TT028. Enhanced Performance of Targeted NGS Assays Using Single-Vial Amplification

S. Barua, *D. Hemnauth*, *S. Hsiao*, *M. Mansukhani*, *H. Fernandes*
Columbia University Medical Center, New York, NY.

Introduction: PCR enrichment for NGS requires multiple reactions to amplify target regions leading to preferential amplification of adjacent overlapping amplicons. Failures due to suboptimal DNA quantity/quality and paucity of neoplastic cells hamper universal adoption of NGS assays. To overcome these limitations, we evaluated single tube Stem-Loop Inhibition Mediated Amplification (SLIMamp) technology (Pillar Biosciences) for detection of variants in solid tumors. The assay was challenged with samples harboring complex genomic variants including homopolymers, indels, promoter and splice-site alterations. **Methods**: The customized panel investigated 47 genes with indications for targeted therapy, immunotherapy and potential clinical trials. DNA libraries with 222 amplicons were prepared in a single tube and sequenced on the Miseq (Illumina). Analytic performance was established using 146 previously tested samples harboring 321 clinically relevant variants. Samples interrogated, originated from lung adenocarcinoma, colorectal and pancreatic cancer, melanoma, gastrointestinal stromal tumors, gliomas and thyroid cancers with neoplastic content =/>10%. The specimen types included formalin-fixed, paraffin-embedded (FFPE), fine needle aspirates, cell smears, Cytolyt solution and frozen tissue. Sensitivity, precision and reproducibility of the assay were evaluated at variant allelic fraction (VAF) of 2.5% with DNA input >2.5 ng. Sequence data were reviewed using two software systems NextGENe (SoftGenetics) and PiVAT (Pillar Biosciences). **Results:** Over 99% of the target areas achieved at least 800X coverage. The results were 100% concordant with orthogonal tests across all specimen types. The assay detected variants with VAF >2.5% in samples where the input DNA was 2.5 ng. The standard deviation of intra and inter-run precision and reproducibility for variants ranged from 0.01 to 0.92. Specificity studies using GIAB NA12878 showed excellent concordance with GetRM project. A correlation of R^2 = 0.98 was observed between the two informatics pipelines. Quality control metrics for monitoring assay performance using a reference control harboring 15 low VAF variants with Levey-Jennings parameters were notable. **Conclusions**: Our results establish that the novel technology enables amplification in a single-tube format with superior quality and uniformity of coverage. By reducing the smaller amplicons that contribute to sequencing errors, SLIMamp is able to generate repeatable, robust and reproducible results with minimal DNA input and neoplastic cellularity. Using a simple workflow, clinically informative results are obtained from FFPE tissue with compromised DNA quality. This method has potential to improve clinical outcome by shortening the turnaround times and overcoming NGS assay failures.

TT032. Improving the Reliability of Buccal Swab Germline Control Sampling with Rapid Cell Culture

E. Degelman[1], *M. Perizzolo*[2], *T. Beattie*[1], *E. Mahe*[1]
[1]*University of Calgary, Calgary, Alberta, Canada;* [2]*Alberta Public Laboratories, Calgary, Alberta, Canada.*

Introduction: With the use of next-generation sequencing in cancer, reliable patient-specific reference materials are becoming increasingly important. Many solid tumor analyses rely on buccal swabs for reference; given the risk of blood contamination, however, these are not adequate for hematological cancers. Other techniques such as skin fibroblast culture require invasive biopsies and potentially complex culturing methods. We surmised that the advantages of cell culture could be extended to buccal swabs and saliva samples, both non-invasive and rapid procurement methods, to isolate buccal epithelial cells for use as germline reference materials. **Methods:** Whole saliva and buccal samples were collected together from healthy controls and washed in phosphate-buffered saline; an aliquot was snap frozen in liquid nitrogen for later comparison. Using a modified explant culture method, samples were distributed onto

the surface of tissue culture flasks in minimal media volumes. Flasks were incubated lying flat in a 37°C, 5% CO2 incubator, allowing the buccal cells to adhere to the surface. Eagle's Minimum Essential media was then added carefully to the flask and incubated for additional time standing upright. Flasks were returned to the flat position, allowing the media to cover the cells and left overnight. Buccal cells were collected the following day using a standard trypsin method and DNA was isolated from the cultured cells and previously frozen whole saliva/buccal samples. **Results:** The use of a modified explant culture method resulted in excellent adherence of buccal epithelial cells to culture flasks without concurrent expansion of contaminating hematopoietic elements. Whole and cultured specimens were further compared using highly sensitive *IGH* and *TCR* (beta and gamma) gene rearrangement studies, inferring from each the degree of B- and T-cell contamination, respectively. In each of the tests, we observed a consistent near absence of *IGH* and *TCR* gene rearrangement amplicons in the cultured buccal samples when compared to the starting materials (these demonstrating definitive molecular evidence of B- and T-cell contamination). DNA quality for each sample was comparable, noting only reduction of total DNA in the cultured samples. **Conclusions:** We describe a non-invasive, rapid culture method of saliva and buccal samples offering a reliable source of germline materials free from hematopoietic cell contamination. As a proof of concept, we used varying buccal collection techniques to simulate contamination by hematopoietic cells and in each we were able to reliably purify epithelial cells. This method provides an excellent alternative when avoidance of invasive procurement methods and rapid turnaround are desired.

TT058. A Novel Approach to Next-Generation Sequencing-Based Assessment of T-cell Clonality

D.A. Oldridge, *C. Gentile*, *J.N. Rosenbaum*, *V.M. Van Deerlin*
Hospital of the University of Pennsylvania, Philadelphia, PA.

Introduction: T-cell receptor gamma (*TRG*) gene rearrangement analysis is useful in the diagnostic work-up of T-cell neoplasms. The current gold standard, capillary electrophoresis (CE), has limitations, most notably the inability to distinguish different *TRG* clonotypes with the same sequence length. Next-generation sequencing (NGS) of *TRG* clonotypes addresses this limitation, enabling more precise estimation of clonotype abundances. Carefully designed computational pipelines are crucial to handle the technical complexity of NGS-based methods and maximize their clinical yield. Here, we present a novel approach for analyzing NGS clonality data in the context of single specimens and paired blood/tissue specimens. **Methods:** *TRG* sequencing data were generated using the LymphoTrack *TRG* Panel-MiSeq (Invivoscribe, San Diego, CA) assay from residual patient specimens with prior CE profiling (n = 62). A computational pipeline was developed in the R programming language to address technical challenges, including NGS-associated sequencing errors, distinguishing prominent clones from background, and differentiating cross-sample contamination from truly related clonotypes on multiplexed runs. The final results are displayed in a custom dashboard with clonotypes grouped by sequence length (a “virtual electropherogram”), as well as by clone rank and V-J segment. Result interpretation was evaluated using multiple criteria and concordance was assessed between NGS and CE assays. **Results:** When accounting for sequencing errors, merging (allowing up to 1 to 2 bp mismatches) across all unique reads yielded higher average clonotype frequencies amongst top 10 clones compared to merging only the top 500 sequences for computational efficiency, as recommended by the manufacturer. Objective criteria for calling prominent clones versus background were established using Poisson statistics and clonotype frequency cutoffs. Comparison of CE and NGS results showed an overall concordance of 57% to 70% depending on choice of interpretive criteria; notably, in patients with a definitive T-cell malignancy, the concordance was considerably higher. Preliminary analysis of paired blood/tissue data versus non-related samples show promising results for distinguishing true relatedness from cross-sample contamination. **Conclusion:** Careful computational pipeline

design has addressed many of the technical and diagnostic complexities of clonality assessment by NGS. Future efforts will focus on limit of detection analyses to refine our criteria for cross-sample clonotype detection, enabling improved determination of minimal residual disease compared to CE. We hope that our experience may serve as a guide to other laboratories that have deployed or are considering transition to clonality assessment by NGS.