Bio-Hacking:

Evolution as a Tool

A short talk on a new technology
for protein engineering

and

Building a PhageStat

A longer talk on building the necessary devices

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MUSEUM of LIFE + SCIENCE

Evolution as a Tool

PACE - Phage Assisted Continuous Evolution

(Esvelt et al. *Nature*, April 2011)

Use a virus to evolve a custom protein

This requires you to ***build a PhageStat*** *to maintain a population of evolving virus (Husimi 1989)*

Off the shelf hardware for about $30,000

\- or -

DIY hardware + open source software ~$1000

Motivation

- Shigella kills 1,000,000 people – mostly children in the developing world – every year
- Shigella without the extracellular proteases **Pic** and **SepA** is harmless
- *Hypothesis*: We can evolve protease inhibitors with strong binding affinity to Pic and SepA to diminish Shigella's virulence
- *Hypothesis*: Engineered proteases produced by lactobacillus or other probiotic could provide inexpensive, long-term immunity

Protein-based pharmaceuticals

- Large proteins have reduced side-effect potential over small-molecule drugs which cross metabolic barriers and have more interaction potential
- Proteins can be more easily metabolized without stressing renal function
- Proteins degrade more quickly in the waste stream
- Binding affinity is the principal characteristic of metabolic processes and pharmaceuticals
- Increased binding affinity would lower dosage for any protein therapeutic

Evolution = *Mutation* + *Selection*

- Virus replicates inside E. coli which uses an error-prone DNA polymerase to increase mutations
- E. coli rewards improvements to evolving protein by providing the fusion protein that increases viral infection

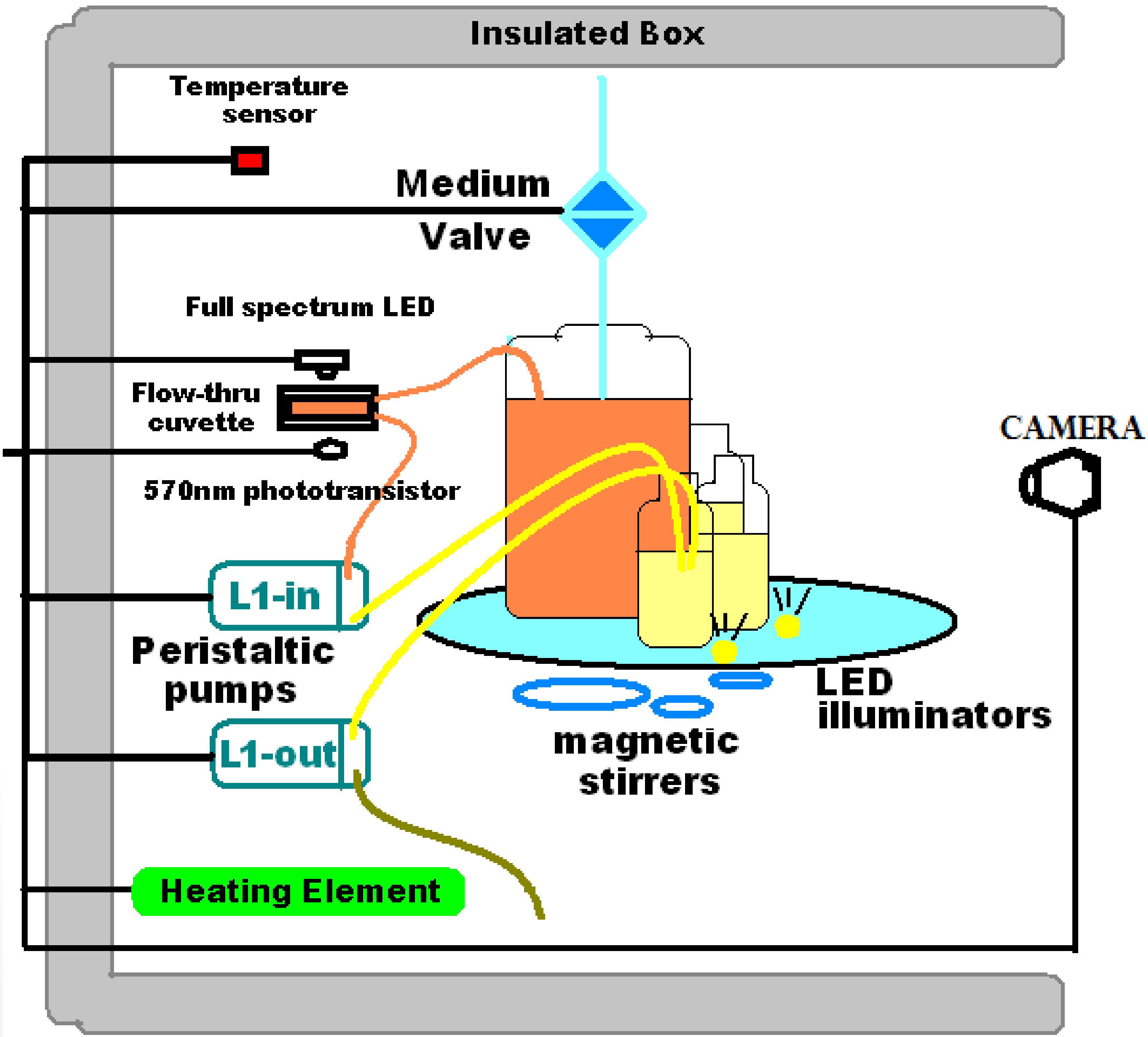
M13 PHAGE

PACE: Phage Assisted Continuous Evolution

- M13 virus (phage) with the sequence to evolve replacing its fusion gene

- E. coli with two extra plasmids (chromosomes)
  - **Mutation**: Arabinose activated mutagenesis (error-prone DNA polymerase)
  - **Selection**: M13 fusion gene activated by a particular protein interaction

- E. coli *host* cells flow through “lagoons” with populations of evolving viruses


Insulated Box
Temperature sensor
Medium
Valve
Full spectrum LED
Flow-thru cuvette
570nm phototransistor
CAMERA
L1-in
Peristaltic pumps
L1-out
LED illuminators
magnetic stirrers
Heating Element

Experiments can run from four to six days requiring reliability and automation

(10^3 generations X 10^9 mutations)

Off-the-shelf solution requires integration as well as more hands-on oversight

(three shifts of lab personnel?)

Building a **PhageStat**

A device to maintain a stable population of bacterial phage (virus)

To build a **Phagestat**, you need:

1. A ***Cellstat*** to produce (E. coli) host cells
2. Control of output and multiple input flows

Inputs: Host cells and inducers

Output: Sampling and waste output

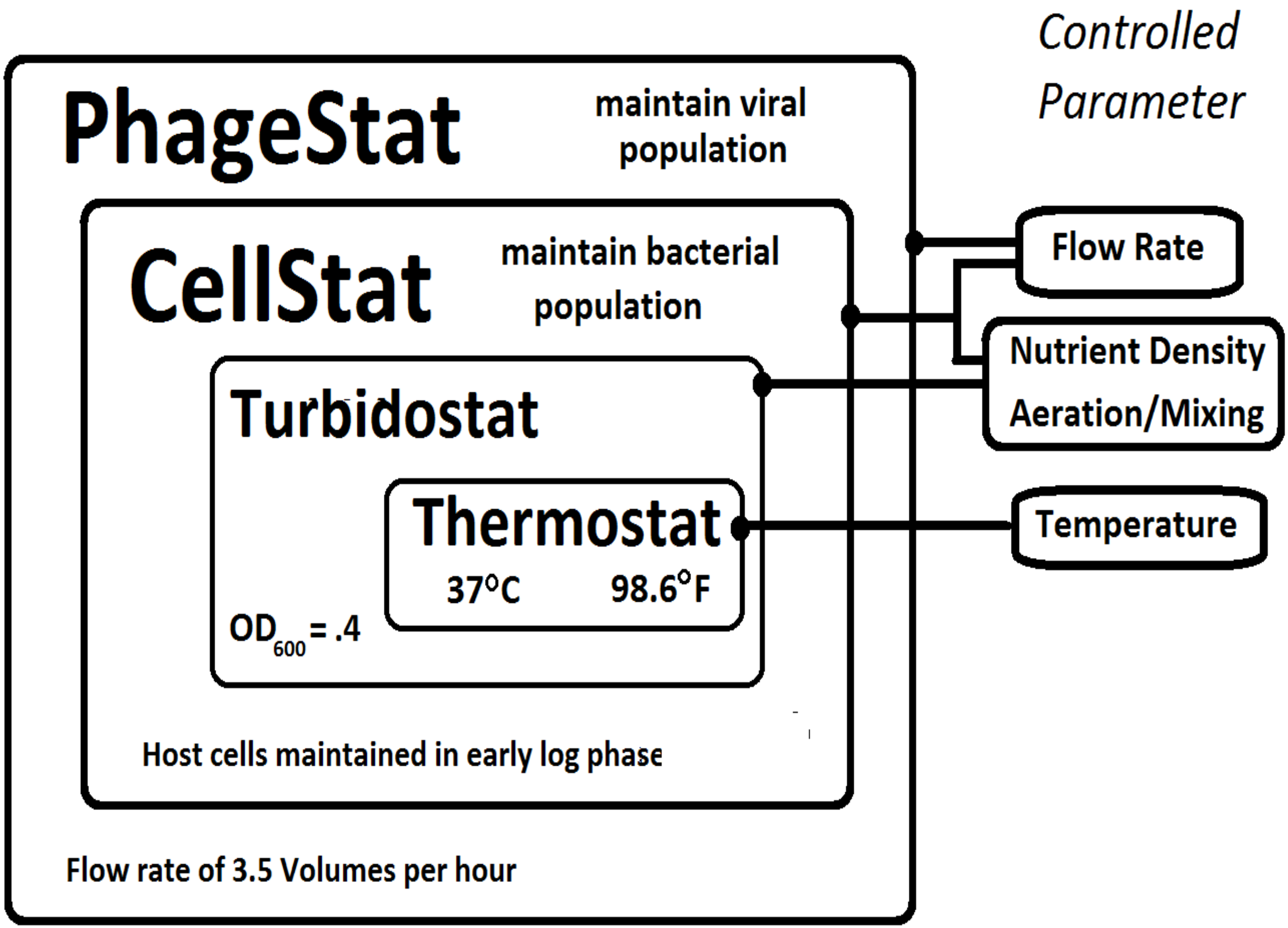
Building a **CellStat**

A device to maintain a

continuous supply of host cells (E. coli)

1. A **Thermostat** to maintain the culture at 37˚C
2. A **Turbidostat** to maintain cell density with nutrient dilution
3. Isolation from bacterial phage:

   Cellstat output is the **Phagestat** input


Controlled
Parameter
PhageStat
maintain viral population
CellStat
maintain bacterial population
Turbidostat
Thermostat
37°C
98.6°F
OD600 = .4
Host cells maintained in early log phase
Flow rate of 3.5 Volumes per hour
Flow Rate
Nutrient Density
Aeration/Mixing
Temperature

Flow rate control

The ideal **Phagestat** has:

1. A flow rate high enough to force host cells through in one cell lifetime, so the only mutations are in the phage sequence

2. A flow rate slow enough to prevent washout of phage even with large variations in the viral reproduction rate

Design Principle

*Replace **expensive,** precision equipment which is not fixable / hackable by team members with extremely simple hardware and sensors and then produce the required precision with computer control*

Raw materials

Arduino micro-controller

Raspberry Pi / Linux

Webcam

Python programming language

OpenCV image processing software

PIR (Passive InfraRed) temp sensor

LEDs, resistors, motors, magnets

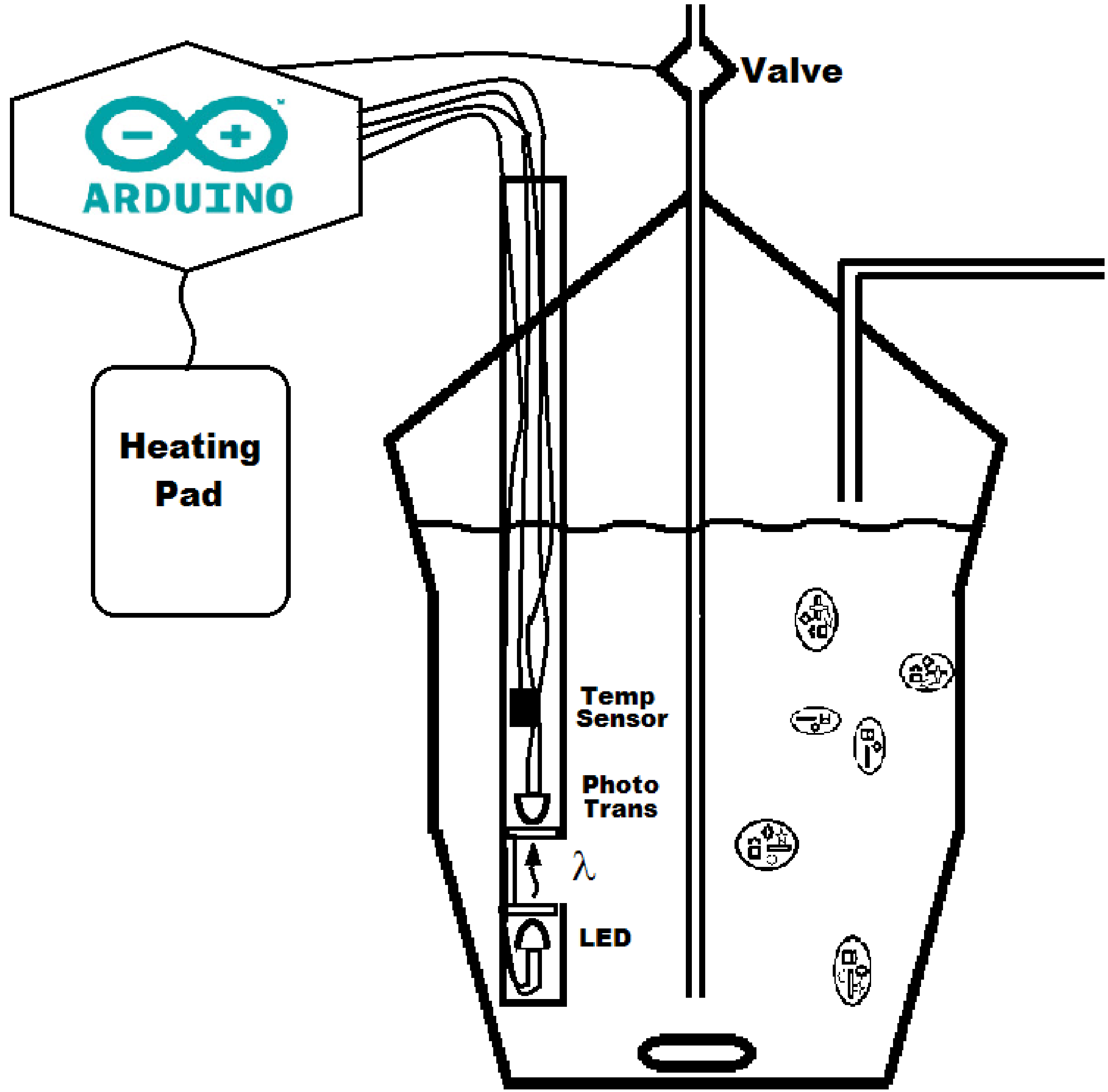
Discarded flatbed scanner

Styrofoam shipping containers

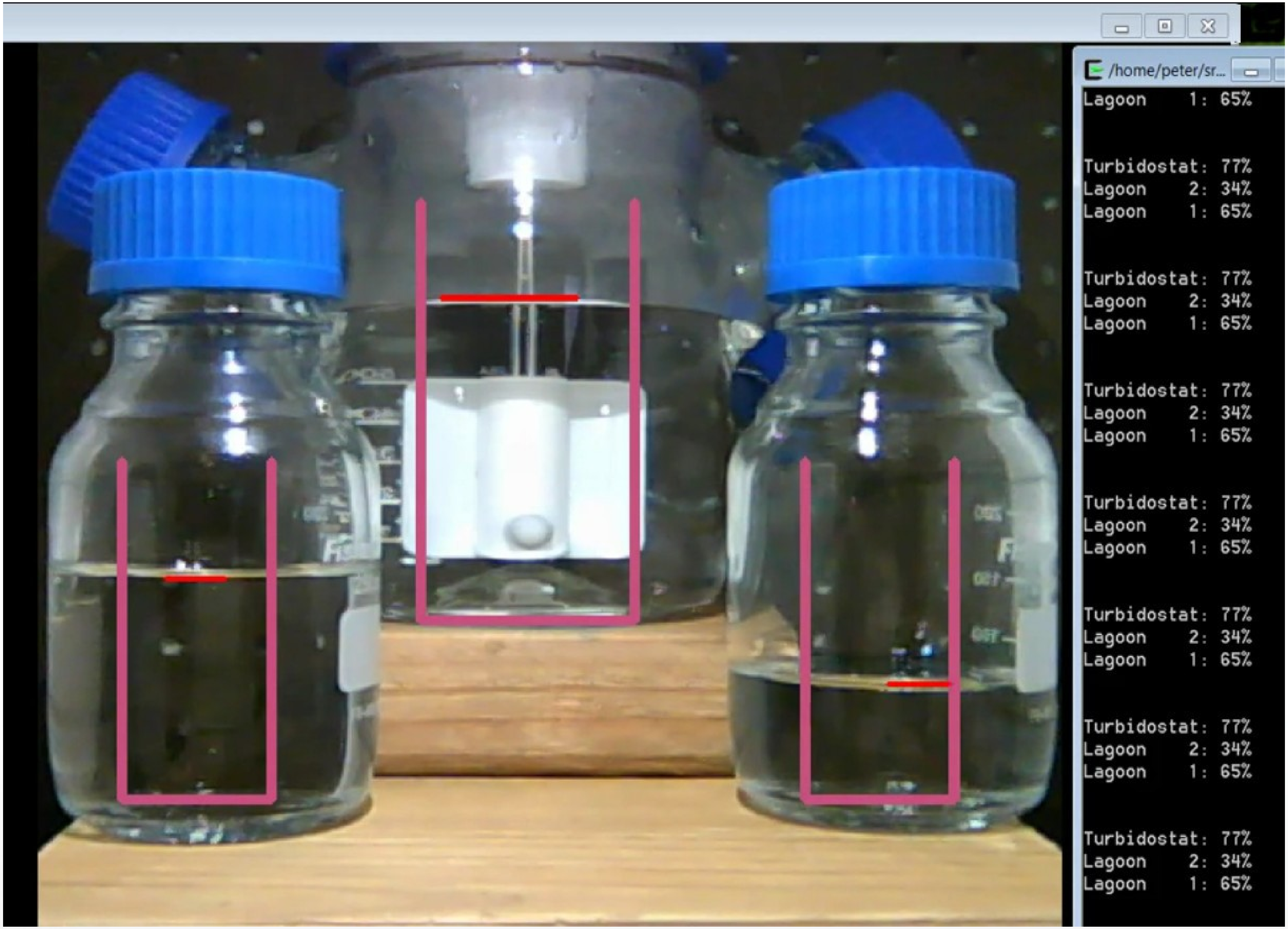
PVC plumbing hardware

3D printer

Turbidostat *version 1.0*



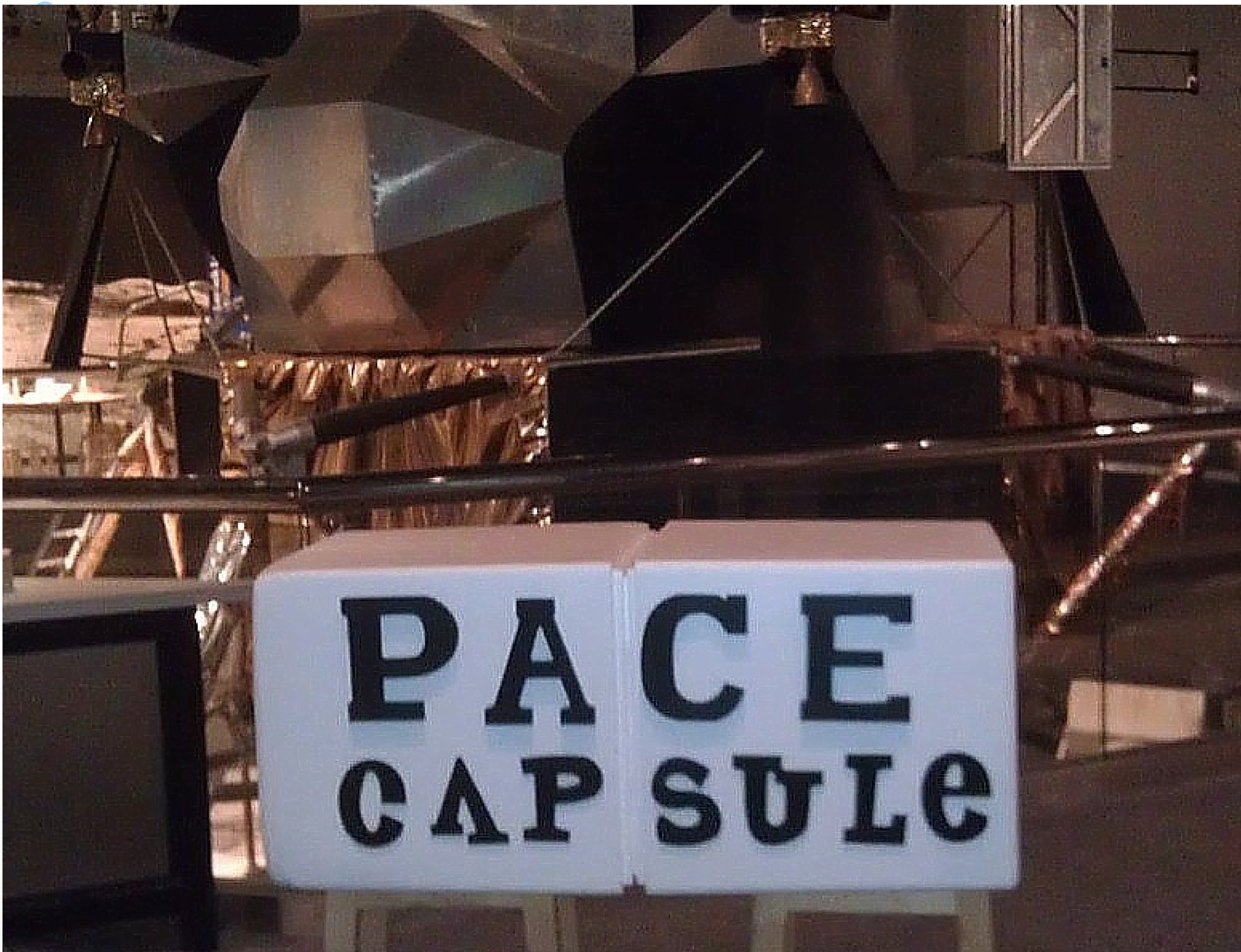
Non-contact level-sensing



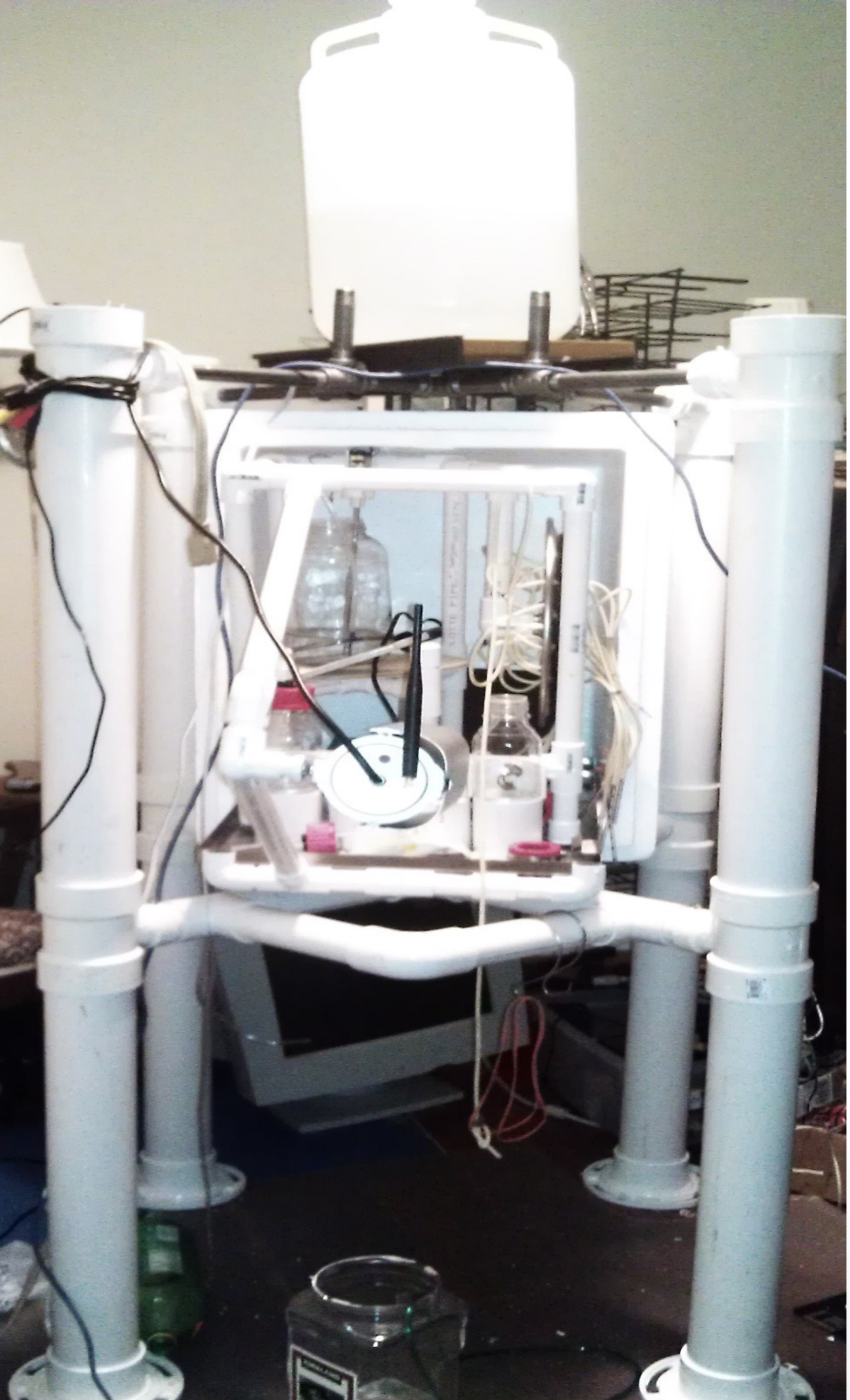
Material Costs for a “PACE capsule” $1035*

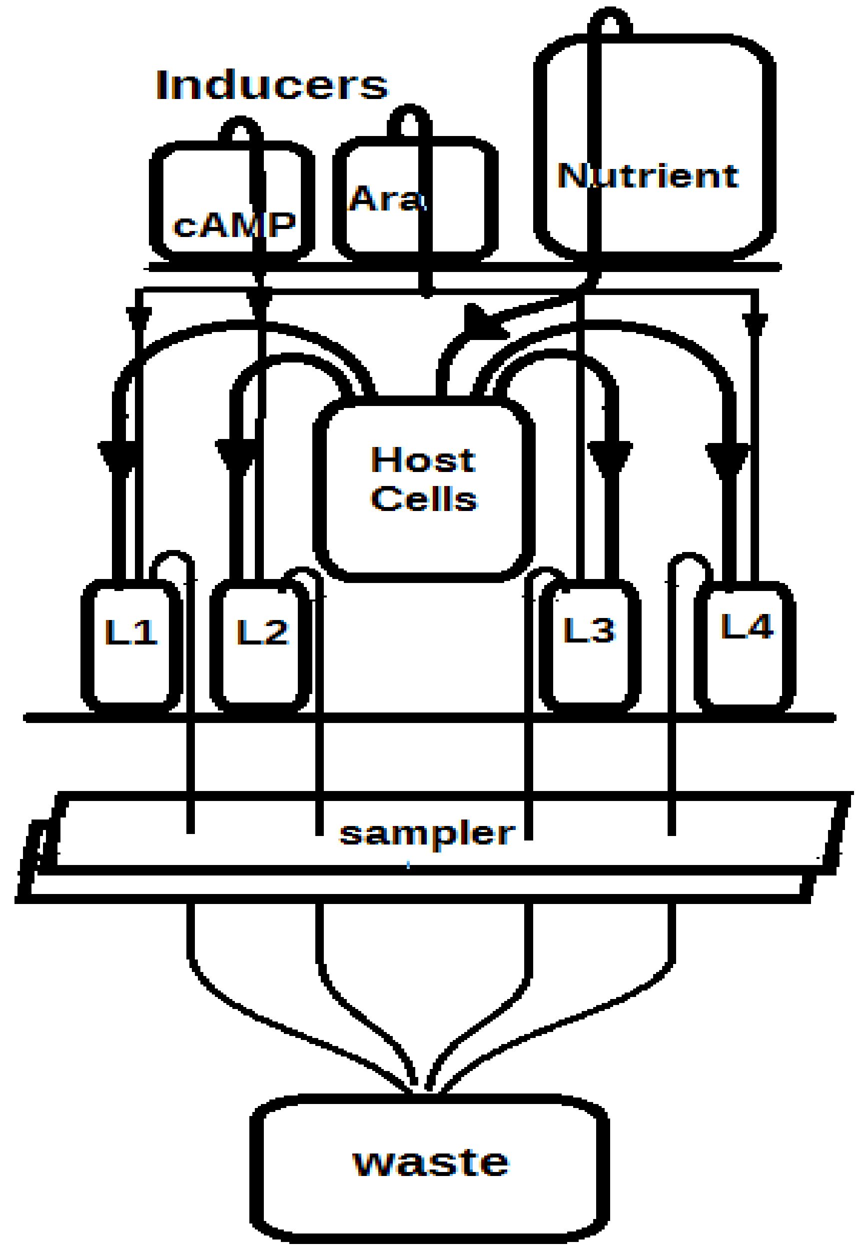
- **Raspberry PI main computer ($40)**
- **Wide-angle USB or IP Camera ($100)**
- **Arduino Mega 2650 ($35)**
- **PIR (non contact) Temperature Sensor ($50)**
- ***Laser, LEDs, Photo transistor ($20)***
- **Insulated boxes (Uline Styrofoam) ($70 X 2)**
- **Heating Elements ($30 + $60)**
- **Stirring Motors w/magnets ($10 X 5)**
- **Aquarium air pump ($35)**
- **Rotary valve motor, valves, peristaltic pumps ($150)**
- **Miscellaneous Hardware-PVC ($100)**
- **5V and 12V Power Supplies ($20)**
- **Glassware ($200)**
- **Tubing + Nutrient (operating cost)**

* *Retail, single quantity prices*


PACE
CAPSULE

More like a 'lander' after the adding $80 worth of PVC





Hardware Projects

- A multi-channel Autosampler from a flatbed scanner plus 3D printed parts
- Computer controlled heater and magnetic mixer for biological samples
- Using a 3D printer to create a 16-channel computer-controlled valve for ~$50
- Low-voltage heating for liquid environments

Software Projects

- Measuring fluid levels with OpenCV
- Detecting bioluminescence with OpenCV and a USB camera
- Writing an Arduino PID controller

(PID = Proportional / Integral / Differential)

Summary:

Styrofoam boxes for thermal isolation

Black garbage bags for optical isolation

Stovetop coils, printer power supplies,

50 ohm 10W power resistors for

low-voltage heating (up to 40ºC)

motor($1),T-nut,2 magnets = Magnetic stirrer

Camera + image processing = sensors

Pizza crisper pan, motor, 3D parts = valves

Flatbed scanner, motor, 3D parts = sampler

$10 Ardweeny controlling a heat gun

Problems:

Bioluminescence detection requires expensive high-voltage photomultiplier tubes and complex plumbing

3D printers can't make stainless steel parts

DIY electronic sensors must be sterile, but fail after being autoclaved repeatedly

The $10,000 environmental chamber failed catastrophically mid-experiment

Silicon/Tyvek tubes ruptured after several days in a constantly running peristaltic pump

Problem:

Bioluminescence detection requires expensive high-voltage photomultiplier tubes and complex plumbing

Solution:

In a dark room, point a heat gun at a $20 webcam and take a picture, this is your camera's noise image

Take 100 pictures in total darkness and add the frames up using: +Green–(Red+Blue)/2 finally, subtract the noise image

Problem:

3D printers can't make stainless steel parts

Solution:

Perhaps you simply need parts with stainless steel edges or surfaces

Buy uncoated stainless steel welding rods $6/lb and design plastic 3D parts with openings for rods and/or plates

Problem:

DIY electronic sensors must be sterile, but fail after being autoclaved repeatedly

Solution:

Use non-contact sensing

PIR (passive InfraRed) Temperature sensor

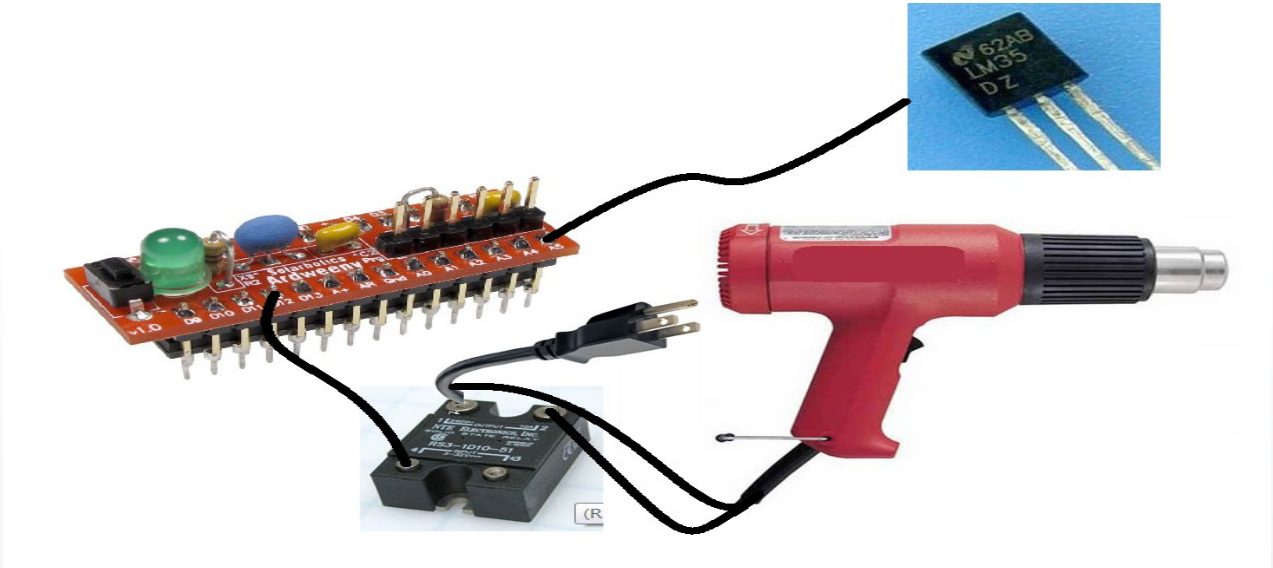
Camera, laser and LED lighting, OpenCV image processing for turbidity(cloudiness), bioluminescence, and level sensing

Problem:

The $10,000 environmental chamber failed catastrophically mid-experiment

Solution:

LM35DZ temperature sensor ($1.50)
Ardweeny ($10) running PID algorithm
1500W heat gun from Lowes ($25)
with a solid-state relay ($10)



Problem:

Tyvek tubes ruptured after several days in a constantly running peristaltic pump

Solution:

Design the system for gravity/siphon flow and use peristaltic pumps for priming
Peristaltic pumps now only run for a fraction of the total experiment duration

BioHacking

- **ApE** – an Open Source plasmid editor

- PCR Thermocycler ($25): *Ardweeny,Peltier Junction, Fan,drilled aluminum block*

- Gibson and Gateway assembly reagents

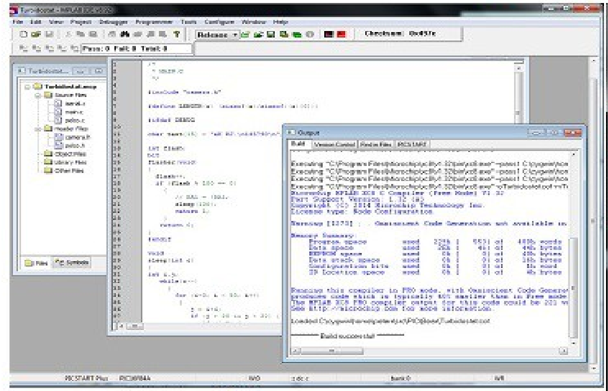
- Phage Assisted Continuous Evolution

- SPATEs*: *The Achilles ~~Heel~~ of Microbial Pathogens?*
  Tendon

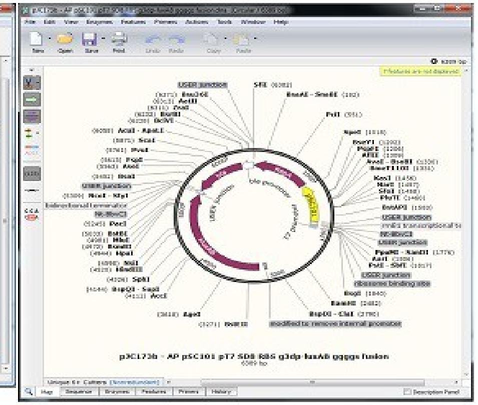
*Serine Protease AutoTransporters of Enterobacteriaceae

Know Wonder Moment

Language

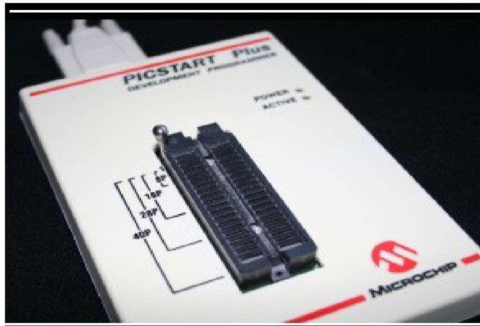


C



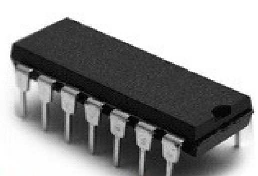
DNA

Programmer



PICStart-Plus

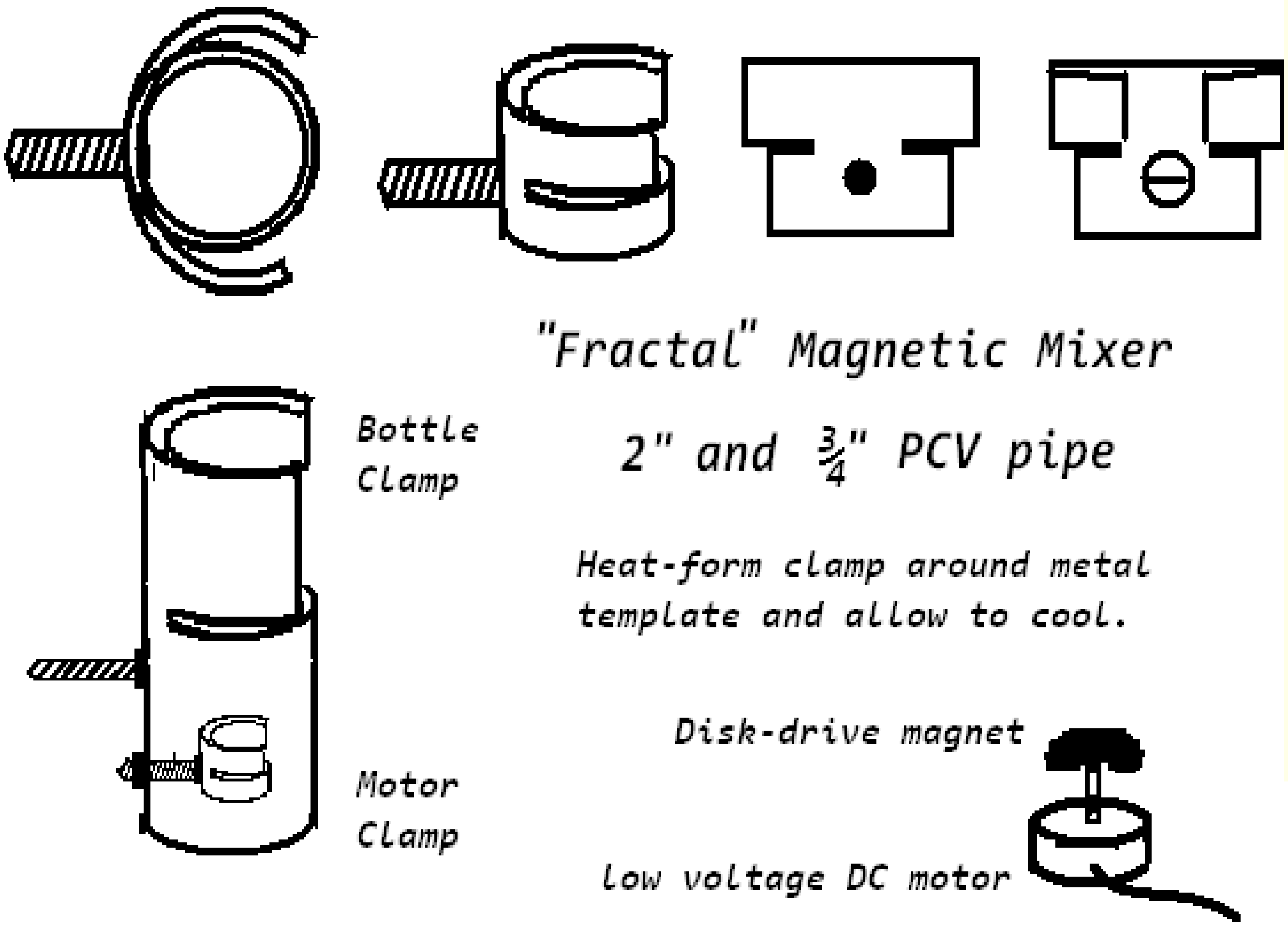
Electroporator 2510

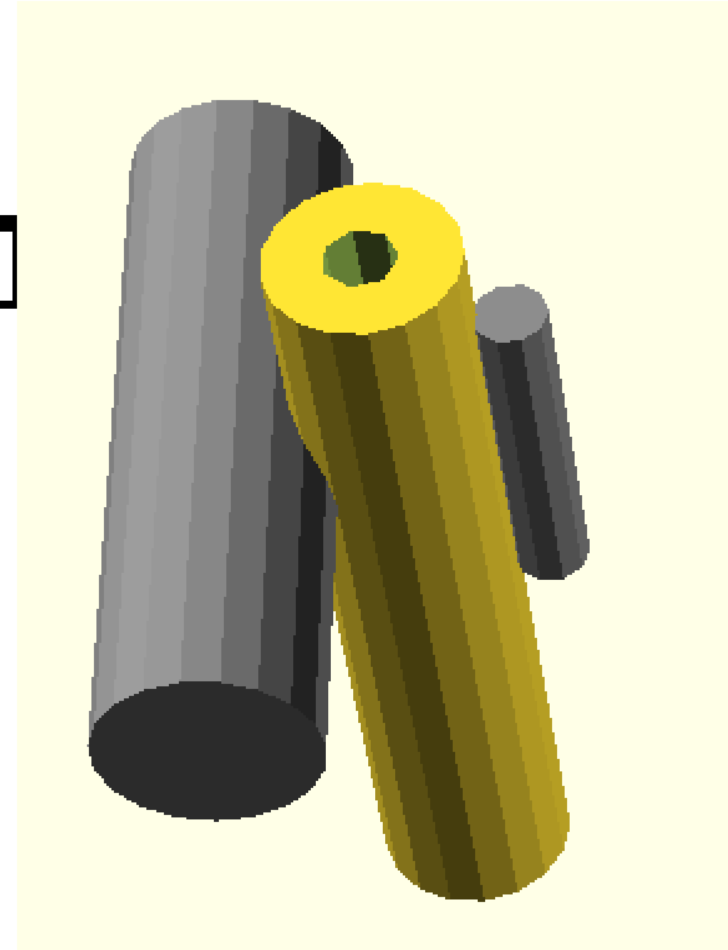


45 cents
per processor

0.0125 nano-cents
per processor

Details





Minimize pressure and stress required for tube-valve