

Geno-Sen's
GAPDH (Rotor Gene)
Real Time PCR Kit

Quantitative

for use with the
Rotor Gene™ 2000/3000/6000
(Corbett Research Australia)



PACK INSERT

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Genome Diagnostics Pvt. Ltd.
(An ISO 13485:2012, 9001:2008 Certified Company)

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Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

GAPDH *Geno-Sen's Real Time PCR Kit* for use with the *Rotor Gene™ 2000/3000/6000** (Corbett Research).

1. Contents of the Kit:

Color Code	Contents	REF 91117001 100 rxns	REF 91117002 50 rxns	REF 91117003 25 rxns
R1 Blue	GAPDH Super Mix.	25 rxns x 4 Vials	25 rxns x 2 Vials	25 rxns x 1 Vials
R2 Yellow	Mg Sol RT.	1 Vial	1 Vial	1 Vial
GAPDH S1 Red	GAPDH Standard 1 1×10^5 copies/ μ l	1 Vial of 300 μ l	1 Vial of 300 μ l	1 Vial of 300 μ l
GAPDH S2 Red	GAPDH Standard 2 1×10^4 copies/ μ l	1 Vial of 300 μ l	1 Vial of 300 μ l	1 Vial of 300 μ l
GAPDH S3 Red	GAPDH Standard 3 1×10^3 copies/ μ l	1 Vial of 300 μ l	1 Vial of 300 μ l	1 Vial of 300 μ l
GAPDH S4 Red	GAPDH Standard 4 1×10^2 copies/ μ l	1 Vial of 300 μ l	1 Vial of 300 μ l	1 Vial of 300 μ l
GAPDH S5 Red	GAPDH Standard 5 1×10^1 copies/ μ l	1 Vial of 300 μ l	1 Vial of 300 μ l	1 Vial of 300 μ l
W White	Molecular Grade Water.	1 Vials of 1 ml	1 Vial of 1 ml	1 Vial of 1 ml

R = Reagents

S = Quantitation Standards

W = Molecular Grade Water.

All Vials have Color Coder tops to distinguish between different reagents.

2. Storage of the Kit.

All the reagents of the kit should be stored at -20°C and is stable till expiry at this temperature. Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay. If the kit is to be used only occasionally, the reagents should be frozen in aliquots. Storage at +4°C is not recommended & should not exceed a period of 2 hours in any case.

* The *Rotor Gene™ 2000/3000/6000* is a registered trademark of Corbett Research, Australia.

3. GAPDH Information

Application

Housekeeping (HK) genes are defined as genes constitutively expressed in all tissues to maintain essential cellular functions. Conversely, tissue specific (TS) genes, are defined as genes only or mainly expressed in a specific tissue or organ, and hence responsible for specific functions and development. Gene expression profiling is a key to characterizing normal and diseased biological states.

Many disciplines need to discriminate between housekeeping and tissue specific genes. In Microbiology, housekeeping genes are known to play a role in enhancing virulence of pathogens and they are studied to find potential drug targets, while slowly diverging housekeeping genes are used in evolutionary studies to discriminate subspecies. In Medicine they are studied to discover if genetic diseases linked to housekeeping genes are more likely to affect multiple organs. In Biology and Physiology housekeeping genes are the key to determine the set of basic functions necessary for cellular life or an organ function. Additionally, many quantitative techniques used for diagnosis and research use housekeeping gene expression as a baseline to normalize numerical values and to detect differential expression. Housekeeping genes like Glyceraldehyde-3-phosphate Dehydrogenase (GADPH), beta-Globin, beta Actin or beta-tubulin are used as standards in assay techniques like microarrays, RT-PCR, Northern blotting (for mRNA levels) and Western blotting (for protein levels).

The sensitivity of qRT-PCR allows to work with a minimal amount of starting material, still achieving an accurate quantification of poorly transcribed mRNAs. Problems associated with the use of this assay are linked to the variability associated with the various steps of the experimental procedure, and could lead to severe misinterpretation of the results: different amounts and quality of starting material, variable enzymatic efficiencies (i.e. efficiency of retrotranscription from RNA to cDNA, and PCR efficiency) or differences between tissues or cells in overall transcriptional activity. Among several strategies proposed, house-keeping genes (HKGs) are commonly accepted and frequently used to normalize qRT-PCR and to reduce possible errors generated in the quantification of gene expression. In this normalization strategy, internal controls are subjected to the same conditions as the RNA of interest and their expression measured by qRT-PCR. The success of this procedure is highly dependent on the choice of the appropriate control genes. Although many studies using qRT-PCR have relied upon only one endogenous control, to date the use of a single HKG appears to be insufficient at times, and normalization by multiple controls might be required. An ideal HKG, exposed to the same experimental protocol of the gene of interest (GOI), should present stable expression levels. If the expression of the reference gene is altered by the experimental conditions or by external factors, such as contamination, and is affected by a large variation, the noise of the assay is increased and detection of small changes becomes unfeasible, producing results that may be entirely incorrect. Several works prove how some of the most commonly used

HKGs can not always be considered as reliable controls and/or they show different behaviour in various tissues, emphasizing the importance of preliminary evaluation studies, aimed at identifying the most stable HKGs in different organisms. The number of articles concerning the evaluation and selection of the best HKGs for each single experiment is rapidly increasing, together with the number of softwares which use statistical methods to evaluate stability of selected HKGs. Some HKGs (those encoding for Act-B, GAPDH, HPRT1 and 18S ribosomal RNA) have been used as reference for many years in Northern blots, RNase protection tests and conventional quantitative PCR (qPCR). With the introduction of qRT-PCR, other ordinarily used HKGs, involved with basic and ubiquitous cellular functions and belonging to different functional classes, have been introduced.

In quantitative real-time Reverse Transcriptase-PCR (RT-PCR) normalization is performed using housekeeping genes as references against the expression level of a gene under investigation. The housekeeping genes are a large group of genes that code for proteins whose activities are essential for the maintenance of cell function.

GAPDH is a housekeeping gene used frequently for normalization of qRT-PCR results.

4. Precautions for PCR

The following aspects should always be taken care of:

- Store positive material (Specimens, Standards or amplicons) separately from all other reagents and add it to the reaction mix in a separate facility.
- Thaw all components thoroughly at room temperature before starting the assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the Cooling Block.
- All the reagents including the NTC (except for standards & specimens) should be mixed & dispensed in pre-mix area.
- All the standards & specimens should be mixed & dispensed in extraction area.
- Use pipette tips with filters only.
- Always use disposable powder-free gloves

5. Additionally Required Materials and Devices

- DNA/RNA isolation kit (see **8.a. DNA/RNA extraction**)

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- 0.2 ml PCR tubes for use with 36-well rotor (Corbett Research, Cat.-Nr.: SE-1003F) alternatively 0.1 ml PCR tubes for use with 72-well rotor (Corbett Research, Cat.-Nr.: ST-1001)
- Micro Pipettes Variable Volume 2-20µl, 10-100µl, 100-1000µl,
- Sterile pipette tips with aerosol barrier 2-20µl, 10-100µl, 100-1000µl,
- Disposable powder-free gloves
- Vortex mixer
- Centrifuge Desktop with rotor for 1.7 ml reaction tubes
- *Rotor Gene™ 2000, 3000 or Rotor Gene™ 6000*, Corbett Research (The Real time PCR Instrument)
- *Depending upon the target being amplified, a separate kit for cDNA synthesis is available Cat No. 110071, 110072, 110073.*

6. Principle of Real-Time PCR

The robust assay exploits the so-called Taqman principle. During PCR, forward and reverse primers hybridize to a specific sequence product. A TaqMan probe, which is contained in the same reaction mixture and which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream, 3'-quencher dye, hybridizes to a target sequence within the PCR product. A Taq polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. The reporter dye and quencher dye are separated upon cleavage, resulting in an increase in fluorescence for the reporter. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

7. Description Of the Product.

The **Geno-Sen's GAPDH PCR** Reagents constitute a ready to use system for detection and quantification of **GAPDH** using Polymerase chain reaction (PCR) in the Rotor Gene 2000/3000/6000 (Corbett Research). *The Specific Master* mix contains reagents and enzymes for the specific amplification of **GAPDH** and for the direct detection of the specific amplicon in fluorescence channel Cycling A.JOE/YELLOW of the *Rotor Gene 2000/3000/6000*. External positive Standards (**GAPDH S 1-5**) are supplied which allow the determination of the gene load. For further information, please refer to section 8.b Quantitation.

8. Procedure

8.a DNA/RNA Extraction

DNA/RNA Extraction kits are available from various manufacturers. Sample volumes for the DNA/RNA Extraction procedure depend on the protocol used.

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Please carry out the DNA/RNA Extraction according to the manufacturer's instructions.

The recommended Extraction kits are the following:

Sample Material	Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
Whole Blood.	Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification	98021 or 98022	Genome Diagnostics Pvt. Ltd. India
	QIAamp Blood DNA Mini extraction Kit (50)	51104	QIAGEN
	Suitable Extraction kit which give a higher yield.		

Above Kits for evaluated in conjunction with the *Geno Sen's® GAPDH Real Time PCR RG Kit on the Rotor Gene 3000/6000..* However the sample size was limited for this study hence for all practical purposes all the validations were conducted with the *Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification.*

However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher DNA yield.

Blood collection tubes coated with anticoagulants may inhibit the PCR, However these inhibitors will be eliminated by the use of the isolation kits given above. It is recommended to avoid the usage of heparin blood.

When using Extraction protocols with ethanol-containing washing buffers, please carry out an additional centrifugation step before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.

The *GAPDH Rotor Gene PCR Reagents* should not be used with phenol based isolation methods.

8.b Quantitation

The quantitation standards provided in the kit (*GAPDH S 1-5*) are treated in the same way as extracted samples and the same volume is used i.e. (5µl) instead of the sample. To generate a standard curve in the *RotorGene™ 2000/3000/6000*, all 5 Standards should be used as defined in the menu window *Edit Samples* of the *RotorGene™* software. The same should also be defined as standards with the specified concentrations (see *RotorGene™* Manual). The standard curve generated

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as above can also be used for quantitation in subsequent runs, provided that at least one standard is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *Rotor Gene™ 2000/3000/6000 Manual*). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs & due to varying Reaction efficiencies.

Attention: The standards are defined as copies/μl. The following formula has to be applied to convert the values determined using the standard curve into Copies/ml of sample material:

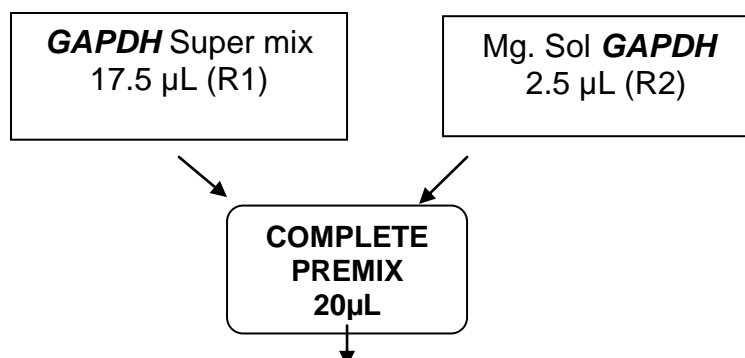
$$\text{Result (Copies/ml)} = \frac{\text{Result (Copies/}\mu\text{l)} \times \text{Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

8.c Preparation for PCR

8. d. Preparation for PCR amplification:

First make sure that the Cooling Block (accessory of the *Rotor Gene™*, Corbett Research) is pre-cooled to +4°C in a Refrigerator or Deep Freezer. Place the desired number of PCR tubes into the Cooling Block. Make sure that the tubes for standards & at least one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied Standards (**GAPDH S 1-5**) for each PCR run. Before each use, all reagents need to be thawed completely and mixed (by pipetting or by brief vortexing).

Please follow the pipeting scheme mentioned below for each sample depending upon the number of samples a mix can be prepared as follows. Each sample



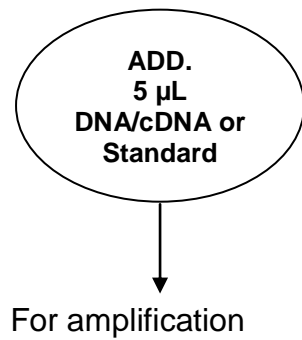


Fig. 4.

Depending upon the number of samples the following pipetting scheme can be followed. e.g. for 10 rxns.

<i>GAPDH</i> MASTER MIX		1 rxns.	10 rxns.
<i>GAPDH</i> Super Mix (R1)		17.5 µL	175 µL
<i>GAPDH</i> Mg Sol. (R2)		2.5 µL	25 µL
Total		20µL	200µL

Fig. 5.

Pipette 20 µl of the Master Mix into each labelled PCR tube. Then add 5 µl of the earlier extracted DNA or cDNA to each sample tube and mix well by pipeting up and down. (Depending upon the initial concentration of the template the volume of the cDNA can be adjusted i.e. reduced and for the balance volume, Molecular Grade Water can be used to complete the volume.) Correspondingly, 5 µl of the Standards (*GAPDH* S1-5) must be used as a positive control and 5 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes and transfer the *GAPDH* tubes into the rotor of the *RotorGene™* instrument. The *RotorGene™* software versions 5.0.53 and higher require a Locking Ring (accessory of the *RotorGene™*, Corbett Research) to be placed on top of the rotor to prevent accidental opening of the tubes during the run.

8.e. Programming the *Rotor Gene™* 2000/3000

The *Rotor Gene™* 2000/3000 PCR program for the detection of *GAPDH* can be divided into following steps:

- A. Setting of general assay parameters & reaction volume
- B. Thermal Profile & Calibration
- C. Cycling profile/ & Initial activation of the Hot Start enzyme
- D. Cycling for Amplification of DNA
- E. Adjustment of the sensitivity of the fluorescence channels
- F. Starting of the *Rotor Gene™* run

[Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000](#)

Program the *RotorGene™ 2000/3000* for these 5 steps according to the parameters shown in Fig. 6-20. All specifications refer to the *RotorGene™* software version 6.0.33. Please find further information on programming the *RotorGene™* in the *RotorGene™ 2000/3000 Operator's Manual*. In the illustrations these settings are shown by arrows

Setting of general assay parameters & Reaction volume.

First, confirm whether the PCR tubes used are No Domed PCR tubes by clicking in the box as shown in the figure. Please note that tubes with domes cannot be used in Rotor gene hence click in the box as shown below.

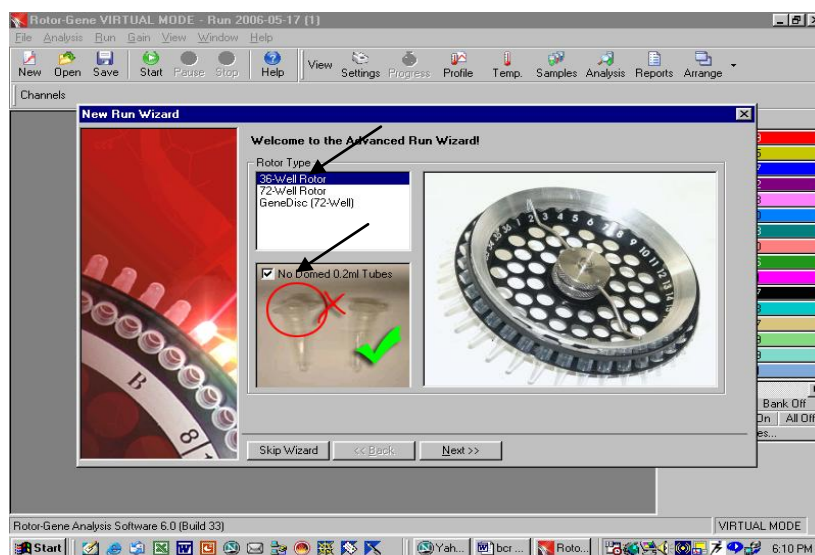


Fig. 6.

Confirmation of Reaction Volume as follows.

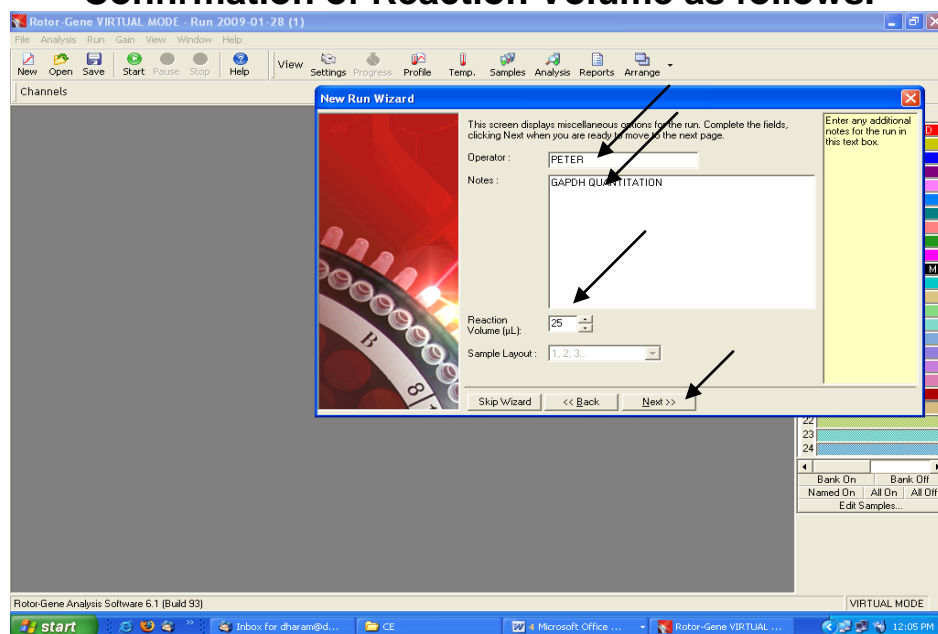


Fig. 7. Setting of general assay parameters.

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- Please click on the volume buttons to make sure that 25 μ l is reflected in the window as shown above.
- Then click next and a new window will open as shown below.

THERMAL PROFILE & CALIBRATION:

Here the thermal profile for the assay will be defined.

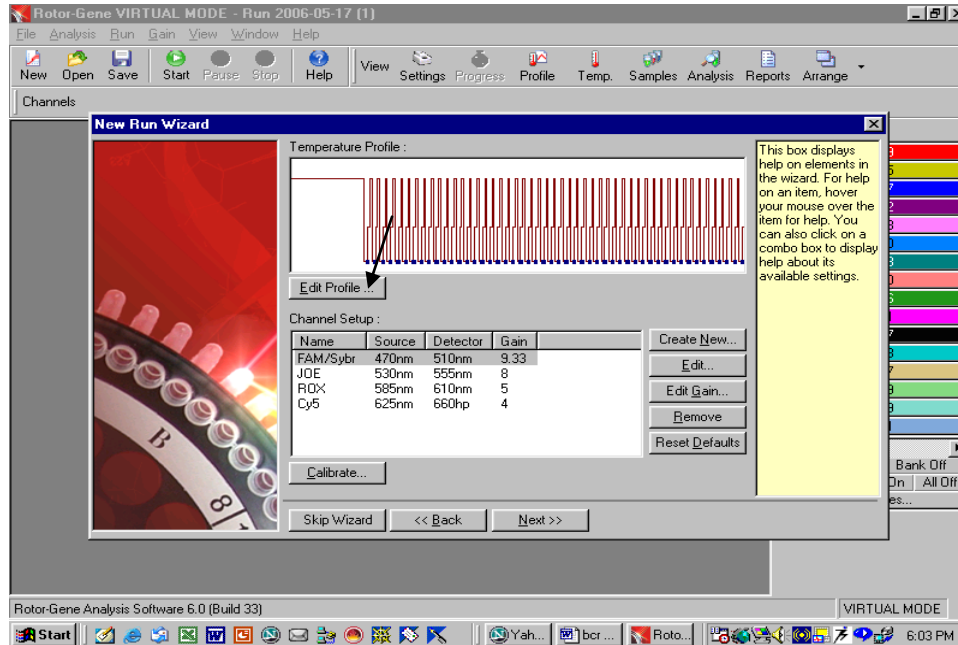


Fig. 8.

Programming the temperature profile is done by activating the button *Edit Temperature Profile* in the next *New Experiment Wizard* menu window as shown above.

CYCLING PROFILE: Hold 95°C for 10 minutes as below

Geno-Sen's *GAPDH* Real Time PCR Kit for Rotor Gene 2000/3000/6000

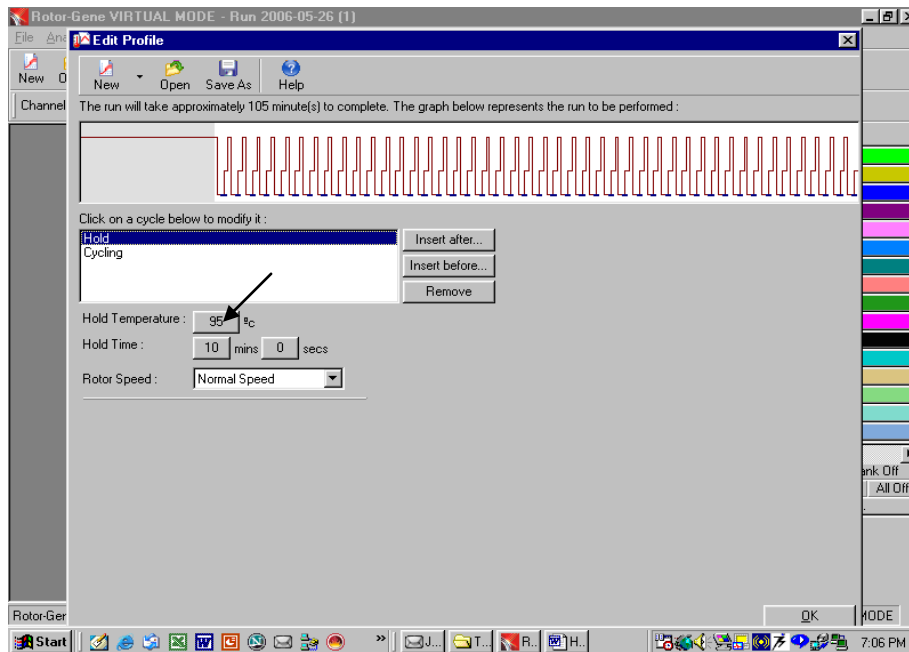


Fig. 9. Initial activation of the Hot Start enzyme.
Setting up of denaturation step in the cycling profile as depicted below i.e. 95°C for 15 seconds.

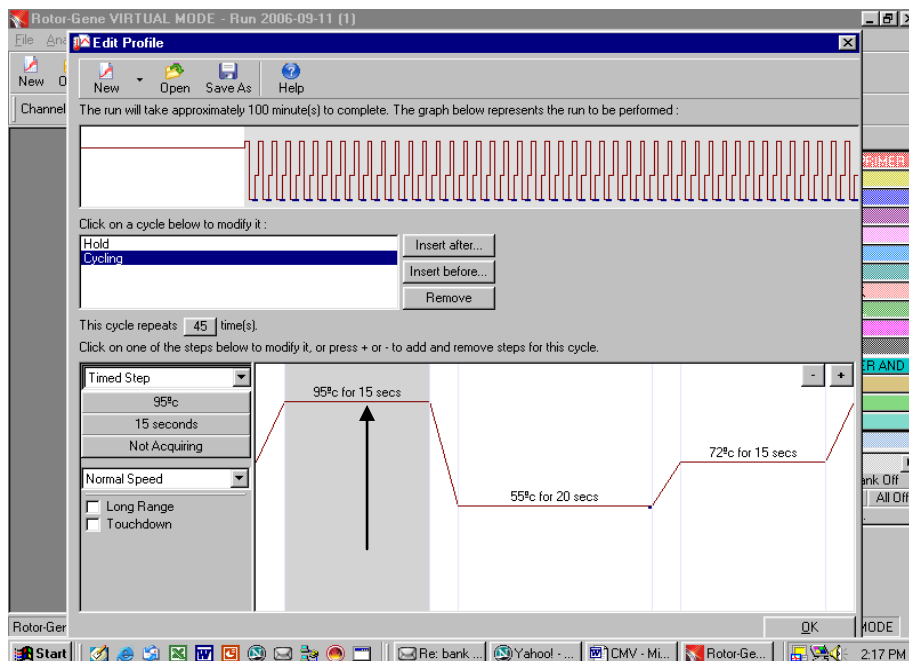


Fig. 10.

Setting up of Anneling step in the cycling profile as depicted below i.e. 55°C for 20 Seconds and defining the Data acquiring channel i.e JOE

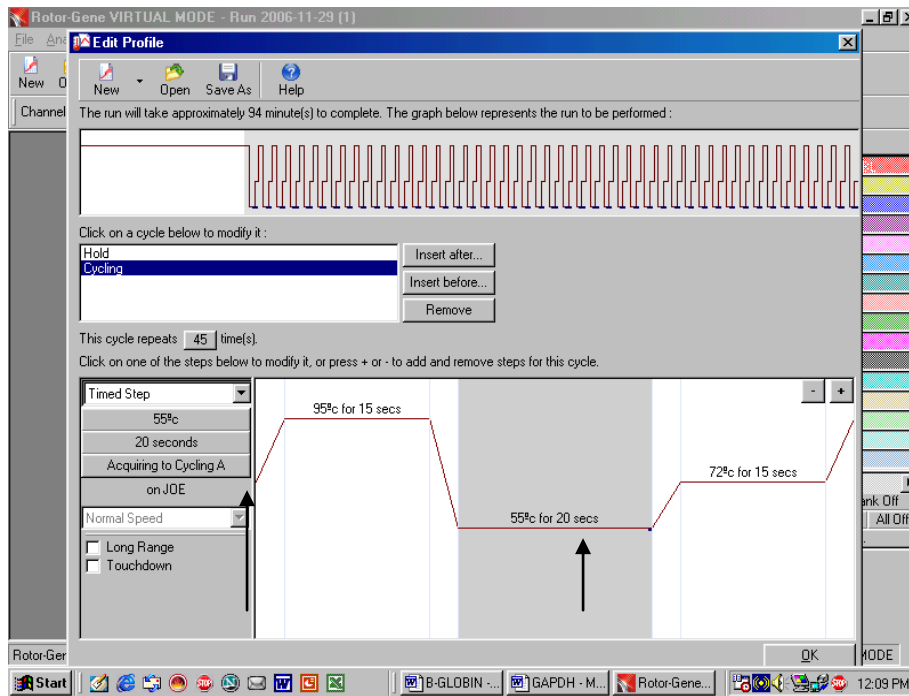


Fig. 11.

Setting up of Extension step in the cycling profile as depicted below i.e. 72°C for 15 Seconds

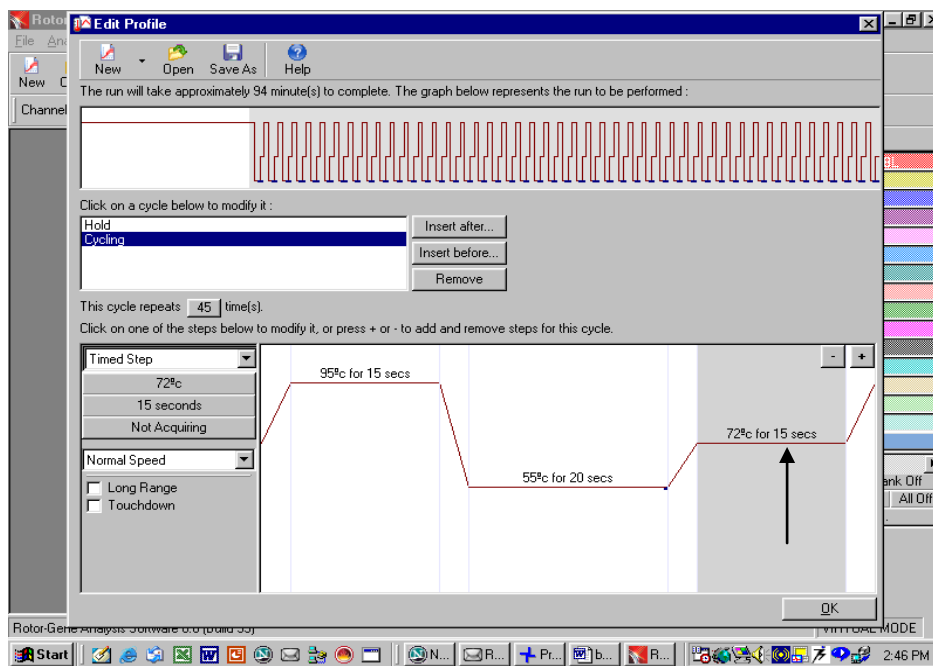


Fig. 12.

Setting up of Number of Cycles to 45 cycles in the cycling profile as depicted below.

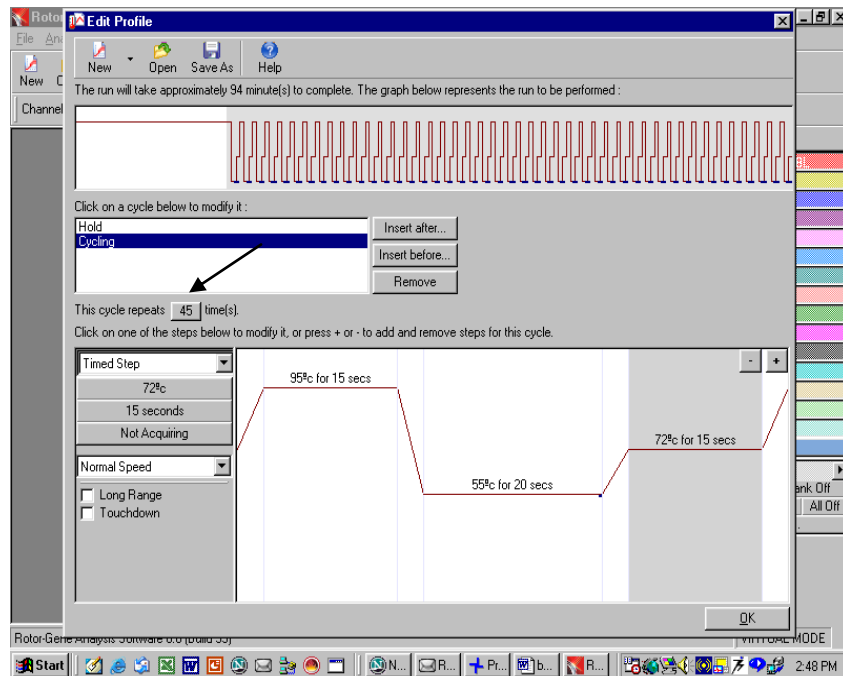


Fig. 13.
Final Confirmation of the Thermal profile by pressing OK button as shown below.

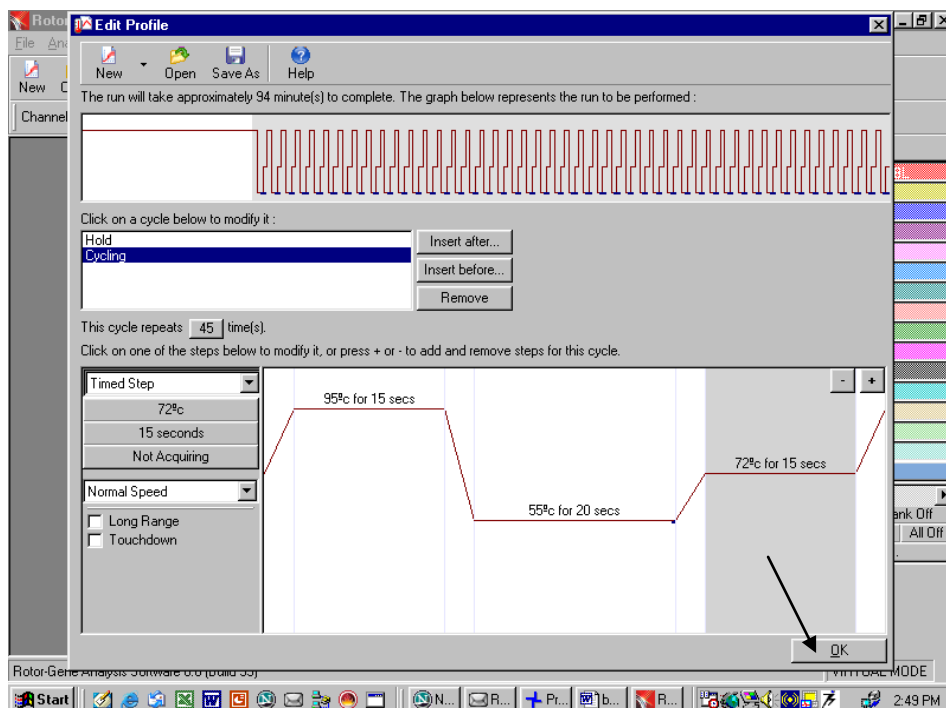


Fig. 14.
Setting the gains for the acquiring channel by clicking at the Calibrate button as shown below.

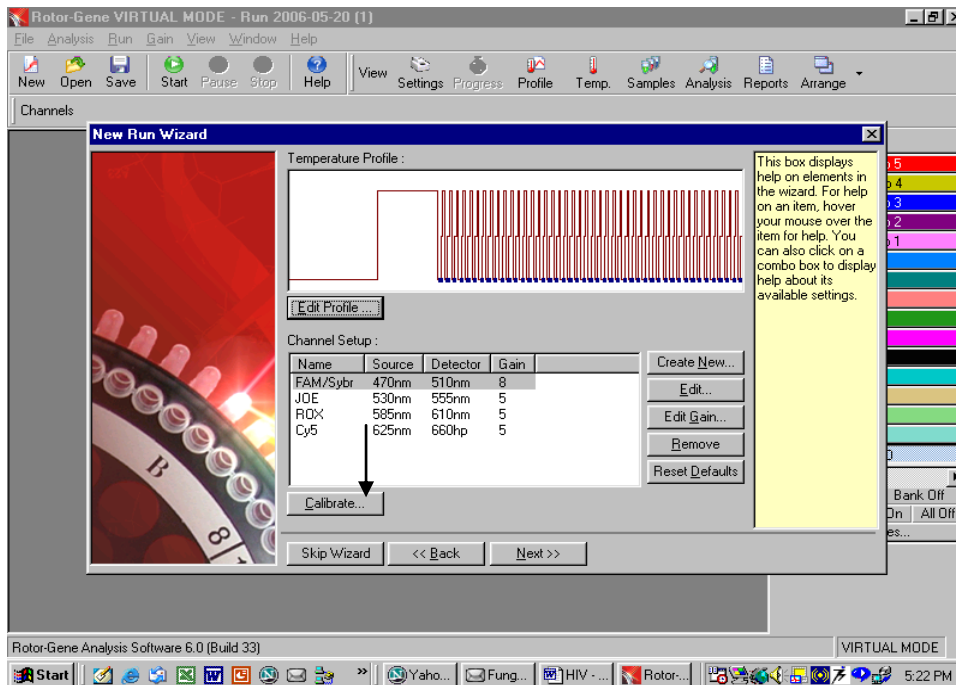


Fig. 15.

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain Calibration Setup* (activation in menu window *New Experiment Wizard* under *Calibrate*). Please set the calibration temperature to the annealing temperature of the amplification program (compare Fig. 16).

Adjustment of the fluorescence channel sensitivity as shown below.

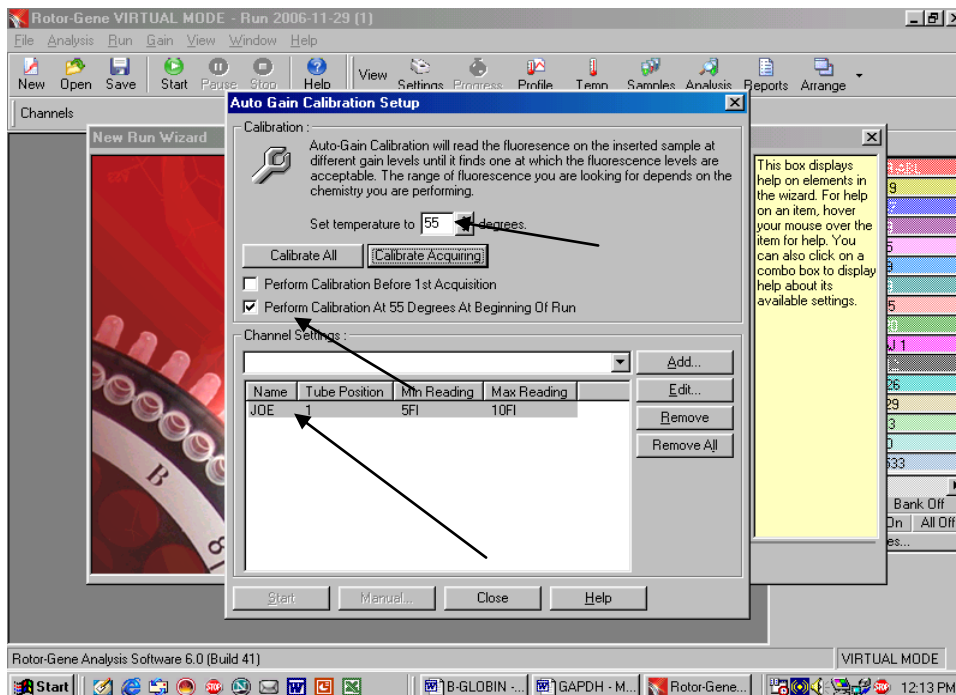


Fig. 16.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

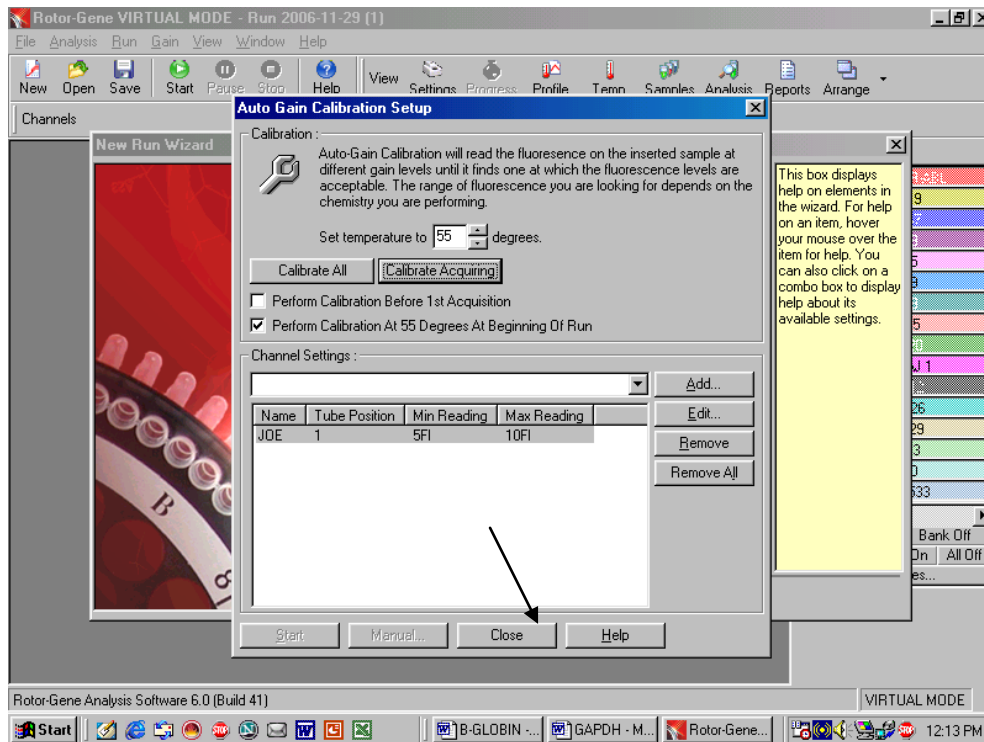


Fig. 17.

Please do not forget to click on the box against “ Perform calibration at 55°C at beginning of the run.” After that press Close and a new window will open as shown below. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure.

PRESS NEXT

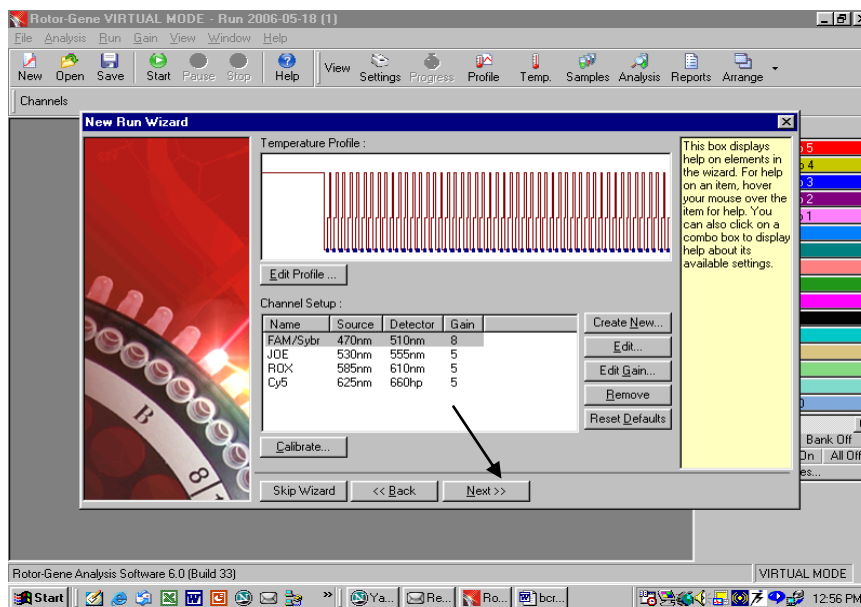


Fig. 18.

Starting of the *Rotor Gene*TM run.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

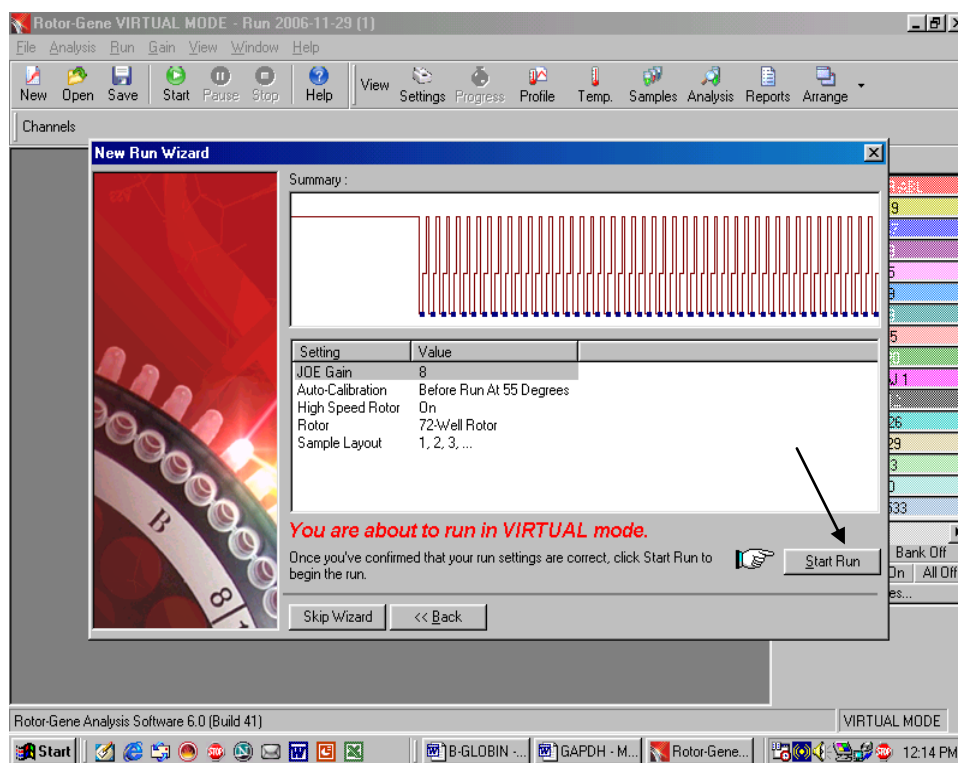


Fig. 19:
Press Start Run Button.

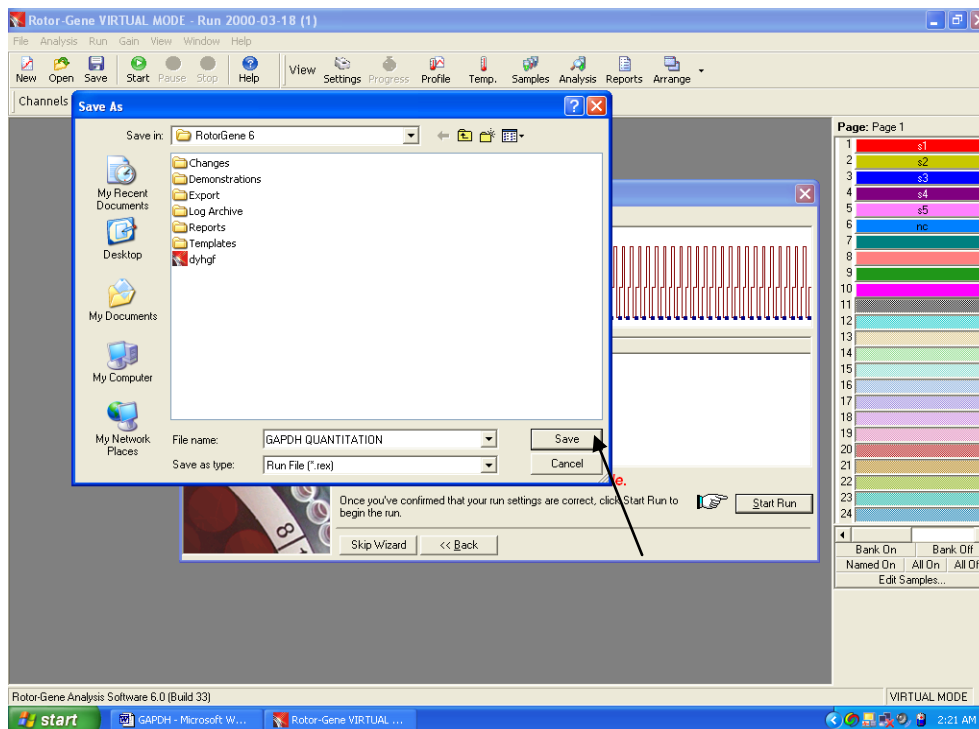


Fig. 20.

SAVING THE RUN FILE AS ABOVE

Store the run file either in my documents or a designated folder as shown above.

[Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000](#)

The moment save button is clicked after the file name, machine will start.

8.f. Programming the *Rotor Gene*[™] 6000

The *RotorGene*[™] 6000 PCR program for the detection of GAPDH can be divided into following steps:

- G. Setting of general assay parameters & reaction volume
- H. Thermal Profile & Calibration
- I. Cycling profile/ & Initial activation of the Hot Start enzyme
- J. Cycling for Amplification of cDNA
- K. Adjustment of the sensitivity of the fluorescence channels
- L. Starting of the *Rotor Gene*[™] run

Program the *RotorGene*[™] 6000 for these 5 steps according to the parameters shown in Figures 21-36 below All specifications refer to the *RotorGene*[™] 6000 software version 1.7 Please find further information on programming the *RotorGene*[™] in the *RotorGene*[™] 6000 *Operator's Manual*,. In the illustrations these settings are shown by arrows

g) Setting of general assay parameters & Reaction volume.

Please see to it that you in advanced mode and then click Hydrolysis Probes. On a double click the software will automatically go to the next function. If there is just a single click then click new after highlighting the Hydrolysis Probes.

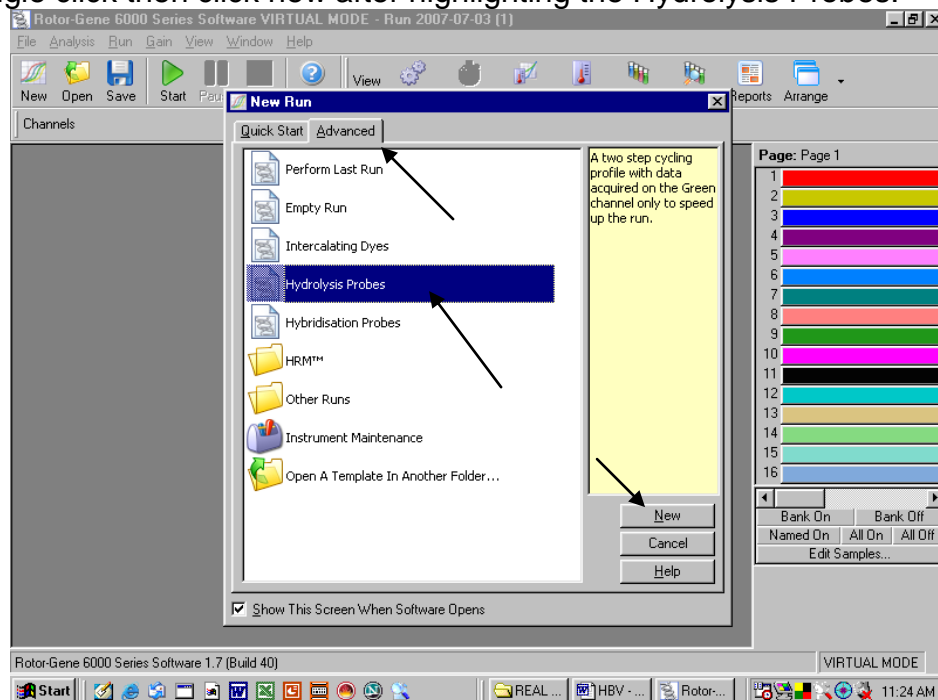


Fig. 21.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

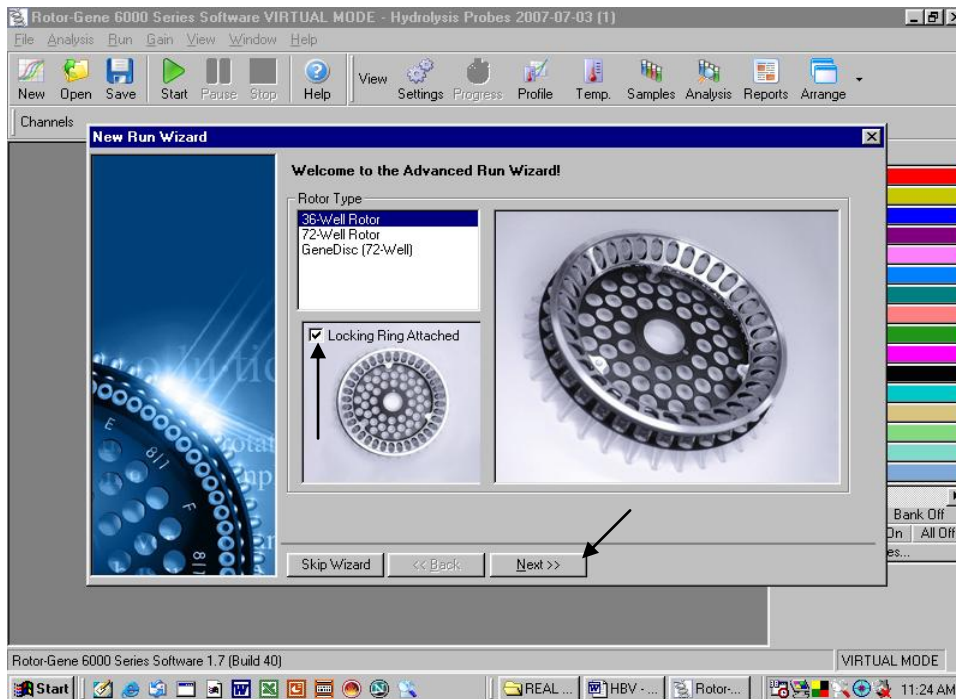


Fig. 22.

First, confirm by clicking in the box as shown in the figure above whether a locking ring has been attached. Then click next to proceed to the next step.

Confirmation of reaction Volume as follows.

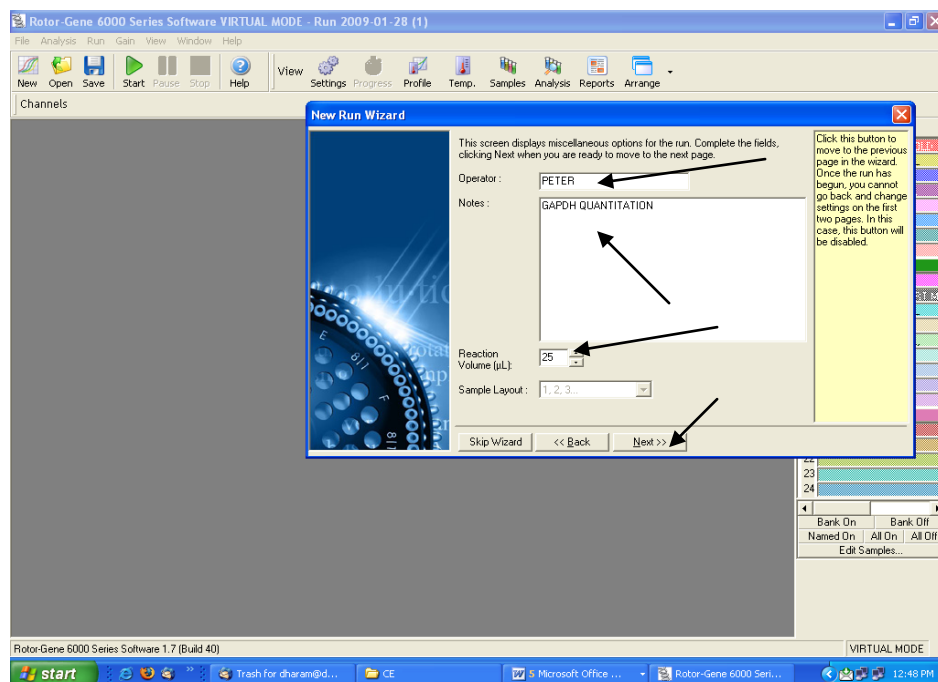


Fig. 23.

- Please click on the volume buttons to make sure that 25µl is reflected in the window as shown above.
- In case required Operators Name can be Fed into the system.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

- Any Notes which need to be either printed into the Report can be typed out in the Box of Notes.
- Then click next and a new window will open as shown below.

h) THERMAL PROFILE & CALIBRATION:

Here the thermal profile for the assay will be defined.

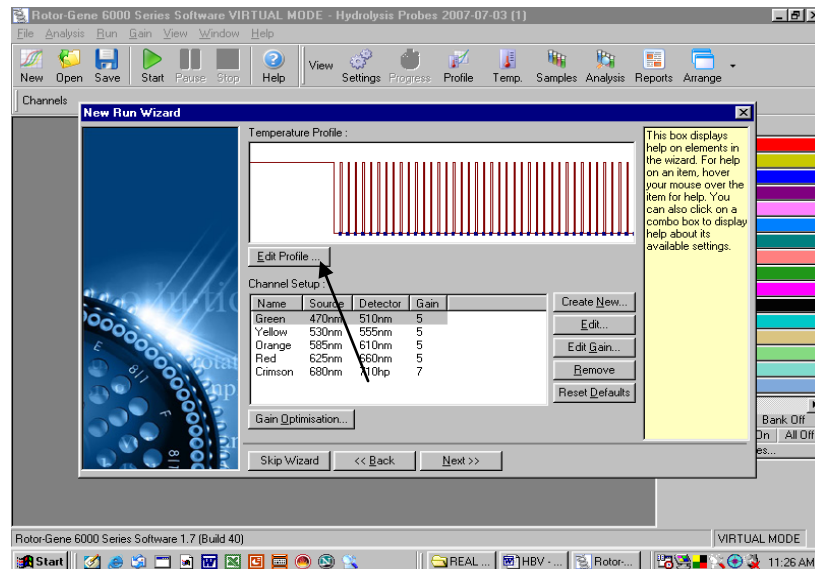


Fig. 24.

Programming the temperature profile is done by activating the button *Edit Profile* in the next *New RUN Wizard* menu window as shown above.

i) CYCLING PROFILE: First hold 95°C for 10 minutes as below

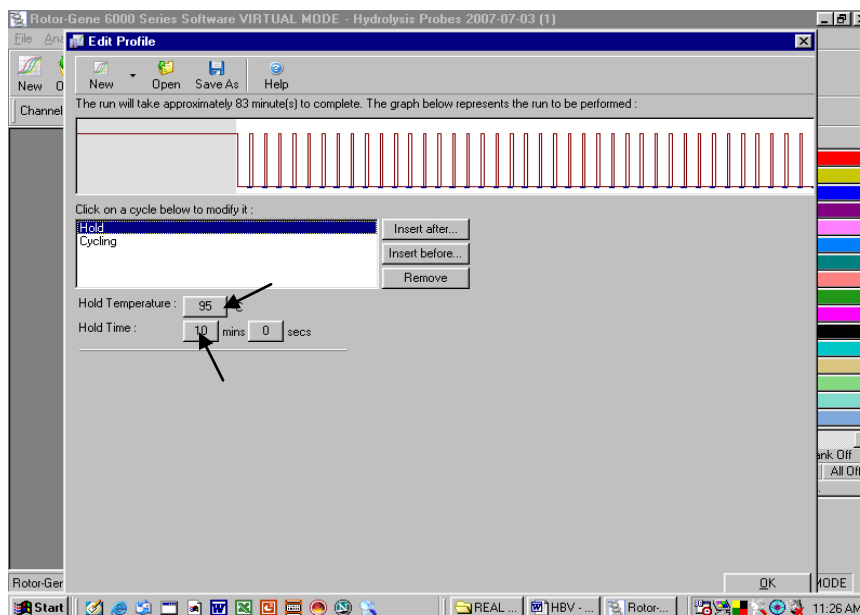


Fig. 25. Initial activation of the Hot Start enzyme. Generally the window will open with the Hold Temp as 95°C and the Hold Time as 10 minutes. In case it is different then set the same to as shown above.

j) Setting the Cycling and acquisition.

When clicked on Cycling the window will open as below.

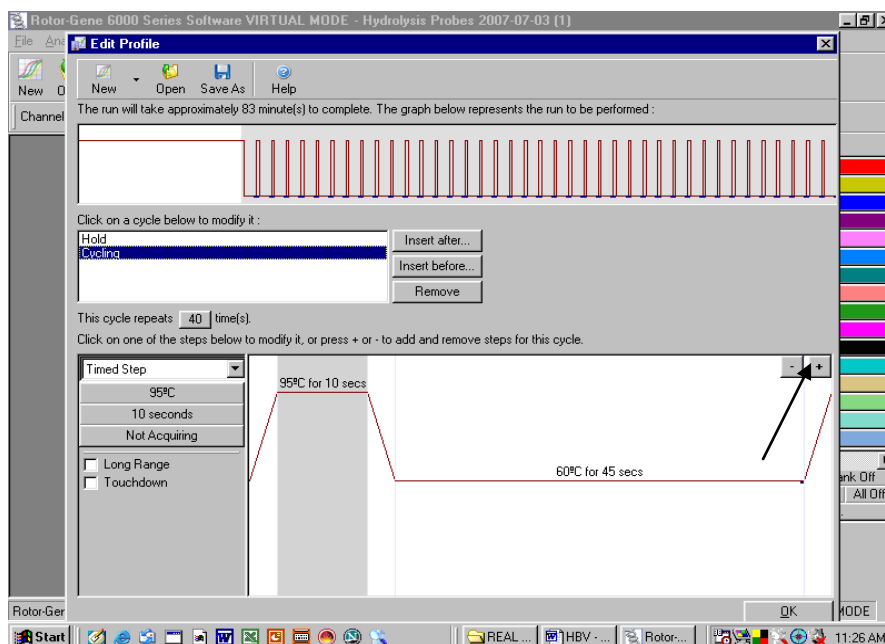


Fig. 26.

Click on the Plus sign at the right hand as shown in the figure to make the Cycling a three step process. A new window as shown below will be there.

Setting up of denaturation step in the cycling profile as depicted below i.e. 95°C for 15 seconds.

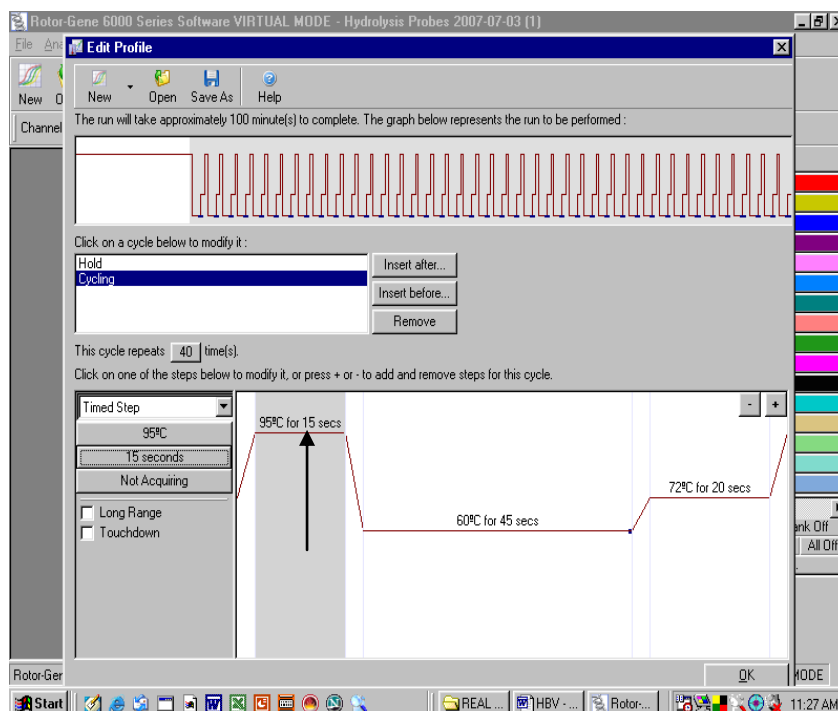


Fig. 27.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

Setting up of Anneling step in the cycling profile as depicted below i.e. 55°C for 20 Seconds

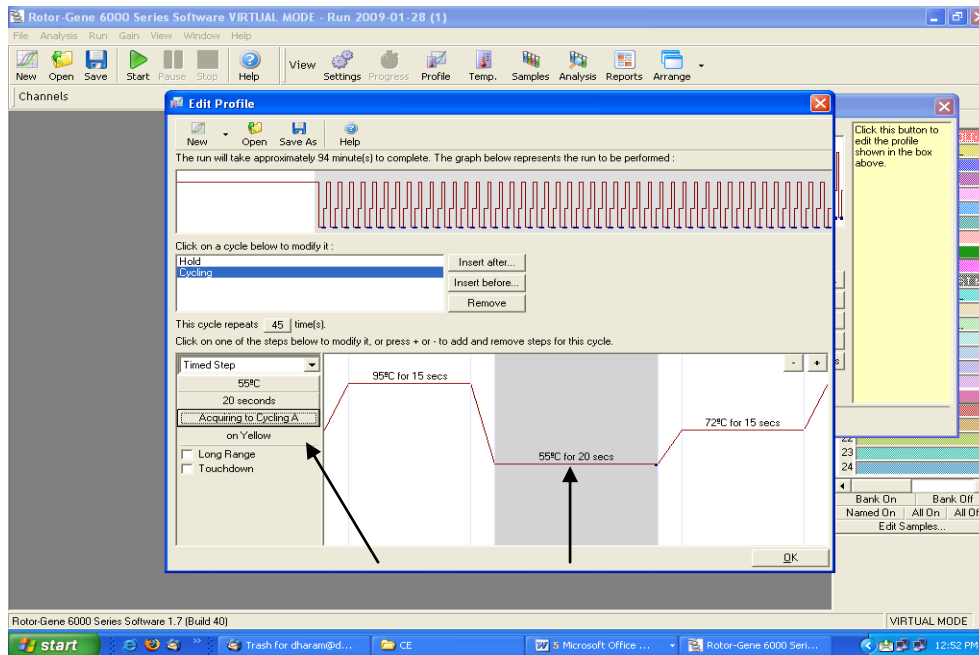


Fig. 28.

After setting the Anneling temperature and the time for anneling click on the "Acquiring to Cycling A" as shown by arrow. A New window will open as shown below.

Defining the Data acquiring channel i.e. YELLOW (JOE)

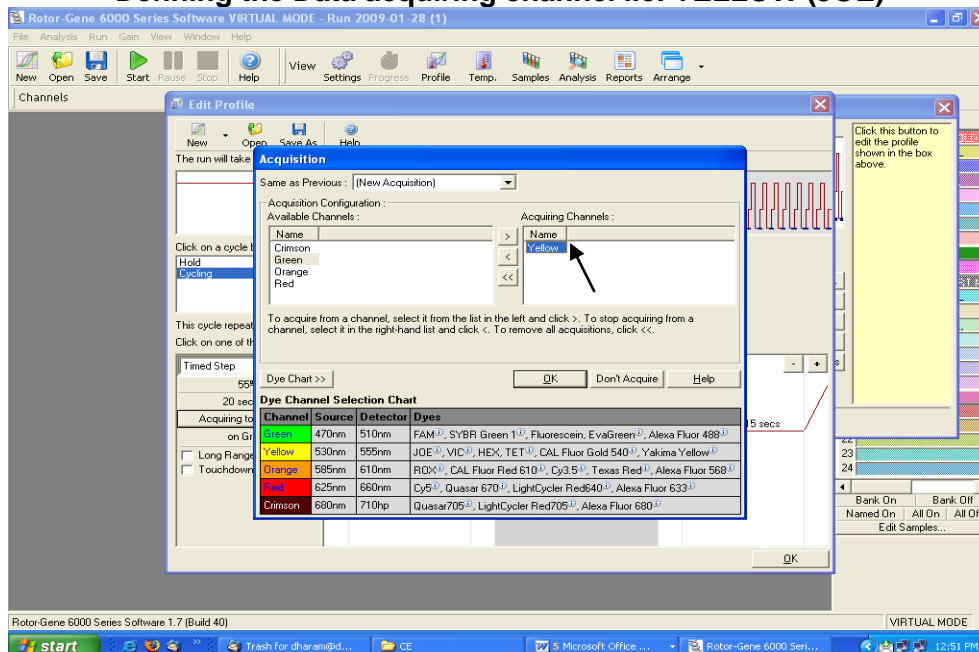


Fig. 29.

Just see there is no other channel in the right except for Yellow. In case any other Channel appears besides Yellow on the right then the same to be shifted to the left.

Confirmation of Channels as shown below.

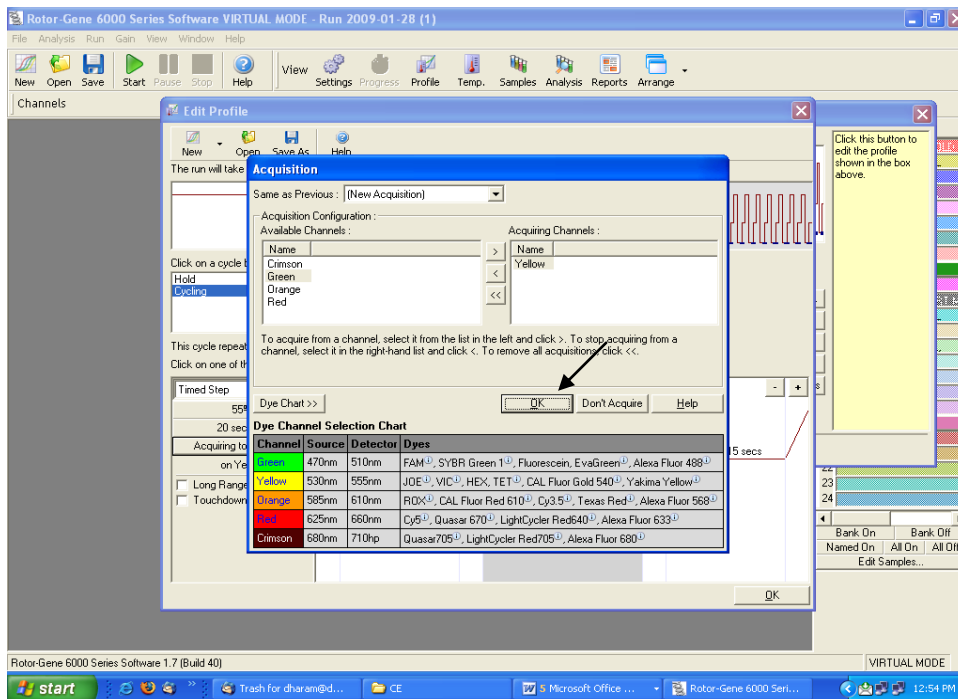


Fig. 30.

Once the Yellow Channels is on the Right side then press OK as shown by the arrow.

Setting up of Extension step in the cycling profile as depicted below i.e. 72°C for 15 Seconds

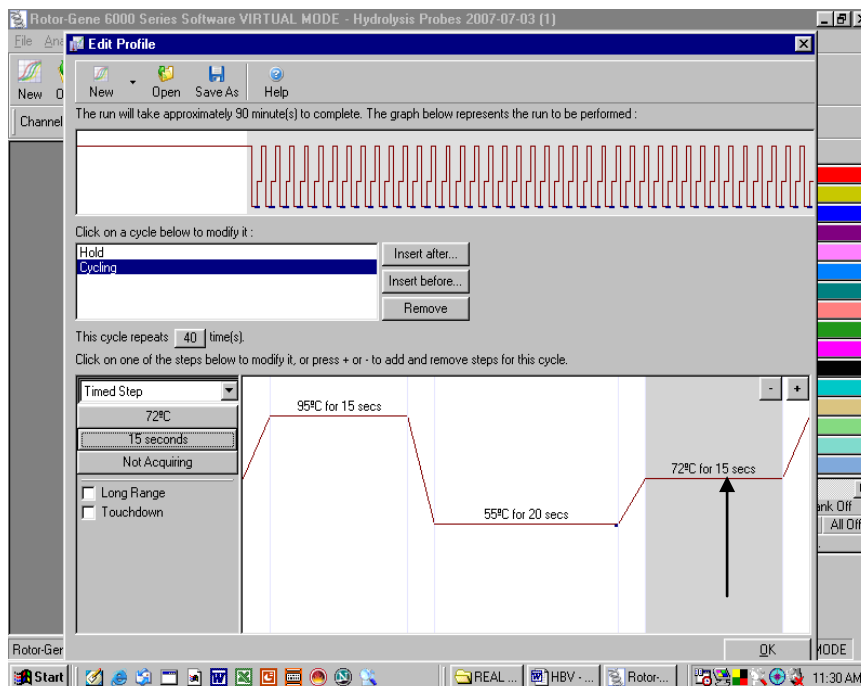


Fig. 31.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

Setting up of Number of Cycles to 45 cycles in the cycling profile as depicted below.

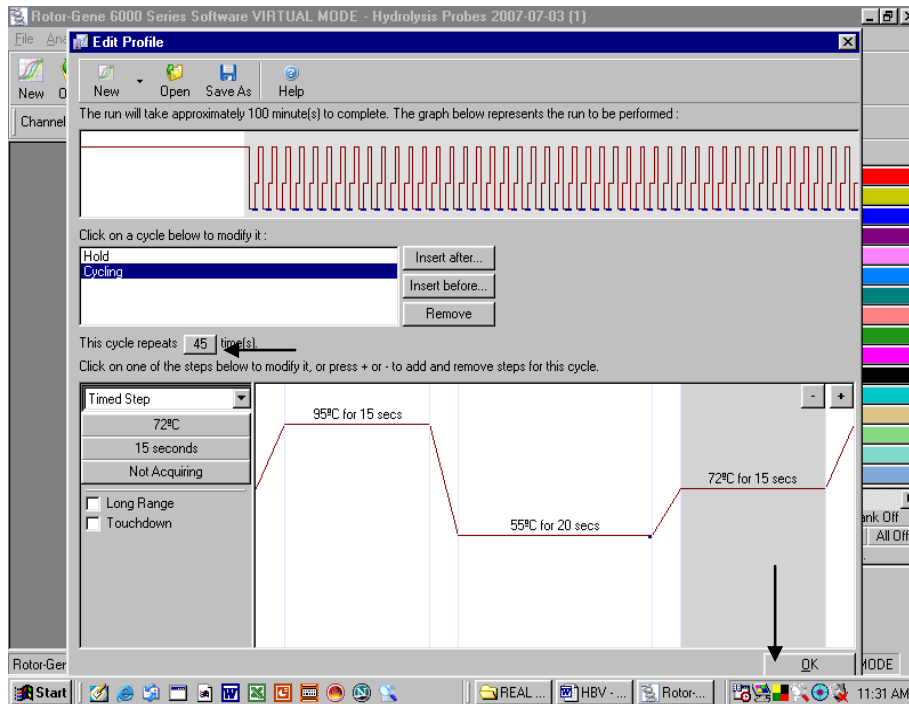


Fig. 32.

After setting the number of Cycles Press OK.

- k) Setting the gains for the acquiring channel by clicking at the Gain Optimisation button as shown below.

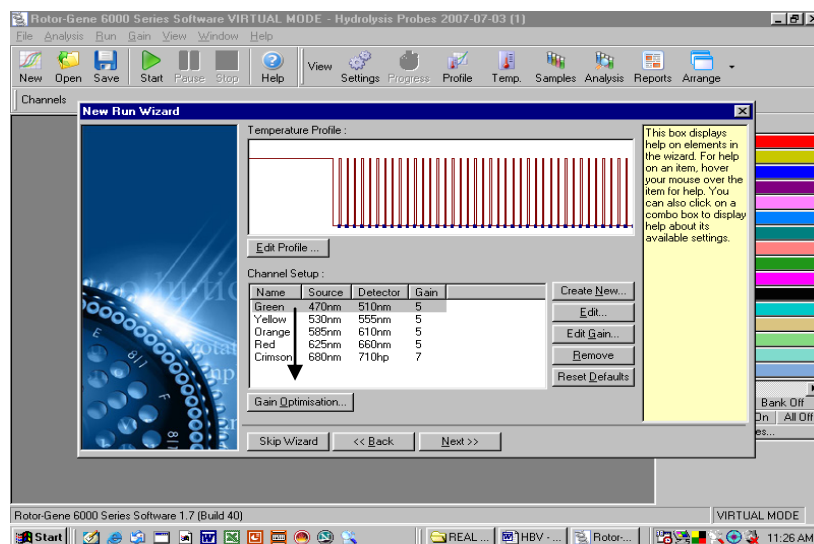


Fig. 33

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain optimization Setup* (activation in menu window *New Experiment Wizard* under Gain Optimization). Please set the temperature to the annealing temperature of the amplification program (compare Fig.34).

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

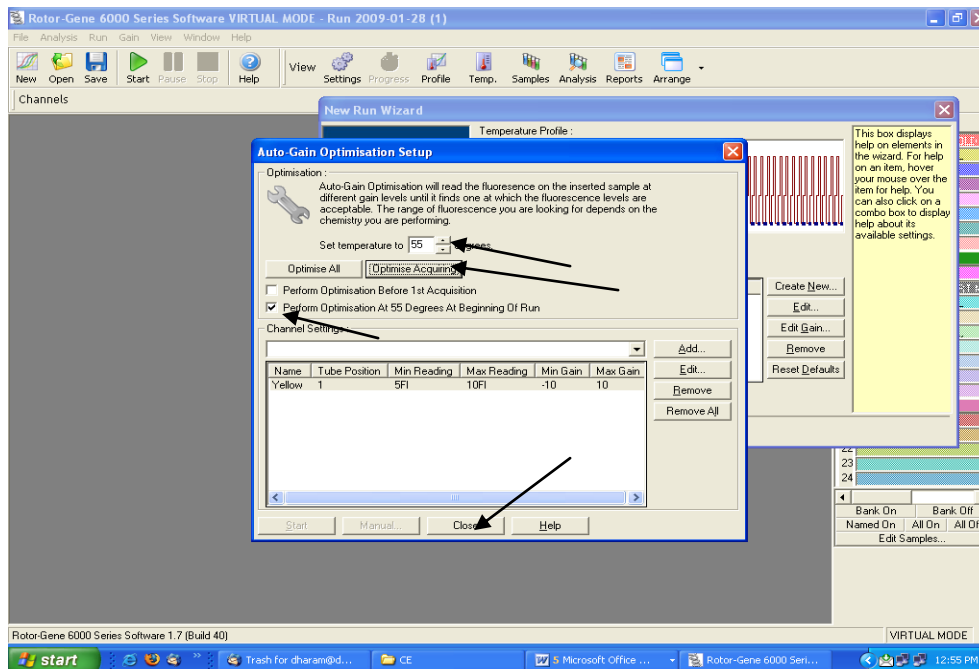


Fig. 34.
The following needs to be done.

- Click on the Optimise Acquiring
- Click on Set temperature To Adjust the Temperature to 55°C.
- Click on the Box Perform Optimisation At 55 degree At beginning of Run.
- Just see that below the channel settings there appear only one channel i.e. Yellow. In case it is different then the channels have not been set properly. Close the window and reset the channels.
- Then Press Close.

L) PRESS Start RUN

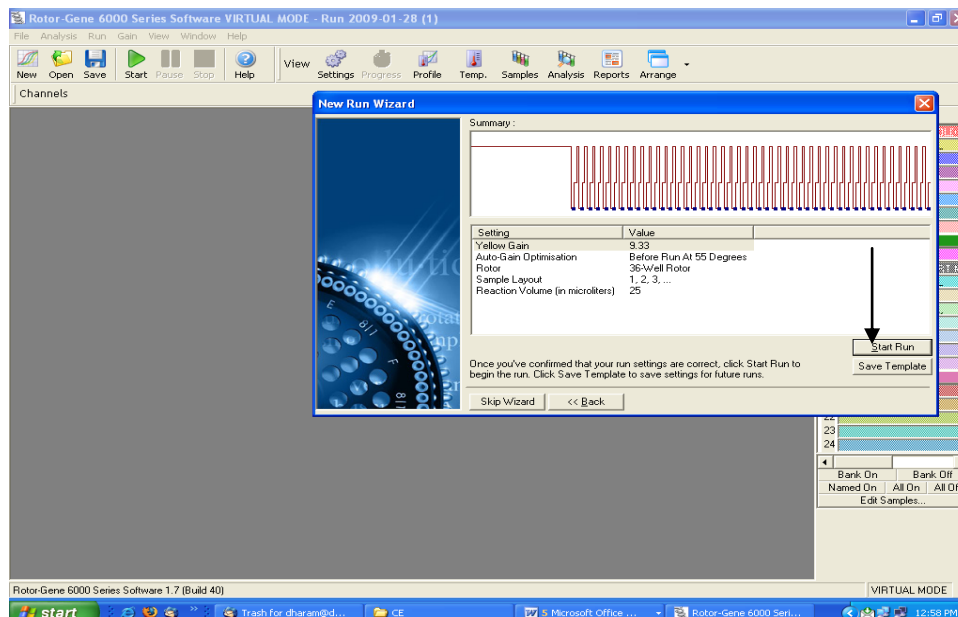


Fig. 35.

Saving the RUN File.

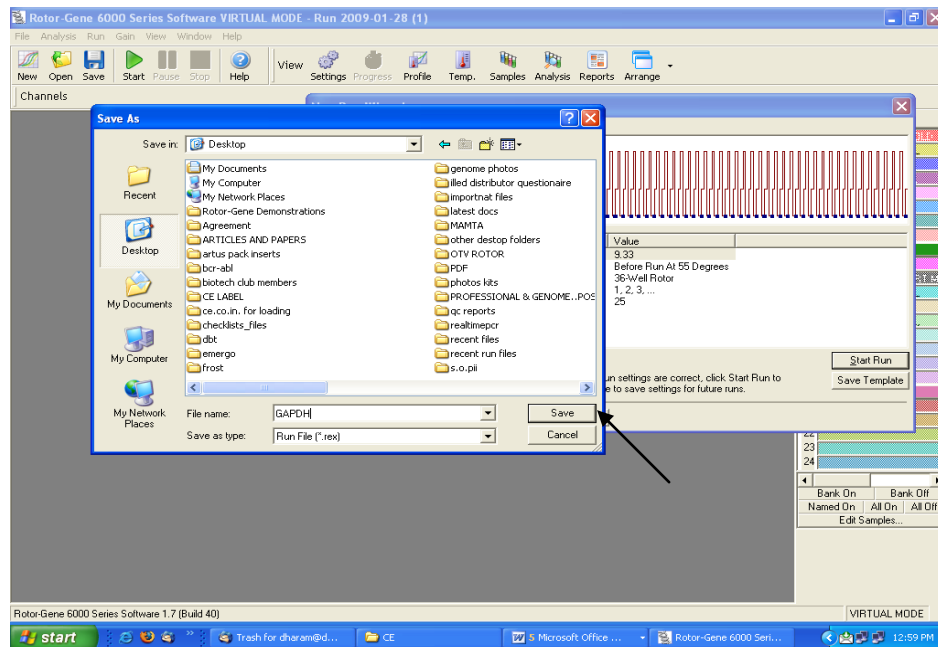


Fig. 36.

Store the run file either in my documents or a designated folder as shown above.

The moment save button is clicked after the file name, machine will start.

9. a) Generated Data Interpretation & Analysis for Rotor Gene 2000/3000

Data analysis is performed with the *Rotor Gene*[™] software according to the manufacturer's instructions (*Rotor Gene*[™] 3000 Operator's Manual).

The following results are possible:

1. A signal is detected in fluorescence channel Cycling A.JOE.

The result of the analysis is positive: The sample contains **GAPDH** DNA/cDNA

2. In fluorescence channel Cycling A.JOE no signal is detected.

No GAPDH DNA/cDNA detectable. It can be considered negative if there is amplification in the Joe Channel. Please refer for details on PCR inhibition in the section PCR inhibition.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

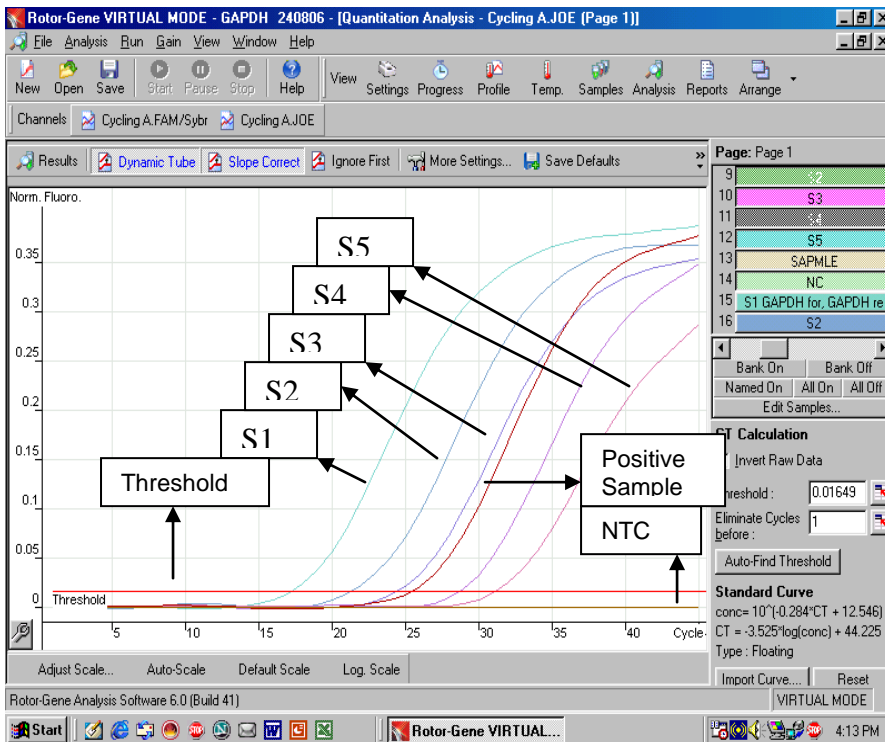


Fig. 37: Detection of the quantitation standards (*GAPDH* S 1-5) in fluorescence channel Cycling A.JOE. NTC: non-template control.

Examples of positive and negative PCR reactions are given in the above figure.

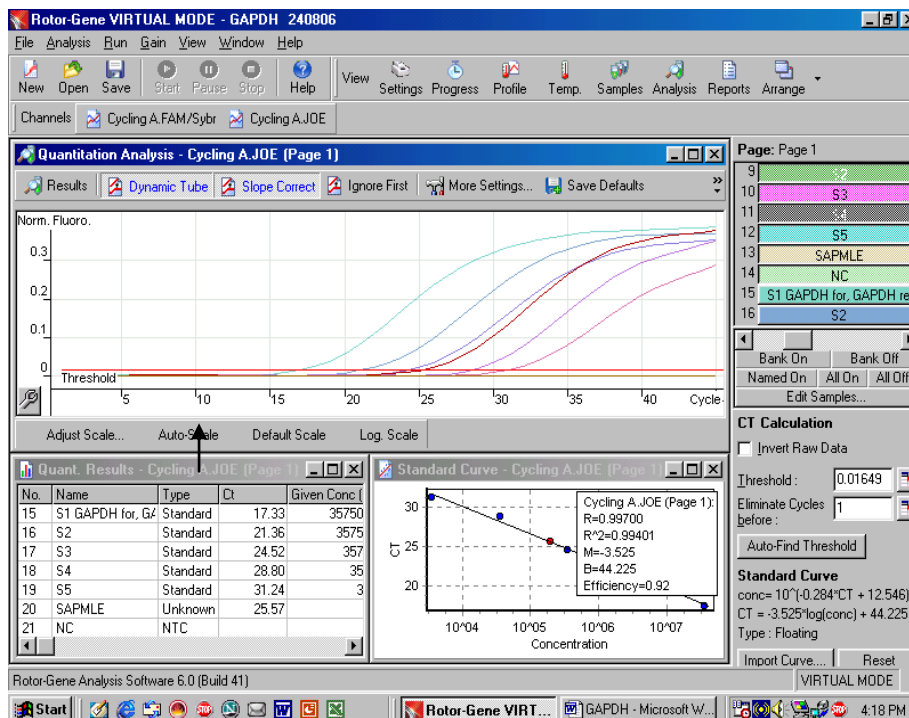


Fig. 38. Example of analysed data for *GAPDH* where all the amplification curves can be seen as well as the standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

9.b) Generated Data Interpretation & Analysis for Rotor Gene 6000

Data analysis is performed with the *Rotor Gene*™ software according to the manufacturer's instructions (*Rotor Gene*™ 6000 Operator's Manual).

The following results are possible:

3. A signal is detected in fluorescence channel Cycling A. Yellow.

The result of the analysis is positive: The sample contains **GAPDH** DNA/cDNA

4. In fluorescence channel Cycling A.Yellow no signal is detected.

No GAPDH DNA/cDNA detectable. It can be considered negative.

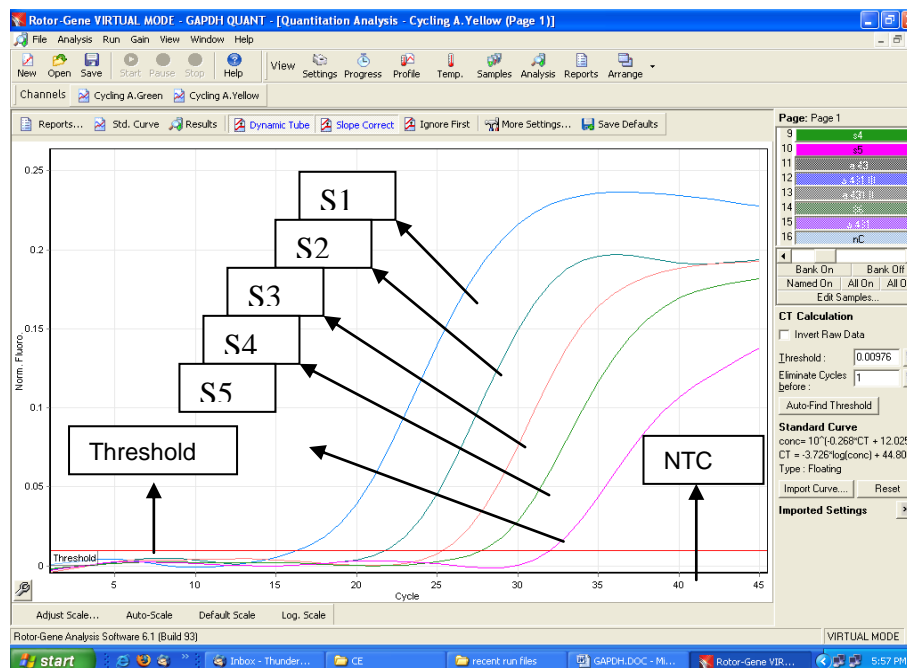


Fig. 39: Detection of the quantitation standards (**GAPDH S 1-5**) in fluorescence channel Cycling A.Yellow. NTC: non-template control.

Examples of positive and negative PCR reactions are given in the above figure.

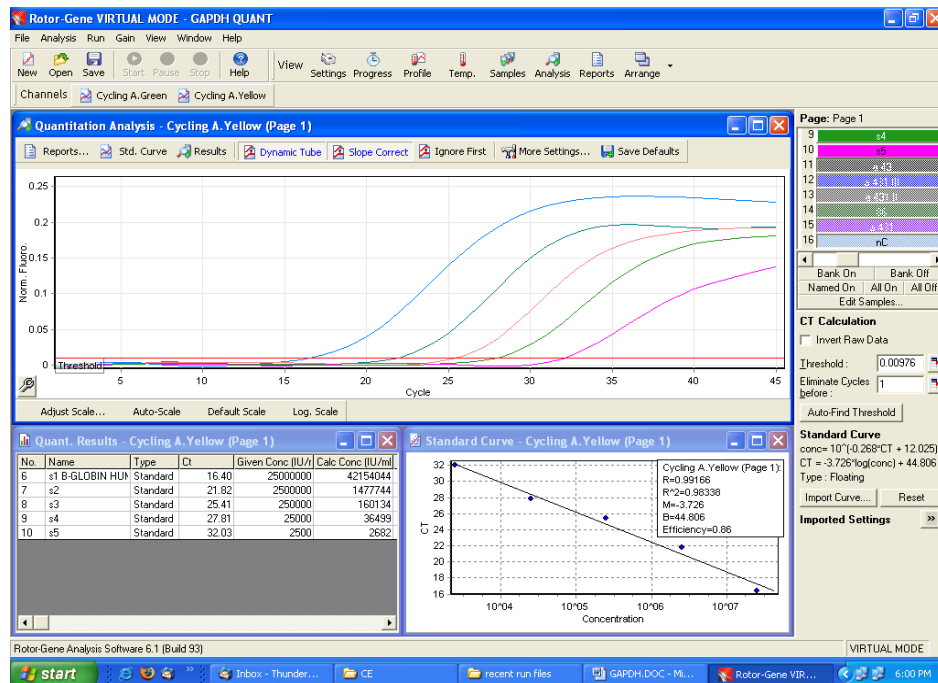


Fig. 40. Example of analysed data for *GAPDH* where all the amplification curves can be seen as well as the standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

10. a) Troubleshooting for rotor Gene 2000/3000

1. No signal with positive Standards (*GAPDH* S 1-5) in fluorescence channel Cycling A.JOE.

- Incorrect programming of the *Rotor-Gene™ 2000/3000*.
➔ Repeat the PCR with corrected settings.

2. Weak or no signal in fluorescence channel Cycling A. JOE:

- The PCR conditions do not comply with the protocol.
➔ Repeat the PCR with corrected settings.
- The *GAPDH* Super Mix R1 has been thawed and frozen too often.
- The *GAPDH* Super Mix R1 has been kept at +4°C for longer than 5 hours.
➔ Please mind the storage conditions given in the **Storage**.
➔ Repeat the assay using a new *GAPDH* super mix (R1).

- **The PCR was inhibited.**

Ample care should be taken while extraction of the samples so that PCR inhibition does not occur. All negative samples should be retested with newly extracted samples as house keeping genes are supposed to be present in general in the system.

- ➔ Make sure that you use a recommended extraction method (see **8.a. DNA extraction**) and stick closely to the manufacturer's instructions.

10. b) Troubleshooting for rotor Gene 6000

1. No signal with positive Standards (*GAPDH S 1-5*) in fluorescence channel Cycling A.Yellow.

- Incorrect programming of the *Rotor-Gene*[™] 6000.
 - ➔ Repeat the PCR with corrected settings.

2. Weak or no signal in fluorescence channel Cycling A. Yellow:

- The PCR conditions do not comply with the protocol.
 - ➔ Repeat the PCR with corrected settings.
- The **GAPDH** Super Mix R1 has been thawed and frozen too often.
- The **GAPDH** Super Mix R1 has been kept at +4°C for longer than 5 hours.
 - ➔ Please mind the storage conditions given in the **Storage**.
 - ➔ Repeat the assay using a new **GAPDH** super mix (R1).
- **The PCR was inhibited.**

Ample care should be taken while extraction of the samples so that PCR inhibition does not occur. All negative samples should be retested with newly extracted samples as house keeping genes are supposed to be present in general in the system.

 - ➔ Make sure that you use a recommended extraction method (see **8.a. DNA extraction**) and stick closely to the manufacturer's instructions.

11. Specifications

11.a Sensitivity and Reproducibility

In order to determine the sensitivity of the **Geno-Sen's GAPDH Real Time PCR Kit**, a dilution series has been set up from 10^7 down to 10^0 Copies/ μ l of **GAPDH** DNA/cDNA and analyzed with the **Geno-Sen's GAPDH Real Time PCR Kit**. The assays were carried out on three different days in the form of 8-fold determinations. The results were determined by a probit analysis. The detection limit as determined for **Geno-Sen's GAPDH Real Time PCR Kit** is consistently 70 copies/ml. This means that there is 95% probability that 70 copies/ml will be detected.

Analytical Sensitivity

Analytical Sensitivity in Conjunction with the **Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification (Cat . No. 98021)** of the **Geno Sen's® GAPDH Real Time PCR RG Kit on Rotor Gene 3000/6000** was determined by Spiking a known negative Serum to a nominal 80 copies/ml. This was subjected to extraction using the **Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification (Cat . No. 98021)** eight times with starting volume of 200 μ l & elution volume of 60 μ l.

All the Eight extractions were then analyzed with the *Geno Sen's® GAPDH Real Time PCR RG Kit* along with the standards & the NTC.

All the eight extractions were amplified correctly & the mean value obtained for the eight replicates was 79 copies/ml.

Hence Analytical Sensitivity in Conjunction with the **Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification (Cat . No. 98021)** of the **Geno Sen's® GAPDH Real Time PCR RG Kit on Rotor Gene 3000/6000** was determined to be 80 copies/ml.

11.b Specificity

In order to check the specificity of the **Geno-Sen's GAPDH Real Time PCR kit**, different DNA & RNA listed below were analyzed with **Geno-Sen's GAPDH Real Time PCR Kit**. None of these led to a positive signal with the **Geno-Sen's GAPDH Real Time PCR kit**. Gene sequence analysis of the amplified region of **GAPDH** shows a pronounced homology among the various **GAPDH** subtypes, and no homology with other DNA/RNA. Besides which utmost care has been taken in selection of the primers & probes being used in the kit.

To further Validate the stringent data In order to check the specificity of the **Geno Sen's® GAPDH Real Time PCR RG Kit**, different RNA & DNA listed below were analyzed with **Geno Sen's® GAPDH Real Time PCR RG Kit**. None of these led to a positive signal with the **Geno Sen's GAPDH Real Time PCR RG Kit**.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

β -Actin	Hepatitis B Virus	N. Meningitis
Human Herpes Virus 1 & 2	Hepatitis C Virus	S. Pneumonia
Epstein barr Virus	Hepatitis E Virus	JEV
Cytomagalovirus	HIV-1	Mycobacterium tuberculosis
Chlamydia pneumonia	HIV-2	Hepatitis A
Parvovirus B 19	West Nile Virus	Staphylococcus aureus
Dengue Virus 1-4	H. Influenza	ChikunGunya Virus
Leprosy	Malaria	Scrub typhus
B.pseudomallie	Filaria	Leptospira interrogans.
β -Globin	Abl gene	TTV

In order to validate the Specificity further with wet lab testing the following Known clinical samples were analyzed using the **Geno Sen's® GAPDH** Real Time PCR RG kit on RotorGene 3000/6000. The extraction was carried out with the **Geno Sen's® Genomic DNA Extraction Mini Kit — for DNA purification (Cat. No. 98021)**

The run was carried out with the known set of standards in order to quantiate the **GAPDH DNA** Gene.

Sample Type	Whole blood
High +ve's	
Medium +ve's	5
Low +ve's	6
Extremely low +ve's	4
Negative samples.	6
	21

All the above samples were correctly identified by the **Geno Sen's® GAPDH** Real Time PCR RG kit & all the 4 extremely low samples were accurately detected by the **Geno Sen's® GAPDH** Real Time PCR RG kit & exhibited copies around 80 copies /ml or less than 80 copies/ml.

Further studies are underway on this aspect.

12. Warranty::

Products are guaranteed to confirm to the quality and content indicated on each vial and external labels during their shelf life. Genome Diagnostics Pvt. Ltd. obligation and purchaser's rights under the warranty are limited to the replacement by Genome Diagnostics Pvt. Ltd of any product that is shown defective in fabrication, and that must be returned to Genome Diagnostics Pvt. Ltd Freight prepaid, or at Genome Diagnostics Pvt. Ltd. option, replacement of the purchasing price. Any complaint on damaged goods during transport must be directed to the handling or transport agent.

In Vitro Diagnostic Medical device, as per the Directive 98/79/EC specifications. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the

product. No other warranties, of any kind, express or implied, including without limitation, implicit warranties of commercialization ability or adequacy for a given purpose, are provided by Genome Diagnostics Pvt. Ltd. Genome Diagnostics Pvt. Ltd will not be responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

13. Limitations of product use:

- a.) All reagents may exclusively be used for *in vitro* diagnostics.
- b.) The product is to be used by personnel specially instructed and trained in for the *in-vitro* diagnostics procedures only.
- c.) It is important to pipet the indicated quantities, and mix well after each reagent addition. Check pipettes regularly.
- d.) Instructions must be followed Correctly in order to obtain correct results. If the user has any questions, please contact our Technical Dept. (dharam@vsnl.com or at pbpl@vsnl.net).
- e.) This test has been validated for use with the reagents provided in the kit. The use of other Reagents or methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for any modifications done in any of the indicated parameters. Compliance with the kit protocol is required.
- f.) Detection of RNA/DNA depends on the number of copies present in the sample, and can be affected by sample collection methods, patient-related factors (e.g. age, symptoms), or for infection stage and sample size.
- g.) False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives. This is achieved by the inhibition Control included in the kit.
- h.) Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.
- i.) Attention should be paid to expiration dates printed on the kit box and labels of all components. Do not use expired components.

If you have any further questions or problems, please contact our technical support at dharam@vsnl.com OR pbpl@vsnl.net.

14. Publication and reference;

1) Detection of Helicobacter pylori in Gastro duodenal Diseases by Real Time PCR

by Ashok kumar, Mohd Imran, Atul kumar Singh, Dipika Chandel and Amitabh Talwar
Dept Of Biotechnology
HNBG University (A central University)
Srinagar Garhwal
Uttarakhand India

15. LIST OF GENO-SEN'S RANGE OF REAL TIME PCR KITS

S.NO.	PRODUCT
1	HIV-1 RG quantitative Real time PCR kit.
2	HBV RG quantitative Real time PCR kit.
3	HCV RG quantitative Real time PCR kit.
4	HCV Genotyping 1/2/3/4 RG qualitative Real time PCR kit.
5	HEV RG quantitative Real time PCR kit.
6	HAV RG quantitative Real time PCR kit.
7	JEV RG quantitative Real time PCR kit.
8	ENTEROVIRUS RG quantitative Real time PCR kit.
9	DENGUE RG quantitative Real time PCR KIT
10	HSV 1 & 2 RG quantitative Real time PCR kit.
11	CMV RG quantitative Real time PCR kit.
12	Hanta Virus RG quantitative Real time PCR kit.
13	Measles Virus RG quantitative Real time PCR kit.
14	West Nile Virus RG quantitative Real time PCR kit.
15	H5 N1 (Bird Flu) RG quantitative Real time PCR kit.
16	Chikungunya RG quantitative Real time PCR kit.
17	TTV RG quantitative Real time PCR kit.
18	SARS RG quantitative Real time PCR kit.
19	JC/BK Virus RG quantitative Real time PCR kit.
20	MTb Complex RG quantitative Real time PCR kit.
21	MTb Complex /MOTT RG quantitative Real time PCR kit.

Geno-Sen's GAPDH Real Time PCR Kit for Rotor Gene 2000/3000/6000

22	Chlamydia pneumonia RG quantitative Real time PCR kit.
23	Streptococcos pneumonia RG quantitative Real time PCR kit.
24	N. Meningitis RG quantitative Real time PCR kit.
25	H. Influenza RG quantitative Real time PCR kit.
26	Leprosy RG quantitative Real time PCR kit.
27	Helicobacter Pylori RG quantitative Real time PCR kit.
28	Scrub Typhus RG quantitative Real time PCR kit.
29	B. Pseudomali RG quantitative Real time PCR kit.
30	Filaria RG quantitative Real time PCR kit.
31	Leptospira(pathogenic) RG quantitative Real time PCR kit.
32	CCL3-L1 RG quantitative Real time PCR kit.
33	Malaria (P. Vivax) RG quantitative Real time PCR kit.
34	Bcr/abl Major RG quantitative Real time PCR kit.
35	Bcr/abl Minor RG quantitative Real time PCR kit.
36	PML/RARA RG quantitative Real time PCR kit.
37	RARA/PML RG quantitative Real time PCR kit.
38	GAPDH RG quantitative Real time PCR kit.
39	β-Actin RG quantitative Real time PCR kit.
40	β-Globin RG quantitative Real time PCR kit.
41	Abl gene RG quantitative Real time PCR kit.
42	Rabies RG quantitative Real time PCR kit.
43	Factor V Leiden detection RG Real time PCR kit.



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Version : 005

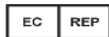
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