Appendix C

Interview 1

He's being recorded. Yes. Perfect. Okay. So my question is, what does what's raw data mean to you?

So, yeah, it's that is kind of the the key question, we want to get to the stage where we'll have custom, in essence, custom functions for as many different qPCR machines. So that whatever format they output, it can then automatically be arranged into a tidy format that that conforms to the way that tidy verse itself defines tidy data. At the moment, we only have a very small number of qPCR machines that actually conform to order actually have custom functions to read in. And we're hoping, again, sort of what we're thinking of as raw data, you have the sort of inferred calculations that the qPCR machines themselves do to work out what the CPUs are from calculating that directly from the amplification curves, etc, etc.

I always let the machine just do that. And it just gives me a CQ value. And I didn't even care to think anything beyond that.

Yeah, at the moment, that's kind of the way tiny qPCR works. Well, it's just assuming that the

I guess, in principle, you could get the fluorescence value in every Well, every cycle. Yeah. And then you could do, you could you could pick how you calculate CQ yourself. But I don't want to do that. That's hard.

Yeah. So we're hoping that in a couple of versions time, to qPCR itself will be able to take the raw fluorescent value and infer any SetCPU values. Because, again, one of the sort of issues are, you know, these machines are doing some form of calculation according to some sort of algorithm. But these algorithms are not published in well, a lot of them are not published at all, and they haven't been peer reviewed. So you just and they could be like 20 years old. So you have no idea exactly what's what, what's what's occurring, and whether or not there is a mistake, numerical error is going on, you just have no control over that. So we've got to be in a stage where we could do as ourselves. But at the moment, we're not quite at that stage that's still in development. Very good. Good, good, good, good. Good. I'm just gonna get my questions up and running. So as you probably had a look. In the PDF that I sent you, the interviews consisted of two separate parts. First is sort of general background questions so we can get an idea of where like, what sort of experience you have, and whether or not tidyqpcr as it's built so far, can cater for as large an audience as possible. So very easy. Start at 10. Tell me about your experience with qPCR experiments. What sort of experiments are you doing? Is it more RNA DNA?

Okay. Short term. Sure. Really, really general. But yeah, RNA DNA hadn't even occurred to me, as I remember from the question, it was RNA. Yep. So I was I haven't done qPCR in maybe a year and a half. I think February 2019, would have been the last time I touched it. Just because that was the way things have been, but I was doing RNA. And essentially, I was trying to do like clip seek, but just with qPCR because clip seq is a lot of overhead. And I kind of had a straightforward question of, does my protein bind to the five prime UTR of an mRNA? More than it does to the open reading frame? So it was an enrichment question, which means I had a shitload of samples So it was, I'd have the IP fraction and like a total fraction, and they were both mild RNA digest. And then from those from those extracted RNA samples, then you'd have to do qPCR with many different probes. So you'd have to have like a five prime probe, an open reading frame probe, and I would do a three prime probe. And then I would do that for many different genes all from the same sample. And then you have to do that in the IP fan samples and in the supernatant samples. And then I looked for, as it was the ratio of five prime over ORF Oh, have the IP compared to the super night? So ratio ratios? Yep. Yeah. And then I would do that at different stress conditions to see if this RNA binding protein changed its binding during stress. Yep. So that was, that was the heart of what I was doing. There was also some like generic heat shock response, which was just kind of a happy byproduct of that, because I was looking at heat shock gene. So I just got the heat shock response. Anyways, see, I did this all with TaqMan probes. So I talked to Edward a little bit about this. When I started doing qPCR, I tried cyber, and like, I couldn't get the standard curves to work, everything, nightmare. And then I tried the attacker and stuff and it did work right out of the box, no problem. So apparently hold grudges. So that's that's just the way in like, you get the added specificity and think like the numbers finally started making sense and behaving controls would work. So I did all tech man stuff. I didn't do multiple colours. Because that was not quite necessary, it would have been much more convenient. I shouldn't have done it would have been expensive. And I was trying to conserve money, even though it's not my job. Yeah.

Yeah. I mean, you seem to have answered a lot of the varying questions, I heard the beginning.

If you want to do a screen share, I actually found some of my old data. So I have raw data from the machine, I can show you what I turned it into. And then I can show you like some graphs that I made an R.

That's, that's perfect. Yeah, do it this way. I think you've got,

you've got to make me co host. And then there still might be some issues, because I'm going to be switching windows and stuff. But let's give it a shot.

I think you should I think you should be able to share your screen already, I think.

Okay, let me try the button share screen. Yeah, that's okay. So, I'm just gonna start my, I have a whole experiment saved in a folder. The end graph kind of looks like that, which I can also go back to in a moment. So I did three biological replicates. Each replicate has one. Oh,

it's got three different plates. So each plate from the BioRad. Let's see if X 96 gave me a thing. And I essentially I did one gene per plate. Gene has three different probes, a five prime, an open reading frame and a three prime. So see, the BioRad boo. This, this is the stuff that was just a gigantic pain in my ass that I never wanted to deal with. So I guess it's got all the information that you would presumably want? Yeah. Okay. And then it just gives you well and CQ value. And right now, I don't actually remember which one was which. But I think I organised. It seems like I organised stuff by row. And I'll write down. Okay, so then I took all of this data, and I took it from three different plates, and I turned it into a text file with a tidy format. So I have biological replicates as a column of which gene which is redundant information with the probe. So I have the five prime probe, the Open Reading probe, and like, I didn't do the three prime probe here, but they're all from the same SSA for gene. See the fraction I have the supernatant versus an IP fraction? I have stress treatment. So 30 degrees 42 degrees recovery, recovery recovery. I was treating them guanidinium chloride. So right after this biological reps had zero millimolar Guam three millimolar. Guam, yeah. And then finally we get to the CQ values. So yeah, I had to make everything tidy myself. Okay. And that was once I had that data, then it was easy enough to go into our and gosh bunch of packages. I don't know what I did. extremely old, but the figure and ends up looking like this. So yeah, ratio of IP to supinate. And, and so you get your five primary share your mid and your three primary show, I guess it's a different experiment than the one I just showed you, but some extremely similar idea. And I did that measurement, three different stress conditions. So that that is what qPCR looks like to me.

Yeah, I mean, seems very, very similar concept to what tidyqpcr is all about? Really,

you'll never guess where I learned it from.

Yeah, yeah. Perfect. That does seem great. So in terms of how you did your original sort of text, plate plan? Sure. How did you go about actually doing that? Was it sort of copying, pasting and just making it a giant text file,

but it was a gigantic pain in the ass? I think Sublime Text lets you do. Um, like vertical highlighting. So I don't get that haste or cons. That's cool. Yeah, that saved me. But there's a while when I think I didn't have that functionality. And it was just a gruelling, copy, paste, copy, paste, copy, paste. Kind of mind numbing. It worked. It was fine. You know, you only have to do it 600 times. It's fine. But yeah, that's that's how I did that. So you're saying more interesting in here? No? Okay. I think I'm gonna stop sharing. I don't think there's anything else interesting to see here. Oh, it's perfect. Yeah, okay. Let's see that describes my qPCR experiments. None of that work was ever published. So perfect. That what you will?

How about papers that you've read, though? And how your experience of other people's work and how they present their qPCR data? Do you ever see anything other than the summary figure? At the very, very end of all the analysis that people typically put in at least a link to the raw data or something? I don't

think I've ever looked for the raw data. I have looked for primer and probe sequences. And those are usually pretty accessible. Usually. And I've also looked for things like what, how did they do the reverse transcription? What diet did they use? What machine did they use? So I looked for a lot of technical details like that. And I think I've, I think I've usually been able to find them. I can't think of any particular instance when one or the other right now. It's often frustrating. Just searching through supplemental materials to find that information. Yeah. But usually I could find primers. thought, I don't think I've ever looked for the raw like Ct values. I assume I can't even I can't imagine that. I don't think I've ever seen them. But I've never looked

well about any sort of quality control or two people put any.

I am very frustrated that no one ever runs a standard curve. And like I know, I know, labs down the hall from me that are not running standard curves. And they looked like me like an idiot whenever I mentioned it. And I talked to other people in the lab and they go Yeah, the qPCR is not working like well. I don't think biology is broken. So that's definitely an issue with that people. Don't take the time to do the curves. Really easy to have shitty probes.

Yep. Yeah, we encounter that problem regularly.

Isn't curious how? How many probes? Do you have to test to get a good one for a gene? On average? You have to guesstimate.

Oh my gosh, I mean, I'm really totally new on that blog. So I'm not necessarily the most experienced qPCR because I obviously just sort of depend on Edward, who's kind of a little bit more experienced than me and our lab manager. Yeah,

I guess I bring it up because I, I was batting something like one in three. already. If you really needed an experimental work, you just buy three different sets of probes, test them, and then probably one of them was good enough and you could continue your experiment without but like 10 Day gap in between testing, realising none of them work, ordering new ones, and then 10 days to have them synthesise actually Have you?

Okay, sorry, I'm not. Great. That's what question. So let's have a think. Yes. So we've talked about first two questions. The next one is kind of a little bit more detail about the general design of your experiments. So we've already had we've already talked about you do was it three biological replicates?

I asked him to do two or three. Yeah, two or three.

And inside them, how many sort of technical how many plates would you how many wells? Would you repeat the same thing

on? I think I was doing duplicates. Yeah. Because three, yeah, I remember three just seeming like too much to ask. Like, there's no way I could fit enough samples onto a plate. We would have been

concerned. Yeah. So how many on that, on the line of thought, how many wells? How big was your plates? What sort of plates were you dealing with?

So I refused to use 384 Well, plates, because that's hurt. My eye hurts my eyes. And like, I get bubbles, and everything's a nightmare. So I was always using 96 well plates. And I'd fill them up entirely, or maybe one or two rows wouldn't be filled up. And often, I would leave rows empty for the sake of organisation. So that's when I was screensharing. Like, I would do all of the pgk one probes on one plate. Yep. The other be one row empty. Like, I'm not going to split up my next gene. I'm just gonna have my next gene all on its own plate. Yeah, that that makes, you know, going back and looking at this data. Now I can actually understand what the hell it is. Instead of having it all jumbled up and mixed together.

Yeah, I'm trying to unpick that. No, that makes perfect sense. Duh. Sorry, we've we've answered multiple questions at different times. So I'm trying to make sure that I'm not repeating ourselves. In terms of just the general plate design, was it normally the different probes were rows? And then, yeah, columns were like combinations of biological replicates? And,

yeah, so I guess it would mix it up, depending on the day and like what I had to do, but usually it would be different samples. I think it's your nomenclature, columns. And then primer sets probes for rows. Because I think I had usually I would have more samples than I have probes. And so it made sense to do it that way. That way, yeah.

Perfect. And when you actually loading the plates, it's a bit of a general question. But what were you actually doing to load the plates? Was it just a single channel? pipette? Or did you have like a multi channel pipette? Or single channel? Oh, fun,

isn't it? We had a multi channel, but I did not trust it. Like, you could, you could play that and then look and see that, like there was one of them still had material in it was not like that.

So what did you do for for quality control?

When, um, I guess I what a what a challenging question to answer, it's very broad, I would check the I'd use DNA template to check the linearity of microbes. Or just to know what range they're fine. And so if, if it's, if they're not linear at very low dilutions, often wasn't a deal breaker, as long as they were linear, where I needed them to be sorry, check them on DNA for linearity. I think for Okay, and then I would my reverse primer, I would actually use for Gene specific priming. So then I would check how efficient my gene specific cramming was by using a dilution series of total RNA. So just with the with that gene specific primer, and then because I was doing gene specific priming, I primed 14 genes at once. So I would try and prime them all in make sure there wasn't too much crosstalk. Just because that's the thing that I sort of worried about. And I think I got lazier about. And that experiment was nice, because it also checks for linearity. If you're doing the dilution series of RNA. If it's linear, then you're happy that your primers and probes are working and you're also happy that your gene specific timing is working. As long as that was linear and whatever range let me let me check my notes. I think that was about it. and citing check on DNA check reverse transcription. Oh yeah. And then I would do a no reverse transcription to control something just to make sure there's no DNA contamination. And that's about it. Those. So those first two standard curves before I get started with the experiment, and then, uh, no, no RT control, kind of every every day

that I didn't expect.

Yes, I think that's I think that's all I did. I don't have anything else written down. That's cool.

In terms of each or looking at a particular run of the qPCR machine, did you ever look at, you know, amplification curves or melt curves or anything like that?

Oh, yeah, sure, I would always stare at the the fluorescence curves, usually, just to avoid doing other work. Like the run only takes 55 minutes, it takes me 15 minutes to walk back to the lab and then walk back again, I'm just gonna sit here and watch it. So I always watch the curves. And it's fairly easy to throw out spurious swells like okay, CQ, of nine, no way that makes absolutely no sense. Or the curve was really jagged or something crazy like that. Those ones are relatively easy to spot by eye, it just goes out, you know, air bubble or whatever. Yeah, start by machine, by machine, watch the curves. Look at the trace, I didn't do melt curves, because that has no meaning when you're doing Takamine probes, melt curve. And the thing that I should have done that I don't know if I ever actually did was when I was testing the primers and probes, run the PCR product on a gel. So I don't think I ever did that. So shame on me, however, will because I was using tack, man, you can get away with a lot more. So even if there were off target amplicons, it doesn't mean that they're being detected. So I rested somewhat easily, but probably not ideal.

That's all good. In terms of the actual, like fluorescent curves, or did you ever? Did you ever print them off? And put them with any work? Or was it more than just sort of like doing it by when the qPCR machine was running? Or did you save it later?

Oh, I believe it is saved on a machine in a building that I could get to right now.

Email, I think I just emailed myself the CQ values. You know, maybe because they my usual form of backup was whenever there was a machine, like the computer that the machine is hooked up to, I made a dedicated gmail account for that computer. Because that prevents people from stuck in or from sticking flash drives in there. So you just email yourself your data every time and it's saved to Google forever. So perfect.

Comp What the hell was that thing called? Eighth floor. Floor qPCR machine. Yeah. Okay. gDNA standard curve? No, I only emailed myself to refer to CQ values. Never forget those data.

So there you go. I do not have the first data. Maybe it could be gotten, I think I usually saved it in like some proprietary format. But when I did like export to CSV, we did seek as I might have done for us, instead of One Time Out of curiosity. We wound up not being worth my effort to plot those. Yep.

That's fine. That's fine. Okay, I think we've kind of talked enough about qPCR experiments. And I just want to talk a little bit more about the other side of things, which is the sort of analysis programming side of things. So it looks like you did your analysis with R anyway. How did you how did you calculate like, how do you do normalise? How do you calculate delta CQ and stuff like that?

Right. So here I guess this is a bit of a sidebar, but I never did corrections. So I just took my CK values as is. If there was some no non linearity. I never I never corrected anything. It's just the way that I got called out for that online. A like thesis defence.

And then everything was relative. Okay, so how do they do it? Delta CQ values? I think I can. Gosh, I mean, I guess I'm staring at the code, so I don't have to think.

Yeah, I guess I don't really know how to answer that question. I think I will I'm staring at things. Yeah. So for each sample and replicates, as I took a mean or something. How did I did calculate delta CQ values? Subtraction? Yeah.

Did you do like a mean of any of the buyer reps or technical reps? Or something? I'll answer that.

I think I took the mean as late as possible. But yeah, so I get it doesn't seek out CQ value for each sample, or Sample Prep combination. And then at the last possible moment, I would get an average. But then I would always do my best to plot the raw value. Okay. So like on my graphs, the line goes to the mean, if I if I'm plotting some change or something, but then I always had the raw data points.

So so it's when you say raw data points? You mean, do you mean technical replicates as well?

Yes, that's all I mean, I guess in this case, no, no, I don't mean technical, I mean, biological replicates. So I would plot delta CQ values of biological replicates. technical replicates? I think I actually took the mean extremely early. And just dealt with it.

Yep, I think that's right. summarise. I think I took it very early. Yeah. Yep. So yes. Like the first thing I did was compute the average of technical replicates. And then that's just the value that I use moving forward.

So were you using when you were doing this? These analysis? Were you using tidy verse already?

Yes. Yep.

Okay, another could be quite a general question. But do you have a biggest frustration when it comes to this sort of analysis? Is there something we've talked a little bit about it before? Or is this something you feel like you should be doing, but you weren't doing?

It now that you bring it up? I was never sure how to handle replicates and stuff. Like when when should I take averages? Or how do I propagate error? I don't know how to do that. I do not know how to do that. So if I wanted to plot technical replicates, like I wouldn't know, you know, because you can't take the ratio of the two different technical replicates to a biological replicate that itself has to technical, I don't know how to do that. So that would be I don't know if that's possible, first of all, but that would be satisfying. Plus, it would give me somebody more points on my on my plot, it would look legit.

Yeah, it's a, it's a difficult one, especially since, I mean, with so few data points, even three technical replicates, you're talking about such a low sample rate, you don't, you can't really plot that data, because the variation in it's gonna be huge. And so you have to do some sort of minimization, which I think, ultimately, you know, taking a mean, or even the median is probably the best, this sort of idea. And basically, I'm glad that it's something that people are considering. Because in tidyqpcr, we normally take the median of the technical replicates quite early on as well.

Yeah, and I guess it doesn't, it's kind of a moot because I think I only did two technical replicates. So like, yeah, taking the mean was, I mean, that that number doesn't exist. No one ever measured to the mean, measure of Alex, whatever. Okay, yeah.

Yeah. completely mute because whatever way you use, you're just choosing the halfway point between those two. Perfect. So would you say that you're quite comfortable with our programming then?

i Yeah. I hesitate to say yes, because I don't want to sound too boisterous and overconfident but yes, I feel pretty confident these days. I mean, it's been a couple of years and I don't really encounter problems that I can't solve any more. So I guess yes. I don't do a lot of things in our if it's hard to just do it in Python. So like string manipulation, I don't touch. Enough. Tiny stuff. I'm pretty, pretty happy.

That's great. Okay, I think that's pretty much the end of the subject questionnaires I've somewhat kind of eerily named. And we can move on to the actual use of tidyqpcr.

Have basically not done this. I think I downloaded

the package. Cool. Can you does it appear? Did you have any errors when you were downloading it in any way? Shape?

Christ, maybe? Yes. Library load slid. I did the dev tools bit and that, you know, worked for a moment. It was it was doing lots of stuff. Creating vignettes. I'm doing I'm doing the dev tools instal GitHub, Edward qPCR. Getting Started but right now. The time the library qPCR step does not work. And I

have you since installing it have you restarted our I have not we probably just need to update. Can we start then it probably just needs to update package information to find it.

restarted restart our Oh, damn. Okay. I'm back. I'm just executing Deb to our when

you share your screen again. And then I can.

That would be just fantastic.

Not used to being in a position

of not knowing what's going on. Please help. Okay, so I ran, I ran this line. And now it's telling me things about creating vignettes. Cool. Is this a reasonable place for it to be thinking?

Yeah, yeah, it's quite a few couple. A few calculations as to do to make all the different vignettes. That's fine. It takes a little while.

Yes, this this is as far as I made it.

Cool. So, um, you haven't had a chance to look at the vignette and itself. That's okay. That's okay. Cool. We can hopefully if this works. Hopeful Yes. To show them.

I think I updated our like a month ago. Hopefully I'm running pretty current version.

Yeah, it's always a pain, especially fairly recently because our did quite a serious update. That makes sense. So a lot of things have been changed, changed. Okay, so it looks like it's they're

testing. Okay. Okay.

Awesome. Okay. That's a better word.

Now, I'm actually completely lost. I don't I don't know what to do.

That's okay. I think the next best thing to do is just type in

just vignette. Yeah, then package equals tidyqpcr. But in quotation marks for qPCR Tyvek. himself.

I am not familiar with whatever is that oh, what the hell is this?

So it just lists all of the available vignettes for this package.

I don't even know anything so hard on this. This is a world of discovery for me. So

most, most packages you download will have, in essence, like a very brief tutorial of all the different things you can do with that package. And for some reason they're called vignettes. I don't know why. So, these are all the vignettes for tidyqpcr There's four of them. You've got the plate setup vignette. Oh, that's one reminder.

So are we just are we doing all of them? What do you wanna do?

Just the place a vignette. The other ones are kind of more detailed about different things. But place out tells you More about the basics and then we can go into more complicated bits and bobs. So if you just do again vignette brackets, and then just in quotation marks place up vignette. Brackets, no quotes. Oh, brackets and quotes. Yeah.

Okay, back back here. Yeah. Oh, just like that.

Just get rid of the package equals just have the quotation marks in itself. Yeah. That'll work.

Doing stuff. Okay. Okay. Okay, so yeah, I think I scrolled through. Now I scroll through one of the other vignettes on GitHub, because markdown. Okay. Introduction designing a plate.

So this is the sort of first step, and it's meant to be a bit more, once you get your head around the ways of repeating the same block over and over again, then you don't have to do as much copying and pasting. So you know how, when you were making your text file for your plate design, and you were just copying and pasting rows, and that sort of thing. The way we're trying to teach people in tidyqpcr is to try and make the simplest example with just for example, your gene names and the the probes that's associated with those genes, and then just duplicate duplicate them for every buyer rep and for every tech technical rep. So if you just make one module and then duplicate it over and over again, I just renamed them to their by rep on technically on because it was cetera, then it simplifies the whole process.

Okay, so if

you'd like to basically follow this tutorial, and make the 48 Well, plate goes through all the different steps.

Okay, so, Mikey, I'm a very terribly slow reader. So this is gonna be painful for both. It's perfectly

fine. You're You're not the first person that has tried this. I'm totally prepared.

I mean, feel free to. I mean, you can talk out loud if if you're trying to get your head around something and then I can interject if I think something's going wrong by you, are you happy with what's being set up so far?

believes? I believe so. I think it's using more. I've never actually had to like deal with the how Tibbals work. They tidy verse just has them and they work and then off to worry about it. So this is slightly more gory detail than I'm used to. But I think I'm getting it. And it's organised really nicely where like, you say what the sample IDs are, and I don't worry about it from there. So I like that so okay, that's right now row key. And conky are just, they're just tables out there in the universe. And they're not actually associated with each other. Somehow I suspect a plate is going to become an object. Does does R do objects I have no idea.

I'm not really not in the sort of object oriented programming kind of way. It's very much a functional programming language that people have tried to adapt.

And mostly it is a complete mess. So we try and stay away from that as much as possible.

Sounds fine. Alright, so I've created a blank plate Okay, I think this was the step that I was expecting. Me Okay, yeah, I like this. So you basically mean just the three chunks with the rows, the columns and you put them together in plate, okay.

That way, and then it has your display thing. So is this, um, considerations about what are efficient and good ways to organise plates like avoiding corners or avoiding edges or jumbling things up in some intelligent way? Or is Was that too much?

Wasn't sure if my mic was muted? Yeah. So we don't have any particular advice in terms of whether or not you should avoid edges or anything like that because it's not something. We normally have issues with that with ovum, like machines like plate readers if you're trying to do protein expression, but don't normally have too many issues with corner effects with qPCR. Expand So I don't know do you have if you if you encountered things like that

I have encountered the, especially for beginners or the bad day for the experienced person, the the plastic seal on top is more likely to fail on edges or corners. And then you just get evaporation and the wells run. Oh, there we go. That's one of my quality controls. If I have a well, that is behaving poorly for the trace, at the end, visibly visually inspect and look for evaporation. Yes, and then it's, I feel much more confident in throwing out a data point because it's, it's clearly broken and wrong. Because there shouldn't be No, there should be no evaporation. So I actually have a visual material reference for why I'm throwing out a data point, instead of it looks funny, and I don't like that.

In terms of like, what's a good plate design, generally, we do training coorporate different types of plate design. So one of the things we're trying to do is so for us, when we're trying to do three or four well plates, but from 96. Well, like samples, and we're trying to do like technical replicates from the same samples we have multiple but it doesn't do the right distances between the 384 well plate and the 96 well plate like the distance between the belt that the each individual well, it doesn't go narrow enough to cover the 384 well plates. Well sorry.

Oh, okay, the multi channel doesn't

Yeah, the multi channel doesn't and so one of the other plate designs we have is kind of staggered. Yeah, like a staggered so it looks really cool. It makes a nice little pretty picture. But it's kind of a bit more hard to automate the labelling of the plates with with the way that we're trying to do with sample IDs and target IDs and automatic labelling like that but it's it's on it's on the list to try and make sure that variety of different ones

possible possible Yeah. Okay I have a quality of life suggestion Yep,

yeah, sorry, I just had a muted, so I was,

okay. Um, well, so I don't know if this is a good idea, but I just want to put it out into the ether, um, when I like, so the design of having a row for a thing or a column for a thing, the beauty of that is I can mindlessly just put that down, or like pipette. If I have a single channel, right, I just, I'm just doing the row, and I'm just doing the column, I do not want to look at every single well, on this image when I'm doing that. So it would be nice instead of ABCD, 12345 12, etc. to actually say, which primer set goes in here? And then which sample goes in here and that that's the salient information often. Okay, I don't quite know the right way to implement that. But

ya know, I think that should be doable.

Yeah, I guess that's, that's how it functionally worked when I was pipetting. Like, I would physically draw on the plate, you know, pgk probe set, and then I could reference that true easily and type that down the row. And that made my life very, very easy. Because I don't I didn't care which column it was. I just needed to know which row I was in for this one to that I'm pipetting out of frequently. Okay. Yeah, does that that makes some sense.

No, I get it. I do get it.

Yes, I don't know if it fits with the rest of your design paradigm, but I'm just throwing that idea out there.

No, I think I think it would work.

And the ABC 123 information is still relevant because that's what's actually physically printed on the plate. So I guess I do still need that information. I just need to know a is x one, B is the FG two. What is the FG? Two? Does that sound like a fake t? So what are those?

Probably? I can't actually remember it's been a while since we made this

I always remember why f g your favourite gene was something Okay? I like that's okay. So something like this would actually already combined well, with the the I made my data tidy already. But for some instances, which I think I didn't show you, when I was first starting, I would also just add well number or like a one a two B five. Yeah, I have that in my tidy information. I usually get rid of it immediately because it wasn't relevant for the analysis. But it would be pretty easy for me to copy paste to well CQ value and just have an interface with this automatically. And now I wouldn't have to do all the manual entry of the various types rep two plus RT Act One, because I could just merge by well, and that would be incredibly convenient.

So I would I would use that feature. Yes. 51 Okay, I think I think I finished the vignette and I learned something I did not know about unite. I only knew separate

is a nice alternative

I have been doing with pace zero, which is effective needless apparently very good.

So now that you've got a idea of how to generally make a plate could you re do this for one of your own plates

in with with constant reference to the vignette but yeah, I think so. Yeah, I'll be pasting and I've just changed rep. 123. To my my stuff, I returned to the jeans to my jeans.

Perfect. Yeah, if you don't mind, I just want to make sure that you know, it's fine following the vignette, but actually using it in a case.

Fair enough. Let's give it a shot. So it's my conditions. I'm gonna steal your nomenclature then because it's effective. So just your nice heat shock national level repeat. I don't think I want that too.

How are we doing?

poorly. This is also probably due to a lack of vision on my part. So I think I want 12 columns. I've got three different treatments. And I want to do them in duplicates. So I think that are worked out, okay. So that means I have six templates, but then I need to prep types. So I tried to get my prep factor as six plus our T's and six minus our T's. So that should give me a total of 12. So now I'm trying to figure out oh, yeah, so the dark three, three treatments, times two biological replicates, six templates that I'm times to prep conditions for 12 columns. So I'm trying to get those into the Call key. System. So well, columns, I think one through 12 is right, by a rep.

Two times four, six times four. Do it. Think I might have done it? Rep one rep to rep. One, two. Yes, but not quite. So I think I still need training can I add sample here? I guess He ignored some part of the paradigm. The no unstuck.

That's that's fine. That's fine. Okay, so let's have a think about the, the columns to start off with. So we know there's three conditions, the three different temperatures, right? That's. And it for each condition, there's two technical replicates.

Yep. Well, so I was going to do biological replicates as columns and then technical replicates as rows. You can do the same difference. Yeah.

Okay. Well, yeah, I mean, we can do it that way. So we've got so buyer reps as

Oh, yeah. Yeah, call the reps, columns,

and tech rep as rows. Okay. So it's going to be to 30 degrees to 42 degrees to 20. Rest or recovery, or whatever it is. And then what happens after that, so we've got six at the moment.

And then the idea was to do plus r t minus R t. So I think

so the first six columns are always plus R T, and the second six columns are always negative, it

was the idea.

So do you need to do two technical replicates for two biological replicates for the neck? It okay. Yeah, okay.

No, you do because they're, they're unique samples.

But you need to do two technical replicates of to make them? Probably not. Let's do it this way, for now, just because it'll be the easiest way instead of like making things very hard. So

at the moment, you've guide so you have 430 30 degrees, then 442 degrees, and then 424. So straight.

I have and that's not okay, then then I've used that route. So I wanted to 30 to 42 to 20. Are and then just duplicate that.

So

that's probably a two. Yes, but then it just needs to be doubled.

Yeah, so you'll just have to do another rep. Cool. And times equals two. Okay, times equals two, you've got each equals to

wait. Oh, not realise that those are different. Okay. Yep, that looks.

So times we'll just put them consecutively. Whereas each will go. Go to the first element in the vector, double it. Second element element, double it. rows times we'll double check whole vector.

So if I put this information in there, so the table doesn't like having three things

intuitively, I just want to put sample ID levels there, but it's more information than me. I don't know. It's right now. Yeah, my buyer reps and prototypes are totally redundant. That's something else is wrong?

Yeah, so I don't think the I don't know when you don't want condition levels you want condition values. Because condition levels are just the three levels whereas values are the key ones.

Hey, there goes okay. That is exactly what I wanted it to look like. So now I need to figure out the rows which are fixed. Humans are more diverse?

So you're almost there. You're almost there. It's just you need to get the tech RX

eight wraps right yep I think this is an E. Who? Yeah maybe it's another rep

think all you need is the first rep. Yep. And times equals three

do you want to you want to Okay, happy with that. And now I just need to make a plate I don't think this is quite 96 wells but cross that bridge and we will create those six calls the same six Alright, so those errors. Oh there's a fine because that's from unite. So a one a two a three.

Tech one rep one. Oh yeah. sample ID needs slightly. Okay, so Sybil ID is the thing. Oh, here we go. Sample condition by Rep. Okay, okay. Some of these names are funny. So condition. sample ID levels. Hi. Why are you like this?

So if you go back to where you're defining row key six. Yes, you're saying condition equals tech rep values. So if you change condition to tech rep.

So condition was not a Yeah, it was not a keyword. It's just in table.

Okay. Yeah, you're just defining the name of that column. And it's the same with cold key 12.

Target ID Target ID is correct. Row is fine. My logical route is about to read prep type is fine. Sample level. Okay?

Called stressed so yeah, you had a error. Because now you're uniting because you've changed the names of different things. You're uniting things that don't exist.

There we go. Thank you. So I think that needs to be condition. sample ID, it still exists I think you've, no yeah, that's

the reason you've got em in by a rep. I don't know why.

How can you how do you spot those? Since I am the worst. Thank you. Sorry to use your time. That's why

and how it's the entire purpose.

technic replicated Target ID stress peptide illogical rep. And simple ID. So simple, simple idea. Was that an automatically generated one? Yeah,

so the first. So if you go back to unite, function unite. The first argument of unite is what the column name will be called. Okay. And then any subsequent subsequent arguments will be what columns are united. See, if you put stress in,

do I want stress there? And all the A's are tech time they're supposed to be technical report. Yeah. Great. Okay, I believe I made a plate.

Perfect, congratulations, you have made your very first own tidyqpcr play. So hopefully you can see like, the difficult here is that we're trying to sort of retro actively make a play design for something that's already been made. But I think if you are planning a future experiment, and you can see how tidyqpcr easily, like how it functions and how it sort of expects the plate to be designed with, you know, columns being different samples and rows being different targets. It's kind of a lot easier to make a play design and be then do your experiment because they correlate with each other and then your analysis will be super quick. So there's one final final thing that we can do, just to show what how to do analysis in tidyqpcr. So it's just going to be very quickly following another vignette which will just be doing sort of the normalisation and showing you some of the some of the plots and stuff you can get out of tidyqpcr Yes, so that vignette now is what's it called? I've got it here somewhere.

Package equals

yes.

Calibration multifactor

the Delta CQ 96 Well vignette that

I spoke to you yet wrong and I don't know how to spell vignette

perfect so this already has this will already give you the play design and it will give you experimental data and you'll just get to see how how you analyse data with data qPCR I don't think that'll work but I'm hoping this piece of code I'm just gonna send you a piece of code in the chat Give me one second. So myself out can you see that

that's The TSB not copy from zoom chat.

Apparently no, it's really annoying. Yeah,

we discovered this during Dungeons and Dragons was

really? Oh yeah, I kind of missed because my my flatmates don't actually play down the dragons but people in the lab do it's kind of a bit of a shame that we can't like meet up at the moment and they're doing it Dungeons and Dragons because in Scotland they've literally just reintroduced some more restrictions are running because cases are starting to spike again. So now you can't have more than two households meeting up

okay, that is a interestingly well phrased rule yes it's very clear

you can probably just copy that from was that the same thing? That's the file name Yeah, it's just the file name yeah

that is 100% where typos are going to happen

CAN'T FIND function read CSV I think you just need to do the tidy verse library that will be full of data we'll look at that perfect

yes correct data instrument comical? Oh, this plate is organised funny,

I know I didn't realise it was so weirdly organised. This is so upsetting this is some data from the lab next door to our lung.

I mean God bless them for being able to not mess this up

bit of an odd shaped graph unfortunately.

Oh, I like that okay normalises everything very easily.

Assuming the gravity if it's a bit too weirdly shaped hopefully it'll be better it's just a very elongated

Windows shaped right now much more sensible looking graph

perfect I think that's pretty much it.

Thank you right? Oh, seems like there's one more interesting one

looks very similar to your plots that's

crazy very nice. Oh yeah get that like okay. Totally works just cruise through Alright, delta delta this looks extremely straightforward to do during wonderful Okay. The last one. One more chunk

Okay, I like that that is massively convenient. Have a consistent, reasonable way to do the normalisation because it did wind up being bespoke every time I come. Yeah. Cool. All right, I think you got it.

Perfect so that you aren't there is tied to qPCR. You've managed to master it pretty much. Make a plate for your own data and see how once you've got your plate sorted out. Once you've got your data you can relatively easily copy and paste exactly the same functions. The key part is getting the design, right. And then all of the functions are the same, just specifying exactly what your reference genes are your housekeeping, Gene size, etc, etc. And then you can get straight to plotting and seeing what the impacts are. Yeah, do you have any particular comments? Are you happy with the way the vignettes are?

I'm happy with the way vignettes are I think I'm going to steal this format for my own purposes. I did not know if it's worth things I'm gonna live in. Yes, we're good. I like because the Yeah, what I what I want to do is copy paste my way through it. And then honestly, the way that we did it, where I have to do it myself, and then I just have to look back through the vignette is actually very useful to see the format and where the things are. At I liked that a lot. Yeah, no, no particular comments about this part.

Great, I think, from my point of view, unless you've got any ideas for new functions, or anything on that sort of that side of things. I think that pretty much concludes our tutorial and our interview, I'm happy to say that tidy verse seems to have work. And we've spent quite a few times you're the first person to try this particular vignette. And actually, so I've done three previous user interviews, but they've only been with the plate design vignette, and trying to make their own play design. And they've had a few comments with that. But I think we've got past it now. And you were the first person to try the actual normalisation.

It was great. Yeah, he makes a fair amount of sense, right? He was named the team that you want to deal with and like that a lot. And everything just kind of works. And it does all the things that I want. So ultimately, this full change this last figure, that is the figure of merit that I want, like, I don't care about CQ, or delta CQ values change. But it takes me all the way there and does the calculation in a way that I don't think I have to worry about because getting the logarithms is always kind of a pain. So this is, this is convenient.

Perfect. What's it gonna say? So do you think? I mean, do you have any qPCR experiments planned in the future? No. Hey,

December, if some sequencing comes back, and I do a follow up experiment, I will be doing some qPCR. So that's, that's my earliest timeline, but I will let you know. And we will work through it together.

Yeah, I'm really, really keen to try and try and get people to actually start using it. I know that it's can be a real pain to start off with because you have to learn a new way of doing things. But we honestly do think that once you've got your head around the basics, it's, it does save you time, and it should save you time. And if it doesn't save you time, save your save you time, then we need to know because we need to change something. So yeah, please feel free to get in contact with Edward or myself. Well,

definitely drop me a line when that comes up. Yeah, that seems a frickin lot of effort.

I'm going to stop the recording now because I think we've got absolutely everything we need.

Interview 2

For the future,

I had a friend who did linguistics and he had to do like these video interviews and then had to go back but at to like, like, type up everything.

And then just

Yeah, because Zoom is really good. Have you ever used record recorded anything with Zoom? No, it automatically makes like a transcript for you. They try it tries to understand what people are saying. And it automatically went to

announce the eight as much as possible.

Now it's fun when it gets it wrong. There it entertains me. Just has no idea what

we did. Is it tied on American it trained on American dataset? Yeah, absolutely. Yeah. I don't think I'm too bad. I haven't got an accent. Laura.

Please don't be terrible at this borrow? No, it's fine. It's charming, I think.

Yes, this is what I want. Okie dokie? So let's start off with the first very general question, which is, tell us a little bit about your qPCR experience talking about, you know, very basic questions. Do you do it with DNA RNA do what sort of probes do you use? How often do you do qPCR? Etc.

Right. So I've got a fair amount of experience with qPCR. So really three main experimental areas that we use qPCR for. So we do. RT, looking at transcript levels, Chip. So we're looking at enrichment of certain, in our case, histone modifications. And I've also been using it a little bit to look at retro transposon copy number, just on genomic DNA. Okay. So I've been using on pretty much all my experience is on the Roche Lightcycler 96 in 96. Well, format, and pretty much all of my experience is setting those up manually. So not using repeat pipettors and stuff like that. So I think in a 96 Well, plate that's, that's manageable. So I do do qPCR quite a lot. So I've been doing it is pretty much our bread and butter experiment that we do this again, went over a lot of our output. So in the lab, we're really interested in looking at centromere formation in fishing yeast. And there are two sort of reciprocal outputs you can look at when you're looking at centromere formation, inefficient use, you can look at how much the centromeric transcripts, how much how much the centromeres essentially transcribed, or how much of that centromeric transcript is produced and persists in your wild type versus your various mutants. And then also how much of a particular histone methyl mark you get. So h3 canine methylation is a sort of hallmark of silent chromatin. So what you would expect to see is if you have a mutant that perturbs Hatori, canine dimethylation You know, you look at like qPCR the levels of canine methylation versus the wild type, you should see if you see a loss there, you should see a reciprocal increase in transcript. So, yeah, so I've been doing that. I've been using the Illumina master pure yeast kits. So what I've been doing is growing up samples for CHIP, and because you only need to use two Melfi, RNA prep, skimming off to mill to do the RT. So then you can be sure that you're looking at an exact a snapshot of exactly the same growth phase and essentially exactly the same culture looking at the the histone marks and also then the corresponding transcript. So yeah, I think since since I've started my PhD I the other day, I think around my 250 play, blimey, so a fair a fair few, but it's all been on the Roche Lightcycler. System.

Okay, and the actual process that you're using, is it just sort of single primers in each like one primer and it's well or Yeah, so it's one kind of, yeah,

so one primer per well. qPCR primers that I designed myself. So you know, between 50 and 150 base pairs long with a standard programme TM is between 55 to 62. But I will go through a round of tests. So what I'll usually do is if I'm looking at new target along with three or four different pairs, do you use standard curve optimise your calibration in that way. Yep, find out your efficiencies, find out

your are squared how well they fit the the leading up the line and then just pick the best ones either the ones that have I think the the ones that you know, closest closest to an efficiency of two as close to one on your square as possible. But, you know, I will go down to, you know, 85% efficiency and above is acceptable ish, because I kind of do a calculation where I take into account, you know, my calculations, it primer efficiency, anything up to, you know, 105% is about where my ballpark is. But yeah, it's not that man or anything, it's just standard, one primate pair per Well, usually looking at quite a lot of primate pairs in parallel, actually, because I'm looking at a different species of fish in yeast that has a slightly different centromeric makeup, in that they're all retrotransposons. So I've got 10 Different families that retro transpose on. And I like to look at two different regions, either in the open reading frame or in the terminal repeats. Yep. For each for all 10. So plus, my controls are usually have a panel of 24 primer pairs that I do, which is why I've run so many so many plates, but it's the fact that all sorts of depending on the conditions and depend on the mutants, different product, different retrotransposons behave differently. Although they all seem to be classes that centromere, there's something we're still we're still looking at what's going on, but I need to get an idea across the whole web of you.

Okay, I think that's covered many of the different points. So, in again, another relatively general question is about how you see qPCR results presented? How are they presented in papers? Is it normally just sort of the end summary figures? Do you see any, I don't know. Any quality control data or the raw data itself, to, to see that often? yourself now?

Yeah. And in so I can, I can basically show you so. So it's essentially 99% of the time in the manuscripts that in our field is bar charts with error bars. If I can kind of share my screen quickly, quickly, the Actually, I'll share that one. So this is our latest NIR paper that came out at the end of last year. And this is a typical sort of panel that you would see when looking at qPCR data in our field. So on the left, you have the results of qPCR a QR t. So this is looking so send dG is the centromeric transcript. And then, so let's transcripts that send the G and then also the corresponding methylation. And you can see what I was talking about with reciprocal. Yeah, on the left, where you have the transcript so Cloudforest, the soul methyl transferase, that puts down all the canine dimethyl wwox. So in a situation where you don't have any cloth or you don't have any methylation, you have an increase in your transcripts, and then dicer is immune also involved. But yeah, this is pretty typical of how it would look for both chip and for for our T experiment. And I don't think because really then the next step that people do is either RNA seek or chip sec. So they this is usually sort of either a first step, and then you do a chip SEC or an RNA Seq or this is the confirmation. Once you've done a chip sec, and you see a pattern across your potential, whatever you knew, then check it by qPCR. But it is bar charts and error bars. We don't really do many box and whisker plots, or it's very rare to see all the data point plotted. And yeah, as for QC, and things like you know, publishing primer efficiencies or looking at actual the actual amplification curves or the melting peaks, that is almost never I don't think I've actually seen it ever published main tech stores or as a supplementary figure.

And what about just sort of the raw, the raw values before the applied as like a supplementary CSV file or something like that. Have you ever seen?

No, no, not for not for qPCR those sorts of things are very commonly included for for RNA seek, you know, you're gonna get your either your raw fescue sanctified also a gz file, but very rarely to people put on either the raw CSV file that or the raw txt file. Because a lot of the time these Lightcycler. Now you can pull them off as TX, TS or CSVs. But a lot in there in LC 96 P format, which all right, Q format. So

crap. Yeah.

Very rarely do people include the actual raw CQ counts.

So what's your I know you're talking about when these results are published, it's normally either the confirmation of something else that has been seen in RNA seek data or to see data. Or the other way around. Yeah, so it's never sort of presented on its own really,

sometimes it is. A lot of the time. But that probably isn't actually true. So actually, a lot of the time it is presented. Just because it's so much cheaper to do, yep, across mutants across, as you know, I know, you can get a lot more information out of our chip secondarily sick. But for our purpose, if you have a single view of that you want to make a double mutant, and, you know, if you're doing an RNA seek to do that and duplicate the cost just spirals, whereas if you know, especially for things, especially in the field we're working in, in Palm Bay, at least not me personally. But the general fishing used RNAi field, we're very focused on a very specific locus that we know the sequence of, don't really care what happens in the chromosome arms, or at genic, low sigh. So doing an RNA seek, you're really wasting quite a lot of the information, you get this big data set, but if you're really focused on a very particular part. So yeah, actually, the chipsets in our sorry, these chip qPCR. And our T qPCR are very commonly used as sort of the sole indicator of transcription or transcript accumulation at centromeres, or changes in histone marks.

So have you ever read a paper that pretty much primarily just uses qPCR? Data? And have you doubted it? Have you read it and thought, I'm not entirely sure what you've done? Or like the results? Show what you're trying to say. But I'm not entirely sure if I believe that results. Yeah, well,

it'd be can become quite opaque when you start doing normalizations. I suppose especially for things like chip. So for that I always struggle a little bit with with Chip, especially for things. So even here, we've we've done it, we've been guilty of it. So this is a dimethyl

and trimethyl. Mark on histone h3 on lysine nine. And that's a repressive mark. So that should if you have that mark, you shouldn't have any transcription. So what you do first is with a chip, which is something but you don't do so with an RT, if you're doing internal normalisation, you is normalised to a control gene so normalised to act in, or NBA or something. So what you do first with the chip is you normalise to an input, which you said was essentially unprecedented chromatin. Okay. And then you so what you do is you take a fraction of that before you add your antibody, and once you do the precipitation, you can then work out sort of how much enrichment you get versus how much you have throughout the genome, if you're if your antibody is putting on a very specific region, and it accounts for things like copy number variation. So you do that, first as your, your first normalisation and then here, what we've done is we've normalised it to the amount of methylation, you have acting, but you don't really have very much methylation active because it's an actively transcribed gene. So you're essentially normalising to what is a zero value? Yeah. Which can become a little bit and then also this has been normalised to the wild type set as one. Okay. So it's, you can be quite tricky with it and kind of make it also than, say, a chipset profile. Yeah, you have your accounts. And it depends whether people showed people aren't always honest about showing standard deviation or standard error.

Yeah. A

cat it can be a little bit opaque but it is much seasickness, this is also one thing i is usually used in conjunctions with these sorts of sorry, I'm not sharing my screen with this sort of Panel B, which are phenotypic assays, which you'll say if you insert a certain marker gene in silent chromatin, if you have normal chromatin, it will be read, if you have a perturbation of that it will be painful. But you don't really know to what extent you can see here that this dice are one, delta and Clark or delta look the same. Actually, the amount of transcript accumulation isn't the same, you know, with these sorts of colour colorimetric phenotypic assays, there's a threshold. But actually having numbers there. You do see, you know, that you do see people drawing conclusions and much more subtler, more subtle, much more subtler, more subtle effects that you can maybe pick up more with with qPCR. But you're not I, I tend, I tend to feel i because there's a lot of time people can Can I trust these phenotypic assays a lot less, because people would say, Oh, this is slightly paler, and it's much more sensitive, but at least with qPCR is a quantitative assessment of your transcript level or level. So I tend to trust that a lot more than either growth assays. It I mean, if it's obviously something that died in it, but when you have these sorts of intermediate things are much more likely to trust, if I see an intermediate growth, that kind of looks a bit sketchy. But then if I see, you know, a 50% reduction in in transcript levels that corresponds to that, via qPCR, I'm much more likely to trust that. Okay, then something a bit more qualitative.

So, yeah, I don't know if that answers your question.

No, it's good. It's good. It I mean, one of the most interesting things about doing these interviews, from my perspective is interviewing different people who are obviously in completely different biological subjects, and are using qPCR differently, and what they use it as, as for the sort of how convinced they are by qPCR data and how they implement qPCR data. And it's typically kind of the the richer labs with lots of high throughput. mechanisms are more likely at well, yeah, to say, Oh, we can just do with RNA seek and maybe qPCR can be supplemental, but, you know, it's never gonna be as good as RNA seek, and it's like, Okay, I think that's,

yeah, I mean, I think I think RNA seek is great, as long as you're looking at something that is essentially single copy that is not plastic, that doesn't change. So I have a problem that I'm looking at retrotransposons that are repetitive multi copy the genome assembly is very bad. Your RNA seek is only your RNA seek data analysis is only as good as your genome assembly. Because that is what you are aligning to. If you have transcripts that come from something that hasn't been sequenced properly, you will just throw them away. And also my transposons, when I in a certain you can mobilise and the copy number expands. So I'm trying to align to a plastic genome. So yeah, I mean, it's great if you're looking at transcriptional changes of a gene within certain conditions, but at least I know here, I don't know if like, if I'm doing my qPCR or my transpose ons, it doesn't really matter how many copies I went does, in essence, but as long as I can pick up a small 150 base pair region, the sequencing doesn't have to be perfect. Like it would be with the SEC experiments. For me to pick something up, but essentially if the if with with an RNA Seq, you know, there are people have done PacBio sequencing on my particular betta fish in yeast and you end up with with contexts that are just totally different to the initial short sequencing. But my qPCR probes are still good enough to pick up across though you as long as you've got a half decent sequence and then because you can validate those against your genomic DNA and you know, Whether or not, yeah, or your your your RT you know whether or not you'd put your primers, if you've got a miss sequencing, and you've just you know, and it's all snips and you've decided prime it doesn't work that will come up very quickly when you do validation. Yeah. So yeah, I'm I tend to, at least in my scenario, which is a little bit unusual. Yeah. Working on very repetitive elements, I do I do tend to trust qPCR. I do value the information that qPCR gives me, I hold it in quite high regard, when compared to,

you know, say, largest seq experiment. Yeah.

Cool. Right. So, next stage is kind of talking a little bit more in detail about how you design your, the actual plates of your qPCR experiments. And then talking a little bit about how you analyse the data and what processes you do for analysing the data yet, so it's just starting off with designing. Yeah. Just general questions about like, how many replicates? Do you do both biological and sort of technical? And, yeah, how you put them on the plate, that sort of thing?

Yeah, so So I noticed when I was looking through tiny qPCR, I do things slightly differently in terms of where it so so if I'm talking about, it depends, if I'm talking about a QR t, it does different to if I'm doing a chip, so with a QR t, I will have so so regardless of whether I'm doing QR T, or chip, I'll always have three biological replicates. Okay. And then, so for a QR T, I'll have three technical replicates for my plus RT, and a single well, for my minus, sure, because I don't, which is the same as the, as you have on the title qPCR for the plants. So it's, for each replica three tech reps for your plus and only one p minus, because it's, it's a waste of resources to do those in triplicate. But I usually do, I don't spread them out as much on the plate. So I will do. So for one sample, I'll do my three pluses, and then my minus my three pluses for the next record, then my minus my three, and then my minus, and I find on a 96 well plate, I can divide it up, you know, into into three, three groups of four wells. One, two, my plus plus plus minus plus, plus, plus, minus, plus, plus plus minus, for replicas, one, two, and three. Yep. Which I find for me, because I set manually is much easier for me to think because I like to sit with a fresh box of tips. And my plate, once I've added my Master Mix. And then each tip corresponds to a well, sure I can switch or have switched off before and you know, where your tip is, corresponds to worked well, which you could go in your shoe, I find it easier to go three concurrent or three, three adjacent world where rather than I think the ideal setup on the tidyqpcr is to have one. And then it's your second tech rep is four well was over. Yeah. And then your third tech rep is another four well was two wells over sorry, yeah. That for me, it just isn't how I've done it. And I've done it so much that I find at least in 96, wells, sort of the variability you get across a plate, in terms of the actual machine itself, Reading isn't, it is not something that I've ever encountered as an issue. And felt that I need to, you know, have my tech reps separated. But yeah, that's how I would do it. And then depending on the number of primers, so normally what I would probably do is what I've been doing, it really depends, it really depends on what I'm looking at the number of primers, sometimes I'll run, you know if I have my wild type, because I've got 24 primer pairs. Yep. If say say I'm doing wild type versus a mutant, three biological replicates. That's three plates per per strain, essentially, for my 24 private pay, so I'll probably do all my wild types. On plates one two and three or plates one three and five and then my probably placed one three or five actually, because once I've got my privates out and I've thought them out, I might as well do plate one wild type and then the same prime appears to you and plate three with my you know, the next eight set of primers. If I'm just sitting there doing you know, I can do pretty much like six plates a day. If I'm just churning through setting up manually. So that's how I set up a QR T within chip is slightly different. So, because for each chip, and for each strain, and for each biological replica, you not only have different primer pairs, you have different and so for a basic chip, you will have one antibody and one input sample. So you need to do those in technical triplicate, so, my inputs and then my antibody underneath. And then sometimes if you're testing a new antibody you haven't tested before, you would do a beads only control and no antibody control. So you'd have that well. And usually, if I'm doing canine methylation, I usually do. So if I do h3 canine methylation, I usually like to look at total h3 as well. So for each sample, I'll have 12 wells essentially guys, step three, so input, canine h3, no antibody. So and then I can fit four across, which they do, which end doesn't doesn't wrappers nicely.

Because if I'm doing a QR T, I have four wells, you know, plus, plus plus minus, in biological triplicate, it always fits very nicely on a 96 Well, plate, I'm doing it shit. Because I have three biological replicates 123, that only takes up nine wells, they either have to move, you know, a different primate pair in the end, whereas with a QR T, I can just go straight along the line, all the same primate pair A, all the same Roby, I'd have to go a one to nine as one, private payer, be I say a one to nine be one to nine, see what did I do and so on all this stuff, a primer pair, and then either for 10 to 12, either have a different strain, or a different primer pair, and it just doesn't doesn't quite all fit on one plate. So I ended up with chips kind of end up leaving gaps, just for me setting it up so that I know that on that primer, rather than having, I don't really like to split over plates, he's just for me setting it up, really, if I've got a strain or a replica that I don't like doing, you know, just the input of the canine on that plate, and then doing the low antibody. And luckily, I like it, although it's probably not the most economical way, in terms of plates, you do end up with some empty wells, because I'm doing so many just for keeping track of what's on what I like to keep them all together. But yeah, Chip is one of the things where it's a bit more, it is a bit different. And it changes every time. At least with an RT, you always know you'll have protec per biological replica per private pay, you're gonna have four wells, you're gonna have your three pluses and your minus, with chips, sometimes I will look at, you know, on a poll too, as well, sometimes I look at, I could be doing something or I'm just looking at GFP, so that changes all the time. So I can't really have a standard one size fits all. Plan for a chip that I can just use again, and again, it kind of takes a little bit of that moving about.

So how do you design it then? Do you just open up like an Excel spreadsheet or something like that? When you when you actually try and make it?

Yeah, pretty much. I will do it in Excel. Just just for the initial play plans. And I also have, yeah, so So when I'm doing the initial play plans, I'll lay out like a 96. Well, plate sales at age one to 12 across the top. And just really see what I can fit. Because it is going to vary from experiment to experiment. Yeah, calculate how many plates you're gonna need.

Yeah, cuz I think I mean, the design plate functions that are part of tidyqpcr that obviously assuming you could you have some sort of pattern that is repeated over and over and over

again. Yeah, I think tiny qPCR is great if you're looking at QR T. Yeah. is brilliant for QR T, it's just if you're looking at chip and that sort of thing. Yeah, you're just not something that's I think

what we're probably going to do is grabber function from another package just to automatically read in Excel spreadsheets, so people do make a more complex map and an Excel spreadsheet, then we can just read it in. Yeah. And then the plate format isn't the same is in a tidy format, but just

Yeah, yeah. So I was just I was initially so that the way that you were making a plate plans entirely, which I would just make In those CSVs, and read CSV and those in then basically exporting my qPCR data from the Lightcycler package as a CSV and joining on just joining BioCarbon. What Cardamom is joining on a well position, I think, okay, yeah. Because I also then wanted to join. So I was essentially joining three data frames together, the third of which was the primer efficiency. Yep. So then I'd had I'd have a fully annotated plate with what's in the wells, the CQ as I was facing throwing away everything else because to me, I the only thing I care about from the output of the Lightcycler machine is the well position and the CQ. For further downstream analysis, and I was just joined in that on well position to the plate plans I've made in CSV. And I was joined in those by so we might play plans, I had a primer pair, and then I was joined in by a primer to my CSV that I had that just contained is basically like a general CSV for every every pair of qPCR. primers, I have just make a data frame that has the name of the primer and its efficiency. So joining that as a third one, and then doing all my calculations from that. Okay, a three part annotated data frame. Yeah, I was using a lot of group by to rather than having necessarily a completely unique id like you have been doing your UCR I was doing a lot of you know, grouped by whether or not it is a plus RT or minus RT. Yep, just felt clutching it a bit like that it works well for me, but if you know, it's not necessarily great, but it does.

Okay, so talking a little bit more about the actual analysis side, which was Yeah, comfortably segue to It seems you're quite comfortable with arm now is that

I wouldn't say I've seen I've kind of got to a point where I'm happy analysing my own data. But I think there are big holes in what I actually like, I've basically gone about it. Probably the wrong way, I've approached it with a problem and just pick the bits that I need to solve that problem. Rather than getting a good grounding in the entire tidy verse. You know, there are probably functions that I miss, that might make my life easier, but I'm reasonably comfortable. Yeah, and I've got a pipeline. Now that works well for me to analyse my qPCR and it's just been basically, Magpie and, and just picking from other people's stuff. There was those quite a nice thing that I found that some done that they were they had, that allows me to do t tests on multiple thing, multiple primate pairs, comparing, you know, wild type to movement at once and getting the data frame out the end as a column, that's just your P value and your T value. Yeah. Which I, which I found really useful. And then you can just plug that in, maybe write a function and plug it in. I mean, I can show you what I've got, if you please do not laugh. Terrible. And how inefficient is but it works.

So I got

so basically what I've been doing

is

so I have these. So this this, this function in Britain is called annotate results plate, which takes three arguments. So it's my results plate, my plate plan, and the primer efficiency. And it just, yeah, it's just, it's just joining them together. So essentially, I have this primer efficiency, which is this template frame, which is every primer pair that I would ever use. And there are certainly efficiency, essentially. So this is for a QR t. And then it's a case of reading all of them in separately. Read in all of my results. So basically, our plate plans look something like this. Yep, position strain. Replicate. So that's by biological replica that, yep. I'm gonna mean, I'm going to have Richard tech reps anyway. Yeah. So at the end, if I'm doing group by, you know, whether or not he's got it or not, I come to do the means later on. I don't really need to say whether it's a tech rep or not. Yeah, biological replicates make are important to me. So then I pass that through. So yeah, so this annotate result plate function that I've written, whichever

not run, no need to run it

Okay, so for example, if I put this this annotate results plate, one ends up like this does take everything from but it just joined. So essentially on position, a one either CQ Did you know In his joints, the plate plan plus the efficiency of the primer I used and then run those because then I basically join all these together is because I'm running it in 96 worlds if I don't have the capacity to do 384, we have 396. Well, yeah, machines is basically is what I do. So in basically, I make this, this new object is called all annotated results, which is probably not great, but it's just binding, using our bind to stick all of the separate ones together, and just transmutate in on basically what I care about. Yeah, so I end up with something like that, which is essentially six qPCR plates together. annotated by strain replica, which primary is the efficiency? And then in this case, because I'm doing this as a TSB, which temperature is that? Yeah. And then it gets a little bit of a kludge. So basically, I deliberately make the data untidy. Because what I want is I want for each observation, depending on which control gene

I have,

I want to create a matching column that is so basic that you need to take a step back, I need to tell you how I how I do the calculation first. So the calculation I do to take into account the efficiency. Basically, what I do is I do for each well, for each private payer if the efficiency

to the power of the CQ. Cool, because what you are assuming is that you have something that is 100%. Efficient efficiency is two, right? So every cycle, you get double the amount of product. Sure. And if you have something that say 95% efficient, every cycle, you get 1.95 times as much product, right? So if you do efficiency to the power of CQ, you end up with an arbitrary number that relates your CQ to your primer efficiency. Sure. But there is an issue with that inherent is that in this case, the larger the C queues will give you larger numbers. Which if you're taking this arbitrary number and say an arbitrary amount is inverse to what you should say. Because larger C queues, a smaller amounts, essentially your starting material, so you just inverse it. So it's just one over efficiency to the power of CQ. Okay. Is the is the calculation. Does that make sense? Yeah, yeah, I get what you mean. Yeah. So that. So basically, that's what I then do is basically take the rotating results plate and create a new

column

that is called corrected value, which is one over the efficiency of the CQ. Take the mean? So I group them by strain, record. Primatte. RT and temperature. Yep. So then basically, I mean, take a mean of the tech reps, essentially. And then what I do is I create two new objects, one called control primers, which I've specified in the function here as well, which genes you want to normalise to, okay. And then I create a second object that is basically everything back and removes basically all of the values that you've specified as your control gene. And then I put, essentially, I rejoin it again. So you have a column. I'll show you actually, what comes out. So if I so if I run this function, I call it QR T, Calc. 25. And then yeah, I'm normalised into this history. Gene. Yep. Yeah. And then basically, yeah, that's just so it works. And I'm basically kicking out at any of the minus r T's because a lot of that a lot of the time my quality control on the minus R t is done by I. Ya know, nobody ever nobody ever plops nobody ever published these two plots of the minus Artis Yeah, when I'm basically looking on the software. I dragged my three tech wreck to make sure they overlay each other, drag it on to the fourth, the minus and as long as that Coming up either not coming up or coming up. Yeah, I thought the onset, right there's a little bit of DNA contamination, which there will be more. So in my case, when you're working on centromeric DNA, it's very difficult to digest away. Celebrate dA. So that's really I don't, I don't ever plot the minus r T's. Okay, so cute. If I run that function, I end up with something like this. So I have a mean, primary with a child, then the standard deviation

of those three.

So essentially, what I want, what I do is, yeah, I end up with this arbitrary value. And then I divide. Because the tech represents really, those don't relate to each other your mean that first for each biological rep, and then divide that by your control primer, you end up this something? I mean, I could I could show you. Yeah, let's go. Now show the working. Can I show you the working? Yeah, essentially, it just creates a column where for each strain, biological replica. And in this case, temperature and primer pair. Yeah. For each strain, biological replica, and temperature. Strain replica, whether or not he's plus or minus RT, it basically moves say I was normalising to act one, takes his colorway and then puts it back again as a separate column. So I've got a column that create that, that contains the arbitrary calculation for the corresponding gene. I'm normalising to, so I can do live columns. Does that make sense? Yeah, so it's, um, it's making the data deliberately untidy, so I can do that division, and that normalisation is probably a very messy way to do it. But then basically, what I end up with yet is something like this, that for each primer. At each strain, I have, essentially the mean value, my primary relative to my control, the standard deviation, got a duplicate column nearby doesn't matter. And then I can plot in something like, sorry, so then, no, that's, that's just Fascinating, isn't it? So say, for example, I wanted to just look at, you know, one particular private payer, I'll just create a new object that only looks at the, you know, the subsets only by those private payers that I'm interested in, and then use that as a to do a bar chart I've dodged, you end up with something like that, essentially. Yeah, and then I've also got this t test function which, which is basically the same up to a point. Basically, for each biological replicates, that lists all three values in a cell

and then spreads it and then you can list

those, I'll show you, I'll show you. So this is something I've just stolen from someone. If I were to if I were to run this t test calculation, right. Like get this essentially as an output which is my mutants versus my wild types test p values all in one go rather than do it individually at a t value, which is quite nice. So yeah, that's basically it and then you know, you can also do I also sometimes if I want to do it versus wild type I'll just I'll just essentially do the same make it untidy again, but spread the column wild type and then divide everything through by the wild type value. So you want our values are essentially divided by themselves and come out with one give you

an idea you can you can, you can substitute it like that. And then for the check it is very similar. Except you have an extra step in there. So it's the same basically as I do Wherever I have the function that takes the control gene I want to normalise to, but it does that for anything. So I have a column called antibody. But in that Li to be input or you know came out. And I just use anything for input, essentially divided by that. And then you can note, you can either just leave it at that just do it via by input, or you can then take that, and again, make it untidy goodbye, then doing it via normalising then to your control primer pair is again, taking that out and sticking it back together. So you've got two columns that you can divide

by each other. Okay,

does that make sense?

Yeah, no, I do get I get exactly the logic. Yeah.

Because that's what because that's the thing I was doing it in, you know, just in Excel. Just, you know, take it and try and mutate in it. So transposing it into the shape of the plate plan, and then just doing the calculations in there by dividing the columns. And it just, it's just so much more room for error. Yeah, when you're manually copy and pasting things across. And it basically I'm starting to look at, look at using our for displaying tracks from RNA seek for publication, happy with how IGV looked, and there are quite a few, like GVS is a really good package. So it started to get a bit more comfortable with R and I thought, you know, if it's, if I can do that, then I don't see why I shouldn't be able to just automate the process of analysis, or just take the or not move. I'm not physically moving cell walls around anymore. It's just as long as the plate plan is correct. Yep, they're all joined properly. The function itself might not be the most efficient, but once it runs through it, it works.

Are you the only one in the lab that

uses this? Yes. So yes, so basically, everyone in labs so. So one person in the lab, who's very, very big into Python, and he's doing something similar with pandas, I think, yeah. But isn't really doing as much qPCR is doing a lot more big, big SEC experiments. So you know, you know, Rob, do come to the crypto games Dutch postdoc,

Oh, yes. Yes. I

know. He's very into Linux. Very, very Python. So he, he is out of all of us probably the most code literate? Yeah. But no, I am. I'm the one who's kind of driving this and trying to get people to adopt it. Because I think that if I, if I could just package this up and annotate it really well. Yeah, people can just plug there, as long as they've got a plate plan and a basically a CSV that has all their primary efficiencies into it. Yep, as long as they understand, you might have to change your strain names. And that sort of thing is a bit more manual Fiddling is not completely hands off. But it is applicable to, to everyone else I was gonna I was gonna write up as a as a notebook. And just see, you know, because we have quite specific applications that we own, we really only ever do. Chip or Artie on centromeric transcripts in Palm Bay, very rarely do we look outside that. So rather than writing something that's all the, like you've written that can handle pretty much most stuff. For our, for our purposes. This is enough, essentially, they don't read, we're not going to do 384 Well, we're not doing 1.36 We aren't doing 96 only. I don't know really, if I know it's more error prone to make your plate plans or CSVs. But I think if the learning curves a little bit steeper, yeah, it's a lot easier to autumn you know, to once you went to understand it to get it, but I guess that's like with anything with R is it's a lot easier. Once you understand it. It's the people do this often enough to to bother putting in the, the effort to learn it or you know, if I could write a just put this in a notebook and see if people would be interested in using it.

Cool. Cool. Cool. Cool. Cool. Yeah, I think we've covered pretty much all the sort of interview questions. Yeah. The next thing is to start to jump on to tidy verse and get you to like, mess around with it. I don't know if you have any direct comments, because I know that you've developed something of your own. That's that is useful for your own particular project. I was just wondering, in more general sense. What is it about Tybee qPCR that you think people will struggle with? Is it is it? Like if we added the functionality just to read in an Excel spreadsheet? Will that solve the problem? Or do you think there's something else? That we're just try to? Yeah, I think, I think I think

the main obstacle is people being familiar with how the tidy verse is, is being familiar with tidy data. Yeah. And knowing how and why it's good practice to have tidy data. And see the thing, the thing that I think is, is a big, it might be an issue is sort of wait, at least for like making plate plans. At least for for novice users, it is easier to make it as a CSV. Yeah. So there's no real cost benefit to learn in Italy least at least, you know, for a novice user using it occasionally, like the downstream analysis stuff, you can see quite easily the cost benefit there. Like like, you know, that's why I wrote this script, because it's so much easier than going through 50 different plates. That is all I've got to do is read images, CSVs, join them all, and then put them through that function. Yeah. And what would have been two hours work is crunched down into minutes. Yeah. That side of it is great. And you know, with tidyqpcr, that's great. It's just but the actual, the actual, making the plate plans that you need.

is a little bit more complex.

Yeah.

Do you see what I mean?

No, I exactly. It's, it's a thing that regularly comes up is trying to get people to realise. So the reason why we're kind of doing it this way is because we know that approaching designing the plate in this way, it takes time, because it is you're in essence making it into a logic puzzle, which you need to solve every time. And, you know, it isn't going to be what everybody wants to do. They just want even if it's monotonous and repetitive, they just want it done. Yeah, and that's why doing it in a CSV file, or literally typing things out in the Excel file, whichever way I know that people are gonna be drawn to that. Yeah, it is trying to show people that the, the cost benefit of learning this particular way of designing plates, as an A, not only for in itself, as a good way of solving issues with qPCR. And making, making your analysis reliable and repeatable. It's also a stepping stone into the larger world of tidy data, the tidy verse, and how, you know,

getting this skill and entering this gate yet means you actually gain the tools of a general data scientist actually get skills that can be applied to other jobs. It's quite a traverse versatile skill. Yeah. So yeah, it's hard. It's our number one task is to convince people this is difficult. Yeah. But once you get ahead of your head around, it is actually really beneficial. Yeah, we're still trying to do that.

Yeah, I think it's it's knowing your audience as well. The thing is, for the for the person who runs two or three qPCR plates a year.

Yeah. For them,

it probably

by the time they come to redo it again, they've forgotten everything. But for people like me who you know, run I think I carry like a plate every 10 days pretty much since I started my PhD.

It's one of those things that yeah, it is we is worth sitting down and learning

Yeah, it's

Yeah, I mean, it's it's definitely something that I wish I had explored more earlier on. And he's only locked down gave me the time really to sit down and focus on it uninterrupted because that's that's a lot of the problems you find with bench sciences. You can sit down for an hour, then you got to get up and you got to do something for two hours and just actually dedicating that time unnecessarily, like convincing your pie that you sit in there working through tutorials or working through vignettes. Yeah. Is a valuable use of your time. Yeah, it's against the delayed gratification

thing that

you may you may not be helping you get results immediately. But what you streamline your pipelines further down down the road? Yeah. It's a no brainer, really.

So talking about vignettes

Yes.

I mean, starting off with looking at the general plate reader vignette,

yes. The plate setup plate setup and yet yeah.

What could you a bring it up? And be?

I've got it on my my laptop Yeah.

I have you been through yourself? If you read through it, and

yeah, yeah. Play through with it.

Do you think it's do you have any particular opinions of it? Do you think it's too heavy? Do you think it's not selling? The tidy verse or the general tidy data very well.

Um, I think it's good. I think this is I think the plate plan one is good. And I like how it bill you know, you like how it builds from a 12 to a 48. And to a full 96.

D? Yeah, what was there some?

Yep, I was just playing about with the it might be might be I know, I know. You've got the rep, you know, help. thing but for when I when I was setting up a plate what I would be doing is rather than times it would be each Yeah. You know, each three never minus RT would just be one and then that's a so I've got Yeah, plus RT three but that's that's fine. If you know that the help she is there. I really I really liked the out the this output. Yeah, the display is really nice. And actually being able to see it like that is really nice.

Yeah, I mean,

maybe maybe you could make a bit more of because essentially here when you're doing you create blank plate in a real world application, no one's I don't think anyone is anyone going to be doing create blank plate not just using create blank plate 96 or 384? Or doing whatever? Because that essentially, that is then redundant, isn't it? You're well row you're well column.

It is to a certain extent, but when if people Yeah, they're using a three or four well plate, but maybe for whatever reason, they're only using the first so many columns or so many rows. It's yeah, it is ultimately redundant. In some matches. You can do it without dysfunction. You can just ignore some of the rows or whatever. But yeah, it kind of depends. Yeah, it depends. And especially for this when we're trying to introduce creating a blank plate plate well actually we start off with only like a 1212 wells and then building and that sort of thing. Do you

think you could just have a go? Designing the 20 The 96 Well, plate now just so I can Yeah, the how easy it is to pick up what you need to pick up

when you want the so basically these sort of two factor.

Yeah, it could be exactly the same. I just want to see how easy it is to pick up the information you need from the vignette. Okay, right to make the 96

Yeah, okay, so I would need to do. Do D Yep. So need primers.

Now you're happy with it being called targets instead of primers.

I see why you've done it as targets, because not everyone is just using, you know, one prime or if you didn't tack man or anything like that. For me, I do think about it as primers. Yeah. But I can see why. It's, it's been done that way. And I think I think targets makes the most sense in terms of

Yeah, how you would

have yeah, there's a naming convention makes sense? I think. So I'm not sure I don't know if I've missed this. So like the the difference between like levels and values? Yeah, like setting condition levels, and then using those to set your values. Yeah. Is that particularly is explained?

Um,

yeah, it's not exactly explained. I suppose it's more of tray trying to teach people how we want them to think which is okay, start off with the deck that you have. Yeah. And think about how you're going to map it.

Yeah. How you're going to say like,

What targets Do you have? Or what products do you have? What? What conditions do you have? What replicates you doing? Write that all down? Then think about? How can I do it in a manageable way that's just reproducible. So you can make your life super easy yet?

Yeah. Cool. All right. So I'm gonna so I am going to set it up. So I'm just gonna change it a little, a little bit.

Just because I want to do it my you know, plus, plus, plus minus, that's just how I so I want our two times three

don't regret that. Anyway. Yep. Okay. And then I want condition levels right, so then I will

So, I can essentially build this plate without having to go in through if I set my Target ID and just and just do my reps there rather than calling new objects is that

work as well? Wouldn't it? Yeah, that would be perfectly fine. We're just trying to explicitly state Yeah, yeah, natural thing.

If I were to go I know I need my roll keys. Yeah, that's it so

one thing I did notice is when I when I moved on to the next vignettes, the row keys were set as data dot frames, not tables.

Really, that's interesting.

I think I think if you go to the I'll just quick I'll go to this. Just want to mention it and think Is it the multi factor?

And I was table there. Is it multifactorial or was it the calibration? Yeah, data frame. Huh.

I know. I know. I saw your push really pushing tables? Yeah. Interesting. Yeah, it's data dot frame.

I don't know if there's any more data dot frame.

No, just just there, but I did know that was a little bit of confusion because

yeah, definitely. Check that out. I don't know why. Yeah Because Edward wrote that one so I don't know why

why yeah

okay so I need Hello Target ID levels is that too then a lung condition condition level SERPs control Drug got some bloody levels let me think about this update differently so

each right

yes and

prototype is

is

time three

up sound yeah perfect and then just a case of building that so I need to Do Yeah.

She I find it much easier to think about the repetition. When I'm building the cotton row key and the column key, right? I don't know, I don't know why I just think if I, if I just have, you know what I have, rather than in making another object and then putting that object in the cold cocking the row key, at least me I find it easier just to take those objects and then do use rep within that so it's all in one place. I don't know. Rather, because that's the thing, because if I'm building these as abstract, separate objects, at least when I'm building the row key, I'm thinking about okay, how many times do I need to repeat the Target ID? How many times do I need to be the condition? At the same time, if you see what I mean, rather than happy to work out separately, yeah, there's disparate objects and upbringing. It's a little thing, but it's just how I

possibly help because I think because a little bit confused, especially if they don't know what Tibbles are, yeah, they don't act to be realised that you can change all of these names, and you can change all of these. Like the inputs, like that's entirely arbitrary. And then for us, separating them into abstract objects. Yeah. It doesn't make it easy to excel to acknowledge the fact that everything can be changed and manipulated how you wish. Yeah, I think that's that could be something quite interesting.

Yeah, at least at least from me, say out the row key and the column keys. Just using what I had, rather than because then I think the names get a little bit confused and in the vignette, not necessarily confusing, but you know, you first make a little bit

so like when you make condition levels, and then may condition values, yeah, I'd rather just do rec on condition levels. However many times I need when I'm making, it just seems like a an extra step that is actually probably more efficient, because you're doing BioRad equals anyway. Yeah, it's actually less verbose to do it within the column key and the row key is it. Do you agree? Yeah, no, I

get what you mean. No, I do I do get what you mean. It's pretty good clue.

Yeah, this works brilliantly. Yeah. As you say, you're doing a repetitive repeating pattern along a row or column, but some, but I guess if you it just takes a bit more. bit more thought.

Yeah, it's fine.

Right. Okay. Thank you very much for that. I think the final sort of vignette, I don't know if you have done the called What's it called now? The Delta CQ 96? Well, vignette, that's the newest vignette.

I haven't done that one actually.

So this basically gives you a dataset and allows you to trial. Because your data set, it gives you how the plate plan and just allows you to use the tidyqpcr functions. So this is the thing we need to make sure it's checked. It makes sense. And people can run all the functions properly with a given data set. realise there's an error already, which I was meant to fix, but I didn't

know what this is for.

Give me one second. Feel free to start reading the vignette. I think how it says to access the data isn't going to work. I'll just have to send you something else. Yeah, if you Yeah, that's cool. And then and then it should work.

typo there we we were sorry, on the organised sample and target date or on the plate. The third paragraph down second sentence we use W we

it's very Scottish. So the only thing about this vignette is how often are people likely to get data in this format? They haven't analysed them. You see what I mean? Because this is essentially

someone else's pre processed raw it's not raw data is is the plate that someone has made a plate plan, joined it to the CPUs and got rid of all the columns, right?

Yeah, I don't know. Because the more the more likely pipeline is, make your own plate plan.

Join it to raw data kicked out of the the sequencer right at the start of the qPCR machine? Yeah.

We should go one step back.

I think considering how long it could sit considering there's the vignette on plate plan preparation. I think it would be good practice here. If you said right, these are the primer pairs. These are the conditions. Yeah. Here's how you would Yeah, I know this. This plate plan isn't quite.

So yeah, it's a bit of an

awkward. So it might be a bit of a pain with this.

He he is just I don't know how, how useful this this is here? Because I feel like it's very unlikely that you would ever find yourself in that situation. Yeah. I mean, I've never found myself in that situation. It's always I'm I'm the person who's who's essentially analysing the qPCR. Yeah. So I would have the raw data from the sequencer and my play plan. This the two inputs essentially that I would want to use. I don't think I've ever come across a scenario where I've had a plague plan. Plus, to see queues all in one file, I would then need to map back to a visual plan. See what I mean? Yep. Yeah, I feel I feel like it might be more useful to have a vignette that at least it might be my leap from my experience, my experience might not be the same as other people's maybe it's good to include this if there are multiple people working on and analysing within a lab, you know, if multiple people collaborating on the same experiments Yeah, but just at least in my experience, this is not a situation that I would find myself in and I don't know if it would be more useful to reinforce what you've you've you know, put forward in the in the pipe construction and the the plate plant building vignette, just briefly here. But you know, if you say Right, these you've got the D strings, these IDs, these target IDs, you know, 96 well plate. Here's how you would in practice, generate a plate plan for real experiment? Yeah. That's the the plate plan building vignette is good, but it's abstract. Sure, at least in this scenario, it does relate to real data. And the only thing is that it might be more worthwhile getting something that is one primer pair

per row. Yeah.

We shouldn't be too hard. I mean, it's Yeah. It's just a bit of an unusual plate layout for a standard.

Yeah, I realised. So that the first time I saw this was last week. It doesn't look quite how it is.

Yeah, that's the thing. And I think that's the thing that that is probably the biggest

obstacle

for people when they're doing and learning things like this, is things changing too much when you walk in incremental steps without necessarily being explained? Yeah. Because they've learned all of this stuff. And if you could, if you could then apply it to a real data set, but it's in the same format. This then jumps on too far. Yeah. And then people go, well, this doesn't look the same. Give up? I don't know, I'll feel a bit disheartened by the fact that they can't get to work. But if this was a one primer pair per row, you know, three tech reps? I know it's, it's not. But if you can change the data, and yeah, it just might be a bit of a shallower learning curve. And it might be it might follow on a bit more in a linear fashion. But that is just my experience. I don't know if there are scenarios where people would have this. Yeah. Yeah, because I know the other one that dealt us the other vignette, the I had to start to have a look at the multi factor but I got a bit more I got the data and they went a bit wrong. But if you actually had the only date you give is the 96. Well, TX T file as it comes off the Lightcycler. Yeah, build your own plate plan. Put them together and get rid of any columns not interested in I think that and then go on to do the delta delta delta. See. I think that might be a bit more of a typical workflow.

Yeah.

That's me. Yes. Okay, that's good. I'm happy with that. I think. We will do that. I have just seen you on a chat the right piece of code to access the data. Okay, cool.

Let me copy from the chat.

I don't know if that's a safety feature. I don't know.

Okay, I'll just I'll just

yeah, obviously, the file name itself you can just copy and paste because it's the same file name, it's just putting it in the system file wrapper.

Okay, so if I copy this exactly, it will

I mean, if you if you start off by just copying what it is in the vignette, and then just altering it so that it's looks right at my code. Okay, external data read underscore tsp.

Re tsp. So Yours Yours should work?

Yeah, so the way Edwards done it, is it assumes that you've downloaded the Yeah, hub repository. Yeah, the way this is doing is it's accessing the downloaded package that is downloaded into your thing

that knows that he's better yeah, because that's the thing again, when you're first using vignettes and that's not really explained, yeah. where the data is, people just give up.

But yeah, if you if you can Is your access directly from the package rather than having people go around and

yes

of it cool.

Yeah. So that's that's in

Okay, cool. So this is basically

okay

you can probably just copy and paste from the vignette. You don't need to.

Okay. I mean, as long as that's within the spirit of the exercise,

yeah, it's it is it's more for the most part just checking that the actually works actually works and everybody's happy.

Alright, so that's that one. So, tenbury Okay, so it splits so keep sampling fo Okay, yeah. blitz it on space.

And then makes a sample ID by joining

strain and OD with an underscore right? Yeah, yep. And then all the same all the sample IDs

gather Yeah. Cool. Yep.

So there's no minus R t controlled in this in this

experiment, and apparently not. Okay.

It kind of goes against everything.

Yeah, I think we're gonna have to find some new data. This was just something somebody in Sanders lab just had lying about? And so we're like, Okay, well, we'll have that. But we probably need to find something new.

Yeah, I think I think if in the plate vignette, you're you're linking out to the guidelines on good, good experimental design. It probably would be best practice to have Yeah. A well designed it, you know, the archetypal perfect experiment in terms of your controls and everything. I know it's only a little thing, but

now it is important like this, this play is terrible play. And I think it's a bad last week also commented on better about it. Yeah.

I think it's not great example, like, it's fine. If this is what you need to do, to get, you know, you need to run your samples. That's fine. But I think especially in a vignette for something that people aren't particularly familiar with, you don't want to get too complex. Because yeah, this is going to be a barrier to people getting a nice plot at the end we just had you know, 123123123 and then you three minuses. Although you might have to then build something into I don't know if if that was done deliberately Is there a deep you know, way where you prop the minuses versus the pluses and then get rid of them? Just to see that they're higher in terms Yeah.

Okay, cool. So yeah, that that works. And then you plot the data.

So Target ID versus CQ? facet Yep. I mean, it doesn't. Yeah, the plot example you've given us been made a lot prettier.

then then what you actually get? So I don't know if people will be put off by the fact that it looks the same. But

yeah.

Yeah, I would just include how it looks.

Yeah, I think it's because I know why it's done that. Yes. Because there's at the very start of the vignette, there's a hidden block that defines how all plots should be outputted. It's not in there, tick tick. It just isn't. If you open the vignette, the code of the vignette, you'll see that there's actually a hidden block. At the very start, that defines what it should look like.

Right, I say, I think the same set.

Exactly. So maybe that's something we should get rid of.

Yeah. I think it just needs to be consistent. Either. Either include it and say, you know, just comment around it. So this will just give you pretty flops. Yeah, just made the plots a bit a bit more aesthetically pleasing. Yeah. You don't really you don't need to understand at least at the first instance, what this is doing. Yeah, I mean, you comment around it a bit. Yeah. As long as I think as long as the output shows the same as in the vignette. Yeah, I think yeah, you shouldn't be different Yeah. Oh, wow. That comes up really quickly. five cycles is very very, very, very, very very, very low.

Wow, okay.

I just never seen anything come up that quick. I mean, I've never looked at 5x but it makes sense. You know, it's an extreme but it makes sense Okay.

Showing a Target ID lipstick

that's not helping. Oh, not helping.

That's why I like having my Mac keyboard so I can just hold if for some reason that the values are slightly different. I know again, it's just it's just minor things. If you look at your CQ column versus actually what the output is

praying, it's just the wells are in a different order.

Yeah. Just yeah, there's just a different order. But again, I'm just saying for consistency sake. If you're expecting to see exactly that, and you don't you know, I mean, I'm just I'm just, you know, the novice user

don't actually know why it's in that order. And then

seeing he's quite

lucky if you look at

that from there it gets it's really different.

That 678 My miners mind

start at one and that one starts a one. I don't have a clue. They want to do three B four or five six minus A 123 F four, five So Mr COVID

Yeah I don't know where it's coming from

it's just it's just different

which is I mean yeah it's fine if it's that way round it just needs to be the same

yeah

so that is it just as you're talking yeah because this is talking about a sanity check. Yeah it's not

yeah that's fine that's cool and that's fine

yeah, the only problem is obviously you've set the level you know you specified while while type is that is that in the hidden bit of code as well you probably it because that's alphabetically first. You'll get a ATR about wildlife theory one wild type first you just have to specify it on the level

Yeah, I don't know why it's I haven't actually seen exactly what's in the code but yeah, is it because the colours will be different Yeah.

Different

so I've just got like a I'm just saying all about detail the I just think it's important it would have been yet like this to have consistency between what you should be seeing and what you asked yeah think that helps reinforce a bit more okay group by summarise to calculate the mean values Yeah Nice. Cool

yeah, again, it's just a neurodiverse it says upside down isn't it

I don't care just wild wild type is second but that's again it's the same values it just

looks different

Yeah. So is this standardly how you would plot your key PCRs?

Um,

it depends. In terms of like the first time I get some qPCR data Yeah, we'll probably do a full run through of just checking. Well, obviously there would be RT minus RT as well. But just running what? What happens when you normalise it pre and post normalisation?

Yeah, I just I just mean as a GM point rather than as a GM bar.

Oh, yeah. Edward hates bar charts.

I really okay. No, that's fair enough. And so yeah, we would never ever,

ever do bar charts, although we've got a lot of qPCR data right now. And I'm like, it would actually be quite nice if it was a bar chart because there's just otherwise

Yeah, because that is one of the things that initially put me off a little bit. being candid about about tidy qPCR Is everyone reports it as bar charts? Yeah, it will be, I don't know. Obviously very easy to do it GT like it's fine once it's more than the, you know, the doing the normalisation and stuff that the package is is powerful for then you can just run it and stick it into your own plotting,

you know, use your own preference. But

yeah, I just feel like it's a lot more common.

When you're looking at RT to plot

your means your median zero, but and your standard deviations as bar charts. Yeah. Because here, that's the thing here. See, I see the point of Jiang point if you're plotting every point. Yeah. I don't really see what the advantage of Gam point is. And it's not to me immediately as as clear if you're just plotting one point rather than having

a bar chart.

I mean, I the answer that question is, when you're applying points, it's very obvious whether or not

they sell.

Well, yeah, it's it's it's very obvious whether or not you're making your deductions on one point, or whether or not you're actually making a do it on multiple points. Yeah. And I think that's kind of what Edward

likes. Yeah. But then then this is important. Everything is this is just putting your meetings.

Yes, is plotting your medians, but is plotting. Yeah, I agree that, I think it probably would look better as a bar chart in that. Yeah. In a sense.

Yeah. And that's the thing. I feel like if you were to see in the literature in this, whenever you're you're plotting, you know, mean, or median of a normalised qPCR. Value, I'd say 90% of time you see it as a bar chart with error bars, which gives you that an indication of your your variance, right.

It does, but, again, with if we were to plot it, we wouldn't plot just the median point on its own. We would plot all the biological replicates. Yes. To be sick. Yeah. all on the same plot. Yeah. And

we've been saying here, you're plotting just the median point in its own right. Yeah. It was distaste for Bob bar charts.

Yeah. He just continues on forever and ever. Amen. To that the bar?

Yeah. Fair enough. Okay. No, that's, that's fine. Yes, does that

work? Yeah, that works.

What's the wisdom on having time point? They're not time points. They're od points, aren't they? As discrete variables. If it was a continuous or continuous axis, but then as you join in and buy a line just the joining discrete variables by a line.

Does that make sense? Right.

I mean, if you're looking like a pattern of change, general pattern, but would it not be better? If it was I that's the thing. It depends whether OD is? I don't think OD is linear. So maybe it wouldn't.

OD is definitely not linear. Yeah, it's kind of It depends what you're trying to imply. Really? Yeah. I think it is ultimately a continuous variable in and of itself odd. But you are explicitly measuring discrete points. You're not actually measuring continuous time on.

Yeah, I get Yeah, I guess I guess if you're looking for a general trend in this case that your client is

In immune generally it generally goes down doesn't spike at as much as 36

Yeah that's fine

right okay upside down

right So is this a note, this note here is that is that for the vignette or is that for you?

That was yeah, that was a comment from Yeah, it's it's been fixed now I just haven't I don't know if I've updated it to the GitHub repository yet but yeah, yes that is a fix. So it's just a minus Yeah. Cool.

Yeah

yeah Edward insists does anyone write in American English? Yeah. Okay, that's fine to me sense.

He likes his ads.

Yeah, how many I think I think it's good. It works. It works. It just is a little bit. The examples in the vignette are slightly different to the outputs. Yeah. Yeah. But apart from that, I think it's yes, it's good. Perfect. Yeah, maybe a bit more of a square. Yeah. dataset might might be more appropriate.

Yeah. It'd be far easier for people who are very, very, very new to things.

Yeah. Which I think is the sort of people that will be

that's what we're aiming at. Exactly. Yeah. Yeah.

Do you want to bring those people in? I think that is that is quite a an important one thing.

Right, I think we've covered everything. Okay. I think I have anything more to ask him. You. You've been very diligent, answered all of my questions and had plenty of comments which we'll have If we improve the vignettes themselves I'm going to stop recording now

Interview 3

Thank you so much. There we go. We'll just get the questions now. So the first question is just a general talk about your past qPCR experiments. So I'm looking for things like, was it more RT qPCR? Or was it with DNA? And you know, when was the last time that you actually did an experiment? That sort of thing?

Okay, so I first started using but it's all been RT qPCR. In about 2000. It hasn't been nine ish. I suppose it was possibly earlier. That doesn't relate? Yeah. That'd be back then.

Yeah, and say got to the stage where you're doing maybe 1000 or so reactions? In a single very busy day. Most weeks went like that. But yeah, I mean, actually, no more than that. So if you put about 400 plates, and I might do 10 plates. So yeah, it's several 1000. Saying, it wasn't pleasant.

I can only imagine. And so with with the plates itself, were you using like probe sets or just

Primus? Oh, do update was just a just a primer. So site green.

Perfect. Yep.

I did play around with with doing the presets. Unfortunately, the species we were looking at was such different abundances. I wasn't sure that would work. And we had a system working. So we just thought, Well, why change it?

Yeah. That makes perfect sense. So how, when was the last time you did an experiment?

Well, that's actually some time ago now. So I guess it's about two years.

Two years ago now? Yeah. Gosh, yeah.

So from several 1000 to zero. Yeah, that's it's quite a shock.

I can I can imagine. And kind of the leading question is, how if you say, we're asked to redo one of those experiments, or well either redo that spring from scratch, or re analyse the data from one of those experiments? Could you could you do that? Could you do that?

Yes. In fact, while doing the experiment, I suspect the first time I did it, I'd make all sorts of mistakes, things, but I think by the time I did it, I'd be be okay. As for reanalyzing, have actually been doing some re analysing everything fairly recently. So, yeah, I wouldn't be able to do that. takes me longer now than it was when I was doing all the time. But yeah, I. Yeah, it's not that long ago. I've done it.

So just another broad sort of question in terms of more looking into how you see qPCR data presented in like papers, that sort of thing. Have you? I mean, number one, how are they normally presented? How do you normally see qPCR results? Do you normally actually see, just like a summary graph of some sort of averages? Or do you see actually, the quality control check checks have a background in appendices or anything like that? Always it normally. very superficial.

It's normally pretty superficial. I have to say. Yeah, I mean, you'd hope that the qPCR as were corresponding to the the minimum qPCR requirements, I forgot the acronym there.

Mike, whatever. Yeah, that's the one.

So So mine, mine were but yeah, it doesn't seem to be like most of them are. Most of what people caught Well, this is certainly the case a few years ago that that qPCR was actually just connotation from ethidium bromide still stay in jail, right. Yeah, but most of the time No, it's it's just a summary graph of how things To change,

yeah. Okay.

To be honest, that's what mostly mine were

to be Yeah, we're thinking kind of one of the things that are kind of changing more and more is the requirement to have the actual raw data and like different degrees of analysis readily available. Yeah, work work its way up. Yeah. And trying to have our code make it really easy to show all the steps. Only one go. Yeah,

that's how I that's how I taught. The people taught directly do it. And I built up to protect us beautiful, but it was a lot of work. And I think most of the people I taught can go

sorry, the internet's just slow down. Can you can you hear me?

I can hear you now. Yeah. Oh, yeah.

Just it's a slow down for a second. So you were talking about the people that you you sort of taught how to do qPCR? Yeah. And then I didn't catch the last bit of what he was saying.

I'll go back, I certainly taught them that you have a table with with your data. And then that is sacrosanct, you then have a sort of working data set, where you can say, okay, that's not why I'll exclude that. And then you go on, and you've got your first bit of normalisation and then your second bit, and then your third bit, and then you start doing double normalizations. And everything's there in one place. So that's what I taught people to use, but I don't think many people actually stuck with that.

Okay. And is that just purely because of like the workload and trying to juggle?

Yeah, I mean, that was that was the workload, I did all of my stuff in Excel, and I had I had macros to help you with this. I mean, I consider myself an Excel, Power User. Yeah, so I knew all the sort of tricks and how to make that actually, really quite easy to set up. But yeah, it wasn't for the faint hearted.

That's fine. So now I'm going to talk a little bit more about how you actually designed your qPCR experiments. Yes. So starting off with, like, some of the fundamentals, you know, when you were designing it, how many technical replicates? Were you thinking per plate? How many biological replicates? And if you typically did, like whole experiment replicates at all? Or if that's not typically a thing that gets them?

What do you mean by whole experiment replicates as opposed to a biological replicates?

So, in terms of biological replicates, it means you know, you will have everything before the plate will be from different colonies. Yep. But they would all be then placed on the same plate. Yeah. And then the experiments where it would be pretty much exactly the same thing, but on a different plate. Yeah.

No, all it was all. It was all complete replicates. So I would quite often do an experiment one day and then with an entirely different set of, of a biological samples, I would do it again in a different day or even a different week. So no, I never combined biological replicates into a single experiment. They are always separate experiments.

Okay, so how did you then compare between plates?

normalisation? Oh, sorry, I think I've missed out the technical replicates. Oh, yeah. So it was always always three technical replicates. That was that was just the way I set things up and therefore it was it was relatively easy to do it that way. Okay. Although I have to say my technical replicates were just PCR technical replicates the reverse transcription reaction was just a single I was felt I should have done that. But that was just so much extra work. It was just just too much extra work. So what was your question?

Oh, I was just comparing if you had two different Yes.

i That's What normalisation was for So, yeah, I had several different normalizations I standardly applied to to all of my experiments. Okay. And yeah, and that I didn't really have a problem between experiment replication unless I've done something wrong. But most of the time Yeah, the, the, they were just normalised. And if they deviated by the cut off it did. I think it was one standard deviation was the absolute cutoff. But most of them were well, within that. I'd have to look back. Yeah, I mean, generally, I only had a problem with with replicates if I've done something wrong. Like qPCR, my RT reaction?

So for the actual plates themselves, how many wells

standardly did three or four people.

Okay. And then when it came to actually designing what's going in which well, what was the process for that to do? Like, try and make it in like an Excel spreadsheet? Or was it sort of hand drawn or something like that?

It was an Excel spreadsheet, I had a template in an Excel spreadsheet that I typed in. And that automatically fed to give me a list of the templates, and the PCR reaction and the time point, then whatever other conditions I was changing, not automatically produce a list that I was already in the order that the qPCR machine gave out at the end. So yeah, I could just take this in and, and it will produce it, I could do things like copy and paste, and then search and replace to get the individual sample differences.

Okay. So typically, was it just, how many different probes did you use in one? One on one? In one plate? Did you use?

That depends on how many samples I've got. Okay. So I quite often did six, six sample experiments were just generally convenient. Because you know, it's an even number, it fits into a centrifuge. If you if you do six, then that easily makes up into 12. Which is sort of like kind of like a row of a 96. Well plates, you can then switch to a 96 well plate. But frequently, I have six samples. Six samples, I would. Yeah. If I just had six samples, I could often have about five different PCR reactions on a single plate. That must have been six actually. This is trying to think back so I would I would lay them out. In some my samples would go across the rows, and I'd have different PCR production sets down now. Yeah, done in. Now, my samples would be in columns generally. That's right. And my, my PCR reaction would be in rows. Okay, yeah. So it really depends how many samples of how many PCR probes I can get in. Okay,

that's, that's, that's perfect. And how did you actually like load your clip plates? Was it just manual?

It was it was using a multi channel pipette generally I did the RT reaction in 96 well plate so I then moved to using the multi channel prepared from the 96 well plate to the three four well plate for the VCR. And I could do that. So that I'm sure you're aware that the 96 Well, plate has the same spacing. Yeah, as three or four will take your system got four. So I would set it up in a group of four in a square group of four. Okay, so that 196 well plate well went to a group of four of the qPCR plate Okay, but the top left hand one was always my it minus control. So the ones around it were the the actual samples. Okay. And I just found that easier to go from a 96 well plate to a three or four website using that system.

Yeah. Okay, that makes that doesn't make sense. I get that Yeah, I think that's slightly different from how we typically Yeah. How we typically organise it in terms of the tiny qPCR software but I think it should be okay. So, what was the actual qPCR instrument that you typically used?

It was a rush LC 470

Okay. Because we just keep a check that whatever they output are suffering in life and the final question in this section is what did you do for quality control? How did you know whether or not it was a good reaction or not?

So okay, again, this was done in the Excel spreadsheet, so an example can you share a screen in zoom and if so, does that get recorded?

Um, yes, I think it does get recorded but if that's a problem I can stop recording

No no no that's that's not a problem right sounds exciting

oh yeah sorry

Of course I'm inevitably going to choose one at random that isn't actually very

good okay

looks like it works really well I'll get rid of the filtering that we can see things it kind of works to show share

Yeah. If you go on the zoom thing it has like a green Share button at the bottom.

So it does it's always because it was green hope disabled participants shins screensharing

Oh, Jesus. Sorry about that. Can we one second see if I can change that. So I'm gonna have to go on the interwebs to change that okay.

See, well, what we're going to do is something a bit more risky. We're going to send you I'm going to make you the host,

right.

Yeah. Let's see if this works. So you should be the host now. I and you should be able to share your screen. I can

share my screen okay, can you see a yes. Rather horrendous? That is scary. Okay. So first column is my replicates, that's generally the date. Yeah. So that was, I can't remember a time I suspect it was the fourth of No, must have been the fifth of April. Okay. So then that's the Well, no, this section was generated from a template where I had the grid layout. So that's just the sample. So it's a total RNA. That's my our t minus, that's my three replicates, taking the replicates oops, then that's the gene I'm looking for. And then that's the particular sub species of that genome. We can ignore that one. That's some experiments to try that I wouldn't go any further from. So these are just sort of things to help me do the normalisation. So this is using a sort of hash, I use pivot tables a lot to do this. So I had to have a unique, unique name for everything that was grouped together to do a normalisation strategy.

Yeah, we do exactly the same thing with Yeah.

That's the individual CPE value from the machine. And that's the the TM. So that is sacrosanct. So then take these and have a working thing that I can actually change. Sure. So everything goes from this, but everything points back to to this value here. Sure. It also has various things like, if I've got nothing in there, then it will paint that cell red. Actually, no, that's what it does. It makes it 41 Because it now it paints it red if it's not an R t minus one. Now, t minus it says, Okay, that's no problem. You have gotten a signal. That's great. Yeah, that's fine. So the first check I have is to check against the the RT minus value. Okay. So if it's anything less than three, see, cycles difference, then it flags it up as as red. Okay. So some are very good. I mean, I would be happier if it was more than five. Yeah. But that's still a significant factor above the PRT minus, yep. I found that as the more I did these more my RT values crept up

on average of my technical replicates, and I then do a standard deviation, short of my technical replicates. And again, it flags it up if it's, if it's okay, yeah, there's a background that fails. So yeah, if like the top was red, says that that's a problem. In fact, most of these get all of my standard deviations look to have been fine. Here. This experiment? Yeah, there are things I just can't do anything. Yeah. So then I start doing the normalizations. So they're all my normalised values, and they're all there. I have a standard step, but I could quite easily add them. And then I start to other quality controls. So anything red is a value that suspicious. And I need to look at and I need to reject. So I've quite clearly got a problem with this sample. Normally, I have screen so yeah, that one looks like it's so either chances are I would exclude this but quite often, if the replicates came out with reasonably tight error bars. You know, I would try and leave in the data even if I'm suspicious about it. But it's just that one that so that's not great in this replica that other replicates are giving me fine. That is not that that's that's my average. So that doesn't have a quality check on it. But yeah, most of it was based on I actually think that's percent CV. Yeah, that's percent CV. So I think 15% That was my limit on my, my percent CV. Okay, yeah. So that was things within within the replica. So when I started to look at things together, okay. So this is this is a pivot table based on the data before. Actually, that's that's the pivot table where I could change things. This was just a copy of the data. So I could actually draw graphs, or else everything changed when I changed the pivot table. So I want that's kind of my fixed pivot table. So here, I've got the the raw Ct values. Here, I've got standard error. And that's based on the standard deviation. You're going to have to trust me on that. Because like I say, it's a copy. And that's the number of samples. Okay, so from that I could work out my standard error. Yep. And when I drew the graph, the error bars here were based on the standard error. Okay. And it was even sometimes, such that I decided that that looked like a fiddle things, because the error bars are too tight. So some times just to actually make them visible. I choose this the standard deviation. Yep. If if I had a really good set of replicas together so yeah, and that's yeah, so normally, I presented the things as a as a log scale. Choose chose to I wonder I chose that's not a log scale. That's why. Yeah, that would do. Yeah, and so I have all of these graphs here. And I would I would just make another sheet and copy the data in when I wanted to do it a different analysis. Obviously, Oh, yeah. I that was experiment that didn't work. So yeah, that's that again, is more like the data expecting. Yeah, that's experiment that didn't work again. It was it was looking all the way along the axon to see if there was a variation of sitting along the exon parking and that wasn't it significant viewing. So yes, this this was, again, I had a standard a standard set of colouring my graphs and that was I could access that by Microsoft, just click a button on the on Excel guarantee it would come up and say and give me everything that bright colours for me. So I very strongly suspect that had I been had I learned how to do this in our before I learned how to do Excel. I would almost certainly have have got this, this using Excel using R rather than Excel. But yeah, just a sec. So. Okay, so familiarity.

Yeah. 100% it? I mean, the way you're explaining it seems almost exactly the way that kind of keeps your mental work. Yeah.

That's what I thought. That's how I tried to teach everybody but not everybody listened. With my system. That's, that's

perfect. That's really good. Thank you so much. Right, I think we can stop sharing the screen Do you think you could just make me host again, so if you click on participants

where is that?

Right next to the share button? Yes. If you hover over my name, it should have like options or some sort of work? Yeah. Perfect. Thanks so much.

So yeah, that's really great. I'm glad that we've we've gone through that is there anything particular that like, when you were doing the Is analysis? Was there something that was consistently like, frustrating or something that you just had to repeatedly do? And could have done with a different workaround or something?

Yes. The thing that I always struggled with, even when I was very close to the end of doing it and been doing it this way for for three or four years, was to get a double normalisation working. Even, I had to think through it from first principles every single time I did it, and even then I'd get it wrong. And I'd have to think hard enough, don't run the game. What I mean, is this. Just because it's it is a difficult thing to get your head around what is unique. What defines a set when you're thinking about it on two criteria? And sometimes I have to admit, I did cheat and wasn't cheating. It was it was it was taking the normalised. Yes, I took the normalised value for one and used it to normalise to the other rather than doing it as a Delta Delta Ct, which is what I should have done. It works out to be exactly the same. But it was it just helped me get my head around it. And if I could have had a system that that helped me get that sorted much more, obviously, then that would have helped.

That's interesting. So it's not something that's in tiny qPCR right now, but it is something we're thinking of. Right. So yeah, that's, that's a good show, we'll definitely focus on getting that sorted.

And it was something like double elimination was something I standardly did to everything. Yep. But if I own I generally only presented that data if something had gone wrong, and I needed that extra normalisation to cover up something that was was a bit a bit screwy in the data. So yeah, generally, single normalisation is what I aim for. And if it worked, I present that. But sometimes one replica would be a bit odd. So I had to turn normalise, say against the exon for all of them. And then I'd have to normalise against the time which was actually shown with the data I wanted, I just had to get rid of the variation in the replicates by by doing one normalisation and then I could do the normalisation they actually wanted. But if I can get away without doing that, then then that was was great. Yeah. Perfect.

Okay, so I think we've kind of done the background stuff, for the most part. Just a quick recap of your sort of our programming. I remember you saying that you've done it. You've definitely been or done some courses, but never like, caught on never like,

it's never stuck. So I would do our and think, okay, yep, I understand the basics. And then I'd go straight from the basics into trying to hack, say something like Bioconductor. And it was okay, this is just too much of a leap.

And then I think, you know, I can do the next this next l it might take me longer and might not be as

it might take me longer every time I have to do it. But the time spent learning the code would not have saved me time in the long run. Yeah, I mean, maybe after 10 years, it would have done but you know, you're never thinking about 10 years time you're thinking.

Right, this experiment? Yeah. Yeah, I mean, Bioconductor is a bit of a mess as well, even. That's that's

so yeah, that's perfectly fine. Again, we'll just trial, the first stage of I don't think we'll get to the stage where we're actually analysing data. But the thing I want to test at the moment is that like, plate design and trying to do it in the way tidy qPCR tries to do it and whether or not that is understandable or whether or not that is quite hard. So if you have the question out and the GitHub link,

yep. Yeah. Among the GitHub link

Perfect so it has some instructions at the bottom of it for how to like getting started and installing tidy qPCR the yep

yes getting started but certainly our tools

to know if you've already got our tools installed or not

don't think I have I installed our yesterday so this probably wasn't helpful I should have I should have seen the attachment

link isn't actually leading to anywhere else which is fun

okay, that's not just

have to check one that's

right, so presumably I can instal it using the under that's so it's our tools 40

If you if the version of our that you've installed is version four Yeah, then it articles 14

It's taking back to this installing our cheap thing our tools presentation that isn't actually telling us what to do it was expecting to scroll slightly chrome projects up to this point but that's done.

Hopefully won't take too long. I haven't used Windows in a long time, so I can't remember trying to instal it with Windows. Yeah. I think the last time I tried to do it was with somebody else from the lab. It was it was like crazy because obviously things are just slightly different with Windows and it's like I can't remember at all how how things instal or how to find anything implements anymore. Yeah, yep, Linux or Yeah, so originally I went from Windows to Mac and then I've now gone from Macs to actual Linux now

Yep. Which is good. I quite like limits right now. I'm big fan. I just like that it seems to work how I expect it to work and slight I want back thing downloaded here. And then I didn't write the line in the terminal.

Yeah. Yeah, I mean, I learned dos originally so you do it. Sorry. Do I need cyclin installed? Oh, I

think so.

My wife's asking me if I've got my bedtime I do have my bed right. My wife is very very tech savvy. So she's perfect. That's the MCU. Standing

so do you do you know do you know Edward How well do you know Edward? Oh, My supervisor, yeah.

Yeah, I know, I do quite well. I send the backslap and he was flicking back slack too, so yeah. Quite well. So I've I've even so geven used to have lab retreats that we used to go up to so I went to all of those and Edward came to a couple of those too.

So yeah, any fun stories

frankly, non nice shirts

i i met Jean. And I did a bit of an interview with her for the schools podcast. And she's so lovely. Like, she's, she's done so many things. Yes, it was just absolutely fascinating talk. And some of the mishaps that's happened, like melting equipment and stuff. It was it was, it was a funny little dialogue. And I also managed to go to the symposium that was yes.

That's true. We've seen each other.

Yeah, I was only there for a short while there because I had to rush up to that to remember why I had to rush off but I remember had to work. But it was just fun to see. Like the group down in his Imperial where she started to build, like, yes, the research group, and the community that's built there. It was just it was so weird to see. Because you as a PhD student, you don't really know what happens next. You know what I mean? So it was so cool. It was so cool.

Right, that's tools. Perfect. So I guess now I instal? Right instal the dev tools package,

have you Sorry, there was just one more step on the page. I don't know if you you saw that on there on the art tools web page, which was just adding a path.

Yeah, interestingly, it didn't give me that option. So it's done. Do I have to add it? I see. No, I have to add it I thought that was part of the instal

now I don't know why it isn't part of the instal but apparently it's not

you should make sure the box is checked to have the installer edit your path to say instal?

Maybe just trial it see what happens.

There's a fine package dev tool. So let's try that. Let's see if that works. In fine package, there's no question Did I miss it?

Maybe if you just just try installing. Yeah, try installing something instal the example it has on webpages JSON light. So if you just instal dot packages JSON light on the our command line, see if that works, and if it works, and it works.

Something is happening. Okay, I've stuck that in and it's yeah, it's downloading stuff. Well, that's

good. That's a good sign.

Set right This is taking some time.

What exactly are you installing?

So that was dev tools that seems to have installed. Okay, cool. So the next message and that is Dev Tools instal git hub ie Wallace tidy PCR building next equals true.

So that's taking some time.

I haven't tried that yet. Okay, guys yep, that's doing that next. Oh, perfect. So what's the next stage? Then? Take a PCI standard package presumably this this stuff I have to do once Yeah.

As soon as it's installed, it's installed just weather and obviously there's an update then you have to

okay, it's asking me if I want to download more recent versions

I don't think so. You should be fine. All right now, it takes a little while just because the that build vignette section on computing it's ticking away the suspense

so how are you finding life working from home?

Yeah, it's it's interesting. Because I live where I live with for the flatmates, but one of them has gone back home to live with their parents well, like eight weeks ago, went back home to live with their parents. And so, you know, it's, it's fine, as long as everyone's getting along with each other. But then when somebody doesn't get along with each other, there's like, a huge amount of tension, and then there's nowhere for it to go. stuck inside the same, same flat. But overall, it's been fine. We've just had a few conversations about, you know, maybe you shouldn't do this, could we get over this, this sort of thing, but it's mostly Yes. But it's actually quite nice having other people just to just to chat with on a regular basis. So that's good. I'm glad that because I know a few of my friends who are either in the student halls or are in like a flat with only one other person and that person has gone home then they're just pretty isolated. And that's,

that's yes. Very cool. Yeah. Sorry, I've got an error. No. Error failed to instal tidy qPCR from GitHub system combined Ark command, xe failed, exit status, one, exit, etc, etc.

Was it does it say whether or not it was the vignettes failing or something?

Error processes and vignettes? Yeah, play setup vignette, r&d failed with diagnostic there is no such package called tidy verse.

God this is going to be a pain so yeah, you need to instal tidy verse double check that there isn't any other packages you need to instal? Yeah, so if you instal tidy verse, and there's probably another package you're going to want to instal as well,

which helps All right, same to you let's tidy verse starting

Yeah, just sent you hope. Yeah, I don't. I don't actually know why it's called kappler at all, but it's just a nice way of drawing multi plot graphs. Yes. Okay, that's those two instals. So I go back to dev tools instal again. Yep.

All right. Hopefully I won't have to download them again next time yeah, I'm home with wife and cat. My children are away. So my daughter's stuck in Glasgow for the flatmate. Really. My son was in Cambridge, but they kicked him out of university so everyone's had to go away and he's currently living with his girlfriend and her

parents. Oh, blimey.

Yeah, I've got another error. And I failed to instal tiny PCI front converted installation of package back ports had nonzero exit status. Back ports. Yes.

How'd you spell it was in back as in as AAC K.

Yep. Ba CK P. O. RT s. Who, interestingly, that was one of the so I had a message saying which one recommended that you update? Sorry, these packages have more recent persons available, it is recommended to update all of them. Which would you like to update? all crammed packages and ignore them? And the last option is backports. Yeah, I suppose. And I think the default was one and yeah, I did I entered upon last time they did it. So it's probably so this time, I will choose non. Yeah. So I had to change the default.

That's fine. It's just a lot of the packages. You can't actually instal that way. You have to, like restart our and instal in different ways, so it's more likely to crash if you're trying to update it at that stage.

Right. Well, I clicked on Nan and this time it gave him the same error. God damn, no, it's a different error. Error package backports does not have a namespace.

Okay. So if you just put instal dot packages back ports on its own it's probably going to ask you to restart our or something along the lines of that

Yeah. All right restarting price or updating these packages is strongly recommended Yep, that's it so many articles isn't currently installed

in your articles isn't installed. Yes.

Oh my gosh.

But how can it not be installed? You've been installing packages.

Yes. So I wonder if interesting so installing to the full error message for backports is our tools is required not currently installed Please download and instal

appropriate version of articles for proceeding before proceeding. Installing package so it then says our studio.com Installing package so it looks like it's trying to instal it there is a binary version available for sources later binary source nice compilation so I wonder if because of instals four I definitely downloaded the fourth version of our tools. Yep, our tools 40 So I've installed the right version of our tools which version of Are you are you using?

So I am currently using our three because our four was only just released very recently and it doesn't actually work for the version of Linux that I have and I wonder whether or not back to back ports isn't actually available for our for

I think it might be easier if I honest I probably don't need to uninstall this version but instal a version of an earlier version of our

maybe maybe before trying that to see. Try and instal it from GitHub instead of from cran might work

okay

send me the code now.

Okay

error felt locked directory D use this documents for modifying try removing removing to use as documents try rooty uses deep as documents are when library 4000 Lockback forts tell you I'll see if I can send this message to you in chat

sounds good I mean ultimately we can go back to just sharing your screen if that's the easiest way

so Claire suggesting that I've got a file open in fact that folder that folder yep my wife's right I do have that a folder in that facility open the interesting thing is that it didn't open it so we'll try that again

maybe before you even try it again we just restart our yeah I think so. And then try it one more time

pasted the error message you just sent downloading error failed to lock directory to use this again think this is also one of the reasons why I stopped using

Yeah, I knew it was always going to be an issue. As soon as they did a major update our things just weren't going to work. And now they have Yep.

Well, I can download it download. Ah, that's a 3.6 I can definitely give that a go. Might be advisable though. For me to go away and do this and see if I can get it working.

Again, yeah, there's there's there's no pressure because obviously this is neither of our faults. There's something weird going on. Yeah. And if you have anything better to do in the short term, get out and get on with that. Okay, but if if you do have some free time to try and instal our frequent six then we can try and we will give this another go another time.

Yeah. Looking at my ice, get you Fridays are generally good. Fridays a Good

Friday afternoon,

so okay. Yeah, Friday afternoons. Okay.

Yeah, because no Friday mornings are normally busy for me, but afternoons are normally okay for me.

Yeah, yeah. Okay, cool. We'll do that.

Thank you so much. Sorry, it hasn't been perfect. I think

During this, but I will now start recording this might be yes. Easy. Yes, you definitely can do that. But pretty much the same with your Excel spreadsheet. Like however you define that sample ID will match which what? What gets normalised to what? So as long as you're careful with how you're defining the sample IDs, you're, you're fine.

Yeah. Controls to Yep, that type all seems perfectly reasonable as well. Like each pro muscle one target each column one sound like yeah. Good old Act One. Yep. Your biological replicates. So, is it assuming that those biological replicates are on the plate? Yes. Like, three technical replicates? Okay. I have to make RNA but ever bothered. We want it?

Yes, you do the three by three. Okay. So the first thing to do is define my target IDs, right? Yep, yep. Okay, do you want me to? Sorry? wasn't on the window? So do you want me to follow the instructions? Yeah, exactly. Or?

So yeah, what I'm going to do is get you to first just follow the instructions exactly. And make sure that you sort of understand what step is going on. And then I'll ask you to do a slightly different version on your own. And it just be a matter of changing around a few few of the things that you've already inputted to make sure that it does actually make sense. And what you expect to happen is happening.

Yep, sorry. I'm having difficulty with quotes. Slippery things. Okay, so I can see the first thing I'm doing is assigning this array to the Target ID.

Maybe it might be easiest if we try and share your screen again. And then at least I can I can see what's going on.

Yep. You have to make the posting.

Yeah, I think so. I don't actually know why it's doing that. It doesn't normally do that. But yes, I

shouldn't make you Yeah. Yeah.

I can just quickly swap over to yourself.

All right. Yeah, we've got the other options. Screen.

Okay, can you see that? Yes. Perfect. Here we go. Okay, area

I always like to check that it is what I'm expecting it to do. So a couple of placements.

Yeah, you should be

able to mind you I was fine. Much. I learned it far better if I actually type

it in and type out yourself. Yeah.

I've no idea. What, what? What Tibble? Is?

I? Yeah, so tipple. It's a particular data structure that's part of the tidy verse. So tidy verse is a series of packages that is created has been created to really allow, in essence to mimic what your what you called them in Excel for the pivot tables lessons. Yes. So it's a way of really easily flipping between like having all of your observations in rows to all of the observations and columns and flipping between the two things. And you can try and do this in a normal data frame. As that comes with of the art itself. But with a table, it's got some extra characteristics that makes it if you want to immediately print that table out, it prints it in a nice format. It's just it's basically a data frame with a few extra bells and whistles on it. So nothing too crazy

Okay, last bit looks a bit weird. I can see that I'm, I'm beginning to define my Yeah, okay, I'm not sure what that's No. Rookie that will tell me

oh yeah, if you you probably need to just I would have thought that running tidy qPCR would have activated tidy verse but apparently it doesn't rhyme reason so if you put in library tidy verse I need to make sure that that happens actually, it didn't see why it doesn't happen they will see if you rerun

okay okay, right. So I guess I would call that a key to array in other languages.

Yeah. Yeah, exactly. It's a it is a Akito array just so that when you start filling in what each column is, which is going to be the different samples, then it's easy just to pair the two up and make a full plate.

Yep. Okay, all right. So let's go okay, I think this one I am just gonna copy and paste and that's perfectly fine

so that's the same from the rats now when I did this, I always put the mathematical symbol at the end. Because in Excel it's treats that as a formula.

I have goals of course

with RT plus RT minus three okay

let's define my columns 123 So it says given the soap ladies in the types Oh, yeah.

Right okay okay let's have a three by three grid. Yes. Okay. Okay,

so yeah, so now you have in essence, I can't remember what did you call it? What was your word for it again? For the tipple. You said in other languages keys key the row? Yes. So you know you've got a key to array for the rows and kill it keyed away for the wells for the columns. And then you can just use that to make everything for your individual plates, if that makes

sense. Yep. Right. So okay, I've established my plate it's interesting. Couldn't you create Extra sort of blank 96 plates and like three or four well plate

yeah very very easily. So to make a

lot of standard

I think there might be I can't actually remember if there are tools to automate that but yes, it's very easy to do. I think there are there is one of the helper functions does automatically make a 96 well plate but we don't have a helper function for 48 Well plates

that's just an example. Okay, so So that's created plate but we haven't put anything into the plate I take yep,

yep. Okay, blank plate and now we just need to associate the actual labels that we created previously into

Yep. Okay, I've actually got a note here that there are default functions to make 1536 Okay so late late it's late don't like doing late doing that again

has named x x where key names? Have no idea why it's doing them it seems to have give me one second I'm just gonna rerun the code on my own and I get exactly the same thing or not

I yeah, I having done these sorts of things myself. I know just so difficult to tears when somebody else comes along, they haven't got all the dependencies that you have. Yeah

yeah, not sure what is going on but we shall check. Right, so I didn't get that I don't get that error message when I run it. I'm not sure why.

Rookie for conky three. It's definitely exactly the right things that you want to be typing in.

Let's try it the long way around then. Okay. And see if that's gonna work for us. So yeah, I'm gonna have to make this up on the fly and see what happens.

So if you start off by just doing 12 equals if I just do my own thing, and then make sure

Start off that on its own and you pain in my yeah okay so we're gonna have to unpeel what's going on

I think there's going to be lots of things that you might not understand but that's because all of these are hidden in the wrapper functions which don't seem to want to work right so let's try that see if that works

right so I'm struggling with Zoom to see if I can get

oh you can't see the chat

not easy but I found it I think Okay, that looks like it worked

okay so that's half of what's going on and I'll just make sure the other thing about the other half

during an image

Oh God that's a big mess now I should have put a few spaces in between

Yeah, I think on the whole spaces would have helped by COVID Like

could you just type in Roki for again for me yeah, just present confused by what's going on with why doesn't it detect that that Well, I was not found

Let me try one more thing I was going to put some dots in so we have some space between what's going on and what's not going on just try it out on its own and see what happens. Right, okay. Type in row key on its own again. Yeah, for and then put in the like dollar sign, or sorry, instead of clicking into sorry, before you click into the dollar sign. Yeah. And then click on that. Which one?

Yeah. Oh, am I just being really silly? I think the first letters are capital. The first letter is lowercase. And well, I think that's what the issue is. Cut down. It is.

I think so. Yeah, it definitely looks like it. I think that's what the issue is.

So if you go back to how you defined well, our if you go here, if you go all the way back to what you'd find rookie,

sorry. Yeah, I think you just need to change for such an annoying thing. Okay. That's that's a fun thing. Change that's lowercase? No, the W in well, uppercase.

spotted that. It's thought that the capital W's are. Capital letters are sort of halfway between the two of them. Yeah. Right. Shall I go back to the original one?

Yeah, I think that'll work now. It just couldn't detect where it was. Cut down.

Yes, any one messages? But

yeah, it's just wanting messages to say that it has to convert things to factors, which is as expected, but it seems to work.

Yeah. is really difficult to spot that that's not the case.

Yeah, no, I think that's a good call. Actually. We probably should just change it. So it's either all uppercase or all lowercase.

Okay, right. Okay, that now looks like you're Yep. Oh, yes. We've got to try to display

Yes, and that's different than the another. I think you you've named it target IDs, plural. Instead of singular. Target ID. So when you were defining the call No, the RA. Yeah.

It does actually say target IDs in in the thing.

Yeah. And then once it goes into the table, it becomes Target ID. So it becomes singular. That's, that's confusing. Okay. That's a good catch. That's a good catch.

Yeah, so if you could again, go back to when you're defining rookie for that's that part is fine. It's it's in the table. It becomes Target ID singular equals target IPs for Yes. Yep. That makes it like this one again? Yep. Yep, yep.

Just in that gave me a few error messages.

Oh, yeah. plate does not have variable Target ID. Yeah. Okay gosh okay

yep yes

that's great yeah, if you get back on help

okay

Where were we Yes, okay. Yeah, I tend to do things by squares and then expanding

and changing what I need to do so the sample ID so we're ready to find the sample IDs.

Yeah, the sample IDs are exactly the same. The difference we're doing now is we've only got one technical replica which is one plus RT for each target in each replica and now we want three technical replicates plus a negative control for each feed sample if that makes sense. Yeah okay so we need to expand the coal key so now it's not just three it's 1212 across

some ID symbol IDs times 15. Blank Slate writes that that's that's quite nested, isn't it? Yeah, so creating the plate giving it the row keys and the column keys and then you're making the plan alright worst thing is kind of scary I guess perfect. Yes.

Great. So I think all it gets to do next Yeah, it's just adding more sampling and that sort of thing. Which I don't think we need to exactly do well actually yeah, I think we can move a little bit onto the task and then I think we can call it a day

okay

because the task kind of links into what's happening next on the on the help sheet. So if you this is where are you now you're on that's fine. If you scroll down past this part. Oh, no, this is the to condition part, isn't it? Yeah. Okay. Okay. So if you scroll back up, sorry. You need to recreate the real key Yes, Okay, so adding experimental conditions so instead of the conditions that they say I want to can you see the questions that I sent you the PDF

like two conditions with and without men die on Yeah Okay,

so you've got the same pretty much basis as before three biological replicates three technical replicates but now you're adding plus or minus Melodyne

does it matter which order I put these in manotaur So that should give me the conditions yep

yeah

okay and

says a probe suppose meant to four probes I keep forgetting to change that

can we just stick with the ones we've got

that's fine that's perfectly fine.

So I replicate the conditions now

yeah yeah if you pretty much just follow the help document it should take you towards to the end

okay, good so far okay, please look at six

so, I think the next episode before you can print display it if you go back to the help, sorry,

I think so, I was just gonna see what

So, unfortunately what you haven't made yet you've met you've set the table out but you need to make sure you make your unique sample IDs which you don't have at the moment. Yeah, okay. Which is just a one the next step to do and then you can so the way we make the unique sample IDs is just combining condition by Rep.

Yes, that looks much more likely look more like it

Oh, it's cool to see a different one and unfortunately have to talk. Yeah, yeah.

Yes,

there we are.

Beautiful. Night I

think that's pretty much it. Lovely thing that says add here, add here remind myself to look at that perfect. Okay, that's all that I need right right now, yes, there might be a time in the future where I could do with some help just testing the actual analysis functions and making sure that they kind of make sense. But for now, like, we're not quite at that stage, we still got some more teething problems to iron out. But yeah, I mean, again, if you're busy, there's no pressure whatsoever. But if you do have any time, some I might email you in a few weeks time to see. See,

I hope my my dumb user Act has been convincing. No.

No, it's been really, really helpful. And I think even just just some of the smaller things that could really help like, for example, you know, we wanted to put the warning messages in but they do actually look quite scary. So maybe we

do and it kind of it. What's the warning message? I really needed to know. Yeah, it did not convey contain the sample variable. The variable, the coercing? I didn't really need to know that. Yeah. But I didn't need to know that it was missing a variable.

Yeah, that's very interesting. And I think I'll definitely take that back to Edward. And we might reverse that. And then obviously, trying to actually settle on cases for some of the variable names because and trying to decide whether or not it should be plural or singular. Yeah. silly things.

Yeah. I mean, I've kind of done this too. And I know that sometimes I start off with it, right, I'm gonna use this convention, and it's gonna be this convention. But then I kind of forget it myself. And then somebody else coming and using it doesn't remember doesn't know that. Yeah, yeah.

Yeah. But overall, do you think it kind of makes sense? Does it follow kind of how you think in terms of making those square blocks and

certainly, making those square blocks is exactly the sort of thing I did. So I would have set up a sort of, okay, this is the sort of thing I want to set up and I will replicate this across the plate just changing what I need to change each time I do replication. I guess I guess mine was more like that. So I would set up an example. So it would just be a single single sample with a single throw a single target and then I'd replicate that in that thing every time just changing what changed between them so I would build up say a row of that way with all different samples them and then I would copy that that row down changing say that the VCR each time

Yeah. Okay, cool. Yeah, is it intimidating?

If I were to try and make my own. There's quite a lot of jargon to remember. Something like these. So if this I guess you gotta say well, these my columns Oops, yeah, that does make sense. Whether I could remember it, for example, or whether I'd have to consult the documentation to help documentation every time they did it. And for at least the first few months that's, I would make mistakes, that's for sure.

Yeah, definitely, I think, because one of the benefits of trying to learn this particular way of thinking about your data is, it's, it's very versatile. So the language we're using at the moment in terms of like, creating these tables, and creating the, like, replicating it in blocks, that sort of thing, that is using a more universal language, which is the, the, the tidy verse language, which is not anything to do with biology at all, that's just general data science, language. So if you wanted to learn how to do data science, generally, whatever, whatever subject you want it in, like, it's quite a nice stepping stone. Yes, kind of, because this is meant to be as well as it's meant to be like a practical tool. It's also meant to be like an educational tool to for people who are trying to learn how to use our and trying to learn how these packages talk to each other, you know what I mean? So it's a little bit of a trying to find a happy medium between like actual functions that are biology related and qPCR related and more general functions that people could try and learn Yes

Yeah, I mean, that's that's something we supposed to be doing is teaching our first year students are although COVID has stopped us doing this year and certainly, I look at my children and and they much more instinctively get it than I do.

I think it's the way things built up in multiple, multiple levels. So individually, they make sense. So is this a sort of the plate label plate roll call? So I presume that's a function you have made you have written?

Yeah, that's something we've created yet.

Right? Six okay. Yes. Okay, yeah, if I can think of it as a sort of parameters and a function then yes, it makes a lot more sense to me now. Okay, is there any way like I don't know if there is an R is there sort of mon file for these things? Like do something like this

the question mark at the start

okay

okay yes no, yes that I can I can understand. So Okay

Does that make sense to you?

Do you think that that makes sense? Having seen save bouncer function, you give it these bits and pieces and that's and yes, you get a plate out of it and can see it the way it fills the Create blank plate

do the same to that. Yeah, Yes, okay

they can create lead placement. Yeah. Okay. I think I don't know if I'm, I'm sure I'm not unusual about this. But if if I have a whole load of commands, and I don't know what they're doing, then it's very easy to get lost. But just maybe, if we could say, Okay, now we're going to use the label plate row Col. thing. If you type the question mark, you can see what it's doing and see how it builds up from what we've got before. Rather than just presenting me with this, just the string of, of things that yeah, that makes. That makes a lot of sense. And I can I can use the these little help things to these little health things to tell me the lock bricks I'm putting in to build up the structure. Okay.

Yeah, that's right. We should definitely do that. Again, thank you so much for your time. I hope your wife feels better very soon.

I think you're gonna check up.

Yeah. Yeah, I'll be in contact maybe in a month's time or something. If just to see if we could just spend a little bit more time on the next few steps. Sure. I've got so much to go through now. You so much.

Okay. Like, look,

thank you so much, and have a nice rest of the weekend. It's almost weekend. Yeah, almost weekend.

Interview 4

We'll I sent you back with the invite just the outline of some of the questions I'm gonna ask, I don't know if you've got that hand? Yes, yes, I do. Perfect. So literally, I'm going to start off just going through the the questionnaire to start off with, and then I've got some extra bits, that I tend to ask people, but it's just more about your experience during qPCR. And then I'll probably just get you to open some of the tidyqpcr vignettes, and just talk me through them. See, if there was anything that you thought needs to be expanded on anything that you think didn't make sense the first time you looked at it, or read it. And just anything generally to improve with either the functions or the explanation of the functions. So, questionnaire, tell me about your experience with qPCR experiments. So things I'm generally looking

into is whether or not you do RNA or RT qPCR. Or DNA qPCR. Whether or not you use primers, or, like more fancy tatman probes, that sort of thing. And then how many experiments you've been up to recently.

So, I, I this this like, qPCR experiment, basically, that I've used cloning qPCR was my first one. So that was exciting. It was RT qPCR. I was looking at looking into different transcript levels basically. And I, yeah, I used I use primers for it as well. So I use cyber green assay, I use the protocol that your lab actually has. Edward was kind enough to share it with me. Perfect, perfect.

And did you have any problems at all with since it was your first time?

Surprising the only problem I had was with the primer design, actually, because we had to we had, we just had two copies of I was ordering super folder, GFP transcript levels. And there were two sequences lying around, which I didn't realise and I use the wrong one the first time, none of the primers seem to work. Fair enough. But that was relatively easy to fix.

So so how many was it just one experiment? And then how many qPCR runs? Were you doing a part of

that experiment? So I say I did. Three biological replicates. Basically, I was measuring, I had six samples. And yeah, I did three separate RNA extractions for each sample, and then I did technical replicates of each one as

well. So and was that all on the same plate? Or did you have different plates with different?

I did it all the same plates? Okay, cool. That makes sense. Yeah, I'm actually going I'm actually doing some more on extractions for another one. Yeah,

I mean, that's the next question. Like, what? What do you have planned in the near future? Are you going to be doing more? qPCR?

Okay, yeah. So I'm, I'm just, I'm looking into some more constructs I have, basically, because the first one worked surprisingly well. That's good. Yeah, I had I had, I hadn't done it before. So I had fairly low expectations, but the data seems really good. And all the controls seemed like they worked and everything. So that's nice. Yeah.

I know that you're eventually going to be going off to do a PhD. Would you be doing Do you know, if you're going to be doing any qPCR stuff with that? Or are you just changing fields entirely?

I think I'll probably be doing qPCR though, everyone else in the lab that I'm going off to is doing qPCR currently, so it seems like something that oh, I'm, I'm kind of changing fields a bit. I'm going to work with some cancer biology instead. But I'm still still looking at RNA. So

be very intrigued by that, how, how they do their analysis. Especially for if it's a lab that does quite a bit of qPCR.

It might have their own pipeline or something. Yeah, that's

what I find. I've interviewed a few people from different labs. And it just the issue is that it varies so much. It's not that like, other people's, like programmes are doing something specifically wrong, but things that people are normally doing like slightly different things. So like how they might choose, they're the targets that they're normalising all of their PCR results to, might be doing that differently. They might be like doing some sort of automatic removal of, you know, outliers, and things like that. And it's just like, I can't say, right, that's the wrong thing to do. But at the very least, it needs to be very clear that you're doing that. And, yeah, I think, basically, the idea of tidyqpcr is not to rewrite the book or anything, it's just hopefully, having everyone reading from the same hymn sheet. And then we can actually see what everyone's doing with their data. That kind of makes sense.

I think I think that'll probably be good.

Yeah. But anyway, it's, it's just interesting to see what people do. And how difficult would it be for you to repeat your qPCR experiment? And then reanalyze the data as well.

Probably relatively easy. I mean, I assume I could just use the same. Because I've just got, I've just got the, the, the script that I wrote for it, using the I mean, I, I just based it off of off of one of the vignettes. Basically, I assume I could just, I'd need to make a different plate plan. Actually, that'd be the thing, because I did one or two of the rows, basically, I put in the wrong order. Or I noticed that actually, for one of them, I noticed that it didn't actually take up enough of the sample, basically. But I had enough spare that I could fix it, essentially. So I need to change the flight plan. But other than that, I could probably just put the file in, and then it would, yeah, I can just run through it.

That's good. That's, that's nice to hear. Is it? Like, is the analysis scripted? Is it available? Like, is it on GitHub or something like that? Or do you just have it locally?

I just got it locally. I might not be super helpful. I'm planning on putting it on, on eventually for the lab to use basically, the lab I'm in cuz I reckon Sofia might do some good Sofia, we'll be doing some qPCR experiments at some point as well. Okay, I can, I can send it over to you if you want. It's not the prettiest thing.

That's fine. I might, instead of like sending it over to me, I might towards the end, just ask you to share your screen and talk me through just so I can see how you've changed the vignettes to do your project. Do you mean just to make sure just to see how people are interacting with it, but we can deal with that later. Okay. Next question. So describe how qPCR experiments are used slash presented and publish papers related to your research. So have you read any papers that have used qPCR results in one of their arguments?

Yeah, I thought I should have probably heard this. I remember I looked, I was looking specifically some. When Sanders suggests this, you mentioned reference gene. So I was having a look at a lot of papers about what reference genes you can use for qPCR and things like that. i One, one interesting thing that I noticed is that a lot of papers tend to present them in like bar graphs. And the results of bar graphs, which I found kind of interesting. I don't know. I would have thought like, box plots would have been slightly better.

Yeah, I mean, so it kind of the issue with box plots that, you know, normally the number of replicates are quite small. So having a boxplot kind of a bad spot is great for summarising like maybe 10 data points or more than 10 data points. But if you've only got like, five data points, then I'm not entirely sure if it's the ideal way of doing it. I'm not I'm definitely not a fan of a bar chart. I think that's, I understand what they will do it because it's so easy then to compare differences, like global differences to the mean between different different samples, whatever. And then sticking some sort of error bar and on top of it or telling you what the difference in p values is it's like that stops people from actually critiquing your work because it's such a summary of a summary. Like was so far distant from your raw data and how your results were added? No, it just seems to cover up with that. Whether or not, it's a clear result or not.

But yeah, I kind of I kind of see that. Yeah.

Which is why Edwards always do like a scatterplot.

Yeah, I can see each Electric Gas plots, actually, that that's something that. Yeah, so I've got from all this data, I've basically, I've got all the individual points plotted as scatter plots as well. So you can see like the range, I think I'm going to include that alongside the box plot. Yeah, I have my thesis.

I just had, I think it's, it's a good idea. As well, especially if you've got if it's small number of data points, then you need to see you need to show whether or not you're seeing anything significant because p values. There, they are a summary of the summary statistic. But that's it, they they summarise a lot of different things. And you can't see all the patterns just from telling someone that P value anyway. Anyway, personal personal feelings aside. So yeah, you saw that the, in the paper, they present it typically as bar, bar charts. Did they show any form of like, you know, the scripts? Were the links to any of the scripts that they use to analyse the data? Was there any, like analyst data or anything like that?

No, I don't I don't really remember seeing much, actually.

Yeah, that's also a very typical.

I think I think that's unfortunate. That just seems quite common, I think. Yeah.

And it's, yeah, it's hard to convince people to do that, because it's, it's a bit more work, and you tend to not get any credit for doing it. However, like, when somebody else comes along, and is using your work, for example, to try and find suitable reference genes, you know, like, that's them has a big effect on somebody else's results. And it would be nice if they could verify your results from the very beginning. Just as a sanity check, hey, hey, how are ya how the world looks? Okay, so you've already done this, already talked about it a little bit, but describe the design of your most recent qPCR experiment. So I know that you did three biological replicates, and then technical replicates as well. Is that right? Yeah, of each biological group.

So they're kind of, I guess, nine data points for each sample.

That's perfect. What was your sort of methodology for how to spread these points onto like, the plate design? How did you spread the technical replicates and spread the biological replicates and the samples across, across

I kind of kept the biological replicates separate? So I did. So in each well, so in each row, I had like, my samples, like one to six basically, and then a repeat of that further along. And then to repeat that again. And then I had the, the, the negative controls after that. And then I did that for the, the primers I was using, and then for the three reference, yeah, like reference transcripts I was measuring as well. I have like a block of that. And then underneath that, I repeated that and then underneath that, I repeated that again. Okay. And then at the bottom, I had one I only had one well for the minus RT controls, basically. But I had one for each sample that I use, basically. Yeah, that makes sense. And then I did that that was the ideal plate. And I met I did mess things up and I had some stairwells on the right that I

loaded, whatever I messed up on that afterwards. So that was a bit of a pain. That's okay. It took a little while. It did take about half an hour 40 minutes to load the plate because of that, but it seemed to work so

are you aware of the Mikey best practices to thinks that it's kind of the paper that tidyqpcr was based on? Have you have you read that or are you aware of what that is?

I know I was not. Not really aware of that.

That's okay, like. So, the Mikey guidelines, best practices for qPCR design It was something published back in like 2008 2009, I think, by

a lot of different

both experts in molecular biology and in medicine, for conducting qPCR experiments. And they give you like a list of things that you should aim to do in your experiment and be report in your paper, once you're writing a paper and the conclusions of the results. And a lot of what we're trying to do in tidyqpcr, certainly in that vignette is to include make it as easy as possible to include everything that there might guidelines says we're a long way off, because we need to start adding like meta data so that people start explaining, you know, exactly what

exactly what what machine can you see on machines and what solutions and stuff? That's extra things. But yeah,

I just wanted to see whether or not you'd been aware of that at all, which it's not a requirement.

I have I have I have I breached one of the guidelines then, by any chance. I mean,

I think in terms of the experiment itself, seems fine. Although it does depend on how you decided what your reference genes are.

I, I just asked Edward, actually, for three, the three that yeah, that would work. This, everything was done under the same conditions as well. So that makes it slightly easier.

Yeah, no, I think overall, at the moment, it doesn't sound like you've done anything wrong. But it does depend on how you publish the results, because it's the minimum information required for the publication of qPCR. Basically, this kind of like what Mike Mikey stands for, just requires you to, to actually try and write it into a manuscript. And then so you can, again, like they are very high standards. And very few people, as you've already seen, very few people actually use them. But if you want somebody if you want your experiment to be repeated or repeatable by anybody else, you do kind of need it. Give all the information that Mikey guidelines say Be true to it doesn't make me think that we should make that more obvious in either the vignettes or somewhere just so that it's clear that

we're basing on the mechanics first,

when did you do any primer efficiency calculations or experiments?

I did. I did do the private validation. Actually. I basically followed the same thing. That was in one of the units as well. That's perfect. Yeah,

because at the moment, in an ideal world, when you're actually doing the experiment, after you've done your prime efficiency calculations and stuff, you should put the prime efficiencies into the equation that normalises and gives you the relative abundances or the Delta Delta CQ, etc, etc. But tidyqpcr doesn't have that functionality at the moment. It's something we need to add.

I think, I think I think it should be a case in my projects as well, because it's kind of I'm always using the qPCR to kind of validate the fluorescence that I got from the Construx. Sure. And the transcript levels seem to follow pretty much exactly, exactly what that shows. So

that's perfect. Yeah. Definitely. validation through a completely different sources is always benefit.

Yeah, it also makes sure that it's it's nothing to do with like, like, like the, the control of seeing is transcriptional control or not some form of translational control basically,

through that make sense? So I'm gonna move on to the next question, even though it seems a bit redundant. Describe the analysis pipeline of your most recent RT qPCR experiments. Obviously, you did use tidyqpcr. But there are some interesting questions that might lead on to the later part, which is, what was your biggest frustration? Was there something that you find found particularly frustrating when you were trying to analyse it

Potato biggest frustration I didn't struggle too much with it, I guess make making having to make the plate plan and I was a bit interesting. So I, I've been using the, the analysing plate reader as adults, I was using the Swain love software for that. And essentially they let you make just like an Excel doc Excel document that you can put a plate plan in for that year. So you can just very quickly like, type it in or if it's the same plate, you can submit the same flight plan instead of having to make it each time. Which I guess I have found I have found to be a lot easier. Like, I mean, I did email you for help with making the playoffs. So yeah,

yeah. Because we're kind of torn on that. So it'd be very easy to have exactly the same functionality with within tidyqpcr. But we're worried that it would take away from our want to encourage people to think of like logical, repeatable blocks of designing their their plate readers. So like actually thinking about, since you're not typing each well, individually, and you're not just copying and pasting it well. Repeatedly, you have to think of the smallest block and then repeat that. And then the next smallest, make a bigger block and then repeat that. And we think trying to enforce that helps people to really think about good experimental design, because like blocked designs, or blocks with some sort of random element get to try and remove technical biases. That's the most efficient way of or the most, the best way of designing an experiment. Because it means a things are tentatively tend to be like logically grouped together. It's easier to load if you're doing the same thing in just blocks, and it's easier to analyse. So, yeah, we're thinking of adding the Excel like read in Excel. But we're just worried that that will encourage people to do silly things, or at least not do the plate plans, the way that we're trying to encourage people to do

it. I can see that that makes sense. Yeah, yeah. Especially because I imagine, I imagine a lot of experiments will use the entire plate. I got quite lucky that I had some wells leftover. So yeah, yeah.

Yeah, well, we'll see. We'll see. I think we all get it ultimately added functionality, because I think it's just such a turn off. When people want to do an odd plate design. They can't do it. So yeah, probably what I did.

What was I going to say?

Is there any functionality you think should be added in any way shape or form apart from reading in Excel? What did you feel like you could do everything that you wanted to do?

I felt like I could, I could fairly easily do everything actually. That's nice. Yeah, it was quite nice. I had to get I had to get used to using R again. But yeah, I had used it about two years. But yeah, once like once I kind of got around remembered like, gg plot was and everything. Yeah.

I mean, that's, that's the next the next question. The final question. The question is, what is your previous art programming slash terminal experience

of programming I used it in two summer projects before this. Not like very limited though. And I was basically just using other people's scripts and modifying them. So in terms of terminal I don't have too much I've kind of been I guess fiddling around a bit with it my own my own project, but not not anything too much. Yeah. I've actually been I've actually been using Python a lot more this last year so that's just because Sander Sunday quite likes it. Yeah, absolutely.

Sandra and Swain lab as well. Yeah. Was gonna say so. Do you think that so for people who may even have a less programming knowledge than then yourself coming to us tidyqpcr Is there quite a steep learning curve? Because we're kind of auto also as well as like teaching people good practices for conducting qPCR experiments, we also want to kind of use it as like, a good entry level, Introduction to different concepts in AR. Just wondered what you thought about how tidyqpcr is, as a lung as a piece of software?

I did find it pretty easy to use, I mean, for a lot of it, I could kind of just take the vignettes and modify them slightly.

And it was quite clear where I could modify. Like, what parts I could modify to suit my experiment. without, like, completely breaking everything, so

that that makes sense. Are you aware generally of what the concept of tidy data it's not really properly talked about and tidyqpcr, but the tidy part of the qPCR? Do you have to understand what that refers to?

I, it refers, I assumed it was a reference to the tidy verse, right, which is just a select, which is, from what I understand the selection of our packages, basically to just make analysis and keeping your data and everything a lot more, a lot easier. So cleaner.

Yeah, I mean, that that is true. So tidyqpcr, is based on the tidy verse universe, and how they have packaged a lot of different functions for general data analysis, absolutely nothing to do with biology, just general data analysis, so that, you know, they feed into each other, and you can build them or build them on top of each other, those different two, but the concept of tidy data is is like language agnostic. It's something that it's just a concept for how to deal with data, splits, specifically how to deal with human readable and machine readable data and trying to meld the two together. And so that if there is such a thing, as you know, tidy data in Python and tidy data in I don't know, C++, etc. It's language agnostic. I just, again, it's not something we try and teach in part of typing qPCR. Because that's not the point. It's more like exposing you. And then maybe you'll be interested in what, hang on, what is the tidy verse hang on what is tidy? It's just something that we wanted to include in tidyqpcr, whether or not people actually pick up on the fact that there's bigger concepts

at play here. I probably I probably would have, I probably would have picked up on that, I think, when I continued using it. Yeah. So I think I think the first it's very, it was just getting to grips with it. Yeah,

because ultimately, we'd love tidyqpcr To be a stepping stone. So people who don't know any programming, but they want to do an experiment. Okay, I'm gonna have to learn programming. Let's try and learn tidyqpcr. And then actually, I want to learn how to properly programme and learning all these concepts. Oh, hang on, I kind of understand these concepts, because they arise in something that I used previously. But again, big wide ambitions. But we'll see. We'll see. We'll see. Great, so do you feel more comfortable with our programming generally, now that you've returned to it again? And do you intend to learn even more or you're going to stick with Python? Do you think in the near future?

I yeah, I do feel comfortable. I mean, I are is I was I, I forgot just kind of how easy it was to I don't know, I guess plot things and just manipulate data in it. Because I guess it kind of is just designed for that. Really? Yeah.

Can we start to something that in mind to start off with, but is quickly already got a lot of functionality?

Yeah. I mean, there's been a lot Yeah. I was just in comparison to Python where in order to do anything, I have to import packages, basically.

Yeah, there is a lot. There's a lot already included in base r that allows you to do a fair amount with before you So we have to like, really import some monster packages, but

also the figures just nicer and are as well. Yeah, I'm not sure why but they do so.

Yeah. Yeah. Yeah. I don't understand why. Certainly now since I've been around for a while, people haven't spent more time trying to learn what art as well and put it in everything. But hey. Cool. I think that's the end of the question there. Anything else to say? Think so. I was wondering whether or not you would be willing to share your screen now. And then we can have a little bit of talk about some of the vignettes. And whether or not there was something that stumped you when you first read the vignettes. And then moving on to some of your own code. And how you kind of adapted the code for your particular problem.

Yeah, sure. You want me to just open up some of the vignettes? I guess, yeah. If you don't mind. Which which vignette? Should we start on?

Just the the plate design one, the

first one? Got what's it called? Just I think it's just like setup. There we go. Yeah. Cool, I think that's sharing.

Perfect.

Yeah. Is it sharing? Yeah.

Yeah. Okay. So scroll down a little bit and just go for the overview. Were you inclined to read this? Or did you just go straight into playing with the code?

I, I'm gonna be honest, I did pretty much just go into the code. I think I'm, that might just be a me thing. I find it quite hard actually to, I don't know, convince myself to do something if there's not like a reason behind it, basically. So going in and just messing around with the code was. Yeah, just something that I don't know what works for me, but that I did. I did actually come back and read this. When I got stuck on something. Yeah. Or if I needed to understand something. So when looking at the Target ID sample ID and perhaps I was like, oh, I should probably go and understand what those are, then. Yeah.

And do you think the vignettes as structured in the way that they are? Does it facilitate being able to do that? Does it facilitate be able to just get stuck into the code? And then being able to find what things mean? Or do you think? Because the way it's set up where it is text heavy at the start, then code? Do you think that makes it hard for you to actually find what you want to find?

I think it was two, I think it was quite well set out. So I could come back. And I'd go to overview and then the, the essential information is all Yeah, it's all kind of like nicely formatted, basically. So it's quite easy to hop back and forth.

Interesting. When you started plotting some of the data a bit further down. You know, because we have sets. So if you if you render the actual vignette with with an R, and you can see the plots. Did you see the fact that, you know, if you run exactly the same code, your plot will look different from the plot that's

in the vignette. Did you notice that at all? I actually didn't that's it. I mean, I don't I didn't say that in the vignette.

Maybe I was just missing it, but I didn't think I could run the plot in the vignette itself. So I was just like what I was just putting this into our I thought was just yeah.

So yeah, if Give me one second. Let me just start my own. Think maybe if I just share my own screen, share my screen. I'm going to do All right.

I didn't I didn't realise I could I could I should have I should have realised like vignette itself into into our that.

That's totally fine. So

I imagine that would have probably been a bit easier

can be one second. Why not working? Yeah. So yeah, if you use the vignette function. First off, if you can just list all the vignettes associated with a particular package. So this is useful for other packages as well. So if you have, if you're using something else, like gg plot, for example, the Yeah, GG, part two has its own vignettes that you can just see. And then, let's go back to and then once you know the name of the vignette, then you could just replace that. Just type it straight in. And then it pops up, that vignette appears here, which you can make bigger or smaller if you want. Okay, and then it fully renders it. But, and starts showing you the outputs. So yes, that's outputting all the figures and stuff. Okay. So what I was trying to imply, was, these figures actually slightly different from what you would do if you ran it yourself? If it's gonna work or not.

So I was I was running things myself. Yeah, no, that's fine. It's just

because if you go back to the actual GitHub page because we do a lot of formatting of the plots and stuff in the setup. Chunk, and that doesn't actually get rendered, you don't see the setup chunk when you look in the vignette. So there's, there's a set of chunk bits here, that doesn't get rendered when you actually make the thing. And that makes the plot different. Slightly different, like different colours orientation, from how you put it in. And people have commented previously saying, Oh, the figures and the vignette are different from what I'm plotting why, but interestingly, I wasn't expecting this. You just copied it straight from the code. So you saw what the setup chunk is, but it's very interesting.

Yeah.

But that's good. That's that's an important thing for me to think about actually in so much as making sure the code if you're just going to look at the underlying code, make sure that's readable as much as the formatted file is fully formatted Markdown file is because people might just circumvent the rendering and just go straight in get stuck in the good Yeah, that's fine. That's fine. Interesting. Okay. Let's try and have a look if I ever go back to you showing screens that's okay. Let's have a look at

hold on i i assume you will have a look at my code if you don't mind yeah, I'm my it's been interesting saying that file has moved. Okay. I just need to check that this is correct London

Okay, yeah, this should be fun. Okay. I know I know what's going on because I back took my laptop. So it'll be it'll be annoyed about that. I think we go perfect. If you're if you're wondering why I'm doing this here and not the Markdown file, basically. Yeah. I think I think it's just I mean, I kind of made the decision to do this just because I use spider a lot in Python. And I quite like how the figures just come up. Yeah. In the box down. Yeah. Fair enough. This is me making this terrible, terrible pipeline. Sure. Which is, yeah, kind of annoying. But it wasn't it wasn't too bad. I just had to copy and paste thing of it and then change the labels. And it does work. By run through everything.

Some errors coming up.

I see. Interesting. I did. I did notice this. That was That's something I've just been reminded of now. Oh, that's because I didn't import things at the start. Oops.

Yeah, it's probably best if you just go up. Sorry, if you just click on source.

To the right. Oh, oh, yeah. Okay. Can't find the right. That's fun. Okay. This is this is fine. This is fine. I'll just run through things quickly. Oh, also, okay. I have I have been getting this warning message a lot.

Do you find that disruptive? It was a conscious decision to put that in. But I initially thought I was doing something wrong. I don't know. That's, that's fine. I mean, it's because you know, has to do a change of variable. So you're putting in a string, which is the well column names. And then because we want to manipulate it later, as you can see, like when you print the plate, and then you've got, well, well, row, well column, the well row, well Column A factors. Because you want to specify that there's, it's actually not a continuous variable, for example, the, the integers for well columns, it's not a continuous variable, you can only hold a set number of values, one to

20 something.

And therefore, you can fit it to a factor to tell the computer that it's actually fixed the number of values can possibly take. And it's, it acts as a a sanity check for later on when you're are combining things together. And B enables you to plot things with colours as like discrete variables rather than as continuous variables. And it's just, it's just a programming thing. And we feel compelled to tell you that were convenient to refactor because it has different behaviour to a string, which is what you're inputting is a string, and then we're comparing it to a factor. And we thought we need to tell you what convenience factor because it's different.

Okay, yeah, I did. I did kind of figure I think kind of figure out figure that was why the warning was coming up. But I mean, because it is in the but yeah. It was. I don't know, it might have just been the fact that it was in red. I was like, Oh, the first not worked, what's gone wrong? And then I looked at it again, it's like, okay, I guess it has worked, then.

Yeah, it is a difficult thing, where I wonder if we can put it in a tweet isn't a warning, measured message. It's like a comment. So it just doesn't look so aggressive.

Yeah, I think I think it's probably fine. It's probably quite important as well, if you're going to do more with the data after this.

Yeah, I think there's a thing. There's definitely a thing that we should do. And it's just come to my mind, which is you can set it so it only shows you the warning message once per our session. So it'll just tell you the first time you run this piece of code, it's comparing two factor and then it won't tell you again until like, you know Maybe you restart your computer and then open it again. And then it will probably tell you again, but then it won't tell you until you say computer again. So then you're not seeing your message all the time. I noticed that with another. I don't remember what what package it was actually. But I have I have noticed that before, actually. And maybe we can change it to that. So it's not quite as aggressive.

I'll look into that. I mean, to be honest, this is this is a probably quite a minor thing.

Yeah, I mean, it isn't it isn't in like, once you understand it, it's such a minor thing. But if you're new to programming, and suddenly you're getting warnings and read messages like that is quite disconcerting. So yeah, I think it's right. And lots of people have commented on it before. So it's something people are noticing.

Okay. So I'll just keep keep running through then I guess.

Like get up to. You mean, you probably just click CTRL A. And then ctrl and run it? Yeah. Just so it's runs everything. There

we go. Lots of fun things Oh, that. Okay. I think I've done something wrong. Because that did not

look the same. Like got Oh, no, maybe it did. Okay. Yeah, cuz the location curves all look normal and everything. Here looks like a very solid

qPCR run.

Yeah, I was always quite, quite surprised. I said that. The why I only did this once. Because this doesn't have the GFP and certain only do one replica of that because I figured if anything showed up in that, then that was just gonna be everything was wrong. Luckily, the GFP primer just shows up where the minus RT is basically. So yeah. For Yeah. It's called Yeah, it's called GST one because weirdly enough, the first primer worked best out of like the five brackets. Unless you've got an NA though.

Where's the NA coming from?

Oh, right. That's from that's from the pipeline. I actually should filter that out. Because I had empty wells. I figured it was just easier to label them as na and then filter them out.

Oh, no, that's fine. That's cool. Just wasn't sure if something was going wrong.

No, no, that's that that was by it. That was by design that those are some of the wells that are messed up, basically.

Perfect. Perfect. Very nice. Yeah. See if that's gone. Yeah, the milk curves look fine. Yeah, the only weird one is the GFP for the BYU the second spike there. But I guess that's kind of to be expected because there's not.

Gonna have this with some work? Yeah, that's that's probably the better one. That's nice. Yeah, it shows pretty much exactly the same pattern as well. Yeah. We'd expected so ps4 and five had the almost almost no fluorescence. Basically, the ps4 sometimes had, like, barely a background as one slightly above that. Interestingly, vs two is slightly higher than I'd expect, in terms of the fluorescence is actually slightly above vs. One. But there's a whole thing of that one doesn't have the spacer sequences in the binding site for the complex protein complex that we're using, essentially. And we already suspected that there was some secondary structure stuff going on. So this kind of confirms that I think, yeah, possibly. Yeah. It's not all we're going to use anyway. And then ps3 And as well, to at least one that has the most has the most fluorescence and Bs threes roughly halfway there. Also, what's quite was quite intense. Last things the I would also notice something where the kind of the tighter the transcriptional control, the less variation that was interesting. Which is you can kind of see here and transcript level as well. So that's Yeah. Kind of interesting to note. Yeah, that's, I mean, yeah, that's a

whole combination of different factors. So some of them might be biological, some of them might be just statistical.

Yeah, I'm gonna, I've got a I mean, I'm also running a PCA, basically, of all of my data. So that includes secondary structure predictions, for essence, and then I'll plug this plug some of the results of this in as well. Is to see if, yeah, just identify clustering, see if there is like a common trend or anything?

Yeah, possibly. I mean, how do you when you're doing PCA? How do you sort of normalise the orders of magnitude? Because,

you know, they all have like,

very different scales. Oh,

yeah. There's a fairly common way of doing that. And that's basically just to set it so that the was it included in in Fikret? I just don't remember the exact parameters. I think it's so that the, the mean if you normalise the data, so the mean is zero and the variance is one, I think that each for each individual data set and then plot that against each other.

Interesting. Okay. Read a little bit on the effects of that. Because that ultimately, yes, okay. Just doing the mean, standard deviation?

I think I think I'm probably Yeah, I think, probably oversimplifying it a bit.

I, I mean, that's kind of fairly standard for any, any sort of machine learning use of data from different sources.

But I don't know, I guess. Yeah. I

don't know how that actually changes things anyway. Yeah. I mean, I guess it's

not it's not super for

machine learning. It's more for just dimensionality reduction. Really? PCA. Yeah, but the idea of like normalising all the different datasets to have

mean, zero.

Standard deviation, one is quite, quite standard technique. But anyway, I'm just intrigued. I think I am done, then I think we've covered everything. I've definitely got some things to go away and think about. Do you have any final comments? I know that you did have to ask for help on changing individual cells or like groups of cells? Because that's not covered in the vignettes. But that could also be covered if we include reading in excels. So something Graham stuff?

Oh, yeah, I mean, actually, I, I just, I, I ended up being figuring out the simplest way was to just label a few wells as Na, make a few different pipelines and join them together, and then just filter out DNA. Corona, Corona. Yeah.

So yeah, well, we'll think a little bit more about that.

But yeah, thank you, because this has been really, really easy to use. Like, I'm surprised how quickly I must. King. Really?

That's good. I am pleased to see. And thank you very much for taking the time to do a bit of an interview.

Yeah, but yeah, I guess thanks for. I mean, I know it's all GitHub. So it's for everyone to use, but I guess Thanks for Thanks for making it.

Yeah, I mean, the majority of the work, certainly the basis was was Edward, but I'm trying to like, make it into a proper package that people can use. So yeah, that's all good. And I'll speak with you soon. I'll stop recording as well now, to do

Interview 5

Oh, that seems to be working hopefully, not entirely sure what it's actually recording. I don't know if it's recording the screen as well. But we shall find out at the very least it's going to be recording the audio. Yeah. Which is mostly what I need. So, yes, just fullscreen. So let's begin with the questionnaire. Very first question. very general, it's just talk a little bit about your experience with qPCR. Have you done many experiments previously? And what have you actually done with qPCR? In those experiments?

Yeah, well, depends how far back you want me to go. I have been doing qPCR for about 20 years. That's a life 10 years. 10 years. Sorry. Yeah. And initially, I just used to do all the analysis in Excel. Because that was before my time of understanding how to work with code. So yeah, it was just basically using some of the basic some of the software on the qPCR machine, which was an epi machine, and then downloading the stuff into Excel and then using. I was using a formula for calculating what the difference was. So it wasn't doing it the proper way for that, like his guidance, although it well, it kind of was because it was taking into account the differences in the inefficiency of the different reactions, but

Okay. That's good. Um, so in terms of because we're trying to, like, separate a little bit. So to talk a bit about the actual experiments that we're doing, and then talk about the software. So in terms of the actual experiments that were we've previously done, is it you know, is it with RNA or was it been with DNA?

sarni? That was all. So it PCR Yeah. Okay,

cool. And the actual assays that you were using? Were did you use, like, probe sets?

Or? Yes, I did a little of Prop sets, but the vast majority of what I did was in, say everything. Okay, cool.

When was the last time that you did? You did actually do it?

And see, I can't remember, it was quite a while ago now. The last time ninth of the seventh 2019. Okay.

And in in sort of the near future? Do you have many planned?

Not at present? Because Because I'm optimised for trying to get this stuff done for the manuscript, and it doesn't require qPCR. for that.

That's kind of one of the key points later on is given, if you picked one of your previous experiments, how easy would it be for you to redo them both in terms of redoing the actual qPCR itself? And redoing the analysis itself? Would you be able to do that quite easily? Or would that would require you to go back and really think about the steps that you took?

Because it's a while since I've done it, I would have to go back a bit. But I, I reckon I should be okay. Because I spent quite a lot of time learning Edwards original code, which wasn't done as a package. But, and I actually had to play and plan my plates myself. So I sort of got my head, I think, gradually got my head around how to set these up, because I had some issues with it initially. So to that extent, I don't think it would take too long, but then who knows?

And what about back when you using the export? Excel spreadsheet was actually relatively easy at all?

It was, it was very easy, but because there was lots of copy paste. Yeah. And the way I designed the spreadsheet, but it was very tedious because I had experiments when I was doing like, umpteen different probes. And I was having to repeat myself a lot. Yeah, just to get the analysis to work. So it was far from ideal. I know it was absolutely shocked me to do it.

That That makes sense.

But ultimately, like it was it was a way it worked. You know what I mean? Yeah, definitely. Yeah, it

worked and it got published. Yeah. Yeah,

Okay, that's fine. And the actual, like you said, underlying it was using, like, almost Mike compliant, fully might compliant analysis underneath. So where the formulas kind of standard, or did you have to make? Did somebody have to make them up? Or were they like,

yeah, it was basically altering the factor depending on how what the efficiency of the slot was so that I could counteract the fact that it wasn't they weren't all the same. But what was bad was the fact that we only had we only used a single control RNA. I mean, we've done lots of analysis that suggested it wasn't changing. Yeah. And you could use some samples from the same sort of, so this was using RNA from different gestations or foetal ovary. Yeah. And you you basically for the same amount of already you essentially got the same the same values and regardless of which sample he is, so to that extent, it was a fairly reliable marker. But I aware of all sorts of potential issues with that if you try and do anything else

so yeah, you said that some of your previous work in the Excel spreadsheets, they were like publishable Yeah. How did you present that data to remember? Was it

yeah and shitty ways? Bar graphs Oh, let the last one I did I did actually at least have the you that had the separate different bits and did them in sort of scatterplot things so that you could actually see what the differences were

and if I was to set up the task of going back and trying to reproduce those graphs or try and reproduce that analysis, would it be easy or would that be hard? Would you still have the Excel spreadsheet?

I'm not actually sure about that. Yeah, I might have some stuff but I'm just I'm not entirely sure where they all are just I've probably got them on a pendrive somewhere

that is perfectly fine. So a few more sort of technical details about experiment itself. Do you know when you were doing it we can go back to what you've done most recently if it's most fresh in your mind. Do you remember what the split is in terms of how many technical replicates on each plate you have and then biological replicates that one

so what did I have let's see so I had Oh, that's not the best one to look at because that was doing that was I had very limited samples while I was trying to compare

it was rip stuff and the RIP stuff was pretty rubbish because I got sort of learning about so it's probably not the best one

see see okay before I did so I had to know

these two axes for a lot of this stuff was actually just doing you weren't getting the analysis to work for doing the calibrations Yeah. I've got

Sorry, I'm struggling to find this. eight sets of Cramer's rate CD any pair as well I was testing sometimes biological replicates.

I should be able to get this which ones this 2018 or seven or nine Okay. Oops, what the hell's happened there? What's it done that for? Don't have a clue. There we are. Anyway, okay, so we had

um be by

So we had three tech reps of the RT plus for each and two buyer reps, I think in that particular one,

okay. I think that's right.

I'm still not 100% Sure. Maybe not. Because that was that's just that is just a calibration. And

what else could I find? Sorry, it's

just so long since I've done this done this, I'm just not

probably going to remember all these small details. It's

yeah. So this is one? One

Yeah, so we had, we had four different treatments. And it was, well, no, so two different treatments to pay to buy reps for each. And then take reps. We did three of each. No, did we? No, we didn't. We didn't even do as many as that. We were just trying to see what was going on. So yeah, I mean, I've tried that. It's basically I've tried various different things, and I really can't remember exactly what I've gotten. Sorry.

That's perfectly fine. What was it gonna say the next thing that I was going to talk about dude? And then many cases or reasons to do like whole experiment replicates? Or do you normally just try and stick to like, one, one plate with everything on?

I prefer to do one plate with everything on? Yeah, but it all depends. I mean, when I was doing my old stuff, we only did 96 Well plates. So it was impossible to do the whole experiment. So basically, we just used to have, we had some something that we used as a sort of standard that you put in every plate. So that you could then refer that compared to the other ones.

But

how many wells are the plates that we're using at the moment? Typically?

And typically, how many probes Do you normally use?

It sort of theories. It can be one well, so I think I think I've always done at least

two.

I've certainly always done to 10 reps of each. So I would say at most so I've read four well plate is is what is 16 by 12. Okay, so I have done so you basically I would have six, at least six eight. Yeah, I would probably do the VIP kits on depends I have done across three as well. So in theory, you could do up to eight groups in two technical advocates, okay, depending on how many samples you have. Or it might depending on whether you do across we're up and down the V and the plane.

So when when you are figuring this out about like, in essence the dimensions of how do you normally pack all that, you know, we're like drawing it out or something. I don't know.

Okay, I can't remember how I was actually doing it generally. Oh, Sorry, I'm really not being very helpful here.

You You are like the pausing and thinking about our tourism porn as the things that the questions you're answering directly, because what you're thinking about is what the user is thinking about, and therefore, it's what we should be thinking about.

Yeah. So, like that one. Yeah. So that that one, I definitely did. 30 tool. So that was it. That's actually one of Adrian's ones, but it's the same sort of things that I will do. So he's got, yeah, he's got three technical totals. So he's got three different samples, right, at 30 and 42. And then he's repeating them four times across the plate. And that's, and then he's doing that. He's just doing that one. So it's one law as each probe in that case. Okay. I have done some more weird ones. It's just trying to remember what was fought.

Have you ever done well, do you regularly do like the actual proficiency primary efficiency calculations and do experiments just to pin down the actual premise themselves? Yes. So

what I mean, so a lot of the stuff I was doing was basically calibrating the primers. So I was trying to work out what the efficiencies were and whether they were near enough to the twofold difference that you would expect?

Sure.

Okay, so I mean, this is more personal question, because I don't, obviously, you've never done this. But for like, do you have to do a prime efficiency calculation every single time? Or is it kind of just, if you're a new lab, you probably have to do it. And then once you're a setup lab, then you probably don't have to do it.

You probably I mean, if you've got if you've got primers, and you're doing them on RNA from the same species, and, and something reasonably similar conditions. Yeah. And I would say, you can probably assume that the primer efficiency is probably okay. But I would still be inclined to check, especially if I haven't done something for a long time. Because you don't know how good your RNA is, either. So you've got to check your RNA. And that's actually quite a good way of telling you how good your RNA is, as well, to some extent. So, you know, because if everything's we off, because the only is just all over the place, then you'll get crap pipe payment efficiencies.

Next time and I've got like a whole list of things that I have to attend to when your actual actually like loading the plate itself, or you typically using like, electronic multi channel puppet thing? Yes, or

definitely. Nowadays, in the past, it was individual. That's why I only did 96 Well, plates, we did have the facility to do 384. And it was like, No, I My eyesight is not good enough to get that right.

Yeah. I just have it the

tiny volumes involved.

Are you interested in using the robot and automating it in any way shape? Or form? Or is that still? Like, are you still unsure about like, whether or not that accurate or?

To be honest, I don't know. Because I don't know how much any of the others have tested to do about for qPCR yet? Yeah. Okay. I'd certainly Yeah, it would need to be it would need to be tested. But to be honest, they, I mean, you could use the robot to do a certain stage. Yeah. But because I don't think the robot set up for doing it set up for 96. Wales. And it'd be tough to use the one in the genome foundry. To do the to do three four Well, plates. Yeah. Yeah. It's really it's more set up for doing a four times that was I can't remember what the number of times it's four times 12. Is it's 1900. And something or 1800. And something. Yeah. And there's no way I would want to look that manually. prepared, we've got it makes it very, very easy. And that's one of the reasons for planning your plate properly. To know how you can actually take your samples and actually make it that it'd be simple to do with multichannel. Okay.

That's very interesting.

So when you use this multi channel prepared like you first things first is do you have to do that weird, alternating because the distance between the pets is can't be the same size as the distance between Wales or something like that. And sometimes you have to do alternating.

Yes. So the way the way that multi channel prepare that we've got works is it Oh, it's got three different spacings. Okay. So you can set it to put it into one and a half mil Eppendorf tubes, which is probably where you've got your master mixes in. Yeah. And then you have other things where you've got your, your cDNA isn't are in 96, or in tubes that have the same sort of sizes and 86. Well, separation has a 96 well plates, you do it in a separate way. But you can see you can suck stuff up, and then you just press a button and it changes it to the 96 format. And then you can just load it and it sucks up multiple lots, and you can just do a little bit and then move it to the next set Wales little bit, move it to the next set Wales a little bit.

But what about like, keeping it even though, so if you're trying to put it in, like so many wells and like you're actually tilted it a little bit. So some of the ones at the end suck up less than some of the ones in the middle.

It's not too bad, I just I just try to make sure that I put it far enough down into the well and keep the tips angle against the, against the wet against the side of the tube when I prepared it. And then that way, you can be sure that it's pulling out as well. Because you get a certain amount of you know, you get certain of the meniscus, that sort of it sucks it. So it's much better if you actually put it against something rather than just dropping it in because they're small volumes.

Okay. The final question of this sort of segment is, once you've done your experiment, what do you do for quality control? How do you know? Typically, how do you know whether or not you've got like a decent, decent RNA to start off with? Or there's something wrong? or something's maybe the primer hasn't isn't particularly efficient? You know? Yeah, what's the first step you do in terms of like, quality control?

So, basically, so it's a mixture of, of doing graphs. So basically, various types of graphs that you want to look at, to see how well things are how well well, things are behaving from SAP from replicate triplicate, and from biological replicates from technical replicates, and also from biological replicates, looking at the slope of the curve for the primaries, making sure that, that that's okay. And then also, you can be, you can quite often tell, sometimes it's very obvious, because if you're, if you're already rubbish, you can usually see it very easily, because you just get shit results, you get next to nothing, whereas your other samples are fine. So you usually know from that, that that's not right. Else,

back when you were using the Excel spreadsheet, could you still do like plot all the graphs,

so that was on the on the machine software, you could get plots of how things were, and you get the slopes of your standard curves and all the rest of it. And if you had the same amount of r&d, you know, at least for your your, you're in control gene that you were using, then if that was way off what you would normally expect, you'd know there was something wrong, whereas if it was about where it should be, and everything was behaving as you were used to, and that would be alright.

So for these, the plots that the machine itself would make. Would you save that someone? Would you print them off?

Yeah, yes, yes. It depends, depending on what I was doing. But yeah, yeah. You but you could also save the whole thing, so that you could basically go back and open it up again.

And so but that would be more for like, your lab book or something? Yeah. So it isn't really like, it seems kind of weird, but it isn't very common to put the quality control graphs or anything like that in, in a paper, is it?

Yeah, I guess probably not. I certainly haven't in the past. Yeah. I might have put one in to show that, you know, it was this is this is what we see. And this is like, this is essentially what we were getting each time sort of thing and, and if it's anything that's much often that then we don't use the material we just ignore because you assume that there's something wrong. Okay.

Perfect. Um, right. Now, this is kind of an odd question, because it's like, describe the analysis pipeline of your most recent recent RT qPCR experiment where your analysis pipeline was pretty much tied to qPCR. So it's

exactly the last all the ones I have done since I've moved and Ted with lab have been using essentially Ah, a version of tidy qPCR.

Perhaps not the most up to date. And it was all done. I was done, but somewhat more manually, but yeah. Okay.

I think the only the only time I've actually used the tidy qPCR package when I was when I was trying to help those get her stuff sorted. That makes sense.

Yeah, so I don't think I'm gonna ask you that question because it seems rather rather bias. Um, but I will do the last question, which is just a brief outline of your previous programming experience, obviously. Experience like, obviously, you are, like, you have experience?

Yes. Yeah. Yeah. So I mean, I did some very basic stuff on my Mac for my master's project with a few others that reflects, I was basically just, I was doing some stuff that I had to try and work out. Oh, god, what's the word. So I took my input from my machine learning stuff through sort of what I was using random forests, I tried to try and get a get a dataset for to use as a training set and stuff like that I was using this thing called V surf, which was a means of identifying the sorts of stuff that you would put in the first place. And so that was basically simple. That was just largely it was fairly simple commands. And the rest of it was doing lots of stuff in gg plot, but not as fancy as the stuff I've learned how to do since then we've toyed with but yeah, I mean, in terms of I'm getting, I'm saying I'm getting to grips with ways of doing stuff working in the in the terminal as well as a console with our studio in perfect so I'm getting there I was just, there's still lots of things I'm not so you know, if things go wrong, I'm not always sure what the problem is. But

so coming from from from your position, which is someone who has previous art experience, but obviously not the specific packages that are used to the extent that they are in tiny qPCR how hard or how, how steep is the learning curve, even coming with your art experience, to use tiny qPCR or whatever elements that you

have. I actually think that wasn't so bad, because you're working from examples that Edward had given, I was able to mostly work out there I must say, I think that we've got the tidy qPCR package now where he gives the different ways of setting up the plate because my problem was that I was setting up plates to suit what I was wanting to do. And it didn't necessarily make it that easy to do the analysis as simply because it was more complicated. So by using the tidy qPCR package, I think it's relatively easy to work out how to do the analysis because it's you know, it's set up to work that way. Okay.

Now, speaking of tidy qPCR we can switch to actually use it so I think the best thing for you to do now is if maybe you just open a blank

script Yep.

File a New File Scripts or markdown because I usually do markdown markdown s and this is for Oh 520 20 Right

perfect.

Oops, I've just I've just started which was not very clever Oh, wait, I have a story. That's the next question.

Dammit. Oh because it's not here. Oh, wait, did I do I can't just start a new one fail you fail or mark dying See, I'm just going to call it that. Okay.

Okay. Whoops. Why is it not done that?

That's what it is a file. See? So that's why I didn't see that. because it was doing it in college I just do here if I can find it

sorry was finals PCR see it was cold okay right. Perfect.

So the first thing I'd like you to do is in the console at down at the bottom just see what vignettes are available for tiny qPCR

Yep. Let me make this a bit smaller so I can actually right

right. Okay, so

if you just type in, vignette,

yes, yeah. Yeah, then brackets.

Package equals. And then in inverted commas Yeah, I'm tidy qPCR right. So what do we have over here?

We've got plate set up. Multifactor vignette calibration vignette, InterDesign plate set up multifactorial example and probe calibration.

100% remember if there was on the intro to design place, I think that's horrible. Whether or not that's the latest ones or not. Whether or not Edward changed their names in any way, shape, or form. Give me one second. I'm

just gonna check. Yeah, that seems right. That seems. Okay. That's fine.

So if you open up the vignette on plate design,

right, okay. Okay, so sorry, I'm just I'm really bad at necessarily doing stuff in the console. I tend to do it in my

fine. If you just go on if you go back, so vignette, if you type vignette again,

yeah. Okay. Right. And then brackets, and then in inverted commas, the intro design

plate setup or whatever it is? Yes.

Okay. Yeah.

I think it's plate setup vignettes. Probably what you want to put in? Oh, yes. Okay. Okay.

Say yes, there we are.

Yeah, and then I see all the information about setting it up in the panel on the right hand side

of it. How have you seen this before? Have you read this before?

I don't know if I picked it up to quite as same as up to date as it is. But I've certainly seen some of this stuff.

Because the next thing I'm going to get you to do, if you see the PDF that I sent you earlier,

yes.

It's just a create a really, really simple, really small, eight by 12. Well, plate. Right. Okay. With these certain holes. It actually says April. It's only meant to be four probes. And just to try and use the vignette, if you get stuck, but just to try and make this plate this plate. Yes. Okay. And obviously, like we did in the test, it's just

No, I'm just stay. I'm wondering with it. Is this just a multifactorial vignette or is this a calibration vignette? Because presumably, it's going to determine which one I'm going to use is going to matter.

So, because typically, to the plate set up towards the end of the plate survey, it's got some ideas on how to actually design the plate, more scale, you can go on multifactor vignette as well. cuz that's got like a very

quick Yeah,

this one has a more detailed overview in designing smaller smaller plates because we kind of want to quote rather than a larger one

yeah okay,

so sorry it's quite hard to read through all this just really quick

but again, I've set aside quite a lot of time for this particular thing so yeah,

I need to set up 96 Feel free to okay

that's up to the insects possible smoke plate setup and lots of photoreal being able to follow okay so

anyway yeah, okay, so

we will have right

yeah, this is where so this is where I'm getting a bit I'm also getting a bit confused because because I always write everything in our markdown on the thing I'm gonna have to mess around creating various various sorts of stuff to make it work because I'm not used to just doing it in the in the console directly.

Um, I mean, you can switch and swap however you want you can you can whatever is easiest for you if you want to like flip back and just do it in Montana. It's fine I mean, whatever is more comfortable for you

Yeah, I'm just trying to remember what I did with the other stuff? Because I've got the same file right yeah I'm sorry I'm just yeah it's just because it's labelling stuff for our please set up that'll do right I'll just do that okay so I need to do I always miss out the extra in life

yes so far the

crops the crops were

seeing on

my typing shirt, so you're gonna have to come up with this

T two and

HT Okay, and then we've got to do looking for sweetness we're doing eight by 12 plates. Meat by 12 So we've got travel eight rows and 12

columns two conditions

multifactor vignette Okay, so I really want to multiply the two vignette makes more sense sorry I'm just know what have I just done I've just typed the note oh just shit that was actually in my stuff I was trying to do right Are we

okay sir. Okay, so we've got three technical problems. snorkellers one strain okay conditions right

that's 116 and I'm not going to do 116 for that so lucky enough to be so 116 So 96 rows I've got

eight so that's

okay

como annoying because this is not as big as I would like it

to be really hard to read this I think we'd be much better if I actually had this out as a PDF

to read it because it's not

a frame there we are that works it might be easier

but well it's our crew people's back to our troops okay right

okay okay so plus or minus this so right now two conditions plus and minus associate sorry six conditions right um

really the one I wanted to use

so, if you go scroll further down on the designing Yes Can yet

yeah that's what I'm trying to do the label and plan plates but it's just it's the ones that Edwards got are so much more complicated and it's just trying to get my head around exactly what I want to do with it, right because I can't remember how to do this eventually but

no, no, it's perfectly fine if you On the note on the multifactor on the design vignette all the way it starts off by telling you how to make a 46 well plate and then later on tells you how to in essence just double it so that you can make a 96 well plate yeah

okay all right. All right okay. Oh yeah manually yeah here we are probes against right okay, there we are right that's fine. Yes. Okay. So pubsey is 14 so biological replicates yes so that we just do yes so I think that's what I need to do sorry yeah

yeah

okay

right so we need for I do that what are we doing so so Byron I need to go see a capitalist and that's not what I want to do Sorry see one three

do that again

okay that's what is right okay yeah so it's that's what is

right we'll hear at all Oh yeah, I've lost where I was part experiment right control okay so see ya liking yeah so that's what I want to do right Okay, so

I'm gonna leave that just and then I'm going to do Yeah.

It's not what I wanted to do.

So if I can,

yeah, you can for a second. Yeah, sorry.

No, no, it's been, I think probably the easiest way. For now. Yeah. Yeah. You pick no treatment section. We can add that in. Yeah. Okay. All right. Keep it there. Keep it there. Alright, okay, we're looking in terms of tackling this problem. Yeah. If you follow the steps on so underline we're nine 150 on the plate set up a vignette.

Right I'm just trying to work out where that's gone now because I seem to have lost it right I'm just going to have to run that again

Yeah, right okay. Yeah

14 So okay goes for yeah so that's a low key because for yeah that's why I wanted to keep was down below we want to do that yeah, okay okay for

now have done that sorry, I'm completely people watching me makes me nervous that I'm just making a mess right okay. And then

yeah, yeah, okay yes you're missing

I know I know loops Well yes. So as paint yeah your mind that didn't work but anyway

so yeah, I think you need to also include the tidy verse package and the

yes, yeah, I thought I Oh, tidy verse. Yes, of course. It's so it's yes. So I do yeah, of course I do. So that's what's wrong yeah

okay

okay real key

levels yes because I haven't actually put any levels in yet it's probably because it's because I did I've done this wrong I haven't done this the way it said so I'm just going to sort that yeah, so Okay, sorry. That's what I did wrong for I actually copy it because props Yeah, I was trying to get myself to think

okay, then

we name it you called it rookie and then rookie for in the press so

I did it yes rookie for that's what it is sorry

right okay it has hasn't printed

if you scroll down

Oh, it does sorry. Yes, of course. Yeah. Something And markdown. So yes. Yes. So that's fine. Yes, sorry. Right. Okay. Right. So now Yes. Okay. So now I can go on with it right I'm get my job done. Oh, right. Okay, so some polls

see come on coma. Coma oops wrong way helps Vansickle you

Titus copyedits Come on yep that's fine and then

oops okay hold information about likely it was likely yesterday Okay so we do IPS IPS C three right okay so yeah you're not seeing it that's because it's four no sleep virus

oh you're missing the E and create

Oh Where am I? Oh yes why I'm class What's wrong next and that should be one to four or no it's noise but yes three that sounds fine I'm talking about starts with tears right okay

yeah I don't

I don't need to print this yeah I need to print it to a table yeah it did sorry Yeah, you can't print it. That's because it's an object so I did I did wonder I'm just like, yeah terrible that was it. Well, our Yeah, that's got it. Yeah, yeah. Okay. Right. Okay. So right. So we've got that then access helpers.

Right. Okay. Play plan. One, two, okay.

Right. Three take reps so that's so I'm just being the 12 Well, many places no, then other than that.

Yeah. You will have done the 12. Well, well mini rep. So the next step would to make it with the negative rt I don't think you've put a negative in our team. No, they've all been positive.

Yeah, because yes, so it should be plus the tape should be plus R T comma

S R T, o r t the de

minus R T. Okay.

Yeah, what my day? That's not what to do okay, no, that didn't like that. What am I doing?

That's because there's only three technical outputs plus minus RT. Yeah.

You haven't made it a vector you have need to put a C. Oh, so I have

to helps. Right? Okay. Yeah. And then yeah, yeah, I've done that before right next, we're happy with that anymore ah three columns. We can call it, but we need to track because we need

it's not C equals. Yeah, no, that's a repeat and then you repeat it.

No, oh, yeah. See? That's me sense. Columns and samples. So

if you see how they are Edwards define types just below. Yes. Yeah, sure. Code. Yeah. Actually written?

Oh, yes. Okay. So he's just he's done it specifically like that. Okay. Yeah. Okay. So

at the moment you've got, yeah, three? Yes. Well, you've got four technical replicates for one of the

Yes, yeah. Okay. So yeah, because no normally Yeah, no, that makes sense. Yeah. That it's just okay. Yeah. Yeah. Okay. So if I run that, sorry. Just what's your take? Because it's after four.

Yeah, I think I've got a few more minutes and then I'll have to disappear. Right? I still haven't been told

there's something else I've done wrong. Samples straight 123.

Yes, types equals that.

It's telling me there's an error. Alright, so now our language error.

So the issue you have now is that you've replicate you've repeated the plus or plus RT and the negative rt. Three plus aunties and one minus RT for each biological replica. Yeah, you haven't multiplied the number of biological replica tags. So that matches the number of Yeah, okay. Hardy tags. Yeah. So if you scroll a bit further down, you scroll further down

on. Okay, right. Yes. cookieless 12.

See, it says sample equals read. samples times. Yes,

yes. Right. Yeah, that's what it is some Yeah.

Okay

I need that further up.

You could probably just put it straight into the so you've got cold key three. Yes. And in that table Yeah, well see which should now Yes. One to 12 Yeah, that's what it is. Yeah. And then that samples there should be sample equals rep samples. Yes. That's

what it is. Sorry. Right. Yeah.

Yeah

I was just I was getting some yeah sorry Yeah, to delete

samples tentacles for

clothes that are fuel. Rockets and a comma.

Yeah. Yes. And then type equals.

type equals pipes. You've got that right.

Yes, that's okay. That's fine. Right. Okay. Right now maybe

I'll run

samples not found? Oh, that's because I put samples

Yeah. Cool. Yeah. estimate a bit different. So okay. Yeah.

That's all good. All right.

Okay. And then I do yeah. And then you just that's,

that's what you've done. That's what you

that's all I need to do. Yeah.

Get rid of that. Okay. So

and then it would be putting that thing together. So you want me to do that now? Yes. Right. Okay.

And then I'll probably have to call it a day because I mean, yes,

obviously yes.

That's because

you just need to rename your cookie. So it's called cookie three.

It's cookie three. So as no, that should be cold. Yeah, that should be cold. That's because it shouldn't be cold should be cold key.

14 paper that

you didn't know.

Did I do the wrong thing? Yeah.

I don't think you actually ran it. I don't think you run that. Did not

know, I'll catch on the whole thing. And that would make sense. That's probably because I haven't done that. I just do it that way. Yeah. Yeah, okay. Sorry. Yeah. Right.

I'm glad that the vignette at least tolerable.

Yes. Yes, it is. I'm just, I'm just, I'm just my head's not in the right place for working out. And then I see it and I've got my playtime there. So that's fine.

Yeah. It seems like you've worked through it. And yeah, you just all it would be a manner of spending an extra 10 minutes to add the extra level of the conditions on top of it, and then you'll have a 96 Well,

plate. Yeah, exactly. Exactly. Right.

It seems relatively logical. I hope.

So. Yeah. No, I think it is. It's just I There's nothing worse I hate I when I'm trying to do something. When somebody else is sitting there, I just get so nervous and my brain just doesn't work properly. It's good. Yeah. So yeah, no, it's fine.

Thank you so much for your time. You're welcome. Yeah, it has been really useful. And I just needed this experience. So I can go to this, this user experience guy and just saying, This is what happened. Do you have any advice for this than the other? Yeah. Hopefully it wasn't too much of an ordeal for you.

It was just embarrassing.

It's been really fun. Yeah, thank you again. You're welcome. And I will stop

Interview 6

Unfortunately, the audio file for interview 6 was corrupted and could not be transcribed. Only hand written notes by myself remain which I have not copied here.