IGEM AM

Synthetic Biology Hub
Amazonas, Brazil

HARDWARE 2019

A cheap DIY CO2 incubator to use in scientific research and cell culture

Introduction:

Hello there, do you want to build an incubator? Come on, let´s begin!
This small guide will offer most of the technical part necessary to build a DIY incubator, but as every engineer will tell, adaptability and resourcefulness are always good skills to have when building anything.

The incubator here described is designed to culture any CO2 dependant cell line, being it cancerous or normal line. It can also be used to culture non-CO2 dependent cell line, just as simple as closing a tube and regulating the temperature according to your need.

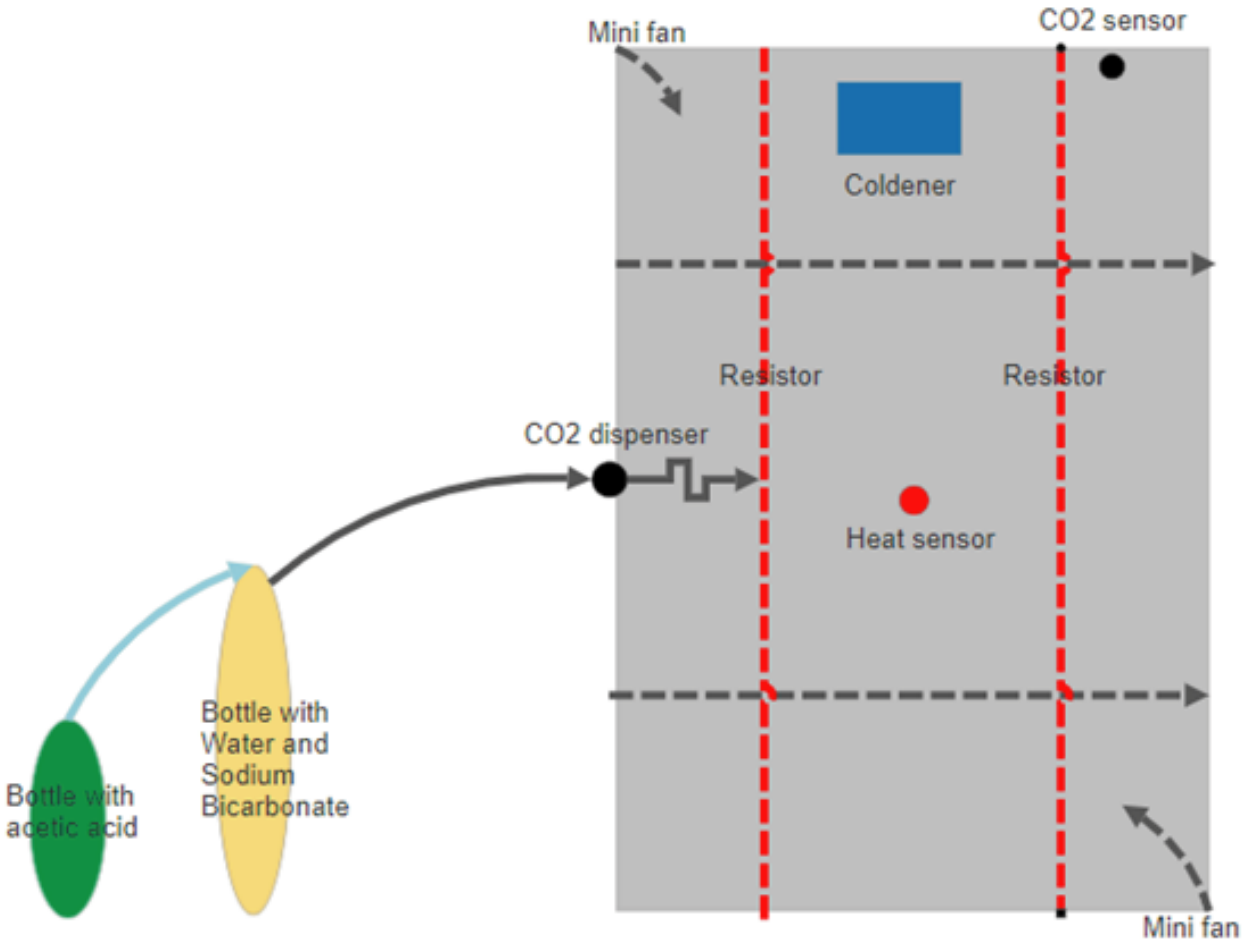
Our focus in this project was to design and build the cheapest option of CO2 incubator and also the easiest one to build; while not perfect it will be good enough to handle the job it was made for. However, if you have more technical or financial resources available to improve our design, please do it and if possible, e-mail us with your results, we look forward to receiving feedback. We are also ready to help if you have and doubts.

Necessary materials	
Ice box- 24L	Silicon glue
Metal grid or Metal sheet	Silver Tape
Heating Resistor	Sodium Bicarbonate
CO2 sensor	Vinegar
Relay module	Connection Cables for Arduino
Arduino UNO	Solder 38Watts
Peltier Coller and dissipators	Scissors
2 Coolers	Pincer and cutting pincer
LCD or LED display	Ruler
Macrodrop	Pencil or Marker
60cm plastic tube	2 2L PETs bottles or similar containers

Recommended Materials
Measuring Tape

Starting it out

The first thing to do is to acquire the material listed above and organize them in your workspace, after that we recommend to measure your ice box, since they aren't made equal. Use a ruler or measuring tape to do it and adjust other parts to it. There's not a perfect way to organize your system, but we recommend you to adjust the sensor as shown in the next image.



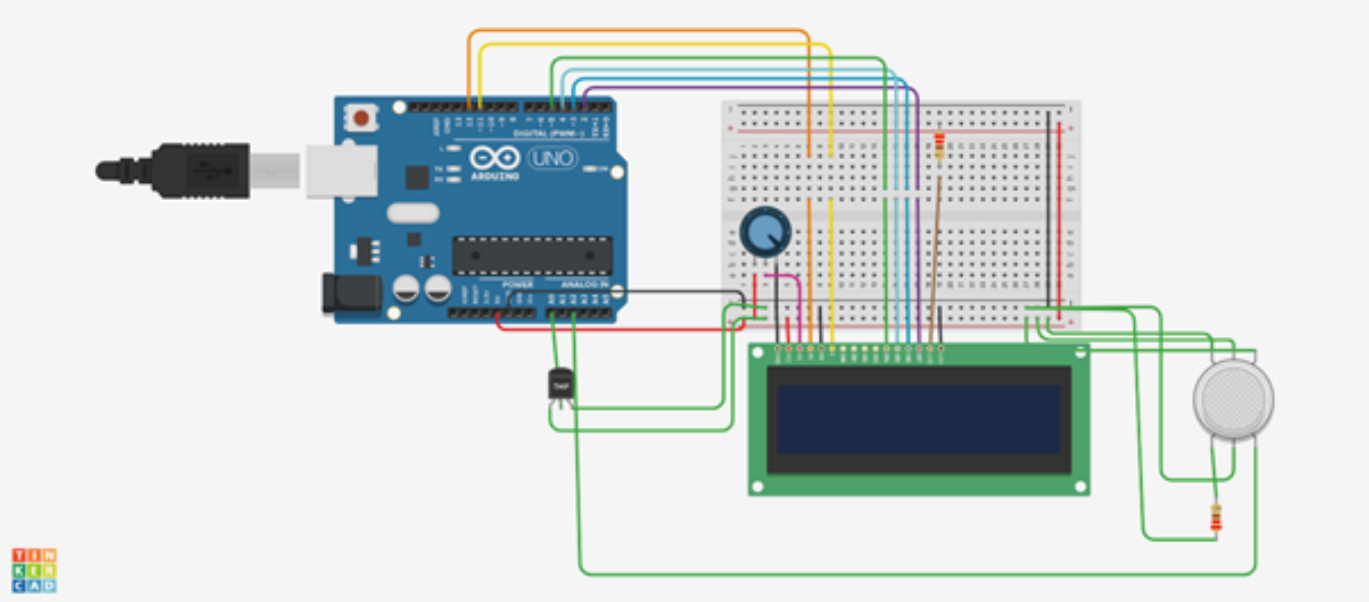
To help organize both this guide and your project, we suggest dividing the project in 3 modules, the Electronic, CO2 production and Incubator modules.

We recommend building first the Electronic module and the CO2 production module and then leaving the Incubator Module last.

Electronic module

PARTS INVOLVED	
CO2 Sensor	2 coolers
Arduino Uno	LCD or LED display
Relay module	Connection Cables
Peltier Cooler with dissipators	Solder 38Watts

This should be the simplest, or the most difficult part of the whole project, since it's extremely dependant of the user ability in welding and programming.



You should weld the cables in the Universal Plate using the 38W solder (They generally come with the Arduino Unit, a 10x10 unit should be enough) and then connect them in the Arduino following the image above, or as you prefer. It should be very simple to master welders, but a bit challenging for novices. We recommend doing things one at a time, and testing them after every successful welding, even if it is a lot slower, it will be the safest approach. And as always, safety should come first.

After that, you should put up the code we have on:

https://github.com/G-cout/Incubator-Code-for-Starters

Or you can make your own code if you want, feel free to use our code as a base.

TIPS and TRICKS:

- Sometimes the sensors take very long to calibrate, so put them in a more stable environment to let them calibrate faster, 10-15 minutes should be enough.

- When welding, do it on a calm and controlled environment, nobody needs a jumpscare when dealing with a solder.

- This should be old news for every coder out there, but please, check your code!

- The sensor we used only showed ppm not the percentage of CO2 inside the incubator, but simple math can solve your problem! To convert from ppm to %, divide ppm by 10,000 To convert from % to ppm, multiply % by 10,000. So 5% should be around 50.000 ppm!

CO2 module

PARTS INVOLVED	
2 2L PETs bottles	Sodium Bicarbonate
Macrodrop	Vinegar
60cm plastic tube	Scissors
Silicon Glue	

PARTS RECOMMENDED
38W Solder

To prove the power of Chemistry, we will slice a molecule in Half! The CO2 producing module, possibly the most important part of an incubator, will have as its core the principle of acid-base reaction. When an acid mix with a base, the resulting product will be a Salt and CO2, in its gaseous form, a deeper explanation will come shortly.

For safety purposes, we will use a weak acid and a weak base, acetic acid in the form of vinegar and Sodium bicarbonate. While a stronger base or a stronger acid would be more efficient, the danger involved made us use a safer and more stable approach. This approach even facilitates the storage and handling of the reagents, opening an opportunity for us to use simple PET bottles.

We used a simple, system to regulate our CO2 making, basing it just on plastic tubes, a Macrodrop simple old pressure. But first, let's prepare our storage facilities. You will need two bottles or at least two containers for the mixtures. Using the PET bottles, make 1 gap in one of the,, and in the other one, make 2 gaps. You can use a simple scissor or if you are fancy and want something more precise, use a solder, we recommend a 38W one, but you can use what is available.

After making that, put the plastics tubes in the gaps, in the cap with the lone gap, put a plastic tube connecting it to a gap in the 2-gap cap. Then in the last gap, you put the tube connecting it to the Macrodrop , you should be careful to put it in a greater Height than the tube coming from the first bottle. Then you need to cease leaks in the gaps, we recommend using Silicon Glue to seal it, but you can use what is available. Then we'll start the chemical part.
In one bottle you will dispense 500ml of Vinegar (Let's call it Bottle 1), and in the other one 250ml of water and 60g of Sodium bicarbonate (Bottle 2). Bottle 1 should have the 1 gap cap and Bottle 2 the 2 gap cap.

In the end, your system should look somewhat like this:

Bottle 1 is the green one (left) and Bottle 2 is the white one (right)

And now you guys should be wondering, what's going on. The answer is simple: Squeeze Bottle 2.

If everything has gone alright, or at least most of it, engineering is tricky, you should see the vinegar in Bottle 1 flow in the plastic tube and then drop onto the solution in Bottle 2, this should immediately cause the solution to start bubbling. Now, you need to release the pressure in the Macrodrop, or if you leave it open, don't worry, just lock it and then release it. Now you should smell a vinegar-like smell coming through the opening in the macrodrop. If you guys have your CO2 sensor working, stuck the opening close to it, of if not, pick a glass of water and stuck it in.

If you used the CO2 sensor, it should show an increase of CO2, if you use the glass of water, you should see some bubbles, this is the CO2 being produced by the acid-base reaction! You can control it's flow by using the macrodrop or by controlling the pressure in the bottles by simply squeezing them! Easy peasy.

The solution of 500ml of Vinegar, 250ml of water and 60g of Sodium bicarbonate should produce roughly 24 hours of CO2 in a rate of 2 bubbles per second, which should be close to 4 L of CO2, more than enough to create a 5% CO2 atmosphere in a 24L ice box!

But how any of that works?

Good question! And to answer it, we need to first look at the chemical equation to an acetic acid X Sodium bicarbonate reaction:

$$CH3COOH(s) + NaHCO3(l) = CH3COOONa(aq) + CO2(g) + H20(l)$$

This reaction produces Sodium Acetate, Water and CO2, but in what quantities? In Theory, 1 mole of acetic acid (mol- 60 g/mol) will react with 1 mole of sodium bicarbonate (mol- 84 g/mol) producing 1 mole of Carbon dioxide (mol- 44 g) . Let's assume the same proportions of the reactants as their molecular weight, which means there's 1 mole for both, their reaction will produce 44 grams of carbon dioxide which is equivalent to 22.4 litres of carbon dioxide at STP conditions. These on perfect theoretical conditions, but our conditions aren't perfect or theoretical.

We will not use pure acetic acid, but if you have access to, it's even better, but the 4% inbued in vinegar. Since we will put 500ml of vinegar, with à weight of 500g, we will have only 4% of it in acetic acid:

500g	100%
?	4%
Answer: 20g of acetic acid, or ⅓ of mol.	

Then we will not use 84g of sodium bicarbonate, but 60g, lowering again our Yield.

84g	100%
60g	?
Answer: 71% or 75% rounding up to facilitate our math, which should be ¾ of the original value.	

Summarising everything, we will use respectively ¼ and ¾ of the theoretical perfect mixture to produce 44g/22,4L of CO2.
Dividing 44g by ¼ we'll get an answer of 11g. Dividing it again by ¾ we will get 8,25g of CO2. Since 44g of CO2 is equal to 22,4L, a simple division waits us with the answer!

44g	22,4L
8,25g	?
Answer: 4,2L or 4L.	

Since our system could safely produce 4L of CO2 and more if we refilled it, we simply need to know how much CO2 is used to create an 5% atmosphere in a 24L ice box. Normally, Earth atmosphere have 0.04% of CO2 in concentration (Which is still a lot), we will count it as Zero.

24L	100%
?	5%
Answer: 1.2L of CO2. And since our system could produce 4,2 L of CO2 in a single charge, this means we could completely fill it up almost 4 times before having to refill!	

TIPS and TRICKS:

- Always put water with the sodium bicarbonate, it helps lower any exo-thermic effect of the mixture and avoids you losing any bottle!

- Gently shake Bottle 2 from time to time, sometimes the acetic acid and the sodium bicarbonate don't mix correctly and don't react properly producing then less CO2.

- If you can, use recycled PET bottles, Go Earth!

- Sometimes the Macrodrop can get coggled, shake it a bit to clean it up.

- If you don't have a Macrodrop , an aquarium/porous stone can be used in its place, but you then lose the capacity to control the CO2 flow.

- The Macrodop will probably smell like vinegar, so pick one you like, the types don't affect the result!

Incubator module:

Necessary materials	
Ice box- 24L	Ruler
Metal grid or Metal sheet	Scissors
Pincer and cutting pincer	

Recommended Materials	
Measuring tape	38W Solder

Being the last part, you should expect that it will be the worst part. While not wrong, it's actually very simple, but with a high chance to go completely into chaos and subsequent failure. So, constant vigilance and great care are needed.

The first thing to do is to pick your ice box and measure it, if you had already done it when starting it, do it again, could be by a ruler or a measuring tape. You should measure both it's external and internal space. This is important because again, there are a lot of differences between ice boxes.

This is important because you will build some floors for your incubator. We recommend using a simple metal grid, common in barbecues, or a metal sheet. Since is almost impossible to buy a sheet with the exact same measures than the internal part of your icebox, but if you do please regard it as a lucky charm, you need to do some modifications.

Using a cutting pincer, or a solder, you should cut enough of the metal grid or sheet to fit on the internal part, because since Styrofoam is somewhat flexible, if it's a little too big, it should still work. You can make as much floors as necessary, but we recommend it to be it between 2 and 4. Put them on a somewhat equal distance between each other. If any floor looks a little fragile, we recommend reinforcing it with silicon glue.

After that, mark where you will put your electronic parts and your CO2 producing parts, could be with a pencil, a marker or an alternative. Don't put them on it, because now the really tricky part will begin.

Separate where you will put your sensors, cables, Macrodrop and other things necessary, then with a scissor or with a solder, make the necessary holes. The holes should be wide enough to fit the necessary components, or a little too wide if needed. This should be done last as any mistake will invalidate the incubator module and you will need to do it all again.

Any sort of leaks should be closed with silicon glue or an alternative one, since now leaks will threaten the whole system, even if you don't found any leaks, silicon glue should be applied nonetheless, since they will help stabilize the components and in the sheet with the solder.

After that, your incubator is finished! You can customize it as you want!

TIPS and TRICKS:

- If you use a metal sheet we recommend putting in it a little deeper in the incubator, leaving some gaps for the air to flow. An alternative could be to make some holes in the sheet with the solder.

- If you use the solder to make the holes in the incubator, do it with care! Styrofoam is very sensible to heat, and as such will make really big craters if you stick it too fast or don't take it out fast enough. Be really careful with that.

- Sometimes the lid of the ice box will be too deep and as such will not close after you insert the "floors", please adapt your floor size if necessary.

General directives:

These are more general tips and tricks, but most of them are important to remember, since dealing with an incubator, should it be this simple model or a professional one.

- **Be Clean:** Since contamination is a major problem in cell culture, we recommend operating your system in the most sterile environment possible, we recommend cleaning the room that houses the incubator 3-4 times per week. Using specific IPEs to this room and cleaning it 2 times per week is very much recommended. Everytime that a major pause in cell culture happens,1-2 weeks, we recommend cleaning the incubator in it's external and internal area before use.

- **Plan Ahead:** Dealing with living cells is a hard work, many times you will face yourself dealing with problems and test in not desired times, like having to change cell media 15 min before giving a lecture to +40 people, as such a comprehensive and well organized planning is of great help, but be ready for surprises!

- **Remember your Training:** Since cell culture is a very delicate business, major care needs to be done in every aspect, from changing media to put them on the incubator. Cleanness and speed are essential, and in doubt, remember your training!

- **Keep Calm and Carry On:** Let's say you have done everything correct, you keep everything clean, you have done your planning and have done your training proud, but still your test is wrong or your culture is growing in a slug pace. Keep calm and revise every step above and everything else. And then Carry on, since dealing with cells, as any living thing is very hard and error-prone.

For feedback or doubts please contact:

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