Q&A: Peter Vallone of NIST Talks Trends in Rapid PCR for Forensics

Jun 03, 2015 | Madeleine Johnson

Premium

Peter Vallone

NEW YORK (GenomeWeb) – The technology to identify people using short tandem repeats, or STRs, is mature, but it is being rejuvenated.

In a recent review article in the journal *Forensic Science International: Genetics*, Peter Vallone, leader of the applied genetics group at the National Institute of Standards and Technology, and his colleague Erica Romsos carefully documented numerous advances in rapid PCR for human identification, including the introduction of commercially available integrated platforms.

Standard workflows can be sped up at various steps in the process, from extraction to amplification and detection. Integrated "swab-in, answer-out" platforms, meanwhile, debuted a few years ago, and the RapidHIT from IntegenX and ANDE from partners NetBio and General Electric, are now commercially available.

However, use of these platforms is limited by the wording of the 1994 law that established the Combined DNA Identification System, or CODIS, which requires tests be run in certified forensics laboratories.

A congressional subcommittee is now mulling over the Rapid DNA Act, or H.R. 320, which, if ratified, would allow DNA testing to be done in "booking stations, jails, prisons, [and] detention centers ... that can perform DNA analysis using sample-to-answer DNA systems."

Indeed, representatives at IntegenX told GenomeWeb this week that they so far have placed more than 140 RapidHIT platforms, about two thirds of them in international markets. The first ever integrated platform data, from a RapidHIT rollout in the state of Arizona, was uploaded to CODIS in May. The Rapid DNA Act is purported to have support on both sides of the aisle, and its passage will likely boost adoption of these platforms.

In an interview with GenomeWeb, Vallone, who also oversees production of the STR reference standard called SRM 2391c, talked about adoption of integrated platforms, drivers of advances in rapid PCR, as well as the potential for next-generation sequencing in forensics applications.

What follows is an edited transcript of that conversation.

Why is a fast turnaround for STR typing so important?

It is usually better to have the information faster, and it is usually more economical. In STR or DNA typing for forensics, there are maybe two to three hours that are needed for PCR. If you can bring that down to a half hour or 15 minutes, you're reducing the overall workflow time. It allows you to have higher bandwidth for getting samples out, you can help reduce backlogs, and improve your turnaround time. Also, the faster you have the information, the faster it can applied. It can be used to search or be uploaded to a database. Then the PCR amplification portion of the process isn't the bottleneck anymore.

From a scientific point of view, it's also fun to see if you can make a process faster or improve upon it from a molecular biology standpoint.

What parts of the workflow have people tried to attack to speed up the STR typing process?

Faster polymerases — ones that bind to the DNA longer so they can process across synthesizing the new strand, giving faster amplification — have been engineered. PCR is a series of heating and cooling steps, so having faster devices — whether those be heat blocks, or a fan method, or microfluidic-based — in combination with the polymerases, will give you more rapid amplification.

There's also direct PCR, in which you bypass DNA extraction and put a blood punch or saliva punch on paper right into the PCR reaction. Polymerases used in those cases can tolerate inhibitors that allow the direct PCR process.

Also integrated devices, with everything on one cartridge, take advantage of the small volumes and other benefits that microfluidics can provide.

Did any trends jump out at you when you reviewed these different strategies in your recent paper?

I think increasing speed is based on the engineering of some of the polymerases. The combination of the faster polymerases and the thermocyclers is what's exciting. With previous polymerases, even if you had a faster cycler, you wouldn't be able to accomplish the fast PCR amplifications.

Once the PCR is down to, let's say 15 minutes or so, it opens up other avenues for making a microfluidic device ... that can do the whole workflow in an hour or 90 minutes.

You mentioned the commercially available integrated platforms in your review. Does anyone

actually use these?

DNA typing labs are just starting to take a look at them now. Some labs can afford them and they're starting to pilot those for rush cases, or if they want to get investigative leads.

They can provide a DNA profile in [about] 90 minutes. It's as simple as putting a swab in a cartridge, popping the cartridge in a machine, and hitting "go." All the steps are on board in the integrated process. You can see how that could be attractive — it's faster, requires less operator training, and you can have it in a lab or it can go out in the field and you can generate profiles.

How much do they generally cost?

I think an instrument would cost [about] $250,000. The integrated cartridge set is approximately $1,000 to $1,500 and you're typically running anywhere from five to seven samples on that cartridge. As it becomes more economical to mass produce them, you may see the price of the instruments go down, as well as the price of the cartridge consumables. That will probably help with adoption.

Do you think swab-in, result-out STR typing is 'the wave of the future,' or will we still need technicians who can run the entire PCR workflow?

In the short-term I think we'll still need to have manual analysis and practitioners running the samples. As the technology becomes more robust or more commonplace, maybe it can become an integral part of the lab itself.

Forensics is somewhat conservative, because the information generated goes into the criminal justice system. You want to make sure that the science performed is reproducible and robust. It's also a matter of cost and throughput, and you're kind of always competing with a 96 well-format type of scaling.

Sequencing is increasingly being developed for forensics applications — Illumina launched a dedicated platform earlier this year, for example, and feasibility studies are underway — but is NGS likely to be adopted by forensics labs in the near future?

Forensic researchers are starting to look at this for human identity applications. Sometimes with sequencing you can get a finer resolution of the STR and it also allows for SNP typing. It is important to make sure things are backward compatible, so you get the concordant results from next-gen sequencing as you get from capillary electrophoresis.

A benefit will be obtaining information that's not included in the traditional STR typing. So the idea of using SNPs to estimate ancestry or to do eye color, hair color, the phenotypic traits, is attractive.

There have been a few interesting cases of phenotypic profiling lately — litterer shaming in Hong Kong, a portraits-from-gum art project in Brooklyn — as well as emerging ethical issues with an ancestry database recently mined for leads in a cold case. What are your thoughts?

That's one of the issues with the next-gen sequencing. STRs are neutral markers. They don't tell you anything about health or phenotype or ancestry. In parallel with adopting next-gen methods will have to be some policy and ethical components.

Do you anticipate sequencing ever replacing STR typing?

I think the next five to ten years will be interesting. Right now there about 12 million STR profiles in the national database, so I could envision over time migrating from CE to next-gen sequencing. It might be a little bit slower in terms of moving those core STRs to a different type of marker. If anything, I would see it being laddered, where you adopt new markers, such as SNPs, but concurrently keep STRs going for a period of time.

Clinical lab researchers seem to be motivated by improving health or saving lives — what motivates forensics researchers?

I think part of it is providing unbiased science that can be properly used within the criminal justice system; trying to do the best science that you can, so that it can be applied appropriately. Information is being used to convict people who are guilty or exonerate those who are innocent, so the science needs to be of the highest quality.

Filed Under PCR | Applied Markets | STR analysis | IntegenX | NetBio

Related Articles

Jan 21, 2015
Illumina Launches MiSeq FGx for Forensic Applications

Sep 11, 2014
FIU Researchers Show Value of qPCR to Evaluate PCR Inhibition, Improve STR Amplification

Oct 14, 2014
Battelle, Forensic Labs to Put NGS Technology through Its Paces for Human Identification

Sep 22, 2014
IntegenX Secures $10M Loan

Jul 10, 2014
Diomics Poised to Grow Biospecimen Collection Business Following Recent Positive Study, US Patents

Sep 09, 2014
Johns Hopkins Team Designs SNP-based Haplotype Method for Human Identity Testing