In Dialogue with Dr. Laurent Potvin-Trottier

iGEM CONCORDIA

ASTROYEAST 2020

Dr. Laurent Potvin Trottier
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PhD Concordia University: The Potvin lab research goals are to engineer reliable synthetic gene circuits suitable for impactful applications, and to use them as models and tools to learn more about biology.Interviewed by Lancia Lefebvre and Hajar El Mouddene of iGEM Concordia

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Dr. Laurent Potvin-Trottier 00:01
I was curious to know what kind of stress expression genes get upregulated.

iGEM Concordia 00:17
Some are upregulated and some are downregulated in simulated microgravity and then some have different expressions in space. And also some genes that experience that change in regulation in space, will come back and carry it. And then others, they lose it when they come back to Earth. There's a variety of things happening.

Dr. Laurent Potvin-Trottier 00:46
By losing it, I guess there's two things right? Usually when we talk about gene expression, it's genes that are expressed at any given time, RNA levels, so that would be RNA sequencing. But you're also talking about permanent change, which would be like genomic DNA mutations. I'm gonna say yes to that. I haven't looked into it enough to say positively that it's DNA mutations, but that some are upheld in microgravity but not on Earth.

iGEM Concordia 01:12
It's the same in humans. There's a large loss of muscle density and bone density, but when they come back, they regain it. In bacteria, we spoke to one researcher, some of the antibiotic resistance when they come back to Earth is maintained and some of it is lost.
I can't say for sure, because there's a lot of research being done. I don't have any

papers to back it up.

Dr. Laurent Potvin-Trottier 01:46
That it's positively mutations, but definitely a good approach to start looking at it. Okay, you're looking both at like temporary gene expression change and permanent gene expression change.

iGEM Concordia 02:10
We are doing our gene selection right now and we have been performing interviews. Things like this are coming up and will definitely go to guide our selection.

Dr. Laurent Potvin-Trottier 02:23
He's talking about the difference between space and simulated microgravity. The difference that I can see, I mean, I know nothing about space research, but you have radiation, right? I don't know how shielded the International Space Shuttle is, or wherever, these experiments are done. There is definitely a higher kind of high energy radiation. I know they did some experiments with the astronauts. They had tons of DNA damage due to the high energy radiation. You could have a response to both microgravity and the kind of radiation. Which would explain some of the mutations, that would accelerate the mutations.

What can I do for you guys?

iGEM Concordia 03:46
Well, one question we've been having lately. For our database we are using the adjusted p value. The experiments are done versus controls in normal gravity. Then they use a log Fold Change to report the results against the control. We were wondering, I guess not necessarily for this research, particularly, but in your research, if you're measuring a change in expression, is there a threshold or standards for which, you consider those an acceptable change? When you would say that the promoter genes are being affected?

Dr. Laurent-Potvin Trottier 04:36
Yeah, I guess it depends really, I mean, I don't do a lot of these kinds of sequencing or high throughput gene sequencing experiments, or micro arrays. I know what they are. More recent are micro RNA-seq and a little bit older one would be micro arrays. And the issue is, we usually look at like a handful of genes at a time. We evaluate a lot of time points. So we have a lot of data for very few genes. The problem with the microarrays and the RNA-seq is you have few data points for like thousands of genes. You have the issue

of multiple hypothesis testing, you're testing for, if any one of these thousands of genes that you're looking at is changing. So this is why they have these adjusted p value.Yeah, and when they do this they look at this log fold change.

iGEM Concordia 05:50
So the adjusted p value is because you have so little data for so many terms. Is that kind of what you're saying?

Dr. Laurent-Potvin Trottier 06:00
Yeah, I mean, it's mostly that you're looking at like, 1000 different hypotheses at the same time, right? Where whether any of these thousands of genes is changing and there are different conditions. So yeah, basically, this is why you need more confidence. Basically, the idea is if you do an experiment with a coin 1000 different times, there's maybe one of these thousand times that it's going to flip like 10 heads in a row, but it's still going to be a fair coin. It's not the best analogy but if you do something 1000 different times, then one of these thousand different times, it's gonna look weird, just by chance. So the best person to talk to about this kind of bioinformatics is Michael Hallett, he's the bioinformatician. I know a little bit of bioinformatics just kind of by default, but this it's not necessarily my expertise. And in terms of fold change, I would say, a rule of thumb would be probably at least a tenfold change. It depends on your expression level and a lot of different things. At least a two fold change, and probably ten fold changes is more significant. But here it's in log, right? So it's a log, I don't know what's the base of your log.

iGEM Concordia 07:54
Log two.

Dr. Laurent-Potvin Trottier 07:55
Okay, so that's two to the four. I guess yeah, one would be a two-fold change. Also in terms of the display itself, that would include everything that a researcher would be looking for in a nutshell quickly.Yeah, I'm kind of playing around with the up and down and don't seem to be doing anything. One thing you want to do is probably look for genes that are most highly up or downregulated. Right? Instead of just looking for one specific gene and in here I only see negative log fold change. I don't know if I'm doing something wrong. The issue with this is always how do you combine multiple data sets together, because the experiments are different, right? Whether it's simulated or actually in space.

iGEM Concordia 10:09
If you're reporting in bacteria is ‘Generations’ a valid parameter, I know for yeast it is,

but is that what you would use as well?

Dr. Laurent-Potvin Trottier 10:23
Yeah, for the length of the experiment. I think generation is always my favorite time scale because it's kind of the relevant timescale for the organism. It enables you to compare from organism to organism.Things change on the timescale of one generation for microbes, whether they are in bacteria or in yeast. Generation is a good timescale.

How many papers are you looking at? Overall, how many papers do you want to include, how many data sets you want to include in your database?

iGEM Concordia 11:16
As many as possible eventually.

Dr. Laurent-Potvin Trottier 11:20
I mean, I don't know anything about space research, how many papers are there? Are there hundreds of data sets? Is there dozens of datasets?

iGEM Concordia 11:34
I mean, it looks probably hundreds but Hajar go for it, go ahead.

I was just gonna say we probably have 20 now but because we're doing this as we go along. We found papers and we added them to the study but there are a lot more. There's a Gene Lab by NASA database and they have quite a lot. There aren't thousands but I'd say hundreds.And it'll also be so that researchers can enter their own data so they can contribute their papers as well.

Dr. Laurent-Potvin Trottier 12:15
Yeah, that'll be nice.It'll be good to look at. I mean, there's some other people concatenating different data sets, but for different purposes, it would be interesting to look at what they do. How do they display the data? How do they integrate the different experiments together. I think one thing would be nice as if you were able to browse by experiment. If you just click like I want to say, 'Oh, I want to look at this experiment' and just show them most upregulated and downregulated genes then do across experiments. I think that's probably what you're doing here. Let's say

iGEM Concordia 13:28
Try SSA1.

Dr. Laurent-Potvin Trottier 13:31
One way you could visualize...one experiment shows the most upregulated and downregulated gene and then across different experiments. You enter one gene and you show the result from different experiments, what happened in all of these experiments. Yeah, also some kind of visualization would be nice. People do different kinds of plots like just ordering the most upregulated and downregulated gene, basically plotting like each gene as one point. Most of them should be around zero, right, all the insignificant ones, and then the significant ones would be up and down regulated.

iGEM Concordia 14:46
Like a heat map?

Dr. Laurent-Potvin Trottier 15:03
I guess people do heat maps too. I was just thinking about just plotting a scatter plot. Have you contacted Mike Hallett?

iGEM Concordia 15:37
We have, his labs are busy but we are in contact with one of his Master's students.

Dr. Laurent-Potvin Trottier 15:45
Yeah, because that's what they do, analyze this kind of gene expression data. They should definitely be able to help you out.this gene here. I am just gonna pick a random gene…

iGEM Concordia 15:58
We're taking a set of around 10 genes, well promoters, and we want to build a reporter circuit. One of our promoters that we're selecting is going to have consistent expression in microgravity. That's going to be our control. That's one goal. And then the others, we're going to use the circuits, perform evolutionary experiments, and they'll help us determine which candidates have taken on the evolutionary resistance. Currently, we're thinking of grouping them in clusters. A cluster of three promoters and then putting them in this reporter circuit, using signaling like GFP and luciferases. Two questions; we're looking at using luciferase because it's a little bit more sensitive for our control. But I have heard that maybe it's not the best in yeast. I'm not sure how much work you've done in yeast? And then is it possible to mix luciferases and GFPs, or is that just a mess?

Dr. Laurent-Potvin Trottier 17:29
I never really worked with luciferase because I do microscopy and luciferase, it's usually not very strong because it's bioluminescence. I've never heard of anyone mixing the two. I guess they have different spectra. So what kind of wavelength are these? They have different colors of luciferase. The ones I'm looking at they're like, around the same

or maybe the red one could potentially work. I haven't used luciferase and I don't really work with yeast. I don't know about luciferase in yeast. When you're making a reporter, how do you want to read this reporter? Is it for use like in space, or is it for use in the lab?

iGEM Concordia 17:59
Space is something we're trying but that's long, long term. It's a collaboration but we'd be building this 3D-Clinostat, measuring, probably stopping the machine, and then using the lab equipment. You'd need a different detector for the two reporters, for luciferase, a fluorometer and because we started with this Promega product, a luciferase. We received a Promega grant. We were kind of riding this luciferase horse and the mentors have definitely been pushing us towards GFP. We're just, I guess, filling out why anyone would use luciferases and if there's benefits to it. You're not working with it and don't seem familiar with it.

Dr. Laurent-Potvin Trottier 20:34
I'm a big GFP fan.I think for luciferase, the advantage is… so luciferase is an enzyme that converts its substrates so it should be more sensitive in theory. One enzyme can convert multiple substrates. I use GFPs and then it's easy to multiplex. You can look at least three colors: CFP, YFP , mCherry. I can have at least three reporters. If you really want you can probably go with four. There's some infrared reporters that are not that great, but they're usable. So it's BFP, GFP, RFP, and infrared fluorescent protein. Those are pretty easy to read in the lab with a plate reader or something like that. Or flow cytometry. We have a bunch of flow cytometry at Concordia. While a bunch, we have flow cytometry that should be accessible for you. I would also recommend going with the fluorescent protein. Well I just want to understand why you're building this reporter. The goal is to validate the targets that you've discovered using your bioinformatic approach.

iGEM Concordia 22:25
Correct. The main goal is to develop microgravity induced stress resistance, to alleviate that effect. The main goal is that these reporters allow us to monitor the change in expression to bring it back down to what we have for normal expression levels. Secondary interest is to also see which ones are affected first. But that's not a primary goal. If they conflict, it's definitely the one we let go. What we imagine is these clusters of promoters and we submit them to experiments. When one cluster becomes responsive, then we can focus on that cluster and do more experiments with that one. That's the current plan. We're researching reporters and definitely looking for any insights when building these circuits.

Dr. Laurent-Potvin Trottier 23:22
How do you plan on making them resistant to the microgravity? Is it a kind of evolution?

iGEM Concordia 23:29
Yeah, directed or adaptive evolution. The only thing is adaptive is a little tricky and kind of long term. We're not positive yet. Those are kind of the two that we've been looking at and we're going to decide, probably a little bit more over the fall.

Dr. Laurent-Potvin Trottier 23:55
If I just look at your website, for example, But there's dozens of genes that are upregulated and if you, let's say measured three genes, they have three reporters for three genes. And then you do some evolution mutants that don't have up or down regulation of some genes. The first thing you could find is mutants that I guess, if you have three because you can find mutants that downregulate these three genes, but still upregulate all of the rest of the genes. Right? I'm not sure if you understand what I mean.

iGEM Concordia 25:08
They have feedback paths and all these interactions, even if one is affected, the others could be adversely affected.

Dr. Laurent-Potvin Trottier 25:22
The idea behind this kind of gene regulation is that there's a few kinds of master regulators and it would somehow up or down regulate all of these genes. And, and a lot of it is known in yeast. You could select.

iGEM Concordia 25:44
Yeah, that could work. The hope is that there's only a few kinds of master regulators, and that all of these genes go up and down, due to one or two things then with only two or three reporters, you could find mutations that make them adaptive to microgravity.

Dr. Laurent-Potvin Trottier 26:15
My point is you have to be smart about picking these reporters. You can do a lot of bioinformatics. I mean, people have probably done some kind of bioinformatics to try to find which regulators are involved in this and tried to go up to this kind of master regulator. I don't know if because the ones that are the most up or down regulated might not be the most interesting for you. I don't know if MSN2 Or MSN4 are involved in this, but they're stress response transcription factors. Those would be the kind of interesting things to look at. Because this represents a whole organism response, and they may regulate 100 genes, but the only thing you care about is whether MSN2 is up or down regulated.

iGEM Concordia 27:28
That is one that's come up quite a lot. So it's good to hear. I'm just going to screenshot our gene criteria really quickly. When somebody had said that we should only look at upregulation instead of downregulation because it's easier to make reporters for upregulation is that true?

Dr. Laurent-Potvin Trottier 28:00
No, I don't think I agree because you can measure downregulation too.You have to look for lower GFP amounts. I mean, it's not harder. It’s less straightforward, but I mean, you measure before, you measure after and you look for change in fluorescence. Whether you expect it to go down or expected to go up. Yeah, so it's fine.

iGEM Concordia 28:43
Is there a certain level of expression that you need for something like that? Is there a threshold that you want that promoter to be expressing at?

Dr. Laurent-Potvin Trottier 28:58
I'm a little bit less familiar with making a transcriptional reporter in yeast, but in bacteria when we make transcriptional reporters usually we just kind of copy the upstream region of the gene. Then we have a ribosome binding site and a fluorescent protein. I can look a little bit about what people do in yeast. I haven't really done that.It depends on your readout, if it's flow cytometry. Then it's a pretty precise readout, the most precise is microscopy, but this is very low throughput, and it's not probably what you want to do. Flow cytometry would be the most precise, and there's just in like, well plates, like during the growth curve, that's kind of less precise. You would need higher fold change for your reporter. Okay, so I guess what I would look for is let's say you're interested in MSN2, then I would look at genes that are influenced by MSN2 and the ones that have the biggest fold change whether it's up or down, and then another, typically only regulated by MSN2. A lot is known about yeast so you can just look up these genes, look up their regulation and see whether they're like typical MSN2, if they represent MSN2 expression, and then pick one that has a big kind of fold change either up or down because that'll be the easiest to see.

iGEM Concordia 31:03
Are you familiar with a database that's a really easy place to get gene expression in yeast from?

Dr. Laurent-Potvin Trottier 31:12
No, I don't really work with yeast.

iGEM Concordia 31:21
Okay, I'm just going to share our promoter criteria.

We want predictability and reproducibility so that the promoters are acting the same way generally across the experiments, because in some experiments it can be upregulated one and then downregulated in another. We are looking that it doesn't have too many interactions or crosstalk.

Dr. Laurent-Potvin Trottier 31:48
This is kind of predictability and reproducibility. In a sense, you mean also specificity, right? That it's specific in the sense that it only changes in microgravity, it's specific to microgravity.

iGEM Concordia 32:08
Yes, that we came to. And we were looking at other stressors, for that reason. So that it's specific to microgravity and especially for the control. But then, like you were mentioning MSN2, it's affected by so many other stressors. It's kind of contradicting to what we're saying. I'm a little on the fence because there's some genes that we're looking at, like RHO1 is a very interesting one, but it's affected by heat shock, cell wall integrity, and osmoregularity. It has a lot of inputs. You have some of these promoters that are interesting, but they definitely do not meet that specificity criteria at that point.Do you have any thoughts on that?

Dr. Laurent-Potvin Trottier 33:03
You could have a mix, right? You could say, let's say, you have three reporters in your stream. You can have one or two that are specific to microgravity. And one which is a general stress response, like something MSN2 related because if somehow you can turn down the microgravity specificificity, but then the cells are still doing general stress response, then you haven't really solved the problem of the stress. So, yeah, you don't have to select all of the promoters in the same way.

iGEM Concordia 33:58
Perfect. It's been really interesting. We've been selecting genes for quite a while. What''s to consider when you're making building a reporter?

Dr. Laurent-Potvin Trottier 34:19
Well it's good to spend time because it's a lot easier to spend time on a computer, then to build something for a few months and then realize that you did it wrong. It's actually good you guys are forced to be off the bench because usually people just jump in and then realize like months later li oh actually I should not have done that. So there's a good side to this.

iGEM Concordia 34:48
I definitely agree after last year being really rushed and going in and having people

forcing through the experiments. It's nice to be able to think through it a little bit and speak with people like yourself, honestly, it helps a lot. Do you have any generalized advice for our process here when we're building this circuit?

Dr. Laurent-Potvin Trottier 35:19
Yeah, you mentioned AND gates and things like that.

iGEM Concordia 35:32
I've been thinking about three genes because, let's say you have three colors to look at. You have three outputs. We can also combine more if, depending on the goals that I'd really think about. So think about the long term if we were to do an evolution experiment, what would we need to read out to? Think about the long term goal because you have the option, as you mentioned, using AND gates, right? So you could say, I want gene ABC to be on to turn on the, let's say, fluorescent protein one. So you may or may not want to do this. And you can build multiple designs too.I like usually knowing what exactly happens.

Having one promoter is one color, then you know exactly what happens. Let's say you pick a mutant, then you say okay, in that strain MSN2 is not responding, but this one is up and this one is down. When you pick it, you know exactly what happened. If you have a bunch of AND gates. Then you say, one of these turned off, one of these genes, but I don't know which one.

iGEM Concordia 37:31
Which would maybe be more useful if they were related, like in a pathway or something like that.

Dr. Laurent-Potvin Trottier 37:37
I guess. Yeah. Here you could have an OR gate, meaning either this gene is on or this gene is on or this gene is on. And then you turn on the promoter. That's useful. It's like having three different promoters and fluorescent proteins. You have gene A express, let's say, GFP, Gene B, express GFP, Gene C Express GFP. So if any one of these three is on, then you have GFP expression. That's kind of useful if you want to, it kind of removes the false positives. Oh, this mutant just downregulated that particular gene, like to downregulate GFP entirely, then you need to shut down expression of all of these different genes. That's kind of a good way to make sure that you have some kind of general response.

iGEM Concordia 38:41
So OR gates would be more specific because AND gates are. They do seem a little messy from what I'm understanding?

Dr. Laurent-Potvin Trottier 38:50
What I'm saying is I'm just thinking about combining promoters. If you say A and B and C, then if one of the three is downregulated then you'll see downregulation of your promoter. You want something that changes multiple things at the same time. You don't want something that just downregulates SPG4 or SPS100 or something that genes I'm just picking there. You want something that's affected in microgravity.

iGEM Concordia 40:22
Is there anything else we should consider that you take in consideration when you're building a reporter circuit?

Dr. Laurent-Potvin Trottier 40:32
Yeah, not that I can think of. Making a reporter circuit is pretty straightforward. I mean, if it's been used in microbiology they've been used for a very long time.[...]

Dr. Laurent-Potvin Trottier 43:48
As a recommendation, really review the literature pretty well. I mean, it takes a lot of time. Have a nice literature review of what people know about a change in gene expression. That’s so that you have the state of the art knowledge before you start doing.

[End]