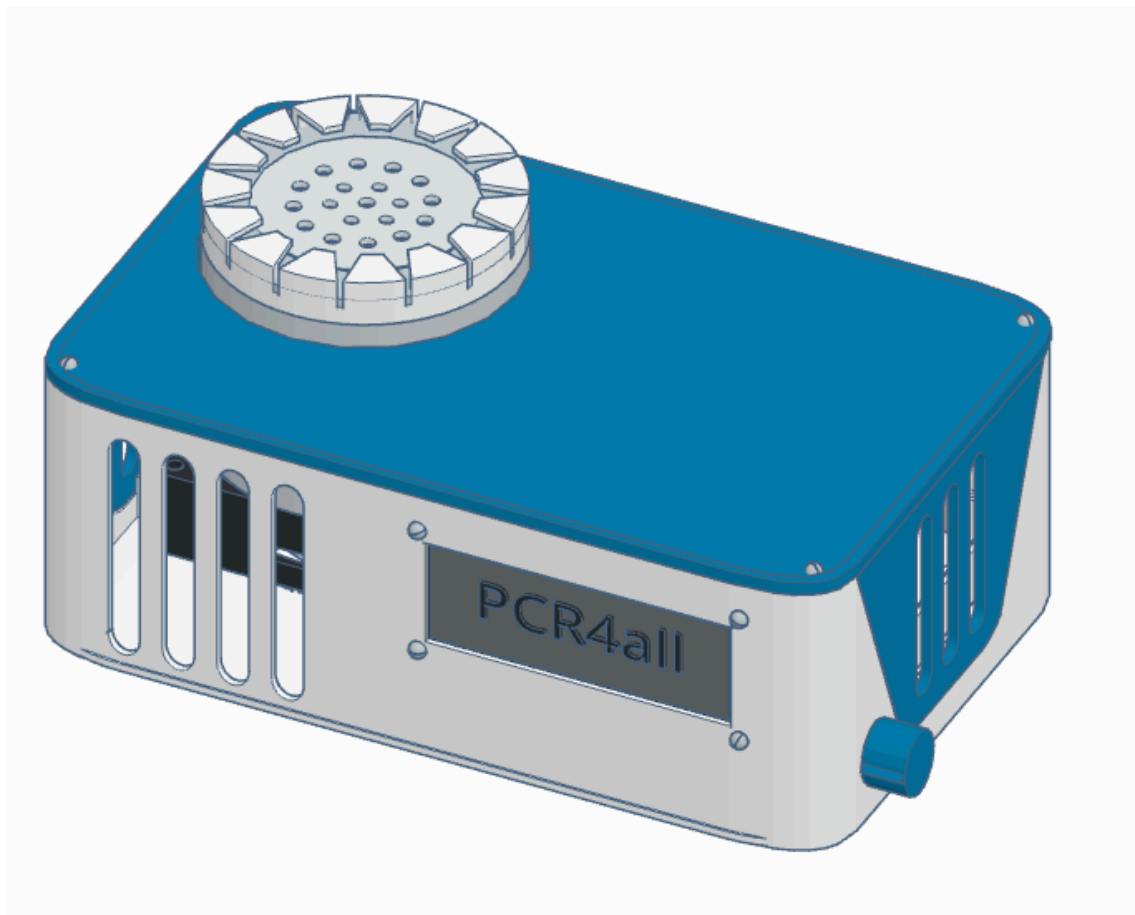


Thermal cycler PCR4all

for DNA technology



[v0 initial release]

Reference Doc

by [Francesc Codony]

About PCR4all

PCR4all started as a personal project from the Francesc Codony several years ago. The initial idea was making an open platform for PCR, with a build cost as low as possible. Francesc Codony, is PhD in Biochemistry and he has multiple publications in the PCR field.

After several attempts following some projects (<https://www.instructables.com>) he decided to find the help of someone with experience in electronics.

The electronics, as well the Arduino program, has been made by Jordi Soley Muntades who was very easy to convince.

Actually exists a better platform named mini PCR (<https://www.minipcr.com/>) and despite of their competitive and low cost (only 585\$) it's more an excellent educational platform than an open project for bio-hackers. Additionally, this price still is enough expensive for some people. This project is in a different wave and stage.

This project still is not enough mature for being considered as a finished project, I have new ideas for improving the whole design. Nevertheless, the current COVID-19 has been the main excuse to release the project to the makers' community. I hope that cleverest people than me can take this development and improve it. (I'm not native speaking English, and this document no has been reviewed, in advance I apologize for the problems and mistakes)

Our next goal is a second design for the current *Arduino* shield which can overcome some electrical limitations, adding new features related with thermal control and making it possible to be controlled with a phone or tablet.

Those are our principles, and if you don't like them... well, we can find others:

(G. marx master)

- Open platform
- DIY
- Crowdfunding
- We like Arduino based electronics
- No business, only sustainable project
- Truly wonderful, the mind of a child is (**Yoda master**)
- "Salid y disfrutad" (**Johan Cruyff**)

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1. Introduction

Thank you for following the PCR4all project.

Before participating, using or form part of the PCR4all environment is essential that you read this user manual carefully. Following the instructions and safety information in this user manual will ensure safe operation and maintain the system in a safe condition.

This is an Open Source platform, we provide the all or some part of this instrument and we share the designs, software as well the molecular biology protocols and reagents in order to made easier your firsts steps in biochemistry. We want to do as easy as possible this learning providing the best of our knowledge and god faith. However once you start this project all the success and failures will be of your responsibility. Please share both with the PCR4all community; we will be happy to congratulate you or if is necessary we will try to help you.

1.1. Intended use of the PCR4all

This thermal cycler unit has been designed for enzymatic DNA amplification that needs multiple cycles of incubation at different temperatures/times.

Follow the next link for more information about PCR

https://en.wikipedia.org/wiki/Polymerase_chain_reaction

1.2. General Information

1.2.1. Scope of delivery

The delivery includes the following items:

- PCR4all BOM & assembly instructions
- User's Guide

This instrument need a 12 v Power supply (AC/DC converter, 1,5A), , please it's important don't use more than 1,5A for power supply.

1.2.2. Technical assistance

The **PCR4all** community we pride ourselves on the quality and availability of our technical support. If you have any questions, experience or any difficulties regarding the PCR4all thermal cycler, do not hesitate to contact us.

PCR4all community and customers are a major source of information regarding advanced or specialized uses of our project. This information is helpful to other customers as well as to the researchers at. We therefore encourage you to contact us if you have any suggestions about product performance or new application and techniques.

1.2.3. Policy statement

It is the policy of PCR4all improving products as new techniques and components become available. PCR4all reserve the right to change the specifications of products at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual.

1.2.4. Requirements for PCR4all users

Table 1 covers the general level of competence for the use and servicing of the PCR4all .


Task	Personnel	Training and experience
Routine use	Laboratory Technicians or equivalent	Training in techniques for laboratory instrument operation
Servicing	Service Specialists only	Trained, certified, and authorized by PCR4all
Education	Students	Training on laboratory safety. Introduction to genetics and PCR (*). A minimum previous training is necessary for pipetting, sample handling and centrifuge use.


(*)https://en.wikipedia.org/wiki/Polymerase_chain_reaction

2. Safety Information

Before using the PCR4all , it is essential that you read this user manual carefully. Following the instructions and safety information in this user manual will ensure safe operation and maintain the system in a safe condition.


The following types of safety information appear throughout the PCR4all *Thermal cycler PCR4all*


<p>WARNING</p> 	<p>The term WARNING is used to inform you about situations that could result in personal injury to you or other persons.</p> <p>Details about these circumstances are given in a box like this one.</p>
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CAUTION 	<p>The term CAUTION is used to inform you about situations that could result in damage to the instrument or other equipment.</p> <p>Details about these circumstances are given in a box like this one.</p>
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The advices given in this manual are intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

2.1. Proper use

WARNING/ CAUTION 	<p>Risk of personal injury and material damage Improper use of the PCR4all thermal cycler may cause personal injury or damage to the instrument.</p> <p>The instrument must only be operated by qualified personnel or under their direct supervision.</p>
--	--


CAUTION 	<p>Damage to the instrument Avoid the use of chemicals with extreme pH. or organic solvents as Acetone or Chloroform.</p>
--	---

CAUTION 	<p>Damage to the instrument Avoid UV exposition</p>
---	---


2.2. Biological safety


Use safe laboratory procedures as outlined in publications such as *Biosafety in Microbiological and Biomedical Laboratories*, HHS:

<http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>

<p>WARNING</p> 	<p>Samples containing infectious agents</p> <p>Some samples used with the PCR4all may contain infectious agents. Handle such samples in accordance with the required safety regulations.</p> <p>The responsible person(s) (e.g., laboratory manager) must take the necessary precautions to ensure that the workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents, as defined in the applicable Safety Data Sheets (SDSs) or OSHA¹ ACGIH² or COSHH³ documents.</p> <p>Venting of fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.</p>
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2.3. Chemicals

<p>WARNING</p> 	<p>Hazardous chemicals</p> <p>Some chemicals used with the PCR4all workflow may be hazardous.</p> <p>Always wear safety glasses, gloves, and a lab coat.</p> <p>The responsible person(s) (e.g., laboratory manager) must take the necessary precautions to ensure that the workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of toxic substances (chemical or biological), as defined in the applicable Safety Data Sheets (SDSs) or OSHA, ACGIH or COSHH documents.</p> <p>Venting of fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.</p>
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<p>WARNING/ CAUTION</p> 	<p>Risk of personal injury and material damage</p> <p>Do not use the PCR4all thermal cycler for handling flammable products. Adequate protection such as ventilation must be provided if dangerous liquids are used in the analytical work. If incidental spillage occurs, carefully clean- up the spillage, taking into account the nature of the spilled liquid, including all required safety measures</p>
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¹ OSHA: Occupational Safety and Health Administration (United States of America).

² ACGIH: American Conference of Government Industrial Hygienists (United States of America).

³ COSHH: Control of Substances Hazardous to Health (United Kingdom).

2.4. Maintenance safety

CAUTION 	Damage to the instrument Do not use disinfectants or chemicals with extreme pH to clean the surface of the PCR4all instrument or its parts. Do not use UV or Autoclave as decontamination method.
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WARNING 	Risk of personal injury and material damage Only perform maintenance that is specifically described in this Thermal cycler PCR4all.
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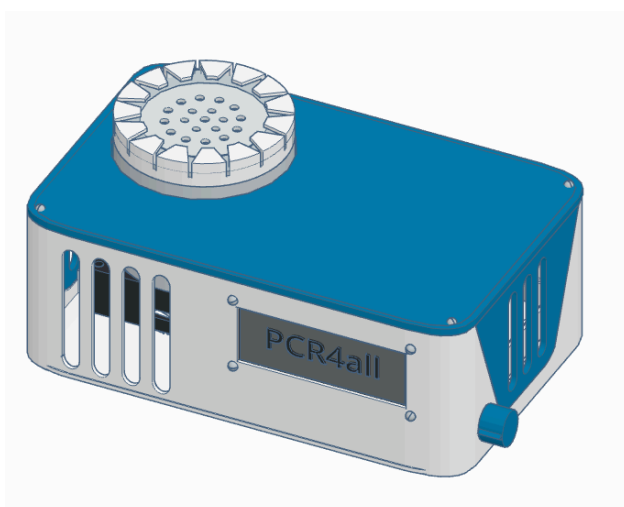
2.5. Waste disposal

Used consumables, such as sample tubes, bottles or liquid-bags, may contain hazardous chemicals or infectious agents. Such waste must be collected and disposed of properly in accordance with local safety regulations.

3. General Description

The PCR4ALL thermal cycler only has been designed as an educational instrument, it's not a professional instrument nor for supporting high workloads. With this instrument and following the appropriate protocol is possible the DNA amplification of a genetic target contained in your sample.

3.1. Features of the PCR4all system



The structure or carcass of this instrument is formed by one box with two internal chambers separation. The first one contains the PCR reactor and the second one all the electronics. The enclosure has been designed in order to improve the airflow and to prevent the overheating.

In the next figure are depicted a drawn of all the components of this instrument, without wires, screws and nuts.

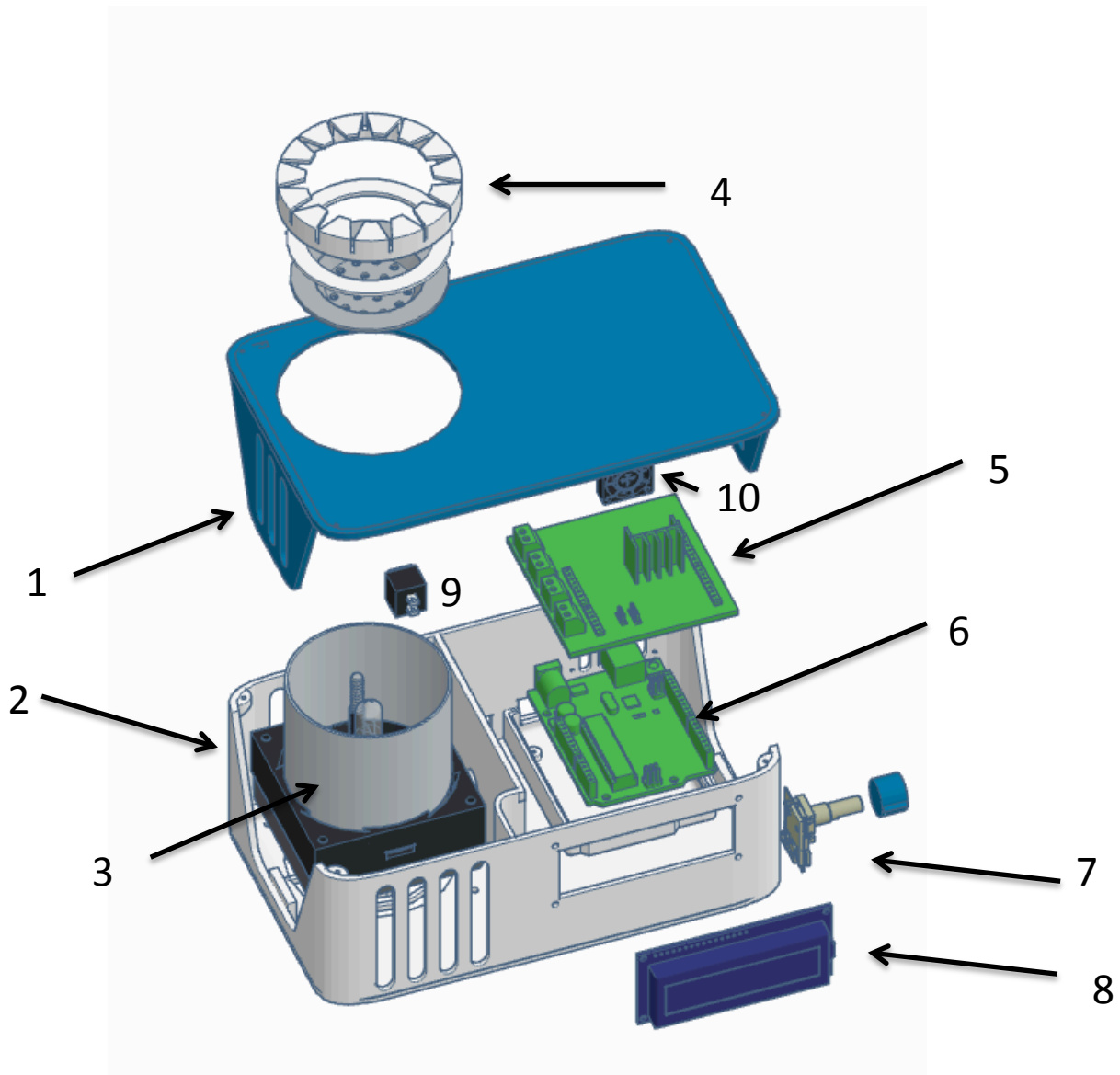


Fig 1. PCR thermal cycler breakdown of components . 1.- cover, 2.- box, 3.- PCR reactor body (see next scheme for more detail), 4.- PCR tubes holder, 5.- Arduino PCR Shield 6.- Arduino Uno board 7.- Rotary encoder and button, 8.- LCD screen 9.- Reset button, 10.- internal fan

In the next figure are depicted a drawn of all the components of the PCR reactor without wires, screws and nuts

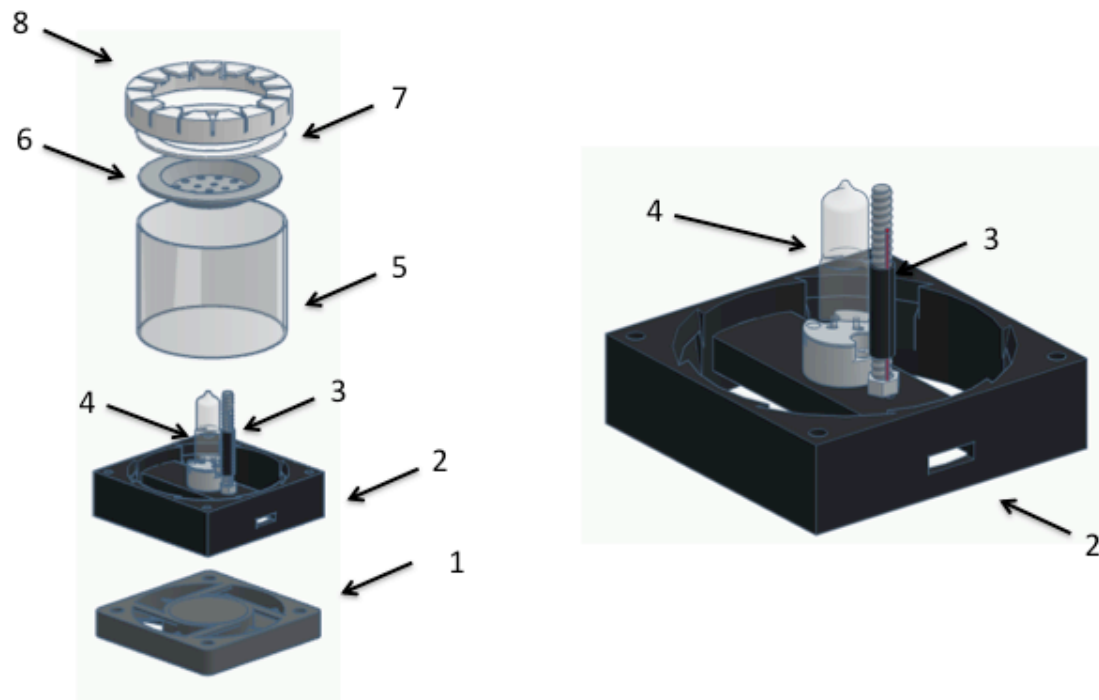
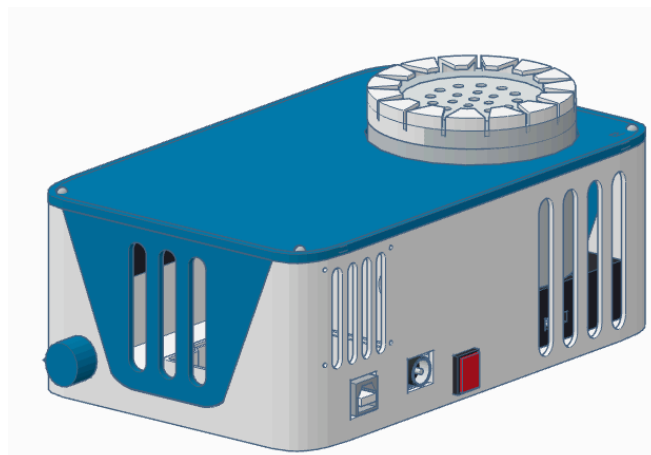



Fig 2. PCR reactor, breakdown of components. 1.-Fan (cooling system) , 2,- Reactor armour , 3.- temperature probe and their support 4.- halogen lamp (heating system) , 5.- reactor wall 6.- PCR tube holder 7.- Silicone ring, 8.- Support for tube holder



Into the back of the PCR4ALL system are located the DC connector, the reset switch and USB connector. The front of the unit contains the LCD display. At the right side is placed the rotary encoder for programing the instrument.

3.2. Complementary accessories (not provided)

- This instruments needs a AD/DC 12v 1,5 A as power source

WARNING/ CAUTION 	Risk of personal injury and material damage Do not use more than 12v, 1,5 A power source, it can seriously damage the electronics and overheat the systems
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4. Installation

Since this instrument is the result of open project, you can receive an assembled unit or some parts of them and incorporate you own design improvements. If you don't receive a complete unit, please follow the assembly instructions before to read the next points.

4.1. Unpack the instrument

The packaging of the PCR4all can be stored for reuse.

4.2. Site requirements

Place the PCR4all instrument on a level, stable surface, ideally a firmly secured. Since PCR products are one of the main contaminants during prior PCR workflow, it's important a physical separation between critical activities.

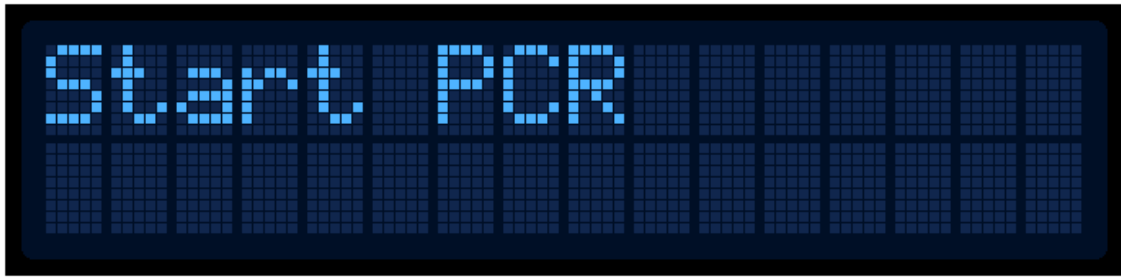
Locate it away from inflammable products.

5. Operating Procedures

5.1. Preparation

Connect the AC/DC jack plug connector to the PCR4all thermal cycler, and plug the AC/DC power supply to mains. Once the system is initialized you can se on the display the next messages. <http://avtanski.net/projects/lcd/>





5.2. Programming the instrument

5.2.1. Starting a previous program

The instrument memory stores the last settings, if you want to repeat the PCR cycle push the button once you see **Start PCR** on the display.

5.2.2. Menu options

By rotating the button you can chose the different menu options

- 1.- Start PCR
- 2.- PCR program
- 3.-Temperature Control

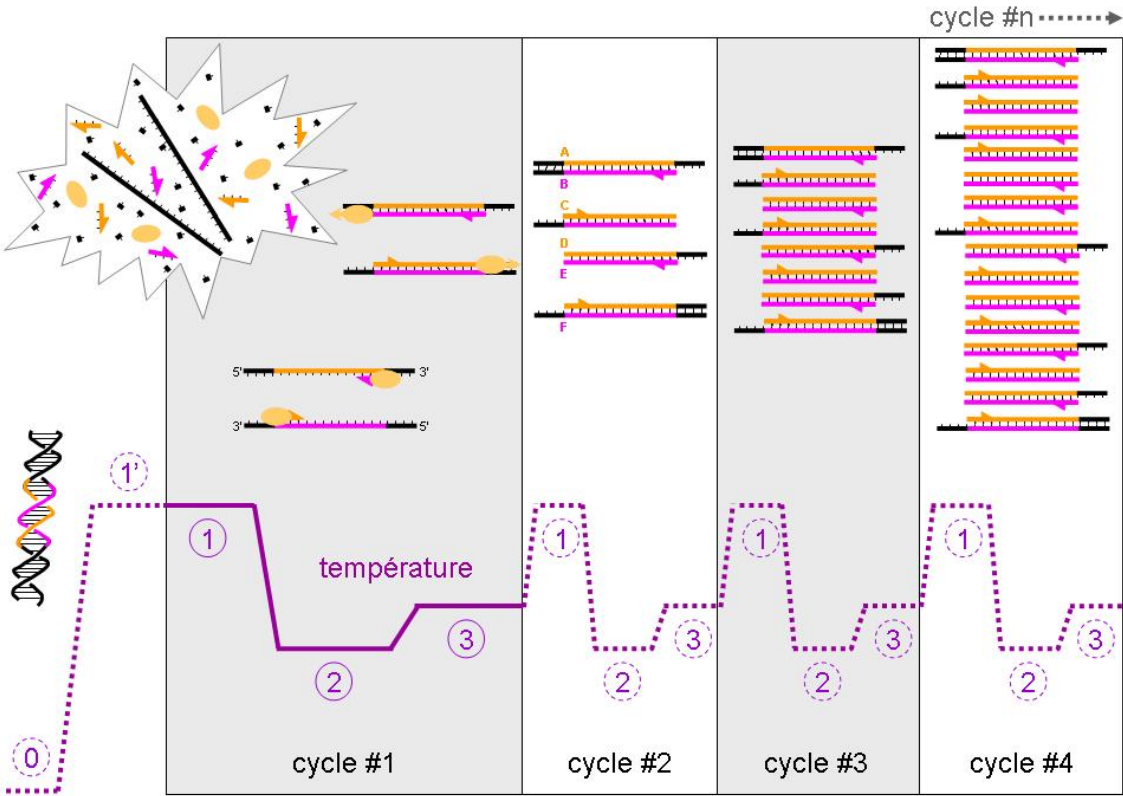
5.2.3. PCR program



The PCR program has 3 stages:

- 1.- Enzyme activation
- 2.- PCR
- 3.- Cooling, it's a default stage and cannot be modified .

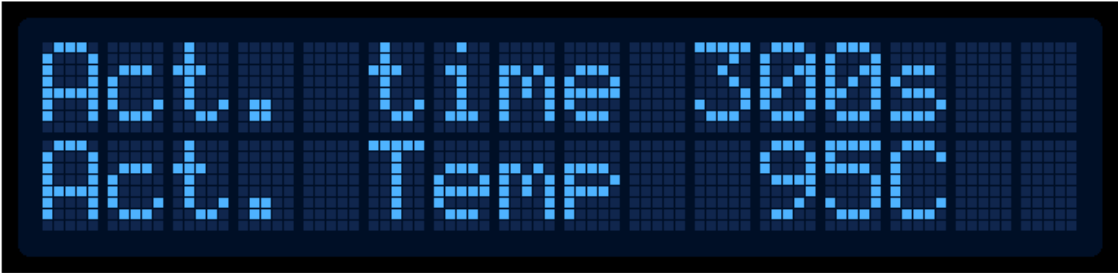
In order to initiate the programming push the button



(source: https://commons.wikimedia.org/wiki/Polymerase_chain_reaction#/media)

5.2.3.1. Enzyme activation (1')

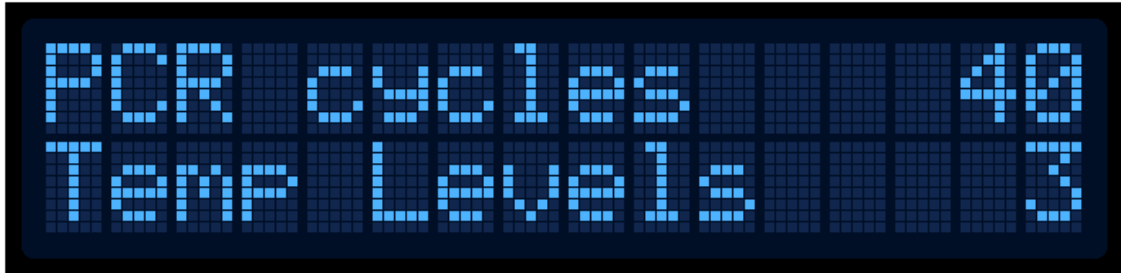
Nowadays the common enzyme technology is based in hot start polymerases wich needs an initial temperature activation step for becoming active. In this stage you can define the time (seconds) and temperature (°C). In most cases ranges is 300-600 seconds and 95°C.



Rotate the button back - forward for changing the **Act. time** (activation time) and push for fix and move to **Act. Temp** set up (Activation Temperature) and proceed in the same way. Once you push the button you'll change to PCR

5.2.3.2. PCR

In the next menu screen you can define the number of PCR cycles (usually 35-40) and the temperature levels (usually 2 or 3)

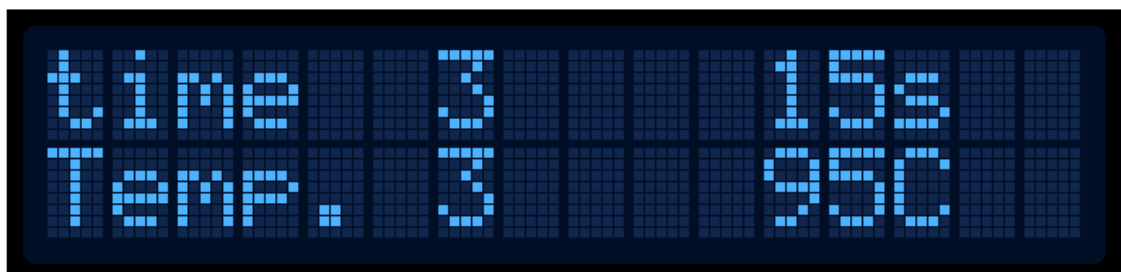


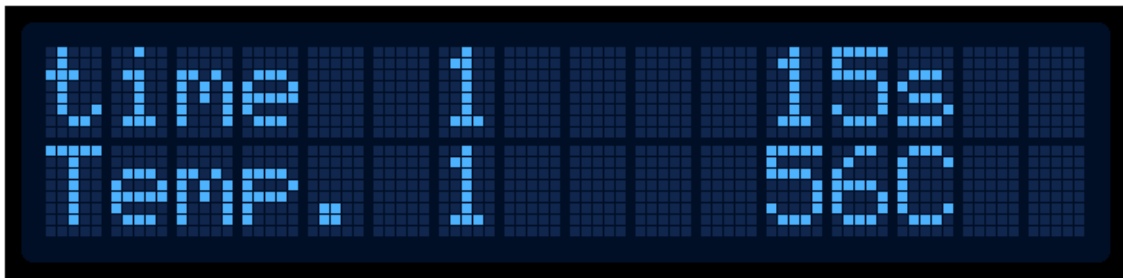
5.2.3.3. Programing each level

Once the temperature levels have been defined, is moment to define the time and Temperature of each of them.

For classical PCR the next examples are quite usual, at least for microbiology application, however the elongation time can be function of the amplicon size:

1. Denaturation: 95°C, 15-30 s
2. Annealing: 55°C-65°C, 13-30 s
3. Elongation: 72°C, 30-60 s





Push the button for start the PCR

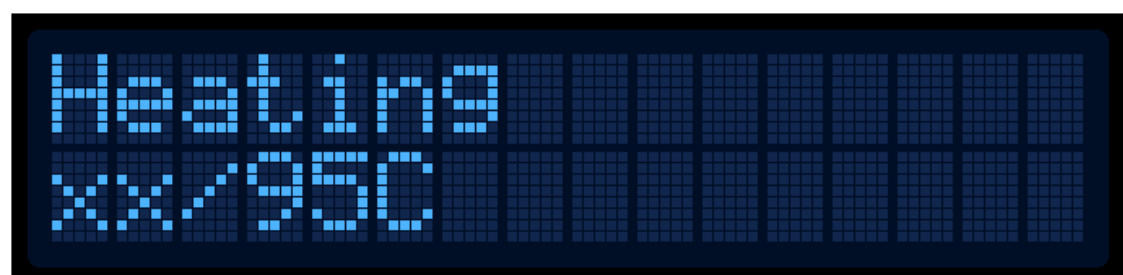


If you need to change or correct some settings press the reset button at the back of the instrument and the process can be began again.

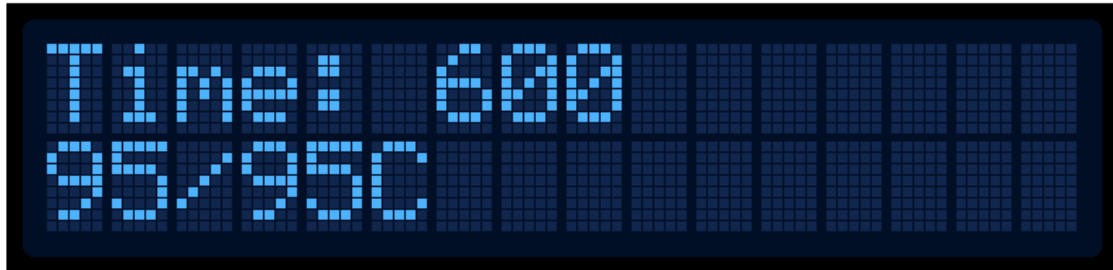
5.2.3.4. Following the PCR

Once the PCR starts,

1st step i.e the instrument increases the temperature from xx up to 95°C for activate DNA polymerase (Hot Start Technology)



Once the system is at the activating temperature, it starts a count down timer.

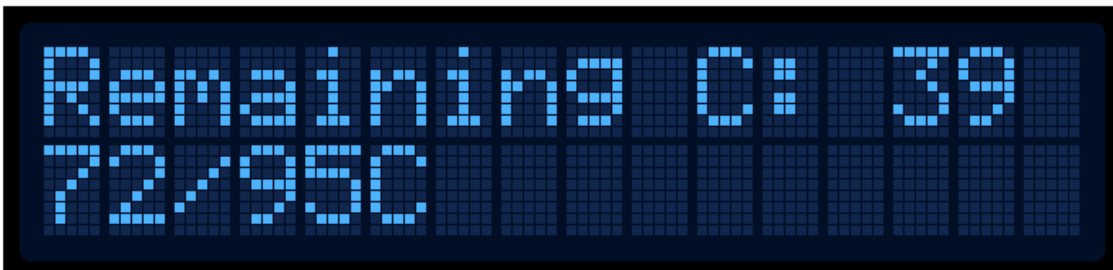


2on step, PCR cycles, at this point will start the PCR you can see the target temperature at the right of the “/” and the current temperature at left.

i.e “6X/95C”, the system is heating from 6x up to 95°C

i.e “6X/55C”, the system is cooling from 6x up to 55°C

At the beginning of each cycle, when the system is moving from elongation temperature to denaturation temperature you’ll see the Remaining Cycles.



5.2.4. Temperature control

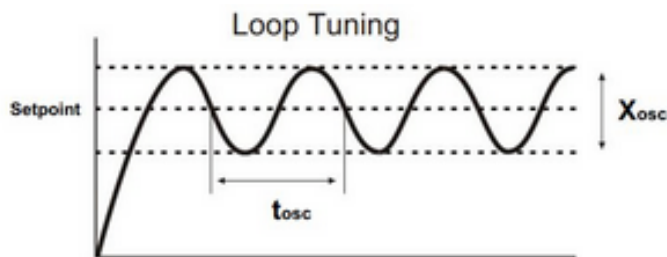
This instrument works with two different approaches for the temperature control. The heating system is modulated by a variable voltage supply, which is operated by a PID controller. Additionally, in order to increase the cooling speed, also exist an active cooling element, in this case a fan. For this reason, in order to don't overpass the target temperature and/or don't interfere in the heating control, is important to define a cut-off temperature for activation.

In this menu area can be adjusted key parameters of temperature control: the controller and the cut temperature for activating the fan.

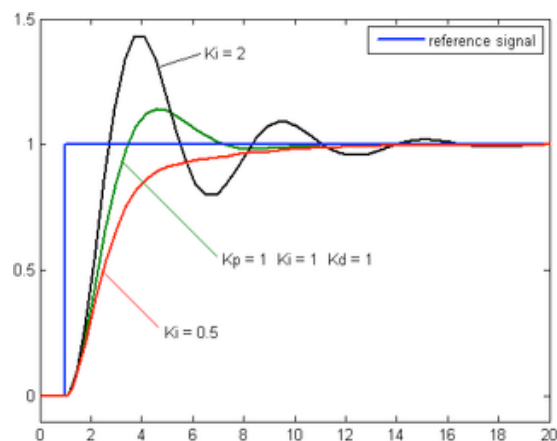
Both options should be used for advanced users or for those makers that develop their own PCR systems from some of the elements of this project.

TEMPERATURE CONTROL

The classical on/of temperature control it's not the better approach for quick temperature stabilization.



The PCR4all thermal cycler has a PID (proportional–integral–derivative controller), which allows a better temperature adjustment.



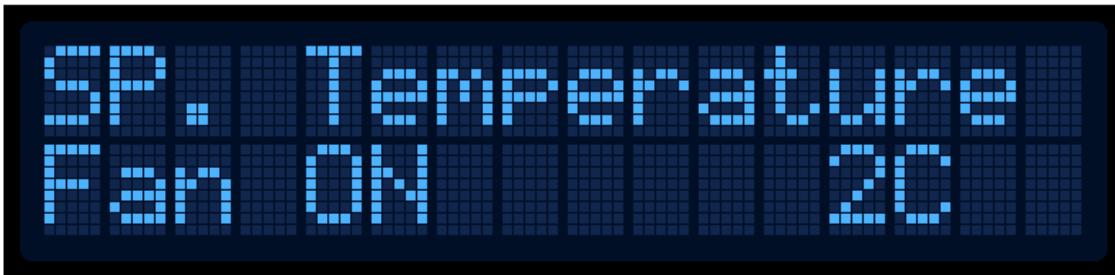
https://en.wikipedia.org/wiki/PID_controller

Kp and Ki can be adjusted in this menu. Push the control button for Kp / Ki adjustment



Moving back/forward the control button you can set up the Kp value, pushing the button it becomes fixed and then you can repeat the same process for the Ki value.

Once the Kp/Ki values are adjusted, you can define the fan activation. Usually it's fixed at + 1-2 °C from target temperature.



Moving back/forward the control button you can set up the temperature difference from target temperature value for activating the fan, pushing the button it becomes fixed.

6. Error messages

This instrument has failure detection system, if some problem is detected will appear on screen the next messages.

E1 appears when the heating system doesn't work, probably as result of bulb failure. Follow the instructions of point 6.1 for replacement.



E2 appears when the internal temperature of PCR reactor is over 98°C. It can be due to:

- Temperature sensor displacement, please review if it's well placed in an equivalent position of rest of tube wells.
- The temperature control is broken; some electronics parts can be damaged.



E3 appears when the system detects a temperature probe failure



7. Maintenance

7.1. Cleaning procedure

Do not use extreme pH reagents for cleaning the PCR4all and their parts. When you need to clean this instrument, use one of the next following methods:

- 1 - A clean dry cloth.
- 2 - A cloth dampened with water.
- 3 - A cloth dampened with soapy water

Only domestic and/or neutral soap can be used. No other form of detergent or chemical may be used. Rinse with water

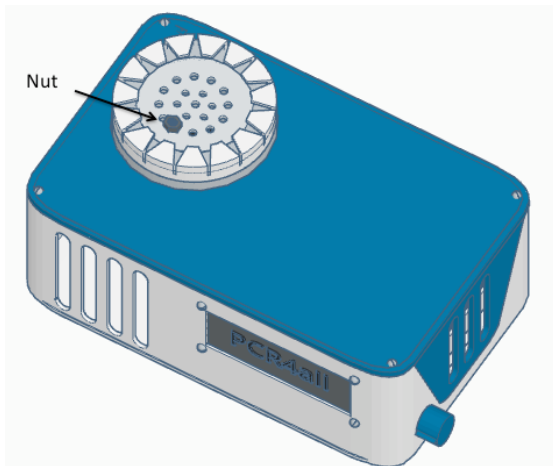
CAUTION**Damage to the instrument**

Do not use disinfectants or chemicals with extreme pH to clean the surface of the PCR4all instrument or its parts.

7.2. Bulb change (Error 1)

WARNING**Risk of burn / electrical cramp**

Please, previous to do any action, unplug the instrument and confirm that PCR reactor is cold.

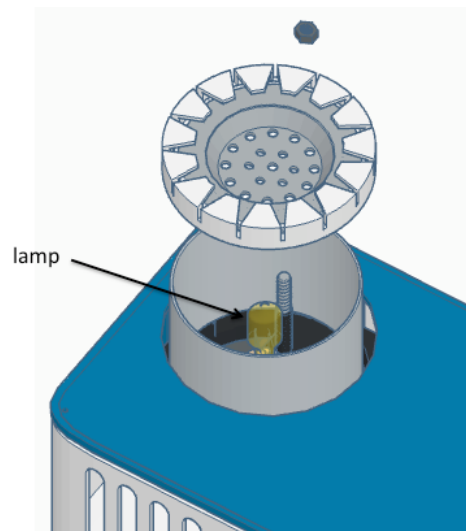


The heating system of this instrument is as simple as effective, and it's a 25w/12v halogen bulb (G4 type).

Please remember that good quality lamps probably can have a longer life than cheapest lamps.

If is necessary to do a lamp change, please follow the next steps.

1. Unscrew the nut of PCR tube holder.
2. Remove the tube holder
3. Change the lamp
4. Try to connect the lamp as centered as possible
5. Cover again the PCR reactor with the PCR tube holder and screw the nut.



8. Technical Data

PCR4all reserve the right to change specifications at any time.

Specifications

Heating ramp \leq °C/s

Cooling ramp \leq °C/s

Temp precision $\pm 1^\circ\text{C}$

Operating conditions

Power range 12V DC

Maximum power 20 W

Overvoltage category II

Air temperature 15-32 °C (59-89°F)

Relative humidity 15-75% (Non-condensing)

Altitude Up to 2000 m. (6500 ft.)

Place of operation For indoor use only

Pollution level 2

Transportation conditions

Air temperature 25°C to 60°C (-13°F to 140°F) in manufacturer's

Relative humidity Maximum of 75% (Non-condensing)

Storage conditions

Air temperature 5°C to 40°C (41°F to 104°F) in manufacturer's packaging

Relative humidity Maximum of 85% (Non-condensing)

Dimensions and Weight

Dimensions Length: 191 mm (7,52 in)

Width: 120 mm (4,72in)

Height: 76 mm (2,99 in)

Weight 0,5 kg (1,10 lb)

9. Ordering codes

Cat. #	Product PCR4all thermal cycler
	Complete and assembled unit
	Complete Electronics set without box
	Arduino uno PCR4all shield

(this project still not have been finished we can't provide physical products)

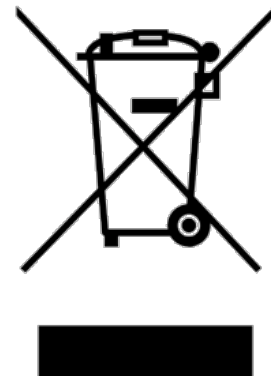
10. Waste Disposal

Waste Electric and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users.

The crossed-out wheeled bin symbol indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.



11. Warranty

The products delivered through the **PCR4all** project are only for education or learning purposes. We made public our basic designs; software and BOM therefore experienced users can make their own developments and improvements. We can sell assembled prototypes as well spare parts in order to facilitate your learning experience, however it's not our mission. From the good faith, we test the products and spare parts before to send, nevertheless it's important to understand that at this stage it's a collaborative project not a business.

12. Acknowledgments

1. This instrument has been inspired in **The Light Bulb PCR Machine** project, which was clever idea from Russell Durrett.

<http://russelldurrett.com/lightbulbpcr.html>

2. The screen shots from the LCD display has been obtained using the simulator developed by **Alexander Avtanski** (<http://www.avtanski.net>)

3. As stated previously, we are very gratefully to **Jordi Soley Muntades**

Notes

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Knowledge for all

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