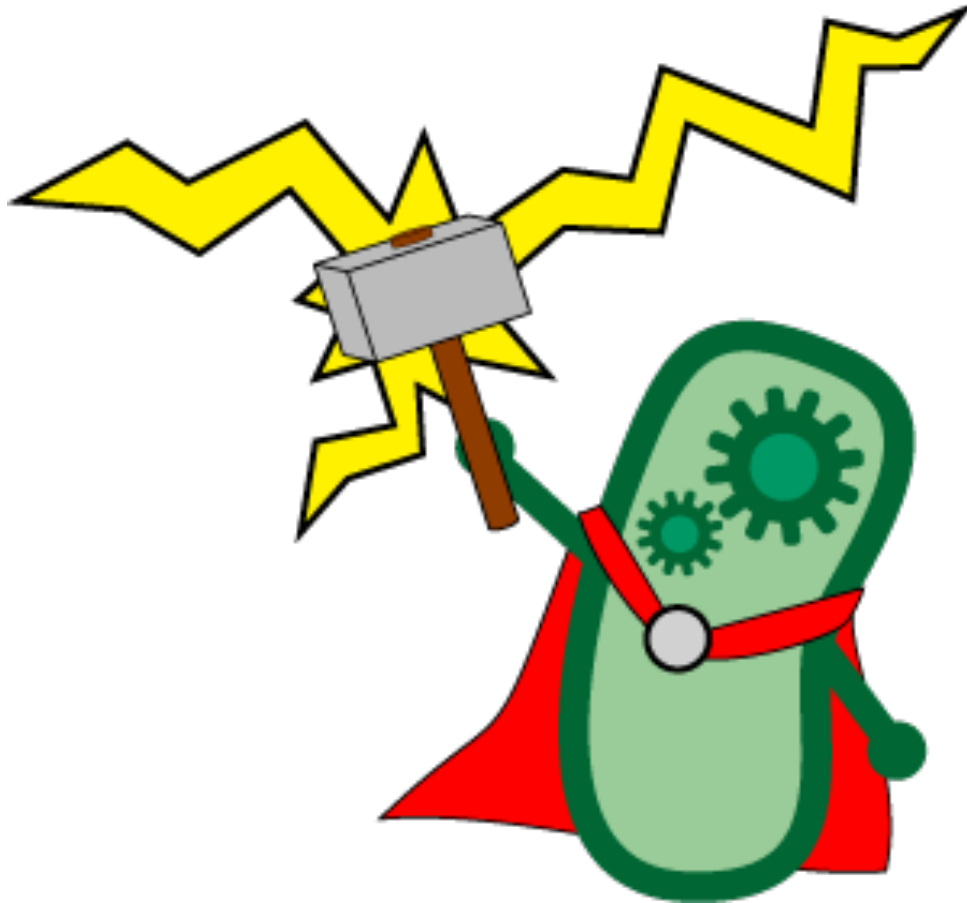# Building a Biosensor!

Do you wish you could put your organisms to good use? Wouldn't it be nice to make your very own **homemade biosensor**? For under $50.00, you can build a functional potentiostat circuit, which is compatible with data acquisition hardware and can be run with a free downloadable software suite that can be run on the MATLAB platform. This will give you the ability to conduct measurement of chemical concentrations in solution using **cyclic voltammetry**, a highly reliable and precise method of chemical detection.

This step-by-step guide contains detailed information about the necessary materials and procedures for building, calibrating and experimenting with a homemade biosensor. Enjoy!

Words of wisdom: soldering irons can get *very* hot... watch your fingers.

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Getting Started

Materials

- **1** x NI USB-6009 DAQ (Data AcQuisition device)
- **4** x LM-741C operational amplifiers
- **4** x op-amp sockets
- **2** x 9 V batteries
- **9** x Resistors:
  - ⇒ **6** x 1.0 kΩ
  - ⇒ **1** x 1.5 kΩ
  - ⇒ **1** x 2.2 kΩ
  - ⇒ **1** x 6.8 kΩ
- **1** x prototyping printed circuit board, or breadboard
- **3** x alligator clips
- **1** x soldering iron and wire solder
- **3 ft** high gauge (~24) wire
- **~20** x patterned strip electrodes
- **Bacteria** containing reporter gene plasmids (*uidA, bglX, lacZ*)
- **Phosphate Buffered Saline** at 0.1M pH7.0
- **LB** broth
- **Substrates** for bacterial reporters (PNPG, PDPG, or CPRG)

Software Requirements

- Windows 7 OS
- 32-bit MATLAB R2010a or later, with additional toolboxes:
  - ⇒ *Data Acquisition*
  - ⇒ *Signal Processing*
  - ⇒ *Instrument Control*
- NI-DAQmx drivers
- NI-DAQmx tools for MathWorks

Software Set-Up

i. Install all software packages in the order listed above
ii. Right-click the MATLAB application icon and select ***Run as Administrator***
iii. In the MATLAB command window, install the adaptor for the National Instruments device with the following code

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```
>> daqregister('nidaq');
```

iv. Move the downloaded files (from Calgary wiki) into the *MATLAB* folder (likely found in your *Documents* folder)

Circuit Construction

1. Use the circuit schematic below to map out your circuit on the prototyping board / breadboard.
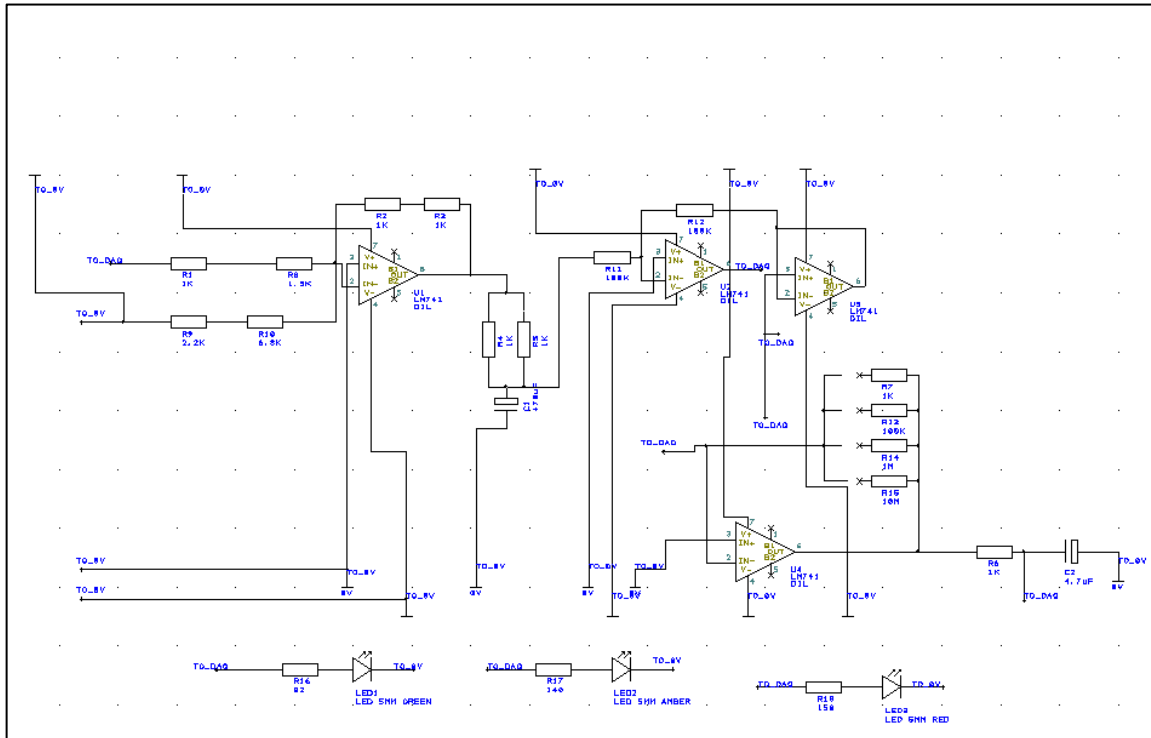
2. Put each of the components (resistors, op-amps, etc.) into their appropriate places, then solder/push them into place.
3. Use the high gauge wire to connect each of the components appropriately.
4. Cut ~30 cm lengths of wire for connections to the counter, reference, and auxiliary electrodes. Solder an alligator clip to one end of each wire, and solder the other end of each wire to the rest of the circuit.
5. Connect the positive terminal of one 9 V battery to the 6.8 kΩ resistor. Connect the negative terminal of the latter battery to the positive terminal of the other battery. Use the node between the batteries as your virtual 'ground' for the rest of the circuit.

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6. Connect the positive terminal of the first 9 V battery to the +V$_{EE}$ ports of each op-amp.Connect the negative terminal of the second 9 V battery to the −V$_{cc}$ ports of each op-amp

7. Now we must connect the 0-5 V Sawtooth waveform voltage source. In this case, the waveform source is a National Instruments device: USB-6009. Connect the 1 kΩ resistor (near the sawtooth source on the schematic) to port 13 (GND), and connect a groundwire to port 14 (AO O).
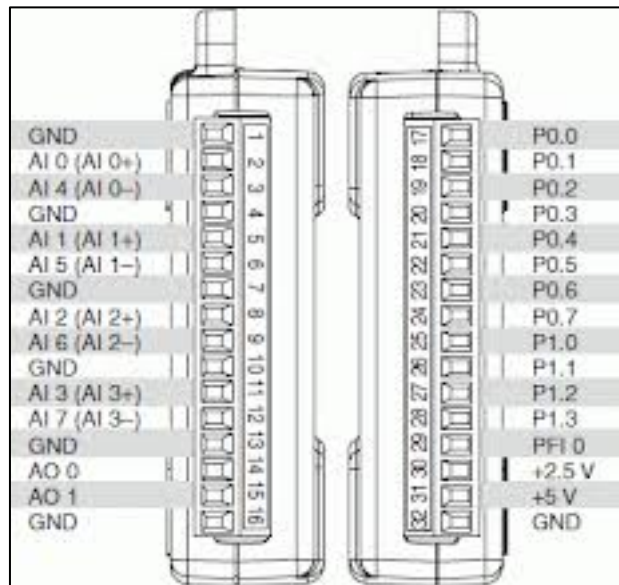
8. The NI USB-6009 is also used for measurement; connect the positive terminal of V (far right on schematic) to port 2 (AI O+) and a groundwire to port 3 (AI O-). This configuration will measure the current in solution.

9. Finally, we need to measure voltage between the reference and auxiliary electrodes. Find a node connected to the reference electrode, and connect that node to port 5 (AI 1+). Connect a groundwire to port 6 (AI 1-)

10. Your circuit is complete, and the DAQ is now properly configured. Connect the USB-6009 to your computer via USB, and proceed with setting up the electrodes.

11. The electrode set-up you use will vary based on the context of your experiment. Use the alligator clips to connect to each of your electrodes in whatever configuration you choose.

12. Run MATLAB, and enter the following code into the command window:

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```
>> test
```

13. A prompt will ask for an experiment name, as well as the agent being detected, and the sample number. Once the fields have been filled appropriately, click 'OK' to proceed.
14. The program 'test.m' is now running. A real-time plot will appear, and you can watch the voltage sweep in motion. This is a plot of the current (in microamps) with respect to voltage across the reference and auxiliary electrodes.
15. The third sweep for every trial will be saved automatically to a .m data file, which can be accessed on the MATLAB sidebar at any time.
16. You have successfully constructed your very own potentiostat circuit, and a system that can be used for electrochemical detection in a biosensor! Below is a list of links to software downloads and resources that may aid you in your conquest.

Testing with Biology

Using the electrochemical reporter genes *uidA, bglX,* and/or *lacZ* this technique can accurately detect gene expression. To do so follow this protocol:

1. Grow up a 3mL culture of the cells to be tested in LB overnight.
2. Pellet the cells at 3,750rpm for 10 minutes.
3. Resuspend in 1mL of 0.1M pH7 Phosphate Buffered Saline (PBS).
4. Add the cells to a 25mL solution of 0.1M pH7 PBS and submerge the three electrodes into the solution.
5. Add the substrate for the reporter gene(s) to a final concentration of 50µM.
6. Run the test and observe the results.

Resources

MATLAB software download
http://www.mathworks.com/products/matlab

NI-DAQmx 9.5.1 (driver) download:
http://joule.ni.com/nidu/cds/view/p/id/2933/lang/en

NI-DAQmx tools for the MathWorks download:
http://lumen.ni.com/nicif/us/evaldaqtools/content.xhtml

Circuits
http://www.khanacademy.org/science/physics/electricity-and-magnetism/v/circuits--part-1

www.**learningcircuits**.co.uk/

http://www.allaboutcircuits.com/

Soldering
http://www.mediacollege.com/misc/solder/

National Instruments USB-6009 User Manual
http://www.tau.ac.il/~electro/pdf_files/computer/ni_6008_ADC_manual.pdf

MATLAB documentation and resources
www.mathworks.com/help/techdoc/

iGEM Calgary 2012 wiki:
http://2012.igem.org/Team:Calgary

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