Clinical Chemistry

Quantitative 1-Step DNA Methylation Analysis with Native Genomic DNA as Template

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

The July issue of *Clinical Chemistry* reports a novel DNA methylation assay that reduces both the hands-on time and potential errors caused by handling a pipetting and allows methylation analysis to be completed within 90 minutes after DNA extraction.

Combined with its precision and reliability, these features make the assay well-suited for diagnostic procedures as well as high throughput analysis.

Dr. Thomas Von Känel, lead author of the paper is a Researcher at the Division of Human Genetics in Bern, Switzerland, and he is our guest in this podcast.

Tell us, Dr. Von Känel, what was the motivation to develop this novel assay?

Dr. Thomas Von Känel: Well, the turning point was to determine the methylation state of a gene that we were interested in. We first tried browser-based protocols, which however initially produced some artifacts in our hands. We thus turned towards methylation sensitives and endonuclease. We first digested and treated genomic DNA with methylation-sensitive endonuclease HBA2, and then determined the number of cut DNA molecules with quantitative PCR.

This approach allowed to conclude on DNA Methylation, and their amplified reaching.

One day I was not particularly motivated for doing lab-work and the idea struck me to directly add the endonuclease to the qPCR mix. I tried this out and started an initial short incubation at 37 degrees to the actual qPCR cycling. First I have indicated that this combined approach works quite well, and this made it the first one-step approach for DNA methylation analysis.

Evidently the combination of native genomic DNA as templates and the one-step approach significantly reduced workload.

Host: So, can you tell us some of the specific applications that you pursued and do you see other applications for it?

Dr. Thomas Von Känel: Well, I think the assay is useful in all those situations where you want to measure DNA methylation at a

specific locus. Accordingly, there are many potential applications for it.

We for example used it to diagnose Prader-Willi Syndrome and Angelman Syndrome. These are neuro-genetic disorders, which are characterized by aberrant methylation patterns at the SNRPN locus.

While Prader-Willi patients will show 100% methylation, Angelman syndrome will show no methylation at this locus.

Other applications of the assay might include cancer diagnostics or it will be used to identify DNA methylation in bacteria. But the assay is not only limited to DNA methylation analysis, evidently it can also be used for genotyping or allele-specific quantification for example you just need an appropriate endonuclease that retains its activity in your qPCR mix.

Host: So now what do you consider to be the utmost important strengths of your new assay?

Dr. Thomas Von Känel: Well, personally I like it because it's easy to establish. You only need two primers and an endonuclease that fits the locus you are interested in. As the assay should work with many endonucleases, one is quite flexible in assay design.

In addition, once you have established a specific assay, you will have to do a little pipetting for sample, and you get your results quickly.

Accordingly, I think the assay is well-suited for high-throughput investigation. This makes it highly suitable for projects investigating many samples at a specific locus.

Host: With that being said, doctor, are there any weaknesses?

Dr. Thomas Von Känel: Yeah, evidently the number of sites that can be analyzed is limited by the number of available endonucleases. In addition you will not get no sequence information like bisulfite sequencing. It's also hard or even possible to achieve full digestion of the DNA unfortunately.

Accordingly, the assay is not suited for all those cases in which you are interested in analyzing very low methylation levels. So I wouldn't for example, rely on the assay to detect residual disease in cancer

using a methylated biomarker, but maybe this problem can overcome one day.

Host: You mentioned that the assay is easy to set up, is it really that straightforward to establish methylation analysis with it? Or are there any pitfalls that potential users should be aware of?

Dr. Thomas Von Känel: We mentioned it in our paper, the assay might be prone to positional effects. This means that your results can be fortified by artifacts produced by the qPCR instrument. This is simply because one is often interested in very GC-rich reach when analyzing DNA methylation and GC-richness of the target region is always a predisposing factor for such positional effects in qPCR.

We addressed this issue in our 2007 letter to *Clinical Chemistry*. If you follow the guidelines given there, you will presumably not get into trouble. In addition, we are currently preparing an additional paper describing how these effects can be overcome on plate-based qPCR instruments.

Host: Is this your only pitfall?

Dr. Thomas Von Känel: If you use DNA extracted with CTAB-column-based protocols, then yes, to our current knowledge. However, with low-quality DNA you might additionally encounter incomplete digestion as described in the paper. However, this can be overcome by applying our correction model, yet, its application should be well validated for every specific assay and every set of DNA samples.

Host: Doctor, what do you see as the future for your assay?

Dr. Thomas Von Känel: Well, evidently I wonder if the assay will find its way into the labs, considering how much it can facilitate DNA methylation analysis, I am quite confident regarding this.

Host: Dr. Thomas Von Känel is a trained biologist and a researcher at the Division of Human Genetics in Bern, Switzerland. He's been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening!

Total Duration: 6 Minutes.