B71 A Novel Workflow for Identifying Phenotypic Polymorphisms in Detoxification Enzymes Associated With Drug Metabolism

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After attending this presentation, attendees will have an alternative workflow to proprietary enrichment and library preparation pipelines provided by the major manufacturers of Massively Parallel Sequencing (MPS) platforms for the identification of Single Nucleotide Polymorphisms (SNPs), small Insertions and Deletions (INDELs), and Copy Number Variants (CNVs) in detoxification enzymes related to drug metabolism.

This presentation will impact the forensic science community by presenting an alternative workflow for identifying genetic polymorphisms associated with drug metabolism that forgo the proprietary workflows developed by manufacturers of next generation sequencing platforms. Alternative approaches have potential cost-saving implications as phenotypic polymorphisms are considered in molecular autopsies.

The capabilities and affordability of MPS are bringing personalized medicine into the realm of possibility for many patients. As costs continue to fall for MPS analysis, pharmacogenomic approaches offer an opportunity to provide insight into personalized drug metabolism rates. Most current efforts in this area have focused on opiate metabolism by members of the cytochrome P450 family of enzymes, typically in regard to accidental overdoses.

Polymorphisms associated with metabolism function are largely classified into the type of genetic lesion and the phenotypic effect associated with each particular mutation. The types of lesions are broken down into SNPs, small INDELs of nucleotide sequence, and additional CNVs from gene duplication events. (Due to being the most prevalent and similar to INDELs, both SNPs and INDELs will be referred to as SNPs for the remainder of the discussion.) As mentioned previously, the mutations are then further classified based on the phenotypic effect they have on enzyme function. These are broken down into silent mutations (in which no effect on metabolism is observed), gain of function mutations (in which an increased rate of metabolism is observed), and reduction/loss of function (in which an ablated or reduced rate of metabolism is observed).

As mentioned above, the expanding capabilities and reduced costs of MPS platforms allow an expansion of this technique to not only provide information for molecular autopsies associated with cardiac arrest or opiate overdoses but could provide insight into the levels of intoxication an individual has based on personalized drug metabolism rates. This information could be pertinent in establishing levels of impairment for culpability associated with drug-induced accidents.

One limitation to wider implementation of such technologies is that the costs are still slightly more than would be feasible for routine testing. To remedy this, a series of iterative price reductions are likely necessary to bring the costs down enough to make this type of testing routine. One area in which large cost savings are possible is the area of target enrichment and library preparation. Currently, manufacturers of MPS platforms have proprietary custom target enrichment/library preparation workflows that ease the design of the assay by taking the work load from the practitioner and exporting it to their production facilities. Without budgetary restraints, this scenario is favorable and desirable.

In situations in which budgetary constraints don't allow the higher price associated with proprietary custom target enrichment/library design, an alternative method that is only slightly more labor intensive is proposed.

Utilizing Primer-BLAST, primers were designed for long Polymerase Chain Reaction (lPCR) around the gene for the human carboxylase enzyme (CES1), which is known from literature to affect methylphenidate metabolism. The gene is encoded over an approximately 31kb region on the q-arm of chromosome 16 in humans. This study designed lPCR primers that broke the gene into two overlapping amplicons of approximately 17kb each. These amplicons were then pooled at equimolar concentrations and served as the enriched target for MPS library preparation. A third party enzymatic library fragmentation kit was used to cleave the lPCR amplicons into fragments of 200bp for MPS suitability. Another enzyme from the same kit was used to repair the cleaved ends and a third enzyme was utilized to ligate adaptors for barcoding/indexing of fragments prior to clonal amplification on an automated library preparation platform. Once the samples were placed onto the automated library preparation platforms, the recommended workflow of the manufacturer was followed through sequencing. SNPs were identified via both the manufacturer's bioinformatics software package as well as independently via generated fastq files and freely available software (IGV).

Analytical thresholds for identified SNPs were established based on depth of coverage in a region and percentage of reads observed for individual SNPs. This was based on an initial training set of 32 individuals sequenced twice for concordance between runs. Additionally, the nature of the mutations was determined based on 172 individual samples that were classified based on their sensitivity to methylphenidate and clustered via principle component analysis.

The method described could be adapted to other targets and purposes, such as molecular autopsy and other detoxification enzymes. Additionally, sonic fragmentation of amplicons could be used in a similar fashion.

Phenotypic SNPs, Detoxification Enzymes, Pharmacogenomics