

Geno-Sen's
Major-Bcr/abl (CML) (Rotor Gene)
Real Time PCR Kit

Quantitative

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



PACK INSERT

Revised June 2016



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

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<table border="1"><tr><td>EC</td><td>REP</td></tr></table>	EC	REP	EMERGO EUROPE Prinsessegracht 20,2514 AP, The Hague The Netherlands	
EC	REP			

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

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

Bcr/abl Major 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 91116001 100 rxns	REF 91116002 50 rxns	REF 91116003 25 rxns
R1 Blue	Bcr-M Super Mix RG.	25 rxns x 4 Vials	25 rxns x 2 Vials	25 rxns x 1 Vials
R2 Yellow	Mg Sol Bcr/abl	1 vials	1 vial	1 vial
R3 Blue	abl Super Mix RG.	25 rxns x 4 Vials	25 rxns x 2 Vials	25 rxns x 1 Vials
Bcr-S1 Red	<i>Bcr-M Standard 1</i> <i>1 X 10⁶ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Bcr-S2 Red	<i>Bcr-M Standard 2</i> <i>1 X 10⁵ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Bcr-S3 Red	<i>Bcr-M Standard 3</i> <i>1 X 10⁴ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Bcr-S4 Red	<i>Bcr-M Standard 4</i> <i>1 X 10³ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Bcr-S5 Red	<i>Bcr-M Standard 5</i> <i>1 X 10² copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Abl-S1 Red	<i>abl Standard 1</i> <i>1 X 10⁶copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Abl-S2 Red	<i>abl Standard 2</i> <i>1 X 10⁵ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Abl-S3 Red	<i>abl Standard 3</i> <i>1 X 10⁴ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Abl-S4 Red	<i>abl Standard 4</i> <i>1 X 10³ copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
Abl-S5 Red	<i>abl Standard 5</i> <i>1 X 10² copies/μl</i>	1 Vial of 200μl	1 Vial of 200μl	1 Vial of 200μl
W White	Molecular Grade Water.	1 Vial 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.

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3. Bcr/abl Information

Chronic myelogenous leukemia (CML) is characterized in 90% to 95% of cases by the Philadelphia (Ph) translocation, t (9;22) (q34;q11). The juxtaposition of 3' sequences of the *abl* gene from chromosome 9 and 5' sequences of the *bcr* gene on chromosome 22 results in a chimerical *bcr-abl* gene, which plays a crucial role in the pathogenesis of the disease. The vast majority of CML patients have breakpoints that result in a fusion mRNA in which either *bcr* exon b2 or b3 is fused to *abl* exon a2 (b2a2 or b3a2, [M-*bcr*]). Both transcripts give rise to a 210kD *bcr-abl* protein. Fusion transcripts containing the minor (m-*bcr*) breakpoint cluster region (e1a2) which are characteristic for both CML and acute lymphoblastic leukemia (ALL) may be measured with a distinct GenoSen Kit.

The kit is optimally suited for quantification of residual amounts of leukemia-specific Ph⁺ cells and for the follow up of CML patients undergoing allogenic stem cell transplantation with reduced conditioning or MUD. The increase or decreases of *bcr-abl* transcripts normalized to *abl* transcripts may be an additional tool for early treatment decisions as donor lymphocyte infusions or immunosuppression withdrawal.

The GenoSen M-*bcr* cDNA Quantification assay is developed for laboratory scale or high-throughput quantitative transcript analysis by real time quantitative fluorescence PCR.

GenoSen's standardized ready-to-use Control and Reaction mix allow fast processing of the samples.

Module specificity

In vitro quantification of *bcr-abl* fusion transcripts harboring the Ph⁺ translocation major breakpoint cluster (M-*bcr*) region b2a2 or b3a2 in whole RNA/mRNA samples which are reverse transcribed into cDNA using random hexanucleotides, optimized for transcripts prepared from crude or purified human white blood cells (WBC).

Samples which can be used for Extraction: Whole Blood or cultured cells.

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.

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- 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

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

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 BCR/ABL Major PCR** Reagents constitute a ready to use system for detection and quantification of BCR/ABL using Polymerase chain reaction (PCR) in the Rotor Gene 2000/3000/6000 (Corbett Research). *The BCR Master* mix


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contains reagents and enzymes for the specific amplification of Bcr translocation, and for the direct detection of the specific amplicon in fluorescence channels Cycling A.FAM/GREEN of the *Rotor Gene 2000/3000/6000* & the *Reference gene on Cycling A.FAM/GREEN* In a separate reaction to determine ratio of BCR Vs ABL gene. External positive Standards (BCR S 1-5) & (ABL S 1-5) are supplied which allow the determination of the gene load. For further information, please refer to section 8.3 Quantitation.

8. Procedure

8.a RNA Extraction

RNA Extraction kits are available from various manufacturers. Sample volumes for the RNA Extraction procedure depend on the protocol used. Please carry out the RNA Extraction according to the manufacturer's instructions. The recommended Extraction kits are the following:

Sample Material	Nucleic Acid Isolation Kit	REF Cat. Num.	
Whole Blood or cultured cells.	QIAamp RNA Blood Mini extraction Kit (50)	52304	QIAGEN
	GenoSen's whole blood RNA Extraction kit (50)	98007	Genome Diagnostics Pvt.Ltd.

However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher RNA 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 *Bcr/abl Rotor Gene PCR Reagents* should not be used with phenol based isolation methods.

8.b Reference gene:

Reference Gene i.e. **abl** allows the user to determine the Bcr/abl ratio & simultaneously control possible PCR inhibition. The Reference gene control reagents

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are provided separately and have to be run separately on FAM channel itself. There is no need to add separate internal control in the reaction mix.

8.c Quantitation

The quantitation standards provided in the kit (*Bcr S 1-5*) & (*abl S 1-5*) are treated in the same way as extracted samples and the same volume is used i.e. (2µl) 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 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:

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

Or else direct conversion can be done keeping in mind the starting volume of the sample & the final eluted Volume e.g.

If the starting volume of the sample while using the Qiagen QIAamp RNA Blood Mini extraction kit is 1.5 ml & the final Eluted Volume is 40µl then to obtain the direct values i.e. copies/ml for the patient samples the following values for the standards can be fed directly into the operating software which is again based on the above formula and will yield direct conversion of per ml of patient sample data.

S1: 10 ⁶ Copies/µl =	26660000 Copies/ml
S2: 10 ⁵ Copies/µl =	2666000 Copies/ml
S3: 10 ⁴ Copies/µl =	266600 Copies/ml
S4: 10 ³ Copies/µl =	26660 Copies/ml
S5: 10 ² Copies/µl =	2666 Copies/ml

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8.d Preparation for PCR

8. e. 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 X 2 (in duplicate). 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 (*BCR S 1-5*) & (*abl 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

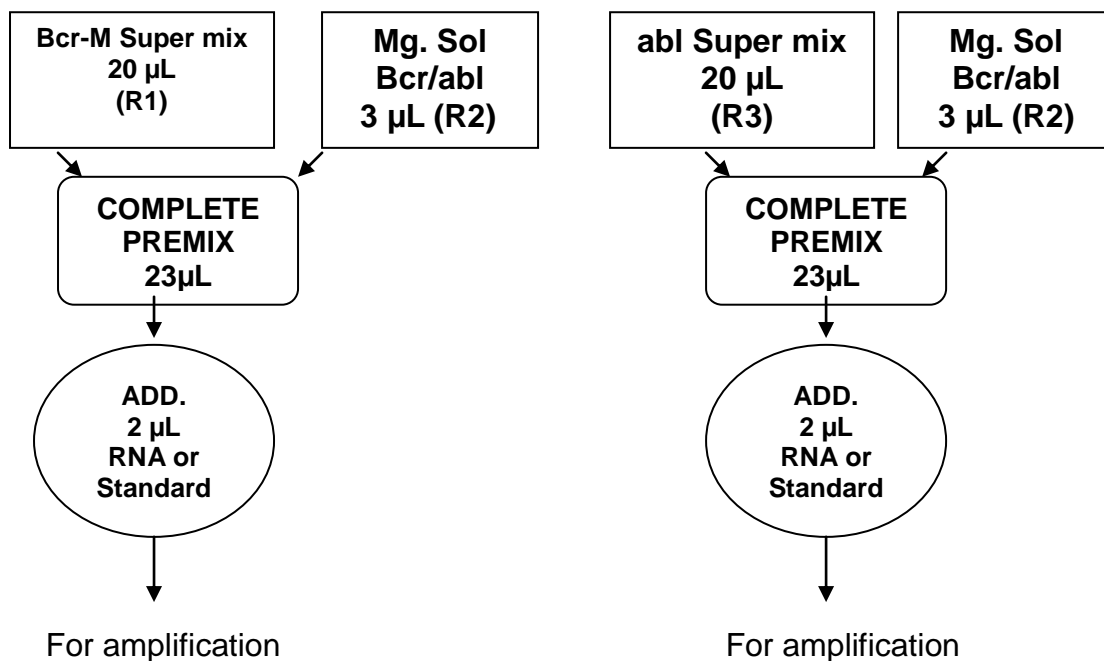


Fig. 4. Depending upon the number of samples the following pipetting scheme can be followed. e.g. for 10 rxns.

For Bcr

Bcr	1 rxns.	10 rxns.
Bcr Super Mix (R1)	20 µL	200 µL
Bcr Mg Sol. (R2)	3 µL	30 µL
Total	23µL	230µL

For abl

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abl	1 rxns.	10 rxns.
abl Super Mix (R3)	20 µL	200 µL
abl Mg Sol. (R2)	3 µL	30 µL
Total	23µL	230µL

Fig. 5.

Pipette 23 µl of the Master Mix into each PCR tube labelled separately for Bcr & *abl* respectively. Then add 2 µl of the Extracted RNA to each sample tube in both sets and mix well by pipeting up and down. Correspondingly, 2 µl of the Standards (Bcr S1-5) & (abl S 1-5) must be used as a positive control and 2 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes and transfer the Bcr & *abl* tubes into the rotor of the *RotorGene*TM instrument in case the number of samples are less whereas if samples are more then transfer the tubes that of *abl* into the deep freezer at –20°C so that the same can be run later. The *RotorGene*TM software versions 5.0.53 and higher require a Locking Ring (accessory of the *RotorGene*TM, Corbett Research) to be placed on top of the rotor to prevent accidental opening of the tubes during the run.

8.f. Programming the *RotorGene*TM 2000/3000

The *RotorGene*TM 2000/3000 PCR program for the detection of Bcr/*abl* can be divided into following steps:

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

Program the *RotorGene*TM 2000/3000 for these 5 steps according to the parameters shown in Fig. 6-20. All specifications refer to the *RotorGene*TM software version 6.0.33. Please find further information on programming the *RotorGene*TM in the *RotorGene*TM 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.

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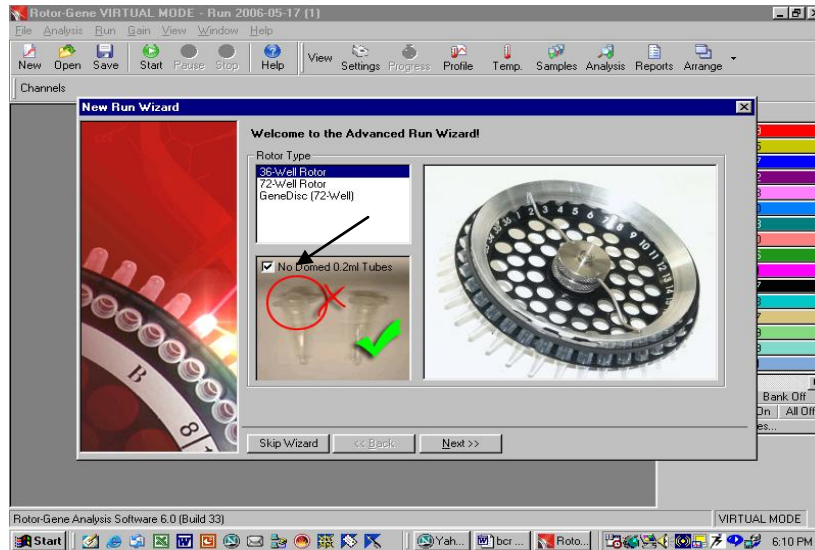


Fig. 6.

Confirmation of Reaction Volume as follows.

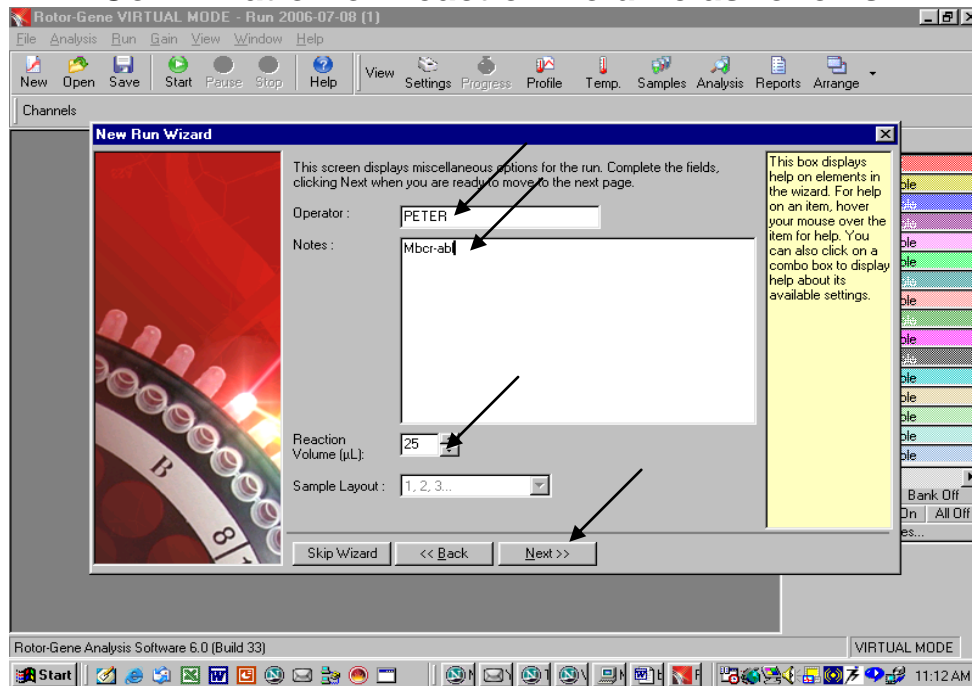


Fig. 7.

Setting of general assay parameters.

- Please click on the volume buttons to make sure that 25µl 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.

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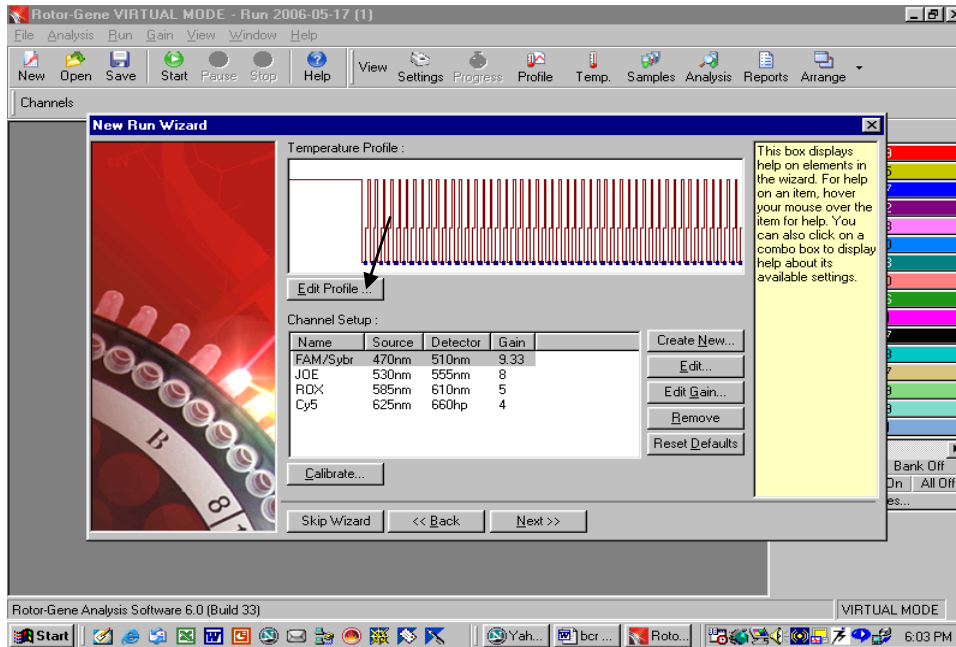


Fig. 8.

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: First hold 50°C for 15 minutes i.e. cDNA synthesis step as below.

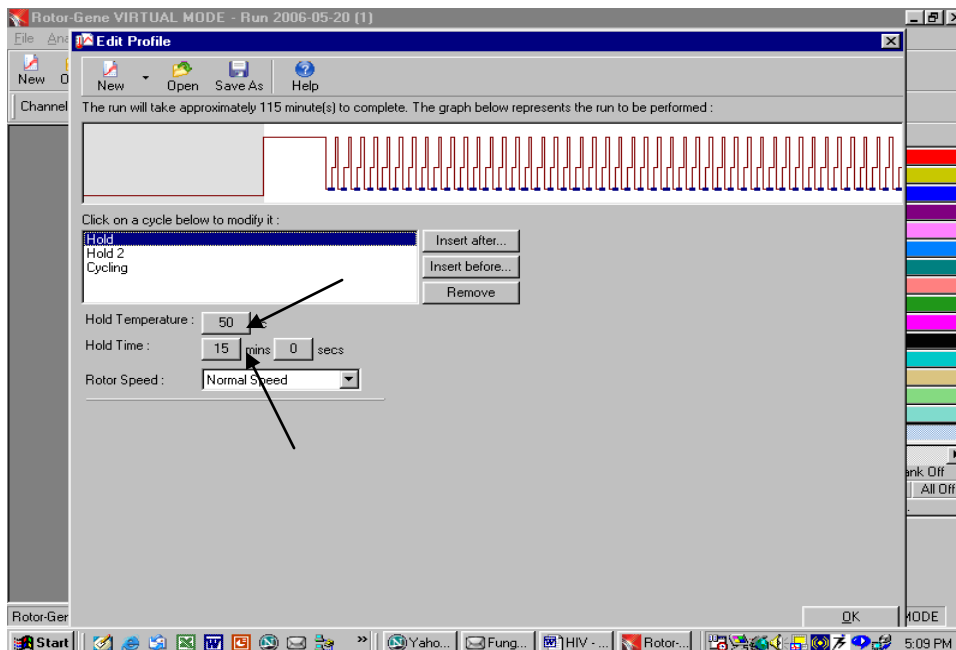


Fig. 9.

CYCLING PROFILE: Second hold 95°C for 10 minutes as below

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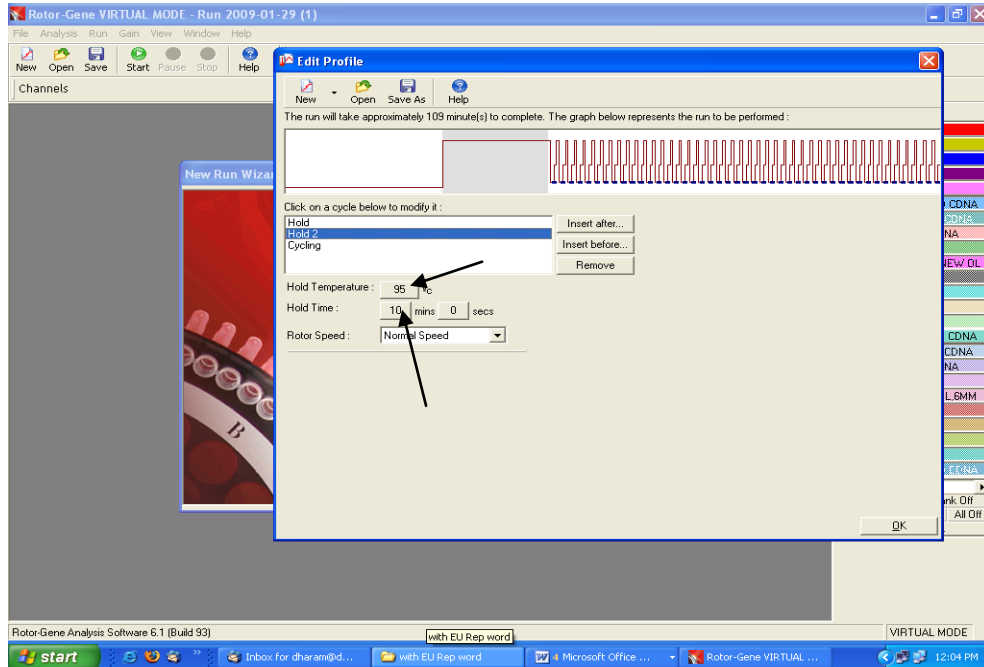


Fig. 10. Initial activation of the Hot Start enzyme.

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

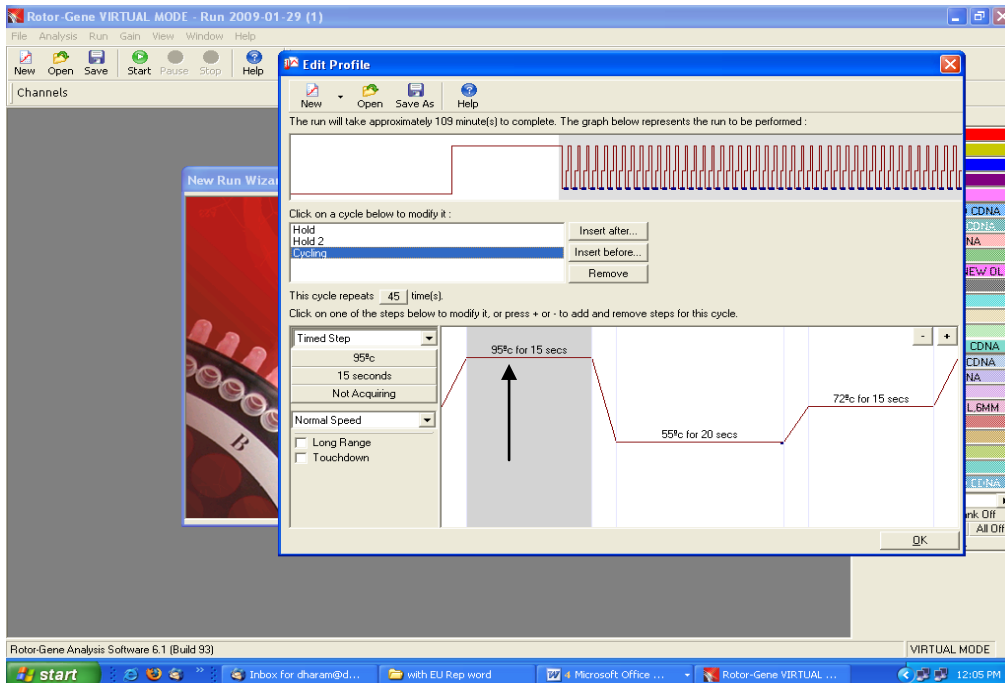


Fig. 11.

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

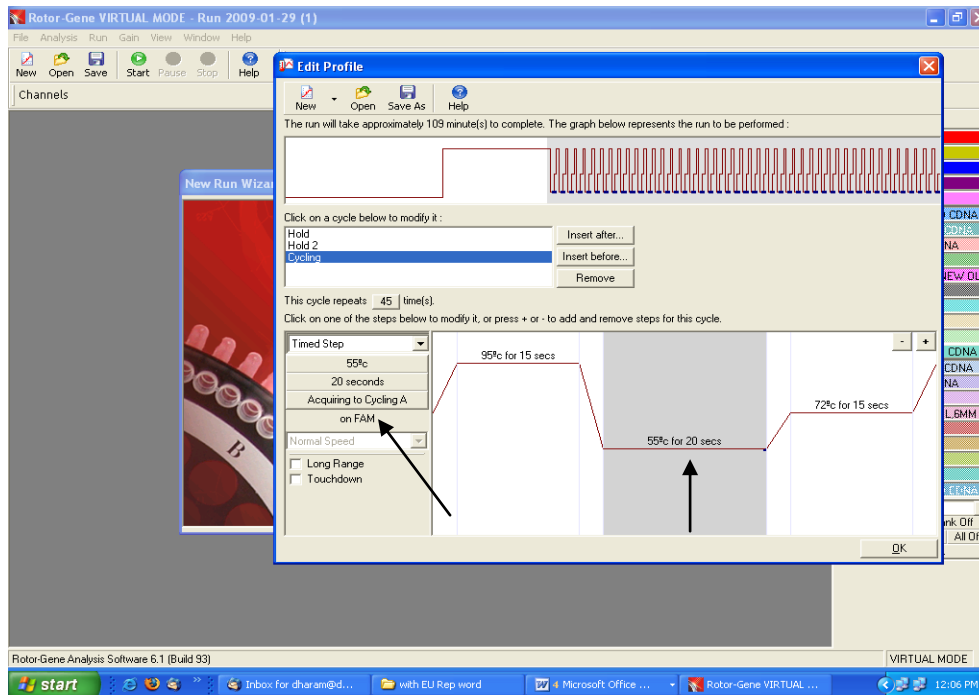


Fig. 12.

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

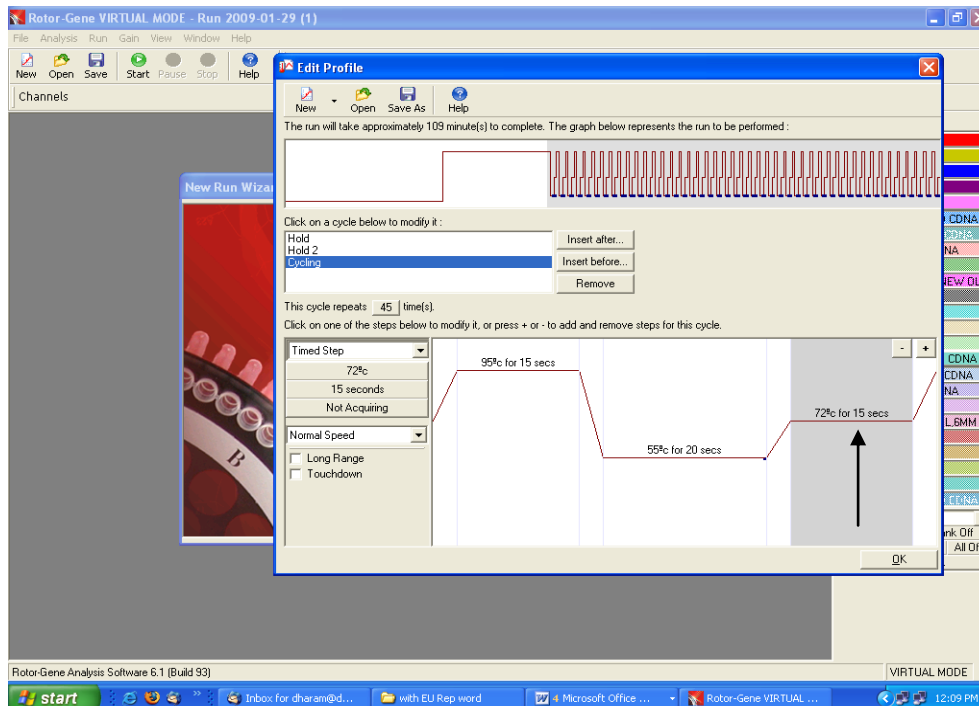


Fig. 13.

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

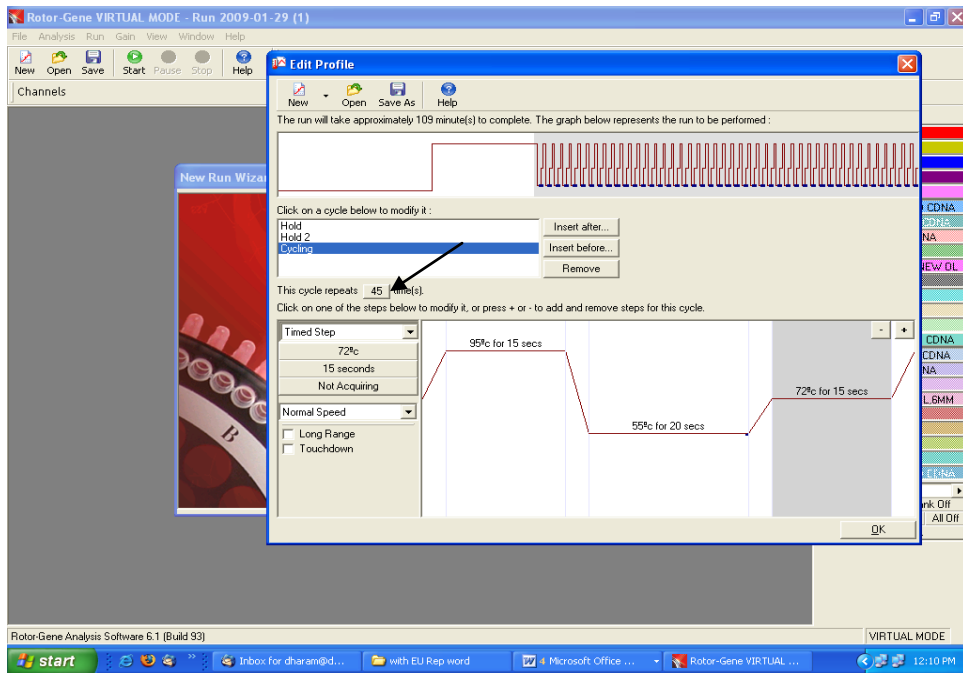


Fig. 14.

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

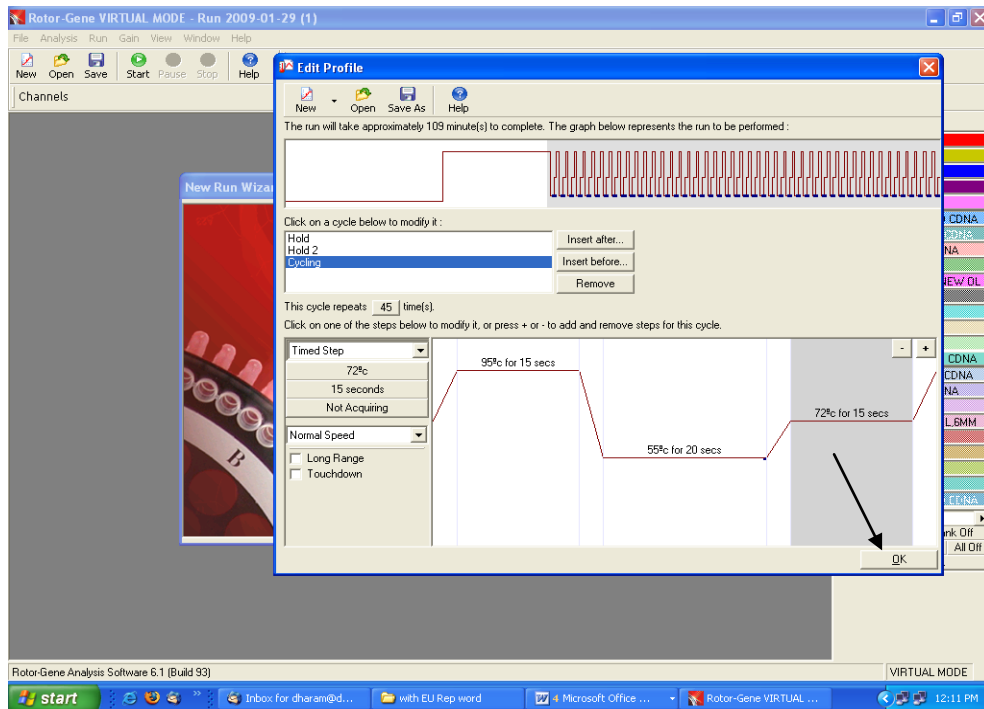


Fig. 15.

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

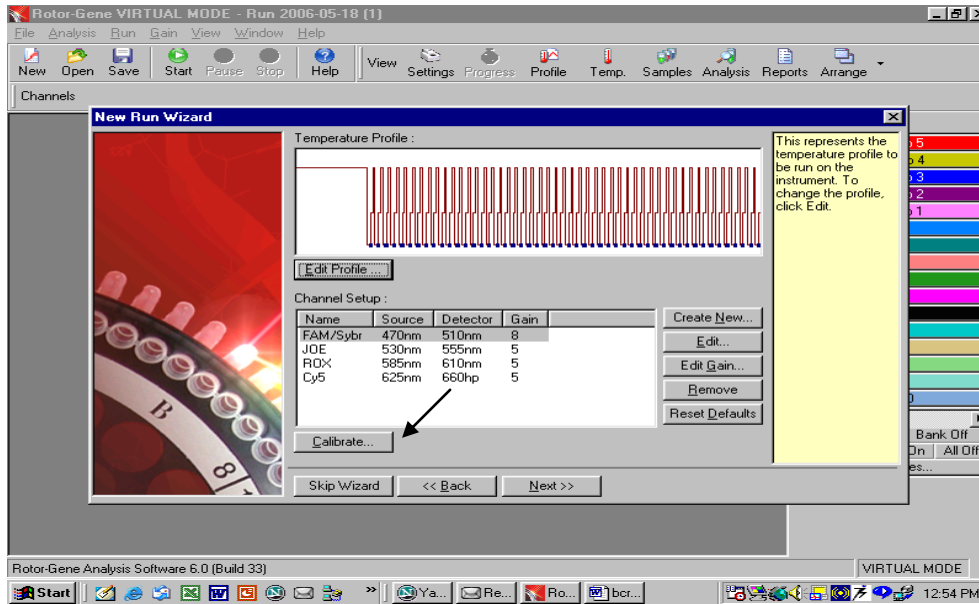


Fig. 16.

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. 17).

Adjustment of the fluorescence channel sensitivity as shown below.

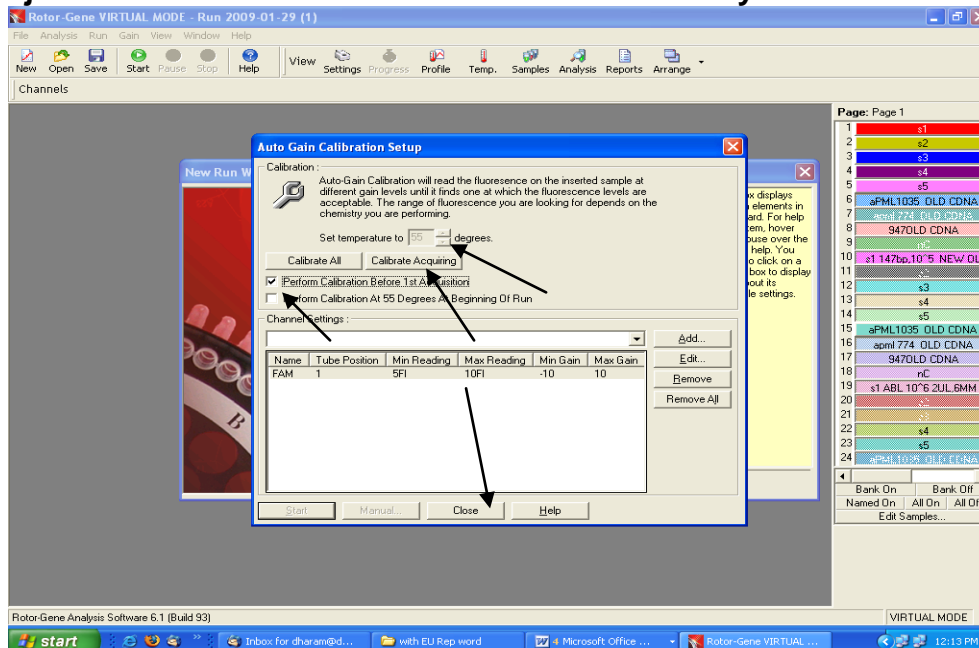


Fig. 17.

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

Please do not forget to click on the box against "Perform calibration before 1st, acquisition." 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 (Fig. 8).

PRESS NEXT

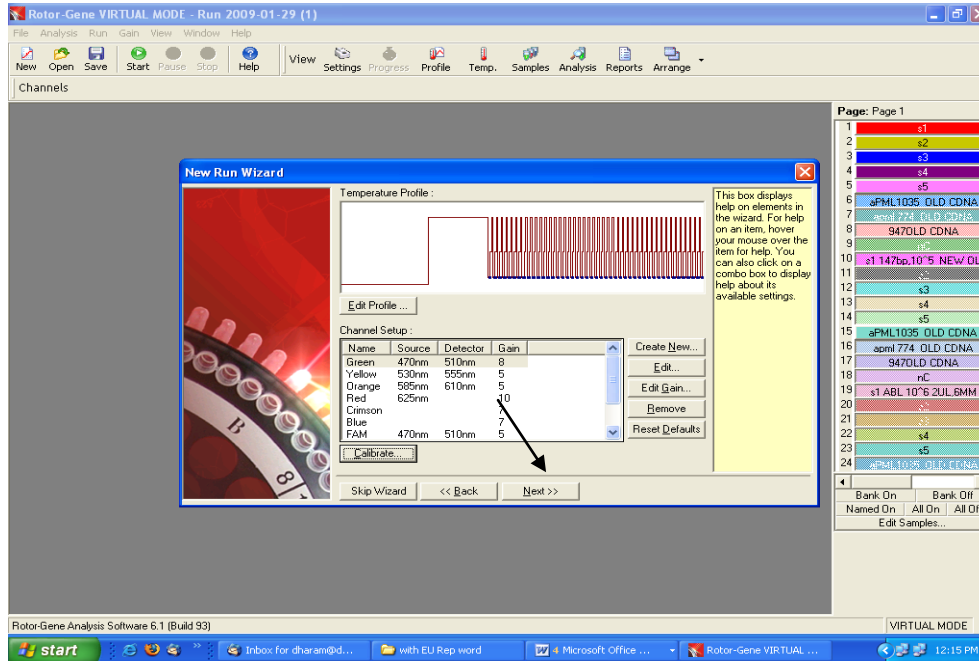


Fig. 18.

Starting of the *Rotor Gene*TM run. **FIG: AMPLIFICATION OF THE cDNA**

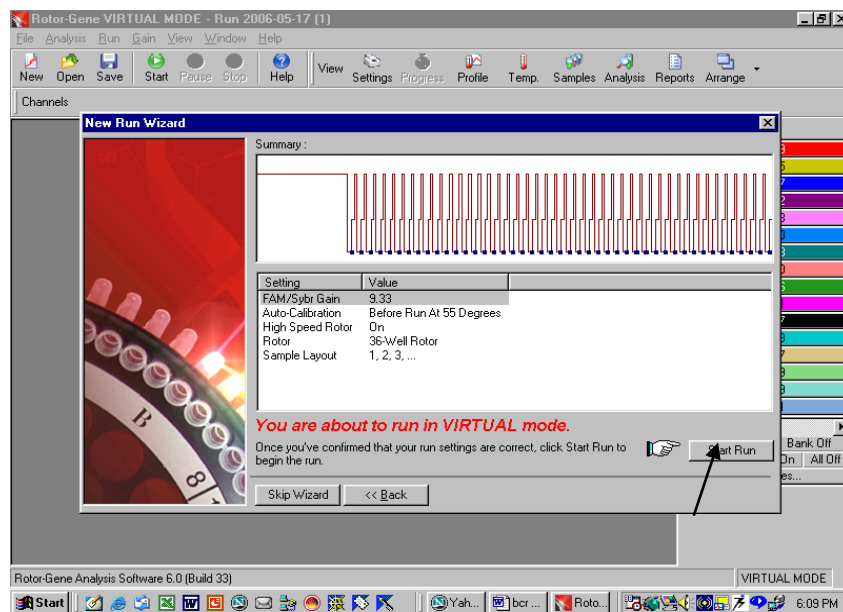


Fig. 19: Press Start Run Button.

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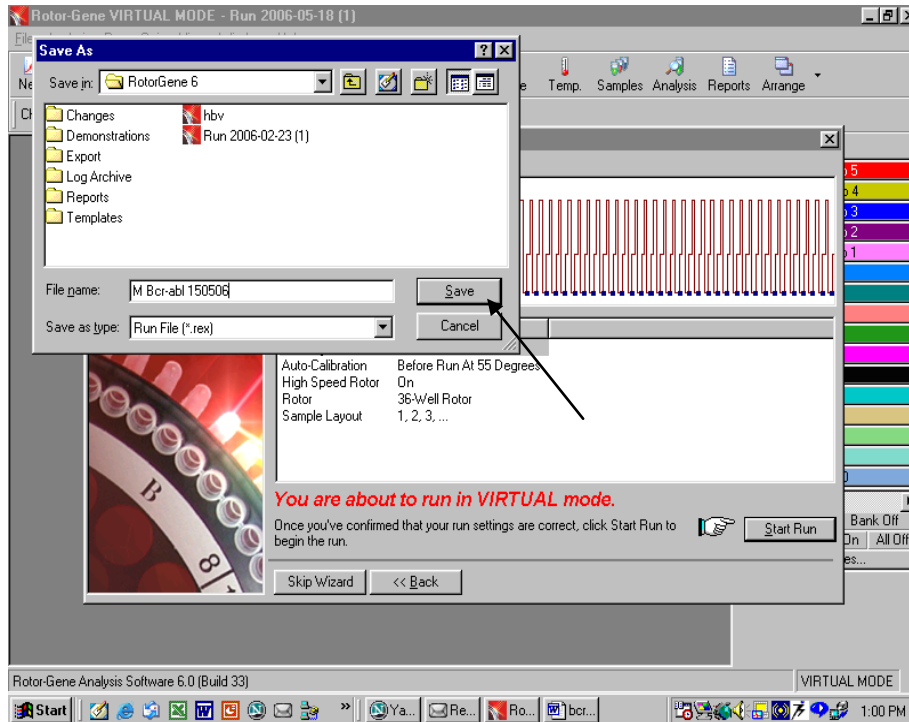


Fig. 20.

SAVING THE RUN FILE AS ABOVE

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.

8.g. Programming the Rotor Gene™ 6000

The RotorGene™ 6000 PCR program for the detection of BCR/abl-Major can be divided into following steps:

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

Program the RotorGene™ 6000 for these 5 steps according to the parameters shown in Figures 21-38 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

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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.

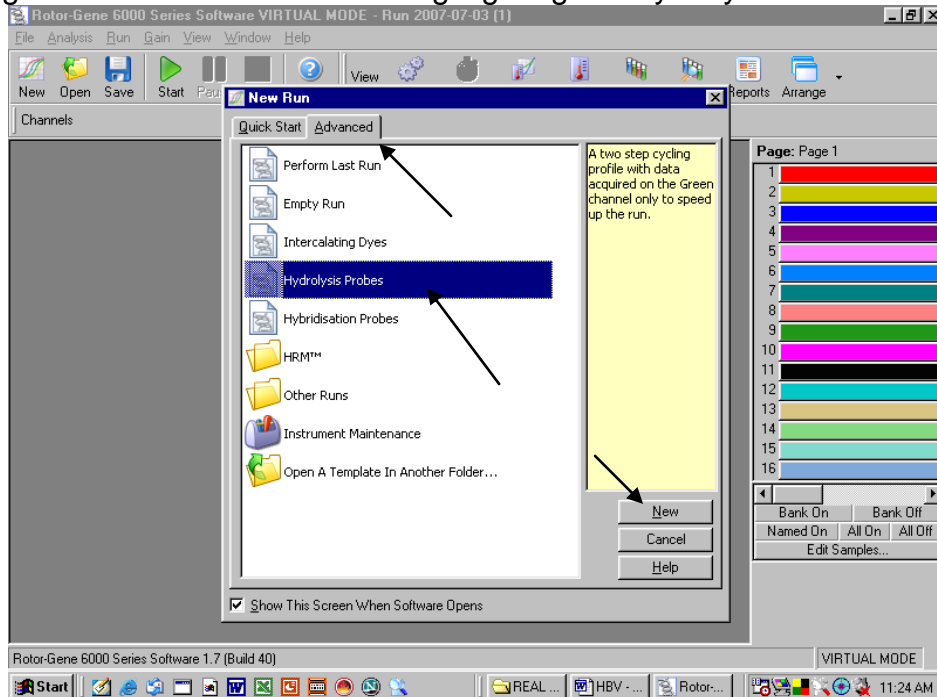


Fig. 21.

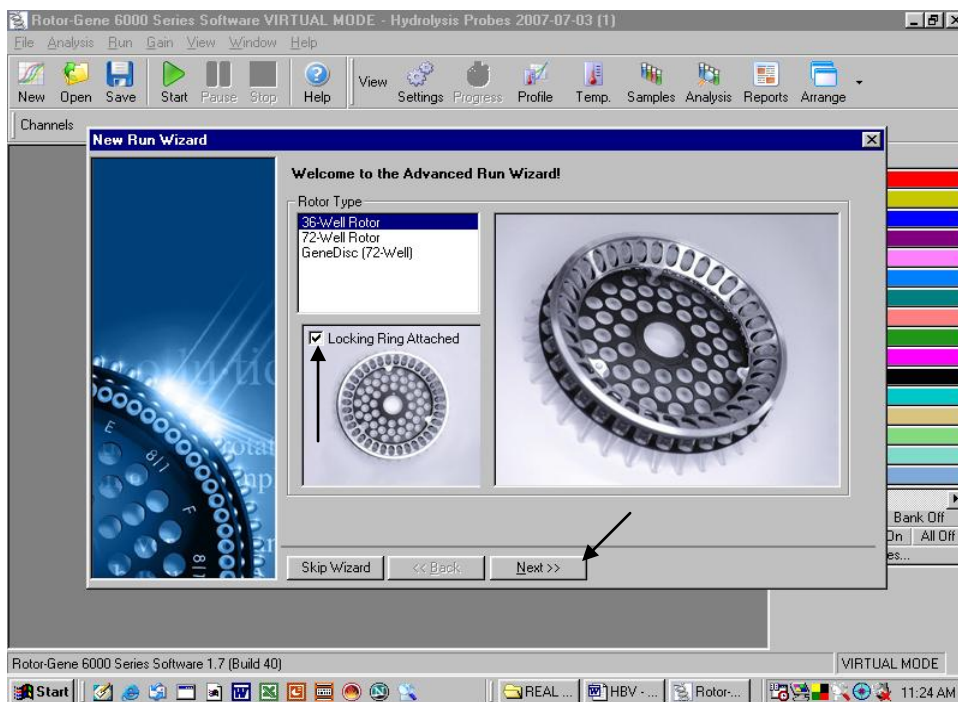


Fig. 22.

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.

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Confirmation of reaction Volume as follows.

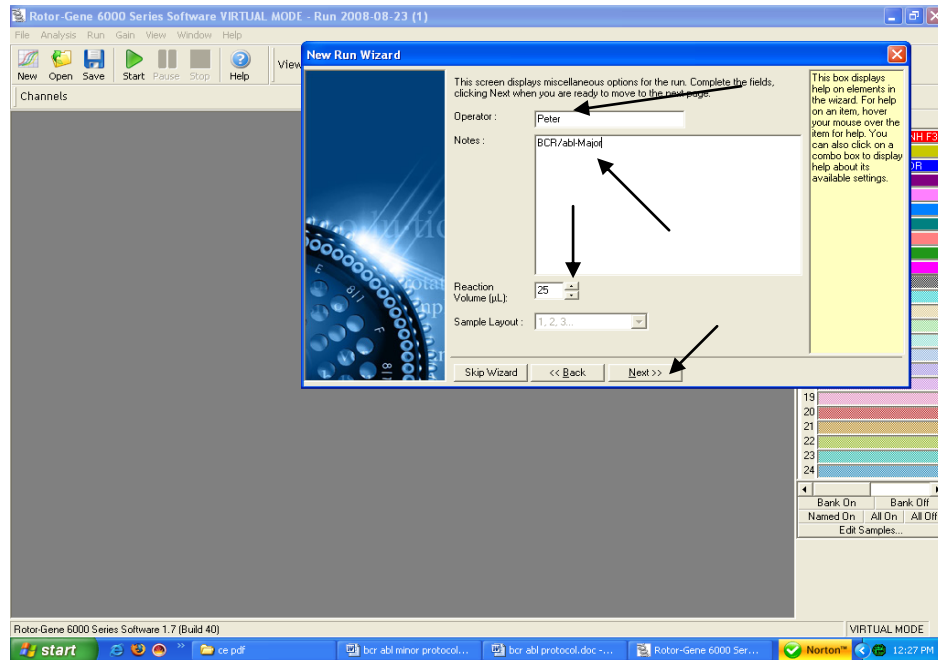


Fig. 23.

- 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.
- 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.

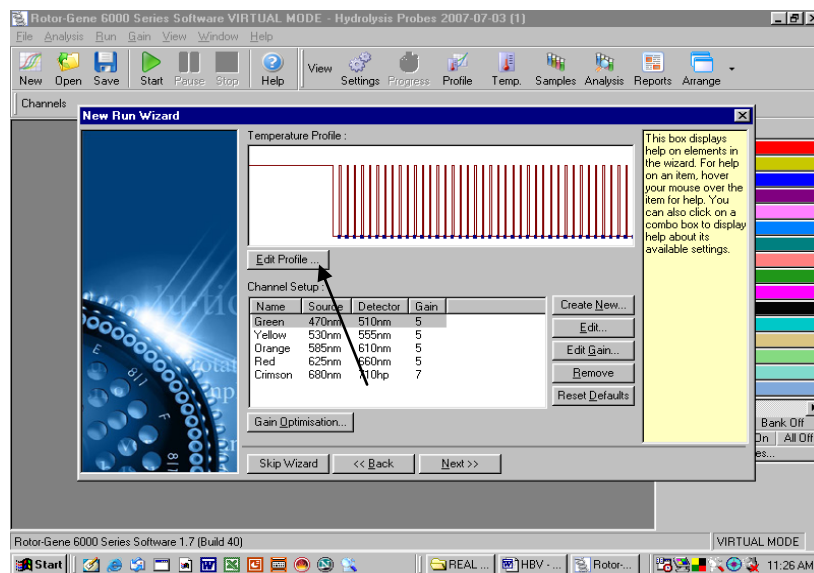


Fig. 24.

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

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 50°C for 15 minutes i.e. cDNA synthesis step as below**

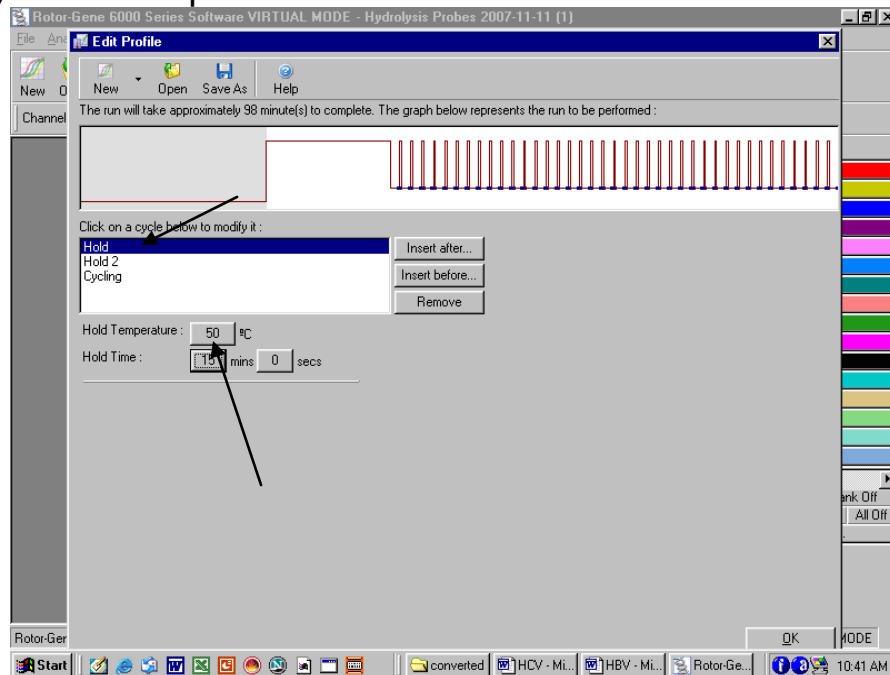


Fig. 25.

Second hold 95°C for 10 minutes as below

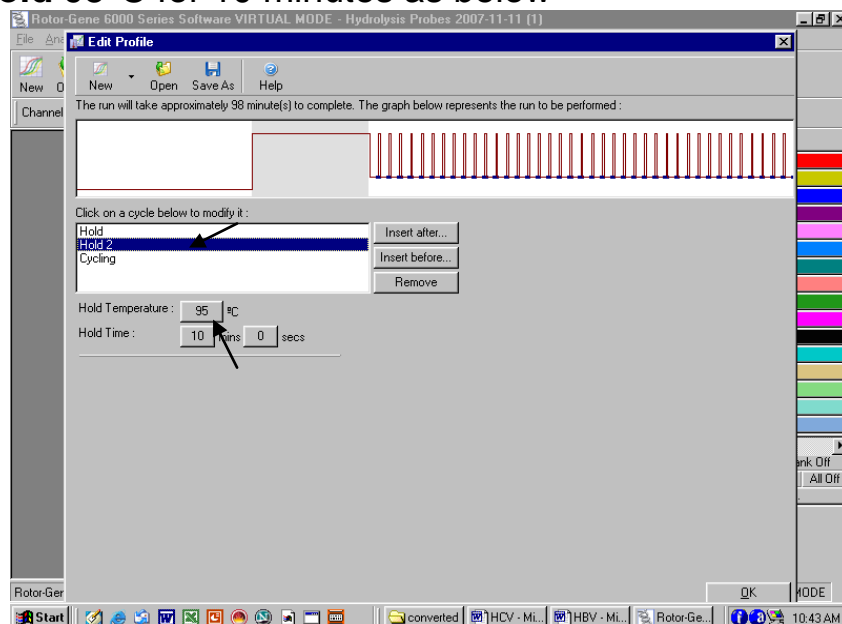


Fig. 26. Initial activation of the Hot Start enzyme. Generally the window will open with the Hold Temp as 95°C and the Hold Time as 10 minutes. In case it is different then set the same to as shown above.

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

j) Setting the Cycling and acquisition.

When clicked on Cycling the window will open as below.

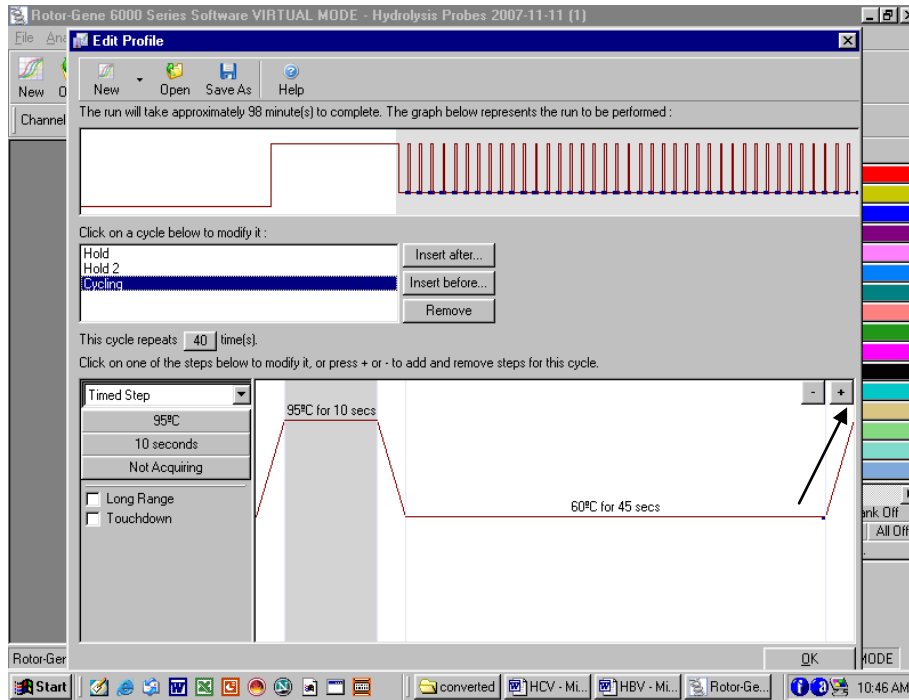


Fig. 27.

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.

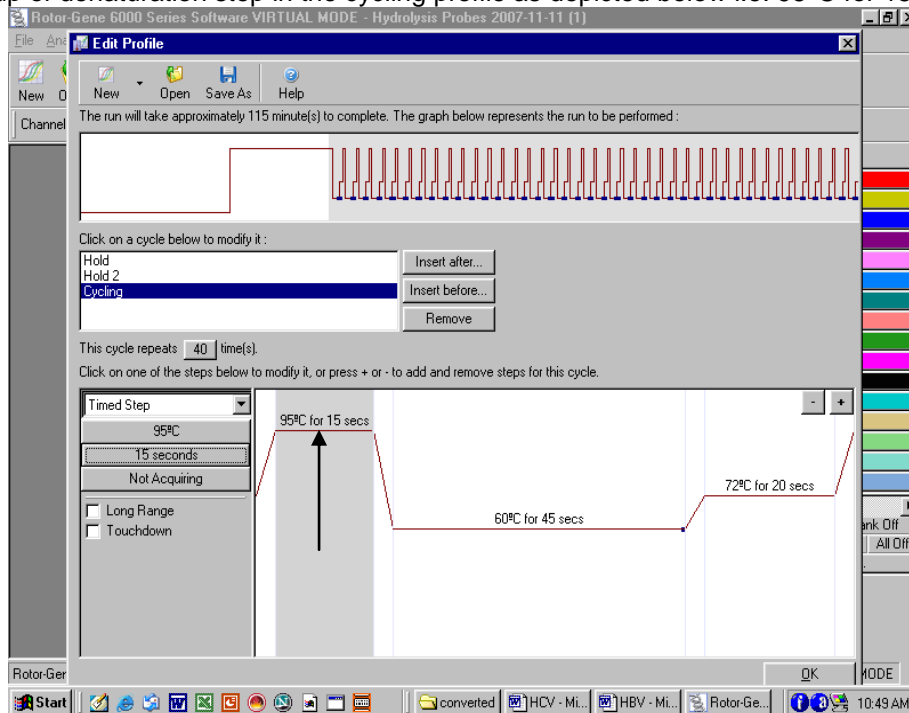


Fig. 28.

[Geno-Sen's Major-Bcr/abl 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

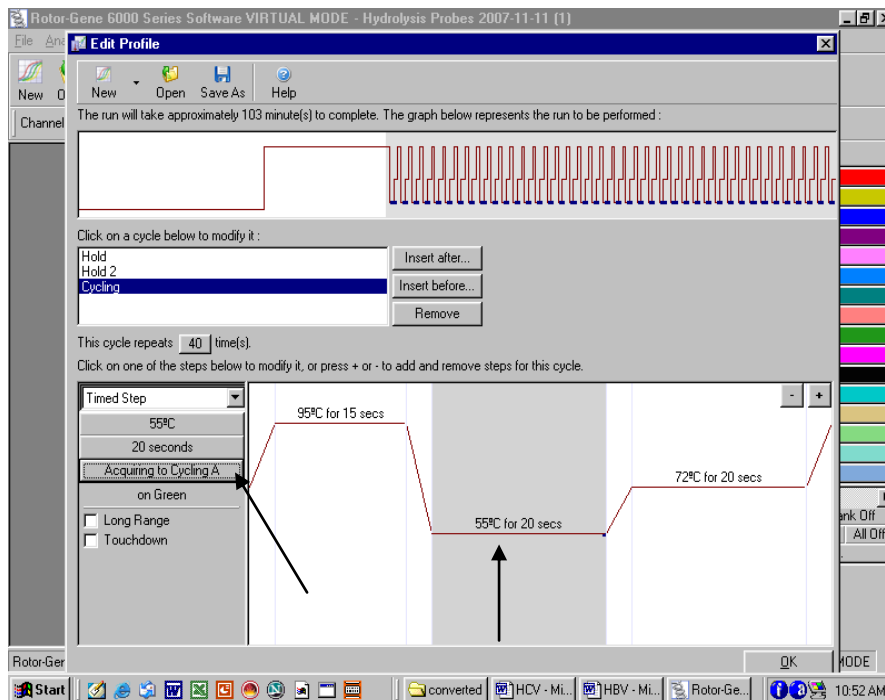


Fig. 29.

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 Green (FAM)

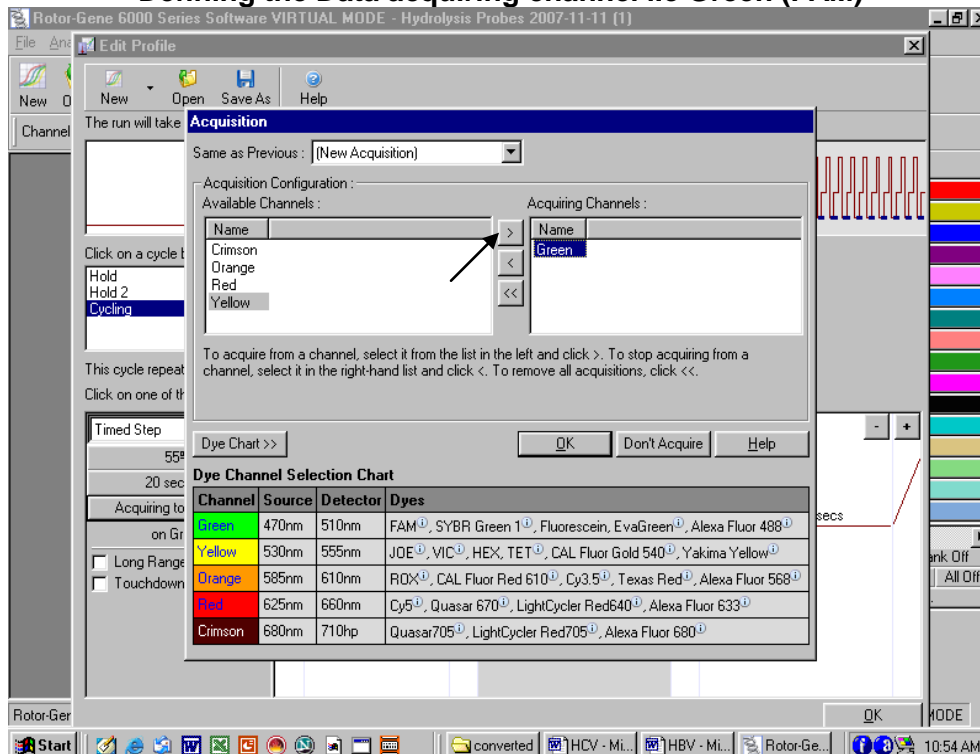


Fig. 30.

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

In case any other Channel appears besides Green on the right then the same be shifted to the left.

Confirmation of Channels as shown below.

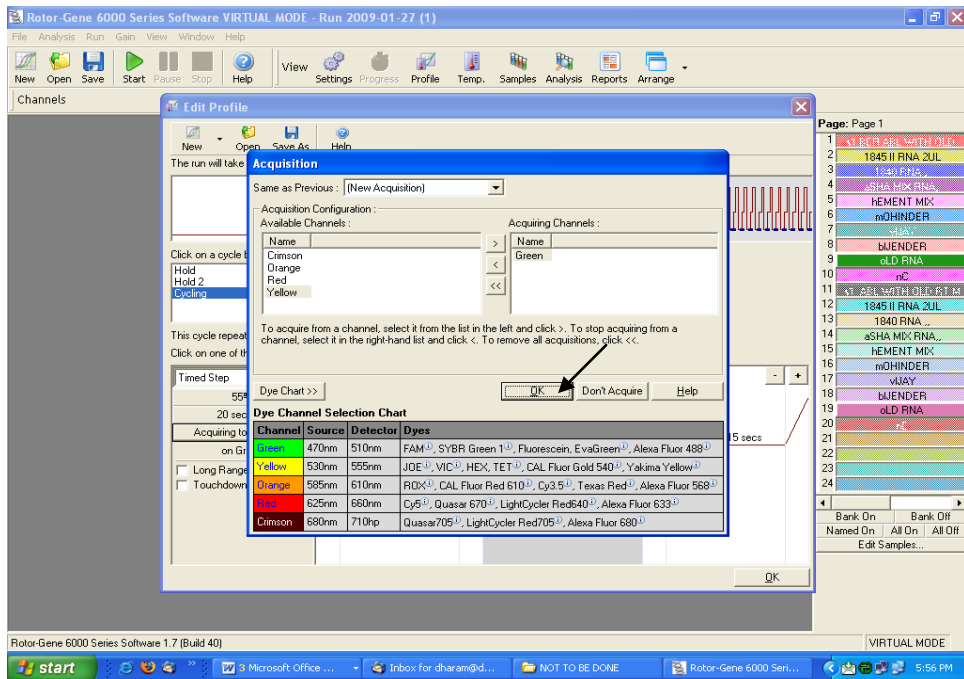


Fig. 31.

Once the Green Channels are 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

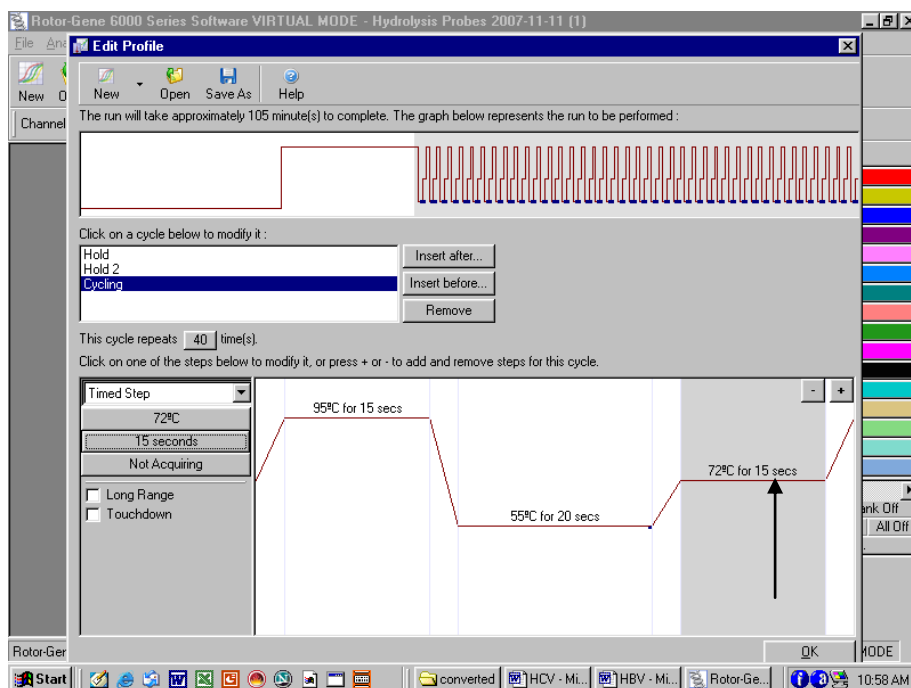


Fig. 32.

[Geno-Sen's Major-Bcr/abl 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.

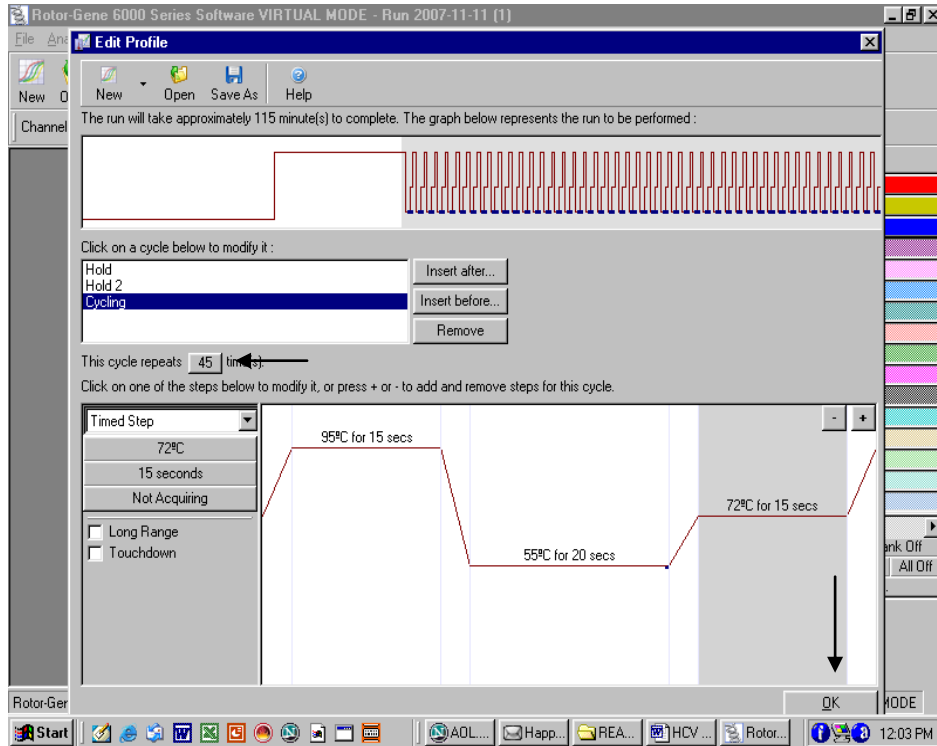


Fig. 33.

After setting the number of Cycles Press OK.

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

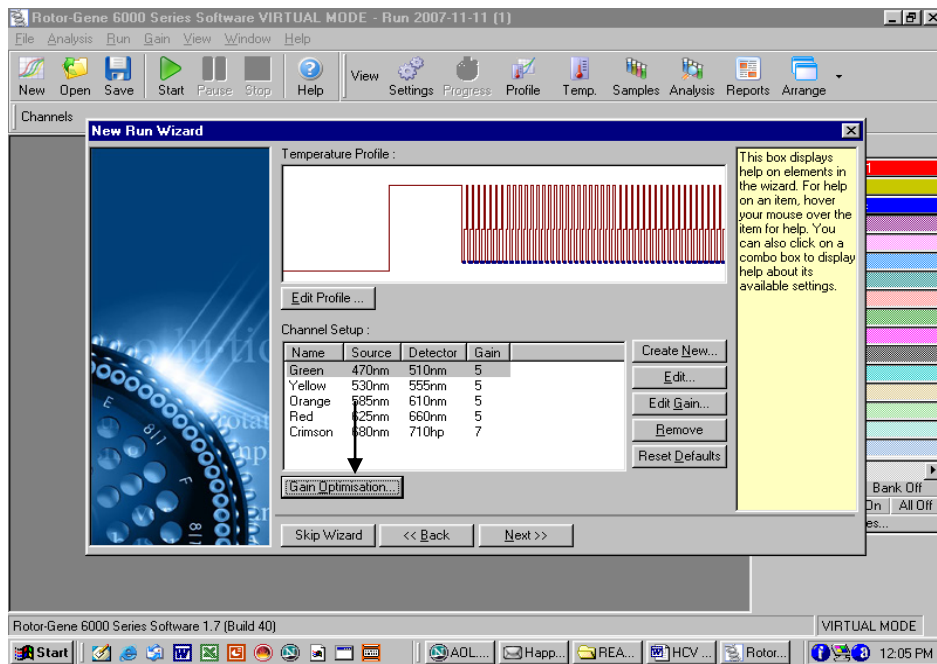


Fig. 34

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

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.35).

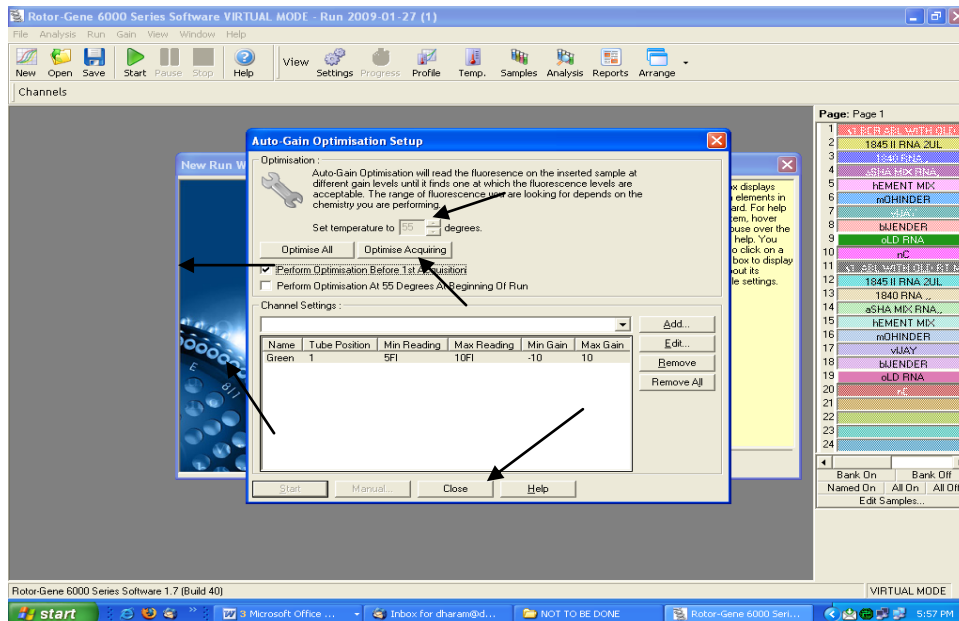


Fig. 35.

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 before 1st Acquisition.
- Just see that below the channel settings there appear only one channels i.e. Green. In case it is different then the channels have not been set properly. Close the window and reset the channels.
- Then Press Close.
- The press Next as shown below.

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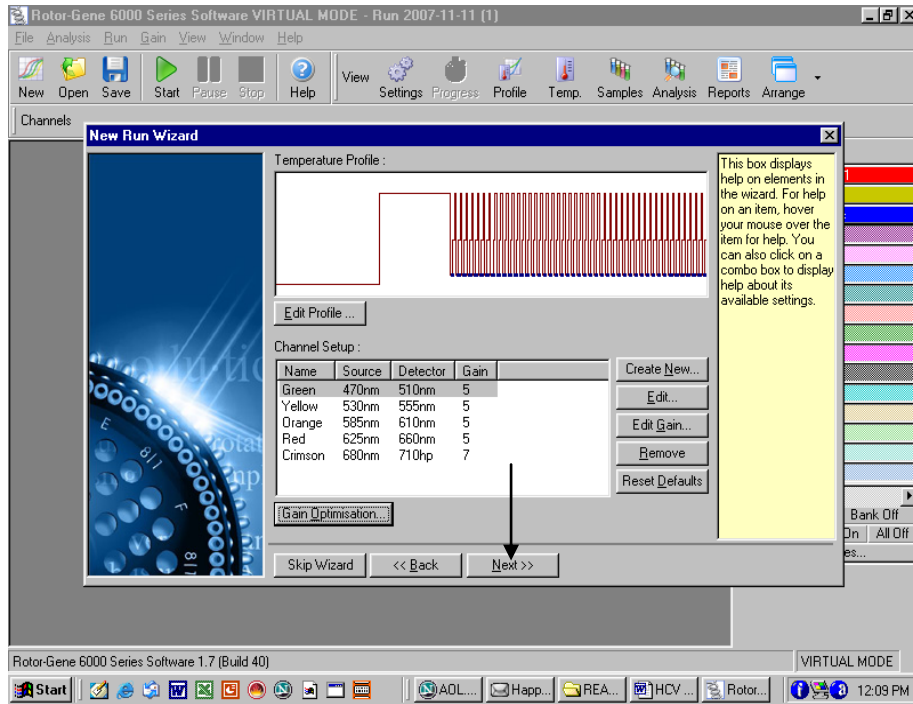


Fig. 36.

N) PRESS Start RUN

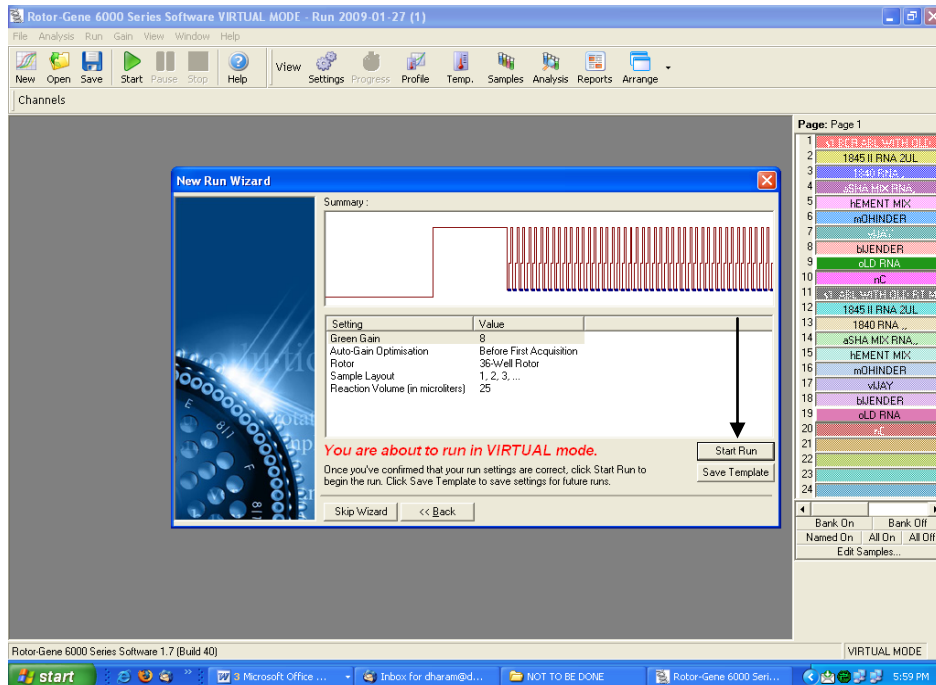


Fig. 37.

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

Saving the RUN File.

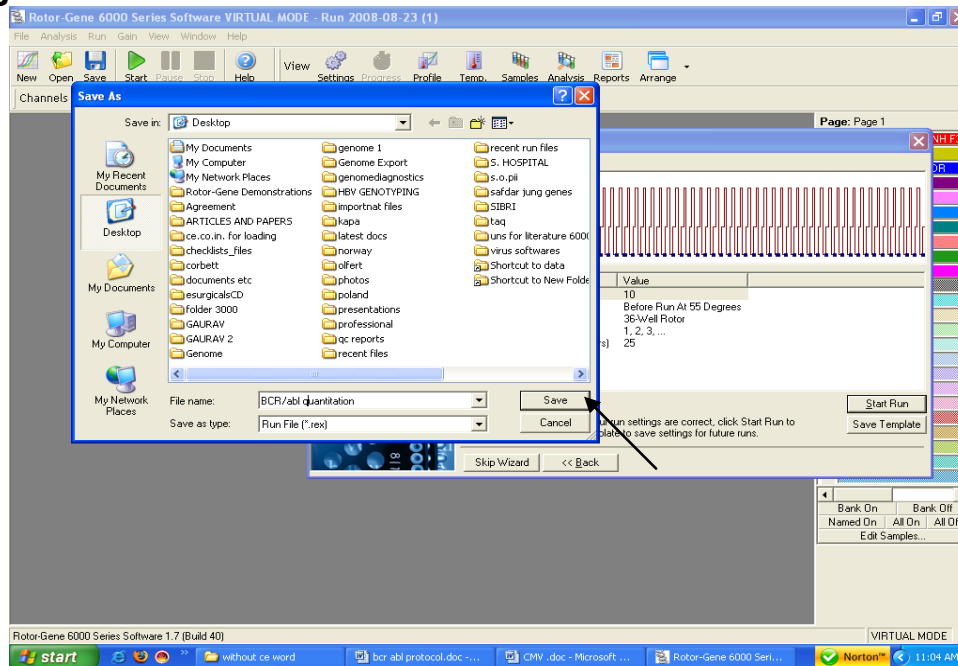


Fig. 38.

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 *RotorGene*TM software according to the manufacturer's instructions (*RotorGene*TM 3000 Operator's Manual).

The following results are possible:

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

The result of the analysis is positive: The sample contains Bcr cDNA.

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

No Bcr cDNA detectable. It can be considered negative if there is amplification in the abl run. Please refer for details on PCR inhibition in the section PCR inhibition.

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

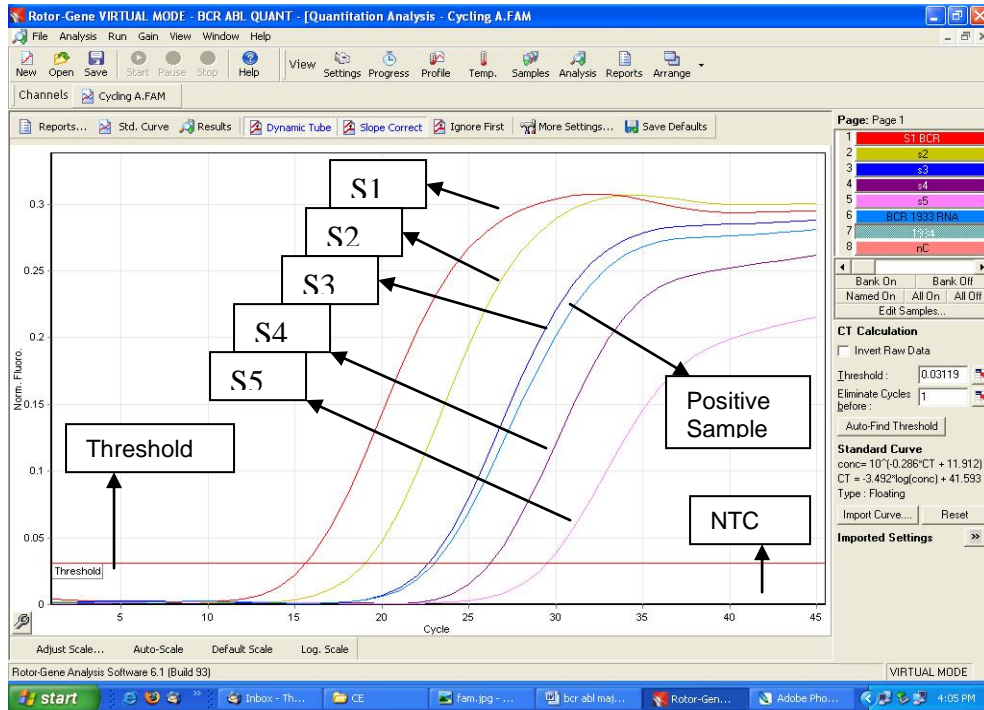


Fig. 39: Detection of the quantitation standards (*Bcr* S 1-5) in fluorescence channel Cycling A.FAM. NTC: non-template control.

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

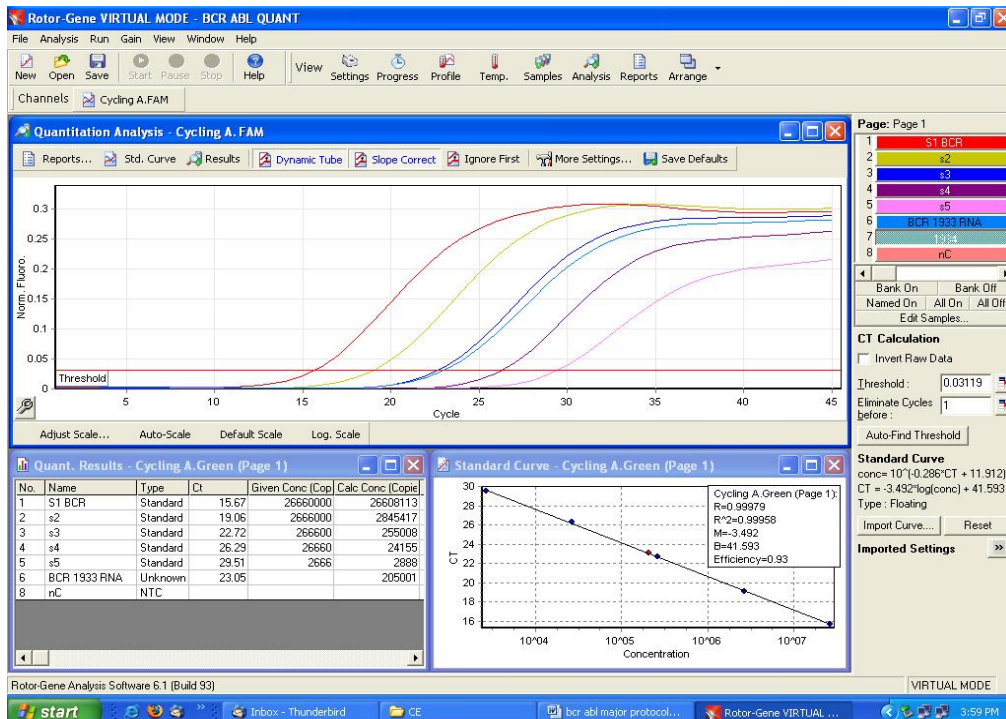


Fig. 40.

Example of analysed data for *Bcr* 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.

Abl reference gene amplification.

Run the diluted samples for abl stored at –20 °C in exactly the similar fashion as described for Bcr so far. After the run the quantitative results for the abl gene can be obtained & then the ratio of Bcr/abl can be determined.

The results of BCR/abl can be expressed as a ratio or can also be expressed as %.

PCR Inhibition

A signal is detected in fluorescence channel Cycling A. FAM in **abl**: **No PCR inhibition**

In fluorescence channel Cycling A. FAM in **abl** no signal is detected.

A possible PCR inhibition has occurred.

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

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

The following results are possible:

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

The result of the analysis is positive: The sample contains Bcr cDNA.

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

No Bcr cDNA detectable. It can be considered negative if there is amplification in the abl run. Please refer for details on PCR inhibition in the section PCR inhibition.

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

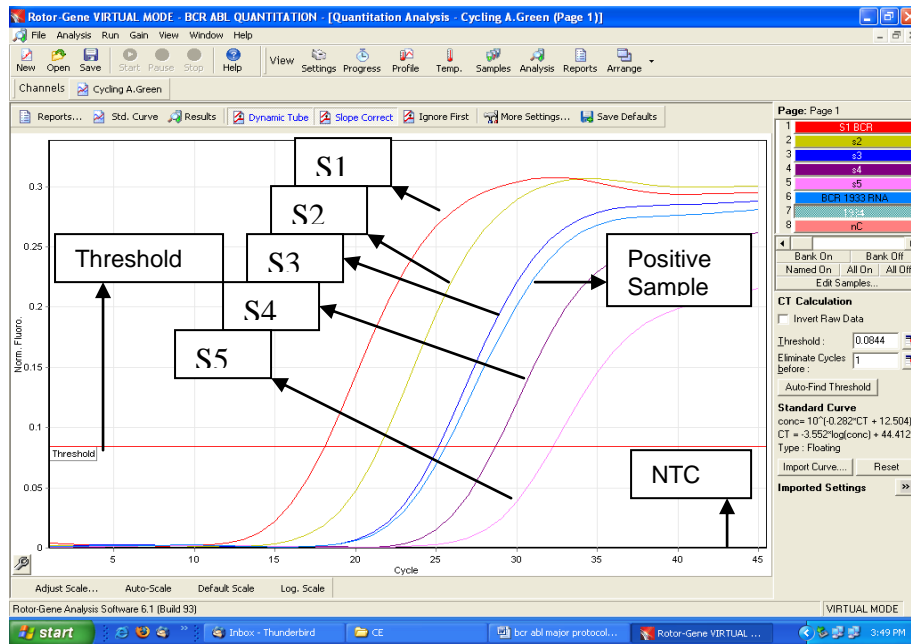


Fig. 41: Detection of the quantitation standards (*Bcr* S 1-5) in fluorescence channel Cycling A.Green. NTC: non-template control. Examples of positive and negative PCR reactions are given in the above figure.

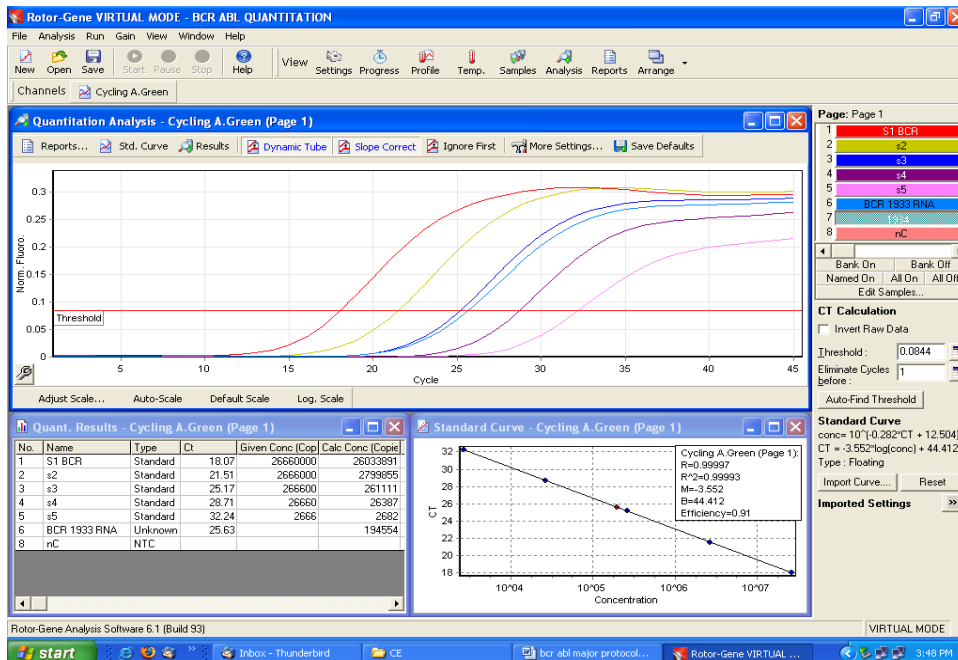


Fig. 42. Example of analysed data for Bcr where all the amplification curves can be seen as well as The standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

Abl reference gene amplification.

Run the diluted samples for *abl* stored at -20°C in exactly the similar fashion as described for BCR so far. After the run the quantitative results for the *abl* gene can be obtained & then the ratio of Bcr/*abl* can be determined.

The results of BCR/*abl* can be expressed as a ratio or can also be expressed as %.

PCR Inhibition

A signal is detected in fluorescence channel Cycling A. Green in **abl**: **No PCR inhibition**

In fluorescence channel Cycling A. Green in **abl** no signal is detected.

A possible PCR inhibition has occurred.

10. a) Troubleshooting for Rotor Gene 2000/3000

1. No signal with positive Standards (Bcr/*abl* S 1-5) in fluorescence channel Cycling A.FAM.

- Incorrect programming of the *Rotor-Gene*[™] 2000/3000.

➔ Repeat the PCR with corrected settings.

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

- The PCR conditions do not comply with the protocol.

➔ Repeat the PCR with corrected settings.

- The Super Mix *R1*, *R3* has been thawed and frozen too often.
- The Super Mix *R1*, *R3* has been kept at $+4^{\circ}\text{C}$ for longer than 5 hours.

➔ Please mind the storage conditions given in the **Storage**.

➔ Repeat the Steps with New vials of Super mix (*R1*) & *R3 super Master Mix*.

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

- **The PCR was inhibited.**

- ➔ Make sure that you use a recommended extraction method (see **8.1. RNA extraction**) and stick closely to the manufacturer's instructions.

10. b) Troubleshooting for Rotor Gene 6000

1. No signal with positive Standards (Bcr/abl S 1-5) in fluorescence channel Cycling A.Green

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

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

- The PCR conditions do not comply with the protocol.
 - ➔ Repeat the PCR with corrected settings.
- The Super Mix *R1, R3* has been thawed and frozen too often.
- The Super Mix *R1, R3* has been kept at +4°C for longer than 5 hours.
 - ➔ Please mind the storage conditions given in the **Storage**.
 - ➔ Repeat the Steps with New vials of Super mix (*R1*) & *R3 super Master Mix*.

- **The PCR was inhibited.**

- ➔ Make sure that you use a recommended extraction method (see **8.1. RNA extraction**) and stick closely to the manufacturer's instructions.

11. Specifications

11.a Sensitivity and Reproducibility

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

In order to determine the sensitivity of the **Geno-Sen's Bcr/abl Major Real Time PCR Kit**, a dilution series has been set up from 10^7 down to 10^0 Copies/ μ l of Bcr & abl cDNA and analyzed with the **Geno-Sen's Bcr/abl Major 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 Bcr/abl Major Real Time PCR Kit** is consistently 80 copies/ml. This means that there is 95% probability that 80 copies/ml will be detected.

Analytical Sensitivity

Analytical Sensitivity in Conjunction with the **Geno Sen's® Whole Blood RNA Extraction Mini Kit — for RNA purification (Cat. No. 98007, 98008)** of the **Geno Sen's® Geno-Sen's Bcr/abl Major Real Time PCR RG Kit on ROTOR GENE 3000/6000** was determined by Spiking a known negative blood to a nominal 90 copies/ml. This was subjected to extraction using the **Geno Sen's® Whole Blood RNA Extraction Mini Kit — for RNA purification (Cat . No. 98007, 98008)** eight times with starting volume of 1.5 ml & elution volume of 40 μ l.

All the Eight extractions were then analyzed with the **Geno Sen's® Bcr/abl Major 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 93 copies/ml.

Hence Analytical Sensitivity in Conjunction with the **Geno Sen's® Whole Blood RNA Extraction Mini Kit — for RNA purification (Cat . No. 98007, 98008)** of the **Geno Sen's® Bcr/abl Major Real Time PCR RG** was determined to be 90 copies/ml.

11.b Specificity

In order to check the specificity of the **Geno-Sen's Bcr/abl Major Real Time PCR** kits, different cDNA and plasmids listed below were analyzed with **Geno-Sen's Bcr/abl Real Time PCR Kit**. None of these led to a positive signal with the **Geno-Sen's Bcr/abl Major Real Time PCR kit**. Gene sequence analysis of the amplified region of Bcr/abl shows a pronounced homology among the various Bcr/abl cDNA, and no homology with other cDNA. 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® Bcr/abl Major Real Time PCR RG Kit**, different RNA & DNA listed below were analyzed with **Geno Sen's® Bcr/abl Major Real Time PCR RG Kit**. None of these led to a positive signal with the **Geno Sen's® Bcr/abl Major Real Time PCR RG Kit**.

TNF α
IFN γ
APML

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

h-tert

In order to validate the Specificity further with wet lab testing the following Known clinical samples were analyzed using the **Geno Sen's®** Bcr/abl Major Real Time PCR RG kit on ROTOR GENE 3000/6000 machine. The extraction was carried out with the QIAamp RNA Blood Mini extraction Kit (**Cat. No. 52304**)

The run was carried out with the known set of standards in order to quantitate the Bcr/abl RNA Gene.

Sample Type	WHOLE BLOOD	CELL CULTURE
High +ve's	6	7
Medium +ve's	8	7
Low +ve's	9	4
Extremely low +ve's	4	4
Negative samples.	4	4
	31	26

All the above samples were correctly identified by the **Geno Sen's®** Bcr/abl Real Time PCR RG kit & all the 8 extremely low samples were accurately detected by the **Geno Sen's®** Bcr/abl 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 conform 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.

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

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 depends on the number of RNA 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:

Phytohemagglutinin-Induced Peripheral Blood Cytogenetics: A Valid Means for Diagnosis and Imatinib Therapy Monitoring of Chronic Phase Chronic Myeloid Leukemia Patients

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

S.NO.	PRODUCT
1	HIV-1 RG quantitative Real time PCR kit.

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

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.
22	Chlamydia pneumonia RG quantitative Real time PCR kit.
23	Streptococcus 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.

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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. Pseudomalle 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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