WEBVTT

NOTE duration:"00:27:46.0500000" language:en-us

NOTE Confidence: 0.937588512897491

00:00:00.490 --> 00:00:34.420 So uh turning now to the topic at hand, which is our shared resources as many of you know, we had our renewal of our Cancer Center. Grant last year. It did extremely well get garnering a 73% funding increase, and there is no question that a great deal of the credit that goes to that success is our shared resources beyond our research programs and we're so pleased to have shared resources that not only provide the needed support to advance the mission of research.

NOTE Confidence: 0.926535606384277

00:00:34.890 --> 00:01:03.880 In their own right or innovators to coming up with new ideas and I think over the course of grand rounds. We're going to review or we're going to have some of our shared resource leaders lead to present their their efforts, but to present the share with us introduce today's speakers. I wanted to turn it over to doctor. David Stern, who is a professor pathology and the associate director for shared resources to introduce our speakers.

NOTE Confidence: 0.896746039390564

00:01:05.260 --> 00:01:07.510 Thank you Charlie.

NOTE Confidence: 0.927432775497437

00:01:08.290 --> 00:01:39.380 I I don't want to cut in much to our speakers time, but I do want to remind all of you that the Cancer Center has a translational mission that comes from the bench to the bed and goes back and forth and ultimately the goal really is to impact patient patient care one of the most highly leveraged investments from the Cancer Center, including CCSG support is investments that subsidized shared resources and also develop new ones, and then.

NOTE Confidence: 0.88299834728241

00:01:39.890 --> 00:01:41.080 Is to make your?

NOTE Confidence: 0.941412448883057

00:01:41.810 --> 00:02:06.320 Basic and Translational and clinical research, most impactful and to that end, we have 14 Cancer Center supported shared resources. I don't have time to enumerate them now but please go to Google or your browser of choice. Search Yale Cancer Center shared resources and you'll see the list of resources.

NOTE Confidence: 0.922864675521851

00:02:07.010 --> 00:02:29.400 That are either subsidized by the Cancer Center or supplied free to you by the Cancer Center and they support a range of cutting

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edge activities. Today we have talks from 2 of our Cancer Center resource leaders. The first from Dave Calderwood is about a brand new Cancer Center supported core.

NOTE Confidence: 0.947439312934875

00:02:30.100 --> 00:02:42.370 That is the functional genomics shared resource Dave is an associate professor in the Department of pharmacology and an active member of the signal transduction research program, thanks Dave.

NOTE Confidence: 0.847605288028717

00:02:45.480 --> 00:02:47.470 Thank you David.

NOTE Confidence: 0.838689804077148

00:02:52.820 --> 00:02:53.430 Sorry.

NOTE Confidence: 0.807256102561951

00:02:54.370 --> 00:02:55.840 I have to find my toe.

NOTE Confidence: 0.888596057891846

00:03:12.160 --> 00:03:14.020 OK, hopefully, we're in business night.

NOTE Confidence: 0.864024758338928

00:03:18.260 --> 00:03:33.820 Yeah, so thanks for the introduction and as David said. I'm here to talk to you about the knew functional genomics, core that I'm running together with Van Turk, who's also in pharmacology.

NOTE Confidence: 0.882953941822052

00:03:34.580 --> 00:04:02.230 And this core grew out of work that we did together through the yield ghillie. Add collaboration where we set up a lab that was interested in running SH. RNA and then in subsequently running crisper cast 9 based screening strategies and the goal of the genomics functional genomics. Core is to support pulled SH. RNA or pulled crisper cast 9 screens that will advance the mission, and cancer research at Yale.

NOTE Confidence: 0.895100891590118

00:04:02.980 --> 00:04:29.970 I think a great many of you in the audience know what I'm talking about when I'm talking about pulled SH. RNA or Christopher screening. But since part of our goal today is to build an audience. I want to take the opportunity to explain a little bit more specifically what this involves and so the idea behind these screens is to identify jeans that iponan activation at Perturber specific cellular phenotype.

NOTE Confidence: 0.875735223293304

00:04:30.870 --> 00:05:00.570 And so the strategy for achieving this screen is to generate a pool of plasma that contains SH. Rnas for knockdown specific jeans or SG. RNA is to knockout specific jeans and that mixture of plasmas, which

2

would number in the hundreds or thousands or many thousands can then be packaged enter lentiviral cool and this mixture of lentivirus can then be applied to the cell line that's being investigated.

NOTE Confidence: 0.910153210163116

00:05:01.270 --> 00:05:09.140 And this results in a complex mixture of cells each of which is knockout or not down for one specific gene in that pool.

NOTE Confidence: 0.918578028678894

00:05:10.100 --> 00:05:13.660 And this population of cells can then be subjected to the screen.

NOTE Confidence: 0.885041952133179

00:05:14.330 --> 00:05:45.640 And that screening modality can really vary. It's limited only in a large extent by the imagination of screening group, the most classical would just be a viability screen where we'd be looking at cell survival. This is being conducted extensively in a variety of groups and no really. Perhaps more interesting aspect of this is to perform these viability screens in the presence of a specific drug or a specific targeted inhibitor to look at agents that would synergise with or antagonize the effect of that inhibit her.

NOTE Confidence: 0.913877844810486

00:05:46.430 --> 00:05:58.750 Alternatively, if you can generate a reporter selling for example, where GFP is activated under certain conditions that you can screen for agents that would disrupt the activation or would enhance that activation.

NOTE Confidence: 0.911899268627167

00:05:59.570 --> 00:06:27.080 And then finally in a slightly more complex set of screens, but ones that are probably of interest to this audience that you can also take these sorts of screens in vivo if for example, you use your infected cell population to initiate tumors in mice and you could then screen for jeans present within the tumor cell that would be important for tumor growth. Tumor sensitivity to specific agent. Sumer sensitivity to immunotherapy or something along those lines.

NOTE Confidence: 0.894105613231659

00:06:28.650 --> 00:06:59.990 Once the screen is being performed you then harvest cells at the far end and using by assessing the abundance of sales containing each specific SH RNA using a lumina sequencing in the starting population and the ending population. You can identify cells that are enriched or depleted depending on which specific hairpin or SG. RNA they control. They contain and that allows you when you apply specific informatics pipelines to generate a ranked list of jeans.

NOTE Confidence: 0.928228497505188

00:07:00.650 --> 00:07:04.270 Practice the phenotype either positively or negatively.

00:07:05.050 --> 00:07:07.740 And so really I hope you can imagine that.

00:07:08.410 --> 00:07:15.940 Using this sort of approach that provides the opportunity to screen for a lot of different interesting things depending on your own research.

00:07:16.610 --> 00:07:28.230 So if you're going to do this at what do you need well obviously you need an SH or an SG RNA library to start with that you need an efficient ideally nonbiased delivery system.

00:07:29.010 --> 00:07:51.950 Need suitable cell or animal based screening strategies and an analysis methods or pipeline and some way of Validating. The hits that come out of the screen to ensure that these are real and valid points and really the goal of our core is to provide you support at all those various key steps along the way of performing one of these screens.

00:07:52.610 --> 00:08:12.640 So what we set out to do is to provide your Cancer Center. Investigators with a variety of resources that will help them perform these screens. We are knowledgeable resource for investigators that are planning screens and we're happy to discuss with you if you have things in mind. In terms of the feasibility or strategies that you would maybe need.

00:08:13.410 --> 00:08:25.640 And perhaps most importantly, we have SHRNEN crisper libraries that are either genome wide or gene family targeted and we can provide those at cost to labs.

00:08:26.670 --> 00:08:41.400 Will also help with the bioinformatics in terms of analyzing that next generation sequencing that will ultimately lead you from the raw data counts to a hit list of jeans that would be of interest to you in the outcome of your screen.

00:08:42.530 --> 00:09:10.820 And then finally and this is an area that we've already been helping quite a few labs with that, we can then provide individual SH. Rnas that can be used in targeted experiments to validate results that come out of screens or to perform mechanistic studies and of course, those sorts of

studies studying mechanisms of specific jeans don't have to be limited to the results of screening strategy. We can provide SH rnas for jeans that you would just be interested in from your lab.

NOTE Confidence: 0.89415568113327

00:09:11.440 --> 00:09:18.980 And finally we've obtained the human orpheum, which is a collection of C DNA's that can be used for rescue overexpression studies.

NOTE Confidence: 0.901540219783783

00:09:20.020 --> 00:09:43.740 So with that summary. I just want to point out that we have now activate the core is actor from live. We have a website. That's up on this website that that's the website that David was talking about earlier and so please visit the site and it will provide a list of facilities and resources that we have available.

NOTE Confidence: 0.901405453681946

00:09:44.480 --> 00:10:14.650 And so I just want to start step through some of the key things that we've got just to make sure that everyone has that information, although as I said more details are available on the library. So are crisper cast 9. SG RNA libraries are ones that we imagine people would be quite interested in these were all originally developed at the Broad Institute and they're pulled libraries for either mouse or human jeans and we can make them available under an umbrella MTA agreement.

NOTE Confidence: 0.848759472370148

00:10:15.580 --> 00:10:35.630 These contain a single vector plenty crisper version 2 plasma that allows expression of both the guide RNA and the CAS name from the same vector and that's important 'cause. It means you don't have to pre generate a cell line initially expresses the cast name.

NOTE Confidence: 0.854108154773712

00:10:36.690 --> 00:10:55.550 We have both their human and most libraries and as well as genome wide libraries, which target more than 19,000 jeans that we also have specific libraries. For example, targeting the Quenum That's to say all canius and Canius related targets of about 700 jeans.

NOTE Confidence: 0.853142201900482

00:10:56.330 --> 00:11:12.070 They're generally 4 SG RNA spurgin, ensuring built in Redundancy in the system and all these will be available either as pulled plasmid with which you can generate your own lentiviral cools or as lentiviral pools.

NOTE Confidence: 0.893979668617249

00:11:14.320 --> 00:11:31.540 The lamp started off doing SH RNA screening that's not as popular anymore. I think most people are wanting to do. Chris

previous screens. Although we do still have the option of doing SH. RNA libraries and for this we use the mission wide human and mouse libraries that we purchased from Sigma.

NOTE Confidence: 0.852050364017487

00:11:32.320 --> 00:11:53.630 Square originally generated by the RNA I consortium. Also at the broad and they have a wide coverage of human and mice jeans at with an average somewhat variable average of about 6:00 SH. RNA is per gene and once again, they to allow stable lentiviral transduction and have been widely used many of SH ironies there are validated.

NOTE Confidence: 0.856727063655853

00:11:54.320 --> 00:12:12.200 And here we can provide as it was the case for the crisper libraries. We can provide them, either, as pulled plasmid. Oras lentivirus and in this case were providing ones that are targeted gene pools that we ourselves have generated either targeting all that kindness is an phosphor teasers.

NOTE Confidence: 0.825384199619293

00:12:12.730 --> 00:12:23.000 Chrome atanor epigenetic related proteins or the ubiquitin ligases or ubiquitin proteases. These pools are available from the core.

NOTE Confidence: 0.913475275039673

00:12:23.530 --> 00:12:24.600 As I mentioned.

NOTE Confidence: 0.848079323768616

00:12:25.200 --> 00:12:44.010 We also had the individual hair pins for all the mouse and all the human jeans and these can be provided as glycerol stocks. Or as cultures and are going to be useful for validation or mechanistic experiments writing out of?

NOTE Confidence: 0.744478285312653

00:12:44.800 --> 00:12:45.480 Screens.

NOTE Confidence: 0.869608581066132

00:12:46.100 --> 00:13:14.030 And then finally in terms of reagent that's available. We have obtained the Orpheum Human Orpheum Collection, which is about 17,000 full length or nearly full length. So you have to check human see Dnas. These are present in a gateway vector. Most of which have an open C Terminus, allowing you to get wake lawn into something with a C terminal tag, which will be very helpful for rescue and overexpression studies.

NOTE Confidence: 0.887153089046478

00:13:14.990 --> 00:13:45.280 So those are the resources that we have available if I have a little bit of time. What I would like to do now is mentioned just a few screens that we have run to provide some sort of background information.

Some of the things that we've done and that will be kick start. Some ideas that of things that you might like to do, if I get a chance. I'll try and touch on all three of these but I'll start off and I'll say most about the more classical viability screens and this.

NOTE Confidence: 0.91411018371582

00:13:45.790 --> 00:13:50.870 So I want to talk about here were performed initially in collaboration with ghillie add Sciences.

NOTE Confidence: 0.885410249233246

00:13:51.490 --> 00:14:13.490 And they focused on a search for modulators of back Promo Domain Inhibitors and as many of you will know the bed. Romo domain inhibitors of class of complain that become considerable interest in recent years because of their potential to alter the expression of important cancer targets that were previously thought to be undruggable for example, make expression.

NOTE Confidence: 0.857890903949738

00:14:14.190 --> 00:14:30.950 And the bedroom would've been inhibitors and the classic tool component is called JQ one at work by binding in a pocket in the bet domain of the BRD 4 protein or BRD four family members and preventing the binding of this protein to acetylated histones.

NOTE Confidence: 0.852399468421936

00:14:31.670 --> 00:14:55.020 And that in turn has an impact on the overall expression level of these target juice and so ghillie. Add like a number of other companies had generated programs to identify back Promo Domain Inhibitors and BRD 4 inhibitors? What what they did with us was they set out to screen for jeans who would modulate that promo domain activity activity.

NOTE Confidence: 0.857460498809814

00:14:55.790 --> 00:15:11.180 And so we did this in a panel of colorectal cancer lines, which were transduced with the K Norm Library for Target all non. Canius is because this is a good target for future drug discovery.

NOTE Confidence: 0.863042294979095

00:15:11.920 --> 00:15:29.290 And so by culturing these transduced cells in the presence of either DMSO control or low and high dose of the ghillie. Add inhibitor or the general tool component inhibit her we could identify jeans that would potentially it or inhibit the activity of these inhibitors.

NOTE Confidence: 0.0855639725923538

00:15:30.080 --> 00:15:30.730 At.

NOTE Confidence: 0.894574522972107

00:15:31.870 --> 00:15:43.690 And this was one of the first screens. We did, and so we were really quite excited. When we first looked at the results and the results here showed that in the presence so this is a reiger analysis of.

NOTE Confidence: 0.87427294254303

00:15:44.340 --> 00:16:06.420 SH Aranese that would drop in that they were enriched in the cells that came out at the end of the screen. And if you compare the conditions in the presence of the Roman Inhibitors or in the absence. The notable factor was that at the top top ranking gene in every kiss in the presence of the inhibitor was this protein trim 33.

NOTE Confidence: 0.876284897327423

00:16:07.520 --> 00:16:30.450 And you can see that if you just look at the rank. The SHINA's across the entire library. You can see that in the DMSO situation trim 33 was not enriched so losing trim 33 protein was not good for growth in that setting but in the presence of the bedroom modern inhibitors.

NOTE Confidence: 0.803176999092102

00:16:31.390 --> 00:16:38.380 SH rnas targeting trim 33, so Los of trim 33 protein at conferta growth advantage.

NOTE Confidence: 0.863311588764191

00:16:39.010 --> 00:17:09.360 So what is trim 33 I don't have time to go into much detail on this. I just want to summarize that its tripartite motif. Protein is part of a large E3. Lady's family. Why is it in the Canius Library in that case it's in the Kennedy's library 'cause it was once annotator does it canius and this of course is the danger of using large scale annotation when you build pools pools of jeans. Nonetheless, lucky for us. It was because it's a really strong hit in this screen.

NOTE Confidence: 0.829067349433899

00:17:09.810 --> 00:17:29.550 We've shown is that Los of trim 33 reduces sensitivity to back primitive men. Inhibitors and it does this in part through effects on mech expression, but it's also through its effect on TGF beta signaling so trim 33 negative negatively regulates TGF leaders signaling and that sensitizes cells to that, Romo Domain Inhibitors.

NOTE Confidence: 0.843855559825897

00:17:30.240 --> 00:17:42.650 I'm suggesting that if we combine inhibitors of TGS feeder signalling with that promo demand inhibitors. We may potentially it effects and those of course, are studies for the future.

NOTE Confidence: 0.86553019285202

00:17:43.590 --> 00:18:09.150 I concentrated there on a drop in that was gene hairpins that were enriched in the population. But you could also look for hair pins that were depleted from the population and we did. This looking across a

large panel or moderately. Large panel of colorectal cancer lines in this case 6 colorectal cancer lines and look for her pins that dropped out of the population.

NOTE Confidence: 0.843540191650391

00:18:09.900 --> 00:18:18.670 Los of this protein in combination with Los of with innovation of back trauma demands resulted in paramount of growth.

NOTE Confidence: 0.89072185754776

00:18:19.260 --> 00:18:40.550 And what you can see from this screen is that among the top 3 hits the most frequent hits that came up. We saw BRD 4:00 and BRD 2, which are the targets of the drug, and those are would be expected. If we've lowered that target then hitting it again with the inhibitor potentiates the effects and the other really strong head with CDK 4 cycle independent canius for.

NOTE Confidence: 0.843704700469971

00:18:41.820 --> 00:19:13.070 And we showed using our individual SH rnas that by combining knockdown of CDK four with innovation of that promo depends. We really strongly inhibited cell growth so this validated, the results of the screens as you will be aware. There are CDK. 46 Inhibitors and so a combination of a CDK 46 inhibitor with the BRD 4 inhibitor also strongly potentiated.

NOTE Confidence: 0.881906688213348

00:19:13.100 --> 00:19:21.670 At the effect the innovation of growth, suggesting that this is a combination treatment at least that's working in vitro.

NOTE Confidence: 0.886084794998169

00:19:22.760 --> 00:19:52.880 And then just uh wanna touch finally in this aspect of some of the additional things that you can do with these sorts of screening strategies here because we've been interested in the effect of that promo demand inhibitors. We generated cell lines that were resistant to background of Inhibitors by dose escalation and culturing cells in the presence of those inhibitors and we then performed another screen to identify agents that would target the growth of those resistant sales.

NOTE Confidence: 0.860036790370941

00:19:53.560 --> 00:20:23.570 At this time, we did it with a kind on crisper library rather than the SH. RNA Library 'cause. This screen was done. Some years after the initial screen and all I want to say about this data, which is not being extensively study. Just yeah is that the top head from this screen once again in the resistant cells and in the crisper based analysis of parental cells is that we once again got top hit of CDK 4. So we seem to have a very robust synergy between CDK 4:00 and.

NOTE Confidence: 0.749879419803619

00:20:23.600 --> 00:20:24.610 Set pro model names.

NOTE Confidence: 0.820154845714569

00:20:26.680 --> 00:20:57.230 So if I have a couple more minutes. I'll just touch on two other classes of screen. I provide very much detail on these these are 2 Chris previous screens that we've run recently so this screen relies on flow cytometry based sorting which hopefully feeds well to our next talk reporter cell line in this cell line was engineered.

NOTE Confidence: 0.768971085548401

00:20:57.880 --> 00:21:05.150 Then why you to express GFP at whenever a Fusion protein fused with K Ross.

NOTE Confidence: 0.857501327991486

00:21:05.930 --> 00:21:21.010 Was translocated into the nucleus and so kyrras processing K races normally post translationally modified such that it targets to the plasma membrane and when it's at the plasma membrane. It's can perform its normal signaling activities.

NOTE Confidence: 0.876806139945984

00:21:21.620 --> 00:21:46.610 However, if that post translational modification doesn't happen and it can't get properly targeted to the membrane it. Instead builds up in the in the nucleus and triggers GFP expression and so using CRISPR CAS, 9:00 to knockout key components along that processing pathway and allows the generation of GFP positive sales and we can screen for key key factors that are modulating that.

NOTE Confidence: 0.845980167388916

00:21:47.460 --> 00:22:14.850 And so this screen. We've done both kind am focused screens and this was done with SH. RNA and crisper, notably in all seven of these screens. We pulled out one key Kania Satkania School TLTL. K2 that we now believe promotes correct chaos localization. We've also performed a genome wide screen only as a crisper screen.

NOTE Confidence: 0.872717320919037

00:22:15.400 --> 00:22:46.350 And this is identified a nuclear scaffold factor. Safi that's acting to regulate transcription of a key enzyme in the grass modification pathway FNTA. The final still transfer is a subunit so while the biology of these systems is still being worked out and we have some support from elsewhere to continue those studies. I think this really points out the utility of flow cytometry based screening.

NOTE Confidence: 0.621502041816711

00:22:47.000 --> 00:22:49.990 Emanation with SH RNA or crisper.

NOTE Confidence: 0.910742998123169

00:22:50.930 --> 00:23:10.530 And then finally I just want to touch on a screen. That's really more or less still in progress. We've done some arms of this screen, but not all of it and this is work. That's done in collaboration with Marcus Bosenberg and relies on his younger 1.7 cancer cells, which many of you will be familiar with.

NOTE Confidence: 0.90837287902832

00:23:11.280 --> 00:23:17.580 And what we've done is we've used the most specific genome wide crisper cast 9 library to generate.

NOTE Confidence: 0.785163700580597

00:23:18.230 --> 00:23:21.810 A library of those cells knockout for the.

NOTE Confidence: 0.696661114692688

00:23:22.430 --> 00:23:24.140 Each mouse.

NOTE Confidence: 0.861038029193878

00:23:25.010 --> 00:23:58.120 Gene and we can then either perform just a regular in vitro growth. Assi of these or we can implant those and I say we really been markers markers can implant those into most models and examine the growth of those cells and ultimately the hope of course, is to take these forward sites that we can look at the effect jeans that would involve the immune checkpoint inhibitors or immunization of mice to identify factors there.

NOTE Confidence: 0.268207371234894

00:23:58.970 --> 00:24:00.300 Do I

NOTE Confidence: 0.852961719036102

00:24:00.970 --> 00:24:32.020 See that David scandal at by anxious staying there, so I'm going to wrap it all up with acknowledgments. The functional genomics. Core is banned myself Jane and clicked healed. The room would've been inhibitor screening that I talked about was done by ELSHINA screaming team extensive team listed here in collaboration with collaborators at ghillie. Add and the two more recent screens. I mentioned at the end, one in collaboration with Mark Phillips.

NOTE Confidence: 0.772690117359161

00:24:32.570 --> 00:24:37.920 The second with Markus Persson work here at Yale, thank you.

NOTE Confidence: 0.870694816112518

00:24:41.430 --> 00:24:51.830 Thank you. Baby, I'm time for a few questions. I do want to point out. It's probably not markets himself, who do you think not agents are in the audience today?

NOTE Confidence: 0.849073946475983

00:25:00.960 --> 00:25:02.150 The questions.

NOTE Confidence: 0.840145647525787

00:25:02.790 --> 00:25:20.950 It was also clear it was also clear it's not taking off in terms of Utilization. That's why I'm here. I think it's starting to ramp up then I think the issue was.

NOTE Confidence: 0.922381818294525

00:25:21.610 --> 00:25:37.410 It turns out that setting up an internal service provider at Yale is a much more involved process than I or ban imagined so it took us a long time to get approval for that, so we only went live at the just before Christmas.

NOTE Confidence: 0.883476734161377

00:25:38.090 --> 00:25:42.080 And so we're now in a position of trying to

NOTE Confidence: 0.88262414932251

00:25:42.900 --> 00:25:55.420 Publicize ourselves and I think in that circumstance we've we've talked within the last week, 2 different groups that are interested in CRISPR screening so I think word is getting out there.

NOTE Confidence: 0.800751984119415

00:25:56.100 --> 00:25:58.370 I just want to point indicating.

NOTE Confidence: 0.770481765270233

00:26:02.110 --> 00:26:04.120 It can be intimidating.

NOTE Confidence: 0.904354691505432

00:26:12.920 --> 00:26:43.400 Yeah, so I mean, the problem is that to have a general model is a little bit different 'cause. Each case is somewhat specific. But what we would imagine is that although the screening will be done in your lab and the PCR and submitting it for sequencing can be done there as well. Will certainly provide a lot of primaries and information that's required for that step and then once the Lumina sequencing comes back. We have pipelines in place, which should allow us.

NOTE Confidence: 0.428499191999435

00:26:44.120 --> 00:26:44.500 That.

NOTE Confidence: 0.887332856655121

00:26:45.400 --> 00:26:58.830 To those rieger best head lists and you know depends really how involved you want to be with that process. But if you don't want to be if all you want is the list at the end, then we can provide the list at the end.

NOTE Confidence: 0.780862033367157

00:27:00.960 --> 00:27:07.270 And did you ever listen OK? Well, this is true? I just want to really emphasize to?

NOTE Confidence: 0.89056259393692

00:27:07.830 --> 00:27:12.430 Some of the greatest value added here is Dave just said their experience with the pipeline.

NOTE Confidence: 0.810554325580597

00:27:13.170 --> 00:27:36.360 They have done the hard parts. They know how to do them. They have the bioinformatics piece down and they have typical libraries for knockdowns. Ansi demons, which is it? It's an unusual asset. All you needed some good ideas and now a potato.

NOTE Confidence: 0.892722308635712

00:27:36.960 --> 00:27:38.250 OK thanks Dave,

NOTE Confidence: 0.507459878921509

00:27:39.290 --> 00:27:40.310 There are.

NOTE Confidence: 0.378107666969299

00:27:43.720 --> 00:27:44.950 So.

NOTE Confidence: 0.339565634727478

00:27:45.720 --> 00:27:46.020 This is uh.