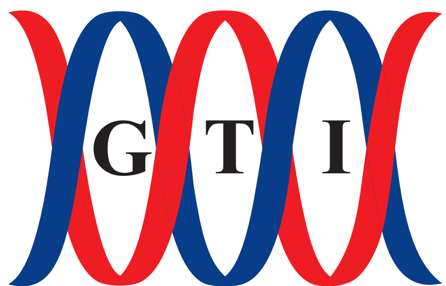
GTI PCR Reader-G

Instruction Manual



Genetic Technology Instrumentation

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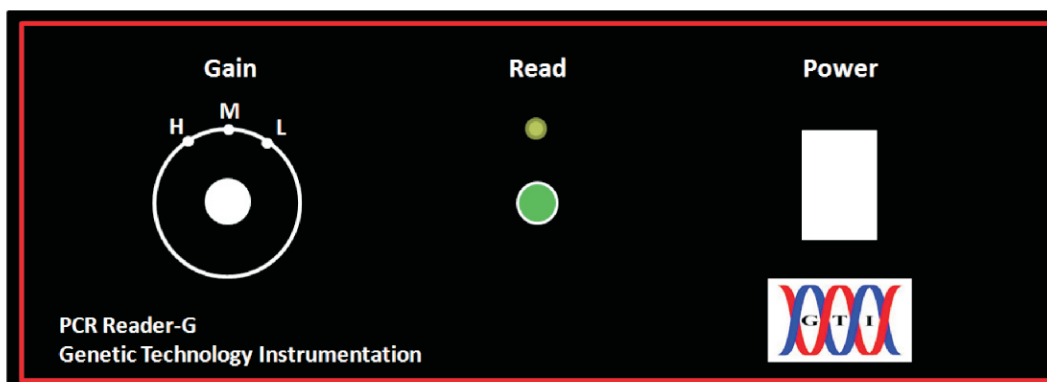
PCR can be used to measure the quantity of DNA or RNA in the sample. The quantitative PCR (Q-PCR) is very useful in diagnosis and monitoring response to treatment in infectious or malignant diseases. Q -PCR is mostly done by real time monitoring of amplification. The amplification is done by a pair of primers and a fluorescent labeled probe. The fluorescence emitted from the PCR reaction is monitored (visualized) after each cycle. The cycle number at which the amplification first appears is dependent on the quantity of target DNA. The amplification appears earlier when the target DNA is more and it appears late with decreasing concentration of target DNA molecules. However, the real time PCR requires expensive equipment that is often beyond the reach of small diagnostic or research labs. A crude method of Q-PCR is by gel electrophoresis of the end products of PCR. The latter is inherent with very low sensitivity and cross contamination of the amplified products from one sample to another.

A cheap alternate to Q - PCR is by measurement of end point fluorescence after PCR done by fluorescent labeled probes. This method does not require expensive real time PCR machine and is also free from the hazard of cross contamination by amplified products because the PCR vial remains closed. The PCR is done in an ordinary thermal cycler with the standard pair of primers and the fluorescent probe as is used in the real time PCR. At the end of the PCR total amount of fluorescence is measured in a mini - fluorometer.

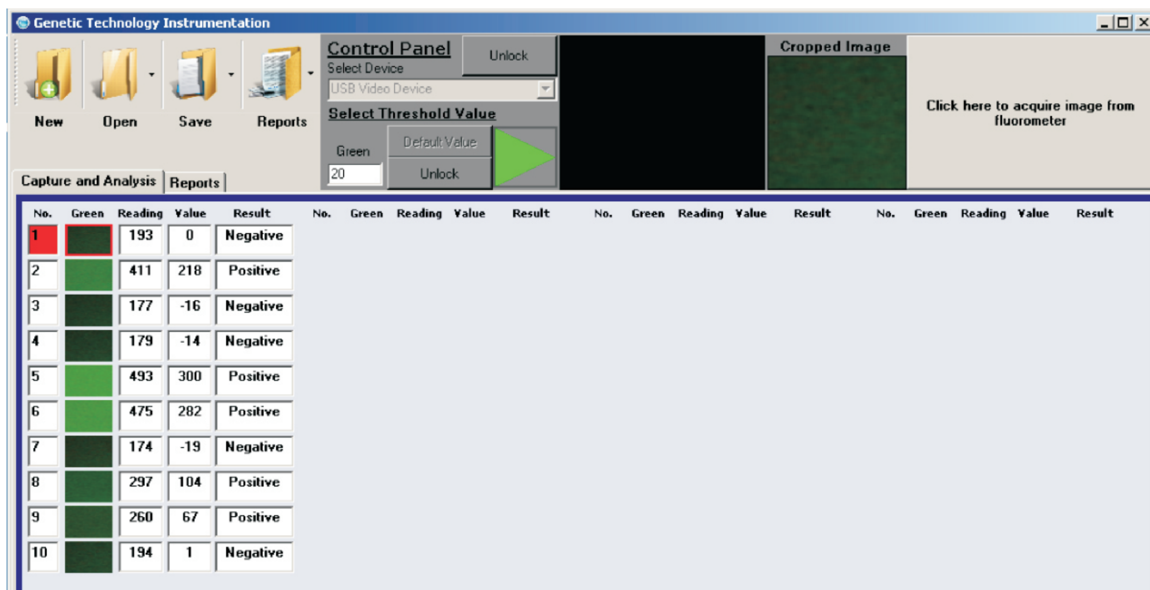
GTI PCR Reader G is a mini - fluorometer that is designed to read the green endpoint fluorescence (512 nm) in a PCR reaction vial with volume as low as 12ul. The fluorescence is measured through data acquisition software installed on a computer. Known positives and negatives can be used to obtain accurate quantitative PCR results. The accuracy of endpoint fluorometry and its correlation with real time PCR results increases with decreasing DNA concentration in the sample. End point fluorometry gives best results in the DNA concentration ranging from 100 copies of DNA to 300,000 copies of DNA in the sample.

PCR Reader -G



Installation:

1. Install the Data Acquisition and Reporting Software (DARS) on a PC.
2. Connect the PCR Reader- G with the PC through the USB port using the data cable provided. The PCR Reader - G does not require separate power source. It derives its power through the USB port of the PC.
3. Turn the unit on.
4. Run DARS by clicking the icon on the computer desktop. If you are running DARS for the first time select the USB video device from the control panel and lock the device. The selected device data is stored in the software and remains available for subsequent operations. The device selection will have to be repeated if the PCR Reader - G is disconnected from the computer.



Reading:

1. Open sample compartment of the PCR Reader - G and insert a known “positive control” PCR reaction vial.
2. Select new from the DARS menu bar. A bright green fluorescence should be visible in the result view window.
3. Gain setting on the PCR Reader -G may be set between H:high - M:medium - L:low depending on the intensity of the green fluorescence from the positive sample. The fluorescence should not be too bright or too low.

4. Take reading of the “positive control” by pressing the read button on the PCR Reader -G or by clicking the sample read button from the DARS menu bar.
5. The recorded image would appear as “cropped image”.
6. The green image and its reading as numerical value in arbitrary fluorescence units also appears in the capture and analysis window. Any sample/control that is read first is automatically assigned serial number “1”. All subsequent samples/controls are assigned consecutive serial numbers.
7. Take reading of the “negative control” and the “unknown samples”. The results appear in a consecutive order in the capture and analysis window. Each batch can store the results of up to 64 samples.
8. The data acquisition software also allows editing of the serial number of each sample. Editing can be done by clicking on the serial number to be edited.

Selection of reference:

1. “Reference” is a “negative control” whose reading is used to calculate the background fluorescence in a PCR reaction. The background fluorescence reading of the “reference” is subtracted from all other samples. Each batch of samples must include a “negative control” for use as “reference.“
2. Select “negative control” as “reference” by clicking on its green image in the capture and analysis window. The selection is highlighted by a red border around the image. The “reference” must be selected out of the first 16 samples.

Threshold value:

1. Threshold value is the cut off limit between a clear negative and a clear positive result. In other words it is the amount of non -specific fluorescence that may be emitted in some PCR reactions. The data acquisition software comes with a default threshold value of 20 that may be changed as desired. It is recommended that each user should define its own threshold value for any particular PCR assay. The threshold value may be ascertained by calculating the $\pm$2SD of the values of several known negatives. The threshold value whether default or user defined may be locked for permanent use.
2. Enter the threshold value in the DARS control panel.

Calculation of value and result:

1. “Value” of each sample is the amount of “specific fluorescence” in a PCR reaction. It is directly proportional to the amount of amplification and hence the amount of target DNA in the sample. The “value” is measured in arbitrary fluorescence units.

2. The “value” of each “sample” and the “positive control” is calculated by subtracting its reading from that of the “negative control”.
3. DARS can calculate the “value” of each “sample” and the “control” by pressing the ▶ button.

Qualitative results:

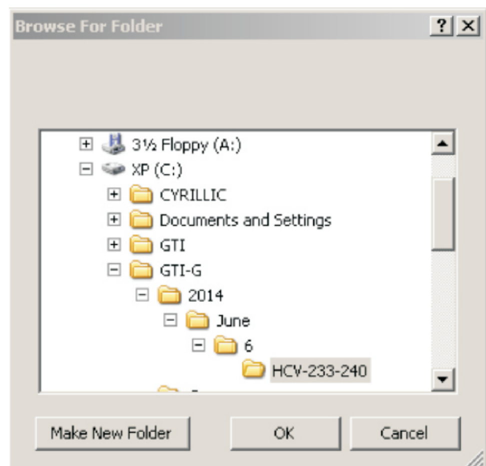
1. The result of each “sample” is categorized as “Positive” or “Negative” depending on the “threshold” value entered. The samples with “values” exceeding the “threshold value” are assigned “Positive” and the others “Negative”.

Saving and opening a result file:

1. DARS allows storage of each batch of results by using the file save option. A maximum of 64 sample results can be included in each file.
2. The file may be stored with any name. DARS stores all files in the default system directory C:\GTI-G with a sub-directory structure for each year, month and day.
3. The stored files may be opened by using the file open menu.

Report generation:

1. DARS allows presentation of the stored data files in a printable report format.
2. Open the stored data file whose report is to be generated.
3. Press the “Report” button from the DARS menu bar.
4. The report opens in the “report window” from where it can be printed.



End Point Analysis Report

Batch Name HCV-233-240
Threshold 20
Date 6/6/2014

Sample No	Green Reading	Green Value	Green Result
1	193	0	Negative
2	411	218	Positive
3	177	-16	Negative
4	179	-14	Negative
5	493	300	Positive
6	475	282	Positive
7	174	-19	Negative
8	297	104	Positive
9	260	67	Positive
10	194	1	Negative

Quantitative results:

1. PCR Reader-G is primarily meant for reporting qualitative results. However, each qualitative result is also expressed in terms of Relative Fluorescence Units (RFU). The Q-PCR results of samples measured at endpoint in RFU show linear correlation with the Q-PCR results obtained by real time PCR. The correlation is highly significant in the DNA copy number ranging from 100 copies/ml to 300,000 copies/ml (r=0.998). However, the degree of correlation becomes poor as the DNA copy number increases above 300,000 copies/ml.
2. PCR Reader-G may be used for Q-PCR by plotting the fluorescence readings of ten fold dilutions of a suitable DNA standard. The standard curve may be used to calculate the approximate DNA copy number in the unknown samples.

Specifications:

End point qualitative and semi-quantitative analysis of FAM and SYBR green labeled PCR products

0.2 ml PCR tube format

Sample volume 12-30 µl

Approximately 10 sample reads per minute

User friendly software for data acquisition, analysis and reporting

Linear correlation between GTI-Green PCR Reader and real time PCR for 100 copies/ml to 300,000 copies/ml (r=0.998)

Wide range of applications in PCR of infectious diseases and malignancies for example HCV, HBV, Dengue, TB, Malaria, Leukemia, Lymphomas etc.

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