Conclusions: The Pan-Cancer 6-Fusion Panel FFPE RNA is a highly characterised, cell-line derived reference material that is commutable with biopsy samples. It serves as an appropriate control for end-to-end validation and optimization of RNA-seq, RT-qPCR or RT-ddPCR assays aimed at detecting *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET,* and *SLC34A2-ROS1* fusions.

G072. **Quantifying Fetal DNA in Maternal Blood Plasma by ddPCR Using DNA Methylation**

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Introduction: The proportion of cell-free DNA (cfDNA) circulating in maternal blood that originates from the fetus, the fetal fraction, is an important quality control metric when performing tests on fetal-derived cfDNA. Epigenetic differences produce dissimilar DNA methylation patterns, allowing for leveraging regions of high methylation contrast using methylation-sensitive restriction enzyme (MSRE) digestion to quantify fetal and maternal DNA via droplet digital PCR (ddPCR). This advancement positions ddPCR as a faster and less expensive alternative to next-generation sequencing (NGS) for fetal fraction estimation. **Methods:** Assays were designed to target MSRE-compatible regions with high methylation contrast between maternal and fetal cfDNA. Fetal assays targeted sites hypermethylated in fetal cfDNA and maternal assays targeted sites hypermethylated in maternal cfDNA. The assay multiplex was tested against contrived and clinical samples using an in-droplet MSRE-ddPCR workflow. The reaction mix was dropletized to create about 20,000 droplets per 24-µL reaction, thermocycled, and analyzed in a QX ONE instrument. The thermocycling profile included a 45-minute MSRE incubation step prior to PCR amplification. Contrived samples were constructed by spiking DNA-free plasma with micrococcal nuclease-digested DNA from an amniotic fluid cell line ("fetal" component) and a B-lymphocyte cell line ("maternal" component). Clinical samples were remnant diagnostic samples with existing NGS non-invasive prenatal testing results attached. DNA was extracted from all samples with the Apostle MiniMax kit on the KingFisher Flex. **Results:** From an initial set of 15 assays, a final five-assay multiplex was produced following amplicon sequencing with NGS and ddPCR screening. Although amplicon sequencing did not completely predict ddPCR performance and non-specific interactions, it was valuable for guiding the final ddPCR screen. The five-assay multiplex, consisting of three fetal assays and two maternal assays, produced an excellent linear response against contrived samples from 0% to 25% fetal fraction (R^2 >0.99). Similarly, a high correlation was observed between ddPCR-estimated fetal fraction and NGS fetal fraction for a set of clinical samples (n = 6, plus two non-pregnant controls, R^2 >0.94). **Conclusions:** As an epigenetic trait, DNA methylation is a useful way to discriminate between otherwise highly similar DNA sequences in an efficient and effective manner. Leveraging DNA methylation may be done with minimal impact to the standard ddPCR workflow. The high sensitivity, speed, and direct quantification of ddPCR make it an attractive alternative to NGS for fetal fraction estimation.

G073. **Higher Resolution and Accurate Breakpoint Determination of a Balanced Translocation by Optical Genome Mapping**

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Introduction: Optical genome mapping (OGM) can detect and characterize structural variants present in the human genome. Whereas fluorescence *in situ* hybridization (FISH) and karyotype are considered standard of care for identifying translocations, OGM can be used to identify otherwise cryptic abnormalities and to better characterize breakpoints and gene content. **Methods:** We present a 12-year-old male with severe sensorineural deafness, reduced DNA repair, and atypical pigmentations. The subject had no history of basal cell or squamous cell carcinomas. The subject was identified to have an apparently balanced translocation between 9 and 22 (46, XY, t[9;22][p22;q11.2]) via routine FISH and karyotype but it was unclear whether it was causative of the clinical features observed. **Results:** OGM was performed to further delineate the translocation and revealed that the breakpoint regions mapped to the *CDKN2A* gene on chromosome 9 and a segmental duplication region (LCR-B) on chromosome 22. A similar translocation was reported previously in the literature. The authors of this case characterized a novel balanced t(9;22)(p21;q11.2) translocation in a patient with melanoma, DNA repair deficiency, and features of DiGeorge syndrome. They identified a unique fusion transcript encoding portions of p14ARF CDKN2A isoform) and a novel gene, *FAM230A*(LCR-B) that was suspected to be causative of the patient's clinical features. **Conclusions:** Importantly, the authors required multiple techniques including karyotype, FISH, bivariate flow sorting, and sequencing to characterize the translocation, whereas in our case, OGM achieved a close level of breakpoint resolution in a single step. This case highlights the benefit of OGM in characterizing complex rearrangements compared to standard of care.

G074. **Invitae's High-Throughput AMP-Based RUO VariantPlex NGS Workflow Offers Scalable and Customizable Targeted-Detection of Germline SNVs, Indels, and CNVs**

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Introduction: Accessible and reliable detection of germline genetic variations has become a key diagnostic tool in personalized healthcare. However, high costs and scalability challenges have limited laboratory adoption of germline testing for both preventative and cascade screening. Next-generation sequencing (NGS) assays designed for platform agnostic automation can improve overall implementation of germline testing by increasing throughput. Invitae's NGS-based anchored multiplex PCR (AMP) enrichment chemistry allows for scalable and customizable target selection. AMP panels enrich target regions using gene-specific primers opposing molecular barcoded adapters containing a universal primer binding site, enabling each primer to function independently. Primers can be added or removed from panels without negatively affecting assay performance, meaning that regions of interest can be effectively targeted. **Methods:** Here, we demonstrate an automation-friendly, platform-agnostic, liquid VariantPlex workflow that enables scalable germline testing with small, medium, or large AMP panels. We tested the equivalence of this liquid-based library prep kit to a commensurate lyophilized workflow, evaluating coverage depth and on-target percent across our small CFTR panel (one gene), and medium cardiomyopathy panel (20 genes), to our large expanded carrier panel (127 genes). Copy number variation (CNV) detection performance was tested using our Expanded Carrier panel and input sample material containing known *SMN1* and *SMN2* CNVs associated with spinal muscular atrophy. **Results:** Our liquid VariantPlex workflow resulted in equivalent target and base coverage compared to the lyophilized workflow across all panel sizes. For the small CFTR and large expanded carrier panel, >99.5% of targeted bases were covered at a depth of >50X for both the liquid and lyophilized assays. The medium cardiomyopathy panel achieved 96.1% and 94.7% coverage of targeted bases at >50X for the liquid and lyophilized assays, respectively. For all panels, >96.3% of all unique fragments were on-target. Finally, we saw high concordance when testing 16 samples containing known *SMN1* and *SMN2* CNVs using the liquid-based workflow. **Conclusions:** Invitae's lyophilized and fully liquid VariantPlex assays reliably detect germline variations across small (<20 gene), medium (20-100 gene), or large (>100 gene) panels without requiring assay re-optimization, primer redesign, or balancing. We have shown strong concordance between the liquid and lyophilized workflows for target coverage and on-target percent. Moreover, the liquid-based workflow is automation compatible and platform agnostic, making it ideal for scalable and customizable germline testing.