Gene Expression Application Note No. 1

Relative Quantification of Multiple mRNA Targets and Reference Genes in Spinocerebellar Ataxia

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1 Introduction

In the research project presented here, we studied the expression of 16 transcripts that might be predictive for the progression stage of spinocerebellar ataxia 1 and 3 (SCA1 + 3), two autosomal-dominant neurodegenerative disorders in humans (see box). We collected a total of 88 blood samples from individuals with either mild, intermediate or severe forms of ataxia. After assay design and optimization cDNAs were generated with the Transcriptor First Strand cDNA Synthesis Kit. For each individual target, the C_p values for all transcripts in each sample were determined by real-time PCR on the LightCycler® 480 System with Universal ProbeLibrary probes. We performed 384-well plate setup using an automated liquid handling system. Using LightCycler® 480 Software 1.5, relative expression values were obtained by determining the ratio between each target and three appropriate reference transcripts, and normalized expression ratios were calculated using a common calibrator cDNA. Using this specific workflow set-up allowed generation of standard curves using five serial two-fold dilutions, which was important because several targets were known to have low expression levels. Statistical analysis was done using the LightCycler® 480 Multiple Plate Analysis Software.

A graphical overview of the entire workflow is presented in Figure 1.

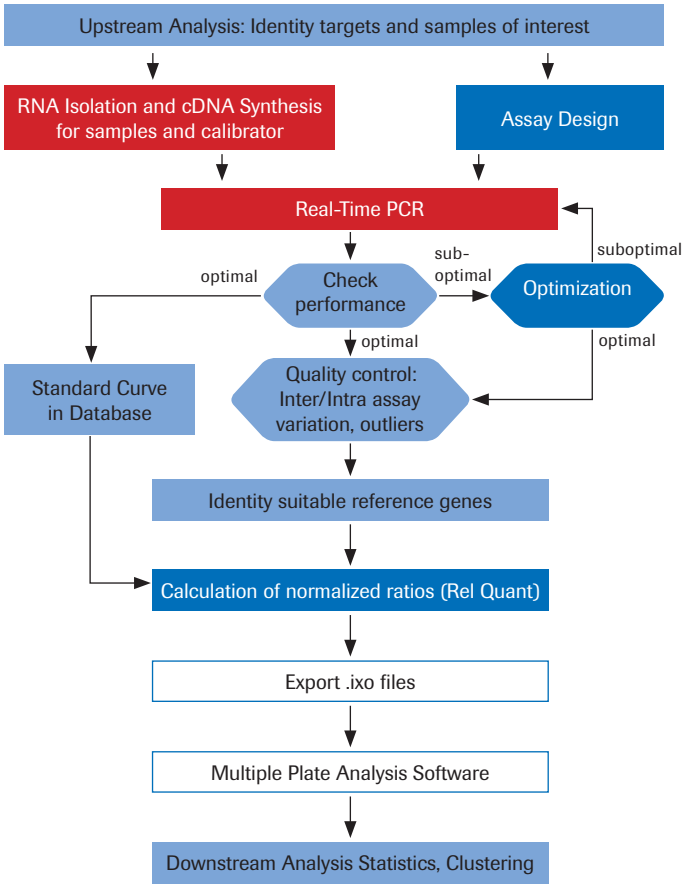

Figure 1: Schematic representation of a typical workflow for relative quantification of mRNA.

BOX 1:

Spinocerebellar ataxia is a group of genetic disorders, characterized mainly by progressive incoordination of gait, poor coordination of hands, speech and clumsy motion of the body due to a failure of the fine coordination of muscle movements. Generally, a person with ataxia retains full mental capacity but may progressively lose physical control. There is no known cure for spinocerebellar ataxia, and although not all types cause equally severe disability, treatments are generally focused on symptoms, not the disease itself. Both onset of initial symptoms and duration of disease can be subject to variation. The most precise means of identifying SCA, including the specific type, is through DNA analysis. The first ataxia gene was identified in 1993 for a dominantly inherited type. It was called "spinocerebellar ataxia type 1" (SCA1). Subsequently, as additional dominant genes were found, they were called SCA2, SCA3, etc. To date, at least 29 different gene mutations have been identified. Ataxias with poly CAG expansions, along with several other neurodegenerative diseases resulting from a poly CAG expansion, are referred to as polyglutamine diseases.

2 Assay Design

The assay design process is the most important and critical part in the workflow of a relative quantification project (shown in Figure 1). To enable high assay sensitivity and circumvent primer-dimer issues, all experiments in this project were designed as 5' nuclease assays with Universal ProbeLibrary probes, making use of the web-based Roche assay design center[1]. To find optimal primer-probe combinations, target sequences were uploaded to the design center. Where applicable, the Ensembl transcript ID was used to identify the target and automatically retrieve the sequence from the Ensembl database[2]. Where possible, the assays were designed with intron-spanning primer pairs or probes (see Table 1). In cases where the assay design center proposed several different possible primers or probes, the top ranking assay was chosen for initial evaluation.

1 https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp
2 www.ensembl.org

3 RNA Extraction and cDNA Synthesis

Blood samples from individuals were collected using PAXgene vacutainer (BD Biosciences). RNA was isolated using the PAXgene RNA Isolation Kit (BD Biosciences) according to the manufacturer's recommendations. Quantification was done spectrophotometrically on a Nanodrop (Thermo Scientific) and quality was checked on a Bioanalyzer 2100 (Agilent). All RNAs had RNA integrity numbers (RIN) greater than 8, indicating good and comparable RNA quality across the entire sample population.
Since a total of 21 different assays was performed for each of the original samples, we used a two-step RT-PCR

approach for cDNA synthesis. Reverse transcription of 1 µg of total RNA was performed with the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science) using a combination of anchored-oligo(dT) primer, binding at the beginning of the poly A tail, and random hexamer primer according to the protocol below. To enable detection when probe binding sites reside several kb upstream of the poly A tail, we used a broader range of primer types (use of oligo-dT primers only can often lead to a dramatic increase in Cp values, because normal oligo-dT can bind somewhere in the often long poly A tail; unpublished observation). We performed one cDNA synthesis reaction per sample and 30 reactions for the calibrator cDNA. cDNAs were stored undiluted at –20°C. To prevent multiple freeze-thaw cycles, the calibrator cDNA was stored in aliquots.

cDNA synthesis protocol:

1 µg	total RNA
1 µl	anchored oligo-dT primer
2 µl	random hexamer primer
ad 13 µl	PCR-grade water
10 min	65°C, then on ice

add[1]:	4 µl	RT buffer
	2 µl	dNTP Mix
	0.5 µl	Protector RNase Inhibitor
	0.5 µl	Transcriptor Reverse Transcriptase
	10 min	25°C
	60 min	50°C
	5 min	85°C → store at –20°C

1 Prepare a master mix and add 7 µl per reaction.

4 Performing the Assays

4.1 Target Gene Assay Evaluation and Optimization

Initially, all assays were tested under standard conditions, *i.e.*, using 300 nM of each primer and probe and a standard PCR protocol (see Figure 2). To establish the standard curves, a rather narrow range of dilutions (five serial two-fold dilutions of a pool of sample cDNAs) was chosen, because several targets were known to have low expression levels. We expected and found the observed fold changes in the study described here to be small, so that a narrow standard curve was sufficient to cover the entire range of observed Cp values.

For assay evaluation, the following PCR performance criteria were considered:

- Amplification efficiency (must be > 1.8)
- Standard deviation of Cp values of replicate reactions < 0.3
- Smooth progression and high plateau signal to background noise ratio of amplification

Initial tests showed satisfactory performance for 10 of the 16 target assays (see Figure 3 A as an example). All target and reference assays were analyzed using the same PCR cycling protocol and conditions. We analyzed the influence of primer and probe concentration on run performance of the remaining 6 assays. In some cases, increasing the concentration of the UPL probe led to a proportional increase in amplification signal in the plateau phase. This yielded an improvement for two assays with high signal-to-noise ratios (Figure 3 C, D). Since the general performance of the remaining four assays could not be improved, the second top ranking assay (as suggested by the UPL Assay Design Center) was tested and gave good results in all four cases (Figure 3 E, F). Table 1 gives an overview on all 16 assays and the run parameters used. The standard curves resulting from each of the final assays were stored as external standard curves and used later for efficiency correction (see below). The relative quantification analysis module of LightCycler® 480 Software 1.5 can automatically load the correct standard curve for each assay, provided the reactions in the run to be analyzed and the standard samples from the standard curve have the same target name assigned in their sample information.

Program Name	Denaturation		
Cycles	1	Analysis Mode	None

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
95	None	00:10:00	4,80		0	0	0

Program Name	Amplification		
Cycles	45	Analysis Mode	Quantification

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
95	None	00:00:10	4,80		0	0	0
60	Single	00:01:00	2,50		0	0	0

Program Name	Cooling		
Cycles	1	Analysis Mode	None

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step size (°C)	Step Delay (cycles)
40	None	00:00:05	2,50		0	0	0

Figure 2: Standard PCR protocol used for all assays in this study.

Gene Symbol	Ensembl	Genbank	UPL	Primer [nmol]	Probe [nmol]	Temp [°C]	Time [s]	Volume [µl]	Rank*	Efficiency
230585_at	–	AI632692	67	**300**	**100**	**60**	**60**	**10**	**1**	**1.994**
232939_at	–	AU152763	11	**300**	**100**				**1**	**2.075**
244218_at	–	AI374686	69	**300**	**100**				**1**	**1.905**
BANK1	ENST00000322953	AA811540	31	**300**	**200**				**1**	**2.013**
FAM3C	ENST00000359943	NM_014888	2	**300**	**100**				**1**	**1.924**
FCRL5	ENST00000368189	AW241983	21	**300**	**100**				**2**	**1.971**
FLJ32866	–	AK057428	37	**300**	**100**				**1**	**1.973**
FLJ36550	–	AW270105	60	**300**	**100**				**1**	**1.931**
IL18RAP	ENST00000264260	NM_003853	1	**300**	**100**				**2**	**2.037**
LOC285463	–	BF984434	44	**300**	**100**				**1**	**1.945**
MRPL13	ENST00000306185	NM_014078	59	**300**	**100**				**2**	**1.853**
MS4A1	ENST00000389941	BC002807	2	**300**	**200**				**1**	**1.902**
PADI2	ENST00000375486	AL049569	11	**300**	**100**				**1**	**1.963**
SIGLEC5	ENST00000222107	NM_003830	9	**300**	**100**				**1**	**1.863**
THAP6	ENST00000311638	BF685315	76	**300**	**100**				**1**	**1.961**
TSPAN13	ENST00000262067	NM_014399	67	**300**	**100**				**2**	**2.050**

*Rank refers to assay list proposed by Universal ProbeLibrary Assay Design Center.

Table 1: Primer and probe concentrations for the 16 target assays.

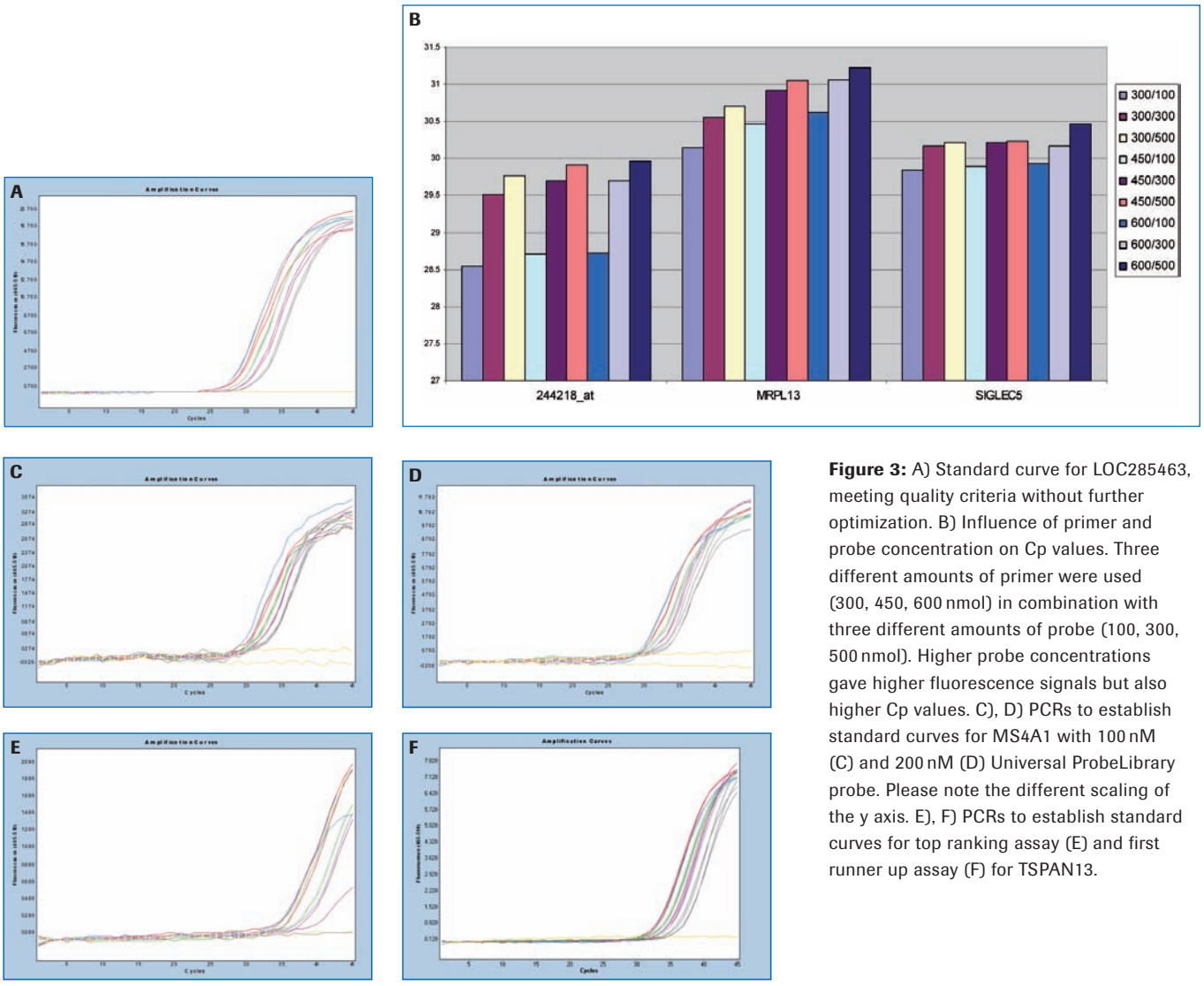

Figure 3: A) Standard curve for LOC285463, meeting quality criteria without further optimization. B) Influence of primer and probe concentration on Cp values. Three different amounts of primer were used (300, 450, 600 nmol) in combination with three different amounts of probe (100, 300, 500 nmol). Higher probe concentrations gave higher fluorescence signals but also higher Cp values. C), D) PCRs to establish standard curves for MS4A1 with 100 nM (C) and 200 nM (D) Universal ProbeLibrary probe. Please note the different scaling of the y axis. E), F) PCRs to establish standard curves for top ranking assay (E) and first runner up assay (F) for TSPAN13.

4.2 Reference Gene Assays

Relative quantification of mRNA requires the use of one or several reference genes. For simple experimental systems, *e.g.*, treatment of cultured cells with different stimuli, a single reference gene might be suitable for normalization. For more complex sample sources, especially human sample material with high degree of genetic heterogeneity, a single reference gene whose expression is uniform throughout the entire sample population, is generally difficult to find. Thus, a combination of several reference genes must be used for normalization. When *a priori* information on reference genes suitable for a given experimental system is not available, it is highly recommended to measure several reference genes and use the most suitable one(s) for later data analysis.

In our case, eight different reference gene assays available in a pre-designed and validated form from Roche Applied Science[1] were tested and could be carried out without further optimization. Five out of these eight (ACTB, G6DH, GAPDH, HPRT, PPIA), spanning the same range of Cp values as the target assays (Cps of approx. 25 to 36) were chosen, and used for all samples. Standard curves for the reference genes were also established and stored (see also section 6).

1 https://www.universalprobelibrary.com

4.3 Study Layout and Plate Preparation

In principal, there are two ways to set up large qPCR studies. One can either do "sample maximization", *i.e.*, analyze as many samples per plate as possible for a given assay (primer/probe combination), or, alternatively for „gene maximization", fill the plate with as many assays as possible for a given sample. Ideally, there is only a single plate containing all reactions for an assay, since no correction for inter-run variability is then required. This is definitely the best choice for studies where all samples can be collected and analyzed in a single batch. The set of transcripts examined in the study presented here was selected to give information on the progression of a disorder. For each individuum investigated, the same set of transcripts can be expected to be analyzed over a long period, initially and later on. Therefore, we decided to choose the gene maximization approach and analyzed all assays for a given sample in a single run on the same plate. To enable comparison of results between different plates, a common cDNA (calibrator) was included in each plate.

Figure 4 shows the layout for the 384-well plates used for qRT-PCR. This experimental design allowed us to analyze four samples plus the common calibrator and negative controls per plate. Twenty-two 384-well plates were required in total to complete the measurement of the 88 samples in this study.

Plates were set up with the aid of an automated liquid handler (Biorobot Universal Platform, Qiagen). For each plate, five master mixes were prepared containing the cDNA for four samples and the calibrator, respectively. A sixth master mix did not contain any template and served as negative control (see below). Eight microliters of master mix were dispensed in each well of the 384-well plate, and 2 µl of primer-probe mix presented in a V-bottom 96-well plate (Sarstedt) was added to the reaction mix (see below). PCR reactions for sample and calibrator cDNA were prepared in triplicates, while no-template control reactions were run only once per plate. The plate was centrifuged 2 min at 1,500 × g prior to PCR amplification.

Pipetting scheme:

Master mix for samples/calibrator

346.5 µl	LightCycler® 480 Probes Master
13.9 µl	cDNA
221.8 µl	PCR-grade water
8 µl/well	+ 2 µl primer probe mix

Master mix for no template control (NTC)

132 µl	LightCycler® 480 Probes Master
79.2 µl	PCR-grade water
8 µl/well	+ 2 µl primer probe mix

Primer-probe mix for target assays:

15 µl	5 µM forward primer
15 µl	5 µM reverse primer
5 µl	UPL probe
65 µl	PCR-grade water

Primer-probe mix for reference assays:

10 µl	Primer (premixed)
10 µl	UPL probe
80 µl	PCR-grade water

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	A1	A1	A1	R1	R1	R1	A1	A1	A1	A1	A1	A1	R1	R1	R1	A1	A1	A1	A1	A1	A1	R1	R1	A1
B	A2	A2	A2	R2	R2	R2	A2	A2	A2	A2	A2	A2	R2	R2	R2	A2	A2	A2	A2	A2	A2	R2	R2	A2
C	A3	A3	A3	R3	R3	R3	A3	A3	A3	A3	A3	A3	R3	R3	R3	A3	A3	A3	A3	A3	A3	R3	R3	A3
D	A4	A4	A4	R4	R4	R4	A4	A4	A4	A4	A4	A4	R4	R4	R4	A4	A4	A4	A4	A4	A4	R4	R4	A4
E	A5	A5	A5	R5	R5	R5	A5	A5	A5	A5	A5	A5	R5	R5	R5	A5	A5	A5	A5	A5	A5	R5	R5	A5
F	A6	Sample 1	A6				A6	Sample 2	A6	A6	Sample 3	A6				A6	Sample 4	A6	A6	Calibrator	A6			A6
G	A7		A7				A7		A7	A7		A7				A7		A7	A7		A7			A7
H	A8		A8				A8		A8	A8		A8				A8		A8	A8		A8			A8
I	A9		A9	R1	R1	R1	A9		A9	A9		A9	R1	R1	R1	A9		A9	A9		A9	R1	R1	A9
J	A10	A10	A10	R2	R2	R2	A10	A10	A10	A10	A10	A10	R2	R2	R2	A10	A10	A10	A10	A10	A10	R2	R2	A10
K	A11	A11	A11	R3	R3	R3	A11	A11	A11	A11	A11	A11	R3	R3	R3	A11	A11	A11	A11	A11	A11	R3	R3	A11
L	A12	A12	A12	R4	R4	R4	A12	A12	A12	A12	A12	A12	R4	R4	R4	A12	A12	A12	A12	A12	A12	R4	R4	A12
M	A13	A13	A13	R5	R5	R5	A13	A13	A13	A13	A13	A13	R5	R5	R5	A13	A13	A13	A13	A13	A13	R5	R5	A13
N	A14	A14	A14				A14	A14	A14	A14	A14	A14				A14	A14	A14	A14	A14	A14			A14
O	A15	A15	A15				A15	A15	A15	A15	A15	A15				A15	A15	A15	A15	A15	A15			A15
P	A16	A16	A16				A16	A16	A16	A16	A16	A16				A16	A16	A16	A16	A16	A16			A16

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	R1	R2								
B	A3	A4	R3	R4								
C	A5	A6	R5									
D	A7	A8										
E	A9	A10										
F	A11	A12										
G	A13	A14										
H	A15	A16										

Figure 4: Layout of white LightCycler® 480 384-well plates (left) and LightCycler® 480 96-well V-bottom plates containing primer probe mixes (right). A1–A16: Target gene assays 1 to 16, R1–R5: Reference gene assays 1 to 5. Position of sample cDNA is colored yellow and pink for target and reference gene assay, respectively. Positions of calibrator cDNA and non-template negative controls are depicted in blue and green, respectively.

5 Quality Control: Outlier Removal, Intra- and Inter-Assay Variability

After completion of each run, a visual inspection of the resulting amplification curves was done and the Cp standard deviations were evaluated. A result was called an outlier when the Cp standard deviation was larger than 0.3 and a single reaction displayed a clearly different amplification curve. All together 111 reactions (1.3 %) were excluded from further analysis.
For the second quality control step, we calculated the Cp values' coefficient of variation (% CV) for the replicates of each sample to determine the intra-assay variability. The % CV values for each assay are displayed as box plots (Figure 5). Intra-assay variability was very low for all assays, with at least 75 % of all triplicate measurements having a CV of less than 1 %.
Since the calibrator cDNA was included in each 384-well plate run, the inter-assay variability could be calculated in a similar way. These values were also found to be very small (data not shown).

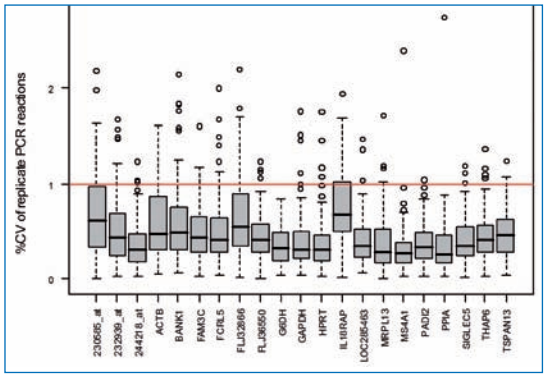

Figure 5: CV (coefficient of variation) values for PCR replicates, represented as box plots for all 21 assays (16 targets, 5 reference genes) included in the study presented here. The horizontal bars indicate the median, the box spans the inter quartile range (25th–75th percentile). Outliers are depicted as open circles; the red horizontal line marks 1% CV.

6 Selection of Reference Genes

The RNAs analyzed in this study were obtained from individuals affected by different progression states of either the SCA1 or SCA3 disorder. Since the variability of expression levels in human samples can be higher than for cell culture systems or animal models, we used three reference genes for normalization of the expression ratios. Since *a priori* knowledge of suitable reference genes individuals with SCA was not available, we measured the expression of five different reference genes in the sample population using the geNorm approach (Vandesompele et al., 2002) to define the three most stably expressed reference genes. With this method, the pair-wise ratios between all reference genes were calculated for all samples. Stably expressed reference genes will show little variation in different samples, while transcripts whose expression changes between samples will show large variation in the ratios. Using this approach, a stability measure (M) is calculated, indicating whether a transcript shows high degree of variation or not (see Vandesompele et al. (2002) for details using this method). Table 2 shows an overview of the M values and % CV of all reference gene assays. GAPDH and PPIA showed the highest degree of variation and were thus not used as reference genes, while ACTB, HPRT and G6DH were used subsequently for normalization.

	CV	M (geNorm)
ACBT	40.61%	1.1096
G6DH	36.94%	1.0530
GAPDH	58.86%	1.4255
HPRT	50.50%	1.1112
PPIA	70.68%	1.4686
Mean	51.52%	1.2336

	CV	M (geNorm)
ACBT	35.36%	0.9467
G6DH	34.61%	0.8769
HPRT	46.66%	0.9860
Mean	38.88%	0.9365

Table 2: M-values and coefficient of variation of all reference genes (left) and after removal of the two most unstable transcripts (right).

7 Relative Quantification Analysis

After identification of the three most suitable reference genes, target-to-reference ratios were calculated using the relative quantification analysis module of the LightCycler® 480 software 1.5. Each sample on a 384-well plate was assigned as either target or reference, cDNA samples were named unknown, the wells containing the calibrator served as positive control/calibrator (Figure 6). Based on this classification, the appropriate target name for each assay was entered in the LightCycler® 480 Software 1.5 sample editor. To allow for efficiency correction during the calculation of expression ratios, the standard curves resulting from the assay evaluation step (see section 4) were stored in the LightCycler® 480 System database using the same target name. This matching of target names allowed the software to automatically detect and extract the standard curve data and use the corrected efficiency value in the expression ratio calculation (Figure 6). Prior to calculation of target/reference ratios, all initially identified outlier reactions (see section 5) were excluded from the underlying absolute quantification analyses. The parameters selected in the relative quantification software dialog box are shown in the right part of Figure 6.

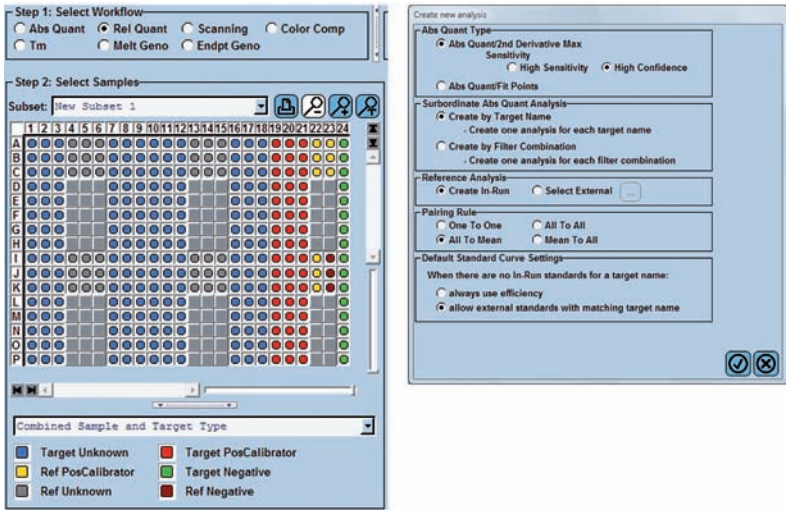

Figure 6: Advanced relative quantification: Left panel: Assignment of sample attributes in sample editor window. Right panel: RelQuant analysis dialog window.

8 Downstream Multiple-Plate Analysis and Statistic Calculations

The entire data set for this study still corresponded to twenty-one 384-well plates. The LightCycler® 480 Multiple Plate Analysis Software was used to easily compile multiple-run data, extract statistical parameters and obtain biologically meaningful information.
Files containing the analysis results from each individual experimental run were exported from the LightCycler® 480 System database as .ixo files (individually or all together in one batch), and then imported into the LightCycler® 480 Multiple Plate Analysis Software. This resulted in a new database object, holding all ixo-files belonging to the same study in a common "study object", thus making any reformatting steps and error-prone copy-paste operations obsolete (see Figure 6).

To enable subsequent data compilation and analysis, so-called properties were defined: *conditions* referring to SCA1 or SCA3; *stage* referring to mild or intermediate or severe forms of ataxia. To analyze each sample set, corresponding values for each property were assigned to each sample and samples were grouped according to type of disorder (SCA1 versus SCA3) and progression category ("mild", "intermediate" and "severe"). As summarized in Figure 7, this procedure led to data compilation that produced a single .xls file holding all the data. Tables included in this file show the normalized ratios with standard deviations for all assays and all samples together with their properties. The provided workflow option "RelQuant Summary" was chosen to display normalized ratios.

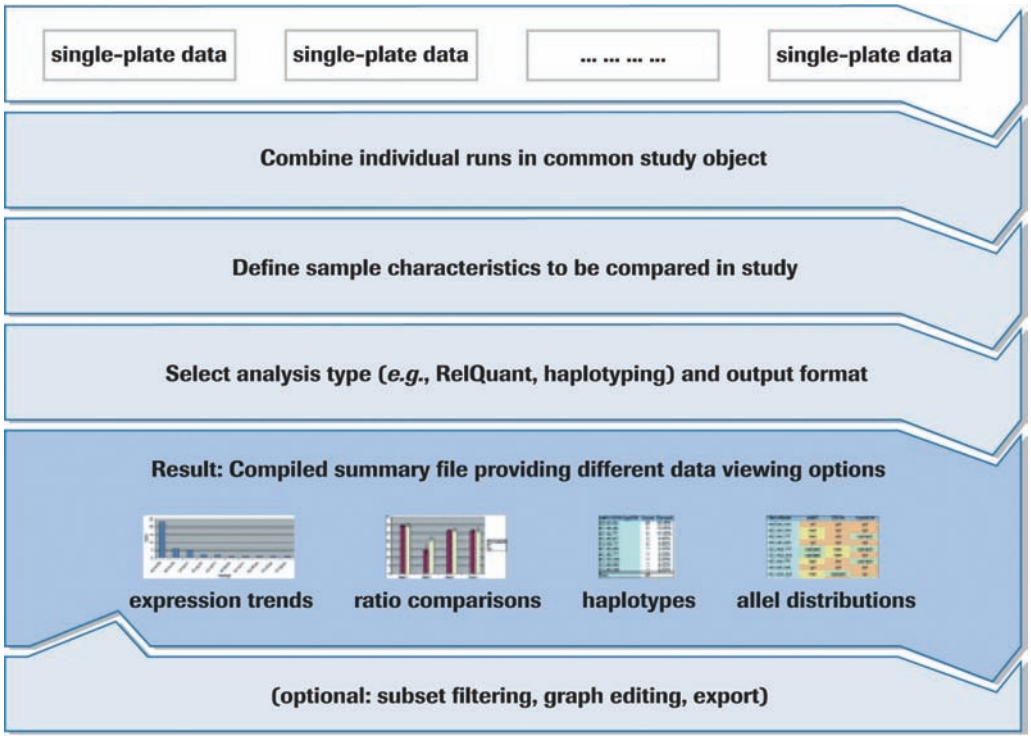

Figure 7: LightCycler® 480 Multiple Plate Analysis Software Workflow

Several advanced options were used to inspect and visualize the target assays individually or in groups in different ways. For instance, the LightCycler® 480 Multiple Plate Analysis Software allows assay distributions to be displayed as box-and-whisker plots. It also allows diagrams to be filtered for any property/value combination, which in turn allows an easy inspection of the range and variation of ratios within the biological replicates, such as type or stage of studied disorder (see Figure 8 A, B). Any filtering applied in an analysis worksheet is applied to all worksheets and all charts are automatically updated. An alternative way to see the distribution of the ratios for individual assays is to display the results as signal histogram. Up to four assays can be displayed in parallel. Additionally, the individual ratios for each sample can be shown as bar plots below the histogram (see Figure 8 C, D).

The most common question addressed when performing real-time PCR experiments is whether the expression of a transcript differs as a function of two or more different physiological conditions. This kind of question could be addressed using the criteria comparison worksheet provided by the LightCycler® 480 Multiple Plate Analysis Software (see Figure 8 E, F). For each assay, the average ratio plus standard deviation for each value of the selected property is calculated and displayed in tabular form. These values are used to graphically display the results in a bar plot. If more than one property is assigned to the data, then an additional filter can be applied to show the results for each subordinate property separately (see Figure 8 E, F). To check if the observed differences are by chance alone, a two-tailed Student's t-test is performed for each combination of values for the selected property based on the filter combination in the criteria comparison (data not shown). For a more complex statistical analysis, the calculated average and standard deviation or the summary table containing the normalized ratios can be easily exported and transferred to more specialized software.

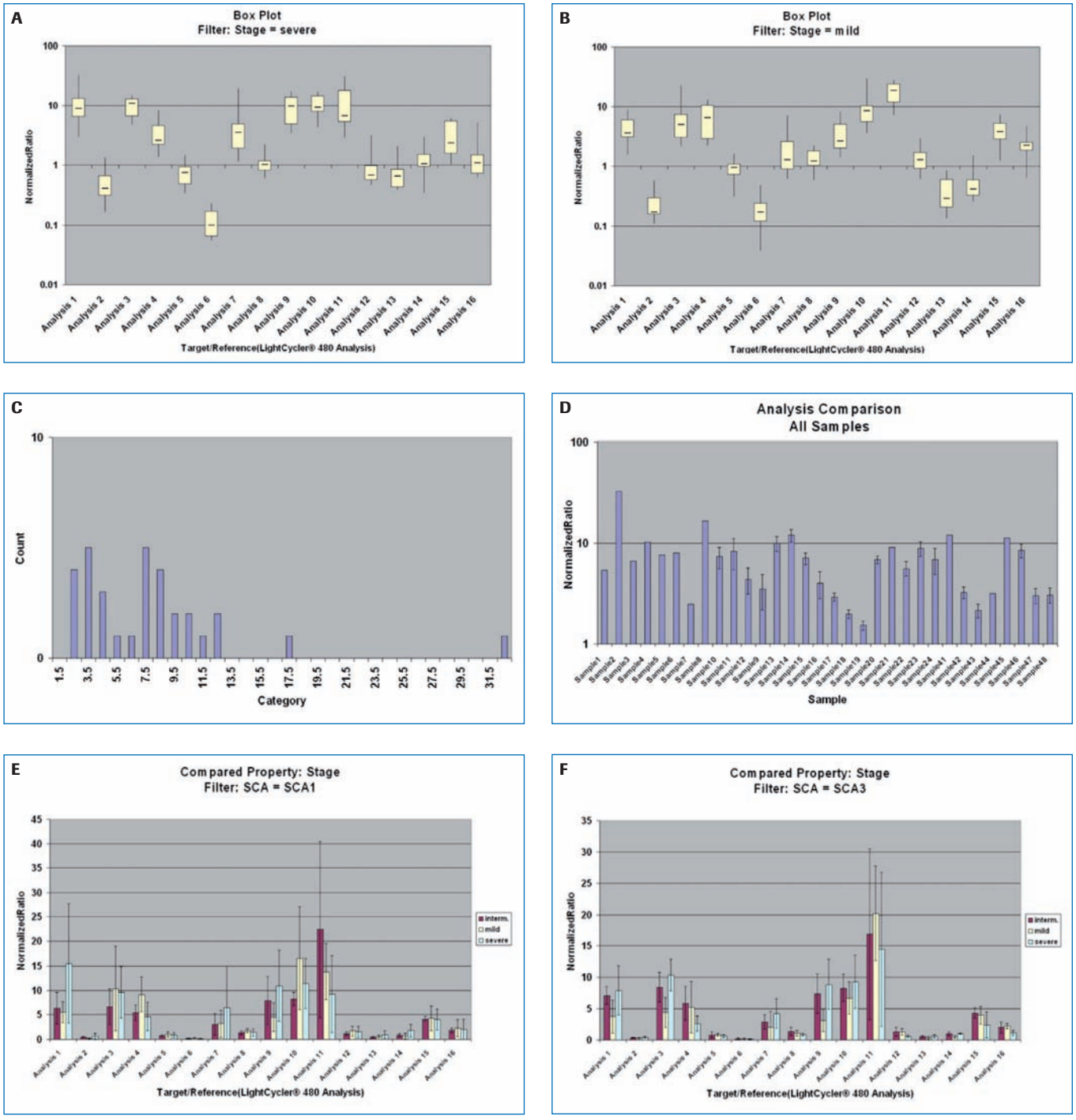

Figure 8: Data evaluation and analysis based on the Multiple Plate Analysis Software output. A, B: Box-and-whisker plots of normalized ratios for individuals with "mild" and "severe" stages of the studied disorder (SCA) for 16 targets assays analysed. The horizontal bars indicate the median, the box spans the inter quartile ratios for individuals and the "whiskers" delineate the minimum and maximum of all data. C) Histogram for a single selected assay with bin width 1 showing a right tailed distribution of a data, which are displayed in D) for all samples individually. E, F) Average ratios and standard deviations for all values within the selected property "Stage". Additional filtering for the two different conditions was applied (E: SCA1, F: SCA3).

9 Conclusion

The aim of the present study was to investigate how the up- and downregulation of certain cellular transcripts is related to the type and severity of spinocerebellar disorders, SCA1 and SCA3. Primers and probes specific for targets that had been previouly identified as candidate markers on array platforms were easily obtained using the free, web-based Roche Universal ProbeLibrary assay design center (www.universalprobelibrary.com). Combining the Transcriptor First Strand cDNA Synthesis Kit, Universal ProbeLibrary probes and the LightCycler® 480 Instrument in a smooth workflow produced high-quality five-point two-fold dilution standard curves crucial for the detection of transcripts expressed at low levels. After rapid real-time PCR, the LightCycler® 480 Multiple Plate Analysis Software provided easy access for the analysis and direct comparison of different progression states of ataxia. Large amounts of high-quality, biologically relevant data were generated in a short period of time.
In summary, the LightCycler® 480 System in combination with the Transcriptor First Strand cDNA Synthesis Kit and Universal ProbeLibrary assays, provides a straightforward and productive workflow for processing large numbers of samples for relative quantification using high-throughput real-time PCR.

10 References

1. Schols, L., P. Bauer, et al. (2004). "Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis." *Lancet Neurol* 3 (5): 291–304.
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Please note: an extended version of this article is available on www.lightcycler480.com for additional information about this application.

Ordering Information

Product	Cat. No.	Pack Size
Transcriptor First Strand cDNA Synthesis Kit [1]	04 379 012 001 04 896 866 001 04 897 030 001	1 Kit (50 reactions) 1 Kit (100 reactions) 1 Kit (200 reactions)
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LightCycler® 480 Multiwell Plate 384, clear	05 102 430 001	50 Plates / 50 Foils
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Universal ProbeLibrary, Human HPRT Gene Assay	05 046 157 001	500 reactions
Universal ProbeLibrary, Human ACTB Gene Assay	05 046 165 001	500 reactions
Universal ProbeLibrary, Human PGK1 Gene Assay	05 046 173 001	500 reactions

1 For detailed information, visit www.roche-applied-science.com/pcr

2 Instrument package includes LightCycler® 480 Instrument, LightCycler® 480 thermal block cycler unit (96- or 384-well), LightCycler® 480 software, LightCycler® 480 Instrument Operator's Manual, LightCycler® 480 Xenon Lamp (spare lamp). A Pentium desktop PC is supplied with the instrument.

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