PNWNCS NCS Research Day Poster Abstract

Optimization of RNA isolation and Processing for RNA-Seq: Utility of Field-Collected and Self-Collected Biological Samples

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This formative research project is evaluating the use of buccal cells and venous blood samples for characterizing environmental response. Compared to microarray and other gene expression platforms, next-generation (next-gen) sequencing technologies produce a broader depth of coverage which we are applying for evaluating NCS specimens. We are exploring the feasibility and cost-effectiveness of using next-gen sequencing for improving the utility of gene expression profiles to evaluate health status and environmental exposure signatures in human populations. In order to meet these goals, new procedures for optimization of RNA isolation and characterization are needed. We used three types of biological samples: whole blood, buffy coat, and buccal swabs. Bioanalyzer and RNA quantification results showed that the two blood-derived samples produced RNA of sufficient quantity, hundreds of nanograms to microgram amounts, and quality, with RNA integrity numbers (RINs) $\geq$7, for direct RNA sequencing. Buccal RNA samples exhibit low yields, less than 100 ng, and RIN values ~2, indicating significant degradation. These results show that buccal-derived RNA is not currently useful for RNA sequencing approaches without further optimization. Several amplification strategies are being implemented to increase the material present for sequencing and to address the degraded nature of the samples. Current results show that ~10 µg of cDNA can be generated with as little as 10 ng of RNA input. Bioinformatic analyses are currently being done to determine if useful gene expression information can be obtained with the amplified products. These results will expand the utility of field-collected samples, particularly non-invasively self-collected specimens.