PONTIFICAL CATHOLIC UNIVERSITY OF GOIÁS

Scientific Initiation

2024-2025

LABORATORY DIARY

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

SNP *TNF-alpha* rs361525

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Participants: Giovanna, João Otávio, Milena, Thayssa, Raquel e Emilly.

Activity carried out: PCR SNP *TNF-alpha* rs361525 standardization, PCR and GENOTYPING.

The PCR was standardized on the day for SNP *TNF-alpha* rs361525, which is why the positive control was not used.

The probe was also diluted 1:1, with 10 microliters of H2O and 10 microliters of probe.

ALEL **X** (**A**): **VIC** [533-580]

ALEL **Y** (**G**): **FAM** [465-510]

We also discovered the savages, mutants and heterozygotes.

WILD	GG
HETEROZYGUS	GA
MUTANT	AA

When we carried out the PCR, we saw that we'd done the math for more reactions than we'd carried out, so we were left with the MIXER that's in the box, along with the probes, identified only with “rs361525”, which was made by Raquel.

NEGATIVE CONTROL	16 microliters of MIXER + 4 microliters of H2O
POSITIVE CONTROL	16 microliters of MIXER + 4 microliters of DS7
WHITE	20 microliters of MASTER MIX

Samples: 12 + 2 NEGATIVE CONTROLS (CN) + 2 WHITE (BR) + 4 FROM PIPETTING ERRORS (EP).

Total number of reactions: 28 reactions.

Accounts:

MASTER MIX

1 reaction ---------- 10 microliters

40 reactions ---------- x

X= 400 microliters

SONDA

1 reaction ---------- 1 microliters

40 reactions ---------- x

X= 40 microliters

H2O

1 reaction ---------- 5 microliters

40 reactions ---------- x

X= 200 microliters

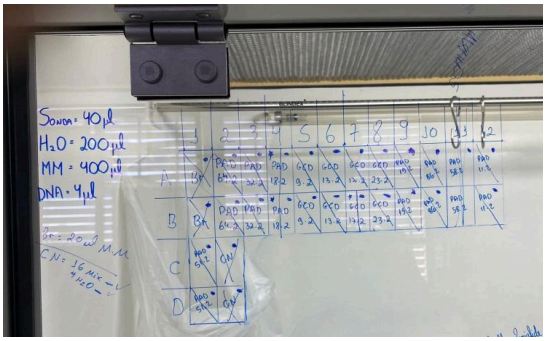
DNA: 4 microliters

The MIXER will have: 400 microliters of Master Mix.

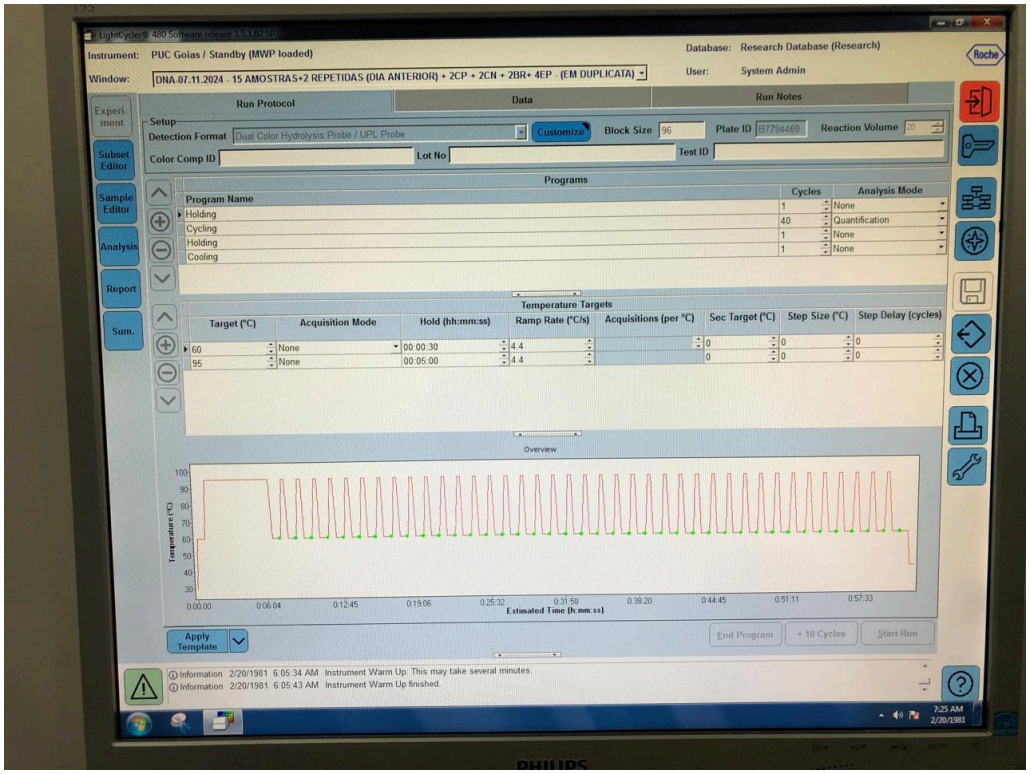
200 '' of H2O.

40 '' of probe.

Pipetting map:



To carry out the PCR, we used the following **Cycling Panel, with 4 programs of 40 cycles:**



Genotyping:

	SAMPLE	ALEL	GENOTYPE
A1	BR	NEGATIVE	-
B1	BR	‘’	‘’
A2	PAD 64.2	GG	WILD
B2	PAD 64.2	‘’	‘’
A3	PAD 32.2	‘’	‘’
B3	PAD 32.2	‘’	‘’
A4	PAD 18.2	GA	HETEROZYGUS
B4	PAD 18.2	No sample	No sample
A5	GCD 9.2	GG	WILD
B5	GCD 9.2	‘’	‘’
A6	GCD 13.2	‘’	‘’
B6	GCD 13.2	GG	WILD
A7	GCD 17.2	‘’	‘’
B7	GCD 17.2	‘’	‘’
A8	GCD 23.2	‘’	‘’
B8	GCD 23.2	‘’	‘’
A9	PAD 19.2	‘’	‘’
B9	PAD 19.2	‘’	‘’
A10	PAD 86.2	‘’	‘’
B10	PAD 86.2	‘’	‘’
A11	PAD 58.2	Sem amostra	Sem amostra
B11	PAD 58.2	‘’	‘’
A12	PAD 11.2	GG	WILD
B12	PAD 11.2	‘’	‘’

C1	PAD 51.2	‘’	‘’
D1	PAD 51.2	‘’	‘’
C2	CN	NEGATIVE	-
D2	CN	‘’	‘’
C3			
D3			
C4			

IMPORTANT OBSERVATIONS:

Samples that finished DNA: PAD 58.2, PAD 18.2, GCD 17.2