

# The essential Biohacker's guide: Begin your journey in the DIY-Bio world

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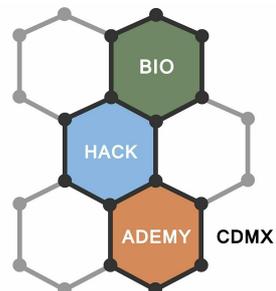
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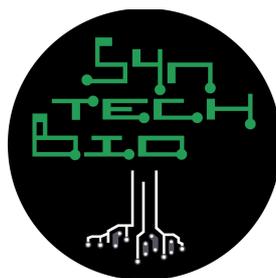
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## **The essential Biohacker's guide (Version 1.1\_English): Begin your journey in the DIY-Bio world**

### **Introduction**

The DIY-Bio (Do-it-yourself biology) movement began as an answer to the necessity for to public access and the democratization of Genetic Engineering. This technology is known by many names, including Molecular Biology, Biotechnology, and Synthetic Biology. Genetic Engineering is key in the production of biomaterials, biofuels, medicines and biosensors, etc. (Table 1).

The beginnings of the movement can be tracked to an early promoter of DIY-Bio, named Rob Carlson, who in a [2005 Wired article](#) showed that \$1,000 was all it cost to start using this technology and pointed to online resources. In 2008, [DIYbio.org](#) launched as a channel of communication for DIYers who wanted to build a community around it. In 2010, the first biohacker spaces opened in California ([BioCurious](#)) and New York ([GenSpace](#)). Today [DIYbio.org](#) maintains a [list](#) of the biohacker spaces around the world: 41 in North America, 28 in Europe, 6 in Latin America, 5 in Asia, and 4 in Oceania (consulted 05/27/2016). This shows that in eight years, the movement has become a global phenomenon.

In 2015 several of the groups in Latin America came together with the aim of promoting the movement in this region, creating the [Biohacker Spaces Network of Latin America - SyntechBio](#). Among the goals of this group is the expansion of knowledge and the creation of tools for DIY-Bio in Latin America. It has driven the creation of this biohacker Guide. This manual seeks to provide simple and punctual information to enable the creation of new spaces dedicated to DIY-Bio/Biohacking, helping with the democratization of this technology. This document is a compendium of the experiences of the pioneering groups in the implementation of these spaces in Latin America.

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## 1. What do I need to start?

The infrastructure and resources that will be needed depend on the activities that will be carried out in the space. Within the DIY-Bio we start with four types of basic activities: Microbiology, Molecular Biology, Cell Biology and Bioinformatics (Table 2). For each one of these types of activity you need certain resources (Table 3).

**TABLE 1 - BIOTECHNOLOGY RELATED APPLICATIONS.**

Product	Technology	Field
<u>Insulin</u>	Recombinant DNA	Medicine
<u>GlowingPlants</u>	Recombinant DNA	Research/Bio-Art
<u>Home-brewing</u>	Fermentation/Microbiology	Food
<u>Biodiesel from microalgae</u>	Fermentation/Biochemistry	Energy
<u>Pleurotus spp</u>	Microbiology	Food
<u>Yeast sounds</u>	Microbiology/Electronics	Bio-Art
<u>Gold precipitation</u>	Synthetic Biology	Recycling
<u>Coliroid</u>	Synthetic Biology	Bio-Art
<u>Tissue</u>	Tissue engineering	Medicine/Research

**TABLE 2 - ACTIVITIES**

Fields	Experiments
Microbiology	<ul style="list-style-type: none"> <li>-Isolation of microorganisms from environmental and other samples.</li> <li>-Growth of bacteria, yeast and other microorganisms.</li> <li>-Bioprospecting and production of compounds of interest as antibiotics, pigments, secondary metabolites, among others.</li> </ul>
Molecular Biology	<ul style="list-style-type: none"> <li>-Extraction of genomic DNA and/or plasmidial and RNA (from microorganisms, animal or plant cells) and analysis of these, including sequencing.</li> <li>-Extraction of proteins and analysis of these.</li> <li>-Copy, multiplication, insertion and modification of DNA to encode, enable or delete functions in microorganisms, plant or animal cells, including the design and synthesis of DNA.</li> </ul>
Cell Biology (animal cells or plant cells)	<ul style="list-style-type: none"> <li>-Cell culture of animal or plant cells.</li> <li>-Measure, observation and testing of physiological and morphological changes on cell cultures under different experiments.</li> <li>-Preservation of reproductive material of species of interest or endangered.</li> <li>-Extraction and analysis of essential oils and secondary metabolites.</li> </ul>
Bioinformatics	<ul style="list-style-type: none"> <li>-Design of DNA to include modifications at the level of DNA, RNA or protein.</li> <li>-Analysis of genetic information using Genomics, Transcriptomics and Proteomics and study of evolution and phylogeny using comparative genomics.</li> <li>-Analysis of interactions between biological molecules in 3D.</li> <li>-Design of pharmaceutical targets.</li> <li>-Analysis of metabolic pathways.</li> </ul>

**TABLE 3 – RESOURCES**

**A- Hardware and Infrastructure**

<b>Microbiology</b>		
<b>Equipment</b>	<b>Description</b>	<b>DIY-Bio alternative</b>
Working area	A designated space for working will be needed, not necessarily large. In this space will be placed only the equipment and materials to be used for DIY-Bio experiments. In some cases it is necessary to divide the area to avoid contaminating some materials when working.	A garage or an empty room can be easily adapted.
Workbench	A workbench that allows working with chemicals without being damaged, that is easy to clean, and has a fire resistant surface is required. It is advisable to have more than one table to work with different organisms.	A workbench appropriate for DIY-Bio can be built following this <a href="#">tutorial</a> for the surface and this other <a href="#">tutorial</a> for the table.
Autoclave	This device uses steam at a pressure of 15 pounds, which allows the chamber to reach a temperature of 121°C. The sterilization time is usually 15 minutes. It serves to sterilize culture media, some solutions, water, glassware, metal, and wood. In the case of plastic and glass, always check if these are resistant to high temperatures before sterilizing them. Always place what will be autoclaved in containers with a screw cap or wrapped in foil paper, do not leave the lid fully closed on the containers because the pressure can force the liquid out causing burns.	A common pressure cooker can be used to replace the autoclave, but will need more time to sterilize the material, about 45 minutes. If you don't have a pressure cooker, you can use a microwave following this <a href="#">protocol</a> . The same care taken to use an autoclave should be taken when using a pressure cooker.
Refrigerator with freezer	In the refrigerator can be stored cultures of microorganisms for short periods of time (weeks) and solutions (months) to prolong their use. In the freezer you can save samples of DNA, RNA and proteins and enzymes for use in molecular biology. Do not use this refrigerator to store food.	A refrigerator that uses arduino and peltier cells can be built using this <a href="#">tutorial</a> .
Bioreactor	It is used to make continuous/batch growth of microorganisms such as yeasts and algae. Also helpful for production of products of interest.	There are tutorials as <a href="#">this</a> and <a href="#">this</a> to build bioreactors.
Microscope	It allows to visualized microorganisms, cells, and some cellular structures.	There are several alternatives to build a microscope using; <a href="#">paper</a> , <a href="#">3D printer</a> , using a <a href="#">Smartphone</a> or purchasing one that can be adapted to a <a href="#">Smartphone</a> .

Balance	It is used to weigh laboratory reagents or different materials with precision. You need one with capacity of weigh grams, if it is possible milligrams.	You can consult this <a href="#">tutorial</a> to mount a balance on a low budget. And this <a href="#">tutorial</a> for a more sophisticated balance, as well as, this <a href="#">other</a> .
Chemical hood	It serves for working with chemicals that are dangerous to inhale, such as acids and strong bases, among others. Please use caution with all dangerous substances.	This <a href="#">tutorial</a> shows how to build one.
Incubator for growth	Used to keep microorganisms in their optimal growth temperature. The incubator must have movement if you plan to make liquid cultures; the movement helps the oxygenation of the cells in the liquid medium. * <i>Escherichia coli</i> (most widely used molecular biology bacteria) grows at 37°C, yeast at 30°C and Agrobacterium at 28°C.	Avoid building incubators with materials such as wood triplex or other woods because they are very vulnerable to moisture. Incubation can be done using a <a href="#">system of hatching eggs</a> . To give movement to the incubator you can put it in a table <a href="#">stir</a> , as described in this <a href="#">tutorial</a> . You can also build one by following this <a href="#">tutorial</a> .
Vortex	It serves to mix small amounts of liquid evenly.	A simple one can be built by following this <a href="#">video</a> . Or a more elaborate as in this <a href="#">other</a> .
Magnetic stirrer	It serves to stir solutions allowing them to be homogeneous.	One can be built using this <a href="#">tutorial</a> . You can choose a version with temperature control, as in this <a href="#">tutorial</a> .
Spectrophotometer	Used to measure concentrations of solutions or the growth of microorganisms. It also serves to measure the concentration of DNA or RNA.	This <a href="#">tutorial</a> explains how to build one to measure growth of cultures of bacteria, while this second <a href="#">tutorial</a> explains how to build one more appropriate to measure concentrations of DNA/RNA.
Bunsen Burner	Helps to maintain sterile material while working (requires a supply of gas), also can be replaced by an alcohol lamp.	This <a href="#">video</a> shows a Bunsen burner that can be built at home.
Laminar flow cabinet	This equipment is optional in microbiology, the good use of a Bunsen burner may be enough to keep the experiments free of contamination. For some experiments it may be that in addition to being sterile they need be anaerobic, and they require a GloveBox.	This <a href="#">tutorial</a> shows how to build a laminar flow cabinet. While this <a href="#">tutorial</a> shows how to build a Glovebox.
pH-meter	Used to measure the pH. It is important in the preparation of culture media and solutions.	We find this <a href="#">kit</a> and this <a href="#">list</a> of pH-meters at reasonable prices. This equipment can also be built, following this <a href="#">guide</a> or any of these tutorials: <a href="#">tutorial_1</a> , <a href="#">tutorial_2</a> .

Other items	<p>-Test tubes (100mL and 1L), pipettes (5mL and 20mL), beakers (500mL and 1L) Erlenmeyer flasks (500mL and 1L), volumetric flasks (100mL, 500mL and 1L), glass beads, glass handles and <u>Neubauer Chamber</u>.</p> <p>-Glass bottles with screw cap to store solutions, water and culture media that were sterilized.</p> <p>-Petri dish. They can be plastic or glass, if they are made of glass can be sterilized using the autoclave or pressure cooker.</p> <p>-Microbiology handle. A wooden or plastic handle subject to a copper wire to handle microorganisms.</p> <p>-Slides and cover slips to place samples in the microscope.</p> <p>* The majority of plastics and glass can be purchased at websites such as amazon or aliexpress.</p>
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<b>Molecular Biology</b>		
<b>Equipment</b>	<b>Description</b>	<b>DIY-Bio alternative</b>
Thermal cycler	This machine makes copies of DNA. It is also used to identify if a sample has a specific region of DNA using specific primers.	There are several low-cost models such as <a href="#">this</a> or <a href="#">this</a> . There are also tutorials to build one like <a href="#">this</a> , which was developed by our group in collaboration with the <i>Lab de Garagem</i> in Brazil.
Centrifuge	Used in almost every experiment, from extraction of DNA to working with proteins. In general, a centrifuge that reaches 11,000rpms is ideal. It is use to quickly separate different components of a solution based on their densities.	It can be constructed by following this <a href="#">tutorial</a> or <a href="#">this</a> .
Water heater	Useful for enzymatic reactions and transformation of cells by heat-shock.	These tutorials: <a href="#">tutorial_1</a> and <a href="#">tutorial_2</a> , explain how to build it. It can also be replaced by a thermal cycler.
Micropipettes and disposable tips	Necessary for all molecular biology experiments (2uL, 20uL, 200uL and 1000uL). Used to measure and move small volumes of liquids.	They can be <a href="#">purchased</a> or built by using this <a href="#">tutorial</a> or this <a href="#">tutorial</a> .
Electrophoresis chamber	Used to separate DNA, RNA and proteins by size and mass, so they can be visualized later. There are vertical (for DNA and RNA) and horizontal chambers (usually proteins).	There are tutorials such as: <a href="#">tutorial_1</a> , <a href="#">tutorial_2</a> and <a href="#">tutorial_3</a> that explain how to build one.
Transilluminator	Used to view electrophoresis gels. It uses UV light. Handle with care.	This <a href="#">tutorial</a> explains how to build one.
Ultrasonic tank	Used to break the cell wall allowing the extraction of some molecules of the cells.	This <a href="#">tutorial</a> shows how to build one. You can also see this <a href="#">other</a> tutorial.
Microcentrifuge tubes	Used for enzymatic reactions and other experiments (0.2ml and 1.5ml) (Brand name: Eppendorf, there are cheaper options on the market)	

<b>Cell biology (with animal or plant cells)</b>		
<b>Equipment</b>	<b>Description</b>	<b>DIY-Bio alternative</b>
Laminar flow cabinet	It is essential for cell culture because these cultures are highly susceptible to contamination by fungi from the environment. It is also necessary to have what was already described for microbiology.	This <a href="#">tutorial</a> shows how to build one.
Incubator with CO <sub>2</sub> and CO <sub>2</sub> tanks	For animal tissues it may be necessary to maintain certain conditions of temperature and concentration of CO <sub>2</sub> .	
Materials of glass for distillation	It varies depending on the method of extraction to be used: fractional distillation, extraction by reflux, percolation, extraction Soxhlet, etc.	Consult this book: <i>L a b o r a t o r y Handbook for the Fractionation of Natural Extracts</i> by Peter J. Houghton and Raman Amala.
Rotary evaporator	It uses low pressure and temperature to separate the molecules extracted using solvents that are volatile.	
Chromatography equipment	There are different methods such as; thin layer chromatography (TLC), liquid chromatography (HPLC), gas chromatography (GC-MS), among others. While some require only a container of glass and solvents (TLC) and others use specialized equipment (HPLC, GC-MS).	<a href="#">Here</a> see how to build DIY chromatography equipment.
Other items	There are systems to grow plants under relatively controlled conditions that can be built like <a href="#">this</a> . Others are already creating <a href="#">robots</a> to help grow their plants or conduct <a href="#">automated</a> laboratory protocols. Still others are already building <a href="#">bioprinters</a> .	

<b>Bioinformatics</b>	
<b>Equipment</b>	<b>Description</b>
Computers and Internet access	Preferably with an emulator of Terminal or an operating system or virtual machine with Linux or MacOs.
Storage in the cloud	Dropbox, Google Drive, Amazon server, etc.
Software	The majority of bioinformatics tools can be used on-line, others are for download, usually free (Mega, PDB Viewer, etc.).
*For people that is going to start working in this area we recommend reading this <a href="#">publication</a> , which was developed by the <a href="#">Leukippos Institute</a> in collaboration with our group.	

## B - Software and Databases

<b>Software and databases</b>	
<b>Application</b>	<b>Tools</b>
DNA	<ul style="list-style-type: none"> <li>-<a href="#">Benchling by Benchling, Inc.</a> - Tool for DNA design (plasmid and CRISPR/Cas) and keeping experiment notes.</li> <li>-<a href="#">Cytostudio by Molecula Maxima</a> - Biolanguage for synthetic biology based on the iGEM database.</li> <li>-<a href="#">Genome Compiler by Genome Compiler</a> - Tool for DNA design (plasmids).</li> <li>-<a href="#">GeneDesigner by DNA2.0</a> - Tool for DNA design (plasmids).</li> <li>-<a href="#">NEB Cutter by New England Biolabs, Inc.</a> - Used to find restriction sites.</li> <li>-<a href="#">ORF Finder by NCBI</a> -Used to find Open Reading Frames.</li> <li>-<a href="#">SnapGene by SnapGene</a> - Tool for DNA design (plasmids).</li> <li>-<a href="#">MEGA by MEGA</a> - Analysis of genomic and proteomic data to generate trees and clusters of sequences that are evolutionarily related.</li> </ul>
RNA	<ul style="list-style-type: none"> <li>-<a href="#">mFold by Michael Zuker</a> - Predicted secondary structures of RNA and DNA using Tm and free energy calculations.</li> </ul>
Proteins	<ul style="list-style-type: none"> <li>-<a href="#">DeepView by GlaxoSmithKline &amp; Swiss Institute of Bioinformatics</a> - Protein structures display.</li> <li>-<a href="#">Molecular Flipbook by Andrej Sali</a> - Used for visualization in 3D of protein structures.</li> <li>-<a href="#">VMD/NAMD by James C. Phillips et al.</a> - Molecular display of protein structures. Molecular dynamics Simulator.</li> <li>-<a href="#">ExPASy Proteomics server by the Swiss Institute of Bioinformatics</a> - Links to calculate protein parameters.</li> <li>-<a href="#">Modeller by Sali Lab</a> - 3D modeler using homology.</li> </ul>
Systems	<ul style="list-style-type: none"> <li>-<a href="#">TinkerCell by Deepak Chandran</a> - Computational models using parts, cells and modules.</li> <li>-<a href="#">Metabolic Tinker by Kent McClymont and Orkun Soyer</a> - Build metabolic pathways using thermodynamic parameters.</li> <li>-<a href="#">Biocompiler by OMIC Tools</a> - It generates genetic regulatory networks, and helps design automation of genetic constructs.</li> </ul>
Databases	<ul style="list-style-type: none"> <li>-<a href="#">Registry of Standard Biological Parts by MIT</a> - Open source repository of BioBricks.</li> <li>-<a href="#">The public instance of the JBEI Registry</a> - Repository of DNA.</li> <li>-<a href="#">GeneBank by National Center for Biotechnology Information</a> - Repository of DNA and RNA.</li> <li>-<a href="#">Protein Data Bank</a> - Repository of protein three-dimensional structures.</li> <li>-We recommend looking at this <a href="#">publication</a>, it describes all the databases of nucleic acids through 2016.</li> </ul>

## C - Chemical and biological substances

<b>Microbiology</b>		
<b>Substance</b>	<b>Description</b>	<b>Formulation</b>
LB medium	It is used to isolate and grow bacteria. You can consult this <a href="#">website</a> for more information about its composition.	The preparation of the LB medium can be seen in this <a href="#">video</a> , an alternative to the LB medium can be consulted <a href="#">here</a> .
PDA culture medium	It is used to isolate and grow yeast. You can consult this <a href="#">website</a> , for more information about its composition.	The preparation of a homemade medium PDA can be seen in this <a href="#">video</a> .
Culture medium for microalgae	It is used to isolate and grow microalgae. For more information you can consult this <a href="#">website</a> .	The preparation of media for micro-algae can be seen in this <a href="#">video</a> and the composition of different media can be consulted in this <a href="#">page</a> .
Agar	It is a <a href="#">polymer</a> non-metabolizable by microorganisms, so it is ideal to formulate solid media.	It can be found in stores as agar-agar, it is used to make jellies. Not to be confused with pectin or gelatin.
Antibiotics	They are used to inhibit the growth of organisms. In the particular case of microbiology and cell cultures, they are used to prevent the growth of bacteria, yeasts and fungi in the culture media. In molecular biology are used to separating those bacterial colonies that have been transformed with a plasmid containing the gene for resistance to a given antibiotic.	Can be available at pharmacies, the most used are ampicillin, and chloramphenicol. In this <a href="#">video</a> it is explained how to make Petri dishes by adding antibiotics to the culture medium. In this <a href="#">other</a> you can find a brief description of the antibiotics classification.
Glycerol	It is used to conserve strains of microorganisms for long periods of freezing (-80°C). Normally, a stock of 50% is used to conserve the microorganisms at a 25% of glycerol.	You can get it in stores as Glycerin. Before use you must sterilize the glycerol using a filter as explained in this <a href="#">video</a> .
Acid solution (HCL)	It is used to acidify the culture media, when it is required to lower the pH. It is usually prepare a stock solution at 1N.	You can get in stores of chemical reagents. Subsequently the 1N solution must be prepared as explained in this <a href="#">video</a> , save the solution properly tagged in amber glass jars.
Basic solution (Sodium hydroxide)	It is used to make basic the culture media, when it is required to raise the pH. It is usually prepare a stock solution at 1N.	You can get in stores of chemical reagents. Subsequently the 1N solution must be prepared as explained in this <a href="#">video</a> , save the solution properly tagged in plastic bottles.

Stains	Used to increase the visibility of cells and cell structures under the microscope, as well as to quantify cell viability. Staining techniques help to identify bacteria. You can consult this <a href="#">website</a> for more information.	Some stains are available in pet stores and also in other stores.
Microorganisms	The most commonly used microorganisms for molecular biology are: <i>Escherichia coli</i> and yeast. There are different strains and genotypes, each with different characteristics.	These organisms can be purchased from <a href="#">official repositories</a> or laboratories in your region.

<b>Molecular Biology</b>		
<b>Substance</b>	<b>Description</b>	<b>Formulation</b>
Restriction enzymes	Used to cut the DNA at specific sites. You can check this <a href="#">video</a> for more information and this <a href="#">website</a> to optimize your reaction. Also check this <a href="#">website</a> and this <a href="#">website</a> to troubleshoot the main problems if your reaction is not working.	The <a href="#">restriction enzymes</a> with best ratio cost/functionality are those described in the <a href="#">standard</a> of Biobricks Assembly: EcoRI, XbaI, SpeI, PstI and NotI. There are also useful: BamHI, BglII, XhoI, NheI and EcoRV.
Molecular weight markers	Used to identify fragments of DNA, RNA, or complete proteins by size or molecular mass using electrophoresis gels. For more information you can refer to this <a href="#">website</a> .	It is necessarily to buy it. Another option for DNA is to prepare a reaction of digestion with a sequence of known DNA that produces a pattern with different sizes of fragments and use it as a marker.
Plasmids	They are used in cloning and expression, both bacteria and plants. This <a href="#">page</a> is a repository of plasmids where you can get more information and order them. You can also check this <a href="#">video</a> to learn more.	They can be donated by a laboratory of molecular biology or purchased and subsequently produced and purified in the biohacker space. As explained in this <a href="#">video</a> or this <a href="#">other</a> .
Water (ultrapure MilliQ)	It is purified and deionized water used in molecular biology. It does not contain salts, microorganisms, and substances that may interfere with experiments. Consult this <a href="#">site</a> for more information.	It can be produced in the biohacker space by passing <a href="#">distilled</a> water through various filtration systems: <a href="#">reverse osmosis</a> , UV light and filter (20 µm).
TAE or TBE buffer	They are used to enable the flow of electric current in the electrophoresis runs and it is used in the preparation of electrophoresis gels. It is usually a prepared stock solution of 50X that is used at a 1X final solution.	It can be prepared in the biohacker space following the instructions on this <a href="#">page</a> for the TAE buffer and <a href="#">this</a> for the TBE buffer. Also, there are alternatives to these two buffers as described in this <a href="#">article</a> .

Agarose	The agarose is a polymer extracted from algae that has the capability of gelling aqueous solutions. It is used to prepare electrophoresis gels.	Agarose remains a powder until used to prepare the electrophoresis gel, as this <a href="#">video</a> shows. To learn more check this <a href="#">page</a> and in this <a href="#">other</a> .
Polyacrylamide	Polyacrylamide is the result of a chemical reaction resulting in the polymerization of its components, generating a gel that unlike the agarose gel is not affected by the temperature and with smaller pore size than the agarose.	Acrylamide solutions should be prepared with extreme caution. The resulting solution must be handled with precaution. To prepare a polyacrylamide gel follow the steps in this <a href="#">video</a> . You can also check this <a href="#">page</a> to learn more about SDS-PAGE, or watch this <a href="#">video</a> and this <a href="#">other</a> .
SDS-PAGE running buffer	This buffer is used to run the polyacrylamide gel in the electrophoresis chamber. Prepare a stock solution at 10X, which can be used at 1X.	It can be prepared as it is explained on this <a href="#">protocol</a> .
Loading SDS-PAGE buffer	This buffer serves to linearize the protein samples to be loaded on the electrophoresis gels.	It can be prepared following this <a href="#">protocol</a> . The steps can also be watched on this <a href="#">video</a> . Consider preparing several vials of this buffer.
Stains for DNA	Usually commonly used dyes to see the DNA after electrophoresis. However, there are low cost alternatives that can be implemented.	Methyl Green is a dye that has been widely used in histology for staining of tissues, but it is also an excellent alternative for double stranded DNA as you can see in this <a href="#">paper</a> . Also, you can use the blue of methylene or the violet of gentian, following this <a href="#">protocol</a> .
Stains for proteins	One of the dyes used to stain proteins is Coomassie blue. However there are more rapid and economic alternatives that may be performed in a biohacker space.	The dye for proteins LAWSONA can be extracted from the leaves of Henna following this <a href="#">protocol</a> . There are dyes such as red #3 (Azorubina) that can also be used following this <a href="#">protocol</a> .
TEMED	Tetramethylethylenediamine (TEMED). It is used to polymerize the polyacrylamide gels. Used with ammonium persulfate.	To do polyacrylamide gels check this <a href="#">protocol</a> for DNA gels or this <a href="#">other</a> for protein electrophoresis.
SDS	Dodecyl sodium sulphate (SDS). Breaks non-covalent links from proteins, which is needed to perform electrophoresis. It is slightly irritating.	It can be consulted <a href="#">here</a> to see the preparation of SDS-PAGE gels.
TRIS-HCl	Trisaminometano and hydrochloric acid. Acid buffer that is used to lower the pH of a solution.	It is prepared from pure HCl by diluting it with distilled water and TRIS.

TRIS-NaOH	Trisaminometano and sodium hydroxide. Alkaline buffer that is used to increase the pH of a solution.	It is prepared from NaOH powder, diluted in distilled water and TRIS.
Enzyme Taq polymerase	This enzyme produces copies of DNA during the PCR, it is resistant to high temperatures.	It can be produced following this <u>protocol</u> .
EDTA	Ethylene diamine tetraacetic acid. Chelating agent that catches minerals as the Mg <sup>2+</sup> to avoid that some enzymes from degradation of DNA or RNA.	
Enzyme ligase	It is used to join DNA fragments, usually to join DNA fragments that want to be cloned in plasmids that have been cut with restriction enzymes.	
dNTP's	Nucleotides (ATCG) that constitute DNA, they are used for the synthesis of copies of DNA during PCR.	
Reagents for <u>Miniprep</u> .	It is used for the purification of plasmids.	
<u>Tubes</u> with resin of silica to purify nucleotides	Tubes covered in treated silica so nucleic acids are trapped in their walls while the other cellular components pass through these.	
Other	Chloroform, acetic acid, calcium alginate, albumin (used to stabilize enzymes/ proteins solutions).	

<b>Cell biology (using plant cells)</b>		
<b>Substance</b>	<b>Description</b>	<b>Formulation</b>
MS medium	Medium of Murashige and Skoog. It contains the basic nutrients for plant tissue cultures, the recipes may vary depending on the type of plant.	There are recipes on the internet with many modifications possible for best results.
Plant growth regulators	Anthocyanins, cytokinins, gibberellins, ethylene, Abscisic acid, etc. They promote the growth and/or differentiation of cells (complete plant or specific organs)	Extracts from plants such as banana or coconut are rich in minerals and plant growth regulators.
Antibiotics	Used to avoid contamination by bacteria and fungi/ yeasts.	Some plant extracts possess antibacterial and/or antifungal properties.
Agar	To give support to the culture media. It is not always necessary.	Kitchen agar can be used.
Solvents	They are used to separate compounds depending on their charges, ranging from polar to apolar. (-) Petroleum ether < pentane < chloroform < dichloromethane < ethyl acetate < methanol < water (+).	They must be always prepared in a chemical hood because they must not be inhaled.

Developers	For thin layer chromatography, allow observing some separate compounds that are not visible to the naked eye: uv light, iodine, vanillin, or others.	In the case of uv light, can be used devices normally used to check money.
Silica	It allows the separation in chromatography column depending on the size of the spheres of silica, or the chemical properties that are given to prepare it.	
<b>Cell biology (using animal cells)</b>		
<b>Substance</b>	<b>Description</b>	
DMEM medium	Basic medium for cell culture.	
Trypsin-EDTA	It is used to lift the cells (which grow adhering on the walls of the flask).	
Phosphate buffer	It maintains pH 7.2.	
Fetal bovine serum	Culture medium for animal cells.	
Antibiotics	Avoid contamination of bacteria and fungi/yeasts.	

Besides these activities several spaces work with hardware such as arduino and 3D printers. The focus of this manual are the Biohacking-related activities, i.e. to understand and modify biological information. With the exception of bioinformatics, all the activities described here should be performed in spaces that comply with the regulation required by each country to work with biological resources. Regulation is discussed in the next chapter.

## **2. Biosafety and biohacker spaces regulation**

The DIY-Bio community continues to grow, thanks to the democratization of technology and the free access to the scientific literature. More people are joining this movement, which is already getting positive results in different countries. However, the Latin American community is still young. Therefore, certain guides to operate out of an academic or business environment, and also to avoid to produce fear in the population are extremely valuable. The DIY-Bio community philosophy includes making sure to not develop anything that could cause damage. For this reason, one of the goals of this guide is to provide basic information regarding regulations and biosafety in the Latin American region.

This manual is focused on the use of beneficial organisms, nothing mentioned in this document can or should be used to work with other types of organisms that could represent biohazards, have toxins or be pathogens. We believe that the good practices regarding biosafety will help to a better diffusion of the biohackers work and towards the democratization of science. In this section we will address good practices within a biohacker space, codes of ethics of the worldwide DIY-Bio community, international and local regulations, and regulatory agencies in Brazil, Mexico and Peru.

### **A- List of good practices for working in a Biohacker space**

- Have the appropriate facilities, these facilities depend on the activity and regulation of each country.
- Dispose the materials and reagents used according to its nature and biosafety regulations of each country.
- Have an internal Biosafety Committee.
- The Biosafety Committee must train new members of the group in the proper use of equipment and experiments.
- Mark all substances and solutions indicating its biological or chemical risk. Always add to the label the date of expiration, and who made it.
- Consult and comply with the biological or chemical risk of substances that are to be used.
- Flammable or toxic products must be worked on, in the chemical hood.
- Do not use your mouth to operate graduated pipettes.
- Do not eat, drink or smoke in the laboratory area, and avoid the use of products for the skin (make-up, sunscreen, etc.) during the activities.
- Wash hands before leaving the laboratory.
- Use clothing and equipment for personal protection (gloves, apron, mask, etc.) in accordance with the activity to be carried out in the laboratory.
- Store the reagents according to their chemical characteristics by isolating the hazardous materials.
- Avoid touching the face, eyes, mouth or hair to avoid contamination.
- For more information see this [Manual](#).

## B - Code of ethics or conduct

We compile a universal code of ethics and/or conduct for Biohackers, using as basis the codes used by the community of North America and of Europe listed in [DIYBio.org](http://DIYBio.org):

- Transparency: Promote the transparency and share ideas, knowledge, data and results.
- Security: Adopt safe practices.
- Democratization: Promote citizen science and decentralize access to biotechnology.
- Education: Help to educate the public about biotechnology, its benefits and its implications.
- Humility: Recognize that you do not know everything.
- Community: Listen carefully to any concerns and question and answer honestly.
- Peaceful purposes: Biotechnology should only be used for peaceful purposes.
- Respect: Respect humans and all living systems.
- Responsibility: Acknowledge the complexity and dynamics of living systems and our responsibility towards them.
- Accountability: Be accountable for your actions and for the defense of this code.
- Environment: Respect the environment.
- Experiment: Experimenting with biology leads to discovery, discover leads to innovation.

## C - International regulations

The most important protocols and conventions at the international level are:

- [Convention on biological diversity \(CBD\)](#).
- [Cartagena Protocol on Biosafety \(PCB\)](#).
- [Nagoya Protocol \(PN\)](#).

<b>Protocols signed by Latin American and Central American countries</b>	
<b>Protocol of Nagoya</b>	<b>Cartagena Protocol</b>
Brazil, Colombia, Ecuador, Guatemala, Mexico, Peru and Panama.	Old and Barbuda, Bahamas, Barbados, Belize, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Ecuador, El Salvador, Grenada, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Saint Kitts and Nevis, Santa Lucia, San Vicente and the Grenadines, Suriname, Trinidad and Tobago, Venezuela.

## D- National regulations

The following are the regulations related to the biohacker spaces in different countries of Latin America:

### I. Brazil - Profile of regulation in [Brazil](#)

The national technical Commission of biosecurity (CTNBio) is the entity responsible for regulating labor in connection with GMO (genetically modified

organisms). This Commission issues The Quality Certificate in Biosecurity (CQB) that regulates institutions (public or private) wishing to conduct the following activities using GMOs:

- The construction.
- The culture.
- Handling.
- Transport or transfer.
- The import or export.
- Storage, release into the environment or disposal.

To do genetic engineering, it is necessary to obtain the CQB. The first step to obtain the CQB is to establish an internal Commission of Biosecurity (CIBIO). The legal representative of the institution shall constitute and appoint the CIBIO, the names of the people who conform this Committee must be attached to the request for the CQB send to the CTNBio. The President of the CIBIO, as well as its members, are responsible in front of the law of the CQB.

Members of the CIBIO must have scientific knowledge and expertise to evaluate and supervise the work with genetically modified organisms. The CIBIO must have at least 3 members, and the legal representative of the institution shall designate one of the members as President, it is allowed a single member external to the scientific community.

The second step for the CQB is to send the specific form to the CTNBio. To fill out this form some points should be clear and defined previously:

- Which areas will be certified?
- Do you have containment equipment?
- Do you have equipment for the prevention and management of accidents?, such as showers to wash body and eyes.
- Which types of GMOs will be used and the risk classification of these?
- What level of risk the lab will have (I, II, III or IV)?
- Does the laboratory meets the minimum requirements listed by the CTNBio?
- Who will be the responsible technician for the laboratory?

After assessing the documentation submitted, the CTNBio can request clarifications, new documents and schedule a visit to the space that is going to receive the CQB. A CQB corresponds to an operating unit of the institution, which can consist of one or more laboratories.

Only after the approval of the CQB, the CIBIO can start the approval of projects that use the certified facilities. The CIBIO can only approve the development of level I of biosafety projects, any project that needs infrastructure or organisms classified as level II must send a requirement by the CIBIO of the institution to the CNTBio to be evaluated. Any experiment involving genetically modified organisms can't be done without having a project approved by the CIBIO.

One of the key points in the spaces is proper waste disposal. This disposal is regulated by the standard PNRS (National Solid Waste Policy), which explains the characteristics of these waste and which treatments or procedures for disposal must be done.

For more information see:

- Resolution rules #1: Regulates the stress of CQB and the CIBIO.
- Resolution rules #2: Regulates the classification of risks of the GMOs and biosafety levels.
- Website of the CNTBio.
- NBR 10.004: Waste sorting.
- NBR 9800: Classification of effluents.
- RDC - 306 and CONAMA 358: Regulation of waste at the area of health.
- NR6: Defines the (EPIs) personal protective equipment.
- NBR 14725-1: Standard for labeling the disposals.

## **II. Mexico** - Profile of regulation in Mexico

In Mexico there is a Commission, dependent of the federal Government, called inter-ministerial Commission CIBIOGEM(Inter-ministerial Commission on biosecurity of genetically-modified organisms) that is responsible for the regulation of the genetically modified organisms in the Mexican territory. The CIBIOGEM was created in the year 2006 as a requirement of the law on biosafety of organisms genetically modified and it is responsible of publishing the regulations and receive the requests of registration of GMO for use, management and production. In addition to the CIBIOGEM, other entities like SAGARPA and SEMARNAT, which are part of the Board of Directors of CIBIOGEM, have applied the use of GMO regulation.

The existing regulations in the country considered the conventions and international protocols, among the most important are the following laws and regulations:

- Law on Biosafety of Genetically Modified Organisms: Regulates the activities of contained use, experimental release, release on pilot program, trade release, commercialization, import and export of genetically modified organisms, in order to prevent, avoid or minimize the potential risks that these activities may cause to human health, the environment and biological diversity or animal, plant health and aquaculture.
- Law on Biosafety of Genetically Modified Organisms.

## **III. Peru** - Profile of regulation in Peru

In the Peru, the agency of evaluation and environmental control (OEFA) is the responsible for monitoring, control, supervision and sanction the use of living modified organisms (LMOS) in the national territory. The moratorium LMOS prevents the import and production in the national territory of living modified organisms (LMOS) for cultivation or breeding purposes, including the marine, to be released to the environment. For more information see the Law N29811.

There is also policy to regulate the entry to the country of living organisms (unmodified), from their natural environments, laboratories or scientific collections. This is regulated in Peru by SENASA. On the other hand, for access to genetic resources it is necessary to revise the rules governing this procedure, which are described in the Regulation to Access Genetic Resources.

Peru follows international biosafety protocols. These involve a series of rules and instructions that allow preserve biodiversity and promote the development of biotechnology in Latin America.

Finally, in Peruvian territory there is a Biosafety Manual that explains how to form a Committee of Biosafety, the rules to follow to ensure the safety of the members of the laboratory, as well as, how to proceed in case of having an accident and standards for waste management. Other entities that are actors within the Peruvian biotechnology field are:

-Ministry of the Environment.

-General Directory of Environmental Health.

### 3. How to open a biohacker space?

Biohacker spaces need some basic elements for allowing the free exploration of the biological sciences. We will describe five basic elements that are common to all biohacker spaces.

**A. Community:** (Biohackers) People who want to share information freely about the biological sciences. To start a biohacker movement in your town or country, you must work to form a critical mass. This minimum group of people will generate an effect on more people and create a community. This interaction should be both virtual and face-to-face.

The community can have a leader or a support team, which will facilitate the organization of the proposed activities (either by the community or the leadership team) and the implementation of them in the best way. It is also important to seek alliances with organizations that promote groups or communities. In Latin America, one of these organizations is the Association of entrepreneurs of Latin America, which has representatives in the countries of Argentina, Chile, Colombia, Mexico and Peru. They promote the entrepreneurship in the region and support to the new actors of this ecosystem. In the field of Biohacking, there is the Network of Biohacker Spaces of Latin America - SyntechBio, which also has representatives in Argentina, Brazil, Chile, Colombia, Mexico, and Peru. This network seeks to inspire and help to create an ecosystem of Biohacking and Synthetic Biology in Latin America. One of the projects of the network is this manual. This document aims to help to open new spaces in the region, and further democratize science in a more open way to society.

**B. Space:** Place where the biohackers gather to share information and build projects, may be public spaces or private spaces. The laboratories set-up will depend on the resources of the group.

A strategy to carry out this idea, is presenting the proposal for the biohacker space to a College, a University, a Research Center, an incubator for business, among others. The advantage is that you won't need to rent a space and you may obtain co-financing from the organization who welcome the project. In addition, professionals of the entity that houses the lab can help more frequently in the activities proposed. The disadvantage is that the space shall be subjected to the policies of the host organization, which is not always a good thing for the biohacker space.

Another strategy is using a space of one of the members of the group. Biohacker spaces recognized at the international level have started in a garage, attic or kitchen, among others. The selection of these areas depends on whether they are public or private, and to how many people it is intended to accommodate. Freedom of decisions and actions is the main advantage of this option. The disadvantage is that it becomes more difficult to raise money for activities or projects, which in the majority of cases comes from personal funds,

family and friends. However, there are other ways to get financing and resources, this is discussed in the rest of this chapter.

**C. Equipment and Instrumentation:** Usually the biohacker spaces do not have top line laboratory equipment, this must be compensated for, with creativity. You can build the necessary equipment, find this information in the **Table3: Resource** (Chapter 1). Another option is get the equipment through donations. This can be done directly from universities and research centers, or indirectly, through platforms on the web as Seeding Labs.

**D. Reagents:** The reagents are used, along with equipment, to carry out experiments and/or reproduce existing protocols, within the biohacker space. Before purchasing any type of reagent, you must assess the following points:

- Corresponding biosafety local and international regulations (Chapter 2).
- Budget of the project or space, to be assigned to the reagents.
- Suppliers.

**E. Financing Resources:** Economic resources are an important aspect to keep in mind for both the projects and the space. Each space should consider a business model that allows its sustainability. It is important to find support for this stage. Advice can be taken from those in the area of business or business incubators.

There are different strategies to finance the biohacker spaces, which range from private to collective financing systems or better known as Crowdfunding. Next, we will share some of the most popular options of financing.

## **I. Crowdfunding**

### Indiegogo

- Source of financing for business ideas, development of products, as well as, for new products that want to enter the market. Social campaigns enjoy a platform with rates of 0% at Generosity.
- Prices: Crowdfunding: 5% platform fees, 3% + 30 c third-party credit card fees.
- Two types of financing. Fixed financing: If you fail the targeted amount of money donations are returned. Flexible financing: It does not require a fixed amount of money to be achieved, if you fail to get all the amount the money is not returned.

### Kickstarter

- It is a platform that accepts any type of project such as art, accessories, events and spaces, ideas and experiences that are new. Projects cannot be used to raise funds for charity.
- When a project involves the manufacture and distribution of something complex, such as a device, the creator must show a prototype to the sponsors. Is not allowed the representations in rendering of realism photo.

-Create a project requires that the entity or organization is registered in the country where the project will take place. The responsibility of finishing a project is exclusive of the creator of the project. Kickstarter does not hold funds on behalf of the creator, does not offer refunds.

-Prices: The fees are only charged if you arrive to the total targeted amount. Kickstarter takes 5% and the payment institution: 3% + \$0.20 per contribution. Less than \$10 contributions incur a Special Commission by "micro-contribution" of 5% + \$0.05 per contribution. This for the USA.

### Experiment.com

-Allows the crowdfunding of science-based research projects.

-Each project must meet the following criteria:

-The experiment must answer a specific research question.

-Processes and results must be shared openly and transparently.

-Researchers must have the knowledge necessary to achieve the objectives.

-Need to explain why this project is unique.

-The funds are distributed as checks or transfers and is included the share of the platform (5%), and the rate of processing of payment (3%).

There are other platforms that can interest the reader, depending on the needs and/or countries where the space is located: Capital Cell, Futsci, Sciencestarter, Fundly, Rocket Hub, Endeavorist, among others.

## **II. Competences and International Funds**

-Hello Tomorrow.

-Get it the ring.

-500 StartUps.

-Start Up Chile.

-Seed Starts.

-Startup Battlefield.

-Pitch Competition.

-Premio Santander.

## **III. Competencies and National Funds**

Brazil:

-BioMinas.

Mexico:

-Startup México.

-Premio Nacional del Emprendedor.

-Premio de Innovación UNAM.

Perú:

-StartUp Perú.

-Ideas Audaces.

#### **IV. Products and Services the Space can offer**

-Training.

-Workshops.

-Broadcast events of science.

-Basic Kits for science activities.

-Coworking space.