2012 Winning Abstracts

G22. A Retrospective Study of 200 Fragile X Samples using AmplideX mPCR: Improved workflow, data quality and concordant results relative to Southern blot analysis

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Introduction: Fragile X (FX) syndrome is caused by an expansion of a CGG trinucleotide repeat at the 5' untranslated region of the fragile X mental retardation 1 gene (*FMR1*). Expansion of CGG repeats beyond 200 (full mutation) become abnormally hypermethylated at CpG islands in the 5'- untranslated region of the gene, leading to *FMR1* gene silencing, the absence of the FMRP protein and consequent phenotype. The gold standard for the detection and determination of methylation status of such expanded alleles is Southern blot (SB) analysis. Southern analysis is expensive, time and labor intensive and requires a high quality and quantity of DNA. A novel methylation PCR (mPCR) assay (Asuragen) based on methylation-sensitive restriction digest of DNA and comparative PCR of digested products has been described to assess methylation as well as (CGG)n sizing of such samples. This high throughput approach has the potential to reduce or replace the need for Southern analysis for the determination of methylation status of such samples. The assay may give a more informative assessment of FXS mosaic samples in determining the percent methylation of subpopulations of expanded alleles. **Methods:** We evaluated 200 FX samples that consisted of 112 females and 88 males and comprised of 36 normal, 36 intermediate, 65 premutation and 63 full mutation alleles. All AmplideX mPCR runs were performed at ARUP Laboratories using 10 µL of 20 ng/uL of DNA. Repeat length and methylation status were calculated using peak heights of digested and undigested DNA. All results were compared directly to in-house PCR and Southern analysis whenever possible. **Results:** The methylation status of the 63 full mutation samples revealed by mPCR was concordant with Southern blot analysis for unmethylated, partially methylated and fully methylated alleles, and included expansions as large as ~1000 CGG. In addition, the resolution of mPCR products after capillary electrophoresis revealed molecular features of expansion and methylation that could not be discerned by Southern blot analysis alone. Premutation and normal samples showed normal methylation patterns as expected. **Conclusions:** We find that mPCR methodology is a potential alternative to Southern blot that offers a high throughput, low DNA input, PCR-only workflow and may clarify the interpretation of complex mosaic samples associated with FXS phenotype.

ID14. SENSITIVITY OF HIGH-THROUGHPUT SEQUENCING FOR VIRAL DETECTION IN BLOOD

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Introduction: Unbiased high-throughput sequencing is a promising method for viral detection in clinical and public health settings. However, optimal library preparation techniques and limits of detection are largely unexplored with this technology. We analyzed the effect of various library preparation protocols on the ability of the Illumina MiSeq instrument to detect viral sequence reads, and performed sensitivity analysis for detection of HIV in human plasma. **Methods:** Plasma containing HIV at various titers was used to prepare multiplexed libraries for sequencing on the MiSeq instrument using a 300 cycle Miseq reagent kit for a 150-cycle paired-end run. We assessed two different library preparation methods (Illumina ScriptSeq and TruSeq-modified) as well as nuclease treatment prior to extraction ("pre-nuclease" / "pre-DNAse") or immediately after extraction ("post-DNAse") to reduce host background contributions to the sequencing reads. Sensitivity was determined using a dilution series containing 10^2 – 10^6 HIV particles spiked into negative matrix sera. Sequences were identified as viral by nucleotide BLAST (BLASTn) alignments to HIV (e-value cutoff of 10^-10), and the number of HIV sequence reads generated for each sample as a percentage of total reads was compared. **Results:** Approximately 1.3 to

4.3 million sequence reads were obtained for each sample. At 10^6 viral copies/mL, pre-nuclease treatment increased viral reads from 0.03% of total reads (445 reads) with no treatment to 3.1% (45,517 reads). ScriptSeq and TruSeq-modified post-DNase protocols generated comparable percentages of 0.07% (2,928 reads) and 0.08% (1,410 reads) HIV reads, respectively. At viral loads of 10^4, 10^3 and 10^2 copies/mL pre-nuclease treatment yielded 1.68% (27,173 reads), 0.28% (3,762 reads) and 0.002% (39 reads), while pre-DNase treatment yielded 1.75% (28,300 reads), 0.14% (2,020 reads) and 0.004% (50 reads) HIV sequence reads. Log-log regression plots of the read percentage against the spiked HIV titer were linear with an R2 of approximately 0.998. **Conclusions:** Pre-nuclease / pre-DNAse treatment of samples significantly increased the number and proportion of viral sequence reads in plasma samples. Two different Illumina library preparation protocols achieved similar yields of viral sequence reads. Our protocol was able to detect viral sequence reads down to at least 100 copies/mL, and is thus a sensitive method to perform unbiased detection of viruses present in clinical samples. Sample analysis for viral detection and discovery using these methods is available as a core laboratory service through the UCSF Viral Diagnostics and Discovery Center (vddc.ucsf.edu).

TT10. Using a "loop-out" Primer for Identification and Prevention of MEN1 Genotyping Errors Caused by G-quadruplex- and i-motif-like Sequences

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Introduction: A common polymorphism in intron 1 of the *MEN1* gene, c.-23-16C>G (rs509606), alters the stability of G-quadruplex- and i-motif-like DNA secondary structures such that they interfere with PCR amplification. In heterozygous carriers of rs509606, the variant allele is preferentially amplified by PCR, and mutations in exon 2 of *MEN1* can either be missed or appear homozygous due to allele dropout. While varying the PCR enzyme and buffer conditions has been reported as one solution to this problem, we sought a methodology that would allow us to maintain consistent PCR conditions between different amplicons. **Methods:** A forward PCR primer for *MEN1* exon 2 was designed to "loop-out" a 10 nucleotide region from the template that included the rs509606 polymorphism. Primers were validated for accuracy with 19 samples that were also characterized with an alternative primer set (external primers) that would amplify through the problematic region. Inter-run and intra-run variation was also assessed. Results: Eight of the 19 samples characterized had the rs509606 polymorphism, one of which also had a known pathogenic mutation, c.249_252delGTCT in exon 2. Of the remaining samples, 10 had normal wild-type sequence, and one had a polymorphism, c.435C>T. When using the external primer set, the sample with both rs509606 and c.249_252delGTCT clearly showed preferential amplification of the allele containing the polymorphism; the deletion on the opposite allele was undetectable. When amplifying the same sample with the noncontiguous primer, c.249_252delGTCT was clearly discernible. The sample containing the c.435C>T polymorphism was clearly heterozygous when amplified by either primer set, because it did not contain the rs509606 polymorphism. **Conclusions:** Allele dropout resulting from the common *MEN1* gene polymorphism, rs509606, can be prevented by using a noncontiguous primer that loops out the polymorphism. Additionally, the use of this design principle for primers may simplify the analysis of other exons that are flanked by sequences that complicate amplification or sequencing.