EUROPEAN LIQUID BIOPSY SOCIETY

1st ctDNA Technology Meeting of 2025: Minutes

WG Leads

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Meeting Minutes / questions to the Speakers & Discussion

Welcome by WG leads:

Ellen Heitzer

It is my pleasure to welcome you all to the first ctDNA technology meeting of 2025. Last year, there was a little bit of silence from our side. We have organised the ctDNA reporting workshop, EQA workshop. Luckily, the first paper of this effort has been published last week. I think we will also highlight this paper on the homepage, right Ed. The summary of the EQA is also in review, so we hope that this will be accepted also soon. Before we kick off, I'd like to also thank Svenja, who has taken over from Claudia. As many of you know, for helping out with the organisation of this meeting. And of course, I'd like to thank all of you for joining today. Let me just. Move. So we all know that basically over the past two decades, decades, the field of ctDNA has really. Really witnessed remarkable evolution and what initially began with targeted analysis of individual genomic hotspots, has really expanded into genome wide investigation that now capture the full complexity of tumour derived cell free DNA. With an increasing position and also resolution and. This progression has been driven not only by a technological innovations, but of course also by a deeper understanding of the cfDNA biology with respect to tissue of origin and fragment tonics feature and so on. And today we are at the point where we can interrogate multiple dimensions of cell free DNA. Including, of course, fragmentation patterns, methylation patterns and also genetic alteration. And to gain insights into tumour origin in the progression and also the tumour heterogeneity. Individually these features have expanded our ability to detect and characterise tumours, but basically it's their integration through multimodal analysis that really holds the greatest promise. And of course we need sophisticated machine learning. Models in order to make sense out of this vast. Of degree of, of of signatures. So throughout this meeting today, we will explore recent advances in methodology, but also data integration strategies and translational applications by presentations from various industry partners and also researchers. And again, I'd like to thank you all for joining in. We don't have too much time for discussions, but still after each presentation, we will have a few minutes for Q&A sessions.

1st Presentation by Dr. Michaël Noë (Nederlands Kanker Instituut, The Netherlands)

Title: DNA methylation and gene expression as determinants of genome-wide cell-free DNA fragmentation

Ellen Heitzer

And with that, I'd like to go ahead and introduce our first speaker. Who is Michael, which is probably also not the correct pronunciation. Noë, who is a pathologist at the Netherlands Cancer Institute in Amsterdam, and he used to work with Victor, Val, Cholesky and his group at Johns Hopkins and now has returned to the Netherlands, obviously, and last year. We published a very nice paper on the connection between epigenetic changes and cell free fragmentation patterns, which we will now present to us. And I'm going to stop sharing. And then Michael, you can share your presentation.

Michaël Noë

Yes. I hope do you see my screen.

Ellen Heitzer

Yes.

Michaël Noë

Perfect. Yeah. Thank you very much for the introduction. I'll talk a little bit more about my work as a postdoc in Victor's lab. Initially Frank, like we all know fragmentation profiles and self read DNA fragments are not random. Phenomena and the start and end positions if if it would be random we would see something like on the top. Wherever we see it was already some time ago that they saw that they were recurrent start sites of of fragments and all fragments started at the same pose. And. And I was intrigued by that because also there was a paper that in in patients with liver transplants where these fragments had different recurrent end positions coming from the white blood cells versus the. Versus the liver that was transplanted. And I thought if it's coming from different tissues and it has. Different recurrent end positions. Maybe there's an influence from the from the methylation of the phenomenon and initially I looked at and motifs. We all know that people have looked at that in the past also and and then they mainly look at the. 1st 4 nucleotides we we looked at that but we saw that there were more differences in the nucleotide right before the fragments and in the two nucleotides in the in the fragment. And so we we looked at the beginning and end of course, the end has a different motif. So we we used to reverse the inverse complement to look at or to investigate the motifs and. We did a thorough analysis by. Pairing these and motifs with the occurrence of the of motifs in the self in the human genome, because human genome is not random, certain motifs occur more than others, so you have to normalise it for for these

differences, and we initially looked at shared DNA, which should be random. And if you normalise it for the occurrence of certain motifs, you can see all the motifs kind of occur. In a random pattern. Which is expected from sheer DNA. But if you look at cell free DNA, you can see that certain motifs especially start starting with CC's and AT outside the fragment or an A outside the fragment these are enriched. And this the third is starting with the CG A with a T or a before the fragment. And then if you look at these recurrent. Start and end sites. These motifs are even way more enriched. Which was interesting, and we all know that CG, if the fragment starts with CG like the third and the fourth most seen motif in the at the fragment ends, CC's are the target of methylation and methyl transferases. And to investigate the influence of methylation methylation on the CCP. These we looked at where are the CpGs positioning themselves in self free DNA fragments when if they're methylated versus if they're not methylated? When CpGs are methylated we can see here a representation of a beginning of a fragment which on the left side the first position. Then you go further into the. Fragments and methylated CPG is seen much more frequently at the beginning of fragments in. In comparison, when you look at the position of unmethylated fragment in methylate CpGs, they are more randomly positioned in cell for DNA fragments. So there is. And enrichment of methylated CpGs at the beginning of fragments. We further subdivided it by looking at the nucleotides before the fragments, and then you can see that methylation has here represented in a more gradual way, has an influence on how. How many fragments you see starting at the CPG one, if it's not mutilated, represented here as a better value of 0, there's much less fragments starting at that C. EG in comparison, when it's methylated and these differences are a little different between the between the different motifs. We used publicly available methylation data for that, but we also use an internal control because on the chromosome X we know that there is differences in methylation between men and women. Women have twice the amount of chromosome X. In comparison with men, and that requires CPG inactivation of genes by methylation. So you see more methylation in on the CpGs on the Chrome, on the CPG islands of the extreme. In women, and this is exactly what we saw. We saw more fragments starting at the CpGs, in women, on the X chromosome compared to men. And we didn't see that difference when we looked only at the autosomes. Here we also expanded the analysis to fragments starting with the CC, because if you add behind to the second behind the second C, ANOTHER G You you encounter another CPG. And we looked at the influence of methylation on on the CPG here when it is in the second position of the. Fragments and here we saw an opposite reaction when if the CPG is methylated, you see less fragments starting at the C before the CPG than when the CPG is not methylated. And this opposite influence is also seen in the X chromosome of women and men, and we don't observe it in the autosomes. So how how can we exploit the signal? We tried to find as many differentially methylated CpGs between cancer and normal cell free DNA. So from like from healthy individuals and we used we tried to look in publicly available data sets however. Most of them use the epic array which only contains 850K probes. So, but we saw one paper, the paper. That's probably everybody knows the paper from Daniel de Carvalho. He did sell free. He looked at pancreatic cancers. He did. The laser capture microdissection, so it were very pure samples and it's. Meet up and you mean precipitation for methylated cytosines and he was able to find a lot of differentially methylated CpGs in the genome between pancreatic cancer and healthy self self free DNA or self free DNA mainly coming from the white blood cells. We split it up in in the direction of the methylation because you can have situation where the normal selfhood is methylated and the cancer is unmethylated, and you also have a situation where. The normal cell free DNA is unmethylated and the cancer is methylated and then we further split it up into the motifs that we initially

already also looked. At. And then so if you count, you have 8 different motifs. You have two different directions. So you would have you need, you will have 16 feet. Years and the paper we only showed the features that caused an increase in the signal between the white blood cells, the from so sulphur DNA from healthy individuals and cell free DNA from patients with pancreatic cancer. And you can see the signal from the people with pancreatic cancer. The highest we also looked with the same differentially method, 8 CpGs of. In cancer versus white blood cells in other cancers, which is not ideal, but it still gave us a signal showing that some of the differential methylation is probably cancer specific and a different part is tissue specific, which results in the best signal for the pancreatic cancer and less signals in the other cancers. We also compared it directly with Delphi and it shows with the you can see the AUC in the rock curve, which shows a very similar performance and combining it with Delphi it's in an ensemble model was give extra performance. So it's the benefit of using this technique is that it's a very simple library prep. It's just low coverage cell for DNA sequencing. You don't need to do any special techniques like using restriction enzymes or bisulfite sequencing that which harms the DNA. We think it's going to like, of course we need to further validate it, but we think because we use specific methylation markers, we think it's more specific for cancer type and potentially less impact from clonal hematopoiesis. It might be a good alternative for tumour in front approaches, because you don't need to sequence the tumour and still have specificity for a specific tumour type. It's sensitive like a shout and it can be used in combination with other techniques like Delphi or even the C2I method. And even other methods like looking at chromosome positions and in combination with Delphi, it's the ensemble model was more sensitive. So why do I think this model this performs well? I've always been taught that you need to look at increases in signal and This is why I think also that Delphi is such a sensitive method because it uses a combination of looking at copy number variations like amplifications which is an increase in signal and then it further enriches the tumour specific. Fragments by looking specifically more at shorter frags. And what this method does is also looking at increases in signal. So we we basically look at regions in the genome where you have very little fragments from the healthy. Like here I show a region that is where you have a lot of recurrent start sites. From different fragments at one position. However, at the position before that you don't see any fragments starting and if you look then at that position and you find certain fragments starting there, it's this might be signal that it's coming from a differentially methylated. Issue and so you go from a signal from zero to 1 and we use only one to 2X low coverage sequencing. So I think and combining multiple regions made it a very strong method. Finally, I'd like to say something about. Why we think that suffer DNA fragments from cancers are shorter. We looked at the the sulphur DNA fragments and their size around the transcription start site and around CPG islands, and we saw that around unmethylated CPG islands you see. Smaller fragments similar and even stronger in around transcription start size of genes that are being expressed. We saw fragments, shorter fragments around these areas. This is a different representation within the middle of the transcription start sites, showing smaller fragments coming from highly expressed genes and larger fragments from around transcription start sites of genes that are less expressed and. The same can be said about CPG islands of low methylation and CPG islands of high methylation, and if you compare it to its mutated fragments and wild type fragment. Is. Probably mainly coming from the from healthy white blood cells. You see, it's kind of a similar shift of a couple of bases to our shorter fragments from the mutated tumours from the mutated fragments. And we did an analysis of the of the TCGA showing that we that tumours. Have less methylation in comparison with black white blood cells and have increased amounts of genes being expressed, and I think we propose this as one of

the potential reasons why cell free DNA from tumours is shorter. In comparison with the healthy or the wild type fragments. So in conclusion, methylation impacts the self DNA fragmentation and results in different motifs around the ends of the cell for DNA fragments. Methylation specific differences in fragmentation can be used to sensitively detect cancer and further improve upon copy number. Variance based methods like Delphi. It requires data. Yeah, you need to have data on the differentially methylated CpGs of. Specific cancer types, so that might be difficult. It might require some. Preparation and increased and using increased cancer specific signals overcome the limits that are associated with very low tumour cell percentage in in the cell free DNA of certain cancer patients. And this methods fits into a set. Of. Different analysis like you mentioned in the in the introduction of low coverage whole genome sequencing of cell free DNA and I think it's makes it stronger by using all these different approaches. Are there any questions?

Ellen Heitzer

Thank you so much. Really interesting work. So I would have plenty of questions. Maybe I start with 1. So basically you said that the, the, the differential size distribution is due to the differential methylation, but is the rather due to the different chromatin organisation as a consequence of methylation. So whether it's tightly packed? Or open chromatin that leads to the different fragmentation in the end.

Michaël Noë

Yeah, it's we. During the review process, we had similar questions. We have shown that like we've used different approaches like using the window Protection score and as maybe more measurement for what you're proposing. But we did see that methylation. An expression still has statistically significant impact on the size differences.

Ellen Heitzer

Independent of the chromatin organisation or the chromatin pattern so.

Michaël Noë

Exactly. Yeah. If you look at that by the window protection score.

Ellen Heitzer

OK, So what you're saying is that based on on a methylated, see the? Nuclear is cuts at a different position. Regardless of whether it's open or closed chromatin.

Michaël Noë

Yeah, yeah. Because and there is some data also from more structural studies showing that if if it's CPG is methylated, the DNA wraps more loosely when it's methylated, it wraps more tightly. Around the nucleosome and tightly, I mean it's closer to the to the nuclear zone, while if it's unmethylated the incoming arms of the DNA are much more loose. And that probably allows its exposure to D nases.

Ellen Heitzer

OK. And you showed us that the the the best signal was achieved with prostate cancer is prostate cancer basically sorry, pancreatic cancer, is this also due to the methylation level of pancreatic cancer compared to others?

Michaël Noë

Same credit cancel. As far as I know, there's no like. I don't know any that pancreatic cancer is known to be very differential or like that. The levels of methylation are so much different from other cancers. But it's I think we had the best signal because we had the most different. We discovered the most differentially. Methylated regions comparison with by blood cells and that's I think more technique based. Thing because most like I said, most of the the publicly available methylation data is based on epic arrays from Illumina which contain internal 50K CPG probes. However, there is a couple of times more CpGs in the in the gene.

Michaël Noë

Like there's yeah, 20 million CpGs or something in the in the whole genome. So if you have if you have. Like I think you probably either need more differentially methylated CpGs or you need to have deeper sequencing. However, we only had one to 2X sequencing and that's why I think it's only worked having a huge amount of differentially methylated regions.

Ellen Heitzer

Which cancer stages did you look at? So you didn't specify whether this was stage 1? Two or three.

Michaël Noë

No, it's the same as in. So that's the data from the first Delphi paper.

Ellen Heitzer

OK, so make sure.

Michaël Noë

Yeah. So. There is a yeah, there's some variation, and that's why we compared it just with Delphi. Also because then you have the same stages and it's easily comparable.

Ellen Heitzer

OK. Are there any questions from the audience?

Patrizio Giacomini

I am not sure how I can raise my hand, but I'll have one. Maybe I'm running too far Michael and it's great data but. Are you aware? Of any attempts by anybody, including yourself, of course, or creating a three-dimensional map, putting together mitigation, fragmentation and transcriptomics. So to to have an Atlas of of the entire status DNA and RNA production.

Michaël Noë

Of cancers.

Patrizio Giacomini

Sure, cancer compared to normal solids.

Michaël Noë

Well. I know there's a lot of data with Epic arrays and that's that was also considered whole genome, however. Whole genome by looking at only. A million probes versus whole genome by looking at 40 million CpGs is is different. So I think like we of course we saw from healthy tissues you had the loafer paper for methylation recently then. For expression I'm I'm not sure. I think in like in certain hospitals here it's being used for tumour classification and of course these they will have more lab. Datasets we I'm I'm in the process of applying for grants and of course this. Yeah. Using this especially now it's upcoming. Inclusion of using self read DNA and certain guidelines, probably for colon cancer in stage 2, I think it will be very interesting to have methylation data on colorectal cancer and to have to see how this approach performs in comparison with tumour and formed approaches. And that's of course, that's part of. Yeah, Grant proposals, but.

Patrizio Giacomini

Good luck. It's a very interesting issue. Congratulations.

Ellen Heitzer

Are there any other questions? So can I just speak up or raise your hand? If this is not the case, I'd like to thank you very much. For presenting this to us and then I would hand over to Ed for the industry presentations.

2nd Presentation by Danielle Goldberg (Global PM, Illumina) and Suzanne Rohrback (R&D, Illumina)

Title: Capturing multiomic cfDNA signatures with Illumina 5-base

Ed Schuuring

We were we were very enthusiastic to see how many reactions we get for people to present, so we had to make a selection. We have a tight schedule, so I would like to invite either Danielle or Susan from Illumina. To share their slides and start off for those we cannot see the because we see more than 115 participants at the moment and we cannot see hands raising. So please put your questions in the chats. I don't know whether we have time to deal with them, but we will take them back to the speakers later

and we have some open discussions in the end. After 6:00. So without further notice, Danielle who's speaking Danielle or Susan, please go ahead.

Danielle Goldberg

Getting back into the zoom where we usually use teams, so thank you so much for for the opportunity to present here today. I'm really excited to introduce our new Illumina 5 based solution and talk about how it enables the capture. Of multi omic cell free DNA signatures. So my name is Danielle Goldberg. I am a senior manager of product management at Illumina and I'm joined here by Suzanne Rohrback, and she is a senior staff bioinformatics scientist in our assay research and development team. So brief introduction on our Illumina 5 base. It is a single assay solution that enables dual insights so enables the simultaneous profiling of methylation and also variant detection and it supports both whole genome and target. Enrichment applications so starting with our library prep and our novel conversion technology, which I'll talk about in. A little bit. We have our single solution for library Prep which incorporates that single step, enzymatic conversion, and then we go into sequencing and what we see is getting very high mapping efficiency and data yield from our library prep. In our novel conversion. It's optimised to give the most simple solution and maximise data yield on the back end. And lastly with our Dragon analysis we are. Doing optimised alignment with our conversion and performing the variant and methylation calling. On the back end and that is what essentially gives us the high accuracy dual outputs from our solutions. So holistically we do library prep in less than a day, incorporating that and somatic conversion sequencing on both our mid throughput and high throughput systems and analysis through Dragon and up and coming is going to be data visualisation with our new Illumina connected. Dynamic solution. So I'm talking through a little bit of our conversion chemistry. Our 5 based technology is based on a novel and somatic conversion that. Methylated cytosines to thymes and this is different from you know, the standard conversions gold standard today where the where they convert unmethylated cytosines to thymine. And this is an improvement because the vast majority of cytosines, so around 95% of cytosines are unmethylated and so they end up converting almost all cytosines to diamines. The result of that is you. End up with a library that is essentially what we call a three base complexity library. And that means that almost all of the bases are A's T's, and there's very few cheese and C's. So these libraries are more difficult to sequence, more difficult to math, and DNA can be damaged in the process. And so. If you also want to assess both the genetic variants and methylation on the same sample, you typically have to do 2 separate libraries or get creative in in sequencing. Two different strands. And so that can be a cumbersome and costly. So with our solution that we have the direct conversion. So it is only converting the methylated side dozens to thines and you get A4 Blade base library complexity out of the conversion. So this essentially means that only a small set of the cytosines are converted to three lines, and they're easier to sequence the libraries. It's also easier to map, and we don't have any. Damage to the DNA and so that makes it a really great technology for cell free to DNA. And we see very high coverage uniformity, superior alignment due to that complexity of the libraries that I mentioned. And we can also get high accuracy calling of both the variants and the METHYLATIONS in. A single opposite. So we're really excited about bringing this to. To the field and I'm going to hand it over to Suzanne, who's going to talk about some data on cell free DNA specifically.

Suzanne Rohrback

Yes. So all of the capabilities that Danielle was just describing are really great benefits, but we do realise at the end of the day it's it's pretty critical to understand how our technology and our faces with cell free DNA inputs and uses. So our single step enzymatic conversion is gentle enough to retain high complexity libraries for low input samples on the top left, I'm showing the deduplicated coverage that you get from 600 million sequencing reads for different nanogram input of cell free DNA. That coverage is extremely stable. With only a 1X difference between 10 nanograms and one nanogram input. Coverage uniformity is also maintained with virtually all of the 28 million CPG dinucleotides in the genome captured with at least 10X coverage for all inputs and very little reduction down to 1 nanogram. And no bias is introduced to the methylation estimates as shown by how the average methylation in CP. The islands is highly correlated between technical replicates of different inputs. Then in the middle and left, we're illustrating how our method is compatible with enrichment, which gives greater flexibility to experimental designs. We've extended our probe design software suite to accommodate the CT conversions that occur in our sample preparation so you can retain 80% or higher enrichment rate. That is achieved with four base DNA sequencing for a range of panel sizes. And the lower middle is showing that our methylation profiles obtained before and after enrichment are highly correlated, which confirms that the additional protocol steps are not introducing inaccuracies and annotations. And on the right we're showing several Uri metrics which corroborate our ability to provide over 2000 X. UI collapse coverage for high sensitivity uses and that our Dragon algorithms have been extended to support both simplex and Duplex UMI collapsing which provides error correction and single molecule resolution of both DNA variants and methylation patterning. And with our last slide, we wanted to illustrate some of our assays performance capabilities in more of an application specific context. Some methylation is primarily regulated in a regional manner and in regulatory regions. CPG's tend to form denser clusters, which means that. Individual molecules which turn into individual reeds. Can have four or five or more CpGs with the same methylation state, and that signal is definitely a focus area for low ultra low sensitivity applications such as MRD, because it increases your confidence in detecting an abnormality. So on the left, we're showing that for reads that have at least three CpGs captured Illumina 5 based technology can achieve a sub 10 parts per million error rate which supports sensitivity to detecting populations at less than one and 100,000. Molecules in the middle, we're looking at how our performance compares to standard DNA sequencing for several forms of somatic variant calling. This includes lower coverage, whole genome sequencing for a 10% or greater variant, allele frequency enrichment with moderate coverage and neonate. Collapsing to detect sub 5% allele frequency and ultra high coverage enrichment with or without hotspot calling enabled for sub 1% thefts. And in all instances, 5 base technology is approaching the sensitivity that's achieved with unconverted libraries. And on the right, we're ending on Fragmento mic profiling our ligation based prep and enzymatic conversion does a very good job of retaining accurate fragment information from cell free DNA while adding in methylation annotations as shown in this example here. From a healthy cell free DNA donor. The Window protection score profiles of the whole genome sequencing and five base converted libraries is tightly overlaid along the bottom track. The more 5 prime region that's shown here has greater distance between nucleosomes and dicating open chromatin, and that is corresponding to an unmethylated CPG island that covers the first few exons of a transcript. That's highly expressed in lymphoid cells. In contrast, they immediately adjacent region. Has more dense nucleosome patterning, high methylation and is covering A silenced muscle specific transcript. So there's a lot of depth and versatility. Our technology is poised to provide and we're looking forward to putting it in everyone's hand.

Ed Schuuring

Thank you very much for this very clear presentation and when when you when are you able to put it in your? Hands is it already available?

Danielle Goldberg

So I can go to that. We are in early access right now and we hope to have it broadly available early next year.

Ed Schuuring

OK, I see one question, a couple of questions coming up. One simple question is will the will you be able to share the? Slides with us.

Danielle Goldberg

Yes.

Ed Schuuring

OK, that's fine. There's another question. How can you differentiate between converted time and time and time and original timelines?

Suzanne Rohrback

Yeah. So, so that would be for how we can capture both the variants and the methylation states, so. Our algorithms are are. Really taking advantage of the fact that methylation is a strand specific occurs. So you always have the opposite strand, where with complementary base pairing it's going to be an original G and does not get converted by our enzyme. So we've extended the Dragon algorithms to be aware of that sort of strand specific pattern and weight the the variables and statistics. Used appropriately so that we can deconvolute those different variant types from a methylation change.

Ed Schuuring

OK. Question for my side. I'm doing a lot of ventilation testing using myself for treatments and we always for an optimal conversion you need quite a. Lot of DNA. So what's probably you've mentioned already, but what's the minimum amount of DNA that you that you need to get an optimal conversion?

Danielle Goldberg

Yeah, yeah.

Suzanne Rohrback

I'll let Danielle talk that one.

Danielle Goldberg

Yeah. So we showed data here going down as low as .1 nanogram and we see only a slight drop off in the methylation for the variant calling. We do recommend a a slightly higher input amount, so. That can range from 10 nanograms to 50 nanograms per variant polling.

Ed Schuuring

Yeah, and. And we also know from the vice of the treatment that you really damage your DNA. So you got quite a lot of degradation and therefore lower loss of your input DNA is that is that better because you're using an an enzyme approach or is that similar, do you know?

Suzanne Rohrback

Yes, we have a lot less loss than occurs with bisulfite sequencing and a lot less damage. So we've seen examples where with with bisulfite you might lose a lot of your insert length from, you know, sort of chewing away at the overhangs of cell free DNA molecules. We retain a a comparable insert size to DNA sequencing libraries. We also retain higher deduplicated coverage. At the same input levels as bisuflite, so I think it's as much as twice as high of coverage.

Ed Schuuring

OK. A final question before we move on. Can the approach does the approach require analysis on Dragon? Of course you show it in your example. But is it needed for any application in the future?

Suzanne Rohrback

Yes. So my team has been spending the past five years integrating new algorithms into Dragons. So in order to take full advantage of what this asset can provide, we do strongly recommend using the Dragon software so that you can get the accurate variant calling. You can get high. Alignment rates. You can get the the built in Uri handling. Our data is not incompatible with third party tools. We've definitely been able to run it through pipelines like Bismarck. So you know that is also there as an option if necessary.

Ed Schuuring

Thank you very much again for your contribution. And and I would like to go on to the next presentation.

3rd Presentation by Dr. Tom Charlesworth (Biomodal)

Title: 5-methylcytosine and 5-hydroxymethylcytosine are synergistic biomarkers for early detection of colorectal cancer

Ed Schuuring

We have scheduled Thomas Charlesworth, but I didn't see him in the list. This, ah there. Yes, yeah. Great. So from by tomorrow floor is yours.

Tom Charlesworth

Alright. Thanks very much. Perfect. And I think my talk kind of nicely follows on from the talk from the from the Illumina folks. So I just while since you zoomed. But I'm going to try and share.

Tom Charlesworth

Thank you. So I'm going to talk to you about not the five base gene with the six base Dino. And I'm gonna talk to you, talk through a bit of data that we put out late last year in a pre print. Applying the six based genome to a liquid biopsy application, the early detection of colorectal cancer from. Cell free DNA. So just briefly, I mean this is probably Yuri will understand this, but the six page genome is is a combination of full genetics. So ATG and C but also the two most important and biologically relevant methylation marks that are present on DNA 5 MC and five HMC. So there's sort of. Two of the three methylation states, the last one being. An unmethylated state. 5 MC as you will no doubt all know. Is generally associated with regions of. The genome that are kind of closed. Switched off low of low expression unmethylated regions of the genome and generally associated with regions that are open chromatin and you know, higher levels of gene expression or or active enhancers and and five agencies. You know it's on. It's a an intermediate on the pathway to demethylation. It's also a sort. Of functional mark. And it's really a mark of areas where there is change. Or areas of. Of sort of active gene expression when it's in the. Gene body. So if you look. Answer regions where you start to see 5. HMC is where you're starting to see that transition from a A methylated 5 MC state of sort of an inactive enhancer. Moving towards that unmethylated state, so an active enhancer and the same is true of other regulatory and and sort of functional areas of the genome. And so we believe that it's really important when you're looking at. The sort of. Totality of information you can derive from the DNA samples that you you get as much as possible and that's why we think the six based genome is is useful. But when you look at. Specifically, we want to sort of move on from a mod C readout, which conflates MC and HMC, and try and distinguish between those two marks that we know are functionally very different and their biological function is is distinct. And. And so we talk about our technology helping you move beyond the limitations of acgt sequencing. And you know what that means is that if you currently want to try and get that complete information, both genetics and complete epigenetic information. Shouldn't you're either using a? Genome workflow, where you have limited insight into methylation and. Therefore, biological change. Or you're using a methylation sequencing approach. Whether that's bisulfite and enzymatic approach and you know or other more other relative approaches that that people are talking about, but those often can't distinguish between 5 MC and five HMC, which we think is is critical. So you have to either run multiple workflows. Or workflows that require

very high input and have unreliable outputs. You might be using. You know if you're using by sulfite as was just. Function. It can be quite damaging to your sample and you're losing information that way. And then if you're running those multiple workflows and you have to go through sort of data integration step where you're trying to match up those you know whole genome read with a with a some sort of genetic sequencing read to try and provide you with those complete information as possible. And that this is more complex. Time consuming and you essentially you end up adding the errors so you end up with with. Guppy information, if you like. So we end up with missing missing regions. Uhm. And so, you know, we've developed a solution which is called Duet multimix evoke and it provides you with the six base genome from a A DNA sample. It's a single sample. You use a single sample. You use one workflow and you push it through our our one solution to get that full six base information you can use as little as 5 nanograms of cell free DNA as input. So it's very amenable to a liquid biopsy application. You process your sample through the essay. Do conventional Illumina sequencing, and then use our software to do what we call resolution and convert a full base class queue file into a six base and just six base data, and you get a resolve what we call a resolved fast queue. You get a BAM file, another kind of more other sort of standard. Outputs as well as a a bespoke data format called Disaster, which integrates with downstream analysis solutions that we're working on. Nothing. So that's the sort of the solution and and with your six base data you get complete and accurate genetic information shown here as the plot on the on the left where we're comparing and snip calling accuracy of our solution 6 space gene M2 conventional Lumina whole genome sequencing 2. Or enzymatic or bisulfite. Epigenetic sequencing methods and what you can see is is because our solution isn't confounded at calling seed mutations like the the the efficiency solutions are our. Our pointing accuracy is equivalent to that that you get from whole genome sequencing, so you. Get complete genetic. Mission. I mean, the other plot is just a simple sensitivity, specificity plot of your MC and your HMC calls. And you can see that you get very high accuracy MPC and HTC calls and and of course the most importantly the the ability to distinguish between those two biologically distinct methylation states. So again, complete genetics complete and accurate genetics and complete and accurate epigenetic information from a single sample in a single workflow. I haven't included. Anything about how the the sort of molecular biology of the of the of the solution and the informatics, but you know we can provide that as as follow up and just wanted to highlight this, this plot which is we we've sequenced a a cohort of. Self free DNA samples from healthy individuals and individuals with either stage 123 or 4 colorectal cancer. These were colonoscopy confirmed diagnosis, and I'm just to demonstrate that because ours is a fully enzymatic workflow. You are the workflow preserves all the sort of the fragment length information. And so we did a. Kind. Of proof of. Concept if you like, where we sequenced to. Base pairs and you can see that across healthy to stage 4 you have that fragment information is present within the sample and you can see the sort of characteristic shortening and the sort of. Waves that you get those. Later stages of cancer the the shorter fragments of kind of the hallmark of of of later stage cancer. You're putting examples. I'm going to kind of continue talking through what we've done with with this coloured pencil cfDNA cohort and what we've done is explored the. Synergy between 5 MC and five HMC as biomarkers for early cancer detection. We've and the hypothesis here is that because we know that five HMC is a is an intermediate on that demethylation pathway, it's sort of early marker of activation of the region when you're looking at regions that lose methylation through the course of disease through the. The course of the progression of cancer. You're going to see an increase of 5H and C when those changes start to start to manifest. So the earliest time points of activation and you're going to see an an increase

of five agency and that's reflected in a in a in a small decrease of five MC. But because the the baseline level of five HMC is quite low. In terms of effect size, that five HMC increase is quite significant and therefore is far easier to detect than the change in five MC. And if you have a sort of traditional five base readout. You'll be reading a mod C signal, and in that signal the the increase of 5H between the decrease of five M here or completed, and so you're unable to to detect these early changes. So this was our hypothesis going in and and. We've we've sequenced 26 stage 1 CRC cell free DNA samples from 32 healthy samples. We used 10 nanograms of self free DNA and and we'll process them through the duet evoke solution. Whole genome sequence to an average depth of 34 X and then we used as our feature set. Differentially methylated regions that we derived from the cancer genome. So this was this was a mod C data set where we we compared to matched normal and stage 4 tumour samples to give us a kind of regions of interest that we were going, we then interrogated in our data set. So we took these eleven 11,704 regions. And calculated the MC and HMC levels in the different in the healthy and and Stage 1 CRC patients and used them as a feature set to build models classifiers. To distinguish between these two states. And the the results of that and that kind of that those classifiers are presented here, we built four different classifiers we used. One where we. We kind of. Were three where we mimicked the kind of five base readout, either with a Mod C signal where we've added together the MC and the HMC, or A5 HMC only signal or A5 MC only signal and they're represented here. These are. Rock curves for classification of health fees to stage 1 using a leave one out approach and you can see the green is the mod C signal. The kind of Reddy coral colour is 55 HMC and the light blue is at 5 MC signal. But then when we used the combination of five MC and five HMC signal as kind of. Independent feature sets. You can see. The performance of the classifier was significantly higher than for those other 3 five base rebounds. So you go from AUC of between 5, 4 and 0.54 and 0.7. Do you know? You see a point? Five and we've put on here as a kind of reference. CMS guidelines in the US for performance for an early stage early early stage CRC classifier and also published page for from the likes of Garden Freedom and Gale Grail. And whilst this is a fairly small cohort, it's a sort of proof of concept. The performance of the classifier that we've we've generated is far higher than that reported. These tests are being commercialised in the US and. And then the. The other figure on this on the right hand side is just sort of showing that classification. And that, you know, for the healthy controls is 1 misclassification and a couple from Stage 4, stage 1 CRC samples. But but broadly we're seeing very high performance for that combined the the model that uses both 5M. C and five HC features. And so I think this this. Sort of validates the hypothesis. It's the. 5 HMC distinguishing 5 MC and five HMC has. Really adds value. For early cancer detection and we think that that can translate to other applications and liquid biopsy space. As well and. We know that that liquid biopsy classifiers can be. Sensitive to the. Cohort. And so we randomly dropped three samples from both the health these controls and the stage 1 CRCS. Reiterated on that classifier building and we see that the the performance of the five MC and five agency. Models. Are consistently higher than the models that were built using either Mod C data. MC data or agency data. So we think that this this. Improve performance is is robust to changes of cohort and therefore is probably fairly generalizable to other cohorts and other applications. And. And then just just. Briefly, I'll touch on some of our customers are doing in the liquidity space. One of the names on who you may recognise, but I'll I'll sort of go through them. So we've got. Uh. Sarah Jane Dawson, who's in Australia. He's looking at early detection of liver cancer. We've got Allen, who we're working with on looking at prostate cancer detection. And we've got other collaborators in, in, in Manchester in the UK with Flora Mulier, who's interested in kinds of unknown primary and Melanoma

immunotherapy response and and whether the six base. And can help there. Sign up free show in Canada who's looking at triple negative breast cancer relapse from self free DNA and also Scott Bradman, who's in Toronto understanding breast cancer response to immunotherapy. And these are all customers who are who are working with self DNA data they're self. Free DNA samples, sorry. So I'm going to finish there. This was the final slide and you know, we think that the six page genome can reveal biological change and has great the value in the library space, especially when thinking about picking up those early changes of either early detection of cancer or early changes in other policy applications. I'll stop there.

Ed Schuuring

Also, thank you very much for this very clear presentation. Interesting data.

Ellen Heitzer

Any questions? There was one question whether it's already commercially available and yeah, it. Is.

Tom Charlesworth

Is yes, we launched the the Six base solution about this time last year. It's nearly a year old and and yeah, as I say we've we've we've got. Sales and support globally. In fact, we've just had Dirk Bartels join our team who's covering most men in Europe. And and we've also got you. Know that field application scientist covering men in Europe as well.

Ellen Heitzer

Can you maybe also comment on the analysis software.

Tom Charlesworth

Sure. So so the the solution is both assay and software software does what we call reader resolution. So it converts the full base class queue that come off sequencer into the six base file formats and it does what we call. So it does read resolution, it does quality filtering, trimming and alignment. And then we're also working on a. A kind of analysis solution that's in a closed beach at the moment I'm looking to to make that sort of broaden out slightly later this year, but that's sort of the idea being that we're providing this, this novel data type and you know our customers will will benefit from some analytical tools that will help them sort of make best use of that data.

Ed Schuuring

So I wonder whether do the applications you show or liquid baked liquid biopsies? But there are some other applications testing for modulation building up ventilation applications like for instance reserve type, fixed material and cervical cancer or better material or sexual circular elaborate a little bit on. Other tools, other monetary see then, than the ones that you mentioned in talk.

Tom Charlesworth

Yeah, absolutely. So I guess a couple of things about talking about the compatibility of the product with different applications. So it's compatible with. Targeted approaches and we've we've demonstrated that

it works well with the twist panels because you know sort. Of the the panels. That work in a three base space if you like, but it's also compatible and works very well with FB samples and and and fresh frozen samples as well and and you know, in terms of other applications we've got, there's a particular interest from people working in the neurology. Case or I mean as a sort of interface between oncology and neurology as well, given that we know that in neural tissues there's an increase of five HMC. So we see that as a as a sort of promising application. And we're seeing that come out in some early collaborations and early customer data.

Ed Schuuring

OK. Very thank you very much again. Because of time we have to proceed.

4th Presentation by Dr. Hélène Bauby (QIAGEN)

Title: QIAseq Targeted Methyl Panels: A liquid biopsy-compatible solution for detection of methyl markers

Ed Schuuring

I would like to go to the next presentation from QIAGEN. Hélène Bauby will be, I hope I speak it. I pronunciate it well.

Hélène Bauby

So hello everyone. Thank you very much for the opportunity to present here with you. We are seeing very interesting. Things OK, I'm going to try to get a pointer here. Alright, so today I wanted to talk to you about the casing targeted methyl panel which are liquid biopsy compatible solution for detection of methyl markers using NGS. My name is Ellen Bobby. I'm a senior. Global product manager at QIAGEN in charge of targeted Genomics and Enterprise Genomics solution. So feel free to interrupt if you have any question or we can take them at the end. We'll see. Alright. Here is a legal disclaimer that this products are intended for molecular biology application. All right, so QIAGEN has quite a broad range of NGS products, among which the classic targeted methyl panels, which are an amplicon based. Uh. Enrichment technology. So we do start with a bisulfite conversion and I notice that a lot of people here are not fond of that technology. But fortunately, these methyl panels are also compatible with enzymatic conversion. So lower is here. You don't absolutely need to use. The bisulfite technology. So following your conversion the you will use the library key to go through DNA and repair adapter ligation and indexing with the I7 index sample cleanup target enrichment using the classic enrichment technologies and using umis as well. Sample clean up universal PCR indexing with the III 5 index and final sample. So it's a one day workflow, so quite a fast processing protocol and we have included optimised chemistry for greater efficiency. The

kit configures itself with a panel kit and an index kit, and obviously we can provide UMI where data analysis pipeline, cloud based or software local based depending on your needs. In terms of methylation profiling, we do have 4 catalogue panels and we have come up with this solution because obviously whole genome sequencing is very informative. But once you have identified a few CPG sites that. Will make the signature of a certain pathology. Is it still necessary to go for such broad analysis? So we came up with based on data from the literature. We came up with catalogue panels that are specific of breast cancer, colorectal cancer, immuno oncology and human tissue infiltration. But what's more is that you can actually customise. Any any possible targets with the tool the the design tool that we have on the GeneGlobe portal or using the Enterprise Genomics Solutions team. So this is. Team of Primer and probe design experts that will go through your targets and define the best possible primers using our design algorithm that we have developed during the past 20. 20 years. OK, so if you look at the complete sample to inside, because obviously here we can talk about NGS specifically, but QIAGEN I'm sure you know QIAGEN is not just about NGS kits because we are very well known for our sample stabilisation and preperation. And here I wanted to highlight the fact that we can we can offer a full workflow for cfDNA analysis from urine or blood. And in terms of stabilisation, we have the PAX gene urine liquid biopsy set for example, and the PAX gene. Blood cfDNA tubes and the urine liquid biopsy tubes as well. So once you have stabilised your samples. You can go through actually nucleic acid extraction and this can be automated. Let's say using the calculate connect easy to connect or casing forming or this can be done manually. Once you have your cfDNA extracted you want to QC that and you can do that using the QIAxcel Connect. Then you can go through your conversion again bisulfite is highlighted here because we do have the epitech fast with DNA protect reagent that actually prevent any damages from happening. This is why this is compatible with liquid biopsy. And the classic methyl panels, once you have constructed your library, you can use again the guides and connect for library QC. The run can be done on any Illumina element or other sequencers with an added conversion step, and for the data analysis we do have the gene globe, which is a cloud based solution and the QIAGEN CLC Genomics Workbench, which is a software local software. And both actually run the same pipeline. So I wanted to show you some performance data that we obtained on cfDNA from urine samples. So we have used the PAX gene urine liquid biopsy set and I wanted to quickly present that solution to you because if you look at unstabilized urine, what you are going to see is that after a few hours of storage up to three. Days the yield of extracted cfDNA goes massively down, whereas if you use a stabilisation reagent, you can actually keep a yield that is pretty consistent even after three days of room temperature storage. The liquid, the stabilisation reagent that we have in the tube in the pathogene tube is non cross linking. And so basically what you do is collect the urine sample in the the part here and then you will without the need to open that collection device, you will apply the tube. And that will actually suck up exactly 10 millilitres of urine into that tubes containing the stabilisation solution. So you will always have. The right amount of urine compared to your stabilisation solution, so always the right ratio, you will not have to open or touch or risk any spillage with that solution and it can avoid any exposure to contaminants. So keep that as sterile as possible. So following the sample collection stabilisation we have done some manual or automated extraction using our or the easy to connect on the same donors. So donors 567 and eight are shown here. You can see that the profiles are quite similar with the manual or automated solution, maybe a bit stronger here. And so when you don't stabilise the urine, you will usually not see the high molecular weight DNA. So here you have the typical cfDNA peak that you would also see in blood. And that's actually coming from blood being filtrated. To the urine. But you always

get higher molecular weight DNA on urine samples. When it's stabilised, so once we have done that, we have used those samples to actually build libraries using the breast cancer panel of the classic targeted methyl panel kits. We have done some experiments and I'm going to talk to you about that first using 50 nanogram of cfDNA. And you can see here that the library profiles are quite consistent. And here it's enrichment metrics after sequencing on the Illumina my 6 system. So in dark blue you have the repairs after trimming in lighter blue, the repairs kept for methylation and in medium blue the percentage of primers. On target umm, I above 0.1 of mean so you can see that again the enrichment metric are quite good and also very. Form across the different samples and across the different manual extraction or automated extraction. The input is actually shown here 15 anagram for. Then the whole point was to actually detect the methylated events in those samples. So here we show first the covered CPG. So a total of more more than 8000 sites. Were covered. And we can see that in dark blue we have the covered CPG and in lighter blue, the covert CPG with over 40 beads. So again, very consistent between manual and automated extraction solution. And here we have determined the methylation degree. In the samples so you can see that we also used the percentage of cytosines methylated in the CHG context and CHH context. Both of those are supposed to be unmethylated in mammalian cells coming from healthy individual, so it's also a control for us that the conversion was working well and here you can see that in the CPG context you have a degree. Of methylated cytosine. That is again very consistent between the two different extraction techniques. Here we wanted to show that we had a good correlation between the samples, so all samples came from healthy individuals, healthy donors, and we can see that we have a quite a good correlation coefficient. And here we can see that from donor to donor, obviously this was very consistent but also in between donors. So then what we wanted to do is we have used some FFP and surrounding healthy tissue from cancer patients to build a database so differentially. Methylated ratio, so to build a database on this FFP versus healthy tissue. And what we wanted to do is use that database. Is to analyse the method profile of those cfDNA samples from healthy individual and we can see that as expected. The the rate of methylated cytosine was actually very low, which is expected in these healthy individual. We have also used those regions to generate the methylated profile and you can see that sorry, I'm moving something here. You can see that it was also very consistent in between the different samples and different techniques. So plasma here. Compared to urine. And finally final.

Ed Schuuring

Please take care that you have only two minutes, right?

Hélène Bauby

Yes, exactly. So this is the the last slide. So I wanted to show also that higher methylated region, our accurately called at lower coverage, so here it's coefficient of variation and you can see that the lower it gets the higher accuracy you get. So obviously when and and you have here the methylation degree and the different colours here are the different coverage rate that you can get from a single. Library and depending on the coverage, obviously you get the lower coefficient of variation, so the more accurate data. So even for really low methylation degree the highest coverage will be the best efficient to call that. And here on this plot you we have actually prepared libraries from 10 nanogram only on cfDNA. And you can see that you can very very precisely call the methylated cytosine ratio in different samples, even with

really low input. So thank you so much for your attention. I don't know if we still have time for some question.

Ed Schuuring

Thank you again for your talk and uh, Claire, we have we have only few minutes. So any questions from the audience? Otherwise, I would like to start because you mentioned in one of the slides, the time it took takes to have the collection of material to extract the DNA and do that by sort of treatment. But for the whole procedure, what what's the time line?

Hélène Bauby

Or procedure would be a day and a half to get from sample collection to ready, ready to sequence libraries.

Ed Schuuring

And to perform your analysis, do you really need also your analysis system, your software, etc.

Hélène Bauby

It can be done with a third party solutions as well. What we like to do in that case is share the rich structure of the library so that anyone that wants to use the. Or party solution can identify the UMI and the insert size and everything that they need to know to to develop their own pipeline.

Ed Schuuring

OK, I'll probably have some more questions, but since time is pressing us, thank you very much again.

Hélène Bauby

Thank you, and feel free to contact us. We'd be happy to. Thank you very much.

5th Presentation by Dr. Krystyna Nahlik (LGC)

Title: Advancing Donor-derived circulating tumor methylated DNA Reference Standards

Ed Schuuring

I would like. We will do that. OK. The next talk last in this session is Krystyna Nahlik from LGC.

Krystyna Nahlik

I'm going to be talking today about the latest developments in our circulating tumour DNA methylated reference standards. We are developing at LDC. My name is Christina and. I'm a commercial manager at LDC clinical diagnostics specialising in. The Saracenic range of the oncology reference materials and just to let you know, most of the data I'm going to be showing today are very new and very fresh off the press. So to give you a bit of background, our current biosynthetic methylation reference standards, which have been available for about a year. Are designed to really address the general analytical validity of methylation assays and different methylation detection approaches and that's irrespective of the exact targets that the assay is looking at. And the reason we started with this approach is that when we are looking at different when you're looking at different genome wide. Methylation assay targets especially for early detection. The targets are usually not disclosed or proprietary, so it's difficult to say what targets you should include in a reference material or reference standard to be. Applicable. There are a few notable exceptions, mainly for assays, which are based on PCR chart as the EPI Pro colon or colour guard or colour shoes. So these are all CRC specific early detection or monitoring assays. So what we have started with and what is already commercially available is what we called our just methylated and unmethylated CTD DNA mutation mix, which are reference materials which are derived from a well characterised genome, unable to. Online to have a good work characterised anaemic background and you can get them in two options as an unmethylated or methylated option and the idea is that they are again not target specific. This is genome wide CPG methylation and the customers can blend these as needed. To see you know what the sensitivity of the assay is to the observed the expected level of genome wide CPG methylation and so. Form. But it cannot really tell you about the ability of any specific assay to detect any specific cancer related patterns in a sample. We have been working with NIST on the both development and evaluation of these reference materials, and there are some manuscript in preparation. So before I move on to our latest developments, so I wanted to show you that how the existing reference materials can be used for assay performance evaluation. So this is a quick example actually using the bio model, the word pipeline where you can see this is the actual percentage of the methylated. In our methylated sample dilution and this is the rate of the detected modified cytosine in CPG. So you can just see in this case it could show a very good correlation between the expected and the detected methylation. Rates and this could be used to calculate the sensitivity and specificity parameters of this pipeline for each of the methylation pollutions. However, like I said, these kind of reference materials are great for initial analytical validation, but if you're looking for standards that are going to tell you more, can I detect patient specific methylation patterns and with what efficiency you need a different approach. So we have started to work on new donor derived reference standards which are designed to really closely mimic patient samples and are actually derived from patient samples rather than from a biosynthetic approach. And the idea is they're going to reproduce the complete landscape of the. Circulating cell free DNA fragments, including the fragment size. Although we don't have data on that yet, the mutational content and most importantly for this also the CPG island methylation status. And we decided to employ a method which is quite similar to the one we use for our donor derived NAPT. Reference materials, which are based on unique patient pregnant patient samples, and we have very recently developed a prototype which is based on a colorectal cancer patient circulating. Cell free DNA. However, the same approach can be also used to create donor draft standards from any different tumour types or any different status, with the idea that one could create a library that could be used for evaluation of different assays. So the workflow is as follows. It starts

with the patient blood collection. From which we extract the cfDNA which is then amplified and processed using a proprietary method which preserves much of the original cfDNA characteristics or the size distribution DNA sequence and the CPG methylation patterns, and the final amplified. Of DNA can be provided in buffer or cephalic process control in plasma if needed and could be used as a. Really as a reproducible and sustainable sample that can be used to compare the performance of the different assays and the different methylation analysis approaches, in theory we could also make the methylation standards using our current method that preserves the mutation starters part. Would apply a genome wide 0% or 100% methylation and the idea is that this completely unmethylated sample from the same patient could be also used to dilute down the patient specific signal to enable limit of detection. Studies. However, the data I'm going to show you for now are just using the original patient derived sample without any modifications as to the tumour content. So to give you a bit of a background, this is the low pass and the Matic methyl sequencing data of our current methylation standards. And as you would expect here, you can see in red. It's the methylated areas in blue, the unmethylated, so the 100% methylation you can see everywhere is red and unmethylated. Everything is blue and this is the typical size profile which is quite similar to patient samples but not identical. However, if you look at the same data of our Donald derived standard prototype, so as I mentioned, this is a colorectal cancer derived sample. So we looked at the several of the loci that I mentioned which are relevant to some of the assays already on the market, so. Exception 9, which is a target of the EpiPen Colon assay. So here you can see this is the GM24385 cell line, which is normal healthy cell line which we use for a lot of our reference material. So we don't expect any cancer related or cancer like methylation pattern. This is. The native original patient sample, this is the reference standard that has been derived from the patient sample, and again the same type of processing for a healthy patient sample, CF. DNA sample and the referenced under developed from the normal from the healthy patient sample. So as you can see the methylation patterns. Are largely preserved around here on the septin side, and they are clearly distinguishable from both the normal and the cell line methylation patterns. And you can see a very similar picture when looking at other loci related to colorectal cancer detection. So the vim is the target of the cholesterol assay, so again you can see a clear you can really clearly distinguish the methylation patterns between the. C or C derived standard the normal derived standard where there is no methylation, and again the normal healthy cell.

Ed Schuuring

For only 1-2 minutes. Yes.

Krystyna Nahlik

Please so this is almost done and finally the same you can see for other from PM 3 which is the target of the colour guard. And just the final two slides showing that you can also look at the original mutational patterns in this sample. So we selected very high tumour fraction. In patient sample to start with the prototype, so you can also detect several clinically important SNV's in these samples, so this can be used as a reference standard for both methylation, but also S&V detection if needed. And that's all. I don't know if we have any time for questions, if yes. I'm happy to take them.

Ed Schuuring

Thank you very much again, Christina, we have time for one quick question. I don't see any questions here for the moment. Thank you. Again, I mean. We speak each other often, so I will discuss this in more detail later, and I thank you again. I would like to go on and hand over to to Patricio for the next session.

Krystyna Nahlik

Thank you.

Ed Schuuring

And I thank all the speakers to keep the time I really appreciate that.

Introduction by Prof. Marc Van Den Bulcke (Sciensano)

Title: OncNGS, Introduction of OncNGS PCP

Patrizio Giacomini

Thank you very much. Yes, indeed. Well, now we switch to the third and final section of our online webinar. So we are talking about other solutions. They are novel for two reasons. First, because obviously they are not yet on the market. And second, because of the technology, the. The approach that's been taken to develop them, it is a pre commercial procurement PCP EU project called OncNGS. That is a special blend of academia and company driven research, but I don't want to say anything more because we will have three talks in the first mark when the book who's the coordinator of this project will outline what's the procedure has been on changes, and then we will have two companies. To present their own solution. So Mark the stage is your welcome.

Marc Van Den Bulcke

Yeah, thank you, Patricio. Thank you very much to give us the opportunity to talk about OncoNGS. As you said, Patricia, it's a PCP and in fact it fits wonderfully well in what we are doing now in the European Beating Cancer Plan and the mission on cancer. But we started before we started, I think thinking about it in 2018 and the project really started in 2020. While the EBCP were only started in 21, but what we really would like to do is provide better cancer diagnostics and treatment for all and the process of the ongoing. Yes, you say it's a pre commercial procurement in. It the the final, I would say the drive is a tender that a number of organisations, institutions are compiling together and that are then designated as the buyers. So we were taking the initiative Ascension and invited a number of key institutions to help us to. To formulate. Tender. And then you open that to the market and you are supposed to have a competitive process in three steps, starting with four operators, economic operators in the beginning. Then after the first period, you select three and then you go to two and then we are now at the stage that we are in at

2. Selected in operators and they will after me present a little bit. But they have found as a solution to our challenge, which we formulated together with all the partners as being an efficient, efficient, molecular Iona, DNA profiling device of tumour derived material in liquid biopsy, it had to be bang cancer, it had to be based on NGS analysis. And integrated with an ICT decision support system that allows, let's say, harmonised test interpretation and harmonised reporting we receive. So for the the Commission provided money for that about 12 million Euro and the project started in 2020 and was supposed to last till 2025, but we got a prolongation so it ends mid next year. These are the partners, so we are since annual, we have in Belgium also institutional. But there in Italy it's the Alliance Secondary Council who is joining. Germany, we have a little bit maximum on in Munchen charity France Echo and Hospice, Civil de Leon and Institute Curie. So we have partners in five countries and we have also in Spain Eco so five countries. Hospitals that are going to buy and supported by the cancer, the Belgian Cancer Register, an IP company, the institutional, the cancer, just as us, a public health institute involved in bringing innovation into the healthcare system to research driven institutions and aquas who is actually, I must say, Patricia. Who learn does everything. Who is the body? Who introduces innovation in the Catalunya region? This is the complex process, so two years we were preparing the tender in phase one. It's a design phase. The four operate we had seven companies that applied and four of them were selected to develop their design. They got six months for that and phase two there was the analytical test. Performance testing with three partners and then in the last phase now and that's where we are today. We are in the phase that people can show clinical performance of the solution. What do we hope to get? Uh, more common guidelines more facilitating the data sharing. We hope that we get cross-border purchase because we set also of a time price limit to that. So we hope that this can also drive let's say. Disrupting the market a bit. We hope that it stimulates the molecular tumour boards, stimulates multicentric clinical trials and in the end develop patient matching tools that are can be operating through Europe and I think these were things that we decided in 2019-2020 and if I look at the situation today. Everything is still very pertinent and now I think, Patricia, I can hand it over to our colleagues first Agilent, and I know colleagues from Carolyn.

Patrizio Giacomini

Thank you. Thank you, mark.

6th Presentation by Dr. Alessandro Agostino (Agilent)

Title: Agilent solution for the OncNGS project

Patrizio Giacomini

So the first was going to speak is Alessandro Augustino on behalf of adjuvant. Alessandro, I see you online, so please go ahead.

Alessandro Agostino

So good afternoon everybody. And before starting, I would like to to thank Patrizio for inviting us and to the organiser for allowing us to give this presentation. I'm Alessandro Agostino, market developer from the life science diagnostic market group at Agilent focusing on cancer application and the biopsy. Has it been stated from by Mark, the OncNGS Challenge has started. I would like to start from the benefits. That comes from this. And that will be development, but also the challenges that the OncNGS project brought us to address. Basically the solution that we we've been developed is focused on a very high quality of data and result is focused on providing simplicity and ease of use with a really. Facilitated war. Low and also is focused on offering a scalable and flexible solution. As you know, the biomarkers are evolving and the possibility of adapting the solution to the upcoming new biomarkers is really a must have requirement. We achieved the development of a really. Compelling solution based on the combination of the most up-to-date disruptive technologies, both in terms of how we capture NGS lab technologies and. The thing that will allow to reach a really high affordable price per sample that will make the solution that argument is being developed longer has really compatible with the healthcare system. Reimbursement in terms of workflow that we. We'll be working on in this slide. You can see the major components of the of it. And we include the some of the well known equipment that we we've been working on for years like tape station that is a device for the quality control of the nucleic acid in specifically here in the client in the in the use of the CFD. Basically day where we can enable the relative quantification. Patient of the cell free DNA versus genomic contamination after preparation. The real innovator here is the technology company that we acquired 2 years ago named Avida through which we empower A newer regiment. I thought that as I will tell you in a in a couple of slides will improve the performance of the hybrid capture performances. This will be combined to our library preparation. Magness Angels persistent, that is fully automated walk away device. And the combination of this new technology for hybrid capture with any any sequence available, but specifically focus on the combination on the new sequence like element ability that is the one that we've been using for the both the analytical and clinical validation is really one of the major. Component of innovation because thanks to this combination, we can cater the highest sensitivity specificity possible at the lowest sequencing price. The entire overflow can be handled in as less as three days, depending of course on the sequencing platform that that has been used, because that is the the bottleneck of the duration of the workflow. The library by itself takes just five hours. Today, the technology has improved for the passes in in liquid biopsy, but there are still some challenges with ours. The workflows that are enabled for liquid biopsy today are sometimes very, very demanding in terms of time in terms of steps, in terms of bias introduced by PCR steps and in terms of recovery, there is a lot of material loss and sometimes the sensitivity is compromised thanks to a video technology that. This is based on a specific probe setting. As you can see below, we enable a workflow that allows the preparation of the libraries just in five hours. Getting rid of the pre captured PCR step. This of course will improve the quality of the libraries by getting rid of the possible bias introduced by the PCR. What is interesting here is that the performance of the of the system is really linear, meaning that the amount of molecules that you get. Out of the library, preparation is directly correlated to the sample input. And we can actually start from very, very low, good amount down to 1 nanogram as a starting material. Predicting the outcome in terms of recoveries and and and and the coverage at the end of the of the process based on this technology and these prop systems we develop. Has three specific partners based on the requirement that the consortium gave us, where are they listed? 2 list of genes. The name has must have genes, and

nice to have genes splitted by the solid versus haematological tumour specific genes based on those lists, we developed one partner. That will be commercially named as Amita DNA, LLB, Panel 164 Panel Cancer panel including all the most relevant genes based on the must have list that was provided and capable of detecting any. Type of barrier. And then we are, we've been working on the B Plus panel. It is a broader panel for 155 genes is including all the gene content from LB Parel, plus additional markers that were in the Nice to have list of genes. Last but not least, we also developed a specific parallel for cancer related to lymphatic system that is named DNA Lymphoma panel, where we put together 8584 genes from the master and the Nice to have genes list that were provided. We are going to present a poster at the ACR to share more technical data on the performance very soon. In terms of library preparation automation, that is. One of the key steps of making the process really easy and for reducing the. Manual error in handling magnis NGS prep system is allowing the the the preparation in just one working day with just 15 minutes hands on time and what we've been doing now is that of adapting the reagents of of avida and our new partners to the. Replated format required for for the liquid handler in terms of. Capability of our workflow as we've been talking today in this meeting about mutilation, it must be mentioned that avida is a neighbour of epigenetic analysis. Thanks to a special workflow where we can simultaneously analyse not just the genetic variant but also. Maybe genetic studies of the. As of today, the the common methods based on the analysis of methylation from cfDNA sample are biassed by the limitation that you need to split the samples if you want to do methylation analysis, or if you want to do. Genetic variant analysis. These result in a loss of signal and the loss of sensitivity when you have a low amount of of DNA, you cannot waste the waste material. So we developed thanks to Avid and thanks to the high sensitivity and specificity of our probe system and our protocol is one single workflow. You start with the first capture of the genetic targets, and then instead of discarding the supernatant, you can utilise it for a second highly capture of the epigenetic. Targets. This will allow you to save as much as possible your purchase samples and at the same time provide not just the the status of the genetic variants of the samples, but also the epigenetic status of the samples. We developed a specific code that was named Mutilation Index that is gives the sense of the presence or absence Our actual ct DNA. This system works for a very limited amount of DNA. We tested even below 3 nanograms. And enables the multi omic analysis of one single plasma sample exploiting the the performance of of a video technology. The workflow in the dual modality takes a bit more than 8 hours, but it's still compatible with one day one day. In terms of sizing the key features of our solution for open JS as I just mentioned that either technology is really a differentiator in terms of efficiency in terms of the capacity of generating results or very high quality in terms of specificity sensitivity, even with very low input amount. And a very a very good limit of detection as we can start from a very, very low input amount as low as 1 nanogram. As I mentioned, the workflow is a workflow that can be. Flexible because it can be handled even manually. There were it's really easy. There's no pre captured PCR so it can be leak. It can be easily handled manually but we will automate it to increase flexibility to reduce the risk of manual error from the operator and also for increasing the reproducibility. The turn around time as mentioned is 3 to 4 days and in terms of other benefits our solution is compatible with the most advanced sequences like, not just the new platforms from Illumina, but also. Tested Elemental media back bio also so the the the bench top devices that have been launched in the last in the last years. The solution is scalable and flexible to be adapted to new biomarkers as mentioned or to the needs of the lab depending on the throughput. And thanks to the features of our. Solution we can be really competitive in terms of paper sample as we combine a really efficient technology with sequencer

where the quality is increasing and and the the the sequencing cost is really, really going going down. And this was my last slide, so open for for question. Thank you.

Patrizio Giacomini

Give us some wrong, very clear and to the point. If there are any questions, we have time for one or two very quick. Things I don't know if anybody wants to bring up anything. I do not see anything in the chat but.

7th Presentation by Prof. Joris Vermeesch (Katholic University of Leuven)

Title: Integrating of fragmentome and mutation based tumor cfDNA profiling

Patrizio Giacomini

So let's move to the next speaker and then we try to meet the 16 PM the the 6:00 PM deadline. And we may have some time later to address the other issue. So the next speaker I can see your. Is the mesh professor here? Is the mesh online and it's about the Catholic University of Living solution. Yaris, please go ahead.

Joris Vermeesch

Thank you very much everyone for the first time in my life. I'm I'm on the. Company side, just the trucks. And it will be a little bit hybrid. OK. So in this call, we participated in the tender with a solution we called Explore Plus build mutations and now hope that's going to be clear. Just who we are basically we are three PI's myself. So we coin our Explore Plus build mutations and I hope that's gonna be clear. What that meant. We submitted a project with three Pis, myself, Isabelle Vanden Bempt who is a PI on Onco-diagnostics and then Yves Moreau, who's a AI machine learning engineer who's joining on the project. And then we see a number of names with Wouter Bossuyt who's leading the genomics course. Ton Broeckx and so forth. The we teamed up recently with Uncle DNA and that's what I'm gonna tell you about. So what is the solution? Basically, we created the focus of the call and the tender was on detecting mutations in cancer. But as we heard already. In this meeting, there's more in the data and cfDNA and just mutations analysis and so we. We we basically split the cfDNA sample in two and for one one subset of the sample we will do what we heard before is basically capturing a panel of genes actually was a list. Provided by the tenders of about 460 genes, if I'm correct weekly that target that capture probes to make sure that we had a very sensitive approach of capturing those. Genes. And then we do deep sequencing and in parallel we do shallow sequencing of the cfDNA fragmentome we call it and then we go into an analysis this process here in the beginning is automated in, in the lab we have we're using adjusted Hamiltons to. Make

this whole process automated and then we will run samples on a sequencer. Typically we use the Illumina 6000 or the X plus for sequencing. And we have a series of analysis pipelines, some of which are proprietary, which we can then use in combined to give you an output and and gather smart results. Let's say I'll briefly zoom in into this. What we exactly are analysing well the whole solution. Can be done in less than one week. Actually just the sequencing and data analysis in for five days. But then you need a day to interpret and sit down with the molecular tumour board so it will take you a week to do everything. So what's beyond the state-of-the-art? So first of all, we have the comprehensive gene panels for capturing the idea. The solution had to be able to be used for both diagnosis, treatment, response analysis, minimal result of disease. So the, the, the, the, the, the aims were quite high. OK. And so I will briefly show you how we go beyond limitations. Sorry. So first of all, we make use of the low pass sequencing where we can do copy number alteration, profiling which we piloted to 10 years more than 10 years ago in non invasive prenatal screening. We not just capturing the redeploy as many tools do like, but we are measuring its scores, which is slightly more sensitive in detecting variations in the methylation profiles. Just to show you here an example, we know we know from many studies that actually the these patterns in cfDNA are congruent or similar to the tumour patterns that we see. If we sample the solid tumours. The nice thing is from the cfDNA we can do more and we showed already a few years ago that we can do a principal component analysis of different samples. If you have samples with and without. And if we do principal component analysis, usually tumour samples split from control samples and then of course we can use classifiers to identify which tumour is or which profile belongs to which tumour. And just this is an old slide from the paper which we published. Long time ago is an example where we actually are mapping multiple myeloma diffuse large B cells or patients. You have DNA from patients with. Large B cell lymphoma as multiple myeloma than Hodgkin's lymphoma, and as you can see. Clustering is not perfect and then grey is the controls. As you can see, there's quite distinctive profiles for each of these tumour carriers with a very high sensitivity and specificity. If you look at the areas under the curve for these different specific leukaemia, I must say the same is true for solid tumours. But the the specificity and sensitivity is somewhat lower. So we believe that we have scalable and flexible design, so we can easily add new genes if that's necessary. We automated everything. Yeah, this is what I mentioned already maybe where the collaboration with ONCO DNA is really very, very valuable. Is they have of course already experienced with all the logistics of of things, and they also have an excellent interface for people who do the interpretation and the molecular tumour boards to help them in interpreting these results. We have a whole suite of analysis that we're incorporating in our in our methodology at SMC calling is we we, we we have a fusion of different variant colours such that we believe that we have a more sensitive approach and accurate more accurate approach than just. Regular what most people do when they just use one colour and then we incorporate the number of other analysis into the CF DNE mining. When we performed this, this is on a series of samples which we created with artificial or samples to test the performance of detection of the different test and visa and indels, different concentrations 3 to 5 around 5 to 25 nanogram. As you can see here and then at different concentrations. 5% two percent, one percent 0.5% tumour fraction and I realise this is only showing the 5% fraction, but we felt that we have very good performance. For the variant. calling. We also tested this not just on artificial samples, but also on first 20 tumour samples which were tested before cfDNA by ddPCR or were positive by ddPCR or FoundationOne liquid biopsy. This is a very small sample set. Do you realise? We're currently extending that. First of all, if we detect the mutation in the cfDNA, the correlation in terms of the concentrations with what was

detected with the ddPCR were, in our opinion, excellent. And these were some of the results. So we with the cfDNA capture. We missed or we had 80%. More than eighty, yeah, 80% positive with just the CNA analysis we had 83% coverage and I realised in the combination there was only one sample that was actually missed by the combination. But was ddPCR. Positive sample. So in the 20 it was OK. So we see that we're on the right track. I just show you what the PCA analysis shows you is. These are the samples that we had in in, in, in our data set and as you can see the colorectal cancer breast cancer and the lung cancers actually segregate to some extent. So we believe that the fragmentome analysis is is a nice addition in and can help in the interpretation of the. Findings if in case, even in the absence of mutation, in the absence of a mutation detected in the cfDNA. Obviously, I'm very excited about that because for now we have also a tool where we can combine both mutation analysis and fragment tone analysis and we're now exploring to use that in the advanced therapeutic settings and and and a number of clinical trials are underway. So in conclusion. We have a powerful multimodal approach because we believe it's cost effective. It's driven by the oncology teams. So we know that it's going to be useful and we're building working woth OncoDNA to make this an option if it has two more minutes, maybe I'll take two more minutes. He also believe he can go beyond and in the in the research lab case. Show that we can actually insert. So we are our Kate show that if you can. So from the window protection scores, we know that the cfDNA is deduced from degraded tissue and we know that that is correlated with the transcriptome of of tissues, and Kate took the tabula sapiens single cell atlas with over 450 cell types and infer to which. The cell types would be present in cfDNA. She could show that we can actually deconvolute the Constitution of the cfDNA. And exciting if you take two populations, male versus female, you can see that certain cell types go up in the male or ranked higher compared to females and amazingly, or at least the mice view. Amazingly, the prostate, we can see prostate derived tissue going up compared to. Uterus derived tissue types which are going. down, and I think that shows the power and the sensitivity also just the fragmentome analysis in that we can deduce tissues of origin. But even tissues which are in low quantity present can be deduced if your population is high enough and you see here you see pregnant versus non pregnant and then if you do that with with. Cancers versus controls. We start seeing cell types which are upranked and downranked in breast cancer. Colorectal cancer and multiple myeloma and these cell types are different in each of these folks. We call these volcano plots, but they're different and they're specific for the tumour biology. So we're very excited if we use classifier, we can use this as a biomarker and use this not only to deduce which. Tissue types are are induced, but also as a buyer market and we hope we can build that in into future versions of the solution. So in conclusion we are imputing single cell Atlas as it enables the deconvolution of cfDNA composition and didn't really show that. We can look in our paper, we can infer tissue changes, which allows us to learn about biology, and it can be used as a cell ranking. So, and if we combine all these multi modal aspects of fragmental mix and mutation capture analysis, I think we will. We're moving towards very sensitive detections of cancers. Thank you very much.

Patrizio Giacomini

Thank you Joris. Thank you. Well, I must say that every time I listen to the sound and yourself, I always learn something. So I don't know whether there are any comments, whether there are any questions for the last two presentations that as you have seen are mainly focused. On you know, cancer diagnostics, but also more in general about what we have talked about today that I think it's a very interesting topic.

Ellen Heitzer

Yeah. Thank you. Amazing talk is is always for the cheap explore. Plus you said that you in for the copy number alterations based on C scores this do you also report a tumour fraction because particularly in the molecular tumour Board, it really helps if you have a mutation agnostic? Measure for the tumour fraction in order to have the context. So you you're not including this because basically. From a Cisco, you cannot infer the tumour fraction. I guess you can only approximate it, probably or.

Joris Vermeesch

Exactly. Yes, it is. It's of course we cannot do that. We would need to do just so it's you know, I of course the the. The programme which most people use that based on sequencing depth, and there you can get a score, but there the limitation is at the cut off at least is 3% and so if you want to go lower.

Joris Vermeesch

I think that's. Where? Where the tricky part comes and no, yes. So we will not.

Ellen Heitzer

But I would just. I would. Just recommend of including. I don't know an ACNA based estimation because it's really it really. It helps in the molecular tumour board and in interpreting the the the result and if if it's only just stratifying into higher than three and lower than three.

Joris Vermeesch

No thanks for the suggestion. I think maybe I completely agree, Alan, and we, yeah.

Patrizio Giacomini

Any other curiosity? Coming up from the audience. Well, can I ask? OK, let let's, let's say and it's it's 6:00 PM. So let's end with the social game. So I'd like to hear from all of you anybody in the audience where do they think that metabolomics and fragmentary, so the epigenomics, cfDNA epigenomics? Can help more in diagnostics. We have seen many applications but name one in your opinion where that would be the biggest leap forward through these approaches in the next few.

Hélène Bauby

I can start, maybe disease monitoring, fragmented mix and methylation signatures might be a very good tool to monitor the disease in cancer patients.

Patrizio Giacomini

You mean the longitudinal monitoring? Yeah. OK. That's. And how about minimal residual disease? Do you think they will be able? To complement the.

Hélène Bauby

Yeah, I think. To complement like single nucleotide variation monitoring. Yeah, at all. I think that depending on the pathology, some biomarkers might might be more suited than others and S&V might not be the right choice depending on the pathology. So.

Closing remarks by WG leads:

Patrizio Giacomini

OK. Well, any other suggestions? If not, I'd like to conclude with Ellen and Ed. Thanking all of you for participating. It was really an interesting, very interesting seminar, I believe. And at the libs, we are very eager to listen from you. And to have suggestions for future webinars. I think this is not the last webinar that we will run on cfDNA epigenomics, because it's a rapidly expanding field. So if anybody has ideas of what about presentations, please refer to Dania. I also thanks Dania for organising so well and I would like to leave the last word well and and then for conclusion.

Ellen Heitzer

I think you nicely concluded. Everything I just want to also make him comment on your question. What also yours showed nicely. I think with fragment tonics and methylation markers we can really also move beyond cancer now because if we know the. The composition of the cell for DNA pool, we can really use this for many different other conditions, and there's great studies out there for various types of conditions. So I think this is only the beginning of a new era. And I also like to thank all the the the, the speakers. It was great. Thank you so much for joining us. Yeah, that's it from my side.

Patrizio Giacomini

Yes, and very brilliant. I mean, this is a very important point that you raised. And I'm also thinking about immunotherapy. I mean the, the, the, the approach of knowing which immune cells are in C DNA or possibilities, I mean other less and another major potential area of interest.

Ed Schuuring

Yeah, I'm not going to repeat everything I I mean, I think I think it's very important that we would like to we we keep on offering this platform to to inform you and get the get good talks here and subjects. So in fact, we are looking forward to the next workshops. We are going to plan this year from the Elbs Citizen Working Group. Thank you for all your attentions.

Patrizio Giacomini

Well, thank you very much. Have a good evening.

Ellen Heitzer

Good evening, everyone. Bye bye.