Geno-Sen's

HCV Genotyping 1/2/3/4 Real Time PCR Kit

Qualitative

for use with the

IVD

PACK INSERT

Revised June 2016

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Genome Diagnostics Pvt. Ltd.

(An ISO 13485:2012, 9001:2008 Certified Company)

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Table of Contents

HCV Genotyping Geno-Sen's Real Time PCR

1. Contents of the Kit:

Color Code	Contents	REF 9111501 100 rxns	REF 9111502 50 rxns	REF 9111503 25 rxns
R1 Blue	HCV *Genotyping* 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
HCV Genotype 1 Red	*HCV Genotyping 1 Positive Control*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
HCV Genotype 2 Red	*HCV Genotyping 2 Positive Control*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
HCV Genotype 3 Red	*HCV Genotyping 3 Positive Control*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
HCV Genotype 4 Red	*HCV Genotyping 4 Positive Control*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
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.

3. HCV Genotyping Information

Application

Even in a single infected individual, HCV does not exist as a homogeneous species. Heterogeneous genomes - "quasispecies" - resulting from mutations due to high error rates in RNA replication are found within the same host. Many important biological features of several viruses are attributable to their quasispecies nature, including vaccination failure, persistent infection, and resistance to antiviral drugs.

The amount of diversity of the quasispecies population has also been found to be related to the progression to liver disease.

The most striking feature of HCV is its ability to persist in the host. The mechanism(s) of viral persistence are unclear but cannot include viral integration into the host genome as with certain other viruses due to the lack of a DNA intermediate in its life cycle. Instead, persistence appears to result from HCV's ability to mutate rapidly under immune pressure, giving rise to related but immunologically distinct variants. Any one of these variants can become the predominant strain, and coexistence of multiple quasispecies allows HCV to escape the host immune response.

Most mutations occur in a short, hypervariable region of the E2/NS1 domain. The region represents only 8% of the domain but accounts for approximately half of the nucleotide changes in the entire envelope region. Because of the occurrence of this hypervariable region within the envelope where it would be most likely to be exposed to antibody, mutations in this region may serve to evade an immune response. It has been reported that the nucleotide substitution rate within the hypervariable region rises during acute infection at a time when HCV RNA levels in the serum are decreasing, possibly due to a host immune response. There is also evidence that HCV can escape immune clearance by down-regulating its replication while persisting quiescently in the liver.

Traditionally, viruses have been classified according to antigenic characteristics, but with recent advances in molecular biology, genotypic classification through the analysis of genomic variation is now possible. Variations in the HCV genome fall into a series of specific patterns that have been classified into genotypes. Among the different HCV genotypes, the sequence of the 5' NC region is relatively conserved and is most often applied for diagnosis of HCV infection by PCR. In contrast, the sequences of NS3, NS5, and core regions are more variable and are therefore often used to define and distinguish among the HCV genotypes. Studies indicate that there are nine major HCV types (according to the general classification) designated 1 through 9.

Some of these types are further divided into subtypes. The potential significance of this becomes apparent when considering virus-host interactions, severity of infection, and sensitivity to treatment. The clinical importance of HCV lies in its persistence and ability to cause chronic liver disease. The dramatic disparities in HCV disease course among infected persons and differences in disease patterns between countries with divergent dominant genotypes raise the possibility that the existence of various strains of HCV may be a critical factor in this variability.

Chronic infection with the hepatitis C virus (HCV) is estimated to affect about 170 million people worldwide, and about 20%-30% of these cases will eventually progress to liver cirrhosis and its sequelae such as hepatocellular carcinoma.Recent studies have indicated that combination of -interferon plus ribavirin is more effective in the treatment of HCV infection than monotherapy with interferon alone. Using logistic regression, Poynard et al. identified five independent factors significantly associated with response to interferon therapy: genotypes 2 or 3; viral load less than 2 million copies/ml; age over 40 years; minimal liver fibrosis stage; and female gender. It is, therefore, important that the genotype of the HCV be determined prior

to therapy, as it has implications for diagnosis, management and response to therapy. Moreover, HCV genotype determination assays can be particularly useful in studying worldwide and local evolutions of the HCV endemics, since the epidemiology of HCV is changing rapidly.

The *Geno-Sen's* HCV Genotyping assay is developed for laboratory scale or high-throughput transcript analysis by real time fluorescence PCR. Geno Sens HCV Genotyping kit detects and differenciates the most Important Genotypes of HCV i.e. 1, 2, 3, & 4.

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

Samples which can be used for Extraction: Serum, plasma, whole blood.

4. Precautions for PCR

The following aspects should always be taken care of:

- Store positive material (Specimens, Controls 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 Control & specimens) should be mixed & dispensed in pre-mix area.
- All the Controls & 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 HCV GENOTYPING*** *PCR* Reagents constitute a ready to use system for detection of HCV Genotypes 1/2/3/4 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 HCV Genotypes 1/2/3/4 and for the direct detection of the specific amplicon in fluorescence channel Cycling A.FAM, JOE, Cy5 & ROX of the *Rotor Gene 2000/3000 or Green, Yellow, Red, & Orange of Rotor Gene 6000.* External positive Control (HCV Genotyping 1,2,3,4) are supplied which confirm the assay is working.

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**	**Cat. Num. REF**	
Serum or plasma.	***Geno Sen's*** ® Viral RNA Extraction Mini Kit (Columns based) OR QIAamp Viral RNA Mini extraction Kit (50)	98001 or 98002 52904	Genome Diagnostics Pvt. Ltd. India. QIAGEN

The ***Geno Sen's***® HCV Genotyping Real Time PCR kits has been optimized with the above mentioned extraction kit. The ***Geno Sen's***® Viral RNA Extraction Mini Kit provides a relatively higher yield than most of the commercial extraction kits available on the world market & hence is the preferred Kit for extraction of Viral RNA. 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 otherwise the low positives will not be detected.

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 *HCV Genotyping Rotor Gene PCR Reagents* should not be used with phenol based isolation methods.

8.b Inhibition Control.

There is no Inhibition in the kit as all the four channels are used for Gene of Interest. Besides which the assay has to be performed on the Known HCV positive samples.

8.c Positive Control

The Positive control provided in the kit are treated in the same way as extracted samples and the same volume is used i.e. (15µl) instead of the sample. The same should also be defined as Positive Control. (See *RotorGene*™ Manual). The positive control provided in the kit is for Genotype 1, Genotype 2, Genotype 3 and Genotype 4 only.

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 Positive Control & at least one negative control (*Water, PCR grade*) are included per 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

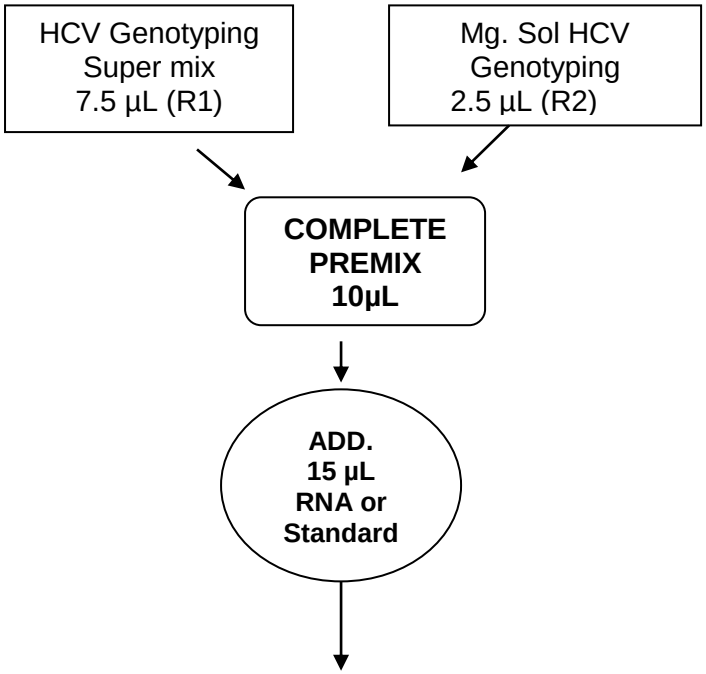


Fig. 4.

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

HCV Genotyping MASTER MIX	**1 rxns.**	**10 rxns.**
HCV Genotyping Super Mix (R1)	**7.5 µL**	**75 µL**
HCV Genotyping Mg Sol. (R2)	**2.5 µL**	**25 µL**
Total	**10µL**	**100µL**

Fig. 5.

Pipette 10 µl of the Master Mix into each labelled PCR tube. Then add 15 µl of the earlier extracted RNA to each sample tube and mix well by pipeting up and down. Correspondingly, 15 µl of the Positive Control (HCV Genotyping) must be used as a positive control and 15 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes and transfer the same 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 *RotorGene*™ 2000/3000

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

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

Program the *RotorGene*™ *2000/3000* for these 5 steps according to the parameters shown in Fig. 6-21. 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.

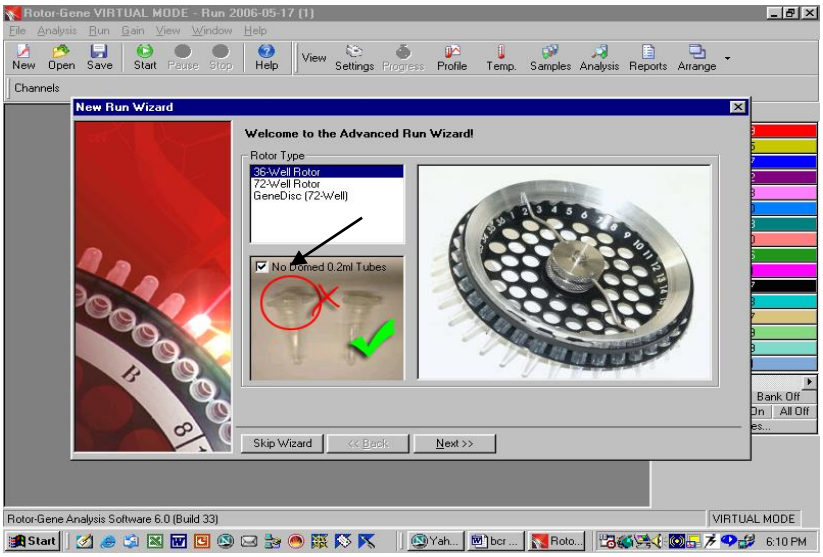


Fig. 6.

Confirmation of Reaction Volume as follows.

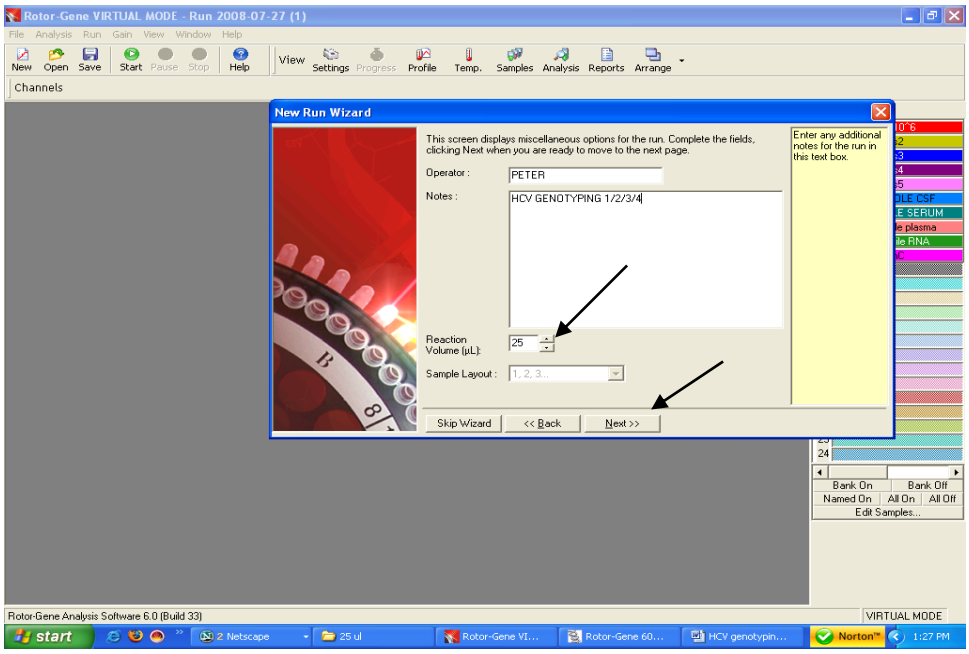


Fig. 7. Setting of general assay parameters.

- 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.

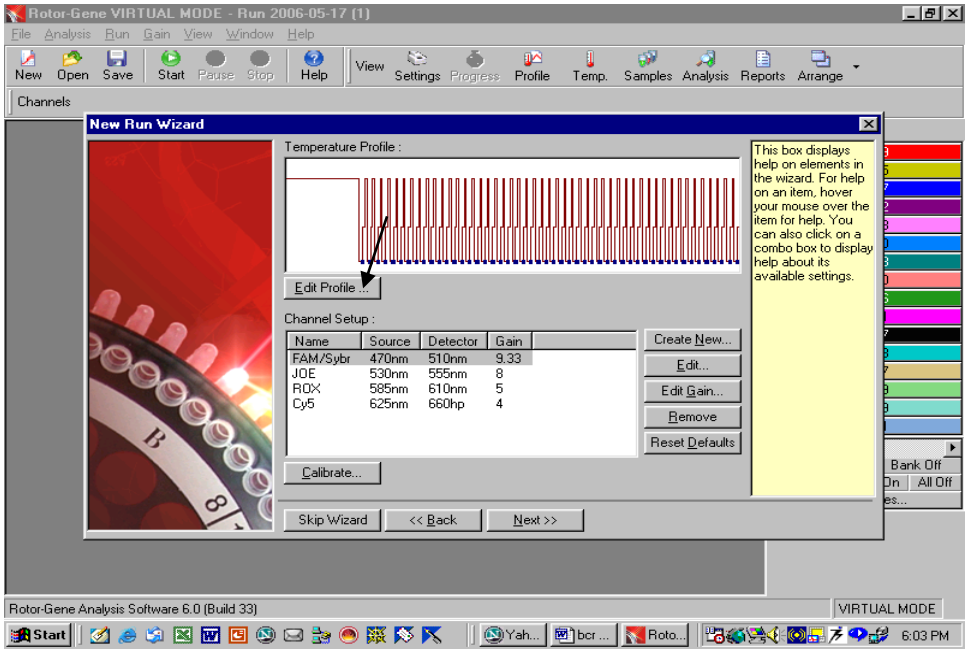


Fig. 8.

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

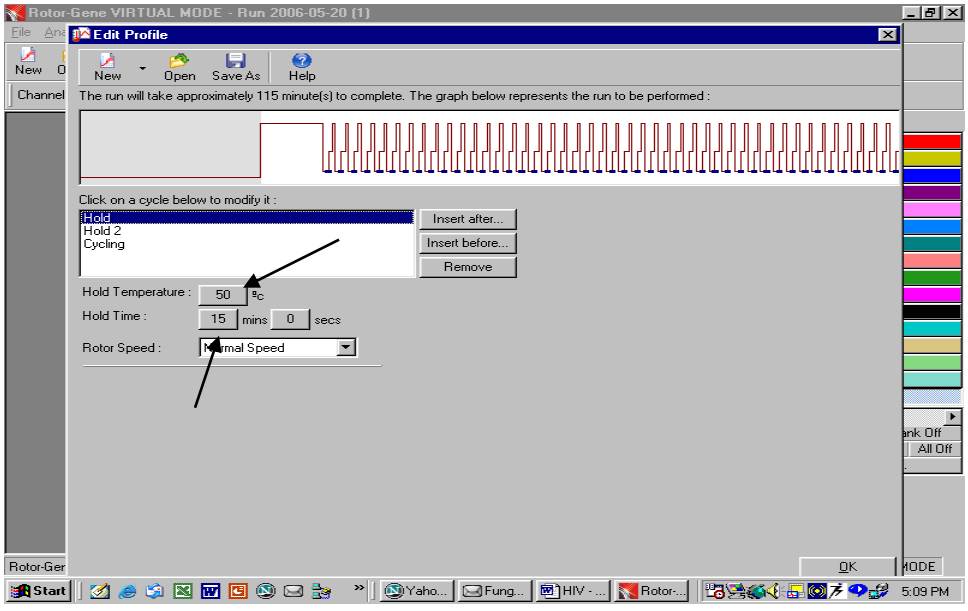


Fig. 9.

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

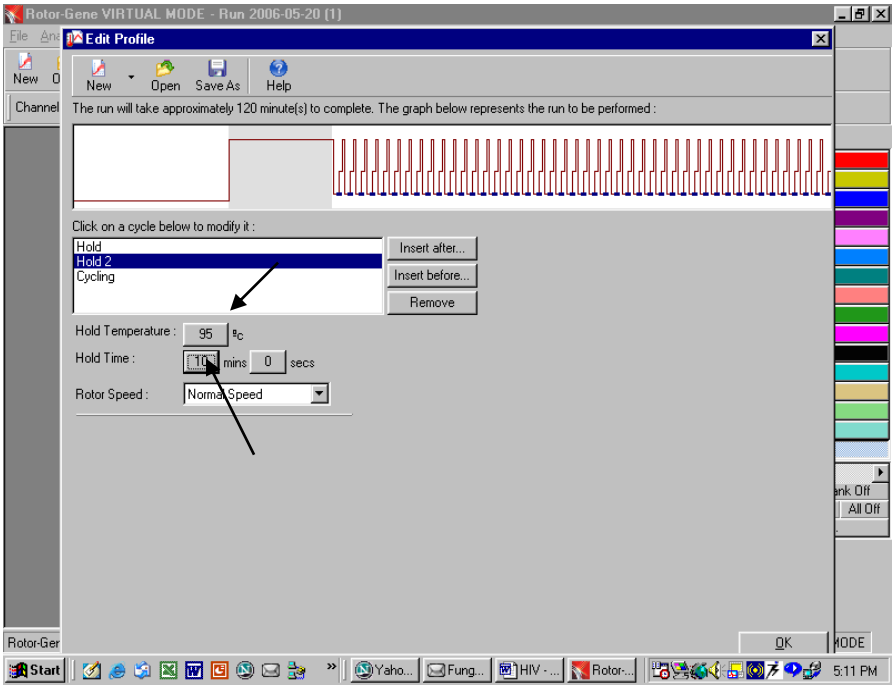


Fig. 10. Initial activation of the Hot Start enzyme.

Setting up of denaturation step in the cycling profile as depicted below i.e.

95°C for 15 seconds.

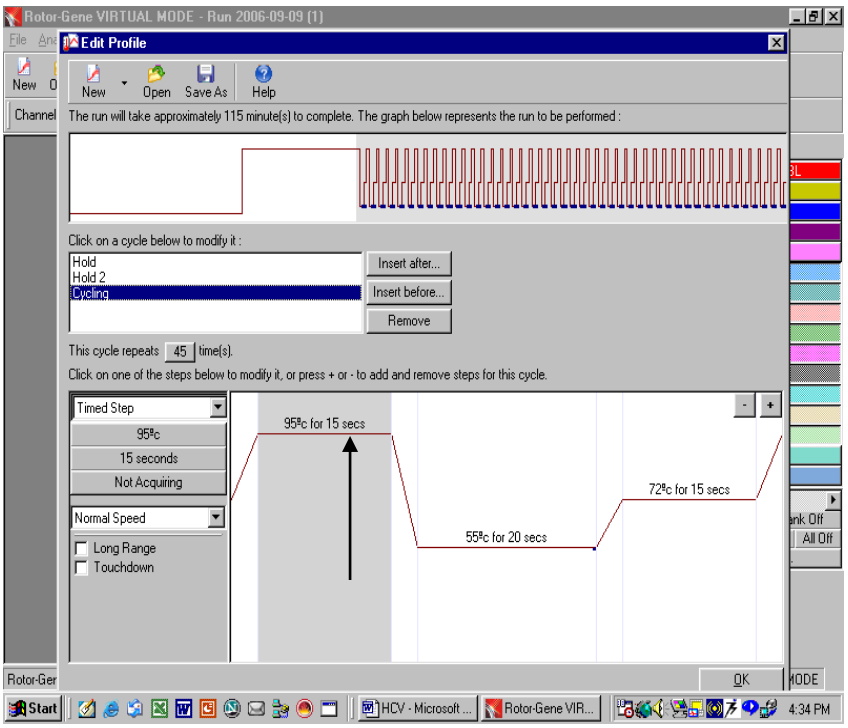


Fig. 11.

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 FAM & JOE, ROX & Cy5**

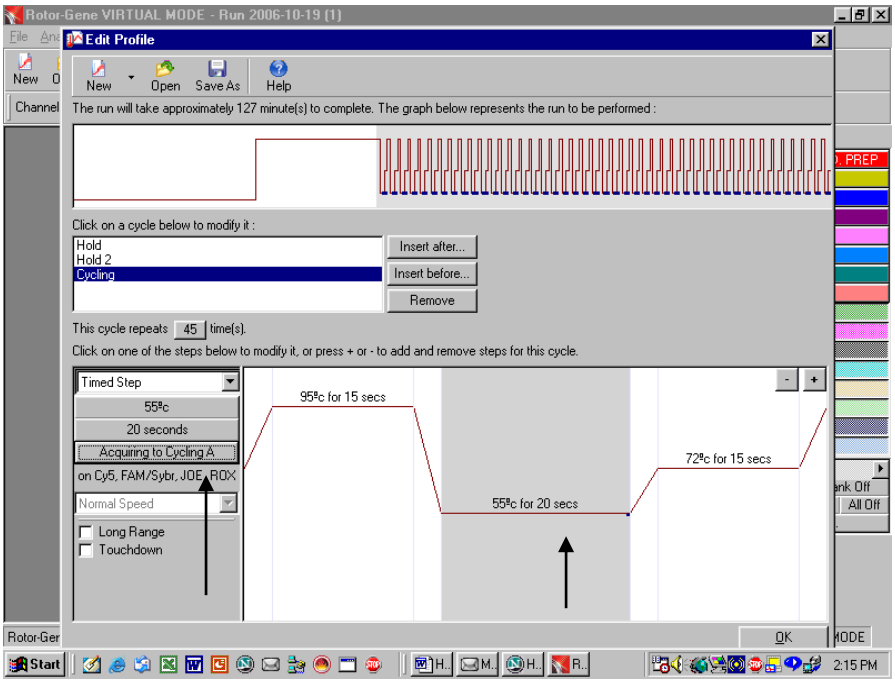


Fig. 12.

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

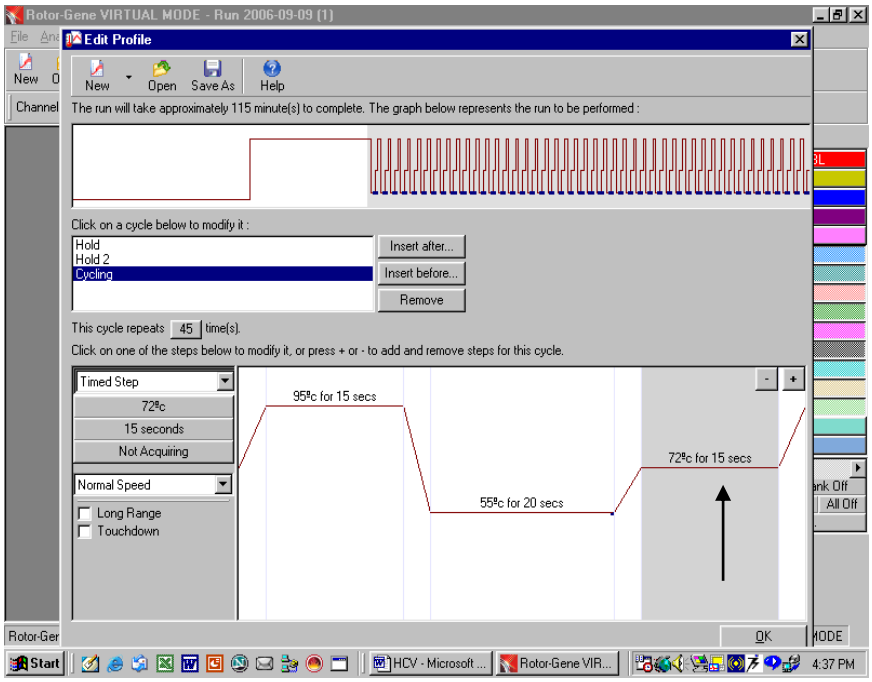


Fig. 13.

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

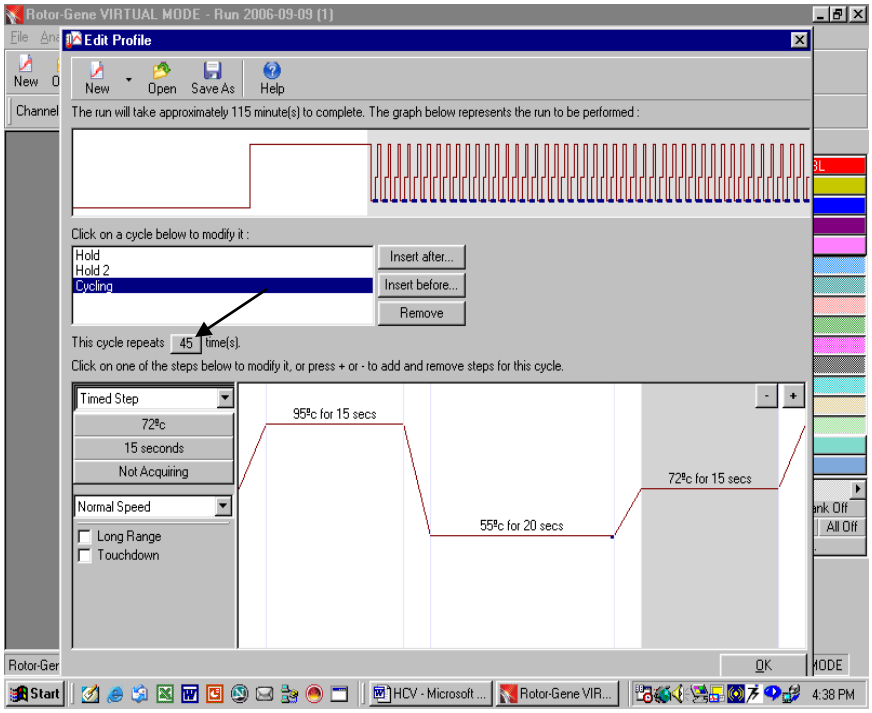


Fig. 14.

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

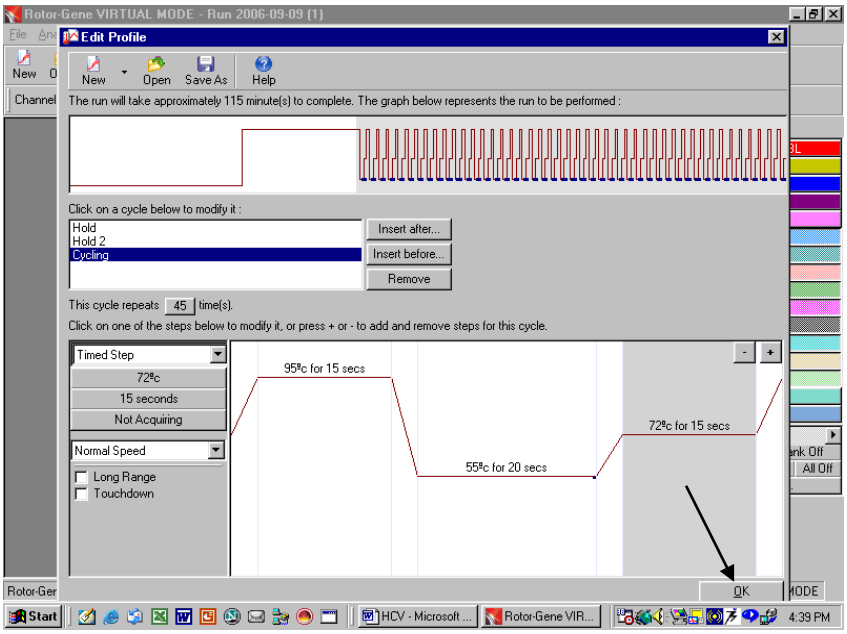


Fig. 15.

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

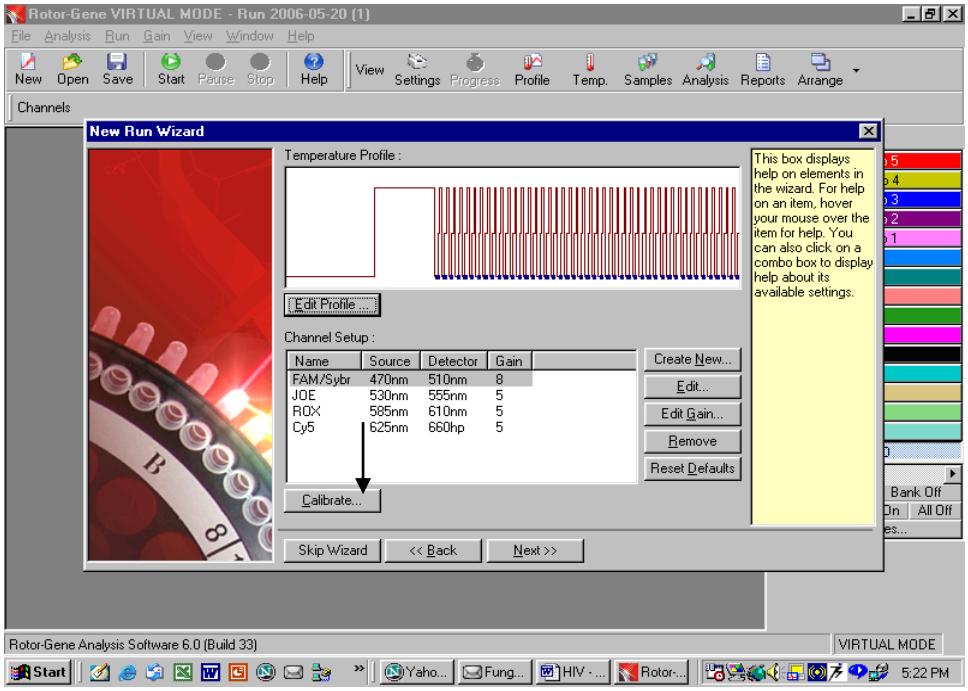


Fig. 16.

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.

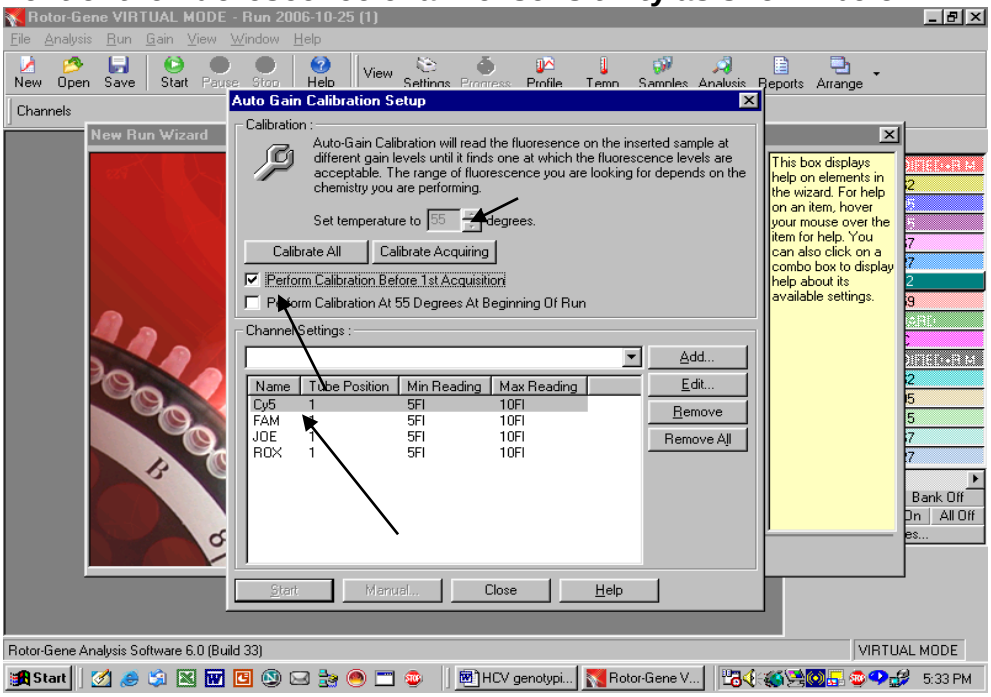


Fig. 17.

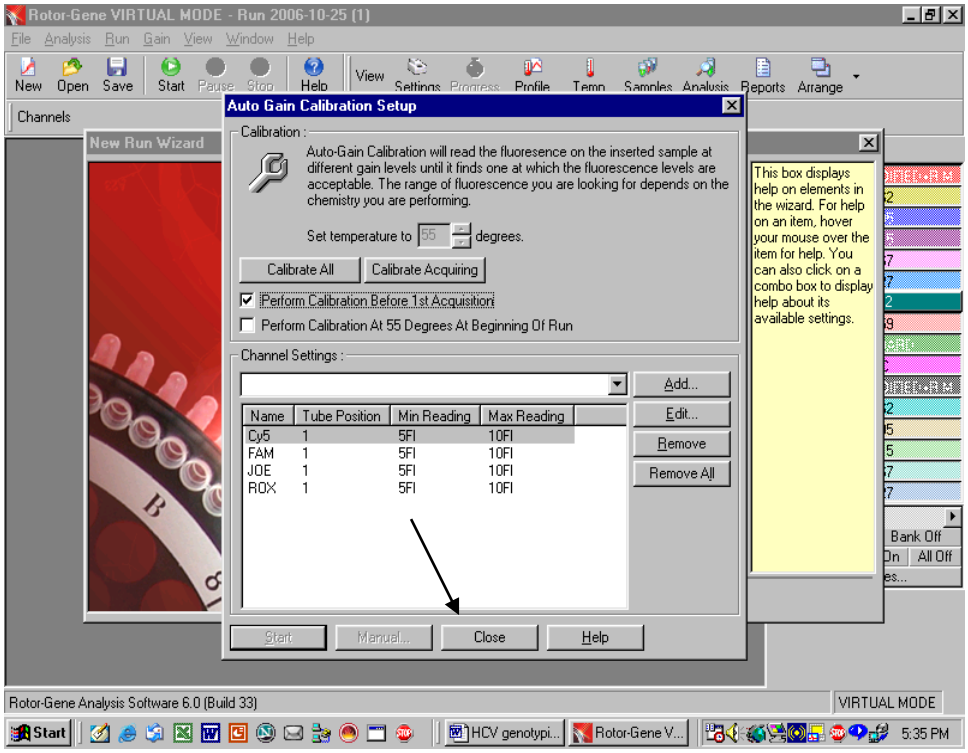


Fig. 18.

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.

PRESS NEXT

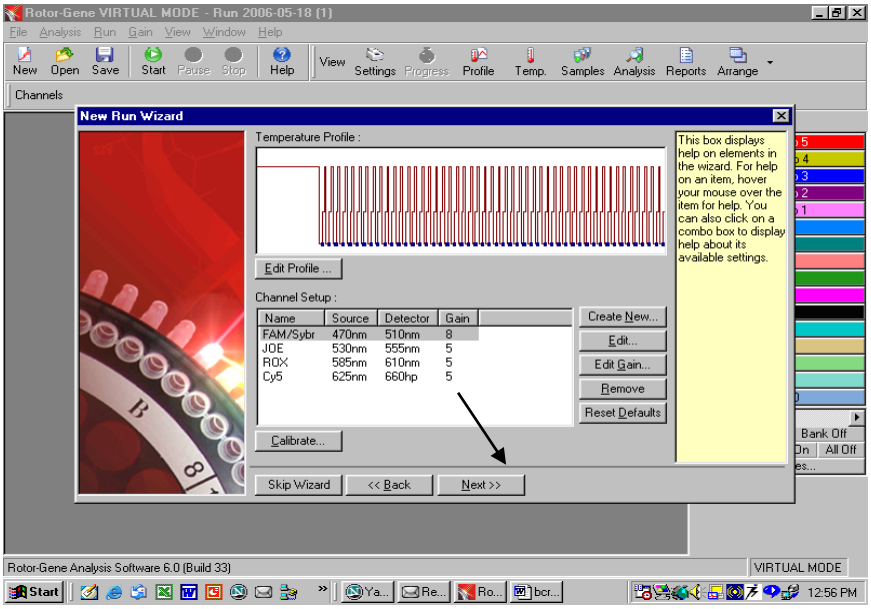


Fig. 19.

Starting of the *Rotor Gene*™ run.

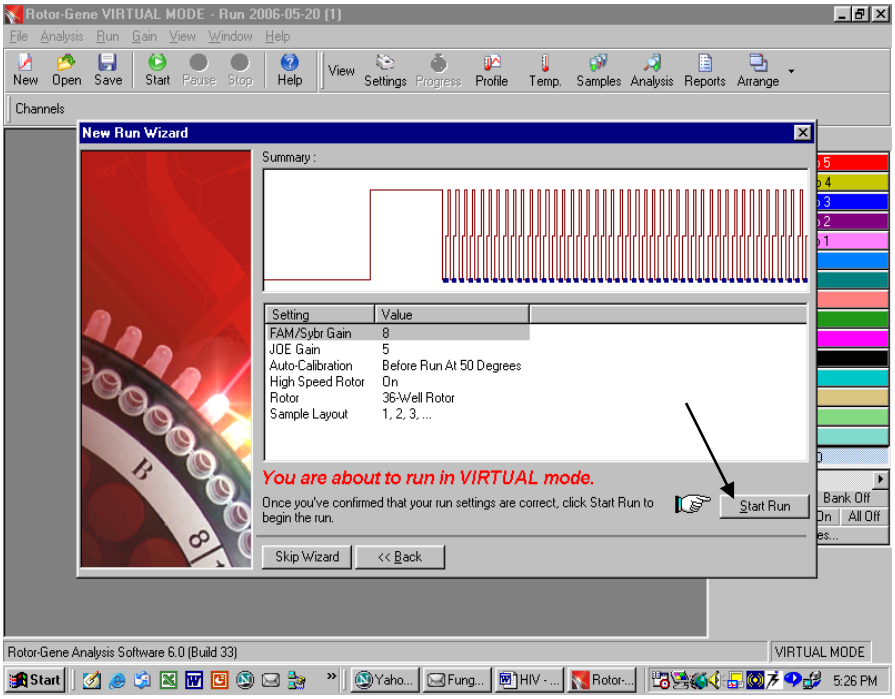


Fig. 20: Press Start Run Button.

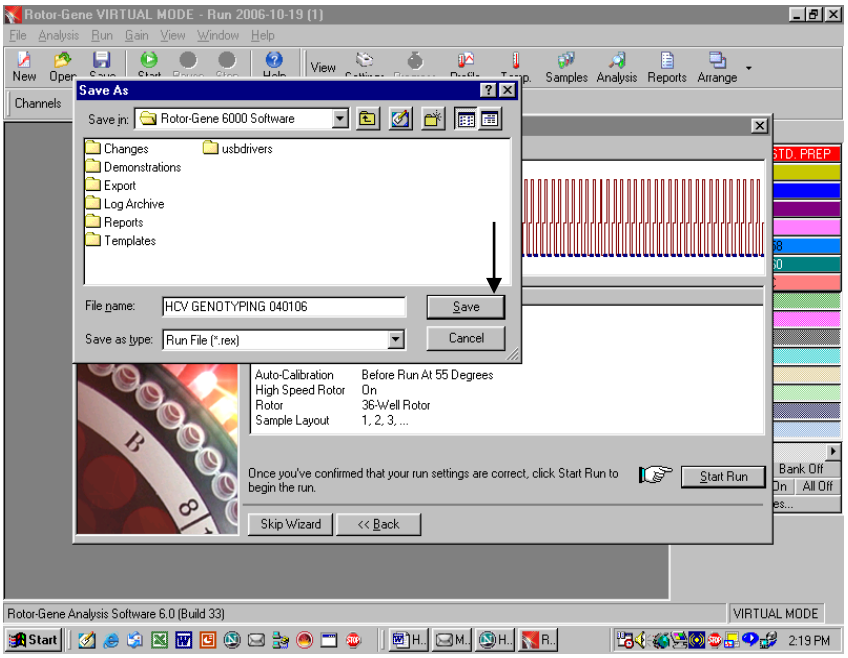


Fig. 21.

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.f. Programming the *RotorGene*™ 6000

The *RotorGene*™ 6*000* PCR program for the detection of HCV Genotyping can be divided into following steps:

G. Setting of general assay parameters & reaction volume
H. Thermal Profile & Calibration
I. Cycling profile/ cDNA Synthesis & 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 22-39 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 are 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.

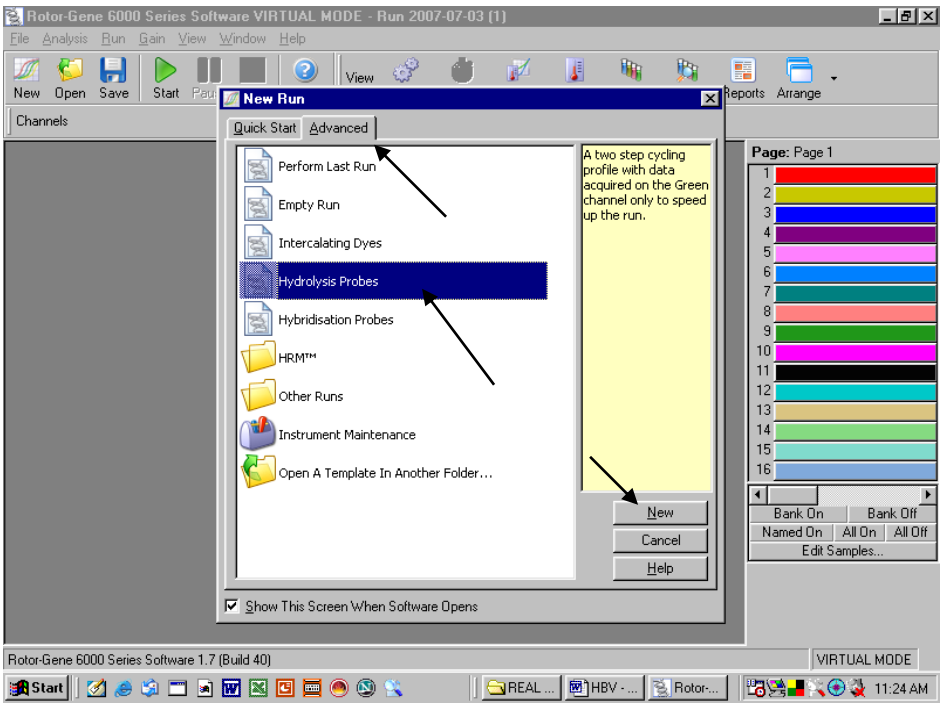


Fig. 22.

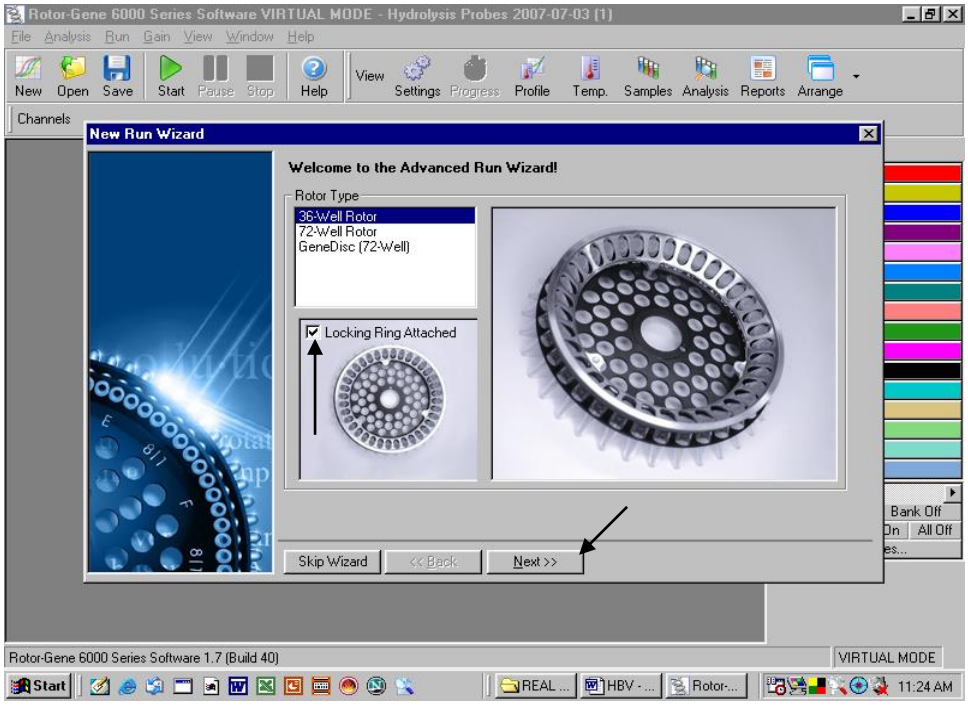


Fig. 23.

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.

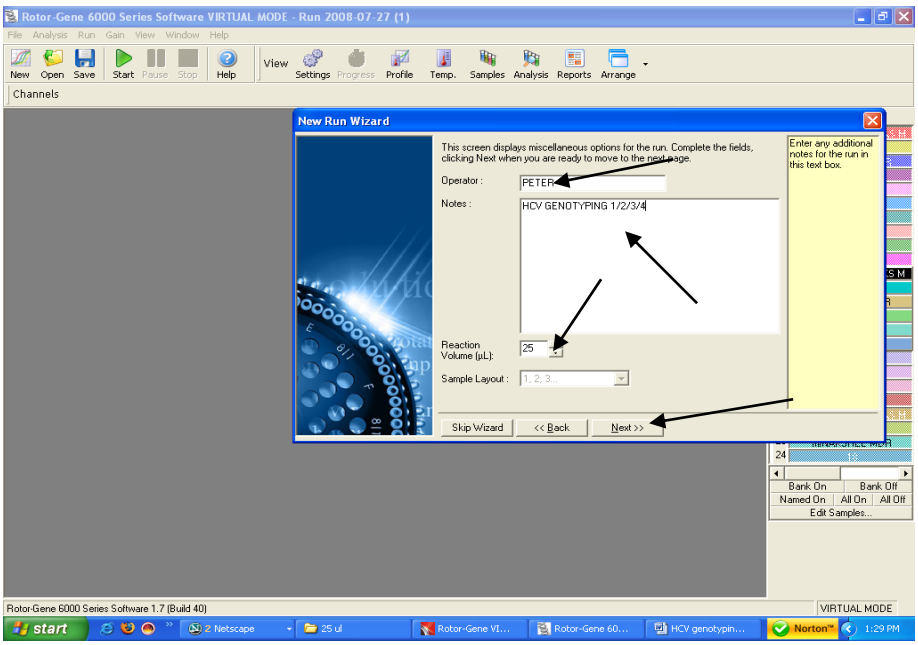


Fig. 24.

- 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.

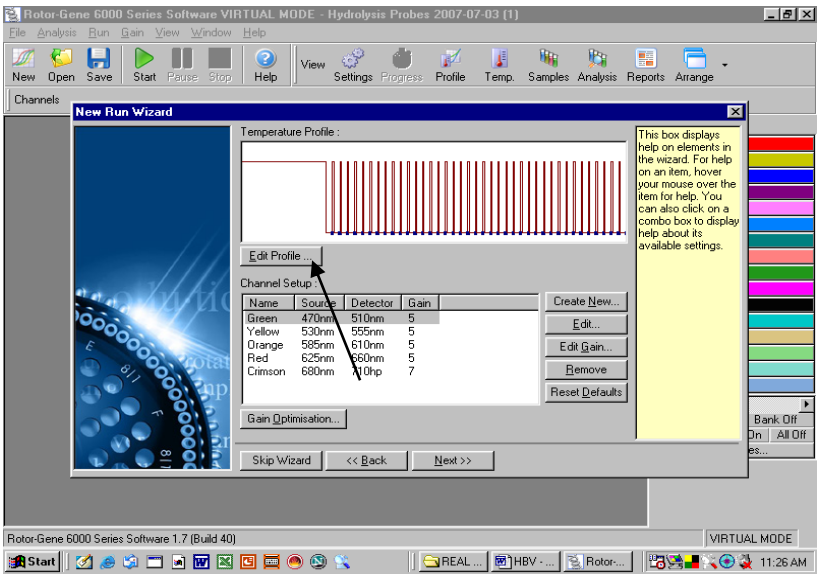


Fig. 25.

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

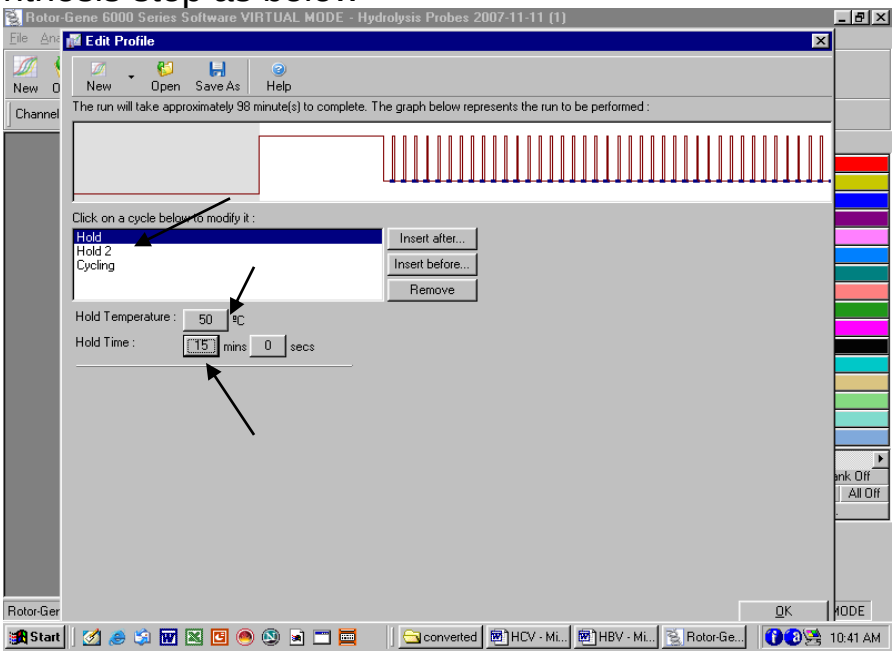


Fig. 26.

Second hold 95°C for 10 minutes as below

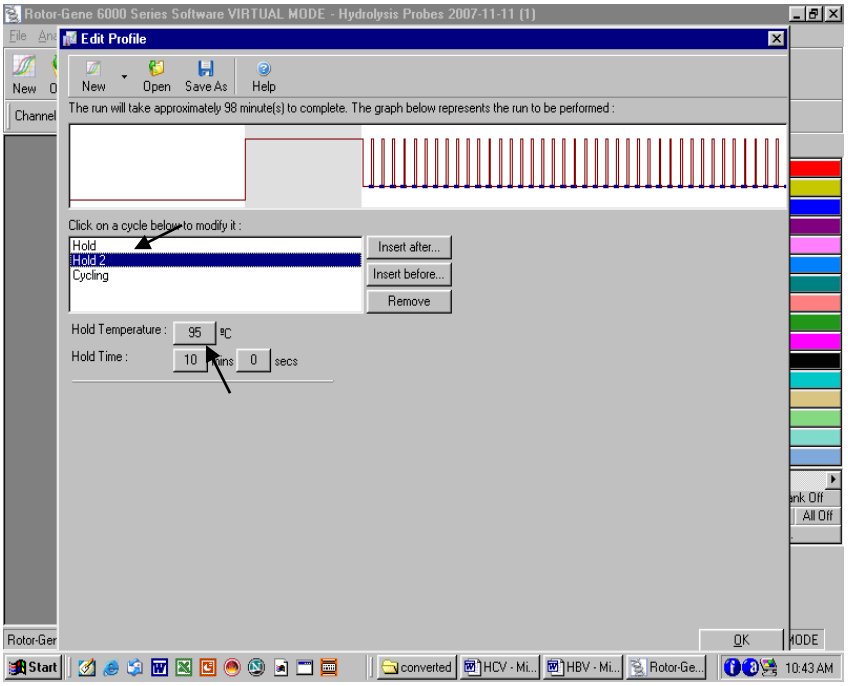


Fig. 27. Initial activation of the Hot Start enzyme. Generally the window will open with the Hold Temp as 95°C and the Hold Time as 10 minutes. In case it is different then set the same to as shown above.

j) Setting the Cycling and acquisition.

When clicked on Cycling the window will open as below.

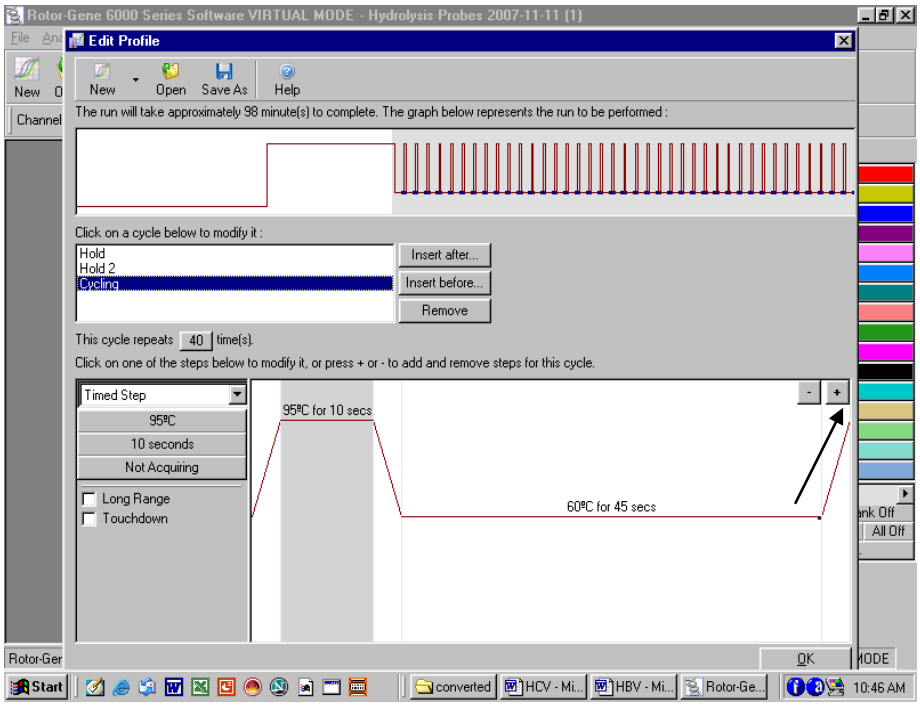


Fig. 28.

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.

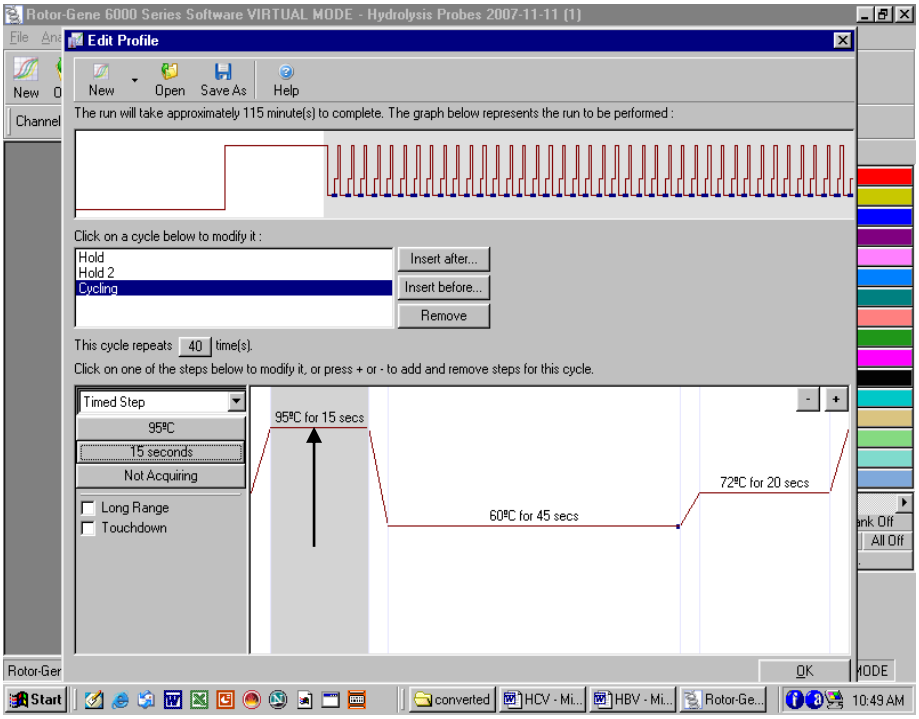


Fig. 29.

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

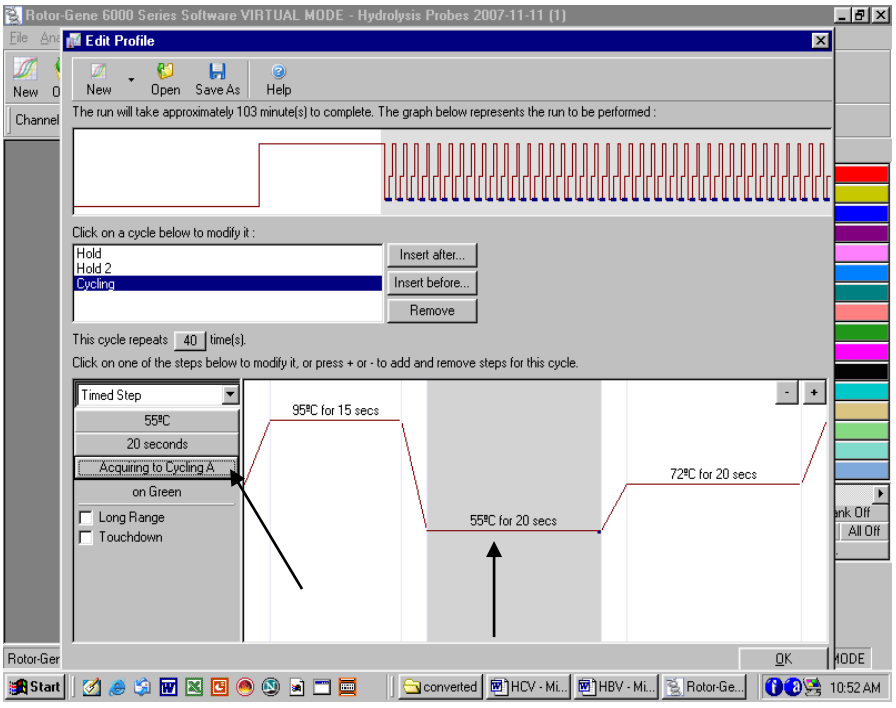


Fig. 30.

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), Yellow (JOE), Orange (Rox), Red (CY5)

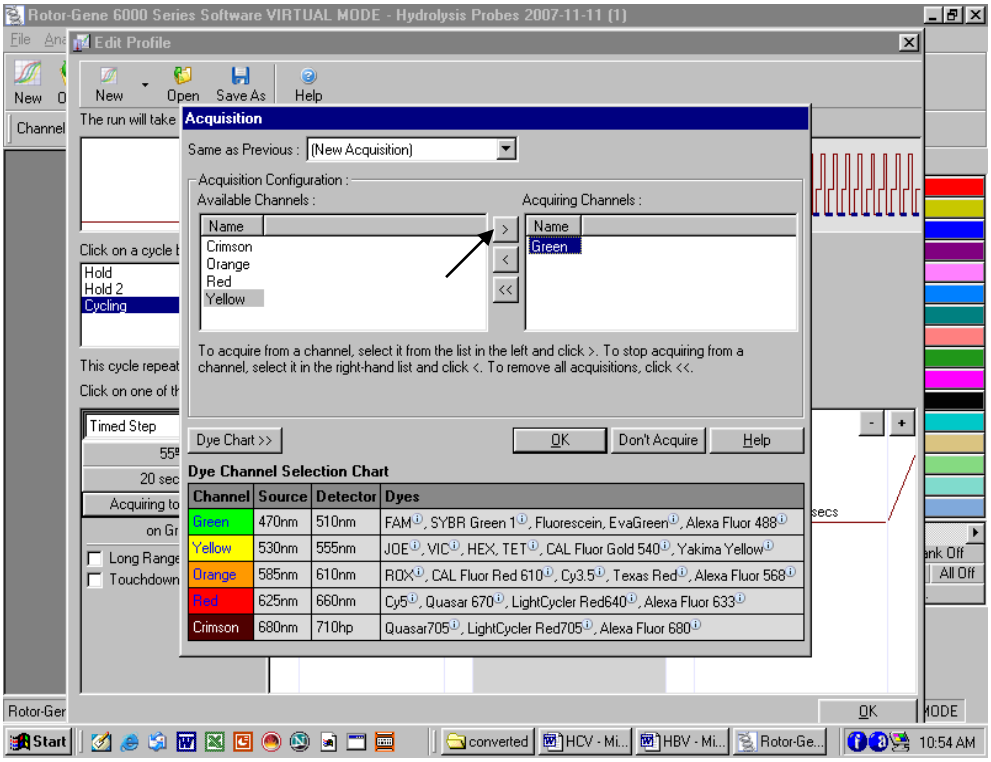


Fig. 31.

Highlight the Yellow and then press the right arrow. High Light Red then press right arrow & finally Highlight Orange & then press right arrow. Just see before shifting the yellow, Red and Orange to right that there is no other channel in the right except for Green. In case any other Channel appears besides Green on the right then the same be shifted to the left first.

Confirmation of Channels as shown below.

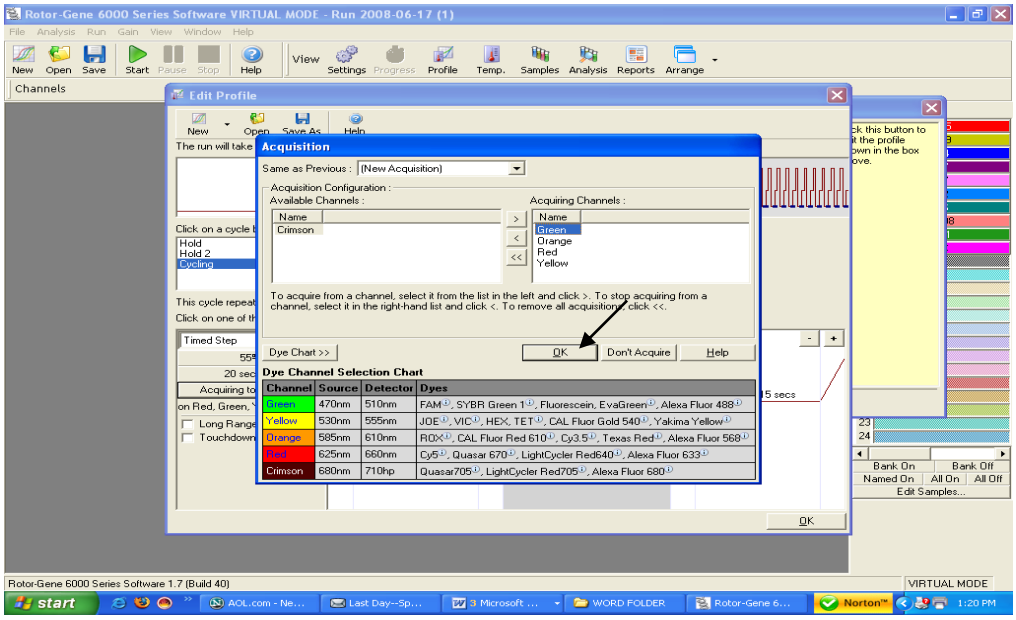


Fig. 32.

Once the Yellow and 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

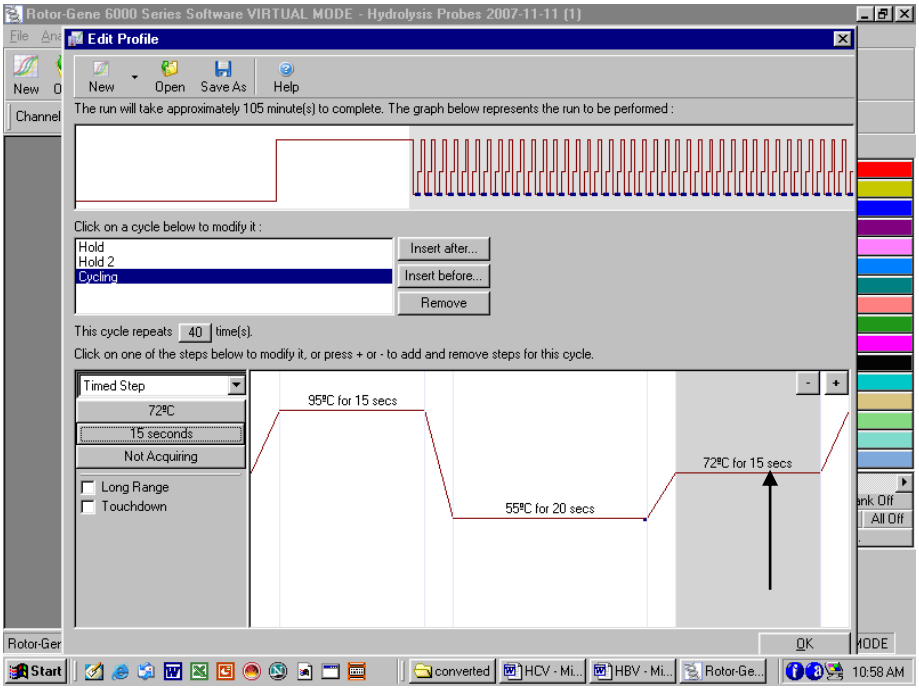


Fig. 33.

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

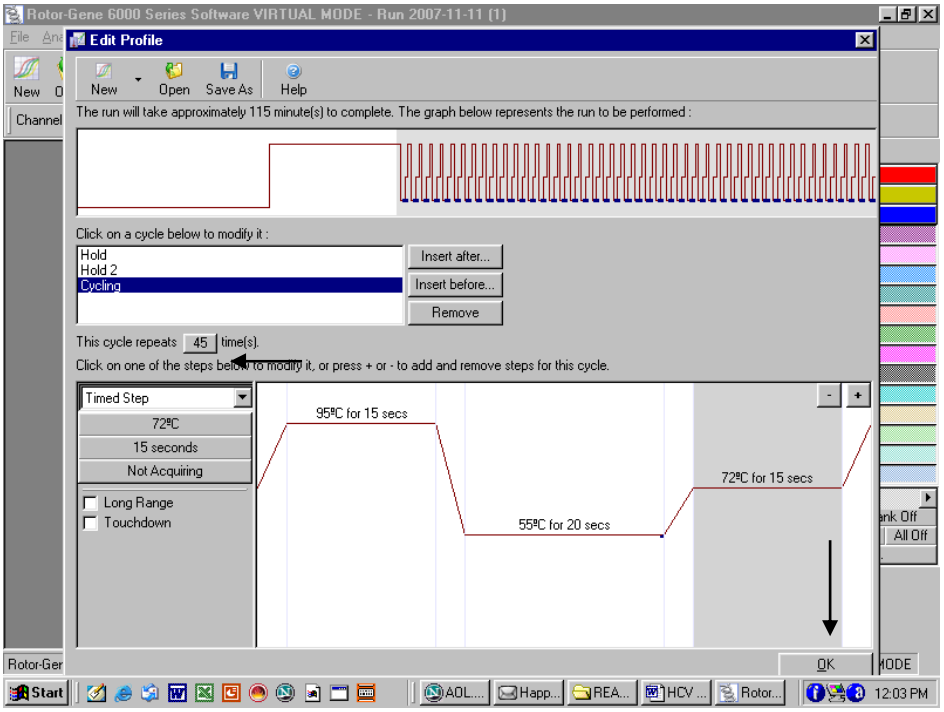


Fig. 34.

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.

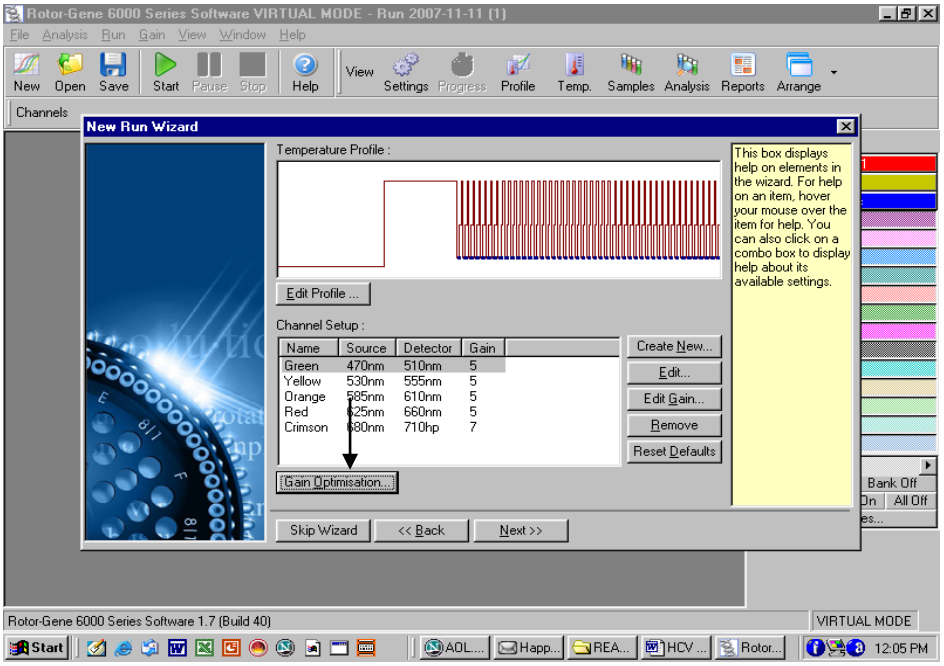


Fig. 35

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.36 .

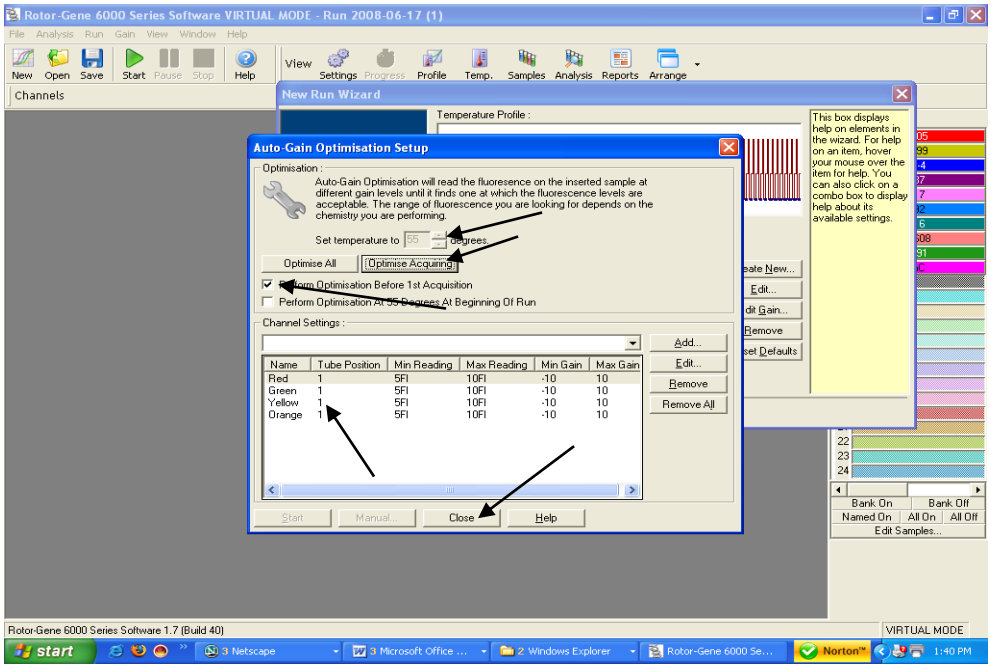


Fig. 36.

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 two channels i.e. Green and Yellow. 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.

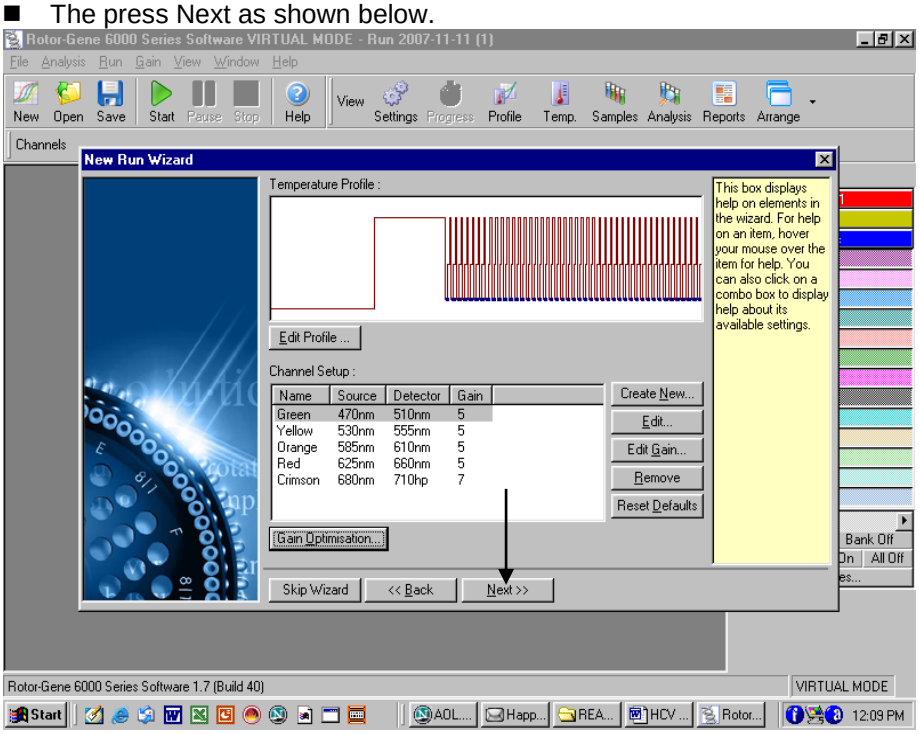


Fig. 37.

L) PRESS Start RUN

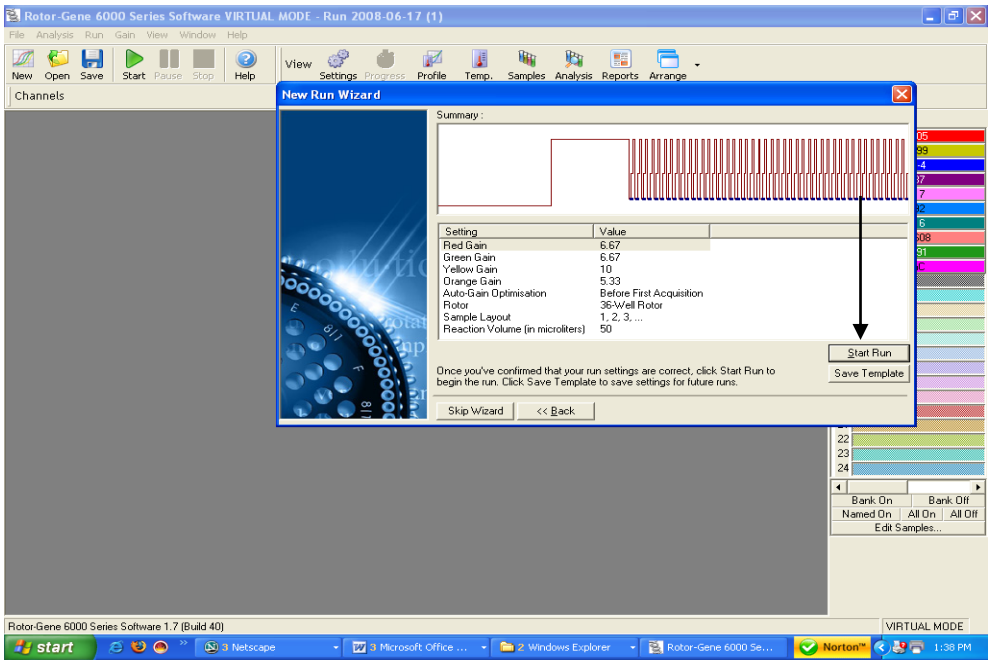


Fig. 38.

Saving the RUN File.

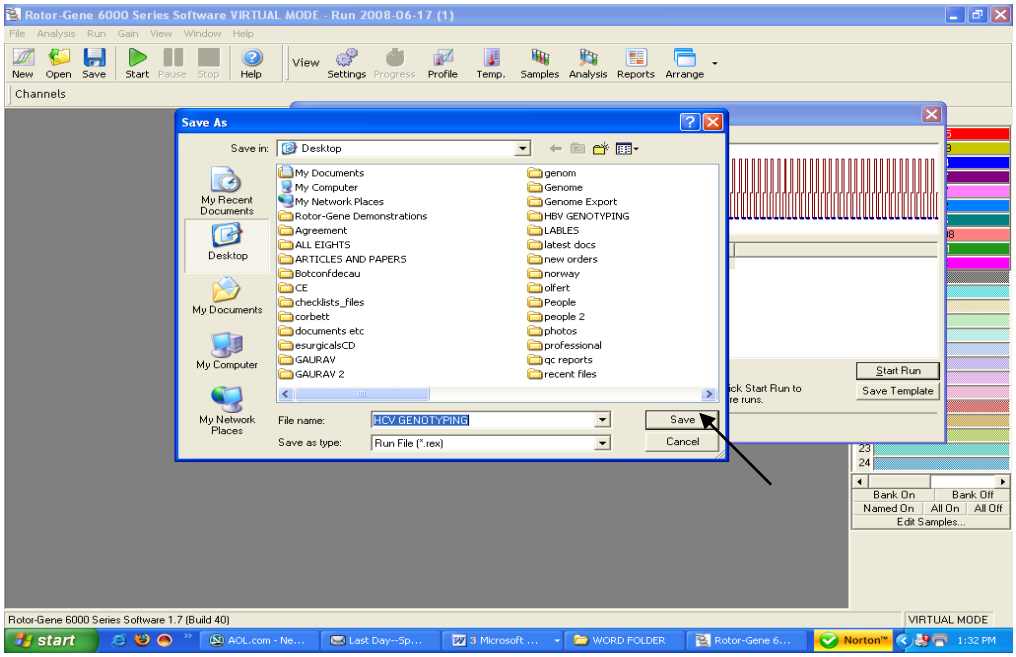


Fig. 39.

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

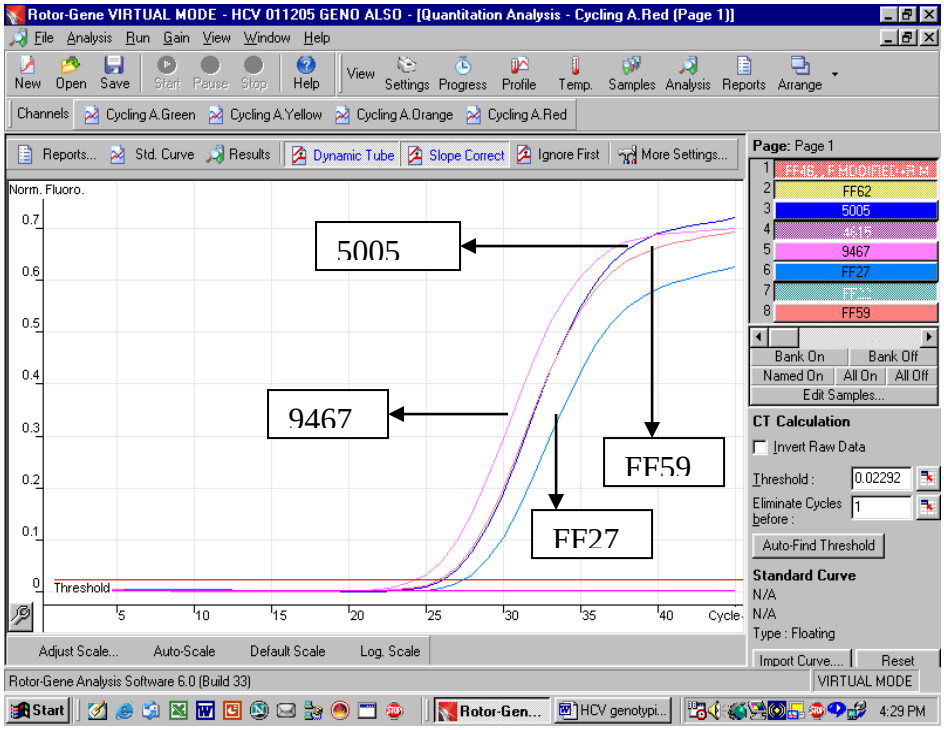
For Rotor Gene 2000/3000

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

The following results are possible:

A signal is detected in fluorescence channel Cycling A.Cy5.

The result of the analysis is positive: The sample contains HCV Genotype 1 RNA.

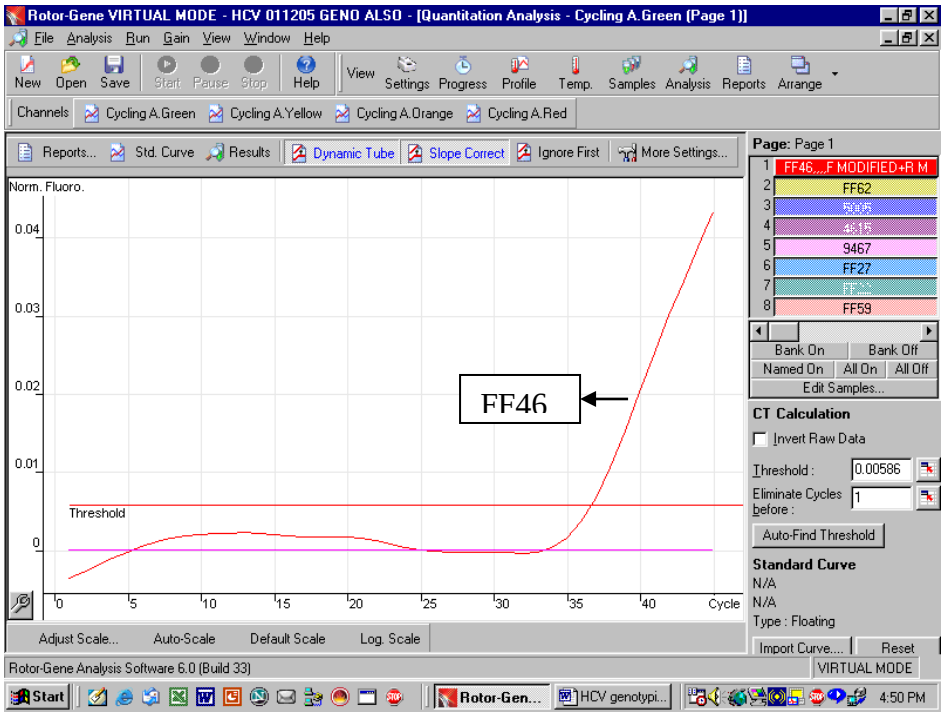


Cy5 Channel results 4 positive samples for HCV GenoType 1.

All Samples turning positive in CY5 channel are positive for HCV Genotype 1.

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

The result of the analysis is positive: The sample contains HCV Genotype 2 RNA.

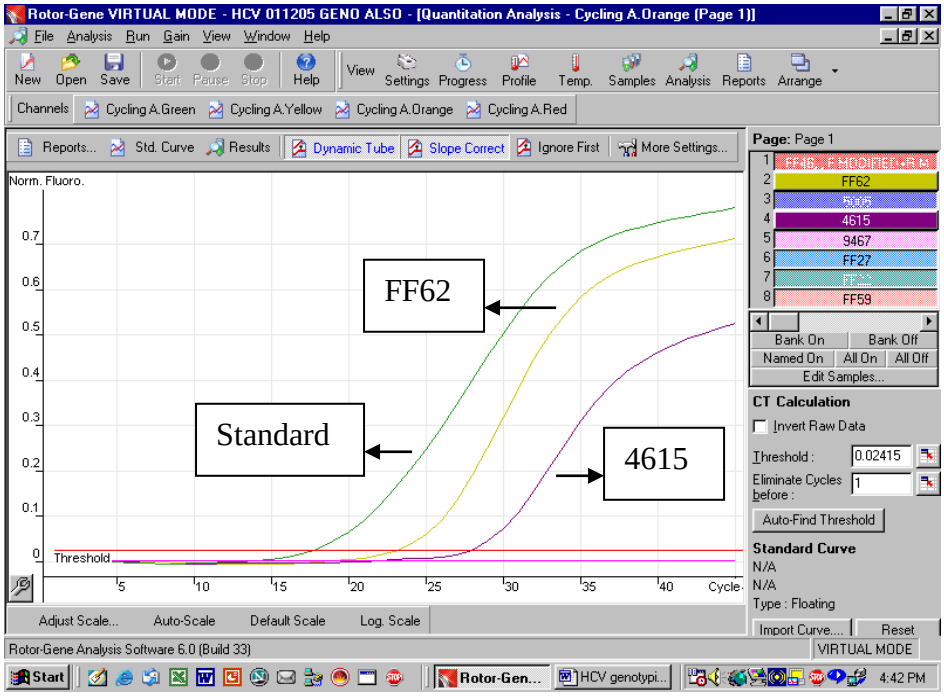


FAM Channel results 1 positive samples for HCV GenoType 2.

All Samples turning positive in FAM channel are positive for HCV Genotype 2.

A signal is detected in fluorescence channel Cycling A.ROX.

The result of the analysis is positive: The sample contains HCV Genotype 3 RNA.

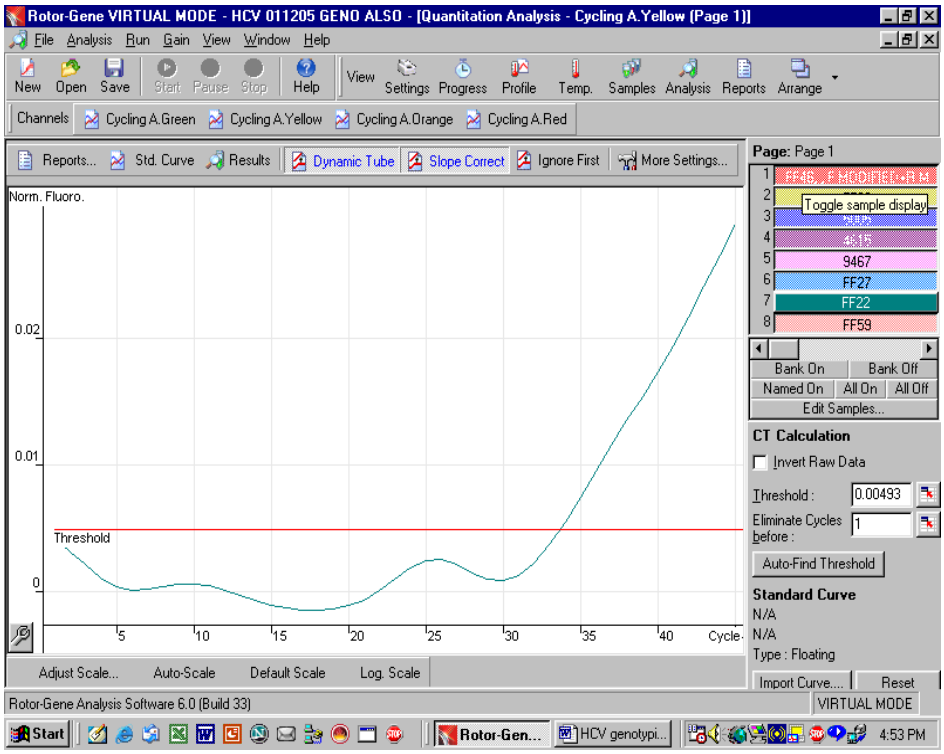


Rox Channel results 2 positive samples for HCV GenoType 3 & One positive control for Genotype 3.

All Samples turning positive in ROX channel are positive for HCV Genotype 3.

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

The result of the analysis is positive: The sample contains HCV Genotype 4 RNA.



Joe Channel results 1 positive samples for HCV GenoType 4.

All Samples turning positive in JOE channel are positive for HCV Genotype 4.

COMMENTS::

1. At times there could be cross signals in one or more channels for some samples due to the mutations occurring in the samples. On such a case the following thumb rule should be followed.

Look for the Ct Value of the sample in all the channels it is amplifying.
The sample should be classified as a Genotype where the Ct value is lower. e.g. if a sample is amplifying in CY5 Channel and ROX Channel both and the Ct value in Cy5 is 32 whereas in ROX it is 30. The sample will be classified in ROX and will be reported as Genotype 3.
2. Genotype 3b sequences are very identical to the other genotypes and can cross amplify in Genotype 1 channel or Genotype 2 channel. Hence look for the Ct value

and apply the thumb rule mentioned above and the sample should be reported accordingly.

NO signal is detected in fluorescence channel Cycling A.FAM, JOE, ROX & Cy5.

The sample contains HCV Genotype RNA other than HCV Genotype 1,2,3 & 4.

9. b) Generated Data Interpretation & Analysis

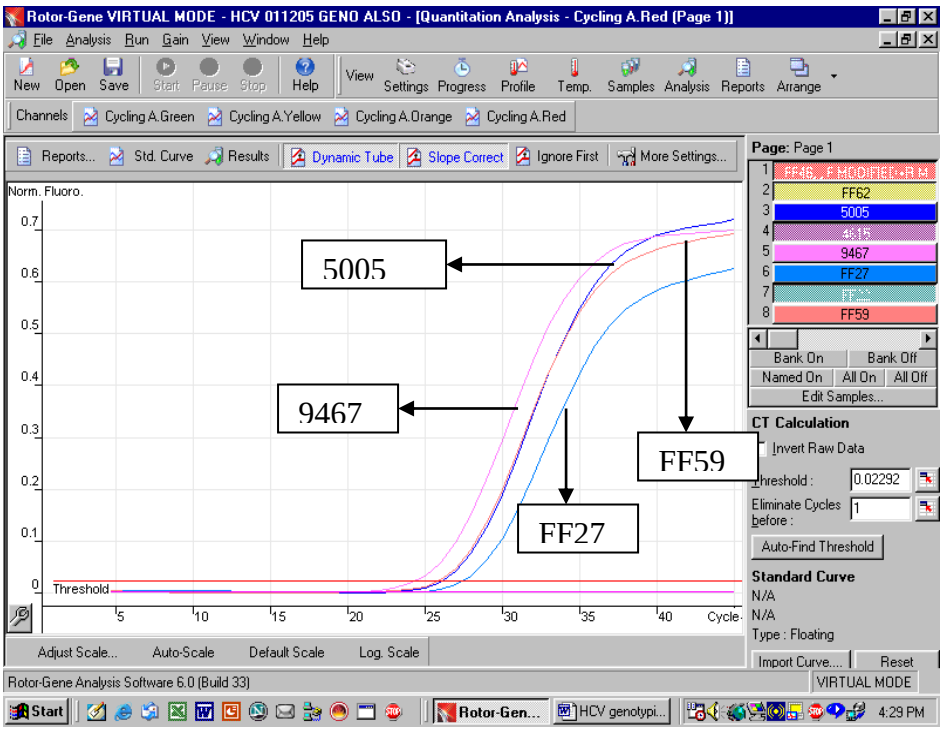
For Rotor Gene 6000

Data analysis is performed with the *RotorGene*™ software according to the manufacturer's instructions (*RotorGene*™ 6*000 Operator's* Manual).

The following results are possible:

A signal is detected in fluorescence channel Cycling A.RED.

The result of the analysis is positive: The sample contains HCV Genotype 1 RNA.

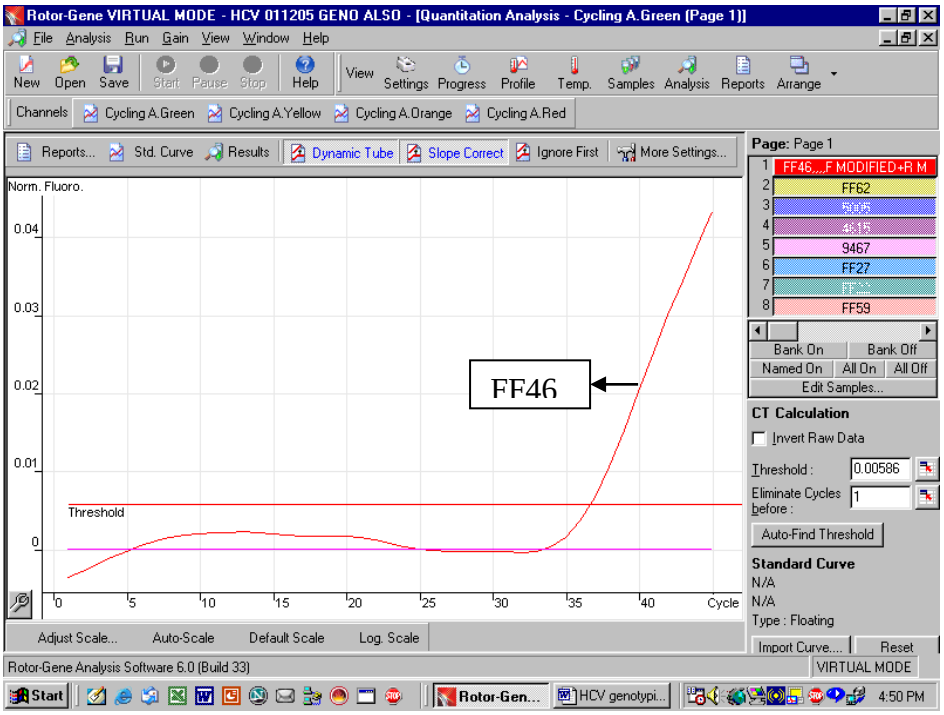


Red Channel results 4 positive samples for HCV GenoType 1.

All Samples turning positive in Red channel are positive for HCV Genotype 1.

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

The result of the analysis is positive: The sample contains HCV Genotype 2 RNA.

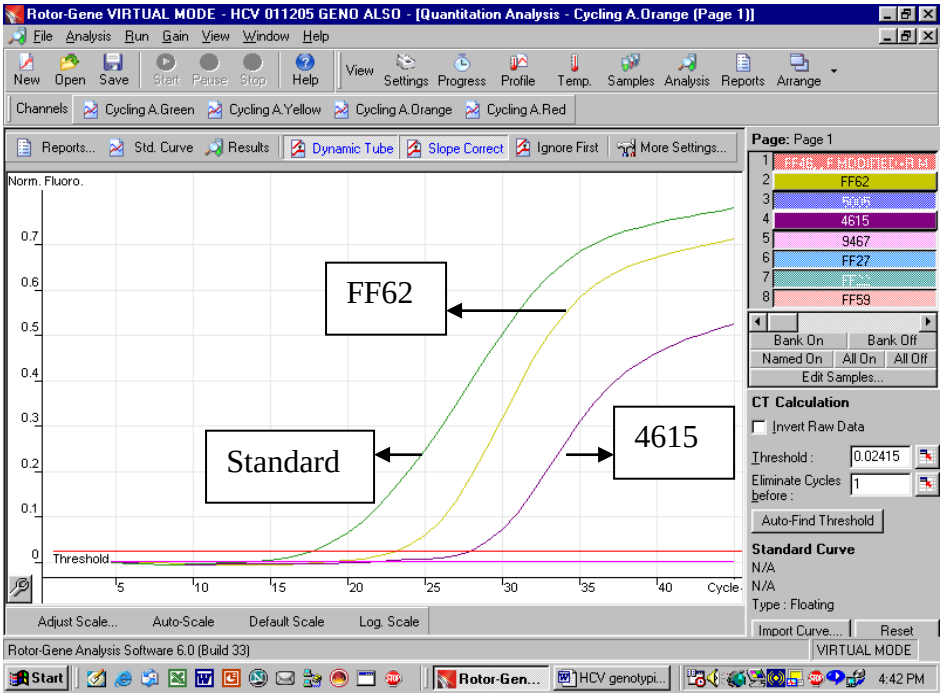


Green Channel results 1 positive samples for HCV GenoType 2.

All Samples turning positive in Green channel are positive for HCV Genotype 2.

A signal is detected in fluorescence channel Cycling A.Orange

The result of the analysis is positive: The sample contains HCV Genotype 3 RNA.

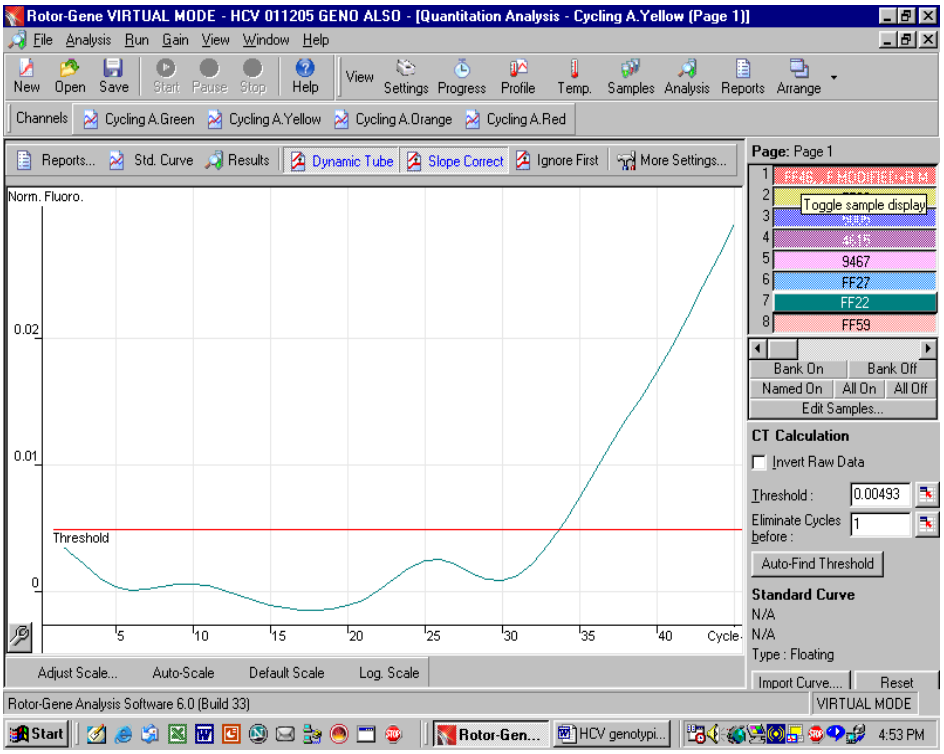


Orange Channel results 2 positive samples for HCV GenoType 3 & One positive control for Genotype 3.

All Samples turning positive in Orange channel are positive for HCV Genotype 3.

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

The result of the analysis is positive: The sample contains HCV Genotype 4 RNA.



Yellow Channel results 1 positive samples for HCV GenoType 4.

All Samples turning positive in Yellow channel are positive for HCV Genotype 4.

COMMENTS::

2. At times there could be cross signals in one or more channels for some samples due to the mutations occurring in the samples. On such a case the following thumb rule should be followed.

Look for the Ct Value of the sample in all the channels it is amplifying.
The sample should be classified as a Genotype where the Ct value is lower. e.g. if a sample is amplifying in Red Channel and Orange Channel both and the Ct value in Red is 32 whereas in Orange it is 30. The sample will be classified in Orange and will be reported as Genotype 3.
2. Genotype 3b sequences are very identical to the other genotypes and can cross amplify in Genotype 1 channel or Genotype 2 channel. Hence look for the Ct value and apply the thumb rule mentioned above and the sample should be reported accordingly.

NO signal is detected in fluorescence channel Cycling A.Green, Yellow, Orange & Red.

The sample contains HCV Genotype RNA other than HCV Genotype 1,2,3 & 4.

10. a.) Troubleshooting

For Rotor Gene 2000/3000

1. No signal with positive Control in fluorescence channel Cycling A. Rox. Green, Yellow & Orange.

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

  ➔ Repeat the PCR with corrected settings.

- The PCR conditions do not comply with the protocol.

  ➔ Repeat the PCR with corrected settings.

- The HCV Genotyping Super Mix *R1* has been thawed and frozen too often.
- The HCV Genotyping 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 HCV Genotyping super mix (R1).

10. b). Troubleshooting

For Rotor Gene 6000

1. No signal with positive Control in fluorescence channel Cycling A. Orange, Red, Yellow, Green

- Incorrect programming of the *Rotor-Gene™ 6000.*

  ➔ Repeat the PCR with corrected settings.

- The PCR conditions do not comply with the protocol.

  ➔ Repeat the PCR with corrected settings.

- The HCV Genotyping Super Mix *R1* has been thawed and frozen too often.
- The HCV Genotyping 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 HCV Genotyping super mix (R1).

11. Specifications

11.a Sensitivity and Reproducibility

In order to determine the sensitivity of the ***Geno-Sen's*** *HCV Genotyping Real Time PCR Kit*, a dilution series has been set up from 10^6 down to 10^0 IU/µl of HCV RNA and analyzed with the ***Geno-Sen's*** *HCV Genotyping 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*** *HCV Genotyping Real Time PCR Kit* is consistently 40 IU/ml. This means that there is 95% probability that 40 IU/ml will be detected.

Analytical Sensitivity in Conjunction with the ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) of the** ***Geno Sen's®*** *HCV GENOTYPING Real Time PCR RG Kit on ROTOR GRNR 3000/6000 was determined by Spiking a known negative Serum to a nominal 70 copies/ml. This was subjected to extraction using the* ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) eight times with starting volume of 150**µl & elution volume of 60µl.

All the Eight extractions were then analyzed with the ***Geno Sen's®*** HCV GENOTYPING *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 67 copies/ml.

Hence Analytical Sensitivity in Conjunction with the ***Geno Sen's*®** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) of the *Geno Sen's*®** HCV GENOTYPING *Real Time PCR RG was determined to be 70 copies/ml.*

11.b Specificity

In order to check the specificity of the ***Geno-Sen's*** HCV Genotyping Real Time PCR kit, different RNA & DNA listed below were analyzed with ***Geno-Sen's*** HCV Genotyping Real Time PCR Kit. None of these led to a positive signal with the ***Geno-Sen's*** HCV Genotyping Real Time PCR kit. Gene sequence analysis of the amplified region of HCV Genotyping shows a pronounced homology within the subtype. Besides which utmost care has been taken in selection of the primers & probes being used in the kit.

Vericella Zoster Virus	Hepatitis B Virus	N. Meningitis
Human Herpes Virus 1 & 2	Hepatitis A Virus	S. Pneumonia
Epstein barr Virus	Hepatitis E Virus	JEV
Cytomagalovirus	HIV-1	Mycobacterium tuberculosis
Chlamydia pneumonia	HIV 2	West Nile Virus
Parvovirus B 19	EnteroVirus	Staphylococcus aureus
Dengue Virus 1-4	H. Influenza	ChikunGunya Virus
Leprosy	Malaria	Scrub typhus
B.pseudomallie	West Nile Virus	Leptospira interrogans.

All the above samples were correctly identified by the ***Geno Sen's*®** HCV Genotyping Real Time PCR RG kit & all the 4 extremely low samples were accurately detected by the ***Geno Sen's*®** HCV Genotyping Real Time PCR RG kit & exhibited copies around 70 copies /ml or less than 70 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 Viral RNA depends on the number of Viruses 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:

i) Distribution and predominance of Genotype 3 in hepatitis C virus carriers in the Province of Kahramanmaras, Turkey

Ahmet Caliskan,Ozlem Kirisci; Esra Ozkaya; Sevinc Ozden; Seray; Tumer Selma Ates Guler; Hande Senol

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Department of Biostatistics, Faculty of Medicine, Pamukkale University, Denizli, Turkey
**Corresponding Author*: Ahmet Caliskan, Medical Microbiology Laboratory, Necip Fazil City Hospital, Kahramanmaras, Turkey. Tel: +90-3442282800, E-mail: ahmetsuna@msn.com
Received: November 5, 2014; **Revised:** March 2, 2015; **Accepted:** April 2, 2015

ii)

Correlation Study Between HCV Genotypes Distribution Pattern and Viral Load in a Tertiary Care Hospital in Kolkata, India

Article *in* Journal of Clinical and Diagnostic Research · January 2015

BHATTACHARJEE, DEBOJYOTI; MUKHERJEE, KHEYA; CHAKROBORTI, GOUTAM; GHOSH, RANADEEP; MANDAL, NABARUN; BOSE, MOHUA

May 2015
Journal of Clinical & Diagnostic Research;May2015, Vol. 9 Issue 5, p15

III) Determining hepatitis C virus genotype distribution among high-risk groups in Iran using real-time PCR

Marzieh Jamalidoust, Mandana Namayandeh, Sadaf Asaei, Nasrin Aliabadi, Mazyar Ziyaeyan
Shiraz, Iran

IV) The Relationship between Distribution of HCV-RNA and ALT-AST Levels with Genotypes of Hepatitis C Virus Infected Patients

Viral Hepat J 2013; 19: 67-70
10.4274 / Vhd.57966

Ahmet ÇALIŞKAN Sümeyra ALKIŞ KOÇTÜRK Pınar ERDOĞMUŞ Mustafa GÜL Assistant Professor

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 quanlitative Real time PCR kit.**
22	**Chlamydia pneumonia RG quantitative Real time PCR kit.**
23	**Streptococcous 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. Pseudomalie 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.**

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

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