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PCR API DOCUMENTATION

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INTRODUCTION

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The **Covmatic + BioRad API**, written in C#, with the .NET framework, is used for controlling the BioRad PCR thermal cyclers **C1000 and C1000 Touch with CFX96 optical reaction module**. The API (a .exe program) communicates with the PCR machines by exchanging XML files that follow a schema defined by Bio-Rad, described in the below documentation. The PCR machines are connected to the PCs through a **USB serial port**

Though the software like CFX manager, CFX Maestro and CFX Dx Manager are tailor-made for BioRad machines, the API gives more flexibility and allows the user to run customized functions for automation.

The Bio-Rad documentation for the original API and XML schema is available on the following link:
https://github.com/covmatic/PCR_station/blob/main/PCR_Documentation/BioRad-CFX%20Manager_Software_API_Reference_Guide.pdf

To understand the PCR hardware (C1000 Touch), refer to the following documentation:
https://github.com/covmatic/PCR_station/blob/main/PCR_Documentation/BioRad_C1000_Touch.pdf

To understand the PCR hardware (C1000), refer to the following documentation:
https://github.com/covmatic/PCR_station/blob/main/PCR_Documentation/BioRad-C1000.pdf

To understand the Bio-Rad CFX Maestro/Manager software, refer to the following documentation:
https://github.com/covmatic/PCR_station/blob/main/PCR_Documentation/BioRad-CFX_MAESTRO-User_Guide.pdf

1. INTRODUCTION

The API supports 3 different kinds of testing kits. The following documents present the biological details about the testing kits.

KHB SARS-CoV-2 kit:

https://github.com/covmatic/PCR_station/blob/main/COVID19_PCR_Test_kits_docs/KHB_kit.pdf

PrimerDesign COVID-19 kit:

https://github.com/covmatic/PCR_station/blob/main/COVID19_PCR_Test_kits_docs/PrimerDesign_kit.pdf

AllPlex SARS-CoV-2 kit:

https://github.com/covmatic/PCR_station/blob/main/COVID19_PCR_Test_kits_docs/AllPlex_kit.pdf

However, new testing kits can be added to the API code.

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You will need the following to run the API:

❑ A Windows OS, preferably Windows 7 or higher

❑ Installed CFX Manager / CFX Dx Manager / CFX Maestro v3.1.1621 or higher (**only one** of these three)
  ▪ Note that the Location of installation must be the default one in C:\ drive. **DO NOT CHANGE THE DEFAULT LOCATION OF INSTALLATION WHILE INSTALLING THE SOFTWARE**

❑ Make sure that the Number Format is the **English (United States)** format
  ▪ Go to Control Panel > Clock and Region > Change date, time or number formats
  ▪ Ensure that the "Format" under the tab "Formats" is set to English (United States)
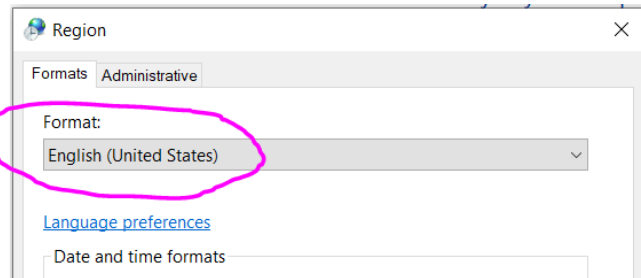
❑ You need permission to access and modify folders in the "C:\" drive
❑ It is Recommended to also use English (United States) as the **display language**

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You will need the following to edit/modify the API:

❑ All the pre-requisites required to "Run/use" the API mentioned in section **1.c**

❑ Microsoft Visual Studio version 2017 or higher with C# .NET framework 4.6.1 or higher

❑ NuGet Package: Csv helper (v15.0.5 or higher)

❑ NuGet Package: Newtonsoft Json (v12.0.3 or higher)

HOW TO USE?

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- ❏ Make sure you have installed **CFX Manager v3.1.1621** or higher. CFX Maestro or CFX Dx Manager will also work. But make sure that you have **only one CFX software** installed to avoid conflicts

- ❏ Ensure that all requirements stated in the section **1.c** "Pre-requisites for using the API" are met

- ❏ The Bio-Rad PCR thermal cycler (C1000 or C1000 touch) must be connected to the local computer via a **USB serial** and must be switched-on.

- ❏ Confirm that the PCR machine is in "IDLE" state before using the API

2.b. Setting up the API

❏ Go to Local Disk Drive "C:\" and copy paste the folder titled "PCR_BioRad" from the link below. The directory on your local computer should look like "C:\PCR_BioRad".

(https://github.com/covmatic/PCR_station/tree/main/PCR_BioRad)

❏ Under the Directory "C:\PCR_BioRad", check if the following directories exist:
1. C:\PCR_BioRad\APIs
2. C:\PCR_BioRad\csv_results
3. C:\PCR_BioRad\json_results
4. C:\PCR_BioRad\pcrd_results
5. C:\PCR_BioRad\plate_files
6. C:\PCR_BioRad\protocol_files
7. C:\PCR_BioRad\sim_protocol_files

❏ If any one of the above-mentioned directories do not exist, then contact the programmer

❏ Go to the directory C:\PCR_BioRad\APIs. You will find the versions of the API here. Please use the latest version ONLY.
❏ For example, in this case, the latest version is **v1.3**
❏ **Open this latest version directory**

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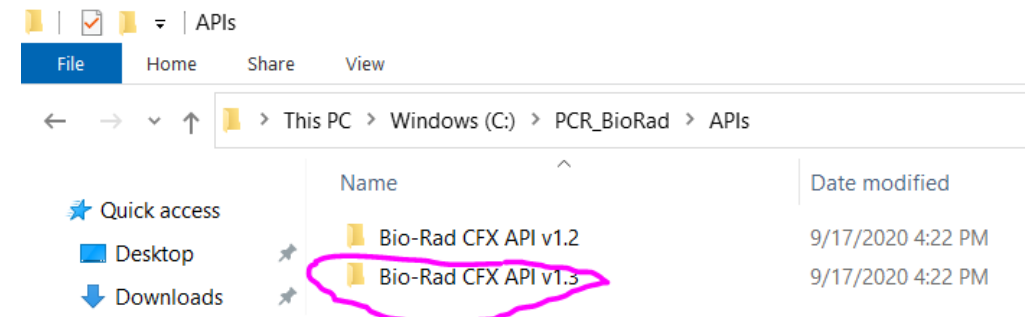
❑ In this directory, search for the executable file named **"BioRad.Example_Application.exe"**. So for example, if you are in version v1.3, the complete path of the executable file will be **"C:\PCR_BioRad\APIs\Bio-Rad_CFX_API_v1.3\BioRad.Example_Application.exe"**

❑ **Create a shortcut** of this executable file and paste it wherever you want. On the desktop for example

❑ You can run the software by double-clicking on the executable file

❑ In case the firewall or the virus and malware protection on your computer blocks it, you will have to make an exception for this API in Firewall Settings or anti-virus settings

❑ Close the API and wait for a few seconds

❑ Launch the CFX Manager or Maestro and Go to **Tools > Options > Data Export Settings**
  ▪ Check **true** on the option "Automatically export all data sheets at the end of run"
  ▪ Select the **Data Format as .csv**
  ▪ Use this folder: "C:\PCR_BioRad\csv_results"

❑ The settings must look like this →

❑ **IMPORTANT: It is not possible to have the CFX Manager or Maestro AND the API running at the same time. If you want to run the API, then close/exit the CFX Manager if you want to run the CFX Manager, close/exit the API**

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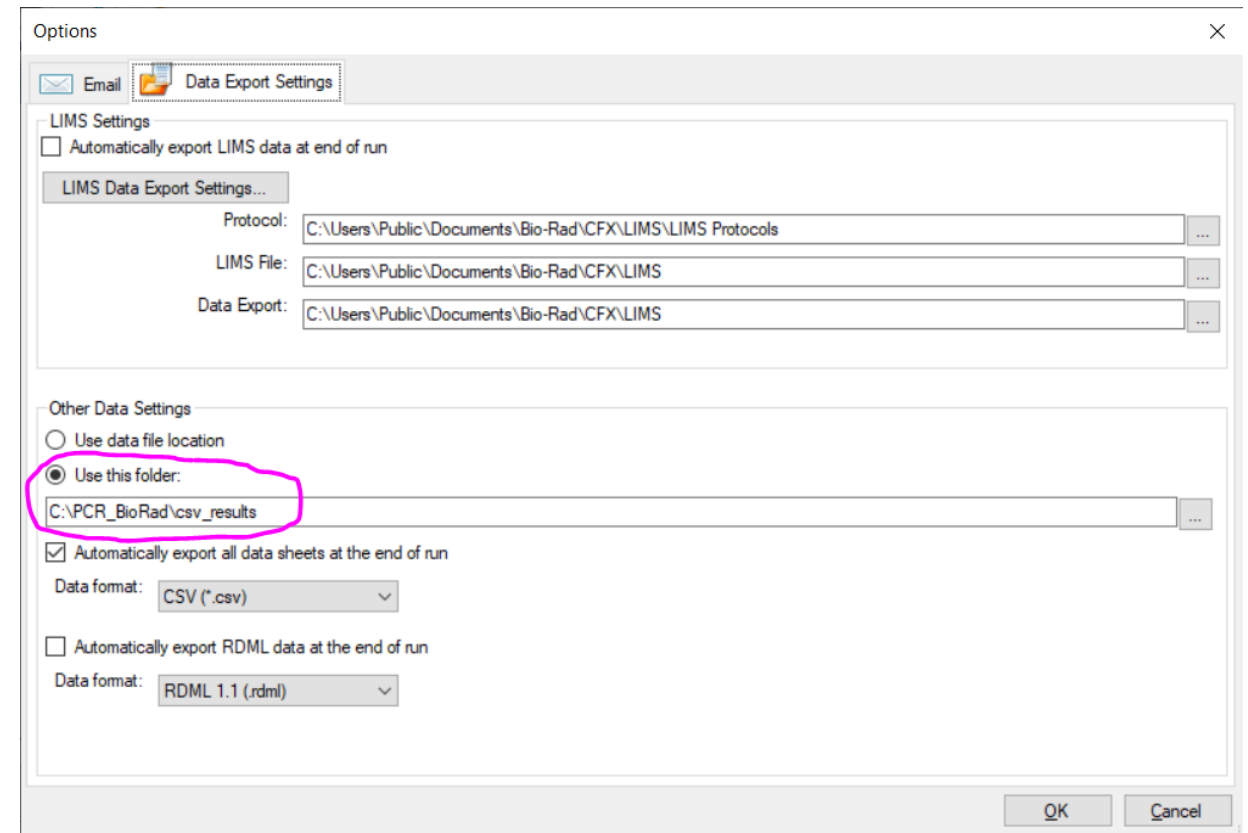
- ❑ The plate files for the following test kits are already created and saved in the "PCR_BioRad\plate_files" folder. The Negative Control in these plate files is at position A01 and the positive control is at position A10
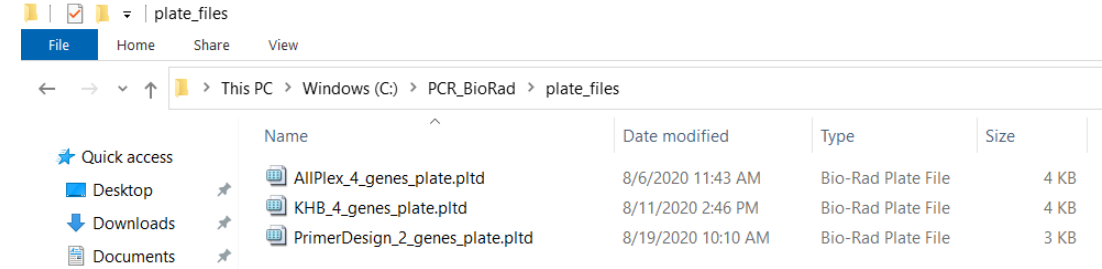

- ❑ If you want to create a new Plate file for another test kit, then follow the instructions provided in the BioRad CFX software documentation. **You can create new plate files only with CFX manager or CFX Maestro, not with the API.**

- ❑ **IMPORTANT:** While creating a plate file, select only those fluorophores that are required. For example, the **KHB test kit** requires 4 fluorophore channels, namely, HEX, FAM, Cy5, and ROX. So make sure that in your plate file, only these 4 fluorophores are selected. **NO OTHER FLUOROPHORES MUST BE SELECTED**
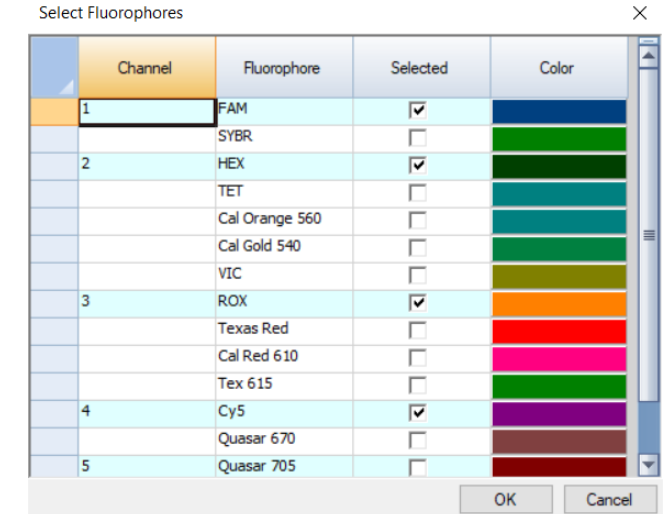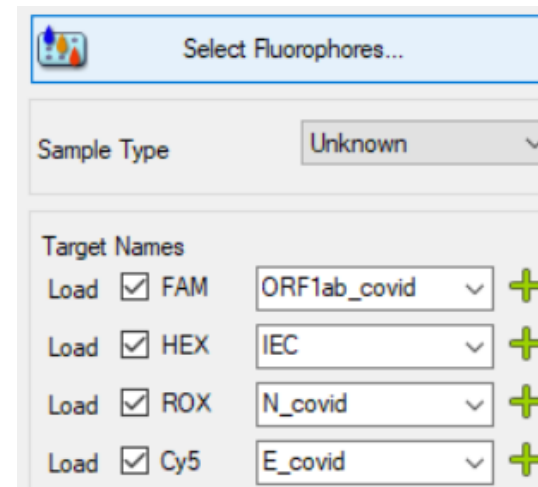

Snapshots from CFX Manager – Edit Plate File

❑ Each plate must have a negative extraction control sample (NEC) and a positive control sample (PCT). You must specify the negative control and positive control samples location in the plate file by setting the Sample Type as shown in the picture →

Snapshots from CFX Manager – Edit Plate File

❑ Each plate file MUST have only one Negative Control and only one Positive Control. NO MORE NO LESS.

❑ If you do not specify the well locations of Negative Control and Positive Control, the API will assume that **A01 is the Negative Control and A10 is the Positive Control**

❑ Also, in the CFX Manager, you can specify the type of Well Plate: Clear or White by going to **Settings > Plate Type**

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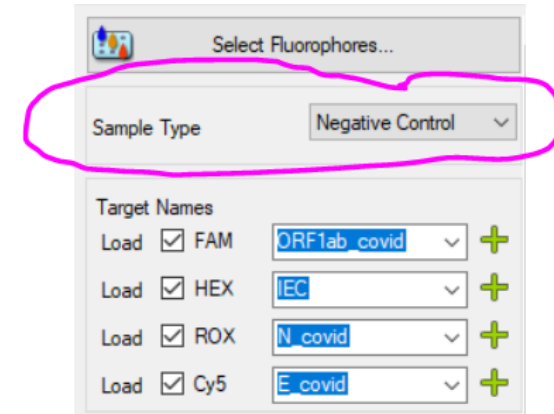
❑ You can create new protocol files from CFX Manager/Maestro by referring to the documentation provided in **section 1.a**

❑ The protocol files for the test kits mentioned in **section 1.b** have already been created and saved in the Directory "PCR_BioRad\protocol_files"

❑ You can launch the API directly from the .exe file or its shortcut (see **section 2.b**

❑ In case you are using the Covmatic digital interface, the web page will launch the API automatically once you have scanned the plate barcode and clicked on Go. Let's say that the plate is loaded into the PCR and the API is launched. It looks like this →

❑ Now, you have to wait for the API to detect the PCR connected to the computer. **PLEASE MAKE SURE THAT THE PCR IS CONNECTED TO THE COMPUTER WITH A USB SERIAL AND DO NOT CLICK ON ANY BUTTONS IN THE API UNTIL IT DETECTS THE CONNECTED PCR.**
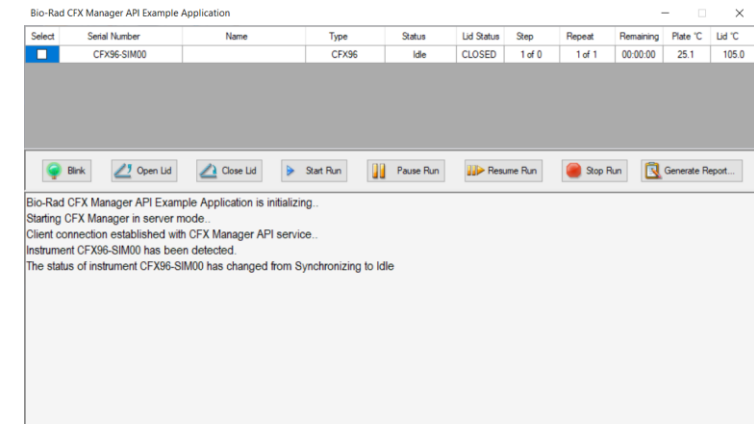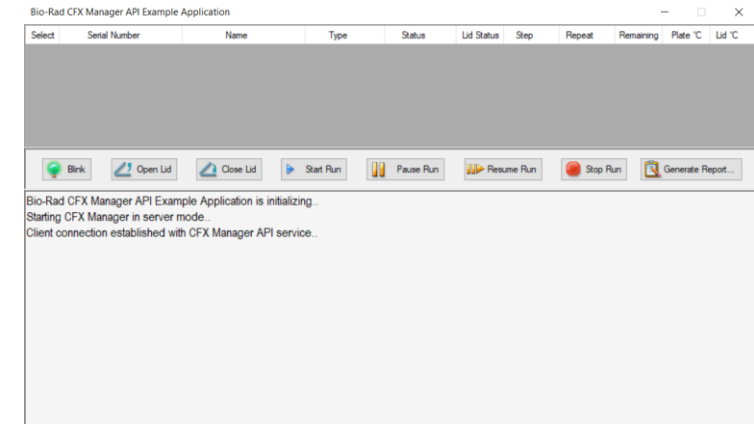
❑ You can see in this picture that the API has detected a connected PCR

❑ Once detected, Click on "Start Run". A new window will Open

❑ From this window, you can select the Protocol File, plate file, and the Folder where you want to store your results and reports

❑ By default, the files and folders shown in the picture are selected

❑ DO NOT CHANGE THE OUTPUT FILE NAME IF YOU ARE USING THE COVMATIC software interface

❑ Click on "Start Run" to start the protocol

❑ After the Protocol is finished or after you have stopped the protocol, **don't do anything The API will shut down by itself. After few seconds. DO NOT ATTEMPT TO CLOSE THE API APPLICATION MANUALLY.** This can cause errors
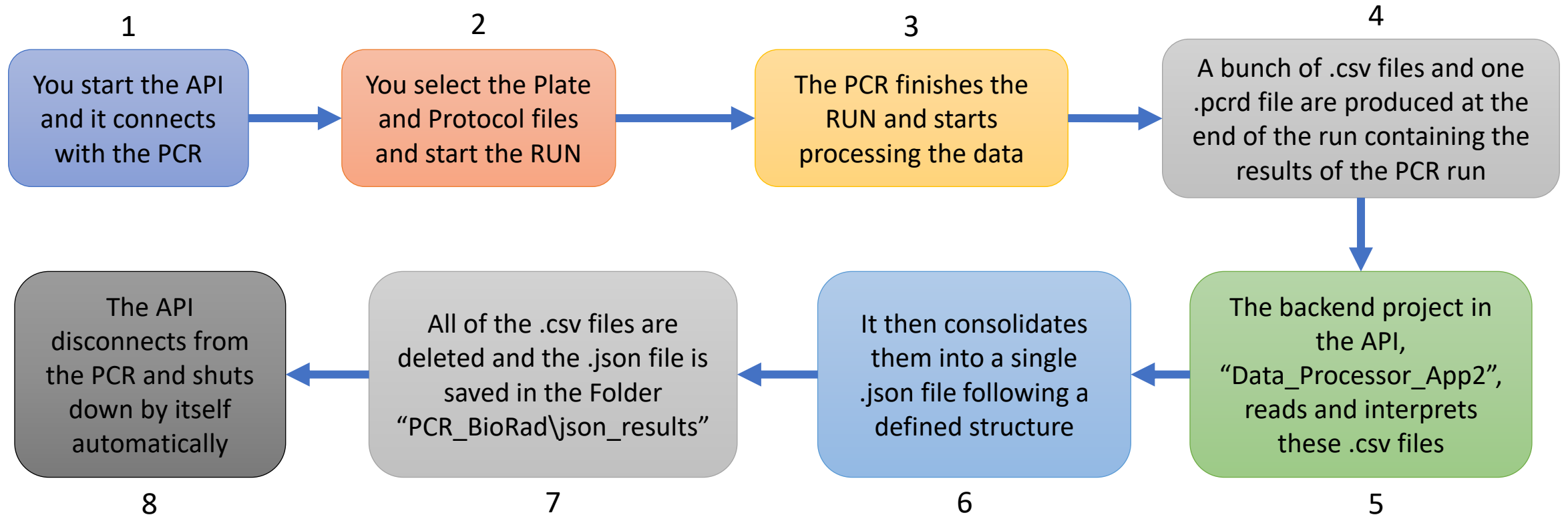
Covmatic
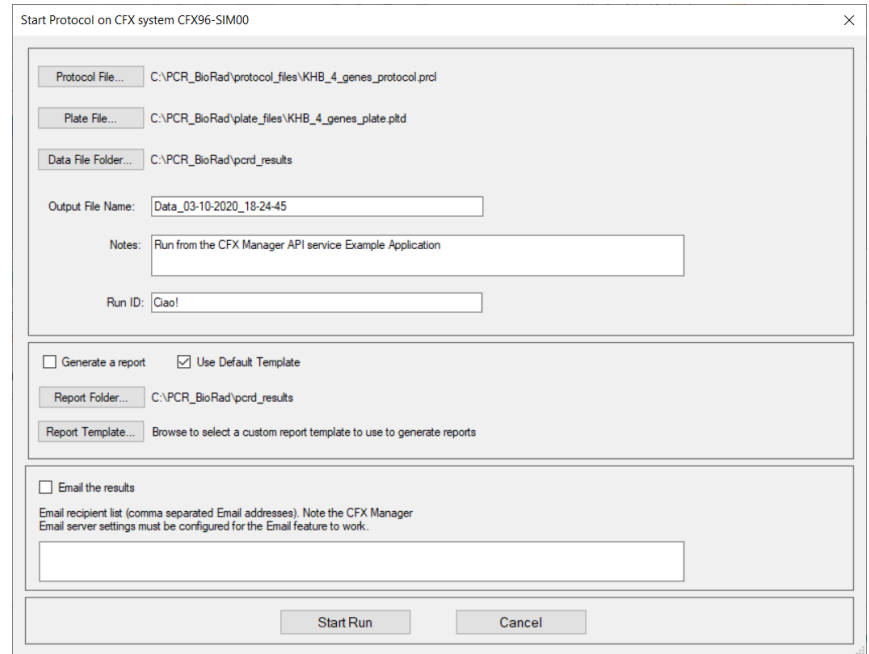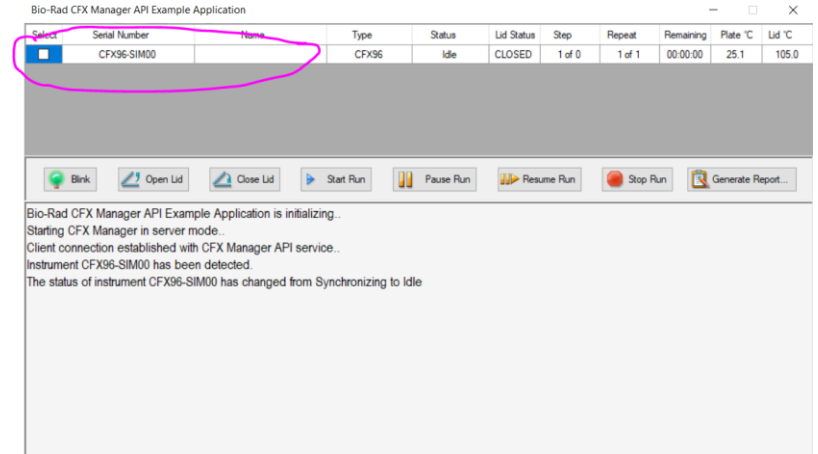
❑ After the Protocol has ended, the API will close automatically after a few seconds. YOU MUST NOT CLOSE IT MANUALLY

❑ After a successful protocol run, PCR produces a ".pcrd" file (readable by the CFX manager) at the location "PCR_BioRad\pcrd_results"

❑ Also, it produces a ".json" file at the location "PCR_BioRad\json_results". This json file is a consolidated file containing results of all 96 wells and the details of the protocol Run.

HOW TO EDIT THE API?

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❑ **The Bio-Rad documentation for the original API and XML schema is available on the following link:**
https://drive.google.com/file/d/1kQMEkg8mJrvs-B46q7s_iiBS7A4iXAmN/view?usp=sharing

❑ **Please go through the above documentation file before trying to edit the API.**

❑ Refer to **section 1.d and 1.c** to know the requirements before editing the API

❑ The API code follows the .NET framework. It is composed of 1 solution (called CFXManagerAPI_Examples) and 4 projects under this solution that are as follows:
   1. **Example_Application:** A windows forms project (from BioRad) that handles the interface
   2. **Example_Client:** A class library project (from BioRad) that communicates with the PCR machine
   3. **Example_Client_Wrapper:** A class library project (from BioRad) that interfaces the Example_Application with the Example_Client
   4. **Data_Processor_App2:** A class library (not from BioRad) that reads and processes the data generated by the PCR machine

❑ New features at the Back-End were added to the BioRad API
   • To analyze the data produced at the end of every run
   • To consolidate the data into one single json file to upload it to the Covmatic Digital storage
   • The "Data_Processor_App2" project in the API solution was developed to execute these tasks at the back-end

❑ Note: The API and the CFX Manager should never be run simultaneously. Close one before launching the other

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Here is a rough sketch of what the API does and its working procedure

1

You start the API and it connects with the PCR

2

You select the Plate and Protocol files and start the RUN

3

The PCR finishes the RUN and starts processing the data

4

A bunch of .csv files and one .pcrd file are produced at the end of the run containing the results of the PCR run

8

The API disconnects from the PCR and shuts down by itself automatically

7

All of the .csv files are deleted and the .json file is saved in the Folder "PCR_BioRad\json_results"

6

It then consolidates them into a single .json file following a defined structure

5

The backend project in the API, "Data_Processor_App2", reads and interprets these .csv files

❑ Steps 1 to 4 are handled by the default BioRad API solution
❑ Steps 5 to 8 are handled by the "Data_Process_App2" project that was added to BioRad API

3. HOW TO EDIT THE API?

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A basic understanding of the PCR process will help the programmers to:
❑ Understand the "Data_Processor_App2" project file which interprets and processes the PCR data
❑ Edit or modify the above project file to improve data interpretation and add functions to handle new test kits

Here is a brief description of the PCR detection process

The PCR (Polymerase Chain Reaction) is a process that rapidly generates millions or billions of copies of the RNA/DNA inside a sample by
▪ First, mixing it with several reagents and
▪ Then, subjecting the mix to repetitive temperature-controlled thermal cycles (typically 45 to 55 cycles)
If there exists a viral RNA, it is replicated millions of times (gradually) by the thermal cycles. This makes the RNA detectable by specific FLUOROPHORE channels (photosensors) within the Reaction Module

Reaction Module

Thermal Cycler

BioRad C1000 touch Real-time PCR

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In this example, the KHB test kit has been used to briefly illustrate the PCR process. The understanding gained from this example can also be extended to other test kits.

KHB test kit works with 4 Fluorophore channels: **1) HEX –** Internal Control to detect human gene
2) FAM – to detect ORF1ab gene – COVID19   **3) Cy5 –** to detect E gene – COVID19
4) ROX – to detect N gene – COVID19

If the gene is present, it gets replicated gradually with the number of thermal cycles. Higher the concentration of gene, higher is the value of **Relative Fluorescence Unit** (RFU) of the Channel corresponding to that gene. The RFU value for each well (remember, there are 96 wells in a plate), and each channel (there are 4 channels in this test kit as mentioned above) is captured at the end of each thermal cycle.
For example, if the ORF1ab gene is present in the well, the FAM channel RFU will show amplification as shown in the picture here →

If the gene is not found, then no amplification is reported/observed

The Cq (also called as Ct) value tells us the number of cycles after which the amplification rises above threshold and the RFU value increases significantly
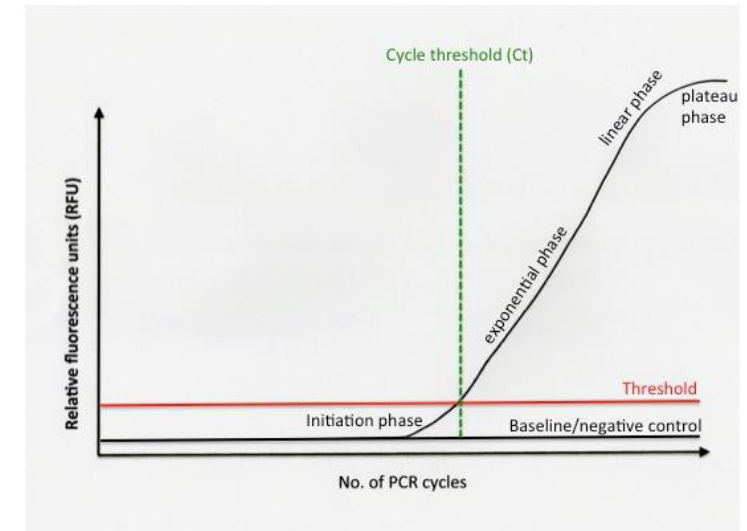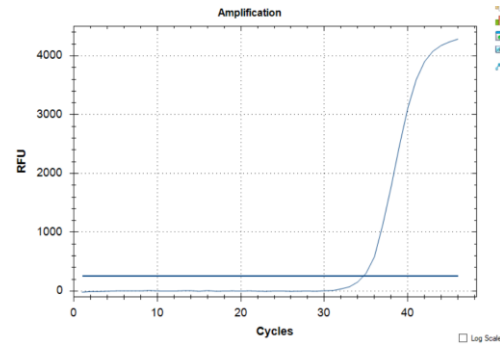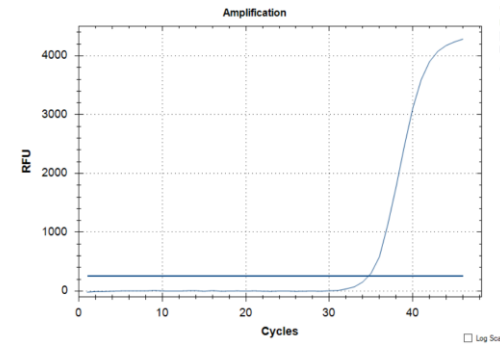
In this example, the KHB test kit has been used to briefly illustrate the PCR process. The understanding gained from this example can also be extended to other test kits.
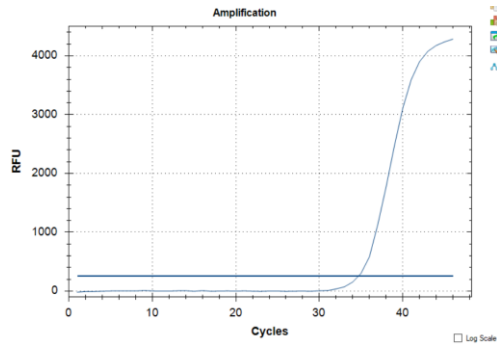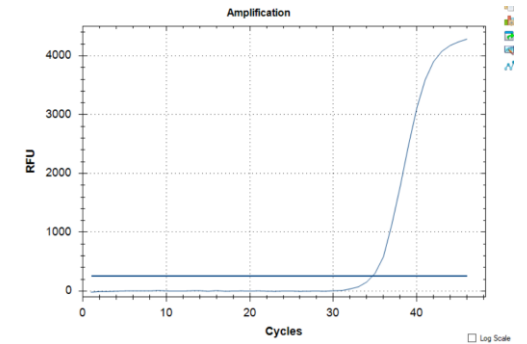

RFU curve for HEX channel for one well

RFU curve for FAM channel for one well

RFU curve for ROX channel for one well

RFU curve for Cy5 channel for one well

Above, you see the sample reaction curves (RFU) for all 4 channels, only for a single well. Each curve in the KHB test kit is generated by 55 points (because the KHB kit has 55 thermal cycles). And these 4 curves are generated for all 96 wells in the well plate.

Here, you see amplification in all of the channels. In real data, that might not be the case.

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To sum up:

1) The Fluorophore channels are used to detect specific genes. Different kits use different combinations of Fluorophore channels. The KHB kit uses HEX, FAM, Cy5 and ROX channels

2) If the gene is present, an amplification in RFU value is observed in the corresponding fluorophore channel. The RFU value is captured at the end of each thermal cycle

3) Cq or Ct is the number of cycles after which the amplification becomes significant

4) If the gene is not present or not detected, then there is no amplification. And the value of Cq is "NaN" or some random garbage value

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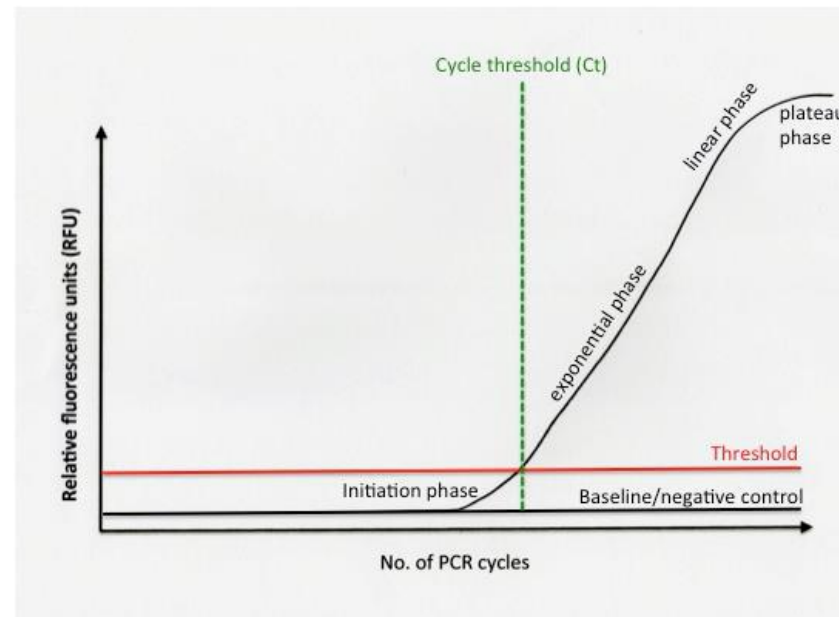
After a PCR run is finished, a bunch of .csv files are generated which carry details of the run and the data generated. [RFU values, Cq/Ct values, etc]. We will focus on only the important files

Data_21-10-2020_22-33-14 - Quantification Amplification Results_Cy5.csv
Data_21-10-2020_22-33-14 - Quantification Amplification Results_FAM.csv
Data_21-10-2020_22-33-14 - Quantification Amplification Results_HEX.csv
Data_21-10-2020_22-33-14 - Quantification Amplification Results_ROX.csv

These files contain the RFU points for each cycle for each well for each channel (FAM, ROX, HEX, Cy5 are the 4 channels). Each well has as many RFU values as there are number of thermal cycles in the protocol. See section 3.b. **"About the PCR process". The files are named based on the date and time of run, respectively.**

Data_21-10-2020_22-33-14 - Quantification Cq Results.csv

This file contains the Cq values for each well for each channel. Each well has only one Cq value.

You will find example .csv files for a sample PCR run in the directory "PCR_station/PCR_BioRad/csv_results/". Here is a link to this directory: https://github.com/covmatic/PCR_station/tree/main/PCR_BioRad/csv_results

The API uses the library "Csvhelper" to read these csv files and load them into "DataTables"

To test your API code, BioRad has provided a PCR simulator, so you don't need an actual PCR machine. This is super helpful, and it speeds up the code-testing and bug-fixing process.

To use the simulator, follow these steps:

1. Open the **"CFXManagerAPI_Examples.sln"** solution file
2. In the project **"Example_Client_Wrapper"**, go to the source code file **"CFXManagerUtilities.cs"**
3. Scroll down to code lines 255 and 256. They are shown in the picture below.
4. Activate the line with the "-simulation" argument and deactivate/comment the line with the "-startup" argument.
5. Run the solution!

```
253         new_process.EnableRaisingEvents = true;
254         new_process.StartInfo.FileName = m_CFXManagerExecutable;
255         new_process.StartInfo.Arguments = string.Format("-startup");    //To run on actual machine: FOR RELEASE
256         //new_process.StartInfo.Arguments = string.Format("-simulation"); //To run a PCR simulation {Edit:Aff23Jun2020}
257         new_process.Start();
258         m_executing_cfx_manager_process = new_process;
```

CONTACTS

To know more about the code or to ask for assistance, send a mail to:

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