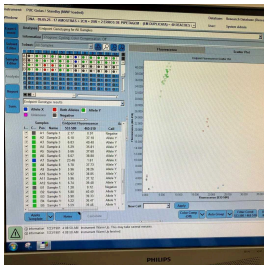
Jun 06, 2025

Genotyping of the rs361525 polymorphism in the *TNF-α* gene in individuals infected with the Dengue virus: A case-control study in the Brazilian Midwest



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We use this protocol and it's working

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Abstract

Dengue is an arboviral disease of major public health importance, particularly in tropical regions such as the Brazilian Midwest. The host's inflammatory response plays a key role in the clinical progression of the disease, with tumor necrosis factor-alpha (TNF-α) being one of the central cytokines in this process. The single nucleotide polymorphism SNP *TNF-α* rs361525, located in the promoter region of the *TNF-α* gene, has been associated with the regulation of this cytokine's expression and susceptibility to infectious diseases with hepatitis C (HCV) and hepatitis B (HBV). This case-control study aimed to investigate the genotypic and allelic frequencies of the rs361525 *TNF-α* SNP in individuals infected with the dengue virus compared to healthy controls residing in the Brazilian Midwest region. Genotyping was performed using real-time PCR, and the data were analyzed for associations between genotypes and infection status. The findings may contribute to a better understanding of the genetic factors involved in the immune response to dengue.

Attachments

PDF

LABORATORY DIARY - T...

451KB

Materials

- Roll of paper for sanitizing the cooling box during movement and protecting the PCR plate after reading;
- PCR cooling box to place the PCR plate in during handling;
- Notebook and pen for making calculations, notes and relevant observations.

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1 **General aspects**

Definition:

Genotyping is a tool in scientific research that allows genetic variability to be studied and associated with specific characteristics. In this way, it participates in genetic tests that make it possible to identify genetic variations in studies focused on single nucleotide polymorphisms (SNPs).

Objectives:

To evaluate the use of qPCR to identify the SNP *TNF-α* rs361525 in DNA samples in order to guarantee the reliability of the results obtained, providing a standardized methodology for the genetic analysis of the presence of this SNP in individuals with dengue.

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2 **Personal Protective Equipment (PPE)**

- Powder-free nitrile gloves;
- Lab coat;
- Closed shoes and pants.

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3 **Materials**

- TaqMan probe (Thermo Fisher) of SNP *TNF-α* rs361525 diluted 1:1 with ultra-pure water (**C___2215707_10**).
- Roll of paper for sanitizing the cooling box during movement and protecting the PCR plate after reading;
- PCR cooling box to place the PCR plate in during handling;
- Notebook and pen for making calculations, notes and relevant observations.

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4 Procedure

Genotyping of the rs361525 polymorphism in the *TNF-α* gene in individuals infected with the Dengue virus: A case-control study in the Brazilian Midwest

4.1 **Check that the stabilizer is connected to the 220V socket.**

4.2 **Lightcycler 480. Switch on the Lightcycler 480.**
Red lights will come on, wait for the green light to appear to turn on the computer.

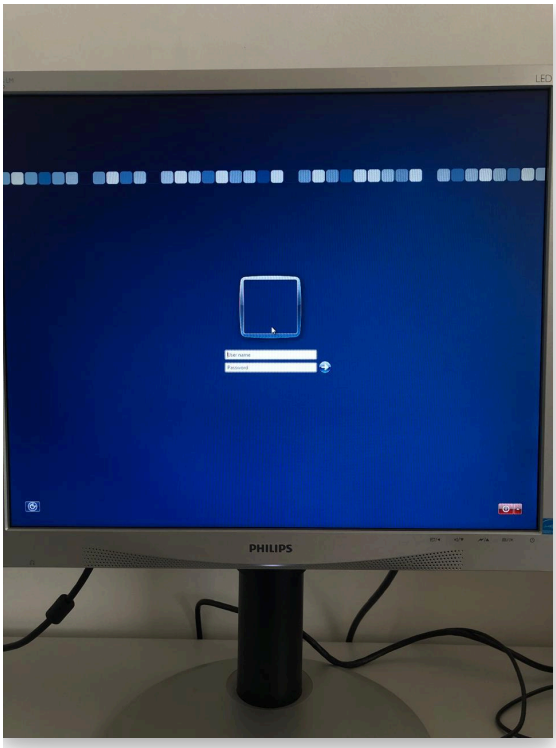
Switching on the equipmen Lightcycler 480.

Lightcycler 480 on.

4.3 **Turn on the computer and wait for the start screen to appear.**
As soon as the screen below appears, click F1.

Turn on the computer to start studying.

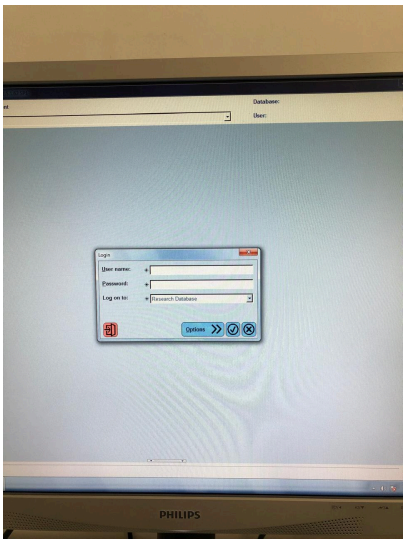
4.4 **Enter the login and password from the first access.**



4.5 **Open the Lightcycler 480 program.**

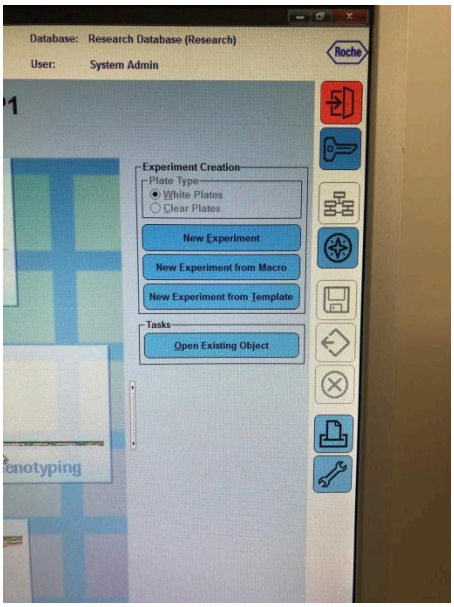
Lightcycler 480 program.

4.6 **Enter the login and password for the second access.**

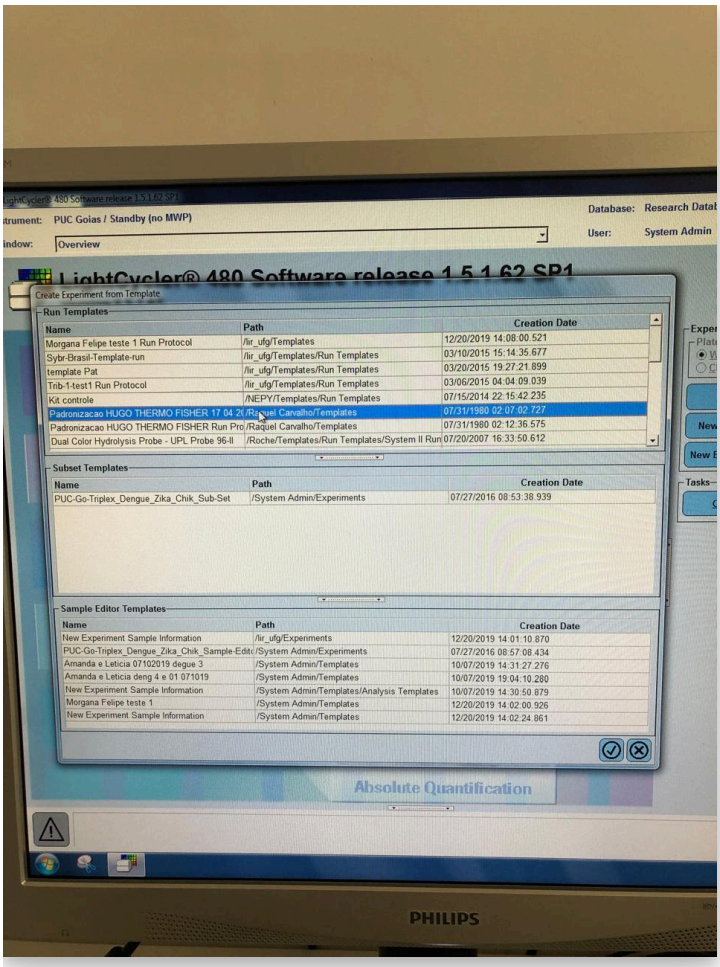


Location of login and password to access the program.

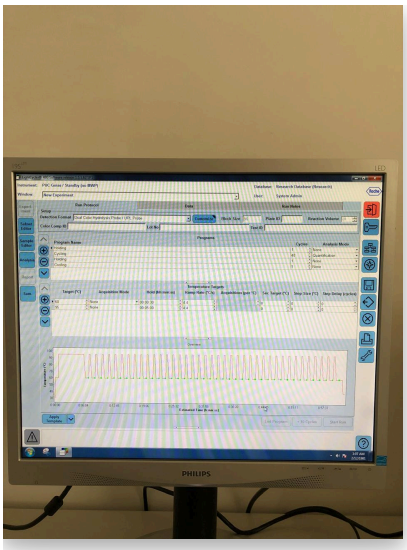
4.7 **Click on “New Experiment from Template”.**



4.8 **Select the following standardization already saved on the computer:**



4.9 **..Check that you are in 4 schedules of 40 cycles each, as below:**
At the end, click on “Apply Template”.



PCR cycles.

4.10 **Click on the side button of the Lightcycler 480 to exit the treadmill.**
Carefully remove the PCR plate from the cooling box, touching only the sides, and place it on the conveyor belt.

ATTENTION:
Observe the edge of the diagonal side and be careful not to touch the barcode.

Lightcycler 480 switched on and running.

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4.11 **Click the button again to close the belt.**

4.12 **At the top, under "Window", identify the PCR that is being made:**

Select the folder.

It is necessary to identify with the type of sample (DNA), number of samples (SAMPLES, CP, CN, BR, TOTAL REACTIONS) and rs361525.

4.13 **Select "Start Run".**
Then wait for the heating graph to appear.

4.14 **Wait an average of 40' to 1 hour for the plate to be read.**

4.15 **Record the fluorescence graph.**

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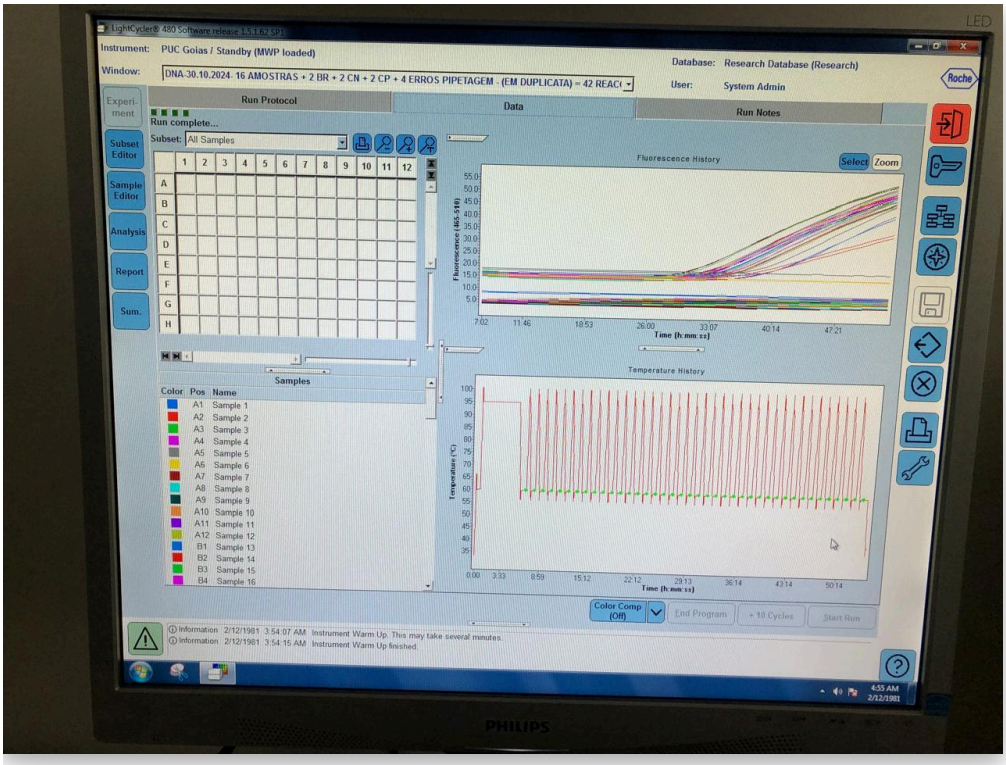
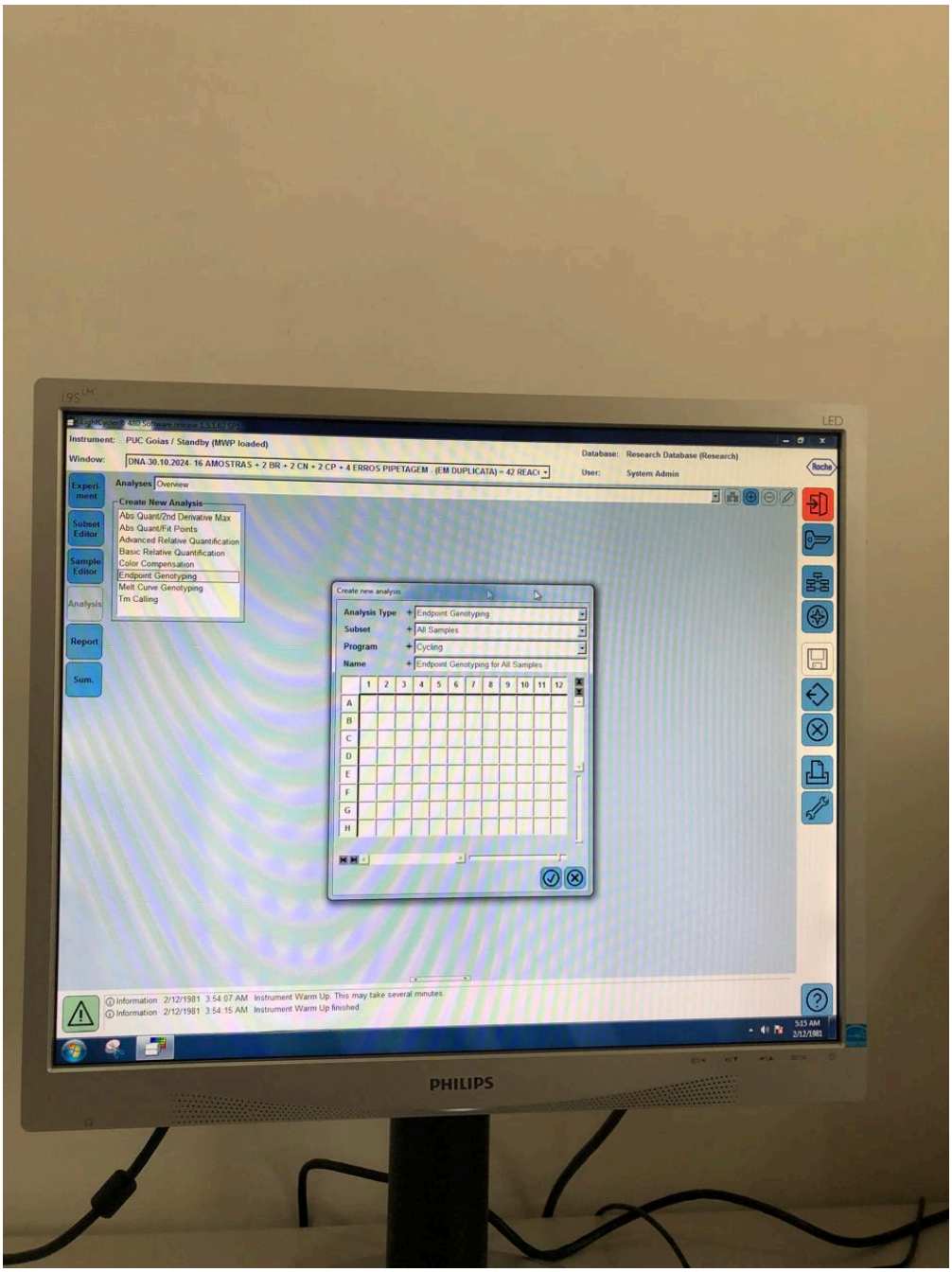
4.16 **End of PCR.**

The photo below shows the end of a qPCR.

4.17 **Click on “Analysis” on the side of the page.**



4.18 **Click on “Endpoint Genotyping” and “OK”.**



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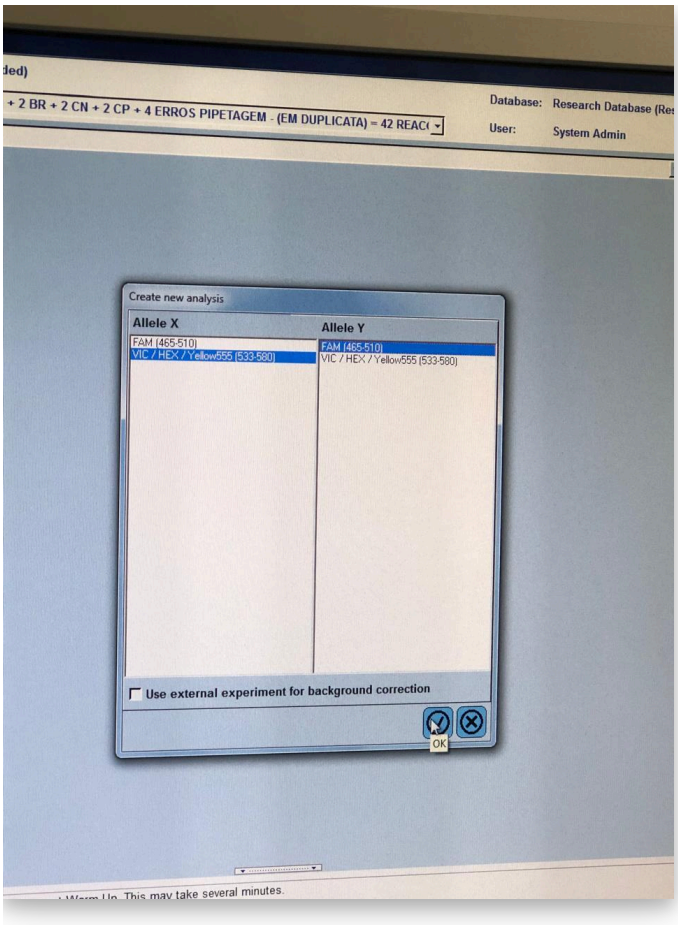
4.19 **Select the colors VIC and FAM:**

VIC (ALEL X): VIC/HEX/Yellow555 (**533-580**)
FAM (ALEL Y): FAM (**465-510**)



Place to choose the colors according to the Thermo Fisher protocol.

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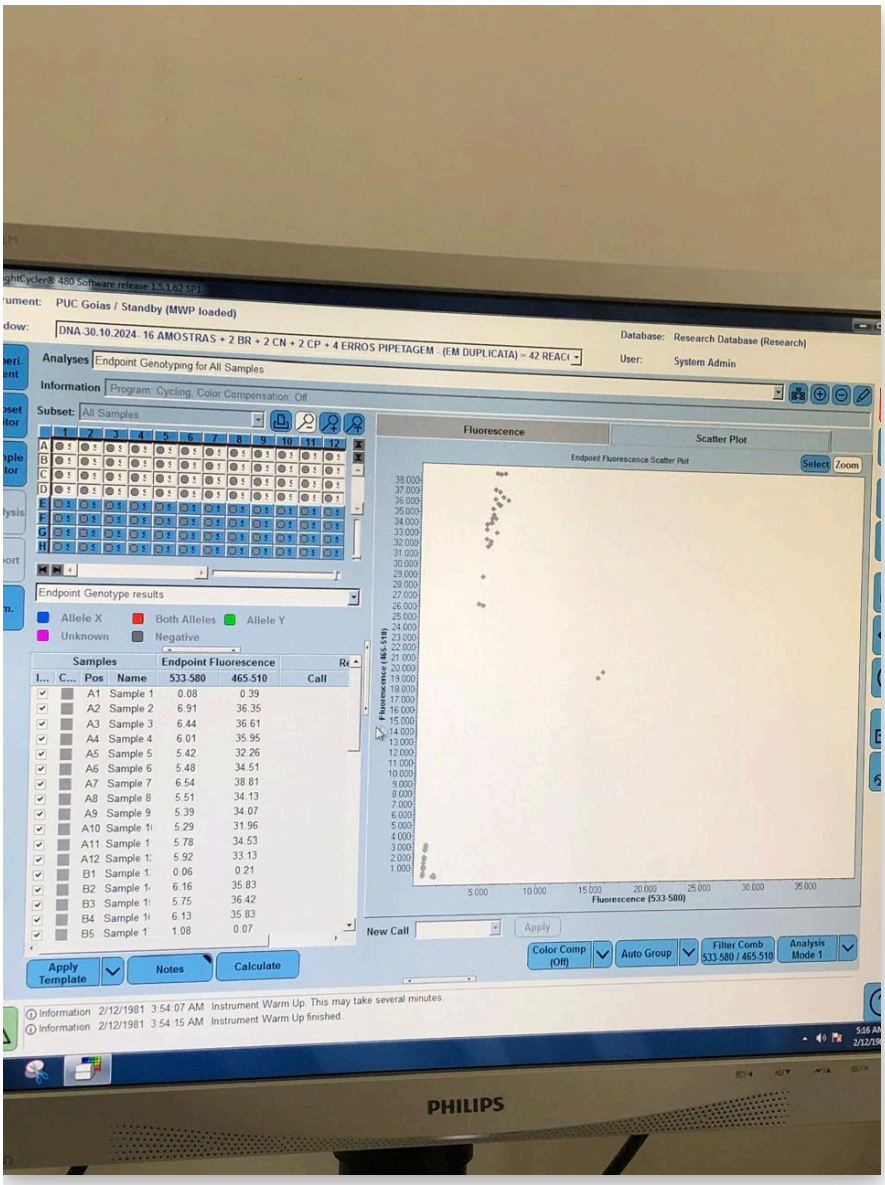
4.20 **Select the wells that have been pipetted and check the filters.**
After selecting the wells, click on “Calculate”.



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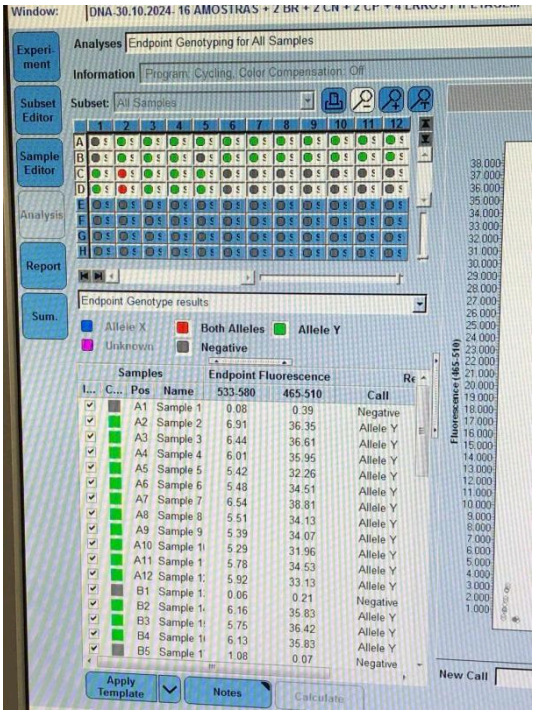
4.21 **Analyze the presence of the alleles and write down their respective genotypes in the notebook.**

In order to genotype the samples, the following data from the duplicates is required:

ALEL X - GENOTYPE A
ALEL Y - GENOTYPE G

Example of genotyping after calculations:



Thus, when analyzing the samples, they can be considered wild, mutant and heterozygous:

ALEL	GENOTYPING
YY-**GG**	WILD
XX-**AA**	MUTANT
XY-**GA**	HETEROZYGUS

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5 **Actions in cases of non-compliance**

- Record any complications in the ATA notebook;
- Contact the counselors;
- Make photographic records;
- If there are any problems with the product or equipment, contact technical assistance.

6 **Send the analysis for statistical study**

The analysis of the genotypes correlated with the symptoms presented by individuals infected with Dengue by statistical study.

Protocol references

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2-SANTOS, N. C. D. *et al*. **Association of single nucleotide polymorphisms in TNF-α (-308G/A and -238G/A) to dengue: Case-control and meta-analysis study**. Cytokine, v. 134, p. 155183, Out. 2020. DOI: 10.1016/j.cyto.2020.155183. Epub 27 Jul. 2020. PMID: 32731142.

3-THERMO FISHER SCIENTIFIC. **Gene expression analysis & genotyping**. Disponível em: https://www.thermofisher.com/br/en/home/life-science/gene-expression-analysis-genotyping/genotyping-genomic-profiling.html. Acesso em: 14 nov. 2024.

4-ZHENG, M. *et al*. **The TNF-α rs361525 and IFN-γ rs2430561 polymorphisms are associated with liver cirrhosis risk: a comprehensive meta-analysis**. *Frontiers in Immunology*, Lausanne, v. 14, p. 1–12, 14 abr. 2023. DOI: 10.3389/fimmu.2023.1129767.

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