06 Jan 2021

* Mark walked us through the “master” spreadsheet and how to adjust the necessary cells to match our experiment
  + This spreadsheet needs to be simplified to be more user friendly
    - Goal: adjust this spreadsheet to be as clean and straight forward as possible so we can lock the cells and verify…
  + For now we will adjust the spreadsheet manually before each experiment and by hand during each experiment while we develop a more efficient system
* The term “Master Mix” is dualistic in that it refers to both the mix as a whole that is used in the experiment and one of the components used in the overall Master Mix
  + This component used in todays experiments is referred to as “5 x PCR MM” in the spreadsheet
  + Personal question, what exactly is the “5 x PCR MM”?
    - Goal: distinguish between the two “Master Mixes” by renaming one of them
* Mark showed us how to fill the pouches for “Kafeneio”, load them into the machine, and run the program
  + Due to Connor’s innovation on the pouches, we may look to adjust the process of filling the pouches. However, for the time being:

1. Test the seal on the pouch by injecting a minimal amount of mineral oil.
2. Draw back as much min. oil as possible and use a second syringe to inject the Master Mix (the total mixture NOT the component) only enough is needed to fill the pouch without expanding it.
3. Use the soldering iron to seal the channel connecting the port to the rest of the pouch.
   * Kafeneio runs on a raspberry pi with an Arduino controller and the program is written in python.
     + There is no power switch on the machine, as soon as power is running to it, it will start up.
     + The code has a few bugs but it gets the job done
   * Adjust code to match spreadsheet and run
   * It will take pictures of every cycle

* Mark also showed me how to run the LS-32
  + Similar principle as Kafeneio but instead of pouches the mix is transferred to capillaries
  + The machine still runs on Windows xp so it is not very efficient
    - We are currently taking screenshots of the data and saving them to a usb so we can transfer it over to the cloud
    - Goal: Transfer the machine to the main building
* IMPORTANT: Do not vortex the Master Mix with the [PCR MM] mixed in. The proteins can denature.
* I had the idea of making a PCR machine where the liquid flows from a hot surface to a cold surface (hot and cold are relative) so I asked mark if that were possible and he explained that there have been a lot of different designs including one where the sol’n travels in a sinusoidal path from each temperature. A professor tried it but the surface area to volume may not have been the correct ratio…
  + From what I understand of Kirk’s idea is he wants the sol’n to move back and forth in a straight line (from one end to the other in a pouch)
* Is it possible to mix the DNA in a solution with a lower heat capacity so the temperature of the container is closer to the temperature of the sol’n?

07 Jan 2021

Log:

* Found gas cylinder company but unsure of which type of holder we need
* Read a paper on differentiating PCR products by analyzing melting curves
* Kirk started explaining how to take apart Kanefeio to clean (got sidetracked)
* Mark helped me understand PCR master spreadsheet and how to adjust it for individual experiments
  + Made a Master mix for a series of cycles for LS32 4 dilutions of DNA (0/1, 1/100, 1/10, 1/1), 6 runs total
* Looked at laser engraving patterns to adjust to fit Connor’s hole punch but decided that while Mark is here I needed his help understanding the master spreadsheet.

Gen Notes:

* As the DNA is amplified in the PCR with dsDNA specific dyes (SYBR Green I), the fluorescence increases (usually around cycle 26).
  + The fluorescence is a good indication of DNA amplification but the fluorescence also increases as a result of primer-dimers (usually around cycle 35)
    - How to distinguish between primer-dimers and target DNA using dye method?
* I hate the lack of a labeling system and that dates are recorded as 20210107 instead of 07 Jan 2021
* I can only connect to the design software if the laser is running… It shouldn’t take too long to redesign the pouches but I need to set aside time specifically for that design.

Experiments:

* 20210107 CF07 JW\_1 hold at 72 and 60 for 5 sec and HRM: 55, 63, 95
* 20210107 CF07 JW\_2 hold at 72 and 60 for 3 sec and HRM: 55, 63, 95

Goals:

Ideas:

* Redo the master spreadsheet to be more user friendly

08 Jan 2021

Log:

* Cleaned Kafeneio and reassembled it. Kirk showed us how to make new bladders.
* Ran 2 more experiments with the same mix
* Found how to export the data points for the graph so we don’t have to just get a screenshot of everything.
  + I’m currently working on the code to plot the data
* Kirk explained his new idea for a product

Gen Notes:

Experiments:

* The CF07 exp I did yesterday amplified well but did not amp soon enough so there is no plateau (the graph looks like a standard exponential curve with no limit)
  + Today I’ll try 72o for the elongating phase at 10 sec rather than 5 or 3 like yesterday and 56o at 4 sec instead of 60o at 5 or 3.
  + I tried one more experiment with 56 and 72 at 7 and 15 respectively. It started to plateau at around 54 RFu but did not achieve an Fmax
  + Also try increasing primer conc.

Goals:

Ideas:

11 Jan 2021

Log:

* Redesigned the template for laser cutting the pouches for Kafeneio so they fit in Connor’s new hole punch
* Redesigned the tri-pouch for Action so the ports are within the circle and you don’t have to cut anything off after the laser cut.
* Adjusted a spreadsheet to make a Master Mix for Action with left over for LS32
* Designed a pattern for the bladder for Kafeneio. The spacing between each component is not quite right so I need to adjust it tomorrow

Gen notes:

Experiments:

Goals:

Ideas:

12 Jan 2021

Log:

* Adjusted the design for the Kafeneio bladder.
* Connor sent files of his design for pouches for Kafeneio and Action
  + His Kafeneio template is cleaner than mine but mine saves more space so I might combine the two
  + His Action template is great but it still has tabs on the end that we would have to cut off
    - My template is more compact but we don’t have the right hole punch yet
* Kirk showed me how to laminate the film

Gen notes:

* We should make a detailed set of instructions for new employees
* Wear gloves to handle sheets of film while laminating to prevent contamination

Experiments:

Goals:

Ideas:

* Connor’s hole punch is great except it’s hard to separate the layers
  + Why not laser cut the port holes in a single sheet of film, then laminate, then laser the rest of the pattern?
    - Mark said I should try it but the problem I might face is how clean the laser cut on the hole is (due to its calibration)

13 Jan 2021

Log:

* Cut port holes in one sheet of film before lamination
  + First tried with action

Gen notes:

* When cutting the port holes before lamination: it’s pretty easy to align the double sheet where the single sheet was
  + Just space it about the same distance from the edge of the hex plate

Experiments:

Goals:

* Design a template to fit in laser to align sheets perfectly

Ideas:

* How to ensure the holes line up with the rest of the design?
  + Need to ensure laminated film is in the same spot as single sheets…

14 Jan 2021

Log:

* Finished designing and printing a sheet of pouches for Kafeneio.
  + Stuck some ports on a few of them
* Aligned the pattern for the Action pouches so you can just line the film with the edge of the hex plate
* Ran 20210114 experiment in Kanefeio and LS-32
* Chris showed me how to run Action

Gen notes:

* My patterns for both the action and Kafeneio pouches work well as long as the laminated film is in the same position as the single layer when the port holes are cut.
  + Align edges of film to edges of honeycomb sheet
* Connor sent the pattern to cut the port adhesives in the laser so I need to figure out the right settings for that

Experiments:

* 20210114
  + I ran this mix (Teal mixed it yesterday) in Kanefeio and LS-32. In Kanefeio it needed more than 40 cycles to amplify but in LS-32 it amplified just fine.
  + I also ran this mix in Action after Chris showed me how to operate the machine

Goals:

* Inject pouches without any air…

Ideas:

25 Jan 2021

Log:

* We received the Oligos for PMMV and MS2 that Mark ordered last week so we resuspended them and mixed the forward and reverse with TE to make a 100uL mix
  + We used the spec sheets that came with the Oligos to determine how much TE to add to the Oligo to resuspend it to make a 100 uM mix
  + We added 60 uL TE to 20 uL of the forward primer and then 20 uL of the reverse primer
* The 20 uL pipette tips fit the 100 uL pipette and were not labeled properly so the first two mixes were not accurate but I threw those out and remixed the oligos with greater precision

Gen Notes:

Experiments:

Goals:

Ideas:

27 Jan 2021

Log:

* Yesterday Mark, Teal, and I utilized the BIO-RAD iScript Select cDNA Synthesis Kit to synthesize cDNA from the MS2 oligos we ordered
* Today I started creating a protocol to run the PCR with the cDNA from yesterday as the template
  + Mark still needs to review the protocol
  + The plan is to run the PCR tomorrow
* I moved all of the equipment from the other building to the new PCR room in Action and organized the room
* Kirk said he wants me to design something on the laser but he needs to draw it out for me first

Gen Notes:

* I think the biggest confusion for me at this point is the vocabulary
  + There are multiple words for each component in a PCR solution so it’s hard to remember if it’s a new component or if it’s another name for one that I already know
  + It’s also hard to learn all the different combinations of things we can/should add to a PCR mix

Experiments:

Goals:

* Find website that sells PTC material kits (talk to Connor)
* Order silicone foam

Ideas:

23 Feb 2021

Log:

* Yesterday I investigated how to create a color compensation file for the LS32 we have been using. It occurred to me that we have not calibrated that machine (just make a color comp. file)
  + Color comp. is not needed if only one dye is being used per capillary.
  + We recently ran a PCR exp. Using a kit from “Eikon” that had multiple dyes in the Master Mix.
    - The data we saw suggested that the signal was bleeding into different channels, indicating a need for color comp.
* We need multiple dyes to create a color comp. file so for now, we decided to create Master Mixes with just one dye, substituting the other dyes with TE and perform TE on those samples.
  + Yesterday we forgot to add the reverse primers and we diluted each component significantly so the LS32 could not detect the samples at the start of the run.
  + Today we corrected the errors.
* I began reading the manual for LS32. There are a lot of settings/abilities in the software that I’m not familiar with.

Gen Notes:

* I think I will need to solve a linear algebra equation with the data from the mock color comp run today. Mark uploaded a paper onto Teams that describes the equation.

Experiments:

* Mock Color Composition (EEEV, SLEV, WNV, NAM)

Goals:

Ideas:

16 Apr 2021

Log:

* Continued to inventory freezer

Gen Notes:

Experiments:

Goals:

Ideas:

**Definitions**

*Denaturing phase:* DNA double strand is separated into two single strands. **Hot**

*Annealing phase:* Primers attach to the single stranded DNA. **Cold**

*Elongating phase:* Primers are stretched to complete DNA sequence (enzyme). **Cool**

*Oligonucleotide (oligo):* Short stranded DNA or RNA. Also called primers.