Automated Characterization Method for Validation of Epigenomic Editors in T Cells

0:05
Can you hear me?

0:05
OK.

0:06
There we go.

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I'm going to go ahead and get started.

0:10
Thank you for being here.

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My name is Dan Imhoff.

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I'm a scientist at Tune Therapeutics.

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I'm grateful to Curiox for asking me to be here to share some of our experience Using their Auto 1000 system.

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We've been working to automate some key flow cytometry methods to support the advancement of our epigenomic editing pipeline and I'm really excited to share that with you today and also talk a little bit about how we at a small biotech evaluate automation platforms to integrate into our smaller, a little bit more fluid research environment.

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So at Tune, we're trying to leverage the power of the epigenome to create the next generation of gene therapies.

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If you're not familiar with the epigenome, think of it having to do more with the way the DNA is packaged rather than the sequence itself.

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And epigenomic editing or EPI editing or genetic tuning is the way that we leverage the mechanism of control of the epigenome to drive gene expression to a desired state to bring about a clinical outcome.

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And we do this using our TEMPO platform.

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And TEMPO is primarily comprised of two main components.

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So we have a DNA binding domain and an effector molecule and together they make what we call an EPI editor.

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The DNA binding domain can be a zinc finger protein or a cast system with a guide RNA.

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And there's a lot of effectors that we have at our disposal and they all act on the epigenome in different ways to different degrees.

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It can be activation of a gene repression, can be transient or it can be more durable and even heritable.

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So we have a lot of flexibility with this platform to bring about the desired outcome and to take that a step further, one of the things that this allows us to do is to not only target single genes, but really get inside complex gene networks and manipulate multiple genes at the same time to bring about that effect.

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And with less than a, it's not so much a binary on off switch, it's more like a volume knob on your guitar amp where you can change that that volume with a lot of precision and you can do that like I said across all these complex networks or on an individual gene.

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One of the main ways that we leverage this technology in T cells are through these large genome wide guide RNA screens.

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If you're not familiar with this process, we synthesize a large guide RNA library typically targeted at any number of potential genes of interest, and we introduce those to the cells such that there's one guide per cell.

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And that allows us to, once we've added our EPI editor, to use flow cytometry to really pick out the cells with the phenotype that we're after, whether that's a surface phenotype, A functional phenotype And flow cytometry has traditionally been a really powerful way to do this.

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But those assays can be complex and require a lot of resources.

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Very, very briefly in case you're not familiar with flow cytometry cell preparation, we have a staining step where we're complexing the cells with fluorescently labeled antibodies targeting our markers of interest.

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So going back to thinking about what phenotypes were after we build panels specifically to target those phenotypes be it surface or functional and then we go through data acquisition where the the cells are pass single file through the pass of a laser, the light is excited, emitted and detected and then pass forward for analysis.

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In the data acquisition phase, we also can sort cells for downstream sequencing and then plug that information back into another screen with a more refined library to validate those targets.

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And when we think about automation where we really need the power of automation is in the staining portion of these assays.

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They're complex, they take a long time to develop and fine tune once they're ready to go.

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They're pretty straightforward and repetitive, but they are time and resource intensive.

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So for us, we saw in a small biotech environment the value we could gain by automating these activities and taking that time and giving it back to our operators to do other functions.

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This is a typical ICS workflow.

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These workflows can be designed in many different ways, but you essentially do a surface blocking stain.

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You're washing, you're fixing and permeabilizing the cells to get at the intracellular markers and then doing another series of blocking and staining steps.

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And one of the big drawbacks of these methods, you can see all the wash steps that we have here.

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And traditionally this has been done with centrifugation, which is I'm sure a lot of you have experienced, contributes to significant cell loss in these assays.

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So at all these different junctures, we're losing cells, losing cells at up to 12 times throughout the assay.

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And for you know traditionally at least in my experience working with healthy T cells, working with PBMCS, the way we account for this is by adding more cells to a well and we just accept 75% loss.

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We get our 250,000 cells and we go on.

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But as the field of cell and gene therapy moves more towards rare and sensitive cell types, things like CAR, T cells, tills, IPSCSNK cells, that loss doesn't really work anymore.

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We can't tolerate that kind of loss.

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Cells are expensive to produce and they're not always available in the kinds of numbers you need to do these large screens.

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So we were experiencing this issue and we realized we needed to try and find a way to get around centrifugation that enabled these high, high dimensional screens to be successful at their current scale or larger.

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And the other part of this I mentioned a little bit before is we were really looking to take that time to give it back to our operators because at a small company we need that those man hours used for other things once these methods get to a point where they can be easily automated.

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So we had first experienced through a demo the power of the Curiox washing system in a previous role, but there was not a liquid handling component at the time and it didn't really fit our need at that point.

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But when we came back to it, we were excited about the liquid handling option.

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And the first thing I wanted to highlight that we noticed was how simple the system was.

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And at a company like Tune, we don't always have the resources to staff departments of automation engineers or even just specific personnel to run these methods.

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So we really needed our solution to be accessible to even automation novices, people that are even new to analytical development, so that we could see these gains without adding additional resources to infrastructure and head count.

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I guess I should say one more thing about that execution.

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We've seen really good success with really low training thresholds for users with this platform and it's been really powerful for us.

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We love that.

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Now the washing platform came with this full automation of our assays from end to end.

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So no pipetting steps have to be performed by an operator.

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Everything is done in the instrument and they can walk away and come back at the end.

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And the biggest thing for us was that they have replaced centrifugation with this laminar wash technology and this ended up being one of the main reasons we went with this platform.

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I'll show some data from some of the work we did to look at the quality of that wash in just a second.

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But I want to explain for those that aren't familiar how this works.

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I do encourage you to go to the booth because they have a lot of people here who know this you know really well as well.

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So they can give you that information.

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But essentially using their proprietary plates, you plate the sample and you let the cells settle by gravity.

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And then the two probes come down and perform the buffer exchange at a really slow rate in such a way that it creates this laminar wash phenomenon where the velocity of the wash is the highest at the top of the bubble and at the lowest or almost zero there at the bottom of the well where the cells are.

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This is a fairly rudimentary image that I put together, but I think it gets the point across.

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You know, the real benefit here is that the cells undergo essentially no stress.

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And a nice benefit too is that there's really not a lot of loss or at least that's what it appeared to us.

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And so we set out to test this in our own hands and we ran our existing manual ICS protocol along with a protocol that we adapted for use on the Auto 1000 to look at cell loss specifically using healthy T cells, which is what we had at our disposal at the time.

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And what we saw was a two fold increase or more in cell retention using the laminar wash system versus our traditional centrifugation protocols.

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So for us this was a big win because we also were dealing with instances where we had low cell inputs and we weren't able to perform some of these methods because we would lose essentially all of our cells.

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So this was a nice win for us.

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And as a secondary win down at the bottom you can see and if you're not familiar with flow cytometry, that's OK.

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The, the key take away here is that we saw really reproducible staining quality in our data with this platform.

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So we didn't see a negative impact from the laminar wash on our staining profile.

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The data was really reproducible, but as you start to get down to the lower plated amounts, you can see the value of the difference between having twice as many cells.

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If you're getting a lot further down in your gating tree and you're getting lower and lower event counts.

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So we're able to resolve our populations a little bit better, which is gonna be really key for some of our main applications.

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We wanted to take this a step further because if you remember from that diagram about the the guide RNA screens, we're really looking for functional phenotypes in these screens and we're trying to identify those, grab them and then take them for downstream sequencing.

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So we wanted to look at cytokines.

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This is one of the main things that we look at when we're working with T cells and this this study we we sort of piggybacked on an existing effort in the lab and we received 50,000 cells for each condition that they were running and just ran them in parallel through the ICS method on the Curiox and let them do their own manual execution.

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We didn't really align on, we didn't have the same person do the analysis for example.

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We just sort of said let's just test it and see where we're at and we'll go from there.

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And we were surprised to see a really nice correlation between these key readouts with very little optimization, very little effort to fine tune or make the system fit within what we were wanting.

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And that reproducibility right out-of-the-box was really helpful for us.

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We could move really quickly.

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We could deploy this and get our operator's time back really, really soon after we implemented the system.

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Now there's we're currently in the process of doing some due diligence optimization.

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So we actually expect these numbers to get tighter which we're already starting to see and hopefully I'll have some data to share on that in the future.

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But this is what our ultimate goal was, was to enable these types of readouts to be achieved with very little operator effort and to have that kind of reproducibility to replace our manual workflows and not have to do large scale comparability studies to justify that switch.

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So to go back to some of these things that we feel like the auto 1000 has given us or helped empowered us to do, you know, the flexibility is something that stands out to me.

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I haven't mentioned this yet, but to host multiple flow panels on this platform and move between them doesn't really require any adjustment to the system.

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The user interface for setting up your assay is really simple and once you've established that method, you can save it and come back to it so you can flow really quickly between the methods.

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It's not complex.

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There's no adjustments needed.

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The ease of use was the best I've seen from a flow automation system that was end to end.

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We have people that are brand new to flow and have no automation experience and it takes us about half a day to get them operating on this instrument.

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So it's a really nice tool for us.

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We can be flexible with everybody kind of running their own experiments on having to take anybody's time to be the person who, the one person who always runs everybody's, everybody's work.

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I've mentioned this a few times, but we've reclaimed a lot of operator time and in a company the size of ours, this is tremendously valuable for us.

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Anytime you're, I feel like you're moving to automation, obviously you're going to reduce that potential for human error.

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And as we've continued to run the assays over and over, we're seeing those improvements in accuracy in real time and we're excited about what that's going to mean for us down the line.

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And I can't overstate the quality of the reproducibility, especially out-of-the-box.

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We were not expecting that to require so little optimization to get us to where we were, but we were really impressed by that.

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So I've taken that same data that I showed a couple slides ago and just sort of gone down one level deeper to illustrate that using these methods we can see the, the magnitude of the, the tuning that we're doing with our Tempo platform.

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And we were able to do this with this new automated method in a way that doesn't compromise data quality, doesn't compromise the integrity of the data.

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And the added cell washing component in replacing centrifugation allows us to expand the use of these functional screens to other cell types we've been wanting to leverage, but we weren't able to before, and that's going to be powerful for us going forward.

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And as we increase the scale and scope of our delivery pipelines, we really feel like this platform is going to allow us to expand our analytical capabilities to match those screening efforts and help drive this platform and these therapies to the clinic a lot faster, which at the end of the day with all the momentum that's sort of popped up behind Epigenome editing, it's going to help us to continue to push the envelope and be at the vanguard of that technology as it moves towards the clinic.

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And that's it.

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I'm happy to take any questions about the platform or our platform at Tune, but I appreciate your attention and feel free to come and grab me afterwards if you have other questions.

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All right.

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Thank you very much.