three parameters can be specified:

a. **Target Dye:** Choose the Target dye from the pull-down menu. The StemElite® Gene Expression Primers are provided for use as a two-color duplex reaction, with the gene-of-interest labeled in fluorescein (this is the target dye).

11.B. Performing Relative Quantitation (RQ) Analysis Using the Plexor® Analysis Software (continued)

b. **Reference Dye:** Click the "Use reference dye" checkbox, and choose the Reference dye from the pull-down menu. The StemElite® Gene Expression Primers are provided for use as a two-color duplex reaction, with the reference gene (GAPDH) labeled in JOE dye.

c. **Efficiency Corrections (optional):** Efficiency correction is useful in RQ analysis if the amplification efficiency is poor, or if the amplification efficiencies of the target transcript and the reference transcript differ greatly. Otherwise, the user may choose to omit this option. The StemElite® Gene Expression Primers should not require efficiency corrections.
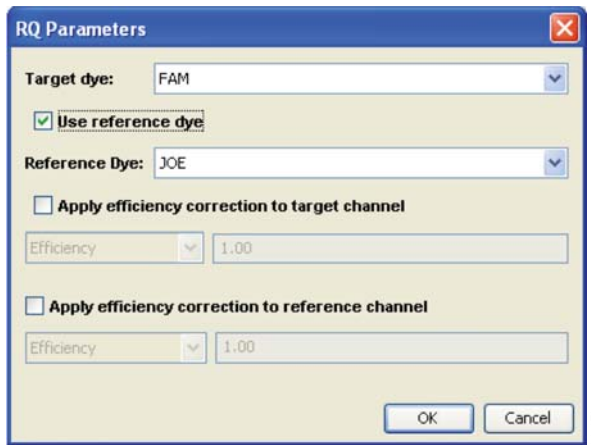
Figure 5. The RQ Parameters dialog box.

If desired, enable the Efficiency Corrections option by clicking the appropriate "Apply efficiency correction..." checkbox. Select the unit from the pull-down menu (either "slope" of the standard curve or "efficiency" of the reaction), and enter the value.
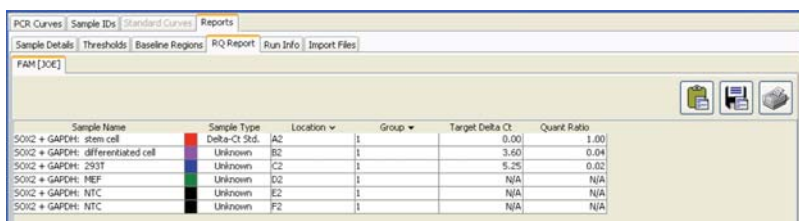
Note: If a standard dilution series was included in the same run for purposes of efficiency correction in RQ Analysis, perform the standard curve analysis before beginning the RQ analysis (refer to Section 11.D of this technical manual for more information regarding standard curves). Alternatively, historic reaction efficiency values from prior runs can be used.

After specifying parameters for the RQ analysis, click "OK" to complete the analysis. The Plexor® Analysis Software will perform the analysis and present the RQ Report for the selected target/dye channel.

11.C. RQ Report

1. **Viewing the RQ Report.** After completion of the RQ Analysis, the results can be viewed by clicking the "Reports" tab, followed by the "RQ Report" tab (Figure 6).
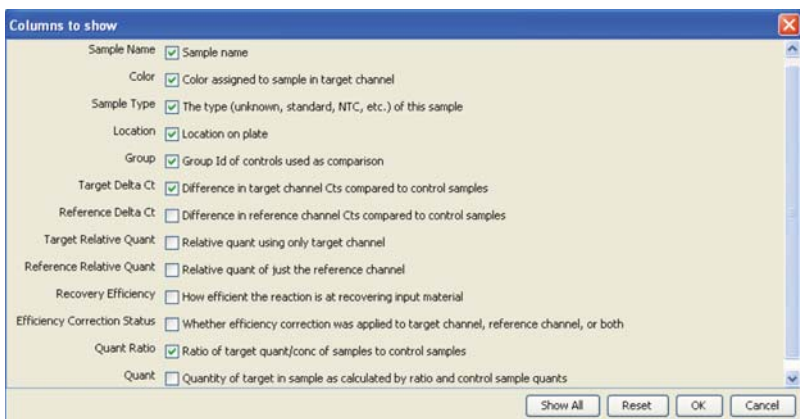
   Note: The data in the RQ Report may be sorted (ordered) by any of the parameters displayed (for example, by Sample Name or Quant Ratio). Click the column header for the desired parameter once to sort in ascending order or click again to sort in descending order. Column order may be rearranged by clicking and dragging the column header right or left to the desired position within the table.

Figure 6. The RQ Report.

2. **Selecting RQ Report data to display.** The RQ Report includes options for displaying a variety of useful metrics related to the RQ analysis (Figure 7). To change the columns displayed, right click in the "RQ Report" tab window and select "change columns to show" in the dialog box that appears. In RQ analysis, the most informative output is the Quant Ratio; this is the ratio of the change in gene expression in each sample, as compared to the reference sample (see Section 11.A). After selecting the appropriate columns to display, this setting may be saved by right clicking in the "RQ Report" tab window and selecting "Save table defaults".

Figure 7. The RQ Report options.

3. **Exporting data.** The data in the RQ Report page can be exported from the Plexor® Analysis Software for further processing or presentation. Select the rows of data to be exported in the RQ Report table, then click one of the three buttons in the upper right corner of the RQ Report window to Copy (to clipboard), Export (save as tab-delimited text file) or Print:

 Copy, Export or Print Selected Data

Copied data can be pasted directly into a spreadsheet program, such as Microsoft Excel®. Likewise, you may open an exported .tab file by first opening the spreadsheet program and then opening the file from within that program.

11.D. Generating a Standard Curve (optional)

If desired, a standard curve may be generated using amplification results from a dilution series of a standard reference template. The StemElite® Gene Expression Primers are designed to amplify RNA transcripts; therefore, the standard reference template used with these primers should be cDNA generated using RNA from a control sample (tissue or cells) that is known to express the transcript of interest.

The Plexor® Analysis Software will plot the C_q vs. log of concentration for the dilution series, then derive a standard curve from a linear regression of the data. This plot, the equation of the regressed line, and the R^2 value are displayed in the "Standard Curves" tab view. The standard curve can be used to determine concentrations of unknown samples or for efficiency/slope correction for RQ analysis.

Refer to the appropriate Instrument Setup and Data Analysis technical manual for detailed instructions on generating a standard curve. The instrument-specific manuals are available at: **www.promega.com/plexorresources/**

12. Reporting and Saving Sample Analysis

1. The Plexor® Analysis Software saves the analysis as an *.aan file. The current analysis can be saved at any time by selecting "Save Analysis File (.aan)" in the File menu.

2. Selected wells can be exported into a new analysis file. In the file menu, select "Export Selected Wells as New Analysis File (*.aan)".

3. The analysis screen can be printed or saved as a screenshot. In the file menu, select "Save a Screenshot (.png)" or "Print a Screenshot".

13. Troubleshooting

Refer to the appropriate Instrument Setup and Data Analysis Technical Manual for detailed instructions regarding troubleshooting. These instrument-specific manuals are available at: **www.promega.com/plexorresources/**

14. Appendix

14.A. Plexor® Analysis Software Operating System Compatibility

The Plexor® Analysis Software is compatible with the following operating systems: Windows® 98, Windows NT® 4, Windows® ME, Windows® XP, and Windows® 2000 and Windows Vista®. Other operating systems are not supported. The Plexor® Analysis Software is not compatible with Macintosh® operating systems.

Be sure that the display settings for the computer are set to 32-bit color, rather than 16-bit color when using the Plexor® Analysis Software.

14.B. Plexor® Analysis Software Installation

The Plexor® Analysis Software is available free-of-charge on CD-ROM by request. It is also available for download at: **www.promega.com/resources/tools/**

To install the Plexor® Analysis Software from CD-ROM:

1. Insert the CD-ROM into the CD-ROM drive.

2. Double-click the "Plexor.exe" installer icon on the CD-ROM, and follow the on-screen instructions to install the software.

3. Following installation, the program can be accessed in the "Start" menu: Programs\Plexor\Analysis Desktop\

   Note: Installation of the software may take several minutes. There is a pause where the computer may appear to be inactive between the launch of the installer and the software installation.

14.C. Reference

1. Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT−PCR. *Nucl. Acids Res.* **29**, e45.

14.D. Related Products

RNA Isolation and Reverse Transcription Systems

Product	Size	Cat.#
SV Total RNA Isolation System	10 preps	Z3101
	50 preps	Z3100
	250 preps	Z3105
SV 96 Total RNA Isolation System	1 × 96 each	Z3500
	5 × 96 each	Z3505
MagneSil® Total RNA mini-Isolation System	4 plate	Z3351
GoScript™ Reverse Transcription System	50 reactions	A5000
	100 reactions	A5001
GoScript™ Reverse Transcriptase	100 reactions	A5003
	500 reactions	A5004
RQ1 RNase-Free DNase	1,000 units	M6101

15. Summary of Changes

The following changes were made to the 7/14 revision of this document:

1. Expired patent or license statements were removed.

2. The document design was updated.